

Yang et al., <http://www.jcb.org/cgi/content/full/jcb.200910126/DC1>

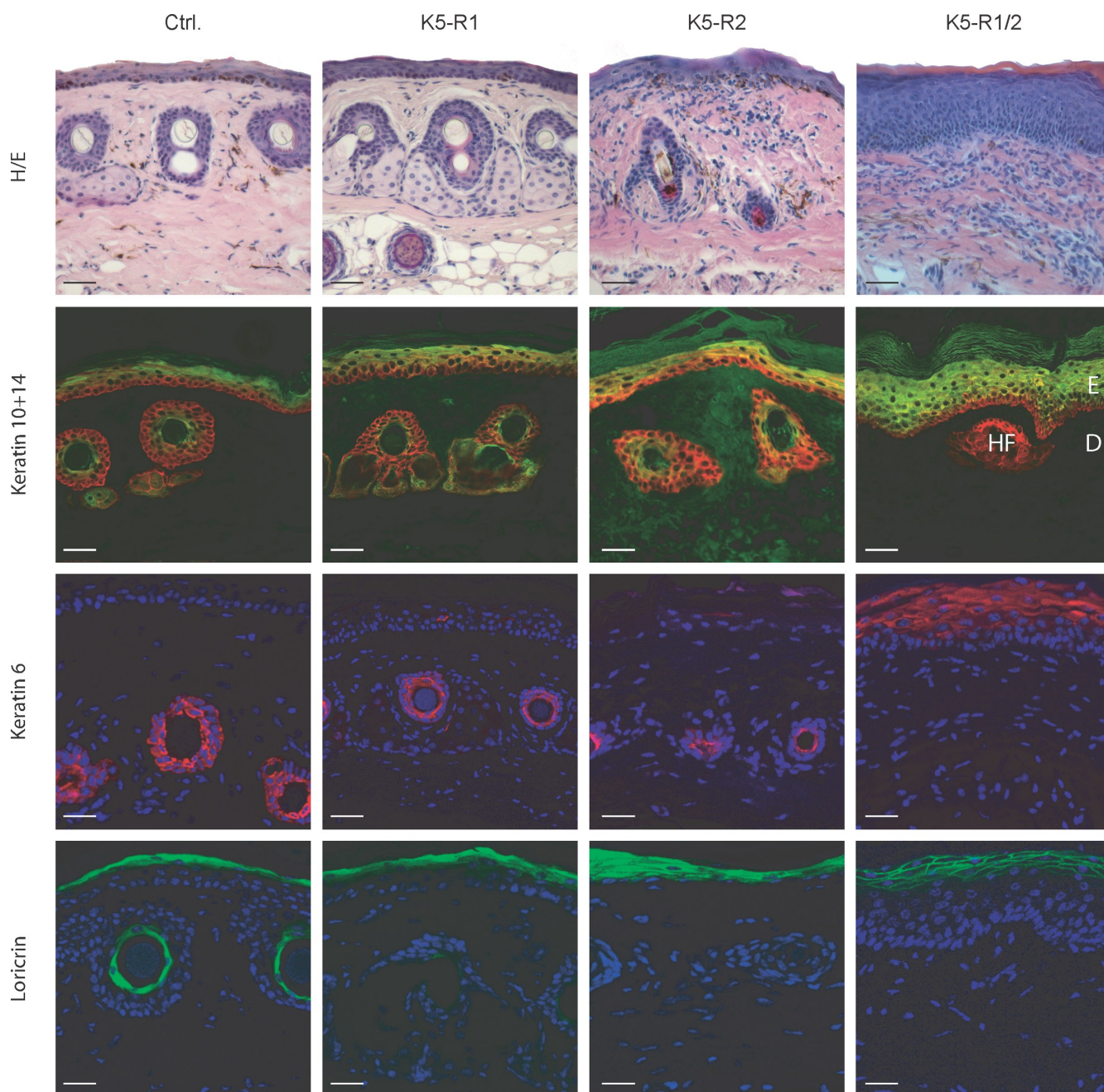


Figure S1. **Keratinocyte differentiation is only mildly altered in K5-R1/R2 mice.** Paraffin sections from tail skin of control and K5-R1, K5-R2, and K5-R1/R2 mice (5 mo of age) were stained with H/E (top) or analyzed by immunofluorescence with antibodies against keratin 10 (green) and keratin 14 (red; second from the top), keratin 6 (red; third from the top), or loricrin (green; bottom). Nuclei were counterstained with Hoechst (blue) in the two bottom panels. E, epidermis; D, dermis; HF, hair follicles. Bars, 100 μ m.

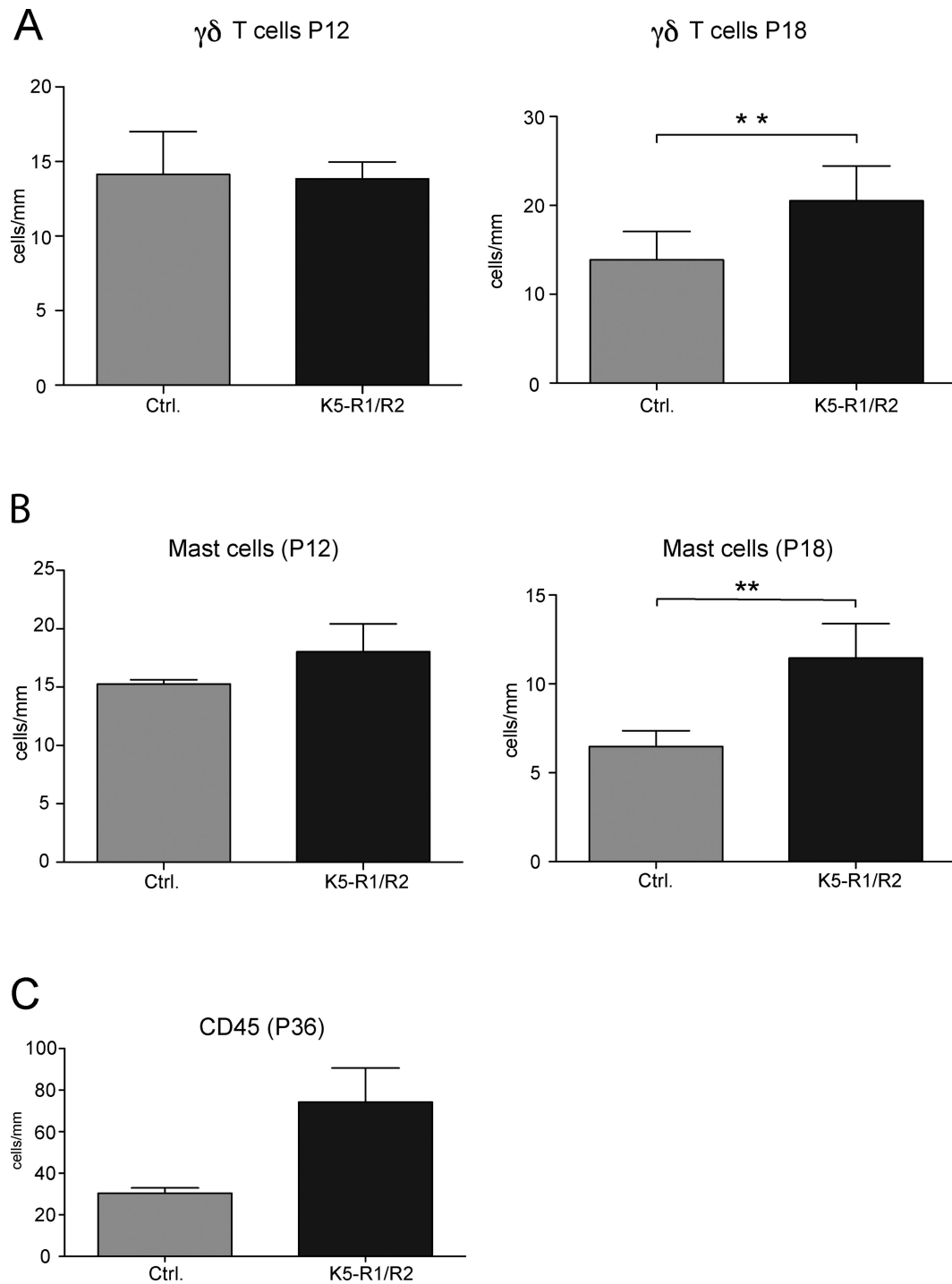


Figure S2. **Quantification of mast cells, $\gamma\delta$ T cells, and CD45-positive cells in control and K5-R1/R2 mice.** (A) $\gamma\delta$ T cells were identified by immunofluorescence with an antibody against the $\gamma\delta$ T cell receptor. Their number in the back skin epidermis was quantified in seven sections per mouse at 200 \times magnification. Error bars indicate mean \pm SD. $n = 3$ control mice and 6 K5-R1/R2 mice at P12, and 7 mice per genotype at P18. (B) Mast cells were stained with toluidine blue. Their number in the dermis of back skin was quantified in eight serial images at 200 \times magnification. Error bars indicate mean \pm SD. $n = 4$ control mice and 6 K5-R1/R2 mice at P12, and 6 mice per genotype at P18. (C) CD45-positive cells were identified by immunofluorescence in P36 back skin from control and K5-R1/R2 mice. Error bars indicate mean \pm SD. $n = 4$ mice per genotype. *, $P \leq 0.05$; **, $P \leq 0.005$; ***, $P \leq 0.001$.

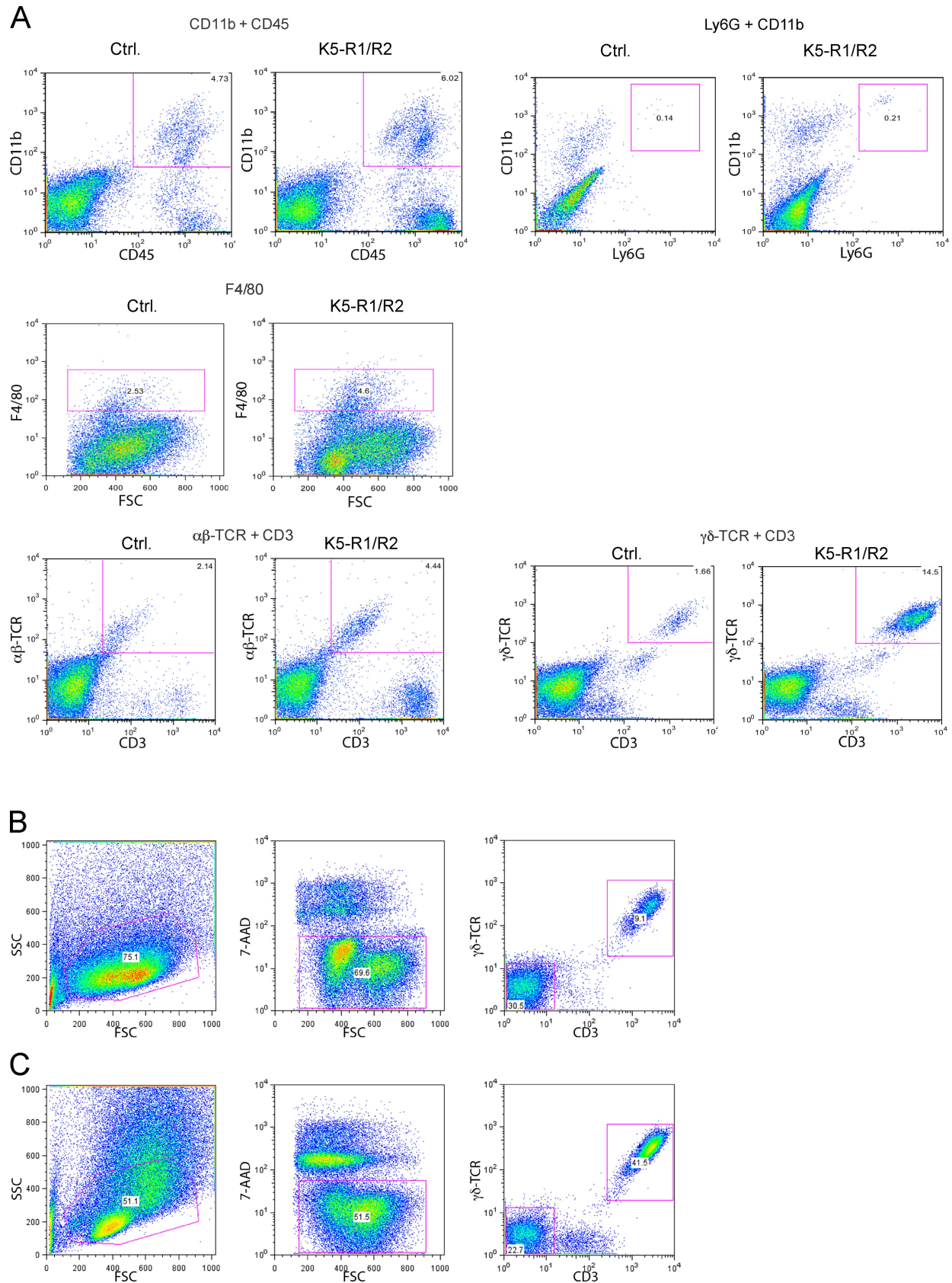


Figure S3. **Analysis of immune cells in the dermis and epidermis of adult control and K5-R1/R2 mice by FACS.** (A) Cells from isolated dermis of 12-mo-old mice were analyzed by FACS using antibodies against different inflammatory cell markers. $n = 4$ mice per genotype. Original FACS data are shown. Results are summarized in Fig. 5 B. (B and C) Cells from isolated epidermis of adult control (B) and K5-R1/R2 (C) mice were sorted by FACS using antibodies against CD3 and the $\gamma\delta$ T cell receptor to separate $\gamma\delta$ T cells from the remaining epidermal cells (mainly keratinocytes). Original FACS data are shown. The sorted cells were used for RNA isolation and subsequent RT-PCR analysis (shown in Fig. 6 D). The percentage of positive cells among viable cells is shown (numbers inside boxes).

Tight junction gene expression in control mice

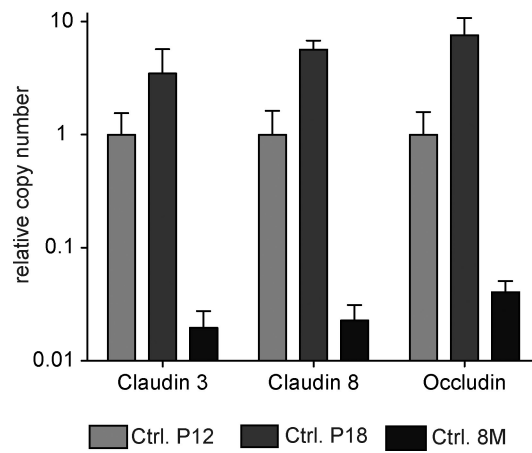


Figure S4. **Time course of claudin 3, claudin 8, and occludin expression during postnatal development.** RNA was isolated from the epidermis of control mice at P12, P18, and 8 mo (8M) and analyzed by real-time RT-PCR for the levels of claudin 3, claudin 8, and occludin mRNAs. Amplification of *Rps29* cDNA was used for normalization. $n = 3-5$ per time point. Expression levels at P12 were arbitrarily set as 1. Error bars represent mean \pm SD.