Supplementary Figure Legends:

Supplementary Figure 1: Regulatory T cells are activated and accumulate in skin during epidermal regeneration after injury. Related to Figure 1.

(a) Schematic of skin barrier disruption. The backs of 7-12 week old mice were shaved and tape stripped 5-7 times per day over 3 consecutive days using Shurtape (3M) (barrier disruption phase). Mice are disrupted to achieve a transepidermal water loss (TEWL) value between 40-75 gH20/m^2/hr averaged over 4 quadrants of back skin by day 0, the last day of skin disruption. Mice recover over an 11-day period (recovery phase).

(b) Transepidermal water loss measurements in WT mice on the indicated days of recovery.

(c) qRT-PCR of the indicated epidermal differentiation genes normalized to *β2m* from skin biopsies harvested on the indicated days of recovery.

(d) Representative flow cytometry plots of Treg cells in the dermis. Plots are pregated on live, $CD45^+$ $CD3^+$ $\gamma\delta^-$ $CD8^-$ cells.

(e) Percent and absolute number of Treg cells in the dermis at the indicated times of barrier recovery.

(f) Representative plots of Ki-67 expression in skin Treg cells at the indicated times of barrier recovery. Plots are pre-gated on $CD45^+$ $CD3^+$ $CD4^+$ $FoxP3^+$ cells.

(g) Quantification of Ki-67 expression at the indicated times of barrier recovery.

(h) Representative IF images of $FoxP3^{GFP}$ localization at the indicated times of barrier recovery.

(i) Percentage of 'HF associated Treg cells' (defined as GFP^+ cell localization < 20μm to nearest HF).

HF – Hair follicle. Data are +/- S.E.M. *p < 0.05; **p < 0.01; ***p < 0.001 ****p < 0.0001 by Student's t-test. Results in b are representative > 10 experiments (n=3-6) and c-e are representative of 4 independent experiments (n = 2-4 mice per group). Results in f & g are representative of 2 independent experiments with 2-4 mice per group.

Supplementary Figure 2: Regulatory T cells are deleted in skin of $FoxP3^{DTR}$ mice and are not required for barrier function in the steady state. Related to Figure 1.

(a) $FoxP3^{DTR}$ mice were injected with DT on days 0,1,3 and 5 and compared to non DT treated controls. Representative flow cytometry plots of Treg cells ($CD4^+$ $FoxP3^+$) in the skin, 11 days after the first injection. Plots are pre-gated on live, $CD45^+$ $CD3^+$ $CD4^+$ cells.

(b) Representative histology of epidermal thickness at the indicated times of recovery of skin barrier disrupted mice. The skin was injured and Treg cells were depleted in FoxP3^{DTR} mice as described in Figure 1a. Mice are compared to Treg cell-sufficient controls (Cntrl). (See also Figure 1d & 1e).
(c) FoxP3^{DTR} were treated with DT to deplete Treg cells as in a. Back skin was shaved on day 0 but not injured by tape stripping. Transepidermal water loss measurements of Treg cell-depleted mice were compared to Treg cell-sufficient controls 7 and 11 days later. Data in c are +/- S.E.M by Student's t-test. n.s. – no significance. Results are representative of 2 independent experiments with 3 mice per group.

Supplementary Figure 3: Representative flow cytometric plots and quantification of the major cytokine-producing T cell subsets in skin of Treg cell-sufficient and Treg cell-depleted mice following epidermal injury. Related to Figure 2.
(a) Representative gating scheme for immune cell phenotyping in skin by flow cytometry. The gating strategy is shown for neutrophils, Ly6C^{+} monocytes, Ly6C^{-} monocytes, CD11c^{+} MHC II^{+} cells, CD4^{+} T effectors (Teffs), Treg cells, CD8^{+} T cells, epidermal $\gamma\delta$ T cells, and dermal $\gamma\delta$ T-cells.
(b) Representative flow cytometry plots of IL-17 and IFN-γ production by CD8^{+} and $\gamma\delta$ T cells from PMA/ionomycin stimulated skin cells comparing Treg cell-depleted to Cntrl mice 4 days after epidermal injury.
(c) Quantification of IFN-γ expression by Teffs, $\gamma\delta$ T cells, and CD8+ T cells 4 days after epidermal injury (See also Figure 2f)
(d) Representative plots and quantification of IL-5 and IL-13 production in the skin by Teffs. For relevant panels, data are S.E.M. by Student's t-test. *$p < 0.05$; n.s. – no significance. Results are representative of 3 experiments with 3 mice per group. Quantification of $\gamma\delta$ T cells shown in panels b - d are combined epidermal and dermal compartments.

Supplementary Figure 4: Kinetics and the effect of diphtheria toxin on Lgr5-derived cell migration during epidermal regeneration. Related to Figure 3.
(a) Schematic for panels a-c. Lgr5-tdTom mice were injected with tamoxifen 7 days before epidermal injury. After tamoxifen has been metabolized and cleared 7 days later, back skin of mice were shaved and disrupted as in Supplementary Figure 1. Mice were harvested 2, 4 and 7 days after barrier injury and skin was processed for IF microscopy.
(b) Representative images and

(c) quantification of Lgr5 labeled cells in the IFE

For panels c &d, the back skin of Lgr5-tdTom mice were injured, $Lgr5^+$ stem cells were labeled using tamoxifen, and mice were administered DT as in Figure 4a. Mice were compared to non-DT treated littermate controls.

(d) Representative images and

(e) quantification of Lgr5 labeled cells in the IFE.

(n = 2-3 mice per group). Scale bar in b and d is 100 μm. Red- $tdTomato^+$ Lgr5 stem cell progeny; Blue – DAPI. n.s. – no significance.

Supplementary Figure 5: Treg cells facilitate loss of the stem cell marker $CD34^+$ on HFSCs and plays a minor role in HFSC proliferation during epidermal repair. Related to Figure 4.

Cntrl and Treg cell-depleted mice were barrier injured and harvested at specific times during recovery. The epidermis was disassociated from the underlying dermis and live cells were stained for flow cytometry.

(a) Representative gating scheme of epidermal cells. The gating strategy is shown for HF bulge keratinocytes ($CD34^+$); interfollicular and infundibular (IFE/IF) keratinocytes ($CD34^-$ $Sca1^+$); isthmus keratinocytes ($CD34^-$ $Sca1^-$ $EpCAM^+$) and immune cells ($CD45^+$).

(b) Quantification of CD34+ cells as proportion of $CD45^-$ cells (keratinocytes) at the indicated times of skin barrier recovery using flow cytometry.

(c) The back skin of Lgr5-tdTom-$FoxP3^{DTR}$ was disrupted. $Lgr5^+$ HFSCs were labelled using Tamoxifen and Treg cells were depleted using DT as in Figure 4a. Mice were compared to tamoxifen-labelled, Treg cell-sufficient littermates. Mice were harvested 4 days after barrier injury. Epidermal cells were analyzed by FACS for co-expression of endogenous Lgr5-GFP, td-Tomato (Lgr5-labelled cells) and CD34 Plots are pre-gated on live $CD45^-$ epidermal cells.

(d) Quantification of tamoxifen induced labeling efficiency (left panel; as percentage of GFP^+ cells) and expression of CD34 in Lgr5-labelled cells (right panel).

(e) Representative IF image of Ki-67 of Lgr5-traced cells in the epidermis of Treg cell sufficient and depleted mice. Small arrows indicate examples of co-expression of Ki-67 and tdTomato

(f) Quantification of the number of $Ki\text{-}67^+$ $tdTom^+$ cells per high powered field (hpf)

(g) FACS purified $tdTom^+$ cells from DT-treated (*i.e.* Treg cell-depleted) or untreated Lgr5-tdTom-$FoxP3^{DTR}$ (*i.e.* Treg cell-sufficient) mice were analyzed by RNA-sequencing. Raw

counts of the indicated cell cycle associated genes. Results in a-b are pooled from 3 independent experiments with 3-5 mice per group. Results in c &g are representative of 2 independent experiments (n=2 mice per group). Results in d & e are from 2 mice per group. Data are +/- S.E.M. n.s. – no significance; *p < 0.05; **p < 0.01. Scale bar in e is 100μm; Red- tdTomato^{+} Lgr5 stem cell progeny; Green – Ki-67; Blue – DAPI.

Supplementary Figure 6: Treg cells do not regulate CXCL5 receptor expression on keratinocytes. Neutralization of IL-17A, CXCL5 or co-depletion of neutrophils partially restores skin barrier function and HFSC differentiation in the absence of Treg cells. Panel a is related to Figure 5 and panels b-e are related to Figure 6.

(a) FACS purified tdTom^{+} cells from DT-treated (*i.e.* Treg cell-depleted) or untreated Lgr5-tdTom-FoxP3^{DTR} (*i.e.* Treg cell-sufficient) mice were analyzed by RNA-sequencing as in Figure 4. Raw counts of the CXCL5 receptor, *cxcr2* are shown. For comparison of absolute raw gene counts, expression levels of *lgr5* are also displayed.

(b) Raw Ct values of *cxcr2* from bulk keratinocytes of Treg cell-sufficient and depleted mice 4 days after barrier disruption. For comparison, C_t values of the housekeeping gene, *actb* are also displayed. n=4 mice per group. Results in a are pooled from 2 independent experiments. n.s. - no significance. n.d. – not detected and is defined as $C_t > 35$.

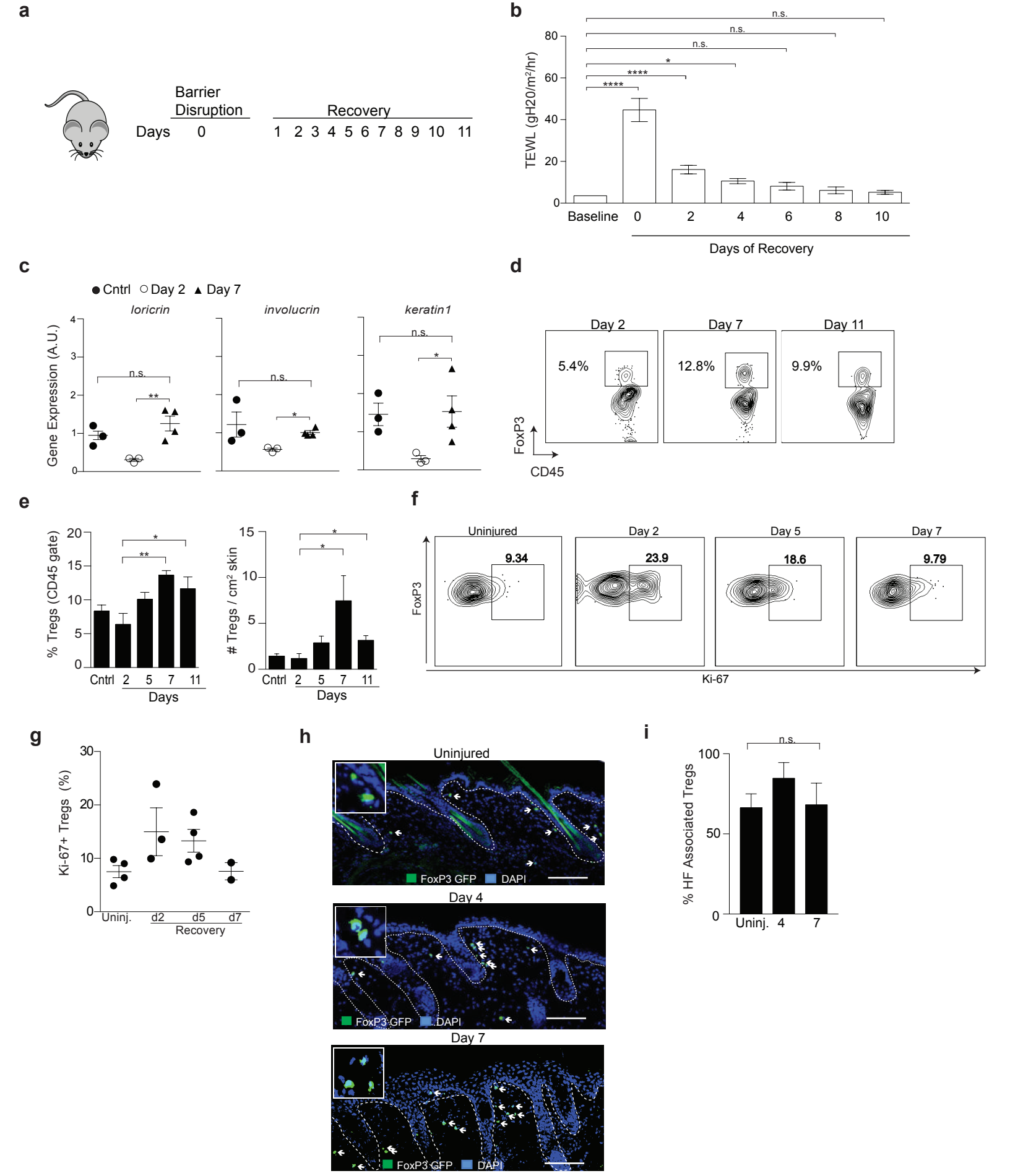
For panels c-e, Lgr5-tdTom-FoxP3^{DTR} mice were treated as in Figure 2a. Mice were co-administered α-CXCL5 mab, α-IL-17A mab, α-Gr1 mab or isotype control with DT on days 0,1 and 3 and harvested on day 4. Mice were compared to Treg cell-sufficient controls.

(c) Representative IF images of Lgr5 traced cells.

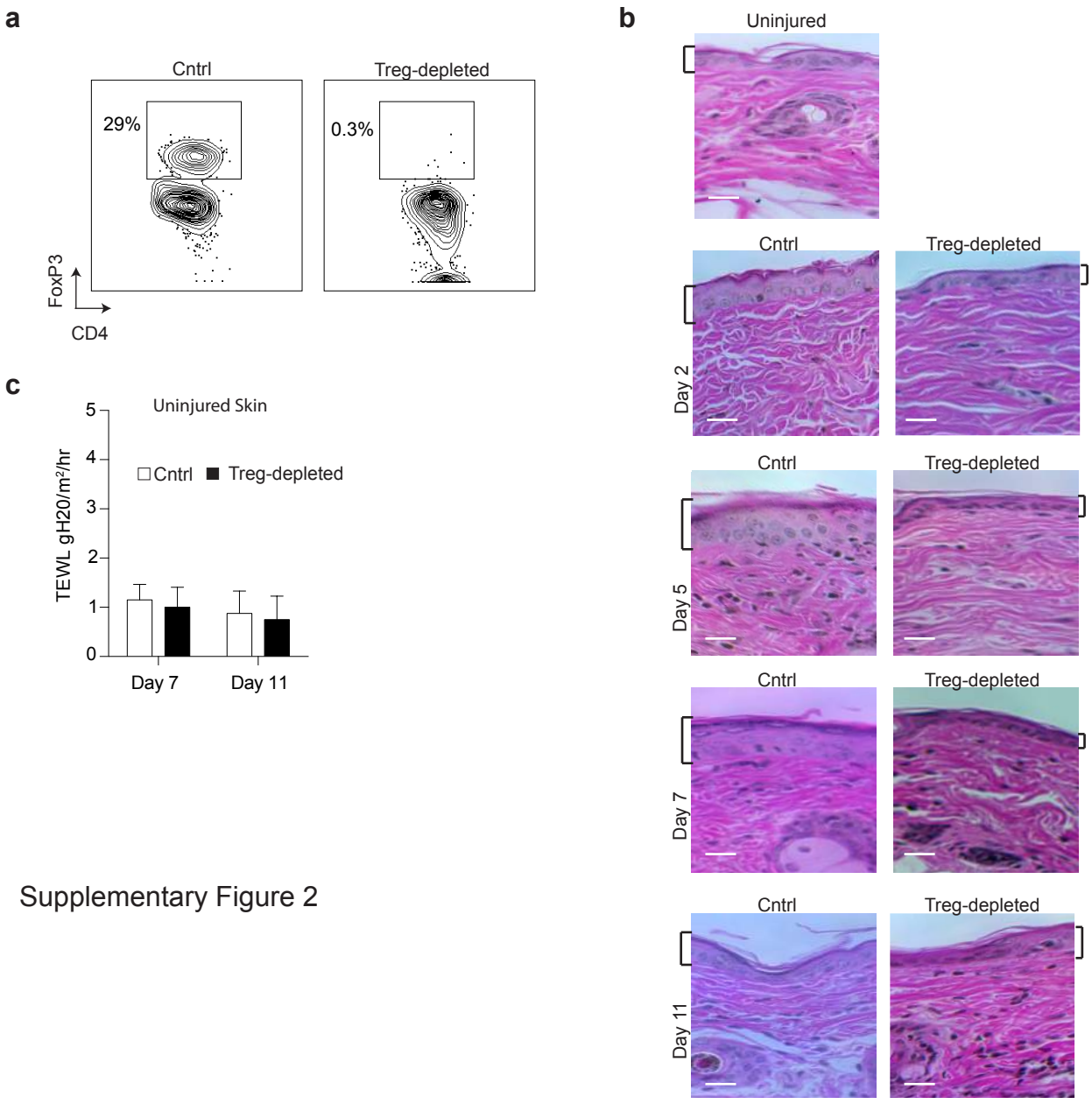
(d) Quantification of Lgr5-derivied cells in the IFE 4 days after skin injury.

(e) TEWL 4 days after injury of the indicated groups.

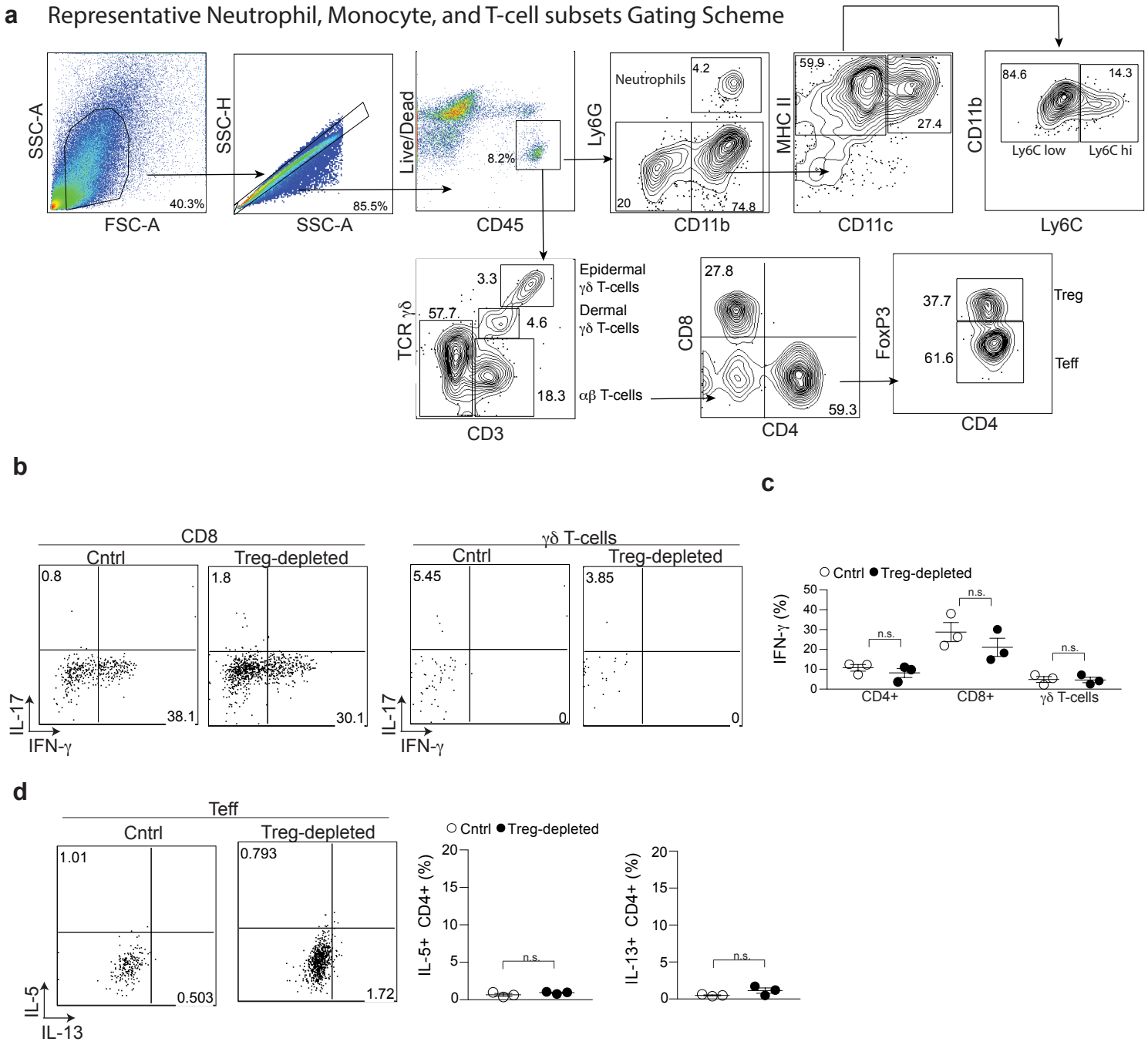
For relevant panels, data are mean ± s.e.m according to Student's t-test. Results are representative of 4 independent experiments with 3-7 mice per group. *p < 0.05; **p < 0.01; ****p < 0.0001. Scale bar in each panel of a is 100 μm. Red - tdTomato^{+} Lgr5 stem cell progeny; Blue – DAPI.



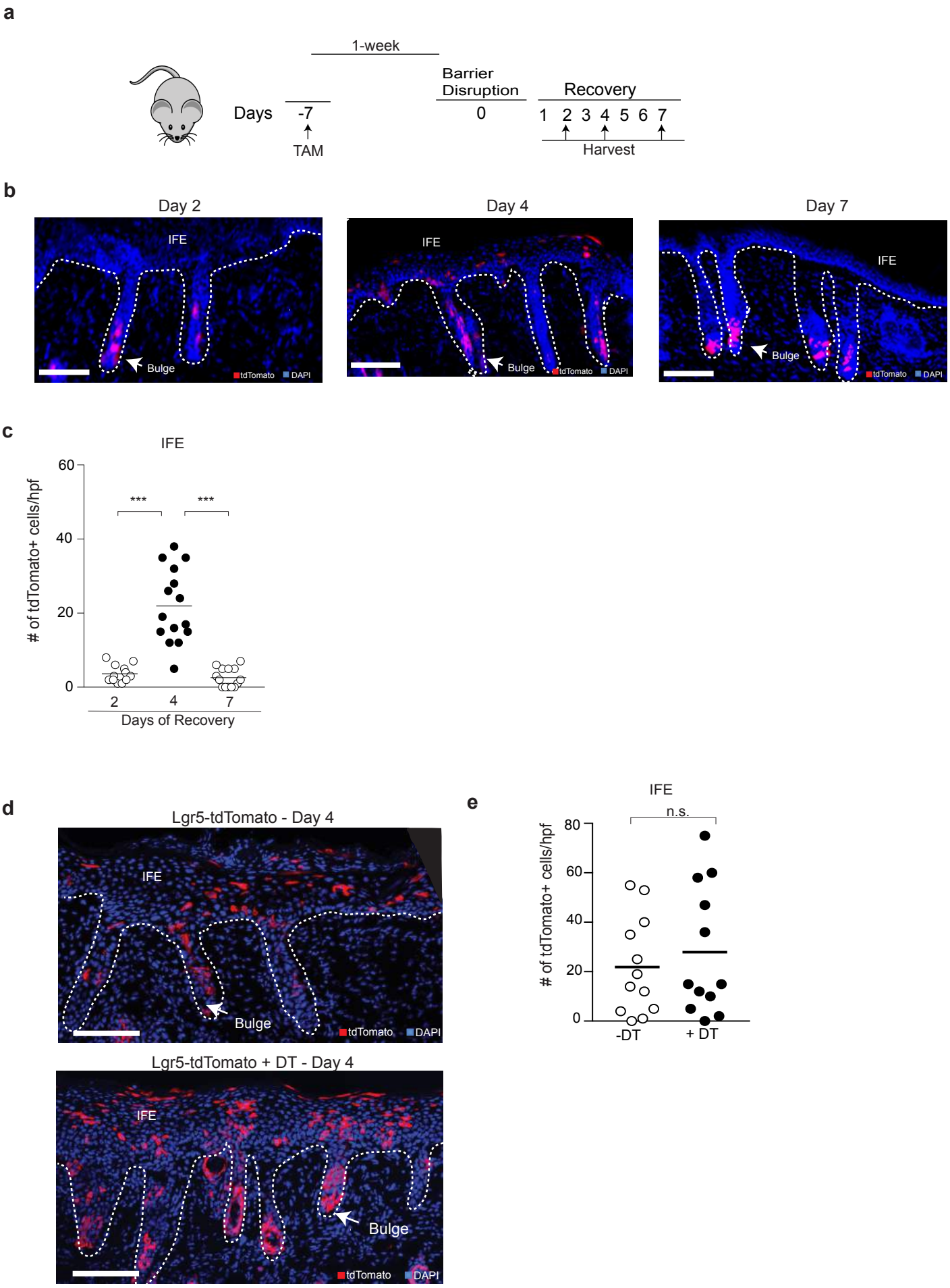
Supplementary Figure 1



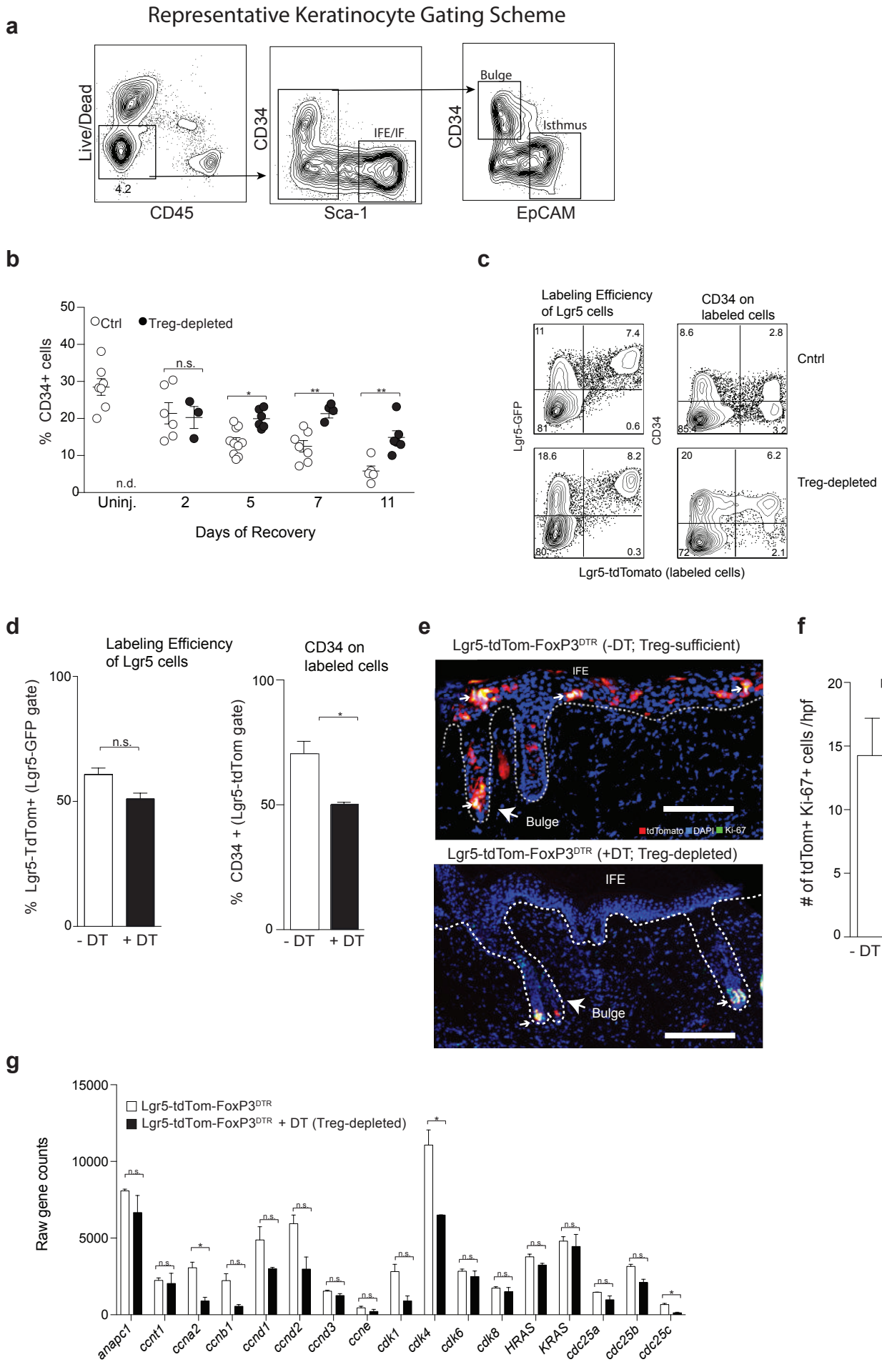
Supplementary Figure 2



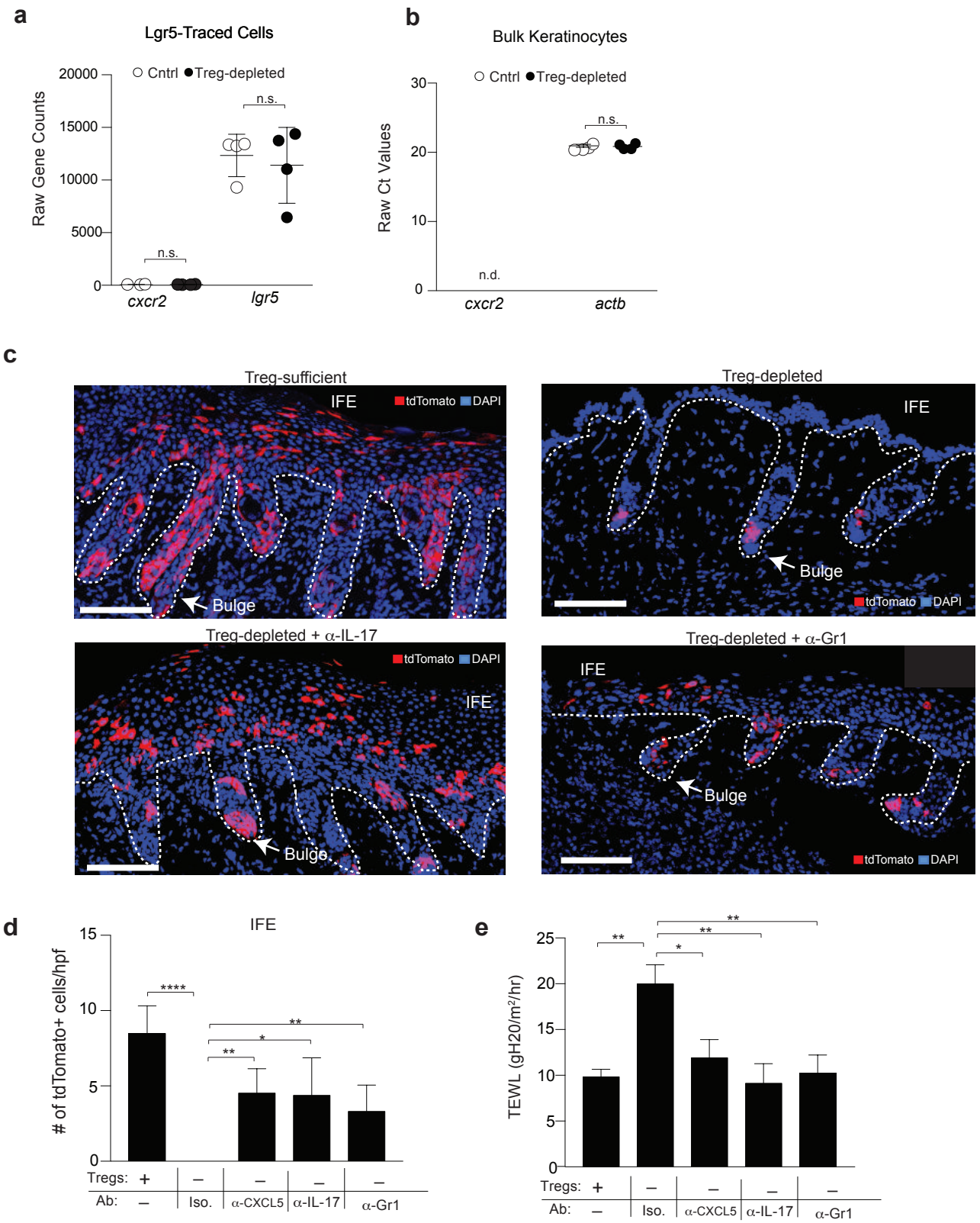
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6