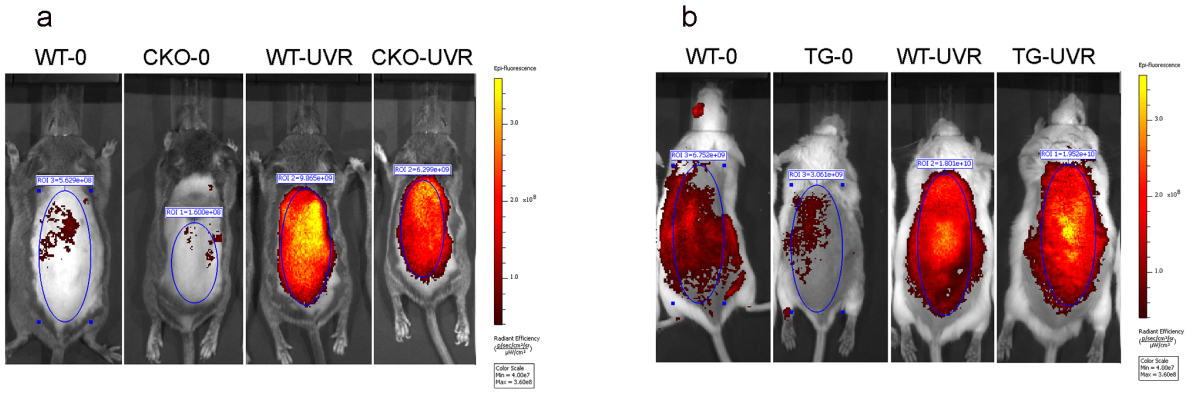


Figure S1. CKO mice have reduced and TG mice have increased neutrophil elastase activity 24 hours following UVR exposure. Representative images of CKO (a) and TG (b) mice injected with a fluorescently labeled neutrophil elastase probe and imaged using the IVIS Spectrum 24 hours after no exposure or exposure to UVR. Blue circles indicate the regions of interest measured.

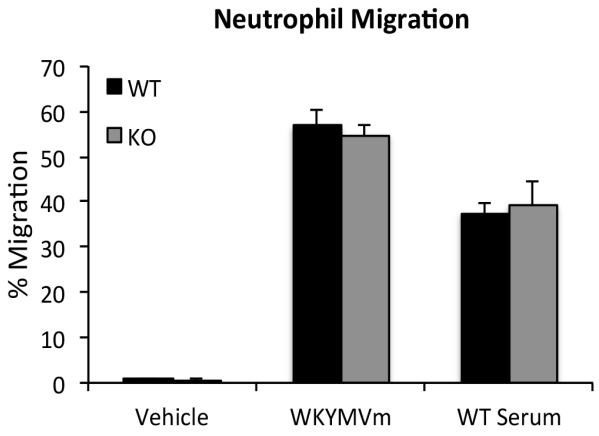


Figure S2. WT and KO neutrophils migrate similarly in response to WKYMVm and normal mouse serum. Neutrophil migration assays were performed as described in Supplementary Material and Methods. For both genotypes, there was little migration in the absence of the neutrophil chemotactant WKYMVm or mouse serum. For both genotypes, the presence of these chemoattractants significantly (P<0.05) enhanced migration; however, there was no significant difference between WT and KO neutrophils under any condition. Data are shown as means of three independent assays ±SD. Groups were compared by ordinary one-way ANOVA followed by Tukey's multiple comparison test.

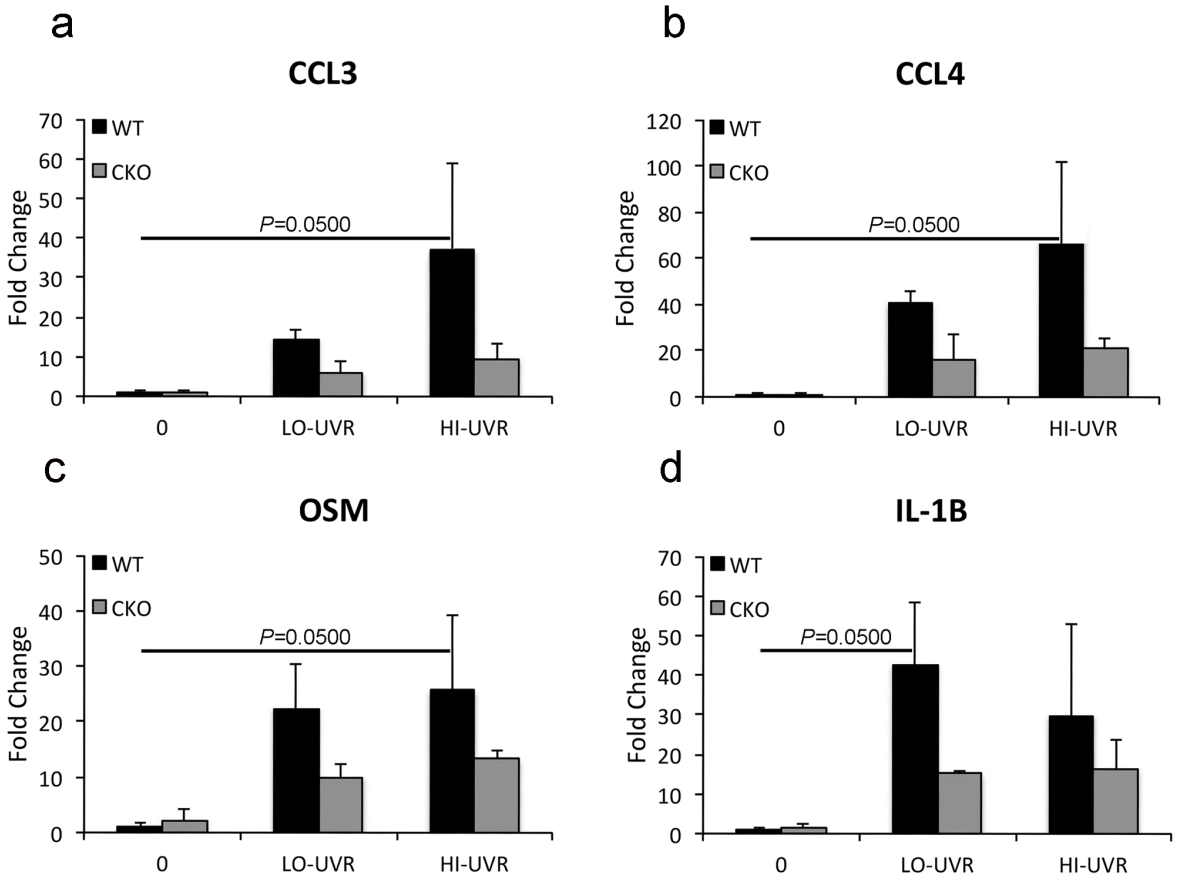


Figure S3. qRT-PCR validates differences between WT and CKO epidermis in UVR induction of proinflammatory cytokines. (a-d) Cytokine induction was determined 24 hours following exposure to 1250 (LO-UVR) or 2500 (HI-UVR) J/m^2 UVR in WT and CKO mice. Expression of the tested cytokines did not differ between unexposed WT and CKO epidermis. Significant induction of cytokines was observed for WT epidermis exposed to UVR compared to unexposed WT epidermis. CKO epidermis did not show significant induction of any cytokine compared to unexposed CKO epidermis. Values shown are the means of values from three mice ±SD. Values were compared by Kruskal-Wallis ANOVA followed by Dunn's multiple comparison test.

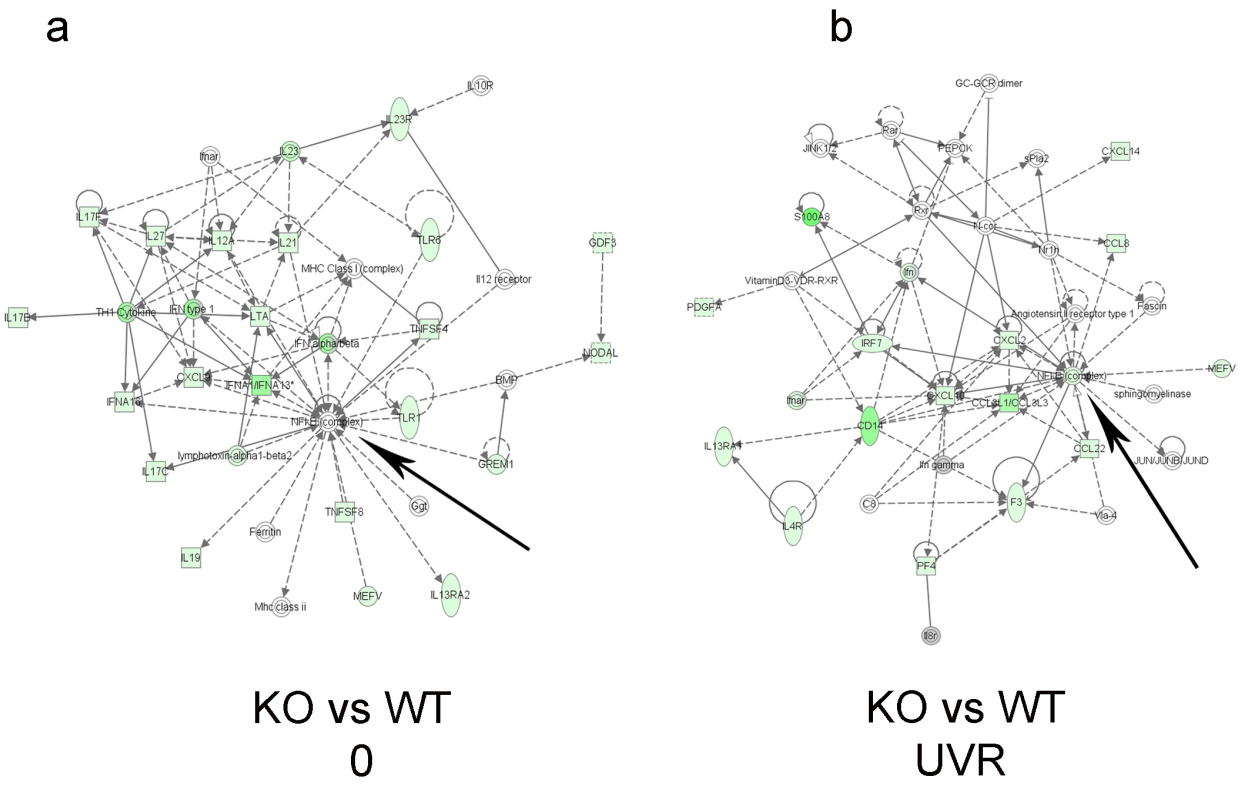


Figure S4. Ingenuity Systems (Redwood, CA, USA) Network Nodes analysis reveals a central role for NF-κB in differences between inflammatory responses in KO and WT mice. (a) Analysis of differences between unexposed epidermis of KO and WT mice using data shown in Table S1. The arrow indicates the NF-κB node. (b) Analysis of differences between UVR-exposed epidermis of KO and WT mice using data shown in Table S1. The arrow indicates the NF-κB node.

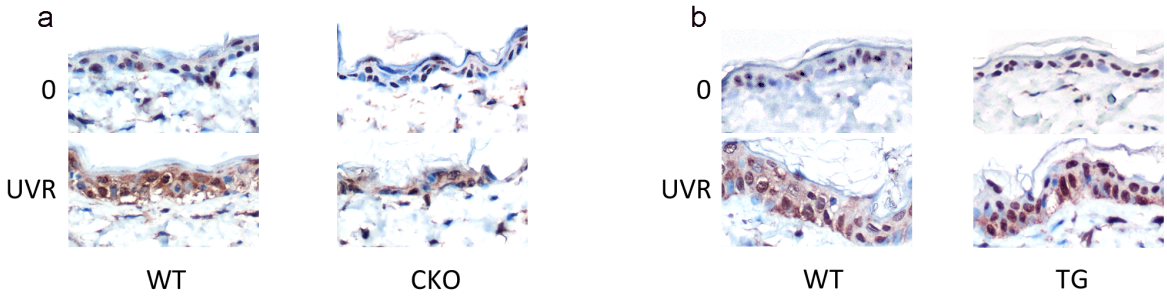


Figure S5. UVR activation of the NF-κB pathway is reduced in CKO compared to WT epidermis and enhanced in TG compared to WT skin. Examples of immunohistochemical staining for phospho-NF-κB in unexposed and UVR-exposed skin. (a) CKO and WT mice were exposed to 0 or 4800 J/m^2 UVR and skin was harvested 24 hours later. (b) TG and WT mice were exposed to 0 or 1500 J/m^2 and skin was harvested 24 hours later.

Table S1. Summary of PAMM 3803 microarray analysis of gene expression in the epidermis of unexposed and UVR-exposed mice

	Fold Change in Expression			
Treatment Group:	WT-0	KO-0	WT-UVR	KO-UVR
Gene Symbol:				
Adora1	1	1.32	1.76	3.02
Ahsg	1	0.75	0.90	1.91
Aif1	1	0.72	1.68	1.75
Apcs	1	0.70	0.66	1.84
Apoa2	1	0.78	1.38	1.74
Apol7a	1	0.62	0.43	0.99
Apol8	1	0.31	0.74	1.18
Areg	1	0.67	3.34	0.98
Bcl6	1	1.51	0.61	0.79
Blnk	1	1.08	0.61	0.79
Bmp1	1	1.53	0.80	0.58
Bmp2	1	1.53	0.46	0.71
Bmp3	1	2.44	0.51	1.24
Bmp7	1	1.49	0.59	0.57
C3	1	2.16	1.41	2.57
C3ar1	1	1.08	3.78	2.83
Cast	1	1.34	0.87	0.99
Ccl1	1	0.56	0.49	0.75
Ccl11	1	1.85	1.06	1.61
Ccl12	1	0.52	2.49	2.41
Ccl17	1	0.60	0.44	0.39
Ccl19	1	0.79	0.82	1.26
Ccl2	1	0.32	2.65	3.86
Ccl20	1	0.15	0.38	0.41
Ccl22	1	0.23	0.29	0.15
Ccl24	1	0.56	0.86	1.37
Ccl25	1	0.83	0.90	1.72
Ccl27a	1	5.20	0.38	0.73
Ccl28	1	0.41	0.56	1.56
Ccl3	1	1.12	8.54	1.67
Ccl4	1	2.41	15.83	2.24
Ccl5	1	0.59	2.19	1.66
Ccl6	1	0.68	3.29	3.33
Ccl7	1	0.27	4.22	4.90
Ccl8	1	0.74	2.04	1.03
Ccl9	1	0.57	1.08	2.06
Ccr1	1	0.84	5.87	3.39
Ccr10	1	1.66	0.48	0.97
Ccr2	1	0.96	1.82	2.64
Ccr3	1	0.63	1.54	2.26
Ccr4	1	0.81	0.30	0.52
Ccr5	1	0.58	2.55	2.26
Ccr6	1	1.18	0.43	0.74
Ccr7	1	0.41	1.25	1.54
Ccr8	1	0.80	0.79	1.19

Ccr9	1	0.60	0.49	1.08
Ccrl1	1	1.57	0.32	0.38
Ccrl2	1	0.87	5.88	2.00
Cd14	1	1.28	22.81	2.87
Cd180	1	0.86	1.52	1.58
Cd27	1	1.08	0.62	1.14
Cd28	1	0.61	0.69	2.00
Cd4	1	0.63	0.66	1.06
Cd40	1	0.48	0.86	1.14
Cd40lg	1	0.31	0.72	1.69
Cd70	1	0.66	0.80	1.73
Cd74	1	2.03	0.76	0.66
Cd86	1	0.71	0.87	0.81
Cd97	1	0.99	1.65	1.66
Cebpb	1	1.19	0.82	0.87
Cer1	1	0.66	0.85	1.76
Cklf	1	1.08	1.01	1.16
Clcf1	1	0.36	3.31	0.88
Cmtm1	1	0.60	1.04	1.89
Cmtm2a	1	0.37	0.66	1.69
Cntfr	1	8.16	2.37	1.49
Crp	1	0.62	0.92	1.90
Csf1	1	1.17	1.07	1.40
Csf2	1	0.47	0.57	1.03
Csf2ra	1	1.63	1.78	1.37
Csf3	1	0.69	3.26	1.90
Csf3r	1	0.42	5.89	3.56
Ctf1	1	1.07	0.65	0.95
Ctf2	1	0.83	0.98	1.69
Cx3cl1	1	1.10	0.66	0.90
Cx3cr1	1	0.78	0.77	1.24
Cxcl1	1	0.47	2.90	2.51
Cxcl10	1	0.58	3.24	1.17
Cxcl11	1	0.85	1.08	1.50
Cxcl12	1	1.76	1.85	1.67
Cxcl13	1	1.37	0.45	1.73
Cxcl14	1	1.41	1.10	1.08
Cxcl15	1	0.68	0.79	1.82
Cxcl16	1	0.56	0.82	0.70
Cxcl2	1	2.03	12.77	2.49
Cxcl5	1	0.45	2.46	4.11
Cxcl9	1	0.53	0.62	1.03
Cxcr3	1	0.78	0.78	1.20
Cxcr4	1	1.14	3.29	1.07
Cxcr5	1	0.58	1.54	2.06
Cxcr6	1	1.28	0.45	0.82
Cybb	1	1.11	1.46	2.31
Cyp26b1	1	1.57	1.27	0.54
D17Wsu104e	1	0.87	1.03	0.82
Dock2	1	0.93	1.14	1.22
Ebi3	1	0.75	0.89	1.38

Eda	1	0.78	0.75	1.38
Ephx2	1	1.51	0.71	1.28
Epo	1	0.74	0.96	2.11
Epor	1	0.78	0.74	1.33
Erbb2	1	1.49	0.60	0.68
Erbb2ip	1	1.30	0.64	0.75
F11r	1	0.99	1.08	0.94
F2	1	0.58	0.86	1.73
F3	1	2.25	1.02	0.60
F8	1	0.43	0.76	1.75
Fasl	1	0.47	0.91	1.82
Fgf1	1	0.98	1.01	1.59
Fgf10	1	1.48	0.74	2.02
Fgf12	1	0.56	0.66	1.92
Fgf2	1	1.16	1.18	2.19
Fgf3	1	0.67	1.72	1.80
Fgf4	1	0.41	0.76	1.52
Fgf5	1	1.68	0.91	1.79
Fgf6	1	0.73	1.14	2.02
Fgf7	1	2.23	0.91	1.78
Fgf8	1	0.70	1.17	1.60
Fgf9	1	0.94	1.07	1.89
Figf	1	0.61	0.63	0.93
Flt3l	1	1.54	0.77	1.09
Fn1	1	2.26	1.90	1.82
Fos	1	0.26	1.95	0.59
Fpr1	1	0.55	3.95	2.40
Gdf1	1	0.95	0.73	1.18
Gdf2	1	0.51	1.15	1.48
Gdf3	1	0.60	0.80	1.73
Gdf5	1	0.66	1.08	2.41
Gdf6	1	0.76	1.20	2.32
Gdf7	1	0.47	0.78	1.39
Gdf9	1	0.71	0.87	1.56
Gfra1	1	0.78	1.07	1.64
Gfra2	1	1.73	1.12	1.60
Ghr	1	1.74	1.20	1.98
Glmn	1	0.93	0.69	0.98
Gpi1	1	1.01	0.88	1.00
Gpr68	1	0.90	0.68	0.87
Grem1	1	0.57	0.73	0.79
Grem2	1	0.94	0.88	1.38
Grn	1	1.30	1.43	1.27
Hdac4	1	1.61	0.78	0.83
Hdac5	1	2.60	0.85	1.22
Hdac7	1	0.94	1.20	0.66
Hdac9	1	0.80	0.72	2.00
Hrh1	1	0.72	0.99	1.69
Ifna11	1	0.45	0.83	1.77
Ifna14	1	0.63	0.72	2.02
Ifna2	1	0.39	0.79	1.99

Ifna4	1	0.66	0.61	2.04
Ifna9	1	0.41	0.72	1.64
Ifnab	1	0.53	0.80	2.13
Ifnar1	1	1.05	1.37	1.11
Ifnar2	1	1.14	1.34	1.76
Ifnb1	1	0.60	0.79	1.86
Ifne	1	0.53	0.82	1.72
Ifng	1	0.74	1.07	1.85
Ifngr1	1	0.83	0.67	0.72
Ifngr2	1	1.04	0.75	0.92
Ifnk	1	1.30	0.88	1.40
Ik	1	1.27	1.01	1.21
Il10	1	0.50	1.44	1.64
Il10ra	1	0.84	2.21	1.45
Il10rb	1	1.17	1.10	1.10
Il11	1	0.66	1.06	1.25
Il11ra1	1	2.15	0.83	1.13
Il12a	1	0.63	1.19	1.49
Il12b	1	0.42	0.73	1.12
Il12rb1	1	0.95	0.69	1.24
Il12rb2	1	1.22	0.26	0.50
Il13	1	0.76	0.72	1.18
Il13ra1	1	0.68	0.62	0.41
Il13ra2	1	0.60	0.66	1.67
Il15	1	1.55	0.75	1.11
Il15ra	1	0.85	0.48	0.57
Il16	1	0.98	1.25	1.29
Il17a	1	0.48	0.88	1.62
Il17b	1	0.57	1.31	1.85
Il17c	1	0.34	0.70	0.93
Il17d	1	0.92	0.82	1.32
Il17f	1	0.61	0.87	1.59
Il17ra	1	0.90	1.60	1.43
Il17rb	1	0.90	0.60	0.90
Il18	1	1.66	0.36	0.30
Il18r1	1	0.93	0.46	0.96
Il18rap	1	0.50	1.86	1.55
Il19	1	0.30	2.29	0.75
Il1a	1	1.01	0.88	0.28
Il1b	1	0.96	10.16	1.42
Il1f10	1	2.46	0.43	0.77
Il1f5	1	17.97	11.62	4.67
Il1f6	1	0.36	0.36	0.37
Il1f8	1	0.52	0.70	0.39
Il1f9	1	0.53	0.80	0.30
Il1r1	1	1.47	0.93	1.50
Il1r2	1	1.15	1.60	1.67
Il1rap	1	0.97	1.40	0.86
Il1rapl2	1	0.61	0.87	2.35
Il1rl1	1	0.61	0.97	1.66
Il1rl2	1	1.87	1.57	1.59

Il1rn	1	0.58	1.12	1.06
Il2	1	0.69	0.76	1.60
Il20	1	1.65	0.60	0.76
Il20ra	1	0.86	0.26	0.31
Il21	1	0.56	0.70	1.51
Il21r	1	0.50	1.38	1.45
Il22	1	0.42	0.72	1.53
Il22ra1	1	1.59	0.53	0.42
Il22ra2	1	0.29	0.33	0.56
Il23a	1	0.75	1.67	1.86
Il23r	1	0.65	0.64	1.61
Il24	1	0.54	4.21	1.59
Il27	1	0.44	1.29	1.51
Il28ra	1	1.43	0.59	0.67
Il2ra	1	0.56	0.85	1.57
Il2rb	1	0.92	0.60	0.84
Il2rg	1	0.68	1.04	0.81
Il3	1	0.57	0.88	1.61
Il31	1	0.66	0.83	1.80
Il31ra	1	1.06	0.93	1.40
Il3ra	1	1.31	1.20	1.45
Il4	1	0.61	1.16	1.25
Il4ra	1	0.63	2.51	1.49
Il5	1	0.59	0.82	1.57
Il5ra	1	0.54	0.78	1.87
Il6	1	0.39	1.18	1.70
Il6ra	1	1.34	0.50	0.58
Il6st	1	1.52	0.86	1.43
Il7	1	0.79	0.77	1.41
Il7r	1	1.06	1.21	0.86
Il8ra	1	0.40	0.90	1.28
Il8rb	1	0.30	1.85	0.81
Il9	1	0.48	0.58	0.96
Il9r	1	0.63	0.65	1.32
Inha	1	0.95	0.83	1.05
Inhba	1	1.11	3.76	2.10
Inhbb	1	1.16	1.35	0.99
Ins1	1	0.56	0.72	1.87
Ins2	1	0.55	1.01	1.71
Irf4	1	0.68	0.50	0.79
Irf7	1	0.64	3.72	2.12
Itgb2	1	1.01	2.14	1.73
Itih4	1	0.49	0.85	1.87
Kitl	1	1.95	1.00	1.65
Kng1	1	0.47	1.05	2.09
Lbp	1	0.54	0.87	2.71
Lefty1	1	0.84	1.03	1.36
Lefty2	1	0.50	1.07	1.37
Lepr	1	1.76	0.85	1.41
Lif	1	0.47	1.23	1.64
Lifr	1	0.62	0.50	0.55

Lta	1	0.58	0.58	0.96
Ltb	1	0.67	1.60	1.18
Ltb4r1	1	0.30	1.69	0.56
Ly75	1	0.52	0.31	0.56
Ly86	1	1.25	1.01	1.71
Ly96	1	0.71	0.78	0.98
Mdk	1	1.14	0.79	0.89
Mefv	1	0.63	3.04	2.03
Mgll	1	1.24	0.45	1.24
Mif	1	0.67	0.87	0.64
Mmp25	1	0.65	0.87	0.58
Mpl	1	0.38	0.87	1.84
Mstn	1	1.40	0.80	1.34
Muc4	1	0.65	0.98	1.88
Myd88	1	0.79	1.19	1.20
Nfam1	1	0.83	2.12	1.59
Nfatc3	1	1.43	0.69	0.68
Nfatc4	1	0.74	1.08	1.26
Nfe2l1	1	0.87	0.67	0.95
Nfkb1	1	1.14	0.86	0.62
Nfrkb	1	1.38	0.76	0.88
Nfx1	1	1.12	0.75	0.87
Nlrp12	1	0.40	1.45	2.15
Nmi	1	0.95	0.93	1.26
Nodal	1	0.55	0.89	1.50
Nos2	1	0.69	1.94	1.87
Nr3c1	1	1.72	0.62	0.80
Nrg1	1	0.53	3.59	1.27
Ntf3	1	0.43	0.38	0.62
Olr1	1	0.57	1.53	2.11
Osm	1	0.92	5.51	1.64
Osmr	1	0.54	0.81	0.87
Parp4	1	1.18	0.84	0.85
Nampt	1	1.19	0.71	0.84
Pdgfa	1	1.40	0.88	0.83
Pdgfb	1	1.48	0.74	0.75
Pdgfc	1	1.28	0.83	1.60
Pf4	1	0.50	6.06	3.46
Pglyrp1	1	0.44	1.82	0.81
Pla2g2d	1	1.25	0.78	0.95
Pla2g7	1	0.92	2.10	1.70
Ppbp	1	0.42	4.78	2.70
Prdx5	1	1.13	0.68	0.64
Prg2	1	0.51	0.81	1.59
Prg3	1	0.55	0.83	1.43
Prl	1	0.68	0.68	2.32
Prlr	1	1.26	0.35	0.78
Procr	1	3.90	4.08	1.24
Prok2	1	0.53	1.27	2.30
Ptafr	1	1.16	3.10	1.50
Ptgs2	1	0.31	3.36	1.35

Ptn	1	0.67	0.67	1.66
Ptpra	1	1.36	0.63	0.62
Ptx3	1	0.60	2.09	5.26
Pxmp2	1	1.29	0.82	1.43
Reg3a	1	0.57	0.92	1.94
Reg3g	1	0.41	0.66	1.87
Ripk2	1	0.88	0.62	0.90
S100a11	1	0.83	1.38	0.96
S100a8	1	0.14	7.41	0.92
S100b	1	1.28	0.72	1.41
Saa4	1	0.72	0.89	1.63
Scg2	1	0.49	0.75	2.22
Scube1	1	1.19	1.12	1.90
Scye1	1	1.06	0.73	0.80
Sdcbp	1	0.94	1.71	1.27
Sectm1b	1	1.27	0.83	1.26
Sele	1	0.46	1.11	2.38
Serpina1a	1	0.93	1.12	1.67
Serpina3n	1	1.07	2.33	3.55
Serpinf2	1	0.96	1.71	28.89
Sftpd	1	0.60	0.92	1.90
Sigirr	1	0.81	0.82	0.86
Siglec1	1	0.85	1.83	1.53
Siva1	1	0.97	0.97	1.27
Slco1a4	1	0.52	0.60	1.81
Slurp1	1	0.82	0.18	0.31
Socs2	1	0.92	0.92	1.51
Spaca3	1	0.73	1.02	1.75
Spp1	1	1.69	11.32	1.54
Spred1	1	1.17	0.90	0.97
Srgap1	1	1.43	0.47	0.86
Stab1	1	0.86	2.19	1.16
Stat3	1	0.50	0.90	0.62
Syk	1	1.02	2.03	0.95
Tacr1	1	0.68	0.83	1.42
Thpo	1	0.84	0.86	1.76
Tirap	1	1.16	1.66	1.82
Tlr1	1	0.61	1.38	1.78
Tlr2	1	0.68	2.88	1.16
Tlr3	1	0.99	0.60	1.43
Tlr4	1	0.88	1.94	2.50
Tlr5	1	1.16	0.88	1.15
Tlr6	1	0.58	1.50	1.59
Tlr7	1	1.01	1.26	2.08
Tlr8	1	0.69	1.24	1.78
Tlr9	1	0.99	1.42	1.24
Tnf	1	0.75	1.68	0.79
Tnfaip6	1	0.52	1.42	1.99
Tnfrsf11b	1	1.26	0.75	1.75
Tnfsf10	1	0.76	0.67	1.11
Tnfsf11	1	0.69	0.67	1.55

Tnfsf13	1	0.73	0.71	1.14
Tnfsf13b	1	0.72	0.77	1.44
Tnfsf14	1	0.66	1.31	2.89
Tnfsf15	1	0.91	0.30	0.82
Tnfsf18	1	0.88	0.87	1.53
Tnfsf4	1	0.47	0.77	1.62
Tnfsf8	1	0.65	0.53	1.57
Tnfsf9	1	0.61	1.12	1.59
Tollip	1	0.99	0.91	0.96
Tpst1	1	1.17	1.08	1.31
Trap1	1	0.99	0.74	0.74
Ttn	1	1.77	0.60	1.95
Tymp	1	0.53	0.77	1.54
Vegfa	1	1.16	1.30	0.87
Vegfb	1	1.69	0.61	1.09
Vps45	1	1.06	0.71	0.82
Xcl1	1	0.67	0.63	1.42
Xcr1	1	0.44	0.59	1.64
Yars	1	1.27	0.77	0.78
Gusb	1	1.01	1.31	1.28
Hprt1	1	1.11	0.81	0.86
Hsp90ab1	1	0.85	0.84	0.73
Gapdh	1	0.83	0.92	1.06
Actb	1	1.43	1.38	1.29

Abbreviations: WT, wild type; KO, knockout; 0, no UVR exposure; UVR, UVR exposure.

SUPPLEMENTARY MATERIALS AND METHODS

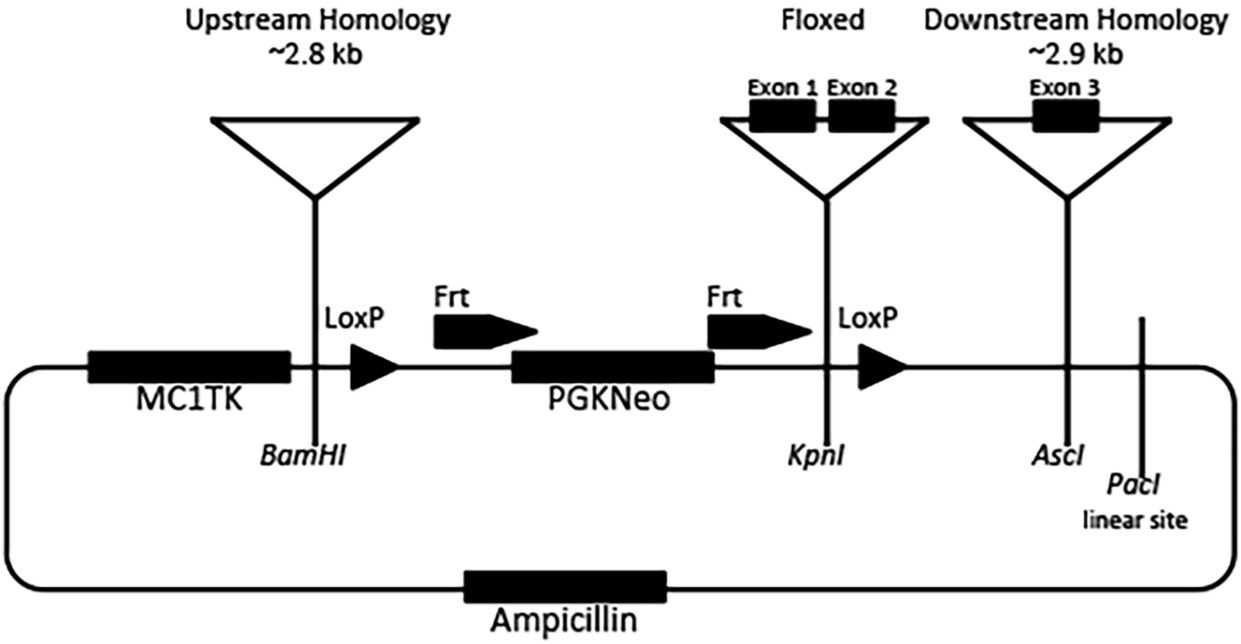
Mice

Animal studies were performed in an animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), in accordance with all applicable state and federal animal welfare regulations and with the NIH Guide for the Care and Use of Laboratory Animals, using protocols approved by the MD Anderson Cancer Center (MDACC) Institutional Animal Care and Use Committee.

Previously described *Slug* knockout (KO) mice (Jiang *et al*., 1998), their wild type (WT) 129 littermates, *Slug* transgenic (TG) mice with exogenous *Slug* expression driven by the bovine K5 promoter (Quan *et al.*, 2014), and their WT FVB littermates were obtained from breeding colonies maintained at MDACC. Knockin mice with a floxed *Slug* gene were generated on a 129 background by the Genetically Engineered Mouse Facility Core at MDACC using standard ES cell electroporation techniques. Homozygous females of this strain ($Slug^{fl/fl}$) were crossed with male K5-Cre mice on an FVB background (Ramirez et al., 2004) to create $K5\text{-}Cre^{+/-}/Slug^{fl/+}$ mice. Male offspring were then crossed with female $Slug^{fl/fl}$ mice to produce CKO mice ($K5\text{-}Cre^{+/-}/Slug^{fl/fl}$) and littermate control WT ($K5\text{-}Cre^{-/-}/Slug^{fl/fl}$) mice. Only agouti CKO offspring were employed for studies.

The vector used for production of conditional Slug knockin mice was constructed by NorClone Biotech Laboratories (London, Ontario, Canada). The backbone of the vector was the pBluescript II KS+ plasmid. It was modified as shown below to include the MC1TK cassette, a loxP cassette containing exons 1 and 2 of the mouse Slug gene amplified from a mouse Slug BAC clone, and upstream and downstream Slug homology domains also amplified from a mouse Slug BAC clone. The homology domains were located outside of the loxP cassette. The

linearized vector was used for construction of knockin ES cells by standard techniques. Correct targeting of the vector in ES cells was confirmed by Southern blotting and ES cells were used to produce knockin mice on a pure 129 background.



Bone marrow transplantation

Female wild type (WT) and Slug knockout (KO) bone marrow recipient mice were placed on prophylactic Clavamox (Zoetis, Florham Park, NJ) antibiotic in drinking water (50mg/kg/day) for two weeks prior to bone marrow transplantation. On the day of transplantation, recipient mice were irradiated with 5 (KO) or 10 (WT) Gy X-ray from a Rad Source RS 2000 Biological Research Irradiator (Rad Source Technologies, Suwanee, GA) to ablate endogenous bone marrow. Each male donor mouse (KO or WT) was killed by CO_2 asphyxiation; bone marrow was flushed from both femurs, filtered to remove bone fragments, pelleted, resuspended in 150 µl sterile PBS, and injected into the tail vein of a single recipient female within four hours of X-irradiation. Transplanted mice were housed in sterile caging, fed autoclaved food and water, and

treated with Clavamox in drinking water for 21 days. Mice that were not successfully engrafted died within the first 10 days following transplantation. At least five weeks later, mice were exposed to three minimal erythemal doses (MED) (4800 J/m^2) UVR as described in the text and euthanized with CO_2 48 hours later, when inflammatory cell infiltration into the skin was expected to be maximal.

UVR exposure

Three to four days prior to UVR exposure, mice were shaved (Oster Technique, McMinnville, TN). The next day, mice were treated with Nair (Church and Dwight, Ewing, NJ) for complete hair removal. Two to three days later, mice were exposed to UVR from Westinghouse FS20 sunlamps (80% UVB, 20% UVA). UVR was filtered through UVT cast acrylic (Polycast Technology, Stamford, CT) to exclude wavelengths below 280 nm. The UV dose was assessed with a radiometer-dosimeter (UV-1360, UB-IR Tech, Dietzenbach, Germany).

In vivo imaging

For imaging, the activatable fluorescent agents used were ProSense 750 FAST (PS750) and Neutrophil Elastase 680 FAST (NE680) (PerkinElmer, Waltham, MA). PS750 is activated by a variety of cathepsins, including B, L, S, K, V and D, while NE680 is activated by neutrophil elastase; both agents produce a fluorescent signal after proteolytic cleavage. Mice were anesthetized by isoflurane inhalation and injected intraorbitally with 100 µl of each fluorogenic agent using a 27-gauge needle. PS750 was injected one hour and NE680 19 hours after UVR treatment. Twenty-four hours following UVR exposure animals were imaged using the IVIS Spectrum system (PerkinElmer). PS750 fluorescence was detected with a combination of 750 nm

excitation and 770 nm emission filters and NE680 fluorescence was detected with 675 nm excitation and 720 nm emission bandpass filters, with an acquisition period ranging from 0.5 to 1 second. Regions of interest within acquired images were analyzed using LivingImage 4.4 software (Caliper Life Sciences, Hopkinton, MA).

Immunohistochemistry

Skin was fixed in neutral buffered formalin for 24-48 hours and stored in 70% ethanol until routine paraffin embedding and sectioning. For immunohistochemistry, endogenous peroxidase activity was blocked by incubation with 3% H_2O_2; antigen retrieval was performed by microwaving the samples in 10 mM citrate buffer (pH 6.0); non-specific antibody binding was blocked with Biocare Blocking Reagent (Concord, CA); and primary antibodies were added for incubation either for 1 hour at room temperature or overnight at 4 °C. Antibodies included a rat monoclonal anti-Ly6G (BD Pharmingen, Franklin Lakes, NJ) diluted 1:200, a goat polyclonal anti-S100A8 (Santa Cruz, Santa Cruz, CA) diluted 1:250, a rat monoclonal anti-CD45R diluted 1:1000 (AbD Serotec, Raleigh, NC), and a rat monoclonal anti-CD3 (AbD Serotec) diluted 1:100. Detection was carried out by incubation for 30 minutes with Biocare Goat HRP or biotinylated rabbit-anti-rat IgG (Vector Laboratories, Burlingame, CA), as appropriate, followed by 3,3'-diaminobenzidine tetrahydrochloride (DAB; DAKO, Carpinteria, CA) for signal detection. For Ly6G immunohistochemistry, the signal was amplified using a tyramide amplification kit (PerkinElmer) according to the manufacturer's instructions. Slides were then counterstained with hematoxylin, dehydrated, and cover-slipped.

Morphometry

T lymphocytes, B lymphocytes, and mast cells were quantified by direct examination of slides stained using appropriate immunohistochemical techniques. Because of the large number of neutrophils and macrophages present, images of immunohistochemically stained skin sections were analyzed using the Cell Profiler program. For each mouse and each cell type, at least 1 mm^2 of dermis and subcutis was analyzed.

Numbers of neutrophils and macrophages were determined using the open-source CellProfiler cell image analysis software program (Broad Institute, Cambridge, MA). Images from immunohistochemically stained slides were captured from slides digitized using the Aperio Scan Scope CS (Leica Biosystems, Buffalo Grove, IL). Images were converted from color to black and white and then reversed, thus showing light positive cells on a dark background. CellProfiler was trained to identify neutrophils or macrophages. For each animal, at least one mm^2 of dermis and subcutis was analyzed. Only regions of skin in telogen or early anagen were evaluated; hair follicles and sebaceous glands were excluded from analysis.

Slides stained for NF-κB, phospho NF-κB, and IKK-α were digitized using an Aperio Scan Scope CS (Leica Biosystems, Buffalo Grove, IL) and analyzed with the manufacturer's GENIE software (Spectrum Version 10.2.2.2315). The standard DAB nuclear analysis program was modified to improve its ability to recognize immunopositive nuclei, and the modified analysis algorithm was applied to all slides. A minimum of 3 mm in length of representative epidermis was evaluated for each mouse.

Neutrophil migration studies

Bone marrow was flushed from the femurs and tibias of wild type and knockout mice with murine neutrophil buffer (MNB; 1X Hank's buffered salt solution, 0.1% bovine serum albumin,

1% glucose), strained thorough a 40 μm filter, rinsed and resuspended in 45% Percoll (Sigma, St. Louis, MO). Cells were applied to a Percoll gradient (50:55:62:81%) and centrifuged at 1600g for 30 minutes at 10° C. The band of cells between 62% and 81% was collected and cells were rinsed twice with MNB. Cells were resuspended in MNB and layered onto Histopaque (Sigma-Aldritch, St. Louis, MO). After centrifugation at 1600g for 10 minutes at 10° C, cells at the boundary between buffer and Histopaque were collected. Cells were rinsed twice in MNB. Throughout the isolation process, extreme care was taken to avoid agitating the neutrophils unnecessarily and centrifugation was performed without braking. Approximately 75,000 neutrophils in 300 μl MNB were placed in the upper chamber of a 24-well migration assay plate (3.0 μm; Corning, Tewksbury, MA); the bottom well contained 1 ml MNB with or without the chemoattractants WKYMVm (Trp-Lys-Tyr-Met-Val-D-Met-NH_2) at 100 nm or 10 μl normal mouse serum. Plates were incubated for 2 hours at 37° C. Sixty μl of 0.5M EDTA was then added to the bottom chamber and incubated 10 minutes at 4° C. Cells in the upper and lower chambers were quantified using a hemocytometer.

Microarray

WT and KO mice were used for these studies. Mice were shaved and depilated before exposure to 0 or three MED (4800 J/m^2) UVR as described in the text. Mice were euthanized with CO_2 and skin collected 24 hours later, when robust proinflammatory cytokine production was expected. Strips of skin were frozen on a liquid nitrogen-cooled aluminum block and stored at -80° C. To obtain RNA from mouse skin, frozen strips of skin were placed, epidermis side down, on 500 μL of cold TRIzol (Life Technologies, Carlsbad, CA, USA) for one minute. Epidermis

was then scraped from the surface, briefly homogenized in a total of 1 ml TRIzol, and frozen in liquid nitrogen for storage at -80° C.

RNA isolation was completed as recommended by the TRIzol supplier and RNA quality was assessed using Agilent's RNA 6000 Nano kit (Santa Clara, CA). No RNA was used if the RIN value was less than 7.5. A total of 1000 ng of RNA was used to synthesize cDNA using the High Capacity cDNA RT Kit from Applied Biosystems (Foster City, CA). Equal amounts of cDNA were added to each well, cDNA was mixed with iTAQ SYBR Green Supermix with ROX from Bio-Rad Laboratories (Hercules, CA), and each plate was run using the Applied Biosystems Prism 7900 HT qPCR protocol (Foster City, CA) as follows: one cycle at 95° C for 10 minutes; 40 cycles at 95° C for 0.15 seconds and at 60° C for 60 seconds; and a final cycle of 15 seconds each at 95° C, 60° C, and 95° C. Analyses of each plate was performed using the SDS 2.3 program from Applied Biosciences, using the same threshold and baseline for all samples. Microarray study results have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database as Study GSE86107 (Accession numbers GSM2293723-GSM2293735).

qRT-PCR

One microgram of epidermal RNA, isolated as described for microarray assays, was used for qRT-PCR detection of CCL3, CCL4, CCrL2, IL-1β, and oncostatin. The High Capacity cDNA Reverse Transcription Kit from Life Technologies (Carlsbad, CA) and random hexamers were used for cDNA synthesis. Quantitative PCR was performed by the M.D. Anderson Science Park Molecular Biology Facility Core with an ABI Prism 7900HT instrument (Applied Biosystems, Foster City, CA) and gene-specific primer pairs, using the Syber Green method. Primers were

designed using File Builder 3.1 software (Applied Biosystems) and MacVector Version 11.0 (Apex, NC). Samples were run in duplicate, and results were normalized to 18S RNA. Data were analyzed using the ΔΔCt method with further normalization to the average value for unexposed WT skin (Schmittgen and Livak, 2008).

Statistics

Differences between and among groups were analyzed using one-way ANOVA followed by Dunnett's, Tukey's, or Sidak's multiple comparison test or the one-tailed Mann-Whitney test, as appropriate. Analysis was carried out with Prism 5.0 software for Mac OS X (GraphPad Software, La Jolla, CA).