Supplemental Material to:

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The DNA binding and accumulation of p53 from breast cancer cell lines and the link with serine 15 phosphorylation

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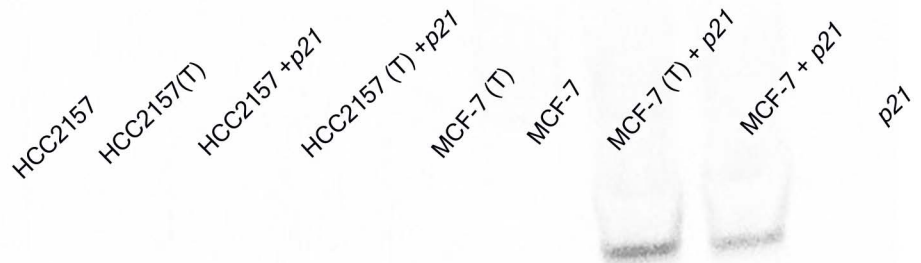
HCC2157
HCC2157(T)
HCC2157 +p21
HCC2157 (T) +p21
MCF-7 (T)
MCF-7
MCF-7 (T) + p21
MCF-7 + p21
p21

Supplemental Figure 1. EMSA analysis of DNA binding to p53 responsive sequences

Aliquots of nuclear extracts from MCF-7 cells or HCC2157 cells with or without treatment with 0.2 mM H_2O_2 for 3 hours (H_2O_2) containing 50 pg of the p53 protein were reacted with 20 pmoles of biotinylated gene regulatory sequences (marked at the top of each lane) and DNA binding performed by gel shift as described in Materials and Methods. The reaction mixtures were loaded on a 5% TBE gel along with no DNA and DNA only controls, transferred to a nylon membrane and probed with streptavidin conjugated to alkaline phosphatase to determine the shift in DNA band. The unbound DNA was allowed to run off the gel. The figure is representative of repeats from 3 individual gel shift experiments.

Supplementary Table 1

	Genes from which regulatory sequences were used											
Cell Line	***bax***	***cdc25C***	***cyclinG***	***fas***	***kai1***	***mdm-2***	***mdr1***	***noxa***	***p21***	***pcna***	***puma***	***survivin***
MCF-7	11+/-4	14+/-5	43+/-2	38+/-6	9+/-3	0+/-0	4+/-4	14+/-2	34+/-3	44+/-3	22+/-5	47+/-1
MCF-7 (T)	46+/-1	45+/-4	46+/-6	62+/-6	27+/-2	43+/-3	18+/-2	56+/-7	83+/-5	58+/-3	10+/-2	76+/-4
ZR-75-1	57+/-4	52+/-1	53+/-5	68+/-5	43+/-6	0+/-0	41+/-5	52+/-4	50+/-3	57+/-2	25+/-5	47+/-3
ZR-75-1(T)	55+/-2	5+/-2	50+/-4	65+/-5	37+/-5	0+/-0	37+/-5	50+/-2	22+/-2	54+/-4	20+/-5	20+/-4
HCC1395	2+/-2	0+/-0	5+/-1	3+/-0	0+/-0	0+/-0	3+/-3	1+/-1	0+/-0	2+/-1	4+/-3	4+/-0
SK-BR-3	5+/-1	2+/-1	2+/-1	2+/-2	1+/-1	0+/-0	5+/-2	3+/-2	2+/-0	7+/-2	10+/-0.4	2+/-1
T-47D	4.0+/-0.2	0+/-0	4+/-1	12+/-1	9+/-1	0+/-0	8+/-7	2+/-2	0+/-0	14+/-2	4+/-4	0+/-0
HCC2157	2+/-2	0+/-0	2+/-1	0+/-0	0+/-0	0+/-0	3+/-3	1+/-1	0+/-0	2+/-1	4+/-2	0+/-0
HCC70	7+/-1	2+/-0	0+/-0	0+/-0	3+/-3	0+/-0	0+/-0	0+/-0	2+/-2	9+/-4	0+/-0	6+/-1
MDA-MB-468	4+/-2	2+/-1	2.0+/-0.3	4+/-1	4+/-1	0+/-0	0+/-0	0+/-0	1+/-1	0+/-0	0+/-0	3+/-1

Percentage of p53 in the bound fraction. Individual streptavidin magnetic bead assays were performed with p53 from nuclear extracts of the indicated breast cancer cell lines using the biotinylated gene regulatory sequences indicated (sequences used presented in Table 2). The nuclear extracts were isolated from 4 cell lines (MCF-7, HCC2157, MDA-MB-468 and T-47D) following treatment with H_2O_2, but only in the case of the MCF-7 cell extracts (MCF-7 (T)) was DNA binding by the p53 detected. The values given are the percent of p53 in the bound fraction and are the average of three or more independent reactions. The binding to *bax*, *cdc25C, fas*, *mdm-2, noxa*, *p21*, and *survivin* gene regulatory sequences was significantly different ($p<0.05$) between untreated and treated MCF-7 cell extracts. The binding to *p21, cdc25C* and *survivin* gene regulatory sequences was significantly different ($p<0.05$) between untreated and treated ZR-75-1 cell extracts. The difference in wild-type p53 binding to *bax*, *cdc25C, fas, kai1*, *mdr1* and *noxa* regulatory sequences was determined to be significant at $p<0.05$ between the p53 from ZR-75-1 and untreated MCF-7 cells. The binding to *fas* and *pcna* regulatory sequences was significantly different ($p<0.05$) between the extracts from T-47D and the MCF-7 (treated or untreated) or the ZR-75-1 cells.

Supplementary Table 2:

Cell Lines	Nuclear p53 level (pg p53/ug total protein)	Total p53 (ng) in nuclear fraction	Cytoplasmic p53 level (pg p53/ug total protein)	Total p53 (ng) in cytoplasmic fraction
MCF-7	2+/-0	0.9+/-0.1	1+/-0	0.9+/-0.2
MCF-7(T)	6+/-1	3.4+/-0.2	1+/-0	1.9+/-0.4
ZR-75-1	1.4+/-0	0.9+/-0.1	0.05+/-0	0.20+/-0.04
ZR-75-1 (T)	3+/-0.2	2.1+/-0.2	0.4+/-0	0.6+/-0.05
HCC1395	35+/-4	7.6+/-0.2	1+/-0	2.5+/-0.5
SK-BR-3	8+/-2	3.8+/-0.2	0.2+/-0	0.30+/-0.01
T-47D	5+/-1	4.0+/-0.3	2+/-0.4	5.6+/-0.7
T-47D(T)	7+/-1	8.3+/-0.4	2+/-0	8.8+/-0.3
HCC2157	15+/-2	8.5+/-0.7	18+/-2	7.9+/-1
HCC2157(T)	84+/-2	8.1+/-0.4	29+/-2	5.8+/-0.8
HCC70	10+/-2	5.3+/-0.1	1+/-0	0.3+/-0.02
MDA-MB-468	14+/-1	6.3+/-0.4	1+/-0	1.7+/-0.4
MDA-MB-468(T)	14+/-1	5.5+/-0.2	1+/-0	1.8+/-0.4

Accumulation of nuclear and cytoplasmic p53 protein in the breast cancer cell lines. The p53 concentrations in individual fractions for each cell line were estimated using ELISA and the total protein determined by BCA assay. The total p53 in the nuclear or cytoplasmic fractions was determined for the cells in a T75 flask. Extracts from some of the cell lines treated with H_2O_2 for 3 hours (T) were also examined. The changes in p53 levels upon H_2O_2 treatment in MCF-7, ZR-75-1 and HCC2157 cells were significant at $p<0.05$.The changes in nuclear localization (total nuclear p53) of p53 from MCF-7 cells between treated and untreated cells was significant at $p<0.05$.