Supporting Information

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SI Methods

Live-Cell Imaging of Dendritic Spine Morphogenesis. Live-cell experiments were performed at 37 °C on a thermostable closed-bath imaging chamber RC30WA (Warner Instruments). Secondary apical dendrites of 14 days in vitro (DIV) hippocampal neurons were selected from EGFP-positive neurons and selected for confocal time-lapse imaging in a Zeiss Axiovert 200M LSM PASCAL Confocal Laser Scanning Microscope. High-resolution (2,048 × 2,048 pixel) fluorescence images were acquired with an oil immersion 63×/1.4AN objective and a LP530 emission filter, with excitation at 488 nm. Confocal stacks (6–7.5 μm) from 20–25 serial images with a Z-step size of 0.3 μm and a pinhole diameter of 200 μm were acquired every 5 min for 1 h in the presence of vehicle (control) and for 2 h after the treatment with recombinant wingless-type family member 5A (Wnt-5a) ligand. Maximal intensity projections of the image stacks in each time were used to quantify the number of dendritic spines in each time lapse.

Electrophysiology. Recordings were filtered at 2.0–3.0 kHz, sampled at 4.0 kHz using an A/D converter, and stored with pClamp 10 (Molecular Devices). Evoked postsynaptic responses were analyzed off-line, using pClampfit analysis software (Molecular Devices) that allowed visual detection of events, computing only events that exceeded an arbitrary threshold. For control of Wnt-5a effect, we used medium for L cells, without Wnt-5a, diluted 1:100.

Whole-Cell Patch Clamp. The hippocampal neurons were stabilized at 22 °C for 30 min before starting the experiments. Current changes in the neurons were detected using the whole-cell patch-clamp technique with an Axopatch-700B amplifier (Axon Instruments, Inc., Molecular Devices) and a Nikon Eclipse TE200-u inverted microscope. The membrane potential was adjusted to −60 mV, and the current was recorded at 50-μs intervals and filtered at 2 kHz using commercially available software (Axon Instruments, Inc.). The patch electrodes were made of borosilicate (World Precision Instruments) and were prepared using a vertical pipette puller (Narishige). The resistance of the electrodes when filled with normal internal solution was less than 5 MΩ.

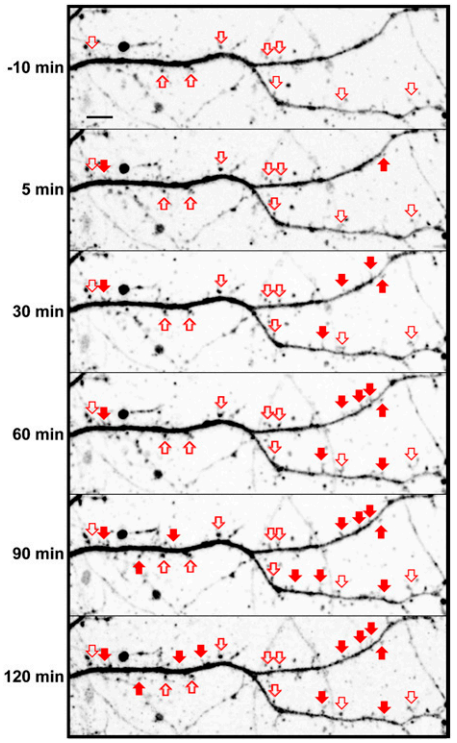


Fig. S1. Wnt-5a induces de novo formation of dendritic spines and modulates preexisting spine volume. Live-cell time-lapse imaging of an EGFP-transfected neuron dendrite shown before (−10 min) and after 5, 30, 60, 90, and 120 min of treatment with recombinant Wnt-5a (rWnt-5a). Filled arrows indicate de novo formation of dentritic spines. Empty arrows indicate modulation of preexisting spines volume. (Scale bar: 5 μm.)

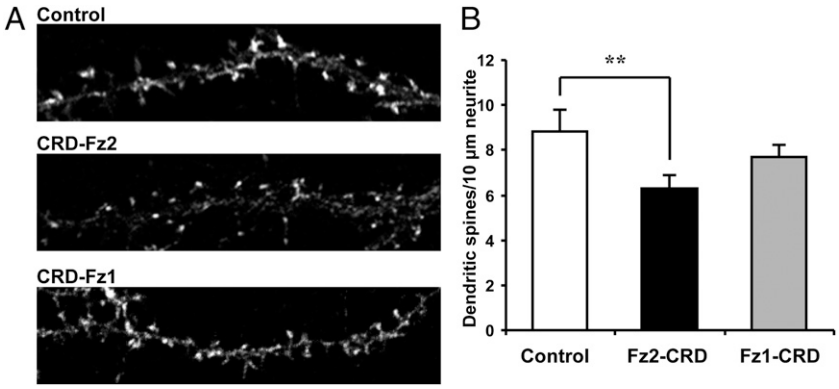


Fig. S2. Treatment with the soluble cysteine-rich domain (CRD) of Frizzled 2 (Fz2) decreases spine density. (*A*) Cultured hippocampal neurons at DIV 12 were incubated with 2 μg/mL Fz2-CRD or Fz1-CRD for 48 h. Dendritic spines were observed by immunodetection of drebrin (1), using a monoclonal anti-Drebrin antibody (Novus Biologicals). (*B*) Quantification of dendritic spine per 10-μm neurite length. Bars indicate mean ± SE from four different experiments. $^{**}P < 0.01$.

1. Takahashi H, et al. (2003) Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. *J Neurosci* 23:6586–6595.

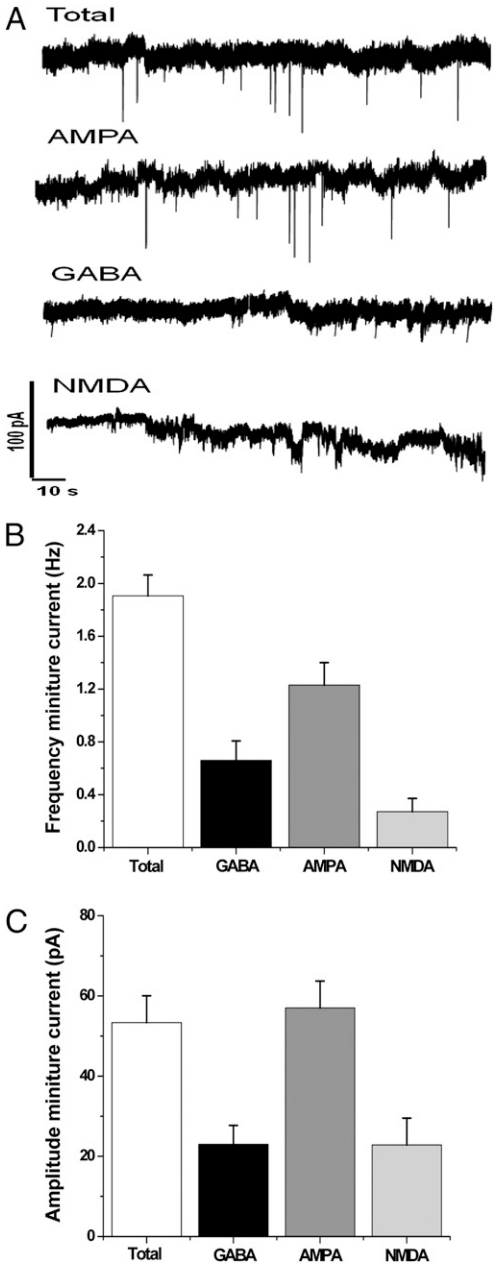
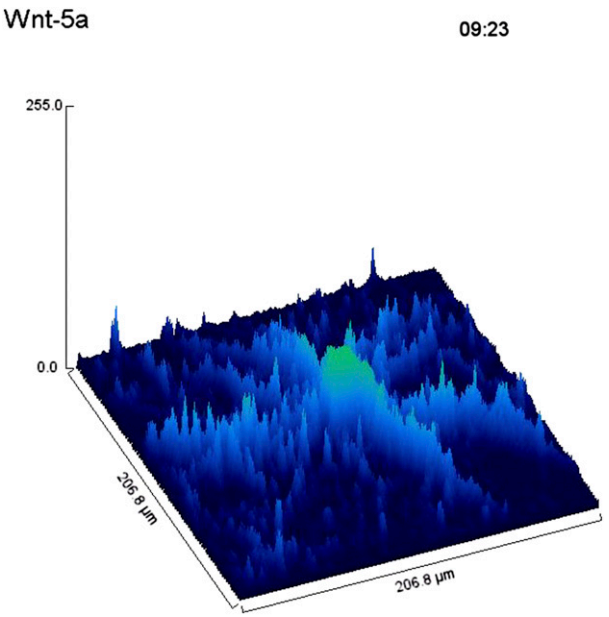
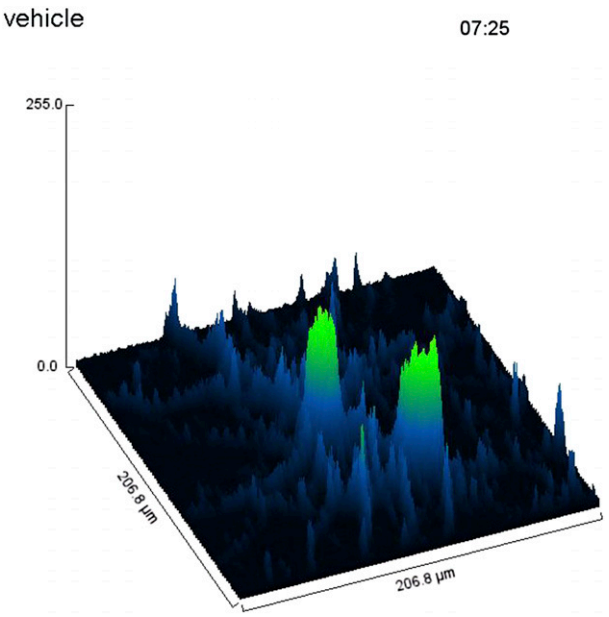


Fig. S3. Endogenous synaptic activity in hippocampus culture at DIV 10. The cultures were recorded in the presence of different drugs for isolated neurotransmission: TTX (100 nM), picrotoxin (PTX) (5 μM), and magnesium (2 mM) for AMPA neurotransmission; TTX (100 nM), PTX (5 μM), and 2,3-dihidroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) (1 μM) for NMDA neurotransmission. (*A*) Miniature current traces in control. (*B*) Plot of frequency miniature of every neurotransmission isolated. (*C*) Plot of amplitude miniature for each neurotransmitter receptor. Bars indicate mean ± SE from nine different cells.



Movie S1. In mature DIV 14 hippocampal neurons, treatment with recombinant Wnt-5a induced a rapid, transient increase in the intensity of the calcium indicator Fluo-3 contained in the dendritic compartments.

Movie S1



Movie S2. The maximal neurite intensity of the calcium-sensitive probe was significantly higher in Wnt-5a–treated neurons than in vehicle-treated neurons.

Movie S2