**Supplementary information**

**Title page**

# Wnt5a promotes hippocampal postsynaptic development and GluN2B-induced expression via the eIF2α HRI kinase

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**Abbreviated title:** Wnt5a modulates PSDs through HRI kinase

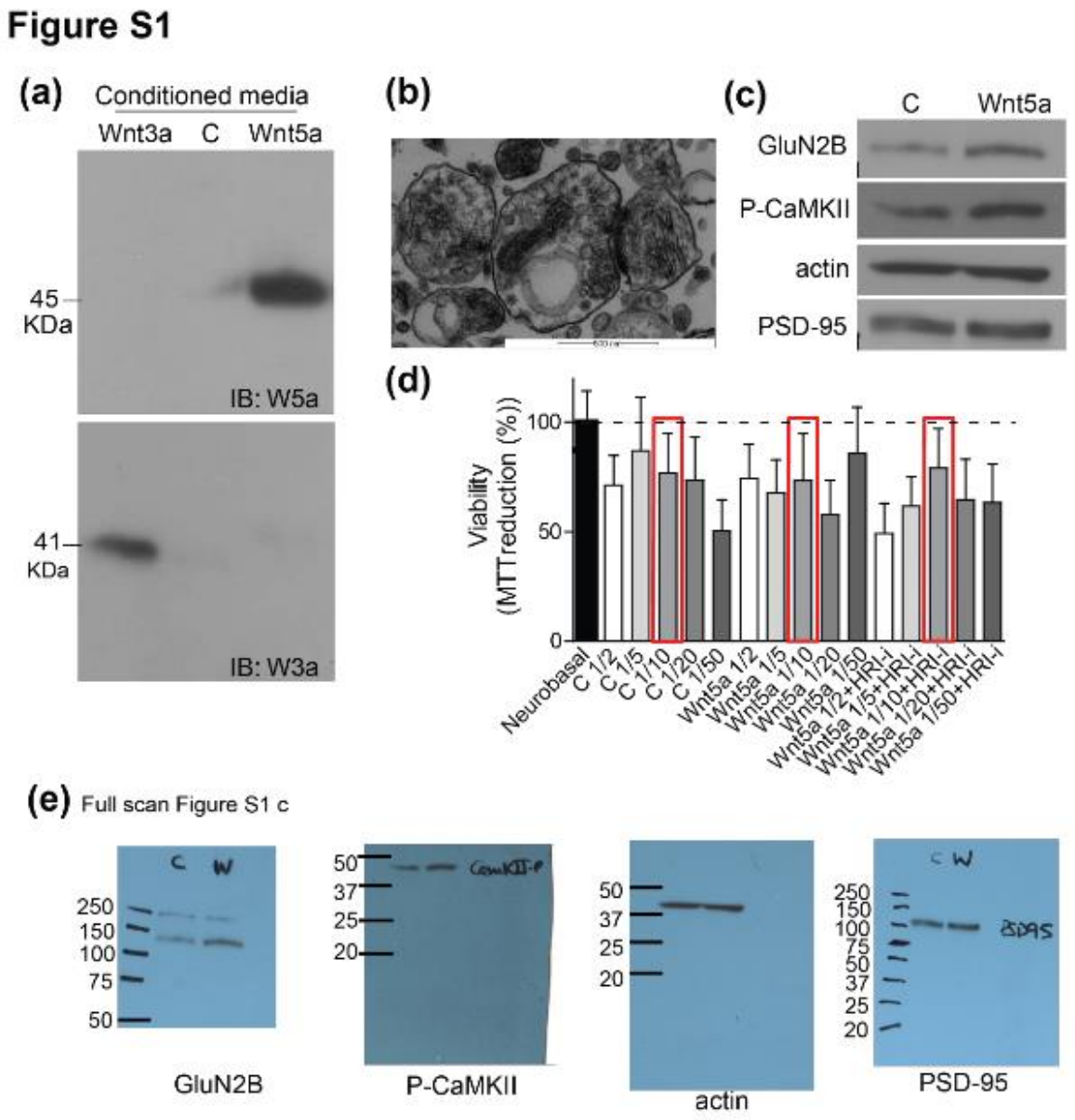


***Figure S1. Reliability of conditioned media and synaptosomes*** *a) Representative western blot of control, Wnt5a-containing and Wnt3a-containing conditioned media. b) Representative transmission EM image of synaptosome isolation. c) Representative western blot of hippocampal neurons treated with Wnt5a and the signaling activation (p-CaMKII). d) Viability assay (MTT reduction %) of hippocampal neurons treated with conditioned media and HRI-i. N=6 independent experiments. e) Full-length blots of Figure S1c.*

## Figure S2

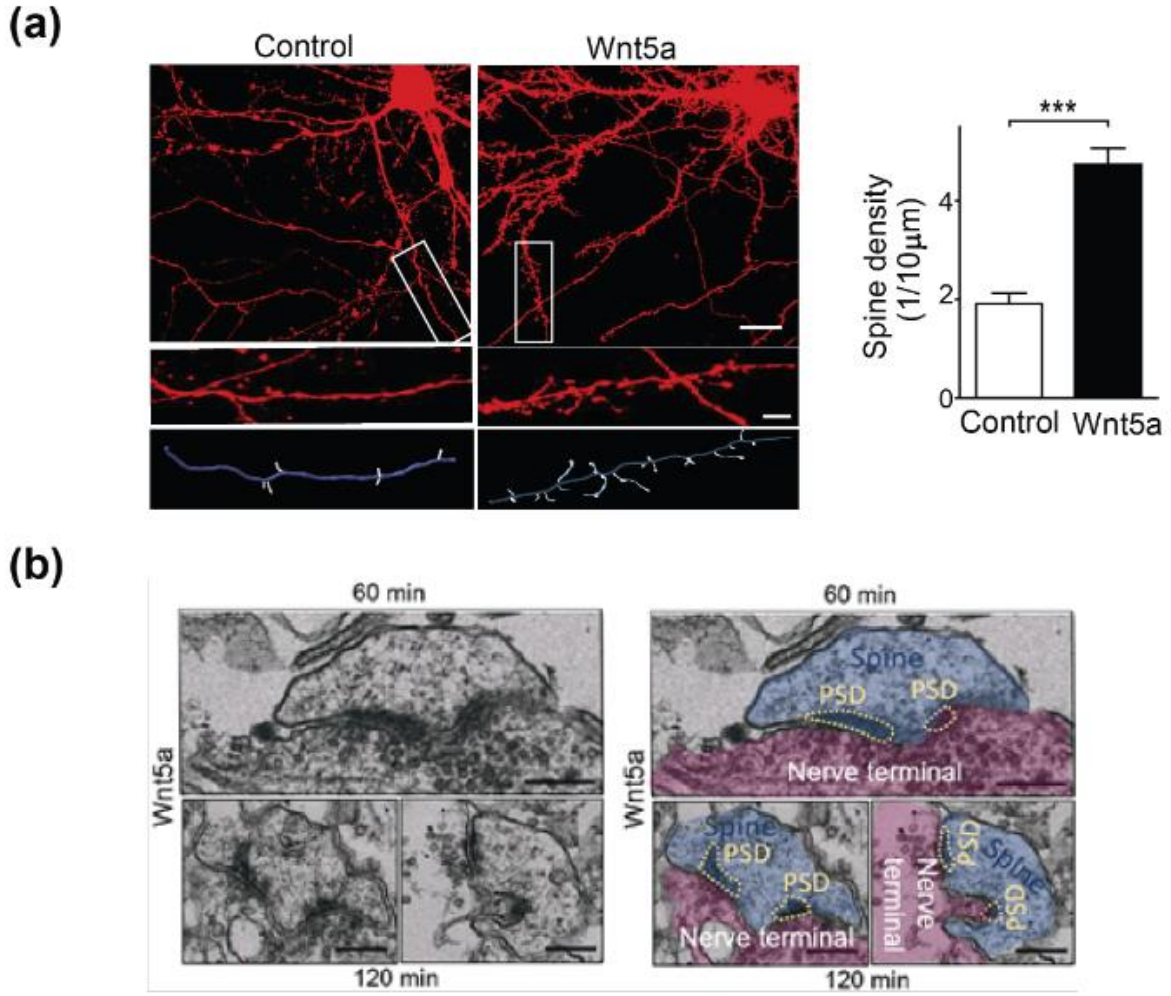


***Figure S2. Wnt5a increases spine density in DIV10 neurons.*** *Representative images of 3D reconstructed dendrites and spines (left panel) from neurons transfected with EGFP (right panel, neurites shown in red) at DIV7 and treated at DIV10 with control and Wnt5a conditioned media. Scale bar: 3 µm. N=5 independent experiments; Control (82); Wnt5a (129) dendrites. ***: p= 0.00009 b) Colored EM images in Figure 2a showing Postsynaptic ending (Spine, in blue), Nerve terminal (pink) and PSD region (yellow).*

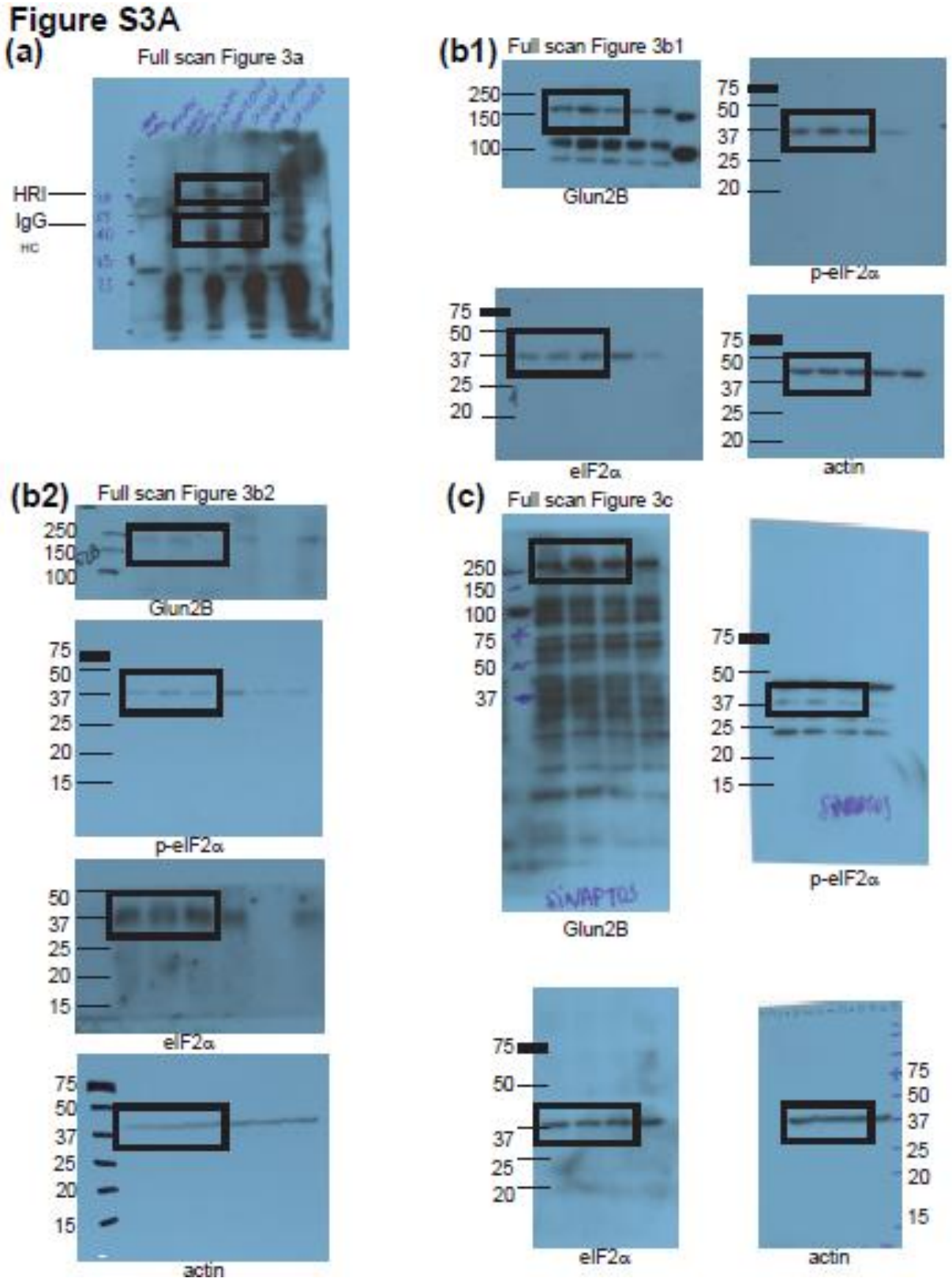


***Figure S3A. Full-length gels of Figure 3***

**Figure S3B**

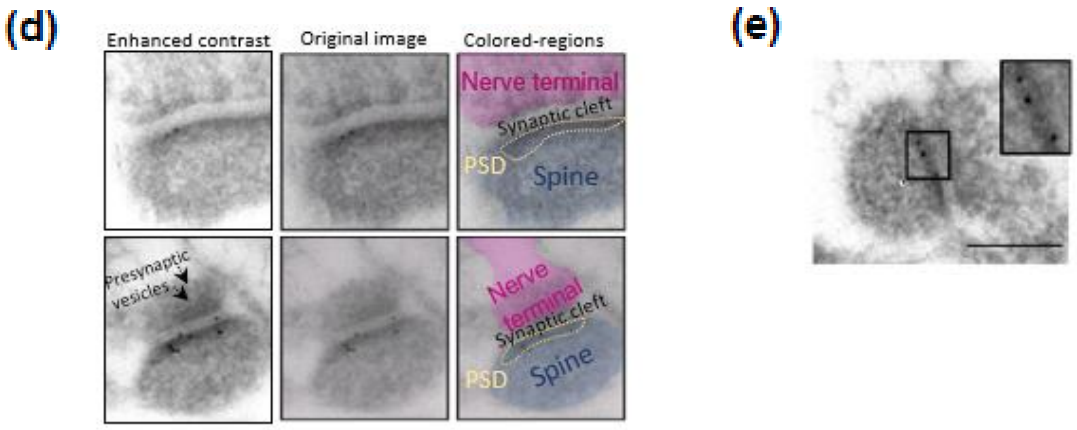


***Figure S3B. Immunogold images.*** *(d) EM images of Figure 3 d with enhanced contrast and colored regions. Nerve terminal (pink), synaptic cleft, presynaptic vesicles, Spine (blue) and PSD region (yellow) are highlighted. (e) Immunogold image showing PSD-95 (larger diameter gold particle, in the middle) and GluN2B (two gold particles at the extremes of PSD).*

## Figure S4

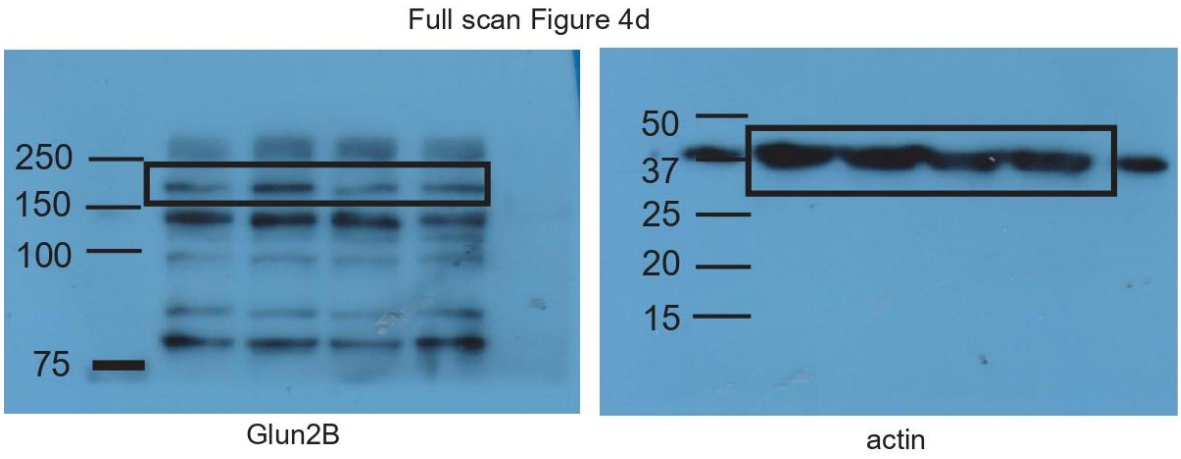


***Figure S4. Full-length blots of Figure 4. The protein ladder was loaded into the extreme wells of the blot.***

## Figure S5

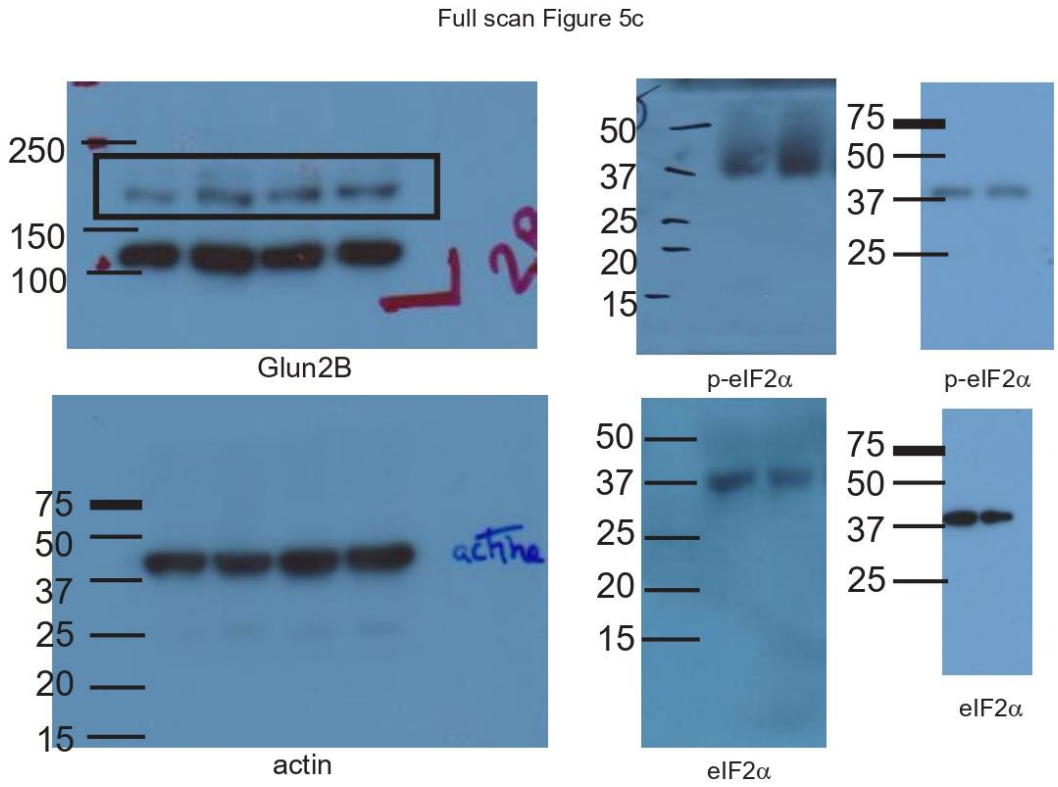


***Figure S5. Full-length blots of Figure 5***

## Figure S6

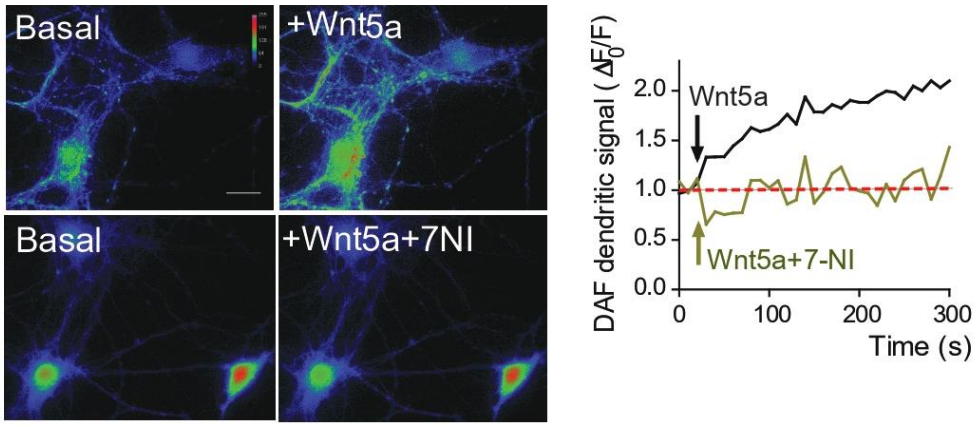


***Figure S6. Wnt5a induces the production of NO.*** *a) Representative images and graph showing dendritic DAF fluorescent signal before and after Wnt5a application. Hippocampal neurons were incubated with the NO molecular probe DAF-FM. An immediate increase in DAF fluorescence was observed after Wnt5a application (right and left panels). This effect was severely prevented by the addition of 7-NI, a specific inhibitor of NO synthase (NOS). N=13-9 (dendrites). Scale bar: 10 µm.*

**Figure S7**

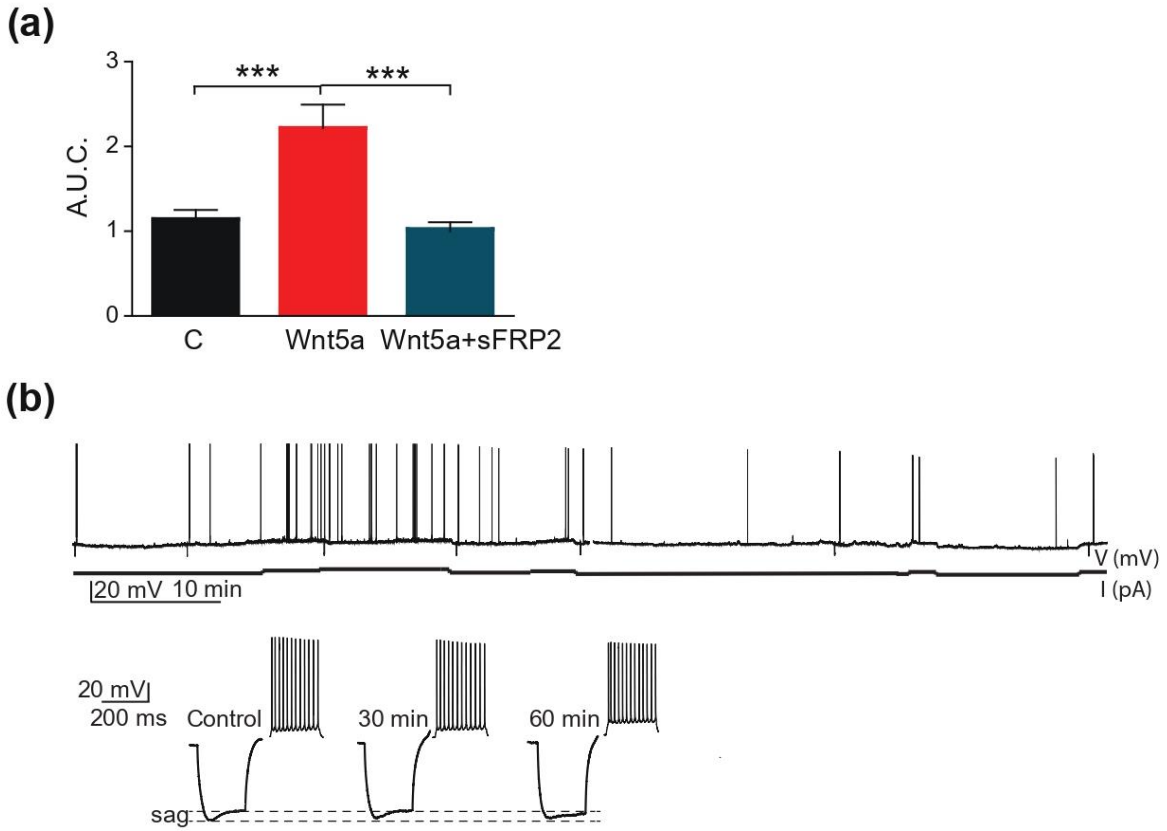


***Figure S7. Calcium experiments of hippocampal neurons co incubated with sFRP2 and electrophysiological recordings in current clamp to demonstrate stability of long-term recordings.*** *a) Graphs depict the normalized AUC of the Fura-2 AM fluorescence ratio (340/380) after NMDA +glycine exposure in hippocampal neurons treated for 1 h with control (C) or Wnt5a (W5a) conditioned medium and/or sFRP2. N=5 independent experiments. C (100), Wnt5a (110), Wnt5a+sFRP2 (111) (***: p= 0.0001). b) Current-clamp recording of a CA1 pyramidal cell, showing the membrane potentials with spontaneous APs generated when purposely apply current through the circuit. Small deflections toward hyperpolarization indicate negative current pulses to determine $R_{in}$, which also evidence the sag component, a characteristic of h-current present in hippocampal pyramidal cells. The presence of sag as well as the APs amplitude and number after 1 h of recording, are an indication of healthy cell.*

## Figure S8

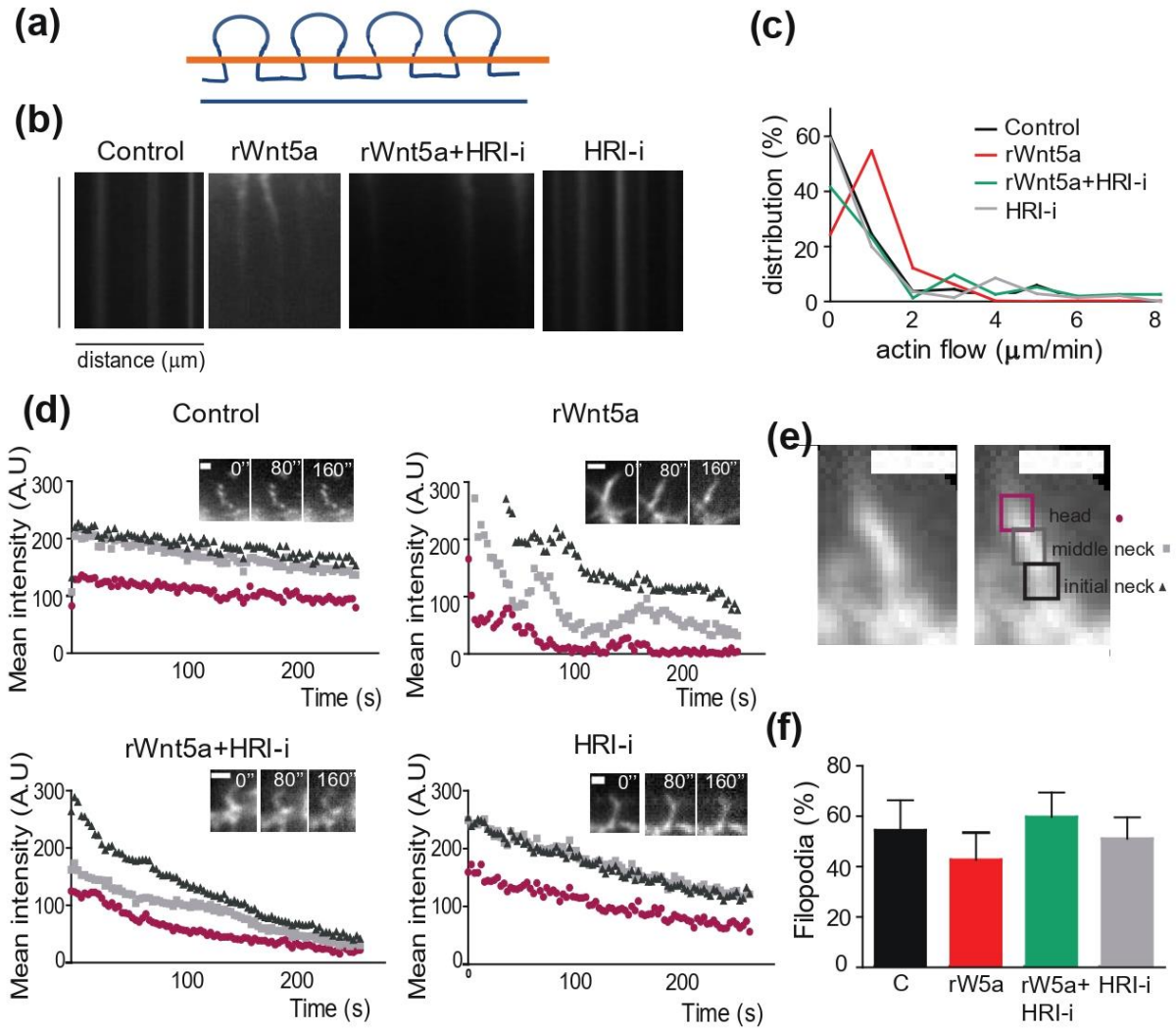


***Figure S8. EGFP-LifeAct dynamics.*** *Kymographs were performed from at least 5 different ROIS (scheme included in (a)) from each video (b). (c) Histogram showing the % distribution of LifeAct-GFP positive protrusions vs the actin flow (µm/min). n=7 (control), n=12 (rWnt5a), n=7 (rWnt5a+HRI-i), n=5 (HRI-i) number of cells from 3 independent experiments. (d) Fluorescence intensity vs time graphs of each condition and insets showing representative protrusions. Scale bar: 1 µm (e) Scheme showing the regions selected for analysis of the distribution of fluorescence (data in d). Scale bar: 1 µm (f) Graph showing the % of filopodia in each condition. N=6 (control), N=8 (rWnt5a), N=6 (rWnt5a+HRI-i), N=6 (HRI-i) number of cells from 3 independent experiments.*

**Supplementary videos legend**

***Video 1 (control), 2 (rWnt5a), 3 (rWnt5a+HRI-i) and 4 (HRI-i).*** *Movies of EGFP-LifeAct-transfected hippocampal neurons recorded each 3 sec during 1:30 min after 1 h with different treatments.*