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Supplemental information

SYNGAP1 deficiency disrupts synaptic neoteny in xenotransplanted human cortical neurons *in vivo*

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Figure S1 related to Figure 1

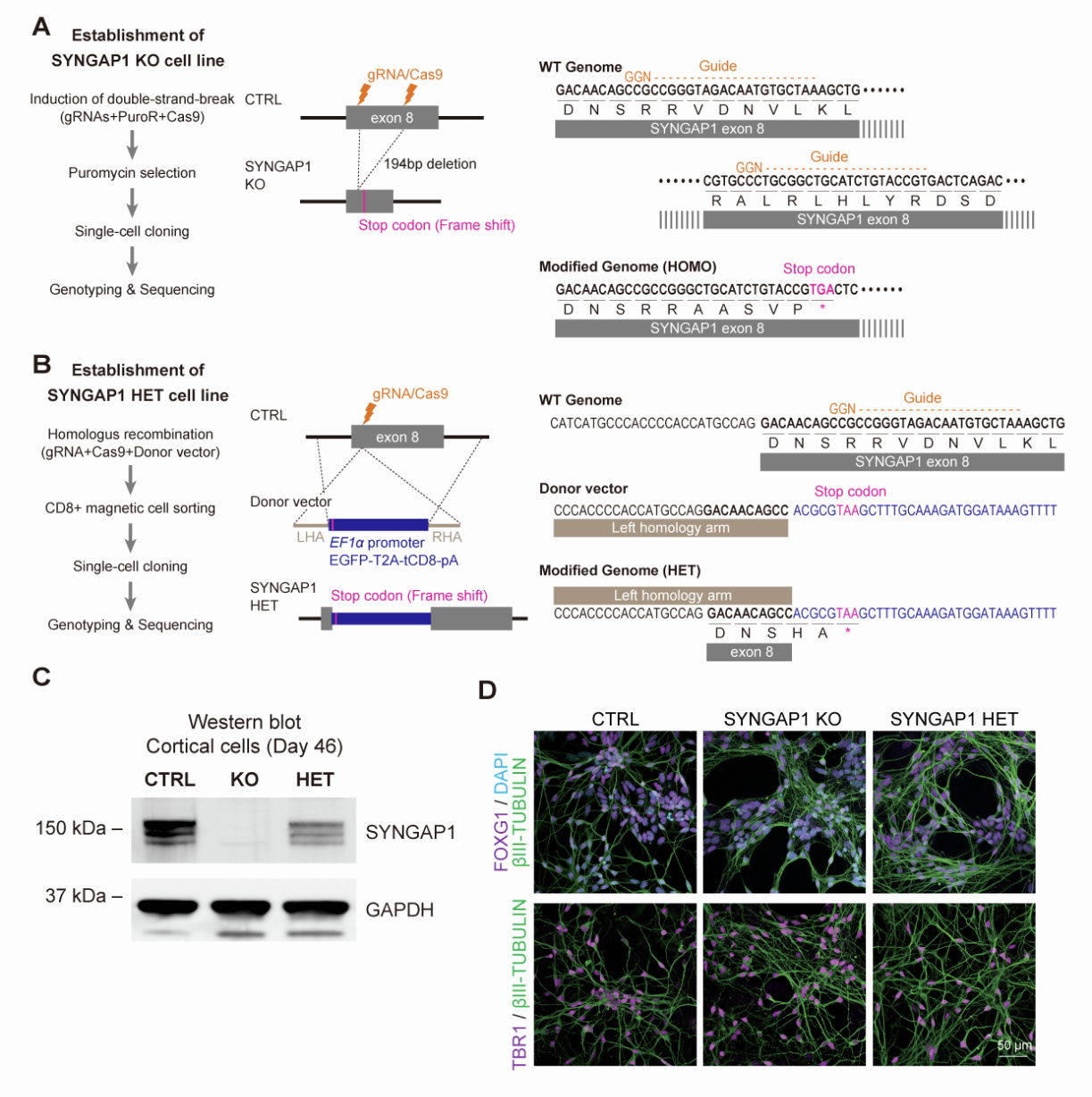

Figure S1. Establishment of SYNGAP1 mutant cell lines.

(A) The scheme of SYNGAP1 KO cell line establishment. Two gRNAs, Cas9 gene and Puromycin resistance (PuroR) gene expressing plasmids were transfected by nucleofection into human PSCs. The cells were selected by Puromycin treatment and

used for single-cell cloning. The 194-bp deletion in exon8 induced a frameshift. *Right*: target sites of gRNAs.

(B) The scheme of SYNGAP1 HET cell line establishment. Single gRNA, Cas9 protein and donor vector were transfected by nucleofection into human PSCs. The cells were used for CD8 positive magnetic sorting. The donor vector sequence in exon 8 induced a frameshift. *Right*: target site of gRNA.

(C) Representative image of Western blot for SYNGAP1 protein from whole cell protein extracts of human PSC-derived cortical cells at day 46 in vitro.

(D) Representative images of cortical marker positive cortical cells at day 32 in vitro. (Top) Telencephalon-specific marker FOXG1. (Bottom) Deep-layer marker TBR1. Scale bar: 50 μm.

Figure S2 related to Figure 1

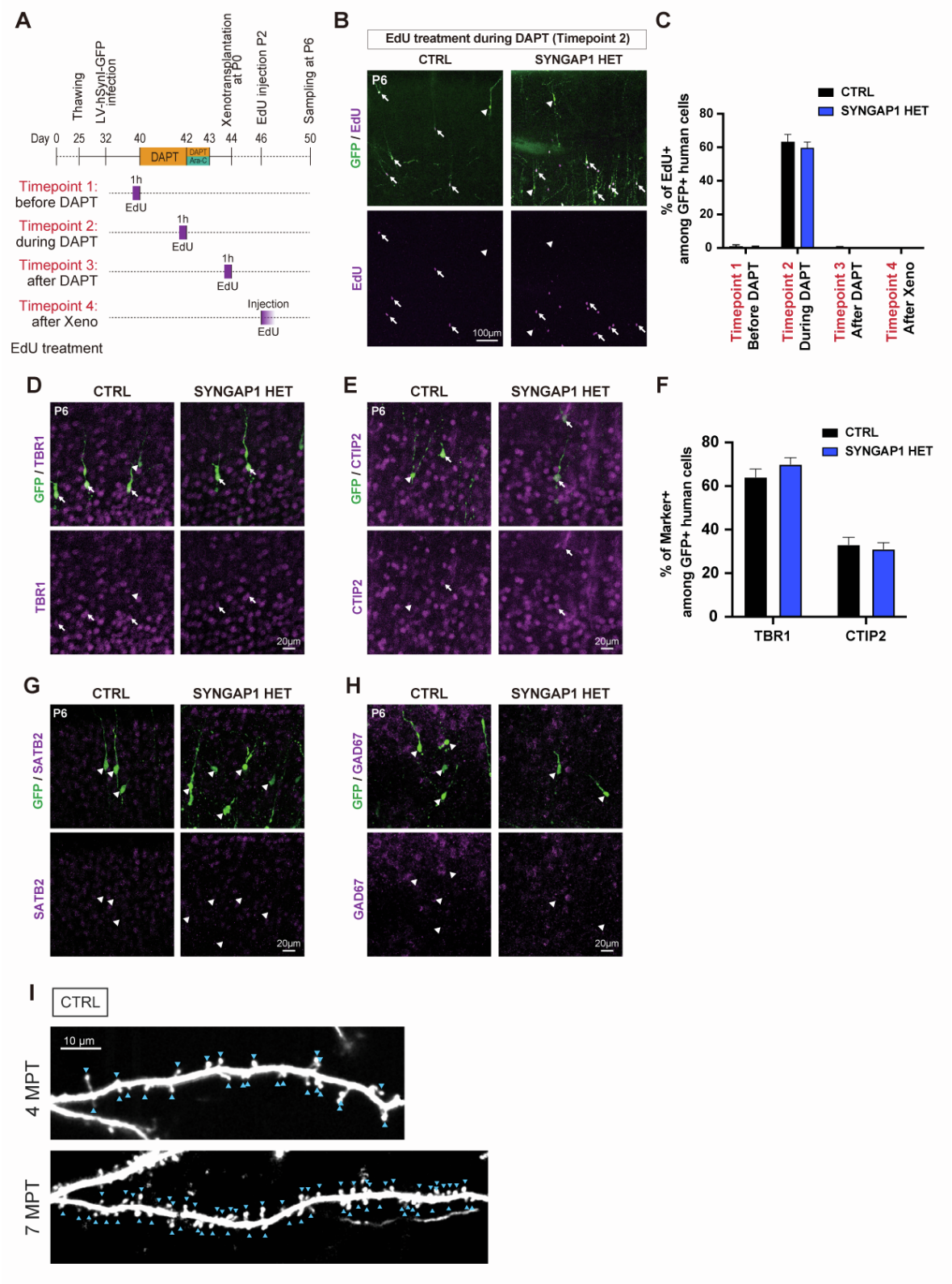

Figure S2: Characterization of transplanted CTRL and SYNGAP1 mutant cells

(A-C) Overview of EdU birth-dating experiment. (**A**) Timeline and (**B**) representative images of EdU-treated human neurons in mouse brain at P6. (**C**) Quantification (Right) of EdU+ cells among GFP+ xenotransplanted human neurons. Arrow: EdU positive cell, Arrow head: EdU negative cell. N=3 mice for each group. Data are shown as mean ± SEM. (**D-H**) Overview of fate marker expression of transplanted neurons. (**D-E**) Representative images (**D**: TBR1, **C**: CTIP2) of marker+ human neurons in mouse brain at P6. (**F**) Quantification of marker+ cells among GFP+ xenotransplanted human neurons. Arrow: marker positive cell, Arrow head: marker negative cell. N=4 mice for each group. Data are shown as mean ± SEM. (**G**) Representative images of human neurons in mouse brain at P6. Arrowhead: SATB2 negative cell. (**H**) Representative images of human neurons in mouse brain at P6. Arrowhead: GAD67 negative cell. **(I)** Example of quantification of dendritic spine density from data obtained using *in vivo* two-photon imaging. Two branches taken from SYNGAP1 CTRL neurons at 4 and 7 MPT are shown with blue arrow heads indicating structures that we identified as dendritic spines.

Figure S3 related to Figure 2

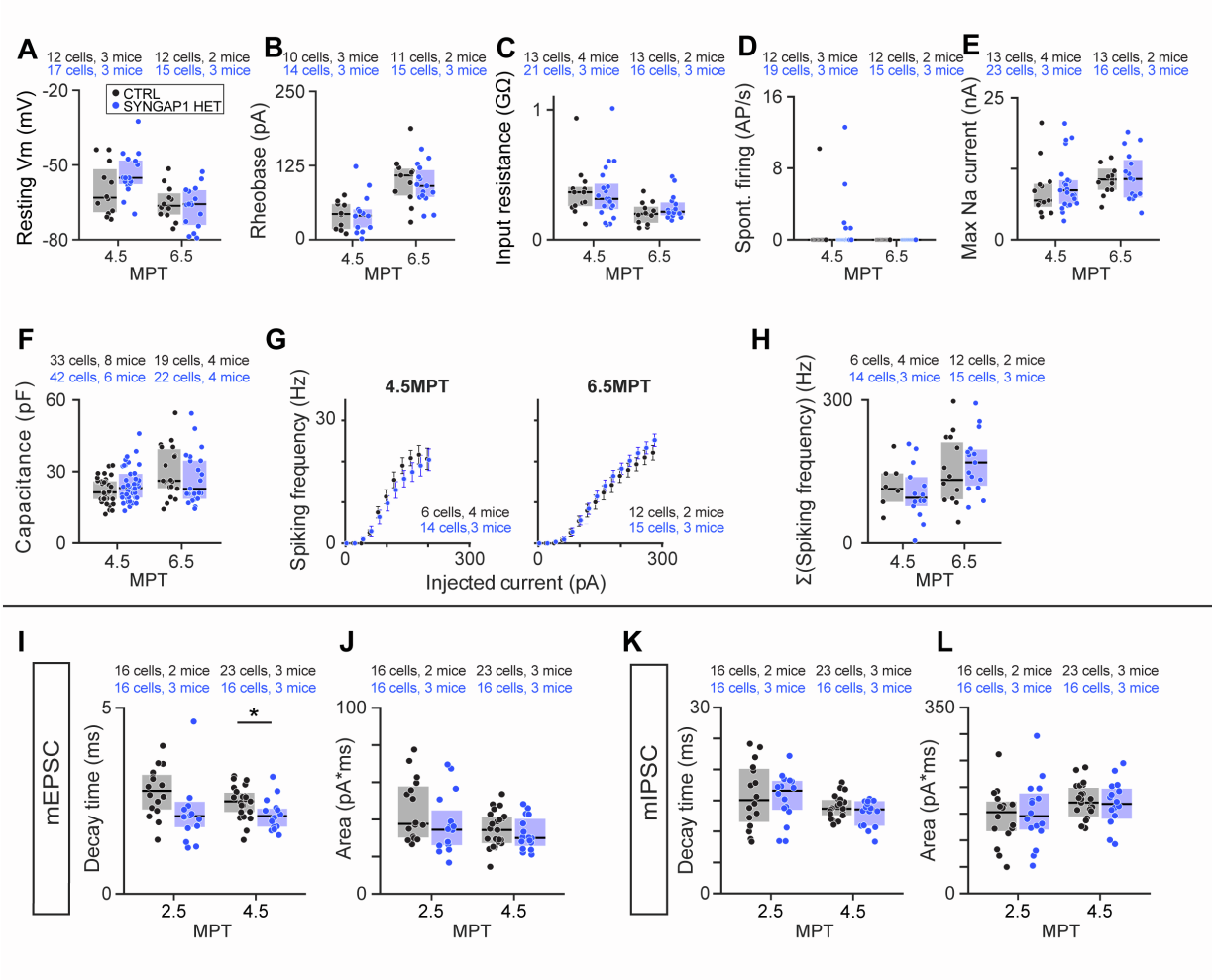

Figure S3. *Ex vivo* patch-clamp recordings of SYNGAP1 HET neurons.

(A-H) Cell intrinsic properties were compared between SYNGAP1 CTRL (black) and HET (blue). (A) Resting membrane potential. Medians per genotype/timepoint: 4.5 MPT: -63.15; -55.20, 6.5 MPT: -66.40; -65.70. (B) Rheobase. Medians per genotype/timepoint: 4.5 MPT: 43.17; 40.38, 6.5 MPT: 108.30; 90.58. (C) Input resistance. Medians per genotype/timepoint: 4.5 MPT: 0.36; 0.31, 6.5 MPT: 0.20; 0.21. (D) Spontaneous firing rate. Medians per genotype/timepoint: 4.5 MPT: 0.0; 0.0, 6.5 MPT: 0.0; 0.0. (E) Max sodium current. Medians per genotype/timepoint: 4.5 MPT: 6.9; 8.7, 6.5 MPT: 10.6; 10.7. (F) Capacitance. Medians per genotype/timepoint: 4.5 MPT: 21.2; 23.1, 6.5 MPT: 26.1;

22.7. (G) f/I curves. (H) Summed spiking frequency, as in shown (G). Medians per genotype/timepoint: 4.5 MPT: 113.5; 94.5, 6.5 MPT: 132.5; 169.0. **(I-L)** Additional properties from mEPSC and mIPSC experiment. (I) mEPSC decay time. Medians per genotype/timepoint: 4.5 MPT: 2.77; 2.09, 6.5 MPT: 2.49; 2.09. (J) mEPSC Area. Medians per genotype/timepoint: 4.5 MPT: 37.6; 34.5, 6.5 MPT: 34.4; 30.1. (K) mIPSC decay time. Medians per genotype/timepoint: 4.5 MPT: 15.1; 16.6, 6.5 MPT: 13.7; 13.6. (L) mIPSC Area. Medians per genotype/timepoint: 4.5 MPT: 37.6; 34.5, 6.5 MPT: 34.4; 30.1.

Figure S4 related to Figure 2.

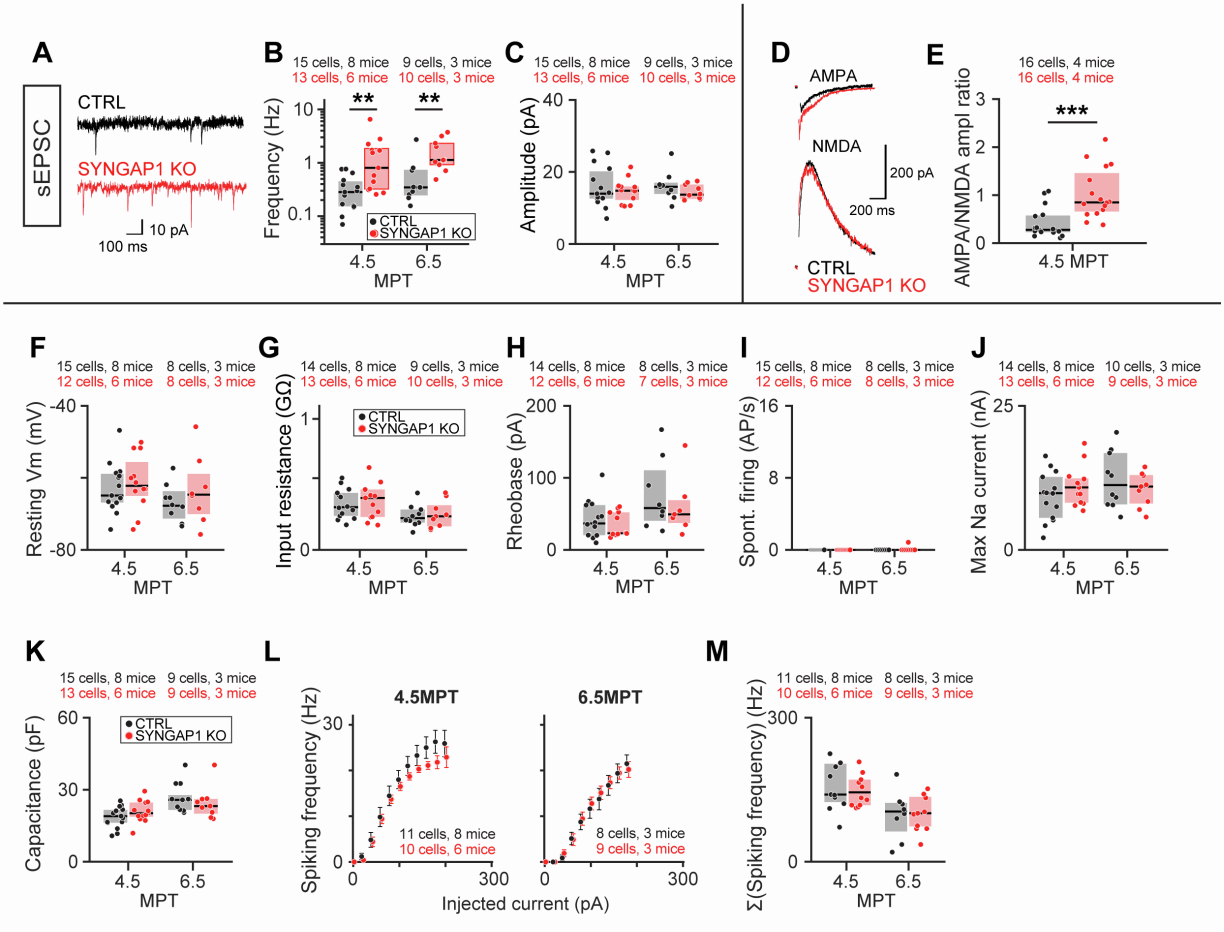

Figure S4: *Ex vivo* patch-clamp recordings of SYNGAP1 KO neurons.

(A) Recording traces of SYNGAP1 CTRL and KO neurons showing example sEPSCs. Note more and larger inflections in the red (KO) trace. **(B-C)** Quantification of synaptic properties across time: (B) mEPSC frequency at 4.5 and 6.5 MPT. Medians per genotype/timepoint: 4.5 MPT: 0.28; 0.80, 6.5 MPT: 0.35; 1.13. (C) sEPSC amplitude at 4.5 and 6.5 MPT. Medians per genotype/timepoint: 4.5 MPT: 14.0; 14.80, 6.5 MPT: 15.9; 13.7. (**D**) Example AMPA (top) and NMDA (bottom) traces for both CTRL (black) and KO (red) genotypes. AMPA currents are increased in KO (red) neurons. **(E)** AMPA/NMDA ratio at 4.5 MPT. Medians per genotype: 0.28; 0.85. Boxes indicate median and interquartile range. Statistical comparison was done using rank sum tests.

(F-M) Additional cell intrinsic properties were compared between SYNGAP1 CTRL and KO (red). (F) Resting membrane potential. Medians per genotype/timepoint: 4.5 MPT: -64.9; -62.2, 6.5 MPT: -67.75; -64.65. (G) Input resistance. Medians per genotype/timepoint: 4.5 MPT: 0.33; 0.40, 6.5 MPT: 0.24; 0.26. (H) Rheobase. Medians per genotype/timepoint: 4.5 MPT: 36.7; 23.0, 6.5 MPT: 58.1; 49.4. (I) Spontaneous firing rate. Medians per genotype/timepoint: 4.5 MPT: 0.0; 0.0, 6.5 MPT: 0.0; 0.0. (J) Max Sodium current. Medians per genotype/timepoint: 4.5 MPT: 9.9; 10.8, 6.5 MPT: 11.2; 11.0. (K) Capacitance. Medians per genotype/timepoint: 4.5 MPT: 19.0; 20.2, 6.5 MPT: 25.8; 23.2. (L) f/I curves. (M) Summed spiking frequency, each dot represents the spikes rates summed over the entire f/I curves shown in (L). Medians per genotype/timepoint: 4.5 MPT: 140.0; 144.5, 6.5 MPT: 104.5; 101.0.

Figure S5 related to Figure 3.

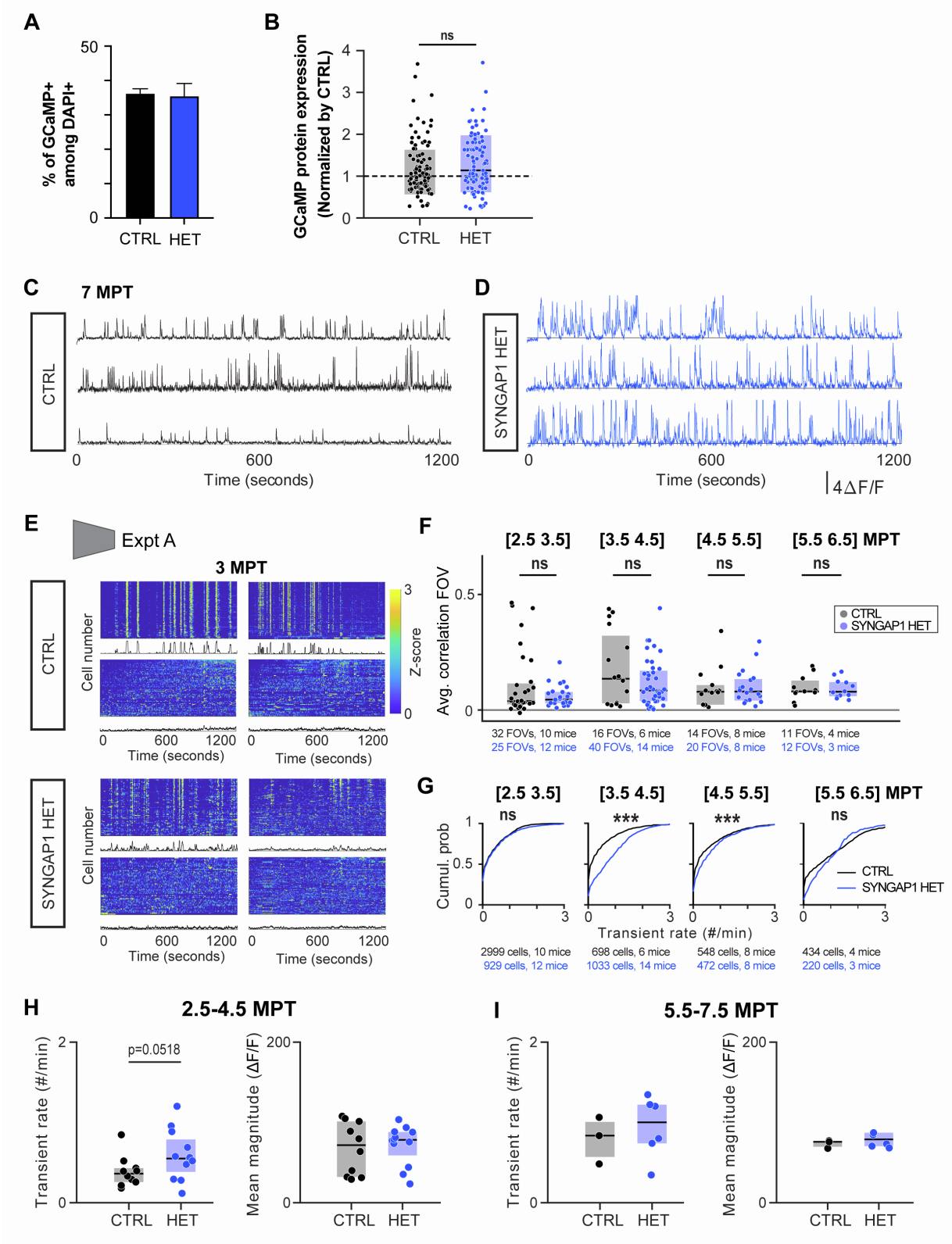

Figure S5. Summary of functional data on calcium activity during visual stimulation

(A) Proportion of GCaMP+ cells among human cortical cells. Human neurons were infected with lentiviral constructs encoding GCaMP at day 32 of in vitro differentiation. The cells were split on day 44 and DOX treatment was started. Quantification of GCaMP+ cells among DAPI+ human cells on day 46. N=4 batches for each group. Data are shown as mean ± SEM. **(B)** Comparison of GCaMP protein expression between CTRL and SYNGAP1 HET cells in xenotransplanted mice. Each dot indicates individual cell GCaMP signal intensity detected by anti-GFP antibody immunostaining. Horizontal bars indicate median values. There is no significant difference between genotypes. N=100 cells from 5 mice for each genotype. **(C-D)** Example traces as in main figure 3C-D. **(E)** Population activity graphs from example FOVs in CTRL and SYNGAP1 HET at 3 MPT shown as heatmaps (brighter colors reflect higher activity). The top left panel shows highly synchronous bursting patterns in CTRL, found rarely in SYNGAP1 HET neurons (bottom). Values shown in rows are the Z-scored df/f calcium traces of individual neurons recorded during 20 minutes. The black trace below each heatmap shows the average over all neurons. Large peaks in these traces indicate strong synchronous activity. No visual stimulus was presented during these experiments. **(F-G)** Quantification of population correlation and evoked activity at 4 different time epochs. (**F**) Dots indicate average pairwise correlation between activity traces of all simultaneously recorded neurons for individual FOVs. Note the elevated correlation for CTRL FOVs in the first two epochs. Boxes indicate median and interquartile range. (**G**) Cumulative probability plots showing the distribution of calcium transient rates per neuron for CTRL or SYNGAP1 HET neurons. **(H)** Transient rate (left) and mean magnitude (right) at 2.5-4.5 MPT, averaged

per animal (n=10,12 for CTRL, HET). **(I)** Per animal average of transient rate (left) and mean magnitude (right) at 5.5-7.5 MPT (n=3, 6 for CTRL, HET). **(H)** Transient rate (left) and mean magnitude (right) at 2.5-4.5 MPT, averaged per animal (n=10,12 for CTRL, HET). **(I)** Per animal average of transient rate (left) and mean magnitude (right) at 5.5-7.5 MPT (n=3, 6 for CTRL, HET).

Figure S6 related to Figure 3.

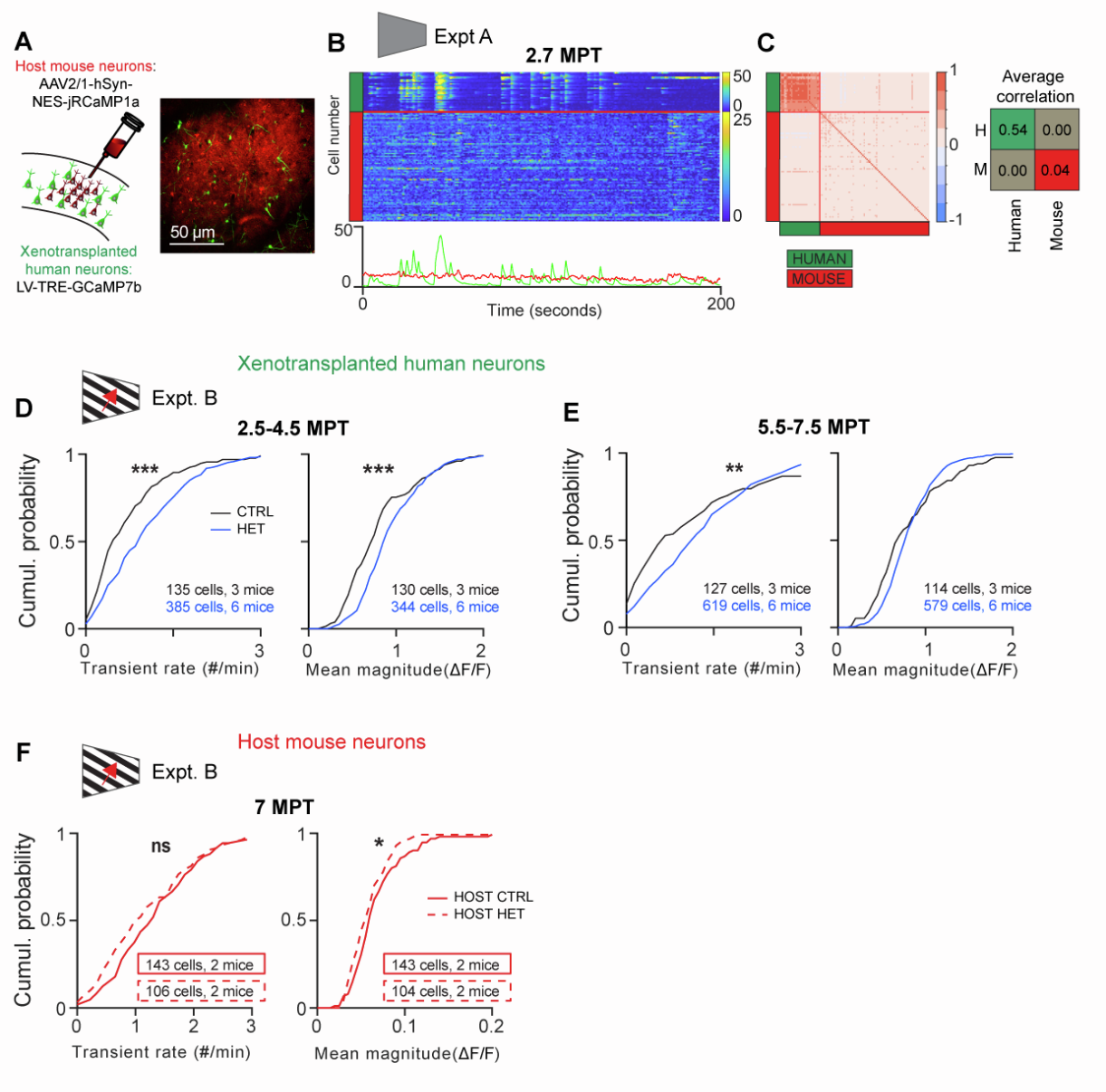

Figure S6: Combined imaging of host and transplanted neurons

(A-C) Dual color imaging of transplanted human and host mouse neurons. (**A**) (left) Experimental design: After locating transplanted human cells, we locally injected AAV-hSyn-NES-jRCaMP1a to infect predominantly mouse cells. (right) Example FOV showing transplanted human neurons expressing inducible TRE-GCaMP7b and host mouse cells

expressing jRCaMP1a. Images were acquired using dual beam two-photon excitation (920 nm and 1073 nm). (**B**) Example experiment recorded at 2.7 MPT showing activity of simultaneously recorded transplanted CTRL human neurons (top) and host mouse (bottom) neurons. Human neurons show strong bursting activity in absence of visual stimulation. (**C**) Correlation matrix showing that CTRL human neurons are highly correlated in time and that they do not synchronize with mouse neurons. **(D)** Quantification of evoked calcium transient rate (left) and average transient magnitude (right) for CTRL and SYNGAP1 HET (blue) neurons recorded between 2.5 and 4.5 MPT. The rightward shift of the blue curve indicates a consistent increase in activity for HET over CTRL. Medians per genotype: transient rate: 0.50; 0.95, avg. transient magnitude: 73.4; 88.0. **(E)** Same parameters as quantified in (A) for neurons recorded between 5.5 and 7.5 MPT. The evoked transient rates for SYNGAP1 HET neurons (blue) are increased compared to CTRL neurons. Medians per genotype: transient rate: 0.62; 1.16, mean magnitude: 68.3; 78.5. **(F)** Mouse neurons labelled with AAV-jRCaMP1a in CTRL or SYNGAP1 HET transplanted animals.

Figure S7 related to Figure 4.

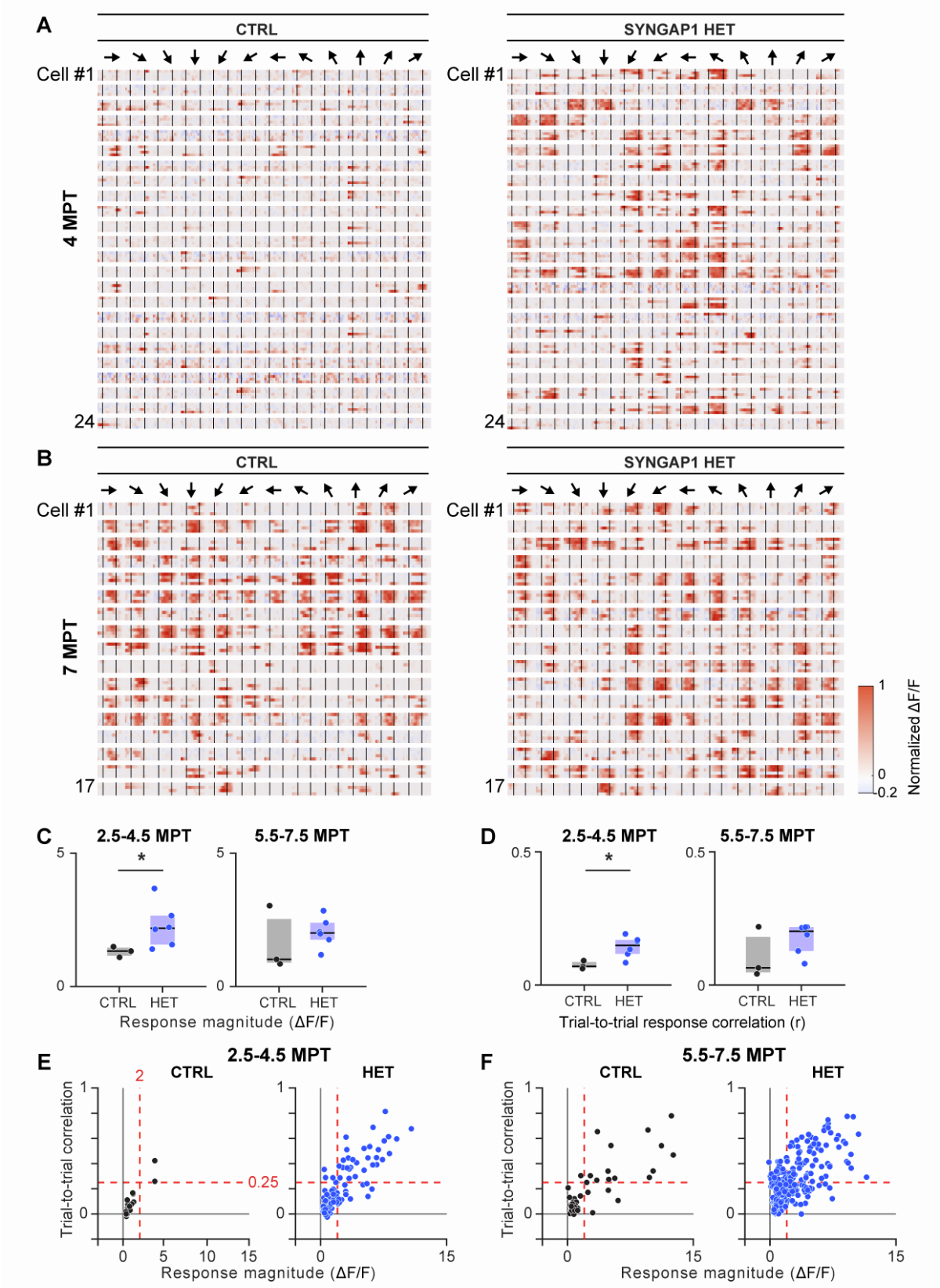

Figure S7: Functional calcium data on visual tuning

(A) Example data at 4 MPT showing single trial responses to 12 directions of the drifting square wave grating at the optimal spatial and temporal frequency for each cell. Each row shows 4 repeats of the same visual stimulus for one of the 24 cells. All cells are recorded simultaneously in CTRL (left) or SYNGAP1 HET (right) transplanted animal. Note the higher occurrence of red boxes in the right panel indicating the SYNGAP1 HET neurons at 4 MPT more frequently respond stronger and with less variability. Black vertical lines indicate onset and offset of the visual stimulus **(B)** Similar example data as shown in (A) recorded at 7 MPT. Note the increase in response strength and reliability in CTRL neurons. **(C)** Response magnitude at both timepoints across genotype, averaged per animal (n= 3, 6 for CTRL, HET). **(D)** Trial-to-trial response correlation at both timepoints across genotype, averaged per animal (n= 3, 6 for CTRL, HET). **(E)** Scatter of response magnitude and trial-to-trial response correlation at 2.5-4.5 MPT. Red dashed lines indicate the cut-off used for both variables to determine visually response neurons. Note the higher number of SYNGAP1 HET neurons at early stages. **(F)** Scatter plot showing similar data shown in (C), now for neurons recorded at 5.5-7.5 MPT. Note the relative increase of neurons that pass both thresholds in CTRL neurons.