Supplementary Figure 1

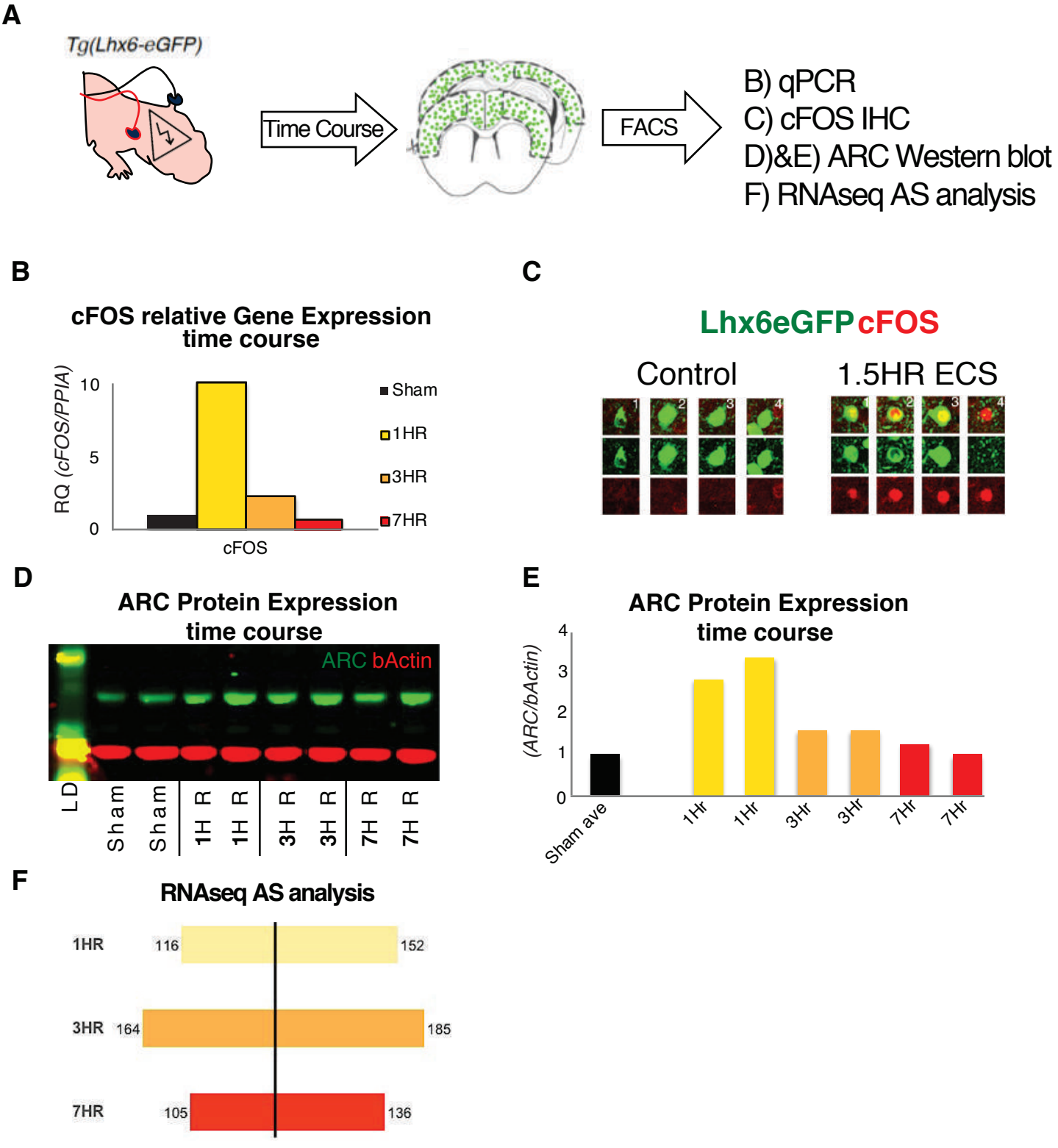



**Figure S1: Acute increases in neuronal activity induces immediate early gene expression and differential splicing within SST+ cINs in vivo (Related to Figure 2)**

A) Schematic of experimental approach: P8 tgLhx6eGFP animals were subjected to ECS (left)' then following a time course of 1 Hr, 3 hr or 7 hrs the S1 cortex was dissected (middle) and GFP+cINs were isolated by FACS for qPCR, Western blot, and RNAseq analysis (right). As well as IHC.

B) Quantification of relative mRNA expression (RQ) of cFOS (normalized to housekeeping gene PPIA) in ctl/sham animals (black), 1hr (yellow), 3hrs (orange), and 7hrs (red) following ECS within cINs.

C) Immunostaining of cFOS in sham (red) and eGFP vs ECS treated animals, showing the expression of cFOS 1.5 hours post ECS activity induction.

D) Representative western blot of ARC protein expression within sham treated (two replicates), 1hr (two replicates), 3hrs (two replicates), and 7hrs (two replicates) following ECS within cINs.

E) Fold of ARC protein expression (normalized to b-actin) in ctl/sham treated (black), 1hr (yellow), 3hrs (orange), and 7hrs (red) following ECS within cINs.

F) Magnitude of differential alternative splicing events from the comparison of sham cINs to cINs 1hr (yellow, 268 events), 3hrs (orange, 349 events), and 7 hrs (red, 241 events) following ECS.

Supplementary Figure 2

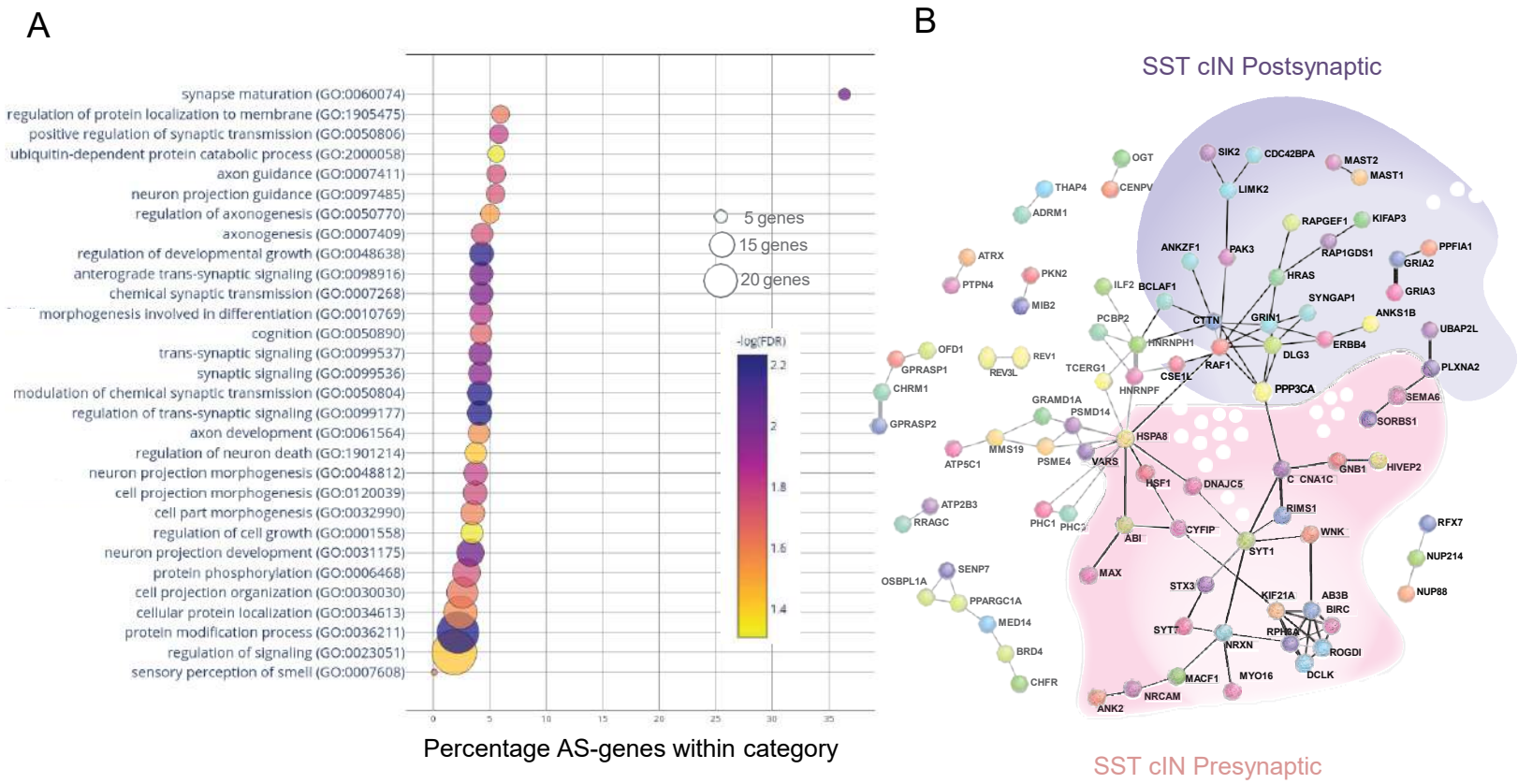



**Figure S2: Neuronal activity influences alternative splicing and Nova expression within SST cINs (Related to Figure2)**
A) Bubble dot plot of gene ontology (GO) most significant terms for the genes subjected to activity-dependent alternative splicing within SST+ cINs (false discovery rate (FDR)<0.05), x-axis is the enrichment of the activity-dependent AS genes in the GO catagory (# of genes in GO category from SST transcriptome/ # of genes activity-dependent AS in category). Color of dot indicates magnitude of significance (-log10 transform FDR, none shown above FDR <0.05) and size corresponds to number of genes in catagory.
B) Protein-protein interaction (PPI) network formed from 312 activity-depdendent spliced genes in SST cINs with Disease Association Protein-Protein Link Evaluator (DAPPLE) (Rossin et al., 2011) and performed over 10,000 permutations (pVal<0.00009). Green shading- post-synaptic gene network, pink shading- pre-synaptic gene network.

Supplementary Figure 3

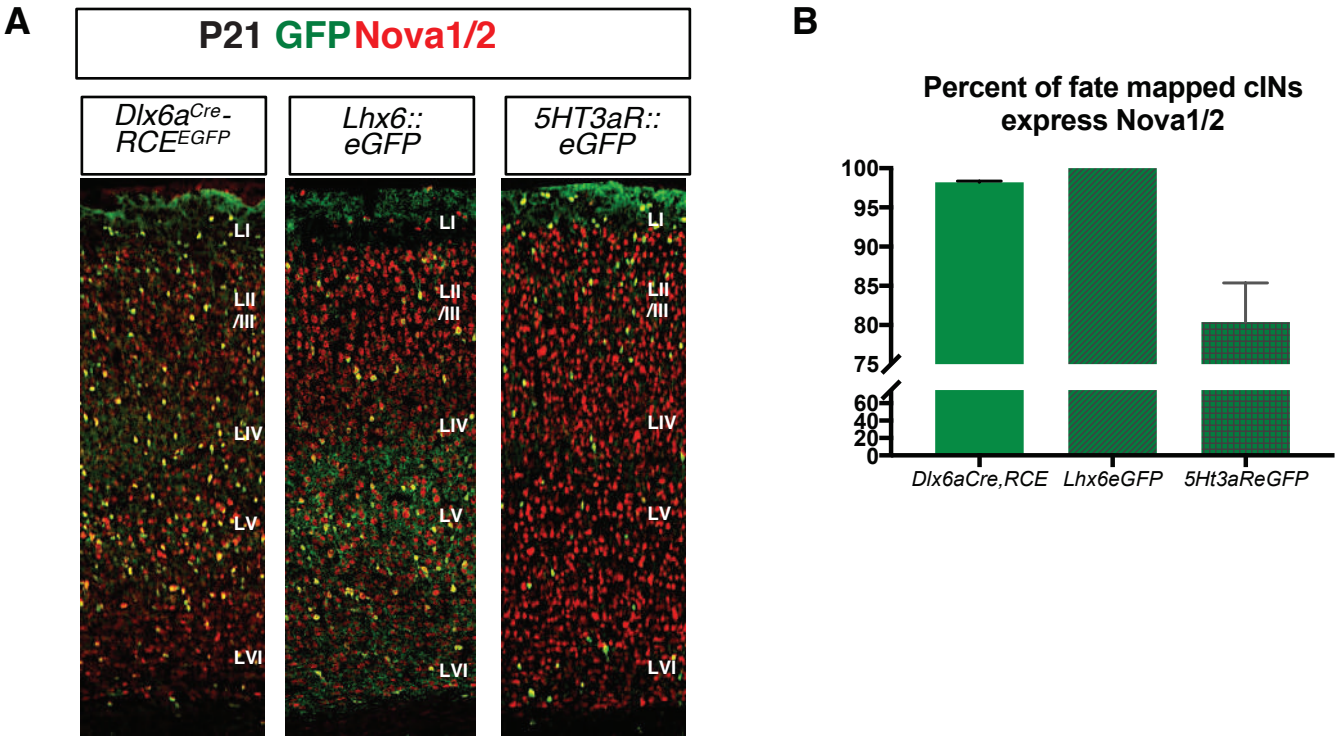



**Figure S3: Nova1/2 AS factors expression within the MGE and cINs (Related to Figure 3)**
A) Representative IHC image of brain section at P21 from Dlx6aCre;RCEeGFP, Lhx6::eGFP and 5HT3aR::eGFP. Anti-Nova1/2 (red); GFP (green).
B) Quantification of eGFP cells expressing Nova1/2. 100% of Lhx6::eGFP cells express Nova1/2.

# Supplementary Figure 4

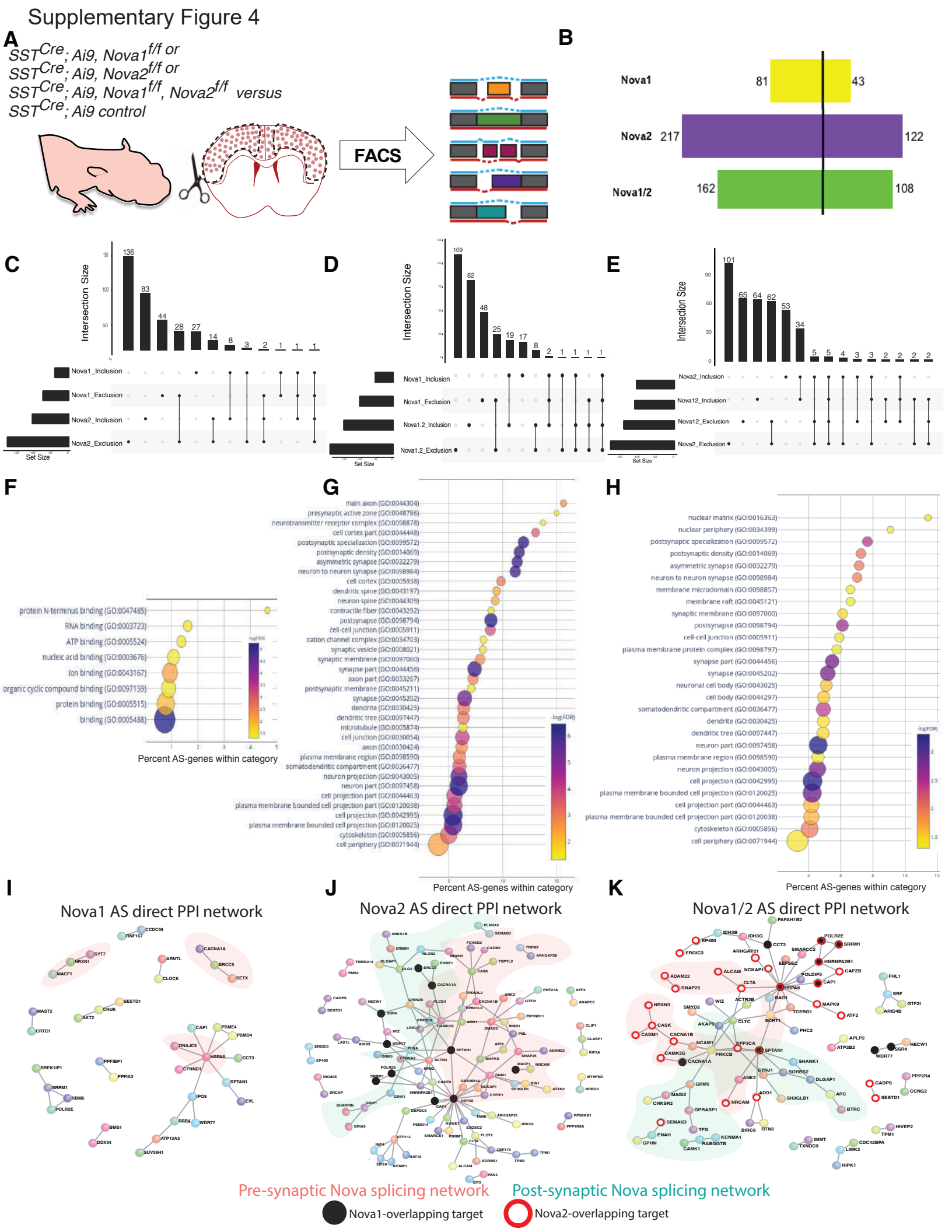


**Figure S4: Nova2 controls the majority of splicing events of the Nova1/2 family within SST+ cINs and these events colecse into GO catagories and direct PPI- networks related to pre- and post-synaptic development of SST cINs.**

A)Model of experimental set up: the S1 cortex from P8 animals (SSTCre;Ai9; Nova1f/f, SSTCre;Ai9; Nova2f/f, SSTCre;Ai9; Nova1f/f; Nova2f/f; or SSTCre;Ai9) were isolated for FACS of SST+Ai9+ cINs. SST cINs were then prepared for RNAseq and splicing analysis was performed.
B) Magnitude of differential splicing events between ctl SST+ cINs and SST-Nova1 SST cINS (Top, yellow, 124 events), ctl SST cINs and SST-Nova2 mutants (Middle, purple, 339), and ctl SST cINs and SST-dKO (Bottom, green, 270) (FDR < 0.05, Exclusion< -0.1,0.1>Inclusion)
C) Quantification of the overlap of SST-Nova1 cIN and SST-Nova2 mutant cIN splicing events: Bottom left horizontal bars indicate the number of genes subjected to AS events for each data set: Nova1_inclusion splicing events (43), Nova1_exclusion splicing events (81), Nova2_inclusion splicing events (122), Nova2_Exclusion splicing events (217). Top histogram bars indicate the magnitude of overlap between the data sets indicated by a filled black circle below (i.e 136 of Nova2_Exclusion splicing events overlap with the other sets, 28 of Nova1_Exclusion splicing events overlap with Nova2_exclusion, etc.)
D) Quantification of the overlap of SST-Nova1-cKO cIN and double SSTNova1/2-dKO SST cIN splicing events: Bottom left horizontal bars indicate the number of genes subjected to AS events for each data set: Nova1_inclusion splicing events (43), Nova1_exclusion splicing events (81), Nova1.2_inclusion splicing events (108), Nova1.2_Exclusion splicing events (162). Top histogram bars indicate the magnitude of overlap between the data sets indicated by a filled black circle below (i.e 25 of Nova1_Exclusion splicing events overlap with Nova1.2_Exclusion, 19 of Nova1_Inclusion splicing events overlap with Nova1.2_Inclusion, etc.)
E) Quantification of the overlap of SST-Nova2-cKO cIN and SST-Nova1/2-dKO SST cIN splicing events: Bottom left horizontal bars indicate the number of genes subjected to AS events for each data set: Nova2_inclusion splicing events (122), Nova2_exclusion splicing events (217), Nova1.2_inclusion splicing events (108), Nova1.2_Exclusion splicing events (162). Top histogram bars indicate the magnitude of overlap between the data sets indicated by a filled black circle below (i.e 62 of Nova2_Exclusion splicing events overlap with Nova1.2_Exclusion, 34 of Nova1_Inclusion splicing events overlap with Nova1.2_Inclusion, etc.)
F) Bubble dot plot of most significant GO terms for the genes subjected to AS within SST-Nova1 mutant cINs, x-axis is the percent enrichment of the AS genes in the GO catagory (/# genes SST-Nova1 AS in category/ # genes in GO category from SST transcriptome), color of dot indicates magnitude of significance (-log10 transform FDR, none shown above FDR <0.05) and size corresponds to number of genes in category.
G) Bubble dot plot of most significant GO terms for SST-Nova2 genes subjected to AS, same parameters as F, but illustrating the substantial enrichment of Nova2-dependent events to synaptic development
H) Bubble dot plot of most significant GO terms for double SST-Nova-dKO genes subjected to AS, same parameters as F, but illustrating the similar catagories of Nova1/2-dependent events to Nova2-dependent events.
I) Protein-protein interaction network formed from 124 Nova1-cKO splices genes in SST cINs with DAPPLE (10,000 permutations, pVal<0.09), pink shading labels genes that belong in Synapse-related GO catagories
J) Protein-protein interaction network formed from 339 Nova2-cKO splices genes in SST cINs with DAPPLE (10,000 permutations, pVal<0.00009), Black dots indicate shared genes with Nova1-cKO, green shading labels post-synaptic that belong in Synapse-related GO catagories, pink shading labels pre-synaptic that belong in Synapse-related GO catagories
K) Protein-protein interaction network formed from 270 double Nova1/2-dKO splices genes in SST cINs with DAPPLE (10,000 permutations, pVal<0.00009), Black dots indicate shared genes with Nova1-cKO, Red outline dots indicate shared gene with Nova2-cKO, green shading labels post-synaptic gene network, pink shading labels pre-synaptic gene network that belong in Synapse-related GO catagories

Supplementary Figure 5

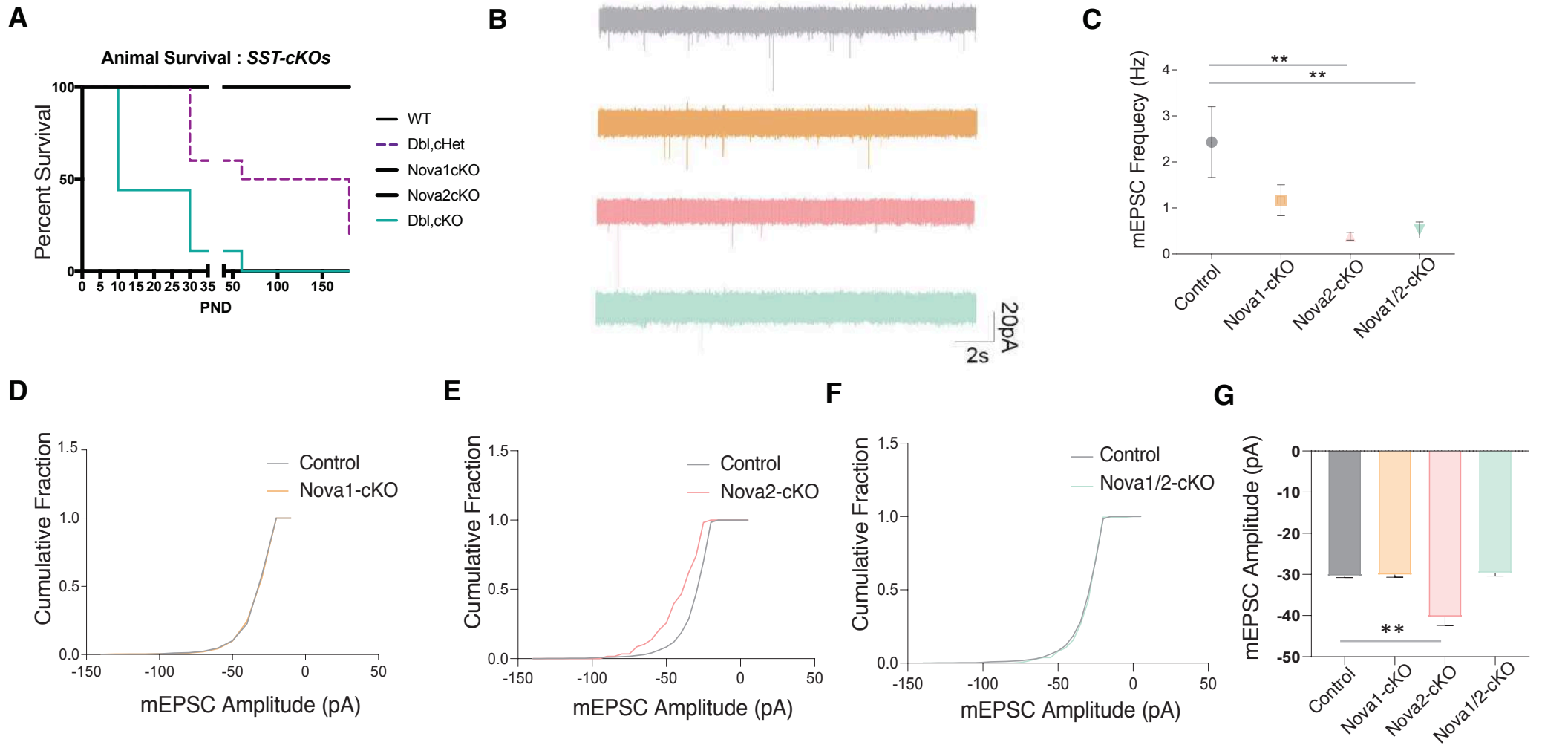



**Figure S5: Conditional loss of Nova1/2 within SST+ cINs impacts animal survival and disrupts their afferent and efferent synaptic connectivity (Related to Figure 4)**

A) Survival plot of conditional knockouts within SSTCre;Ai9 animals (WT SSTCre; Ai9 animals black line, double heterozygous (het) SSTCre; Ai9, Nova1f/+, Nova2f/+ slashed purple line (Dcl,cHet), SSTCre; Ai9, Nova1f/f black line, SSTCre; Ai9, Nova2f/f black line, double SSTCre;t Ai9, Nova1f/f Nova2f/f turquoise line) at P8 50% of double conditional SSTCre;t Ai9, Nova1f/f Nova2f/f (Dbl,cKO) animals have deceased by P50 90% of double conditional animals have deceased. Whereas, at P30 40% of Dbl, cHet animals and by P60 50% have deceased. WT and singular conditional mutants do not exhibit decreased survival.

B) Representative mEPSCs recordings from SST cINs from: Top to bottom: wt animals, grey traces (SSTCre; Ai9 or SSTCre; Ai9, Nova1f/+ or SSTCre; Ai9, Nova2f/+), Nova1-cKO animals, orange traces (SSTCre;t Ai9, Nova1f/f), Nova2-cKO animals, pink traces (SSTCre; Ai9 Nova2f/f), and double Nova1/2-cKO, turquoise traces (SSTCre; Ai9, Nova1f/f Nova2f/f). Scale bar:20pA and 2 seconds.

C) Quantification of mEPSC frequencies recorded from SST cINs in control animals, grey dot (SSTCre; Ai9 or SSTCre; Ai9, Nova1f/+ or SSTCre; Ai9, Nova2f/+), Nova1-cKO animals, orange square (SSTCre; Ai9, Nova1f/f), Nova2-cKO animals, pink triange (SSTCre; Ai9, Nova2f/f), and double Nova1/2-cKO, turquoise upside-down triangle. **pVal= >0.005 for wt vs. Nova2-cKO and wt vs. Nova1/2-cKO,

D) Cumulative probablility distributions of mEPSC amplitudes from recordings of wt SST cINs, grey line, and Nova1-cKO SST cINs, orange line, exhibiting no difference.

E) Cumulative probablility distributions of mEPSC amplitudes from recordings of wt SST cINs, grey line, and Nova2-cKO SST cINs, pink line, exhibiting a significant increase in the amplitude of mEPSCs in Nova2-cKO SST cINs.

F) Cumulative probablility distributions of mEPSC amplitudes from recordings of wt SST cINs, grey line, and Nova1/2-cKO SST cINs, turquoise line, exhibiting no difference.

G) Quantification of mEPSC amplitude in SST cINs from each mutant, illustrating significant increase in amplitude within Nova2-cKO SST cINs. **pVal>0.005.

Supplementary Figure 6

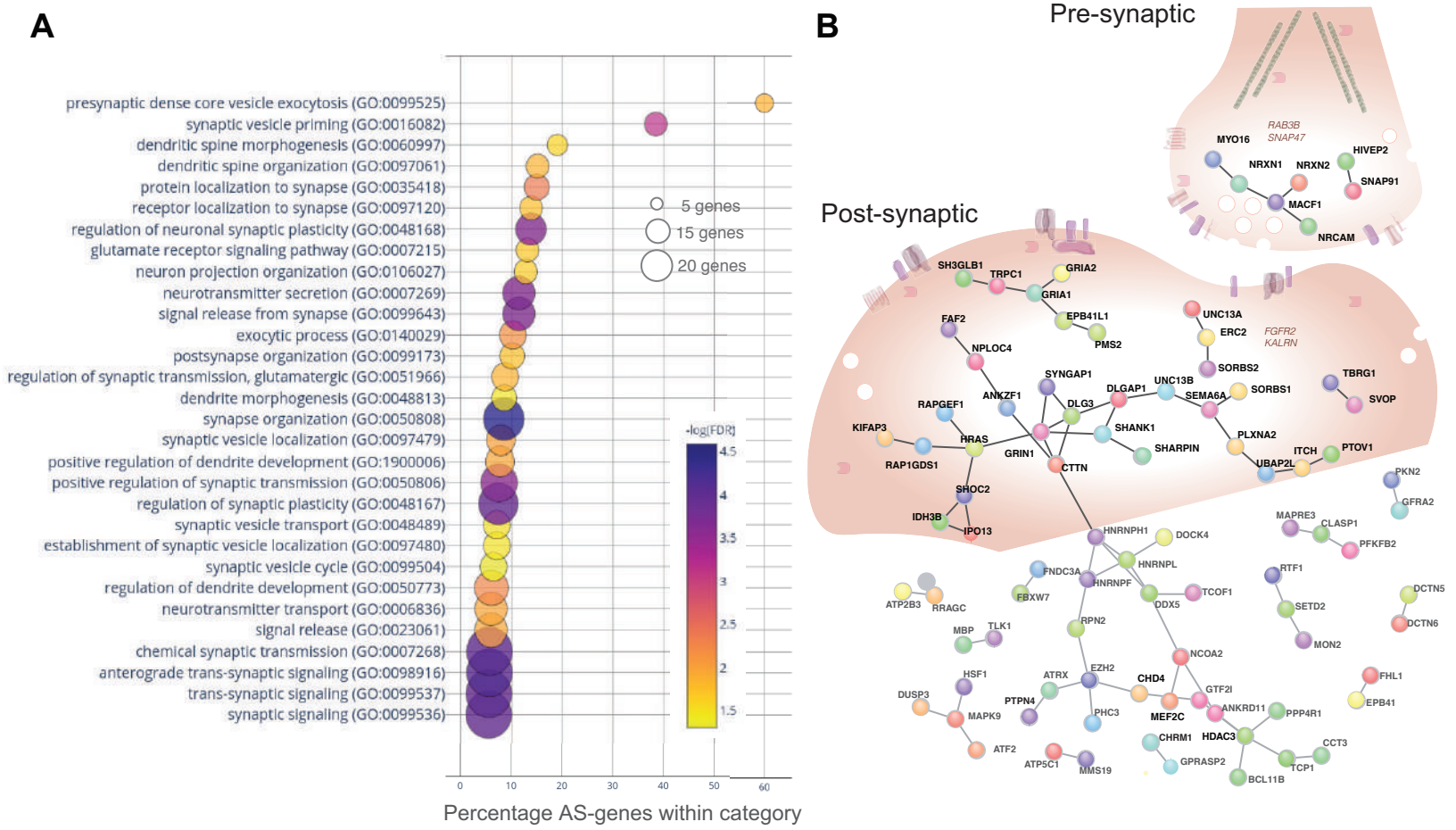


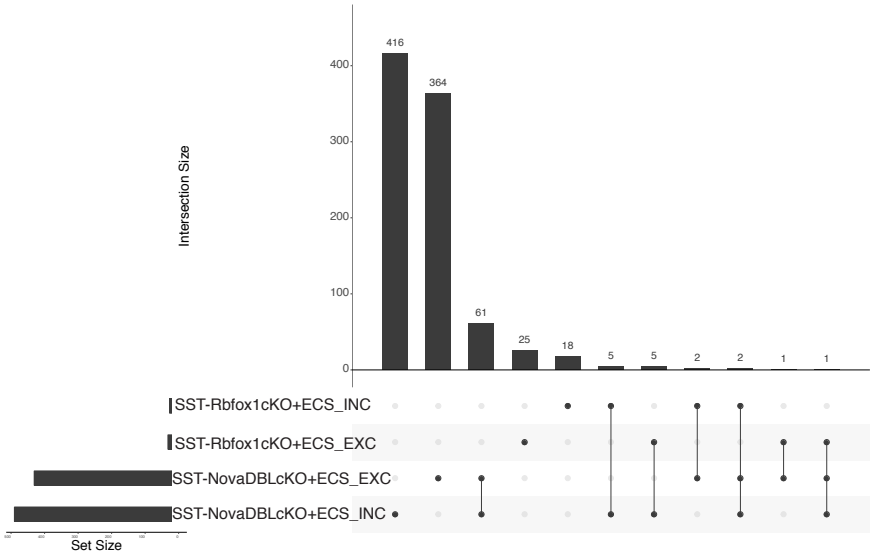

**Figure S6: Nova RNA binding proteins control activity dependent AS in SST cINS during development (Related to Figure 5)**

A) Bubble dot plot of the most significant GO terms for the genes undergoing Nova1/2 activity-dependent AS within SST+ cINs (all shown GO terms FDR<0.05) (Same graph parameters as Figure S2A).

B) Schematic of a SST+ cIN pre-synaptic inhibitory axonal puncta (top right) and a SST+ cIN excitatory post-synaptic density (middle left) overlaid on top of the significant DAPPLE generated PPI diect network from the 346 genes undergoing Nova1/2-dependent activity induced AS (***pVal=0.00009, 10000 permutations).

Supplementary Figure 7

**A**

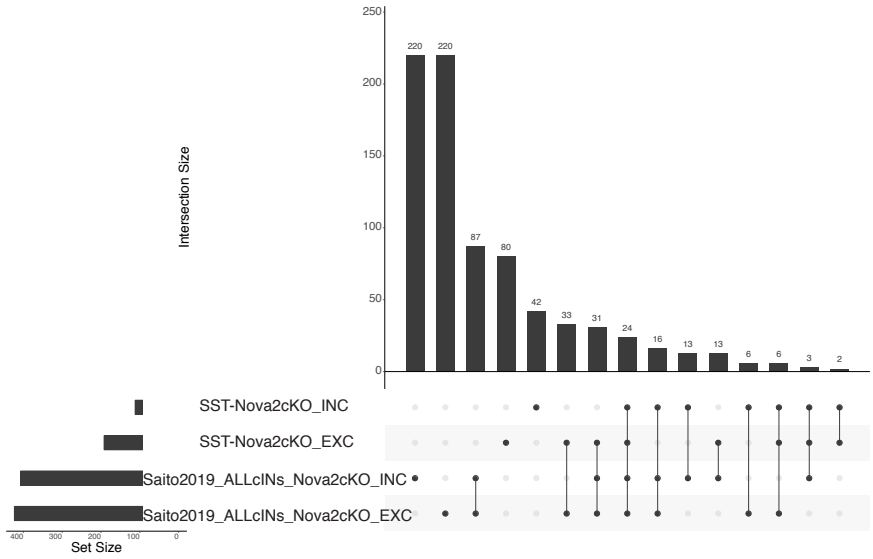



Data set Comparison

**SST-Rbfox1cKO+ECS**:
*$SST^{Cre}$; Ai9, $Rbfox1^{f/f}$* +ECS *versus*
*$SST^{Cre}$; Ai9 control* +ECS

**SST-dKO+ECS**:
*$SST^{Cre}$; Ai9, $Nova1^{f/f}$, $Nova2^{f/f}$*+ECS *versus*
*$SST^{Cre}$; Ai9 control*+ECS

**B**




Data set Comparison

**SST-Nova2cKO**:
*$SST^{Cre}$; Ai9, $Nova2^{f/f}$ versus*
*$SST^{Cre}$; Ai9 control*

**Saito2019_ALLcINs_Nova2cKO**:
*$Gaba^{Cre}$; $Nova2^{f/f}$ versus*
*$Gaba^{Cre}$; control*

**Figure S7: Nova1/2 controls the activity-dependent splicing of a large and unique pool of mRNAs compared to Rbfox1 within SST cINs and SST-specific Nova2 AS genes overlap well with pan-cIN Nova2 AS genes**.

A) Quantification of the overlap of SST-Rbfox1-cKO cIN +ECS (differential splicing events from the comparison of SST cIN +ECS to SST-Rbfox1cKO+ECS) and SST-dKO (differential splicing events from the comparison of SST cINs
+ECS to SST-NovadKO+ECS): Bottom left horizontal bars indicate the number of genes subjected to AS events for each data set. Top vertical bars indicate the number of overlapping genes cooresponding to the black dot below indicating the data set identity.

B) Quantification of the overlap of SST-Nova2 cIN splicing events (differential splicing events from the comparison of ctl SST cINs to SST-Nova2cKO) with the dataset generated by Saito et al 2019 utilizing a mouse cross of GadCre and Nova2f/f (differential splicing events from Ctl cINs versus cINs-Nova2cKO). Bottom left horizontal bars indicate the number of genes subjected to AS events for each data set. Top vertical bars indicate the number of overlapping genes cooresponding to the black dot below indicating the data set identity