Endocrine aryl hydrocarbon receptor signaling is induced by moderate cutaneous exposure to ultraviolet light

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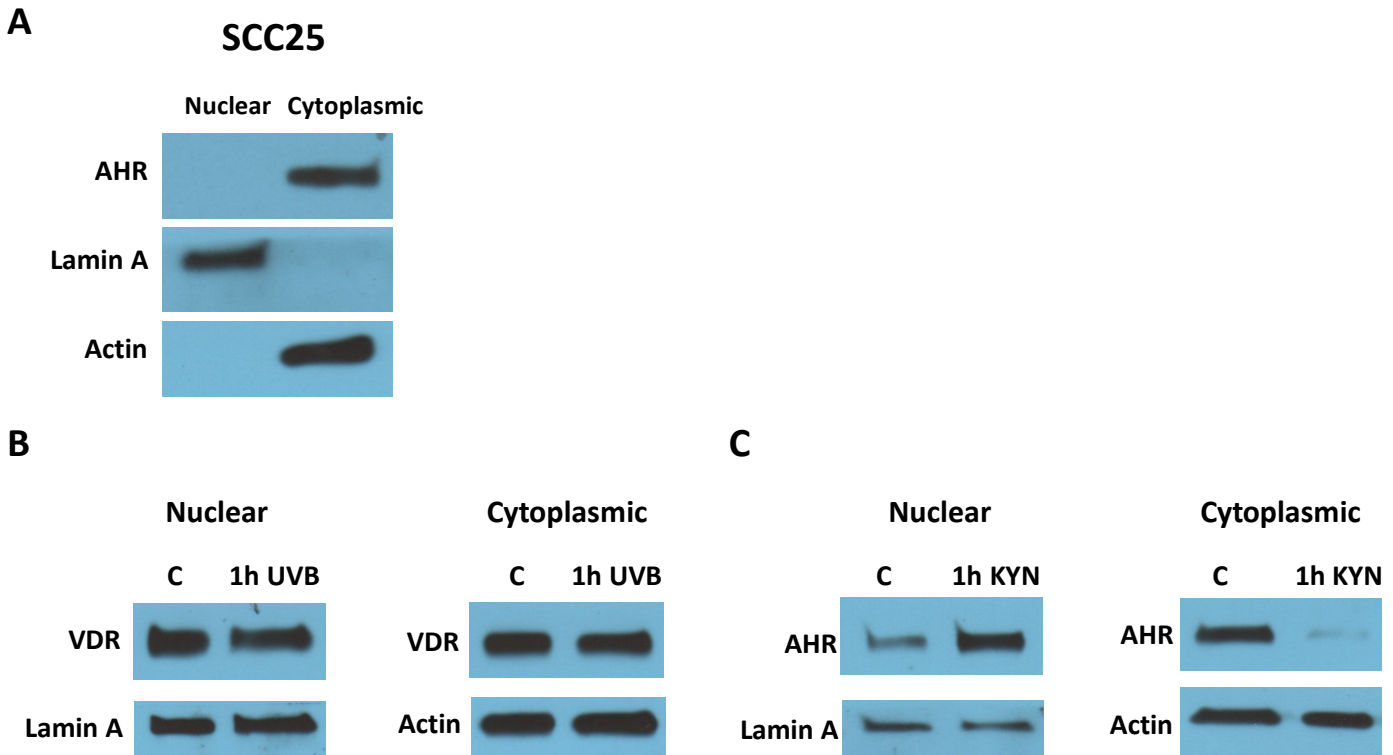
A

SCC25

Nuclear Cytoplasmic

AHR

Lamin A

Actin

B

Nuclear

C   1h UVB

VDR

Lamin A

Cytoplasmic

C   1h UVB

VDR

Actin

C

Nuclear

C   1h KYN

AHR

Lamin A

Cytoplasmic

C   1h KYN

AHR

Actin

Figure S1. A) Western Blot analysis of distribution of nuclear and cytoplasmic proteins. Actin was probed as a cytoplasmic marker; lamin A was probed as a nuclear marker. AHR and the internal controls were taken from the same blot. Blot images are provided in the Supplementary Fig S5A. **B)** Western blot analyses of VDR protein in nuclear and cytoplasmic fractions in SCC25 cells 1 hr following irradiation with UVB (15 min). VDR and the internal controls were taken from the same blot. Blot images are provided in the Supplementary Fig S5B. **C)** Western Blot analyses of AHR from nuclear and cytoplasmic fractions in SCC25 cells, 1 hr following treatment with 50 uM Kynurenin. AHR and the internal controls were taken from the same blot. Blot images are provided in the Supplementary Fig S5C.
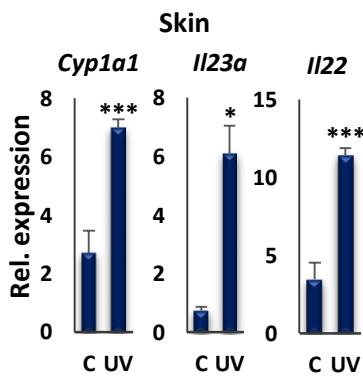
Skin

Cyp1a1   *Il23a*   *Il22*

Rel. expression

C UV   C UV   C UV

Figure S2. Effect of a single 15-min dose of cutaneous UVB irradiation on expression of AHR target genes *Cyp1a1, Il23a*, and *Il22* in mouse skin (n = 3 per group). RNA was extracted 4hr after UVB exposure.*P ≤ 0.05, ***P ≤ 0.001 as determined by one-way ANOVAs followed by Tukey's post hoc test for multiple comparisons.
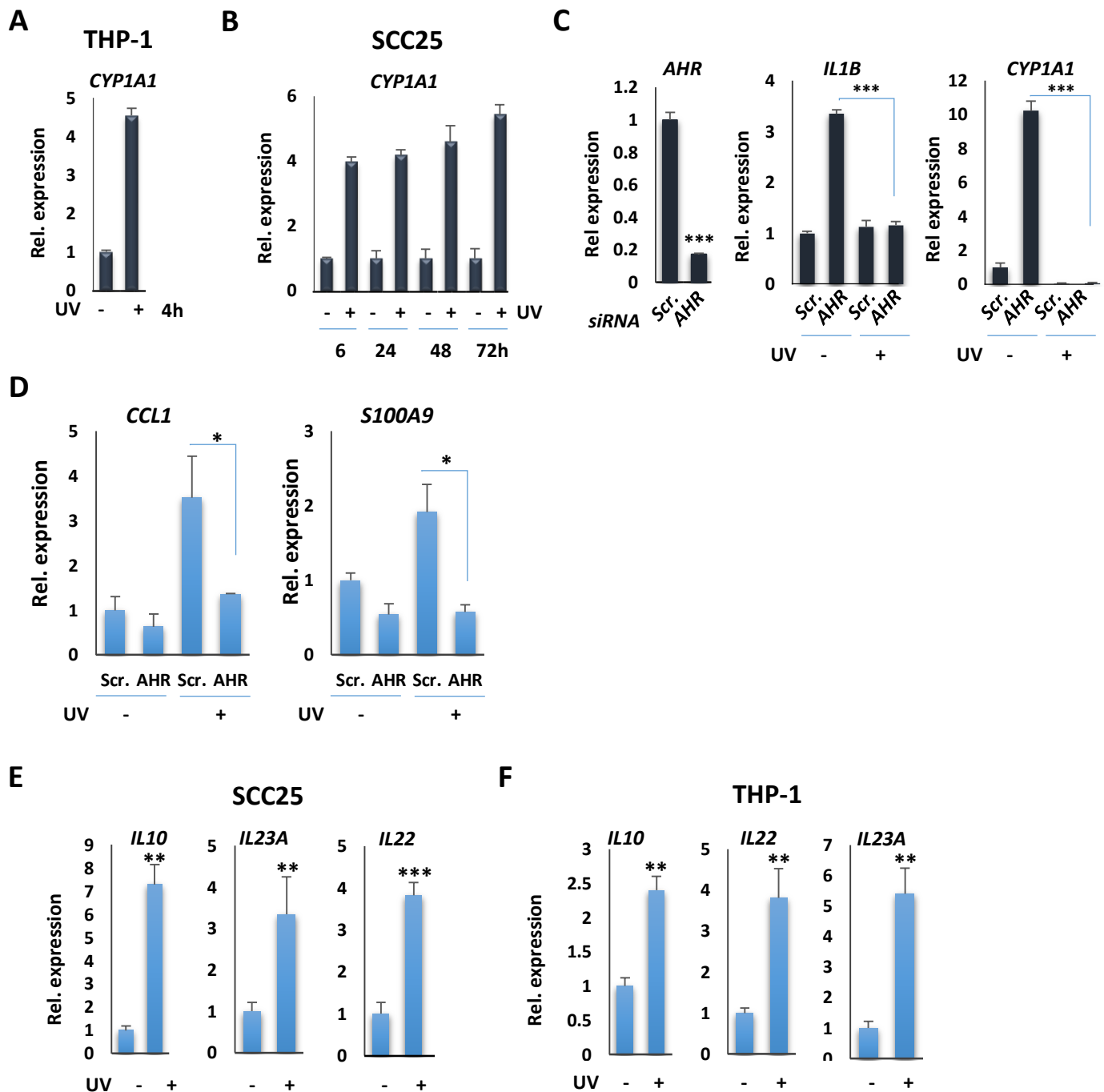
Figure S3: A) RT-qPCR analysis of *CYP1A1* transcription in THP-1 cells 4 hr following UVB exposure (15 min). **B)** RT-qPCR analysis of *CYP1A1* transcription in SCC25 cells 6, 24, 48, and 72 hr following irradiation with UVB (15 min). The expression of CYP1A1 in UV-exposed cells is a fold expression relative to the control cells, and is set as 1 in each time point. **C)** (left panel) RT-qPCR assay of *AHR* mRNA expression after knockdown of its gene with pooled siRNA #2 in SCC25 cells. (center and right panel) RT-qPCR analysis of *CYP1A1* and *IL1B* transcription in SCC25 cells following knockdown of the *AHR* gene and 4 hr after exposing cells to UVB for 15 min. **D)** RT-qPCR analysis of CCL1 and S100A9 transcription in SCC25 cells following knockdown of AHR expression and 4 hr after exposing to UVB for 15 min. **E)** RT-qPCR assay of *IL10*, *IL23A* and *IL22* mRNAs in SCC25 cells, 4 hr after 15 min of UVB irradiation. **F)** RT-qPCR assay of *IL10*, *IL23A* and *IL22* mRNAs in THP-1 cells, 4 hr after 15 min of UVB irradiation. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 as determined by one-way ANOVAs followed by Tukey's post hoc test for multiple comparisons.
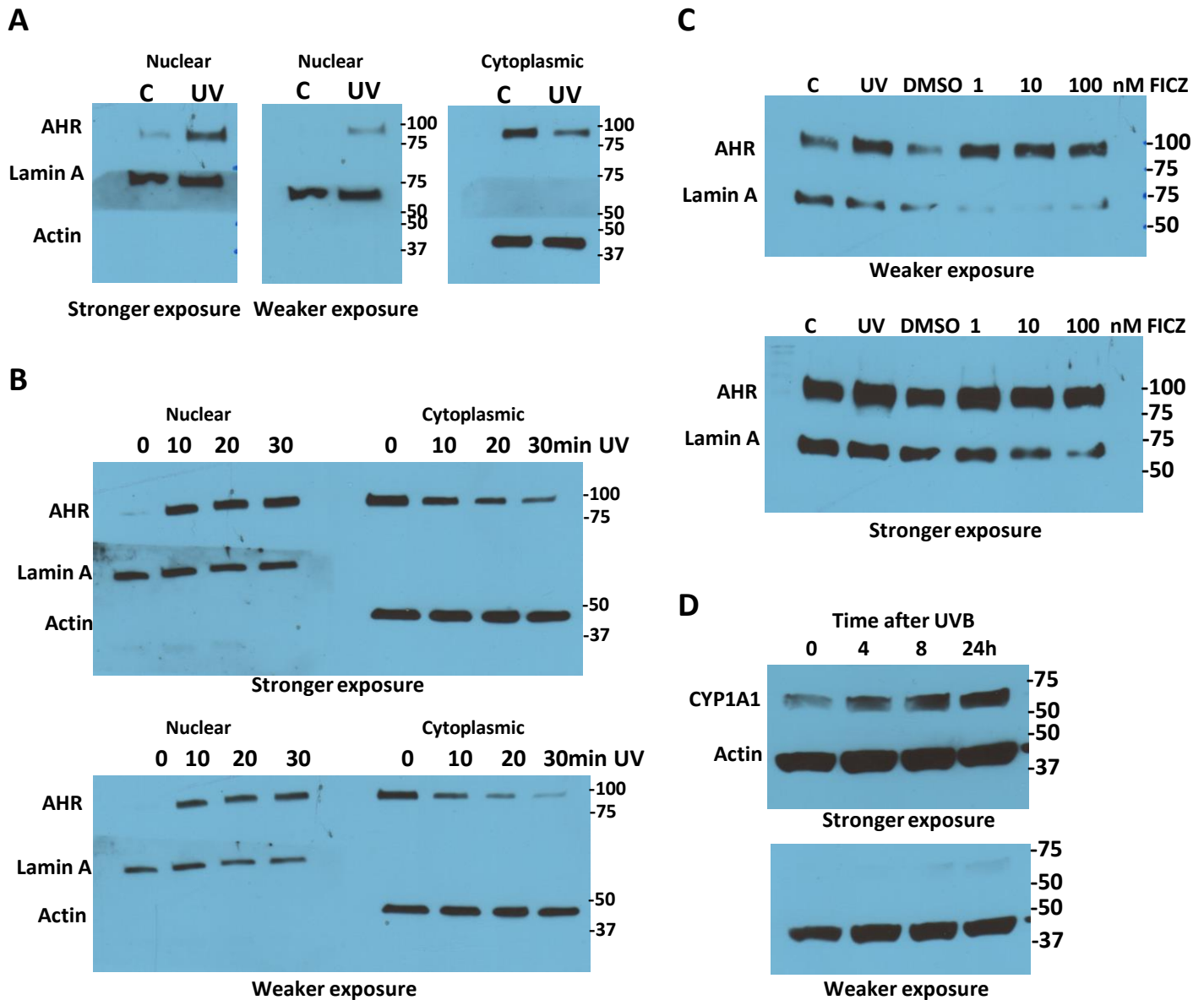
Figure S4: A) The blot corresponding to figure 1A. The blot was cut in three, up the 75 and 50 KD ladder bands. **B)** The blot corresponding to figure 1B. The blot was cut in two, up the 75 KD ladder band in the nuclear fraction part; and was cut in two, up the 50 KD ladder band in cytoplasmic part. **C)** The blot corresponding to figure 1C. The blot was cut in two, up the 75 KD ladder band. **D)** The blot corresponding to figure 1E. The blot was cut in two, at the 50 KD ladder band.
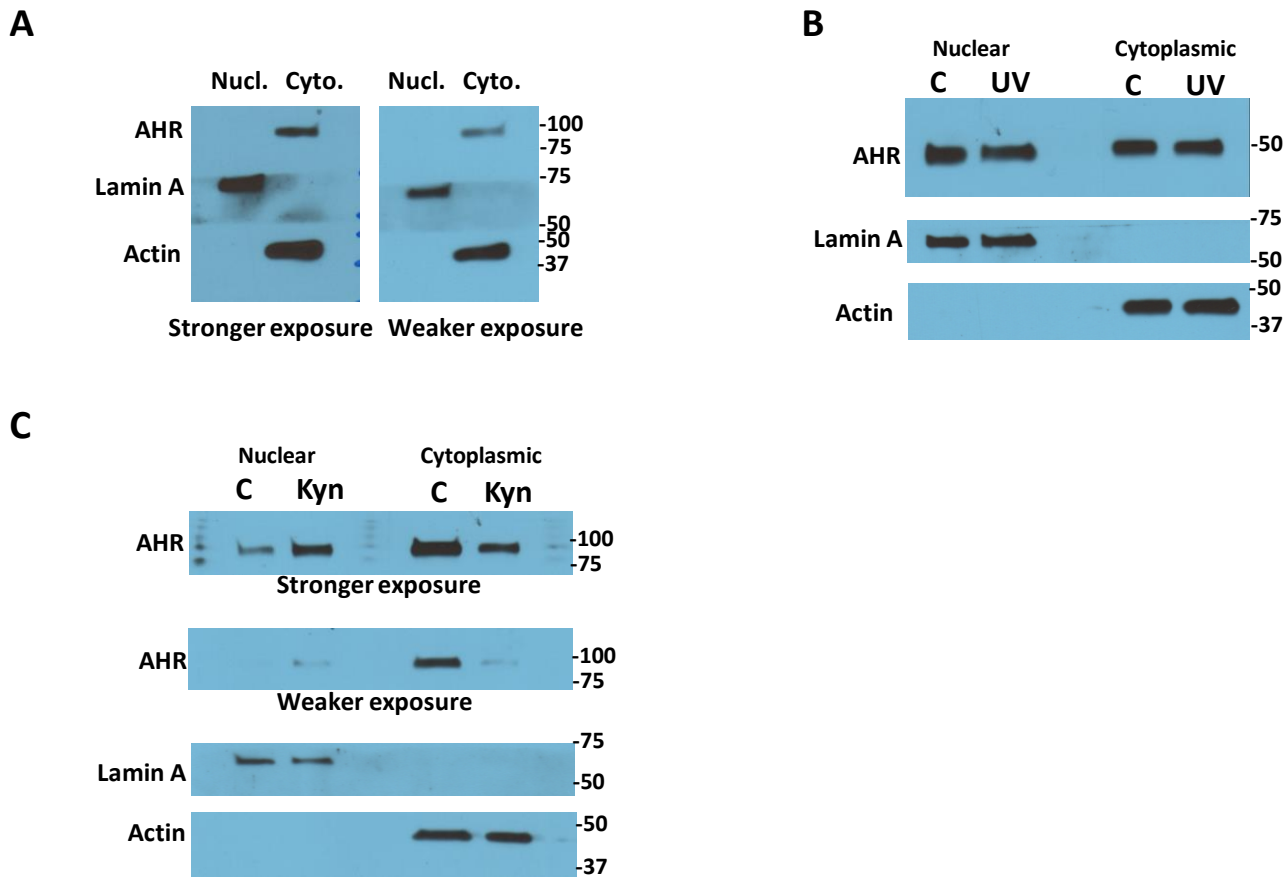
Figure S5: A) The blot corresponding to figure S1A. The blot was cut in three, up the 75 and 50 KD ladder bands. **B)** The blot corresponding to figure S1B. The blot was first probed for VDR and then stripped and cut in two, up the 75 and 50 KD ladder bands and probed for Lamin A and Actin. **C)** The blot corresponding to figure S1C. The blot was cut in three, up the 75 and 50 KD ladder bands.

Table S1: Primers for gene expression analysis		
Human AHR	F: CTTCCAAGCGGCATAGAGAC	R: AGTTATCCTGGCCTCCGTT
Human IL1B	F: AGATGAAGTGCTCCTTCCAGG	R: GGTCGGAGATTCGTAGCTGG
Human CYP1A1	F: CTTGGAACCTTCCCTGATCCTTG	R: GATCTTGGAGGTGGCTGAGGTA
Human actin (ATCB)	F: CACCTTCACCGTTCCAGTTTT	R: AACCTAACTTGCGCAGAAAACAA
Human RNA18S5	F: ACCCGTTGAACCCCATTCGTGA	R: GCCTCACTAAACCATCCAATCGG
Mouse Hmbs	F: GTGCCTACCATACTACCTCCTG	R: ACTCTCCTCAGAGAGCTGGTTC
Mouse Ahr	F: TGATGCCAAAGGGCAGCTTA	R: TGAACTGGTACCCCGATCCT
Mouse Il23a	F: ACCAGCGGGACATATGAATCT	R: AGACCTTGGCGGATCCTTTG
Mouse Il22	F: CTGTTGACACTTGTGCGATCTCTG	R: TTGACGGGCAGCGCATTTG
Mouse Nqo1	F: GATGGGAGGTACTCGAATCTGAC	R: AGCTCACCTGTGATGTCATTTCT
Mouse Cyp1a1	F: TTTAAAACACGCCCGCTGTG	R: CAGGCACAATGTCCCAGGAT
Primer sequences used for ChIP analysis:		
Human AHR on IL23A	F:GTCAGTTGTAGCCCTGGATGTA	R:TTGGGTAGGAAGAAGGGTTGGT
Human AHR on CYP1A1 (ref 1)	F: GCGCGAACCTCAGCTAGT	R:TTCCCGGGGTTACTGAGTC
Human AHR on IL10 (ref 2)	F: GTCTTGGGTATTCATCCCAGGTTGGGG	R:CTGTGGGTTCTCATTCGCGTGTTCCTA
Mouse AHR on Cyp1a1 (ref 3)	F: TATCCGGTATGGCTTCTTGC	R: CACCTTCAGGGTTAGGGTGA
Mouse AHR on Il22	F: ACAGTGATTTTCATGACTTCGCGTTCT	R: TCCCAGATAGCACCTGACAACTAGACT
Mouse AHR on Ahr	F: GTGTGTGCGCTCCCTTTGAC	R: GAGTCCGTCCACCAGTTCGTC

1. Vorrink, SU et al. (2013) Hypoxia perturbs aryl hydrocarbon receptor signaling and CYP1A1 expression induced by PCB 126 in human skin and liver-derived cell lines. *Toxicology and Applied Pharmacology* **274**, 408-416.
2. Gandhi R et al. (2010) Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell–like and Foxp3+ regulatory T cells. *Nature Immunology* **11**, 846–853.
3. Amenya HZ et. al. (2016), Dioxin induces Ahr-dependent robust DNA demethylation of the Cyp1a1 promoter via Tdg in the mouse liver. *Scientific Reports* **6**, 34989.