Retrograde semaphorin–plexin signalling drives homeostatic synaptic plasticity

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Homeostatic signalling systems ensure stable but flexible neural activity and animal behaviour1–4. Presynaptic homeostatic plasticity is a conserved form of neuronal homeostatic signalling that is observed in organisms ranging from Drosophila to human5,6. Defining the underlying molecular mechanisms of neuronal homeostatic signalling will be essential in order to establish clear connections to the causes and progression of neurological disease. During neural development, semaphorin–plexin signalling instructs axon guidance and neuronal morphogenesis7–10. However, semaphorins and plexins are also expressed in the adult brain11–16. Semaphorins have been shown to be synaptic signalling proteins, but the activity of semaphorins has been limited to the control of neuroanatomical synapse formation and elimination15–17. Here we demonstrate that semaphorin–plexin signalling achieves retrograde, trans-synaptic control of presynaptic neurotransmitter release and homeostatic plasticity.

We use a well-documented assay to induce presynaptic homeostatic plasticity (PHP), applying a sub-blocking concentration of the glutamate-receptor antagonist philanthotoxin-433 (PhTx; 15 µM) to significantly decrease the amplitude of average miniature excitatory postsynaptic potentials (mEPSPs; 0.3 mM [Ca²⁺]e) or miniature excitatory postsynaptic currents (mEPSCs; 1.5 mM [Ca²⁺]e). This postsynaptic perturbation induces a significant increase in presynaptic neurotransmitter release (the quantal content) that offsets the postsynaptic perturbation and restores normal muscle excitation (Fig. 1a–c; see also Extended Data Fig. 1 for a further description of the gene mutations used in this study). Consistent with this being a loss-of-function phenotype, heterozygous mutations (either sema2b/+ or PlexB+/−) have normal PHP (Fig. 1d, e). Remarkably, a double-heterozygous mutant combination of sema2b/+ and PlexB+/− blocks PHP, consistent with both genes acting in concert to drive the expression of PHP (Fig. 1d, e).

Figure 1 | sema2b and PlexB are necessary for PHP. a, Representative traces (left, 0.3 mM [Ca²⁺]e; right, 1.5 mM [Ca²⁺]e) or miniature excitatory postsynaptic potentials (mEPSPs) and quantal content (QC) for each genotype expressed as the percentage change in the presence of PhTx compared to the baseline (absence of PhTx). b, Average mEPSPs and quantal content (QC) for each genotype compared to the genotype control. Data are mean ± s.e.m. and individual data points are shown.
We subsequently investigated the long-term maintenance of PHP and the involvement of other semaphorin or Plexin gene family members. Deletion of a non-essential glutamate-receptor subunit (GluRIIA) induces a long-lasting form of PHP. We find that long-term PHP is blocked in a sema2b;GluRIIA double mutant as well as in GluRIIA transgenic larvae expressing transgenic RNA interference (RNAi) to knockdown sema2b and PlexB. Muscle-specific expression of sema2b RNAi is sufficient to potentiate baseline release, and this effect is also dependent upon PlexB. Finally, application of Sema2b protein (100 nM) completely restores PHP in the sema2b mutant following PhTx treatment to induce PHP. We found that Sema2b protein (100 nM) completely restores PHP in the sema2b mutant following PhTx treatment to induce PHP. The involvement of other semaphorin or Plexin genes encoded in the Drosophila genome (Extended Data Fig. 3) for additional controls). In addition, application of Sema2b protein is acutely applied to the neuromuscular junction (NMJ) of presynaptic nerve terminal (Fig. 2d). Taken together, our data indicate that Sema2b is a ligand originating in the muscle that acts via presynaptic PlexB to drive expression of PHP.

If Sema2b is a retrograde signal that acts upon the presynaptic PlexB receptor, then it should be possible to reconstitute this retrograde signalling by acute application of Sema2b protein. Purified Sema2b protein was acutely applied to the neuromuscular junction (NMJ) of sema2b mutants following PhTx treatment to induce PHP. We found that Sema2b protein (100 nM) completely restores PHP in the sema2b mutant, but fails to restore PHP in the PlexB mutant (Fig. 2e–h; see Extended Data Fig. 3 for additional controls). In addition, application of Sema2b protein is sufficient to potentiate baseline release, and this effect is also dependent upon PlexB. Finally, a membrane-tethered UAS-sema2b transgene, expressed in muscle, fails to rescue PHP (Extended Data Fig. 4). These results indicate that Sema2b is a secreted, postsynaptic ligand that acts upon presynaptic PlexB to enable the expression of PHP. We acknowledge the possibility that PlexB could require a presynaptic co-receptor of, as yet, unknown identity.

Given that acute application of Sema2b protein rescues PHP in the sema2b mutant, the failure of PHP in sema2b-mutant larva cannot be
a secondary consequence of altered NMJ development. Nonetheless, Sema2b–PlexB signalling is required for normal NMJ growth. Axon-targeting errors are rare at muscles 6/7, analysed at the third instar larval stage (Extended Data Table 1). We demonstrate that the NMJs in *sema2b* and *PlexB* mutants are composed of fewer, larger synaptic boutons (Fig. 3a–d) with no change in total NMJ area (Fig. 3b). The abundance of the active-zone-associated protein Bruchpilot (Brp) is unaltered in the *sema2b* mutant and the *sema2b/+;PlexB/+* double-heterozygous larvae (Fig. 3e, ‘trans-het’), both of which block PHP (Fig. 1a, d, e). There is a significant decrease in total Brp staining in the *PlexB* mutant, an effect of unknown consequence (Fig. 3e; see also below). Qualitatively, the ring-like organization of Brp staining was similar across all genotypes, indicative of normal active-zone organization (Fig. 3a, inset, arrows). Finally, there is no consistent difference in synapse ultrastructure across genotypes (Fig. 3f–i). Therefore, the Sema2b–PlexB-dependent control of bouton size may be a separate function of Sema2b–PlexB signalling, analogous to anatomical regulation by semaphorins in mammalian systems. 

PHP occurs through the potentiation of the readily releasable pool (RRP) of synaptic vesicles (see Methods). Application of PhTx induces a doubling of the apparent RRP in wild-type larvae, an effect that is disrupted in both *sema2b* and *PlexB* mutants (Fig. 4). Failure to potentiate the RRP is also shown as a failure to maintain the cumulative EPSC amplitude after PhTx application (Fig. 4c–e). We subsequently show a strong genetic interaction with a mutation in the presynaptic scaffolding gene *rab3-interacting molecule (rim)*, a PHP gene. Heterozygous mutations in *rim*, or in *sema2b* or *PlexB* have no effect on PHP (Fig. 4h, i). However, double-heterozygous combinations of *rim/+* with either *sema2b/+* or *PlexB/+* strongly impaired the expression of PHP (*sema2b/+;rim/+*) or abolished PHP (*rim/+;PlexB/+*) (Fig. 4h, i). These data do not, however, reflect direct signalling between PlexB and Rim (Extended Data Fig. 3e).

To define how PlexB could modulate the RRP, we tested known downstream signalling elements. We discovered that *mical* is necessary for PHP (Fig. 4). In *Drosophila* a single *mical* gene encodes a highly conserved multi-domain cytoplasmic protein that mediates actin depolymerization, achieved through redox modification of a specific methionine residue (Met44) in actin. Notably, prior genetic evidence has placed Mical downstream of both PlexA and PlexB signalling during axon guidance.

An analysis of multiple *mical* mutations in larvae as well as transgenic rescue animals demonstrates that *mical* is necessary presynaptically for PHP (Fig. 4a, b, f, g). Mical protein is present presynaptically (Extended Data Fig. 5) and presynaptic expression of a Mical-resistant *UAS-Act5C* transgene, which interferes with Mical-mediated actin depolymerization, blocks PHP (Fig. 4a, b). This transgenic protein also concentrates within presynaptic boutons (Extended Data Fig. 5). Additional experiments reveal that the homeostatic expansion of RRP is blocked in *mical* mutants and when Mical-resistant *UAS-Act5* is expressed presynaptically (Fig. 4f, g and Extended Data Fig. 6). We find strong genetic interactions between *mical* and both the *PlexB* and *rim* mutants (Extended Data Fig. 4b–d). Finally, anatomical experiments demonstrate that active zones are normal in the *mical* mutant, including in both light and electron microscopy experiments (Fig. 3). We propose that Mical enables PlexB-mediated control of the RRP through the regulation of presynaptic actin.

For half a century, evidence has underscored the importance of target-derived, retrograde signalling that controls presynaptic neurotransmitter release. Gene discovery, based on forward genetics, indicates that PHP is controlled by the coordinated action of at least three parallel signalling systems (see Extended Data Fig. 7). If our data regarding Sema2b, PlexB and Mical can be generalized, then semaphorin–plexin signalling could represent a platform for retrograde, trans-synaptic, homeostatic control of presynaptic release.

**Figure 3 | Altered NMJ growth with normal active-zone number and integrity.** a, Structured illumination microscopy images of NMJ. Inset, single confocal sections; arrows indicate Brp rings. Scale bars, 5 μm and 0.5 μm (inset). b–e, Quantification of morphology; n = 12, except micalR12, n = 10. f, Representative active zones. Scale bar, 70 nm. g–i, Quantification of the ultrastructure. *P < 0.05, **P < 0.01; two-tailed Student’s t-test, pairwise comparison to wild type larvae. Two larvae per genotype; wild-type, n = 16 active zones; *sema2b*, n = 29 active zones; *PlexB*, n = 30 active zones; *mical*, n = 13 active zones. Data are mean ± s.e.m. and individual data points are shown.
Figure 4 | Sema2b, PlexB and Mical control the homeostatic potentiation of the RRP. a. The mical mutants (green) in 0.3 mM [Ca^{2+}]_c (left) and 1.5 mM [Ca^{2+}]_c (right) and larvae overexpressing a Mical-resistant UAS-Act5C transgene (red) show blocked PhTx DI, deficiency. b. Expression of UAS-mical in motor neurons (MN mical rescue) rescues PhTx mical RNAi in motor neurons blocks PhTx. mical RNAi in muscle has no effect (right). c, Representative traces from wild-type (black) and PlexB-mutant (blue) larvae (1.5 mM [Ca^{2+}]_c) with or without PhTx (stimulation frequency 60 Hz, 30 stimuli). Cumulative EPSCs and back extrapolation from steady state (red line) is shown below each trace. d, Quantification of the percentage change in RRP (open) and mEPSPs thereby stabilizing synaptic transmission and information transfer throughout the nervous systems of organisms ranging from Drosophila to humans.

Online Content  Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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METHODS

Fly stocks and genetics. For all experiments, the w1118 strain of Drosophila melanogaster was used as the wild-type control. Male and female larvae were used. Larvae were maintained at 22˚C. When performing rescue, RNAi or overexpression experiments with the Gal4/UAS expression system, progeny were raised at 25˚C. The following Drosophila stocks were used: sema2b21 (ref. 21), UAS-sema2b-RNAi (Bloomington stock 28932), UAS-sema2b-TM-GFP (ref. 21), UAS-sema2b flies were a gift from A. Kohdokin (Johns Hopkins University), P[Gal4]/UAS-Myc (Kyoto stock 112237), Sema2bGFP (Bloomington stock 65752), P[Scout-P; P]PlexaEGFP8 (ref. 20), UAS-Myc-PlexaEGFP8 (ref. 21), UAS-Myc-PlexaB (ref. 20), PlexaEGFP8 (Bloomington stock 23097), UAS-Plexa-RNAi (ref. 18), PlexaEGFP8 (Bloomington stock 7083), GluRIIA (ref. 22), OK371-Gal4 (ref. 23), rpl13A (ref. 24), micalEGFP (ref. 6), Df(3R)wp1, mical deficiency37, micalEGFR518 (ref. 6), UAS-mical-RNAi (Bloomington stock 31148), UAS-MicalmCherry (ref. 25), Mical-GFP Protein Trap (Bloomington stock number 60203), UAS-Cas9-GFP 3744 (ref. 7).

Electrophysiology. Recordings were made from muscle 6 in abdominal segments 2 and 3 of male and female third-instar larvae in current-clamp (0.3 mM CaCl2) or voltage-clamp (1.5 mM CaCl2) mode as indicated, without randomization26,27. Haemolymph-like (HL3) saline was used (70 mM NaCl, 5 mM KCl, 10 mM MgCl2, 10 mM NaHCO3, 115 mM sucrose, 4.2 mM trehalose, 5 mM HEPES). Quantal content was calculated by dividing the average EPSP amplitude by the average mEPSP amplitude for each muscle recording (EPSP/mEPSP) and averages were made across muscles for a given genotype. For acute pharmacological induction of PHP, larvae were incubated in philanthotoxin-433 (PhTx; 10–20 μM; Sigma-Aldrich) for 10 min according to previously published methods28,29. Two-electrode voltage-clamp recordings were done as previously described in 1.5 mM CaCl2, HL3 saline30. EPSC analyses were conducted using custom-written routines for Igor Pro 5.0 (Wavemetrics) available as published28, and mEPSCs were analysed using Mini Analysis v.6.0.0.7 (Synaptosoft). Recordings were excluded if the resting membrane potential was more depolarized than –60 mV. Each experiment was repeated for at least two independent crosses. Key experiments demonstrating the blockade of synaptic homeostasis were performed by an independent investigator who was blinded to the genotype. All controls and experimental genotypes were independently replicated for each experiment in each figure. Experimental sample sizes equal to or greater than seven were considered sufficiently powered to detect a blockade in homeostatic plasticity, an effect size of ~80–120% compared to controls.

Anatomical analyses. Third-instar larval preparations (muscles 6/7) were filleted and fixed in 4% paraformaldehyde, washed and incubated overnight at 4˚C with primary antibodies. Secondary antibodies were applied at room temperature for 2 h. The following primary antibodies were used: anti-Myc (1:500, mouse; 9E10 Santa Cruz), anti-GFP 3E6 (1:500, mouse; Life Technologies), anti-NC82 (1:100, mouse; Developmental Studies Hybridoma Bank), anti-HA antibody (1:1,000, rabbit; Cell Signaling Technology) and anti-Dlg (1:10,000, rabbit; ref. 28). Alexa-conjugated secondary (488, 555) antibodies and Cy3-conjugated goat anti-HP-RP were used at 1:500 (Life Technologies; Molecular Probes). Larval preparations were mounted in Vectorshield (Vector) and imaged with an Axiosvert 200 (Zeiss) inverted microscope, a 100× Plan Achromat objective (1.4 NA) and a cooled charge-coupled device camera (CoolSNAP HQ, Roper). Slidebook 5.0 Intelligent Imaging Innovations (JI) software was used to capture, process and analyse images. Structured illumination microscopy imaging was performed using the N-SIM Nikon system, consisting of a Nikon Ti-E microscope equipped with an Apo TIRF 100×/1.49 oil objective and an Andor DU9897 camera.

Sema2b ligand generation and application. We used the Drosophila S2 expression system to express the Sema2b-AP ligand for the bath application of Sema2b ligand to the Drosophila NMJ for in vivo electrophysiological recordings. Drosophila S2 cells (obtained from the Vale laboratory, UCSF for additional source information, see http://flybase.org/reports/FBbt000006.html). Cells are mycoplasma negative, tested by MycoAlert (2017). We co-transfected UAS-sema2B-AP and actin-Gal4 plasmids in S2 cells using Effectene (QIAGEN) and incubated the cells at 27˚C for four days in serum-free Schneider’s medium. We then collected the medium and diluted the Sema2b-AP ligand in HL3 (0.3 mM CaCl2) to a concentration of 100 nM. To record from the NMJ in the presence of bath-applied Sema2b-AP ligand, we prepared the larval fillet as described above and incubated the preparation in Sema2b-AP HL3 for 10 min. To assess Sema2b-AP ligand rescue, we bath-applied HL3 (0.3 mM CaCl2) containing both PhTx15 (15 μM) and Sema2b-AP ligand (100 nM) to the preparation for a 10-min incubation. Next, we removed the PhTx/Sema2b-AP ligand HL3 saline and replaced it with HL3 containing only the 100 nM Sema2b-AP ligand to record in the presence of Sema2b.

Transmission electron microscopy. Transmission electron microscopy samples for all third-instar larvae were prepared and imaged according to methods that have been previously published31.

Data availability. The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.
Extended Data Figure 1 | Mutations in additional semaphorin and Plexin gene family members do not alter the rapid induction of PHP.

(a) Gene diagrams indicating the mutations used in this study. Colours match scale bars.

(b) Quantification of the percentage change in mEPSPs (solid bars) and quantal content (open bars) in the presence of PhTx for the following genotypes: wild-type (w1118), sema1aK13702, sema1bEY21782, sema2aEY08184, sema5cMI10577 and PlexAEY1654. Each genotype was either previously described as, or predicted to be, a strong loss-of-function mutant: sema1aK13702 (ref. 17), sema1bEY21782 (ref. 30), sema2aEY08184 (ref. 31), sema5cMI10577 (ref. 32) and PlexAEY1654 (ref. 30). All recordings were made in 0.3 mM [Ca\textsuperscript{2+}]. Data are mean ± s.e.m. *P < 0.05; **P < 0.01; two-tailed Student’s t-test.

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**Extended Data Figure 2 | Evidence of sema2b gene expression in larval muscle.**

**a**, Left, representative images of a sema2b-promoter–Gal4 fusion driving UAS-cd8-GFP at low magnification; images show multiple NMJs in the peripheral musculature, all expressing GFP. Muscles 4, 6 and 7 are labelled. Segmental boundaries are indicated by the horizontal lines and the middle segment is indicated as abdominal segment 3 (A3). Right, a higher magnification image taken of muscle 6/7, the muscles in which all recordings were made in this study, revealing expression of sema2b-promoter–Gal4. The NMJ is labelled with anti-Dlg (pink). Muscle identity is indicated. **b**, Images were taken at an identical exposure to those in **a** showing that there is no background GFP immunofluorescence in the absence of the UAS-cd8-GFP reporter as a control. Scale bars, 200μm (left) and 10μm (right). **c**, Data displayed as in **a**. Expression of sema2b-HA is controlled by endogenous promoter sequences. Scale bars, 200μm (left) and 10μm (right). **d**, Structured illumination, super-resolution microscopy was used to image Sema2b-HA expressed as in **c**. A single optical section (single plane) is shown revealing close proximity between Sema2b (green; anti-HA) and the presynaptic membrane (purple) labelled with anti-HRP. Scale bar, 2μm.
Extended Data Figure 3 | Effects of exogenous application of Sema2b protein on baseline transmission. a, Raw data and analysis of additional control genotypes for the electrophysiological analysis of the effects of application of exogenous Sema2b protein (0.3 mM [Ca\(^{2+}\)]), Wild type (wt; \(n = 5\)), wt + sham (\(n = 12\)), wt + Sema2b (\(n = 23\)), PlexB + sham (\(n = 7\)), PlexB + Sema2B (\(n = 6\)). b, A silver-stained protein gel of supernatant collected from S2 cells transfected with both Actin-Gal4 and UAS:Sema2b-AP, or the Actin-Gal4 plasmid alone (sham). The red box highlights that the Sema2b-AP ligand is present at the correct size when both plasmids were transfected together, but absent when the Actin-Gal4 plasmid is transfected alone (sham). Bottom, BSA standards. c, Representative traces (0.3 mM [Ca\(^{2+}\)]). d, Raw data (0.2 mM [Ca\(^{2+}\)]) for indicated genotypes without (filled bars) and with (open bars) application of exogenous Sema2b protein (100 nM protein). Application of Sema2b protein causes a 40% increase in quantal content in controls (\(n = 6\)) and this effect is blocked in larvae that overexpress a PlexB dominant-negative transgene in motor neurons (OE MN PlexB DN) (control, \(n = 6\); OE MN PlexB DN, \(n = 14\)). e, Effects of applying Sema2b protein to the rim\(^{103}\) mutant (compare rim baseline to rim with Sema2b). Experiments were performed in 0.4 mM [Ca\(^{2+}\)] to achieve comparable levels of absolute baseline vesicle release to the experiments shown in a. Sema2b protein has no effect on mEPSP amplitudes, but potentiates both the average EPSP and quantal content in rim\(^{103}\), demonstrating a significant (\(P < 0.05\)) potentiation of release. Sema2b protein rescues the blockade of PHP observed in the rim\(^{103}\)-null mutant. Application of PhTx reduces mEPSP amplitudes in rim\(^{103}\) (\(P < 0.01\)) and there no significant (n.s.) increase in quantal content resulting in average EPSP amplitudes that are smaller than baseline. When Sema2b is co-applied with PhTx (rim + PhTx + Sema2b), the homeostatic potentiation of quantal content is significantly potentiated (\(P < 0.01\)) consistent with a rescue of PHP (rim\(^{103}\) baseline, \(n = 6\); rim\(^{103}\) + Sema2b, \(n = 13\); rim\(^{103}\) + PhTx, \(n = 7\); rim\(^{103}\) + PhTx + Sema2b \(n = 10\)). Data are mean ± s.e.m. *\(P < 0.05\), **\(P < 0.01\); two-tailed Student's t-test.
Extended Data Figure 4 | Genetic interactions. a, Averaged mEPSP and quantal content in the absence and presence of PhTx for the indicated genotypes. Both genotypes (A and B) expressed the membrane-tethered UAS-sema2b-GFP (UAS-sema2b<sup>TM</sup>-GFP) in muscle (BG57-GAL4). Expression of membrane-tethered UAS-sema2b-GFP has no deleterious effects on neurotransmission or the expression of PHP in control larvae with a heterozygous mutation in the sema2b gene (sema2b<sup>/+</sup>) (<i>n</i> = 8 without PhTx and <i>n</i> = 8 with PhTx; genotype A). Muscle expression of membrane-tethered UAS-sema2b-GFP in the sema2b homozygous mutant background failed to rescue PHP (<i>n</i> = 8, <i>n</i> = 9; genotype B). This is in contrast to the observation that expression of wild-type UAS-sema2b in muscle fully restores PHP in the sema2b mutant background (Fig. 1). We conclude that a membrane-tethered Sema2b protein is unable to signal to the presynaptic terminal without being secreted from the postsynaptic membranes. These data are consistent with Sema2b being a secreted ligand, originating in muscle, for the induction and expression of PHP. b–d, Averaged mEPSPs and quantal content in the absence and presence of PhTx for the indicated genotypes. Heterozygous mutations in rim<sup>/+</sup> (<i>n</i> = 8, <i>n</i> = 8; without and with PhTx, respectively), PlexB<sup>/+</sup> (<i>n</i> = 9, <i>n</i> = 9) and micalK584<sup>/+</sup> (<i>n</i> = 8, <i>n</i> = 8; note micalK584 shortened to micalK5 in the figure) show normal PHP following PhTx-dependent inhibition of mEPSP amplitudes. Double-heterozygous combinations of rim<sup>/+</sup> with micalK584<sup>/+</sup> (<i>n</i> = 9, <i>n</i> = 11) or PlexB<sup>/+</sup> with micalK584<sup>/+</sup> (<i>n</i> = 8, <i>n</i> = 13) results in complete blockade of PHP. These genetic interactions indicate that mical, rim and PlexB all participate in a common process that is directly required for PHP. Data are mean ± s.e.m. *<i>P</i> < 0.05; **<i>P</i> < 0.01; two-tailed Student’s t-test.
Extended Data Figure 5 | Synaptic localization of Mical and Act5C.

a, Transgenic expression of UAS-Act5C^{M44L} (middle; green in merge on the right) localizes throughout the presynaptic terminal marked with anti-HRP (left; magenta). Scale bar, 5 μm.
b, The transgenic expression of UAS-mical-mCherry (green), used to rescue PHP in the mical mutant, localizes to the presynaptic boutons of motor neurons labelled with anti-HRP (magenta). Scale bar, 10 μm.
c, Image of a Mical protein trap (see Methods) showing endogenous localization of Mical protein in the postsynaptic muscle and enrichment at the NMJ, which is labelled with anti-HRP (magenta). Projections in the z plane (y–z or x–z planes) indicate the presence of Mical protein within the presynaptic bouton of the protein trap.
d, To selectively image presynaptic Mical–GFP in the protein-trap background, UAS-mical-RNAi was selectively expressed in muscle (BG57-GAL4) in the protein-trap background, greatly reducing muscle Mical–GFP and revealing strong presynaptic Mical–GFP originating from the protein trap at the endogenous gene locus. The NMJ is defined by anti-HRP (magenta, left) and by anti-Dlg (magenta, right).
Extended Data Figure 6 | Representative data showing that homeostatic expansion of the RRP fails in sema2b and mical mutants and following expression of mutant UAS-Act5C. Representative traces and graphs indicating cumulative EPSCs and back extrapolation from steady state (red line) for the indicated genotypes. a, Data are shown for wild type. b, Data are shown for sema2b. c, Data are shown for micalKG. d, Data are shown for larvae expressing UAS-DN-Act5C. We note that sema2b mutants have a baseline synaptic transmission defect, with a smaller baseline initial EPSC and correspondingly smaller RRP compared to both wild-type larvae and PlexB mutants (Fig. 4). Because there is no change in the number of active-zone-associated vesicles in sema2b mutants (Fig. 3), this defect must reflect a change in the allocation of vesicles to the RRP at baseline that parallels the failure to homeostatically potentiate the RRP during PHP. However, because PlexB mutants also block PHP without a change in baseline RRP, it seems unlikely that there is a causal link between reduced baseline RRP and failed PHP in the sema2b mutant.
Extended Data Figure 7 | A schematic of retrograde, trans-synaptic Sema2b–PlexB signalling. Signalling is schematized in the context of other mechanisms that have been recently demonstrated to be necessary for PHP. Sema2b–PlexB signalling (red) is a coherent trans-synaptic, retrograde signalling system that is conveyed, via Mical, to modify presynaptic actin and potentiate the readily releasable synaptic vesicle pool. Other genes have been shown to be necessary for PHP, but none can be connected into a coherent, trans-synaptic signalling cascade. In brown, presynaptic Deg/ENaC channels are inserted into the presynaptic plasma membrane, causing sodium leak and potentiation of presynaptic calcium influx through presynaptic calcium channels (CaV2.1)33. In blue, two components residing in the synaptic extracellular matrix have been implicated in PHP. The α2δ3 auxiliary subunit of the presynaptic calcium channel is necessary for PHP34. The matrix-derived signalling protein Endostatin, a cleavage product of the collagen homologue Multiplexin, is also necessary for PHP35. In orange, the innate immune receptor, peptidoglycan-recognition protein (PGRP), is essential for PHP29. Signalling downstream of PGRP is hypothesized to reach the neuronal nucleus and could thereby mediate the long-term maintenance of PHP. A major task for the future will be to define how these diverse signalling mechanisms participate in a coordinated response that rapidly, accurately and persistently regulates presynaptic neurotransmitter release following disruption of postsynaptic glutamate receptor function.
P, phosphorylation; TF, transcription factor; Ub, ubiquitination.
### Extended Data Table 1 | Axon guidance defects for type 1s and type 1b motor neurons

| Genotype     | 1s Guidance defects | 1b Guidance defects | n   |
|--------------|---------------------|---------------------|-----|
| Sema2bAC     | 2                   | 1                   | 20  |
| Sema2bAC/+   | 1                   | 0                   | 20  |
| PlexB        | 0                   | 0                   | 20  |
| PlexB/+      | 0                   | 0                   | 20  |

Note, the small number of guidance defects at the third instar NMJ may represent the action of compensatory or overlapping mechanisms that promote correct target selection.
Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

1. Sample size
   Describe how sample size was determined.

   As stated in the methods section under 'Electrophysiology', "All controls and experimental genotypes were independently replicated for each experiment in each figure". Note that we are working with an effect size that is very large - often equal to or exceeding a 100% change. This greatly enables our ability to discern significant differences as data distributions often have little or no overlap, enabling small sample sizes to be used in some instances. Experimental sample sizes equal to or greater than 7 were considered sufficiently powered to detect a block in homeostatic plasticity, an effect of size of ~80-120% compared to controls. In some instances, where controls are independently replicated and the data are in agreement across figures, sample sizes are 5-7. The combined sample size, if these controls are grouped, far exceeds the estimated sample size necessary to power our results.

2. Data exclusions
   Describe any data exclusions.

   As stated in the methods section under 'Electrophysiology' - 'Recordings were excluded if the RMP was more depolarized than -60mV. " The average resting membrane potential of postsynaptic muscle is approximately -65mV. We exclude samples that are more depolarized than -60mV as a means to select against recordings where the electrode has damaged the muscle fiber upon insertion. This has been a standard exclusion in all of our papers for the past 20 years as good practice, and is consistent with accepted practices in synaptic electrophysiology.

3. Replication
   Describe whether the experimental findings were reliably reproduced.

   We state in the methods section under "Electrophysiology" "Each experiment was repeated from at least two independent crosses. Key experiments demonstrating a block of synaptic homeostasis were performed blind to genotype by an independent investigator. All controls and experimental genotypes were independently replicated for each experiment in each figure."

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.

   This does not apply. We compare independent genotype. As we state in the methods section, "All controls and experimental genotypes were independently replicated for each experiment in each figure. As a consequence, control genotypes are interleaved with the corresponding experimental genotypes when making an assessment for how each experiment alters synaptic homeostasis.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   We state in the methods section under "electrophysiology", "Key experiments demonstrating a block of synaptic homeostasis were performed blind to genotype by an independent investigator."

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☑️ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☑️ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑️ | A statement indicating how many times each experiment was replicated |
| ☑️ | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☑️ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☑️ | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| ☑️ | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ☑️ | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Policy information about availability of computer code

Describe the software used to analyze the data in this study. We used previously published software and provide the citation for this software (Gavino et al., 2015). The code has already been made publicly available.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Policy information about availability of materials

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No restrictions. We include a statement on data availability in our manuscript, as required by Nature policy.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Each antibody that we use refers to the original reference. Drosophila-specific antibodies are in common use in the Drosophila community and have been validated by showing that the immunofluorescence is eliminated in the absence of the target protein, through genetic analysis of the null mutation. This is the case for anti-NC82 (1:100; mouse; Developmental Studies Hybridoma Bank) used here. All other antibodies are in common use, and we provide the catalogue number and source with method of validations included therein, as requested.
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. We provide this information in our Methods Section under "Sema2b ligand
generation and application." We state "We utilized Drosophila S2 expression
system to expressed Sema2b AP ligand21 for the bath application of Sema2b ligand
to the Drosophila NMJ for in vivo electrophysiological recordings. Drosophila S2
cells (obtained from the Vale laboratory, UCSF with additional source information
at http://flybase.org/reports/FBtc9000006.html). Cells are mycoplasma negative,
tested by MycoAlert (2017)."

   b. Describe the method of cell line authentication used. Drosophila S2 cells (obtained from the Vale laboratory, UCSF with additional source
information at http://flybase.org/reports/FBtc9000006.html). Cells are
mycoplasma negative, tested by MycoAlert (2017).

   c. Report whether the cell lines were tested for
      mycoplasma contamination. Drosophila S2 cells (obtained from the Vale laboratory, UCSF with additional source
information at http://flybase.org/reports/FBtc9000006.html). Cells are
mycoplasma negative, tested by MycoAlert (2017).

   d. If any of the cell lines used are listed in the database
      of commonly misidentified cell lines maintained by
      ICLAC, provide a scientific rationale for their use. Not Applicable

— Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
   Provide details on animals and/or animal-derived materials used in the study. The insect Drosophila melanogaster was the only model organism used in our
study. "Recordings were made from muscle 6 in abdominal segments 2 and 3 of
male and female third-instar larvae."

Policy information about studies involving human research participants

12. Description of human research participants
   Describe the covariate-relevant population characteristics of the human research participants. Not Applicable.