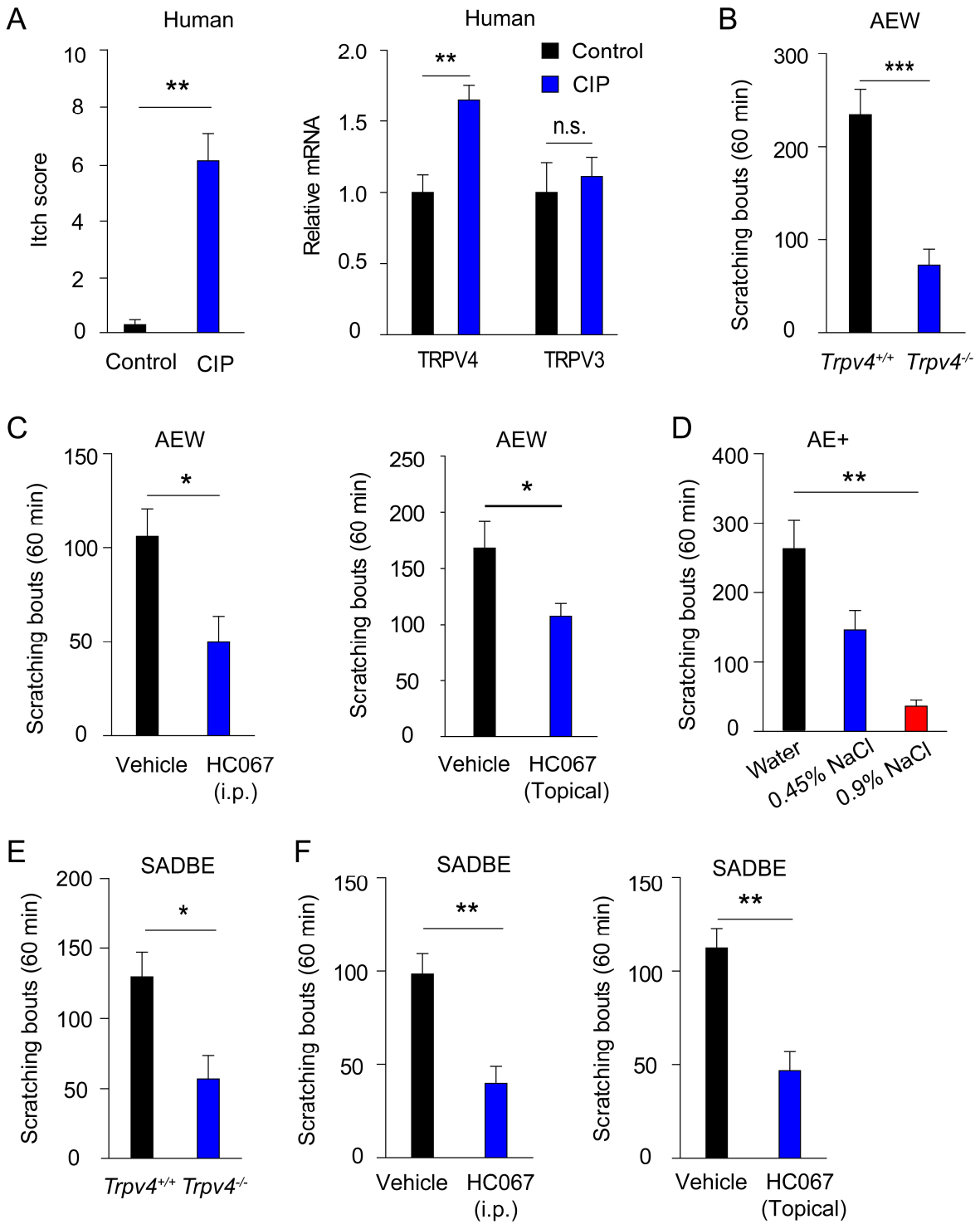
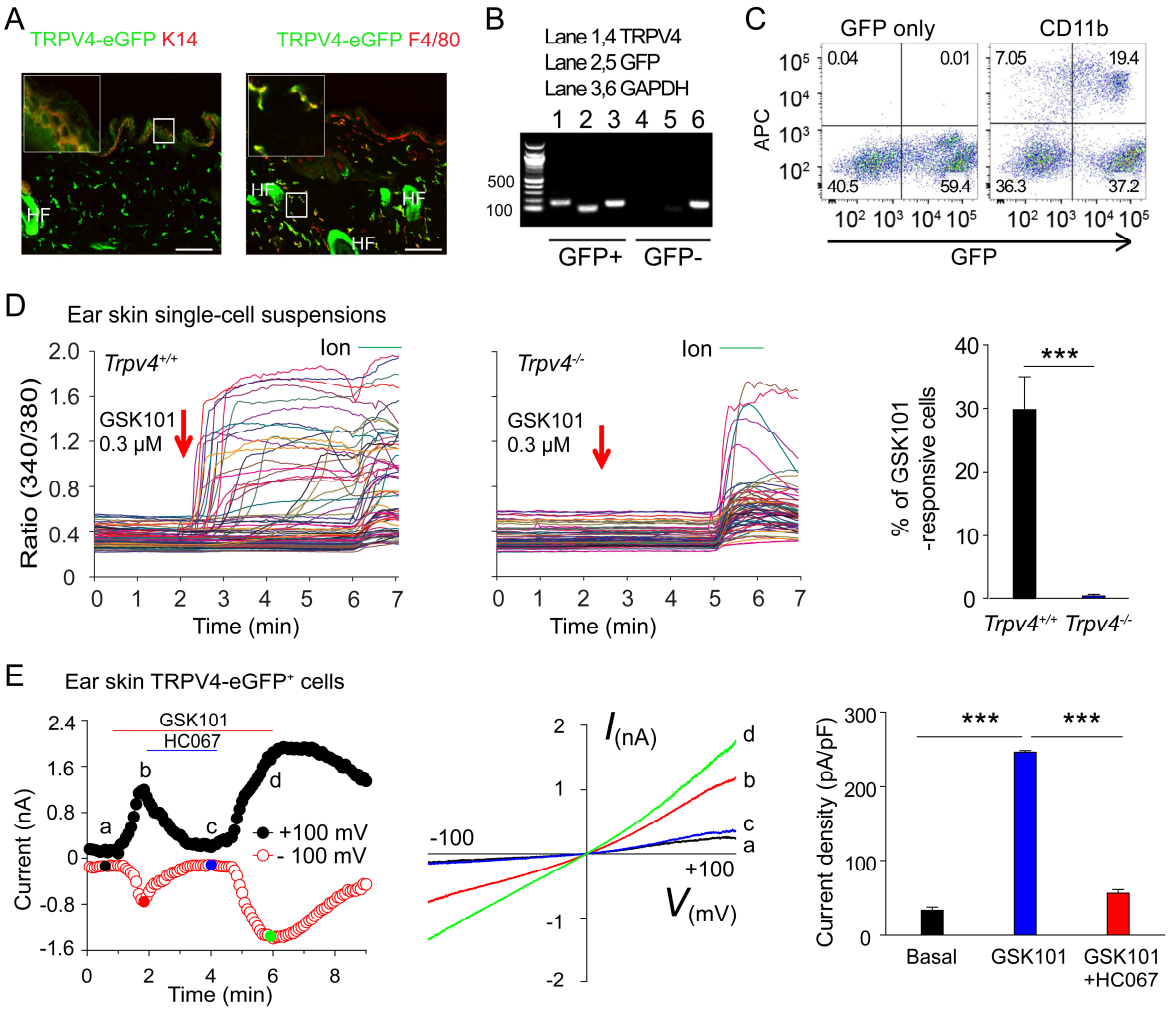
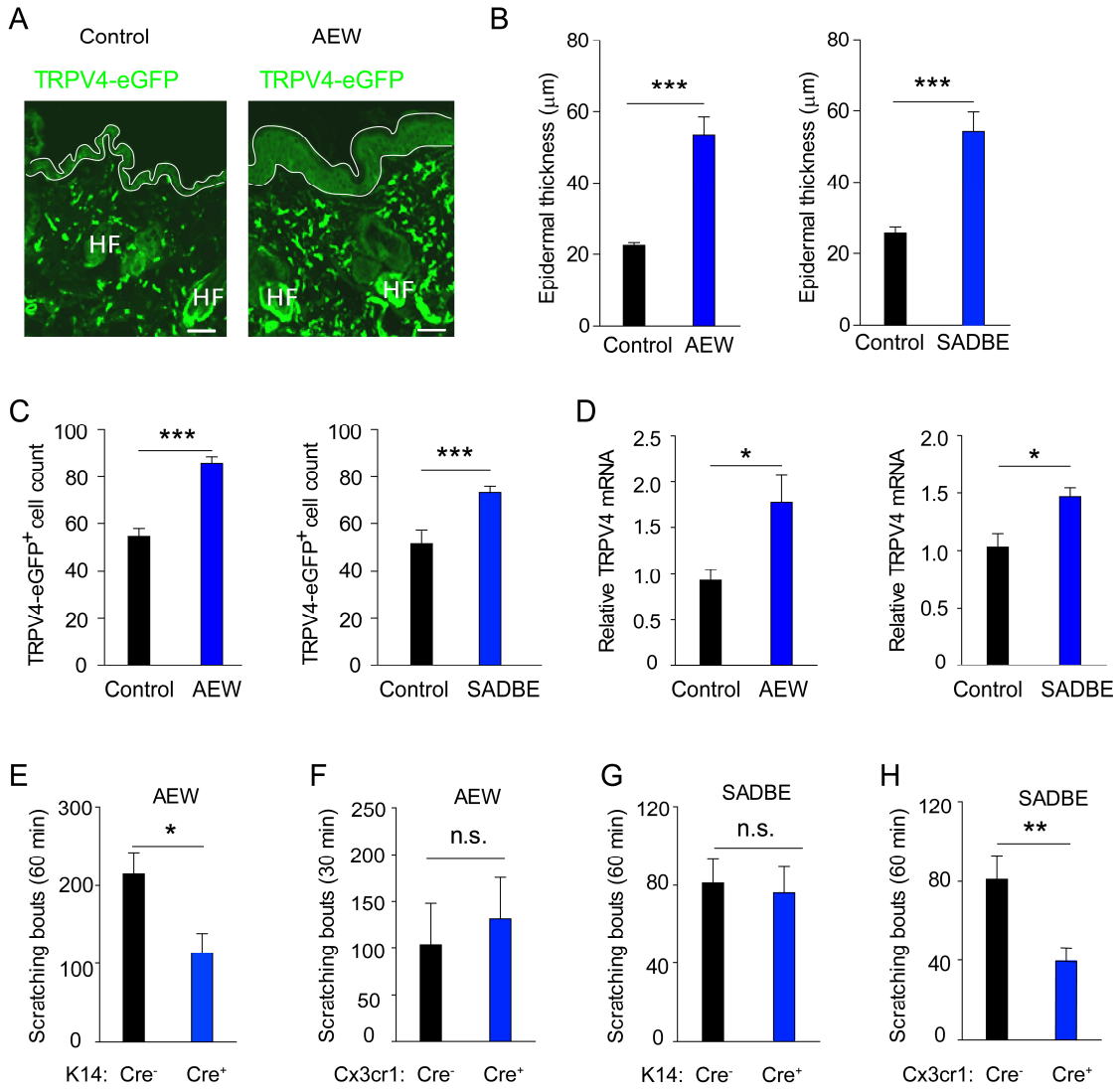
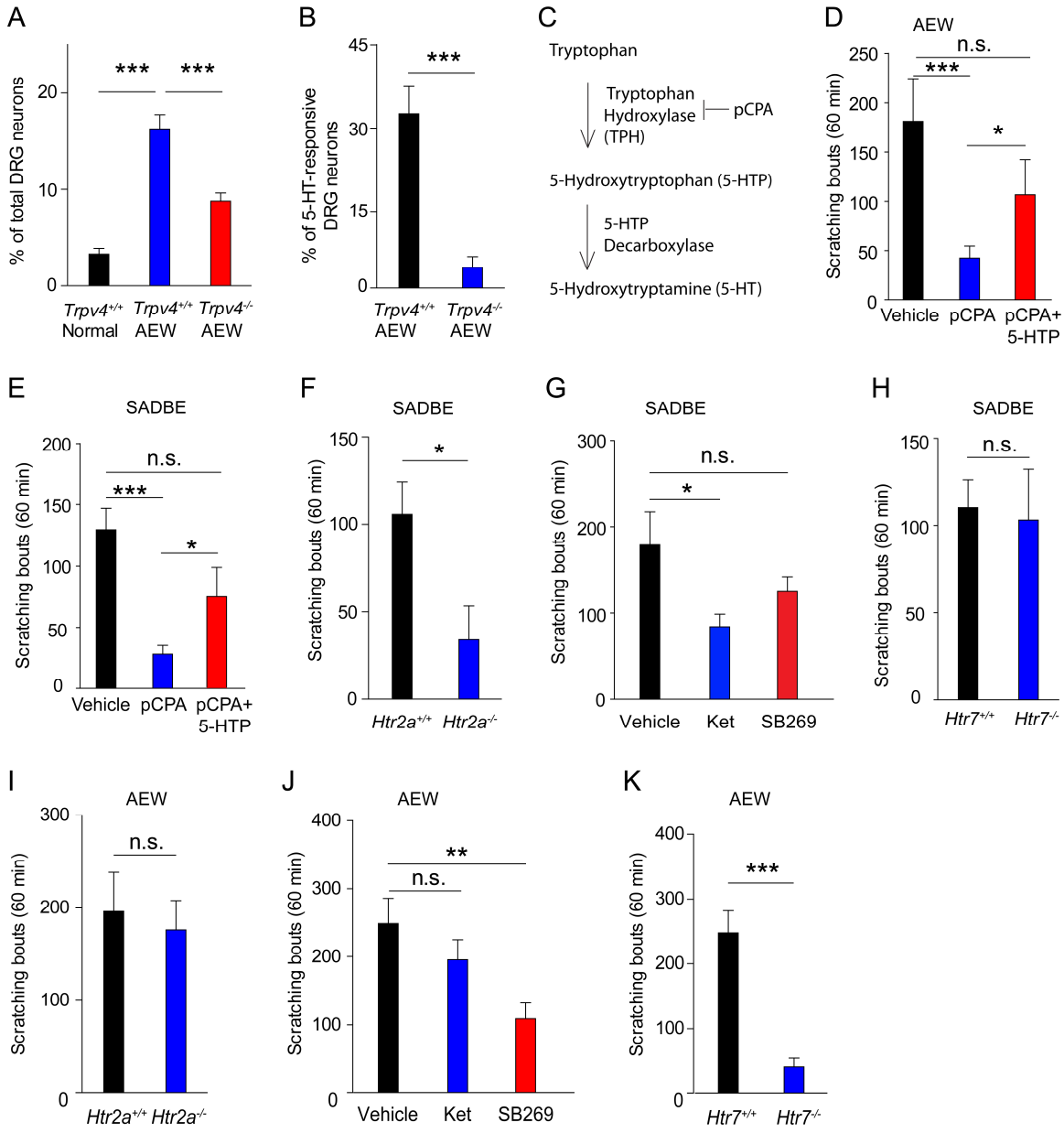
Table E1 Primary antibodies used for immunofluorescent staining and flow cytometry

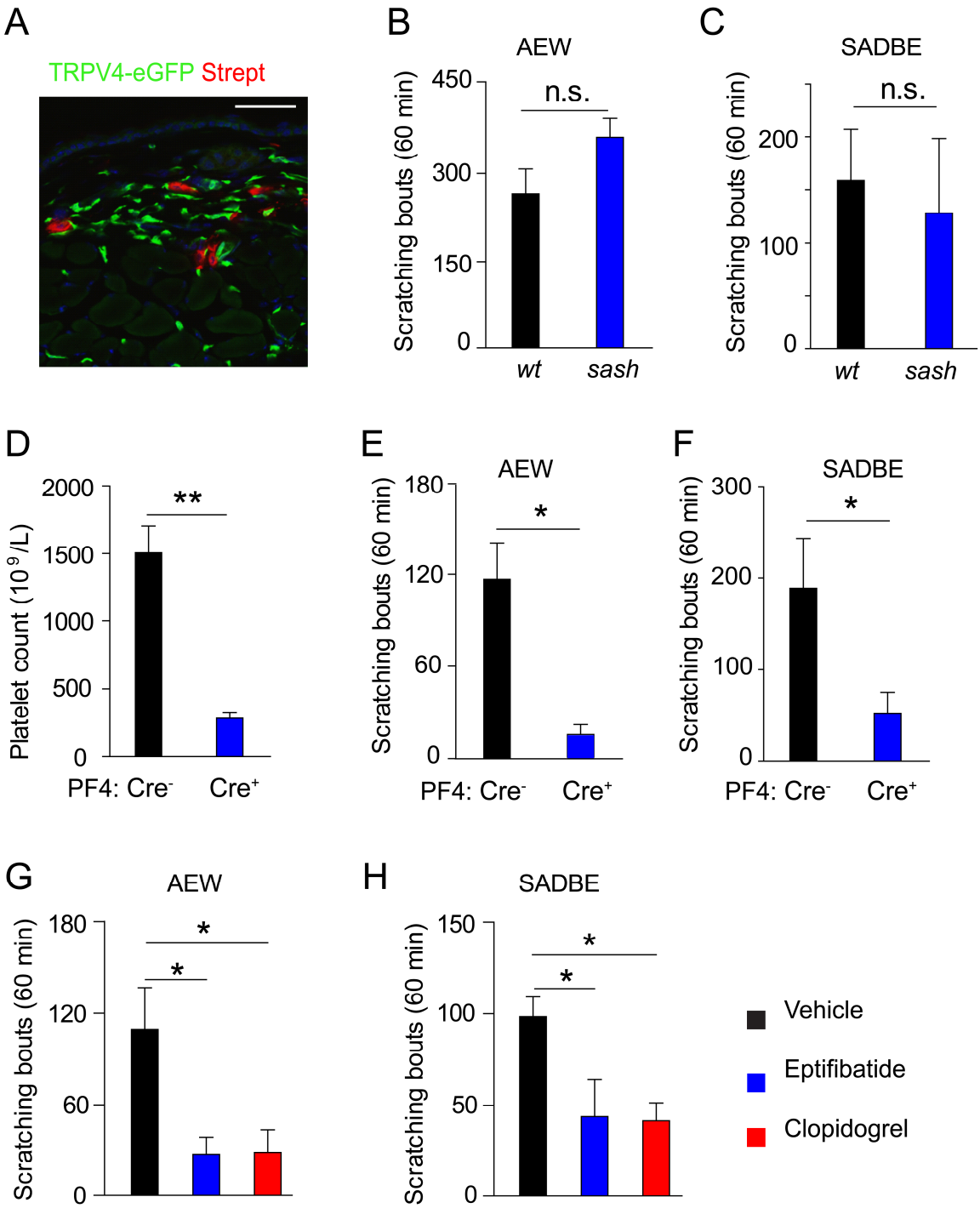
Antibody	Host species	Dilution	Source/cat. #
GFP	Chicken	1:500	Aves Labs Inc Cat #: GFP-1020
Purified anti-K14	Rabbit polyclone	1:500	Covance Cat #: PRB-155P
Purified anti-F4/80	Rat (clone # BM8)	1:500	Biolegend Cat #: 123101
Purified anti-CD11b	Rat (clone # 5C6)	1:500	AbD serotec Cat #: MCA711GT
Purified anti-CD206	Rat (clone # MR5D3)	1:500	AbD serotec Cat #: MCA2235T
Purified anti-CD68	Rat (clone # FA-11)	1:200	Biolegend Cat #: 137001
Purified anti-CD163	Rabbit polyclone	1:100	Santa cruz Cat #: sc-33560
Rhodamine conjugated Avidin	N/A	1:1000	Rockland antibodies & assays Cat #: A003-00
APC anti-CD11b	Rat (clone # M1/70)	1:300	eBioscience Cat #: 17-0112
PE anti-CD11c	Hamster (clone # N418)	1:300	Biolegend Cat #: 117307
PE anti-CCR2	Rat (clone # 475301)	1:300	R&D Systems Cat #: FAB5538P
PerCP Cy5.5 anti-CD3e	Hamster (clone # 145-2C11)	1:300	eBioscience Cat #: 45-0031
PerCP anti-B220	Rat (clone RA3-6B2)	1:300	BD Pharmingen™ Cat #: 553933

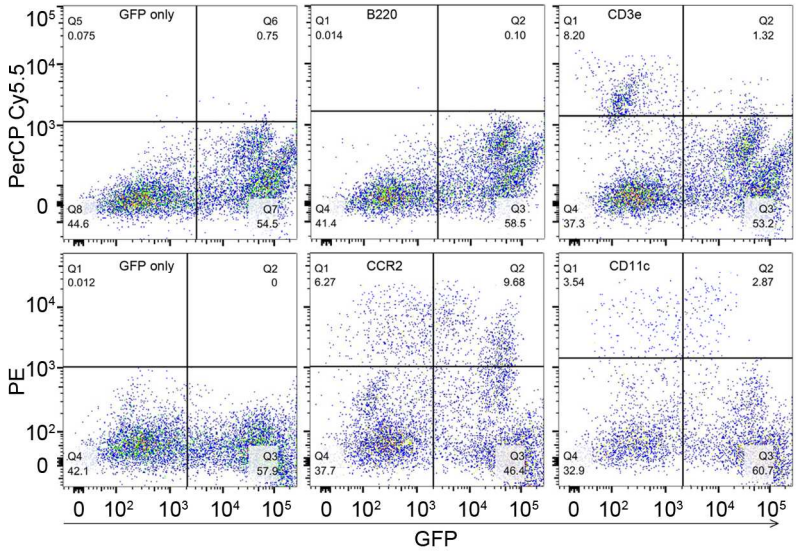

A
Human
Itch score
10
8
6
4
2
0
**
Control
CIP
Human
Relative mRNA
2.0
1.5
1.0
0.5
0
**
n.s.
Control
CIP
TRPV4
TRPV3
B
AEW
Scratching bouts (60 min)
300
200
100
0
***
Trpv4+/+
Trpv4-/-
C
AEW
Scratching bouts (60 min)
150
100
50
0
*
Vehicle
HC067
(i.p.)
AEW
Scratching bouts (60 min)
250
200
150
100
50
0
*
Vehicle
HC067
(Topical)
D
AE+
Scratching bouts (60 min)
400
300
200
100
0
**
Water
0.45% NaCl
0.9% NaCl
E
SADBE
Scratching bouts (60 min)
200
150
100
50
0
*
Trpv4+/+
Trpv4-/-
F
SADBE
Scratching bouts (60 min)
150
100
50
0
**
Vehicle
HC067
(i.p.)
SADBE
Scratching bouts (60 min)
150
100
50
0
**
Vehicle
HC067
(Topical)

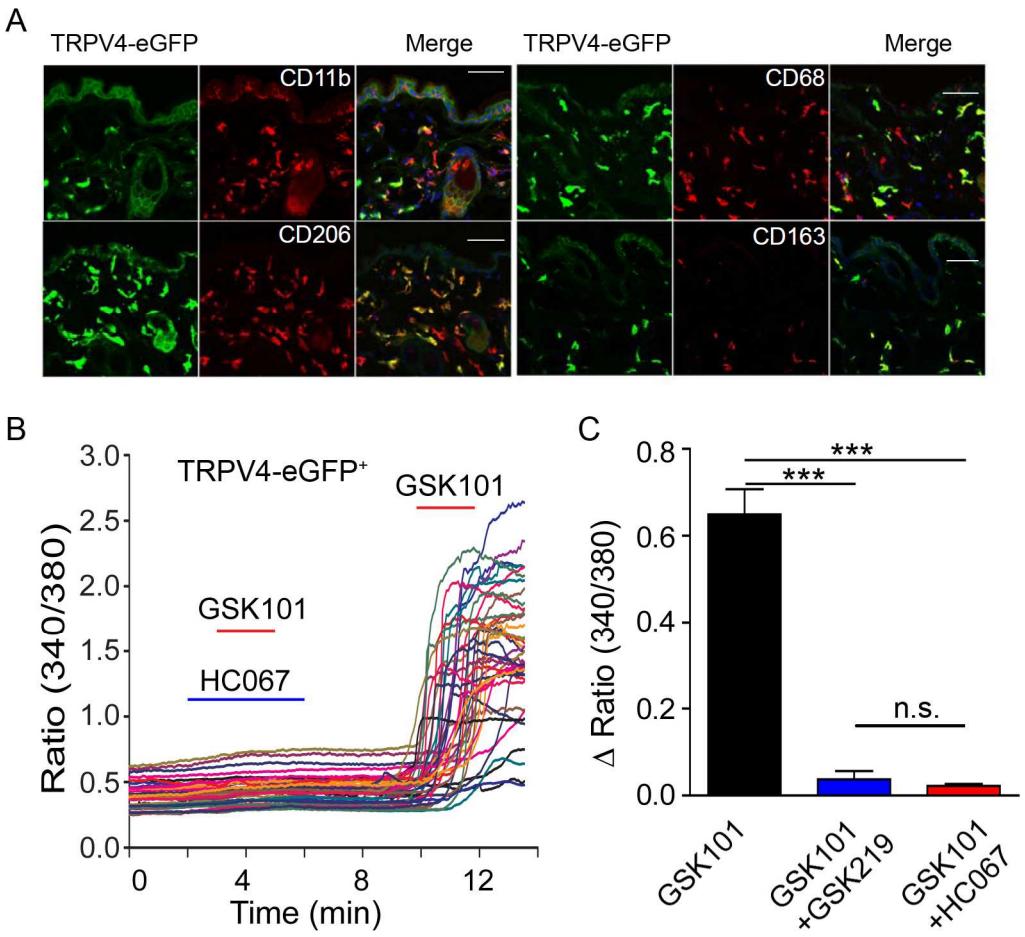

A
TRPV4-eGFP K14
TRPV4-eGFP F4/80
HF
B
Lane 1,4 TRPV4
Lane 2,5 GFP
Lane 3,6 GAPDH
1 2 3 4 5 6
500
100
GFP+ GFP-
C
GFP only
CD11b
APC
0.04
0.01
40.5
59.4
7.05
19.4
36.3
37.2
GFP
D
Ear skin single-cell suspensions
Trpv4+/+
Ion
GSK101
0.3 μM
Ratio (340/380)
Time (min)
Trpv4-/-
% of GSK101 -responsive cells
***
E
Ear skin TRPV4-eGFP+ cells
GSK101
HC067
+100 mV
- 100 mV
Current (nA)
I(nA)
V(mV)
-100
+100
Current density (pA/pF)
Basal
GSK101
GSK101 +HC067

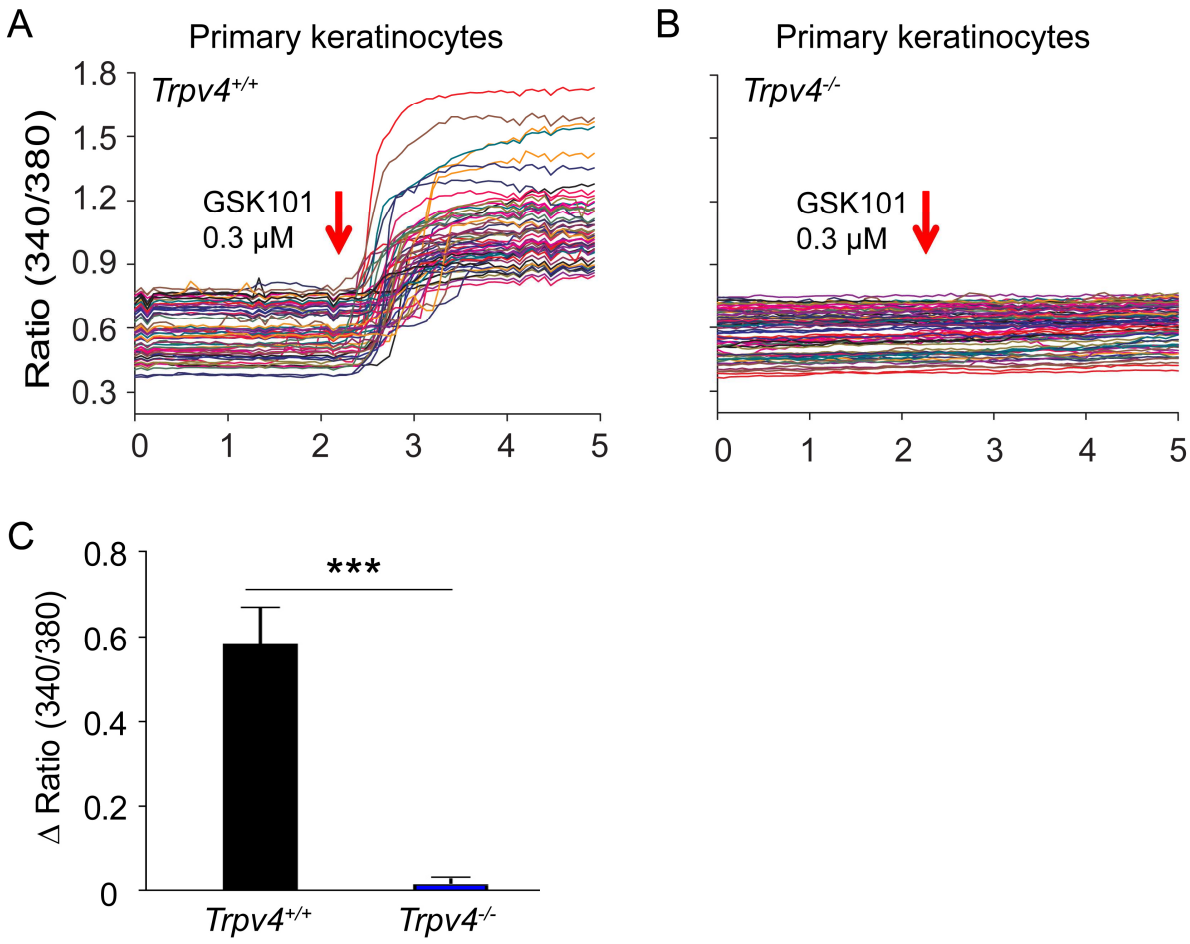

A
Control
AEW
TRPV4-eGFP
TRPV4-eGFP
HF
HF
HF
HF
B
Epidermal thickness (μm)
***
Control AEW
Epidermal thickness (μm)
***
Control SADBE
C
TRPV4-eGFP+ cell count
***
Control AEW
TRPV4-eGFP+ cell count
***
Control SADBE
D
Relative TRPV4 mRNA
*
Control AEW
Relative TRPV4 mRNA
*
Control SADBE
E
AEW
Scratching bouts (60 min)
*
K14: Cre- Cre+
F
AEW
Scratching bouts (30 min)
n.s.
Cx3cr1: Cre- Cre+
G
SADBE
Scratching bouts (60 min)
n.s.
K14: Cre- Cre+
H
SADBE
Scratching bouts (60 min)
**
Cx3cr1: Cre- Cre+

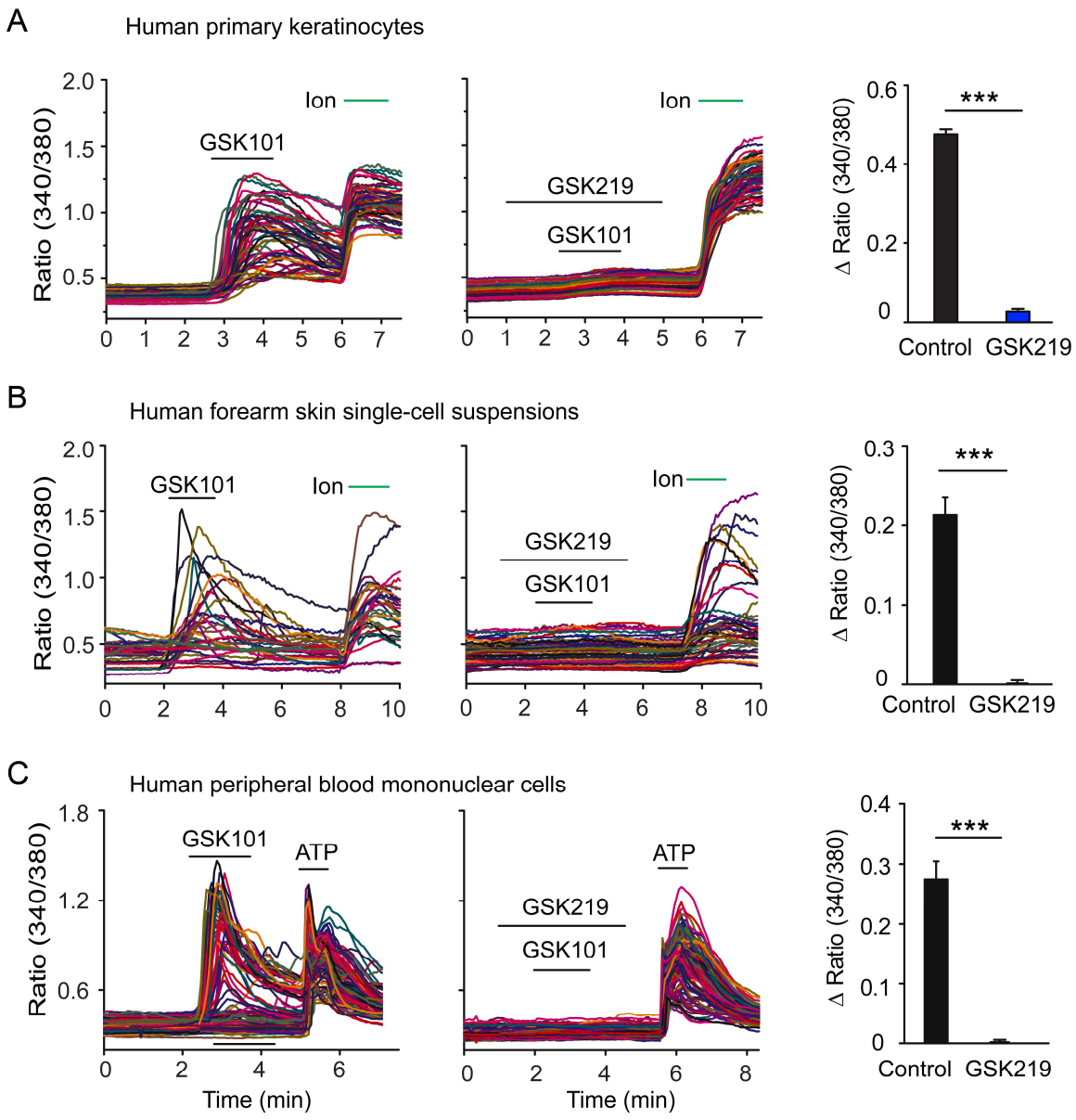

A
% of total DRG neurons
***
***
Trpv4+/+ Normal
Trpv4+/+ AEW
Trpv4-/- AEW
B
% of 5-HT-responsive DRG neurons
***
Trpv4+/+ AEW
Trpv4-/- AEW
C
Tryptophan
Tryptophan Hydroxylase (TPH)
pCPA
5-Hydroxytryptophan (5-HTP)
5-HTP Decarboxylase
5-Hydroxytryptamine (5-HT)
D
AEW
n.s.
***
*
Scratching bouts (60 min)
Vehicle
pCPA
pCPA+ 5-HTP
E
SADBE
n.s.
***
*
Scratching bouts (60 min)
Vehicle
pCPA
pCPA+ 5-HTP
F
SADBE
*
Scratching bouts (60 min)
Htr2a+/+
Htr2a-/-
G
SADBE
n.s.
*
Scratching bouts (60 min)
Vehicle
Ket
SB269
H
SADBE
n.s.
Scratching bouts (60 min)
Htr7+/+
Htr7-/-
I
AEW
n.s.
Scratching bouts (60 min)
Htr2a+/+
Htr2a-/-
J
AEW
**
n.s.
Scratching bouts (60 min)
Vehicle
Ket
SB269
K
AEW
***
Scratching bouts (60 min)
Htr7+/+
Htr7-/-

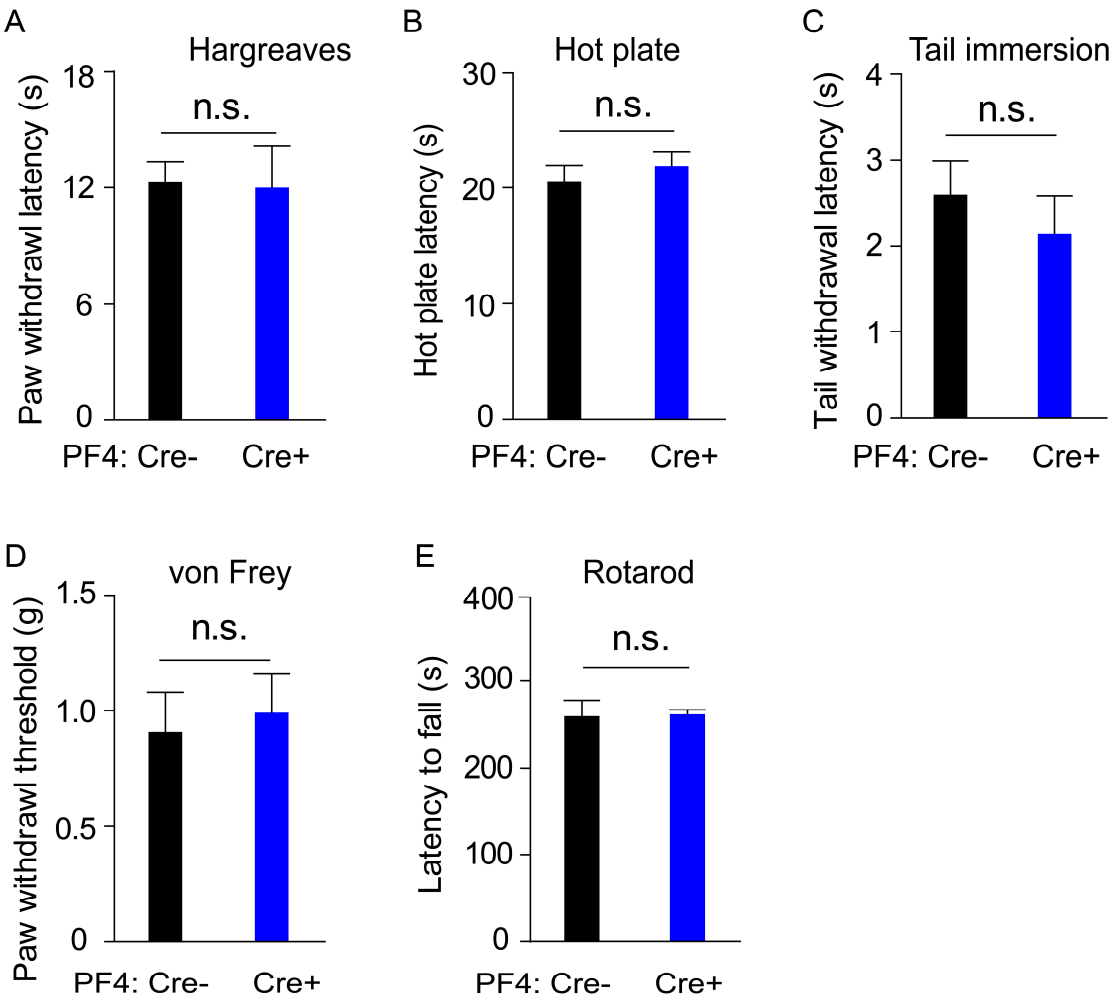

A
TRPV4-eGFP Strept
B
AEW
Scratching bouts (60 min)
450
300
150
0
n.s.
wt
sash
C
SADBE
Scratching bouts (60 min)
200
100
0
n.s.
wt
sash
D
Platelet count (10^9/L)
2000
1500
1000
500
0
**
PF4: Cre-
Cre+
E
AEW
Scratching bouts (60 min)
180
120
60
0
*
PF4: Cre-
Cre+
F
SADBE
Scratching bouts (60 min)
300
200
100
0
*
PF4: Cre-
Cre+
G
AEW
Scratching bouts (60 min)
180
120
60
0
*
*
H
SADBE
Scratching bouts (60 min)
150
100
50
0
*
*
Vehicle
Eptifibatide
Clopidogrel

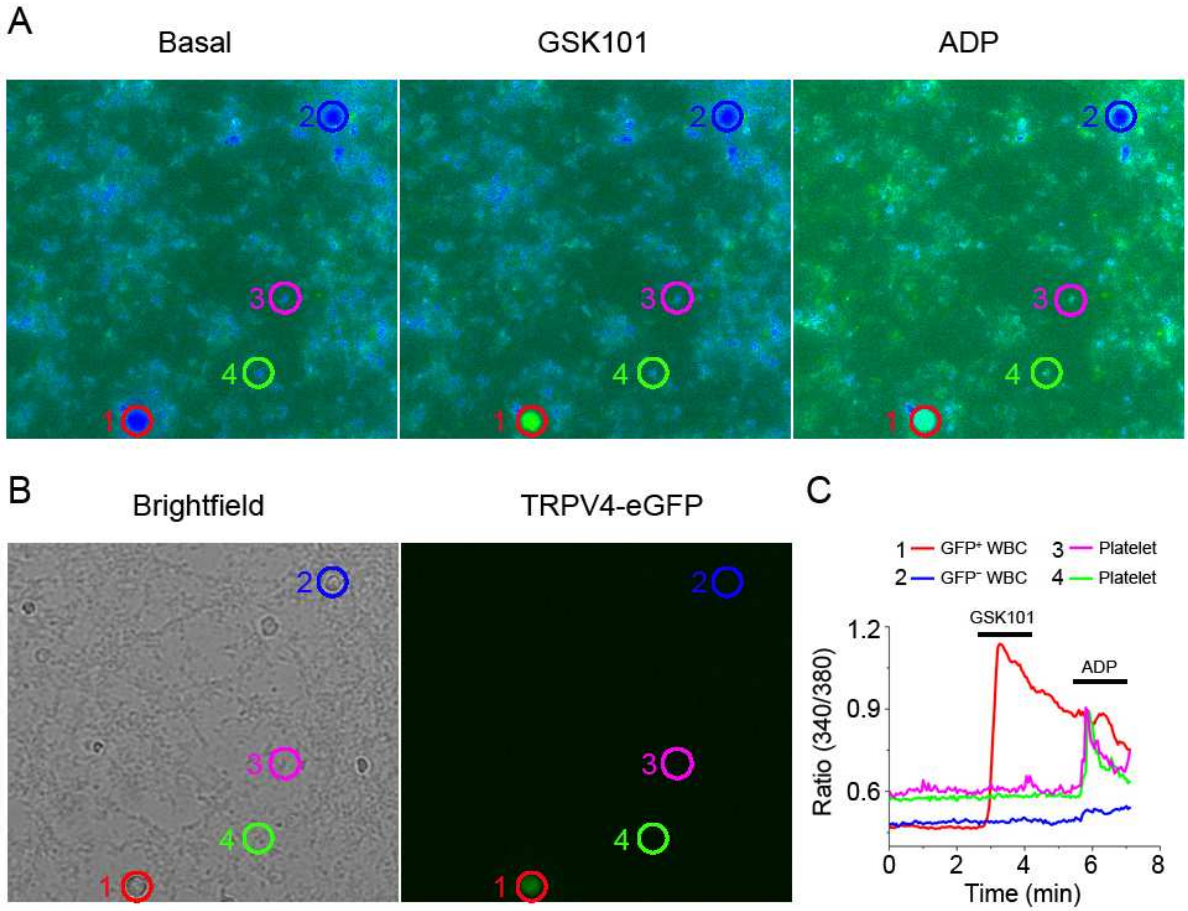

GFP only
Q5
0.075
Q6
0.75
Q8
44.6
Q7
54.5
B220
Q1
0.014
Q2
0.10
Q4
41.4
Q3
58.5
CD3e
Q1
8.20
Q2
1.32
Q4
37.3
Q3
53.2
GFP only
Q1
0.012
Q2
0
Q4
42.1
Q3
57.9
CCR2
Q1
6.27
Q2
9.68
Q4
37.7
Q3
46.4
CD11c
Q1
3.54
Q2
2.87
Q4
32.9
Q3
60.7
PerCP Cy5.5
PE
GFP
0
10²
10³
10⁴
10⁵


A
TRPV4-eGFP
Merge
CD11b
CD206
TRPV4-eGFP
Merge
CD68
CD163
B
TRPV4-eGFP+
GSK101
GSK101
HC067
Ratio (340/380)
Time (min)
C
Δ Ratio (340/380)
***
***
n.s.
GSK101
GSK101 +GSK219
GSK101 +HC067


A
Primary keratinocytes
Trpv4+/+
GSK101
0.3 μM
Ratio (340/380)
1.8
1.5
1.2
0.9
0.6
0.3
0
1
2
3
4
5
B
Primary keratinocytes
Trpv4-/-
GSK101
0.3 μM
0
1
2
3
4
5
C
***
Δ Ratio (340/380)
0.8
0.6
0.4
0.2
0
Trpv4+/+
Trpv4-/-


A
Human primary keratinocytes
Ratio (340/380)
Ion
GSK101
GSK219
Δ Ratio (340/380)
***
Control
GSK219
B
Human forearm skin single-cell suspensions
C
Human peripheral blood mononuclear cells
ATP
Time (min)


A
Hargreaves
Paw withdrawl latency (s)
18
12
6
0
n.s.
PF4: Cre-
Cre+
B
Hot plate
Hot plate latency (s)
30
20
10
0
n.s.
PF4: Cre-
Cre+
C
Tail immersion
Tail withdrawal latency (s)
4
3
2
1
0
n.s.
PF4: Cre-
Cre+
D
von Frey
Paw withdrawl threshold (g)
1.5
1.0
0.5
0
n.s.
PF4: Cre-
Cre+
E
Rotarod
Latency to fall (s)
400
300
200
100
0
n.s.
PF4: Cre-
Cre+


A
Basal
GSK101
ADP
2
3
4
1
B
Brightfield
TRPV4-eGFP
C
1 GFP+ WBC
2 GFP- WBC
3 Platelet
4 Platelet
GSK101
ADP
Ratio (340/380)
1.2
0.9
0.6
0
2
4
6
8
Time (min)

Supplementary Table:

Table E1.

Primary antibodies used for immunofluorescent staining and flow cytometry.

Supplemental Figure Legends:

Figure E1

TRPV4-eGFP is expressed by dermal macrophages but not T or B cells. Flow cytometry analysis using ear skin single-cell suspensions from the *Trpv4*^{eGFP} mice reveals that TRPV4-eGFP is expressed by a subpopulation of CCR2^{+} or CD11c^{+} macrophages but not by the CD3e^{+} T cells or B220^{+} B cells. The experiment was repeated 4 times.

Figure E2

Expression of cellular markers for macrophages and dermal macrophages in the skin of *Trpv4*^{eGFP} mice. **A,** Double labeling experiments show that CD11b, CD206, CD68, and CD163 were co-expressed with the TRPV4-eGFP^{+} cells in the skin. Bar=50 μm. **B** and **C,** Representative traces (Fig E2, B) and summarized data (Fig E2, C) illustrate that 0.3 μM GSK101 elicited a $[Ca^{2+}]_i$ response in freshly isolated TRPV4-eGFP^{+} ear skin single cell suspensions, which was inhibited by 0.3 μM GSK219 or 5 μM HC067. ***p<0.001, ANOVA; n=11 coverslips for GSK101 and 5 coverslips for GSK219 and HC067.

Figure E3

Functional expression of TRPV4 in primary cultured mouse kereatinocytes. **A** and **B,** Representative traces showing the GSK101-elicited $[Ca^{2+}]_i$ responses in cultured keratinocytes from *Trpv4*$^{+/+}$ (Fig E3, A) and *Trpv4*$^{-/-}$ (Fig E3, B) mice. **C,** Summarized data showing the averaged response evoked by GSK101 in cultured keratinocytes from the *Trpv4*$^{+/+}$ and *Trpv4*$^{-/-}$ mice. ***p<0.001, Student's *t* test; n=5 coverslips in each group.

Figure E4

GSK101 elicits $[Ca^{2+}]_i$ responses in human primary keratinocytes, forearm skin cell suspensions, and human peripheral blood mononuclear cells. **A-C,** Representative traces showing the GSK101 (0.3 μM)-elicited $[Ca^{2+}]_i$ response in the absence (left) and presence (middle) of GSK219 (0.3 μM) in human primary keratinocytes (Fig E4, A), human forearm skin cell suspensions (Fig E4, B), and human peripheral blood mononuclear cells (Fig E4, C). The bar graphs on the right show that GSK219 abolished the GSK101 responses in all cell types tested. *** p<0.001, Student's *t* test; n=5 coverslips in each group. Ionomycin (Ion) and ATP were used as positive controls.

Figure E5

Conditional depletion of platelets does not affect thermal and mechanical sensations and motor function in mice. **A-E,** Paw withdraw latency (Fig E5, A), Hot plate latency (Fig E5, B), Tail withdraw latency (Fig E5, C), Paw withdraw threshold (Fig E5, D), and the latency to fall (Fig E5, E) in the *Pf4-Cre*$^{-}$ mice (n=5 mice) mice were not significantly different from that of the *Pf4-Cre*$^{+}$ mice (n=6 mice). p>0.05, Student's *t* test. n.s. not significant versus *Pf4-Cre*$^{-}$ group.

Figure E6

TRPV4 is not functionally expressed by platelets. **A,** Representative images showing the $[Ca^{2+}]_i$ response elicited by 0.3 μM GSK101 and 100 μM ADP. Cell number 1 represents a GFP-positive white blood cell. Cell number 2 represents a GFP-negative white blood cell. Cells number 3 and 4 represent platelets. **B,** Brightfield and GFP images show that TRPV4-eGFP is present in a white blood cell (cell number 1) but not in platelets. **C,** Representative traces show that GSK101 elicited a $[Ca^{2+}]_i$ response in the GFP^+ white blood cell (cell number 1) but not the GFP^- white blood cell (cell number 2) or platelets (cells number 3 and 4). ADP serves as a positive control. The same experiment was repeated at least 3 times.