### **Supplementary information**

# **Structure of the nutrient-sensing hub GATOR2**

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reconstruction, and model refinement.

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#### Fig. 4a - KPTN (cell lysate)



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



Fig. 4a - FLAG (cell lysate)





Fig. 4c - DEPDC5 (IP)

250 -	
180 —	
130 -	

#### Fig. 4c - HA-Sestrin2 (IP)



#### Fig. 4c - FLAG (IP)





-

6000 -

1.14

-

26



72

55

43

34

26

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









Ext. Data Fig. 1c - S6K pT389

-	-		
72 –	 -	-	
55 –		-	

#### Ext. Data Fig. 1c - S6K



Ext. Data Fig. 1c - Raptor



Ext. Data Fig. 1d - SYPRO Ruby





Ext. Data Fig. 1e - DEPDC5 (cell lysate)
Ext. Data Fig. 1e - NPRL3 (cell lysate)
72 -
55 -
Ext. Data Fig. 1e - NPRL2 (cell lysate)
43 -
Ext. Data Fig. 1e - KPTN (cell lysate)
55 - 43 -
Ext. Data Fig. 1e - WDR24 (cell lysate)
95-
72 –
Ext. Data Fig. 1e - MIOS (cell lysate)
95 - 72 -
and the ers and the
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate) 43 - 34 - Ext. Data Fig. 1e - SEC13 (cell lysate) 43 -
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)
Ext. Data Fig. 1e - SEH1L (cell lysate)







Ext. Data Fig. 6b - Coomassie (MIOS ΔCTD)



Ext. Data Fig. 6b - Coomassie (WDR24 ΔCTD)









#### Ext. Data Fig. 6c - SEH1L (cell lysate)



Ext. Data Fig. 6c - SEC13 (cell lysate)



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













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



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

130 -		
95 —		
72 –		

Ext. Data Fig. 6e - WDR59 (IP)



Ext. Data Fig. 6e - SEH1L (IP)



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



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





Ext. Data Fig. 6f - S6K pT389 (HA IP)



Ext. Data Fig. 6f - S6K (HA IP)



Ext. Data Fig. 6f - DEPDC5 (FLAG IP)

250 -	
180 –	-
130 —	

Ext. Data Fig. 6f - KPTN (FLAG IP)



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



Ext. Data Fig. 6f - DEPDC5 (cell lysate)



Ext. Data Fig. 6f - KPTN (cell lysate)



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









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



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

95 -

72 -

55 -



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



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











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



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



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



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









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



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

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



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



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









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



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



#### Ext. Data Fig. 8c - Coomassie



Ext. Data Fig. 8d - Ubiquitin





95

Ext. Data Fig. 8e - Coomassie



Ext. Data Fig. 8f - Ubiquitin conjugates



Ext. Data Fig. 8f - UBE2D







Ext. Data Fig. 8f - NRF2





Ext. Data Fig. 8g - Raptor



Ext. Data Fig. 8h - S6K pT389



Ext. Data Fig. 8h - S6K



Ext. Data Fig. 8h - FLAG



Ext. Data Fig. 8h - Raptor







Ext. Data Fig. 10b - myc-MIOS (IP)
95-
Ext. Data Fig. 10b - FLAG-MIOS (IP)
95-72-
Ext. Data Fig. 10b - FLAG-METAP2 (IP)
72 - <b>•</b>
Ext. Data Fig. 10b - myc-MIOS (cell lysate)
Ext. Data Fig. 10b - FLAG-MIOS (cell lysate)
95
Ext. Data Fig. 10b - FLAG-METAP2 (cell lysate)
72 -
55 -

Ext. Data Fig. 10d - Coomassie (MIOS A560E)







Ext. Data Fig. 11g - WDH24 (cell lysate)
130 -
95 -
72 -
1234 1231
Ext. Data Fig. 11g - MIOS (cell lysate)
95
72 -
Ext. Data Fig. 11g - SEH1L (cell lysate)
43 -
34
schill input _s
Ext. Data Fig. 11g - SEC13 (cell lysate)
43 -
34 -
Ext. Data Fig. 11g - Raptor (cell lysate)
Ext. Data Fig. 11h - S6K pT389
55 -
Ext. Data Fig. Thi - Son
72 -
<b>5</b> 5 -
<b>600 600</b>
34 -
Ext. Data Fig. 11h - Raptor
130-

Ext. Data Fig. 11i - S6K pT389	
95 - 72 -	
Ext. Data Fig. 11i - S6K	
55 -	
Ext. Data Fig. 11i - SEC13	
	4
Ext. Data Fig. 11i - Baptor	
180 - 130 -	





Ext. Data Fig. 12d - HA-Sestrin2 (cell lysate)



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



Ext. Data Fig. 12e - HA (IP)



Ext. Data Fig. 12e - WDR59 (IP)



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



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



Ext. Data Fig. 12e - WDR59 (cell lysate)





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



Ext. Data Fig. 12f - S6K pT389 (HA IP)



Ext. Data Fig. 12f - S6K (HA IP) 95 -72 -

Ext. Data Fig. 12f - MIOS (FLAG IP)

95

72

Ext. Data Fig. 12f - WDR24, WDR59 (FLAG IP)



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



Ext. Data Fig. 12f - MIOS (cell lysate)



Ext. Data Fig. 12f - WDR24, WDR59 (cell lysate)



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



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



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



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



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

130 -95 -72 -55 -

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



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



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



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



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



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

250 -	
180 -	
130 -	

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



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











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



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 (FCS) for the full GATOR2 map (top) and local maps used for generation of a composite map.





Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	EKDIATKMRLRALSRYGL EKDIATKMRLRALSRYGL EKDIATKMRLRALSRYGL DRDIATKMRLRALSRYGL EKDIATKMRLRALSRYGF ELDLVDETRQRALEDYGI GSDIQGTILDRVKKGYQF EKDISVIMRTRASLGYGL	DTEQVWR NHI LAGNEI DTEQVWR NHI LAGNEI DTEQVWR NHL LAGNEI DTEQVWR NHL LAGNDI DTVQVWR NHV LAGGDI KPDNKRF G ELHL DSQKN SELVS DPMNTVEMIDSSKNLQNI	DPOLKSLWYTLHFMKO DPOLKSLWYTLHFMKO DPOLKSLWYTLHFMKO DPOLKSLWYTLHFMKO DPOLRSLWYTLHFMKO TPYLRNVWSTLNNV 1 DLYLKDLWSWIHLSHRO NAYIRNTWRWIAIAKAS	(TEDMDQKSPGN (TEDNDQKSPGN (TEDMDQKLSGN (AEDIDQKSSGN (AEDIDQKQSSGN (SEDRLTGLKAT QSESLFGDTGDTDF SVDDGT - MVSGDLDL	KGSLVYAGI KGSLVYAGI KVPLVYAGI KVPLVYAGI KQLLVYAGI KQSLIYSGI LGI - NLCH SYQGALGI - WFMD GYEGVIGI - WNCI
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	aS5 457K 467V KSIVKSSLGMVESSRH KSIVKSSLGTTENLRH KSIVKSSLGTTENLRH KNIVKSSSGTTET-RR TSEALMASSRIESQVL TELTSMSDVFEAK NGISNQDRYRQETILSDK	473- 475- 	480- 48 DK QSDI - QNLNE DK QADI - QNLNE DR QADI VHYSE DR QADI VHYSE NSP SNKLI CYRSE R LDLDI FTSI QTKRPI DRNSP I ANAAGSPKY VC	aS6 BE 498G RILALQLCGWIKKG RSLALQLCGWIKKG RSLALQLCGWIKKG RSLALQLCGWIKKG RSLALQLCGWAFE RSLALQLCGWAFE RSLALQLCGWAFE RSLALQLCGWAFE RQLALLACGLGMSN DRLCLIISGWDLSR	aS7 508V T D V D VG P F L NS L VQ T D V D VG P F L NS L VQ T D L D VE P F L NS L EQ P D T D VE P F L KS L EQ - Q E L D R F I D Q L Y A D - D L L L E I R R L I R S - D YE D K Y N I I MK
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces cerevisiae	aS8 518E 528A EGEWE RAAAVALFNLDIF EGEWE RAAAVALFNLDIF EGEWE RAAAVALFNLDIF EGEWE RAAAVALFNLDIF EGEWE RAAAVALFNLDIF EGEWE RAAAVALFNLDIF KNEHYKAAGLALFHGKIE NGHYEKAAAWAVFFGDIF	aS9 538 548E IRA I Q L NEGASSE KGDLI IRA I Q L NEGASSE KGDLI IRA I Q L NKGASSG KGDLI IRA I Q L NKGASSE KGDLI IRA I Q L NKGASSE KGDLI IRA I Q L NKGASSE KGDLI IRA I Q L SSAADNMRDPSI NVVR L SSANE - L VA VE L SSAKKER	aS10 558A 56 NLNVVAMALSGYT - D NLNVVAMALSGYT - D NLNVVAMALSGYT - D NLNVVAMALSGYT - D NLNVVAMALSGYT - D NLNVVAMALSGYT - D EKTISTAVAGY ITSOC LRLIATA IAGY LAYKE	aS11	aS12 581L STLRLQLNNPYLCV STLRLQLNNPYLCV STLRLQLNNPYLCV SSLRLQLKKPYLCI SSANMQIHDPHLRA RNLSTELEDPYLRA RKMSSELDDPYLRV
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces cerevisiae	aS12 591M 601S MFAFLTSE TGSYDGVLYE MFAFLTSEAGAYDGVLYE MFAFLTSEAGSYDGVLYE MFAFLTSEPGVYDGVLYE MFAFLTSEPGAYDGVLYE VFSFLTMEKDNFDAVLKE IFAYVSN - SDWRDVLDE IFAFIAD - NDWWDILYE	aS14 611V 621C NKVAVRDRVAFACKFLS NKVAVRDRVAFACKFLS NSMAVRDRVAFACKFLS NSMAVRDRVAFACKFLS SRVAVRDRVAFACKFLS EGVSLSDRMAFACKFLN EGVSLSDRMAFACKYLS VSLSLKDRLGIALRFLP PAISLRERLGVALRFLN	aS15 631R 64 7 QL NRY I EKLTNEMKE AQL NRY I EKLTNEMKE AQL SRY FOKLTNEMKE AQL PRY I DKLTNEMKE EKLADY VAQQ I QAA I D DDL SNY LCDL CHTTVG TDL TTFLDRTSSTV I E	αS16 IK 651L AGNLEGILLTGLTK AGNLEGILLTGLTK AGNLEGILLTGLTK AGNLEGILLTGLTK AGNLEGILLTGLTK AGNLEGILLTGLTK AGNLEGILLTGLTP NGELEGLILTGITP	aS17 661L DGVDL MESYVDRTG DGVDL MESYVDRTG DGVDL MESYVDRTG DGVDL IESYVDRTG DGVDL MESYVDRTG DGVDL MESYVDRTG DGVDL MESYVDRTSF LGMEL LQSYMDTSF NGIDL LQSYVNKTS
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	aS18 aS19 671D 681Q DVQTASYCMLQGSPLDVL DVQTASYCMLQGSPLDVL DVQTASYCMLQGSPSDVL DVQTASYCMLQGSPSDVL DVQTASYCMLQGSPSDVL DVQTASFCMLKGSPGEVL DVQTASFCMLKGSPGEVL DVQTALIAAFVVPK-KF DVQTALIAAFVVPK-KF	aS20 691E 701R KDERVQYWIENYENLLD/ KDERVQYWIENYENLLD/ KDERVQYWIENYENLLD/ KDERVQYWIESYENLLD/ KDPRVQCWIENYENLLD/ KDPRVQCWIENYENLLD/ CORVDEWIENYENLLD/ CORVDEWIENYENLLD/ CORVDEWIENYENLL/ CORVDEWIENYENL/ CORVDEWIENYEN	aS21 711H 72 WRFWHKRAEFDIHRS WRFWHKRAEFDIHRS WRFWHKRAEFDIHRS WRFWHKRAEFDIHRS WRFWHKRAEFDIHRS WRFWHKRAEFDIHRS WRFWHKRAEFDIHRS WKLYRERAKFDIFRTE WKLYRERAKFDIFRTE	1S 728- CLDPSS KP CLDPSS KP CLDPSS KP CLDPSS KP CLDPSS KP SIRPSS KP SIRPSS KP SIRPSS KP SIRPSS KP SIRPSS KP SIRPSS KP SIRPSS KP SIRPSS KP SIRPSS	βC1 734F LAQVFVSCNFCGKS LAQVFVSCNFCGKS LAQVFVSCNFCGKS LAQVFVSCNFCGKS SRTVFLSCNFCGKS SRTVFLSCNFCGKS EPSIRIICNFCRKP PRQIYIQCQNCKQN
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Dania rerio	744I 749S ISYSCSAVPHQGF ISYSCSAVPHQGF	759S 760- IGFS	764S 77 QYGVSGSPTKS QYGVSGSPTKS	RII BC2 DS 780K VTSCPGCRKPLPRC VTSCPGCRKPLPRC	ALCL I NMGTPVSSC

RING Loop L2 aC1 βC4 βC5 aC2 aC3 810D 820F 850P 860Q 830C 840L 800F 808 SACTCKCMQLDTTGNLVP TWCHNCRHGGHAGHMLS TWCHNCRHGGHAGHMLS PVSAC PG - GTKSDE PG - GSKSDE Homo sapiens KVDLSKDKKLAQFNNW RD KCMQLDTTGNLVP KCMQLDTTGNLVP RCFDMPO KVDLSKDKKLAQ FNNV RDHAE Mus musculus PG-GSKSDE KVDLSKDKKLAQ<mark>F</mark>NN TWCHNCR нб<mark>б</mark>н G MLS Alligator mississippiensis RD SΑ SLFY-KVDLSKEKKVAKFNNV KADLTRDKKLAQFNNV P - - DVPGWQTKPFSKV TWCHNCRHCGHAGHMLS TWCHNCRHCGHAGHMLS SWCQTCRHCGHAGHMLS SFCLNCGHGAHASHASE SACS Xenopus laevis PG - ASKSDE KDHSE - TGKADE RDHSE РG Danio rerio NG-ETPTTT---KQNSE N D Drosophila melanogaster SS -QNYVKEVNLQKSRFGLV Schizosaccharomyces nombe KDDFSQKP - -V P N Е STHTI KLK\*-----VINGTQSRDPMQTEDSQDGANRELVSRKLKLNE<mark>WF</mark>SFCLSCNHGMHAGH</mark>AEE Saccharomyces cerevisiae DRHNV PTPG

	870A
Homo sapiens	A E T <mark>V</mark> Q P
Mus musculus	A E T <mark>V</mark> Q P
Alligator mississippiensis	
Xenopus laevis	AENVQP
Danio rerio	SDS <mark>V</mark>
Drosophila melanogaster	RDIS
nizosaccharomyces pombe	
Saccharomyces cerevisiae	

ZnF

Scł

RING

**B**-propeller

donor β-blade
α-solenoid
C-terminal domain
unresolved

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 gray rectangles indicate regions unresolved in our cryo-EM structure.

33 | Nature | Valenstein and Rogala et al. (2022) | https://doi.org/10.1038/s41586-022-04939-z



Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	NL I FN I DKGTR I NGWD	HDPYFLLS HDPYFLLS HDPYFLLS DSHCLLS ELLS INKEPTVLEN	GSKDSTLYQ GSKDSTLYQ GSKDSTLYQ ISKDSTIYK CSKDGIFSQ L <mark>SK</mark> NT <mark>T</mark> TWR	H I FKDASQP I H I FRDARRP I HMFKDASRPV HAFKDATRPA TRVENAFNC I DLDGNGLLSV	D	RANPEGL RANPEGL KANPEGL KANAQGA KANAQGA MLPRATS	.СҮ .СҮ .СF .SL .SW .SW
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	346- 346- 	350- G	βD3 354A - DLA F AA - S L V F SSNP G LTS F TPE -	356- 	356- 	360V - KESLVAAE - - KESLISSO - KESLISSO - KESLMSAPA - KIKEYEPKT SLKSALYASQN - KSLTLASGA	
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	371- RKPYT-GDRRHP RKPYA-GDRRHP RKPYI-GDRRHP RKPYDRRHP RKTYPGGDRRYP NTKSS GSTSNP SASLTSLTPQTENREE	380K IFFKRKLDPA IFFKRKLDPA IFFKRKLDPT IFFLRRPDPT SFIRRKTNVA VFEVPHN IAIEPP	390- E P F E Q F E Q F G S I Q F H L D H F C I I T L D I	390A 	βD4 394S 	403E (FETEPG-GGG (FETESS-GGS (FETDVE-SGS (FEAG-GGE (FESEADSAG SESVSRPTQE LAYRSEE SESSSMKNSF	412F 412F MRWEVD SMSWEVD MDWEVR MDWEVA MDWEIN HESFIG VAQECY VEKEKY
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	422A TAERYALAG TAERYVLAG TAKQYVLAG TAERYALAG TAQSYLLSG CARELVISG LAKNYRISG LARQLKFSYIREHNVS	424-	424-	424-	424-	424-	424-
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	424- 42	24- 4; 	24-	424- 	424- 4 SASRQSHDRAF	24- 4 	24-
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	426L 436V + PLAELCDHNAKVAR - RPLAELCDHNAKVAR - RPLAELCDHNAVAK - RPLAELCDHNAVAK - RPLAELCDHNAVAK - KKLSELCEHNAHVAK - KKLSELCEHNAEVSK - D - ISSACKENAFFAR LVDLISIATHNASVYL	446A E LGRNQVAQT E LGRNQVAQT D LNRWQVAQT S LNRPQESTT KYGKHNATTL NVGAE FAYQI S I DD LTNFK I	aS3 WTMLRIIYC WTMLRIIYC WTMLRIIYS WTMLRIMFS WNFIKLFYG WDALYFSLG WILIRDSLL	Y 466 SPGLVPTANL SPGLVSSANL SPGTVSSANL SPGTVPPANP EPAN-PSLST SNHFEPPFEH VLNNT	N 476S NHSVGKGSSCG NHSVGKGSSCG NHSMGKGS-AS NHSLGKSG-TS NHNLNKS-GN RSSFSNQK SDKGISELIN SSQISSDN	486N LPLMNSFNLK LPLMNSFNLK LPLMNSFNMK LPLVNSFSMK - NPMNSRRAT I PFVSANNS- ASNMDANESS	DMAPGL DMGPGL DIQSGM ELPSGI EMSGAL QVASDW - MADDE SDFEAGE
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	495G 503- - GS - ETRL DR - SKGD - GS - ETRL DR - SKGD - GS - ESRL DR - SKGE - VG - ESRL EH - SKGD - NE - RNKENR - PQQRTE L GQ DLNG KGRNLKNL TQ ISTS NLKTGKE FPEEDGAGT	512V ARSDTV LLDS TRSDAALLDS SRPENILMDS SRADSILMDP - QDN - IHSL NTPET - AHEI STP AHDN SGAESLVEER	522T SATLITNED SATLVTNED SSTLINNDD A - AINNDE ETNLNNNDE DV - ANVDDD LSLNDF PQAFR	532S NEETEGSDVP NEETEGSDVP NEETEGSDVP NEETEGSDVP NEETEGSEGV TLGSSGVEHP FEPR-EASTP ANSDEP	538- AD	539- - Y LL - Y LL - Y LL - Y LF SETQ LF SEDNLDKAVLN - KEQLKNTE I I	.G DVE .G DVE .G DVE .G DVE .G DVE .V DDE .S E I T JK QSQ PYAQPN
Homo sapiens Mus musculus Alligator mississipiensis Xenopus laevis Dario rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	553L GEEDE - LYLLD GEEDE - LYPLD GDEDE - LYMMD GDEDE - LYMMD LDDDE - LYSME FDNFE - LL WHDLE NPIVLKK EDSDEVLTKLKELQNQ	555- 	555- 	556E PE HAHPE TE HV HSE HE NP HA QE QP HTE HD NQ PAE HD NQ PAE RN STD SRAS I E DE HE HQ - E	566V DPECVLP EQEYVLP EQEYVLP ESEYLP GFIYVGPTEC- NSSYLDSAAS EQPHDSPTKSA	569- QEA QEA QEA QEA QEA 	575- (FPL (FPL )FPL )FPL (FPL (FPA 1PILQK



Supplementary Fig. 6 | Sequence alignment of WDR24. Sequence alignment of WDR24 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 gray rectangles indicate regions unresolved in our cryo-EM structure.

WDR59							
	-211	-201	-191	-181	-171	-161	-151
Homo sapiens							
Mus musculus Alligator mississiopiensis							
Xenopus laevis							
Danio rerio Drosophila melanogaster							
Schizosaccharomyces pombe	MRELQGDDSSRKS	PPSDSVVKNSSP	IDYEHSLKSL	QDERTLNYPN	KQFNSENPSS	YYNMDDSGEL	CNFELEKDSLESMIH
Saccharomyces cerevisiae							
	_131	-101	_111	-101	-01	-81	-71
Homo sapiens					-31	-01	-, '
Mus musculus							
Xenopus laevis							
Danio rerio Drosophila melanogaster							
Schizosaccharomyces pombe	ESSTALSAQSNTA	QDGDQLASSSTI	SKDHSETLDN	KLNDSKILIK	KASNSFANFSI	ESVRSSTIVA	YSQTPTQRLQGLTSI
Saccharomyces cerevisiae							
	-51	-41	-31	-21	-11	-,1	10V
Homo sapiens						<sup>-</sup> MAARW	SSENVVVEFRDSQAT
Mus musculus Alligator mississippiensis				· · · · · · · · · · · · ·		MAARW <mark>MAA</mark> RW	SSENVVVEFRDSQAT SSENVVVEFRDSQAT
Xenopus laevis Danio rerio						<mark>MAA</mark> HW	SSENVVVEFR <mark>D</mark> SQAT
Drosophila melanogaster			MPPTET	LRPGERGTAG	GPG ,	AGAPEQTYIV	RQSNKYYEHRDSQAT
Schizosaccharomyces pombe Saccharomyces cerevisiae	SSSSFDDGSYGSR	R I S F NSQG V SG R 	LRRNMDST-V	IIPSEDEDLQ	LPSNSNSNVE MCSSINI	YGPFDSTTF- EGPYNSPTF-	DRQLSIEVNQPVG GKSLSLKVDGGFN
,							_
	30H	40Y	50G	60W	70H	80S	90W
Homo sapiens Mus musculus	AMSVDCLGQHAVL	SGRRFLYIVNLD SGRBFLYIVNLD	AP FEGHRKIS	RQSKWDIGAV	QWNPHDSFAH' OWNPHDSFAH'	YFAAS <mark>SNQ</mark> RV YFAASSNORV	DLYKWK - DGSGEVGT
Alligator mississippiensis	AMSVDCLGQHAVL	SGRRFLYIVNLD	VPTEGHRKIS	RQSKWDVGAV	QWNPH <mark>DSCAY</mark>	FAASSNORV	DLYKWK - DGNGEVCT
Xenopus laevis Danio rerio	AMSVDCHGQLAVL AMSVDCLGQHAVL	SGRRFLYIVNLD SGRRFLY <mark>V</mark> VNLE	TPIDGHRKIS TPSEAPRKIS	RQSKWDIGIV	QWNPHESCAH QWNPH <mark>KSD</mark> AH	FAASSNORV FAAS <mark>S</mark> NORV	DLYQWK - DGIGDINI DLYTWR - DGSGKSHT
Drosophila melanogaster	AMSVDYSGQWVLL	AGRGHLALQRLG		RQSKYEVSVA		Y CAIATSOHI	DIVRWG - TAEPHYEM
Saccharomyces cerevisiae	AVSINPSGRDIVL	ASRQGLYIIDLD	DPFTPPRWLH	HITPWQVADV	QWSPHPAKPY	VIVST <mark>SNO</mark> KA	IIWNLAKSS <mark>S</mark> NAIEF
Homo coniona					149-		
Mus musculus	TLQGHTRVISDLD	WAVFEPDLLVTS WAVFEPDLLVTS	SVDTYIYIWD	IKDTRKPTVA	LSAV AGAS	Q V KWNKKNAN	YLATSHDGDVRIWDK
Alligator mississippiensis Xenopus laevis	SLQGHTRVISDLD	WAVFEPDLLVTS WSVYEPDVLVTS	SVDTYIYIWD	I KDTRKPTVS	LSAV AGASO	QVKWNKKNAN QVKWNKKNAN	CLATSHDGDVRIWDK CLATSHDGDVRIWDB
Danio rerio	SLQGHTRVISDLD	WSWFEPEFLVTS	SVDTYIYIWD	TSDTRRPTVT	LSAV AGAS	V KWN RRNHY	CLASSHDGDVRIWDK
Drosopnila melanogaster Schizosaccharomyces pombe	MLHGHTRAVTDMN	WHRQNPDVLATC	SIDSSVHCWD	LREPREPALS LRSPRFPVNS	FYDW-HNGAT	QVGFNRVSGN QVKWNYKNPH	I LASSHGRLVRIWDD
Saccharomyces cerevisiae	VLHGHSRAITDIN	FNPQH <mark>PDVL</mark> ATC	SVDTYVHAWD	MRSPHR <mark>P</mark> FY <mark>S</mark>	TSSW-RSAAS	QVKWNYKDPN	V <mark>LA</mark> S <mark>SH</mark> GN <mark>D</mark> IFVWDL
	4.071	40511	00511	0450	005B	0040	0.145
Homo saniens	187L						
Mus musculus	RKPSTAVEYLAA -	-HLSKIHGLDWH	PDSEHIFATS	SQDNSVKFWD	YRQPR - KYLN	ILPCQVPVWK	ARYTPFSNGLVTVMV
Alligator mississippiensis Xenopus laevis	RKPNTAVEYLAA - RKPSTAVEYIAA -	- HLYKIHGLDWH - HLYKIHGLDWH	PDNEYTLATS PDSEYILATS	SQDNSV <mark>R</mark> FWD SQDNSVKFWD	YRQPR - KYLN SRQPR - KCLN'	ILSCQVPVWK /LPCQVPVWK	ARYTPFSSGLVTVMV ARYTPF <mark>N</mark> NGLVTVMV
Danio rerio	RKSNTAVEYVAA -	- HLSKINGLDWH	PDNEYILATS	SQDNSV <mark>R</mark> FWD			ARYTPFSNGLVTVMV
Schizosaccharomyces pombe	RYGSAPLHTIKTS	ENITKINGLE FN	RACETRLLTC	AMDRTVKFWN	YEKSTEEPEH	LITTDS <mark>PVW</mark> K	ARFTPFGDGVILM
Saccharomyces cerevisiae	RKGSTPLCSLKG-	- HVSSVNSIDFN	RFKYSEIMS <mark>S</mark>	SNDGTVKFWD	YSKSTTESKR <sup>®</sup>	TVTTNF <mark>P</mark> I <mark>W</mark> R	G <mark>RY</mark> L <mark>PF</mark> GE <mark>G</mark> YCIM
	2631	971_	979T	283D	2030	JOBK	308D
Homo sapiens	PQLR - RENSLLLW	NV F D	LNT <u>PVH</u> TF	VGHDDVVLEF	QWRKQKEG	SKDYQLV	TWSRDQTLRMWRVDS
Mus musculus	PQLR - RENSLLLW	NASD	LNAPVHTF	VGHDDVVLEF		SKDYQLV	TWS RDQ T L RMWRV DY
Anigator mississippiensis Xenopus laevis	PQLR - RENSLLLW	NVCD	LQAPVHIF	VGHDDVVLEF	QWRKQKEA	LKDYQLV	TWSRDQTLRMWRVDS
Danio rerio Drosophila melanogaster	PQLR-RENSLLLW PHLGRGENSLLLW	STLE	LNSPVHAF	VGHDDVVYEF VGHTDVILDE	QWRPQKEG AWRPNRES	SKDC <mark>QL</mark> V SNE   ELV	TLSRDQTLRIWRVDP TWSRDRTLRVWKIDD
Schizosaccharomyces pombe	PQRG - DNSVHMY -	- DCRNL D	KEGPRAVHRF	AGHTDQVKEF			TWSKDHHVRLWPIGN
Saccnaromyces cerevisiae	PINIV G - G NNAVY []	NLODDDSEQNK	RIKLQPIYA <mark>F</mark>		LWRSKHICDGI	υ τ UURE F <mark>QEV</mark>	

	328-	335F	339-	348T	358-	358-	358-	
Homo sapiens	QMQRLCAND ILD	G <mark>VDE</mark> FIES	I <mark>S L</mark> L <mark>P</mark> E	PEKTLHTED	TDHQ			
Mus musculus	QMQRLCAND I L D	G <mark>VDE</mark> FIES	I <mark>S L</mark> L <mark>P</mark> E	PEKTPHPQD	I D H Q			
nieciecinniancie					9590			

Alligator mississipplerisis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	QLQRLCAND QLQKLCCAND NMLKLCCANE NMLKLCEPSAEE DILNSMGHDRTK SIYGKVNFDRGK	IMDPVDELMD IVDEL IESRFEPDLS (PVPFKLT (RLEEKLP	G LS G LR E LR RLGAKYRTYS DY DYCSYNH	I PEQEKSLQPP TTETEKTLRSC VPTPPEFLHPF EPLKQSLINTE EPENRENVQK-	EQISG DSEPP SILVA CDSSDAMNSF	DSNFGAERAN - NEF	rsdlsrgfva - RRLR	FANRKK
	3619	371-	370-	370-	388D	3081	408E	
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	Solo 	EEEALKE - DP EEEALKE - DP DEEALKE - DF EDATFGE - ES ADNSLDQFDG ACNTLPMARS ITRSTKS TN ITRSTKS KN	975- PRNLLE PSSLLE PTTSLI PQS G PSFGGGYYRRE PMTPLNWLRG KTNHI TWLSG		DQ LG LPOTLQ DQ LG LPOTLQ DQ PG LPOTLQ EQ LG LPOTLQ AKND IPOTLQ LTDQPTCSLH - WEVPONLG FNETKIQNLG	QEFSLINVQIF QEFSLINVQIF QEFSLINVQIF QEFSLINVQIF QEFSLVNLQIF HEFSLNTNMF EELSWIGQKYS EEVSAIGHKFF	NVNVE - MDA NVNVE - MDA NVNVE - MDA NVNE - MDA NVNE - MDA NVNE - MDA NVNE - MDA NVSE NVSFE I D VVVFE KISV	ADRSCT ADRSCT GNRSCT MNRSCT VNRSCF IKRYAC AERTCT STRELC
Homo sapiens Mus musculus Alligator mississippiensis	423- VSVHC VSVHC VSVHC	429M - SNHRVKMLV - SNHRVKMLV - GSHIVTMLV	439N K FPAQYPNNA T FPAQYPNNA MFPVQYPNNA	4481 APSFQFINPTT APSFQFINPTT APSFQFVQHTN	458K TTSTMKAKLL TTSAVKAKLL TTPPMKTKLL	468T KILKDTALOKI KILKDTSLOKI KVLQDTSLOKI	478S /KRGQSCLEP /KRNQSCLEP /KRNQSCLEP	CLRQLV CLRQLV CLRQLV
Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	VSVHC VSADC FKICA ISLNAPILPD LTLNGPWSEENF	- GNHRVRMLV - GANRVRLVV - GGHTVILQV DGYAYIRLHV 2DDYIFLRISI	MFPVQYPNNA KFPAQYPNNA TFTTSYPSPS/ YFPNNYPISA NFPLNYPNKGI	APSFQFVHPTT APSFQFVPPTN PPDFQLCQGTT - PVFQLERSSA OPP <mark>K</mark> F <mark>TIEENSN</mark>	ISSAMKAKLL ISSSMKIKIQ LSSEVSGVLL FNDEQFNYVF ILTMSKRQEIL	KILKDTSLOKI KILTDTSLOKI KVLRCNALGRI NTLTSISDOC SNLATIGQKY	KRNQSCLEP KRNQNCLEP KKSRTCLEQ SSHKYCMDA DSNLYCLEP	CLRQLV CVRQLV CLRALV CLSYLS CIRFVL
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	498E SCLESFVNQEDS SWLESVVNQEDS SCLESFVNQESS SCLESDGVMED- AAMKKKVAAVGG GNLSVDEIWKLG GEKVSLEDIEG	503- A S	503- - FQKDNS DHIDFEELSSI	503- 	506- 	512V A L P NSV T P P L F A L Q NSV T P P L F A L A NSV T P P L F N L Q G P I T P P L F V L T N P V T P A L F L L Q S P R L E F Q D V F P S M Y K E A L V F P D T -	522V TFARVTTAYO TFARVTNAYO TFARVSNAYO TFARVPNAYO AFPRVTNTY OFROGODRG DFROGODRG SNQG-LDFG	GSYQDA GSYQDA GSYQDS GSYQDA GSYQDA STLHDA STLHDA LSHKHQ RNLALD
	βD1 542A	βD2 552Y	562A	566-	571R	581T	590R	
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	NIPFPRTSGARF NIPFPRTSGARF NIPFPRTSGARF NIPFPRTSGARF CIPYPRTSGVHF NIPLPKTCAAIF TTPVPNGCGSCV	CGAGYLVYFT CGAGYLVYFT CGAGYLVYFT CGAGYLVYFT CGTGSLVYFT NAIGMLTTFA CGNDELVCFF VTATGELFCF	RPMTMHRAVS RPMTMHRAVS RPMTMHRAVS RPMTMHRAVS RPMTMHRAVS QPVNNKRLTLF TIKADESAQA ANEKKPEKKQI		- EPTPRSLSA - EPTPRSLSA - EPTPRSLSA - EPTPRSLSA - EPTPRSLSA - ALTPRTFSS RQ-KLFES VEKHPFKIEP	LSAYHTGLIAF LSAYHTGLIAF LSAYHSGLITF LSAYHTGVMAF LSAYHSGVMTF IN-GSGLLGT FGVLDSS QVLYDKEVDSS	MKI - RTEAP MKI - RTEAP MKI - RTETP MKI - RTEAP MKM - RTEAP MKM - RTESQ VWMA - T SNSV AD	3NLRLY 3NLRLY GNLRLY GNLRIY TTLRLY SDSTNY ARPKRY
	609K	619Y	623-	623-	623-	623K	633G	_
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces ceravisias	SGSP - TRSEKEG SGSP - TRSEKEG SGSP - TRSEKEG SGSP - TRSEKEG SGSP - TRTDKDC AQF DDENSLNRGGTS	2VSISSFYYKE 2VSISSFYYKE 2VSISSFYYKE 2VSISSFYYKE 2VSISSFYYKE 2VSISSFYYKE 2EANASFYLQE 2ESDSEFIWDV 2ESDSEFIWDV	R	ARRRWSIQVI P-KSKTSINL-N	IN I NSASAS I M	KSRRW KSRRW -NDFPKSRRW KSRRW KSRRW GSRFGAADIFN	(SKREGSDSG (SKREGSDSG (SKREGTDAN (SKRENSDPN (GKREGGDYS - SGKPGKQRA NSKRPSTKGS - SSESKTV-	NRPSIL
Guerna on yees ceremsae	β	D3 aD1	aS1	αS	2	aS3		
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	646K - NRQ I KAAGKVI - NRP I KAAGKVI - NRP I KAAGKVI - SRP I KAAGKVI - NRP I KLAGKVI - I ROMNGSPVVI LSKPSHDFHVVF - ESTKKNKNLVI	GENERAL CONTRACT OF CONTRACT O	HKSLGELYIL HKSLGELYIL HKSLGELYIL HKTLGEMYIL HKTLGEMYIL SRRMAREFSL KRALAAEYVVI RKELALEYLFN	676T IVNDIQETCQKN IVNNIQETCQKN IHGNLQETCQKN IVDNIQTCQKN IVDIQDTCQKN IVNDIQDTCQKN IVNDIQETCRKN IVCNIAETCRKN IGDKVTVCNKN IDATPEGFARNN	686L AASALLVGRK ATSAMLVGRK AASALAIGRR ATAALSVGRR GAAALAVARR GEICRQHGRL AEVSAKHNYY ALVAEKFDLD	696W DLVQVWSLAT\ DLVQVWSLAT\ DLVQVWSLAT\ DLVQVWSLAT\ DLVQVWALAAA DLAKVWALASA DLVPVWLLAEI RLAKVWLMLGF EISHCWQILSI	706C / ATDLCLG / ATDLCLG / ATDLRLG ANDVCLS ASTNLNLS - I ATPQIP - 3 LLGHLSRTE DML I DQSDYD	PKSD PKSD PKPD PKSD PDPD HET - NKDHIN
	722H	731S	αS4 733-	7,33L	aS5 7,43Q	7 <mark>53E</mark>	7,63N	_
Homo sapiens Mus musculus Alligator mississippiensis Xenopus laevis Danio rerio Drosophila melanogaster Schizosaccharomyces pombe Saccharomyces cerevisiae	PDLETPWARHPF PDLETPWARHPF PDAETPWARHPF PDAETPWARHPF DAGTPWAKHPF - LNDLLFYKDPF DEEAFPWLKSPL - PYTTIWNNHPM	GRQ - LLESL- GRQ - LLESL GRQ - LLESL GRQ - LLESL GRH - LLE TL KKS - LLESL - AKWVVNSL GIKWFIKEA -	GDRESTRVCGF	PLSGARLAHY PLSGARLAHY LAHYS LAHYS LAHYS LHYS LEHYS LHYYA LHYYA LUYYA	RLRDVOTLAM QLRDVOTLAM QLHDVOTLAM GLSDVOSLAM ISGDIOTAVL SQCNTOMLAM RQQNLOMLAM	LCSV FEAQSRF LCSV FEAQSRF LCSV FEAQSRF LCSV FAQSRF LCSV FRGHGSF LACL FDKCPTC LACL FDKCPTC LACV LD I PNPF LCCV I LSARRF	VQGLPNP-FG VQGLPNP-FG LQGCPNS-CA QGGQTTCP VQEYFTL-YG VQGYTHEY VGGAIMEKTV (KQSGNT-SA (KIPARY-YG)	P F P N R - P F P N R - P F P Q R - T F T S R - H Q Q P R - R L P P Q L  Q E L E N M
	777-	780-	780-	780-	7905	798-	7991	
Homo sapiens	SSNLV V	'S <mark>H</mark>	<mark>SR</mark>	( <mark>PSFTSS</mark>	GSC - SS	M <mark>SD</mark> P	<mark>G</mark> LNTGG	<mark>WN I</mark> AG -



Supplementary Fig. 7| Sequence alignment of WDR59. Sequence alignment of WDR59 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 gray rectangles indicate regions unresolved in our cryo-EM structure.



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 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
Data collection and								
processing Magnification (calibrated) Voltage (kV) Electron exposure (e <sup>-</sup> /Å <sup>2</sup> ) Exposure time (s) Defocus range (μm)	76,415 300 44 2.4 0.8 - 2.0	74,176 300 47 4.0 0.8 - 2.0						
Pixel size (A)	0.530	0.546	C1	C1	C1	C1	C1	namida C2
Symmetry imposed Initial particle images (no.)	013.619	6 324 254	CI	CI	CI	CI	CI	pseudo C2
Final particle images (no.)	913,019 0,324,234 778,084 combined datasets		666,069	486,346	413,540	191,791	337,214	-
Map resolution (Å) FSC threshold = 0.143	3.66		3.53	3.37	3.38	3.84	4.45	-
Map resolution range (Å)	2.5	- 6.5						
Refinement Model resolution (Å) FSC threshold = 0.5 Model composition Non-hydrogen atoms Protein residues Ligands <i>B</i> factors (Å <sup>2</sup> ) Protein Ligand / ion R.m.s. deviations Bond lengths (Å) Bond angles (°) Validation MolProbity score Clashscore EMRinger Poor rotamers (%) Ramachandran plot Favored (%) Allowed (%) Disallowed (%)								3.48 $51810$ $7314$ $32 (Zn2+)$ $185.85$ $159.73$ $0.003$ $0.434$ $1.21$ $4.34$ $2.21$ $0.35$ $98.12$ $1.88$ $0.00$
EMDB accession code	26 addi m	519 tional ap	26519 additional map	26519 additional map	26519 additional map	26519 additional map	26519 additional map	26519 main map
PDB accession code		1	· ···r	· ~ <b>r</b>	· · · · r	r	··· <b>T</b>	7UHY

 $Supplementary\,Table\,1 |\,Summary\,of\,cryo\text{-}EM\,data\,collection,\,3D\,reconstruction,\,and\,model\,refinement.$