Motor axons receive retrograde signals from skeletal muscle that are essential for the differentiation and stabilization of motor nerve terminals. Identification of these retrograde signals has proved elusive, but their production by muscle depends on the receptor tyrosine kinase, MuSK (muscle, skeletal receptor tyrosine-protein kinase), and Lrp4 (low-density lipoprotein receptor (LDLR)-related protein 4), an LDLR family member that forms a complex with MuSK, binds neural agrin and stimulates MuSK kinase activity. Here we show that Lrp4 also functions as a direct muscle-derived retrograde signal for early steps in presynaptic differentiation. We demonstrate that Lrp4 is necessary, independent of MuSK activation, for presynaptic differentiation in vivo, and we show that Lrp4 binds to motor axons and induces clustering of synaptic-vesicle and active-zone proteins. Thus, Lrp4 acts bidirectionally and coordinates synapse formation by binding agrin, activating MuSK and stimulating postsynaptic differentiation, and functioning in turn as a muscle-derived retrograde signal that is necessary and sufficient for presynaptic differentiation.

Postsynaptic muscle cells provide signals to motor axons, and these signals regulate the formation, maturation, stabilization and plasticity of neuromuscular synapses. During development, motor axons approach and form synapses with muscle in a prepatterned region, marked by elevated expression and clustering of key postsynaptic proteins, including acetylcholine receptors (AChRs). Muscle prepatterned depends on MuSK and Lrp4, which forms a complex with MuSK and stimulates MuSK kinase activity. Stabilization of developing synapses requires motor-neuron-derived agrin, which binds Lrp4, stimulates further association between Lrp4 and MuSK, and increases MuSK kinase activity, leading to anchoring of key proteins in the postsynaptic membrane and elevated transcription of "synaptic genes" in myofibre synaptic nuclei.

Lrp4 and MuSK are both required for presynaptic as well as postsynaptic differentiation, as motor axons grow beyond the prepatterned region and fail to cluster synaptic vesicles in mice deficient in either gene. How agrin, Lrp4 and MuSK control presynaptic differentiation is poorly understood. Because Lrp4 activates MuSK, the presynaptic defects in Lrp4-mutant mice could be a consequence of inadequate MuSK activation and a failure to produce novel retrograde signals. Alternatively, Lrp4 may have a direct role in regulating motor axon growth and differentiation. To distinguish between these possibilities, we established a cell culture assay to determine whether Lrp4 is sufficient to induce presynaptic differentiation. First, we co-cultured motor neurons, dissected from HB9-GFP (green fluorescent protein) transgenic mice, with skeletal muscle cells and established culture conditions that were permissive for presynaptic differentiation. Under these conditions, synapsin, a protein that is associated with synaptic vesicles, accumulated in motor axons at sites that were apposed to AChR clusters in muscle (Fig. 1a, b). We then co-cultured motor neurons with NIH 3T3 cells or 3T3 cells (mouse embryonic fibroblast cell lines) expressing Lrp4 and stained for synapsin. Figure 1 shows that synapsin is distributed homogenously in axons of motor neurons co-cultured with control 3T3 cells, whereas synapsin accumulated in motor axons at sites of contact with Lrp4-expressing 3T3 cells (Fig. 1c). We also co-cultured motor neurons with HEK 293 cells that expressed a Flag-tagged version of Lrp4, allowing us to visualize cell surface Lrp4. Lrp4 was clustered on the cell surface, and synapsin accumulation in motor axons was often apposed to these clusters of Lrp4 (Figs 1d and Supplementary Fig. 2; see below). In addition, we transfected 293 cells with truncated forms of Lrp4 and found that the LDLa repeats from the extracellular region of Lrp4, in the absence of the EGF-like and β-propeller domains, are sufficient to induce presynaptic differentiation (Supplementary Fig. 2).

These experiments indicated that Lrp4 is sufficient to trigger presynaptic differentiation but left open the possibility that Lrp4 acted together with other proteins expressed in 3T3 and 293 cells to induce presynaptic differentiation. Therefore, we treated HB9-GFP motor neurons with an Lrp4–Fc fusion protein that contained the LDLa repeats and was attached to polystyrene microspheres, and stained for synapsin. We found that synapsin, as well as synaptophysin and SV2, bona-fide synaptic vesicle proteins, were clustered at contact sites with Lrp4-LDLa–Fc beads (Figs 2a and Supplementary Fig. 3). We also stained for bassoon, a protein that is concentrated at synaptic vesicle fusion sites in nerve terminals, called active zones, and found that bassoon was similarly clustered with Lrp4-LDLa–Fc beads (Fig. 2b). In contrast, neither Fc alone nor the LDLa repeats of Lrp1, another Lrp-family member, induced clustering of synapsin or bassoon, indicating that presynaptic differentiation is induced selectively by the LDLa repeats from Lrp4 (Figs 2b, c and Supplementary Fig. 3). Moreover, addition of soluble, dimeric Lrp4-LDLa–Fc, unattached to beads, failed to induce presynaptic differentiation (Supplementary Fig. 4), indicating that a large number of interactions, conferred by the attachment of the extracellular region of Lrp4 (ecto-Lrp4) to polystyrene microspheres, cooperate to mediate presynaptic differentiation.

Because MuSK, like Lrp4, is required for presynaptic differentiation in vivo, and because MuSK activation causes clustering of MuSK as well as Lrp4 at synapses, we tested whether the extracellular region of MuSK could also induce presynaptic differentiation. Although microspheres with ecto-MuSK–Fc or ecto-Lrp4–Fc attached equally well to motor axons (Supplementary Fig. 5), only ecto-Lrp4–Fc induced clustering of synaptic vesicle and active-zone proteins (Fig. 2b). Moreover, Myc-tagged MuSK, expressed in 293 cells, failed to induce clustering of synapsin (Supplementary Fig. 2). Thus, although Lrp4 and MuSK are both required for the differentiation of motor nerve terminals in vivo, only Lrp4 is sufficient to stimulate presynaptic differentiation.

Because Lrp4 binds neural agrin, we asked whether agrin was required for Lrp4 to induce presynaptic differentiation. We crossed agrin-null (Aggrn−/−) and HB9-GFP mice and treated Aggrn−/− explants with Lrp4-LDLa–Fc. Wild-type and Aggrn−/− motor neurons were equally responsive to Lrp4-LDLa–Fc beads (Fig. 2d), indicating that Lrp4 induces presynaptic differentiation in a manner that does not depend on agrin.

1Molecular Neurobiology Program, Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, New York University Medical School, 540 First Avenue, New York, New York, USA.
To determine whether Lrp4 induced functional release sites, we measured recycling of synaptic vesicles using the styryl dye FM 4-64FX. Depolarization of motor neurons treated with Lrp4-LDLa–Fc beads, caused uptake of FM 4-64FX, and further depolarization led to the release of dye (Fig. 2e, f). In contrast, depolarization of motor neurons treated with Lrp4-LDLa–Fc beads, led to low and uniform axonal uptake of FM 4-64FX (Fig. 2e). Thus, Lrp4 induced both morphologically and functionally specialized neurotransmitter release sites.

Our experiments indicate that Lrp4 interacts with a protein expressed by motor axons to promote presynaptic differentiation. To determine whether motor axons express an Lrp4-binding protein, we cultured explants from the ventral neural tube, which contains motor neurons, and probed the explants with an alkaline phosphatase (AP)–ecto–Lrp4 fusion protein. We stained for AP activity and found that increasing expression in muscle of Agrn-mutant mice is not significantly different from wild-type motor neurons (the response of Agrn-mutant motor neurons is 106% that of wild-type motor neurons). Scale bar, 10 μm.

The gradual and linear increase in binding from proximal to distal regions is probably due to an increase in number rather than affinity of Lrp4-binding sites, as preferential binding to distal segments is evident at the highest concentration (25 nM) of AP–ecto–Lrp4 that we tested (Supplementary Fig. 6). Binding of ecto-Lrp4 to motor axons is independent of agrin and mediated by the LDLa repeats from Lrp4 regions is approximately 30-fold on distal motor axons (Supplementary Fig. 6).

We next sought to determine whether Lrp4 is essential for motor axons to terminate and differentiate in vivo. Previously, we showed that increasing Msk expression in muscle of Agrn-mutant mice is
AChR clusters (Fig. 4 and Supplementary Fig. 9). Moreover, Musk overexpression did not rescue the neonatal lethality of Lrp4-mutant mice, which is caused by a failure to form neuromuscular synapses. These findings show that Lrp4 has an essential and early role, independent of MuSK activation, in presynaptic differentiation in vivo, as Lrp4 is required to arrest motor axon growth and induce clustering of synaptic vesicles.

We have a good, although incomplete, understanding of the signals and mechanisms for postsynaptic differentiation at neuromuscular synapses, and this knowledge has led to the identification of genes responsible for congenital myasthenia and the synaptic proteins that are targeted in autoimmune myasthenia gravis. In contrast, discovery of the signals and mechanisms by which muscle cells control the differentiation of motor nerve terminals has proved more challenging and remains one of the notable gaps in our understanding of neuromuscular synapses.

Here we show that Lrp4 acts in a bidirectional manner, coordinating synaptic development, as Lrp4 not only binds agrin and regulates postsynaptic differentiation but also functions as a muscle-derived retrograde signal for early steps in presynaptic differentiation. This dual role of Lrp4 in presynaptic and postsynaptic differentiation represents a parsimonious means for mediating reciprocal signalling between adjacent cells and resembles the dual roles that Eph receptors and ErbB receptors have in responding to their respective ligands and in stimulating signalling in ligand-presenting cells. Our findings suggest that Lrp4 functions as a critical check-point at three steps during synapse formation (Supplementary Fig. 1): first, before innervation, Lrp4 forms a complex with MuSK to establish muscle prepatterning; second, as motor axons approach muscle, Lrp4, clustered as a consequence of MuSK activation, acts as a retrograde signal to promote their differentiation; and third, once motor axons establish contact with muscle, Lrp4 binds agrin, which is released from motor nerve terminals, stimulating further MuSK phosphorylation and stabilizing neuromuscular synapses.

Other ligands, including members of the FGF7, FGF10 and FGF22 family, laminin β2, collagen IV and SIRPα, stimulate clustering of synaptic vesicles in cultured motor neurons and have a role in synaptic maturation in vivo. Nevertheless, motor axons terminate and differentiate to a considerable extent in the absence of these signalling components, indicating that additional retrograde organizers regulate earlier steps in presynaptic differentiation. Because motor axons fail to stop and display any signs of presynaptic differentiation in mice lacking Lrp4, Lrp4 must act at an early stage in presynaptic differentiation.

Auto-antibodies to AChRs, MuSK or Lrp4 are responsible for myasthenia gravis. The clinical and pathological manifestations of anti-Lrp4 myasthenia have not been described in detail, but our studies indicate that auto-antibodies to Lrp4 have the potential to obstruct synaptic function not only by blocking binding between Lrp4 and agrin, or Lrp4 and MuSK, but also by interfering with binding between Lrp4 and Lrp4 receptors on nerve terminals. Because the premature withdrawal of motor nerve terminals, which causes muscle denervation, is an early step in amyotrophic lateral sclerosis and a characteristic feature of muscle wasting during ageing, defects in retrograde signalling may underlie or contribute to neuromuscular diseases and sarcopenia.

Lrp4, like Musk, is expressed in the cerebellum, cortex, hippocampus and olfactory bulb (see http://www.brainatlas.org), raising the possibility that Lrp4 may regulate synaptic differentiation in the central nervous system (CNS). Although Lrp4-mutant mice die at birth, well before the peak period of synapse formation in the CNS, Lrp4-mutant mice rescued for Lrp4 expression in muscle survive as adults, and should provide a good model system for studying the role of Lrp4 in synapse formation in the CNS.
METHODS SUMMARY

Muscles from wild-type, agrin-mutant and Lrp4-mutant mice were stained with antibodies to neurofilament and synaptophysin to assess presynaptic differentiation and with J–o-bungarotoxin to measure postsynaptic differentiation. Explants of neural tube, containing motor neurons, were grown in cell culture together with muscle, control non-muscle cells or non-muscle cells expressing Lrp4 or MuSK. Alternatively, motor neurons were treated with polystyrene microspheres, which had the extracellular region of Lrp4, Lrp1 or MuSK attached to the beads. Presynaptic differentiation was measured by staining with antibodies to presynaptic proteins and by quantifying vesicle recycling with the styryl dye FM4-64FX. Binding of AP–ecto-Lrp4 to the cell surface of motor axons was visualized and quantitated by staining for alkaline phosphatase activity.

Full Methods and any associated references are available in the online version of the paper.

Received 20 January; accepted 22 June 2012.

Published online 1 August 2012.

1. Sanes, J. R. & Lichtman, J. W. Induction, assembly, maturation and maintenance of a presynaptic apparatus. Nature Rev. Neurosci. 2, 791–805 (2001).
2. DeChiara, T. M. et al. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. Cell 85, 501–512 (1996).
3. Weatherbee, S. D., Anderson, K. V. & Niswander, L. A. LDL-receptor-related protein 4 is crucial for formation of the neuromuscular junction. Development 133, 4993–5000 (2006).
4. Kim, N. et al. Lrp4 is a receptor for Agrin and forms a complex with MuSK. Cell 135, 334–342 (2008).
5. Zhang, B. et al. LRP4 serves as a coreceptor of agrin. Neuron 60, 285–297 (2008).
6. Arber, S., Burden, S. J. & Harris, A. J. Patterning of skeletal muscle. Curr. Opin. Neurobiol. 12, 100–103 (2002).
7. Yang, X. et al. Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. Neuron 30, 399–410 (2001).
8. Yang, X., Li, W., Prescott, E. D., Burden, S. J. & Wang, J. C. DNA topoisomerase IIbeta and neural development. Science 287, 131–134 (2000).
9. Lin, W. et al. Distinct roles of nerve and muscle in postsynaptic differentiation of the neuromuscular synapse. Nature 410, 1057–1064 (2001).
10. Panzer, J. A. S., o. n. g. Y. & Balice-Gordon, R. J. In vivo imaging of preferential motor axon outgrowth to and synaptogenesis at prepatterned acetylcholine receptor clusters in embryonic zebrafish skeletal muscle. J. Neurosci. 26, 934–947 (2006).
11. Flanagan-Steet, H., Fox, M. A., Meyer, D. & Sanes, J. R. Neuromuscular synapses can form in vivo by incorporation of initially aminergic postsynaptic specializations. Development 132, 4471–4481 (2005).
12. Burden, S. J. SnapShot: neuromuscular junction. Cell 144, 826a1 (2011).
13. Kummer, T. T., Misgeld, T. & Sanes, J. R. Assembly of the postsynaptic membrane at the neuromuscular junction: paradigm lost. Curr. Opin. Neurobiol. 16, 74–82 (2006).
14. Zhang, W., Coldefy, A. S., Hubbard, S. R. & Burden, S. J. Agrin binds to the N-terminal region of Lrp4 and stimulates association between Lrp4 and the first Ig-like domain in MuSK. J. Biol. Chem. (2011).
15. Gautam, M. et al. Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. Cell 85, 525–535 (1996).
16. Lin, W. et al. Neurotransmitter acetylcholine negatively regulates neuromuscular synapse formation by a Cdk5-dependent mechanism. Neuron 46, 569–579 (2005).
17. Misgeld, T., Kummer, T. T., Lichtman, J. W. & Sanes, J. R. Agrin promotes synaptic differentiation by countering an inhibitory effect of neurotransmitter. Proc. Natl Acad. Sci. USA 102, 11088–11093 (2005).
18. Kim, N. & Burden, S. J. MuSK controls where motor axons grow and form synapses. Nature Neurosci. 11, 19–27 (2008).
19. Gomez, A. M. & B. u. r. d. e. n. S. J. The extracellular region of Lrp4 is sufficient to mediate neuromuscular synapse formation. Dev. Dynam. 240, 2626–2633 (2011).
20. Engel, A. G., Ohno, K. & Sine, S. M. Sleuthing molecular targets for neurological diseases at the neuromuscular junction. Nature Rev. Neurosci. 4, 339–352 (2003).
21. Higuchi, O., Hamuro, J., Motomura, M. & Yamanashi, Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. Ann. Neurol. 69, 418–422 (2011).
22. Dresscher, U. The Eph family in the patterning of neural development. Curr. Biol. 7, R799–R807 (1997).
23. Bao, J., Wolpowitz, D., Role, L. W. & Talmage, D. A. Back signaling by the Nrg-1 intracellular domain. J. Cell Biol. 161, 1133–1141 (2003).
24. Fox, M. A. et al. Distinct target-derived signals organize formation, maturation, and maintenance of motor nerve terminals. Cell 128, 179–193 (2007).
25. Higuchi, O., Hamuro, J., Motomura, M. & Yamanashi, Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. Ann. Neurol. 69, 418–422 (2011).
26. Pun, S., Santos, A. F., Saxena, S., Xu, L. & Caroni, P. Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. Nature Neurosci. 9, 408–419 (2006).
27. Valdez, G. et al. Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. Proc. Natl Acad. Sci. USA 107, 14863–14868 (2010).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank L. Landmesser and K. Hata for helping us to establish a motor-neuron and muscle co-culture system. W. Zhang for constructs encoding Fc- and AP-tagged forms of Lrp4, T. Jessell and J. Dasee for HB9:GFP mice, J. Sanes for approximately 15% of AChR clusters are contacted by motor axons; these contacts may be incidental, as motor axons grow and branch extensively throughout muscle of Lrp4-mutant mice, inevitably placing axons in the vicinity of AChR clusters (mean ± s.e.m., n = 3). The insets show higher-magnification views of AChR clusters that are innervated by motor axons (h) or devoid of contact from motor axons (e, a). NF, neurofilament; Syn, synaptophysin.
agrin-mutant mice, K. Anderson and L. Niswander for Lrp4-mutant mice, and D. Littman and R. Lehmann for comments on the manuscript. We are grateful to P. Lopez and M. J. Sunshine and personnel in the transgenic mouse core for their assistance. The flow cytometry and transgenic mouse cores are supported by a New York University Cancer Institute Center Support Grant (National Institutes of Health (NIH)/National Cancer Institute (NCI) 5 P30CA16087-31). This work was supported with funds from the National Institutes of Health (NS36193 to S.J.B.), the Skirball Institute and postdoctoral training support to N.Y. from New York State Stem Cell Science (NYSTEM).

Author Contributions N.Y. designed and carried out all of the experiments in Figs 1, 2 and 3. N.K. designed and carried out the experiments in Fig. 4. S.J.B. helped to design and interpret experiments. All authors wrote and edited the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to S.J.B. (burden@saturn.med.nyu.edu).
METHODS

Co-culture of motor neurons and muscle or non-muscle cells. Explants of neural tube from HB9-GFP transgenic mice at embryonic day 11.5 (E11.5) – E13 were dissected and cultured in Neurobasal medium (Invitrogen), supplemented with B27 and GlutaMax (Invitrogen), 2 ng ml−1 BDNF, 2 ng ml−1 GDNF (Cell Sciences), 2 ng ml−1 CTNF, 1 ng ml−1 NGF (Sigma) and antibiotics. The ventrolateral portion of the neural tube, containing motor neurons, was dissected and isolated based on HB9-GFP expression; the dissected dorsal region of the neural tube lacked GFP expression. Explants were cultured on poly-L-ornithine- and laminin-coated tissue culture dishes for 4 to 6 days before application of microspheres or addition of myotubes or non-muscle cells (NIH 3T3 or HEK 293 cells). Mouse myotubes were generated from primary myoblasts in a separate culture dish and transferred to explant cultures by non-enzymatically detaching myotubes, as described previously28. Non-muscle cells were transfected with Flag–Lrp4, Flag–Lrp4–mCherry or mCherry4, sorted by flow cytometry for mCherry or cell-surface Flag expression, using M2 antibodies (Sigma). We monitored Lrp4 expression either by viewing mCherry expression in cells transfected with Flag–Lrp4–mCherry (Fig. 1c) or by staining for Flag in cells transfected with Flag–Lrp4 (Figs 1d and Supplementary Fig. 1). Non-muscle cells were co-cultured with explants for 20 to 24 h in supplemented Neurobasal medium together with conditioned medium from rat Schwann cells or E12.5 mouse neural tube cells. Half of the medium was replaced every other day.

Assays for presynaptic differentiation. Co-cultures were fixed with 3.7% formaldehyde and stained with antibodies to synapsin (Synaptic Systems), GFP (Abcam), bassoon (Stressgen), SV2 (Developmental Studies Hybridoma Bank), Synaptophysin (Invitrogen) and Alexa 647-conjugated α-bungarotoxin (α-BGT; Invitrogen). Human Fc (Jackson ImmunoResearch), Lrp1-LDLa–Fc (Cluster II of Lrp1 from R&D Systems) or Lrp4-LDLa–Fc4 were attached to Protein A microspheres (Bangs labs) and incubated with explants for 20 to 24 h in the co-culture growth medium described above. Some Lrp4 beads were inadvertently removed during washing, which may explain the absence of beads at some synapsin clusters. Uptake of the styryl dye FM4-64FX, a tracer for recycling synaptic vesicles, was assessed by incubating cells for 2 min in a depolarizing buffer (90 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 10 mM glucose and 20 mM HEPES, pH 7.2) containing 10 μM FM4-64FX (Invitrogen). After washing in a non-depolarizing buffer, dye release was monitored by depolarizing cells further for 2 min, mainly as described previously118,119. Images were acquired on a Zeiss 510 confocal microscope and analysed using Velocity 3D imaging software (Perkin Elmer). We defined synaptic puncta as synapsin clusters that were ≥3 μm² in size for co-cultures of motor neurons and HEK 293 or NIH 3T3 cells and ≥1.5 μm² in size for motor neurons treated with microspheres. FM4-64FX clusters that were ≥1.5 μm² in size were designated as puncta. We determined the number of puncta in a field of 1.44 × 10⁴ μm².

Staining with AP–Lrp4. AP–Lrp4 fusion proteins were generated as described previously28, and their concentrations were determined by measuring alkaline phosphatase activity. Explants were incubated for 90 min at room temperature (23–28 °C) with culture medium containing alkaline phosphatase fusion proteins (10 nM) in binding buffer (150 mM NaCl, 2 mM CaCl2, 1 mM MgCl2, 0.2% BSA and 20 mM HEPES, pH 7.2, 0.1% NaNO3). After washing 5 times in binding buffer, the explants were fixed for 10 min in 3.7% formaldehyde, washed three times in HBS (HEPES-buffered saline) (150 mM NaCl and 20 mM HEPES, pH 7.2) and incubated for 30 min at 65 °C to inactivate endogenous alkaline phosphatase activity. After 3 washes in reaction buffer (100 mM NaCl, 50 mM MgCl2 and 100 mM Tris, pH 9.5), alkaline phosphatase activity was revealed by overnight incubation in reaction buffer with NBT/BCIP (Roche) at room temperature (23–28 °C). Images were acquired with a charge-coupled device (CCD) camera (Princeton Instruments) and were analysed with MetaMorph or Image J. To quantitate binding along the proximal–distal axis, we measured staining along short axon segments at varying distances from the soma, and we subtracted the values for binding of alkaline phosphatase alone from the values for AP–ecto-Lrp4.

Mice. Mice that are agrin-null, mutant for Lrp4-LDLa, lack Lrp4 and Lrp4-LDLa, as described previously14. Similar results were found in mice that overexpress Lrp4-LDLa–Fc (Fig. 1c) with culture medium containing alkaline phosphatase fusion proteins (10 nM) in binding buffer (150 mM NaCl, 2 mM CaCl2, 1 mM MgCl2, 0.2% BSA and 20 mM HEPES, pH 7.2), and were designated as puncta. We determined the number of puncta in a field of 1.44 × 10⁴ μm².

28. Hata, K., Polo-Parada, L. & Landmesser, L. T. Selective targeting of different neural cell adhesion molecule isoforms during motoneuron myotube synapse formation in culture and the switch from an immature to mature form of synaptic vesicle cycling. J. Neurosci. 27, 14481–14493 (2007).

29. Umemori, H., Linhoff, M. W., Ornitz, D. M. & Sanes, J. R. FGF22 and its close relatives are presynaptic organizing molecules in the mammalian brain. Cell 118, 257–270 (2004).

30. Umemori, H. & Sanes, J. R. Signal regulatory proteins (SIRPS) are secreted presynaptic organizing molecules. J. Biol. Chem. 283, 34053–34061 (2008).