SUPPLEMENTARY METHODS AND LEGENDS TO FIGURES AND MOVIES

SUPPLEMENTARY METHODS

MS/MS methodology and analyses

The samples were washed with acetone, resuspended in 50mM Hepes buffer, pH 7.2, reduced and alkylated using 2 mM Tris(2-carboxyethyl) phosphine (Fisher, AC36383) at 65°C for 30 minutes and 5 mM iodoacetamide (Fisher, AC12227) at 37°C in dark for 30 minutes, respectively. The proteins were digested with 1 ug trypsin (Roche, 03 708 969 001) at 37°C overnight.

Automated 2D nanoflow LC-MS/MS analysis was performed using LTQ tandem mass spectrometer (Thermo Electron Corporation, San Jose, CA) employing automated data-dependent acquisition. An Agilent 1100 HPLC system (Agilent Technologies, Wilmington, DE) was used to deliver a flow rate of 300 nL min^{-1} to the mass spectrometer through a splitter. Chromatographic separation was accomplished using a 3 phase capillary column. Using an in-house constructed pressure cell, 5um Zorbax SB-C18 (Agilent) packing material was packed into a fused silica capillary tubing (200µm ID, 360 um OD, 10 cm long) to form the first dimension RP column (RP1). A similar column (200µm ID, 5 cm long) packed with 5 um PolySulfoethyl (PolyLC) packing material was used as the SCX column. A zero dead volume 1µm filter (Upchurch, M548) was attached to the exit of each column for column packing and connecting. A fused silica capillary (200µm ID, 360 um OD, 20 cm long) packed with 5um Zorbax SB-C18 (Agilent) packing material was used as the analytical column (RP2). One end of the fused silica tubing was pulled to a sharp tip with the ID smaller than 1µm using a laser puller (Sutter P-2000) as the electro-spray tip. The peptide mixtures were loaded onto the RP1 column using the same in-house pressure cell. To avoid sample carry-over and keep good reproducibility, a new set of three columns with the same length was used for each sample. Peptides were first eluted from RP1 column to SCX column using a 0 to 80% acetonitrile gradient for 150 minutes. The the peptides were fractionated by the SCX column using a series of 6 step salt gradients (0mM, 20mM, 40mM, 60mM, 100mM, 1M ammonium acetate for 20 minutes), followed by high resolution reverse phase separation using an acetonitrile gradient of 0 to 80% for 120 minutes.

The mass spectrometer was operated in positive ion mode with a source temperature of 150°C and a spray voltage of 1500V. Data-dependent analysis and gas phase separation were employed. The full MS scan range of 300-2000 m/z was divided into 3 smaller scan ranges (300-800, 800-1100, 1100-2000Da) to improve the dynamic range. Each MS scan was followed by 4 MS/MS scans of the most intense ions from the parent MS scan. A dynamic exclusion of 1 minute was used to improve the duty cycle of MS/MS scans.

Raw data were extracted and searched using Spectrum Mill (Agilnet, version A.03.02.060b). MS/MS spectra with a sequence tag length of 1 or less were considered as poor spectra and discarded. The rest of the MS/MS spectra were searched against the protein sequences from dictyBase (Version: May 2007, 2005). A concatenated forward-reverse database is constructed to calculate the in-situ identification false discovery rates (FDR). The enzyme parameter was limited to full tryptic peptides with a maximum mis-cleavage of 1. All other search parameters were set to SpectrumMill's default settings (carbamidomethylation of cysteines, +/- 2.5 Da for precursor ions, +/- 0.7 Da for fragment ions, and a minimum matched peak intensity of 50%). Search results for individual spectra were first automatically validated using the filtering criteria listed in Supplemental Table 1. Due to the natural sequence redundancy in the protein database, the same peptide sometimes may appear in multiple protein sequences. In order to address this protein redundancy issue protein sequences containing the same set or subset of valid peptides were grouped together into protein groups. Using above criteria, a total of 169 protein groups from the forward database were identified, while 5 proteins (3% protein FDR) from the reverse database were identified. Proteins with 1 or 2 unique peptides were manually validated.

Supplemental Table 1. Filtering Criteria for autovalidation of database search results using SpectrumMill.
* SPI: percent scored peak.

mode	Protein score	1+ peptide (score, % SPI*)	2+ peptide (score, % SPI*)	3+ peptide (score, % SPI*)
Protein Details	>20	>9, >50%	>9, >50%	>11, >50%
Peptide	NA	>12, >50%	>13, >50%	>15, >50%

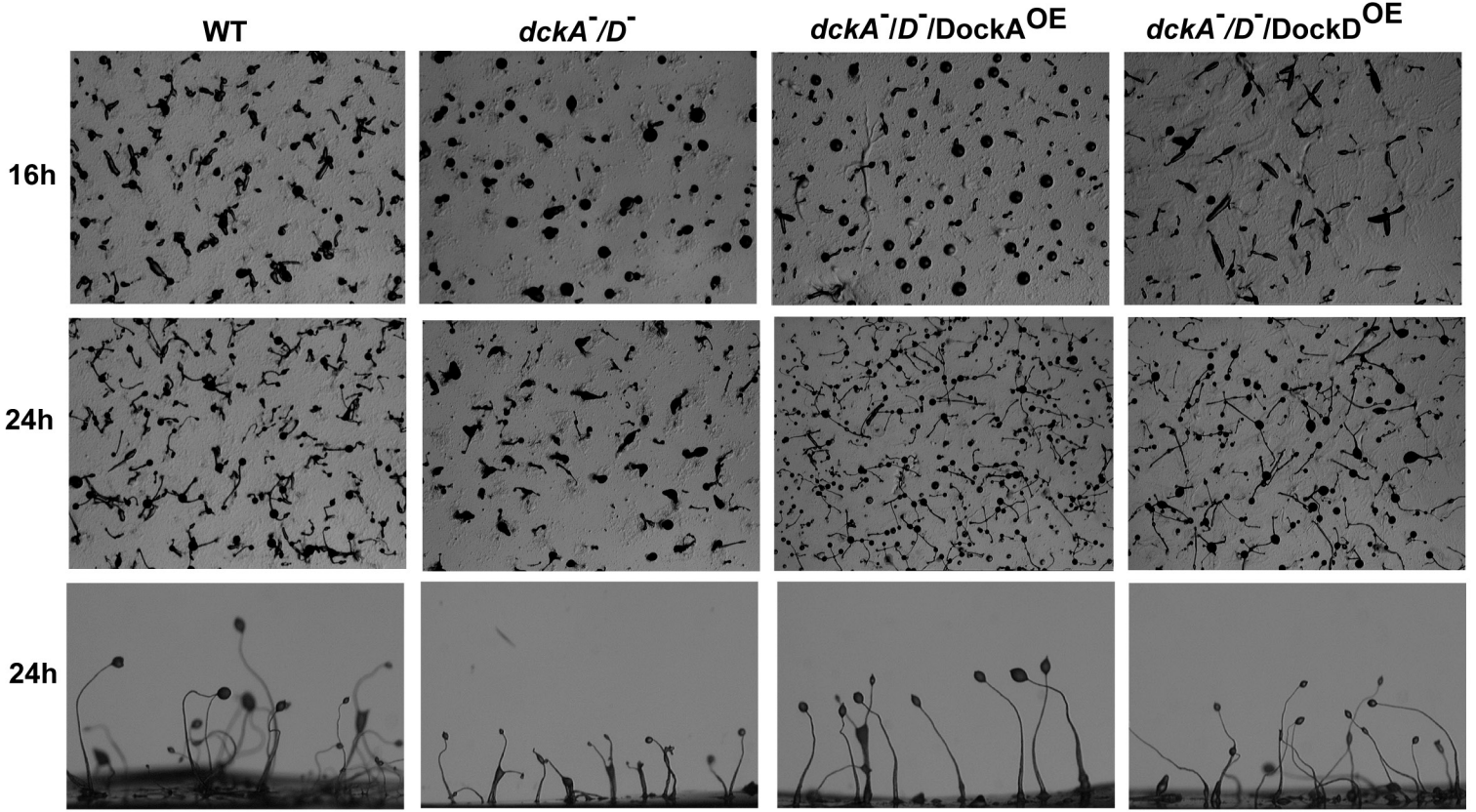
Figure S1. Developmental morphology for wild-type, *dckA⁻/D⁻*, *dckA⁻/D⁻*/GFP-DockD, and *dckA⁻/D⁻*/GFP-DockD cells. Cells were plated on 12 mM Na/K PO_4 containing agar.

Figure S2. Kinetics of F-actin polymerization in wild-type, *dckA⁻*, *dckD⁻*, and *dckA⁻/D⁻* cells in response to chemoattractant stimulation.

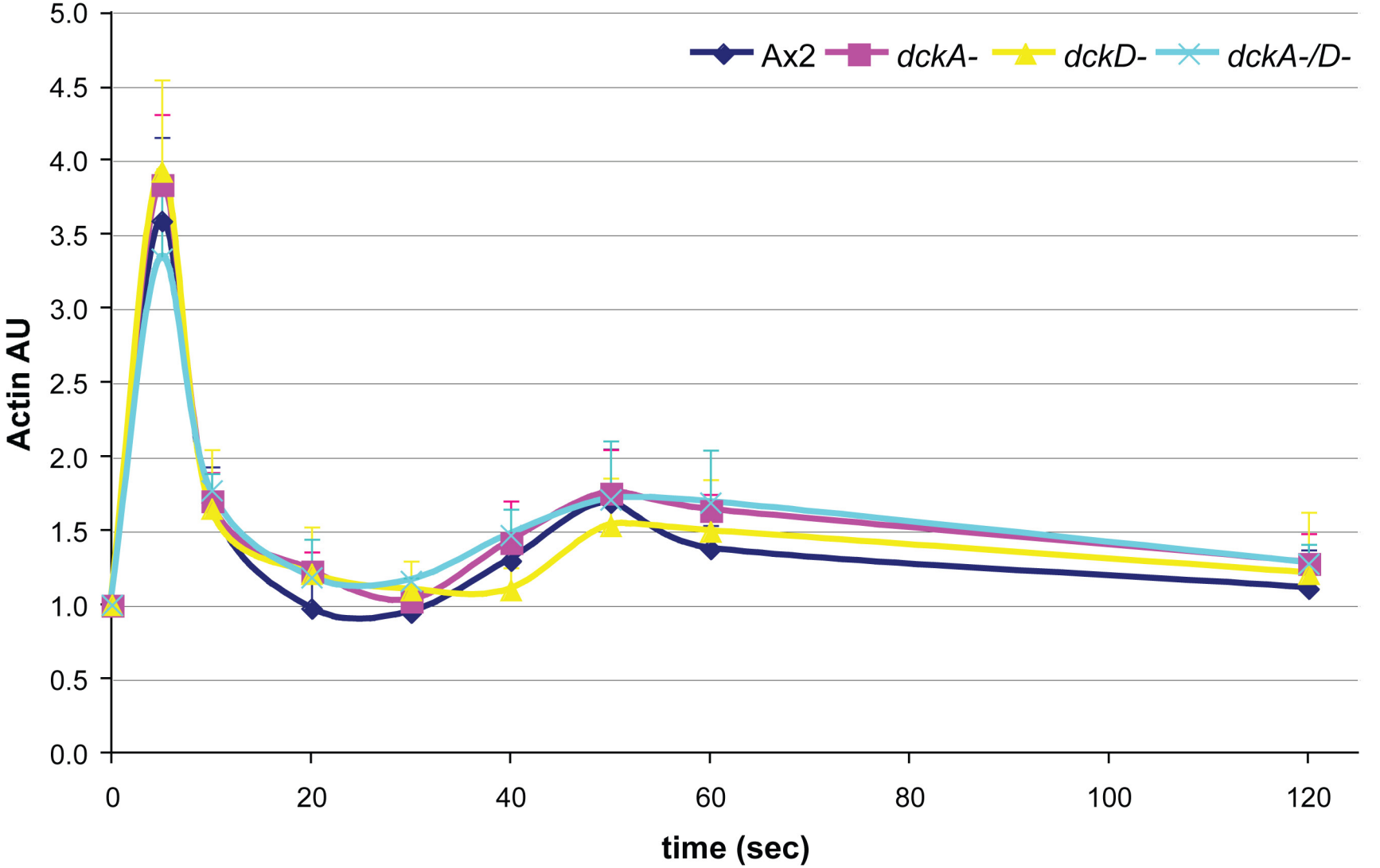
Figure S3. Domain organization of the *Homo sapiens* and *Dictyostelium discoideum* ELMO proteins. The domain composition of *Homo sapiens* ELMO1 is shown on top. Triangle: PH domain. *Dictyostelium discoideum* ELMO proteins are indicated by the corresponding dictyBase accession number on the left. White circles: asparagines rich domain; arrows: ankyrin repeats; white and light blue circles: glutamine/asparagine - rich domain.

Movies M1 and M2. Chemotaxis of $DockD^{OE}$ (M1) and wild-type (M2) cells labeled with GFP-ABP.

Movies M3 and M4. Global stimulation of wild-type cells expressing GFP-DockD (M3) and wild-type cells expressing the F-actin reporter GFP-limEΔCoil (M4).

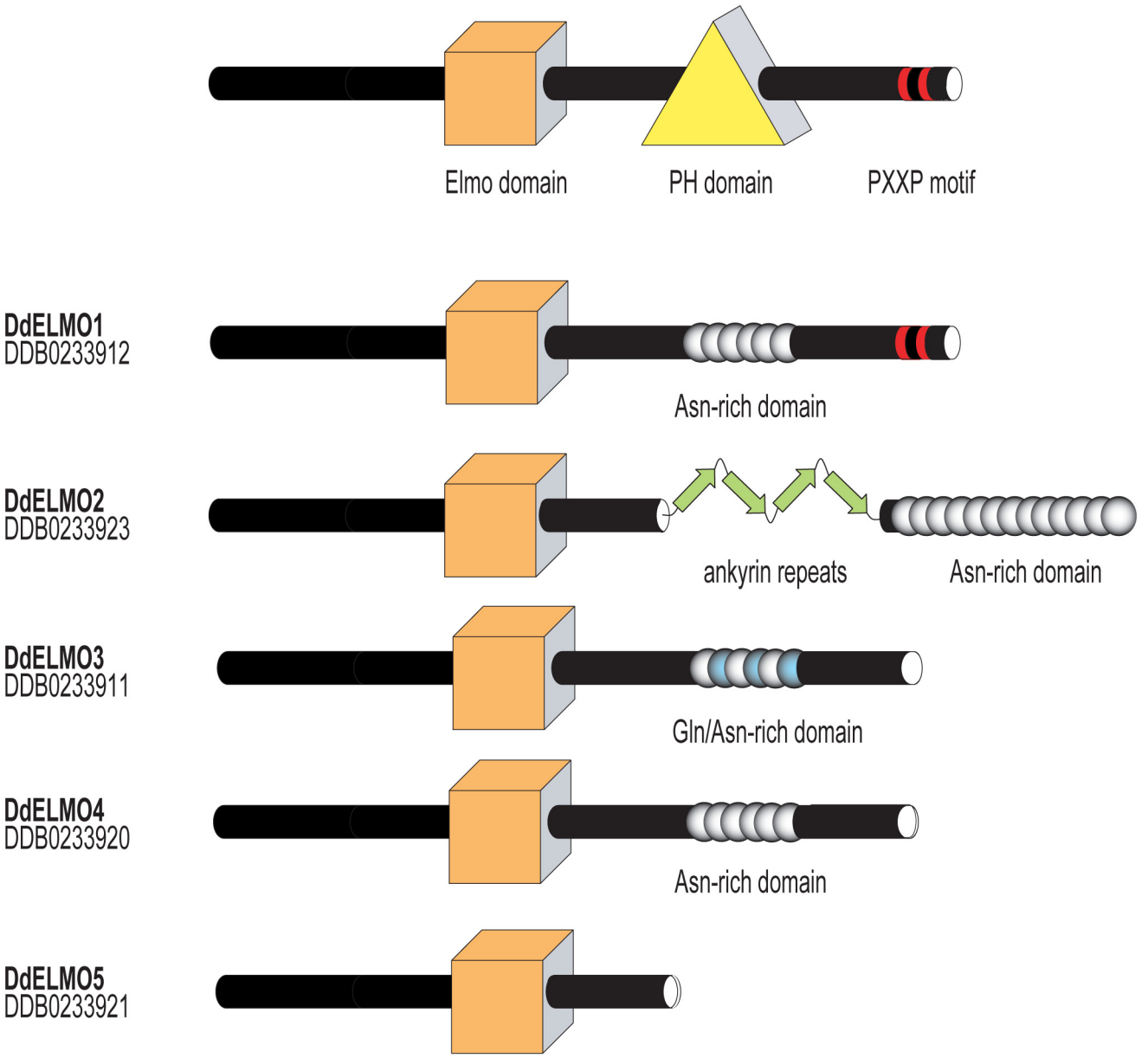


Para *et al*, Supplemental Figure S1



Para *et al*, Supplemental Figure S2

A



B

Elmo1 (human)	DMMSDLTRNDLDTLLSMEIKLRLLDLENIQI**P**DA**PPP**I**P**KE**P**SNYDFVYDCN
DdElmo1	GQEIKSQEGLDDYHSLLSINTSVKLLDLDGIDI**P**KET**P**QI**P**IL**P**DNFDFRTV
DdElmo2	QKKADSVVNVRRLLNESLCLVDLICSGPEKQKENVKKLNEALKSCLKNVKNF
DdElmo3	KSPSIKHLVTFFEVKSSELKDNSSTVSGSVNNNLNVGIPQLKREKSFKSDF
DdElmo4	QDQPSTIKVDKLISLFGEKVGFSLKRKESQRDLQHQASSSNLKFSLASSSK
DdElmo5	QFNELYSLVFISFDRFYQSKKPKSIMEFNTIKKEFETKISQNSDLVQLLRN

Para *et al*, Supplemental Figure S3