Adapter Proteins for Opposing Motors Interact Simultaneously with Nuclear Pore Protein Nup358

Heying Cui[1], Crystal R Noell[1], Rachael P Behler, Jacqueline B Zahn, Lynn R Terry, Blaine B Russ and Sozanne R Solmaz[*]

Department of Chemistry, Binghamton University, PO Box 6000, Binghamton, NY 13902.

SUPPORTING INFORMATION

Supporting information contains 3 Tables and 7 Figures. **Page**

Table S1 Molar masses determined by SEC-MALS for Fig. 5. **S2**

Table S2 Molar masses determined by SEC-MALS for Fig. 7. **S3**

Table S3 Molar masses determined by SEC-MALS for Fig. 8. **S4**

Figure S1 The W-acidic motif with the sequence LEWD in Nup358 is conserved in vertebrates, but lost in insects. **S5**

Figure S2 Sequence alignment of Nup358 from Figure 2 showing a larger region of Nup358. **S6**

Figure S3 SDS-PAGE analysis of purified proteins used in this study. **S7**

Figure S4 Load controls for all analytical size exclusion chromatography experiments. **S8**

Figure S5 A very weak interaction with KLC2 ^{TPR} is observed with a large excess of the Nup358-min/W2224A/D2225A mutant. **S9**

Figure S6 BicD2-CTD and KLC2^{TPR} do not interact. **S10**

Figure S7 The W-acidic motif is not required for the interaction of Nup358-min with BicD2-CTD. **S11**

SUPPORTING TABLES

Table S1 Molar masses (MW) determined by SEC-MALS for Fig. 5

Protein	**Conc. (mg/ml)**	**MW (kDa) Peak**	**Conc. at Peak* (mg/ml)**	**Replicates**	**Fig. Panel**
Nup358-min	5	10.6 ±0.5	1.0 ±0.03	3	A
Nup358-min	1	10.6 ±0.5	0.2 ±0.1	3	
Nup358-min/W2224A/D2225A	5	10.0 ±0.5	1.3 ±0.2	3	B
Nup358-min/W2224A/D2225A	1	11.2 ±0.6	0.3 ±0.1	2	
KLC2 $^{TPR\text{-}trunc}$	3	37.9 ±1.9	0.1 ±0.004	2	C
Nup358-min + KLC2^{TPR}	4	70.2 ±3.5	0.2 ±0.06	4	D
Nup358-KLC2-fusion	4	68.9 ±3.4	0.4 ±0.01	3	E
Nup358-min + KLC2^{TPR}	4	70.2 ±3.5	0.2 ±0.06	4	
Nup358-KLC2-fusion	8	72.1 ±3.6	0.7 ±0.01	2	F
Nup358-KLC2-fusion	1	56.3 ±2.8	0.1 ±0.01	2	
Nup358/W2224A/D2225A-KLC2	8	45.1 ±2.3	1.1 ±0.01	2	
Nup358/W2224A/D2225A-KLC2	1	43.2 ±2.2	0.1 ±0.02	3	

* Protein concentration at the apex of the elution peak, determined by the refractive index and averaged from all replicates; standard deviations are shown. Note that in panels D and E the same dataset of Nup358-min + KLC2^{TPR} is shown.

Table S2 Molar masses (MW) determined by SEC-MALS for Fig. 7

Protein	Conc. (mg/ml)	MW (kDa) Peak 1 & 2 & 3	Conc. at Peak 1 & 2 & 3*	Replicates	Fig. Panel
Nup358-min + $KLC2^{TPR}$ + BicD2-CTD	5	125.1 ±6.3	0.1 ±0.02	3	A
Nup358-min + $KLC2^{TPR}$	4	70.2 ±3.5	0.2 ±0.06	4	
Nup358-KLC2-fusion + BicD2-CTD	5	117.4 ±5.9	0.1 ±0.02	3	B
Nup358-KLC2-fusion	4	68.9 ±3.4	0.4 ±0.01	3	
Same data as in panels A & B					C
Nup358/W2224A/ D2225A-KLC2 + BicD2-CTD	5	113.4 ±5.7 & 53.7 ±2.7 & 43.2 ±2.2	0.02 ±0.002 & 0.2 ±0.08 & 0.2 ±0.05	2	D
Nup358/W2224A/ D2225A-KLC2	4	44.0 ±2.2	0.6 ±0.1	2	

* Protein concentration at the apex of the elution peaks 1 & 2 & 3 in mg/ml, determined by the refractive index and averaged from all replicates; standard deviations are shown. Elution peaks are numbered from highest to lowest molar mass. Conc.: Protein concentration. Note that for panels A and B the datasets of Nup358-min + $KLC2^{TPR}$ and Nup358-KLC2-fusion from Fig. 5 are shown again.

Table S3 Molar masses (MW) determined by SEC-MALS for Fig. 8

Protein	Conc. injected (mg/ml)	MW (kDa) Peak 1	MW (kDa) Peak 2	Conc. at Peak 1 & 2 (mg/ml)*	Replicates	Fig. Panel
Nup358-KLC2/BicD2-CTD	5	118.4 ±5.9	64.7 ±3.2	0.4 ±0.01 & 0.2 ±0.01	2	A
Nup358-KLC2	5	67.9 ±3.4		0.4 ±0.1	3	
Nup358-KLC2/BicD2-CTD	2.5	119.3 ±6.0	64.9 ±3.2	0.1 ±0.01 & 0.1 ±0.01	4	B
Nup358-KLC2	2.5	63.2 ±3.2		0.2 ±0.01	2	
Nup358/W2224A/D2225A-KLC2/BicD2-CTD	2.5	119.2 ±6.0	51.1 ±2.6	0.02 ±0.01 0.2 ±0.01 &	3	C
Nup358/W2224A/D2225A-KLC2	2.5	43.6 ±2.2		0.4 ±0.01	3	

* Protein concentration at the apex of the elution peaks 1 & 2, determined by the refractive index and averaged from all experiments; standard deviations are shown. Elution peaks are numbered from highest to lowest molar mass. Conc.: protein concentration. For all experiments in this Table, pre-assembled and purified Nup358-KLC2/BicD2-CTD complexes were analyzed by SEC-MALS (rather than mixtures of these proteins).

SUPPORTING FIGURES

***Drosophila melanogaster* Nup358**

MFTTRKEVDAHVHKMLGKLQPGRERDIKGLAVARLYMKVQEYPKAIEYLNGYLRVRDDAVGHNMIATCYSRLNPPDVTEALQHYQRSIQIDPRQSEVVIDA
CELLVKENNASITECARYWLDQANSLDLSGNKQVFNLRMRVNLADSNGERDDTSGGDGEQNTLEILMYKELQARPQDVNIRIQLLRSYVEKMKIDQAFNYA
LKTELESKNCTSQSNEWYEQIWMVLFKIEMAKDVKKNWRFWHFALHTLDRLVQLSLEGSGLADSSKQLFRLDQYLFKFSTSIERSGDAPQRDLHQACIDHF
TGQLLLHAVTLIFKREVLANKNKWMSTLRSALPLLLLGYQVRPIDDSSTNQWIKHCDAEQKQLIQMWRPQGAFRCAQLGRTLLGCLDRSQMEIKNDRENAE
FDENKNSGNSMPGLFADSEELLASAHQQCLDKSWRSQIYQQLFTHAEHKLKDTSSHLVRNLRLQLPLFEWPNLAHIENYELQALVLPPHSLAQHVYLALGT
DPNKLGDAPRVVFYEGFQRDVKQNLNYCGQDSISQVDVDLYLYATTIQTRRKLQIQREVYDSSNLGNRNAAARPHMMPFANLVGQLGAPEQSNWWDLVV
RLNSNQLITEGNRAEQRAQLQHGLEAVRGVNGPKADAIIIFQLGKILNSRSDRSSLEARIDTLYRQGFSILRHQHNQQMESYVRVFKYGSAGSTAAWQDLQ
SLAEEAVTYFSEKMFRIGQYEQFLDEVRGLHLPMAYFLQSEACHHLEESSKLPRTSRDRYSERRRECLQKTQKLIKNDDKHPLIAAMHRHQQDRNSRGIDN
SFGSPDVHNNSSAYEDAEDDFYSHAAFSANRSRRQLEVTPVTPIVMAQPSQEMEQAVKQISKSLCVLKDDVSVGMEAMRQDIKVLTEKFTGLEDLLKKIKI
SSRDTPTRDVDPAAALGLDDLFIIEDALAEHQQQQQHQQQQSHNQGAIHPVVPNPYTSGFYNGMPNTPSAQERFLQGPYGSPMFNQNQMYNYYAAQAQ
AQAQAQFLRTPPAPGSIPPPNMFGPRNPNFGLPSMFPPPTVPSVAPYIDAMGNFTQPPPSLIPPPAQPAAPPAPLNILESKPVVALPTPGFFNTTTPVFGAS
PIQVPQSKPLTVPTVPIPSTAPAPPIAGTVNPPATTAVPPPVHIPQVAPSVPAQPPAPAPVSVPSMFNRALNNQPVEKEPPANVVITSSDPLPKPTTASVQPT
LSVTIPAQHIKPSLVQAPEQPAQSAQPAQPSVSGVGSLSFNFGSKSSESPFSFKTQVAKAAAEKQKEQEEAEQNQSGATDPNKTLPQDTSADDYDPRPDF
KPIIPLPDEVEVRTGEEGEDIKFTSRAKLFRYVDKEWKERGTGVIKILCDKATGVSRVLMRRDQTHKVCANHTITADITINVANQDKDKKSLLWAANDFADEQ
VTLERFLVRFKTGELAEEFRVAFTKASEAAKSKETVKPTVNTAEKGSTATAPAAFKSFVTSTPAANSLINKPQEQTKTQPNPDPPATAAKSLFGTLSVSAAP
ATSAPASATPFASFSFTPNGSSGFGTSTASPFGNLSFGTASAVGSGNNTTLFTTALIKDNTVQGKTLQQESQLNKSNSSDAEEEYVPTAQFVPVIALPDIVE
VVTGEENEDVLFEHRAKLLRWDKEANEWKERGLGNMKLLRDRTDPNKVRLLMRREQVHKLCCNQRLLPETKFTYATNCKAAVTWGAQDYSDEELTTALL
AVRFKSQDICQQFLEAVQKAQQSIGNEPKKEEVPSAAGEKEKPIKGFGDAFKPKAGSWNCQACYTNNGQDQLYCLACQEPKDATVPPKQSGLDQGNALN
LTTSSSNKFSFGFASSATLPATGGFSFGGATQPKEKPAVAVVTASASAPTSVQTAALGFGKSSMTSGFGDAFKPAVGSWSCSACYVNNPGESLYCSACD
APKNDTVPQKEKSLGSGLNLPPTSKFSFGFGAAAAGDKDQAGDGATFNFAAMPAAVAPTTSIGSSSFTFSMTKPKPDQQQPNSTAAKEDEDNDSQEVEE
EENNTYFSPVIPLPDKIDVKTGEEDEELLYVHKAKLYRLNESDWKERGLGDVKILRHRQTKKLRVVMRREQVFKICLNHVLNENVVYREKTETSWMFAVHD
FSEGESVLERFTLRFKNKEVAQGFMEAIKNALNETAKPIEDSPVVGSVSQSTEANKPSQKNDGAAKSRGGESEVLDVGKTSSVRPTTHEVIPPLPMTLPLLT
LPQPLAKPNDYQTPATILFKGSSLSRNNSSASEASKTPSSAFIFGSTDKSEPGKDAGPLANLQKLASGEGQGNVLGSIFRSGSSNENSSDGSVKFFFGGGN
KAAEQQKKDSSESVFGGNKADSQSPATQEAPKLAFGGIAAPVFGDANPFGGHKVNLQKSDGKEEPKSIIGGTPLLFGGSNAFGIPKIETQSPAKDFVFGSA
PAFGQMATFSFTAAKNEKEKDITSNNTTDLKAEGKEKKELVPETTSTFADLAKTGSTFADLASNPGGTFADLANKTGNDFANLSANSQGTTVGFNKSAGG
GFYNLTHQNAFKNFESPQATEECDDDGDATTDDNYDPHYDAIVELPDEIVVTTGEENETKLFGERAKLYRYDAESKQWKERGVGEIKVLEHPELQTFRLIM
RQEQIHKLVLNMNISASLQMDYMNAQMKSFLWAGYNYAVDAEGKVDTEGVLERLACRFAKEEIASEFLNTVNSCIKRAKALQGDEENKNDDAPEEQASS

***Apis mellifera* Nup358**

MFRSKKDVDRHVKDIFIKLKSSEEKKLRCYNIAKLYYQVGDNESAKKYVSNYLEIRDKSAGAHKLLGQALEGLGQKEAAFTQYKISLELEPKQDDLLLKVCEL
LSNMDINIANDKIKHLVERADKKFPHHPIVFELKEKLITLEKPNGNDDDLEKLIISELSVRQTDVNLQVKLLKHYIGNHRLEDAYNKATGIEATHSHRNNIIWYQS
LSELLMKCKESKQSDWTFWIFYISVLERYAALCLKEQGNIIKKSITDVTQAVFNFDQSLFEFKSKNFSNHPAFIEHMLLHMWGQLHFHLACLILRKIKAGEDS
WSEAGRLCGPLLLTALHVTPIDPTAVWTMHLKDRLKNQTHIWYREGSYRCSQAGHVLQDYARDNTKKLLDKIDKFCTSSWRERIYQRIFIGRLYQDGKKTS
FFTNSLISNPPLRLCSYNELKRFDEISEEAWPDSLHHQVWLGLRARPQGSQNKNNGLHPNQTSHVFYELQFSVYNLHQAAPDSLSRLDIDAFLNAAICTSSII
EEQHGGLLNPEKLPTLPADLTNTLCTCAQEQWWSYAYKVYSMDKQKPLDDDLGELRLELQRGLEVVRCLGNHGLHPVLLVHLARIFHHRAETLKEINQEN
SDIPYLEARSEMYWSAAIPLLERLQNNQVIRVTNSKFFNYQGKDMNNAELIKALEEGKLLLAQRFVRSKQYEKAINALQALKCPEASFQQGQMYEKLADEIIS
SIAKENLTSEMRSQHIIMLSKARECFYLTLDRLRSPETNPKHPLNSELSTYIADIENKLKRIDPDLSRGDLSRNECDGMSDESYSSAHSAVDQAVTKIALPTLT
GLNTSVNVLSTPQKNIHRTPKQSSTPYKLQHQDILDLSRNRSEARPSPERLDAQIRAINQMIHSKDNMIQSIAEQTKTILELNKTVMEIVERLAKEMTELRKEI
QKQRTQTVNVNPNLEEDLCILSEDDYNDLNYNANQSGASSISGNMFQSSHRHPYSHLVYPSATFQGYYPTGMSFNDPNAQAIPSLYPPNIYSMPVLYPNT
RSKIPENILQQSLFMPPTNQPLQIPLQKLETSKPESTIKDAPVNKIPPVNVAITTSDTLPTTVPIVQPTLSVTIPPHFRQGSSSTPVTTEQSAPHCYQISMPSQA
TIPTTVNLPPLSNTFTTTPANLSTSETSKQNTTICSTGSSNSSDHEHDPIPDFVPVIPLPAEIKVTTGEEGQETLFCARAKLYRFVDNEWKERGIGNVKLLKNE
EGKVRLLMRREQVLKVCANHYLVPDMELTAKSNNEKAWFWVAHDFADGELKLEKFCIRFKTVEEGISFKEHFDKAKASLTFEKVKIENVDKISNKDNIAISKK
TEKIKSEQKIIEQTPLFISGQTIQKPIDVTASTDSIISEKLQNNITPTVIGGFSFSSTPIIQKAITTESSKISTKSEISPFAGFTFNKTVTNAENFITTTQKSTTIGTITT
SQFTFSFTKTNTPLQSATTTTVSTVPSSIHSTSISSSQPNNTAGTNENISANTSQFSLRRPHAPAPGASVSTSTIAGIQDTEKSALQTEYEEVLFNAKISLQYY
NNDNKQWENRAIGQMKILWNSKTNKIRLLMVDENNLKIFYNYNVFAKMSFTYKSNSTVVNWNMHCGSDNKTGMFAATFKTSNQASQFYNIITSNQQKMVK
DCIIAENLTKLETIQVVQTHKSENSLSEMFKPPEGSWECNGCYIRNNVSDVKCIACEKSRFSESDESIISSIPSNQISLSQMSKPSTGSWKCKCCNIFNAAES
NYCVTCDSPKDPSLPPKPKIDGFQINSNSSGITSTFTFGIPQDTTKKGTNVFSFRLPTSNDVSKSASLVATKSDAKTDASNAKFVFGTPGKSFGFNFVTKSS
PTKSGGEENSEEEVVENDDIHFSPTIPLPDKIEVKTGEEEEEVLYSHRAKLFRYNKPANEWKERGIGDIKLLRHNETGKLRLVMRREQILKLCLNHFVLPNFE
LNSKDERTWIWNAADYSEGEIEPTLFACRFKTSDIANNFKEIIDESKKALPPIEKTNDTKIETFTEVSSIQDIEVVYEMKVTPEEKQAALKLQLPKNFYAYKQKP
DCPGCRGCKESKLSLFTDESSEKSKISAENRKSLTTTSTMQFLPKSTTFSTASNEVTTPISSTSTIVSSIFKPSLYGTNTLQFATNSISTTTSTISFGNVDSITSS
DKKTGFSFVMPQISSNEIQKSMIDKQNISISENLKICSPITSQTQVTSTTCATSFSFITSTSTIPTSSIFGMAKLDNTGKNIFGGISKKDSSSLLKPSSINVCTNSE
LVFSNNFNTQPKTITNTTITSVLSNATPIFGSSVLKANSENTNTGSLEVSVKSTNSVFPNTSVPFSYNLFSGVRTNTSSFTISNPVTTNAFETITPTTSDSQKES
DVAFLPMTDLSFSTLAAKSSQSAFKIDPNFSFAGAGTAVFGTKSNTITNKCTEKSKEMVEKKDEDEEEDDQVDENDHDYDPHFEPIIPMPDIVEVHTGEEEE
EKIFSERAKLYRYDSDTREWKERGVGEMKILHHAKYNRYRLLLRRDQVYKVVCNFLLTPDITFSRLRTSDRAWMWAGMNHAEEQPCLESLSVKFKSPVLA
TKFKDTIDKIQQTLSESREKNVKDSPVIEEHGDEENEDENCEEVGAVDDIKEEDEEEDDDDDDDDDDDEDEDDDDEDDDDDDDDDDDDDDDDQNSITFEK
RATLLTRTNKDSQWEPVALGNLIIYYDSNIFGERIILKADKTDEIVSNTIISMNTKMEVNEKECVWTAIDYALTPPTKRTLCAVFSSVHVAREMHKHFQEGVEY
AYQADISEPFYEE

Figure S1. The W-acidic motif with the sequence LEWD in Nup358 is conserved in vertebrates, but lost in insects. Protein sequences of Nup358 from *Drosophila melanogaster (Dm)* (Uniprot database identifier A0A0B4K7J2-1) and *Apis mellifera* (Uniprot database identifier A0A088AHI9-1). Tryptophan residues are highlighted in cyan. Note that the sequences lack a W-acidic LEWD motif. It should be noted that the sequence conservation between human Nup358 and *Dm* Nup358 is very low, therefore we were despite performing various sequence alignments unable to identify any region in *Dm* Nup358 that would be homologous to the minimal BicD2 and kinesin-1 binding site in human Nup358 (residues 2147-2240).

Figure S2. The sequence alignment of Nup358 from **Fig. 2** is shown for a larger portion of Nup358.

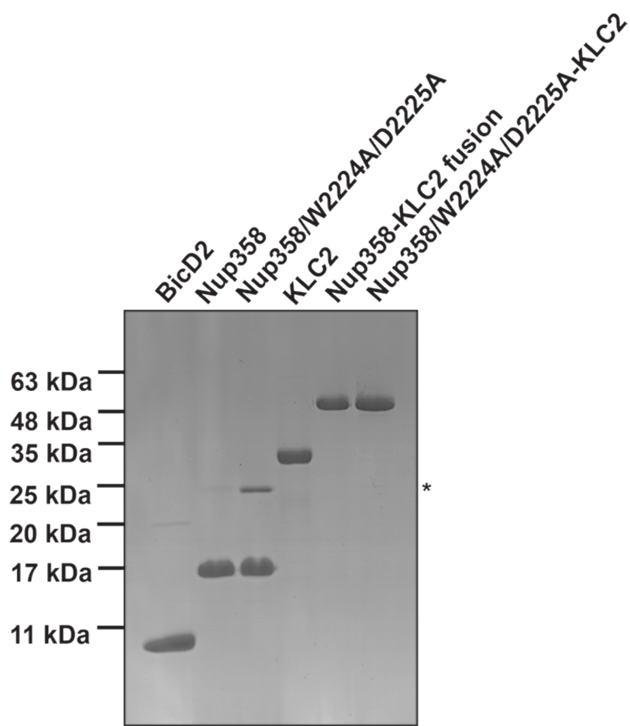

Figure S3. SDS-PAGE analysis of all purified proteins, stained by Coomassie Blue. Molar masses of standards are depicted on the left. From left to right: BicD2-CTD, Nup358-min, Nup358-min/W2224A/D2225A, $KLC2^{TPR}$, Nup358-KLC2-fusion protein, Nup358/W2224A/D2225A-KLC2-fusion protein. The asterisk indicates the location of GST bands. Multiple batches of purified proteins were used for this study, this SDS-PAGE shows a representative result. See also **Fig. 3**.

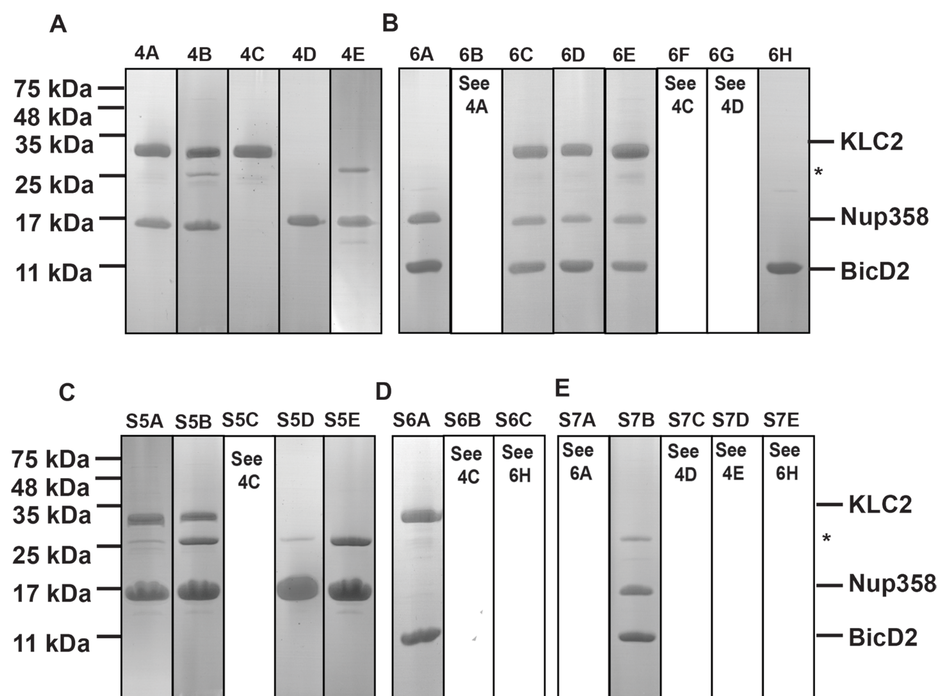

Figure S4. Load controls for all analytical size exclusion chromatography experiments (see **Figs. 4, 6, S5, S6** and **S7**). Coomassie-stained SDS-PAGE analyses of 10 µL of the samples that were injected onto the column are shown, which is the same amount as the elution fractions that were analyzed. Gel lanes are grouped by figures and labeled on top with the figure panel showing the corresponding experiment. The locations of the bands of the molecular weight standards are indicated on the left. The asterisk indicates the location of the GST band. Multiple batches of purified proteins were used for the analytical size exclusion chromatography experiments in this study, the figure shows a SDS-PAGE analysis of representative samples. See also **Fig. S3**.

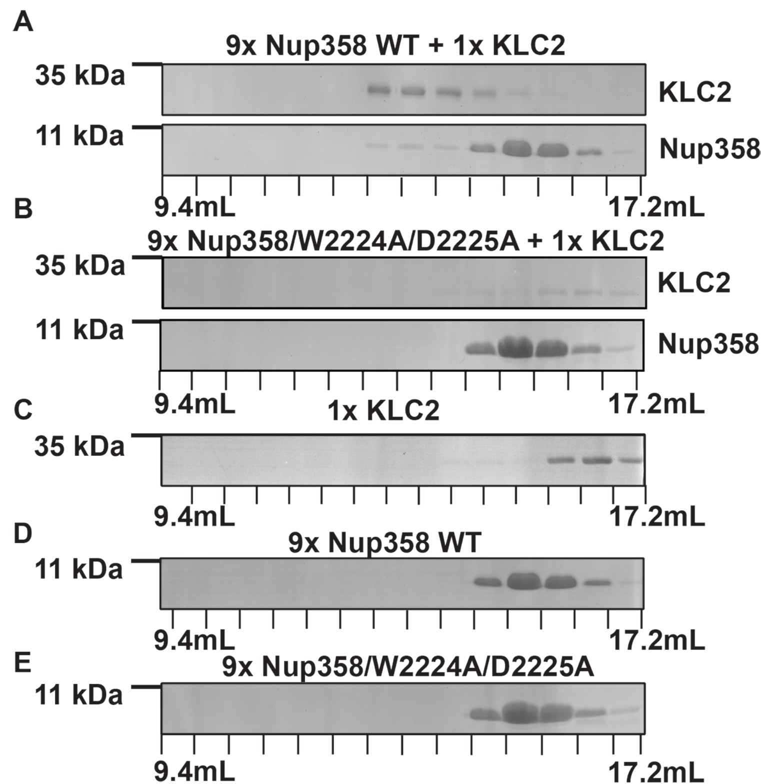

Figure S5. A very weak interaction with KLC2^{TPR} is observed with a large excess of the Nup358-min/W2224A/D2225A mutant. (A-E) To assess binding, purified Nup358-min (WT or W2224A/D2225A mutant) and KLC2^{TPR} were mixed in 9:1 molar ratio and analyzed using gel filtration chromatography. The elution fractions were separated by SDS-PAGE. The individual proteins were analyzed as well. (A) KLC2^{TPR} and Nup358-min. (B) KLC2^{TPR} and Nup358-min/W2224A/D2225A mutant. (C) KLC2^{TPR}. (D) Nup358-min. (E) Nup358-min/W2224A/D2225A mutant. Note that with addition of the large excess of Nup358-min/W2224A/D2225A, KLC2^{TPR} is shifted slightly towards higher mass compared to the individual KLC2^{TPR}, suggesting a weak interaction between these proteins. The interaction is however much weaker compared to Nup358-min WT. The experiments were repeated and very similar results were obtained. The number of replicates for experiments shown in each figure panel were: (A) 2, (B) 2, (C) 3, (D) 3, (E) 2. See also **Figs. S3** and **S4**. Note that KLC2 has a higher solubility when mixed with Nup358-min (resulting in thicker gel bands) and that solubility of KLC2 is decreased when mixed with Nup358-min/W2224/D2225A.

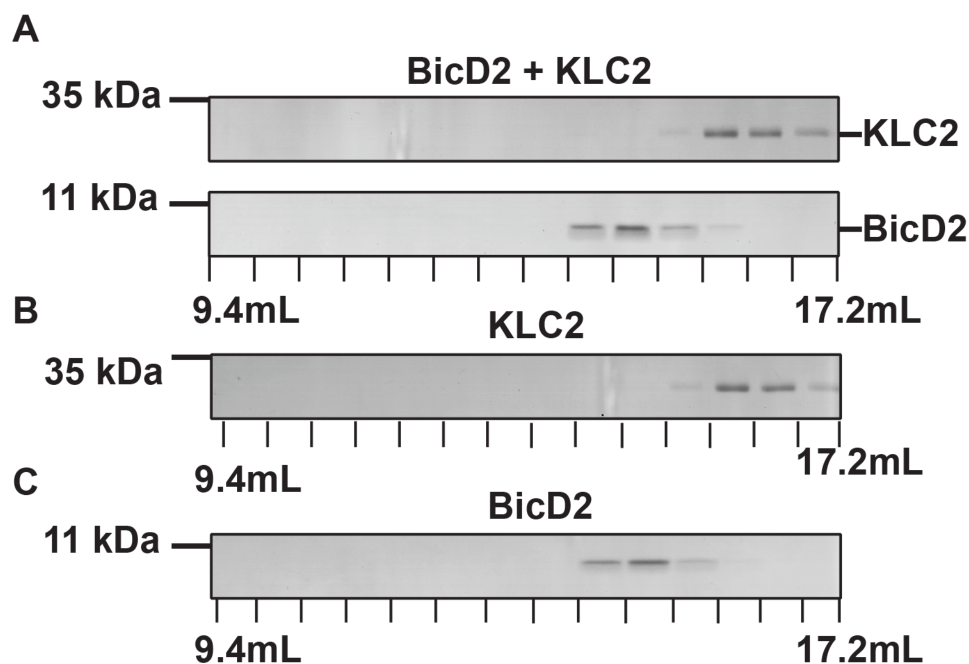

Figure S6 BicD2-CTD and KLC2^{TPR} do not interact. (A-C) To assess binding, purified BicD2-CTD and KLC2^{TPR} were mixed (1:1 molar ratio) and analyzed by gel filtration chromatography. The elution fractions were separated by SDS-PAGE. BicD2-CTD and KLC2^{TPR} were also analyzed individually. (A) BicD2-CTD and KLC2^{TPR}. (B) KLC2^{TPR}. (C) BicD2-CTD. Note that BicD2-CTD and KLC2^{TPR} elute at the same elution volumes when mixed as when individually analyzed, suggesting that they do not interact. The experiments were repeated and very similar results were obtained. The number of replicates for experiments shown in each figure panel were: (A) 3, (B) 3 (C) 5. See also **Figs. S3** and **S4**.

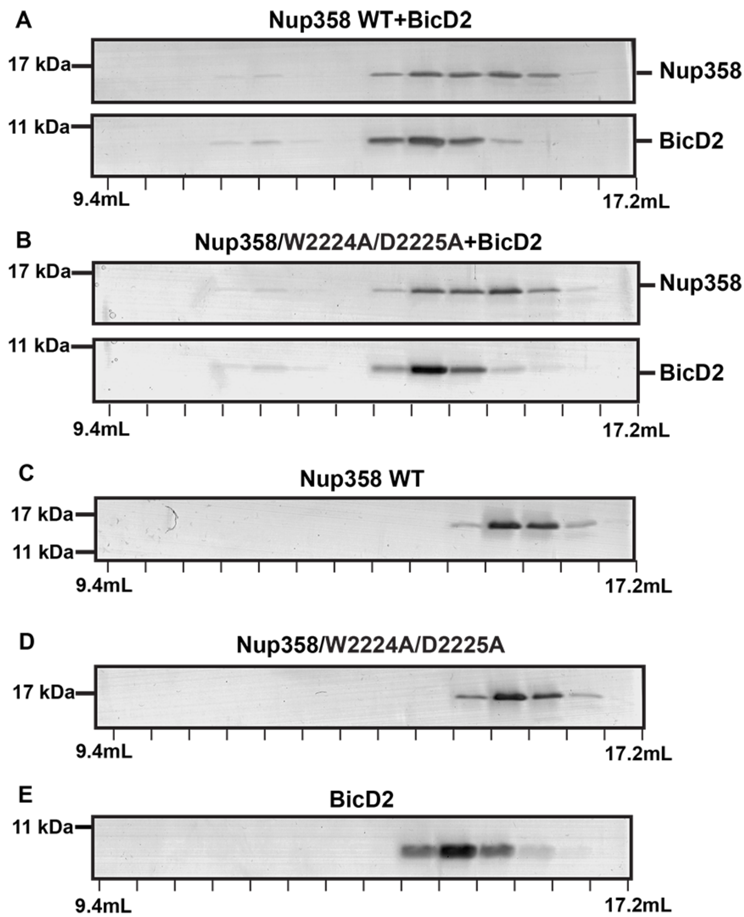

Figure S7. The W-acidic motif is not required for the interaction of Nup358-min with BicD2-CTD. (A-E) To assess binding, purified Nup358-min (WT or W2224A/D2225A mutant) and BicD2-CTD were mixed (1:1 molar ratio) and separated by gel filtration chromatography. The elution fractions were separated by SDS-PAGE. The individual proteins were analyzed as well. (A) Nup358-min and BicD2-CTD. (B) Nup358-min/W2224A/D2225A mutant and BicD2-CTD. (C) Nup358-min WT. (D) Nup358-min/W2224A/D2225A mutant. (E) BicD2-CTD. Note that both Nup358-min WT and the W2224A/D2225A mutant coelute with BicD2-CTD. Furthermore, the elution peaks are shifted towards higher mass when mixed compared to the individual proteins. The effect is comparable for Nup358-min WT and the W2224A/D2225A mutant, suggesting that the W2224A/D2225A mutation does not affect the interaction of Nup358-min with BicD2-CTD. The experiments were repeated and very similar results were obtained. The number of replicates for experiments shown in each figure panel were: (A) 3, (B) 2 (C) 4, (D) 2, (E) 5. See also **Figs. S3** and **S4**.