SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. ERG downregulation of CHK1 is AR independent. A, Expression profile analysis of HEK 293 cells overexpressing ERG showing CHK1 mRNA downregulation. The results of the two different Affimetrix probe sets for CHK1 are shown. **B,** Western blot analysis of HEK293 cell line overexpressing either ERG or ETV1 showing robust downregulation of CHK1 levels. **C,** Western blot analysis of PC3 cell line overexpressing either ERG or ETV1 showing robust downregulation of CHK1 levels. **D,** Western blot analysis of VCaP cells shows reduced amount of CHK1 but not CHK2 after 10 nM DHT treatment for 24 hours. **E,** Expression profile analyses of ERG and CHK1 transcripts in VCaP cells either untreated or treated with 10 nM DHT for 16 hours. The results of the two different Affimetrix probe sets for CHK1 are shown. Experiments were performed in triplicate, data were analyzed using unpaired t-test. Values of $P < 0.05$ were considered statistically significant (*p<0.05, **p<0.01). **F,** Immunohistochemistry analysis for ERG and CHK1 on serial sections of a human primary CaP TMA. Statistical significance was determined by Fisher's exact test.

Supplementary Figure S2. ETV1 represses CHK1 expression. A, Western blot analysis of HEK-293 cell line overexpressing AR either treated or not with 10 nM DHT for 24 hours showing no sizable difference in CHK1 amount. **B,** Western blot analysis of VCaP and 22RV1 prostate cell lines either treated with 10 nM DHT or untreated for 24 hours showing reduction of CHK1 levels in VCaP but not in 22RV1. **C,** RT-qPCR analysis of LnCaP cells showing downregulation of CHK1 transcript following induction

of ETV1 with 10 nM DHT for 24 hours. **D,** Western blot analysis of LnCaP cells untreated or treated with DHT and proteasome inhibitor MG132 as single treatments or in combination. **E,** RT-qPCR analysis of LnCaP cells showing upregulation of CHK1 transcript following ETV1 silencing. **F,** Western blot analysis of LnCaP cells showing increased amount of CHK1 protein in the same samples described in **E**. **G,** Expression profile analysis of ETV1 and CHK1 transcripts in RWPE1 cells overexpressing ETV1. The results of the two different Affimetrix probe sets for CHK1 are shown. Error bars in graphs indicate mean±s.d. **H,** Western blot analysis of LnCaP cells showing downregulation of CHK1 protein following ERG overexpression. 10 nM DHT treatment for 24 hours decreases CHK1 levels in the control (line 1 compared to line 3) but not in ERG overexpressing LnCaP cells (line 2 compared to line 4). Experiments were performed in triplicate, data were analyzed using unpaired t-test. Values of $P < 0.05$ were considered statistically significant (*p<0.05, **p<0.01).

Supplementary Figure S3. ERG binding to *CHK1* promoter. A, ChIP-seq datasets analysis showing ERG and AR binding sites on CHK1 gene locus. The two sequence of *CHK1* promoter identified by Yu and colleagues (1) are listed. **B,** 1290 bp of CHK1 promoter upstream the ATG with the 20 putative ERG binding sites in red. Underscored, the two sequence identified by Yu and colleagues (1). **C,** On the left, scheme of wild type and two shorter forms of *CHK1* promoter progressively loosing ERG binding sites in the distal part of the promoter. On the right, luciferase assay in HEK293T showing similar repressive activity of ERG on the three constructs. **D,** Luciferase assay in HEK293T showing on the left the absence of AR activity on *CHK1* promoter (Wt), while on the

right the induction of an AR reporter (4xARE) is shown as positive control for AR function.

Supplementary Figure S4. Chk1 heterozygosity accelerates classical Pten$^{+/-}$ driven phenotypes. A, Western blot analysis showing PTEN and CHK1 levels in VCaP cells 48 hours after transfection of a specific PTEN siRNA. GAPDH was used as loading control. **B,** Cytosolic fractionation of *wild type* and *Pten*$^{+/-}$ mouse prostate tissue. Laminin B1 and GAPDH were used as markers of the nuclear and cytosolic fractions respectively. **C,** Survival of *wild type, Chk1*$^{+/-}$*, Pten*$^{+/-}$*, Pten*$^{+/-}$*;Chk1*$^{+/-}$ mice are shown as Kaplan Meier curves. No significant differences were described for males. **D,** a statistically significant increase of lethality was reported for the *Pten*$^{+/-}$*;Chk1*$^{+/-}$ females. **E,** Comparison of spleens, adrenal glands and submandibular lymph nodes in 5 month-old *wild type, Chk1*$^{+/-}$*, Pten*$^{+/-}$*, Pten*$^{+/-}$*;Chk1*$^{+/-}$ male mice showing the exacerbation of splenomegaly, pheochromocytoma, and benign lymphoproliferation in the *Pten*$^{+/-}$*;Chk1*$^{+/-}$ mice. **F,** Percentage of B- (B220+) and T- (CD3+) cells in the lymph nodes of 5 month-old *wild type, Chk1*$^{+/-}$*, Pten*$^{+/-}$*, Pten*$^{+/-}$*;Chk1*$^{+/-}$ male mice. **G,** phospho-53BP1 staining in *Pten*$^{+/-}$ and *Pten*$^{+/-}$*;Chk1*$^{+/-}$ mouse prostates. Percentage of positive cells is shown in the graph (n=3 mice per genotype on a total of 10,000 cells analyzed).

Supplementary Figure S5. ERG overexpression induces p53 activation through excessive DNA damage. A, Overexpression of ERG results in nuclear fragmentation and apoptosis in p53 wild type 22Rv1 prostate cell line. **B,** ERG overexpression drives p53 activation (P-Ser15) in p53 proficient cells. **C,** Overexpression of ERG prevents colony

formation in cells harboring wild-type p53 (LnCaP and 22RV1) but not in cells harboring p53-null PC3. Bar graphs show quantifications of colonies formed after overexpression of ERG compared to that formed following transfection with an empty vector in the indicated cell lines.

REFERENCES

1. Yu J, Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, et al. An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. Cancer cell. 2010;17(5):443-54. Epub 2010/05/19. doi: 10.1016/j.ccr.2010.03.018. PubMed PMID: 20478527; PubMed Central PMCID: PMC2874722.