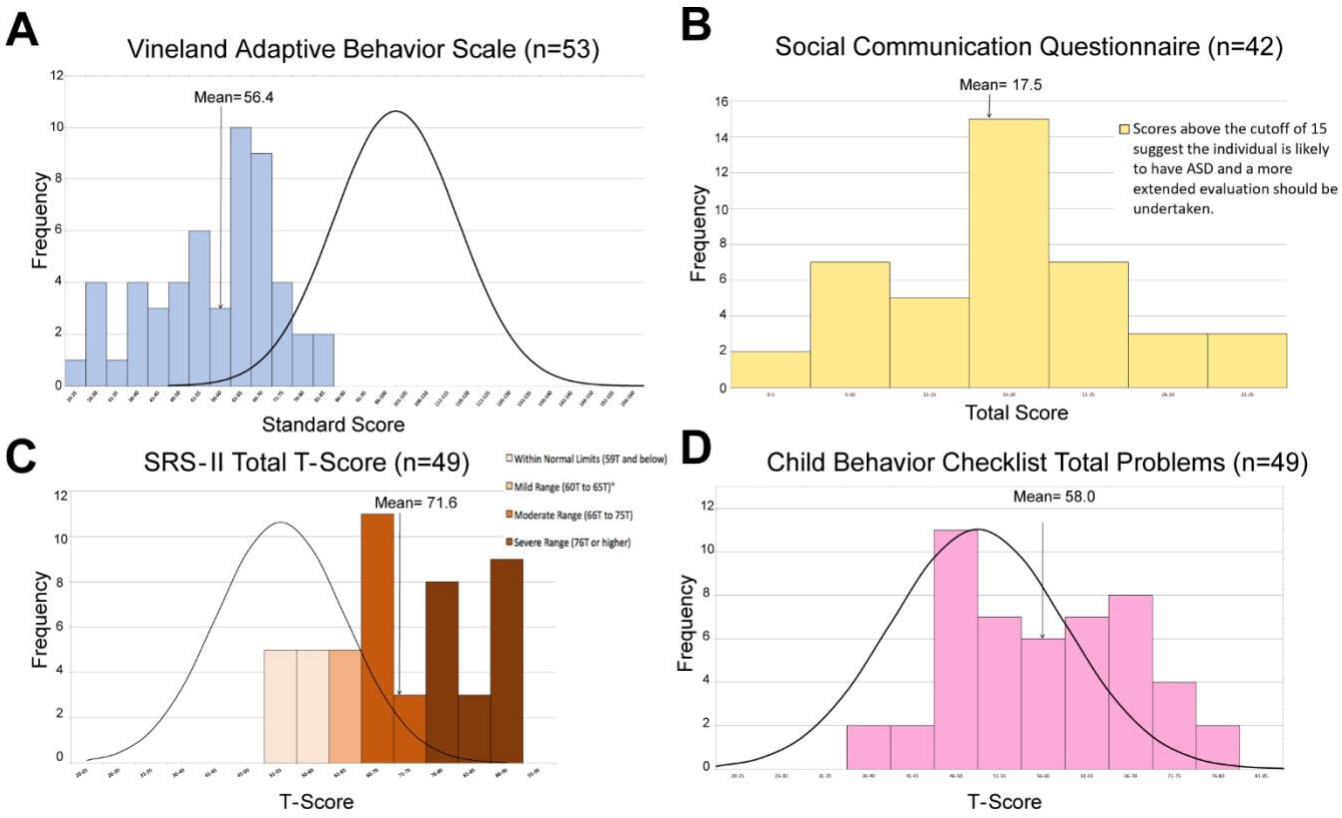
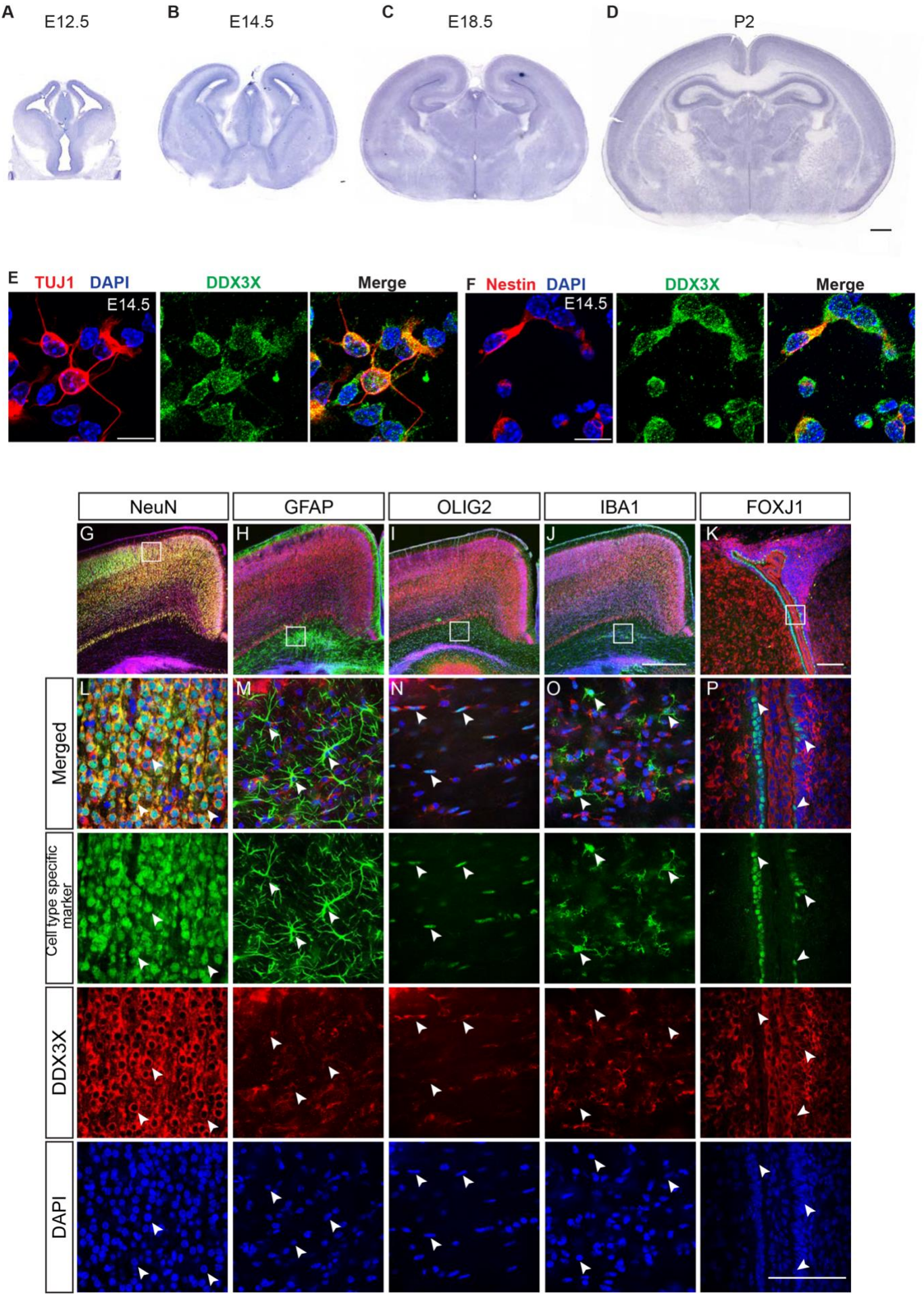
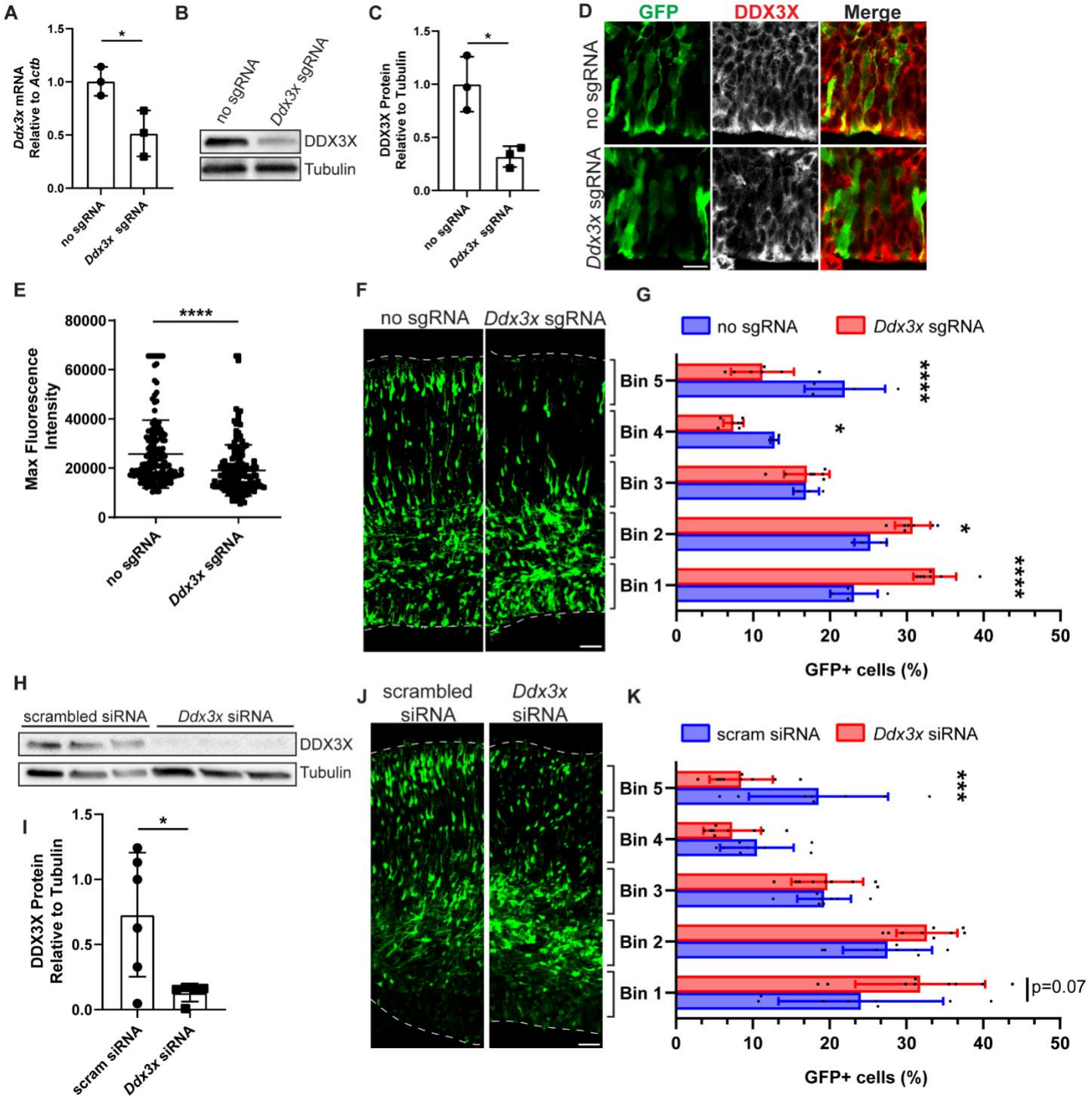
Supplemental Figures and Legends:



Supplemental Figure 1. Scores on four measures of clinical assessment, Related to Figure 2. A, the Vineland Adaptive Behavior Scales (VABS), **B,** the Social Communication Questionnaire (SCQ), **C,** the SRS-II, and **D,** the Child Behavioral Checklist (CBCL). If a standard curve for neurotypical individuals was available (VABS, SRS-II, and CBCL) it was displayed alongside the binned data for the *DDX3X* participants. The number of individuals for whom data was available for each test is indicated in the title and the graph also indicates the mean for the *DDX3X* cohort. There is a significant difference between *DDX3X* patients and neurotypical controls on all of these measures. For example, the VABS score had a mean of 56.4, which is nearly three standard deviations below the mean for controls (mean = 100).

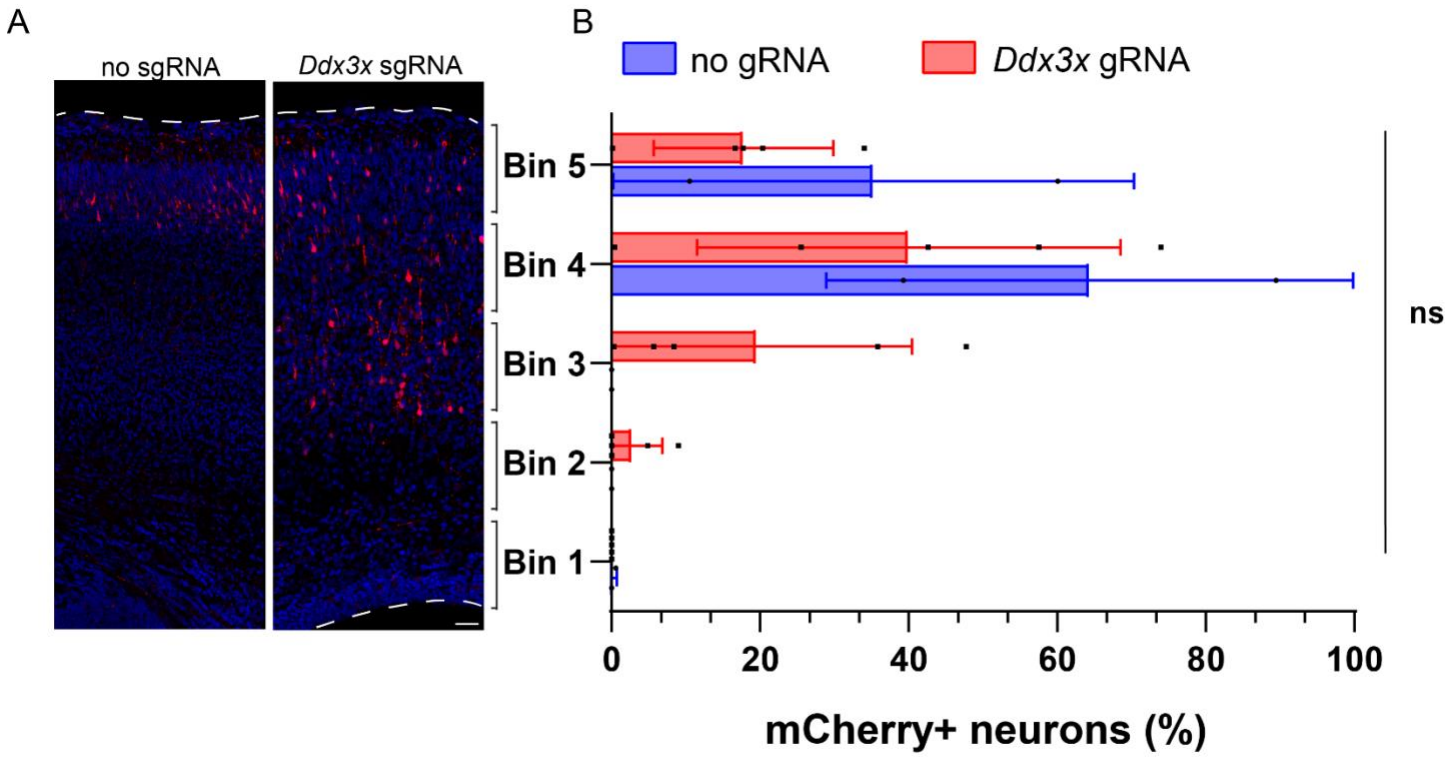

A
E12.5
B
E14.5
C
E18.5
D
P2
E
TUJ1
DAPI
E14.5
DDX3X
Merge
F
Nestin
DAPI
E14.5
DDX3X
Merge
NeuN
GFAP
OLIG2
IBA1
FOXJ1
G
H
I
J
K
L
M
N
O
P
Merged
Cell type specific marker
DDX3X
DAPI

Supplemental Figure 2. Immunohistochemistry depicting DDX3X embryonic and postnatal expression, Related to Figure 3. A-D, *In situ* hybridization against *Ddx3x* mRNA in coronal sections in E12.5 (A), E14.5 (B), E18.5 (C), and P2 (D) brains. **E-F,** Immunofluorescence for DDX3X (green) in primary cells isolated from E14.5 cortices co-stained for the neuron marker TUJ1 (red) (**E)** or the progenitor marker Nestin (red) (**F**) and DAPI (blue). **G-K,** Immunohistochemistry on coronal vibratome sections of P5 CD1 brains (n = 4) with antibodies against DDX3X (red) and indicated specific cell type markers NeuN (neurons), GFAP (astrocytes), OLIG2 (oligodendrocytes), IBA1 (microglia) or FOXJ1 (ependymal cells). **L-P,** Low power images (20x) showing regions of the neocortex and corpus callosum where cingulate cortex is to the right. (I) Low power image (20x) showing ependymal cells lining the lateral ventricle and choroid plexus within the ventricle. High power images (60x) for the insets (boxed regions) respectively. Arrow heads represent examples of co-expression. Scale bar = 15 µm (**E,F**); 500 µm (**G-K**);100 µm (**L-P**).

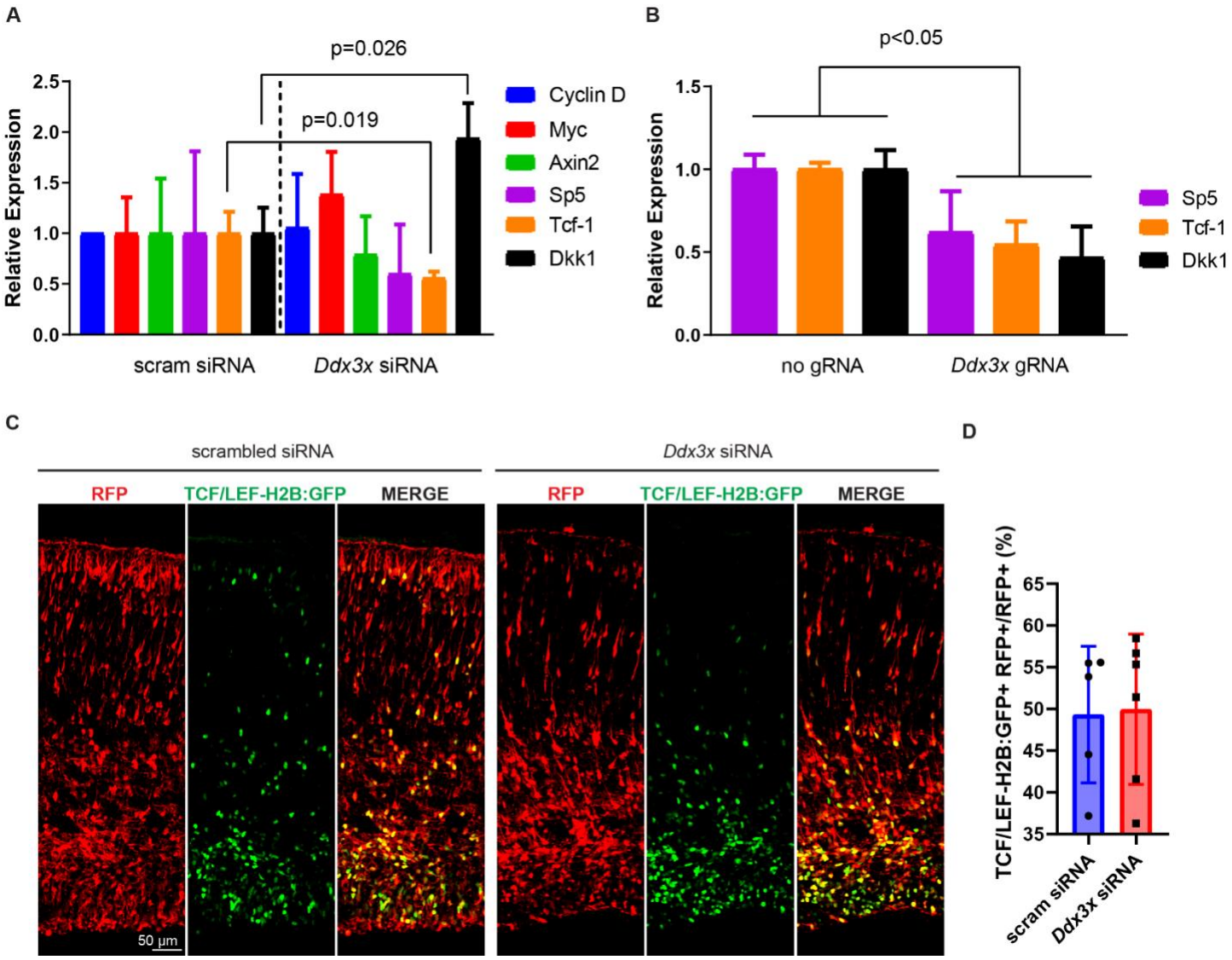


Supplemental Figure 3. Analyses of 3 day *in utero* electroporation of *Ddx3x* gRNA and siRNA, Related to Figure 3. A-C, Validation of *Ddx3x* sgRNA efficacy in Neuro2A cells assessed by depletion of mRNA and protein levels 3 days after transfection. Approximately 60% of cells were transfected. **A,** qPCR of *Ddx3x* mRNA in exons 5-6 normalized to *Actb* (n=3 biological replicates/condition, Student's t-test p=0.038). **B,** Representative western blot of Neuro2A cells treated with no sgRNA or *Ddx3x* sgRNA and probed for DDX3X and Tubulin. **C,** Quantification of western blots by densitometry, following normalization to Tubulin (n=3 biological

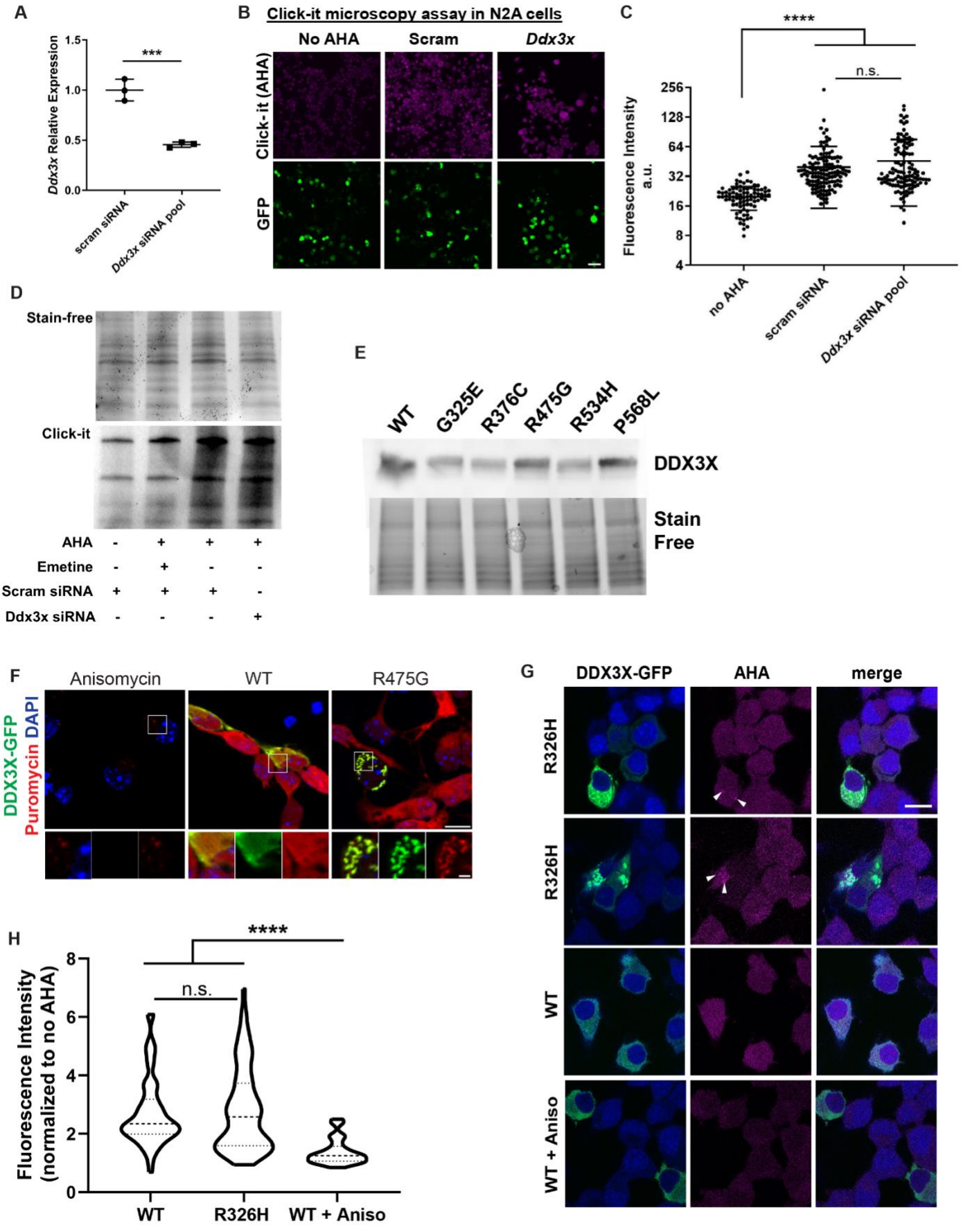
replicates/condition, Student's t-test, p=0.032). **D,** Representative magnified images of E17.5 brains *in utero* electroporated at E14.5 with pCAG-GFP and pX330-Cas9 no sgRNA or pX330-Cas9 *Ddx3x* sgRNA. Sections were stained with anti-GFP antibody (green) and anti-DDX3X antibody (white or red) to quantify DDX3X knockdown. **E**, Quantification of DDX3X protein knockdown assessed by change in fluorescence intensity in GFP+ cells (n=4 embryos (no gRNA) or 6 embryos (*Ddx3x* gRNA) with 2-3 slides/brain and 40 cells/slide). **F**, Representative magnified images of E17.5 brains *in utero* electroporated at E14.5 with pCAG-GFP and pX330-Cas9 no sgRNA or pX330-Cas9 *Ddx3x* sgRNA. Sections were stained with anti-GFP antibody (green). Dotted lines represent ventricular and pial surfaces, and brackets on the right refer to the bins. **G,** Quantitation of distribution of GFP-positive cells in five evenly spaced bins with Bin1 at the ventricle and Bin5 at the pia (n=4 embryos (no gRNA) or 7 embryos (*Ddx3x* gRNA), Two-way ANOVA (Sidak's) p<0.0001(Bin1); 0.0205(Bin2); >0.9999(Bin3); 0.0244(Bin4); <0.0001(Bin5)). **H,** Validation of *Ddx3x* siRNA efficacy in Neuro2A cells assessed by western blot three days after transfection. Representative western blot for DDX3X and Tubulin **I,** Quantification of western blots by densitometry, following normalization to Tubulin (n=3 biological replicates, Student's t-test p=0.047). **J,** Representative coronal sections of E17.5 brains *in utero* electroporated at E14.5 with pCAG-GFP and scrambled or *Ddx3x* siRNAs stained with anti-GFP (green). **K,** Quantification of distribution of GFP-positive cells in five evenly spaced bins (n=8 embryos (scrambled), or 9 embryos (*Ddx3x)*, Two-way ANOVA (Sidak's) p=0.0668(Bin1); 0.4015 (Bin2); 0.9999(Bin3); 0.8320(Bin4); 0.0076(Bin5)). Scale bars: 15 μm (**D);** 50 μm (**F,J**). Error bars = standard deviation. *p≤0.05, **p≤0.01, *** p≤0.001, ****p≤0.0001.



Supplemental Figure 4. Neuron-specific depletion of *Ddx3x* does not significantly impact neuronal migration, Related to Figure 4. A, Representative coronal sections of P2 brains *in utero* co-electroporated at E14.5 with *pDcx*-mCherry and either pX330-*Dcx*-Cas9 or pX330-*Dcx*-Cas9 plus *Ddx3x* sgRNA. Sections were stained with anti-RFP antibody (red). Dotted lines represent ventricular and pial surfaces, and brackets on the right refer to the bins. **B,** Quantitation of distribution of RFP-positive cells in five evenly spaced bins with Bin1 at the ventricle and Bin5 at the pia (n=2 embryos, no gRNA; 5 embryos, *Ddx3x* gRNA), Two-way ANOVA (Sidak's) p=0.9999(Bin1); 0.9997 (Bin2); 0.4056(Bin3); 0.8514(Bin4); 0.8951(Bin5). Scale bar: 50 μm. Error bars = standard deviation.



Supplemental Figure 5. Impact of *Ddx3x* depletion in the developing cortex upon Wnt signaling, Related to Figure 4. A, RT-qPCR analysis of Wnt signaling targets in Neuro2A cells treated with scrambled or *Ddx3x* siRNAs (n=3 biological replicates). **B**, RT-qPCR analysis of Wnt signaling targets in FACS isolated GFP+ cells from E15.5 embryos electroporated at E13.5 with pCAG-GFP and pX330-Cas9 no sgRNA (n= 5 embryos) or pX330-Cas9 *Ddx3x* sgRNA (n= 4 embryos).**C**, Representative images of coronal sections of E17.5 brains *in utero* electroporated at E14.5 with pCAG-mCherry, TCF/LEF-H2B:GFP, and scrambled or *Ddx3x* siRNAs. Sections were stained with anti-RFP (red) and anti-GFP (green). **D,** Quantification of percentage of RFP+ cells also positive for TCF/LEF H2B:GFP (n=5 embryos (scrambled) or 6 embryos (*Ddx3x)*, Student's *t*-test p=0.9045). Scale bars: 50 µm (**C**). Error bars = standard deviation.


A
Ddx3x Relative Expression
1.5
1.0
0.5
0.0
***
scram siRNA
Ddx3x siRNA pool
B
Click-it microscopy assay in N2A cells
No AHA
Scram
Ddx3x
Click-it (AHA)
GFP
C
****
n.s.
Fluorescence Intensity a.u.
256
128
64
32
16
8
4
no AHA
scram siRNA
Ddx3x siRNA pool
D
Stain-free
Click-it
AHA - + + +
Emetine - + - -
Scram siRNA + + + -
Ddx3x siRNA - - - +
E
WT
G325E
R376C
R475G
R534H
P568L
DDX3X
Stain Free
F
Anisomycin
WT
R475G
DDX3X-GFP
Puromycin DAPI
G
DDX3X-GFP
AHA
merge
R326H
R326H
WT
WT + Aniso
H
****
n.s.
Fluorescence Intensity (normalized to no AHA)
8
6
4
2
0
WT
R326H
WT + Aniso

Supplemental Figure 6. *Ddx3x* missense mutations or reduced expression does not globally impair translation, Related to Figures 6 and 7. A, RT-qPCR validation of *Ddx3x* knockdown in Neuro2A cells transfected with scrambled or *Ddx3x* siRNAs (n=3 biological replicates). **B**, FUNCAT/Click-it assay in Neuro2A cells transfected with GFP and scrambled or *Ddx3x* siRNAs and subsequently incubated in methionine-free media and supplemented with a methionine analog, L-azidohomoalaine (AHA) or no AHA. **C**, Quantification of fluorescent-based readout of AHA incorporation in GFP+ Neuro2A cells (N=2 biological replicates with >50 cells per condition; ****p<0.0001 for scrambled and *Ddx3x* siRNAs relative to no AHA). **D**, FUNCAT/Click-it assay (western-based readout) in Neuro2A cells transfected with GFP and scrambled or *Ddx3x* siRNAs and subsequently incubated in methionine-free media and supplemented with AHA or no AHA or AHA + translation elongation inhibitor, emetine (N=3 biological replicates with 1 representative image shown). **E**, Protein levels of Neuro2A cells transfected with WT or missense mutant *DDX3X*. **F**, Puromycin incorporation assay to monitor translation in primary cortical cells 24 hours after transfection with R475G GFP-DDX3X (green). Puromycin signal (red) is blocked with the translation inhibitor, anisomycin. **G,** FUNCAT/Click-it assay in Neuro2A cells transfected with GFP and WT or R326H DDX3X subsequently incubated in methionine-free media and supplemented with AHA or no AHA or AHA + translation inhibitor, anisomycin (N=3 biological replicates with 1 representative image shown). **H**, Quantification of fluorescent-based readout of AHA incorporation in GFP+ Neuro2A cells (N=3 biological replicates with >20 cells per condition). Scale bars= 50 μm (**B**); 10 μm (upper panels, **F**), 2 μm (lower panels, **F**); 15 μm (**G**). Student's unpaired, two-tailed t-test (**A**) and One-way ANOVA with multiple correction's (Tukey's) (**C, H**). Error bars = standard deviation. *p≤0.05, **p≤0.01, *** p≤0.001, ****p≤0.0001. Panels D and E were adjusted with white boxes and rotated to correct for unevenness.