Zhang et al., http://www.jcb.org/cgi/content/full/jcb.200801157/DC1

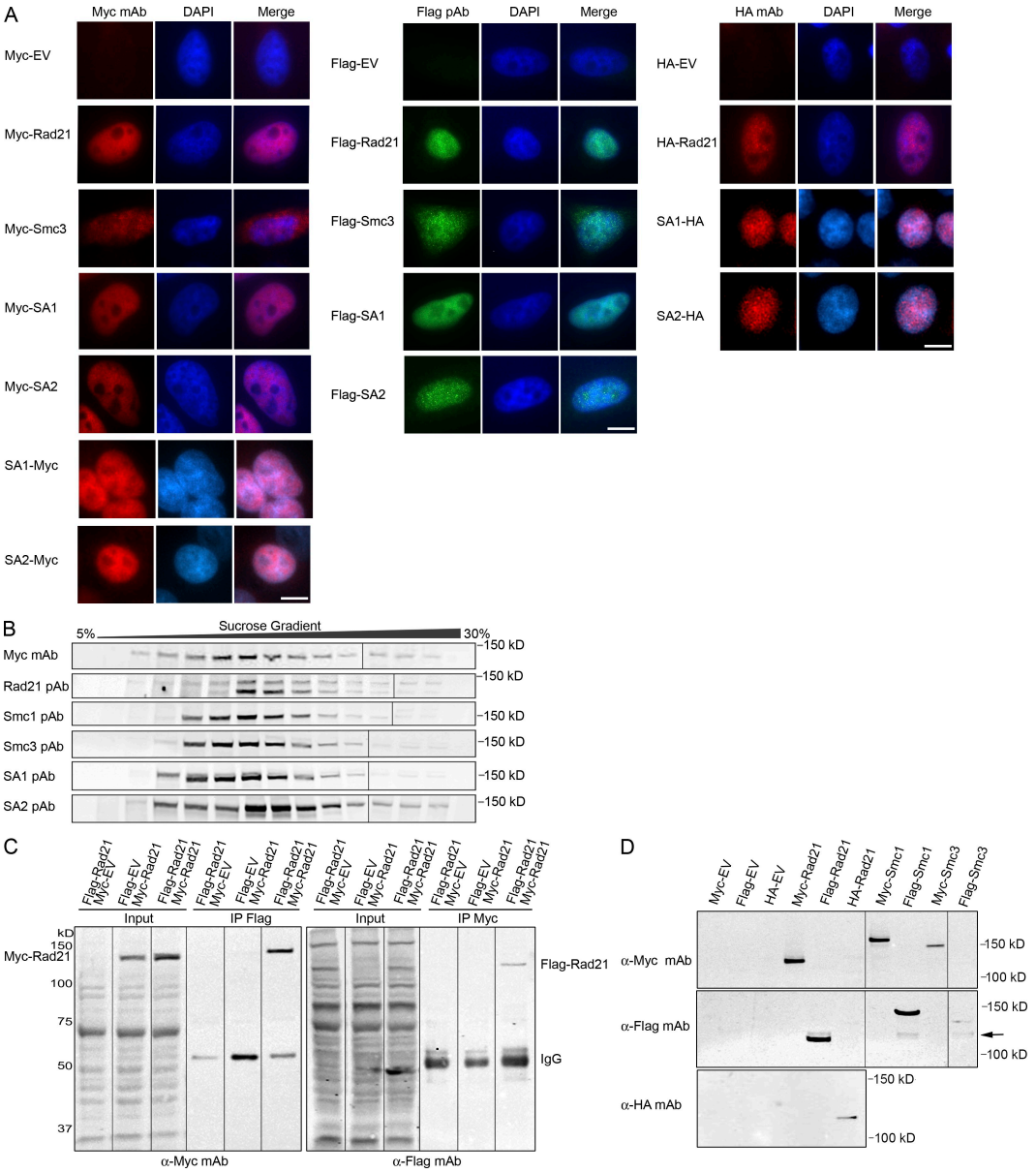
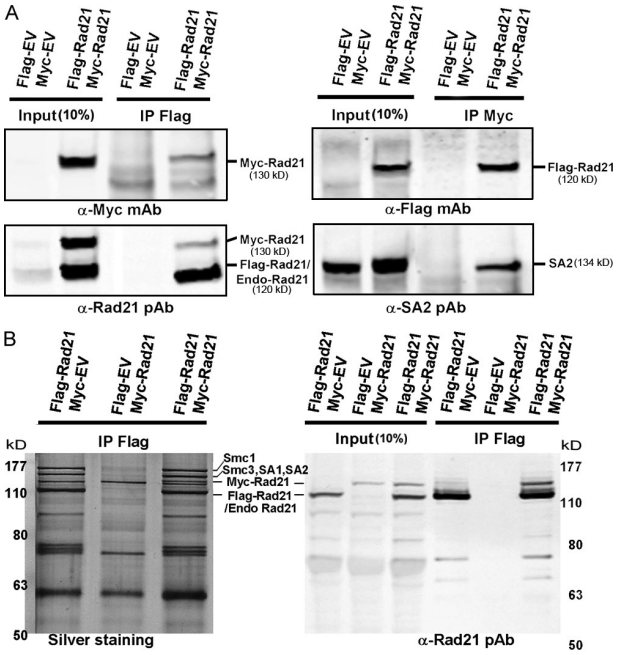


Figure S1. **Validation of epitope-tagged cohesin proteins with immunofluorescence microscopy, sucrose gradient centrifugation, and co-IP.** (A) Immunofluorescence showing the localization of Myc-, Flag-, and HA-tagged cohesin subunits in HeLa cells. Myc, Flag, and HA mAb were used to stain the cohesin proteins according to their respective tags. DAPI staining of the nuclear material is shown in blue, whereas Myc and HA epitopes are shown in red and Flag epitope is shown in green. (B) Sucrose gradient centrifugation of ectopically expressed Myc-Rad21 and endogenous cohesin subunits. 293T cells were transfected with Myc-Rad21, and whole cell lysate was prepared 48 h later. An aliquot of the cell extract was loaded to 5–30% of the sucrose density gradient and spun at 36,000 rpm for 16 h in an ultracentrifuge (L8-M; Beckman Coulter) with an SW-40 rotor. The fractions were taken manually and analyzed by WB using cohesin subunit antisera. (C and D) Validation of the co-IP experiments using ectopically expressed epitope-tagged cohesin subunits in 293T cells. (C) Two proteins with different tags are either expressed together or one protein along with a blank vector of the opposite tag, and an IP was performed against each of the tags. This is an example that validates the specificity of the co-IP of Flag-Rad21 and Myc-Rad21. (D) Myc, Flag, and HA mAbs do not cross react with any cohesin components other than the respective tagged protein immunoprecipitated by antibody-conjugated beads. Myc and HA antibody–conjugated agarose beads specifically immunoprecipitated the respective tagged cohesin proteins. Along with the Flag-tagged cohesin proteins, Flag antibody–conjugated agarose beads also precipitated a faint nonspecific band (arrow). This nonspecific band does not affect the co-IP analysis because antibody against the other tag (e.g., Myc or HA) was used to determine the co-IP. Black lines indicate that intervening lines have been spliced out. EV, empty vector. Bars, 10 µm.



C

Protein	Probability	Score	Peptide Number	Sequence Cover (%)
Rad21	3.19E-13	148	46	50.4
Myc-tag	NA	NA	19	NA
Smc1	5.70E-12	158	20	30.9
Smc3	2.76E-11	190	31	33.9
SA2	3.55E-14	20	3	7.3

Figure S2. **Flag-Rad21 and Myc-Rad21 coimmunoprecipitated each other in protein solution released from chromatin or whole cell lysate.** (A) Logarithmically growing 293T cells were transfected with appropriate Rad21 plasmids or empty vector (EV) for 48 h. Chromatin was isolated and digested with micrococcal nuclease to release chromatin-bound proteins that were used for IP. Input (10% of IP) and IP samples were resolved by 7% SDS-PAGE and blotted with the indicated antibodies. (B) SDS-PAGE gel stained with silver staining (left) and WB (right) show a distinct band of Myc-Rad21 that was coimmunoprecipitated by Flag-Rad21. Flag-Rad21 has a similar migration rate as endogenous Rad21. (C) Mass spectrometry analysis of Flag immunoprecipitates from 293T cells cotransfected with Flag- and Myc-tagged Rad21. Cell lysate was treated with DNase I and RNase A before IP. Rad21 co-IP bands on an SDS-PAGE were analyzed using nano-HPLC/mass spectrometry/mass spectrometry (Jung, S.Y., A. Malovannaya, J. Wei, B.W. O'Malley, and J. Qin. 2005. *Mol. Endocrinol.* 19:2451–2465). Probability, Xcorr scores, number of peptides, and sequence coverage are shown. Because Myc-Rad21 contains 6×Myc, it is impossible to determine whether the Myc peptides detected in mass spectrometry are from the same Myc repeat or from different ones (Jung, S.Y., A. Malovannaya, J. Wei, B.W. O'Malley, and J. Qin. 2005. *Mol. Endocrinol.* 19:2451–2465).

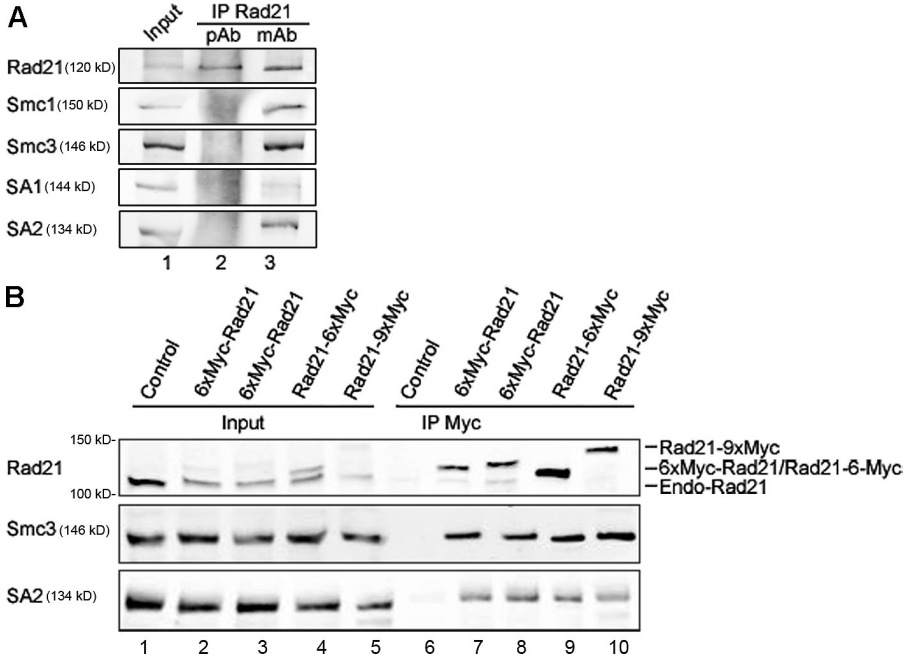


Figure S3. **Myc epitope tagging to the C-terminal but not the N-terminal Rad21 hinders Rad21–Rad21 interaction.** Input samples were loaded equivalent to 10% of IP samples. (A) Endogenous Rad21 of 293T cells was immunoprecipitated using Rad21 pAb (raised against the last 14 aa) and mAb (raised against the middle part of Rad21). (B) 293 cells were stably transfected with 6×Myc tagged to Rad21 NT (6×Myc-Rad21) or CT (Rad21-6×Myc) and expressed from the native promoter using a bacterial artificial chromosome–engineered clone. HeLa Tet-On cells that stably transfected with Rad21-9×Myc (provided by J.-M. Peters) and untransfected 293 cells (control) were used as a comparison. The expression of Rad21-9×Mcy is controlled by the Tet-responsive cytomegalovirus promoter. Myc pAb–conjugated agarose beads were used to IP Myc-tagged Rad21. The immunoblots were probed with the respective antibodies.

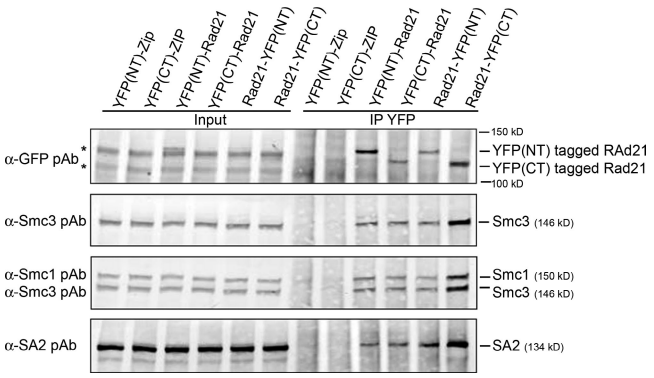


Figure S4. **Immunoprecipitation of YFP(NT)- and YFP(CT)-tagged Rad21.** 293T cells were transfected with YFP(NT)- or YFP(CT)-tagged Rad21 constructs. YFP was immunoprecipitated by anti-GFP pAb 48 h after transfection. YFP(NT)-Zip and YFP(CT)-zip constructs were used as controls. WBs were probed with the antibodies shown on the left. *, nonspecific bands.

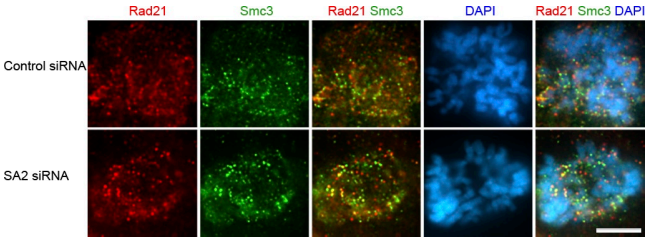


Figure S5. **Immunofluorescence microscopy of Rad21 and Smc3 in SA1 and SA2 knockdown HeLa cells.** HeLa cells were transfected with control siRNA or SA2 siRNA for 48 h. Rad21 (red) and Smc3 (green) were stained with anti-Rad21 mAb and anti-Smc3 pAb, respectively. Bar, 10 μm.

Table S1. **The mammalian expression constructs utilized in this study and the strategy used for cloning the human cohesin proteins**

Constructs	cDNA insert	Vectors	Cloning sites
Flag-Rad21	Rad21 full length	pFlagCMV2	HindIII and NotI
Myc-Rad21	Rad21 full length	pCS2MT	XhoI and XbaI
HA-Rad21	Rad21 full length	pCruz HA	EcoRV and XbaI
GAL4AD-Rad21	Rad21 full length	pC86	BglII and NotI
GAL4BD-Rad21	Rad21 full length	pC97	BglII and NotI
GAL4AD-Rad21 NT-L	Rad21 (1–283 aa)	pC86	BglII and NotI
GAL4BD-Rad21 NT-L	Rad21 (1–283 aa)	pC97	BglII and NotI
GAL4AD-Rad21 CT	Rad21 (451–631 aa)	pC86	BglII and NotI
GAL4BD-Rad21 CT	Rad21 (451–631 aa)	pC97	BglII and NotI
GAL4AD-Rad21 CT-L	Rad21 (254–631 aa)	pC86	BglII and NotI
GAL4BD-Rad21 CT-L	Rad21 (254–631 aa)	pC97	BglII and NotI
Flag-Smc1	Smc1 full length	pFlagCMV2	EcoRI and BamHI
Myc-Smc1	Smc1 full length	pCS2MT	EcoRI and SnaBI
Flag-Smc3	Smc3 full length	pFlagCMV2	EcoRI and BamHI
Myc-Smc3	Smc3 full length	pCS2MT	EcoRI and SnaBI
Flag-SA1	SA1 full length	pFlagCMV2	EcoRI and EcoRV
Myc-SA1	SA1 full length	pCS2MT	EcoRI and SnaB1
Flag-SA2	SA2 full length	pFlagCMV2	XhoI
Myc-SA2	SA2 full length	pCS2MT	BamHI and ClaI
SA1-HA	SA1 full length	pCruz HA	BamHI
SA1-Myc	SA1 full length	pCS2MT	BamHI
SA2-HA	SA2 full length	pCruz HA	BamHI
SA2-Myc	SA2 full length	pCS2MT	BamHI