

SUPPLEMENTARY TABLES:

Table S1: Primers used for RT-PCR (this table is related to Figure S2).

Genes	Primer sequence (5'-3')
p27 ^{KIP1}	F: TCTCTTCGGCCCGGTCAAT R: GGGGCTTATGATTCTGAAAGTCG
p53	F: TGAAACGCCGACCTATCCTTA R: GGCACAAACACGAACCTCAA
p21 ^{CIP/WAF}	F: CTGAGGATGAACAGTAACAACCG R: CTGGGAAGATAGAGCGAAGCC
p16 ^{INK4a}	F: GCTGCAGACAGACTGGCCA R: GTCCTCGCAGTTCGAATCTG
FGF receptor 1 (FGF-R1)	F: GGTGCTTCATCTACGGAATGTC R: TGATGGGAGAGTCCGATAGAGT
GAPDH	F: ACGACCCCTTCATTGACCTCAACT R: ATATTTCTCGTGGTTCACACCCAT

Table S2: Primer pairs used for ChIP analysis (this table is related to Figure 3).

Primers	Primer Sequence (5'-3')
D1	F: CTTCTCATCCCAAGTCCACA R: AGACTACCTGGGTAGGTAATTTGG
D2	F: GGCTGGAGTCATCAGAAAGC R: GGCCATAAGACAAAGGAGGA
P1	F: TGAAGAGATTGGGGGTATGG R: AGCCCCAACTGTGTCTGAAG
P2	F: CAGTTGGGGCTCATAATTGC R: AGAACACCTGCGTGGAAGAT
Dc	F: AGACACTGAAAATTATACTAA R: GTAACAATAATTGGCATCTTT

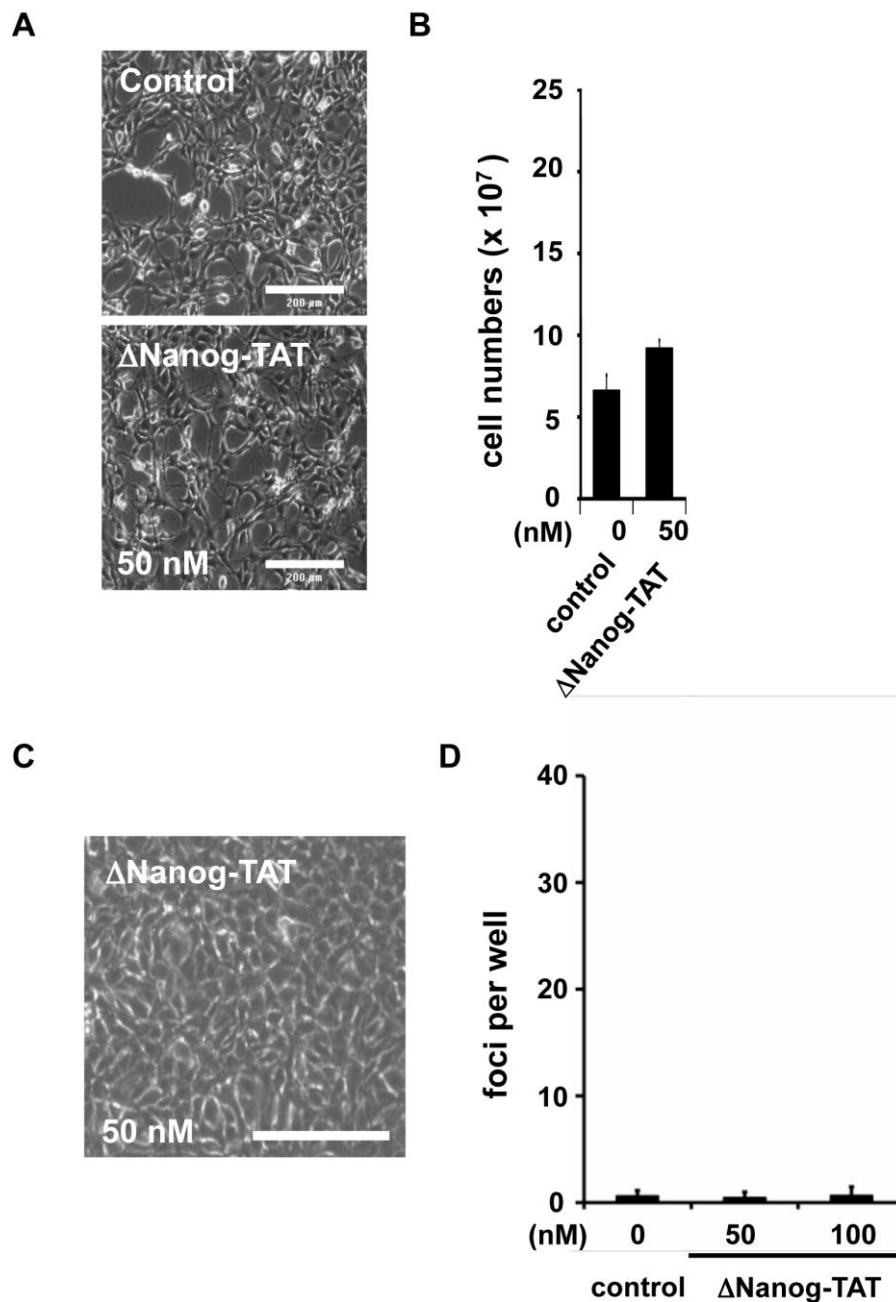


Figure S1: Δ Nanog-TAT fails to enhance proliferation or generate cell-foci in NIH3T3 cells.

The deletion of the 65 aa long homeobox domain should attenuate binding of the deletion mutant to the DNA, i.e. Δ Nanog-TAT should not exhibit functionality when compared to Nanog-TAT. (A,B) Effect of Δ Nanog-TAT on the proliferation of NIH 3T3 cells. To investigate the effects of Δ Nanog-TAT on the proliferation of NIH 3T3 cells, 7.5×10^4 cells were plated in 3.5 cm² cell culture dishes with both control medium and 50 nM of Δ Nanog-TAT recombinant protein for 10 days and were replated on day 3 and 7. Normal medium served as control. After 10 days, phase contrast images (A) were taken and the cumulative cell number was determined and shown in (B). Data are means \pm s.e.m. n = 3. Scale: 200 μ m. (C,D) NIH 3T3 cells could not form foci in the presence of Δ Nanog-TAT. (C) Phase contrast images of NIH 3T3 cells cultivated with 50 nM Δ Nanog-TAT for 10 days. (D) NIH 3T3 cells were cultivated with 50 and 100 nM concentrations of Δ Nanog-TAT and numbers of foci per well were determined after 8 days; normal medium served as control. Data are means \pm s.e.m. n = 3.

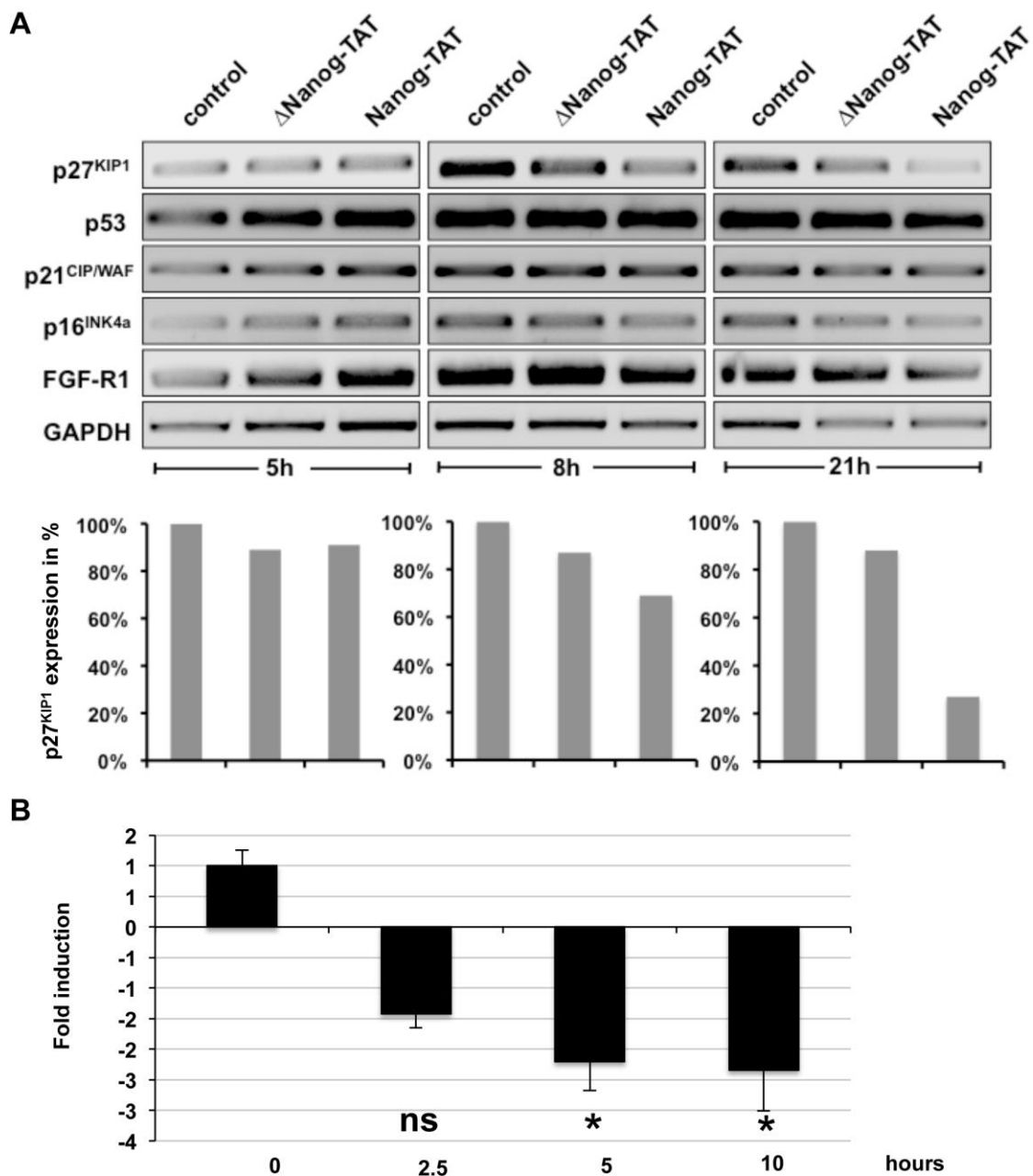


Figure S2: Nanog-TAT delivery results in decreased expression levels of p27^{KIP1}.

(A) MEFs were synchronized and cultivated with control medium, Δ Nanog-TAT and Nanog-TAT, respectively. After 5, 8 and 21h the fibroblasts were harvested and the expression levels of different key molecules involved in proliferation as well as cell cycle regulation were assessed via RT-PCR. No change in transcription levels is apparent for p53, p21^{CIP/WAF}, p16^{INK4a} and FGF-receptor 1. Modulation of p27^{KIP1} mRNA expression is apparent in Oct4-GiP MEFs upon cultivation with Nanog-TAT (top). The transcriptional expression level of p27^{KIP1} was quantified densitometrically (bottom). Results are normalized to GAPDH. FGF-R1: FGF receptor 1; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase. (B) Fold change expression levels of p27^{KIP1} transcripts by qPCR. Oct4-GiP MEFs were synchronized and subsequently cultivated with control medium or 100 nM Nanog-TAT. After 0, 2.5, 5 and 10h, fibroblasts were harvested and the expression levels of p27^{KIP1} were assessed via qPCR. Before harvesting fibroblasts, cells were washed with heparin to remove extracellularly bound Nanog-TAT protein. Expression levels of p27^{KIP1} were down-regulated after Nanog-TAT treatment compared to untreated fibroblasts. p27^{KIP1} gene expression levels are expressed as Log₂ fold changes based on Ct calculation using GAPDH as a house-keeping gene and non-transduced fibroblasts (0 hours) as a negative control. n = 2. Statistical analysis to calculate p-value was carried out using a two-tailed test; p-value: ns - not significant; * p < 0.05.