PNAS

Supplementary Information for

A ferroptosis defense mechanism mediated by glycerol 3-phosphate dehydrogenase 2 in mitochondria

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This PDF file includes:

Figures S1 to S5 (not allowed for Brief Reports)
Tables S1 to S2 (not allowed for Brief Reports)

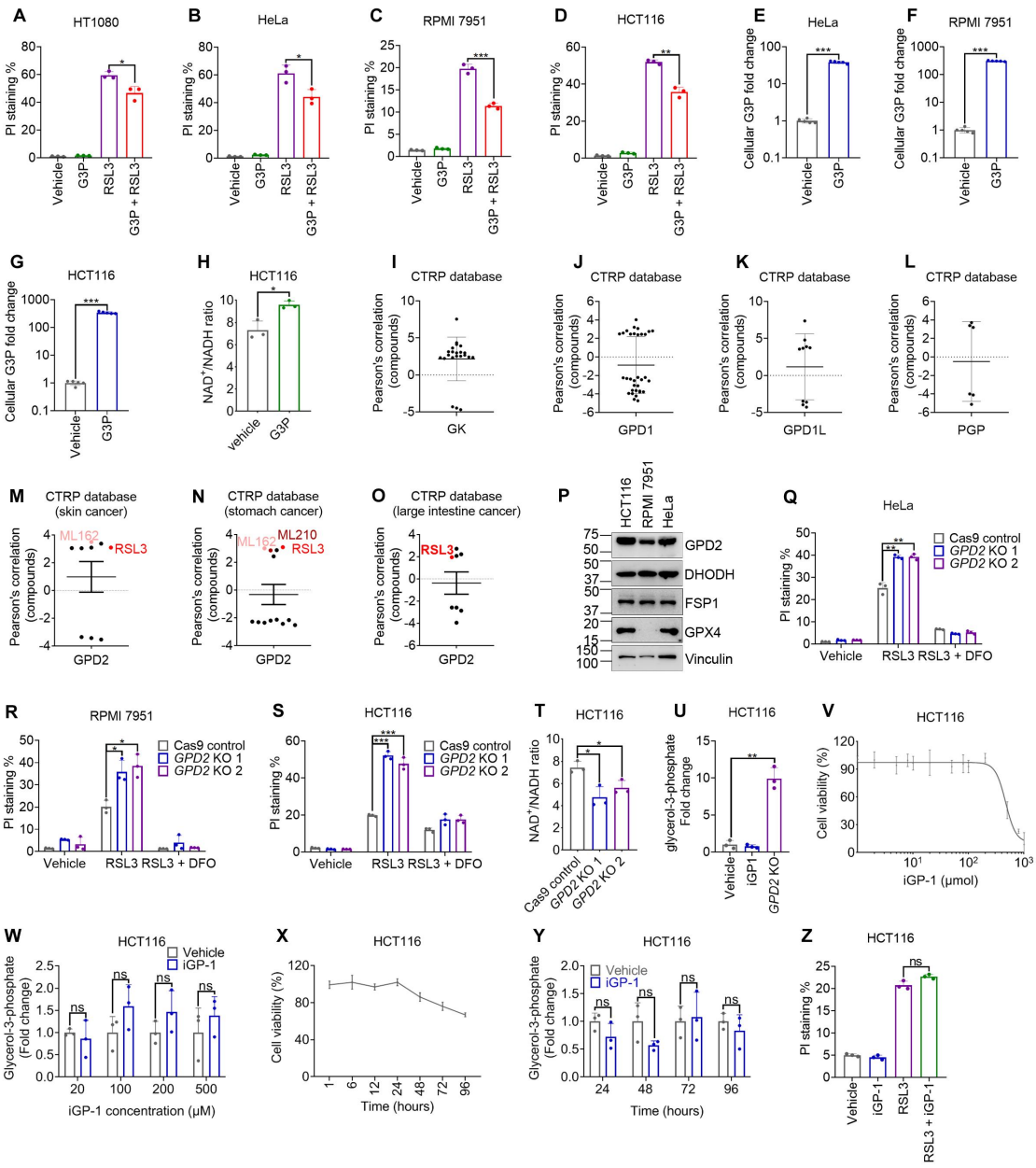

Fig. S1. Metabolomic analysis reveals a role for GPD2 in regulation of ferroptosis. **A-C,** Results of PI staining of HT1080 (**A**), HeLa (**B**), and RPMI 7951 (**C**) cells treated with RSL3 (10 μM) for 8 h following pretreatment with a vehicle or G3P (1 mM) for 24 h. **D,** Results of PI staining of HCT116 cells treated with RSL3 (20 μM) for 24 h following pretreatment with a vehicle or G3P (1 mM) for 24 h. **E-G,** Intercellular G3P concentrations after treatment with G3P (1mM) in HeLa (**E**), RPMI 7951 (**F**), and

HCT116 (**G**) cells. **H**, The effect of G3P treatment on the NAD^+/NADH ratio in HCT116 cells. **I-O,** Expression levels for glycerol kinase (GK; **I**), GPD1 (**J**), GPD1L (**K**), and phosphoglycolate phosphatase (PGP; **L**) do not correlate with sensitivity to treatment with GPX4 inhibitors (RSL3, ML162, and ML210) in cancer cells. High GPD2 expression correlates with resistance to these inhibitors in skin (**M**), stomach (**N**), and large intestine (**O**) cancer cells. The plotted data were mined from the CTRP database. Plotted values are Pearson's correlation coefficients. The scatter plots show medians, 10th and 90th percentiles, and minima and maxima of the distributions. **P,** Western blot of GPD2, FSP1, DHODH, and GPX4 protein levels in HCT116, RPMI7951, and HeLa cells. **Q-S,** Results of PI staining of Cas9 control and *GPD2*-KO (numbers 1 and 2) HeLa (**Q**), RPMI 7951 (**R**), and HCT116 (**S**) cells treated with RSL3 (10 μM) and/or deferoxamine (DFO; 5 μM) for 6 h (**Q, R**) or 24 h (**S**). **T**, Measurement of the NAD^+/NADH ratio in Cas9 control and *GPD2*-KO HCT116 cells. **U,** The relative fold changes in intracellular G3P levels upon treatment with iGP-1 (200 μM) for 24h or *GPD2* KO in HCT116 cells. **V-Y**, Dose dependent (**V, W**) and time course (**X, Y**) analyses of iGP-1 treatment on cell viability and intracellular G3P levels. 24 hour treatment was used in **V, W** and 20 uM iGP-1 was used in **X, Y**. **Z,** Results of PI staining of HCT116 cells treated with iGP-1 (200 μM) and/or RSL3 (10 μM) for 24 h. Data are presented as means (± s.d.) for three independent repeats. Unpaired, two-tailed *t*-test; **P*< 0.05, ***P*< 0.01, ****P*< 0.001. ns, not significant.

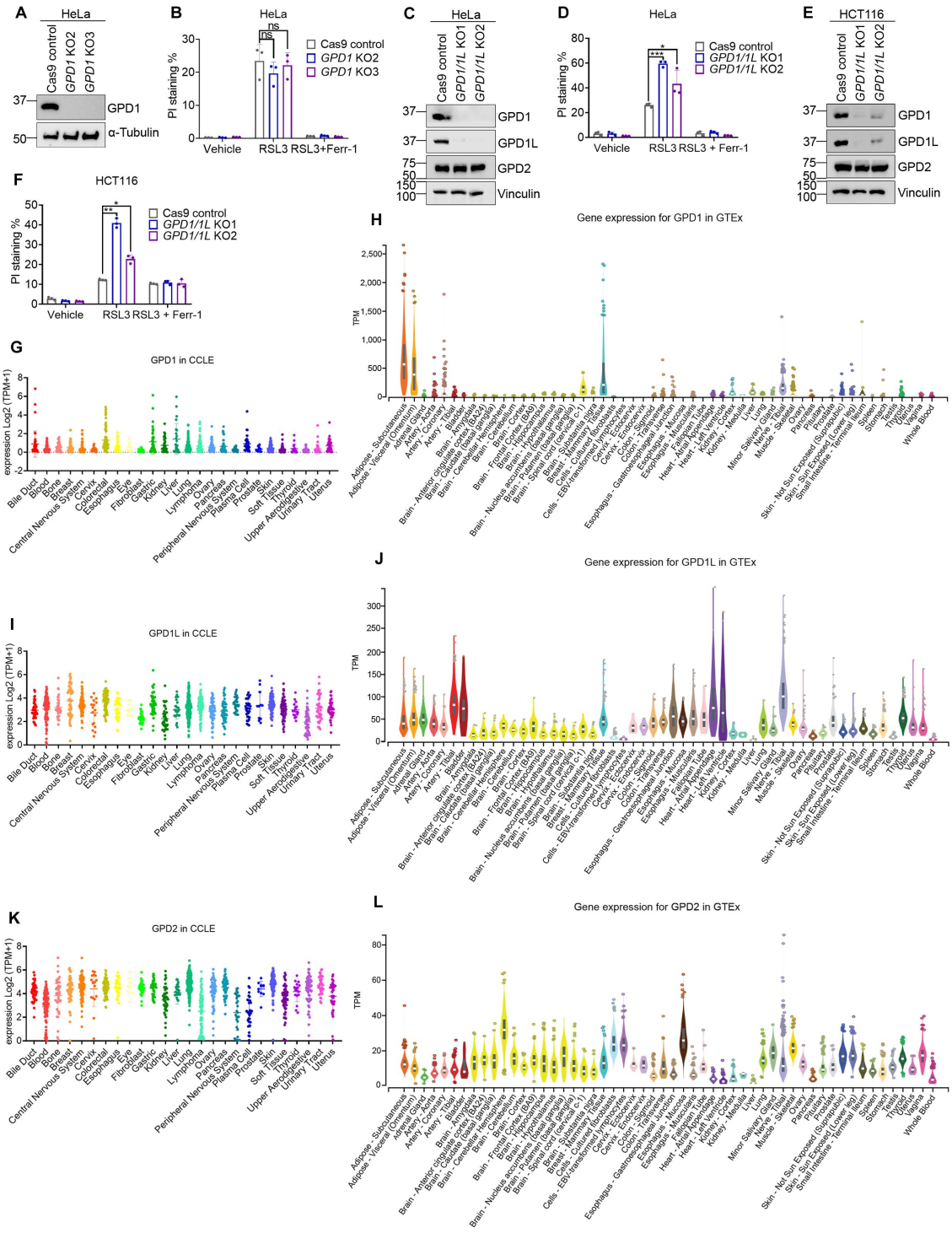

Fig. S2. The G3P shuttle inhibits ferroptosis. **A,** GPD1 protein levels in Cas9 control and *GPD1*-KO HeLa cells. **B,** Results of PI staining of Cas9 control and *GPD1*-KO HeLa cells. **C,** GPD1, GPD1L, and GPD2 protein levels in Cas9 control and *GPD1/1L* DKO

HeLa cells. **D,** Results of PI staining of Cas9 control and *GPD1/1L* DKO HeLa cells. **E,** GPD1, GPD1L, and GPD2 protein levels in Cas9 control and *GPD1/1L* DKO HCT116 cells. **F,** Results of PI staining of Cas9 control and *GPD1/1L* DKO HCT116 cells. **G-L,** mRNA expression levels for *GPD1* (**G**), *GPD1L* (**I**), and *GPD2* (**K**) in different cancer cell lines from the Cancer Cell Line Encyclopedia database and mRNA expression levels for *GPD1* (**H**), *GPD1L* (**J**), and *GPD2* (**L**) in different tissues from the Genotype-Tissue Expression database.*$P< 0.05$, **$P< 0.01$, ***$P< 0.001$.

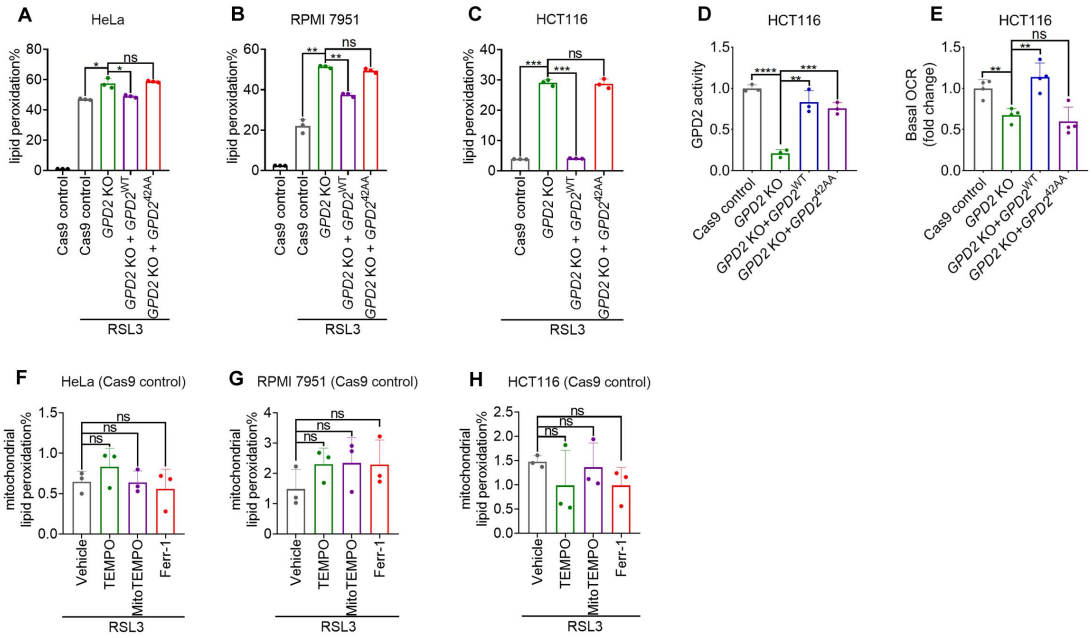

Fig. S3. GPD2 suppresses mitochondrial lipid peroxidation. **A-C,** Lipid peroxidation levels in Cas9 control and *GPD2*-KO HeLa (**A**), RPMI 7951 (**B**), and HCT116 (**C**) cells expressing the indicated GPD2 constructs upon treatment with RSL3 (10 μM) for 3 h (**A, B**) or RSL3 (1.5 μM) for 24 h (**C**). **D, E**, Measurement of GPD2 activity (**D**) and basal oxygen consumption rate (OCR; **E**) in HCT116 cells with corresponding genotypes as indicated. **F-H,** Mitochondrial lipid peroxidation levels in Cas9 control HeLa (**F**), RPMI 7951 (**G**), and HCT116 (**H**) cells treated with RSL3 (1 μM) for 2 h (**F, G**) or 24 h (**H**) following pretreatment with a vehicle, TEMPO (10 μM), MitoTEMPO (10 μM), or ferrostatin-1(Ferr-1; 5 μM) for 24 h. Data are presented as means (± s.d.) for three independent repeats. Unpaired, two-tailed *t*-test.**P*< 0.05, ***P*< 0.01, ****P*< 0.001, ****P < 0.001. ns, not significant.

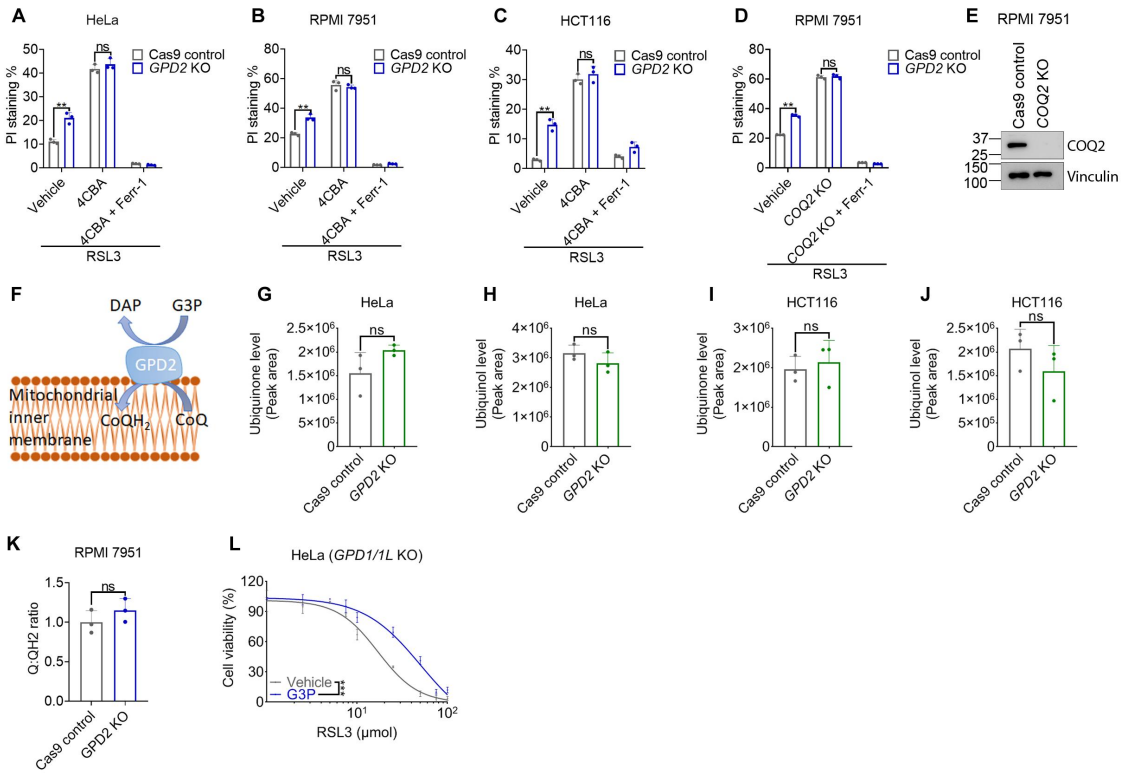

Fig. S4. GPD2 inhibits ferroptosis through generation of $CoQH_2$ in mitochondria. **A-C**, Results of PI staining of Cas9 control and *GPD2*-KO HeLa (A), RPMI 7951 (B), and HCT116 (C) cells treated with RSL3 (10 μM) for 6 h following pretreatment with a vehicle, 4CBA (5 mM), or 4CBA (5 mM) plus ferrostatin-1(Ferr-1; 5 μM) for 24 h. **D**, Results of PI staining of Cas9 control and *GPD2*-KO RPMI 7951 cells with or without *CoQ2* deletion treated with RSL3 (10 μM) for 6 h following pretreatment with a vehicle or Ferr-1 (5 μM) for 24 h. **E,** COQ2 protein levels in Cas9 control and *COQ2*-KO RPMI 7951 cells. **F**, Schematic of the GPD2 reaction to couple G3P oxidation to DAP with CoQ reduction to $CoQH_2$ in the inner mitochondrial membrane. **G-J**, Ubiquinone (CoQ) and ubiquinol ($CoQH_2$) measurement in control and *GPD2* KO HeLa (**G, H**) or HCT116 (**I, J**) cells. **K,** CoQ/$CoQH_2$ ratios in *GPD2*-KO RPMI 7951 cells. **L**, The effect of G3P treatment on RSL3-induced ferroptosis in *GPD1/1L* KO HeLa cells. Data are presented

as means (± s.d.) for three 3 independent repeats. Unpaired, two-tailed t-test; *P < 0.05, **P < 0.01, ***P < 0.001. ns, not significant.

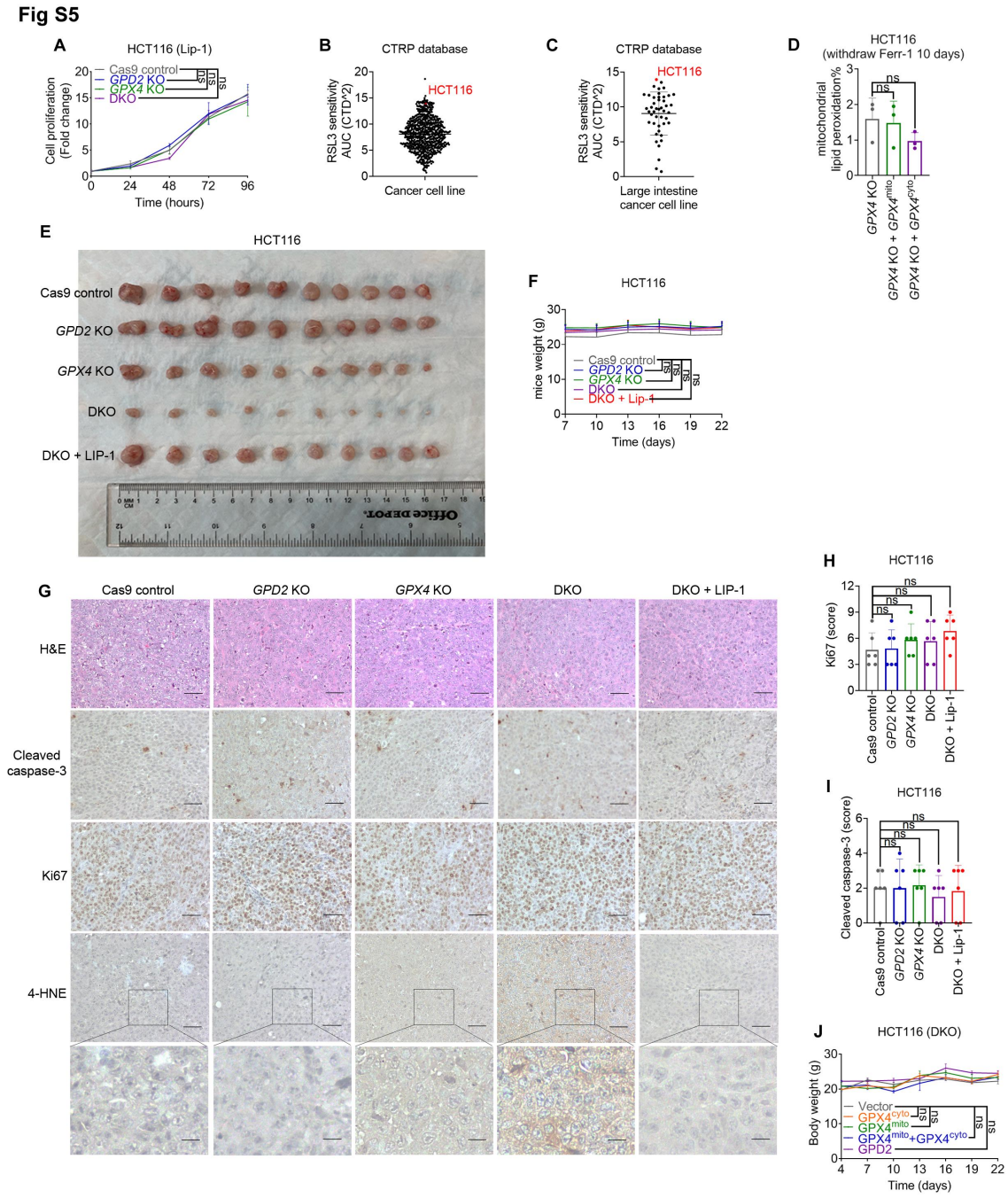

Fig. S5. GPD2 acts in parallel with mitochondrial GPX4 to suppress ferroptosis. **A,** Cell proliferation measurement in *GPD2* and *GPX4* single or double KO cells under the treatment with the ferroptosis inhibitor liproxstatin-1. **B, C,** The RSL3 sensitivity of all cancer (**B**) and large intestine cancer (**C**) cell lines from the CTRP database. Plotted values are Pearson's correlation coefficients. The scatter plots show medians, 10th and

90th percentiles, and minima and maxima of the distributions. **D,** Mitochondrial lipid peroxidation measurement in *GPX4*-KO HCT116 cells expressing the indicated GPX4 constructs 10 days after ferrostatin-1 removal. **E**, Representative images of Cas9 control, *GPX4*-KO, *GPD2*-KO, and DKO HCT116 xenograft tumors with and without treatment with liproxstatin-1 (LIP-1) at the experimental endpoints. **F,** Weights of mice with Cas9 control, *GPD*2-KO, *GPX4*-KO, and DKO HCT116 xenografts with or without treatment with liproxstatin-1 (Lip-1) at different time points (days). **G,** Representative immunochemical images of Cas9 control, *GPX4*-KO, *GPD2*-KO, and DKO HCT116 xenograft tumors with the indicated treatments (upper scale bars, 20 μM; lower scale bars, 5 μM). H&E, hematoxylin and eosin. **H, I,** Immunohistochemical scores for Ki67 and cleaved caspase-3 staining of Cas9 control, *GPD*2-KO, *GPX4*-KO, and DKO HCT116 xenograft tumors with the indicated treatments. **J**, Weights of mice of *GPX4/GPD2* DKO HCT116 xenografts with indicated genotypes at different time points (days). Data are presented as means (± s.d.) for three independent repeats. Unpaired, two-tailed *t*-test; **P*< 0.05, ***P*< 0.01, ****P*< 0.001. ns, not significant.

Table S1. The list of primers used in this study.

GPD2 knockout primers-1 (human) Fwd-:	CACCGACTCGCCCATCACTGGTCGC
GPD2 knockout primers-1 (human) Rev-:	AAACGCGACCAGTGATGGGCGAGTC
GPD2 knockout primers-2 (human) Fwd-:	CACCGAAGTGCGTGTGAGCGGCGCA
GPD2 knockout primers-2 (human) Rev-:	AAACTGCGCCGCTCACACGCACTTC
GPX4 knockout primers-1 (human) Fwd-:	CACCGGGTGAAGCGCTACGGACCCA
GPX4 knockout primers-1 (human) Rev-:	AAACTGGGTCCGTAGCGCTTCACCC
GPD1 knockout primers-2 (human) Fwd-:	CACCGGTGTACAAGGTGTGCTACGA
GPD1 knockout primers-2 (human) Rev-:	AAACTCGTAGCACACCTTGTACACC
GPD1 knockout primers-3 (human) Fwd-:	CACCGAAGGCGGCAGTGATCCGGCT
GPD1 knockout primers-3 (human) Rev-:	AAACAGCCGGATCACTGCCGCCTTC
GPD1L knockout primers-1 (human) Fwd-:	CACCGCAAAGCGGCCGTCATCCGCC
GPD1L knockout primers-1 (human) Rev-:	AAACGGCGGATGACGGCCGCTTTGC
GPD1L knockout primers-2 (human) Fwd-:	CACCGGTACCGCATCCTCAAACAGA
GPD1L knockout primers-2 (human) Rev-:	AAACTCTGTTTGAGGATGCGGTACC
COQ2 knockout primers-1 (human) Fwd-:	CACCGATGCTGGGCTCGCGAGCCGC
COQ2 knockout primers-1 (human) Rev-:	AAACGCGGCTCGCGAGCCCAGCATC
DHODH knockout primers-2 (human) Fwd-:	CACCGCATCTTATAAAGTCCGTCCA
DHODH knockout primers-2 (human) Rev-:	AAACTGGACGGACTTTATAAGATGC
FSP1 knockout primers-1 (human) Fwd-:	CACCGGGAGATGGGGTCCCAGGTCT
FSP1 knockout primers-1 (human) Rev-:	AAACAGACCTGGGACCCCATCTCCC

Table S2. The annotation of common metabolites detected from metabolomics analyses described in Fig. 1A-D.

metabolite name	Chromatography method	Ionization mode	m/z	retention time (min)	Pubchem ID	Formula	SMILES
2-keto-isovalerate	C18 reversed phase	negative	115.0392	13.3	49	C5H8O3	CC(C)C(=O)C(=O)O
alpha-ketoglutarate	C18 reversed phase	negative	145.0135	13.5	51	C5H6O5	OC(=O)CCC(=O)C(O)=O
citrate	C18 reversed phase	negative	191.0194	14.2	311	C6H8O7	OC(=O)CC(O)(CC(O)=O)C(O)=O
3-phosphoglycerate	C18 reversed phase	negative	184.9852	13.8	724	C3H7O7P	C(C(C(=O)O)O)OP(=O)(O)O
glutathione	C18 reversed phase	negative	306.077	8	745	C10H17N3O6S	NC(CCC(O)=NC(CS)C(O)=NCC(O)=O)C(O)=O
pyruvate	C18 reversed phase	negative	87.00787	8.7	1060	C3H4O3	CC(=O)C(=O)O
sarcosine	C18 reversed phase	negative	88.03945	2.9	1088	C3H7NO2	CNCC(O)=O
succinate	C18 reversed phase	negative	117.0185	12.9	1110	C4H6O4	O=C(O)CCC(=O)O
urate	C18 reversed phase	negative	167.0205	7	1175	C5H4N4O3	O=C1NC2=C(N1)C(=O)NC(=O)N2
isocitrate	C18 reversed phase	negative	191.0194	15.3	1198	C6H8O7	C(C(C(C(=O)O)O)C(=O)O)C(=O)O
NADP+	C18 reversed phase	negative	742.0698	14.1	5886	C21H29N7O17P3+	NC(=O)C1=C[N+](=CC=C1)[C@@H]1O[C@H](COP([O-])(=O)OP(O)(=O)OC[C@H]2O[C@H]([C@H](OP(O)(O)=O)O2)C@@H]2O)N2C=NC3=C2N=CN=C3N)[C@@H](O)[C@H]1O
NAD+	C18 reversed phase	negative	662.1031	8.9	5893	C21H28N7O14P2+	C1=CC[=C[N+](=C1)[C@H]2[C@@H]([C@@H]([C@H](O2)COP(=O)(O)OP(=O)(O)OC[C@@H]3C@H]([C@H]([C@@H](O3)N4C=NC5=C(N=CN=C54)N)O)O)O)O)C(=O)N
NADH	C18 reversed phase	negative	664.1188	14.2	439153	C21H29N7O14P2	NC(=O)C1=C[N+](=CC=C1)[C@@H]1O[C@H](COP([O-])(=O)OP(O)(=O)OC[C@H]2O[C@@H]([C@@H](O)[C@@H]2O)N2C=NC3=C2N=CN=C3N)[C@@H](O)[C@H]1O
glucose-6-phosphate	C18 reversed phase	negative	259.0225	7.4	5958	C6H13O9P	C([C@@H]1[C@H]([C@@H]([C@H](C(O1)O)O)O)OP(=O)(O)O
inosine	C18 reversed phase	negative	267.0738	3.4	135398641	C10H12N4O5	OC[C@H]1O[C@H]([C@H](O)[C@@H]1O)N1C=NC2=C(O)N=CN=C12
uridine	C18 reversed phase	negative	243.0622	2.7	6029	C9H12N2O6	OC[C@H]1O[C@H]([C@H](O)[C@@H]1O)N1C=CC(=O)NC1=O
UMP	C18 reversed phase	negative	323.029	10.4	6030	C9H13N2O9P	O[C@H]1[C@@H](O)[C@@H](O[C@@H]1COP(O)(O)=O)N1C=CC(=O)NC1=O
UDP	C18 reversed phase	negative	402.9954	13.9	6031	C9H14N2O12P2	O[C@H]1[C@@H](O)[C@@H](O[C@@H]1COP(O)(=O)OP(O)(O)=O)N1C=CC(=O)NC1=O
folate	C18 reversed phase	negative	440.1329	14.3	135398658	C19H19N7O6	NC1=NC(=O)C2=NC(CNC3=CC=C(C=C3)C(=O)N[C@@H](CCC(O)=O)C(O)=O)=CN=C2N1
AMP	C18 reversed phase	negative	346.0561	12.1	6083	C10H14N5O7P	NC1=C2N=CN([C@@H]3O[C@H](COP(O)(O)=O)[C@@H](O)[C@H]3O)C2=NC=N1
CMP	C18 reversed phase	negative	322.0449	9.1	6131	C9H14N3O8P	NC1=NC(=O)N(C=C1)[C@@H]1O[C@H](COP(O)(O)=O)[C@@H](O)[C@H]1O
CDP	C18 reversed phase	negative	402.0112	13.7	6132	C9H15N3O11P2	NC1=NC(=O)N(C=C1)[C@@H]1O[C@H](COP(O)(=O)OP(O)(O)=O)[C@@H](O)[C@H]1O
UTP	C18 reversed phase	negative	482.9619	15.2	6133	C9H15N2O15P3	O[C@H]1[C@@H](O)[C@@H](O[C@@H]1COP(O)(=O)OP(O)(=O)OP(O)(O)=O)N1C=CC(=O)NC1=O
CTP	C18 reversed phase	negative	481.9779	15.1	6176	C9H16N3O14P3	NC1=NC(=O)N(C=C1)[C@@H]1O[C@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)[C@@H](O)[C@H]1O
pantothenate	C18 reversed phase	negative	218.1032	11.4	6613	C9H17NO5	CC(C)(CO)[C@@H](O)C(=O)NCCC(O)=O
4-pyridoxic acid	C18 reversed phase	negative	182.0454	14.3	6723	C8H9NO4	CC1=NC=C(CO)C(C(O)=O)=C1O
guanosine	C18 reversed phase	negative	282.0846	3.4	135398635	C10H13N5O5	NC1=NC2=C(N=CN2[C@@H]2O[C@H](CO)[C@@H](O)[C@H]2O)C(=O)N1
GTP	C18 reversed phase	negative	521.9839	15.2	135398633	C10H16N5O14P3	NC1=NC2=C(N=CN2[C@@H]2O[C@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)[C@@H](O)[C@H]2O)C(=O)N1
N-acetyl-glucosamine-1/6-phosphate	C18 reversed phase	negative	300.0493	7.8	7115	C18H14	CC(=O)N[C@@H]1C@H](C@H](C@H](OC1OP(=O)(O)O)CO)O)O
pyroglutamate	C18 reversed phase	negative	128.0344	7.4	7405	C5H7NO3	OC(=O)[C@@H]1CCC(=O)N1
UDP-D-glucose	C18 reversed phase	negative	565.0485	13.3	8629	C15H24N2O17P2	OC[C@H]1O[C@H](OP(O)(=O)OP(O)(=O)OC[C@H]2O[C@H]([C@H](O)[C@@H]2O)N2C=CC(=NC2=O)[C@H](O)[C@@H](O)[C@@H]1O
GDP	C18 reversed phase	negative	442.0176	13.9	135398619	C10H15N5O11P2	NC1=NC(=O)C2=C(N1)N(C=N2)[C@@H]1O[C@H](COP(O)(=O)OP(O)(O)=O)[C@@H](O)[C@H]1O
fructose-1-6-bisphosphate	C18 reversed phase	negative	338.9891	14.1	10267	C6H14O12P2	O[C@H]1[C@H](O)[C@@](O)(COP(O)(O)=O)O[C@@H]1COP(O)(O)=O
D-gluconate	C18 reversed phase	negative	195.0505	5.3	10690	C6H12O7	OC[C@@H](O)[C@@H](O)[C@H](O)[C@@H](O)C(O)=O
dATP	C18 reversed phase	negative	489.994	15.5	15993	C10H16N5O12P3	NC1=NC=NC2=C1N=CN2[C@H]1C[C@H](O)[C@@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)O1
ADP-D-glucose	C18 reversed phase	negative	588.0754	13.6	16500	C16H25N5O15P2	NC1=C2N=CN([C@@H]3O[C@H](COP(O)(=O)OP(O)(=O)O[C@H]4O[C@H](CO)[C@@H](O)[C@H](O)[C@H]4O)[C@@H](O)[C@H]3O)C2=NC=N1
UDP-D-glucuronate	C18 reversed phase	negative	579.0279	15.2	17473	C15H22N2O18P2	O[C@@H]1[C@@H](COP(O)(=O)OP(O)(=O)O[C@H]2O[C@@H]([C@@H](O)[C@H](O)[C@H]2O)C(O)=O)O[C@H]([C@@H]1O)N1C=CC(=O)NC1=O
dTTP	C18 reversed phase	negative	480.9825	15.4	64968	C10H17N2O14P3	CC1=CN([C@H]2C[C@H](O)[C@@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)O2)C(=O)NC1=O
dCTP	C18 reversed phase	negative	465.9829	15.1	65091	C9H16N3O13P3	NC1=NC(=O)N(C=C1)[C@H]1C[C@H](O)[C@@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)O1
glutathione disulfide	C18 reversed phase	negative	611.1455	12.9	65359	C20H32N6O12S2	N[C@@H](CCC(=O)N[C@@H](CSSC[C@H](NC(=O)CC[C@H](N)C(O)=O)C(=O)NCC(O)=O)C(=O)NCC(O)=O)C(O)=O
glucose-1-phosphate	C18 reversed phase	negative	259.0227	8.3	65533	C6H13O9P	OC[C@H]1O[C@H](OP(O)(O)=O)[C@H](O)[C@@H](O)[C@@H]1O
indole-3-carboxylate	C18 reversed phase	negative	160.043	13.8	69867	C9H7NO2	OC(=O)C1=CNC2=CC=CC=C12
N-acetyl-glutamate	C18 reversed phase	negative	188.056	13.4	70914	C7H11NO5	CC(=O)N[C@@H](CCC(O)=O)C(O)=O
N-Acetyl-L-alanine	C18 reversed phase	negative	130.0503	8.4	76406	C5H9NO3	CC(=O)NCCC(O)=O
ribose-phosphate	C18 reversed phase	negative	229.0118	8	77982	C5H11O8P	C([C@H](C@H](C@H](C=O)O)O)O)OP(=O)(O)O
6-phospho-D-gluconate	C18 reversed phase	negative	275.0177	13.7	91493	C6H13O10P	O[C@H](COP(O)(O)=O)[C@@H](O)[C@H](O)[C@@H](O)C(O)=O
N-carbamoyl-L-aspartate	C18 reversed phase	negative	175.0355	13	93072	C5H8N2O5	NC(=O)N[C@@H](CC(O)=O)C(O)=O
lactate	C18 reversed phase	negative	89.02343	7.4	107689	C3H6O3	C[C@H](O)C(O)=O
CDP-ethanolamine	C18 reversed phase	negative	445.0536	6.8	123727	C11H20N4O11P2	NCCOP(O)(=O)OP(O)(=O)OC[C@H]1O[C@H]([C@H](O)[C@@H]1O)N1C=CC(N)=NC1=O
octulose-bisphosphate	C18 reversed phase	negative	399.0103	14.2	130470	C8H18O14P2	C([C@@H]([C@H]([C@H]([C@H]([C@@H](C(=O)COP(=O)(O)O)O)O)O)O)O)OP(=O)(O)O
kynurenine	C18 reversed phase	negative	207.0771	3.4	161166	C10H12N2O3	N[C@@H](CC(=O)C1=CC=CC=C1N)C(O)=O
sedoheptulose-bisphosphate	C18 reversed phase	negative	368.9997	14.2	164735	C7H16O13P2	C([C@H](C@H](C@H](C@@H](C(=O)COP(=O)(O)O)O)O)O)O)OP(=O)(O)O
malate	C18 reversed phase	negative	133.0135	13.1	222656	C4H6O5	O[C@@H](CC(O)=O)C(O)=O
4-aminobutyrate	C18 reversed phase	negative	102.0551	5.3	223130	C5H11NO2	NCCCC(=O)O
glycerol-3-phosphate	C18 reversed phase	negative	171.0058	7.7	439162	C3H9O6P	C([C@H](COP(=O)(O)O)O)O
S-methyl-5-thioadenosine	C18 reversed phase	negative	296.0825	11.5	439176	C11H15N5O3S	CSC[C@H]1O[C@H]([C@H](O)[C@@H]1O)N1C=NC2=C1N=CN=C2N
2-hydroxy-2-methylbutanedioate	C18 reversed phase	negative	147.0291	13.1	441696	C5H8O5	CC(CC(=O)O)(C(=O)O)O
glutathione-S-sulfonate	C18 reversed phase	negative	386.0338	13.4	444104	C10H17N3O9S	C(CC(=O)N[C@@H](CS(=O)(=O)O)C(=O)NCC(=O)O)[C@@H](C(=O)O)N
acetyl-CoA	C18 reversed phase	negative	808.1203	16.1	444493	C23H38N7O17P3S	CC(=O)SCCNC(=O)CCNC(=O)[C@@H](C(C)(C)COP(=O)(O)OP(=O)(O)OC[C@@H]1[C@H]([C@H]([C@@H](O1)N2C=NC3=C2N=CN=C3N)O)OP(=O)(O)O)O
fumarate	C18 reversed phase	negative	115.0028	13.6	444972	C4H4O4	OC(=O)\C=C\C(O)=O
UDP-N-acetyl-glucosamine	C18 reversed phase	negative	606.0753	13.4	445675	C17H27N3O17P2	CC[=O]N[C@@H]1[C@@H](O)[C@H](O)[C@@H](CO)O[C@@H]1OP(O)(=O)OP(O)(=O)OC[C@H]1O[C@H]([C@H](O)[C@@H]1O)N1C=CC(=O)NC1=O
riboflavin	C18 reversed phase	negative	375.1313	12.3	493570	C17H20N4O6	CC1=C(C)C=C2N(C[C@H](O)[C@H](O)[C@H](O)CO)C3=NC(=O)NC(=O)C3=NC2=C1
aconitate	C18 reversed phase	negative	173.0087	14.2	643757	C6H6O6	OC(=O)C\C(=C\C(O)=O)C(O)=O
citraconic acid	C18 reversed phase	negative	129.0186	13.1	643798	C5H6O4	C/C(=C/C(=O)O)/C(=O)O
FAD	C18 reversed phase	negative	784.1512	15.4	643975	C27H33N9O15P2	CC1=CC2=C(C=C1C)N(C[C@H](O)[C@H](O)[C@H](O)COP(O)(=O)OP(O)(=O)OC[C@H]1O[C@H]([C@H](O)[C@@H]1O)N1C=NC3=C1N=CN=C3N)C1=NC(=O)NC(=O)C1=N2
FMN	C18 reversed phase	negative	455.0979	14.8	643976	C17H21N4O9P	CC1=CC2=C(C=C1C)N(C[C@H](O)[C@H](O)[C@H](O)COP(O)(O)=O)C1=NC(=O)NC(=O)C1=N2
deoxycholate	C18 reversed phase	negative	448.3074	18	3035026	C26H43NO5	C(C@H](CCC(=O)NCC(=O)O)[C@H]1CCC2[C@@]1([C@H](CC3C2CC[C@H]4[C@@]3(CC[C@H](C4)O)C)O)C
2-dehydro-D-gluconate	C18 reversed phase	negative	193.035	6.8	3035456	C6H10O7	C([C@H](C@H](C@@H](C(=O)C(=O)O)O)O)O)O
FGAR	C18 reversed phase	negative	313.0447	7.9	16048611	C8H15N2O9P	O[C@H]1[C@@H](O)[C@H](NC(=O)CNC=O)O[C@@H]1COP(O)(O)=O
Octulose-8/1-P	C18 reversed phase	negative	319.0439	8	90657269	C8H17O11P	C([C@H](C@H](C@H](C@H](C@@H](C(=O)CO)O)O)O)O)O)OP(=O)(O)O
sedoheptulose-1/7-phosphate	C18 reversed phase	negative	289.0334	8.3	92042786	C7H15O10P	OC[C@]1(O)O[C@H](COP(O)(O)=O)[C@@H](O)[C@H](O)[C@@H]1O
alanine	amide HILIC	negative	88.03951	14.3	5950	C3H7NO2	C(C@@H](C(=O)O)N
arginine	amide HILIC	negative	173.1037	18.5	6322	C6H14N4O2	C(C[C@@H](C(=O)O)N)CN=C(N)N
aspartate	amide HILIC	negative	132.0297	15.5	5960	C4H7NO4	C([C@@H](C(=O)O)N)C(=O)O
valine	amide HILIC	negative	116.0708	11.9	6287	C5H11NO2	CC(C)[C@@H](C(=O)O)N
Glucose	amide HILIC	negative	179.056	12.2	5793	C6H12O6	C([C@@H]1[C@H]([C@@H](C[C@H](C(O1)O)O)O)O)O
glutamate	amide HILIC	negative	146.0452	15.3	33032	C5H9NO4	C(CC(=O)O)[C@@H](C(=O)O)N
glutamine	amide HILIC	negative	145.0609	15	5961	C5H10N2O3	C(CC(=O)N)[C@@H](C(=O)O)N
glutathione	amide HILIC	negative	306.0767	15.4	124886	C10H17N3O6S	C(CC(=O)N[C@@H](CS)C(=O)NCC(=O)O)[C@@H](C(=O)O)N
glycine	amide HILIC	negative	74.0238	14.8	750	C2H5NO2	C(C(=O)O)N
histidine	amide HILIC	negative	154.0615	15.3	6274	C6H9N3O2	C1=C(NC=N1)C[C@@H](C(=O)O)N
leucine	amide HILIC	negative	130.0866	9.7	6106	C6H13NO2	CC(C)C[C@@H](C(=O)O)N
isoleucine	amide HILIC	negative	130.0866	10.3	6306	C6H13NO2	CC[C@H](C)[C@@H](C(=O)O)N
lysine	amide HILIC	negative	145.0974	18.5	5962	C6H14N2O2	C(CCN)C[C@@H](C(=O)O)N
methionine	amide HILIC	negative	148.0432	11	6137	C5H11NO2S	CSCC[C@@H](C(=O)O)N
phenylalanine	amide HILIC	negative	164.0711	9.5	6140	C9H11NO2	C1=CC=C(C=C1)C[C@@H](C(=O)O)N
proline	amide HILIC	negative	114.0553	12.4	145742	C5H9NO2	C1C[C@H](NC1)C(=O)O
serine	amide HILIC	negative	104.0349	15.3	5951	C3H7NO3	C([C@@H](C(=O)O)N)O
threonine	amide HILIC	negative	118.0501	14.5	6288	C4H9NO3	C(C@H](C@@H](C(=O)O)N)O
tryptophan	amide HILIC	negative	203.082	9.7	6305	C11H12N2O2	C1=CC=C2C(=C1)C(=CN2)C[C@@H](C(=O)O)N
tyrosine	amide HILIC	negative	180.0661	12.2	6057	C9H11NO3	C1=CC(=CC=C1C[C@@H](C(=O)O)N)O