Protein phosphatase 2A-B55β mediated mitochondrial p-GPX4 dephosphorylation promoted sorafenib-induced ferroptosis in hepatocellular carcinoma via regulating p53 retrograde signaling

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Supplementary Materials

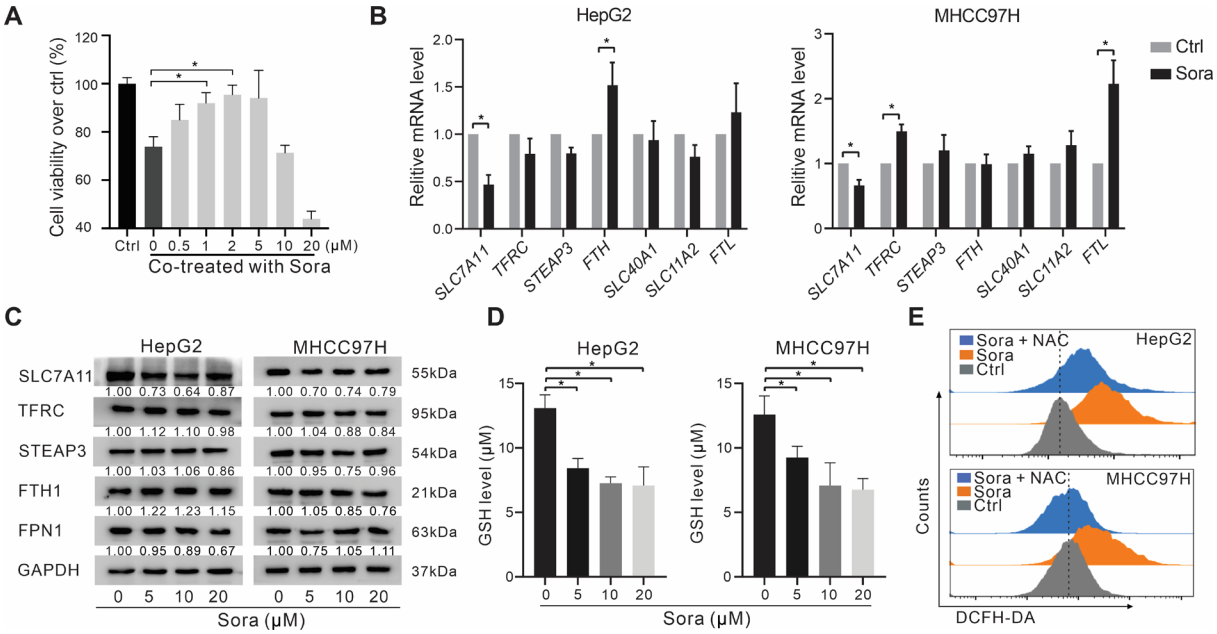

Figure S1. Sorafenib induced GPX4-related ferroptosis in HCC cells. A. HepG2 cells were treated with sorafenib (Sora, 10 μM) and co-treated with different concentrations of the ferroptosis inhibitor Ferrostatin-1 (Fer-1, 1 μM) for 24 h. Cell viability was measured with the MTS assay. **B.** The mRNA levels of *SLC7A11*, *TFRC*, *STEAP3*, *FTH*, *SLC40A1*, *SLC11A2*, and *FTL* in HCC cells upon Sora treatment (10 μM, 24 h). **C.** The protein levels of SLC7A11, TFRC, STEAP3, FTH1, and FPN1 in HCC cells upon Sora treatment (5, 10, 20 μM, 24 h). **D.** GSH level in HCC cells upon Sora treatment (5, 10, 20 μM, 24 h). **E.** The ROS level in Sora-treated HCC cells. HCC cells were individually treated with Sora (10 μM, 24 h) or co-treated with ROS inhibitor NAC (10 μM, 12 h). Cells was stained with a DCFH-DA fluorescent probe and tested with FCM. * $P < 0.05$.

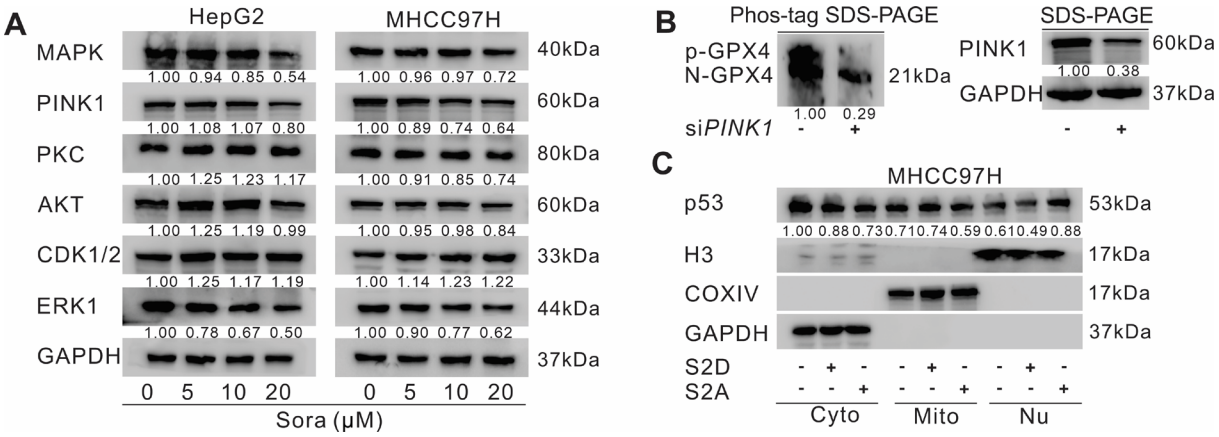

Figure S2. Dephosphorylation of mitochondrial p-GPX4^{Ser2} triggered ferroptosis and induced mitochondrial p53 translocation. A. Protein levels of MAPK, PINK1, PKC, AKT, CDK, and ERK in HCC cells treated with Sora (5, 10, 20 μM, 24 h). **B.** HepG2 cells were treated with si*PINK1* (50 nM, 24 h). Whole cell lysates were isolated and subjected to Phos-tag SDS-PAGE, and the protein levels of the phosphorylated GPX4 (p-GPX4) and non-phosphorylated GPX4 (N-GPX4) were tested. **C.** Protein levels of p53 in cytosolic (Cyto), mitochondrial (Mito), and nucleus (Nu) fractions of WT cells and the constructed S2D or S2A MHCC97H cells, n = 3.

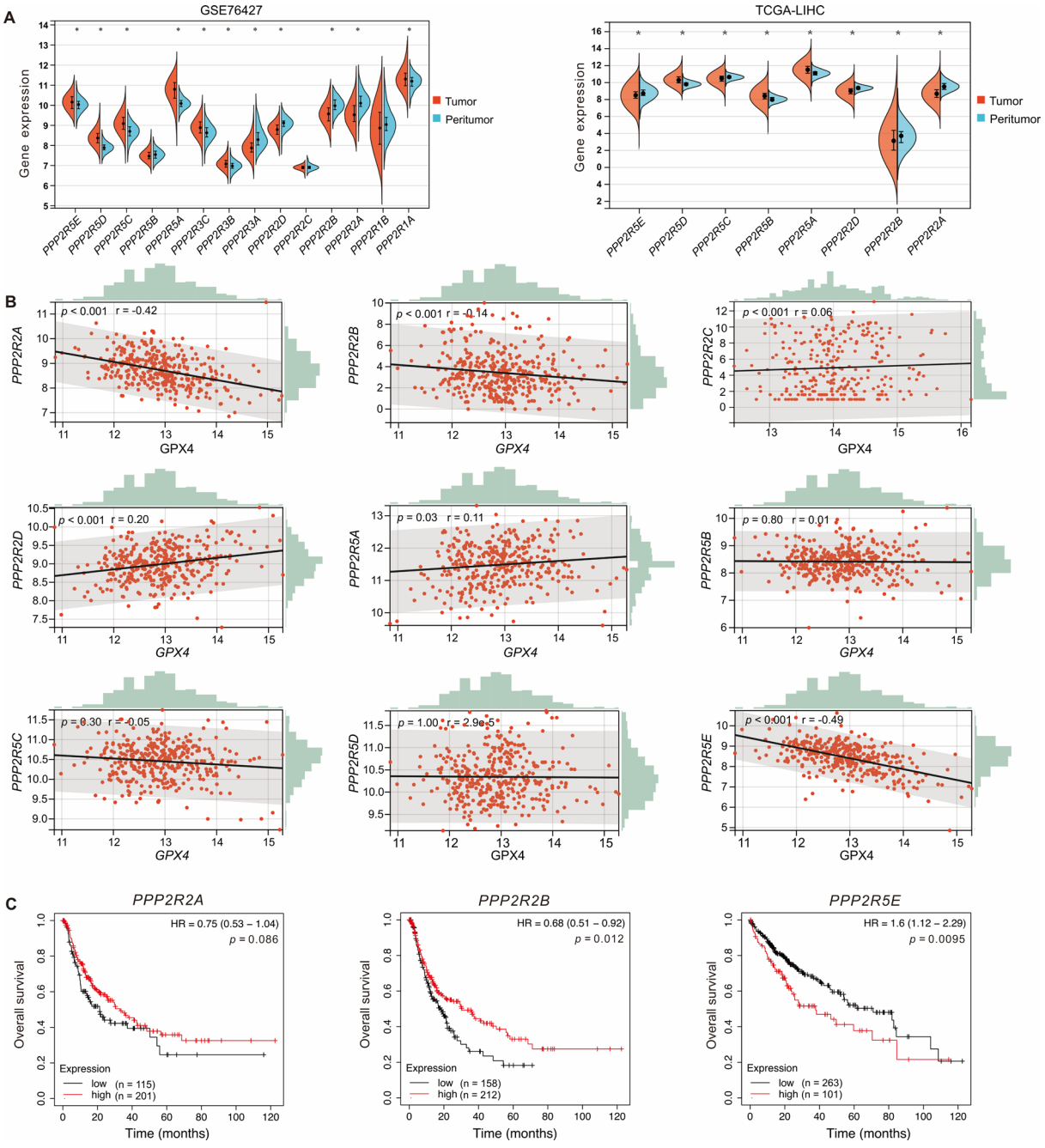

Figure S3. *PPP2R2B* associated with the development of HCC via the negative modulating on GPX4 expression. A. The expression of various regulatory B subunits of PP2A in tumor tissues and peritumor tissues of HCC patients from the GEO database (GSE76427, n = 243) and TCGA-LIHC database (n = 423). **B.** The correlation between the expression of *GPX4* and regulatory B subunits of PP2A in HCC patients from the TCGA-LIHC database (n = 423). **C.** Kaplan-Meier analysis showed the overall survival of HCC patients from TCGA-LIHC with different expressions (low or high level) of *PPP2R2A*, *PPP2R2B*, and *PPP2R5E*.

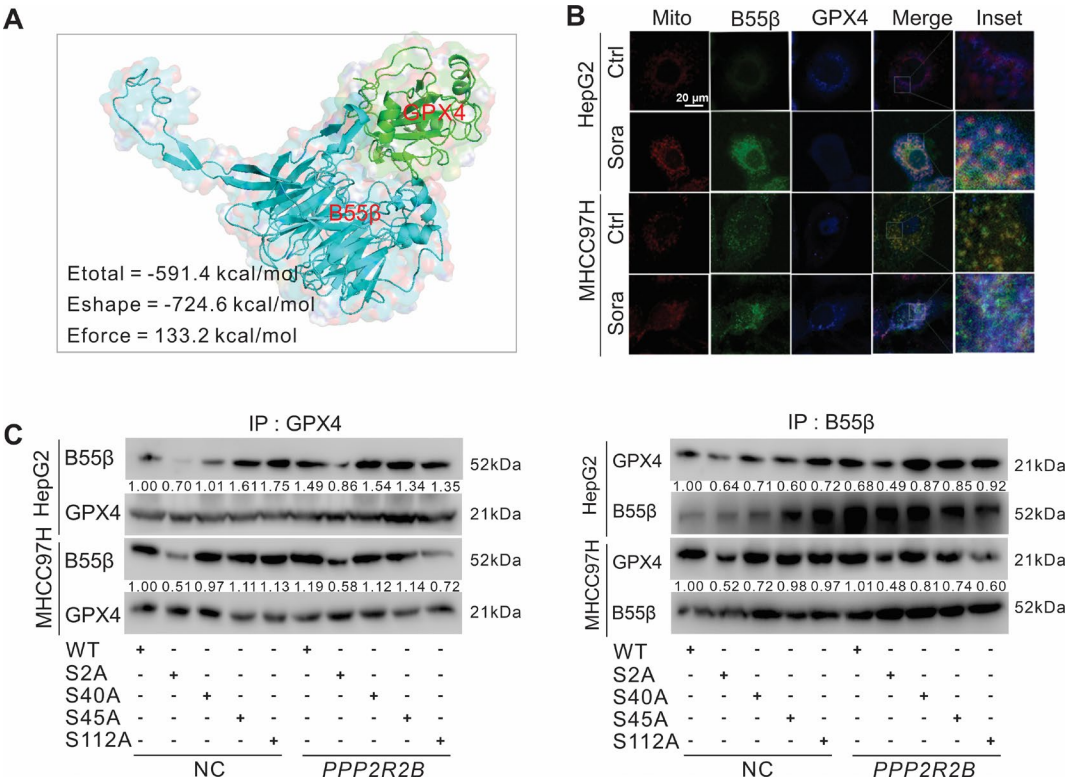

Figure S4. PP2A-B55β interacted with GPX4 and potentially targeted dephosphorylation regulation.

A. Molecular docking between GPX4 and B55β was performed using the Hex Protein Docking software (Hex 8.0.0). **B.** The distribution of B55β and GPX4 in mitochondria of HCC cells treated with Sora (10 μM, 24 h). Scale bars, 20 μm. **C.** HCC cells (HepG2 and MHCC97H cells) were transfected with the pBabe-*PPP2R2B* plasmid to construct the B55β-overexpression cells following site-directed mutation of the serine (S) 2, S40, S45, and S112 sites of GPX4, while S to A (alanine) mimics the dephosphorylation. The pBabe plasmid-transfected cells were as negative control (NC). The potential target proteins in whole cell lysates were pulled down by GPX4 (Left) and B55β (Right) antibodies. The interaction between B55β and GPX4 was detected by Co-IP.

Table S1. Information on the primary antibodies used in the present study

Primary antibodies	Manufacturers	Code number	Dilution/Concentration				Molecule weight	Species
			WB	IF/PLA	IP	IHC		
GPX4	Santa Cruz	sc-166570	1:400	1:100	5 μg	1:100	21 kDa	Mouse
p53	Abcam	ab26	1:1000	1:400	5 μg		53 kDa	Mouse
B55α	Abcam	ab197194	1:1000				52 kDa	Rabbit
B55β	Abcam	ab251885	1:1000	1:400	5 μg	1:200	52 kDa	Rabbit
B56ε	Abcam	ab198290	1:1000				55 kDa	Rabbit
Phosphoserine	IMMUNECHEM	ICP9806	1:250		5 μg		45 kDa	Rabbit
FLAG	HUABIO	HA601080	1:5000		5 μg		35 kDa	Mouse
Histone 3(H3)	Cell Signaling Technology	#4499	1:2000				17 kDa	Rabbit
COXIV	Abcam	ab16056	1:2000				17 kDa	Rabbit
GAPDH	LABLEAD	G0100-100UL	1:5000				37 kDa	Mouse
TOM20	Santa Cruz	sc-17764	1:400				20 kDa	Mouse
SLC7A11	Abcam	ab307601	1:1000				55 kDa	Rabbit
FPN1	Santa Cruz	sc-518125	1:400				63 kDa	Mouse
STEAP3	Abcam	ab151566	1:1000				54 kDa	Rabbit
TFRC	Santa Cruz	sc-393719	1:300				95 kDa	Mouse
FTH1	Cell Signaling Technology	#4393	1:1000				21 kDa	Rabbit
MAPK	Cell Signaling Technology	#8690	1:1000				40 kDa	Rabbit
PINK1	Cell Signaling Technology	#6946	1:1000				60 kDa	Rabbit
PKC	Santa Cruz	sc-17769	1:400				80 kDa	Mouse
AKT	Cell Signaling Technology	#4685	1:1000				60 kDa	Rabbit
CDK1/ CDK2 (CDK1/2)	Santa Cruz	sc-53219	1:400				33 kDa	Mouse
ERK1	Cell Signaling Technology	#4372	1:1000				44 kDa	Rabbit

Table S2. Information of the paired primers in the present study

Experiment	Genes	Primers	Sequences (5'- 3')
Real-time PCR	*SLC7A11*	FP	TCCTGCTTTGGCTCCATGAACG
		RP	AGAGGAGTGTGCTTGCGGACAT
	SLC40A1	FP	TGAGCCTCCCAAACCGCTTCCATA
		RP	GGGCAAAAAGACTACAACGACGACTT
	STEAP3	FP	TGCAAACTCGCTCAACTGGAGG
		RP	AGGCAGGTAGAACTTGTAGCGG
	SLC11A2	FP	AGCCACTCAGGTATCCACCAT
		RP	CCAGGGGACTGTGAAAGAGAG
	FTH1	FP	TGAAGCTGCAGAACCAACGAGG
		RP	GCACACTCCATTGCATTCAGCC
	FTL	FP	AGCCTTCTTTGTGCGGTCGGGTAA
		RP	ACGCCTTCCAGAGCCACATCAT
	TFRC	FP	ACCATTGTCATATACCCGGTTCA
		RP	CAATAGCCCAAGTAGCCAATCAT
Site-directed mutagenesis	S2A	FP	ATGGCCCTCGGCCGCCTTTGCCGCCTACTG
		RP	GAGCAGCGCCGGCTTCAGTAGGCGGCAAAG
	S2D	FP	ATGGATCTCGGCCGCCTTTGCCGCCTACTG
		RP	GAGCAGCGCCGGCTTCAGTAGGCGGCAAAG
	S40A	FP	GCCATGCACGAGTTTTCCGCCAAGGACATC
		RP	AACCATGTGCCCGTCGATGTCCTTGGCGGA
	S45A	FP	GCCGCCAAGGACATCGACGGGCACATGGTT
		RP	GTACTTGTCCAGGTTAACCATGTGCCCGTC
	S112A	FP	GCCAACGAAGAGATCAAAGAGTTCGCCGCG
		RP	TTTGACGTTGTAGCCCGCGGCGAACTCTTT
	S112D	FP	GATAACGAAGAGATCAAAGAGTTCGCCGCG
		RP	TTTGACGTTGTAGCCCGCGGCGAACTCTTT