Dual inhibition of ABCE1 and LCP1 by microRNA-96 results in an additive effect in breast cancer mouse model

SUPPLEMENTARY MATERIALS

Supplementary Table 1: SYBR green RT-PCR primers for mRNA quantification

Primer name	Sequence	
M Lep1 For	TCTCACTGTCACGGTGGTTGGC	
M Lcp1 Rev	CTCAAATCTCCAGAGAAGCAAGCAC	
H Lep1 For	CGTTGGCACCCAACACTCCTA	
H Lcp1 Rev	GGGATGACATGCCGACAATCAG	
M β-Actin-For	ACCAGAGGCATACAGGGACA	
M β-Actin-Rev	CTAAGGCCAACCGTGAAAAG	
H GAPDH-For	AGCCACATCGCTGAGACA	
H GAPDH-Rev	GCCCAATACGACCAAATCC	

Abbreviations: *H-human, M-mouse.

Supplementary Table 2: Primary antibody used for Western blot analysis

Antibody	Manufacturer	catalog number	Dilution
Mouse anti-LCP1	Santa Cruz	sc-133219	1:1000
Mouse anti-Actin	Millipore	clone C4, MAB1501	1:5000

Supplementary Table 3: Secondary antibody used for Western blot analysis

Antibody	Manufacturer	Catalog number	Dilution
HRP-conjugated AffiniPure Goat- anti-Mouse IgG	Jackson ImmunoResearch Laboratories, Inc.	115-035-166	1:10000



Supplementary Figure 1: LCP1 mRNA expression in 53 non-diseased tissue sites. LCP1 mRNA expression levels were determined in 53 non-diseased tissue sites collected from nearly 1,000 individuals using data from the GTEx project [19]. TPM- transcripts per million kilobases.



Supplementary Figure 2: LCP1 and ABCE1 mRNA and protein expression in cells that stably underexpress Abce1, Lcp1, Abce1+Lcp1, or scrambled. (A) Expression levels of ABCE1 and LCP1 were calculated for 4T1 cells stably transfected with shRNAs against Abce1/ Lcp1/Abce1+ Lcp1/ scrambled. ABCE1 RNA expression was reduced in ABCE1 KD and ABCE1+LCP1 KD tumors, while LCP1 was decreased in LCP1 KD and ABCE1+LCP1 derived tumors. (B) Western blot analysis of protein extracted from the cells in (A) revealed reduced protein expression of Abce1 in Abce1 KD and dual Abce1+Lcp1 KD groups compared to the scrambled group, while the Lcp1 protein expression level was reduced in the Lcp1 KD and the dual Abce1+ Lcp1 KD groups compared to the scrambled group. Data is presented as mean \pm SEM. **p < 0.01.



Supplementary Figure 3: LCP1 and ABCE1 mRNA expression in mouse tumors. Expression levels of ABCE1 and LCP1 were calculated for breast tumors originating from Abce1 KD, Lcp1 KD, Abce1 KD+Lcp1 KD, or scrambled 4T1 cells. ABCE1 RNA expression was reduced in ABCE1 KD and ABCE1+LCP1 KD tumors, while LCP1 was decreased in LCP1 KD and ABCE1+LCP1 derived tumors. Data is presented as mean \pm SEM. **p < 0.01.