

Supplemental Figures legend

Figure S1: CRISPRi identifies expressed pan-essential transcripts. (A-E)

Distribution of sgRNAs targeting high- or low-expressed pan cell-essential transcripts.

Figure S2: CRISPRi screen quality control. (A-E)

Distribution of sgRNAs targeting negative controls or core essential genes.

Figure S3: Essential transcripts that score due to a bidirectional promoter. (A)

All GC-essential transcripts that are expressed from a bidirectional promoter (defined as a second transcript at a distance of <2,000bp). (B) Proliferation scores (CERES scores) from project Achilles for genes exhibiting bidirectional promoters. *ARPC5L*, *ATP5F1A* and *KIAA08595* score due to close proximity to a cell essential gene and are excluded.

Figure S4: Validation of GC-essential transcripts.

Quantitative RT-PCR in YCC3 cells 5 days post infection with sgRNAs targeting (A) CIT (B) CCNE1 (C) MTA3. For MTA3 sgRNAs targeting isoform P1 and P2 are shown. CIT and CCNE1 only express one isoform. Results are plotted as an average $\pm$ SD n = 2. pValue calculated using two tailed unpaired t-test. (*, $p \leq 0.05$).

Figure S5: CIT dependency in GC. (A)

CIT and KIF14 CERES scores from project Achilles plotted against each other. (B) RNA-Seq following suppression of CIT expression in YCC3 cells. (C,D) pNDRG1 levels following 24h treatment with the

indicated concentration of Phorbol 12-myristate 13-acetate (E,F) pNDRG1 levels following 24h treatment with the indicated concentration of prostratin.

Figure S6: ZFH3 isoform P2 expression following CRISPRi-mediated suppression of transcript P2. Results are plotted as an average $\pm$ SD, n = 2. pValue calculated using two tailed unpaired t-test. (*, $p \leq 0.05$).

Figure S7: In vivo validation of ZFH3 and CIT. (A) CIT protein levels from tumour xenografts. (B) ZFH3 mRNA levels from tumour xenografts. Results are plotted as an average $\pm$ SD. Each dot represents a different xenograft tumour. pValue calculated using two tailed unpaired t-test. (*, $p \leq 0.05$). (C) Kaplan-Meier survival plot of GC patients expressing CIT using the ACRG cohort or the (D) TCGA cohort. (E) Kaplan-Meier survival plot and (D) subclass by patient type of ZFH3 expression using the TCGA patient cohort.

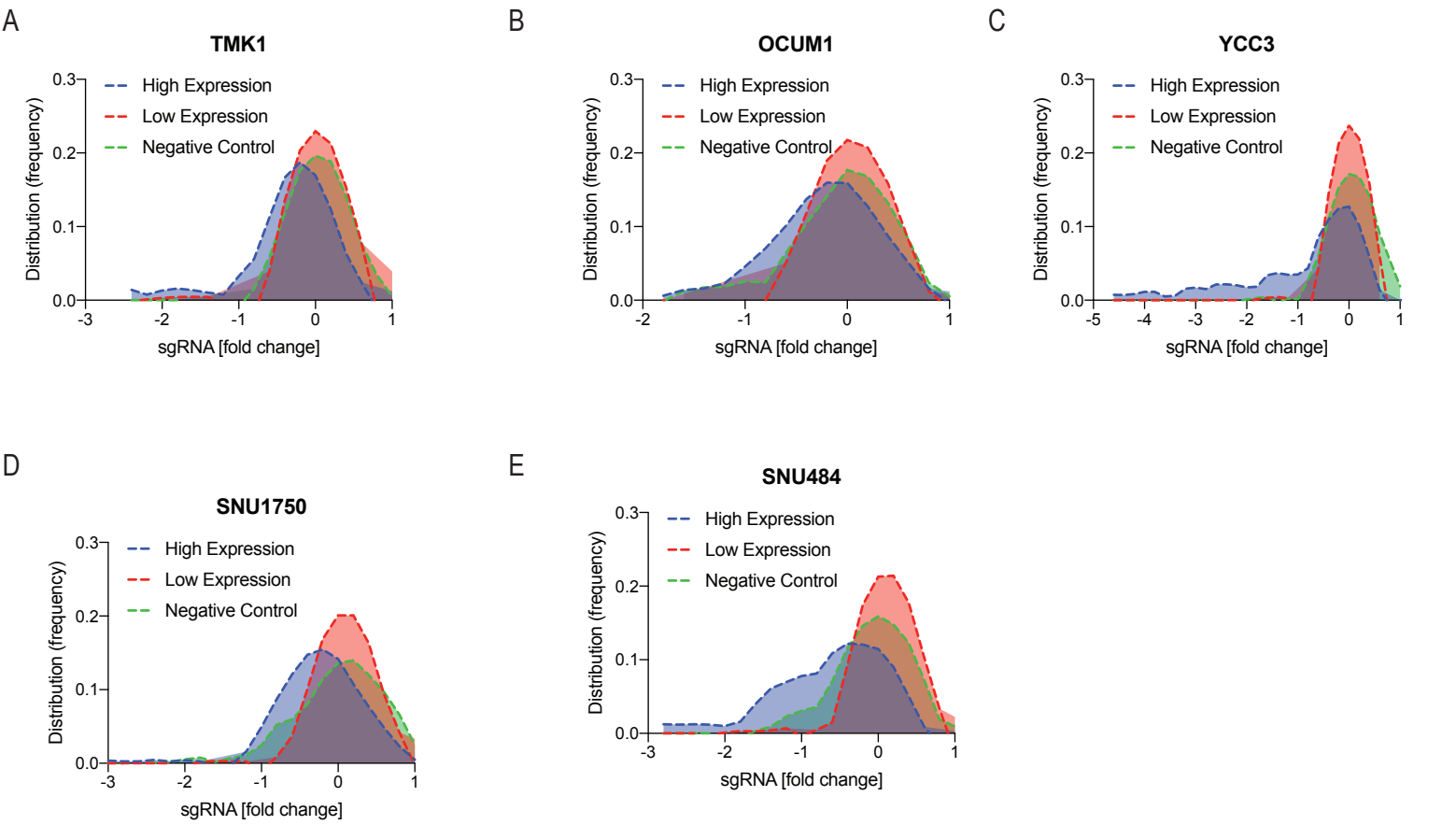


Figure S1

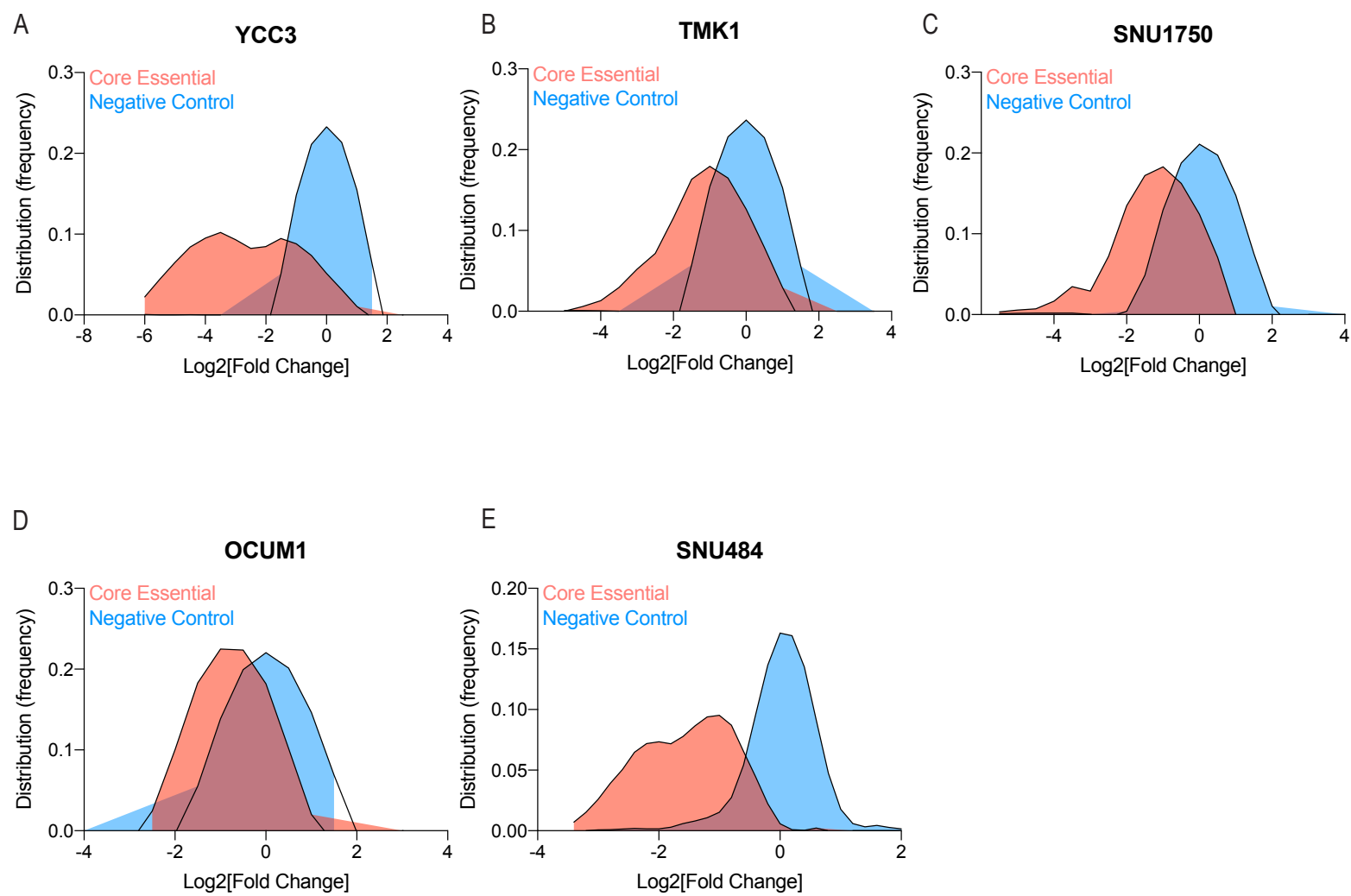
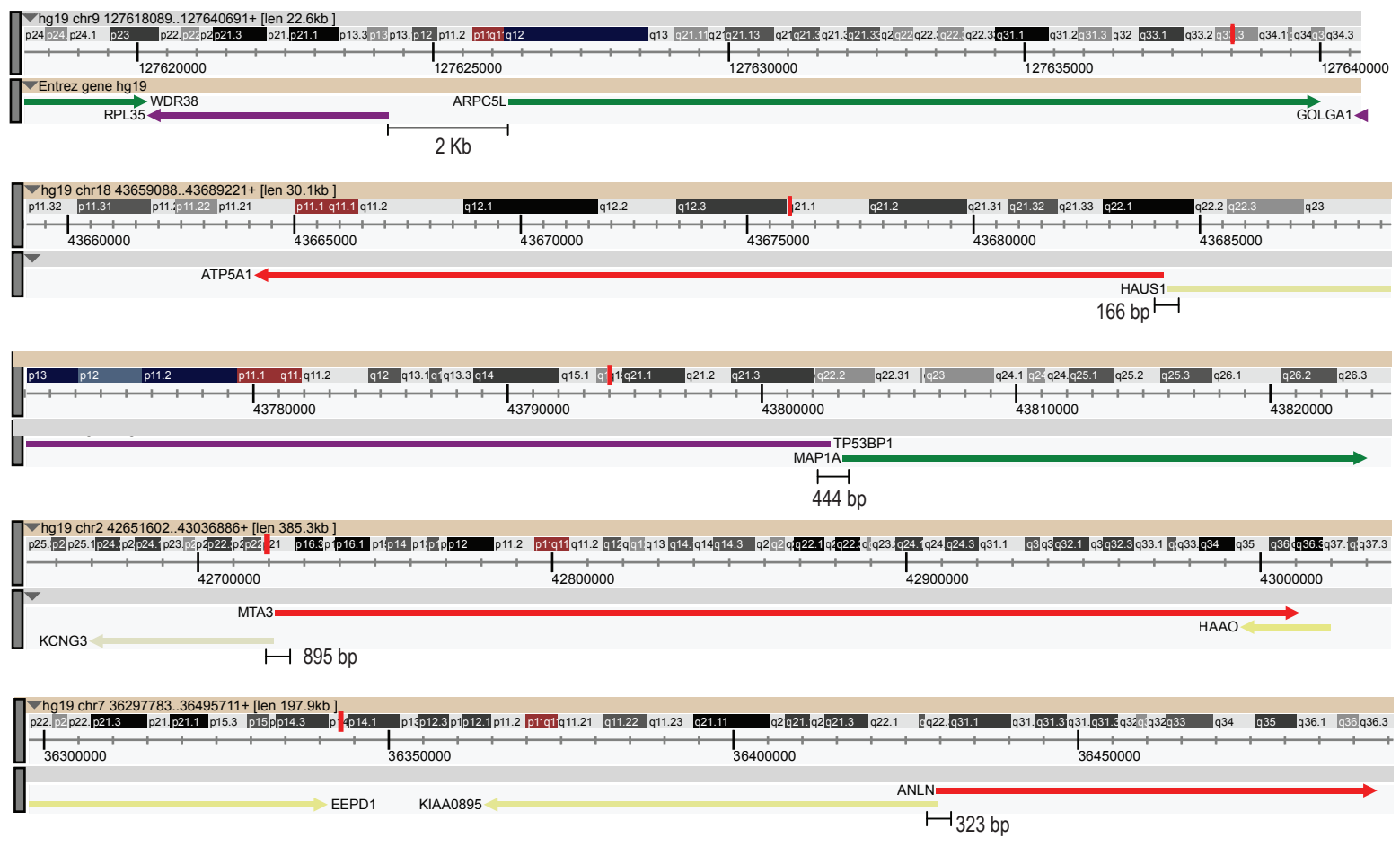


Figure S2

A



B

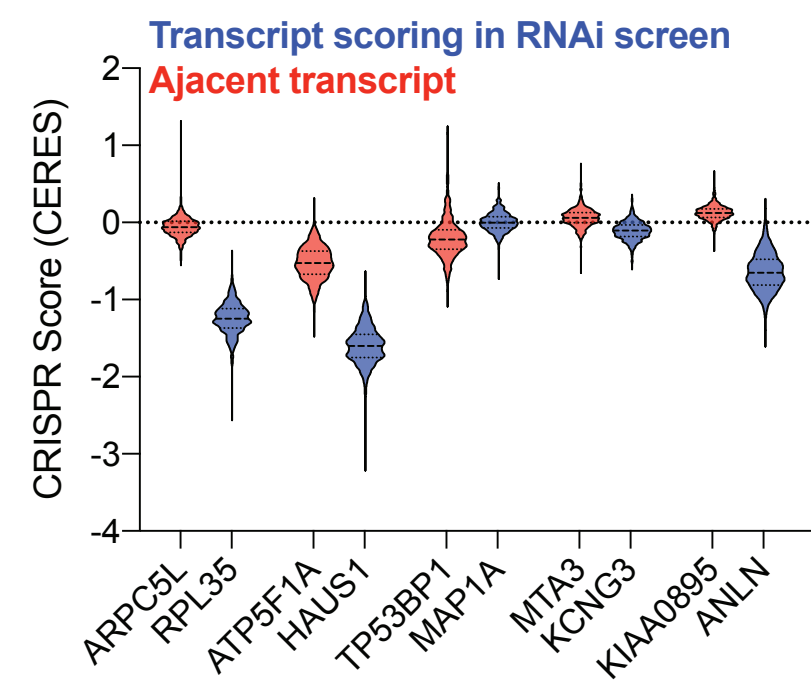
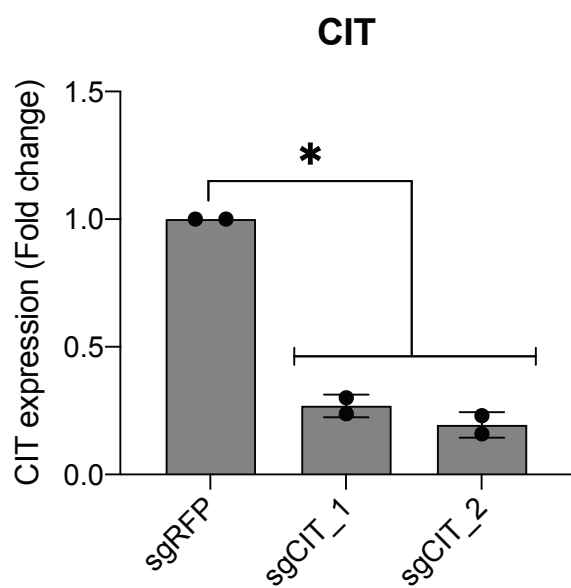
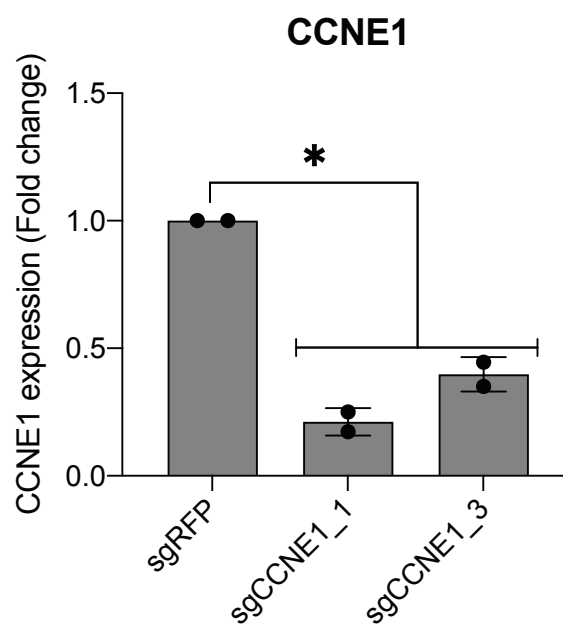


Figure S3

A



B



C

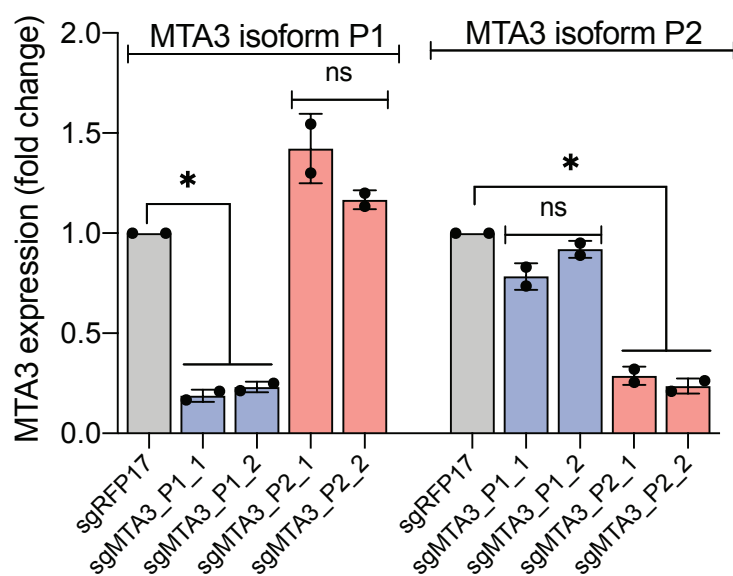
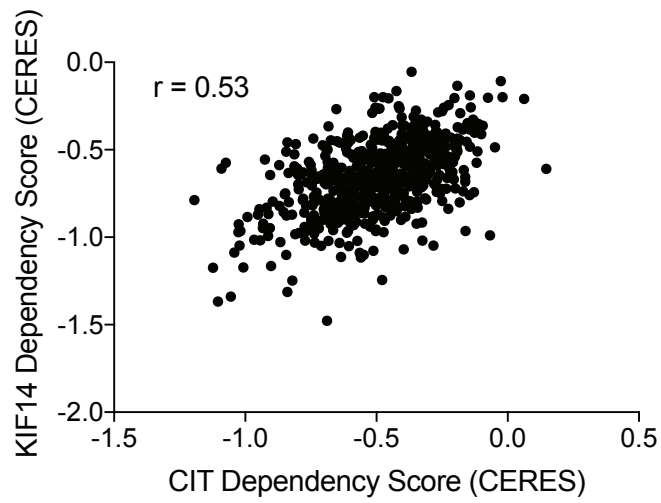
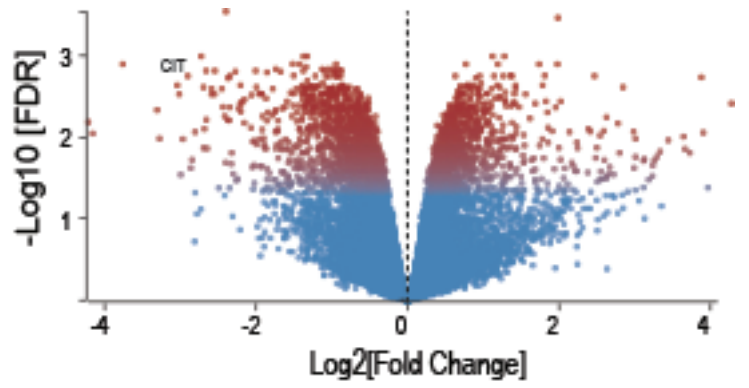


Figure S4

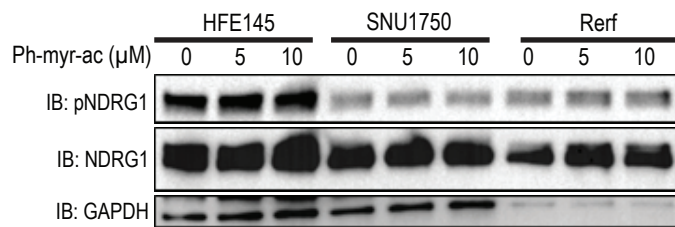
A



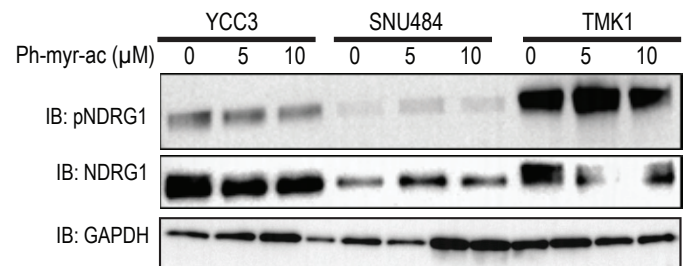
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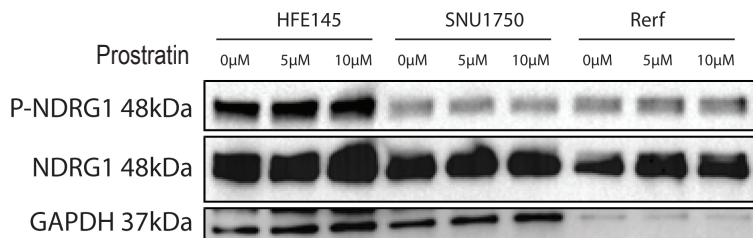
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D



E



F

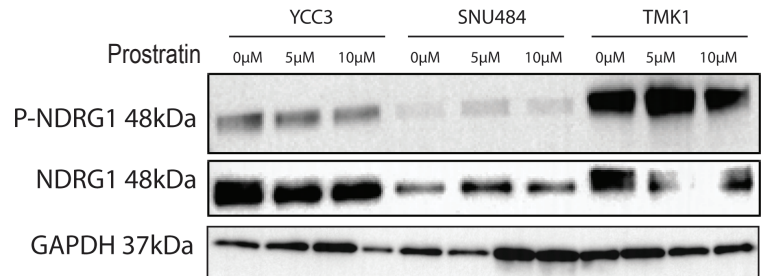


Figure S5

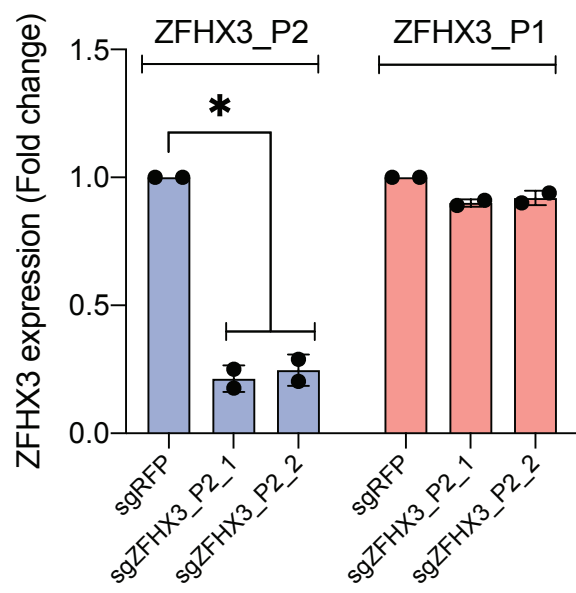


Figure S6

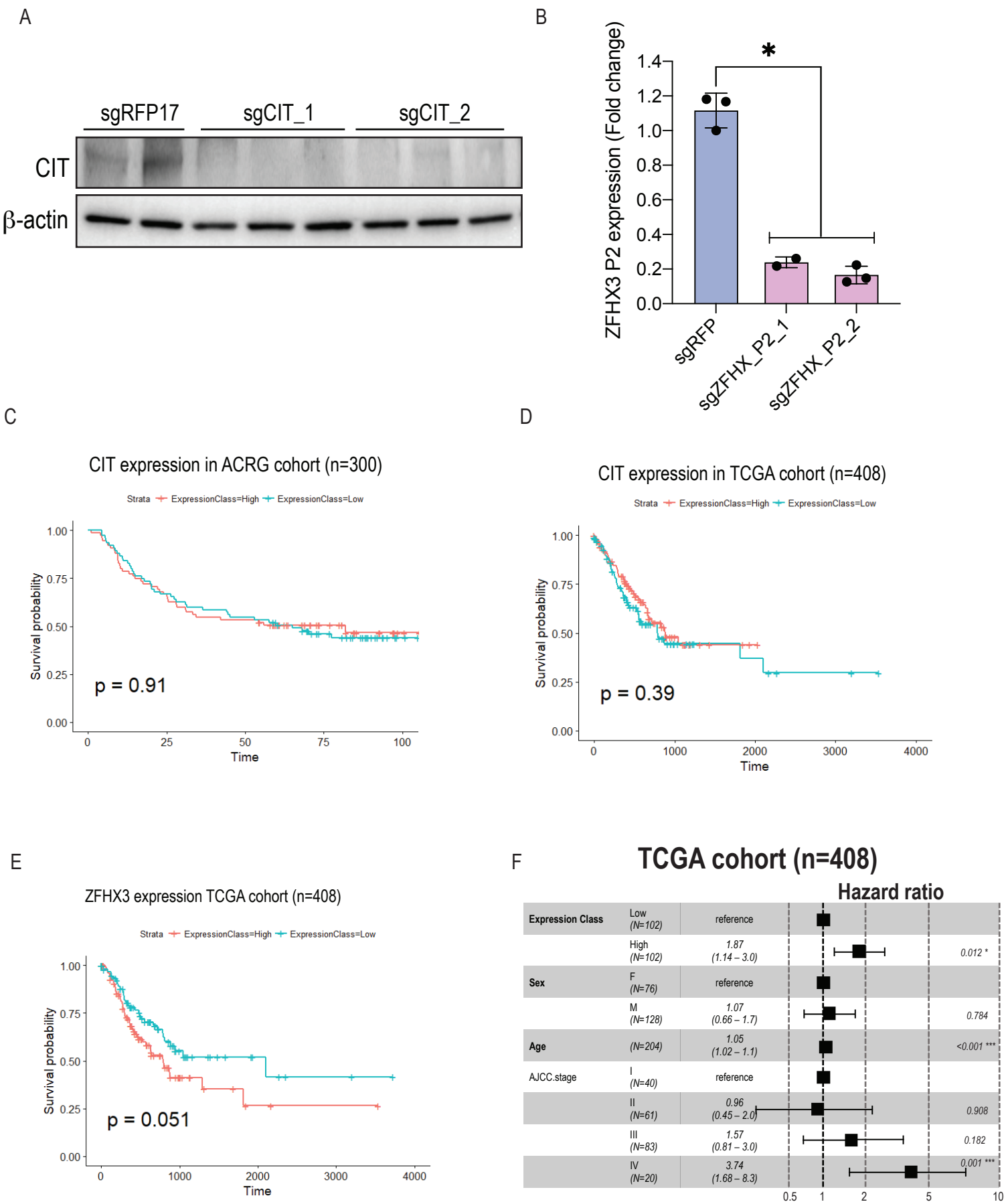


Figure S7