Supplementary Materials and methods

Construction of baculovirus expression vectors

hSMC4/hCAP-C

pIO4 (no tag): A full-length cDNA clone for hSMC4/hCAP-C (1,288 amino-acid long) was obtained from RZPD German Resource Center for Genome Research (clone ID: DKFZp434F205Q2). The two PCR primers used for subcloning were: HC101, 5’-GATGAATTCATGCCCCGTAAAGGCACCCA-3’ (EcoRI site underlined); HC102, 5’-GCCGAATTCTCAACAAAGTCCCTTAGATG-3’ (EcoRI site underlined). The predicted molecular mass was 147.2 kDa whereas the apparent molecular mass on SDS-PAGE was ~167 kDa.

hSMC2/hCAP-E

pFE101 (no tag): A full-length cDNA clone for hSMC2/hCAP-E (1,197 amino-acid long) was a gift of K. Yokomori (UC Irvine). The two PCR primers used for subcloning were: HE101, 5’-GCCGAATTCTGATGTAAGTCTGAAGTGCTTCCCTTAGATG-3’ (EcoRI site underlined); HE102, 5’-GCCGAATTCTCAACAAAGTCCCTTAGATG-3’ (EcoRI site underlined). The predicted molecular mass was 135.8 kDa whereas the apparent molecular mass on SDS-PAGE was ~135 kDa. Additional modifications were made to put a hexahistidine tag at the C-terminus of hCAP-E/hSMC2. phE135 (short tag) expressed the full-length polypeptide with an extra sequence of LEHHHHHHH, whereas phE133 (long tag) expressed the full-length polypeptide with an extra sequence of ASSVDKLAALEHHHHHHH.

hCAP-D2
pFD101 (no tag): A full-length cDNA clone for hCAP-D2 (1,401 amino-acid long) was obtained from Kazusa DNA Research Institute (Clone name, HA03574; Gene name, KIAA0159). The two PCR primers used for subcloning were: HD101, 5’-GCCTCTAGAATGGCTCCCCAAATGTATGA-3’ (*Xba*I site underlined); HD102, 5’-GAATCTAGACTAGGATCTGTGCCTGCGAG-3’ (*Xba*I site underlined). The predicted molecular mass was 157.1 kDa whereas the apparent molecular mass on SDS-PAGE was ~155 kDa.

hCAP-G

pFG101 (no tag): A full-length cDNA clone for hCAP-G (1,015 amino-acid long) was described by Kimura et al (2001). The two PCR primers used for subcloning were: HG101, 5’-GAATCTAGAATGGGAGCGGAAAGGAGGCT-3’ (*Xba*I site underlined); HG102, 5’-GGCTCTAGACTAGGATCTGTGCCTGCGAG-3’ (*Xba*I site underlined). Additional modifications were made to put a hexahistidine tag at the C-terminus of hCAP-G. pFG102 (short tag) expressed the full-length polypeptide with an extra sequence of LEHHHHHHH, whereas pFG104 (long tag) expressed the full-length polypeptide with an extra sequence of SASSVDKLAASEHHHHHH. The predicted molecular mass of the no-tag version was 114.3 kDa whereas the apparent molecular mass on SDS-PAGE was ~115 kDa.

hCAP-H

pFH101 (no tag): A full-length cDNA clone for hCAP-H (730 amino-acid long) was obtained from Kazusa DNA Research Institute (Clone name, HA01438; Gene name, KIAA0074). The two PCR primers used for subcloning were: HH101, 5’-
GCCGAATTACATGAATAACTCTTCTTCAGAG-3’ (*Eco*RI site underlined); HH102, 5’-GCCGAATTCTCAATCTCCTTGCTCACAA-3’ (*Eco*RI site underlined). The predicted molecular mass was 81.6 kDa whereas the apparent molecular mass on SDS-PAGE was ~100/102 kDa. Additional modifications were made to construct pFH102 that expressed the full-length polypeptide with an extra sequence of SRACSLEHHHHHH at its C-terminus.

Alternatively, HH101 was combined with HH1173xba-rev (5’-AAATCTAGACCTCTGCCAGCTCTGAACCTG-3’; *Xba* I site underlined) to make a construct (pIO18) that produced an N-terminal domain of hCAP-H (amino acid 1-393), followed by an extra sequence of DSRACSLEHHHHHH. Finally, HH1232ecori (5’-CAGGAATTACATGATTCTTGGGAGATGGAG-3’; *Eco*RI site underlined) was combined with HH102 to make a construct (pIO6) that expressed a C-terminal domain of hCAP-H (amino acid 395-730), followed by an extra sequence of SRACSLEHHHHHH.

**hCAP-D3**

pTH126 (no tag): A full-length cDNA clone for hCAP-D3 (1,498 amino-acid long) was obtained from Kazusa DNA Research Institute (Clone name, HA01062; Gene name, KIAA0056). The two PCR primers used for subcloning were: HT127, 5’-ACATGCATGCATGTTGGCGTTGGGAGCTT-3’ (*Sph*I site underlined); HT128, 5’-ACATGCATGCATTGTTGGCTTGGGGCCT-3’ (*Sph*I site underlined). The predicted molecular mass was 168.9 kDa whereas the apparent molecular mass on SDS-PAGE was ~167 kDa.

**hCAP-G2**
phGT125 (short tag): A full-length clone for hCAP-G2 (1,143 amino-acid long) was purchased from OriGene Technologies (AK092008). Only a tagged version was constructed that expresses the full-length polypeptide with an extra sequence of LEHHHHHH at its C-terminus. The predicted molecular mass was 132.1 kDa (with the tag) whereas the apparent molecular mass on SDS-PAGE was ~122 kDa.

hCAP-H2
	pHHT107 (no tag): A full-length clone for hCAP-H2 (605 amino-acid long) was amplified from a human lambda gt10 library by using the following two primers: HHT105, 5’-ACGAATTCCATGGAGGACGTGGAGGCGCG-3’ (EcoRI site underlined); HHT106, 5’-ACGAATTTCTCAGGGCTGGGCCATGGAGG-3’ (EcoRI site underlined). The predicted molecular mass was 68.2 kDa whereas the apparent molecular mass on SDS-PAGE was ~88/91 kDa. An additional modification was made to add an extra sequence of AAAFSRACSLHHHH at the C-terminus of hCAP-H2 by using the primers HHT105 and HHT112 (5’-ATAGTTTAGCGGCCGCGGGCTGGGCCATGGAGGGGG-3’; NotI site underlined) (pIO19). Alternatively, HHT105 was combined with HHT113 (5’-CCCGCTCCGCGCGCGGTACCTCCTGGGCCAGGGCAG-3’; NotI site underlined) to make a construct (pIO20) that expressed an N-terminal domain of hCAP-H2 (amino acid 1-295) followed by an extra sequence of AAAFSRACSLHHHH. Finally, the primer HHT111 (5’-CCGGAATTCATGCTGGAGGACGGGAG-3’; EcoRI site underlined) was combined with HHT112 to make a construct (pIO21) that produced a C-terminal domain of hCAP-H2 (296-605) followed by an extra sequence of AAAFSRACSLHHHH.
**Site-directed mutagenesis**

The primers used for mutagenesis of the two SMC subunits were as follows (only the sense sequences are shown here; the mutation sites are underlined):

- pIO8: hSMC2 Walker A (K38I)
  
  \[
  5'-\text{GGCTTAAATGGTAGTGGGATATCCAACATATTGGACTCC-3'}
  \]

- pIO9: hSMC2 C-motif (S1086R)
  
  \[
  5'-\text{GAAAACCTAACTGAACCTCCTGGTGGTGCAGAGGTCTTTAG-3'}
  \]

- pIO10: hSMC2 Transition (E1114Q)
  
  \[
  5'\text{-CCAATTTATATCCCTTGATCAAGTATGCAGCCTTGG-3'}
  \]

- pIO11: hSMC4 Walker A (K119I)
  
  \[
  5'-\text{GGCCAAATGGCAGTGGCATATCCAATGTTATGATTCTATG-3'}
  \]

- pIO12: hSMC4 C-motif (S1192R)
  
  \[
  5'-\text{TTGGAAAAAGATCTTCAACCCTTAGGGGAGGAGAGAAAACACTTAG-3'}
  \]

- pIO13: hSMC4 Transition (E1220Q)
  
  \[
  5'-\text{CCCTTTACTTCATGGATCAGATTGATGCAGCCTTGG-3'}
  \]

**Construction of TnT expression plasmids**

The primers used for subcloning into the pTnT vector (Promega) were as follows (*Xba* I and *EcoR*I sites used for subcloning are underlined, whereas initiation and termination codons artificially introduced are italicized):

- hCAP-D2
pAD103 (amino acid 1-1401):

HD101 (forward) 5'-GCCTCTAGAATGGCTCCCCAAATGTATGA-3';
HD102 (reverse) 5'-GAATCTAGA GTTAGATCTGTGCTCGAG-3'.

pAD104 (amino acid 560-1401)

HD122 (forward), 5'-GCCTCTAGAA7GACCAGGCTCTTTGAATATATT-3';
HD102 (reverse), 5'-GAATCTAGACTAGGATCTGTGCTCGAG-3'.

pAD105 (amino acid 920-1401)

HD123 (forward) 5'-GCCTCTAGAA7GACTTTTCTGTTGATGAACCT-3';
HD102 (reverse) 5'-GAATCTAGACTAGGATCTGTGCTCGAG-3'.

pAD106 (amino acid 1-564)

HD101 (forward) 5'-GCCTCTAGAATGGCTCCCCAAATGTATGA-3';
HD120 (reverse) 5'-GCCTCTAGACTATTCAGAAGCCTGGTCTCCT-3'.

pAD107 (amino acid 1-925)

HD101 (forward) 5'-GCCTCTAGAATGGCTCCCCAAATGTATGA-3';
HD121 (reverse) 5'-GCCTCTAGACTAGTTCATCAACAGAAAGTTGG-3'.

hCAP-G

pAG103 (amino acid 1-1015)

HG101 (forward) 5'-GAATCTAGAATGGAGCGGAAGGAGGCG-3';
HG102 (reverse) 5'-GGCTCTAGACTAACTTAGATCTTTGAG-3'.

pAG104 (amino acid 334-1015)

HG124 (forward) 5'-GGCTCTAGAA7GAAATTGCTTTTGATTGGTG-3';
HG102 (reverse) 5'-GGCTCTAGACTAACTTAGATCTTTGAG-3'.
Purification of sub- and holo-complexes of condensin I for complementation assay

5.4x10^8 Sf9 cells were co-infected with recombinant viruses in different combinations. Cells were resuspended in 12 ml of buffer N, lysed by sonication, and supplemented with Tween 20 at a final concentration of 0.05%. The lysate was clarified by centrifugation at 3,000 x g at 4°C for 10 min, mixed with 0.5 ml of Ni-NTA beads (Qiagen), and incubated under gentle rotation at
4°C for 4 h. The beads were washed 4 times with 10 ml of buffer N and the proteins were eluted from the beads by adding 2 ml of buffer E (20 mM HEPES [pH 7.7], 100 mM KCl, 2.5 mM MgCl₂, 10% glycerol and 250 mM imidazole). The eluate was supplemented with 0.1 mg/ml ovalbumin and 1 mM DTT, and concentrated by Centricon YM-30 (Millipore) to a final volume of 50 µl. During this process, the KCl, glycerol and imidazole concentrations in the sample were reduced to 50 mM, 2% and 25 mM, respectively. Hexahistidine-tagged versions of hSMC2 and hCAP-G were used for the reconstitution of the SMC dimer and the non-SMC trimer, respectively. Both of the tagged subunits were used for the reconstitution of the holocomplex. In mock purification, Ni-NTA beads were mixed with uninfected Sf9 cell extract, and treated as above.

**Purification of hSMC2 for limited proteolysis**

~1.8-3.6 x 10⁸ Sf9 cells expressing histidine-tagged hSMC2 (short tag) were resuspended in 10-20 ml of buffer P (20 mM HEPES [pH 7.7], 100 mM KCl, 2.5 mM MgCl₂ and 10% glycerol), and lysed as described above. The lysate was clarified by centrifugation, and loaded onto a 2-ml cellulose phosphate column (Whatman). After washing the column with 10 ml of buffer P, proteins were eluted with 6 ml of buffer P containing 600 mM KCl. The 600 mM KCl-eluted fraction was diluted two-fold with buffer P containing no KCl, 40 mM imidazole and 10 mM 2-mercaptoethanol, mixed with 0.5 ml of Ni-NTA beads (Qiagen), and incubated under gentle rotation at 4°C for 4 h. The beads were washed once with 10 ml of buffer N, packed in a column (0.8 x 2.0 cm), and washed sequentially with 10 ml of buffer N, 1.5 ml of buffer N containing 1 M KCl, and 1.5 ml of buffer N containing 100 mM KCl. Finally, proteins were eluted from the column with 1.5 ml of buffer E. The eluate was supplemented with 1 mM DTT and concentrated.
by Centricon YM-30 (Millipore) to a final volume of 100 µl. During this process, the KCl concentration in the sample was reduced to 50 mM.