



Supplementary Figure S2.

The requirement of MdmX for full Mdm2 mRNA levels after NCS treatment does not depend on the p53: Mdm2 interaction.

MCF7 cells were transfected with control (Luc) or MdmX siRNA (50 nM) for 48 hr prior to treatment with NCS (300 ng/mL) for 4 hr. In the experiment with Nutlin + NCS (Nut + NCS) Nutlin-3 (10 μ M) was added for 12 hr at 36 hr after siRNA transfection followed by co-treatment with Nutlin-3 (10 μ M) and NCS (300 ng/mL) together for 4 hr. Nutlin-3 alone (10 μ M) was administered for total of 16 hr in two applications, first for 12 hr followed by an additional 4 hr, 36 hr after transfection with siRNA. **A.** Panels show levels of Mdm2 (left) and p21 (right) mRNAs in the different conditions as measured by qRT-PCR. **B.** Immunoblot analysis using antibodies against MdmX, Mdm2, p53, p21 and actin as indicated. **C.** Co-immunoprecipitation experiment was performed with lysates prepared from MCF7 cells that were transfected with control (Luc) or MdmX siRNA (50 nM) for 48 hr followed by NCS treatment (300 ng/mL) for 90 min. p53 was immunoprecipitated with monoclonal antibody 1801. Input and immunoprecipitated (p53 IP) samples were analyzed by immunoblotting using antibodies against Mdm2, MdmX, p53 and actin.