Figure S1. *Otx1* expression in *Otx2 RxCre* cKOs (Refers to Figure 1). ISH showing *Otx1* expression in rostral-caudal series of sections from (A-C) *Otx2f/+* and (D-F) *Otx2f/-; RxCre* forebrains at E11.5. Note that the expression of *Otx1* complements that of *Otx2* in the early telencephalon (see Figure 1), and that *Otx1* is not markedly changed in cKOs. *Scale bars: 0.5mm.*

Figure S2. Otx2 and cortical patterning (Refers to Figure 3). (A-B) *Otx2* expression is positively regulated by Fgf8 from the RPC, as demonstrated by diminished expression in $Fgf8^{CreER/neo}$ (effectively $Fgf8^{null/neo}$) embryos at E10.5. (C-F) E13.5 ISH showing effects of Otx2 loss of function on cortical patterning. (C-D) *COUP-TF1* expression is reduced and (E-F) *SP8* is upregulated in the dorsal cortex of *RxCre* cKOs. Abbreviations: Di, diencephalon, rTel, rostral telencephalon, RPC, rostral patterning center. *Scale bars: 0.4mm.*

Figure S3 (Refers to Figure 7). Abnormal MGE and POA development in *Nkx2.1Cre* cKOs at E13.5 and P15 (Refers to Figure 7). ISH show expression of (A-C') *Nkx5.1*, (D-F') *COUP-TF1*, and (G-I') *Nkx2.1* in (A-I) *Otx2f/+* and (A'-I') *Otx2f/-; Nkx2.1Cre* forebrains. (J-L') Anti-ChAT IHC on P15 coronal sections from (J-L) *Otx2f/+* and (J'-L') *Otx2f/-; Nkx2.1Cre* brains. Abbreviations as in Figure 8. *Scale bars: 0.5mm.*

Figure S4. Postnatal deficits in MGE derivatives in *Otx2* cKOs (Refers to Figure 7). (A-L') P0 ISHs on (A-L) *Otx2f/+* and (A'-L') Otx2f/-; RxCre brains with probes to (A-C') *Lhx6*; (D-F') *Lhx8*; (G-H') *TrkA*, (I-I') *Gbx1*. (J-L') Anti-ChAT IHC on (J-L) *Otx2f/+* and (J'-L') *Otx2f/-; RxCre* brain sections from two week old mice. Abbreviations: VP, ventral pallidum; AcS, shell of accumbens; DBB, diagonal band of Broca; NB, nucleus basalis; MS, medial septum. *Scale bars: A-I' = 0.25mm, J-L' = 0.5mm.*

Figure S5 (Refers to Figure 2). Anti-OTX2 ChIP-seq peaks occupy basal ganglia enhancers. Coronal sections of E11.5 embryos showing expression of telencephalic enhancers driving *β-galactosidase* expression, visualized with X-Gal staining, from transient transgenic mice (A, C, E, G, I K). The genomic location of each enhancer (green rectangle) is shown along with the called peaks from 3 separate Anti-OTX2 ChIP-seq experiments (B, D, F, H, J, l; Otx2 R1-3 respectively, red-yellow bars). (M) Venn diagram showing overlap of called peaks between each of the OTX2 ChIP replicates R1-

R3. The intersection of all three replicates (590 peaks, white) was used for motif and GO analysis in Figure S6. (N) Graph showing the distribution of the distances from each of the OTX2 ChIP peaks to the TSS of its associated gene. Abbreviations: cortex: Cx, lateral ganglionic eminence: LGE, medial ganglionic eminence: MGE.

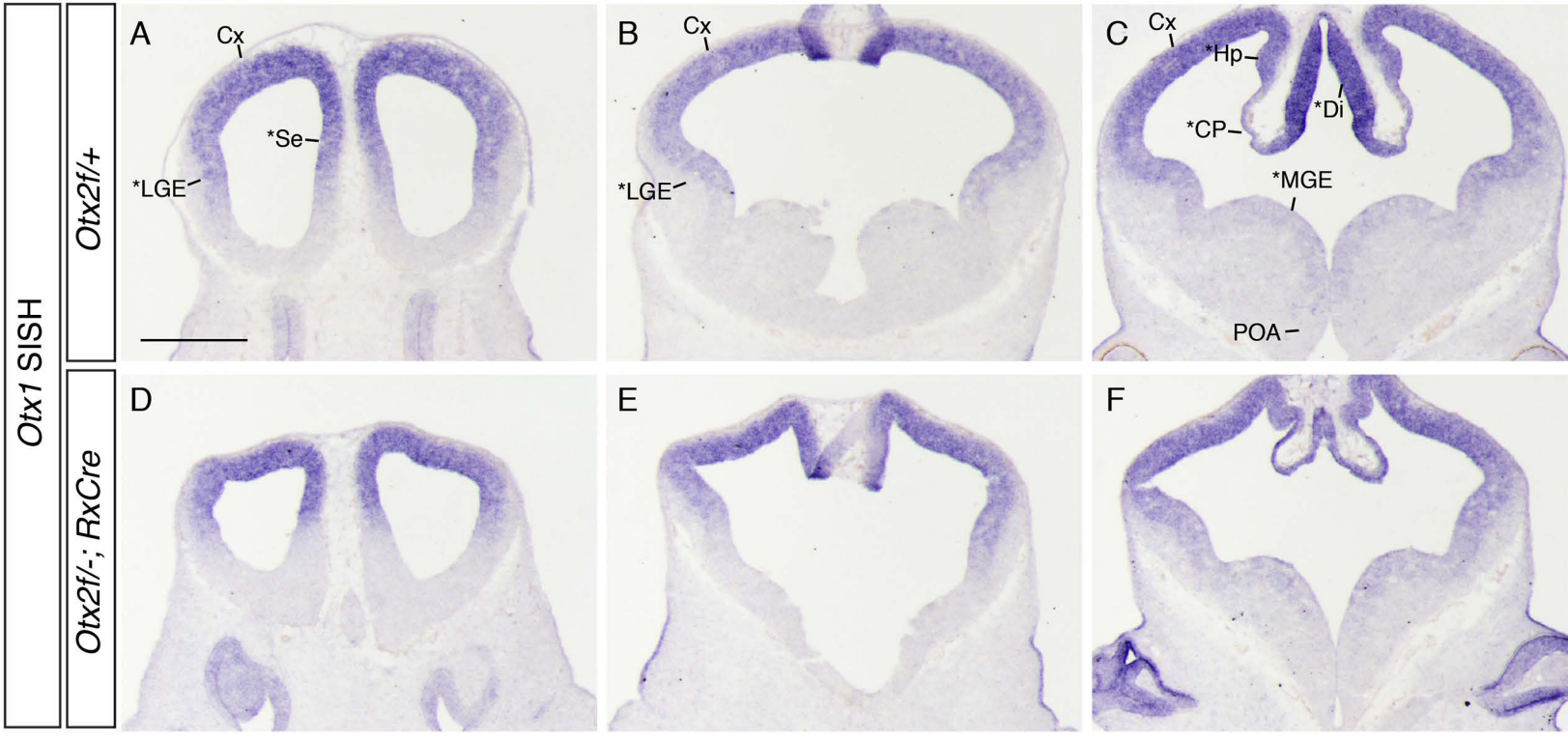
Figure S6 (Refers to Figure 2). Genomic sequence motifs and gene ontology derived from anti-OTX2 ChIP-seq. Top ten sequence motifs derived from the intersection of all three Anti-OTX2 ChIP–seq replicates by RSAT(A), displaying the associated transcription factor, number and percentage of 590 total peaks, and the average number of motifs per peak. Top results from gene ontology analysis of peaks using the GREAT annotation tool for molecular function (B) and biological process (C) for terms with a FDR Q-Val < 1E-3.

Figure S7 (Refers to Figures 4, 5 and 6). Diagram summarizing Otx2 effects on POA and MGE patterning. At E11.5, *Otx2* cKOs lack expression of vMGE VZ markers (*Tal2*, *Tll2*) as well as vMGE expression of *Olig1* and *Zic1*. POA genes expressed in the VZ (*COUP-TF1*, *Dbx1*) and MZ (*Nkx5.1*, *Nkx5.2*, *Sox14*) expand dorsally and rostrally into the MGE. In addition, some vLGE genes (*COUP-TF1*, *Sizn1*) expand or shift ventrally into the dorsal MGE. Together, these changes result in mis-specification of the vMGE toward a POA-like fate. At later stages, this results in agenesis or altered molecular properties of the globus pallidus and a severe reduction in cholinergic neurons. In addition to these changes in regional MGE patterning, *Otx2* cKOs have reduced neurogenesis and reduced numbers of oligodendrocyte progenitors in the MGE at this stage, which are not shown in the diagram.

Table S1 (Refers to Figures 1-7). Genes discussed in text that were identified in microarray as significantly de-regulated in Otx2; RxCre cKOs. ChIP-seq data designations (see also Figure 2): '+' reproducible peaks in or near locus; '(+)' peaks not reproducible, of low amplitude, >1 MB from locus, or in gene-dense region near locus; '-' no peaks in or near locus. Genes having two numbers assigned were deregulated on two independent microarray spots. Some B<0 genes were investigated because of biological relevance, existence of outliers in sample group, or absolute intensity values

close to detection threshold. See Supplementary Table 1 for the complete microarray (B>0) dataset.

GROUP	GENE	FOLD CHANGE	B VALUE	ChIP-Seq peaks?
Otx family	*Otx2*	2.756	3.45	+
	Otx1	1.318	0.21	+
Midbrain/hindbrain	*En2*	4.776	-2.66	-
	Pax3	1.784	-2.42	+
Oligodendrogenesis	*Olig1*	0.369	4.782	+
	Olig2	0.593	1.412	+
MGE neurogenesis	*Gbx2*	0.289	2.97	+
	Arx	0.597	0.42	+
	Robo2	0.659	-2.56	+
	Plxna4	0.483	0.11	(+)
	NeuroD6	0.600	-1.845	(+)
	NeuroD2	0.58/0.586	-1.112/-3.235	-
	Hes1	1.413	-1.975	+
	Id4	1.331	-2.303	(+)
POA patterning	*Nkx5.1 (Hmx3)*	4.062	2.829	+
	Nkx5.2 (Hmx2)	2.993	3.539	+
	Sox14	2.021	3.715	+
	Dbx1	2.172	-1.743	+
	Arhgap22	3.214	2.79	-
	Slit2	1.897	0.146	-
	Sox3	1.603	-0.474	-
	mShisa (Shisa2)	1.404	0.305	(+)
	Spry1	0.734	-5.579	(+)
MGE patterning	*Tal2*	0.271	1.83	+
	Tll2	0.584	0.818	+
	Tgfb3	0.605	-0.19	(+)
	brevican	0.137	2.182	-
	Sall3	1.424	0.432	+
	Spry2	1.378	-4.057	(+)
Septum	*Msx3*	2.12	2.233	-
Interneuron genes	*Lhx6*	0.597/0.453	0.623/1.79	(+)
	Lhx8	0.247/0.222	3.888/4.325	+
	maf-a	0.598	2.043	(+)
	maf-b	0.686	0.319	+
	c-maf (Maf)	0.566	-4.143	-
	Somatostatin	0.090	1.137	(+)
	Neuropeptide Y	0.366	1.776	(+)
	Gad1	0.651	0.558	+
Other genes of interest	*Gbx1*	0.429	2.023	(+)
	Nkx2.9	0.509	0.692	+
	Sox10	0.486	1.82	(+)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Otx2f/+
Otx2f/-; RxCre
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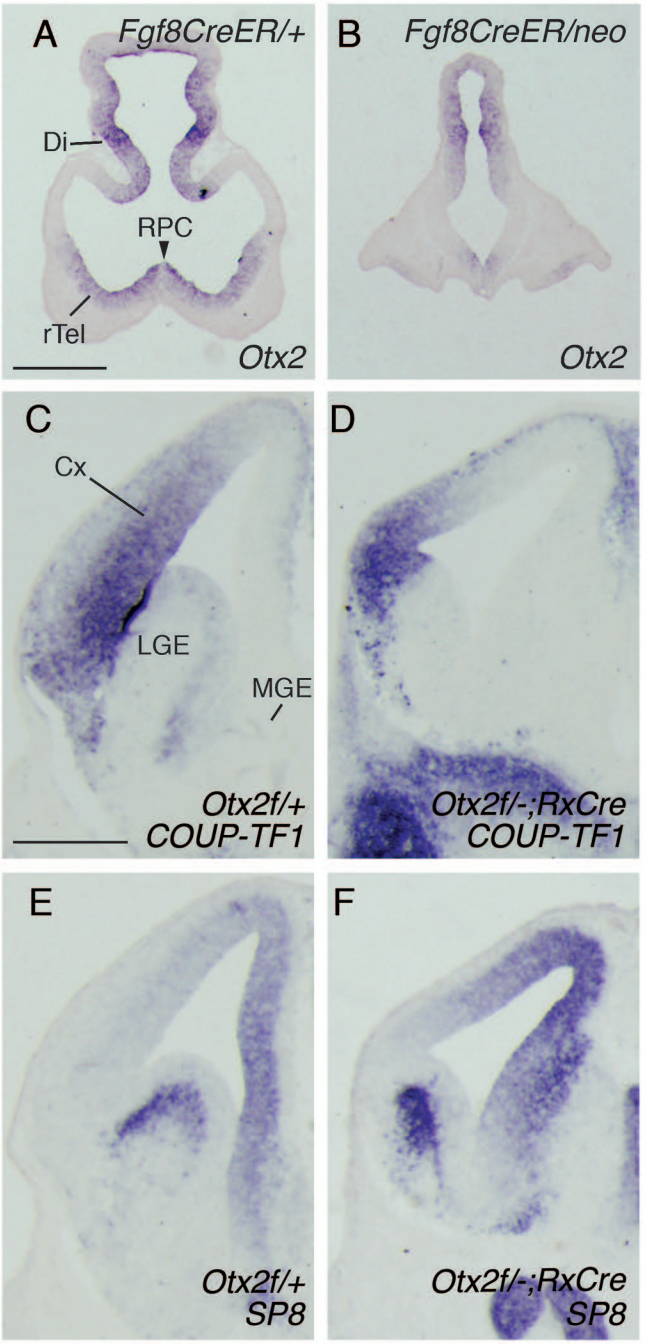
*LGE
B
Cx
*LGE
C
Cx
*Hp
*Di
*CP
*MGE
POA
D
E
F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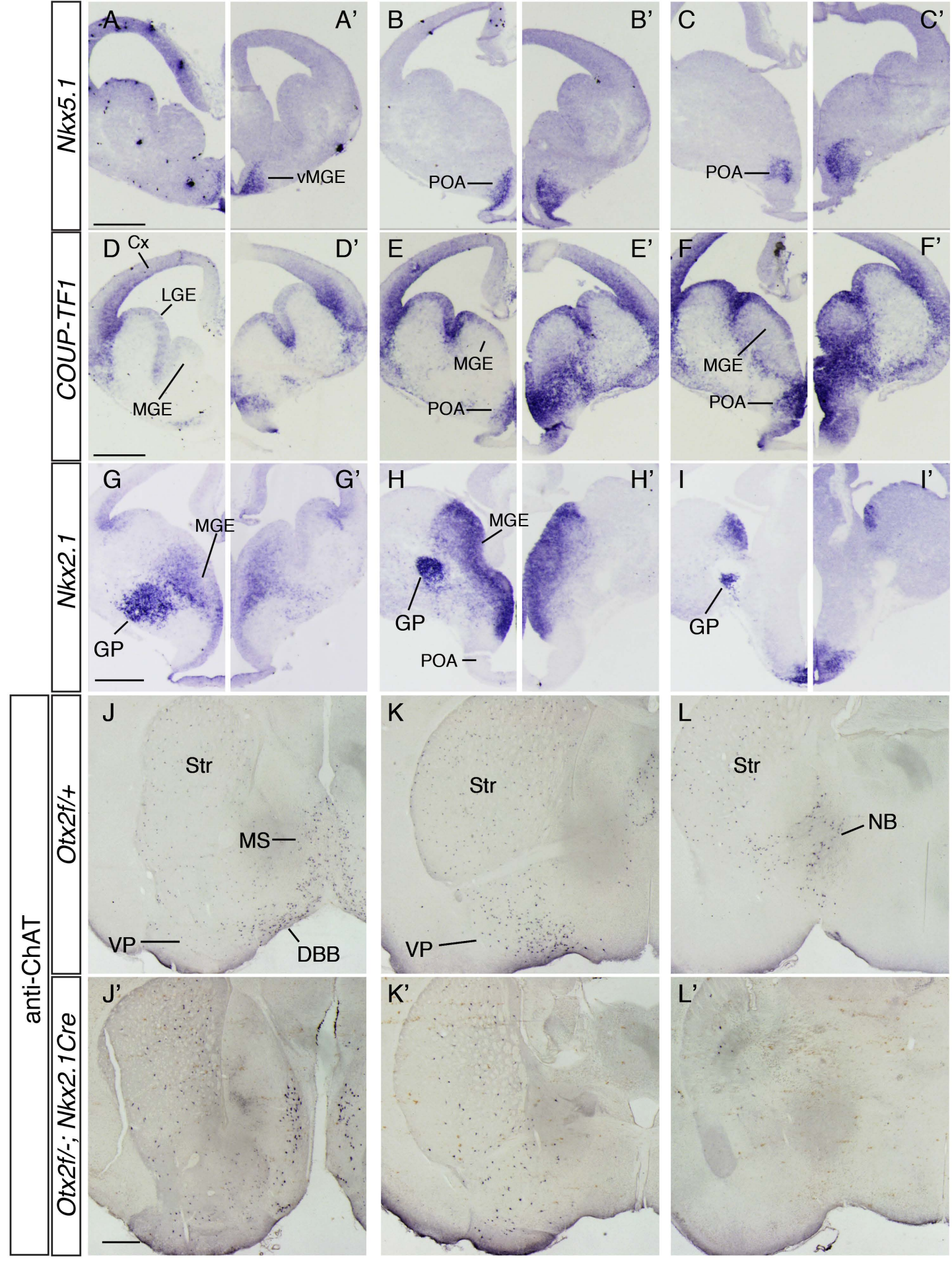
Di
RPC
rTel
Otx2
B
Fgf8CreER/neo
Otx2
C
Cx
LGE
MGE
Otx2f/+
COUP-TF1
D
Otx2f/-;RxCre
COUP-TF1
E
Otx2f/+
SP8
F
Otx2f/-;RxCre
SP8


Nkx5.1
COUP-TF1
Nkx2.1
anti-ChAT
Otx2f/+
Otx2f/-; Nkx2.1Cre
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A'
B
B'
C
C'
vMGE
POA
POA
D
D'
E
E'
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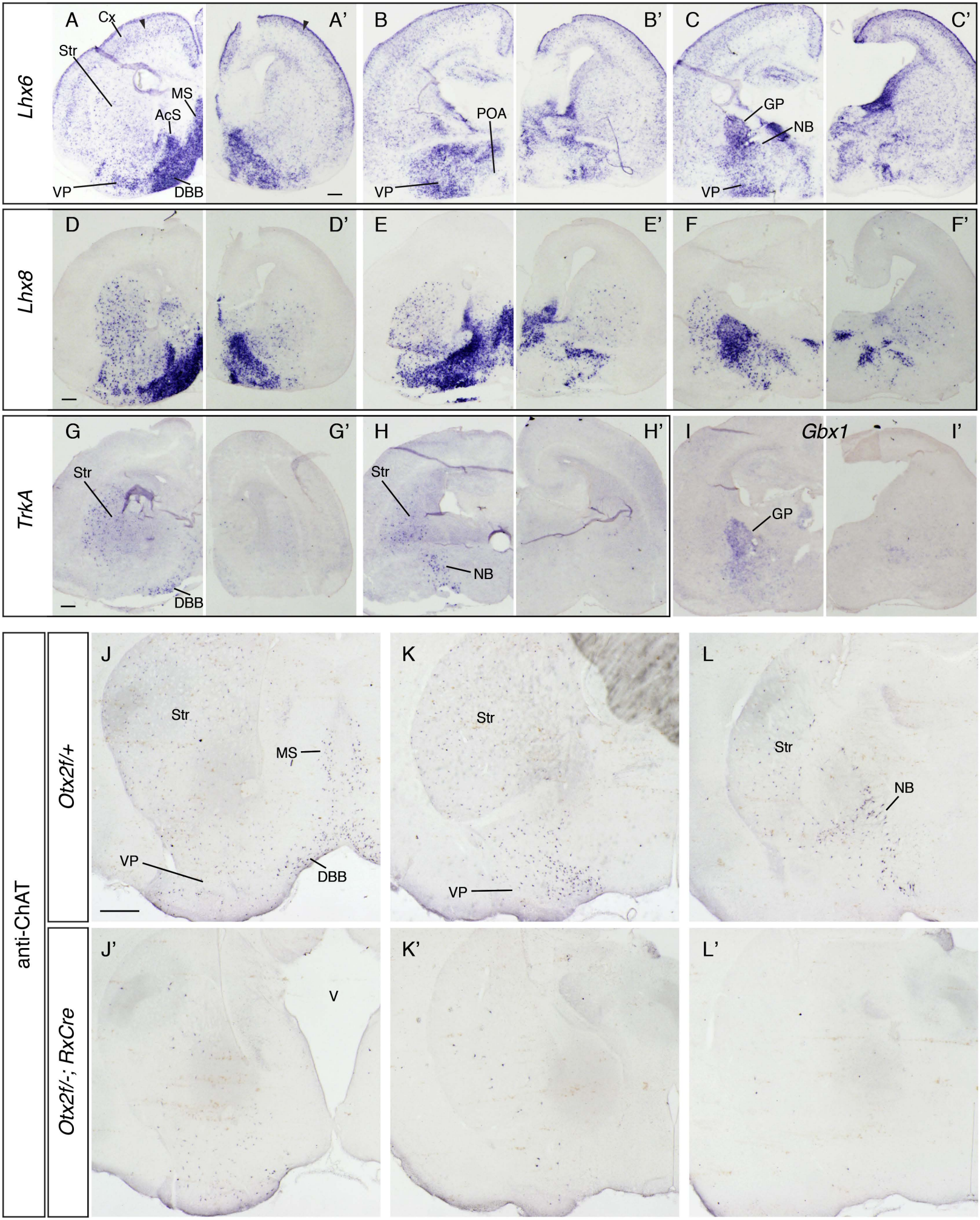
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D'
E
E'
F
F'
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G
Str
DBB
G'
H
Str
NB
H'
I
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GP
I'
anti-ChAT
Otx2f/+
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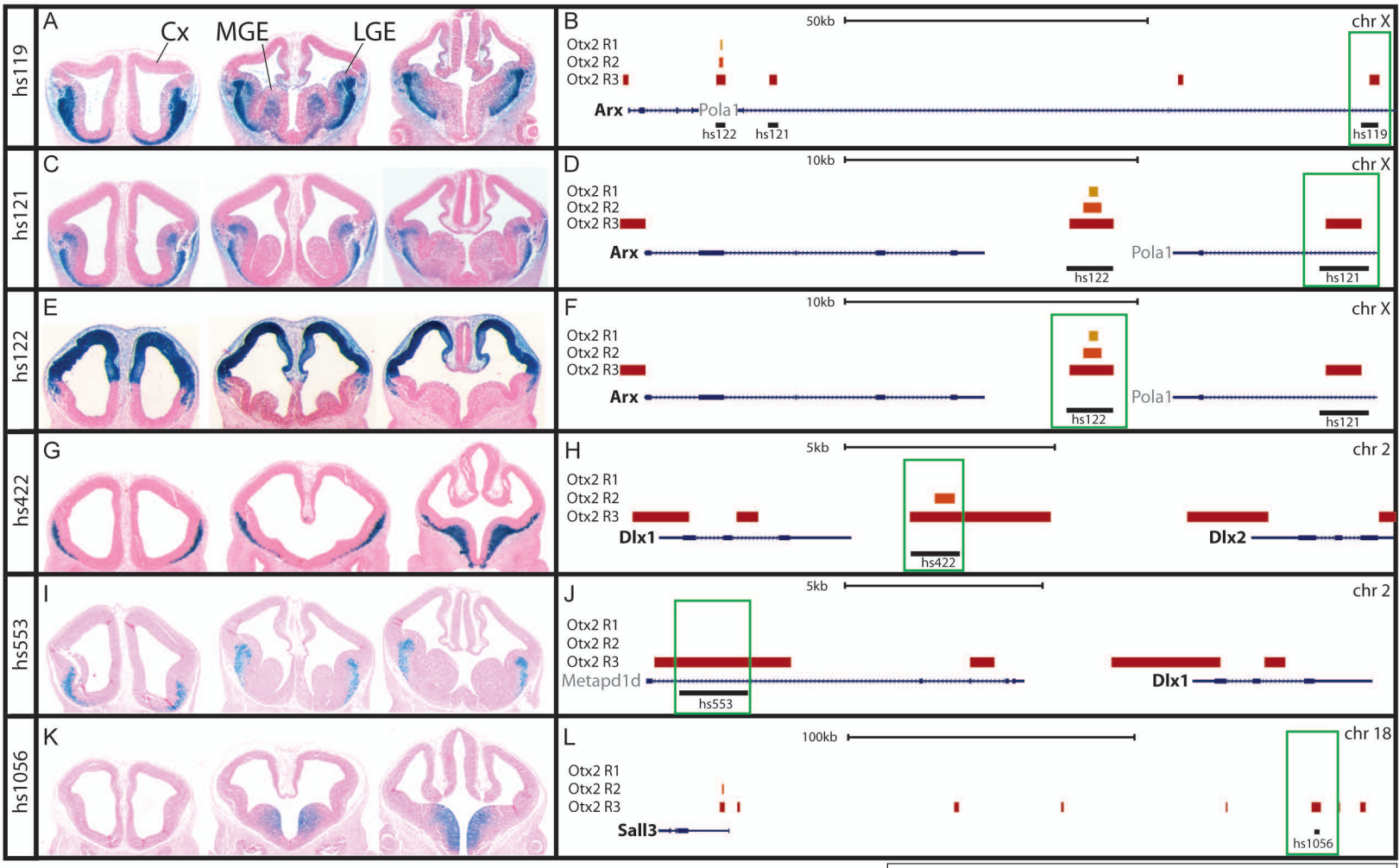
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B
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Otx2 R2
Otx2 R3
Arx
Pola1
hs122
hs121
hs119
hs121
C
D
10kb
chr X
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Otx2 R2
Otx2 R3
Arx
Pola1
hs122
hs121
hs122
E
F
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Otx2 R2
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Otx2 R2
Otx2 R3
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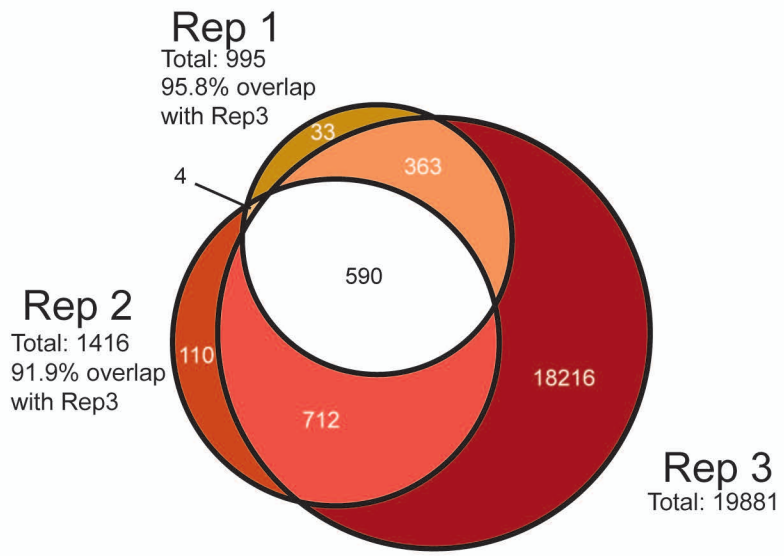
Otx2 R1
Otx2 R2
Otx2 R3
Sall3
hs1056
Legend
ChIP Replicate 1
ChIP Replicate 2
ChIP Replicate 3
Arx Gene of interest
Pola1 Adjacent gene
hs122 VISTA Regulatory Region

M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Rep 2
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with Rep3
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712
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Rep 3
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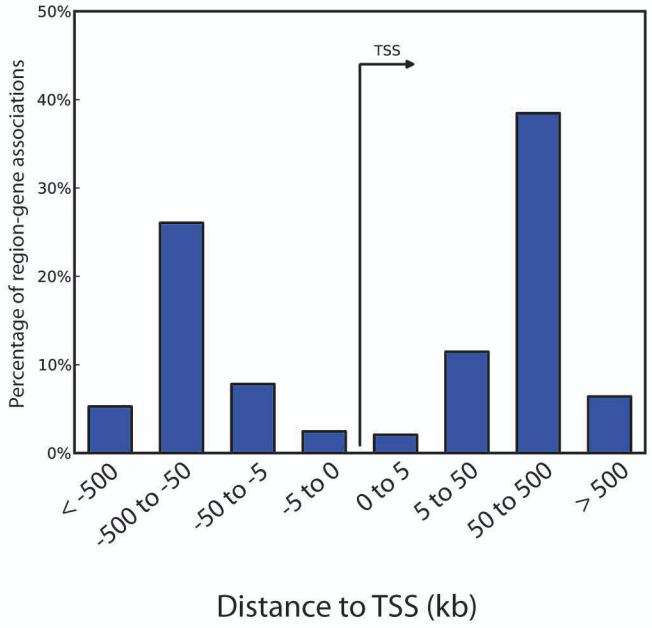
N


50%
40%
30%
20%
10%
0%
TSS
Percentage of region-gene associations
< -500
-500 to -50
-50 to -5
-5 to 0
0 to 5
5 to 50
50 to 500
> 500
Distance to TSS (kb)

A

Name	# of peaks with motif	% of peaks with motif	Ave. # motifs per peak	
Otx2	236	40.00%	1.10	
Otx2	172	29.15%	1.09	
Otx2	156	26.44%	1.05	
Otx2	129	21.86%	1.05	
Nobox	74	12.54%	1.19	

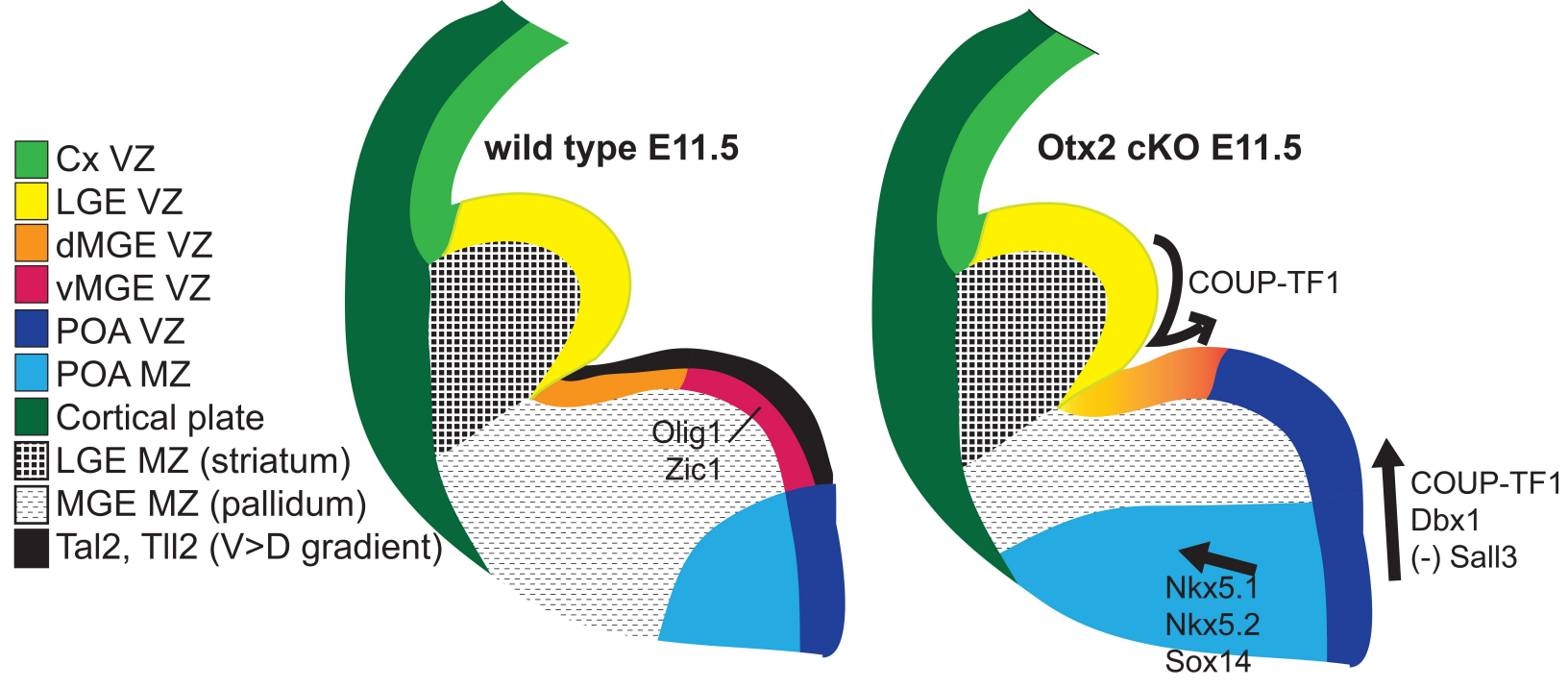
Name	# of peaks with motif	% of peaks with motif	Ave. # motifs per peak	
Sox2	71	12.03%	1.11	
Sox2	121	20.56%	1.15	
HoxA5/ Pdx1	134	22.71%	1.20	
HoxA5/ Pdx1	88	14.92%	1.08	
Unknown	89	15.08%	1.17	

B GO Molecular Function

Term Name	Rank	Raw P-Val	FDR Q-Val	Fold Enrichment	Region Hits	Region Set Coverage
sequence-specific DNA binding	1	6.58E-12	2.14E-08	2.1863	86	18.18%
transcription regulatory region DNA binding	8	1.48E-07	6.02E-05	2.4164	43	9.09%
regulatory region DNA binding	9	2.20E-07	7.95E-05	2.3809	43	9.09%
transcription regulatory region sequence-specific DNA binding	12	8.78E-07	2.38E-04	3.3187	23	4.86%
chromatin binding	14	9.23E-06	2.15E-03	2.3286	33	6.98%
RNA polymerase II regulatory region sequence-specific DNA binding	16	1.44E-05	2.92E-03	4.0547	14	2.96%
RNA polymerase II regulatory region DNA binding	17	1.69E-05	3.24E-03	3.9942	14	2.96%
Wnt-protein binding	18	1.77E-05	3.19E-03	6.3177	9	1.90%
sequence-specific DNA binding RNA Pol II transcription factor activity	19	2.57E-05	4.40E-03	2.5578	25	5.29%
core promoter proximal region sequence-specific DNA binding	21	1.44E-04	2.23E-02	4.7881	9	1.90%
core promoter proximal region DNA binding	24	2.15E-04	2.91E-02	4.5342	9	1.90%
axon guidance receptor activity	25	2.48E-04	3.23E-02	5.8158	7	1.48%
basal RNA polymerase II transcription machinery binding	30	3.46E-04	3.75E-02	8.5478	5	1.06%
ephrin receptor activity	31	3.66E-04	3.84E-02	4.2105	9	1.90%
transmembrane receptor protein kinase activity	34	5.26E-04	5.03E-02	2.5423	17	3.59%

C GO Biological Process

Term Name	Rank	Raw P-Val	FDR Q-Val	Fold Enrichment	Region Hits	Region Set Coverage
central nervous system development	1	5.9129E-19	5.0751E-15	2.455	111	23.47%
regulation of transcription from RNA polymerase II promoter	2	2.6546E-18	1.1392E-14	2.2624	123	26.00%
brain development	3	1.1766E-17	3.3663E-14	2.613	94	19.87%
forebrain development	8	5.6142E-16	6.0233E-13	3.0487	68	14.38%
generation of neurons	12	1.1237E-15	8.037E-13	2.0985	122	25.79%
neurogenesis	14	2.8489E-15	1.7466E-12	2.0465	125	26.43%
neuron differentiation	15	3.2737E-15	1.8732E-12	2.2889	101	21.35%
negative regulation of transcription, DNA-dependent	16	4.3757E-15	2.3473E-12	2.3739	94	19.87%
negative regulation of RNA metabolic process	18	7.8523E-15	3.7442E-12	2.3508	94	19.87%
negative regulation of gene expression	19	1.9571E-14	8.841E-12	2.2877	96	20.30%
negative regulation of nitrogen compound metabolic process	20	3.2804E-14	1.4078E-11	2.268	96	20.30%
negative regulation of transcription from RNA polymerase II promoter	21	5.1473E-14	2.1038E-11	2.6695	72	15.22%
negative regulation of nucleobase-containing compound metabolic prc	23	7.8758E-14	2.939E-11	2.2475	95	20.08%
negative regulation of cellular macromolecule biosynthetic process	24	8.5473E-14	3.0567E-11	2.2574	94	19.87%
negative regulation of macromolecule biosynthetic process	26	1.1034E-13	3.6425E-11	2.2346	95	20.08%


Cx VZ
LGE VZ
dMGE VZ
vMGE VZ
POA VZ
POA MZ
Cortical plate
LGE MZ (striatum)
MGE MZ (pallidum)
Tal2, Tll2 (V>D gradient)
wild type E11.5
Olig1
Zic1
Otx2 cKO E11.5
COUP-TF1
COUP-TF1
Dbx1
(-) Sall3
Nkx5.1
Nkx5.2
Sox14