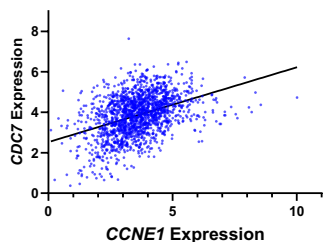
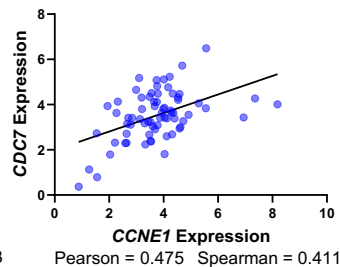


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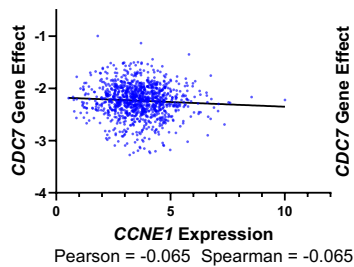


Breast

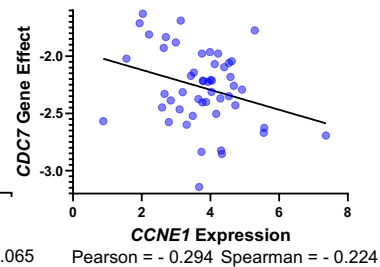


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Pan-cancer

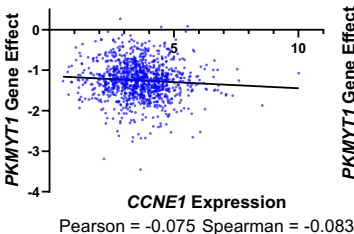


Breast

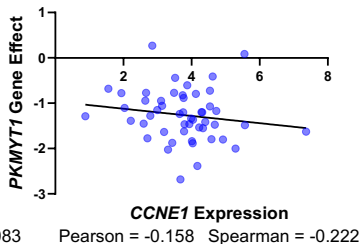


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Pan-cancer

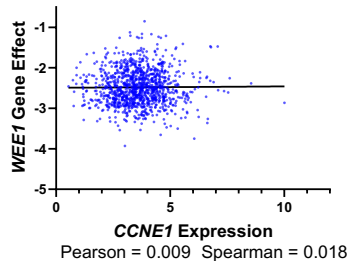


Breast

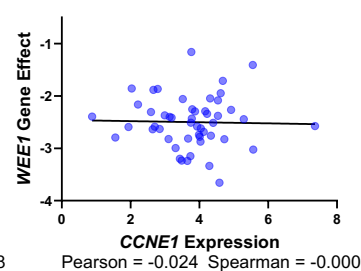


D.

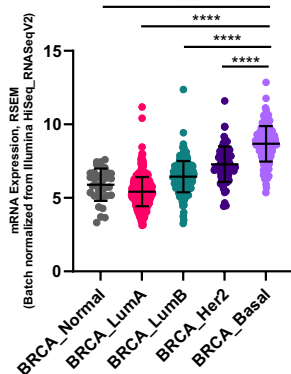
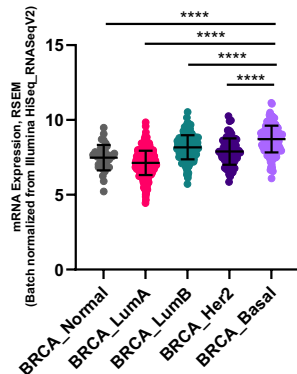
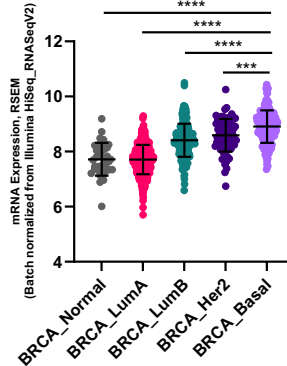
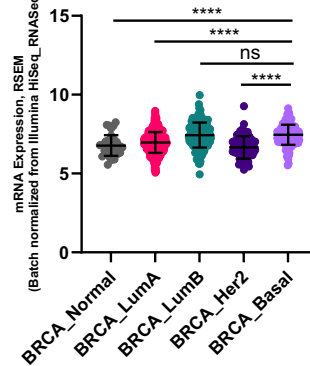
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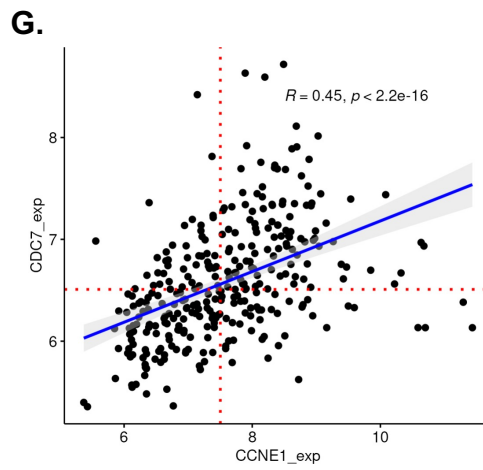
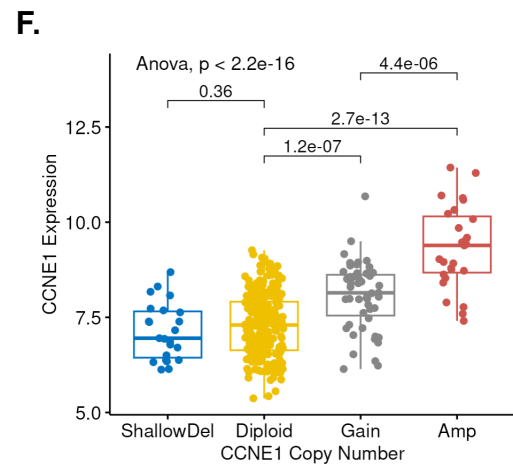


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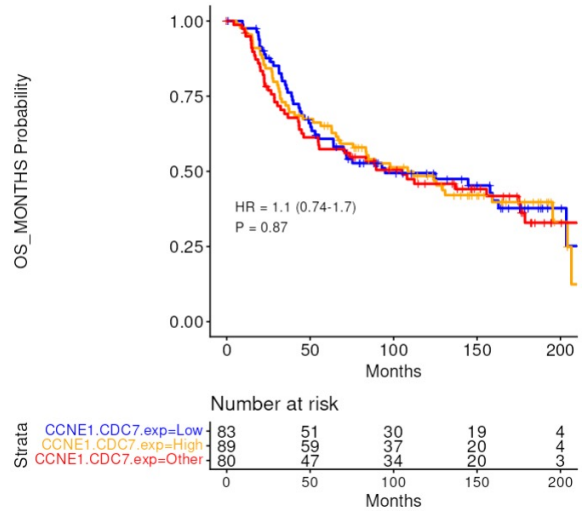
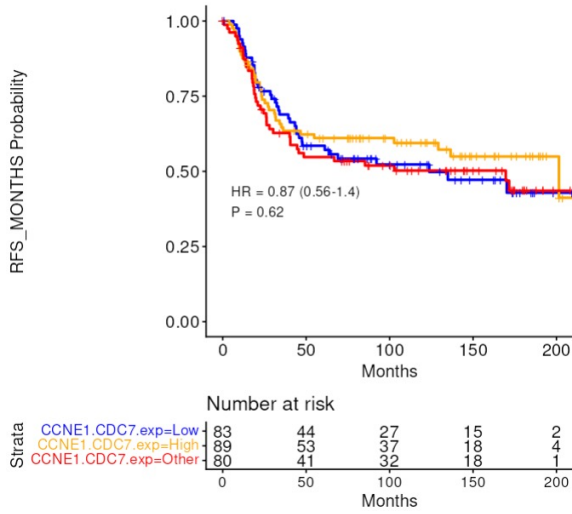


E.

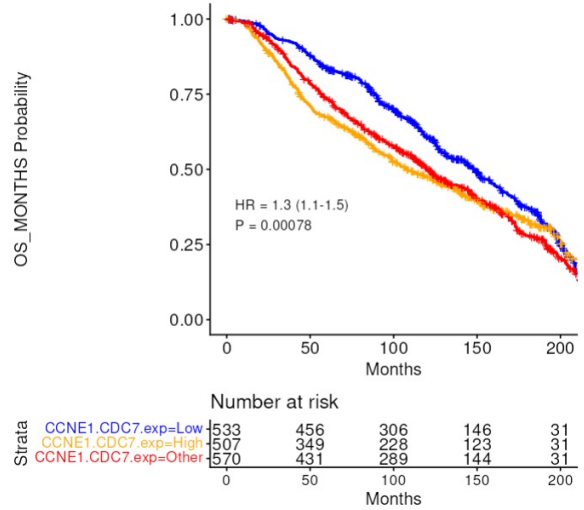
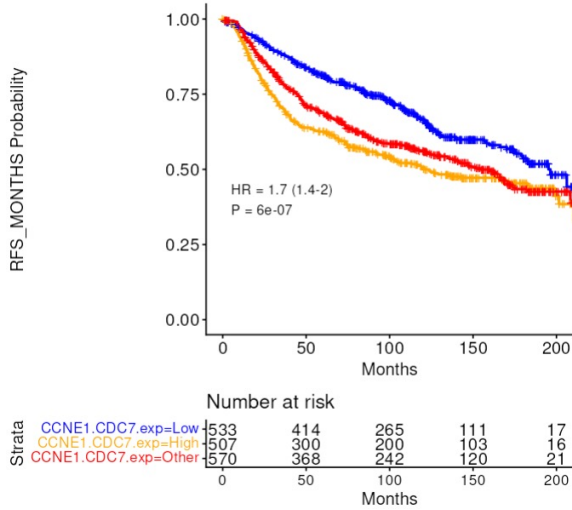
CCNE1*CDC7**DBF4**DBF4B*



H. TNBC



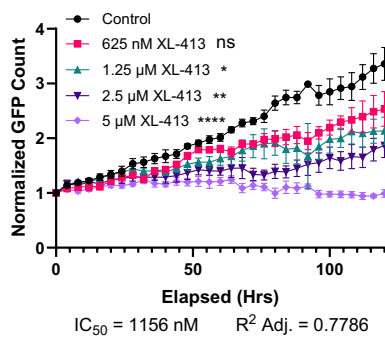
All Subtypes



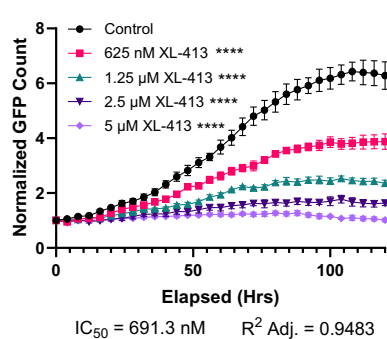
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CCNE1 Amplified

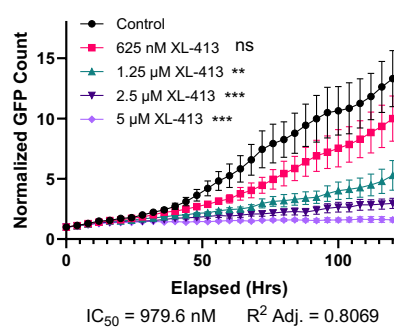
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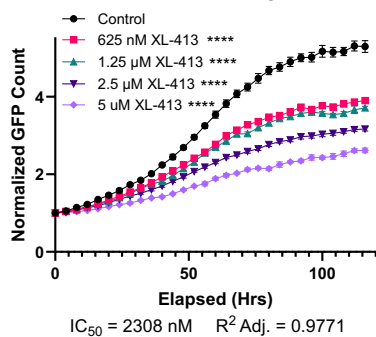
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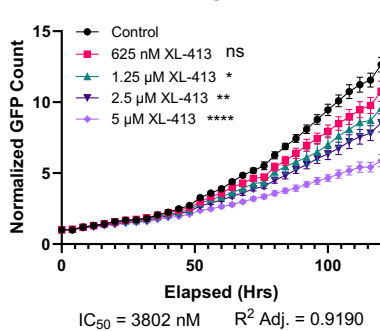
HCC-1806

Non-CCNE1 Amplified

MDA-MB-231



MCF-7

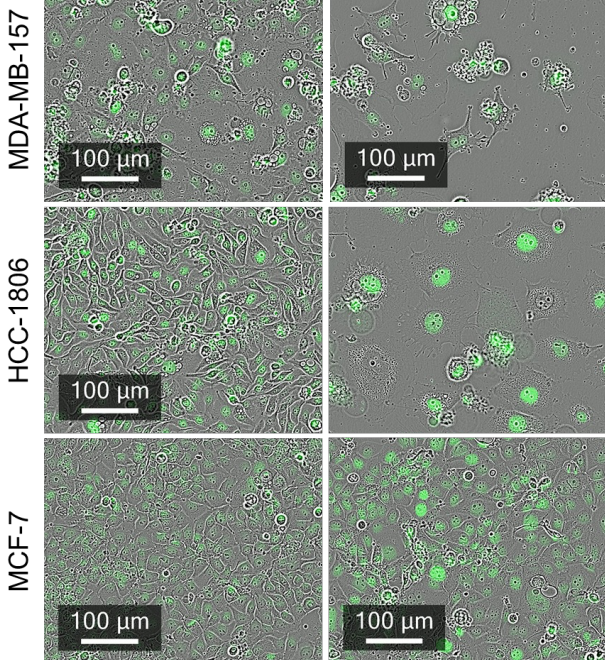


J.

TAK-931

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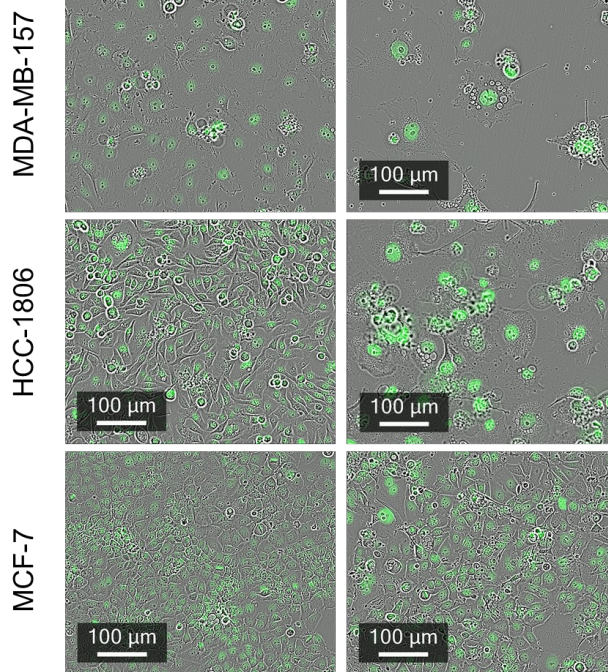
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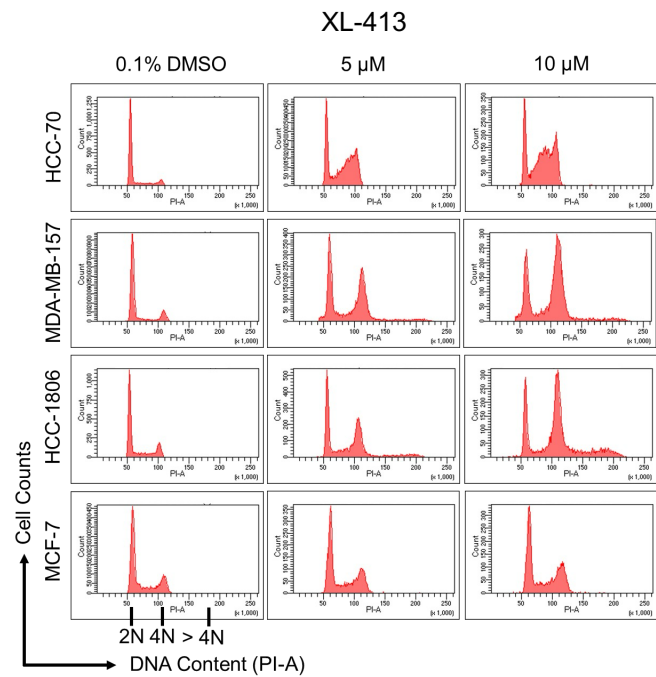
XL-413

0.1% DMSO

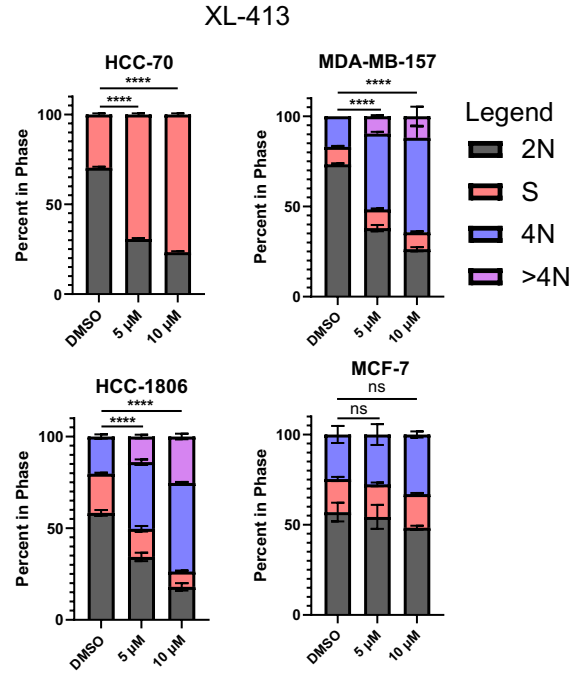
5 μM



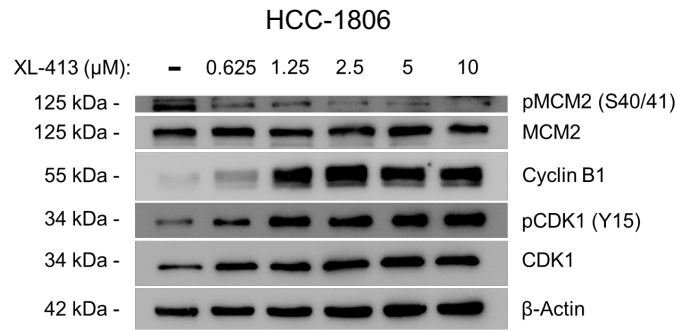
K.



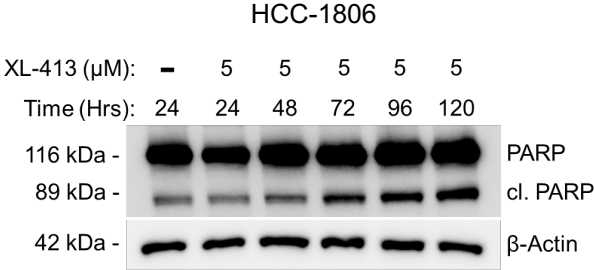
L.



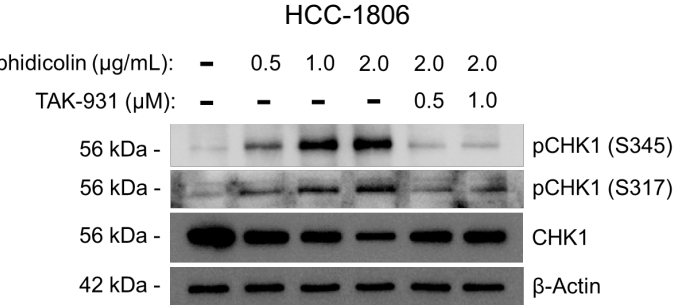
M.



N.



O.



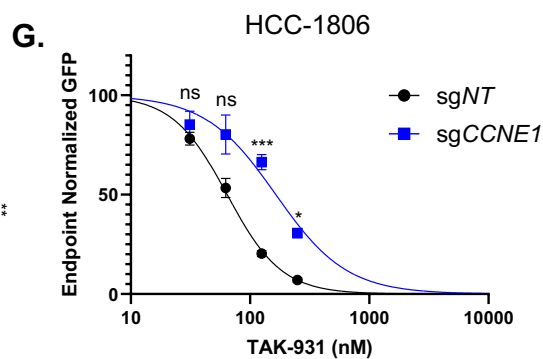
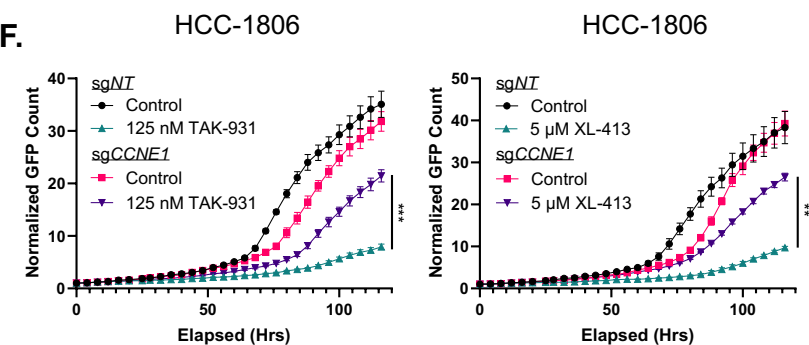
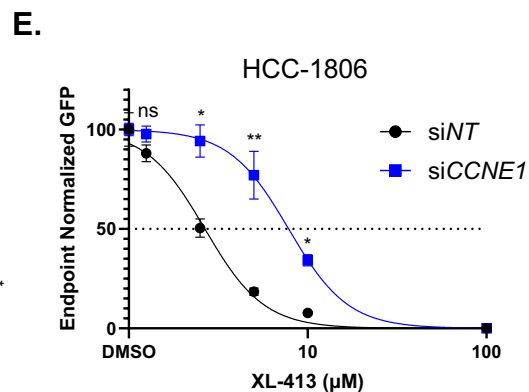
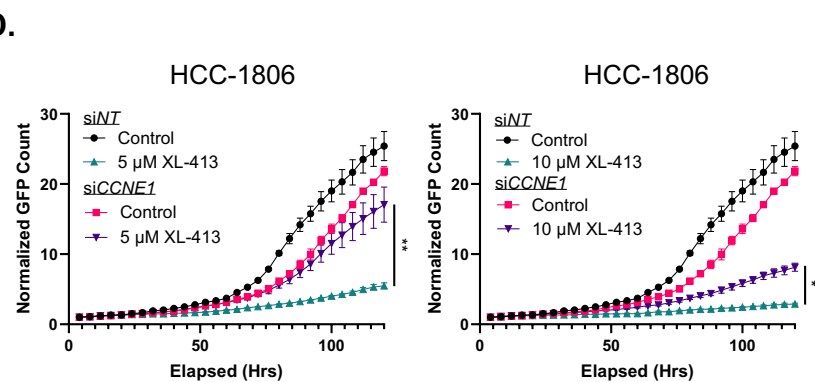
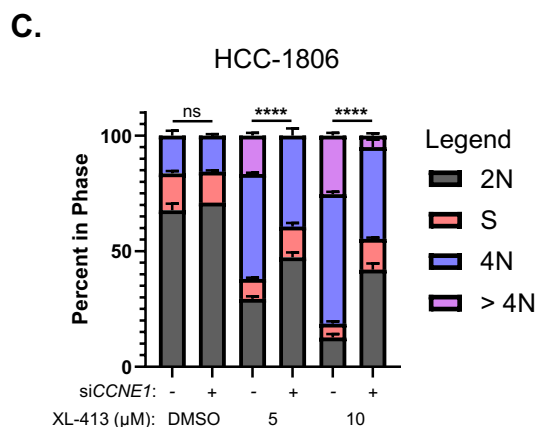
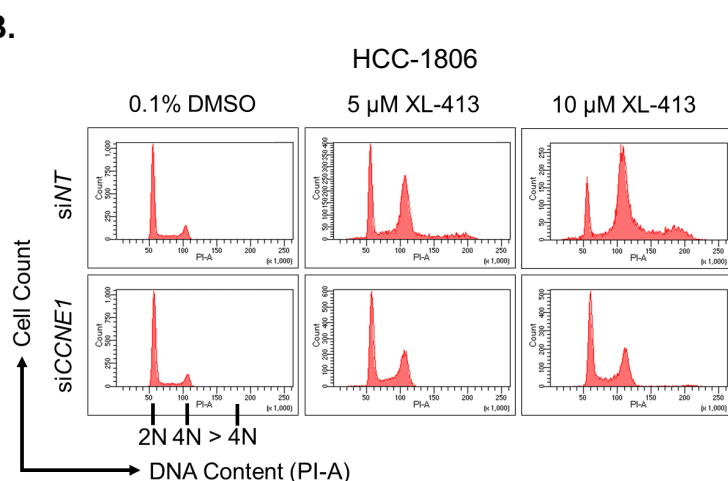
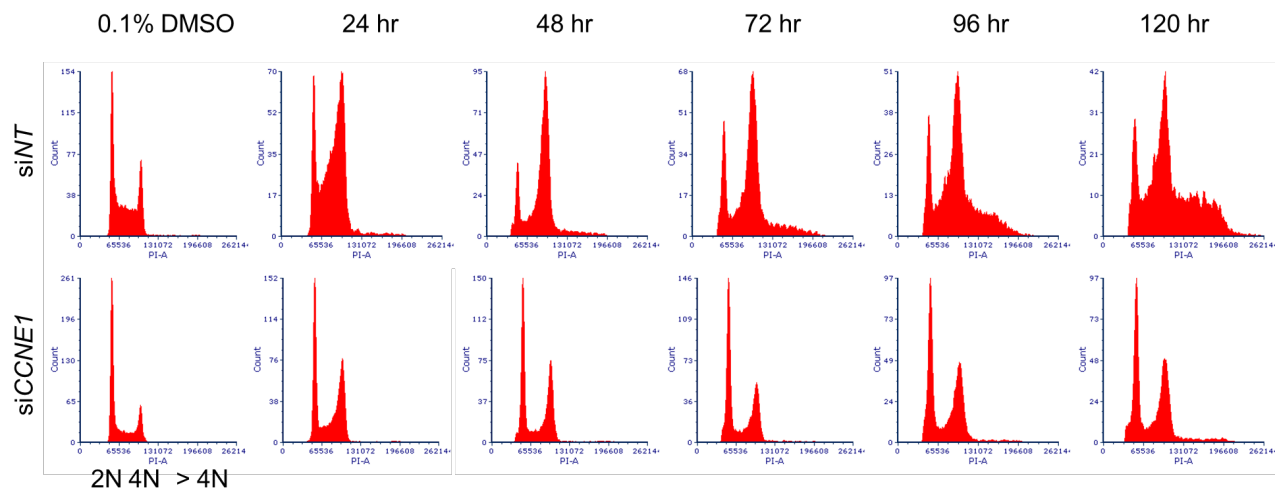
Supplemental Figure legends

Figure S1. CDC7 Inhibition is Efficacious in *CCNE1* Amplified TNBC.

A, Line graph derived from the DepMap project for *CDC7* expression as a function of *CCNE1* expression in the pan-cancer (left) or breast cancer (right) settings. **B**, Line graph derived from the DepMap project for *CDC7* gene effect as a function of *CCNE1* expression in the pan-cancer (left) or breast cancer (right) settings. **C**, Line graph derived from the DepMap project for *PKMYT1* gene effect as a function of *CCNE1* expression in the pan-cancer (left) or breast cancer (right) settings. **D**, Line graph derived from the DepMap project for *WEE1* gene effect as a function of *CCNE1* expression in the pan-cancer (left) or breast cancer (right) settings. **E**, Bar graphs derived from the cancer genome atlas for *CCNE1*, *CDC7*, *DBF4*, and *DBF4B* transcript levels across different breast cancer subtypes (BRCA_Normal, n=36; BRCA_LumA, n=499; BRCA_LumB, n=197; BRCA_Her2, n=78; BRCA_Basal, n=171; one-way ANOVA with Tukey's multiple comparisons test; lines indicate mean with SD). **F**, *CCNE1* status and associated expression in TNBC patients from Metabric. **G**, Correlation of *CCNE1* and *CDC7* expression in TNBC patients from Metabric. **H**, Kaplan-Meier plots of recurrence-free survival (RFS, left) and overall survival (OS, right) of TNBC (top) and all breast cancer patients (bottom) dichotomized by median *CCNE1* and *CDC7* expression. HR, hazard ratio. **I**, *CCNE1* amplified or non-amplified breast cancer cells treated with serial concentrations of XL-413 for five days and monitored with Cellcyte or Incucyte live cell proliferative software (n=4; one-way ANOVA with Dunnett's multiple comparisons test comparing each concentration to DMSO control using endpoint normalized GFP counts; error bars represent SEM). **J**, Representative images captured at the end of five-day live cell proliferative analyses from cells treated with 250 nM TAK-931 (left) or 5 μ M XL-413 (right). **K**, Representative cell cycle profiling of the indicated cell lines following 48 hr treatment with XL-413. **L**, Quantification of cell cycle state from indicated cell lines treated with 48 hr XL-413 (n=3; one-way ANOVA with Dunnett's multiple comparisons

test comparing each concentration to DMSO control with significance determined for $\geq 4N$ DNA (for HCC-70, significance determined for $>2N$ DNA); error bars represent SD). **M**, Representative western blot of HCC-1806 cells for pMCM2 (S40/41) and mitotic inhibition following 48 hr XL-413 treatment. **N**, Representative western blot of HCC-1806 cells for cleaved PARP following time-dependent exposure to 5 μM XL-413. **O**, Representative western blot of HCC-1806 cells for pCHK1 (S317, S345) following 24 hr aphidicolin treatment with or without TAK-931.

HCC-1806 (250 nM TAK-931)



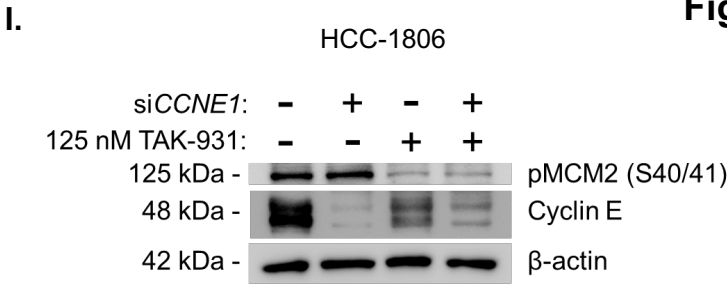
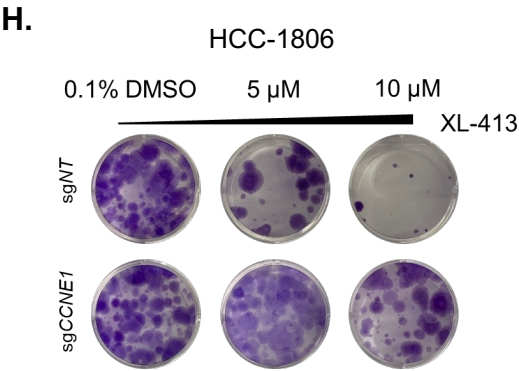
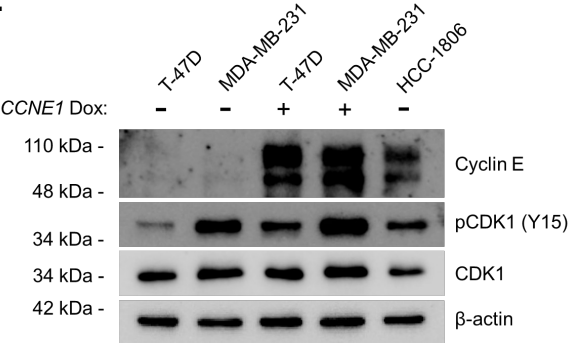


Figure S2. *CCNE1* Knockdown Reverses Sensitivity to CDC7 Inhibition.

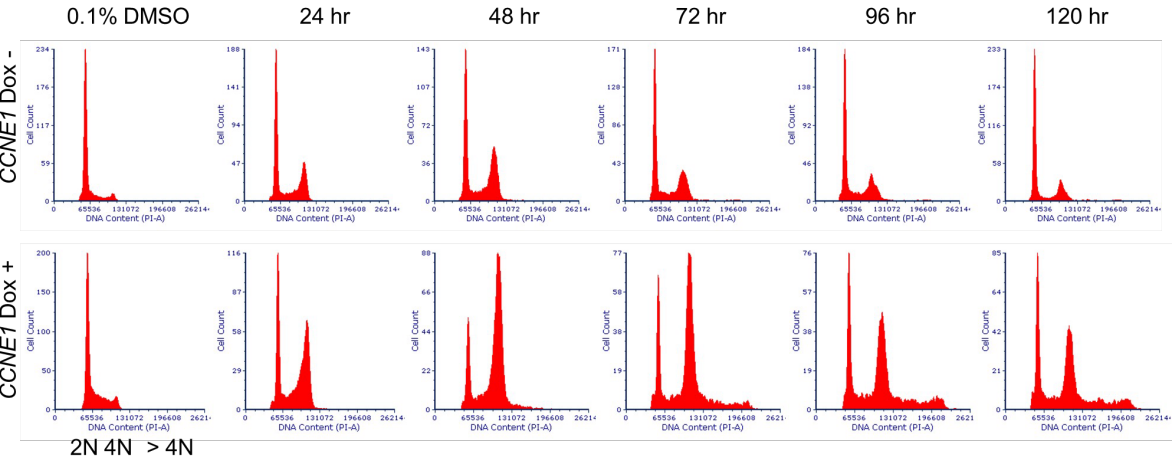
A, Representative cell cycle profiling of HCC-1806 cells following *CCNE1* transcriptional knockdown and time-dependent exposure to 250 nM TAK-931. **B**, Representative cell cycle profiling of HCC-1806 cells following *CCNE1* transcriptional knockdown and 48 hr treatment with indicated concentrations of XL-413. **C**, Quantification of cell cycle state from HCC-1806 cells following *CCNE1* transcriptional knockdown and 48 hr treatment with indicated concentrations of XL-413 (n=3; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect with significance determined for $\geq 4N$ DNA; error bars represent SD). **D**, HCC-1806 cells following *CCNE1* knockdown and treatment with 5 μ M (left) or 10 μ M (right) XL-413 for five days and monitored with Cellcyte or Incucyte live cell proliferative software (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM). **E**, IC₅₀ curves derived from live cell proliferative analyses as described in panel **D** (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM). **F**, HCC-1806 CRISPR *CCNE1* isogenic pair following treatment with 125 nM TAK-931 (left) or 5 μ M XL-413 (right) for five days and monitored with Cellcyte or Incucyte live cell proliferative software (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM). **G**, IC₅₀ curves derived from TAK-931 live cell proliferative analysis as described in panel **F** (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM). **H**, Representative colony outgrowth assay from HCC-1806 cells with CRISPR deleted *CCNE1* or non-targeting control and treatment with indicated concentrations of XL-413 for three cycles, with treatment replenishment every five days. **I**, Representative western blot of HCC-1806 cells for pMCM2 (S40/41) and cyclin E1 following *CCNE1* transcriptional knockdown and 24 hr treatment with 125 nM TAK-931.

A.



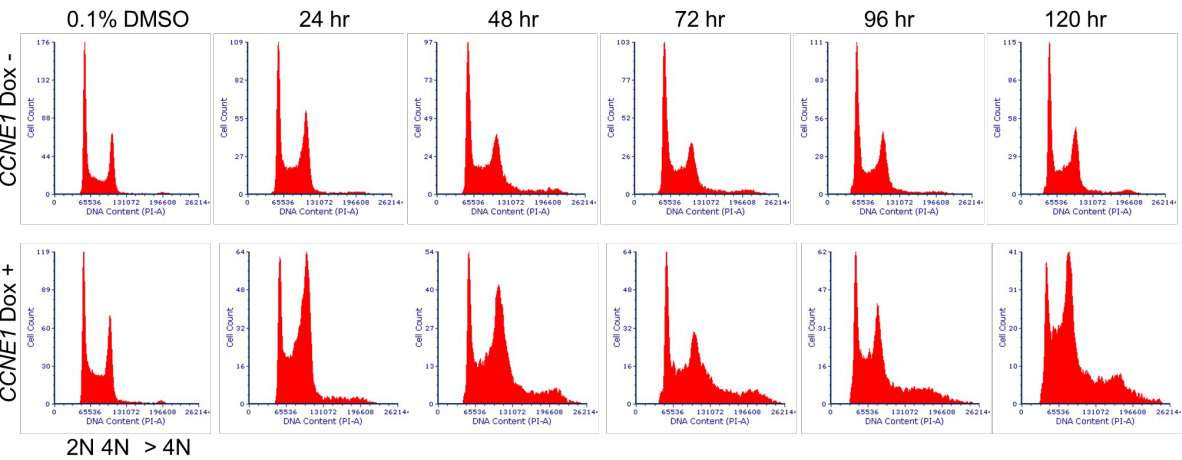
B.

T-47D (62.5 nM TAK-931)

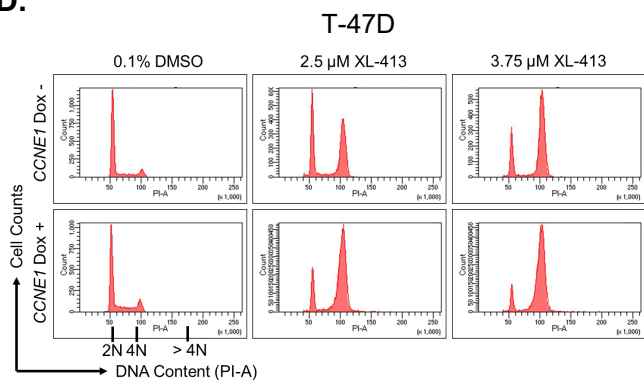


C.

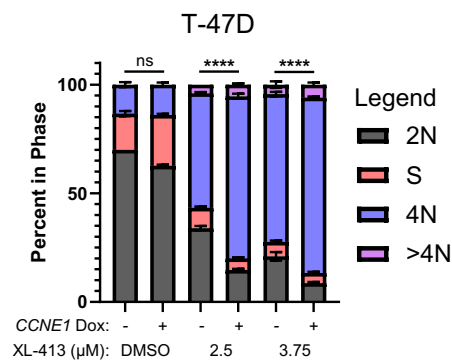
MDA-MB-231 (250 nM TAK-931)



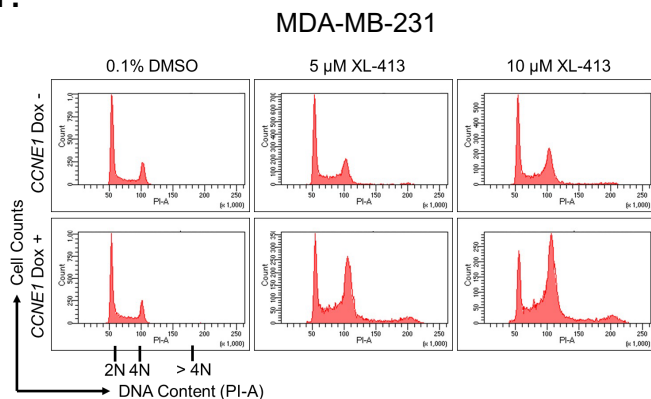
D.



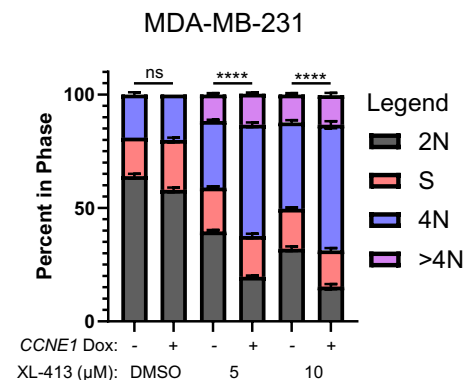
E.



F.

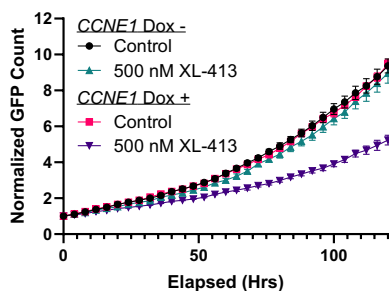


G.

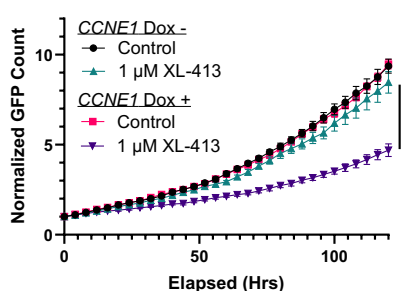


H.

T-47D

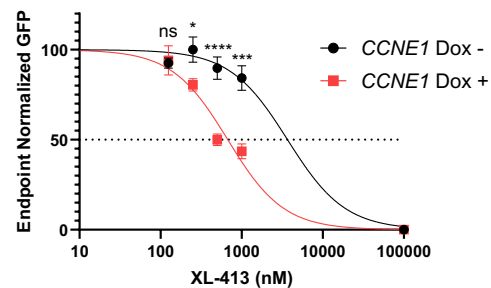


T-47D



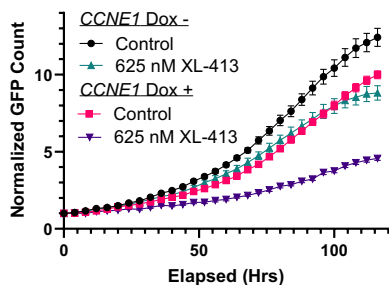
I.

T-47D

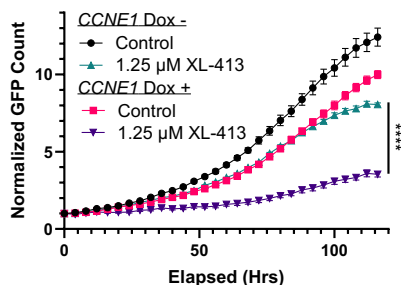


J.

MDA-MB-231

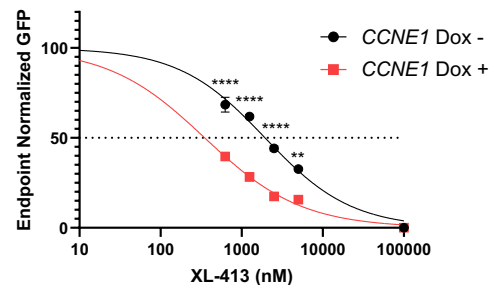


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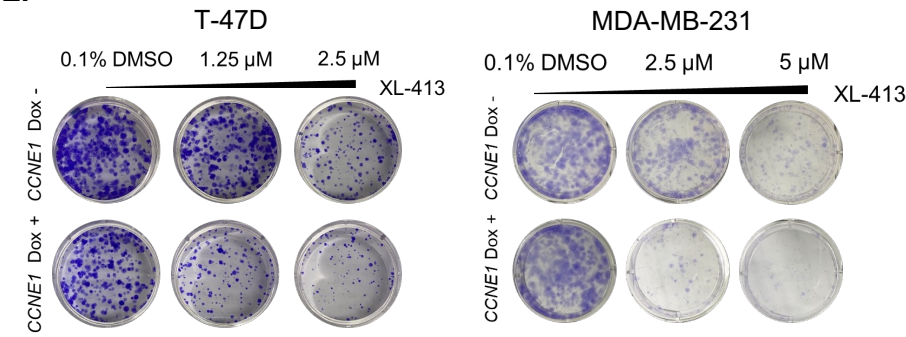


K.

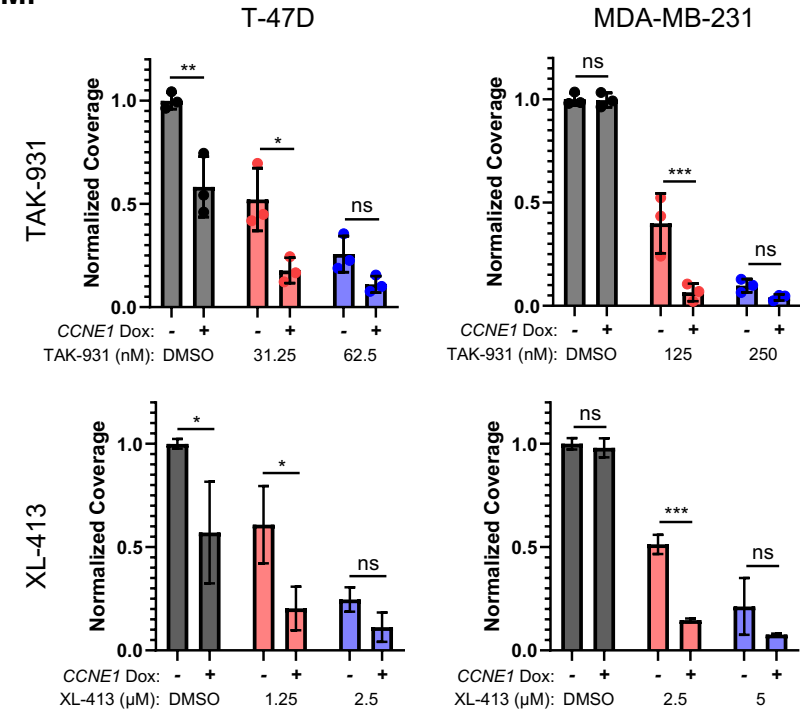
MDA-MB-231



L.



M.



N.

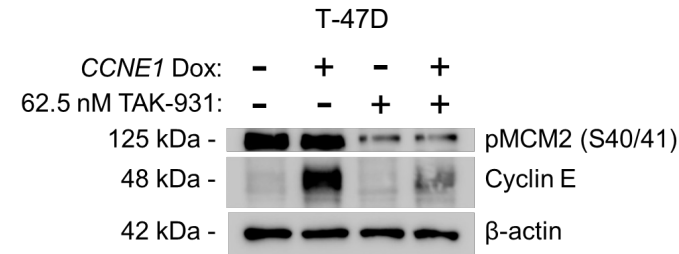


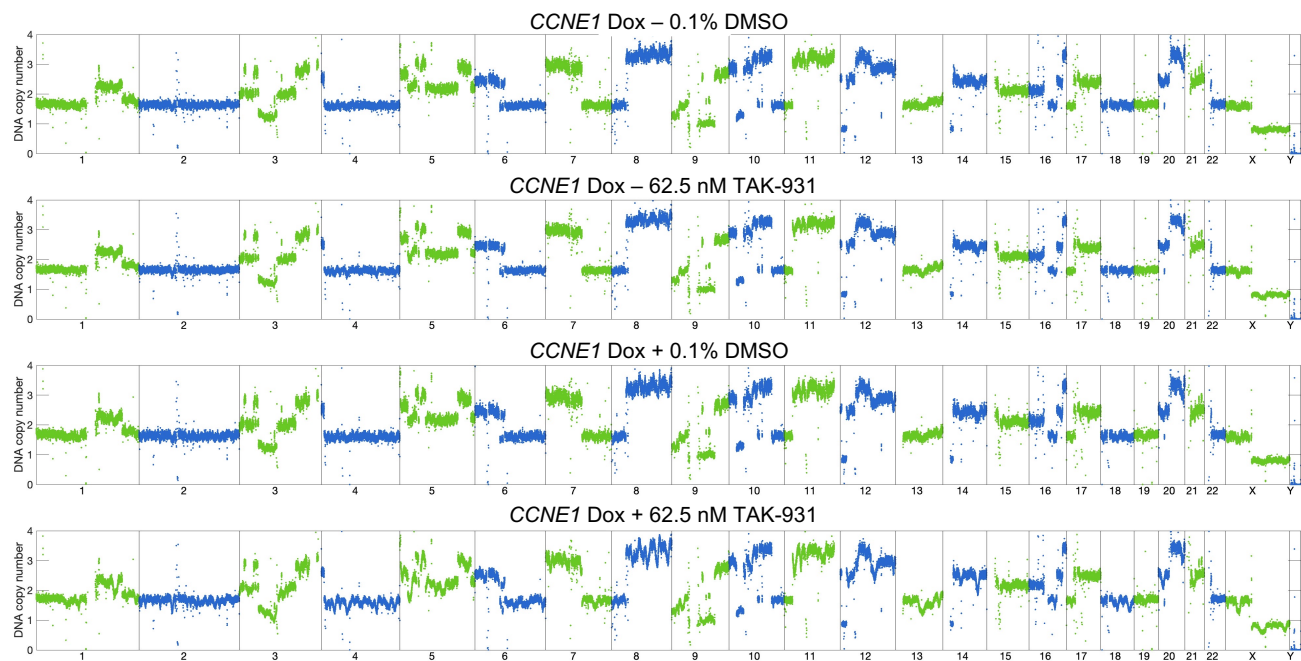
Figure S3. Cyclin E Overexpression Enhances Sensitivity to CDC7 Inhibition.

A, Representative western blot of indicated cell lines for pCDK1 (Y15) and cyclin E1 following 24 hr induction of *CCNE1*. **B**, Representative cell cycle profiling of T-47D cells following cyclin E overexpression and time-dependent exposure to 62.5 nM TAK-931. **C**, Representative cell cycle profiling of MDA-MB-231 cells following cyclin E overexpression and time-dependent exposure to 250 nM TAK-931. **D**, Representative cell cycle profiling of T-47D cells following cyclin E overexpression and 48 hr treatment with indicated concentrations of XL-413. **E**, Quantification of cell cycle state from T-47D cells following cyclin E overexpression and 48 hr treatment with indicated concentrations of XL-413 (n=3; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect with significance determined for $\geq 4N$ DNA; error bars represent SD). **F**, Representative cell cycle profiling of MDA-MB-231 cells following cyclin E overexpression and 48 hr treatment with indicated concentrations of XL-413. **G**, Quantification of cell cycle state from MDA-MB-231 cells following cyclin E overexpression and 48 hr treatment with indicated concentrations of XL-413 (n=3; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect with significance determined for $\geq 4N$ DNA; error bars represent SD). **H**, Representative T-47D cells following cyclin E overexpression and treatment with 500 nM (left) or 1 μ M (right) XL-413 for five days and monitored with Cellcyte or Incucyte live cell proliferative software (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM). **I**, IC₅₀ curves derived from live cell proliferative analyses as described in panel **H** (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM). **J**, Representative MDA-MB-231 cells following cyclin E overexpression and treatment with 625 nM (left) or 1.25 μ M (right) XL-413 for five days and monitored with Cellcyte or Incucyte live cell proliferative software (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM). **K**,

IC₅₀ curves derived from live cell proliferative analyses as described in panel **J** (n=4; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized GFP counts; error bars represent SEM) . **L**, Representative colony outgrowth assays from T-47D (left) and MDA-MB-231 (right) cells following cyclin E overexpression and treatment with indicated concentrations of XL-413 for three cycles, with doxycycline and treatment replenishment every five days. **M**, Normalized well coverage by cell colonies from TAK-931 and XL-413 colony outgrowth assays in T-47D and MDA-MB-231 cells (n=3; two-way ANOVA with Tukey's multiple comparisons test accounting for gene and drug effect using endpoint normalized well coverage; error bars represent SD). **N**, Representative western blot of T-47D cells for pMCM2 (S40/41) and cyclin E1 following cyclin E overexpression and 24 hr treatment with 62.5 nM TAK-931.

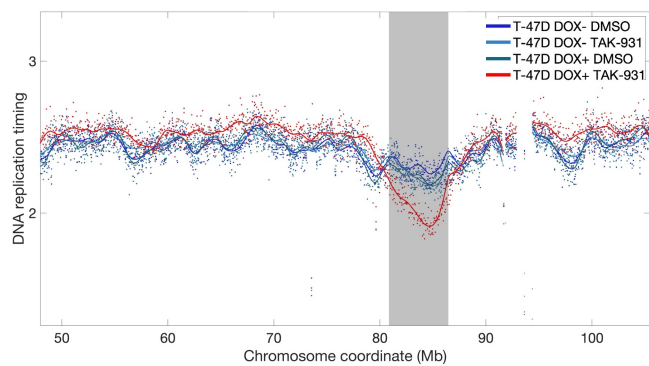
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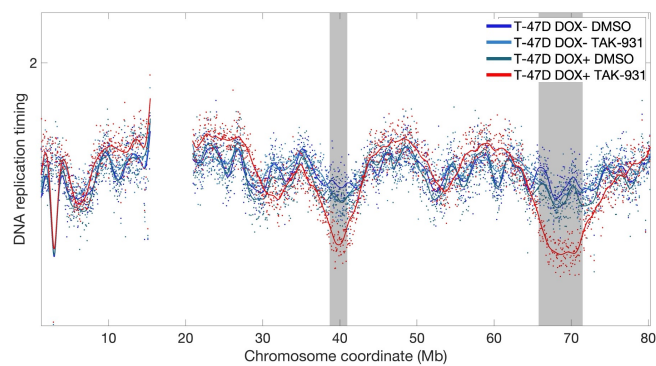


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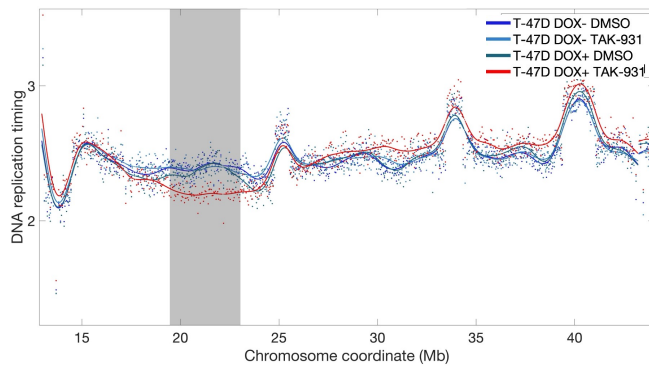
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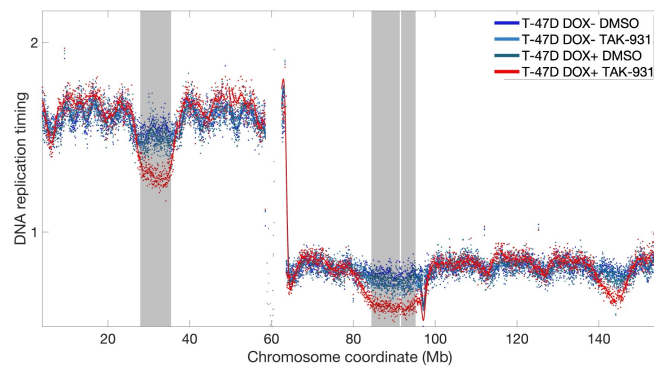
Chromosome 18



Chromosome 21

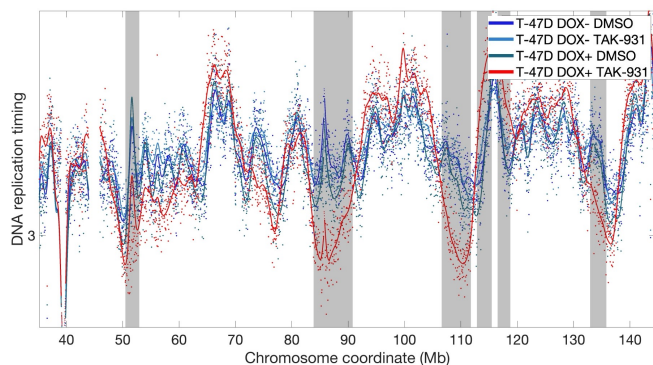


Chromosome X

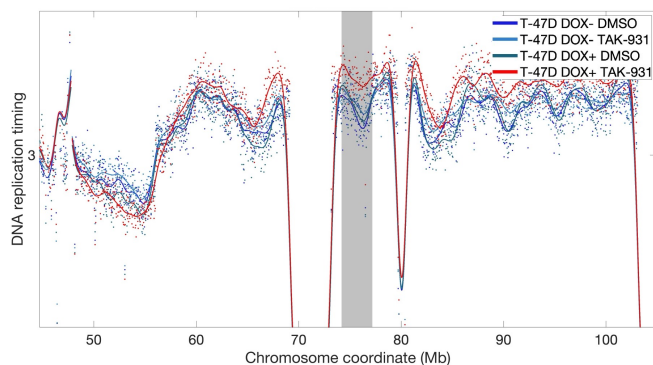


c.

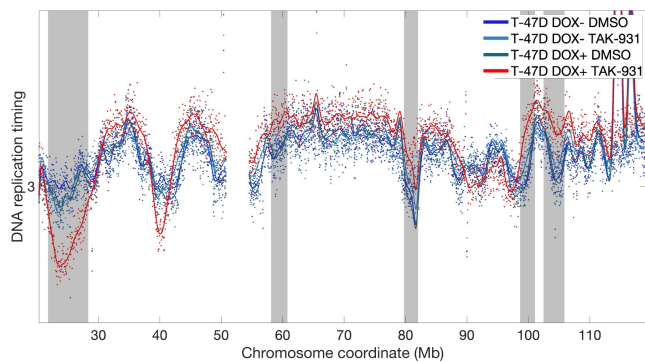
Chromosome 8



Chromosome 10



Chromosome 11



Chromosome 12

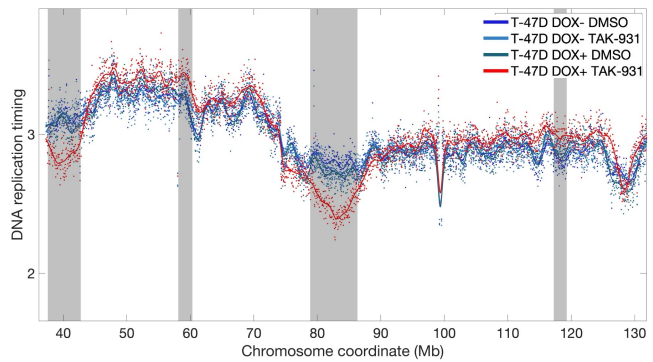
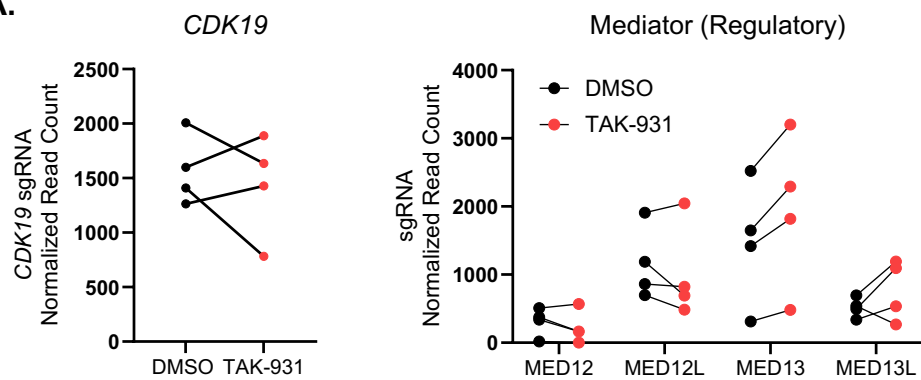


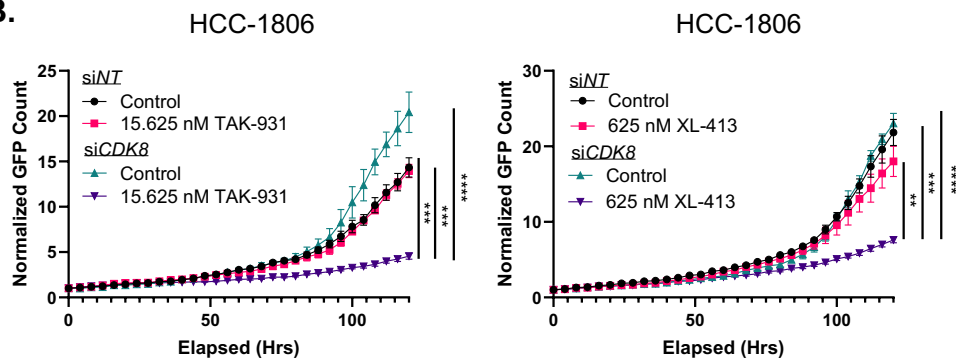
Figure S4. CDC7 Inhibition Associates with Delayed Replication Timing in Cyclin E Deregulated Cells.

A, Representative individual genome-wide replication timing profiles from T-47D cells following cyclin E overexpression and 48 hr treatment with 62.5 nM TAK-931. **B,** Representative individual chromosome replication timing profiles from T-47D cells following cyclin E overexpression and 48 hr treatment with 62.5 nM TAK-931. **C,** Representative individual chromosome replication timing profiles from T-47D cells following cyclin E overexpression and 48 hr treatment with 62.5 nM TAK-931.

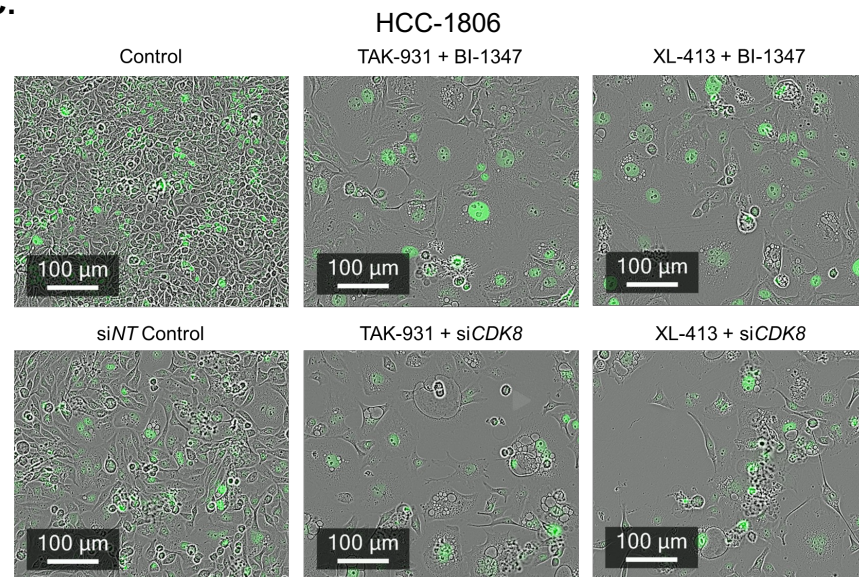
A.



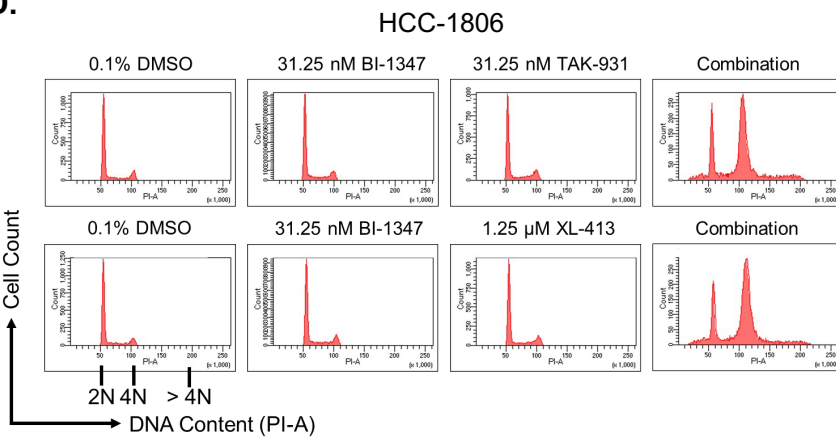
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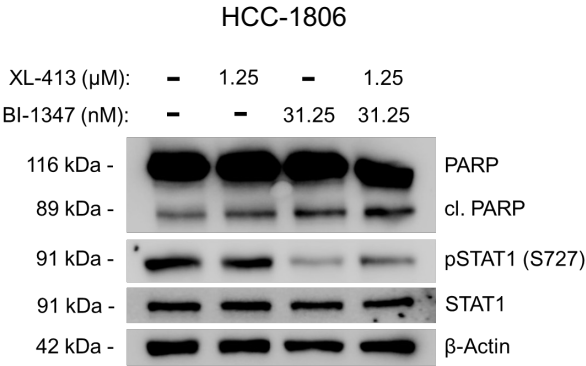
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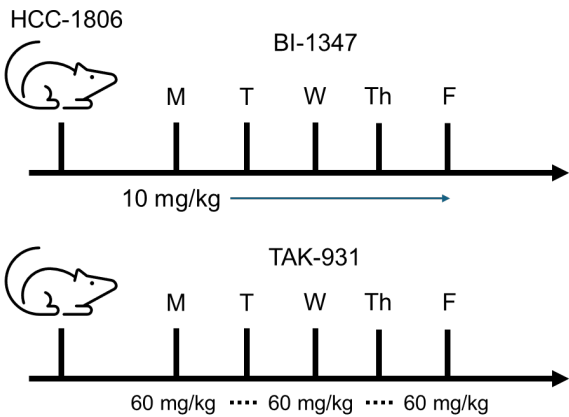
D.



E.



F.



G.

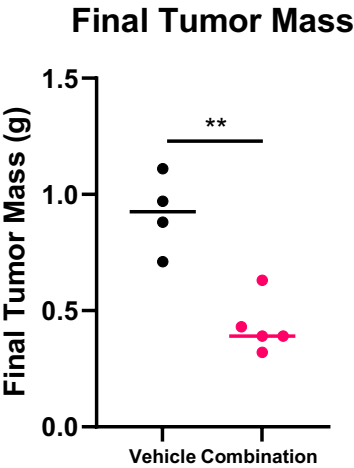


Figure S5. CDK8 Inhibition Synergizes with CDC7 Inhibition in *CCNE1*-amplified Cells.

A, Individual guide dropout rates for *CDK19* (left) and regulatory mediator complex members (right) from HCC-1806 cells following CRISPR screening. **B**, Representative HCC-1806 cells following *CDK8* knockdown and treatment with 15.625 nM TAK-931 (left) or 625 nM XL-413 (right) for five days and monitored with Cellcyte or Incucyte live cell proliferative software (n=4; one-way ANOVA with Tukey's multiple comparisons test comparing each treatment condition using endpoint normalized GFP counts; error bars represent SEM). **C**, Representative images captured at the end of five-day live cell proliferative analyses from HCC-1806 cells following treatment with BI-1347 and TAK-931 or XL-413 (top) or *CDK8* knockdown and treatment with TAK-931 or XL-413 (bottom). **D**, Representative cell cycle profiling of HCC-1806 cells following 48 hr treatment with BI-1347, TAK-931 or combination (top) or BI-1347, XL-413 or combination (bottom). **E**, Representative western blot of HCC-1806 cells for cleaved PARP and pSTAT1 (S727) following treatment with combined XL-413 and BI-1347 for 120 hrs. **F**, Schematic depicting treatment regimen for HCC-1806 cell line derived xenograft study for *in vivo* efficacy of combined TAK-931 and BI-1347. **G**, Final tumor mass from *in vivo* HCC-1806 xenograft models orally administered combined TAK-931 and BI-1347 (n=4 per vehicle group and 5 per combination group; unpaired t-test with Welch's correction (two-tailed)).