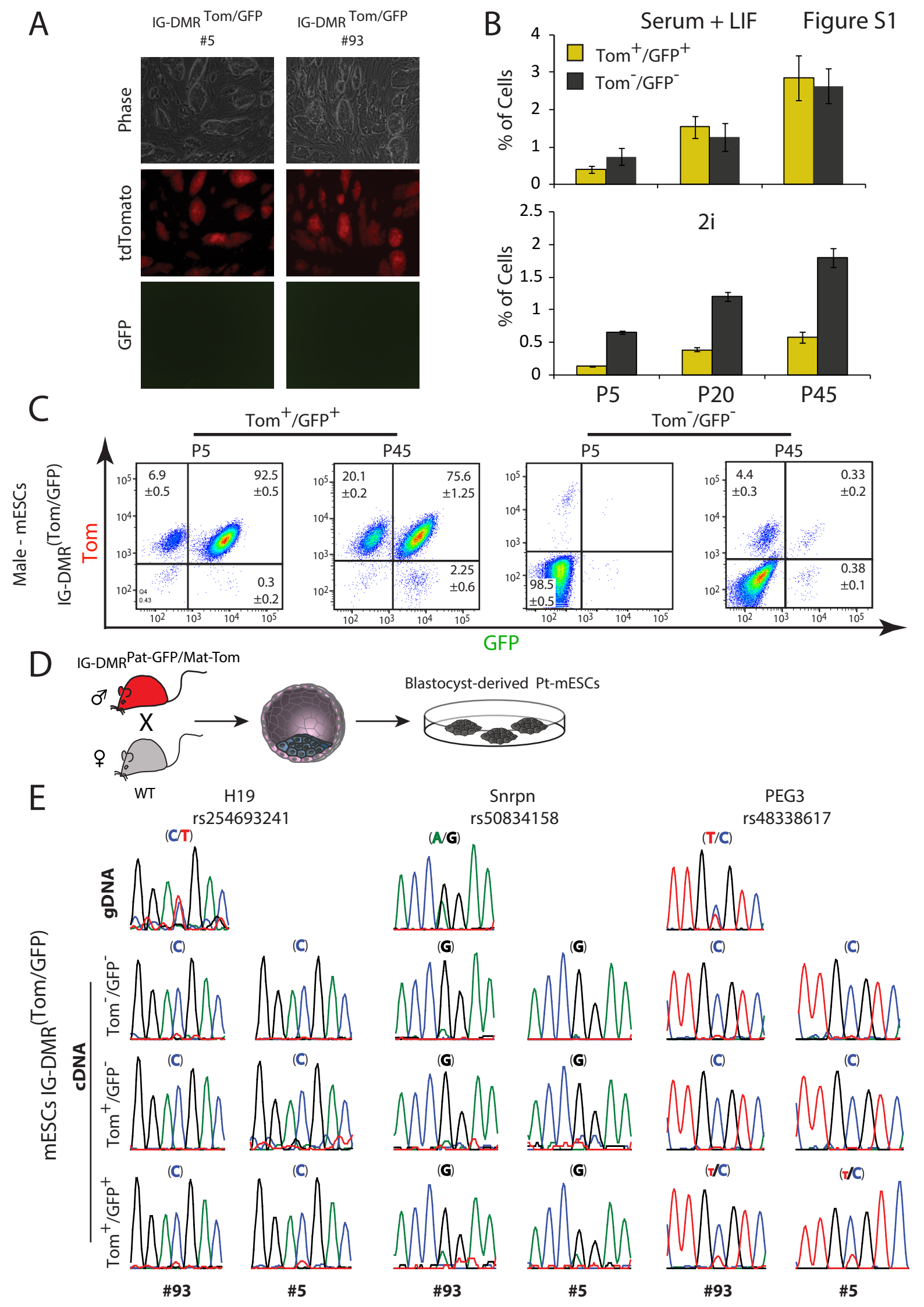

A
IG-DMR Tom/GFP #5
IG-DMR Tom/GFP #93
Phase
tdTomato
GFP
B
Serum + LIF
Figure S1
Tom+/GFP+
Tom-/GFP-
% of Cells
2i
P5
P20
P45
C
Tom+/GFP+
Tom-/GFP-
P5
P45
Male - mESCs
IG-DMR(Tom/GFP)
Tom
GFP
6.9 ±0.5
92.5 ±0.5
0.3 ±0.2
20.1 ±0.2
75.6 ±1.25
2.25 ±0.6
98.5 ±0.5
4.4 ±0.3
0.33 ±0.2
0.38 ±0.1
D
IG-DMRPat-GFP/Mat-Tom
WT
Blastocyst-derived Pt-mESCs
E
H19
rs254693241
Snrpn
rs50834158
PEG3
rs48338617
gDNA
cDNA
mESCs IG-DMR(Tom/GFP)
Tom-/GFP-
Tom+/GFP-
Tom+/GFP+
(C/T)
(A/G)
(T/C)
#93
#5

Figure S1. Related to Figure 1. Analysis of mES IG-DMR$^{(Tom/GFP)}$ cells.

(A) Representative images of two established mESC IG-DMR$^{(Tom/GFP)}$ lines.

(B) Bar-graph summarizing changes in the percentage of Tom^{+}/GFP^{+} and Tom^{-}/GFP^{-} cells at different passages. Shown is the average of 3 independently targeted cell lines ± s.d.

(C) Flow cytometric analysis of the proportion of GFP/Tom-positive cells in sorted mESC IG-DMR Tom^{+}/GFP^{+} and mESC IG-DMR Tom^{-}/GFP^{-} lines during prolonged culture with serum+LIF. Shown are mean ± s.d of two independent targeted cell lines.

(D) Schematic diagram of the mating scheme used for establishment paternally-transmitted (Pt) mESCs from ICM-blastocysts.

(E) Sequencing of three representative imprinted genes in sorted IG-DMR$^{(Tom/GFP)}$ mESC lines. Heterozygous SNPs were identified in the coding regions and analyzed in the cDNAs of two independently targeted cell lines. Note the minor T allele identified in Peg3 for mESC IG-DMR Tom^{+}/GFP^{+}, suggesting mosaicism in the sorted cell populations.

Figure S2

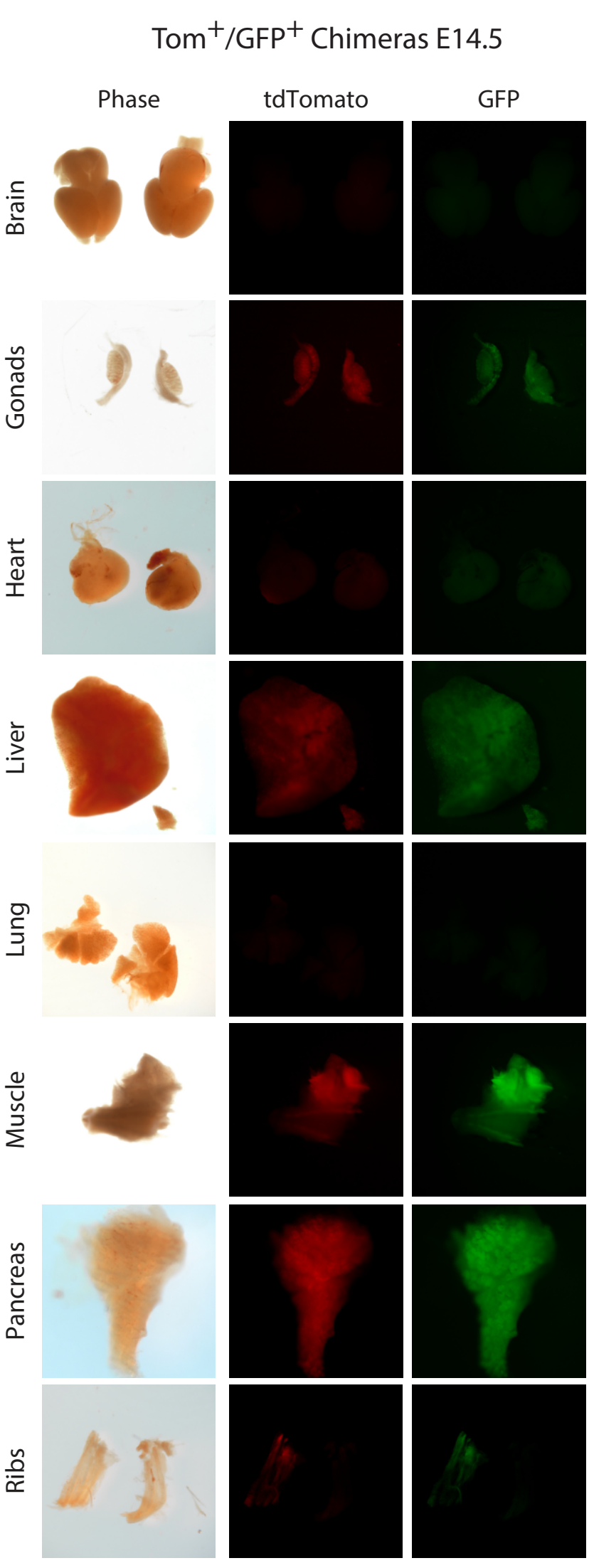

Tom+/GFP+ Chimeras E14.5
Phase
tdTomato
GFP
Brain
Gonads
Heart
Liver
Lung
Muscle
Pancreas
Ribs

Figure S2. Related to Figure 2. Organ analysis in E14.5 IG-DMR Tom^{+}/GFP^{+} chimeric embryos.

Organs images obtained from E14.5 IG-DMR Tom^{+}/GFP^{+} chimeric embryos, demonstrating co-expression of Tom and GFP reporters in some tissues, and complete repression of reporter activity in other tissues. Representative images were consistent in 13 analyzed embryos between E12.5-14.5.

Figure S3

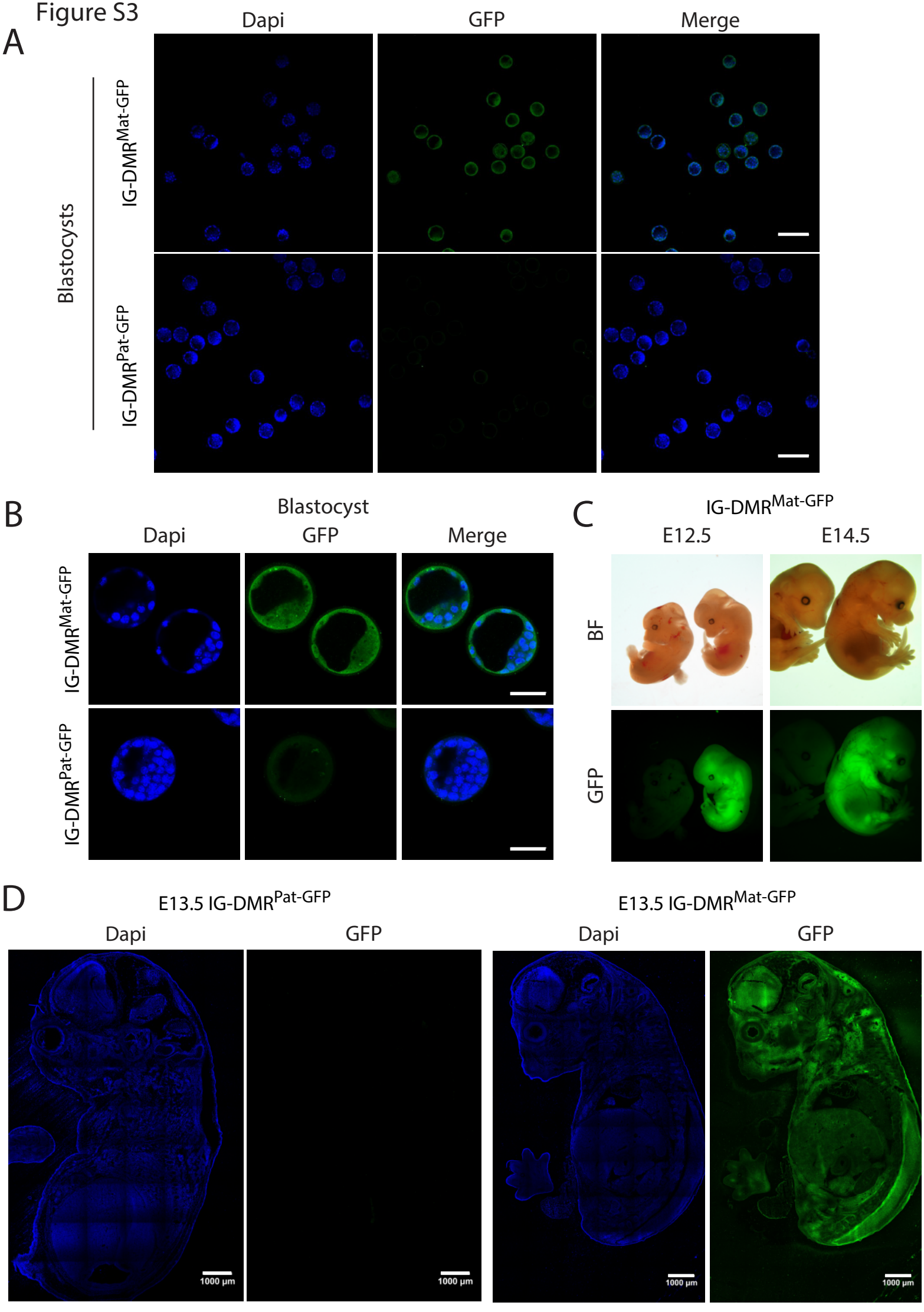

A
Dapi
GFP
Merge
Blastocysts
IG-DMRMat-GFP
IG-DMRPat-GFP
B
Blastocyst
Dapi
GFP
Merge
IG-DMRMat-GFP
IG-DMRPat-GFP
C
IG-DMRMat-GFP
E12.5
E14.5
BF
GFP
D
E13.5 IG-DMRPat-GFP
E13.5 IG-DMRMat-GFP
Dapi
GFP
Dapi
GFP
1000 µm
1000 µm
1000 µm
1000 µm

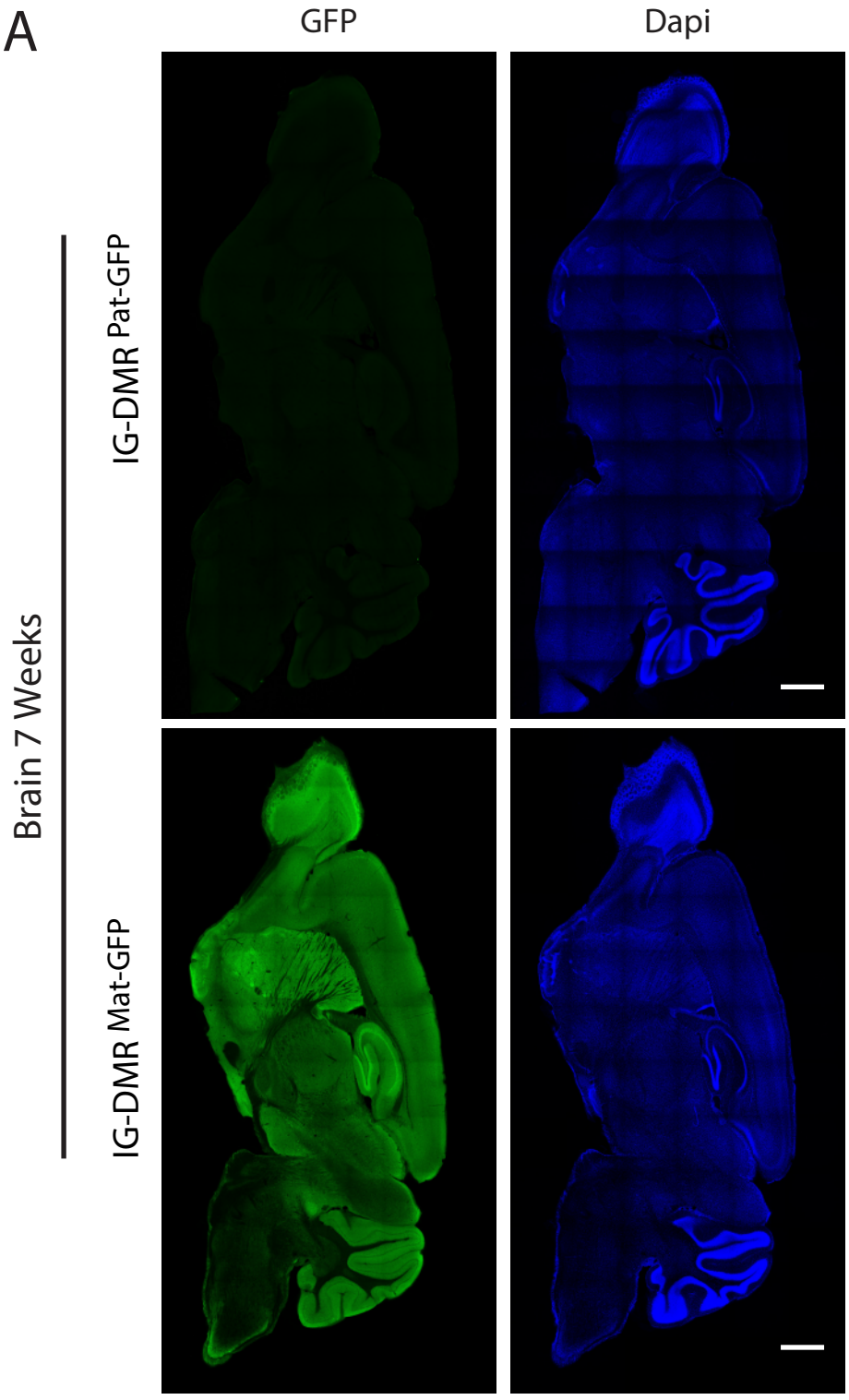
Figure S3. Related to Figure 3. Analysis of IG-DMR$^{Mat\text{-}GFP}$ and IG-DMR$^{Pat\text{-}GFP}$ reporter mice during embryonic development.

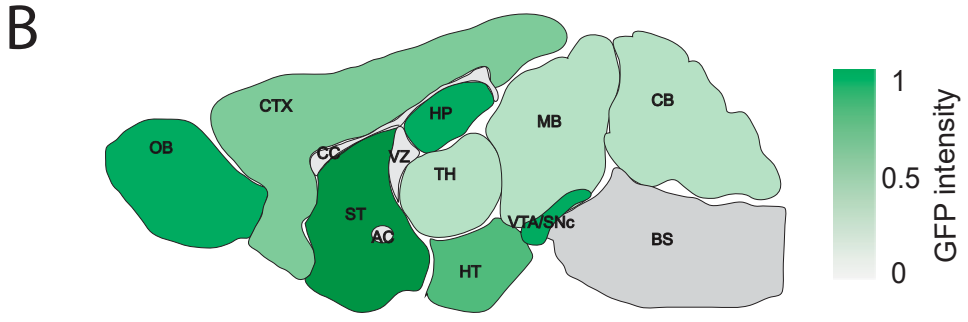
(A and B)**,** Maternally and paternally transmitted IG-DMR^{GFP} blastocysts were stained with Dapi and anti-GFP; (A) bar = 250µm; (B) bar = 50µm.

(C) Representative images of F2 IG-DMR$^{Mat\text{-}GFP}$ embryos at different developmental stages; Left are control embryos.

(D) Sagittal stitched sections of maternally and paternally transmitted IG-DMR^{GFP} E13.5 embryos stained with Dapi and anti-GFP; bar = 1000 µm.

Figure S4





OB: Olfactory Bulb
CTX: Cortex
ST: Striatum
TH: Thalamus
HT: Hypothalamus
HP: Hippocampus
VTA: Ventral Tegmental Area

MB: Mid Brain
CB: Cerebellum
BS: Brain Stem
CC: Corpus Collosum
AC: Anterior Commission
VZ: Ventricular Zone
SNc: Substantia Nigra compacta

Figure S4. Related to Figure 5. Analysis of adult brains in maternally and paternally transmitted IG-DMR^{GFP} mice.

(A) Representative image of IG-DMR$^{Mat\text{-}GFP}$ and IG-DMR$^{Pat\text{-}GFP}$ whole-mount stitched sagittal brain sections stained with Dapi and anti-GFP; bar = 1000 µm.

(B) Heat map of GFP intensities in different brain anatomical regions, as measured in 6 independent 5-7 weeks old IG-DMR$^{Mat\text{-}GFP}$ mice.

Figure S5

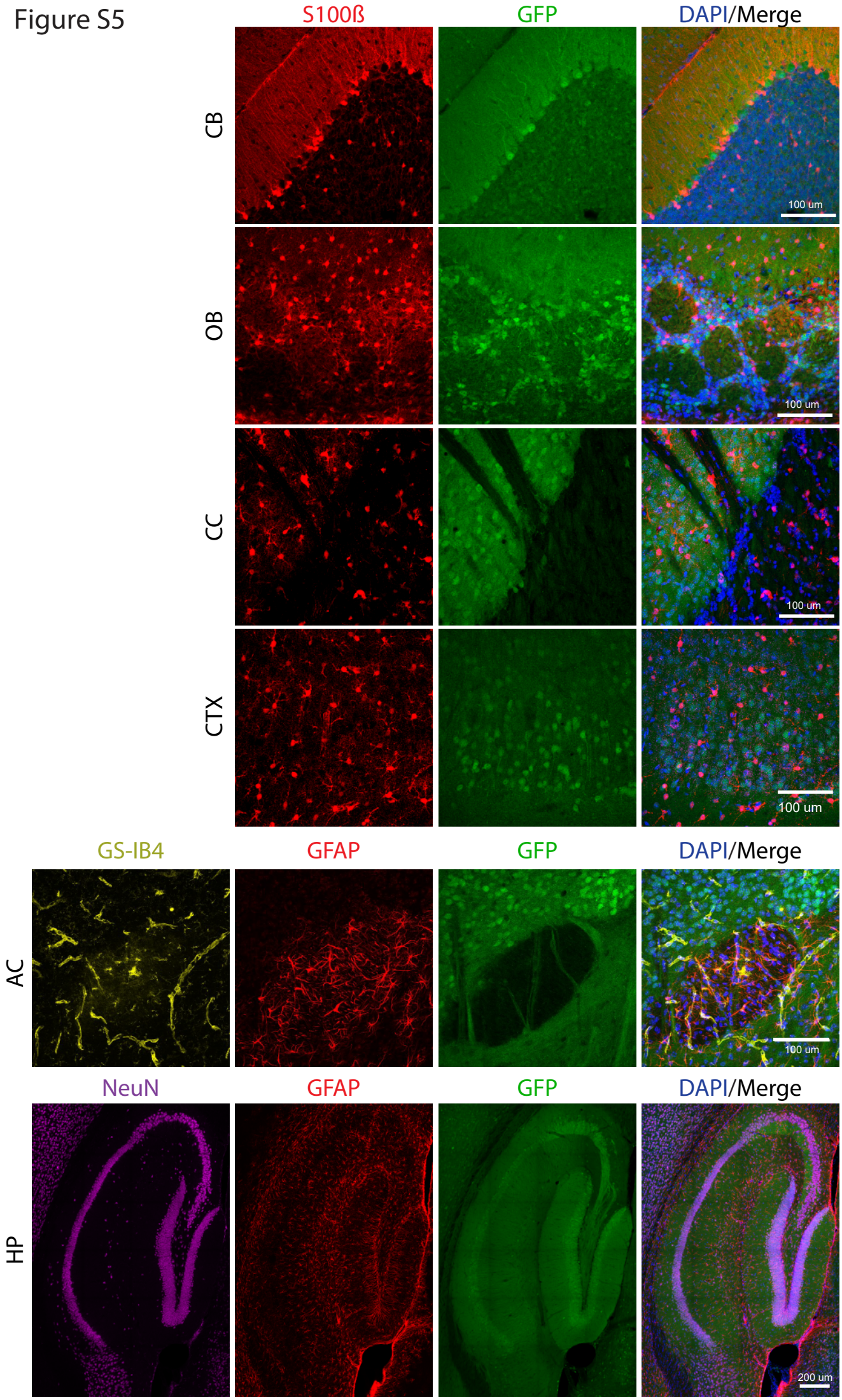

S100ß
GFP
DAPI/Merge
CB
OB
CC
CTX
100 um
GS-IB4
GFAP
AC
NeuN
HP
200 um

Figure S5. Related to Figure 5. Analysis of astrocytic markers in IG-DMR$^{Mat\text{-}GFP}$ adult brains.

Images of 5-7 weeks old brain sections stained with Dapi (blue), anti-GFP (green), anti-S100β (red), anti-Glial Fibrillary Acidic Protein (GFAP, red), anti-GS-IB4 (yellow) and anti-NeuN (purple); Shown are representative images obtained from different anatomical brain regions that include: Cerebellum (CB), Olfactory Bulb (OB); Corpus Collosum (CC), Cortex (CTX), Anterior Commission (AC) and stitched images of Hippocampus (HP). Note that GFP expression is mutually exclusive with the expression of bona-fide astrocytic markers S100β and GFAP, as well as with blood vessel endothelial cells (yellow) in all regions analyzed.

Figure S6

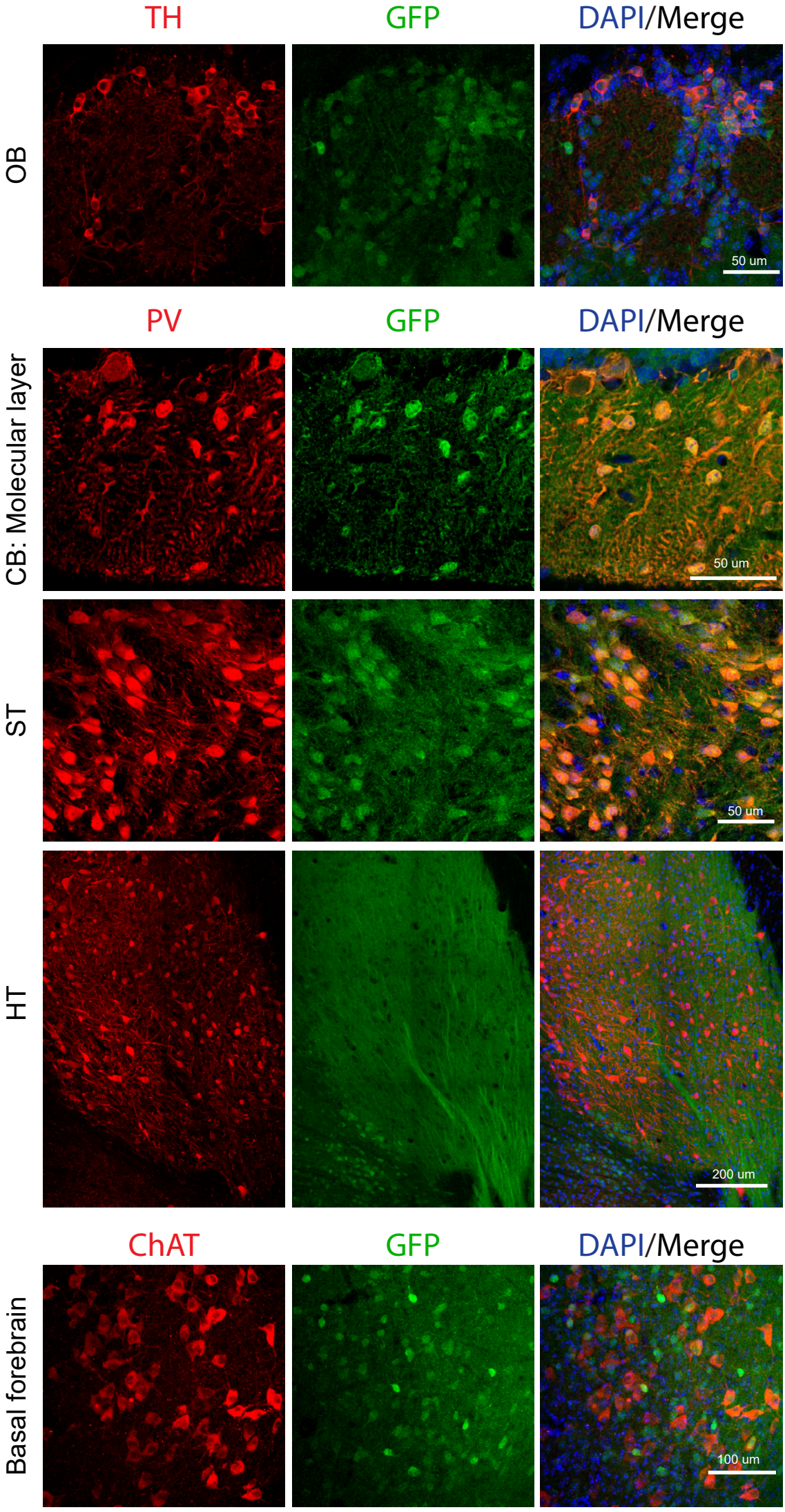

TH
GFP
DAPI/Merge
OB
50 um
PV
GFP
DAPI/Merge
CB: Molecular layer
50 um
ST
50 um
HT
200 um
ChAT
GFP
DAPI/Merge
Basal forebrain
100 um

Figure S6. Related to Figure 5. Analysis of different neuronal markers in IG-DMR$^{Mat\text{-}GFP}$ adult brains.

Brain sections from 5-7 week old mice stained with DAPI (blue), anti-GFP (green), anti-Tyrosine Hydroxylase (TH, red), anti-Parvalbumin (PV, red) and anti-Choline Acetyltransferase (ChAT, red); Shown are representative images obtained from different anatomical brain regions that include: Olfactory Bulb (OB); Cerebellum (CB) molecular layer, Striatum (ST), stitched images of Hypothalamus (HT) and the Basal forebrain. Note that while complete overlap between GFP expression and TH; and between GFP and PV in the CB and ST regions was apparent, neurons stained with ChAT and PV (on the HT region) exhibited only partial overlap with GFP expression.

Figure S7

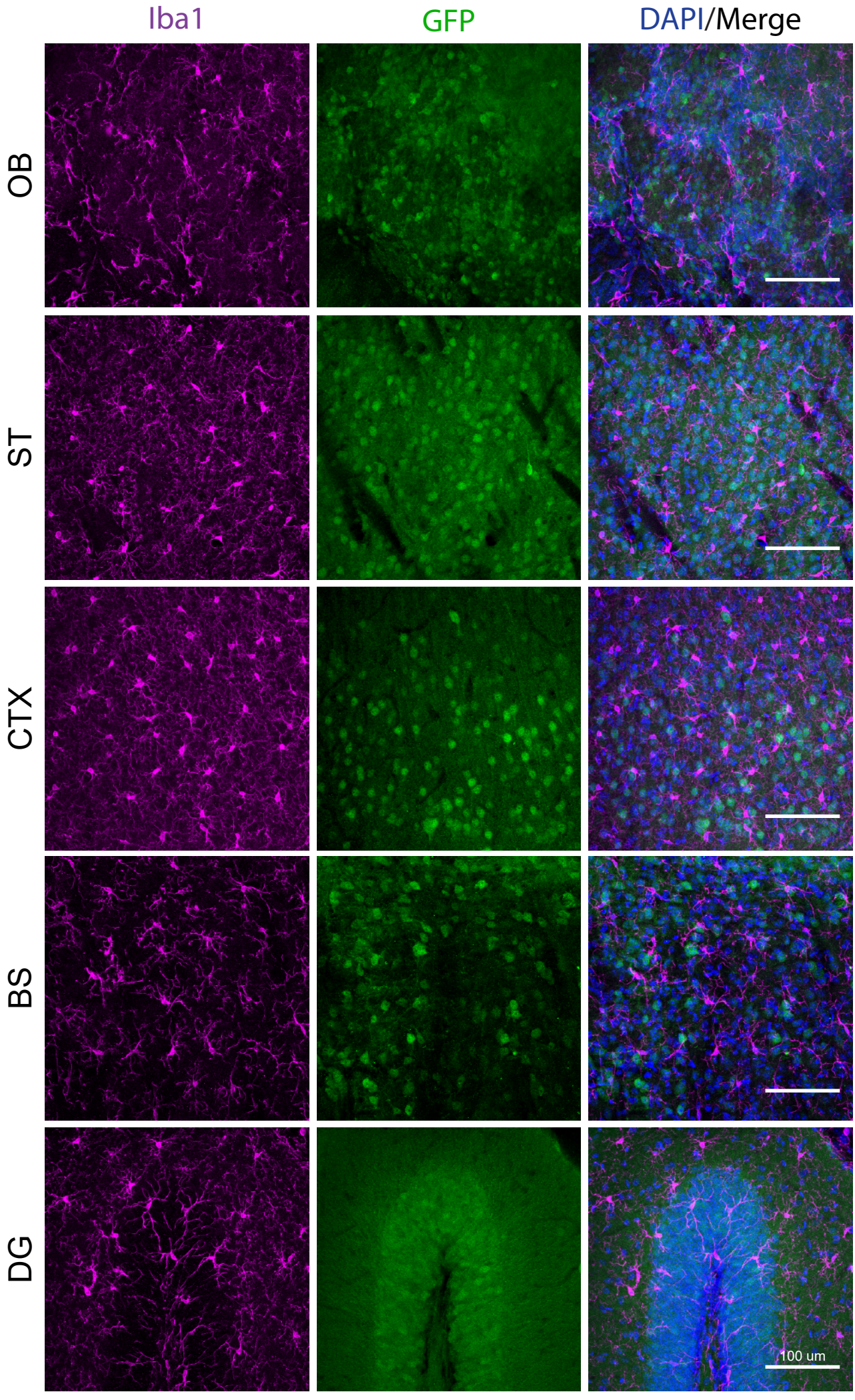


Figure S7. Related to Figure 5. Analysis of microglia IG-DMR$^{Mat\text{-}GFP}$ adult brains.

Brain sections from 5-7 weeks old mice stained with Dapi (blue), anti-GFP (green) and the microglia marker anti- Ionized calcium binding adapter molecule-1 (Iba1, purple); Shown are representative images obtained from different anatomical brain regions that include: Olfactory Bulb (OB), Striatum (ST), Cortex (CTX), Brain Stem region (BS) and the Dentate Gyrus (DG). Note that GFP expression is mutually exclusive with the expression of Iba1, in all regions analyzed.

Figure S8

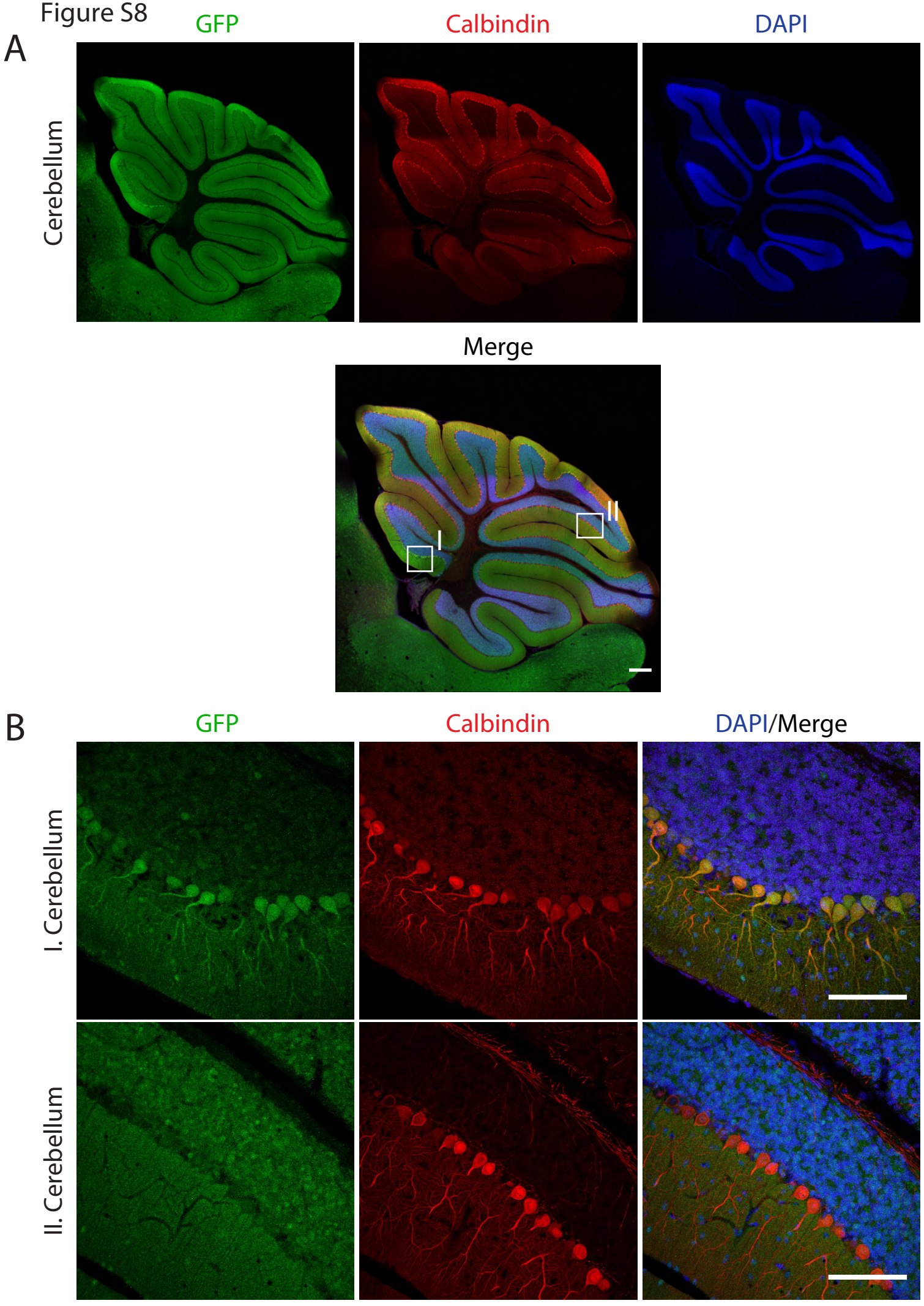

A
GFP
Calbindin
DAPI
Cerebellum
Merge
I
II
B
GFP
Calbindin
DAPI/Merge
I. Cerebellum
II. Cerebellum

Figure S8. Related to Figure 5. GFP heterogeneity in Cerebellum Purkinje cell in IG-DMR$^{Mat\text{-}GFP}$ adult brains.

Images of 5-7 weeks old brain sections stained with Dapi (blue), anti-GFP (green) and Purkinje cell marker Calbindin (red). (A) Shown is whole mount stitching Cerebellum images (bar = 500 µm) with blowup of (B) two representative lobe regions, demonstrating that while most cells are Calbindin^{+}GFP^{-} (as indicated in region II), some lobes contain Calbindin^{+}GFP^{+} cells (as indicated in region I); bar = 100µm.

Figure S9

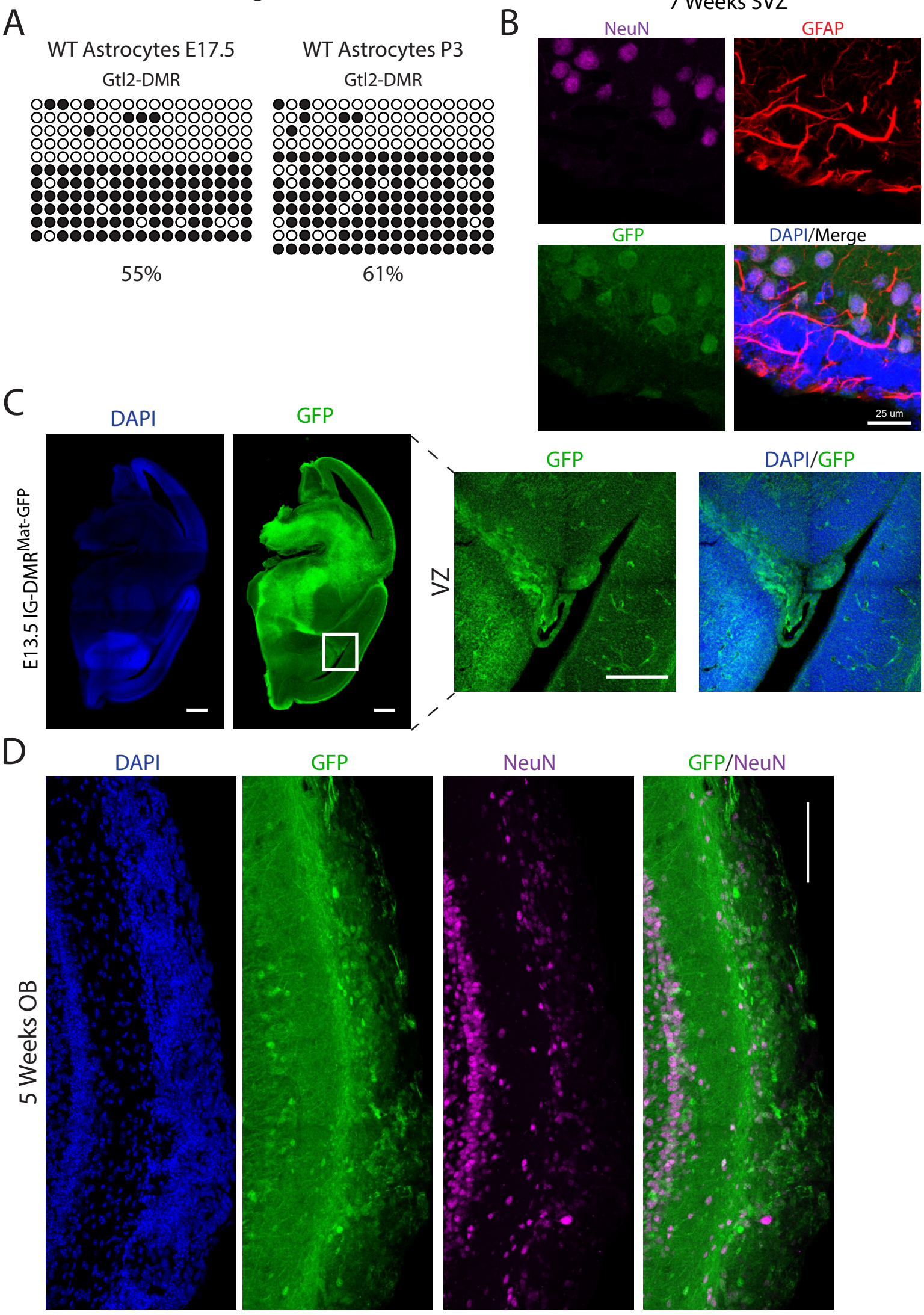

A
WT Astrocytes E17.5
Gtl2-DMR
55%
WT Astrocytes P3
Gtl2-DMR
61%
B
7 Weeks SVZ
NeuN
GFAP
GFP
DAPI/Merge
25 um
C
DAPI
GFP
E13.5 IG-DMR Mat-GFP
VZ
GFP
DAPI/GFP
D
DAPI
GFP
NeuN
GFP/NeuN
5 Weeks OB

Figure S9. Related to Figure 6. Analysis of IG-DMR$^{Mat\text{-}GFP}$ brains.

(A) Bisulfite sequencing was performed on the Gtl2 promoter DMR region in WT astrocytes isolated from E17.5 and P3 brains. Shown are percentages of methylated CpGs. Note the presence of completely methylated and unmethylated alleles suggesting proper parent-specific monoallelic methylation.

(B) Representative images of the Subventricular Zone (SVZ) ependyma wall stained with Dapi (blue), anti-GFP (green), anti-Glial Fibrillary Acidic Protein (GFAP, red) and anti-NeuN (purple); bar=25µm.

(C) Representative whole mount stitched sagittal section of E13.5 IG-DMR$^{Mat\text{-}GFP}$ brain stained with Dapi (blue) and anti-GFP (green); bar = 500 µm. Right panel include Dapi and GFP blowup images of the E13.5 Ventricular Zone (VZ), demonstrating overall GFP positivity in this region; bar = 100 µm.

(D) Representative stitching images of the Olfactory Bulb (OB) in 5 weeks IG-DMR$^{Mat\text{-}GFP}$ brain. Sections were stained with Dapi (blue), anti-GFP (green) and anti-NeuN (purple), demonstrating mosaicism in GFP expression in Neun positive cells. bar = 100µm.