

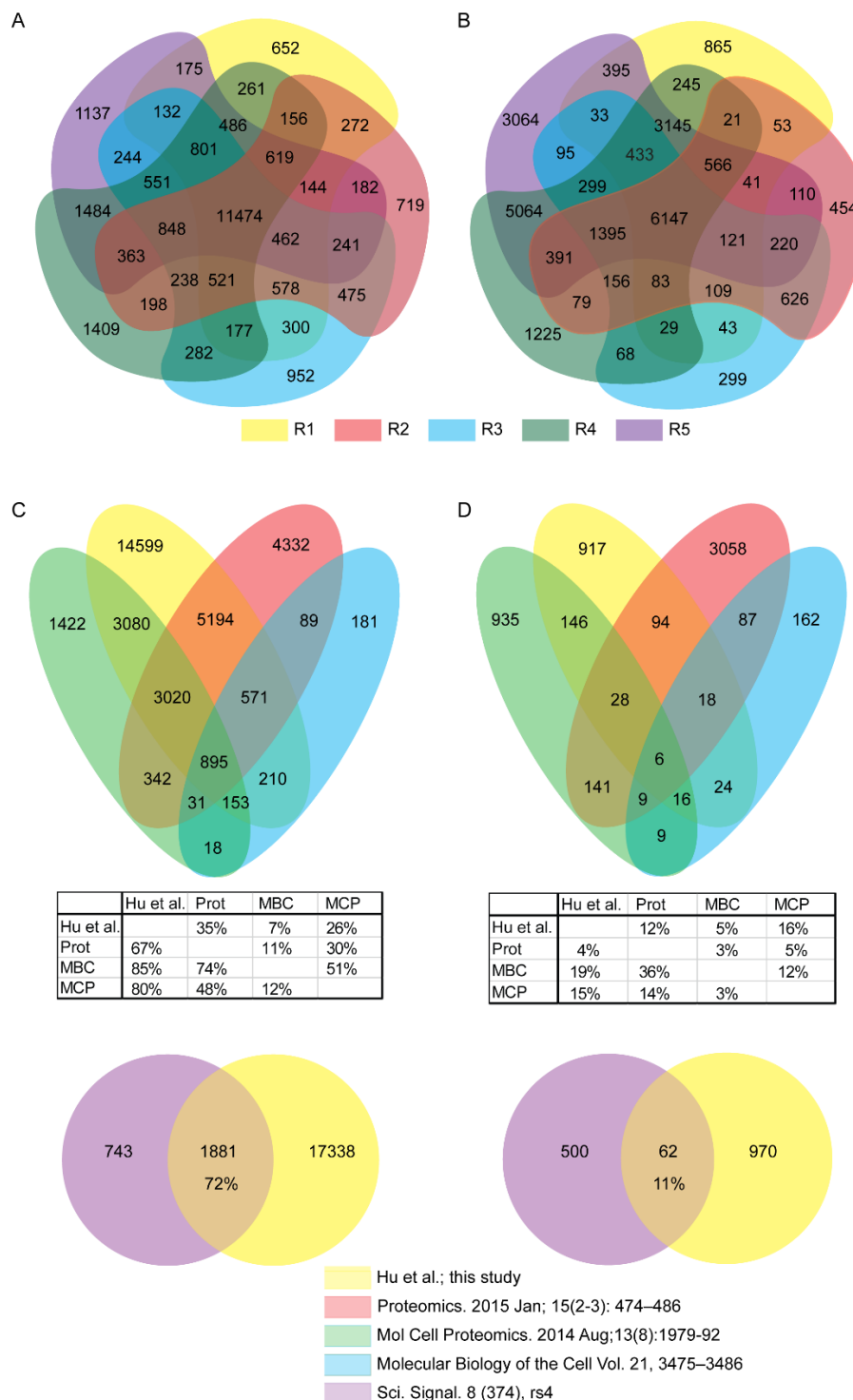
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## **Supplemental Information**

### **Multilayered Control of Protein**

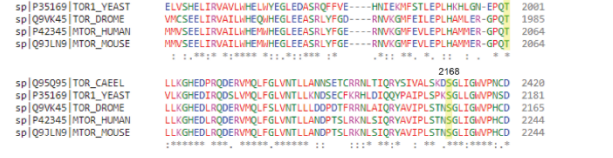
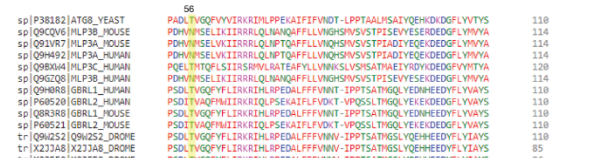
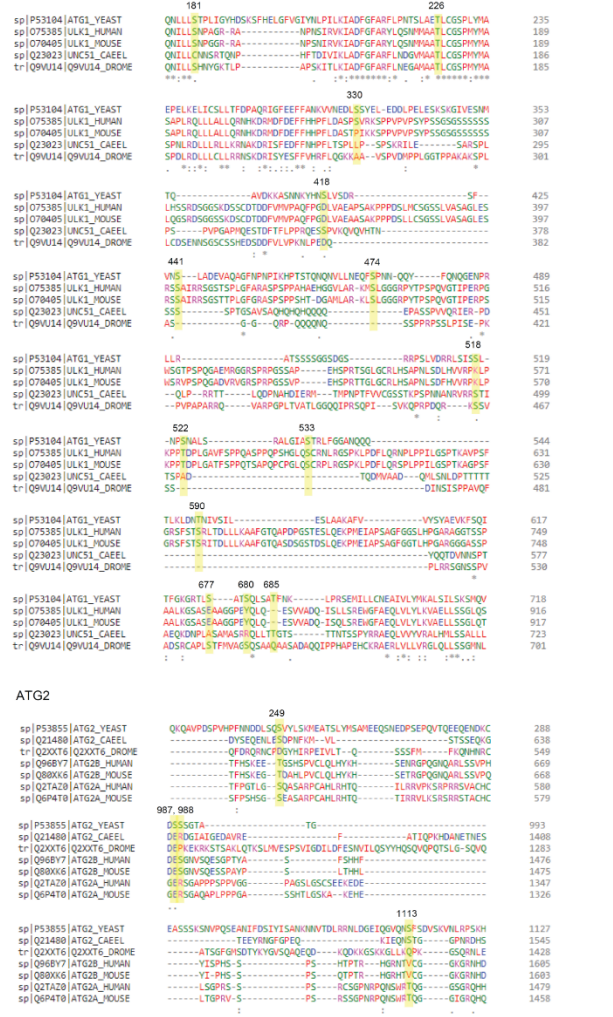
### **Turnover by TORC1 and Atg1**

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**Supplemental Figure S1: Reproducibility of phosphosite identifications and comparison with published datasets, related to Figures 1 and 2.** Overlap between phosphosite identifications of five biological replicates comparing **(A)** rapamycin-treated WT with non-treated WT cells, and **(B)** rapamycin-treated WT cells with rapamycin-treated and non-treated *atg1Δ* cells (see STAR Methods for labeling scheme). With respect to **(C)** quantified phosphosites and **(D)** rapamycin-sensitive, significantly regulated phosphosites, the current dataset enlarges the number of identified/quantified phosphosites the most while utilizing stringent criteria for defining rapamycin-sensitivity. Due to the output data the studies published in Proteomics, Molecular and Cellular Proteomics and Molecular Biology of the Cell were compared on phosphosite-level. The study published in Science Signaling could only be compared on phosphopeptide level, not taking site information into account.





**Supplemental Figure S3: Alignment of canonical Atg and TORC1 proteins, related to Figure 4.** Atg and TORC1 proteins from yeast *S. cerevisiae*, *C. elegans*, *D. melanogaster*, mouse and human origin were aligned using Clustal Omega (Madeira et al., 2019). Phosphosites that were identified in yeast proteins using *in vivo*- or *in vitro*-based screens and that were conserved in at least one additional species are highlighted (see supplemental Figure S3 for graphical representation).

**Supplemental Table S2: Published TORC1 and Atg1 target sites.** The table lists source proteins and respective amino acid residues being reported to be phosphorylated by TORC1 and Atg1, respectively. Sites highlighted in green were identified in the referenced and in the current study. Sites highlighted in red were exclusively found in the referenced study, and sites highlighted in blue were exclusively found in the current study. Black sites were found in the current study and the literature data was complex. \*: significant  $p < 0.05$ , but less than 2-fold regulated.

**Proximal TORC1 effectors:**

Protein	Residues	Ref.
Sch9	T711, T723, T737, S758, S765 [S163, S300, T721]	(Urban et al., 2007)
Sfp1	S181, S183*, S39, S170, T227, S228, T446 [S84, S209, S451]	(Lempiainen et al., 2009)
Gln3	[S223, S224, S252, S256, S257, S262, T275, S276, S288, S289, S400, S619, T641]	(Bertram et al., 2000)
Ypk3	S513 [S72, T82, S90, S94, S105, T107, S501, S519]	(Gonzalez et al., 2015; Yerlikaya et al., 2016)
Atg13	S437, S438, S646, S649*, S348, S496, S535, S541, [S355, S428, T441, S484, S554, S644]	(Kamada et al., 2010)
Lst4	S523, S547*, S550*, S483, S484, S497, S498, S525, S548, S549,	(Péli-Gulli et al., 2017)
Vps27	S155*, S157, T159, S274, S277, S279, S280	(Hatakeyama et al., 2019)

**(Predicted) proximal Atg1 effectors:**

Protein	Residues	Ref.
Aim43	S117	(Papinski et al., 2014)
Alg14	S85	(Papinski et al., 2014)
Aly1	S157	(Papinski et al., 2014)
Atg1	T226, S517	(Papinski et al., 2014; Yeh et al., 2010)
Atg2	S249 [S98, S1133]	(Papinski et al., 2014)
Atg4	S307	(Sanchez-Wandelmer et al., 2017)
Atg9	S802, S864, S969, S657 [S135]	(Feng et al., 2016; Papinski et al., 2014)
Atg29	S201, S197, S199 [S125, S106]	(Mao et al., 2013)
Cdc16	S734	(Papinski et al., 2014)
Cep3	S532	(Papinski et al., 2014)
Fig4	S257	(Papinski et al., 2014)
Fks3	S1632	(Papinski et al., 2014)
Gal10	S47	(Papinski et al., 2014)
Hsl7	S343	(Papinski et al., 2014)
Hxt1	S46	(Papinski et al., 2014)
Irc7	S109	(Papinski et al., 2014)
Msb2	S1266	(Papinski et al., 2014)
Msp1	S106	(Papinski et al., 2014)
Nde2	S499	(Papinski et al., 2014)
Pet130	S257	(Papinski et al., 2014)
Pig1	S446	(Papinski et al., 2014)
Plc1	S223	(Papinski et al., 2014)
Qdr3	S125	(Papinski et al., 2014)
Rpo41	S1322	(Papinski et al., 2014)
Sgv1	S144	(Papinski et al., 2014)
Snu114	S351	(Papinski et al., 2014)
Snu56	S160	(Papinski et al., 2014)
Sro7	S203	(Papinski et al., 2014)
Ubp12	S982	(Papinski et al., 2014)
Yel1	S155	(Papinski et al., 2014)

<i>YHR151C</i>	<b>S187</b>	(Papinski et al., 2014)
<i>YJR039W</i>	<b>S1057</b>	(Papinski et al., 2014)
<i>YPR084W</i>	<b>S203</b>	(Papinski et al., 2014)
<i>Zrc1</i>	<b>S331</b>	(Papinski et al., 2014)

#### Distal TORC1 effectors:

Protein	Rap	Branch	Residues	Ref.
Stb3	-	Sch9	<b>S254, S285, S286</b> [S146, S231, S234, S235, S241, T336, S337, T340, S341, S345, S348, S350, T370]	(Huber et al., 2009; Huber et al., 2011)
Dot6	-	Sch9	<b>S368*</b> , S247, S282, S313, S335, [S6, S253, S431, S432, S553, S580]	(Huber et al., 2009; Huber et al., 2011)
Tod6	-	Sch9	<b>S308*, S318*, S333*, S208, S280, S346 S203, S226, S300]</b>	(Huber et al., 2009; Huber et al., 2011)
Rim15	-	Sch9	<b>S1061</b> [S632*]	(Wanke et al., 2008)
Rim15	-	Tap42-PP2A	<b>T1075</b>	(Wanke et al., 2005)
Gcn2	-	Tap42-PP2A	<b>S577</b> [S569]	(Cherkasova and Hinnebusch, 2003)
Msn2	-	Tap42-PP2A	-	
Gln3	-	Tap42-PP2A/Sit4	S223, S224, S251, S256, S257, S262, T275, S276, S288, S289, S400, S619, S641	(Tate et al., 2018; Tate et al., 2019)
Rtg3	-	Tap42-PP2A?	S123, S230, S231, S239, S240, S241, S269	
Maf1	-	Sch9 or PKA	S90, S209, S210	(Huber et al., 2009; Huber et al., 2011; Lee et al., 2009)
Gis1	+	Igo1-PP2A	<b>S425</b>	(Bontron et al., 2013)
Sui2	+	Gcn2	<b>S52</b>	(Dever et al., 1992)
Igo1	+	Rim15	<b>S64</b> [S32*]	(Talarek et al., 2010)
Igo2	+	Rim15	<b>S63</b> [S106*]	(Talarek et al., 2010)
Npr1	+	Npr1	[S51, S125, S136, S137, S141, S169, S177, S251, T301, T305]	(Gander et al., 2008)
Orm1	+	Npr1	<b>S29, S32, S34, S35, S36</b>	(Breslow et al., 2010; Shimobayashi et al., 2013)
Orm2	+	Npr1	<b>S9, S15, T18, S22, S29, S31, T36</b>	(Breslow et al., 2010; Shimobayashi et al., 2013)
Par32	+	Npr1	<b>S34, S36, S39, S49, S206, S246, S249, S250, T253</b> [S134, S138, S141]	(Boeckstaens et al., 2015; Varlakhanova et al., 2018)
Art1	+	Npr1	<b>S79, S82</b>	(MacGurn et al., 2011)
Aly2	+	Npr1	[S639]	(O'Donnell et al., 2010)
Mep2	+	Npr1	<b>S457</b>	(Boeckstaens et al., 2014)
Sec7	+	Npr1	-	(MacGurn et al., 2011)
Trs120	+	Npr1	[S358*]	(MacGurn et al., 2011)
Vcx1	+	Npr1	-	(MacGurn et al., 2011)
Agp1	+	Npr1	-	(MacGurn et al., 2011)
Itr1	+	Npr1	-	(MacGurn et al., 2011)
Bem3	+	Npr1	-	(MacGurn et al., 2011)
Nus1	+	Npr1	-	(MacGurn et al., 2011)
Pib2	+	Npr1	[T56, S70, S82, S96, S97, S113, S118, S132, S135]	(MacGurn et al., 2011)
Tom22	+	Npr1	-	(MacGurn et al., 2011)
Ylr257W	+	Npr1	[S197*, S223*]	(MacGurn et al., 2011)

Ynt1	+	Npr1	S244, S246	(Martín et al., 2011)
Bcy1	+	Mpk1	T129	(Soulard et al., 2010)
Sic1	+	Mpk1	T173	(Moreno-Torres et al., 2015)

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**Supplemental Table S5. Strains and plasmids used in this study, related to STAR Methods.**

Strain	Genotype	Source	Figure
TB50a	<i>MATa; trp1, his3, ura3-52, leu2-3,112, rme1</i>	Schmelzle et al., 2004	
RL170-2C	[TB50a] <i>TCO89-TAP::TRP1</i>	Hatakeyama et al., 2019	4
BY4741	<i>MATa; his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	Euroscarf	4
Y14547	[BY4741] <i>atg1Δ::kanMX4</i>	Euroscarf	4
MP5102	[BY4741] <i>atg13Δ::kanMX4</i>	Euroscarf	4
Y258	<i>MATa; his4-580, leu2-3,112, ura3-52, pep4-3</i>	Zhu et al., 2001	4
SR5190	[Y258] pBG1805-GAL1-ATG2-TAP	Open Biosystems	4
SR5192	[Y258] pBG1805-GAL1-ATG3-TAP	Open Biosystems	4
SR5194	[Y258] pBG1805-GAL1-ATG5-TAP	Open Biosystems	4
SR5195	[Y258] pBG1805-GAL1-ATG6-TAP	Open Biosystems	4
SR5193	[Y258] pEGH-GAL1-GST-ATG4	Open Biosystems	4
SR5196	[Y258] pEGH-GAL1-GST-ATG7	Open Biosystems	4
SR5197	[Y258] pEGH-GAL1-GST-ATG8	Open Biosystems	4
SR5198	[Y258] pEGH-GAL1-GST-ATG9	Open Biosystems	4
SR5199	[Y258] pEGH-GAL1-GST-ATG10	Open Biosystems	4
SR5200	[Y258] pBG1805-GAL1-ATG11-TAP	Open Biosystems	4
SR5201	[Y258] pEGH-GAL1-GST-ATG12	Open Biosystems	4
SR5202	[Y258] pEGH-GAL1-GST-ATG13	Open Biosystems	4
SR5203	[Y258] pEGH-GAL1-GST-ATG14	Open Biosystems	4
SR5204	[Y258] pBG1805-GAL1-ATG15-TAP	Open Biosystems	4
SR5205	[Y258] pEGH-GAL1-GST-ATG16	Open Biosystems	4
SR5206	[Y258] pEGH-GAL1-GST-ATG17	Open Biosystems	4
SR5207	[Y258] pBG1805-GAL1-ATG18-TAP	Open Biosystems	4
SR5208	[Y258] pBG1805-GAL1-ATG19-TAP	Open Biosystems	4
SR5209	[Y258] pBG1805-GAL1-ATG20-TAP	Open Biosystems	4
SR5210	[Y258] pBG1805-GAL1-ATG21-TAP	Open Biosystems	4
SR5211	[Y258] pBG1805-GAL1-ATG22-TAP	Open Biosystems	4
SR5212	[Y258] pEGH-GAL1-GST-ATG23	Open Biosystems	4
SR5213	[Y258] pEGH-GAL1-GST-SNX4	Open Biosystems	4
SR5214	[Y258] pBG1805-GAL1-ATG26-TAP	Open Biosystems	4
SR5215	[Y258] pBG1805-GAL1-ATG27-TAP	Open Biosystems	4
SR5216	[Y258] pEGH-GAL1-GST-ATG29	Open Biosystems	4
SR5217	[Y258] pBG1805-GAL1-ATG31-TAP	Open Biosystems	4
SR5218	[Y258] pEGH-GAL1-GST-ATG32	Open Biosystems	4
SR5219	[Y258] pBG1805-GAL1-ATG33-TAP	Open Biosystems	4
SR5220	[Y258] pBG1805-GAL1-ATG34-TAP	Open Biosystems	4
SR5221	[Y258] pBG1805-GAL1-ATG36-TAP	Open Biosystems	4
SR5222	[Y258] pBG1805-GAL1-ATG38-TAP	Open Biosystems	4
SR5223	[Y258] pBG1805-GAL1-ATG39-TAP	Open Biosystems	4
SR5224	[Y258] pEGH-GAL1-GST-ATG40	Open Biosystems	4
SR5225	[Y258] pEGH-GAL1-GST-ATG41	Open Biosystems	4
TS139	[TB50a] <i>pho8Δ60</i>	Schmelzle et al., 2004	4
SR4934	[TS139] <i>atg29Δ::kanMX</i>	This study	4
SR4991	[BY4741] <i>arg4Δ::URA3, lys2Δ, ATG29-3HA-kanMX</i>	This study	4
MJ5682	[BY4741] <i>arg4Δ::His3-MX6, lys2Δ::HphNT1</i>	This study	4



MJ5691	[BY4741] <i>arg4Δ::His3-MX6, lys2Δ::HphMX, atg1Δ::kanMX</i>	This study	4
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Plasmid	Genotype	Source	Figure
p1613	[pRS316] <i>HA-ATG1</i>	Kawamata et al., 2008	4
p1614	[pRS316] <i>HA-atg1<sup>D211A</sup></i>	Kawamata et al., 2008	4
p3577	[pR316] <i>ATG13-2HA</i>	Yamamoto et al., 2016	4
p3632	[pRS416] <i>atg13<sup>S554A</sup>-3HA</i>	This study	4
p3425	[pRS416] <i>ATG29-3HA</i>	This study	4
p3473	[pRS416] <i>atg29<sup>3SA</sup>-3HA</i>	This study	4
p3504	[pRS416] <i>atg29<sup>T115A</sup>-3HA</i>	This study	4
p3541	[pRS416] <i>atg29<sup>T115E</sup>-3HA</i>	This study	4
pRS413	CEN, ARS, <i>HIS3</i>	Sikorski et al., 1989	4
pRS414	CEN, ARS, <i>TRP1</i>	Sikorski et al., 1989	4
pRS415	CEN, ARS, <i>LEU2</i>	Sikorski et al., 1989	4
pRS416	CEN, ARS, <i>URA3</i>	Sikorski et al., 1989	4