Supplementary Materials

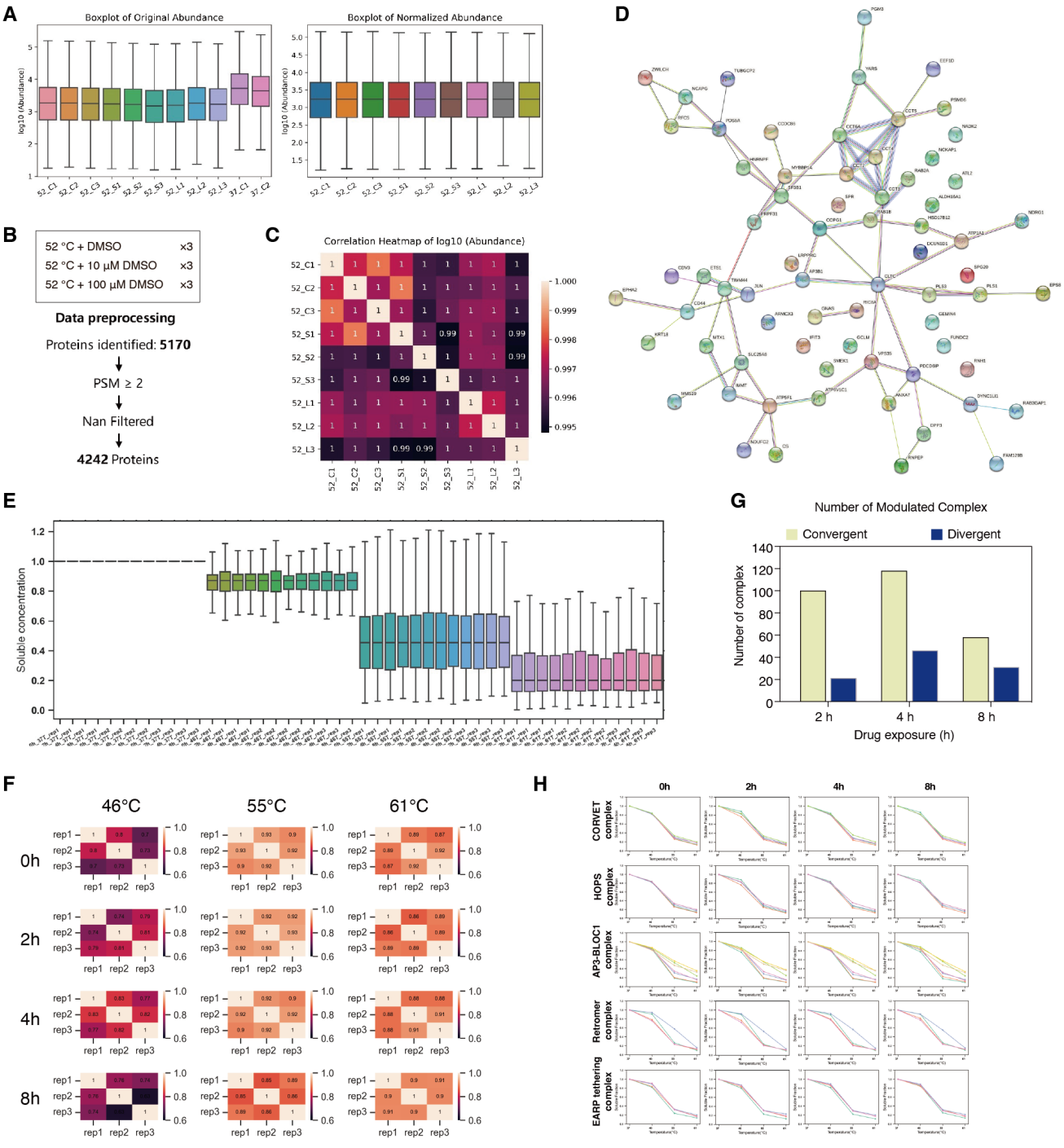

Figure S1. Quality control and extended results of TPP-TPCA. (A) The abundance of proteins was normalized by the median value. 9 TMT channels were divided into three groups, control (Ctrl), low dose (S) and high dose (L). **(B)** Workflow of data preprocessing. After PSM filtering and null removal, there were 4242 valid proteins. **(C)** The correlation between abundance in the 9 TMT channels was evaluated using Pearson's correlation coefficient. **(D)** PPI map of 68 differential proteins were analyzed by STRING database. **(E)** The abundance of proteins in TPCA was normalized by the median value. 37, 46, 55, 61 represent the heating 37 degrees, 46 degrees, 55 degrees, 61 degrees, respectively. **(F)** Correlation of peptide abundance in three

replicates was evaluated using Pearson's correlation coefficient. **(G)** The quantity of associated and dissociated complexes observed at various time intervals. **(H)** TPCA profiling of dynamic protein complexes at various time points during VAM exposure (CORVET and HOPS complex, AP3-BLOC1 complex, Retromer complex, EARP tethering complex).

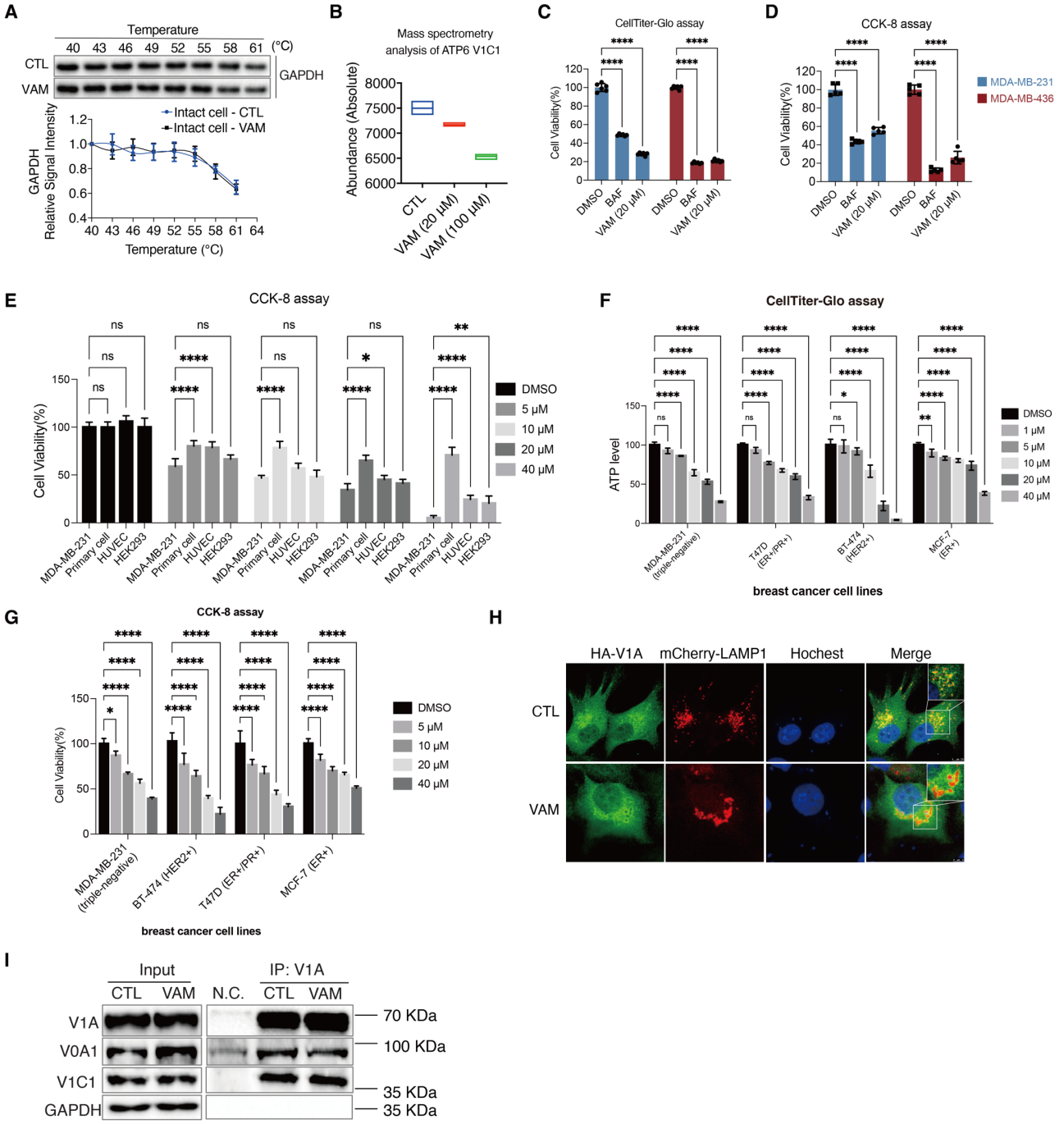

Figure S2. VAM did not affect V-ATPase assembly. (A) Melting curve of GAPDH under VAM or DMSO treatment. **(B)** Abundance of ATP6V1C1 in lysates treated with DMSO or VAM was quantified by MS. **(C)** CCK-8 assay assessing the effects of 100 nM BAF and VAM treatment for 48 hours. **(D)** CellTiter-Glo® 2.0 luminescent cell viability assay evaluating the effects of 100 nM BAF and VAM treatment for 48 hours. **(E)** CCK-8 assay was used to evaluate the effects of different doses of VAM treatment on primary cells or non-cancer cell lines for 72 h. **(F)** CellTiter-Glo® 2.0 luminescent cell viability assay was used to evaluate the effect of VAM on different subtypes of breast cancer cells. **(G)** CCK-8 assay was used to evaluate the effect of VAM on the viability of different subtypes of breast cancer cells. **(H)** Co-distribution of V-ATPase peripheral domain (ATP6V1A) and lysosome (LAMP1). **(I)** MDA-MB-231 cells were treated with DMSO or VAM for 24 h. Following treatment, co-immunoprecipitation was conducted using V1A and IgG to evaluate the interaction with ATP6V1C1 and ATP6V0a1.

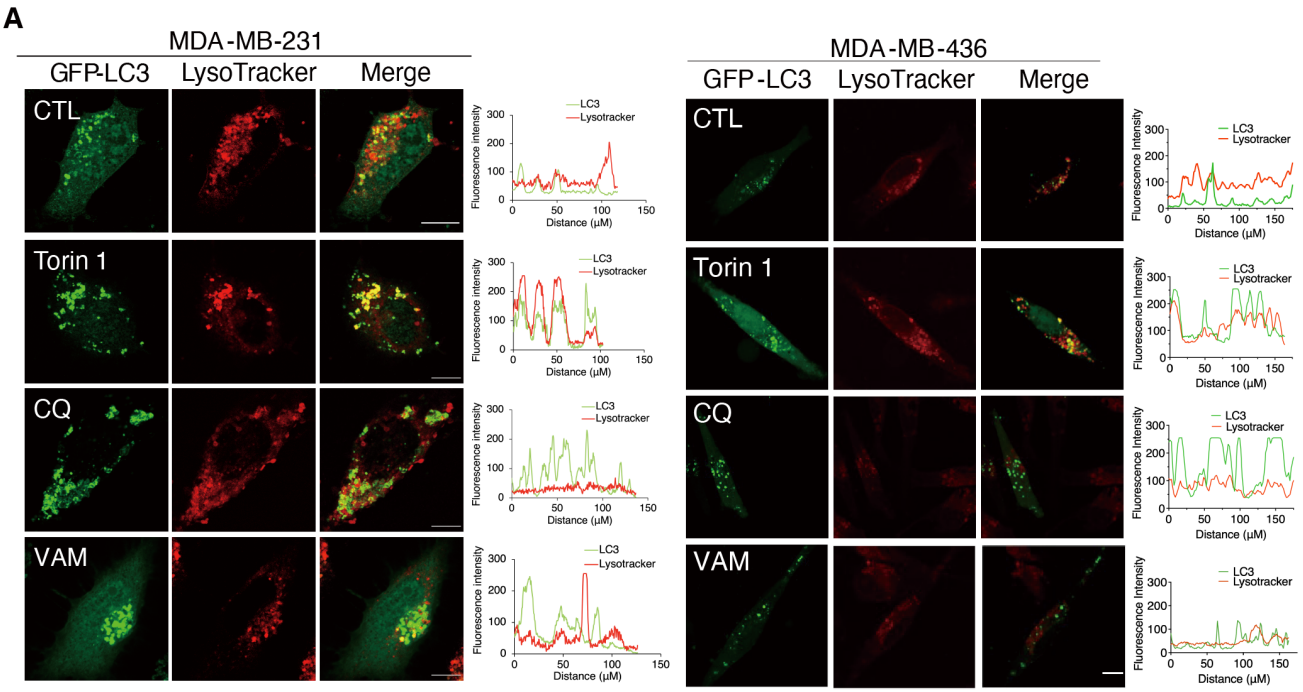

Figure S3. VAM blocked autophagosome-lysosome fusion. (A) Colocalization analysis of the autophagosomes and lysosomes. EGFP-LC3 Hela cells were treated with 10 μM VAM, 100 nM Torin1, 30 μM CQ or DMSO for 24 h and stained with LysoTracker Red (50 nM) for 30 min. The fluorescence images of LC3 and lysotracker were scanned via Leica TCS SP8 Confocal Laser Scanning Microscope System. Scale bar: 10 μm.

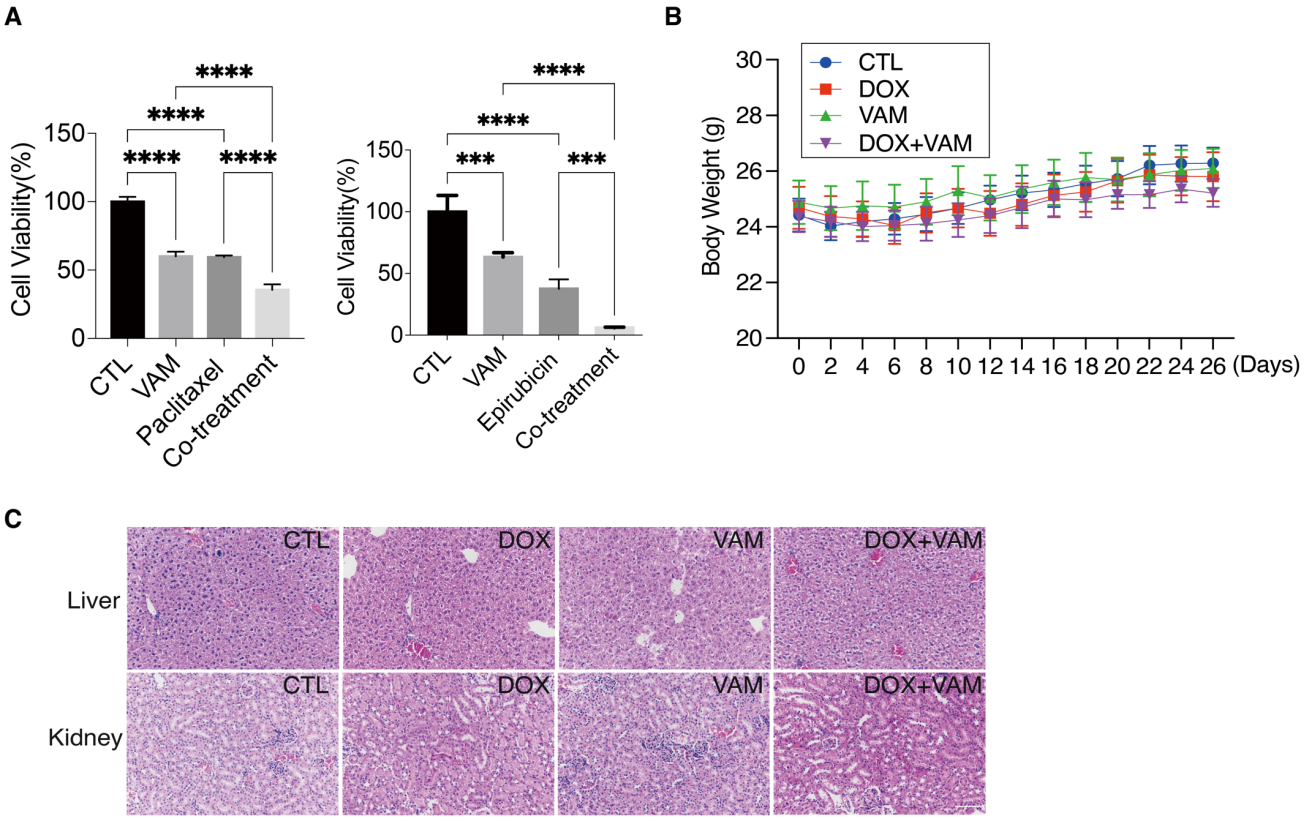

Figure S4. Toxicity evaluation. (A) MDA-MB-231 cells were exposed to paclitaxel (10 nM) or epirubicin (0.5 μM) either with or without VAM (20 μM) for 72 h. Cell viability was evaluated using CCK-8 assays (n=4). **(B)** Body weight changes of mice during 26 days of exposure. **(C)** H&E staining assays of the liver and kidneys from all experimental groups (n = 3). Scale bar=100 μm.

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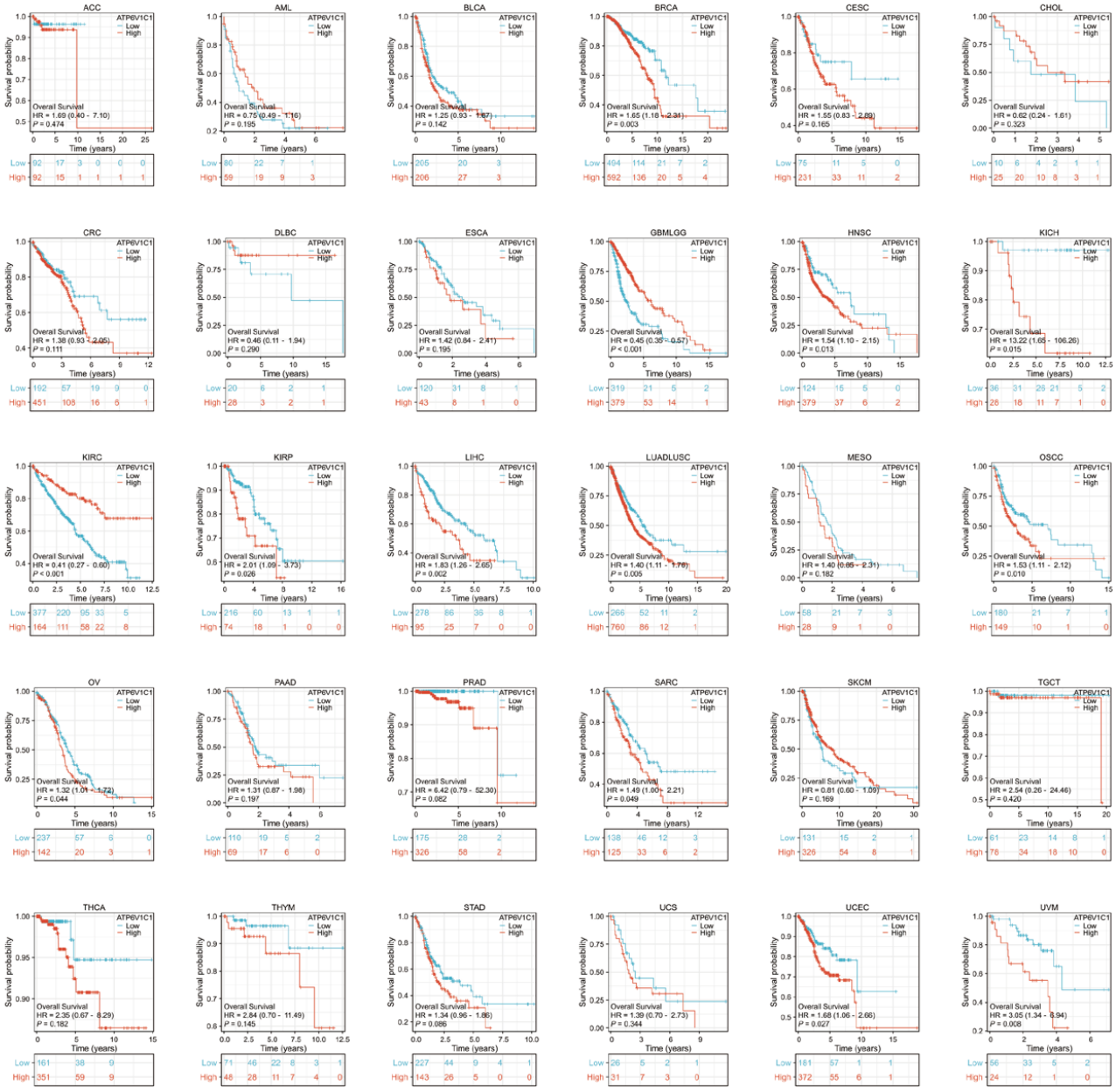

Figure S5. (A) Overall survival (OS) comparison between high and low expression of the *atp6v1c1* gene in 33 common malignant tumors.

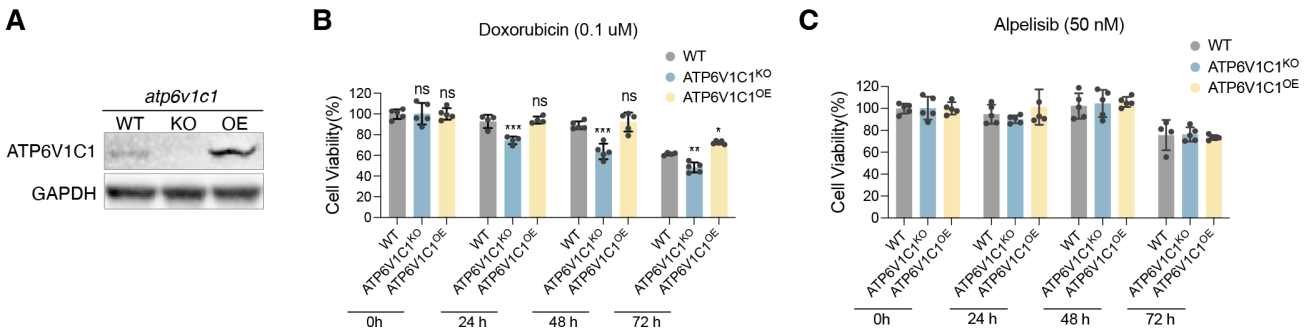

Figure S6. Influence of cytosolic ATP6V1C1 levels on tumor viability. (A) Western blot analysis to assess cytosolic ATP6V1C1 levels in WT, KO, and OE MDA-MB-231 cell lines. **(B)** Evaluation of cell viability in ATP6V1C1 WT, KO, and OE cell lines following doxorubicin treatment. **(C)** Assessment of cell viability in ATP6V1C1 WT, KO, and OE cell lines upon paclitaxel treatment (n=5).

Supplemental Table S1

Table S1: 20 functional targets of VAM identified in TPP-TPCA profiling based on soluble protein abundance.

Entry	Gene Name	Protein Complex	Adjust P value (DMSO v.s. VAM)
1	ATP6V1C1	V-type proton ATPase catalytic subunit A	0.0147
2	DYNC1LI1	Dynein-dynactin complex	0.0427
3	AP3B1	AP3-BLOC1 complex	0.0490
4	VPS35	Retromer complex	0.0141
5	PDS5A	Sororin-cohesin complex	0.0327
6	ZWILCH	ACTB-ANP32A-C1QBP-PSMA1-PTMA-PSMA1 complex	0.0497
7	NCAPG	Condensin I-PARP-1-XRCC1 complex	0.0141
8	ATP5F1	F1F0-ATP synthase	0.0065
9	NDUFC2	Respiratory chain complex I	0.0427
10	NCKAP1	Wave-2 complex	0.0284
11	PSMD6	PA700 complex	0.0026
12	MYBBP1A	Nop56p-associated pre-rRNA complex	0.0141
13	TIMM44	HSPA9-GRPEL1-GRPEL2-TIMM44 complex	0.0261
14	HNRNPF	C complex spliceosome	0.0427
15	SF3B1	17S U2 snRNP，Spliceosome	0.0331
16	CCT7	CCT complex	0.0355
17	CCT6A	CCT complex	0.0427
18	CCT5	CCT complex	0.0141
19	CCT4	CCT complex	0.0142
20	CCT3	CCT complex	0.0142

Proteins listed had (1) a calculated P value in 3 replicates; (2) were either stabilized or destabilized in 3 replicates; (3) had an intensity shift of ≥ 10%.