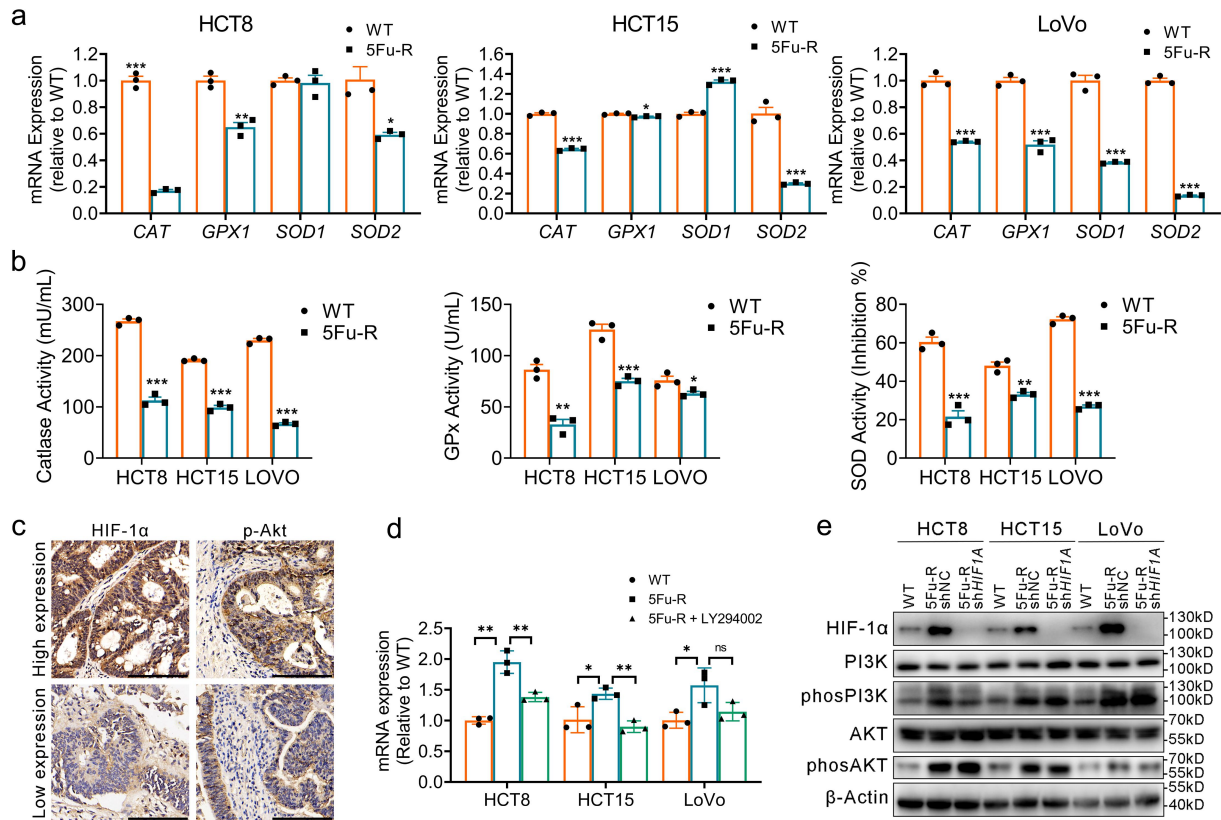




Additional file 6: Fig. S6. ROS/PI3K/AKT pathway activation boosts HIF-1α levels and induces 5-FU resistance, related to Fig. 7.

a. RT-qPCR analysis for ROS scavenging enzymes genes in 5-FU-R CRC cells relative to WT CRC cells. *ACTB* was used as the internal reference.

b. The activities of CAT, GPx, and SOD enzymes of 5-FU-R CRC cells relative to WT CRC cells as determined by colorimetric analysis. Enzyme activity was normalized to total protein concentration.

c. Representative IHC staining images of p-Akt and HIF-1α in CRC patients received preoperative fluorouracil analog-based chemotherapy. Scale bar = 100 μm.

d. 5-FU-R cells were treated with 25 μM LY294002 for 48 hours. RT-qPCR analysis for the gene expressions of *HIF1A*. *ACTB* is used as an internal reference.

e. Effect of *HIF1A* knock-down on PI3K/AKT pathway. β-Actin was used as an

internal reference.

For all studies $n \geq 3$. Data are presented as means $\pm$ SEM. Data were analyzed by Student's t-test or ANOVA (ns = not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).