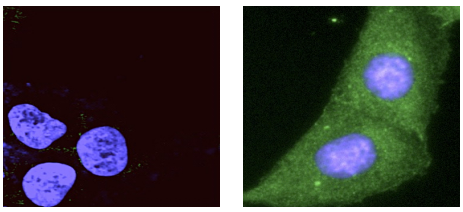
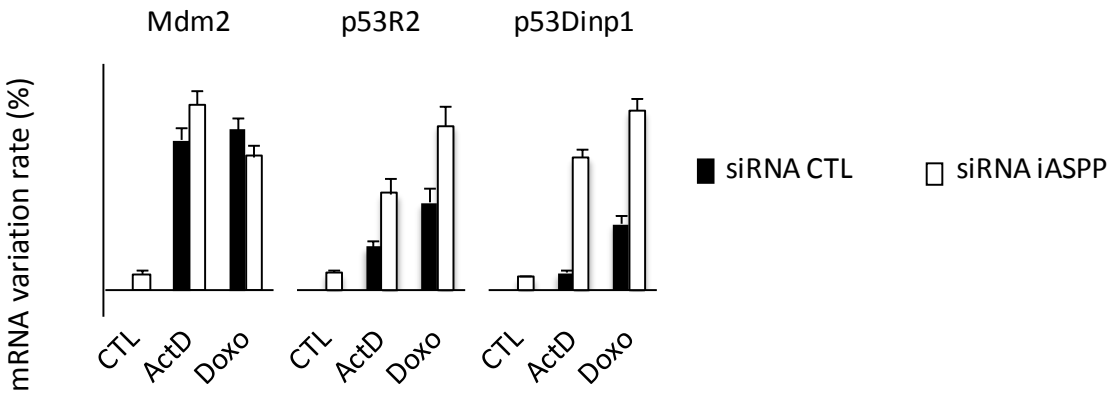
U2OS



U2OS
ASPP1

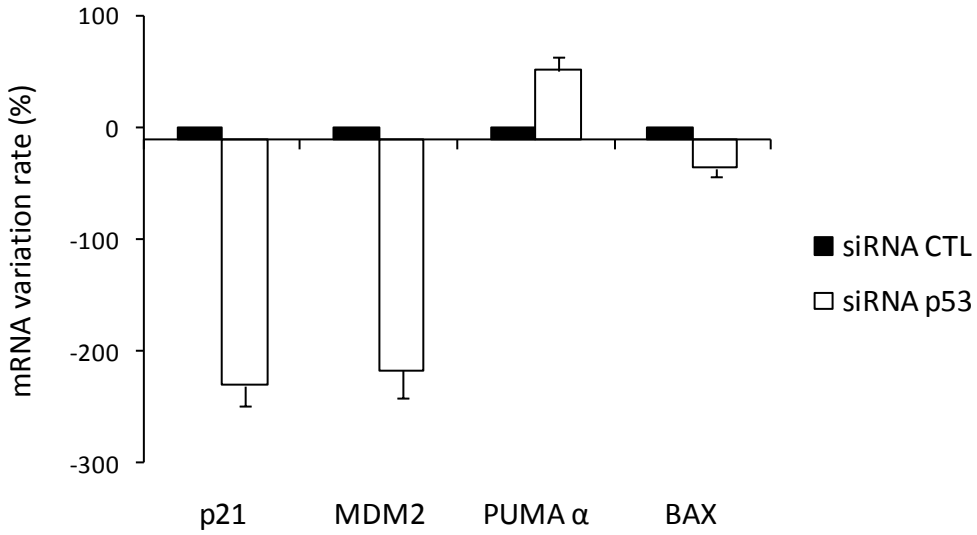
Supplementary Figure S1:

Expression and localization of ASPP1 in U2OS cells infected with a retroviral construct coding for ASPP1 or control, using an antibody directed against ASPP1. Nucleus is counterstained with DAPI.



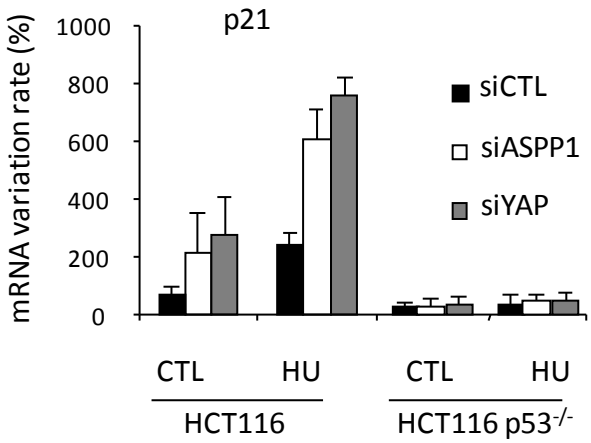
Supplementary Figure S2:

U2OS cells were transfected with control siRNA or specific siRNA directed against iASPP. mRNA expression of indicated p53 target genes was determined by RT-QPCR using specific primers after incubation of these cells 24 hours with 5nM Actinomycin D (ActD) or 200ng/ml Doxorubicin (Doxo). The results were normalized against two different standard genes, and the graphs represent the mean of three independent experiments.



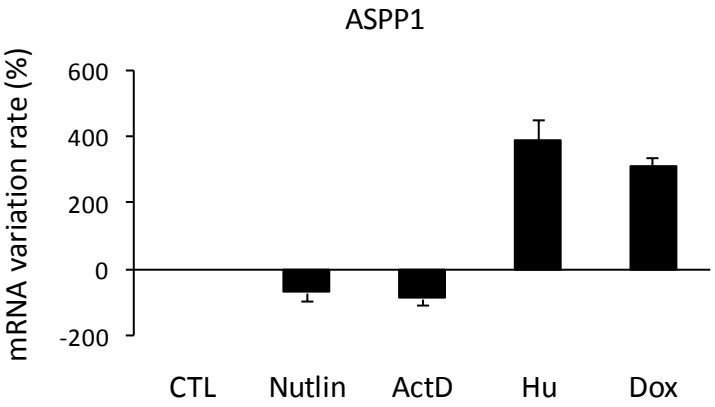
Supplementary Figure S3:

mRNA expression of different p53-target genes in HCT116 cells treated with control siRNA or specific siRNA targeting p53 mRNA. mRNA expression was quantified by RT-QPCR using specific primers. The results were normalized against two different standard genes, and the graphs represent the mean of three independent experiments.



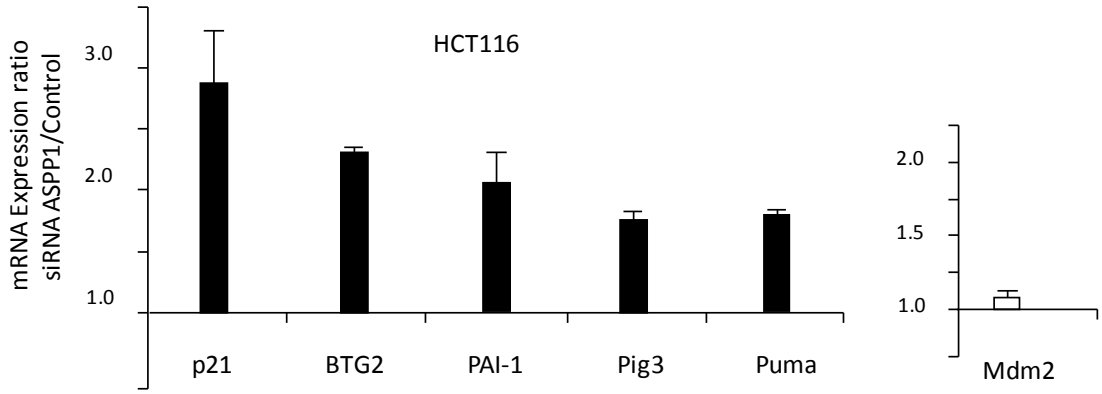
Supplementary Figure S4:

HCT116 or HCT116 p53 -/- transfected with control siRNA or siRNA targeting ASPP1 or YAP were treated for 24h with 400µM hydroxyurea. p21 mRNA expression was quantified by RT-QPCR using specific primers. The results were normalized against two different standard genes, and the graphs represent the mean of three independent experiments.



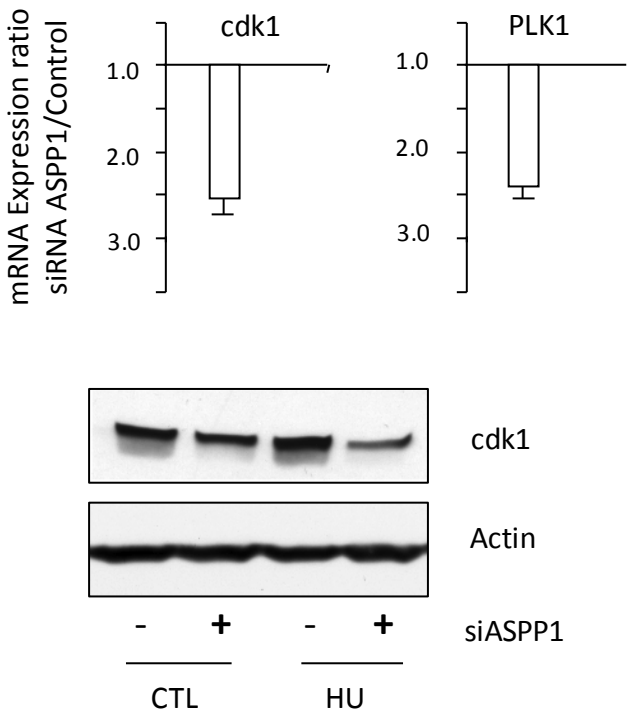
Supplementary Figure S5:

HCT116 cells untreated or treated with 10μM Nutlin, 5nM Actinomycin D, 400μM hydroxyurea or 150ng/ml doxorubicin were lysed and mRNA expression of ASPP1 was quantified by RT-QPCR using specific primers. The results were normalized against two different standard genes, and the graphs represent the mean of three independent experiments.



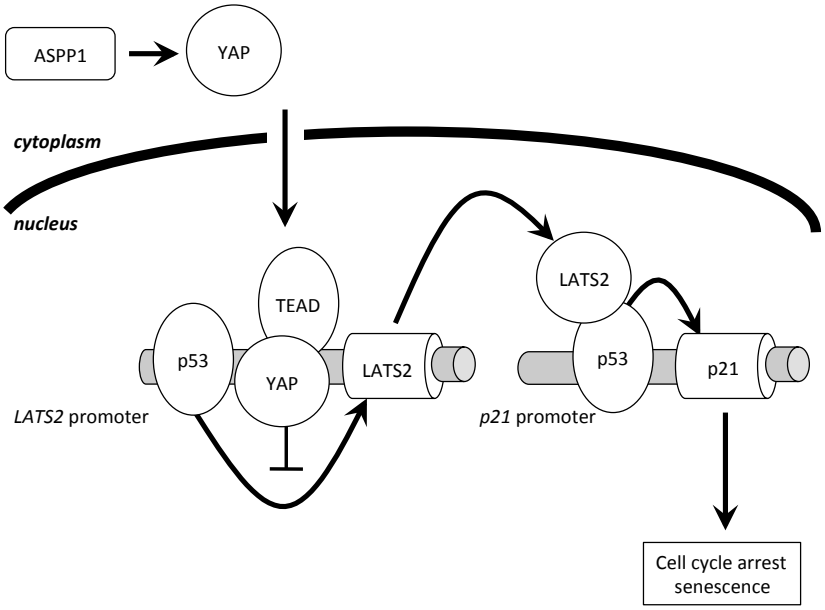
Supplementary Figure S6:

Indicated p53 target gene expression measured by RT-QPCR with specific primers in HCT116 cells transfected with control or ASPP1 siRNA and treated with 400μM hydroxyurea for 24 hours. The results were normalized against two different standard genes and presented as a ratio between the values obtained under siRNA ASPP1 and control conditions. The graph represents the mean of 5 independent experiments.



Supplementary Figure S7:

Cdk1 mRNA expression measured by RT-QPCR with specific primers in HCT116 cells transfected with control or ASPP1 siRNA and treated with 400μM hydroxyurea for 24 hours and release 48hours in normal medium. The results were normalized against two different standard genes and presented as a ratio between the values obtained under siRNA ASPP1 or control conditions. The graph represents the mean of 3 independent experiments. Lower panel shows cdk1 protein expression measured by western blot with a specific antibody against cdk1. Actin is used as a loading control.



Supplementary Figure S8:

Model depicting the p21 gene regulation by the p53/LATS2 and ASPP1/YAP pathways.