SUPPLEMENTAL MATERIAL

Unprocessed interleukin-36α regulates psoriasis-like skin inflammation in co-operation with interleukin-1

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Running title: Co-operation between IL-36 and IL-1 in psoriasis

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Gene	Targeting strategy and validation
IL-36α	Generation initiated at GSK through standard homologous recombination eliminating the mRNA nucleotides 121-263, NM_019450.3. Consequently, part of the 5'UTR and the first 34 codons are absent from the targeted gene (Fig. S1). Backcrossed to C57BL/6 4 times at GSK, after which it was transferred to our lab. Backcrossed to C57BL/6 for >6 generations in our lab. IL-36α mRNA levels reduced 30-fold. (Fig. S2a). No IL-36α protein, or fragment thereof, detected in skin (Fig. S2b).
IL-36β	Strain was generated as part of the Knockout Mouse project (KOMP) using a VelociGene KOMP Definitive Null Allele Design ablating the entire Il1f8 coding region, i.e., no mRNA can be expressed. The strain was generated directly on the C57BL/6 background. Phenotypic characterization was not performed by the KOMP.
IL-36γ	Origin: Genentech/Lexicon Pharmaceuticals (repository: MMRRC). Targeting approach: Standard homologous recombination deleting coding exons 1-3 (1645 bp region, first 124 amino acids). Phenotype characterization: http://mmrrc.mousebiology.org/phenotype/Genentech/SEC544N1-treeFrame.html. The strain was backcrossed to C57BL/6 for >10 generations. IL-36γ mRNA not detected.

Table S1: Targeting strategies, sources and verification of IL-36 KO strains. The three IL-36 KO strains were developed and validated as indicated. Because the three *Il1f* genes are localized within a 73 Kbp region on chromosome 2, we confirmed that ablation of one *Il1f* did not significantly affect basal expression of one of the other IL-36 mRNAs (data not shown). IL-36 KOs were born at an expected ratio from het x het matings, and appeared normal at birth through life. Homozygous KO breeders lived beyond one-year of age without any health disparities. The three strains breed well and overall have no apparent defects or obvious phenotypes. Average morning glucose levels (130-140 mg/dL) were not statistically different from those of wild type mice (data not shown). Proportions of peripheral neutrophils, monocytes and lymphocytes did not deviate from those of wild type mice (data not shown).

a

b

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  1 MNKEKELRAA SPSLRHVQDL SSRVWILQNN ILTAVPRKEQ TVPVTITLLP CQYLDTLETN
 61 RGDPTYMGVQ RPMSCLFCTK DGEQPVLQLG EGNIMEMYNK KEPVKASLFY HKKSGTTSTF
121 ESAAFPGWFI AVCSKGSCPL ILTQELGEIF ITDFEMIVVH
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Figure S1: Targeting strategy to ablate IL-36α expression. (**a**) Coding and non-coding sequences of the mouse *Il1f6* gene are shown in dark and light green, respectively. An HSV-TK/neomycin resistance cassette was inserted through homologous recombination to replace the 3' 40 bp of exon 2 (starting in the non-coding region) through the first 86 bp of exon 3. (**b**) The full-length mouse IL-36α protein sequence is shown. Truncated region of the successfully targeted *Il1f6* is underlined. Predicted pro-domain of IL-36α is highlighted in blue.

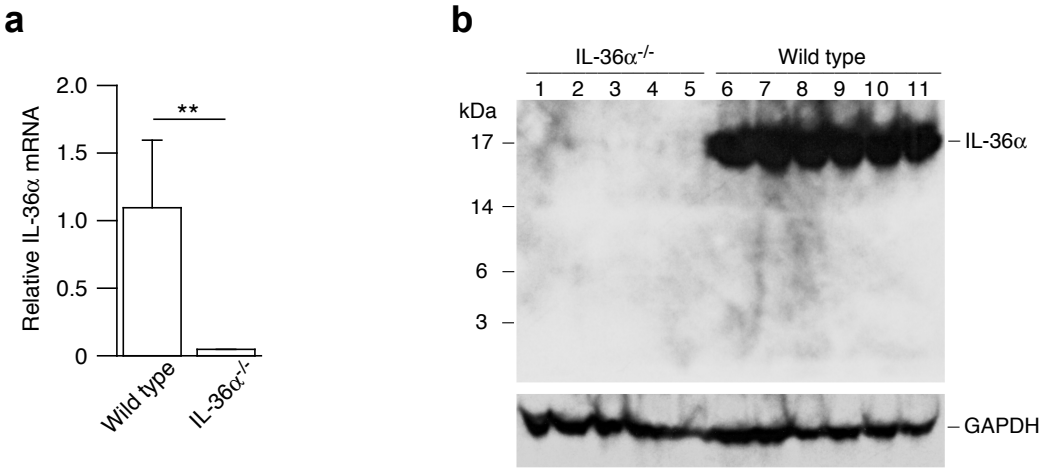

Figure S2: Confirmation of *Il1f6* knockout targeting. Wild type (n=6) and IL-36α KO (n=5) mice were treated with imiquimod 4 times and RNA (**a**) and total protein (**b**) isolated from full thickness skin. (**a**) Relative expression of IL-36α mRNA was examined using real-time PCR (mean + SD). (**b**) Levels, and potential size, of IL-36α protein were examined by Western blotting of total protein extracts from skin. Data from one of two independent experiments with similar outcomes are shown. **, $P < 0.01$.

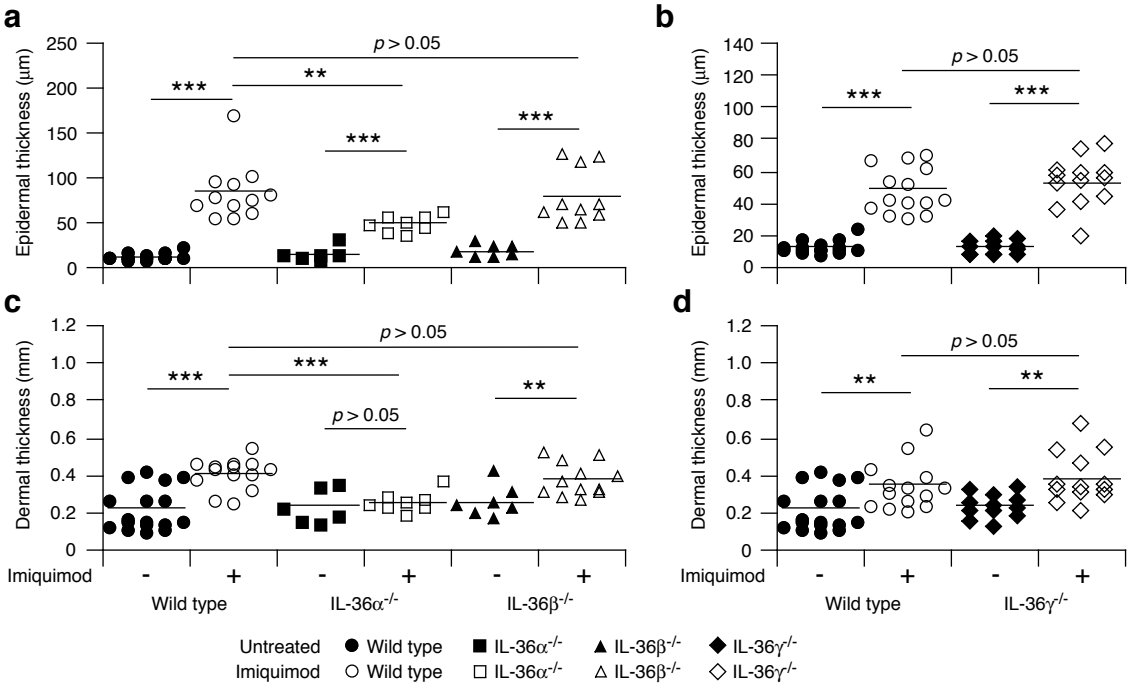

Figure S3. IL-36α plays a significant role in psoriasiform skin disease induced by imiquimod. Wild type (circles), IL-36$\alpha^{-/-}$ (squares), IL-36$\beta^{-/-}$ (triangles) and IL-36$\gamma^{-/-}$ (diamonds) mice were treated with imiquimod for 4 days (open symbols) and skin collected the day after the last application. Control mice (filled symbols) were denuded and left untreated until skin collection. Skin sections were H&E stained (Fig. 1) and epidermal (**a**-**b**) and dermal (**c**-**d**) thickness determined using Image J. Each symbol represents a single mouse. Pooled data from 5 (**a** and **c**) and 3 (**b** and **d**) independent imiquimod experiments are shown. **, $P < 0.01$; ***, $P < 0.001$.

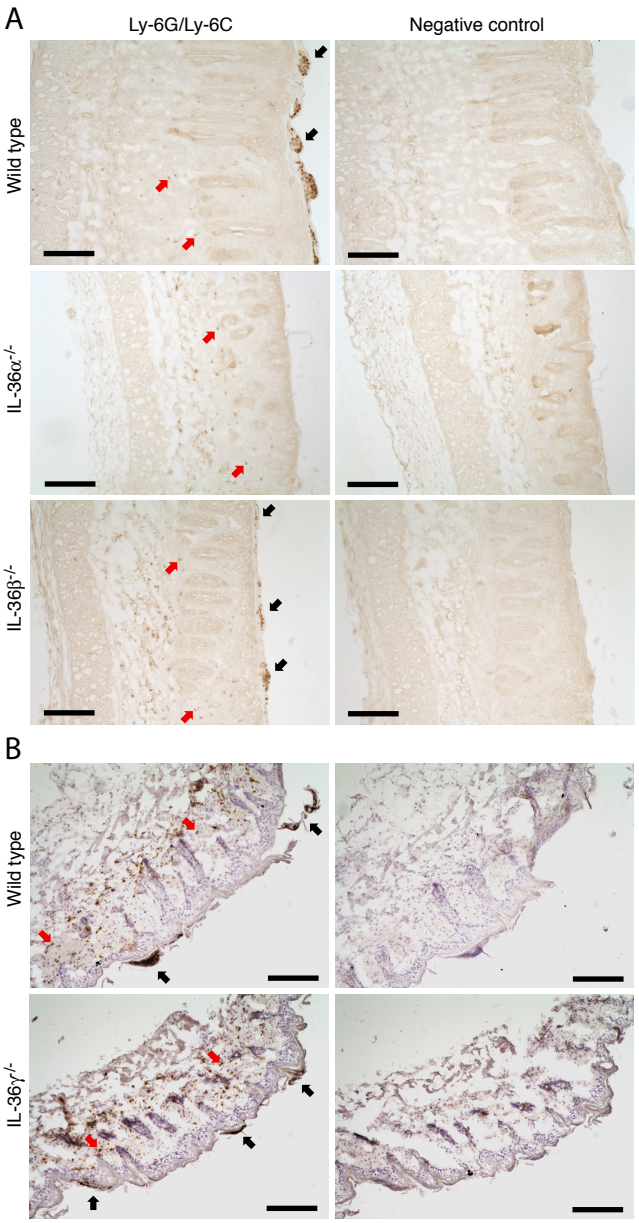

Figure S4: Neutrophil recruitment to the skin is IL-36α dependent. Wild type (**a** and **b**), IL-36$\alpha^{-/-}$ (**a**), IL-36$\beta^{-/-}$ (**a**) and IL-36$\gamma^{-/-}$ (**b**) mice were treated with imiquimod 4 times. Neutrophils recruited to the skin were detected by immunohistochemistry for Ly-6G/Ly-6C. Red arrows indicate individual neutrophils in the dermis. Black arrows indicate clusters of neutrophils (Munro's microabscesses) at the top of the epidermis. Scale bars = 200 μm.

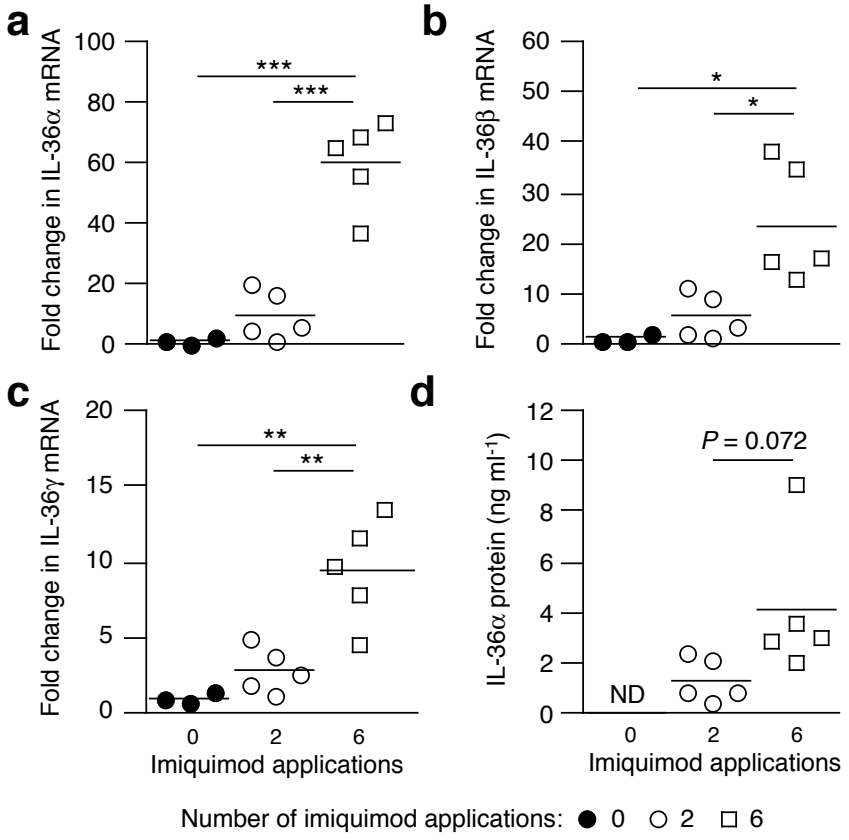

Figure S5: Topical imiquimod treatment leads to IL-36 mRNA induction and IL-36α secretion. Wild type mice (n = 3-5 per time point) were treated with imiquimod 0 (black circles), 2 (open circles) or 6 (open squares) times. Skin was collected and used for 4 mm explant cultures or RNA isolation. Relative expression of IL-36α (**a**), IL-36β (**b**) and IL-36γ (**c**) mRNA was determined using real-time PCR and the ΔΔCt method with GAPDH as the housekeeping gene. (**d**) Levels of IL-36α secreted into culture medium were examined by ELISA. ND, below detection limit. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

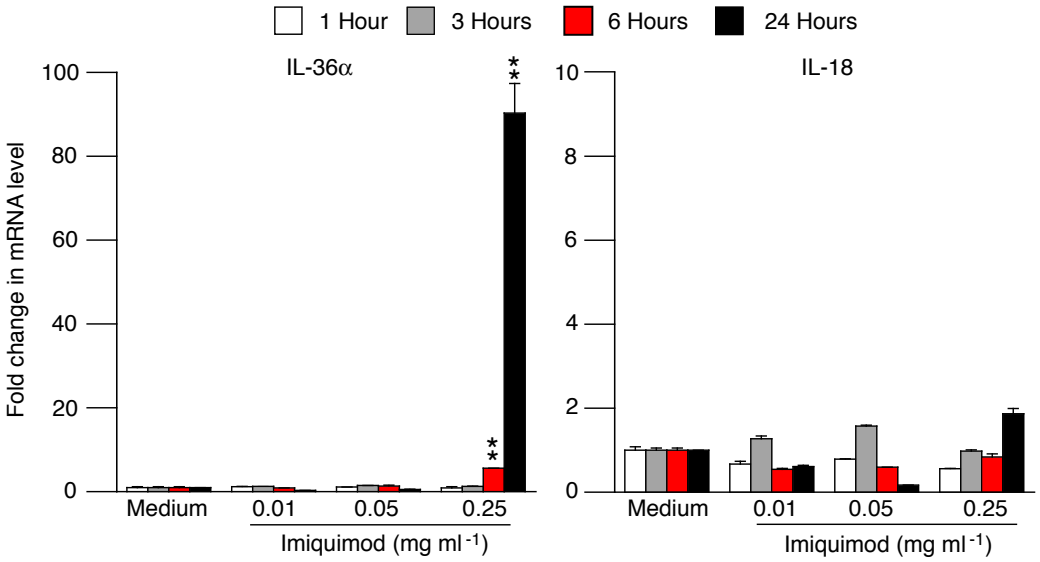

Figure S6: Imiquimod induces IL-36α but not IL-18 mRNA expression *in vitro.* Human primary keratinocytes were treated with imiquimod at indicated concentrations. Cells were harvested at indicated time points and IL-36α and IL-18 mRNA levels evaluated using real-time PCR (mean + SD). **, $P < 0.01$.

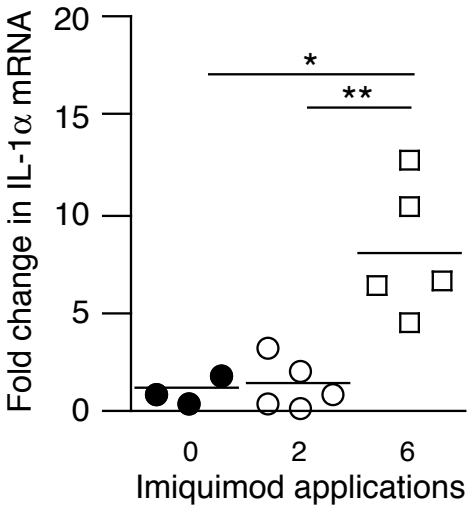

Figure S7: IL-1α mRNA expression is induced in the imiquimod-model. Wild type mice were treated with imiquimod 0 (black circles), 2 (white circles) or 6 (white squares) times and expression of IL-1α mRNA examined as described in Fig. 4.

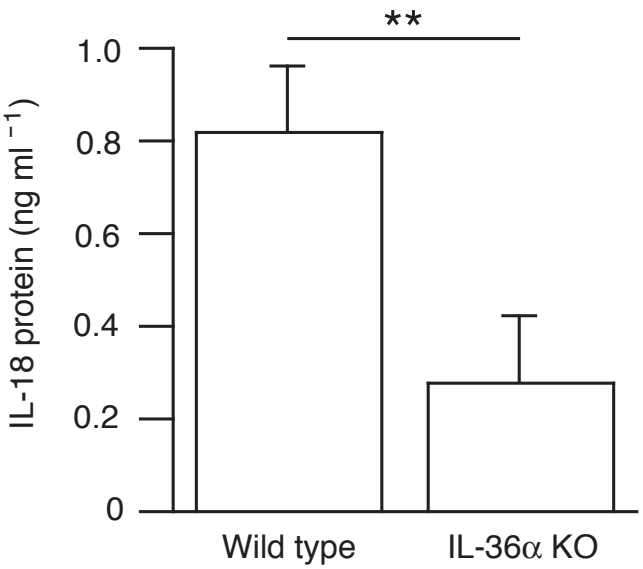

Figure S8: IL-36α KO mice have reduced secretion of IL-18. Wild type (n=5) and IL-36α (n=3) KO mice were treated with imiquimod for 4 consecutive days. Mice were sacrificed 24 hours after the last application. Medium from explanted skin was examined for presence of IL-18 protein by ELISA (mean + SD). **, $P < 0.01$.

Protocol	Gene/mRNA	Primers (5' – 3')
Genotyping	*Il1f6*	GTCACAGTTAAGGCGTTCACC (forward)
		AAGGGCCAGGGCTACTCAC (wild type reverse)
		CTTAATATGCGAAGTGGACCTG (KO reverse)
	Il1f8	CTTAGGGATTGCTGTCCTTG (forward)
		GTGTTATGATTCGGTTCCCAC (wild type reverse)
		GATAGGTCACGTTGGTGTAG (KO reverse)
	Il1f9	CTGGGCTATTTGTATCTTCA (wild type forward)
		CACACCTGCTGGTCCAAGTC (wild type reverse)
		GGCGGATTTCTGAGTTGGAG (KO forward)
		GCAGCGCATCGCCTTCTATC (KO reverse)
Real-time PCR	Mouse IL-36α	ATCTGGACACTCTTGAGACG (forward, exon 4)
		GAGAGGCTTTTACAGGTTCC (reverse, exon 5)
	Human IL-36α	GACCAGACGCTCATAGCAG (forward)
		CTTTAGCACACATCAGGCAG (reverse)
	Mouse IL-36β	CACTATGCATGGATCCTCAC (forward)
		TGTCTCTACATGCTATCAAGC (reverse)
	Mouse IL-36γ	ATGGACACCCTACTTTGCTG (forward)
		CAGGGTGGTGGTACAAATC (reverse)
	Mouse IL-1α	TGAGTCGGCAAAGAAATCAAG (forward)
		AGTGAGCCATAGCTTGCATC (reverse)
	Mouse IL-17A	TTTTCAGCAAGGAATGTGGA (forward)
		TTCATTGTGGAGGGCAGA (reverse)
	Mouse IL-18	TGACCCTCTCTGTGAAGGATAG (forward)
		TTTCAGGTGGATCCATTTCCTC (reverse)
	Human IL-18	TGACTGATTCTGACTGTAGAGATAATG (forward)
		CATACCTCTAGGCTGGCTATCT (reverse)
	Mouse CXCL1	AGCCACACTCAAGAATGGTC (forward)
		GCCATCAGAGCAGTCTGTC (reverse)
	Mouse actin	CATCACACCCTGGTGCCTA (right)
		CACCAGTTCGCCATGGAT (left)

Table S2: Primers used for genotyping and real-time PCR.

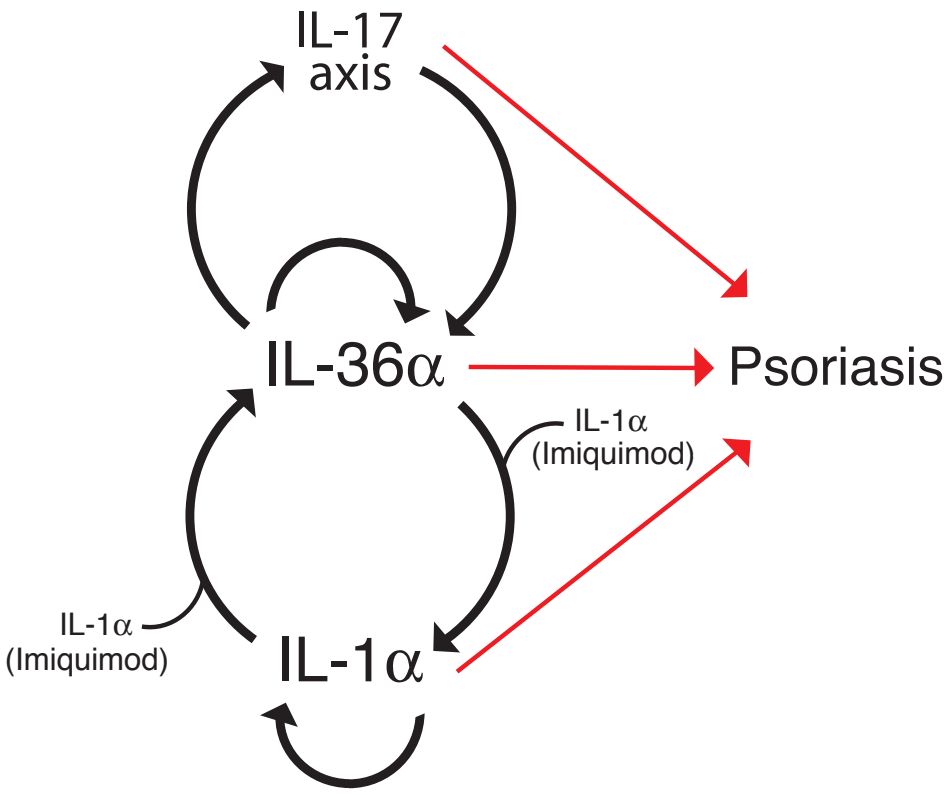

Figure S9: Through mutual regulation IL-36α and IL-1α maintain psoriasiform skin inflammation. Black arrows indicate cytokine interactions. Red arrows indicate progression to disease. See text for details.