The aryl hydrocarbon receptor controls mesenchymal stromal cell-mediated immunomodulation via ubiquitination of eukaryotic elongation factor-2 kinase

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Supplement Figure 1

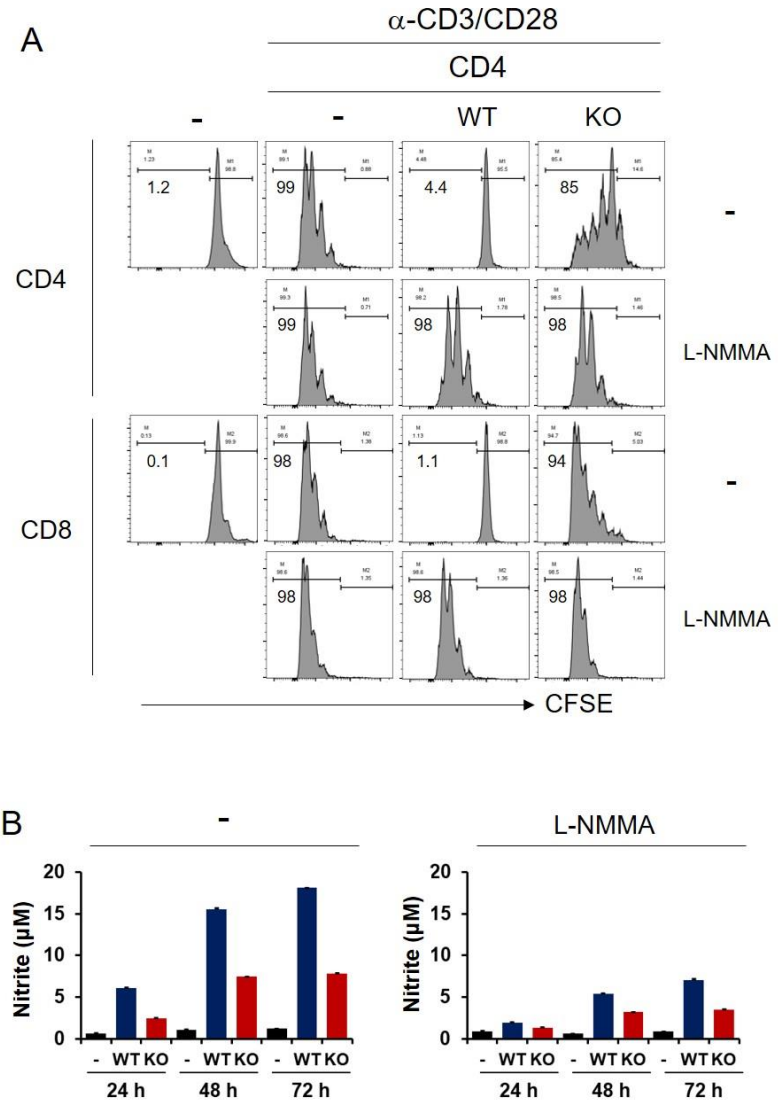

Fig. 1 Effect of *Ahr*$^{-/-}$ MSC on T cell proliferation in the presence of iNOS inhibitor

Lymphocytes were stained with CFSE and stimulated with 1 μg/mL anti-CD3 and anti-CD28 Abs in the presence of 1 mM L-NMMA, an iNOS inhibitor. (A) After 72 h, T-cell divisions were analyzed by flow cytometry. (B) NO production was measured in the culture media using ELISA.

Supplement Figure 2

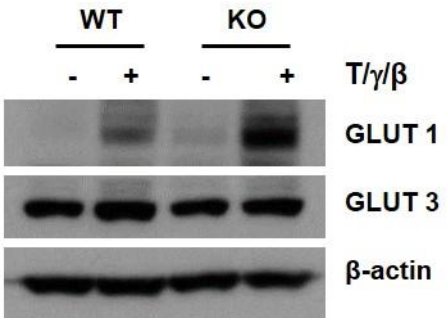

Fig. 2 Effect of *Ahr* deficiency on glucose transporters

WT- and KO-MSCs were stimulated by TNF-α, IFN-γ, and IL-1β. After 24 h, the expression of GLUT1 and GLUT3 proteins was measured by western blotting.

Supplement Figure 2

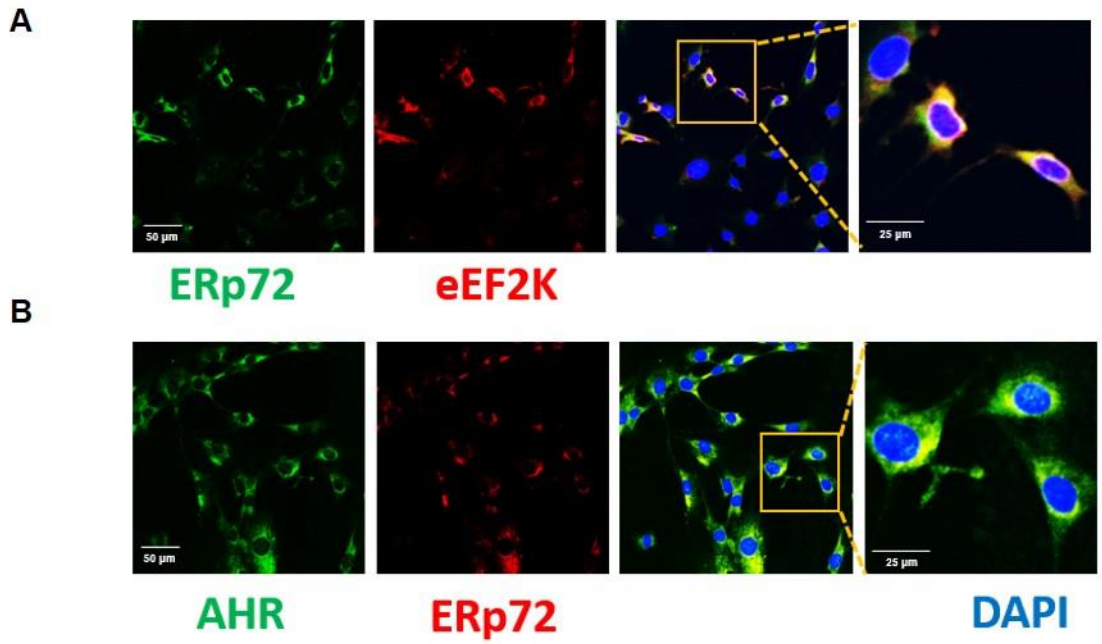

Fig. 3 Co-localization of AHR and eEF2K with ER marker ERp72

(A) MSCs were stained with anti-AHR (green) and anti-eEF2K (red) antibodies and co-localization was measured by immunofluorescence. (B) MSCs were stained with anti-AHR (green) and anti-ERp72 (red) antibodies and immunofluorescence measured co-localization. DAPI was used to count nuclear staining. All experiments were repeated at least twice, and similar results were obtained.

Supplement Figure 4

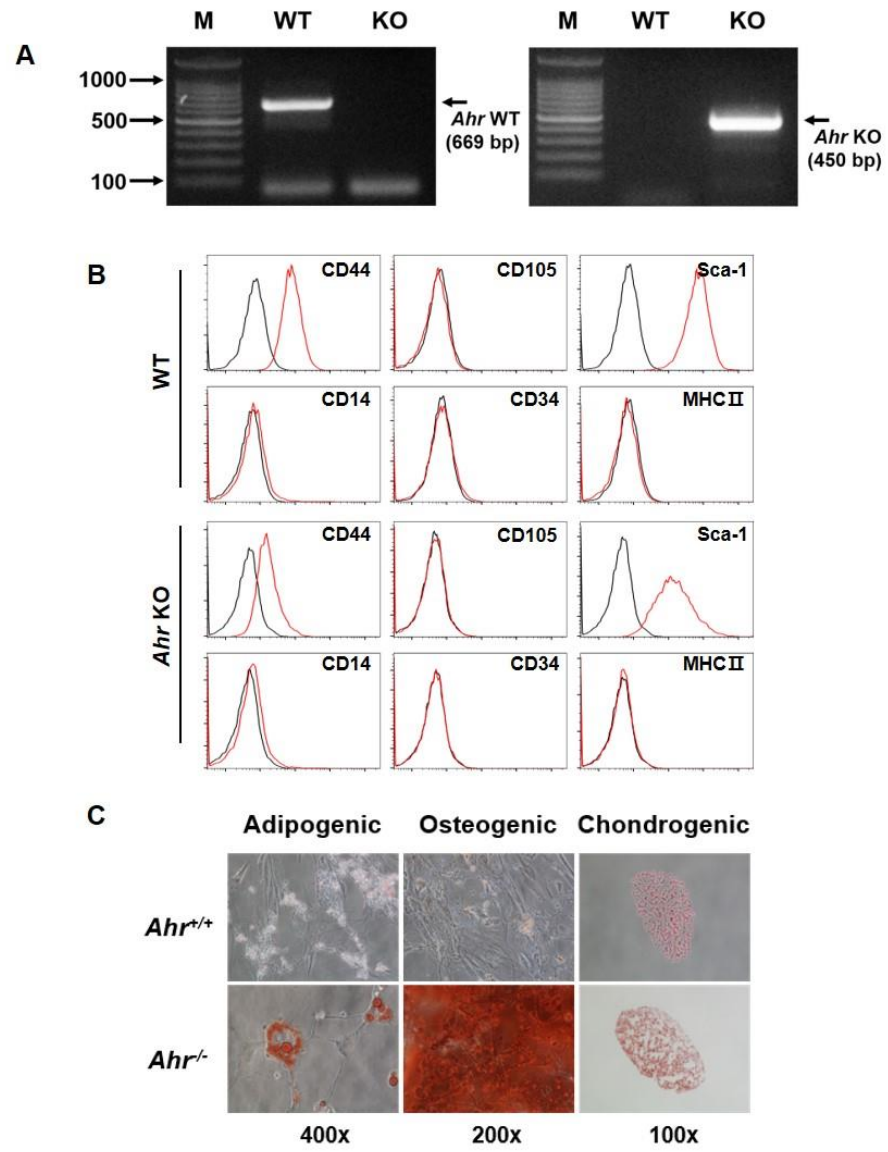

Fig. 4 Genotyping, characterization, and differentiation of MSCs

(A) Genotyping of *Ahr*. Using phenol extraction, genomic DNA was isolated from *Ahr* wild-type (WT)- and knockout (KO)-MSCs. *Ahr* gene deficiency was confirmed by genomic PCR. (B) MSCs were characterized according to cell surface markers, such as positive for CD44, CD105, and Sca-1 and negative for CD14, CD34, and MHC class II. (C) Three lineage differentiation of MSCs was performed. All experiments were repeated at least twice, and similar results were obtained.

Supplement Figure 5

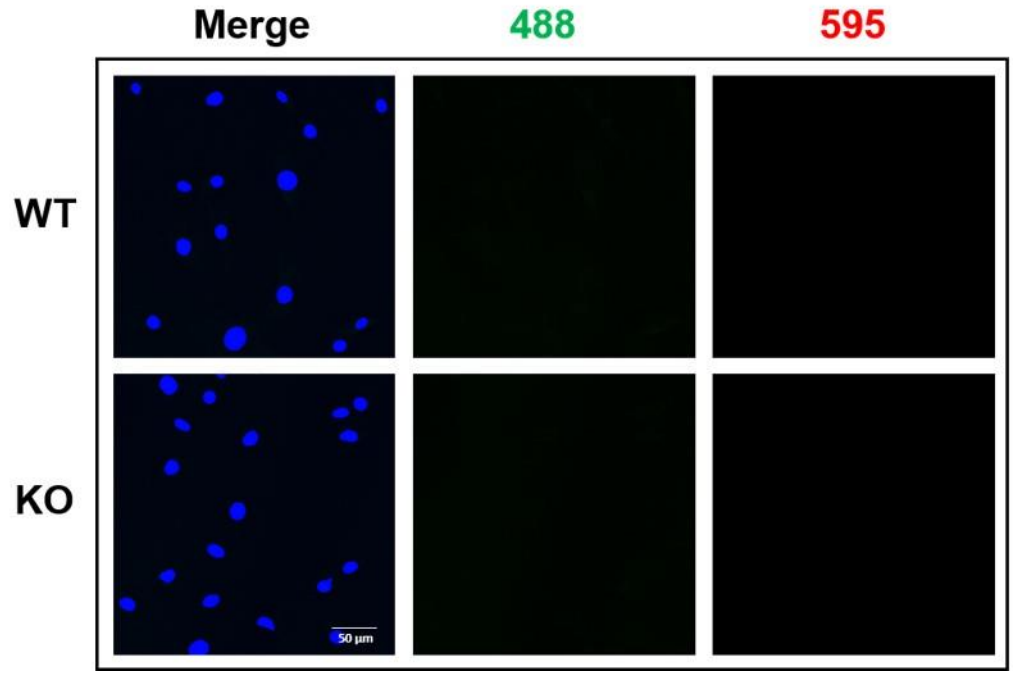

Fig. 5 Immunofluorescence staining control

2nd Antibodies were stained alone as a negative control.

Supplemental Table 1 Antibodies

Antibody	Source	Identifier	Application
Actin	Santa Cruz (CA, USA)	#47778	WB (1/1,000)
AhR	Proteintech (Rosemont, IL, USA)	#17840-I-AP	WB (1/1,000), IF (1/100)
b-TRCP	Cell Signaling (Boston, MA, USA)	#11984S	WB (1/1,000)
CD14	BD Biosciences (NJ, USA)	#560638	FC (1/100)
CD34	Biolegend (CA, USA)	#128611	FC (1/100)
CD44	BD Biosciences (NJ, USA)	#553133	FC (1/100)
CD105	Biolegend (CA, USA)	#120407	FC (1/100)
CUL4B	Proteintech (Rosemont, IL, USA)	#12916-1-AP	WB (1/1,000), IP (4 μg)
eEF2	Cell Signaling (Boston, MA, USA)	#2332S	WB (1/1,000)
eEF2k	Santa Cruz (CA, USA)	#390710	WB (1/500), IP (4 μg), IF (1/100)
eIF2a	Cell Signaling (Boston, MA, USA)	#9722S	WB (1/1,000)
ERp72	Cell Signaling (Boston, MA, USA)	#5033S	IF 1/100
GLUT1	Abcam (Cambridge, UK)	#115730	WB (1/1,000)
GLUT3	Proteintech (Rosemont, IL, USA)	#20403-1-AP	WB (1/1,000)
INOS	Cell Signaling (Boston, MA, USA)	#13120	WB (1/2000), IP (4 μg)
MHC II	BD Biosciences (NJ, USA)	#553623	FC (1/100)
p-eEF2 (Thr56)	Cell Signaling (Boston, MA, USA)	#2331S	WB (1/1,000)
p-eEF2k (S366)	Cell Signaling (Boston, MA, USA)	#3691	WB (1/1,000)
p-eIF2a (S51)	Cell Signaling (Boston, MA, USA)	#3597S	WB (1/1,000)
STAT1	Cell Signaling (Boston, MA, USA)	#14944	WB (1/1,000)
p-STAT1 (S727)	Cell Signaling (Boston, MA, USA)	#8826	WB (1/1,000)
Ubiquitin	Santa Cruz (CA, USA)	#8017	WB (1/1,000)
Mouse IgG1-HRP	Cell Signaling (Boston, MA, USA)	#7076S	WB (1/1,0000)
Rabbit Ig-G1-HRP	Invitrogen (Carlsbad, CA, USA)	#31460	WB (1/1,0000)

Supplemental Table 2. Chemical lists

Name	Source	Identifier
A-484954	Sigma (St Louis, MO, USA)	#324516
Choloroquine diphosphate salt	Sigma (St Louis, MO, USA)	#C6628
CH-223191	Sigma (St Louis, MO, USA)	#C8124
Cycloheximide	Sigma (St Louis, MO, USA)	#C7698
6-formylindolo [3, 2-b] carbazole	Sigma (St Louis, MO, USA)	#SML1489
L-NMMA	Sigma (St Louis, MO, USA)	#53308-83-1
MG132	Sigma (St Louis, MO, USA)	#474790
3-Methylcholanthrene	Sigma (St Louis, MO, USA)	#442388
Adipogenic differentiation		
Dexamethasone	Sigma (St Louis, MO, USA)	#D8893
Indomethacin	Sigma (St Louis, MO, USA)	#17378
Insulin	Sigma (St Louis, MO, USA)	#19278
Isobutylmethylxanthine	Sigma (St Louis, MO, USA)	#17018
Chondrogenic differentiation		
Ascorbic acid	Sigma (St Louis, MO, USA)	#A4403
Dexamethasone	Sigma (St Louis, MO, USA)	#D8893
ITS Premix	BD Bioscience	#354352
L-Proline	Sigma (St Louis, MO, USA)	#P5607
TGF-β1	R&D Systems	#240-B
TGF-β3	R&D Systems	#243-B
Osteogenic differentiation		
Ascorbic acid	Sigma (St Louis, MO, USA)	#A4403
β-glycerolphosphate	Sigma (St Louis, MO, USA)	#G9422
Dexamethasone	Sigma (St Louis, MO, USA)	#D8893
dibutyryl cyclin AMP	Sigma (St Louis, MO, USA)	#D067

Supplemental Table 3. GVHD Clinical scores

	Severity scores		
Symptoms	normal	mild	severe
Ruffled hair	0	1	2
Hunched back	0	1	2
Diarrhea	0	1	2
Damaged skin	0	1	2
Ascites	0	1	2
Total			10
Dead			10