Supplementary Material

Recipient Glycemic Micro-environments Govern Therapeutic Effects of Mesenchymal Stem Cell Infusion on Osteopenia

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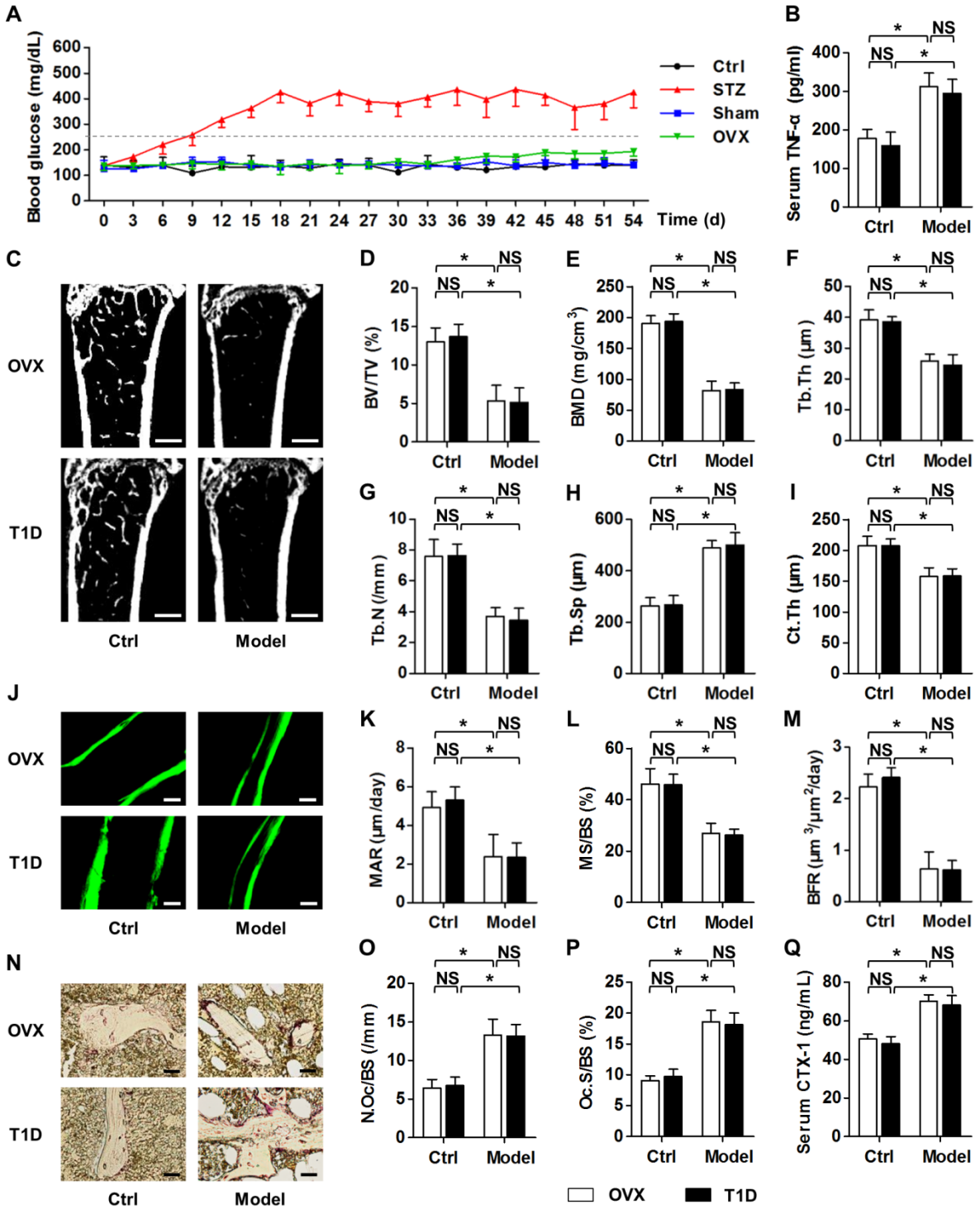

Figure S1. Establishment of osteopenias induced by ovariectomy (OVX) and type 1 diabetes (T1D). (**A**) Non-fasting blood glucose levels of OVX and T1D mice (Model) and their respective control (Ctrl). Mice underwent bilateral Sham or OVX at D1, or received daily injections of buffer or 50 mg/kg streptozotocin (STZ) from D1 to D5 for diabetic induction. Grey dashed line indicates diabetic criterion of 250 mg/dL. (**B**) Enzyme-linked immunosorbent assay (ELISA) analysis of inflammatory cytokine tumor necrosis factor-alpha (TNF-α) in serum. (**C-I**) Representative micro-computed tomography (micro-CT) images (**C**) and quantitative analysis of trabecular (**D-H**) and cortical (**I**) bone microarchitecture in the distal metaphyses of femora. Bars:

500 μm. BV/TV, bone volume per tissue volume; BMD, bone mineral density; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Ct.Th, cortical thickness. (**J-M**) Representative images of calcein double labeling (**J**) with quantification (**K-M**) of bone formation rates in distal femora. Bars: 50 μm. MAR, mineral apposition rate; MS/BS, mineralized surface per bone surface; BFR, bone formation rate. (**N-P**) Representative images of tartrate resistant acid phosphotase (TRAP) staining (red) (**N**) with parameters (**O, P**) of bone resorption rates in distal femora. Bars: 25 μm. N.Oc/BS, number of osteoclasts per bone surface; Oc.S/BS, osteoclast surface over bone surface. (**Q**) ELISA analysis of bone resorption marker in serum. CTX-1, cross linked C-telopeptide of type 1 collagen. n = 6 per group. Data represents mean ± SD. *P < 0.05; NS, not significant (P > 0.05). Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc tests.

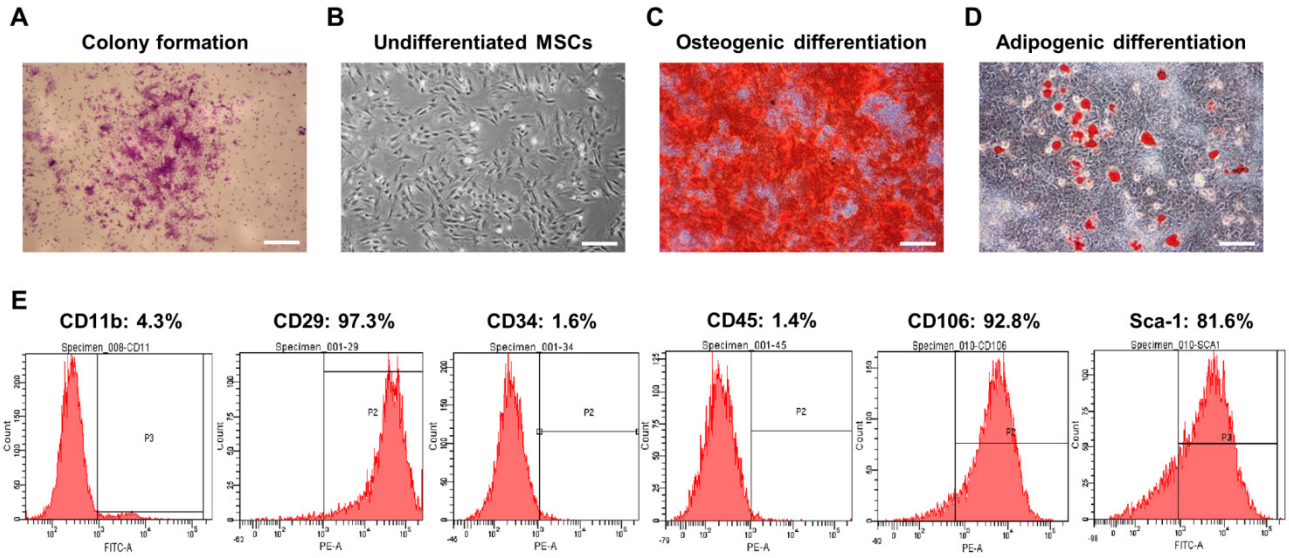

Figure S2. Characterizations of MSCs for infusion. (**A**) Colony formation of MSCs stained with crystal violet. MSCs expanded in a fashion that started from a single cell to a colony with over 50 cells. Bar: 500 μm. (**B**) Undifferentiated MSCs expanded readily in a spindle shape. Bar: 500 μm. (**C**) Osteogenic differentiation of MSCs stained with alizarin red. Bar: 200 μm. (**D**) Adipogenic differentiation of MSCs stained with oil red O. Bar: 200 μm. (**E**) Flow-cytometric analysis on surface marker profiling of MSCs. Sca-1, stem cell antigen-1.

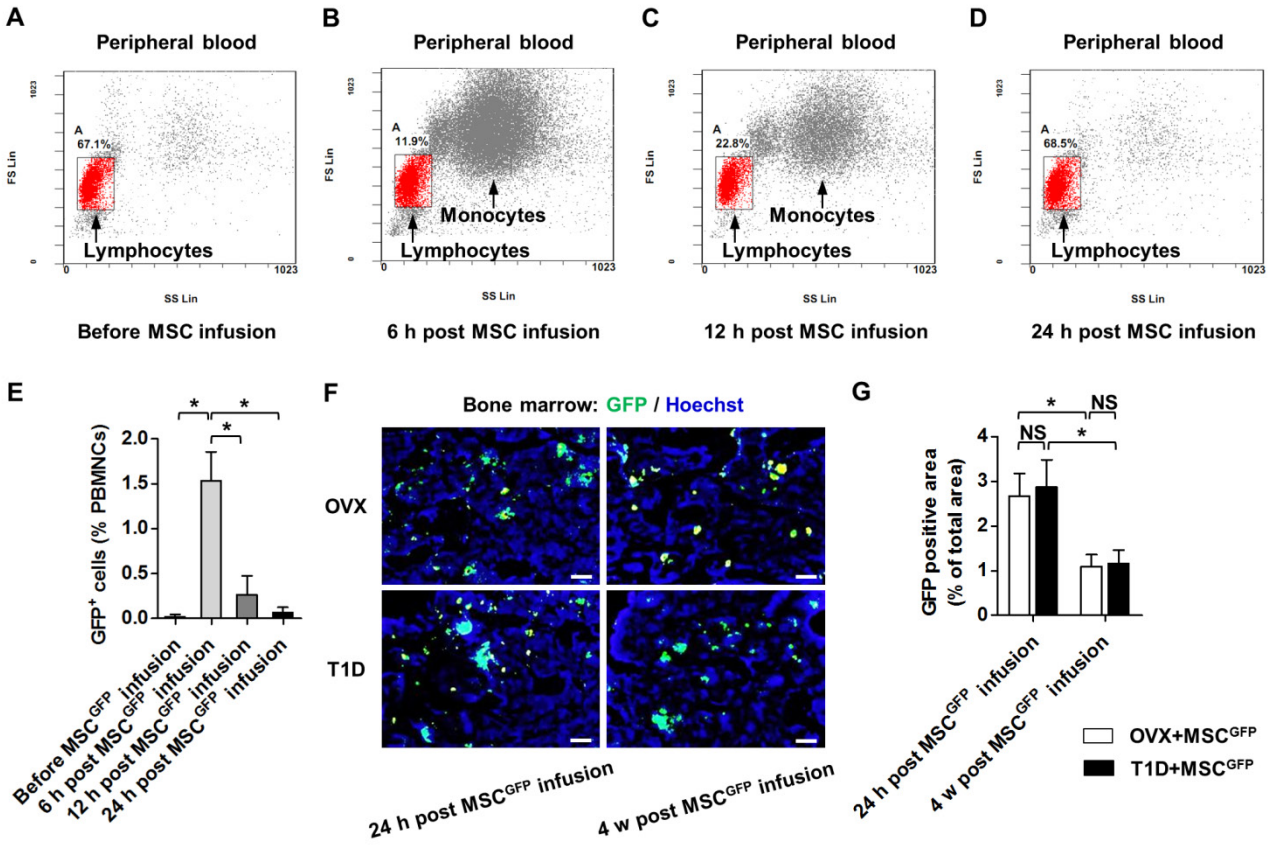

Figure S3. Fates of MSCs after systemic transplantation regarding survival and the homing capacity. (**A-D**) Flow-cytometric analysis on cell components in peripheral blood at indicated time points. (**E**) Flow-cytometric quantification of green fluorescent protein $(GFP)^+$ cell percentages in peripheral blood mononuclear cells (PBMNCs) at indicated time points. Mice accepted GFP-labeled MSC (MSC^{GFP}) infusion. (**F, G**) Representative images of immunofluorescent staining of GFP (green) and Hoechst (blue) (**F**) with quantification (**G**) of GFP^+ area percentages in bone marrow, from OVX and T1D mice accepting MSC^{GFP} infusion at indicated time points. Bars: 100 μm. n = 3 per group. Data represents mean ± SD. *P < 0.05; NS, not significant (P > 0.05) (ANOVA followed by Newman-Keuls post-hoc tests).

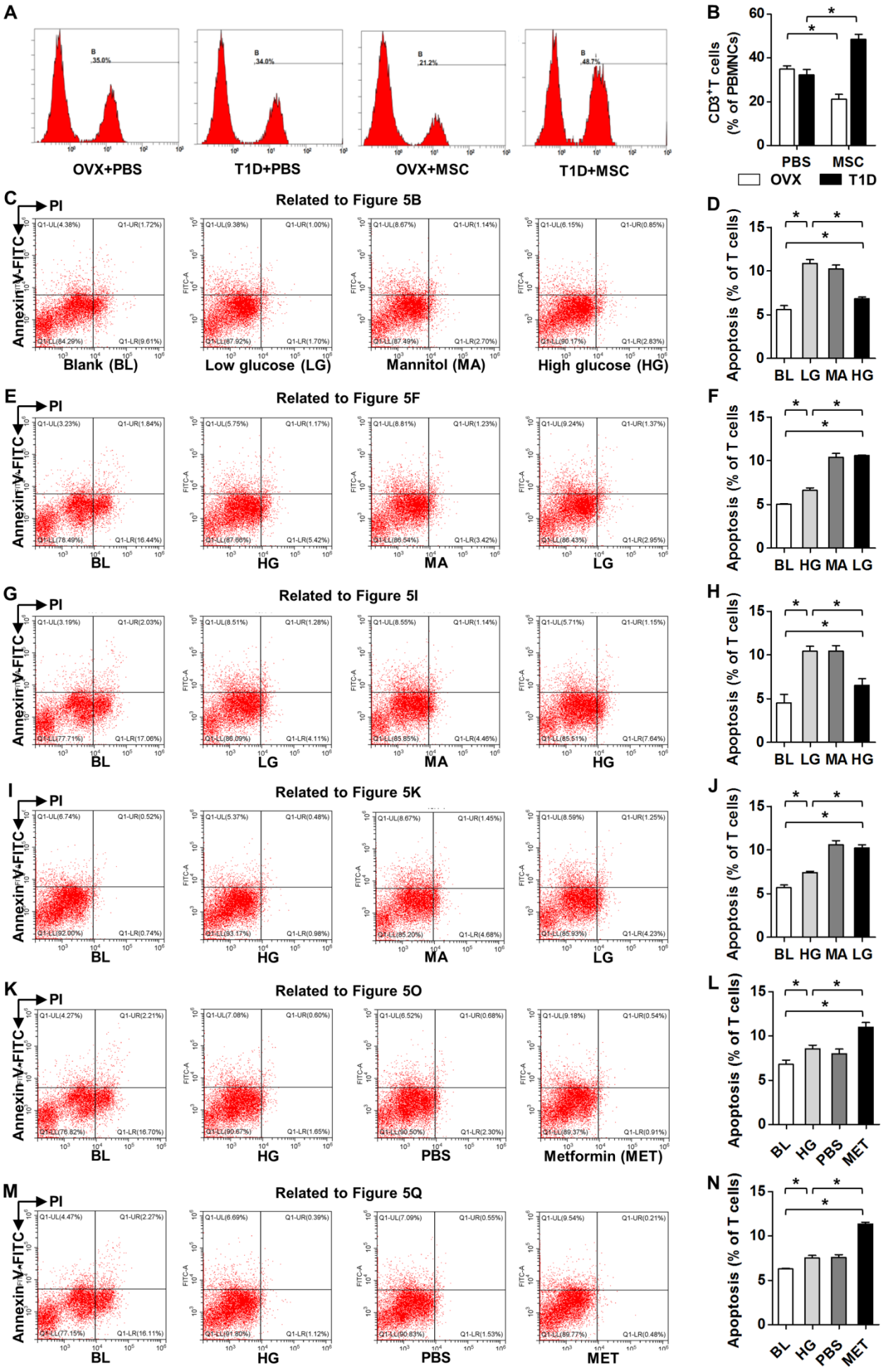

Figure S4. Anti-inflammatory capacity of MSCs, high glucose (HG) effects and rescuing effects of

metformin (MET) are dependent on induction of T lymphocyte apoptosis. (**A, B**) Representative flow-cytometric images (**A**) and quantification of $CD3^+$T cell percentages over PBMNCs (**B**), in OVX and T1D mice that accepted PBS or MSC infusion. *n* = 3 per group. (**C-N**) Apoptosis analysis of T cells (shown as $FITC^+PI^+$ plus $FITC^+PI^-$ percentages) in the respective experiments related to Figure 5. *n* = 3 per group. Data represents mean ± SD. **P* < 0.05. (ANOVA followed by Newman-Keuls post-hoc tests).

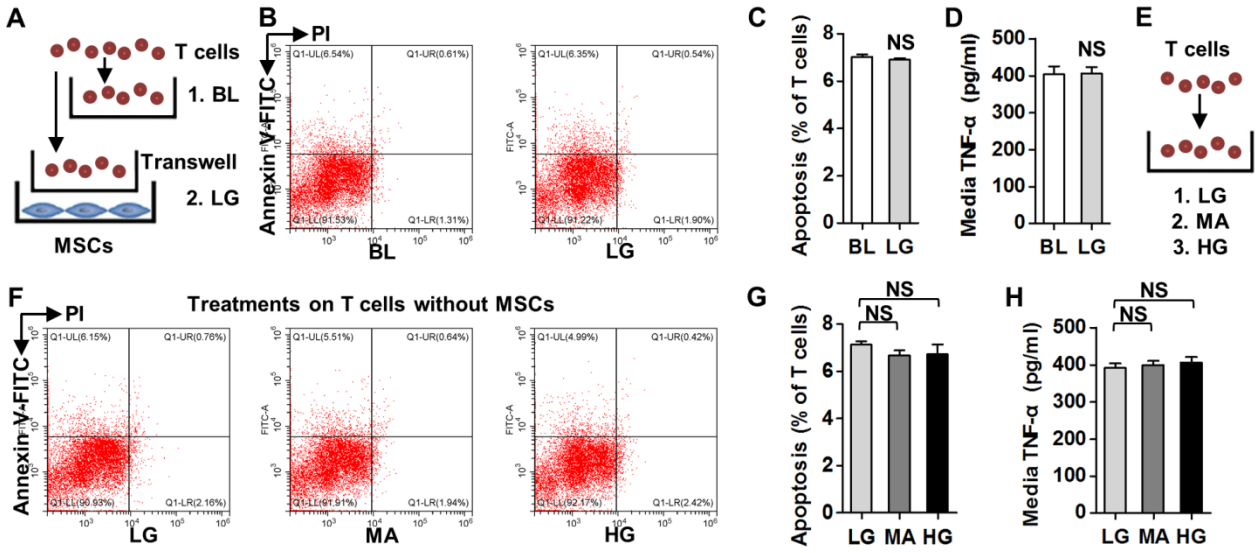

Figure S5. Anti-inflammatory capacity of MSCs and high-glucose effects are dependent on cell contact between MSCs and T lymphocytes. (**A-D**) Design of Transwell co-culture of T cells with MSCs (**A**), the apoptosis (**B**) and quantifications (**C**), and ELISA analysis of TNF-α in media (**D**). *n* = 3~4 per group. (**E-H**) Design of direct treatments of T cells with HG (**E**), the apoptosis (**F**) and quantifications (**G**), and ELISA analysis of TNF-α in media (**H**). *n* = 3~4 per group. Data represents mean ± SD. **P* < 0.05; NS, not significant (*P* > 0.05) (Student's t tests for **C** and **D**; ANOVA followed by Newman-Keuls post-hoc tests for **G** and **H**).

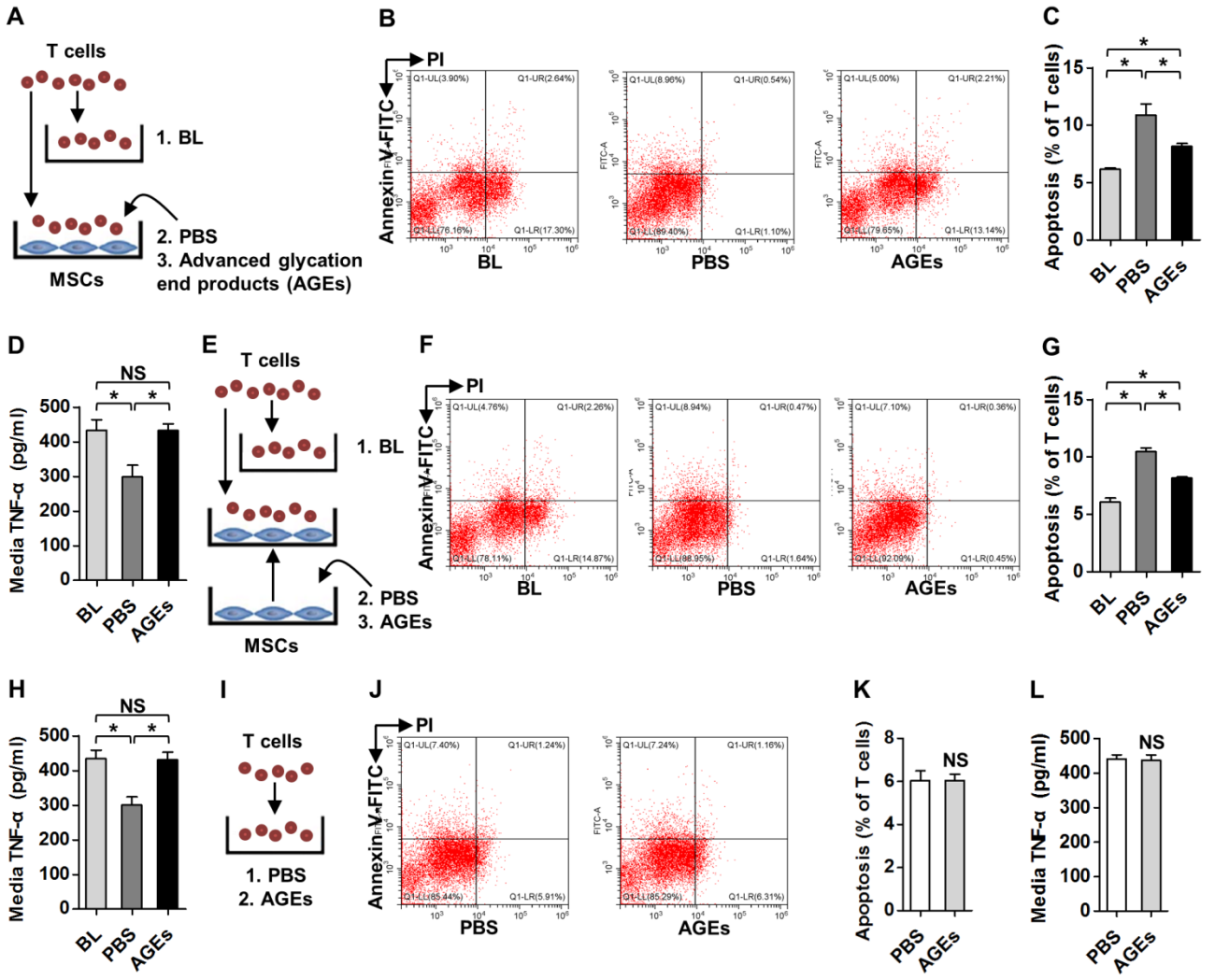

Figure S6. Advanced glycation end products (AGEs) treatments reduce anti-inflammatory capacity of MSCs. (**A, E**) Experimental designs to investigate potential effects of AGEs on anti-inflammatory/immunomodulatory capacity of MSCs in (**A**) and before (**E**) co-culture with T cells. AGEs was dissolved in PBS and was applied at 200 μg/ml. (**B-D, F-H**) The apoptosis (**B, F**) and quantifications (**C, G**), and ELISA analysis of TNF-α in media (**D, H**) from the respective experiments of **A** and **E**. *n* = 3~4 per group. (**I-L**) Design of direct treatments of T cells with AGEs (**I**), the apoptosis (**J**) and quantifications (**K**), and ELISA analysis of TNF-α in media (**L**). *n* = 3~4 per group. Data represents mean ± SD. **P* < 0.05; NS, not significant (*P* > 0.05) (ANOVA followed by Newman-Keuls post-hoc tests for **C, D, G** and **H**; Student's t tests for **K** and **L**).

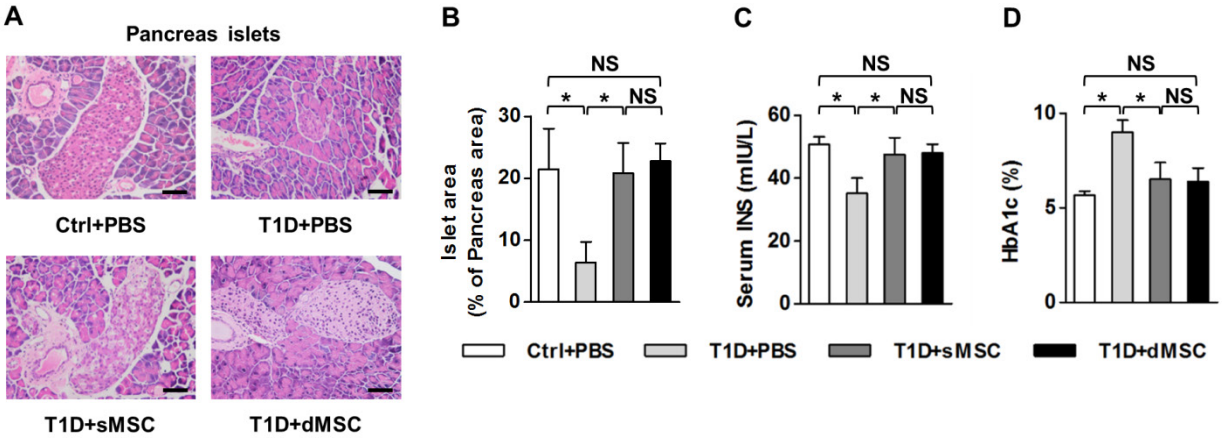

Figure S7. Therapeutic potential of MSCs on glucose homeostasis in recipient diabetes mellitus. (**A, B**) Representative images of H&E staining (**A**) with quantification (**B**) of islet area percentages in pancreas, from Ctrl and T1D mice accepting PBS infusion and T1D mice accepting single MSC (sMSC) and double MSC (dMSC) infusion. Bars: 50 μm. (**C, D**) ELISA analysis of insulin (INS, **C**) and glycated hemoglobin (HbA1c, **D**) percentages in serum. *n* = 6 per group. Data represents mean ± SD. **P* < 0.05; NS, not significant (*P* > 0.05) (ANOVA followed by Newman-Keuls post-hoc tests).

Table S1. Primer sequences in the present study.

Gene	Primer sequences
Gapdh	Forward: 5'-CCAATGTGTCCGTCGTGGATCT-3'
	Reverse: 5'-GTTGAAGTCGCAGGAGACAACC-3'
Fasl	Forward: 5'-GGCTCTGGTTGGAATGGGAT-3'
	Reverse: 5'-AAATGGGCCACACTCCTCG-3'
Hgf	Forward: 5'-CCACCATAATCCCCCTCACA-3'
	Reverse: 5'-GGCTGGGGCTACACTGGATT-3'
Ido	Forward: 5'-CCAGTGCAGTAGAGCGTCAA-3'
	Reverse: 5'-TCCCAGACCCCCTCATACAG-3'
Il10	Forward: 5'-GCCGGGAAGACAATAACTGC-3'
	Reverse: 5'-AAGGCTTGGCAACCCAAGTA-3'
Inos	Forward: 5'-CAGATCGAGCCCTGGAAGAC-3'
	Reverse: 5'-CAACCTTGGTGTTGAAGGCG-3'
Mmp2	Forward: 5'-CCCCATGAAGCCTTGTTTACC-3'
	Reverse: 5'-GAAGGGGAAGACACATGGGG-3'
Mmp9	Forward: 5'-CCATGCACTGGGCTTAGATCA-3'
	Reverse: 5'-GGCCTTGGGTCAGGCTTAGA-3'
Tgfβ1	Forward: 5'-AGGGCTACCATGCCAACTTC-3'
	Reverse: 5'-CCACGTAGTAGACGATGGGC-3'