Endothelial progenitor cell-derived conditioned medium mitigates chronic cerebral ischemic injury through macrophage migration inhibitory factor-activated AKT pathway

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Short title: **The role of MIF in the EPC-CM in chronic cerebral ischemia**

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Supporting information

Table S1 The human cytokine array results from conditioned medium of EPCs

	Protein name	EPC-CM (<5%)	EPC-CM (5-30%)		Protein name	EPC-CM (<5%)	EPC-CM (5-30%)		Protein name	EPC-CM (<5%)	EPC-CM (5-30%)
1	PAI-1	3.65	18,637.18	47	DKK-1	0.52	25.10	93	Dtk	6.80	0.93
2	ICAM-2	71.66	10,729.11	48	TREM-1	16.35	24.68	94	EGF R	0.00	0.91
3	MIF	13.33	8,018.72	49	Galectin-7	72.36	22.35	95	MIP-1d	0.00	0.80
4	IGFBP-4	641.66	2,295.08	50	DR6	0.50	21.91	96	EG-VEGF	3.81	0.78
5	Angiostatin	1,887.46	1,914.17	51	IL-2 Ra	34.12	20.28	97	bFGF	4.68	0.73
6	TIMP-2	2.28	1,421.55	52	I-TAC	22.97	20.22	98	BTC	5.22	0.67
7	TIMP-1	0.00	1,317.97	53	XEDAR	5.10	20.04	99	VEGF	0.00	0.65
8	IL-13 R2	244.11	979.02	54	Eotaxin	2.15	16.03	100	TARC	0.56	0.46
9	VEGF R1	0.00	728.14	55	TNF RII	3.05	14.28	101	NRG1-b1	2.98	0.32
10	TRAIL R3	4.04	539.34	56	IL-15	32.48	13.22	102	NT-4	3.59	0.29
11	IGFBP-2	3.58	533.83	57	SCF R	0.00	12.54	103	MIG	0.31	0.24
12	MCP-1	5.82	481.05	58	Cathepsin S	5.82	10.53	104	BDNF	0.14	0.21
13	HCC-1	1.50	325.24	59	IL-6	4.19	10.42	105	IL-12p70	0.32	0.18
14	IL-17B	76.01	239.46	60	Shh-N	0.00	9.75	106	BLC	0.04	0.14
15	DAN	0.00	236.07	61	LIMPII	0.30	9.41	107	MIP-1b	0.93	0.13
16	ICAM-1	0.32	177.37	62	IL-17R	18.36	8.98	108	MIP-3a	0.15	0.12
17	GDF-15	0.20	156.55	63	CEACAM-1	6.21	8.86	109	IL-11	7.30	0.11
18	PIGF	0.04	134.45	64	IL-6R	2.61	8.57	110	GCP-2	0.00	0.10
19	IL-13 R1	303.09	124.85	65	Endoglin	1.78	8.16	111	MCP-3	0.10	0.02
20	PECAM-1	9.32	110.14	66	TSLP	10.27	7.38	112	IL-9	17.60	0.00
21	E-Selectin	0.04	78.41	67	CXCL16	0.00	7.36	113	IL-18 BPa	2.73	0.00
22	OPG	0.00	71.39	68	ST2	6.17	7.14	114	IL-31	0.47	0.00
23	gp130	0.00	71.21	69	MICA	4.42	7.09	115	LIGHT	1.04	0.00
24	Activin A	253.30	67.88	70	ErbB3	1.37	4.65	116	MCP-4	0.16	0.00
25	TGFb1	170.68	67.13	71	Cripto-1	2.69	4.56	117	MIP-3b	0.00	0.00
26	E-Cadherin	34.58	66.18	72	MCSF	0.24	4.51	118	MSP	13.89	0.00
27	Follistatin	8.43	64.12	73	L-Selectin	47.30	4.04	119	PARC	2.55	0.00
28	LYVE-1	2.96	63.13	74	IL-2 Rg	12.83	4.00	120	SDF-1a	0.37	0.00
29	IL-13	68.25	62.40	75	BMP-4	4.35	3.63	121	BMP-5	307.48	0.00
30	IL-8	2.09	61.65	76	TGFb2	2.54	3.63	122	GH	3.71	0.00
31	LAP(TGFb1)	3.06	57.85	77	GRO	1.44	3.49	123	VEGF-D	0.15	0.00
32	VCAM-1	97.70	55.79	78	IGFBP-1	5.00	3.47	124	GM-CSF	2.70	0.00
33	ANG-1	64.41	50.56	79	ALCAM	0.00	2.64	125	I-309	1.66	0.00
34	uPAR	36.23	47.88	80	IL-1a	1.96	2.21	126	IL-1b	0.02	0.00
35	TNF RI	2.88	45.80	81	IL-1ra	0.00	2.16	127	IL-7	0.86	0.00
36	TNFa	33.03	42.78	82	HGF	0.00	1.75	128	IL-10	0.79	0.00
37	PDGF-AB	27.77	40.14	83	OPN	2.60	1.69	129	IL-12p40	0.24	0.00
38	Tie-2	28.63	39.03	84	IL-17	0.00	1.68	130	IL-16	0.49	0.00
39	AR	25.25	38.99	85	RANTES	0.57	1.64	131	TNFb	16.04	0.00
40	IL-2 Rb	52.72	37.19	86	Lipocalin-2	1.97	1.52	132	CD40L	6.44	0.00
41	Eotaxin-3	43.81	35.89	87	IP-10	1.22	1.48	133	ICAM-3	4.32	0.00
42	NrCAM	12.12	35.22	88	VEGF R2	0.00	1.46	134	MICB	17.11	0.00
43	AgRP	38.27	31.93	89	IFNg	0.00	1.34	135	PDGF Rb	82.19	0.00
44	Axl	0.00	30.30	90	RAGE	2.31	1.22	136	TIM-1	12.39	0.00
45	Angiogenin	32.72	25.96	91	BCMA	6.29	1.08	137	Trappin-2	2.63	0.00
46	TRAIL R4	58.60	25.14	92	IL-2	0.59	1.03				

The medium was collected from young EPCs and filtered by a Tangential Flow Filtration (TFF) membrane filter system unit with a 30 kDa cut-off (Millipore). The filtered and concentrated medium was studies with the cytokine array by using Quantitative Cytokine Quantibody Human Array 4000 (RayBiotech, Norcross, GA) following the manufacturer's protocol.

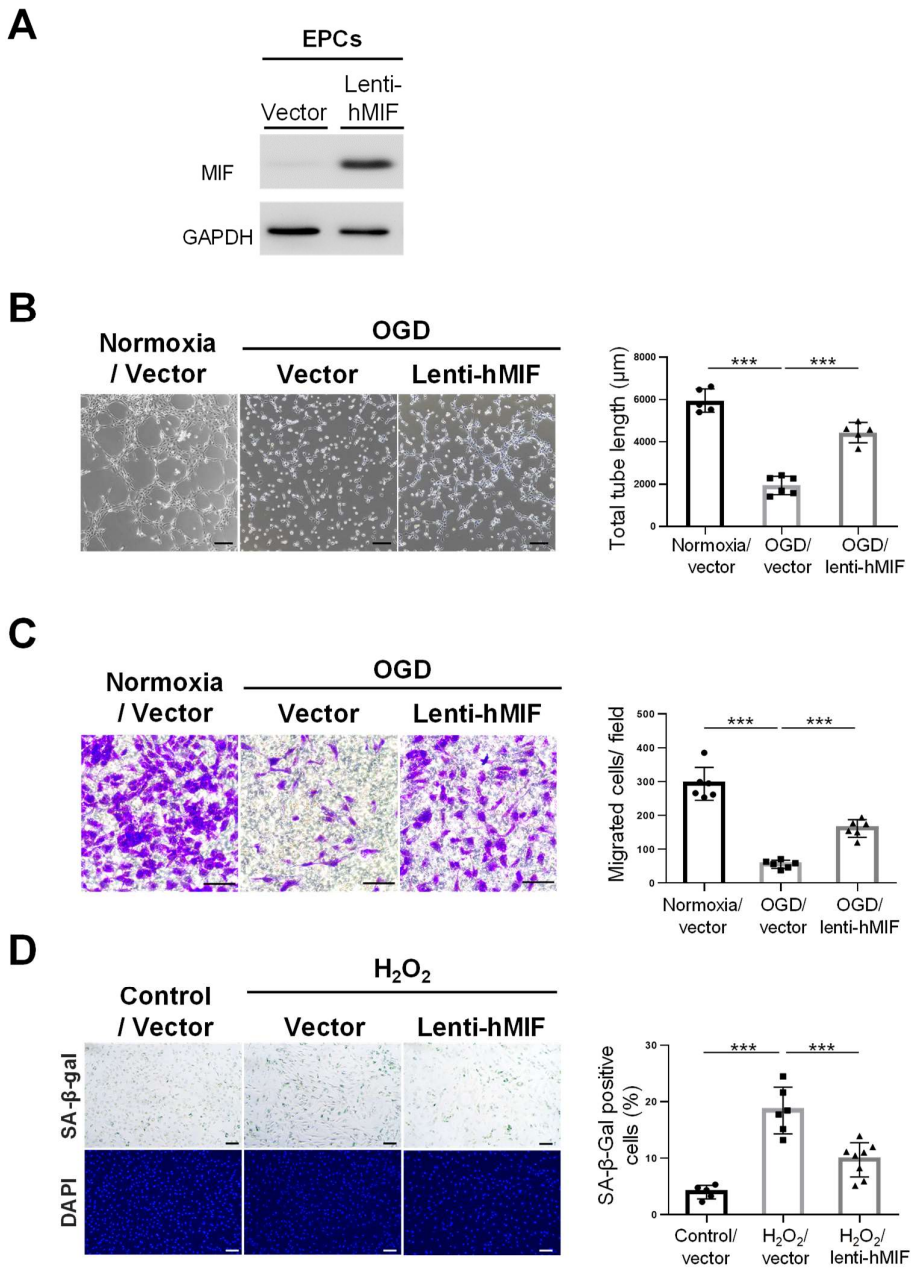

Fig. S1 Transduction of EPCs with lenti-hMIF increases cells angiogenesis and anti-senescence *in vitro*

A Detection of the MIF protein expression of the lenti-hMIF virus transduced cells. EPCs were transduced with lenti-hMIF virus for three days. The MIF expression was analyzed by Western blotting using anti-MIF Ab. The lentivirus vector-transduced cells

were used as control. **B** Determination of the tube formation in EPCs expressed vector or hMIF under OGD treatment by Matrigel assay (left panel). The EPCs incubated in the normorxia were used as control. The average total tube length in five random fields was quantitatively analyzed and showed as bar graph (right panel). Scale bar = 100 μm. **C** Images of the EPCs passed through the transwell membrane and stained by crystal violet stain (left panel). The migrated cell numbers were quantitatively analyzed by 6 fields randomly (right panel). Scale bar = 100 μm. **D** Images of the SA-β-gal staining in H_2O_2 treated EPCs. Young EPCs (passage <10) was transduced with lenti-hMIF or lenti-vector control for three days. The transduced EPCs were treated with H_2O_2 to induce the senescence, and then incubated in 5% FBS EGM-2 for three days (left panel). The SA-β-gal positive cells were analyzed and normalized with DAPI as senescence ratio. The data wad comparted with the H_2O_2/vector group (right panel). Scale bar = 100 μm. The data was shown as the mean ± SD, and analyzed using One-way ANOVA. ** $p < 0.01$, *** $p < 0.001$.

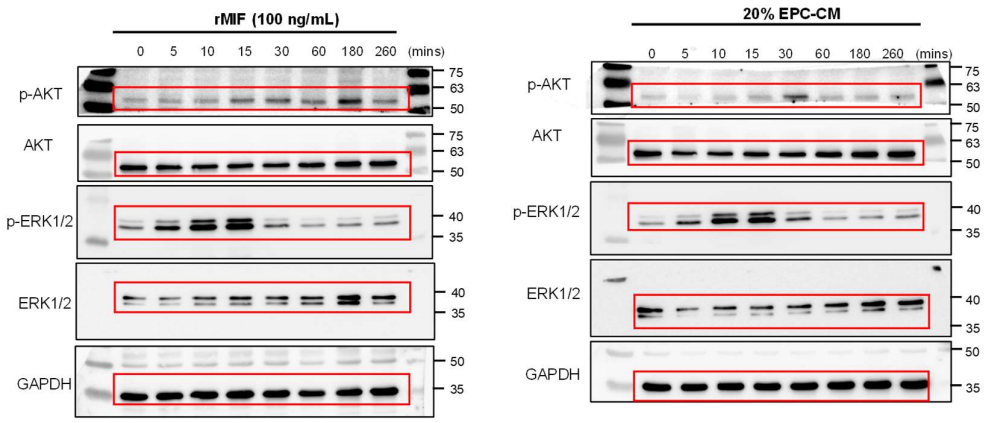

Fig. S2 Corresponding full-length immunoblot of Figure 6A

Western blot analysis of p-AKT, AKT, p-ERK1/2, ERK1/2, and GAPDH in Figure 6A.

The red rectangles indicate the cropped immunoblot.

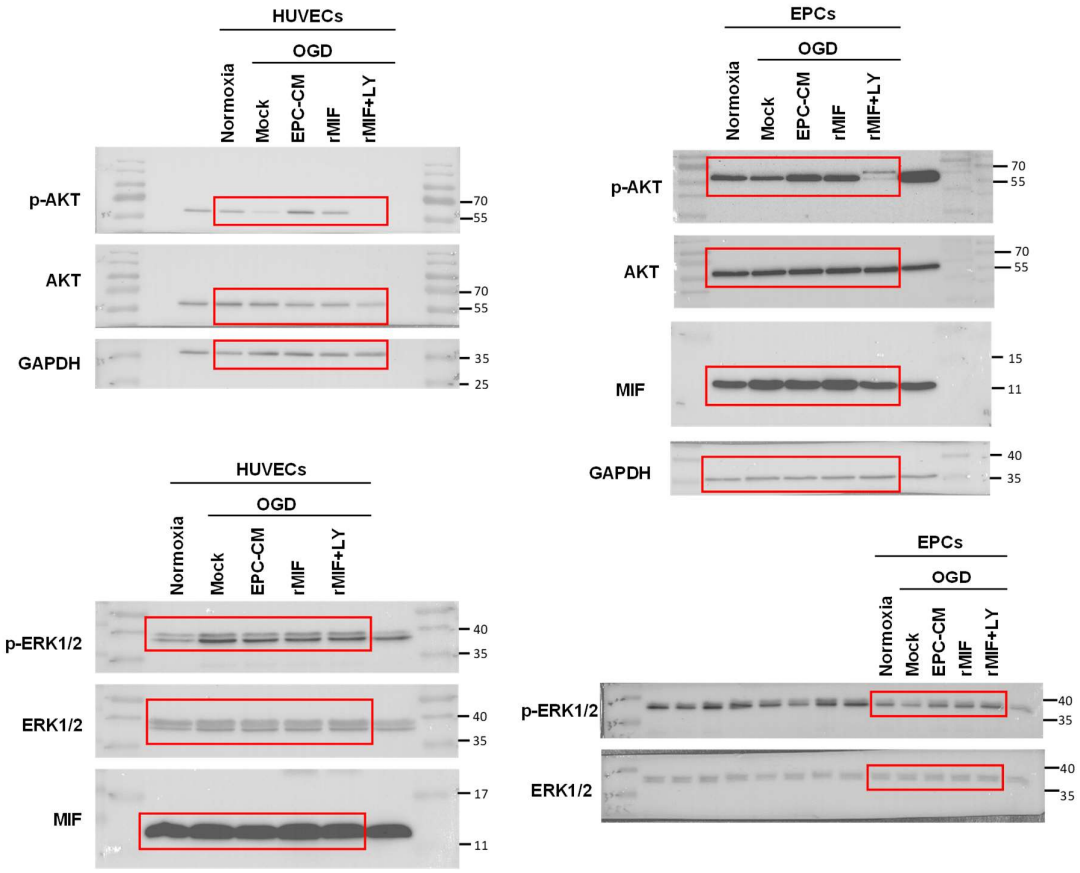

Fig. S3 Corresponding full-length immunoblot of Figure 6B

Western blot analysis of p-AKT, AKT, p-ERK1/2, ERK1/2, MIF, and GAPDH in Figure 6B. The red rectangles indicate the cropped immunoblot.