

A Quantitative Model for BicD2/Cargo Interactions

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES

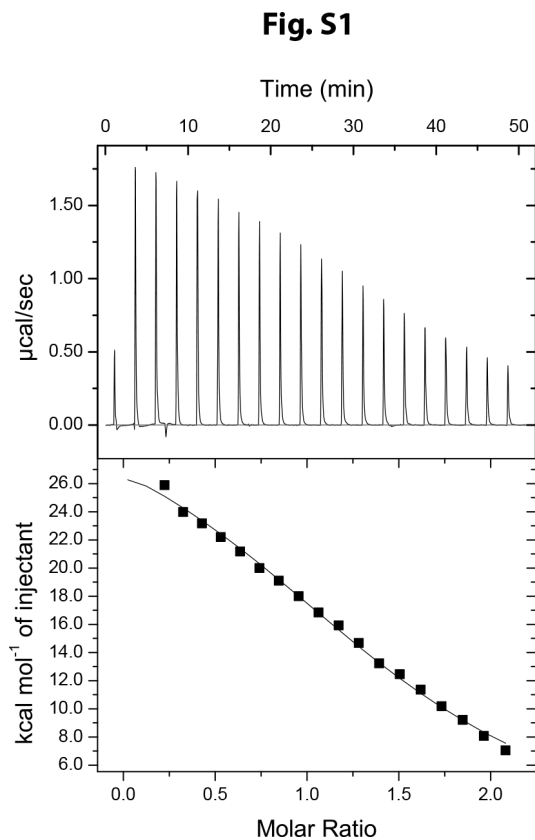


Fig. S1 The affinity between $\text{Rab6}^{\text{GTP}}_{\text{min}}$ and BicD2-CTD was determined by ITC to $12 \pm 1.2 \mu\text{M}$. The ITC titration curve of BicD2-CTD with $\text{Rab6}^{\text{GTP}}_{\text{min}}$ is shown. It should be noted that the titration of the truncated $\text{Rab6}^{\text{GTP}}_{\text{min}}$ into buffer was exothermic, whereas it was endothermic for the full-length protein.

SUPPLEMENTARY TABLES

Table S1 Protein concentrations used for ITC experiments

Nup358-RBD2	88 μ M	BicD2-CTD	8.8 μ M
Nup358-RBD2/RanGTP	150 μ M	BicD2-CTD	15 μ M
Rab6 ^{GTP}	300 μ M	BicD2-CTD	3 μ M
Rab6 ^{GTP_{min}}	300 μ M	BicD2-CTD	3 μ M

Table S2 Published posttranslational modifications in interacting domains of human Nup358, Rab6 and BicD2

Protein	Nup358-RBD2	BicD2-CTD	Rab6
Residues included	2006 - 2443	715 – 804	13 – 174
Phosphorylated residues (Cdk1 sites in bold)	2008 2153 2246 2251 2270 2276 2280 2290 2293 2297	None found	None found ^a
Sumoylated residue	2022		

The Swiss-Prot database (1) was used to identify published posttranslational modification sites (2-6). Non-physiological modifications were excluded. Note that most of the listed phosphorylation sites are specific for G2 phase or mitosis; Cdk1-specific phosphorylation sites are highlighted in bold. It was shown that these Cdk1-specific phosphorylation sites increase binding of Nup358 to BicD2 in G2 phase (4). However, the cell cycle stage in which phosphorylation sites identified in refs (2,3) appeared is unknown, and whether these sites modulate the Nup358/BicD2 interaction remains to be investigated. ^aRab6 is lipidated at its C-terminus (S-geranylgeranyl cysteine at residues 206 and 208 and a cysteine methyl ester at residue 208).

SUPPLEMENTARY REFERENCES

1. UniProt-Consortium. (2017) UniProt: the universal protein knowledgebase. *Nucleic Acids Res* **45**, D158-D169
2. Huttlin, E. L., Jedrychowski, M. P., Elias, J. E., Goswami, T., Rad, R., Beausoleil, S. A., Villén, J., Haas, W., Sowa, M. E., and Gygi, S. P. (2010) A Tissue-Specific Atlas of Mouse Protein Phosphorylation and Expression. *Cell* **143**, 1174-1189
3. Villén, J., Beausoleil, S. A., Gerber, S. A., and Gygi, S. P. (2007) Large-scale phosphorylation analysis of mouse liver. *Proc Natl Acad of Sci U S A* **104**, 1488-1493
4. Baffet, A D., Hu, D J., and Vallee, R B. (2015) Cdk1 activates pre-mitotic nuclear envelope dynein recruitment and apical nuclear migration in neural stem cells. *Dev Cell* **33**, 703-716
5. Dephoure, N., Zhou, C., Villén, J., Beausoleil, S. A., Bakalarski, C. E., Elledge, S. J., and Gygi, S. P. (2008) A quantitative atlas of mitotic phosphorylation. *Proc Natl Acad of Sci U S A* **105**, 10762-10767
6. Hendriks, I. A., Lyon, D., Young, C., Jensen, L. J., Vertegaal, A. C. O., and Nielsen, M. L. (2017) Site-specific mapping of the human SUMO proteome reveals co-modification with phosphorylation. *Nat Struct Mol Biol* **24**, 325-336