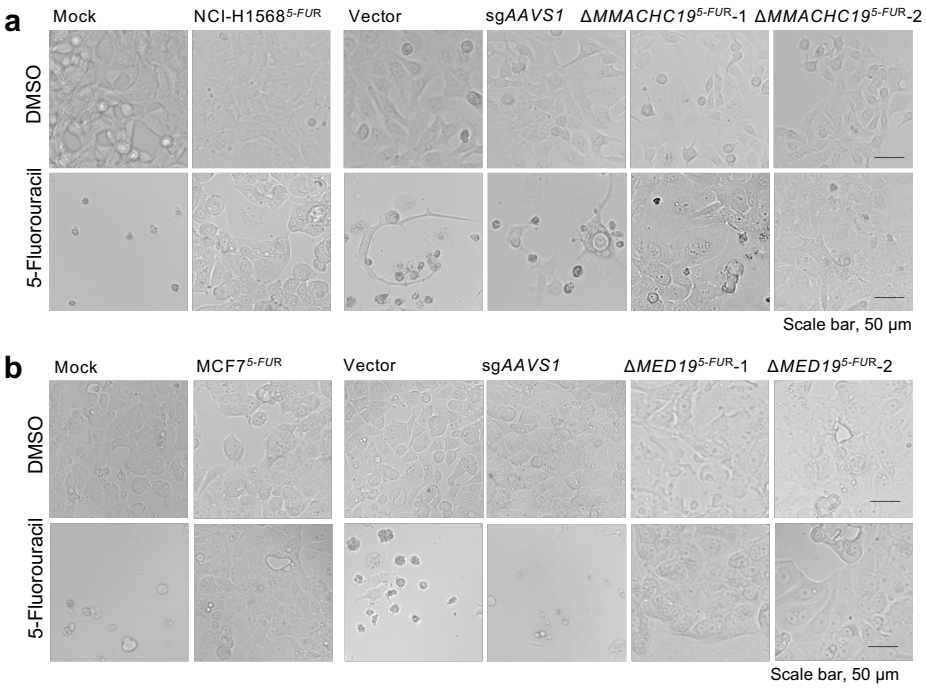
Supplementary Information

Chemoresistance mechanisms to 5-Fluorouracil and reversal strategies in lung and breast cancer

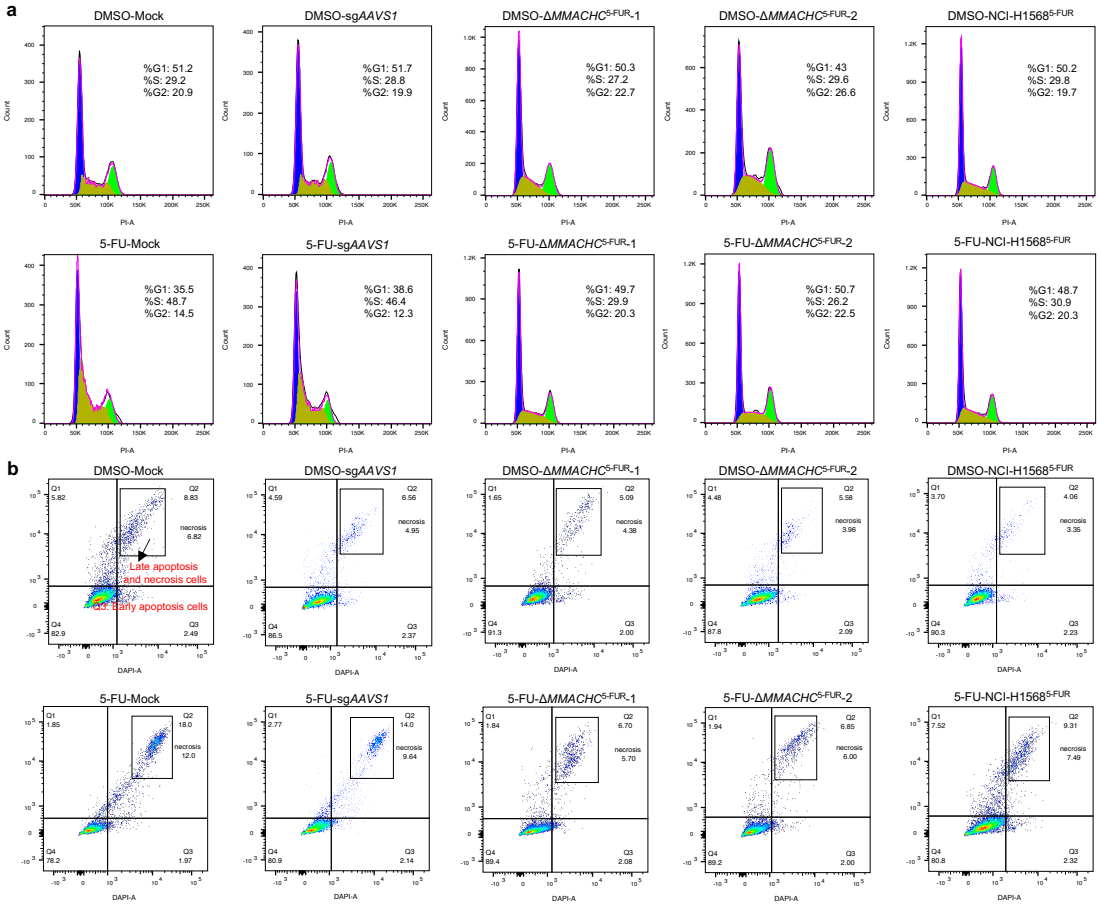
Chunge Zhong[1,2,3,4], Shengnan Wang[1,2,3,4], Wen-Jie Jiang[5], Zexu Li[1,2,3,4], Xiaofeng Wang[1,2,3,4], Shuangshuang Fan[1,2,3,4], Jun Huang[6], Hua-Jun Wu[7,8,9], Ren Sheng[1], Teng Fei[1,2,3,4,*]

Supplementary Information includes:

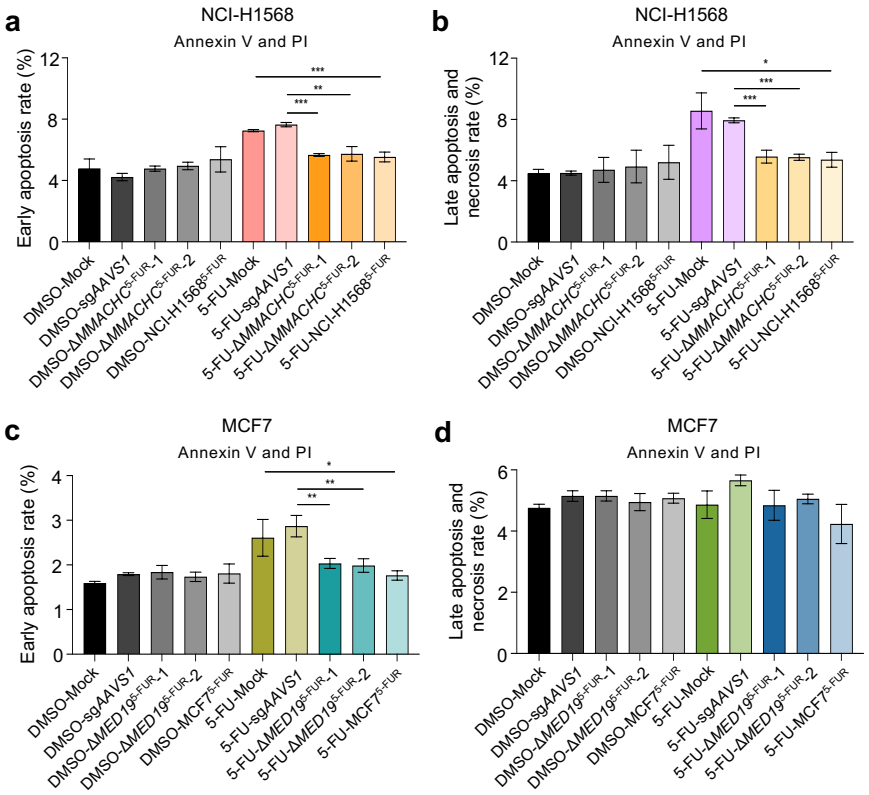
Supplementary Figs. 1-10.
Supplementary Table 1. RNA-seq data. (attached dataset)



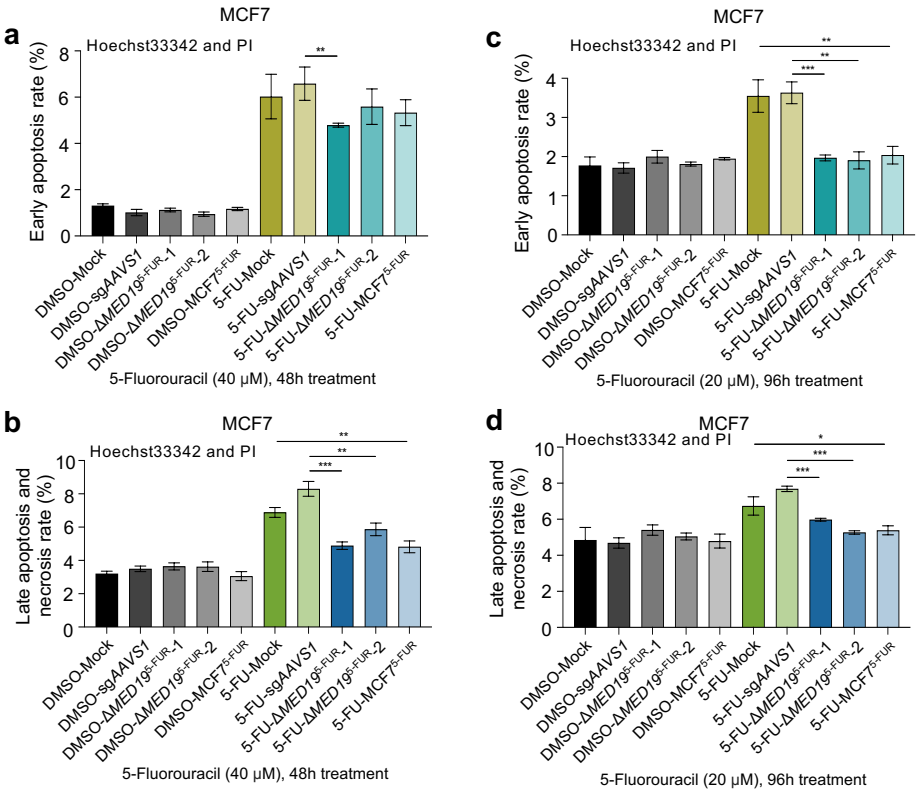
Supplementary Fig. 1. The morphology of 5-FU-sensitive and -resistant cells. (**a-b**) Cell morphology of multiple 5-FU-sensitive (Mock, Vector and sg*AAVS1*) and -resistant cell lines in the absence (DMSO) or presence of 5 μM 5-FU for 11 days (NCI-H1568) (**a**) or 20 μM 5-Fluorouracil for 9 days (MCF7) (**b**). Scale bar, 50 μm.



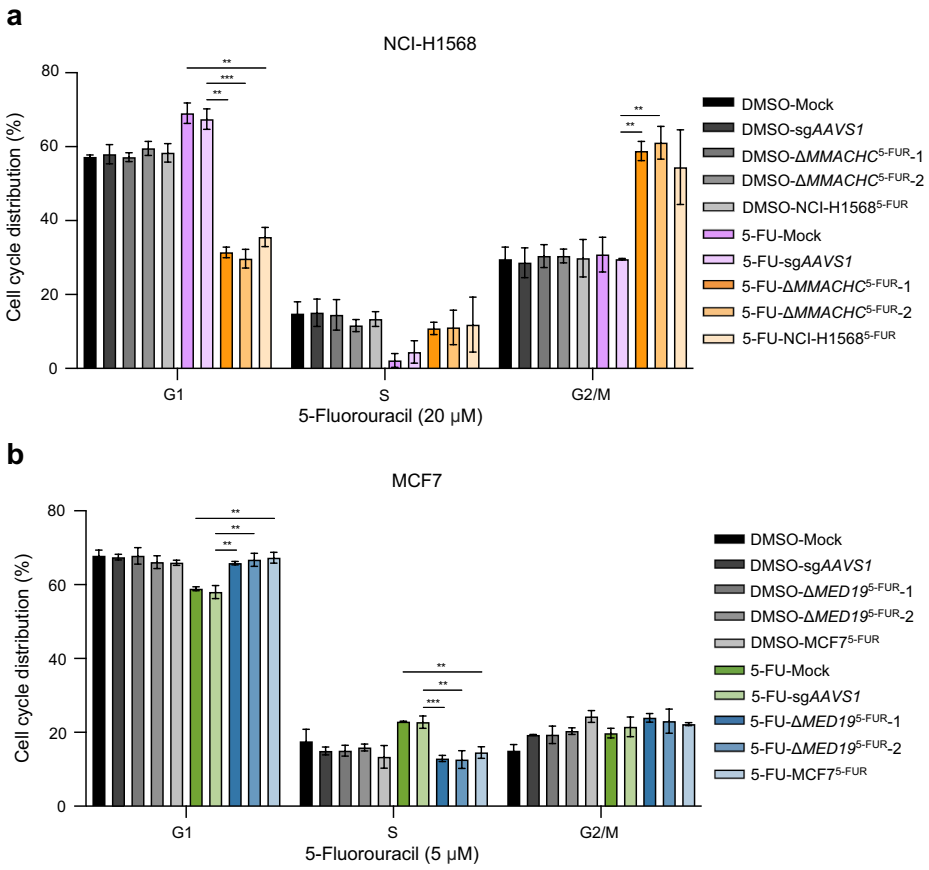
Supplementary Fig. 2. Representative histograms for FACS data. (**a**) The representative single-staining histograms for cell cycle analysis in Fig. 2a. (**b**) The representative FACS plots for apoptosis and necrosis analysis using PI and Hoechst staining method in Fig. 2b,c.



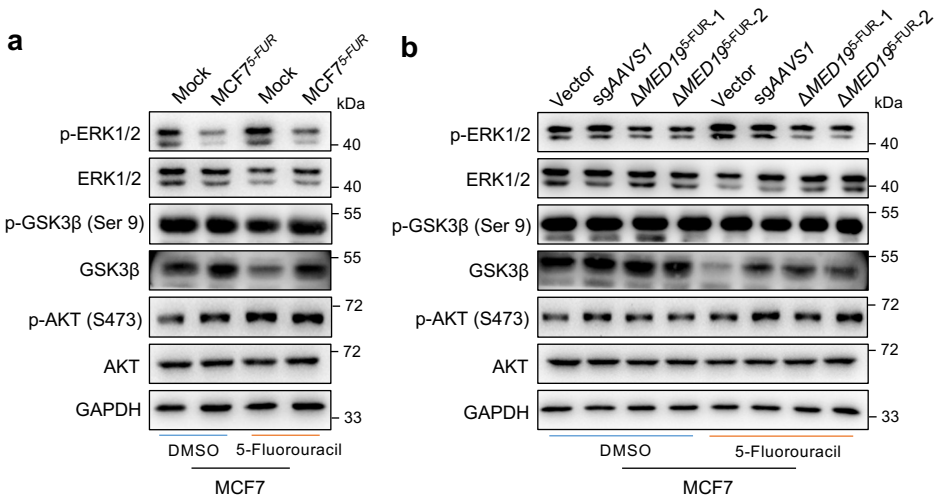
Supplementary Fig. 3. Apoptosis analysis by Annexin V-FITC and PI staining method. (**a**-**b**) Early apoptosis (**a**) or late apoptosis and necrosis (**b**) analysis of indicated NCI-H1568 cells in the absence (DMSO) or presence of 5 μM 5-FU for 48 h by Annexin V-FITC and PI staining method. Mean ± SD with n = 3. Unpaired t test, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. (**c**-**d**) Early apoptosis (**c**) or late apoptosis and necrosis (**d**) analysis of indicated MCF7 cells in the absence (DMSO) or presence of 20 μM 5-FU for 48 h by Annexin V-FITC and PI staining method. Mean ± SD with n = 3. Unpaired t test, *$p < 0.05$ and **$p < 0.01$.



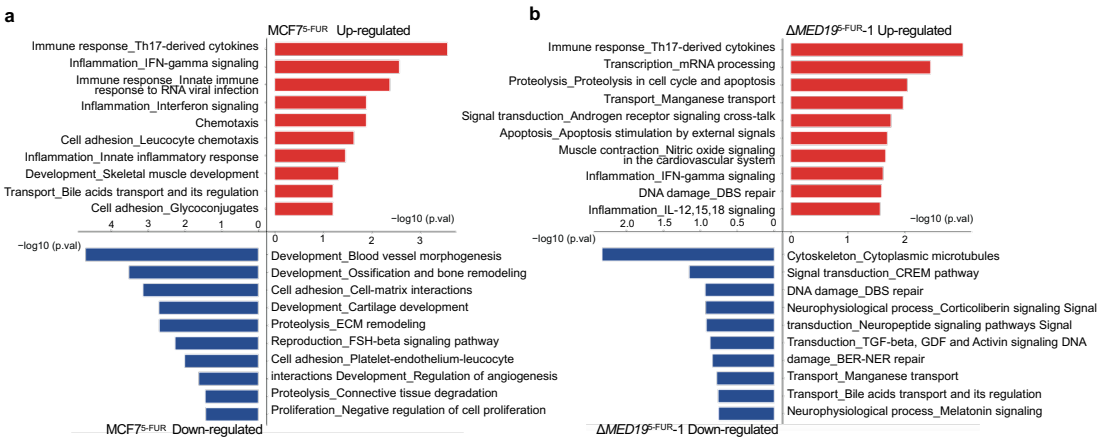
Supplementary Fig. 4. Additional apoptosis analysis by Hoechst 33342 and PI staining method. (**a-b**) Early apoptosis (**a**) or late apoptosis and necrosis (**b**) analysis of indicated cells in the absence (DMSO) or presence of 40 μM high dose 5-FU for 48 h by Hoechst 33342 and PI staining method. Mean ± SD with n = 3. Unpaired t test, $^{**}p < 0.01$ and $^{***}p < 0.001$. (**c-d**) Early apoptosis (**c**) or late apoptosis and necrosis (**d**) analysis of indicated cells in the absence (DMSO) or presence of 20 μM 5-FU for 96 h longer time duration by Hoechst 33342 and PI staining method. Mean ± SD with n = 3. Unpaired t test, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.



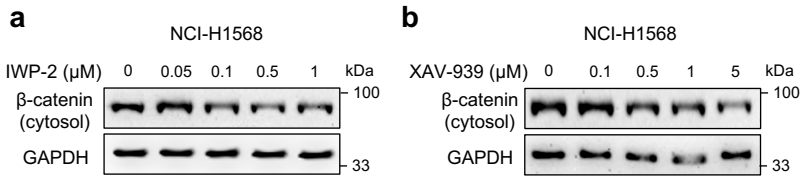
Supplementary Fig. 5. Additional cell cycle analysis. (**a**) Cell cycle analysis by propidium iodide (PI) staining of indicated NCI-H1568 cells in the absence (DMSO) or presence of 20 µM 5-FU for 48 h. Mean ± SD with n = 3. Unpaired t test, $^{**}p < 0.01$ and $^{***}p < 0.001$. (**b**) Cell cycle analysis of indicated MCF7 cells in the absence (DMSO) or presence of 5 µM 5-FU for 48 h. Mean ± SD with n = 3. Unpaired t test, $^{**}p < 0.01$ and $^{***}p < 0.001$.



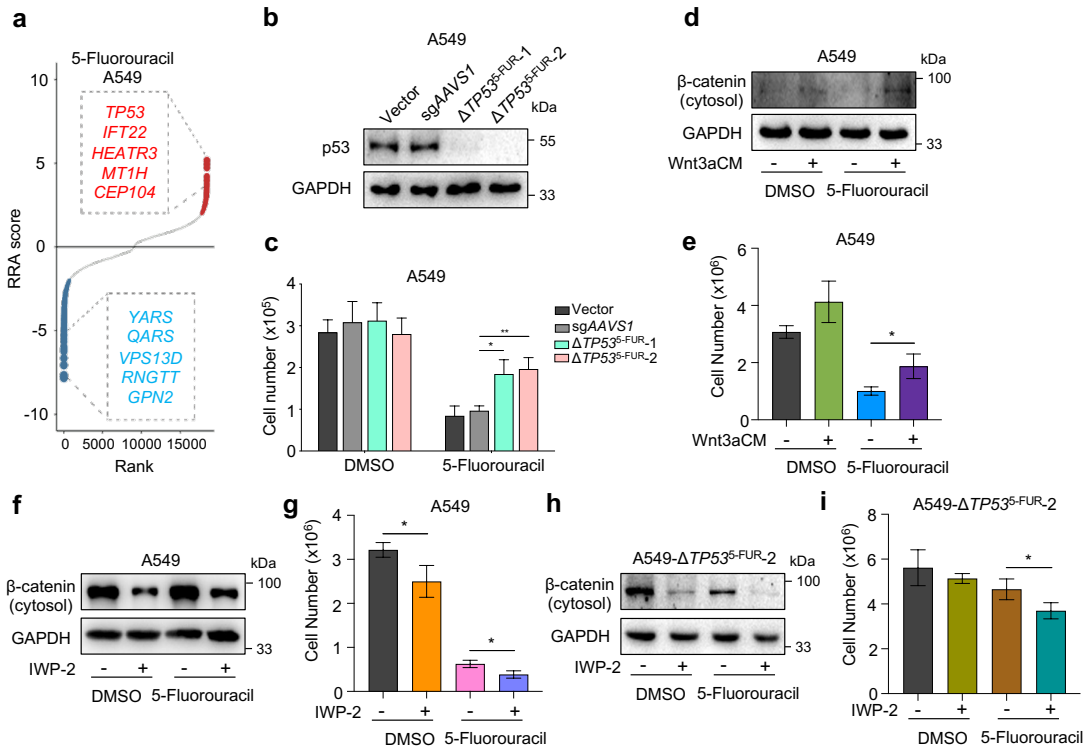
Supplementary Fig. 6. Immunoblotting analysis of MCF7 cells. (**a**-**b**) Immunoblotting analysis of indicated proteins for multiple 5-FU-sensitive or -resistant MCF7 cells lines in the absence (DMSO) or presence of 20 μM 5-FU for 48 h.



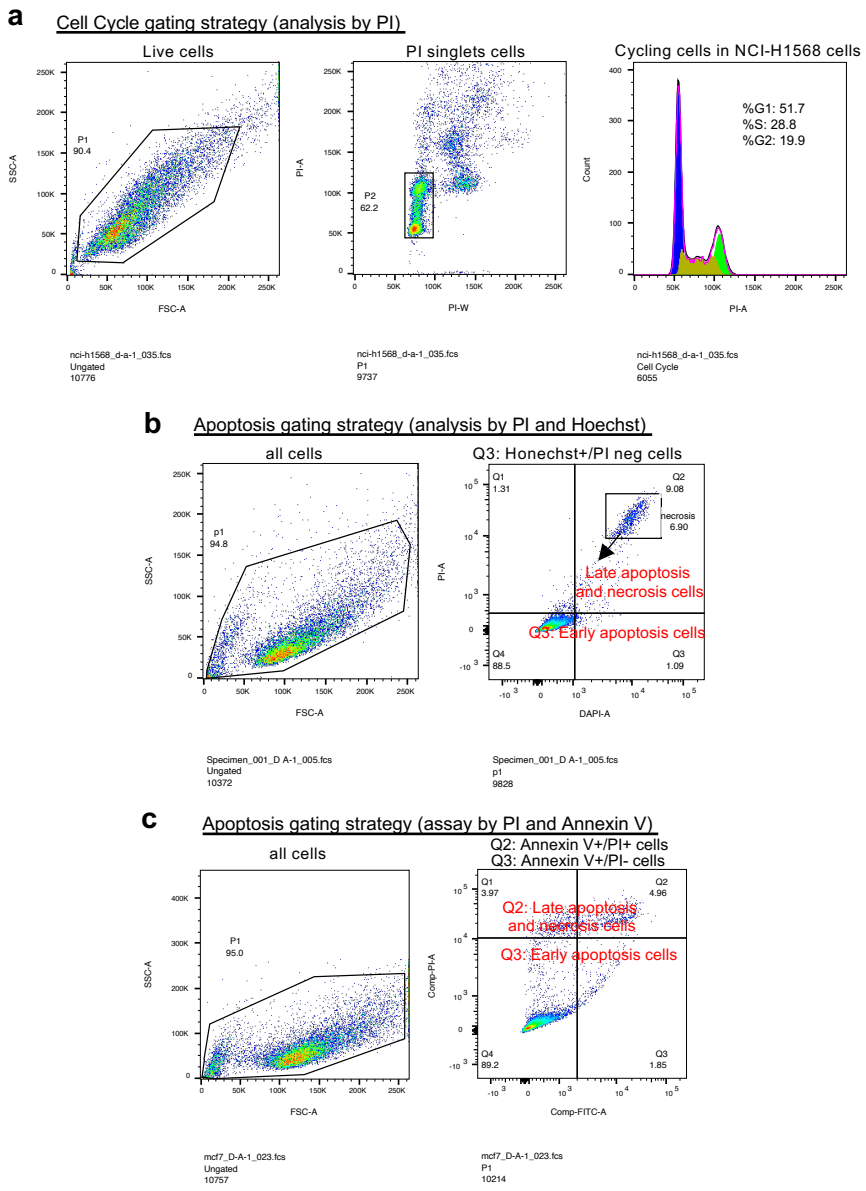
Supplementary Fig. 7. Functional enrichment analysis for 5-FU resistance signature genes in MCF7 cell models. (**a**) Bar graph showing the enriched functional terms for up-regulated or down-regulated genes between MCF7$^{5\text{-FUR}}$ vs. untreated Mock cells. (**b**) Bar graph showing the enriched functional terms for up-regulated or down-regulated genes between Δ*MED19*$^{5\text{-FUR}}$-1 vs. Vector control cells.



Supplementary Fig. 8. Inhibition of WNT/β-catenin signaling by two indicated inhibitors. (**a**) Immunoblotting analysis of active β-catenin upon different doses of IWP-2 inhibitor in NCI-H1568 cells. (**b**) Immunoblotting analysis of active β-catenin upon different doses of XAV-939 inhibitor in NCI-H1568 cells.



Supplementary Fig. 9. Validation of targeting WNT/β-catenin signaling in another lung cancer 5-FU-resistant A549 cell line model. (**a**) Ranked genes from genome-wide CRISPR knockout screen in A549 cells against 5-FU. Red highlighted gene indicates a resistance phenotype upon gene knockout while blue indicates lethal upon 5-FU treatment. (**b**) Immunoblot of p53 for control and *TP53* knockout A549 cells. (**c**) Cell growth evaluation of control (vector, sg*AAVS1*) and 5-FU-resistant (5-FUR) A549 cells derived from *TP53* knockout. Mean ± SD with n=3. Unpaired t test, **p* < 0.05 and ***p* < 0.01. (**d**) Immunoblot analysis of cytoplasmic β-catenin in A549 cells treated with Wnt3aCM and/or 5-FU (5 μM) for 6 days. (**e**) Cell growth evaluation of indicated A549 cells after 6 days treatment of Wnt3aCM and/or 5-FU (5 μM). Mean ± SD with n = 3. Unpaired t test, **p* < 0.05. (**f-i**) Immunoblot analysis of cytoplasmic β-catenin in A549 cells (**f**) or A549-Δ*TP53*$^{5\text{-FUR}}$-2 cells (**h**) and cell growth evaluation of A549 cells (**g**) or A549-Δ*TP53*$^{5\text{-FUR}}$-2 cells (**i**) treated with IWP-2 (0.5 μM for panel f,g or 2 μM for panel h,i) and/or 5-FU (5 μM) for 7 days. Mean ± SD with n = 3. Unpaired t test, **p* < 0.05.



Supplementary Fig. 10. The gating strategy for FACS analysis**.** (**a**) The gating strategy for cell cycle analysis (PI staining). Cells were first gated on SSC-Area and FSC-Area for morphology to remove debris. Doublets and cell aggregates were excluded by gating in single cells (PI-Width vs. PI-Area). The cell population gated in after debris and doublet exclusion was then used to create single-staining histograms. This gating strategy was used for Fig. 2a,d and Supplementary Fig. 5. (**b**) The gating strategy for apoptosis analysis (PI + Hoechst staining). Cells were gated on SSC-Area and FSC-Area for morphology to remove debris. Cells were plotted for PI and Hoechst parameters to identify apoptosis and necrosis cells. This gating strategy was used for Fig. 2b,c,e,f and Supplementary Fig. 4. (**c**) The gating strategy for apoptosis analysis (PI and Annexin V staining). Cells were gated on SSC-Area and FSC-Area for morphology to remove debris. Cells were plotted for PI and Annexin V parameters. This gating strategy was used for Supplementary Fig. 3.