

AHR signaling dampens inflammatory signature in neonatal skin $\gamma\delta$ T cells.

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Supplementary Data

Supplementary Materials

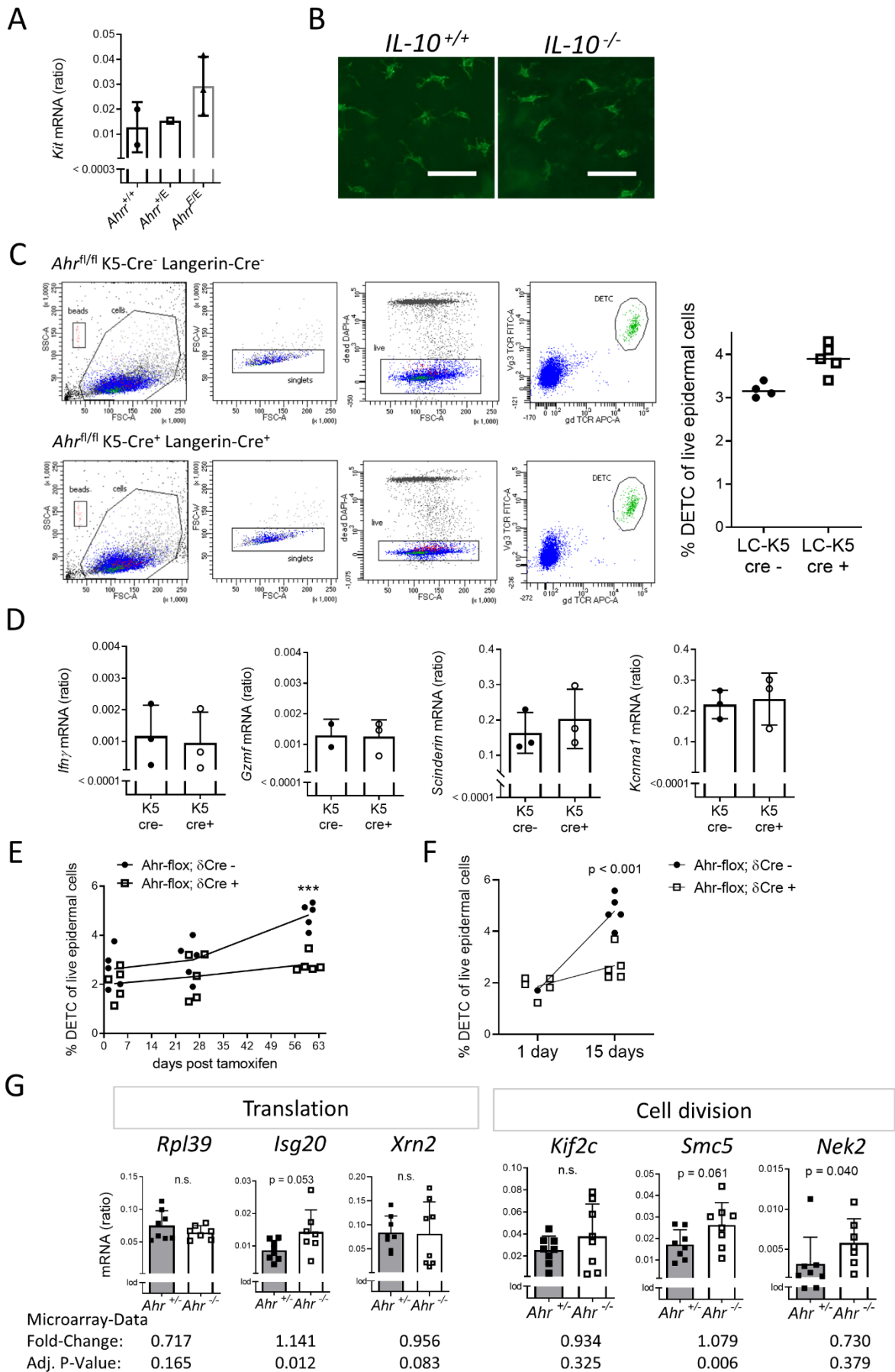
Supplemental Table 1

Primer sequences for quantitative real-time PCR

<i>Rps6</i>	(TACTGTGCCTCGTCGGTTG,	antisense:	TGAATCTTGGGTGCTTTGGT),
<i>Ifnγ</i>	(GGCCATCAGCAACAACATAA	, antisense:	ATCAGCAGCGACTCCTTTTC),
<i>Pdl1</i>	(AAGTCAATGCCCCATAACCGC,	antisense:	CTCTTCCCCTCACGGGTTG),
<i>Ccr5</i>	(AGACATCCGTTCCCCCTACA,	antisense:	GCAGGGTGCTGACATACCAT),
<i>Ccl1</i>	(GCATGCTTACGGTCTCCAAT,	antisense:	AGGCGCAGCTTTCTCTACCT),
<i>Ccr2</i>	(CAGAAGAGGGCATTGGATTC,	antisense:	CAAGGCTCACCATCATCGTA),
<i>Ccr1</i>	(GTTGGGACCTTGAACCTTGA,	antisense:	TCCTTTGCTGAGGAACTGGT),
<i>Kenmal</i>	(ATATCCGCCAGACTGAC,	antisense:	ATCGTTGGCTGCAATAAACC),
<i>Camk2d</i>	(TCCATCGGGACCTAAAGCCT,	antisense:	TGCCAGCAAACCAAACCAT),
<i>Fermt2</i>	(CCCAGAGATCCCACAAAGAA,	antisense:	TATCCCAGGATTGCCTTCTG),

Arhgap6 (TTTGACCGTGGGGTTGATGT, antisense: GGCTCCAACAGGAGAGTGTT),
Rhod (AACCTGTGCACCTCCAAATC, antisense: CACAACAATGATGGGCACTC),
Advillin (GTGGAGGTGGATCAGCAGTT, antisense: GTTGGAGTTGAGCGAGGAAG),
Il10ra (GCCCTCAAACAGTACGGAAA, antisense: AAGCGAGTCTCAGTGGTGGT),
Scinderin (CAGGGTGCCTATGGTGACTT, antisense: CTTGCCACCCAAATAGTCGT),
GranzymeF (TGACCCTTCTTCTGCCTCTC, antisense: TCATTGAGCTTCCAGTGCAG),
C-Kit (ATCCCGACTTTGTCAGATGG, antisense: AAGGCCAACCAGGAAAAGTT),
Rpl39 (CAAAATCGCCCTATTCCTCA, antisense: TTCAAGACACGAAGAGTCTCCA),
Isg20 (CCATGACCTGAAGCACGACT, antisense: CCCGCCAGTTGTTCTGGAT),
Xrn2 (ACAGCAACTGTATTACCCCAGG, antisense: GGCTGGGCTCTTTGTCTTCT),
Kif2c (GCCGACACATCTAGTGCTGA, antisense: GAGTTCTCCCCAATGAACGA),
Smc5 (AATCCTTCGGTGGTTGAGGAG, antisense: CCACCTTTCTTTCACCTGCG),
Nek2 (CCTGGACAGCAAACACAATG, antisense: CAAGGACCAGATGTCCGACT),
Cyp11a1 (ATATCCCCAAGGGGTGCTGT, antisense: TTCAGGCCGGAACCTCGTTTG)

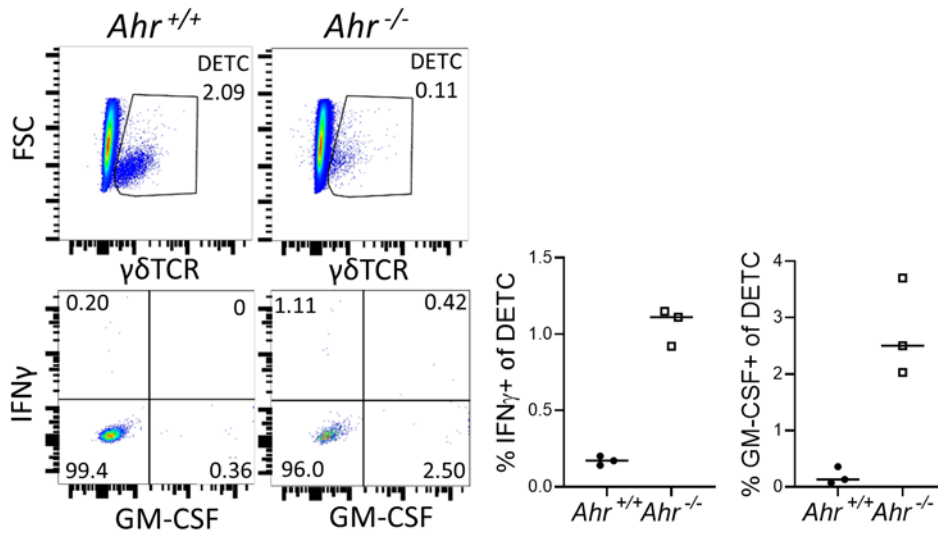
Supplementary Figure 1



Supplementary Figure 1

(A) RNA from sorted DETC from 6 – 32 weeks old *Ahrr*^{+/+}, *Ahrr*^{+E} or *Ahrr*^{E/E} mice was isolated and expression of *Kit* was measured by qPCR. Shown are ratios referred to the housekeeper gene *Rps6* (n = 1-3 mice) (B) Representative pictures of epidermal ear sheets from 9-20 weeks old *Il-10*^{+/+} or *Il-10*^{-/-} mice stained with anti-V γ 3 (scale bar = 50 μ m; n = 3 mice) (C) Epidermal cells from female, 9 weeks old, *Ahr*^{fl/fl} Cre- or *Ahr*^{fl/fl} K5-Cre+Langerin-Cre+ mice (LC-K5-Cre+) were stained for DETC (V γ 3+, $\gamma\delta$ TCR+) and were analyzed by flow cytometry for the frequency of DETC (n = 4-5 mice). (D) Epidermal cells from male and female, 13-16 weeks old, *Ahr*^{fl/fl} Cre- or *Ahr*^{fl/fl} K5-Cre+ mice were stained for DETC (V γ 3+, $\gamma\delta$ TCR+) and DETC were FACS-sorted to over 98 % purity and their RNA was isolated. Expression of interferon- γ (*Ifn γ*), granzyme F (*Gzmf*), scinderin (*Scin*) and *Kcnma1* was measured by qPCR; shown are ratios referred to the housekeeper gene *Rps6* (n = 3 mice) (E) 8 week old Ahr-flox; δ Cre+ and δ Cre- mice were treated with tamoxifen as described in the methods section. Epidermal cells were isolated 3, 26 and 60 days after the last tamoxifen-injection and stained for DETC by anti- $\gamma\delta$ TCR and anti-V γ 3 antibodies. The percentage of DETC from live cells is displayed (2-way-ANOVA with Sidak's test for multiple comparisons, *** p < 0.001; n = 5 mice) (F) Newborn mice (1 day old) were treated with tamoxifen as described in the method section. Epidermal cells were isolated 1 day and 15 days after the last tamoxifen-treatment and stained for DETC by anti- $\gamma\delta$ TCR and anti-V γ 3 antibodies. The percentage of DETC from live cells is displayed (t-test for 15 days timepoint; n = 1-5 mice) (G) RNA of DETC, FACS-sorted from mice two weeks of age, was isolated. Expression of indicated genes was measured by qPCR; shown are ratios to the housekeeper gene *Rps6* (n = 8; Mann-Whitney-test for *Nek2*, t-tests for remaining genes)

Supplementary Figure 2



Supplementary Figure 2

Epidermal cells were isolated from 8 weeks old *Ahr*^{+/+} and *Ahr*^{-/-} mice and stained for DETC by anti- $\gamma\delta$ TCR-BV711 followed by fixation and intracellular staining for IFN γ by anti-IFN γ -APC and for GM-CSF by anti-GM-CSF-PE. One representative plot for DETC-gating from live cells and IFN γ /GM-CSF proportion among DETC is shown per genotype. The plots were not standardized to a certain cell number. The percentage of IFN γ ⁺ or GM-CSF⁺ DETC of all DETC is displayed as graph (n = 3 mice)