Supplementary Methods
Recombinant proteins, cell lines and mice

GDNF, GFRα1-Fc and GFRα2-Fc were purchased from R&D, NTN from Peprotech, and NGF from Promega. BDNF was provided by Regeneron Pharmaceuticals. Soluble GDNF, NTN or BDNF were used at 100 ng ml\(^{-1}\). MG87 fibroblasts expressing GFRα1 have been described previously\(^{43}\). Ret knock-out mice\(^{44}\) were obtained from Vassilis Pachnis; Gfrα1 mutant mice\(^{45}\) from Arnon Rosenthal; and Ncam knockout mice\(^{46}\) from the Jackson Laboratory (Maine).

RT-PCR

Total RNA was isolated from hippocampus using RNA-easy columns (Qiagen) according to the manufacturer’s instructions. Single stranded cDNA was synthesized using Multiscribe reverse transcriptase and random hexamers (Perkin Elmer). The cDNA was amplified using the following primer sets: GFRα1: forward, 5’-GAC CGT CTG GAC TGT GTG AAA G-3’; reverse, 5’-TTA GTG TGC GGT ACT TGG TGC T-3’; RET: forward, 5’-ATG ATG ATG AAG ACG ACT CCC C-3’; reverse, 5’-CGC TTA AAC TCC ACC ACA GCA-3’; NCAM: forward, 5’-CCT AGA CTG GAA CGC CGA GTA C-3’; reverse, 5’-GAA GTG AGC TGC CTT GGA TTT T-3’; GAPDH: forward, 5’-TGG GTG TGA ACC ACG AGA AAT A-3’; reverse, 5’-GCT AAG CAG TTG GTG GTG CAG-3’; mouse GDNF: forward, 5’-GGT GCG TTT TAA CTG CCA TAC A-3’; reverse, 5’-AAG ATC AGT TTC TCC TTC TGT TGT TCA-3’; rat GDNF: forward, 5’-ATG TCA CTG ACT TGG GTT GGG G-3’; reverse, 5’-GCT TCA CAG GAA CCG CTA CAA-3’. Real-time PCR was performed using a LightCycler rapid thermal cycler system (Perkin Elmer) according to manufacturer’s instructions using Master SYBR Green I mix (Perkin Elmer).

Primary neuronal cultures

Rat hippocampal and cortical neurons from embryonic day (E)18.5 and mouse hippocampal neurons from E16.5 embryos were dissociated by trituration and cultured in Neurobasal medium (Gibco) supplemented with B27 (Gibco). Twenty thousand cells were plated on poly-lysine coated glass coverslips and cultured in 24 well plates. Localization of synaptic markers and GFRα1 in naïve cultures was done 48-72 hours after plating.
Colocalization of pre- and post-synaptic markers

Colocalization of pre- and post-synaptic markers was analysed in hippocampal neurons cultured for 12 days in the presence or absence of GDNF (added every 4 days at 100 ng/ml). Quantification of colocalization of Synapsin I and PSD-93 was done by counting the number of double-labeled puncta in 4 different 50 m dendritic segments per neuron. Puncta were defined as distinct spots of high intensity visualized at high magnification by confocal microscopy. At least ten neurons were analyzed per well in triplicate wells. Quantification of synaptophysin clustering on proximal dendrites of hippocampal neurons was done by counting the number of synaptophysin puncta in the most proximal 50 m segment of individual dendrites as stained with anti-MAP-2 antibodies (Sigma). Restricting these measurements to a fixed dendritic length made it independent of possible changes in neurite outgrowth.

Total cell lysates, Western blotting, pull-down and cross-linking assays

For total lysates, cells were lysed at 4°C in buffer containing 0.5% Triton X-100, 1% β-octylglucoside plus protease and phosphatase inhibitors. Protein lysates were clarified and analyzed by Western blotting as previously described\(^2^\). All blots were scanned in a Storm 840 fluorimager (Molecular Dynamics) and analyzed with ImageQuant software (Molecular Dynamics). Antibodies were obtained from various sources as follows: anti-GFRα1 rabbit antiserum (#1371) was kindly provided by Michele Sanicola (Biogen-Idec); anti-NCAM\(^{ICD}\) (12F11) and anti-NCAM\(^{ECD}\) (N-CAM13) were purchased from BD PharmMingen; anti-RET from Santa Cruz Biotechnology, anti-synapsin I, anti-synaptophysin, anti-synaptotagmin, anti-syntaxin and anti-GAP-43 from Chemicon; anti-PSD-95 from Affinity BioReagents; anti-NMDA 2A and 2B from Affinity BioReagents; monoclonal anti-HA from Covance; and anti-TrkA was kindly provided by D. Kaplan. For pull-down assays, COS cells were transfected with pcDNA3 or HA-tagged GFRα1. After 48 h, the cells were incubated with recombinant GFRα1-Fc (R&D, 300 ng/ml) in the presence or absence of GDNF (100 ng ml\(^{-1}\)). After 20 min at 37°C, the cells were lysed at 4°C in buffer containing 0.5% Triton X-100, 1% β-octylglucoside plus protease and phosphatase inhibitors. Samples were incubated with gentle rocking for 2 h at 4°C with protein G Sepharose beads. After washing, the beads were resuspended in loading buffer and separated by SDS-PAGE. Bound protein was detected on immunoblots using a mouse
monoclonal anti-HA (Covance). Cross-linking assays to SPM fractions were performed with EDAC (Pierce) using GDNF iodinated by the lactoperoxidase method as previously described⁵⁰.

**Immunofluorescence and microscopy**

For immunocytochemistry, cells were washed, permeabilized with 0.3% Triton X-100 at room temperature, fixed in 4% paraformaldehyde, and stained with the indicated antibodies by overnight incubation at 4°C. An affinity purified anti-GFRα1 rabbit anti-serum (#1371) provided by Michele Sanicola (Biogen-Idec) was used at 1:300 dilution. MAP-2 antibody (Sigma) was used 1:750; VGlut2 and VGAT antibodies (Synaptic Systems) 1:1000; antibodies to synapsin I, synaptophysin and synaptotagmin (Chemicon) 1:1000; to PSD-95 (Affinity BioReagents) 1:100; and to PSD-93 (Synaptic Systems) 1:500. Anti-HA staining was done on non-permeabilized, PFA-fixed Jurkat cells with 1:100 dilution of the anti-HA (COVANCE) for 2 h at room temperature. Hippocampal cryostat sections (14 μm) were obtained from PBS-perfused P15 mice and probed with antibodies against synaptophysin (Chemicon) at 1:1000 dilution. Synaptophysin puncta were quantified in 3 different fields of the stratum radiatum of 5 animals of each genotype. Density of dendritic fields was assessed with antibodies against MAP-2 (Sigma). In all cases, secondary antibodies conjugated to the fluorescein isothiocyanate (FITC) or rhodamine (TRITC) were from Jackson Immunoresearch Lab. After immunostaining, confocal microscopy was performed in a Zeiss LSM 510 confocal microscope using laser excitation wavelengths 488 and 543 nm. Quantification of synaptophysin puncta in hippocampal sections was performed in 48 x 48 μm micrographs of the stratum radiatum of CA1 (three fields per animal in 4-5 animals of each genotype) using OpenLab software (Improvision).