Supporting Information

Drexler et al. 10.1073/pnas.1209171109

SI Materials and Methods

Two-Step Carcinogenesis. A single dose of DMBA [7,12-Dimethylbenz(a)anthracene] (100 nmol in acetone/100 μL acetone; Sigma-Aldrich) was topically applied on the shaved back skin, followed by biweekly TPA (12-O-Tetradecanoylphorbol-13-acetate) applications (100 nmol in DMSO/100 μL acetone; Sigma-Aldrich) (1). Some mice cohorts were treated with a batch of DMBA with reduced mutagenic potency (indicated as "low dose DMBA" in Fig. 1*B* and Fig. S3). Tumor number was assessed once a week. A tumor was defined as a dome-forming lesion of at least 1-mm diameter that persisted for two consecutive weeks or more (2). Trials were terminated after about 6 mo or when disease reached the endpoint criteria. Tumor and tissue samples were collected, immediately frozen in liquid nitrogen, and kept frozen until use. For histological analyses, tissues were fixed with 4% paraformaldehyde in PBS, pH 7.4, and then embedded in paraffin.

Preparation of Primary Murine Cells. Tail skin was peeled off the underlying tissue and digested overnight at 4 °C in 8 mg/mL Dispase II (Roche). The epidermal sheets were separated from the underlying dermis and both were digested in 0.05% trypsin, 1 mM EDTA (Gibco) for 10 min at 37 °C. The resulting dermal and epidermal cells were either prepared for Western blot analysis or the epidermal suspension was filtered through a 100-μm cell culture mesh and cultured in Cnt-57 basal keratinocyte medium (Cellntec) on collagen-coated dishes (Purecol; Nutacon).

Bone marrow-derived macrophages and dendritic cells were differentiated from tibial and femoral bone marrow cells, as described previously (3, 4)

Immunohistochemistry. For immunohistochemistry, 4-μm sections were cut from the paraffin blocks of the paraformaldehyde-fixed human tissue samples obtained from University Hospital Tübingen after approval from the local ethics committee (University of Tuebingen; 547/2011BO2). After pretreatment with 10 mM citrate buffer (100 °C, 5 min) for epitope retrieval, sections were incubated with rabbit anti-apoptosis-associated speck-like protein containing a CARD (ASC) (2 μg/mL; Adipogen) followed by HRP-labeled secondary antibody and visualized by HRP (DAKO). Nuclei were counterstained with hematoxylin.

1. Fürstenberger G, Kopp-Schneider A (1995) Malignant progression of papillomas induced by the initiation—Promotion protocol in NMRI mouse skin. *Carcinogenesis* 16 (1):61–69.
2. Gebhardt C, et al. (2008) RAGE signaling sustains inflammation and promotes tumor development. *J Exp Med* 205(2):275–285.
3. Didierlaurent A, et al. (2006) Tollip regulates proinflammatory responses to interleukin-1 and lipopolysaccharide. *Mol Cell Biol* 26(3):735–742.
4. Gross O, et al. (2006) Card9 controls a non-TLR signalling pathway for innate antifungal immunity. *Nature* 442(7103):651–656.

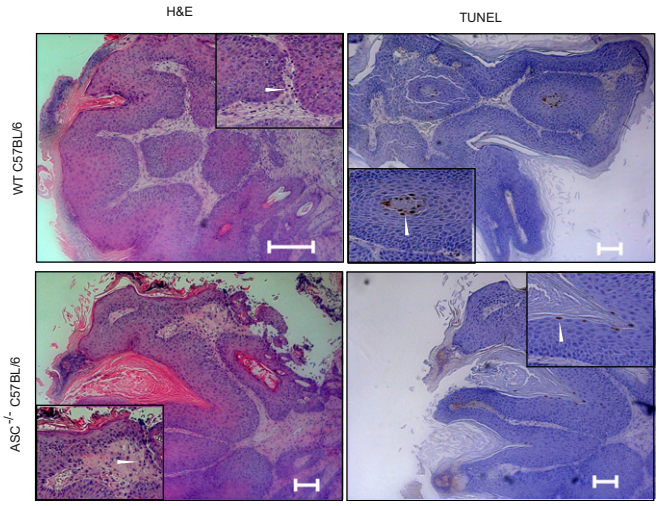

Fig. S1. Tumors from ASC knock-out mice do show no difference to WT tumors in inflammatory cell infiltration and cell death. Histology was performed on tumors from WT and ASC$^{-/-}$ mice at trial termination. H&E and TUNEL stainings were performed to visualize cellular infiltrate into skin and apoptotic cells. (Scale bars, 100 μm.)

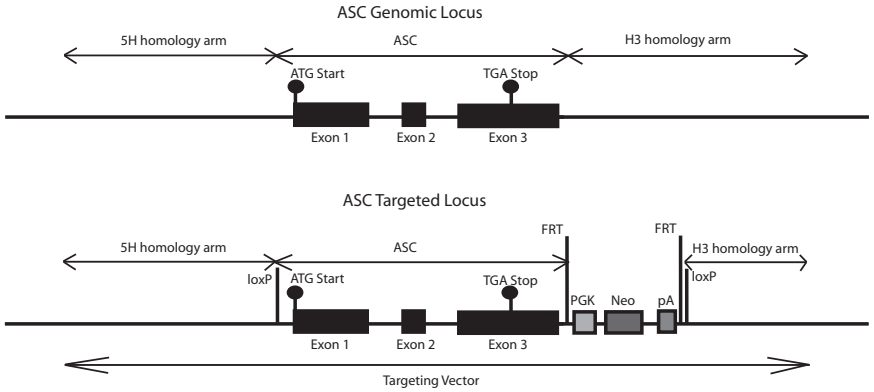

Fig. S2. Generation of floxed ASC mice. The genomic and the targeted locus are shown. Exons are highlighted as black boxes. The targeting vector contains a PGK-neomycin resistance cassette (gray boxes) and introduces loxP sites to excise the entire ASC gene.

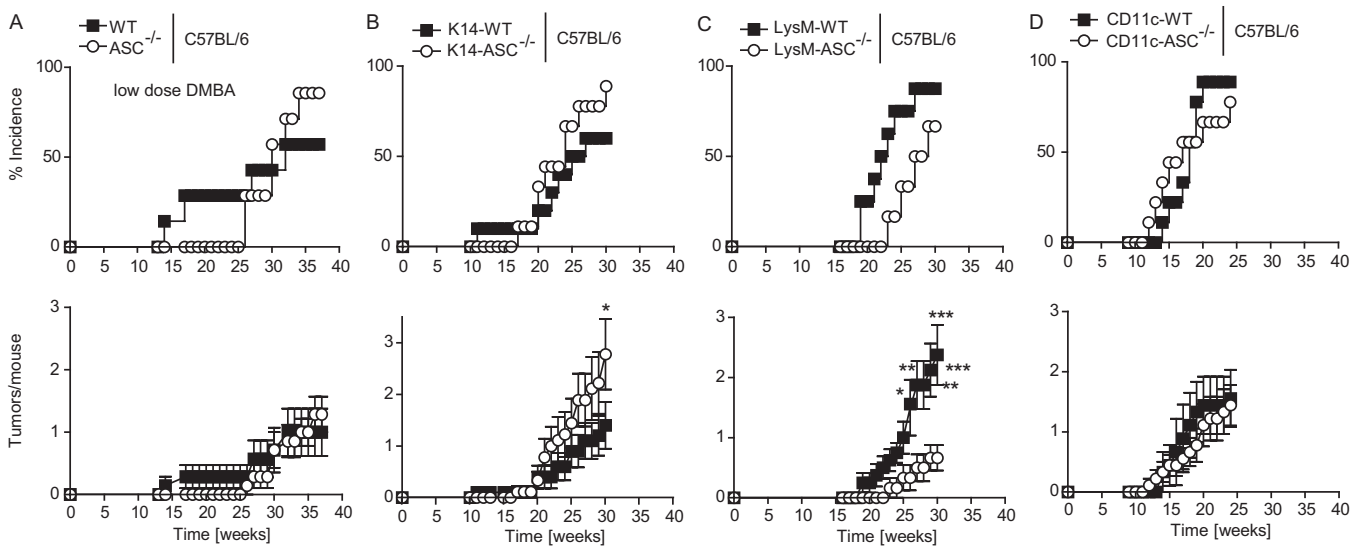

Fig. S3. The phenotype of susceptibility to epithelial skin cancer of ASC$^{-/-}$, K14-ASC$^{-/-}$, and LysM-ASC$^{-/-}$ is reproducible in independent experiments. CD11c-ASC$^{-/-}$ do not show a phenotype in the susceptibility to epithelial skin cancer. (*A*) ASC$^{-/-}$ (littermate WT $n = 7$, ASC$^{-/-}$ $n = 7$), (*B*) K14-ASC$^{-/-}$ (littermate K14-WT $n = 10$, K14-ASC$^{-/-}$ $n = 9$), (C) LysM-ASC$^{-/-}$ (littermate LysM-WT $n = 8$, LysM-ASC$^{-/-}$ $n = 6$), and (*D*) CD11c-ASC$^{-/-}$ (littermate CD11c-WT $n = 9$, CD11c-ASC$^{-/-}$ $n = 9$) mice were treated with the DMBA/TPA protocol, and tumors were recorded. Tumors larger than 1 mm were counted. Data are expressed as mean ± SEM, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

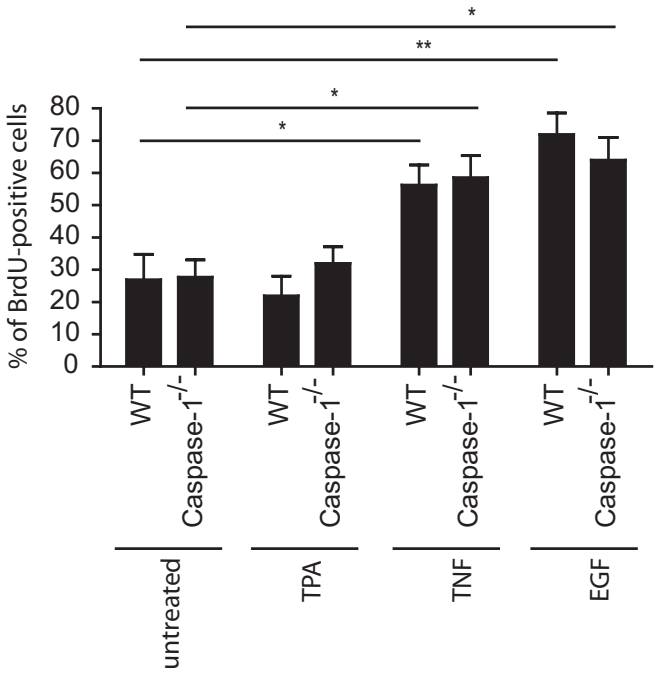

Fig. S4. Caspase-$1^{-/-}$ keratinocytes do not show differences in BrdU staining. After 5 d in culture in the presence or absence of TPA, TNF, or EGF WT and caspase-$1^{-/-}$ primary murine keratinocytes were incubated for 30 min BrdU, stained with an anti-BrdU antibody, and analyzed by fluorescent microscopy. *$P \leq 0.05$; **$P \leq 0.01$.

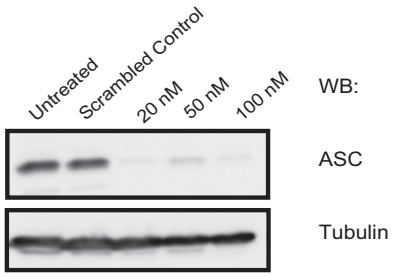

Fig. S5. ASC knock-down achieved through siRNA transfection in HaCaT cells. HaCaT cells were transfected with 20 nM, 50 nM, and 100 nM of siRNA against ASC and analyzed at time 72 h for ASC expression by Western blot. Tubulin was used as a loading control.