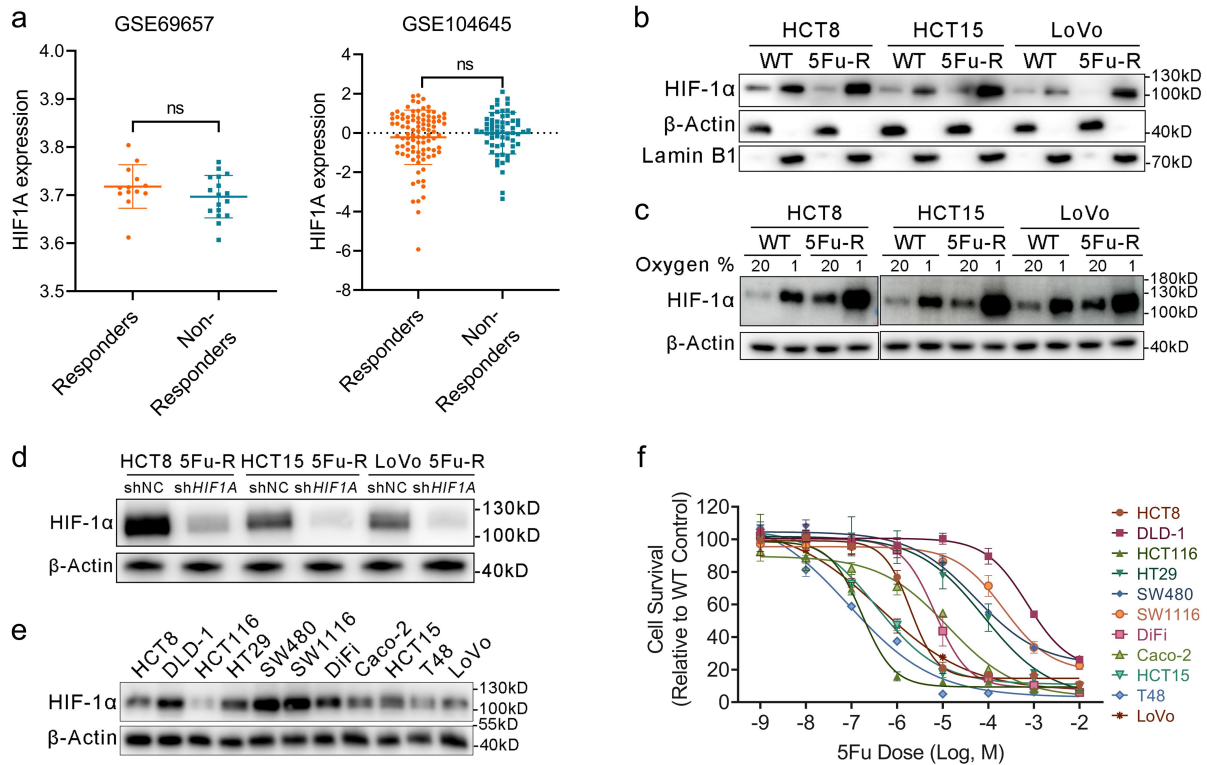




99 **Additional file 4: Fig. S4. HIF-1α is a prognostic biomarker and regulator of**

100 **5-FU resistance in CRC, related to Fig. 4 and Fig. 5.**

101 **a.** *HIF1A* mRNA expressions were obtained and compared from 2 GEO datasets.

102 **b.** Nucleus and cytoplasm distribution of HIF-1α was analyzed by Western blots.

103 Lamin B1 is a nuclear protein reference, and β-Actin is a cytoplasmic marker.

104 **c.** Expression of HIF-1α in WT CRC cells versus 5-FU-R CRC cells. Cells were

105 cultured under normoxia (20% oxygen) or hypoxia (1% oxygen). β-Actin was used as

106 an internal reference.

107 **d.** WT and 5-FU-R CRC cells were stably knocked down for *HIF1A* using shRNA.

108 HIF-1α was confirmed by Western blotting, using β-Actin as a loading control.

109 **e.** Western blots of HIF-1α in 11 CRC cell lines (HCT8, DLD-1, HCT116, HT29,

110 SW480, SW1116, DiFi, Caco-2, HCT15, T84, and LoVo). β-Actin was used as the

internal reference.

f. CCK8 assays to assess 5-FU sensitivity of 11 CRC cell lines, and cells were treated with an increasing concentrations of 5-FU for 72 hours. All experiments were performed with 6 replicates.

Data are presented as means $\pm$ SEM. Bar chart data were compared by Student's t-test (ns = not significant).