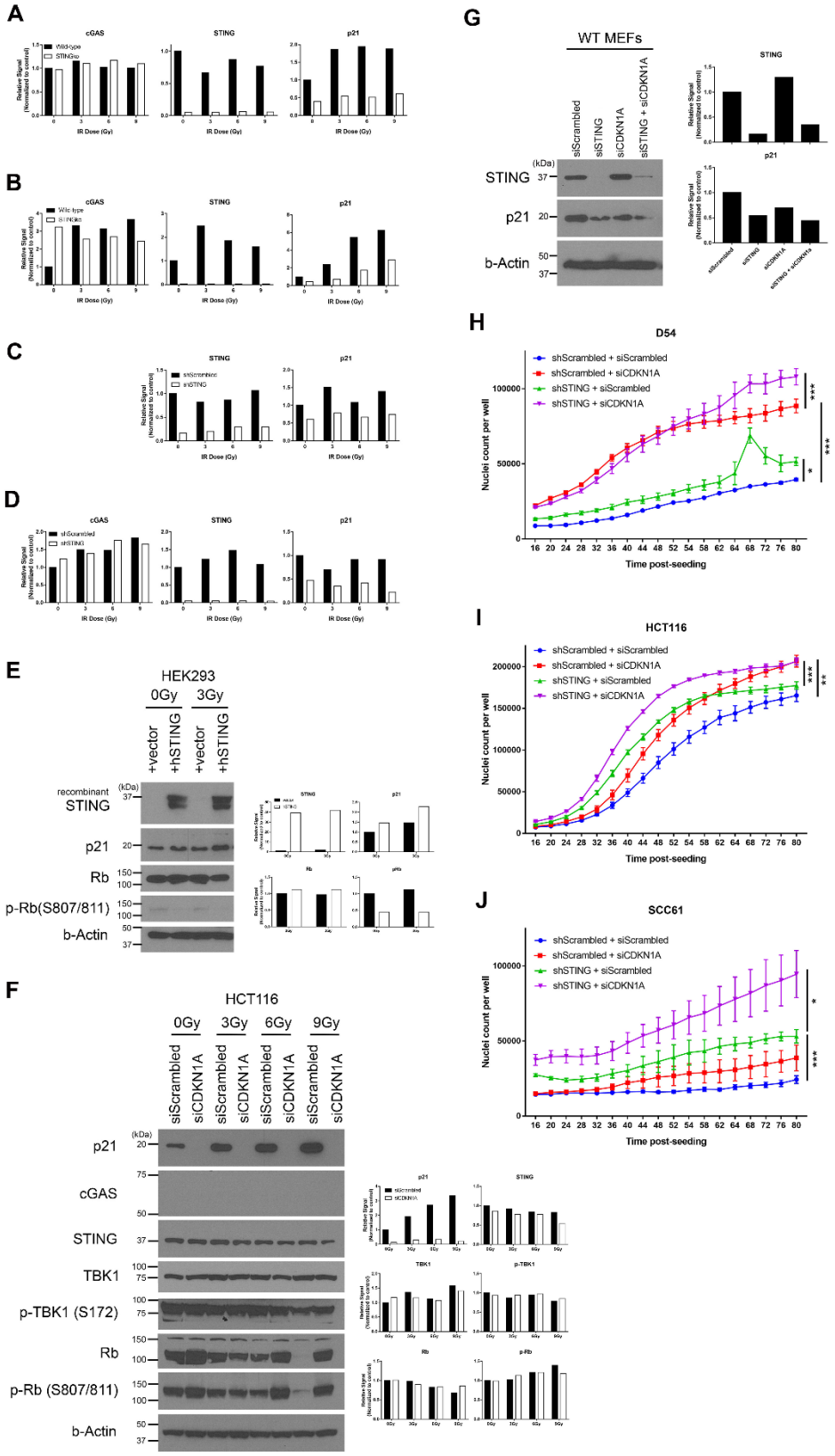
Supplementary Figure S4



Supplementary Figure S4. STING is upstream of CDKN1A signaling, but only has partial control of CDKN1A. (A-D) Quantification of bands from Western gels shown in Main Figures 3H **(A)**, 3I **(B)**, 3J **(C)**, and 3K **(D)** using Image J. The pixel units obtained for each protein band was normalized to the pixel units calculated from their respective b-Actin loading control. **(E)** Western analysis of lysates harvested from HEK293 cells that were transiently transfected with either an empty vector of recombinant STING at 48 hours following exposure to 3Gy IR show that the protein levels of p21 and cyclin D1 were restored following overexpression of STING in HEK293 cells. **(F)** Western analysis of lysates harvested from siCDKN1A HCT116 cells at 48 hours following exposure to increasing dose of IR demonstrate that depletion of p21 did not affect STING expression, and only affected proteins that it regulates downstream of the pathway. (**G**) Western analysis of lysates harvested from MEFs that were transiently transfected with siRNA targeting STING and/or p21 at 48 hours reveal that depletion of STING led to down-regulation of p21, but not vice versa. Quantified bands are shown right next to each Western blot panels. **(H-J)** Kinetic analysis of STING-depleted human tumor cell lines D54 **(H)**, HCT116 **(I)**, and SCC61 **(J)** that were transiently transfected with siRNA targeting p21. Proliferation of nuclei-stained cells were measured in vitro over time using the IncuCyte live cell imaging system. The number of nuclei-stained cells were calculated using the Incucyte software and data are representative of three experiments, each with n = 3 per group. P-values were determined using unpaired Student's t-test. Error bars are SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.