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Glycosylation of Skp1 Promotes Formation of Skp1/Cullin-1/F-box Protein Complexes in *Dictyostelium*

M. Osman Sheikh, Yuechi Xu, Hanke van der Wel, Paul Walden, Steven D. Hartson, Christopher M. West

SUPPLEMENTAL FIGURES AND TABLE LEGENDS

Suppl. Fig. 1. **Interaction of CulE-FLAG with subunits of the COP9 signalosome and the 19S regulatory particle of the proteasome.** Strains whose *culE* locus was 3'-FLAG-tagged were immunoprecipitated with anti-FLAG, and the protein composition was analyzed by mass spectrometry. Total spectral counts associated with subunits of the COP9-signalosome (CSN) and the 19S-regulatory particle of the 26S-proteasome (PSM) are shown in panels *A* and *B*, respectively. Base and lid subparticle assignments are inferred from sequence-based annotations in dictyBase.org and ref. 33. Data are from single co-IP experiments, analyzed in triplicate, of growth stage (vegetative) and slug stage (developing) cells. Total spectral counts observed from the 3 analyses are shown here; average spectral counts (total divided by the number of subunits detected) are shown in Fig. 4.

Suppl. Fig. 2. **Sequence similarities between *Dictyostelium* CulE and CulA, and Cullin-1.** Alignments of human cullin protein sequences, that lie at the interface with substrate binding adaptors or subunits and differentiate mammalian cullins 1-5 (7), with corresponding cullins from *D. melanogaster*, *C. elegans*, and *S. cerevisiae*, are shown. Sequences are aligned using the 'framework' amino acids highlighted in yellow. Other conserved amino acid identities or similarities that differentiate cullin types are highlighted in different colors. The 5 predicted *Dictyostelium* cullins are assigned according to their best matches.

Suppl. Fig. 3. **Chaperone proteins interacting with FLAG-FbxD.** Hsp- and Cct/TriC-type chaperone proteins that were not detected in control co-IPs are grouped according to class, and plotted according to their abundance based on total or average spectral counts from Suppl. Table 4. Hsp20-like proteins included HspG-1, -2, -4, -5, -6, -7, -8, -9, and -12, HspL and HspM. Cct proteins included Tcp1 and Cct2-8. Protein genes are listed in Suppl. Table 1, annotations are according to www.dictyBase.org, and data are derived from Suppl. Table 4.

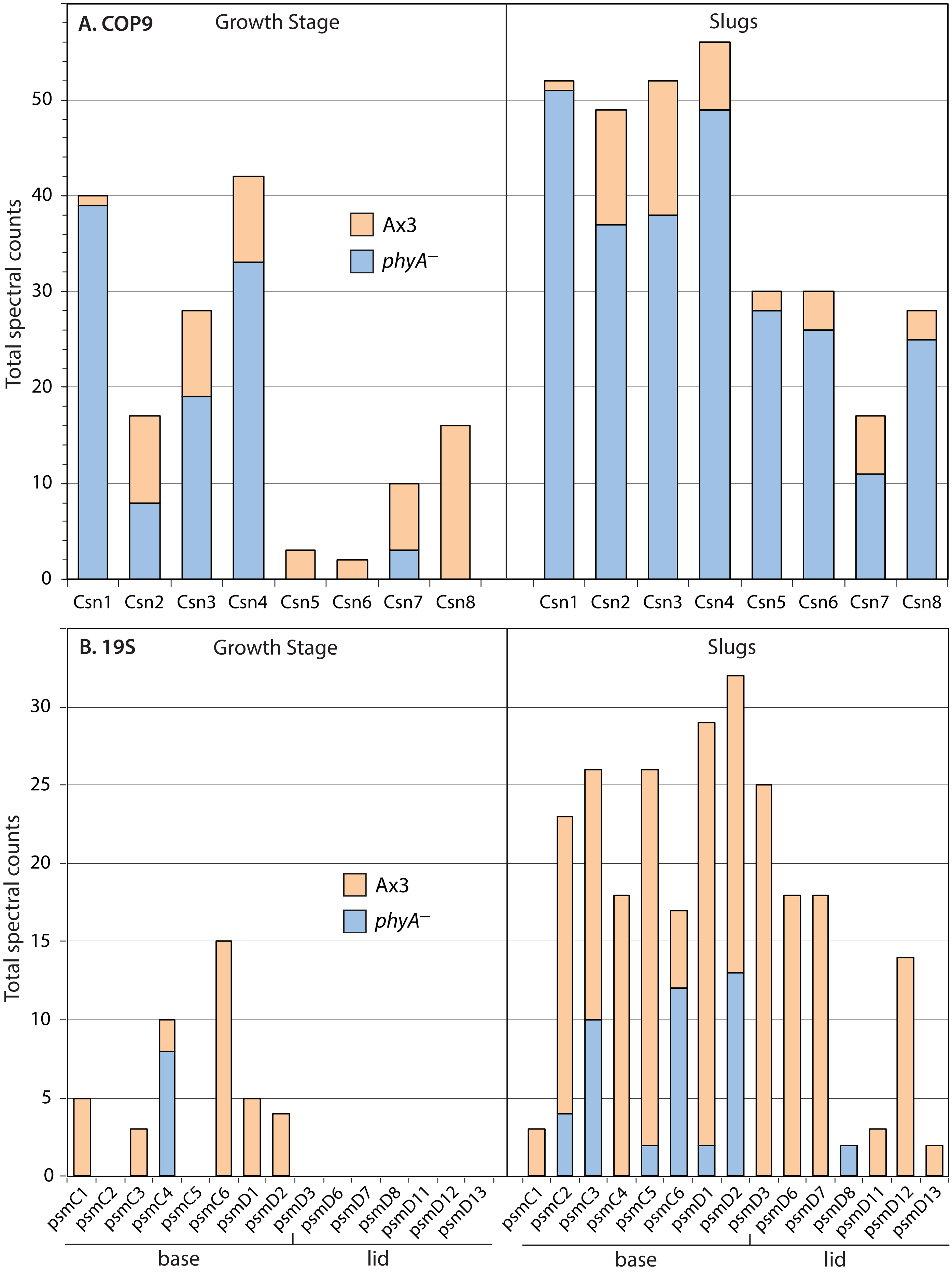
Suppl. Table 1. **Observed and predicted SCF-associated proteins in *D. discoideum*.** Proteins identified in the co-IPs shown in Figs. 4-6, listed by protein target. Gene and protein IDs from www.dictyBase.org and UniProt, predicted polypeptide M_r values, functional predictions, and expression levels during growth (0 h) and development (4-24 h), as inferred from RNAseq studies (35), are shown.

Suppl. Table 2. **Spectral counts report from CulE-FLAG co-IPs.** Summary of data exported from the Scaffold report of the two independent CulE-FLAG co-IPs. Columns in which the CulE-FLAG bait is targeted are shaded in gray; control co-IPs are unshaded. Technical replicates (3 each) reside within the same shading regime. Proteins and peptides (minimum of 2) were each identified at 1% FDR. Only proteins with ≥2 spectral counts for each of the 3 triplicate analyses in at least one condition are reported. See Fig. 4 and Suppl. Fig. 1 for summary after exclusions were applied.

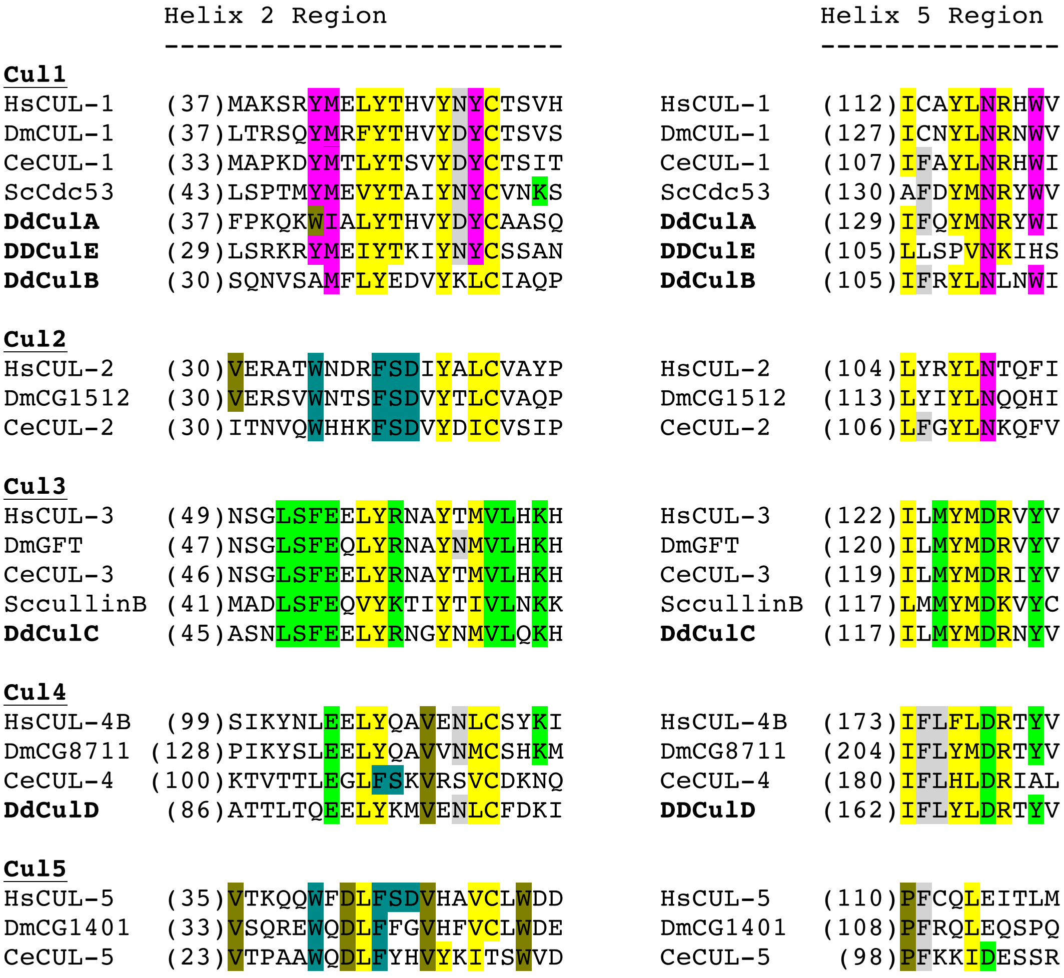
Suppl. Table 3. **Spectral counts report from Skp1 co-IPs.** Summary of data exported from the Scaffold report of the four independent Skp1 co-IPs. Columns in which Skp1 is targeted are shaded in gray; control co-IPs are unshaded. Technical replicates (3 each) reside within the same shading regime. Proteins and peptides (minimum of 2) were each identified at 1% FDR. Only proteins with ≥2 spectral counts for each of the 3 triplicate analyses for at least one condition are listed. See Fig. 5 for summary after exclusions were applied.

Suppl. Table 4. **Spectral counts report from FLAG-FbxD co-IPs.** Summary of data exported from the Scaffold report of the three independent FLAG-FbxD co-IPs. Columns in which the FLAG-FbxD bait is targeted are shaded in gray; those targeting mutant FLAG-FbxD(LP-AA) are in light green; control co-IPs are unshaded. Technical replicates (3 each) reside within the same shading regime. Proteins and peptides (minimum of 2) were each identified at 1% FDR. Only proteins with ≥2 spectral counts for each of the 3 triplicate analyses for at least one condition are shown. See Fig. 6 and Suppl. Fig. 3 for summary after exclusions were applied.

Supplemental Figure 1



Supplemental Figure 2



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              Helix 2 Region                              Helix 5 Region
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Cul1
HsCUL-1     (37)MAKSRYMELYTHVYNYCTSVH       HsCUL-1     (112)ICAYLNRHWV
DmCUL-1     (37)LTRSQYMRFYTHVYDYCTSVS       DmCUL-1     (127)ICNYLNRNWV
CeCUL-1     (33)MAPKDYMTLYTSVYDYCTSIT       CeCUL-1     (107)IFAYLNRHWI
ScCdc53     (43)LSPTMYMEVYTAIYNYCVNKS       ScCdc53     (130)AFDYMNRYWV
DdCulA      (37)FPKQKWIALYTHVYDYCAASQ       DdCulA      (129)IFQYMNRYWI
DDCulE      (29)LSRKRYMEIYTKIYNYCSSAN       DDCulE      (105)LLSPVNKIHS
DdCulB      (30)SQNVSAMFLYEDVYKLCIAQP       DdCulB      (105)IFRYLNLNWI

Cul2
HsCUL-2     (30)VERATWNDRFSDIYALCVAYP       HsCUL-2     (104)LYRYLNTQFI
DmCG1512    (30)VERSVWNTSFSDVYTLCVAQP       DmCG1512    (113)LYIYLNQQHI
CeCUL-2     (30)ITNVQWHHKFSDVYDICVSIP       CeCUL-2     (106)LFGYLNKQFV

Cul3
HsCUL-3     (49)NSGLSFEELYRNAYTMVLHKH       HsCUL-3     (122)ILMYMDRVYV
DmGFT       (47)NSGLSFEQLYRNAYNMVLHKH       DmGFT       (120)ILMYMDRVYV
CeCUL-3     (46)NSGLSFEELYRNAYTMVLHKH       CeCUL-3     (119)ILMYMDRIYV
SccullinB   (41)MADLSFEQVYKTIYTIVLNKK       SccullinB   (117)LMMYMDKVYC
DdCulC      (45)ASNLSFEELYRNGYNMVLQKH       DdCulC      (117)ILMYMDRNYV

Cul4
HsCUL-4B    (99)SIKYNLEELYQAVENLCSYKI       HsCUL-4B    (173)IFLFLDRTYV
DmCG8711   (128)PIKYSLEELYQAVVNMCSHKM       DmCG8711    (204)IFLYMDRTYV
CeCUL-4    (100)KTVTTLEGLFSKVRSVCDKNQ       CeCUL-4     (180)IFLHLDRIAL
DdCulD      (86)ATTLTQEELYKMVENLCFDKI       DDCulD      (162)IFLYLDRTYV

Cul5
HsCUL-5     (35)VTKQQWFDLFSDVHAVCLWDD       HsCUL-5     (110)PFCQLEITLM
DmCG1401    (33)VSQREWQDLFFGVHFVCLWDE       DmCG1401    (108)PFRQLEQSPQ
CeCUL-5     (23)VTPAAWQDLFYHVYKITSWVD       CeCUL-5      (98)PFKKIDESSR
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Supplemental Figure 3