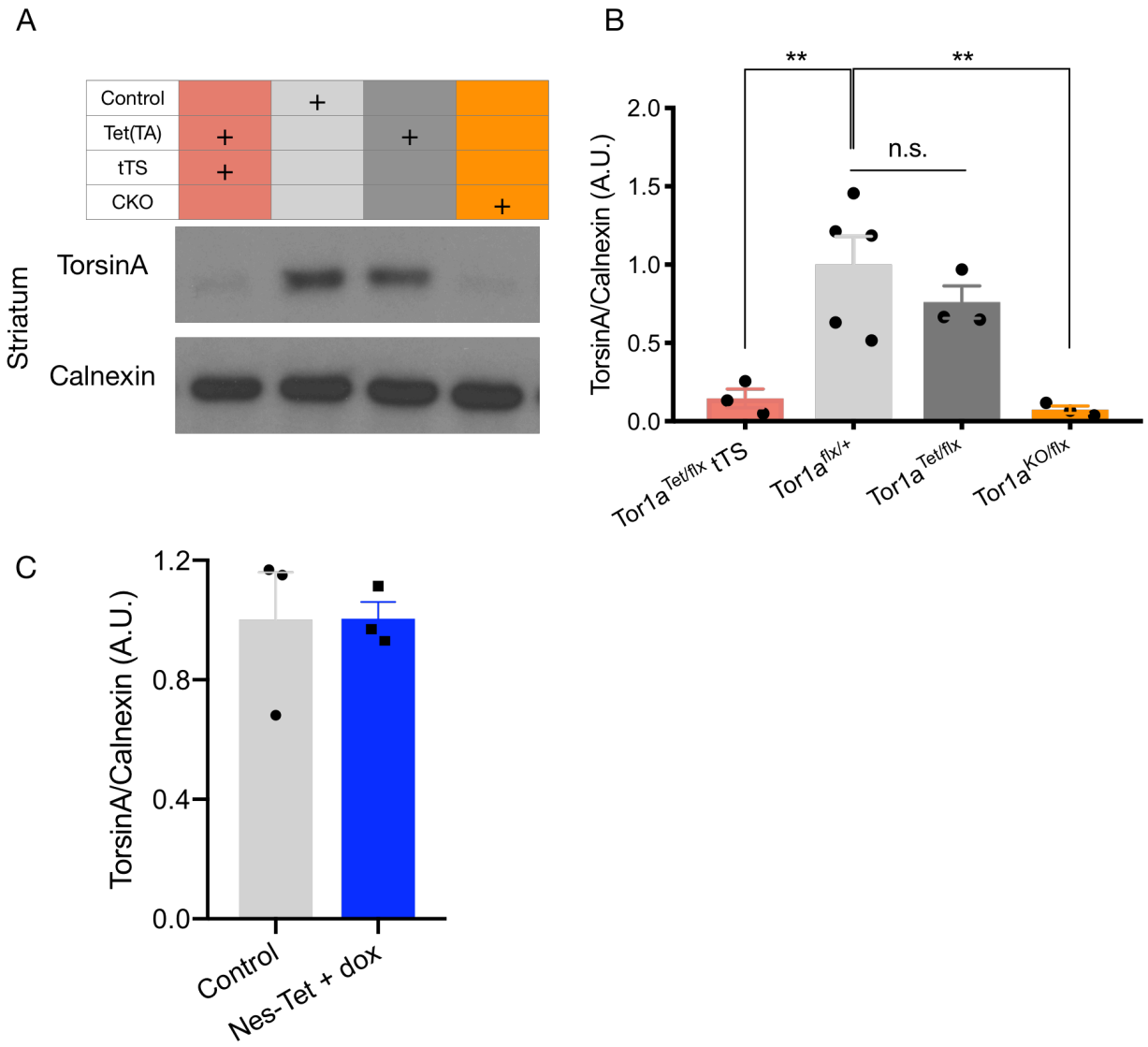
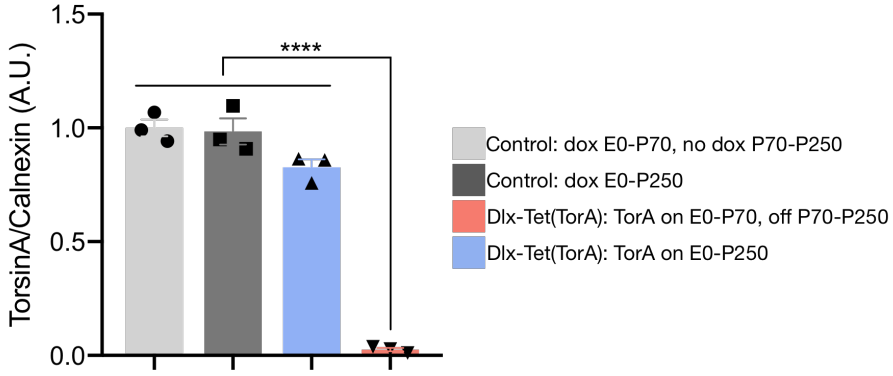
Associated with Figure 1

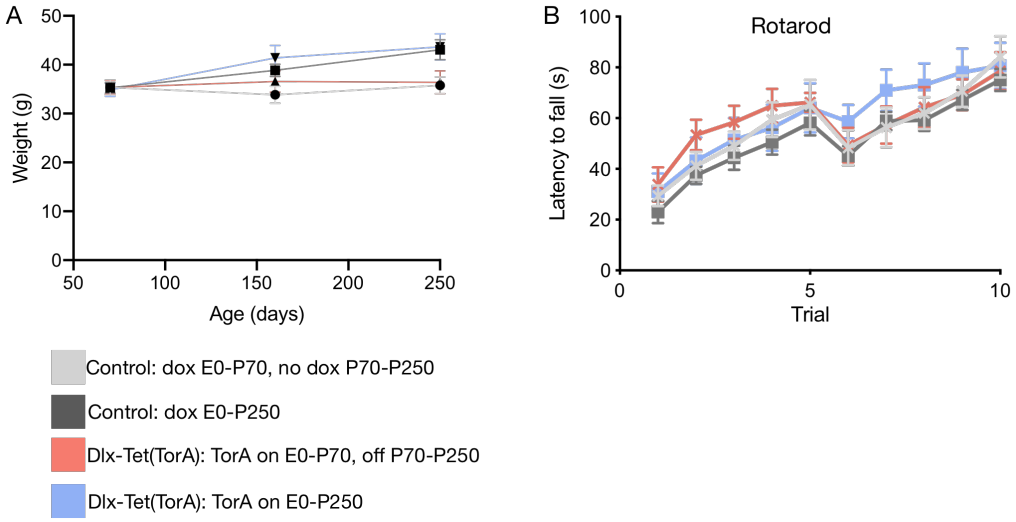


Supplemental Figure 1. Further analysis and quantification of tetracycline-inducible torsinA gene expression. (A) Western blot analysis of torsinA expression in striatal lysates from Dlx-Tet(TorA) mice with and without *ß-actin tTS* in the absence of doxycycline. **(B)** Quantification of western blot in Figure 1D. *ß-actin* tTS suppresses torsinA expression from the Tet(TorA) allele to levels comparable to that of Dlx-CKO mice. The Tet(TorA) allele expresses to levels comparable to that of *Cre* control mice in the absence of tTS (one-way ANOVA; $p = 0.0021$; $n = 3$-5 per group). **(C)** Quantification of western blot in Figure 1C. Doxycycline administration relieves tTS suppression, enabling full torsinA expression from the Tet(TorA) allele. Data for **(C)** analyzed by unpaired t-test. No significant differences were found and $n = 3$ for all groups.

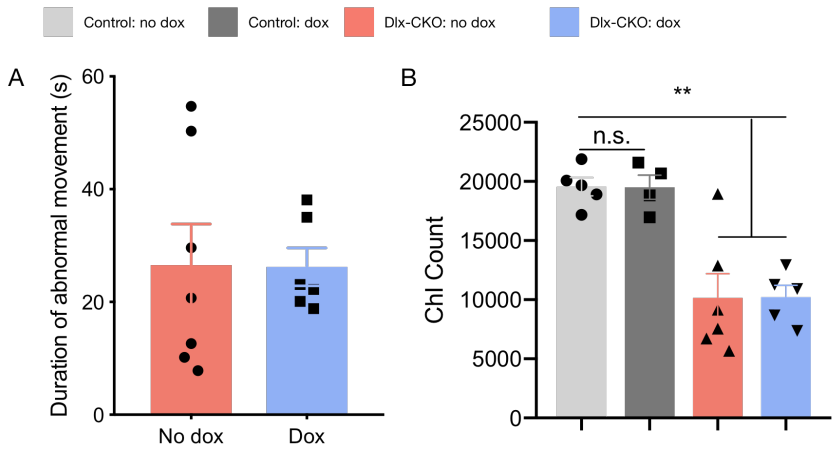
Associated with Figure 2



Supplemental Figure 2. Doxycycline withdrawal at P70 results in loss of striatal torsinA expression in Dlx-Tet(TorA) mice. Quantification of western blot in Figure 2E. TorsinA is suppressed following doxycycline withdrawal in Dlx-Tet(TorA) mice (two-way ANOVA; **** = $p < 0.0001$; $n = 3$ for all groups).

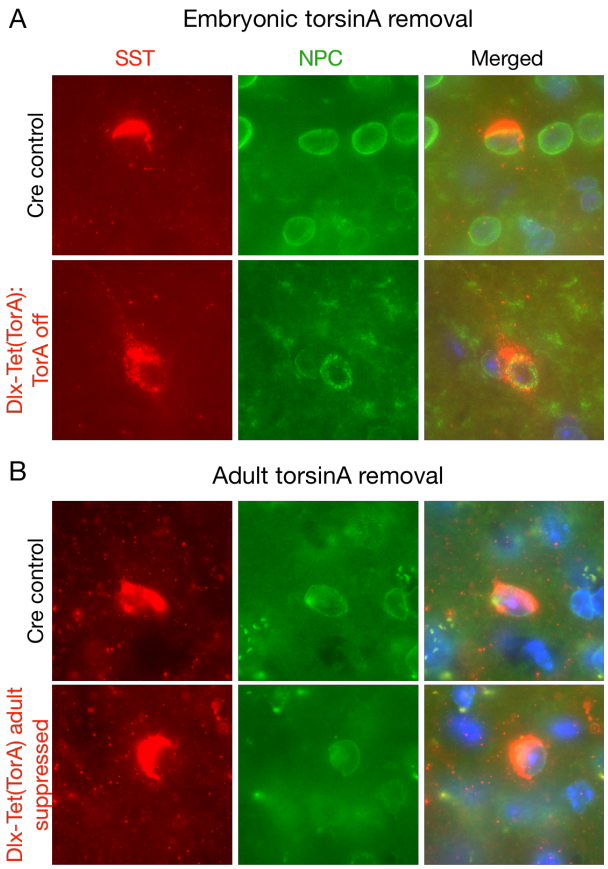


Supplemental Figure 3. Weight and motor learning are not impacted by adult forebrain torsinA suppression. (A) Weights of Dlx-Tet(TorA) mice following adult torsinA suppression. Forebrain torsinA inactivation starting at P70 does not alter weight (two-way ANOVA main effect of experimental group $F_{3,40} = 1.914$, $p = 0.1428$, time $F_{1.429, 57.18} = 17.50$, $p < 0.0001$, interaction $F_{6,80} = 118.8$, $p < 0.0003$; no significant differences between experimental groups at each timepoint by Tukey's multiple comparisons tests; *Cre* control dox off at P70 n = 10, *Cre* control dox continued at P70 n = 12, Dlx-Tet(TorA) dox off at P70 n = 10, Dlx-Tet(TorA) dox continued at P70 n = 12). **(B)** Rotarod performance of Dlx-Tet(TorA) mice following adult torsinA suppression. TorsinA suppression in adult forebrain does not impair motor learning (two-way ANOVA main effect of experimental group $F_{3,40} = 0.4702$, $p = 0.7048$, trial $F_{9.360} = 65.03$, $p < 0.0001$, interaction $F_{27, 360} = 1.291$, $p = 0.1547$; same sample sizes as panel **(A)**).

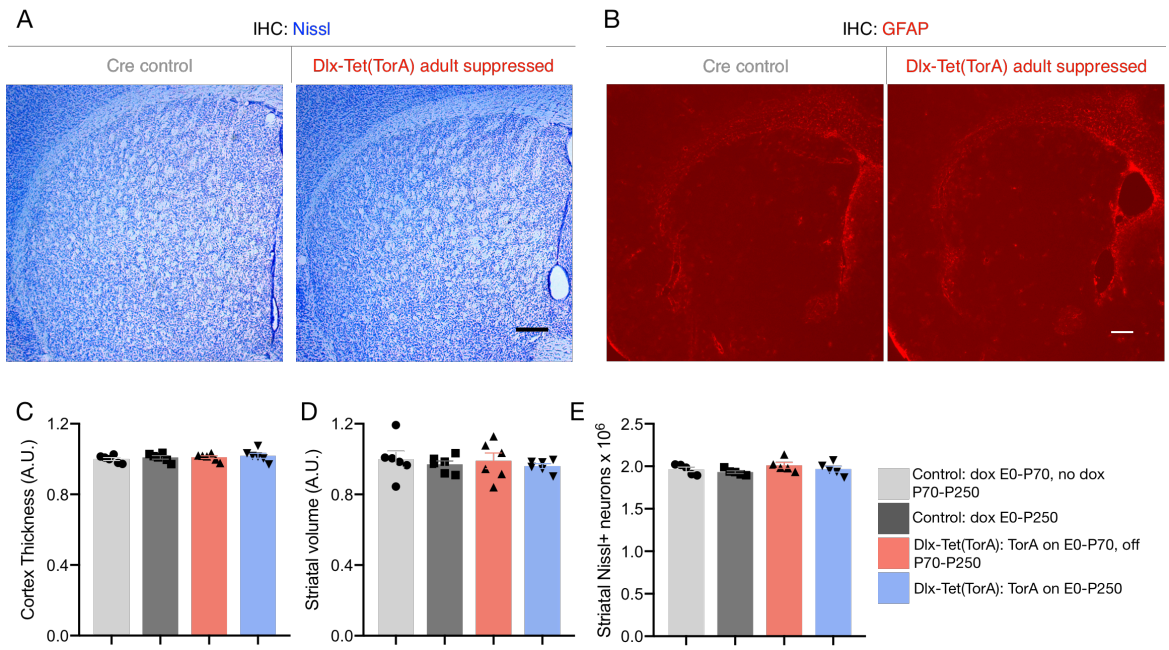


Supplemental Figure 4. Doxycycline does not change the motor phenotype of Dlx-CKO mice. **(A)** Duration of abnormal movements during one minute of tail suspension in Dlx-CKO mice, which lack a tetracycline-responsive allele, treated with doxycycline for their entire lives. Data analyzed by unpaired t-test. No significant differences were found and n = 6-7 per group. **(B)** ChI counts from Cre control and Dlx-CKO (no tetracycline-responsive allele) mice in the presence of absence of doxycycline. Doxycycline does not influence ChI degeneration in a torsinA-independent manner (two-way ANOVA; $p < 0.0001$; n = 4-6 per group).

Associated with Figure 3

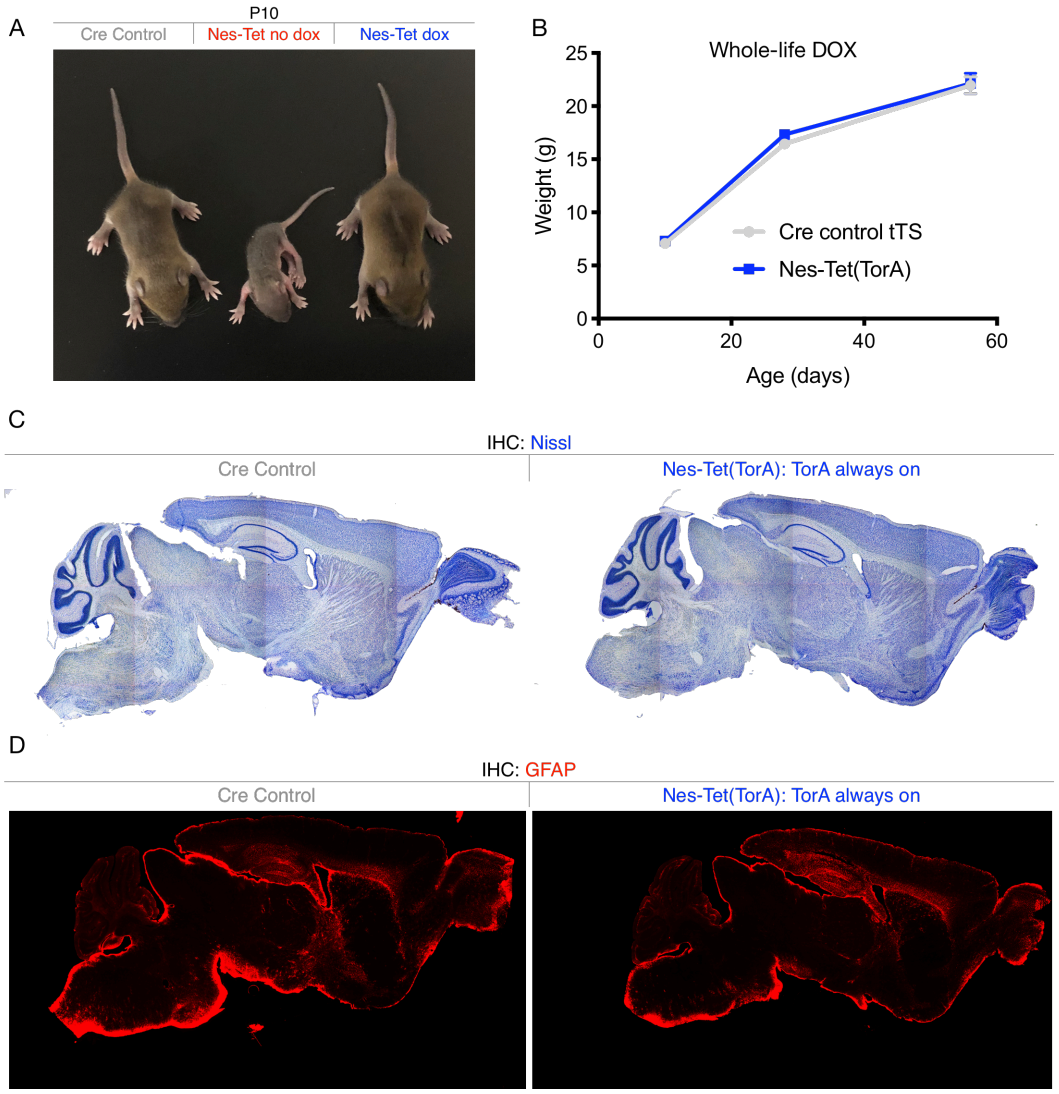


Supplemental Figure 5. Representative images of nuclear pore immunoreactivity following prenatal and adult suppression of torsinA. (A) Nuclear pore complex (mab414) staining in SST+ cortical neurons in P70 Dlx-Tet(TorA) mice following prenatal torsinA suppression. Embryonic forebrain torsinA suppression causes abnormal nuclear pore clustering. **(B)** Nuclear pore complex (mab414) staining in SST+ cortical neurons in Dlx-Tet(TorA) following adult torsinA suppression. Adult forebrain torsinA suppression does not cause abnormal nuclear pore clustering.

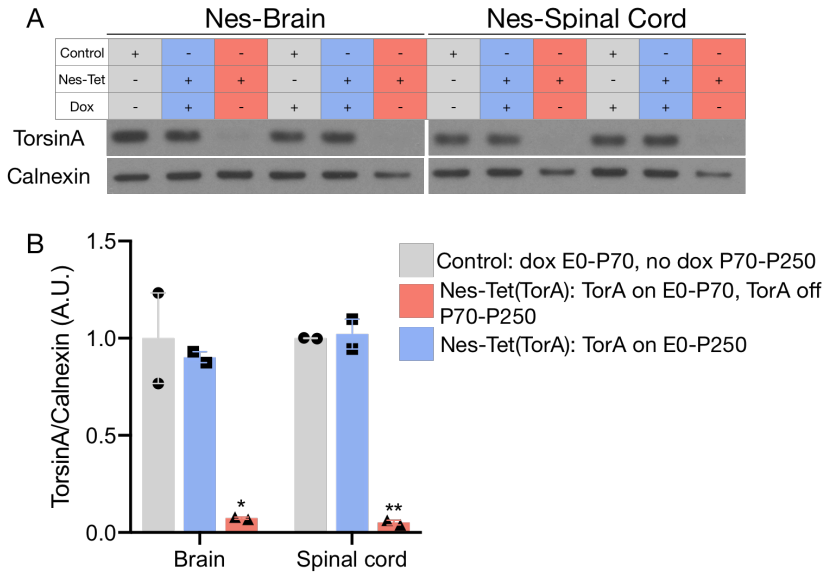


Supplemental Figure 6. Morphological and histological analysis following adult removal of torsinA from the forebrain. (A) Representative image of Nissl stained coronal section from Dlx-Tet(TorA) mice following adult torsinA suppression. Scale bar represents 200 µm. **(B)** Representative image of GFAP stained coronal section from Dlx-Tet(TorA) mice following adult torsinA suppression. Scale bar represents 200 µm. **(C)** Cortical thickness of Dlx-Tet(TorA) mice following adult torsinA suppression. Adult forebrain torsinA suppression does not alter cortical thickness (two-way ANOVA main effect of genotype $F_{1, 20} = 0.9041$, $p = 0.3530$, treatment $F_{1, 20} = 0.7355$, $p = 0.4013$, interaction $F_{1, 20} = 0.0027$, $p = 0.9593$; $n = 6$ for all groups). **(D)** Striatal volume of Dlx-Tet(TorA) mice following adult torsinA suppression. Adult forebrain torsinA suppression does not change the volume of the striatum (two-way ANOVA main effect of genotype $F_{1, 20} = 0.08528$, $p = 0.7733$, treatment $F_{1, 20} = 0.7626$, $p = 0.3929$, interaction $F_{1, 20} = 3.33 \times 10^{-6}$, $p = 9986$; $n = 6$ for all groups). **(E)** Striatal Nissl+ small and medium neuron counts in Dlx-Tet(TorA) mice following adult torsinA suppression. Adult forebrain torsinA suppression does not change total striatal neuron count, assessed by unbiased stereology (two-way ANOVA main effect of genotype $F_{1, 20} = 1.981$, $p = 0.1784$, treatment $F_{1, 20} = 1.745$, $p = 0.2051$, interaction $F_{1, 20} = 0.0372$, $p = 0.8495$; $n = 5$ for all groups).

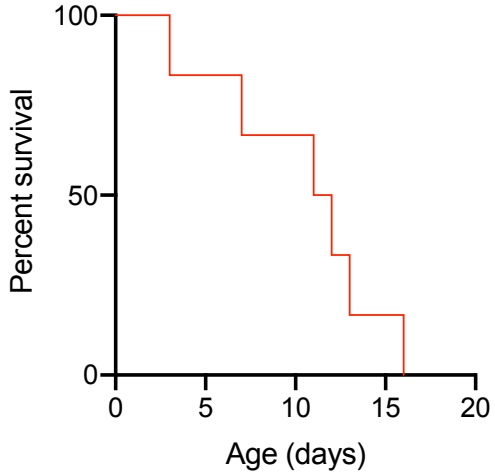
Associated with Figure 4



Supplemental Figure 7. Characterization of Nes-Tet(TorA) mice with torsinA expression continuously activated by doxycycline. (A) Image of P10 *Cre* control, Nes-Tet(TorA) torsinA suppressed, and Nes-Tet(TorA) torsinA activated pups. **(B)** Growth curves of Nes-Tet(TorA) mice maintained on doxycycline chow for their entire lives (two-way ANOVA main effect of genotype $F_{1, 14} = 0.5615$, $p = 0.4661$, age $F_{2, 28} = 390$, $p < 0.0001$, interaction $F_{2, 28} = 0.2573$, $p = 0.7750$; *Cre* control n = 9, Nes-Tet(TorA) n = 7). **(C)** Representative Nissl stained sagittal sections from P70 Nes-Tet(TorA) mice maintained on doxycycline chow for their entire lives. **(D)** Representative GFAP immunoreactivity in sagittal sections from P70 Nes-Tet(TorA) mice maintained on doxycycline chow for their entire lives.

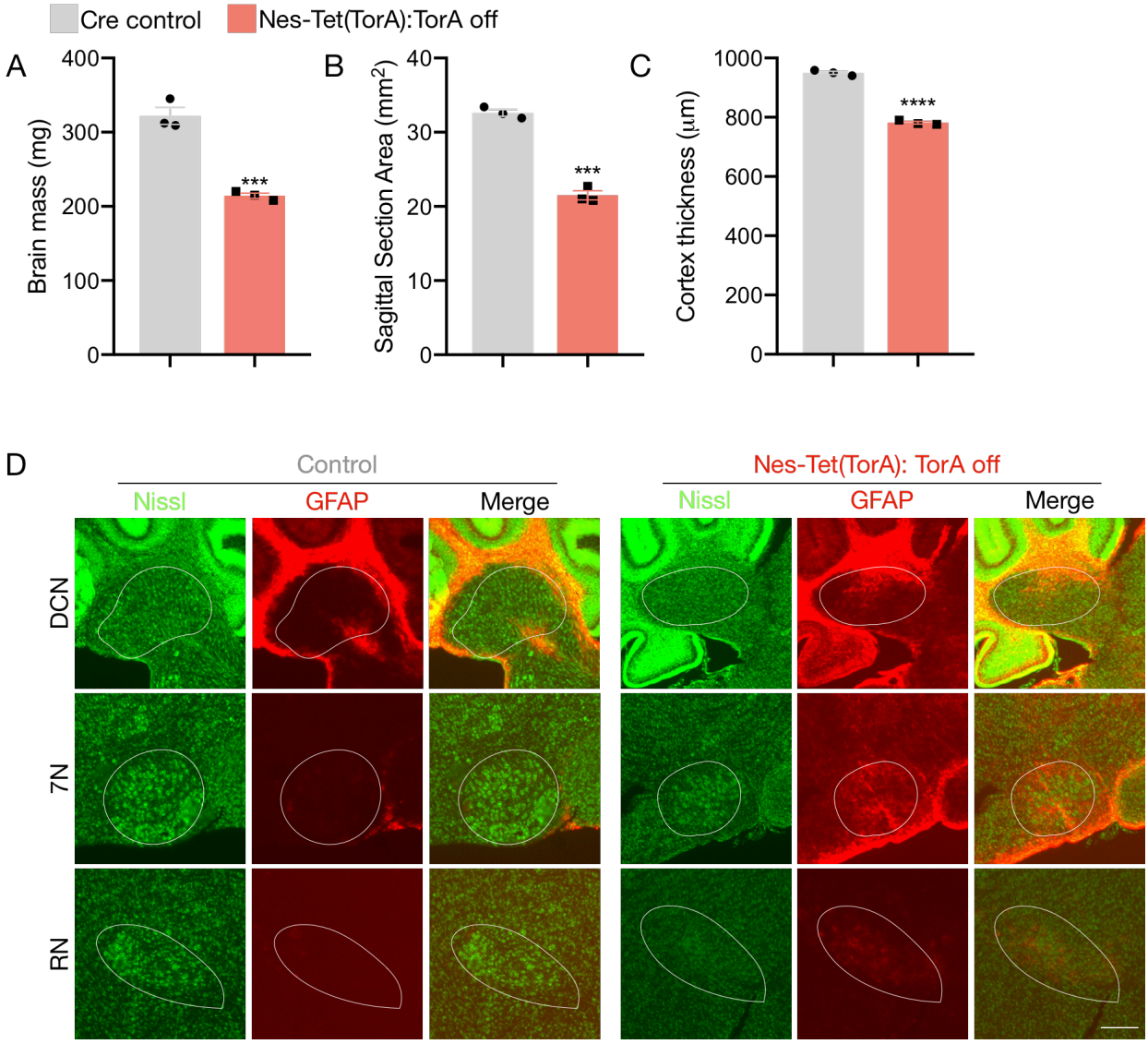


Supplemental Figure 8. Withdrawal of doxycycline at P70 results in loss of CNS torsinA expression in Nes-Tet(TorA) mice. (A) Western blot analysis of torsinA expression in whole brain and spinal cord lysates from Nes-Tet(TorA) mice following adult torsinA suppression. TorsinA is expressed in Nes-Tet(TorA) mice fed doxycycline chow, but is suppressed in Nes-Tet(TorA) mice switched to regular chow in adulthood. **(B)** Quantification of western blot in Figure S8A. TorsinA is suppressed following doxycycline withdrawal in Nes-Tet(TorA) mice.n = 2 per group. Data analyzed by one-way ANOVA with Dunnett's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$.

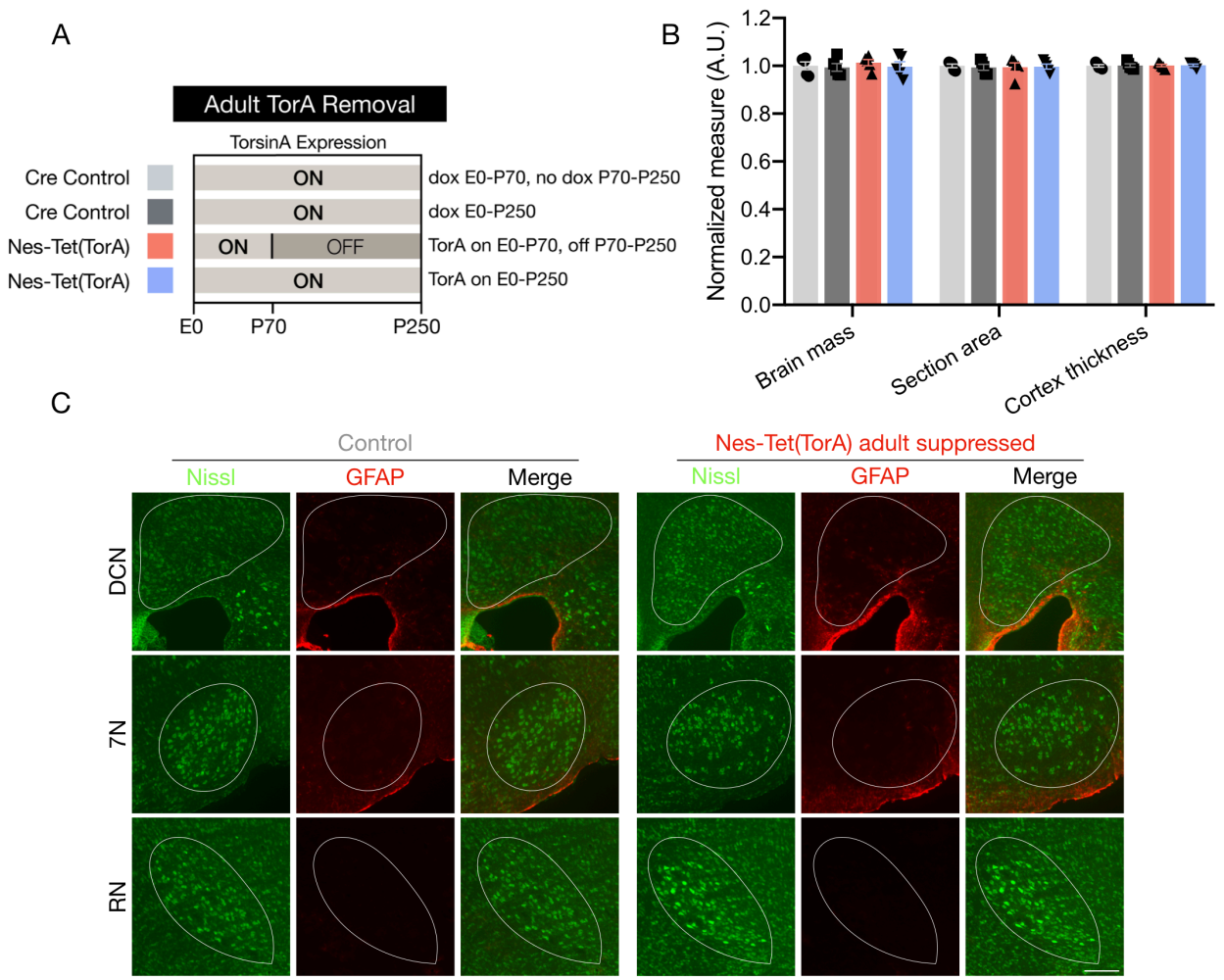


Supplemental Figure 9. Doxycycline does not rescue early lethality in N-CKO mice. Survival curve of Nes-CKO mice, which lack a tetracycline-responsive allele, treated with doxycycline starting at E0. Doxycycline does not rescue lethality. n = 6.

Associated with Figure 5

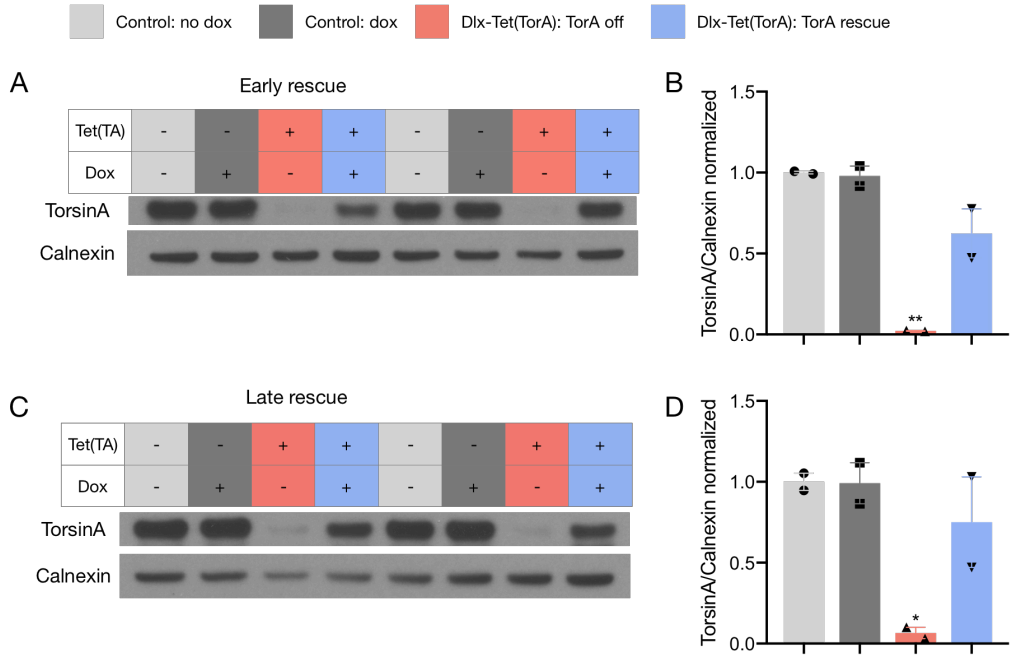


Supplemental Figure 10. Analysis of morphological and gliotic changes in the brains of Nes-Tet(TorA): TorA off mice at P8. (A) Brain mass of control and Nes-Tet(TorA) mice following prenatal torsinA suppression. Brain mass is reduced in Nes-Tet(TorA) mice. **(B)** Sagittal section area of control and Nes-Tet(TorA) mice following prenatal torsinA suppression. Sagittal section area is reduced in Nes-Tet(TorA) mice. **(C)** Cortex thickness of Nes-Tet(TorA) mice following prenatal torsinA suppression. Cortex thickness is reduced in Nes-Tet(TorA) mice. **(D)** Representative images of DCN, 7N, and RN from Nes-Tet(TorA) mice following prenatal torsinA suppression immunostained with an antibody targeted to GFAP. These regions exhibit reactive astrogliosis in Nes-Tet(TorA) mice. Scale bar represents 200 µm. Data analyzed by unpaired t-test. *** $p < 0.001$, **** $p < 0.0001$. $n = 3$ for both groups.



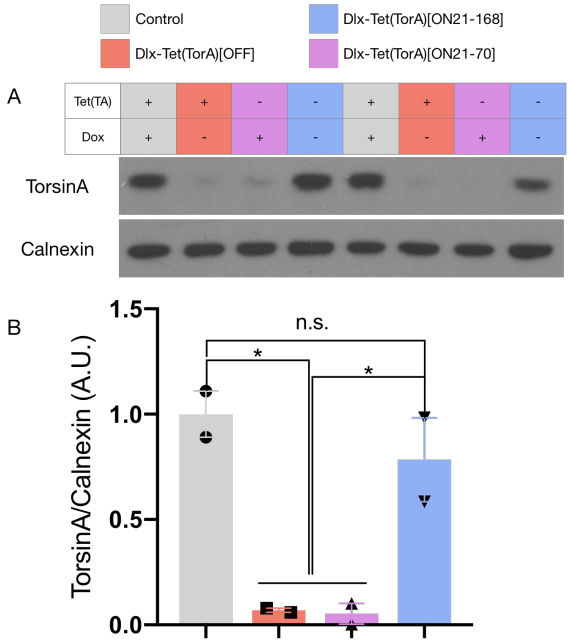
Supplemental Figure 11. Further analysis of the brains of Nes-Tet(TorA) mice following adult torsinA suppression. (A) Schematic of experimental design for adult torsinA suppression in the Nestin-Cre field. Light gray (ON) areas of bars represent ages when torsinA is expressed and dark gray (OFF) areas represent ages when torsinA is suppressed. Each color corresponds to an experimental group in subsequent graphs. **(B)** Morphological analyses of the brains of Nes-Tet(TorA) mice following adult torsinA suppression. Brain mass (two-way ANOVA main effect of genotype $F_{1, 16} = 0.2529$, $p = 0.6219$, treatment $F_{1, 16} = 0.4823$, $p = 4973$, interaction $F_{1, 16} = 0.08258$, $p = 0.7775$), sagittal section area (two-way ANOVA main effect of genotype $F_{1, 16} = 0.006184$, $p = 0.9383$, treatment $F_{1, 16} = 0.02658$, $p = 0.8725$, interaction $F_{1, 16} = 0.1002$, $p = 0.7557$), and cortex thickness (two-way ANOVA main effect of genotype $F_{1, 16} = 0.04771$, $p = 0.8299$, treatment $F_{1, 16} = 0.05195$, $p = 0.8226$, interaction $F_{1, 16} = 0.001596$, $p = 0.9686$) are not altered by adult torsinA suppression in the entire nervous system ($n = 5$ for all groups). **(C)** Representative images of DCN, 7N, and RN from Nes-Tet(TorA) mice following adult torsinA suppression immunostained with an antibody targeted to GFAP. These regions do not exhibit reactive astrogliosis (quantified in Figure 5F). Scale bar represents 200 μm.

Associated with Figure 6



Supplemental Figure 12. TorsinA expression analysis of Dlx-Tet(TorA) juvenile and adult torsinA rescued mice. (A) Western blot analysis of Dlx-Tet(TorA) juvenile rescue mice. **(B)** Quantification of the western blot presented in **(A)**. TorsinA is expressed following doxycycline administration in Dlx-Tet(TorA) rescue mice. n = 2 per group. **(C)** Western blot analysis of Dlx-Tet(TorA) adult rescue mice. **(D)** Quantification of the western blot presented in **(C)**. TorsinA is expressed following doxycycline administration in Dlx-Tet(TorA) rescue mice. n = 2 per group. Data analyzed with one-way ANOVA and Tukey's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$.

Associated with Figure 7



Supplemental Figure 13. TorsinA expression analysis of P168 Dlx-Tet(TorA) mice treated with doxycycline from P21 until P70 or until P168. (A) Western blot analysis of striatal lysates from Dlx-Tet(TorA) critical therapeutic period mice. **(B)** Quantification of the western blot presented in **(A)**. Doxycycline treatment from P21 to P168 results in robust torsinA expression, while Dlx-Tet(TorA) mice never given doxycycline or taken off doxycycline from P70 to P168 do not express torsinA. n = 2 per group. Data analyzed with one-way ANOVA and Tukey's multiple comparisons test. * $p < 0.05$.

Supplemental Methods

Mice

Nestin-Cre and *Dlx5/6-Cre* mice were obtained from the Jackson Laboratory. Mice were maintained at the University of Michigan and the University of Texas Southwestern Medical Center. Mice were genotyped for *Tor1a*, *Cre*, and *tTS* as previously described [1, 2]. Genotyping of the novel Tet(TorA) line is described below:

Gene	Primer name	Primer sequence	Cycle	Band sizes
Tet(TorsinA)	Tet-F	AAA GTG AAA GTC GAG CTC GGT A	94°C, 3 min; 94°C, 30 sec; 62°C, 30 sec; 72°C, 30 sec, 30 cycles; 72°C, 5 min	WT – 313 bp Mutant– 300 bp
	WT/Tet-R	GCA GTA GAG ACG AGG GTA GGA GAT A		
	WT-F	AGA AGC AGA AGG GAC TTT GCC CTT A		

Mice were housed in temperature and light-controlled colony rooms and provided access to food and water ad libitum. Doxycycline was administered P.O. using 200 mg/kg doxycycline rodent pellets from Bio-Serv. Mice of all genotypes were housed together in cages of 2-5 mice to prevent environmental bias. Colony rooms were maintained on a 12-hour light/dark cycle. Behavioral testing occurred exclusively during the light cycle. Age- and sex-matched littermates were used as controls. Controls utilized were *Cre* controls (generally Cre;*Tor1a*$^{flx/+}$;B-actin tTS unless otherwise noted).

Western blotting

Mice were anesthetized with isoflurane, decapitated, and tissues were rapidly removed. Brain, spinal cord, striatum, and/or liver were dissected and placed into tubes containing lysis buffer consisting of TBS with 1% sodium dodecyl sulfate, 1 mM dithiothreitol, and Halt protease inhibitor cocktail (Thermo Scientific #78437). Tissues were homogenized using a plastic plunger, then centrifuged at 12,000 rpm for 5min. Supernatants were collected and transferred to a new tube, and BCA protein assay was performed to determine protein concentration. Final lysates were prepared at 1 μg/μL including sample buffer (Invitrogen NP0007). 5 μg protein samples and Dual Precision Plus standards were run on a 4-12% Bis-Tris gels (Invitrogen NP0323). Transfer was performed at 350 mA for 1-hour at 4°C onto a 0.22 μm PVDF membrane. Membranes were washed in TBS with 0.1% Tween-20 (TBS-T) and blocked with 5% non-fat dry milk in TBS-T. They were then incubated with primary antibody (torsinA 1:10,000, rabbit anti-calnexin 1:20,000) overnight at 4°C. Membranes were then incubated with HRP-conjugated anti-rabbit secondary antibody (1:20,000) for 1-hour at room temperature (RT). Bands were visualized using Supersignal West Pico PLUS substrate (Thermo Scientific #34580) and exposed to x-ray film (Thermo Scientific #34090). Films were scanned and band intensity was quantified in ImageJ.

Immunohistochemistry

Mice were anesthetized with a lethal dose of ketamine/xylazine and transcardially perfused with 10 mL of 0.1 M phosphate buffered saline (PBS) followed by 10 mL of 4% paraformaldehyde in

0.1 M phosphate buffer (PB). After 2 hours of postfixation in 4% paraformaldehyde in 0.1 M PB, brains were transferred to 20% sucrose in 0.1 M PB for cryoprotection. Serial 40 μm frozen sections were generated on a cryostat and stored in PBS. For immunofluorescence, sections were permeabilized in PBS with 0.1% Triton-X (PBS-Tx), blocked in PBS-Tx with 5% normal donkey serum (NDS), and incubated with primary antibodies diluted in PBS-Tx with 1.5% NDS overnight at 4°C. Sections were then washed with PBS-Tx, incubated with secondary antibodies diluted in PBS-Tx for 1-hr at RT, washed in PBS-Tx, and mounted on glass slides. For DAB (3,3' Diaminobenzidine) staining, sections were permeabilized with PBS-Tx, quenched with 0.3% H_2O_2 in PBS, and blocked in PBS-Tx with 5% normal donkey serum. They were then incubated with primary antibodies diluted in PBS-Tx with 1.5% NDS overnight at 4°C. Sections were washed with PBS-Tx, incubated with biotinylated secondary antibody diluted in PBS-Tx for 1-hr at RT, and incubated with ABC (Vectastain Elite ABC-HRP Kit, Peroxidase (Standard); Vector laboratories PK-6100) for 1-hr at RT. Sections were exposed to DAB and quenched and washed with PBS after chromogenic reaction. Sections were then mounted on glass slides.

Stereology parameters

Region and cell type	Counting frame (μm)	Grid size (μm)
Striatal ChIs	100 × 100	250 × 250
Striatal medium and small neurons	20 × 20	600 × 600
Medial DCN neurons	90 × 90	150 × 150
7N neurons	90 × 90	200 × 200

References

1. Liang, C.C., et al., *TorsinA hypofunction causes abnormal twisting movements and sensorimotor circuit neurodegeneration.* J Clin Invest, 2014. **124**(7): p. 3080-92.
2. Mallo, M., B. Kanzler, and S. Ohnemus, *Reversible gene inactivation in the mouse.* Genomics, 2003. **81**(4): p. 356-60.