

Preclinical Characterization of a Bispecific Bridging Antibody for the Treatment of LAMA2-Related Congenital Muscular Dystrophy

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Abstract

LAMA2-related congenital muscular dystrophy (LAMA2-CMD) is a devastating pediatric disease caused by the loss of laminin- α 2 for which there is no disease-modifying treatment. Laminin- α 2 is the α chain of laminin-211, the heterotrimer that assembles the basement membrane around each muscle fiber and anchors it to the sarcolemma. Muscle lacking laminin- α 2 overexpresses a related laminin, laminin- α 4, but the protein fails to self-polymerize and binds α -dystroglycan only weakly, and thus fails to compensate for the missing laminin- α 2. We hypothesized that a bispecific antibody bridging laminin- α 4 to β -dystroglycan (β -DG), the transmembrane subunit of dystroglycan, would restore basement membrane anchorage and sarcolemma stability. We engineered such bispecific bridging antibodies in several formats and studied two in depth, one bivalent and one tetravalent. Both bound their targets with nanomolar affinity and bridged dystroglycan-overexpressing cells to a recombinant fragment of laminin- α 4. In a pilot study in laminin- α 2-deficient *dy*^W mice, the bivalent antibody was recovered from muscle days after last dose, at concentrations exceeding those required for bridging *in vitro*. Meanwhile, mice treated with the tetravalent construct developed about twice the peak force and grip strength of vehicle-treated mice. These data identify antibody design as a major determinant of bridging efficiency, suggest that reaching translationally relevant antibody exposures in muscle is feasible, and provide proof of principle for the therapeutic approach, supporting the continued progression of Anthropic's bispecific bridging antibody programs in LAMA2-CMD and α -dystroglycanopathy.

Children with LAMA2-related congenital muscular dystrophy (LAMA2-CMD), caused by the loss of laminin- α 2 (ref. 1), are hypotonic and weak from birth or early infancy.² In one cohort of 42 children with complete deficiency, a single child walked independently, about half needed nocturnal ventilation and one in six died before adulthood.³ Care remains supportive, and no disease-modifying treatment is available.^{4–6} Laminin- α 2 is the α chain of laminin-211 (α 2 β 1 γ 1),⁷ the heterotrimer that assembles the basement membrane around each muscle fiber and anchors it to the sarcolemma. Laminin-211 polymerizes through the laminin N-terminal (LN) domains at the tips of its short arms, and each node of the polymer joins one α , one β and one γ LN domain from neighboring molecules.⁸ At the opposite end, laminin-211 binds integrin α 7 β 1 and, through the laminin globular (LG) domains of its α chain, α -dystroglycan (α -DG), whose transmembrane partner β -dystroglycan (β -DG) binds dystrophin.^{9–11} Loss of laminin- α 2 therefore removes both polymerization and anchorage, leaving a disrupted basement membrane around fibers that degenerate from the first months of life.^{12,13}

Muscle that lacks laminin- α 2 instead carries laminin-411 (α 4 β 1 γ 1) around its fibers in the *dy/dy* and *dy*^{3K} mouse models^{7,14} and in patients from infancy to adulthood.^{15,16} In one series of biopsies, laminin- α 4 was increased in all patients with laminin- α 2 deficiency.¹⁷ Laminin- α 4 normally surrounds embryonic mouse myotubes and disappears from the fiber surface within the first postnatal week, persisting at synapses and in capillaries and perineurium. Its presence around laminin- α 2-deficient fibers was therefore first described as compensatory retention.⁷ Consistent with retention, immunoblots showed similar amounts of laminin- α 4 in muscle of wild-type and laminin- α 2-deficient *dy*^W mice at birth and a decline over 8 weeks

only in the wild type,¹⁵ and *Lama4* RNA transcripts were more abundant than wild type in the muscle of *dy*^W mice.¹⁸ Another study, however, found laminin- α 4 increased in blood vessels, perineurium and isolated regenerating fibers of *dy/dy* mice.¹⁹ Whether laminin- α 4 expression persists from development or is induced anew, for instance by regeneration, remains unsettled. In either case, laminin- α 4 has no LN domain, so laminin-411 does not polymerize on its own, and its LG domains bind α -DG only weakly.^{15,20} Laminin-411 therefore does not functionally compensate for laminin-211, and muscle that carries it still degenerates.

Polymerization and anchorage can nevertheless be supplied to laminin-411 in trans by two engineered linker proteins, α LNNd, which gives laminin-411 a polymerizing arm, and mini-agrin, which links laminin to α -DG.^{15,21,22} Each linker fits one adeno-associated virus (AAV) vector,^{23,24} whereas the *LAMA2* coding sequence is about twice the packaging capacity of these vectors.²⁵ In *dy*^W mice, a single intravenous injection of the two vectors on postnatal day 1 brought weight gain, gait speed and muscle mass relative to body mass close to normal at 8 weeks (ref. 24). Injection at 3 weeks of age, at half the dose per kilogram, was less effective but still conferred substantial benefit.²⁴ When the linkers were expressed from transgenes in *dy*^W mice, mini-agrin alone raised median survival from 15.5 to 50 weeks, and the two linkers together extended it to 81 weeks (ref. 15). These experiments showed that the laminin remaining in deficient muscle is a usable scaffold, and that restoring anchorage alone more than tripled survival in this model. Other approaches for repairing the basement membrane have included activation of the paralog *LAMA1* with AAV vectors^{26,27} and injection of laminin-111 protein, which improved strength and survival of *dy*^W mice.²⁸ Neither

the linkers nor these other approaches have entered an interventional trial (ClinicalTrials.gov, October 2026).

A bispecific bridging antibody designed to reconnect laminin to the sarcolemma has already been tested in the α -dystroglycanopathies, a related group of disorders in which α -DG lacks the glycan that binds laminin-211 (ref. 29). This antibody carried two binding sites each for the last two LG domains (LG4-5) of laminin- α 2 and for the ectodomain of β -DG, and it improved grip strength and treadmill running in adult *Large*^{myd-3J} mice, a model of these disorders.³⁰ In LAMA2-CMD, the missing connection between the remaining laminin and the sarcolemma is likewise extracellular and in principle accessible to a circulating protein. We therefore hypothesized that a bispecific bridging antibody targeting β -DG and LG4-5 of laminin- α 4 would bridge laminin-411 to the sarcolemma and restore anchorage. We kept β -DG as the anchor because it appears to persist in laminin- α 2-deficient muscle of mice³¹ and of two patients with residual laminin- α 2 (ref. 32), and because the same anchor could then serve LAMA2-CMD and the α -dystroglycanopathies.

Here, we set out the rationale for a bispecific bridging antibody in LAMA2-CMD and test the hypothesis with two tool molecules built on published variable regions. We show that both antibodies bridge dystroglycan-overexpressing cells to a recombinant fragment of laminin- α 4 *in vitro*, which we take as a proxy for the anchorage they are meant to restore. We also report proof of principle for the therapeutic approach with one of the two antibodies in *dy*^w mice. Finally, we describe how we now work with Claude, a large language model, on this program.

Background and rationale

Genetics, pathophysiology and natural history

Genetics and disease phenotype

Merosin, whose heavy chain is now called laminin- α 2, was first described in 1988 as a basement membrane protein of striated muscle, Schwann cells and trophoblast.³³ Its absence from muscle of 13 patients with congenital muscular dystrophy (CMD) was reported in 1994 (ref. 34), and a year later the first mutations in *LAMA2*, which encodes laminin- α 2, were identified.¹ LAMA2-related muscular dystrophies are autosomal recessive and span a clinical spectrum from early-onset “classical” LAMA2-CMD, formerly called merosin-deficient CMD (MDC1A), to a limb-girdle muscular dystrophy (LGMD R23) with ambulation preserved for decades.^{5,35}

Disease severity correlates with the class of *LAMA2* variant. Of 309 distinct disease-associated variants in the international *LAMA2* database, 54% were truncating (nonsense or frameshift) variants, predicted to abolish the protein, and 14% were missense variants or small in-frame deletions, predicted to spare most of it (Fig. 1A).³⁶ The remaining splice-site and copy-number variants can abolish laminin- α 2 or leave residual protein.^{37–39} Accordingly, patients with truncating variants on both alleles usually have no detectable laminin- α 2 in muscle and classical LAMA2-CMD.^{5,36,40,41} Conversely, in a Russian series, 4 of 7 patients with milder disease and none of 83 with the classical form carried a missense variant.⁴²

Among variants that spare the protein, disease severity appears to depend on which function of laminin- α 2 is lost. Laminin- α 2 is a chain of 3,122 residues whose LN domain mediates polymerization and whose five LG domains bind α -DG and integrins (Fig. 1A).^{43,44} Missense variants in the LN domain are thought to impair polymerization, and one of them, p.Gln167Pro, lowered it

by about 60% *in vitro*.⁴⁵ Patients with these variants usually have reduced but detectable laminin- α 2 in muscle and walk, some into adulthood, even when the second allele is truncating.^{5,35,36,40,42,46} In contrast, missense variants and in-frame deletions in the LG domains, such as p.Gly2889Arg in LG4, can cause severe congenital disease although residual laminin- α 2 is detected at the sarcolemma.^{5,38,42,47} These observations suggest that anchorage of the basement membrane to α -DG and integrins through the LG domains is more important for normal muscle function than polymerization through the LN domain, and anchorage is the function that a bispecific bridging antibody would restore.

Disease severity also depends on how much laminin- α 2 remains in muscle.^{3,48} In a London series, 2 of 33 patients (6%) with no detectable laminin- α 2 walked independently, compared with 5 of 13 patients (38%) with residual staining ($P = 0.02$).³⁷

Natural history

The early-onset form is best documented in 42 London children classed as completely deficient in laminin- α 2 (ref. 3; Fig. 1B, C). Weakness and hypotonia are present at or soon after birth.^{2,3} Most children learn to sit, at about one year, but only 1 of the 42 walked independently. As contractures and scoliosis progress through the first decade, some children lose the ability to sit.^{2,3} Because intercostal and accessory muscles are weak,⁵ forced vital capacity was below 60% of predicted in 23 of 25 children at first spirometry (median age 8.7 years). Half were ventilated at night by 11.7 years, and seven had died by 17 years.³ In a Chinese series in which few children were ventilated, 23 of 116 died, mostly of respiratory failure after pneumonia.²

Nerve, brain and heart are also involved but seldom dominate the clinical picture (Fig. 1B). White-matter T2 hyperintensity appears after about six months in nearly all patients and is compatible with normal cognition.^{52,53} About one fifth of patients have a cortical malformation,^{2,41} and 10 to 36% develop epilepsy.^{3,54} Motor nerve conduction is often slowed,³ but neuropathy rarely manifests clinically and is thought to contribute far less to weakness in patients than in *Lama2*-mutant mice.^{5,55,56} Cardiac findings are usually subclinical.^{2,3} Skeletal muscle therefore limits function and survival in childhood.

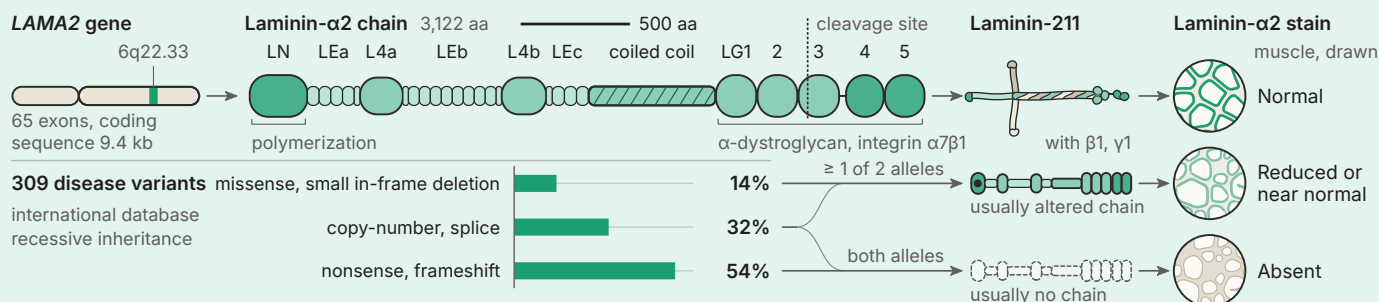
Laminin-211 and the muscle basement membrane

Assembly of the basement membrane

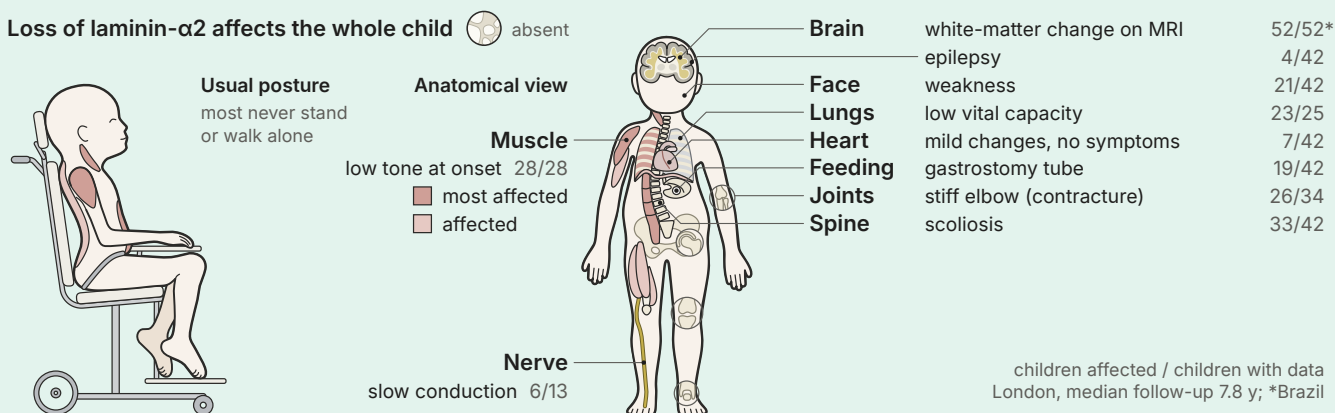
The sarcolemma of each muscle fiber is coated by a basement membrane whose outer face merges with the endomysial collagen that neighboring fibers share (Fig. 2A).⁵⁷ Contractile force is transmitted to the endomysium laterally, across the sarcolemma and the basement membrane, as well as longitudinally at the ends of the fiber.⁵⁷ Lateral transmission may account for the severity of the disease that follows loss of a single basement membrane protein, laminin- α 2. Consistent with this view, tetanic stress of muscles from *dy/dy* mice was depressed under uniaxial but not under biaxial load, which suggested that laminin transmits force between adjacent fibers.⁵⁸ The basement membrane is therefore part of a load-bearing composite, although its share of the load has not been measured.

Laminin-211 is the main extrasynaptic laminin of the adult muscle fiber and is the organizer of the basement membrane.⁷ Its three short arms are each tipped by an LN domain, and its long arm, about 80 nm, ends in the five LG domains of laminin- α 2 (ref. 63). In the model of basement membrane assembly, both ends of the molecule play a role (Fig. 2B).⁶⁵ The LG domains bind sulfated glycolipids and receptors, which concentrates laminin on

A Most LAMA2 disease variants are predicted to abolish laminin- α 2



B Loss of laminin- α 2 affects the whole child



C Onset in infancy, complications through childhood

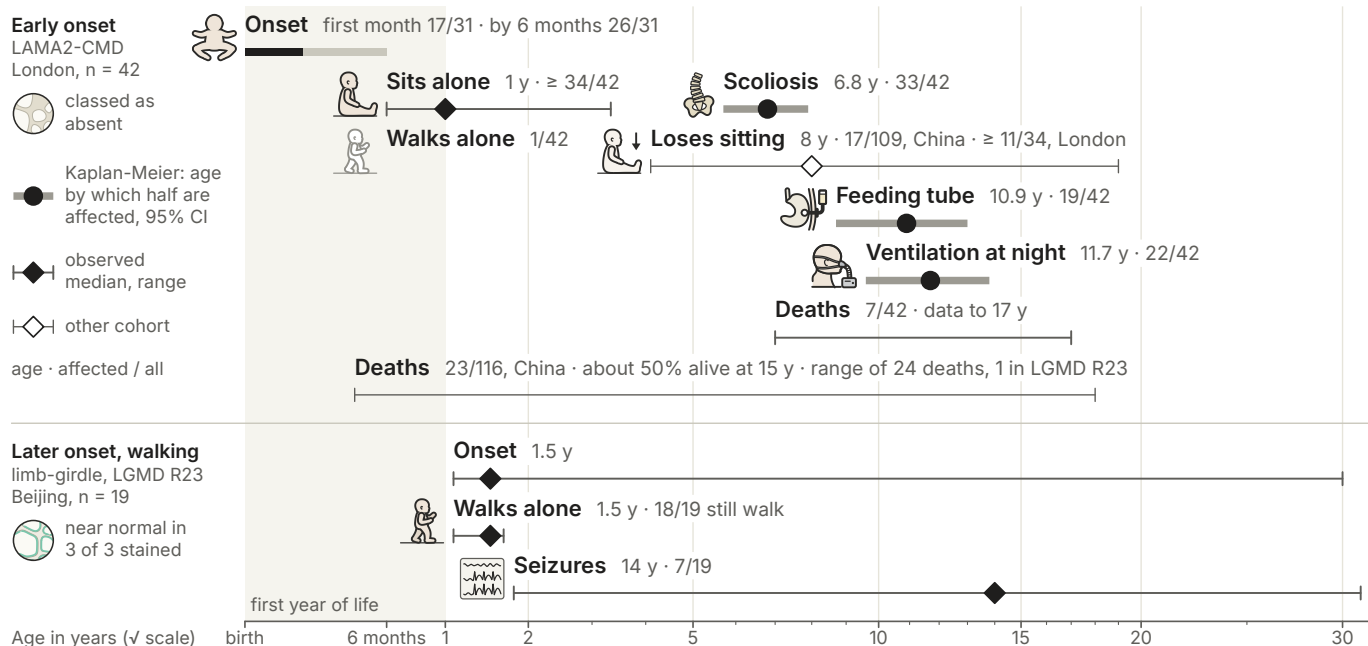
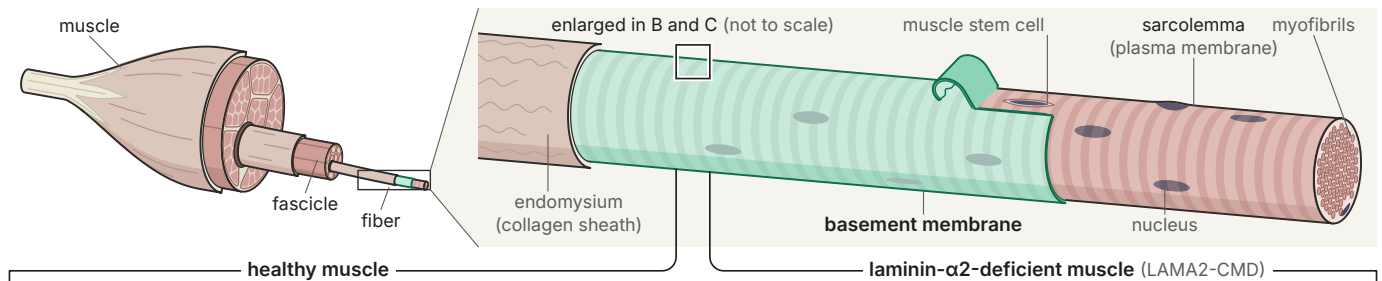


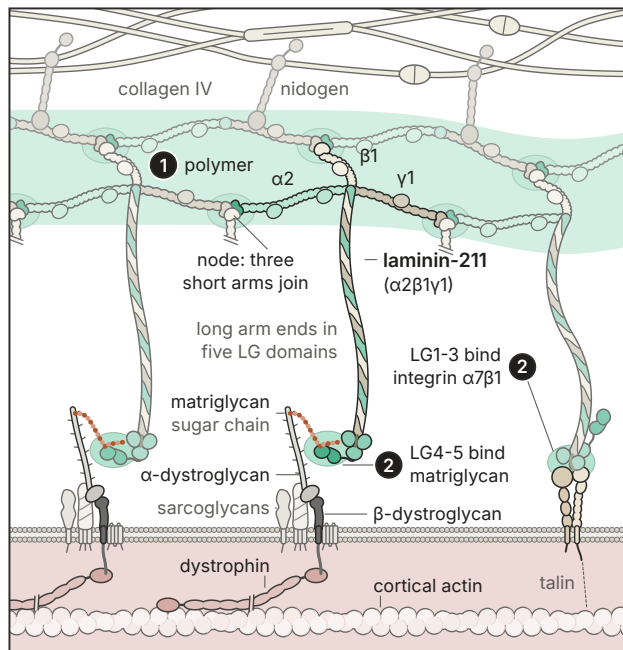
Figure 1. Loss of laminin- α 2 causes a multisystem disease established in infancy. (A) Laminin- α 2, to scale in residues of the original cDNA,⁴⁹ with LG domains and cleavage site placed schematically.^{43,50} Bars show percentages of 309 distinct disease-associated variants.³⁶ Dot, missense variant. Stains are drawings. Arrows give the usual outcome. One family with a homozygous missense variant had complete deficiency,³ and two siblings with two truncating variants had near-normal staining.⁵¹ (B) Schematic, including shading of axial and proximal muscles. Fractions refer to 42 London children classed as

completely deficient ("absent") by staining or, without biopsy, by genotype and presentation.³ Asterisk, Brazilian series of 52 patients across the clinical spectrum.⁴¹ (C) Upper lane, London children. Open diamond and thin lines, 116 Chinese children with early onset.² Lower lane, 19 patients enrolled with onset after 1 year and walking before 2 years.⁴⁶ Partial deficiency, mostly of early onset, is not shown. Ages at ventilation and gastrostomy reflect local practice. CI, confidence interval.

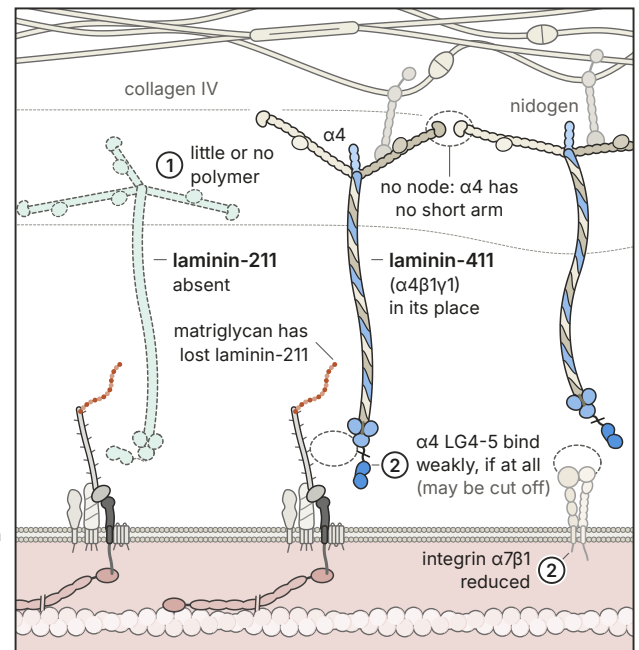
A Every muscle fiber is wrapped in a basement membrane



B Laminin-211 ① polymerizes, ② anchors to the fiber



C Laminin-411 barely ① polymerizes or ② anchors



D Fibers die, repair fails and scar replaces them

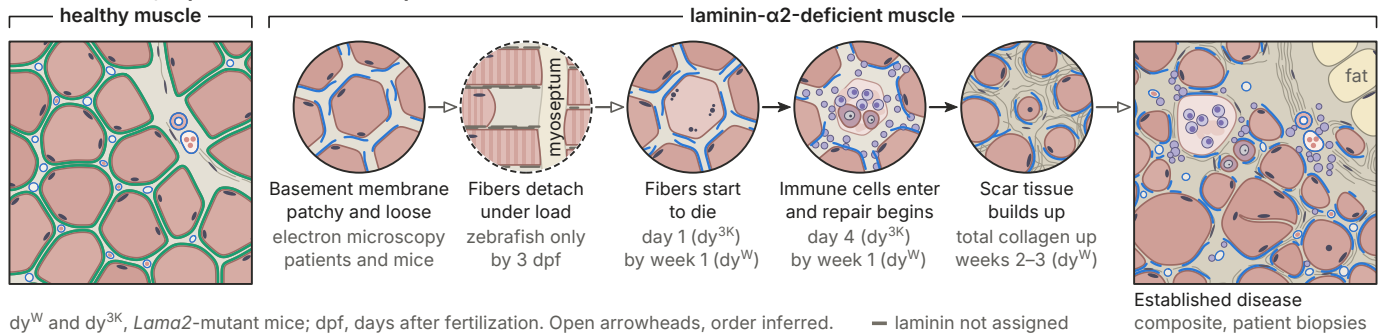


Figure 2. Laminin-211 polymerizes and anchors the basement membrane to the fiber, and polymerization and anchorage fail without laminin-α2. (A) Enlargements lead from muscle to a patch of fiber surface. (B) Laminin-211 (laminin-α2, green) polymerizes through nodes of three LN domains, and nidogen can link it to collagen IV. Its LG domains bind integrin α7β1 and matriglycan on α-DG, which β-DG (charcoal) links to dystrophin and actin. The polymer sheet is viewed obliquely, with receding arms foreshortened. (C) Without laminin-211 (dashed outline), laminin-411 (laminin-α4, blue) forms little or no polymer and binds integrin α7β1 and α-DG weakly, if at all (open disks).

Slashes mark where LG4-5 of laminin-α4 can be cleaved.²⁰ (D) Stages are shown with model and age.^{12,15,59-62} Solid arrowheads join stages timed in the same model. Gaps in blue rings depict disruption seen by electron microscopy, and “loose” denotes that laminin-411 is extracted more easily than laminin-211. The zebrafish basement membrane (gray) is assigned no laminin. Laminins, the extracellular core of the dystrophin-glycoprotein complex and actin are to scale (bar, 20 nm). All else, including the upright orientation of laminin,⁶³ is schematic. Perlecan, agrin, laminin-511, sulfated glycolipids and the furin-released N-terminal domain of α-DG⁶⁴ are omitted.

the cell surface.⁶⁶ There, LN domains of three molecules join in a node of one α, one β and one γ domain,⁸ and laminin-111 (α1β1γ1) lacking any one of the three formed no polymer.⁶⁷ Nidogen can link the laminin polymer to the collagen IV network,⁶⁸ and the proteoglycans perlecan and agrin add collateral links.⁶⁵ Of the

three chains, only laminin-α2 takes part in both polymerization and receptor binding.

Anchorage to the fiber through dystroglycan and integrin $\alpha 7 \beta 1$

The LG domains of laminin-211 bind integrin $\alpha 7 \beta 1$ and dystroglycan (Fig. 2B), which is cleaved from a single precursor into extracellular α -DG and transmembrane β -DG.⁶⁹ α -DG binds laminin through matriglycan, a polymer of xylose and glucuronic acid that the glycosyltransferase LARGE1 synthesizes on the mucin-like region of α -DG.^{70,71} α -DG binds laminin- $\alpha 2$ at LG4-5 and also at LG1-3 (ref. 10). Laminins bind integrins through LG1-3 and the C-terminal tail of $\gamma 1$ (refs. 9,72). In crystals of LG4-5 with a matriglycan oligosaccharide, a single disaccharide chelates the calcium ion of LG4. Affinity nevertheless rose with chain length, from a dissociation constant (K_D) of 3.7 μ M for three sugars to 0.23 μ M for five sugars, and long chains were predicted to favor rebinding.⁷³ Accordingly, mouse muscle with only short matriglycan bound laminin but was not protected from eccentric contraction.⁷⁴ This finding suggests that a link able to withstand contraction depends on avidity, that is, on multiple binding sites that allow laminin to rebind along a long matriglycan chain. The same requirement may apply to an antibody that substitutes for this link.

The cytoplasmic tail of β -DG binds dystrophin,¹¹ and along with the sarcoglycans and sarcospan, these proteins form the dystrophin-glycoprotein complex (DGC).⁷⁵ Cryo-electron microscopy maps of the native DGC^{76,77} suggest how load is transmitted through the complex. The cleavage site between α - and β -DG lies within a single folded domain to which both subunits contribute, and a flexible linker tethers this domain to the transmembrane helix of β -DG. The adjoining immunoglobulin-like domain of α -DG attaches to the edge of a tower, about 13.5 nm tall, formed by β -, γ - and δ -sarcoglycan. Load on matriglycan must therefore be transmitted along the mucin-like region to the immunoglobulin-like domain, where it could divide between the tower and the tether of β -DG.

Although dystroglycan and integrin $\alpha 7$ both contributed to force production in mouse muscle, only the loss of dystroglycan detached the basement membrane from the sarcolemma and rendered mice prone to contraction-induced injury.⁷⁸ The muscle of *Large^{myd}* mice, in which the DGC is intact but matriglycan is missing, displays the same defects.⁷⁸ Protection from injury therefore depends on the bond between matriglycan and the basement membrane. A laminin- $\alpha 1$ transgene without LG4-5, the sole dystroglycan site of laminin- $\alpha 1$, corrected diaphragm and heart of laminin- $\alpha 2$ -null *dy^{3K}* mice, whereas limb muscles degenerated.⁷⁹ Polymerization and integrin binding, which this truncated chain retains, thus suffice in the diaphragm and heart, but limb skeletal muscle uniquely requires the link to dystroglycan.

Consequences of laminin- $\alpha 2$ deficiency

Without laminin- $\alpha 2$, the $\beta 1$ and $\gamma 1$ chains persist, predominantly assembled with laminin- $\alpha 4$ to form laminin-411. Laminin-411 forms little or no polymer and is poorly anchored to the sarcolemma (Fig. 2C).^{14,15} Consistent with these properties, the basement membrane of the muscle fibers was severely disrupted on electron microscopy in three patients (Fig. 2D).¹² The two laminin receptors are affected differently by the loss of their ligand. Integrin $\alpha 7$ was markedly reduced in the muscle of patients and mice,⁸⁰ whereas staining for dystroglycan was increased in *dy^{3K}* mice.^{14,31} In patients, β -DG and dystrophin stained normally in two cases with residual laminin- $\alpha 2$ (ref. 32), and their staining was deranged on the degenerating fibers of infants.¹³ These immunostaining data suggest that the DGC persists on the

sarcolemma until a fiber degenerates, although its main ligand is absent.

Animal models indicate that a laminin- $\alpha 2$ -deficient fiber fails by detaching from the surrounding matrix, whereas a dystrophin-deficient fiber fails when its sarcolemma becomes permeable. Evans blue, a dye that normal fibers exclude, labeled groups of fibers in *mdx* mice, which lack dystrophin, but only occasional single fibers in hypomorphic *dy/dy* and *dy^{2J}* mice.⁸¹ Failure of laminin- $\alpha 2$ -deficient fibers has been imaged only in zebrafish, in which loaded fibers of the *lama2* mutant *candyfloss* detached from the myoseptum with membranes still impermeable and underwent apoptosis later (Fig. 2D).⁵⁹ Sparing of the sarcolemma is unexpected, because loss of dystroglycan or matriglycan renders it fragile.⁷⁸ We suspect that other ligands of α -DG, such as perlecan, still occupy matriglycan,^{10,14} in which case the plane of failure would lie within the basement membrane and the DGC would remain bound to the matrix. The sarcolemma and the DGC of a laminin- $\alpha 2$ -deficient fiber would thus remain intact until the fiber detaches. Restoring the connection between the DGC and the remaining laminin would therefore address the primary defect of the disease.

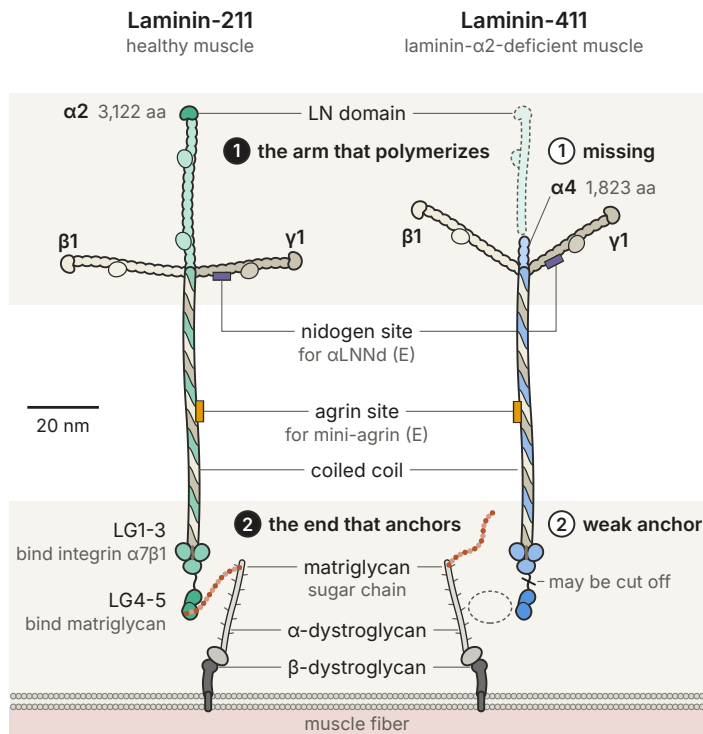
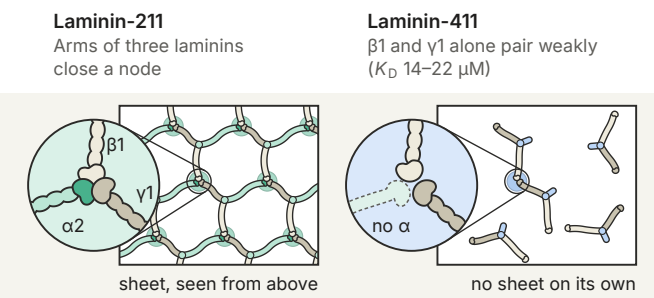
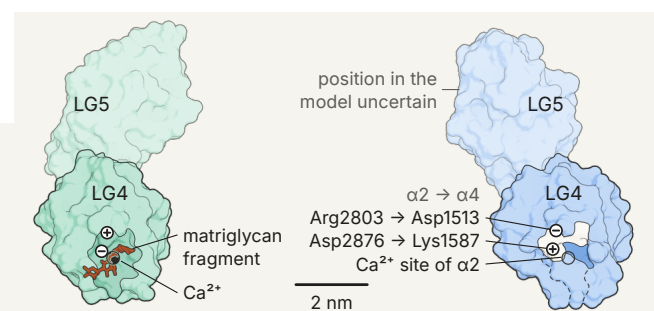
Cell death is the earliest postnatal event recorded after loss of laminin- $\alpha 2$ (Fig. 2D). In *dy^{3K}* mice, the most severely affected strain,⁵⁶ limb muscle was normal at embryonic day 18.5 and contained apoptotic, caspase-3-positive cells on postnatal day 1, and degenerating fibers, mononuclear infiltrates and collagen III deposits were present by day 4 (ref. 60). Inflammation and matrix deposition thus followed cell death and are most simply explained as responses to it. By week 4, the tibialis anterior of hypomorphic *dy^w* mice⁸² contained one third of the normal number of fibers.⁶¹ Regeneration also requires laminin- $\alpha 2$, because injured muscle of *dy^w* mice rarely completed repair unless the mice carried a *LAMA2* transgene.⁶²

The downstream processes in muscle follow from the loss of a single extracellular connection, as restoration of laminin- $\alpha 2$ to the muscle of *dy^w* and *dy^{2J}* mice with a *LAMA2* transgene greatly improved morphology and longevity.⁸³ These mice nevertheless remained lame in the hindlimbs, most likely because Schwann cells also depend on laminin- $\alpha 2$, whose loss results in partial myelination of spinal roots.⁸⁴ This peripheral neuropathy is a feature of the mouse models. In patients, it rarely manifests clinically, and its contribution to weakness is considered minimal.^{5,56} Persistent hindlimb lameness in mice that receive a muscle-directed treatment therefore reflects disease of the nerve and has little counterpart in patients.

Laminin-411 in laminin- $\alpha 2$ -deficient muscle

Expression and function of laminin- $\alpha 4$

Laminin- $\alpha 4$ is normally a component of vascular, neural and synaptic basement membranes. All endothelial cells produce laminin-411 (ref. 85), and *Lama4*-null mice have perinatal hemorrhages and discontinuous capillary basement membranes, which indicates a role in the maturation of microvessels.⁸⁶ In peripheral nerve, laminin-411 accompanies laminin-211 in the basement membrane of Schwann cells, and its loss impairs the sorting and myelination of axons.^{87,88} At the neuromuscular junction, laminin-421 ($\alpha 4 \beta 2 \gamma 1$) aligns the active zones of the nerve terminal with the folds of the postsynaptic membrane.⁸⁹ Laminin- $\alpha 4$ also surrounds developing muscle fibers,^{7,86} but in adult muscle it is very low or undetectable outside vessels and synapses.¹⁵ An anti-laminin- $\alpha 4$ antibody will therefore also encounter its target in vessels, nerves and synapses.

A Laminin-411 ① lacks the α short arm and ② anchors weakly**B** ① A polymer node needs an α LN domain**C** ② $\alpha 4$ reverses two charges of the matriglycan site**D** Laminin-411 is deficient in both functions

	211	411	+ linker
Found around the fiber in			
healthy muscle	●	○	
$\alpha 2$ -deficient muscle	○	●	
Functions			
① Polymerizes	●	○	● α LNNd
② Binds α -dystroglycan	●	○	● mini-agrin
② Binds integrin $\alpha 7 \beta 1$	●	○	
Docking sites for the linkers			
Nidogen site on $\gamma 1$	●	○	● α LNNd
Agrin site on $\gamma 1$	●	○	● mini-agrin

● yes ○ present, loosely held ● weak ○ little or none

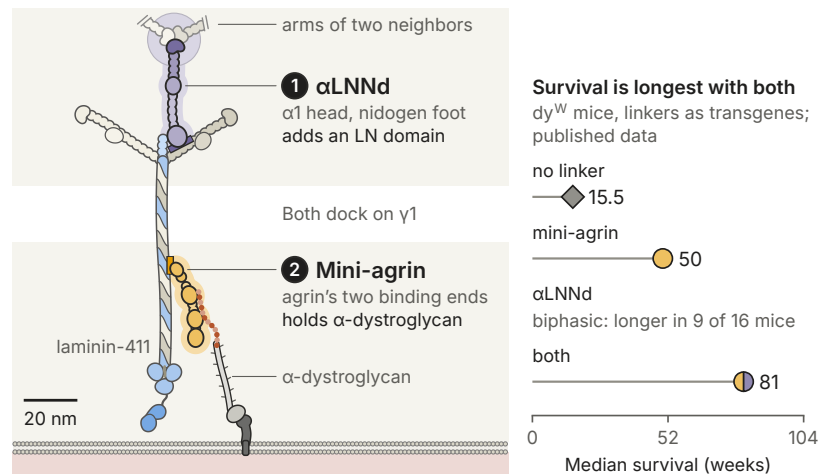
E Two linker proteins supply ① an arm and ② an anchor

Figure 3. Laminin-411 lacks the polymerizing arm and receptor affinity of laminin-211, and linker proteins supply an arm and a link to α -DG. Disks mark polymerization (1) and anchorage (2), open where deficient. (A) Laminins are to scale. Lengths follow laminin-111 (short arms of laminin- $\alpha 2$ and laminin- $\alpha 4$ estimated).^{44,63} Dashes mark missing parts, and bars mark linker-binding sites. (B) A node is formed by the LN domains of one α , one β and one γ chain.^{8,91} Nets are idealized. (C) LG4-5 of mouse laminin- $\alpha 2$ (PDB 5IK8)⁷³ and human laminin- $\alpha 4$ (AlphaFold model, no ions)⁹² are shown after superposition of

LG4, with charges of aligned residues. Shaded residues of laminin- $\alpha 2$ contact Ca^{2+} or matriglycan. On laminin- $\alpha 4$, aligned residues are replaced (white), conserved (dark blue) or uncertain (dashed). (D) Circles score presence or activity (integrin, adult $\alpha 7 \times 2 \beta 1$).^{7,9,10,15,20,93,94} The third column shows laminin-411 with equimolar linker *in vitro*. (E) Linkers bind $\gamma 1$ (lengths estimated, poses schematic).^{21,95,96} The plot shows median survival of dy^W mice with muscle-specific transgenes ($n = 16$ for α LNNd and for both linkers, no median reported for α LNNd).¹⁵

Laminin- $\alpha 4$ is increased in the laminin- $\alpha 2$ -deficient muscle of mice and patients, where it occupies the basement membrane of the fiber. It stained intensely in the extrasynaptic basement membrane of adult dy/dy mice,⁷ was redistributed from capillaries to the sarcolemma in the muscle of dy^{3K} mice,¹⁴ and colocalized with $\beta 1 \gamma 1$, its partner chains in laminin-411, in biopsies of patients.¹⁵ Because laminin- $\alpha 4$ was increased in all laminin- $\alpha 2$ -deficient biopsies, whereas laminin- $\alpha 5$ was increased in many

dystrophies, the increase in laminin- $\alpha 4$ was proposed to be a specific compensatory response to the loss of laminin- $\alpha 2$ (ref. 17).

The increase in laminin- $\alpha 4$ is several-fold in magnitude and extends to most fibers. *Lama4* RNA transcripts were about 4-fold more abundant than wild type in the muscle of dy^W mice,¹⁸ in which immunoblots suggested retention of the embryonic laminin- $\alpha 4$ chain.¹⁵ In muscle extracts from dy^{3K} mice, laminins that contain laminin- $\alpha 4$ reached about 30% by mass of the laminins that contain laminin- $\alpha 2$ in wild-type muscle, mostly

in the detergent-soluble fraction.¹⁴ Muscle proteomes of four patients contained 5.4-fold more laminin- α 4 than those of four controls.⁹⁰ In 49 biopsies from 43 patients aged 2 months to 27 years, fibers with sarcolemmal laminin- α 4 occupied a median of 80% of the sample area and at least half of it in 40 of the 47 biopsies that could be scored, whereas sarcolemmal staining was absent from nine age-matched controls.¹⁶ Laminin-411 is thus broadly distributed on the muscle fibers of most patients from infancy to adulthood, which suggests that it offers sufficient substrate for bridge formation.

Polymerization and receptor binding

Laminin-211 and laminin-411 differ only in the α chain (Fig. 3A). Human laminin- α 2 (3,122 residues) carries a full short arm capped by an LN domain, whereas in laminin- α 4 (1,823 residues) this arm is truncated to EGF-like modules.^{43,49} Because a polymer node requires an α LN domain to stabilize the bond between the β 1 and γ 1 LN domains,⁸ laminin-411 alone is not expected to polymerize (Fig. 3B). Accordingly, it has been shown to form little to no polymer in *in vitro* sedimentation assays.¹⁵

The LG domains of laminin- α 4 are also poor ligands for the receptors of the fiber (Fig. 3A). Recombinant LG fragments of laminin- α 4 have 30- to 100-fold lower affinity for α -DG than LG1-3 of laminin- α 2 (ref. 20), and full-length laminin-411 exhibits minimal binding to integrin α 7 β 1 or α -DG,¹⁵ the latter of which may be explained by the absence of a Ca^{2+} site. In the crystal structure of LG4-5 of laminin- α 2, matriglycan binds through the Ca^{2+} ion of LG4 (ref. 73), and no equivalent site is predicted from the laminin- α 4 sequence.^{43,97} When we superposed LG4 of the AlphaFold model of human laminin- α 4 (ref. 92) on this mouse structure, laminin- α 4 residues of opposite charge aligned with Asp2876, one of two aspartates that bind the ion, and with Arg2803, on which the xylose stacks (Fig. 3C). Binding to integrin α 7 β 1 is limited by splicing, because only the X1 variant bound laminin-411 (ref. 9), whereas adult mouse muscle expresses X2 (ref. 94). Consistent with these properties, sequential extraction of muscle released laminin- α 4 of patients with collagenase, whereas laminin- α 2 of controls was released by EDTA, which dissolves the laminin polymer and Ca^{2+} -dependent receptor bonds.^{10,14,15} Laminin-411 is thus only loosely attached, being neither polymerized nor firmly anchored to the fiber (Fig. 3D), and it does not functionally compensate for laminin-211.

Therapeutic approaches for LAMA2-CMD

Linker proteins

Because laminin-411 is abundant in laminin- α 2-deficient muscle but lacks polymerization and anchorage, two linker proteins were engineered to supply these functions (Fig. 3A, E). Both bind its γ 1 chain, at the sites that nidogen occupies on the short arm and agrin on the coiled coil.^{95,96} Mini-agrin joins the laminin-binding N-terminal domain of agrin to its LG domains,^{22,98} which bind matriglycan on α -DG.²⁹ *In vitro*, mini-agrin increased the binding of laminin-411 to α -DG (Fig. 3D), and when expressed from a muscle-specific transgene it raised the median survival of dy^{W} mice from 15.5 to 50 weeks (ref. 15). The transgene also raised tetanic force of the extensor digitorum longus to 4-fold that of untreated dy^{W} mice, or 37% of control.⁹⁹

The second linker, α LNNd, fuses the LN-bearing end of laminin- α 1 to the laminin-binding part of nidogen-1 (ref. 21) and supplies polymerization, which mini-agrin does not restore. With α LNNd as a third arm, laminin-411 polymerized like laminin-111 *in vitro* (Fig. 3D), and an α LNNd transgene alone prolonged

survival in 9 of 16 dy^{W} mice.¹⁵ Both linkers together raised median survival to 81 weeks (Fig. 3E) and restored a basement membrane indistinguishable from wild type by electron microscopy.¹⁵ In $\text{dy}^{3\text{K}}$ mice, mini-agrin alone approximately doubled survival and the two linkers tripled it.¹⁴ Anchorage and polymerization therefore each contribute to rescue.

Mini-agrin, alone or together with α LNNd, was also effective when first supplied after birth. Mini-agrin delivered alone by AAV on day 2 or 3 extended mean lifespan of dy^{W} mice from 10.2 to 42.1 weeks (ref. 100). α LNNd was shortened to fit one AAV genome together with the ubiquitous promoter needed to treat the peripheral neuropathy of the mice,^{23,24} and both linkers delivered by AAV at 3 weeks of age improved grip strength and reduced fibrosis.²⁴ When a doxycycline-regulated mini-agrin transgene was induced at about day 28, survival rose 2- to 3-fold, but motor performance improved more with earlier onset and existing fibrosis was not reversed.⁹⁸

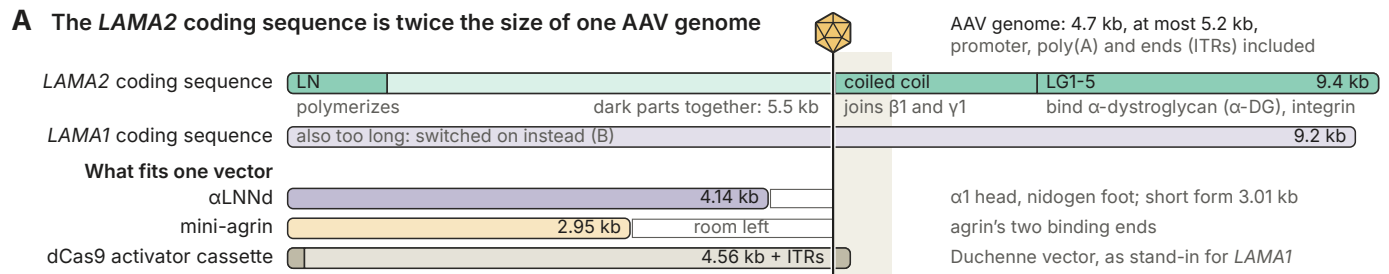
An antibody that bridges LG4-5 of laminin- α 4 to β -DG would add no polymerizing arm, and mini-agrin is therefore the linker closest in mechanism to such a bispecific bridging antibody. In dy^{W} mice, mini-agrin alone partly restored basement membrane structure and stable binding of β 1 γ 1 but did not change the extractability of laminin- α 4 (ref. 15). Because mini-agrin binds γ 1, it also anchors laminin-511, which may account for part of its benefit.¹⁵ No linker binds LG4-5 of laminin- α 4, a segment that is released by proteolysis in cell culture and was not detected in normal tissues.²⁰ Because a bispecific bridging antibody would depend on this segment, we examined whether it is retained in the muscle of dy^{W} mice (Results).

Other approaches

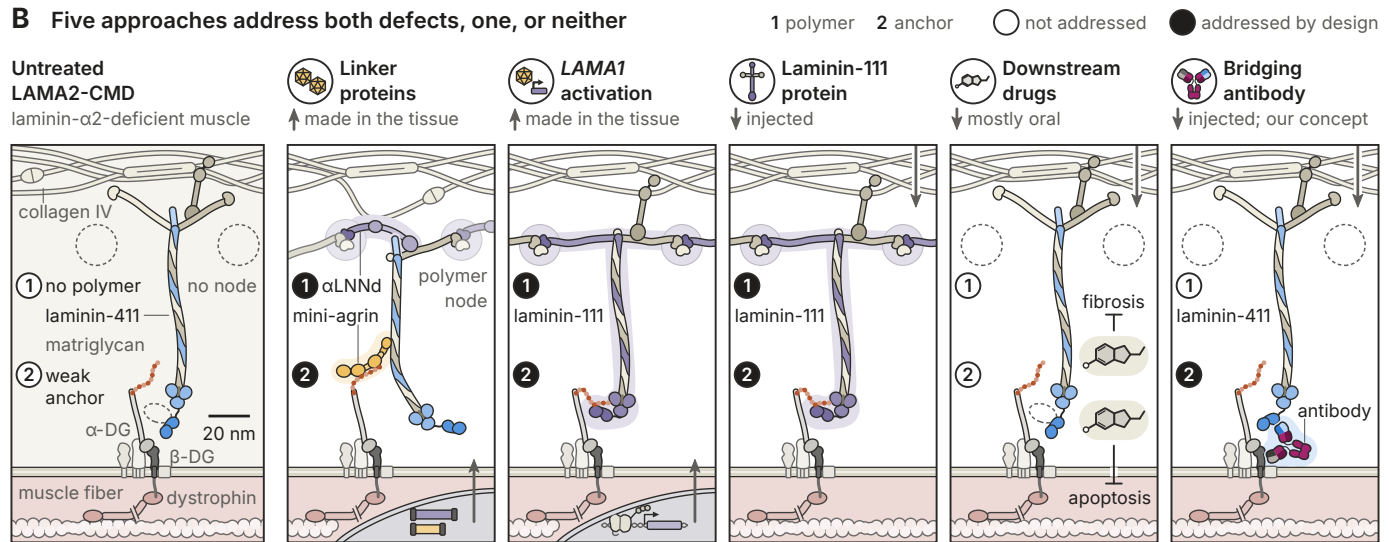
Direct replacement of LAMA2 would restore polymerization and anchorage at once but is hindered by size, because its coding sequence spans about 9.4 kb and an AAV vector accommodates about 4.7 kb (Fig. 4A).^{23,101} Although the rod domain of dystrophin can be shortened,¹⁰² laminin- α 2 offers no comparably dispensable region.^{24,100} Its LN domain, coiled coil and LG domains together account for about 5.5 kb,⁴⁹ which exceeds the capacity of one vector. Direct correction of LAMA2 by gene editing is likewise hindered by mutational heterogeneity, because truncating and splice-site variants are distributed throughout the gene without clear hotspots and only 14 of the 309 disease-associated variants in the international database had 10 or more independent entries.³⁶ Vectors have thus far restored full-length laminin- α 2 only in $\text{dy}^{2\text{J}}$ mice, a mild model,¹⁵ either from three AAV genomes whose products are joined by split inteins²⁵ or through Cas9 repair of the splice-site variant specific to this strain.¹⁰³ Like linker proteins, the other strategies that address the primary defect in mice substitute for laminin- α 2 (Fig. 4C).

Laminin- α 1 is the laminin chain most closely related to laminin- α 2 (ref. 28). It is expressed in early embryonic muscle as laminin-111, which laminin-211 replaces during development, and LAMA1 remains silent in muscle after birth.^{26–28} When expressed from a transgene, laminin- α 1 largely replaced laminin- α 2 in $\text{dy}^{3\text{K}}$ mice.¹¹⁷ Because LAMA1 also exceeds the capacity of AAV, vectors instead deliver catalytically inactive Cas9 (dCas9) fused to transcriptional activators, which reactivate the endogenous gene (Fig. 4B).²⁶ However, activation appears to be inefficient. Uniform staining for laminin- α 1 in $\text{dy}^{2\text{J}}$ mice required two vectors at a combined dose of 6×10^{14} vector genomes per kg.²⁶ In juvenile non-human primates, a single vector at 1×10^{14} vector genomes per kg raised LAMA1 mRNA in muscle to 3% to 6% of the level of the housekeeping gene *HPRT*.²⁷

A The LAMA2 coding sequence is twice the size of one AAV genome



B Five approaches address both defects, one, or neither



C A bridging antibody restores anchorage and is adjustable

● yes ● partly ○ no — not reported

Modality	Linker proteins Two AAV vectors	LAMA1 activation One or two AAV vectors	Laminin-111 protein Heterotrimeric protein	Downstream drugs Mostly small molecules	Bridging antibody Bispecific IgG
Restores polymerization	①	①	①	①	①
Restores anchorage	② α-DG only	② α-DG, integrin	② α-DG, integrin	②	② laminin-411 to β-DG
Adjustable, can be stopped	○	○	●	●	●
No preexisting immunity	○	○	●	●	●
One-and-done	●	●	○	○	○
Reaches peripheral nerve	● ubiquitous promoter	●	—	● doxycycline, glatiramer	○
Preclinical efficacy	● dyW mice	● dy2J mice, one severe model	● dyW mice	● dyW, dy2J mice	● dyW mice (this study)

Figure 4. Five approaches for LAMA2-CMD address polymerization and anchorage, anchorage alone, or neither. (A) Coding sequences (mini-agrin, mouse⁹⁸) and one activator cassette (vector for Duchenne muscular dystrophy¹⁰⁴) are to scale against one AAV genome (ITRs, inverted terminal repeats).^{23,49,101,105,106} (B) Scenes depict intended mechanisms on one patch of sarcolemma and do not indicate extent of repair. Dashed outlines mark absent polymer nodes and the position LG4-5 would occupy on matriglycan of α-DG, which laminin-α4 binds weakly.²⁰ Pale patches mark agents (gray, nucleus; tan rod, nidogen). The bispecific bridging antibody is our concept. Lengths are to scale (estimated for linkers and matriglycan), and widths and

antibody are enlarged 1.35-fold. Drugs and icons are symbols. Integrin α7β1 is omitted, as is laminin-411 where laminin-111 is shown. Fibrosis arises outside the basement membrane. (C) Numbered disks are as in B. Green disk, yes, by design or as reported; half disk, partly, in the cases named; open ring, no; dash, not reported. The strategies were not compared side by side. For laminin-111 protein, strength and survival were shown with laminin-111 from a mouse tumor and activity with recombinant human laminin-111. Sources: linker proteins,^{15,23,24} LAMA1 activation,^{26,27,107} laminin-111 protein,^{28,108,109} downstream drugs,¹¹⁰⁻¹¹⁴ bridging antibody.^{30,115,116}

All vector-based strategies share the properties of systemic AAV, in that some children carry anti-capsid antibodies that preclude treatment¹¹⁸ and the antibody response to the first dose impedes a second.¹¹⁹ In addition, a child treated with linker proteins would receive two vectors, because each linker fits one AAV genome (Fig. 4A).^{23,24}

Laminin-α1 has also been supplied without a vector, as injected laminin-111 protein. Weekly injections of 10 mg/kg laminin-111

purified from a mouse tumor increased the median survival of dy^W mice from 82.5 to 291.5 days and improved their strength.²⁸ Recombinant human laminin-111 at 1 mg/kg weekly increased the distance traveled by immunodeficient dy^W mice about 1.6-fold but did not change their grip strength.¹⁰⁸

Other treatments leave the basement membrane defect uncorrected and target its downstream consequences. Doxycycline, used to inhibit apoptosis, increased the median survival of

laminin- $\alpha 2$ -deficient mice from about 32 to about 70 days (ref. 110), and angiotensin receptor blockers reduced fibrosis in dy^{2J} and dy^w mice.^{112,113} Omigapil, another inhibitor of apoptosis, is the only drug tested in patients thus far, but only for pharmacokinetics and safety.¹²⁰ In mice, it added to the benefit of mini-agrin,⁹⁹ and such drugs may therefore complement a treatment that restores anchorage.

Potential role for a bispecific bridging antibody

An antibody that bridges laminin-411 to β -dystroglycan follows the principle of mini-agrin but would be given by subcutaneous or intravenous injection rather than expressed from a vector (Fig. 4B). Linkers injected as proteins would bind matrix proteins that are not specific to the muscle basement membrane.¹⁵ We reasoned that an arm targeting laminin- $\alpha 4$, which in normal adult muscle is confined to capillaries, perineurium and the synaptic cleft,⁷ would encounter a smaller antigen sink than the linker proteins, which bind the $\gamma 1$ chain that laminin-411 shares with other laminins. Such a bispecific bridging antibody would restore anchorage irrespective of the *LAMA2* variant, provided that laminin-411 is present.²⁴ In fact, such an approach has been explored in related muscular dystrophies. A bispecific bridging antibody targeting laminin-211 and β -dystroglycan improved muscle function in a mouse model of α -dystroglycanopathy.³⁰

From a translational perspective, exposure to an injected antibody can be measured and adjusted, whereas a vector is given once at a fixed dose.¹¹⁹ Dose and interval can be titrated against plasma concentration and response, as shown for adalimumab in children,¹²¹ and adapted to growth.¹²² The distribution of IgG is also predictable. Its concentration in muscle is about 4% of that in plasma, and such ratios predicted tissue concentrations in mice, rats, monkeys and humans within 2-fold.¹¹⁶ Accordingly, the published bispecific bridging antibody was detected in limb muscles, diaphragm and heart of wild-type mice after a single intravenous dose.³⁰ By comparison, most genomes of an intravenous AAV9 vector accumulated in the liver of dy^{2J} mice.²⁶ Whether an antibody reaches muscles and fibers more uniformly than a vector has not been tested thus far.

For patients and physicians, an antibody would differ from a vector chiefly in that treatment can be stopped (Fig. 4C). Intravenous AAV at 10^{14} vector genomes per kg or more, the range of the doses cited above, has been followed by fatal liver failure in X-linked myotubular myopathy¹²³ and Duchenne muscular dystrophy,¹²⁴ by a fatal innate immune reaction¹⁰⁴ and by thrombotic microangiopathy.¹²⁵ Such outcomes are rare,¹²⁶ but a vector cannot be withdrawn, and recipients receive corticosteroids for one to several months.¹²⁷ In contrast, an antibody can be interrupted if it is not tolerated, its effect is reversible,³⁰ and gene therapy remains available afterward, because no anti-capsid antibodies are induced. We consider this flexibility valuable in *LAMA2*-CMD, in which more than half of the children with complete deficiency were admitted to the hospital for respiratory complications within their first two years.³ The safety of a bispecific bridging antibody itself remains to be established.

Therapeutic hypothesis

We therefore propose that a bispecific bridging antibody comprised of binding arms targeting LG4-5 of laminin- $\alpha 4$ and the ectodomain of β -DG would behave like a laminin- $\alpha 2$ mimetic, bridging laminin-411 to the DGC and restoring basement membrane anchorage (Fig. 5A). Transgenic mini-agrin shows that a protein that restores anchorage need not resemble laminin.²² Because mini-agrin also partly restored basement membrane

structure in dy^w mice,¹⁵ we expect the antibody to concentrate the loosely attached laminin-411 (Fig. 3D) at the fiber surface until it is incorporated into the matrix through its binding site for nidogen, which links laminins to collagen IV.^{68,96}

We chose dystroglycan as the membrane anchor because it alone among the laminin receptors keeps the basement membrane attached to the sarcolemma⁷⁸ and appears to persist without laminin- $\alpha 2$ (Fig. 2C). We targeted its transmembrane subunit, β -DG, as in the published study in α -dystroglycanopathy mice (Fig. 5B),³⁰ so that force on the β -DG ectodomain is transmitted through the cytoplasmic tail to dystrophin. By comparison, α -DG would be a less dependable anchor, because it is often secondarily lost^{30,131} or heterogeneously glycosylated^{29,132} in dystrophic muscle (Fig. 5C). Since the binding of an anti- β -DG antibody is matriglycan-independent, the same arm could also serve in the α -dystroglycanopathies.

The laminin arm targets LG4-5 of laminin- $\alpha 4$, the counterpart of the domain pair through which laminin- $\alpha 2$ binds matriglycan,⁷³ on the working assumption that an antibody bound to LG4-5 would hold laminin-411 as α -DG holds laminin-211. LG4-5 also contains the epitope of a published anti-laminin- $\alpha 4$ antibody that supplied the variable regions for our tool molecules (Methods).

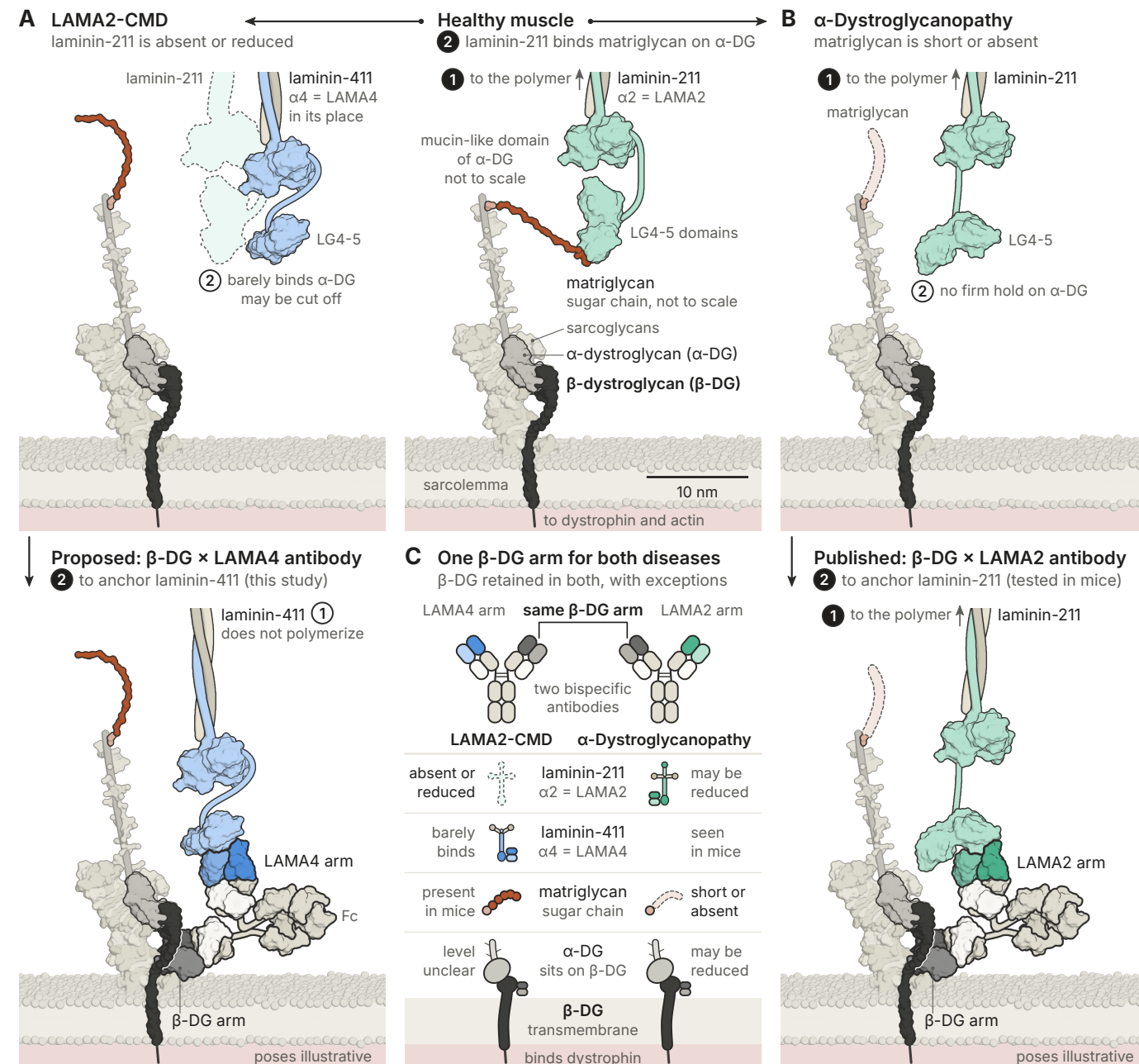
The reach and valency of the antibody and the local density of its targets matter as much as its affinity. The epitope reported for the β -DG arm lies within a few nanometers of the cell membrane (Methods), beside the sarcoglycan tower of about 13.5 nm (refs. 76,77). Because the antigen-binding sites of an IgG lie ~12–13 nm apart,¹³³ the laminin arm of a 1+1 bispecific (one arm per target) would extend only slightly beyond this tower, and binding probably relies on the flexible linker that tethers LG4-5 to LG1-3 (ref. 97).

Valency matters because a single bridge is short-lived. By surface plasmon resonance (SPR), the tool arms release β -DG with half-lives of 4 to 9 min and laminin- $\alpha 4$ over tens of minutes (Results), and mechanical load shortens the lifetime of such bonds further.¹³⁴ A fiber can therefore remain attached only through many bridges in parallel, each of which reforms soon after it breaks,¹³⁵ as proposed for the bond between matriglycan and laminin-211 (ref. 74). A second arm for the same target should favor this, because it holds the antibody in place while the released arm rebinds. This reasoning led us to compare formats from 1+1 to 2+2 that carry the same arms.

Proof of principle for this mechanism of action comes from the α -dystroglycanopathies (Fig. 5B). In these disorders, variants in at least 18 genes leave matriglycan short or absent,¹³⁶ and α -DG then holds laminin-211 too weakly to withstand load, or not at all.^{29,74} By contrast, β -DG is retained in the muscle of Fukuyama CMD patients and *Large*^{myd-3J} mice,^{30,128} and laminin- $\alpha 2$ is present (Fig. 5C). Because laminin-211 polymerizes,⁹³ it needs only to be anchored. An antibody targeting β -DG and LG4-5 of laminin- $\alpha 2$ would bypass matriglycan, whichever glycosylation gene is affected, so these disorders test directly whether an antibody can replace the missing link.

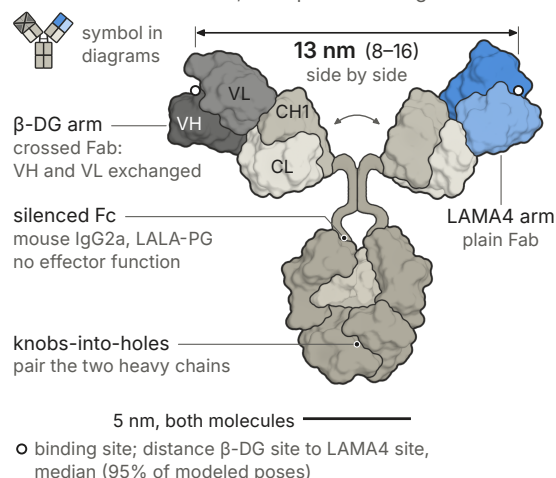
Such an antibody improved muscle function in adult *Large*^{myd-3J} mice, which received a tandem bispecific immunoglobulin with two sites per target at 30 mg/kg twice weekly for 7 weeks (ref. 30). Grip strength reached nearly 70% of wild type, treadmill time doubled and fewer tibialis anterior fibers took up Evans blue. An antibody can thus partly replace the link between laminin and β -DG in living muscle.

We expect the same mechanism to operate in *LAMA2*-CMD, where laminin-411 takes the place of laminin-211 as the laminin to be anchored (Fig. 5A). Laminin- $\alpha 2$ -deficient muscle retains a laminin in its basement membrane and a laminin receptor in its sarcolemma, but the two are only weakly connected. Mini-agrin

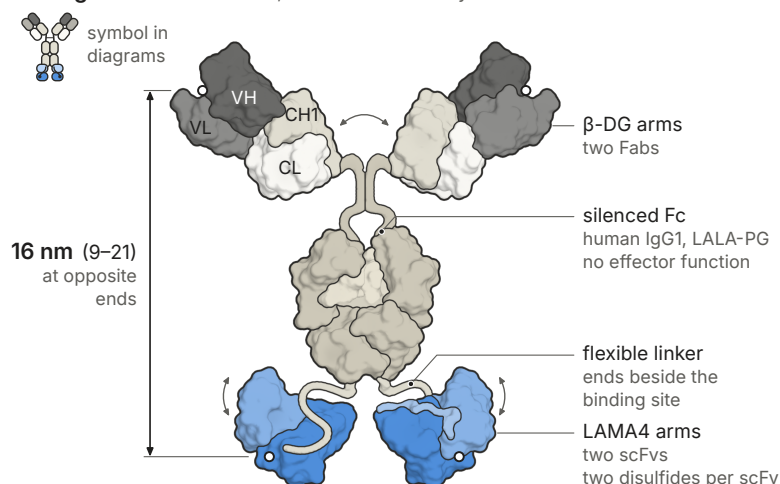


A Each tool has arms for β -dystroglycan (β -DG) on the muscle fiber and for laminin- α 4 (LAMA4) in the matrix

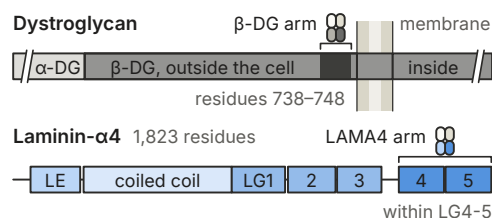
◆ **1+1 CrossMab** murine, for repeated dosing in mice



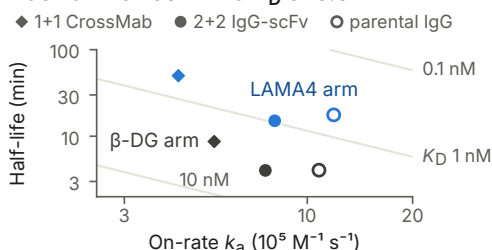
● **2+2 IgG-scFv** humanized, to ask what valency adds



B Where the arms bind as published (Methods)

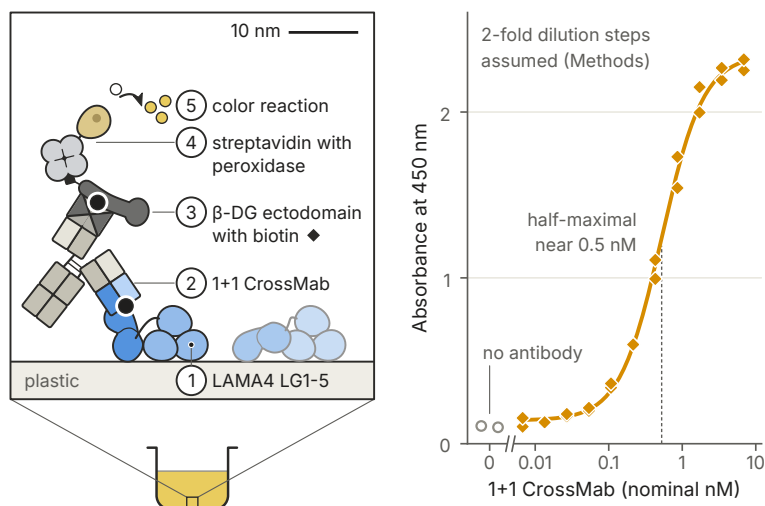


C Each arm binds with a K_D of 0.5–4 nM



D An ELISA designed to require both arms detects the 1+1 CrossMab

color needs both bonds ● on one molecule; points are single wells



E Experiments in which each tool molecule was used

	<i>in vitro</i>			<i>in vivo</i>		
	SPR Fig. 6C	β -DG flow cytometry Fig. 7	Cell adhesion assay Fig. 8	Wild-type mouse PK Fig. 9	Muscle biodistribution Fig. 9	Disease model efficacy Fig. 11
◆ 1+1 CrossMab	●	●	●	—	●	●
● 2+2 IgG-scFv	●	●	●	●	—	●

Figure 6. A murine 1+1 CrossMab and a humanized 2+2 IgG-scFv bind both antigens with K_D of 0.5 to 4 nM. (A) Models at one scale, each in one open pose (arcs, flexible joints; lines span the pose drawn; direction of scFv paratopes not predicted). Numbers, median and 95% range of the distance between β -DG and LAMA4 paratopes (rings; 20,000 modeled conformers). In the symbols, a cross marks the crossed pair and angular domains a murine construct. (B) Schematic maps, each at its own scale. (C) SPR, one run per

point. Half-life of the complex, $\ln 2/k_d$ (k_d , dissociation rate constant); lines, equal K_D . The parental anti-LAMA4 IgG is a re-humanized variant. (D) Dual-antigen ELISA (poses illustrative; numbers, order of addition; α -DG part of the antigen not drawn) and calibration curve. One plate; line, four-parameter fit. (E) Filled circle, performed; dash, not done. Flow cytometry of the 1+1 CrossMab is not shown. PK, pharmacokinetics.

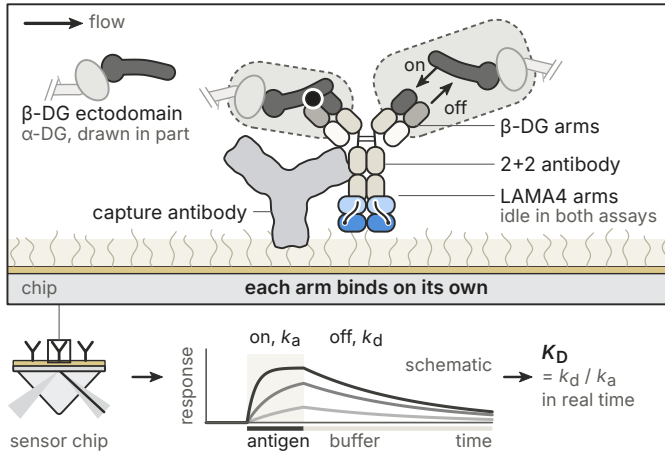
and laminin- α 4 (Fig. 6A, B; Methods). We depict valency as the number of β -DG arms plus the number of laminin- α 4 (LAMA4) arms, so that a 2+1 bispecific carries two β -DG arms and one LAMA4 arm. Two of ten total tool designs (Supplementary Fig. S1, Supplementary Table S1) were used in most experiments (Fig. 6E).

The first molecule is a murine 1+1 CrossMab (Fig. 6A), which combines the parental, mouse-derived variable regions with mouse constant domains to limit anti-drug antibodies during about six weeks of dosing in dy^w mice. Knobs-into-holes substitutions in its IgG2a Fc pair the two heavy chains,¹³⁷ and a VH-VL crossover in the β -DG Fab directs each light chain to its own heavy

A SPR reads one arm on one free antigen, flow cytometry the whole antibody on a cell

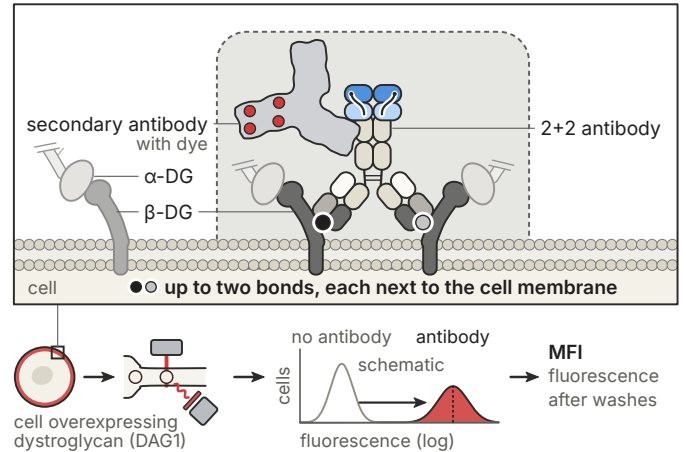
what each assay reads

■ **SPR** surface plasmon resonance: antibody on a chip, β -DG in solution



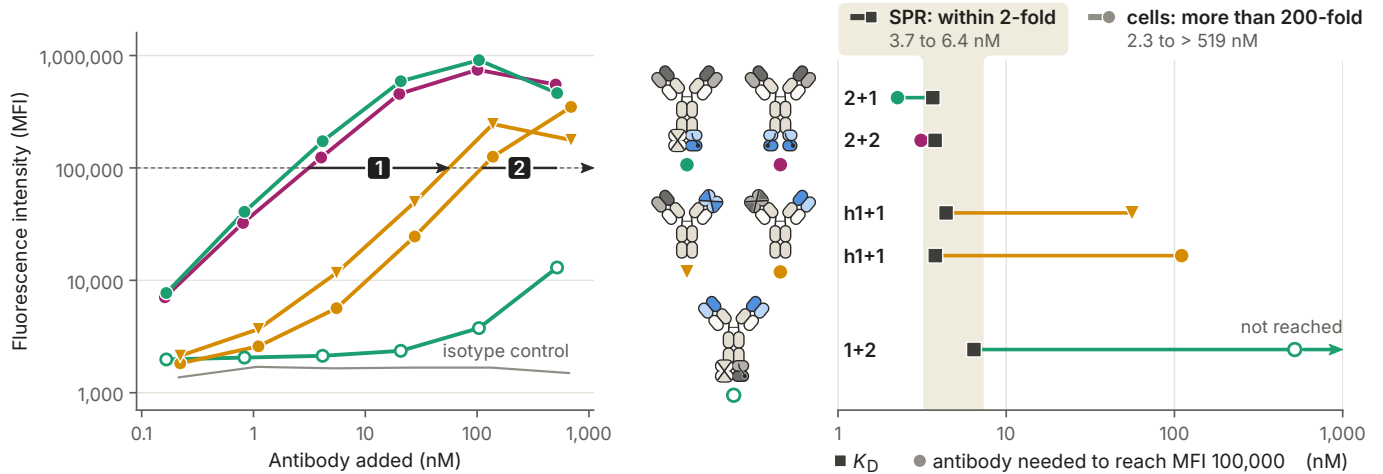
● **Flow cytometry** β -DG on a cell, antibody in solution

10 nm



B Similar affinity by SPR, far apart on cells

name = ● β -DG arms + ● LAMA4 arms; X = crossed Fab



C Interpretation: number and position of the β -DG arms

schematic; molecules to scale 10 nm

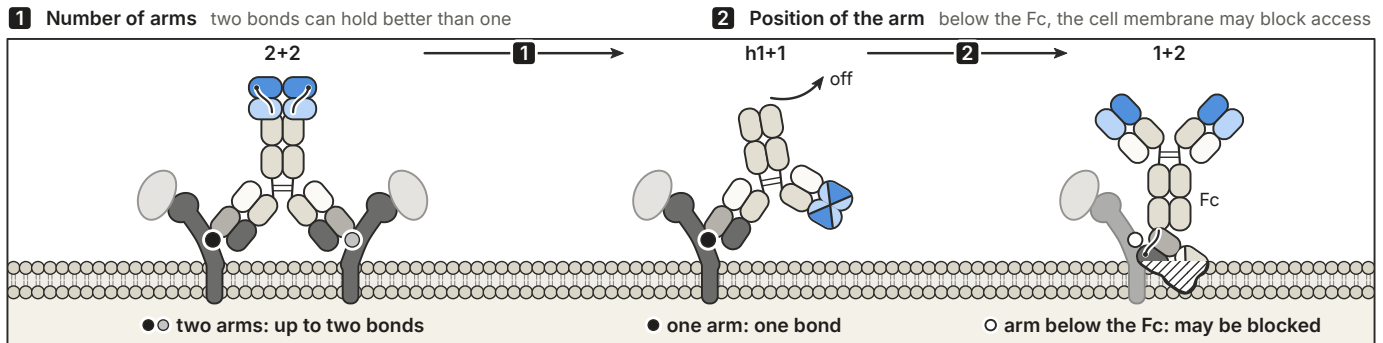


Figure 7. Among humanized constructs, affinity per arm did not predict binding to cells. All five constructs carry the same humanized arms and Fc. (A) Molecules are to scale. Poses, receptor spacing and number of bonds are illustrative, and chip surface, icons and traces are schematic. Filled disk, bond; pale disk, second bond, not shown to form. Several secondary antibodies (one drawn) can bind the heavy and light chains of one molecule, so signal may rise with format size. (B) Left, titration on cells, one experiment; symbols, means of duplicate wells. Right, one row per construct: K_D for β -DG in SPR (squares on the shaded column; one run per construct, the 2+2 in a separate session),

joined to the antibody concentration at which its curve reached an MFI of 100,000 (dashed line at left; symbols as at left), which the 1+2 did not reach at 519 nM. Arrows 1 and 2, the shifts interpreted in C. X, crossed Fab (VH-VL or CH1-CL pair). h1+1, humanized 1+1 CrossMap with the LAMA4 Fab (triangle) or the β -DG Fab (circle). h1+1, humanized 1+1 CrossMap with the LAMA4 Fab (triangle) or the β -DG Fab (circle). Open symbols, 1+2. (C) Proposed explanations, not mutually exclusive (schematic, molecules to scale). Open ring, free epitope. Hatched, part of the Fab that would overlap the cell membrane. MFI, fluorescence intensity (Methods).

chain.¹³⁸ The Fc is silenced by L234A/L235A/P329G (LALA-PG),¹³⁹ because the antibody is intended to remain bound to living fibers.

The second molecule is a humanized 2+2 IgG-scFv, an anti- β -DG IgG with a disulfide-stapled anti-LAMA4 single-chain variable fragment (scFv) fused to the C terminus of each heavy chain,^{140,141} and as a homodimer it requires neither knobs-into-holes nor a crossover. It belongs to a series that varies the number and position of the arms for each target, in which both arms are humanized and the human IgG1 Fc carries LALA-PG. We built this series because β -DG in the sarcolemma and laminin-411 in the basement membrane are both surface-bound targets, for which a second arm of the same specificity could add avidity. The 2+2 was one of two humanized designs that supported strong adhesion (Supplementary Fig. S1D) in the assay described below, in which an antibody bridges dystroglycan-overexpressing cells to immobilized LAMA4 LG4-5. We therefore advanced it to *dy*^W mice despite its human sequences.

Both tool molecules bound β -DG and LAMA4 with K_D values of 0.5 to 4 nM, as measured by SPR with captured antibody and soluble antigen (Fig. 6C, Supplementary Table S1). Each arm of the 2+2 bound within 2-fold of the same arm in an IgG or a 1+1 CrossMab, so the 2+2 format preserved affinity. For both molecules, the complex with β -DG dissociated faster than that with LAMA4, so that the membrane end of a bridge should open first. As Fabs, the humanized arms bound the murine antigens about as well as the human antigens or better (Methods), which supports the use of the 2+2 in mice.

The 1+1 CrossMab engaged both antigens simultaneously, as bridging requires. In a dual-antigen ELISA, which captures the antibody on plate-bound LAMA4 and detects it with biotinylated β -DG ectodomain, it gave a saturable signal (Fig. 6D). The 2+2 was not measured in this ELISA, and its simultaneous binding is inferred from its activity in the adhesion assay.

In size-exclusion chromatography (SEC), the main peak was at least 98% for all lots except the *in vitro* lots of the 1+1 CrossMab (90 to 92%; Supplementary Table S1, Methods). This difference is relevant when formats are compared, because aggregates are multivalent. Apart from the calibrator of the ELISA, the lots dosed in *dy*^W mice were not those tested *in vitro*.

Binding to membrane-bound β -dystroglycan

The affinities above were measured by SPR, which presents β -DG as a soluble protein, whereas a bispecific bridging antibody encounters membrane-bound β -DG. On cells, part of the β -DG ectodomain is folded together with α -DG,^{76,77} and the epitope published for the β -DG arm (Fig. 6B) lies in a poorly ordered stretch next to the cell membrane. Because access to this epitope may depend on antibody format, we compared five constructs carrying the same humanized arms in different numbers and positions, in solution and on cells. Two are humanized 1+1 CrossMabs (h1+1), with the VH-VL crossover in the LAMA4 or the β -DG Fab. The 2+1 and the 2+2 are anti- β -DG IgGs with one or two LAMA4 arms, each fused to a heavy-chain C terminus below the Fc, and the 1+2 is an anti-LAMA4 IgG with a single crossed β -DG Fab in the same position.

On cells, binding varied with the number and position of the β -DG arms. We titrated all five constructs side by side on HEK293-derived cells engineered to overexpress human dystroglycan (DAG1). After staining at 4 °C and washing, bound antibody was detected by flow cytometry with a fluorescent anti-human IgG (Fig. 7A, B). The curves of the 2+1 and the 2+2 nearly coincided, and those of the h1+1 CrossMabs were parallel but shifted to higher concentrations, whereas the 1+2 remained near the human IgG1 isotype control up to 104 nM. Because no curve

held a plateau, we compared the concentration at which each curve first reached a fluorescence intensity (MFI) of 100,000. This concentration was 2.3 and 3.1 nM for the 2+1 and the 2+2, 56 and 110 nM for the h1+1 CrossMabs and more than 519 nM for the 1+2. Constructs whose K_D for β -DG in SPR agreed within 2-fold (Supplementary Table S2) thus differed more than 200-fold (2+1 versus 1+2) in the concentration needed to stain cells (Fig. 7B).

A single humanized arm appears to bind β -DG far more weakly on cells than in solution. Both h1+1 curves rose in proportion to concentration up to 138 nM. This concentration exceeds the K_D by more than 30-fold and would nearly saturate a binding site of that affinity. The continued rise cannot be attributed to dissociation during the washes, which would lower a curve without delaying its saturation. Because the soluble antigen bound in SPR also contains α -DG (Methods), the obstacle on cells is more plausibly the adjacent cell membrane than α -DG. Given a single contact this weak, avidity is the simplest explanation for the difference between constructs with one and two N-terminal β -DG arms (Fig. 7C). The two Fabs of an IgG can engage antigens 3 to 17 nm apart,¹⁴² a spacing that overexpressing cells are likely to offer, and we attribute the 18- to 35-fold shift between the h1+1 CrossMabs and the 2+2 to such a second contact.

We propose that access to β -DG may be sterically hindered for an arm below the Fc (Fig. 7C), because avidity does not explain the weak staining by the 1+2. The 1+2 also has one β -DG arm but needed 36- to 82-fold more antibody than the h1+1 CrossMabs to reach an MFI of 10,000. The crossover of the constant domains (CH1-CL) in the β -DG Fab of the 1+2 may add to the effect of its position. Consistent with hindered access, each arm below the Fc had a lower on-rate in SPR (Supplementary Table S2) than the same arm at the N terminus. The β -DG Fab of the 1+2 is joined to the Fc by its paratope end and can reach its epitope only if the Fc approaches the cell membrane. Together, these data suggest that humanized arms engage membrane-bound β -DG best when presented as two N-terminal Fabs.

Cellular adhesion as a proxy for antibody function

Because flow cytometry measures only one side of the bridge (Fig. 7), we used a cell adhesion assay to test whether the same constructs can hold cells on a surface coated with LAMA4 (Fig. 8A, B). Wells coated with the LG4-5 fragment of human LAMA4 (the LG4-5 coat) were incubated with antibody and washed before dystroglycan-overexpressing cells were added. Cells that withstood two centrifugal washes were quantified and expressed as a percentage of the positive control (poly-L-lysine, PLL). A cell remains attached only if enough bridges share the force of the wash.^{135,143} We therefore take the EC_{50} , the concentration that gave half-maximal retention, to reflect the functional potency of each antibody.

Both tool molecules bridged cells to the LG4-5 coat, and the murine 1+1 CrossMab retained them as fully as the 2+2 (Fig. 8C; Supplementary Table S3). Its EC_{50} values of 7.67 to 32.9 nM, compared with 2.40 to 9.69 nM for the 2+2 (Fig. 8C), suggest that the 2+2 is more potent. However, no plate held both tool molecules, and EC_{50} values from different days could be distinguished only when they differed by more than about 5-fold (Supplementary Fig. S2A, B).

Adhesion required both the antibody and the LAMA4 coat (Fig. 8D). Wells without coat, without antibody or with a control protein retained medians of 1.9, 3.8 and 6.2% of PLL. On a coat of the corresponding LG4-5 fragment of laminin- α 2, the 1+1 CrossMab and the 2+2 retained 3.2 and 1.9% of PLL, compared with 106 and 110% on the LG4-5 coat. Adhesion is therefore specific to the antibody and to its laminin target.

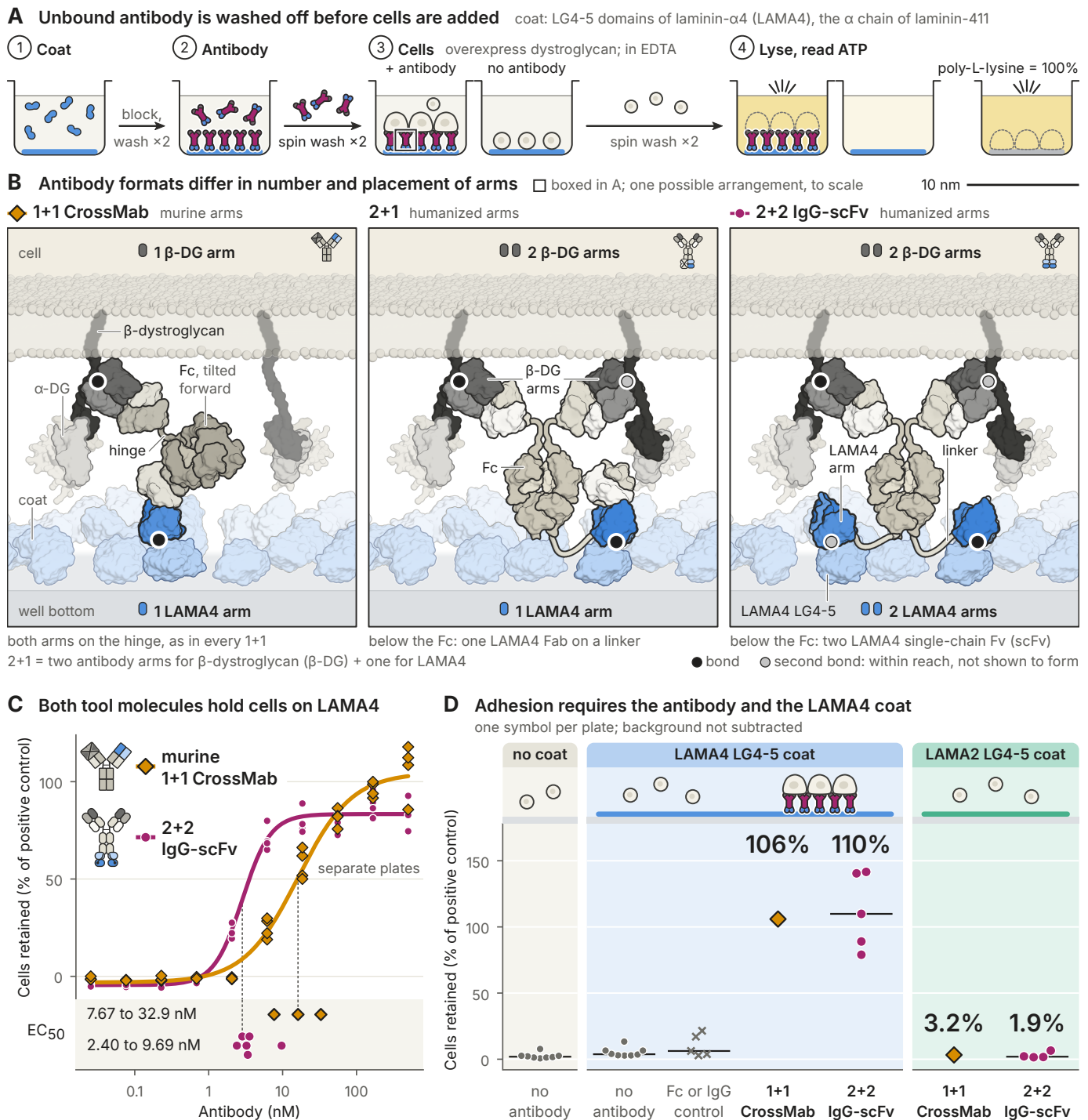


Figure 8. Both tool molecules bridge cells to LAMA4, and adhesion requires the antibody and the LAMA4 coat. (A) Assay steps, schematic. (B) Rendered to scale from structures and models; bystander molecules paled. One possible arrangement; orientation and density of the adsorbed fragment are unknown, no stoichiometry was measured and poses are illustrative. (C) Cells retained on the LG4-5 coat, as percent of the positive control (poly-L-lysine) after subtraction of wells without antibody. Points, wells (4 per dose); lines, four-parameter

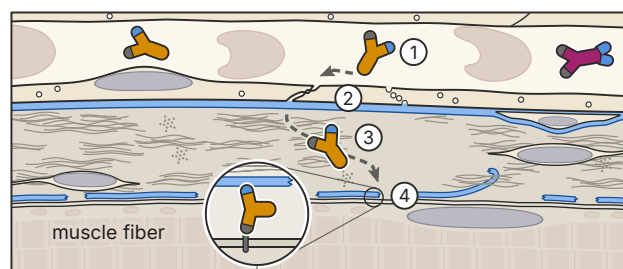
fits. The two tool molecules were titrated on separate plates. Strip, EC₅₀ of every fitted curve; dotted, the curves shown. (D) One symbol per plate (1 to 8 per column; plates with a failed positive control omitted): highest dose mean or mean of control wells, without subtraction. Lines and numbers, medians (1+1 CrossMap, one plate per coat). Control, Fc or human IgG. LAMA2, laminin- α 2. Schematics as in A.

Only the 2+1 and the 2+2 retained cells efficiently under stress when the constructs that share humanized arms and Fc were titrated on one plate (Supplementary Fig. S1D), and these two constructs had also bound cells best (Fig. 7B). On this plate, the 2+1 and the 2+2 reached 78 and 88% of PLL. Although the 2+2 carries a second LAMA4 arm, the two EC₅₀ values were similar

on the LG4-5 coat (ratio 1.13; 95% CI 0.81 to 1.57). They differed 2.10-fold on a commercial laminin-411 fragment that may lack LG4-5 (Supplementary Table S3, Methods). Unlike the humanized 1+1 CrossMaps, both carry a second β -DG Fab, which adds avidity at β -DG, the end of the bridge that dissociates faster (Fig. 6C).

A From blood to fiber surface: what each assay samples

schematic, not to scale


1+1 CrossMab, intended bridge: laminin-411 to β -DG

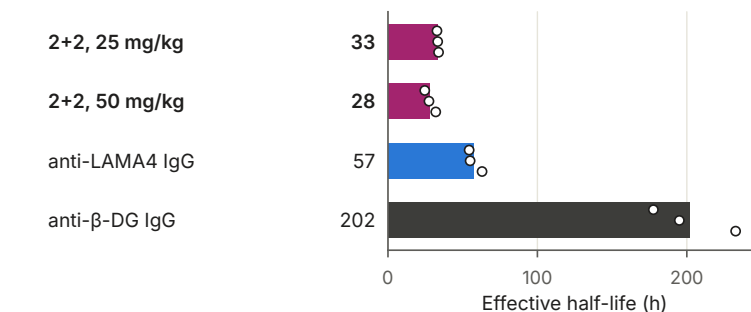
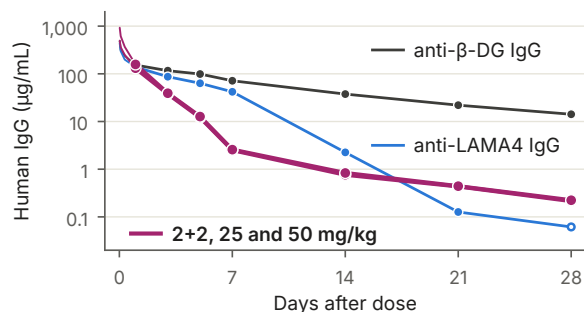
① Plasma after one intravenous dose

2+2 IgG-scFv and parental IgGs human Fc

Wild-type mice

B The 2+2 is cleared faster than its parental IgGs

C The 2+2 has an effective half-life of about 30 h



① ② ③ ④ Whole muscle

1+1 CrossMab mouse Fc

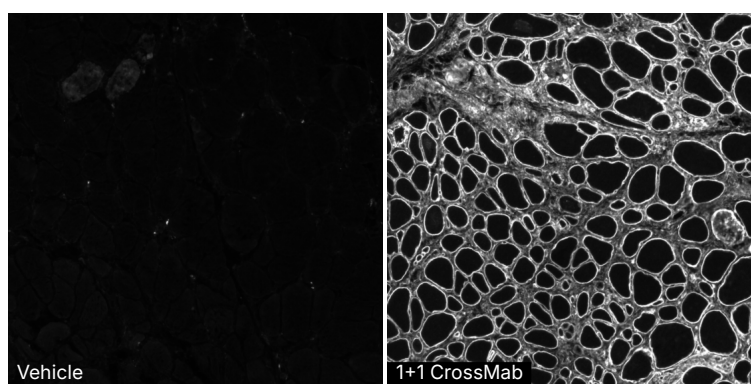
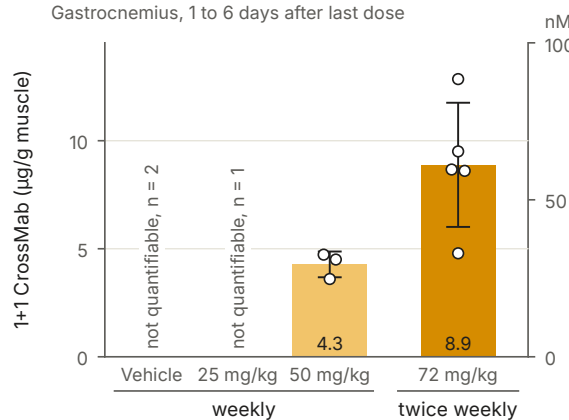
dy^W mice laminin- α 2 deficient

D Antibody in muscle increases with dose

Gastrocnemius, 1 to 6 days after last dose

E Antibody outlines the muscle fibers throughout the section

Stained with anti-mouse IgG



F Antibody in muscle lies between the estimated levels of its two targets

1+1 CrossMab in gastrocnemius

50 mg/kg weekly

72 mg/kg twice weekly

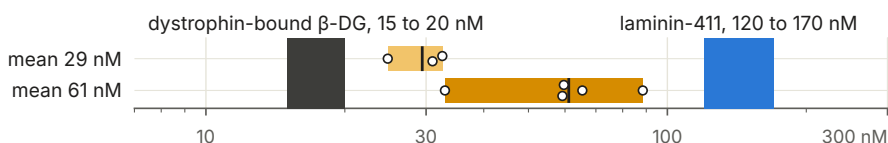


Figure 9. The 2+2 IgG-scFv is cleared faster than its parental IgGs, and the 1+1 CrossMab is recovered from muscle days after last dose. (A) Schematic of stations 1 to 4 between plasma and fiber surface. Brackets, what each assay samples. Dashed arrows, presumed path. (B) Total human IgG in plasma after one intravenous dose (parental IgGs, 25 mg/kg). Symbols, geometric means from day 1 (n = 3 mice per group). Open symbol, below the lowest quality control. Both 2+2 doses coincide from day 3. anti-LAMA4 IgG, re-humanized variant. (C) Effective half-life of the mice in B. Bars and numbers, means (h). Circles, mice. (D) Antibody binding both antigens in unperfused gastrocnemius of mice given the 1+1 CrossMab intraperitoneally at 25 mg/kg weekly (n = 1), 50 mg/kg weekly (n = 3) or 72 mg/kg twice weekly (n = 5), collected 1 to 6 days after last dose. Bars and numbers, means (μ g/g). Whiskers, SD. Circles, mice. Right scale, nM (1 g taken as 1 mL). Not quantifiable, below the lowest standard (0.78 μ g/g). A fourth mouse given 50 mg/kg (13.7 μ g/g) was excluded as an outlier (Methods). (E) Hindlimb sections of one vehicle-treated and one treated mouse of a preceding study (Methods), stained with the anti-mouse IgG secondary antibody alone. (F) Concentration of the 1+1 CrossMab in the mice of D (boxes, range, colored as the bars in D; vertical lines, means; circles, mice) between the whole-tissue concentrations of dystrophin-bound β -DG and of laminin-411 (bands), estimated from published abundances (Methods). Logarithmic scale.

6 days after last dose. Bars and numbers, means (μ g/g). Whiskers, SD. Circles, mice. Right scale, nM (1 g taken as 1 mL). Not quantifiable, below the lowest standard (0.78 μ g/g). A fourth mouse given 50 mg/kg (13.7 μ g/g) was excluded as an outlier (Methods). (E) Hindlimb sections of one vehicle-treated and one treated mouse of a preceding study (Methods), stained with the anti-mouse IgG secondary antibody alone. (F) Concentration of the 1+1 CrossMab in the mice of D (boxes, range, colored as the bars in D; vertical lines, means; circles, mice) between the whole-tissue concentrations of dystrophin-bound β -DG and of laminin-411 (bands), estimated from published abundances (Methods). Logarithmic scale.

Bridging depends on the placement of the arms as well as on their number. The reversed 2+2, an anti-LAMA4 IgG with C-terminal anti- β -DG scFvs, and the 1+2 retained at most 3.8 and 20% of PLL on the LG4-5 coat, although their K_D values were within 2-fold of those of the 2+2 (Supplementary Fig. S1B, D, Supplementary Tables S1 and S3). Both constructs carry their β -DG arms below the Fc, where access to β -DG may be sterically hindered (Fig. 7C). We conclude that a bispecific bridging antibody can hold dystroglycan-overexpressing cells on a LAMA4 fragment under stress, and that among humanized constructs of similar affinity this bridging is most efficient with two N-terminal β -DG Fabs and LAMA4 arms below the Fc, as in the 2+2.

Pharmacokinetics and muscle exposure

To bridge β -DG to laminin- α 4 *in vivo*, a dosed antibody must leave the blood, reach the surface of the muscle fiber and persist there between doses (Fig. 9A). We therefore interrogated the plasma pharmacokinetics and biodistribution of our tool molecules across wild-type and *dy*^w mice.

Wild-type mice received one intravenous dose of the 2+2 IgG-scFv (25 or 50 mg/kg) or of either parental IgG (anti- β -DG or anti-LAMA4; 25 mg/kg), after which total human IgG in plasma was followed for 28 days (Fig. 9B). The 2+2 was eliminated from plasma faster than either monospecific parental IgG. Its effective half-life was about 30 h, compared with 202 and 57 h for the anti- β -DG and anti-LAMA4 IgGs, respectively (Fig. 9C, Supplementary Fig. S3A). At equal mass dose (25 mg/kg), it was cleared 3.9- and 1.7-fold faster than these two IgGs, respectively (Supplementary Table S4). Moreover, exposure to the 2+2 increased less than in proportion to dose (Supplementary Fig. S3B), which suggests that the doses administered may have been too low to saturate its clearance. Plasma exposure to the 2+2 therefore lasts days rather than weeks in wild-type mice. Wild-type mice, however, do not share the laminin- α 4 expression pattern of *dy*^w mice, and we have not characterized the extent to which this influences pharmacokinetics.

To identify the arm responsible for this clearance, we compared the two parental IgGs, which share format and Fc but bind different targets. The clearance of the anti- β -DG IgG lay within the 3 to 20 mL/day/kg reported for human IgG given subcutaneously to mice,¹⁴⁴ suggesting that β -DG is unlikely to be a major sink at 25 mg/kg. The anti-LAMA4 IgG, by contrast, was cleared 2.3-fold faster, and its clearance accelerated as concentration declined (Supplementary Fig. S3C), consistent with target-mediated clearance. Because elimination of the 2+2 did not slow at twice the dose, this sink appears to have a high capacity. A candidate is the laminin- α 4 of vascular basement membranes,⁷ which the antibody encounters before it reaches the fiber (Fig. 9A). The format may also contribute, as IgG-scFv fusions are cleared faster than their parental antibodies in cynomolgus monkeys, independently of target binding.¹⁴⁵ We conclude that binding to laminin- α 4, rather than β -DG, is likely the limiting variable for 2+2 IgG-scFv exposure.

Plasma antibody levels are not indicative of antibody exposure in the muscle tissue. We therefore analyzed muscle biodistribution of the 1+1 CrossMab in *dy*^w mice of the pilot study described below (Fig. 10C, Supplementary Table S6). Muscle levels of the 2+2 were not measured. The dual-antigen ELISA (Fig. 6D), which by design requires both arms to bind, detected the 1+1 CrossMab in unperfused gastrocnemius of all five mice given 72 mg/kg twice weekly, at 4.8 to 12.9 μ g/g, and in neither of two vehicle-treated mice (Fig. 9D). Mean levels increased with dose, from below the limit of quantification at 25 mg/kg weekly ($n = 1$) to 4.3 μ g/g at 50 mg/kg weekly ($n = 3$) and 8.9 μ g/g at 72 mg/kg

twice weekly. Immunofluorescence corroborated this result. In hindlimb muscle from a treated mouse, an anti-mouse IgG secondary antibody outlined the fibers throughout the section, consistent with broad distribution of the 1+1 CrossMab and its localization at the sarcolemma (Fig. 9E). The Fab of the murine LAMA4 arm likewise outlined the fibers of a vehicle-treated *dy*^w mouse, whereas in a wild-type mouse it labeled mainly bright points between the fibers, the position of capillaries (Supplementary Fig. S5), consistent with retention of LG4-5 at the fiber surface. The group mean at 72 mg/kg twice weekly corresponds to about 60 nM averaged over the tissue, which exceeds the K_D of either arm (Fig. 6C) and the EC_{50} of the 1+1 CrossMab in the adhesion assay (Fig. 8C).

Because the concentration required *in vivo* also depends on the abundance of the two targets, we estimated both from published protein abundances, assuming one β -DG per dystrophin (Fig. 9F, Methods). Dystrophin-bound β -DG was estimated at 15 to 20 nM and laminin-411 in laminin- α 2-deficient muscle at 120 to 170 nM. At about 30 nM (50 mg/kg weekly) and 60 nM (72 mg/kg twice weekly), the 1+1 CrossMab lay between the two, the range in which a bridging molecule with high-affinity arms is predicted to form the most bridges.¹⁴⁶ These estimates suggest that, in *dy*^w mice, the doses administered may have supplied the 1+1 CrossMab in molar excess over dystrophin-bound β -DG without reaching the surplus at which antibody caps both targets separately and bridging declines (hook effect). A systemically dosed bispecific bridging antibody can therefore be recovered from dystrophic muscle days after last dose, retains the ability to bind both antigens, and is present at concentrations exceeding those required for bridging *in vitro*. Delivery to muscle is thus unlikely to be a fundamental or existential constraint for the application of bispecific bridging antibodies in muscle-related diseases.

Functional translation in a LAMA2-CMD animal model

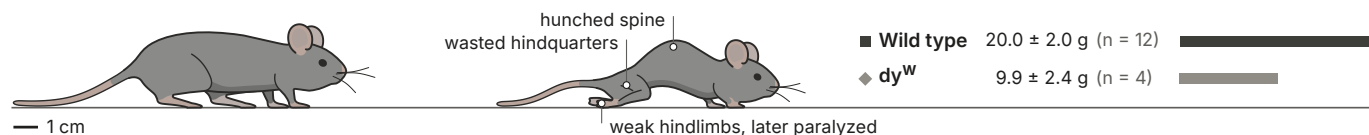
The *dy*^w mouse is the workhorse model of the LAMA2-CMD field.¹⁴⁷ Linker proteins and laminin-111 protein were evaluated in it,^{15,28,100} and its phenotype has been compared among laboratories to standardize preclinical testing.¹⁴⁷ To test whether a bispecific bridging antibody improves muscle function, we chose this model, because it is deficient in laminin- α 2, retains laminin- α 4 around its fibers and lives long enough for several weeks of dosing.¹⁵ The *dy*^w mouse shares early onset, failure to thrive, inflammation and fibrosis with severe LAMA2-CMD (Fig. 10A, B).^{5,56} The disease begins before birth, with small muscles at embryonic day 18.5 (ref. 148), growth fails from week 3 (ref. 61), and at 6 to 7 weeks our vehicle-treated *dy*^w mice weighed half as much as wild-type mice. Even dosing from birth, therefore, begins after disease onset, and the severe course leaves a short window in which to detect a benefit.

The peripheral neuropathy of *dy*^w mice, in contrast, is a feature of the mouse models that rarely manifests clinically in patients.^{5,56} Because hindlimb paralysis was not prevented when laminin function was restored in muscle alone,^{15,24,83} we expected the neuropathy to persist under antibody treatment (Fig. 4C) and, as a result, prioritized forelimb-centric functional measures.

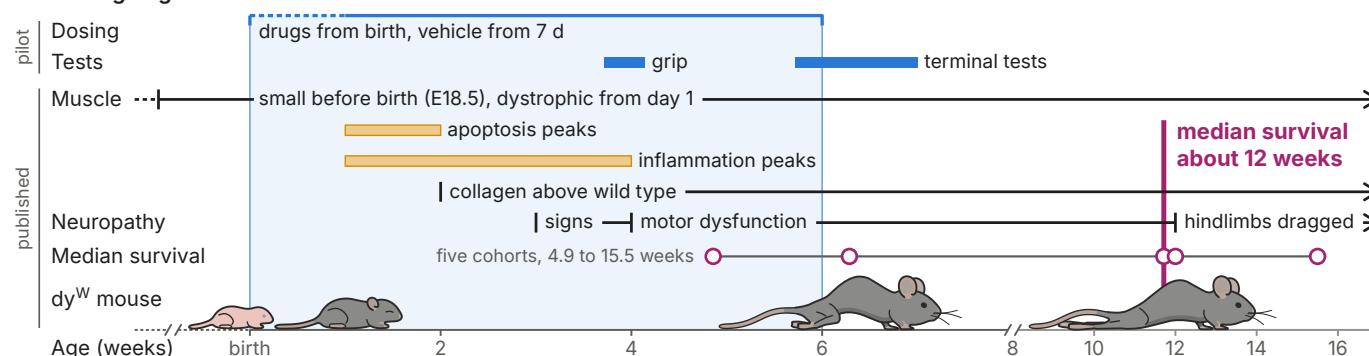
We evaluated our tool molecules in a pilot study, in which *dy*^w mice received drug for six weeks and were compared with vehicle-treated *dy*^w and wild-type mice (Fig. 10C). The positive control was AAV9 mini-agrin given once at birth, because mini-agrin sets the benchmark for anchorage alone in this model (Supplementary Table S5).^{15,100} We measured this benchmark in the same colony, because untreated median survival has ranged

A dy^w mice reach half the mass of wild type

6 to 7 weeks of age, body mass as mean $\pm$ SD



B Dosing began after disease onset and covered six weeks



C Five groups, two tool molecules at equimolar doses

Group	Dose, mg/kg (μ mol/kg)	Regimen	Started	Tested (males/females)
■ Wild type, vehicle		weekly from 7 d of age	12	12 (6/6)
◆ Vehicle		weekly from 7 d of age	8	4 (1/3)
● 1+1 CrossMab	72 (0.496)	twice weekly from birth	27	5 (1/4)
● 2+2	100 (0.503)	twice weekly from birth	9	7 (4/3)
▲ AAV9 mini-agrin	1.5×10^{14} vg/kg	once at birth	5	4 (0/4)

D Both tests load only the forelimb

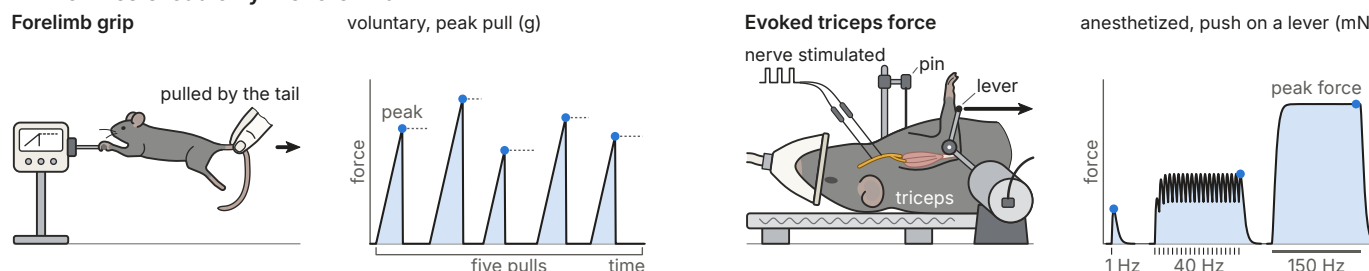


Figure 10. dy^w mice are dystrophic from day 1 and live about 12 weeks; five groups were treated for six weeks and compared in two forelimb tests. (A) Bars, mean body mass of vehicle-treated mice. The scale bar assumes a body length of 8.5 cm in wild-type mice. (B) Published signs (also in A) and their course.^{15,24,56,61,113,148,149} Blue, the pilot study (dashed, vehicle not yet given). Ticks, earliest age reported; arrowheads, no published end. Open circles, median survival of untreated cohorts; vertical line, median of these five

medians.^{15,147} (C) Groups of the pilot study (symbols as in Fig. 11). All groups are dy^w mice except wild type, and antibodies and vehicle were injected intraperitoneally. Doses are nominal, per injection. Bars, mice started (pale gray) and tested (group color). Band, tool molecules. Started, mice enrolled, including those collected early (vehicle, 1; 1+1 CrossMab, 16). Tested, mice with a force test at week 6. vg, vector genomes. (D) The two tests, done in all groups. Mice, rigs and traces are schematic.

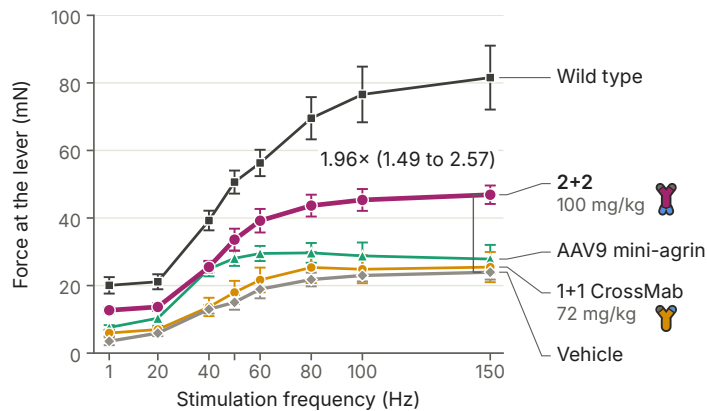
from 4.9 to 15.5 weeks among five published cohorts, with a median of about 12 weeks (Fig. 10B).^{15,147}

The 1+1 CrossMab at 72 mg/kg and the 2+2 IgG-scFv at 100 mg/kg were injected intraperitoneally twice weekly from birth. These two doses were nominally equimolar (Fig. 10C), and the tetravalent 2+2 therefore delivered twice as many binding sites per injection as the 1+1 CrossMab.

The groups were compared in two forelimb tests (Fig. 10D). Grip strength was measured in awake mice, and isometric triceps force, which requires no cooperation, was evoked through the radial nerve under anesthesia. We also recorded body mass, an endpoint recommended for dy^w mice, for which three mice per

group suffice to separate the genotypes from week 5 (ref. 147), as well as rotarod performance and plethysmography (Methods). Because overt paralysis is confined to the hindlimbs,¹¹³ grip strength and triceps force, which load only the forelimb, are the most informative tests in this model.

We first asked whether either tool molecule increased nerve-evoked triceps force, which was recorded at eight stimulation frequencies. From 40 to 150 Hz, force was about twice as high in the 2+2 IgG-scFv group as in the vehicle group, whereas the curves of both the 1+1 CrossMab group and the AAV9 mini-agrin group followed that of the vehicle group at the highest frequencies (Fig. 11A).

A Force in the 2+2 group exceeded vehicle at every frequencyGroup mean $\pm$ SEM, n in B**B Peak force in the 2+2 group was about twice vehicle**

Largest response of each mouse, with group means

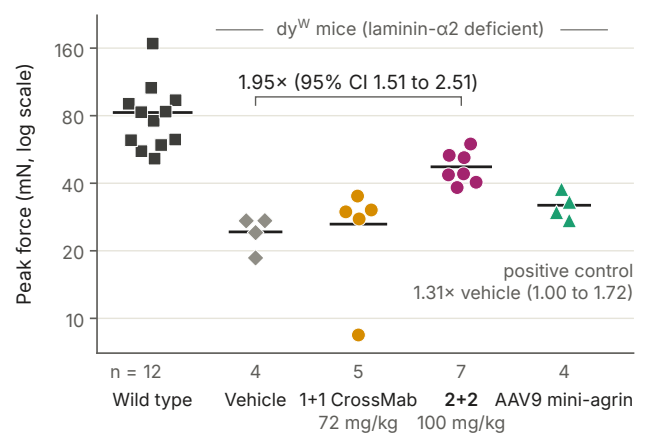
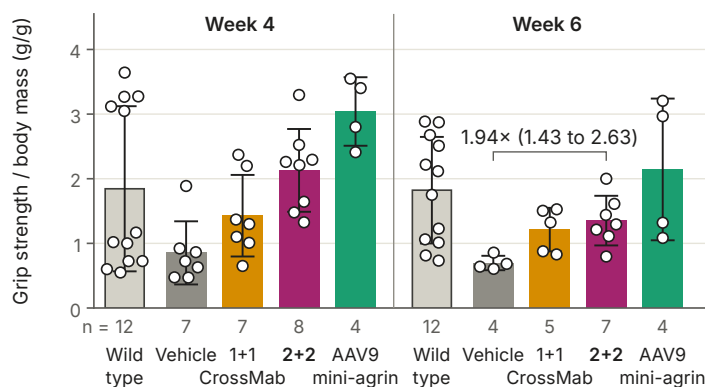
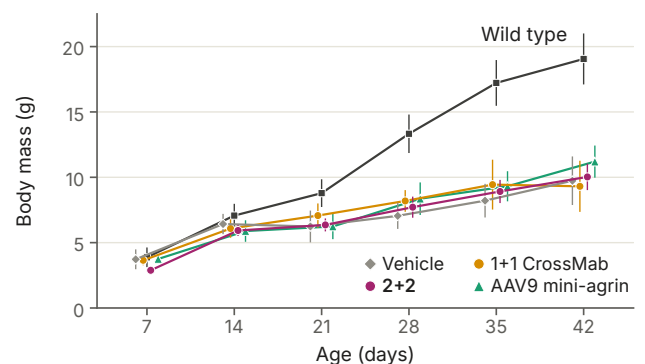
**C Grip strength was also higher in the 2+2 group**Mean $\pm$ SD, one circle per mouse**D No treatment changed body mass detectably**Mean $\pm$ SD of the mice that reached week 6

Figure 11. Evoked forelimb force was higher in dy^w mice treated with the 2+2 than in vehicle-treated mice. (A) Triceps force against stimulation frequency (means; whiskers, SEM; ratio, 2+2 to vehicle at 150 Hz). (B) Peak force of all 32 mice; 72 and 100 mg/kg are the same nominal molar dose, 0.50 μ mol/kg (Fig. 10C). Ticks mark means. (C) Grip strength per body mass at weeks 4 and 6 (bars, means; whiskers, SD; circles, mice). Week 6, the mice of B; week 4,

the same mice and six dy^w mice not tested again (vehicle, 3; 1+1 CrossMab, 2; 2+2, 1). (D) Body mass by age of the mice of B (means; whiskers, SD). In B to D, overlapping symbols are moved sideways. Ratios of means carry 95% CIs. Peak force, 2+2 against vehicle, $P = 0.057$ (exact rank test on litter means; by mouse, Welch's t test, $P = 0.0001$, unadjusted). Antibody signs are schematic.

Groups were then compared by peak force, the largest response of each mouse (Methods). The 2+2 group developed 1.95-fold the peak force of the vehicle group (95% CI 1.51 to 2.51; $n = 7$ and 4; Fig. 11B, Supplementary Table S7). Because each litter received a single treatment, we repeated the comparison on litter means,¹⁵⁰ which gave the same ratio and $P = 0.057$ by the exact rank test. This difference equals 39.5% of the deficit of the vehicle group relative to wild-type mice. By comparison, mice given the positive control, AAV9 mini-agrin, developed 1.31-fold the peak force of the vehicle group (1.00 to 1.72). These data suggest a partial restoration of force following bispecific antibody treatment.

We next compared the two tool molecules at the same nominal molar dose (Fig. 10C). Mice given the 1+1 CrossMab did not differ detectably in peak force from the vehicle group (1.08-fold, 0.67 to 1.76; Fig. 11B), while the 2+2 IgG-scFv group developed 1.80-fold their peak force (1.12 to 2.91; Supplementary Fig. S4B). Weekly regimens of the 1+1 CrossMab at lower doses likewise showed no detectable benefit (Supplementary Tables S6 and S7). Because this antibody was recovered from muscle (Fig. 9D), the lack of a detectable benefit may reflect insufficient bridging rather than insufficient delivery, consistent with the higher apparent potency of the 2+2 *in vitro*.

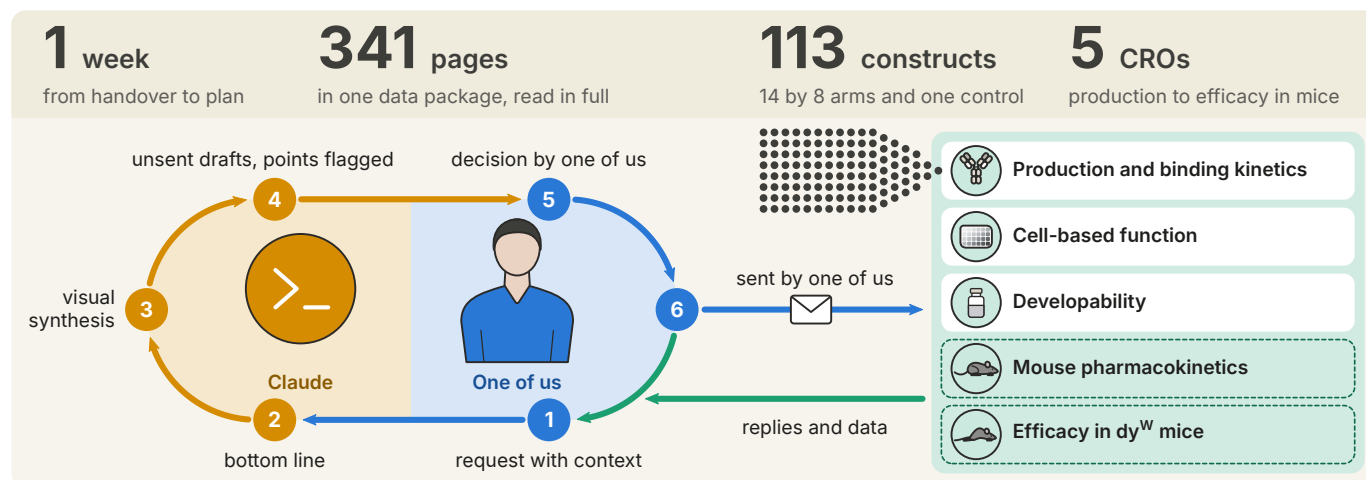
Among the other endpoints, grip strength per body mass was higher in the 2+2 group than in the vehicle group at weeks 4 and 6 (week 6, 1.94-fold, 1.43 to 2.63; Fig. 11C), as was absolute grip strength at week 6 (2.13-fold, 1.64 to 2.78; Supplementary Table S7). However, absolute grip strength also rose 2.65-fold in the wild-type mice (Supplementary Fig. S4D). Body mass of the dy^w mice remained about half that of wild-type mice, irrespective of treatment (Fig. 11D).

Overall, both measures of forelimb function, evoked triceps force and grip strength, were about twice as high in the 2+2 IgG-scFv group as in the vehicle group. Because the paralysis of dy^w mice is confined to the hindlimbs, the forelimbs are the limbs in which a gain in muscle function can be measured. We take this consistency across functional measures as *in vivo* proof of principle for the therapeutic approach. These results set a strong foundation for testing and optimizing novel, proprietary bispecific bridging antibodies.

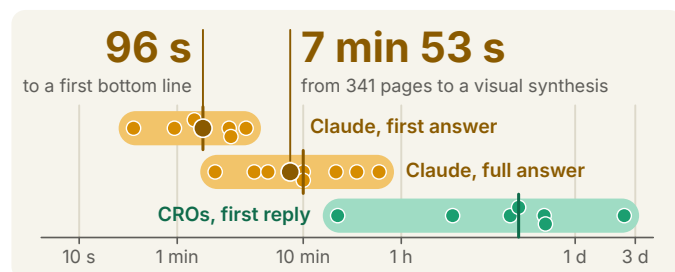
Working with Claude

Testing and optimizing novel constructs requires screens far larger than the work with two tool molecules. We therefore had a panel of 113 bispecific antibodies produced, in which each of 14

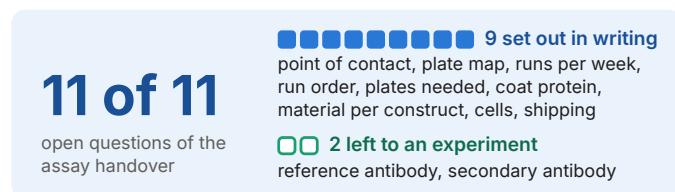
A In one week, one of us, working with Claude, took a data package to one plan across five CROs



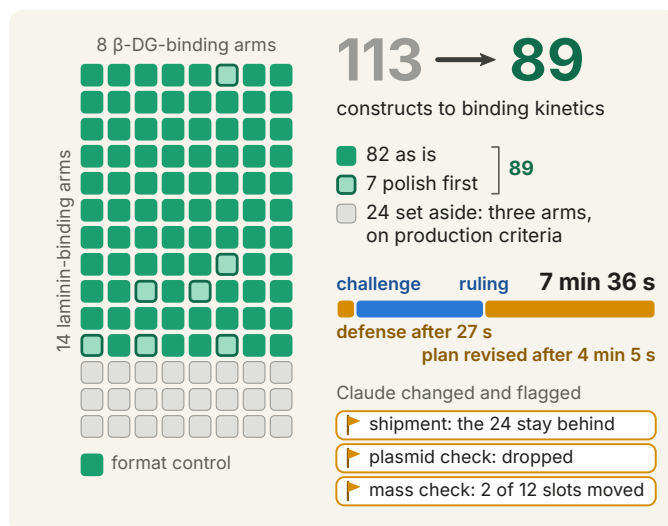
B Claude answered in a median of 96 s, CROs in 8.5 h



C Eleven questions were addressed in four working days



D One ruling revised the plan from 113 to 89 constructs



E We kept proposals for controls from the draft emails

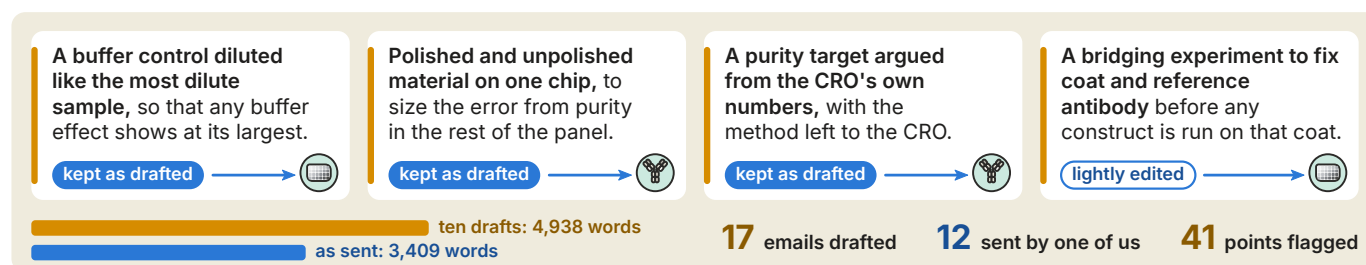


Figure 12. In one week, one of us, working with Claude, turned a 341-page data package on 113 constructs into one plan across five CROs. (A) Schematic of the loop and of the five CROs, by function (amber, Claude; blue, the team; green, CROs). Dots, constructs. Dashed outlines, no email sent. (B) Time from a request to Claude's first answer ($n = 7$) and full answer (plan, page or set of drafts; $n = 9$), and from an email to the first reply of a CRO ($n = 7$). Logarithmic axis. Thick ticks, medians. Dark points, the two answers called out.

(C) State of the eleven questions of day 1 after four working days. (D) The 113 constructs, including the format control, by triage class. Rows, laminin-binding arms. Columns, β -DG-binding arms. Bar, the exchange of day 7, to scale. Flags, further steps that Claude changed. (E) Four passages of Claude's drafts, paraphrased, with the fate of each passage and the CRO addressed. Bars, words of ten emails as drafted and as sent.

laminin-binding arms is paired with each of 8 β -DG-binding arms (112 constructs) and which includes one format control. Our team had designed and ordered these constructs, and the production CRO had made them, before the week analyzed here. The characterization of the panel, planned across five contract research organizations (CROs), is the largest screen of the program thus

far, and the two cell-based assays are to take more than ten times as many constructs as in any earlier round. We carry out this work with Claude (Anthropic), which reads the email and data of the program, runs code and saves drafts of emails in the mailbox of one of us. We asked whether this arrangement extends to a screen of this size, and analyzed the week in which the production

data arrived and were turned into one plan across the five CROs. Throughout this week, one of us passed what arrived from the CROs to Claude with a request, decided on the plans that Claude returned in answer, and edited and sent the emails that Claude had drafted for us (Fig. 12A). Claude sent no email.

The week opened with the handover of two cell-based assays, cell adhesion and flow cytometry, within our team, while eleven questions remained open with the assay CRO. In two sentences, one of us asked Claude to review the latest emails between the outgoing assay lead and the assay CRO and to recommend next steps for the handover, and gave it the link to the handover folder. Claude returned a bottom line after 96 s and a full plan with five drafted emails after 18 min (Fig. 12B). For the full plan it read six email threads, the handover folder, two slide decks and two reports, all in the mailbox and file store to which we had given it access. Four working days later, nine of the eleven questions had an answer in writing and the other two had been left to pilot experiments (Fig. 12C). The adhesion screen was thereby set out in writing. The CRO drew the plate map to the format that our team had requested, Claude compiled the run order and the material calculation from the production data, and one of us reviewed and approved them.

On day 4, the production CRO delivered its data package, a report of 341 pages, which one of us forwarded to Claude in two sentences, with the request to review it, share observations and recommend next steps. Claude returned a bottom line after 2 min 40 s and, once it had read every page, a visual synthesis of all 113 constructs after 7 min 53 s (Fig. 12B). The synthesis arranged the constructs on the grid of 14 by 8 arms. Claude marked 82 constructs for screening as is, seven for additional polishing, and the 24 constructs of three laminin-binding arms to be held back from the cell-based assays on production criteria (Fig. 12D). Each of the 113 constructs thus had a proposed next step in less than 8 min.

Claude had recommended binding kinetics for all 113 constructs, and on day 7 one of us challenged this plan. Claude defended it within 27 s, arguing that affinity would show whether the three arms merit further work. One of us overruled this recommendation and set the three arms aside on production criteria. Their material remains in storage. Four minutes later, Claude had revised its memo, the working page, the run order and three unsent drafts from 113 to 89 constructs. It had also applied the ruling to three dependent steps in the unsent drafts, each flagged for our veto (Fig. 12D). The exchange took 7 min 36 s from challenge to new plan, and every affected email was still an unsent draft. After the ruling, eight emails drafted by Claude went out on the same day to three CROs and a consultant.

Over the whole week, Claude drafted 17 emails, each in answer to a request from one of us, and we sent 12 of them, each after editing. With its drafts, Claude listed 41 points, such as tube fills, shipping conditions, recipients and assay settings, for which it had found no written statement of ours, so that we could confirm, change or strike each before sending. We kept some, struck others and changed one. Among the passages that we kept were proposals for controls (Fig. 12E). One draft proposed a comparison of polished and unpolished material on one sensor chip, to size the error that impurities cause in the rest of the panel. Another requested a buffer control diluted like the most dilute sample, so that any effect of the buffer appears at its largest. These proposals concerned the measurement of constructs that had already been produced, and each reached a CRO only after one of us had reviewed and approved it.

Claude also made errors, and we examined six of them in detail. Claude found five of them, one after a question from a CRO and two before the affected email was sent, while at least

three reached a CRO in emails that we had edited and sent. One of these, a request for less material per tube than the assay CRO had asked for, was corrected within a day, before any tube was filled. Two others, both statements in Claude's review of the production data, went unnoticed for more than 4 days, and one remains unsettled. These cases suggest that our editing tests the decisions in an email more reliably than the details and premises that it carries.

Despite these errors, all 89 constructs were shipped for both cell-based assays within a week of the ruling. Claude's first and full answers took medians of 96 s and 10 min, respectively, while the first reply of a CRO took a median of 8.5 h (Fig. 12B). The pace of this work was therefore set by the replies of the CROs and by our decisions. Claude performed the work that grows with the size of a panel, namely reading, calculation, drafting and tracing a decision through every dependent step. The judgments remained with us, and one ruling on three arms decided 24 constructs. This division of labor suggests that the size of a screen need not be limited by the size of the team that directs it.

Discussion

We hypothesized that a bispecific bridging antibody targeting β -DG and LG4-5 of laminin- α 4 would act as a laminin- α 2 mimetic, bridging laminin-411 to the sarcolemma and restoring anchorage in laminin- α 2-deficient muscle fibers. We designed and characterized two tool molecules, a murine 1+1 CrossMab and a humanized 2+2 IgG-scFv, which bound their respective targets with nanomolar affinity and facilitated cellular adhesion *in vitro*. In dy^w mice, treatment with the 2+2 IgG-scFv was associated with about twice the evoked forelimb force and grip strength of vehicle-treated mice. We regard this functional activity as proof of principle for the therapeutic approach.

In vivo proof of principle for a bispecific bridging antibody

Bridging *in vivo* requires delivery to muscle, which is unlikely to be a fundamental constraint. In dy^w mice, the 1+1 CrossMab recovered from muscle days after last dose bound both antigens, and its mean concentration exceeded the K_D of either arm and its EC_{50} in the adhesion assay (Fig. 9D). Immunofluorescence was consistent with its localization at the sarcolemma (Fig. 9E). Moreover, antibody may reside longer in muscle than in plasma, because the published antibody targeting laminin-211 and β -DG was detected in perfused muscle for up to 14 days, despite a serum half-life of about 30 h (ref. 30). The 2+2 IgG-scFv had a similar half-life in wild-type mice and was cleared faster than its parental IgGs (Fig. 9B, C), and binding to laminin- α 4 is likely the limiting variable for its exposure.

The 2+2, which appeared more potent than the 1+1 CrossMab *in vitro*, was also associated with about twice the peak triceps force and grip strength of vehicle-treated mice (Fig. 11B, C), whereas a nominally equimolar dose of the 1+1 CrossMab had no detectable effect on force (Fig. 11B). Because the 1+1 CrossMab exceeded its EC_{50} in muscle, bridging of fibers may require higher potency or valency than retention of dystroglycan-overexpressing cells. The simplest explanation for an effect of the 2+2 on force is a restored connection between the basement membrane and the sarcolemma, which allows fibers to transmit more force and protects them from degeneration. A circulating antibody would then have acted as a laminin- α 2 mimetic, as we hypothesized. This interpretation requires a concurrent, blinded replication that includes unlinked parental IgGs as a control³⁰ and provides

the histology and target-engagement data that were beyond the scope of our work here.

Anchorage without polymerization

Laminin-211 has two functions, polymerization and anchorage, and a bispecific bridging antibody only restores the latter. Evidence from human genetics and animal models suggests anchorage alone can deliver disease-modifying effects. Patients with missense variants in the LN domain, which impair polymerization, usually walk, whereas variants in the LG domains cause severe congenital disease despite the presence of laminin- α 2 (refs. 5,36,47). Normal muscle function thus appears to depend more on anchorage than polymerization. In mice, mini-agrin is the closest precedent, and it alone more than triples the lifespan of dy^w mice.¹⁵

We suspect that anchorage acts largely through assembly of the basement membrane. Mini-agrin partly restored the structure of this membrane,¹⁵ and the sarcolemma is largely spared in laminin- α 2-deficient mice.⁸¹ However, mini-agrin did not change the extractability of laminin- α 4 (ref. 15), and direct evidence that laminin-411 becomes linked to nidogen and collagen IV is lacking. If anchorage acts through assembly, a bridge would need to hold laminin-411 at the sarcolemma densely enough for these links to form.

A bispecific bridging antibody may anchor laminin-411 more effectively than mini-agrin, for several reasons. First, the antibody binds β -DG directly, whereas mini-agrin binds matriglycan on α -DG.^{29,98} The bridge is therefore independent of the state of α -DG in dystrophic muscle (Fig. 5C), and it leaves matriglycan free for perlecan and other ligands.^{10,14} Second, the antibody holds laminin-411 at LG4-5, the counterpart of the domains through which laminin-211 is anchored,⁷³ whereas mini-agrin binds the coiled coil of the long arm.⁹⁵ Third, the affinity, valency and geometry of an antibody can be engineered. Among constructs that share arms and Fc, format alone raised cell retention from at most 20% to 78 and 88% of the positive control (Supplementary Fig. S1D), and bridging was most efficient with two β -DG Fabs. Fourth, exposure to an antibody can be raised until its targets are saturated, whereas a vector is given once at a fixed dose.¹¹⁹ Consistent with these arguments, peak force was 1.95-fold that of vehicle-treated mice in the 2+2 IgG-scFv group and 1.31-fold in the AAV9 mini-agrin group (Fig. 11B).

The antibody carries no LN domain and would therefore leave laminin-411 unpolymerized. Patients whose variants impair only polymerization would not be expected to benefit,²³ and nerve and brain would remain untreated, because IgG enters both poorly.^{115,116} The fullest rescue of dy^w mice required both mini-agrin and α LNNd.¹⁵ In these mice, α LNNd also prevented hindlimb paralysis, a feature of the mouse models that rarely manifests clinically in patients,^{5,56} whereas ubiquitously expressed mini-agrin did not.²⁴ The antibody could therefore be combined with a single AAV vector for α LNNd.²³

α -Dystroglycanopathies

The same β -DG arm and format could serve the α -dystroglycanopathies, for which fewer questions are open than for LAMA2-CMD. Laminin-211 is largely retained and polymerized, so the missing function is anchorage, which a bridge would supply to muscle but not to brain or eye. LG4-5 of laminin- α 2 is accessible, since antibodies targeting it stain unfixed muscle.³⁰ Staining for laminin- α 2 and β -DG was, however, reduced in small biopsy series.^{129,151} Because the placement of the arms was as important as their number *in vitro*, an anti- β -DG IgG

with laminin- α 2 arms below the Fc should be compared with its reversed counterpart and a 1+1 bispecific with the same arms in *Large^{myd-3J}* mice.

Limitations

The main limitations concern the pilot study, in which groups were enrolled in sequence, vehicle followed a different regimen (Fig. 10C) and each litter received one treatment without randomization. Statistical support therefore depends on whether mice or litters are the unit of analysis (Fig. 11, Methods), and litter means gave $P = 0.057$, the smallest value possible with seven litters. Losses before week 6 left four to seven treated mice per group (Fig. 10C), so groups may differ by survival as well as by treatment. Because six treated groups (Supplementary Table S6) and at least 11 endpoints were examined, a chance finding is possible.

Exposure to the 2+2 cannot be related to its effect, because it was measured only in the plasma of wild-type mice, and muscle levels were measured only for the 1+1 CrossMab (Fig. 6E). Neither the intact 2+2 nor the anti-LAMA4 IgG, whose re-humanized arm differs from that of the 2+2, was tested on murine antigens, and the persistence of the 2+2 in muscle over the 6 or 7 days between last dose and force test was not measured (Methods). The pharmacokinetic comparison confounds format, Fc substitutions, target binding and anti-drug antibodies, which were not measured in any study, and it does not explain why exposure to the 2+2 increased less than in proportion to dose. The 1+1 CrossMab recovered from muscle may have been unbound, because these muscles were not perfused and plasma was not measured. Whole muscle holds about 4% of the plasma concentration of a non-binding IgG,¹¹⁶ so about 200 μ g/mL in plasma, which a conventional IgG could reach on this regimen (Fig. 9B), would account for the mean levels in muscle. The stoichiometric estimate (Fig. 9F) compares whole-tissue totals and does not measure occupancy. It is uncertain by more than an order of magnitude, because published amounts of dystrophin correspond to 2 to 730 nM (refs. 152,153). At the 466 nM reported for mouse muscle,¹⁵⁴ the 1+1 CrossMab would match only about one eighth of dystrophin-bound β -DG, which would then exceed laminin-411. A molar excess also need not result in occupancy, because a single humanized Fab engaged membrane-bound β -DG only weakly (Fig. 7B) and laminin- α 4 outside the basement membrane of the fiber^{7,14} may hold antibody out of its reach.

The *in vitro* assays described here are simplified systems that lack several controls. The adhesion assay, with dystroglycan-overexpressing cells on a fragment of laminin- α 4, reproduces neither repeated stress nor the native density of β -DG. Arms below the Fc had lower on-rates than at the N terminus, which may in part reflect capture of the antibody through its Fc in SPR. Most comparisons of humanized formats rest on a single experiment with one lot, so we interpret only large differences.

Both targets also remain incompletely characterized in diseased muscle. The density of laminin-411 on the fibers of patients has not been measured, so the number of bridges that an antibody could form is unknown. Evidence that β -DG persists in patients rests on two cases with residual laminin- α 2 (ref. 32). Safety was not addressed, although both targets are expressed in tissues well beyond the muscle fiber.^{7,155}

Conclusion

In vitro, bispecific bridging antibodies bridged dystroglycan-overexpressing cells to LG4-5 of laminin- α 4, most efficiently with two N-terminal β -DG Fabs. In dy^w mice, the 1+1 CrossMab was

recovered from muscle, and the 2+2 IgG-scFv was associated with higher evoked force and grip strength than in vehicle-treated mice, a consistency across forelimb measures that we take as proof of principle for the therapeutic approach. Together with human genetics and the precedent of mini-agrin, these findings support the hypothesis that anchorage restored by a bispecific bridging antibody can benefit laminin- α 2-deficient muscle. Beyond the tool molecules, discovery campaigns for each target gave diverse binders for both arms, from which more than 100 bispecific candidates covering all pairings were designed and produced. The next experiments should show in dystrophic muscle that the difference in force withstands concurrent controls, and that the antibody engages LG4-5 of laminin-411 together with β -DG and stabilizes the basement membrane. Confirmation of the hypothesis would make loss of anchorage, a primary defect of LAMA2-CMD, treatable with an antibody that can be titrated and stopped.

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Methods

Study design

All experiments were designed and overseen by the Mithril Medicine team and performed at CROs, which are referred to by role. The work was exploratory and not conducted under Good Laboratory Practice. Unless stated otherwise, experiments were neither randomized nor blinded, group sizes were not set by a sample-size calculation, and no primary endpoint, analysis or acceptance criterion was prespecified. Molar concentrations and doses were derived from calculated molecular masses.

Animal welfare and oversight. All animal studies were performed by CROs under the oversight of their Institutional Animal Care and Use Committees. The study protocols on file specify housing in an AAALAC International-accredited vivarium holding a Public Health Service Animal Welfare Assurance and compliance with the Animal Welfare Act, the CIOMS International Guiding Principles for Biomedical Research Involving Animals and the AVMA Guidelines for the Euthanasia of Animals.

Structural models, illustrations and registry search

Superposition of LG4 (Fig. 3C). LG4 of the AlphaFold model of human laminin- α 4 (AF-Q16363-F1, version 4)⁹² was superposed on LG4 of mouse laminin- α 2 (crystal structure, PDB 5IK8, molecule B)⁷³ by iterative C α -based structural alignment (custom script; 159 pairs within 4 Å, root-mean-square deviation 1.2 Å, 24% identity over 170 aligned residues). This alignment and three others (super, cealign and align in PyMOL 3.2) paired Arg2803 of laminin- α 2 with Asp1513 of laminin- α 4, and Asp2876 with Lys1587 (Ile1588 in cealign). Contacts were residues within 4.0 Å of the Ca²⁺ ion or the glycan. Tips of exposed side chains more than 10 Å from the ion were omitted from surfaces.

Molecular scenes (Fig. 5). All scenes shared the DGC of a rabbit cryo-electron microscopy structure (PDB 9C3C, 4.3 Å; β -DG residues 720 to 740 traced tentatively),⁷⁷ drawn without the N-terminal domain of α -DG, which furin releases.⁶⁴ The two structures above supplied LG4-5, and LG1-3 of the laminin- α 4 model also represented LG1-3 of laminin- α 2. Modeled variable domains of the tool arms were grafted onto human IgG1 (crystal structure, PDB 1HZH).¹⁵⁶ The variable fragment (Fv) of the LAMA4 arm kept an unrefined docked pose. The same Fab, recolored, served as the laminin- α 2 arm and, like the β -DG Fab, was placed free of clashes.

Rendering and scale. Figs. 3C, 5, 6A and 8B and Supplementary Fig. S1A were rendered in PyMOL, and all other illustrations were composed in Matplotlib. In Fig. 8B, bystander molecules were faded and each LAMA4 arm was colored a single blue after rendering, without change of outlines or positions. Where a legend states “to scale,” molecules share the scale of the bar, and lengths follow published electron microscopy, crystal structures or the coordinate files above, whereas number, spacing and poses of molecules are schematic.

Registry search. ClinicalTrials.gov was searched in October 2026 for interventional studies of the conditions LAMA2, merosin, MDC1A or “laminin alpha 2” (nine records). The Japanese (JRCT) and European Union (CTIS) registries were not searched.

Tool molecules

Tool arms. The β -DG arm was derived from hybridoma clone AS30, whose epitope has been mapped to residues 738 to 748 of human β -DG,¹⁵⁷ and the LAMA4 arm from mouse clone 15F7, which binds within LG4-5 (ref. 158). Murine constructs carried the

parental sequences AS30 S/S (SEQ ID NOs 172 and 173) and 15F7 (16 and 17), and humanized constructs carried AS30SS_Hu6 (314 and 330) and Hu15F7 H2L1 (58 and 59). The variable regions were taken from the patent sequence listings and gene-synthesized at the tool-molecule CRO, unchanged apart from the engineered cysteines of the stapled scFv and one or two C-terminal residues of V domains in crossed arms and scFv arms. The LAMA4 parental IgG was a re-humanized variant, which differs from the tool arm at 21 positions of the variable regions.

Formats and production. We report ten bispecific bridging antibodies made at the tool-molecule CRO (Supplementary Table S1), namely two murine 1+1 CrossMabs on mouse IgG2a LALA-PG and eight humanized constructs on human IgG1/ κ LALA-PG with the half-life-extending mutations M252Y/S254T/T256E (YTE).¹⁵⁹ In heterodimers, heavy chains were paired by knobs-into-holes and light chains by a VH-VL or CH1-CL crossover in one Fab. In the murine 1+1 constructs, the LAMA4 heavy chain carried the knob and the β -DG heavy chain the hole. All ten constructs and the parental IgGs were first expressed in CHO-K1 cells (20 mL; 1 mL for the anti-LAMA4 IgG) and purified on protein A and, except for the murine constructs and the anti-LAMA4 IgG, by SEC. A second construct of the 2+2, identical except that it lacks YTE (2+2 without YTE), was made likewise at the production CRO in CHO-S cells (200 mL). We omitted YTE from the construct dosed in dy^W mice because Fc mutations of this kind had lowered serum concentrations of IgG in mice.¹⁶⁰ The first construct (2+2 with YTE) was used for SPR, flow cytometry, adhesion and PK in wild-type mice, and the second for adhesion and dosing of dy^W mice. Assembly was assessed as the share of the target mass in liquid chromatography-mass spectrometry (LC-MS) of intact, denatured, deglycosylated protein, which cannot detect a swap of the two light chains (Supplementary Table S1).

Antigens. Antigens were produced in CHO-K1 cells at the tool-molecule CRO, and their oligomeric state was not determined. All carried a C-terminal His₈-Avi tag, and biotinylated lots were biotinylated during expression. The human antigen called β -DG ectodomain spanned DAG1 residues 30 to 749 (81.5 kDa) and comprised α -DG and the ectodomain of β -DG, which ran as separate bands in SDS-PAGE, and the murine version ended at V747. LAMA4 antigens were human LG4-5 (43.9 kDa), human LG1-5 (residues 833 to 1823, 113.8 kDa) and murine LG1-5 (Q828 to A1816, 113.9 kDa).

SPR. Antibody (0.8 to 3 μ g/mL) was captured for 30 s at 10 μ L/min on a Biacore 8K (CM5 chip, HBS-EP+, 25 °C) by amine-coupled anti-mouse Fc antibody (Cytiva; regenerated with glycine, pH 1.7) or human Fc (Jackson ImmunoResearch; glycine, pH 1.5). The antigen of the matching species (for LAMA4, human LG4-5 or murine LG1-5) was injected at 30 μ L/min for 180 s in 2-fold steps from 0.781 to 50 nM (25 nM for the anti- β -DG IgG), dissociation was followed for 600 s (1,200 s for murine LAMA4 and the parental IgGs), and a 1:1 model was fitted. Each antibody-antigen pair was run once, and the parental IgGs, the 2+2 and the other constructs of Fig. 7 were run in separate sessions. Because capture reagents¹⁶¹ and antigens differed, K_D was compared only within the murine or humanized set. For β -DG and LAMA4, the association rate constant (k_a) was 5.42 and $4.28 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and the dissociation rate constant (k_d) was 1.33×10^{-3} and $2.31 \times 10^{-4} \text{ s}^{-1}$ for the 1+1 CrossMab. For the parental IgGs, k_a was 10.8 and $11.9 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, k_d 2.85×10^{-3} and $6.55 \times 10^{-4} \text{ s}^{-1}$ and K_D 2.65 and 0.549 nM (humanized constructs, Supplementary Table S2). The half-life of the complex ($\ln 2/k_d$) with β -DG and LAMA4 was 8.7 and 50 min for the 1+1 CrossMab and 4.0 and 15 min for the 2+2, and the values for LAMA4 are extrapolated beyond the 10 or 20 min recorded. The on-rate of the C-terminal β -DG scFvs of the reversed 2+2 was

5 to 21% below that of the N-terminal Fabs. The first, small-scale lot of each construct was used, and maximal response ($R_{\max}$) and fit statistics were not reported.

Dual-antigen ELISA. Plates were coated overnight with human LAMA4 LG1-5 (2.0 $\mu\text{g/mL}$) and blocked with 2% bovine serum albumin (BSA). Captured antibody was detected with biotinylated human β -DG ectodomain (0.3 $\mu\text{g/mL}$, 1 h), streptavidin-peroxidase (30 min) and 3,3',5,5'-tetramethylbenzidine (TMB; 450 nm). The bioanalysis CRO adapted this protocol of the tool-molecule CRO to 96-well plates. The calibrator in Fig. 6D was diluted in duplicate from 1,000 ng/mL and fitted with a four-parameter logistic function (EC_{50} 0.52 nM; 0.83 nM on a second plate). Its concentrations assume 2-fold steps between standards, and with 1.6-fold steps its EC_{50} would be 1.2 nM.

Fab ELISAs. Fabs of the humanized arms were titrated on directly coated antigens. Murine-to-human EC_{50} ratios of the β -DG arm were 0.95 to 1.5 (periplasmic extracts, three experiments), and the EC_{50} of the LAMA4 arm (purified Fab) was 0.13 and 0.26 nM on murine and 0.44 to 1.49 nM on human LG1-5.

Modeled distances. Fab, scFv and Fc were treated as rigid bodies (PDB 1HZH, also for the mouse IgG2a construct), with one modeled Fv per arm and fixed elbow, CH2 and lower hinge. The upper hinge and the connector below the Fc were freely rotating chains with a persistence length of 0.45 nm, measured for Gly/Ser linkers¹⁶² and assumed for the hinge. Distances between paratope centers were taken from 20,000 Monte Carlo conformers without C α clashes (< 5.0 Å).

Binding to cells

Cells. HEK293-derived cells engineered to overexpress DAG1 were grown at the assay CRO in DMEM with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 1 $\mu\text{g/mL}$ puromycin.

Flow cytometry. Each construct of Fig. 7 was expressed once, and its SEC-polished lot was used for SPR and flow cytometry (Supplementary Table S2). Cells (1×10^5 per well, V-bottom plates) were labeled with LIVE/DEAD Fixable Near-IR dye (Invitrogen; 30 min, room temperature) and incubated for 45 min at 4 °C with 100 μL of antibody or human IgG1 isotype control (Sino Biological; taken as 150 kDa), in duplicate, at six 5-fold dilutions from 100 $\mu\text{g/mL}$. After two washes in PBS, they were stained with Alexa Fluor 647 goat anti-human IgG (H+L) (Jackson ImmunoResearch; 30 min, 4 °C; dilution not recorded) and washed twice, and live single cells were read on a NovoCyte Quanteon (Agilent). Whether MFI is a mean or a median was not recorded. The concentration that reached a given MFI was interpolated on the rising limb of each curve, between the two flanking means on logarithmic axes and without background subtraction.

Composition of the lots. Semi-quantitative LC-MS detected a homodimer of the hole chain, by design a bivalent anti- β -DG IgG, in the lots of the h1+1 CrossMabs (LAMA4 crossed, 1.1%; β -DG crossed, 0.2%). If this species stains like the 2+1 or the 2+2, it accounts for 22 to 28% and 8 to 9%, respectively, of the isotype-subtracted signal at 138 nM. The shifts of the h1+1 CrossMabs from the 2+2 are then lower bounds, and the shift of the 1+2 from the h1+1 CrossMabs is an upper bound.

Specificity controls and murine 1+1 CrossMab. Because the titration included no parental cells, specificity was judged from the isotype control and the low signal of the 1+2. The earlier run that included the 2+2, one of six, was excluded (secondary antibody alone, MFI 2.7×10^5). The murine 1+1 CrossMab (*in vitro* lot; SEC main peak, 90%) gave an MFI of 2.8 to 8.7×10^5 at 69 nM in three runs with goat anti-mouse IgG conjugates diluted 1:50 (isotype controls, MFI 690 to 10,098; parental cells, 2.7 to 27% of this signal, unsubtracted).

Cell adhesion assay

Coats. White 384-well plates (OptiPlate-384 HB, Revvity) received 20 μL coating solution in DMEM with 25 mM HEPES for 1 to 3 d at 4 °C. Coats, at 10 $\mu\text{g/mL}$ unless stated, were recombinant human LAMA4 or laminin- α 2 LG4-5 (biotinylated, from the tool-molecule CRO, adsorbed passively), the commercial laminin-411 fragment (ACROBiosystems LAT-H5263), full-length laminin-411 (BioLamina LN411, one lot) or PLL (Sigma P4832, 50 $\mu\text{g/mL}$). We suspect that the commercial fragment is an E8-type truncation (coiled coil and LG1-3, without LG4-5).

Procedure. DAG1-overexpressing cells (see “Binding to cells”) were thawed 2 to 5 d before use into DMEM with 10% FBS. At room temperature, wells were blocked for 1 h with 80 μL assay buffer (DMEM, 25 mM HEPES, 10% FBS) plus 2% BSA. Antibody (20 μL , 90 min) and then cells (50,000 in 30 μL assay buffer with 10 mM EDTA, 60 min) were added, and each of the three steps ended with two washes in 50 μL assay buffer on a centrifugal washer (BlueWasher, BlueCatBio; 2,000 rpm, 10 s). EDTA abolishes binding of integrins and α -DG to laminin.¹⁰ CellTiter-Glo (25 μL , 20 min; Promega G7573) was added, and plates were read on one of two EnVision 2105 readers (Revvity).

Layout and controls. A standard plate held, in fixed positions, 48 uncoated wells without antibody, 28 PLL wells, 68 coated wells without antibody and six curves of ten 3-fold doses from 500 nM in quadruplicate. Variant plates, the plates of two preliminary days with differently prepared cells and the plates with full-length laminin-411 departed from this layout or protocol. Negative controls, on 14 of 39 plates but not on that of the humanized constructs in Supplementary Fig. S1D, were an Fc protein (ACROBiosystems FCC-H5214) or polyclonal human IgG (Jackson ImmunoResearch 009-000-003). In Fig. 8D, the control proteins retained at most 21% of PLL (5 plates) and the 2+2 on laminin- α 2 LG4-5, coated at 10 or 30 $\mu\text{g/mL}$, at most 6.5% (4 plates). The panel shows the plates whose PLL wells failed neither criterion (a mean below 2×10^6 counts/s or a coefficient of variation above 25%), on which antibody was incubated for at least 60 min and cells were added directly after the wash. The 1+1 CrossMab is shown on standard plates only, hence one plate per coat. Its two other standard days on the LG4-5 coat failed a PLL criterion, and on a plate of the preliminary days, which contributes to the three control columns, it retained 65% of PLL. Without an input-cell standard, percent of PLL is not a fraction of the cells added.

Curve fitting. Raw counts of all wells of a curve were fitted with an unweighted, unconstrained four-parameter logistic (SciPy, multiple starts), with 95% profile-likelihood confidence intervals (CIs). A fit was accepted if R^2 was at least 0.5, one dose mean reached 10% of PLL and the CI was bounded. For percent of PLL, the mean of wells without antibody on the same coat and plate half was subtracted from each well and from the PLL mean of the plate, except in Fig. 8D, Supplementary Fig. S2D and where marked in Supplementary Table S3. The reader exported two blocks of counts, which differed in 463 wells on five plates. We fitted the first block without excluding wells, whereas the assay CRO had fitted the second after excluding 32 wells, for which no criterion was recorded. The three highest control curves lay on plates whose two blocks differed.

Coat titration and binding ELISA. Molar concentrations were calculated with 43.9 kDa for the LG4-5 fragment and about 575 kDa (5,218 residues at 110 Da) to 650 kDa (sum of chains on gels¹⁶³) for laminin-411, assuming equal adsorption per mole. For the plates of Supplementary Fig. S2D, full-length laminin-411 was coated at 30 $\mu\text{g/mL}$ (46 to 52 nM) and the LG4-5 fragment at 10 $\mu\text{g/mL}$ (228 nM). The LG4-5 coat was titrated on one plate with 60 nM 1+1 CrossMab and the first of two batches of fragment, and the

assay CRO fitted the curve. Retention was already lost at 3.1 $\mu\text{g/mL}$ (71 nM; Supplementary Fig. S2C). Because muscle presents LG4-5 as part of the laminin-411 heterotrimer, we also tested the 2+2 on two plates coated with full-length laminin-411. Retention remained close to that of the coat alone, although the antibody bound this laminin in an ELISA (Supplementary Table S3). This laminin was coated at a molar concentration below that at which retention on the LG4-5 coat was already lost, and the orientation of LG4-5 was not controlled, so these plates do not test bridging to the heterotrimer. For the ELISA, coats on separate plates (10 $\mu\text{g/mL}$, overnight, 4 °C; blocked with 3% BSA) were probed with the 2+2 without YTE or an isotype IgG (ten 3-fold doses from 10 nM, in duplicate) and goat anti-human IgG-peroxidase.

Pharmacokinetics and exposure in muscle

Test articles. The 2+2 with YTE (198.5 kDa), the anti- β -DG IgG (144.5 kDa) and the anti-LAMA4 IgG (144.9 kDa) were formulated in 20 mM histidine, 150 mM NaCl, pH 5.5 (SEC main peaks, 96.4 to 99.4%; parent lots, less than 0.1 endotoxin units/mg). Both parental IgGs carry wild-type human IgG1/ κ constant regions, whereas the Fc of the 2+2 carries LALA-PG and YTE. Within the Fc we suspect YTE as a cause of faster elimination, because Fc variants with raised affinity for the neonatal Fc receptor also bound the murine receptor more tightly at pH 7.4 and had lower serum concentrations in mice.¹⁶⁰ If YTE shortens exposure in mice, the half-life measured here understates that of the 2+2 without YTE, which was dosed in dy^{W} mice. The Fc is probably not the only cause of faster elimination, because a tetravalent antibody targeting laminin-211 and β -DG, with a murine Fc, and its parental antibodies also had half-lives near 30 h in mice.³⁰

Plasma study. Male C57BL/6J mice aged 6 to 8 weeks ($n = 12$) were allocated at random to four groups of three according to the study protocol. Each mouse received one intravenous bolus (5 mL/kg of a 5 or 10 mg/mL solution) and was bled from the orbital venous plexus after 0.5, 2, 8, 24, 72, 120, 168, 336, 504 and 672 h, without a pre-dose sample. The matrix, reported as K2-EDTA plasma and as serum, is called plasma here. Anti-drug antibodies were not measured.

Total human IgG ELISA. Human IgG was captured on Fc-specific goat anti-human IgG (Sigma I2136). Samples, pre-diluted 10- to 1,000-fold if needed, were diluted 50-fold in PBS-Tween with 0.5% BSA, incubated for 1.5 h and detected with monkey-adsorbed goat anti-human IgG (H+L)-peroxidase (Bethyl A80-319P, 1 h) and TMB (450 minus 630 nm). Each article served as its own standard (nine 2-fold calibrators from 5 $\mu\text{g/mL}$, in duplicate; four-parameter logistic weighted by $1/Y^2$), with quality controls at 2, 0.5 and 0.1 $\mu\text{g/mL}$. The assay was not validated and detects any species with a human Fc. All 42 quality controls lay within $\pm 20\%$, and 117 of 120 samples lay within the range they bracket (except the anti-LAMA4 IgG at 672 h). Because each group had its own plates, CIs of group ratios do not include plate-to-plate error. The 336 h samples of the anti-LAMA4 IgG were assayed twice, the second run was used, and the ratio between runs differed about 5-fold among mice.

Noncompartmental analysis. Each mouse was analyzed with nominal times, the linear-up/log-down trapezoidal rule and log-linear back-extrapolation to the initial concentration (C_0). The terminal slope came from the unweighted regression of $\ln$ concentration on time over the three or more last points with the highest adjusted R^2 . Clearance was $\text{dose}/\text{AUC}_{\text{inf}}$, mean residence time (MRT) was $\text{AUMC}_{\text{inf}}/\text{AUC}_{\text{inf}}$ and effective half-life was $\ln 2 \times \text{MRT}$ (AUMC, area under the first-moment curve; inf, extrapolated to infinity). We report the effective half-life because the slow tail of the 2+2, with a half-life of about 180 h from day 14, accounted

for about 2% of total exposure (AUC_{inf} ; Supplementary Fig. S3A). Doubling the dose of the 2+2 from 25 to 50 mg/kg raised AUC_{inf} 1.31-fold (95% CI 1.11 to 1.54; Supplementary Fig. S3B). Phase half-lives were fitted over fixed windows (24 to 168, 168 to 504 and 336 to 672 h), and shares of exposure were increments of cumulative AUC relative to AUC_{inf} . Groups were compared by ratios of geometric means, with 95% CIs from Welch's t on the $\ln$ scale. The PK CRO's analysis gave clearances up to 10% lower.

Antibody in muscle homogenate. Right gastrocnemius, tibialis anterior and triceps were frozen at necropsy without perfusion. The bioanalysis CRO homogenized each muscle in 19 volumes (0.05 g/mL) of 10 mM HEPES, 0.5% Triton X-100 with 4 $\times$ Halt protease inhibitor, and cleared the homogenate (12,000 $\times$ g, 10 min, 4 °C). It ran the dual-antigen ELISA (see "Tool molecules") with the protocol and antigens of the tool-molecule CRO, in 96-well plates read at 450 nm. Duplicate 10-fold dilutions were read against duplicate standards of the dosed lot of the 1+1 CrossMab, diluted in 2-fold steps from 1,000 ng/mL. We took the lowest standard as the limit of quantification (0.78 $\mu\text{g/g}$), and all quantified values exceeded the limit set by the tool-molecule CRO (1.46 $\mu\text{g/g}$). The assay was not validated for muscle and was run in 10% matrix (tested by the tool-molecule CRO up to 5%), without quality controls or spike recovery, and dilutional linearity was not established. One of the four mice dosed weekly at 50 mg/kg had 13.7 $\mu\text{g/g}$ in gastrocnemius, 3.2-fold the mean of the other three (4.3 $\mu\text{g/g}$). It met the Grubbs criterion for an outlier ($G = 1.49$; critical value for $n = 4$ at $\alpha = 0.05$, 1.48) and was excluded from Fig. 9D and F; Supplementary Table S4 gives the group with and without it. Levels in triceps were similar to those in gastrocnemius, and levels in tibialis anterior were lower (Supplementary Table S4).

Stoichiometric estimate. Whole-tissue concentrations of the two targets were estimated from published amounts per mass of muscle protein. Amounts were converted with 212 mg protein per mL of muscle (density 1.06 g/mL,¹⁶⁴ protein assumed at 20% of wet mass), so that 1 pmol per mg protein corresponds to 212 nM. For β -DG we assumed one β -DG per dystrophin, as in the structures of the dystrophin-glycoprotein complex,^{76,77} and 427 kDa for dystrophin. Six published amounts of dystrophin, 4.8 ng/mg,¹⁵² 0.002% of muscle protein,¹⁶⁵ 29.5 ng/mg,¹⁶⁶ 93.2 fmol/mg,¹⁶⁷ 75 ng/mg¹⁶⁸ and 3,440 fmol/mg,¹⁵³ correspond to 2.4, 10, 15, 20, 37 and 729 nM, and we report the two central values. In wild-type mouse muscle, dystrophin was reported at 83.4 ng/mg (41 nM)¹⁶⁶ and at 2.20 nmol per g protein (466 nM).¹⁵⁴ The chains of laminin-211 and laminin-221 in a proteome of wild-type mouse triceps add up to 1,621 ng per mg protein,¹⁶⁹ or 477 nM at 720 kDa per heterotrimer, in agreement with 466 nM by quantitative immunoblot.¹⁵⁴ We took laminin-411 as 25 to 35% of 477 nM, because laminins that contain laminin- $\alpha 4$ amounted to about 30% by mass of the wild-type amount of laminins that contain laminin- $\alpha 2$ in muscle extracts of 3-week-old $\text{dy}^{3\text{K}}$ mice.¹⁴ The 1+1 CrossMab was converted with 145.3 kDa and 1 g taken as 1 mL, so that the group means in gastrocnemius (4.3 and 8.9 $\mu\text{g/g}$, Fig. 9D) correspond to 29 and 61 nM, and the values of single mice (3.6 to 12.9 $\mu\text{g/g}$) to 25 to 88 nM (Fig. 9F).

Immunofluorescence. Frozen sections of left hindlimb muscle were stained with an anti-mouse IgG secondary antibody alone, without primary antibody or counterstain. It was the only reagent used to detect the 1+1 CrossMab and also detects endogenous mouse IgG. Sections came from a preceding study, from one dy^{W} female given the 1+1 CrossMab intravenously at 50 mg/kg twice weekly from birth, chosen among mice read as responders, and from one vehicle-treated male. The imaging CRO reported secondary-antibody signal in all muscles of dy^{W} mice examined, highest in treated mice, whereas the image shown for the vehicle-treated mouse is nearly blank. Companion images were labeled

gastrocnemius (treated mouse) and tibialis anterior (vehicle-treated mouse). Panels show whole frames, resampled without intensity adjustment. The secondary-antibody product, microscope, exposure, display range and pixel size (hence no scale bar) were not reported.

LG4-5 staining (Supplementary Fig. S5). The imaging CRO incubated hindlimb sections of one vehicle-treated dy^w mouse of the preceding study and of one wild-type mouse with the His-tagged Fab of the murine LAMA4 arm, followed by rabbit anti-His and AF568-conjugated anti-rabbit antibodies. Without Fab, the outlines were fainter in the dy^w mouse. In the wild-type mouse they were similar with and without Fab, and only the bright points between fibers were absent without Fab. No capillary marker was used. The stain does not distinguish LG4-5 attached to laminin-411 from released LG4-5 held in the matrix. Whole frames are shown as supplied, without intensity adjustment. Concentrations, exposure, display range and pixel size were not reported.

Treatment and testing of dy^w mice

Study and groups. The pilot study began with weekly intraperitoneal injections of the 1+1 CrossMab, chosen to limit the handling of neonates. We later added two groups dosed twice weekly, first the 1+1 CrossMab at 72 mg/kg and then the 2+2 at 100 mg/kg. The main text reports these two groups and the three control groups (Fig. 10C), together with muscle levels in mice dosed weekly (Fig. 9D), and all groups are listed in Supplementary Table S6. Groups were enrolled and tested in sequence, because each litter received one treatment and the colony was small. As a result, the 2+2 group was tested last, 126 and 161 days after the two sessions of the vehicle group (Supplementary Fig. S4A), and the colony moved to a new facility in the interval.

Animals. B6.129S1(Cg)-*Lama2*^{tm1Eeng/J} mice (dy^w ; The Jackson Laboratory, stock 013786) were bred at the *in vivo* CRO from heterozygous pairs. Homozygotes and wild-type littermates or colony mates of both sexes were used (Supplementary Table S6). Mice were fed Teklad 2018 diet ad libitum, received moistened food and DietGel 76A daily and were weighed three to four times per week. The humane endpoint was the veterinarian's judgment, and one mouse was euthanized as moribund. Concentrations from two single-dose studies (1+1 CrossMab, 15 dy^w mice; 2+2, seven dy^w and six wild-type mice) are not available.

Allocation and attrition. Three females and three males were planned per group. Each litter received one treatment, only wild-type and vehicle-treated dy^w mice shared litters, and no vehicle-treated litters were enrolled with the 2+2 group. As a result, 3 of the 28 treated mice shared a session with vehicle-treated mice, and none of them had received 72 mg/kg, AAV9 mini-agrin or the 2+2. Litters were enrolled whole, before genotyping, only in groups dosed from birth. Losses before week 6 amounted to 26 of 58 dy^w mice, not counting mice collected early (Supplementary Table S6). In the vehicle group, three mice were found dead and one was collected early for a single-dose test of tissue distribution, and in the 2+2 group one was found dead and one was missing. At 72 mg/kg, enrollment exceeded the supply of antibody, and dosing of 18 surplus pups was stopped (16 collected early for tissue, one found dead, one of unrecorded fate).

Test articles and vehicle. The dosed lots of the 1+1 CrossMab and the 2+2 (145.3 and 198.7 kDa) contained < 0.050 and 0.005 endotoxin units/mg. SEC main peaks were 98.3% (1+1 CrossMab) and > 99% (2+2), and 98.6% for the lot of the 2+2 dosed in wild-type mice. Vehicle was 20 mM histidine acetate, 150 mM NaCl, pH 5.5. AAV9 encoding mini-agrin under the hybrid chicken β -actin (CBh) promoter^{22,170} was supplied by the vector CRO (4.24×10^{13}

vector genomes/mL). Expression of mini-agrin in muscle was not measured.

Dosing. Antibodies and vehicle were injected intraperitoneally, at 5 mL/kg in the wild-type, vehicle and weekly 1+1 CrossMab groups and 6.77 mL/kg for the undiluted 2+2 (14.78 mg/mL). The study began with weekly injections, and we planned to move to twice-weekly dosing if early body mass did not improve. The 2+2 followed once it had been produced. By the schedule, twice-weekly regimens comprised 13 doses at 72 mg/kg and 12 of the 2+2. Terminal tests followed the last injection by 1 to 10 d (vehicle, 1 or 5; 2+2, 6 or 7), at 41 to 52 d of age (vehicle, 45 to 47; 2+2, 47).

Choice of endpoints. Grip strength depends on motivation and operator.¹⁷¹ Rotarod performance and plethysmography are less specific for skeletal muscle than grip strength and evoked triceps force, because the rotarod requires hindlimb stepping²⁴ and quiet breathing draws on about one tenth of the pressure that a healthy diaphragm can generate.¹⁷² Survival was not an endpoint, and histopathology was not assessed.

Grip strength. Mice held by the tail grasped a bar with the forepaws and were pulled horizontally until release (weeks 4 and 6). The score, in grams-force, was the mean of two sets of five pulls, each without its highest and lowest value. For Fig. 11C, it was divided by body mass, as entered with the test at week 4 and as measured at necropsy on the following day at week 6.

Rotarod and plethysmography. After 3 d of acclimation (20 rpm, 5 min), mice ran on the rotarod (Ugo Basile) at 20 rpm for 3 min and then under acceleration until they fell (weeks 4 and 6; AAV9 mini-agrin, week 6 only). The rate of acceleration was not recorded. Minute volume (mL/min) was taken from unrestrained whole-body plethysmography (DSI; three periods of 2 min, week 6). Per gram of body mass, it did not differ between wild-type mice and dy^w mice given vehicle (4.18 ± 1.12 and 5.07 ± 3.12 mL/min/g; $n = 12$ and 4).

Nerve-evoked triceps force. On the day after the tests without sedation and before necropsy, mice were anesthetized with isoflurane and taped to a heated table. A pin was pressed against the humeral head, and the forelimb rested against a bar on the arm of a dual-mode lever (Aurora Scientific 305C). The radial nerve was stimulated through two percutaneous needle electrodes, with muscle and tendon left in place. The current was raised to the optimal isometric twitch (at least 30 s between twitches), and trains of 500 ms (0.2 ms pulses) followed at 1, 20, 40, 50, 60, 80, 100 and 150 Hz. Force is given in mN at the lever, without conversion to torque. Elbow angle, lever length and rest between trains were not recorded.

Analysis of functional endpoints

Animals and unit of analysis. All 44 mice that reached the terminal tests, 32 of them in the five groups of the main text, were analyzed without exclusion, with the mouse as unit. Peak force was the largest of a mouse's eight responses, which the *in vivo* CRO supplied as the maximum of each train, without the recordings. In 17 of 44 mice (vehicle, 3 of 4; 2+2, 3 of 7) the series was repeated, according to the numbering of recordings, and a later series was analyzed. In the 2+2 group, four mice with a first series averaged 47.4 mN and three with a later series averaged 47.2 mN.

Dating of test sessions. Force tests were dated by tissue collection, and the grip strength test at week 6 was placed one day earlier, as scheduled by the protocol. Days are counted from the first session of the vehicle group (day 0). The band that marks the colony's move in Supplementary Fig. S4A and C to E extends from day 61, when the *in vivo* CRO was moving and mice were quarantined, to day 153, by which time mice were housed in

the new facility. The site of sessions from day 61 onward was not recorded.

Group comparisons. Effects were expressed as ratios of arithmetic group means, with 95% CIs by the delta method on the log scale and Welch-Satterthwaite degrees of freedom. The 2+2 and vehicle groups were compared by Welch's *t* test on mice and by the exact Wilcoxon rank-sum test on litter means. At single frequencies, force in the 2+2 group was 1.96- to 2.23-fold that of the vehicle group from 40 to 150 Hz, and the ratio was larger below 40 Hz. The two groups differed in sex ratio (Fig. 10C), and among females alone the ratio of peak forces was 2.10 (1.78 to 2.48; *n* = 3 per group). As sensitivity analyses, each mouse was omitted in turn (ratio, 1.81 to 2.03), and the five groups of Fig. 11B were compared by one-way ANOVA with Tukey's test on raw values (*P* = 0.43), whose pooled variance is dominated by wild-type mice (coefficient of variation 38%), and on log-transformed values (*P* = 0.023). The group given 72 mg/kg was also compared without its smallest mouse (6.2 g; peak force, 8.4 mN), which gave 1.27-fold the peak force of the vehicle group (0.98 to 1.63).

Derived measures. Effects were also expressed as a share of the deficit relative to wild type, (treated – vehicle)/(wild type – vehicle) on group means.¹⁷³ Peak force was also divided by the wet mass of the triceps. In the 2+2 group, this measure was 1.79-fold that of the vehicle group (1.35 to 2.38) and 1.28-fold that of wild-type mice (1.03 to 1.58). Only the 2+2 group was perfused before muscles were weighed.

Association with time. Between the two sessions of five wild-type mice, 61 d apart and at equal body mass, peak force changed 0.84-fold (0.61 to 1.16) and grip strength 2.65-fold (2.07 to 3.39; Supplementary Fig. S4C, D). Session means of peak force of the dy^w mice outside the 2+2 group lay between 20.3 and 26.3 mN in all nine sessions up to day 75 (30.8 mN on day 53 without the mouse with 8.4 mN). On day 84, the last mouse of the groups dosed weekly with the 1+1 CrossMab reached 42.5 mN and was the only other dy^w mouse within the range of the 2+2 group. The four mice given AAV9 mini-agrin, tested on day 119, averaged 31.9 mN. Peak force was correlated with day of testing (Spearman's ρ) among the 25 dy^w mice outside the 2+2 group (0.53, *P* = 0.007), among these mice without the positive control, which was tested last (0.34, *P* = 0.13), and on the 11 session means (0.62, *P* = 0.04).

Body mass and leg length. Body mass curves pooled weighings within 1 d of the nominal day, which can run 1 to 2 d ahead of age. Leg length was examined because the force recorded at a lever depends on where the limb meets the bar. It was greater in each of the seven mice given the 2+2 than in any of the 18 other dy^w mice measured, and its mean exceeded that of wild-type mice of almost twice their body mass (Supplementary Fig. S4E). Leg length was noted without unit or landmark, and not for the five mice given 72 mg/kg and two mice of a group dosed weekly with the 1+1 CrossMab, including the mouse with 42.5 mN.

Analysis of the work with Claude

Workspace. Each task has its own thread in a shared workspace, where a session of Claude (an instance) can read the program's email and data, run code, prepare pages and save drafts in the mailbox of one of us. Claude sent no email, and further instances reviewed plans and drafts before they were handed over.

Period. The week comprised seven consecutive days (five working days), from the first request on the handover of the assays (day 1) through the arrival of the data package (day 4) to the ruling and the eight emails (day 7). We selected it as the best recent example of our work with Claude, and one member of the team made every request.

Times. Times were calculated as differences between message stamps. A first answer was Claude's first message on the substance of a request (*n* = 7), and a full answer was the plan, page or set of drafts that fulfilled it (*n* = 9). Twelve requests had at least one of these times, and five of the nine full answers could be timed to the minute at best. The full plan of day 1 corrected five points of the first answer, and between the two answers on the data package the list of constructs to polish shrank from 41 to 7. A first reply had arrived for 7 of the 11 emails to CROs by the evening of day 7, and these times include nights, a weekend and a difference of 12 h in time zone.

Counts. Emails, words and send status were read from the mailbox on the evening of day 7. Sixteen drafts were addressed to CROs and one to a consultant, and both versions were available for ten sent emails (4,938 words drafted, 3,409 sent). One of us set the list of eleven questions, and of the nine answers in writing, five were confirmed by both sides, three stood in our emails without a reply, and one, on shipping, lacked a date. The unsettled statement concerns the evidence on two of the three arms that were set aside. Instances compiled all times and counts.

State at the end of the week. Three CROs had active email threads, while mouse pharmacokinetics and efficacy in dy^w mice had one unsent draft each. No plate of either cell-based assay had been run. The production CRO had accepted the scope of the binding kinetics, which it scheduled in the following week. The 24 constructs had already been held off the plates, so their removal left the number of plates and the timeline unchanged.

Statistics and software

Tests were two-sided, *P* values are uncorrected for multiplicity, and intervals are 95% CIs. Analyses and data panels were produced in Python 3.13 with NumPy 2.5, SciPy 1.18, pandas 3.0 and Matplotlib 3.11.

Data handling and use of Claude

Under our direction, Claude (Anthropic) searched the literature, extracted CRO reports and instrument exports into tables, re-analyzed and plotted the data, and drafted text and figures. Printed values were traced to these tables, and all values of earlier drafts and some of the new values were recomputed by instances that had not produced them. SPR and purity values are those reported by the CROs.

Data and code availability

The values per curve, per well and per animal behind Supplementary Tables S1 to S7, the values plotted in each data panel and the code that draws it are available from the corresponding author. Antibody sequences and the raw files of the CROs are not provided.

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Competing interests Anthropic, which acquired Mithril Medicine in July 2026, owns the antibody program described here, develops and sells Claude and employs the corresponding author, who founded Mithril Medicine. Members of the Mithril Medicine team have been paid consultants or advisers to the company. The contract research organizations were paid for their work.

Use of AI tools Under our direction, Claude searched the literature, performed the re-analyses, prepared the figures and drafted the manuscript (Methods). The section on working with Claude was compiled by Claude from its own threads and notes and therefore carries a risk of bias, which we have tried to limit by reporting its errors and the limits of the counts.

Supplementary information

Figure S1. Among the humanized constructs, cell binding and adhesion varied with format although each arm retained its affinity.

Figure S2. EC₅₀ values varied 2.5- to 4.3-fold between days, and adhesion rose steeply with the coat.

Figure S3. Plasma exposure to the 2+2 accrues within a week of a dose, and a doubled dose adds to it only during the first 3 days.

Figure S4. The 2+2 group was tested last and differed from the other groups of dy^w mice in evoked force and in measured leg length.

Figure S5. The Fab of the LAMA4 arm outlines the muscle fibers of a dy^w mouse.

Table S1. Molecules built from the two tool arms, ordered by adhesion on the LG4-5 coat (2+2 constructs together).

Table S2. Purity and binding of the five humanized constructs, all with YTE (one lot each, one SPR run per arm, means of duplicate wells in one experiment).

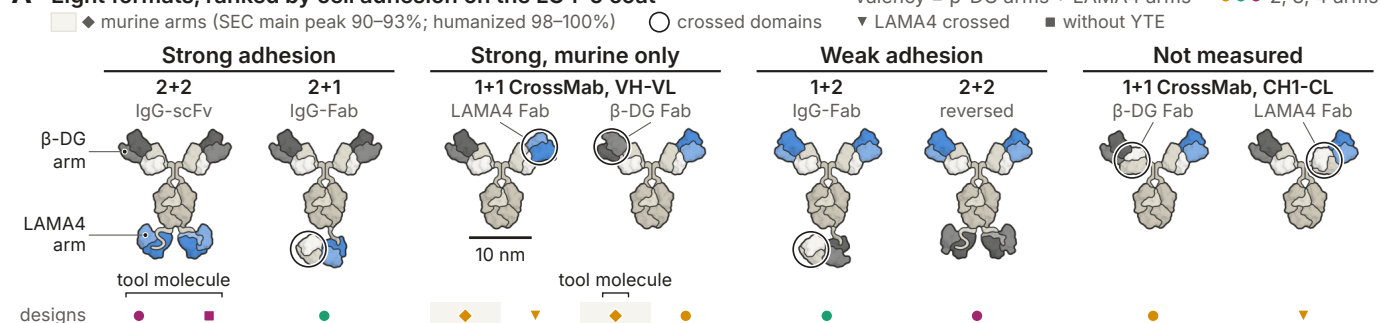
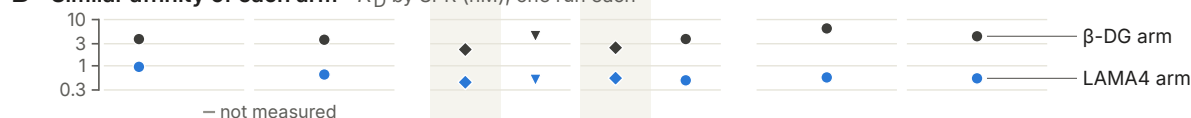
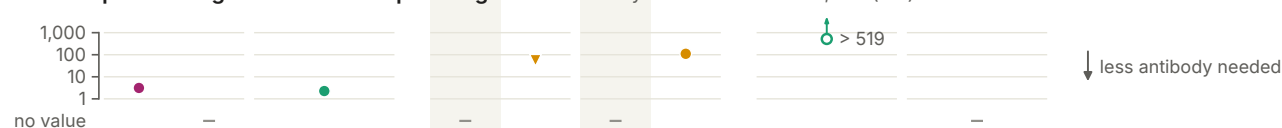
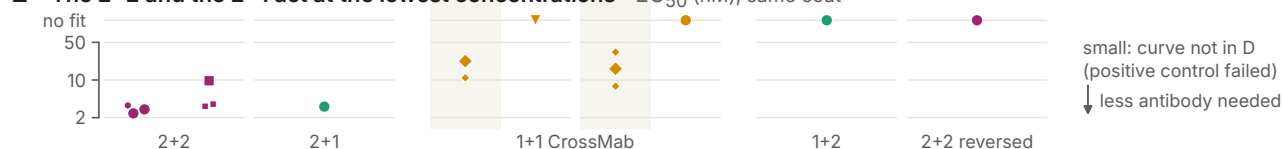
Table S3. Potency and maximal retention of the constructs in the adhesion assay.

Table S4. Plasma pharmacokinetics and antibody concentration in muscle.

Table S5. Survival, force, grip strength and body mass of dy^w mice under published treatments.

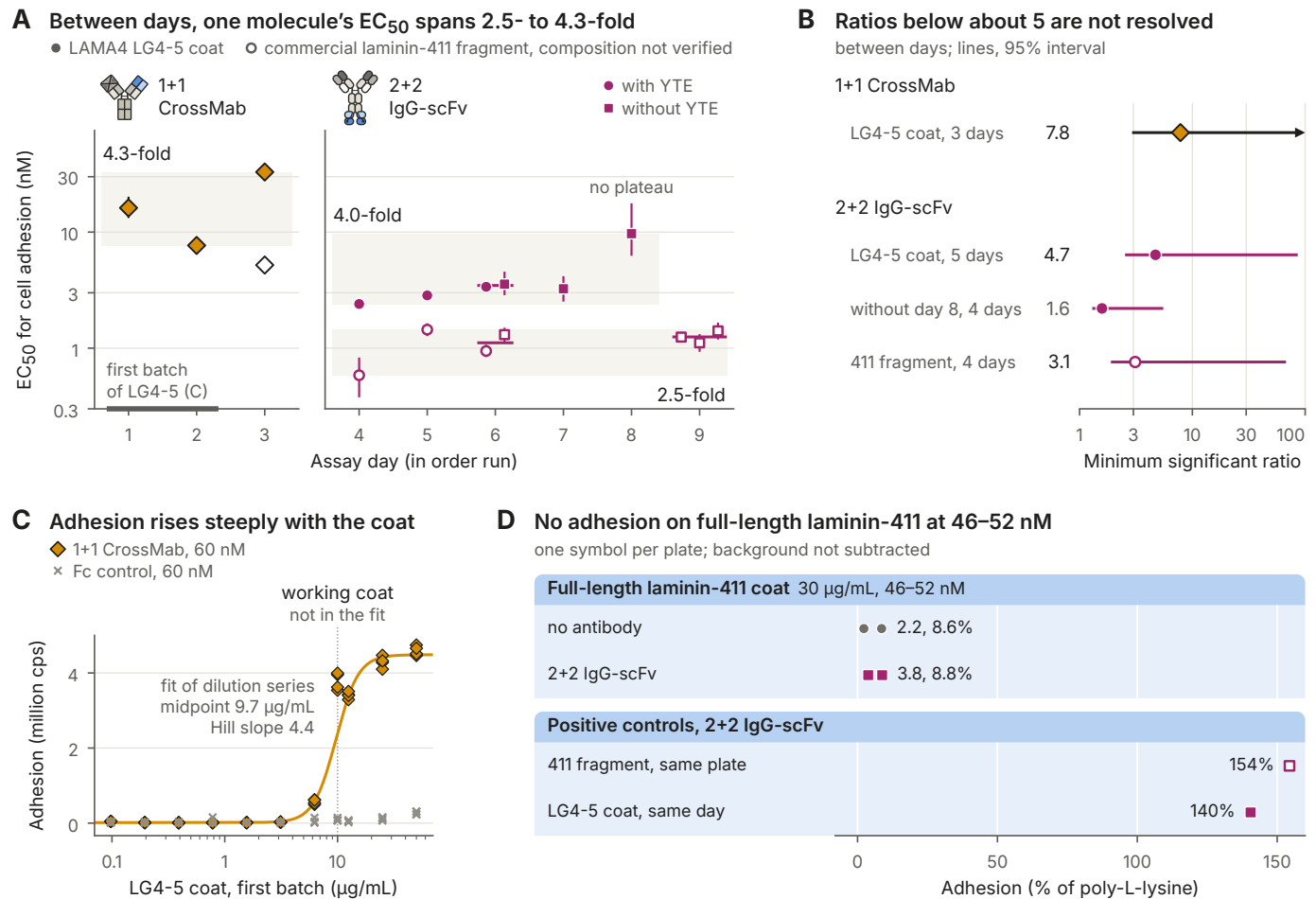
Table S6. All groups of the pilot study.

Table S7. Functional endpoints in dy^w and wild-type mice by group.

A Eight formats, ranked by cell adhesion on the LG4-5 coat**B Similar affinity of each arm** K_D by SPR (nM), one run each**C Unequal binding to DAG1-overexpressing cells** antibody needed for MFI 100,000 (nM)**D Among humanized constructs, only the 2+2 and the 2+1 retain cells** highest dose mean, % of poly-L-lysine wells**E The 2+2 and the 2+1 act at the lowest concentrations** EC_{50} (nM), same coat

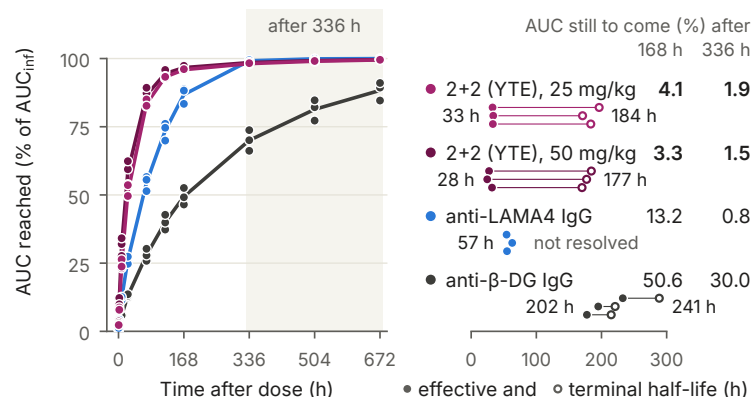
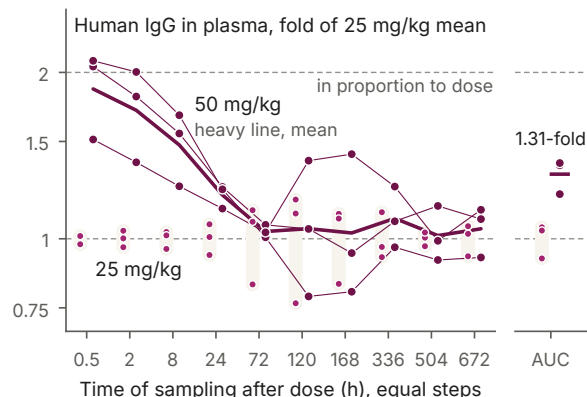
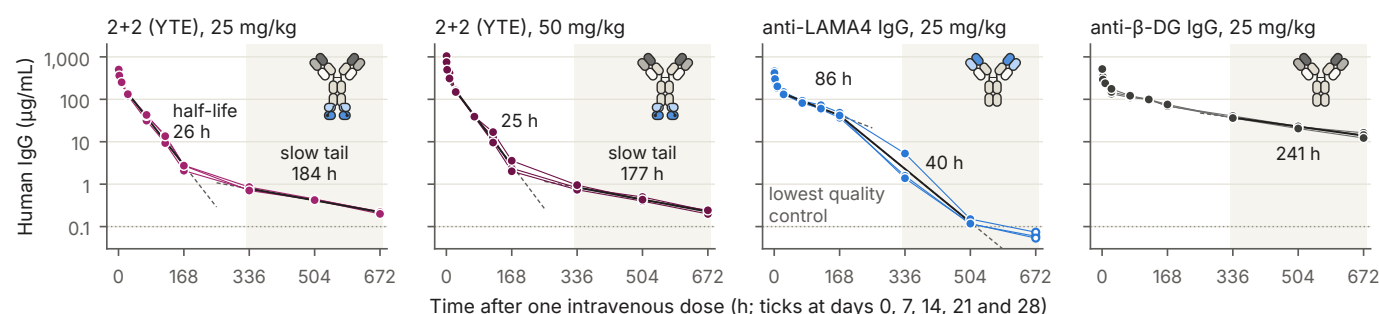
Supplementary Figure S1. Among the humanized constructs, cell binding and adhesion varied with format although each arm retained its affinity. (A) Models at one scale, each in one open, extended pose (ten designs; the 2+2 in two constructs). One picture represents all constructs of a format. Rings, domains crossed to direct chain pairing. Murine and humanized constructs differ in arms, Fc and lot purity, and no matched pair was made. (B) One SPR

run per arm. (C) One experiment; open symbol, threshold not reached; dashes, construct not included. (D) Highest background-subtracted dose mean per curve; dashed line, highest of two controls, which were run on other plates. (E) Accepted fits, from different plates and days. Values, Supplementary Tables S1 and S2.



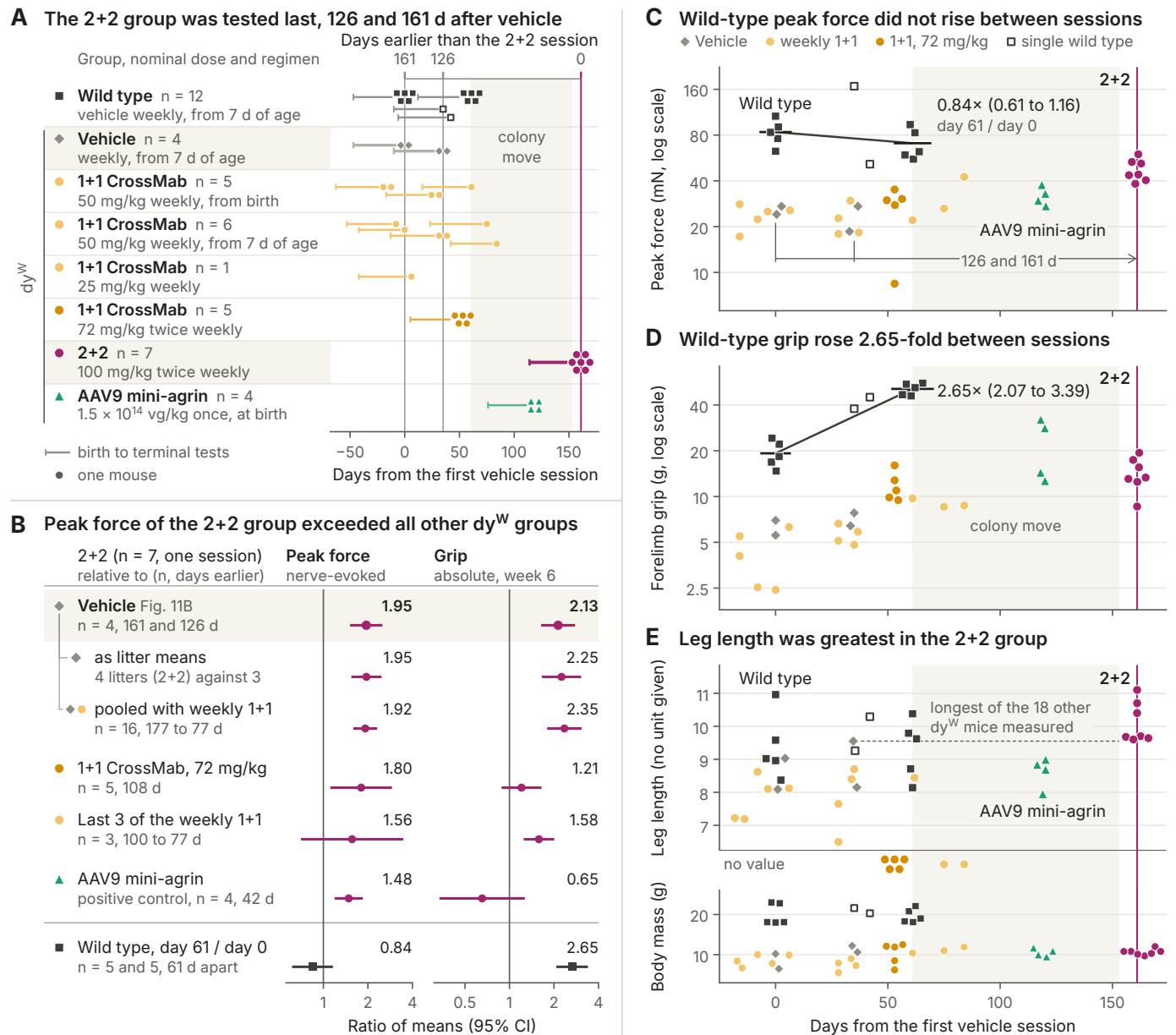
Supplementary Figure S2. EC₅₀ values varied 2.5- to 4.3-fold between days, and adhesion rose steeply with the coat. (A) EC₅₀ of every fitted curve of the two tool molecules on standard plates (17 curves, nine days). Filled, LG4-5 coat; open, commercial fragment. Bars, 95% profile-likelihood CIs; marks, geometric mean per day; shading, range of day values. (B) Minimum significant ratio between days, the smallest EC₅₀ ratio that can be resolved,¹⁷⁴ with 95% interval; arrow, upper limit above 100. One row omits day 8 (post hoc). (C) Titration of the LG4-5 coat on one plate. Symbols, wells; line, fit of the 2-fold

series; dotted line, wells coated separately at 10 µg/mL. (D) Coat of full-length laminin-411 (30 µg/mL, 46 to 52 nM, below the 71 nM at which the LG4-5 coat lost retention in C; one lot, two plates). Percent of poly-L-lysine wells without subtraction. One symbol per plate, highest dose mean or mean of the wells without antibody. Positive controls, the same 2+2 on the commercial fragment (same plate) and on the LG4-5 coat (same day), each coated at 10 µg/mL. Shapes as in A; open, commercial fragment.

A The slow tail of the 2+2 holds little plasma AUC**B The gain from a doubled dose fades by 72 h****C Only the anti-LAMA4 IgG declines faster after 168 h**

Supplementary Figure S3. Plasma exposure to the 2+2 accrues within a week of a dose, and a doubled dose adds to it only during the first 3 days. Three wild-type mice per group; 2+2, construct with YTE. (A) Left, share of AUC_{inf} reached by each sampling time (points, mice; lines, group means). Shading (also in C) marks the window of the terminal fit of the 2+2, its slow tail. Right, share to come after 168 and 336 h, and effective (filled) and terminal (open) half-life of each mouse, with group means. (B) Concentration in each

mouse given the 2+2, and AUC_{inf} , as fold of the geometric mean at 25 mg/kg. Pale bars, range at 25 mg/kg; heavy line, ratio of geometric means; dashed lines, 1 and 2. (C) All profiles (thin lines, mice). Black lines, log-linear fits with mean half-lives (2+2, 24 to 168 h and 336 to 672 h; anti-LAMA4 IgG, 24 to 168 h and 168 to 504 h; anti-β-DG IgG, terminal phase); dashed, their extensions; dotted, lowest quality control; open symbols, values below it.



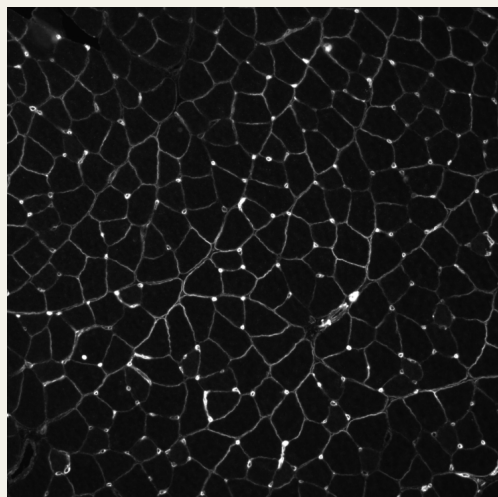
Supplementary Figure S4. The 2+2 group was tested last and differed from the other groups of dy^w mice in evoked force and in measured leg length. (A) Calendar of the pilot study, one lane per group, for the 44 mice that reached the terminal tests. Vertical lines, vehicle and 2+2 sessions. (B) Ratio of the 2+2 mean (seven mice or four litters) to that of each other group, with 95% CI. The tinted row, as in A, is the contrast of Fig. 11B. Indented rows include the vehicle group. Bottom row, later over earlier session of wild-type mice. (C) Peak force

of all 44 mice by day of testing. Arrow, from the vehicle sessions to the 2+2 session. (D) Absolute grip strength at week 6, as in C. In C and D, the line joins the means of the two sessions of five wild-type mice, and their ratio carries a 95% CI. (E) Leg length (no unit given) and body mass at necropsy, as in C. Open squares, wild-type mice tested singly. Band, window of the colony's move (Methods). Overlapping symbols are shifted sideways.

The Fab of the LAMA4 arm outlines the muscle fibers of a dy^w mouse

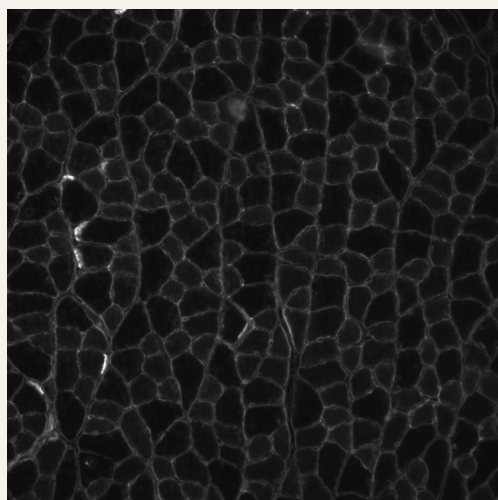
On hindlimb sections, the Fab was detected through its His tag with rabbit anti-His and a fluorescent anti-rabbit antibody

With Fab
His-tagged Fab of the
murine LAMA4 arm



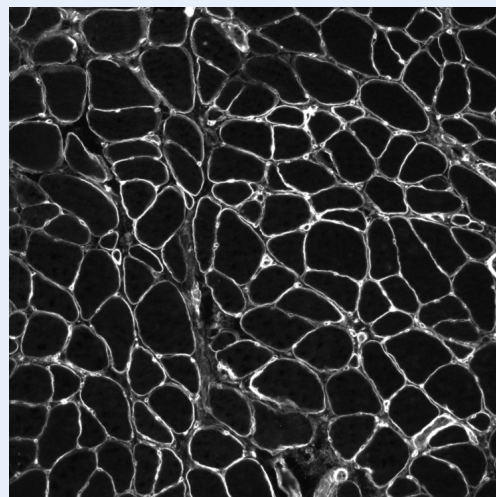
bright points between fibers

Without Fab
detection antibodies
only

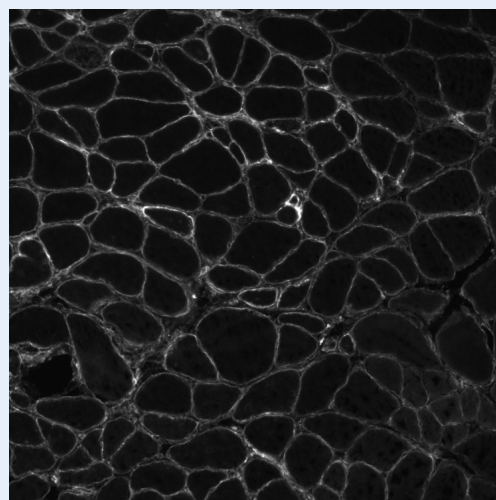


similar outlines, no bright points

dy^w mouse, vehicle-treated



outlines around the fibers



fainter outlines

Supplementary Figure S5. The Fab of the LAMA4 arm outlines the muscle fibers of a dy^w mouse. Hindlimb sections incubated with (top) or without (bottom) the His-tagged Fab of the murine LAMA4 arm and detected through the His tag. Without Fab, the outlines were fainter in the dy^w mouse and

similar in the wild-type mouse, in which only the bright points between fibers were absent. One mouse per genotype. Whole frames as supplied, intensities unchanged. Exposure, display range and magnification were not reported.

Supplementary Table S1. Molecules built from the two tool arms, ordered by adhesion on the LG4-5 coat (2+2 constructs together). K_D , single SPR runs on antigens of the matching species. EC_{50} , geometric mean (range; number of accepted fits). Peak (no accepted fit), highest background-subtracted dose mean in percent of PLL wells. –, not measured.

Molecule (arms humanized unless stated)	Format (modified or appended arm)	SEC main peak, % first lot (adhesion lot, if another)	LC-MS target mass, % first lot	K_D , β -DG / LAMA4, nM	Adhesion on LG4-5: EC_{50} , nM, or peak, %
2+2 IgG-scFv, with YTE	anti- β -DG IgG, LAMA4 scFv	98.2	–	3.78 / 0.949	2.85 (2.40 to 3.38; 3)
2+2 IgG-scFv, without YTE	anti- β -DG IgG, LAMA4 scFv	(100)	–	–	4.82 (3.25 to 9.69; 3)
2+1	anti- β -DG IgG, LAMA4 Fab	99.1	97.3	3.66 / 0.647	3.21 (1)
1+1 CrossMab (LAMA4 crossed), murine	VH-VL crossed, LAMA4	96.5 (93.3)	90.2	2.25 / 0.440	15.7 (11.0 to 22.4; 2)
1+1 CrossMab, murine	VH-VL crossed, β -DG	91.6 (90.2)	91.3	2.46 / 0.540	16.0 (7.67 to 32.9; 3)
1+2	anti-LAMA4 IgG, β -DG Fab	100.0	89.6	6.43 / 0.560	peak 20
h1+1 CrossMab (β -DG crossed)	VH-VL crossed, β -DG	99.4	98.6	3.79 / 0.485	peak 7
Reversed 2+2	anti-LAMA4 IgG, β -DG scFv	97.7	–	4.35 / 0.535	peak 4
h1+1 CrossMab (LAMA4 crossed)	VH-VL crossed, LAMA4	100.0	97.5	4.39 / 0.496	peak 2
h1+1 CrossMab (β -DG crossed, CH1-CL)	CH1-CL crossed, β -DG	100.0	85.1	–	–
h1+1 CrossMab (LAMA4 crossed, CH1-CL)	CH1-CL crossed, LAMA4	100.0	65.9	–	–
β -DG parental IgG	IgG (2+0)	100.0	–	2.65 / –	–
LAMA4 parental IgG	IgG (0+2), re-humanized	79.5	–	– / 0.549	–

Supplementary Table S2. Purity and binding of the five humanized constructs, all with YTE (one lot each, one SPR run per arm, means of duplicate wells in one experiment). Concentrations read from either replicate series agreed within 11%. The 1+2 lot contained 9.8% of species without a correctly paired β -DG Fab. k_a , association rate constant.

Parameter	2+1 (LAMA4 Fab below the Fc)	2+2 (LAMA4 scFvs below the Fc)	h1+1 CrossMab (LAMA4 crossed)	h1+1 CrossMab (β -DG crossed)	1+2 (β -DG Fab below the Fc)
SEC main peak, %	99.1	98.2	100.0	99.4	100.0
Target mass by LC-MS, % (semi-quantitative)	97.3	not determined	97.5	98.6	89.6
β -DG, k_a , $10^5 M^{-1} s^{-1}$	7.88	7.59	6.55	6.83	3.96
β -DG, k_d , $10^{-3} s^{-1}$	2.88	2.87	2.87	2.59	2.54
β -DG, K_D , nM	3.66	3.78	4.39	3.79	6.43
LAMA4, k_a , $10^5 M^{-1} s^{-1}$	9.48	8.07	15.2	15.5	13.2
LAMA4, k_d , $10^{-4} s^{-1}$	6.13	7.66	7.53	7.50	7.41
LAMA4, K_D , nM	0.647	0.949	0.496	0.485	0.560
nM that reaches MFI 10,000	0.21	0.23	4.48	10.3	369
nM that reaches MFI 100,000	2.26	3.12	56.0	110	> 519
Highest mean MFI (at nM; highest tested, 504 to 692)	910,000 (104)	747,000 (101)	245,489 (138)	348,827 (692)	13,007 (519)

Supplementary Table S3. Potency and maximal retention of the constructs in the adhesion assay. EC_{50} , curves on standard plates. Highest signal, highest dose mean in percent of PLL wells after subtraction of wells without antibody. Flat, below 10% at every dose; no fit, acceptance criteria not met; nr, not reported (PLL wells failed a criterion); –, not applicable. Semicolons separate items in the order named. ^aComposition not verified (Methods). ^bWithout subtraction.

Coat (10 μ g/mL unless stated)	Construct (days; curves)	EC_{50} (nM), geometric mean (range)	Highest signal (% of PLL)
LAMA4 LG4-5	1+1 CrossMab (3; 3)	16.0 (7.67 to 32.9)	106 (2 nr)
	2+1 (1; 1)	3.21	78
	2+2 with YTE (3; 3)	2.85 (2.40 to 3.38)	88 to 110 (1 nr)
	2+2 without YTE (3; 3)	4.82 (3.25 to 9.69)	78 (2 nr)
	h1+1 CrossMab, β -DG crossed; LAMA4 crossed; 1+2; reversed 2+2 (2; 4)	flat; flat; no fit; flat	6.9; 2.1; 20; 3.8
	Control proteins (8; 10, all plates)	flat, 9; no fit, 1	–0.7 to 17 (median 2.4; 4 nr)
Commercial laminin-411 fragment ^a	1+1 CrossMab (1; 1)	5.20	nr
	2+1 (1; 1)	3.04	120
	2+2 with YTE (3; 3)	0.927 (0.584 to 1.45)	112 to 150 (1 nr)
	2+2 without YTE (2; 4)	1.26 (1.11 to 1.41)	169 to 209 (1 nr)
	Same four constructs (2; 4)	5.24; 13.2; flat; no fit	34; 22; 1.3; 48 (control IgG, same plate, 14)
Full-length laminin-411, 30 μ g/mL	2+2 without YTE, to 125 nM (1; 1)	flat	3.8 (no antibody, 2.2) ^b
Same coat; commercial fragment ^a ; laminin- α 2 LG4-5	2+2 without YTE, 20 or 200 nM (1 plate; 10 wells per dose, 2 without antibody)	–	8.8; 154; 1.5 (no antibody, 8.6; 5.9; 1.3) ^b
ELISA: LAMA4 LG4-5; full-length laminin-411; commercial fragment ^a	2+2 without YTE (1; 3)	0.071; 0.17 (apparent); not readable	OD ₄₅₀ 3.446; 3.290; 3.390 (isotype, 0.228; 0.825; 3.374)

Supplementary Table S4. Plasma pharmacokinetics and antibody concentration in muscle. Upper block, mean $\pm$ SD after one intravenous dose, and ratios of geometric means. Lower block, mean (range) of antibody able to bind both antigens in unperfused muscle. n.d., below the lowest standard (0.78 $\mu\text{g/g}$). Outlier, the mouse excluded from Fig. 9D and F (Methods).

Plasma, wild-type mice, n = 3 per group (dose in nmol/kg)	Plasma AUC _{inf} (h· $\mu\text{g/mL}$)	Clearance (mL/day/kg)	Clearance relative to anti- β -DG IgG (95% CI)	Clearance relative to anti-LAMA4 IgG (95% CI)	Effective half-life (h)	Terminal half-life (h; slow tail of the 2+2); share of AUC _{inf} after 336 h (%)
anti- β -DG IgG, wild-type Fc, 25 mg/kg (173)	43,054 $\pm$ 762	13.9 $\pm$ 0.2	1		202 $\pm$ 28	241 $\pm$ 41; 30.0
anti-LAMA4 IgG, wild-type Fc, 25 mg/kg (173)	18,893 $\pm$ 2,073	32.0 $\pm$ 3.3	2.29 (1.77 to 2.95)	1	57.4 $\pm$ 4.8	not resolved; 0.8
2+2, LALA-PG andYTE, 25 mg/kg (126)	11,131 $\pm$ 778	54.1 $\pm$ 3.9	3.87 (3.28 to 4.57)	1.69 (1.36 to 2.11)	33.3 $\pm$ 0.5	184 $\pm$ 13; 1.9
2+2, LALA-PG andYTE, 50 mg/kg (252)	14,570 $\pm$ 1,030	82.6 $\pm$ 6.1	5.92 (5.01 to 7.00)	2.59 (2.08 to 3.22)	28.0 $\pm$ 3.7	177 $\pm$ 7; 1.5
Muscle, dy ^w mice, antibody from birth (nominal dose)	n	Days since last dose	Gastrocnemius ($\mu\text{g/g}$)	Triceps ($\mu\text{g/g}$)	Tibialis anterior ($\mu\text{g/g}$)	
Vehicle, weekly	2	1, 5	n.d.	n.d.	n.d.	
1+1 CrossMab, 25 mg/kg weekly	1	4	n.d.	n.d.	n.d.	
1+1 CrossMab, 50 mg/kg weekly	4	1 to 5	6.64 (3.60 to 13.72)	6.76 (3.28 to 12.43)	3.68 (2.46 to 6.27)	
1+1 CrossMab, 50 mg/kg weekly, without the outlier	3	1 to 5	4.28 (3.60 to 4.73)	4.87 (3.28 to 5.92)	2.81 (2.46 to 3.05)	
1+1 CrossMab, 72 mg/kg twice weekly	5	3 to 6	8.88 (4.79 to 12.85)	8.58 (3.41 to 12.66)	5.83 (2.32 to 9.46)	

Supplementary Table S5. Survival, force, grip strength and body mass of dy^w mice under published treatments. Values are published means, or medians where stated, with n in parentheses where given (muscles for force, otherwise mice). Force of isolated muscles is not comparable with force evoked *in situ* (Fig. 11).

Treatment	Endpoint (age), unit	Untreated	Treated	Healthy control
Mini-agrin, muscle transgene ¹⁵	median survival, weeks	15.5	50	
α LNNd and mini-agrin, muscle transgenes ¹⁵	median survival, weeks	15.5	81 (16)	
Mini-agrin, muscle transgene ⁹⁹	tetanic force, isolated extensor digitorum longus (4 weeks), mN	20.9 (6)	84.2 (5)	227.5 (6)
	specific force, same muscles, mN/mm ²	42.3 (6)	146.2 (5)	297.2 (6)
	body mass (4 weeks), g	8.4	14.3	21.3
α LNNd and mini-agrin, ubiquitous transgenes ¹⁷⁵	forelimb grip (8 weeks)		more than 2-fold untreated	
AAV9 mini-agrin (cytomegalovirus promoter), postnatal day 2 to 3 (ref. 100)	mean survival, weeks ($\pm$ as published)	10.18 $\pm$ 7.12 (22)	42.1 $\pm$ 10.6 (9)	
	body mass (13 weeks), g ($\pm$ as published)	11.31 $\pm$ 1.41 (7)	15.65 $\pm$ 1.3 (7)	
AAV9 α LNNd and mini-agrin (muscle promoter), postnatal day 1 (ref. 24)	body mass (8 weeks)		about 2-fold untreated	
AAV9 micro-laminin, one of two constructs ¹⁸	forelimb grip per body mass (3 months), g/g ($\pm$ as published)	2.5 $\pm$ 0.1 (24)	3.1 $\pm$ 0.2 (12)	
	hindlimb grip, ambulation, body mass		no significant change	
Recombinant human laminin-111, 1 mg/kg weekly, weeks 2 to 6, immunodeficient mice ¹⁰⁸	forelimb grip		no significant change (6)	
Losartan, weeks 2 to 7 (ref. 176)	body mass (7 weeks), g ($\pm$ SD)	10.11 $\pm$ 3.39 (8)	10.35 $\pm$ 1.85 (4)	18.05 $\pm$ 2.91 (6)

Supplementary Table S6. All groups of the pilot study. The main text reports five of these groups (Fig. 10C) and muscle levels in mice dosed weekly (Fig. 9D). All groups are dy^w mice except wild type, and antibodies and vehicle were injected intraperitoneally. Doses are nominal, per injection. Started, mice enrolled, including those collected early (vehicle, 1; 72 mg/kg, 16). Tested, mice with a force test at week 6. Days, from the first vehicle session (one mouse unless n is given). vg, vector genomes.

Group	Dose, mg/kg ($\mu\text{mol/kg}$)	Regimen	Started	Tested (males/females)	Terminal session, day (n)
Wild type, vehicle		weekly from 7 d of age	12	12 (6/6)	0 (5), 35, 42, 61 (5)
Vehicle		weekly from 7 d of age	8	4 (1/3)	0 (2), 35 (2)
1+1 CrossMab	50 (0.344)	weekly from birth	11	5 (1/4)	-16 (2), 28 (2), 61
1+1 CrossMab	50 (0.344)	weekly from 7 d of age	10	6 (2/4)	-8, 0, 35 (2), 75, 84
1+1 CrossMab	25 (0.172)	weekly from birth	5	1 (1/0)	6
1+1 CrossMab	72 (0.496)	twice weekly from birth	27	5 (1/4)	53 (5)
2+2	100 (0.503)	twice weekly from birth	9	7 (4/3)	161 (7)
AAV9 mini-agrin	1.5 $\times 10^{14}$ vg/kg	once at birth	5	4 (0/4)	119 (4)

Supplementary Table S7. Functional endpoints in *dy^w* and wild-type mice by group. Upper block, mean ± SD of the n mice that reached the terminal tests. Lower block, ratio of the mean of the group given the 2+2 to that of another group (mouse as unit; Methods). These groups were tested 42 to 161 d before the 2+2 group. n.d., no data.

Group	n (lit- ters)	Peak triceps force (mN)	Grip strength, week 6 (g)	Body mass, necropsy (g)	Rotarod latency, week 6 (min)	Minute volume, week 6 (mL/ min)
Wild type	12 (7)	82.6 ± 31.8	36.18 ± 15.84	20.0 ± 2.0	5.72 ± 2.46	82.5 ± 18.7
Vehicle	4 (3)	24.3 ± 4.1	6.69 ± 0.94	9.9 ± 2.4	0.14 ± 0.13	45.1 ± 18.0
1+1 CrossMab, 50 mg/kg weekly from birth	5 (4)	21.6 ± 4.3	6.21 ± 2.17	7.8 ± 1.8	0.07 ± 0.03	39.0 ± 12.9
1+1 CrossMab, 50 mg/kg weekly from 7 d of age	6 (6)	27.4 ± 8.3	5.49 ± 2.78	9.5 ± 1.8	0.09 ± 0.05	56.3 ± 22.1
1+1 CrossMab, 25 mg/kg weekly	1 (1)	25.6	6.30	9.9	0.05	48.3
1+1 CrossMab, 72 mg/kg twice weekly	5 (3)	26.3 ± 10.3	11.82 ± 2.67	10.2 ± 2.8	0.10 ± 0.05	46.4 ± 24.7
2+2, 100 mg/kg twice weekly	7 (4)	47.3 ± 7.8	14.26 ± 3.55	10.6 ± 0.7	n.d.	n.d.
AAV9 mini-agrin, positive control	4 (2)	31.9 ± 4.5	21.83 ± 9.73	10.5 ± 1.0	0.18 ± 0.15	n.d.
Ratio of the 2+2 group to (95% CI)						
Wild type		0.57 (0.44 to 0.75)	0.39 (0.28 to 0.55)			
Vehicle		1.95 (1.51 to 2.51)	2.13 (1.64 to 2.78)			
1+1 CrossMab, 72 mg/kg twice weekly		1.80 (1.12 to 2.91)	1.21 (0.88 to 1.65)			
AAV9 mini-agrin, positive control		1.48 (1.19 to 1.85)	0.65 (0.34 to 1.27)			