Supplements to Höglund et al

Experimental procedure

Materials. The proteasome inhibitor Z-Leu-Leu-Phe-CHO was from Sigma. Cdc2 and p-Cdc2 antibodies were from Cell signalling.

Cell culture. *Arf*, *Atm* or *Arf/Atm* knockout MEFs were kind gifts from Dr Kirsteen Maclean (formerly at St Jude Children's Research Hospital, Memphis, TN) and were cultured in Dulbecco's Modified Eagle Medium with 10% fetal calf serum (FCS), 2 mM L-glutamine, 1 mM sodium pyruvate and antibiotics.

Flow cytometry methods. For cleaved caspase 3 measurements, cells were fixated in 3% formaldehyde and permeabilized by resuspension in ice-cold methanol. Cells were then incubated overnight with a primary antibody directed against cleaved caspase-3 (Cell Signaling) and stained with a FITC conjugated secondary antibody. Cells were stained with 7-aminoactinomycin D (7-AAD; Sigma) and analyzed in a FACScalibur flow cytometer (BD Biosciences) using the FL1 (Cleaved Caspase 3) and FL2 (7-AAD) channels.

Immunophenotyping of tumors arising in our Myc retrovirus-induced lymphoma model was made by first generating single-cell suspension of the tumors with scalpels and cell strainers. After red cell lysis with ammonium chloride, cells were pelleted by centrifugation, washed in PBS containing 1% FCS and then stained using RPE-conjugated anti-CD3, anti-B220 or anti-IgM. Following washing the cells were analyzed in a FACScalibur flow cytometer (BD Biosciences).

Figure Legends

Figure S1. (A) MycER^{TAM}-expressing NIH3T3 fibroblasts were infected with shRNA against *Atm, Atr* and *Prkdc* (encoding DNA-PK) and *mRNA* knockdown was verified using qRT-PCR. (B) WB analysis of *p53* knockout, *Arf* knockout or *Arf/ATM* double knockout MEFs

expressing inducible MycER^{TAM}. Myc was activated with 4-hydroxytamoxifen (4-HT) and 24 h later whole cell lysates were analysed with Western blot (WB) using antibodies against Chk1, Cdc2 and p-Cdc2. Actin was used as a loading control.

Figure S2. Coexpression analyses of *MYC* and *CHEK1*. (A) The Basso lymphoma study were searched for genes which coexpressed with *MYC* in Burkitt lymphoma (BL) in the web based resource Oncomine (www.oncomine.com). In this study, *CHEK1* and *MYC* are co-expressed and elevated in the cluster containing Burkitt lymphomas (BL). (B) Affymetrix microarray expression profiling data from the Basso study was downloaded from NCBI (accession number GSE2350). Expression data of the indicated genes were analyzed in Excel for Spearman's rho (R) value. This correlation is not identical to that of Oncomine since this database also takes into account clustering effects generating higher R-values.

Figure S3. (A) Colon or small intestine adenomas from *Apc*^{Min} mice were harvested at around 120 days of age. Tumor material was snap frozen and prepared for WB as described in the main text. Normal tissue from every animal was harvested and used as control for Chk1 expression. (B) qRT-PCR analysis of *Chek1* and c-*Myc* transcript levels in adenomas or normal tissue of colon or small intestine from *Apc*^{Min} mice.

Figure S4. WB analysis of Chk1 expression in all tumors developed following transplantation of lymphoma cells expressing non-target shRNA or *Chek1* #48 shRNA. Chk1 expression in the cells before transplantation and in a lymph node (LN) was assessed as a control.

Figure S5. A) The human lymphoma cell lines Akata, BJAB, DG75, KemI and Raji were treated for 72 h with 1 μM of Chekin. The cells were counted daily using trypan-blue staining in a counting chamber. Asterisk indicates *$P<0.05$;DMSO versus Chekin-treatment. B) KemI cells were further analyzed by measuring ^{3}H-thymidine uptake for the last three hours of 48 h incubation in the presence of indicated concentrations of Chekin or vehicle (DMSO). Following incubation cells were harvested by filtration onto glass fiber filters and counted in a

96-well format TopCount scintillation counter (Perkin-Elmer). C) WB analysis of human B-cell lymphoma lines treated with Chekin using indicated antibodies.

Figure S6. DNA histograms of cells treated as in Figure 4. Their *TP53* and *EBV* status are shown in the bottom panel.

Figure S7. (A) A *λ-Myc* (p53 mutant) mouse lymphoma cell line was treated for 12 h with 1 μM Chekin with or without 10 μM of the proteasome inhibitor Z-Leu-Leu-Phe-CHO (z-LLF) present in the growth media during the last 3 h of Chekin treatment. WB analysis shows increased Chk1 levels after z-LLF treatment alone (Chk1 short exposure) and in combination with Chekin (Chk1 long exposure). Myc was used as a positive control for proteasome inhibition since it is short-lived. (B-C) Chekin-induced apoptosis involves caspase-3. Caspase-3 activity was measured using an antibody that specifically recognizes caspase-3. Data in *B* is total caspase-3 active cells whereas in *C* the caspase-3 activity is shown in different cell cycle phases. (D) Cell cycle distribution of propidium iodide-stained cells treated with 1 μM Chekin in the presence or absence of the pan-caspase inhibitor QVD-OPH.

Figure S8. Two tumors arising in mice transplanted with *p53* deficient bone-marrow derived B cells were immunophenotyped by flow cytometry using the indicated antibodies.