Supporting Information

Dual inhibition of glutaminase and carnitine palmitoyltransferase decreases growth and migration of glutaminase inhibition–resistant triple-negative breast cancer cells

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Running Title*: CPT1 compensates GLS inhibition in TN breast cancer*

Figure S1

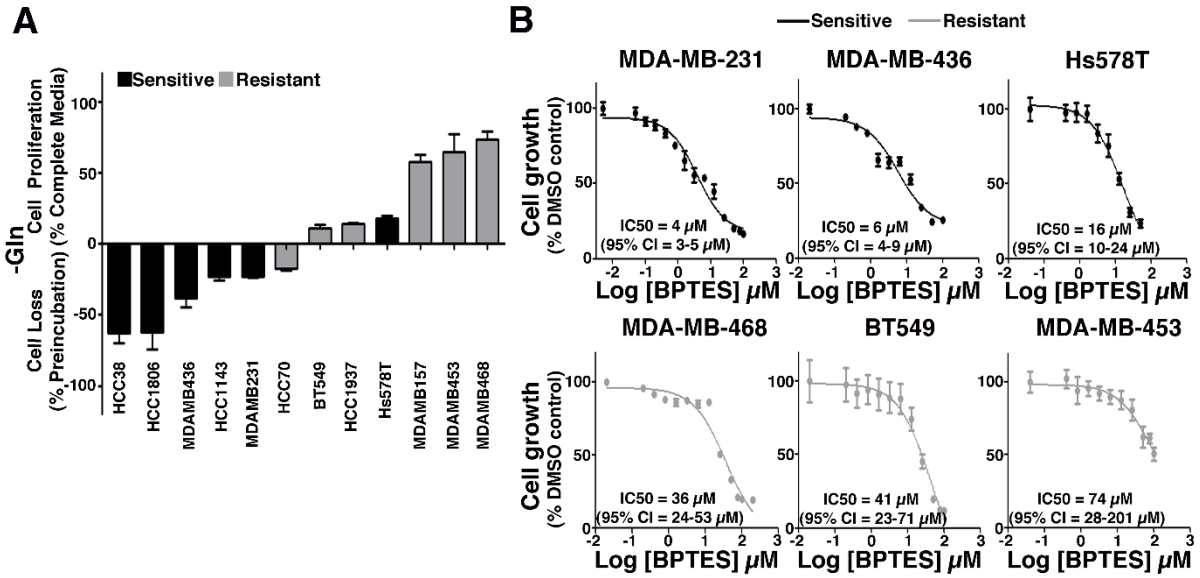

Fig. S1. TNBC cell lines have heterogenous glutamine-withdraw sensitivity and BPTES IC50 over cell growth. *A,* Growth response of 12 TNBC cell lines incubated with glutamine-deprived RPMI medium for 4 days. B, Growth response of 6 TNBC incubated with increasing amounts of BPTES, another glutaminase inhibitor, for 2 days. IC50 value and 95% confidence interval (CI) is presented as an inset. Graphics in *A-B* represent the mean ± standard deviation (SD) of n = 4.

Figure S2

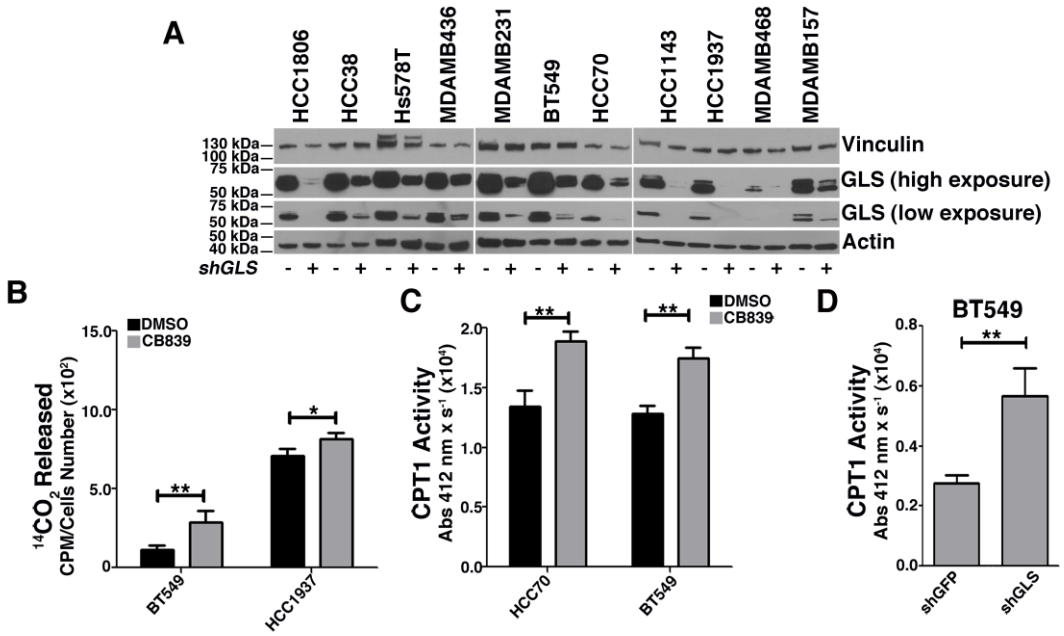

Fig. S2. Resistant cell lines respond to GLS attenuation by increasing beta-oxidation. *A*, *GLS* knockdown efficiency in 11 TNBC cell lines as shown by western blot (except MDA-MB-453). *B*, Beta-oxidation was directly measured by quantifying $^{14}CO_2$ released from uniformly labeled (^{14}C)-palmitic acid. The resistant cell lines BT549 and HCC1937 released more $^{14}CO_2$ when treated with CB-839 compared to DMSO. *C*, CPT1 activity increase in the resistant BT549 and HCC70 cell lines after CB-839 incubation compared to DMSO. *D*, CPT1 activity increased in the resistant BT549 cell line after *GLS* knockdown. Graphics in *B-D* represent the mean ± SD of n = 3. Student t-test was applied; $p < 0.05$ (*), $p < 0.01$ (**).

Figure S3

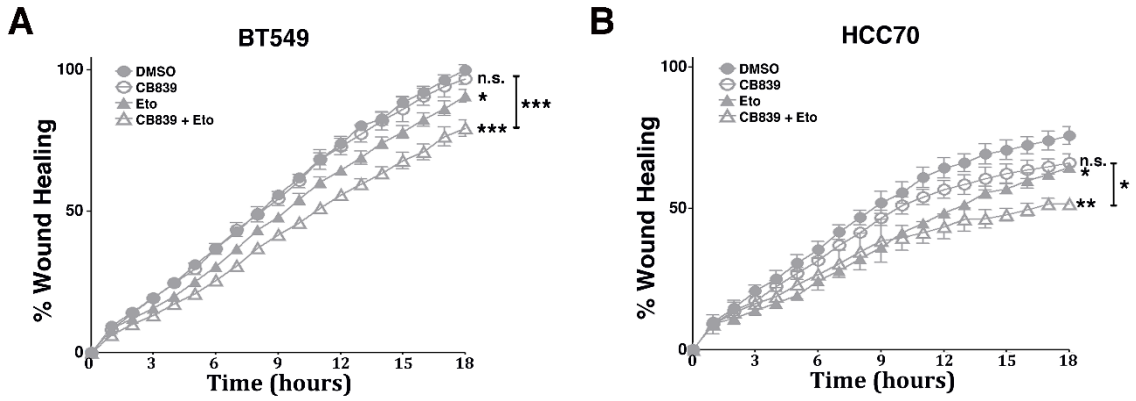

Fig. S3. Etomoxir and CB-839 combined treatment promoted a further decrease in the migration of resistant cell lines. Combined CB-839 + etomoxir treatment further decreased cell migration in the resistant BT549 (*A*) and HCC70 (*B*) cell lines compared to individual treatments. Graphics in *A-B* represent the mean ± SD of n = 4. Student t-test was applied; $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), n.s., non-significant. Whenever not indicated comparison was made against DMSO control.

Figure S4

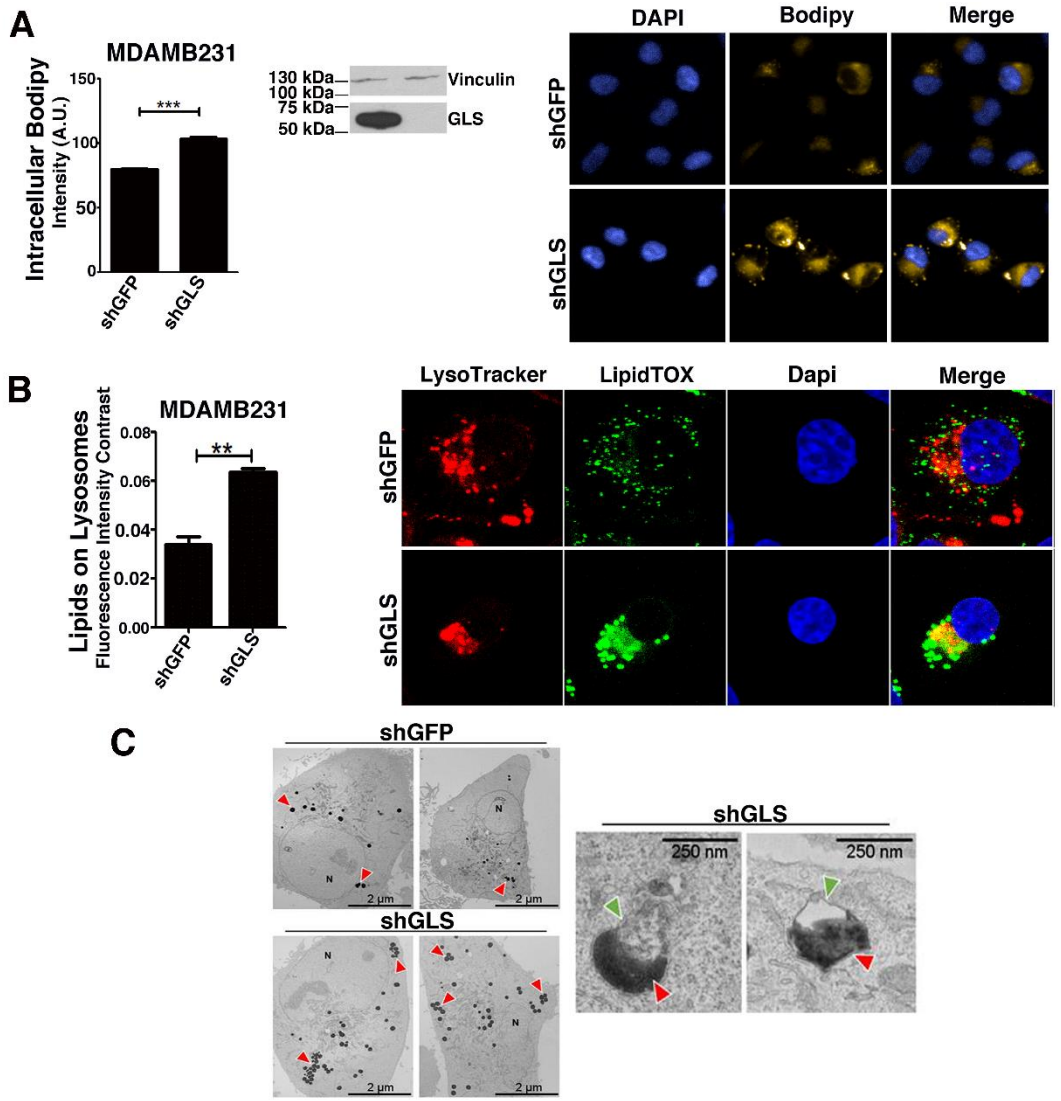

Fig. S4. MDA-MB-231 cell line responds to *GLS* knockdown by increasing FA intake and lipid droplets mobilization to lysosomes. *A*, MDA-MB-231 responded to *GLS* knockdown by capturing more C1-BODIPY 500/510 C12 fatty acid (Bodipy) from the medium. Fluorescence microscopy images on the right depicting a higher signal in cells with knocked down *GLS*. *B*, *GLS* knockdown drove cells to a higher mobilization of neutral lipid droplets (as identified by the specific marker Lipidtox) to lysosomes (stained by Lisotracker), implying lipophagy in these cells. Confocal fluorescence microscopy maximum projection images depicting lysosome and neutral lipid droplet co-staining (in yellow) in cells with *GLS* knocked down; Nuclei were stained with DAPI. *C*, On the left, transmission electron micrographs (TEM) of shGFP and shGLS cells showing a visual enrichment on lipid droplets (red arrowheads) on cells with *GLS* knocked down. N = nucleus. On the right, TEM displaying fusion between lipid droplets (red arrowheads) and autophagosomes (green arrowheads) found on shGLS cells.

Table S1. List of genes DE between resistant and sensitive cell lines and enriched pathways which are related to lipid metabolism (log 2 FC ≥ + 1 or log2 FC ≤ -1, padj ≤ 0.05).

GO pathway term	padj	DE genes
Lipid metabolic process GO:0006629	0.00008	*ABCG1, ACOT1, ACSS2, ALDH1A2, ANKRD1, APOBR, CHKB-CPT1B, CISH, CLN3, CPT1B, CRAT, CYP26A1, CYP26B1, CYP27B1, DGKG, EFR3B, FGF21, GGPS1, GPC2, INPP5A, ISYNA1, OSBPL5, PAFAH2,CK2, PDSS1, PIK3C2B, PIK3R1, PLA2G3, PLA2G4B, PLA2G6, PRLR, SERINC4, SESN2, SLC16A11, SLC44A4, STARD10, SULT1E1, TBXAS1, TM7SF2, TMEM150A, TMEM86A, TRIB3, TTC39B, XBP1*
Lipid biosynthetic process GO:0008610	0.00120	*ABCG1, ACSS2, ADGRF5, ALDH1A2, CYP27B1, GGPS1, ISYNA1, PDSS1, PIK3C2B, PIK3R1, PLA2G6, PRLR, SERINC4, TBXAS1, TM7SF2, TRIB3, XBP1*
Cellular lipid metabolic process GO:00044255	0.00131	*ABCG1, ACSS2, ACOT1, ADGRF5, ALDH1A2, APOBR, CLN3, CPT1B, CRAT, CYP26A1, CYP26B1, EFR3B, GGPS1, GHR, GPC2, HELZ2, INPP5A, ISYNA1, PDSS1, PDPN, PIK3C2B, PIK3R1, PLA2G3, PLA2G4B, PLA2G6, SERINC4, SESN2, TBXAS1, TMEM150A, TRIB3, XBP1*
Phospholipid biosynthetic process GO:0008654	0.00400	*ADGRF5, GGPS1, ISYNA1, PIK3C2B, PIK3R1, PLA2G6, SERINC4, XBP1*
Phospholipid metabolic process GO:0006644	0.00691	*ADGRF5, CLN3, EFR3B, INPP5A, ISYNA1, GGPS1, PIK3C2B, PIK3R1, PLA2G3, PLA2G4B, PLA2G6, SERINC4, TMEM150A, XBP1*
Fatty acid derivative metabolic process GO:1901568	0.00808	*ACSS3, GGTA1P, PLA2G3, PLA2G4B, TBXAS1*
Phospholipid transport GO:0015914	0.01141	*ABCG1, ATP10A, OSBPL5*
Positive regulation of phospholipid biosynthetic process GO:0071073	0.01384	*ADGRF5, XBP1*
Fatty acid metabolic process GO:0006631	0.02225	*ACOT1, ACSS2, CPT1B, CRAT, GHR, PDPN, PLA2G3, PLA2G4B, SESN2, TBXAS1, TRIB3, XBP1*
Acyl carnitine transport GO:0006844	0.02472	*CPT1B, CRAT*
Acyl carnitine transmembrane transport GO:1902616	0.02472	*CPT1B, CRAT*

Cholesterol biosynthetic process GO:0045540	0.03002	*ABCG1*
Regulation of phospholipid biosynthetic process GO:0071071	0.03731	*ADGRF5, XBP1*
Carnitine metabolic process GO:0009437	0.03967	*CPT1B, CRAT*
Unsaturated fatty acid metabolic process GO:0033559	0.04735	*PDPN, PLA2G4B, TBXAS1*
Lipid transport GO:0006869	0.04841	*ABCG1, APOBR, ATP10A, CPT1B, DISP3, OSBPL5, PLA2G6, SLCO2A1, STARD10, FZD4*

Table S2. List of genes DE between resistant and sensitive cell lines, which were related to the pathways listed in Table S1. Genes related to beta-oxidation and lipid catabolism are in bold.

Gene	log2(FC) (Resistant/Sensitive)	padj	Gene	log2(FC) (Resistant/Sensitive)	padj
ATP10A	-5.14	3.3E-12	*TM7SF2*	2.24	0.010333
IGFBP1	-4.32	0.001869	*ISYNA1*	2.28	0.012792
PDPN	-4.03	0.005988	*EFR3B*	2.28	0.023631
ANKRD1	-3.84	0.004141	***PLA2G6***	2.28	0.007354
ADGRF5	-3.72	0.009985	*SERINC4*	2.39	0.033476
DGKG	-3.45	0.018184	*CHKB-CPT1B*	2.39	0.009758
CYP26A1	-3.01	0.035812	*GPC2*	2.40	0.004141
ACSS3	-2.95	0.031423	*APOBR*	2.40	0.035456
TBXAS1	-2.25	0.005988	*CYP27B1*	2.40	0.017153
CYP26B1	-2.05	0.028926	***CRAT***	2.46	0.026845
PDSS1	-1.46	0.002857	*OSBPL5*	2.47	0.0052
INPP5A	-1.40	0.004229	*SLC16A11*	2.57	0.040341
PAFAH2	1.02	0.003687	*TMEM86A*	2.59	0.022815
ACSS2	1.13	0.044733	*PRLR*	2.62	0.013213
GGPS1	1.24	0.025419	*STARD10*	2.71	0.004409
ACOT1	1.40	0.031423	*GNA14*	2.78	0.045759
CLN3	1.51	0.014323	*FGF21*	2.85	0.045759
TMEM150A	1.56	0.030933	*CISH*	2.86	0.003657
FZD4	1.58	0.033288	*XBP1*	2.86	0.000323
SESN2	1.60	0.026989	*CREB3L4*	3.02	0.000323
CPT1B	1.68	0.049406	*SLCO2A1*	3.06	0.008565
PIK3C2B	1.73	0.046818	*ABCG1*	3.25	0.010536
HELZ2	1.82	0.04533	*GHR*	3.30	0.028926
TTC39B	1.89	0.043085	*DISP3*	3.37	0.00075
TRIB3	1.95	0.013852	***PLA2G3***	3.90	0.003395
PIK3R1	1.95	0.003009	*SULT1E1*	3.92	0.002022
PLA2G4B	2.02	0.047481	*ALDH1A2*	3.98	0.00103
PCK2	2.18	0.000678	*SLC44A4*	4.40	0.000433

Table S3. List of genes DE between low and high *GLS* breast tumors related to the "fatty acid oxidation process" (intersection between GO #0006631 and GO # 0019395).

GeneSymbol	log2FC (low/high)	padj	GeneSymbol	log2FC (low/high)	padj
SLC27A2	2.69	2.88E-31	*SCP2*	0.42	7.24E-07
ACOX2	2.67	2E-36	*ADIPOR1*	0.42	4.95E-09
PLIN5	2.48	1.54E-26	*ETFDH*	0.39	1.25E-08
ACSM1	2.14	6.77E-36	*ACOT8*	0.37	7.11E-06
CRAT	2.10	1.73E-36	*AUH*	0.34	4.23E-06
ECI1	1.47	1.1E-45	*CPT1B*	0.33	0.012091
IVD	1.35	2.33E-47	*ACAT1*	0.33	0.001647
ECI2	1.32	1.01E-27	*AKT2*	0.33	3.62E-07
DECR2	1.22	2.92E-29	*HADH*	0.31	0.000157
ACADS	1.18	3.16E-19	*HACL1*	0.23	0.001316
CROT	1.16	1.11E-16	*PECR*	0.21	0.035404
ETFB	0.95	1.9E-10	*PEX2*	0.18	0.008166
PEX7	0.86	3.59E-26	*SLC25A17*	0.17	0.012334
ECHDC2	0.80	5.19E-14	*MLYCD*	0.17	0.029296
ADIPOR2	0.79	8.96E-13	*MTOR*	-0.19	0.008311
ACOX3	0.76	3.97E-16	*ACOX1*	-0.24	0.003667
AMACR	0.76	8.54E-16	*HIBCH*	-0.32	0.000182
CPT1A	0.75	2.53E-09	*PHYH*	-0.32	0.006093
ECH1	0.75	1.23E-15	*PEX13*	-0.34	4.51E-07
ECHS1	0.74	1.94E-17	*EHHADH*	-0.35	0.003127
HSD17B4	0.74	6.41E-15	*PPARD*	-0.36	1.26E-05
IRS1	0.73	5.13E-06	*PRKAG2*	-0.39	5.09E-07
ACAA1	0.72	3.6E-18	*MAPK14*	-0.42	1.59E-12
ALDH3A2	0.71	1.2E-08	*ABCD1*	-0.42	0.000509
ETFBKMT	0.66	8.34E-14	*ACAT2*	-0.43	0.000287
TYSND1	0.65	9.82E-16	*ACAA2*	-0.44	1.81E-05
ABCD3	0.63	6.24E-11	*PRKAA1*	-0.59	1.59E-08
ACACB	0.60	4.23E-05	*ACAD11*	-0.70	1.48E-08
APPL2	0.60	1.63E-14	*ECHDC1*	-0.81	2.78E-13
AKT1	0.58	1.27E-13	*ALOX12*	-0.88	4.04E-09
ACAD10	0.55	2.31E-17	*NR4A3*	-1.16	3.17E-11
ACOXL	0.55	0.006364	*DGAT2*	-1.20	2.09E-13
ACADVL	0.54	7.56E-10	*ADH7*	-1.46	0.011757
LONP2	0.53	2.13E-09	*PPARA*	-1.62	1.31E-52
CPT2	0.48	1.57E-12	*ABCD2*	-2.25	2.43E-36
GCDH	0.48	6.12E-08	*PPARGC1A*	-2.64	1.45E-24
SESN2	0.47	1.06E-06	*HAO1*	-6.64	1.97E-54
MCAT	0.43	8.8E-07			