

Induction of alternative proinflammatory cytokines accounts for sustained psoriasiform skin inflammation in IL-17C+IL-6KO mice

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Supplemental Figures

Figure S1. Uninvolved skin from 10-week old IL-17C+KO mice has decreased cutaneous inflammatory immune cell infiltrate compared to age matched IL-17C+ mice. This difference is abrogated by 14-weeks of age. Representative images of uninvolved skin of 10 and 14 week old IL-17C+ and IL-17C+KO mice demonstrating a reduction (or delay) in the number of skin infiltrating immune cells in IL-17C+KO mice at 10-weeks of age which is abrogated at 14 weeks. Scale bar = 100µm.

Figure S2. KC-Tie2 psoriasiform mice have elevated cutaneous expression of *Il17c*, *Tnf* and *Il6*; introduction of additional exogenous IL-6 or TNFα elicits further inflammatory gene response in the skin of KC-Tie2 x IL-6KO mice. (a) qRT-PCR analyses of *Il17c*, *Tnf* and *Il6* transcripts in the KC-Tie2 transgenic mouse model of psoriasis (n=6 each). (b) KC-Tie2xIL-6KO mice injected with intradermal recombinant IL-6 or TNFα develop increased epidermal thickness (acanthosis; µm), a surrogate measure of inflammation. Each line represents an individual mouse. *P<0.05.

Figure S3. IL-17C, IL-6 and TNFα elicit additive and synergistic production of keratinocyte-derived proinflammatory transcripts. Primary wildtype mouse keratinocytes were stimulated for 8 hours with 10ng/ml of IL-17C, IL-6, TNFα alone or in combination, and harvested for transcript analyses using qRT-PCR. IL-17C-IL-6-TNFα together induce the highest levels of many of the candidate transcripts measured, including *Il17c*, *Tnf*, *Il36α/β/γ*, *Defb3* and *S100a8/a9*. (n= 3/group performed in triplicate). Line between groups indicates P<0.05.

Figure S4. IL-17C, IL-6 and TNF α elicit additive and synergistic production of keratinocyte-derived proinflammatory transcripts. (a) Primary adult human keratinocytes were stimulated for 24 or 48 hours with recombinant human IL-17C (100 ng/ml), TNF α (20 ng/ml) and IL-6 (20 ng/ml) alone or in combination, and harvested for transcript analyses using qRT-PCR. IL-17C-IL-6-TNF α together induce the highest levels of many of the candidate transcripts we measured, including *IL17C*, *TNF*, *IL36RA/B*, *DEFB4A*, *IL8*, *CCL20*, and *SI00A8/A9*. (n= 3/group performed in triplicate). (b) Statistics table comparing differences between groups.

Figure S5. Heat map analyses of uninvolved skin comparing changes in 14-week old IL-17C+KO and IL-17C+ mice. Heat map of transcripts that change between uninvolved skin comparing IL-17C+ vs. controls at 14-weeks of age and IL-17C+KO vs. controls at 14-weeks of age (n=4/5/group). IL-17C+KO vs. IL-17C+ mice were then compared with each other to identify key up- and down-regulated transcripts altered in IL-17C+KO mice.

Figure S6. Heat map analyses of uninvolved skin comparing changes in IL-17C+KO mice between 10- and 14-weeks of age. Heat map of gene transcripts that change between uninvolved skin of IL-17C+KO vs. control mice at 10-weeks of age and IL-17C+KO vs. control mice at 14-weeks of age (n=4/5/group) and then 10-week vs. 14-week transcript changes in IL-17C+KO were evaluated.

Figure S7. Heat map analyses of involved skin comparing changes in IL-17C+ and IL-17C+KO at 14-weeks of age. Heat map of gene transcripts that change between involved skin comparing IL-17C+ vs. controls at 14-weeks of age and IL-17C+KO vs. controls at 14-weeks of age (n=4/5/group). IL-17C+KO vs. IL-17C+ were then compared with each other to identify key up- and down-regulated transcripts altered in IL-17C+KO mice at 14-weeks of age.

Figure S8. Exogenous introduction of TNF α and IL-6 into control mouse skin and IL-17C+ uninvolved mouse skin promotes a preferential proinflammatory response in IL-17C+ mice. (a) Epidermal thickness measures (acanthosis; μm) following intradermal injections of either BSA or recombinant TNF α and IL-6 (500ng of each), every other day for 16 days. (b) Representative images from C57Bl/6 mouse skin and IL-17C+ uninvolved skin injected with either BSA or TNF α /IL-6 immunostained using antibodies specific to CD4 demonstrates increases in skin-infiltrating CD4⁺ T cells following cytokine injections. Quantification of skin-infiltrating CD4⁺ (c) and F4/80⁺ (d) cells (mean number of cells/field of view) in BSA and TNF α /IL-6 injected control mouse skin (n=6-8) and uninvolved IL-17C+ skin (n=7). *P<0.05 vs. control +BSA; ** P<0.05 vs. IL-17C+BSA. Scale bar in b = 100 μm .

Figure S1

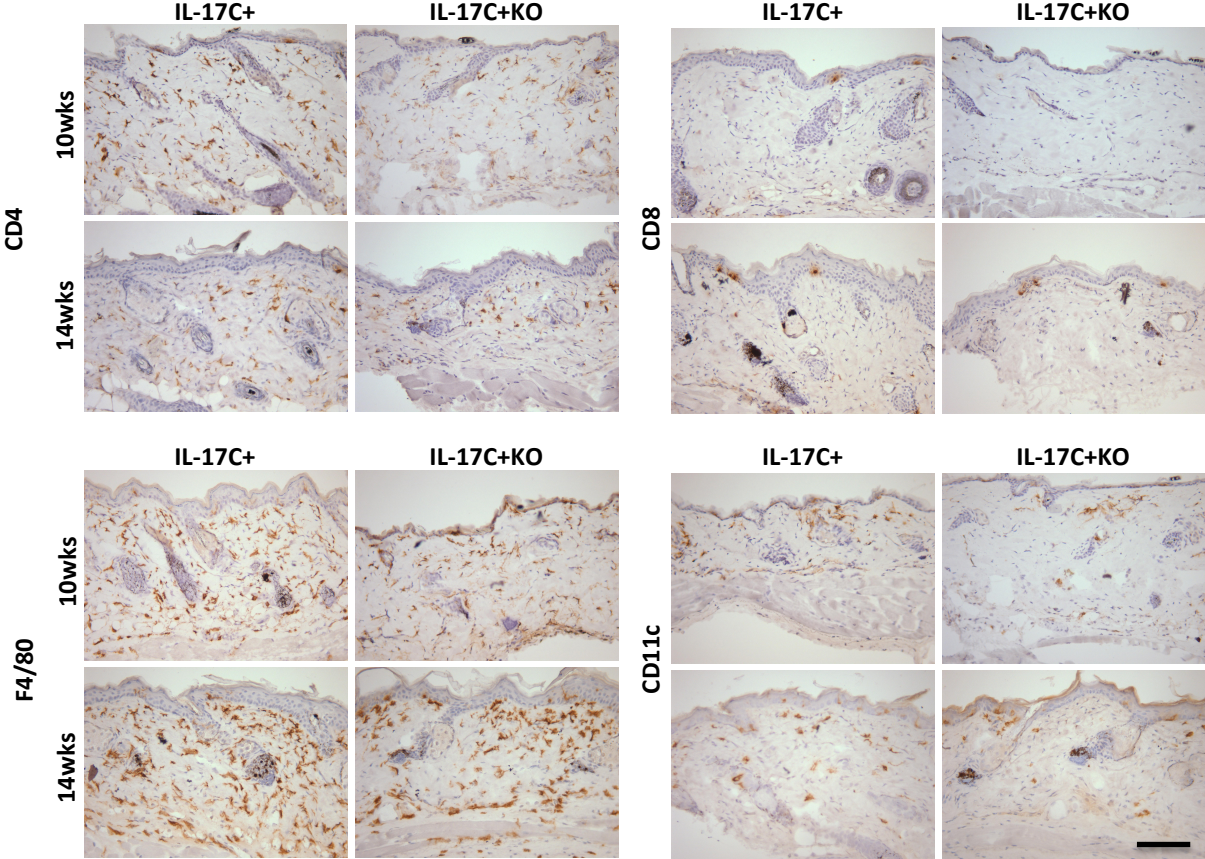
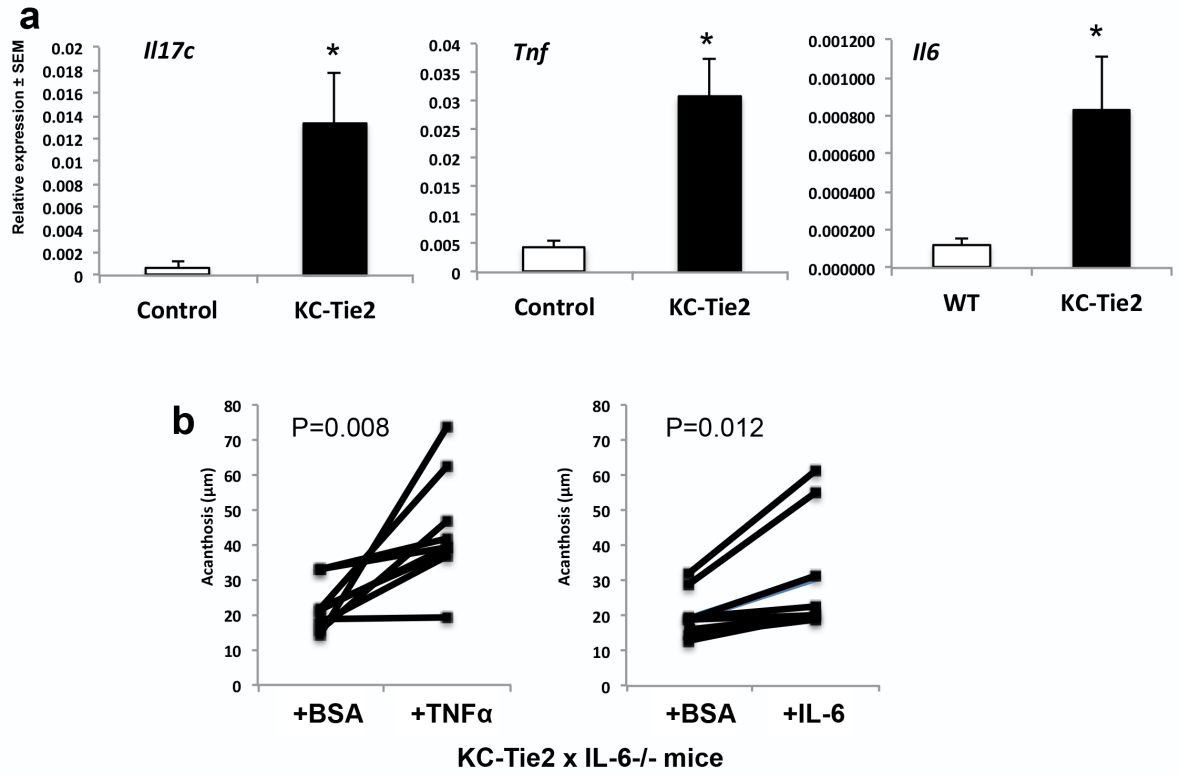
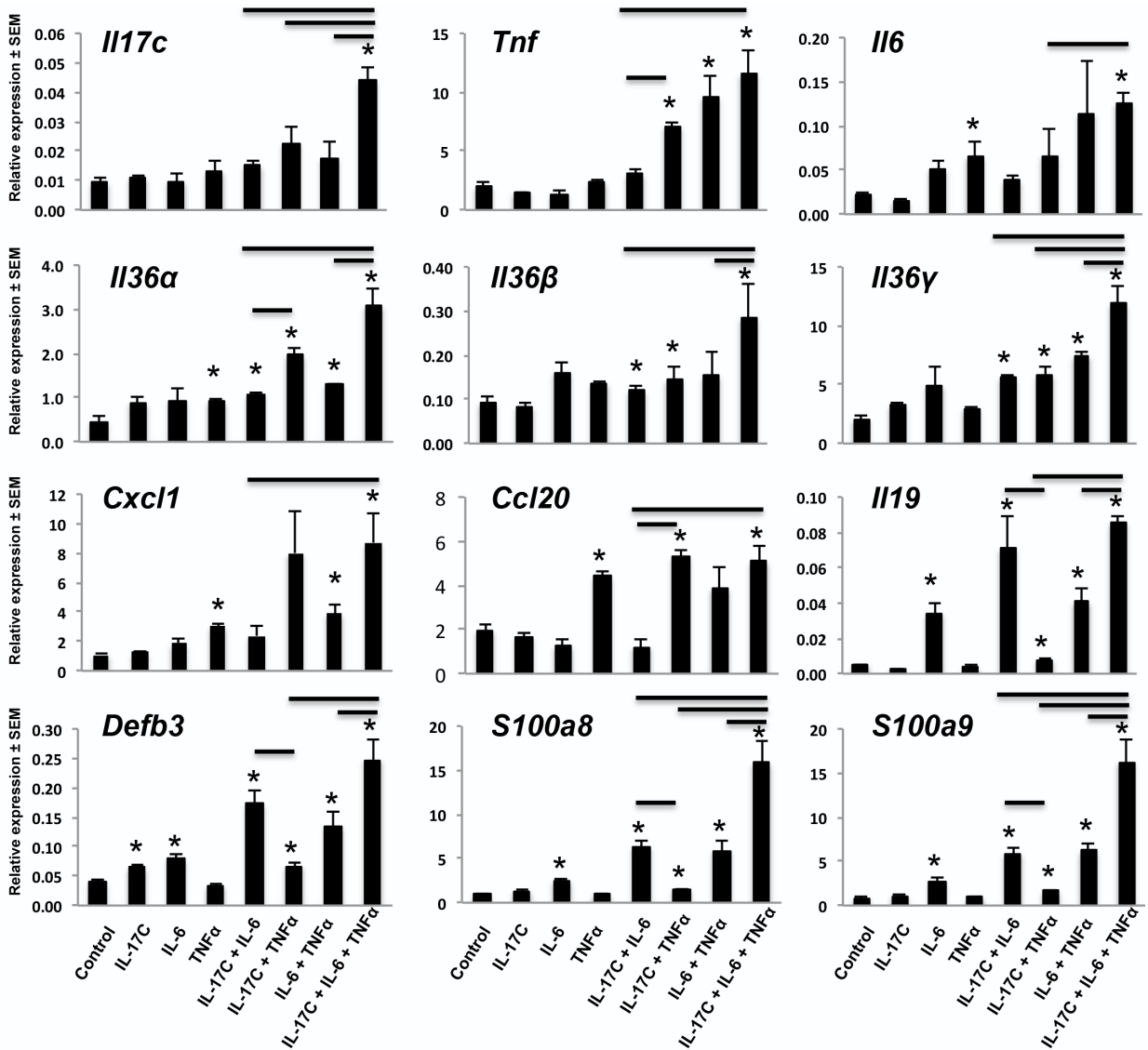


Figure S2



Mouse primary keratinocytes



b**Statistics on all primary human KC stimulation qRT-PCR data.**

IL-17C	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.54957477	0.01346675	1	1												
IL-6	0.09899463	0.00302248	0.22296357	0.27533358	1	1										
TNF	1.6248E-05	0.03962814	4.4503E-05	0.57472178	0.00115601	0.13079114										
IL-17C + IL-6	0.00328966	0.00394579	0.01042568	0.02454939	0.16761083	0.10075759	0.01079592	0.02363376	1	1						
IL-17C + TNF	0.00116612	0.17925261	0.00096301	0.07827843	0.00504688	0.01107839	0.43098627	0.23041535	0.02329788	0.00457519						
IL-6 + TNF	0.00010512	0.00073728	8.7804E-05	0.01211396	0.00064378	0.07825103	0.12497846	0.00962286	0.00315037	0.68979319	0.63255903	0.00102855	1	1		
IL-17C+IL-6+TNF	0.00060387	0.00171252	0.00025858	0.14049559	0.00048331	0.68054493	0.00365527	0.06828783	0.00092723	0.11858759	0.0099824	0.00535566	0.01314705	0.11578702	1	1

TNFalpha	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.8884317	0.26191838	1	1												
IL-6	0.06551695	0.03146449	0.05351874	0.08957311	1	1										
TNF	0.00546865	0.00679037	0.00541316	0.00765676	0.00821399	0.01184477	1	1								
IL-17C + IL-6	0.00702554	0.09315023	0.00402251	0.14458751	0.80271664	0.53870671	0.00853016	0.01841924	1	1						
IL-17C + TNF	0.00781434	0.01620771	0.00777282	0.01800819	0.00984063	0.02621092	0.29716839	0.9658818	0.01010291	0.03737025	1	1				
IL-6 + TNF	0.01182994	0.00700172	0.01178997	0.00573664	0.01378042	0.00979605	0.14194893	0.30151984	0.01404516	0.01253621	0.48945327	0.30707513	1	1		
IL-17C+IL-6+TNF	0.01206693	0.02245575	0.01201887	0.02359205	0.0144176	0.02802635	0.20721987	0.2543617	0.01473395	0.03250129	0.70386945	0.25548001	0.74776571	0.70993314	1	1

IL-6	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.33526075	0.20920827	1	1												
IL-6	0.23926214	0.55251441	0.82350954	0.43535766	1	1										
TNF	0.04443248	0.12561883	0.15549857	0.10977222	0.09948135	0.11681544	1	1								
IL-17C + IL-6	0.22744316	0.17967056	0.72401136	0.11231748	0.85084221	0.14018133	0.08362324	0.26266123	1	1						
IL-17C + TNF	0.01758375	0.03625938	0.03808071	0.02137316	0.02861857	0.02735116	0.23241673	0.39390327	0.02581809	0.48439638	1	1				
IL-6 + TNF	0.04170131	0.06343896	0.08094664	0.04274082	0.0645339	0.3704117	0.43781718	0.05918486	0.4595384	0.83122105	0.89622089	1	1			
IL-17C+IL-6+TNF	0.00592669	0.02657148	0.01906988	0.01791756	0.01169981	0.02150924	0.22846738	0.56952076	0.00992989	0.24953829	0.83470117	0.56327225	0.96663195	0.68188272	1	1

IL-1F5	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.80573104	0.04373081	1	1												
IL-6	0.93461361	0.23190144	0.75092203	0.68708101	1	1										
TNF	0.00010529	0.00076541	0.00011507	0.00129443	0.00010883	0.00120332	1	1								
IL-17C + IL-6	0.81524614	0.0997055	0.95149224	0.46112541	0.75276806	0.36329226	8.8868E-05	0.00249945	1	1						
IL-17C + TNF	1.7764E-07	7.4783E-06	1.7127E-07	1.0731E-05	2.1697E-07	1.0527E-05	0.13208094	0.02002573	8.06E-08	1.938E-05	1	1				
IL-6 + TNF	4.0854E-05	0.0001129	4.3875E-05	0.00015976	4.5892E-05	0.00015227	0.23038293	0.0729683	2.458E-05	0.00024886	0.00264475	0.70274116	1	1		
IL-17C+IL-6+TNF	1.5375E-05	0.00045112	1.6142E-05	0.0006779	1.7659E-05	0.00063716	0.37417494	0.34241574	8.5384E-06	0.000110976	0.00522501	0.17013243	0.64152974	0.36219074	1	1

IL-1F8	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.78217943	0.17958187	1	1												
IL-6	0.35824178	0.05269938	0.42892572	0.33334484	1	1										
TNF	0.00011003	0.01904847	0.00011381	0.02150018	0.00016591	0.02458985	1	1								
IL-17C + IL-6	0.67837321	0.09392553	0.8159112	0.29658893	0.61738864	0.72692473	0.00013187	0.02674146	1	1						
IL-17C + TNF	0.00129307	0.01273959	0.00134779	0.01470305	0.00183483	0.01724887	0.67105632	0.86473222	0.00150032	0.01912003	1	1				
IL-6 + TNF	0.00016342	0.00435611	0.00016857	0.00490077	0.00021897	0.00559102	0.33863233	0.55134967	0.00018532	0.00609258	0.22165771	0.42042291	1	1		
IL-17C+IL-6+TNF	1.4592E-05	0.01784575	1.4929E-05	0.01908193	2.2738E-05	0.02054716	0.59658651	0.29371706	1.7856E-05	0.02147134	0.36990038	0.23515939	0.56489619	0.51014709	1	1

IL-1F9	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.00207235	0.00460862	1	1												
IL-6	0.02593677	0.56349457	0.07497716	0.01116469	1	1										
TNF	3.1579E-06	0.00328845	3.7978E-05	0.00876289	9.0985E-06	0.00365467	1	1								
IL-17C + IL-6	2.5477E-06	0.05617052	0.91613539	0.43687555	0.00933145	0.0726124	1.9047E-05	0.02285636	1	1						
IL-17C + TNF	8.0808E-06	0.00014582	2.8825E-05	0.00029241	1.4066E-05	0.00015712	0.01539901	0.06599347	2.2993E-05	0.00066669	1	1				
IL-6 + TNF	1.3639E-05	0.00018507	8.6752E-05	0.00067659	3.033E-05	0.00021276	0.37323751	0.93981084	5.9709E-05	0.00377893	0.07787918	0.04647144	1	1		
IL-17C+IL-6+TNF	7.885E-05	1.3038E-10	0.0002328	2.1751E-08	0.00012729	2.8751E-10	0.03726778	0.66672791	0.00020464	0.00021584	0.94069624	0.01203352	0.12130703	0.45232312	1	1

CXCL1	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.05102011	0.5380899	1	1												
IL-6	6.0452E-07	0.13494861	6.9776E-05	0.30823464	1	1										
TNF	0.00174257	0.14536979	0.00243089	0.15246346	0.00732234	0.17147774	1	1								
IL-17C + IL-6	6.5865E-05	0.10561646	0.00061703	0.16051664	0.28621127	0.38834226	0.01069958	0.21039766	1	1						
IL-17C + TNF	0.00598526	0.02524172	0.00736665	0.03017769	0.01470766	0.04688505	0.45505863	0.49277096	0.01829054	0.10774088	1	1				
IL-6 + TNF	0.00764487	0.01486722	0.00885916	0.01707952	0.0145	0.02413508	0.19663083	0.72125421	0.01686803	0.04748497	0.49881429	0.50657144	1	1		
IL-17C+IL-6+TNF	0.010455	0.01527983	0.01180683	0.01924871	0.01773334	0.03403332	0.15167375	0.43283484	0.02005297							

DefB4a	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.01397318	0.00143326	1	1												
IL-6	0.00235755	0.0002675	0.12486418	0.00635042	1	1										
TNF	0.00181668	0.00143497	0.00406447	0.00302021	0.0084621	0.01548581	1	1								
IL-17C + IL-6	0.00081858	0.01329545	0.00247808	0.01739339	0.00700164	0.03016732	0.51743661	0.1875576	1	1						
IL-17C + TNF	1.3827E-06	0.00215679	2.5597E-06	0.00273359	4.7818E-06	0.00448794	0.00981873	0.02846877	0.00106477	0.36891173	1	1				
IL-6 + TNF	4.0591E-05	0.00013849	7.1228E-05	0.00019074	0.00012146	0.00038391	0.03796893	0.00764319	0.0077755	0.41359127	0.72381657	0.78893168	1	1		
IL-17C+IL-6+TNF	3.9825E-06	0.00016903	5.057E-06	0.00021199	6.2962E-06	0.00034391	8.1221E-05	0.00253887	3.5092E-05	0.09277979	0.00076225	0.4240667	0.00081729	0.21727247	1	1

S100A8	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.08927914	4.1721E-05	1	1												
IL-6	7.1738E-05	0.00018307	0.00079182	0.00106847	1	1										
TNF	0.00012139	2.9095E-05	0.00071779	0.00011086	0.38984317	0.13697122	1	1								
IL-17C + IL-6	3.2625E-06	0.0023206	2.446E-05	0.00563026	0.04356839	0.21769232	0.3034373	0.77082612	1	1						
IL-17C + TNF	1.5946E-05	4.6014E-05	5.8434E-05	0.00011738	0.01135788	0.01745054	0.06340639	0.16139407	0.21710247	0.42722786	1	1				
IL-6 + TNF	2.8728E-08	0.00049315	1.4133E-07	0.00093422	3.4105E-05	0.01796688	0.00042169	0.07707234	0.00086227	0.17473523	0.04401997	0.38788757	1	1		
IL-17C+IL-6+TNF	2.5285E-08	0.00076442	1.1982E-07	0.0014265	2.524E-05	0.02405582	0.00030103	0.09547162	0.00057703	0.19892429	0.0300437	0.42829863	0.77075655	0.97151353	1	1

S100A9	Control		IL-17C		IL-6		TNF		IL-17C + IL-6		IL-17C + TNF		IL-6 + TNF		IL-17C+IL-6+TNF	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1	1														
IL-17C	0.20367018	0.01558752	1	1												
IL-6	0.0033167	0.00035768	0.00812101	0.00085257	1	1										
TNF	0.00047936	1.0103E-06	0.00133814	3.0329E-06	0.666053	0.41255549	1	1								
IL-17C + IL-6	9.0464E-06	6.2777E-05	2.6787E-05	0.00013691	0.08593508	0.37425718	0.14873209	0.78752851	1	1						
IL-17C + TNF	0.00025119	2.8337E-08	0.00044815	6.517E-08	0.05155588	0.0048498	0.07971371	0.0036146	0.39783267	0.03291515	1	1				
IL-6 + TNF	2.4684E-07	4.5191E-05	5.1027E-07	6.6919E-05	0.00031958	0.00604146	0.00034555	0.00883893	0.0020018	0.01771422	0.06214993	0.15094206	1	1		

Figure S5

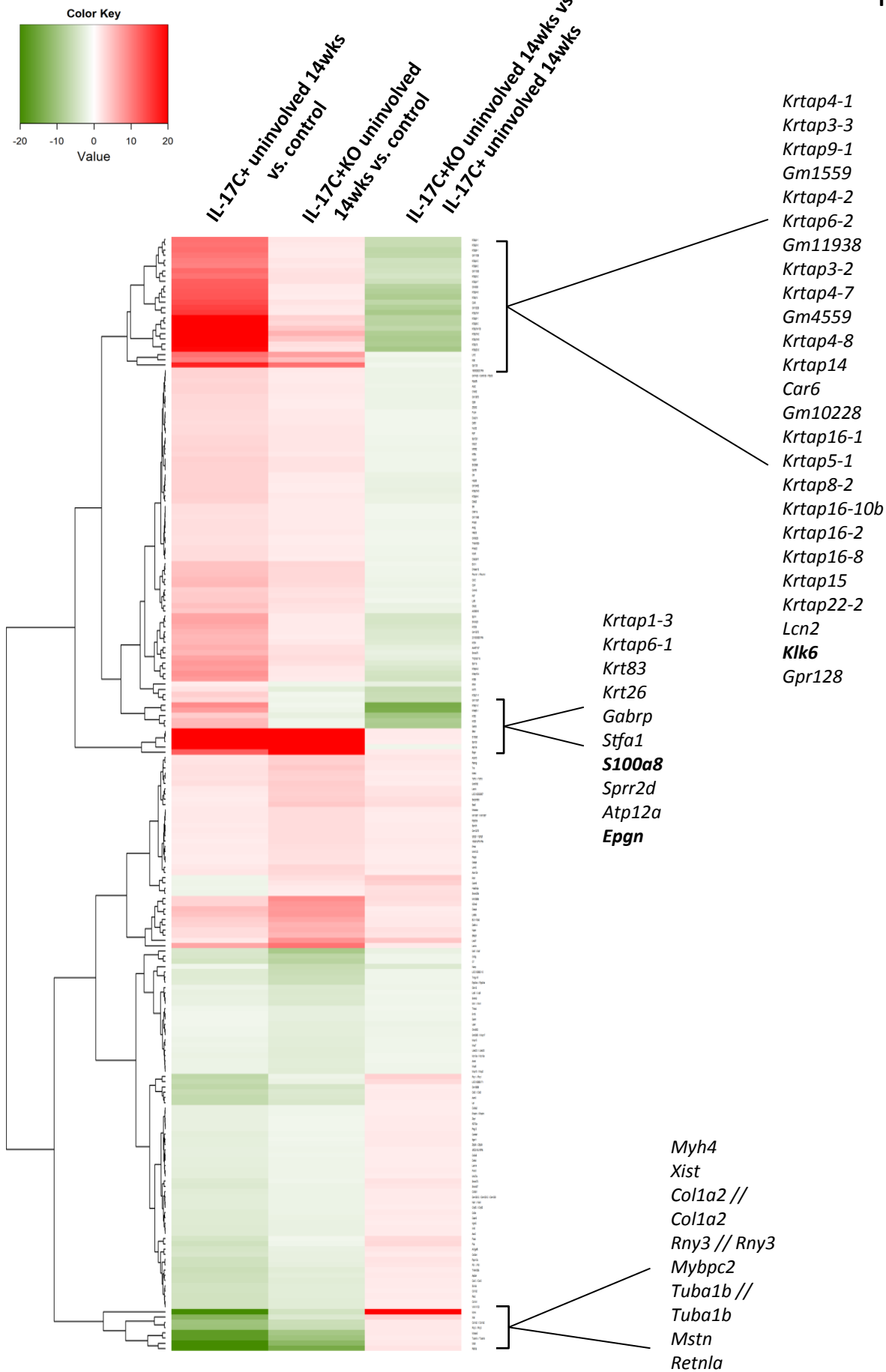
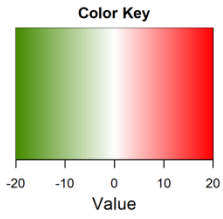


Figure S6



IL-17C+KO uninvolved 10wks
vs. control

IL-17C+KO uninvolved 14wks
vs. control

IL-17C+KO uninvolved 14wks vs.
IL-17C+KO uninvolved 10wks

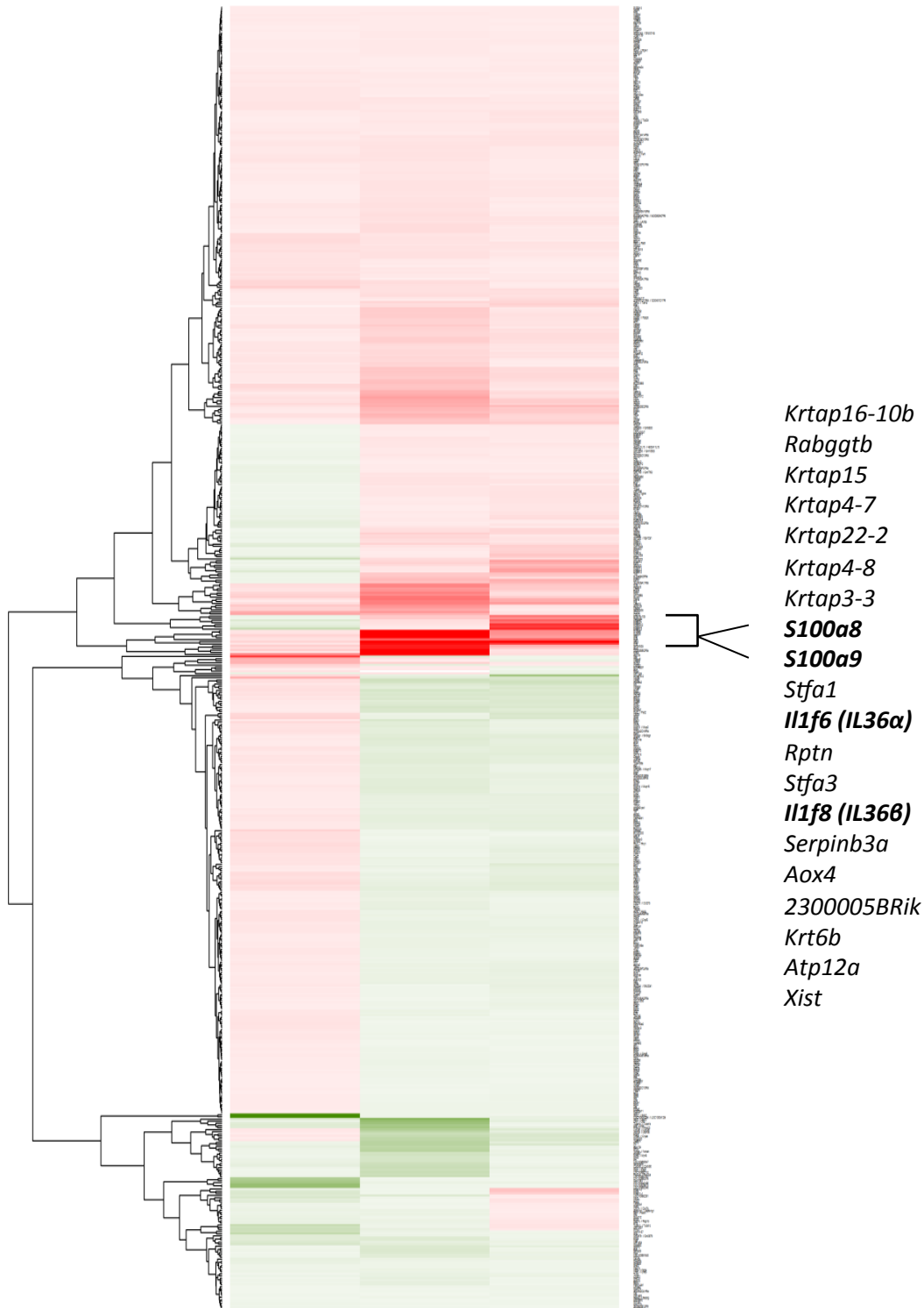
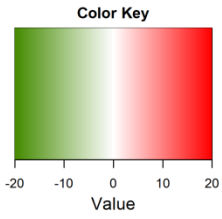


Figure S7



IL-17C+ involved 14wks vs. control
IL-17C+KO involved 14wks vs. control
IL-17C+ involved 14wks vs. IL-17C+KO involved 14wks

