SI Appendix

Cell culture preparation

Aplysia co-cultures (an L7 gill motor neuron and a pleural sensory neuron) were prepared as described previously (1,2). L7 gill motor neurons were isolated from juvenile (1-3 g) animals and pleural sensory neurons were isolated from adults (70 – 120 g). The animals were purchased from the Howard Hughes Medical Institute Mariculture Facility (Miami, FL). The cell culture medium consisted of 50 % filtered hemolymph and 50 % L15 medium supplemented with salts (2). The hemolymph was collected from wild caught adults (> 500g, Marinus, California) and kept in a -80° C freezer.

Miniature EPSC (mEPSC) and evoked EPSP (eEPSP) recording

All recordings were performed as described previously (2). Briefly, 5 min after whole cell patching of the MN we started the first 10 min session of recording spontaneous mEPSCs. We then added 5HT or mApNT and started the second 10 min recording session 5 min later (Figure 1A main text). mEPSCs were recorded in voltage clamp mode at high gain, filtered at 300Hz (-3dB), and AC coupled. At the end of each mEPSC recording session we switched to current clamp mode and checked the eEPSP by stimulating the SN with an extracellular stimulating electrode.

ApTrk-Fc or anisomycin (Sigma) were added to the bath 30 min before the first recording session. Likewise, antisense-ApTrk, siRNA-ApNT, PKAi and PKCi (Calbiochem), or Calcium Orange (Molecular Probes) were pressure injected intracellularly at least 30 min before the first session. mApNT, ApTrk-Fc, antisense ApTrk, and siRNA-ApNT were constructed based on the Aplysia sequences. The mammalian reagents have been characterized previously in Aplysia (3,4).

Expression of Constructs for Fluorescent Fusion Proteins and Image Analysis

All the constructs for fluorescent fusion proteins (e.g. ApNT-pHluorin, ApTrk-mem-GFP, OAR-GFP) were cloned into the *Aplysia* expression vector pNEX3 (5). Purified plasmid DNAs were microinjected into the sensory or motor neuron, which was examined 1 d later with an Olympus FV1000 scanning unit coupled to an IX81 inverted microscope with a 40x objective. That microscope was also used to view immunoreactivity and Calcium Orange. The images in the figures were acquired from optical sections of the bottom 20 µm of SNs and the bottom 30 µm of MNs, and image analysis was performed on the bottom 3 µm. Fluorescence intensity was measured in the initial segment area of both neurons.

A Hybrid system for Activating Pre- or Postsynaptic ApTrk receptors selectively

For ApTrk-Mem, a DNA fragment encoding a N-myristoylation signal and 2xDmrB was PCR-amplified from pHom-Mem1 (Clontech), digested with SphI and SalI (encoded in the primer sequences), and then inserted at the SphI and SalI sites of pNex-ApTrk-GFP. The resulting plasmid, pNex ApTrk-Mem-GFP, expresses a fusion protein consisting of the N-myristoylation signal, 2xDmrB (dimerization domain), ApTrk (after the transmembrane domain), linker

sequence, and EGFP (**Figures S1, S2**). ApTrk-Mem was expressed in the SN or MN and activated with an artificial ligand (BB dimers, Clontech) (1μl/dish).

Immunocytochemistry

Immunocytochemistry was performed as previously described (6). The anti-ApNT antibody (M50) was raised against the mature domain. Anti-eIF4E (phospho 209) monoclonal antibody and the antibody against the catalytic unit of PKA were purchased from Abcam, and antibodies against phospho-Akt (Ser473) and phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) were purchased from Cell Signal. The mammalian antibodies against p-eIF4E and p-MAPK have been characterized previously in Aplysia (7,8). The preparations were incubated with primary antibodies (diluted 1:100) at 4°C overnight, and with secondary antibodies (Invitrogen, diluted 1:1000) at room temperature for 1 hr.

Statistical Analysis

Drug conditions are compared with interleaved saline or vehicle controls. The data were normalized to the control or pretest value and presented as mean +/- SEM. The normalized data were analyzed with Student's *t*-tests (two groups) or ANOVAs (more than two) with one repeated measure (time) followed by planned or post hoc comparisons of the groups of interest. All the comparisons are two tailed unless stated otherwise. Control groups from experiments in the same figure were pooled if they were not significantly different from each other. $p < 0.05$ is considered significant.

References

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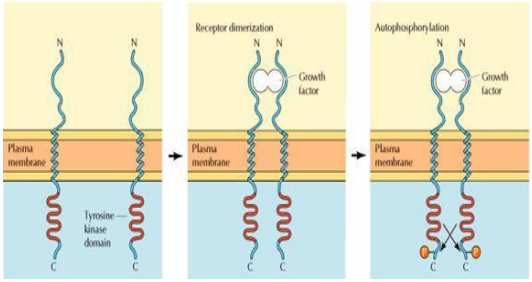
Figure Legends

Figure S1. ApTrkmem/BB system: a tool for selective activation of ApTrk receptors in either the presynaptic or postsynaptic neuron
We adopted the small molecule dimerizer (Clontech), which consists of two parts, a Dmr (dimerizer) protein with a binding site for a specific ligand and the ligand (BB ligand). We cut off the extracellular ligand binding domain of the original ApTrk receptor and inserted two Dmr domains in the intracellular portion of the receptor. When this construct is expressed in a cell, application of an artificial ligand (BB dimer) can activate downstream signaling of ApTrk receptors exclusively in that cell without directly affecting other cells. To keep the construct inside the cell membrane we added a myristylation signal, and to visualize it we added EGFP.

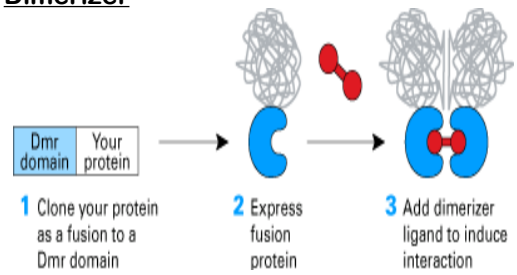
Figure S2. How the construct works: (1) The ApTrk-mem construct is expressed in the cell of interest. (2) BB ligands (cell permeable) are applied to the cell. (3) The BB ligands bind to Dmr domains of ApTrk-mem inside the cell, which induces dimerization of the receptor monomers. (4) Each receptor phosphorylates its partners' tyrosine residue, leading to the activation ApTrk receptor signaling in the cell.

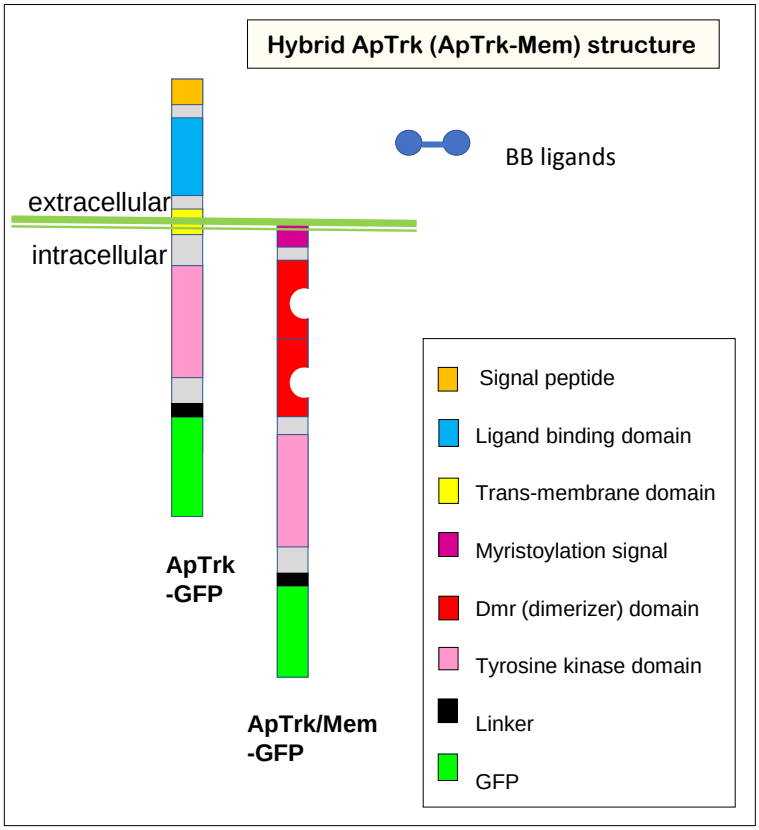
ApTrkmem/BB system:
a tool for selective activation of ApTrk receptor

Receptor Tyrosine kinase activation



Small Molecule Dimerizer





Small molecule dimerizer:
a tool for selective activation of ApTrk receptor