

**Manuscript Title: Diabetic kidney disease alters the transcriptome and function of human adipose-derived mesenchymal stromal cells but maintains immunomodulatory and paracrine activities important for renal repair**

## **Supplemental Materials**

### **MSC Harvesting and Phenotyping Methods**

MSC were isolated from abdominal subcutaneous fat (0.5-2 g), cultured, and characterized as previously described.<sup>1-5</sup> In brief, adipose tissues were minced and digested in 2% collagenase type I at 37°C for 45 minutes (Gibco, NY). Afterward, the suspension was filtered through a 100 µm cell strainer (BD Biosciences, San Jose, CA) to remove remaining tissue pieces and then centrifuged at 1000 RPM for 5 minutes (233 relative centrifugal force) to pellet cells. Cells were cultured in Advanced minimum-essential-medium (MEM; Thermo Fisher Scientific, Waltham, MA) supplemented with 5% platelet lysate (PLTMax, Mill Creek Life Sciences, Rochester, MN) and 2 mM L-glutamine (Invitrogen, Carlsbad, CA) in a 37°C incubator with 5% CO<sub>2</sub> incubator for 3–4 days. When 60-80% confluent, cells were passaged using TrypLE (Trypsin-like Enzyme, Invitrogen). The 3<sup>rd</sup> passage was collected and kept in Gibco Cell Culture Freezing Medium (Life Technologies, Carlsbad, CA) at -80°C. Cells were then characterized<sup>6</sup> by surface marker positivity to CD73, CD90, CD105, and negativity to CD34 and CD45 (according to the manufacturer's recommended dilution; Abcam, Cambridge, MA; BD Pharmigen, San Jose, CA; BioLegend, San Diego, CA) and analyzed by flow cytometry (FlowSight, Amnis, Seattle, WA) using Amnis<sup>®</sup> Image Data Exploration and Analysis Software (IDEAS v.6.2). MSC phenotype was further confirmed by trilineage differentiation into osteocyte (mouse anti-human osteocalcin), adipocyte (goat anti-mouse FABP-4 antibody), and chondrocyte (goat anti-human

aggrecan antibody) lineages using a Human MSC Functional identification kit (R&D Systems, Minneapolis, MN) following manufacturer's instructions.

**Supplemental Table 1. Demographic characteristics of participants with diabetic kidney disease and controls at time of adipose tissue sampling for mesenchymal stem/ stromal cell harvest -Migration and Proliferation subset**

MSC Migration and Proliferation			
	<b>Controls</b> n=16	<b>DKD</b> n=38	<b>p-value</b>
<b>Age, years</b>	63.3 (3.4)	64.8 (6.8)	0.12
<b>Female Sex</b>	9 (56.3%)	17 (44.7%)	0.44
<b>White Race</b>	16 (100%)	33 (86.8%)	0.51
<b>Diabetes Type 1</b>	--	8 (21.1%)	--
<b>Diabetes Type 2</b>	--	30 (78.9%)	--
<b>Diabetes Duration, years</b>	--	23.9 (16.0)	--
<b>BMI, kg/ m<sup>2</sup></b>	29.8 (3.1)	34.1 (6.0)	0.009
<b>eGFR, ml/min/1.73m<sup>2</sup></b>	77.6 (12.5)	44.7 (19.7)	--
<b>Glucose, mg/dL</b>	108.6 (20.0)	167.0 (64.8)	--
<b>HbA1c, %</b>	--	7.8 (1.2)	--
<b>UACR, mg/g</b>	--	507.5 (1202.0)	--
<b><u>Anti-Diabetes therapy*</u></b>			
<b>Insulin only</b>	--	25 (65.8%)	--
<b>Oral/other</b>	--	22 (57.9%)	--
<b>Insulin+Oral/Other</b>	--	11 (28.9%)	--

Data are mean (SD) or n (%)

BMI: body mass index; DKD: diabetic kidney disease; eGFR: estimated glomerular filtration rate; HbA1c: hemoglobin A1c; UACR: urine albumin:creatinine ratio: random sample; Rx: therapy; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; NGSP HbA1c converter <http://www.ngsp.org/convert1.asp> can be used to calculate HbA1c values from % to mmol/mol.\*Values do not add up to 100% of sample total.

HbA1c 7.8 % NGSP = 62 mmol/mol IFCC = 177 mg/dL eAG = 9.8 mmol/l eAG  
Change or SD of 1.20 % NGSP = 13.1 mmol/mol IFCC = 34.4 mg/dL eAG = 1.91 mmol/l eAG

**Supplemental Table 2. Demographic characteristics of participants with diabetic kidney disease and controls at time of adipose tissue sampling for mesenchymal stem/ stromal cell harvest – MSC RNA Sequencing subset**

	MSC RNA Sequencing		p-value
	Controls n=9	DKD n=29	
<b>Age, years</b>	63.9 (3.9)	65.4 (8.0)	0.2
<b>Female Sex</b>	4 (44.4%)	9 (31.0%)	0.46
<b>White Race</b>	9 (100%)	24 (82.8%)	0.41
<b>Diabetes Type 1</b>	--	3 (10.3%)	--
<b>Diabetes Type 2</b>	--	26 (89.7%)	--
<b>Diabetes Duration, years</b>	--	20.7 (13.8)	--
<b>BMI, kg/ m<sup>2</sup></b>	28.8 (3.63)	35.4 (5.8)	0.004
<b>eGFR, ml/min/1.73m<sup>2</sup></b>	80.5 (8.5)	39.0 (15.5)	--
<b>Glucose, mg/dL</b>	112.2 (18.5)	179.3 (87.2)	--
<b>HbA1c, %</b>	--	7.9 (1.3)	--
<b>UACR, mg/g</b>	--	585.3 (1307.8)	--
Data are mean (SD) or n (%)			

BMI: body mass index; DKD: diabetic kidney disease; eGFR: estimated glomerular filtration rate; HbA1c: hemoglobin A1c; UACR: urine albumin:creatinine ratio: random sample; Rx: therapy; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; NGSP HbA1c converter <http://www.ngsp.org/convert1.asp> can be used to calculate HbA1c values from % to mmol/mol.

HbA1c 7.9 % NGSP = 63 mmol/mol IFCC = 180 mg/dL eAG = 10.0 mmol/l eAG  
 Change or SD of 1.30 % NGSP = 14.2 mmol/mol IFCC = 37.3 mg/dL eAG = 2.07 mmol/l eAG

**Supplemental Table 3. Demographic characteristics of participants with diabetic kidney disease and controls at time of adipose tissue sampling for mesenchymal stem/ stromal cell harvest – MSC Conditioned Medium subset**

	MSC Conditioned Medium		
	<b>Controls</b> n=10	<b>DKD</b> n=38	<b>p-value</b>
<b>Age, years</b>	63.6 (3.1)	65.1 (6.9)	0.2
<b>Female Sex</b>	6 (60.0%)	17 (44.7%)	0.39
<b>White Race</b>	10 (100%)	33 (86.8%)	0.69
<b>Diabetes Type 1</b>	--	8 (21.1%)	--
<b>Diabetes Type 2</b>	--	30 (78.9%)	--
<b>Diabetes Duration, years</b>	--	23.9 (16.0)	--
<b>BMI, kg/ m<sup>2</sup></b>	30.7 (2.9)	34.1 (6.0)	0.08
<b>eGFR, ml/min/1.73m<sup>2</sup></b>	75.3 (13.6)	44.7 (19.7)	--
<b>Glucose, mg/dL</b>	111.6 (20.4)	167.0 (64.8)	--
<b>HbA1c, %</b>	--	7.8 (1.2)	--
<b>UACR, mg/g</b>	--	507.5 (1202.0)	--
Data are mean (SD) or n (%)			

BMI: body mass index; DKD: diabetic kidney disease; eGFR: estimated glomerular filtration rate; HbA1c: hemoglobin A1c; UACR: urine albumin:creatinine ratio: random sample; Rx: therapy; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; NGSP HbA1c converter <http://www.ngsp.org/convert1.asp> can be used to calculate HbA1c values from % to mmol/mol.

HbA1c 7.8 % NGSP = 62 mmol/mol IFCC = 177 mg/dL eAG = 9.8 mmol/l eAG  
 Change or SD of 1.20 % NGSP = 13.1 mmol/mol IFCC = 34.4 mg/dL eAG = 1.91 mmol/l eAG

**Supplemental Table 4. Demographic characteristics of participants with diabetic kidney disease and controls at time of adipose tissue sampling for mesenchymal stem/ stromal cell harvest – MSC Senescence Associated (SA)- $\beta$ -galactosidase Activity subset**

	MSC SA- $\beta$ -galactosidase (SA $\beta$ -gal) activity		p-value
	Controls n=14	DKD n=27	
<b>Age, years</b>	63.6 (3.4)	64.3 (7.8)	0.46
<b>Female Sex</b>	7 (50.0%)	10 (37.0%)	0.42
<b>White Race</b>	14 (100%)	22 (81.5%)	0.23
<b>Diabetes Type 1</b>	--	14 (14.8%)	--
<b>Diabetes Type 2</b>	--	23 (85.2%)	--
<b>Diabetes Duration, years</b>	--	20.7 (13.9)	--
<b>BMI, kg/ m<sup>2</sup></b>	29.7 (3.3)	35.8 (5.8)	0.0006
<b>eGFR, ml/min/1.73m<sup>2</sup></b>	77.7(11.8)	38.7 (16.1)	--
<b>Glucose, mg/dL</b>	108.6 (21.2)	172.4 (72.5)	--
<b>HbA1c, %</b>	--	8.0 (1.3)	--
<b>UACR, mg/g</b>	--	581.1 (1335.9)	--

Data are mean (SD) or n (%)

BMI: body mass index; DKD: diabetic kidney disease; eGFR: estimated glomerular filtration rate; HbA1c: hemoglobin A1c; UACR: urine albumin:creatinine ratio: random sample; Rx: therapy; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; NGSP HbA1c converter <http://www.ngsp.org/convert1.asp> can be used to calculate HbA1c values from % to mmol/mol.

HbA1c 8.0 % NGSP = 64 mmol/mol IFCC = 183 mg/dL eAG = 10.2 mmol/l eAG  
Change or SD of 1.30 % NGSP = 14.2 mmol/mol IFCC = 37.3 mg/dL eAG = 2.07 mmol/l eAG

**Supplemental Table 5. Univariate comparison between MSC *in vitro* studies and gender among participants with diabetic kidney disease (DKD)**

MSC studies in participants with DKD				
	<i>In vitro</i> Study	Gender		
		Females	Males	
MSC Function		N=19	N=30	
	Migration	0.51 (0.12)	0.51 (0.11)	0.8
	Proliferation	0.39 (0.04)	0.38 (0.05)	0.4
MSC Secretome		N=19	N=29	
	<b>Proangiogenesis, Anti-apoptosis, Antifibrosis</b>			
	VEGF-A	2069.19 (1092.50)	2345.90 (1931.04)	0.8
	HGF	4560.90 (3013.77)	4676.14 (3274.47)	0.9
	SDF	97.02 (101.02)	144.58 (119.25)	0.3
	<b>Immunomodulatory</b>			
	IDO	0.27 (0.16)	0.29 (0.18)	0.9
	PGE2	1.68 (1.11)	1.58 (1.11)	0.8
	IL-6	1261.73 (350.56)	1214.60 (369.62)	0.6
MSC Senescence		N=10	N=17	
	SA- $\beta$ -gal	25186.55 (2765.60)	25101.79 (2502.17)	0.9
	Data are mean (SD)			

\*PGE2 (n=10 Female; n=14 Male). VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; SDF: stromal cell derived factor-1 (or CXCL12); IDO: indoleamine 2,3 deoxygenase-1; PGE2: prostaglandin E2; IL: interleukin; SA- $\beta$ -gal: senescence-associated beta-galactosidase; UACR: Urine albumin:creatinine ratio (per mg/g creatinine). Units of measurement: ng/mL for all IDO, PGE2, and activin A; pg/mL for VEGF-A, VEGF-C, HGF, and IL-6. Other studies are expressed as OD: optical density (Migration), ABS 490: Absorbance 490 nm wavelength (Proliferation); and RFU: relative fluorescence unit (SA- $\beta$ -gal). Additional studies: epithelial growth factor (angiogenic), bone morphometric protein-7 (anti-fibrosis), and TNF $\alpha$  (proinflammatory) were undetectable in culture medium by ELISA/Luminex.

**Supplemental Table 6. Univariate comparison between MSC *in vitro* studies and diabetes type or metformin use among participants with diabetic kidney disease (DKD)**

MSC studies in participants with DKD						
<i>In vitro</i> Study	Diabetes Type			Metformin Therapy		
	Type 1	Type 2	p value	Non-Use	Use	p value
<b>MSC Function</b>	N=10	N=39		N=33	N=16	
Migration	0.54 (0.13)	0.50 (0.11)	0.3	0.51 (0.12)	0.51 (0.09)	0.7
Proliferation	0.39 (0.04)	0.38 (0.05)	0.5	0.38 (0.05)	0.40 (0.04)	0.1
<b>MSC Secretome</b>	N=10	N=38		N=32	N=16	
<b>Proangiogenesis, Anti-apoptosis, Antifibrosis</b>						
VEGF-A	1970.44 (1168.02)	2306.35 (1752.22)	0.6	2150.12 (1352.11)	2408.85 (2150.72)	0.8
HGF	4306.34 (3452.41)	4715.83 (3097.06)	0.7	4849.51 (3036.42)	4192.55 (3396.83)	0.4
SDF	111.75 (87.37)	133.37 (124.17)	0.7	143.75 (106.10)	87.09 (127.00)	0.1
<b>Immunomodulatory</b>						
IDO	0.25 (0.26)	0.30 (0.14)	0.07	0.28 (0.19)	0.30 (0.12)	0.4
PGE2*	0.56 (0.28)	1.77 (1.08)	0.2	1.53 (1.17)	1.75 (1.00)	0.7
IL-6	1027.82 (308.74)	1287.31 (355.34)	0.06	1176.37 (347.11)	1347.02 (366.71)	0.2
<b>MSC Senescence</b>	N=4	N=23		N=10	N=17	
SA-β-gal	26904.38 (2270.54)	24825.15 (2512.32)	0.1	<b>25868.18</b> <b>(2555.24)</b>	<b>23883.70</b> <b>(2102.69)</b>	<b>0.03</b>
<b>Data are mean (SD)</b>						

\*PGE2 (n=3 Type 2; n=21 Type2; n=14 Metformin Non-Use; n=10 Use). VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; SDF: stromal cell derived factor-1 (or CXCL12); IDO: indoleamine 2,3 deoxygenase-1; PGE2: prostaglandin E2; IL: interleukin; SA-β-gal: senescence-associated beta-galactosidase; UACR: Urine albumin:creatinine ratio (per mg/g creatinine). Units of measurement: ng/mL for all IDO, PGE2, and activin A; pg/mL for VEGF-A, VEGF-C, HGF, and IL-6. Other studies are expressed as OD: optical density (Migration), ABS 490: Absorbance 490 nm wavelength (Proliferation); and RFU: relative fluorescence unit (SA-β-gal). Additional studies: epithelial growth factor (angiogenic), bone morphometric protein-7 (anti-fibrosis), and TNFα (proinflammatory) were undetectable in culture medium by ELISA/Luminex.



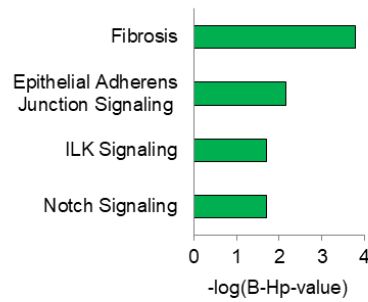
**Supplemental Table 7. Univariate correlation coefficients between MSC *in vitro* studies and patient characteristics among Control participants**

MSC studies in Controls								
<i>In vitro</i> Study	Age		BMI		Glucose		eGFR	
	$r_s$	p-value	$r_s$	p-value	$r_s$	p-value	$r_s$	p-value
<b>MSC Function</b>								
Migration	0.182	0.5	-0.144	0.6	-0.293	0.3	-0.292	0.3
Proliferation	0.379	0.1	-0.049	0.9	-0.055	0.08	-0.028	0.9
<b>MSC Secretome</b>								
<b>Proangiogenesis, Anti-apoptosis, Antifibrosis</b>								
VEGF-A	0.544	0.1	<b>-0.661</b>	<b>0.04</b>	0.311	0.4	-0.200	0.6
HGF	0.030	0.9	<b>-0.709</b>	<b>0.02</b>	0.433	0.2	-0.212	0.6
SDF	0.240	0.5	-0.294	0.4	0.041	0.9	-0.587	0.07
<b>Immunomodulatory</b>								
IDO	<b>-0.671</b>	<b>0.04</b>	0.558	0.09	-0.179	0.6	0.018	0.9
PGE2	-0.006	0.9	0.467	0.2	-0.409	0.2	<b>0.709</b>	<b>0.02</b>
IL-6	-0.106	0.8	-0.127	0.7	0.030	0.9	-0.261	0.5
<b>MSC Senescence</b>								
SA- $\beta$ -gal	-0.372	0.2	0.125	0.7	0.018	0.9	0.305	0.3

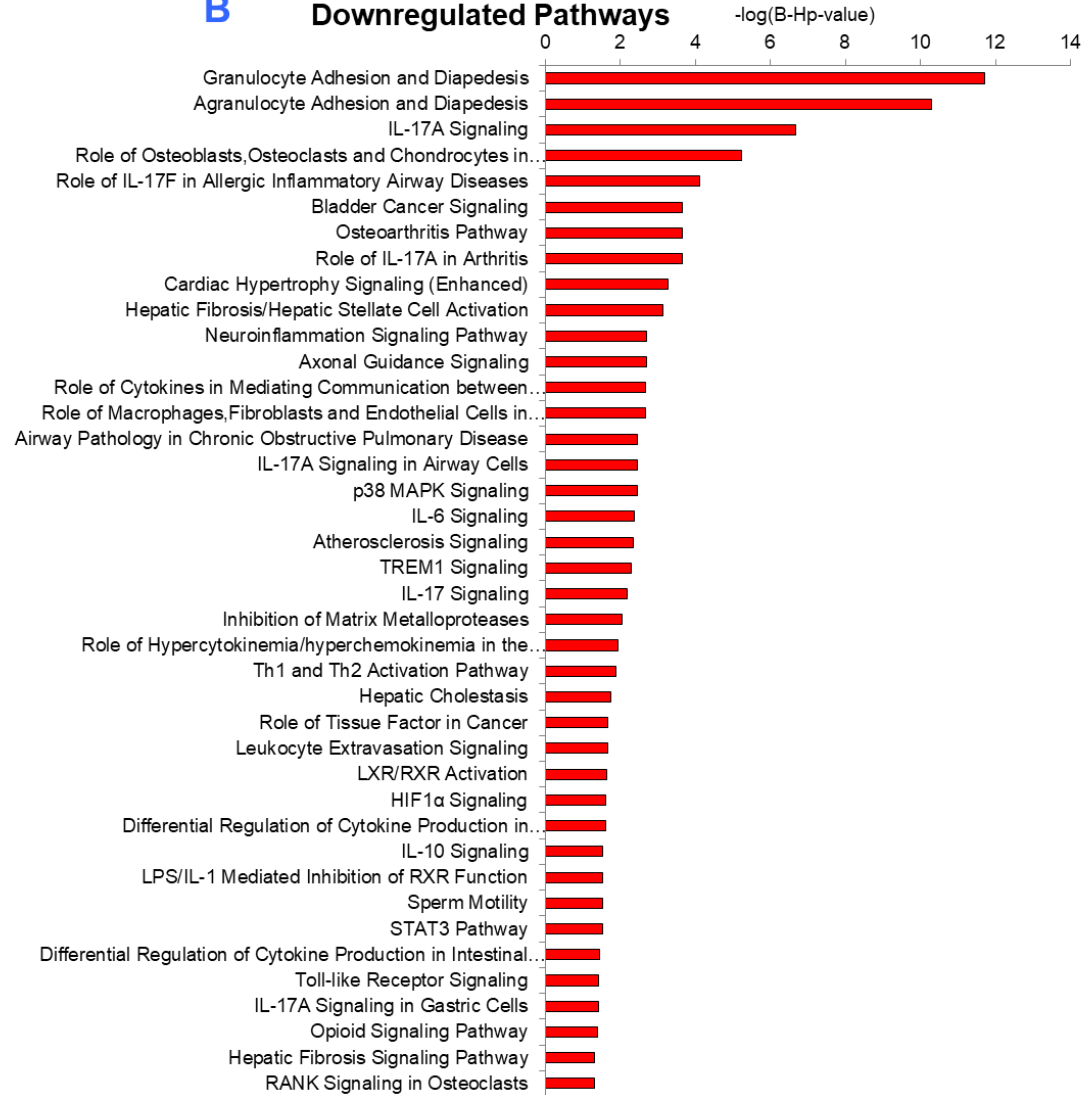
Migration and proliferation n=16, SA- $\beta$ -gal activity: n=14; all MSC secretome studies n=10. Age (per year); BMI: body mass index (per kg/m<sup>2</sup>), glucose (per g/dL); eGFR: estimated glomerular filtration rate (per mL/min/1.73m<sup>2</sup>); UACR urine albumin:creatinine (per mg/g); eGFR: estimated glomerular filtration rate (per mL/min/1.73m<sup>2</sup>); UACR (per mg/g);  $r_s$ : Spearman's Rank Correlation coefficient; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; SDF: stromal cell derived factor-1 (or CXCL12); IDO: indoleamine 2,3 deoxygenase-1; PGE2: prostaglandin E2; IL: interleukin; SA- $\beta$ -gal: senescence-associated beta-galactosidase; UACR: Urine albumin:creatinine ratio (per mg/g creatinine). Units of measurement: ng/mL for all IDO, PGE2, and activin A; pg/mL for VEGF-A, VEGF-C, HGF, and IL-6. Other studies are expressed as OD: optical density (Migration), ABS 490: Absorbance 490 nm wavelength (Proliferation); and RFU: relative fluorescence unit (SA- $\beta$ -gal). Additional studies: epithelial growth factor (angiogenic), bone morphometric protein-7 (anti-fibrosis), and TNF $\alpha$  (proinflammatory) were undetectable in culture medium by ELISA/Luminex.

# Supplemental Figure 1. Upregulated and Downregulated Ingenuity Pathways for Differentially Expressed MSC Genes

## A Upregulated Pathways



## B Downregulated Pathways



$-\log(B-Hp\text{-Values}) > 1.3 = p < 0.05$

Supplemental Figure 1 Legend. Functional annotation clustering analyses were performed in differentially expressed MSC genes consisting of **A**) upregulated (n=4) and **B**) downregulated (n=40) pathways with statistical significance.  $-\log(\text{B-H pvalues}) > 1.3$  represent pathways with  $p < 0.05$ . ILK (integrin-linked kinase); IL-17A (interleukin-17A); IL-17F (interleukin-17F); MAPK (mitogen-activated protein kinases); IL-6 (interleukin-6); TREM1 (triggering receptor expressed on myeloid cells 1); IL-17 (interleukin-17); Th1 (T helper type 1); Th2 (T helper type 2); LXR/RXR (liver X receptors and retinoid X receptors); HIF1 $\alpha$  (hypoxia inducible factor 1 $\alpha$ ); IL-10 (interleukin-10); LPS/IL-1 (lipopolysaccharide/ interleukin-1); STAT3 (signal transducer and activator of transcription 3); RANK (receptor activator of nuclear factor  $\kappa$  B)

## **REFERENCES:**

1. Eirin A, Zhu XY, Krier JD, et al. Adipose tissue-derived mesenchymal stem cells improve revascularization outcomes to restore renal function in swine atherosclerotic renal artery stenosis. *Stem cells (Dayton, Ohio)*. 2012;30(5):1030-1041.
2. Eirin A, Zhu XY, Puranik AS, et al. Mesenchymal stem cell-derived extracellular vesicles attenuate kidney inflammation. *Kidney international*. 2017;92(1):114-124.
3. Zhu XY, Ma S, Eirin A, et al. Functional Plasticity of Adipose-Derived Stromal Cells During Development of Obesity. *Stem cells translational medicine*. 2016;5(7):893-900.
4. Saad A, Dietz AB, Herrmann SMS, et al. Autologous Mesenchymal Stem Cells Increase Cortical Perfusion in Renovascular Disease. *Journal of the American Society of Nephrology : JASN*. 2017;28(9):2777-2785.
5. Saad A, Zhu XY, Herrmann S, et al. Adipose-derived mesenchymal stem cells from patients with atherosclerotic renovascular disease have increased DNA damage and reduced angiogenesis that can be modified by hypoxia. *Stem cell research & therapy*. 2016;7(1):128.
6. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-317.