Abnormal development of NG2^{+} PDGFRα^{+} neural progenitor cells leads to neonatal hydrocephalus in a ciliopathy mouse model.

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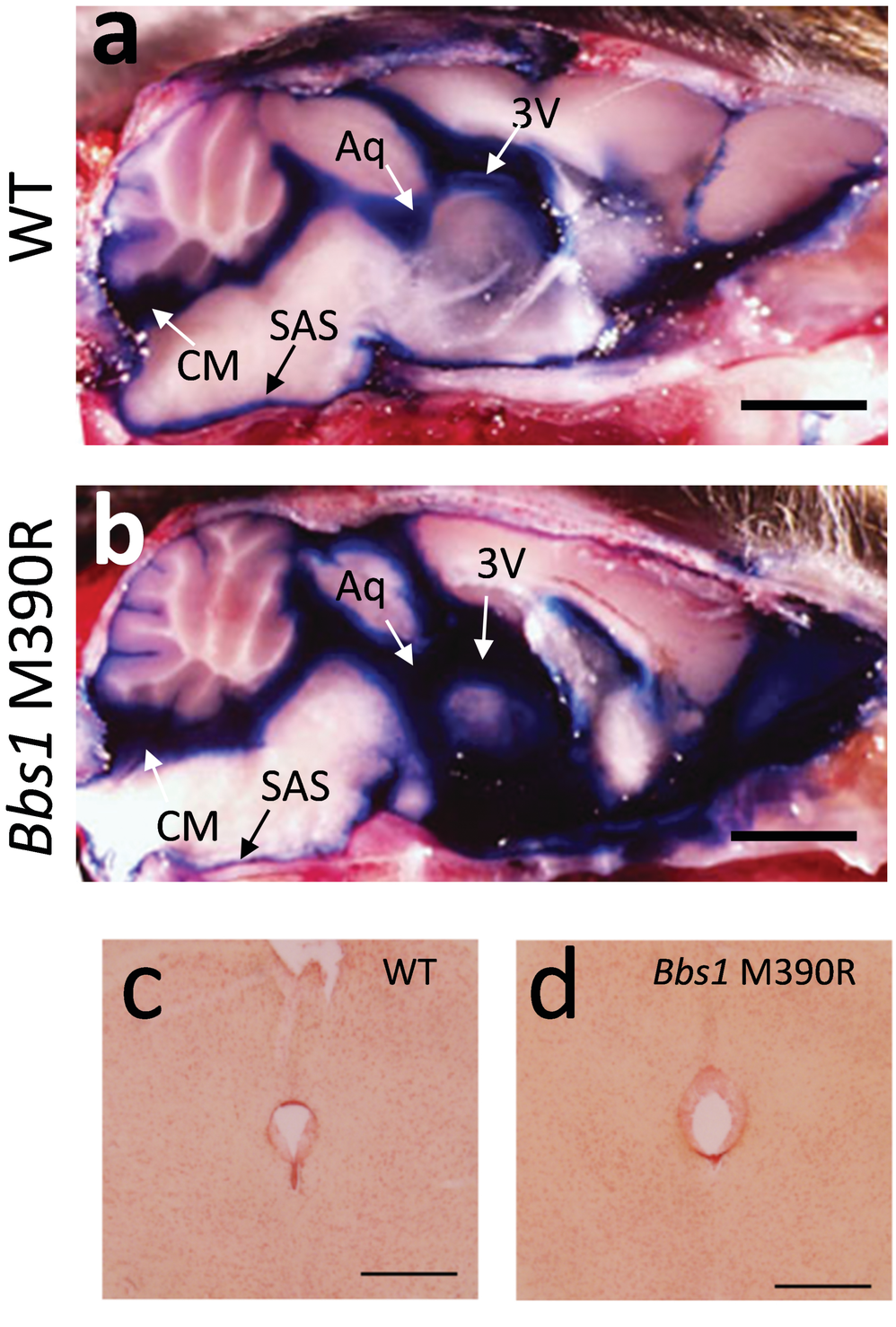
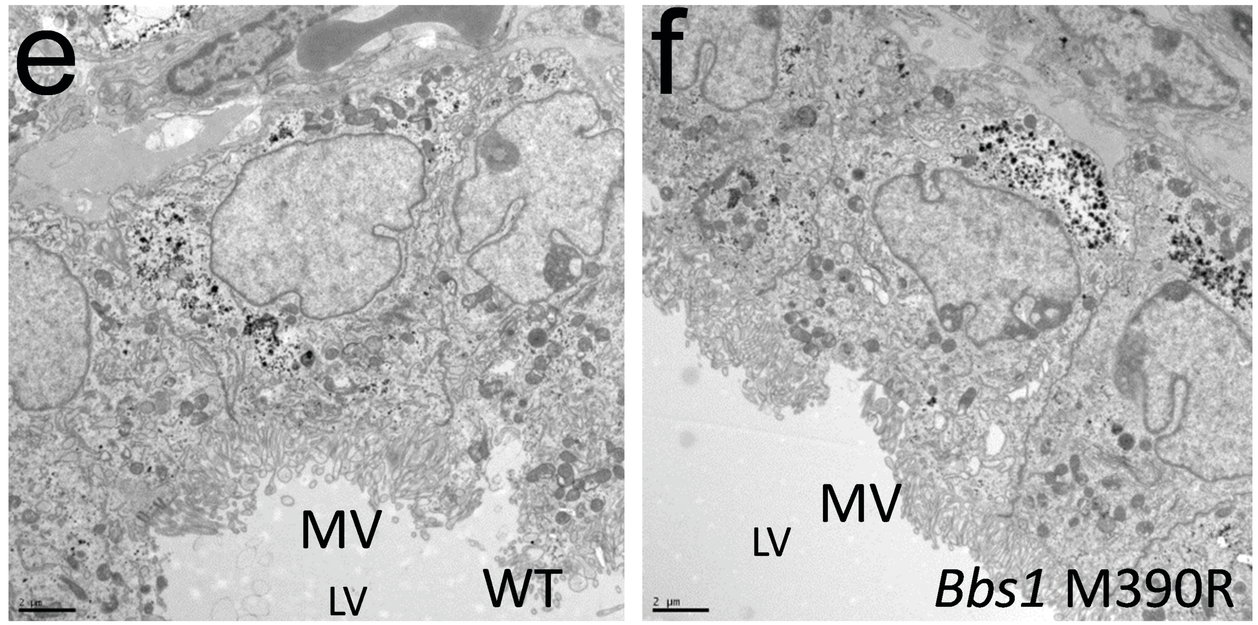
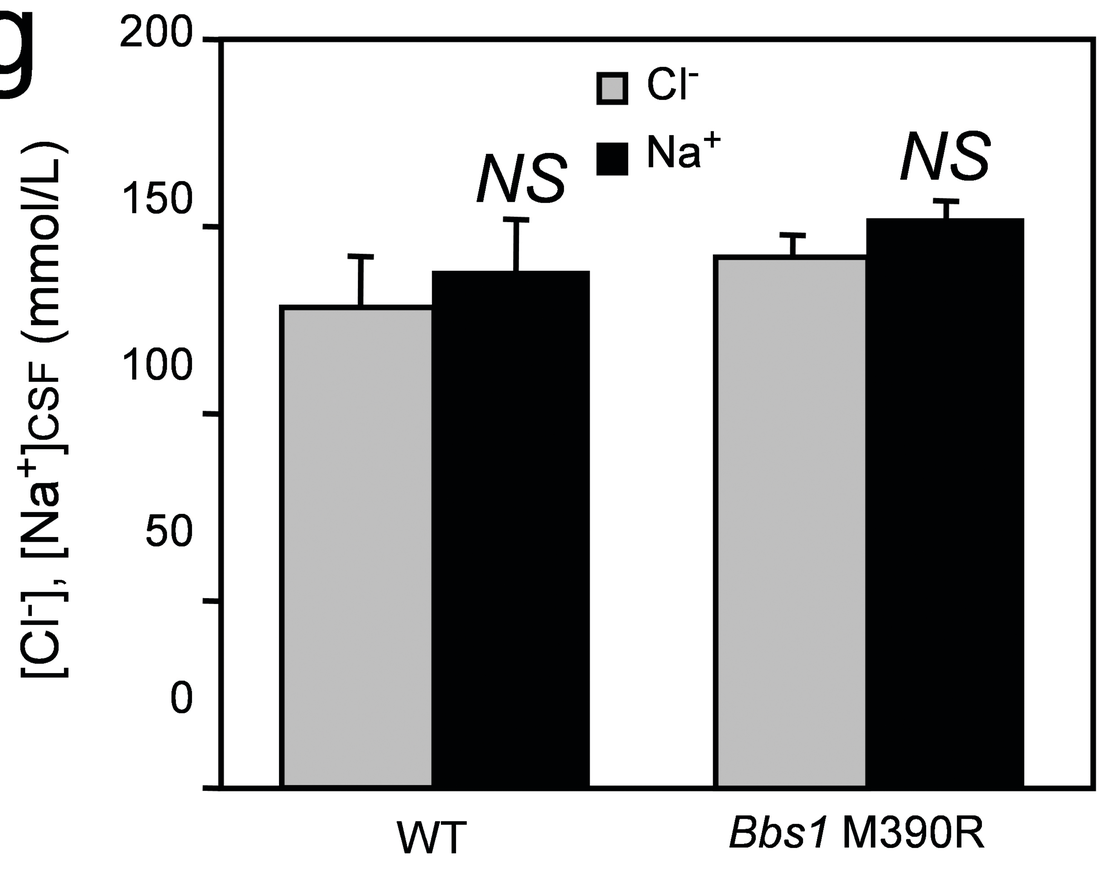






Supplementary Figure 1

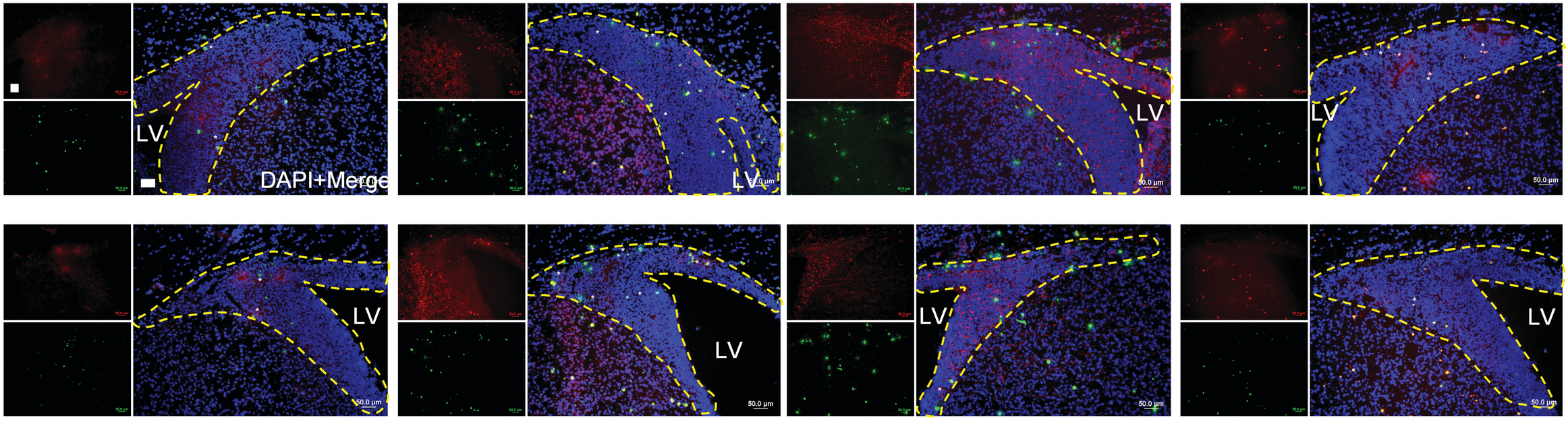
Supplementary Figure 1 Hydrocephalus in *Bbs1*$^{M390R/M390R}$ mice is communicating. Sagittal view of adult WT (**a**) and *Bbs1*$^{M390R/M390R}$ (**b**) mice injected with Evan's Blue dye, showing normal CSF circulation. (**c,d**) Histology showing patent cerebral aqueduct in WT (**c**) and *Bbs1*$^{M390R/M390R}$ (**d**) brains. (**e,f**) TEM micrographs of P0 brains showing normal ultrastructure of the choroid plexus epithelium in both WT (**e**) and *Bbs1*$^{M390R/M390R}$ (**f**) mice. *Bbs1*$^{M390R/M390R}$ cells appear healthy with microvilli protruding from the apical surface into the lateral ventricle. (**g**) Quantitations of CSF concentrations of Cl^- and Na^+ showing normal ion concentrations in *Bbs1*$^{M390R/M390R}$ mice ([Na^+] P=0.35, [Cl^-] P=0.10). n= at least 3 mice per group and genotype for all experiments. All error bars represent s.e.m and statistical results derive from unpaired t tests. Scale bars equal 2 mm (**a**,**b**), 1 mm (**c**,**d**) and 2 µm (**e**,**f**). 3V, third ventricle; Aq, cerebral aqueduct; CM, cisterna magna; LV, lateral ventricle; MV, microvilli; SAS, subarachnoid space.

a

Nestin/TUNEL | NeuN/TUNEL | GFAP/TUNEL | O4/TUNEL

WT

Bbs1 M390R

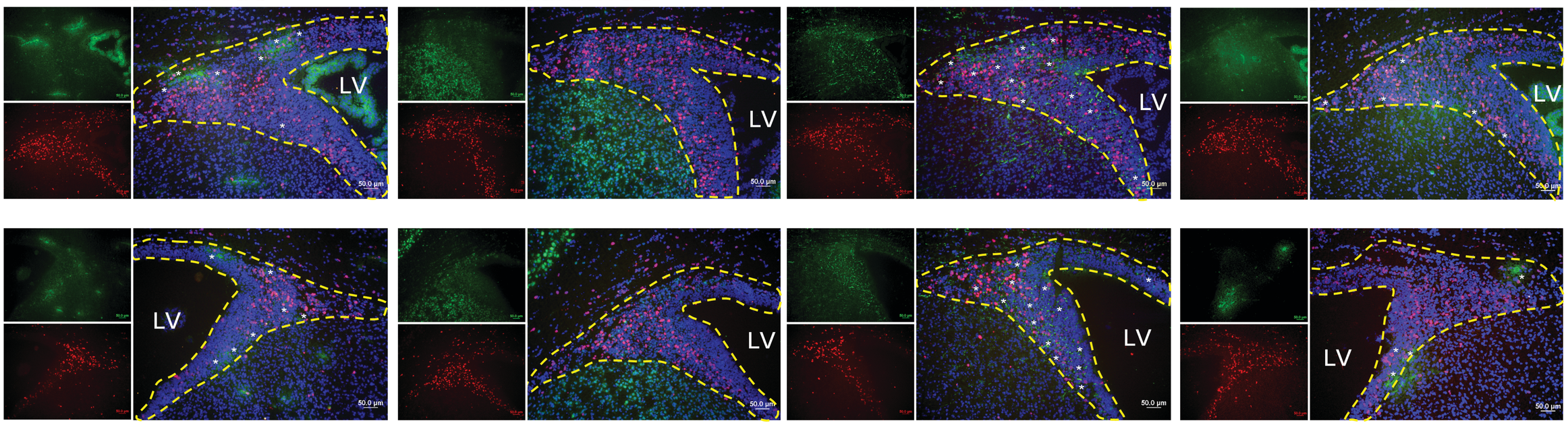


b

Nestin/BrdU | NeuN/BrdU | GFAP/BrdU | O4/BrdU

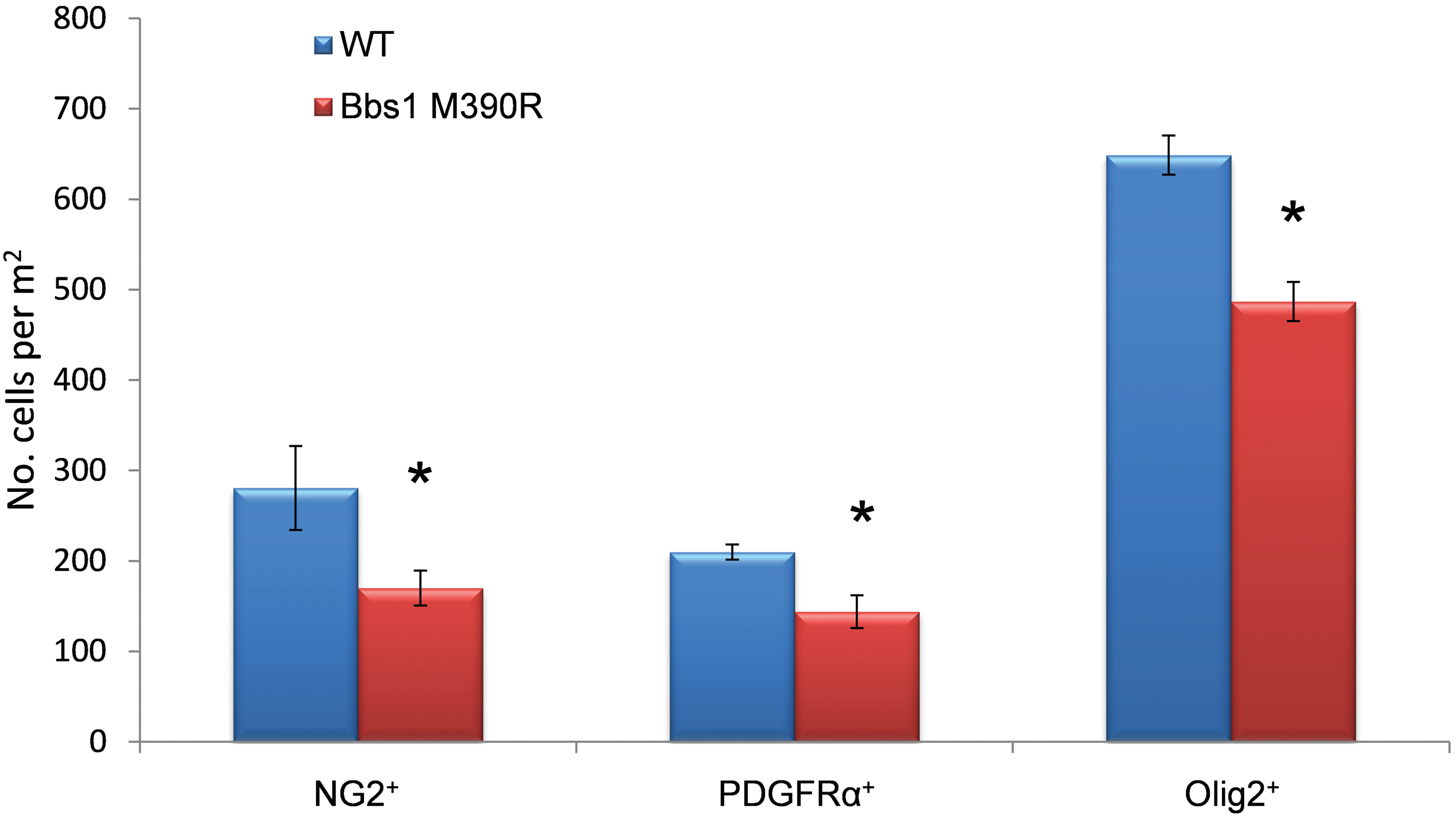
WT

Bbs1 M390R



Supplementary Figure 2

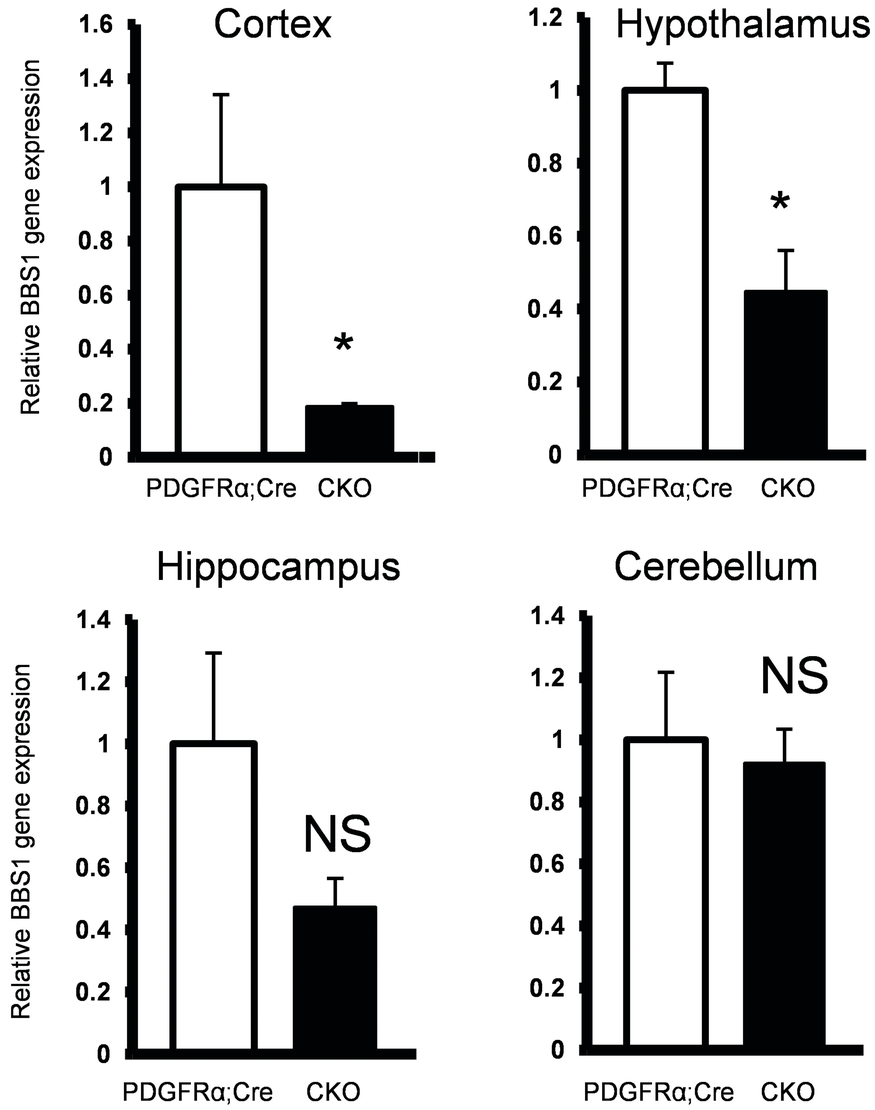
Supplementary Figure 2 Normal apoptosis and cell proliferation of Nestin, NeuN, GFAP and O4 positive cells in the SVZ of BBS mutant mice. (**a,b**) Representative immunofluorescent images showing the SVZ in brain tissue from post-natal day 3 pups labeled with TUNEL (**a**, green) or BrdU (**b**, red) and Nestin, NeuN, GFAP and O4. No significant differences were found between WT and $Bbs1^{M390R/M390R}$ (**Fig. 3**). $BrdU^{+}$ cells also expressing NeuN were not analyzed because post-mitotic neurons ($NeuN^{+}$) do not divide and no significant overlap was observed. We analyzed at least 3 mice per group and genotype. Scale bars equal 50 μm. LV, lateral ventricle; SVZ, subventricular zone.



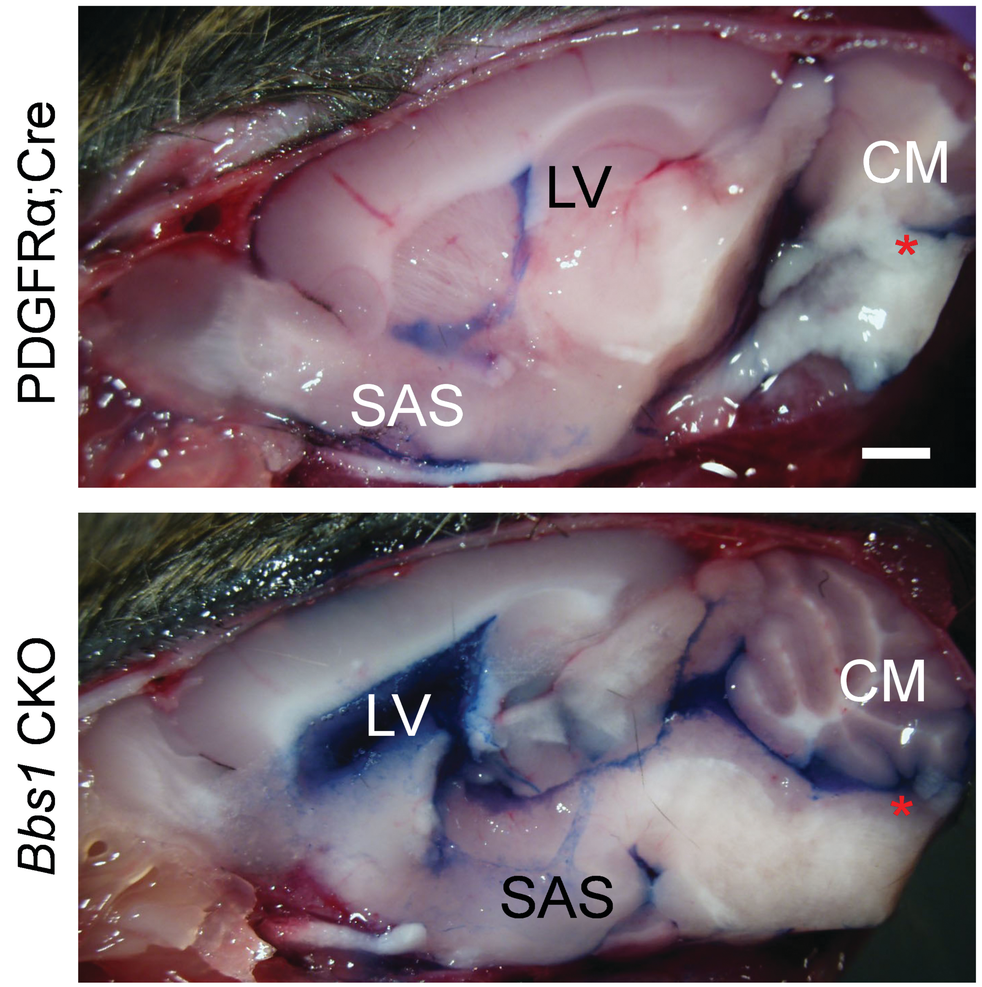
Supplementary Figure 3

Supplementary Figure 3 Reduced populations of NG2^{+}, PDGFRα^{+} and Olig2^{+} cells in the SVZ of Bbs1$^{M390R/M390R}$ mice. Quantitations of NG2^{+}, PDGFRα^{+} and Olig2^{+} cells per area in the SVZ of post-natal day 3 Bbs1$^{M390R/M390R}$ mice reveal significantly fewer populations of all three cell types relative to WT mice. Representative images for these analyses may be observed in **Fig. 3b** (NG2^{+}, PDGFRα^{+} and Olig2^{+} cells in green). We analyzed at least 3 mice per group and genotype. All error bars represent s.e.m. *$p<0.05$, results from unpaired *t* tests. LV, lateral ventricle; SVZ, subventricular zone.

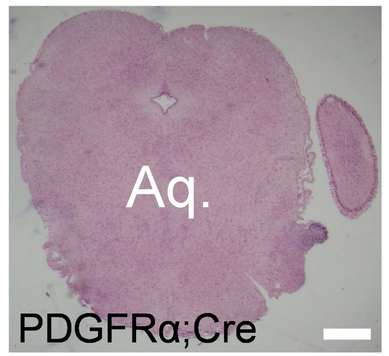
a

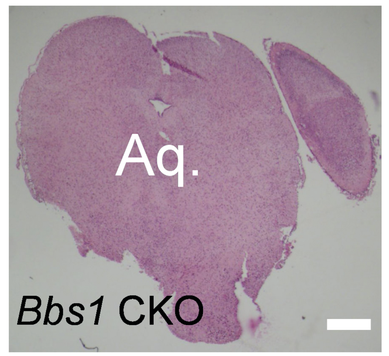


c



d





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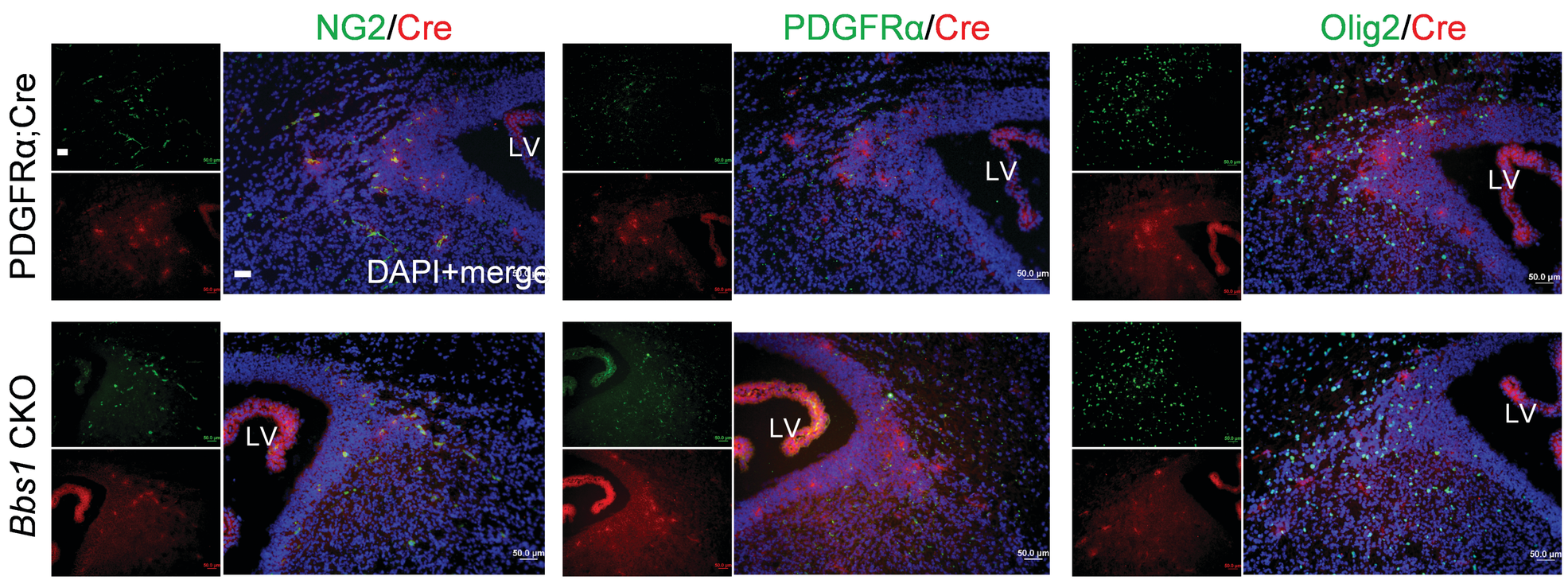
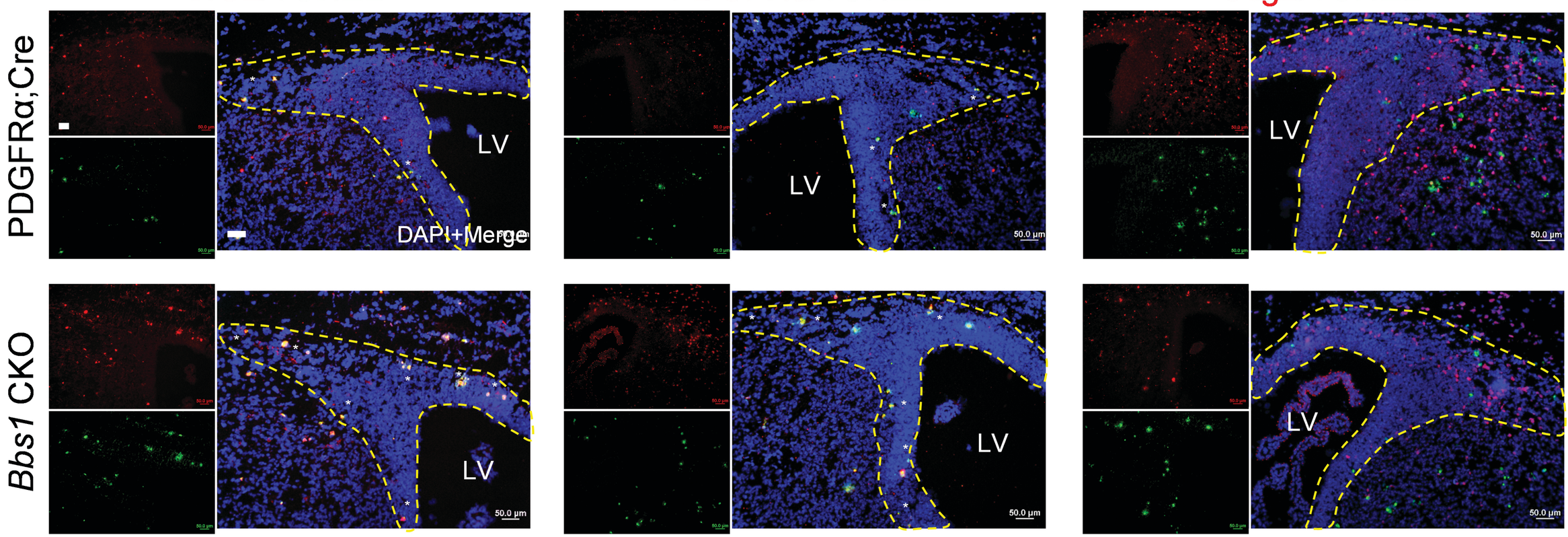


Supplementary Figure 4

Supplementary Figure 4 Conditional *Bbs1* knockout in neural progenitors leads to communicating hydrocephalus. (**a**) Quantitations of *Bbs1* mRNA levels in four brain regions showing significant reductions in the cortex and hypothalamus in adult *Bbs1*^{CKO} mice relative to PDGFRα;Cre (control). (**b**) Representative immunofluorescent images showing that Cre protein (red) is expressed in periventricular regions, specifically in neural progenitor cells expressing NG2, PDGFRα and Olig2 in both post-natal day 3 PDGFRα;Cre and *Bbs1*^{CKO} mice. Cre is not present in ependymal cells lining the ventricles. (**c**) Representative images following Evan's blue dye injections into the lateral ventricles. Dye is present throughout the ventricular system and subarachnoid spaces in both the PDGFRα;Cre and the hydrocephalic *Bbs1*^{CKO} adult brains indicating normal ventricular outflow in both mice and communicating (non-obstructive) hydrocephalus in *Bbs1*^{CKO} mice. (**d**) Histology in P0 pups showing patent cerebral aqueduct. Scale bars equal 50 μm (**b**), 1 mm (**c**) and 500 μm (**d**). All error bars represent s.e.m. *$p<0.05$, results from unpaired *t* tests. 3V, third ventricle; CKO, conditional knockout; CM, cisterna magna; LV, lateral ventricle; P, postnatal day

a



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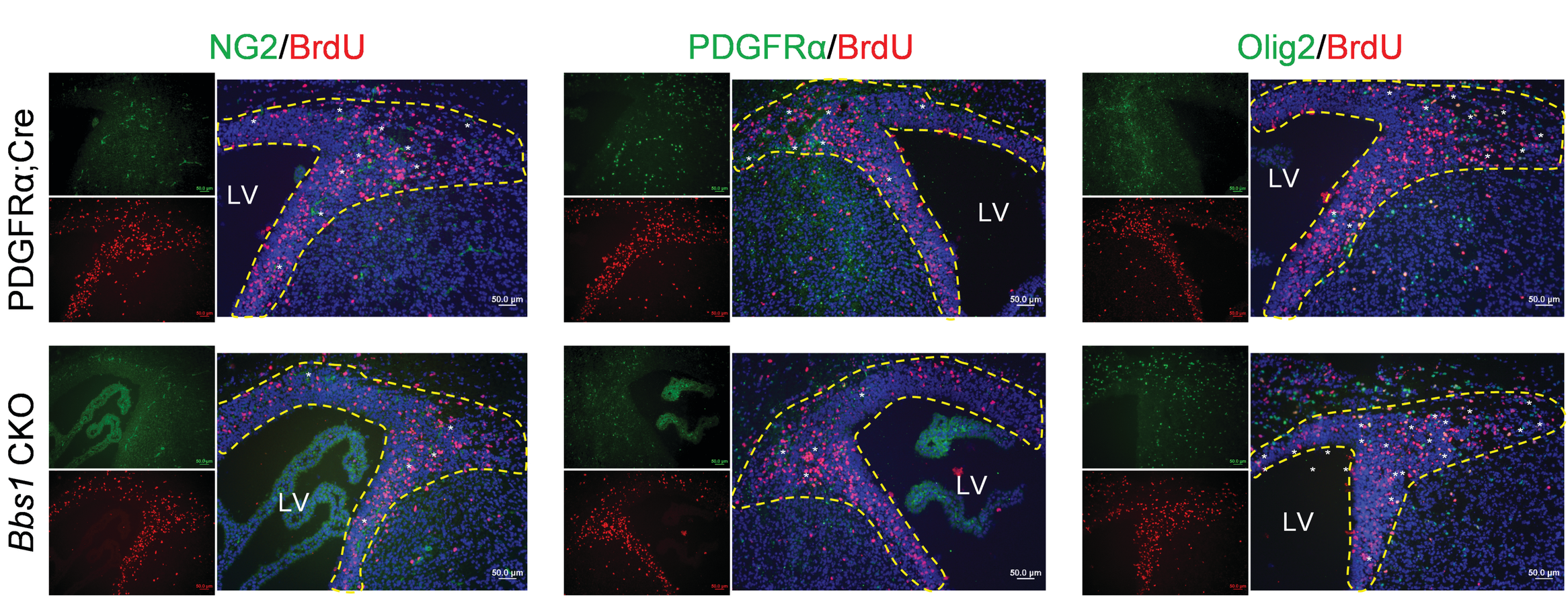
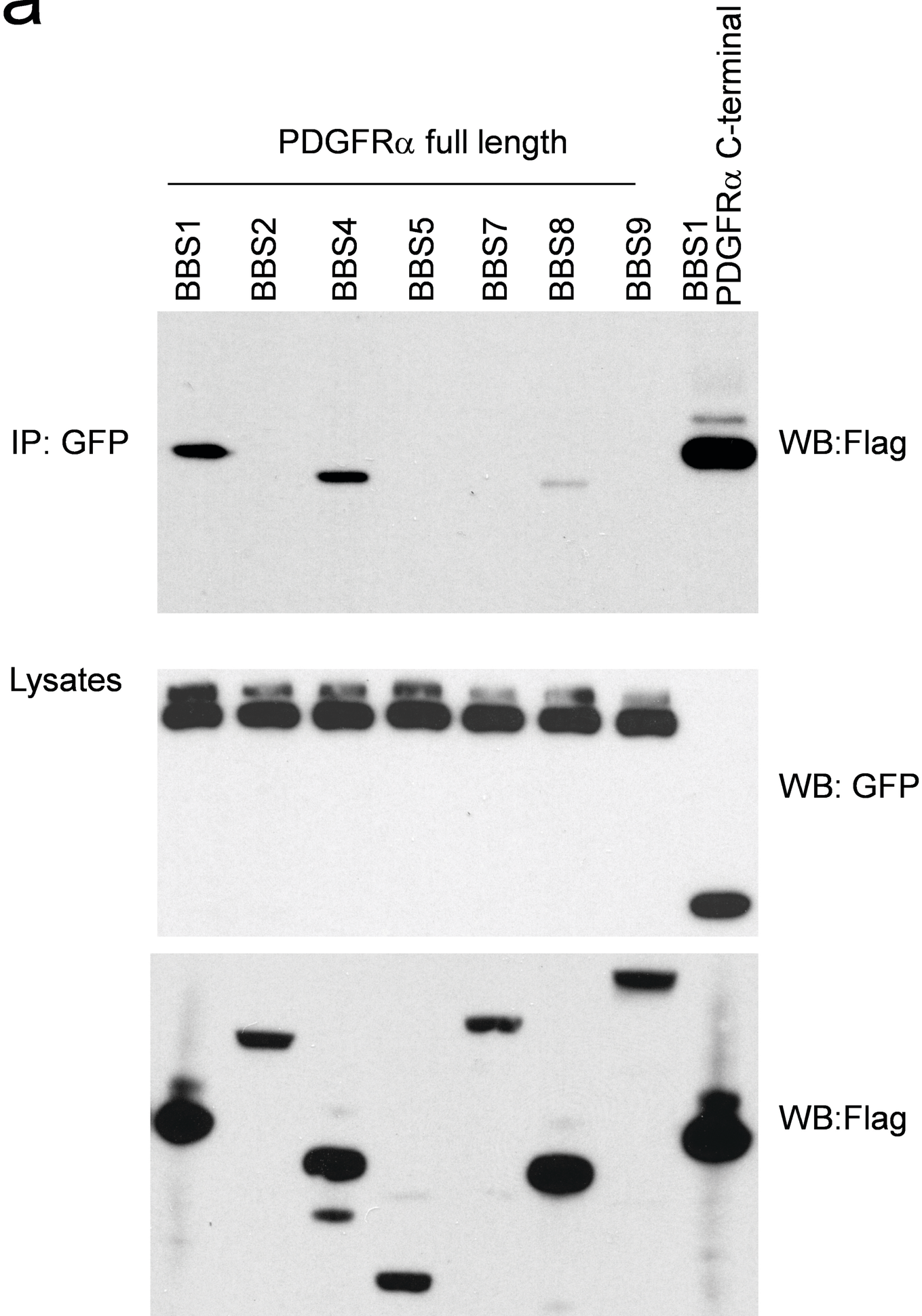


Supplementary Figure 5

Supplementary Figure 5 Conditional *Bbs1* knockout disrupts development of $NG2^{+}PDGFR\alpha^{+}$ neural progenitor cells. (**a,b**) Representative immunofluorescent images showing more $NG2^{+}$ and $PDGFR\alpha^{+}$ cells labeled with TUNEL (**a**, green) and fewer $NG2^{+}$ and $PDGFR\alpha^{+}$ cells labeled with BrdU (**b**, red) in the SVZ of post-natal day 3 PDGFRα;Cre and $Bbs1^{CKO}$ brains. No difference was observed between PDGFRα;Cre and $Bbs1^{CKO}$ mice in the number of $Olig2^{+}$ cells (**a**, red and **b**, green) also labeled with TUNEL (**a**) or BrdU (**b**). All quantitations are in **Fig. 4f** and **i, bottom**. Scale bar equals 50 μm (**a**,**b**). CKO, conditional knockout; SVZ, subventricular zone.

a



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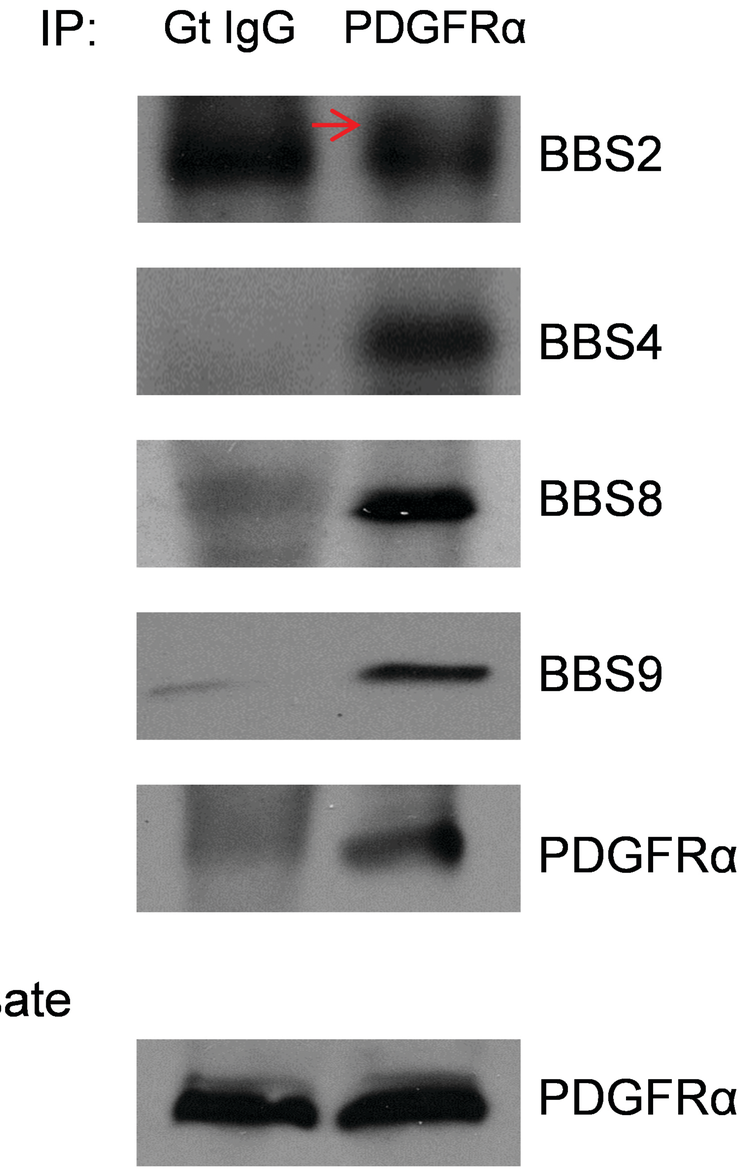


Supplementary Figure 6

Supplementary Figure 6 PDGFRα interacts with BBS proteins. (**a**) Co-transfection of GFP tagged PDGFRa full length and cytoplasmic tail in addition to Flag tagged BBSome subunits and subsequent western blot in 293T cells illustrating an interaction between PDGFRα and several BBSome subunits. The interaction between PDGFRα and the BBSome occurs through the C-terminal cytoplasmic tail of PDGFRα (**b**) Endogenous PDGFRα immunoprecipitation in P3 mouse cortices and subsequent western blot analyses using endogenous antibodies showing BBS proteins present in the PDGFRα immunoprecipitated but not IgG (control) lanes. These results illustrate an endogenous interaction between PDGFRα and BBS 2, 4, 8 and 9. Immunoblots from total lysates using anti-PDGFRα show equal protein loading in both samples. IP, immunoprecipitation; WB, western blot.

NG2/BrdU

PDGFRα/BrdU

Olig2/BrdU

WT

NaCl

LiCl

Bbs1 M390R

NaCl

LiCl

LV

50.0 μm

Supplementary Figure 7

Supplementary Figure 7 Lithium treatment rescues $NG2^{+}PDGFR\alpha^{+}$ cell proliferation in *Bbs1*$^{M390R/M390R}$ mice. Representative immunofluorescent images showing fewer BrdU labeled cells also expressing NG2 and PDGFRα in the SVZ of post-natal day 3 NaCl treated *Bbs1*$^{M390R/M390R}$ mice relative to WT mice. Lithium treatment increased the number of $BrdU^{+}$ cells expressing NG2 and PDGFRα in the SVZ of WT and *Bbs1*$^{M390R/M390R}$ mice relative to NaCl treated WT and *Bbs1*$^{M390R/M390R}$ mice (quantitations in **Fig. 6e, right**). Scale bars equal 50 μm. LiCl, lithium chloride; SVZ, subventricular zone.

NG2/TUNEL

PDGFRα/TUNEL

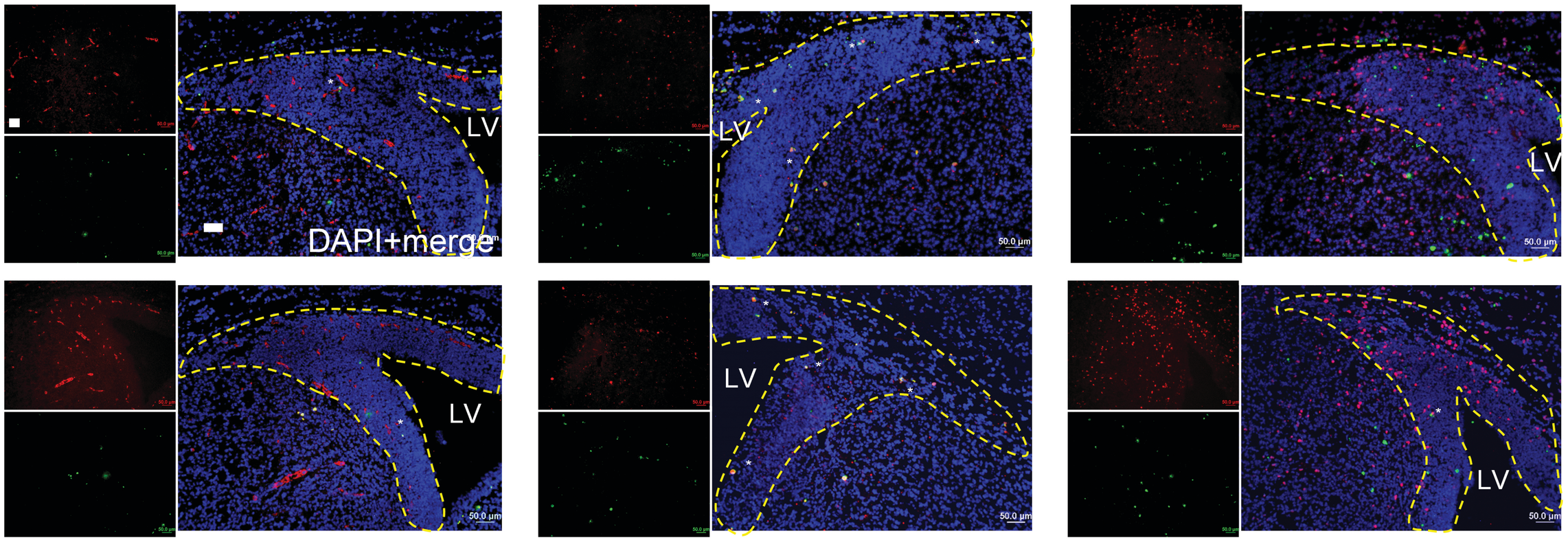
Olig2/TUNEL

WT

Bbs1 M390R

NaCl

LiCl



Supplementary Figure 8

Supplementary Figure 8 Lithium treatment does not modify apoptosis of neural progenitor cells. Representative immunofluorescent images showing more TUNEL labeled cells (green) also labeled with NG2 and PDGFRα (red) in the SVZ of post-natal day 3 NaCl and LiCl treated *Bbs1*$^{M390R/M390R}$ mice relative to NaCl and LiCl treated WT mice. Lithium treatment did not alter the number of TUNEL^{+} cells also expressing NG2 and PDGFRα in *Bbs1*$^{M390R/M390R}$ relative to NaCl treated *Bbs1*$^{M390R/M390R}$ mice (quantitations in **Fig 6g, right**). Scale bars equal 50 μm. LiCl, lithium chloride; SVZ, subventricular zone.