Supplemental Information

Perivascular Stem Cell-Derived Cyclophilin A

Improves Uterine Environment with Asherman's

Syndrome via HIF1α-Dependent Angiogenesis

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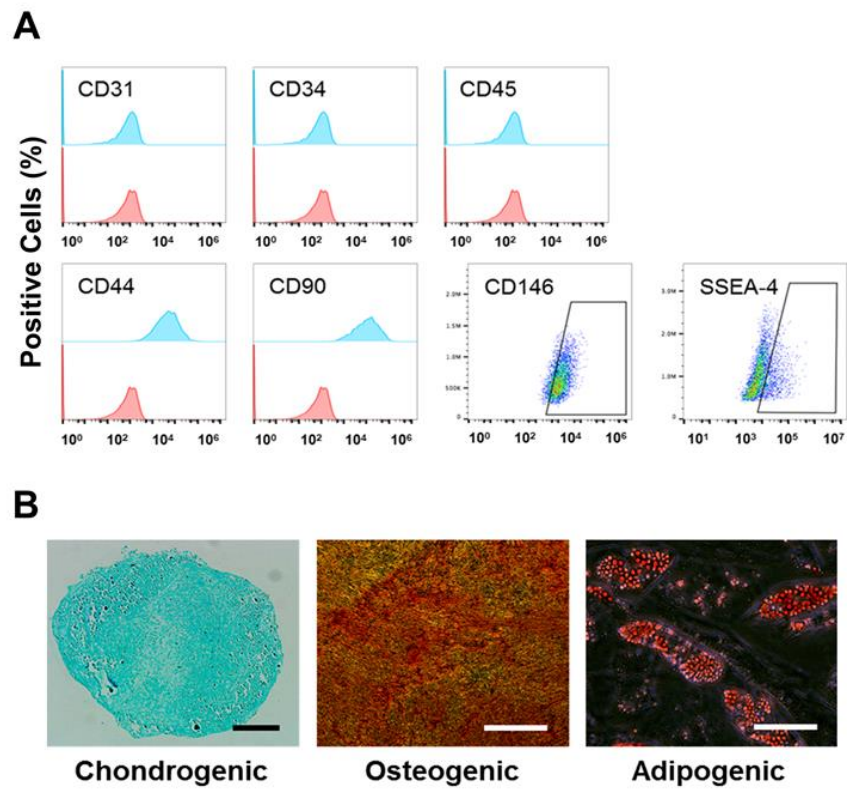
Figure S1. Molecular and cellular characterization of hPVSCs as MSCs. (A) Representative flow cytometry histograms and dot plots for phenotypic analyses of hPVSCs (passage 2). The pink histograms show the isotype control. (B) Multilineage differentiation of hPVSCs. Representative images of Alcian blue staining of chondrocytes, Alizarin Red S staining of osteocytes, and Oil Red O staining of adipocytes. Scale bar: 100 μm.
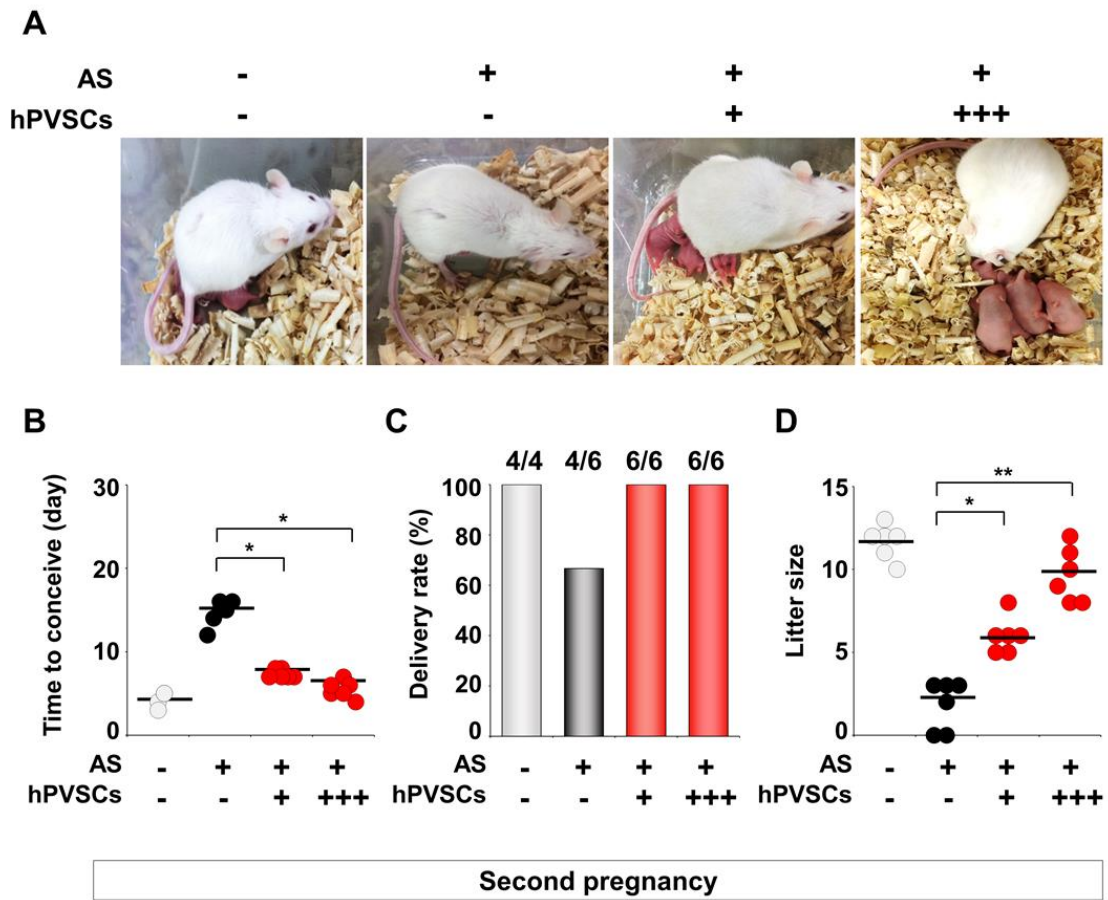
Figure S2

Figure S2. Therapeutic actions of hPVSCs on chronic reproductive impairments in mice with AS. (A) Representative photographs of mice with AS after the second delivery. +; IU delivery of hPVSCs (1×10^6), +++; a single IU followed by 3 IP delivery of hPVSCs (1×10^6). Therapeutic effects of hPVSCs on time to conceive (B), delivery rate (C), and litter size (D) in the second pregnancies of mice with AS. The horizontal black lines represent median values. Numbers above the bars indicate the number of mice that delivered live pup(s)/total mice examined. *; $P<0.05$, **; $P<0.01$.
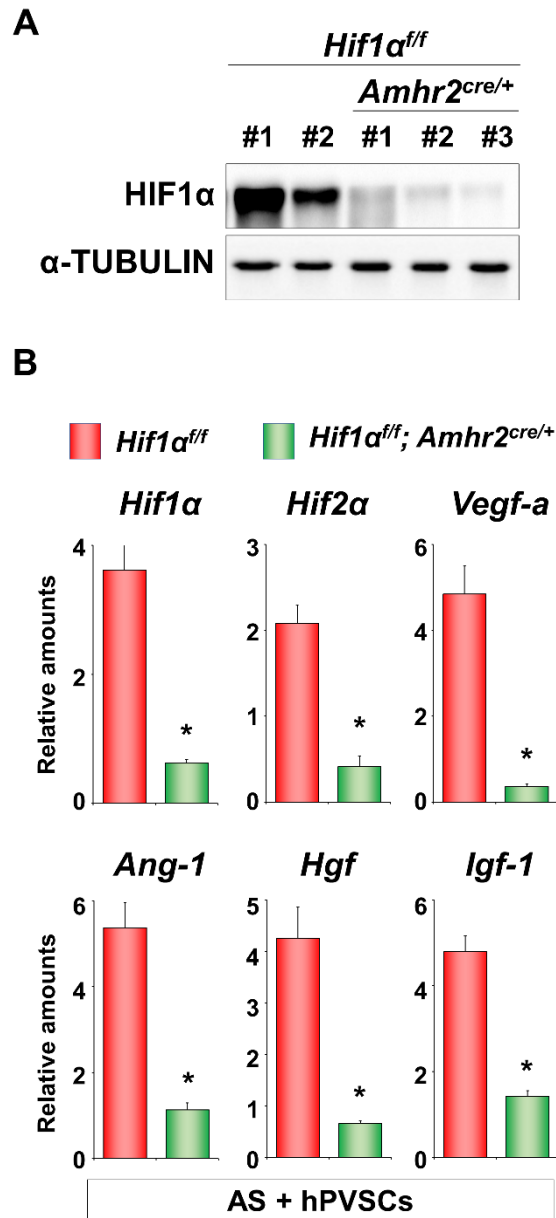
A

B

AS + hPVSCs

Figure S3. Conditional deletion of uterine Hif1α using AmhR2-Cre and expression of angiogenic factors in *Hif1α^f/f^;Amhr2^cre/+^* mice with AS after hPVSCs therapy. (A) Western blotting for the HIF1α deletion in the uteri of *Hif1α^f/f^; Amhr2^cre/+^* mice. α-TUBULIN was used as the loading control. (B) Real-time RT-PCR analyses of expression of angiogenesis-related factors in *Hif1α^f/f^;Amhr2^cre/+^* mice with AS after hPVSCs transplantation.
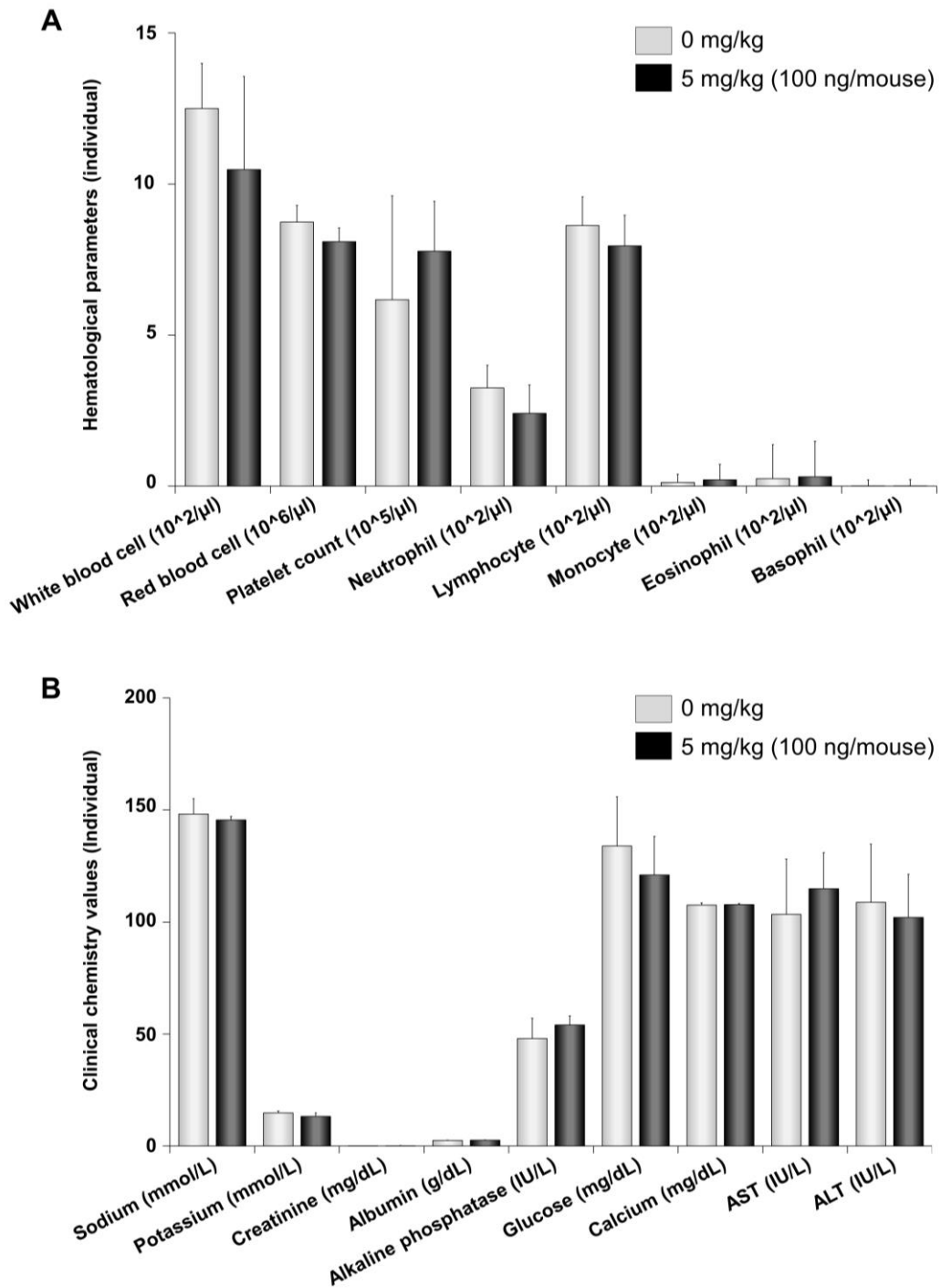
Figure S4

Figure S4. Immune cell profiles and general toxicity in mice after intrauterine administration of CYP-A at 100 ng/mouse. Cell count analyses of various immune cells of whole blood (A) and biochemical assays of serum (B) 24 h after CYP-A treatment. AST; aspartate transaminase, ALT; alanine transaminase.

Table S1. List of secreted proteins from human perivascular stem cells

No.	Protein name	NCBI BLAST	Score	Mass
1	Profilin-1	NP_005013.1	1071	15225
	Cystatin-C precursor	NP_000090.1	70	16029
2	Peptidylprolyl isomerase A (Cyclophilin A)	AAH07104.1	1025	18239
	hCG2016877, isoform CRA_c	EAW74247.1	81	44298
	Transgelin	NP_003177.2	73	22674
	Hypothetical protein, partial	CAH18175.1	46	199821
	Collagen, type V, alpha 2, isoform CRA_b	EAX10907.1	41	86488
	Collagen alpha-2(V) chain	CAA28454.1	41	106111
	Mucin short variant	AAK30142.1	40	18378
3	Tropomyosin beta chain isoform Tpm2.1sm/cy	NP_998839.1	389	33050
4	Cofilin-1	NP_005498.1	182	18724
	Cofilin-2 isoform 1	NP_068733.1	60	18848
	PEX5-related protein isoform X8	XP_011511190.1	41	66711
5	Crystal Structure of Anthrax Edema Factor	1XFU_O	126	16839
	Macrophage expressed 1	AAI12231.1	42	79660
6	Cadherin 2, type 1, N-cadherin (neuronal)	EAX01239.1	113	94379
	Keratin 13 (KRT13), transcript variant 1	BAF82933.1	67	49869
7	Transgelin variant, partial	BAD92792.1	68	12321
	UTP15 protein	AAH13064.1	47	32546
	Apolipoprotein B mRNA editing enzyme	BAD92216.1	46	25657
	cDNA FLJ13705 fis, clone PLACE2000302	BAB14672.1	40	23050
8	Plasminogen	AAH60513.1	47	93333
9	Coiled-coil domain-containing protein 180	Q9P1Z9.2	43	192570
	Actin-related protein 2/3 complex	NP_005708.1	41	16381

Table S2. Primer sequences for genotyping

Genotype		Sequence (5'-3')	Size (bp)
Hif1α^{f/f}	Forward	TGCATGTGTATGGGTGTTTTG	WT: 99
	Reverse	GAAAACTGTCTGTAACTTCATTTCC	f/f: 120
Amhr2^{cre/+}	Forward	GGACATGTTCAGGGATCGCCAGGC	Cre: 219
	Reverse	CGACGATGAAGCATGTTTAGCTG	

Table S3. Primer sequences for RT-PCR and real-time RT-PCR

Gene		Sequence (5'-3')	Size (bp)
Col1a1	Forward	CTGGCGGTTCAGGTCCAAT	141
	Reverse	TTCCAGGCAATCCACGAGC	
Timp1	Forward	GGGTTCCCCAGAAATCAACGAG	139
	Reverse	ACAGAGGCTTTCCATGACTGGGGTG	
Tgfβ1	Forward	GTGAAACGGAAGCGCATCGAAG	193
	Reverse	CATAGTAGTCCGCTTCGGGCTCC	
Tnfα	Forward	CTGAACTTCGGGGTGATCGG	122
	Reverse	GGCTTGTCACTCGAATTTTGAGA	
Hif1α	Forward	ACAAGTCACCACAGGACAG	168
	Reverse	AGGGAGAAAATCAAGTCG	
Hif2α	Forward	AATGACAGCTGACAAGGAGAAAAA	257
	Reverse	GAGTGAAGTCAAAGATGCTGTGTC	
Vegf-a	Forward	GCAGGCTGCTGTAACGATGA	105
	Reverse	GCATGATCTGCATGGTGATGTT	
Ang-1	Forward	GGGACAGCAGGCAAACAGA	110
	Reverse	TGTCGTTATCAGCATCCTTCGT	
Hgf	Forward	CTGACCCAAACATCCGAGTTG	125
	Reverse	TTCCCATTGCCACGATAACAA	
Igf-1	Forward	TGCTTCCGGAGCTGTGATCT	125
	Reverse	CGGGCTGCTTTTGTAGGCT	