nature biomedical engineering

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41551-018-0328-5

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Interleukin-31-mediated photoablation of pruritogenic epidermal neurons reduces itch-associated behaviours in mice

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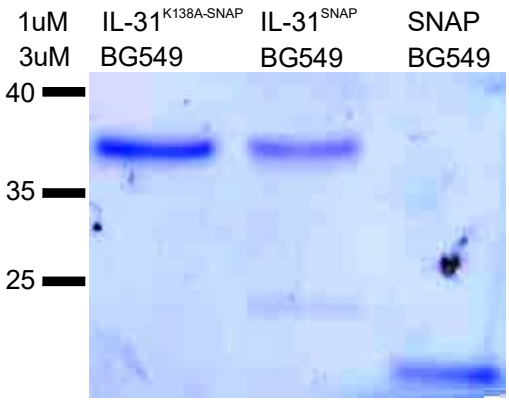
Supplementary Figure 1

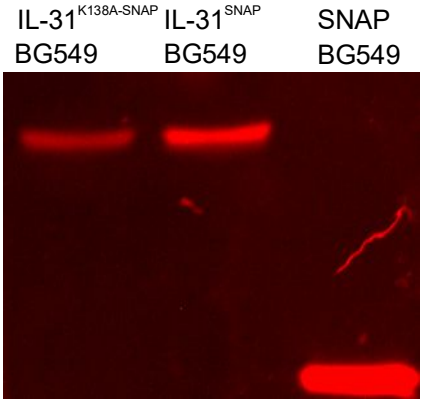
a

	1	2	3	Average	Conc. M	Conc mM	% of SNAP-activity
	~280 nm	~280 nm	~280 nm				
IL31$^{SNAP-K138A}$	0.007	0.011	0.007	0.00833333	0.000001964	1.96355639	28.80%
IL31^{SNAP}	0.004	0.007	0.004	0.005	0.000001178	1.17813384	35.37%
SNAP	0.007	0.013	0.01	0.01	0.000004740	4.74045982	79.11%
	~550 nm	~550 nm	~550 nm				
IL31$^{SNAP-K138A}$	0.006	0.007	0.006	0.00633333	0.000000565	0.56547619	
IL31^{SNAP}	0.004	0.005	0.005	0.00466667	0.000000417	0.41666667	
SNAP	0.039	0.046	0.041	0.042	0.000003750	3.75	

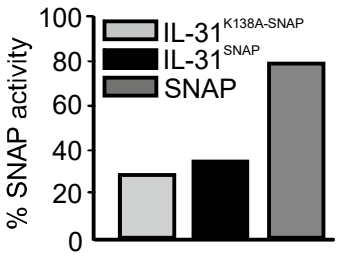
b



c

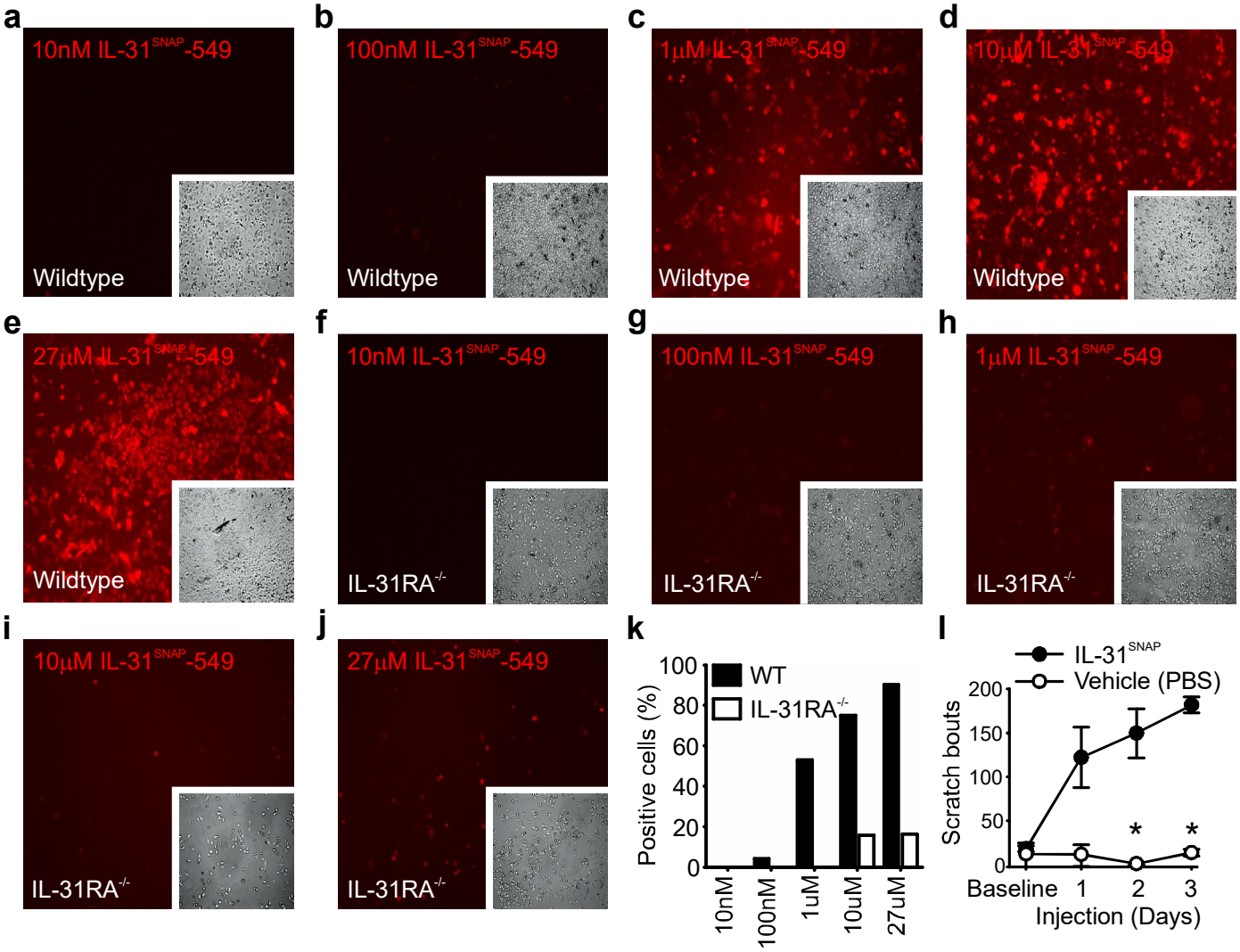


d



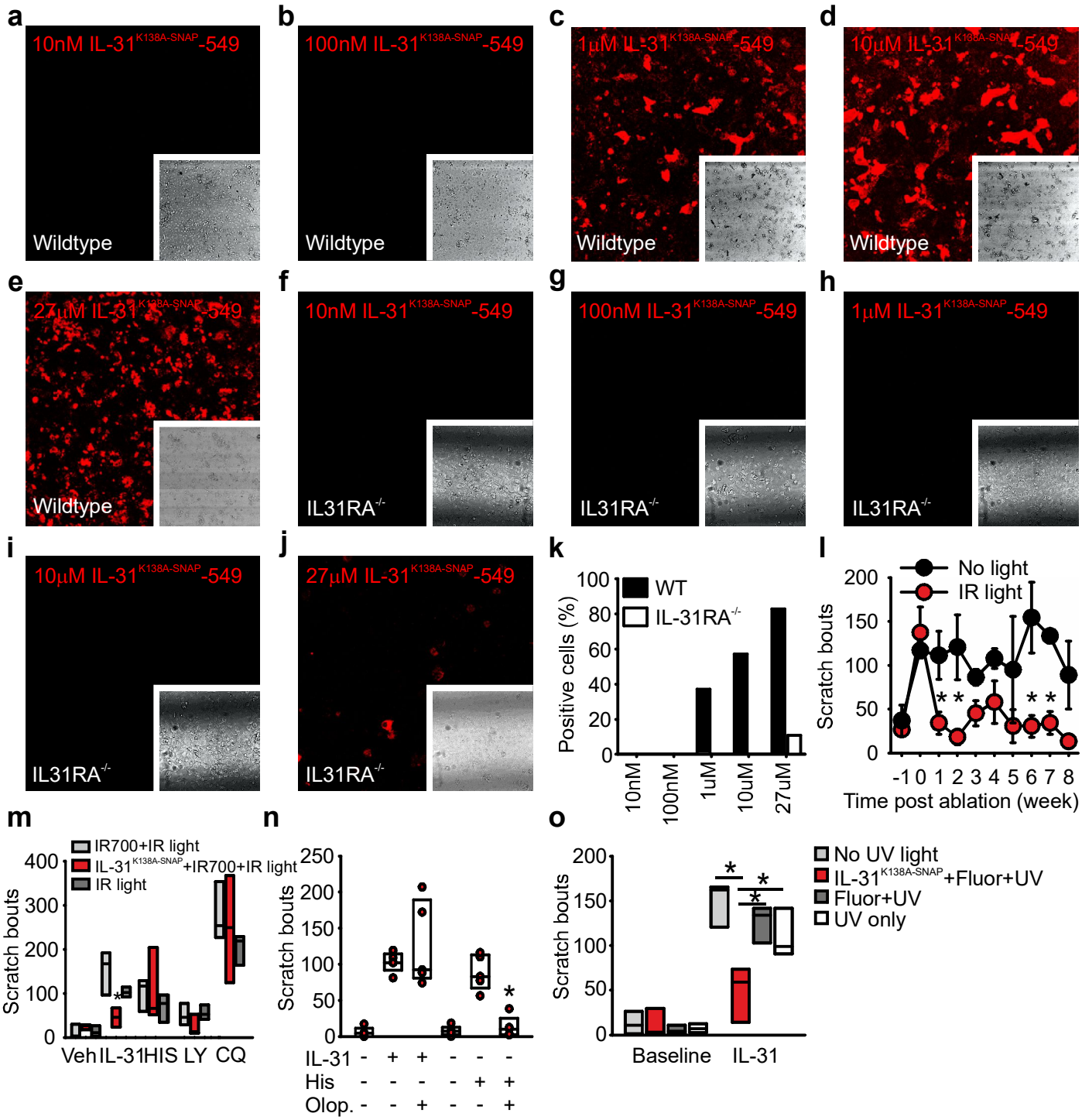
Supplementary Figure 1. SNAP-tag activity. (**a**) Spectrophotometer measurements of the binding activity of IL-31$^{K138A-SNAP}$, IL-31^{SNAP} and SNAP protein to the fluorescent substrate BG-549 at a 1:3 ratio. Readings were done at 280nm and 550nm. (**b**) Representative Coomassie and (**c**) fluorescence gels showing the binding of IL-31$^{K138A-SNAP}$, IL-31^{SNAP} and SNAP to BG-549 (n=3 independent experiments).(**d**) Graph showing the percentage binding activity as determined from spectrophotometer measurements.

Supplementary Figure 2



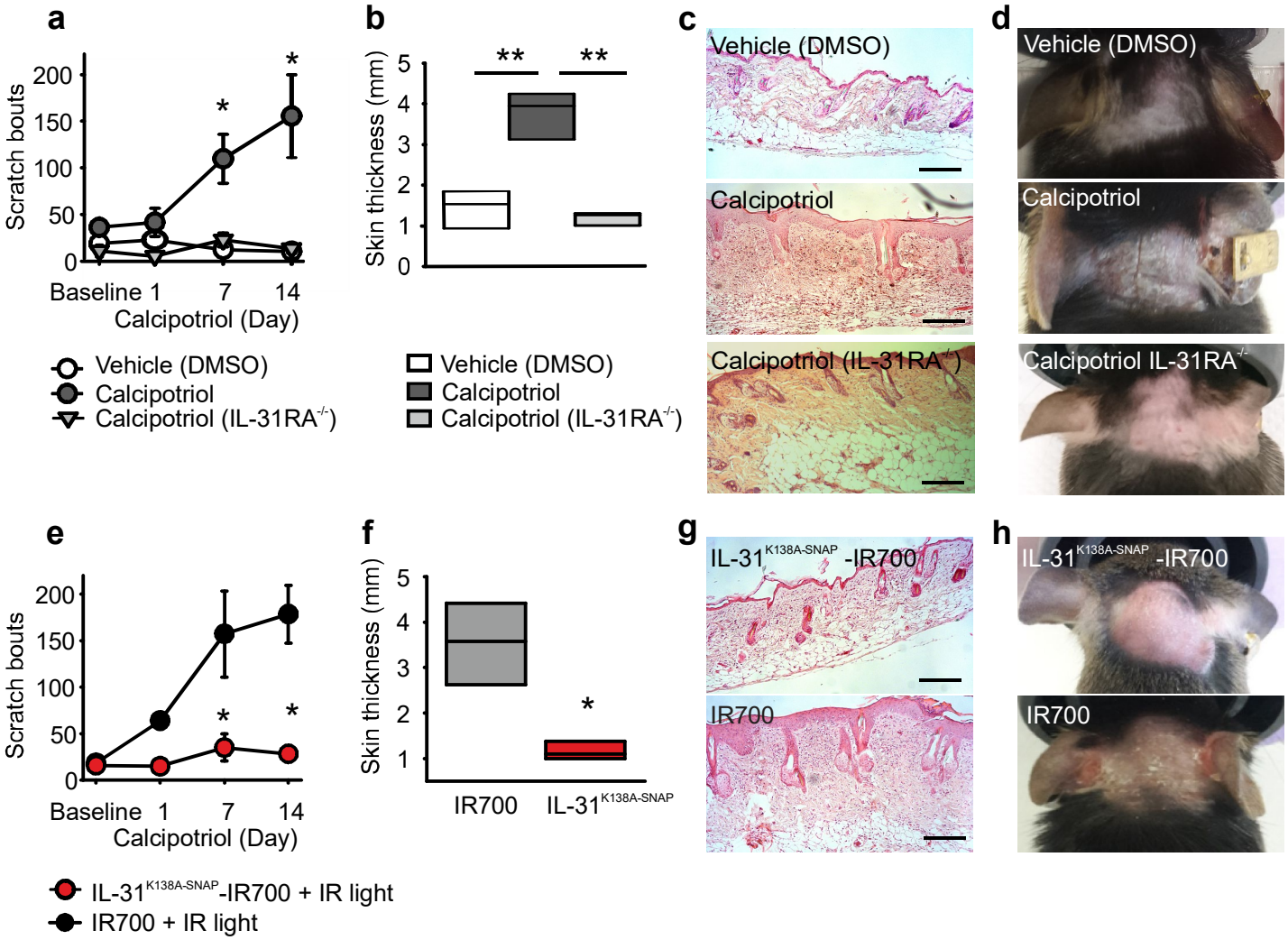
Supplementary Figure 2. Functional analysis of IL-31^{SNAP}. Concentration-dependent staining of primary keratinocytes from wild-type (**a-e**) and IL-31RA$^{-/-}$ mice (**f-j**) (n=3 animals). Cells were labelled with different IL-31^{SNAP} concentration (10 nM, 100 nM, 1 μM, 10 μM and 27 μM) coupled with the fluorescent substrate BG549 (in red) at a 1:3 molar ratio. The insets represent the brightfield image of the stained cells. (**k**) The number of cells was counted and IL-31^{SNAP}-BG549 positive-cells were expressed as percentage of the total cells. Black bars represent the positive cells in wild type mice and white bars represent the positive cells in IL-31RA$^{-/-}$ mice. (**l**) IL-31^{SNAP} evokes progressive increase in scratching behaviour over 3 consecutively days of injection (n=4 animals) whereas vehicle (PBS) injections does not induce any scratching. Baseline refers to spontaneous scratching behavior before injection. Error bars indicate SEM. *p<0.05 (Two-Way ANOVA, followed by Bonferroni test).

Supplementary Figure 3



Supplementary Figure 3. Functional analysis of IL-31$^{K138A-SNAP}$. Concentration-dependent staining of primary keratinocytes from wild-type (**a-e**) and IL-31RA$^{-/-}$ mice (**f-j**) (n=3 animals). Cells were labelled with different IL-31$^{K138A-SNAP}$ concentration (10 nM, 100 nM, 1 µM, 10 µM and 27 µM) coupled with the fluorescent substrate BG549 (in red) at a 1:3 molar ratio. The insets represent the brightfield image of the stained cells. (**k**) The number of cells was counted and IL-31$^{K138A-SNAP}$-BG549 positive-cells were expressed as percentage of the total cells. Black bars represent the positive cells in wild type mice and white bars represent the positive cells in IL-31RA$^{-/-}$ mice. (**l**) Long-term monitoring of IL-31$^{K138A-SNAP}$-IR700 mediated ablation over the course of 8 weeks (n=4 animals). Red circles indicate scratching responses from mice injected with IL-31$^{K138A-SNAP}$-IR700 and illuminated, black circles indicate injection but without illumination. * p<0,05 (Two-Way ANOVA, followed by Bonferroni test). (**m**) Light and IR700 plus light controls for behavioral responses to pruritogens, * p=0,008 (two-tailed t-Test, n=5 animals. (**n**). The histamine H1 receptor antagonist Olopatadine reverses scratching evoked by histamine but not by IL31. * p<0,01, n=5 animals. (**o**) Injection of IL-31$^{K138A-SNAP}$ coupled to BG-Fluorescein and subsequent UV illumination reduces IL31 evoked scratching behavior. Control UV illumination, UV + BG-Fluorescein, or IL-31$^{K138A-SNAP}$ + BG-Fluorescein in the absence of illumination are without effect. * p<0,05, n=5 animals. All error bars indicate standard error SEM. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile.

Supplementary Figure 4



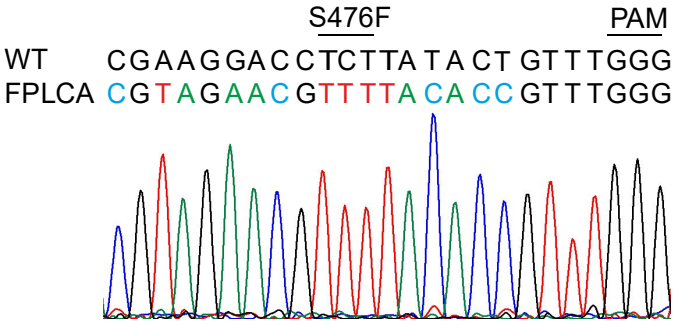
Supplementary Figure 4. Calcipotriol model of atopic dermatitis. (**a**) Scratching behaviour over 14 days of treatment with vehicle DMSO (white circles, n=4 animals), Calcipotriol in wildtype mice (dark grey circles, n=5 animals) and Calcipotriol in IL-31RA$^{-/-}$ mice (light grey triangles, n=8 animals). * p<0.05 (Two-Way ANOVA, followed by Bonferroni test). (**b**). Skin thickness after 14 days of treatment with DMSO (n=4 animals, white box), Calcipotriol in wildtype mice (n=5 animals, dark grey box) and Calcipotriol in IL-31RA$^{-/-}$ mice (light grey box, n=8 animals). ** p=0.001(two-tailed t-Test). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. (**c**) Representative Hematoxylin and Eosin staining of skin sections of mice treated with vehicle (DMSO, top panel, n=2 animals), Calcipotriol in wildtype mice (middle panel, n=2 animals) or Calcipotriol in IL-31RA$^{-/-}$ mice (lower panel, n=2 animals) for 14 days. (**d**) Representative skin pictures of a mouse treated for 14 days with DMSO (top panel, n=4 animals), Calcipotriol in wildtype mice (middle panel, n=5 animals) or Calcipotriol in IL31RA$^{-/-}$ mice (lower panel, n=4 animals). (**e**) Scratching behaviour over 14 days of Calcipotriol treatment of mice pre-injected for 3 days with IL-31$^{K138A-SNAP}$+ IR700 (n=4 animals, red circles) or IR700 only (n=4 animals, black circles), followed by near IR illumination. * p<0.05 (Two-Way ANOVA, followed by Bonferroni test). (**f**) Skin thickness of mice injected with IR700 (n=4 animals, grey box) or IL-31$^{K138A-SNAP}$+ IR700 (n=4 animals, red box), followed by 14 days of Calcipotriol treatment. * p=0.029 (two-tailed t-Test). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. (**g**) Hematoxylin and Eosin staining of representative sections of skin from mice pre-injected with IL-31$^{K138A-SNAP}$+ IR700 (top panel, n=2 animals) or IR700 alone (lower panel, n=2 animals), and then topically treated for 14 days with Calcipotriol. (**h**) Representative skin pictures (n=4 animals). Error bars indicate SEM, scale bars 200 µm.

Supplementary Figure 5

a

Embryos transferred	Pups born	Genotype	efficiency HDR (%)
90	9	9 wt	0%
100	14	1 FPLCA 1 KO	7%

b

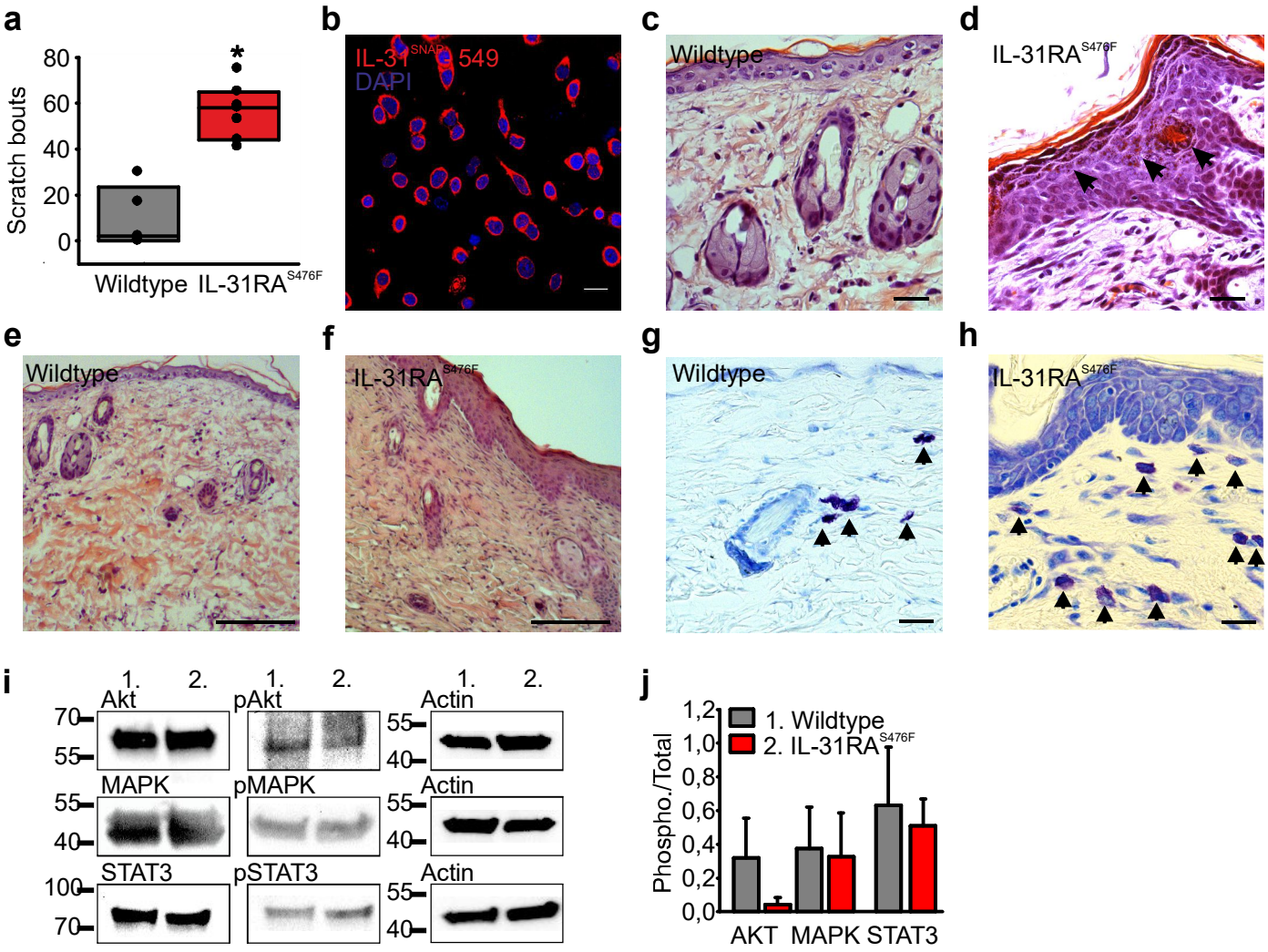


c

TracrRNA	5'AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU 3'
crRNAIL-31RA	5'GAAGGACCUCUUAUACUGUUGUUUUAGAGCUAUGCUGUUUUG 3'
ssODN	5'CCAAGACTGTTAACTCTCATGCCCTGAAGTGTGACCTGGAGTCTCTGACACGTAGAACGTTTTACACCGTTTGGGTCATGGCAAGCACCAGAGCTGGAGGTACCAACGGGGTGAGAATAAAC 3'

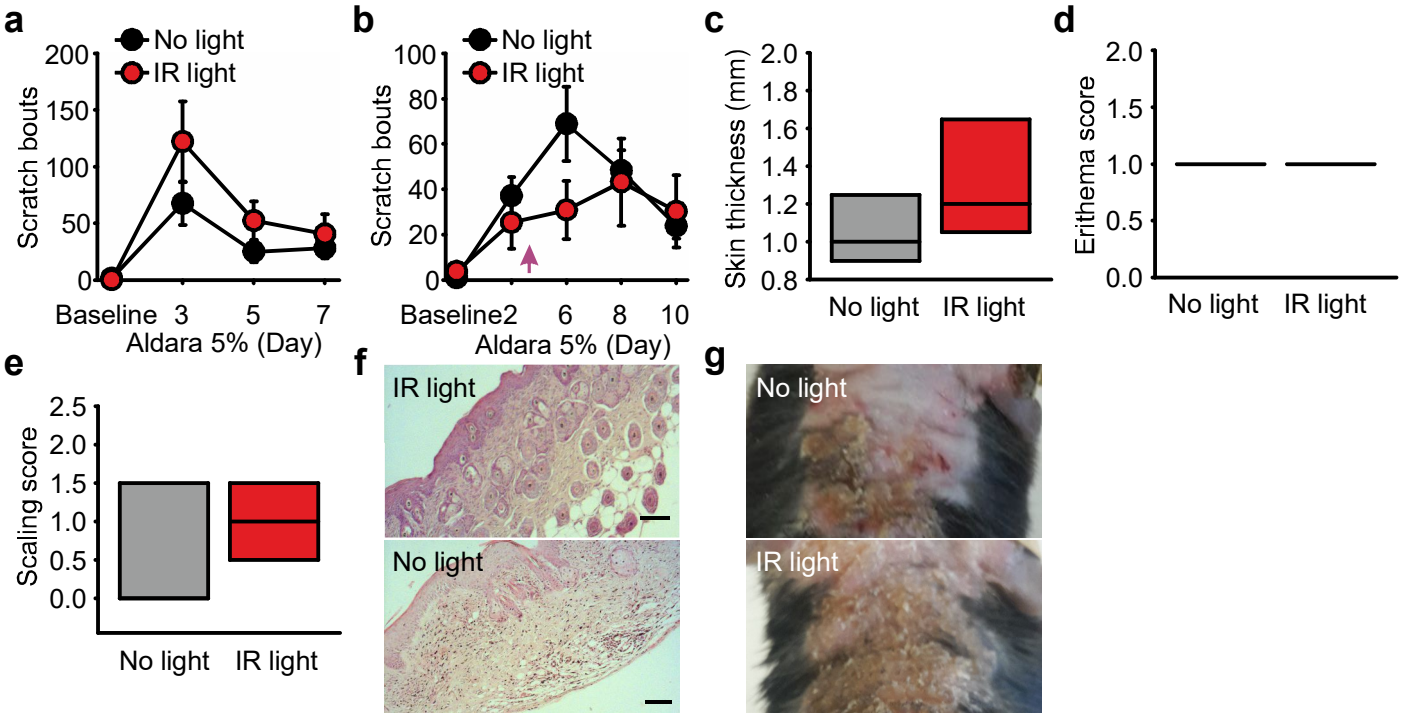
Supplementary Figure 5. Generation of a mouse model of Familiar Primary Localized Cutaneous Amyloidosis (**a**) Table showing the results of the pronuclear injections performed to generate FPLCA mice using CRISPR-Cas9. (**b**) Sequencing chromatogram showing the mutation site in exon 14 of IL-31RA gene (TCT>TTT) corresponding to the amino acid substitution S476F. PAM region (GGG) is also shown. (**c**) Table showing the synthetic RNAs (TracrRNA and crRNA IL-31RA) and single strand oligoDNA (ssODN) sequences used for the cloning-free Cas9 strategy.

Supplementary Figure 6



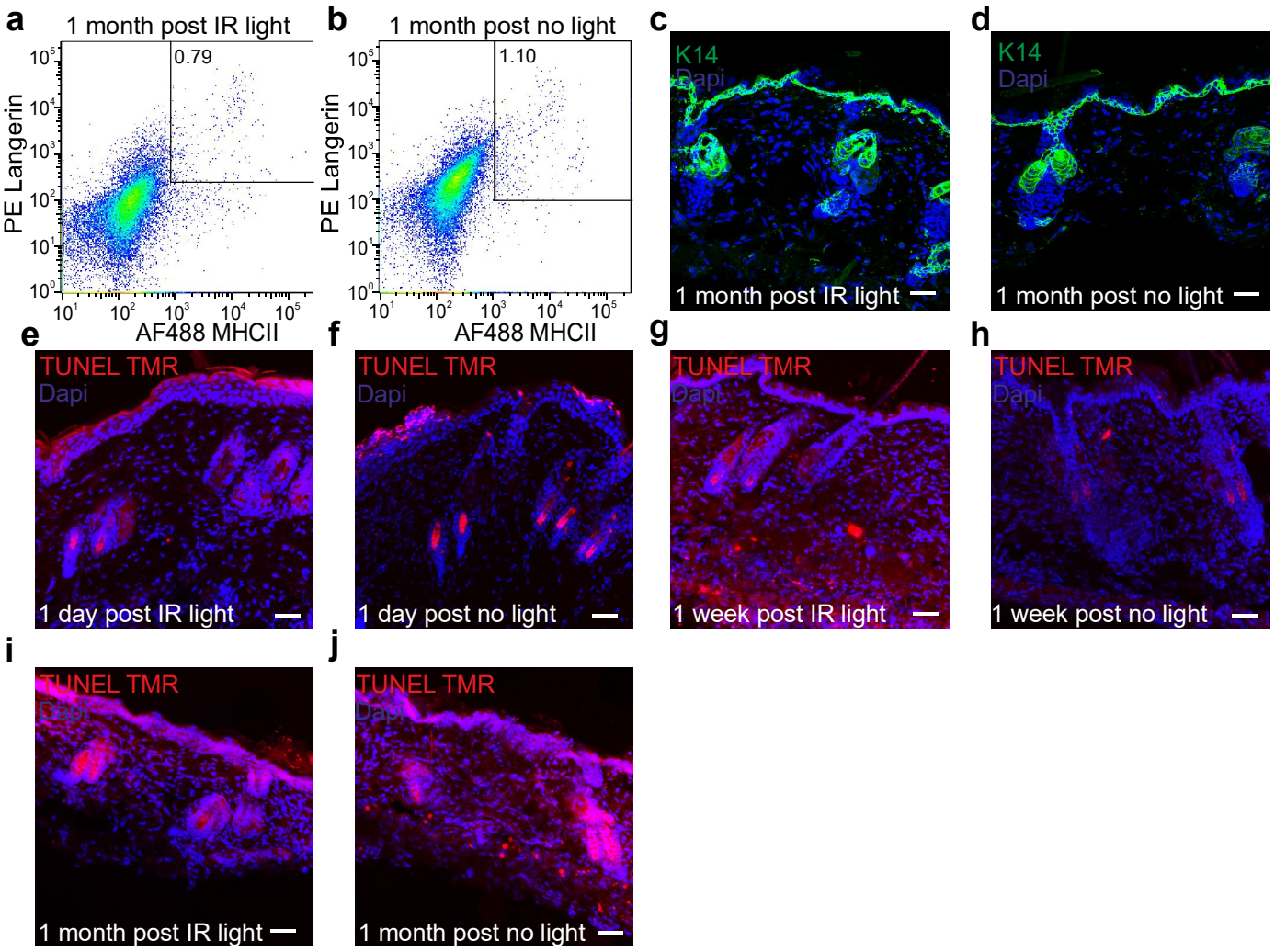
Supplementary Figure 6. Characterization of FPLCA mouse model. (**a**) Spontaneous scratching in wildtype and IL-31RA^{S476F} littermates (n=5 animals) over a 30-minute-recording period. * p<0.001 (Two-tailed t-Test). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. (**b**) Primary keratinocyte culture from IL-31RA^{S476F} newborn mice (n=3 animals) shows the binding of IL-31^{SNAP}-BG549 to the mutant receptor. Nuclei were stained with Dapi (in blue). Scale bar 20 µm. (**c–d**) Congo Red staining for amyloid deposit in skin sections of wildtype and IL-31RA^{S476F} littermate mice (n=3 animals). Arrows indicate Congo red positive amyloid deposits. Scale bar 50 µm. (**e-f**) Hematoxylin & Eosin staining of skin sections of wildtype and IL-31RA^{S476F} littermate mice (n=3 animals). Cellular infiltration represented by dense eosinophilic material at dermis level and higher epidermal thickness are observed in IL-31RA^{S476F} mice compared to wildtype littermate. Scale bar 200 µm. (**g-h**) Toluidine blue staining for mast cells in skin sections of wildtype and IL-31RA^{S476F} littermate mice (n=3 animals). Arrows indicate toluidine blue positive mast cells. Scale bar 50 µm. (**i-j**) IL-31 receptor mediated signaling in skin of FPLCA mice. (i) Representative western blots showing total and phosphorylated Akt, MAPK and STAT3 in . (**j**) Quantification of blots showing Phospho/Total signal normalized to actin, n=2 independent experiments. Error bars indicate SEM.

Supplementary Figure 7



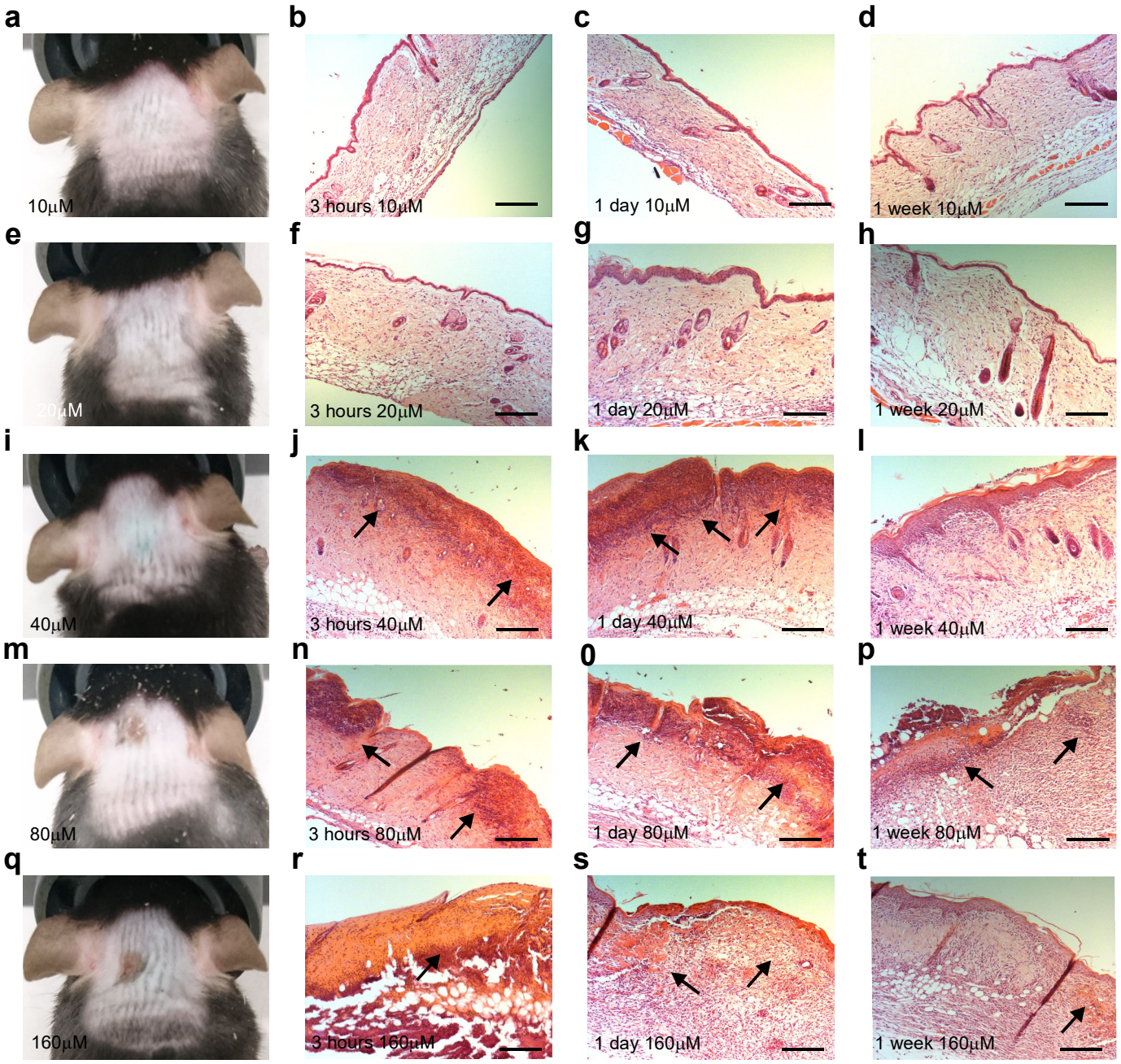
Supplementary Figure 7. IL-31$^{K138A\text{-}SNAP}$ mediated photoablation is ineffective in a mouse model of psoriasis. (**a**) Number of scratch bouts over 7 days of 5% Aldara application in mice pre-injected with IL-31$^{K138A\text{-}SNAP}$+ IR700 for 3 days, with (n=5 animals, red circle) and without (n=5 animals, black circle) near IR illumination. (**b**). Number of scratch bouts over 10 days of 5% Aldara application in mice post-treated with IL-31$^{K138A\text{-}SNAP}$+ IR700 at day 3 (arrow), with (n=8 animals, red circle) and without (n=8 animals, black circle) near IR illumination. **(c)** Skin thickness after 10 days of 5% Aldara application in mice post-treated with IL-31$^{K138A\text{-}SNAP}$+ IR700 (n=8 animals). (**d, e**) Skin erythema and scaling scored from 0 to 4, according to the Psoriasis Area and Severity Index (PASI) after 10 days of 5% Aldara application in mice post-treated with IL31$^{K138A\text{-}SNAP}$+ IR700 (n=8 animals). (**f**) Representative Hematoxylin and Eosin staining of skin sections after 10 days of 5% Aldara treatment and post-treated with IL31$^{K138A\text{-}SNAP}$+ IR700 (n=3 animals). Scale bar 200 µm. (**g**) Representative skin pictures of mice treated for 10 days with 5% and post-treated with IL-31$^{K138A\text{-}SNAP}$+ IR700 (n= 4 animals). Error bars indicate SEM. In box plot graphs, the boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile.

Supplementary Figure 8



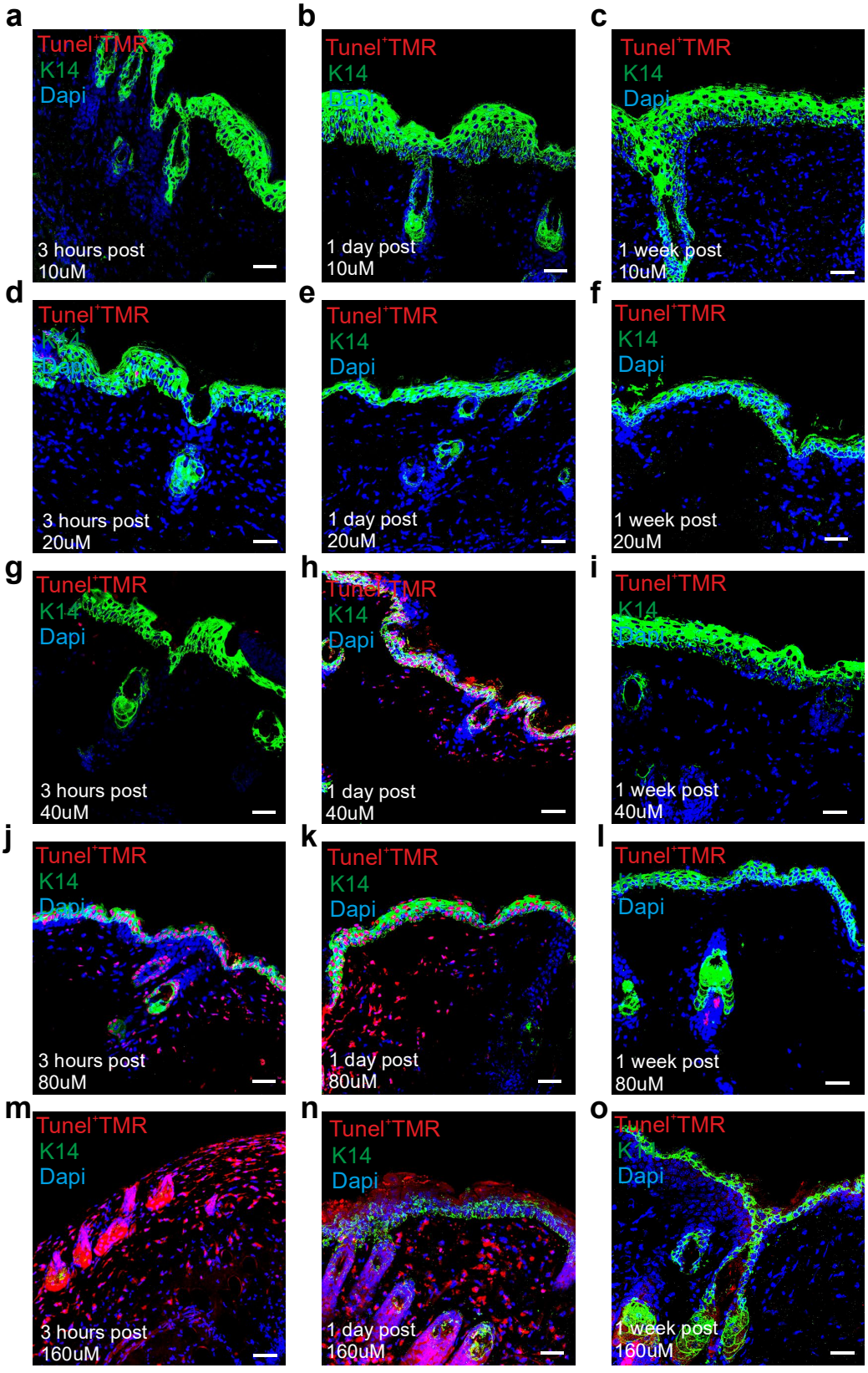
Supplementary figure 8. Cellular effects of IL-31$^{K138\text{-}SNAP}$ guided photoablation in the skin (**a, b**) No loss of epidermal migratory dendritic cells 1 month post IL-31$^{K138A\text{-}SNAP}$ mediated photoablation. Representative flow cytometry plots showing cutaneous dendritic cells isolated from the back skin of mice 1 month after topical application of IL-31$^{K138A\text{-}SNAP}$+BGIR700, with (**a**) and without (**b**) near IR illumination. Percentage of total events is indicated in the box. (**c, d**) No loss of basal keratinocytes 1 month after IL-31$^{K138A\text{-}SNAP}$ mediated photoablation. Representative immunofluorescence staining of back skin with a K14 antibody 1 month after topical application of IL-31$^{K138A\text{-}SNAP}$+BGIR700, with (**c**) and without (**d**) near IR illumination. (**e-j**) No increase in apoptosis upon IL31$^{K138A\text{-}SNAP}$ mediated photoablation. Cell death was assessed using the TUNEL assay at 1 day (**e**, **f**), 1 week (**g**,**h**) and 1 month (**i**,**j**) post topical IL-31$^{K138A\text{-}SNAP}$-IR700 application, with (**e**,**g**,**i**) and without (**f**,**h**,**j**) near IR illumination. Scale bars indicate 50 µm. n=3 animals.

Supplementary Figure 9



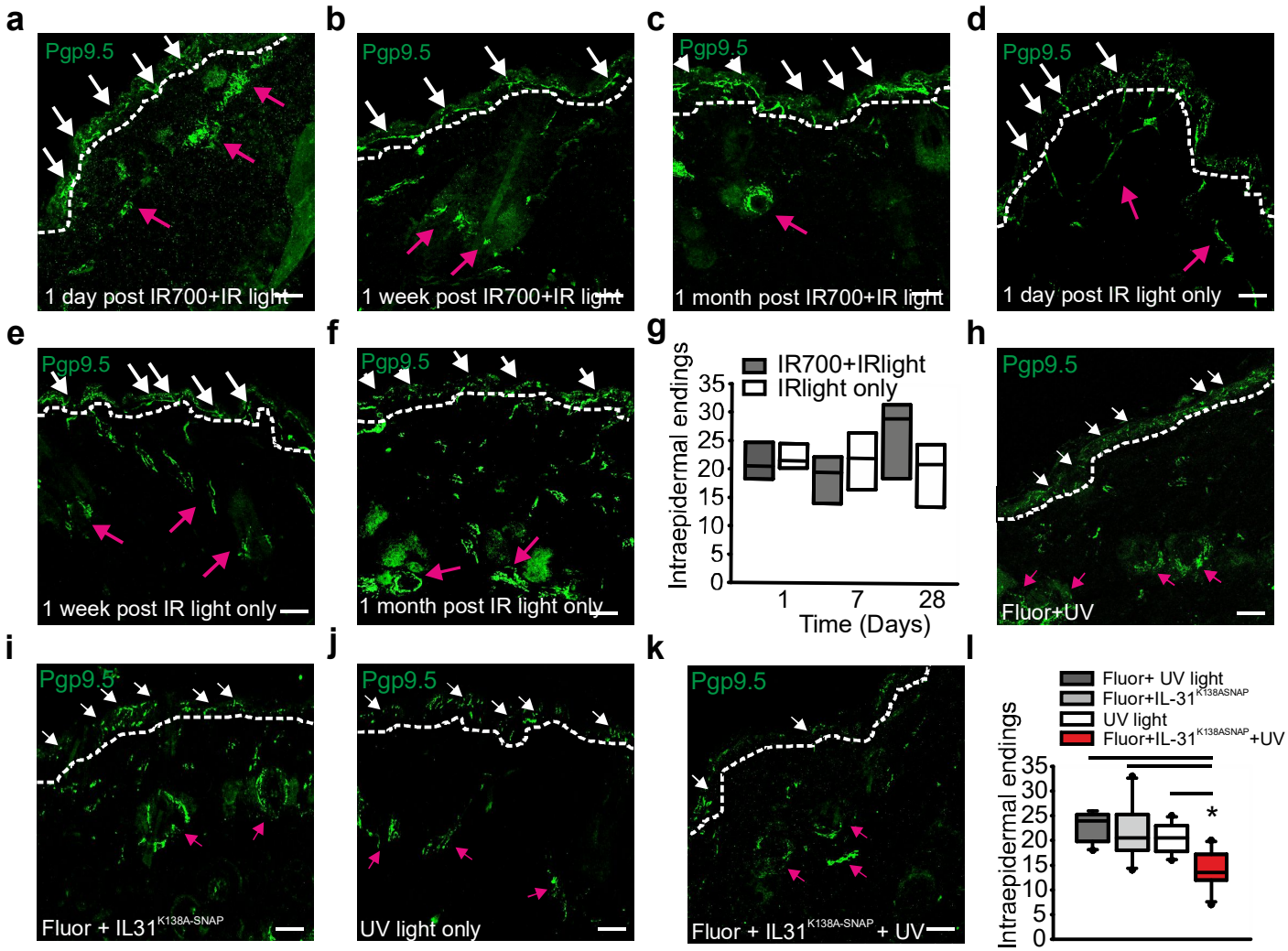
Supplementary Figure 9. Increasing concentrations of IL-31$^{K138A\text{-}SNAP}$ induces tissue damage upon near IR illumination. (**a, e, i, m and q**) Mouse skin after 3 days of injection of IL-31$^{K138A\text{-}SNAP}$ +IR700+near IR illumination at the indicated concentration. Tissue damage is clearly visible at 80μM (**m**) and 160μM (**q**). Representative Hematoxylin & Eosin staining of back skin sections at 3 hours (**b, f, j, n and r**), 1 day (**c, g, k, o and s**) and 1 week (**d, h, l, p and t**) post IL-31$^{K138A\text{-}SNAP}$ +IR700+near IR illumination at the indicated concentrations of IL-31$^{K138A\text{-}SNAP}$ (10, 20, 40, 80, 160 μM). Cellular infiltration and tissue damage are clearly visible at 40, 80 and 160 μM as indicated by black arrows. Scale bars indicate 200 μm. n=3 animals.

Supplementary Figure 10



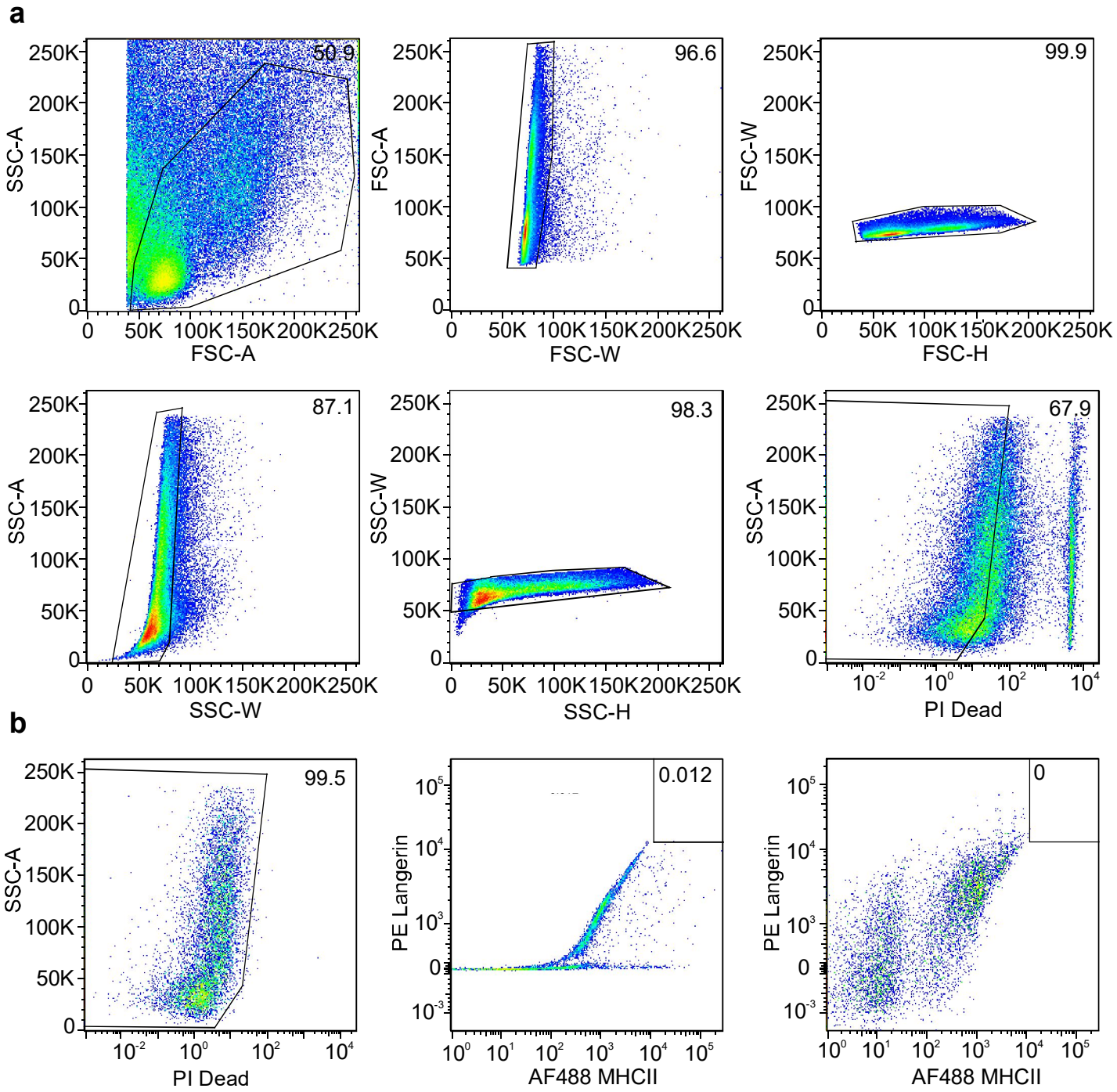
Supplementary Figure 10. Increased concentrations of IL-31$^{K138A\text{-}SNAP}$ induces cell death upon near IR illumination. (**a-o**) Representative Tunel (in red) and K14 (in green) staining of back skin sections at 3 hours (**a, d, g, j, m**), 1 day (**b, e, h, k, m**) and 1 week (**c, g, l, i, o**) post IL-31$^{K138A\text{-}SNAP}$ +IR700+near IR illumination at the indicated concentrations of IL-31$^{K138A\text{-}SNAP}$ (10, 20, 40, 80, 160 μM). Nuclei are stained with Dapi (in blue). Scale bars indicate 50 μm. n=3 animals.

Supplementary Figure 11



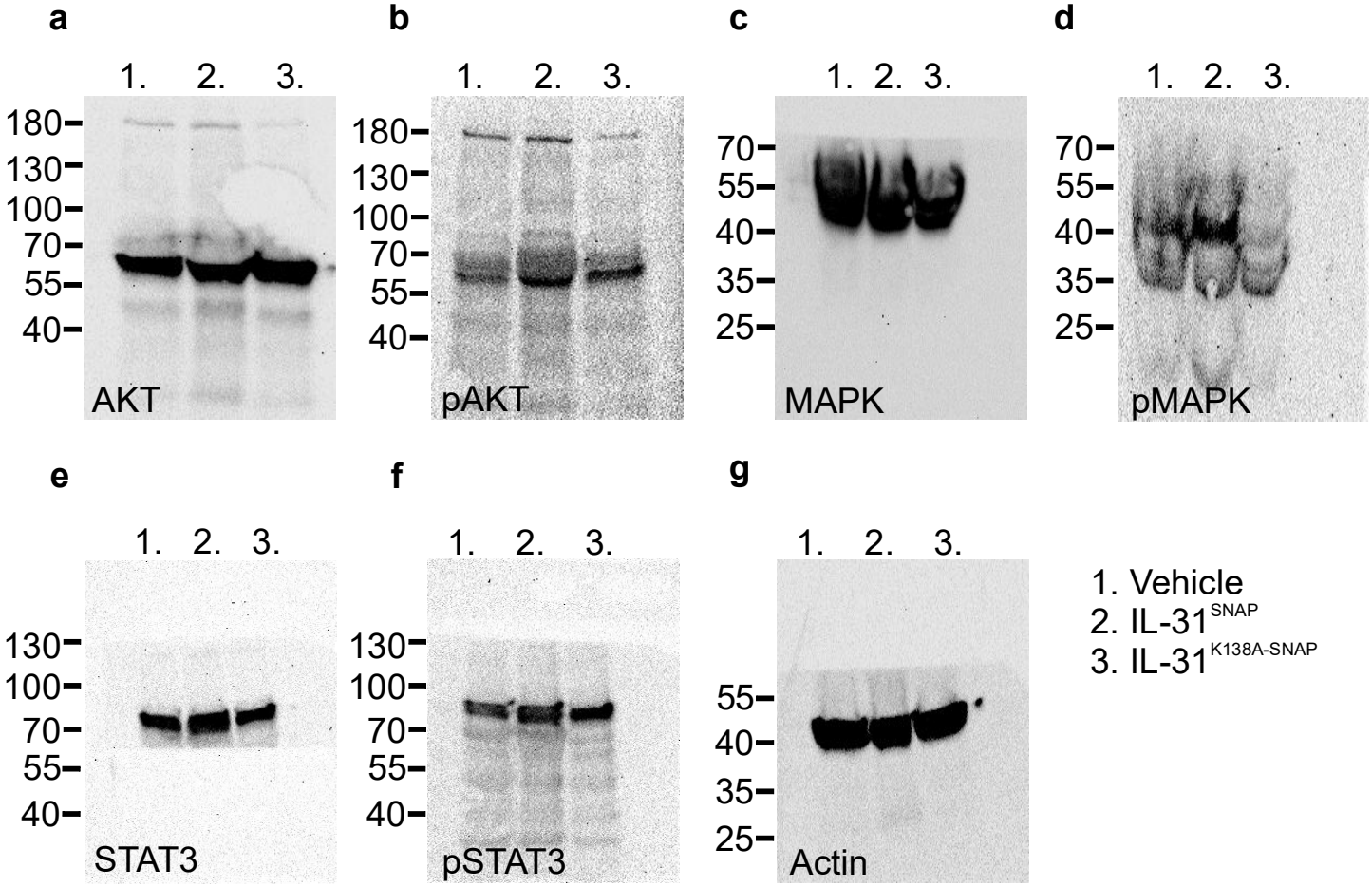
Supplementary Figure 11. Pgp9.5 staining. (**a-g**) Epidermal free nerve endings are preserved upon near IR illumination with and without the photosensitizer IR700. Representative Pgp9.5 staining (in green) of nerve fibers innervating the back skin at 1 day (**a** and **d**), 1 week (**b** and **e**) and 1 month (**c** and **e**) post near IR illumination with (**a-c**) and without (**d-f**) IR700 (n=3 animals). Dotted line represents the dermal-epidermal junction, white arrows indicate epidermal free nerve endings; magenta arrows indicate dermal innervations around hair follicles. (**g**) Quantification of the epidermal free nerve endings (n=3 animals). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. (**h-l**) Loss of epidermal free nerve endings upon IL-31$^{K138A\text{-}SNAP}$ mediated photoablation using Fluorescein as a photosensitizer. Representative immunofluorescence staining of Pgp9.5 positive nerve fibers innervating back skin at 1 week after Fluorescein+UV light (**h**), Fluorescein + IL-31$^{K138A\text{-}SNAP}$ (**i**), UV light only (**j**) and IL-31$^{K138A\text{-}SNAP}$ + Fluorescein +UV light application (**k**) (n=3 animals). Dotted line represents the dermal-epidermal junction, white arrows indicate epidermal free nerve endings which are lost upon IL-31$^{K138A\text{-}SNAP}$ mediated photoablation; magenta arrows show innervations around hair follicles. (**l**) Quantification of the epidermal free nerve endings. (* $p<0.001$, two-tailed t-Test; n=3 animals). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Scale bars indicate 50 µm, error bars indicate SEM.

Supplementary Figure 12



Supplementary figure 12. Cell Sorting Strategy. (**a**) Hierarchical gating strategy for selecting live single cells from suspensions of skin. (b) FMOs for determining gate placement.

Supplementary Figure 13



Supplementary figure 13. IL-31^{SNAP} and IL-31$^{K138A-SNAP}$-mediated signalling in wildtype mouse skin. (**a-g**) Full blots showing the expression of AKT (a), pAKT (b), MAPK (c), pMAPK (d), STAT3 (e), pSTAT3 (f) and Actin (g). Lanes are labelled as 1 for the vehicle (PBS), 2 for IL-31^{SNAP} and 3 for IL-31$^{K138A-SNAP}$ treatment.