Legends to Supplemental Figures and Tables

Figure S1:

(A)Correlation plots between the Area and the Height of ChIP-seq p53 peaks (upper left), Width and Area (upper right), Width and Height (down left) and the frequency distribution of the width of the peaks (down right). Coefficients of correlation are presented inside of the corresponding plots. (B) percentage of peaks with predicted p53 consensus site (p53Scan)

Figure S2:

The frequency of p53 peaks according to their position relative to TSS was assessed for 2432 peaks common for all three treatments.

Figure S3:

(A) Intersection of the ChIP-seq peaks common for nutlin3a, RITA, and 5FU treatments (stringency area 20) with mock-treated MCF7 cells: all peaks (left Venn diagram) and peaks within 10 kbp from TSS (right Venn diagram). (B) Scatter plot for Area and fold change of the size of the p53 peaks located within 10 kbp from TSS of the protein-coding genes, which were detected in mock-treated samples as well as in all treated samples, i.e. in nutlin3a, RITA and 5FU. Red-, green- and black coloured dots correspond to peaks located in genes, whose expression is induced, repressed or not changed upon nutlin3a treatment, respectively. Black circles around dots mark the peaks that contain p53 consensus motif as revealed by p53Scan program. See also Table S11.

Figure S4:

A. Unsupervised hierarchical clustering analysis of gene expression profiles of class I canonical genes (Fig 2G) obtained in MCF7 cells treated with nutlin3a for 0, 2, 4, 6, 8, 10 and 12 hours.

B. Expression heatmap of class I canonical genes (Fig 2G) upon stable p53-depletion from MCF7 cells[23].
C. Unsupervised hierarchical clustering analysis gene expression profiles of class II noncanonical genes (Fig 2G) obtained in of MCF7 cells treated with nutlin3a for 0, 2, 4, 6, 8, 10 and 12 hours.
D. Expression heatmap of class II noncanonical genes (Fig 2G) upon stable p53-depletion from MCF7 cells[23].

Figure S5:

A. Box plots show that the enrichment of p53 is higher in induced than in repressed genes.

B. Boxplot of the probabilities to find p53 binding sites in ChIP-seq p53 peaks for genes induced and repressed by nutlin3a, calculated by p53MH program. *p*-values were obtained using student t-test.

Figure S6:

Light microscope analysis (A) and a long term viability assay (crystal violet staining in B) demonstrated enhanced growth suppression by nutlin of the MCF7 cells with depleted STAT3 .

Figure S7:

Clinical importance of novel p53 target genes.

Cluster analysis for 214 upregulated genes (A) and 66 downregulated genes (B) in breast tumor samples is visualized as a heatmap. Patient tumor samples formed two major clusters C1 (in black) and C2 (in red). Mutation status of p53 (p53 mt) and the development of metastasis within 5 years (DM<5yr) are marked with black bars above the heatmap. Tumor grades are marked with green bars (Grade 1) and red bards (Grade 3). Light blue bars in the rows for "DM<5yr" correspond to missing data. *P* values are calculated by the chi-squared test. (C) The survival prognosis for 236

patients is classified according to clustering analysis and visualized using Kaplan-Meier plots (left panel for induced genes and right for repressed). *P* values were calculated by the likelihood-ratio test. (D) Expression profiles of the *AURKA* and *ATAD2* genes in the clusters C1 and C2 of tumor samples are visualized by boxplots.

Table S1-S4, related to Figure 1. p53 ChIP-seq peaks obtained from mock-treated, RITA, nutlin3a and 5FU treated MCF7 cells mapped to human genome.

Table S5, related to Figure 1: Known p53 target genes bound by p53 within +/- 10kbp of TSS upon all three treatments (46 genes, pre-filtered)

Table S6, related to Figure 2: Known p53 target genes bound upon all three treatments within TSS and differentially expressed upon nultin3a (40 genes). Expression level after nutlin3a treatment: black - no changes; red - induced; green - repressed

Table S7, related to Figure 2: All known p53 target genes bound and differentially expressed upon nultin3a. Expression level after nutlin3a treatment: black - no changes; red - induced; green - repressed

Table S8, related to Figure 2: Known p53 target genes bound upon nutlin3a treatment, but not differentially expressed (39 genes, not pre-filtered). NA in the columns Log2FC and Adj.p.Val corresponds to the genes, which either not present on expression microarray chip or weakly expressed

Table S9, related to Figure 2: Known p53 target genes differentially expressed upon nutlin3a, but not bound by p53 (18 genes). Expression level after nutlin3a treatment: black - no changes; red - induced; green - repressed

Table S10, related to Figure 2. Functional categories of novel p53 target genes identified by ChIP-seq, which were differentially expressed after nutlin3a. Expression level after nutlin3a treatment: black - induced, green – repressed. Genes marked with a star were found also in previously published p53 ChIP-chip study (Smeenk et al, 2008)

Table S11, related to Figure S3 and S4. List of class II noncanonical p53-bound and regulated genes.

Table S12, related to Figure 1. RITA- and nutlin3a-induced expression of genes bound by p53 only upon RITA.

Table S13, related to Figure 3. List of the primers used for RT-PCR, ChIP-PCR and cloning experiments.