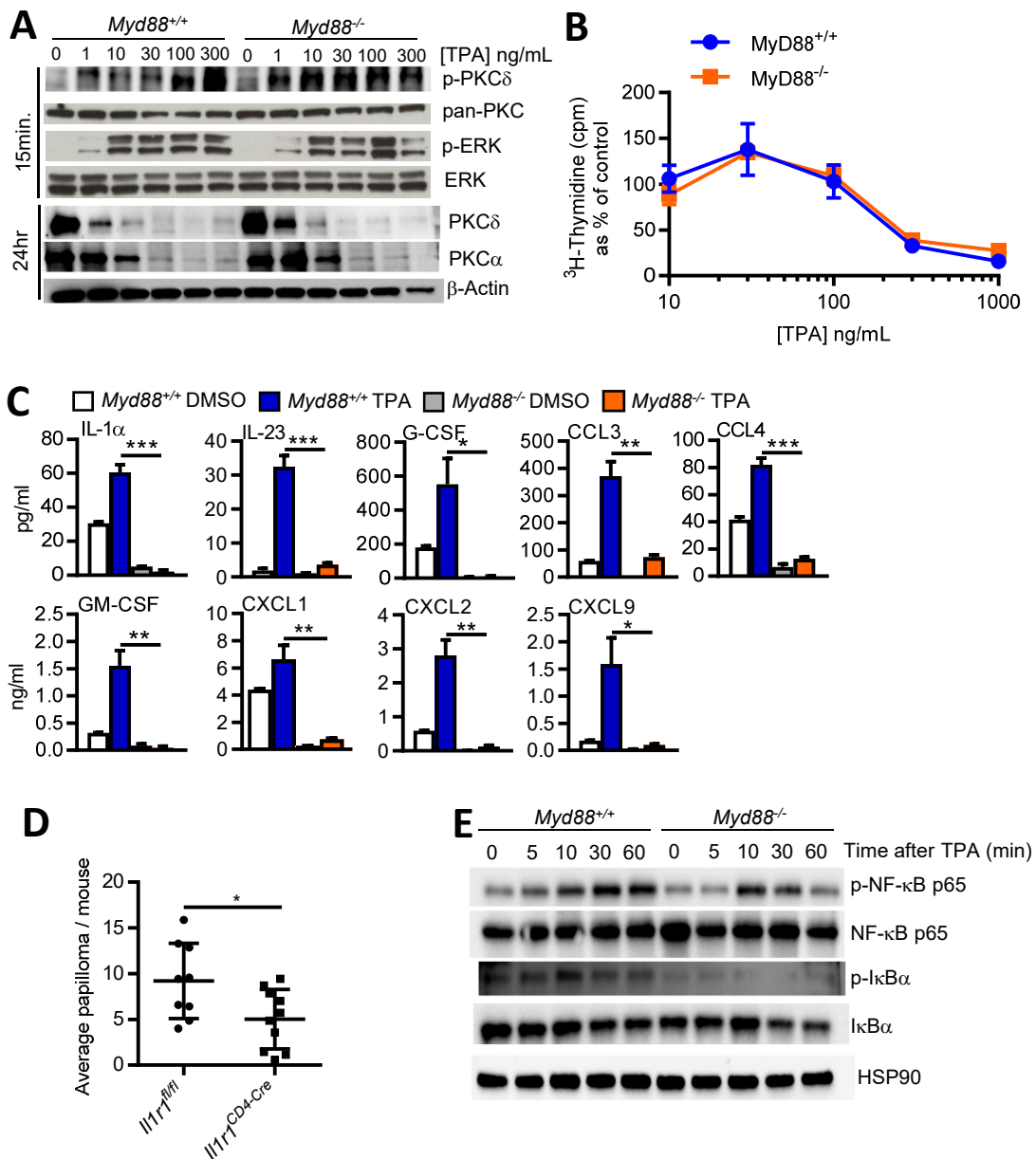
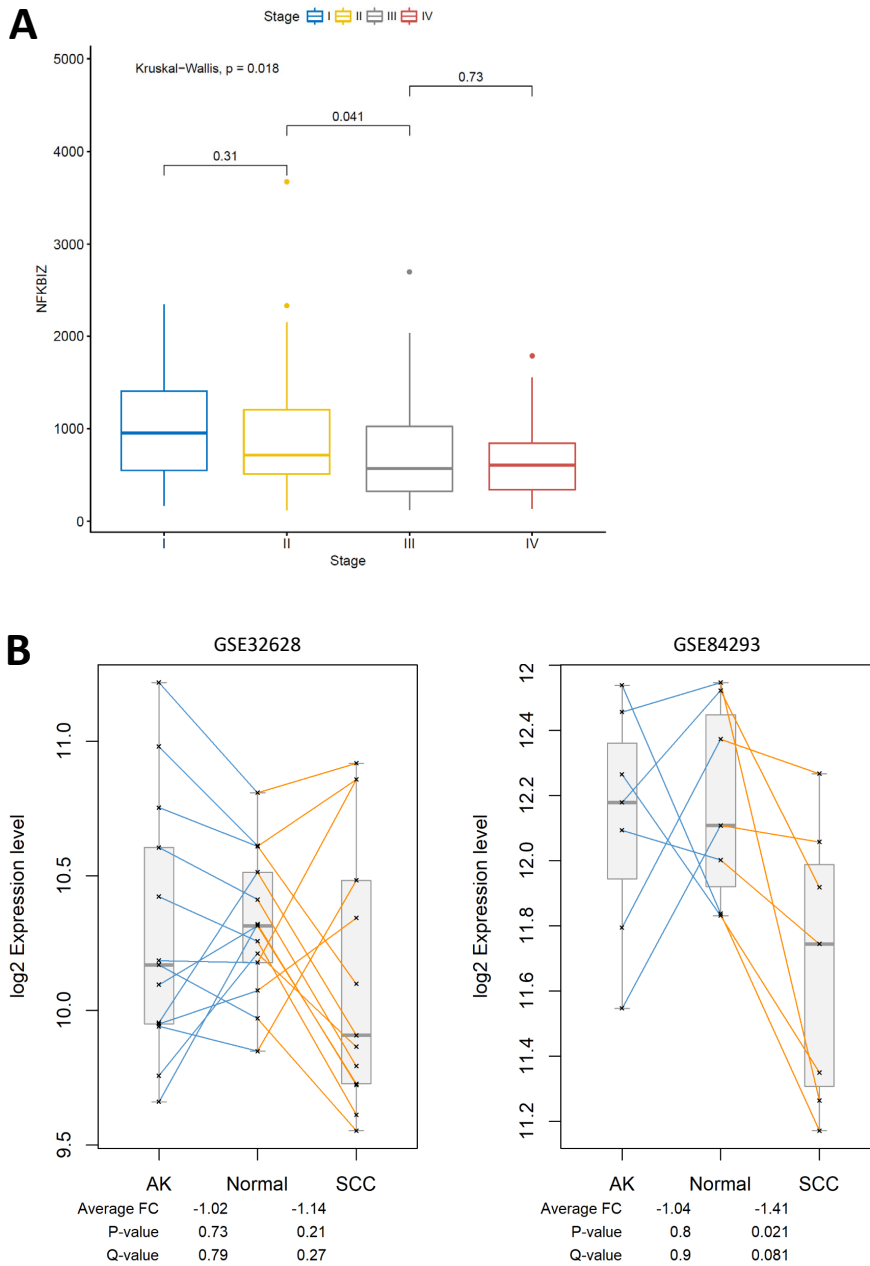


Supplemental figure 1: MyD88 deficiency diminishes the induction of pro-inflammatory factors in keratinocytes. (A) Immunoblotting of total cell extracts from cultured primary WT or MyD88^{-/-} keratinocytes treated with indicated TPA doses for 15 minutes or 24 hours. p-, phosphorylated. (B) Tritiated thymidine incorporation was measured in WT or MyD88^{-/-} keratinocyte cultures treated with TPA for 24 hours. Data shown are representative of two independent experiments, and bars represent the mean ± SEM value of four replicates. (C) Cytokine and chemokine concentrations (ELISA) in culture supernatants from WT and MyD88^{-/-} collected 24h after DMSO or TPA treatment. (D) Deletion of IL-1R in T cells (CD4-cre⁺/Il1r1^{fl/fl} mice) reduces the yield of tumors in DMBA/TPA treated mice. Plot represent the mean number of skin tumors per mouse (mean ± SEM) at week 20 post-initiation. Mice were treated with DMBA/0.2 mL acetone at time 0 then with 10 nmol TPA/0.2 mL acetone twice a week for up to 20 wk. Il1r1^{fl/fl} (n = 9), Il1r1^{CD4-Cre} (n = 10), * P < 0.05. (E) Immunoblotting of total cell extracts from primary WT or MyD88^{-/-} keratinocytes treated with TPA for the indicated period. HSP90, heat shock protein 90.



Supplemental figure 2: *NFKBIZ* expression in colon adenocarcinoma according to stage (A) and expression in cutaneous SCC, normal skin (NS) and actinic keratoses (AK) from GSE 32628 and GSE84293 (B).



Footnote: GSE32628 is from Hameetman L, Commandeur S, Bavinck JN, Wisgerhof HC *et al.* Molecular profiling of cutaneous squamous cell carcinomas and actinic keratoses from organ transplant recipients. *BMC Cancer* 2013 Feb 5;13:58. [PMID: 23379751](https://pubmed.ncbi.nlm.nih.gov/23379751/). GSE84293 is from Chitsazzadeh V, Coarfa C, Drummond JA, Nguyen T *et al.* Cross-species identification of genomic drivers of squamous cell carcinoma development across preneoplastic intermediates. *Nat Commun* 2016 Aug 30;7:12601. [PMID: 27574101](https://pubmed.ncbi.nlm.nih.gov/27574101/)

Supplemental figure 3: Schematic depiction of tissue autonomous and systemic contributions for the development of squamous skin tumors.

It is well known that initiation of keratinocytes through *Hras* mutations is not sufficient to evoke tumor formation in the context of the intact skin environment. The current study reveals how signaling through NF- κ B in multiple compartments amplifies the elaboration of factors required for a tumor promoting environment. We now show that the release of NF- κ B dependent cytokines and chemokines from RAS initiated keratinocytes combined with similar factors released by TPA treated normal keratinocytes is not sufficient for tumor eruption in the absence of host-derived IL-17. Factors released by RAS initiated keratinocytes and TPA treated normal keratinocytes, and particularly IL-1 α , attract and polarize IL-17 producing T cells to the skin through a NF- κ B dependent process. The release of IL-17 in the tumor microenvironment and its action on both normal and initiated keratinocytes (depicted in red as RAS* and light grey as normal) provides sufficient signal amplification to fully evoke tumor emergence and growth. While NF- κ B is required for every step in the process, it appears that oncogenic RAS, TPA and IL-17 signaling through I κ B ζ provide crucial selection for expression of pro-tumorigenic molecules from each contributing cell type.

