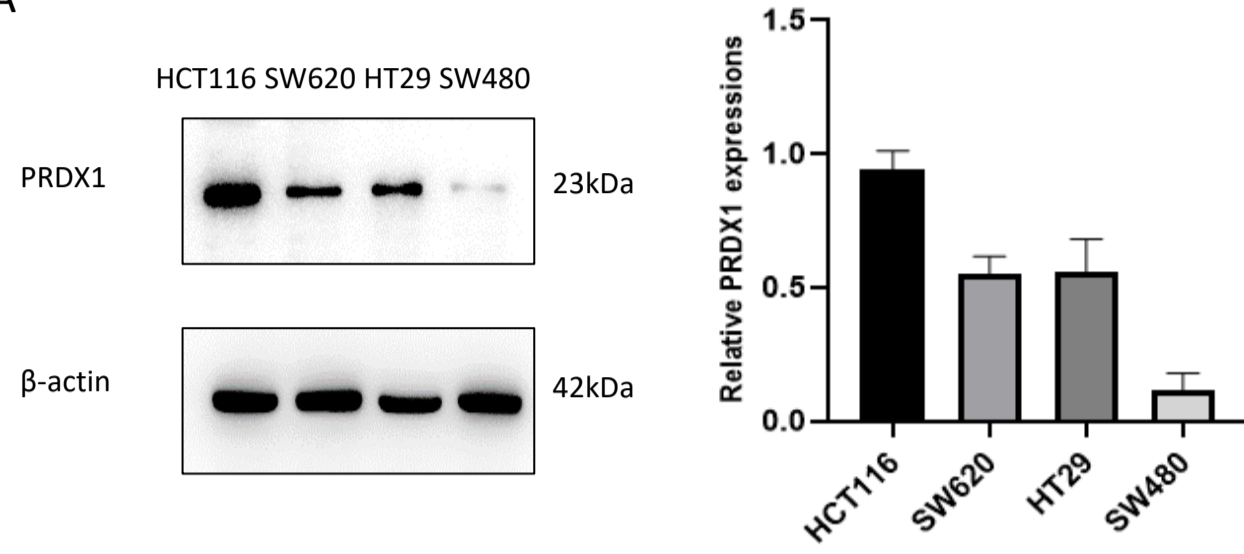
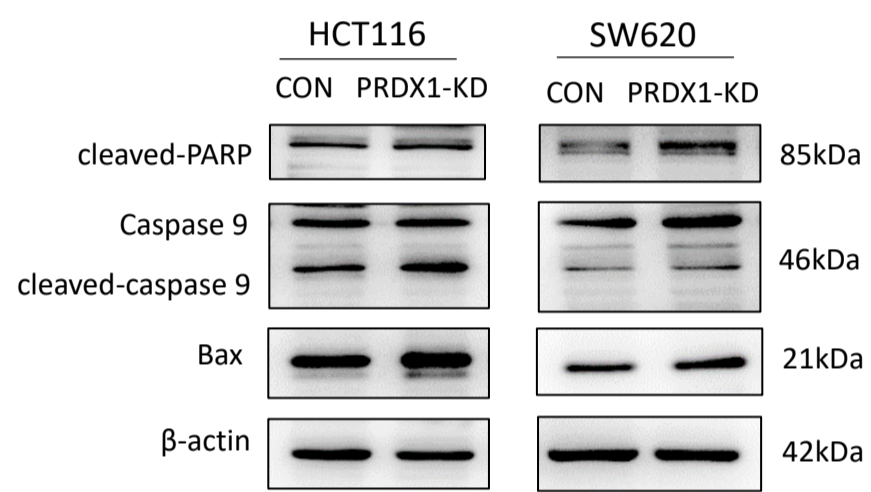


Fig. S1

A



B



C

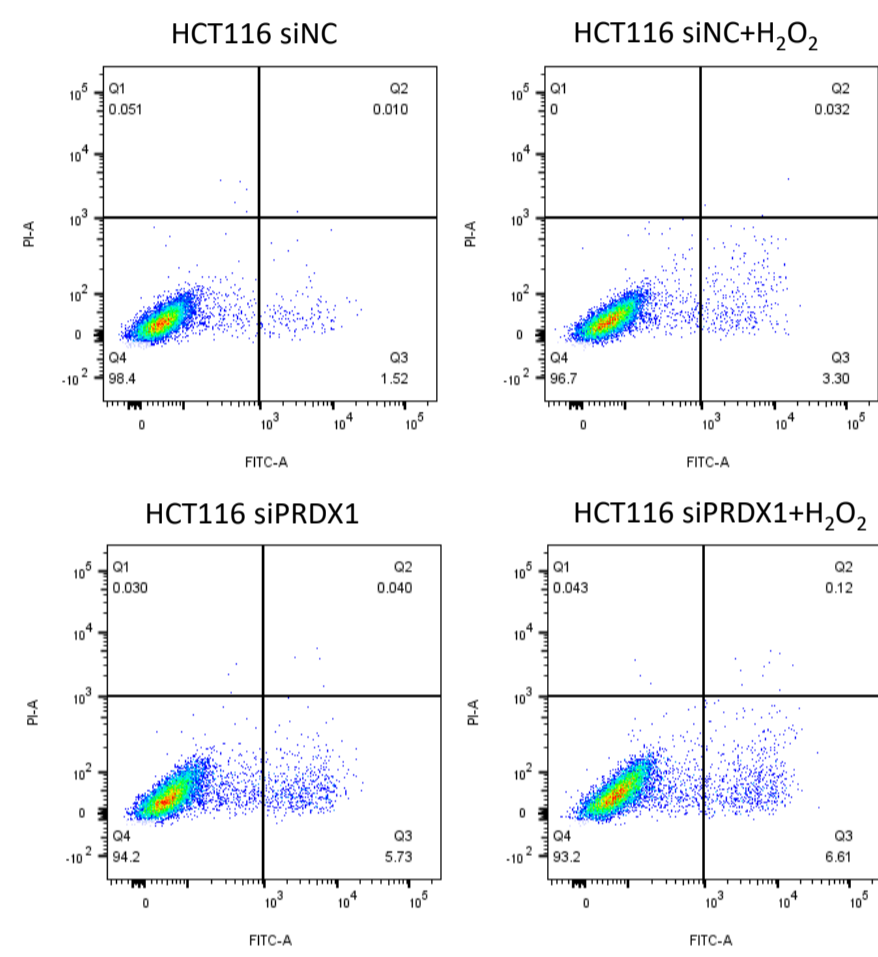
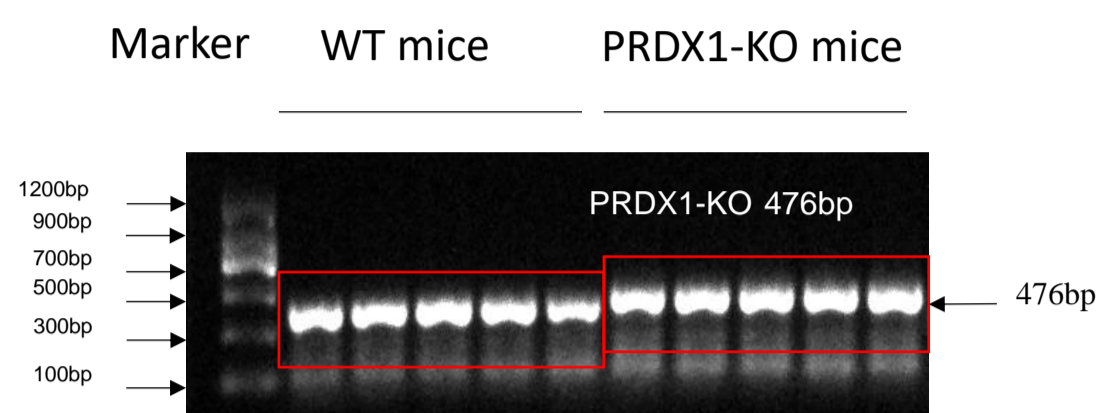
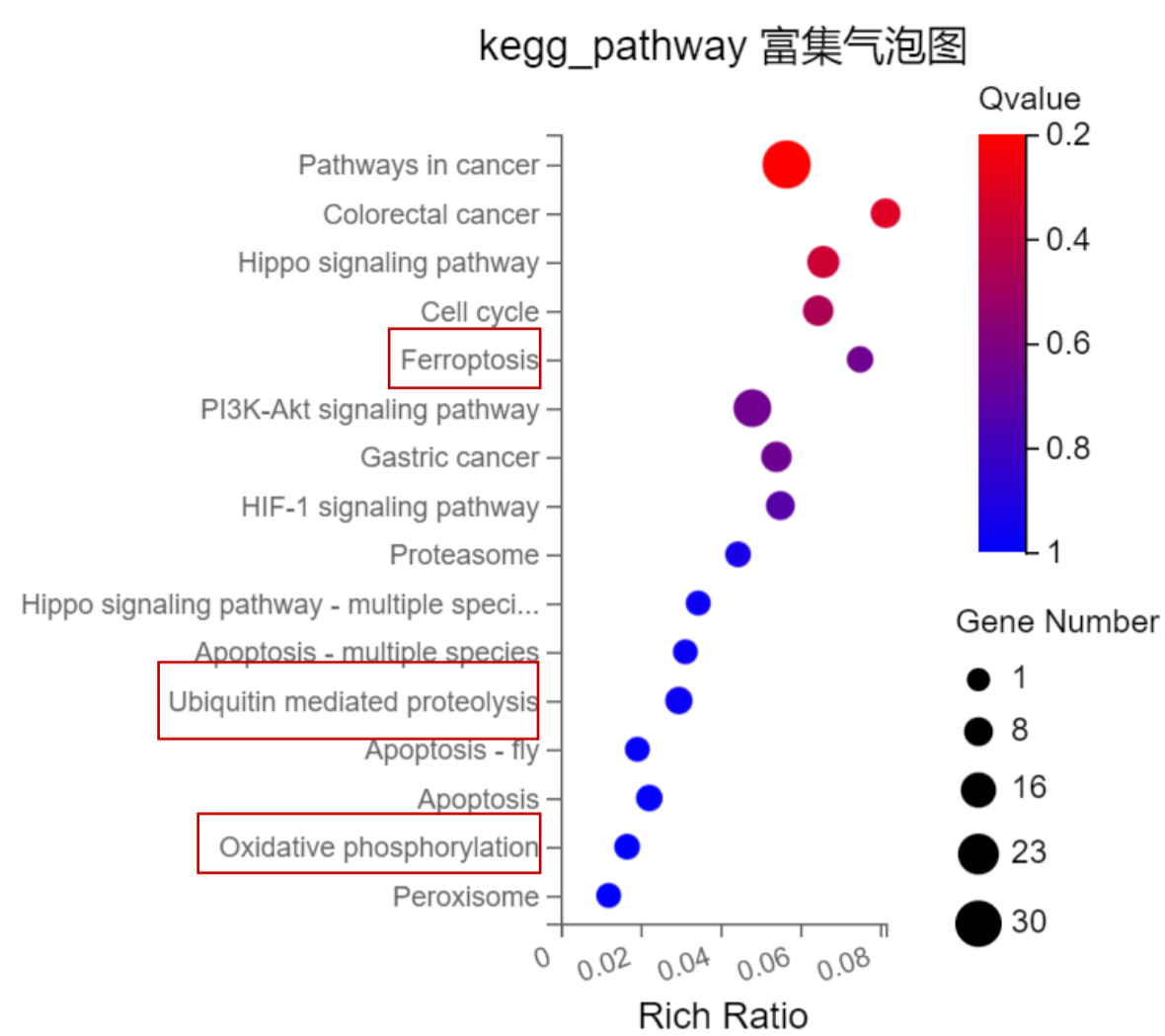


Fig. S2

A



B



C

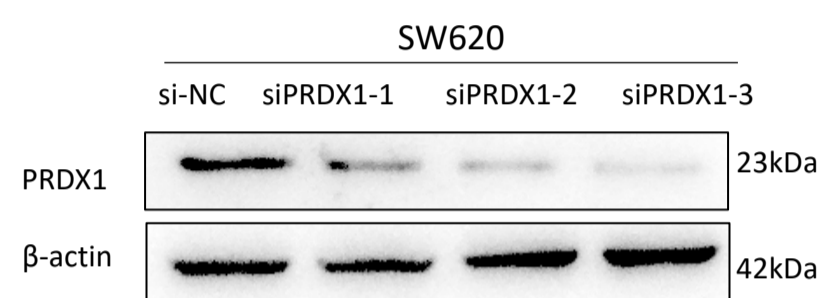
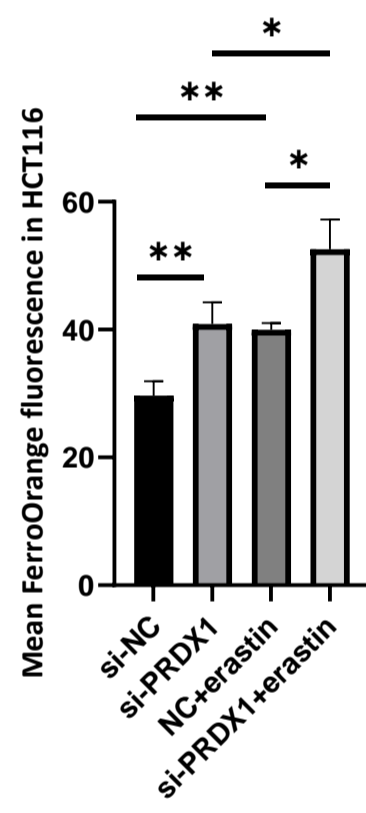
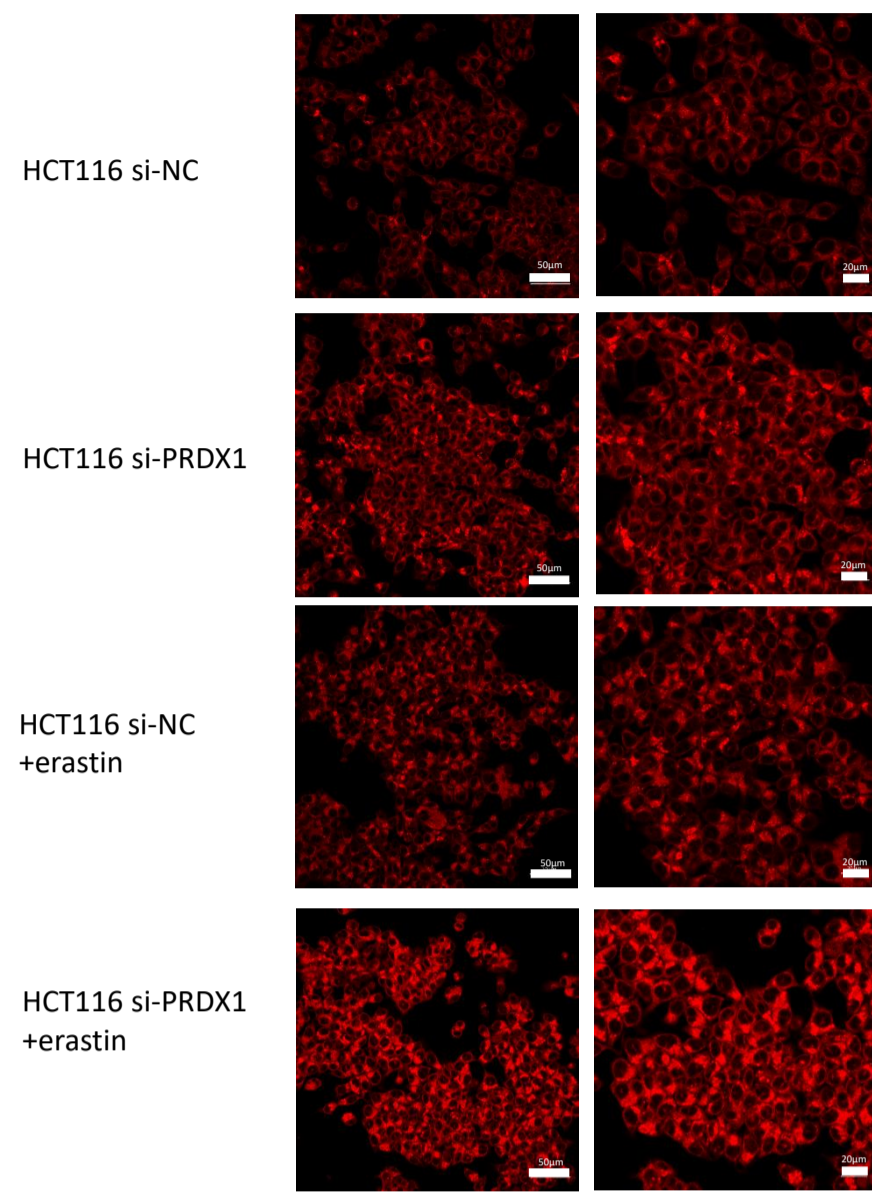


Fig. S3

A



B

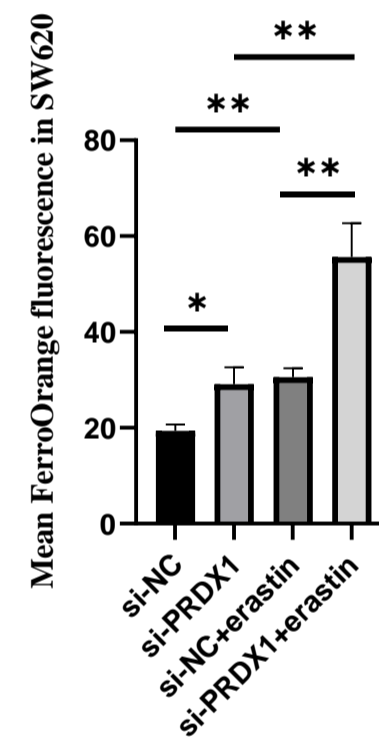
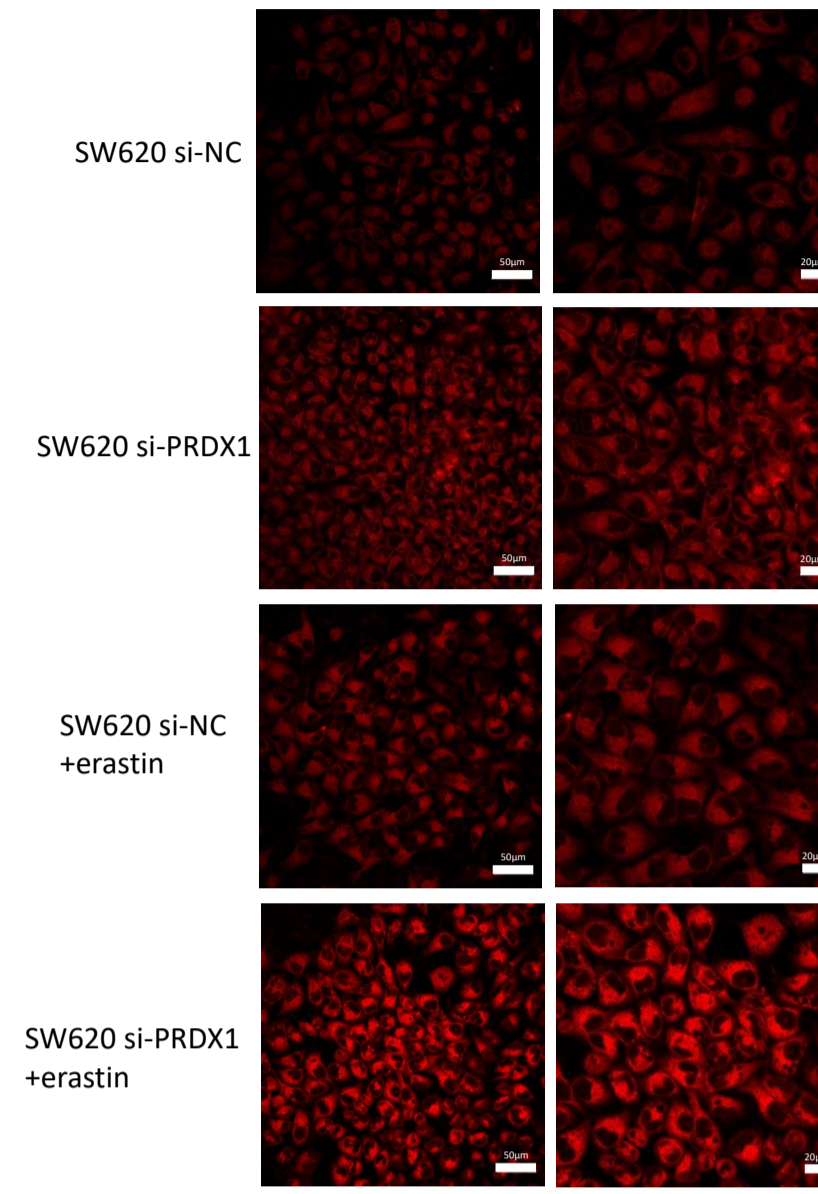
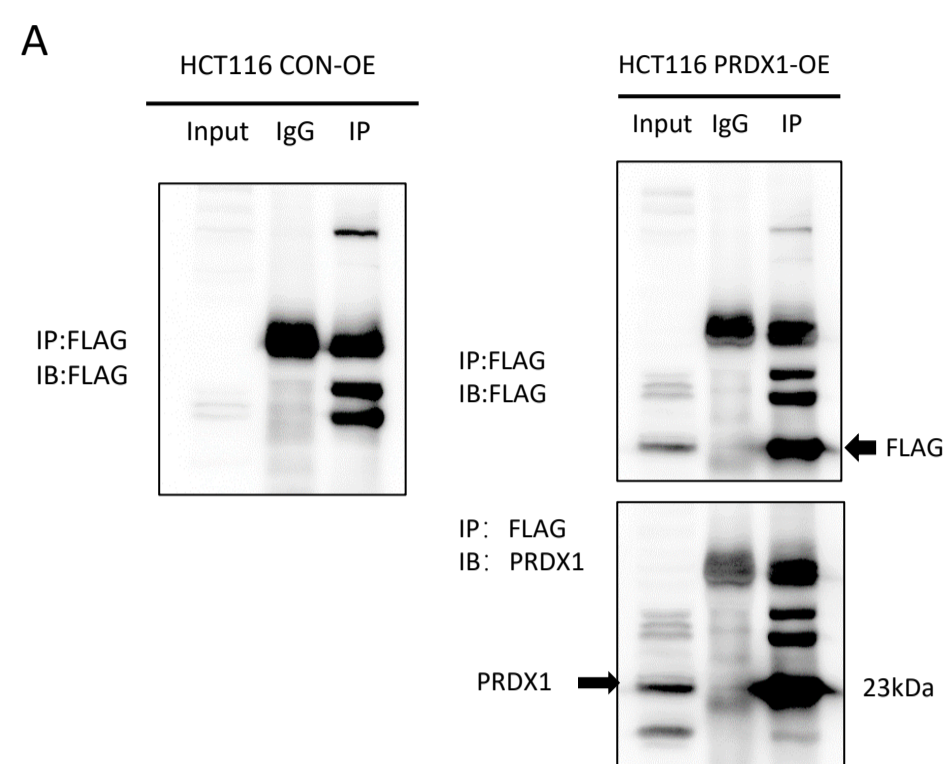


Fig. S4



B

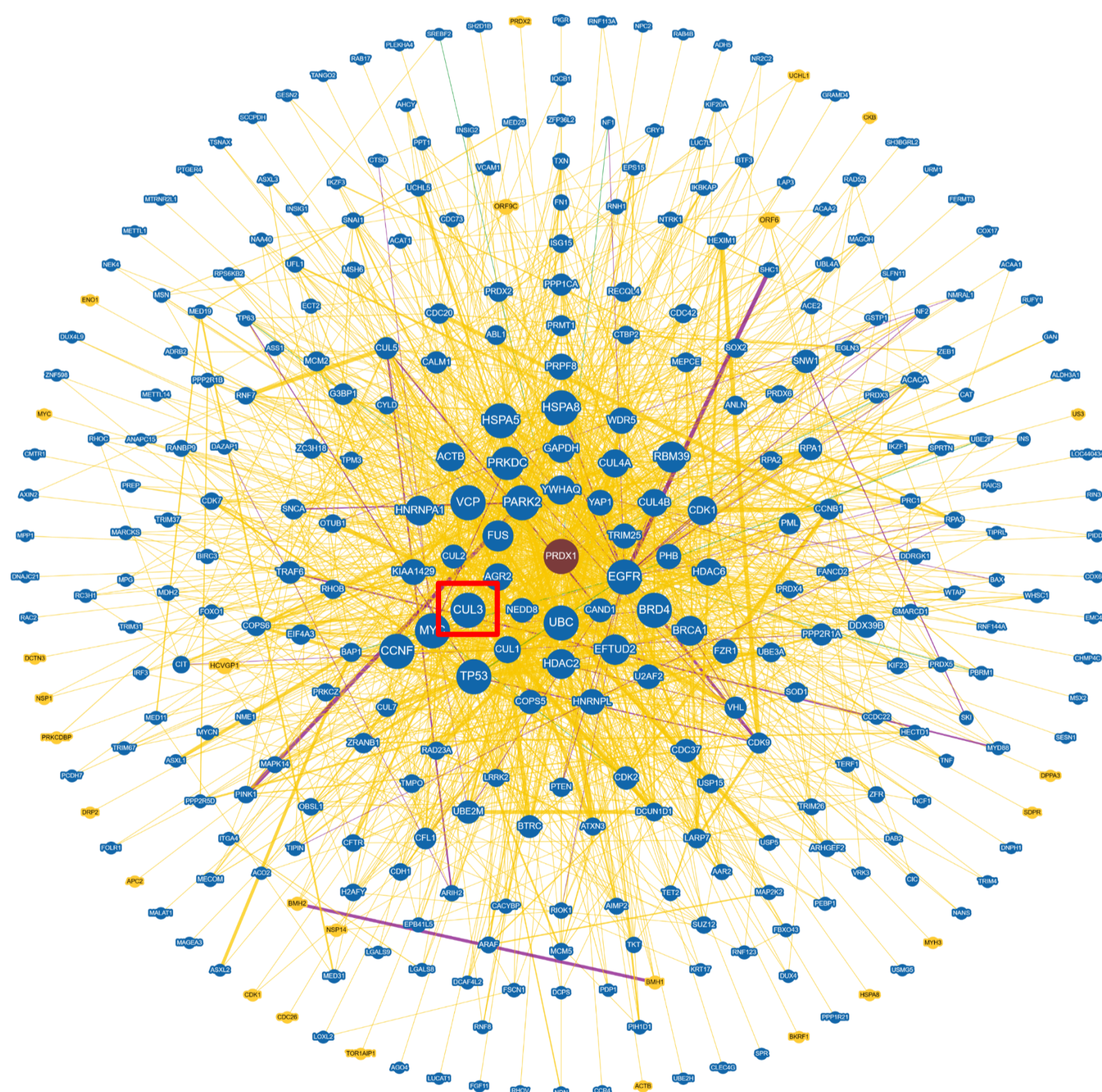
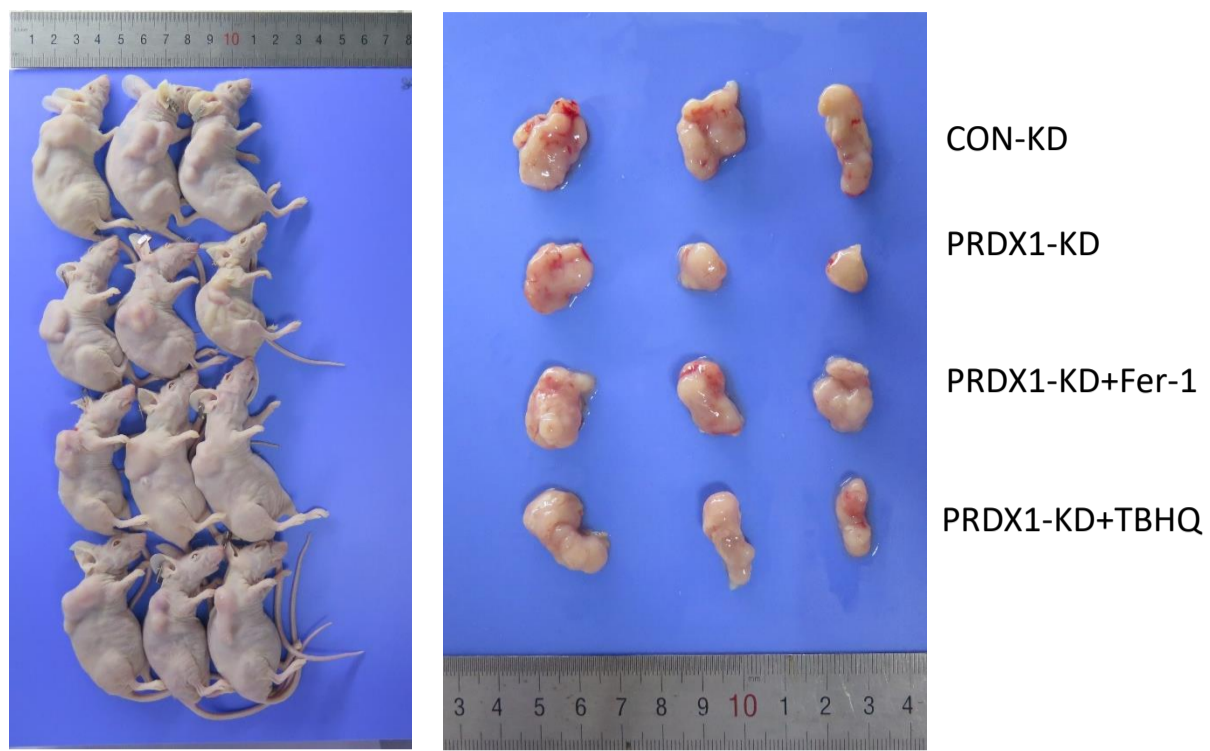
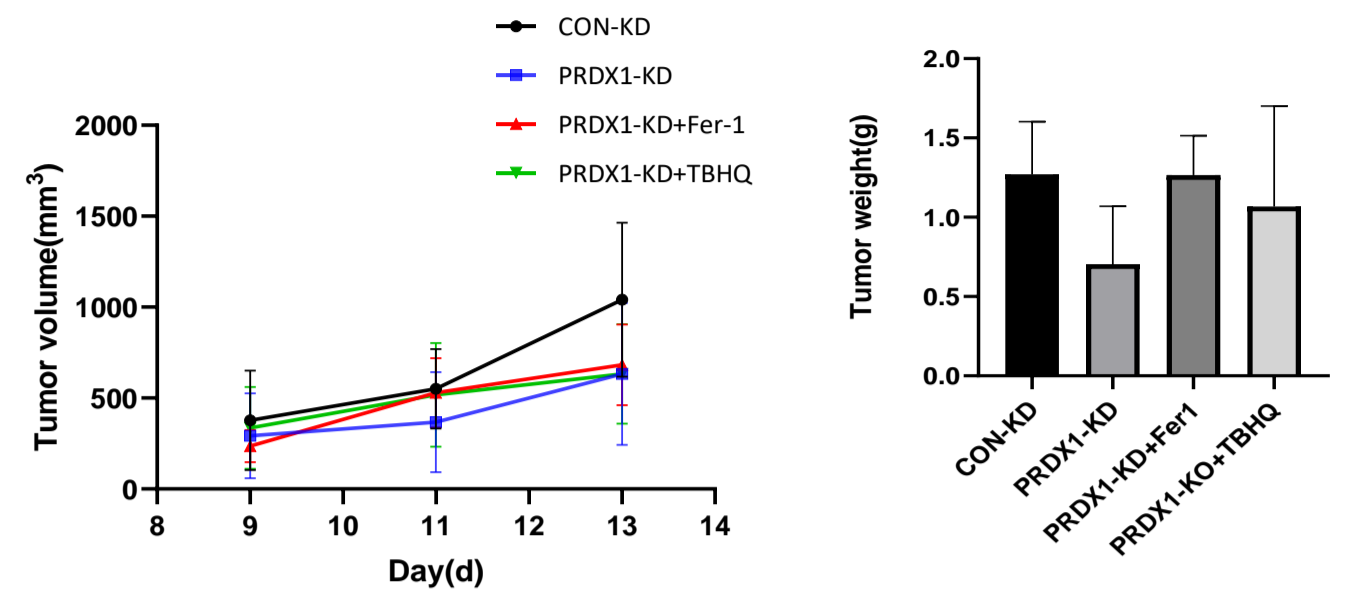


Fig. S5

A



B



C

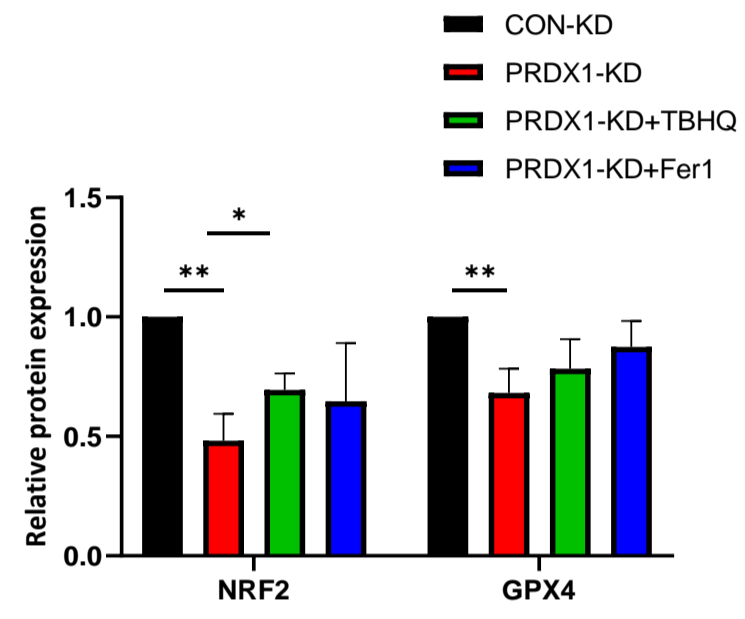
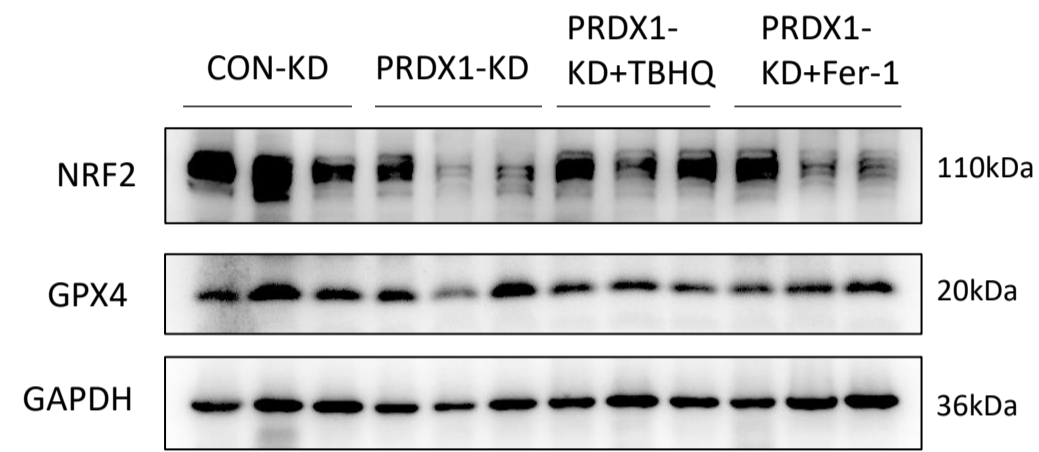


Fig. S1 (A) Western blot analysis of the expression of PRDX1 in HCT116, SW620, HT29 and SW480 cells. β -actin was used as an internal control. (B) Western blot analysis of the expression of cleaved-PARP, cleaved caspase 9, Bax expression in HCT116^{PRDX1-KD} and SW620^{PRDX1-KD} cells compared to controls. β -actin was used as an internal control. (C) Cell apoptosis was determined using Annexin V-FITC and PI staining by flow cytometry analysis.

Fig. S2 (A) Genotyping of the PRDX1-KO mice by allele-specific PCR analysis. The target band is 476bp. Genotyping of WT mice was used as a control. (B) KEGG pathway analysis of the differentially expressed genes between HCT116 siPRDX1 and siNC groups. The main pathways were highlighted in red. (C) Western blot analysis of the expression of PRDX1 in SW620 cells transfected with three different PRDX1 siRNA.

Fig. S3 The FerroOrange fluorescent probe (1 μ M) was used to determine intracellular Fe²⁺ levels in HCT116 or SW620 cells transfected with siPRDX1 and siNC with or without the treatment with erastin (10 μ M). Statistical results of mean FerroOrange fluorescence intensity were indicated.

Fig. S4 (A) HCT116^{CON-OE} and HCT116^{PRDX1-OE} cells were subjected to IP with anti-FLAG antibody and Western blot analysis of FLAG-PRDX1 expression in HCT116^{PRDX1-OE} cells before Mass-Spectrometry. (B) The interaction between PRDX1 and CUL3 was predicted by BioGRID

(<https://thebiogrid.org>).

Fig. S5 (A) Representative images of subcutaneous tumors transplanted with MC38^{CON-KD} and MC38^{PRDX1-KD} cells in nude mice plus the treatment with Fer-1 or TBHQ as indicated (n = 3). (B) The tumor growth curve and weight of subcutaneous tumors among the groups. (C) The expression levels of NRF2 and GPX4 in subcutaneous tumors were determined by Western blot analysis.

Table S1. Demographic information of colorectal cancer patients

	Variables	Number	Percentage (%)
Age	<=70	27	60.0
	>70	18	40.0
Gender	Male	26	57.8
	Female	19	42.8
Location	Colon	34	75.6
	Rectum	11	24.4
Depth of invasion	T ₁₋₂	11	24.4
	T ₃₋₄	34	75.6
Lymph node metastasis	N ₀	21	46.7
	N ₁₋₂	24	53.3
Distant metastasis	M ₀	25	55.6
	M ₁	20	44.4

Table S2. The sequences of primers for RT-qPCR

Gene	Primer Sequence (5' to 3')	Species
PRDX1	F: CAACTGCCAAGTGATTGGTG	<i>Homo sapiens</i>
	R: TGATCTGCCGAAGAATACCC	
NRF2	F: GCGACGGAAAGAGTATGAGC	<i>Homo sapiens</i>
	R: TGGGAGTAGTTGGCAGATCC	
GPX4	F: TCAGCAAGATCTGCGTGAAC	<i>Homo sapiens</i>
	R: GGGGCAGGTCCTTCTCTATC	

Table S3. The antibodies and application

Antibodies	Company	Application
β -actin (66009-1-Ig)	Proteintech	WB
PRDX1 (15816-1-AP)	Proteintech	WB/IHC
NRF2 (16396-1-AP)	Proteintech	WB/IHC/IF
GPX4 (67763-1-Ig)	Proteintech	WB/IHC
NQO1 (A180)	Santa Cruz	WB
PARP (L080950)	PTM Bio	WB
Caspase 9 (10380-1-AP)	Proteintech	WB
Bax (50599-2-Ig)	Proteintech	WB
Ubiquitin (L020707)	PTM Bio	Co-IP/WB
FLAG tag (20543-1-AP)	Proteintech	Co-IP/WB
GAPDH (60004-1-Ig)	Proteintech	WB
HA tag (51064-2-AP)	Proteintech	Co-IP/WB
Histone H3 (4499)	Cell Signaling Technology	WB
Normal Rabbit IgG (2729)	Cell Signaling Technology	Co-IP

Table S4. The downregulated genes in HCT116 siPRDX1 normalized to siNC

Gene ID	Gene Symbol	log ₂ (siPrdx1/siNC)	Q value (siPrdx1/siNC)	P value (siPrdx1/siNC)
5052	'PRDX1'	-3.408226577	1.18E-57	6.68E-62
154865	'IQUB'	-2.573033624	0.04213658	3.89E-04
400966	'RGPD1'	-1.675634422	0.029279443	2.34E-04
1591	'CYP24A1'	-1.59564471	0.00207419	6.00E-06
9498	'SLC4A8'	-1.514491282	0.026866287	1.99E-04
389524	'GTF2IRD2B'	-1.470700195	0.040453887	3.67E-04
143686	'SESN3'	-1.401628064	0.012288167	6.97E-05
81575	'APOLD1'	-1.300341423	0.045366703	4.35E-04
5874	'RAB27B'	-1.173962631	0.008178988	4.13E-05
3800	'KIF5C'	-1.084905562	0.003477128	1.16E-05
6513	'SLC2A1'	-1.064049212	0.004750001	1.86E-05
595	'CCND1'	-1.0639592	0.024556043	1.70E-04
22989	'MYH15'	-0.979167931	1.51E-04	2.06E-07
50515	'CHST11'	-0.951082588	0.02073292	1.33E-04
23531	'MMD'	-0.920192692	0.02073292	1.33E-04
10046	'MAMLD1'	-0.899185834	0.022379321	1.51E-04
10903	'MTMR11'	-0.89237316	0.026866287	2.06E-04
89927	'C16orf45'	-0.858208328	0.042422848	3.94E-04
9949	'AMMECR1'	-0.855438343	0.017886309	1.09E-04
360023	'ZBTB41'	-0.840555193	0.010124988	5.40E-05
1.01E+08	'NOTCH2NLB'	-0.828983403	0.026866287	1.98E-04
160897	'GPR180'	-0.82767099	0.006558552	2.90E-05
10783	'NEK6'	-0.811653125	0.005108144	2.06E-05
5865	'RAB3B'	-0.807377855	0.028251608	2.21E-04
85414	'SLC45A3'	-0.796951175	0.026866287	1.94E-04
79739	'TTLL7'	-0.792909854	0.010740117	5.85E-05
221656	'KDM1B'	-0.777258472	0.015250908	9.08E-05
6480	'ST6GAL1'	-0.762763565	0.047048275	4.61E-04
57613	'FAM234B'	-0.724012378	0.04736613	4.70E-04
84888	'SPPL2A'	-0.71382001	0.004264728	1.57E-05
22848	'AAK1'	-0.712743044	0.020393987	1.27E-04
22858	'ICK'	-0.708348343	0.026866287	2.04E-04
224	'ALDH3A2'	-0.706597313	0.0357682	3.00E-04
283431	'GAS2L3'	-0.701288474	0.035469913	2.96E-04
26579	'MYEOV'	-0.684551374	0.009090272	4.74E-05
214	'ALCAM'	-0.665687456	0.045276951	4.31E-04
284565	'NBPF15'	-0.66318288	0.04007252	3.49E-04
151230	'KLHL23'	-0.635181335	0.035781545	3.02E-04
5937	'RBMS1'	-0.621510984	0.021696188	1.41E-04
4780	'NFE2L2'	-0.612022025	0.003033304	9.80E-06

57590	'WDFY1'	-0.568784956	0.011303261	6.28E-05
7003	'TEAD1'	-0.553077176	0.041201418	3.78E-04
51361	'HOOK1'	-0.540224624	0.047048275	4.61E-04
835	'CASP2'	-0.517516	0.046687521	4.53E-04

Table S5. Proteomic mass spectrometry based on PRDX1 reactivity in HCT116^{PRDX1-}

OE v.s. HCT116 ^{CON-OE}		
Protein	Name	No. of peptides
PLEC	Isoform 2 of Plectin	153
ACTG1	Actin, cytoplasmic 2	138
MYH11	Myosin heavy chain 11 smooth muscle isoform	74
MYO1C	Unconventional myosin-Ic	54
POTEF	POTE ankyrin domain family member F	37
MYO18A	Unconventional myosin-XVIIIa	35
POTEI	POTE ankyrin domain family member I	30
HSPA8	Heat shock cognate 71 kDa protein	27
POTEJ	POTE ankyrin domain family member J	27
SPTAN1	Spectrin alpha chain, non-erythrocytic 1	24
ACTN1	Alpha-actinin-1	22
BUB3	Mitotic checkpoint protein BUB3 (Fragment)	20
NUSAP1	Nucleolar and spindle-associated protein 1	20
MPRIP	Myosin phosphatase Rho-interacting protein (Fragment)	19
DBN1	Drebrin (Fragment)	19
MPRIP	Myosin phosphatase Rho-interacting protein	19
PLEC	Plectin (Fragment)	18
MLLT4	Afadin	17
KRT17	Keratin, type I cytoskeletal 17	17
TPM3	Tropomyosin alpha-3 chain	17
TUBB2B	Tubulin beta-2B chain	15
TUBB2A	Tubulin beta-2A chain	15
PRMT5	Protein arginine N-methyltransferase 5	15
SLC25A6	ADP/ATP translocase 3 (Fragment)	14
RBM33	RNA-binding protein 33 (Fragment)	14
SEPT2	Septin-2	14
SYNCRIP	Heterogeneous nuclear ribonucleoprotein Q	14
TPM2	Tropomyosin beta chain	13
EPB41L5	Band 4.1-like protein 5	13
TUBA3C	Tubulin alpha-3C/D chain	13
CCT2	T-complex protein 1 subunit beta	13
HSPA1L	Heat shock 70 kDa protein 1-like	13
PKM	Pyruvate kinase PKM	13

DHX35	Probable ATP-dependent RNA helicase DHX35	11
TPM2	Tropomyosin beta chain	11
SPECC1L	Cytospin-A (Fragment)	11
FARSA	Phenylalanine--tRNA ligase alpha subunit	11
TUBA3E	Tubulin alpha-3E chain	11
SPTY2D1	Protein SPT2 homolog	11
FLII	Protein flightless-1 homolog	11
TPM2	Tropomyosin beta chain	11
RPL7	60S ribosomal protein L7	10
AURKB	Aurora kinase B	10
ZC3HAV1	Zinc finger CCCH-type antiviral protein 1	10
TUBA3C	Tubulin alpha-3C/D chain	10
RDX	Radixin	10
TMPO	Thymopentin	9
UHRF1	E3 ubiquitin-protein ligase UHRF1	4
SMURF2	E3 ubiquitin-protein ligase SMURF2	4
ERBB2	Receptor tyrosine-protein kinase erbB-2	3
CUL2	Cullin-2	3
TCF7L2	Transcription factor 7-like 2	3
CAND1	Cullin-associated NEDD8-dissociated protein 1	3
UBAP2	Ubiquitin-associated protein 2	3
MID1	E3 ubiquitin-protein ligase Midline-1	3
UBP5	Ubiquitin carboxyl-terminal hydrolase 5	3
RNF20	E3 ubiquitin-protein ligase BRE1A	2
UBP36	Ubiquitin carboxyl-terminal hydrolase 36	2
KCMF1	E3 ubiquitin-protein ligase KCMF1	2
CUL1	Cullin-1	2
TXNRD1	Thioredoxin reductase 1	2
KAT7	Histone acetyltransferase	2
GSK3B	Glycogen synthase kinase-3 beta	2
