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Supplemental Information

PDGF Restores the Defective Phenotype of Adipose-Derived Mesenchymal Stromal Cells from Diabetic Patients

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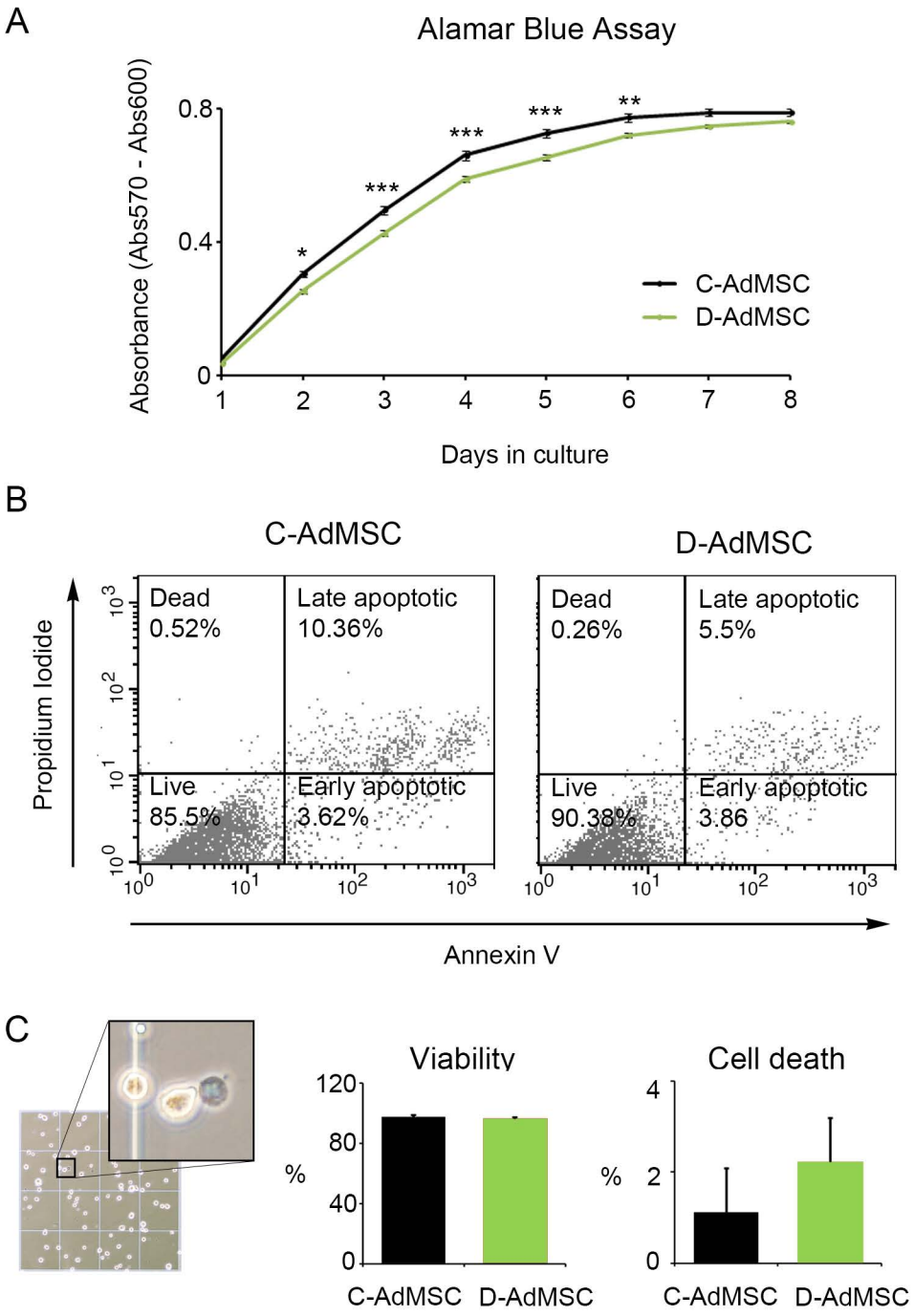

Figure S1. Metabolic activity and viability of AdMSCs derived from T2DM patients and healthy subjects. (A) Metabolic activity of AdMSCs was measured daily by Alamar Blue assay over an 8-day culture period. (B) Apoptosis detection using Annexin V and the non-vital dye propidium iodide and analysis by flow cytometry. (C) Optical microscopic image of the AdMSCs in a hemocytometer after trypan blue staining. Quantification of viable and dead cells is represented by bar graphs. Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 (Two-tailed T-test, Two-way ANOVA).

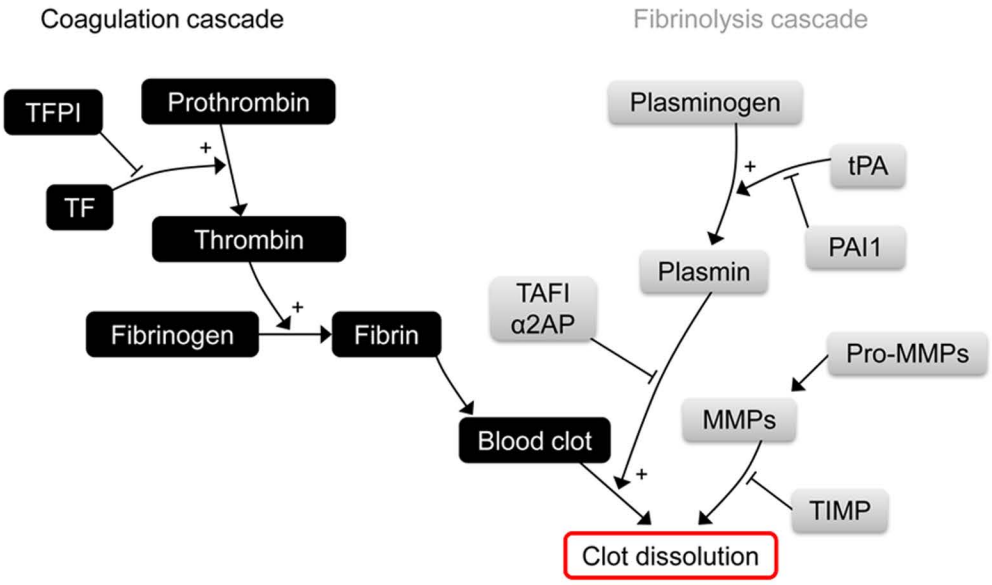

Figure S2. Summary diagram integrating the coagulation and fibrinolysis cascades. Coagulation and fibrinolysis cascades integrate multiple factors. Tissue Factor (TF) and Tissue Factor Pathway Inhibitor (TFPI) are key initiators of blood clotting. Metalloproteases (MMPs) and tissue inhibitors of MMPs (TIMPs) are involved in extracellular matrix degradation to favor dissolution of blood clots. α2-antiplasmin is an inhibitor of plasmin that degrades blood clots. The plasminogen activator inhibitor type 1 (PAI-1) and tissue plasminogen activator (tPA) are master regulators of the degradation of fibrin present in the blood clots.

A

Sample		PDGF-AA (pg/ml)	PDGF-BB (pg/ml)
Conditioned media	C-AdMSC	0	0
	D-AdMSC	0	0
Serum	C-serum	8084 ± 1029	563 ± 32
	D-serum	15458 ± 909*	2177 ± 1.5*

B

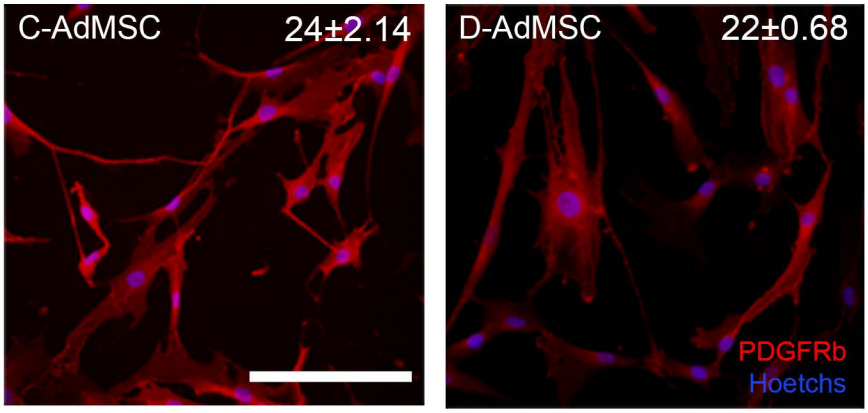

Figure S3. Expression levels of PDGF ligand and its receptor in AdMSCs. (A) Human PDGF-AA and PDGF-BB concentrations in the conditioned media of C-AdMSCs and D-AdMSCs, as well as in serum derived from healthy (C-serum) and diabetic (D-serum) donors. (B) Immunocytochemistry staining for PDGFRβ (red) in D-AdMSCs and C-AdMSCs. Cell nuclei were stained with Hoechst dye (blue). Fluorescence was measured as the mean gray/100 nuclei and did not reveal significant differences. Scale bar: 100 µm. Data are represented as mean ± SEM. *$p<0.05$ (Two-tailed T-test).

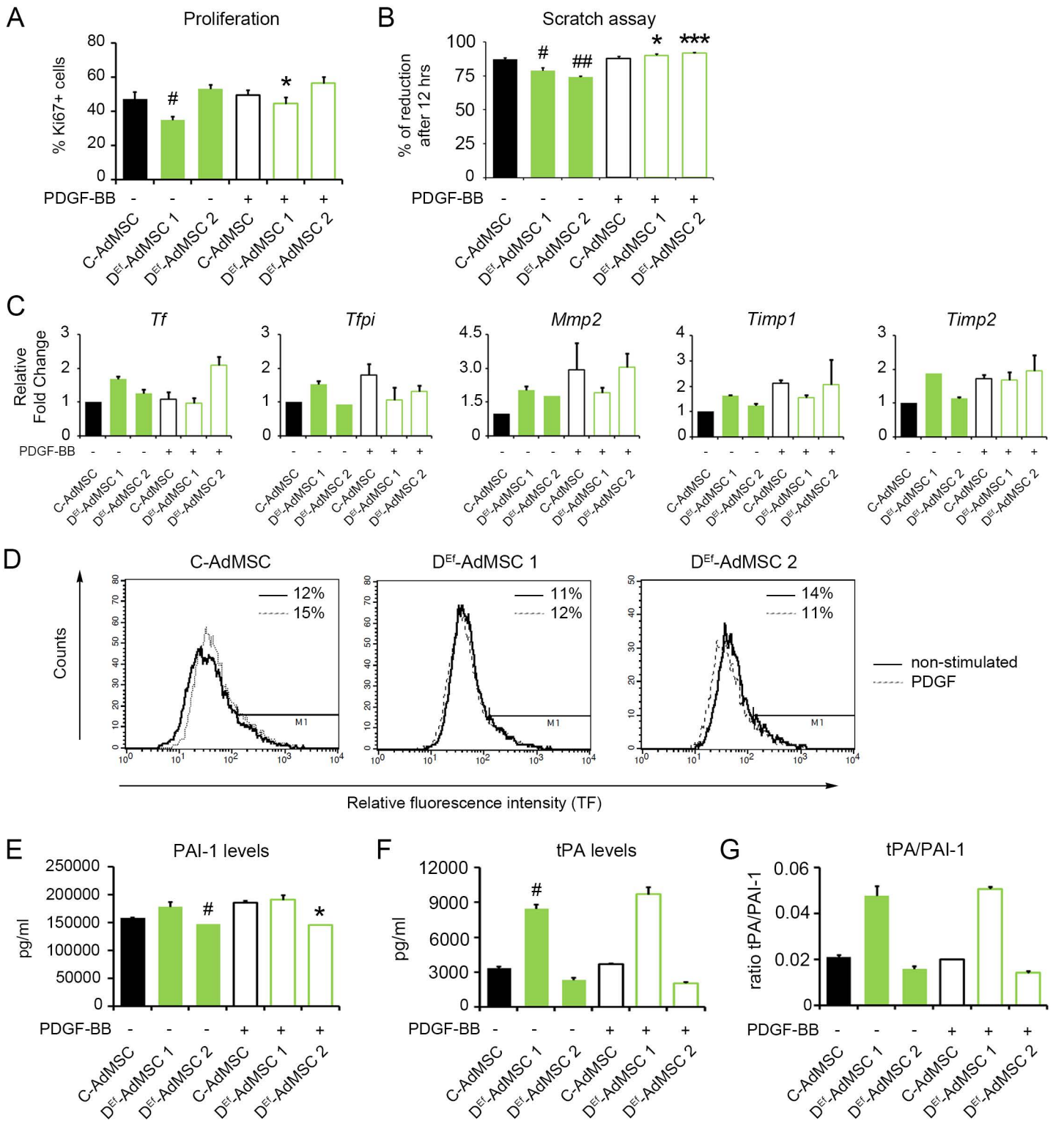

Figure S4. Pathological phenotype of AdMSCs differs across multiple T2DM patients successfully treated with autologous cell therapy for CLI. Data were collected from diabetic patients with CLI enrolled in the NCT01257776 trial, that did not undergo adverse reaction after AdMSCs infusion (i.e., therapeutically effective AdMSCs; DEf-AdMSCs). (A) Bar graph depicting the percentage of Ki67+ cells 5 hrs after PDGF-BB stimulation in AdMSCs derived from healthy and diabetic donors. (B) Scratch assay was performed to determine differences on migration, using medium enriched or not with PDGF-BB. Bar graph depicts the percentage of reduced scratch area after a time period of 12 hrs. (C) RT-qPCR measurements for a selected set of genes involved in coagulation and fibrinolysis. Results were normalized to the internal control Ppia and Rplp0. Results for the healthy donor without PDGF-stimulation were set us 1. (D) Flow cytometry analysis determining the percentage of AdMSCs expressing the pro-coagulant marker TF. (E) Bar graphs depicting the levels of tPA secreted in the medium by cultured AdMSCs. (F) Bar graphs depicting the levels of PAI-1 secreted in the medium by cultured AdMSCs. (G) Bar graphs depicting the tPA/PAI-1 ratio. Data are represented as mean ± SEM. #p<0.05 compared to C-AdMSCs, *p<0.05 compared to non-stimulated, ##p<0.01 compared to C-AdMSCs, ***p<0.001 compared to non-stimulated (One-way ANOVA).

Table S1. Information of patient samples used in this study.

Donor	Condition	Treatment (dose of infused cells)	Adverse reaction	Sample obtained	Figures where samples were used
Donor 1	healthy	none	no	C-AdMSC	1, 2, 3, 4, 5, 6, S1, S3, S4
Donor 2	T2DM and CLI	$5 \cdot 10^5$ cells/kg	yes	D-AdMSC	1, 2, 3, 4, 5, 6, S1, S3
Donor 3	T2DM and CLI	$5 \cdot 10^5$ cells/kg	no	D^{Ef}-AdMSC 1	S4
Donor 4	T2DM and CLI	$5 \cdot 10^5$ cells/kg	no	D^{Ef}-AdMSC 2	S4

Table S2. List of primary antibodies used in this study

Antibody	Species, type	Dilution	Application	Cat. number, manufacturer
CD105	mouse IgG1 k	1:20	Flow cytometry	560839, BD Biosciences (San Jose, CA)
CD13	mouse IgG1, k	1:20	Flow cytometry	347406, BD Biosciences
CD29	mouse IgG1 k	1:20	Flow cytometry	555443, BD Biosciences
CD31	mouse IgG1 k	1:20	Flow cytometry	555445, BD Biosciences
CD34	mouse IgG1 k	1:20	Flow cytometry	555822, BD Biosciences
CD45	mouse IgG1 k	1:20	Flow cytometry	345808, BD Biosciences
CD73	mouse IgG1 k	1:20	Flow cytometry	550257, BD Biosciences
CD90	mouse IgG1 k	1:20	Flow cytometry	555595, BD Biosciences
HLA II	mouse IgG2a, k	1:20	Flow cytometry	555558, BD Biosciences
TF	mouse IgG1 k	1:5	Flow cytometry	550312, BD Biosciences
Human nuclei	mouse IgG1	1:200	Immunocytochemistry	MAB1281, Millipore (Billerica, MA)
Ki67	mouse IgG1	1:150	Immunocytochemistry	NCL-L-Ki67-MM1, Leica (Barcelona, Spain)
TF	mouse, IgG1	1:250	Immunocytochemistry	Sc-393657, Santa Cruz (Santa Cruz, CA)
PDGFRβ	rabbit	1:100 1:1000	Immunocytochemistry Western blot	Sc-432, Santa Cruz
pSMAD 2 (Ser465/467)	rabbit	1:350 1:1000	Immunocytochemistry Western blot	3103, Cell Signaling
ERK 1/2	rabbit	1:1000	Western blot	9102, Cell Signaling (Beverly, MA)
GAPDH	rabbit, IgG	1:1000	Western blot	2118, Cell Signaling
pERK 1/2 (Thr202/Tyr204)	mouse IgG	1:2000	Western blot	9106, Cell Signaling
pPDGFRβ (Tyr716)	rabbit	1:1000	Western blot	Sc-16569, Santa Cruz
SMAD 2/3	rabbit, IgG	1:1000	Western blot	8685, Cell Signaling

Table S3. Sequences of RT-qPCR primers used in this study

Genes	Forward primer	Reverse primer
Mmp2	TATTTGATGGCATCGCTCAG	ACAGTCCGCCAAATGAACC
Ppia	TTCATCTGCACTGCCAAGAC	CACTTTGCCAAACACCACAT
Rplp0	TCGACAATGGCAGCATCTAC	GCCAATCTGCAGACAGACAC
Tf	CAGCCCGGTAGAGTGTATGG	CCACAGCTCCAATGATGTAGAA
Tfpi	GGTTCCCAGCCTTTTTGAAT	TGGCACGACACAATCCTCT
Timp1	CTGTTGTTGCTGTGGCTGAT	AACTTGGCCCTGATGACG
Timp2	GAAGAGCCTGAACCACAGGT	CGGGGAGGAGATGTAGCAC
α2-antiplasmin	TGAAACACCAAATGGACCTG	GGCCTGGAACAACTCCTG