

Supplemental Materials

Molecular Biology of the Cell

Sugden et al.

SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL TABLE S1. Identified phosphorylation sites. Output table from MaxQuant listing the 3,667 phosphorylation sites identified.

SUPPLEMENTAL TABLE S2. Class I DIF-1 regulated phosphorylation sites. DIF-1 regulated phosphorylation sites observed at each of the four time points (1, 5, 8 and 15 min) in more than one biological replicate.

SUPPLEMENTAL TABLE S3. Class II DIF-1 regulated phosphorylation-sites. DIF-1 regulated phosphorylation sites observed in more than one biological replicate (3σ replicate filter cut off) at the same time point with an average 1.5-fold change.

SUPPLEMENTAL TABLE S4. GO term analysis of class II DIF-1 regulated phosphorylation-sites. GO term enrichment ($p \leq 0.05$) of class

SUPPLEMENTAL FIGURE S1. Examples of the assignments of phosphorylation site location from tandem mass spectra data. (A) EppA (DDB0233660) pS325. (B) PKBA (DDB0191195) pT435. (C) cAR1 (DDB0185024) pS324 and pS325. (D) ERK2 (DDB0191457) pY178.

SUPPLEMENTAL FIGURE S2. DIF-1 Regulated phosphorylation sites in Ca^{2+} /CaM related proteins. (A) Summary of Ca^{2+} /CaM related proteins with class I DIF-1 regulated phosphorylation sites. (B) Temporal profile for DIF-1 induced phosphorylation changes to class I phosphorylation sites in Ca^{2+} /CaM related proteins.

SUPPLEMENTAL FIGURE S3. DIF-1 Regulated phosphorylation sites in proteins upstream of PKB. (A) Summary of DIF-1 regulated phosphorylation sites in the TorC2 complex, which phosphorylates PKB; the Sca1C complex, which regulates RasC/RasG; and potential regulators of heterotrimeric G protein regulation. (B) Temporal profile for DIF-1 induced phosphorylation changes for proteins in the Sca1C complex. Solid lines represent averaged data for class I sites and dashed lines from class III sites from a single experiment. (C) Temporal profile for DIF-1 induced phosphorylation changes for class I sites in potential regulators of heterotrimeric G proteins. See Fig. 5 for graphical summary of the signalling pathways between cAR1 and PKB.

SUPPLEMENTAL FIGURE S4. DIF-1 regulated phosphorylation sites in myosin and its regulators. (A) Temporal profile for DIF-1 induced phosphorylation changes in class I sites on myosin heavy chains. (B) Summary of all DIF-1 regulated phosphorylation sites on myosin and its regulators.

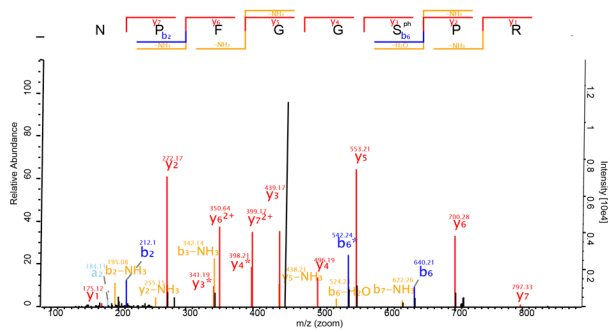
SUPPLEMENTAL FIGURE S5. Temporal profile for DIF-1 induced phosphorylation changes in proteins with multiple class I sites. (A) Nine class I sites on AbcF4 (DDB0232240). (B) Five class I sites on uncharacterized protein DDB0232240. (C) Three class I sites on protein kinase NdrB (DDB0219947). (D) Five class I sites on the polyketide synthase PKS16 (DDB0230068).

Supplemental Table S4

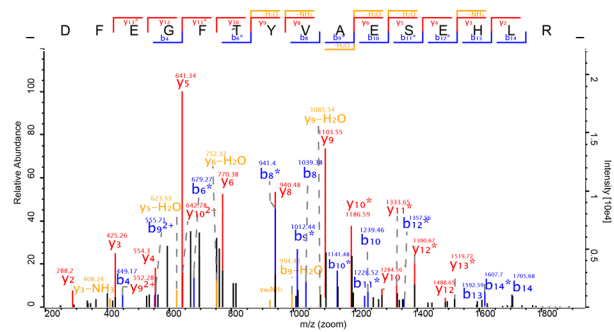
| GO Term enrichment at 1 min | P-value | Sample frequency n=105 | Background frequency n=1234 |
|---|----------|---------------------------|--------------------------------|
| GO Term Biological Process | | | |
| GO:0050896 response to stimulus | 8.62E-06 | 48.6% | 24.1% |
| GO:0044700 single organism signalling | 1.90E-03 | 32.4% | 15.1% |
| GO:0007165 signal transduction | 1.90E-03 | 32.4% | 15.1% |
| GO:0023052 signaling | 1.90E-03 | 32.4% | 15.1% |
| GO:0051716 cellular response to signalling | 3.45E-03 | 35.2% | 17.6% |
| GO:0007154 cell communication | 4.73E-03 | 32.4% | 15.6% |
| GO:0050789 regulation and biological process | 9.85E-03 | 44.8% | 26.1% |
| GO Term Molecular function | | | |
| GO:0030234 enzyme regulator activity | 1.96E-03 | 25.7% | 10.5% |
| GO:0060589 nucleoside-triphosphate regulator activity | 3.45E-03 | 23.8% | 9.5% |
| GO:0030695 GTPase regulator activity | 3.45E-03 | 23.8% | 9.5% |
| GO Term enrichment at 15 min | P-value | Sample frequency n=105 | Background frequency n=1234 |
| GO Term Biological Process | | | |
| GO:0044424 intracellular part | 2.49E-02 | 55.2% | 41.8% |
| GO:0005623 cell | 4.37E-02 | 60.7% | 47.6% |
| GO:0044464 cell part | 4.37E-02 | 60.7% | 47.6% |

Supplemental Figure S1

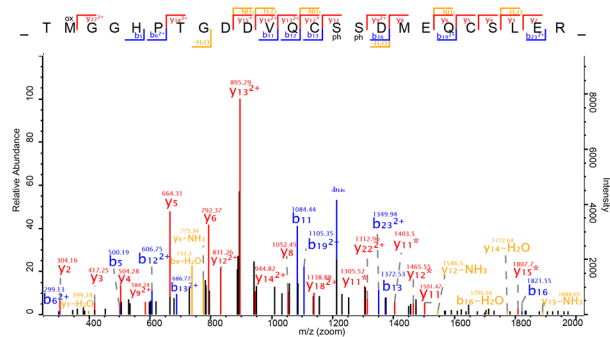
A



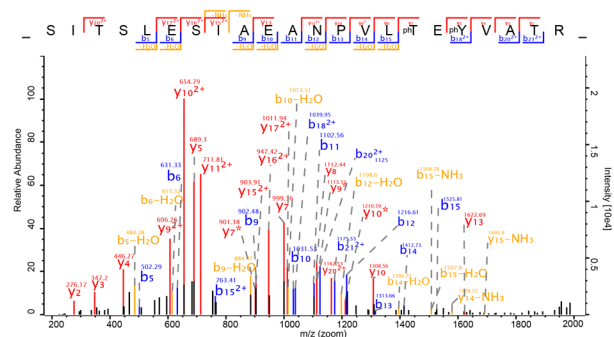
B



C



D



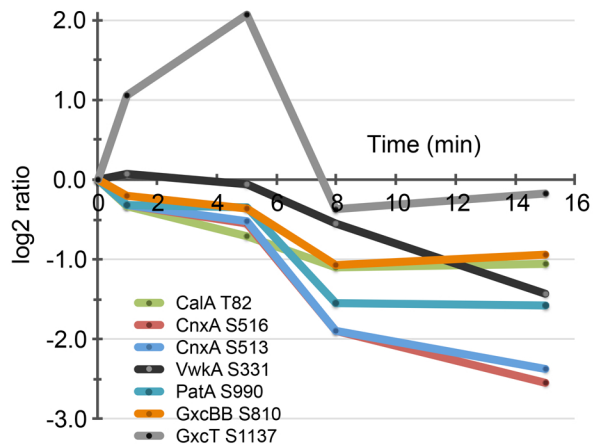
Supplemental Figure S2

A

| Protein | Annotation | Class I sites | Ca ²⁺ CaM binding? | dictyBase ID |
|---------|--|------------------------|-------------------------------------|--------------|
| CalA | Calmodulin | T82 | EF | DDB0214955 |
| CnxA | Calnexin | S516, S513 | - | DDB0215348 |
| PatA | Ca ²⁺ -ATPase | S990 | - | DDB0214945 |
| GxcBB | RhoGEF, PH, CH, IQ domains | S810 | IQ | DDB0233182 |
| GxcT | RhoGEF, PH, IQ, C2H2 zinc finger domains | S1137 | IQ | DDB0233444 |
| VwkA | Von Willebrand factor Kinase A | S331 | CaMBD | DDB0216405 |
| CanA | Calcineurin A, PP2B catalytic subunit | S534, S543, S550, S552 | CaMBD | DDB0185021 |

IQ: calcium independent calmodulin binding motif, CaMBD: calcium dependent CaM binding domain, EF: EF hand Ca²⁺ binding

B



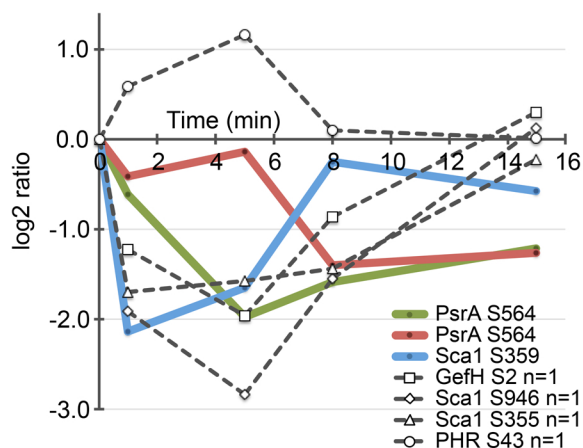
Supplemental Figure S3

A

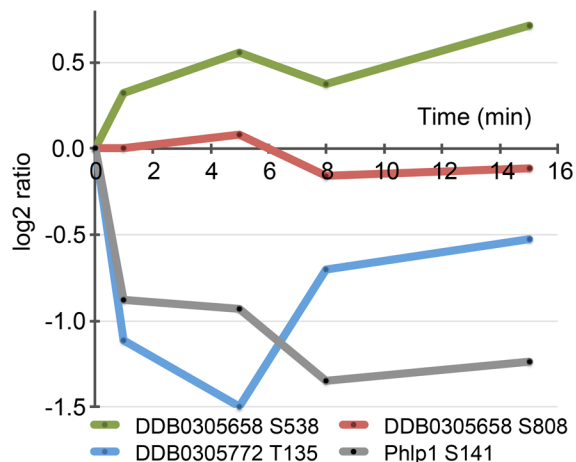
| | Protein | Annotation | No. Class I-IV sites | Class I sites | Other sites |
|----------------------|------------|---------------------------|----------------------|-------------------|--|
| TORC2 complex | Tor | protein kinase TORC | 5 | - | S2282 ^{II} , S254, S249, S245, S242 |
| | RipA | Ras Interacting Protein | 1 | - | S301 |
| Sca1 Complex | PHR | RAS and PH domain protein | 2 | - | S43 ^{III} , S46 |
| | Sca1 | Sca1/DG1105 | 9 | S263, S359 | S355 ^{III} , S946 ^{III} , S920, S403, S261, S259, S225 |
| | GefH | RAS GEF | 1 | - | S2 ^{III} |
| | PsrA | PP2A regulatory | 1 | S564 | - |
| G protein regulation | GpaB | G-protein alpha 2 | 1 | - | S113 |
| | GpgA | G-protein gamma | 1 | - | S4 |
| | Phlp1 | Phosducin-like protein | 1 | S141 | - |
| | DDB0305772 | G protein regulator, RGS | 1 | T135 | - |
| | DDB0305658 | RGS domain protein | 2 | S808, S538 | - |

RGS: Regulator of G protein signalling, ^{II} Class II sites, ^{III} Class III sites

B

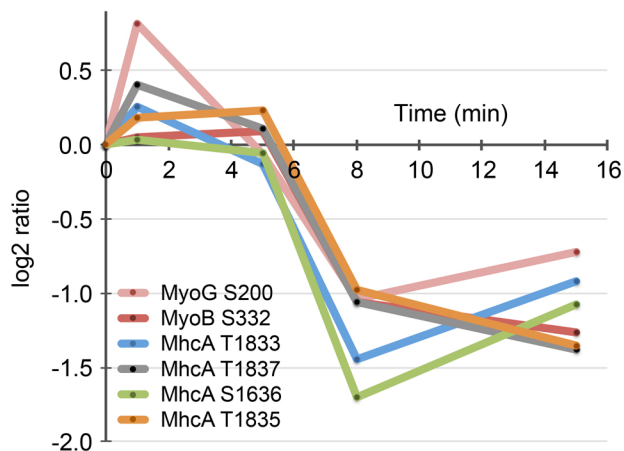


C



Supplemental Figure S4

A



B

| Protein | Annotation | No. Class I-IV sites | Class I Phosphorylation-sites | Other sites | dictyBase ID |
|-------------|--------------------------|----------------------|---|----------------------------|--------------|
| MyoB | myosin I HC | 2 | S332*† | T333 | DDB0191351 |
| MyoG | myosin (orphan) HC | 4 | S200 | S197, S1505, S1769 | DDB0232322 |
| MyoC | myosin I HC | 1 | - | T341† | DDB0215355 |
| MyoE | myosin I HC | 1 | - | S334† | DDB0216200 |
| MyoI | myosin (orphan) HC | 4 | - | T1824, T1829, T1845, T1879 | DDB0185049 |
| MhcA | myosin II HC | 7 | T1833*, T1835, T1837, S1636, S1637 | T2029*, T1823* | DDB0191444 |
| MlcR | myosin (II) LC | 2 | S13*, S14 | - | DDB0185146 |
| MhKB | myosin II HCK | 4 | S395 | T339, S246 | DDB0191333 |
| MhKC | myosin II HCK | 2 | S356, S330 | - | DDB0216199 |
| MhKD | myosin II HCK | 3 | S224 | S225, S234 | DDB0220109 |
| PakA | regulator of mhKs | 2 | S408, S315 | - | DDB0191313 |
| CalA | potential myosin LC | 2 | T82 | T84 | DDB0214955 |
| PakC | potential myosin I HCK | 3 | S177 | T364, S175 | DDB0267078 |
| PakH | potential myosin I HCK | 1 | S7 | - | DDB0229408 |
| PakB | myosin D HCK | 1 | - | T729 | DDB0191345 |
| PakE | potential myosin I HCK | 1 | - | S622 | DDB0229414 |
| GbpC | regulator of mlcR & mhcA | 1 | - | T2312 | DDB0191359 |

*previously identified as phosphorylation site in response to cAMP, † TEDS site phosphorylation on myosin I HC-Heavy Chain, HCK-HC Kinase, LC-Light Chain

Supplemental Figure S5

