Tead transcription factors differentially regulate cortical development

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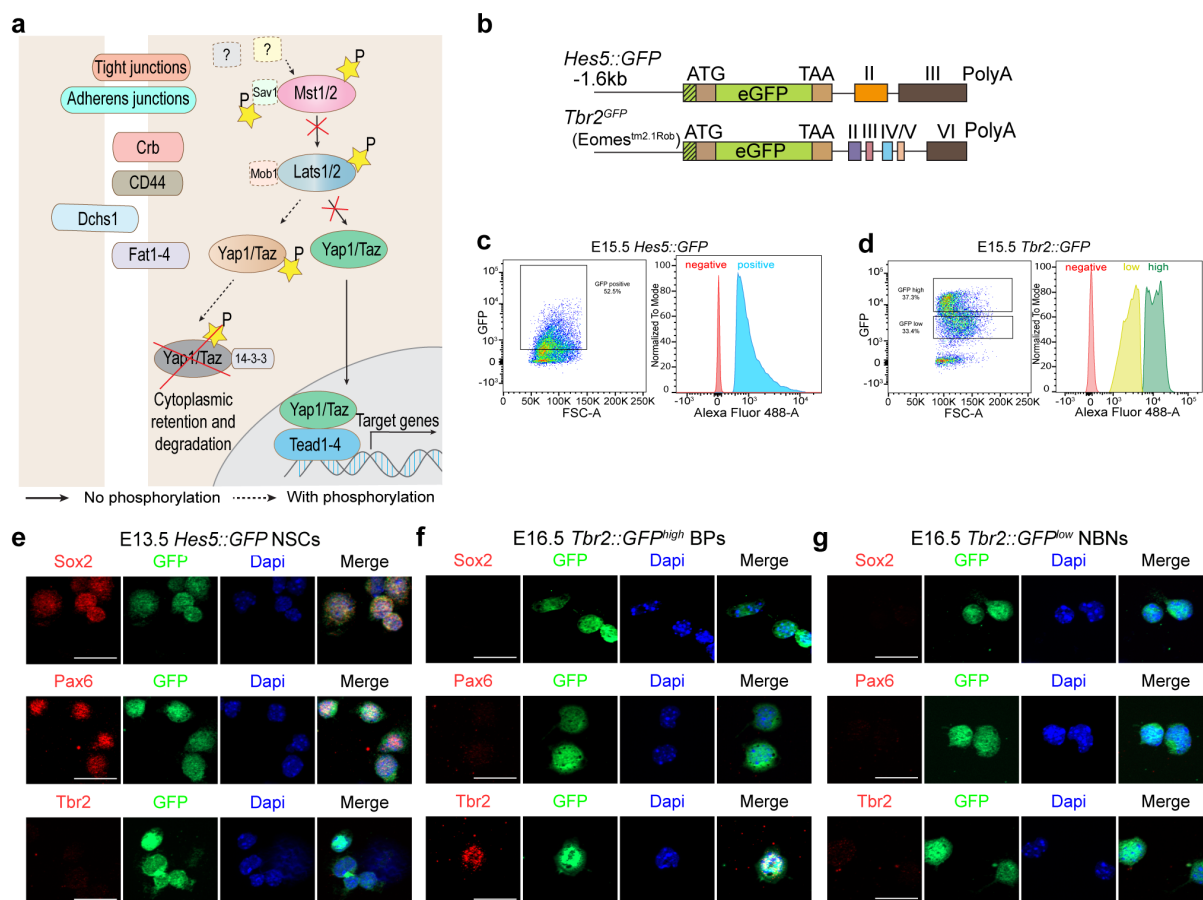# Supplementary Figures and Figure Legends

Figure S1

Figure S1: Validation for RNA sequencing paradigm and transgenic mice.

a. Schematic representation of Hippo signaling cascade. Illustration depicts series of sequential phosphorylation steps for co-activators Yap1/Taz, mediated by Mst1/2 and Lats1/2 kinases. If Hippo signaling is on, Yap1/Taz is phosphorylated, retained in the cytoplasm and degraded. If Hippo signaling is off, Yap1/Taz escapes phosphorylation and translocates to the nucleus, where it binds its partner TFs, e.g. Teads, to regulate transcription.

b. *Hes5::GFP* and *Tbr2::GFP* transgenic mice used for cell isolation.

c. Example of FACS plot for GFP$^+$ cell sorting from *Hes5::GFP* dorsal cortex at E15.5. Left plot shows the gating used to select GFP$^+$ cells. Right plot shows the GFP$^-$ (red) and GFP$^+$ (blue) cell populations.

d. Example of FACS plot for GFP$^+$ cell sorting from *Tbr2::GFP* dorsal cortex at E15.5. Left plot shows the gates used to select the two populations of GFP$^+$ cells. Right plot shows the GFP$^-$ (red), GFP^{low} (yellow) and GFP^{high} (green) cell populations.

e-g. Expression of progenitor markers (Sox2 and Pax6) and basal progenitor marker Tbr2 by *Hes5::GFP$^+$* neural stem cells (NSCs) and *Tbr2::GFP^{high}* basal progenitors (BP) and *Tbr2::GFP^{low}* newborn neurons (NBNs) cells after FAC sorting. Scale bar = 20μm.
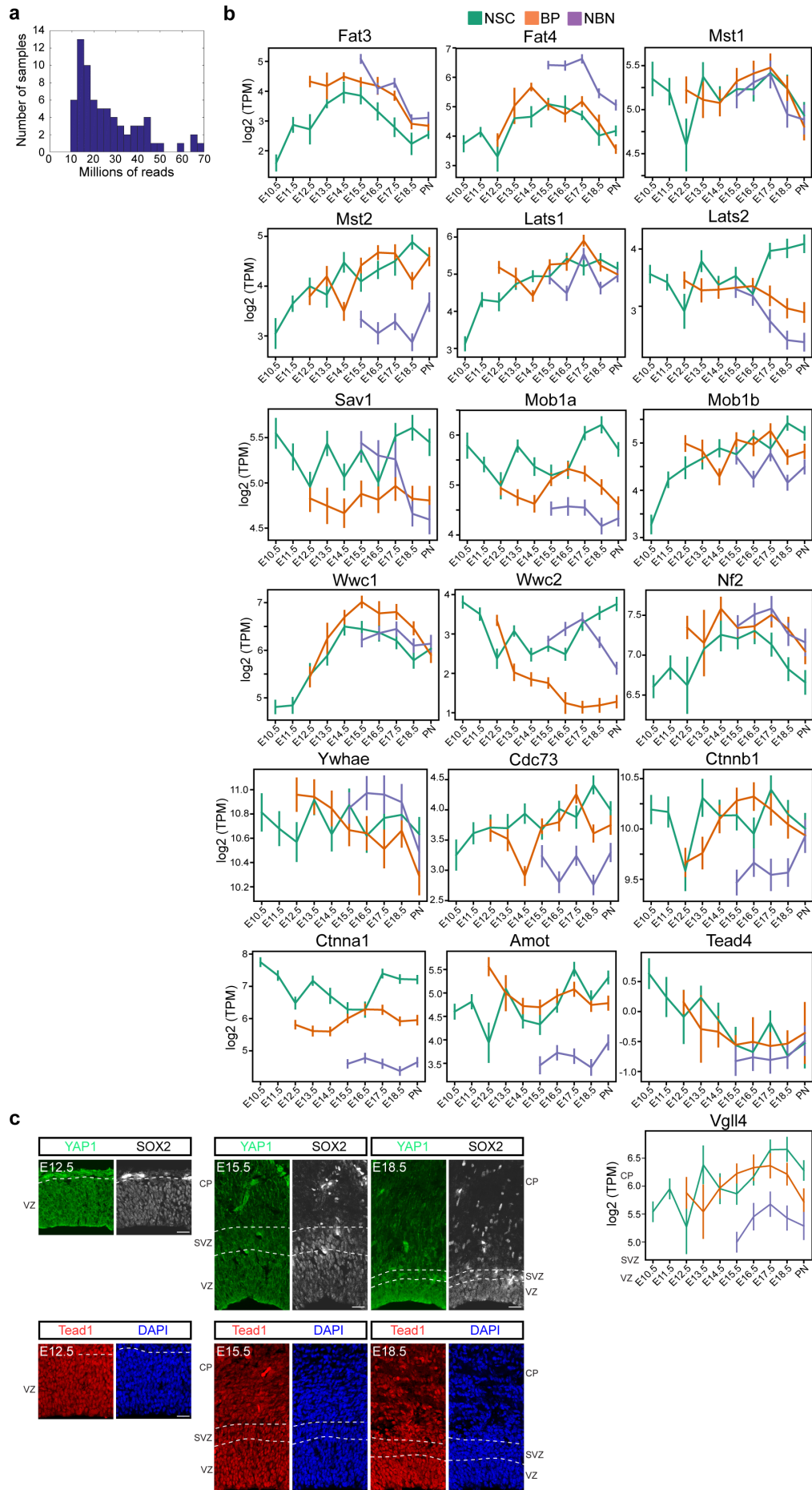
Figure S2

Figure S2: Transcriptional Dynamics of additional Hippo signaling effectors in NSCs, BPs and NBNs from RNA sequencing.

a. Graphical representation of number of total RNA-seq reads over the samples.

b. Expression profiles of components and intermediate effectors of Hippo signaling. Y-axis: mRNA level expressed as log2 TPM (transcripts per million).

c. Expression of Yap1 and Tead1 compared to the progenitor marker Sox2 in the developing cerebral cortex at E12.5, E15.5 and E18.5. Scale bars = 20μm.

Figure S3

a Neural progenitors

b Distribution of transfected cells

c Distribution of Pax6⁺ cells

d Cell Proliferation

e VZ   SVZ/IZ

f

g VZ

h Cell death

i SVZ/IZ

j Basal progenitors

k Distribution of Tbr2⁺ cells

m Upper layer neurons

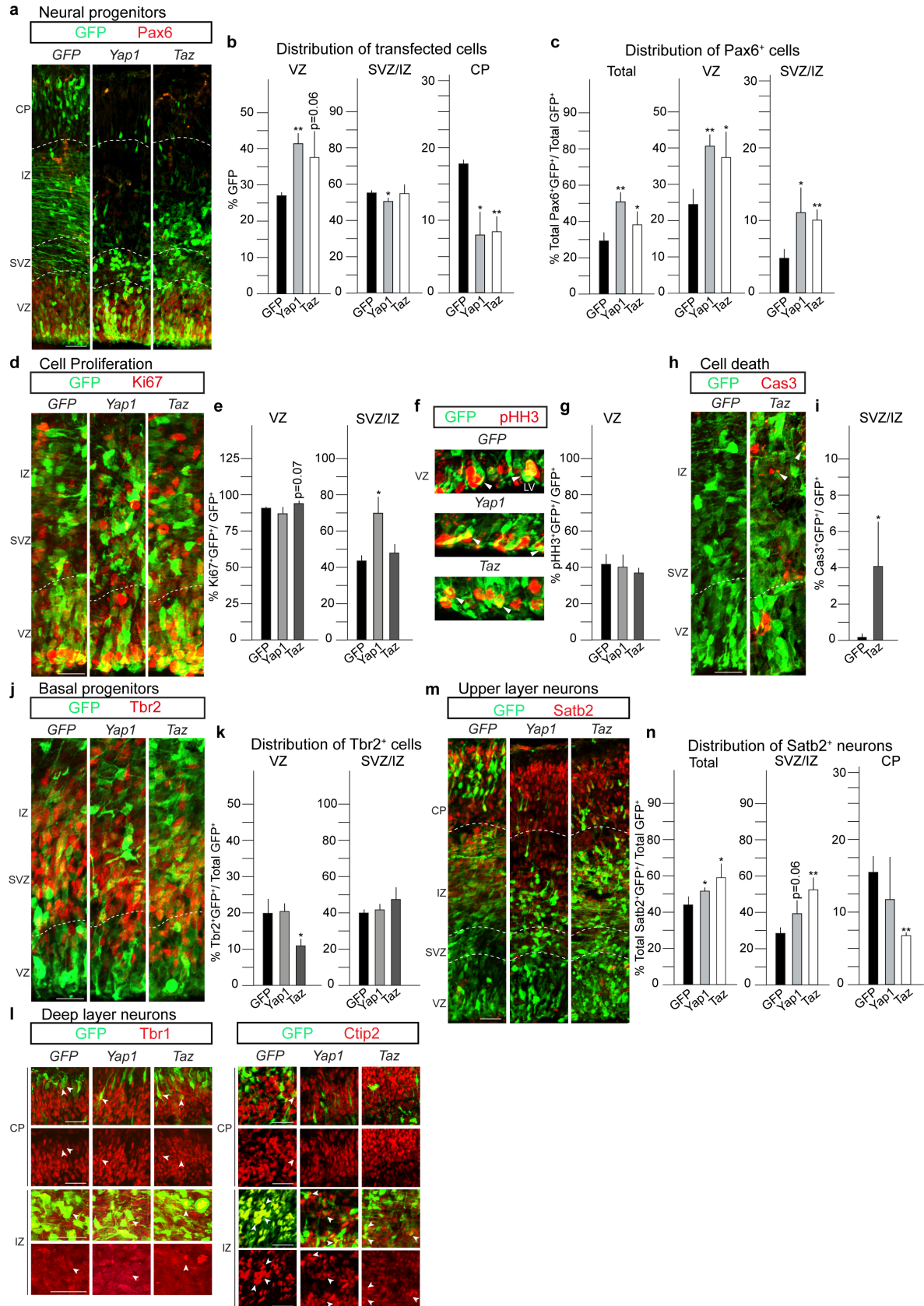**n** Distribution of Satb2⁺ neurons

l Deep layer neurons

Figure S3: Co-activators Yap1 and Taz overexpression increase percentage of NSCs and reduce differentiation.

a. Coronal sections of transfected cortices immunostained for GFP and Pax6.

b. Quantification of distribution of GFP$^+$ transfected cells shows both Yap1 and Taz overexpression induce a similar phenotype.

c. Quantification of Pax6$^+$GFP$^+$ cells shows an increase in total Pax6$^+$ cells upon Yap1 and Taz overexpression, compared to GFP control in VZ, SVZ and total.

d. Coronal sections of transfected cortices immunostained for GFP and Ki67.

e. Quantification of Ki67$^+$GFP$^+$ cells shows no change in total Ki67$^+$ cells upon Yap1 and Taz overexpression, compared to GFP control in VZ, increase is observed in SVZ upon Yap1 overexpression.

f. Coronal sections of transfected cortices along the apical surface immunostained for GFP and pHH3.

g. Quantification of pHH3$^+$GFP$^+$ cells with their cell bodies lining the VZ shows no change in total pHH3$^+$ M-phase cells upon Yap1 and Taz overexpression, compared to GFP control in the apical VZ.

h. Coronal sections of transfected cortices immunostained for GFP and Cas3.

i. Quantification of Cas3$^+$GFP$^+$ cells shows increase in total Cas3$^+$ cells upon Taz overexpression, compared to GFP control in SVZ/IZ.

j. Coronal sections of transfected cortices immunostained for GFP and Tbr2.

k. Quantification of Tbr2$^+$GFP$^+$ cells shows a reduction in Tbr2$^+$ cells upon Taz overexpression compared to the GFP control.

l. Transfected cortices immunostained for GFP and Tbr1 or Ctip2, showing GFP$^+$ deep layer neurons marker positive cells in the CP and IZ.

m. Coronal sections of transfected cortices immunostained for GFP and Satb2, showing impaired migration of GFP$^+$ cells.

n. Quantification of distribution of GFP$^+$ transfected cells shows both Yap1 and Taz overexpression increase total Satb2$^+$GFP$^+$ cells in all cortical zones.

Summaries of the quantifications are in Table S1. Scale bars = 20 μm in d and j, 50 μm in a, h, l and m. Data are shown as average $\pm$ SEM, *p= 0.05, **p= 0.01.
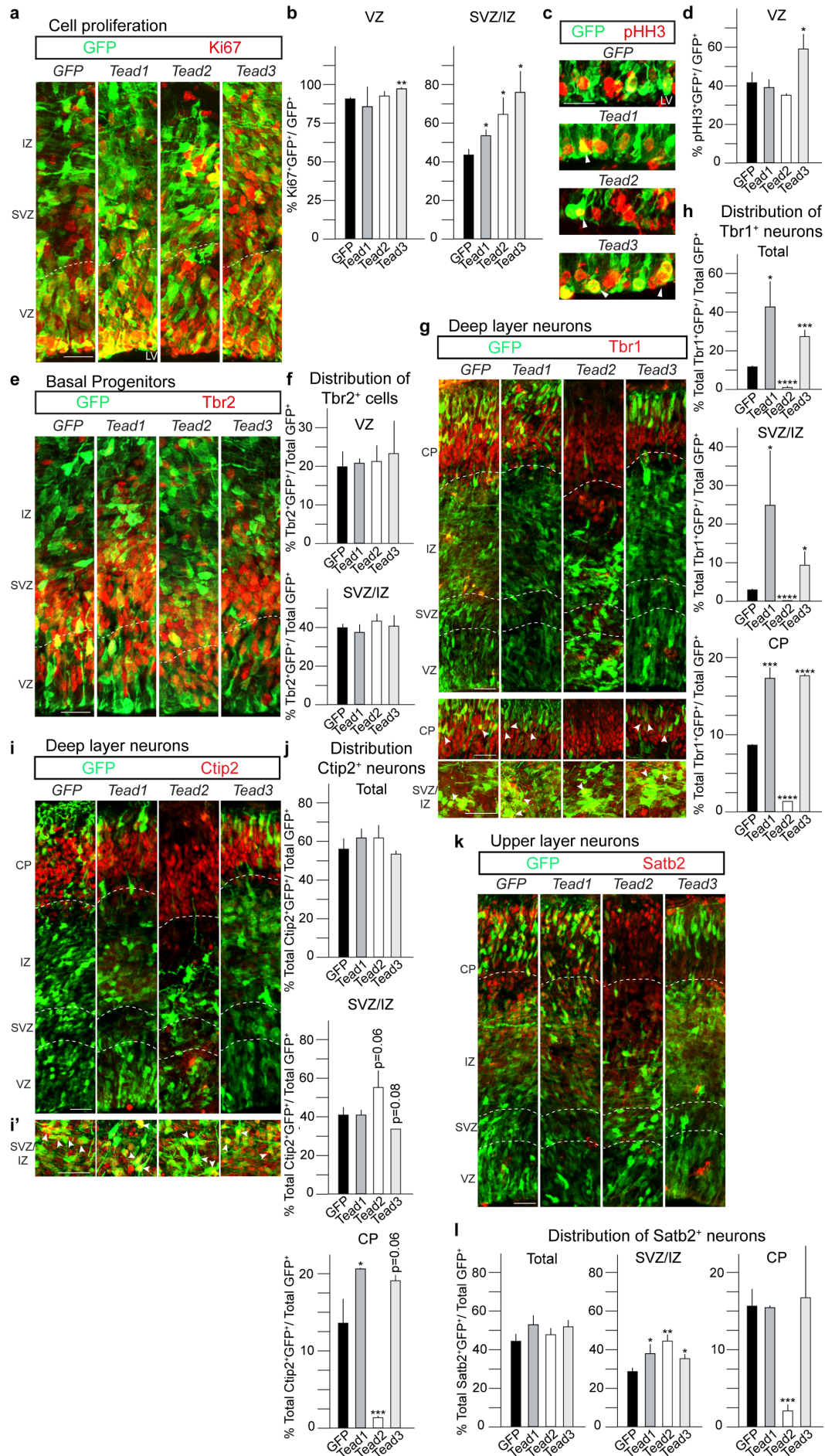
Figure S4

Figure S4: Tead1, Tead2 and Tead3 overexpression affects cell fate and cortical layering.

a. Coronal sections of transfected cortices immunostained for GFP and Ki67.

b. Quantification of Ki67$^+$GFP$^+$ cells shows an increase in proliferation upon overexpression of Tead3 in VZ; and increase in proliferation in SVZ/IZ upon overexpression of all Tead1, Tead2 and Tead3.

c. Coronal sections of transfected cortices along the apical surface, immunostained for GFP and pHH3.

d. Quantification of pHH3$^+$GFP$^+$ cells with their cell bodies lining the VZ upon Tead1, Tead2 and Tead3 overexpression, compared to GFP control in the apical VZ.

e. Coronal sections of transfected cortices immunostained for GFP and Tbr2.

f. Quantification of Tbr2$^+$GFP$^+$ cells shows no change in BPs upon overexpression of Tead TFs.

g. Coronal sections of transfected cortices immunostained for GFP and Tbr1.

h. Quantification of distribution of GFP$^+$ transfected cells shows that both Tead1 and Tead3 overexpression induce a similar phenotype, an increase of Tbr1$^+$GFP$^+$ cells in SVZ/IZ, CP and total. Tead2 overexpression shows an opposite phenotype to Tead1 and Tead3, with less Tbr1$^+$GFP$^+$ cells in the CP and also a significant decrease in total.

i-i". Coronal sections of transfected cortices immunostained for GFP and Ctip2.

j. Quantification of distribution of GFP$^+$ transfected cells shows both Tead1 and Tead3 overexpression induce a similar phenotype, an increase of Ctip2$^+$GFP$^+$ cells in CP and no changes in total.

k. Coronal sections of transfected cortices immunostained for GFP and Satb2.

l. Quantification of Satb2$^+$GFP$^+$ cells over total GFP$^+$ cells shows a decrease in Satb2$^+$GFP$^+$ cells in CP upon Tead2 overexpression, compared to GFP control. Satb2$^+$GFP$^+$ cells remain unchanged upon overexpression of Tead1 and Tead3.

Summaries of the quantifications are in Table S2. Scale bars = 50 μm. Data are shown as average $\pm$ SEM, *p= 0.05, **p= 0.01, ***p=0.001, ****<p=0.0001.

Figure S5

a Deep layer neurons

GFP   Tbr1

GFP   Tead1 DN   Tead2 DN   Tead3 DN

CP
IZ
SVZ
VZ

b Distribution of Tbr1⁺ neurons

Total   SVZ/IZ   CP

% Total Tbr1$^+$GFP$^+$/ Total GFP$^+$

GFP Tead1 DN Tead2 DN Tead3 DN

c Deep layer neurons

GFP   Ctip2

GFP   Tead1 DN   Tead2 DN   Tead3 DN

CP
IZ
SVZ
VZ

d Distribution of Ctip2⁺ neurons

Total   SVZ/IZ   CP

% Total Ctip2$^+$GFP$^+$/ Total GFP$^+$

p=0.08
p=0.06

GFP Tead1 DN Tead2 DN Tead3 DN

e Upper layer neurons

GFP   Satb2

GFP   Tead1 DN   Tead2 DN   Tead3 DN

CP
IZ
SVZ
VZ

f Distribution of Satb2⁺ neurons

Total   SVZ/IZ   CP

% Total Satb2$^+$GFP$^+$/ Total GFP$^+$

p=0.09
p=0.08
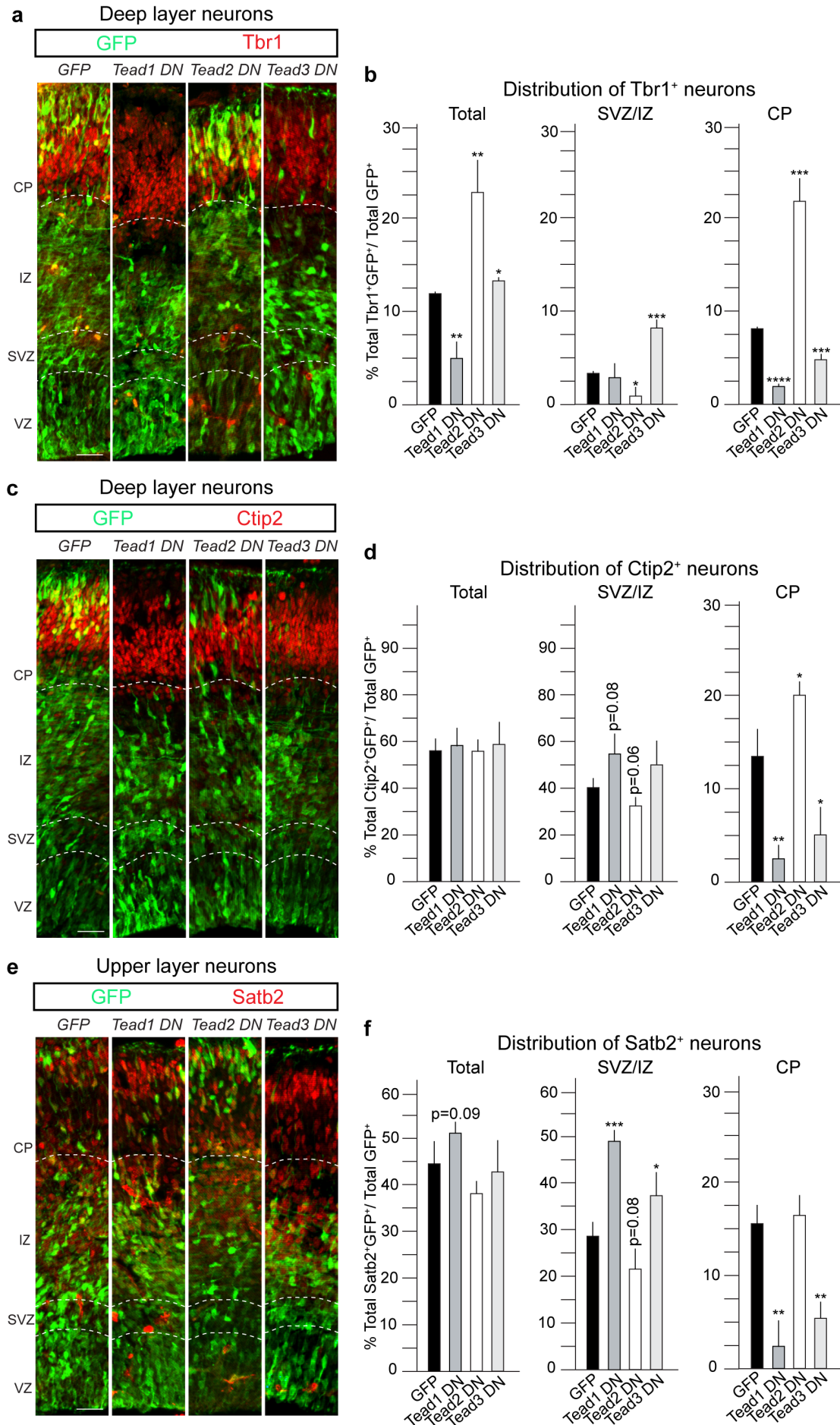
GFP Tead1 DN Tead2 DN Tead3 DN

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Figure S5: Tead1 DN, Tead2 DN and Tead3 DN constructs affect cell fate and cortical layering.

a. Coronal sections of transfected cortices immunostained for GFP and Tbr1.

b. Quantification of fate changes in deep layer Tbr1$^+$ neurons, shows a decrease of Tbr1$^+$GFP$^+$ cells in CP upon Tead1 DN and Tead3 DN transfection. This is opposite to the phenotype with Tead2 DN, with an increase of Tbr1$^+$GFP$^+$ cells in CP. Similar changes are observed in total Tbr1$^+$GFP$^+$ cells.

c. Coronal sections of transfected cortices immunostained for GFP and Ctip2.

d. Quantification of fate changes in deep layer Ctip2$^+$ neurons, shows a decrease of Ctip2$^+$GFP$^+$ cells in CP upon Tead1 DN and Tead3 DN transfection. This is opposite to the phenotype with Tead2 DN, with an increase of Ctip2$^+$GFP$^+$ cells in CP. The total cell numbers remain unchanged; hence the manipulations only affect the migration of Ctip2$^+$GFP$^+$ cells.

e. Coronal sections of transfected cortices immunostained for GFP and Satb2.

f. Quantification of distribution of GFP$^+$ transfected cells shows both Tead1 and Tead3 overexpression induce a similar phenotype, a decrease of Satb2$^+$GFP$^+$ cells in CP and no changes in total.

Summaries of the quantifications are in Table S3. Scale bars = 50 μm. Data are shown as average $\pm$ SEM, *p= 0.05, **p= 0.01, ***p=0.001, ****<p=0.0001.

Figure S6

Figure S6: Additional ISMARA predicted targets tested by RT-qPCR and ChIP-PCR.

a. ChIP-qPCR does not pull-down Ntn4, Axin2 and Tead4 with both Tead1 and Tead2. An empty mCherry vector was used as the negative control.

b. Relative expression of Reln, Dab2ip, Ntn4, ApoER2, Dab1, Lis1, Nde1, Yap1 and Dtna show no significant changes upon overexpression of both Tead1 and Tead2.

Summaries of the quantifications are in Table S5. Data are shown as average ± SEM.

Figure S7

a   Neural progenitors

b   Distribution of Pax6⁺ progenitors

c   Deep layer neurons

d   Distribution of Ctip2⁺ neurons

e   Upper layer neurons

f   Distribution of Satb2⁺ neurons

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Figure S7: Overexpression of ApoE, Cyr61 and Dab2 affects cell fate and cortical layering.

a. Coronal sections of transfected cortices immunostained for GFP and Pax6.

b. Quantification of Pax6$^+$GFP$^+$ cells shows an increase in total Pax6$^+$ cells upon ApoE overexpression.

c. Coronal sections of transfected cortices immunostained for GFP and Ctip2.

d. Quantification of fate changes in deep layer Ctip2$^+$ neurons, shows a slight increase of Ctip2$^+$GFP$^+$ cells in total upon Cyr61 and Dab2 overexpression.

e. Coronal sections of transfected cortices immunostained for GFP and Satb2.

f. Quantification of distribution of GFP$^+$ transfected cells shows ApoE overexpression decreased Satb2$^+$GFP$^+$ cells in CP and no changes in total. Dab2 overexpression increases the Satb2$^+$GFP$^+$ cells in SVZ/IZ.

Summaries of the quantifications are in Table S6. Scale bars = 50 μm. Data are shown as average $\pm$ SEM, *p= 0.05.
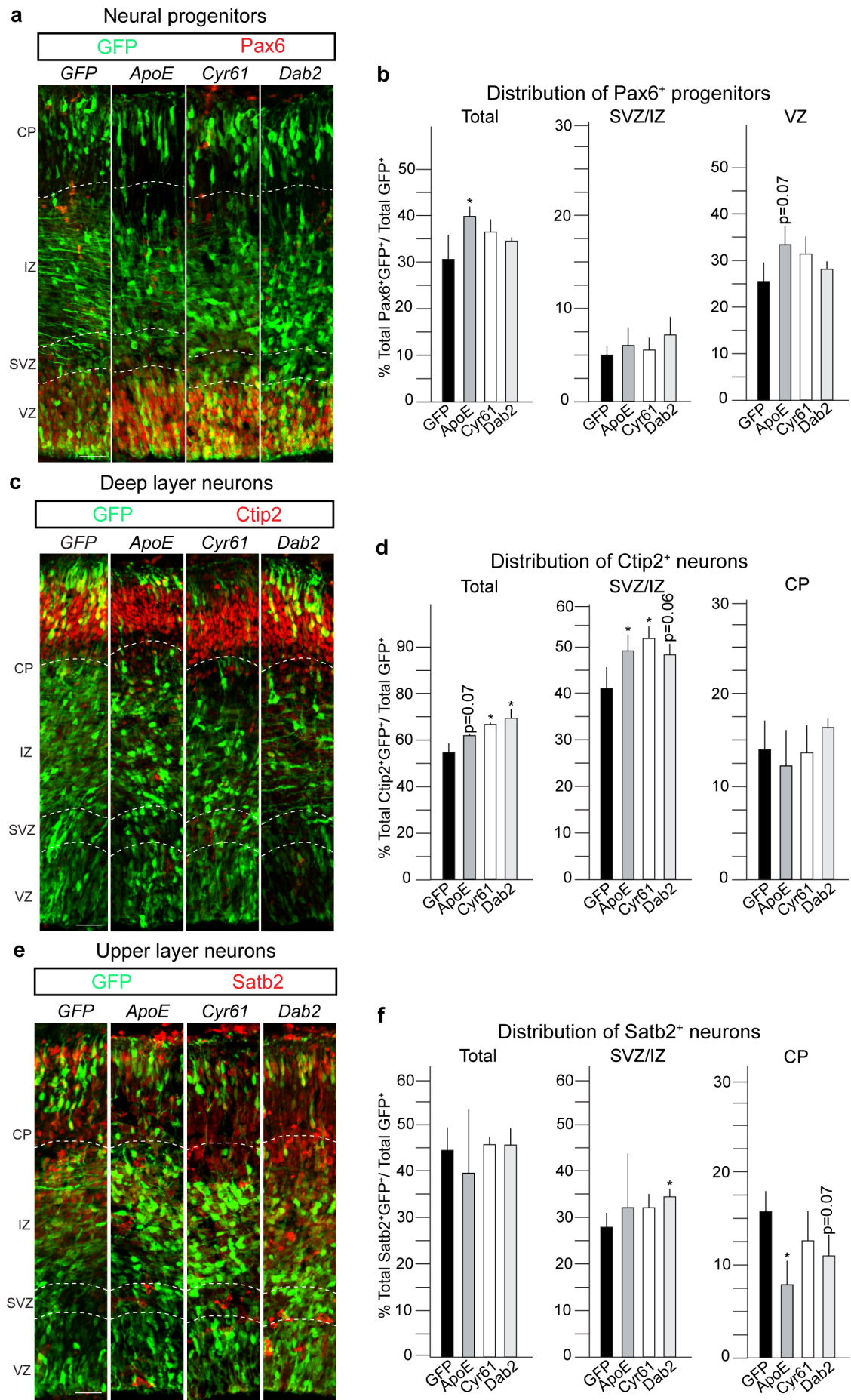
Figure S8

a

GFP Control

Gain of function-
Tead1, Tead3, Dab2 and
Tead2 DN

Gain of function-
Yap1, Taz, Tead2, ApoE and
Tead1 DN, *Tead3* DN

b

Binding partners of Tead1 and Tead2

c

IP Anti-Flag

1- mCherry (negative control)
2- Tead1-flag + Yap1-HA
3- Tead2-flag + Yap1-HA

IP Anti-Flag

1- Tead1-flag + Taz-HA
2- Tead2-flag + Taz-HA

d

1- mCherry (negative control)
2- Tead1-flag + Yap1-HA
3- Tead2-flag + Yap1-HA

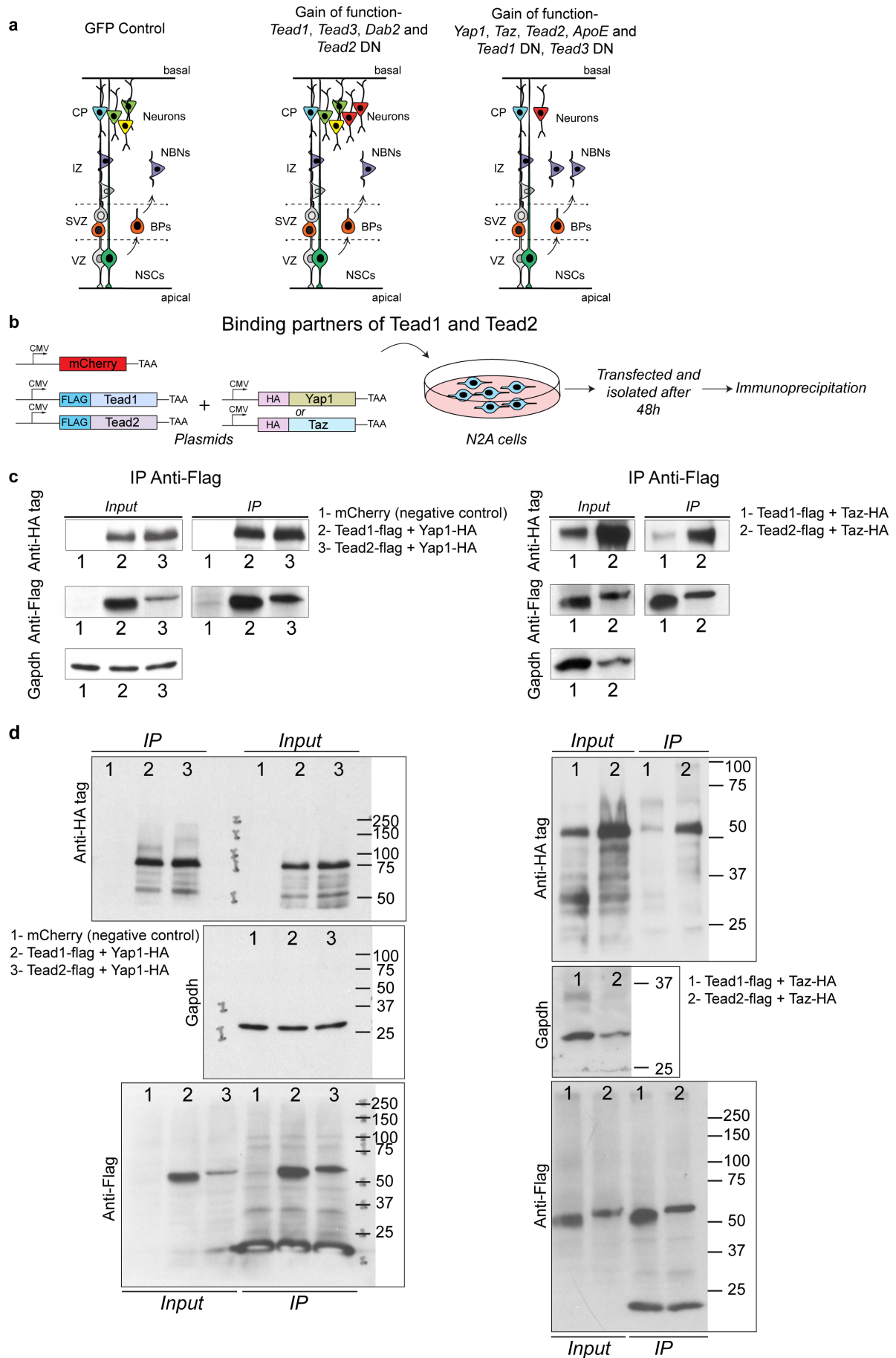1- Tead1-flag + Taz-HA
2- Tead2-flag + Taz-HA

Figure S8: Tead2 preferentially binds Yap1, in an overexpression paradigm.

a. Summary of the phenotypic effects upon gain and loss of function of Hippo effectors and targets on neuronal migration in the CP.

b. N2A cells were transfected with flag tagged-Tead1 or Tead2 expression constructs; co-transfected with HA-tagged Yap1 expression construct. Cells were lysed after 48 hours and immunoprecipitation was performed using Anti-Flag antibody.

c. Western blot detection revealed that Yap1 preferentially binds Tead2 in N2A cells. Cropped images of the complete blots shown in **d**.

d. Images of the full-length blots including, molecular weight markers of the, of the cropped blots shown in **c**.

Supplementary Data Tables

Table S1

a

		Average ± SEM (GFP⁺ cells) E13.5-E15.5, 48 hour chase		
		VZ	SVZ/IZ	CP
GFP Control		28.11 ± 0.46	54.04 ± 1.00	17.85 ± 0.55
	n	n=3	n=3	n=3
Yap1		52.45 ± 3.07	49.16 ± 1.41	8.35 ± 1.69
	n	n=3	n=3	n=3
Taz		48.94 ± 4.65	53 ± 2.95	8.55 ± 1.31
	n	n=3	n=3	n=3
P-Values				
Yap1 v's Control		0.0017 (**)	0.0466 (*)	0.0102 (*)
Taz v's Control		0.066 (*)	0.7571 (ns)	0.0049 (**)

b

		Average ± SEM (GFP⁺Pax6⁺ cells)/Total GFP+ E13.5-E15.5, 48 hour chase		
		VZ	SVZ/IZ	Total
GFP Control		25.71 ± 2.40	4.90 ± 0.65	30.61 ± 2.87
	n	n=3	n=3	n=3
Yap1		41.22 ± 1.57	11.24 ± 2.05	52.45 ± 3.10
	n	n=3	n=3	n=3
Taz		38.45 ± 4.19	10.48 ± 0.90	48.94 ± 1.31
	n	n=3	n=3	n=3
P-Values				
Yap1 v's Control		0.0066 (**)	0.0299 (*)	0.0069 (**)
Taz v's Control		0.05 (*)	0.006 (**)	0.0283 (*)

c

		Average ± SEM (Ki67⁺GFP⁺ cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase	
		VZ	SVZ/IZ
GFP Control		92.71 ± 0.43	43.7 ± 2.16
	n	n=3	n=3
Yap1		90.75 ± 3.73	52.8 ± 1.75
	n	n=3	n=3
Taz		96.96 ± 1.206	47.59 ± 2.8
	n	n=3	n=3
P-Values			
Yap1 v's Control		0.6293 (ns)	0.0308 (*)
Taz v's Control		0.06 (ns)	0.322 (ns)

d

		Average ± SEM (pHH3⁺GFP⁺cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase
		VZ
GFP Control		42.01 ± 3.131
	n	n=3
Yap1		40.83 ± 3.816
	n	n=3
Taz		37.69 ± 1.404
	n	n=3
P-Values		
Yap1 v's Control		0.8231 (ns)
Taz v's Control		0.2761 (ns)

e

		Average ± SEM (Cas3$^+$GFP$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase
		SVZ/IZ
GFP Control		0.18 ± 0.11
	n	n=3
Taz		3.97± 1.37
	n	n = 3
P-Values Taz v's Control		0.05 (*)

f

		Average ± SEM (GFP$^+$Tbr2$^+$ cells)/Total GFP+ in respective zones E13.5-E15.5, 48 hour chase	
		VZ	SVZ/IZ
GFP Control		20.52 ± 2.19	40.64 ± 1.02
	n	n=3	n=3
Yap1		21.29 ± 1.16	42.46 ± 1.73
	n	n=3	n=3
Taz		11.73 ± 1.01	48.39 ± 3.57
	n	n=3	n=3
P-Values Yap1 v's Control Taz v's Control		0.7714 (ns) 0.0219 (*)	0.4165 (ns) 0.1052 (ns)

g

		Average ± SEM (GFP$^+$Tbr1$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase		
		SVZ/IZ	CP	Total
GFP Control		3.66 ± 0.08	8.68 ± 0.07	12.34 ± 0.09
	n	n=3	n=3	n=3
Yap1		2.88 ± 1.48	5.20 ± 0.36	8.08 ± 1.70
	n	n=3	n=3	n=3
Taz		3.44 ± 0.91	4.83 ± 0.70	8.28 ± 0.26
	n	n=3	n=3	n=3
P-Values Yap1 v's Control Taz v's Control		0.2402 (ns) 0.7319 (ns)	0.011 (**) 0.0099 (**)	0.0838 (*) 0.0002 (***)

h

		Average ± SEM (GFP$^+$Ctip2$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase			
		VZ	SVZ/IZ	CP	Total
GFP Control		1.87 ± 1.06	41.39 ± 2.47	13.98 ± 1.82	57.24 ± 3.02
	n	n=3	n=3	n=3	n=3
Yap1		7.58 ± 2.90	53.40 ± 3.2	6.46 ± 1.36	67.44 ± 4.23
	n	n=3	n=3	n=3	n=3
Taz		6.96 ± 1.68	56.21 ± 5.42	5.06 ± 1.10	70.32 ± 5.27
	n	n=3	n=3	n=3	n=3
P-Values Yap1 v's Control Taz v's Control		0.1082 (ns) 0.07 (ns)	0.041 (*) 0.05 (*)	0.0289 (*) 0.0126 (*)	0.1206 (ns) 0.1046 (ns)

i

		Average ± SEM (GFP^+Satb2^+ cells)/Total GFP^+ E13.5-E15.5, 48 hour chase		
		SVZ/IZ	CP	Total
GFP Control		28.87 ± 1.81	15.89 ± 1.39	44.76 ± 2.78
	n	n=3	n=3	n=3
Yap1		40.59 ± 4.45	12.00 ± 3.40	52.59 ± 1.05
	n	n=3	n=3	n=3
Taz		53.15 ± 3.89	6.90 ± 0.31	60.05 ± 4.20
	n	n=3	n=3	n=3
P-Values				
Yap1 v's Control		0.06 (ns)	0.3423 (ns)	0.05 (*)
Taz v's Control		0.0047 (**)	0.0021 (**)	0.04 (*)

Table S1: Quantification of phenotypic changes upon overexpression of co-activators Yap1 and Taz (refers to Fig. 2).

a. Table showing the distribution of transfected cells upon overexpression of Yap1 and Taz, compared to GFP control. **b.** Table showing quantification for $Pax6^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control **c.** Table showing quantification for $Ki67^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control. **d.** Table showing quantification for $pHH3^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control. **e.** Table showing quantification for $Cas3^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control. **f.** Table showing quantification for $Tbr2^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control. **g.** Table showing quantification for $Tbr1^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control. **h.** Table showing quantification for $Ctip2^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control. **i.** Table showing quantification for $Satb2^+GFP^+$ cells upon overexpression of Yap1 and Taz, compared to GFP control.

Table S2

a

	Average ± SEM (GFP⁺ cells) E13.5-E15.5, 48 hour chase		
	VZ	SVZ/IZ	CP
GFP Control	28.11 ± 0.46	54.04 ± 0.99	17.85 ± 0.55
n	n=3	n=3	n=3
Tead1	33.38 ± 1.87	43.99 ± 1.57	24.44 ± 1.33
n	n=3	n=3	n=3
Tead2	35.34 ± 1.54	64.08 ± 1.70	0.59 ± 0.22
n	n=3	n=3	n=3
Tead3	37.43 ± 1.62	36.33 ± 1.89	26.25 ± 0.75
n	n=3	n=3	n=3
P-Values			
Tead1 v's Control	0.05 (*)	0.0057 (**)	0.009 (***)
Tead2 v's Control	0.3129 (ns)	0.0072 (**)	<0.0001 (****)
Tead3 v's Control	0.0048 (**)	0.0012 (**)	0.0008 (***)

b

	Average ± SEM (GFP⁺Pax6⁺ cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase		
	VZ	SVZ/IZ	Total
GFP Control	25.71 ± 2.40	4.90 ± 0.65	30.61 ± 2.87
n	n=3	n=3	n=3
Tead1	31.74 ± 1.21	3.20 ± 0.63	34.95 ± 1.48
n	n=3	n=3	n=3
Tead2	32.21 ± 3.49	7.06 ± 1.98	39.26 ± 5.46
n	n=3	n=3	n=3
Tead3	37.43 ± 1.62	3.73 ± 0.57	41.15 ± 1.99
n	n=3	n=3	n=3
P-Values			
Tead1 v's Control	0.0927 (ns)	0.1396 (ns)	0.2512 (ns)
Tead2 v's Control	0.2016 (ns)	0.4088 (ns)	0.2361 (ns)
Tead3 v's Control	0.0173 (*)	0.2385 (ns)	0.0412 (*)

c

	Average ± SEM (GFP⁺Ki67⁺ cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase	
	VZ	SVZ/IZ
GFP Control	92.71 ± 0.43	43.7 ± 2.16
n	n=3	n=3
Tead1	88.99 ± 5.17	52.8 ± 1.75
n	n=3	n=3
Tead2	94.94 ± 1.95	65.29 ± 4.89
n	n=3	n=3
Tead3	98.49 ± 1.27	75.48 ± 5.78
n	n=3	n=3
P-Values		
Tead1 v's Control	0.5135 (ns)	0.0308 (*)
Tead2 v's Control	0.3271 (ns)	0.0156 (*)
Tead3 v's Control	0.0126 (*)	0.0068 (**)

d

		Average ± SEM (GFP^+pHH3^+ cells)/Total GFP^+ E13.5-E15.5, 48 hour chase
		VZ
GFP Control		42.01 ± 3.131
	n	n=3
Tead1		39.87 ± 2.112
	n	n=3
Tead2		36.02 ± 0.5645
	n	n=3
Tead3		60.03 ± 4.433
	n	n=3
P-Values		
Tead1 v's Control		0.6015 (ns)
Tead2 v's Control		0.133 (ns)
Tead3 v's Control		0.0294 (*)

e

		Average ± SEM (GFP^+Tbr2^+ cells)/Total GFP^+ in respective zones E13.5-E15.5, 48 hour chase	
		VZ	SVZ/IZ
GFP Control		20.52 ± 2.188	40.64 ± 1.025
	n	n=3	n=3
Tead1		21.47 ± 0.6866	38.61 ± 2.317
	n	n=3	n=3
Tead2		22.13 ± 2.39	44.46 ± 2.01
	n	n=3	n=3
Tead3		24.61 ± 4.768	42.03 ± 3.251
	n	n=3	n=3
P-Values			
Tead1 v's Control		0.6983 (ns)	0.4678 (ns)
Tead2 v's Control		0.6445 (ns)	0.166 (ns)
Tead3 v's Control		0.4784 (ns)	0.7043 (ns)

f

		Average ± SEM (GFP^+Tbr1^+ cells)/Total GFP^+ E13.5-E15.5, 48 hour chase		
		VZ	SVZ/IZ	Total
GFP Control		3.66 ± 0.08	8.68 ± 0.07	12.34 ± 0.09
	n	n=3	n=3	n=3
Tead1		25.71 ± 8.39	17.53 ± 0.88	43.24 ± 8.36
	n	n=3	n=3	n=3
Tead2		0.00 ± 0.00	1.04 ± 0.52	1.57 ± 0.02
	n	n=3	n=3	n=3
Tead3		10.22 ± 1.96	17.93 ± 0.20	28.14 ± 2.10
	n	n=3	n=3	n=3
P-Values				
Tead1 v's Control		0.0382 (*)	0.0003 (***)	0.014 (*)
Tead2 v's Control		<0.0001 (****)	<0.0001 (****)	<0.0001 (****)
Tead3 v's Control		0.0145 (*)	<0.0001 (****)	0.001 (***)

g

Average ± SEM (GFP⁺Ctip2⁺ cells)/Total GFP⁺
E13.5-E15.5, 48 hour chase

		VZ	SVZ/IZ	CP	Total
GFP Control		1.87 ± 1.06	41.39 ± 2.47	13.98 ± 1.82	57.24 ± 3.02
	n	n=3	n=3	n=3	n=3
Tead1		1.18 ± 1.18	27.91 ± 13.99	20.96 ± 0.06	64.59 ± 3.4
	n	n=3	n=3	n=3	n=3
Tead2		6.44 ± 1.42	56.21 ± 5.42	1.758 ± 0.26	64.40 ± 4.23
	n	n=3	n=3	n=3	n=3
Tead3		0.44 ± 0.44	35.33 ± 0.95	18.98 ± 0.47	54.75 ± 1.03
	n	n=3	n=3	n=3	n=3
P-Values					
Tead1 v's Control		0.6279 (ns)	0.7329 (ns)	0.0255 (*)	0.08 (ns)
Tead2 v's Control		0.07 (ns)	0.06 (ns)	0.001 (***)	0.2402 (ns)
Tead3 v's Control		0.3258 (ns)	0.08 (ns)	0.06 (ns)	0.479 (ns)

h

Average ± SEM (GFP⁺Satb2⁺ cells)/Total GFP⁺
E13.5-E15.5, 48 hour chase

		SVZ/IZ	CP	Total
GFP Control		28.87 ± 1.81	15.89 ± 1.39	44.76 ± 2.78
	n	n=3	n=3	n=3
Tead1		37.24 ± 2.51	15.44 ± 0.18	52.68 ± 2.68
	n	n=3	n=3	n=3
Tead2		45.79 ± 2.31	2.19 ± 0.56	47.97 ± 2.24
	n	n=3	n=3	n=3
Tead3		35.53 ± 1.50	16.9 ± 3.86	52.43 ± 2.40
	n	n=3	n=3	n=3
P-Values				
Tead1 v's Control		0.05 (*)	0.7957 (ns)	0.1097 (ns)
Tead2 v's Control		0.0045 (**)	0.0008 (***)	0.4181 (ns)
Tead3 v's Control		0.047 (*)	0.8589 (ns)	0.1051 (ns)

Table S2: Quantification of phenotypic changes upon overexpression of TFs Tead1, Tead2 and Tead3 (refers to Fig. 3).

a. Table showing the distribution of transfected cells upon overexpression of Tead1, Tead2 and Tead3 compared to GFP control. **b.** Table showing quantification for Pax6⁺GFP⁺ cells upon overexpression of Tead1, Tead2 and Tead3, compared to GFP control. **c.** Table showing quantification for Ki67⁺GFP⁺ cells upon overexpression of Tead1, Tead2 and Tead3, compared to GFP control. **d.** Table showing quantification for pHH3⁺GFP⁺ cells upon overexpression of Tead1, Tead2 and Tead3, compared to GFP control. **e.** Table showing quantification for Tbr2⁺GFP⁺ cells upon overexpression of Tead1, Tead2 and Tead3, compared to GFP control. **f.** Table showing quantification for Tbr1⁺GFP⁺ cells upon overexpression of Tead1, Tead2 and Tead3, compared to GFP control. **g.** Table showing quantification for Ctip2⁺GFP⁺ cells upon overexpression of Tead1, Tead2 and Tead3, compared to GFP control **h.** Table showing quantification for Satb2⁺GFP⁺ cells upon overexpression of Tead1, Tead2 and Tead3, compared to GFP control.

Table S3

a

		Average ± SEM (GFP⁺ cells) E13.5-E15.5, 48 hour chase		
		VZ	SVZ/IZ	CP
GFP Control		28.11 ± 0.46	54.04 ± 0.99	17.85 ± 0.55
	n	n=3	n=3	n=3
Tead1 DN		38.59 ± 1.29	57.53 ± 1.93	3.89 ± 0.88
	n	n=3	n=3	n=3
Tead2 DN		32.36 ± 0.90	38.46 ± 1.07	29.19 ± 1.68
	n	n=3	n=3	n=3
Tead3 DN		36.59 ± 3.14	54.58 ± 3.10	8.832 ± 1.07
	n	n=3	n=3	n=3
P-Values				
Tead1 DN v's Control		0.0014 (**)	0.1833 (ns)	0.0006 (***)
Tead2 DN v's Control		0.013 (*)	0.0004 (***)	0.0024 (**)
Tead3 DN v's Control		0.05 (*)	0.8746 (ns)	0.0028 (**)

b

		Average ± SEM (GFP⁺Pax6⁺ cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase		
		VZ	SVZ/IZ	Total
GFP Control		25.71 ± 2.40	4.90 ± 0.65	30.61 ± 2.87
	n	n=3	n=3	n=3
Tead1 DN		32.36 ± 0.90	6.30 ± 0.79	39.29 ± 1.23
	n	n=3	n=3	n=3
Tead2 DN		38.58 ± 1.29	8.42 ± 1.05	47.00 ± 2.08
	n	n=3	n=3	n=3
Tead3 DN		36.59 ± 3.14	5.06 ± 0.56	41.65 ± 3.10
	n	n=3	n=3	n=3
P-Values				
Tead1 DN v's Control		0.06 (*)	0.1637 (ns)	0.05 (*)
Tead2 DN v's Control		0.0107 (*)	0.0413 (*)	0.0108 (*)
Tead3 DN v's Control		0.05 (*)	0.8484 (ns)	0.06 (*)

c

		Average ± SEM (GFP⁺Tbr1⁺ cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase		
		SVZ/IZ+	CP+	Total+
GFP Control		3.66 ± 0.08	8.68 ± 0.06	12.34 ± 0.09
	n	n=3	n=3	n=3
Tead1 DN		3.21 ± 1.01	2.22 ± 0.19	5.42 ± 1.02
	n	n=3	n=3	n=3
Tead2 DN		1.04 ± 0.63	22.44 ± 1.40	23.48 ± 2.00
	n	n=3	n=3	n=3
Tead3 DN		8.53 ± 0.64	5.20 ± 0.27	13.73 ± 0.38
	n	n=3	n=3	n=3
P-Values				
Tead1 DN v's Control		0.6025 (ns)	<0.0001 (****)	0.006 (**)
Tead2 DN v's Control		0.041 (*)	0.0003 (***)	0.0036 (**)
Tead3 DN v's Control		0.0009 (***)	0.0004 (***)	0.224 (ns)

d

		Average ± SEM (GFP⁺Ctip2⁺ cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase			
		VZ	SVZ/IZ	CP	Total
GFP Control		1.87 ± 1.06	41.39 ± 2.47	13.98 ± 1.82	57.24 ± 3.02
	n	n=3	n=3	n=3	n=3
Tead1 DN		3.45 ± 0.69	54.43 ± 5.15	2.57 ± 0.89	59.59 ± 4.99
	n	n=3	n=3	n=3	n=3
Tead2 DN		1.00 ± 0.51	33.09 ± 2.25	20.74 ± 0.85	57.30 ± 2.66
	n	n=3	n=3	n=3	n=3
Tead3 DN		2.84 ± 1.03	50.45 ± 6.00	5.22 ± 1.68	60.02 ± 5.14
	n	n=3	n=3	n=3	n=3
P-Values					
Tead1 DN v's Control		0.2817 (ns)	0.08 (ns)	0.0054 (**)	0.7061 (ns)
Tead2 DN v's Control		0.6334 (ns)	0.06 (ns)	0.035 (*)	0.9913 (ns)
Tead3 DN v's Control		0.5067 (ns)	0.2355 (ns)	0.0285 (*)	0.6585 (ns)

e

		Average ± SEM (GFP⁺Satb2⁺ cells)/Total GFP⁺ E13.5-E15.5, 48 hour chase		
		SVZ/IZ	CP	Total
GFP Control		28.87 ± 1.81	15.89 ± 1.39	44.76 ± 2.78
	n	n=3	n=3	n=3
Tead1 DN		49.14 ± 1.31	2.51 ± 1.56	51.65 ± 1.52
	n	n=3	n=3	n=3
Tead2 DN		21.99 ± 2.32	16.75 ± 1.25	38.74 ± 1.55
	n	n=3	n=3	n=3
Tead3 DN		37.68 ± 2.87	5.55 ± 1.04	43.24 ± 3.90
	n	n=3	n=3	n=3
P-Values				
Tead1 DN v's Control		0.0066 (**)	0.0299 (*)	0.095 (ns)
Tead2 DN v's Control		0.08 (ns)	0.6678 (ns)	0.1312 (ns)
Tead3 DN v's Control		0.05 (*)	0.04 (**)	0.7638 (ns)

Table S3: Quantification of phenotypic changes upon expression of dominant negative forms of TFs Tead1, Tead2 and Tead3 (refers to Fig. 4).
a. Table showing the distribution of transfected cells upon expression of dominant negative forms of Tead1, Tead2 and Tead3, compared to GFP control. **b.** Table showing quantification for Pax6⁺GFP⁺ cells upon expression of dominant negative forms of Tead1, Tead2 and Tead3, compared to GFP control. **c.** Table showing quantification for Tbr1⁺GFP⁺ cells upon expression of dominant negative forms of Tead1, Tead2 and Tead3, compared to GFP control. **d.** Table showing quantification for Ctip2⁺GFP⁺ cells upon expression of dominant negative forms of Tead1, Tead2 and Tead3, compared to GFP control. **e.** Table showing quantification for Satb2⁺GFP⁺ cells upon expression of dominant negative forms of Tead1, Tead2 and Tead3, compared to GFP control.

Table S4

a

	Average ± SEM (GFP$^+$ cells) E13.5-E15.5, 48 hour chase		
	VZ	SVZ/IZ	CP
GFP Control	28.11 ± 0.46	54.04 ± 0.99	17.85 ± 0.55
n	n=3	n=3	n=3
Tead1 VP16	46.8 ± 4.55	45.36 ± 4.66	7.72 ± 0.08
n	n=3	n=3	n=3
Tead2 VP16	48.34 ± 17.68	33.63 ± 13.64	1.89 ± 0.61
n	n=3	n=3	n=3
P-Values Tead1 VP16 v's Control Tead2 VP16 v's Control	0.0136 (*) 0.32 (ns)	0.1426 (*) 0.2091 (ns)	<0.0001 (****) 0.0002 (***)

b

	Average ± SEM (GFP$^+$Pax6$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase		
	VZ	SVZ/IZ	Total
GFP Control	25.71 ± 2.40	4.90 ± 0.65	30.61 ± 2.87
n	n=3	n=3	n=3
Tead1 VP16	46.92 ± 4.59	4.19 ± 1.03	51.12 ± 3.60
n	n=3	n=3	n=3
Tead2 VP16	48.22 ± 17.76	29.13 ± 14.14	77.35 ± 15.58
n	n=3	n=3	n=3
P-Values Tead1 VP16 v's Control Tead2 VP16 v's Control	0.0144 (*) 0.278 (ns)	0.5473 (ns) 0.1153 (ns)	0.0115 (*) 0.0476 (*)

Table S4: Quantification of phenotypic changes upon expression of transactive forms of TFs Tead1 and Tead2 (refers to Fig. 5).

a. Table showing the distribution of transfected cells upon expression of transactive forms of Tead1 and Tead2, compared to GFP control. **b.** Table showing quantification for Pax6$^+$GFP$^+$ cells upon expression of transactive forms of Tead1 and Tead2, compared to GFP control.

Table S5

a

Chromatin Immunoprecipitation	Negative	Tead1-flag	Tead2-flag
ApoE P-Values (t-test)	0.0002373 ± 0	0.002739 ± 0.00019 0.0063	0.004509 ± 0.00029 0.0048
n	n=2	n=2	n=2
Dab2 P-Values (t-test)	0.0007925 ± 4.35e-8	0.00274 ± 4.4e-7 <0.0001	0.00335 ± 0.001357 0.2002
n	n=2	n=2	n=2
Cyr61	0.001553 ± 2.55e-8	0.003421 ± 2.65e-7	0.00335 ± 0.001357 0.08
P-Values (t-test)		<0.0001	
n	n=2	n=2	n=2

b

Gene	Relative expression ± SEM (NSCs)		
	GFP Control	Tead1	Tead2
ApoE	1.11 ± 0.11	16.67 ± 0.70	31.43 ± 3.31
P-Values (t-test)		<0.0001	<0.0001
n	n=4	n=3	n=4
Dab2	0.65 ± 0.16	26.80 ± 9.85	38.95 ± 4.80
P-Values (t-test)		0.0378	0.0002
n	n=4	n=3	n=4
Cyr61	0.989 ± 0.12	5.99 ± 1.11	5.66 ± 1.14
P-Values (t-test)		0.0001	<0.0001
n	n=4	n=3	n=4
Dtna	1 ± 0.18	0.81 ± 0.03	1.01 ± 0.17
P-Values (t-test)		0.2332	0.9455
n	n=3	n=5	n=3
Reln	1 ± 0.14	0.80 ± 0.13	1.8 ± 0.9
P-Values (t-test)		0.3777	0.4939
n	n = 3	n = 5	n = 4
Dab2ip	1 ± 0.14	1.17 ± 0.15	0.77 ± 0.10
P-Values (t-test)		0.4339	0.2133
n	n = 5	n = 7	n = 5
Ntn4	1 ± 0.23	0.38 ± 0.024	0.666 ± 0.16
P-Values (t-test)		0.128	0.2978
n	n=3	n=2	n=3
ApoER2	1 ± 0.35	0.88 ± 0.10	1.76 ± 0.52
P-Values (t-test)		0.7007	0.3163
n	n=3	n=5	n=4
Dab1	1 ± 0.22	1.51 ± 0.14	1.18 ± 0.11
P-Values (t-test)		0.0821	0.4571
n	n=3	n=5	n=4
Lis1	1 ± 0.35	1.41 ± 0.18	0.87 ± 0.07
P-Values (t-test)		0.2891	0.681
n	n=3	n=5	n=4
Nde1	1 ± 0.33	0.74 ± 0.10	0.62 ± 0.08
P-Values (t-test)		0.3788	0.249
n	n=3	n=5	n=4
Yap1	1 ± 0.19	1.043 ± 0.06	0.99 ± 0.082
P-Values (t-test)		0.7952	0.9787
n	n=3	n=5	n=4

Table S5: Quantification of ChIP and relative expression for predicted Tead target genes (refers to Fig. 6).

a. Table summarizes ChIP results for flag tagged-Tead1 and flag tagged-Tead2 compared to negative control. **b.** Table summarizes relative expression of predicted target genes upon overexpression of Tead1 and Tead2 *in vivo*, compared to a GFP control.

Table S6

a

		Average ± SEM (GFP$^+$ cells) E13.5-E15.5, 48 hour chase		
		VZ	SVZ/IZ	CP
GFP Control		28.11 ± 0.46	54.04 ± 0.99	17.85 ± 0.55
	n	n=3	n=3	n=3
ApoE		33.85 ± 2.37	53.83 ± 1.40	12.32 ± 0.99
	n	n=3	n=3	n=3
Dab2		28.35 ± 0.88	51.16 ± 0.91	20.49 ± 0.42
	n	n=3	n=3	n=3
Cyr61		31.44 ± 2.19	50.08 ± 0.33	16.69 ± 0.73
	n	n=3	n=3	n=3
P-Values				
ApoE v's Control		0.07 (ns)	0.0906 (ns)	0.0101 (*)
Dab2 v's Control		0.82 (ns)	0.0986 (ns)	0.0194 (*)
Cyr61 v's Control		0.214 (ns)	0.0189 (*)	0.273 (ns)

b

		Average ± SEM (GFP$^+$Pax6$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase		
		VZ	SVZ/IZ	Total
GFP Control		25.71 ± 2.40	4.90 ± 0.65	30.61 ± 2.87
	n	n=3	n=3	n=3
ApoE		33.85 ± 2.37	5.99 ± 1.19	39.84 ± 1.18
	n	n=3	n=3	n=3
Dab2		28.35 ± 0.88	7.04 ± 1.19	35.39 ± 0.60
	n	n=3	n=3	n=3
Cyr61		31.44 ± 2.19	5.55 ± 0.79	36.99 ± 1.45
	n	n=3	n=3	n=3
P-Values				
ApoE v's Control		0.07 (ns)	0.5027 (ns)	0.0441 (*)
Dab2 v's Control		0.3585 (ns)	0.195 (ns)	0.1817 (ns)
Cyr61 v's Control		0.1563 (ns)	0.5511 (ns)	0.1216 (ns)

c

		Average ± SEM (GFP$^+$Tbr1$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase		
		SVZ/IZ	CP	Total
GFP Control		3.66 ± 0.08	8.67 ± 0.07	12.34 ± 0.09
	n	n=3	n=3	n=3
ApoE		5.49 ± 0.80	10.53 ± 2.33	16.02 ± 3.00
	n	n=3	n=3	n=3
Dab2		6.18 ± 1.13	16.66 ± 1.35	22.83 ± 2.01
	n	n=3	n=3	n=3
Cyr61		6.00 ± 0.96	15.91 ± 0.14	21.9 ± 0.91
	n	n=3	n=3	n=3
P-Values				
ApoE v's Control		0.08 (ns)	0.4972 (ns)	0.2927 (ns)
Dab2 v's Control		0.08 (ns)	0.0026 (**)	0.0043 (**)
Cyr61 v's Control		0.06 (ns)	<0.0001 (****)	0.0003 (***)

d

		Average ± SEM (GFP$^+$Ctip2$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase			
		VZ	SVZ/IZ	CP	Total
GFP Control		1.87 ± 1.06	41.39 ± 2.47	13.98 ± 1.82	57.24 ± 3.02
	n	n=3	n=3	n=3	n=3
ApoE		2.74 ± 0.37	50.29 ± 1.58	11.28 ± 1.83	64.31 ± 0.12
	n	n=3	n=3	n=3	n=3
Dab2		6.01 ± 1.51	48.51 ± 1.35	16.49 ± 0.55	71.01 ± 2.58
	n	n=3	n=3	n=3	n=3
Cyr61		2.78 ± 0.77	52.22 ± 1.66	13.66 ± 1.67	68.66 ± 0.37
	n	n=3	n=3	n=3	n=3
P-Values					
ApoE v's Control		0.5394 (ns)	0.03 (*)	0.03 (*)	0.078 (ns)
Dab2 v's Control		0.09 (ns)	0.06 (ns)	0.2646 (ns)	0.0262 (*)
Cyr61 v's Control		0.4552 (ns)	0.022 (*)	0.9106 (ns)	0.0189 (*)

e

		Average ± SEM (GFP$^+$Satb2$^+$ cells)/Total GFP$^+$ E13.5-E15.5, 48 hour chase		
		SVZ/IZ	CP	Total
GFP Control		28.87 ± 1.81	15.89 ± 1.39	44.76 ± 2.78
	n	n=3	n=3	n=3
ApoE		32.79 ± 6.51	7.90 ± 1.58	40.68 ± 7.70
	n	n=3	n=3	n=3
Dab2		35.09 ± 0.88	11.2 ± 1.44	46.29 ± 2.13
	n	n=3	n=3	n=3
Cyr61		33.27 ± 1.76	12.87 ± 2.00	46.13 ± 0.48
	n	n=3	n=3	n=3
P-Values				
ApoE v's Control		0.6346 (ns)	0.0208 (*)	0.6831 (ns)
Dab2 v's Control		0.0376 (*)	0.07 (ns)	0.6831 (ns)
Cyr61 v's Control		0.1549 (ns)	0.2897 (ns)	0.6491 (ns)

Table S6: Quantification of phenotypic changes upon overexpression of predicted Tead targets, ApoE, Dab2 and Cyr61 (refers to Fig. 7).

a. Table showing the distribution of transfected cells upon overexpression of ApoE, Dab2 and Cyr61, compared to GFP control. **b.** Table showing quantification for Pax6$^+$GFP$^+$ cells upon overexpression ApoE, Dab2 and Cyr61, compared to GFP control. **c.** Table showing quantification for Tbr1$^+$GFP$^+$ cells upon overexpression of ApoE, Dab2 and Cyr61, compared to GFP control. **d.** Table showing quantification for Ctip2$^+$GFP$^+$ cells upon overexpression of ApoE, Dab2 and Cyr61, compared to GFP control. **e.** Table showing quantification for Satb2$^+$GFP$^+$ cells upon overexpression of ApoE, Dab2 and Cyr61, compared to GFP control.