Ligand-mediated cytoplasmic retention of the Ah receptor inhibits macrophage mediated acute inflammatory responses

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Table S1. Primers for real-time PCR

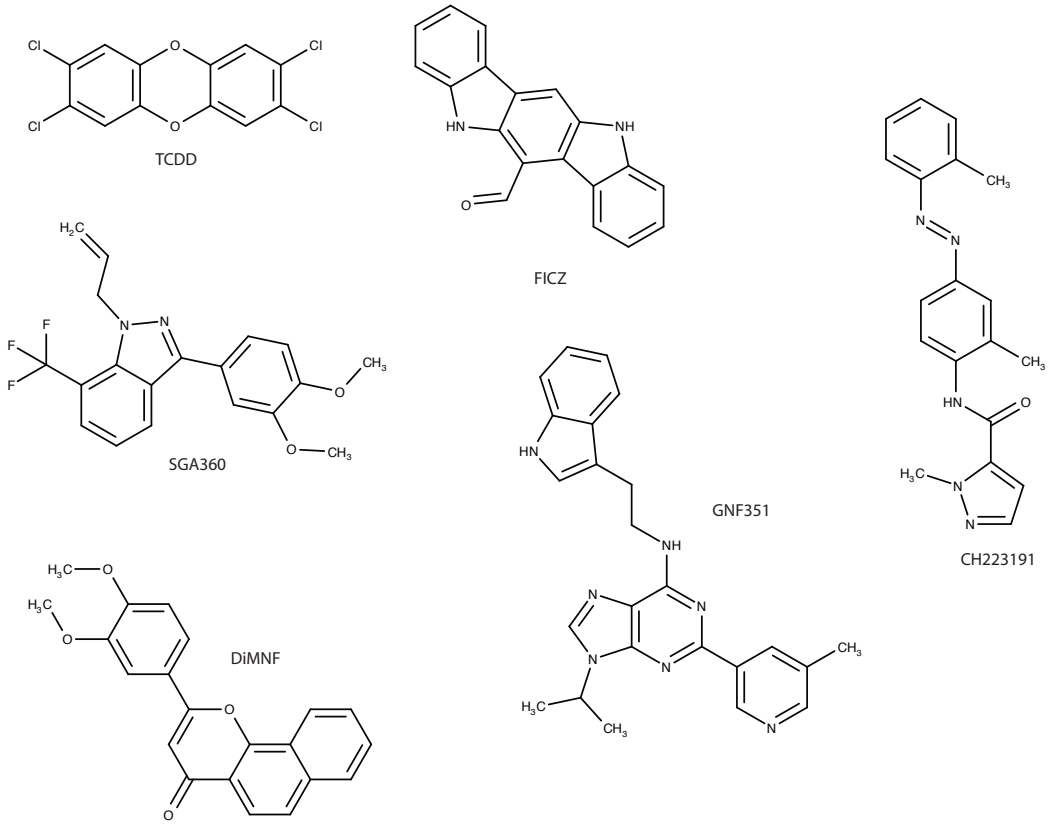
Primer name	Primer sequence
Ahrr	*5 '-CAGAAGCGGAGGCTTACCAT-3 '* *5 '-CTCTGTATTGAGGCGGTCCC-3 '*
Ccl3	*5 '-TACAAGCAGCAGCGAGTACC-3 '* *5 '-TCAGGAAAATGACACCTGGCT-3 '*
Ccl4	*5 '-CAAGCCAGCTGTGGTATTCCT-3 '* *5 '-AGCAAGGACGCTTCTCAGTG-3 '*
Cxcl1	*5 '-GCTGGGATTCACCTCAAGAA-3 '* *5 '-TCTCCGTTACTTGGGGACAC-3 '*
Cxcl2	*5 '-AGACAGAAGTCATAGCCACTCTCAAG-3 '* *5 '-CCTCCTTTCCAGGTCAGTTAGC-3 '*
Cyp1a1	*5 '-CTCTTCCCTGGATGCCTTCAA-3 '* *5 '-GGATGTGGCCCTTCTCAAATG-3 '*
Cyp1b1	*5 '-GCTAGCCAGCAGTGTGATGATATT-3 '* *5 '-GGTTAGCCTTGAAATTGCACTGAT-3 '*
Gapdh	*5 '-AAGAGGGATGCTGCCCTTAC-3 '* *5 '-CGGGACGAGGAAACACTCTC-3 '*
Ier3	*5 '-AAGATGATGGCGAACAGGAG-3 '* *5 '-GAAGGGTGCTCTACCCTCG-3 '*
Il1a	*5 '-CCAGAAGAAAATGAGGTCGG-3 '* *5 '-AGCGCTCAAGGAGAAGACC-3 '*
Il1b	*5 '-AGCTTCCTTGTGCAAGTGTCT-3 '* *5 '-GACAGCCCAGGTCAAAGGTT-3 '*
Il6	*5 '-ATCCAGTTGCCTTCTTGGGACTGA-3 '* *5 '-TAAGCCTCCGACTTGTGAAGTGGT-3 '*
Mrc1	*5 '-AGGGACCTGGATGGATGACA-3 '* *5 '-AGGGAAGGGTCAGTCTGTGT-3 '*
Ptgs2	*5 '-CTGACCCCCAAGGCTCAAAT-3 '* *5 '-ACCTCTCCACCAATGACCTGA-3 '*
Rpl13a	*5 '-TTCGGCTGAAGCCTACCAGAAAGT-3 '* *5 '-GCATCTTGGCCTTTTTCCGTT-3 '*
Tnfa	*5 '-GGTGCCTATGTCTCAGCCTCTT-3 '* *5 '-GCCATAGAACTGATGAGAGGGAG-3 '*

Table S2. Primers for ChIP-qPCR

Primer name	Primer sequence
Ptgs2	*5 '-CGTCTCTCATTTGCGTGGGTA-3 '* *5 '-CCGCTTAGGCTTTCCCCAA-3 '*

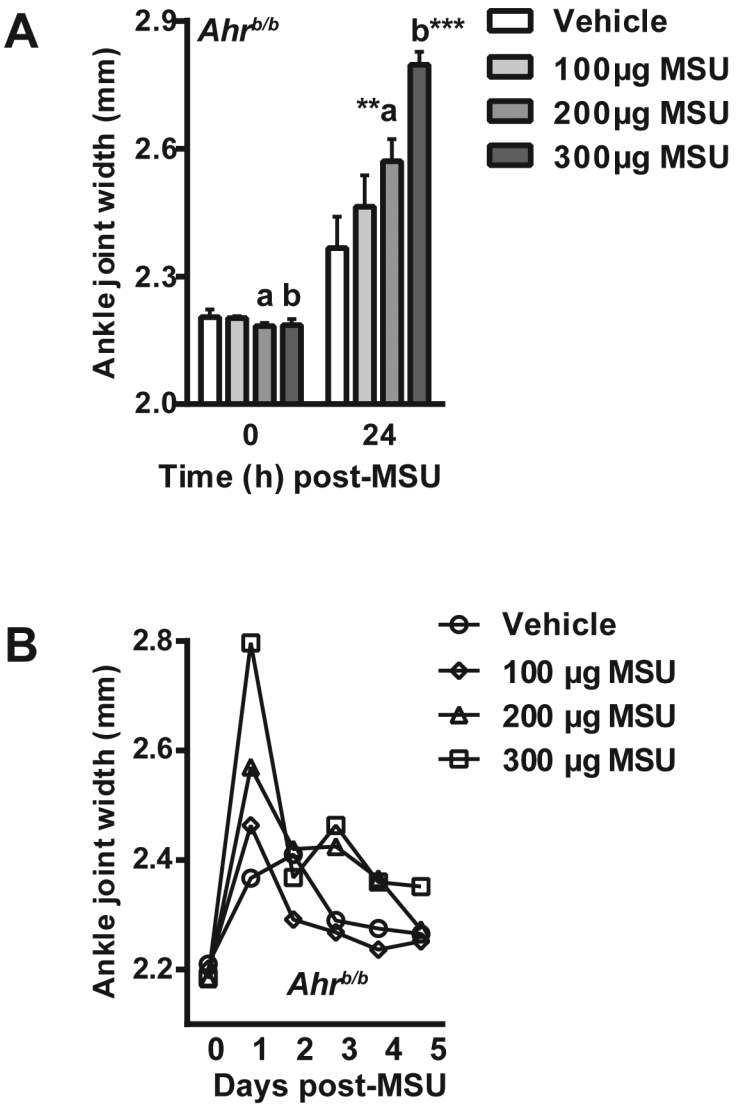
Table S3. Alignment scores

	Veh1	Veh2	LPS1	LPS2	LPS_SGA1	LPS_SGA2
input	37377503	31428251	29347575	29089028	30436943	27496891
mapped	14226751	14516693	12737106	15746862	13255485	15107846
multiple alignments	293414	240890	261413	335952	257224	305645



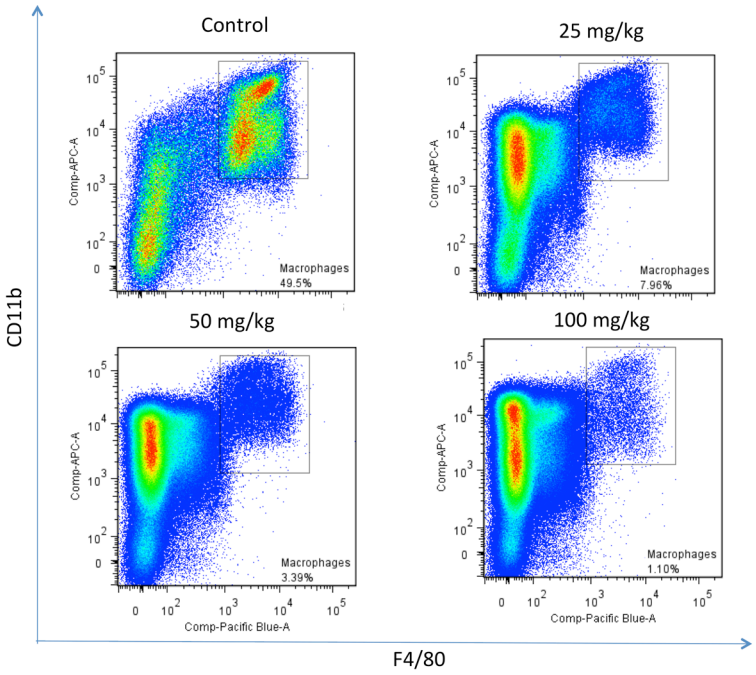
Supplemental Figure 1.

Structures of AHR ligands. TCDD and FICZ are agonists; SGA360 and DiMNF are selective Ah receptor modulators and GNF351 and CH223191 are antagonists.



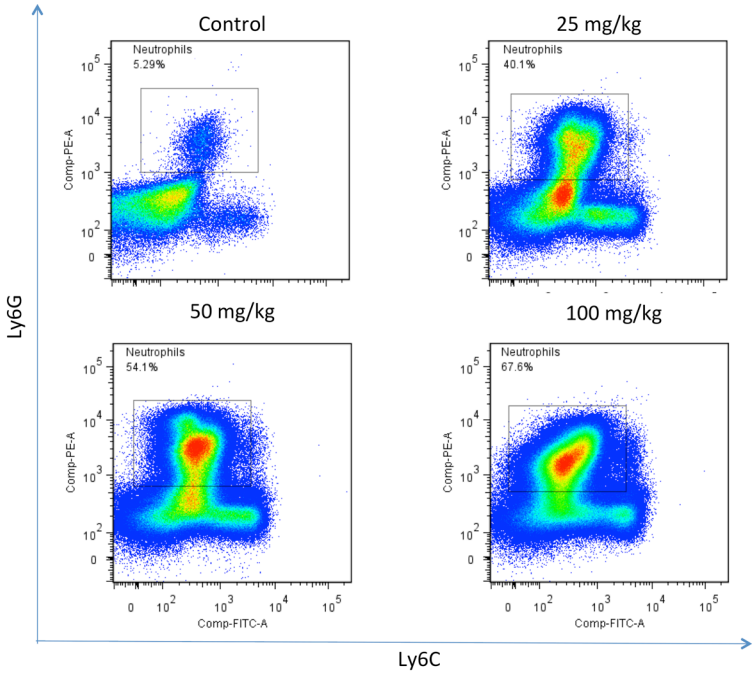
Supplemental Figure 2.

The introduction of monosodium urate crystals into an angle joint induces a dose-dependent increase in joint thickness. A, Wild-type C57BL6/J mice were administered 10 μl PBS or the indicated amount of MSU suspension through injection into the articular space of the ankle joint. Joint edema was assessed 24 h post injection through micrometry. Data represent mean joint width (mm) ± SEM. B, Wild-type C57BL6/J mice were administered 10 μl PBS or the indicated amount of MSU suspension through injection into the articular space of the ankle joint. Joint edema was assessed at 24 h intervals post injection through micrometry. Data represent mean joint width (mm) ± SEM.



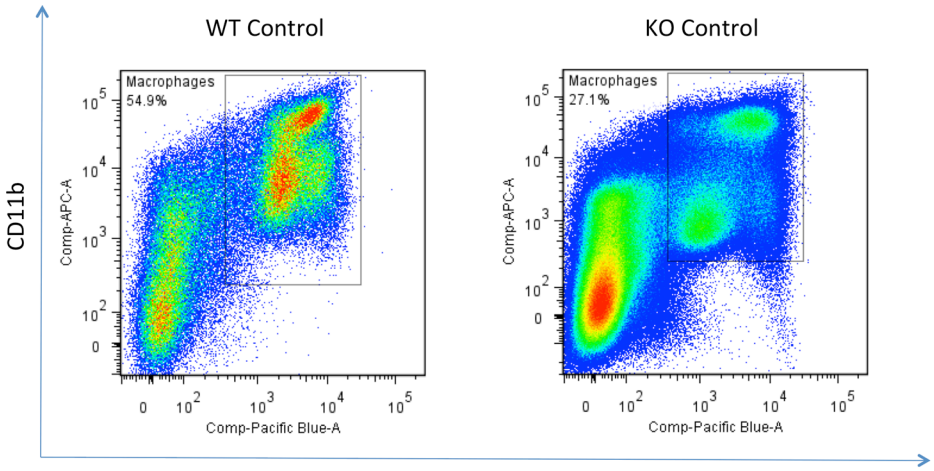
Supplemental Figure 3.

Peritoneal exposure to MSU crystals leads to a dramatic decrease in macrophages in peritoneal wash in a dose dependent manner. C57BL6/J mice were IP injected with MSU crystals after 6 h lavage fluid collected and cells subjected in flow analysis as outlined in methods section.



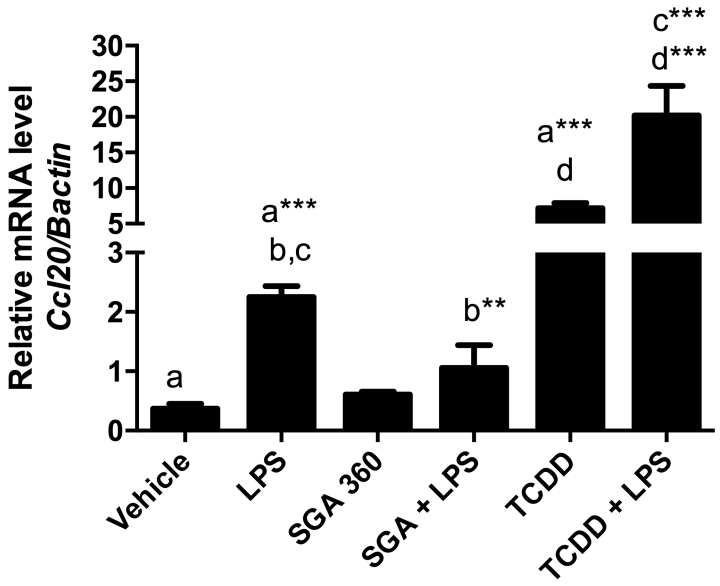
Supplemental Figure 4.

Peritoneal exposure to MSU crystals leads to a dramatic increase in neutrophils in peritoneal wash in a dose-dependent manner. C57BL6/J mice were IP injected with MSU crystals after 24 h lavage fluid was collected and subjected in flow analysis as outlined in methods section.



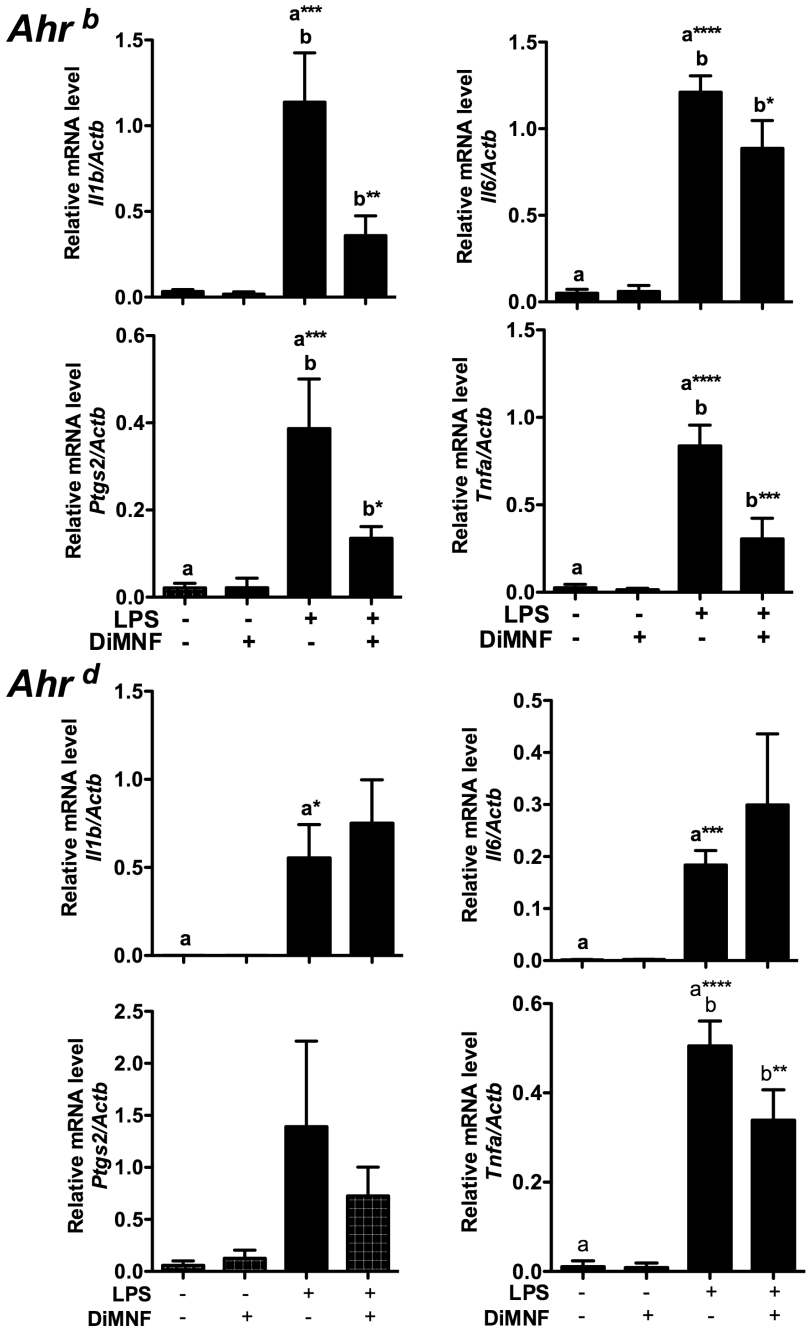
Supplemental Figure 5.

Lack of AHR expression in mice leads to a greatly reduced resident peritoneal macrophage population. Peritoneal lavage fluid from C57BL6/J and *Ahr*$^{-/-}$ mice were subjected in flow analysis as outlined in methods section.



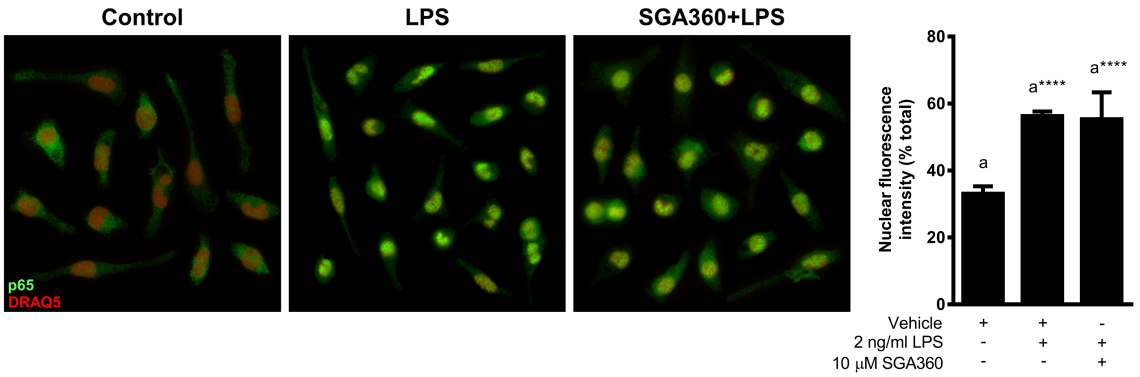
Supplemental Figure 6.

SGA360 represses and TCDD induces *Ccl20* expression in the presence of LPS. Primary peritoneal macrophage cultures were pre-treated for 1 h with AHR ligands prior to exposure to LPS for 5 h. RNA was isolated and qRT-PCR performed and *Ccl20* expression was normalized to *Bactin*.



Supplemental Figure 7.

DiMNF attenuates inflammatory signaling in primary macrophages from C57BL6/J-Ah^b but not in C57BL6/J-Ah^d mice. Primary peritoneal macrophages cultures were treated with DiMNF as indicated, after 1 h cells were treated with LPS or phosphate buffered saline for 4 h. Total RNA was isolated and the relative mRNA levels of *Ptgs2, Il1b, Il6*, and *Tnfa* were assessed through qRT-PCR. The data represents mean mRNA level normalized to *Actb* mRNA expression ± SD. (n=3/treatment group; *P<0.05, **P<0.01, ***P<0.001).



Supplemental figure 8.

SGA360 does not inhibit LPS-mediated p65 translation into the nucleus. Primary macrophages cultures were pre-treated with SGA360 for 1 h prior to exposure to LPS, after an additional 1 h cells were fixed; the presence of p65 visualized by indirect immunofluorescent and confocal microscopy. Cells were counterstained with DRAQ5 to visualized nuclei.

METHODS

Indirect immunocytochemistry

Immunolocalization of p65 was performed as described previously [1]. Briefly, cells were blocked with 10% normal goat serum in PBS, incubated with anti-p65 primary antibody (Cell Signaling Technology, Danvers, MA), and secondary antibody, Alexa Fluor® 488 Conjugate anti-rabbit IgG (Cell Signaling Technology, Danvers, MA). Nuclei were counterstained with DRAQ5 (Cell Signaling Technology, Danvers, MA) before mounting with ProLong® Gold Antifade Reagent (Cell Signaling Technology, Danvers, MA). Cells were visualized using a Keyence All-in-One Fluorescence Microscope BZ-9000 Generation II.

1. Lahoti TS et al. Aryl hydrocarbon receptor activation synergistically induces lipopolysaccharide-mediated expression of proinflammatory chemokine (c-c motif) ligand 20. *Toxicol. Sci.* 2015; 148:229-40.