Supplemental figure 1, related to Figure 1

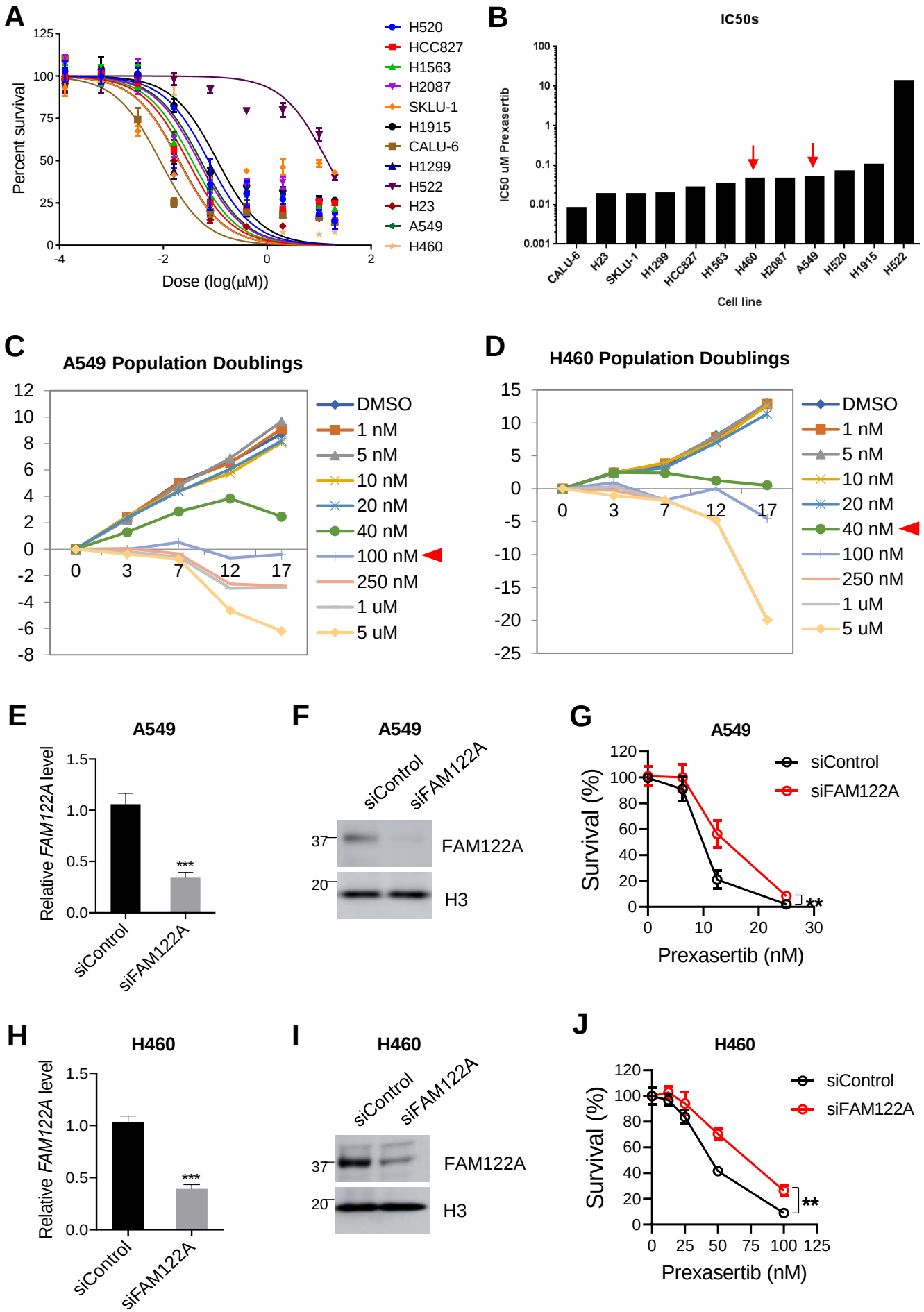


Figure S1. Genome-wide CRISPR screening reveals FAM122A loss as a mechanism of resistance to CHK1i, related to figure 1.

(A) Survival curves of the indicated NSCLC cells treated with graded concentrations of prexasertib.

(B) Quantification of the IC_{50}s of prexasertib sensitivity of indicated NSCLC cells based on the survival curves in panel A.

(C) Population doubling of A549 cells treated with indicated concentrations of prexasertib.

(D) Population doubling of H460 cells treated with indicated concentrations of prexasertib.

(E) FAM122A mRNA expression in A549 cells at 48 hrs after transfection with siRNA against a control gene or FAM122A. ***P<0.001, statistical analysis was performed using student t-test.

(F) Western blots of the lysates from A549 cells at 48 hrs after transfection with siRNA against control gene or FAM122A.

(G) Survival curves of A549 cells treated with graded concentrations of prexasertib after siControl or siFAM122A treatment. Data are shown as mean ± SD from three independent experiments. **P<0.01, statistical analysis was performed using two-way ANOVA.

(H) FAM122A mRNA expression in H460 cells at 48 hrs after transfection with siRNA against a control gene or FAM122A. ***P<0.001, statistical analysis was performed using student t-test.

(I) Western blots of the lysates from H460 cells at 48 hrs after transfection with siRNA against a control gene or FAM122A.

(J) Survival curves of H460 cells treated with graded concentrations of prexasertib after siControl or siFAM122A treatment. Data are shown as mean ± SD from three independent experiments. **P<0.01, statistical analysis was performed using two-way ANOVA.

Supplemental figure 2, related to Figure 2

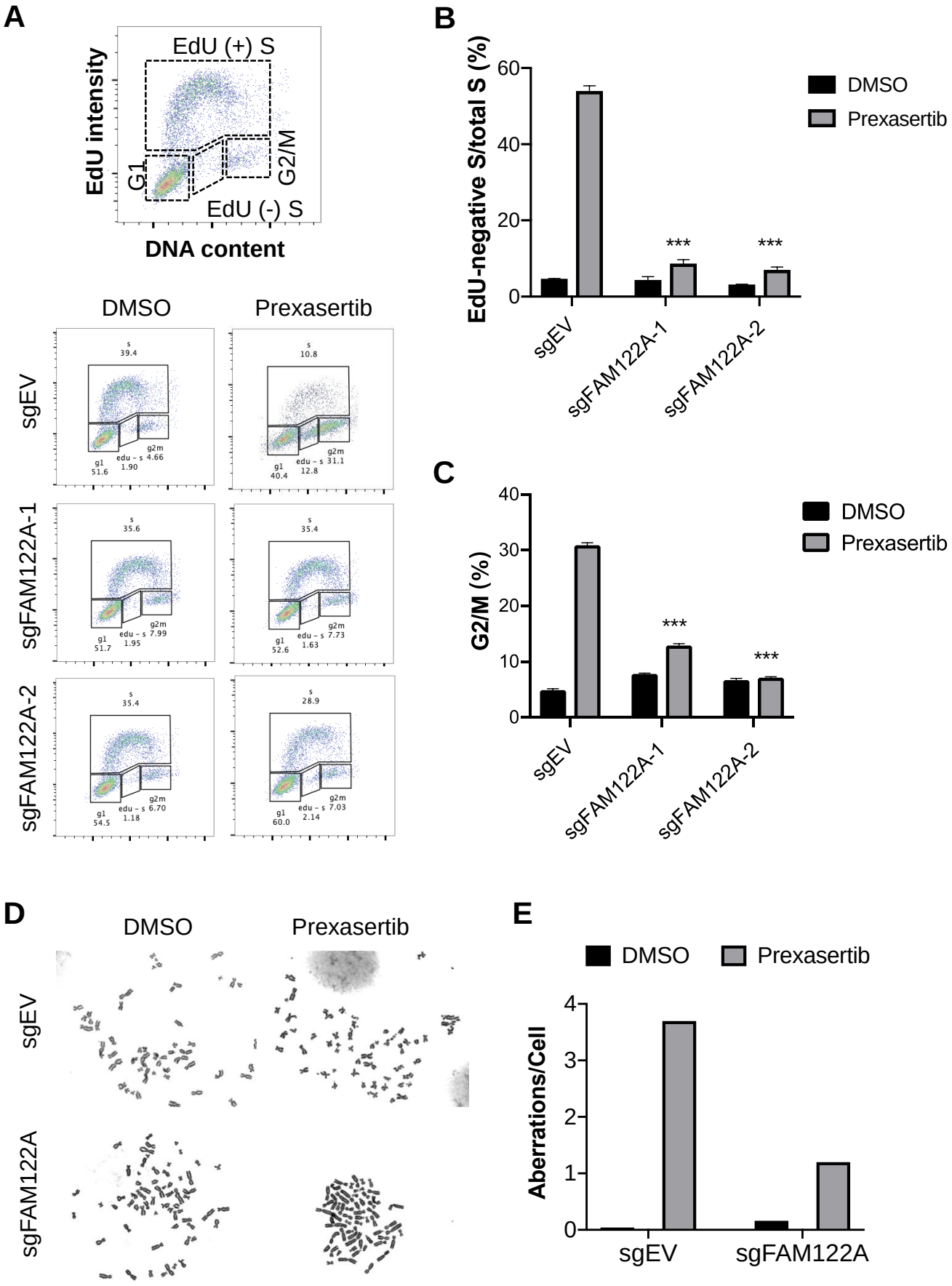


Figure S2. FAM122A loss rescues replication stress, DNA damage and G2/M arrest caused by CHK1i in A549 cells, related to figure 2.

(A) FACS analyses after pulse EdU labeling in control and two different clones of FAM122A knockout (sgFAM122A-1 and sgFAM122A-2) cells. The vertical axis indicates the EdU intensity and the horizontal axis indicates the DNA content as shown in the example at the top panel.

(B) Quantitation of the FACS plots in panel A showing percentage of EdU-negative S phase cells of the total S phase cells. Error bars indicate standard errors and P-values were calculated using the Student t-test (n=3, ***$p < 0.001$).

(C) Quantitation of the FACS plots in panel A showing percentage of cells in G2/M. Error bars indicate standard errors and P-values were calculated using the Student t-test (n=3, ***$p < 0.001$).

(D) Representative images of chromosomal aberrations on metaphase spreads of control and FAM122A-KO A549 cells after treatment with prexasertib (100nM) for 24hrs.

(E) Quantification of chromosomal aberrations per cell in panel D. At least 50 cells were counted in each experiment.

Supplemental figure 3, related to Figure 3

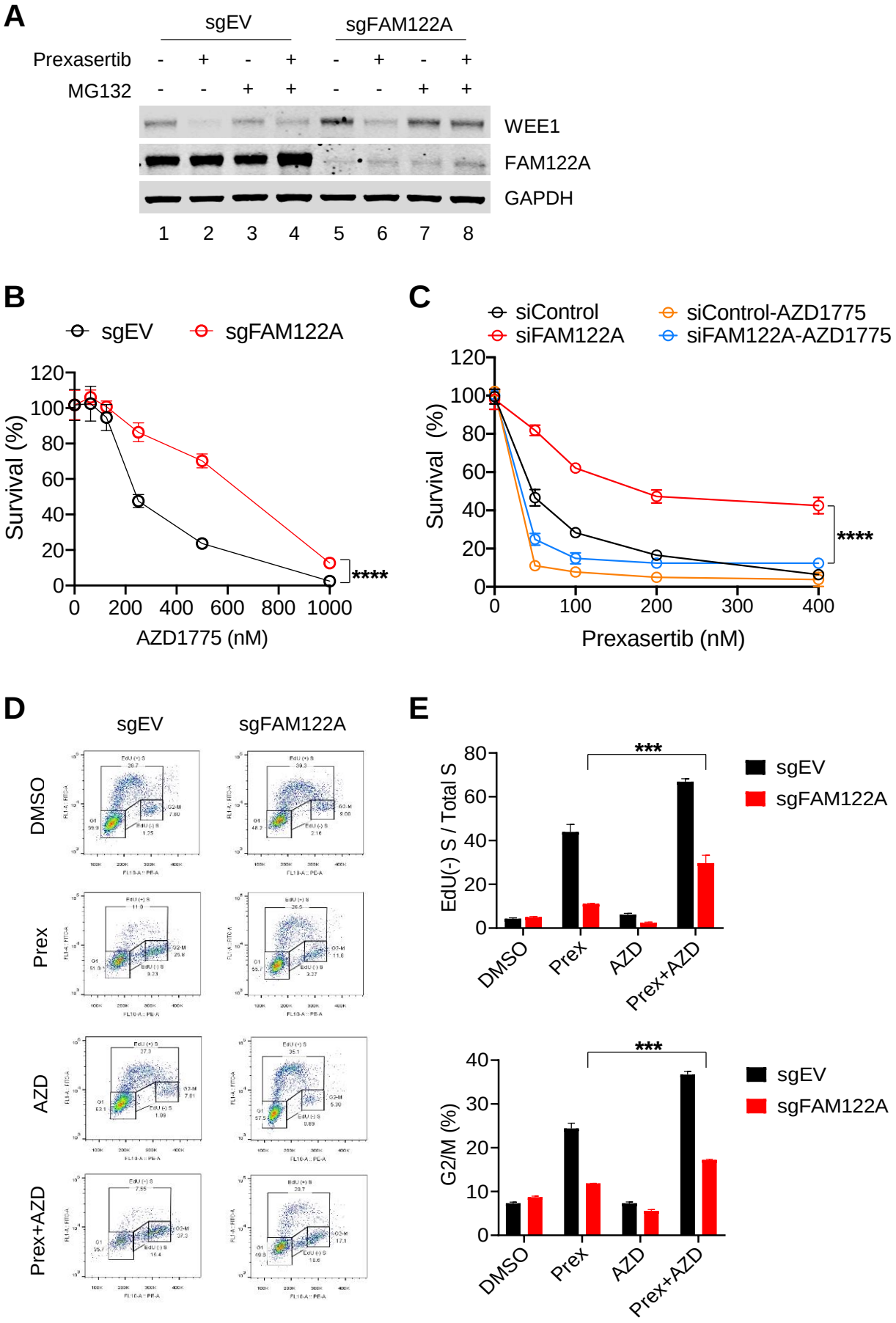


Figure S3. Depletion of FAM122A promotes WEE1 stability, related to figure 3.

(A) Western blots of the lysates from control and FAM122A-KO A549 cells treated with/without prexasertib (100nM) and MG132 for 16 hour.

(B) Survival curves of control and FAM122A-KO A549 cells treated with WEE1i (AZD1775). ****P<0.0001, statistical analysis was performed using two-way ANOVA.

(C) Survival curves of A549 cells treated with graded concentrations of prexasertib along with AZD1775 (50 nM) following transfection with siControl or siFAM122A. siFAM122A versus siFAM122A+AZD1775, ****P<0.0001, statistical analysis was performed using two-way ANOVA.

(D) Representative FACS plots of pulse EdU labeling in control and FAM122A-KO A549 cells after exposure to prexasertib (100 nM), AZD1775 (50 nM) or combination of prexasertib plus AZD1775 for 24hrs. The vertical axis indicates the EdU intensity and the horizontal axis indicates the DNA content.

(E) Quantification of the FACS data in panel C. (Top panel) Graphs of the percentage of BrdU-negative S phase cells of the total S phase cells. (Bottom panel) Graphs of the percentage of cells in G2/M. Error bars indicate standard errors and P-values were calculated using the Student t-test (n=3, ***p < 0.001).

Supplemental figure 4, related to Figure 4

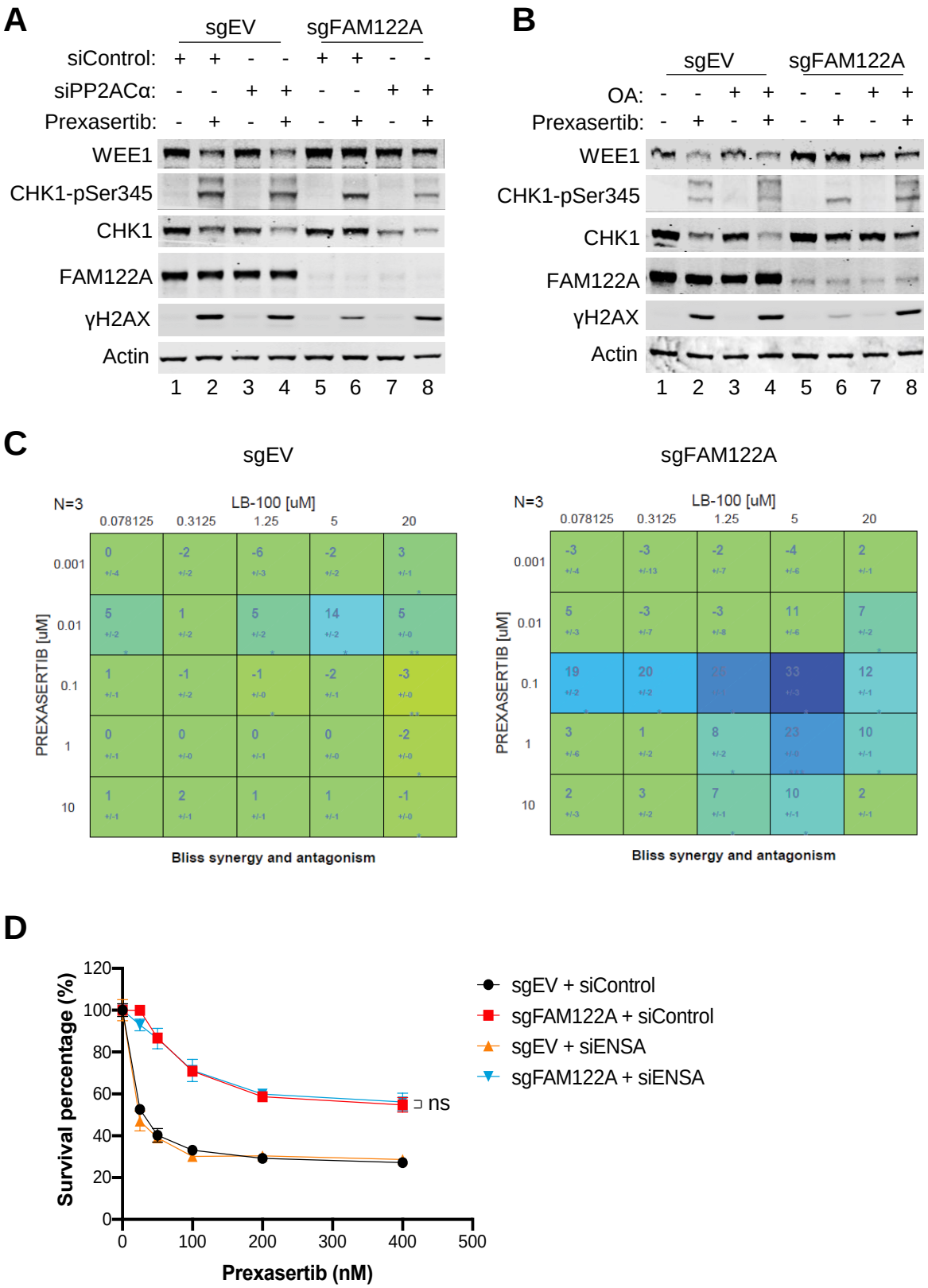


Figure S4. FAM122A regulates WEE1 stability by targeting PP2A-B55α, related to figure 4.

(A) Western blots of the Control and FAM122A-KO A549 cells treated with prexasertib (100 nM) for 24hrs after treatment with siControl or siPP2ACα.

(B) Western blots of the control and FAM122A-KO A549 cells treated with prexasertib (100nM) and Okadaic Acid (OA, 1uM).

(C) Synergy between prexasertib and PP2A inhibitor (LB-100) in control (left panel) and FAM122A-KO (right panel) A549 cells. Control and FAM122A-KO A549 cells were exposed to the graded concentrations of LB-100 and prexasertib for 5days. The cell survival was analyzed using CellTiter-Glo reagent and synergy/antagonism plots were generated using Combenefit. Bliss synergy/antagonism levels in a matrix format are shown.

(D) Survival plots of Control and FAM122A-KO A549 cells treated with prexasertib for 5 days, following transfection with siControl or siENSA. Statistical analysis was performed using two-way ANOVA.

Supplemental figure 5, related to Figure 5

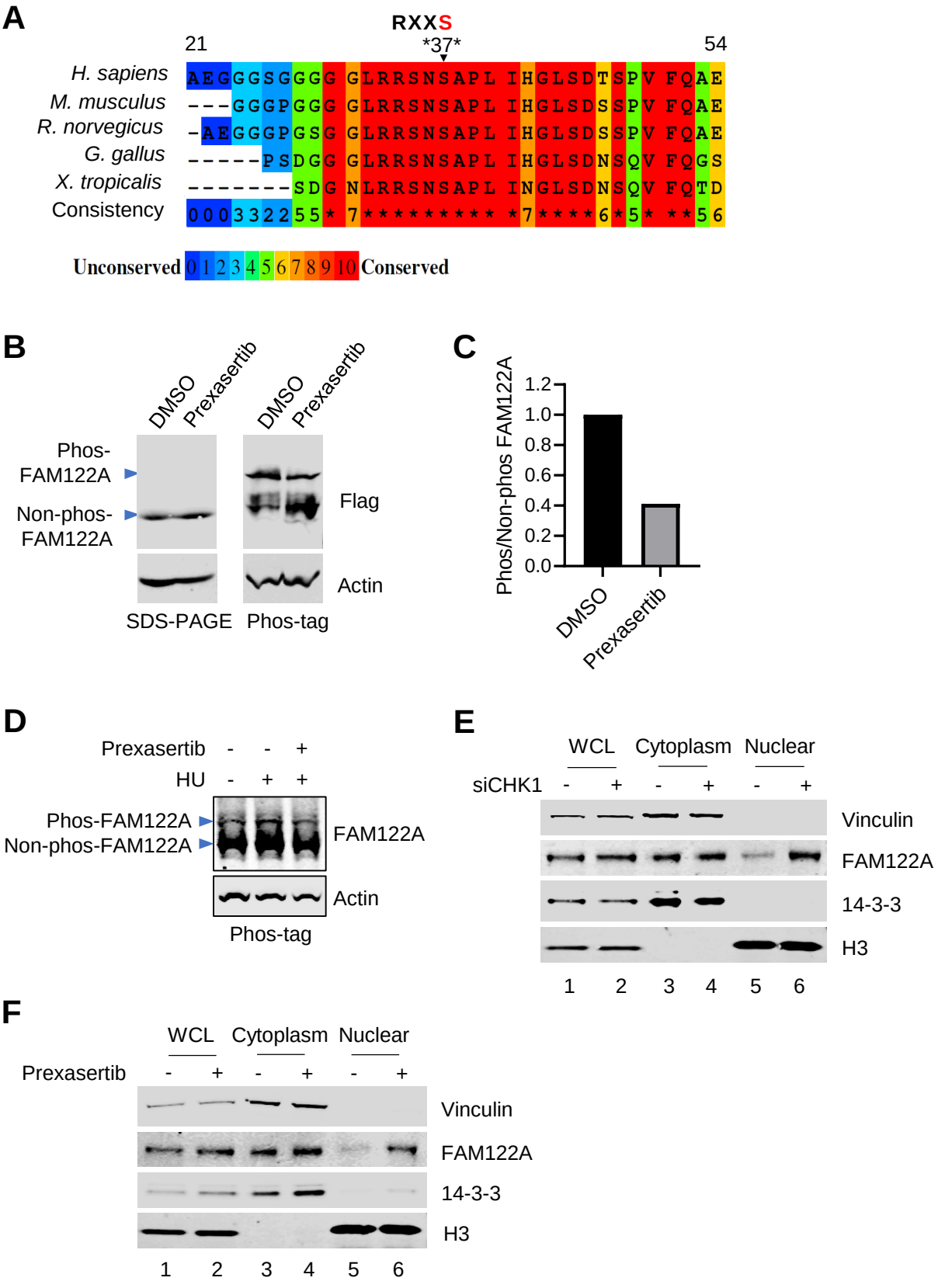


Figure S5. Chk1 phosphorylates FAM122A at Ser37, related to figure 5.

(A) Alignment of FAM122A protein sequence from different species. Conserved CHK1 phosphodegrons (Ser37) of FAM122A are shown.

(B) Western blots of stably expressing FAM122A-Flag 293T cells treated with prexasertib (50 nM) for 24hrs. Samples were separated on a normal SDS-PAGE gel (left) and a Phos-tag labeled SDS-PAGE gel (right). Phosphorylated and unphosphorylated FAM122A are labeled.

(C) Quantification of the data in panel B. showing the ratio of phosphorylated FAM122A compared to the non-phosphorylated FAM122A.

(D) Western blots of the lysates from A549 cells treated with HU (1uM) or/and prexasertib (100nM), Samples were separated on a 12% Phos-tag-SDS PAGE gel. Phosphorylated and unphosphorylated FAM122A are labeled.

(E) Western blots of the indicated proteins in subcellular fractions of A549 cells following transfection with siControl or siFAM122A for 48hrs.

(F) Western blots of the indicated proteins in subcellular fractions of A549 cells following prexasertib (100nM) treatment for 24hrs.

Supplemental figure 6, related to Figure 6

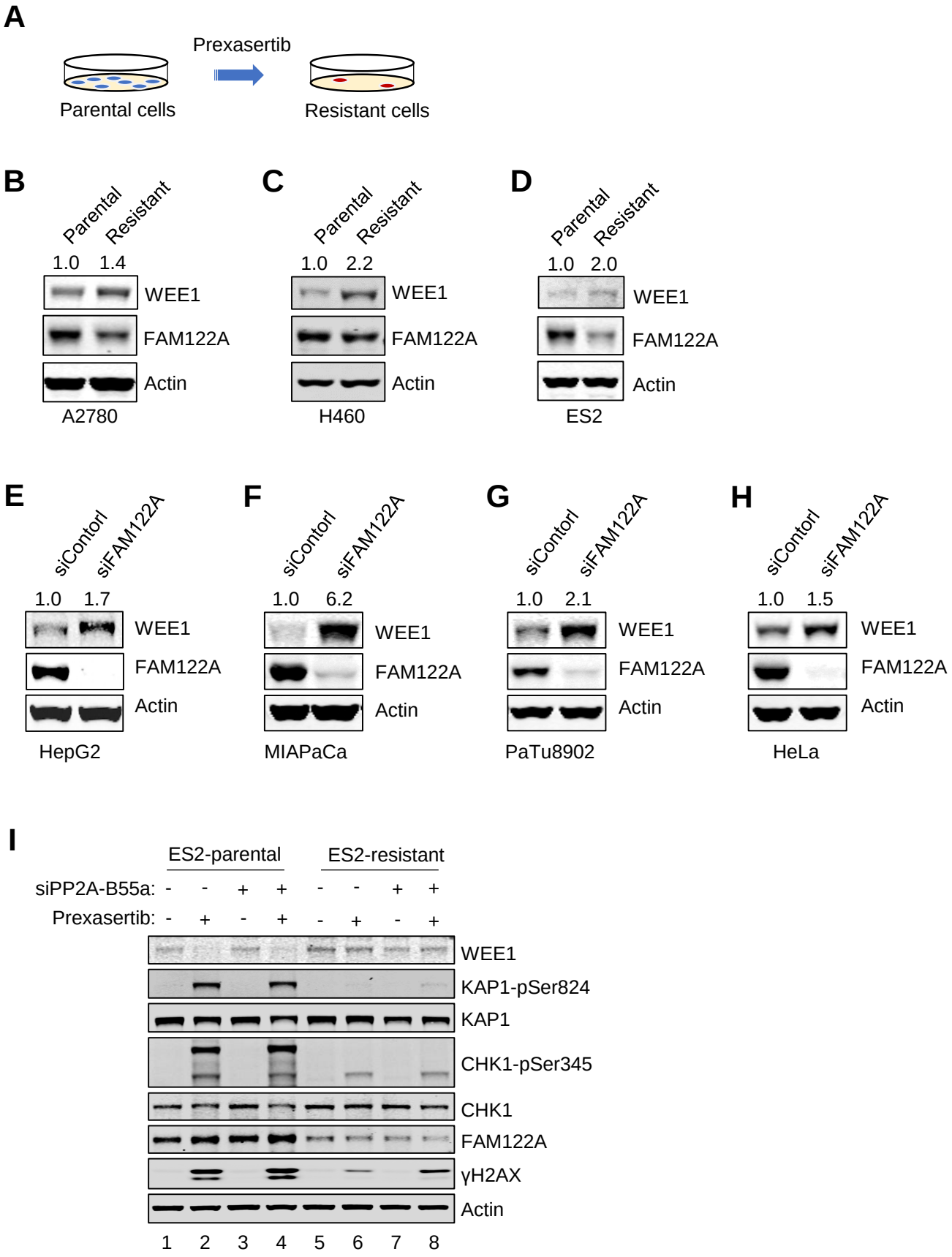


Figure S6. Cancer cells with acquired CHK1i resistance exhibit low levels of FAM122A and high levels of WEE1, related to figure 6.

(A) Schematic of acquired CHK1i (Prexasertib) resistance in cancer cells. Prexasertib sensitive parental cells were exposed to prexasertib for 2-3 months and resistant cells were derived.

(B) Western blots of the lysates from parental and prexasertib resistant A2780 cells. The relative level of WEE1 were quaitified as indicated.

(C) Western blots of the lysates from parental and prexasertib resistant H460 cells. The relative level of WEE1 were quaitified as indicated.

(D) Western blots of the lysates from parental and prexasertib resistant ES2 cells. The relative level of WEE1 were quaitified as indicated.

(E) Western blots of the lysates from HepG2 cells treated with siControl or siFAM122A for 48hrs. The relative level of WEE1 were quaitified as indicated.

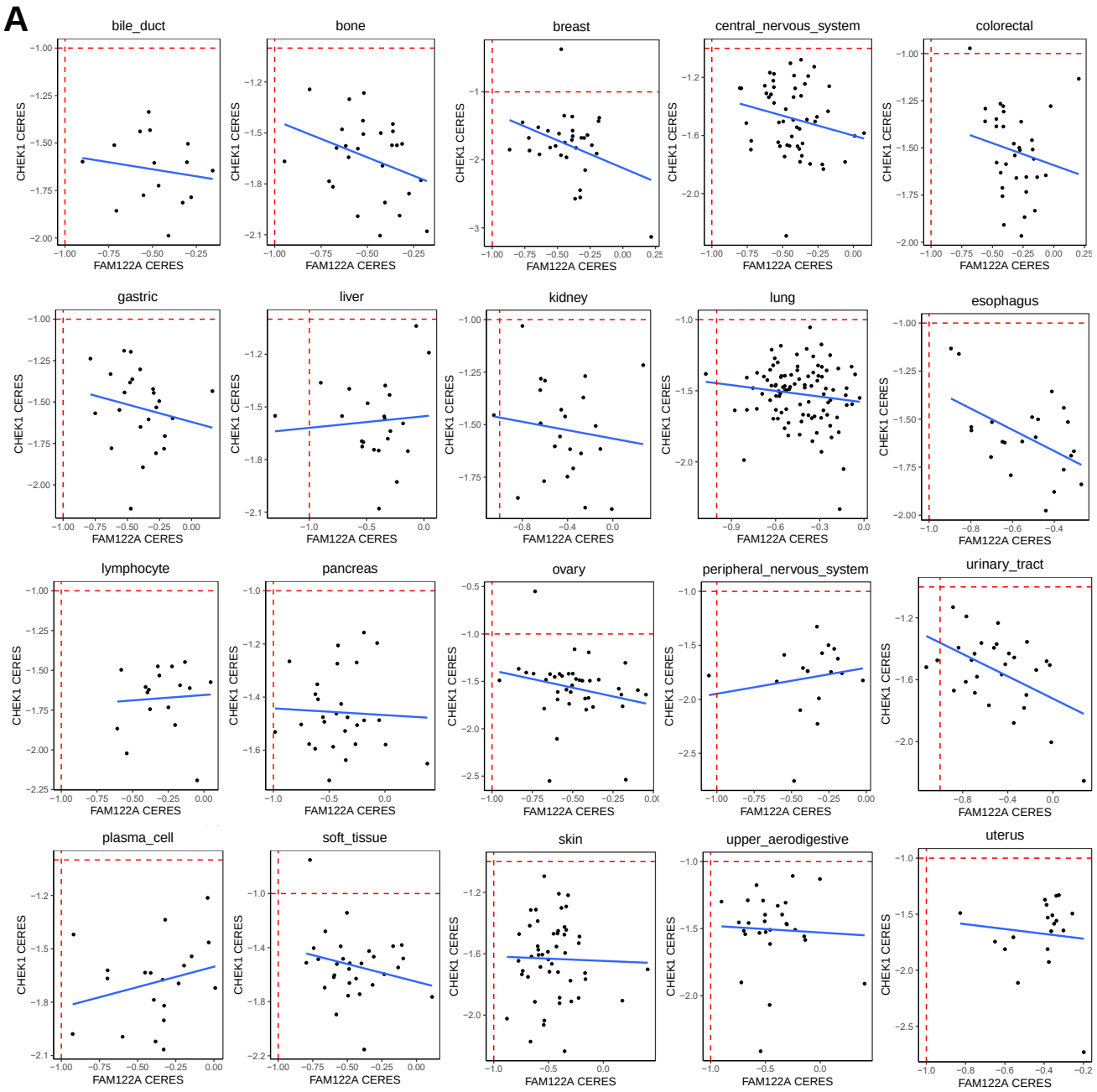
(F) Western blots of the lysates from MIAPaCa cells treated with siControl or siFAM122A for 48hrs. The relative level of WEE1 were quaitified as indicated.

(G) Western blots of the lysates from PaTu8902 cells treated with siControl or siFAM122A for 48hrs. The relative level of WEE1 were quaitified as indicated.

(H) Western blots of the lysates from HeLa cells treated with siControl or siFAM122A for 48hrs. The relative level of WEE1 were quaitified as indicated.

(I) Western blots of the parental and prexasertib resistant ES2 cells treated with prexasertib (100 nM) for 24hrs after treatment with siControl or siPP2A-B55α.

Supplemental figure 7, related to Figure 7



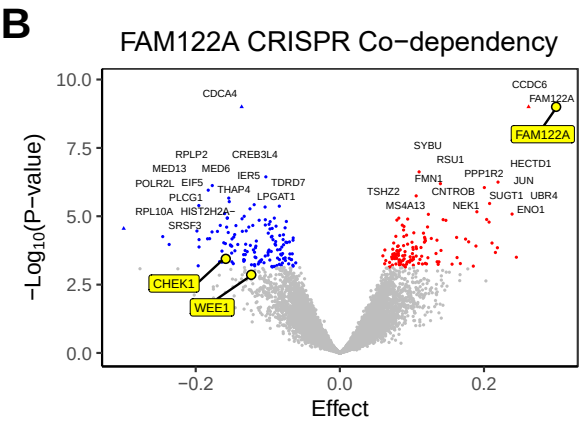


Figure S7. CHEK1 dependency inversely correlates with FAM122A dependency, related to figure 7.

(A) Score patterns of 20 cancer types show the relationship of FAM122A dependency and CHEK1 dependency.

(B) Volcano plot of FAM122A CRISPR co-dependency genes. Value >0 means positive FAM122A co-dependency, value <0 means inverse FAM122A co-dependency.