

SUPPLEMENTAL TABLE OF CONTENTS

Supplemental Method:

Gating strategy for flow Cytometry to confirm the expression of MSC-specific surface markers.

Using the aspect ratio intensity and area of the brightfield channel on the Amnis Flow sight we were able to visualize and gate the single cells. Thresholds on the Amnis flow sight were determined by histograms generated by single color stained positive and negative beads. Using this data, baseline thresholds were determined to run our samples. The threshold generated by the control beads was used to visualize the images of cells in a scatterplot and determine the single cells that were positively stained¹.

Supplemental Figures

Supplemental Figure 1: Mesenchymal stem cell (MSC) showing typical morphological appearance in normoxic and hypoxic conditions under the microscope as spindle-shaped, fibroblast-like cells in culture. **(A, B respectively)**

Supplemental Figure 2: Mesenchymal stem cell (MSC) characterization in normoxia conditions. **(A)** Positive MSC markers (CD73, CD90 and CD105) and **(B)** negative MSC markers (CD14, CD34, CD45).

Supplemental Figure 3: Mesenchymal stem cell (MSC) characterization in hypoxia conditions. **(A)** Positive MSC markers (CD73, CD90 and CD105) and **(B)** negative MSC markers (CD14, CD34, CD45).

Supplemental Figure 4: MSC characterization by with fluorescently conjugated antibodies against CD73, CD90, CD105. **(A)** . Cells strongly positive for markers CD73, CD90 and CD105.

Supplemental Figure 5: MSC Morphology and differentiation. Tri-lineage differentiation into adipocyte (FABP4), chondrocyte (Aggrecan) and osteocytes (osteocalcin) and their quantification.

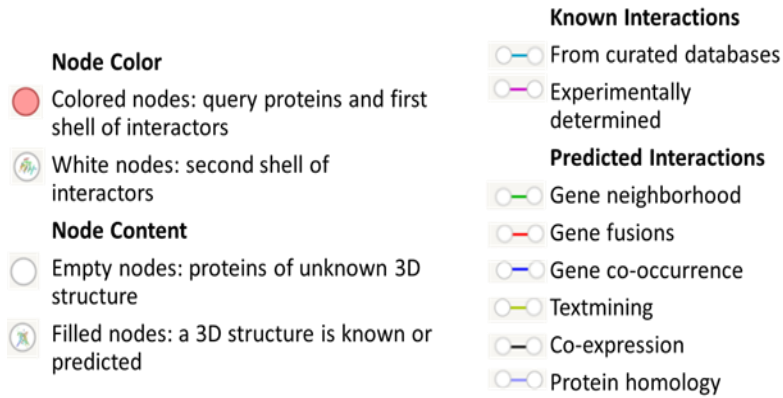
Supplemental Figure 6: Normoxia HTN vs Normoxia Healthy Control MSCs. Normoxic-MSC of hypertension vs. healthy control mapped a total of 13,469 genes, with 463 significant dysregulated genes (n=306 upregulated & n=157 downregulated).

Gene ontology analysis showed that upregulated genes were implicated in modulation of vasculature development, migration, response to growth factor and negative regulation of proliferation, whereas downregulated genes participated in insulin like growth factor binding proteins **(A, B, respectively)**. Volcano plot demonstrated the distribution of differentially expressed genes, with downregulated and upregulated genes based on p-value and \log_2fc **(C)**. Heatmap showed angiogenic and inflammatory genes upregulated in HTN MSCs compared to HC MSCs and an equal dysregulation of senescence genes in MSCs of both the groups **(D)**.

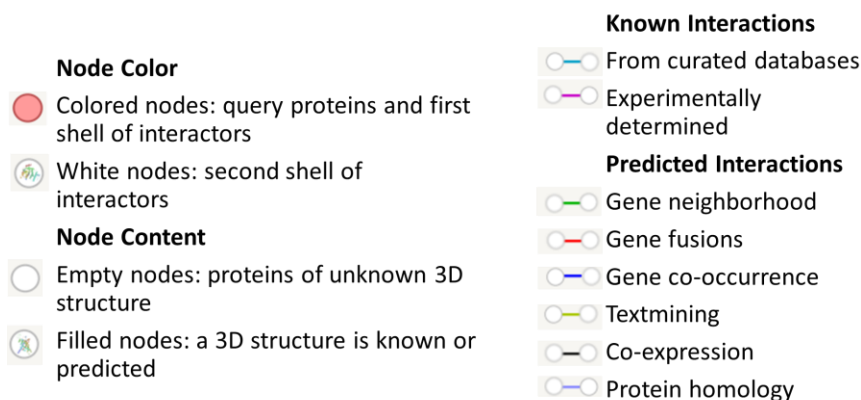
Supplemental Figure 7: Normoxia Healthy Control vs Hypoxia Healthy Control MSCs. In healthy control-MSCs, a total of 13138 genes were mapped, with 261 significant dysregulated genes (n=60 upregulated & n=201 downregulated). Gene ontology analysis showed that gene upregulated participated in modulation of angiogenesis and methylation, whereas downregulated genes were involved in cellular response to tumor necrosis factor **(A, B, respectively)**. Volcano plot demonstrated the distribution of differentially expressed genes, with downregulated and upregulated genes based on p-value and \log_2fc **(C)**. Heatmap showed dysregulated genes significant for angiogenesis, and inflammation upregulated more in hypoxic HC MSCs compared to normoxic HC MSCs **(D)**.

Supplemental Figure 8: Gene ontology (GO) analysis pathways (A) Biological Process (B) Molecular Functions (C) Cellular Components of Upregulated gene sets with significant changes between Hypoxia HKD and Normoxia HKD MSCs.

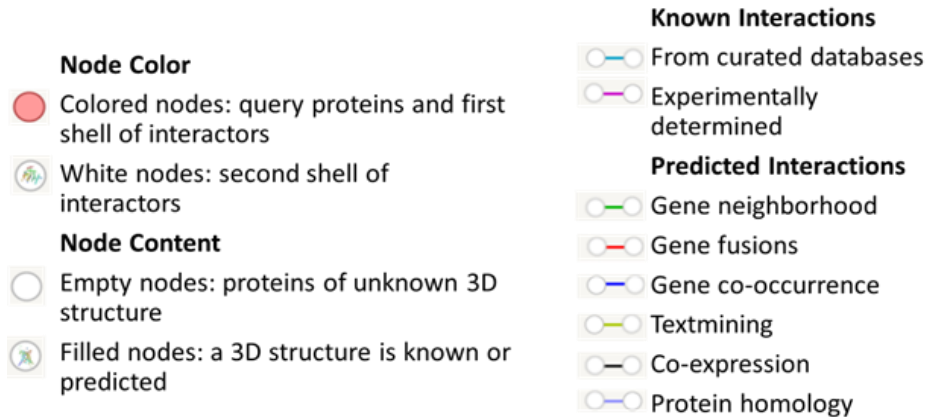
Supplemental Figure 9: Genes and Transcription Factor (TF) network Interactions between Normoxia HKD and Normoxia HC mRNA targets derived from STRING. Color lines represent interactions between Normoxia HKD and Normoxia HC mRNA targets according to the functional association networks. Red circles indicate common (TF) mRNA targets between Normoxia HKD MSCs and Normoxia HC MSCs.



Supplemental Figure 10: Genes and Transcription Factor (TF) network Interactions between Normoxia HKD and Normoxia HTN mRNA targets derived from STRING. Color lines represent interactions between Normoxia HKD and Normoxia HTN mRNA targets according to the functional association networks. Red circles indicate common (TF) mRNA targets between Normoxia HKD MSCs and Normoxia HTN MSCs.



Supplemental Figure 11: Genes and Transcription Factor (TF) network Interactions between HKD normoxia and hypoxia mRNA targets derived from STRING. Color lines represent interactions between HKD normoxia and hypoxia mRNA targets according to the functional association networks. Red circles indicate common (TF) mRNA targets between HKD normoxia and hypoxia.



Supplemental Figure S12: Inflammatory markers

Inflammatory factors under normoxic and hypoxic state in IFN- γ , IL-1 α , IL-6, IL-8, and TNF- α in HC(n=12)-, HTN(n=9)- MSCs, and HKD(n=12)- MSCs. At baseline normoxic conditions, there was no difference in inflammatory cytokines released among the groups (**Fig S12A-E**). However, under HPC, there was an increase of IFN- γ levels only in HC-MSCs (p-value=0.04) (**Fig S12-A**). Among the baseline of the HC, HTN and HKD, there was no difference (**Fig S13**).

Supplemental Table S1: Angiogenesis secretome markers

Under normoxic conditions the expression of the pro-angiogenic factors VEGF and EGF were similar among the groups and HPC did not exert any significant effect in these specific angiogenic markers. There was a significant increase in HC MSCs EGF and HGF (Elisa) after hypoxia. We also saw a significant decrease in HKD MSCs HGF after hypoxia.

Supplemental Table S2: Senescence markers

Under normoxic conditions the expression of P16, P21 and SA β GAL was non-significant but there was a trend decrease in HC MSCs SA β GAL activity after treatment with hypoxia.

Supplemental Table S3: PCR markers

At baseline normoxic conditions, there was no difference in TGF- β , PGE, iNOS, GAPDH, IDO, IL10, IL4 among the groups.

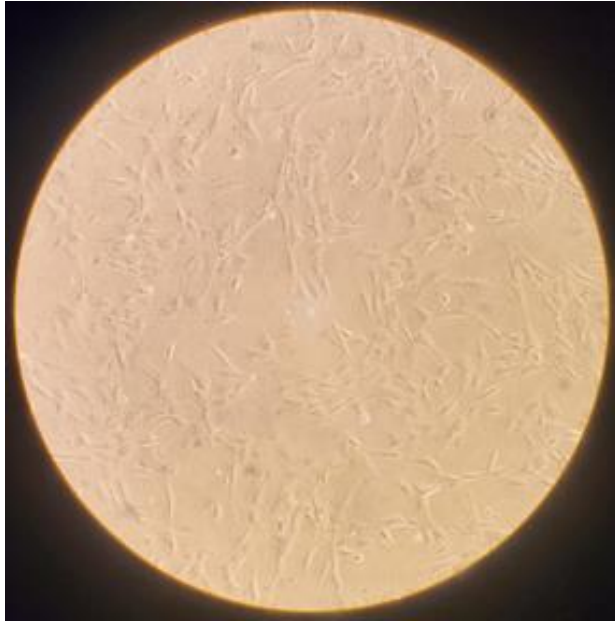
Supplemental Table S4A:

Correlation between MSC Function (normoxia) and Age, MSC Function (hypoxia) and Age

At the baseline and in hypoxic conditions, there was no correlation found between the function of MSCs and the age of the patients.

NORMOXIA

A.



**Low Resolution
4x objective**

HYPOXIA

B.



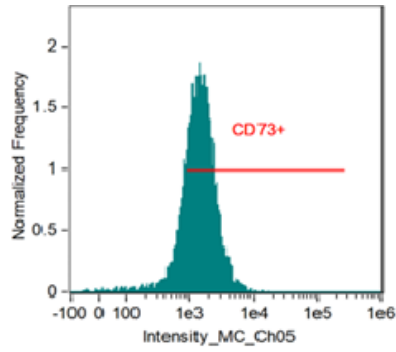
**Low Resolution
4x objective**

Fig. S1. Spindle-shaped morphology of cells isolated from adipose tissue of humans, characteristic of mesenchymal stem cells.

- A. AMSC grown to confluence in Normoxia (20% O₂)**
- B. Cells grown to confluence and subjected to 48 hours of HPC (1% O₂)**

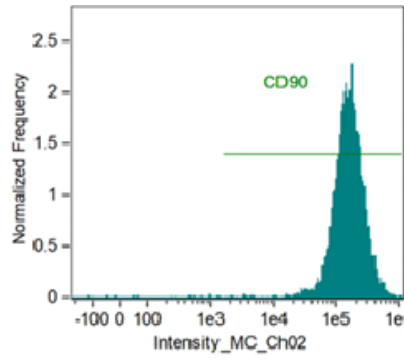
AMSC, Adipose tissue derived mesenchymal stem cells; HPC, Hypoxia preconditioning, O₂, Oxygen

A.



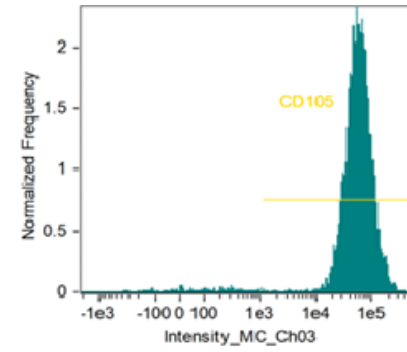
Intensity_MC_Ch05

Population	Count	%Gated
Single	10200	100
CD73+ & Single	8282	81.2



Intensity_MC_Ch02

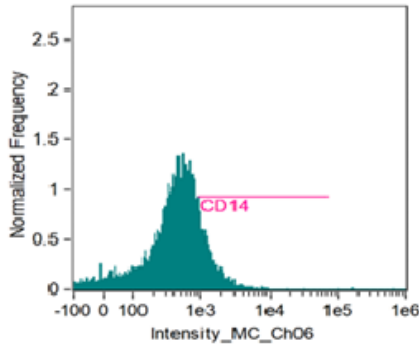
Population	Count	%Gated
Single	10200	100
CD90 & Single	10180	99.8



Intensity_MC_Ch03

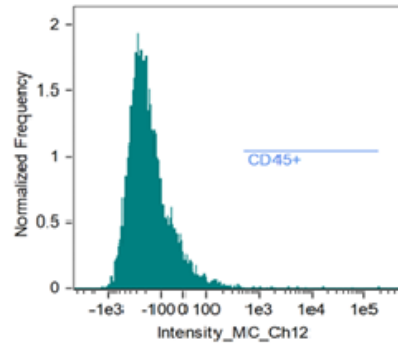
Population	Count	%Gated
Single	10200	100
CD105 & Single	10007	98.1

B.



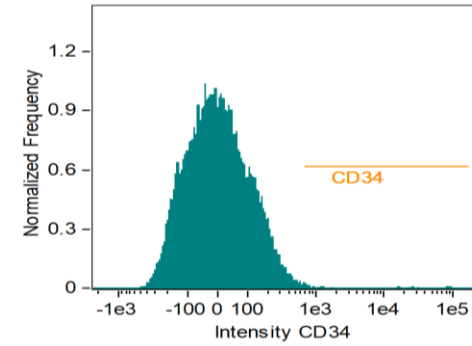
Intensity_MC_Ch06

Population	Count	%Gated
Single	9729	100
CD14 & Single	1712	17.6



Intensity_MC_Ch12

Population	Count	%Gated
Single	9729	100
CD45+ & Single	12	0.12



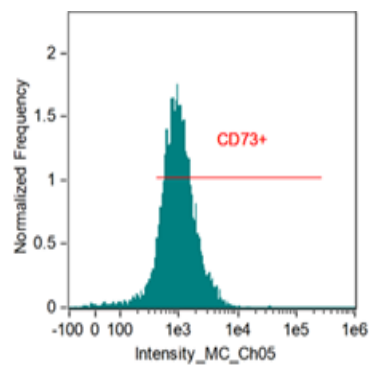
Intensity CD34

Population	Count	%Gated
Single	37798	100
CD34 & Single	111	0.29

Fig. S2. Flowcytometry characterizing the cell surface markers confirming that isolated cells were adipose tissue derived mesenchymal stem cells in Normoxia conditions.

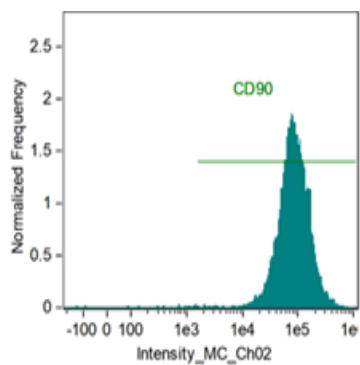
- A. Cells strongly positive for markers CD73, CD90 and CD105
- B. Cells negative for markers CD14, CD34 and CD45

A.



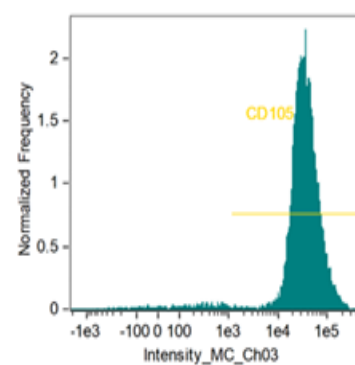
Intensity_MC_Ch05

Population	Count	%Gated
Single	9878	100
CD73+ & Single	8963	90.7



Intensity_MC_Ch02

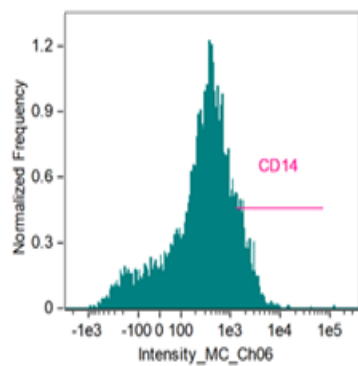
Population	Count	%Gated
Single	9878	100
CD90 & Single	9859	99.8



Intensity_MC_Ch03

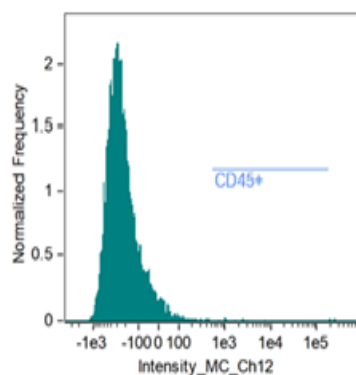
Population	Count	%Gated
Single	9878	100
CD105 & Single	9610	97.3

B.



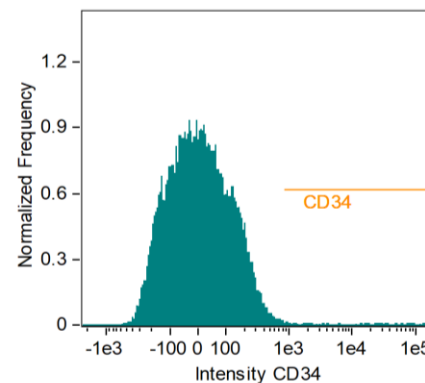
Intensity_MC_Ch06

Population	Count	%Gated
Single	9463	100
CD14 & Single	1126	11.9



Intensity_MC_Ch12

Population	Count	%Gated
Single	9463	100
CD45+ & Single	8	0.08



Intensity CD34

Population	Count	%Gated
Single	28559	100
CD34 & Single	60	0.21

Fig. S3. Flowcytometry characterizing the cell surface markers confirming that isolated cells were adipose tissue derived mesenchymal stem cells in Hypoxia conditions.

A. Cells strongly positive for markers CD73, CD90 and CD105

B. Cells negative for markers CD14, CD34 and CD45

A.

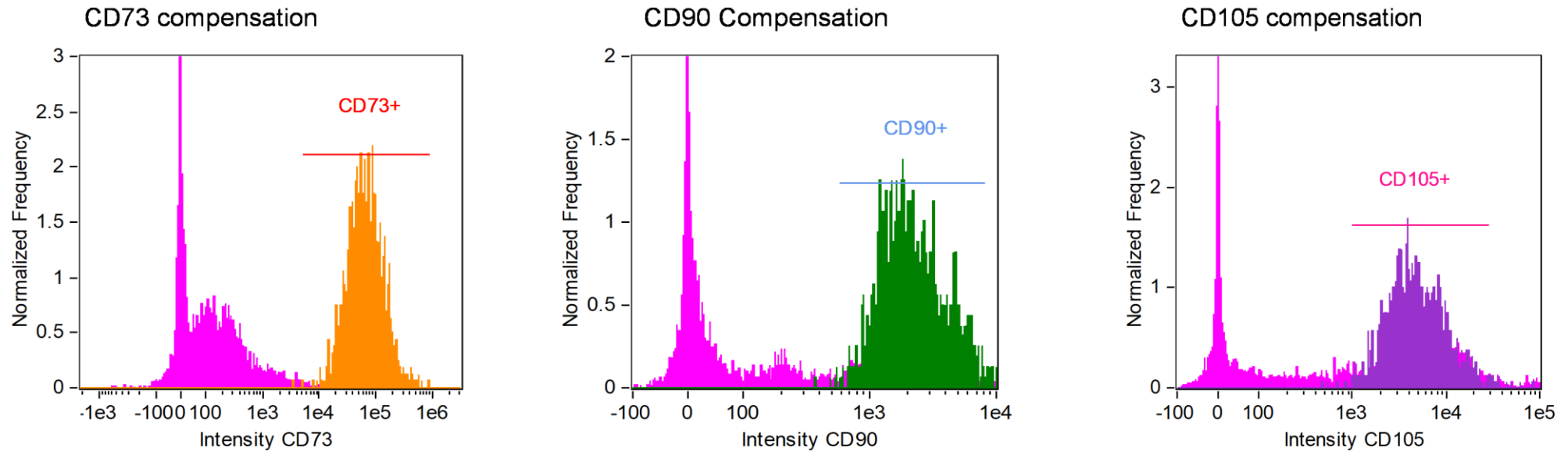
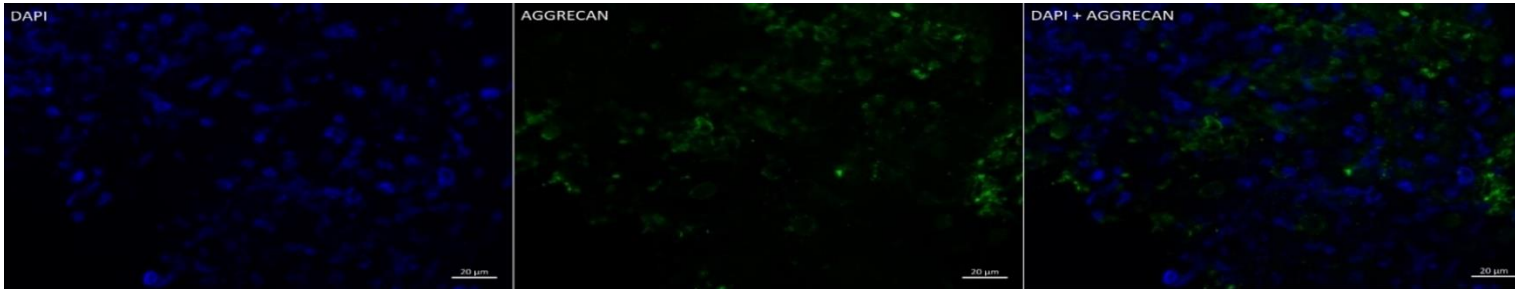
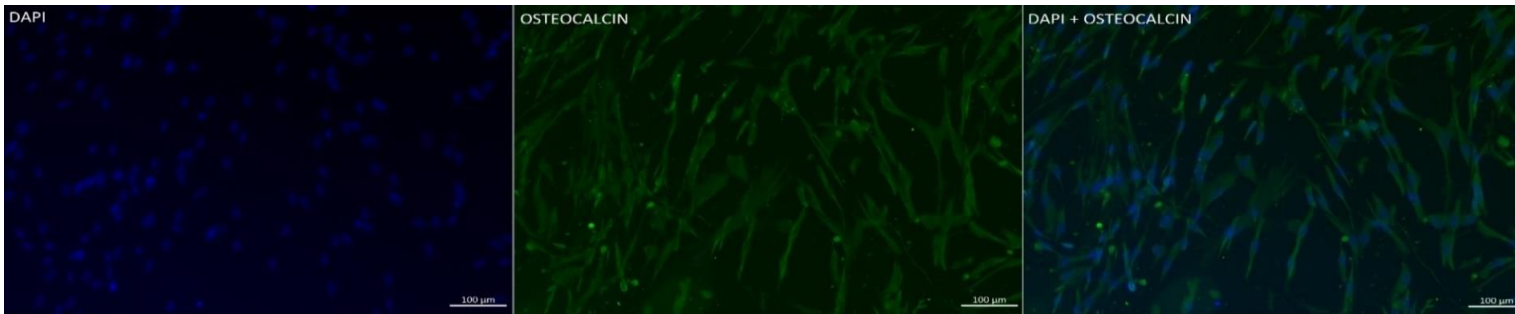


Fig. S4. A. Adipose tissue derived mesenchymal stem cells were stained with fluorescently conjugated antibodies against CD73, CD90, CD105 and the expression was analyzed by flow cytometry. The histogram in pink represents the isotype control and the histogram in orange, green, purple respectively represents the stained sample. Cells strongly positive for markers CD73, CD90 and CD105

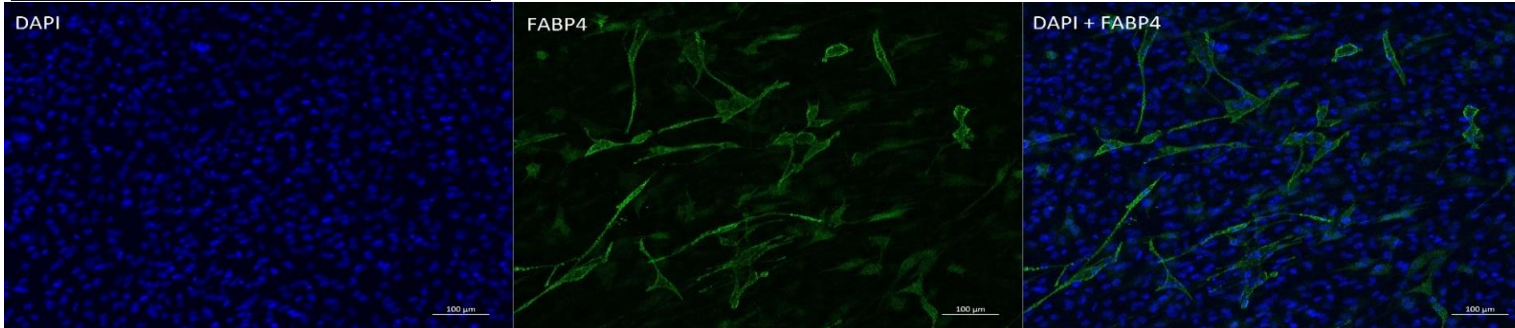
A. ADIPOCYTE LINEAGE



B. OSTEOCYTE LINEAGE



C. CHONDROCYTES LINEAGE



D. MSC TRILINEAGE DIFFERENTIATION

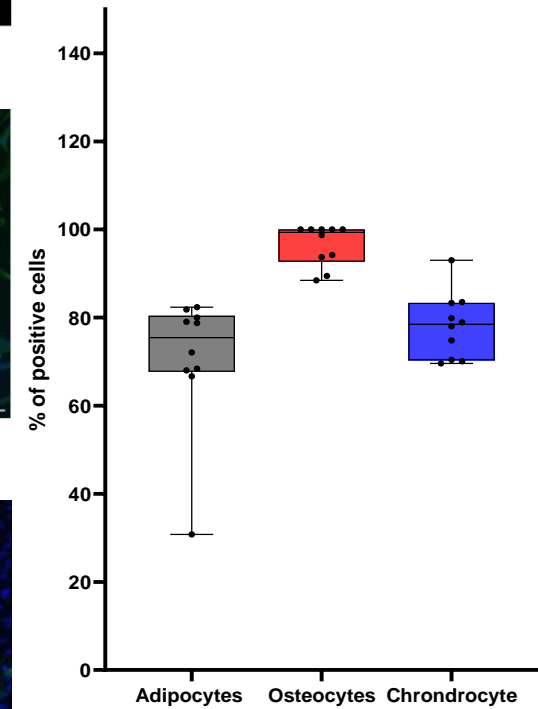
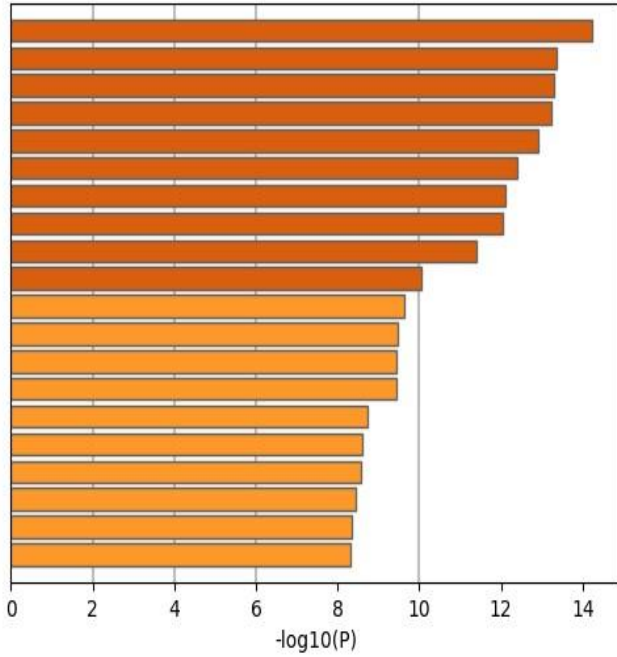


Fig. S5. Differentiation of cells grown after isolation from adipose tissue into adipocytes (A), osteocytes (B), chondrocytes (C), and quantification of adipocytes, osteocytes, and chondrocytes (D) quantification confirming their mesenchymal origin and regenerative properties. Images captured after fluorescent staining of differentiated cells.

Figure: S6

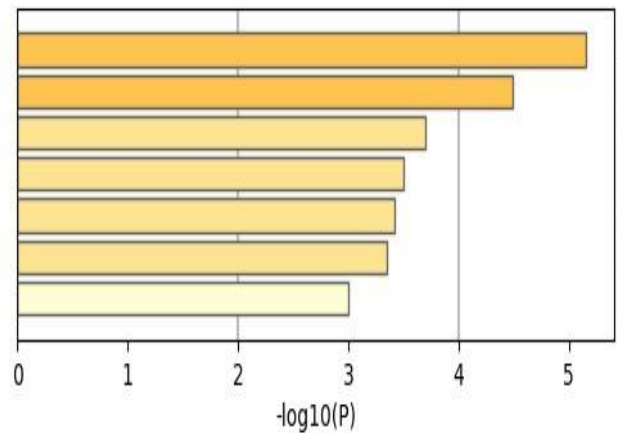
A.

Upregulated genes between HTN and HC (Normoxia)

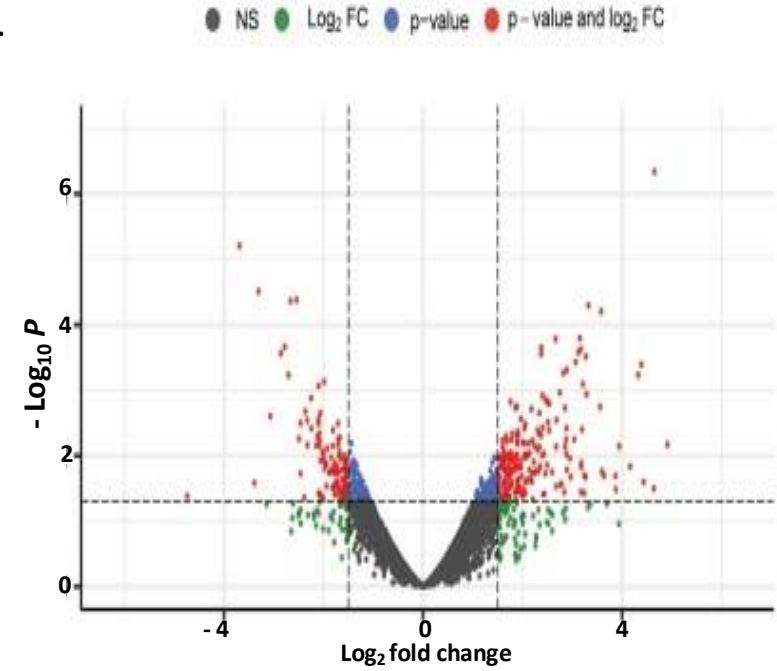


B.

Downregulated genes between HTN and HC (Normoxia)



C.



D.

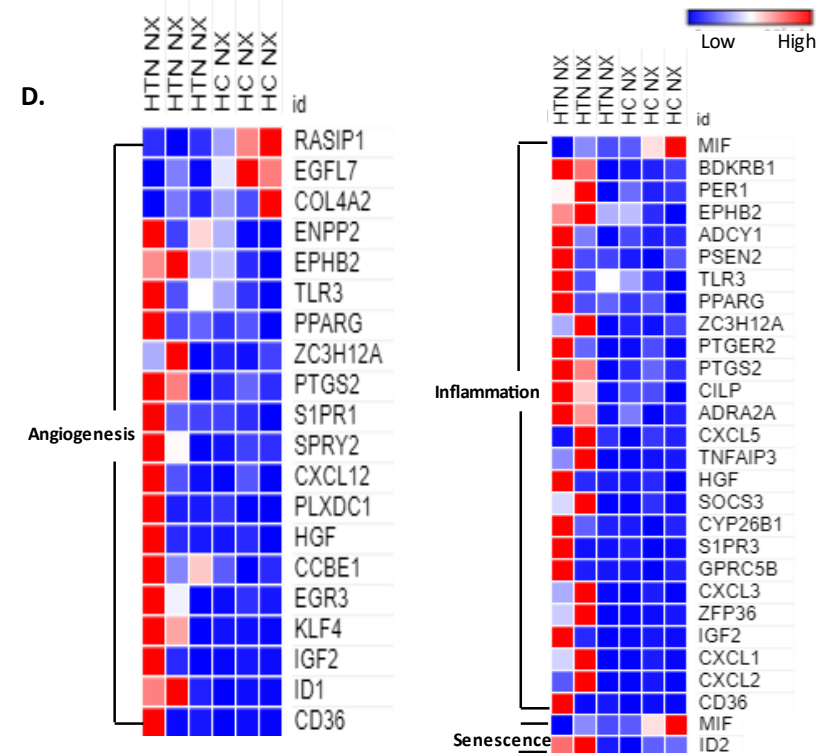
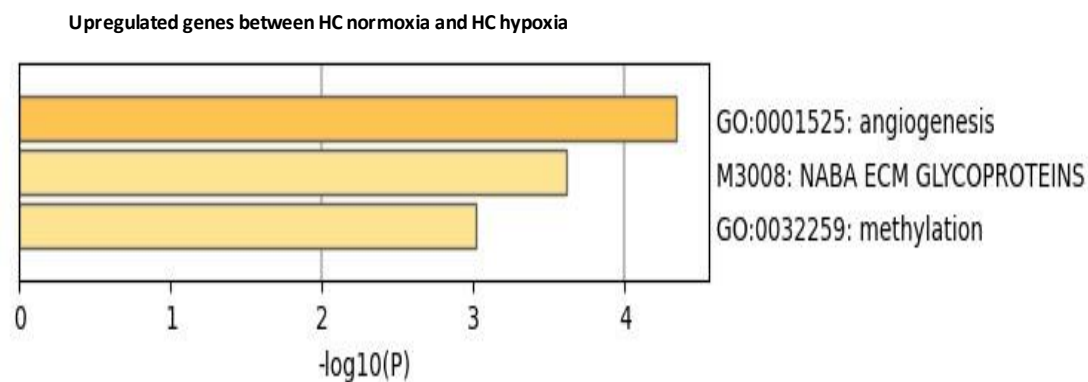
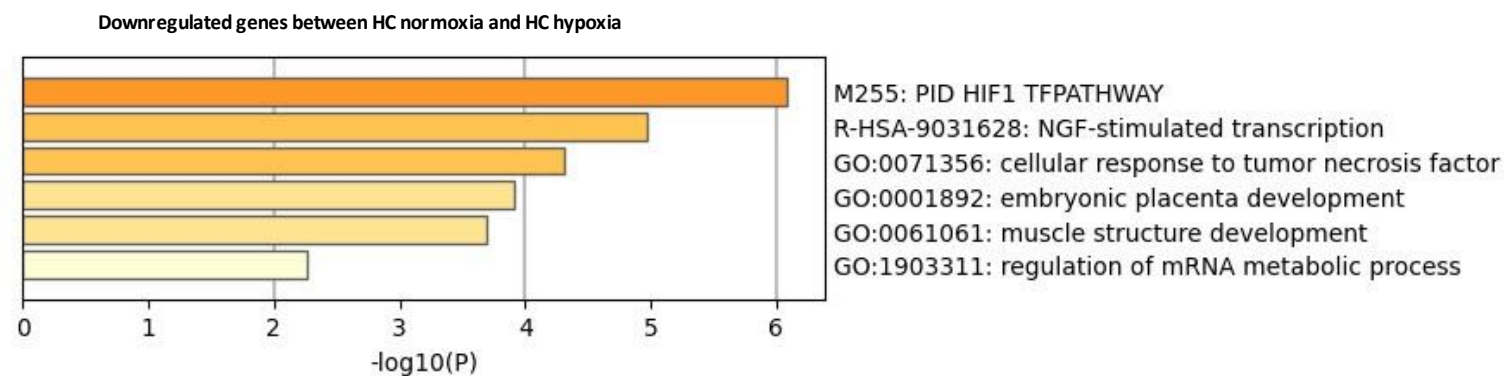


Figure: S7

A.

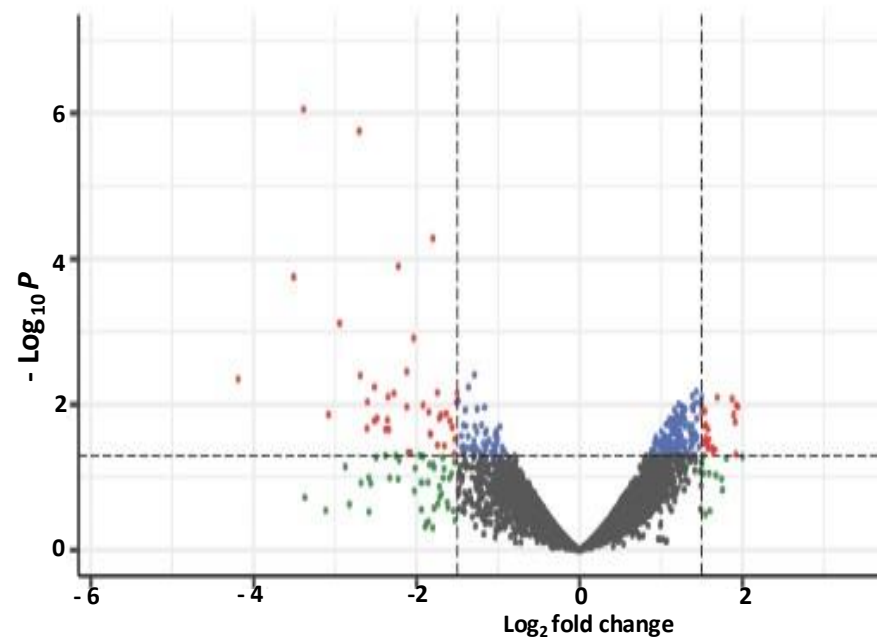


B.

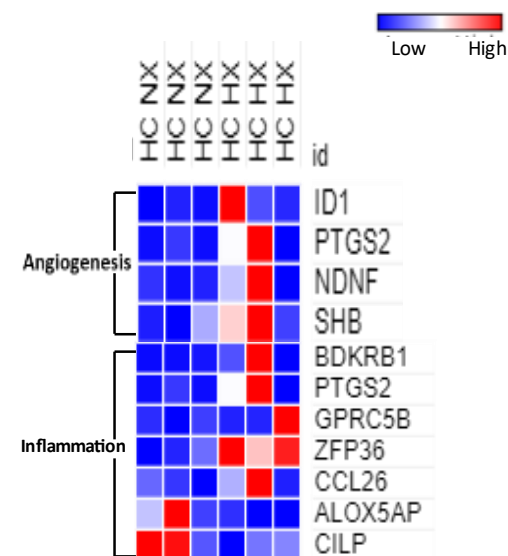


C.

● NS ● Log₂ FC ● p-value ● p-value and log₂ FC



D.



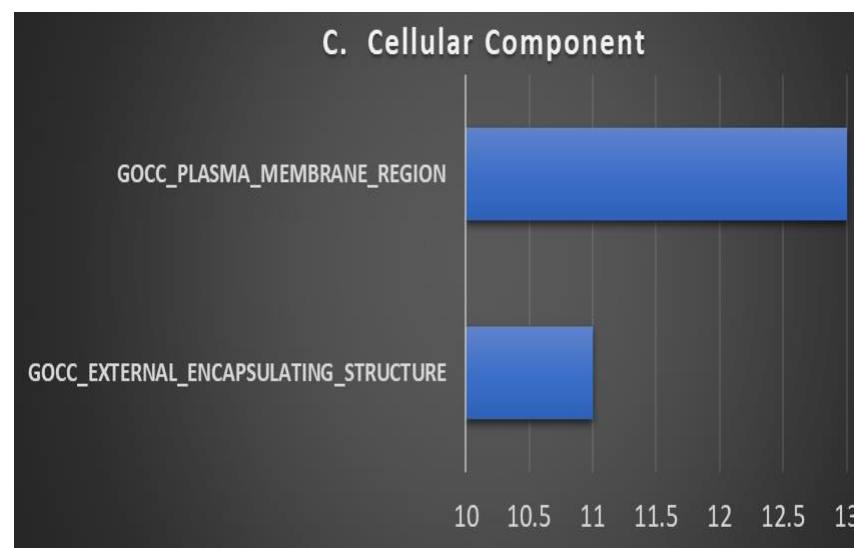
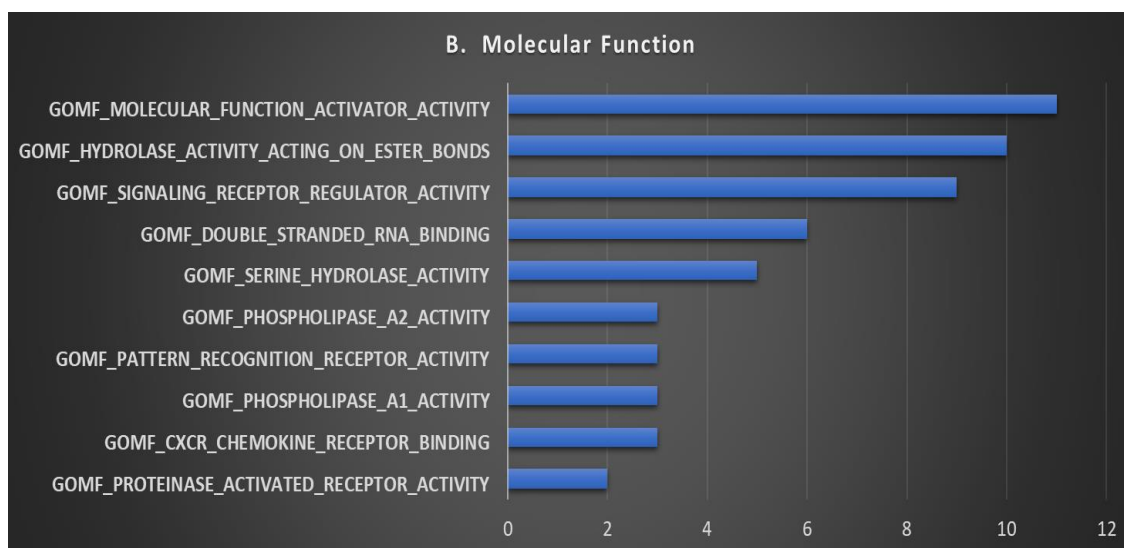
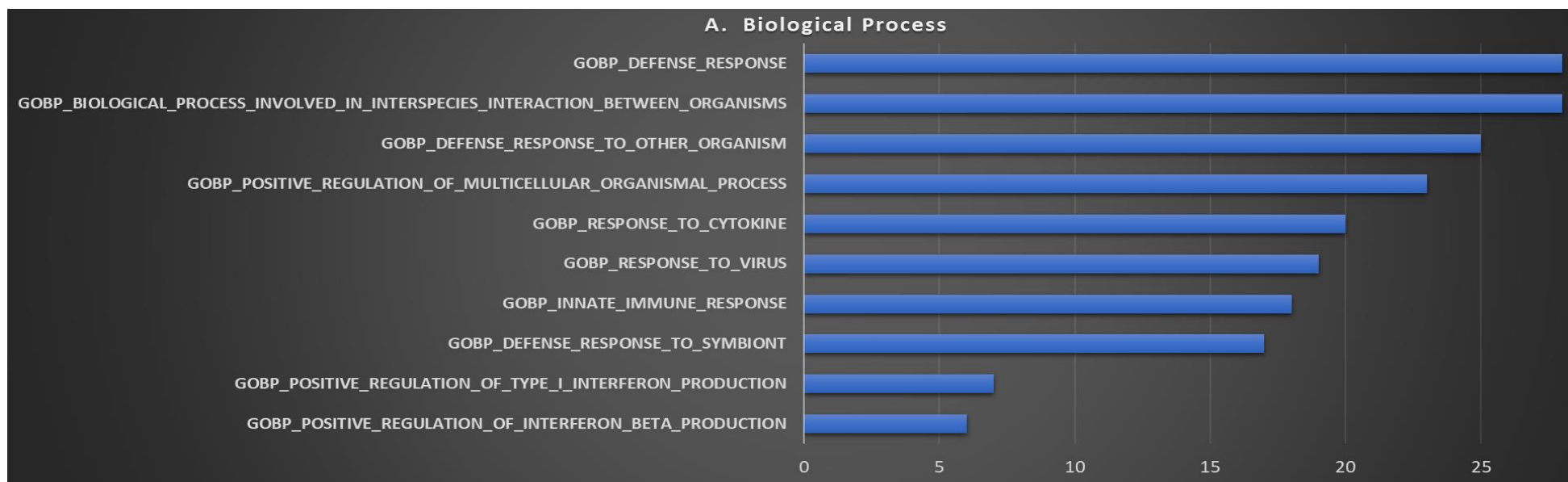


Fig.S8. Hypoxia HKD vs Normoxia HKD MSCs. Gene ontology (GO) analysis pathways (A) Biological Process (B) Molecular Functions (C) Cellular Components of Upregulated gene sets with significant changes between Hypoxia HKD and Normoxia HKD MSCs.

Figure: S9
Gene and transcription factors network interactions between HKD and HC (Normoxia)

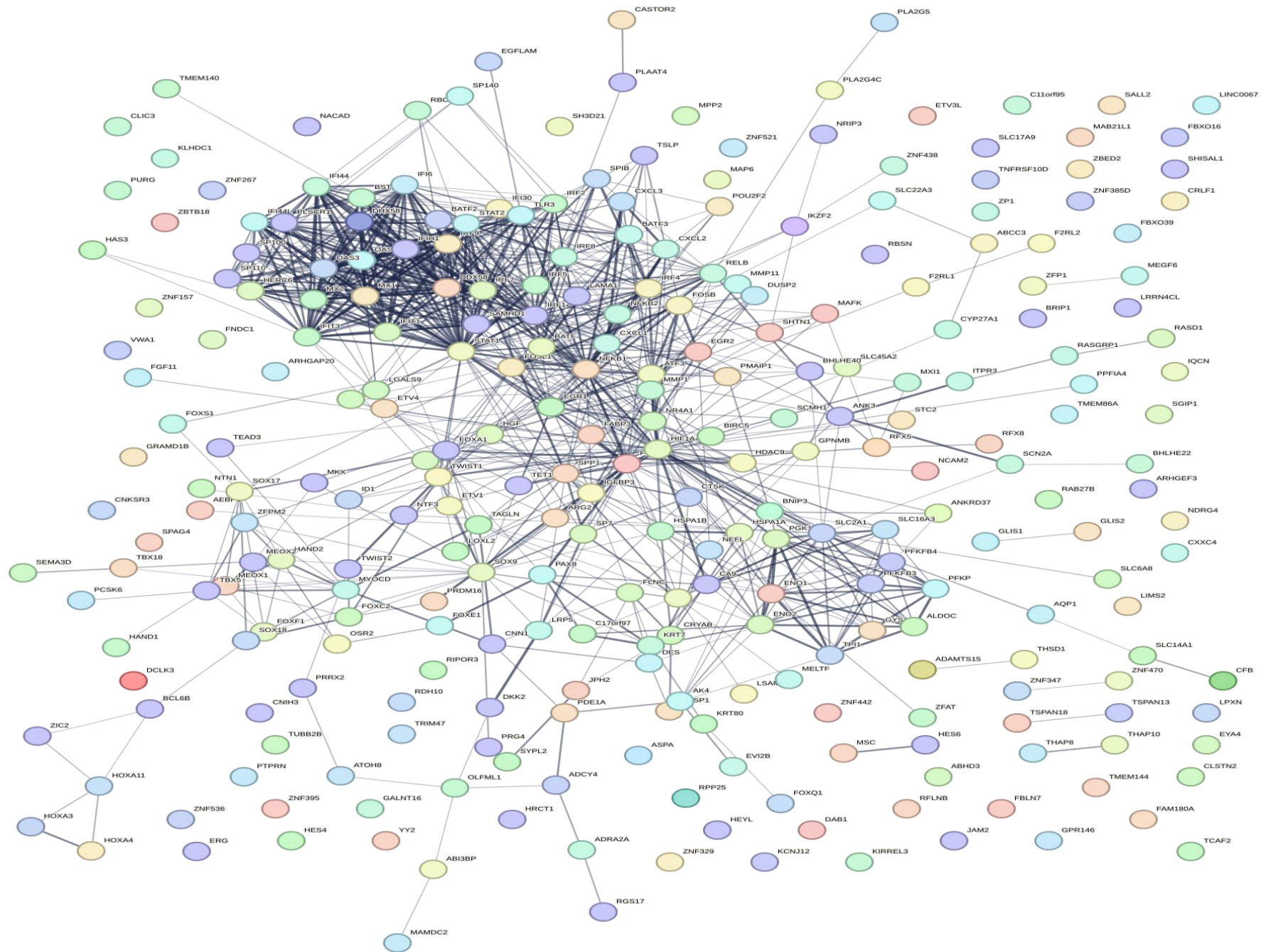


Figure: S10
Gene and transcription factors network interactions between HKD and HTN (Normoxia)

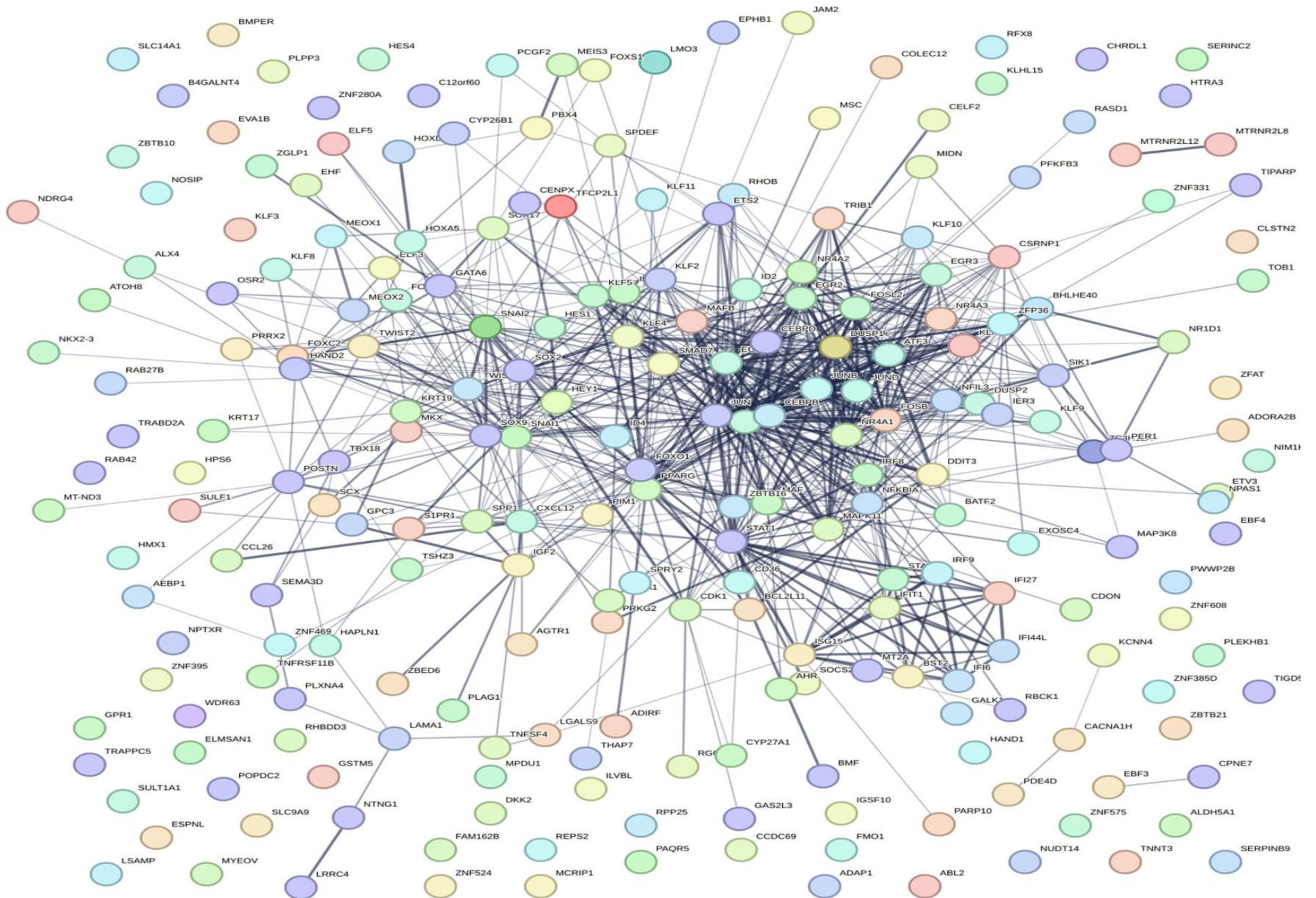


Figure S12

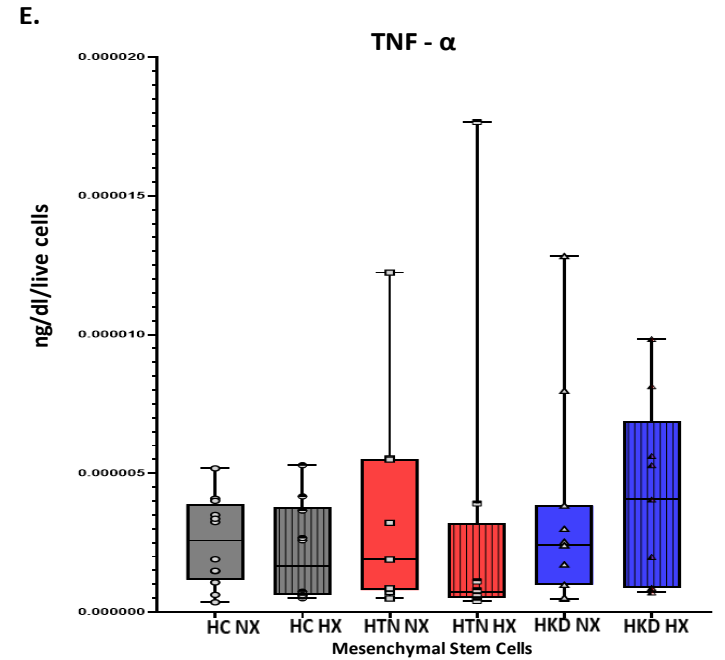
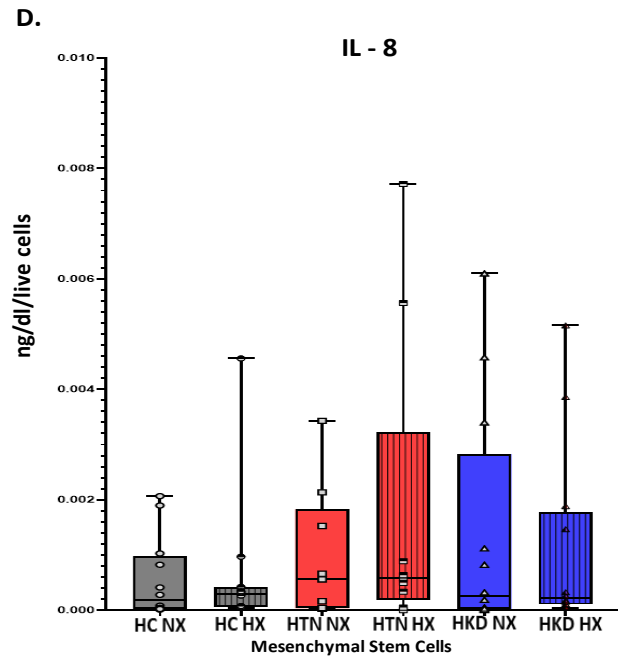
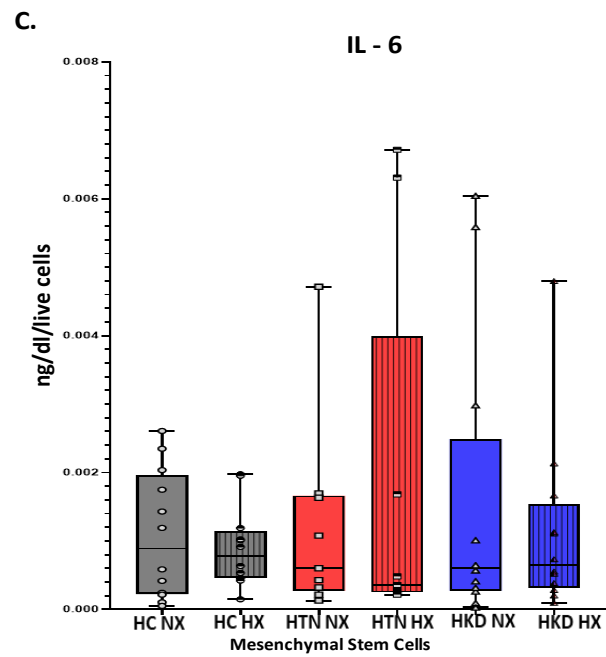
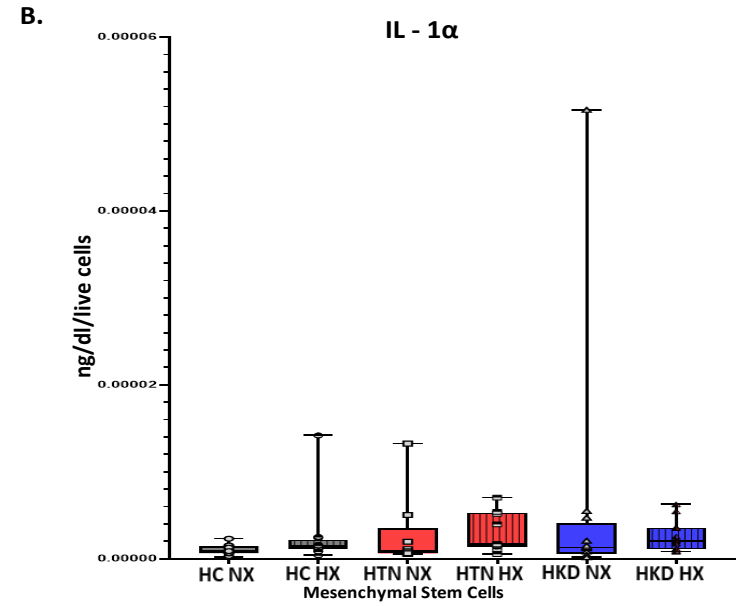
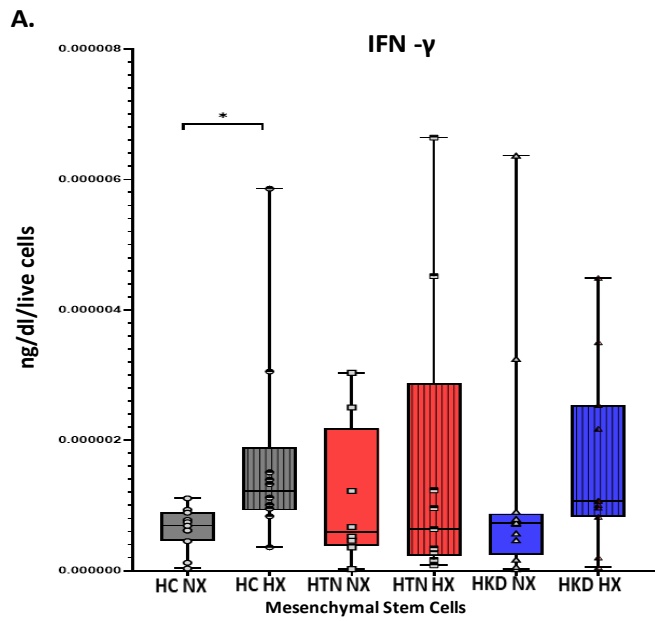


Table S1- Angiogenesis secretome markers at baseline normoxia

VARIABLES	HC (12)	HTN (9)	HKD (12)	P value
EGF	7.94108e ⁻⁹ (5.97554e ⁻⁹ - 1.00493e ⁻⁸)	4.98339e ⁻⁹ (3.44828e ⁻⁹ - 1.5619e ⁻⁸)	7.19277e ⁻⁹ (5.9043e ⁻⁹ - 1.10211e ⁻⁸)	NS
HGF	7.0609e ⁻⁸ (3.90828e ⁻⁸ - 2.02851e ⁻⁷)	7.03846e ⁻⁸ (3.43478e ⁻⁸ - 2.77636e ⁻⁷)	3.7796e ⁻⁸ (2.8515e ⁻⁸ -2.111e ⁻⁷)#	NS
VEGF	0.00018 (5.88315e ⁻⁵ - 0.000262995)	0.000338342 (8.01248e ⁻⁵ - 0.001057009)	0.000119132 (4.22891e ⁻⁵ - 0.000373018)	NS
VEGF (at gene level)	0.977101 (0.55706725- 1.3536075)	1.07 (0.64-1.435)	1.21 (0.79-1.46)	NS
EGF (at gene level)	0.97 (0.705- 2.1325)	3.06 (0.24- 5.68)	1.344779401 (0.425334961 - 3.110220509)	NS

Abbreviations: HC: healthy control; HTN: hypertension; HKD: Hypertensive kidney disease

Table S2- Senescence markers at baseline normoxia

VARIABLES	HC (12)	HTN (9)	HKD (12)	P value
P16	1.063 (0.430- 1.696)	1.160 (0.570- 1.480)	0.866 (0.472- 2.445)	NS
P21	0.810 (0.623- 1.190)	0.870 (0.610- 1.490)	0.895 (0.640- 1.113)	NS
SA β GAL	15765.5 (10393.500- 29550.500)	35322.5 (25168.500- 52886.000)	14956.5 (6672.875- 19085.000)	*0.04

Abbreviations: HC: healthy control; HTN: hypertension; HKD: Hypertensive kidney disease

Table S3 – PCR Markers at baseline normoxia

VARIABLES	HC (12)	HTN (9)	HKD (12)	P value
TGF - β	24.214 (23.488-25.148)	24.800 (24.441, 25.324)	25.182 (24.504, 25.484)	NS
PGE	26.827 (26.414- 27.859)	27.621 (26.550- 28.666)	27.020 (26.817- 27.341)	NS
iNOS	36.454 (35.355- 37.295)	38.396 (37.897- 39.677)	36.625 (36.351- 37.391)	NS
IDO	36.603 (34.912- 39.251)	38.831 (36.902- 39.447)	37.380 (35.873- 38.867)	NS
IL 10	38.702 (35.725- 39.716)	35.699 (34.445- 39.221)	36.374 (34.541- 38.427)	NS
IL 4	39.178 (37.917- 40.000)	39.067 (37.522- 40.000)	38.511 (36.663- 40.000)	NS
GAPDH	20.156 (19.052- 21.981)	21.375 (20.167- 22.746)	20.847 (20.310- 21.231)	NS

Abbreviations: HC: healthy control; HTN: hypertension; HKD: Hypertensive kidney disease; NS: non-significant

Table S4- Correlation between age and MSC function in hypoxic and normoxic conditions

MSC function	Age	
	r	P
NX Proliferation	0.16	0.36
HX Proliferation	0.17	0.33

Abbreviations: MSC: mesenchymal stem cell; NX: normoxia; HX: hypoxic

REFERENCE

[1] Isik B, Thaler R, Goksu BB, Conley SM, Al-Khafaji H, Mohan A, Afarideh M, Abumoawad AM, Zhu XY, Krier JD, Saadiq IM, Tang H, Eirin A, Hickson LJ, van Wijnen AJ, Textor SC, Lerman LO, Herrmann SM: Hypoxic preconditioning induces epigenetic changes and modifies swine mesenchymal stem cell angiogenesis and senescence in experimental atherosclerotic renal artery stenosis. *Stem Cell Res Ther* 2021, 12:240.