



Figure S1. ASCIZ/CHK2 interaction. ~3 million clones of a human placental cDNA library in pGAD10 (Clontech) were screened on reporter plates lacking Leu, Trp, His, Ade, and containing 10 mM 3-amino-triazole (3AT). The FHA bait construct in pAS2 contained CHK2 residues 1-221. All 25 most strongly interacting clones contained in-frame ASCIZ fusions with the Gal4 activation domain. (A,B) Yeast two-hybrid assays. Equal dilutions of yeast strains cotransformed with empty pAS2, or pAS2 containing wildtype or mutant CHK2 and full-length ASCIZ or truncated ASCIZ-SCD (residues 276-667) constructs as indicated were restreaked on control plates (top panel in B) or -His -Ade reporter plates (bottom panel), or were analyzed for β -galactosidase activity as a third reporter assay (A). Note that the two-hybrid interaction is abolished by Ala substitution of the critical Arg117 residue in the FHA phospho-threonine binding site suggesting that it is phosphorylation-dependent, and by introduction of the R145W mutation linked to familial cases of the Li-Fraumeni multi-cancer syndrome. (C) Pull-down assay. Western blot analysis of Ni^{2+} -bound complexes isolated from U2OS cells co-transfected with GFP-ASCIZ and pCDNA4 or pCDNA4-His₆-CHK2, using the indicated antibodies. Note that CHK2 and ASCIZ interact in a DNA damage-independent manner. (D) ASCIZ is phosphorylated under basal conditions and further phosphorylated after MMS treatment. GFP-ASCIZ was immunoprecipitated from control and MMS-treated cells with or without wortmannin or caffeine addition and treated with phosphatase as indicated before Western blotting. We speculate that constitutive ASCIZ phosphorylation involves phospho-threonine as a basis for its interaction with the FHA phospho-threonine binding site already under basal conditions. (E) Autoradiograph of in vitro phosphorylation of ASCIZ by yeast ATR. The ATR homologue Mec1 was immunoprecipitated using anti-myc antibodies from yeast strains containing a myc-tag at the chromosomal *MEC1* locus, and untagged wildtype (WT) as negative control, and used in kinase assays with a recombinant ASCIZ SCD fragment (residues 285-477). Similar kinase assays using the SCD of the yeast Mec1 substrate Mdt1 are shown as control (Pike et al., 2004). The data indicate that ASCIZ can be directly phosphorylated by ATM/ATR-like kinases.