Interleukin-6 increases matrix metalloproteinase-14 (MMP-14) levels via down-regulation of p53 to drive cancer progression

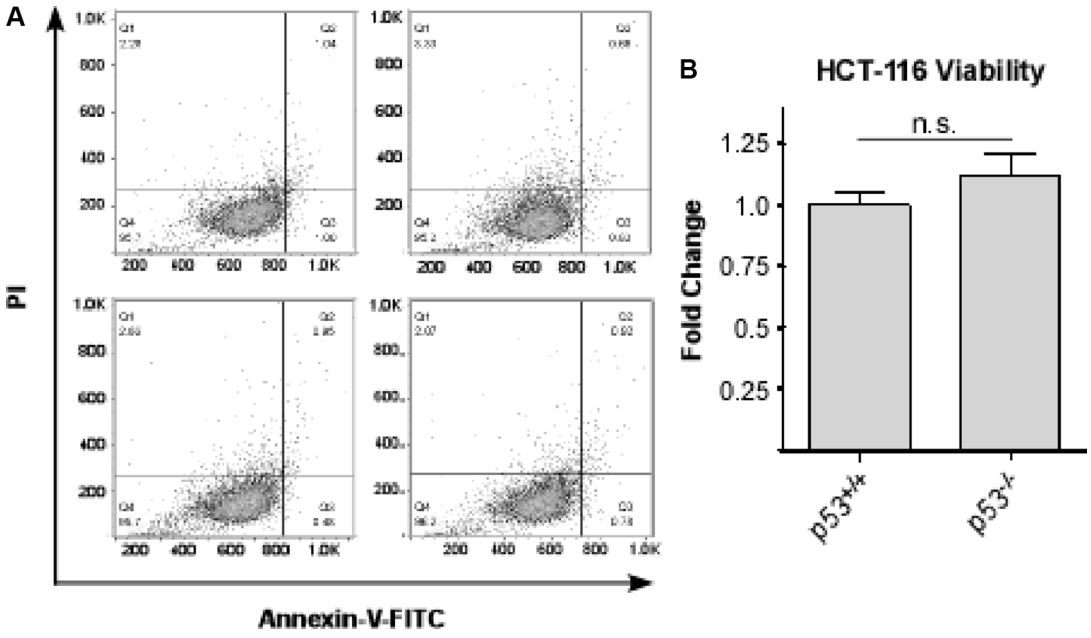
Supplementary Materials

Supplementary Table S1: Primers used for cloning, site-directed mutagenesis, and real time PCR

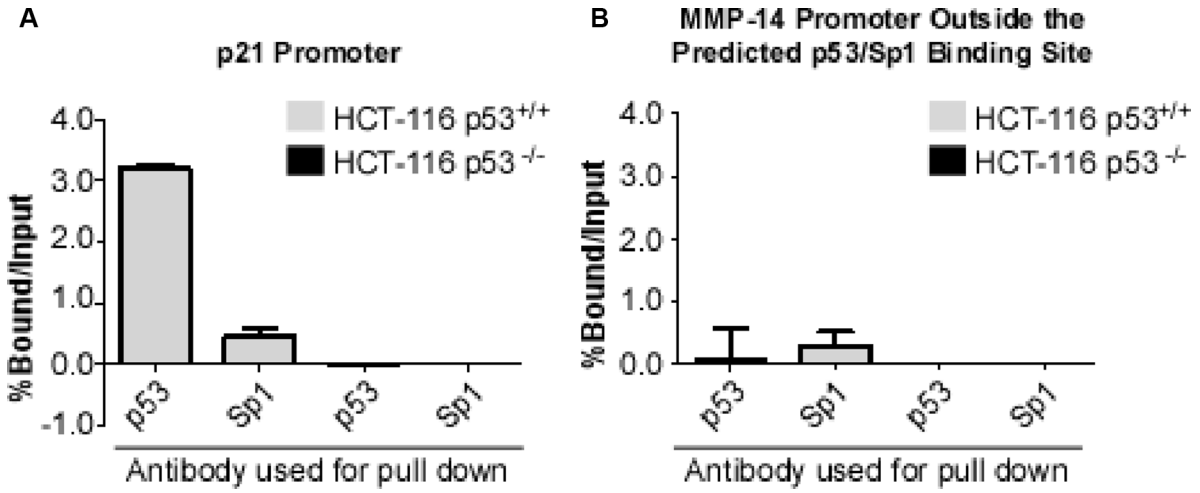
Primer Description	Forward (5′ > 3′)	Reverse (5′ > 3′)
Cloning of 1.2 Kb Promoter from HT-1080 Cells (A)	ATGGTACCAACATGGTGAAACCCTGTCC	ATCCCGGGTTTAACTCCAAGCCG ACAGC
Truncation B	ATGGTACCCTCCCACACTTTTCCTGC	ATCCCGGGTTTAACTCCAAGCCG ACAGC
Truncation C	ATGGTACCCAGATCCCACGGCCTTG	ATCCCGGGTTTAACTCCAAGCCG ACAGC
Truncation D	ATGGTACCGACCCCACCACCATCCCACAC	ATCCCGGGTTTAACTCCAAGCCG ACAGC
Deletion 1 to Truncation D (D1)	ACATAGCCCCCAATAATTC	GGTACCTATCGATAGAGAAAAG
D2	AATACCAGAGGAATCAAGC	AGCATTTGTCTCACCTCAG
D3	CCCTGGATCCCCCTACAG	CAGCATTTGTCTCACCTCAG
D4	ATGGCAGCCTGCACCACA	GACGGGATGTGGGAGACTTTG
D5	TTTTTTTCCTTCCAGTTCTTGGTTGTAATTGG	CTGCAGGGGTTGAGGCCG
D6	ACGTCCCCAACCAGGAAA	AAAAAAACACCTCTAAGTTGCC
D7	TGTGGGAGAAGGGAGGGA	GGTTGTTTTAGCCTGAATCCAATT AC
D8	AGAGAGGGAACCAGACCCC	GCCTCTCCTCCGTCCCCG
Real Time Primers for HPRT-1	ACCCCACGAAGTGTTGGATA	AAGCAGATGGCCACAGAACT
Real Time Primers for MMP-14	GAGCTCAGGGCAGTGGATAG	GGTAGCCCGGTTCTACCTTC
Real Time Primers for p53	ATCTTGGGCCTGTGTTATCTCC	TCCAGTGTGATGATGGTGAGG

Supplementary Table S2: Primers used for chromatin immunoprecipitation (ChIP)

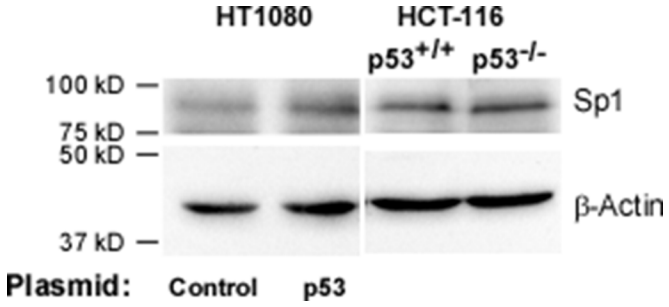
Primer Description	Forward (5′ > 3′)	Reverse (5′ > 3′)	Amplicon Length (bp)
Amplifying the MMP-14 promoter region in which p53 and Sp1 are predicted to bind	CCTTCCAGTTCTTGGTTGTAATTGG	CTCCCACAGCCTCTCCTCC	101
Amplifying the MMP-14 promoter region in an area where p53 and Sp1 are not predicted to bind	ACCCTGAGGTGAGACAAATGC	GACGGGATGTGGGAGACTTTG	86
Amplifying p53 predicted binding site in p21 promoter	GAGGTCAGCTGCGTTAGAGG	CATCTCAGGCTGCTCAGAGT	75



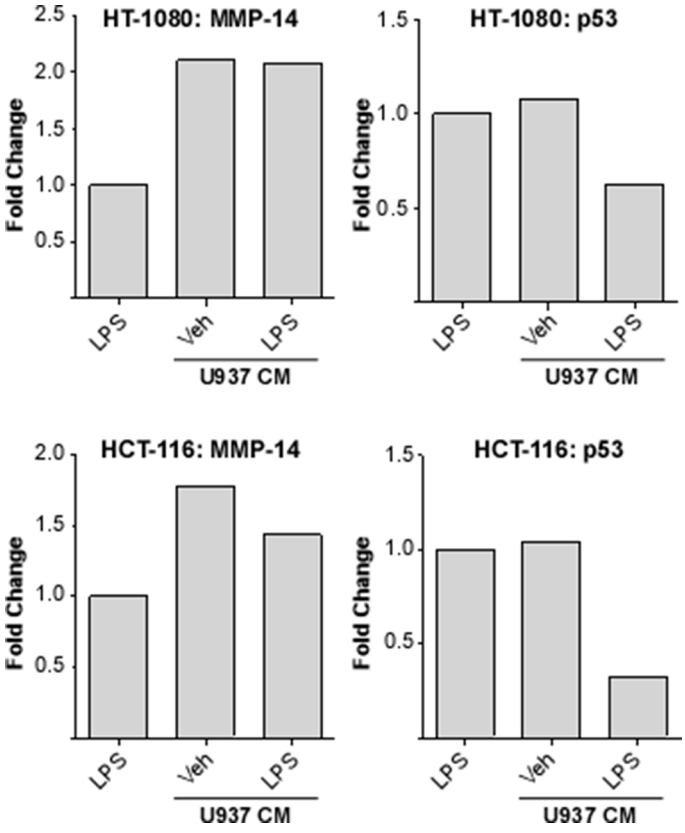
Supplementary Figure S1: The observed phenotypic changes are not a result in differences in cell viability. (**A**) HT-1080 cells were transiently transfected to overexpress p53 or vector control. After 24 or 48 hours post-transfection cells were stained with Annexin V-FITC and propidium iodide (PI) and subjected to flow cytometric analysis. No differences in apoptosis were observed.(**B**) Differences in viability of $p53^{+/+}$ and $p53^{-/-}$ HCT-116 cells was measured using MTT assay. No significant differences in cell viability were observed.



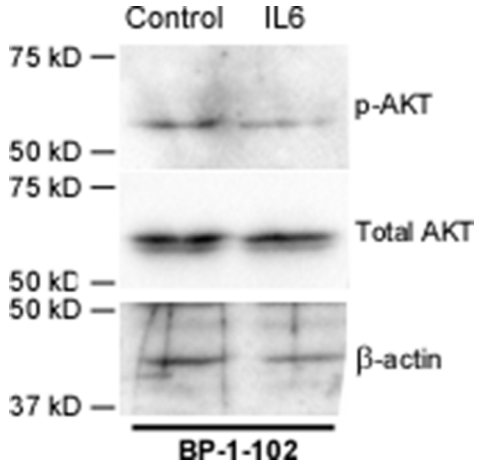
Supplementary Figure S2: Validation of ChIP assay. (**A**) p53 binds to the p21 promoter in HCT-116 WT cells but not in the p53 null cells. There is minimal binding of Sp1 to the p21 promoter in either strain. (**B**) There is minimal binding of p53 or Sp1 to the MMP-14 promoter in a region outside the predicted binding site.



Supplementary Figure S3: Sp1 levels are consistent in both HT-1080 and HCT-116 cells regardless of p53 status.



Supplementary Figure S4: Densitometric analysis of MMP-14 and p53 levels in HT-1080 cells (top) and HCT-116 cells (bottom) treated with LPS alone or with conditioned media collected from U937 macrophage cells stimulated with LPS to induce pro-inflammatory cytokines or vehicle control.



Supplementary Figure S5: Inhibition of p-Stat3 in HCT-116 $^{+/+}$ cells using 10 ug/mL BP1-102 for one hour prior to 15 minutes of IL-6 stimulation abrogates phosphorylation of AKT.