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Supplementary Information for

Multivalent interaction of ESCO2 with the replication machinery is required for sister chromatid cohesion

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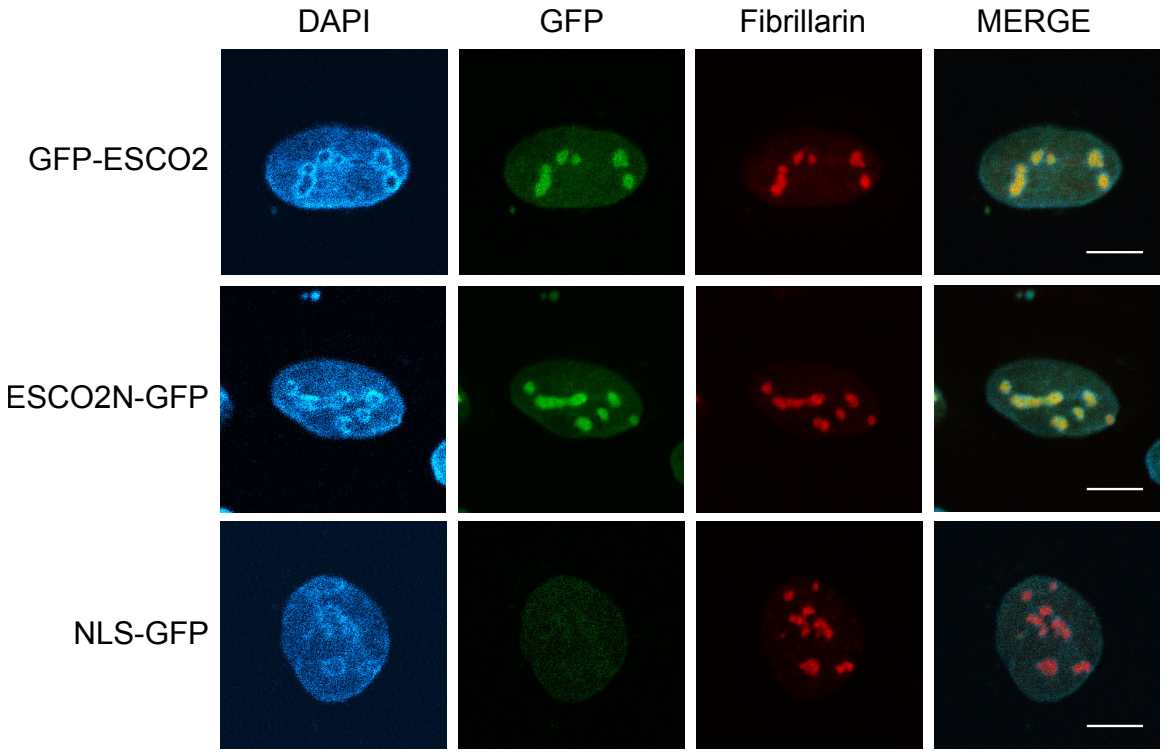

Figure S1. Nucleolar localization of ESCO2

Constructs encoding either full length GFP-ESCO2 (top), or the N terminus of ESCO2 fused to GFP (middle) or nuclear localized GFP (bottom) were cotransfected with a construct encoding mCherry tagged nucleolin and cells were imaged by confocal microscopy. The intensity of nucleolar staining varied depending on the experiment, but was evident for both full-length ESCO2 and ESCO2N.

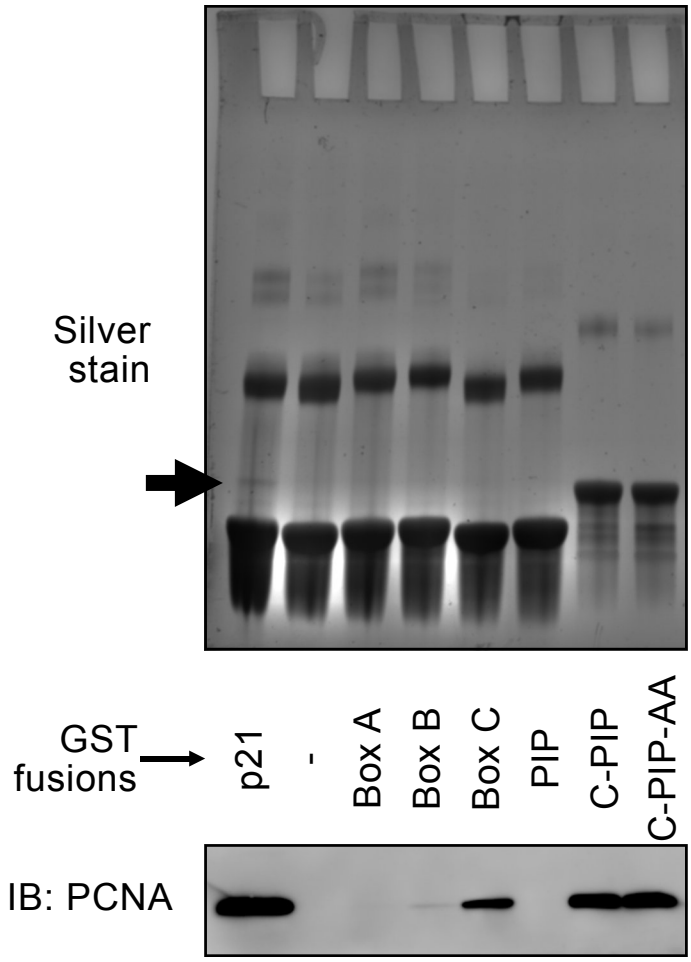

Figure S2. Silver stained gel of GST-fusion protein pull-downs.

GST fusions to the indicated peptides were incubated in *Xenopus* egg extract and retrieved using glutathione beads (as in Figures 4 and 5). The samples were eluted in sample buffer and run on two gels: one for silver stain (top) and the second for immunoblot analysis (at bottom). PCNA can be seen in the p21 lane (heavy black arrow). PCNA is not clearly detectable in the other lanes, where it is easily detected by immunoblot (box C). No additional bands are seen, although multimers of GST fusions are present. This experiment was repeated more than six times, with similar results.

Table S1.

Accession numbers from alignment in Figure 3A.

Accession	organism	common name	Length (a.a.)
NP_001003872	Danio rerio	zebrafish	609
NP_001133441	Salmo salar	salmon	631
NP_001089603	Xenopus laevis	African clawed frog	702
NP_001121462	Xenopus tropicalis	tropical clawed frog	770
XP_014343155	Latimeria chalumnae	coelacanth	702
XP_006132649	Pelodiscus sinensis	turtle	685
XP_420012	Gallus gallus	chicken	691
XP_026702143	Athene cunicularia	owl	624
NP_082315	Mus musculus	mouse	592
XP_006252234	Rattus norvegicus	rat	595
XP_011542723	Homo sapiens	man	601
XP_004382239	Trichechus manatus latirostris	manatee	614
XP_022265548	Canis lupus familiaris	dog	598
NP_001094652	Bos taurus	cattle	610
XP_014593313	Equus caballus	horse	612
XP_007664914	Ornithorhynchus anatinus	platypus	639
XP_007475966	Monodelphis domestica	opossum	610
XP_020839651	Phascolarctos cinereus	koala	607

Table S2. Cohesion assay results for various mutant alleles of ESCO2. In support of Figure 3 D-E.

Exp. #	WT	BoxA	*p*	Box B	*p*	Box C	*p*	PIP	*p*	Comments
E1181	17.9	59.8	<.0004	56.1	<.0004	33.0	0.0548	-		
E1182	8.7	56.6	<.0004	36.8	<.0004	47.0	<.0004	-		
E1183	17.7	74.4	<.0006	66.4	<.0006	35.3	0.0168	-		
E1563	12.5					64.0	0.0384	91.0	<.0006	PIP mutant didn't express well.
E1539	14.5	-		-		48.3	<.0004	84.5	<.0004	
E1595	23.8	-		-		58.9	<.0008	89.1	<.0008	3 ind. ΔPIP clones
								89.5	<.0008	
								91.6	<.0008	
E1621	11.5					29.4	0.0078	81.1	<.0006	2 ind. ΔC clones
						31.5	0.0018	-		
E1635	9.0	89.0	<.0008	66.0	<.0008	45.0	<.0008	97.0	<.0008	

Table S2. Summary of cohesion assays. Individual experiments (named at left) were scored to determine levels of sister chromatid cohesion in knock-down and rescue experiments as described for Figure 3. For statistical analysis, the level of cohesion rescue for a given ESCO2 mutant was compared to the rescue achieved by expression of the wild-type protein under the same conditions. P value was determined by Fisher exact test with Bonferroni's correction for multiple comparisons.

Table S3. Accession numbers of sequences analyzed in Figure 5A.

Accession	organism	common name	Length (a.a.)
KZV11629.1	Saccharomyces cerevisiae	baker's yeast	281
XP_011542723	Homo sapiens	man	601
NP_082315	Mus musculus	mouse	592
NP_001089603	Xenopus laevis	African clawed frog	702
NP_001003872	Danio rerio	zebrafish	609
XP_420012	Gallus gallus	chicken	691
XP_007664914	Ornithorhynchus anatinus	platypus	639
XP_007475966	Monodelphis domestica	opossum	610

Table S4. Plasmids used in this work

Plasmid Name	#	Source	Cat #	Reference/Comments
pCS2/GFP-PCNA	pSR312	Gift from Sansam lab		The PCNA-GFP fusion from (1) was cloned into pCS2+
mRuby-PCNA-19-NLS-4		Addgene	55876	mRuby-PCNA-19-NLS-4 was a gift from Michael Davidson
mEmerald-MCM4-C-19		Addgene	54167	mEmerald-MCM4-C-19 was a gift from Michael Davidson (2)
pET28/6HiS-Pre-GFP-PCNA	pZR07	This work		Bacterial expression of 6His-GFP-PCNA.
pCS2/mCherry-LacI-NLS	pAP18	This work		mCherry-lacI was taken from plasmid containing Brg1 (3) and inserted into pCS2+
pCS2/mCherry-LacI-NLS-ESCO2	pAP20	This work		Derivative of pAP18; mCherry-lacI fused to full length ESCO2
pCS2/mCherry-LacI-NLS-ESCO2ΔA	pAP37	This work		Derivative of pAP20; ESCO2 ΔP97-E105
pCS2/mCherry-NLS-LacI-ESCO2ΔB	pAP38	This work		Derivative of pAP20; ESCO2 ΔG209-F213
pCS2/mCherry-NLS-LacI-ESCO2ΔC	pAp39	This work		Derivative of pAP20; ESCO2 ΔV326-S331
pCS2/mCherry-NLS-LacI-ESCO2ΔPIP	pEL17	This work		Derivative of pAP20; ESCO2 Q375A, I378A,
pCDNA5/FRT-TO- sirESCO2	pAP11	This work		siRNA-resistant ESCO2: bp510-AGTGATCTATAAG to tGTcATtTAcAAaCCt
pCDNA5/FRT-TO-sirESCO2ΔA	pAP12	This work		Derivative of pAP11; ESCO2 ΔP97-E105
pCDNA5/FRT-TO-sirESCO2ΔB	pAP13	This work		Derivative pAP11; ESCO2 ΔG209-F213
pCDNA5/FRT-TO-sirESCO2ΔC	pAP14	This work		A derivative pAP11; ESCO2 ΔV326-S331
pCDNA5/FRT-TO-sirESCO2ΔPIP	pEL18	This work		A derivative pAP11; ESCO2 Q375A, I378A,
pCS2/ESCO2N-eGFP	pJC179	This work		N terminal 375 amino acids of ESCO2 fused to eGFP
pCS2/ESCO2NΔA-eGFP	pJC180	This work		Derivative of pJC179; ESCO2 ΔP97-E105
pCS2/ESCO2NΔB-eGFP	pJC181	This work		A derivative of pJC179; ESCO2 ΔG209-F213
pCS2/ESCO2NΔC-eGFP	pJC182	This work		A derivative of pJC179; ESCO2 ΔV326-S331

pGEX/4T-2		Invitrogen	28954550	Bacterial expression of GST or GST-fusion proteins
pGEX/Box A	pJC183	This work		GST-Box A fusion: G_{225}SSFYNQNKWYLNPLERKLIKE.
pGEX/Box A5A	pJC184	This work		GST Box A-5A fusion: G_{225}SSAANQNKWYLNPLAAALIKE.
pGEX/Box B	pJC185	This work		GST-Box B fusion: G_{225}SQKIKPQVTLQGGAAFFVRKKS.
pGEX/Box B2A	pJC186	This work		GST-Box B-2A fusion: G_{225}SQKIKPQVTLQGGAAAAVRKKS.
pGEX/Box C	pJC187	This work		GST Box C fusion: G_{225}SPKSTVYPIFSASS.
pGEX/Box C2A	pJC188	This work		GST Box C-2A fusion: GSPKSTVYPAASASS.
pGEX/PIP	pJC189	This work		GST-ESCO2 PIP: G_{225}SIPKDQLIIDAGQKHFGA.
pGEX/PIP2A	pJC190	This work		GST-ESCO2 PIP-2A fusion: G_{225}SIPKDALIADAGQKHFGA.
pGEX/P21-PIP	pJC192	This work		GST-p21 PIP fusion: G_{225}SIPRRQTSMTDFYHSKRR.
pGEX/P21-PIP2A	pJC193	This work		GST-p21 PIP-2A fusion: G_{225}SIPRRATSATDFYHSKRR.
pCS2+				(4)
pCDNA5/FRT-TO		Invitrogen	V652020	
mRuby-Fibrillarin-7		Addgene	55862	mRuby-Fibrillarin-7 was a gift from Michael Davidson

Table S5. Antibodies used in this work

Antigen	Source	No	Lot	Concentration
PCNA (FL-261)	Santa Cruz	Sc-7907	F1711	IF 1:500
MCM4	Bethyl Labs	A300-193A		IF 1:500
$SMC3^{Ac}$	Gift from KatsuhikoShirahige (5)			IB 1:1500
SMC3	(6)			
NCK	NeoMarkers	RB0024		IB 1:1000
ESCO2	Bethyl Labs	A301-689A	multiple	IB 1:1000
HA-Tag	Cell Signaling	C29F4	8	IF 1:500
Flag	Sigma	F1804	101M6216	1:1000
GST	Lab made			
ScPCNA	Gift from Paul Kaufman	#82 (7)		1:1,000

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