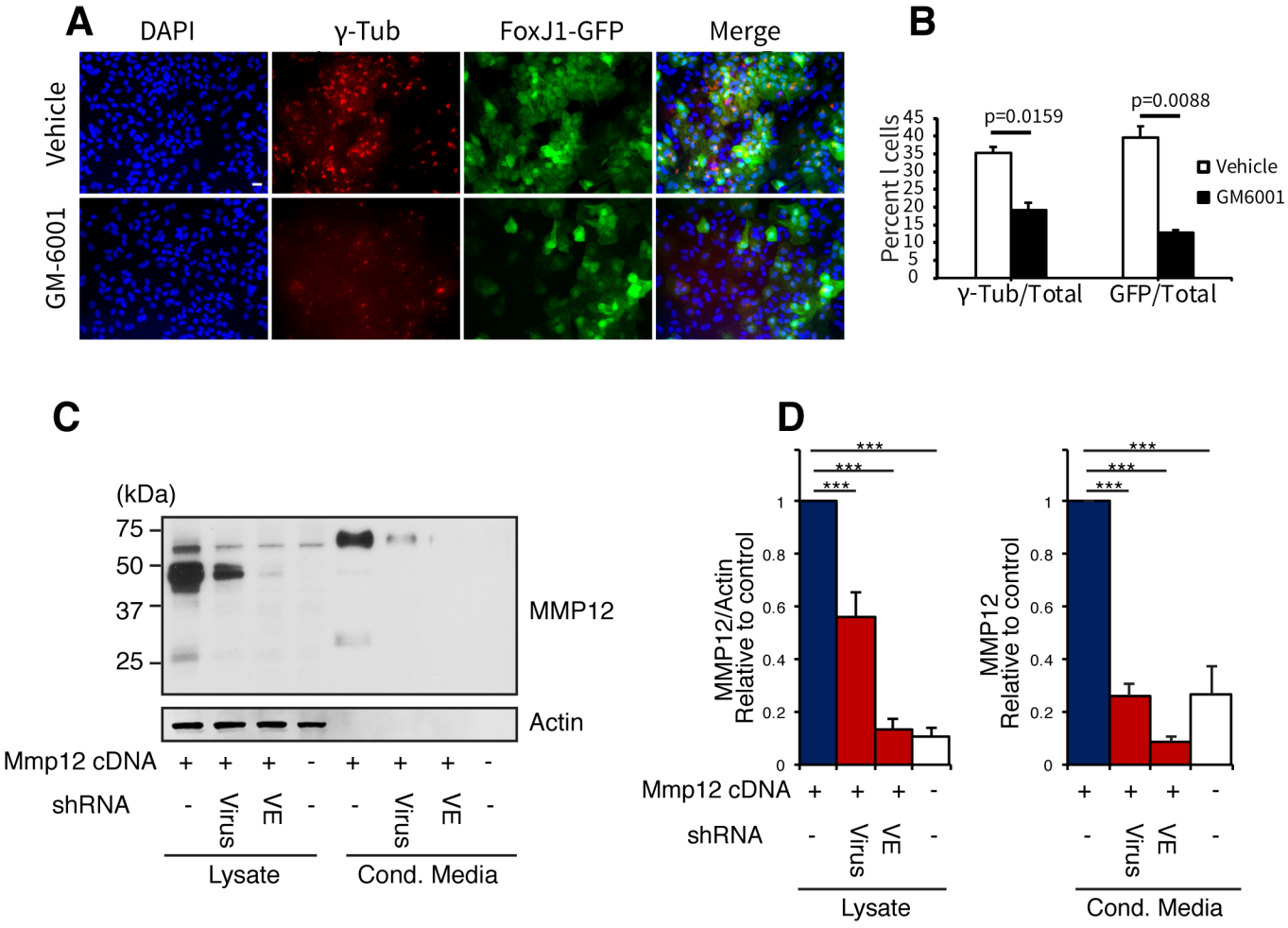
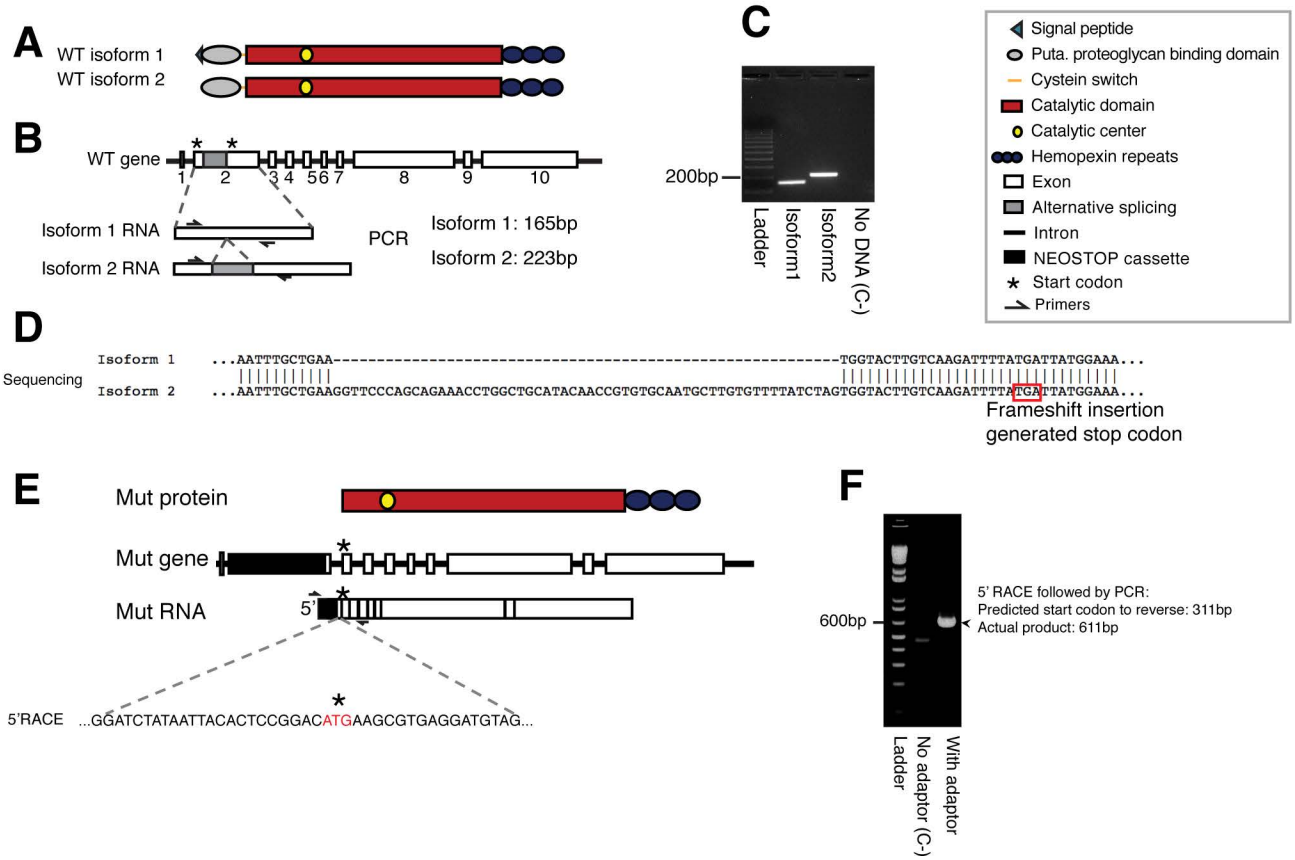
Stem Cell Reports, Volume *10*

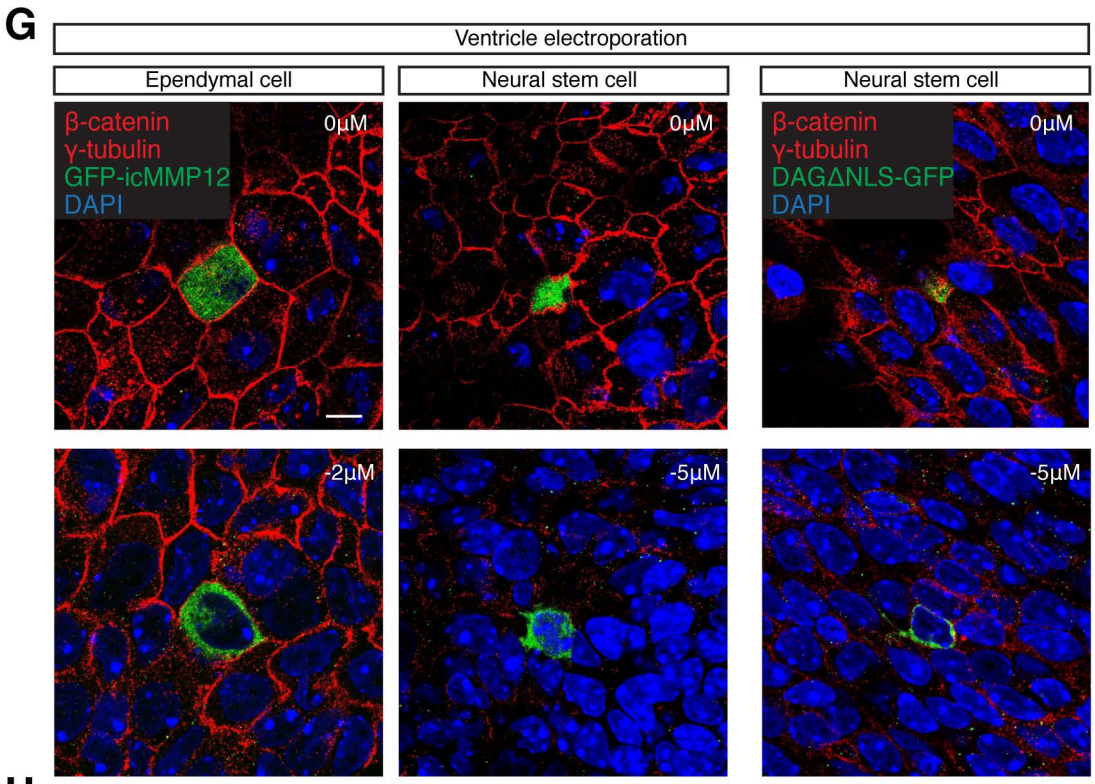
Supplemental Information

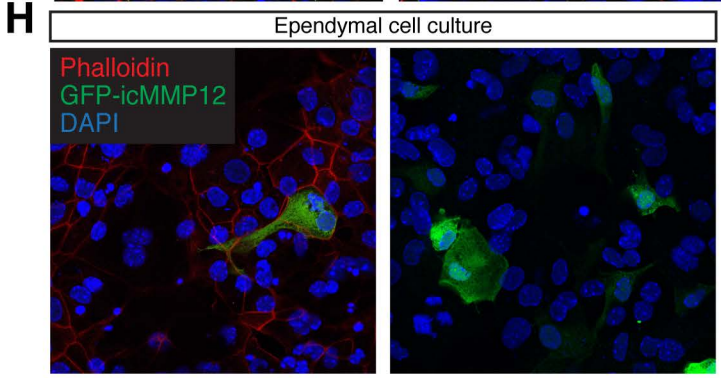
Distinct Requirements for Extracellular and Intracellular MMP12 in the Development of the Adult V-SVZ Neural Stem Cell Niche

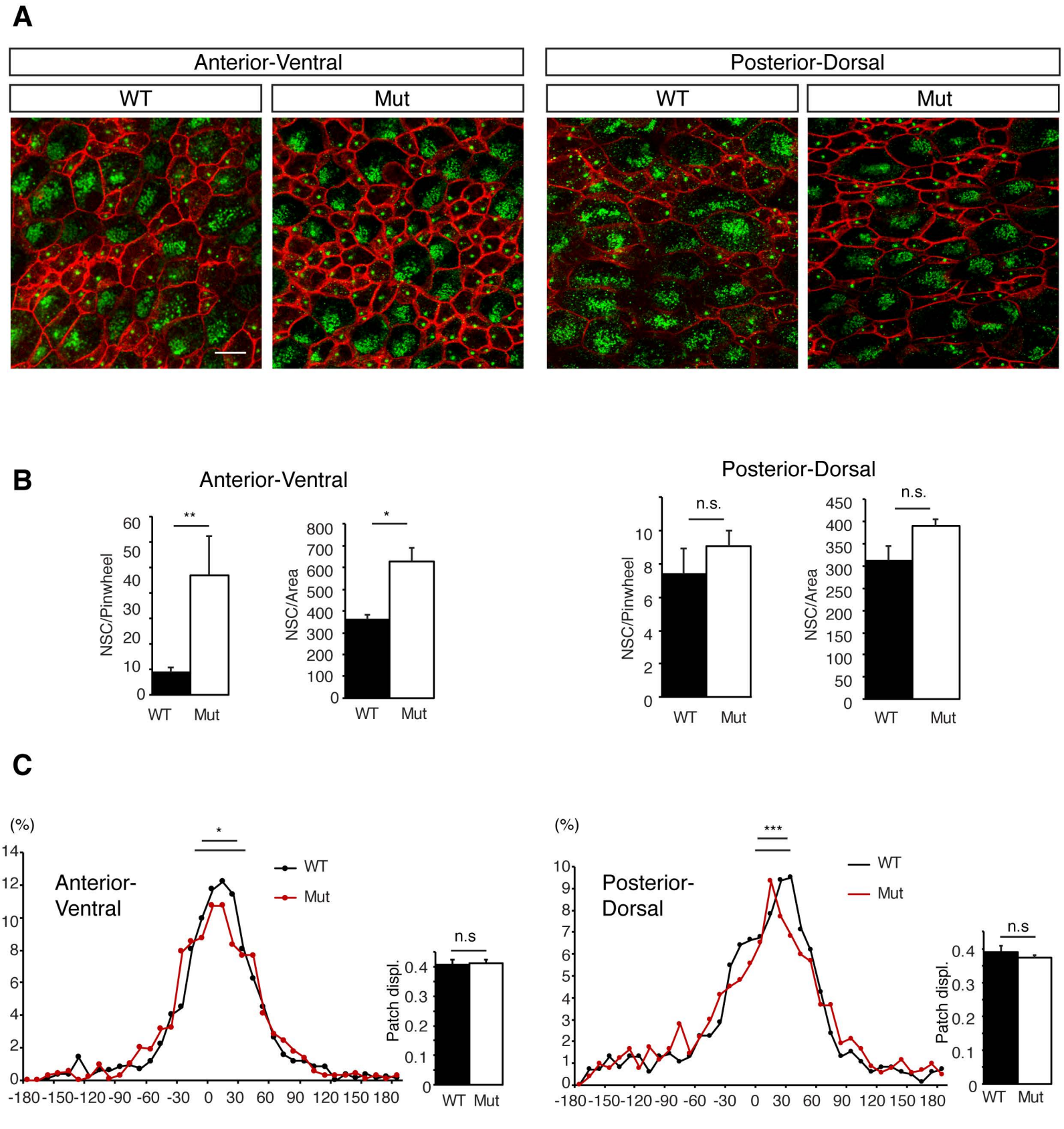
Xiwei Shan, Lyl Tomlinson, Qian Yang, and Holly Colognato

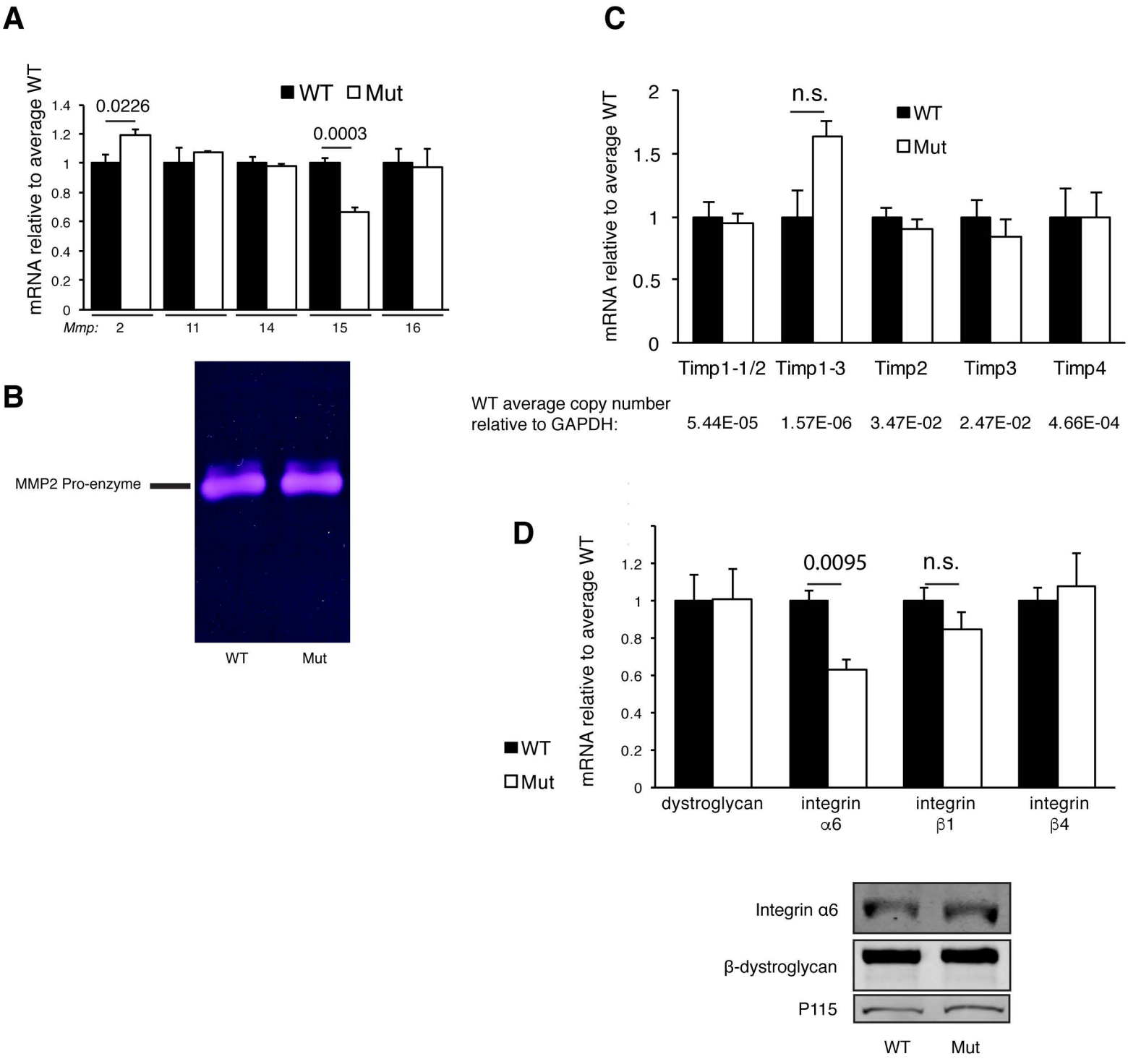

A
DAPI
γ-Tub
FoxJ1-GFP
Merge
Vehicle
GM-6001
B
Percent l cells
p=0.0159
p=0.0088
Vehicle
GM6001
γ-Tub/Total
GFP/Total
C
(kDa)
75
50
37
25
MMP12
Actin
Mmp12 cDNA
shRNA
Virus
VE
Lysate
Cond. Media
D
MMP12/Actin
Relative to control
MMP12
Relative to control
***

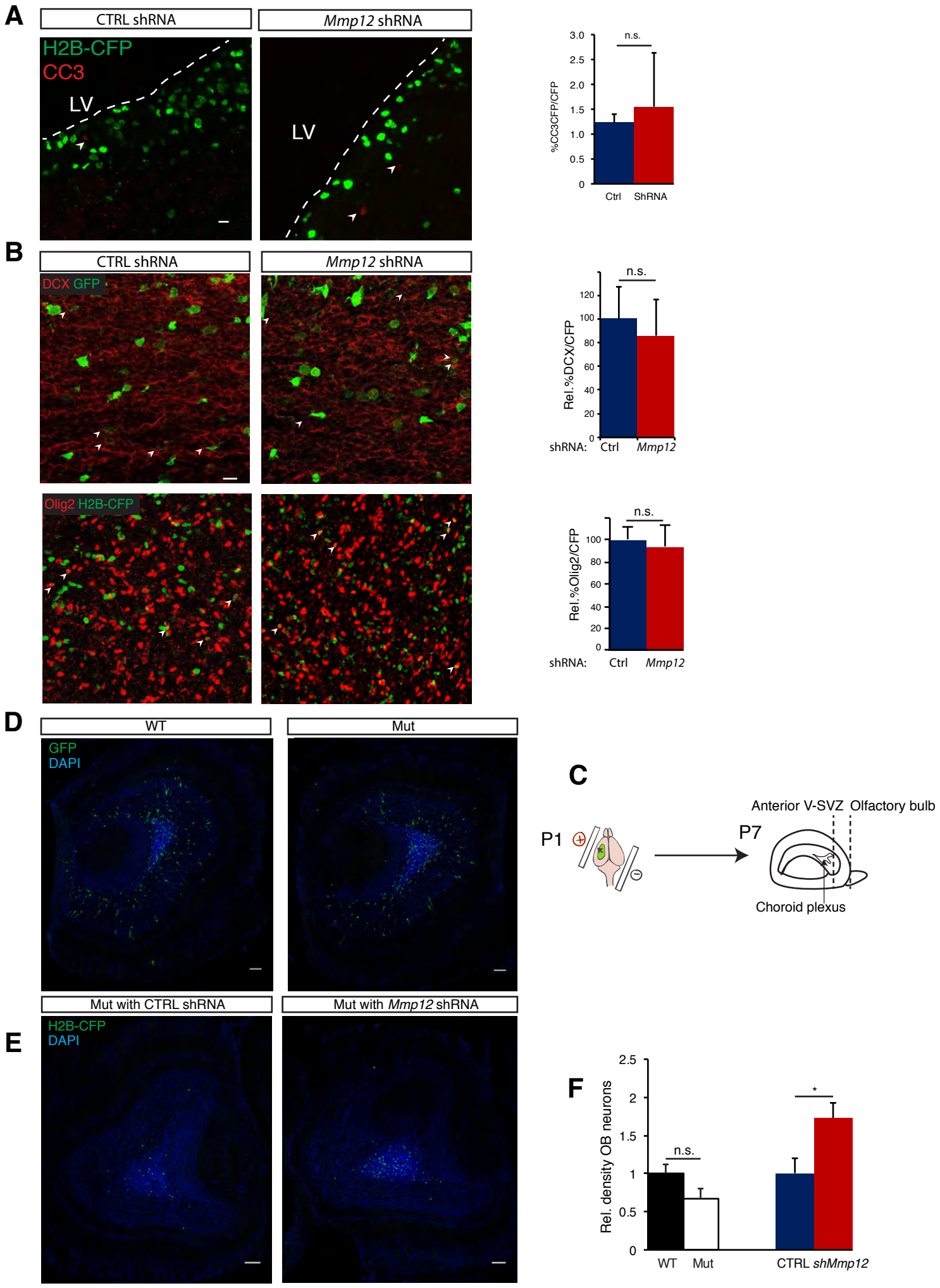

A
WT isoform 1
WT isoform 2
B
WT gene
1 2 3 4 5 6 7 8 9 10
Isoform 1 RNA
Isoform 2 RNA
PCR
Isoform 1: 165bp
Isoform 2: 223bp
C
200bp
Ladder
Isoform1
Isoform2
No DNA (C-)
Signal peptide
Puta. proteoglycan binding domain
Cystein switch
Catalytic domain
Catalytic center
Hemopexin repeats
Exon
Alternative splicing
Intron
NEOSTOP cassette
Start codon
Primers
D
Sequencing
Isoform 1 ...AATTTGCTGAA----------TGGTACTTGTCAAGATTTTATGATTATGGAAA...
Isoform 2 ...AATTTGCTGAAGGTTCCCAGCAGAAACCTGGCTGCATACAACCGTGTGCAATGCTTGTGTTTTATCTAGTGGTACTTGTCAAGATTTTATGATTATGGAAA...
Frameshift insertion
generated stop codon
E
Mut protein
Mut gene
Mut RNA
5'
5'RACE ...GGATCTATAATTACACTCCGGACATGAAGCGTGAGGATGTAG...
F
600bp
5' RACE followed by PCR:
Predicted start codon to reverse: 311bp
Actual product: 611bp
Ladder
No adaptor (C-)
With adaptor


G
Ventricle electroporation
Ependymal cell
Neural stem cell
Neural stem cell
β-catenin
γ-tubulin
GFP-icMMP12
DAPI
0μM
β-catenin
γ-tubulin
DAGΔNLS-GFP
DAPI
0μM
-2μM
-5μM
-5μM


H
Ependymal cell culture
Phalloidin
GFP-icMMP12
DAPI


A
Anterior-Ventral
Posterior-Dorsal
WT
Mut
WT
Mut
B
Anterior-Ventral
Posterior-Dorsal
NSC/Pinwheel
NSC/Area
**
*
n.s.
n.s.
WT
Mut
C
(%)
*
***
Anterior-
Ventral
Posterior-
Dorsal
WT
Mut
Patch displ.
n.s
-180 -150 -120 -90 -60 -30 0 30 60 90 120 150 180


A
mRNA relative to average WT
WT
Mut
0.0226
0.0003
Mmp:
2
11
14
15
16
B
MMP2 Pro-enzyme
WT
Mut
C
mRNA relative to average WT
n.s.
WT
Mut
Timp1-1/2
Timp1-3
Timp2
Timp3
Timp4
WT average copy number relative to GAPDH:
5.44E-05
1.57E-06
3.47E-02
2.47E-02
4.66E-04
D
mRNA relative to average WT
0.0095
n.s.
WT
Mut
dystroglycan
integrin α6
integrin β1
integrin β4
Integrin α6
β-dystroglycan
P115
WT
Mut


A
CTRL shRNA
Mmp12 shRNA
H2B-CFP
CC3
LV
LV
n.s.
%CC3CFP/CFP
3.0
2.5
2.0
1.5
1.0
0.5
0
Ctrl
ShRNA
B
CTRL shRNA
Mmp12 shRNA
DCX GFP
n.s.
Rel.%DCX/CFP
120
100
80
60
40
20
0
shRNA:
Ctrl
Mmp12
Olig2 H2B-CFP
n.s.
Rel.%Olig2/CFP
100
80
60
40
20
0
shRNA:
Ctrl
Mmp12
D
WT
Mut
GFP
DAPI
C
Anterior V-SVZ
Olfactory bulb
P1
P7
Choroid plexus
E
Mut with CTRL shRNA
Mut with Mmp12 shRNA
H2B-CFP
DAPI
F
Rel. density OB neurons
2.5
2
1.5
1
0.5
0
n.s.
*
WT
Mut
CTRL shMmp12

Supplemental Figure Legends

Figure S1. General inhibition of MMP activity blocks FoxJ1 transcription and ciliogenesis in ependymal cells. Related to Figs. 1 and 5 (shRNA confirmation). **A-B.** Ependymal cell cultures generated from FoxJ1-promoter driven GFP reporter mice were treated with vehicle (DMSO) or 25μM GM-6001 during differentiation, and analyzed via immunohistochemistry (IHC) at differentiation day 6. A. Representative images. B. Quantification. The percentages of multiciliated cells and GFP+ cells are both significantly reduced in GM-6001-treated group. n=3 for each group; scale bar = 20μm **C-D.** Lentivirus shRNA ("virus") plasmid and ventricle electroporation (VE) shRNA plasmids were transfected into 293T cells expressing full-length MMP12 cDNA. Corresponding plasmids without insert (designated as "-" in each condition) were used as transfection controls. Western blots indicate a substantial reduction of MMP12 protein in both cell lysates and conditioned media. **C.** Representative blot. **D.** Quantification (n = 5 for cell lysates, n = 3 for conditioned media. Conditioned media loading volume was adjusted based on protein quantification of cell lysate from the corresponding dish, and quantification was normalized to the actin of this corresponding lysate. *** $p<0.001$ by one-way ANOVA followed by multicomparison with Turkey-Kramer correction).

Figure S2. Characterization of icmmp12 transcripts in WT and Mut and icMMP12 subcellular localization in the V-SVZ. Related to Fig. 2. **A-F.** Both WT and *mmp12* mutant ependymal cells contain icMMP12-encoding transcripts. **A**. WT isoform 1 contains signal peptide sequence and therefore can be secreted. Isoform 2 has an inserted sequence in exon 2 resulting in a frameshift and alternative translational initiation. **B.** PCR using primers targeting common sections of isoform 1 and 2 yields products of different sizes. **C.** Agarose gel of the PCR products that were cloned into pcDNA3.1(+). Band sizes agree with the predicted produce sizes of isoform 1, and 2. **D.** Sequencing results of the PCR products that were cloned into pcDNA3.1(+), results are aligned and as predicted, corresponding with isoform 1 and 2 respectively. **E.** 5' RACE identified an intracellular *mmp12* mRNA, with the indicated start codon (asterisks) as the only in-frame ATG. The mRNA contains a small portion of the neo-STOP cassette inserted into mutant genome (black box). **F.** Agarose gel of the 5' RACE products without (middle lane) or with (right lane) the 5' adaptor. **G-H.** icMMP12 in V-SVZ cells translocate into nucleus. **G.** WT neonatal mice were electroporated with GFP-icMMP12 or DAGΔNLS-GFP (negative control) and assessed by wholemount immunohistochemistry at P7. Confocal images indicate modest nuclear localization of icMMP12 in ependymal cells and more extensive nuclear localization in neural stem cells. In contrast DAGΔNLS-GFP was not observed in the nucleus. **H.** Ependymal cell cultures were transfected with GFP-icMMP12 using XtremeGene-HP. Nuclear staining was observed in GFP+ cells at differentiation day 6.

Figure S3. Assessment of neural stem cell niche pinwheel organization and planar cell polarity in anterior-ventral (AV) and posterior-dorsal (PD) V-SVZ. Related to Figs. 3 (C, D) and 4. **A-C.** Left panel: AV V-SVZ. Right panel: PD V-SVZ. **A.** Representative images of AV and PD V-SVZ in WT and *mmp12* mutant (Mut) mice at P7. **B.** There are more NSCs per pinwheel and NSCs per area in the AV, but not PD, V-SVZ of *mmp12* mutant mice. (*$p<0.05$, **$p<0.01$ by t-test) **C.** Cilia patch angle distribution in both the AV and PD V-SVZ are disturbed in *mmp12* mutant mice (*$p<0.05$, ***$p<0.001$ by Watson's U2 test). Patch displacement is unchanged in either the AV or PD V-SVZ (t-test). AV-WT n = 842, AV-Mut n = 948, PD-WT n = 851, PD-Mut n= 1038, WT: 4 animals, Mut: 4 animals. Scale bar = 10μm.

Figure S4. Analysis of MMP and TIMP expression in *mmp12* mutant ependymal cells. Related to Fig.3 (E-G). **A.** RT-qPCR results of *mmp2, 11, 14, 15*, and *16* in ependymal cell (EC) cultures from WT and *mmp12* mutant (Mut) mice at differentiation day 6. *Mmp12* transcription is upregulated while *mmp15* (*mt2-mmp*) is downregulated (WT, n=6; Mut, n=8) **B.** On-gel gelatin zymography of conditioned media from WT and Mut EC cultures. No noticeable band is present for activated MMP2, and the pro band appears similar in both genotypes. **C.** RT-qPCR indicates mRNA level of timps are not changed in Mut. Note that the Timp1 transcription variant 3 (Timp1-3) has much lower copy number compare to variant 1 and 2 (Timp1-1/2). WT, n = 7; Mut, n = 8. **D.** RT-

qPCR indicates mRNA level of dystroglycan, integrin β1, and integrin β4 genes are not changed in Mut ECs. While mRNA level of integrin α6 is lower in Mut ECs ($p=0.0095$, $n=3$), western blotting did not indicate any change in integrin α6 or β-dystroglycan protein levels.

Figure S5. Assessment of additional niche features upon loss of extracellular and intracellular MMP12. Related to Figs. 5 and 6. **A.** V-SVZ apoptosis following loss of icMMP12. *mmp12* mutant mice were electroporated with control shRNA or *mmp12* shRNA coexpressing H2B-CFP at p1 and coronal brain sections were assessed at p7. Apoptotic cells were labeled with cleaved caspase 3 (CC3) antibody. CFP and CC3 double positive cells were quite rare (less than 2%) and never observed in the ependymal cell layer. No significant increase was observed in the *mmp12* shRNA group (by t-test, n = 3 for each group). Scale bar = 10μm. **B.** Neurogenesis and gliogenesis following loss of icMMP12. *mmp12* mutant mice were electroporated with control shRNA or *mmp12* shRNA coexpressing GFP (for DCX co-IHC) or H2B-CFP (for Olig2 co-IHC) at p1 and wholemounts were assessed at p7. Left: Representative images of DCX+GFP (upper) and Olig2+H2B-CFP (lower) wholemount IHC. Arrowheads: double positive cells. Right: Quantification of %DCX/GFP and %Olig2/CFP cells in the entire wholemounts. No significant differences were found (by t-test). DCX+GFP: *ctrl* shRNA n = 3, *mmp12* shRNA n = 3, Olig2+H2B-CFP: *ctrl* shRNA n = 5, *mmp12* shRNA n = 4. Scale bar = 20μm. **C-F**. Rostral migration to the olfactory bulb (OB). **C.** Mice were electroporated at p1 and coronal sections of anterior V-SVZ and OB were analyzed at p7. Representative images of OBs from WT and *mmp12* mutant (Mut) mice electroporated with GFP plasmid. **E.** Representative images of OBs from Mut mice electroporated with control (ctrl) shRNA or *mmp12* shRNA co-expressing H2B-CFP. F. Quantification of relative GFP or CFP+ cells in the OB. Electroporation efficiency is normalized by number of GFP+ or CFP+ cells in the anterior SVZ. Normalized number of CFP+ cells in the OB is significantly increased in *mmp12* shRNA group compared to control. WT n = 4; Mut n = 4; *ctrl* shRNA n = 5; *mmp12* shRNA n = 4. *$p<0.05$ by t-test. Scale bar = 100μm.

Table S1. MMP transcript levels in differentiating ependymal cell cultures (related to Fig.1B). Results are generated from pooled samples of 3 indipendent experiments for each time point.

Max<	MMP	Day 1	Day 6	Day 12
10^-2	14	5.56E-03	4.42E-03	3.88E-03
	12	1.12E-04	1.27E-03	4.65E-03
	15	1.77E-03	1.74E-03	1.62E-03
	16	1.68E-03	1.21E-03	1.22E-03
10^-3	2	7.23E-04	2.87E-04	1.79E-04
	11	4.96E-04	5.50E-04	6.07E-04
10^-4	24	1.32E-04	3.82E-05	3.45E-05
	17	1.29E-04	5.17E-05	3.06E-05
	23	6.22E-05	6.46E-05	5.50E-05
	21	5.50E-05	3.71E-05	4.83E-05
	28A	3.95E-05	2.24E-05	2.91E-05
	28B	3.57E-05	3.43E-05	3.66E-05
	19B	1.55E-05	2.77E-05	3.29E-05
	9	2.34E-06	4.93E-06	2.17E-05
	13	1.38E-06	3.80E-06	1.04E-05
10^-5	19A	4.23E-06	5.29E-06	8.12E-06
	7	4.15E-06	2.27E-06	2.88E-06
	27	1.50E-07	1.28E-06	3.07E-06
	3	3.28E-07	1.41E-06	2.85E-06
	25	6.25E-07	9.02E-07	1.07E-06
10^-6	10	6.53E-07	7.21E-07	8.20E-07
	8	8.15E-08	2.72E-07	2.55E-07
	20	1.64E-07	1.35E-07	9.85E-08
	1	3.35E-08	1.21E-07	1.06E-07

Supplemental Experimental Procedures

Planar cell polarity analysis

```
%% Run each section separately.
%% UI Import: Arrange results from each image into a single column. Import as the form of numeric matrix.For wilde type name import as WT, knockout name KO.

%% WT
[NumRow, NumClmn] = size(WT);
FixedWT = WT;
% M is an array of means of each image.
M = nanmean(WT);

% Loop for going through each image(column).
for c = 1:NumClmn;

 %To prevent shift of mean after fixing number,
 %Possibly need several fixes.
 while abs(M(c)) >= 0.1;
        FixedWT(:,c) = FixedWT(:,c) - M(c);
  %Going through each number in an image.
    for r = 1:NumRow
       %Shift overflowing numbers
        if FixedWT(r,c) >= 180
        FixedWT(r,c) = FixedWT(r,c) - 360;
          elseif FixedWT(r,c) < -180
            FixedWT(r,c) = FixedWT(r,c) + 360;
            %else includes NaN.
                else FixedWT(r,c) = FixedWT(r,c);
        end

    end

      M(c) = nanmean(FixedWT(:,c));
 end


end
% Combine array into a single vector and get rid of NaN.
FixedWTstrng = FixedWT(:);
FixedWTstrng(isnan(FixedWTstrng(:,1)),:)=[];

%% KO
[NumRow, NumClmn] = size(KO);
FixedKO = KO;
% M is an array of means of each image.
M = nanmean(KO);

% Loop for going through each image(column).
for c = 1:NumClmn;

 %To prevent shift of mean after fixing number,
 %Possibly need several fixes.
 while abs(M(c)) >= 0.1;
```

```
        FixedKO(:,c) = FixedKO(:,c) - M(c);
  %Going through each number in an image.
    for r = 1:NumRow
       %Shift overflowing numbers
        if FixedKO(r,c) >= 180
        FixedKO(r,c) = FixedKO(r,c) - 360;
          elseif FixedKO(r,c) < -180
            FixedKO(r,c) = FixedKO(r,c) + 360;
            %else includes NaN.
                else FixedKO(r,c) = FixedKO(r,c);
        end

    end

      M(c) = nanmean(FixedKO(:,c));
 end


end
% Combine array into a single vector and get rid of NaN.
FixedKOstrng = FixedKO(:);
FixedKOstrng(isnan(FixedKOstrng(:,1)),:)=[];

%% plot histogram of W|T/K|O.

Hst = zeros(37,2);
[NumWT,~] = size(FixedWTstrng);
[NumKO,~] = size(FixedKOstrng);
N = [NumWT, NumKO];


 for i = (1:36);
       Find = and(FixedWTstrng >= -180 + (i-1)*10,...
                FixedWTstrng < -180 + i*10);
       Perc = sum(Find)/N(1,1);
       Hst(i,1) = Perc;
 end
 for i = (1:36);
       Find = and(FixedKOstrng >= -180 + (i-1)*10,...
                FixedKOstrng < -180 + i*10);
       Perc = sum(Find)/N(1,2);
       Hst(i,2) = Perc;
 end

Y1 = 100*Hst(:,1);
Y2 = 100*Hst(:,2);
X = -180: 10: 180;
plt = plot(X, Y1, 'k',X, Y2, 'r');
 % line styles.
 plt(1).LineWidth= 2;
 plt(1).Marker='o';
 plt(1).MarkerSize= 4;
 plt(1).MarkerEdgeColor= 'k';
 plt(1).MarkerFaceColor= 'k';
 plt(2).LineWidth= 2;
 plt(2).Marker='o';
```

```
 plt(2).MarkerSize= 4;
 plt(2).MarkerEdgeColor= 'r';
 plt(2).MarkerFaceColor= 'r';


 % Axis range and style.
axis([-180 180 0 10])
set(gca, 'XTick', [-180, -150, -120, -90, -60, -30, 0,...
    30, 60, 90, 120, 150, 180])
set(gca,'box','off')

 % Axis label.
x = xlabel('(o)');
set(x, 'position', get(x, 'position')+[190,0,0]);
y = ylabel('(%)', 'rot', 0);
set(y, 'position', get(y, 'position')+[-10,5,0]);

 % Legend
Lg = legend('WT', 'KO');
set(Lg,'box','off')

%% U2 test
[p,~,~] = watsons_U2_perm_test(FixedWTstrng, FixedKOstrng, 1000);
```

Primers and DNA oligonucleotides

Name	Forward	Reverse	Product size
MMP1a	GCAGGAAGGAGGCCACTGGTGAT	TCCACATTTTGCCGGTCTCCGTTG	135
MMP1b	GCTTCCTGCCAGAACCTTTGATCG	TGCTGCCTTTGAAAAAGCGGAGT	120
MMP2	TCTGCCCCCATGAAGCCTTGT	GTGCCCTGGAAGCGGAACGG	82
MMP3	CCTGCTGTGGCTGTGTGTGGTT	TCCCTGTCATCTCCAACCCGAGGA	215
MMP7	CAGGCCTAGGCGGAGATGCTCA	GCAATGGAGGACCCAGTGAGTGC	297
MMP8	TGGCCCTTCCTACCCAACGGT	AGAGCCCAGTACTGTCTGCCTTT	92
MMP9	GCTGGGCAAAGGCGTCGTGAT	CGTCTGTGGTGCAGGCCGAA	110
MMP10	GTGACACAAGGCCACCCAAGCA	CACCACACCTGGGCTTGTGCAT	211
MMP11	GCCTGTCTCCTCCGCGCGAT	ACGGTGACTCTCCGGTGGCCT	120
MMP12	TGCATTTGGAGCTCACGGAGAC	TGCTGCAGCCCCAAGGAATGG	195
MMP13	AAGACAGATTCTTCTGGCGCCTG	AGTGAACCGCAGCGCTCAGTC	252
MMP14	AAAACCCCGCCTATGGGCCC	GCCAGAACCATCGCTCCTTGA	100
MMP15	GGGTACATGTGAAAGCCAACCT	GGTAATTGTTCCATGCCTTTCC	75
MMP16	CCCGGAGAGCAATTCGCCGT	TCGCCTCCAATTCCGGGTCCA	214
MMP17	CCGTGGGTGACCCGCTACGC	CCCGCACACCGTACAACTGCC	78
MMP19a	GGCTGGCATTCTTACTTCCCATGAC	TCTCCAGTGGCCCAGAAGCAGG	299
MMP19b	AGCAAAGACCTGGAGGATTACCTGT	GCTGCTTCATACGGGCCCTTGT	175
MMP20	ACAGATGGCCCTGCATGCGT	TCCGGGGTCCGTATAATGCTTGG	395
MMP21	CAA AGG CAA CTC GTA CTG GA	AAC CAC TTC TCG GAA ACA GG	108
MMP23	TGATTTCTGTTATGAATTCCCATTTC	TCTCTCACCAACCTTGTTTTCGT	81
MMP24	GTG ATG AGG CCA GTA TGG TG	AAC AGG ATG CCC GCT AGT AT	95
MMP25	AGAAGACGTGGATGCTGTGTTCT	TTTGACCTCGGATCAGGTATGTC	65
MMP27	TTCTGTCTTGCCCGGTTACC	CAGACGGCTGCATCAATTTTC	81
MMP28a	TGGCAATGTTTCAGAGCCTCGTCCA	GTGCGAACCCCCACACGGAC	161
MMP28b	CCATCACTGTAGAGCCTCGTCCAC	GTGCGAACCCCCACACGGAC	159
MMP12 Exon1	GAACTTGCAGTCGGAGGGAA	TGTCATTCATGGGAGCAGCC	127
MMP12 Exon4/5	TTGCATTTGGAGCTCACGGA	ATGTCATCAGCAGAGAGGCG	277
MMP12 Exon7/8	TCCATATGGCCAAGCATCCC	TTGGCGAAGTGGGTCAAAGA	204
GAPDH	GCACAGTCAAGGCCGAGAAT	GCCTTCTCCATGGTGGTGAA	151
5'RACE Outer	GCTGATGGCGATGAATGAACACTG	ATGTCATCAGCAGAGAGGCG	n.a.
5'RACE Inner	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG	TGCTGCAGCCCCAAGGAATGG	611
Isoform ID Outer	GCTGCTCCCATGAATGACAGT	GATGCTGTACATCGGGCACT	229/287/147
Isoform ID Inner	GCTGCTCCCATGAATGACAGT	TTGCCCAGTTGCTTCTAGCC	165/223/83

Biotinylated oligos for Streptavidin-agarose pull-down assays:
Biotin-5'-ACAAACATTAACACCTTGCTTTACTTGGGAAACAAAAAAAATCATGGTCC
Biotin-5'-GGACCATGATTTTTTTTGTTTCCCAAGTAAAGCAAGGTGTTAATGTTTGT

shRNA targeting sequences:
Mmp12 shRNA#1 target (ventricle electroporation): CATTTCGCCTCTCTGCTGATGACATACGT
Mmp12 shRNA#2 target (lentivirus transduction): TACTACATCTTCCAAGGAGCCTATCAATT
Mmp14 shRNA target (lentivirus transduction): GACACAGAGAACTTCGTGTTGCCTGATGA
Scrambled sequence: GTCCGTCTTCGCGCCATATCTCTAGTATA

Antibodies

Antigen	Company	Catalog#	Species	Applications	Dilution
MMP12	R&D Systems	AF3467	Goat	WB	1:1000
MMP12	Abcam	ab66157	Rabbit	IHC	1:100
Sox2	Abcam	ab97959	Rabbit	IHC	1:200
Sox2	R&D Systems	245610	Mouse	IHC	1:100
Mash1	Gift from Dr. David Anderson	N.A.	Mouse	IHC	1:2
Ki67	eBioscience	14-5698	Rat	IHC	1:500
FoxJ1	eBioscience	14-9965-82	Mouse	IHC	1:100
GFP	Aves Labs	GFP-1020	Chicken	IHC	1:600
GFP	Molecular Probs	A11122	Rabbit	WB	1:1000
β-catenin	BD Biosciences	610153	Mouse	IHC	1:300
β-catenin (non-phospho)	CST	19807	Rabbit	IHC	1:1000
γ-tubulin	Sigma	T5192	Rabbit	IHC	1:1000
Acetylated tubulin	Sigma	T6793	Mouse	IHC	1:1000
CD24	BD Biosciences	557436	Rat	IHC	1:100
Pan-laminin	Sigma	L9393	Rabbit	WB, IHC	1:1000
DCX	Abcam	AB18723	Rabbit	IHC	1:1000
Olig2	IBL	18953	Rabbit	IHC	1:100
CC3	Cell Signaling	9661	Rabbit	IHC	1:250
p115	BD Biosciences	612261	Mouse	WB	1:5000
β-actin	Sigma	A5441	Mouse	WB	1:5000
Phalloidin-FITC	Thermo Fisher	F432	N.A.	IHC	1:100
DAPI	Sigma	D9542-1MG	N.A.	IHC	10ug/ml

DNA constructs and molecular cloning
The following plasmids were used in this study: pH2B-GFP-SUPER (Colognato et al., 2004), pEF1-V5-HisA (invitrogen), pCDNA3.1(+) (invitrogen), pEGFP-C1(BD Biosciences, #6084-1), pCIG2 (Hand et al., 2005), PLB (Addgene, #11619)(Kissler et al., 2006). Full length *mmp12* and *ic-mmp12* cDNA was cloned into pEF1-V5-HisA at BamH1 and XhoI sites. Full length *mmp12* and *ic-mmp12* catalytic domain containing an artificial Kozak sequence and tethered with C-terminal 7xHis was cloned into pCDNA3.1(+) at BamH1 and XhoI. *ic-mmp12* cDNA was cloned into pCIG2 at XhoI and XmaI. *ic-mmp12* cDNA was cloned into pEGFP-C1 at XhoI and SpeI. shRNA was cloned into pH2B-GFP-SUPER by BglII and HindIII, into pLB by HpaI and XhoI. All cloning reagents were from NEB.

Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR)
Cells were lysed in 500µl of Qiazol (Qiagen) and stored in -80°C until RNA extraction. Total mRNA was extracted using an RNeasy Mini Kit (Qiagen) with on-column DNase treatment (Qiagen). Eluted RNA was quantified with NanoDrop 1000. A260/280>2.0, A260:230>2.0. 500-1000ng of total RNA per 20µl reaction was used for reverse transcription with Protoscript M-MuLV First Strand cDNA Synthesis Kit (NEB). qPCR was performed with Fast SYBR Green Master Mix (Applied Biosystems) with a StepOnePlus Real-Time PCR System. Between 1 and 50ng of cDNA was loaded per µL reaction, depending on target abundance.

5' Rapid Amplification of cDNA Ends (RACE)
Total RNA was extracted from *mmp12* mutant ependymal cell cultures at differentiation day 12, and 5' RACE was carried out using FirstChoice RLM-RACE Kit. PCR was carried out with Phusion High-Fidelity PCR Master Mix and cleaned with QIAquick PCR Purification Kit (Qiagen) before sequencing.

Protein purification
270 µg pCDNA3.1+-FL*mmp12*Cat-7His, pCDNA3.1+-ic*mmp12*Cat-7His were transfected into 10x15cm tissue culture dishes of 90% confluent 293T cells seeded the day before, with 810mg polyethylenimine (Polyscience), respectively. 48h later, cells were lysed with 1% Triton-X100 in 0.5x phospho-buffered saline (PBS) supplemented with 1mM Phenylmethanesulfonyl Fluoride (PMSF). Cleared cell lysate were incubated with 2.5ml Ni-NTA agarose beads (Qiagen) for 1h at 4°C with 10mM imidazole in phosphate buffer containing 300mM NaCl, PH = 8, then column washed (20mM imidazole in same buffer) and eluted (250mM imidazole in same buffer) in native conditions according to manufacturer's instructions. The eluted material was switched to PBS and concentrated to ~50µL at 4^{o}C with Amicon Ultra centrifugal filters (10kD cutoff, Millipore).

Primary lung fibroblast cell culture
Primary lung fibroblast cell culture was performed as described previously (Seluanov et al., 2010). Briefly, WT and *mmp12* mutant mice of matching age were anesthetized with isoflurane and sacrificed by cervical dislocation. Lungs were removed and cut into small pieces with a stabbing knife under a dissection hood, then digested with Trypsin-EDTA in HBSS (Sigma) for 20min and further dissociated by passing 10 times through a 1000µl micropipette tip. The dissociated cells and tissue chunks were plated on 10cm tissue culture dishes (one per mouse) in DMEM supplemented with 20%FBS and 1% pen-strep. One week later, cells were passaged 1:4 and plated in EMEM supplemented with 20%FBS, 0.584mg/ml glutamine, and 1% pen-strep. Cells were then passaged at confluency up to two times before being lysed at the same degree of confluency (70%).

Immunohistochemistry on cryostat sections
Brains from mice younger than P14 were fixed in 4% PFA overnight at 4°C. Fixed tissue was washed 3 times with PBS and cryoprotected with 30% sucrose in PBS, then frozen at -80°C in Tissue-Tek OCT Compound (Sakura). Coronal sections of 20µm were cut by cryostat (Leica CM1900) at -18^{o}C and mounted on glass slides, air-dried for 1h before being stored in -80^{o}C. Antigen retrieval was performed with Dako Cytomation Target Retrieval solution, Citrate pH 6 (Dako). Sections were blocked in 10% donkey serum in PBS with 0.5% TritonX-100 for 1h, then incubated with 1° antibodies diluted in blocking solution at 4°C overnight. After washing with PBS, sections were incubated with 2° antibodies diluted in blocking solution at room temperature for two hours.

Sections were then washed with PBS with 0.1% TritonX-100 for 3 times and then stained with DAPI for 10 min. Sections were mounted in SlowFade Gold Antifade and coverslips.

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