

SUPPLEMENTAL FIGURES FOR

FGF13 is not secreted from neurons

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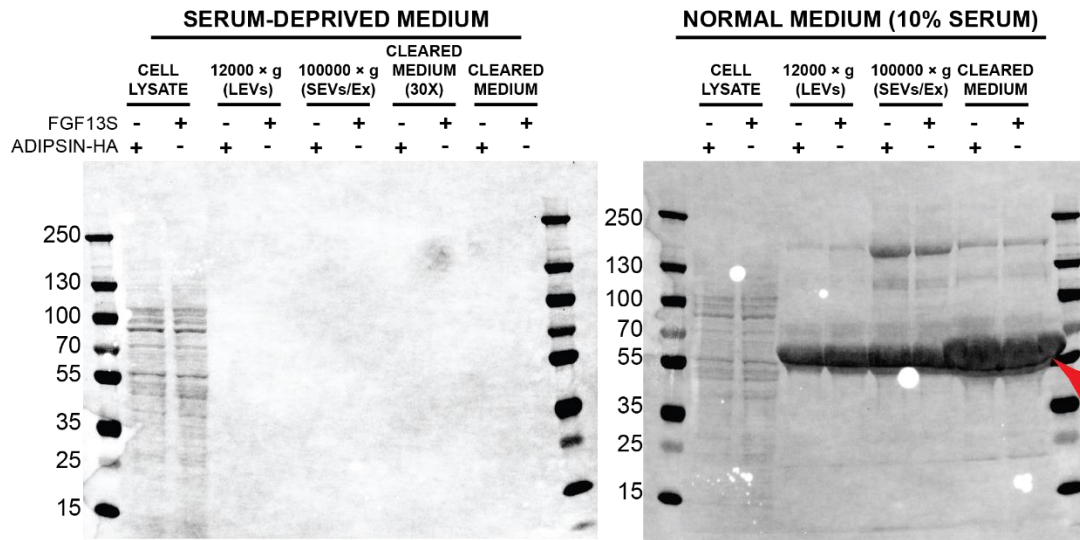
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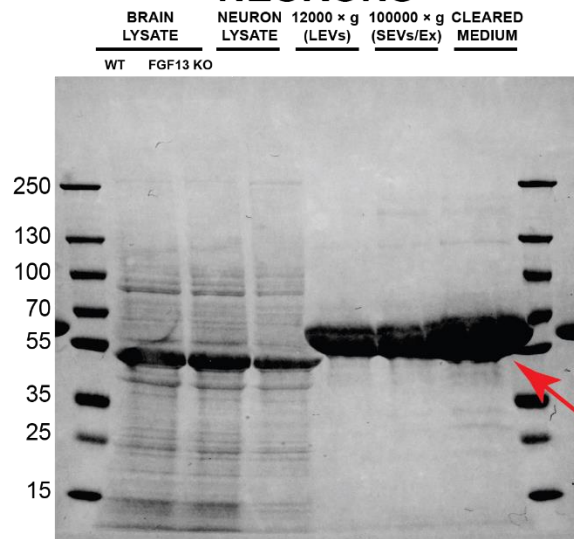
A

HEK293 cells



B

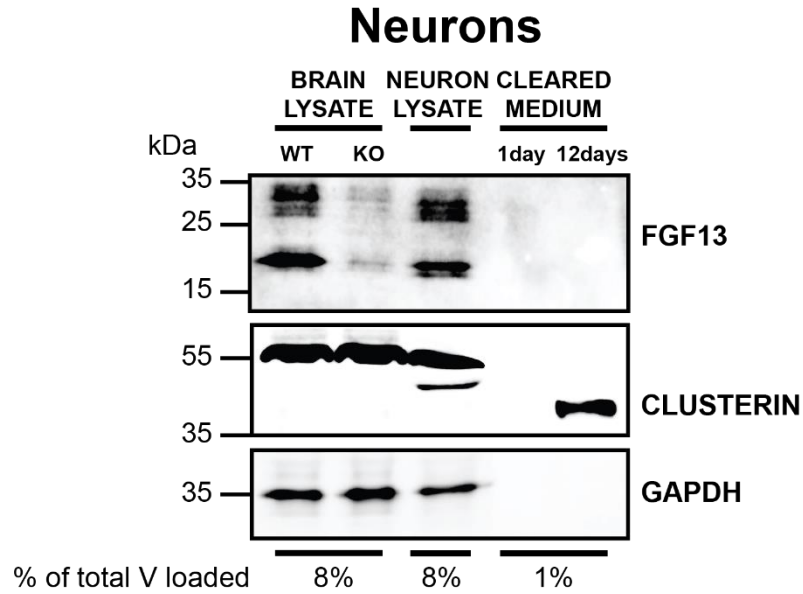
NEURONS



Supplementary Figure 1: Gel artifacts caused by serum and medium supplements.

Ponceau-S staining of western blot membranes showing medium clearing experiments from HEK293 (**A**) and cultured mouse hippocampal neurons (**B**) of the blots shown in Fig. 1. In the presence of 10% serum (normal HEK293 culture conditions, **A**, right) and 1% serum and 2% B27 supplement (normal neuronal culture conditions, **B**) the high amounts of albumin and other supplements cause gel artifacts (red arrows) even without medium concentration. This precludes media concentration in comparison to serum-deprived conditions (**A**, left) where media is concentrated 30×.

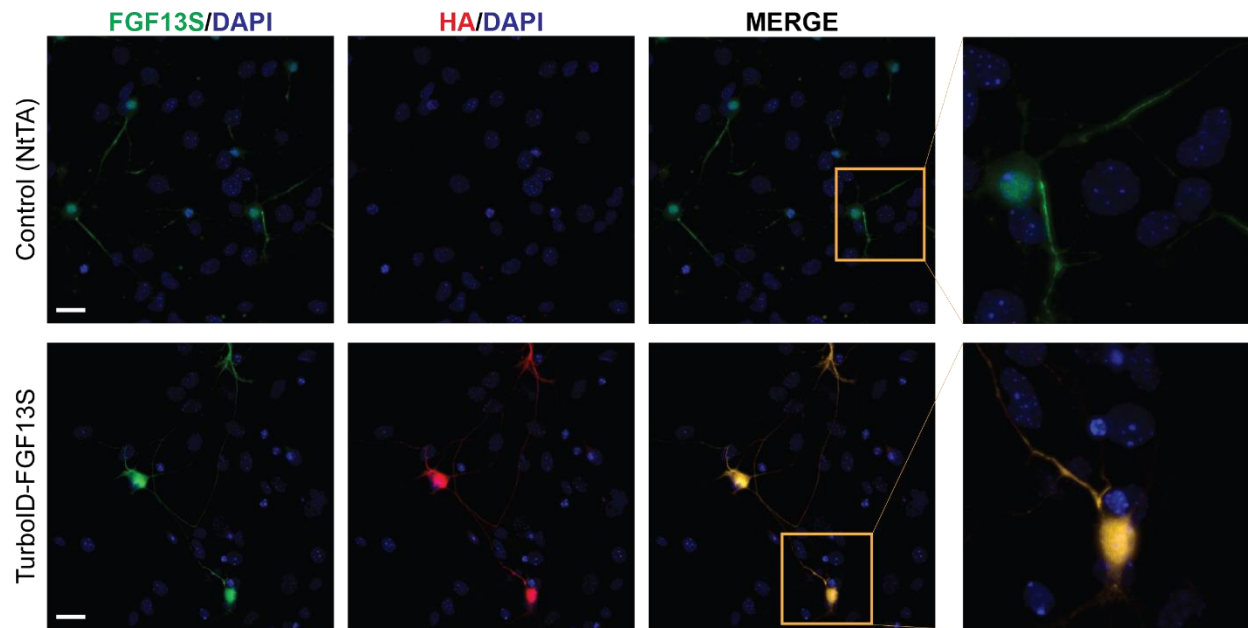
A



Supplementary Figure 2. Time-dependent secretion of clusterin in cultured neurons.

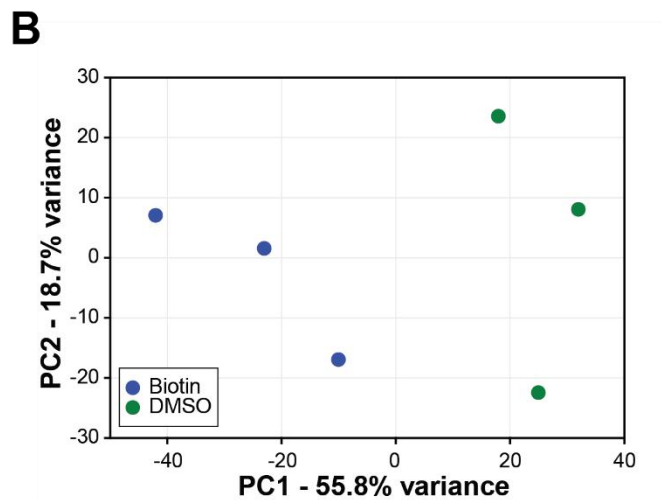
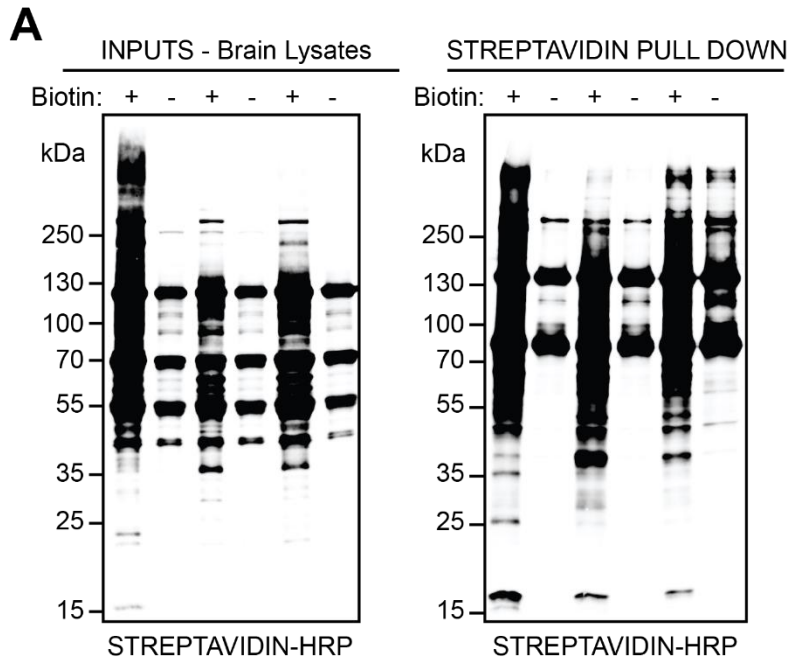
Western blot of cell lysates and extracellular medium derived from cultured mouse hippocampal neurons. Medium was either left untouched for 12 days or changed the day before (11th day in culture). Whole brain lysates from wild-type (WT) and *Fgf13* brain knockout (KO) mice were used to validate the detection of FGF13 isoforms. GAPDH (36 kDa) is used as intracellular marker. Clusterin is used as a positive control for secretion in neurons, and it is only detected in cells cultured for 12 days but not 1 day, consistent with its time-dependent processing and secretion. 15 µg of brain or neuronal lysate were loaded. % of total V loaded indicates the fraction of the sample loaded on the gel relative to the total volume, allowing comparison among lanes.

A



Supplementary Figure 3. Cellular localization of the TurbolD-FGF13S transgene in mouse neurons.

A. Representative images from immunofluorescence staining of cultured hippocampal neurons isolated from control mice (NtTA background, top) and mice expressing the TurbolD-FGF13S in the brain (bottom). Green: FGF13S; Red: Hemagglutinin (HA) tag. Blue: DAPI. Scale bars: 30 μm .

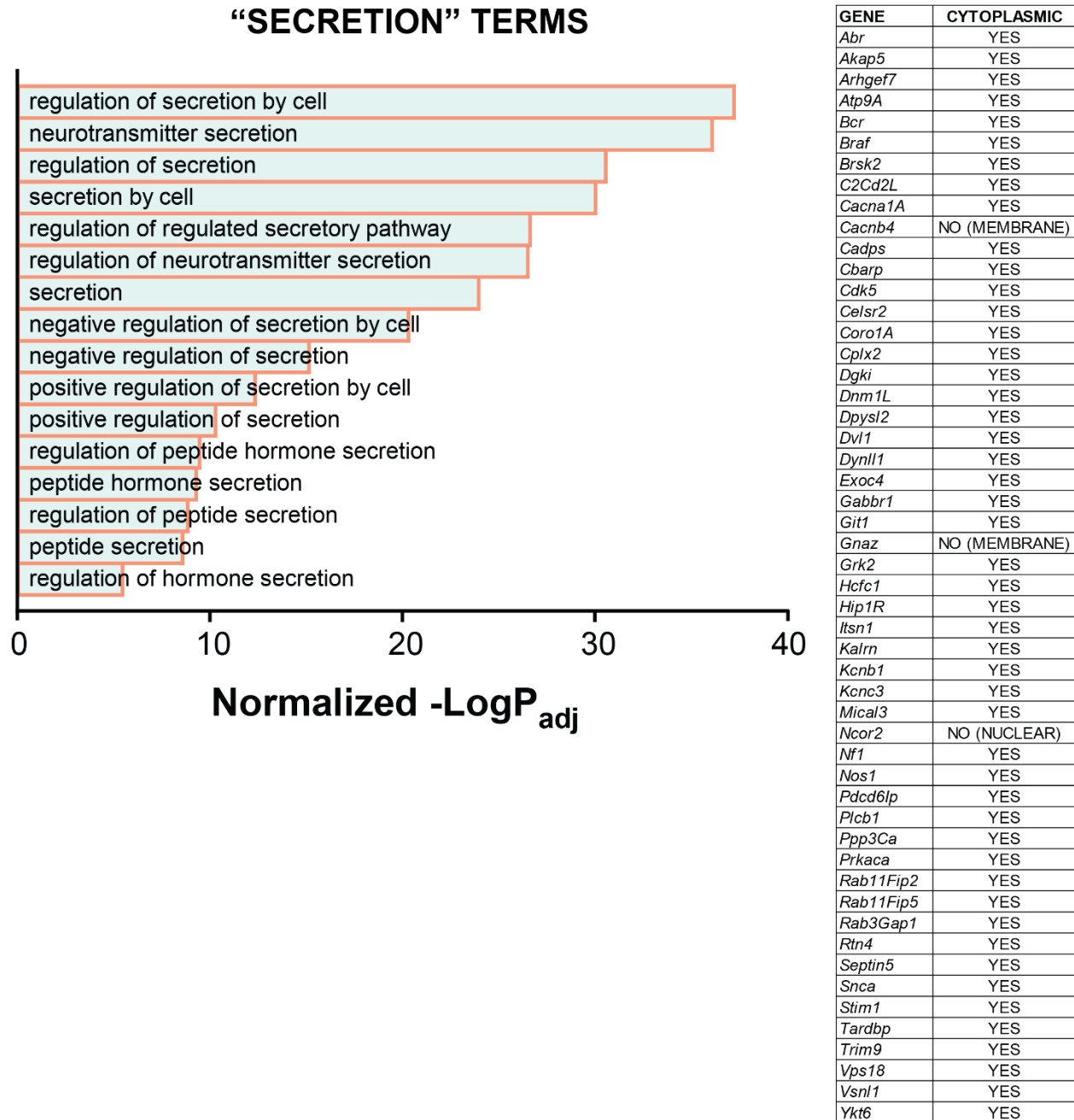


Supplementary Figure 4. *In vivo* labeling efficiency of TurboID-FGF13S in mouse brain.

A. Streptavidin-HRP blots of brain lysates and corresponding streptavidin pull-down samples from DMSO- or biotin-injected TurboID-FGF13S mice, showing robust biotin labeling. The same samples were then processed and analyzed by mass spectrometry.

B. Principal Component Analysis (PCA) plot from mass spectrometry analysis of samples shown in **A**.

A

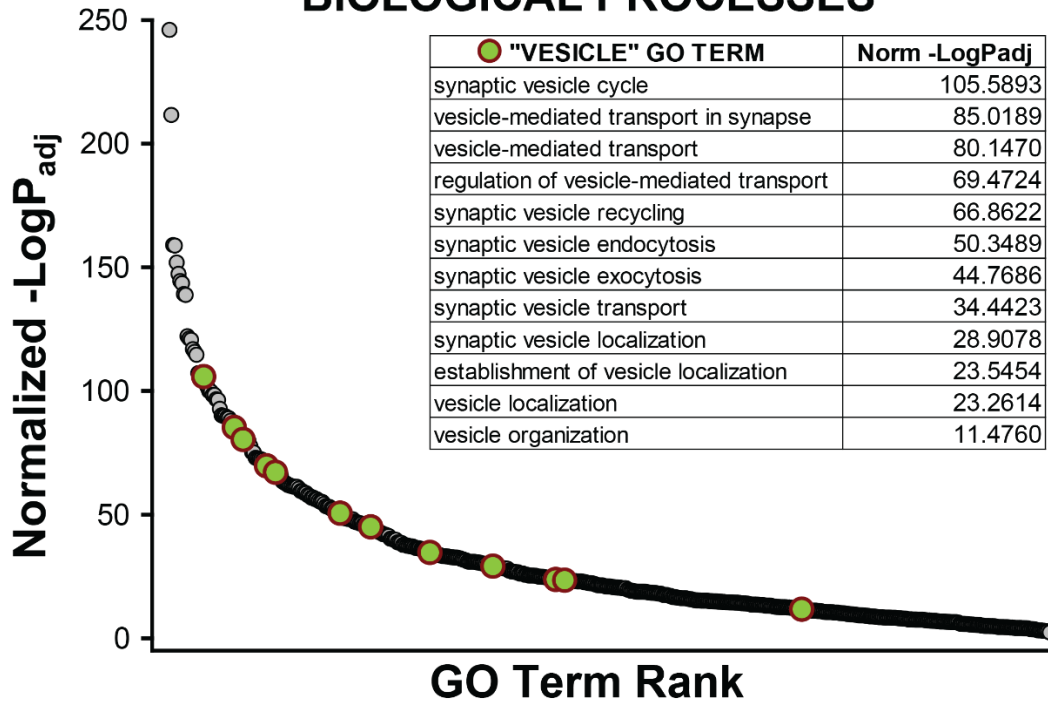


Supplementary Figure 5. GO term analysis of “secretion” terms.

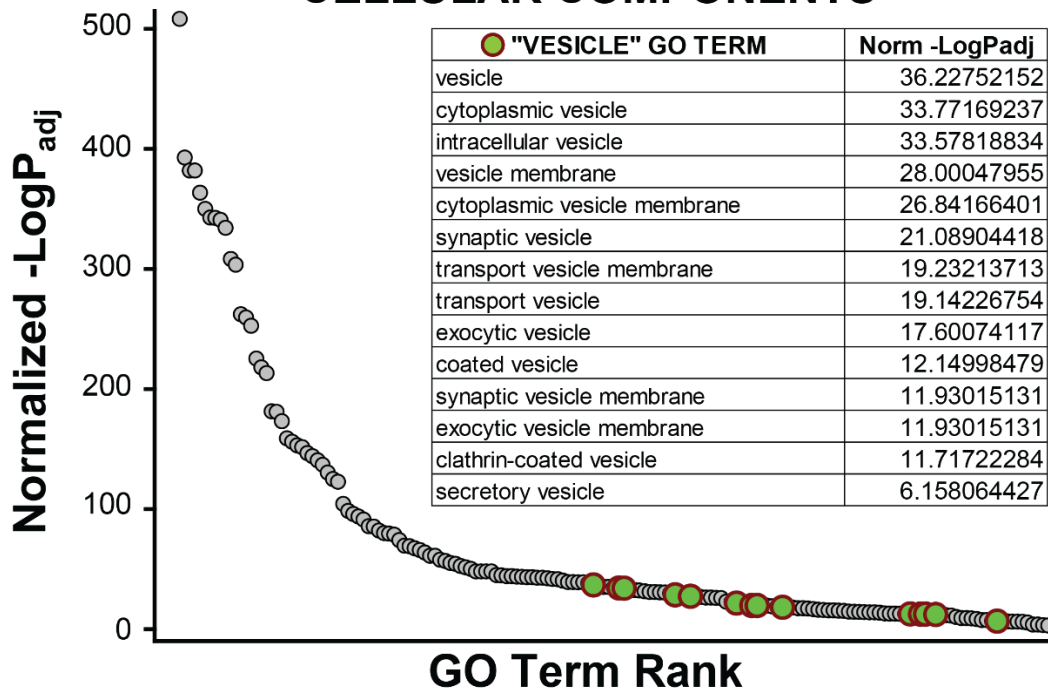
A. The full list of GO terms with “secretion” present in their identifiers. Right table shows cytoplasmic localization of all but one protein (NCOR2). *p*-value normalization details are provided in the *Methods* section.

A

BIOLOGICAL PROCESSES



CELLULAR COMPONENTS



Supplementary Figure 6. GO term analysis of “vesicle” terms.

Biological Processes (**A**) and Cellular Components (**B**) GO terms (grey). Terms with “vesicles” in their identifiers are highlighted in green, and the full list is shown in the tables. The GO term rank is defined by the normalized $-\text{LogP}_{\text{adj}}$ (descending). p -value normalization details are provided in the *Methods* section.