Supplemental methods

Cell viability

Cells were treated with IAA (Sigma-Aldrich, France) at concentrations of 5, 10, 25, and 50µM during 24 hours. HUVEC viability was assessed by the XTT method (Sigma-Aldrich, France) that measures the mitochondrial desydrogenase activity of living cells. The absorbance of the formazan product was measured at 450 nm and corrected with background value measured at 690 nm.

The effect of IAA on cell viability was confirmed with the ANNEXIN V-FITC / 7-AAD Kit (Beckman-Coulter, France). After a 24-hour incubation with IAA at 50µM, HUVECs detached by trypsin-EDTA solution were incubated in binding buffer containing annexin V–FITC and 7-AAD, and analyzed using a Gallios flow cytometer (Beckman-Coulter, France). Cells incubated during 10 min with 3% formaldehyde were used as positive control of apoptosis (not shown). Viable cells were defined as Annexin V–FITC and 7-AAD negative cells.

Cytotoxicity measurement

Cellular cytotoxicity was assessed by measuring LDH release using the Cytotoxicity Detection Kit (LDH) from Roche Diagnostics (France) after overnight HUVEC incubation with IAA at 50 µM.

Efficiency of AhR transfection

The efficiency of transfection by magnetofection was determined 24h post-transfection using a fluorescein-labeled dsRNA oligomer (BLOCK-iT, Life Technologies, France) and compared with the unlabelled control siRNA (Negative Universal Control, Stealth™ RNAi, Life Technologies). The

BLOCK-iT fluorescence was visualized by microscopy with X100 magnification and the percentage of fluorescent transfected HUVEC was quantified using a Gallios flow cytometer (Beckman-Coulter).

Supplemental Table 1. Inhibitors used in experiments

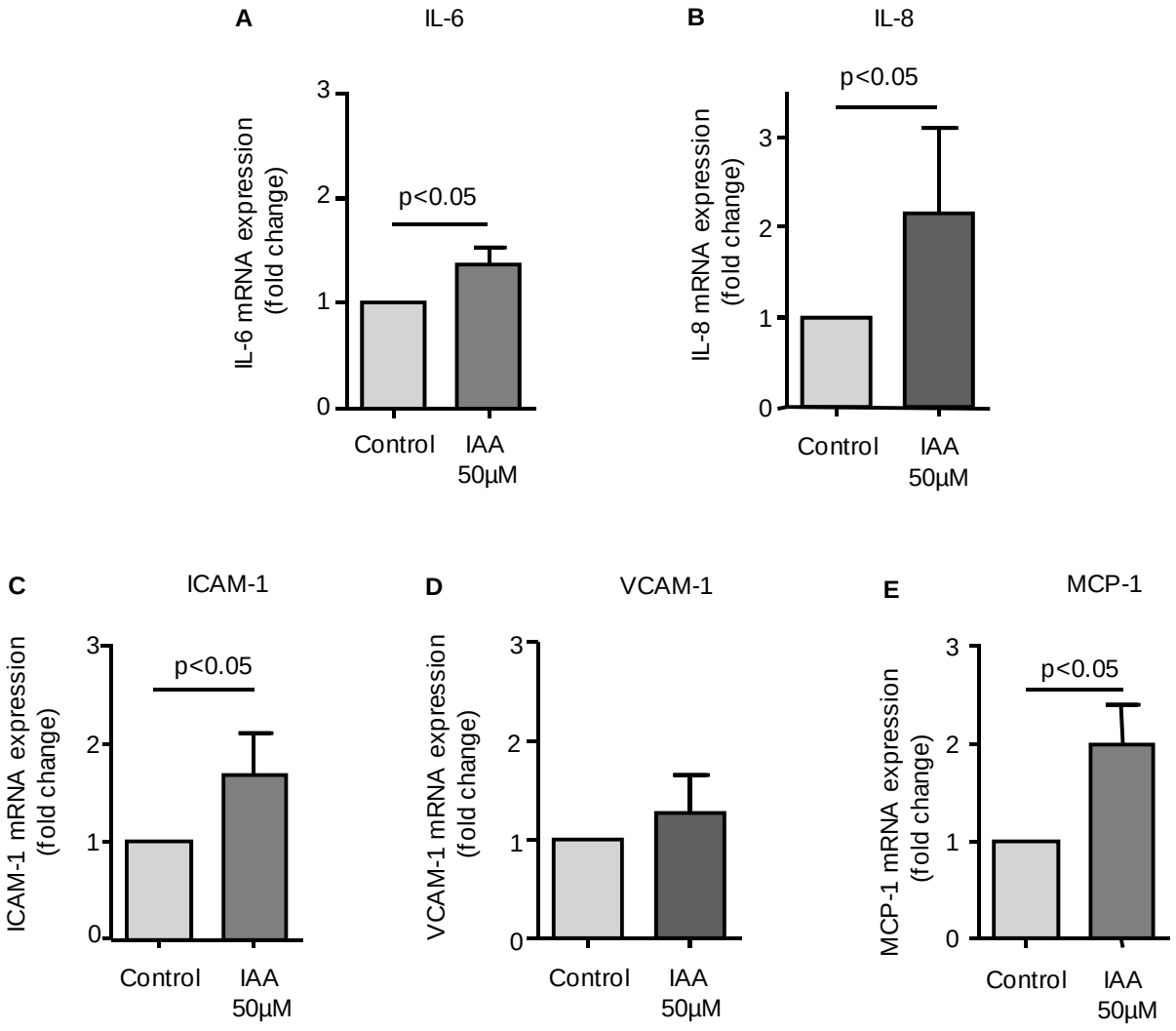
Inhibitor	Target	Concentration	Manufacturer
CH-223191	AhR	10 µM	Sigma- Aldrich
SB203580	P38	10 µM	Merck Chemicals
PD98059	ERK1/2	10 µM	Sigma- Aldrich
BAY 11-7082	NF-κB	20 µM	Merck Chemicals
N-acetylcysteine	ROS scavenger	10mM	Sigma- Aldrich

Supplemental Table 2. Sequences of primers used in RT-qPCR experiments

Gene	Primer reverse	Primer forward
COX-2	(5' ATTGACCAGAGCAGGCAGAT 3')	(5' CAGGATACAGCTCCACAGCA 3')
COX-1	(5' AACATGGACCACCACATCCT 3')	(5' TCCAGGGTAGAACTCCAACG 3')
Cyp1A1	(5'ATAGCACCATCAGGGGTGAG 3)'	(5'GACAGATCCCATCTGCCCTA 3')
Cyp1B1	(5' CCACGACCTGATCCAATTCT 3')	(5' TGATGGACGCCTTTATCCTC 3')
AHRR	(5' CTTTGTGGGTCCTGGAGTCT 3')	(5' GAAGGAGCAGCAGAGAGAGC 3')
HPRT	(5' GAGCTATTGTAATGACCAGTCAACAGG3')	(5'GGATTATACTGCCTGACCAAGGAAAGC 3')
AHR	(5' TGGTGCCCAGAATAATGTGA 3')	(5' TGTTGACGTCAGCAAGTTC 3')
IL-6	(5' CAGGGGTGGTTATTGCATCT 3')	(5'AGGAGACTTGCCTGGTGAAA -3')
IL-8	(5' TGCCAAGGAGTGCTAAAG 3')	(5' CTCCACAACCCTCTGCAC 3')
ICAM-1	(5' AGCTTCTCCTGCTCTGCAAC 3')	(5' CATTGGAGTCTGCTGGGAAT 3')
VCAM-1	(5' AGCACGAGAAGCTCAGGAGA 3')	(5' AAAAGCGGAGACAGGAGACA 3')
MCP-1	(5' CCCCAGTCACCTGCTGTTAT 3')	(5' TGGAATCCTGAACCCACTTC 3')

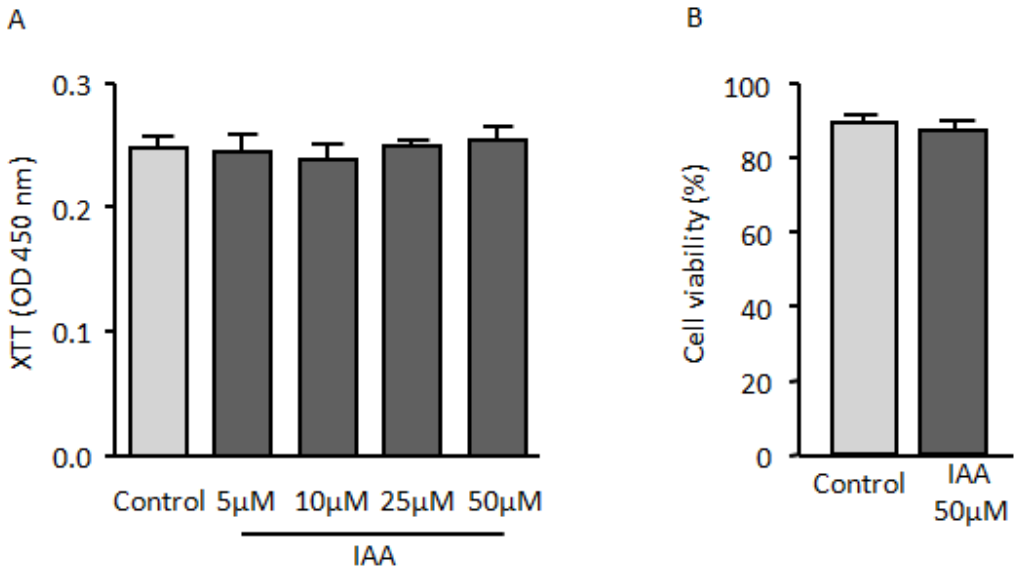
Supplemental figure 1. Effect of IAA on endothelial inflammatory molecules

The increase in IL-6 (A), IL-8 (B), ICAM-1 (C), VCAM-1 (D), and MCP-1 (E) mRNA expression induced by IAA in HUVEC after a 24-h incubation was studied by comparative quantitative RT-PCR and expressed in mRNA fold change vs. control. Data represent the mean ± SEM of 5 independent experiments. * p<0.05.



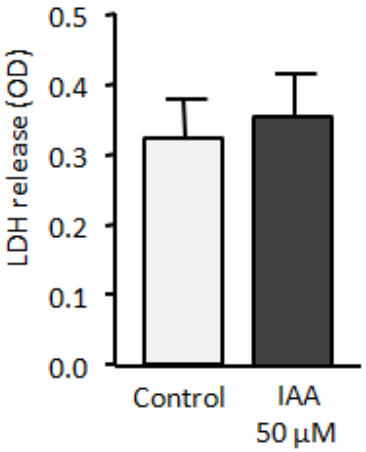
Supplemental figure 2. IAA did not modify endothelial cell viability.

The viability of HUVEC was studied by the XTT method (A) and by Annexin V/7-AAD staining (B). Data are expressed as mean ± SEM of 5 independent experiments.



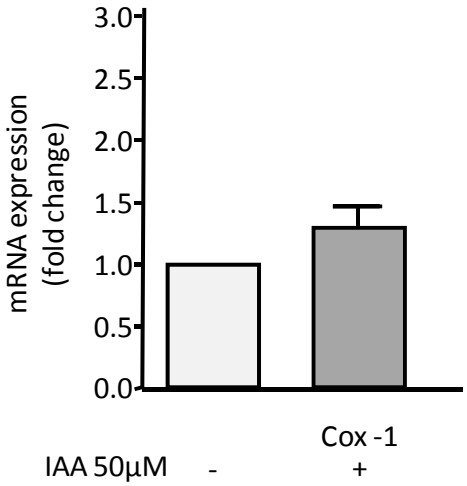
Supplemental figure 3. IAA did not induce endothelial cytotoxicity.

IAA-induced cytotoxicity was evaluated by measuring LDH release by endothelial cells. Data are expressed as mean ± SEM of 5 independent experiments.



Supplemental figure 4. IAA did not modify mRNA expression of COX-1.

mRNA expression of COX-1 after 4-h incubation of endothelial cells with IAA was studied by comparative RT-qPCR and expressed in mRNA fold change vs. control. Data are expressed as mean ± SEM of 5 independent experiments.

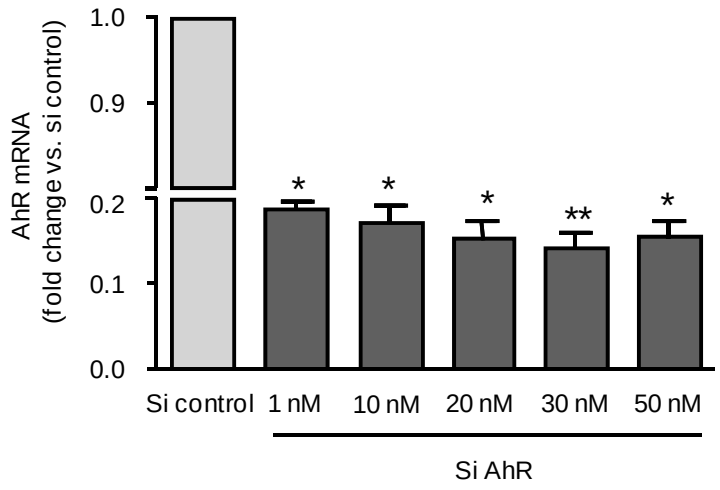


Supplemental figure 5.

(A) Dose-response of AhR siRNA. AhR mRNA levels were studied 72h post-transfection in HUVEC transfected with AhR siRNA and compared to HUVEC transfected with control siRNA. Results are expressed as means ± SEM of 4 independent experiments. *P< 0.05

(B-G) Efficiency of AhR siRNA transfection. The efficiency of transfection by magnetofection was determined 24h post-transfection using a fluorescein-labeled dsRNA oligomer (BLOCK-iT, Life Technologies). Representative images in white light (B) and fluorescence (C) with X100 magnification are displayed. **(D)** The percentage of HUVEC transfected with unlabelled control siRNA or fluorescent BLOCK-iT labelled AhR siRNA was measured by flow cytometry A representative experiment (from n=3) show the percentage of transfected HUVEC. The mRNA expression of AhR-regulated genes Cyp1A1 (E), Cyp1B1 (F), and AHRR (G) after a 4-h incubation with IAA in HUVEC transfected with control siRNA or AhR siRNA was analyzed 72h post-transfection. Data represent the mean ± SEM of 5 independent experiments. *p<0.05, ***p<0.001.

A



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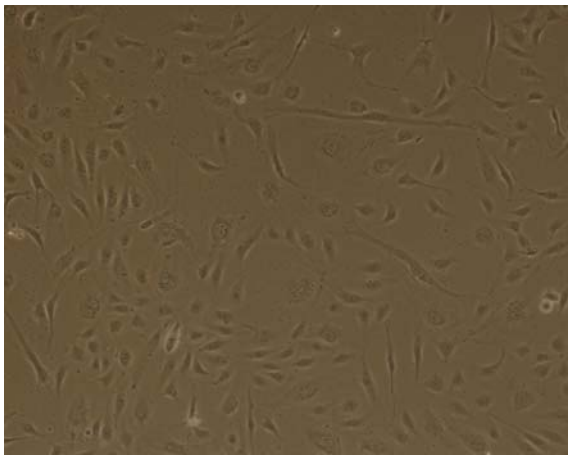

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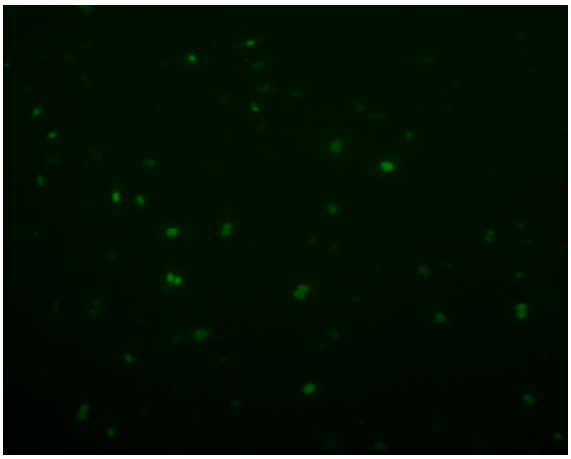

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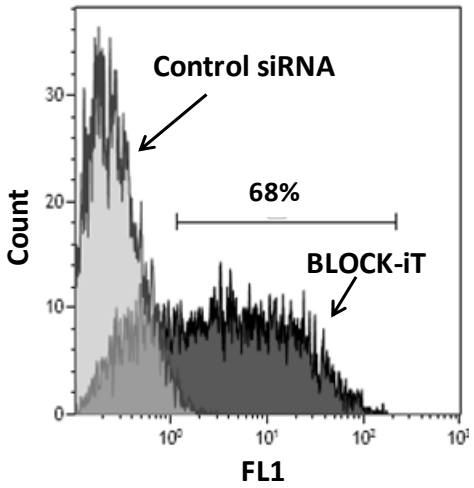
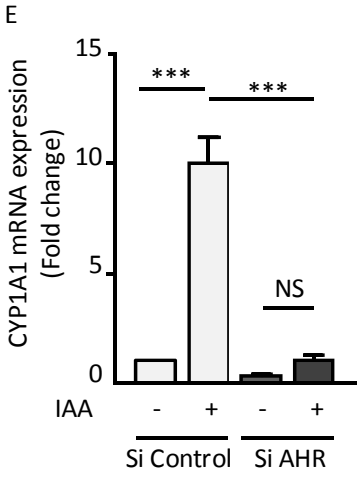
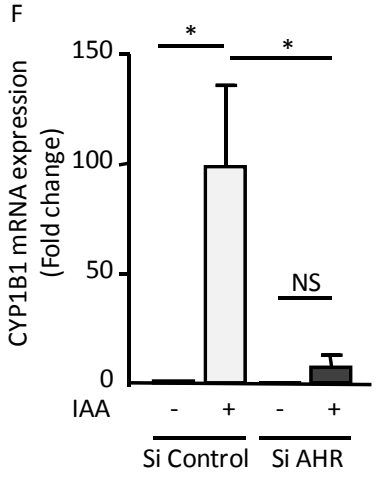

Control siRNA
68%
BLOCK-iT
Count
FL1

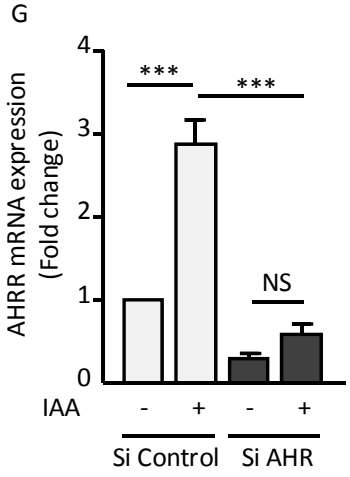
E


CYP1A1 mRNA expression
(Fold change)
15
10
5
0
***
***
NS
IAA
-
+
-
+
Si Control
Si AHR

F


CYP1B1 mRNA expression
(Fold change)
150
100
50
0
*
*
NS
IAA
-
+
-
+
Si Control
Si AHR

G


AHRR mRNA expression
(Fold change)
4
3
2
1
0
***
***
NS
IAA
-
+
-
+
Si Control
Si AHR