# Supplementary Information

Supplementary table 1: Primer sequences used for PCR genotyping of experimental animals in autism auditory habituation experiments.

| Gene	Forward primer sequence	Reverse primer sequence
fmr1	5' – CTA AAT GAA ATC GTC ACA TTA GAG	5' – TCC ATG ACA TCC TGC ATT AG
scn1lab	5' – CAG CAA ATA AAT GAA CGC CTT A	5' – AGA GAG TTA CCA CAA ACA CAC TCG
mecp2	5' – AAA GGA AAG GCA TGA TGT GG	5' – ATA CAT TGG GCC TCT GTC CC
cntnap2a	5' – ACC CTT AAA ATT GAT AAA AGA ACA CG	5' – GCA GAA AAG GGG CTA AAT TAA AA
cntnap2b	5' – TGC GAT GTG TAT CAT ATG TTC TTT T	5' – AAA AAG GTA GCT CAA ACT GTA ATT G
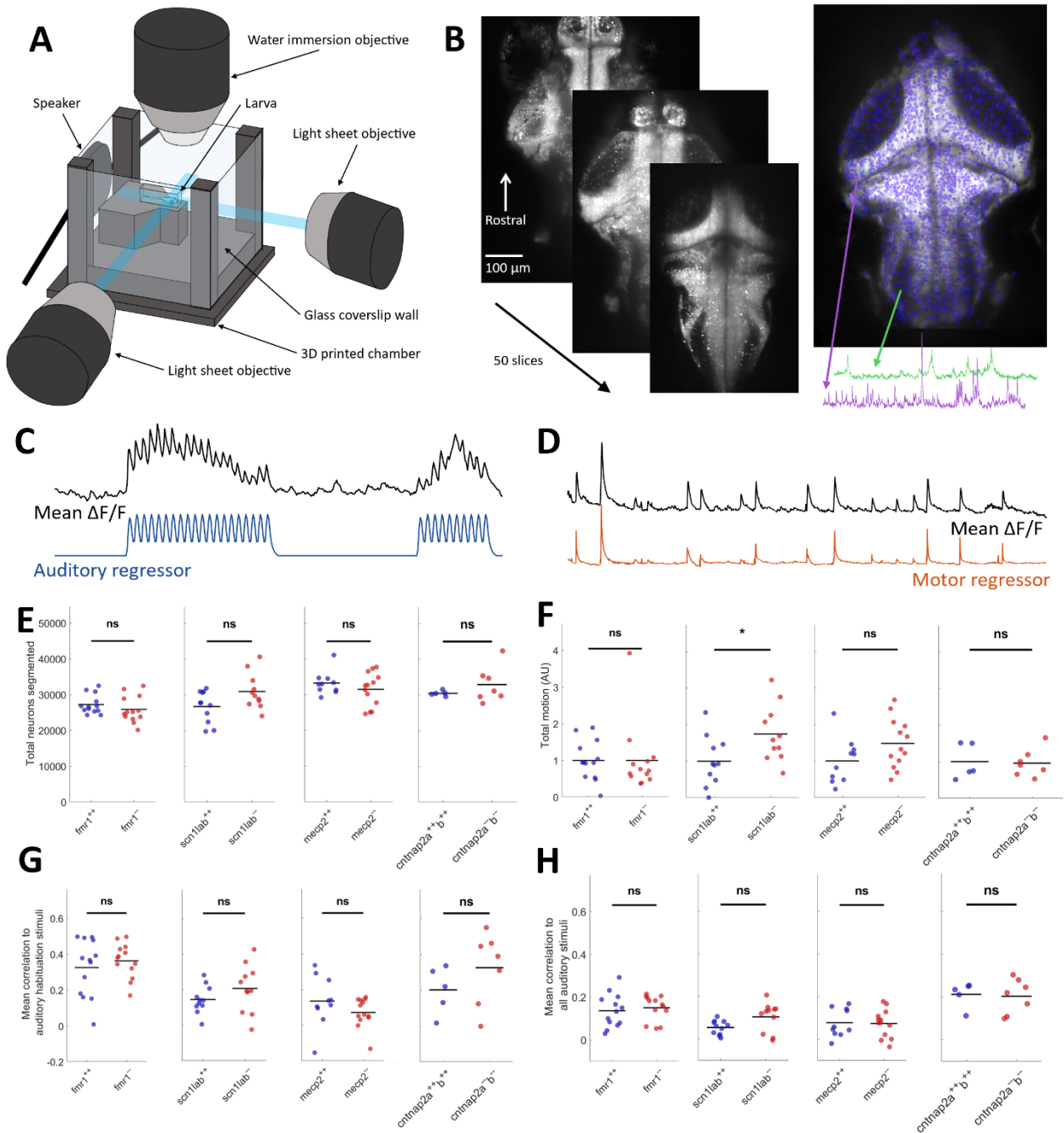
Supplementary Figure 1: Imaging of brain-wide neuronal activity during auditory habituation. A) Selective plane illumination microscope set-up. Larva mounted in low melting point agarose is illuminated by two perpendicular sheets of light. Acoustic stimuli are delivered by a speaker affixed to the back wall. Calcium activity is captured with a water-immersion objective. B) Image processing pipeline. 50 z-planes spanning the dorsoventral axis are acquired at a rate of 2Hz. Individual neurons are segmented using Suite2p, and their fluorescent traces extracted (examples shown in green and purple). C) Example auditory regressor and mean trace from auditory-classified neurons from a single fish. D) Example motor regressor and mean trace from motor-responsive neurons from a single fish. E) Total number of neurons segmented from each fish in each dataset. F) Total area under the curve of the motor regressor, normalized to the mean value for wild types of each dataset. * = p < 0.05. G) Mean correlation to the auditory stimuli in the habituation period from all neurons. H) Mean correlation to all auditory stimuli. For E-H, each dot represents one fish, black lines show means.
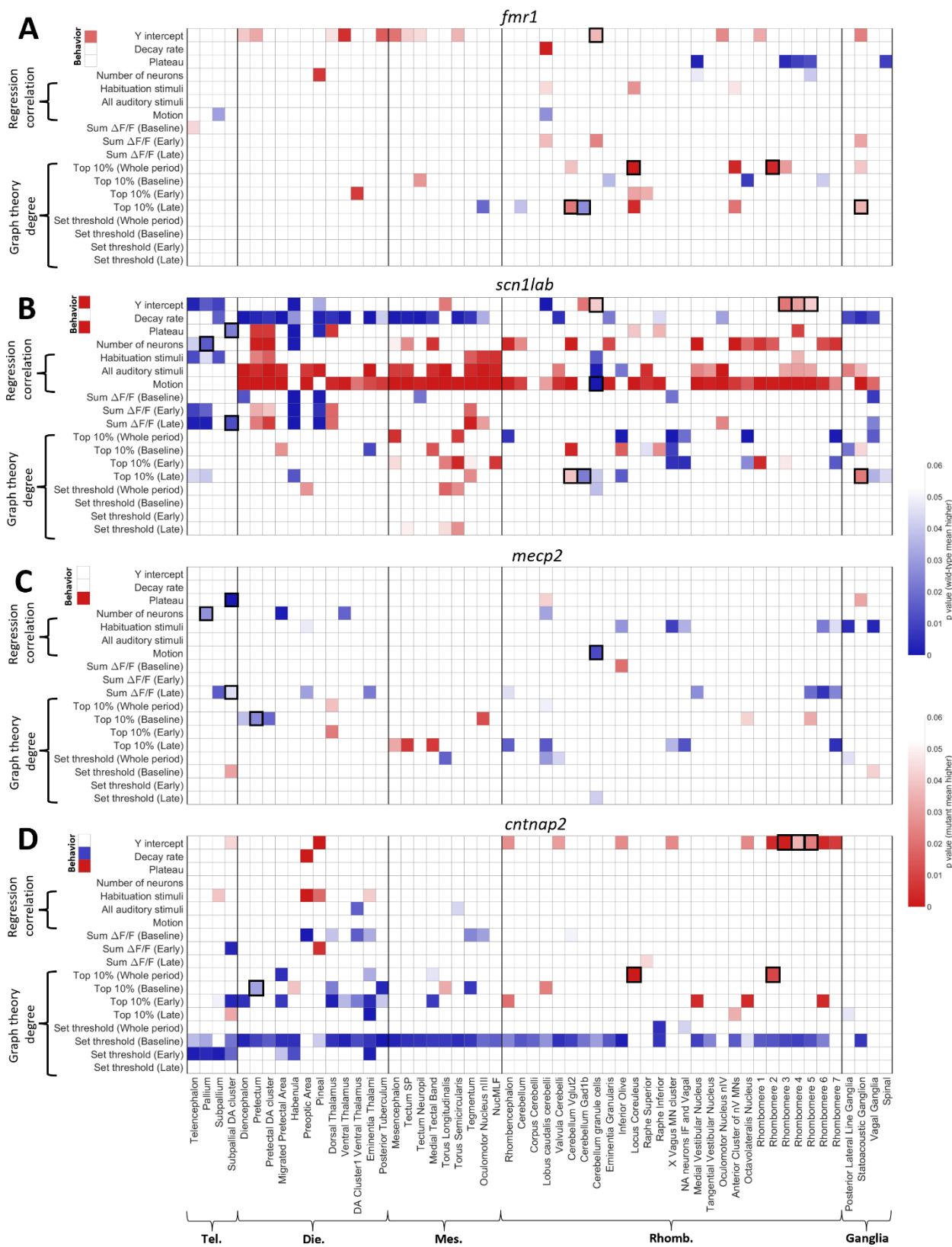
Supplementary Figure 2: Summary graphs of comparisons of different metrics across different brain regions using linear mixed effects models. The same comparisons are presented for each of the four genes. Grid locations are colored by the p-value of the effect of genotype, all p > 0.05 are white. Red colored squares indicate the metric is significantly higher in the mutant, blue indicates the metric is significantly higher in the wild type. Full p values can be found in supplementary material. Dark outlines indicate phenotypes present in two genes. Insets display the p-values from curve fit comparisons of behavioral data. DA = dopaminergic, NA = noradrenergic. SP = Stratum Periventriculare, Tel. = Telencephalon, Die. = Diencephalon, Mes. = Mesencephalon, Rhomb. = Rhombencephalon. MN = motorneuron. NucMLF = Nucleus of the medial longitudinal fascicle. IF = Interfascicular
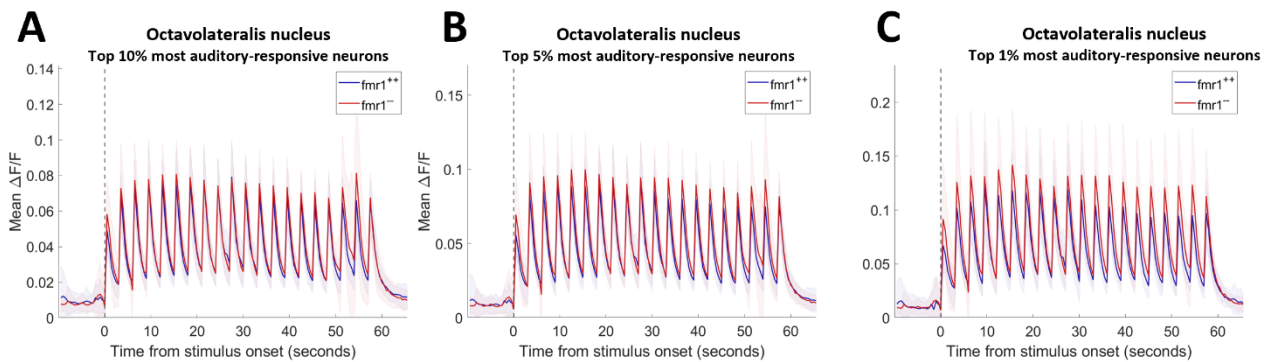
Supplementary Figure 3: Mean traces of neurons in the ON of the *fmr1* group, with increasing threshold stringency for correlation to all auditory stimuli. Mean traces are shown for the top 10% (A), 5% (B) and 1% (C) of auditory neurons in the ON of each fish. Shading indicates std.
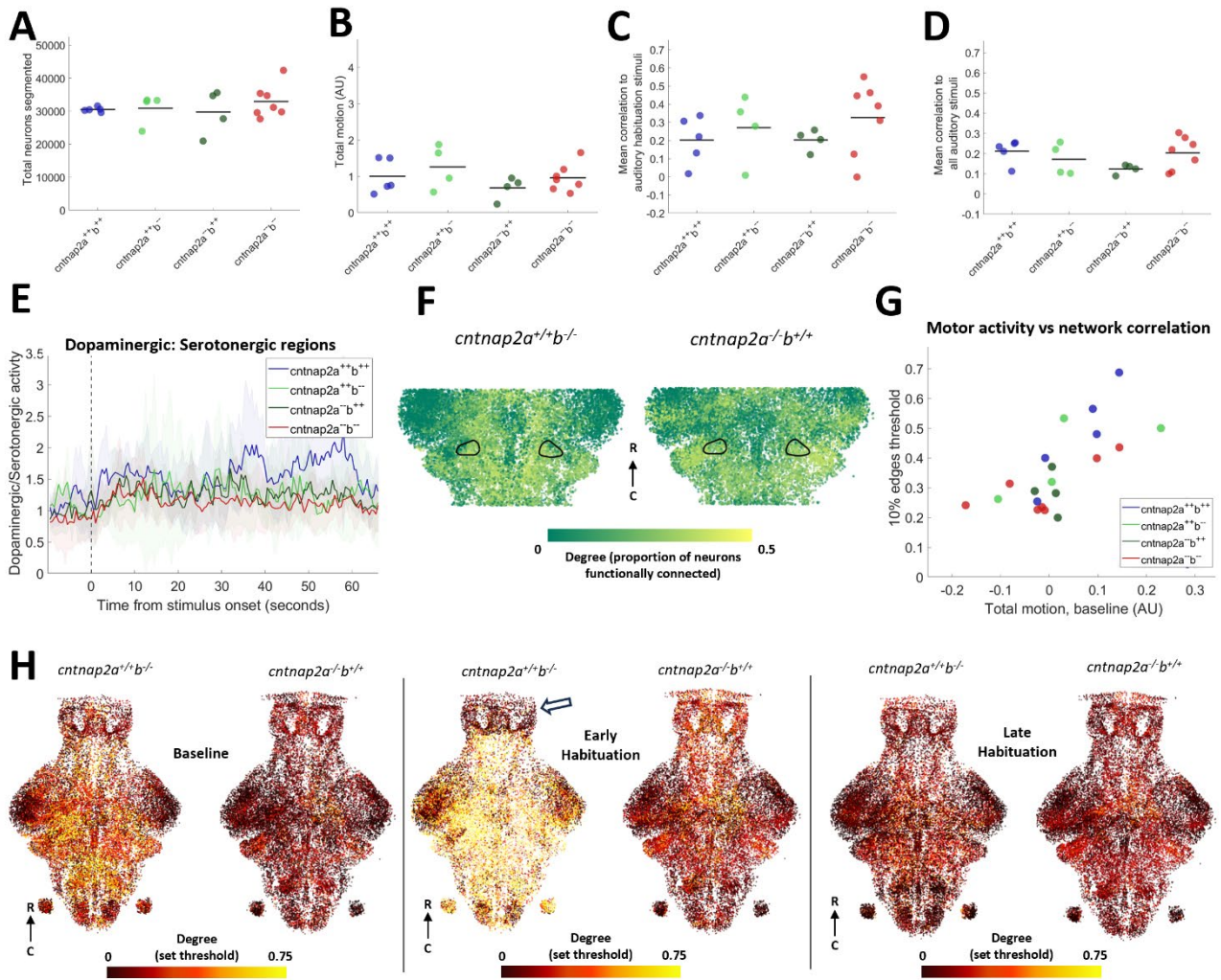
Supplementary Figure 4: Auditory habituation in *cntnap2* single mutants. A) Total number of neurons segmented from each fish in each dataset. B) Total area under the curve of the motor regressor, normalized to the mean value for wild types of each dataset. C) Mean correlation to the auditory stimuli in the habituation period from all neurons. D) Mean correlation to all auditory stimuli. For A-D, each dot represents one fish, black lines show means. E) Mean ratio between activity in all dopaminergic regions and all serotonergic regions. Shading indicates SD. F) Sub-selection of neurons, colored by their degree, as determined using the top 10% of edges from correlation during the whole habituation period. Black outlines indicate the locus coeruleus. G) Sum motor activity compared to correlation threshold required for the top 10% of edges during the baseline period. Each dot represents one fish. Linear mixed-effect model indicates the amount of motor activity affects the overall correlation throughout the brain (p = 0.0004), but the effect of genotype is also strong (p < 0.0001). H) All neurons from all fish in a region of interest in the z-dimension, between 50 – 75 μm depth. Each neuron colored by its degree as defined by a set correlation threshold during the baseline, early habituation period and late habituation period.