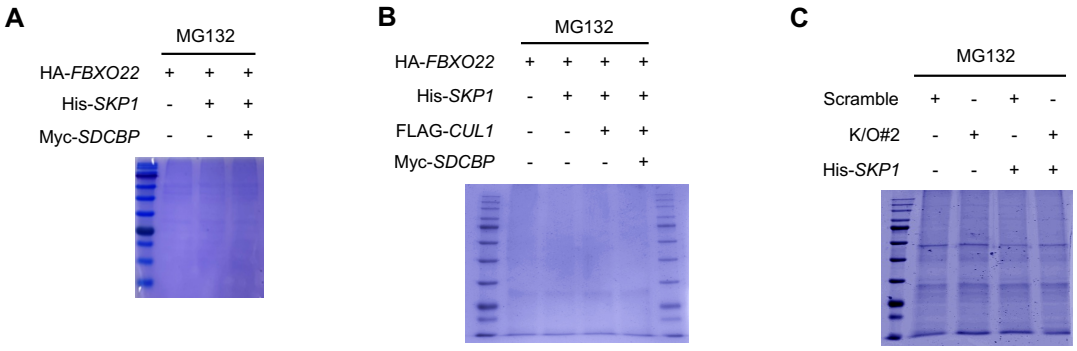
APPENDIX FIGURES

Appendix Figure Contents

Appendix Figure S1

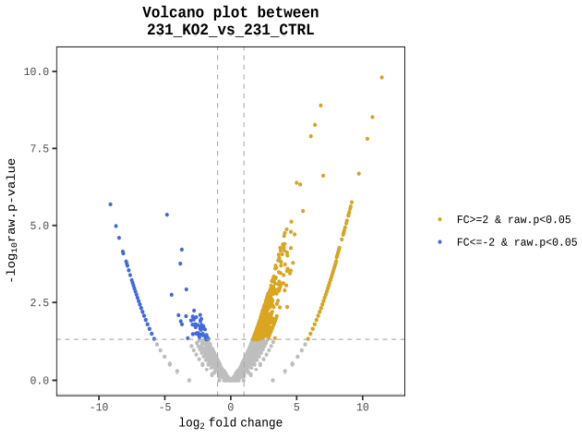


Appendix Figure S1. SDCBP associates with FBXO22 and impairs SCF^{FBXO22}-targeted substrates for K48-linked degradative ubiquitination.

(A) Coomassie blue-stained SDS-PAGE gel of His-pulldown assay in Fig EV5E. (B) Coomassie blue-stained SDS-PAGE gel of His-pulldown assay in Fig 4D. (C) Coomassie blue-stained SDS-PAGE gel of His-pulldown assay in Fig 4E.

Appendix Figure S2

A



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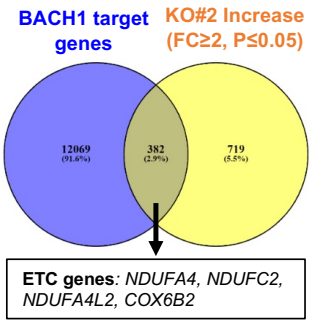

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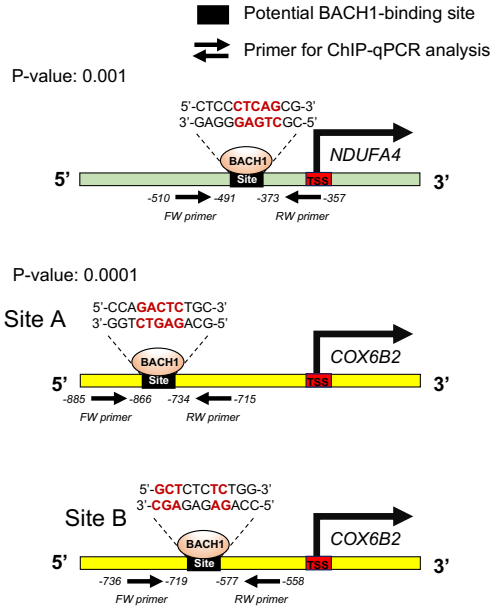

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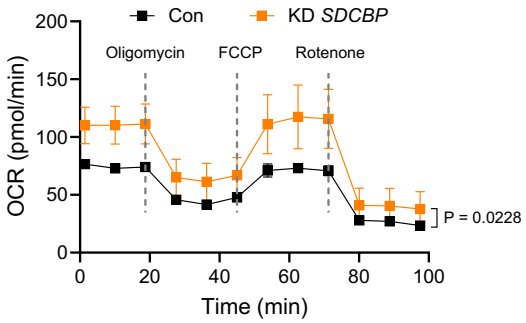

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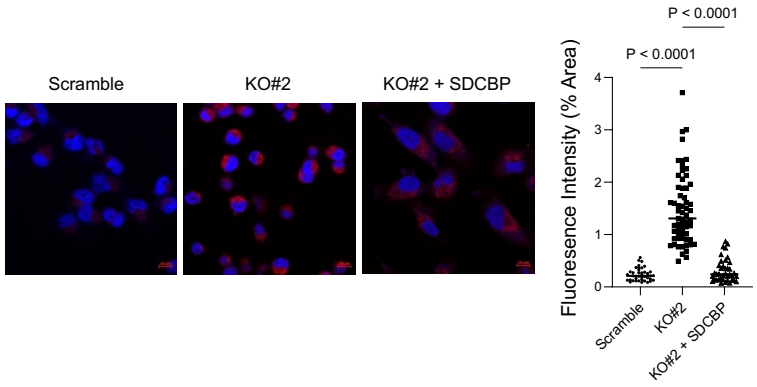


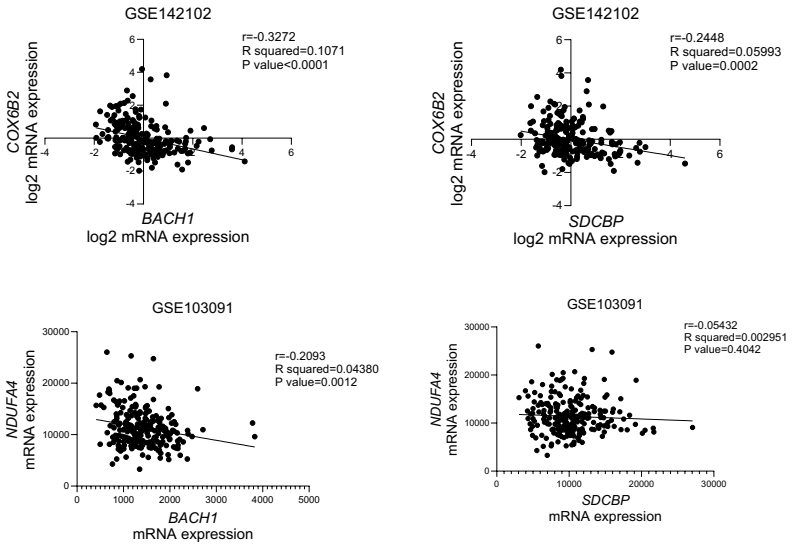
Appendix Figure S2. Identification of *NDUFA4* and *COX6B2 as ETC genes regulated by the SDCBP-BACH1 axis.*

(A) RNA sequencing-derived volcano plot identifying the differentially expressed genes (DEGs) in scramble and in SDCBP-KO MDA-MB-231 cells (n=3); The negative-log10 of the *P*-value and the log2 FC were plotted for gene expression in SDCBP-KO relating to the control. Blue and orange-yellow dots indicate downregulated and upregulated DEGs, respectively. Gray dots indicate non-altered genes or non-significant changes. (B) Venn diagram showing the overlap of 382 potential gene candidates identified from the BACH1 target gene library database and upregulated DEGs from RNA-seq. (C) Schematic showing the BACH1-binding sites for approximately 1000 base pairs upstream of the transcriptional start site (TSS) of *NDUFA4* and *COX6B2*. Black arrows indicate primers used for ChIP-qPCR. BACH1-binding sites on the promoters of genes of interest were bioinformatically predicted using the open EPD promoter database with a *P*-value of at least 0.001. (D) Oxygen consumption rate (OCR) showing the mitochondrial activity of MDA-MB-231 cells transfected with scramble siRNA or SDCBP siRNA (n=3). (E) Left, immunofluorescence staining and confocal imaging of the fluorescent signals for TMRE (orange-red color) in the scramble control, SDCBP-KO MDA-MB-231 cells, and SDCBP-KO MDA-MB-231 cells transfected with SDCBP after incubation with TMRE. DAPI (blue color) was used to stain the nucleus. Representative confocal images are shown; scale bars = 20 μm. Right, fluorescent levels of the TMRE were quantified based on their spectral densities (n=36). Data are expressed as the mean ± SEM and analyzed using two-tailed Student's *t* test with Welch's correction (D), or one-way ANOVA (E). All experiments were repeated at least three times unless otherwise indicated. P values less than 0.05 were considered

statistically significant.

Appendix Figure S3

A



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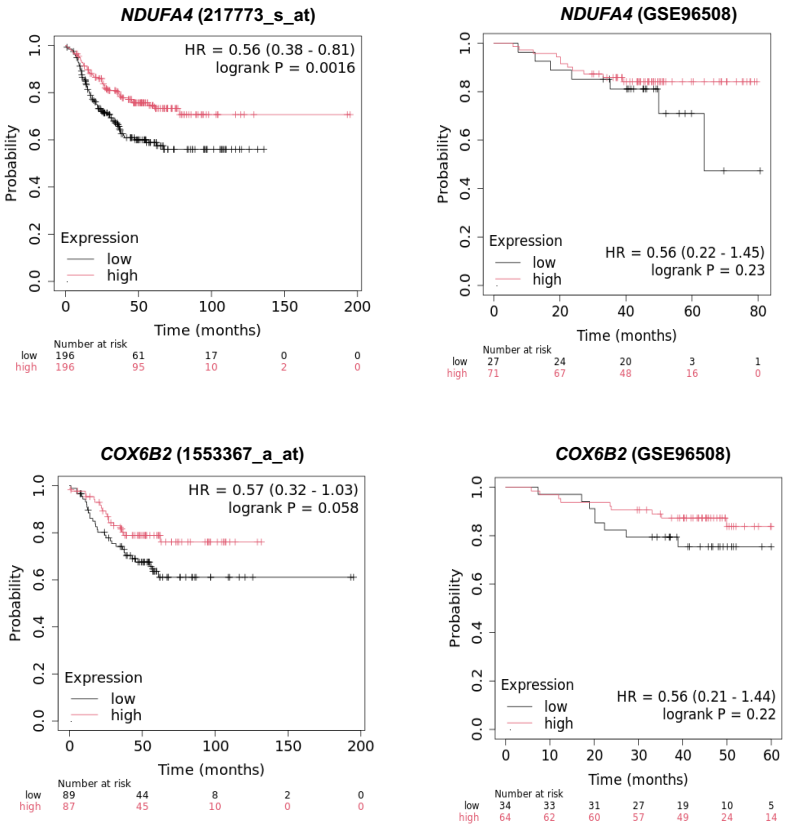
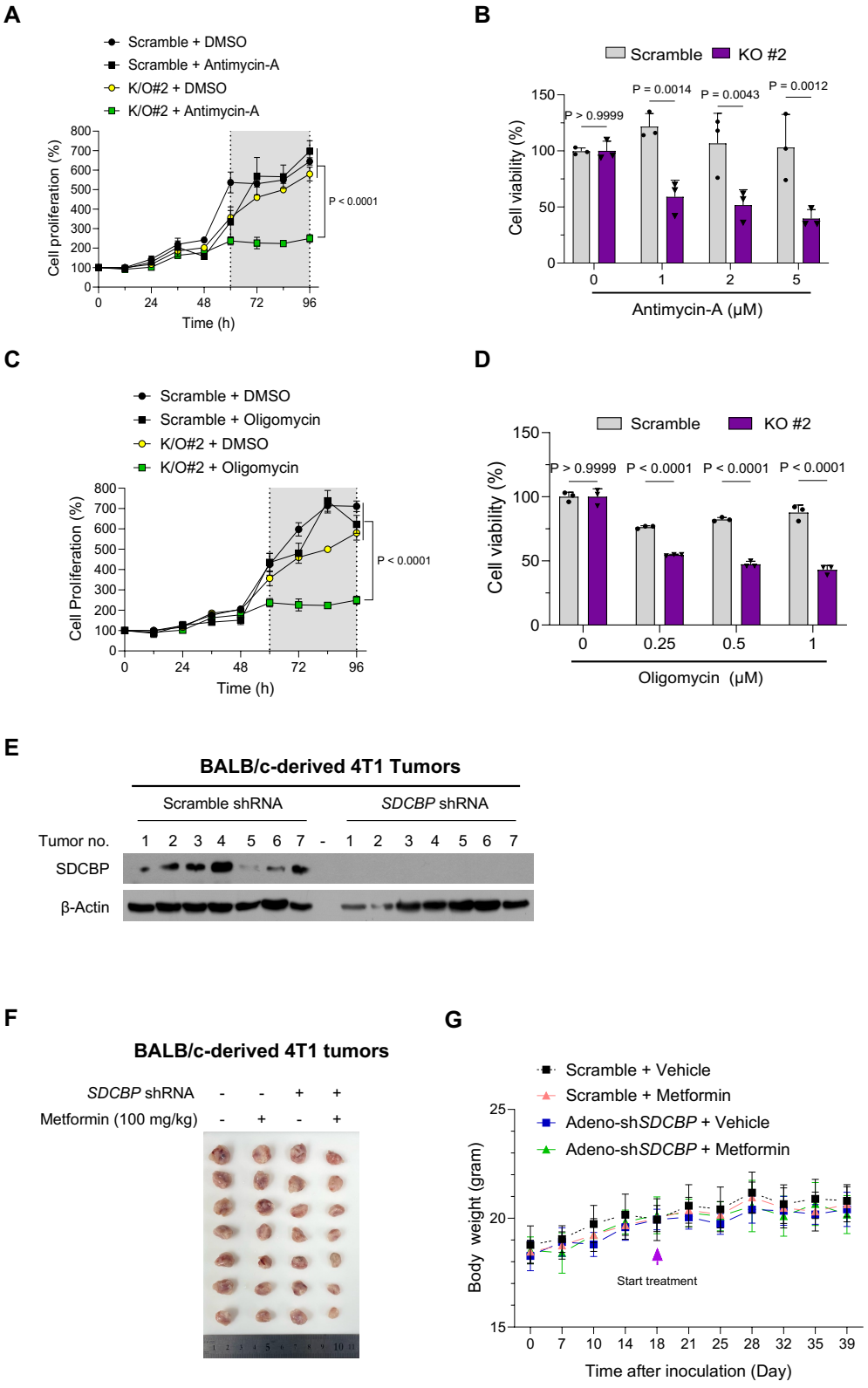


Appendix Figure S3. TCGA data analysis of SDCBP, BACH1, COX6B2, and NDUFA4 MRNA expression in TNBC patients.

(A) Upper, TCGA data analysis showing negative correlation between *BACH1* mRNA and *COX6B2* mRNA (Pearson correlation coefficient r = -0.3272, P<0.0001), and between *SCBPB* mRNA and *COX6B2* mRNA (Pearson correlation coefficient r = -0.2448, P=0.0002) in GSE142102 (n = 226) dataset of TNBC patients. Lower, TCGA data analysis showing negative correlation between *BACH1* mRNA and *NDUFA4* mRNA Pearson correlation coefficient = -0.2093, P=0.0012), and between *SCBPB* mRNA and *NDUFA4* mRNA (Pearson correlation coefficient r = -0.05432, P=0.4042) in GSE103091 (n = 238) dataset of TNBC patients. (B) TCGA data analysis showing a negative association between overall survival and *NDUFA4* mRNA and *COX6B2* mRNA expression in TNBC patients.

Appendix Figure S4



Appendix Figure 4. Targeting SDCBP enhances anti-tumor efficiency of the mitochondrial ETC inhibitors and anti-tumor effect of metformin in TNBC cells.

(A) Cell proliferation of the scramble control and SDCBP-KO MDA-MB-231 cells after treatment with antimycin-A for the indicated periods of time. All experiments were performed in triplicate. (B) Cell viability of the scramble control and SDCBP-KO MDA-MB-231 cells after treatment with antimycin-A for 96 h. All experiments were performed in triplicate. (C) Cell proliferation of the scramble control and SDCBP-KO MDA-MB-231 cells after treatment with oligomycin for the indicated periods of time. All experiments were performed in triplicate. (D) Cell viability of the scramble control and SDCBP-KO MDA-MB-231 after treatment with oligomycin for 96 h. All experiments were performed in triplicate. (E) Western blot showing SDCBP protein expression in 4T1 tumors isolated from BALB/c mice at the end of the experiment in Fig 6D. (F) Images of 4T1 tumors isolated from BALB/c mice at the end of the experiment in Fig 6D. (G) Body weight change of 4T1-bearing BALB/c mice during the treatments in Fig 6D (n= 7 mice/group). Data are expressed as the mean ± SEM and analyzed using two-way ANOVA (A, B, C, D). All experiments were repeated at least three times unless otherwise indicated. P values less than 0.05 were considered statistically significant.