FoxP1 orchestration of ASD-relevant signaling pathways in the striatum

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Supplemental Information

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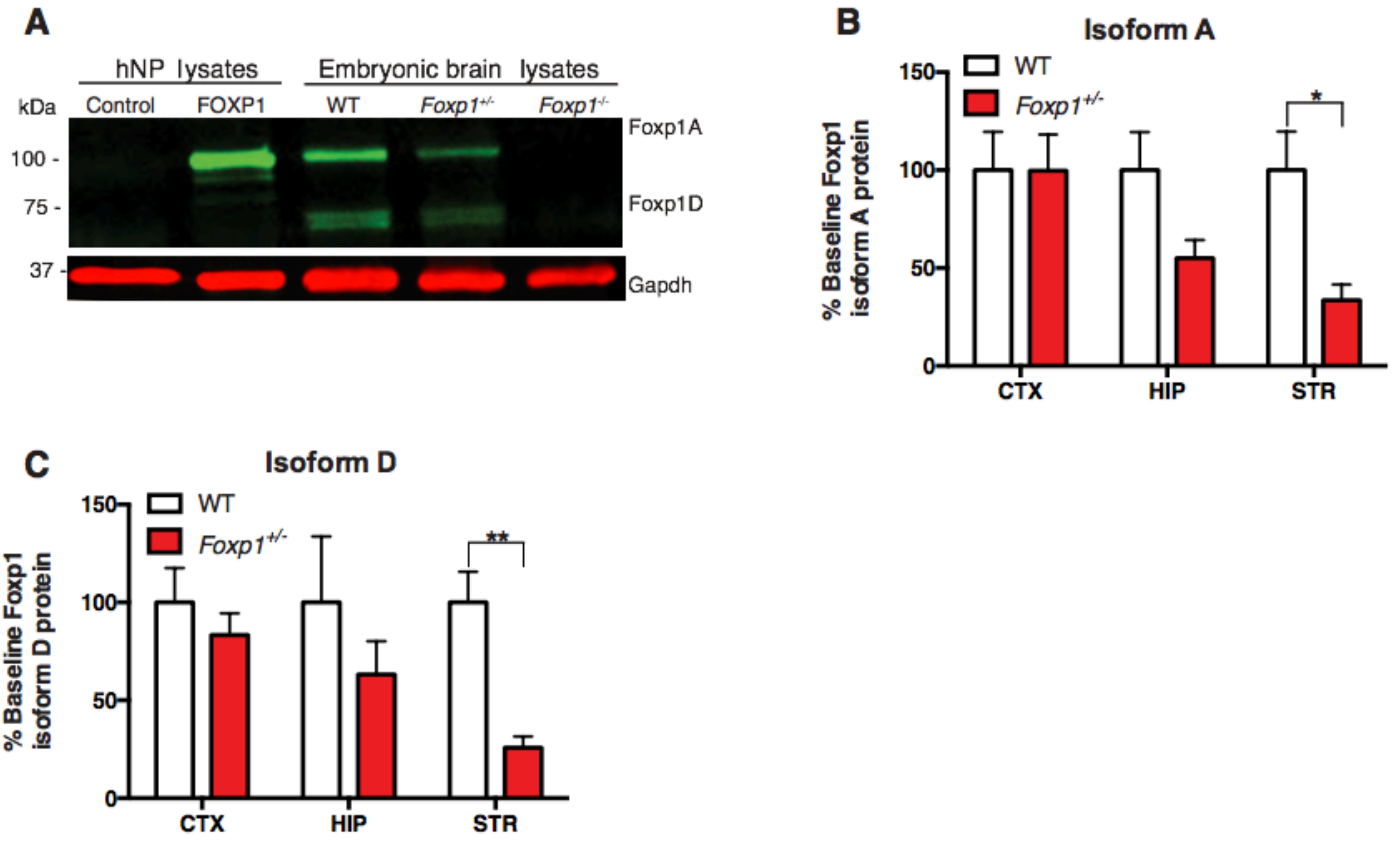
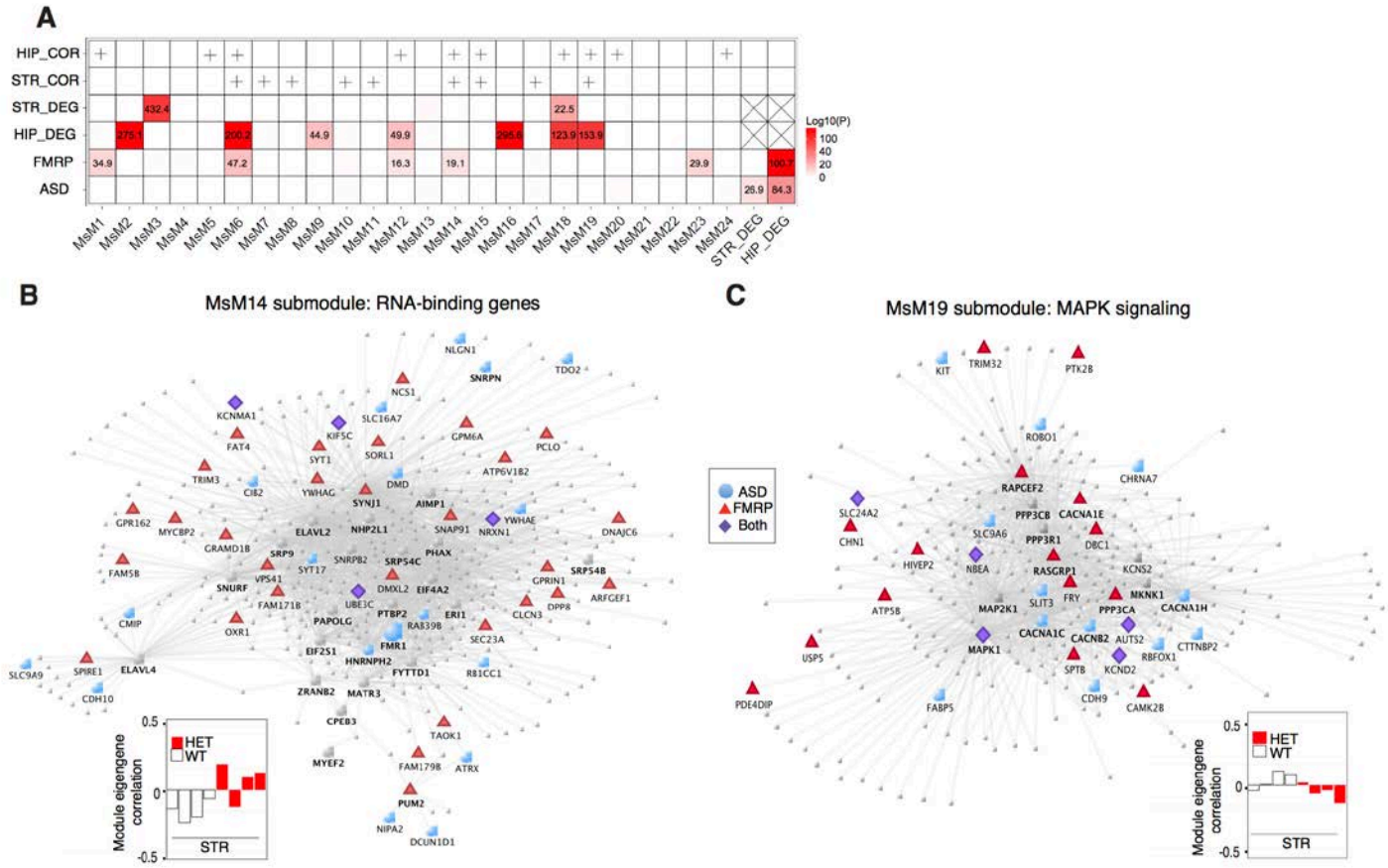
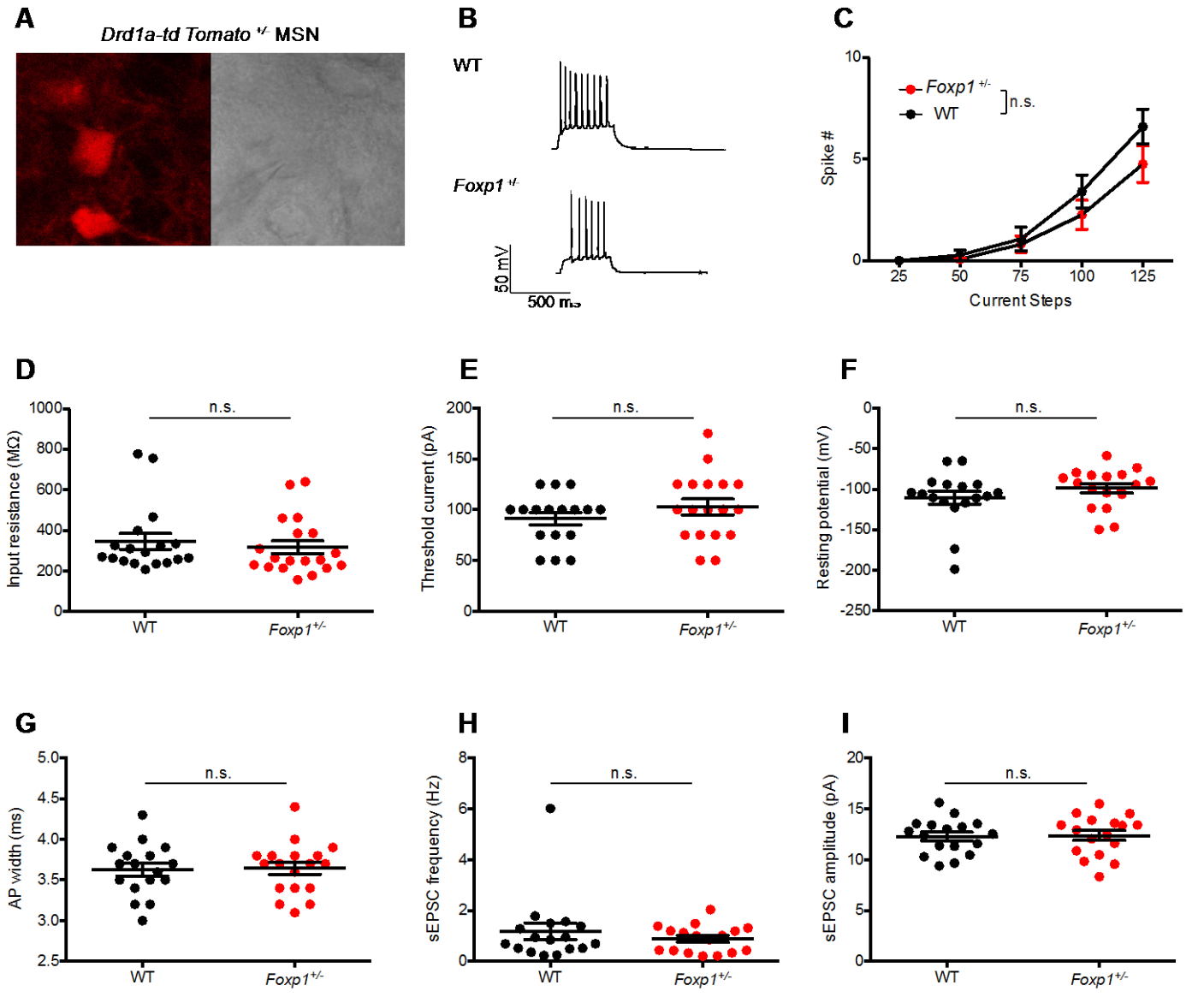


Supplemental Figure 1. Immunoblot for Foxp1 demonstrating antibody specificity. (**A**) hNP samples expressing FOXP1 and E13.5 mouse brain lysates from control and *Foxp1*$^{+/-}$ mice demonstrate expression whereas hNPs with GFP expression and brain lysate from *Foxp1* KO embryos do not demonstrate expression. (**B**) Foxp1A is significantly reduced only in the STR of *Foxp1*$^{+/-}$ mice. Data are represented as means (±SEM). N=4 mice/genotype for each region. *P=0.02 (Student's t-test, compared to wildtype (WT) levels normalized to Gapdh). (**C**) Foxp1D is significantly reduced only in the STR of *Foxp1*$^{+/-}$ mice. Data are represented as means (±SEM). N=4mice/genotype for each region. *P=0.004 (Student's t-test, compared to wildtype (WT) levels normalized to Gapdh).

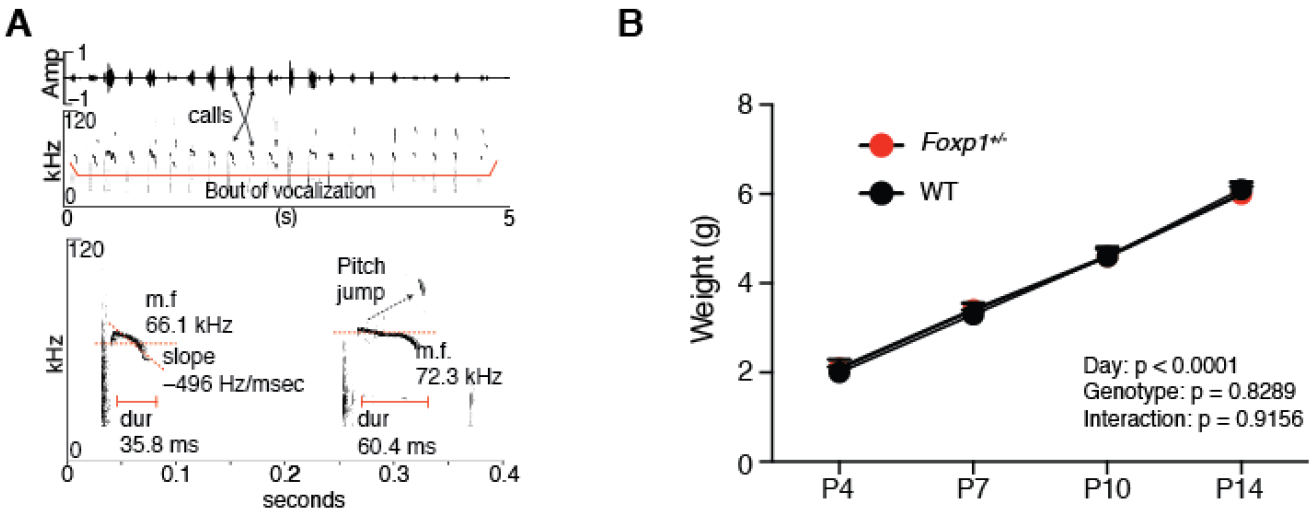


Supplemental Figure 2. Overlaps between mouse WGCNA modules and gene lists. (**A**) Heatmap displaying *Foxp1*$^{+/-}$ mouse RNA-seq WGCNA modules that contain significant enrichments of DEGs, ASD genes and/or FMRP targets. Plus signs indicate a genotype correlation of modules within specific brain regions. Log-transformed adjusted P-values from Benjamini-Hochberg false-discovery test (hypergeometric test). (**B** and **C**) Visualization of MsM19 containing genes significantly enriched in GO categories (using DAVD bioinformatics tool, http://david.abcc.ncifcrf.gov) for MAPK signaling (MsM19). Inserts: eigengene correlation plots show that genotype correlates negatively for MsM19 within the striatum.



Supplemental Figure 3. D_1 positive medium spiny neurons of *Foxp1*$^{+/-}$ mice have no change in excitability. (**A**) Example image of a recorded tdTomato+ (D_1+) neuron. (**B**) Example recordings depicting spiking in response to a 125 pA current step in control and *Foxp1*$^{+/-}$ MSNs. (**C**) Firing rate versus input curves are not significantly changed in *Foxp1*$^{+/-}$ MSNs. Data are represented as means (±SEM). N=15 WT cells, 16 *Foxp1*$^{+/-}$ cells. P=0.26 (two-way ANOVA with repeated measures for current step, compared between genotypes). (**D**) Input resistance is not significantly different in *Foxp1*$^{+/-}$ MSNs. Data are represented as means (±SEM). N=18 WT cells, 19 *Foxp1*$^{+/-}$ cells. P=0.58 (Student's t-test, compared between genotypes). (**E**) The minimum threshold current required for evoking an action potential is not significantly altered in *Foxp1*$^{+/-}$ MSNs.

Data are represented as means (±SEM). N=17 WT cells, 18 *Foxp1*$^{+/-}$ cells. P=0.25 (Student's t-test, compared between genotypes). (**F**) Resting potential is not significantly changed in *Foxp1*$^{+/-}$ MSNs. Data are represented as means (±SEM). N=17 WT cells, 18 *Foxp1*$^{+/-}$ cells. P=0.24 (Student's t-test, compared between genotypes). (**G**) Action potential width is not significantly altered in *Foxp1*$^{+/-}$ MSNs. Data are represented as means (±SEM). N=17 WT cells, 18 *Foxp1*$^{+/-}$ cells. P=0.89 (Student's t-test, compared between genotypes). (**H**) Spontaneous EPSC frequency is not significantly changed in *Foxp1*$^{+/-}$ MSNs. Data are represented as means (±SEM). N=17 WT cells, 17 *Foxp1*$^{+/-}$ cells. P=0.40 (Student's t-test, compared between genotypes). (**I**) Spontaneous EPSC amplitude is significantly decreased in *Foxp1*$^{+/-}$ MSNs. Data are represented as means (±SEM). N=17 WT cells, 17 *Foxp1*$^{+/-}$ cells. P=0.88 (Student's t-test, compared between genotypes).



Supplemental Figure 4. USV analysis parameters and weight gain of *Foxp1*$^{+/-}$ mice. (**A**) Illustration marking all major USV parameters measured including bouts, calls, mean frequency (m.f.), call duration (dur), slope, and jumps. (**B**) *Foxp1*$^{+/-}$ mice do not weigh significantly less than control littermates. Data are represented as means (±SEM). N=38 WT pups, 22 *Foxp1*$^{+/-}$ pups. P=0.83 (two-way ANOVA with a Sidak multiple comparison test, compared between genotypes). The main effects for genotype and postnatal day, and the interactions between these two variables, are reported at the bottom of the panel.

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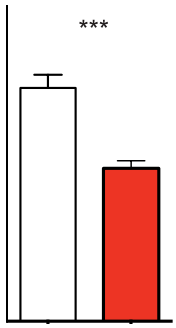
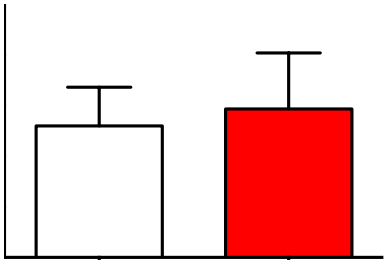




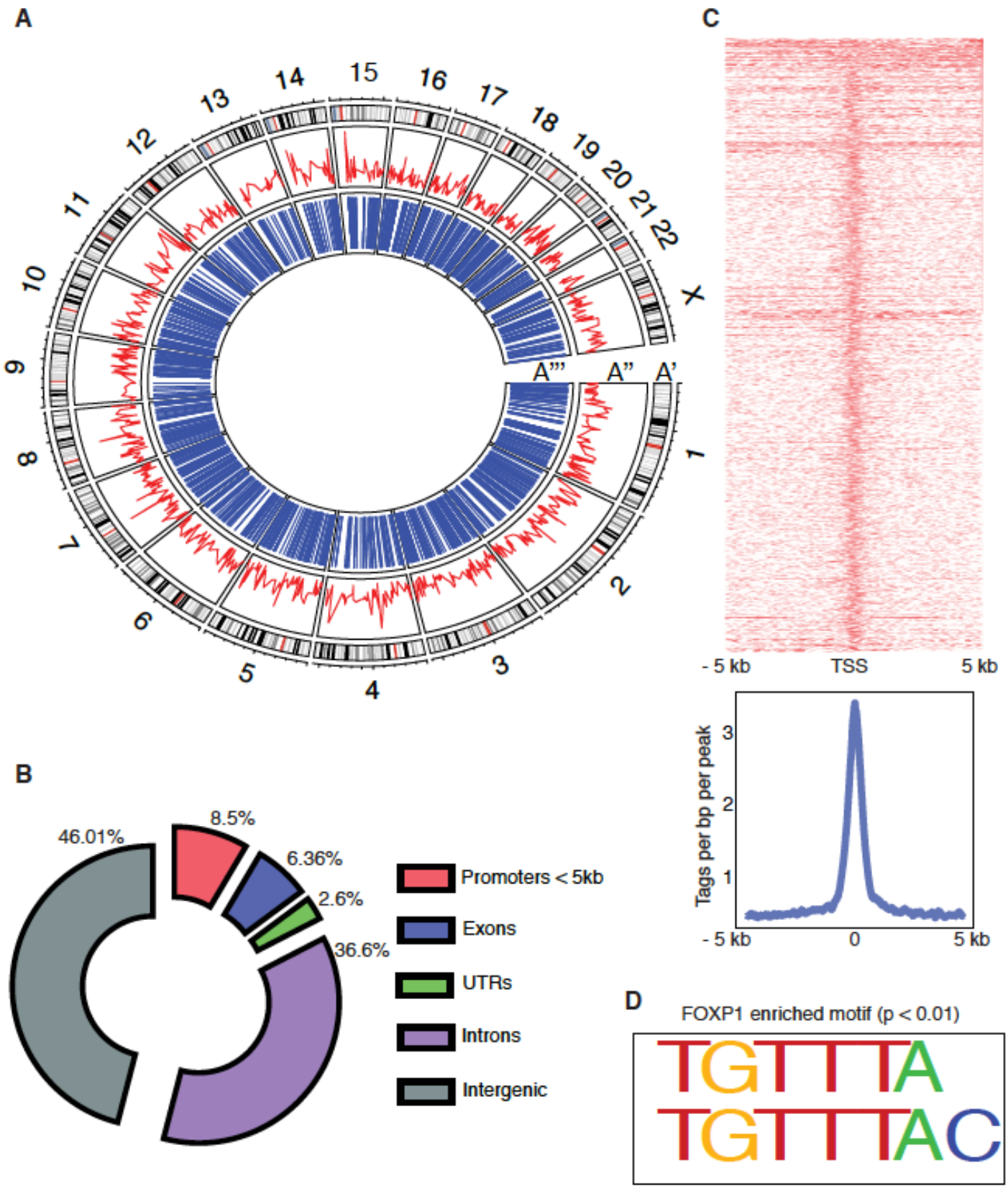
Supplemental Figure 5. Behavioral characterization of *Foxp1*$^{+/-}$ mice.

(A) Righting reflexes in *Foxp1*$^{+/-}$ pups at P4, P7, and P10. Data are represented as means (±SEM). N=11 *Foxp1*$^{+/-}$ pups, 15 WT pups. P=0.22 (two-way ANOVA with a Sidak multiple comparison test, compared between genotypes). (**B and C**) *Foxp1*$^{+/-}$ mice display hyperactivity in the open field test. (**B**) *Foxp1*$^{+/-}$ mice display increased total distance moved and (**C**) an increased average velocity in the open field test compared to WT mice. Data are represented as means (±SEM). N= 27 *Foxp1*$^{+/-}$ mice, 39 WT mice. P=0.0006, P=0.0007, respectively (unpaired Student's t-test, compared between genotypes). (**C**) *Foxp1*$^{+/-}$ mice do not exhibit deficits in motor coordination as measured by the latency to fall during the Rotorod behavioral test. Data represented as means (±SEM) of 4 trials per day. N=9 *Foxp1*$^{+/-}$ mice*,* 7 WT mice (two-way ANOVA with a Sidak multiple comparison test, compared between genotypes). **(D**) *Foxp1*$^{+/-}$ mice exhibit deficits in grip strength in both forelimbs and (**E**) hindlimbs. Data represented as means (±SEM). N=9 *Foxp1*$^{+/-}$ adults*,* 7 WT adults. **P=0.0058, ***P<0.0001 (unpaired Student's t-test, compared between genotypes). (**F**) *Foxp1*$^{+/-}$ mice show no difference in nesting behavior. Data represented as means (±SEM). N=9 *Foxp1*$^{+/-}$ mice*,* 7 WT mice. P=0.7667 (unpaired Student's t-test, compared between genotypes). (**G**) *Foxp1*$^{+/-}$ mice show no difference in grooming behavior. Data represented as means (±SEM). N=5 *Foxp1*$^{+/-}$ mice, 5 WT mice. P=0.81 (unpaired Student's t-test, compared between genotypes).

Test	*Foxp1*$^{+/-}$	WT	*p-value*
1. Body position	1 (±0)	1 (±0)	NS
2. Tremor	0 (±0)	0 (±0)	NS
3. Palpebal closure	0 (±0)	0 (±0)	NS
4. Coat appearance	0.11 (±0.99)	0 (±0)	NS
5. Skin color	1 (±0)	1 (±0)	NS
6. Whiskers	0 (±0)	0 (±0)	NS
7. Lacrimation	0 (±0)	0 (±0)	NS
8. Defecation	0.22 (±0.44)	0.57 (±0.53)	NS
9. Gait	0 (±0)	0 (±0)	NS
10. Tail elevation	0 (±0)	0 (±0)	NS
11. Touch escape	1.89 (±0.33)	2 (±0)	NS
12. Trunk curl	0.22 (±0.44)	0.14 (±0.38)	NS
13. Limb grasping	0.89 (±0.33)	1 (±0)	NS
14. Pinna reflex	1 (±0)	1 (±0)	NS
15. Corneal reflex	1 (±0)	1 (±0)	NS
16. Contact righting reflex	0 (±0)	0 (±0)	NS
17. Evidence of biting	0 (±0)	0 (±0)	NS
18. Vocalization (audible)	0 (±0)	0.14 (±0.38)	NS
19. Startle response	1 (±0)	1 (±0)	NS
20. Positional passivity	0.78 (±0.83)	0.43 (±0.53)	NS

Supplemental Figure 6. SHIRPA battery results.

Foxp1$^{+/-}$ mice underwent a modified SHIRPA behavioral screen and no differences were found between the 20 different categories tested. Individual tests were scored between 0-1, 0-2, or 0-3. Data represented as means (±SEM). N=9 *Foxp1*$^{+/-}$ mice, 7 WT mice.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- 5 kb
0
5 kb
B
46.01%
8.5%
6.36%
2.6%
36.6%
Promoters < 5kb
Exons
UTRs
Introns
Intergenic
D
FOXP1 enriched motif (p < 0.01)
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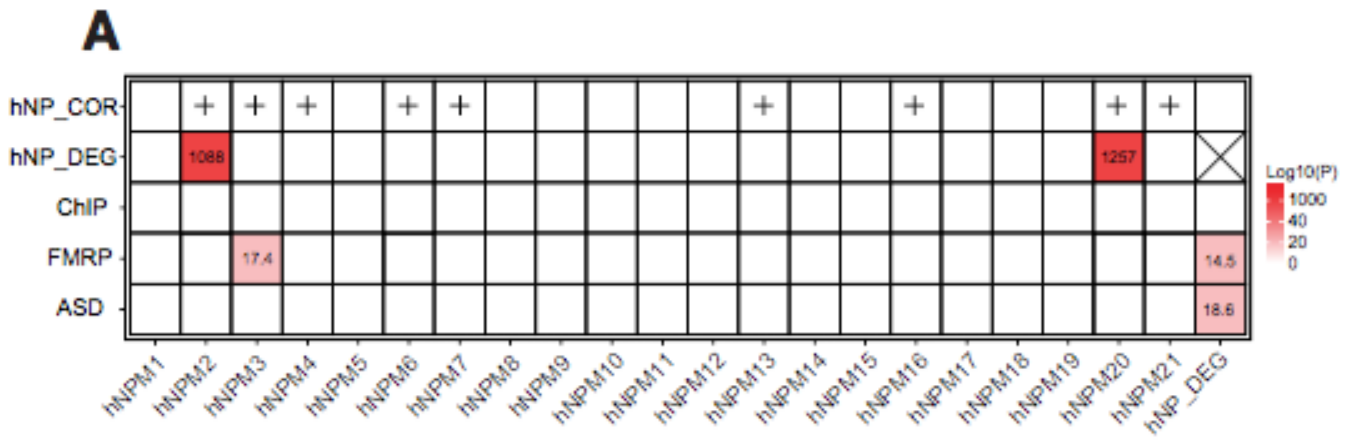
TGTTTAC

Supplemental Figure 7. FOXP1 ChIP-seq characterization. (**A**) Circular visualization of FOXP1 ChIP-seq. A' represents the chromosomal cytoband, A'' represents the FOXP1 peak height, and A''' represents the genomic distribution of FOXP1 binding sites. (**B**) Distribution of all FOXP1 binding site peaks in relation to gene structure. (**C**) Heat map of FOXP1 ChIP-seq enrichment within gene promoters. Each row represents a 10-kb window extending 5kb upstream and 5kb downstream of the transcriptional start site (TSS). Bottom panel shows the average FOXP1 ChIP-seq enrichment across 5kb upstream and 5kb downstream of the TSS. (**D**) Enriched FOXP1 motifs within the detected peaks compared with GFP control.



Supplemental Figure 8. Overlaps between hNP WGCNA modules and gene lists. (**A**) Heatmap displaying hNP^{FOXP1} WGCNA modules that contain significant enrichments of DEGs, ASD genes. ASD scored genes, and/or FMRP targets. Log-transformed adjusted P-values from Benjamini-Hochberg false-discovery test (hypergeometric test). hNP_COR positive modules correlate with FOXP1 genotype. hNP_DEG indicates enrichment of FOXP1 differentially expressed genes.

Supplemental Table 1. Differential gene expression, module membership, and overlap information for both human and mouse FoxP1 datasets. Separate tabs are included for human and mouse data. Only data for expressed genes are included in the table. Columns can be sorted to obtain the list of DEGs, ASD genes, overlap with Foxp2, etc. ModuleColor, ModuleName, and kWithin indicate WGCNA membership and connectivity. “IP” in hNP_ChIP_target column indicates genes with an enriched FOXP1 peak compared to control. “ASD” in SFARI column indicates genes associated with autism based on gene.sfari.org. “FMRP” in FMRP (Darnell et al., 2011) column indicates FMRP targets. “Parikshak_M17” in the Module (Perikshak et al., 2013) column indicates a gene with module membership in the M17 module of that paper. “Voineagu_asdM16” in the Module (Voineagu et al., 2011) column indicates a gene with module membership in the M16 module of that paper. “Enard_deg” in Foxp2 Hets (Enard et al., 2009) column indicates differentially expressed genes in Foxp2 heterozygous striatums. logFC_hNP and FDR_hNP columns indicate the fold change and significance of DEGs in hNPs with FOXP1 expression compared to controls. logFC_STR and FDR_STR columns indicate the fold change and significance of DEGs in *Foxp1*$^{+/-}$ striatum compared to controls. logFC_HIP and FDR_HIP columns indicate the fold change and significance of DEGs in *Foxp1*$^{+/-}$ hippocampus compared to controls. Genes without values or text in any of these columns did not reach significance.

Supplemental Table 2. Foxp1 and Foxp2 target genes within D_1+ and D_2+ enriched MSNs.

Supplemental Table 3. Gene ontology results for human RNA-seq and ChIP-seq.