

Supporting Figures and Tables

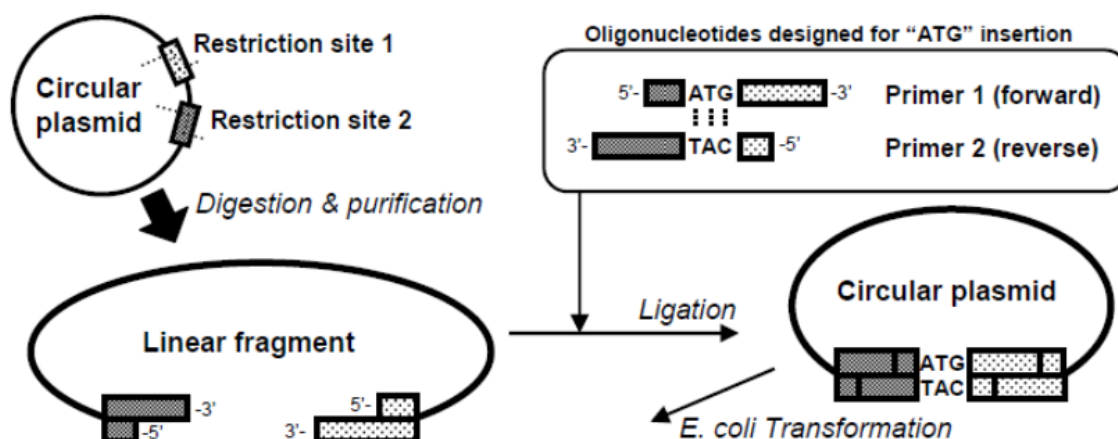


Figure S1. Schematic illustration of the one-step construction method for plasmids (OSCoM-P). The digested linear fragments of the plasmids were ligated with the designed primers at a dry weight ratio of 1:1–1:10 (linear plasmid:each primer). The reaction solution was directly transformed into *E. coli* DH5 α competent cells (Toyobo). The purchased oligonucleotides were dissolved in dH₂O at 10 ng/ μ L before use. TE buffer was not used because OSCoM-P was severely inhibited by the presence of TE buffer (data not shown). Maximum length of fragments to be inserted into plasmids using OSCoM-P is 45 bp (data not shown).

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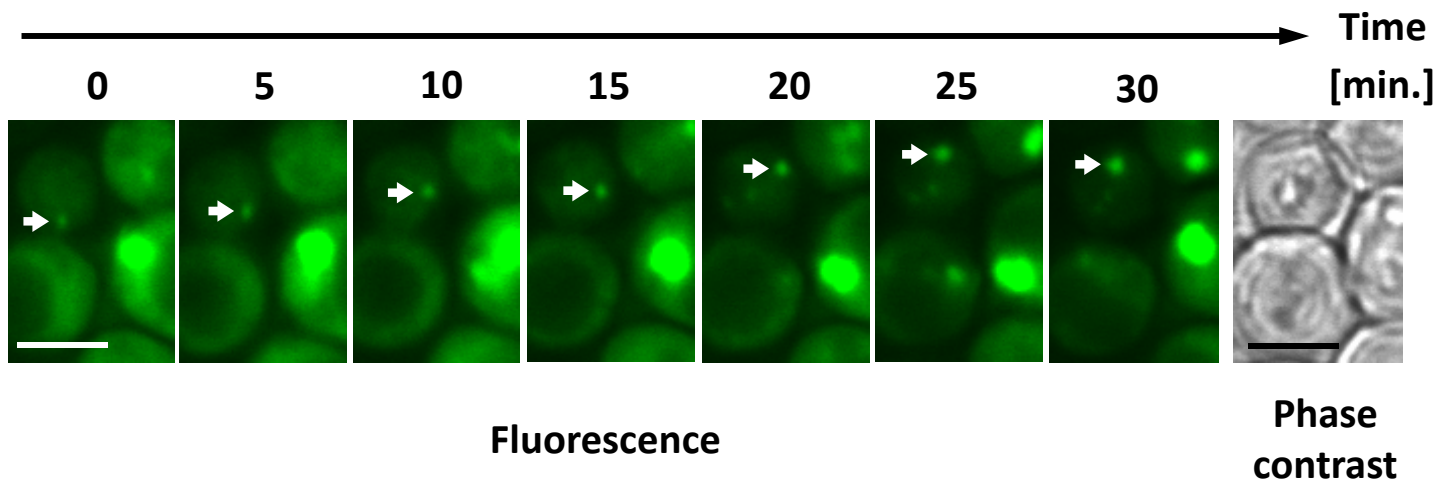


Figure S2. Time-dependent localization change of the eno(1–28) fragment fused to EGFP.

Scale bar: 5 μ m.

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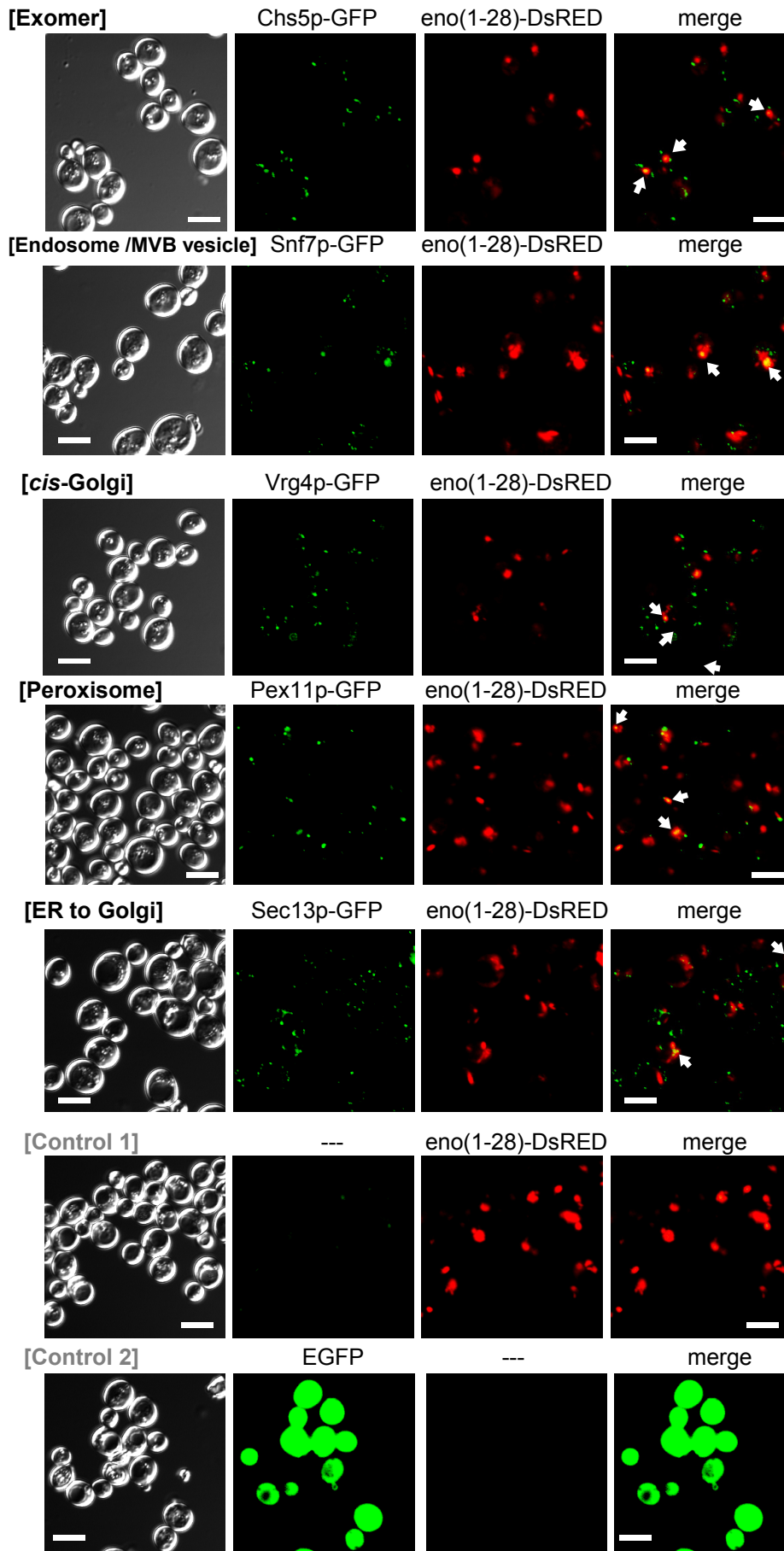


Figure S3. Co-localization of the enolase fragment conjugated to DsRED and the organelle markers conjugated to GFP (continued from Fig. 3). Control 1, wild-type cells transformed with pULR-eno(1-28); control 2, wild-type cells transformed with pUL-ATG-EGFP. Scale bar: 5 μ m.

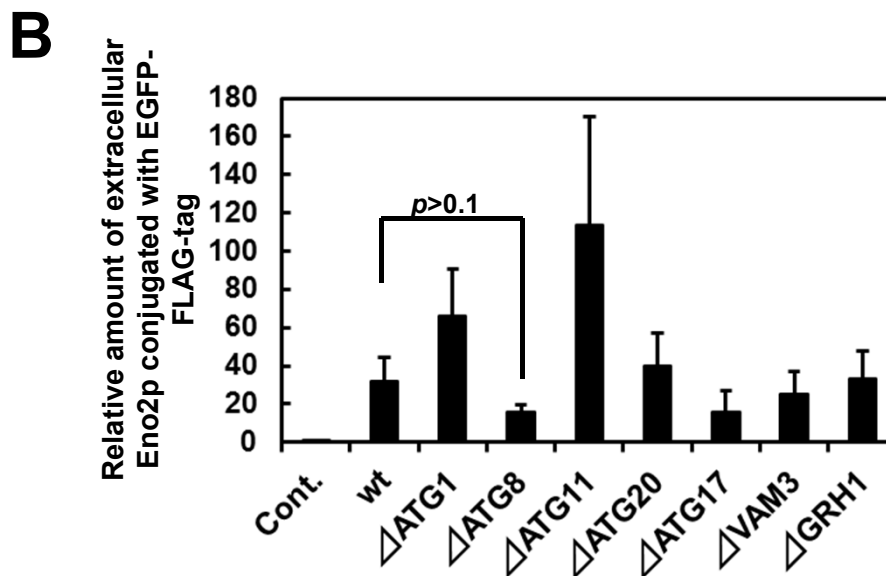
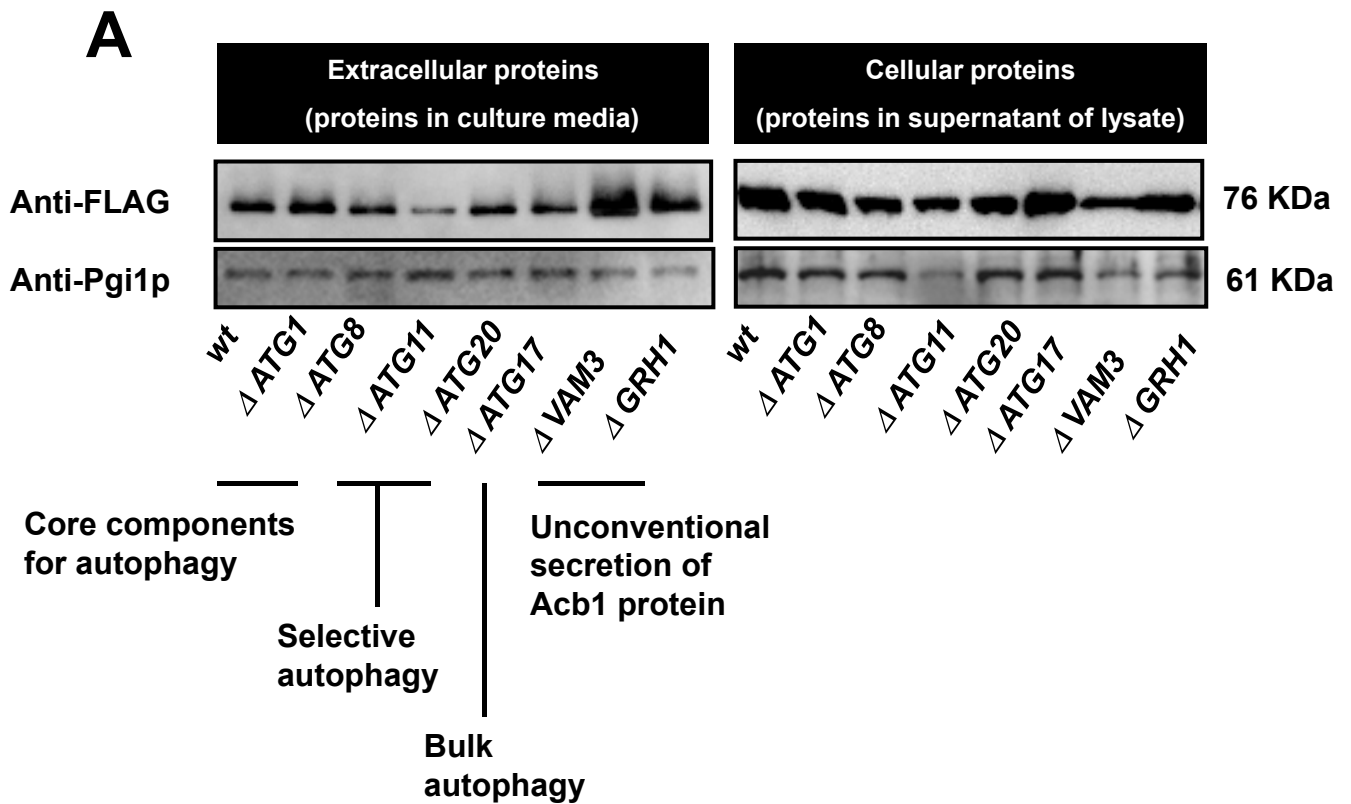


Figure S4. Autophagy independency of Eno2p and Pgi1p secretion. A: Western blots. B: Calculated amounts of secreted Eno2p by comparison to the Pgi1p secreted. Values are the mean \pm SEM of 3 independent experiments.

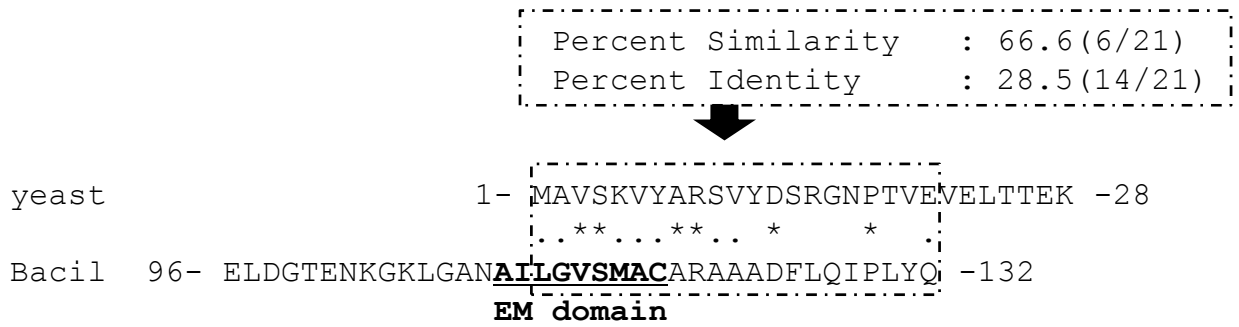


Figure S5. Similarity of eno(1-28) region with EM domain. Percent similarity and percent identity between the two sequences in the box with dotted line are shown. Yeast, *S. cerevisiae* enolase (1-28) aa sequence; Bacil, *Bacillus subtilis* enolase (96-132) aa sequence including EM domain (56); underline, EM domain; asterisk, identical amino acid residues; dot, similar amino acid residues.

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Table S1. List of identified noncovalently-bound cell surface proteins

The data is available in the PRIDE database (57, www.ebi.ac.uk/pride) under accession number 26880. The data was converted using PRIDE Converter (58, <http://pride-converter.googlecode.com>).

Cellular process	Accession	SGDID	Name	Description	# AAs	MW [kDa]	calc. pI	ΣCover age	Σ# PSMs	Σ# Peptides			
Metabolism	Glycolysis	YHR174W	S000001217	ENO2	Enolase II, a phosphopyruvate hydratase	437	46.9	6.0	31	41	9		
		YGR192C	S000003424	TDH3	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3	332	35.7	7.0	40	53	8		
		YCR012W	S00000605	PGK1	3-phosphoglycerate kinase	416	44.7	7.6	32	28	8		
		YJL052W	S000003588	TDH1	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 1	332	35.7	8.3	32	32	7		
		YDR050C	S000002457	TPI1	Triose phosphate isomerase	248	26.8	6.0	35	38	6		
		YJR009C	S000003769	TDH2	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 2	332	35.8	7.0	26	33	6		
		YGR254W	S000003486	ENO1	Enolase I, a phosphopyruvate hydratase	437	46.8	6.6	20	32	6		
		YKL152C	S000001635	GPM1	Tetrameric phosphoglycerate mutase	247	27.6	8.8	26	21	5		
		YAL038W	S000000036	CDC19	Pyruvate kinase	500	54.5	7.7	13	10	5		
		YBR196C	S000000400	PGI1	Phosphoglucose isomerase	554	61.3	6.5	10	6	4		
		YKL060C	S000001543	FBA1	Fructose 1,6-bisphosphate aldolase	359	39.6	5.8	13	5	3		
		Amino acid biosynthesis	Methionine biosynthesis	YER091C	S000000893	MET6	Cobalamin-independent methionine synthase	767	85.8	6.5	17	21	10
				YIL051C	S000001313	MMF1	Mitochondrial protein required for transamination of isoleucine	145	15.9	9.3	49	15	5
			Methionine and threonine biosynthesis	YDR158W	S000002565	HOM2	Aspartic beta semi-aldehyde dehydrogenase	365	39.5	6.7	19	13	5
			Glycine catabolic process	YAL044C	S000000042	GCV3	H subunit of the mitochondrial glycine decarboxylase complex	170	18.8	4.7	30	12	4
			Lysine biosynthesis	YIL094C	S000001356	LYS12	Homo-isocitrate dehydrogenase	371	40.0	8.0	15	7	4
			Histidine biosynthesis	YOR202W	S000005728	HIS3	Imidazoleglycerol-phosphate dehydratase	220	23.8	6.4	15	5	2
		TCA cycle	YKL085W	S000001568	MDH1	Mitochondrial malate dehydrogenase	334	35.6	8.5	10	3	2	
		Pentose phosphate pathway	YPR074C	S000006278	TKL1	Transketolase	680	73.8	7.0	4	3	2	
Alcoholic fermentation	YLR044C	S0000004034	PDC1	Pyruvate decarboxylase	563	61.5	6.2	33	36	13			
Fatty acid metabolism	YGR037C	S000003269	ACB1	Acyl-CoA-binding protein	87	10.1	4.9	59	7	3			
Protein binding	YAL005C	S000000004	SSA1	ATPase involved in protein folding and nuclear localization signal (NLS)-directed nuclear transport	642	69.6	5.1	5	5	3			
	YMR186W	S000004798	HSC82	Cytoplasmic chaperone of the Hsp90 family	705	80.8	4.8	4	8	2			
	YDR155C	S000002562	CPR1	Cytoplasmic peptidyl-prolyl cis-trans isomerase	162	17.4	7.4	17	6	2			
	YLL050C	S000003973	COF1	Cofilin (Actin binfing)	143	15.9	5.2	22	4	2			
	YBR109C	S000000313	CMD1	Calmodulin	147	16.1	4.3	21	3	2			
	YJL034W	S000003571	KAR2	ATPase involved in protein import into the ER	682	74.4	4.9	3	2	2			
	YML028W	S000004490	TSA1	Thioredoxin peroxidase	196	21.6	5.1	31	10	4			
Homeostasis	YLR043C	S0000004033	TRX1	Cytoplasmic thioredoxin isoenzyme	103	11.2	4.9	49	8	4			
	YGR209C	S000003441	TRX2	Cytoplasmic thioredoxin isoenzyme	104	11.2	4.9	49	8	4			
	YFL014W	S000001880	HSP12	Heat Shock Protein	109	11.7	5.4	23	4	3			
	YHR008C	S000001050	SOD2	Mitochondrial manganese superoxide dismutase	233	25.8	8.5	17	2	2			
	YJL138C	S000003674	TIF2	Translation initiation factor eIF-4A	395	44.7	5.1	10	8	3			
Translation	YEL034W	S000000760	HYP2	Translation elongation factor eIF-5A	157	17.1	5.0	19	12	2			
	YDR385W	S000002793	EFT2	Elongation factor 2	842	93.2	6.3	3	2	2			
	YDR382W	S000002790	RPP2B	Ribosomal protein P2 beta	110	11.0	4.1	24	2	2			
	YCL043C	S000000548	PDI1	Protein disulfide isomerase	522	58.2	4.5	27	28	10			
Signaling	YMR116C	S000004722	ASC1	G-protein beta subunit and guanine nucleotide dissociation inhibitor for Gpa2p	319	34.8	6.2	34	28	8			
Traffic	Endocytosis	YIL041W	S000001303	GVP36	BAR domain-containing protein	326	36.6	5.0	20	6	4		
Unknown	YOL154W	S000005514	ZPS1	Zinc- and pH-regulated surface protein	249	27.5	5.0	27	17	5			
	YDR519W	S000002927	FPR2	Membrane-bound peptidyl-prolyl cis-trans isomerase (PPIase).	135	14.5	5.5	34	8	3			
	YPL225W	S00000061	YPL225W	Unknown	146	17.4	5.3	16	4	2			

Table S2. Primers used in this research (continuing)

Plasmid	Oligo name	Description	Sequence (5'-3')
pUL1	GAP-Pro-F	Primer for amplification of MCS-tGAPDH (forward)	CTTAAACTTCTAAATCTACTTTTATAGTTAGTC
	KpnI-TGAP-R	Primer for amplification of MCS-tGAPDH (reverse)	ATGCTGGTACCTCAATCAATGAATGAAATGTCAATAAATAG
pUL-ATG-EGFP	EcATGXhOSCoM_F	Oligonucleotide for OSCoM (forward)	AATTCATGC
	EcATGXhOSCoM_R	Oligonucleotide for OSCoM (reverse)	TCGAGCATG
pRS423-ATG-EGFP	GAPDH promoter -F	Primer for amplification of pGAPDH-EGFP-FLAGtag-tGAPDH (forward)	GGTACGGGATTCAACAGTCTCACAGGAACACCAC
	GAPDH terminator -R	Primer for amplification of pGAPDH-EGFP-FLAGtag-tGAPDH (forward)	ATAAGAATCGGGCGCTCAATCAATGAATCGAAAATGTCAAT
pUL-ATG-DsRED	DsRed-EcoRIATGXho1 F	Primer for amplification of DsRED from pKRD4 (forward)	TAAACAGAAATTCATGCTCGAGGGTGGATCTGGTGCGACAACACCGA
	DsRed-Sal1 R	Primer for amplification of DsRED from pKRD4 (reverse)	GTTCAACCAAGTGCAGTACTAGTGGAGCGGAGTGGCGGGCCCTCG
	seqDsRED(27-34)	Primer for sequencing DsRED	CGAGTTCATGCGCTTCAAGG
pULGI2	pULSG1mcskaIF	Oligonucleotide for OSCoM (forward)	AATTCGGATCCCGGCTCGAGCGGAGATCTCGGGAGCTCCCGG
	pULSG2mcskaIR	Oligonucleotide for OSCoM (reverse)	TCGACCGGGAGCTCCCGAGATCTCCGCTCGAGCCGGGATCCG
pULGI2-ATG-HA	ATG-HAtagF (pULGI2)	Oligonucleotide for OSCoM (forward)	AATTCATGTACCCATACGATGTTCCAGATTACGGTG
	ATG-HAtagR (pULGI2)	Oligonucleotide for OSCoM (reverse)	GATCCAGGGTAACTCGGAACATCGTATGGGTACATG
pULGI2-ENO2/ pULGI2-HA-ENO2	pULIG-eno2 F (<i>Bam</i> H1)	Primer for amplification of ENO2 (forward)	GAATTCGGATCCATGGCTGTCTCAAAGTTTACGCT
	pULIG-eno2all R (<i>Sac</i> I)	Primer for amplification of ENO2 (reverse)	CGACCGGGAGCTCCAACCTGTCCACCGTGGTGGAAAT
	eno2-1 F	Primers for cloning ENO2 from genome and for sequencing	ATACCAAGTCAGCATACACC
	eno2-2 F		TTATCTTCTACCAGAGTTG
	eno2-3 F		AAGTCGAATTAACCCCGAA
	eno2-4 F		TCTTGTGTCTTTGGATGGT
	eno2-5 F		GTTCCGAAGTTTACCACAAC
	eno2-6 F		TTCAAGGACGGTAAAGTACGA
	eno2-7 F		ACTGGGAAGCTTGTCTCAC
	eno2-8 F		CTGGTGAACCTGAAGACACT
	eno2-9 F		TTTTCAAAGACTCGTCTGT
pULGI2-CDC19	CDC19- <i>Bam</i> H1 F	Primer for amplification of CDC19 (forward)	AACAGAATTCGGATCCATGTCTAGATTAGAAAGATTGACCTCATTA
	CDC19- <i>Xho</i> I R	Primer for amplification of CDC19 (reverse)	GAGATCTCCGCTCGAGAACGGTAGAGACTTCCAAAGTGTGGAGTG
	CDC19 (291-310) seqF	Primers for sequencing CDC19	CGATGTTGACTACCAATCC
	CDC19 (681-700) seqF		CTTGGTGAACAAGTAAGG
	CDC19 (1078-1097) seqF		GCTTACTTGCCAACTACGA
	CDC19 (1306-1325) seqF		TACAGAGGTGTCTCCATT
pULGI2-PYK2	PYK2- <i>Bam</i> H1 F	Primer for amplification of PYK2 (forward)	AACAGAATTCGGATCCATGCCAGATCCAGATTGCAGAGACTAGCT
	PYK2- <i>Xho</i> I R	Primer for amplification of PYK2 (reverse)	GAGATCTCCGCTCGAGGAATCTTGACCAACAGTAGAAATGCGTAA
	PYK2 (211-230) seqF	Primers for sequencing PYK2	AAATCGGAACAGCAATCC
	PYK2 (592-611) seqF		ATGAAGGACTTGAATTCGG
	PYK2 (993-1012) seqF		TATGCTTCTCGAGAACCGG
pULGI2-GPM1	GPM1- <i>Bam</i> H1 F	Primer for amplification of GPM1 (forward)	AACAGAATTCGGATCCATGCCAAAGTAGTTTGTAGTACACCGGT
	GPM1- <i>Xho</i> I R	Primer for amplification of GPM1 (reverse)	GAGATCTCCGCTCGAGTTCTTACCTTGGTGGCAACAGCAGCCGGC
	GPM1 (281-300) seqF	Primer for sequencing GPM1	AAGGTAAGGACAAGGCTGAA
pULGI2-TPI1	TPI1- <i>Bam</i> H1 F	Primer for amplification of TPI1 (forward)	AACAGAATTCGGATCCATGGTGAAGACTTCTTTGTGGTGGTAAC
	TPI1- <i>Xho</i> I R	Primer for amplification of TPI1 (reverse)	GAGATCTCCGCTCGAGTTCTTCAAGTGTATGATATCAACAATTC
	TPI1 (243-262) seqF	Primer for sequencing TPI1	CCAAATCAAGGATGTTGGTG
pULGI2-PGI1	PGI1- <i>Bam</i> H1 F	Primer for amplification of PGI1 (forward)	AACAGAATTCGGATCCATGCCAAATCACTTCACTCAACTTCAA
	PGI1- <i>Xho</i> I R	Primer for amplification of PGI1 (reverse)	AACAGAATTCGGATCCATGACTGTTACTACTCTTTTGTGAATGTT
	PGI1 (252-271) seqF	Primers for sequencing PGI1	TAACGTCACCGTGTGAGAG
pULGI2-PGK1	PGK1 (697-716) seqF		GCCAAGAAGTGGTCTTGTGTC
	PGI1 (1178-1197) seqF		CTACCAACGCTCAACACTCT
	PGK1- <i>Bam</i> H1 F	Primer for amplification of PGK1 (forward)	AACAGAATTCGGATCCATGCTTTATCTCAAAGTGTCTGTCCAA
	PGK1- <i>Xho</i> I R	Primer for amplification of PGK1 (reverse)	GAGATCTCCGCTCGAGTTCTTTCGGATAAGAACCAACACCTGG
	PGK1 (221-240) seqF	Primers for sequencing PGK1	AATACTCTTTGGCTCCAGTT
pULGI2-FBA1	PGK1 (510-529) seqF		TCACTCTTCTATGGTCGGTT
	PGK1 (848-867) seqF		ACTTCATCTGCTGATGCT
	FBA1- <i>Bam</i> H1 F	Primer for amplification of FBA1 (forward)	AACAGAATTCGGATCCATGGTGTGAACAAATCTTAAAGAGAAG
	FBA1- <i>Xho</i> I R	Primer for amplification of FBA1 (reverse)	GAGATCTCCGCTCGAGTAAAGTGTAGTGGTACGGAAAGTTTCCAA
pUL-eno(1-17)	FBA1 (296-315) seqF	Primers for sequencing FBA1	CAGCTTACGGTATCCAGTT
	FBA1 (627-646) seqF		TTTGACCCAACTCTCCCAA
	eno2(-17)_ <i>Xho</i> I R	Primers for cloning ENO2 fragments	CACCAGATCCACCCTCGAGGTTACCCGGGAGTGTAGACGGATCTAGC
pUL-eno(1-28)/ pULR-eno(1-28)	eno2(-28)_ <i>Xho</i> I R		CAGATCCACCCTCGAGCTTTTCGGTGGTTAATTCGACTT
pUL-eno(1-30)	eno2(-30)_ <i>Xho</i> I R		CACCAGATCCACCCTCGAGAACACCTTTTCGGTGGTTAATTCGACTTC
pUL-eno(1-50)	eno2(-50)_ <i>Xho</i> I R		CACCAGATCCACCCTCGAGTCTCAATTTCCAAAGCTTCTGT
pUL-eno(1-110)	eno2(-110)_ <i>Xho</i> I R		CACCAGATCCACCCTCGAGAGGTTAGCACCCAACTTGG
pUL-eno(1-169)	eno2(-169)_ <i>Xho</i> I R		AGATCCACCCTCGAGTCTTTCGAAAGCCAAAGCACC
GAPDH promoter	pGAP_F	Primer for sequencing	AGACGGTAGGATTGATTGTAATTCGT

Table S2. Primers used in this research (continued)

Plasmid	Oligo name	Description	Sequence (5'-3')
p413-ADH-TLG2	<i>Bam</i> H1-TLG2 F	Primer for amplification of TLG2 (forward)	AGAACTAGTGAATTCATGTTTAGAGATAGAACTAATTAT
	<i>Xho</i> 1-TLG2 R	Primer for amplification of TLG2 (reverse)	ACATGACTCGAGTCAAAGTAGGTCATCCAAAGCATCATTG
	ADH pro F	Primer for sequencing p413-ADH plasmid	ATGAGCAACGGTATACGG
	TLG2 (140-164) seqF	Primers for sequencing TLG2	ACGATATATCGGCCAGATTGACAGA
	TLG2 (444-466) seqF		GCAATTAAGTCGAGAAGAGCTGA
	TLG2 (786-807) seqF		AGAAGTGAGCACTATTTTCAGG
ΔTLG2 (genome)	TLG2 (coloP) F	Primers for checking the sizes of coding regions	GCAAGACAGGAAAGTCTCCAA
	TLG2 (coloP) R		GCAACAGGTCATATGAACGCT
<i>KanMX4</i> (gene)	<i>KanB</i> -R	Primers for checking genotypes	CTGCAGCAGGAGCCGTAAT
ΔSEC22 (genome)	SEC22 (coloP) F		AATTTTCAACCAAAGTGTGTACT
ΔGOS1 (genome)	GOS1 (coloP) F		AAAGATGCTTATGAAAAATCTCAGG
ΔPEP12 (genome)	PEP12 (coloP) F		TGCCTTAGCTGAAAATTGTATTTA
ΔVPS51 (genome)	VPS51 (coloP) F		AATCTACCCAGGCCCTAAAAGTATG
ΔBTN2 (genome)	BTN2 (coloP) F		CGAGAGTTGTATCCAGTTTCTTGT
ΔSNC2 (genome)	SNC2 (coloP) F		AGTGATCTTGGTCACATGATATACG
ΔSNX4 (genome)	SNX4 (coloP) F		AATAACAGTTCAAATCTATGCCG
ΔSSO1 (genome)	SSO1 (coloP) F		CACACTAACGACAAAAGCAGATATG
ΔSSO2 (genome)	SSO2 (coloP) F		TACCCATAAGAGAGCTGGAATATG
ΔATG1 (genome)	ATG1 (coloP) F		AAGTTAAGTACCAAGGCCATCTTT
ΔATG20 (genome)	ATG20 (coloP) F		ATAAGGCTTGGCTTATTGCTTACT
ΔATG17 (genome)	ATG17 (coloP) F		CTTGAATTATTATCTTCTCATCGC

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Table S3. List of genes and proteins used in this research.

Category	Accession	Name	Description
Organelle marker proteins	YGL008C	PMA1	Plasma membrane H ⁺ -ATPase, pumps protons out of the cell
	TDL116W	NUP84	Subunit of the nuclear pore complex (NPC)
	YKL029C	MAE1	Mitochondrial malic enzyme
	YLR330W	CHS5	Component of the exomer complex
	YLR025W	SNF7	One of four subunits of the endosomal sorting complex required for transport III (ESCRT-III)
	YGL225W	VRG4	Golgi GDP-mannose transporter; regulates Golgi function and glycosylation in Golgi
	YOL147C	PEX11	Peroxisomal membrane protein required for medium-chain fatty acid oxidation and peroxisome proliferation
	YLR208W	SEC13	Component of the Nup84 nuclear pore sub-complex, the Sec13p-Sec31p complex of the COPII vesicle coat, and the SEA (Seh1-associated) complex; required for vesicle formation in ER to Golgi transport and nuclear pore complex organization
SNAREs	YPL232W	SSO1	Plasma membrane t-SNARE involved in fusion of secretory vesicles at the plasma membrane and in vesicle fusion during sporulation
	YMR183C	SSO2	Plasma membrane t-SNARE involved in fusion of secretory vesicles at the plasma membrane
	YLR268W	SEC22	R-SNARE protein; assembles into SNARE complex with Bet1p, Bos1p and Sed5p; cycles between the ER and Golgi complex; involved in anterograde and retrograde transport between the ER and Golgi
	YOR327C	SNC2	Vesicle membrane receptor protein (v-SNARE) involved in the fusion between Golgi-derived secretory vesicles with the plasma membrane
	YOL018C	TLG2	Syntaxin-like t-SNARE that forms a complex with Tlg1p and Vti1p and mediates fusion of endosome-derived vesicles with the late Golgi
	YGR142W	BTN2	v-SNARE binding protein that facilitates specific protein retrieval from a late endosome to the Golgi
	YOR036W	PEP12	Target membrane receptor (t-SNARE) for vesicular intermediates traveling between the Golgi apparatus and the vacuole
	YKR020W	VPS51	Component of the GARP (Golgi-associated retrograde protein) complex, which is required for the recycling of proteins from endosomes to the late Golgi
	YHL031C	GOS1	v-SNARE protein involved in Golgi transport, homolog of the mammalian protein GOS-28/GS28
	YOR106W	VAM3	Syntaxin-like vacuolar t-SNARE that functions with Vam7p in vacuolar protein trafficking; mediates docking/fusion of late transport intermediates with the vacuole
Transport	YDR517W	GRH1	Acetylated, cis-golgi localized protein involved in ER to Golgi transport; homolog of human GRASP65; forms a complex with the coiled-coil protein Bug1p; mutants are compromised for the fusion of ER-derived vesicles with Golgi membranes
Autophagy-related proteins	YGL180W	ATG1	Protein ser/thr kinase required for vesicle formation in autophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway
	YBL078C	ATG8	Component of autophagosomes and Cvt vesicles; undergoes conjugation to phosphatidylethanolamine (PE)
	YPR049C	ATG11	Adapter protein for pexophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway
	YLR423C	ATG17	Scaffold protein responsible for phagophore assembly site organization; regulatory subunit of an autophagy-specific complex that includes Atg1p and Atg13p
	YDL113C	ATG20	Sorting nexin family member required for the cytoplasm-to-vacuole targeting (Cvt) pathway and for endosomal sorting
Cell wall protein	YDR077W	SED1	Major stress-induced structural GPI-cell wall glycoprotein in stationary-phase cells

Supporting References

56. **Yang CK, Ewis HE, Zhang X, Lu CD, Hu HJ, Pan Y, Abdelal AT, Tai PC.** 2011. Nonclassical protein secretion by *Bacillus subtilis* in the stationary phase is not due to cell lysis. *J. Bacteriol.* **20**: 5607-5615.

57. **Vizcaíno JA, Côté R, Reisinger F, Barsnes H, Foster JM, Rameseder J, Hermjakob H, Martens L.** 2010. The Proteomics Identifications database: 2010 update. *Nucleic Acids Res.* **38**: D736-742.

58. **Barsnes H, Vizcaíno JA, Eidhammer I, Martens L.** 2009. PRIDE Converter: making proteomics data-sharing easy. *Nat. Biotechnol.* **27**: 598-599.