Supplementary Information

Supplementary Methods

Proliferation Assay and FACS Analysis

For the proliferation assays, 2000-2500 cells were seeded in triplicate in 96-wells plates; treatments were started 24 hours after seeding. Cells were incubated with WST-1 reagent (Roche) for 1-4 hours and absorbance (450 nm) was measured in a microplate reader (Victor3 Multilabel Counter 1420-042, Perkin-Elmer).

For the FACS analysis, 100,000 cells were seeded in 6-cm dishes 24 hours before the mock or Nutlin-3 treatments. Cells and media were harvested after 48 hours, fixed and analysed on a flow cytometer (Beckton Dickinson, Franklin Lakes, NJ USA) using FACS DIVA software after incubation in PBS with 50 µg/ml propidium-iodide (PI) and 50 µg/ml RNase.

P53 sequencing of soft tissue sarcoma samples

Genomic DNA was isolated from fresh frozen tissue sections. One section of each tumor sample was stained with H&E in order to confirm that the majority of the tissue was comprised of tumor cells. We used PCR to amplify exons 4 to 10 of the p53 gene. The PCR products were analyzed by direct sequencing in both sense and antisense direction using the BigDye Terminator Cycle Sequencing 3.1 Kit (Applied Biosystems, Darmstadt, Germany). The sequencing reactions have been carried out according to the manufacturer's instructions. Primers sequences: Exon 4 fw: 5'-ATCTACAGTCCCCCTTGCCG-3';
Exon 4 rev: 5'-GCAACTGACCGTGCAAGTCA-3'; Exon 5 fw: 5'-TTCCTCTTCCTACAGTACTC-3'; Exon 5 rev: 5'-GCAAATTTCCTTCCACTCGG-3'; Exon 6 fw: 5'-ACCATGAGCGCTGCTCAGAT-3'; Exon 6 rev: 5'-AGTTGCAAACCAGACCTCAG-3'; Exon 7 fw: 5'-GTGTTATCTCCTAGGTTGGC-3' Exon 7 rev: 5'-CAAGTGGCTCCTGACCTGGA-3'; Exon 8/9 fw: 5'-CCTATCCTGAGTAGTGGTAA-3'; Exon 8/9 rev: 5'-CCAAGACTTAGTACCTGAAG-3'; Exon 10 fw: 5'-

GATGTTGCTTTTGATCCGTCATT-3'; Exon 10 rev: 5'-TCCTATGGCTTTCCAACCTAGG-3'.

Supplementary Legends

Supplementary Figure S1. Nutlin-3 response in a set of osteosarcoma cell lines.

Fig. S1A Osteosarcoma cell lines were tested for Nutlin-3 responsiveness by treating the cells for 0, 8 or 24 hours with 10 μM Nutlin-3, after which protein extracts were made and analysed with immunoblotting using antibodies for Hdm2, p53 and USP7. **Fig. S1B** Indicated osteosarcoma cell lines were mock- or Nutlin-3 (10 μM) treated for 24 hours; survival was measured 72 hours later and here displayed as relative survival compared to the untreated cells.**Fig. S1C** FACS analysis of mock- or Nutlin-3 (10 μM, 24 hours) treated KPD, U2OS and OSA cells. Indicated are percentages of cycling cells in G1, S or G2/M phase and percentages of single cells in SubG1.

Supplementary Figure S2. Analysis of *HDMX-S* mRNA expression in the NCI60 cell panel.

Fig. S2A Cell lines were examined for *HDMX-FL* and *HDMX-S* mRNA expression using primers for HDMX exon 3 (Fw) to exon 8 (Rev). **Fig. S2B** A column graph depicting the number of cells with a given *HDMX-S/HDMX-FL* ratio.

Supplementary Figure S3. High *HDMX-S/HDMX-FL* ratios, but not p53 gene mutation associate with worse clinical outcome in 46 soft tissue sarcoma patients with known *HDMX-S/HDMX-FL* ratios and p53 status.

Fig. S3A A Kaplan-Meier plot that displays the overall survival for those patients whose tumours contained either wild-type or mutant p53 (n=46). The p-value depicted on the plot is derived from a log rank test. **Fig. S3B** A Kaplan-Meier plot that displays the overall survival for those patients whose tumours contained high, intermediate or low *HDMX-S/HDMX-FL* ratios (n=46). The first p-value depicted on the plot is derived from a log rank test comparing the patients with higher, equal and lower ratios. The second p-value is derived from a log rank test comparing the patients with higher ratios to those

with low ratios, as is depicted by the * symbol. **Fig. S3C** A Kaplan-Meier plot that displays the overall survival for those patients whose tumours contained high, intermediate or low *HDMX-S/HDMX-FL* ratios and wild-type p53 (n=36). The first p-value depicted on the plot is derived from a log rank test comparing the patients with higher, equal and lower ratios. The second p-value is derived from a log rank test comparing the patients with higher ratios to those with low ratios, as is depicted by the * symbol.

Supplementary Table S1. Clinicopathological data of 51 osteosarcoma patients

Supplementary Table S2. Clinical and histopathological characteristics of the soft tissue sarcoma patients.

Supplementary Table S3. Summary of p53 status, Hdm2 and Hdmx levels in 22 osteosarcoma cell lines.

Supplementary Table S4. Ratio of *HDMX-S and HDMX-FL* mRNA in 51 osteosarcomas.

Supplementary Table S5. Summary of the p53 status, the Hdm2 and Hdmx levels in 37 breast cancer cell lines.

Supplementary Table S6. Summary of the p53 status, the Hdm2 and Hdmx levels in the NCI60 cell panel.