

# Structure of the nutrient-sensing hub GATOR2

Max L. Valenstein<sup>1,2,8,\*</sup>, Kacper B. Rogala<sup>1,3,5,6,7,8,\*</sup>, Pranav V. Lalgudi<sup>1,2</sup>, Edward J. Brignole<sup>2,4</sup>, Xin Gu<sup>1,2</sup>, Robert A. Saxton<sup>1,2</sup>, Lynne Chantranupong<sup>1,2</sup>, Jonas Kolibius<sup>1</sup>, Jan-Philipp Quast<sup>1</sup>, David M. Sabatini

<sup>1</sup>Whitehead Institute for Biomedical Research, Cambridge, MA, USA.

<sup>2</sup>Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA.

<sup>3</sup>Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA, USA.

<sup>4</sup>MIT.nano, Massachusetts Institute of Technology, Cambridge, MA, USA.

<sup>5</sup>Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA.

<sup>6</sup>Department of Structural Biology, Stanford University School of Medicine, Stanford, CA, USA.

<sup>7</sup>Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA, USA.

<sup>8</sup>These authors contributed equally to this work.

\*Correspondence: mvalenst@wi.mit.edu, rogala@stanford.edu

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# Supplementary Figure 1 | part 1

Fig. 1b - Coomassie Stain

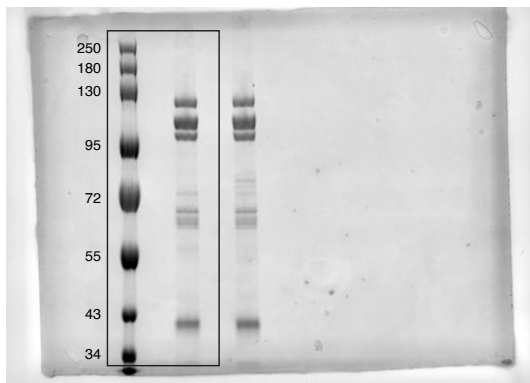


Fig. 2d - HA (IP)



Fig. 1e - S6K pT389

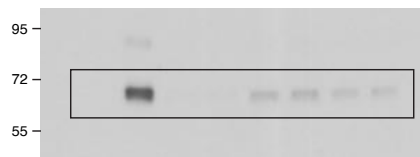


Fig. 2d - FLAG-METAP2 (IP)

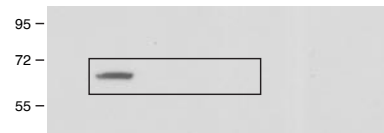


Fig. 1e - S6K

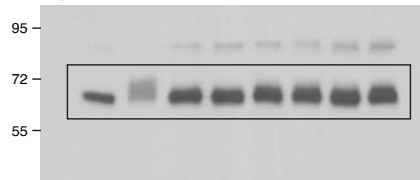


Fig. 2d - FLAG-MIOS-CTD (IP)

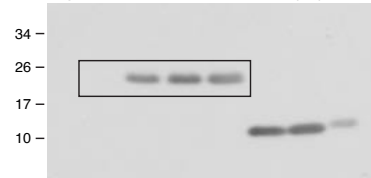


Fig. 1e - WDR24

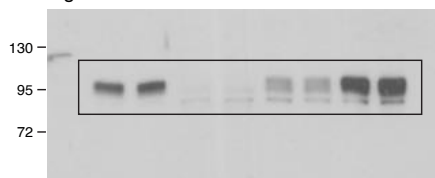


Fig. 2d - HA (cell lysate)

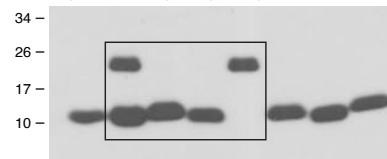


Fig. 1e - MIOS

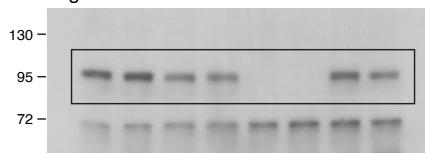


Fig. 2d - FLAG-METAP2 (cell lysate)

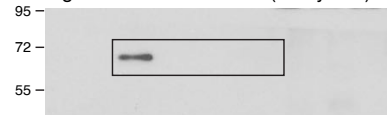


Fig. 1e - WDR59

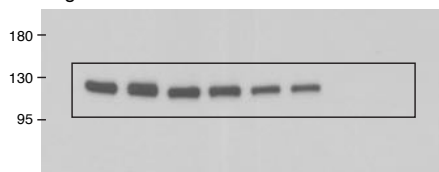


Fig. 2d - FLAG-MIOS-CTD (cell lysate)

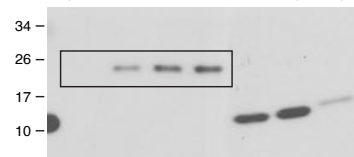
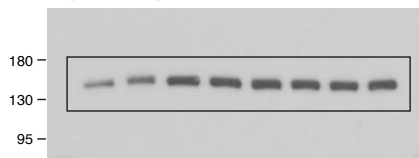
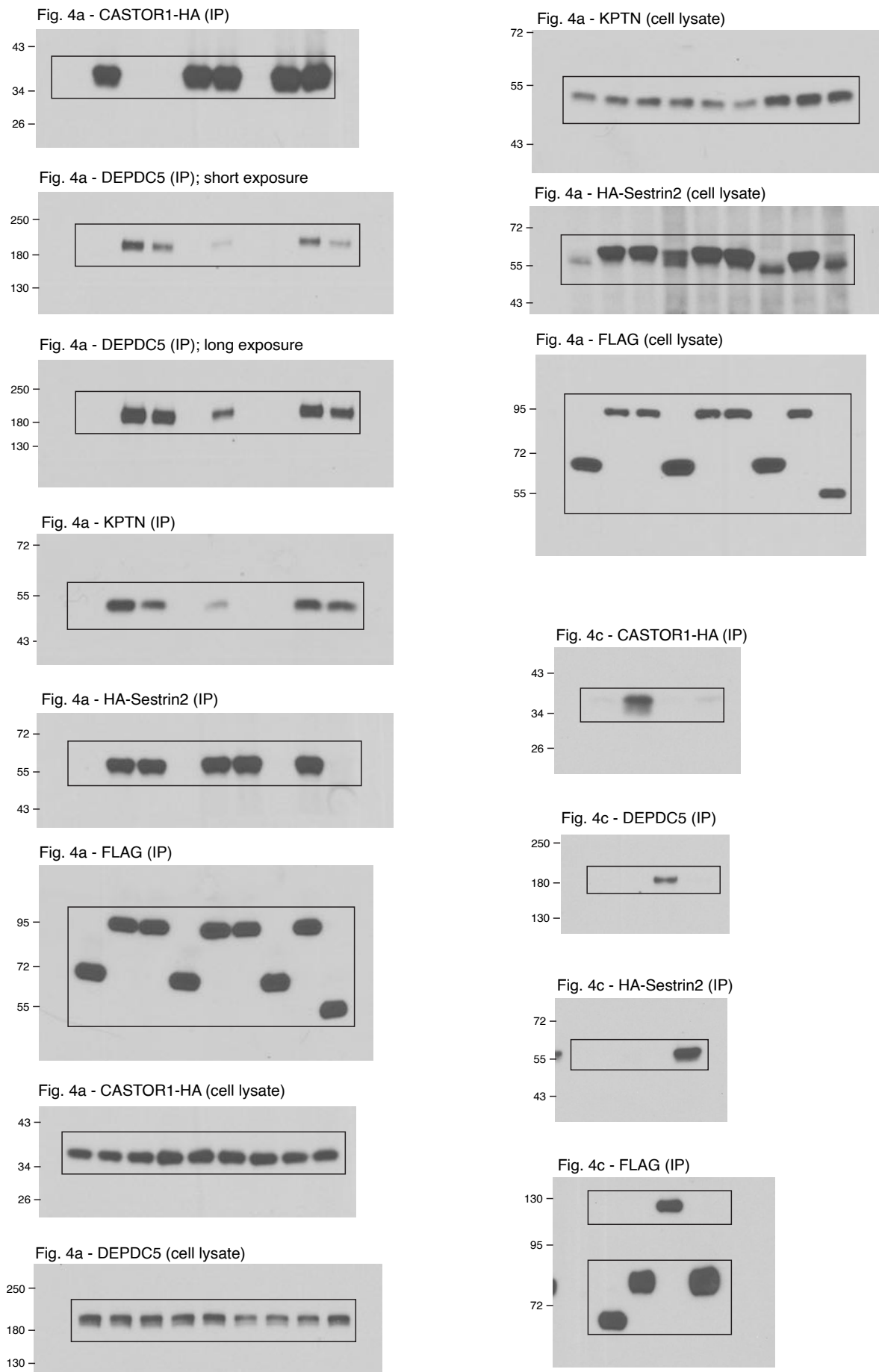


Fig. 1e - Raptor



## Supplementary Figure 1 | part 2



# Supplementary Figure 1 | part 3

Fig. 4c - CASTOR1-HA (cell lysate)

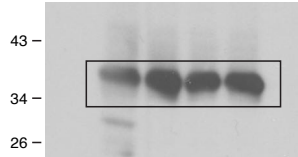


Fig. 4c - DEPDC5 (cell lysate)

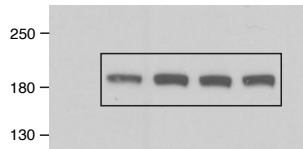


Fig. 4c - HA-Sestrin2 (cell lysate)

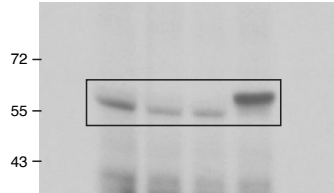


Fig. 4c - FLAG (cell lysate)

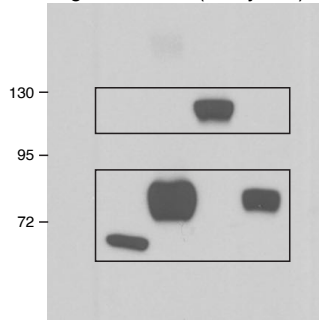


Fig. 4d - FLAG (cell lysate)

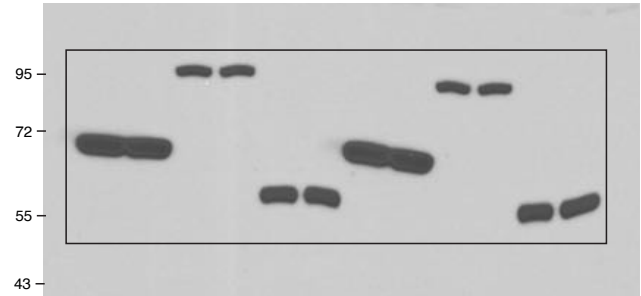


Fig. 4d - Raptor (cell lysate)

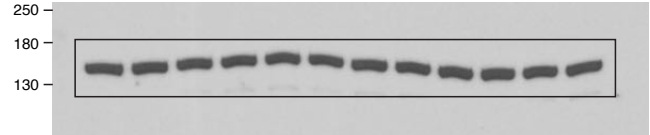


Fig. 4e - S6K pT389 (HA IP)

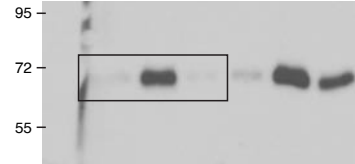


Fig. 4e - S6K (HA IP)

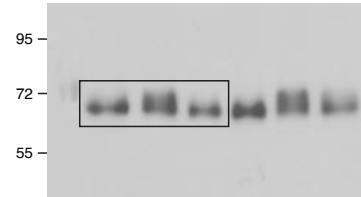


Fig. 4d - S6K pT389 (IP)

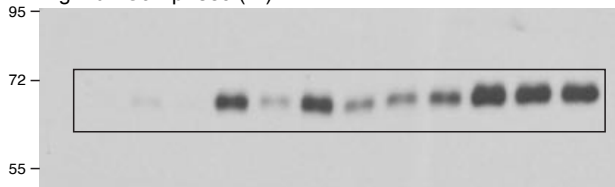


Fig. 4d - S6K (IP)

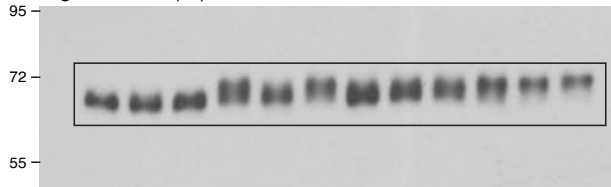


Fig. 4d - CASTOR1-myc (cell lysate)

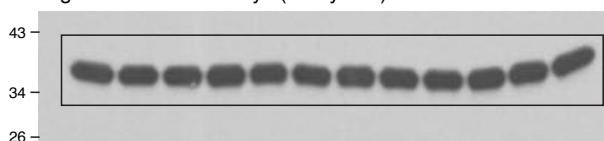


Fig. 4e - DEPDC5 (FLAG IP)

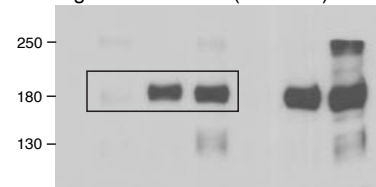


Fig. 4e - KPTN (FLAG IP)

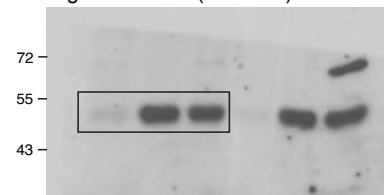




Fig. 4e - FLAG (FLAG IP)

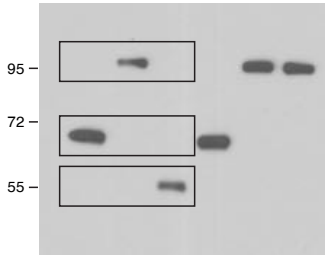


Fig. 4e - DEPDC5 (cell lysate)

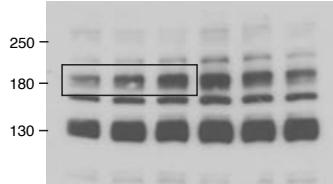


Fig. 4e - KPTN (cell lysate)

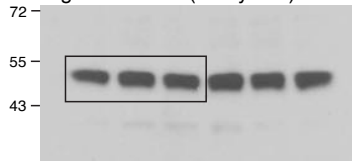
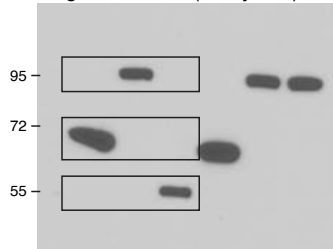
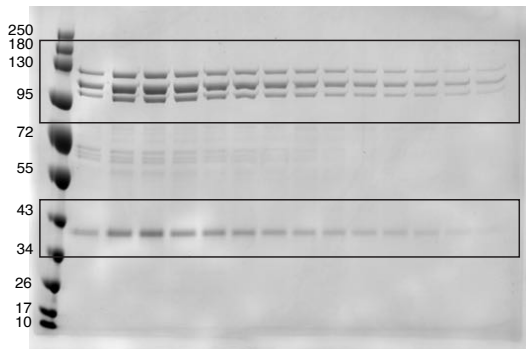


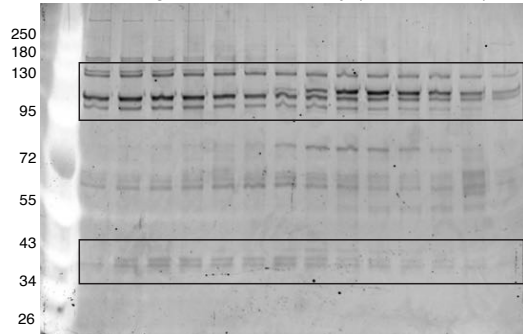
Fig. 4e - FLAG (cell lysate)



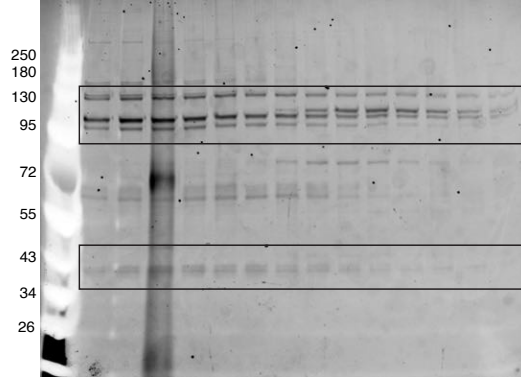
Ext. Data Fig. 1a - Coomassie (top)



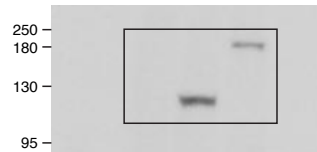
Ext. Data Fig. 1a - SYPRO Ruby (-AAs, middle)



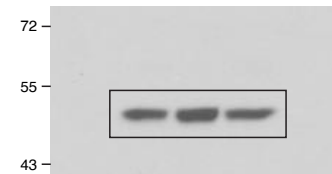
Ext. Data Fig. 1a - SYPRO Ruby (+AAs, bottom)



Ext. Data Fig. 1b - 3xFLAG



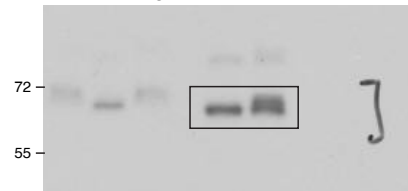
Ext. Data Fig. 1b - KPTN



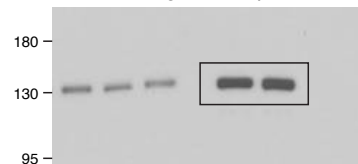
Ext. Data Fig. 1c - S6K pT389



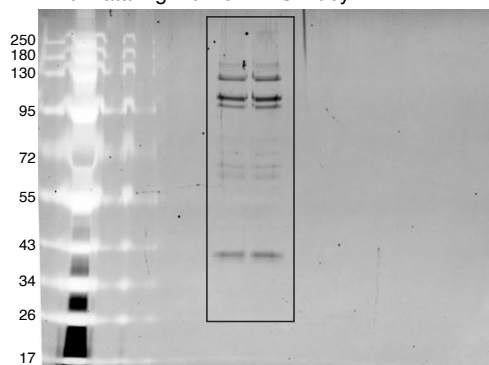
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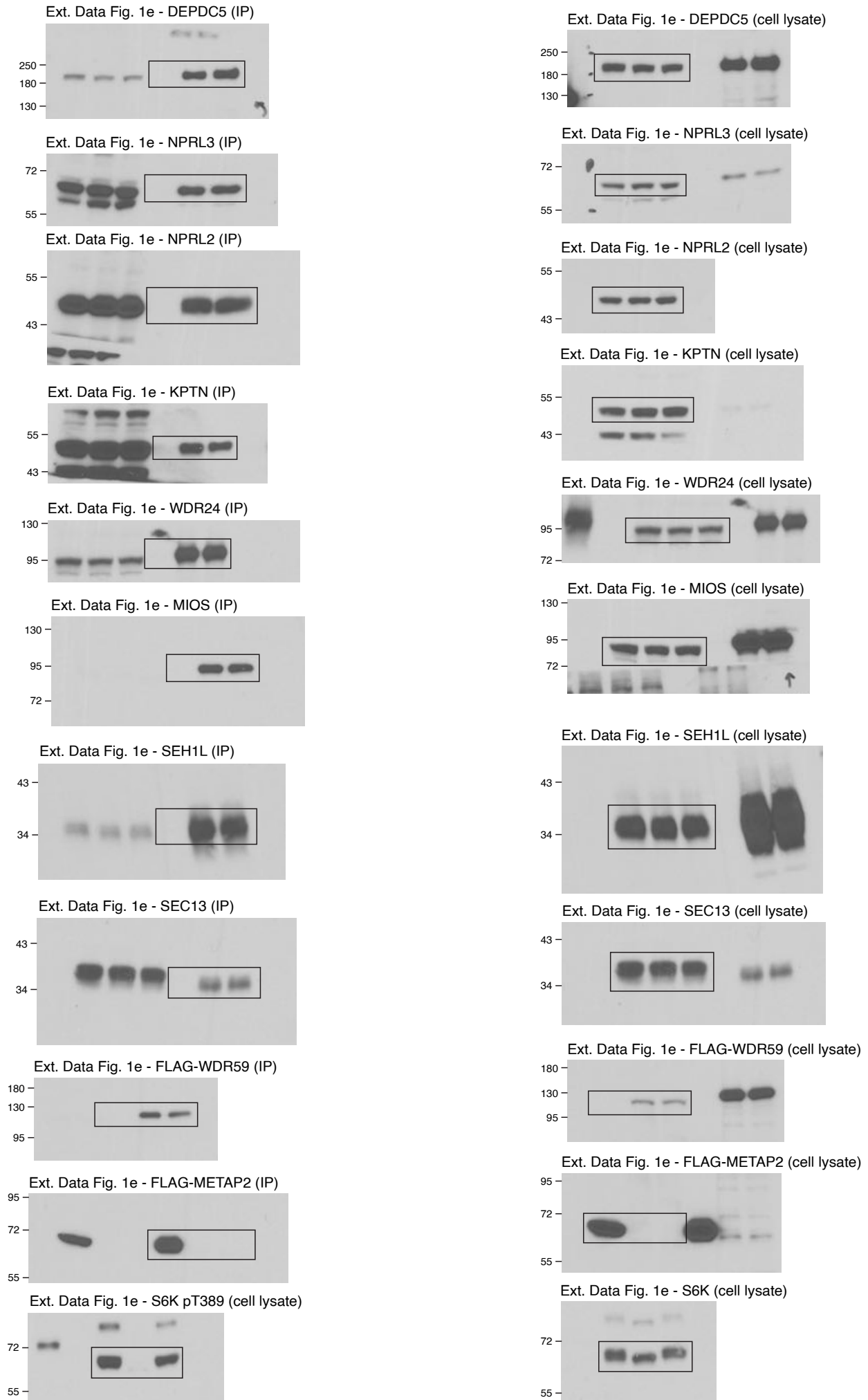
Ext. Data Fig. 1c - Raptor



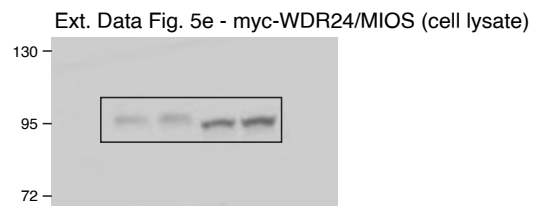
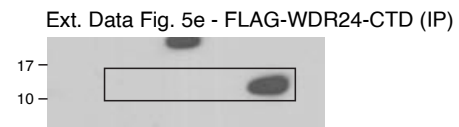
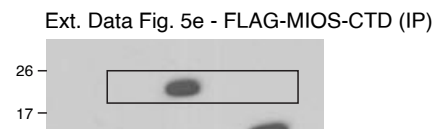
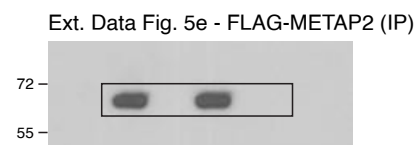
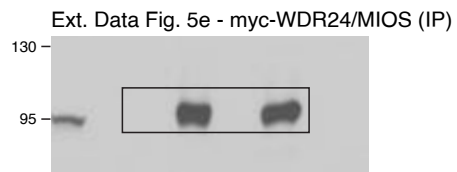
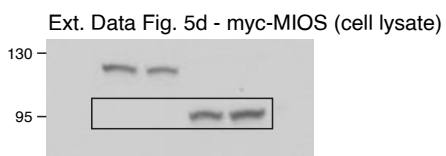
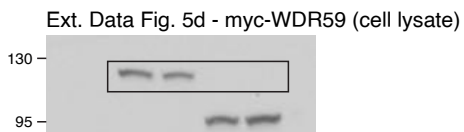
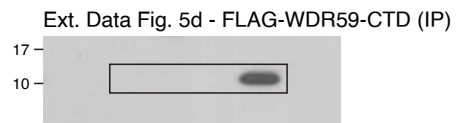
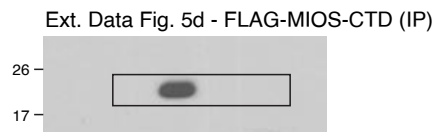
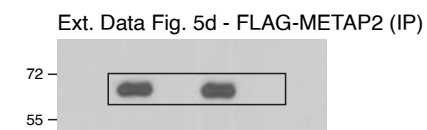
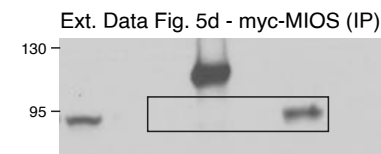
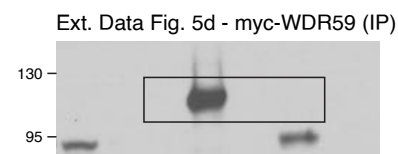
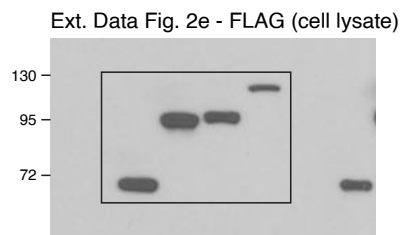
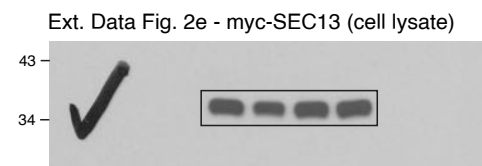
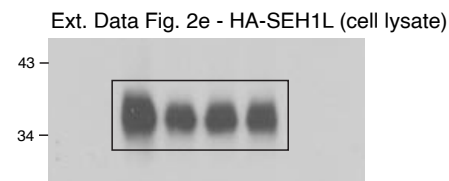
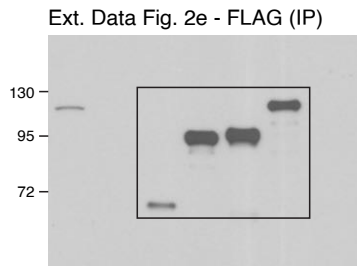
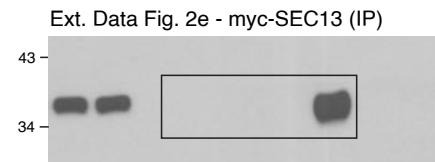
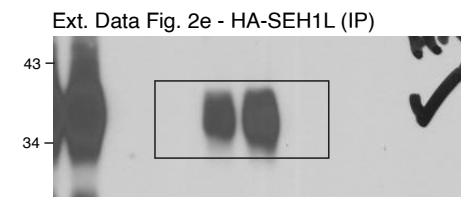
Ext. Data Fig. 1d - SYPRO Ruby

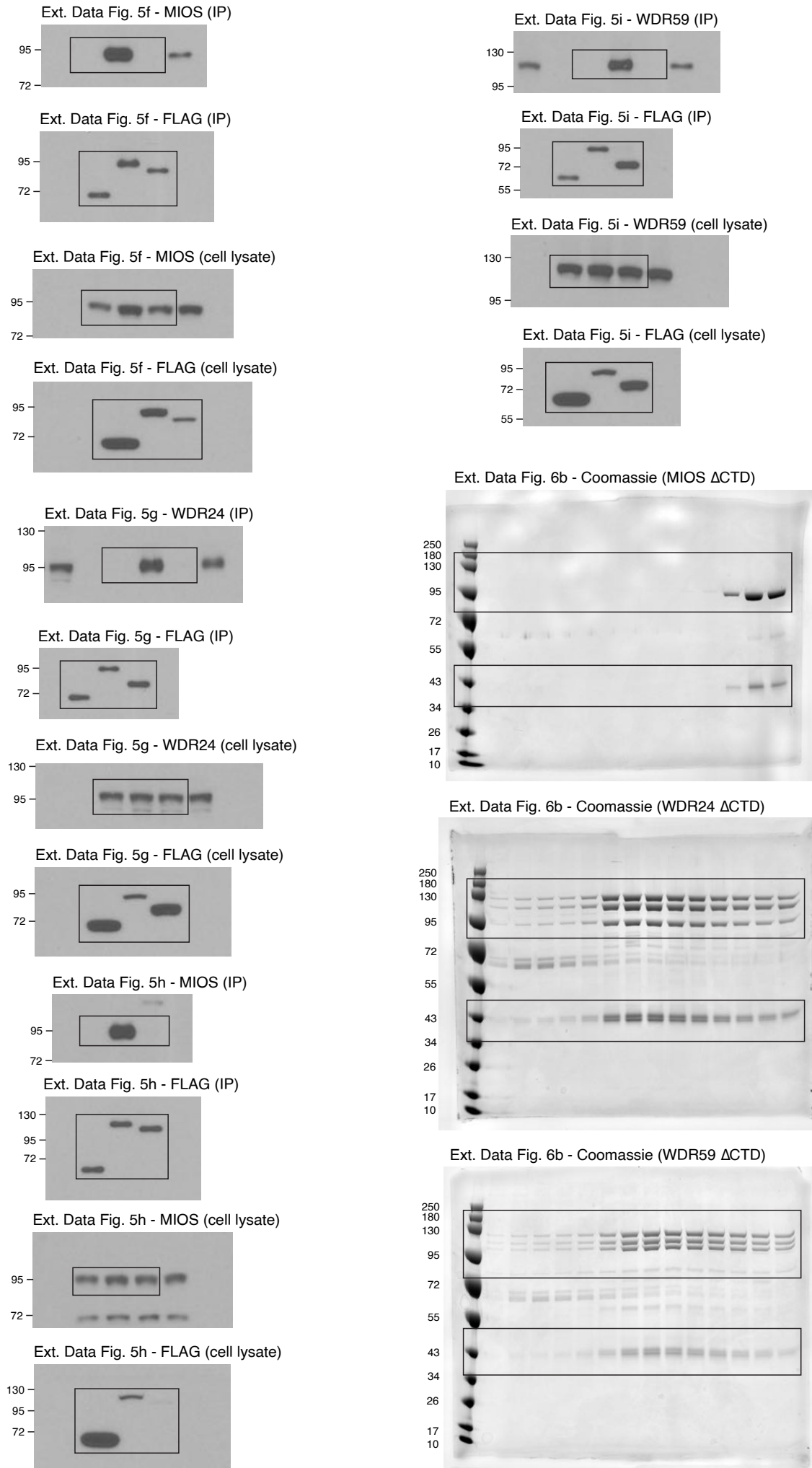


# Supplementary Figure 1 | part 5

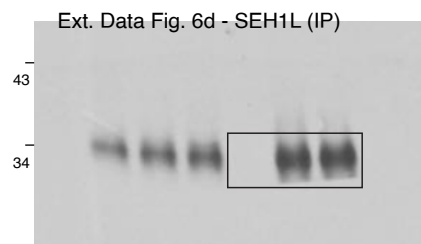
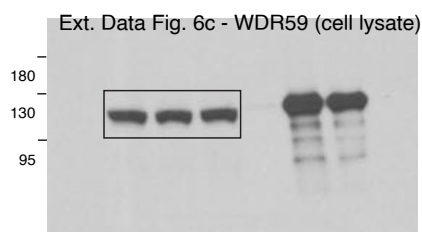
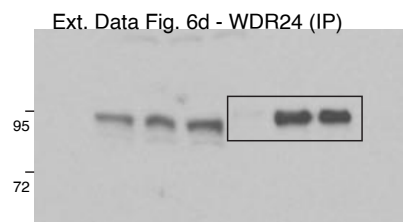
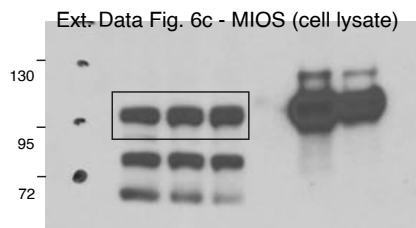
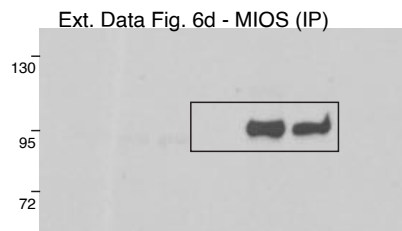
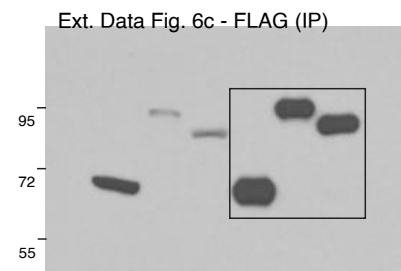
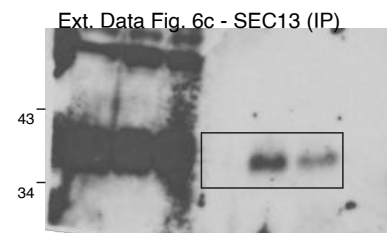
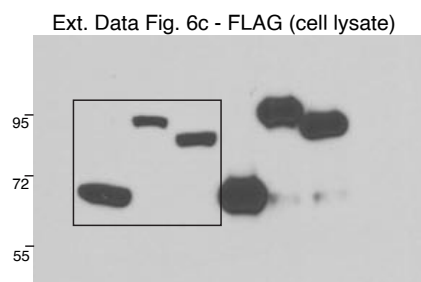
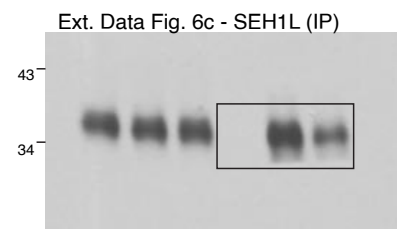
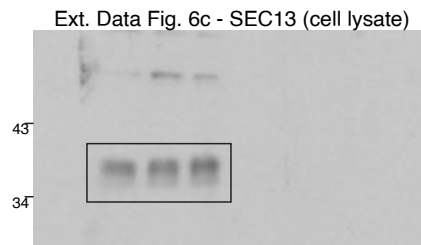
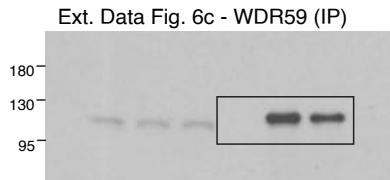
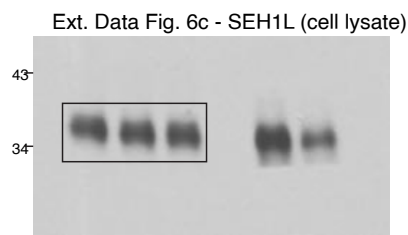
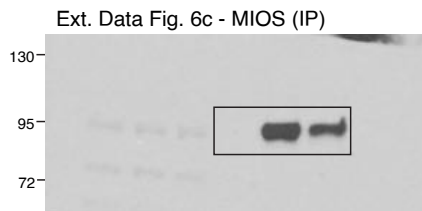


# Supplementary Figure 1 | part 6

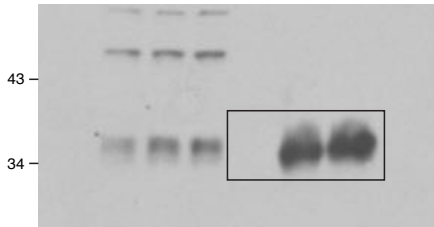




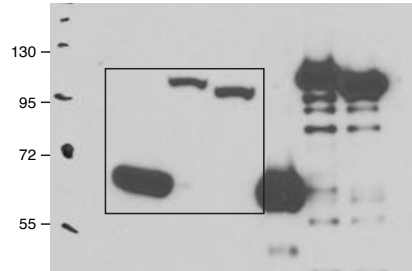
# Supplementary Figure 1 | part 8



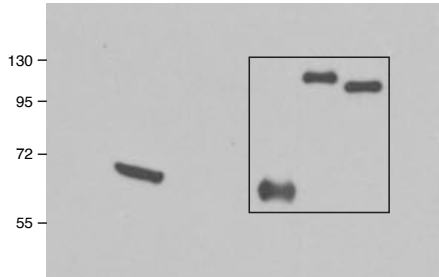
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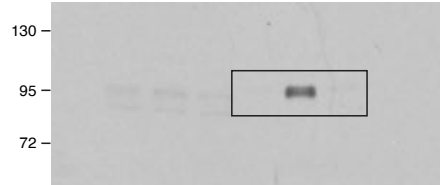
Ext. Data Fig. 6d - FLAG (cell lysate)



Ext. Data Fig. 6d - FLAG (IP)



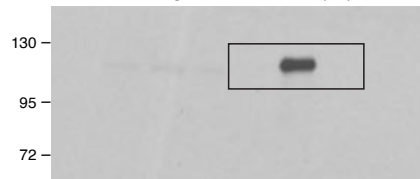
Ext. Data Fig. 6e - WDR24 (IP)



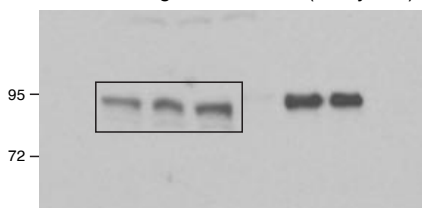
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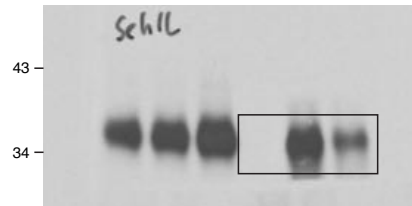
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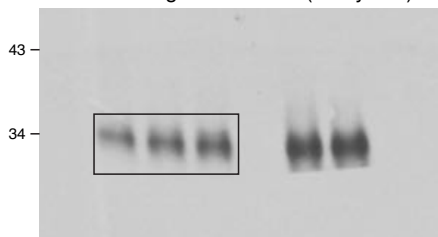
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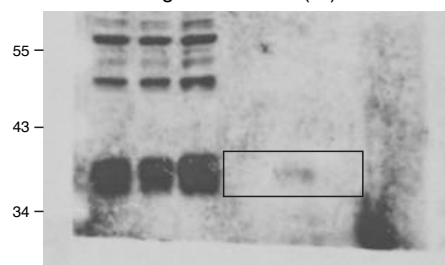
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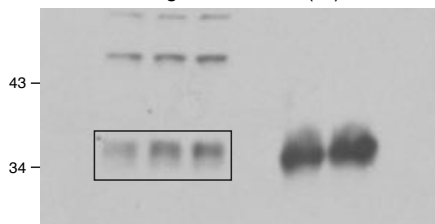
Ext. Data Fig. 6d - SEH1L (cell lysate)



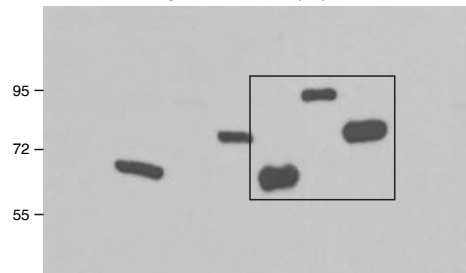
Ext. Data Fig. 6e - SEC13 (IP)



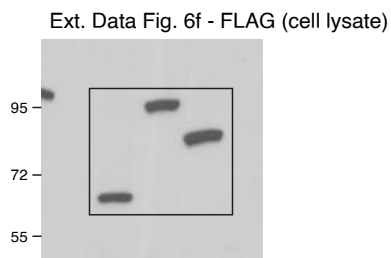
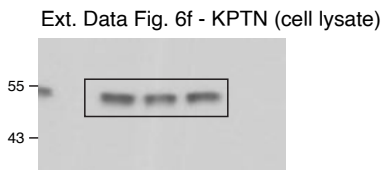
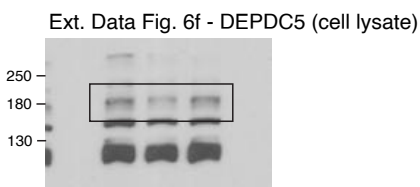
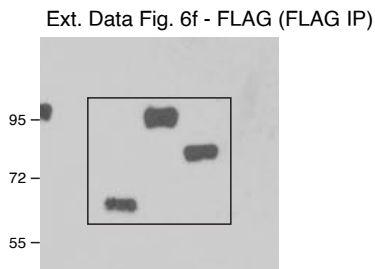
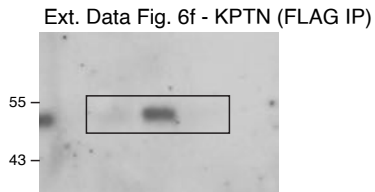
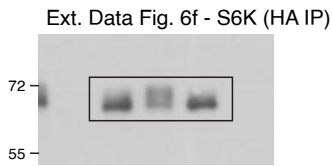
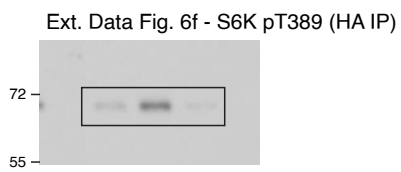
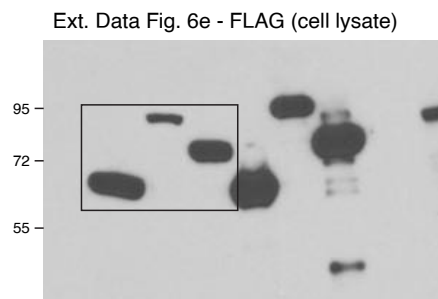
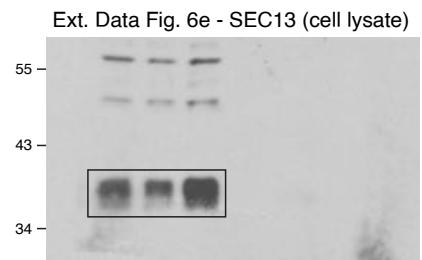
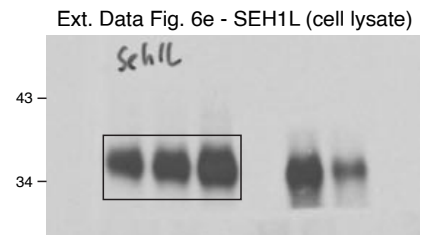
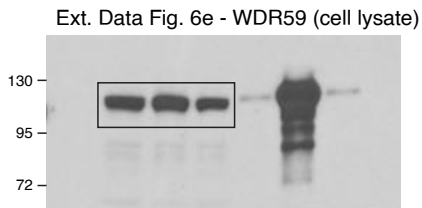
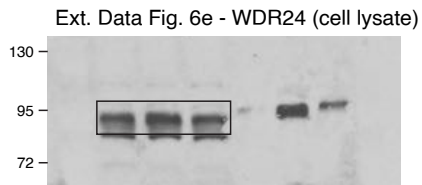
Ext. Data Fig. 6d - SEC13 (IP)

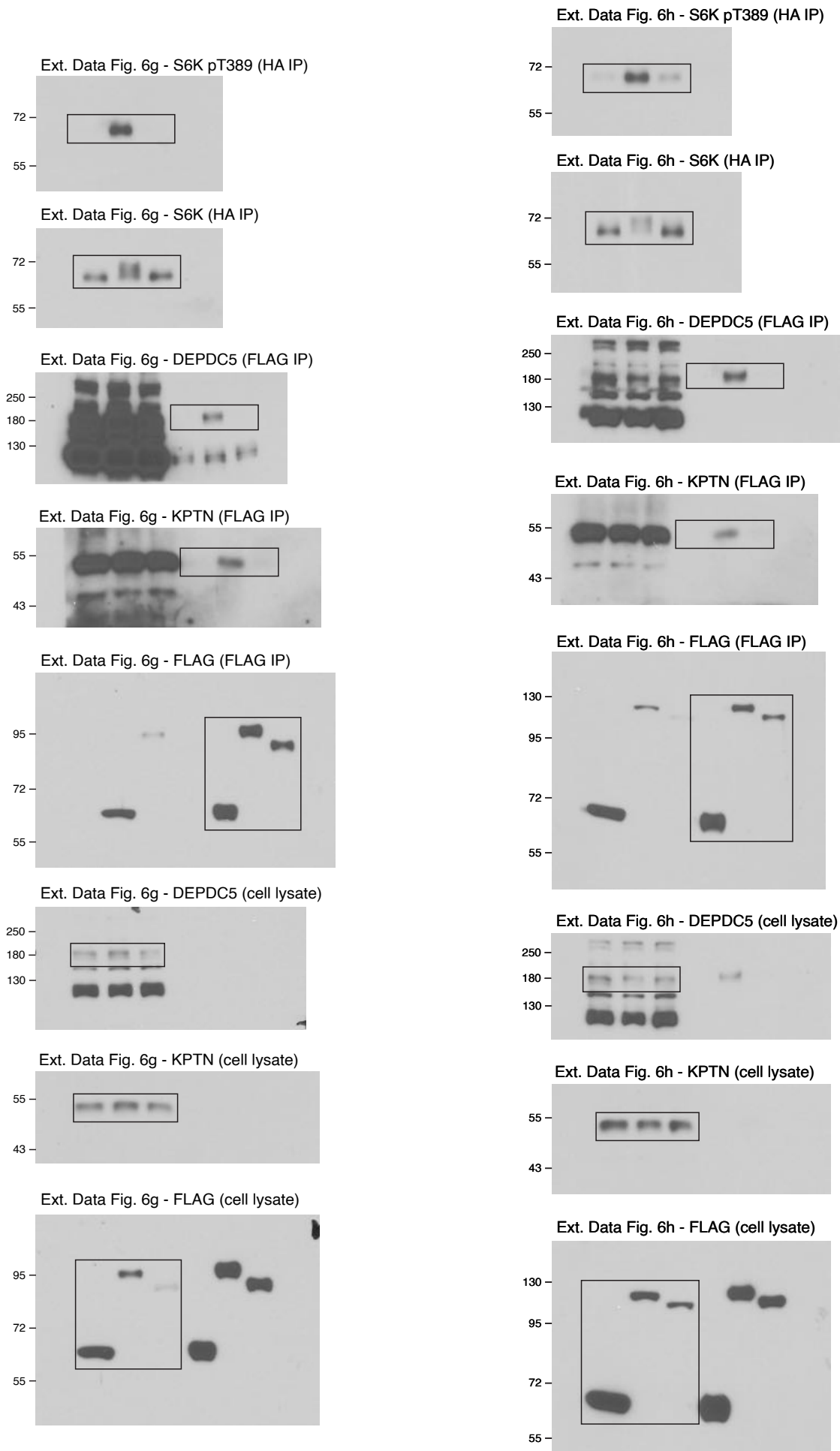


Ext. Data Fig. 6e - FLAG (IP)



# Supplementary Figure 1 | part 10

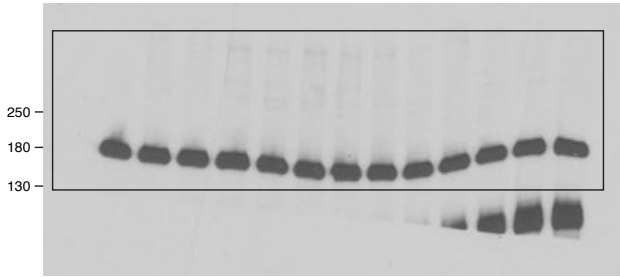




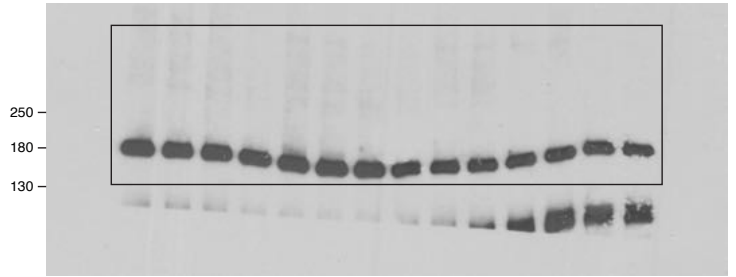


# Supplementary Figure 1 | part 12

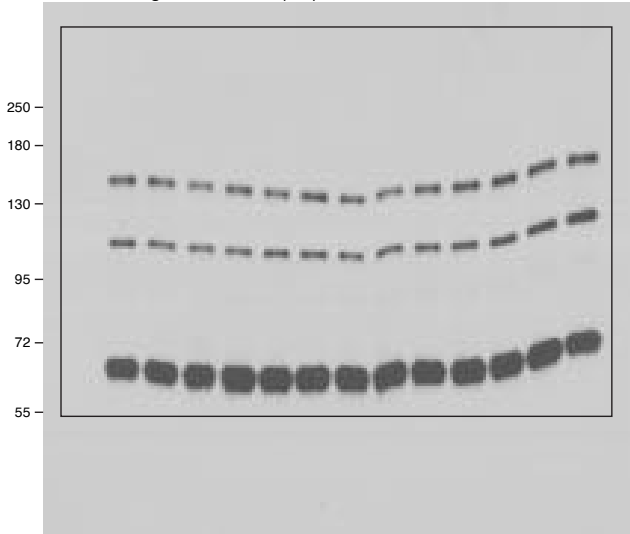
Ext. Data Fig. 8a - DEPDC5 (left)



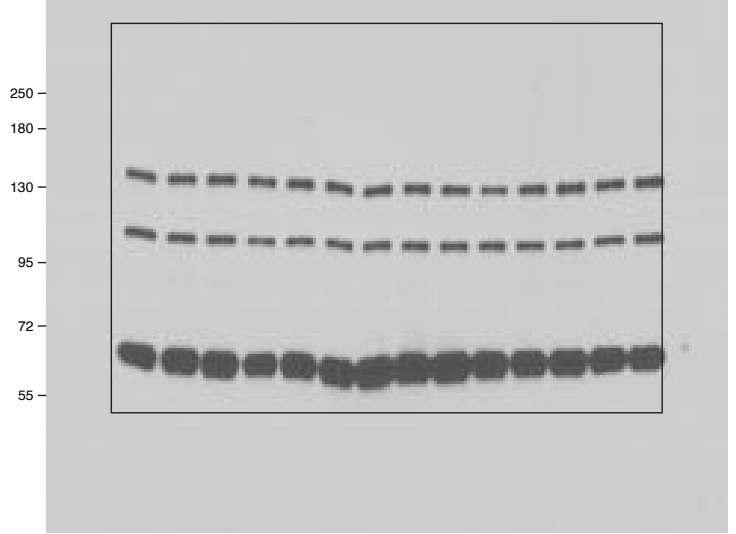
Ext. Data Fig. 8a - DEPDC5 (right)



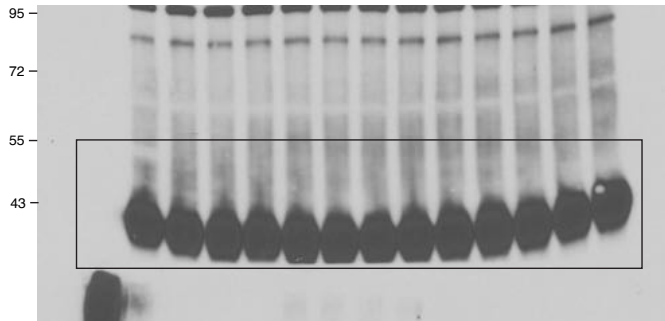
Ext. Data Fig. 8a - NPRL3 (left)



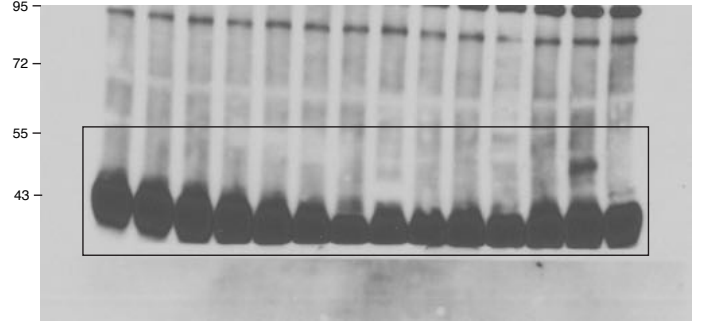
Ext. Data Fig. 8a - NPRL3 (right)



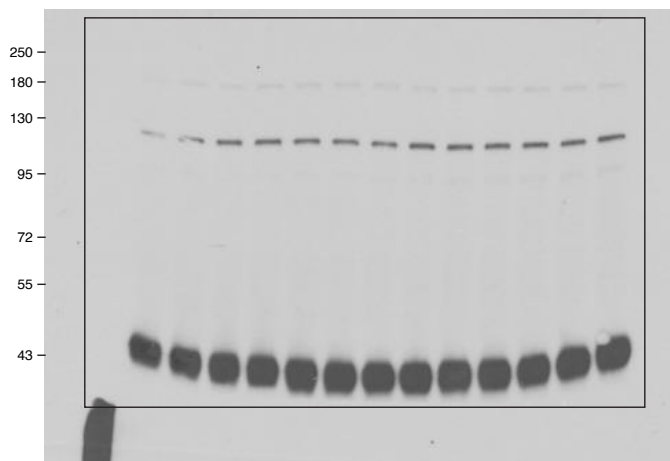
Ext. Data Fig. 8a - NPRL2 (left, long exposure)



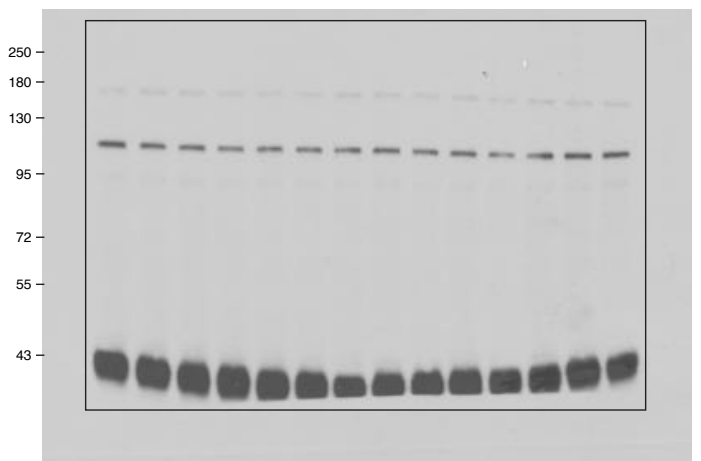
Ext. Data Fig. 8a - NPRL2 (right, long exposure)



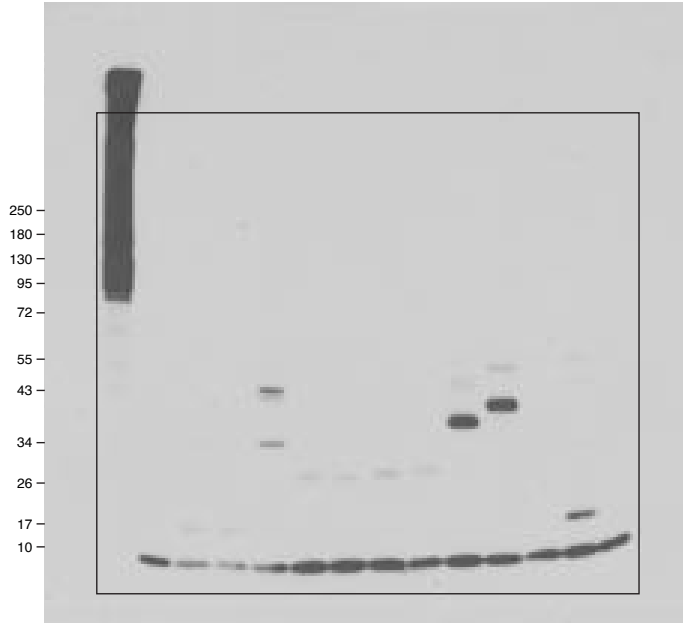
Ext. Data Fig. 8a - NPRL2 (left, short exposure)



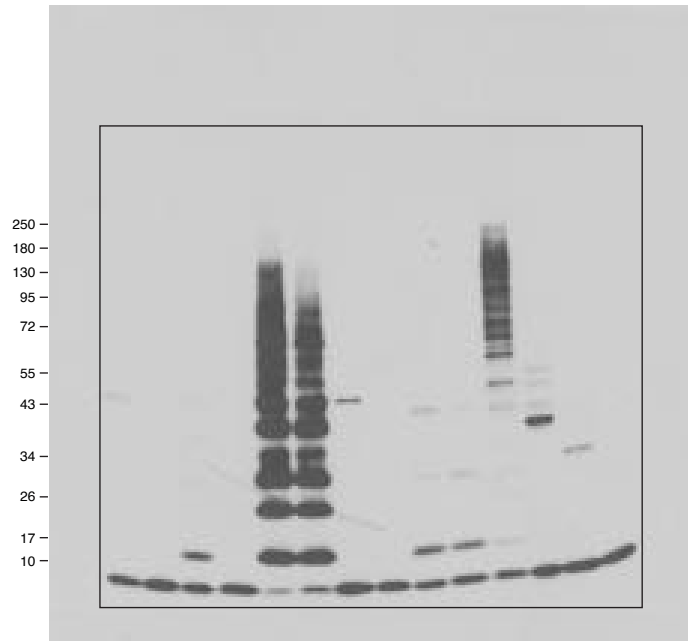
Ext. Data Fig. 8a - NPRL2 (right, short exposure)



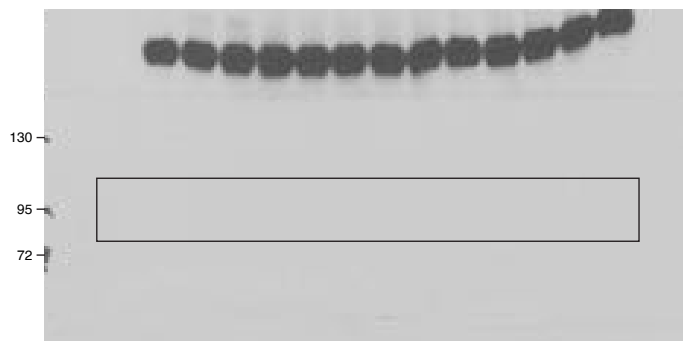
Ext. Data Fig. 8a - Ubiquitin (left)



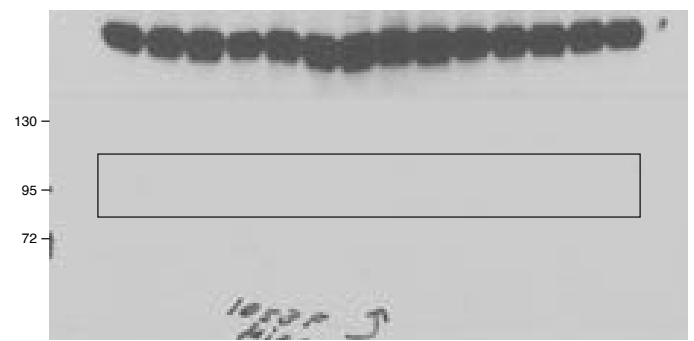
Ext. Data Fig. 8a - Ubiquitin (right)



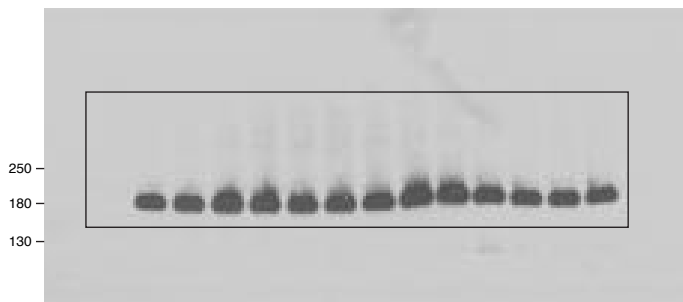
Ext. Data Fig. 8a - MIOS (left)



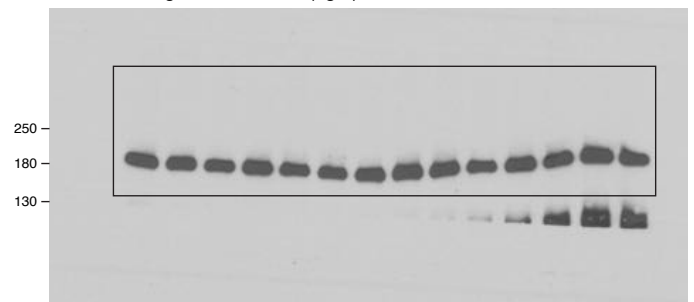
Ext. Data Fig. 8a - MIOS (right)



Ext. Data Fig. 8b - DEPDC5 (left)

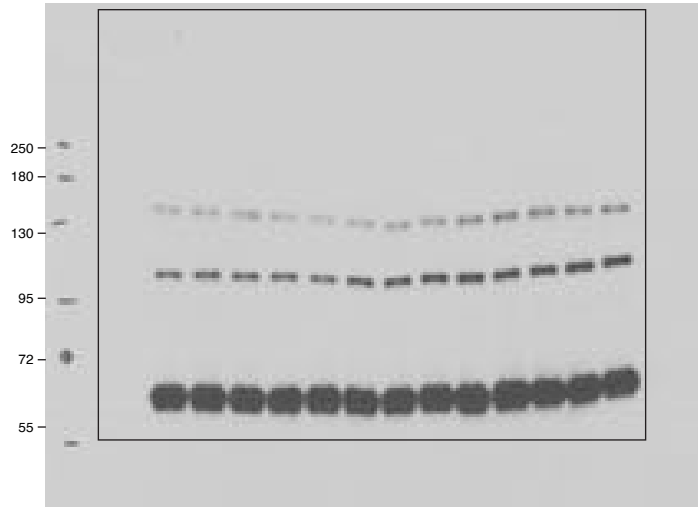


Ext. Data Fig. 8b - DEPDC5 (right)

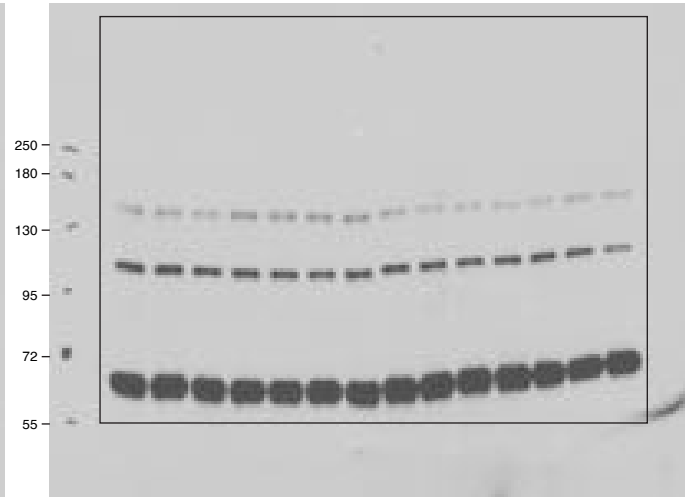


# Supplementary Figure 1 | part 14

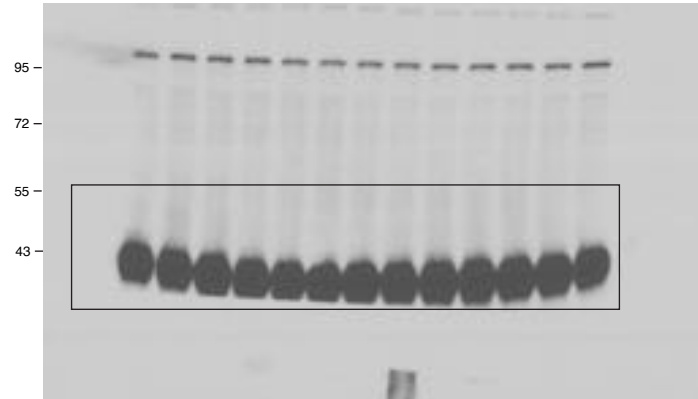
Ext. Data Fig. 8b - NPRL3 (left)



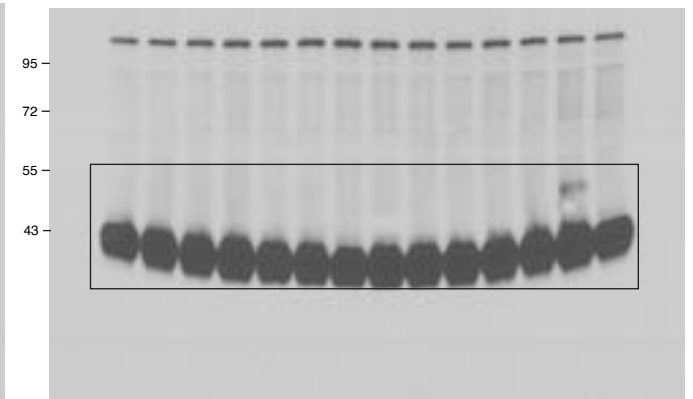
Ext. Data Fig. 8b - NPRL3 (right)



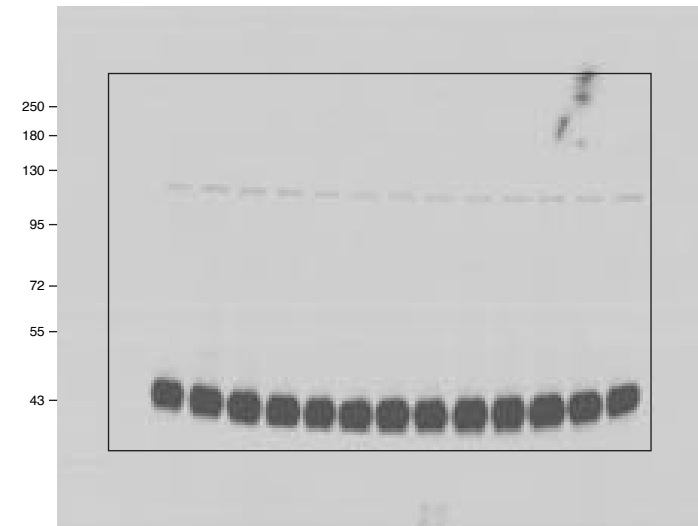
Ext. Data Fig. 8b - NPRL2 (left, long exposure)



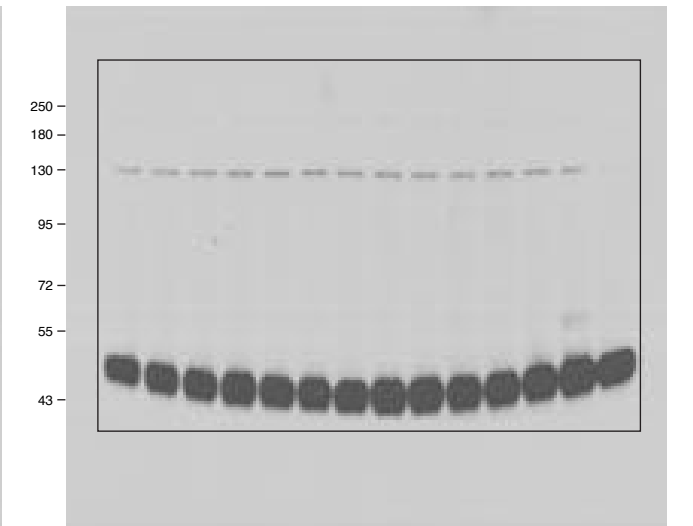
Ext. Data Fig. 8b - NPRL2 (right, long exposure)



Ext. Data Fig. 8b - NPRL2 (left, short exposure)

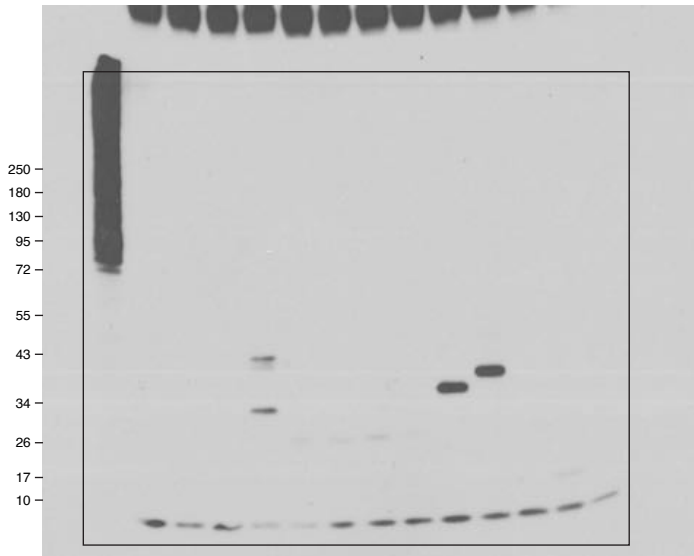


Ext. Data Fig. 8b - NPRL2 (right, short exposure)

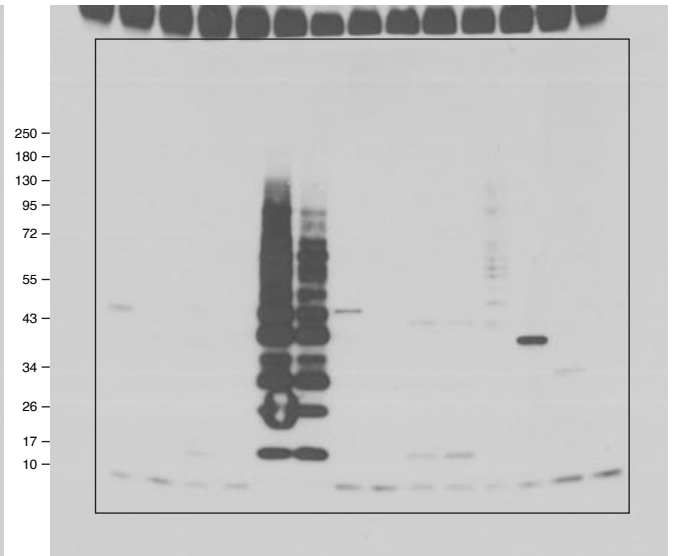


# Supplementary Figure 1 | part 15

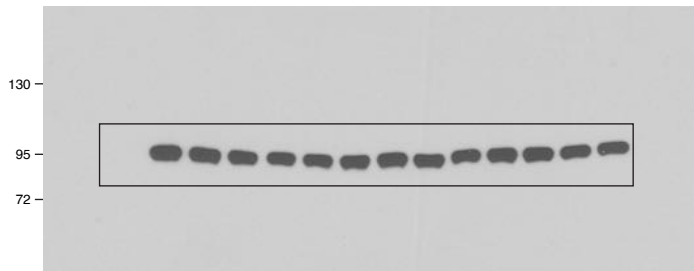
Ext. Data Fig. 8b - Ubiquitin (left)



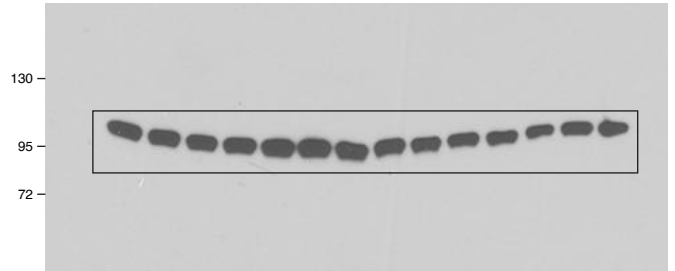
Ext. Data Fig. 8b - Ubiquitin (right)



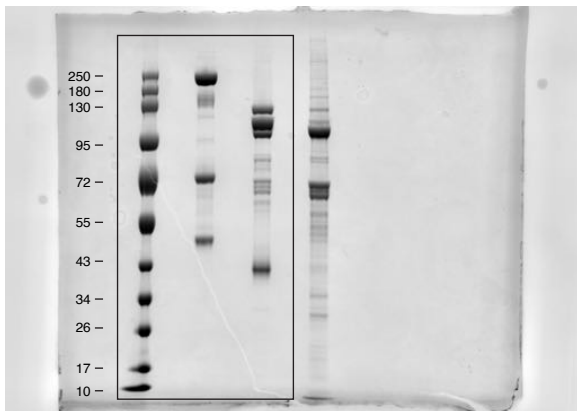
Ext. Data Fig. 8b - MIOS (left)



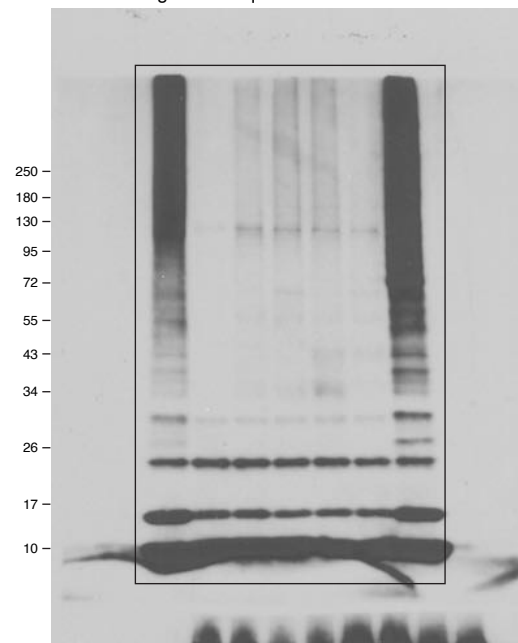
Ext. Data Fig. 8b - MIOS (right)



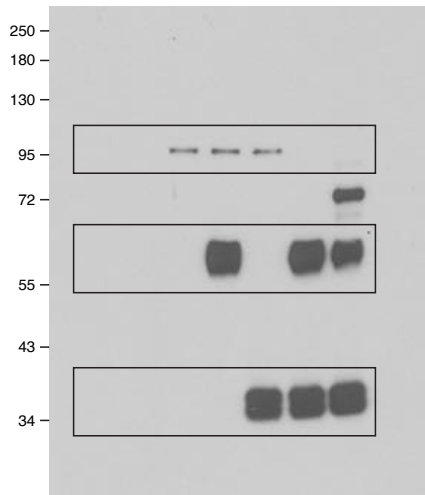
Ext. Data Fig. 8c - Coomassie



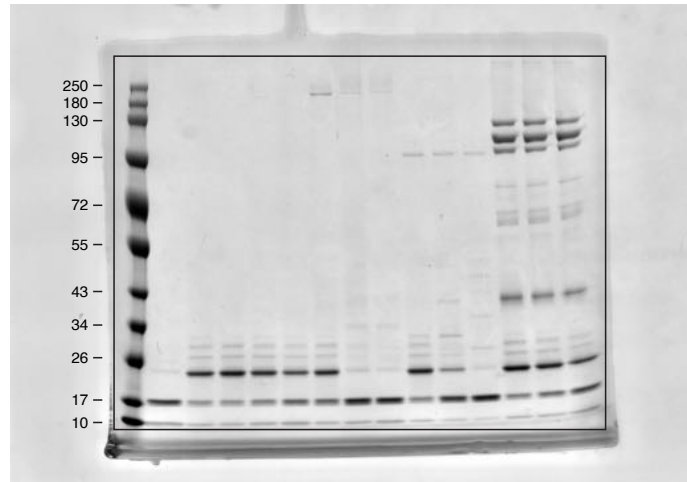
Ext. Data Fig. 8d - Ubiquitin



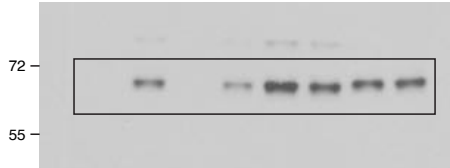
Ext. Data Fig. 8d - FLAG



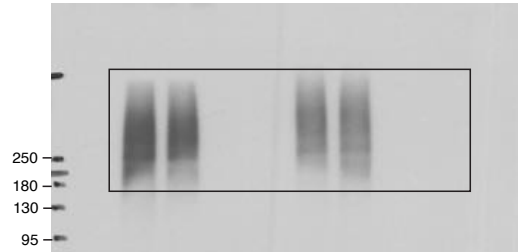
Ext. Data Fig. 8e - Coomassie



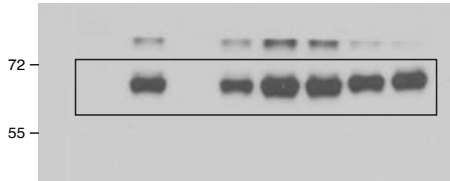
Ext. Data Fig. 8f - S6K pT389 (short exposure)



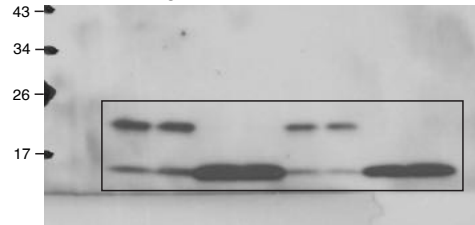
Ext. Data Fig. 8f - Ubiquitin conjugates



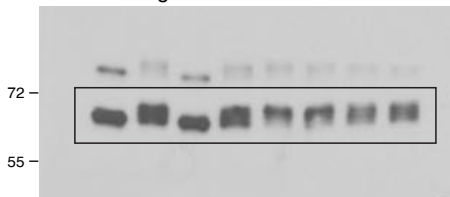
Ext. Data Fig. 8f - S6K pT389 (long exposure)



Ext. Data Fig. 8f - UBE2D



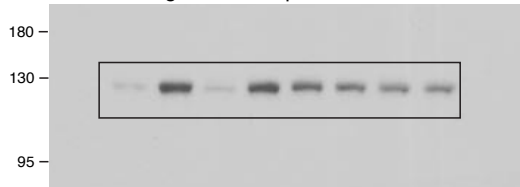
Ext. Data Fig. 8f - S6K



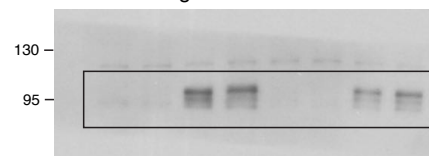
Ext. Data Fig. 8f - HIF1α



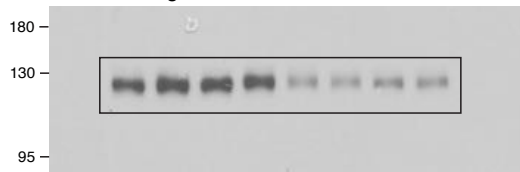
Ext. Data Fig. 8f - ULK1 pS757

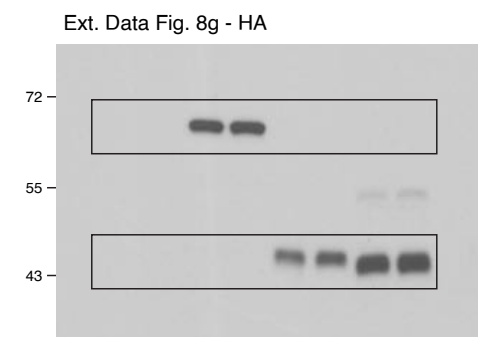
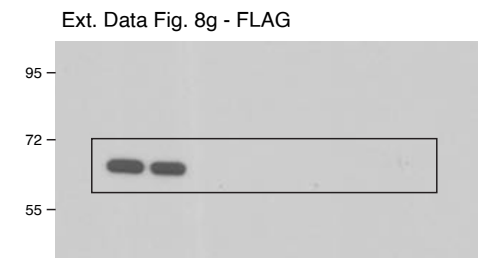
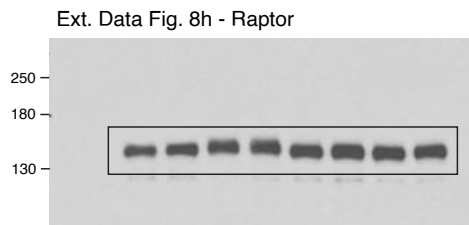
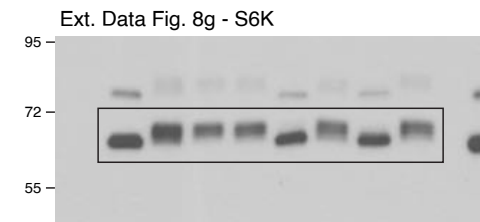
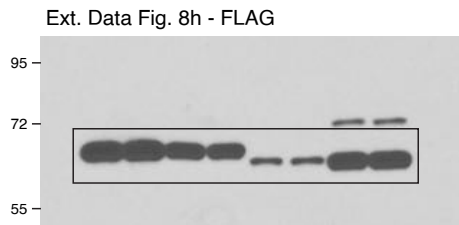
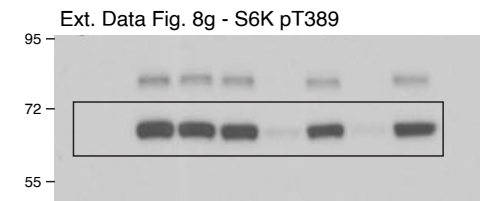
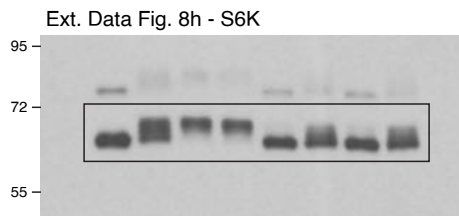
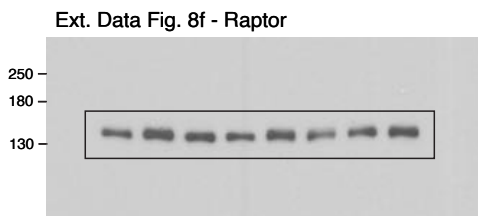
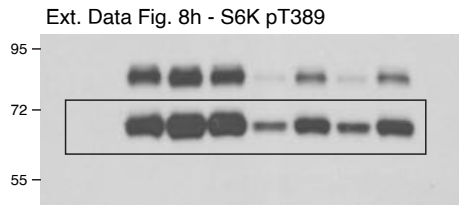
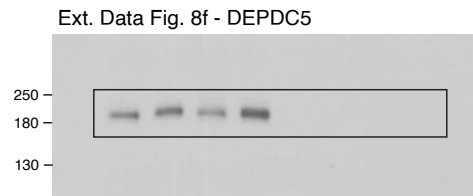
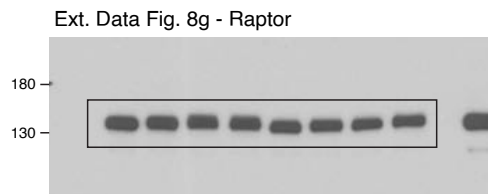
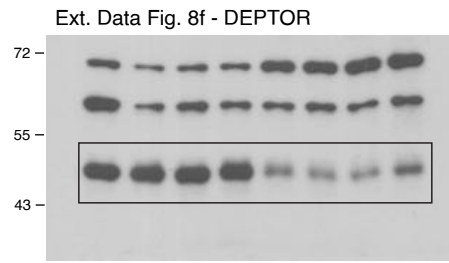


Ext. Data Fig. 8f - NRF2

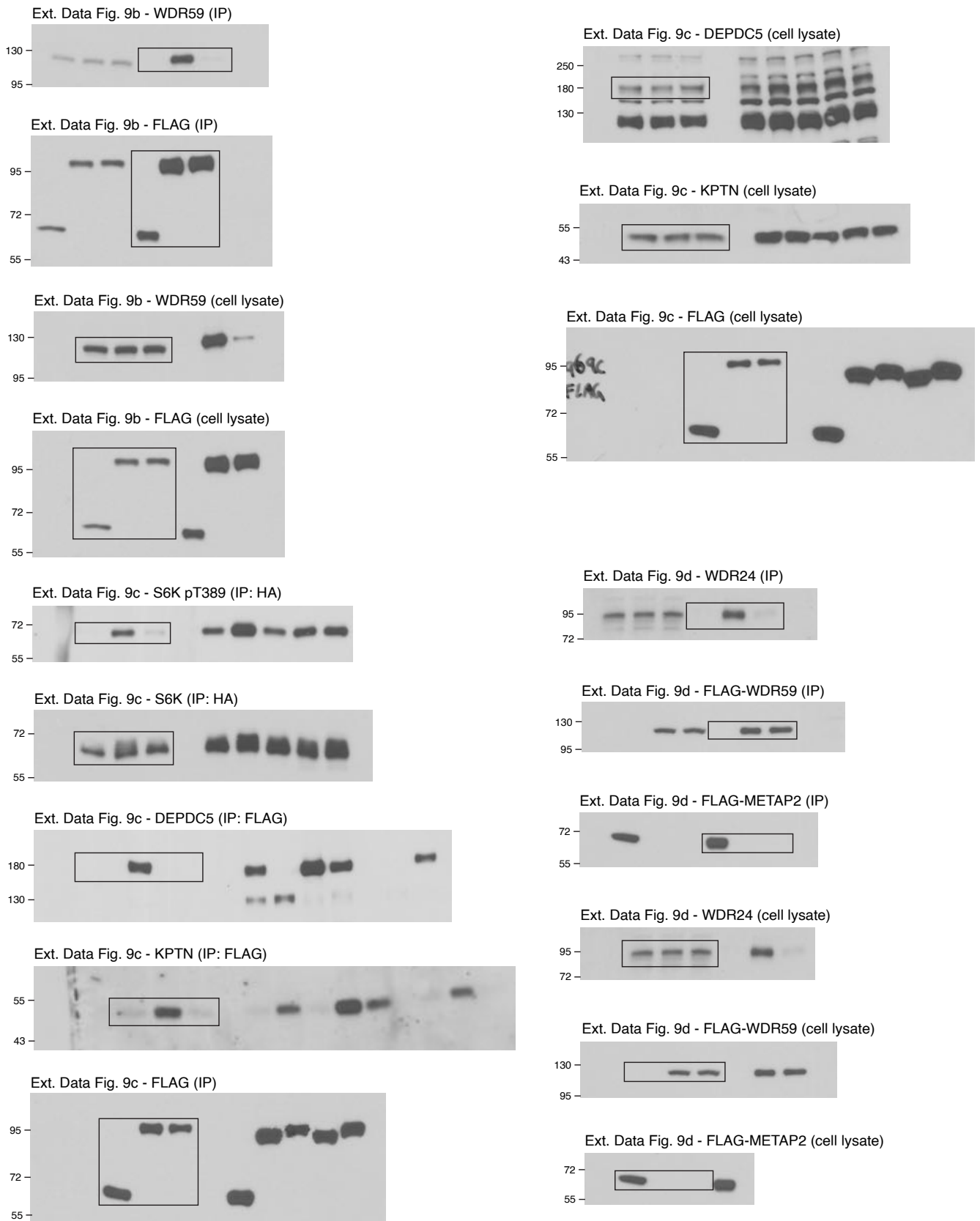


Ext. Data Fig. 8f - ULK1

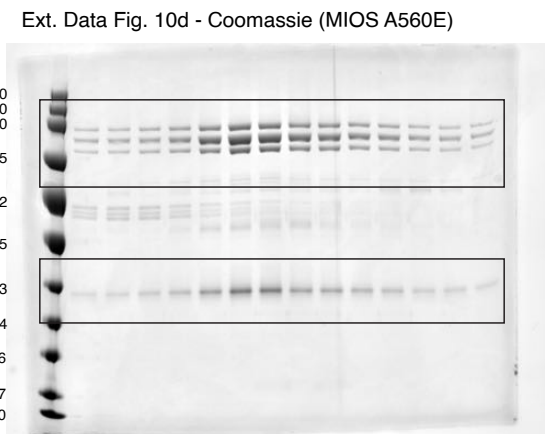
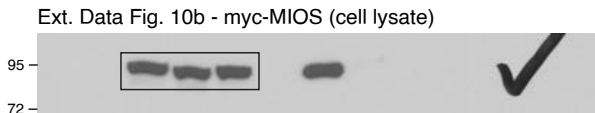
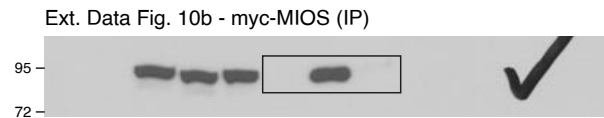
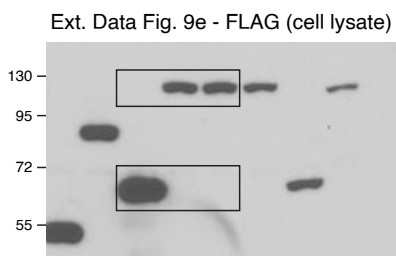
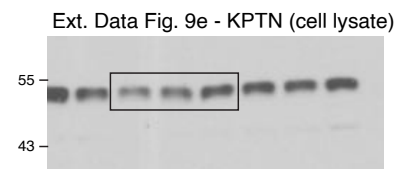
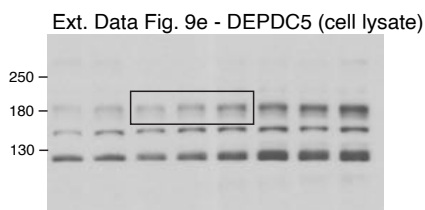
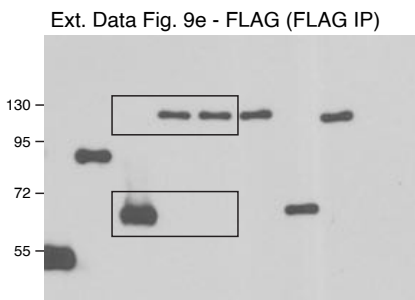
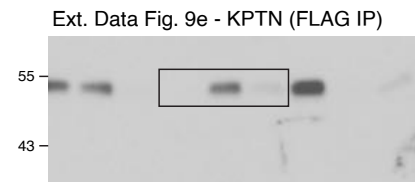
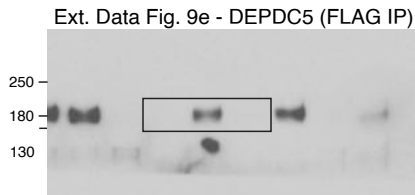
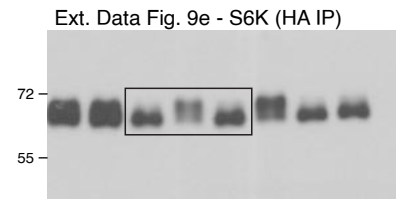
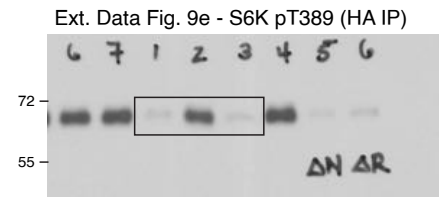




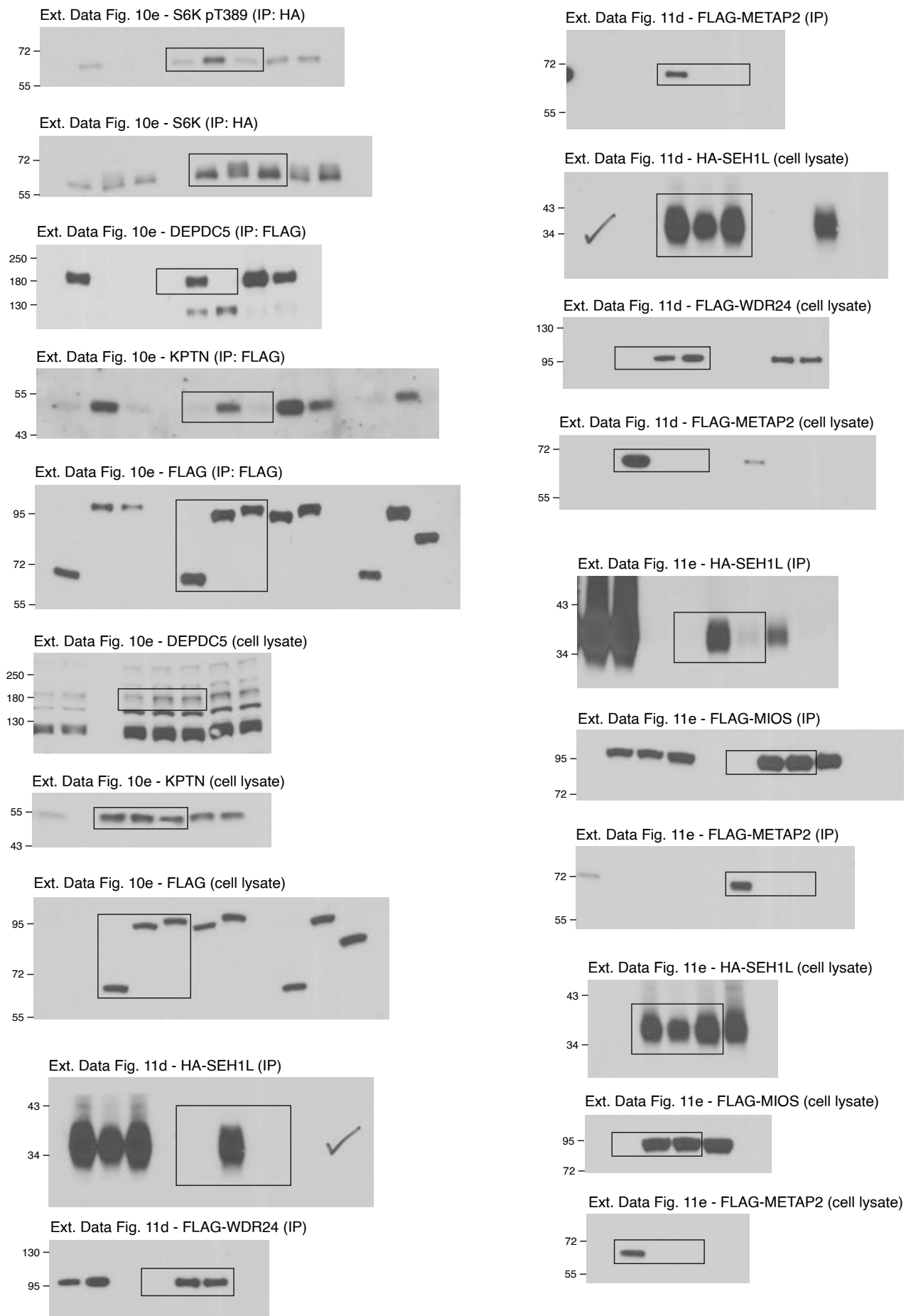
# Supplementary Figure 1 | part 18



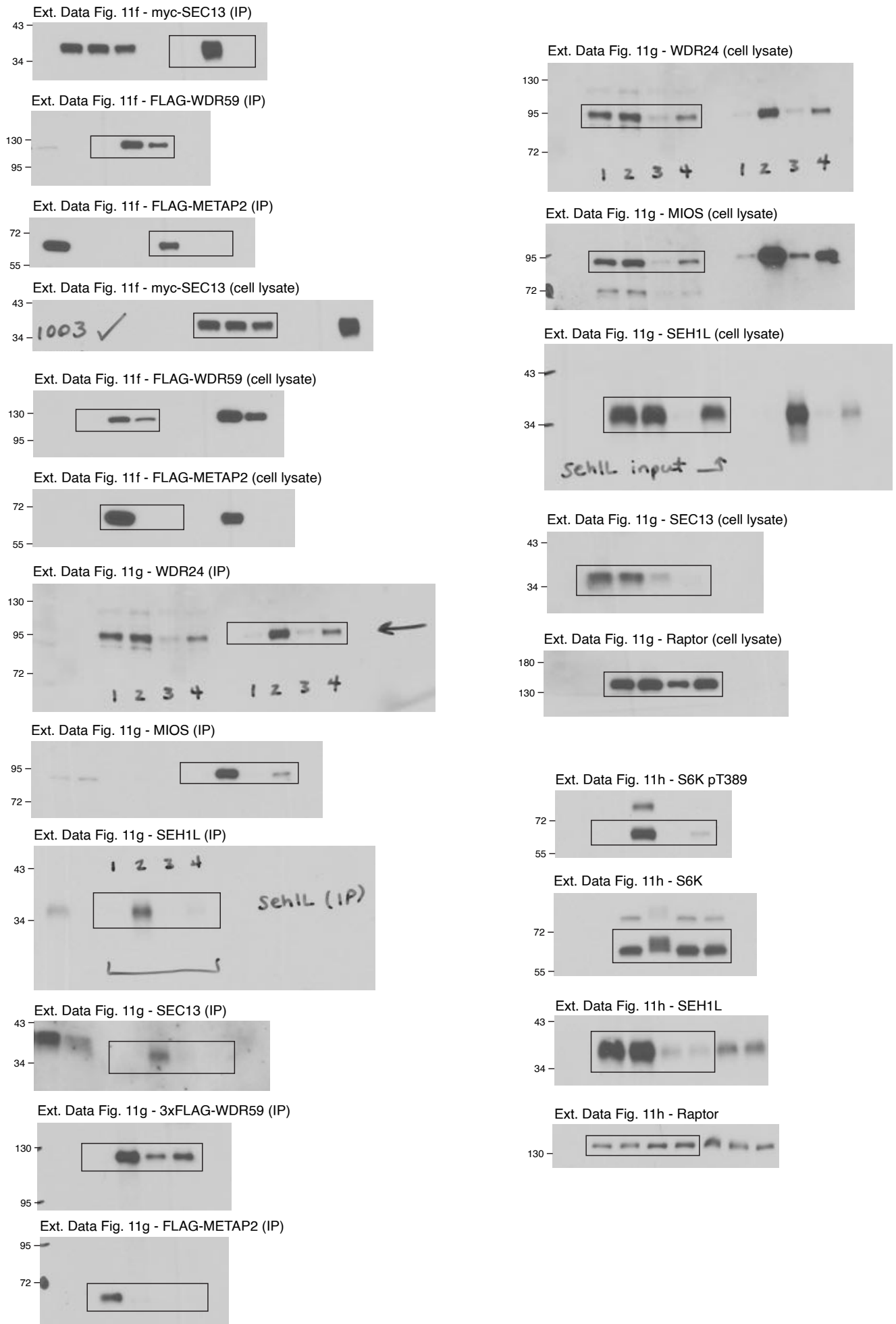
# Supplementary Figure 1 | part 19





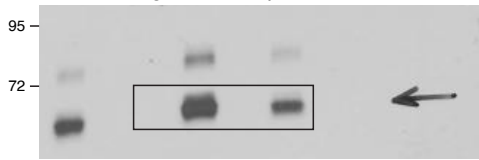


# Supplementary Figure 1 | part 21

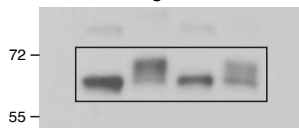


# Supplementary Figure 1 | part 22

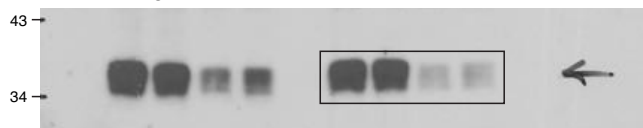
Ext. Data Fig. 11i - S6K pT389



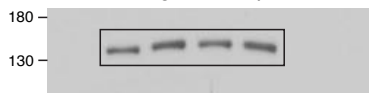
Ext. Data Fig. 11i - S6K

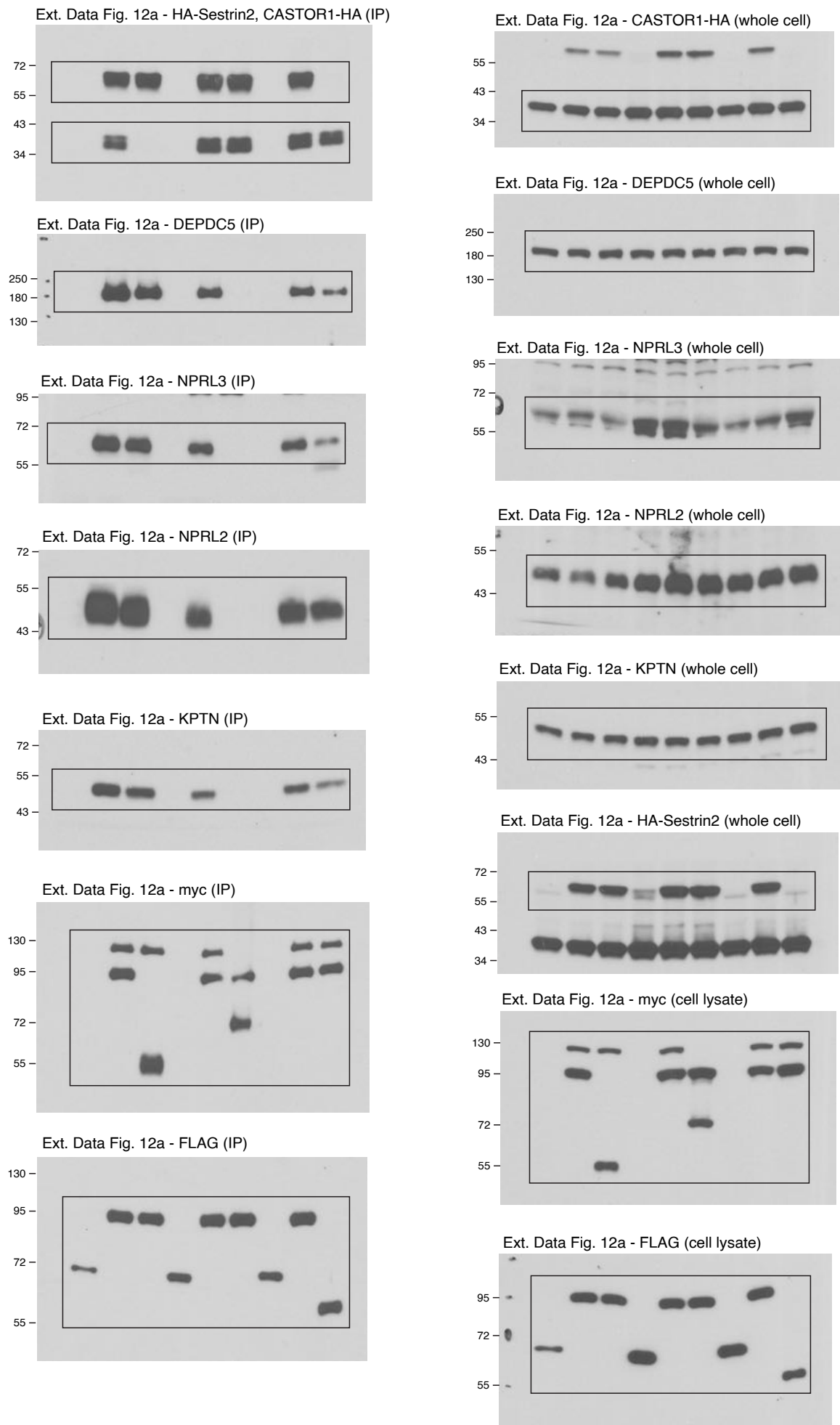


Ext. Data Fig. 11i - SEC13

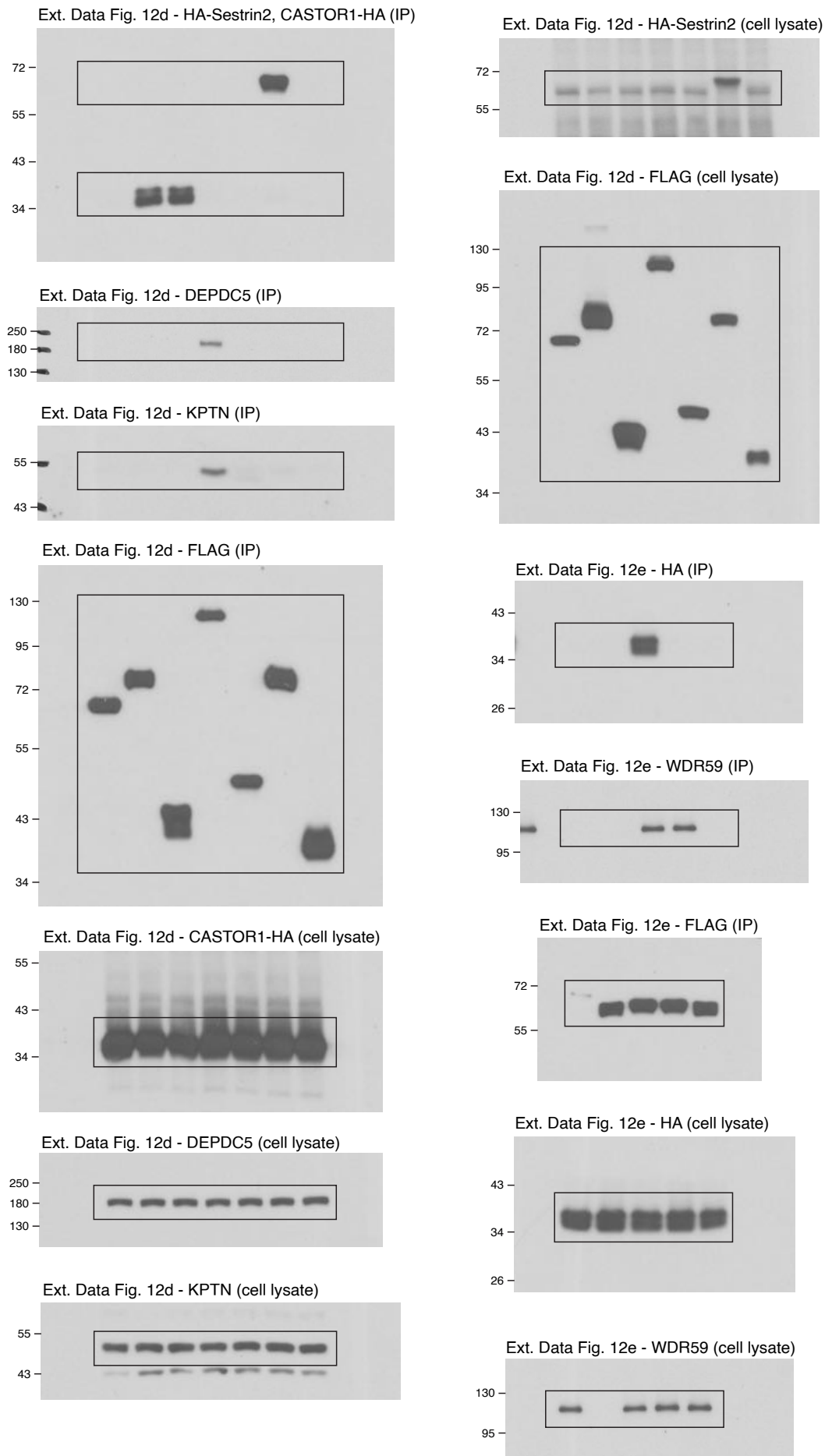


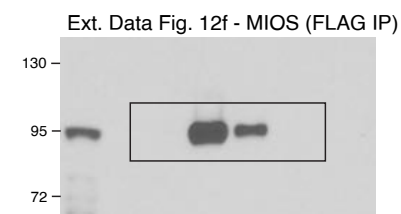
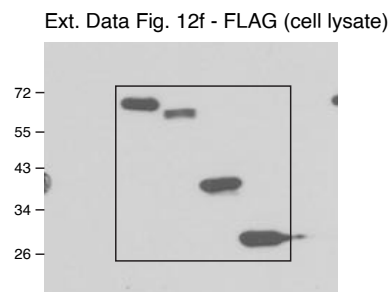
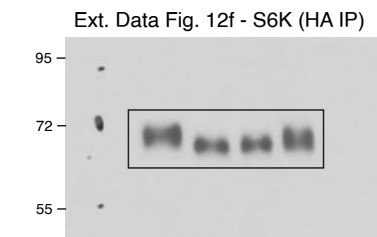
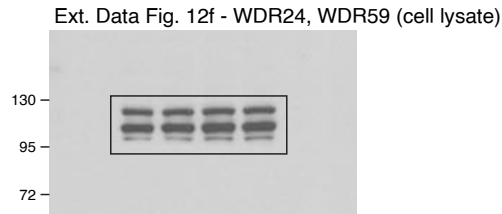
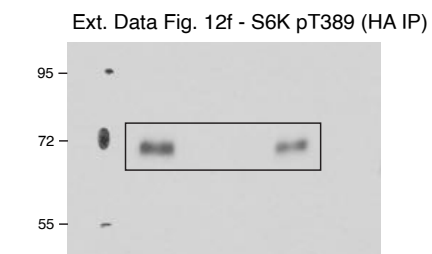
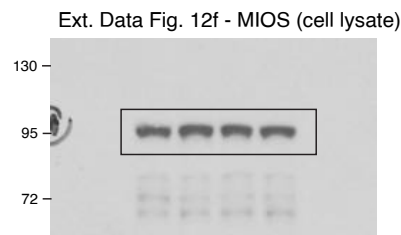
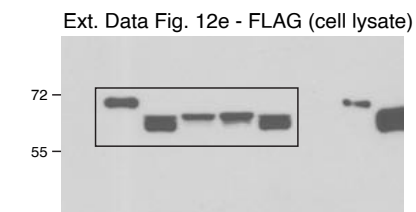
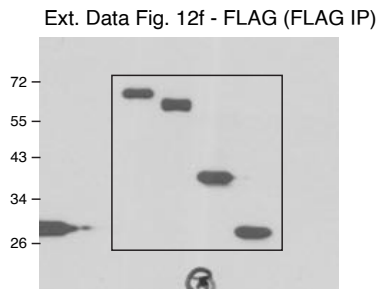
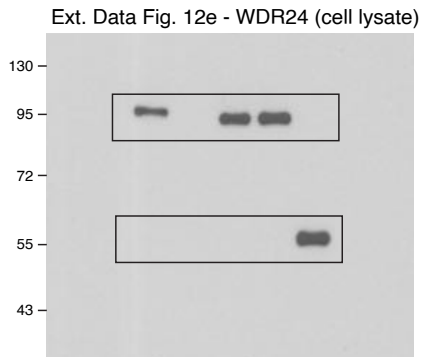
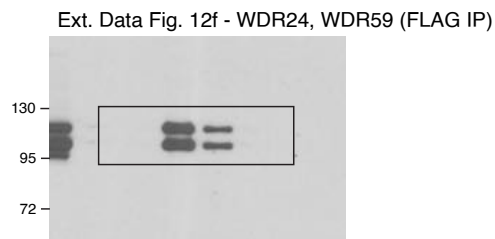
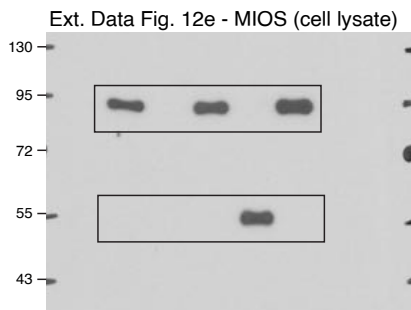
Ext. Data Fig. 11i - Raptor



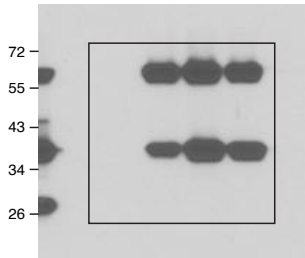


# Supplementary Figure 1 | part 24

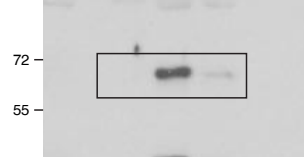




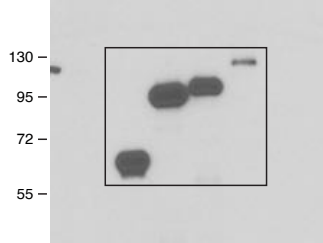
Ext. Data Fig. 12g - HA (FLAG IP)



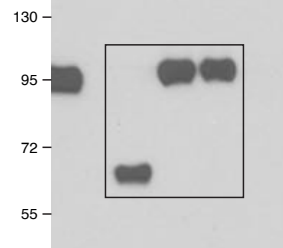
Ext. Data Fig. 12h - NPRL3 (FLAG IP)



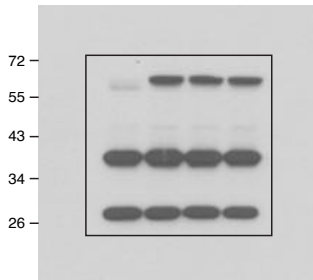
Ext. Data Fig. 12g - FLAG (IP)



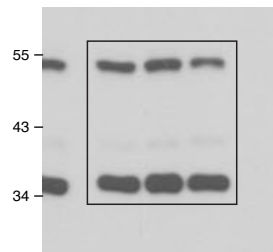
Ext. Data Fig. 12h - FLAG (FLAG IP)



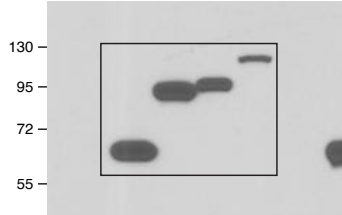
Ext. Data Fig. 12g - HA (cell lysate)



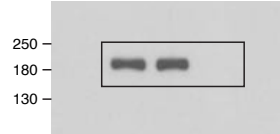
Ext. Data Fig. 12h - RagA, RagC (cell lysate)



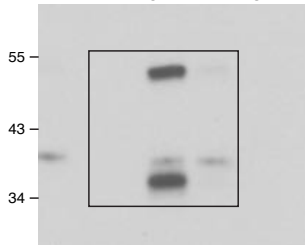
Ext. Data Fig. 12g - FLAG (cell lysate)



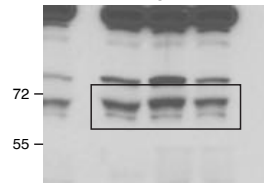
Ext. Data Fig. 12h - DEPDC5 (cell lysate)



Ext. Data Fig. 12h - RagA, RagC (FLAG IP)



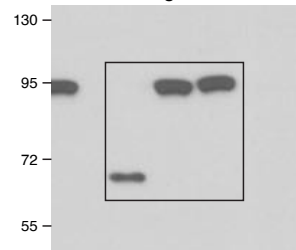
Ext. Data Fig. 12h - NPRL3 (cell lysate)



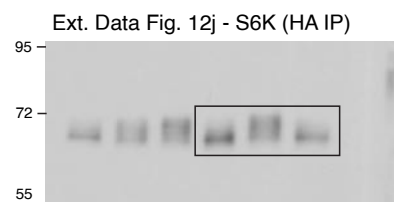
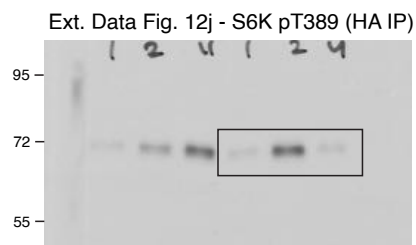
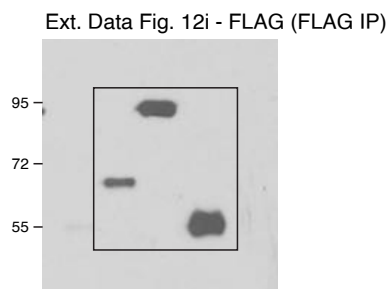
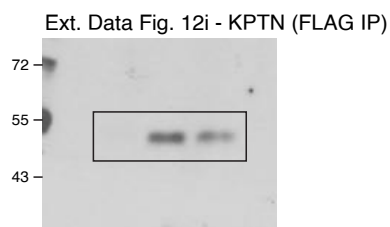
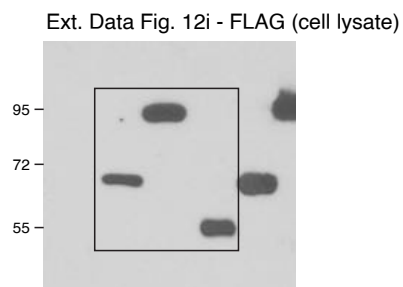
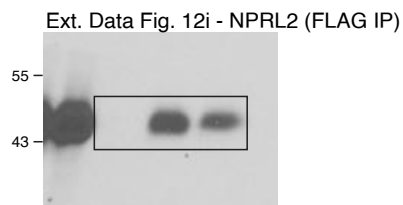
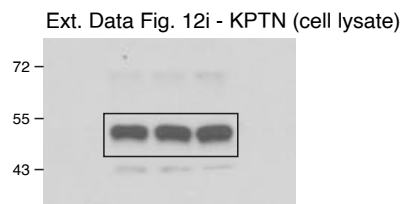
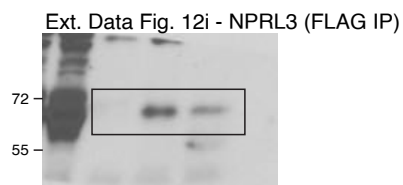
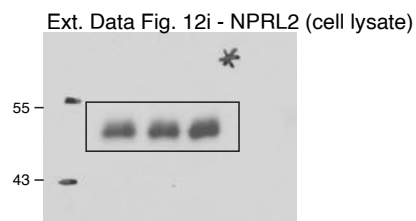
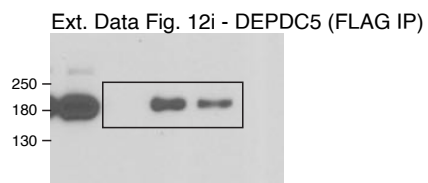
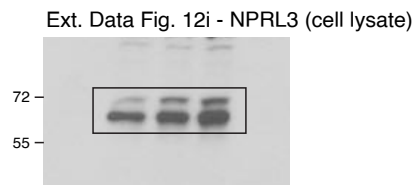
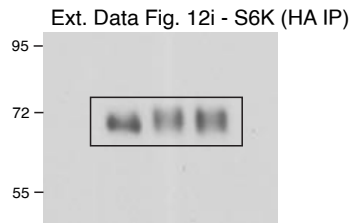
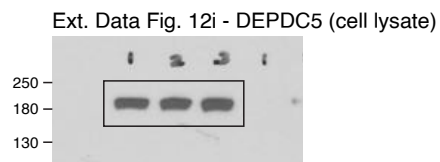
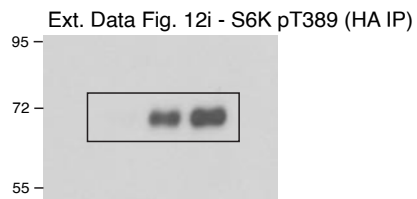
Ext. Data Fig. 12h - DEPDC5 (FLAG IP)



Ext. Data Fig. 12h - FLAG (cell lysate)



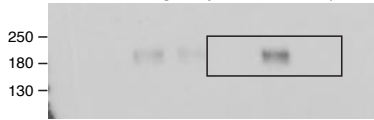
# Supplementary Figure 1 | part 27



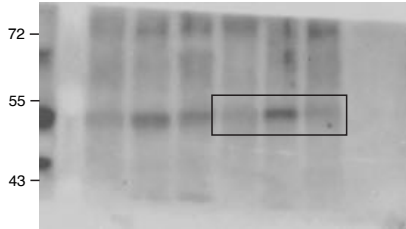


# Supplementary Figure 1 | part 28

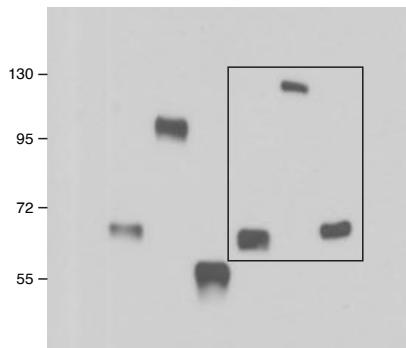
Ext. Data Fig. 12j - DEPDC5 (FLAG IP)



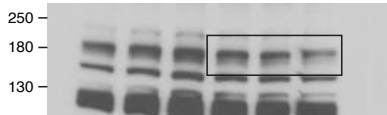
Ext. Data Fig. 12j - KPTN (FLAG IP)



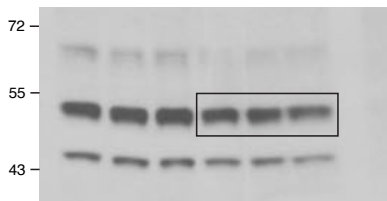
Ext. Data Fig. 12j - FLAG (FLAG IP)



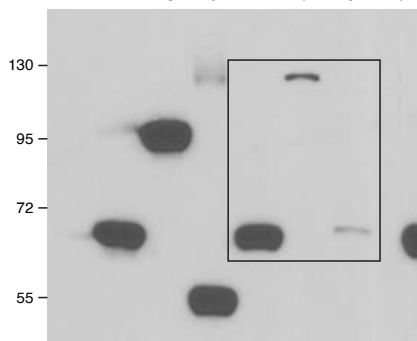
Ext. Data Fig. 12j - DEPDC5 (cell lysate)



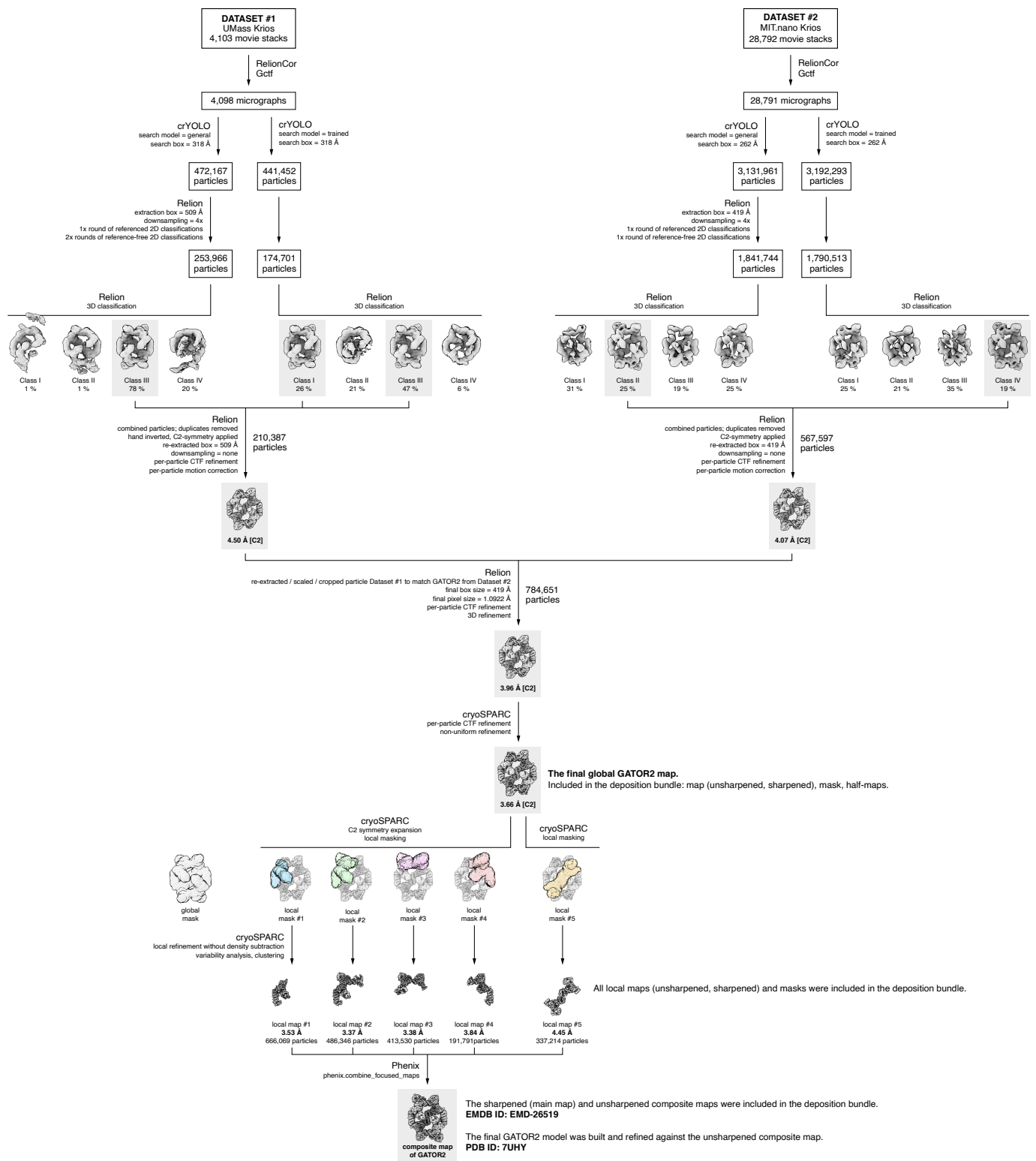
Ext. Data Fig. 12j - KPTN (cell lysate)



Ext. Data Fig. 12j - FLAG (cell lysate)

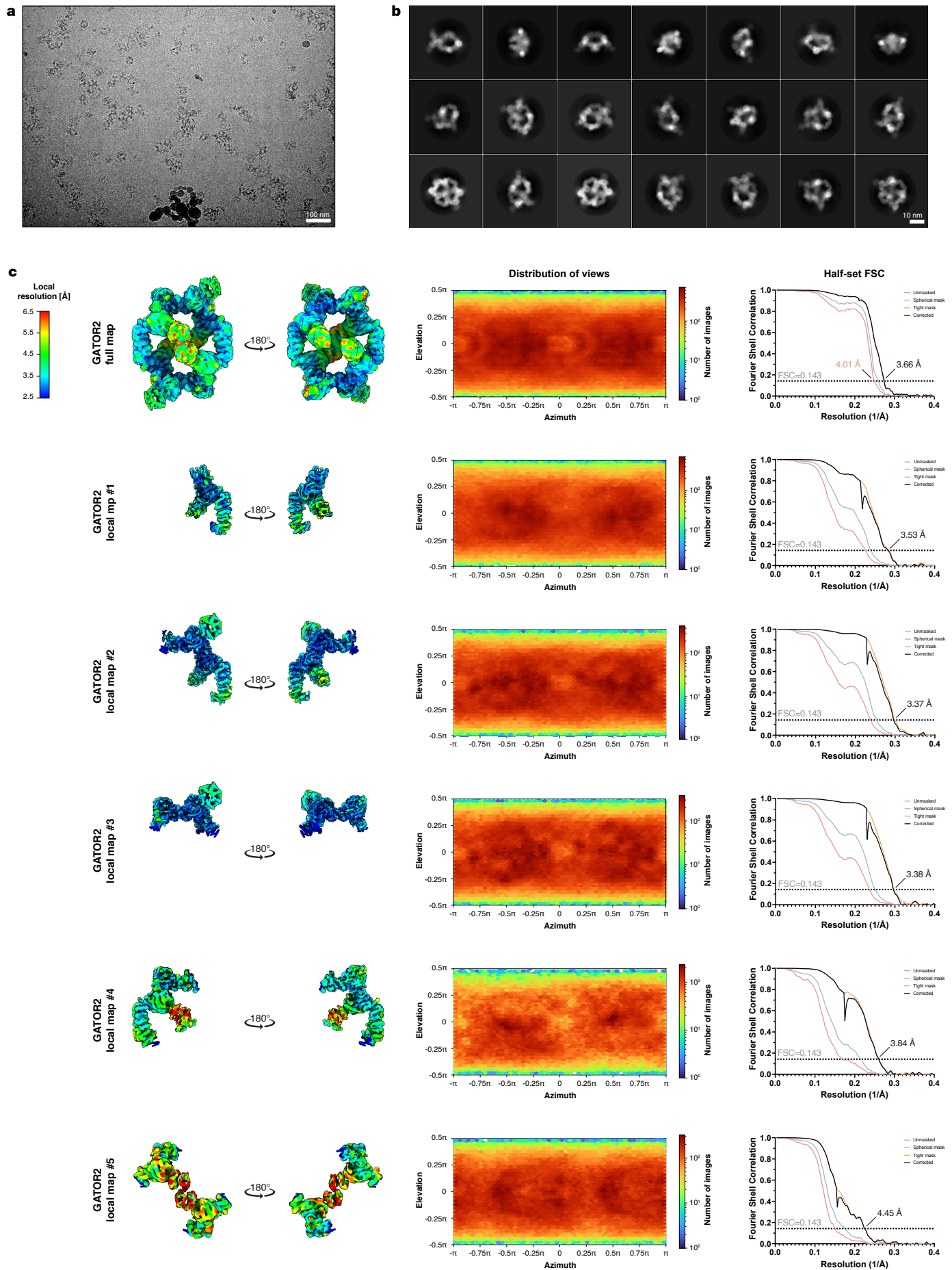


# Supplementary Figure 2



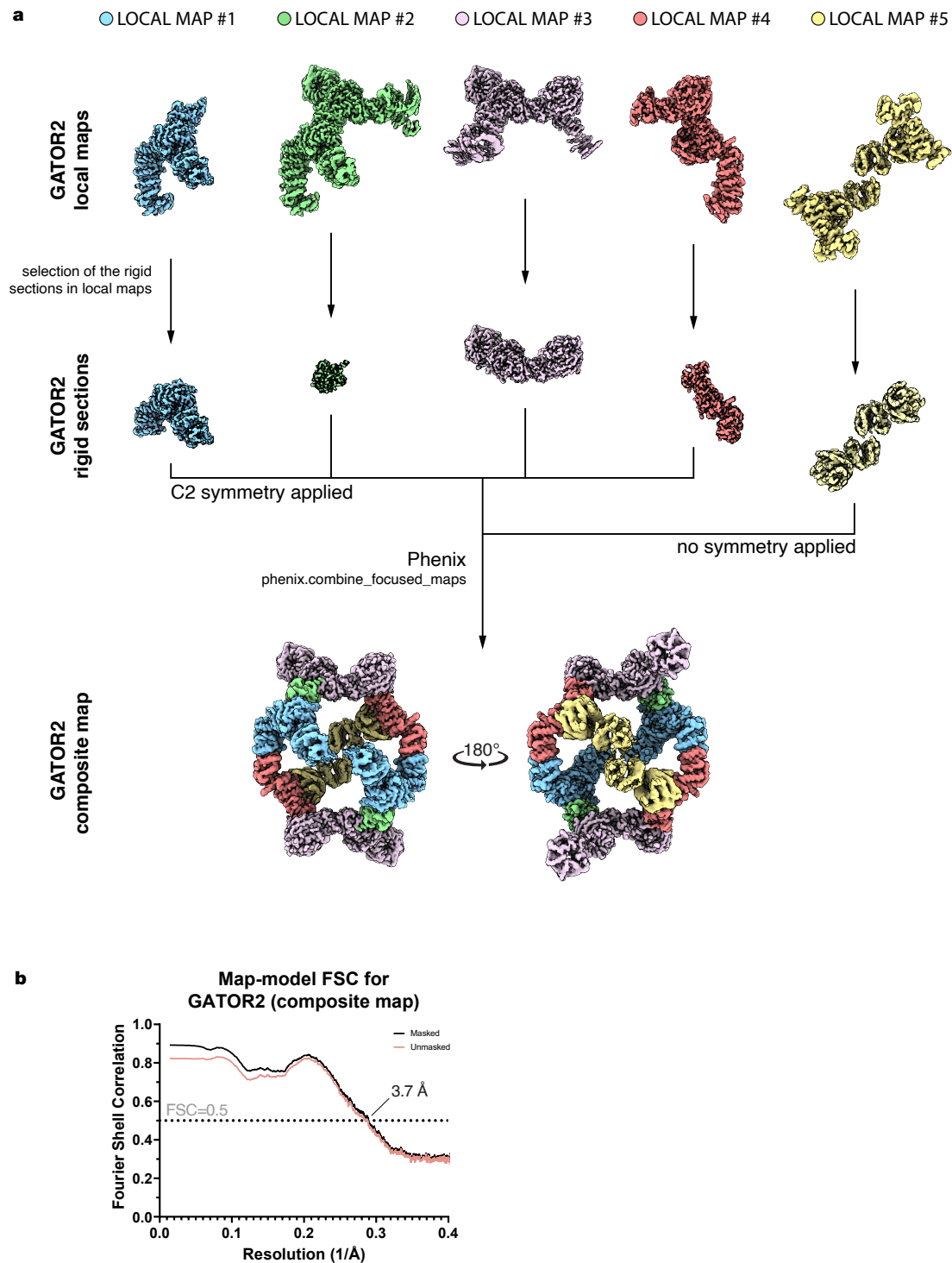
**Supplementary Fig. 2 | Cryo-EM data collection and processing.** Workflow for data collection and processing of the GATOR2 datasets.

# Supplementary Figure 3



**Supplementary Fig. 3 | Structural determination of GATOR2. a**, Representative micrograph of grids spotted with GATOR2. **b**, Representative two-dimensional class averages of the GATOR2 complex. **c**, Local resolution, view angle distribution, and half-set gold-standard Fourier shell correlation (FSC) for the full GATOR2 map (top) and local maps used for generation of a composite map.

## Supplementary Figure 4

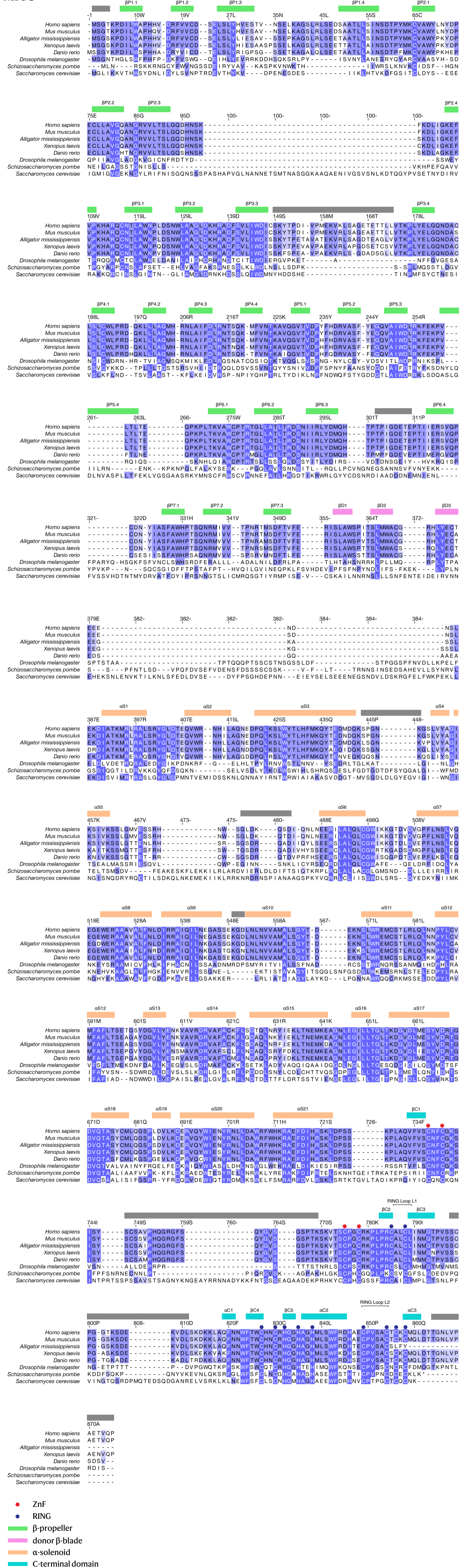


**Supplementary Fig. 4 | Generation of a composite GATOR2 map. a,** Flowchart depicting the assembly of a composite, high occupancy, GATOR2 map from rigid sections of local maps. **b,** Map-model FSC for the GATOR2 composite map.



# Supplementary Figure 5

## MIOS

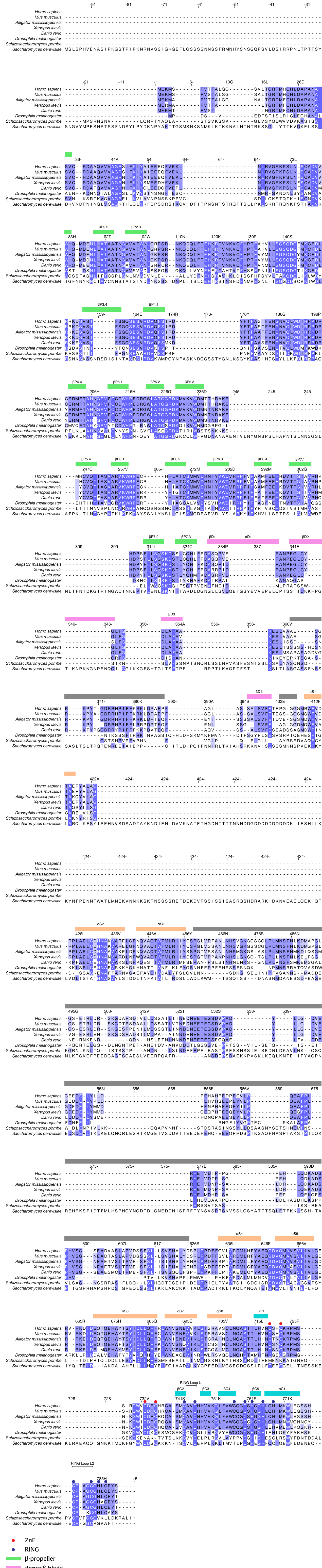


**Supplementary Fig. 5 | Sequence alignment of MIOS.** Sequence alignment of MIOS from various organisms. Residues are colored by percentage identity. Numbering refers to the human protein sequence. Secondary structure boundaries are indicated above the sequence alignment and are colored by domain. Residues that participate in zinc ion coordination are labeled with either red (ZnF) or blue circles (RING). Dark grey rectangles indicate regions unresolved in our cryo-EM structure.



# Supplementary Figure 6

## WDR24

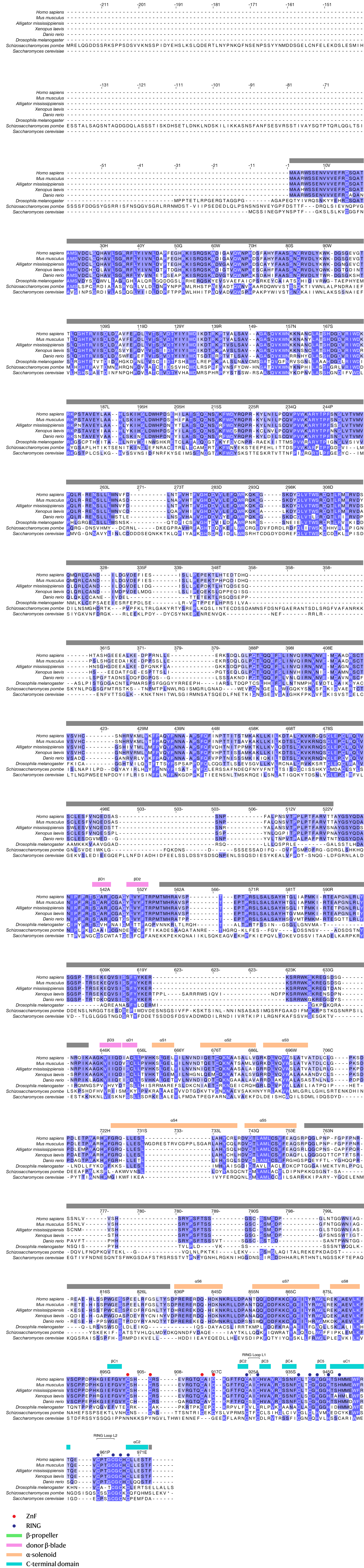


**Supplementary Fig. 6** Sequence alignment of WDR24. Secondary structure alignment of WDR24 from various organisms. Residues are colored by percentage identity. Numbering refers to the human protein sequence. Second structure boundaries are indicated above the sequence alignment and are colored by domain. Residues that participate in zinc ion coordination are labeled with either red (ZnF) or blue circles (RING). Dark gray rectangles indicate regions unresolved in our cryo-EM structure.



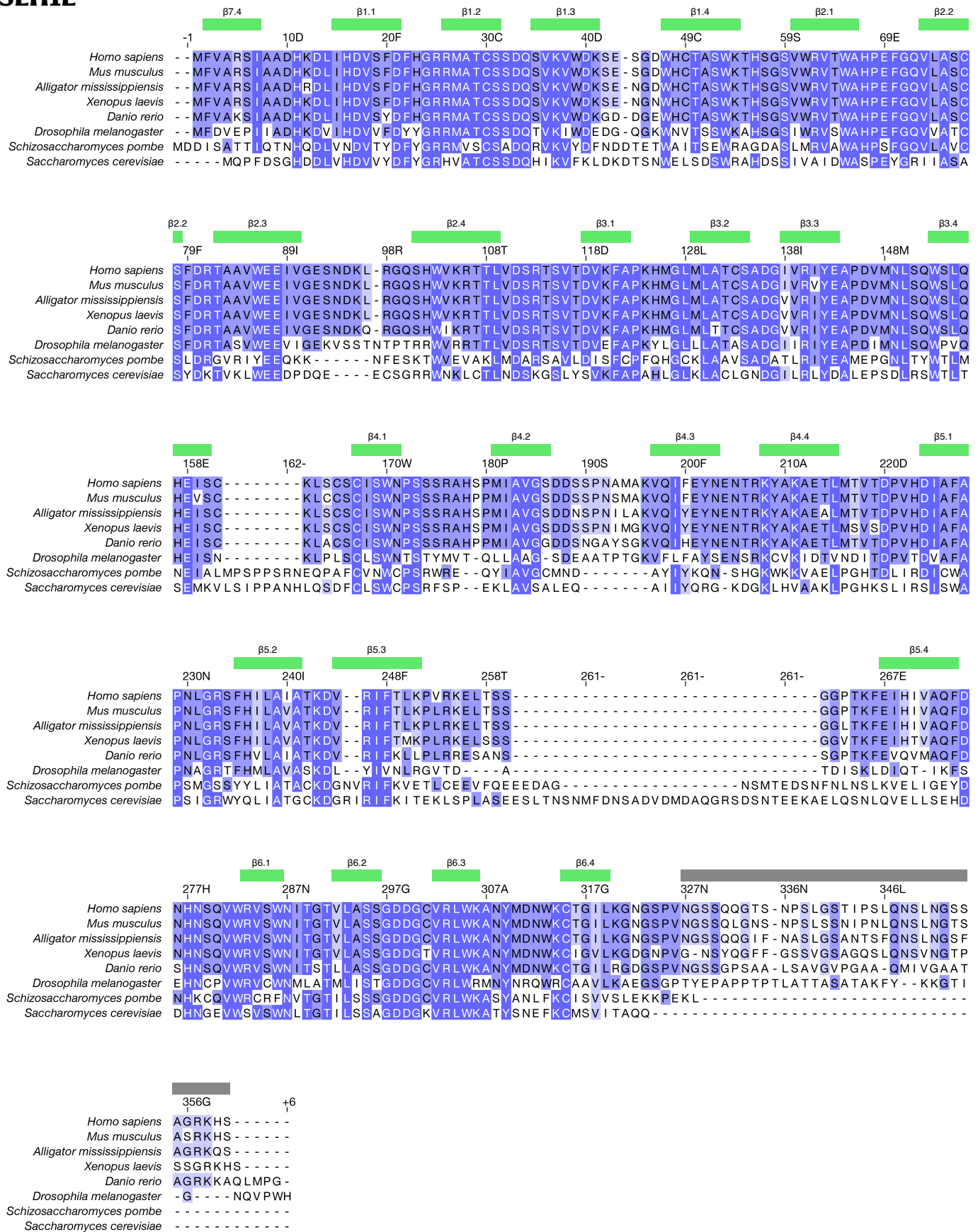
# Supplementary Figure 7

## WDR59



# Supplementary Figure 8

## SEH1L

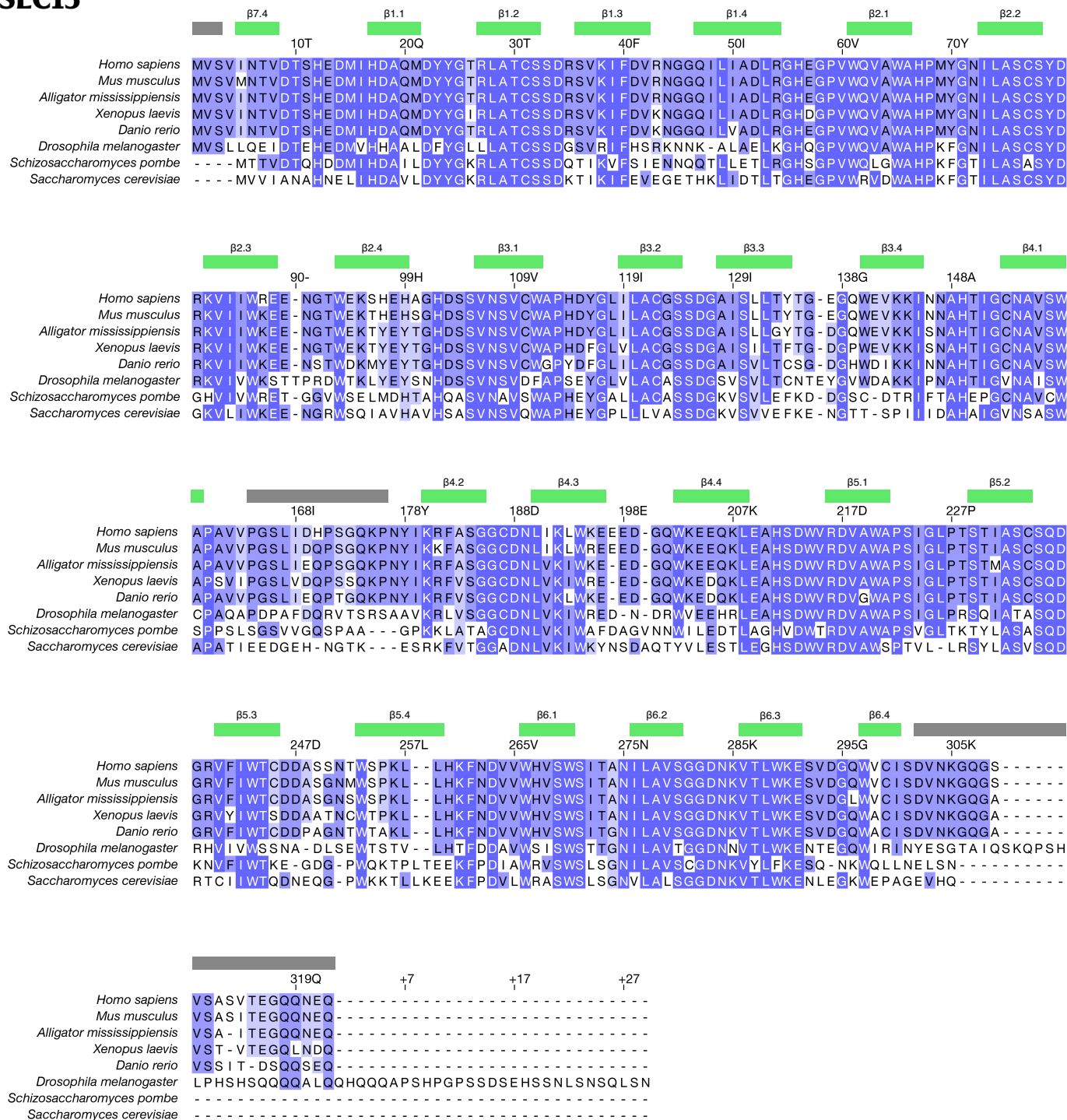


**Supplementary Fig. 8 | Sequence alignment of SEH1L.** Sequence alignment of SEH1L from various organisms. Residues are colored by percentage identity. Numbering refers to the human protein sequence. Secondary structure boundaries are indicated above the sequence alignment. Dark gray rectangles indicate regions unresolved in our cryo-EM structure.



# Supplementary Figure 9

## SEC13



**Supplementary Fig. 9 | Sequence alignment of SEC13.** Sequence alignment of SEC13 from various organisms. Residues are colored by percentage identity. Numbering refers to the human protein sequence. Secondary structure boundaries are indicated above the sequence alignment. Dark gray rectangles indicate regions unresolved in our cryo-EM structure.

# Supplementary Table 1

## Cryo-EM data collection, refinement and validation statistics

	GATOR2 global		GATOR2 local #1	GATOR2 local #2	GATOR2 local #3	GATOR2 local #4	GATOR2 local #5	GATOR2 composite
	#1 UMass	#2 MIT.nano	combined #1 and #2 C2-expanded	combined #1 and #2 C2-expanded	combined #1 and #2 C2-expanded	combined #1 and #2 C2-expanded	combined #1 and #2	composite of local maps
<b>Data collection and processing</b>								
Magnification (calibrated)	76,415	74,176						
Voltage (kV)	300	300						
Electron exposure (e <sup>-</sup> /Å <sup>2</sup> )	44	47						
Exposure time (s)	2.4	4.0						
Defocus range (μm)	0.8 - 2.0	0.8 - 2.0						
Pixel size (Å)	0.530	0.546						
Symmetry imposed	C2	C2	C1	C1	C1	C1	C1	pseudo C2
Initial particle images (no.)	913,619	6,324,254						
Final particle images (no.)	778,084 <i>combined datasets</i>		666,069	486,346	413,540	191,791	337,214	-
Map resolution (Å)	3.66		3.53	3.37	3.38	3.84	4.45	-
FSC threshold = 0.143								
Map resolution range (Å)	2.5 - 6.5							
<b>Refinement</b>								
Model resolution (Å)								3.48
FSC threshold = 0.5								
Model composition								
Non-hydrogen atoms								51810
Protein residues								7314
Ligands								32 (Zn <sup>2+</sup> )
<i>B</i> factors (Å <sup>2</sup> )								
Protein								185.85
Ligand / ion								159.73
R.m.s. deviations								
Bond lengths (Å)								0.003
Bond angles (°)								0.434
Validation								1.21
MolProbity score								4.34
Clashscore								2.21
EMRinger								0.35
Poor rotamers (%)								
Ramachandran plot								98.12
Favored (%)								1.88
Allowed (%)								0.00
Disallowed (%)								
EMDB accession code	26519		26519	26519	26519	26519	26519	26519
	<i>additional</i>		<i>additional</i>	<i>additional</i>	<i>additional</i>	<i>additional</i>	<i>additional</i>	<i>main map</i>
	<i>map</i>		<i>map</i>	<i>map</i>	<i>map</i>	<i>map</i>	<i>map</i>	
PDB accession code								7UHY

Supplementary Table 1 | Summary of cryo-EM data collection, 3D reconstruction, and model refinement.