Mannefeld et al.

Supplemental Figure Legends

Figure S1: Cell cycle distribution of HCT-116 wt, p53 -/-, and p21 -/- after doxorubicin treatment and of MCF-7 cells after Nutlin-3 treatment. Cell cycle profiles of the experiments shown in Figure 2 B, C and D as analyzed by flow cytometry (A) HCT-116 wt and p21 -/- cells were treated with 1 μM doxorubicin for 2 hours and analyzed at the indicated time points. (B) MCF-7 cells were treated with 4.3 μM Nutlin-3 for 24 hours (C) HCT-116 wt and p53 -/- cells were treated as described in A.

Figure S2: Gene expression and pattern of LINC-binding to the promoters of G2/M genes after DNA damage. Analysis of additional genes and time points from the experiment shown in Fig. 3 (A) HCT-116 p53+/+ (black bars) or p53-/- cells (red bars) were treated with 1 μM doxorubicin for 2 hours. Gene expression was analyzed by quntitative real-time PCR and normalized to S14. (B) Crosslinked chromatin HCT-116 wt and p53 -/- cells from cells 24 hours after doxorubicin-treatment was immunoprecipitated with the indicated antisera. Promoters were detected by real-time PCR. GAPDH serves as a control. (C) ChIP assays 48 hours after doxorubicin-treatment. Analysis of additional promoters in the experiment shown in Figure 3B. (D) Cell cycle distribution of doxorubicin treated p53+/+ and p53-/- cells from the experiments shown in B), C) and Fig. 3B was determined by FACS analysis.

Figure S3: DNA-damage induced downregulation of G2/M genes and increased binding of E2F4 and decreased binding of B-MYB to the cyclin B1 promoter in human BJ fibroblasts. (A) BJ fibroblasts were treated with 0.34 μM doxorubicin for the indicated time periods. Cell cycle distribution was analyzed by FACS. (B) BJ cells were treated as in A. Expression of the indicated genes was analyzed by real-time PCR. (C) Chromatin from

untreated or doxorubicin-treated BJ cells was immunoprecipitated with the indicated antisera. Nonspecific IgG was used as a control. The cyclin B1 promoter was detected by real-time PCR. Binding is expressed relative to input DNA.

Figure S4: DNA-damage induced downregulation of G2/M genes and increased binding of E2F4/p130 and decreased binding of B-MYB to G2/M promoters in MCF-7 cells

(A) MCF-7 cells were treated with 1 μM doxorubicin for 2 hours. 48 hours later, expression of the indicated genes was analyzed by quantitative RT-PCR and normalized to GAPDH. (B) Chromatin from doxorubicin-treated (red bars) and untreated (black bars) MCF-7 cells was immunoprecipitated with the indicated antisera. Nonspecific IgG was used as a control. Promoters of the indicated genes were detected by real-time PCR. Binding is expressed relative to input DNA and to IgG.

Figure S5: Binding of LINC to the promoters of G2/M genes upon activation of p53 by Nutlin-3. (A) MCF-7 cells were treated with 4.3 μM Nutlin-3 for 24 hours. Cell cycle distribution was analyzed by FACS analysis. (B) Chromatin of Nutlin-3-treated and untreated MCF-7 cells was immunoprecipitated with the indicated antisera. Bound promoters were analyzed using real-time PCR and are expressed relative to input DNA.

Figure S6: LIN9 and B-MYB are required for the DNA-damaged mediated activation of G2/M genes in p53 mutant cells. Analysis of additional genes from the experiment shown in Figure 4 A and B. (A) HCT-116 p53+/+ and p53-/- cells transfected with a B-MYB specific siRNA or a control siRNA were treated with doxorubicin for 2 hours. RNA was isolated 24 or 48 hours later as well as from untreated cells as a control. The expression of the indicated genes was analyzed be real-time RT-PCR and normalized to S14 (B) HCT-116 p53-/- cells transfected with a control siRNA or with siRNAs specific for LIN-9 or B-MYB were treated

with doxorubicin for 2 hours. 48 hours later the expression of the indicated genes was analyzed by real-time RT-PCR. The expression of S14 was analyzed as a control.

Figure S7: Model summarizing the findings.

A) In p53+/+ cells, DNA-damage or direct activation of p53 by Nutlin-3 switches LINC from B-MYB to p130/ E2F4. This results in dissociation of B-MYB from promoters of G2/M genes and in association of p130/E2F4 and it contributes to the repression of G2/M genes. This could contribute to a stable cell cycle arrest. B) In p53-/- cells B-MYB does not dissociate from LINC upon DNA-damage and remains associated with G2/M promoters. B-MYB/LINC enhances the expression of G2/M gene in p53-/-cells and promotes escape from the G2 checkpoint.