NF-Y loss triggers p53 stabilization and apoptosis in HPV18positive cells by affecting E6 transcription

SUPPLEMENTARY FIGURES



Supplementary Figure S1: A-C. NF-YA inactivation with a pool of NF-YA-targeting shRNAs. A. SubG1 events determined by Propidium Iodide staining of shCTR and shNF-YA cells upon 96h from lentiviral transduction. **B.** Western blot analysis of NF-YA and p53 expression in control and NF-YA-inactivated cells. **C.** qRT-PCRs of the indicated transcripts. Relative expression of transcripts has been calculated in shNF-YA versus shCTR, arbitrarily set at 1. **D-F. Effects of NF-YA inactivation in C4-I cells. D.** SubG1 events in shCTR and shNF-YA upon 96h from viral transduction. **E, F.** Protein and mRNA levels in NF-YA inactivated cells compared to control cells.

А



Supplementary Figure S2: Representation of the non canonical (upper box) or wt (lower box) status of the two identified NF-Y-motives within the LCR in the HPV18 phylogenetic tree (modified from Chen Z. et al. [9]).



Supplementary Figure S3: A. DNA distribution analysis of Propidium Iodide-stained cells treated with increasing doses of Doxorubicin. The percentages of SubG1 events are indicated. **B.** p53 expression analysis in whole cell extract following Doxorubicin administration. Actin was used as loading control.

HPV18	-431	CACTATTGCAAACTTTAATCTTTTGGGCACTGCTCCTACATATTTT	-386
HPV16	-479	I IIIIIII IIIIIIII IIIIIIIII IIIIIIIII	-444
		NF-YNF-I NF-I E2F E-Box AP-1	
HPV18	-385		-350
HPV16	-443	GCCATGCGTGCCAAATCCCTG	-423
	-349		-314
HPVIO	-040		-014
HPV16	-422	ŤŤŤŤĊĊŤĠĂĊĊŤĠĊĂĊŦĠĊŦŦĠĊĊĂĂĊĊĂŤŤĊĊĂŤŤĠŦŦŦŦŦŦĂĊ	-378
HPV18	-313	-CATAACTATATCCACTCCCTAAGTA	-289
HPV16	-377	ACTGCACTATGTGCAACTACTGGAATCACTATGTACATTGTGTCATATAAA AP-1	-328
HPV18	-288	ATAAAACTGCTTTTAGGCACATA-TT	-264
HPV16	-327	ATAAATCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTT YY1+C/EBP OCT1/NF1NF-Y	-278
HPV18	-263	TTAGTTTGTTTTACTTAAGCTAATTGCATACTTGGCTTGTACAACT	-217
HPV16	-277	TTGGCTTGTTTTAAC-TAACCTAATTGCATATTGGCATAAGGTTTAA	-231
HPV18	-216	ACTTTCATGTCCAACATTCTGTCTACCCTTAACATGAACTATAA	-173
HPV16	-230	ACTTCTAAGGCCAACTAAATGTC-ACCC-TAGTTCATACATGAACTGT-G	-184
HPV18	-172	TATGACTAAGCTGTGCATACATAGTT	-147
HPV16	-183	II III E-Box TAAAGGTTAGT-CATACATTGTTCATTTGTAAAAACTGCACATGGG CPE VV1	-140
HPV18	-146	TATGC-AACCGAAATAGGTTGGGCAGCACATACTATACTTTCA	-104
HPV16	-139	I.III I.IIII IIIIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	-95
HPV18	-103	TTAATACTTTTAACAATTGTAGTATATAAAAAAAGGGAGTAACCG	-60
HPV16	-94	TAATAATACTAAACTACAATAATTCATGTATAAAACTA <mark>AGGGCG</mark> TAACCG	-45
HPV18	-59	V-MYB V-MYB TATAbox AAAACGGTCGGGACCGAAAACGGTGTA-TATAAAAGATGTGAGAAACACA	-11
HPV16	-44	AAATCGGT-TGAACCGAAACCGGT-TAGTATAAAAGCAGA	-7
HPV18	-10	CCACAATACTATG +3	
	.6	 CATTTTATG +3	
	-0		

Supplementary Figure S4: Conservation of Transcription Factor Binding Sites (TFBS) within the URR of HPV18 and HPV16 integrated genomes. Sequence alignment of URR sequences from HPV18 (NC_001526) and HPV16 (AY262282) integrated genomes. The ATG sequence of E6 ORF has been indicated as +1. TFBS are highlighted in red boxes, NF-Y binding sites in green boxes.



Supplementary Figure S5: Effect of NF-YA inactivation on p63 and p73 expression in Hela cells. A. mRNA expression levels of total p63 and p73 in control and NF-YA inactivated cells following 96h from viral infection. qRT-PCRs have been represented as expression levels of shNF-YA versus shCTR, arbitrarily set at 1. Statistical significance was calculated with independent t-test (* p < 0.05; ** p < 0.01). Error bars indicate SD. **B.** Western blot analysis of p63 protein levels in shCTR and shNF-YA cells.