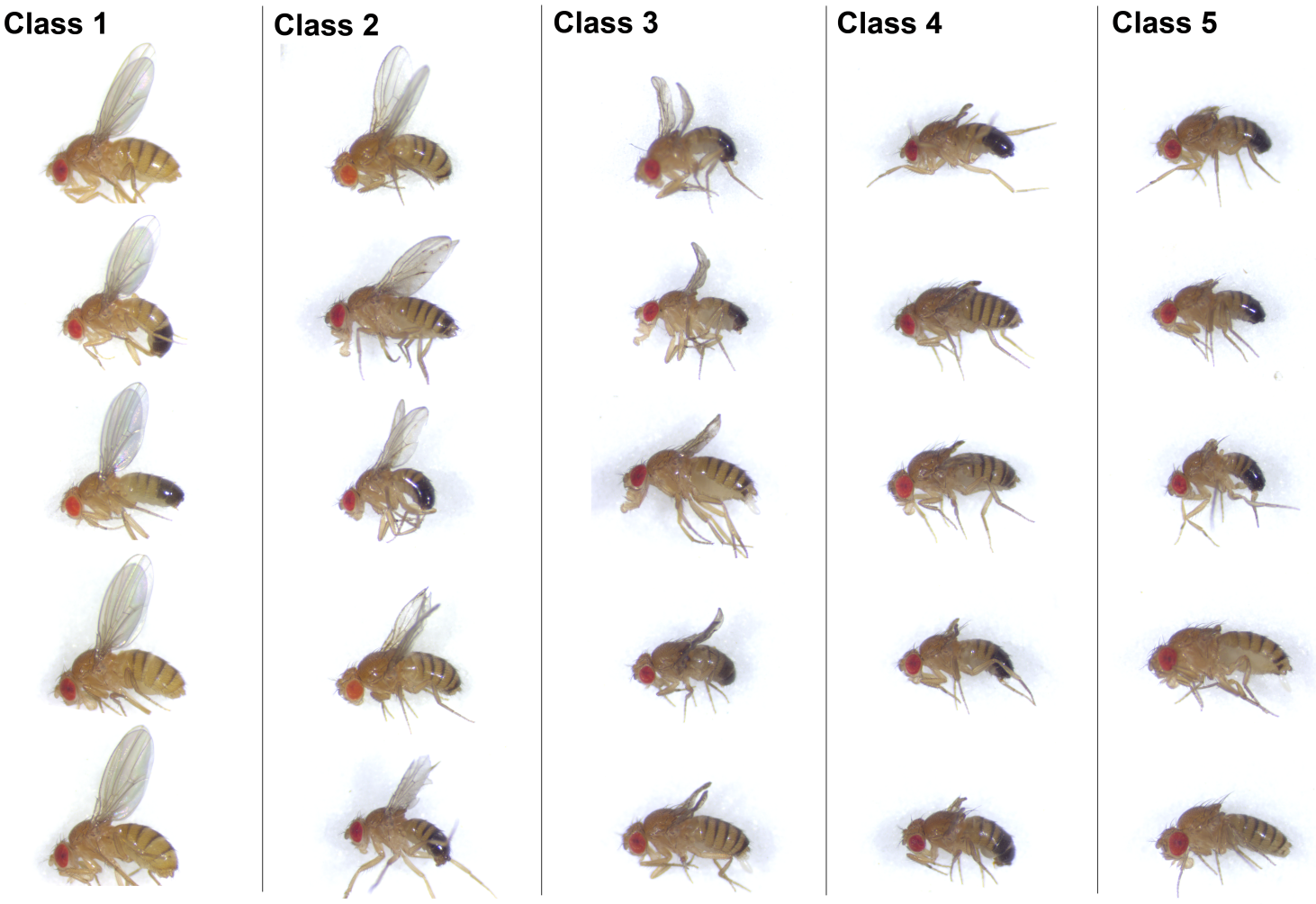
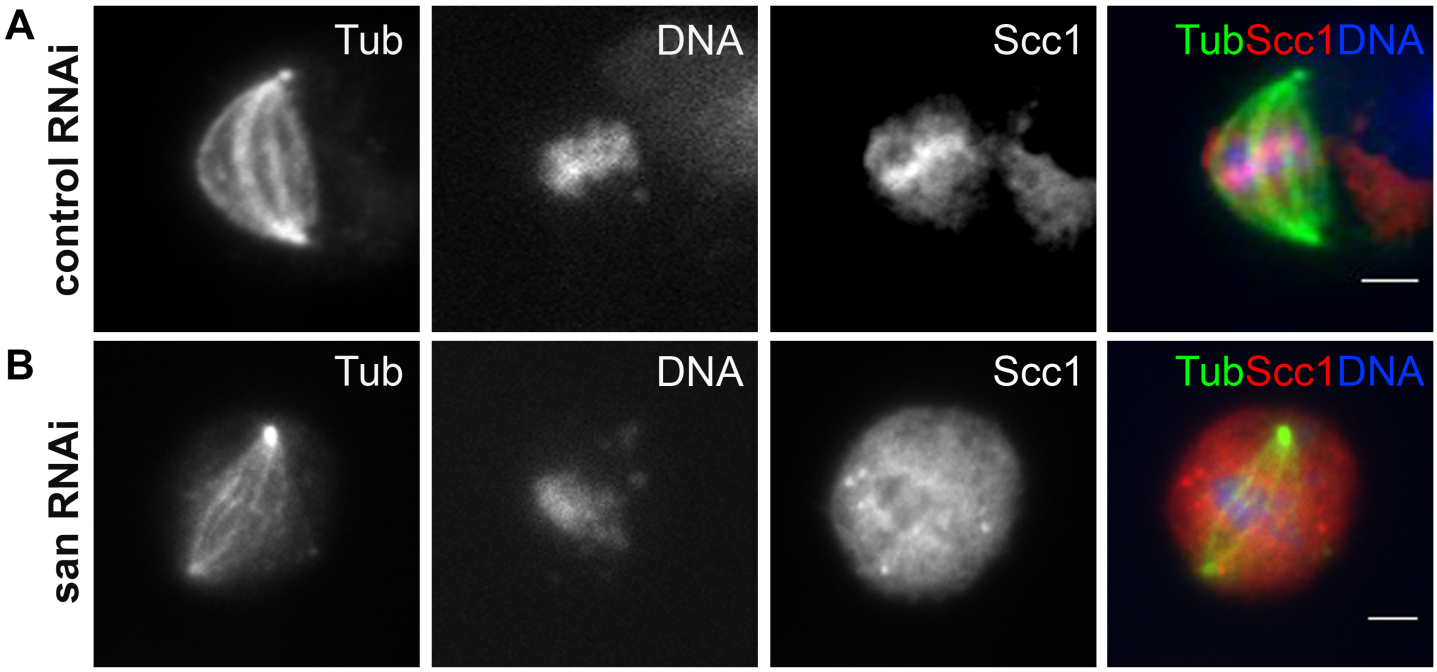
Naa50/San-dependent N-terminal acetylation of Scc1 is potentially important for sister chromatid cohesion.

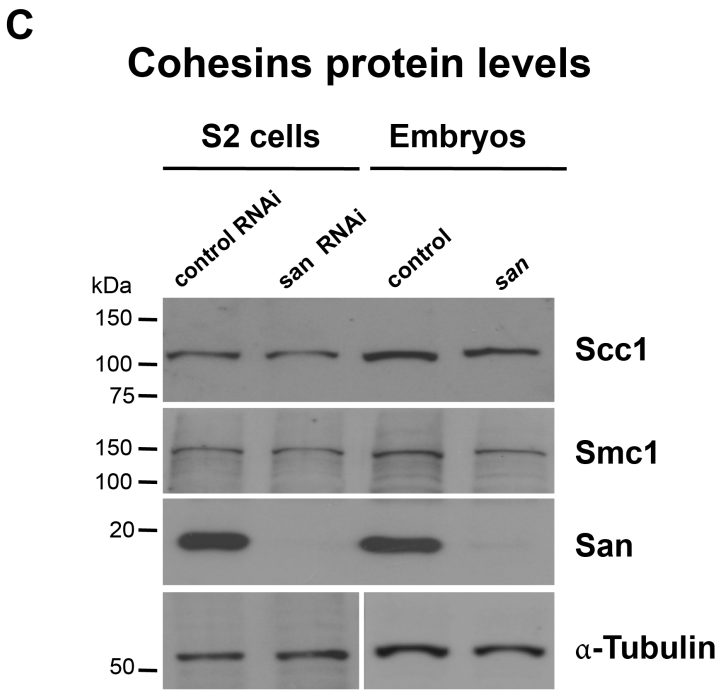
Ana L. Ribeiro[1,2], Rui D. Silva[1,2], Håvard Foyn[4], Margarida N. Tiago[1,2], Om S. Rathore[1,2], Thomas Arnesen[4,5], Rui Gonçalo Martinho[1,2,3,#].

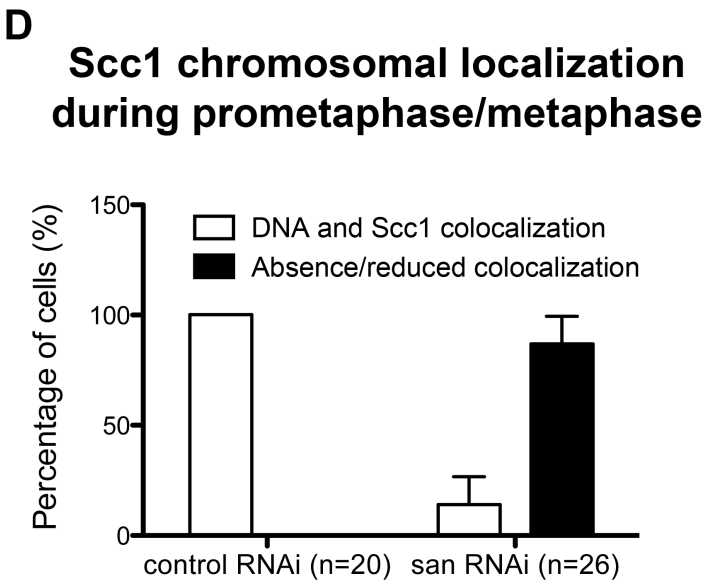
Supplementary Information:

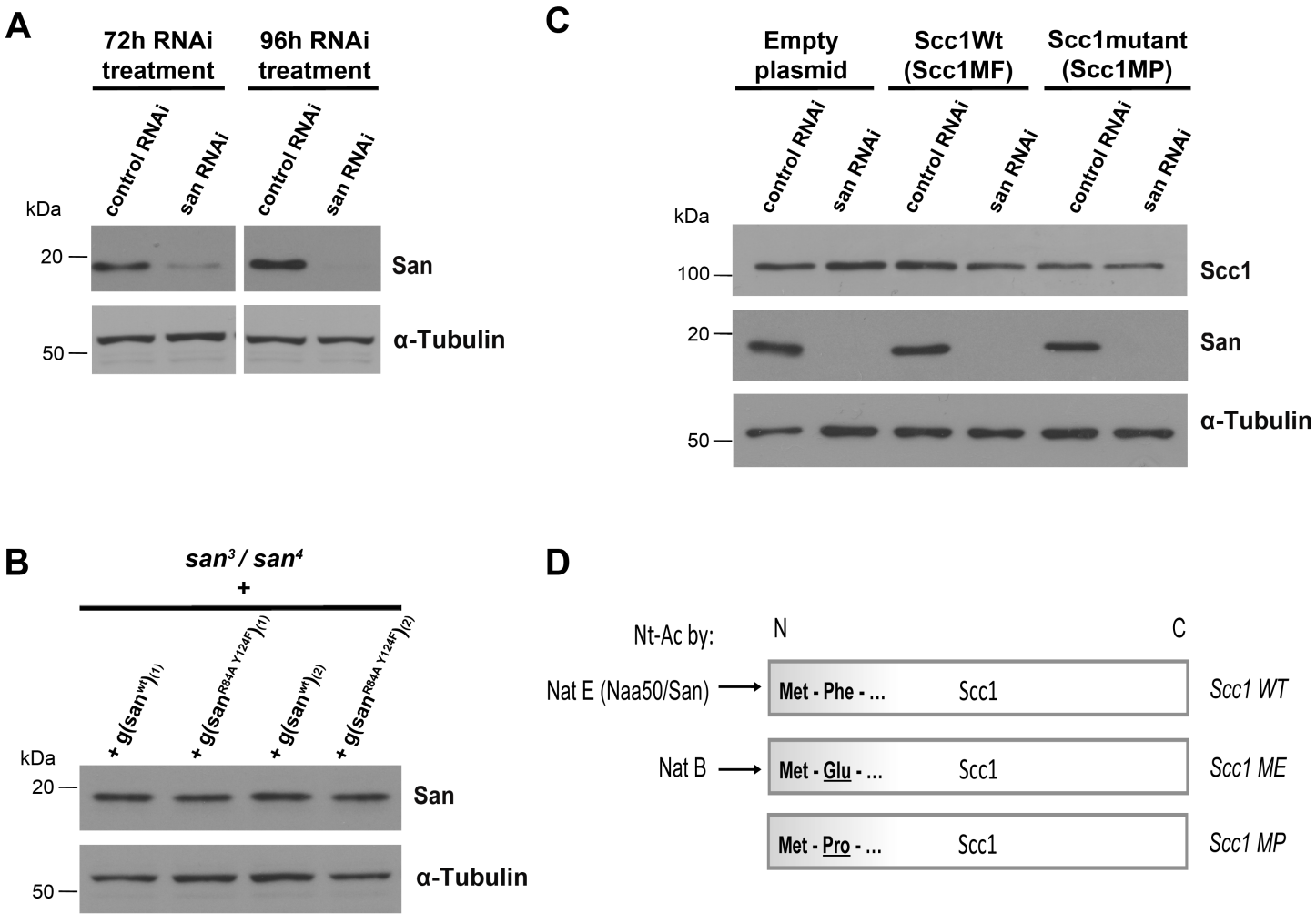
Supplementary Figure 1



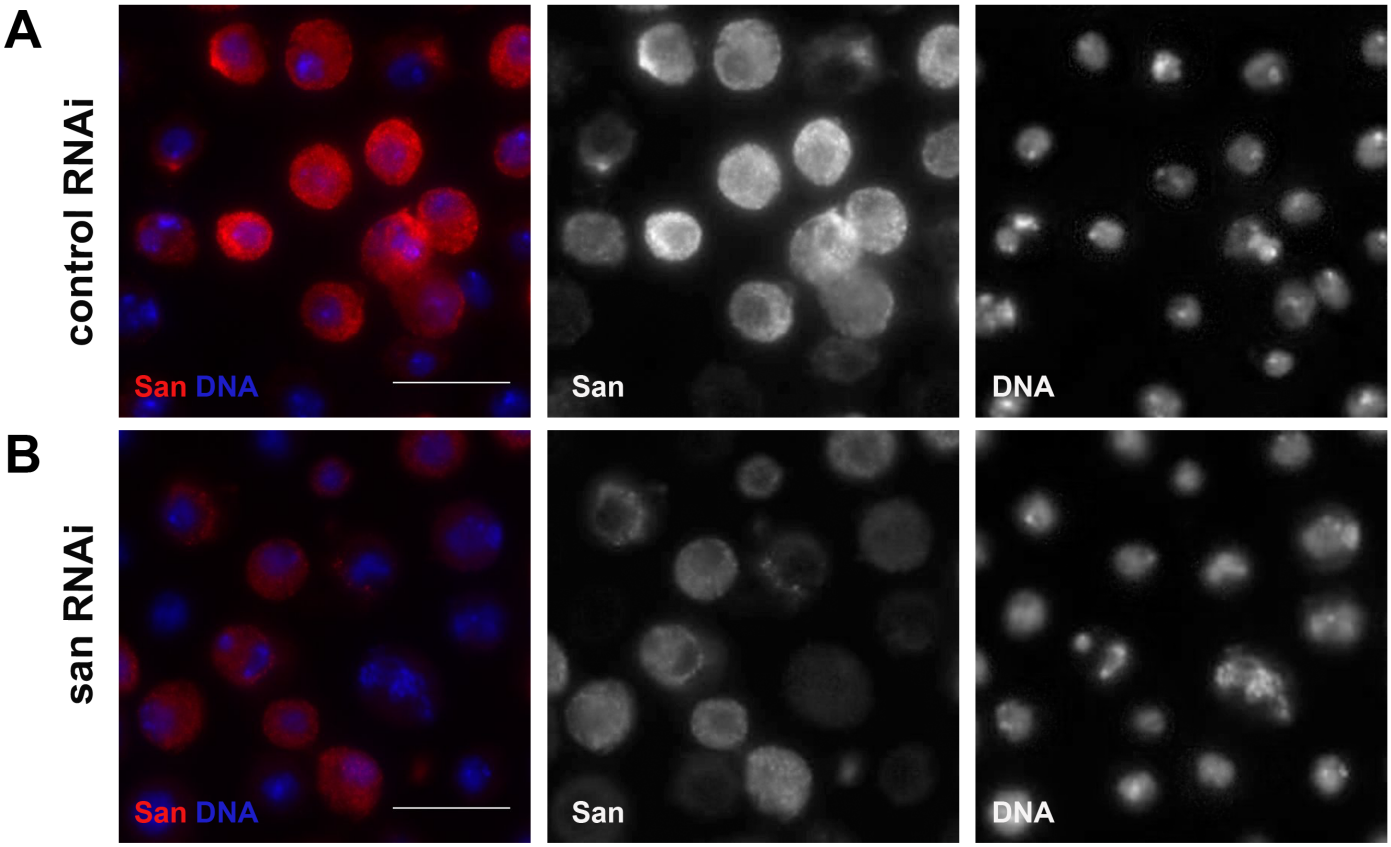

A
control RNAi
Tub
DNA
Scc1
TubScc1DNA
B
san RNAi
Tub
DNA
Scc1
TubScc1DNA

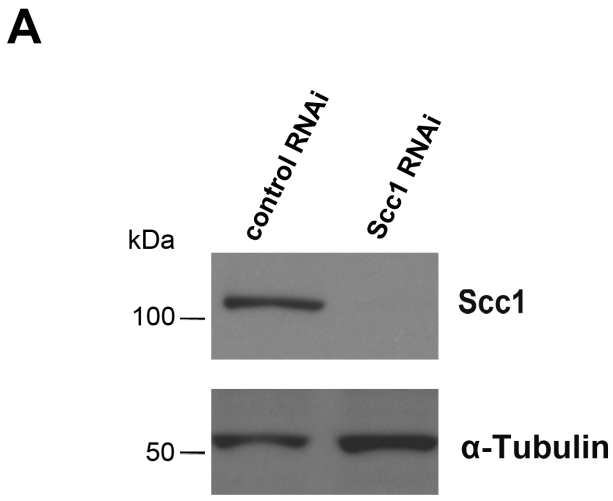

C
Cohesins protein levels
S2 cells
Embryos
control RNAi
san RNAi
control
san
kDa
150
100
75
Scc1
150
100
Smc1
20
San
50
α-Tubulin


D
Scc1 chromosomal localization during prometaphase/metaphase
Percentage of cells (%)
150
100
50
0
DNA and Scc1 colocalization
Absence/reduced colocalization
control RNAi (n=20)
san RNAi (n=26)


A
72h RNAi treatment
96h RNAi treatment
control RNAi
san RNAi
control RNAi
san RNAi
kDa
20
50
San
α-Tubulin
C
Empty plasmid
Scc1Wt (Scc1MF)
Scc1mutant (Scc1MP)
control RNAi
san RNAi
control RNAi
san RNAi
control RNAi
san RNAi
kDa
100
20
50
Scc1
San
α-Tubulin
B
san3 / san4
+
+ g(san wt)(1)
+ g(san R84A Y124F)(1)
+ g(san wt)(2)
+ g(san R84A Y124F)(2)
kDa
20
50
San
α-Tubulin
D
Nt-Ac by:
N
C
Nat E (Naa50/San)
Met - Phe - ...
Scc1
Scc1 WT
Nat B
Met - Glu - ...
Scc1
Scc1 ME
Met - Pro - ...
Scc1
Scc1 MP

Supplementary Figure 4




A
control RNAi
Scc1 RNAi
kDa
100
Scc1
50
α-Tubulin

Supplementary Figure 6

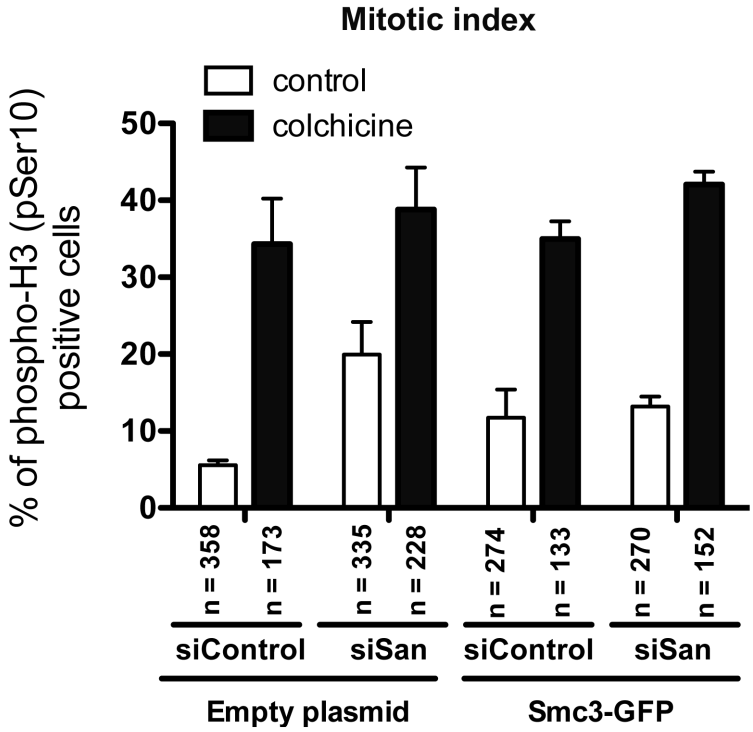
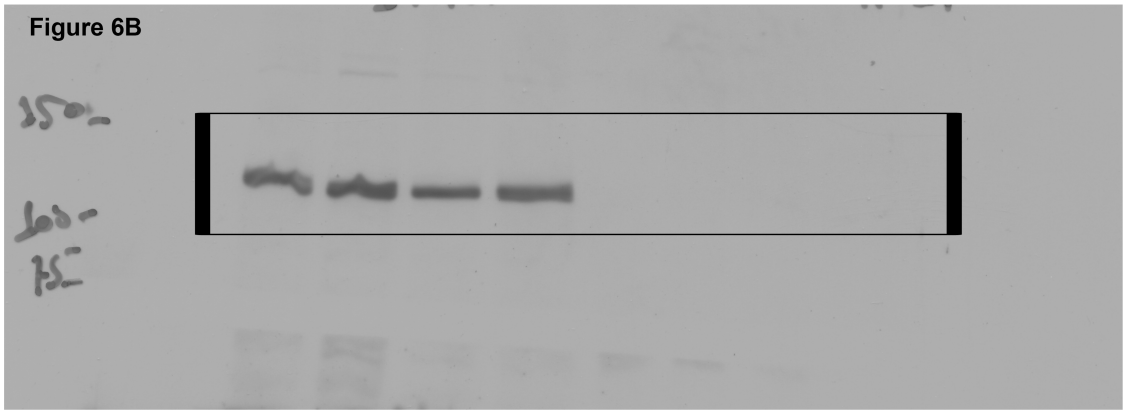


Figure 6 Dalmatian



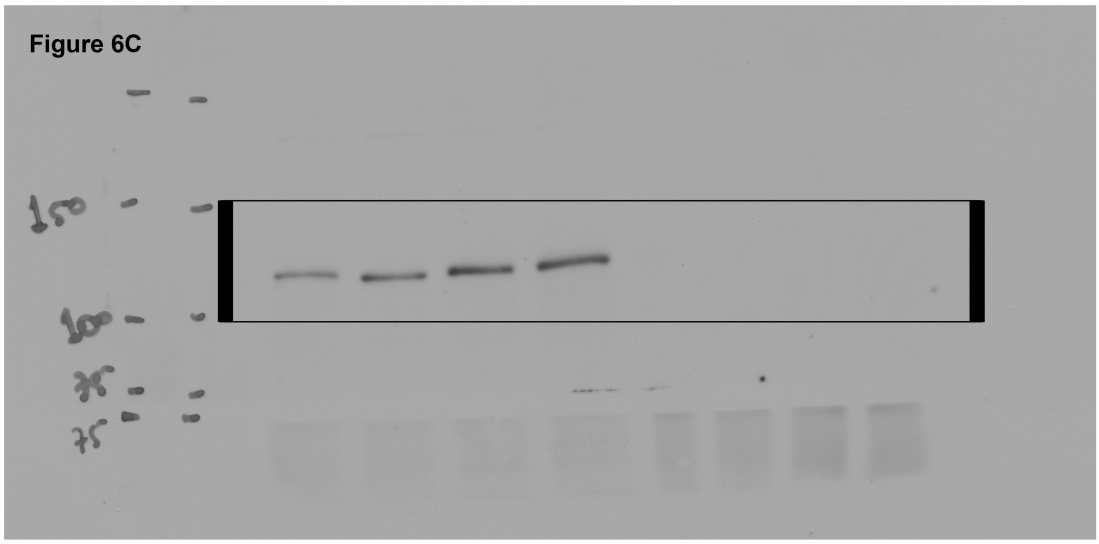


Figure 6B

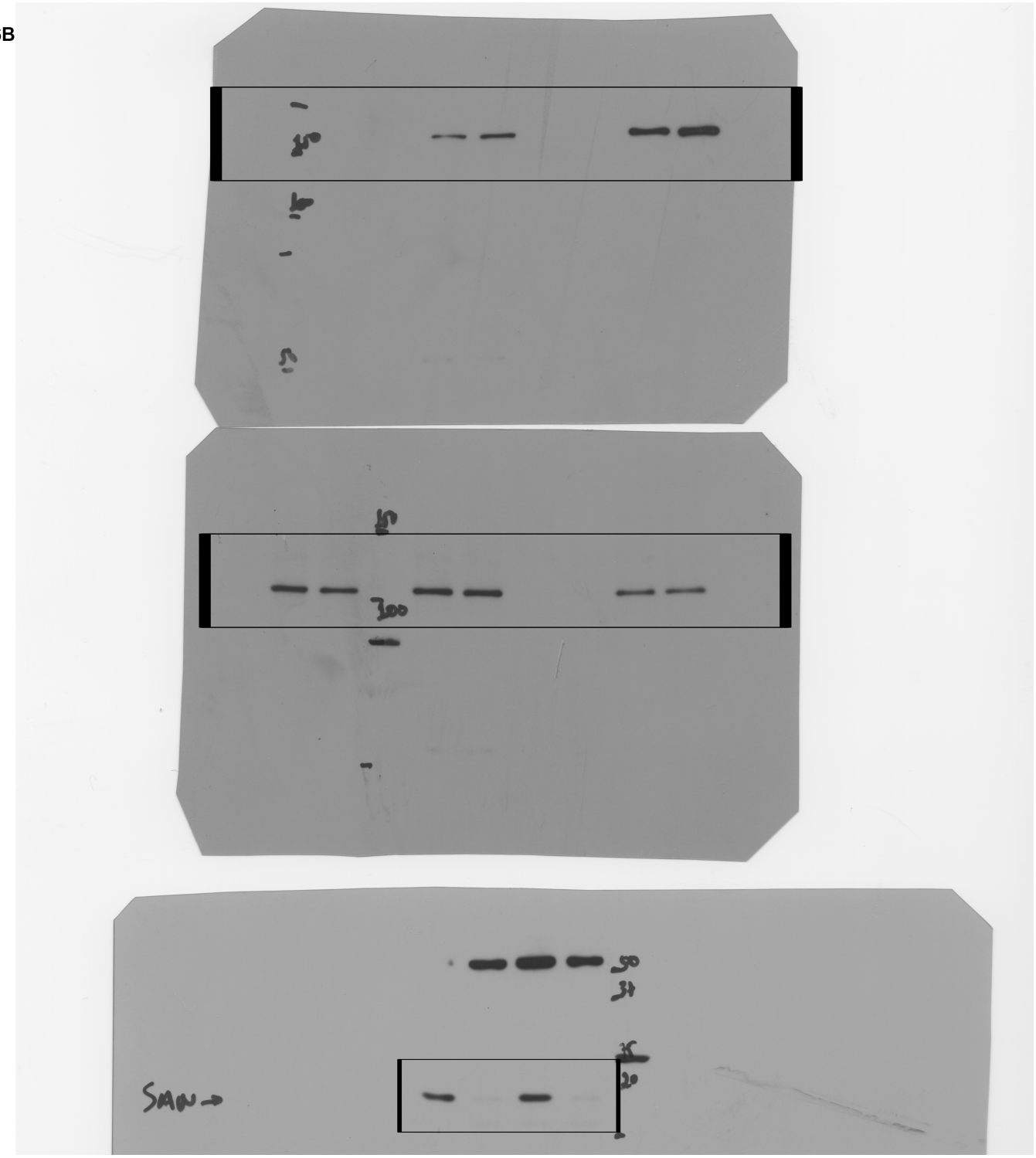
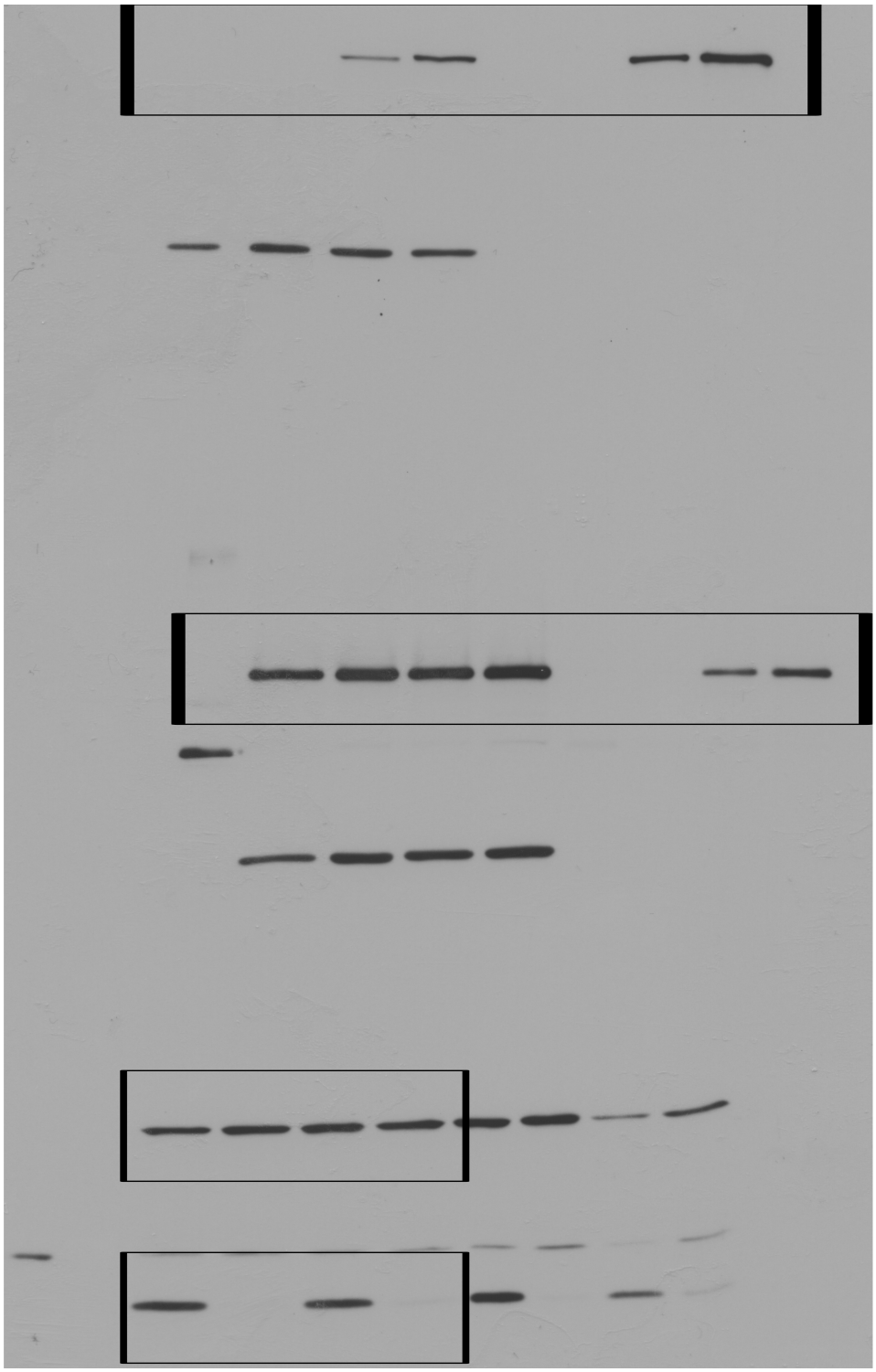
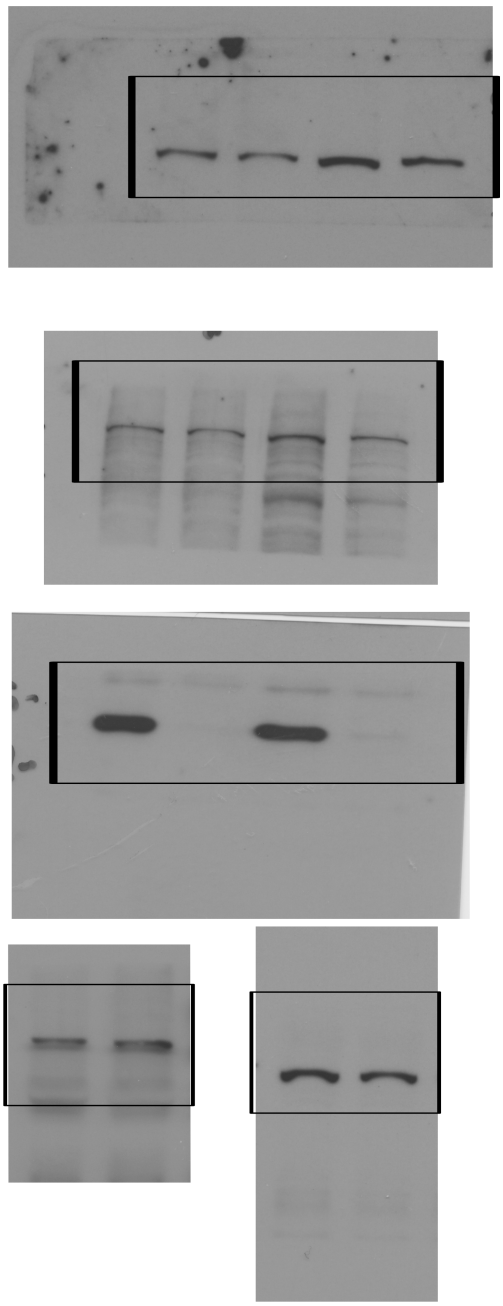


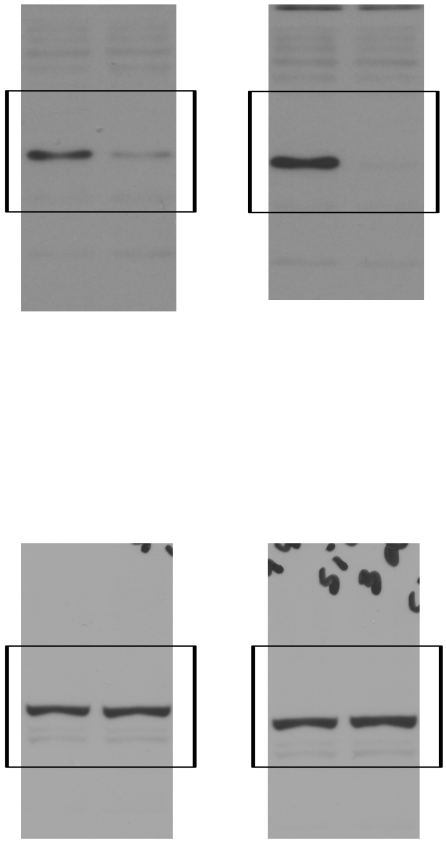
Figure 6C



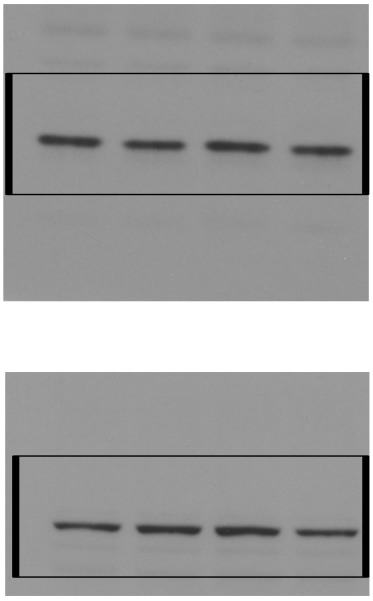
Supplementary Fig.2C



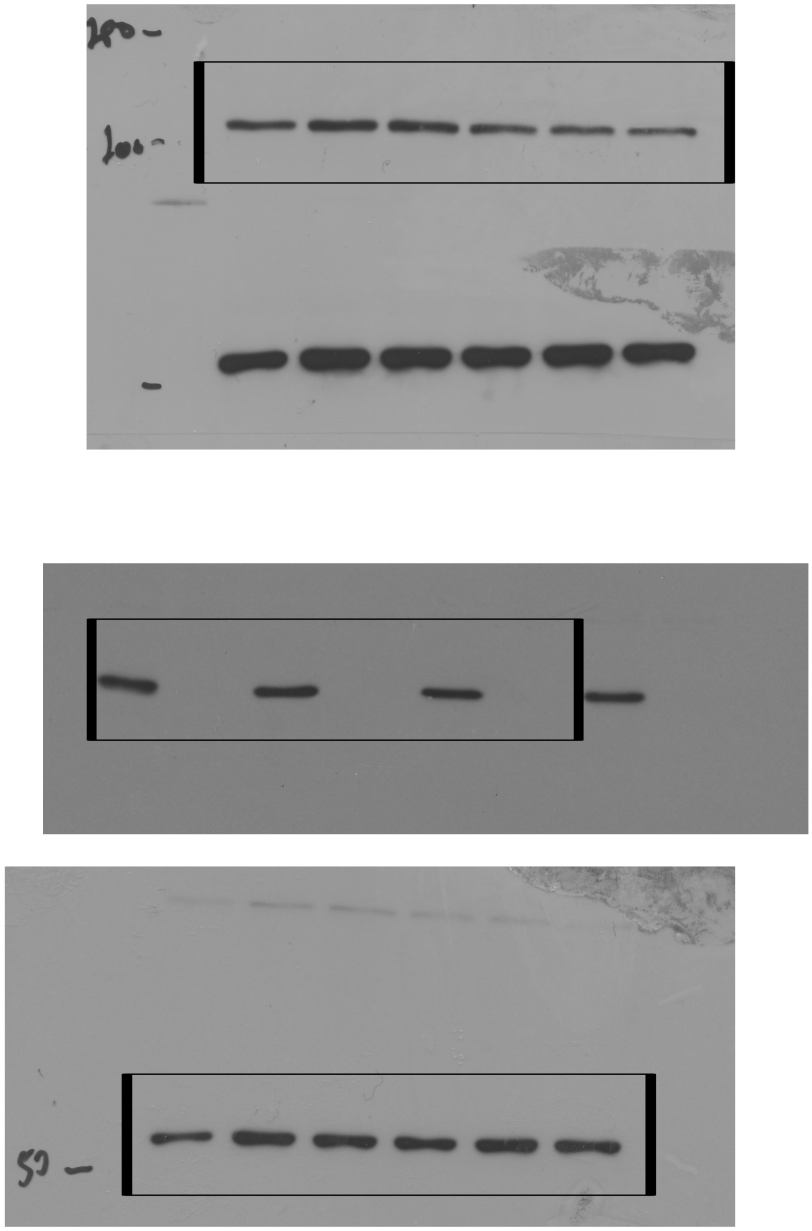
Supplementary Fig.3A



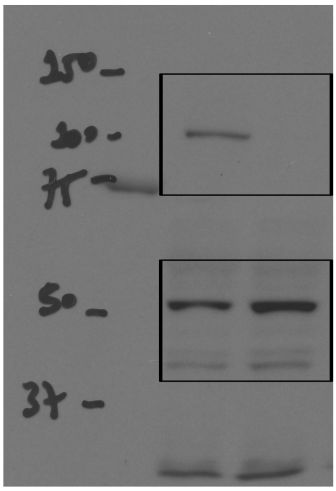
Supplementary Fig. 3B



Supplementary Fig. 3C



Supplementary Fig. 5



Suppl. Table 1. *Drosophila* stocks used in this study.

Name	Genotype	Description	Source/ Reference
Oregon R (OR)		Wild type stock.	
Nubbin-Gal4	y , w^{1118}; P{w^{+}, nubbin-GAL4}	Contains the Nubbin-Gal4 driver for specific expression in the pouch of wing imaginal disc.	BDSC
UAS-San RNAi	y w^{1118}; P{KK101696, y^{+}, $w^{3'}$}	Contains a dsRNA under UAS control for RNAi of san.	VDRC
UAS-mCherry RNAi	y^{1} sc^{*} v^{1}; P{$y^{+t7.7}$ $v^{+t1.8}$=VALIUM20-mCherry}attP2	Contains a dsRNA under UAS control for RNAi of mCherry.	BDSC/TRiP
UAS-deco RNAi	y^{1} sc^{*} v^{1}; P{ $y^{+t7.7}$ $v^{+t1.8}$=TRiP.GL00528}attP2	Contains a dsRNA under UAS control for RNAi of deco.	BDSC/TRiP
UAS-vtd RNAi	y^{1} sc^{*} v^{1}; P{ $y^{+t7.7}$ $v^{+t1.8}$=TRiP.GL00522}attP2/TM3, Sb^{1}	Contains a dsRNA under UAS control for RNAi of vtd/ scc1.	BDSC/TRiP
UAS-mau-2 RNAi	y^{1} sc^{*} v^{1}; P{ $y^{+t7.7}$ $v^{+t1.8}$=TRiP.HMS02374}attP2	Contains a dsRNA under UAS control for RNAi of mau-2.	BDSC/TRiP
UAS-Smc3-Scc1-fusion	UAS-Smc3-Scc1-GFP fusion	Contains smc3 c-terminally fused with the N-terminus of vtd/scc1 under UAS control.	(Eichinger et al., 2013)
UAS-dmt	w^{1118}; P{w^{+}, UAS-Dmt}	Contains dmt/soronin under UAS control.	(Kerman and Andrew, 2010)
dmt^{l184}	dmt^{l184}/Tm3	Carries a loss of function allele of dmt/soronin.	(Kerman and Andrew, 2010)
Nubbin-Gal4 UAS-san RNAi	w^{1118}; P{w^{+}, nubbin-GAL4}, P{KK101696, y^{+}, $w^{3'}$}/ CyO	Contains the Nubbin-Gal4 driver and a dsRNA under UAS control for RNAi of san.	This study
san^{3}	w/w; FRT42B B16-79/ CyO	Loss of function allele of san.	Pimenta-Marques et al., 2008
san^{4}	w/w; FRT42B B50-26/ CyO	Loss of function allele of san.	Pimenta-Marques et al., 2008
Wild type san genomic construct in *san^{4}* background	w/w; FRT42B B50-26/ CyO; P{w^{+}=WALIUM22-San}attP2/ TM6B	Carries a loss of function allele *san^{4}* and a genomic construct that expresses a wild type Naa50/San under the control of its endogenous promoter.	This study
$san^{R84A\ Y124F}$ genomic construct in *san^{4}* background	w/w; FRT42B B50-26/ CyO; P{w^{+}=WALIUM22-S $san^{R84A\ Y124F}$ }attP2/TM6B	Carries the loss of function allele san^{4} and expresses $san^{R84A\ Y124F}$ under control of the endogenous promoter.	This study

BDSC - Bloomington Drosophila Stock Center; VDRC - Vienna Drosophila RNAi Center; TRiP - Transgenic RNAi Project.

Suppl. Table 2 – Complete list of primers used in this study.

Complete list of primers

Primer name	Primer sequence	Comment
san RNAi Fw	TAATACGACTCACTATAGGGAGAA GCAGCATCGAACTGGGC	For san (CG12352) siRNA
san RNAi Rv	TAATACGACTCACTATAGGGAGAC GCTTATAGTATTGCTCCTTGGT	For san (CG12352) siRNA
sanAmp2 RNAi Fw	TAATACGACTCACTATAGGGAG ACAACACTGAGAACCAGCGGC	For san (CG12352) siRNA second amplicon
sanAmp2 RNAi Rv	TAATACGACTCACTATAGGGAGAC GTAGTAAAGCACGACAGTTCAC	For san (CG12352) siRNA second amplicon
deco RNAi Fw	GGATCCTAATACGACTCACTA TACGCTGGCCCGGATGTT	For deco (CG8598) siRNA
deco RNAi Rv	GGATCCTAATACGACTCACTATAC AACCTCCCGTCCACTATT	For deco (CG8598) siRNA
gfp RNAi Fw	TAATACGACTCACTATAGGGAGAC TTCAGCCGCTACCCC	For control siRNA
gfp RNAi Rv	TAATACGACTCACTATAGGGAGAT GTCGGGCAGCACG	For control siRNA
Scc1(Wt) Fw	GGGGACAAGTTTGTACAAAAAAGC AGGCTAAATGTTCTATGAGCACAT TATTTTGGC	For attB PCR to allow expression of native *Drosophila* Scc1 (CG17436) in pDONR221.
Scc1(MP-) Fw	GGGGACAAGTTTGTACAAAAAAGC AGGCTAAATGCCCTATGAGCACAT TATTTTGGC	For attB PCR to allow expression of native *Drosophila* Scc1 (CG17436) with a proline residue after the methionine in pDONR221.
Scc1(ME-) Fw	GGGGACAAGTTTGTACAAAAAAGC AGGCTAAATGGAGTATGAGCACAT TATTTTGGC	For attB PCR to allow expression of native *Drosophila* Scc1 (CG17436) with a glutamate residue after the methionine in pDONR221.
Scc1 Rv	GGGGACCACTTTGTACAAGAAAGC TGGGTCTTAAATTTTTGGGTTTTCG	For attB PCR to allow expression of *Drosophila* Scc1 (CG17436) in pDONR221.
a371t_Rv	CGAAACCAAACTTCTTAAAGAACT CGATGGCTCCG	To generate pETM41-san $^{R84A, Y124F}$ mutant plasmid with pETM41-san plasmid as template
a371t	CGGAGCCATCGAGTTCTTTAAGAA GTTTGGTTTCG	To generate pETM41-san $^{R84A, Y124F}$ mutant plasmid with pETM41-san plasmid as template
c250g_g251c_rev	GATGCCCAGGCGCGCGTACGGGGA GAGG	To generate pETM41-san $^{R84A, Y124F}$ mutant plasmid with pETM41-san plasmid as template
c250g_g251c	CCTCTCCCCGTACGCGCGCCTGGGC ATC	To generate pETM41-san $^{R84A, Y124F}$ mutant plasmid with pETM41-san plasmid as template

Suppl. Table 3 – Adult *Drosophila* zygotic viability

cross	Total number of F1 flies (n)		*Cy+*	*Cy*
$\frac{w}{w}$; $\frac{san^4}{CyO}$; $\frac{\text{san genomic}^{Wt}}{MKRS}$ x $\frac{w}{\supset}$; $\frac{san^3}{CyO}$; $\frac{+}{+}$	n = 173	***Sb*⁺**	**48 (28%)**	125 (72%)
	n = 97	***Sb***	0 (0%)	97 (100%)
$\frac{w}{w}$; $\frac{san^3}{CyO}$; $\frac{+}{+}$ x $\frac{w}{\supset}$; $\frac{san^4}{CyO}$; $\frac{\text{san genomic}^{Wt}}{TM6B}$	n = 155	***Tb*⁺**	**47 (30%)**	108 (70%)
	n = 77	***Tb***	0 (0%)	77 (100%)
$\frac{w}{w}$; $\frac{san^4}{CyO}$; $\frac{\text{san genomic}^{R84AY124F}}{TM6B}$ x $\frac{w}{\supset}$; $\frac{san^3}{CyO}$; $\frac{+}{+}$	n = 84	***Tb*⁺**	**0 (0%)**	84 (100%)
	n = 43	***Tb***	0 (0%)	43 (100%)
$\frac{w}{w}$; $\frac{san^3}{CyO}$; $\frac{+}{+}$ x $\frac{w}{\supset}$; $\frac{san^4}{CyO}$; $\frac{\text{san genomic}^{R84AY124F}}{TM6B}$	n = 64	***Tb*⁺**	**0 (0%)**	64 (100%)
	n = 77	***Tb***	0 (0%)	77 (100%)

Supplementary Table 4

A

Detailed LC-MS analysis of endogenous Scc1 co-immunoprecipitation assays.

Proteins immunoprecipitated with endogeonus Scc1				anti-Scc1 / siGFP (control RNAi)						anti-Scc1 / siSan (Naa50/San RNAi)						pre-imune control (-) / siGFP (control RNAi)						pre-imune control (-) / siSan (Naa50/San RNAi)					
				Rep 1			Rep 2			Rep 1			Rep 2			Rep 1			Rep 2			Rep 1			Rep 2		
CG	*Drosophila*	*Human*	Mass (kDa)	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences
Cohesion complex																											
CG6057-PA	SMC1	SMC1	143298	1797	35	32	1280	29	28	1084	27	27	1283	28	25	-	-	-	-	-	-	-	-	-	-	-	-
CG9802-PA	SMC3	SMC3	140409	1729	36	33	1452	35	28	1702	35	28	1653	37	33	-	-	-	-	-	-	-	-	-	-	-	-
CG3423-PA	SA	STAG1	130999	942	20	19	1019	22	20	584	13	12	971	21	19	-	-	-	-	-	-	-	-	-	-	-	-
CG17436-PA	vtd	Rad21/Scc1	80033	711	15	14	534	12	12	676	14	14	531	11	11	-	-	-	-	-	-	-	-	-	-	-	-
Miscellaneous																											
CG1404-PB	Ran	RAN	24877	244	5	3	89	1	1	69	2	2			-	-	-	-	-	-	-	-	-	-	-	-	-
CG31611-PA	His4	HIST1H4	11374	210	4	4	123	2	2	182	4	4	128	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG9888-PA	Fib	FIB	34662	210	5	5	59	1	1				131	3	3	-	-	-	-	-	-	-	-	-	-	-	-
Ribosomal																											
CG5502-PA	RpL4	RPL4	45090	754	13	10	229	6	6	644	13	10	460	10	9	-	-	-	-	-	-	-	-	-	-	-	-
CG5920-PA	RpS2	RPS2	29065	580	11	9	213	4	4	282	5	5	169	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG6779-PA	RpS3	RPS3	27638	574	9	7	77	2	2	380	6	5	178	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG1524-PB	RpS14a	RPS14	16301	566	9	7	115	1	1	287	4	4	216	4	4	-	-	-	-	-	-	-	-	-	-	-	-
CG11276-PA	RpS4	RPS4	29208	533	9	5	139	4	4	325	8	6			-	-	-	-	-	-	-	-	-	-	-	-	-
CG17521-PC	RpL10	RPL10	25925	502	11	8	171	4	4	142	3	3	188	5	5	-	-	-	-	-	-	-	-	-	-	-	-
CG7977-PA	RpL23A	RPL23A	29445	490	9	7	156	3	3	246	6	6	132	3	2	-	-	-	-	-	-	-	-	-	-	-	-
CG1883-PD	RpS7	RPS7	22156	462	8	7	213	4	4	298	5	5	223	4	3	-	-	-	-	-	-	-	-	-	-	-	-
CG7808-PC	RpS8	RPS8	23837	434	6	5	161	2	2	259	3	3	183	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG4897-PA	RpL7	RPL7	29580	419	9	9	164	6	6	321	7	7	186	5	5	-	-	-	-	-	-	-	-	-	-	-	-
CG3314-PA	RpL7A	RPL7A	30851	418	9	9	168	3	3	378	8	7	243	5	5	-	-	-	-	-	-	-	-	-	-	-	-
CG8415-PB	RpS23	RPS23	16053	313	6	5	83	2	2	122	2	2			-	-	-	-	-	-	-	-	-	-	-	-	-
CG7939-PB	RpL32	RPL32	16056	287	7	5	128	3	2	238	5	4	80	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG17420-PA	RpL15	RPL15	24402	285	6	5	90	2	2	354	8	7	198	5	5	-	-	-	-	-	-	-	-	-	-	-	-
CG4651-PA	RpL13	RPL13	25028	278	7	7	150	4	4	216	5	5	120	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG9282-PA	RpL24	RPL24	17601	265	6	5	188	4	4	150	3	3	165	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG2746-PA	RpL19	RPL19	24121	258	6	5	62	2	2	239	6	5	56	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG4046-PA	RpS16	RPS16	16856	257	6	6	80	2	2	181	4	4	83	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG6510-PA	RpL28	RPL18A	21062	245	7	7	91	2	2	171	4	4	110	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG12740-PB	RpL28	RPL28	16019	241	5	4	145	4	4	78	3	3	127	4	4	-	-	-	-	-	-	-	-	-	-	-	-
CG3395-PA	RpS9	RPS9	22610	240	6	6	72	2	2	94	3	3	60	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG8615-PA	RpL18	RPL18	21789	211	5	5	185	6	5	180	3	3	82	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG10944-PB	RpS6	RPS6	28528	200	5	3	75	1	1	138	2	2	121	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG8857-PC	RpS11	RPS11	18232	195	5	5	87	2	2	148	4	4	132	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG1263-PA	RpL8	RPL8	27967	189	4	4	147	4	3	74	2	2	218	5	5	-	-	-	-	-	-	-	-	-	-	-	-
CG6141-PA	RpL9	RPL9	21516	173	3	3	71	1	1	128	3	3	41	1	1	-	-	-	-	-	-	-	-	-	-	-	-

B

Detailed LC-MS analysis of Scc1-Myc co-immunoprecipitation assays.

Proteins immunoprecipitated with Scc1-Myc				anti-c-Myc Dynabeads & Scc1-Myc / siGFP (control RNAi)						anti-c-Myc Dynabeads & Scc1-Myc / siSan (Naa50/San RNAi)						anti-Myc & control (-) / siGFP (control RNAi)						anti-Myc & control (-) / siSan (Naa50/San RNAi)					
				Rep 1			Rep 2			Rep 1			Rep 2			Rep 1			Rep 2			Rep 1			Rep 2		
CG	*Drosophila*	*Human*	Mass (kDa)	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences	Score	Matches	Sequences
Cohesion complex																											
CG17436-PA	vtd	RAD21	80033	592	13	11	1296	24	19	808	19	16	1122	23	19	-	-	-	-	-	-	-	-	-	-	-	-
CG9802-PA	SMC3	SMC3	140409	303	8	8	696	19	19	149	7	7	740	18	16	-	-	-	-	-	-	-	-	-	-	-	-
CG3423-PA	SA	STAG1	130999	242	6	6	478	11	11	106	2	2	609	11	10	-	-	-	-	-	-	-	-	-	-	-	-
CG6057-PA	SMC1	SMC1	143298	160	5	5	830	20	20	118	4	4	793	20	18	-	-	-	-	-	-	-	-	-	-	-	-
Miscellaneous																											
CG4264-PE	Hsc70-4	HSPA8	71317	508	11	10	710	12	11	95	10	10	1130	20	16	-	-	-	-	-	-	-	-	-	-	-	-
CG4147-PB	Hsc70-3	HSPA5	72308	162	3	3	182	3	3	728	2	2	256	4	4	-	-	-	-	-	-	-	-	-	-	-	-
CG1913-PA	αTub84B	TUBA3D	50428	370	6	5	560	11	9	352	6	6	347	6	5	-	-	-	-	-	-	-	-	-	-	-	-
CG9277-PA	βTub56D	TUBB4B	51632	259	4	4	463	9	6	593	11	9	696	11	10	-	-	-	-	-	-	-	-	-	-	-	-
CG16944-PA	sesB	SLC25A4	33072	59	1	1	143	5	4	121	3	3	299	8	7	-	-	-	-	-	-	-	-	-	-	-	-
CG1404-PB	Ran	RAN	24877	79	1	1	40	1	1	50	1	1	39	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG11154-PA	ATPsynβ	ATP5B	54074	50	2	2	106	1	1				135	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG31611-PA	His4	HIST1H4	11374	37	1	1	161	3	3	106	2	2			-	-	-	-	-	-	-	-	-	-	-	-	-
CG32743-PA	nonC	SMG1	365311	33	1	1	39	2	1				30	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Ribosomal																											
CG6779-PA	RpS3	RPS3	27638	173	5	4	566	10	8	266	7	7	504	8	7	-	-	-	-	-	-	-	-	-	-	-	-
CG7808-PC	RpS8	RPS8	23837	128	2	2	223	4	3	120	2	2	178	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG3203-PB	RpL17	RPL17	21855	100	3	2	51	1	1	53	1	1	178	3	2	-	-	-	-	-	-	-	-	-	-	-	-
CG1883-PD	RpS7	RPS7	22156	98	2	2	219	6	6	104	2	2	125	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG6684-PA	RpS25	RPS25	13193	97	3	3	92	1	1	47	1	1			-	-	-	-	-	-	-	-	-	-	-	-	-
CG11522-PA	RpL6	RPL6	27727	87	2	2	116	2	2	141	3	3	295	6	4	-	-	-	-	-	-	-	-	-	-	-	-
CG9282-PA	RpL24	RPL24	17601	87	1	1	44	2	2	90	1	1			-	-	-	-	-	-	-	-	-	-	-	-	-
CG1524-PB	RpS14a	RPS14	16301	85	2	2	162	2	2	87	2	2	217	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG7939-PB	RpL32	RPL32	16056	79	1	1	138	4	4	66	1	1	157	3	2	-	-	-	-	-	-	-	-	-	-	-	-
CG17420-PA	RpL15	RPL15	24402	74	2	2	55	1	1	108	2	2	58	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG5920-PA	RpS2	RPS2	29065	74	2	2	168	3	3	143	2	2	138	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG6253-PA	RpL14	RPL14	19208	73	1	1	117	3	3	100	2	2			-	-	-	-	-	-	-	-	-	-	-	-	-
CG17521-PC	RpL10	RPL10	25925	70	1	1	120	3	3				91	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG6846-PA	RpL26	RPL26	17270	68	1	1	114	4	3						-	-	-	-	-	-	-	-	-	-	-	-	-
CG10423-PA	RpS27	RPS27	9634	66	2	1	119	3	2				65	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG11276-PA	RpS4	RPS4X	29208	60	1	1	76	3	3	84	2	2	54	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG8857-PC	RpS11	RPS11	18232	56	2	2	174	5	5	45	1	1	98	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG3922-PB	RpS17	RPS17	15321	55	1	1	125	4	3				76	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG7434-PA	RpL22	RPL22	30638	55	1	1	167	2	2	81	2	2			-	-	-	-	-	-	-	-	-	-	-	-	-
CG10305-PB	RpS26	RPS26	13488	54	1	1	74	1	1	358	1	1	65	1	1	-	-	-	-	-	-	-	-	-	-	-	-
CG4651-PA	RpL13	RPL13	25028	53	1	1	138	3	3	107	3	3	120	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG15442-PC	RpL27A	MRPL27	17057	52	4	3	115	5	4	34	1	1	64	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG7977-PA	RpL23A	RPL23A	29445	47	1	1	162	3	3	151	3	3	104	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG5502-PA	RpL4	RPL4	45090	47	1	1	193	4	4	124	4	4	78	2	2	-	-	-	-	-	-	-	-	-	-	-	-
CG4863-PA	RpL3	RPL3	47208	37	1	1	47	1	1	45	1	1	143	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CG8415-PB	RpS23	RPS23	16053	34	1	1	172	4	4						-	-	-	-	-	-	-	-	-	-	-	-	-

Supplementary Figure 1. Examples of the scored adult wing phenotypic classes. Class 1 only includes flies with wild type wings, class 2 includes flies with weak wing phenotype, class 3 includes flies with *san* RNAi-like wing phenotype, class 4 includes highly abnormal wings and class 5 only includes flies with absent or vestigial adult wings.

Supplementary Figure 2. Naa50/San is required for chromosomal localization of Scc1 in *Drosophila* S2 cells.

Scc1 association to chromosomes, but not its protein stability, is impaired after depletion of Naa50/San **(A-D).** Representative images of *Drosophila* S2 cells analyzed 96 hours after control RNAi **(A)** or *san* RNAi **(B)**. S2 cells were stained for DNA (blue), Scc1 (red) and α-Tubulin (green). Scc1 colocalized with DNA during prometaphase/metaphase in control RNAi-treated cells **(A),** but not in *san* RNAi-treated cells **(B)**. **(C)** Western blot of total protein extracts from *san* RNAi-treated S2 cells and embryos mutant for *san* (maternal mutants) showed no clear reduction of Scc1 and Smc1 protein levels. α-Tubulin was used as a loading control. **(D)** Quantification of Scc1 mislocalization phenotype during prometaphase/metaphase after depletion of Naa50/San. Reduced colocalization between Scc1 and DNA: control RNAi-treated S2 cells (0%, n=20), *san* RNAi-treated S2 cells 86.9 % ± 10.2 (n=26). Scale bars equal 5 µm.

Supplementary Figure 3. Naa50/San is efficiently depleted by *san* RNAi-treatment in *Drosophila* S2 cells. Absence of Naa50/San in embryos mutant for *san* (maternal mutants). Naa50/San expression levels are identical for a wild type (San^{wt}) and a catalyticaly dead-version ($San^{R84A\ Y124F}$). Levels of Scc1 after ectopic expression of Scc1MF (wild-type) or Scc1MP are equivalent.

(A) Western blot analysis of total protein extracts from *Drosophila* S2 cells treated with *san* RNAi for 72 hours (left) and 96 hours (right), showed reduced levels of Naa50/San protein when compared with control RNAi-treated cells. Depletion of Naa50/San was more efficient 96 hours after *san* RNAi-treatment.

α-Tubulin was used as loading control. **(B)** Wild type and catalytically dead genomic constructs expressed similar levels of Naa50/San. Western blot analysis of total protein extracts from *san* mutant embryos with one copy of a wild type genomic construct (*san*^{wt}), and *san* mutant embryos with one copy of a catalytically dead genomic construct (*san*$^{R84A\ Y124F}$). α-Tubulin was used as loading control. *san* mutant embryos (maternal mutants) were obtained after the induction of germ line clones using the FRT/*ovo*^{D} system{Chou, 1992 #67} and a previously published loss-of-function allele of *san* (*san*^{3}){Pimenta-Marques, 2008 #5}. Both wild type (*san*^{wt}) and catalytically dead genomic constructs (*san*$^{R84A\ Y124F}$) contain the gene endogenous promoter and were integrated in the same attP2 site (for more details see material and methods). ^{1} and ^{2} indicate that the genomic constructs although identical are from two independently generated *Drosophila* stocks. **(C)** Western blot analysis of total protein extracts from control RNAi and *san* RNAi-treated S2 cells, transfected with an empty plasmid (pHW), wild type Scc1 (Scc1Wt), or a non acetylatable mutant Scc1 (Scc1MP), showed identical levels of Scc1. There were no reproducible variations of Scc1 protein levels for any of the tested mutant alleles. A reduction in Naa50/San protein levels was always observed in *san* RNAi-treated cells. α-Tubulin was used as loading control. **(D)** Representation of the different Scc1 constructs used: a NatE (Naa50/San)-like substrate (Wild-type Scc1; Scc1Wt), a NatB-like substrate Scc1 (Scc1ME mutant variant), and a non-acetylatable mutant Scc1 (Scc1MP mutant variant).

Supplementary Figure 4. Naa50/San is not enriched in the nucleus of *Drosophila* S2 cells. (A and B). Representative images of *Drosophila* S2 cells analyzed 96 hours after control RNAi **(A)** or *san* RNAi **(B)**. S2 cells were stained for DNA (blue) and San (red). San is not enriched in the nucleus during interphase **(A)**{Williams, 2003 #4;Aksnes, 2015 #200}**.** In *san* RNAi-treated cells there is, as expected, a reduction of San protein levels which confirms signal specificity **(B)**. Scale bars equal 5 μm.

Supplementary Figure 5. Scc1 antibody is specific for Scc1 protein. (A) Western blot analysis of total protein extracts from *Drosophila* S2 cells treated with control RNAi (left) or *scc1* RNAi for 96 hours (right). *scc1*-RNAi treated cells showed reduced levels of Scc1 protein when compared with control RNAi-treated cells. α-Tubulin was used as loading control.

Supplementary Figure 6. The mitotic index of cells treated with colchicine is significantly increased. Mitotic index (% of phospho-H3 (pSer10) positive cells) for control RNAi and *san* RNAi-treated cells (96 hours after RNAi-treatment) transfected with an empty plasmid or a GFP-tagged Smc3 was, respectively, 5.6% ± 0.6 (n=358), 19.9% ± 4.2 (n=335), 11.7% ± 3.6 (n=274) and 13.2% ± 1.3 (n=270). The mitotic index for control RNAi and *san* RNAi-treated cells transfected with an empty plasmid or a GFP-tagged Smc3 and treated with 25μM of colchicine for 12 hours was, respectively, 34.3% ± 5.9 (n=173), 38.8% ± 5.5 (n=228), 34.9% ± 2.2 (n=133) and 42.1% ± 1.7 (n=152).

Supplementary Figure 7. Uncropped images of all protein blots shown in this manuscript.

Supplementary Table 1. *Drosophila* stocks used in this study.

Supplementary Table 2. Complete list of primers used in this study.

Supplementary Table 3. Naa50/San catalytic activity is essential for adult *Drosophila* viability. A genomic construct carrying a wild type copy of *san* (san^{Wt}), but not a catalytically dead allele of *san* ($san^{R84A\ Y124F}$), efficiently rescued the zygotic lethality of two loss-of-function alleles of *san* (san^{3} and san^{4}){Pimenta-Marques, 2008 #5}. Given the indicated crosses, and since *Drosophila* flies homozygous for the CyO balancers are not viable, full complementation of *san* mutant viability corresponds to 33% of the total number of progeny (F1) *Drosophila* flies with the genomic transgene.

Supplementary Table 4. Detailed LC-MS analysis of endogenous Scc1 and Scc1-Myc tagged co-immunoprecipitation assays.

All subunits of the cohesin complex, but not Dalmatian/Soronin, were efficiently immunoprecipitated with endogenous Scc1 **(A)** or with Myc-tagged Scc1 **(B)** after depletion of Naa50/San. Co-immunoprecipitations with an anti-Scc1 antibody or with anti-c-Myc Magnetic beads (Invitrogen, Grand Island, NY, USA) were performed, respectively, using total protein extracts from *Drosophila* S2 cells or expressing a Myc-tagged Scc1. Both sets of cells were either treated with control RNAi or *san* RNAi before immunoprecipitation. Score, number of detected peptides (matches) and non-repeated peptides (sequences) for each immunoprecipitated protein is indicated. None of the proteins shown in this table were detected in the negative controls (respectively, **(A)** pre-immune serum or **(B)** *Drosophila* S2 cells expressing an empty plasmid). Two biological replicas (Rep1 and Rep2) are shown for each experimental condition.

Movie S1. Control S2 cells (96h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red).

Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 96 hours of control RNAi treatment. For more experimental details please see methods.

Movie S2. San depleted S2 cells (96h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red).

Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 96 hours of *san* RNAi treatment. For more experimental details please see methods. The arrowhead is labeling a single chromatid at the different time points.

Movie S3. San depleted S2 cells (96h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red).

Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 96 hours of *san* RNAi treatment. For more experimental details please see methods. The arrowhead is labeling a single chromatid at the different time points.

Movie S4. San depleted S2 cells (96h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red), using a distinct non-overlapping dsRNA.
Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 96 hours of *san* RNAi treatment with a non-overlapping dsRNA. For more experimental details please see methods. The arrowhead is labeling a single chromatid at the different time points.

Movie S5. Control S2 cells (96h RNAi treatment), GFP-α-Tubulin (green) and CID-mCherry (red). Time-Lapse microscopy of S2 cells stably expressing GFP-α-Tubulin (green) and CID-mCherry (red) after 96 hours of control RNAi treatment. For more experimental details please see methods.

Movie S6. San depleted S2 cells (96h RNAi treatment), GFP-α-Tubulin (green) and CID-mCherry (red). Time-Lapse microscopy of S2 cells stably expressing GFP-α-Tubulin (green) and CID-mCherry (red) after 96 hours of *san* RNAi treatment. For more experimental details please see methods. The arrowhead is labeling a single chromatid at the different time points.

Movie S7. Control S2 cells (72h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red).
Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 72 hours of control RNAi treatment. For more experimental details please see methods.

Movie S8. Deco depleted S2 cells (72h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red). Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 72 hours of *deco* RNAi treatment. For more experimental details please see methods.

Movie S9. San depleted S2 cells (72h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red). Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 72 hours of *san* RNAi treatment. For more experimental details please see methods.

Movie S10. San and Deco co-depleted S2 cells (72h RNAi treatment), GFP-Histone H2B (green) and α-Tubulin-mCherry (red). Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red) after 72 hours of *san* and *deco* RNAi co-treatment. For more experimental details please see methods. The arrowhead is labeling a single chromatid at the different time points.

Movie S11. Control S2 cells, GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW.
Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW (empty plasmid), and treated with control RNAi for 96 hours. For more experimental details please see methods.

Movie S12. San depleted S2 cells, GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW.
Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW (empty plasmid), and treated with *san* RNAi for 96 hours. For more experimental details please

see methods. The arrowhead is labeling a single chromatid at the different time points.

Movie S13. Control S2 cells, GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW(Scc1Wt). Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with Scc1Wt, and treated with control RNAi for 96 hours. For more experimental details please see methods.

Movie S14. San depleted S2 cells, GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW(Scc1Wt). Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with Scc1Wt, and treated with *san* RNAi for 96 hours. For more experimental details please see methods.

Movie S15. Control S2 cells, GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW(Scc1MP). Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with Scc1MP, and treated with control RNAi for 96 hours. For more experimental details please see methods. The arrowhead is labeling a single chromatid at the different time points.

Movie S16. San depleted S2 cells, GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with pHW(Scc1MP). Time-Lapse microscopy of S2 cells stably expressing GFP-Histone H2B (green) and α-Tubulin-mCherry (red), transfected with Scc1MP, and treated with *san* RNAi for 96 hours. For more experimental details please see methods. The arrowhead is labeling a single chromatid at the different time points.