

The *In Vitro* Human Fracture Hematoma Model – A Tool for Preclinical Drug Testing

Supplementary Data

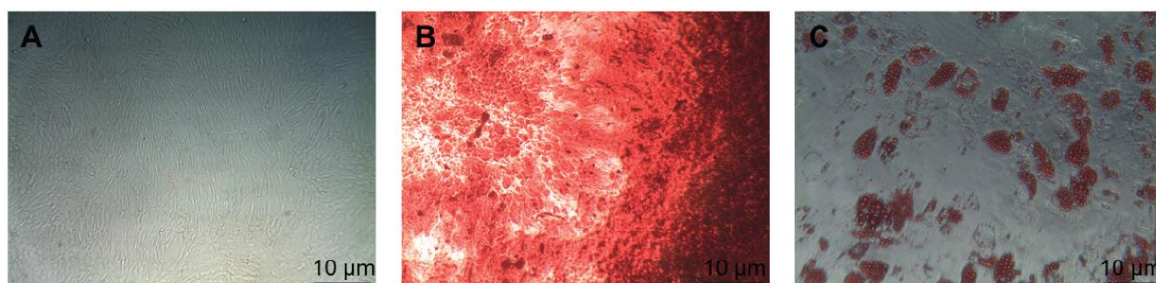


Fig. S1: MSCs in monolayer to prove the capacity to adhere to plastics (A), the differentiation of the MSCs towards the osteogenic lineage, demonstrated by Alizarin Red staining (B) and towards the adipogenic lineage, demonstrated by Red Oil staining (C)

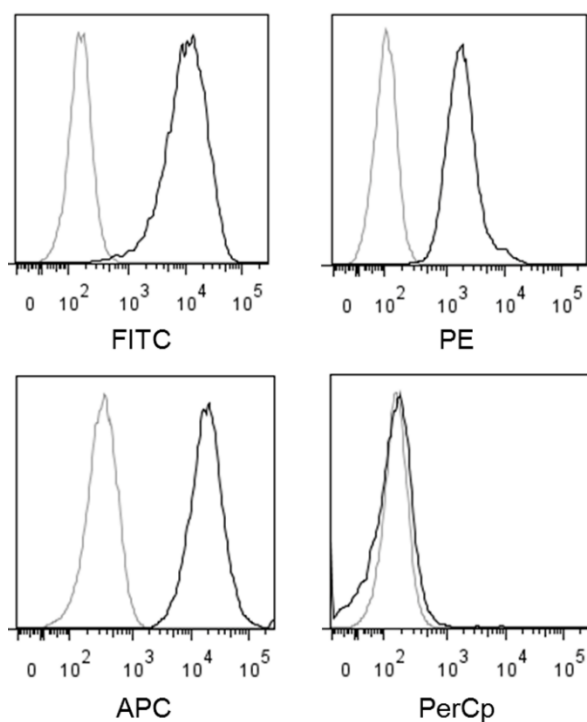


Fig. S2: Surface marker-dependent characterization of hMSCs according to the “minimal criteria” for a common definition of hMSCs as defined by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) (Dominici et al., 2006)

Only hMSCs stained positive for CD73, CD90 and CD105 and negative for CD45, CD34, CD14, CD20 and HLA-DR surface antigens were used in the study as determined by flow cytometry after applying the MSC Phenotyping Kit (Miltenyi, Bergisch-Gladbach, Germany) according to the manufacturer’s protocols based on the phenotyping antibody cocktail including the following antibody conjugates: anti-CD73-FITC, anti-CD105-PE, anti-CD90-APC, anti-CD45-PerCp, anti-CD34-PerCp, anti-HLA-DR-PerCp, anti-CD20-PerCp, anti-CD14-PerCp and a corresponding isotype control cocktail.

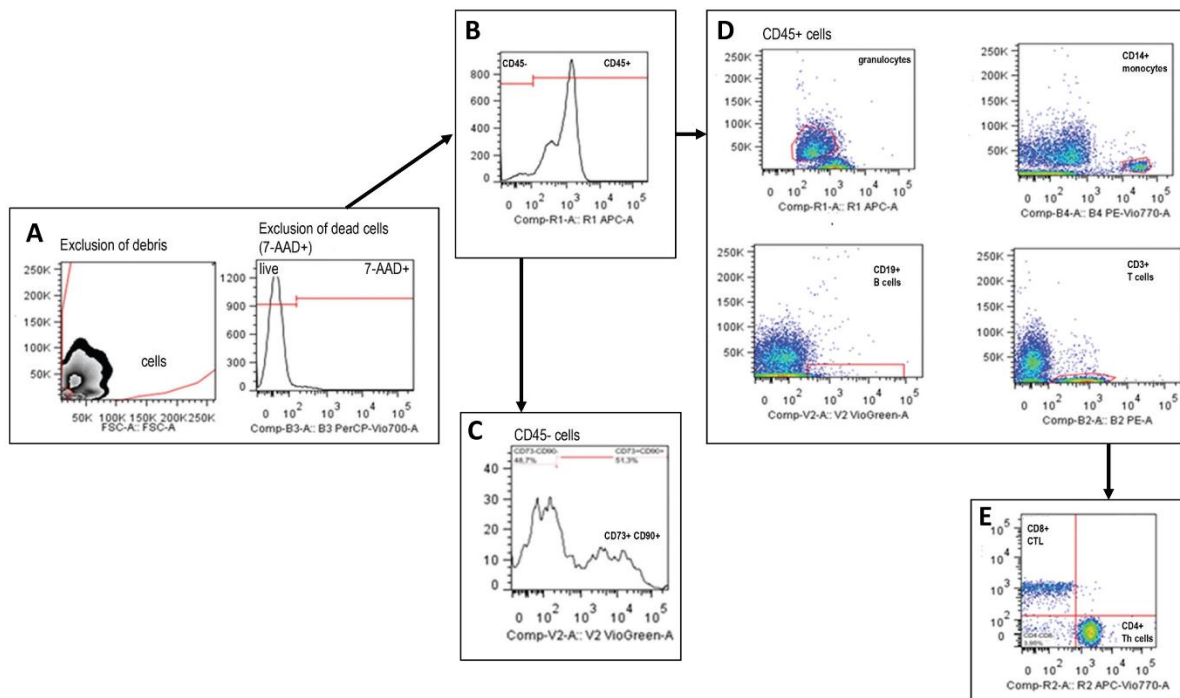


Fig. S3: Gating strategy for evaluation of the cell composition within the FH models

As a first step, cell debris and dead cells were excluded (dead cells positive for 7AAD, A). Then we differentiated between CD45⁺ and CD45⁻ cells (B). The CD45⁺CD73⁺CD90⁺ cells were considered MSCs (C). Within the CD45⁺ gate, we characterized granulocytes based on size and granularity. CD45⁺CD19⁺ cells were considered B-cells, while CD45⁺CD14⁺ cells were considered monocytes (D). CD45⁺CD3⁺ cells were differentiated into CD45⁺CD3⁺CD4⁺ and CD45⁺CD3⁺CD8⁺ cells, considered as T helper or cytotoxic T cells respectively (E).

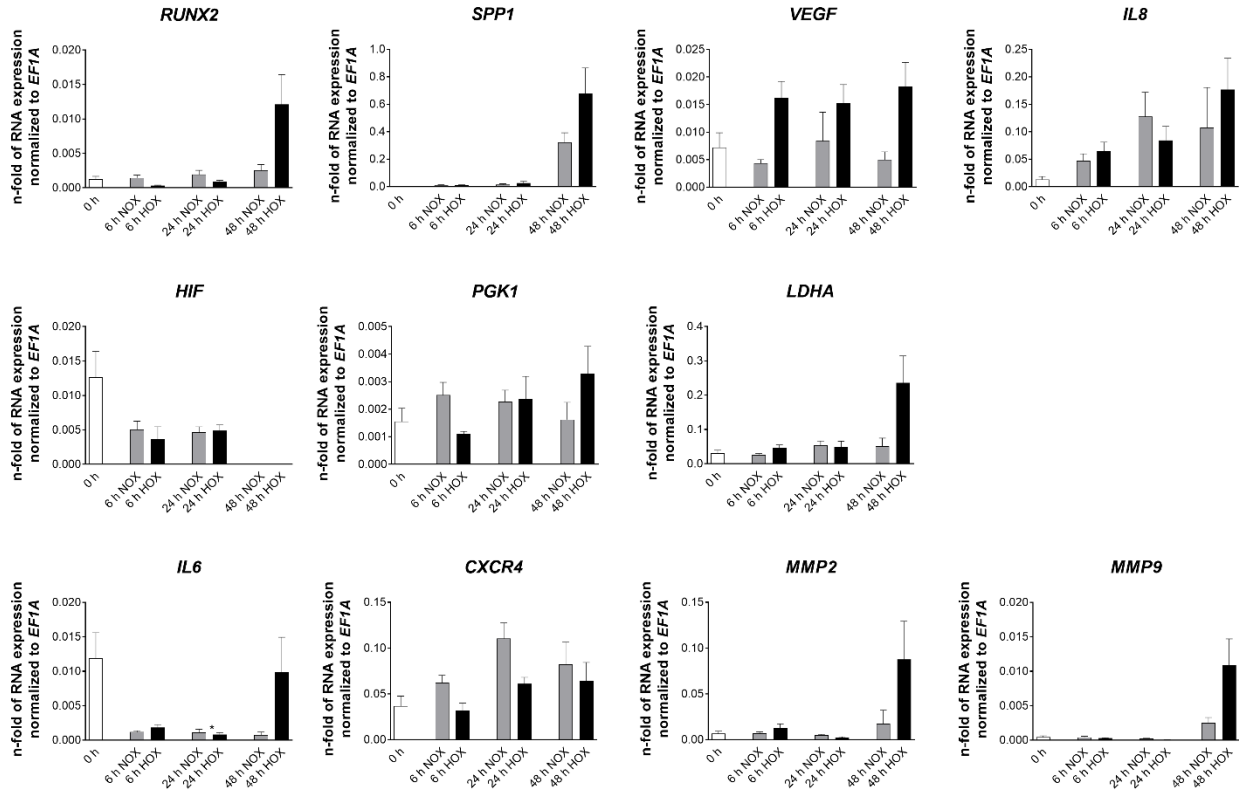


Fig. S4: Time-dependent RNA expression of FH models incubated for 6, 12, 24 or 48 h under either normoxic (NOX) or hypoxic conditions (HOX; n = 6)
The n-fold of the RNA (mean $\pm$ SEM) is depicted. RNA expression was normalized to the gene expression of *EF1A* (ΔCt).

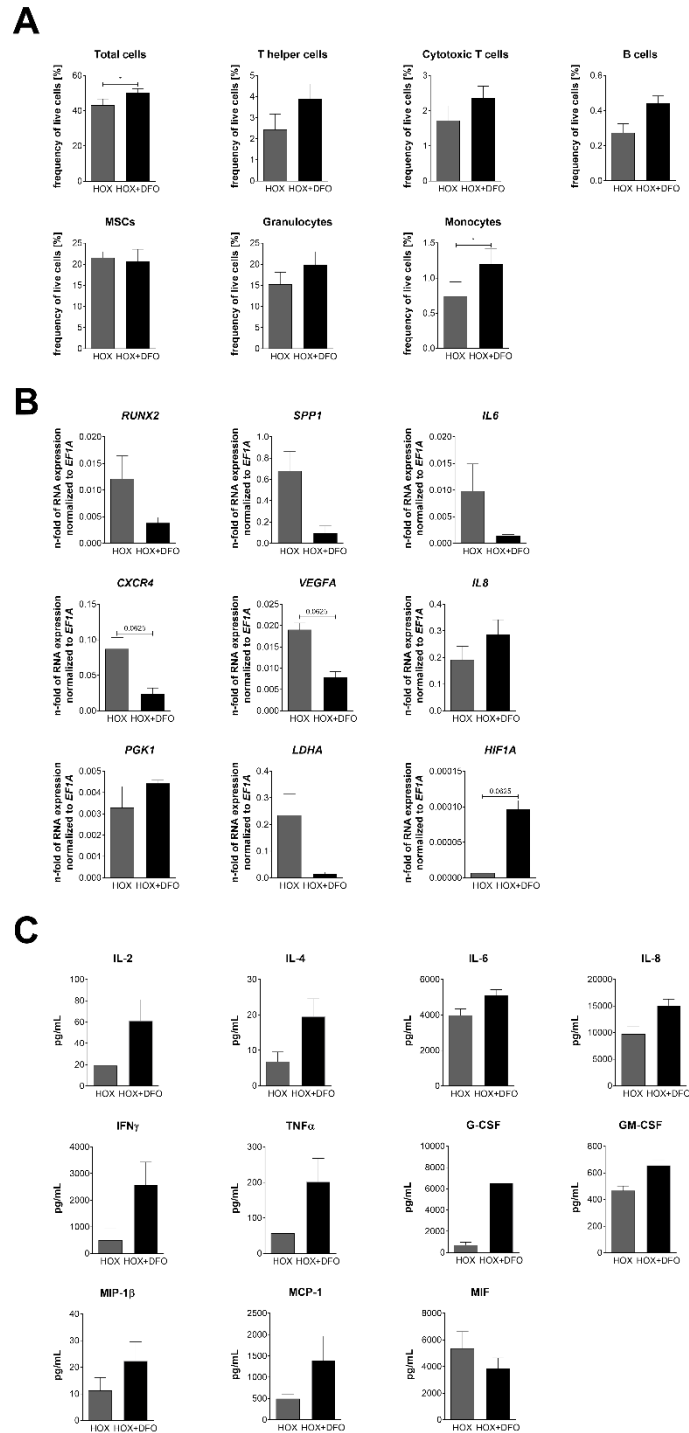


Fig. S5: *In vitro* FH models were incubated under hypoxia (HOX; grey bars) or hypoxia with supplementation of 250 μ M DFO (NOX; dark grey bars) for 48 h in osteogenic medium (A) Frequency of total cells and single cell populations negative for 7-AAD in the FH, (B) relative RNA-expression of relevant genes normalized to *EF1A*, (C) concentration of secreted protein (pg/mL) in the supernatant. Median $\pm$ range ($n = 6$) for the cell composition analysis and mean $\pm$ SEM ($n = 6$) for the RNA-expression and the secreted proteins are shown. Statistical analysis was conducted using the Wilcoxon signed rank test.

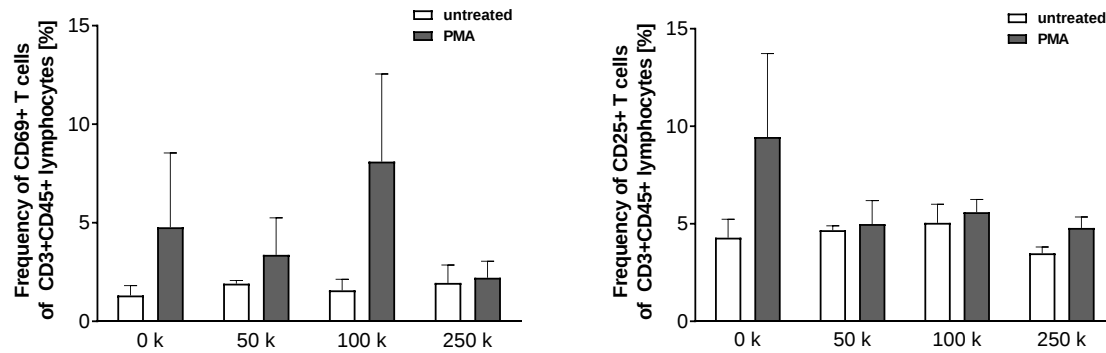


Fig. S6: Expression of early activation marker CD69 and late activation marker CD25 for CD45+CD3+ T cells after incubation of the FH models for 48 h in osteogenic induction medium as determined by flow cytometry (n = 3, mean ±SD)
 FH models were produced by combining either 0, 50,000, 100,000 or 250,000 human allogenic MSCs and 100 µL whole blood and subsequent coagulation of the combined cell mixtures. FH models were treated with PMA (50 ng/mL) or remained untreated.

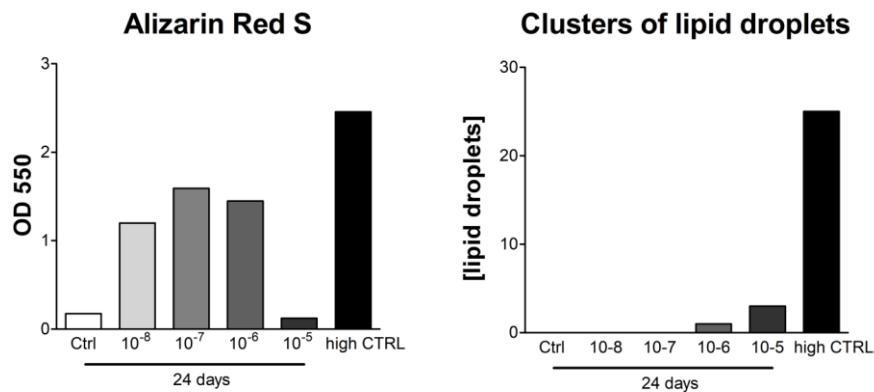


Fig. S7: OD550 values and count of lipid droplet clusters after 24 days of differentiation of monolayer MSCs in either OD or AD (pilot experiment for n = 1)
 Here 10⁴ MSCs per well were plated in a 96-well plate and incubated for up to 24 days in the respective induction medium. As a high control, MSCs were incubated with STEMMac Osteo Diff or STEMMac Adipo Diff medium respectively for 1 week. Osteogenic differentiation was measured at 550 nm using an ELISA plate reader. Adipogenic differentiation was analyzed microscopically.