

Signaling pathway STAT1 is strongly activated by IFN- β in the pathogenesis of osteoporosis

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Additional file 1

Claudine Seeliger^{1*§}, Lilianna Schyschka^{1*}, Zienab Kronbach², Angela Wottge¹, Martijn van Griensven¹, Britt Wildemann², Helen Vester¹

¹ Department of experimental Trauma Surgery, Klinikum rechts der Isar, Technical University Munich, Munich, Germany

² Berlin-Brandenburger Centrum für Regenerative Therapien, Julius Wolff Institut, Charité-Universitätsmedizin Berlin, Berlin, Germany

Corresponding author contact information:

Dr. rer. nat. Claudine Seeliger
Email: seeliger@uchir.me.tum.de

Additional figures:

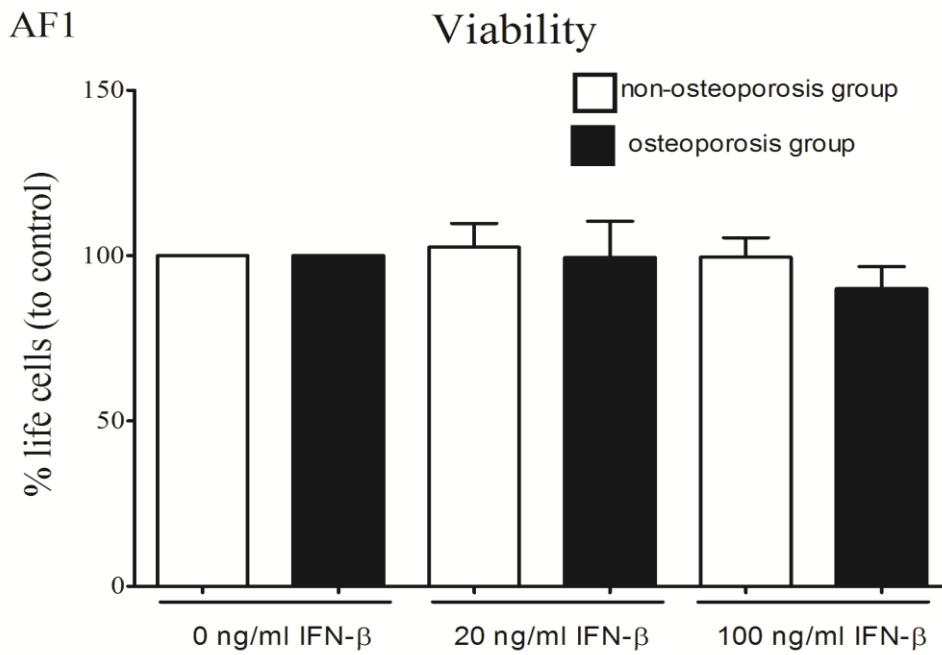


Figure AF1 - Viability of osteoblasts stimulated with IFN-β

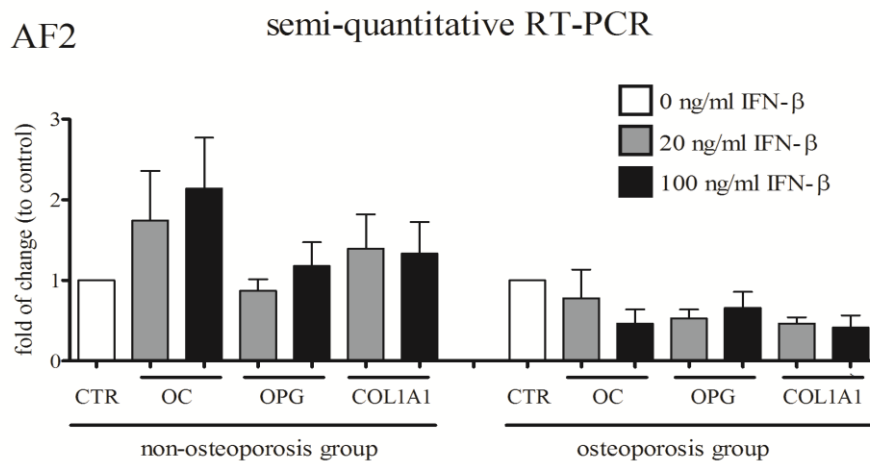


Figure AF2 - semi-quantitative RT-PCR results of stimulated osteoblasts osteocalcin (OC), osteoprotegerin (OPG) and collagen1A1 (COL1A1)

AF3

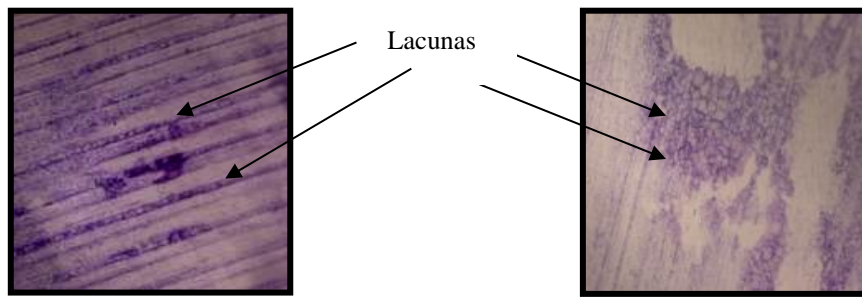


Figure AF3 - Toluidine blue staining of on dentin chips seeded and differentiated osteoclasts. Osteoclasts remove the bone matrixes and on the dentin chip resorption-lacunae get visible

AF4

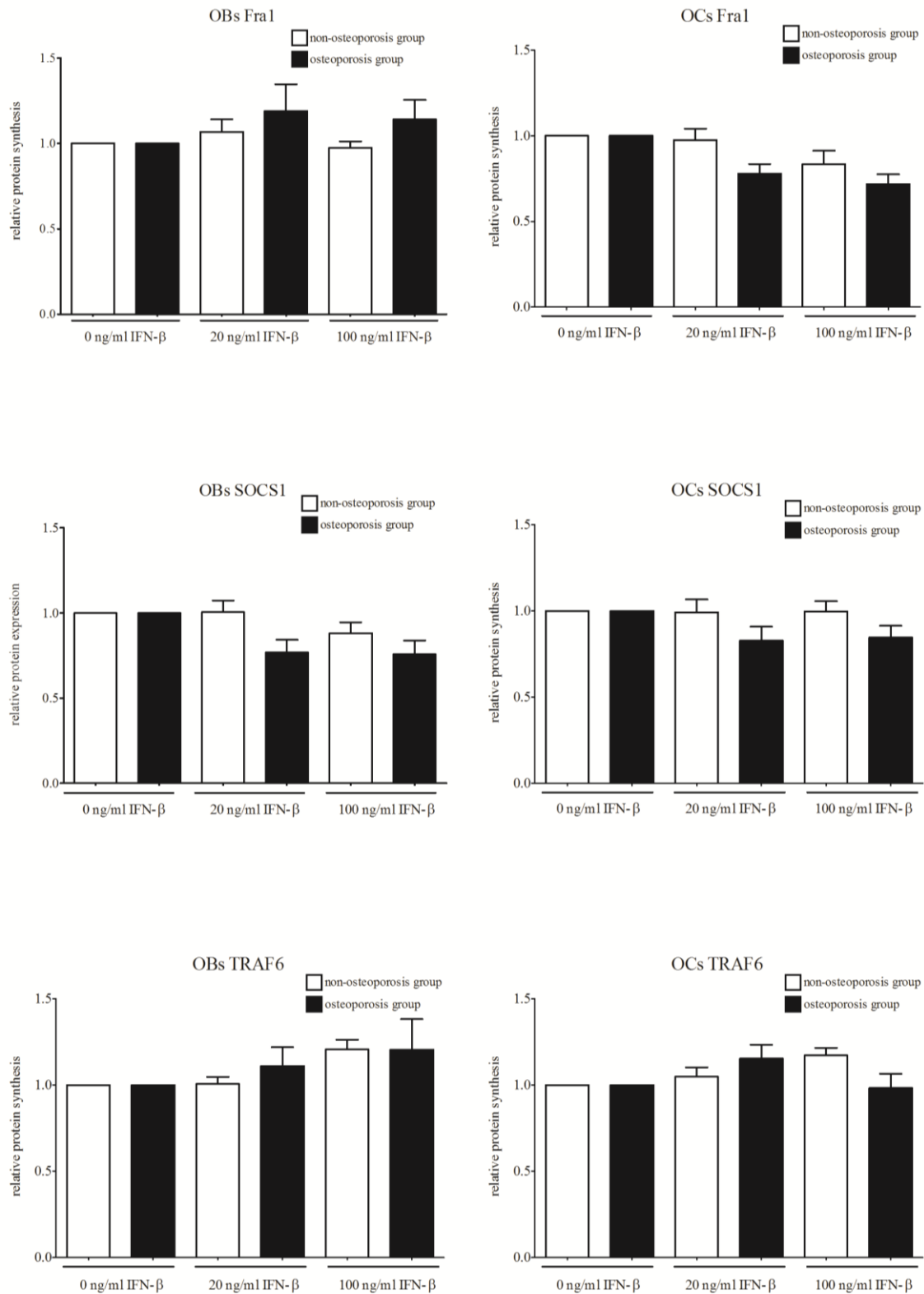


Figure AF4 - Protein levels of Fra1, SOCS1 and TRAF6 in osteoblasts and osteoclasts stimulated with IFN-β. fos-related antigen 1 (Fra1), suppressor of cytokine signaling 1 (SOCS1), TNF receptor-associated factor 6 (TRAF6), osteoblasts (OBs), osteoclasts (OCs)

Tables:

Table 1 - Demographical characteristics of the samples

	osteoporosis group	non-osteoporosis group
included patients (N)	12	11
Age (y), mean \pm SD	78.7 \pm 8.1	70.3 \pm 10.4
Body mass index, \pm SD	24.7 \pm 2.2	28.0 \pm 4.8
Gender (m:f)	4:8	6:5
main diagnosis (beside osteoporosis)	femoral neck fracture, coxarthrosis	femoral neck fracture, coxarthrosis
Bone density (mg/dl) \pm SD	75.1 \pm 12.3	217.2 \pm 27.6

Table 2 - Primer sequences and PCR conditions

osteocalcin (OC), osteoprotegerin (OPG) and collagen1A1 (COL1A1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Name	Accession No.	Forward Primer 5' \rightarrow 3'	Reverse Primer 5' \rightarrow 3'	T _m (°C)	Size (bp)
OC	NM_199173.3	CCAGCGGTGCAGAGTCCAGC	GACACCCTAGACCGGGCCGT	60	236
OPG	NM_002546.3	CCGGAAACAGTGAATCAACTC	AGGTTAGCATGTCCAATGTG	60	313
COL1A1	NM_000088.3	AGCGGACGCTAACCCCTCC	CAGACGGGACAGCACTCGCC	60	340
GAPDH	NM_002046.3	GTCAGTGGTGGACCTGACCT	AGGGGTCTACATGGCAACTG	60	420

Table 3 - Antibodies description and using conditions

fos-related antigen 1 (Fra1), suppressor of cytokine signaling 1 (SOCS1), signal transducer and activator of transcription 1 (STAT1), TNF receptor-associated factor 6 (TRAF6)

Name	Host species	Size (kDa)	Dilution	Company
β -actin	Mouse	43	1:1000	Merck Milipore
Fra1 (FOSL1)	Rabbit	38	1:1000	Sigma
SOCS1	Rabbit	38	1:1000	Sigma
p-STAT1	Rabbit	84/91	1:1000	Cell Signaling
STAT1	Rabbit	84/91	1:1000	Cell Signaling
TRAF6	Mouse	60	1:500	Santa Cruz