TRIM32 dependent transcription in adult neural progenitor cells regulates neuronal differentiation.

- Supplementary Information -

Anna-Lena Hillje[1, 2#], Maria Angeliki S. Pavlou[1, 2#], Elisabeth Beckmann[1, 3#] , Maik M.A. Worlitzer[1], Lamia'a Bahnassawy[1, 2], Lars Lewejohann[3], Thomas Palm[1], Jens C. Schwamborn[1, 2*]

[1]Westfälische Wilhelms-Universität Münster, ZMBE, Institute of Cell Biology, Stem Cell Biology and Regeneration Group, Von-Esmarch-Str. 56, D-48149 Münster, Germany

[2] Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-Belval, Luxembourg

[3]Westfälische Wilhelms-Universität Münster, Department of Behavioural Biology, Badestrasse 13, D-48149 Muenster, Germany

* Correspondence: Jens Schwamborn, Westfälische Wilhelms-Universität Münster, ZMBE, Institute of Cell Biology, Stem Cell Biology and Regeneration Group, Von-Esmarch-Str. 56, D-48149 Münster, Germany; Tel.:0049-2 51- 83 – 57183; Fax: 0049-2 51- 83 – 58616; E-Mail: jschwamb@uni-muenster.de

Supplementary Figure Legends

Supplementary figure 1: Only a small fraction of TRIM32 expressing cells is in the cell cycle.

a) Electrophoresis following RT-qPCR (of figure 1b). RNase free water was used as a negative control.

b) Immunostainings of mouse brain sections showing the SVZ (upper panel) or the RMS (lower panel) labeled with the indicated antibodies. Right columns show higher magnification of indicated areas. Scale bars 20 µm, for high magnifications 10 µm. c) Diagram showing the percentage of Ki67+ cells that are either TRIM32 positive (TRIM32 pos.) or TRIM32 negative (TRIM32 neg.) (mean +/- SEM, , t-test. $P<0.05$, $n \geq 330$ cells, $N \geq 3$ mice). d) Immunostainings of mouse brain sections showing the SVZ labeled with the indicated antibodies. Upper right columns show higher magnifications of indicated areas. Scale bars 20 µm, for high magnifications 10 µm. e) Diagram showing the percentage of PH3+ cells that are either positive (TRIM32 pos.) or TRIM32 negative (TRIM32 neg.) (mean +/- SEM, $n \geq 40$ cells, t-test. $P<0.05$).

f) – h) Immunostainings of mouse brain sections f) cortex; g) Olfactory bulb; h) corpus callosum, labeled with the indicated antibodies. Middle and right columns show higher magnification of indicated areas. Scale bars 20 µm, for high magnifications 10 µm.

i) – m) Immunostainings of mouse brain sections from the indicated region labeled with the indicated antibodies . The right columns show higher magnifications of

selected adult generated (EdU positive) cells. Scale bars 15 µm for i), 20 µm for j) – m). For high magnification 20 µm.

n) - o) Confocal images of the GCL or GL from immunostainings of mouse OB sections labeled with the indicated antibodies. In p) the middle and right columns show higher magnification. Scale bars 40 µm for n) and o). For p) 10 µm.

q-s) Immunostainings of mouse brain sections labeled with the indicated antibodies are shown. The right columns show higher magnification of selected cells. Scale bars 10 µm, for q) higher magnification 5 µm.

t) - u) Immunostainings of Marmoset (t) and Macaque (u) OB sections labeled with the indicated antibodies. Right columns show higher magnification of indicated areas. Scale bars 10 µm.

Supplementary figure 2: Loss of TRIM32 does not affect the amount of newborn cells in the GL. Relate to Figure 4.

a) Schematic drawing of the experimental paradigm. For three days mice received a daily injection of BrdU, 14 days later the brains were fixed and analyzed. b) Immunostainings of sections from wild-type and TRIM32 knock-out mice OB-GL labeled with the indicated antibodies. Scale bars 25 µm, high magnifications 10 µm.

Supplementary figure 3: After knock-down of TRIM32, dividing cells are detectable even in the distal RMS. Relate to Figure 5.

a) – b) N2a cells were transfected with plasmids coding for scrambled shRNA sequences (control) or shRNAs against TRIM32 (shRNA-1, shRNA-2) or with

retroviruses carrying the scrambled shRNA sequences (control) or shRNAs against TRIM32 (shRNA-1, shRNA-2). All constructs express GFP under an independent promoter. Protein levels of TRIM32 can successfully be reduced by knockdown with shRNA. Reduction of TRIM32 protein levels by two different shRNAs shown by Western Blot (a) and immunostainings of cultured N2A cells (b). For Western Blot and immunostainings the indicated antibodies have been used. In b) the right columns show higher magnification of indicated areas. Scale bars 10 µm, for higher magnifications 5 µm.

c) Following the verification that the retroviruses are able to transduce cells *in vitro*, they were injected to the SVZ of adult mice. Eight days after viral injection brain sections were obtained and immunostainings were performed in order to examine TRIM32 levels. On the right part of figure c, higher magnification of the indicated area is shown. Scale bars 20 µm, higher magnifications 10 µm. These results indicate that the utilized shRNAs mediate a knockdown of TRIM32 and that the used anti-TRIM32 antibodies are specific for TRIM32.

d) Viruses for expression of a scrambled shRNA sequence (control), TRIM32-shRNA or overexpression of TRIM32 were injected in the SVZ. Eight days after injection brain sections were analyzed. Immunostainings of mouse brain sections, taken from the distal RMS and labeled with the indicated antibodies are shown. Boxed areas are shown in higher magnification. Scale bars 35 µm, for high magnifications 10 µm (overlay) and 15 µm (individual channels). Only after knock-down of TRIM32 mitotic cells (condensed chromatin in the DNA staining) are detectable in the distal RMS.

Supplementary figure 4: Correct induction of neuronal differentiation depends on presence of TRIM32. Relate to Figure 5.

Viruses for expression of a scrambled shRNA sequence (control), TRIM32-shRNA or overexpression of TRIM32 were injected in the SVZ. All constructs express GFP under an independent promoter. Eight days after injection brain sections were analyzed. a) TuJ1 was used as an early neuronal differentiation marker. A quantification of GFP positive cells that are also positive for TuJ1, summarizing all brain regions is shown. b) – d) Immunostainings of mouse brain sections, taken from the SVZ and labeled with the indicated antibodies. Boxed areas are shown in higher magnification. Red arrows indicate transduced cells that are positive for TuJ1. Scale bars 30 µm, for higher magnifications 10 µm. e) Quantification of GFP positive cells in the SVZ which are also positive for TuJ1 (mean+/- SEM, $n \geq 500$; $N \geq 8$ mice, Mann-Whitney U test, *P<0.05; **P<0.01).

f) – h) Immunostainings of mouse brain sections, taken from the proximal RMS and labeled with the indicated antibodies. Boxed areas are shown in higher magnification. Red arrows indicate transduced cells that are positive for TuJ1. Scale bars 30 µm, for higher magnifications 10 µm. i) Quantification of GFP positive cells in the proximal RMS which are also positive for TuJ1 (mean+/- SEM, $n \geq 500$; $N \geq 8$ mice, Mann-Whitney U test, *P<0.05).

Supplementary figure 5: Loss of TRIM32 leads to TuJ1 negative cells in the RMS. Relate to Figure 6.

Viruses for expression of a scrambled shRNA sequence (control), TRIM32-shRNA or overexpression of TRIM32 were injected in the SVZ. All constructs express GFP

under an independent promoter. Eight days after injection brain sections were analyzed. Immunostainings of mouse brain sections, taken from the middle RMS and labeled with the indicated antibodies are shown in a) and b). Boxed areas are shown in higher magnification. Scale bars 30 µm, for high magnifications 10 µm. Red arrows indicate transduced cells that are positive for TuJ1. Only after knock-down of TRIM32 TuJ1 negative cells (usually with a round shape) are detectable in the middle RMS (b).

Supplementary figure 6: Gene Ontology (GO) term analysis of the 676 transcripts exclusively associated with loss of TRIM32. Relate to Figure 7.

a) 676 transcripts were exclusively regulated in TRIM32 knock-out mice and are therefore specifically associated to loss of TRIM32 (see Figure 7). A gene Ontology (GO) term analysis of the 676 transcripts exclusively associated to loss of TRIM32 is shown. The bar diagram represents the log(1/p-value). The p-value was calculated based on the Fisher's exact test.

b) Immunostainings of brain sections (wild-type and TRIM32 knock-out as indicated), taken from the OB (GCL, EPL and GL) and labeled with the indicated antibodies. Scale bars 45 µm, for high magnifications 10 µm. Only in the TRIM32 knock-out mitotic cells (condensed chromatin) are detectable. These cells are shown in higher magnification.

Supplementary Table Legends

Supplementary Table 1: TRIM32 expression is upregulated in cell types committed to the neuronal lineage. Relate to Figure 1 and Supplementary Figure S1.

Quantification of double-positive cells of immunostainings co-stained for TRIM32 with the according cell type markers in the indicated areas of the SVZ –OB system (mean +/- STDE), n>140 cells for each marker. Abbreviations are as follows: SVZ= subventricular zone, RMS= rostral migratory stream, PRMS= proximal migratory stream, MRMS= middle rostral migratory stream, DRMS= distal migratory stream, GCL= granular cell layer, CC= corpus callosum, OB= olfactory bulb.

Supplementary Table 2: Loss of TRIM32 results in an increase of newborn neurons and a decreased rate of apoptosis. Relate to Figure 4.

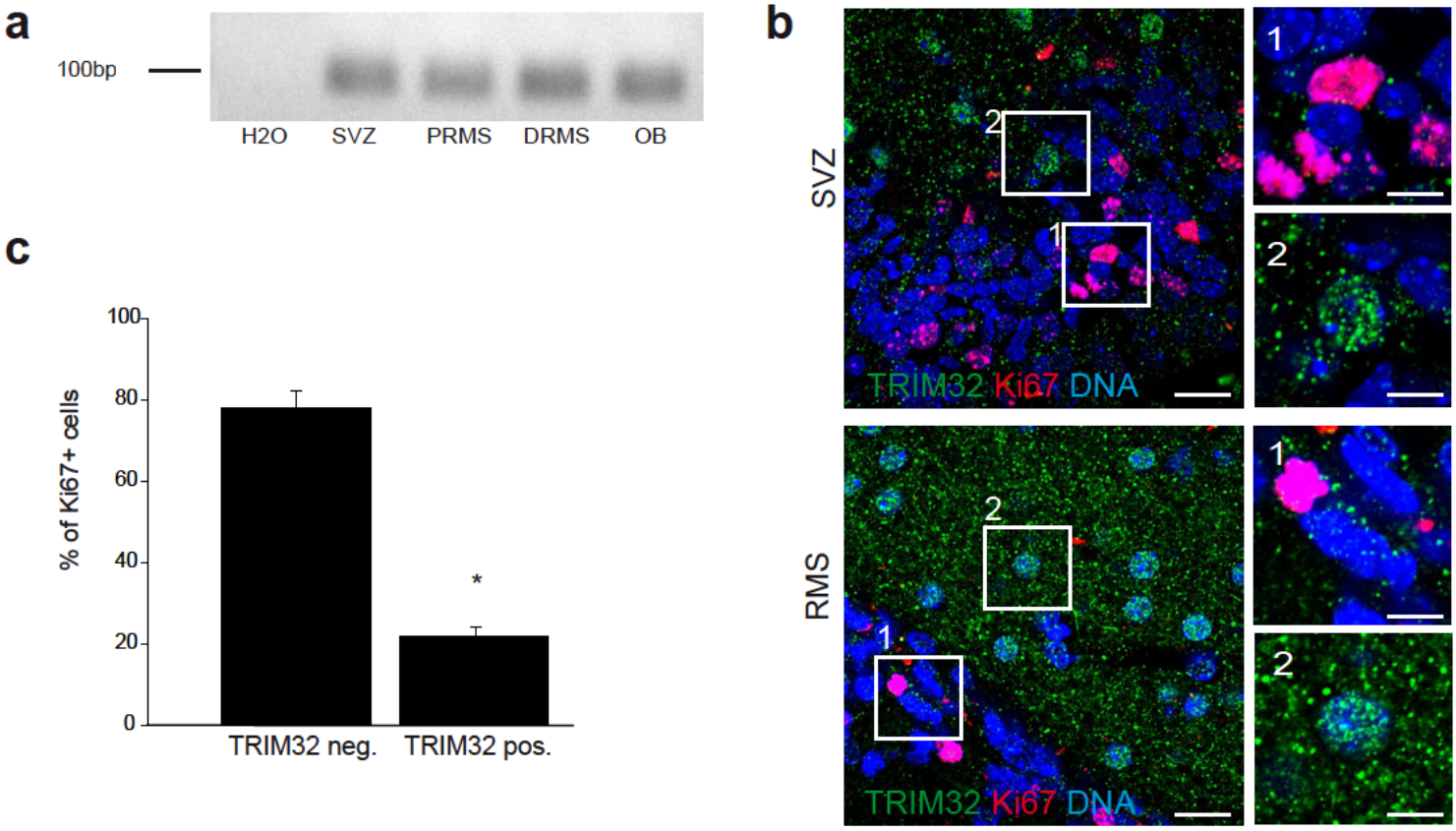
a) Absolute numbers of BrdU positive cells per GCL in wild-type (+/+) and TRIM32 knock-out mice (-/-). b) Quantification of cells that are double positive for BrdU and the neuronal marker NeuN per GCL. Quantification of cells that are positive for TUNEL (c) or Casp3 (d) in the GCL of wild-type and TRIM32 knock-out mice. N= 4 mice.

Supplementary Table 3: Loss of TRIM32 leads to more mitotically active cells. Relate to figure 7.

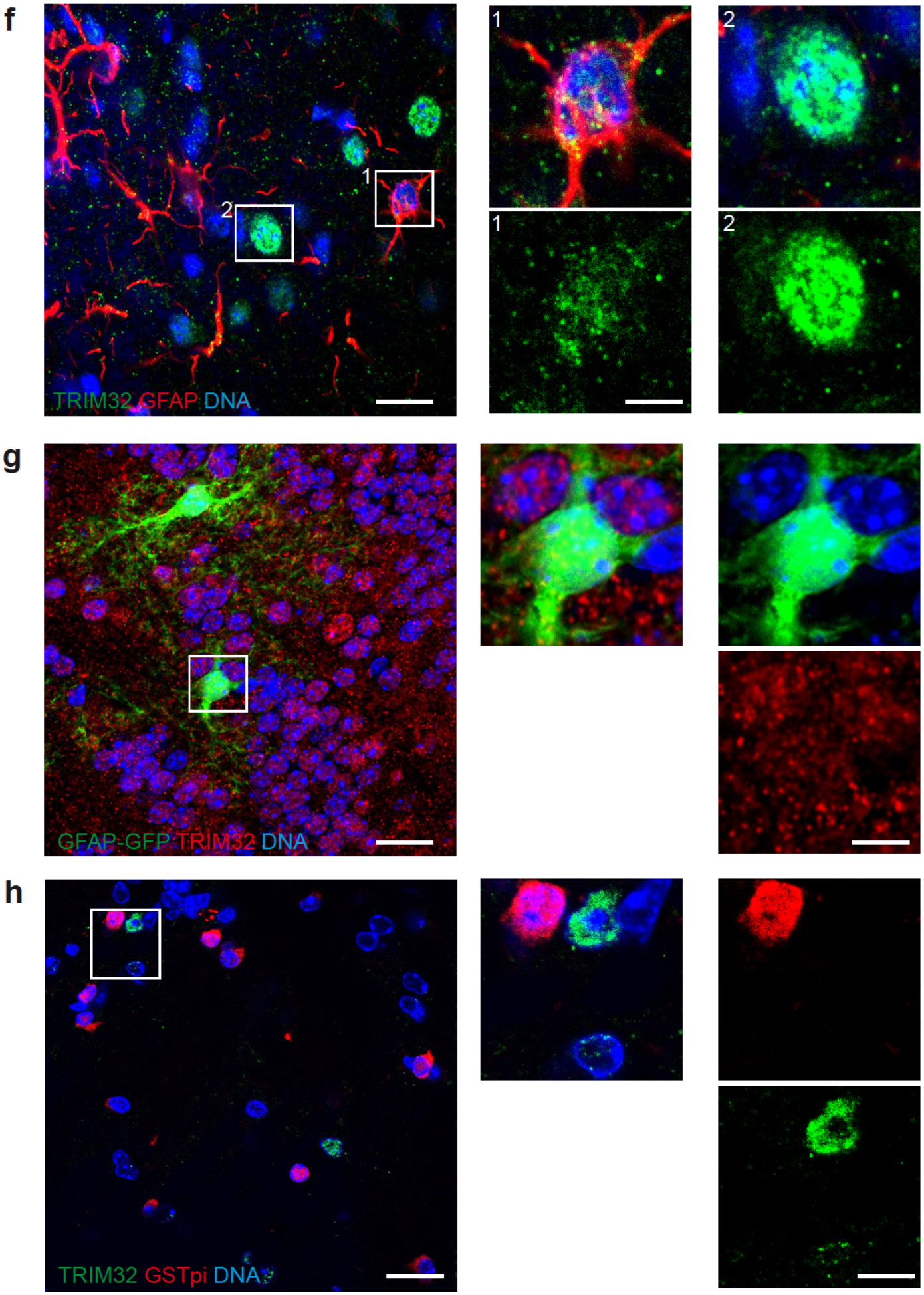
Quantification of absolute cell number for Ki67 positive cells on wt and TRIM32 ko mice through the different brain regions. N= 4 mice.

Supplementary Table 4: Loss of TRIM32 leads to deregulated expression of Cylins, CDKs and Bcl-2 family members. Relate to figure 7.

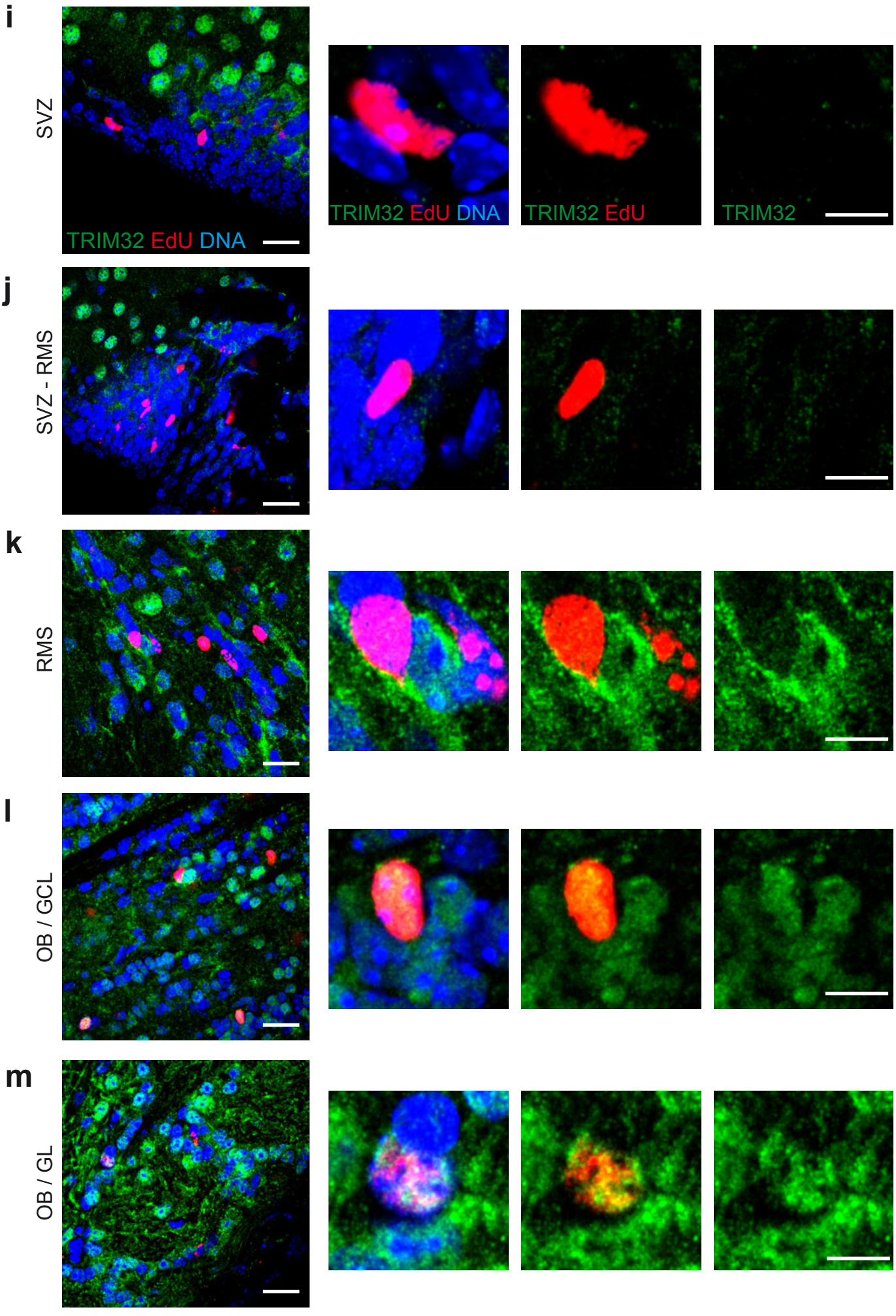
Loss of TRIM32 leads to a deregulation of genes involved in cell cycle control and apoptosis. Based on microarray data, the relative expression difference (comparison in the expression change in the RMS from proximal to distal in wild-type versus TRIM32 knock-out) was calculated. First, for each genotype (i.e. WT or Trim32 knock-out), the proximal expression level was set to 100% and distal expression changes were calculated in relation. In a second step, these relative changes, were compared between both genotypes [cut-off difference 20%]. Expression changes for cyclin dependent kinase (cdk) inhibitors, cyclins and Bcl-2 family members are shown.



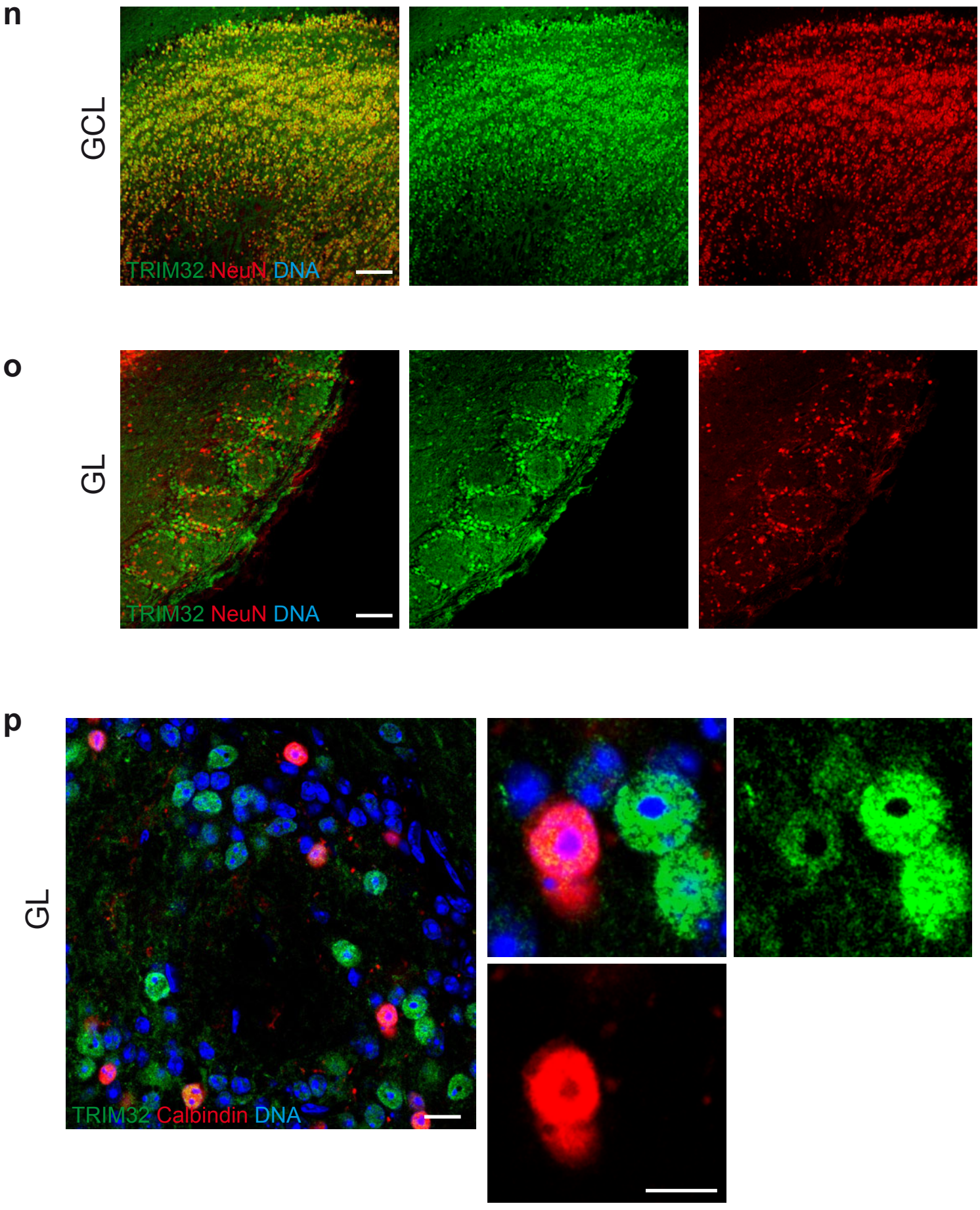
Supplementary Figure 1



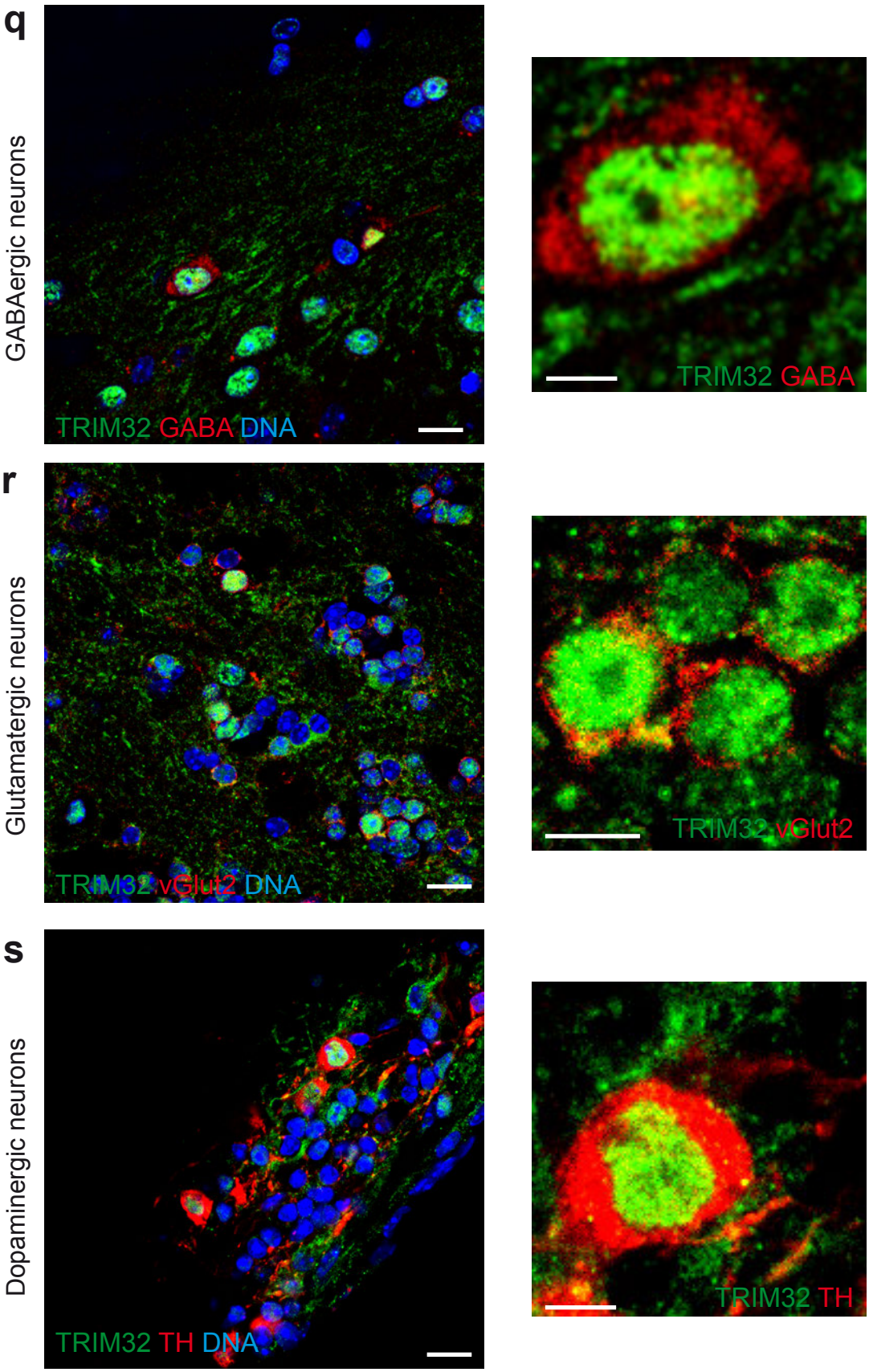
Supplementary Figure 1



Supplementary Figure 1



Supplementary Figure 1



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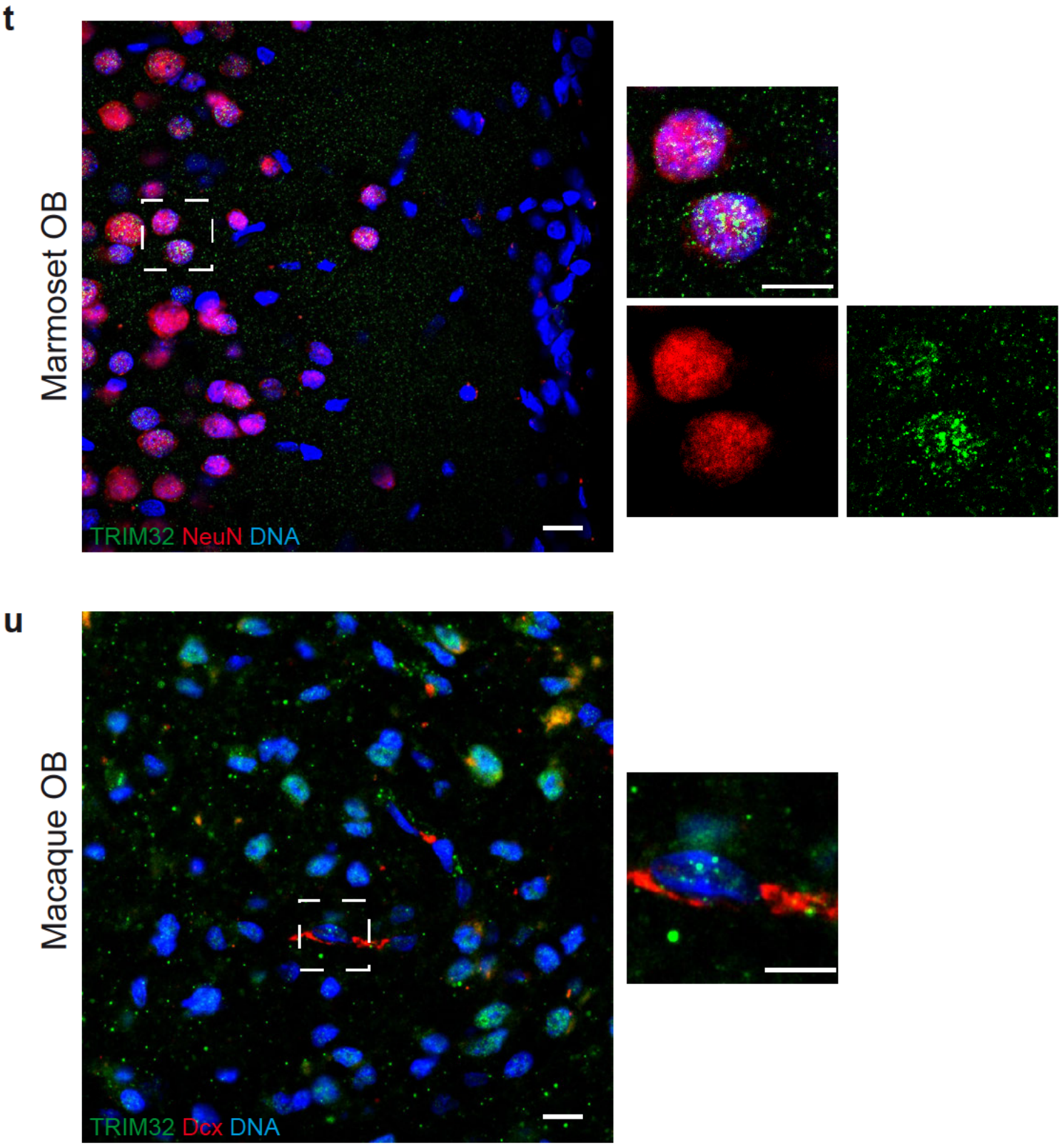

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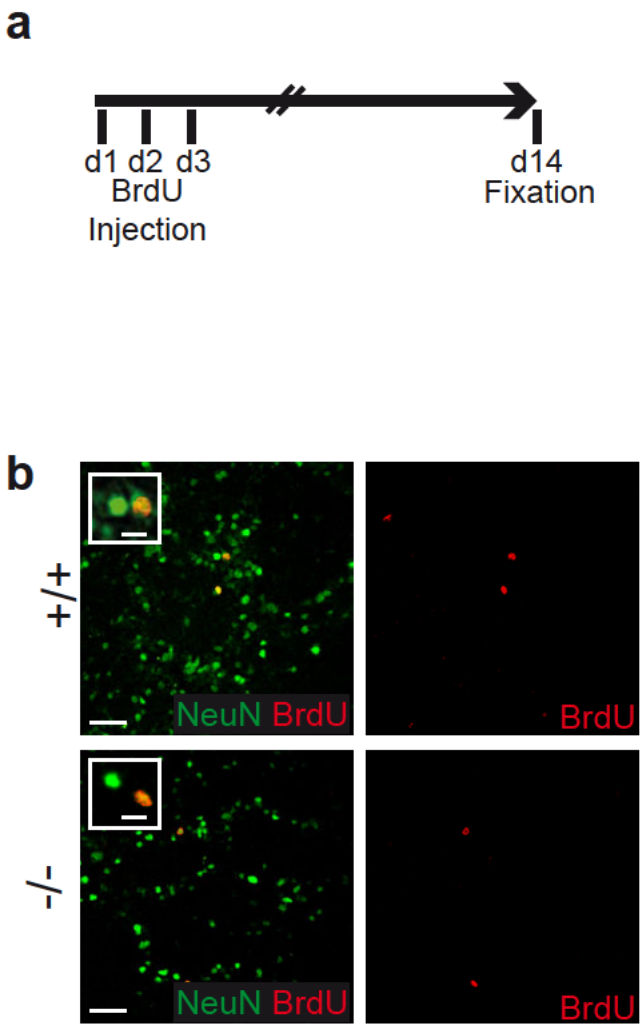

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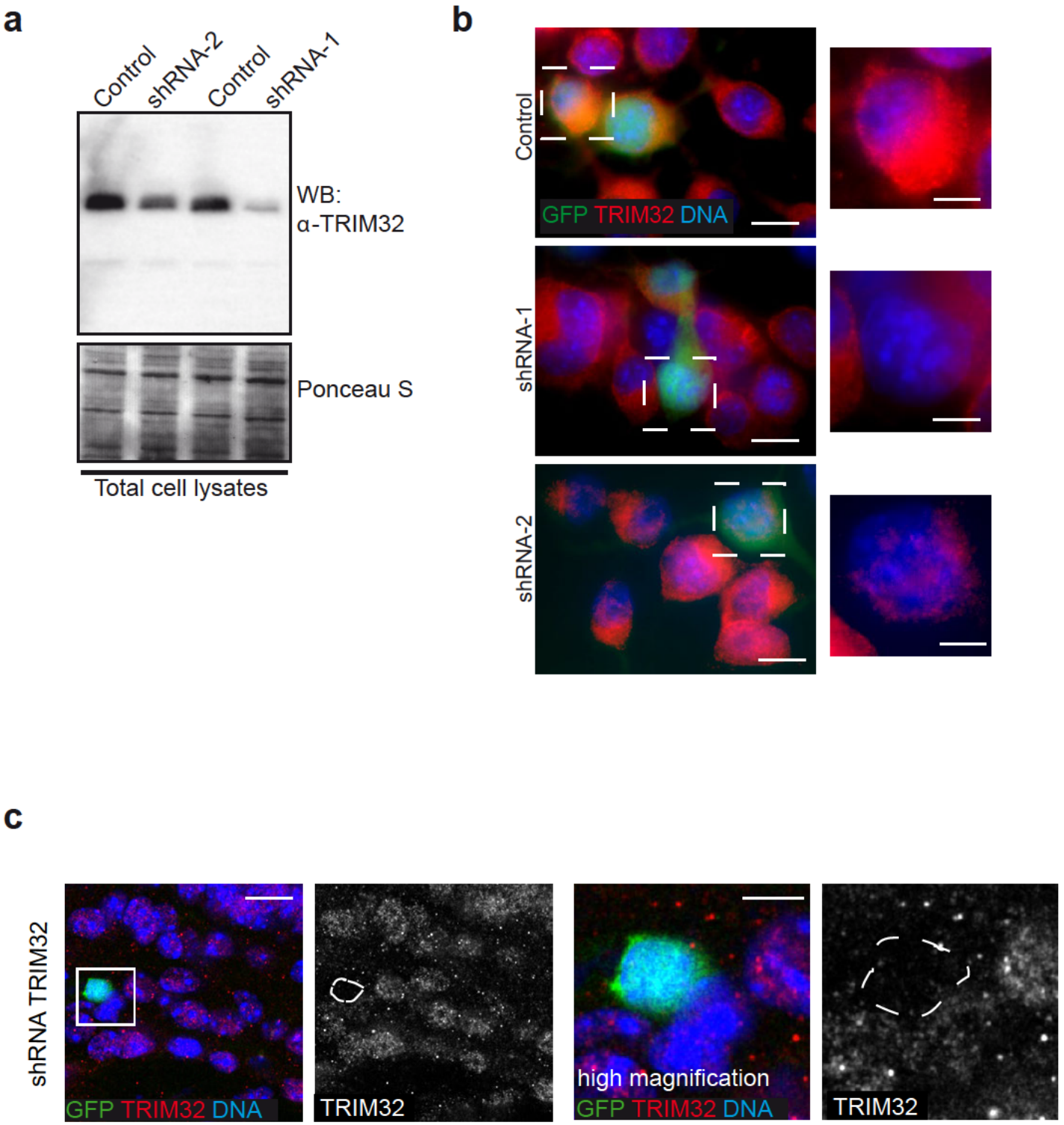

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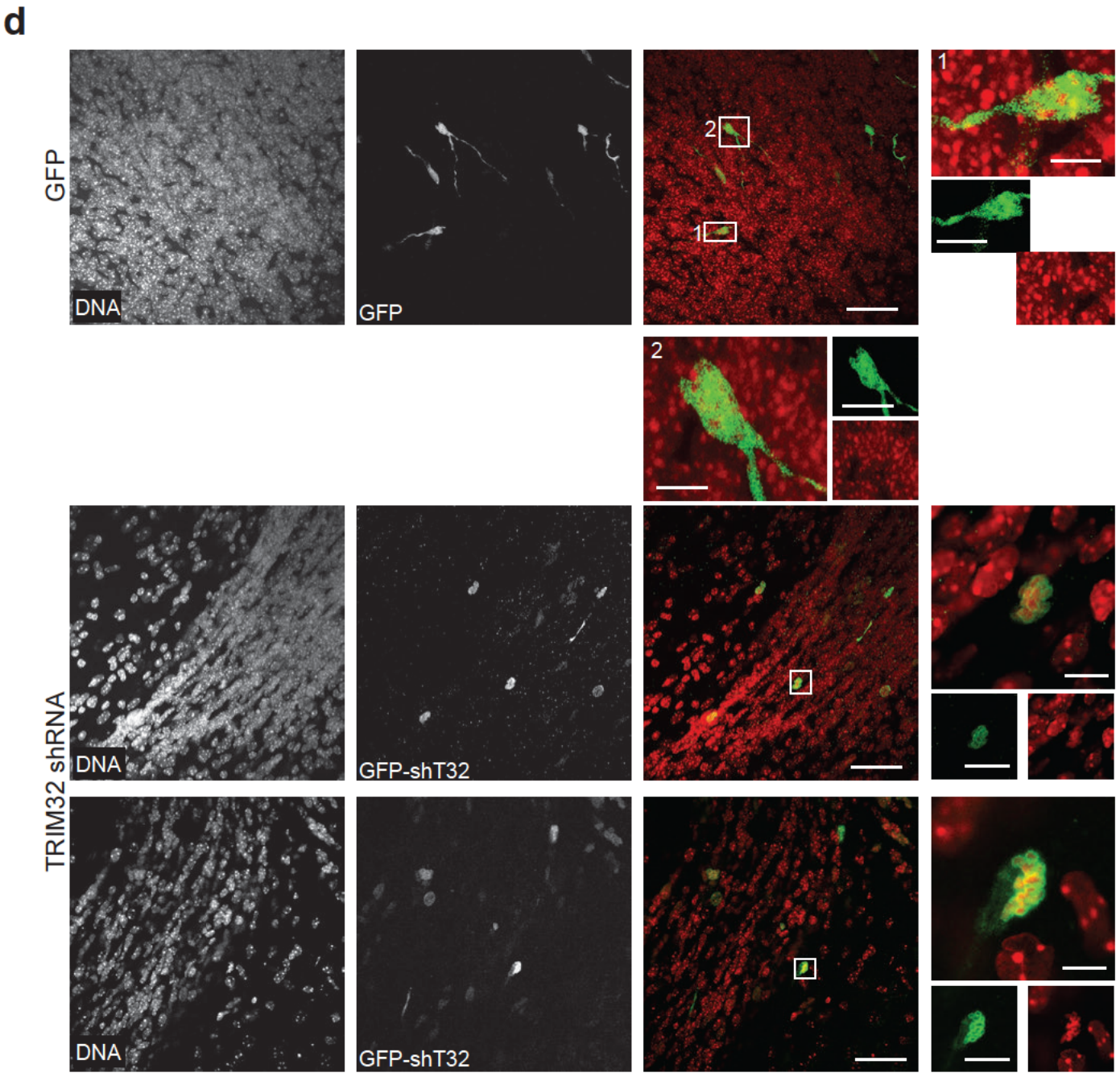
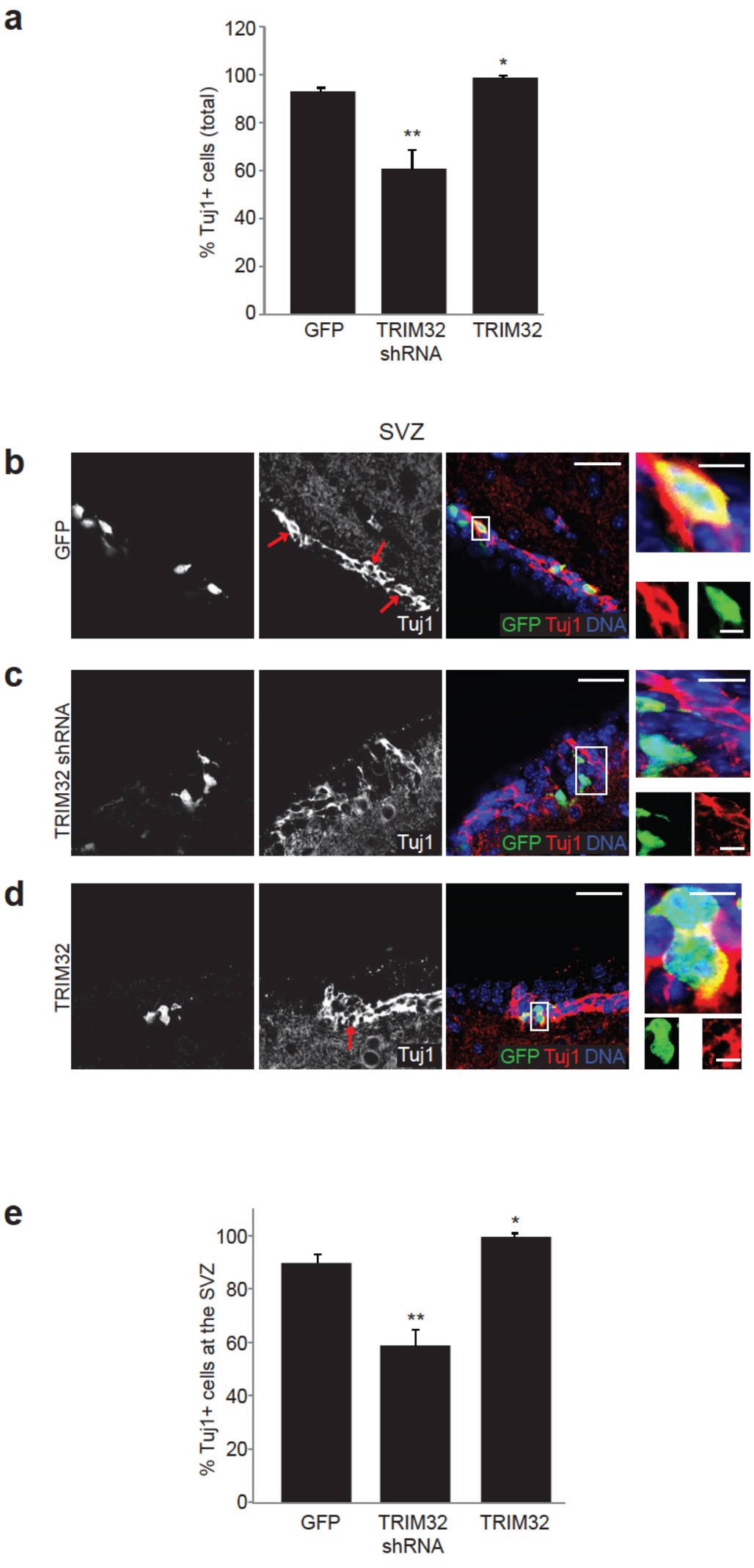
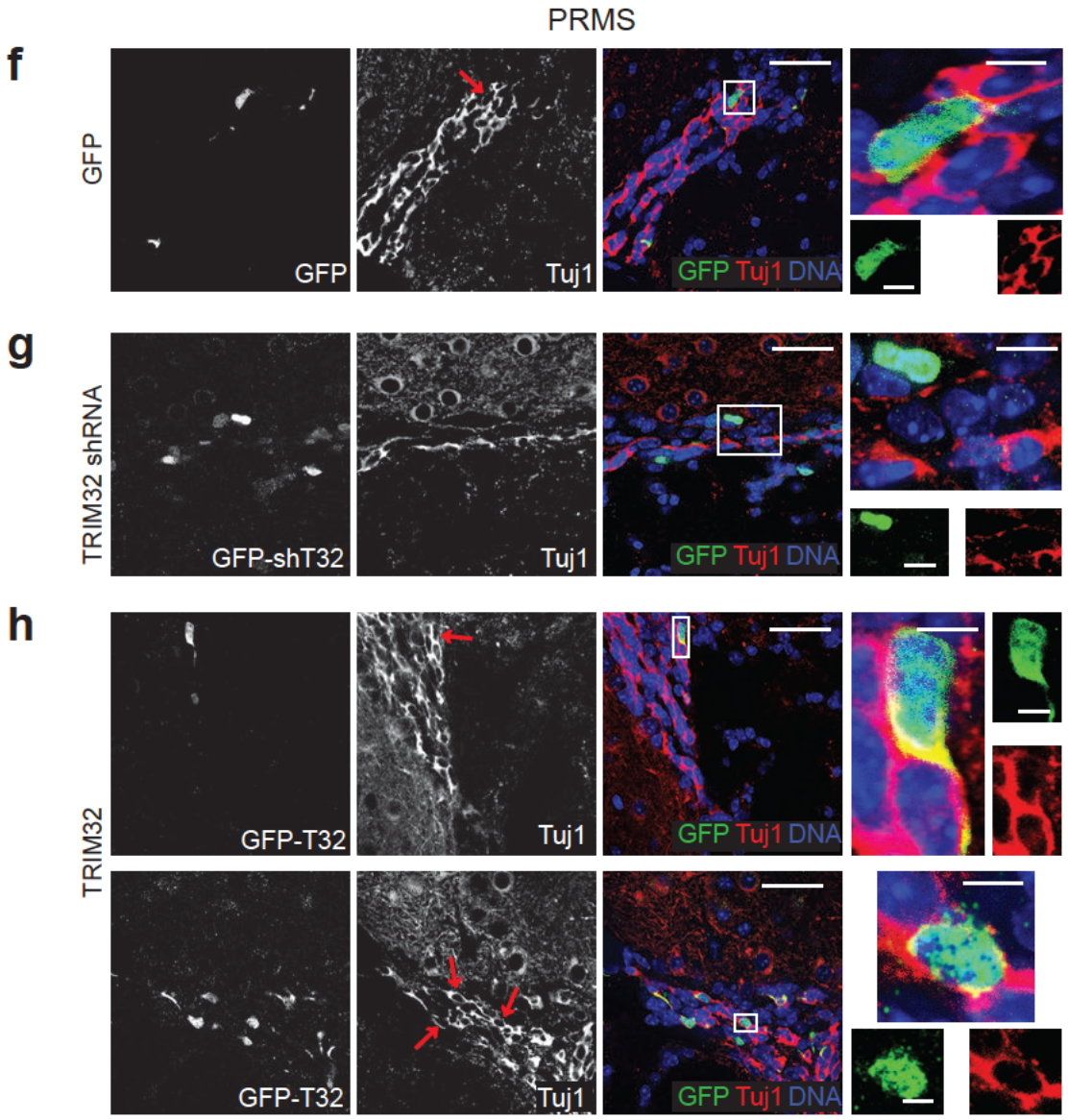
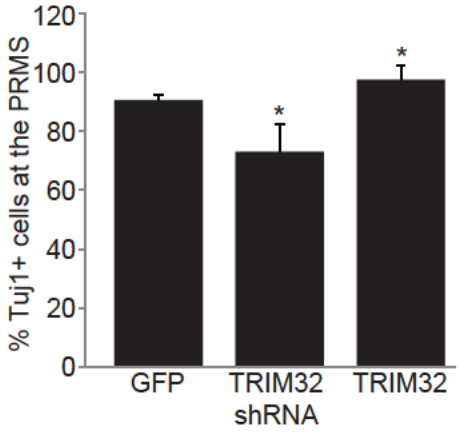


Supplementary Figure 3

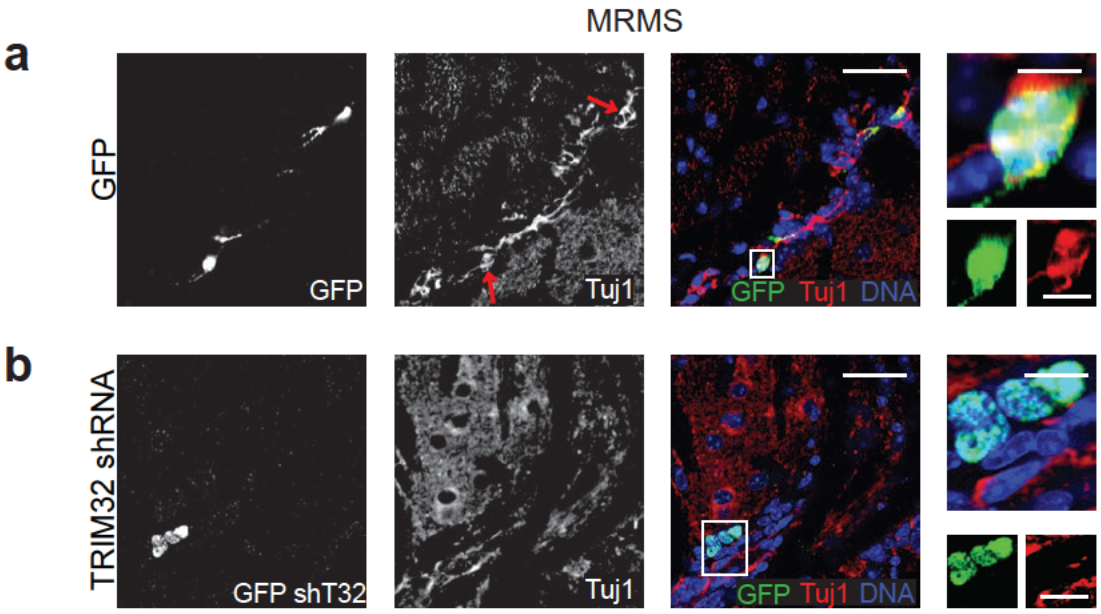


Supplementary Figure 4



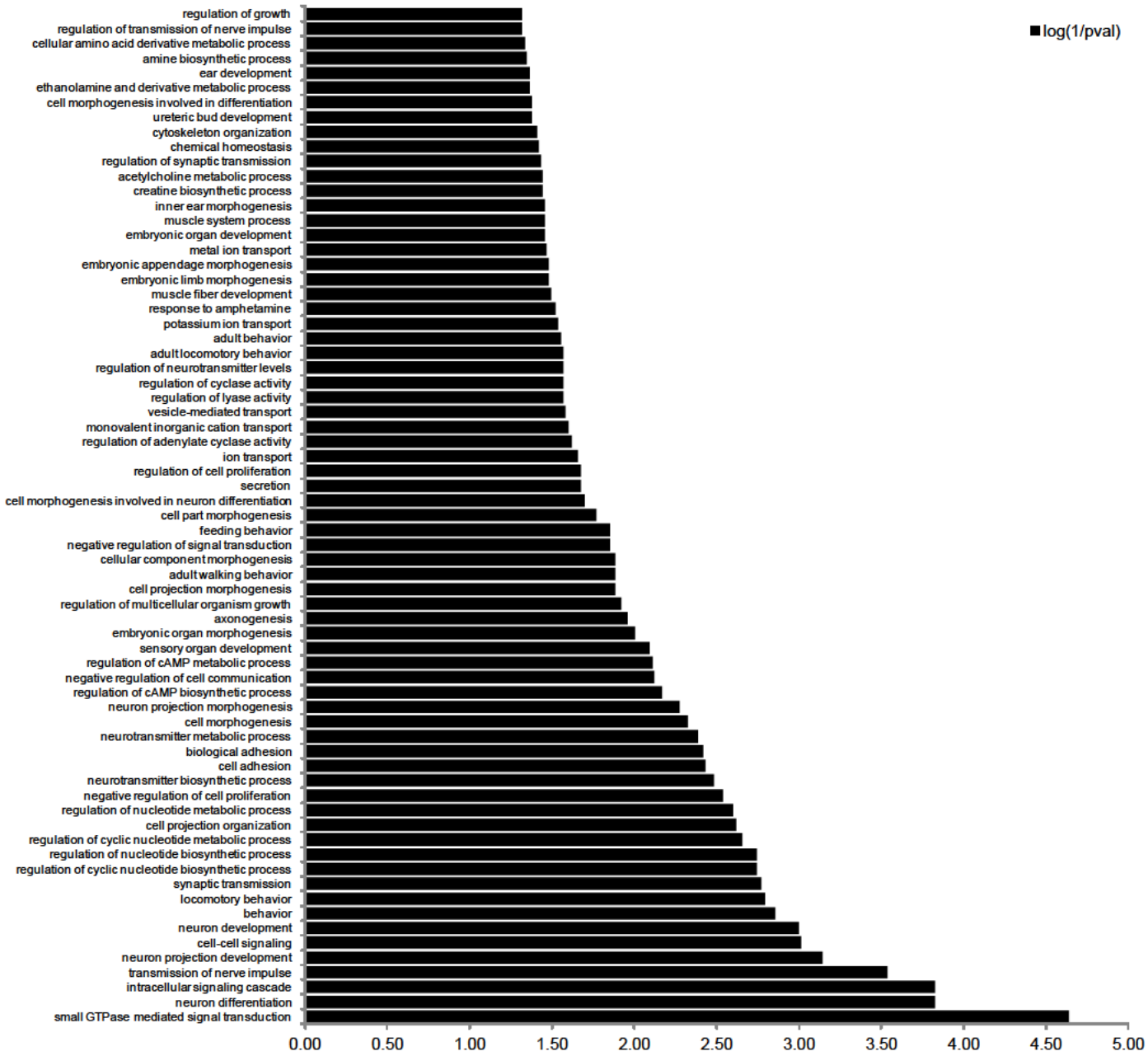


Supplementary Figure 4

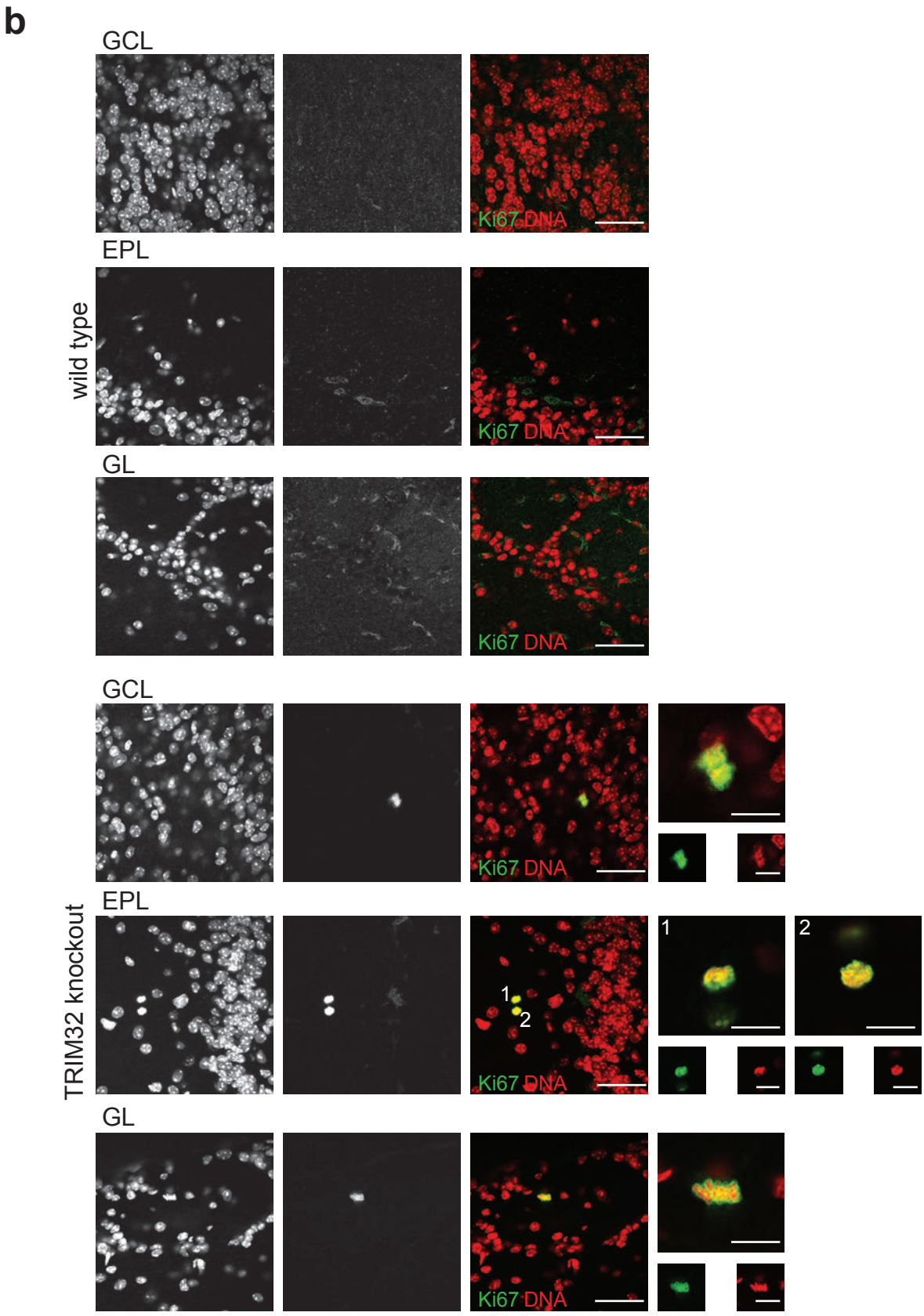


Supplementary Figure 5

a



Supplementary Figure 6



Supplementary Figure 6

Cell type (Marker, Region)	% of TRIM32 negative cells (STDE)	% of TRIM32 weak positive cells (STDE)	% of TRIM32 strong positive cells (STDE)
Type B cells/ Astrocytes (GFAP; SVZ/ OB)	90,71 (2,64)	10,00 (2,60)	0,00 (0,00)
Type C cells (Mash-1; SVZ/ RMS)	83,71 (3,05)	0,00 (0,00)	16,29 (3,05)
Mitotically active cells (Ki67; SVZ/ RMS)	78,05 (4,07)	4,76 (1,70)	17,20 (2,75)
Neuroblasts (PSA-NCAM, DCX; SVZ)	65,31 (4,09)	6,21 (2,13)	23,47 (3,07)
Neuroblasts (PSA-NCAM, DCX; PRMS)	67,67 (6,90)	7,73 (0,79)	16,90 (2,12)
Neuroblasts (PSA-NCAM, DCX; MRMS)	36,82 (10,80)	73,62 (4,88)	16,13 (2,26)
Neuroblasts (PSA-NCAM, DCX; DRMS)	3,01 (0,44)	1,87 (1,32)	91,56 (5,01)
Neurons (NeuN ; GCL)	4,13 (3,55)	0,00 (0,00)	95,87 (3,55)
Newborn cells (EdU; GCL)	33,58 (1,98)	0,00 (0,00)	66,42 (1,98)
Oligodendrocytes (GST-π ; CC, OB)	97,22 (1,96)	2,78 (1,96)	0, 00 (0,00)

Supplementary Table 1

a

	BrdU (per GCL)	
Mouse	+/+	-/-
1	439	766
2	338	605
3	235	851
4	751	1163
Sum:	1763	3385

b

	BrdU (part of GCL)	BrdU/NeuN
Mouse	+/+	+/+
1	140	135
2	80	66
3	62	58
4	131	121
Sum:	413	380

	BrdU (part of GCL)	BrdU/NeuN
Mouse	-/-	-/-
1	91	110
2	143	156
3	109	119
4	172	176
Sum:	515	561

c

+/+	
Mouse	TUNEL + cells/mm^2
1	14081446
2	13214532
3	19039591
4	9800462
Average	14034007

-/-	
Mouse	TUNEL + cells/mm^2
1	5637544
2	5258251
3	6427305
4	5902187
Average	5806322

d

+/+	
Mouse	Casp3+ cells/mm^2
1	6934481
2	7637175
3	7341206
4	6414739
Average	7081900

-/-	
Mouse	Casp3+ cells/mm^2
1	2928571
2	2910053
3	2248876
4	2521008
Average	2652127

cdk inhibitors	
Gene	rel. expression difference ($Wt_{prox \rightarrow dist}$ vs.TRIM32 $ko_{prox \rightarrow dist}$)
Cdkn1a	32,86
Cdkn2b	20,50
Cdkn2a	20,13

cyclins	
Gene	rel. expression difference ($Wt_{prox \rightarrow dist}$ vs.TRIM32 $ko_{prox \rightarrow dist}$)
Ccnb3	37,77
Ccne1	22,80
Ccnd1	20,86

bcl2-family members and regulators	
Gene	rel. expression difference ($Wt_{prox \rightarrow dist}$ vs.TRIM32 $ko_{prox \rightarrow dist}$)
Bcl6	43,57
Bcl11b	34,22
Bok	27,47
Camkk1	23,76
Bcl2l11	23,01
Tox3	22,13
Bcl2a1c	22,05
Bmf	21,97
Mcts1	21,91

Supplementary Table 3

Brain region	Wild type absolute Ki67+ cell number sum	Wild type absolute Ki67+ cell number mean	S.E.	TRIM32 knockout absolute Ki67+ cell number sum	TRIM32 knockout absolute Ki67+ cell number mean	S.E.
SVZ	1036	259	49.53	2009	502.25	47.51
PRMS	621	155.25	22.57	1867	466.75	98.1
MRMS	169	42.25	5307	729	182.25	21.5
DRMS	10	2.5	2.18	74	18.5	5.63
OB	5	1.25	0.48	127	31.75	5.81

Supplementary Table 4