

A Plasma Membrane E-MAP Reveals Links Between the Eisosome, Sphingolipid Metabolism and Endosomal Trafficking

Pablo S. Aguilar^{1,#}, Florian Fröhlich^{2,#}, Michael Rehman^{2,#}, Mike Shales^{3,#}, Igor Ulitsky⁴, Agustina Olivera-Couto¹, Hannes Braberg³, Ron Shamir⁴, Peter Walter⁵, Matthias Mann⁶, Christer S. Ejsing⁷, Nevan J. Krogan^{3*}, Tobias C. Walther^{2*}

1 Institut Pasteur de Montevideo, Uruguay

2 Max Planck Institute of Biochemistry, Organelle Architecture and Dynamics, Martinsried, Germany

3 Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA

4 The Blavatnik School of Computer Science, Tel Aviv University, Tel Aviv, Israel

5 Department of Biochemistry and Biophysics, University of California and Howard Hughes Medical Institute, San Francisco, CA, USA

6 Max Planck Institute of Biochemistry, Proteomics and Signal Transduction, Martinsried, Germany

7. University of Southern Denmark, Department of Biochemistry and Molecular Biology, Odense, Denmark

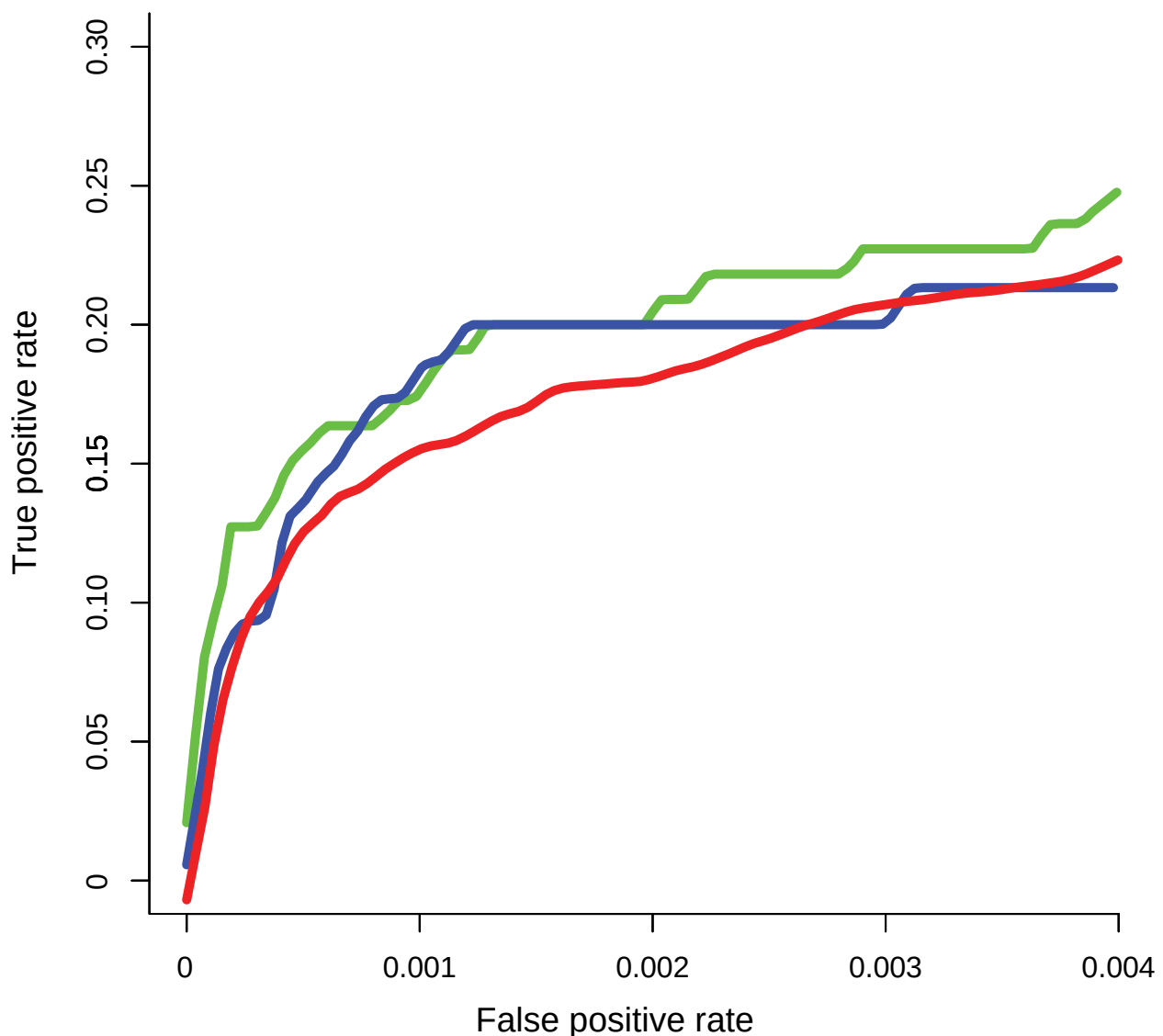
These authors, listed in alphabetical order, contributed equally

* Correspondence to:

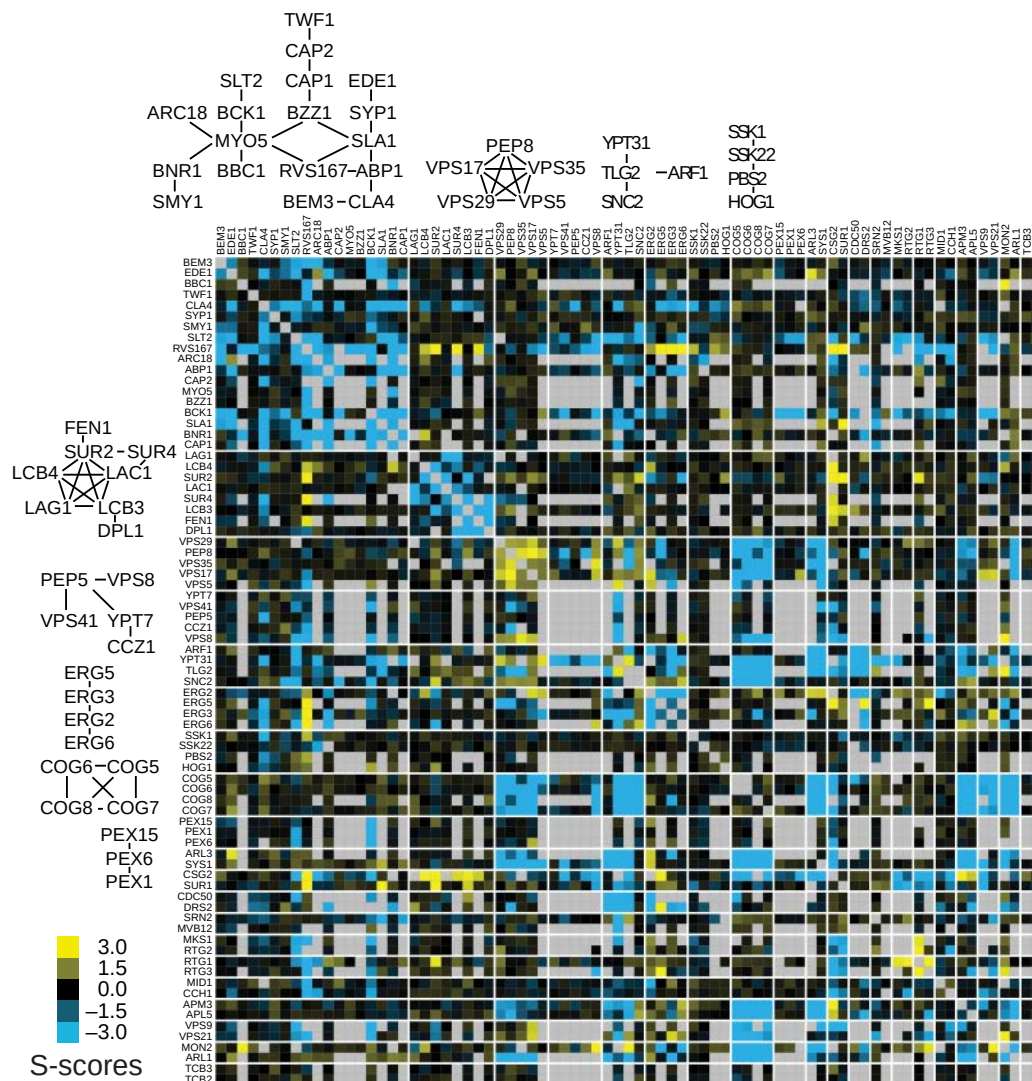
Nevan Krogan
Department of Cellular and Molecular Pharmacology
University of California, San Francisco
1700 4th Street, Beyers Hall 208D
San Francisco, CA 94158
USA
krogan@cmp.ucsf.edu

or

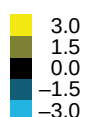
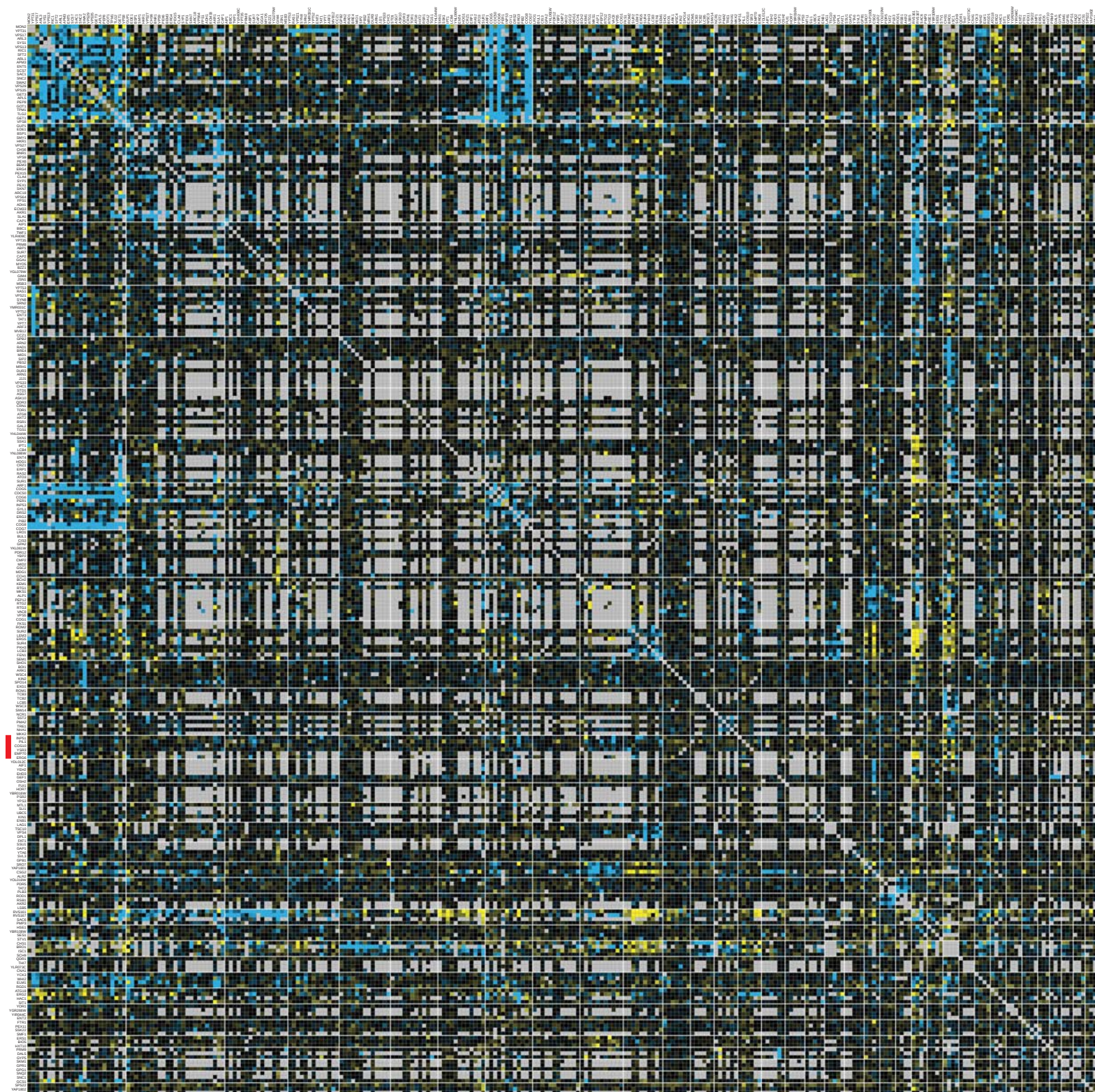
Tobias C. Walther
Max Planck Institute of Biochemistry
Organelle Dynamics and Architecture
Am Klopferspitz 18
82152 Martinsried/Munich
Germany
twalther@biochem.mpg.de



Supplementary Figure 1 - Receiver operating characteristic (ROC) curves gauging the power of pairwise genetic profile correlations to predict physical interactions between pairs of proteins. The predictive power of the plasma membrane E-MAP (blue) is comparable to that of previously published E-MAPs, focused on chromosome biology (¹, red) and the early secretory pathway (², green). The slope of the initial portion of the curve serves as a measure of the predictor's discriminatory power at its minimal false positive rate. Physical interaction definitions were collected from ³, imposing a threshold of PE score > 2.

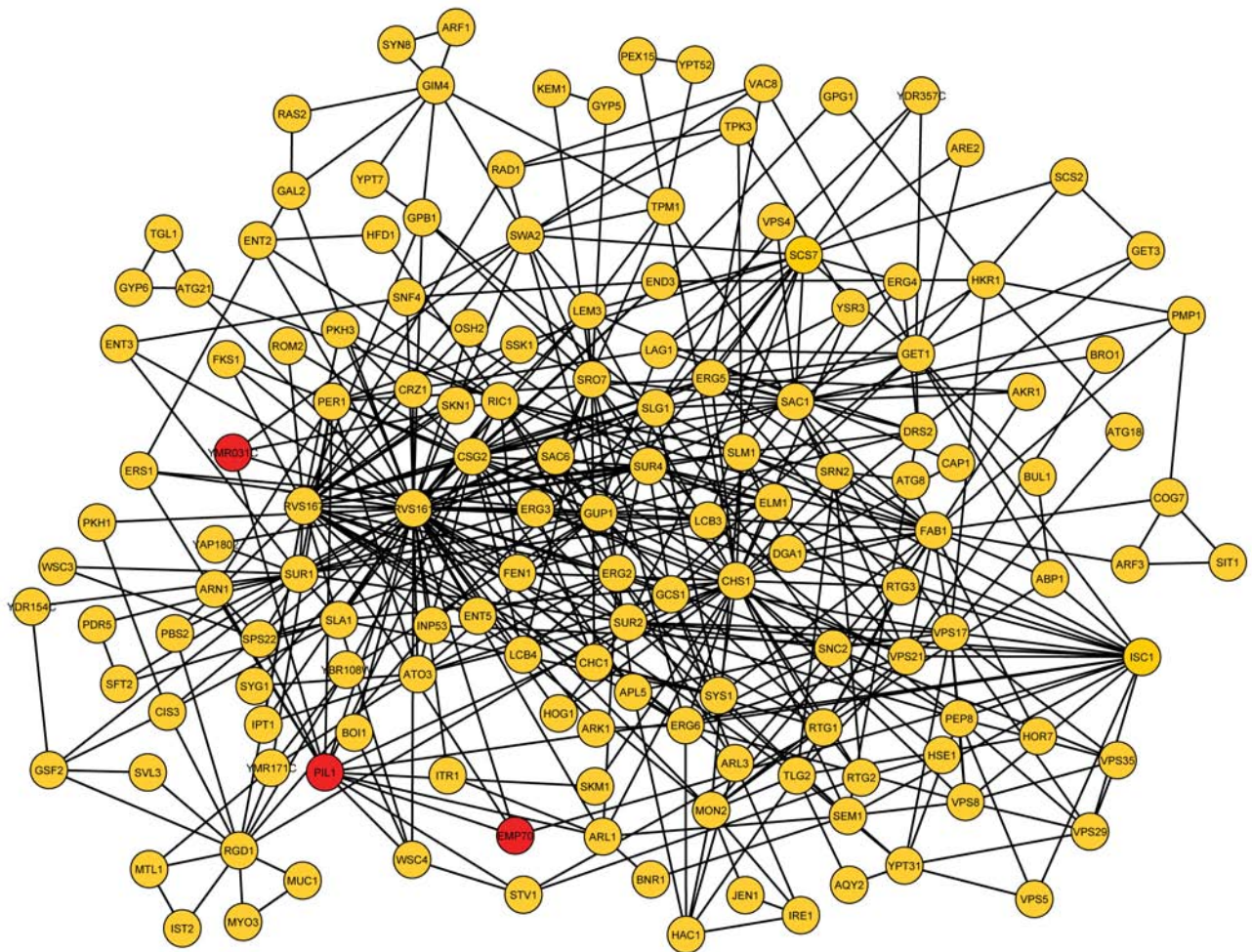


Supplementary Figure 2 - Functional modules identified using the plasma membrane E-MAP data and protein-protein interactions. Modules were identified as described in 4. White lines separate modules, which are ordered by size. Missing values are in grey. The protein-protein interaction sub-network is shown next to each module with at least three genes.

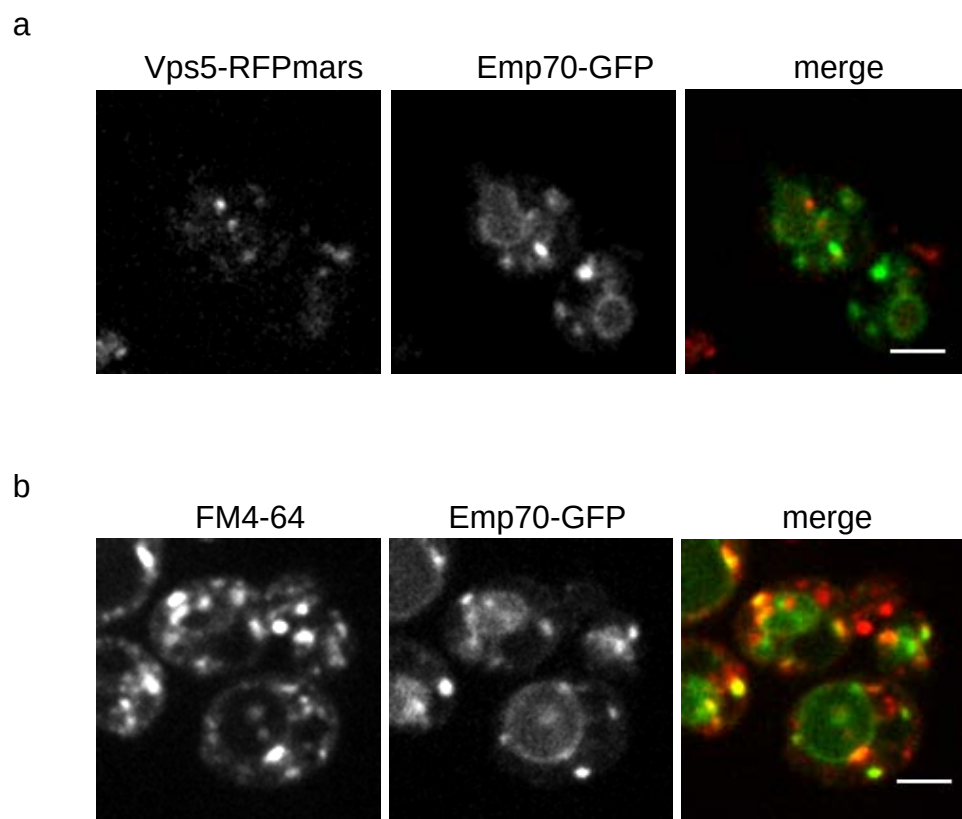


S-scores

Supplementary Figure 3 - Functional modules identified using the plasma membrane E-MAP data for pairs without PPIs. Modules were identified as described in ⁴, but without requiring PPI network connectivity. White lines separate modules, which are ordered by size. Missing values are in grey. The *PIL1-EMP70* module is marked by a red bar.



Supplementary Figure 4 - Map of type I TGMs in the plasma membrane E-MAP. Overview of all Type I TGMs found in the plasma membrane E-MAP. *PIL1*, *YMR031c* and *EMP70* are highlighted in red.



Supplementary Figure 5 - Emp70 localization in respect to endosomes. (a) Strains expressing Emp70-GFP and Vps5-RFPmars from the endogenous locus were imaged by confocal microscopy. Representative images are shown. (b) FM4-64 uptake experiment was performed at 16°C and representative confocal midsections are shown. Bar = 2.5 μ m.

Module #	Gene symbols	ORFs
1	CLA4,ABP1	YNL298W,YCR088W
2	EDE1,SYP1,SLA1	YBL047C,YCR030C,YBL007C
3	SLT2,BCK1	YHR030C,YJL095W
4	CAP1,CAP2	YKL007W,YIL034C
5	RTG3,RTG1	YBL103C,YOL067C
6	ENT3,GGA2,ENT5	YJR125C,YHR108W,YDR153C
7	PEX15,PEX6	YOL044W,YNL329C
8	GET1,GEF1,GET3	YGL020C,YJR040W,YDL100C
9	SYS1,ARL3	YJL004C,YPL051W
10	IMH1,MON2,ARL1	YLR309C,YNL297C,YBR164C
11	SNC2,TLG2	YOR327C,YOL018C
12	VPS17,VPS35,PEP8,VPS29	YOR132W,YJL154C,YJL053W,YHR012W
13	VPS41,APM3,APL5	YDR080W,YBR288C,YPL195W
14	COG8,COG7,COG5,COG6	YML071C,YGL005C,YNL051W,YNL041C
15	VPS21,VPS9	YOR089C,YML097C
16	HOG1,SSK22,SSK1,PBS2	YLR113W,YCR073C,YLR006C,YJL128C
17	TSC10,DPL1,SUR2,LAC1, LCB3,SUR4,LAG1	YBR265W,YDR294C,YDR297W,YKL008C, YJL134W,YLR372W,YHL003C
18	ERG5,ERG3,ERG2	YMR015C,YLR056W,YMR202W

Supplementary Table 3 - List of modules with PPIs found in the plasma membrane EMAP.

Module #	Gene symbols	ORFs
1	INP52,ERG3,ERG2	YNL106C,YLR056W,YMR202W
2	SRO77,FUI1,SHO1,BOI2,RGD1	YBL106C,YBL042C,YER118C,YER114C,YBR260C
3	MYO3,YCK3,YAP1801,TOR2	YKL129C,YER123W,YHR161C,YKL203C
4	GYP5,PRM9,DAL5	YPL249C,YAR031W,YJR152W
5	AIP1,BNR1,IST2,ECM33,YBR108W,CLA4,HSE1,ABP1,PMP3,MYO5	YMR092C,YIL159W,YBR086C,YBR078W,YBR108W,YNL298W,YHL002W,YCR088W,YDR276C,YMR109W
6	YOL019W,SRN2,INP54,FTH1,VPS4,ATG21,GUP1,VPS27	YOL019W,YLR119W,YOL065C,YBR207W,YPR173C,YPL100W,YGL084C,YNR006W
7	FIG4,LEM3	YNL325C,YNL323W
8	SPO14,SNC1	YKR031C,YAL030W
9	EDE1,SLT2,CAP1,BBC1,CHS6,ARC18,CAP2,SLA1,SMY1,MID2,BCK1	YBL047C,YHR030C,YKL007W,YJL020C,YJL099W,YLR370C,YIL034C,YBL007C,YKL079W,YLR332W,YJL095W
10	FPS1,VPS41,SIT1,RTG3,CCZ1,SAC6,RTG1,VAC8,MKS1,WHI2,SST2,MID1	YLL043W,YDR080W,YEL065W,YBL103C,YBR131W,YDL129C,YOL067C,YEL013W,YNL076W,YOR043W,YLR452C,YNL291C
11	DUR3,GGA2,SKM1,WSC4,WSC3,YPT53,ARN1,ARN2	YHL016C,YHR108W,YOL113W,YHL028W,YOL105C,YNL093W,YHL040C,YHL047C
12	CRN1,SRO7,YNL208W,SIP2,RAS2,YDC1	YLR429W,YPR032W,YNL208W,YGL208W,YNL098C,YPL087W
13	BCH1,YPT52,SYN8,RGT2	YMR237W,YKR014C,YAL014C,YDL138W
14	PEX15,BSP1,PEX6,RSR1	YOL044W,YPR171W,YNL329C,YGR152C
15	KIN2,JSN1,SSK22,YDR357C,HUA2,YNL086W,YPT35,PRM8	YLR096W,YJR091C,YCR073C,YDR357C,YOR284W,YNL086W,YHR105W,YGL053W
16	SFK1,BZZ1,SNQ2	YKL051W,YHR114W,YDR011W
17	YKL061W,OSH2,YDL012C,AIF1,GEF1,ALP1,SKN7,YEH2	YKL061W,YDL019C,YDL012C,YNR074C,YJR040W,YNL270C,YHR206W,YLR020C
18	VPS17,ENT3,GOT1,APM3,SAC1,VPS21,MON2,GET1,LCB5,CSG2,VPS29,GET3,VPS9,TLG2,SFT2,SNC2,VPS35,RCY1,SWA2,CUP5,PEP8,VPS8,TPM1,APL5	YOR132W,YJR125C,YMR292W,YBR288C,YKL212W,YOR089C,YNL297C,YGL020C,YLR260W,YBR036C,YHR012W,YDL100C,YML097C,YOL018C,YBL102W,YOR327C,YJL154C,YJL204C,YDR320C,YEL027W,YJL053W,YAL002W,YNL079C,YPL195W
19	HOG1,IMH1,SY51,ELM1,COG8,CCH1,IPT1,ENT5,ARL1,COG5,COG6,SUR1,ARL3,TCB2,PIB2,ATO3,COG7,INP53,GSC2,CRZ1	YLR113W,YLR309C,YJL004C,YKL048C,YML071C,YGR217W,YDR072C,YDR153C,YBR164C,YNL051W,YNL041C,YPL057C,YPL051W,YNL087W,YGL023C,YDR384C,YGL005C,YOR109W,YGR032W,YNL027W
20	YLR073C,SMF1,YPS3	YLR073C,YOL122C,YLR121C
21	TSC10,DPL1,SUR2,LCB3,SUR4,FAT1,LAG1	YBR265W,YDR294C,YDR297W,YJL134W,YLR372W,YBR041W,YHL003C
22	PMP1,ERS1,HXT10,NHA1,HSP30	YCR024C-A,YCR075C,YFL011W,YLR138W,YCR021C
23	HSP12,PSR1,SCP1	YFL014W,YLL010C,YOR367W
24	ROD1,RSB1,PEP12,STD1,AKR2	YOR018W,YOR049C,YOR036W,YOR047C,YOR034C
25	YPL150W,STV1,STL1,TWF1	YPL150W,YMR054W,YDR536W,YGR080W
26	YSR3,INP51,SUR7,PIL1,ERG6,EMP70	YKR053C,YIL002C,YML052W,YGR086C,YML008C,YLR083C
27	YMR086W,WSC2,LCB4,ERP1	YMR086W,YNL283C,YOR171C,YAR002C-A
28	MDG1,SKN1,YIR044C	YNL173C,YGR143W,YIR044C
29	GGA1,TOR1,GAP1,HOR7,SSK1	YDR358W,YJR066W,YKR039W,YMR251W-A,YLR006C

Supplementary Table 4 - List of modules without PPIs found in the plasma membrane E-MAP.

		PM E-MAP (this study)	ESP E-MAP (Schuldiner et al., 2005, ²)
E-MAP size (genes)		374	423
PPI-based modules	Number of modules	19	16
	Genes in module (% of the E-MAP)	14%	22.4%
	% of modules enriched with GO "biological process" set (FDR<0.01)	63%	79.4%
Modules identified without using PPIs	Number of modules	29	36
	Genes in module (% of the E-MAP)	72.4%	62.5%
	% of modules enriched with GO "biological process" set (FDR<0.01)	19.4%	23.2%
% of GO "biological process" sets enriched in at least one module		23.2%	23.0%

Supplementary Table 5 - Comparison of modules detected in the plasma membrane (PM) E-MAP and ESP E-MAP ².

Lipid lass (Normalized by levels in wild-type BY4742)	WT	<i>sur2Δ</i>	<i>sur4Δ</i>	<i>lcb3Δ</i>	<i>csg2Δ</i>	<i>rom2Δ</i>	<i>rom2Δ</i> <i>sur2Δ</i>	<i>rom2Δ</i> <i>lcb3Δ</i>	<i>rom2Δ</i> <i>csg2Δ</i>
LCB	1	65.78	270.22	16.60	0.97	100.40	409.41	14.07	261.34
Cer	1	0.84	0.49	0.80	0.81	0.30	0.20	1.09	0.32
IPC	1	0.19	0.96	0.77	2.48	0.64	0.10	0.86	1.03
MIPC	1	3.80	0.00	1.26	0.00	1.10	0.78	2.10	0.00
M(IP) ₂ C	1	1.81	0.16	0.63	0.04	0.67	0.81	1.06	0.03
PA	1	1.03	1.61	0.64	0.75	1.12	0.98	1.09	1.55
PS	1	0.88	0.26	0.89	0.76	0.75	0.64	0.80	0.38
PE	1	1.07	0.73	1.18	1.06	0.73	0.54	1.12	0.64
PC	1	0.80	0.71	0.74	0.85	0.66	0.60	0.81	0.69
PI	1	1.00	1.11	1.12	1.06	1.07	1.14	1.03	1.10
DAG	1	0.89	0.73	0.72	0.89	0.72	0.58	0.82	0.74
TAG	1	1.24	1.30	1.42	1.28	1.87	1.53	1.31	1.49
SE	1	0.84	3.06	0.84	1.43	1.11	1.89	1.18	1.96

Abbreviations: PC (phosphatidylcholine), PE (phosphatidylethanolamine), PA (phosphatidic acid), PS (phosphatidylserine), PI (phosphatidylinositol) DAG (diacylglycerol), TAG (triacylglycerol), SE (sterol ester) Cer (ceramide), LCB (long-chain base), IPC (inositolphosphoceramide), MIPC (mannosyl-inositolphosphoceramide), M(IP)₂C (mannosyl-diinositolphosphoceramide)

Supplementary Table 6 - Lipidomic data for *rom2Δ* and mutants in the sphingolipid synthesis pathway as shown in Figure 8b

Strain	Genotype	Reference
TWY1194	<i>Mat a KEX2-RFPmars::NAT^R, EMP70-GFP::KAN^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1044	<i>Mat a EMP70-GFP::KAN^R SNF7-RFPmars::NAT^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1063	<i>Mat a EMP70-GFP::KAN^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY871	<i>Mat a EMP70-GFP::KAN^R, LSP1-RFPmars::NAT^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1319	<i>Mat a YLR413w-RFPmars::NAT^R, EMP70-gfp::KAN^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1320	<i>Mat a YLR413w-RFPmars::NAT^R, EMP70-gfp::KAN^R, pil1Δ::KAN^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1195	<i>Mat a KEX2-GFP::KAN^R, emp70Δ::NAT^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY138	<i>Mat a ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1360	<i>Mat a emp70Δ::NAT^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1223	<i>Mat a vps1Δ::HPH^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1230	<i>Mat a tmn1Δ::NAT^R tmn2Δ::HPH^R tmn3Δ::HPH^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1183	<i>Mat a VPS5-RFPmars::NAT^R, EMP70-GFP::KAN^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
	<i>Mat a his3ΔI; leu2Δ0; met15Δ0; ura3Δ0; can1Δ::MATaPr-HIS3; lyp1Δ::MATαPr-LEU2</i>	Fiedler et al., 2009
TWY1770	<i>Mat a rom2Δ::KAN^R csg2Δ::NAT^R his3ΔI; leu2Δ0; met15Δ0; ura3Δ0; can1Δ::MATaPr-HIS3; lyp1Δ::MATαPr-LEU2</i>	This study
TWY1771	<i>Mat a rom2Δ::KAN^R sur2Δ::NAT^R his3ΔI; leu2Δ0; met15Δ0; ura3Δ0; can1Δ::MATaPr-HIS3; lyp1Δ::MATαPr-LEU2</i>	This study
TWY1772	<i>Mat a rom2Δ::KAN^R lcb3Δ::NAT^R his3ΔI; leu2Δ0; met15Δ0; ura3Δ0; can1Δ::MATaPr-HIS3; lyp1Δ::MATαPr-LEU2</i>	This study
	<i>Mat a lcb3Δ::KAN^R his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	Winzeler et al., 1999
	<i>Mat a sur4Δ::KAN^R his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	Winzeler et al., 1999
	<i>Mat a csg2Δ::KAN^R his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	Winzeler et al., 1999
	<i>Mat a rom2Δ::KAN^R his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	Winzeler et al., 1999
	<i>Mat a sur2Δ::KAN^R his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	Winzeler et al., 1999
TWY1122	<i>Mat a PIL1-TEV-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY70	<i>Mat a his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	Walther et al., 2006
TWY790	<i>Mat a pil1Δ::NAT^R LSP1-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY791	<i>Mat a pil1Δ::NAT^R YMR031C-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY726	<i>Mat a PIL1-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY787	<i>Mat a ymr031cΔ::KAN^R PIL1-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY788	<i>Mat a ymr031cΔ::KAN^R LSP1-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
	<i>Mat a ymr031cΔ::KAN^R his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	Winzeler et al., 1999
TWY743	<i>Mat a YMR031C-GFP::HIS PIL1-RFPmars::KAN^R his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY727	<i>Mat a YMR031C-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY786	<i>Mat a Lsp1-GFP::HIS his3ΔI; leu2Δ0; ura3Δ0; lys2Δ0</i>	This study
TWY637	<i>Mat a LSP1-TAP::KAN^R his3ΔI; leu2Δ0; ura3Δ0; met15Δ0</i>	This study
TWY642	<i>Mat a YMR031C-TAP::KAN^R his3ΔI; leu2Δ0; ura3Δ0; met15Δ0</i>	This study
TWY450	<i>Mat a pil1Δ :: KAN^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1184	<i>Mat a tmn2Δ :: HPH^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1182	<i>Mat a tmn3Δ :: HPH^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1207	<i>Mat a emp70Δ :: NAT^R, tmn3Δ :: HPH^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1206	<i>Mat a emp70Δ :: NAT^R, tmn2Δ :: HPH^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1814	<i>Mat a tmn2Δ :: HPH^R, tmn3Δ::NAT^R ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study
TWY1064	<i>Mat a EMP70-GFP :: KAN^R, LSP1-RFPcherry::HIS ura3-1 trp1-1 leu2 his3-11,15 ade2-1 can1-100</i>	This study

Supplementary Table 7- List of strains used in this study

Supplementary Material References

1. Collins, S.R. et al. Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature* **446**, 806-10 (2007).
2. Schuldiner, M. et al. Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. *Cell* **123**, 507-19 (2005).
3. Collins, S.R. et al. Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*. *Mol Cell Proteomics* **6**, 439-50 (2007).
4. Ulitsky, I., Shlomi, T., Kupiec, M. & Shamir, R. From E-MAPs to module maps: dissecting quantitative genetic interactions using physical interactions. *Mol Syst Biol* **4**, 209 (2008).