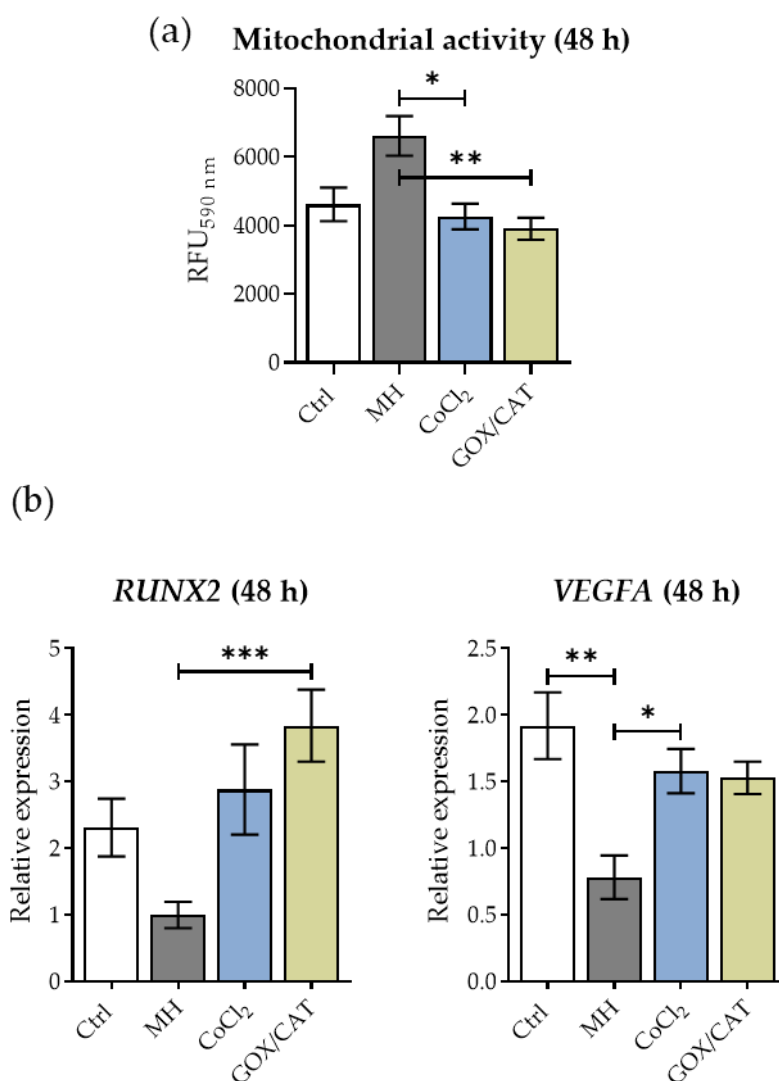


The art of inducing hypoxia

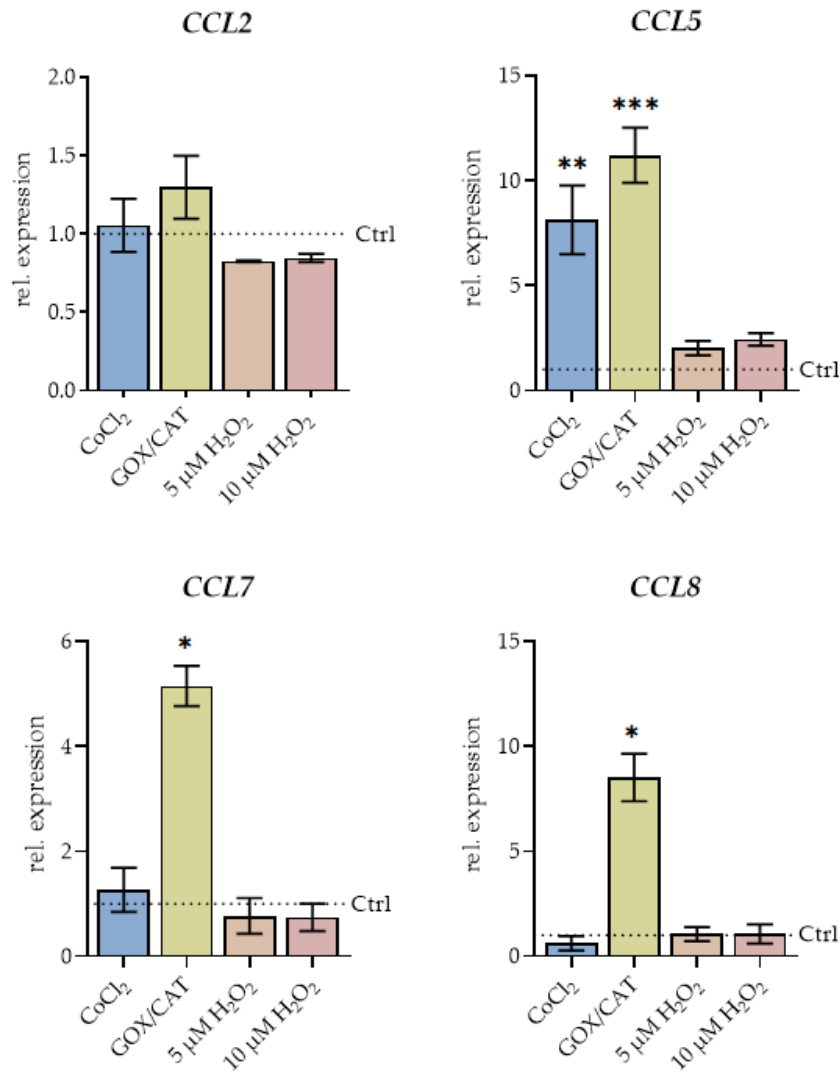
Helen Rinderknecht ¹, Sabrina Ehnert ^{1*}, Bianca Braun¹, Tina Histing ¹, Andreas K. Nussler ¹ and Caren Linne-mann ¹

¹ Siegfried Weller Research Institute, BG Trauma Center Tübingen, Department of Trauma and Reconstructive Surgery, University of Tübingen, Schnarrenbergstr. 95, D-72076 Tübingen, Germany

* Correspondence: Sabrina.ehnert@med.uni-tuebingen.de

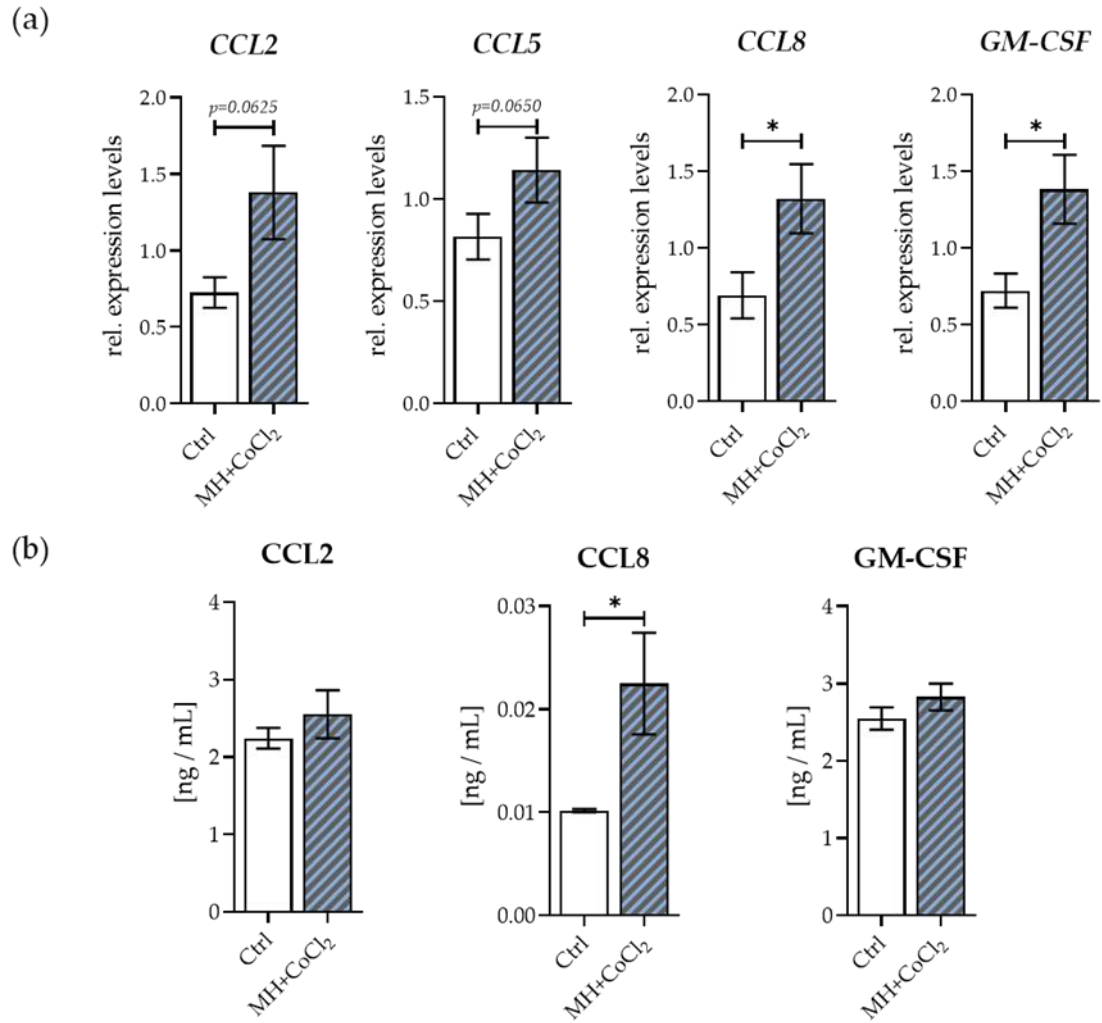


Supplementary Figure S1: 48 h measurements of *in vitro* fracture hematomas (mitochondrial activity, *RUNX2* and *VEGFA* expression). *In vitro* hematomas were cultivated in common aerobic conditions without additional stimulus (Ctrl), with increased MH, 0.4 mM CoCl₂ and the enzymatic GOX/CAT system for 48 h. (a) Mitochondrial activity after 48 h in *in vitro* fracture hematomas (b) Gene expression analysis for targets *VEGFA*, and *RUNX2* after 48 h in *in vitro* fracture hematomas (N=3, n≥3). Statistics were made using non-parametric Kruskal-Wallis Tests with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure S2: Cytokine expression of osteogenic cells in response to H₂O₂.

Osteogenic cells were cultivated in common aerobic conditions without additional stimulus (Ctrl), with 0.1 mM CoCl₂, enzymatic GOX/CAT system and 5/10 μM H₂O₂ for 24 h. Gene expression was analyzed for *CCL2*, *CCL5*, *CCL7* and *CCL8*. (N=3, n=3). Statistics were done by non-parametric Dunn's multi-ple comparison test comparing the Ctrl condition with each stimulus with * p≤0.05, ** p≤0.01, *** p<0.001. Data are shown as mean ± SEM.



Supplementary Figure S3: Chemokine expression and release of osteogenic cells by combination medium height and CoCl₂ to induce hypoxia. SCP-1 cells were cultivated in common aerobic conditions without additional stimulus (Ctrl) and with increased medium height for 24 h and afterwards for 2 h with 0.1 mM CoCl₂. (a) Gene expression analysis of *CCL2*, *CCL5*, *CCL8* and *GM-CSF* (N=3, n=3). (b) Release of cytokines *CCL2*, *CCL8* and *GM-CSF* was analyzed by ELISA (N=3, n=3). Statistics were done by non-parametric Whitney-U tests with * $p \leq 0.05$. Data are shown as mean \pm SEM.