



Supplementary Figure S3. Generation of an siRNA-resistant version of ATRIP. **a**, 293T cells were mock transfected or transfected with siRNAs targeting luciferase or ATRIP. Twenty-four hours post-transfection, extracts were prepared and immunoblotted for ATR and ATRIP. **b**, 293T cells were sequentially transfected (see Methods) with ATRIP siRNA and/or HA-ATRIP* as indicated. Twelve hours following the last transfection, extracts were prepared and immunoblotted as indicated.

Recently, it has been shown that down-regulation of ATRIP also leads to loss of ATR protein¹. We also observed this phenomenon; however, not until 48 hr after transfection with the siRNA and approximately 32 hr after loss of ATRIP protein (data not shown). This therefore provided us with a time-window in which ATRIP-dependent events could be examined.

1. Cortez, D., Guntuku, S., Qin, J. & Elledge, S. J. ATR and ATRIP: partners in checkpoint signaling. *Science* **294**, 1713-1716 (2001).