Supplementary Figure 1. The RAD51-GEMIN2 interaction

(A) The two-hybrid interaction between RAD51 and GEMIN2. Plasmids used in each experiment are indicated at the top of the panel. Yeast strains grown on SD plates with histidine and adenine (top row), and without histidine and adenine (middle row) are shown. The β-galactosidase activities, which were induced by the RAD51-GEMIN2 interaction, were detected using the X-Gal filter assay, with the results shown in the bottom rows.

(B) Purification of human GEMIN2. Proteins (1 μg) from each purification step were analyzed by 15% SDS-PAGE with Coomassie Brilliant Blue staining. Lane 1 indicates the molecular mass markers. Lanes 2 and 3 indicate the whole cell lysates of the E. coli cells before and after induction with isopropyl-1-thio-D-galactopyranoside, respectively. Lanes 4-7 indicate the samples from the peak ProBond fraction, the peak Superdex75 fraction, the fraction after the removal of the His6 tag, and the peak MonoQ fraction, respectively.

(C) Pull-down assay. Proteins bound to the RAD51-conjugated beads were analyzed for their association with purified GEMIN2 or bovine serum albumin (BSA) using 15% SDS-PAGE with Coomassie Brilliant Blue staining.

(D) Association of GEMIN2 and RAD51 in vivo. HeLa cells were transiently transfected with Flag-tagged human GEMIN2, with (+) or without (-) 2 hours pretreatment with 10 μM Camptothecin (CPT) prior to harvest. Lanes labeled “Con” represent the control precipitates with no antibody. Immunoprecipitates with the RAD51 antibody were analyzed using anti-RAD51 in western blots (Upper left panel). Immunoprecipitates with the Flag antibody (for GEMIN2) were analyzed using anti-Flag in western blots (Upper right panel). Immunoprecipitates with the RAD51 antibody were analyzed using anti-Flag in western blots (Lower left panel). Immunoprecipitates with the Flag antibody were analyzed using anti-RAD51 in western blots (Lower right panel).
Supplementary Figure 2. GEMIN2 does not affect topology of supercoiled DNA.

The indicated amounts of GEMIN2 or RAD51 were preincubated at 37°C for 10 min. The ϕX174 dsDNA (20 μM), which was previously treated with wheat germ topoisomerase I, was then added to the reaction mixture. After 30 minutes reaction at 37°C, the reactions were stopped by the addition of 0.1% SDS and 1.97 mg/ml proteinase K (Roche Applied Science), and were further incubated at 37°C for 15 minutes. After adding 6-fold loading dye, the deproteinized reaction products were separated by 1% agarose gel electrophoresis in 1x TAE buffer. The products were visualized by SYBR Gold (Invitrogen) staining.
Supplementary Figure 3. Generation of GEMIN2⁻/⁻tetGEMIN2 Mutants

(A) GEMIN2 gene-disruption in DT40 cells. Disruption of a part of the GEMIN2 locus and the targeted allele. We used the GEMIN2 replacement construct, which contains the HisD⁺ or Puro⁺ selection marker gene, to target the GEMIN2 locus. The filled box represents the GEMIN2 exons. Relevant EcoRV sites and the position of the probe used in the Southern-blot analysis (B) are indicated. The two arrows indicate primers for the RT-PCR shown in (C).

(B) GEMIN2 gene-targeting was confirmed by Southern-blot analysis of EcoRV-digested genomic DNA. Positions of hybridizing fragments of the wild-type (WT) and targeted loci are indicated.

(C) RT-PCR analysis of transcripts from the endogenous GEMIN2 gene in GEMIN2⁻/⁻ tetGEMIN2 cells, using the primers shown in (A). β-actin transcripts are shown as a control.

(D) Western-blot analysis of human GEMIN2 transgene expression under the control of the tetracycline-repressible promoter. GEMIN2⁺ indicates culture without tetracycline (doxycycline), and GEMIN2⁻ indicates culture with doxycycline for the indicated days. Cell extracts from HeLa cells and wild-type DT40 cells are shown as controls. Anti-RAD51 antibody was used as a positive control for the western-blot analysis.
Supplementary Figure 4. GEMIN2-Deficient Cells are Incapable of Proliferating

(A) Growth kinetics of GEMIN2−/−tetGEMIN2 clones. The relative cell number is plotted for wild-type (WT) cells and for the GEMIN2−/−tetGEMIN2 clones, without doxycycline (GEMIN2+) and following the addition of doxycycline at time zero (GEMIN2−). Note that doxycycline has no effect on the cellular growth of wild-type cells. Each value represents the averaged results from three separate clones.

(B) Cell-cycle distribution of GEMIN2−/−tetGEMIN2 cells at the indicated time after addition of doxycycline. Cells were pulse-labeled with BrdU for 10 minutes and subsequently stained with FITC-conjugated anti-BrdU antibody (Y axis, log scale) and propidium iodide (PI) (X axis, linear scale).
| Clones   | Wild type | GEMIN2<sup>+</sup> | RAD54<sup>+</sup> | XRCC3<sup>+</sup> | RAD51<sup>+</sup> | GEMIN2<sup>-</sup> | RAD51<sup>-</sup> |
|----------|-----------|---------------------|-------------------|-------------------|-------------------|---------------------|-------------------|
|          |           | GEMIN2<sup>+</sup>  | GEMIN2<sup>-</sup> | RAD51<sup>-</sup> |       | RAD51<sup>-</sup> |
| Total aberrations (Total ± SE) | 1.3 ± 0.9 | 2.0 ± 1.4 | 7.0 ± 2.6 | 3.3 ± 1.5<sup>a</sup> | 26 ± 4.2<sup>b</sup> | 3.0 ± 1.7<sup>c</sup> | 69 ± 8.3<sup>c</sup> |

Cells were treated with colcemid for 3 hours to enrich mitotic cells. The actual numbers of cells analyzed were 150 for wild type, RAD54<sup>-</sup>, and XRCC3<sup>-</sup>, 100 for GEMIN2<sup>-</sup> and RAD51<sup>-</sup>. GEMIN2<sup>+</sup> indicates culture without doxycycline and GEMIN2<sup>-</sup> indicates culture with doxycycline 4 days; RAD51<sup>+</sup> indicates culture without doxycycline and RAD51<sup>-</sup> indicates culture with doxycycline 12 h. The total number of aberrations per cell ± the standard error was calculated as described previously (Takata et al., 1998).

<sup>a</sup> Data from reference (Takata et al., 2000).

<sup>b</sup> Data from reference (Takata et al., 2001).

<sup>c</sup> Data from reference (Sonoda et al., 1998).
Supplementary References

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