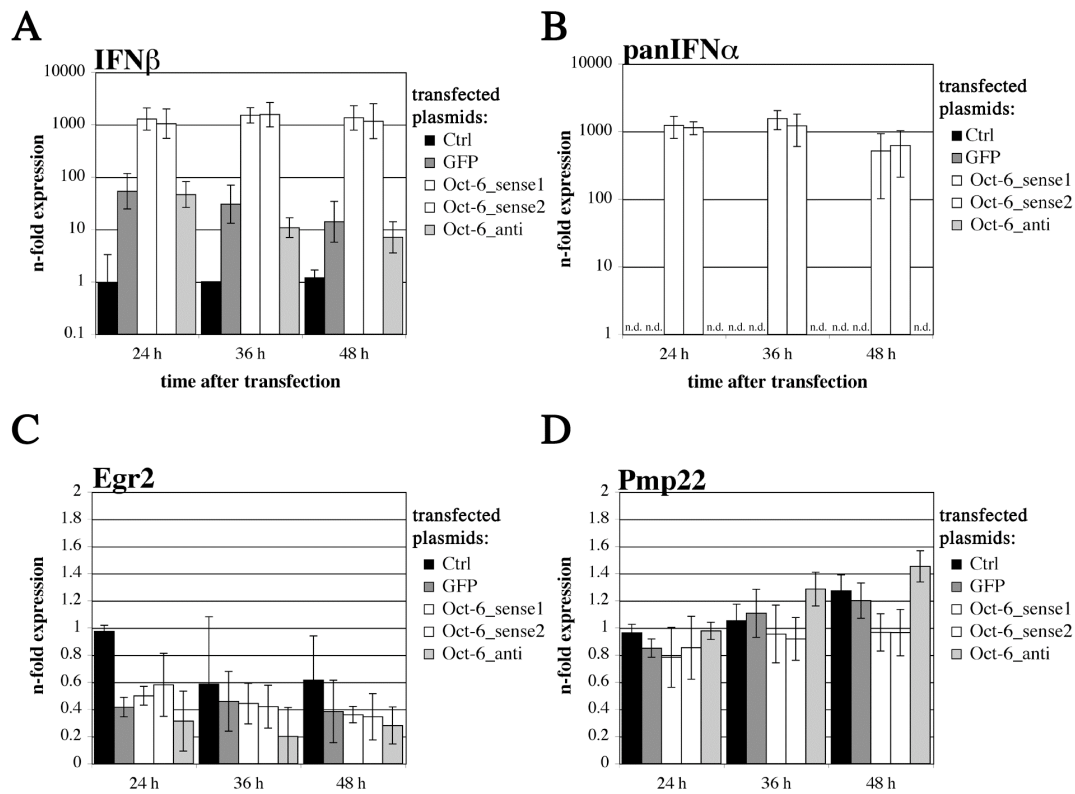




Additional file 4.

Overexpression of Oct-6 in pMEFs enhances the expression of IFN β and IFN α mRNAs, but does not influence the expression of Egr2 and Pmp22 mRNAs. MEFs were transfected with the following plasmids (10 μ g each): enhanced-GFP expression vector (GFP), two different Oct-6 expression plasmids (Oct-6_sense1/2), and a plasmid containing the Oct-6 cDNA in antisense direction (Oct-6_anti). As a control untransfected cells (Ctrl) were analysed. Expression levels of (A) IFN β , (B) panIFN α (all IFN α subtypes), (C) Egr2 and (D) Pmp22 mRNAs were determined 24 h, 36 h, and 48 h after transfection by RT-qPCR using Ube2d2 as endogenous control. (A, C, D) Data are depicted relative to the Ctrl at 24 h. (B) panIFN α mRNA could not be detected reliably in untreated cells (n.d.) and thus data normalised to the endogenous control are depicted (not additionally calibrated to untreated cells). Mean values $\pm$ SD of three independent experiments are shown.

Plasmids

The CMV enhancer-driven Oct-6 expression plasmid was described previously (based on pEVRFO plasmid (1), Oct-6_sense1). Sense and antisense sequence of the Oct-6 cDNA were cloned into the EF1 α -promoter driven expression vector pEFZeo (a kind gift from Pavel Kovarik, MFPL, University of Vienna (2); Oct-6_sense2 and Oct-6_anti). The Oct-6 coding sequence was PCR-amplified from genomic DNA with primers containing restriction sites for BamHI at the 5'ends of the forward (Oct6_BamHI-F: TACGGATCCCGCAGACGGAGCGAGGCG) and reverse (Oct6_BamHI-R: TAGGGATCCCGAACCAGTCCGCAGGGTCAC) primer under the following conditions: in 50 μ l final volume 300 nM primer (Invitrogen), 5% DMSO (Sigma), 200 μ M dNTPs (Fermentas), 5 U Pfu DNA polymerase, 1x Pfu buffer incl. MgSO₄ (2 mM final concentration; all MBI Fermentas) were used, running following PCR program: 95°C for 3 min, 35 cycles of 95°C for 30 sec, 67°C for 30 sec, 72°C for 2 min, and a final extension step of 72°C

for 7 min. The 1.4 kb insert was blunt-ligated into EcoRV digested pEFZeo. Sense and antisense insertion was checked by XhoI digestion. Correct amplification and cloning was confirmed by sequencing analysis (reference sequence accession number NM_011141.1). The enhanced GFP expression plasmid (GFP) was supplied with the transfection kit (Amaxa/ Lonza) as a positive control. Plasmids were purified using the Jetstar Plasmid Midiprep 2.0 Kit (Genomed). Plasmids were transfected into primary fibroblasts using the manufacturer's protocols (MEF1 nucleofector kit, Amaxa/Lonza)

References

- (1) **Meijer, D., A. Graus, and G. Grosveld.** 1992. Mapping the transactivation domain of the Oct-6 POU transcription factor. *Nucleic Acids Res* **20**:2241-7.
- 2) **Kovarik, P., M. Mangold, K. Ramsauer, H. Heidari, R. Steinborn, A. Zotter, D. E. Levy, M. Muller, and T. Decker.** 2001. Specificity of signaling by STAT1 depends on SH2 and C-terminal domains that regulate Ser727 phosphorylation, differentially affecting specific target gene expression. *EMBO J* **20**:91-100.