Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Table 1.

Process	Component	Genes	P-value
Vacuolar transport	ESCRT I complex	STP22, VPS28	9.26E-03
	ESCRT II complex	SNF8, VPS36, VPS25	6.39E-05
	ESCRT III complex	VPS20, SNF7	9.26E-03
Golgi transport	GARP complex	VPS52, VPS51	9.26E-03
Protein glycosylation	Endoplasmic reticulum	ALG3, ALG5, ALG6, ALG8, OST3, PMT1, CWH41, VAN1	4.88E-04
Membrane invagination	Membrane	OSH3, RVS161, VAM7, GVP36, RVS167, VPS41, LDB17, END3	7.78E-03
Lipid metabolic process		LCB3, BST1, FAT1, ALG3, SCS2, ALG6, VPS4, SUR2, OPI1, ALG8, FEN1, ERG4	7.78E-03

GO analysis of chemical-genetic homologous deletion profiling by BINGO software showed that most of genes identified as hits are significantly enriched in ESCRT complex and proteins involove in vesicle trafficking.

Supplementary Figure 1

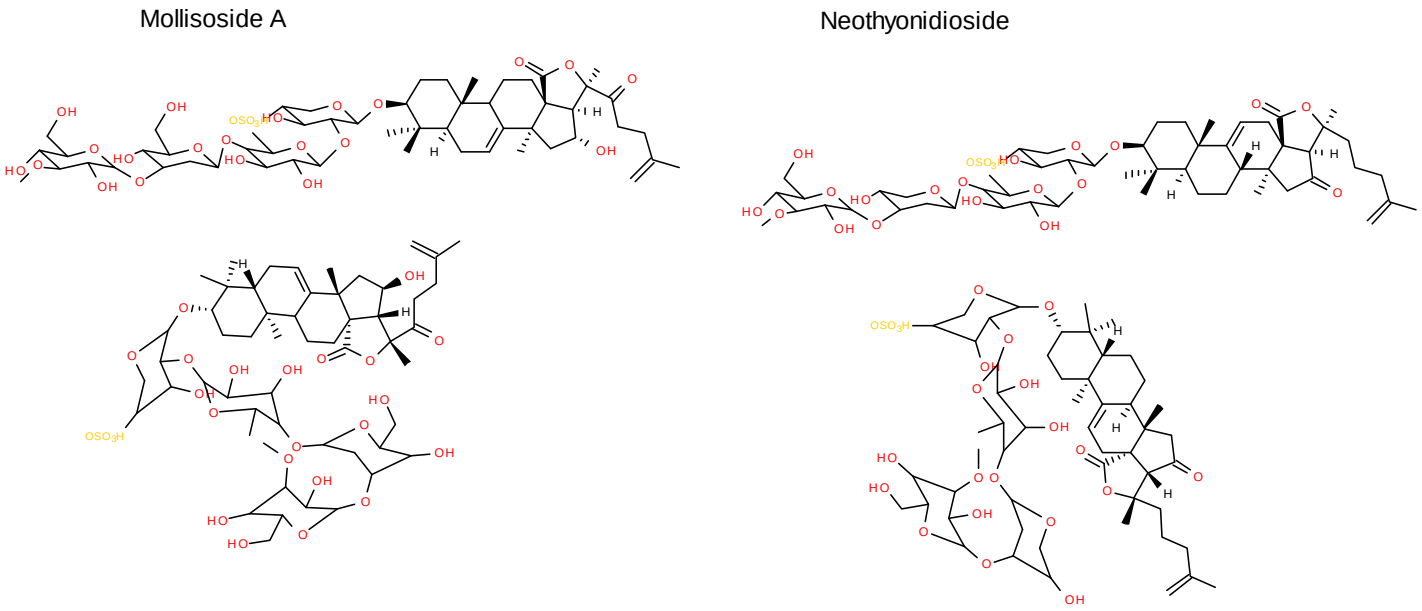

Figure S1. Structure of neothonidioside and mollisoside A

Supplementary Figure 2

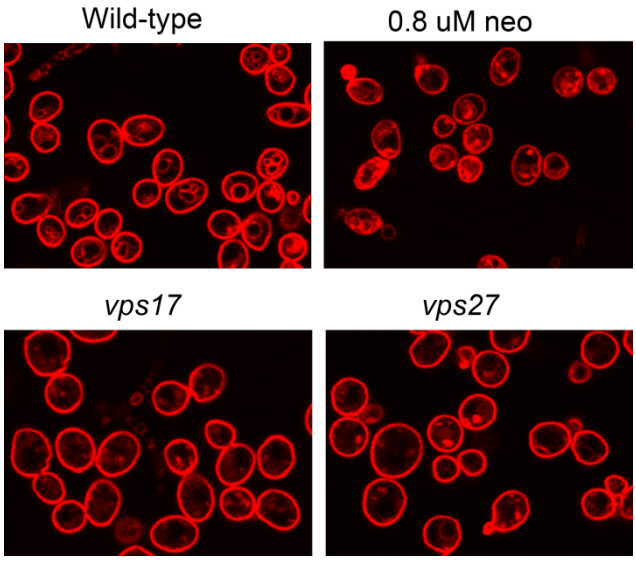

Figure S2. Vacuole morphology of wild-type cells, cells treated with neothionidioside and vacuolar protein sorting (*vps*) mutants staining with FM-4-64 FX as mentioned in experimental section. Cells treated with sublethal concentration of neothionidioside showed defect in vacuole formation similar to *Δvps17* and *Δvps27* . Both of these genes were identified as hits in chemical-genetic homologous deletion profiling (HOP) assay. Vps17p is a subunit of the membrane-associated retromer complex. It

assembles with Vps5p to promote vesicle formation and is essential for endosome-to-Golgi retrograde transport. Vsp27p is an endosomal protein and a member of ESCRT-0 complex which is required for recycling Golgi proteins.