Supporting Information

Liu et al. 10.1073/pnas.1217781110

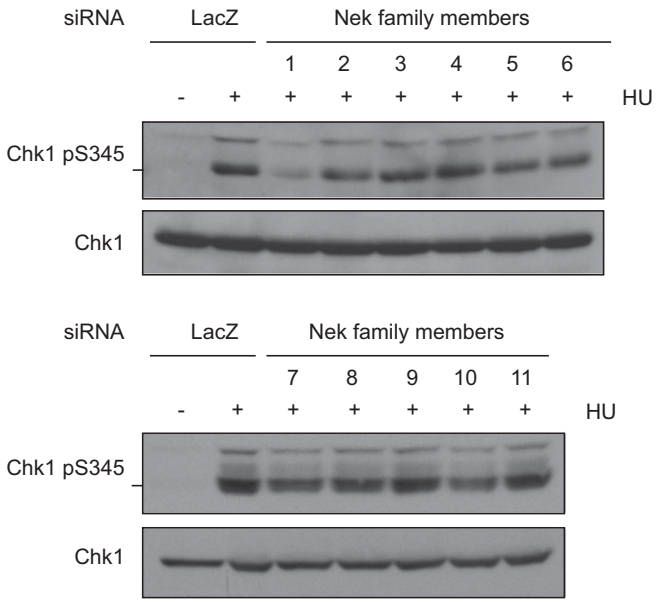

Fig. S1. Nek1 is required for HU-induced Chk1 phosphorylation. HCT116 cells were transfected with siRNAs targeting each of the 11 human Nek kinases. Transfected cells were treated with 1 mM HU for 2 h, and Chk1 phosphorylation was analyzed with phospho-specific antibody.

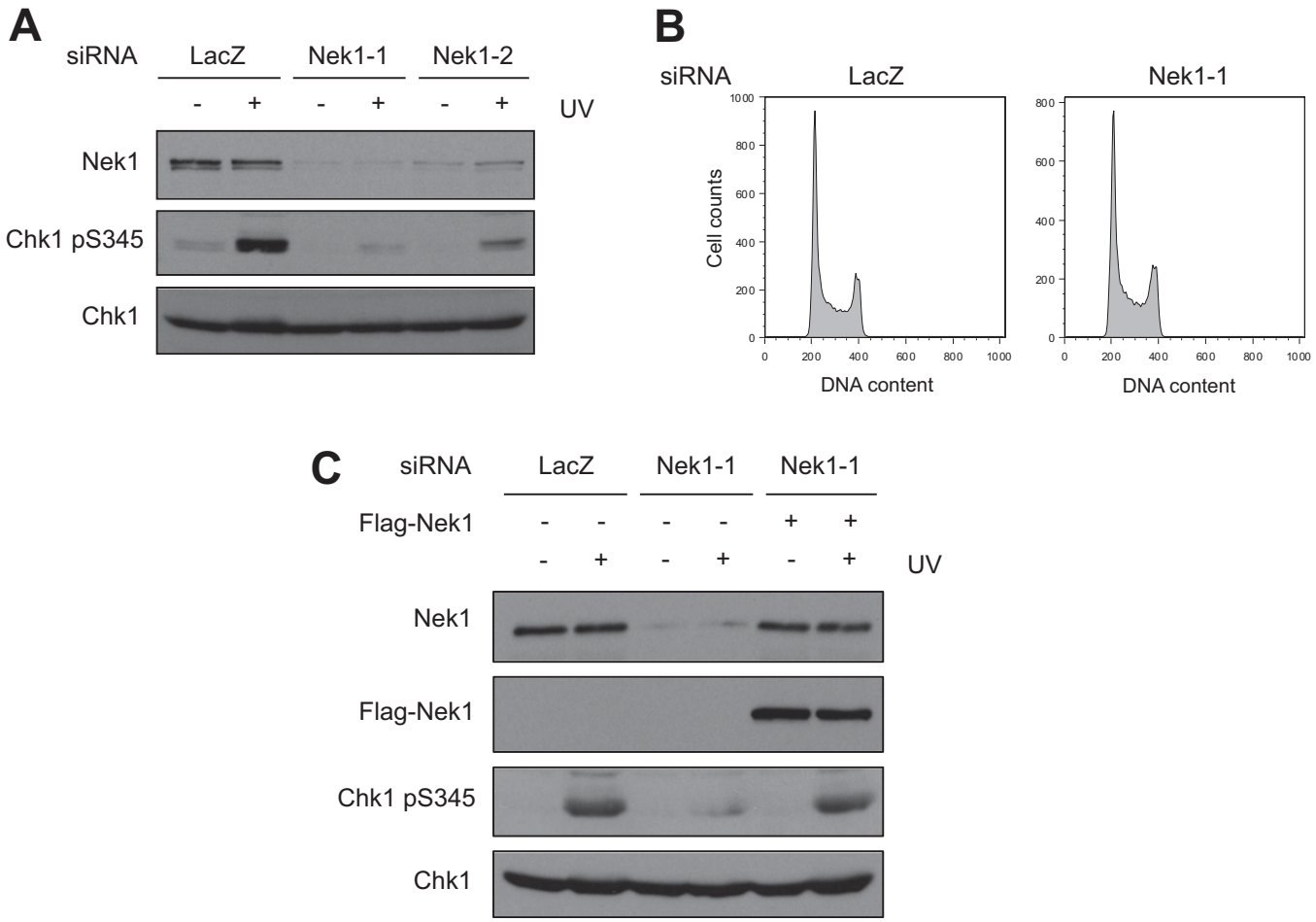

Fig. S2. Nek1 is required for UV-induced Chk1 phosphorylation. (*A*) HCT116 cells were transfected with LacZ, Nek1-1, or Nek1-2 siRNA. Transfected cells were irradiated with UV (15 J/m^2), and Chk1 phosphorylation was analyzed 2 h after UV treatment. Knockdown of endogenous Nek1 was confirmed with Nek1 antibody. (*B*) HCT116 cells were transfected with LacZ or Nek1-1 siRNA as in *A*. Transfected cells were subsequently fixed, stained with propidium iodide, and analyzed by FACS. (*C*) HCT116 cells were first transfected with LacZ or Nek1-1 siRNA, and then transfected again with empty vectors or plasmids expressing siRNA-resistant Flag–Nek1. UV-induced Chk1 phosphorylation was analyzed as in *A*.

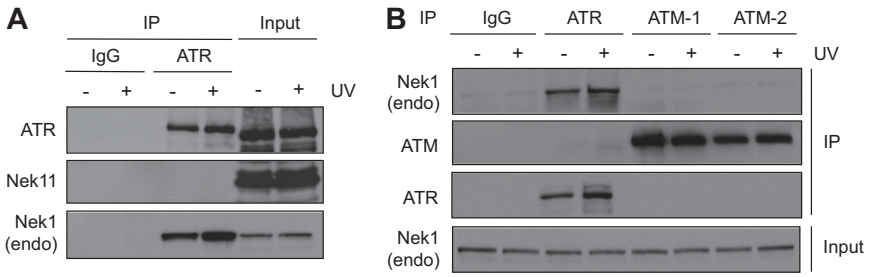

Fig. S3. Nek1 interacts with ATR–ATRIP specifically. (*A*) HCT116 cells were irradiated with UV or left untreated. Immunoprecipitates of ATRIP antibody or control antibody (IgG) were analyzed by Western blot using the indicated antibodies. (*B*) Cells were treated with UV or untreated as in *A*. Immunoprecipitates of ATR and ATM antibodies were analyzed by Western blot using the indicated antibodies.

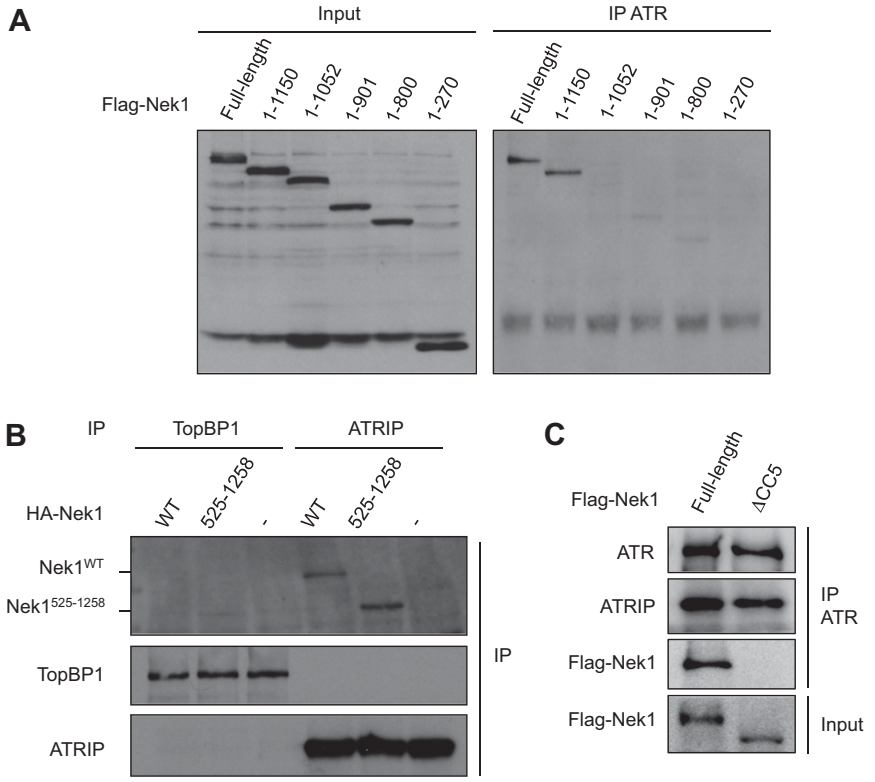

Fig. S4. Mapping the ATR–ATRIP-interacting domain of Nek1. (*A*) Flag-tagged full-length Nek1 and its truncated derivatives were transiently expressed in 293T cells and analyzed by ATR immunoprecipitation. The Flag-tagged Nek1 proteins present in the inputs and ATR immunoprecipitates were analyzed using anti-Flag antibody. (*B*) HA-tagged wild-type Nek1 and a N-terminal truncation Nek1 mutant (525–1258) were transiently expressed in 293T cells. Immunoprecipitation of TopBP1 and ATRIP were performed with respective antibodies. The presence of TopBP1, ATRIP, and HA–Nek1 proteins in the immunoprecipitates were analyzed with the indicated antibodies. (*C*) Cells were transfected with plasmids expressing Flag–Nek1^{WT} or Flag–Nek1$^{\Delta CC5}$, and subjected to ATR immunoprecipitation. The levels of ATR, ATRIP, and Flag–Nek1 proteins in the imunoprecipiates and input extracts were analyzed by Western blot.

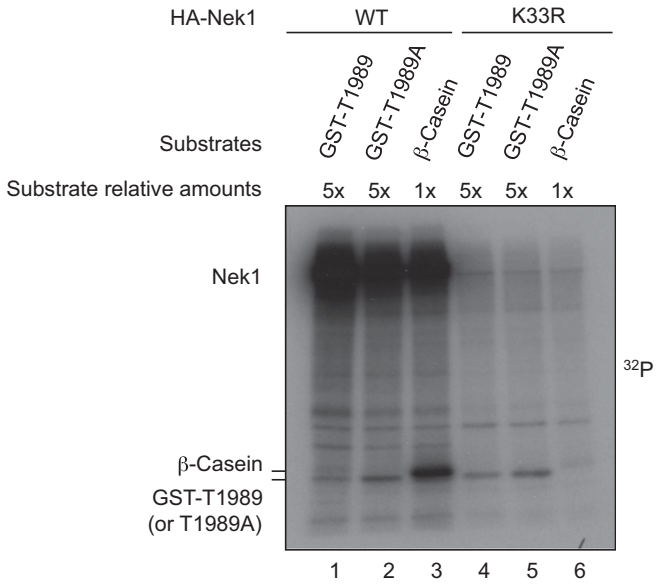

Fig. S5. Nek1 does not phosphorylate ATR Thr-1989 directly. HEK 293T cells were transfected with plasmids expressing HA–Nek1^{WT} or HA–Nek1^{K33R}. HA–Nek1 proteins were immunoprecipitated with HA antibody. The ability of HA–Nek1 to phosphorylate purified GST-T1989, GST–T1989A, and β-casein was tested using in vitro kinase assays. Approximately 50 ng/μL of GST–T1989 or GST–1989A, or 10 ng/μL of β-casein was used as substrate as indicated.

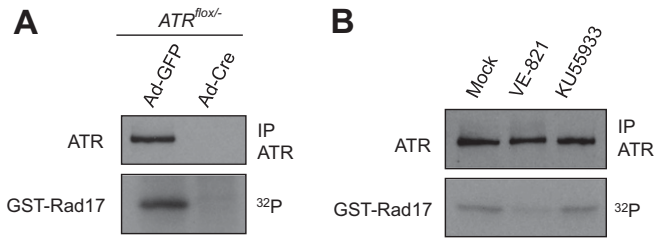

Fig. S6. Analysis of ATR kinase activity. (*A*) HCT116-derived *ATR*$^{flox/-}$ cells were infected with adenovirus expressing the Cre recombinase (Ad–Cre), or with control adenovirus expressing GFP (Ad–GFP). Endogenous ATR was immunoprecipitated from cell extracts. The levels of ATR in immunoprecipitates were analyzed by Western blot, and their ability to phosphorylate GST-Rad17 was analyzed using in vitro kinase assays. (*B*) HCT116 cells were treated with VE-821 (10 μM), KU55933 (10 μM), or DMSO before irradiated with UV. Endogenous ATR was immunoprecipitated from cell extracts, treated with the indicated inhibitors again, and tested with in vitro kinase assays using GST–Rad17 as substrate.