Cell Stem Cell, Volume *22*

Supplemental Information

Epithelial Sodium Channel Regulates Adult Neural Stem Cell Proliferation in a Flow-Dependent Manner

David Petrik, Michael H. Myoga, Sofia Grade, Niklas J. Gerkau, Melanie Pusch, Christine R. Rose, Benedikt Grothe, and Magdalena Götz

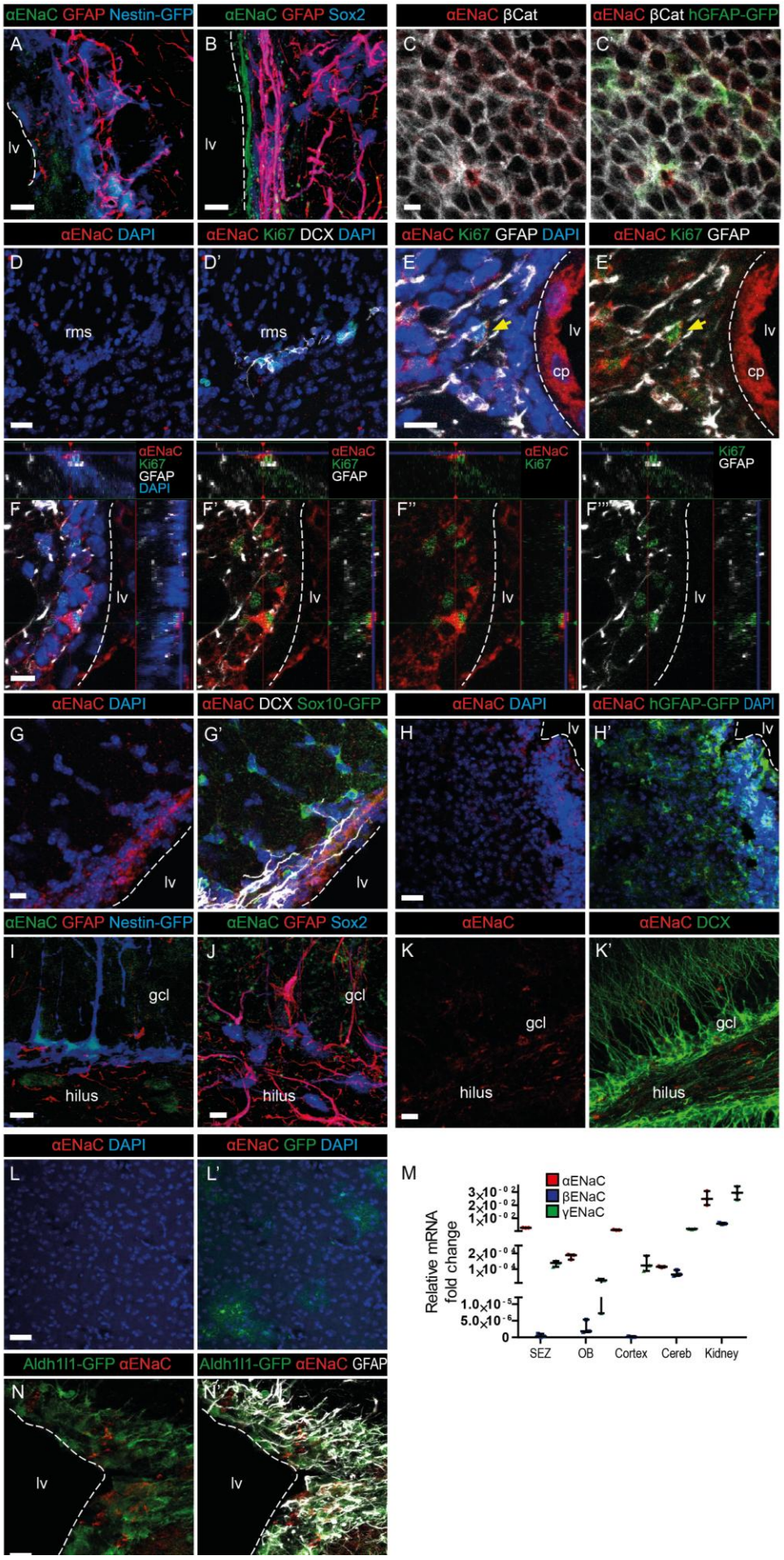
αENaC GFAP Nestin-GFP
αENaC GFAP Sox2
αENaC βCat
αENaC βCat hGFAP-GFP
A
B
C
C'
lv
αENaC DAPI
αENaC Ki67 DCX DAPI
αENaC Ki67 GFAP DAPI
αENaC Ki67 GFAP
D
D'
E
E'
rms
cp
αENaC Ki67 GFAP DAPI
αENaC Ki67 GFAP
αENaC Ki67
Ki67 GFAP
F
F'
F''
F'''
αENaC DAPI
αENaC DCX Sox10-GFP
αENaC DAPI
αENaC hGFAP-GFP DAPI
G
G'
H
H'
αENaC GFAP Nestin-GFP
αENaC GFAP Sox2
αENaC
αENaC DCX
I
J
K
K'
gcl
hilus
αENaC DAPI
αENaC GFP DAPI
L
L'
M
αENaC
βENaC
γENaC
Relative mRNA fold change
SEZ
OB
Cortex
Cereb
Kidney
Aldh1l1-GFP αENaC
Aldh1l1-GFP αENaC GFAP
N
N'

Figure S1. Related to Figure 1. Expression of αENaC in the Brain.

Representative confocal images of SEZ immunostained for αENaC, GFAP and nestin-GFP in (A) and for GFAP and Sox2 in (B); lv = lateral ventricle. (C) En-face whole-mount SEZ stained for αENaC, βCatenin and hGFAP-GFP. (D) Rostral migratory stream (RMS) stained as indicated. (E) SEZ immunostained for αENaC, Ki67 and GFAP showing a proliferating NSC positive for αENaC (arrow), cp = choroid plexus. (F) Orthogonal view of SEZ stained as in (E) showing another proliferating NSC positive for αENaC. (G) SEZ labeled for αENaC, DCX, GFP from Sox10-CreERT2/CAG-GFP mice, and DAPI. (H) SEZ and niche astrocytes from an hGFAP-GFP mouse labeled for αENaC. (I-K) Staining in the subgranular zone (SGZ) of the hippocampus. Staining for αENaC, GFAP and nestin-GFP depicting the granule cell layer (gcl) and hilus (I). Staining for αENaC and for GFAP and Sox2 (J) and for DCX (K). (L) Parenchymal astrocytes in cerebral cortex Grey Matter from a $GLAST^{WT/CreERT2}$/CAG-GFP/$ENaC^{wt/wt}$ mouse 10 DPT labeled for αENaC, GFP and DAPI. (M) Relative mRNA expression of all three ENaC subunits in cDNA from dissected tissues. (N) SEZ stained for αENaC, Aldh1l1-GFP, and GFAP. Scale bars, all 10 μm; (K) 20 μm. Animals were tested at 6-8 weeks of age, N = 3. Data are presented as median ± IQR.

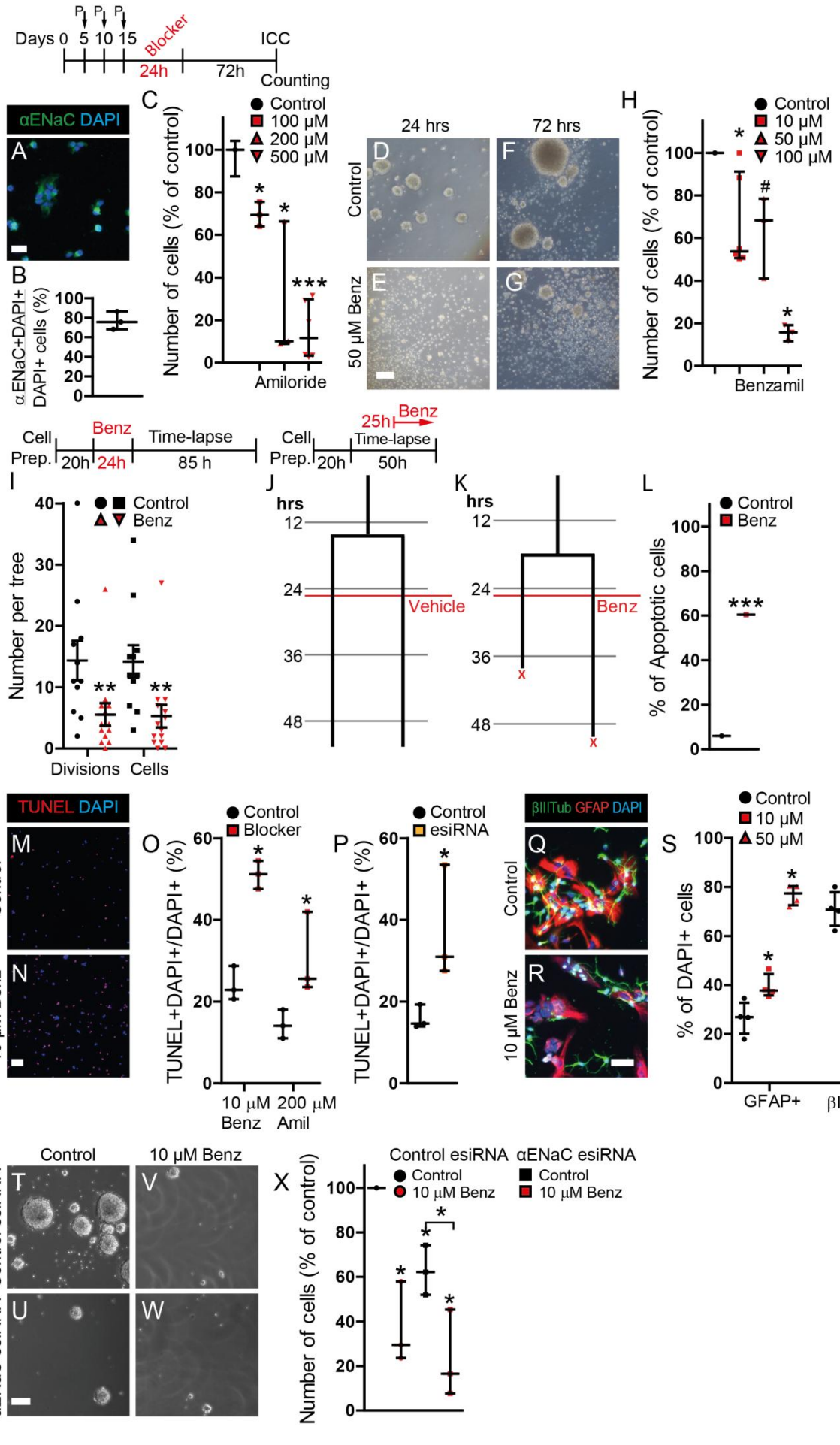
Days 0 5 10 15
Blocker
24h
72h
ICC
Counting
αENaC DAPI
A
B
αENaC+DAPI+/DAPI+ cells (%)
C
Control
100 μM
200 μM
500 μM
Number of cells (% of control)
Amiloride
24 hrs
72 hrs
D
F
E
G
Control
50 μM Benz
H
Control
10 μM
50 μM
100 μM
Benzamil
Cell Prep.
20h
Benz
24h
Time-lapse
85 h
25h
50h
I
Number per tree
Divisions
Cells
J
K
hrs
Vehicle
Benz
L
% of Apoptotic cells
TUNEL DAPI
M
N
O
Blocker
TUNEL+DAPI+/DAPI+ (%)
10 μM Benz
200 μM Amil
P
esiRNA
βIIITub GFAP DAPI
Q
R
S
% of DAPI+ cells
GFAP+
βIII-Tub+
Control esiRNA
αENaC esiRNA
T
V
U
W
X
Control esiRNA
αENaC esiRNA
Number of cells (% of control)

Figure S2. Related to Figure 2. Proliferation and Cell Death in SEZ Neurosphere-derived Cells after Blocking or Knocking-down ENaC.

(A) Immunocytochemistry for αENaC and DAPI in neurosphere-derived cells after 3 passages in proliferating medium. (B) Proportion of neurosphere-derived cells immuno-positive for αENaC. (C) Analysis of number of neurosphere-derived cells 72 hours after incubation in different concentrations of Amiloride for 24 hours normalized to control. Representative images of neurospheres in control (D,F) and after 50 μM Benzamil (E,G) at two time points. (H) Quantification of number of neurosphere-derived cells 72 hours after incubation in different concentrations of Benzamil for 24 hours. (I) Quantification of the number of cell divisions and cells per division tree from 85 hours of time-lapse imaging in control or after 24 hours incubation in different concentrations of Benzamil. Schematics of experimental protocol for time-lapse imaging of SEZ primary cells before and during application of Benzamil into medium and examples of division trees from control (J) or Benzamil (K) treated cells (time of vehicle or Benzamil depicted by a red line). (L) Quantification of percentage of dead cells from time-lapse imaging. Representative images of neurosphere-derived cells stained for TUNEL and DAPI in control (M) or after Benzamil (N). Proportional analysis of TUNEL-positive cells after exposure to blockers (O). (P) Proportion of TUNEL-positive cell transfected with control or αENaC esiRNA. Representative images of primary SEZ cells grown in differentiating conditions stained as indicated in control (Q) or after Benzamil (R). (S) Quantification of proportion of cells positive for GFAP or βIII-tubulin. (T-W) Representative images of neurospheres 72 hours after being exposed to knock-down of αENaC by esiRNA (for 4 hours) followed by treatment with Benzamil (for 24 hours). Top row: control esiRNA (T,V), bottom row: αENaC esiRNA (U,W). Left panels (T,U): neurospheres not exposed to Benzamil, right panels (V,W): Benzamil treated neurospheres. (X) Quantification of neurosphere-derived cells 72 hours after Benzamil that had been treated with control or αENAC esiRNAs. Scale bars, 20 μm (A), 100 μm (D-G, T-W), 50 μm (M-N, Q-R). Animals were tested at 6-8 weeks of age, N = 3-6. * $P < 0.05$, *** $P < 0.001$ Data are presented median ± IQR or mean ± SEM (panel I).

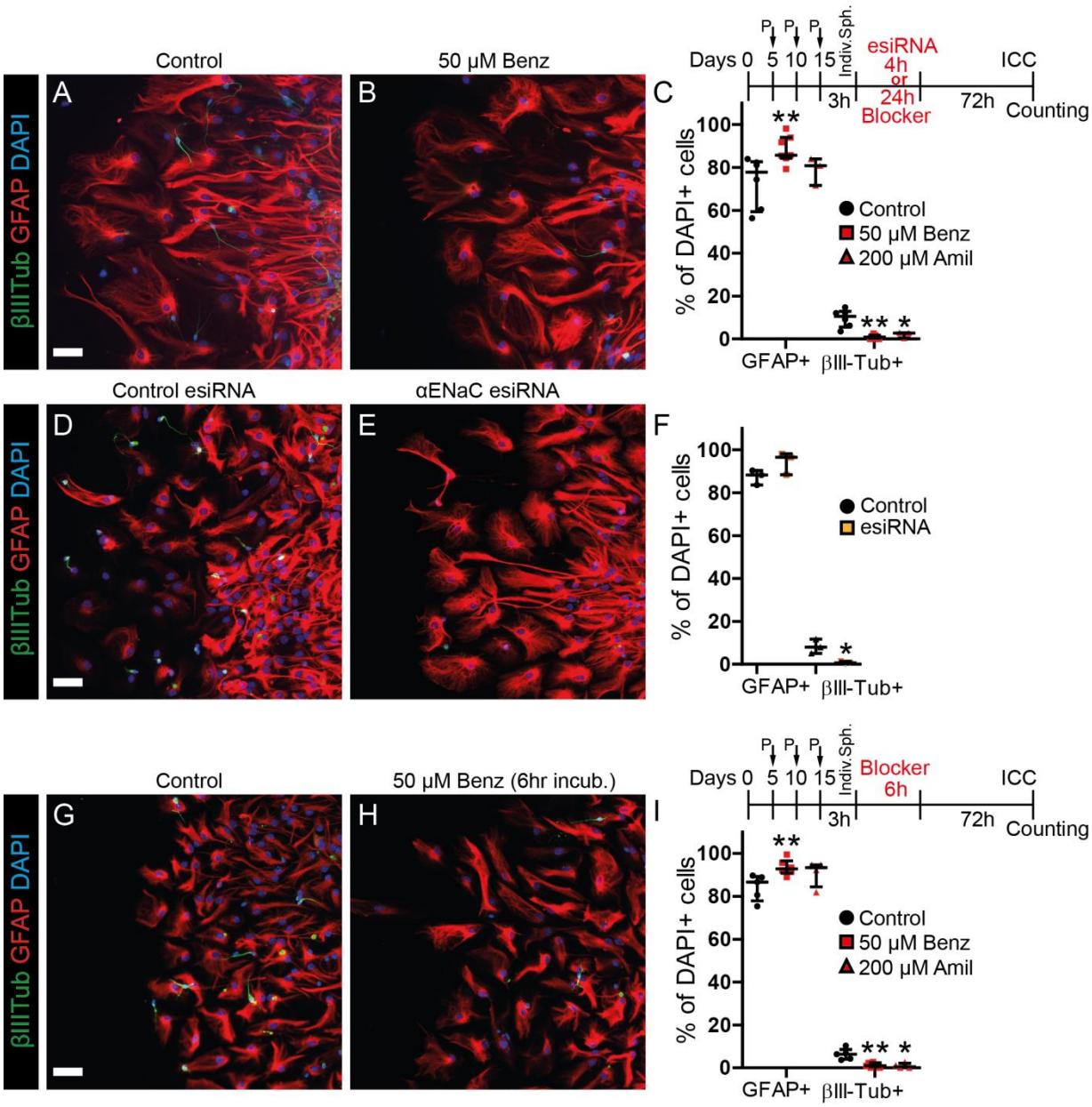

Figure S3. Related to Figure 2. Differentiation of SEZ Neurospheres after Blocking or Knocking-down ENaC.

(A-B,D-E,G-H) Representative images of cells stained for βIII-tubulin, GFAP and DAPI after the treatment indicated on top of the panels. The cells are spreading from a single SEZ neurosphere in control (A) and after Benzamil (B). (C) Schematics of experimental paradigm and proportion of GFAP and βIII-tubulin-positive cells from control or after incubation in Benzamil or Amiloride (C), after control esiRNA and αENaC esiRNA (F), after 6-hour incubation in vehicle or Benzamil or Amiloride (I). Scale bars, 20 μm. Animals were tested at 6-8 weeks of age, N = 3-6. * $P < 0.05$, ** $P < 0.01$ Data are presented as median ± IQR.

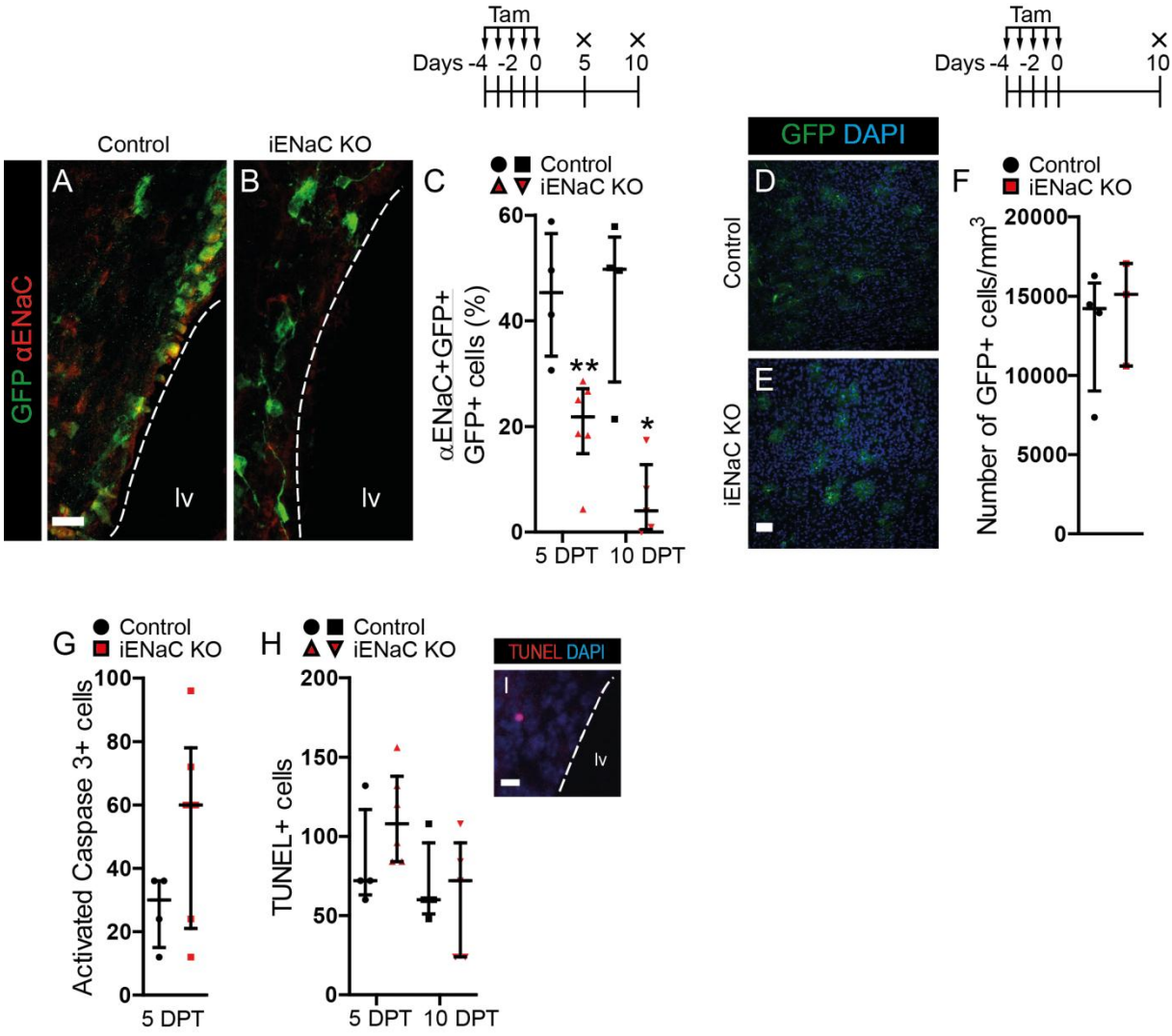

Figure S4. Related to Figure 3. Recombination and Knock-out Efficiency of iENaC KO mice 5 and 10 Days after Tamoxifen. Representative confocal images of SEZ (s.b. 20 µm) from control (A) and iENaC KO mice (B) stained for αENaC and GFP at 10 DPT (lv = lateral ventricle). (C) Quantification of proportion of αENaC+GFP+ cells among GFP+ cells in SEZ of control and iENaC KO mice 5 and 10 DPT. Representative confocal images of brain cortex (s.b. 50 µm) from control (D) and iENaC KO (E) 10 DPT. (F) Quantification of number of GFP-positive cells per mm^3 of cerebral cortex Grey Matter in control and iENaC. (G) Number of cells in SEZ positive for activated caspase 3 at 5 DPT(H) Number of TUNEL+ cells in SEZ at 5 and 10 DPT. (I) Representative image of a TUNEL+ nucleus in SEZ (s.b. 10 µm). Animals were tested at 9-10 weeks of age, N=4-6 (5 DPT), N = 4-5 (10 DPT). * $P < 0.05$ Data are presented as median ± IQR.

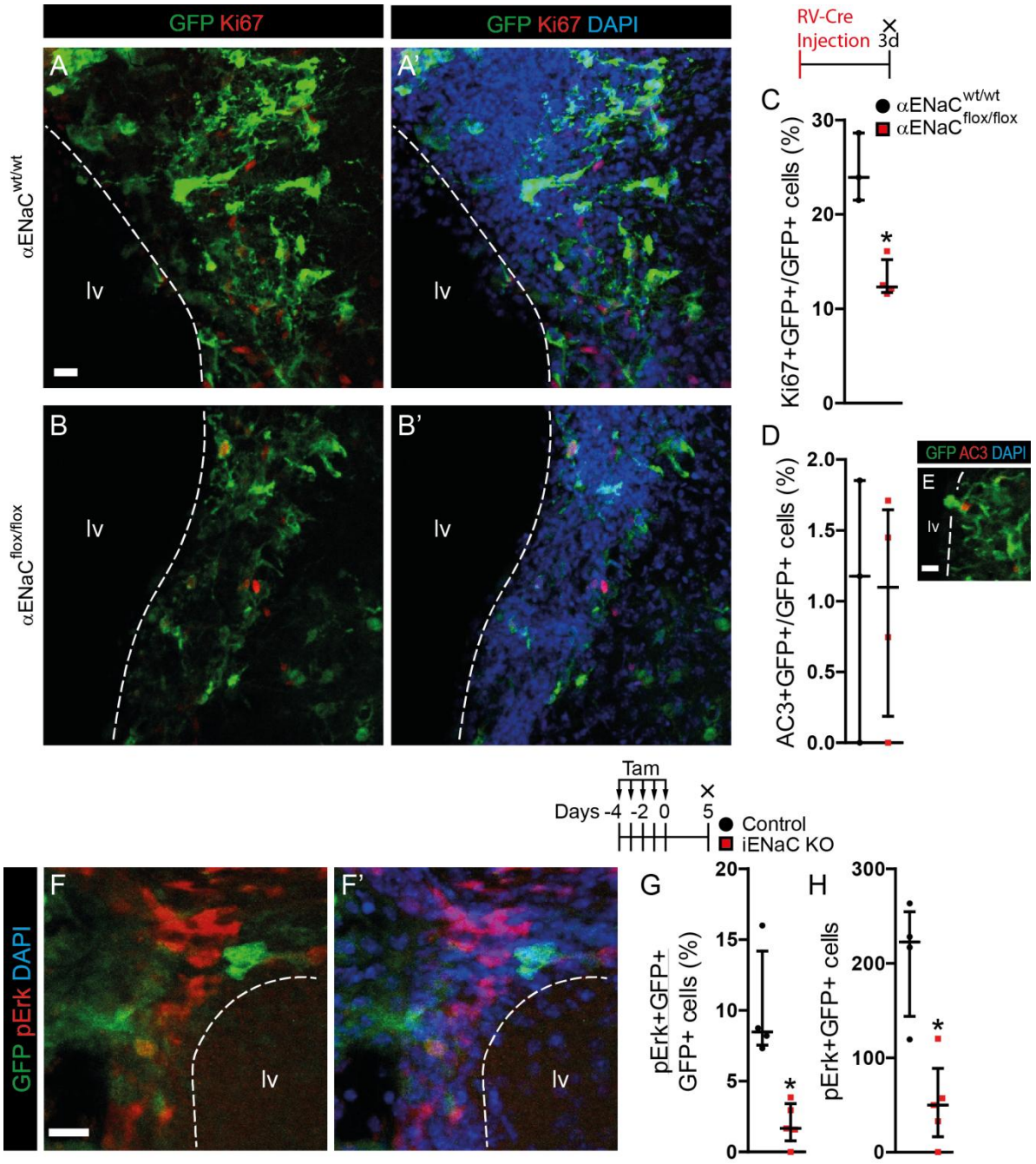

Figure S5. Related to Figure 4. Effects of Retrovirally Delivered Cre into SEZ of control and ENaC fl/fl mice.

Representative images of SEZ from αENaC$^{wt/wt}$/CAG-GFP (A) and αENaC$^{flox/flox}$/CAG-GFP (B) mice stained for GFP, Ki67 and DAPI (s.b. 20 μm, lv = lateral ventricle). (C) Experimental paradigm and quantification of proportion of GFP+ cells expressing Ki67. (D) Quantification of proportion of GFP+ cells expressing activated caspase 3. (E) A representative image of a GFP+ and AC3+ cell in SEZ (s.b. 10 μm). (F) Representative images of SEZ stained for

pErk, GFP and DAPI at 5DPT (s.b. 20 μm). Quantification of proportion (G) and absolute number (H) of pErk+GFP+ cells at 5 DPT. Animals were tested at 9-10 weeks of age, N = 3-4 (RV-Cre), N=4-6 (5 DPT). * $P < 0.05$ Data are presented median ± IQR.

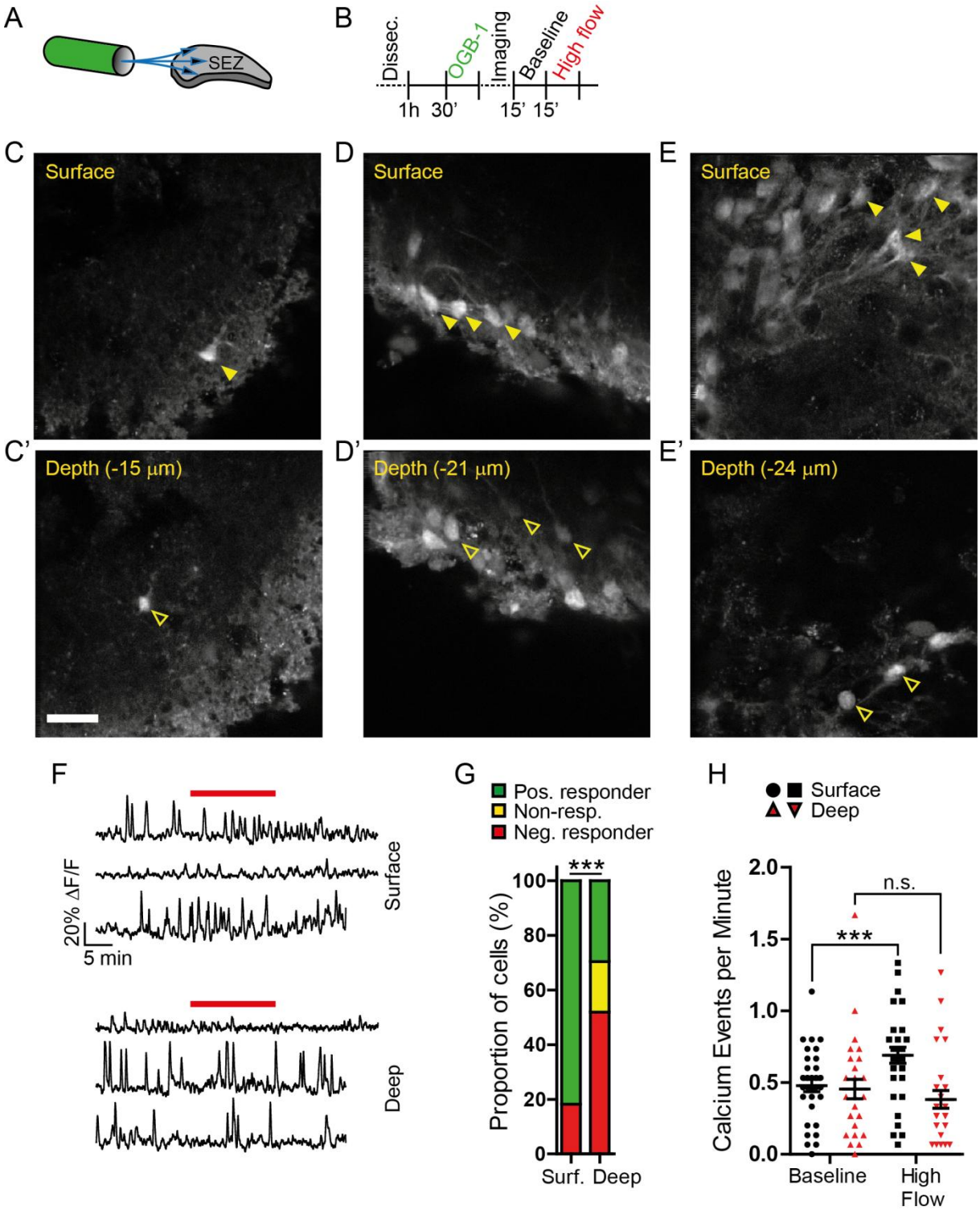

Figure S6. Related to Figure 6. Calcium Imaging in the SEZ Whole-mount Under Different Flow Conditions.

(A) Schematic representation of the SEZ whole-mount subjected to ACSF flow. (B) The experimental design for calcium imaging. Three representative images of surface (C,D,E) and deep focal planes (C',D',E') of SEZ whole-mounts from 2-photon z-stacks used for the

calcium imaging. OGB-1 loaded surface stem cells/progenitors in the surface of the lateral wall (full arrowheads) and deeper in the SEZ (empty arrowheads) are shown. (F) Representative traces of change in fluorescence (ΔF/F) as a function of time in OGB1-loaded SEZ cells from surface (top 3 traces) and deep (bottom 3 traces) cells. The red bar indicates exposure to high flow. (G) Proportion of stem cells/progenitors sorted by their responsiveness to high flow as indicated in the legend in surface and deep cells. (I) Frequency of calcium events per minute in baseline or elevated shear stress in stem cells/progenitors in surface and deeper in SEZ. Scale bar, 20 μm. Animals were tested at 7-10 weeks of age, N = 6, cells N = 27-33. *** $P < 0.001$. Data are presented as mean ± SEM.

Table S1. qPCR Primers and esiRNA, related to STAR Methods

Oligonucleotides		
Mission esiRNA for EGFP	Sigma Aldrich	Cat# EHUEGFP
Mission esiRNA for murine Scnn1a	Sigma Aldrich	Cat# EMU055921
Scnn1a Fwd: CCCTCTGTCACGATGGTCAG	Sigma Aldrich	N/A
Scnn1a Rev: TCCGGAACCTGTGCAGTAAC	Sigma Aldrich	N/A
Scnn1b Fwd: GCAGCTTCCTAAACAGCAGGTG	Sigma Aldrich	N/A
Scnn1b Rev: GCCGTGGGTGTTGGTGTTAT	Sigma Aldrich	N/A
Scnn1g Fwd: GCCAATCAGTGTGCAAGCAA	Sigma Aldrich	N/A
Scnn1g Rev: GGCCAGGTCAGTCTTGTTGA	Sigma Aldrich	N/A
GAPDH Fwd: TTCACCACCATGGAGAAGG	Sigma Aldrich	N/A
GAPDH Rev: CACACCCATCACAAACATGG	Sigma Aldrich	N/A
Flox Scnn1a Fwd: GTCACTGTGTGCACCCTTAA	Sigma Aldrich	N/A
Flox Scnn1a Rev: GCACAAAGATCTTATCCACC	Sigma Aldrich	N/A