SUPPLEMENTAL MATERIAL

Schuster et al., http://www.jem.org/cgi/content/full/jem.20081747/DC1

Table S1. Composition of whole embryonic and adult skin single cell suspensions

	Embryonic skin	Adult skin
CD45$^+$ cells	1.13 ± 0.43% SD (n=18)	11.59 ± 3.51% SD (n=7)
CDCP1$^+$ cells	8.28 ± 2.16% SD (n=3)	60.53 ± 19.60% SD (n=3)
Dermal CD45$^-$ cells	90.59 ± 2.16% SD (n=3)	27.64 ± 19.05% SD (n=5)

Percentage of CDCP1$^+$ (CD318) keratinocytes, CD45$^+$ leukocytes, and CD45$^-$ dermal cells was determined by flow cytometry, and at least 100,000 cells were acquired per experiment.

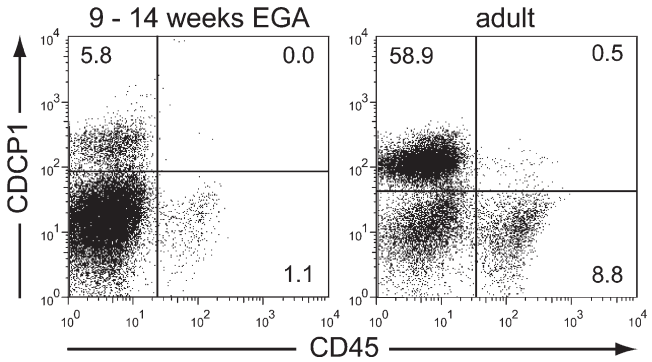

Figure S1. Cellular composition of whole embryonic and adult skin single cell suspensions. Percentage of CDCP1 (CD318)-expressing keratinocytes and CD45-expressing leukocytes was determined by flow cytometry. Dot plots are representative of three to five experiments per group.

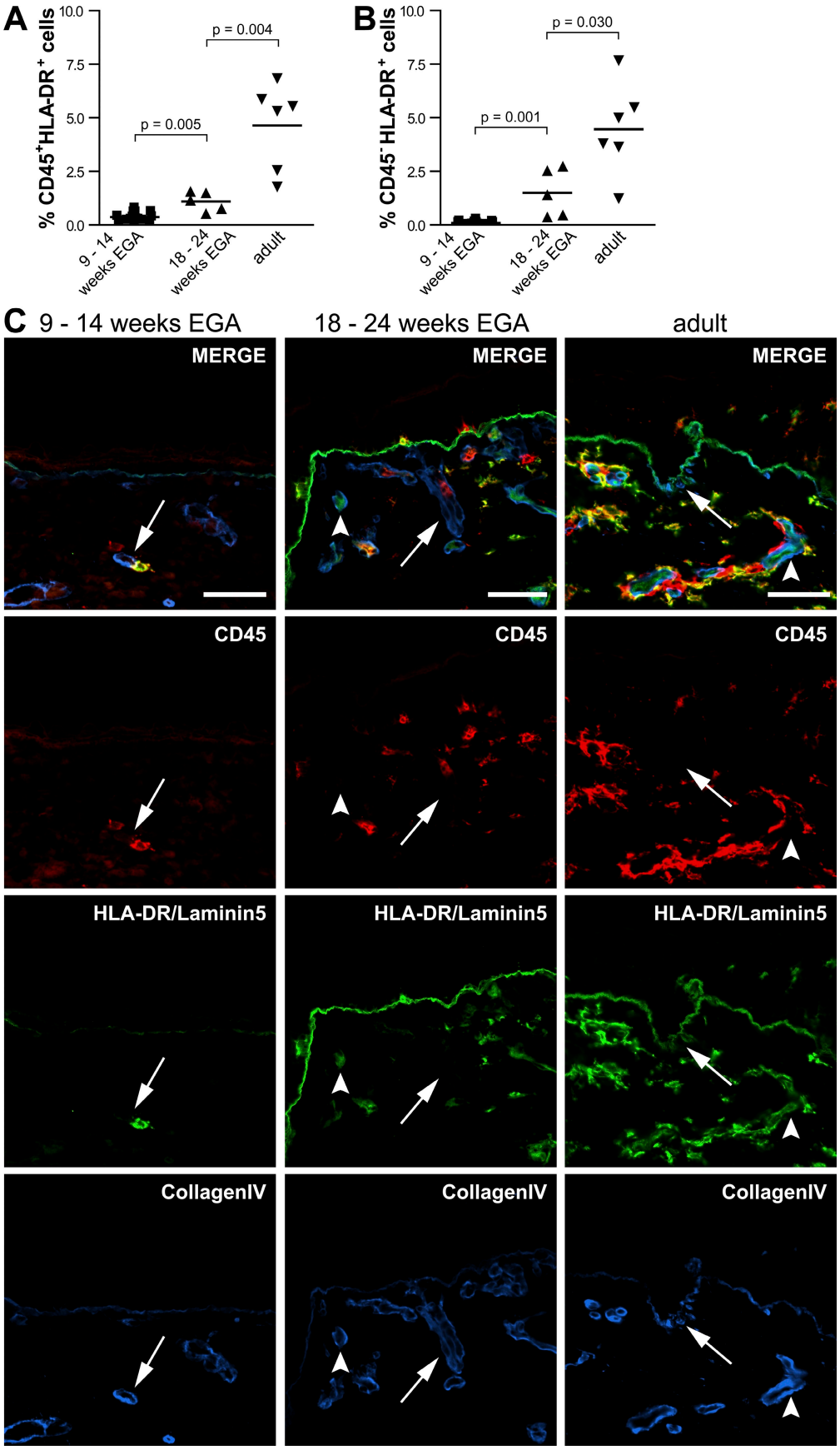

Figure S2. HLA-DR expression on endothelial cells appears in fetal skin. Dot graph shows the percentage of (A) $CD45^{+}HLA\text{-}DR^{+}$ and (B) $CD45^{-}HLA\text{-}DR^{+}$ cells among total skin cells at different stages of development. 9–14 wk EGA: A and B, n=15. 18-24 wk EGA: A and B, n=5. Adult: A and B, n=6. (C) Immunofluorescence triple labeling identified HLA-DR expression on some endothelial cells on the luminal sides of collagen type IV^{+} vascular basement membranes of vessels in fetal skin (arrowheads). Arrows denote vessels which show no HLA-DR staining. Data are representative of at least three experiments per group. Bars, 50 µm.

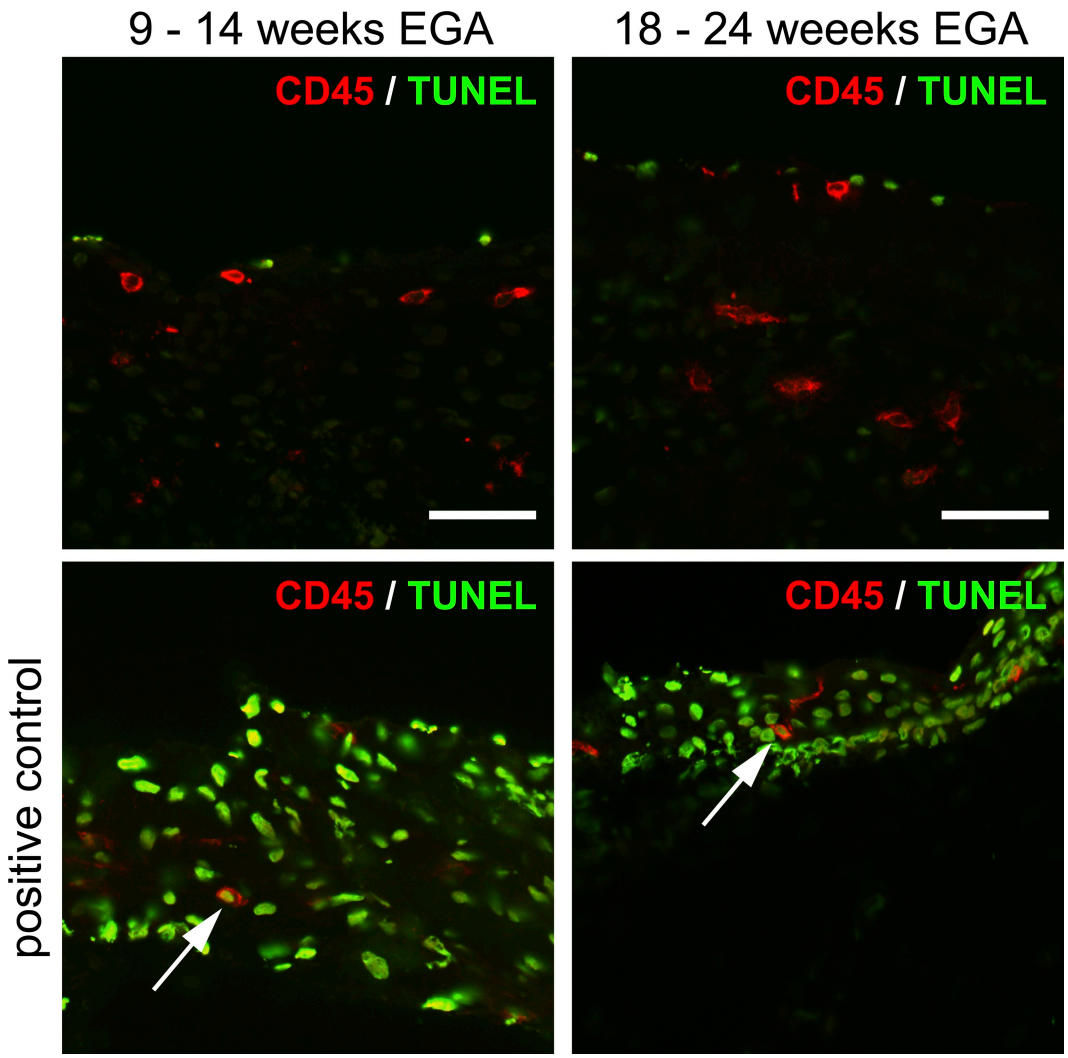

Figure S3. Leukocytes do not undergo apoptosis during skin development. CD45/TUNEL double immunofluorescence staining was performed on cryostat sections of embryonic and fetal skin. Arrows in the positive control denote TUNEL$^+$/CD45$^+$ cells. Data are representative of at least three experiments per group. Bars, 50 μm.

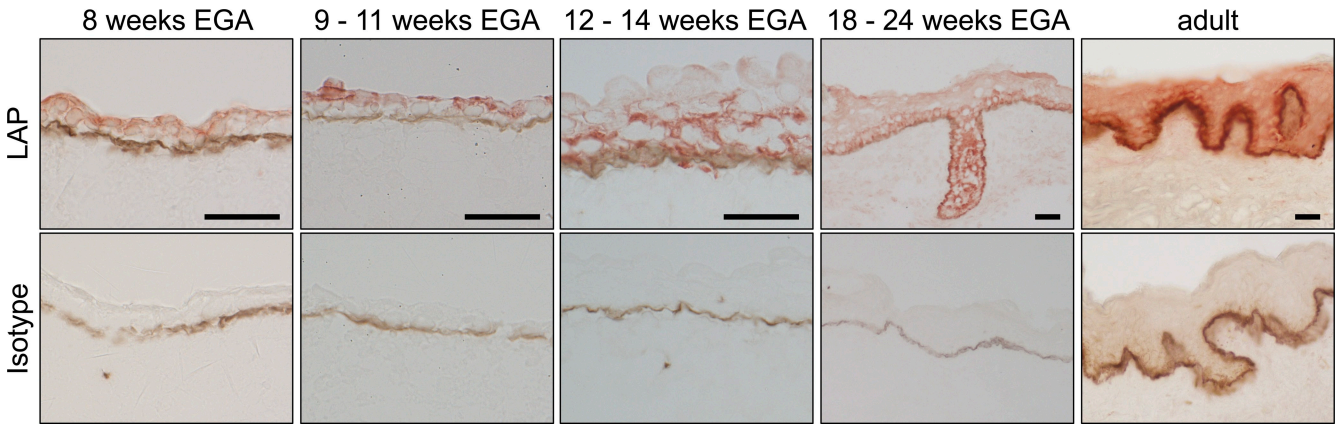

Figure S4. Epidermal LAP production precedes TGF-β1 production. Double immunohistochemical staining was performed on cryostat sections of embryonic, fetal and adult skin. LAP was visualized with AEC (red color). The dermo-epidermal junction was identified both in the TGF-β1 staining and in the controls with mAb directed against collagen type IV until 14 wk EGA and Laminin5 in the remaining age groups and visualized with DAB (brown line). Data are representative of at least three experiments per group. Bars, 50 µm.