Supplementary information online

Structure-function analysis of core STRIPAK: a signalling complex implicated in Golgi polarization

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List of Supplementary Online Figures and Tables

Supplementary References

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A

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                                                                                    57
STRN3   1   ...PGMAAPPRQQQGPGGNLGLSPGGNGAAGGGGPPASEGAGPAAGPELSR
STRN    1   -MDEQAGPG---------------VFFSNNHPGAGGAKGLGPLAEAAAAGDGAAAAGAA
STRN4   1   MMEERAAAAVAAAASSCRPLGSGAGPGPTGAAPVSAPAPGPGPAGKGGGGGGSPGPTAG-

             45                                              90
STRN3  60   PQQYTIPGILHYIQHEWARFEMERAHWEVERAELQARIAFLQGERKGQENLKKDLVRRIK
STRN   44   RAQYSLPGILHFLQHEWARFEVERAQWEVERAELQAQIAFLQGERKGQENLKKDLVRRIK
STRN4  60   PEPLSLPGILHFIQHEWARFEAEKARWEAERAELQAQVAFLQGERKGQENLKTDLVRRIK

                                                          169
STRN3 120   MLEYALKQERAKYHKLKYGTELNQGDLKMPTFESEETKDTEAPTAPQNSQLTWKQGRQLL
STRN  104   MLEYALKQERAKYHKLKYGTELNQGDMKPPSYDSDEGNETEVQPQ-QNSQLMWKQGRQLL
STRN4 120   MLEYALKQERAKYHKLKFGTDLNQGEKK-ADVSEQVSNGPVESVTLENSPLVWKEGRQLL

                                                 219
STRN3 180   RQYLQEVGYTDTILDVRSQRVRSLLGLSNSEPNGSVETK--------------NLEQIL
STRN  163   RQYLQEVGYTDTILDVKSKRVRALLGFSSDVTDREDDKN--------------QDSVVN
STRN4 179   RQYLEEVGYTDTILDMRSKRVRSLLGRSLELNGAVEPSEGAPRAPPGPAGLSGGESLLVK

STRN3 225   NGGESPKQKGQEIKRSSGDVLETFNFLENADDSDEDEENDMIEGIPEGKDKHRMNKHKIG
STRN  208   GTEAEVKETAMIAKSELTDSASVLDNFKFLESAAADFSDEDEDDDVDGREKSVIDTSTIV
STRN4 239   QIEEQIKRNAAGKDGKERLGGSVLGQIPFLQNCEDEDSDEDDELDSVQHKKQRVKLPSKA

STRN3 285   NEGLAADLTDDPDTEEALKEFDFLVIAEDGEGA-GEARSSGDGTEWAEP-----------
STRN  268   RKKALPDSGEDRDTKEALKEFDFLVISEEGD---NESRSAGDGTDWEKEDQCLMPEAWNV
STRN4 299   LVPEMEDEDEDDSEDAINEFDFLGSGEDGEGAPDPRRCTVDGSPHELESRRVKLQGILA

STRN3 333   -----------------------------------------------------------
STRN  325   DQGVITKLKEQYKKERKGKKGVKRPNRSKLQDMLANLRDVDELPSLQPSVGSPSRPSSSR
STRN4 359   DLRDVDGLPPKVTGP--------------------------------------------

                              388
STRN3 333   --------------ITFPSGGKSFIMGSDDVLLSVLGLGDLADLTVTNDAD-YSYDLPA
STRN  385   LPEHEINRADEVEALTFPPSGKSFIMGADEALESEIGLGELAGLTVANEADSLTYDIAN
STRN4 374   ------------PPGTPQPRPHEDVFIMDTIGGGEVSLGDLADLTVTNDND-LSCDLSD
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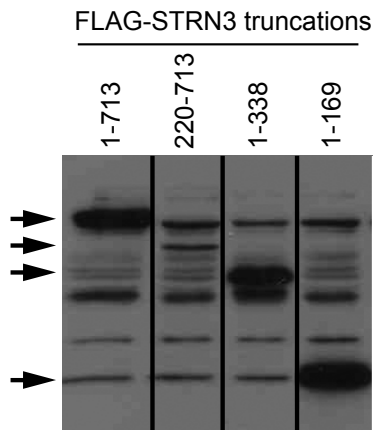
B

FLAG-STRN3 truncations

1-713   220-713   1-338   1-169

Supplementary Figure 1. Striatin alignment and expression levels of FLAG-STRN3 truncation mutants.
A) Sequence alignment of the N-terminus of mouse Strn, human STRN3 and human STRN4. Identical amino acids are highlighted in black; amino acid similarity is indicated in grey. The boundaries of the truncation mutants are indicated by vertical bars for mouse Strn (purple) and human STRN3 (green). The sequence boxed in yellow corresponds to the region mapped as directly binding to the PP2A_A_ subunit; this region also contains a coiled-coil dimerization domain. The region boxed in pink corresponds to the minimal binding peptide for CCM3 binding. B) Expression levels of FLAG-STRN3 1-713, 220-713, 1-338 and 1-169 in the stable human cell lines used for AP-MS.
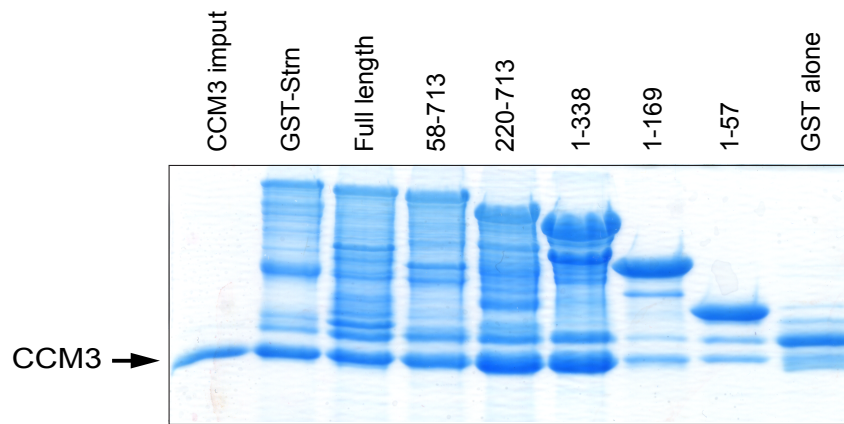
Supplementary Figure 2. Association of full length Strn and STRN3 truncation mutants with CCM3.
Bacterially expressed recombinant soluble CCM3 was tested for its ability to be pulled-down by GST alone, GST-Strn full length or GST-STRN3 full length or indicated deletion mutants. Bound proteins were analysed by SDS-PAGE followed by Coomassie staining. The position of CCM3 is indicated.

STRN3 220-338 peptide array
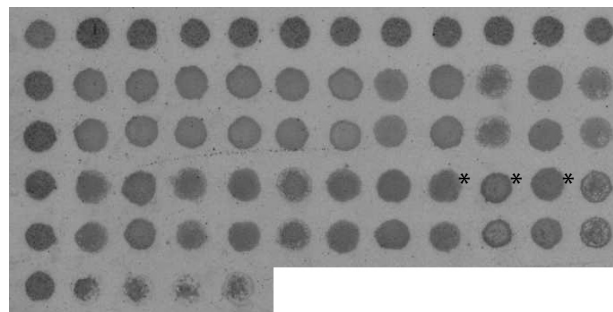
Supplementary Figure 3. Coomassie stain of STRN3 220-338 peptide library spotted on Whatman membrane.
A peptide library of STRN3 220-338 (15 mers) was produced by automatic SPOT synthesis on a cellulose membrane. Coomassie staining was performed to evaluate peptide amounts prior to use in the overlay assays. The interaction of spotted peptides with GST alone and GST-CCM3 is shown in Figure 2E; spots indicated by an asterisk were shown to interact with GST-CCM3 but not with GST alone.
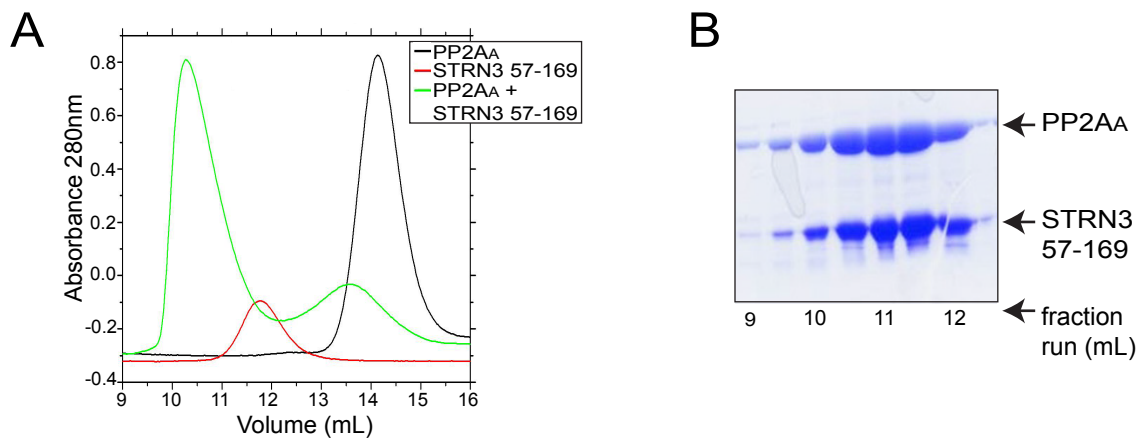
Supplementary Figure 4. Chromatogram and gel of PP2A$_A$ and STRN3 57-169.
A) Elution profile of PP2A$_A$ (black), STRN3 57-169 (red) and PP2A$_A$ combined with STRN3 57-169 (green) from a 24 mL Superdex S200 gel filtration column. STRN3 57-169 encompasses the coiled coil domain of STRN3 and multimerizes. B) Fractions between 9 mL and 12.5 mL from the PP2A$_A$ + STRN3 57-169 (green) run were resolved on a SDS gel and Coomassie stained.

FLAG-MST4   α–Giantin   Overlay
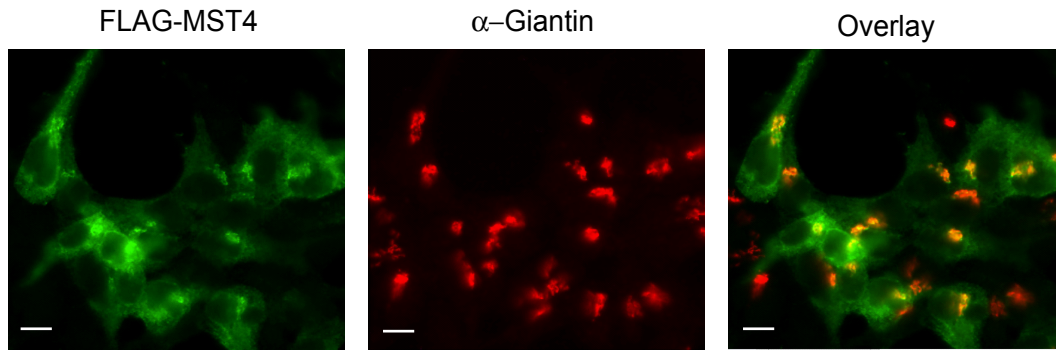
Supplementary Figure 5. FLAG-MST4 localizes to the Golgi.
In stable HEK293 cell pools, the FLAG-tagged kinase MST4 (green) partially co-localizes with the Golgi marker giantin (a fraction of MST4 also localizes to the cytosol as punctae). Scale bar = 10 μm.
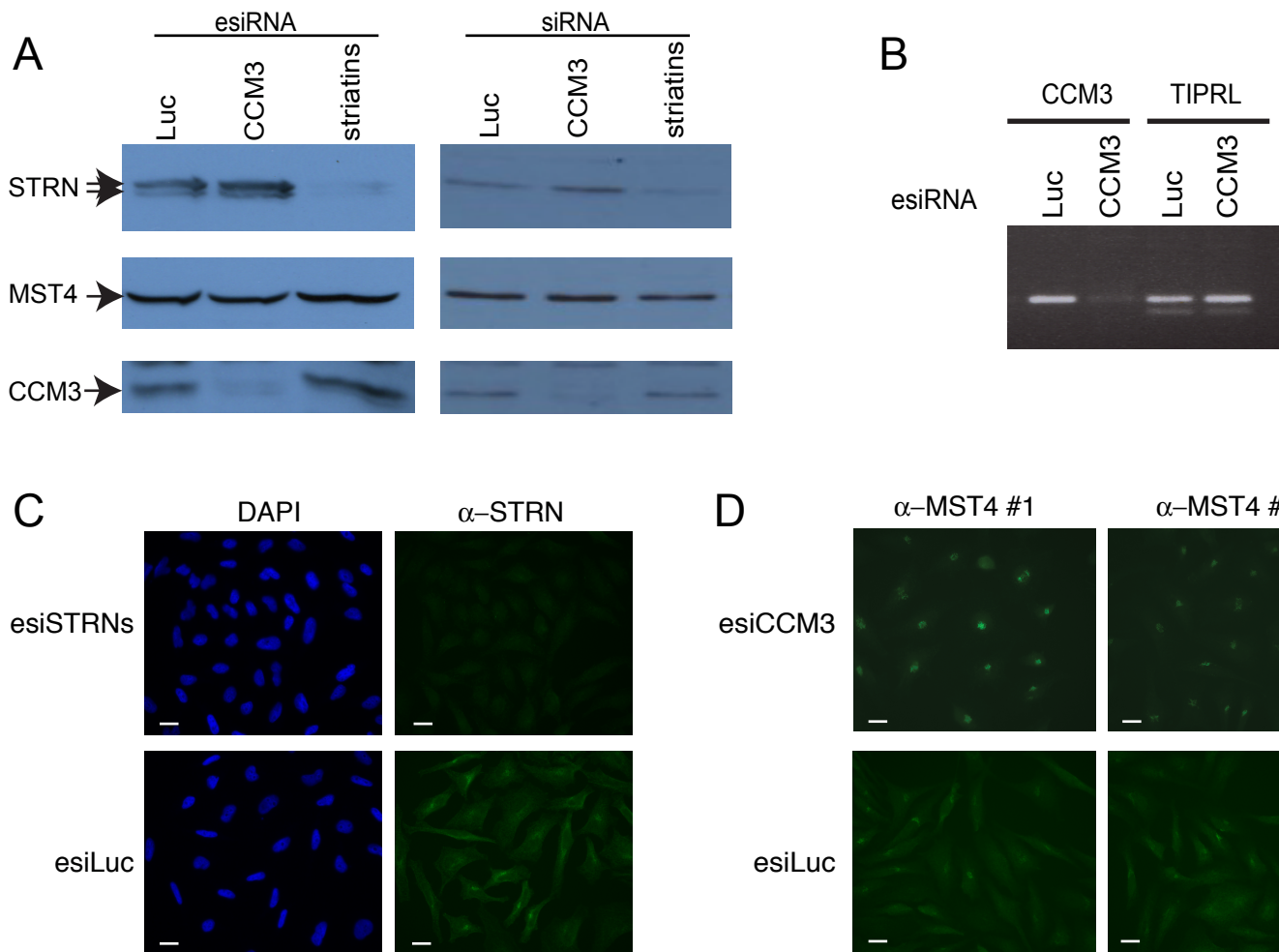
Supplementary Figure 6. Knock-down efficiency.
A) Efficiency of esiRNA-mediated knockdown of striatins and CCM3 in Hela cells and siRNA mediated knockdown in 293 cells, as shown by immunoblot against both proteins (note that knockdown of CCM3 does not affect expression of STRN and vice versa). MST4 is used as a loading control. B) Efficiency of CCM3 knockdown by esiRNA in HeLa cells, as detected by RT-PCR. Primers amplifying the unrelated gene TIPRL were used as loading controls. C) Knockdown of STRNs by esiRNA in HeLa cells is uniform and efficient, as determined by immunofluorescence straining with α-STRN (nuclei are stained with DAPI). D) Indirect assay for the knockdown efficiency of CCM3 via immunofluorescence detection of the localization of MST4 (our endogenous antibodies to CCM3 are not appropriate for immunofluorescence). Two separate fields (labeled #1 and #2) are shown to illustrate efficiency of knockdown. Note that MST4 is uniformly relocalized to the Golgi in all cells observed, indicating a uniform knockdown efficiency of CCM3. Scale bar = 10 μm
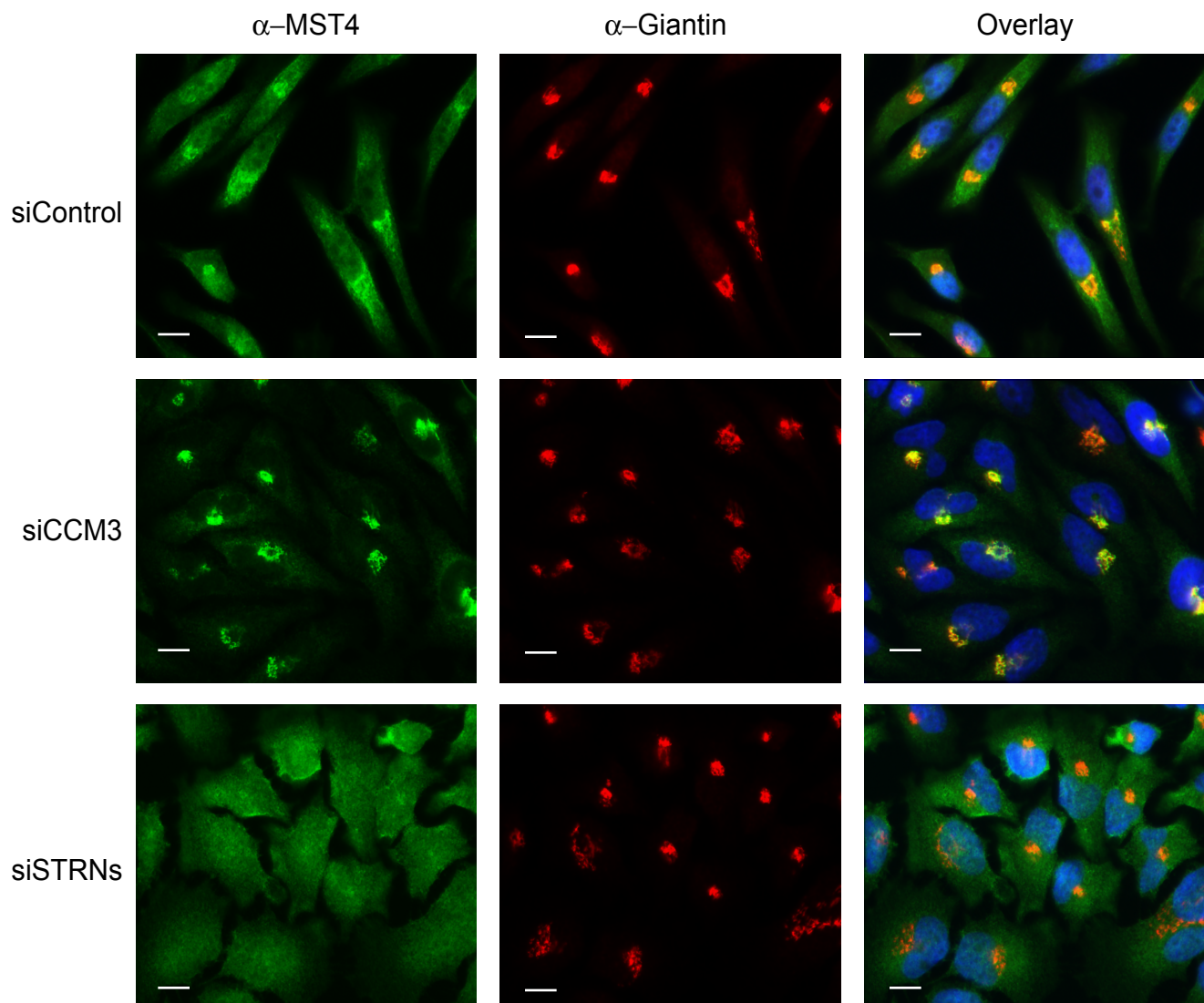
Supplementary Figure 7. Knockdown of CCM3 and STRNs using siRNAs induce an opposite effect on the localization of MST4.
siRNA mediated depletion of CCM3 induces near complete localization of MST4 to the Golgi while depletion of striatins using siRNAs prevents Golgi localization. These data are consistent with the esiRNA data presented in Figure 5. Scale bars = 10μm.

Supplementary Table I. Mass spectrometry data used to create Figure 1C. AP-MS analysis of Strn or STRN3 (full length or indicated deletion mutants) was performed in biological duplicates. Data corresponding to STRIPAK components and to the CCT chaperonin complex (as defined in Goudreault et al., *Mol Cell Proteomics*, 2009) was extracted. "ProHits ID" is the unique identifier for the sample in our internal interaction database, "Mascot" is the score from the Mascot search engine, "n specs" is the total number of peptides, or spectral counts, "n peps" is the number of unique peptides (as defined by the Mascot search engine) and "% cover" is the proportion of the protein sequence that has been detected by mass spectrometry.

ProHits ID	Bait Name	Hit Name	Hit Gene ID	gi number	Mascot	n specs	n peps	% cover
5341	STRN3 -1	CCT2	10576	5453603	632	23	10	29
5344	STRN3 -2	CCT2	10576	5453603	720	21	12	33.1
5341	STRN3 -1	CCT3	7203	58761486	545	14	9	19.1
5344	STRN3 -2	CCT3	7203	58761486	389	12	6	17.5
5341	STRN3 -1	CCT4	10575	38455427	200	6	4	10.2
5344	STRN3 -2	CCT4	10575	38455427	627	24	12	30.6
5341	STRN3 -1	CCT5	22948	24307939	274	8	6	10.5
5344	STRN3 -2	CCT5	22948	24307939	570	19	8	26.6
5341	STRN3 -1	CCT6A	908	4502643	250	10	5	10.4
5344	STRN3 -2	CCT6A	908	4502643	665	20	14	39.9
5341	STRN3 -1	CCT7	10574	5453607	351	8	5	12.9
5344	STRN3 -2	CCT7	10574	5453607	514	15	9	21.9
5341	STRN3 -1	CCT8	10694	48762932	641	19	12	23.5
5344	STRN3 -2	CCT8	10694	48762932	651	25	12	23
5341	STRN3 -1	CTTNBP2	83992	16975496	383	10	6	4.3
5344	STRN3 -2	CTTNBP2	83992	16975496	554	16	10	7.9
5341	STRN3 -1	CTTNBP2NL	55917	24308179	1022	38	16	28.2
5344	STRN3 -2	CTTNBP2NL	55917	24308179	1357	72	21	41.8
5341	STRN3 -1	FAM40A	85369	56790935	1409	61	26	37
5344	STRN3 -2	FAM40A	85369	56790935	1803	159	30	49.8
5341	STRN3 -1	FAM40B	57464	55742705	461	16	6	11
5344	STRN3 -2	FAM40B	57464	197245444	586	20	7	16.1
5341	STRN3 -1	FGFR1OP2	26127	24308111	180	9	4	23.3
5344	STRN3 -2	FGFR1OP2	26127	24308111	655	26	10	54.2
5341	STRN3 -1	MOBKL3	25843	41349449	372	20	6	40.9
5344	STRN3 -2	MOBKL3	25843	41349449	490	63	7	41.3
5341	STRN3 -1	PDCD10	11235	20127517	401	16	7	48.1
5344	STRN3 -2	PDCD10	11235	20127517	694	42	11	66
5344	STRN3 -2	PPP2CA	5515	4506017	469	65	7	31.1
5341	STRN3 -1	PPP2CB	5516	4758952	439	20	9	31.7
5341	STRN3 -1	PPP2R1A	5518	21361399	798	32	15	29.5
5344	STRN3 -2	PPP2R1A	5518	21361399	1342	84	22	43.6
5341	STRN3 -1	PPP2R1B	5519	32455244	228	6	1	7
5344	STRN3 -2	PPP2R1B	5519	32455244	407	23	4	14.5
5341	STRN3 -1	MST4	51765	15011880	512	18	10	30.8
5344	STRN3 -2	MST4	51765	15011880	973	39	15	36.5
5341	STRN3 -1	SIKE1	80143	156151377	460	13	7	45.4
5344	STRN3 -2	SIKE1	80143	156151377	782	24	12	62.3
5341	STRN3 -1	SLMAP	7871	56550043	1477	55	26	35.8
5344	STRN3 -2	SLMAP	7871	56550043	1807	82	32	43.4
5341	STRN3 -1	STK24	8428	20070158	441	16	3	23
5344	STRN3 -2	STK24	8428	20070158	935	41	6	33.2

5344 STRN3 -2	STK25	10494	21361358	494	17	1	18.3
5341 STRN3 -1	STRN	6801	51242945	1066	27	16	30.3
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5341 STRN3 -1	STRN3	29966	142976686	1685	118	24	42.2
5344 STRN3 -2	STRN3	29966	142976686	2318	314	31	48.1
5341 STRN3 -1	STRN4	29888	89886477	893	31	12	25.5
5344 STRN3 -2	STRN4	29888	89886477	1674	125	19	52.2
5341 STRN3 -1	TCP1	6950	57863257	479	16	9	19.1
5344 STRN3 -2	TCP1	6950	57863257	475	11	7	20.5
4701 Strn -1	CCT2	10576	5453603	570	14	8	26
8738 Strn -2	CCT2	10576	5453603	1261	51	18	53.8
4701 Strn -1	CCT3	7203	58761486	315	7	5	14.5
8738 Strn -2	CCT3	7203	58761486	972	47	16	41.4
4701 Strn -1	CCT4	10575	38455427	340	7	6	20
8738 Strn -2	CCT4	10575	38455427	905	51	17	39
4701 Strn -1	CCT5	22948	24307939	481	12	7	21.3
8738 Strn -2	CCT5	22948	24307939	1200	61	22	50.5
4701 Strn -1	CCT6A	908	4502643	314	8	4	13.4
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4701 Strn -1	CCT7	10574	5453607	381	9	4	11.8
8738 Strn -2	CCT7	10574	5453607	814	32	14	30.9
4701 Strn -1	CCT8	10694	48762932	388	7	6	13.9
8738 Strn -2	CCT8	10694	48762932	1384	61	26	45.6
4701 Strn -1	CTTNBP2	83992	16975496	46	4	1	0.8
8738 Strn -2	CTTNBP2	83992	16975496	260	9	5	3.8
4701 Strn -1	CTTNBP2NL	55917	24308179	716	18	12	29.7
8738 Strn -2	CTTNBP2NL	55917	24308179	1570	76	27	49
4701 Strn -1	FAM40A	85369	56790935	1446	67	21	37.4
8738 Strn -2	FAM40A	85369	56790935	1766	176	29	43.1
4701 Strn -1	FAM40B	57464	197245444	242	6	3	9.6
8738 Strn -2	FAM40B	57464	55742705	658	24	10	20.1
4701 Strn -1	FGFR1OP2	26127	24308111	556	20	7	37.5
8738 Strn -2	FGFR1OP2	26127	24308111	864	37	13	64.4
4701 Strn -1	MOBKL3	25843	41349449	414	40	5	38.2
8738 Strn -2	MOBKL3	25843	41349449	454	150	6	40.9
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8738 Strn -2	PDCD10	11235	20127517	440	32	8	56.1
4701 Strn -1	PPP2CA	5515	4506017	433	22	7	33.3
8738 Strn -2	PPP2CA	5515	4506017	485	56	9	33.7
4701 Strn -1	PPP2R1A	5518	21361399	950	46	14	33.1
8738 Strn -2	PPP2R1A	5518	21361399	1459	116	24	48.2
8738 Strn -2	PPP2R1B	5519	32455246	441	26	2	15.5
4701 Strn -1	MST4	51765	15011880	630	24	9	23.3
8738 Strn -2	MST4	51765	15011880	538	31	4	30.3
4701 Strn -1	SIKE1	80143	156151377	497	9	6	36.2
8738 Strn -2	SIKE1	80143	156151377	658	26	11	66.2
4701 Strn -1	SLMAP	7871	56550043	1058	62	16	27.7
8738 Strn -2	SLMAP	7871	56550043	2024	138	40	48
4701 Strn -1	STK24	8428	20070158	570	26	4	22.6
8738 Strn -2	STK24	8428	20070158	681	33	12	28.7
4701 Strn -1	STRN	6801	51242945	2087	627	27	45.6
8738 Strn -2	STRN	6801	51242945	2942	1319	46	73.8
4701 Strn -1	STRN3	29966	142976686	893	23	13	28.4
8738 Strn -2	STRN3	29966	142976686	1803	156	24	43
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8738 Strn -2	STRN4	29888	89886477	1721	225	21	50.1
4701 Strn -1	TCP1	6950	57863257	497	9	7	25.9
8738 Strn -2	TCP1	6950	57863257	1209	56	21	50.7
5350 Strn 45-780 -1	CCT2	10576	5453603	1600	62	21	54.6
5694 Strn 45-780 -2	CCT2	10576	5453603	345	9	5	18.5
5350 Strn 45-780 -1	CCT3	7203	58761486	1202	49	19	43.4
5694 Strn 45-780 -2	CCT3	7203	58761486	603	16	8	21.7
5350 Strn 45-780 -1	CCT4	10575	38455427	1112	39	21	48.1
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5350 Strn 45-780 -1	CCT5	22948	24307939	1629	60	27	57.3
5694 Strn 45-780 -2	CCT5	22948	24307939	541	20	8	29.6
5350 Strn 45-780 -1	CCT6A	908	4502643	879	40	15	37.7
5694 Strn 45-780 -2	CCT6A	908	4502643	248	17	4	11.1
5350 Strn 45-780 -1	CCT7	10574	5453607	755	26	13	30.4
5694 Strn 45-780 -2	CCT7	10574	5453607	405	10	6	16.9
5350 Strn 45-780 -1	CCT8	10694	48762932	1358	52	23	43.2
5694 Strn 45-780 -2	CCT8	10694	48762932	789	25	14	30.5
5350 Strn 45-780 -1	COPA	1314	148536853	35	1	1	1.8
5350 Strn 45-780 -1	CTTNBP2	83992	16975496	309	8	5	3.5
5350 Strn 45-780 -1	CTTNBP2NL	55917	24308179	1280	36	22	38.3
5694 Strn 45-780 -2	CTTNBP2NL	55917	24308179	863	50	14	27.5
5350 Strn 45-780 -1	FAM40A	85369	56790935	1622	88	28	43.7
5694 Strn 45-780 -2	FAM40A	85369	56790935	1523	122	25	43.7
5350 Strn 45-780 -1	FAM40B	57464	55742705	452	17	6	12.9
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5350 Strn 45-780 -1	FGFR1OP2	26127	24308111	875	28	13	61.7
5694 Strn 45-780 -2	FGFR1OP2	26127	24308111	762	29	11	57.7
5350 Strn 45-780 -1	MOBKL3	25843	41349449	388	34	6	40.9
5694 Strn 45-780 -2	MOBKL3	25843	41349449	367	35	5	36.4
5350 Strn 45-780 -1	PDCD10	11235	20127517	534	24	8	60.8
5694 Strn 45-780 -2	PDCD10	11235	20127517	414	19	7	55.2
5694 Strn 45-780 -2	PPP2CA	5515	4506017	388	42	6	28.5
5350 Strn 45-780 -1	PPP2CB	5516	4758952	514	29	9	34
5350 Strn 45-780 -1	PPP2R1A	5518	21361399	1264	63	20	44.8
5694 Strn 45-780 -2	PPP2R1A	5518	21361399	824	75	12	29
5350 Strn 45-780 -1	PPP2R1B	5519	32455246	410	23	1	16
5350 Strn 45-780 -1	MST4	51765	15011880	541	17	2	22.6
5694 Strn 45-780 -2	MST4	51765	15011880	556	45	10	27.6
5350 Strn 45-780 -1	SIKE1	80143	156151379	707	23	11	57.8
5694 Strn 45-780 -2	SIKE1	80143	156151377	260	6	4	30.9
5350 Strn 45-780 -1	SLMAP	7871	56550043	1709	75	34	42.3
5694 Strn 45-780 -2	SLMAP	7871	56550043	1159	53	20	31.1
5350 Strn 45-780 -1	STK24	8428	20070158	628	23	10	26.2
5694 Strn 45-780 -2	STK24	8428	20070158	417	47	1	12.9
5350 Strn 45-780 -1	STRN	6801	51242945	2322	311	35	56.2
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5350 Strn 45-780 -1	STRN3	29966	142976686	1488	80	22	41.9
5694 Strn 45-780 -2	STRN3	29966	142976686	1643	158	21	43.5
5350 Strn 45-780 -1	STRN4	29888	89886477	1383	68	20	47.1
5694 Strn 45-780 -2	STRN4	29888	89886477	1655	171	20	50.6
5350 Strn 45-780 -1	TCP1	6950	57863257	1286	47	18	48.2
5694 Strn 45-780 -2	TCP1	6950	57863257	492	19	9	25.9
5349 Strn 90-780 -1	CCT2	10576	5453603	194	5	4	6.7
5695 Strn 90-780 -2	CCT2	10576	5453603	360	16	8	11.6
5349 Strn 90-780 -1	CCT3	7203	58761486	373	13	7	15.6

5695 Strn 90-780 -2	CCT3	7203	58761486	225	11	4	7.4
5349 Strn 90-780 -1	CCT4	10575	38455427	206	8	4	6.5
5695 Strn 90-780 -2	CCT4	10575	38455427	325	15	7	12.8
5349 Strn 90-780 -1	CCT5	22948	24307939	215	8	4	9.2
5695 Strn 90-780 -2	CCT5	22948	24307939	308	12	7	9.1
5349 Strn 90-780 -1	CCT6A	908	4502643	195	7	4	8.3
5695 Strn 90-780 -2	CCT6A	908	4502643	432	22	12	17.1
5349 Strn 90-780 -1	CCT7	10574	5453607	211	5	4	7.7
5695 Strn 90-780 -2	CCT7	10574	5453607	319	13	8	12.7
5349 Strn 90-780 -1	CCT8	10694	48762932	558	22	11	18.1
5695 Strn 90-780 -2	CCT8	10694	48762932	437	16	9	16.1
5695 Strn 90-780 -2	COPA	1314	148536853	69	3	1	1.2
5349 Strn 90-780 -1	MOBKL3	25843	41349449	245	7	5	36.4
5695 Strn 90-780 -2	MOBKL3	25843	41349449	153	8	3	22.2
5349 Strn 90-780 -1	PDCD10	11235	20127517	381	13	7	41.5
5695 Strn 90-780 -2	PDCD10	11235	20127517	220	12	5	18.9
5349 Strn 90-780 -1	PPP2R1A	5518	21361399	59	1	1	1.7
5695 Strn 90-780 -2	PPP2R1A	5518	21361399	81	3	2	3.4
5349 Strn 90-780 -1	MST4	51765	15011880	369	9	6	16.3
5695 Strn 90-780 -2	MST4	51765	15011880	291	21	2	12
5349 Strn 90-780 -1	STK24	8428	20070158	292	8	1	11.7
5695 Strn 90-780 -2	STK24	8428	20070158	291	19	7	13.1
5695 Strn 90-780 -2	STK25	10494	21361358	71	2	2	3.8
5349 Strn 90-780 -1	STRN	6801	51242945	1272	59	22	36.5
5695 Strn 90-780 -2	STRN	6801	51242945	1053	66	21	32.8
5349 Strn 90-780 -1	STRN3	29966	142976675	367	10	7	18.5
5349 Strn 90-780 -1	TCP1	6950	57863257	175	7	4	7
5695 Strn 90-780 -2	TCP1	6950	57863257	216	13	5	7.4
5340 STRN3 1-169 -1	CCT2	10576	5453603	368	11	6	16.6
5345 STRN3 1-169 -2	CCT2	10576	5453603	654	16	12	33.8
5340 STRN3 1-169 -1	CCT3	7203	58761484	200	7	4	8.7
5345 STRN3 1-169 -2	CCT3	7203	58761486	392	13	6	17.5
5340 STRN3 1-169 -1	CCT4	10575	38455427	191	6	4	8.3
5345 STRN3 1-169 -2	CCT4	10575	38455427	438	11	8	22.4
5340 STRN3 1-169 -1	CCT5	22948	24307939	186	3	2	4.8
5345 STRN3 1-169 -2	CCT5	22948	24307939	459	12	6	21.8
5340 STRN3 1-169 -1	CCT6A	908	4502643	179	5	4	10
5345 STRN3 1-169 -2	CCT6A	908	4502643	556	16	9	24.3
5340 STRN3 1-169 -1	CCT7	10574	5453607	166	4	2	6.4
5345 STRN3 1-169 -2	CCT7	10574	5453607	375	11	7	13.6
5340 STRN3 1-169 -1	CCT8	10694	48762932	332	8	6	11.7
5345 STRN3 1-169 -2	CCT8	10694	48762932	646	17	10	21.7
5340 STRN3 1-169 -1	CTTNBP2	83992	16975496	384	11	7	4.7
5345 STRN3 1-169 -2	CTTNBP2	83992	16975496	242	8	5	3.6
5340 STRN3 1-169 -1	CTTNBP2NL	55917	24308179	1188	42	19	32.9
5345 STRN3 1-169 -2	CTTNBP2NL	55917	24308179	1433	87	24	43.2
5340 STRN3 1-169 -1	FAM40A	85369	56790935	1686	81	27	40
5345 STRN3 1-169 -2	FAM40A	85369	56790935	1843	163	30	49.8
5340 STRN3 1-169 -1	FAM40B	57464	55742705	769	25	10	17.5
5345 STRN3 1-169 -2	FAM40B	57464	55742705	880	34	12	24.5
5345 STRN3 1-169 -2	FGFR1OP2	26127	24308111	130	3	2	9.1
5340 STRN3 1-169 -1	MOBKL3	25843	41349449	491	34	7	44.4
5345 STRN3 1-169 -2	MOBKL3	25843	41349449	568	73	8	50.7
5340 STRN3 1-169 -1	PDCD10	11235	20127517	404	18	7	39.6
5345 STRN3 1-169 -2	PDCD10	11235	20127517	637	34	10	60.8

5340 STRN3 1-169 -1	PPP2CA	5515	4506017	441	31	8	29.4
5345 STRN3 1-169 -2	PPP2CA	5515	4506017	528	53	9	42.7
5340 STRN3 1-169 -1	PPP2R1A	5518	21361399	1010	55	17	35.1
5345 STRN3 1-169 -2	PPP2R1A	5518	21361399	1265	93	20	40.9
5340 STRN3 1-169 -1	PPP2R1B	5519	32455244	507	16	5	18.7
5345 STRN3 1-169 -2	PPP2R1B	5519	32455244	730	32	7	22.6
5340 STRN3 1-169 -1	MST4	51765	15011880	545	19	4	30.5
5345 STRN3 1-169 -2	MST4	51765	15011880	616	27	4	26
5345 STRN3 1-169 -2	SIKE1	80143	156151377	231	5	4	26.6
5340 STRN3 1-169 -1	SLMAP	7871	56550043	126	4	2	2.8
5345 STRN3 1-169 -2	SLMAP	7871	56550043	988	30	18	28.7
5340 STRN3 1-169 -1	STK24	8428	20070158	556	21	11	28.4
5345 STRN3 1-169 -2	STK24	8428	20070158	700	27	12	33
5340 STRN3 1-169 -1	STK25	10494	21361358	278	8	1	14.1
5345 STRN3 1-169 -2	STK25	10494	21361358	383	16	1	14.3
5340 STRN3 1-169 -1	STRN	6801	51242945	1803	70	29	51.4
5345 STRN3 1-169 -2	STRN	6801	51242945	2457	140	34	67.9
5340 STRN3 1-169 -1	STRN3	29966	142976686	1781	170	24	42
5345 STRN3 1-169 -2	STRN3	29966	142976686	2518	272	35	49.3
5340 STRN3 1-169 -1	STRN4	29888	89886477	1410	76	18	40.2
5345 STRN3 1-169 -2	STRN4	29888	89886477	1988	224	23	56.6
5340 STRN3 1-169 -1	TCP1	6950	57863257	102	3	2	4.9
5345 STRN3 1-169 -2	TCP1	6950	57863257	606	14	10	29.9
5346 STRN3 1-388 -1	CCT2	10576	5453603	35	1	1	1.9
5346 STRN3 1-388 -1	CCT3	7203	58761484	37	1	1	2.2
5346 STRN3 1-388 -1	CCT4	10575	38455427	47	2	1	1.5
5574 STRN3 1-388 -2	CCT4	10575	38455427	69	4	1	2.8
5346 STRN3 1-388 -1	CCT5	22948	24307939	257	7	5	10
5574 STRN3 1-388 -2	CCT5	22948	24307939	37	1	1	1.5
5346 STRN3 1-388 -1	CCT6A	908	4502643	95	3	2	3.8
5346 STRN3 1-388 -1	CCT7	10574	5453607	59	2	1	1.7
5574 STRN3 1-388 -2	CCT7	10574	5453607	115	2	2	3.9
5346 STRN3 1-388 -1	CCT8	10694	48762932	163	6	4	7.3
5574 STRN3 1-388 -2	CCT8	10694	48762932	120	4	3	4.6
5346 STRN3 1-388 -1	CTTNBP2	83992	16975496	138	5	3	1.8
5574 STRN3 1-388 -2	CTTNBP2	83992	16975496	329	12	8	4.6
5346 STRN3 1-388 -1	CTTNBP2NL	55917	24308179	520	19	10	15.6
5574 STRN3 1-388 -2	CTTNBP2NL	55917	24308179	600	23	11	15.8
5346 STRN3 1-388 -1	FAM40A	85369	56790935	583	31	11	19
5574 STRN3 1-388 -2	FAM40A	85369	56790935	625	36	13	19
5574 STRN3 1-388 -2	FAM40B	57464	55742705	258	13	2	5.4
5346 STRN3 1-388 -1	FGFR1OP2	26127	24308111	153	5	4	24.1
5574 STRN3 1-388 -2	FGFR1OP2	26127	24308111	116	4	3	8.3
5346 STRN3 1-388 -1	MOBKL3	25843	41349449	348	18	6	40
5574 STRN3 1-388 -2	MOBKL3	25843	41349449	192	12	4	32
5346 STRN3 1-388 -1	PDCD10	11235	20127517	487	19	9	46.2
5574 STRN3 1-388 -2	PDCD10	11235	20127517	349	15	7	27.4
5346 STRN3 1-388 -1	PPP2CA	5515	4506017	314	17	5	21.7
5574 STRN3 1-388 -2	PPP2CB	5516	4758952	265	10	6	22
5346 STRN3 1-388 -1	PPP2R1A	5518	21361399	577	24	10	21.7
5574 STRN3 1-388 -2	PPP2R1A	5518	21361399	818	35	16	27.3
5346 STRN3 1-388 -1	PPP2R1B	5519	32455244	297	9	2	8.5
5574 STRN3 1-388 -2	PPP2R1B	5519	32455244	295	7	2	8.5
5346 STRN3 1-388 -1	MST4	51765	15011880	353	10	2	15.1
5574 STRN3 1-388 -2	MST4	51765	15011880	333	10	4	19.2

5346 STRN3 1-388 -1	SIKE1	80143	156151379	174	4	3	14.7
5574 STRN3 1-388 -2	SIKE1	80143	156151379	214	5	4	20.4
5346 STRN3 1-388 -1	SLMAP	7871	56550043	677	32	13	17.8
5574 STRN3 1-388 -2	SLMAP	7871	56550043	969	57	21	20.5
5346 STRN3 1-388 -1	STK24	8428	20070158	362	11	6	16.5
5574 STRN3 1-388 -2	STK24	8428	20070158	379	15	6	15.8
5574 STRN3 1-388 -2	STK25	10494	21361358	178	5	2	9.6
5346 STRN3 1-388 -1	STRN	6801	51242945	1077	32	16	27.9
5574 STRN3 1-388 -2	STRN	6801	51242945	573	23	12	18.5
5346 STRN3 1-388 -1	STRN3	29966	142976686	1540	70	22	38.5
5574 STRN3 1-388 -2	STRN3	29966	142976675	1200	96	18	34.4
5346 STRN3 1-388 -1	STRN4	29888	89886477	875	33	14	27.8
5574 STRN3 1-388 -2	STRN4	29888	89886477	566	31	9	16.1
5343 STRN3 219-713 -1	CCT2	10576	5453603	373	9	6	20.4
5563 STRN3 219-713 -2	CCT2	10576	5453603	609	17	10	28.2
5343 STRN3 219-713 -1	CCT3	7203	58761486	440	12	6	16.9
5563 STRN3 219-713 -2	CCT3	7203	58761486	425	11	7	20.6
5343 STRN3 219-713 -1	CCT4	10575	38455427	371	9	6	13.4
5563 STRN3 219-713 -2	CCT4	10575	38455427	235	9	5	9.3
5343 STRN3 219-713 -1	CCT5	22948	24307939	366	8	6	13.1
5563 STRN3 219-713 -2	CCT5	22948	24307939	403	13	6	17.6
5343 STRN3 219-713 -1	CCT6A	908	4502643	171	4	3	11.7
5563 STRN3 219-713 -2	CCT6A	908	4502643	342	9	6	19.6
5343 STRN3 219-713 -1	CCT7	10574	5453607	360	10	6	15.7
5563 STRN3 219-713 -2	CCT7	10574	5453607	408	11	5	17.3
5343 STRN3 219-713 -1	CCT8	10694	48762932	380	10	7	13.1
5563 STRN3 219-713 -2	CCT8	10694	48762932	555	13	10	21.4
5343 STRN3 219-713 -1	MOBKL3	25843	41349449	310	12	5	36.4
5563 STRN3 219-713 -2	MOBKL3	25843	41349449	429	29	7	47.1
5343 STRN3 219-713 -1	PDCD10	11235	20127517	175	5	2	21.2
5563 STRN3 219-713 -2	PDCD10	11235	20127517	205	5	3	25.9
5343 STRN3 219-713 -1	PPP2R1A	5518	21361399	32	1	1	3.4
5563 STRN3 219-713 -2	MST4	51765	15011880	105	4	2	6.3
5343 STRN3 219-713 -1	STK24	8428	20070158	183	5	3	8.8
5343 STRN3 219-713 -1	STRN3	29966	142976675	785	24	11	22.3
5563 STRN3 219-713 -2	STRN3	29966	142976675	1223	68	15	25.7
5343 STRN3 219-713 -1	TCP1	6950	57863257	400	11	7	18.2
5563 STRN3 219-713 -2	TCP1	6950	57863257	517	14	9	29.3

Supplementary Table II. Data corresponding to the fluorescence polarization assay in Figure 2E. STRN3 (aa 286-310) interacts with wild type CCM3 and substitution of K132, K139, K172 and K179 in CCM3 by alanines completely abrogated association. The values below are reported in micromolar and are the average of 9 measurements from three independent binding experiments.

	CCM3 wildtype	CCM3 K132-139-172-179A
	One site binding (hyperbola)	Does not converge
Best-fit values		
BMAX	233.7	
KD	0.1323	
Std. Error		
BMAX	1.18	
KD	0.003396	
95% Confidence Intervals		
BMAX	231.4 to 236.1	
KD	0.1256 to 0.1390	
Goodness of Fit		
Degrees of Freedom	118	
R^2	0.9905	
Absolute Sum of Squares	7780	

Supplementary Table III. SAINT-filtered interactions for GM130. AP-MS of FLAG-tagged GM130 full-length or a deletion mutant encompassing amino acids 75-271 was performed in biological replicates. The mutant GM130 75-271 was shown to be sufficient for MST4 interaction (Preisinger et al., *J Cell Biol*, 2004). Data was analyzed using SAINT (Choi et al., *Nature Methods*, 2010), using eight negative control runs (AP-MS from cells transfected with the empty vector). Proteins detected with an averaged probability ≥0.7 (AvgP) and with ≥10 spectra in at least one of the 2 replicates are listed below. In the table, "Sample ID" refers to the unique identifiers for the sample in our database; "Spectra" indicates the total number of peptides for the prey; "iProb" lists the SAINT probability in the individual analyses; "AvgP" lists the final probability. "|" is a delimiter.

Bait	Prey	Sample ID	Spectra	iProb	Spectra in controls	AvgP
GM130	CCT3	7105|8698	2|10	0.75|0.95	0|0|0|0|0|0|0|0	0.8525
GM130	GOLGA2	7105|8698	1815|3270	0.96|0.94	0|0|0|0|0|0|0|0	0.949
GM130	GORASP1	7105|8698	18|19	0.99|0.99	0|0|0|0|0|0|0|0	0.9865
GM130	GORASP2	7105|8698	6|20	0.93|0.96	0|0|0|0|0|0|0|0	0.9435
GM130	MST4	7105|8698	52|104	1.00|0.99	0|0|0|0|0|0|0|0	0.9945
GM130	NDUFA8	7105|8698	6|16	0.95|0.96	0|0|0|0|0|0|0|0	0.955
GM130	PTPN13	7105|8698	6|33	0.68|0.96	0|0|0|0|0|0|0|0	0.8215
GM130	SEC16A	7105|8698	20|26	0.98|0.99	0|0|0|0|0|0|0|0	0.986
GM130	TUBA4A	7105|8698	46|62	1.00|1.00	0|0|0|0|0|0|0|0	0.996
GM130	TUBB2B	7105|8698	68|97	1.00|1.00	37|29|0|0|0|0|0|0	0.9965
GM130	TUBB4	7105|8698	68|97	1.00|1.00	30|0|0|0|0|0|0|25	0.998
GM130	UBR4	7105|8698	9|11	0.73|0.74	0|0|0|0|0|0|0|0	0.737
GM130	USO1	7105|8698	42|34	0.99|0.99	0|0|0|0|0|0|0|0	0.9885
GM130_75_271	CCT2	7108|8739	4|10	0.74|0.84	0|2|0|0|0|0|0|0	0.788
GM130_75_271	CCT3	7108|8739	3|10	0.84|0.96	0|0|0|0|0|0|0|0	0.9005
GM130_75_271	CCT4	7108|8739	5|13	0.94|0.94	0|0|0|0|0|0|0|0	0.94
GM130_75_271	COPA	7108|8739	81|16	0.86|0.80	0|0|0|0|0|0|0|0	0.827
GM130_75_271	COPB2	7108|8739	47|13	0.90|0.88	0|0|0|0|0|0|0|0	0.8925
GM130_75_271	COPE	7108|8739	22|10	0.97|0.96	0|0|0|0|0|0|0|0	0.963
GM130_75_271	GOLGA2	7108|8739	996|1343	0.93|0.93	0|0|0|0|0|0|0|0	0.928
GM130_75_271	IRS4	7108|8739	4|14	0.80|0.95	0|0|0|0|0|0|0|0	0.875
GM130_75_271	MST4	7108|8739	30|19	0.97|0.50	0|0|0|0|0|0|0|0	0.733
GM130_75_271	TUBB4	7108|8739	72|40	1.00|0.68	30|0|0|0|0|0|0|25	0.841

Supplementary Table IV. Detailed mass spectrometry data for the GM130 interactions. Complete mass spectrometric information for interaction partners with SAINT AvgP ≥0.7, for any components of the CCT complex or for any STRIPAK component (as defined in Goudreault et al.*, Mol Cell Proteomics*, 2009) is listed below. "ProHits ID" is the unique identifier for the sample in our internal interaction database, "Mascot" is the score from the Mascot search engine, "n specs" is the total number of peptides, or spectral counts, "n peps" is the number of unique peptides (as defined by the Mascot search engine) and "% cover" is the proportion of the protein sequence that has been detected by mass spectrometry. The mutant GM130 75-271 was shown to be sufficient for MST4 interaction (Preisinger et al.*, J Cell Biol*, 2004). STRIPAK proteins below the SAINT threshold are highlighted in grey.

ProHits ID	Bait Name	Hit Name	Hit Gene ID	gi number	Mascot	n specs	n peps	% cover
7105	GM130 -1	CCT2	10576	5453603	42	1	1	2.8
8698	GM130 -2	CCT2	10576	5453603	354	8	5	19.8
7105	GM130 -1	CCT3	7203	58761484	68	2	1	2.2
8698	GM130 -2	CCT3	7203	58761486	339	10	6	23.3
7105	GM130 -1	CCT4	10575	38455427	203	4	4	7.8
8698	GM130 -2	CCT4	10575	38455427	163	6	3	11.5
7105	GM130 -1	CCT5	22948	24307939	152	3	2	8.5
8698	GM130 -2	CCT5	22948	24307939	313	10	5	20.7
7105	GM130 -1	CCT6A	908	4502643	69	1	1	3.2
8698	GM130 -2	CCT6A	908	4502643	171	9	3	10.9
7105	GM130 -1	CCT7	10574	58331185	37	1	1	2.9
8698	GM130 -2	CCT7	10574	5453607	112	3	2	6.6
7105	GM130 -1	CCT8	10694	48762932	194	6	5	9.7
8698	GM130 -2	CCT8	10694	48762932	140	6	3	5.1
7105	GM130 -1	GOLGA2	2801	194097392	4149	1815	61	63.6
8698	GM130 -2	GOLGA2	2801	194097392	3084	3270	42	57.6
7105	GM130 -1	GORASP1	64689	13994253	223	18	4	16.8
8698	GM130 -2	GORASP1	64689	13994253	140	19	2	6.4
7105	GM130 -1	GORASP2	26003	29826294	86	6	1	5.3
8698	GM130 -2	GORASP2	26003	29826294	260	20	4	15.7
8698	GM130 -2	MOBKL3	25843	41349449	44	2	1	6.2
7105	GM130 -1	NDUFA8	4702	7657369	177	6	3	18
8698	GM130 -2	NDUFA8	4702	7657369	263	16	5	34.3
8698	GM130 -2	PDCD10	11235	20127517	172	5	3	22.6
7105	GM130 -1	PPP2R1A	5518	21361399	31	1	1	3.4
7105	GM130 -1	PTPN13	5783	5453992	284	6	6	3.8
8698	GM130 -2	PTPN13	5783	5453992	985	33	21	14.1
7105	GM130 -1	MST4	51765	15011880	721	52	12	34.1
8698	GM130 -2	MST4	51765	15011880	791	104	12	38
7105	GM130 -1	SEC16A	9919	124378039	713	20	14	10.1
8698	GM130 -2	SEC16A	9919	124378039	664	26	16	13.3
8698	GM130 -2	STK25	10494	21361358	358	59	1	14.1
7105	GM130 -1	TCP1	6950	57863257	34	1	1	2.2
8698	GM130 -2	TCP1	6950	57863257	53	1	1	4.1
7105	GM130 -1	USO1	8615	4505541	1480	42	26	33.4
8698	GM130 -2	USO1	8615	4505541	667	34	11	18.5
7108	GM130 75-271 -1	CCT2	10576	5453603	143	4	2	5.6
8739	GM130 75-271 -2	CCT2	10576	5453603	438	10	6	15.7
7108	GM130 75-271 -1	CCT3	7203	58761484	133	3	3	9.7
8739	GM130 75-271 -2	CCT3	7203	58761486	353	10	7	23.3
7108	GM130 75-271 -1	CCT4	10575	38455427	232	5	4	9.8

8739 GM130 75-271 -2	CCT4	10575	38455427	372	13	6	16.9
7108 GM130 75-271 -1	CCT5	22948	24307939	148	3	2	8.5
8739 GM130 75-271 -2	CCT5	22948	24307939	288	10	4	13.3
7108 GM130 75-271 -1	CCT6A	908	4502643	153	3	3	13.7
8739 GM130 75-271 -2	CCT6A	908	4502643	202	7	4	13.4
7108 GM130 75-271 -1	CCT7	10574	5453607	75	2	1	2.6
8739 GM130 75-271 -2	CCT7	10574	5453607	192	5	3	7.7
7108 GM130 75-271 -1	CCT8	10694	48762932	184	5	4	8
8739 GM130 75-271 -2	CCT8	10694	48762932	466	16	8	17.7
7108 GM130 75-271 -1	COPA	1314	148536855	2118	81	38	36.2
8739 GM130 75-271 -2	COPA	1314	148536853	393	16	9	11.8
7108 GM130 75-271 -1	GOLGA2	2801	194097392	1136	996	17	14.6
8739 GM130 75-271 -2	GOLGA2	2801	194097392	1147	1343	16	15.3
7108 GM130 75-271 -1	MST4	51765	15011880	782	30	14	37.5
8739 GM130 75-271 -2	MST4	51765	15011880	351	19	8	26
7108 GM130 75-271 -1	TCP1	6950	57863259	42	1	1	5.2
8739 GM130 75-271 -2	TCP1	6950	57863257	381	10	8	20.1

Supplementary Table V. Mass spectrometry data used to quantify the recovery of STRIPAK components and GM130 in FLAG-MST4 immunoprecipitates following silencing of CCM3 or STRNs by esiRNA. Columns 2-4 list the spectral counts for each of the identified proteins across the different samples (ProteinPilot, 80% confidence). The number of spectral peaks used for quantification is listed in column 5; all peptides were manually validated.

hit	Specs esiLuc	Specs esiSTRNs	Specs esiCCM3	spectral peaks used for MS1 quant
MST4	59	100	60	11
CCM3	37	56	19	8
STRN	41	12	7	7
STRN3	31	21	10	6
CTTNBP2NL	18	10	7	3
PP2A cat	17	11	5	2
MOB3	12	6	5	3
STRIP1	16	6	3	2
STRN4	10	4	2	1
PP2A A	5	2	1	1
GM130	0	1	1	1