

Functional genomics reveals an off-target dependency of drug synergy in gastric cancer therapy

Supplementary Figures

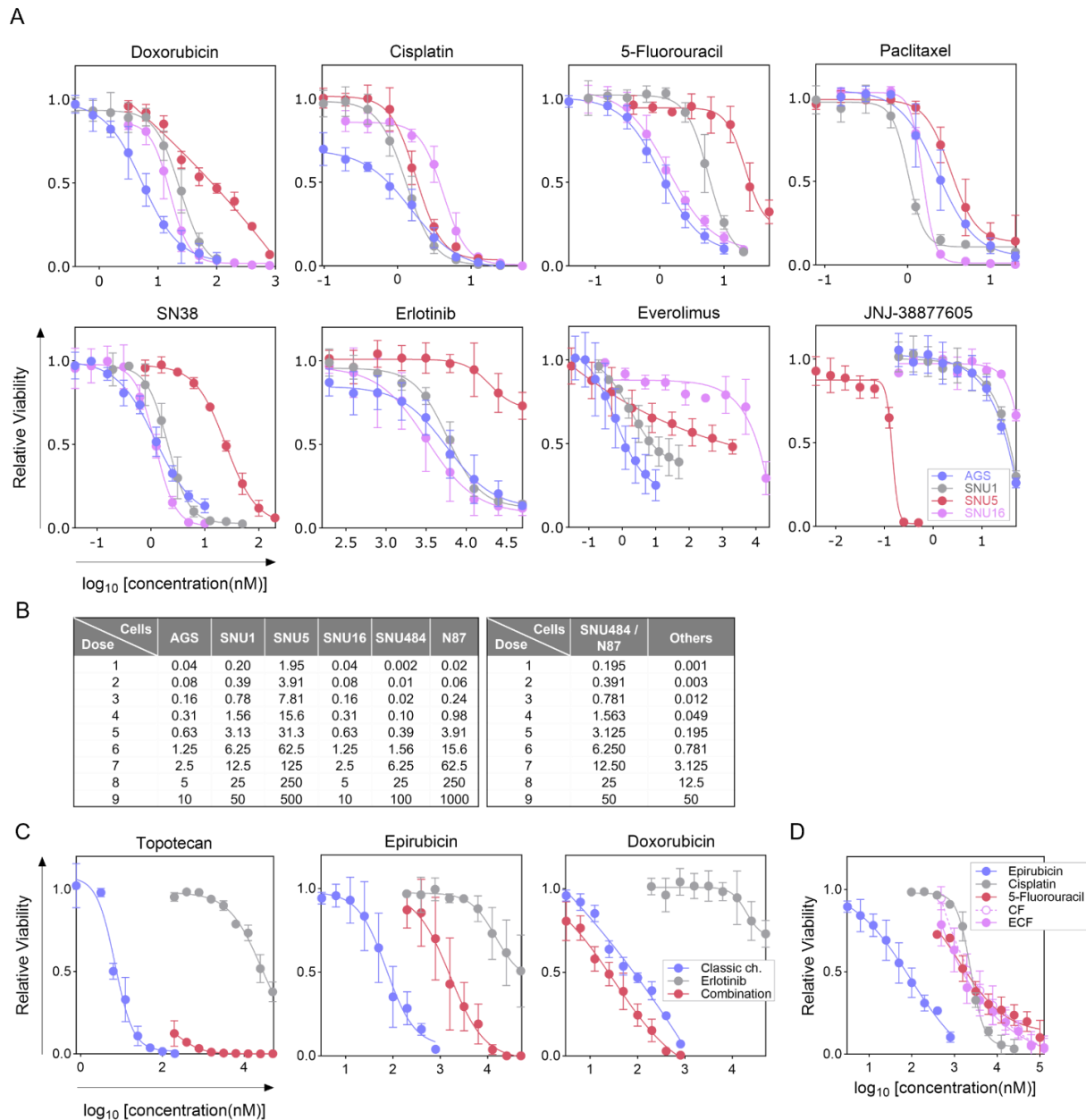


Figure S1. (A) The dose-response curves for conventional chemotherapeutics and molecular-targeted agents in gastric adenocarcinoma cells. **(B)** The relative doses for SN38 in nM (left) and erlotinib in μ M (right) used in the combination experiments presented in Figure 1C. **(C)** The dose-relative viability curves for the combination of erlotinib with topotecan, epirubicin, and doxorubicin in SNU5 cells. **(D)** The dose-relative viability curves for epirubicin, cisplatin, 5-fluorouracil, CF (cisplatin and 5-fluorouracil combination), and ECF (epirubicin, cisplatin, 5-fluorouracil) regimen.

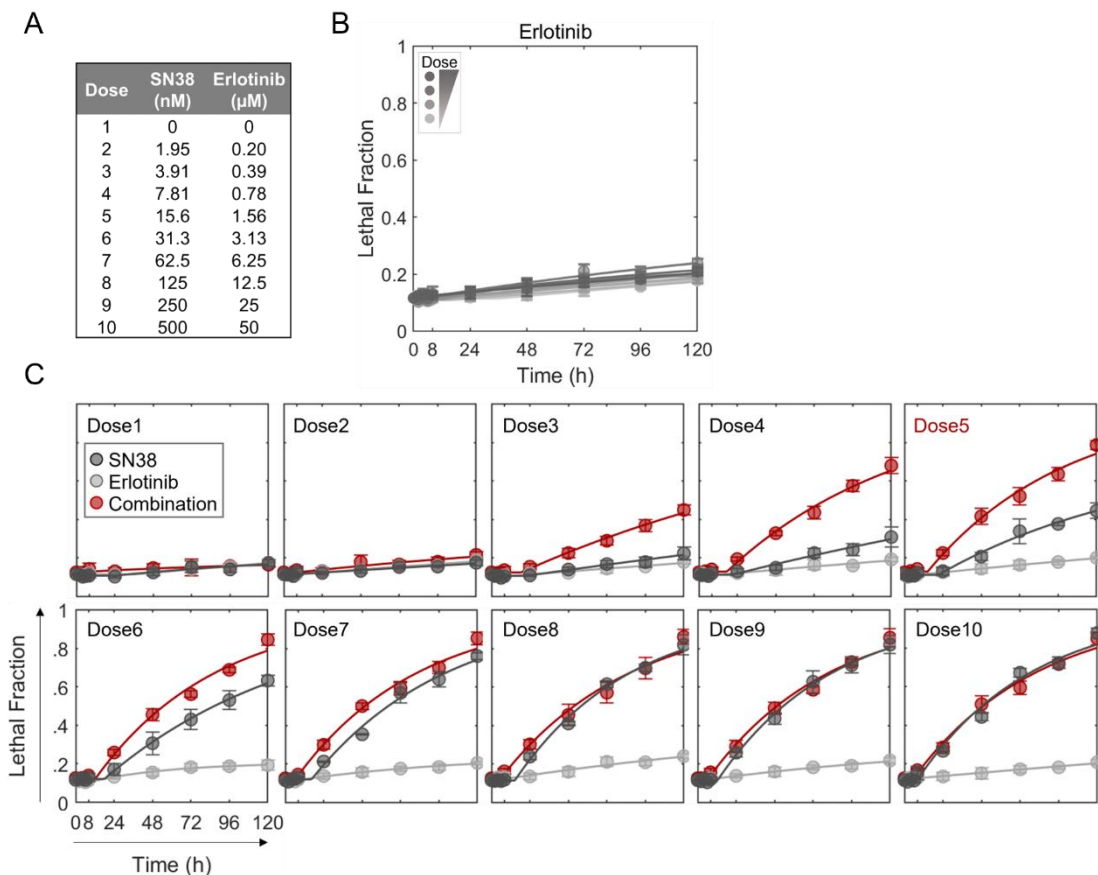


Figure S2. (A) The relative doses of SN38 and erlotinib used in the FLICK assay presented in Figure 2B-D, 4A, and 7E. **(B)** Analysis of the lethal fraction of erlotinib alone for all doses tested. Treatment with erlotinib alone did not substantially induce cell death in SNU5 cells. **(C)** Comparison of lethal fraction curves of the SN38/erlotinib combination and single-agent treatments at each relative dose. Curves at relative dose 5 highlighted are presented in Figure 2E.

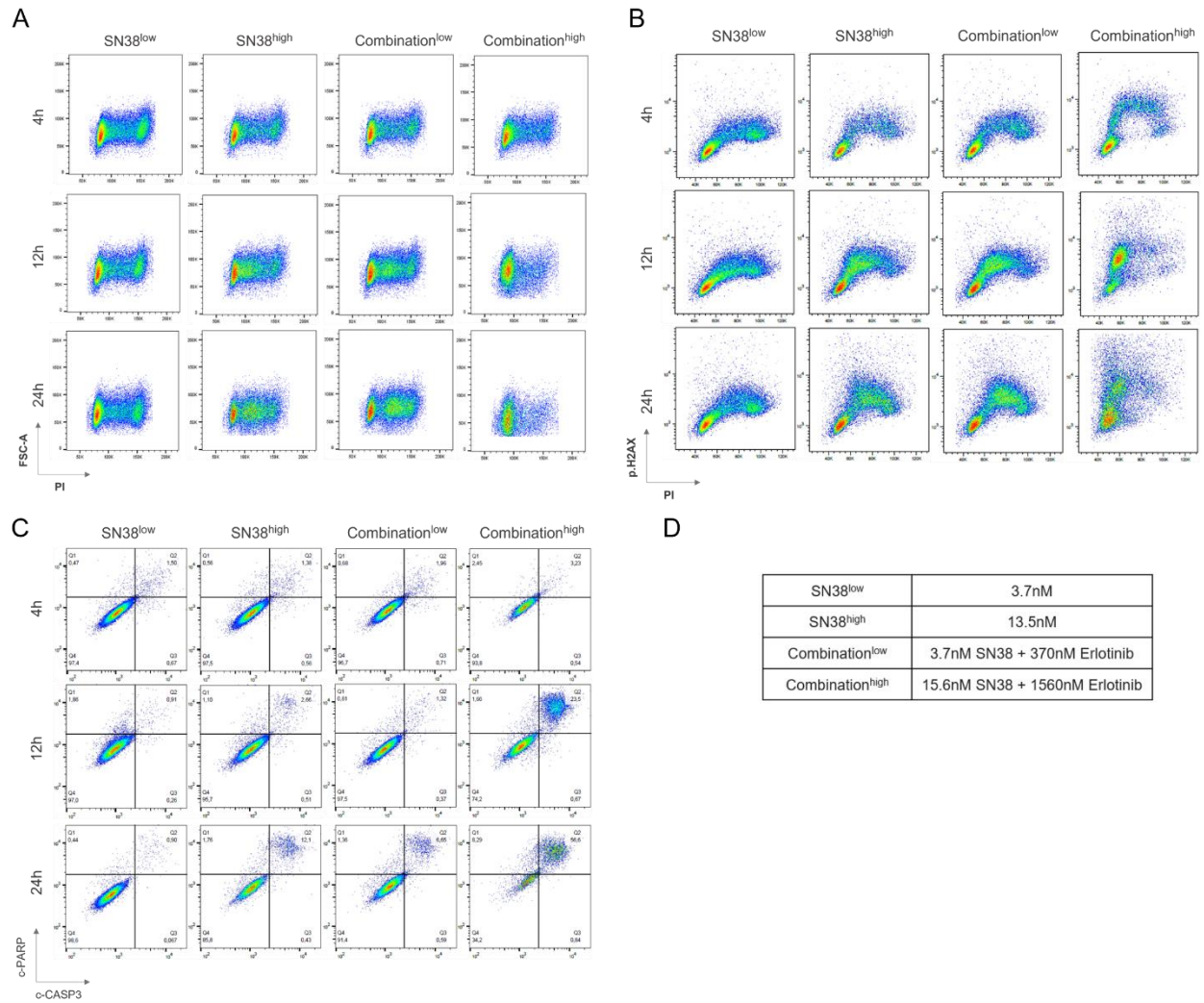


Figure S3. Representative flow cytometry plots for **(A)** cell cycle analysis, **(B)** DNA damage profiling coupled with cell cycle analysis, and **(C)** apoptotic cell death analysis in SNU5 cells. c-PARP: cleaved-PARP, c-CASP3: cleaved-caspase3, p.H2AX: phospho-H2AX, PI: propidium iodide. **(D)** Concentrations of erlotinib and SN38 used in (A-C).

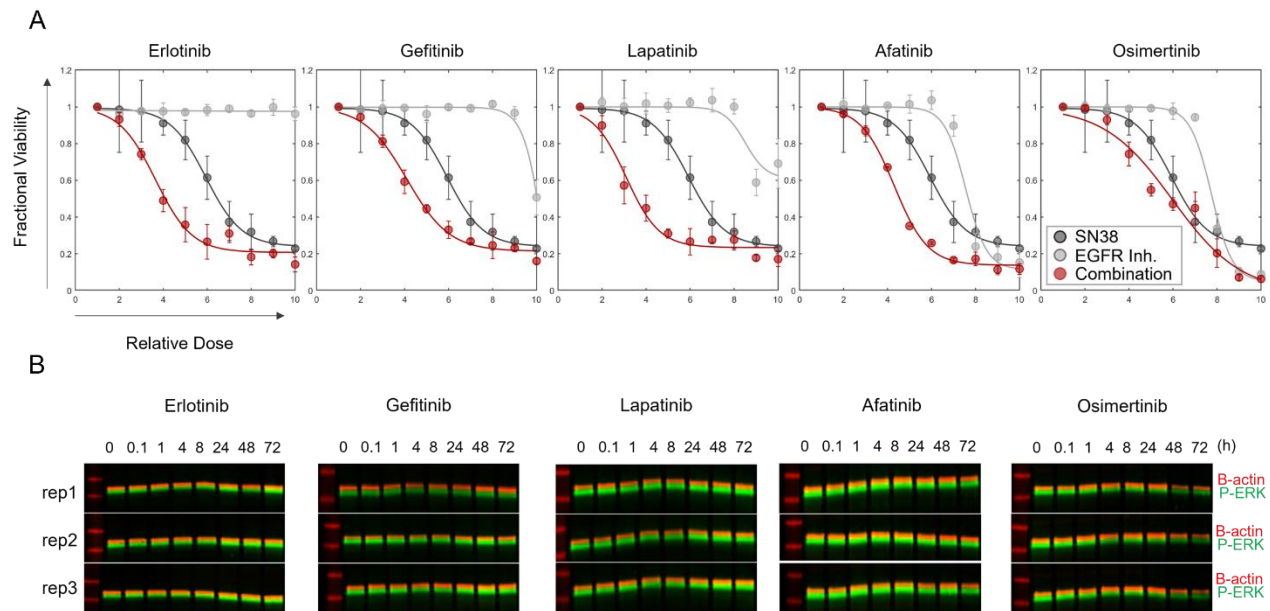


Figure S4. (A) Dose-fractional viability curves for the combination of SN38 with EGFR inhibitors erlotinib, gefitinib, lapatinib, afatinib, and osimertinib. **(B)** The immunoblots (as 3 biological replicates) to assess the effect of EGFR inhibitors on ERK phosphorylation.

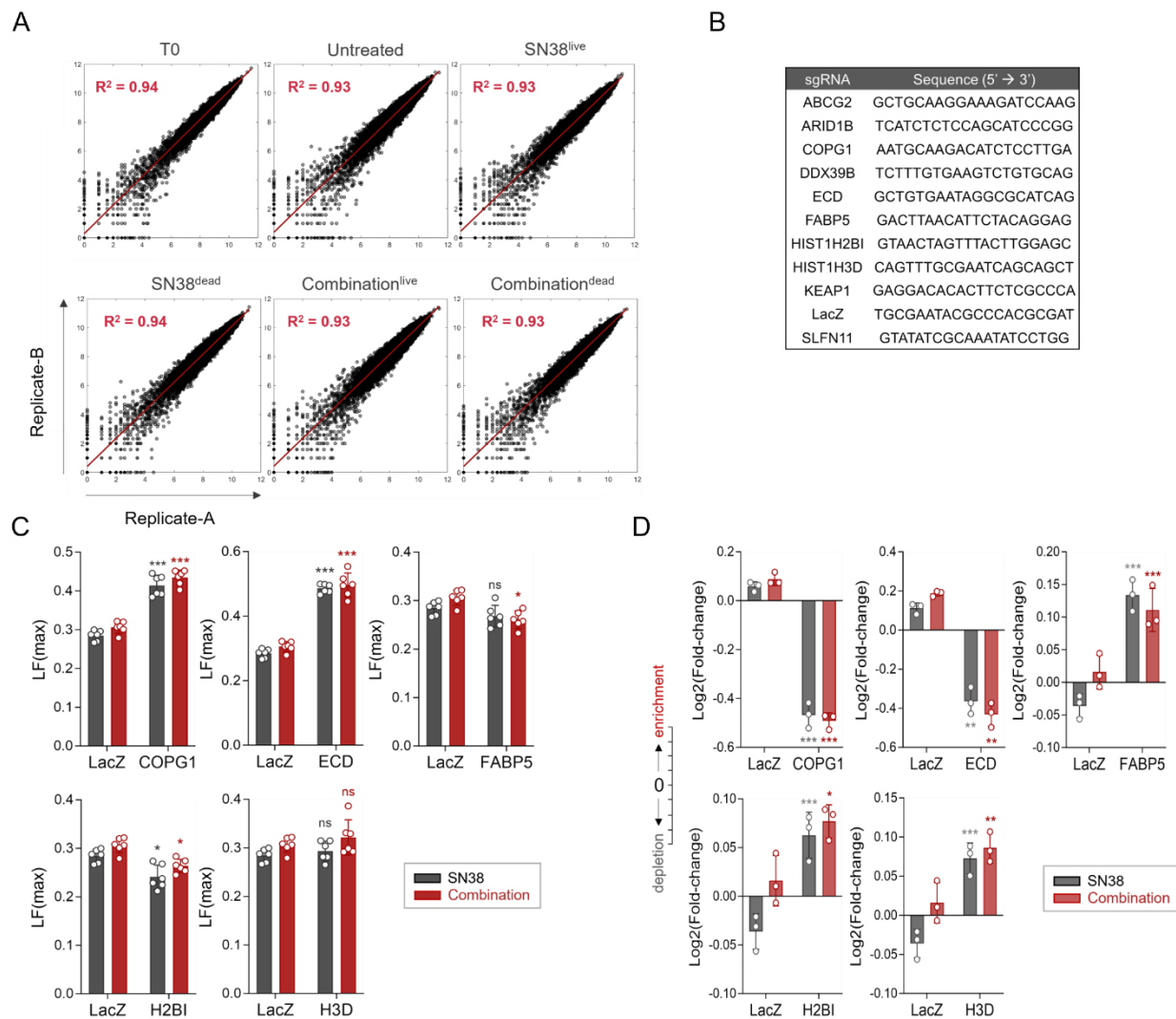


Figure S5. (A) Log₂ count level correlation between the biological replicates of each experiment group. x-axis: replicate A at log₂ count level. y-axis: replicate B at log₂ count level. **(B)** sgRNA sequences used to validate the CRISPR screen hits. **(C)** LF (max), and **(D)** Log₂ fold change plots for COPG1, ECD, FABP5, HIST1H2BI (H2BI), or HIST1H3D (H3D) knockout SNU5 cells or untargeted SNU5 cells (LacZ) under SN38 or SN38/erlotinib combination treatment.

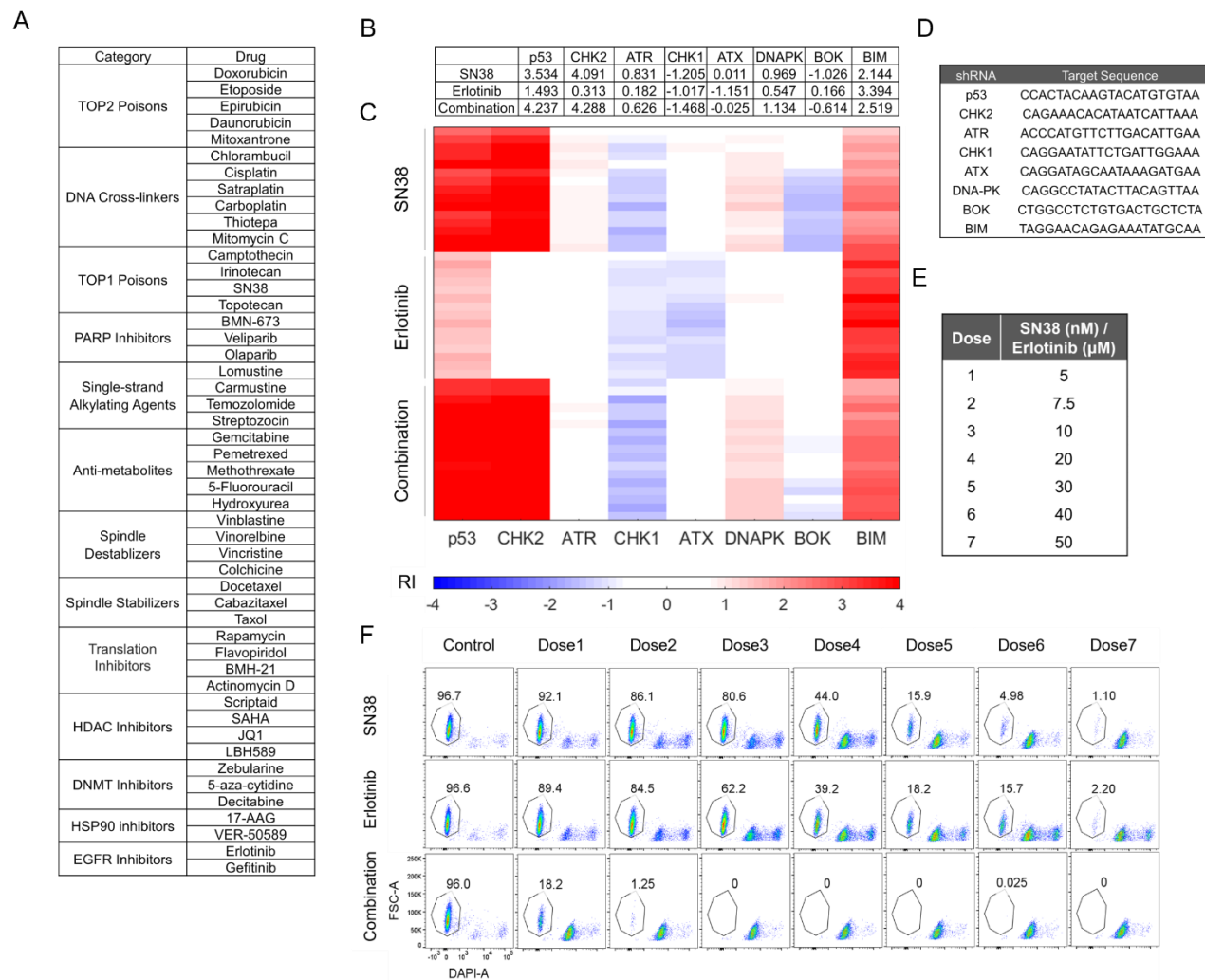


Figure S6. (A) The reference list of drug groups and drugs used to establish shRNA-based signature assay by Pritchard et al. **(B)** Average resistance index (RI) values for each cell population expressing the specified shRNA treated with SN38, erlotinib or SN38/erlotinib combination. **(C)** The heatmap of the signatures for all replicates of each treatment condition generated by assembling the RI values. **(D)** the shRNA sequences used in the signature assay. **(E)** The relative doses of SN38 and erlotinib applied for the dose-response analyses in Eμ-Myc Cdkn2a^{Arf} cells, presented in Figure 6D. **(F)** Representative flow cytometry plots of live cell fractions for each tested dose of SN38, erlotinib, or SN38/erlotinib combination in the signature assay.

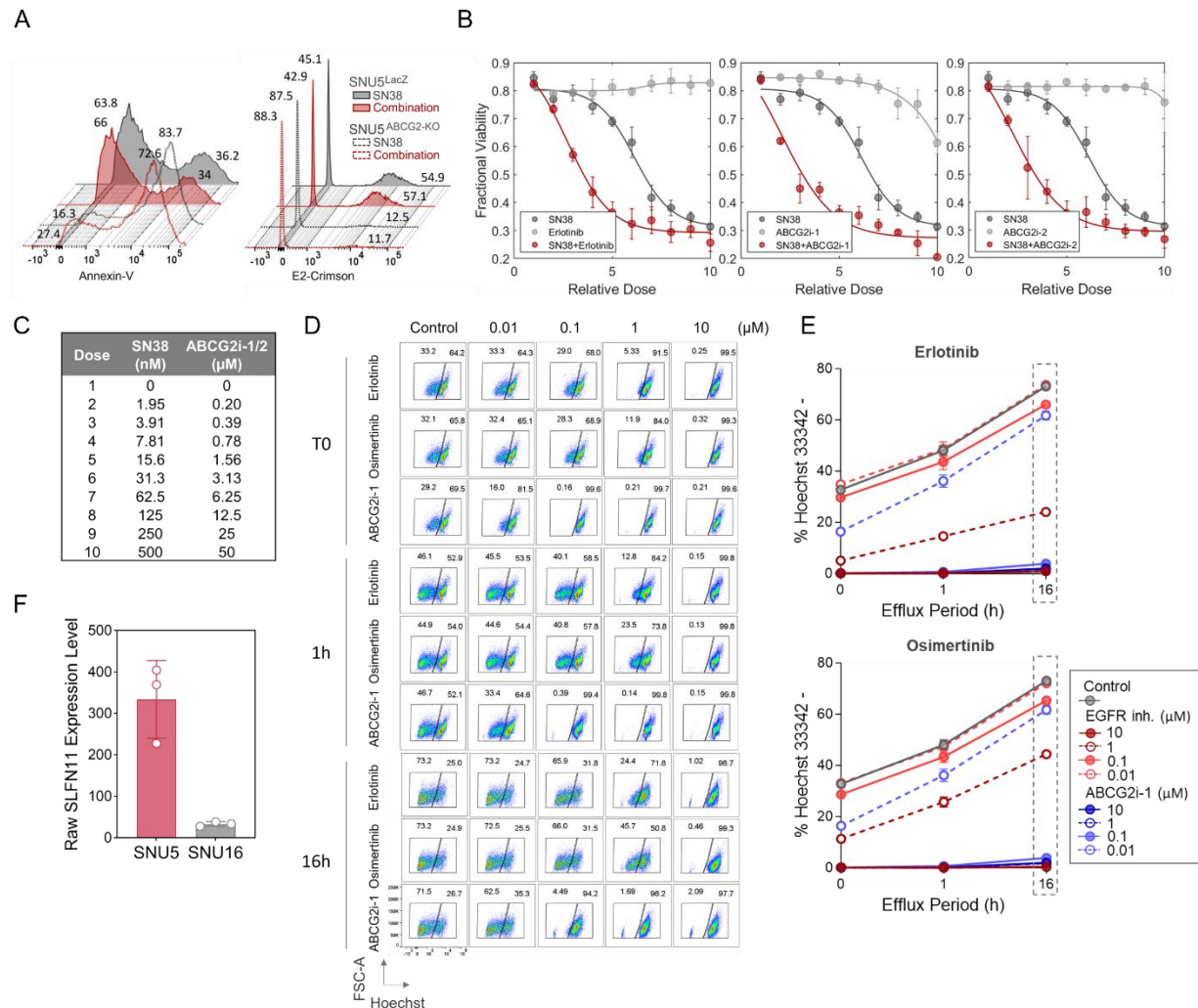


Figure S7. (A) Representative histogram plots of annexin-V (left) and e2-crimson (right) positivity in SNU5^{LacZ} and SNU5^{ABCG2-KO} cells treated with SN38 or SN38-erlotinib combination. **(B)** Dose-fractional viability curves for the dual combination of SN38 with erlotinib, ABCG2i-1, or ABCG2i-2 in SNU5 cells. **(C)** The concentrations of SN38 and ABCG2 inhibitors for the curves in B and Figure 7F. **(D)** Assessment of the impact of ABCG2 and EGFR inhibitors on the efflux of Hoechst over time in SNU5 cells via flow cytometry. **(E)** Percent inhibition of Hoechst efflux by ABCG2 and EGFR inhibitors over time in SNU5 cells calculated from the flow cytometry plots in D. **(F)** The gene expression level of SLFN11 in SNU5 and SNU16 cells. Expression data was exported from merav.wi.mit.edu/ (Shaul et al.2015).

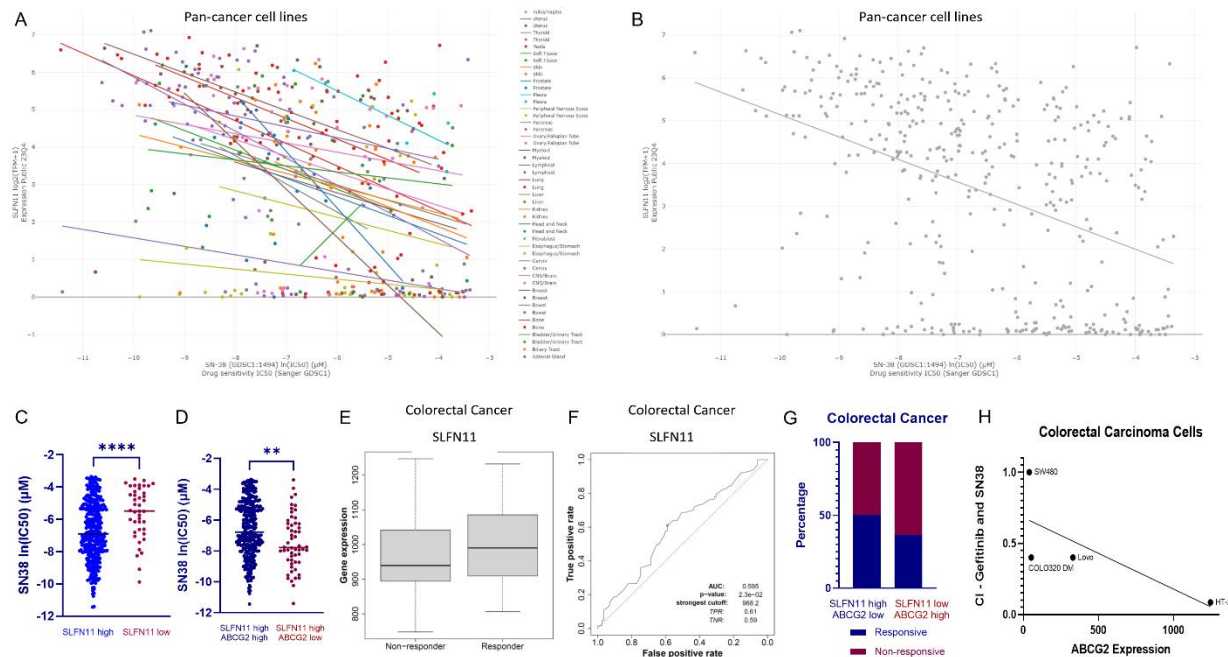


Figure S8. (A) Cell lineage-specific correlation between the expression of SLFN11 and sensitivity to SN-38 in 411 pan-cancer cell lines. (B) The general correlation between the expression of SLFN11 and sensitivity to SN-38 in 411 pan-cancer cell lines. (C) IC₅₀ of SN38 in cells with high SLFN11 expression vs. low SLFN11 expression. (D) IC₅₀ of SN38 in cells with high SLFN11 and high ABCG2 expression vs. high SLFN11 and low ABCG2 expression. (E) The expression profile of SLFN11 in colorectal cancer patients responsive vs. non-responsive to irinotecan. (F) The ROC plot shows the predictive value of SLFN11 in colorectal cancer patients who were treated with irinotecan. (G) The percent distribution of responders vs. non-responders to irinotecan treatment in colorectal cancer patients with high SLFN11 and low ABCG2 expression vs. low SLFN11 and high ABCG2 expression. (H) The correlation between ABCG2 expression and combination index (CI) of SN38-gefitinib combination in colorectal carcinoma cell lines with similar SLFN11 expression. In A and B, the graphs generated by DepMap Portal (<https://depmap.org/portal/>) were downloaded (DepMap Broad, 2019; Ghandi et al. 2019). In C and D, the gene expression and SN-38 IC₅₀ data downloaded from the DepMap Portal were analyzed and used to generate the bar graphs in GraphPad Prism 10. The medians of SLFN11 and ABCG2 expression among 411 cell lines were used as a cut-off to classify cell lines as SLFN11 and ABCG2 high or low. In E and F, the graphs were downloaded from RocPlotter (<https://rocplot.org/>) (Fekete & Györfy 2023). In G, data for colorectal cancer patients downloaded from the RocPlotter was analyzed. In H, ABCG2 gene expression levels of cell lines were downloaded from merav.wi.mit.edu/ (Shaul et al. 2015). The CI of SN38-gefitinib combination in HT-29 cells was retrieved from Azzariti et al. 2004, and in COLO320 DM, Lovo, and SW480 cells, the CI values were retrieved from Kouzimu et al. 2004. The CI values at fa=0.5 (fraction of affected cells =0.5) were used in the analysis.

References

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