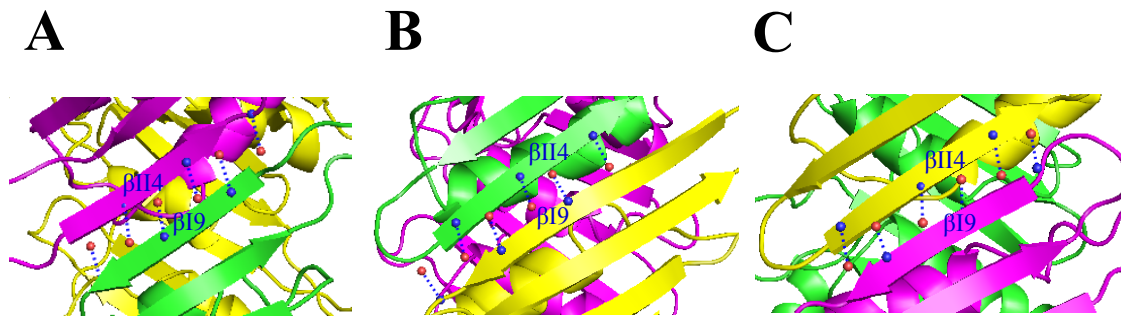
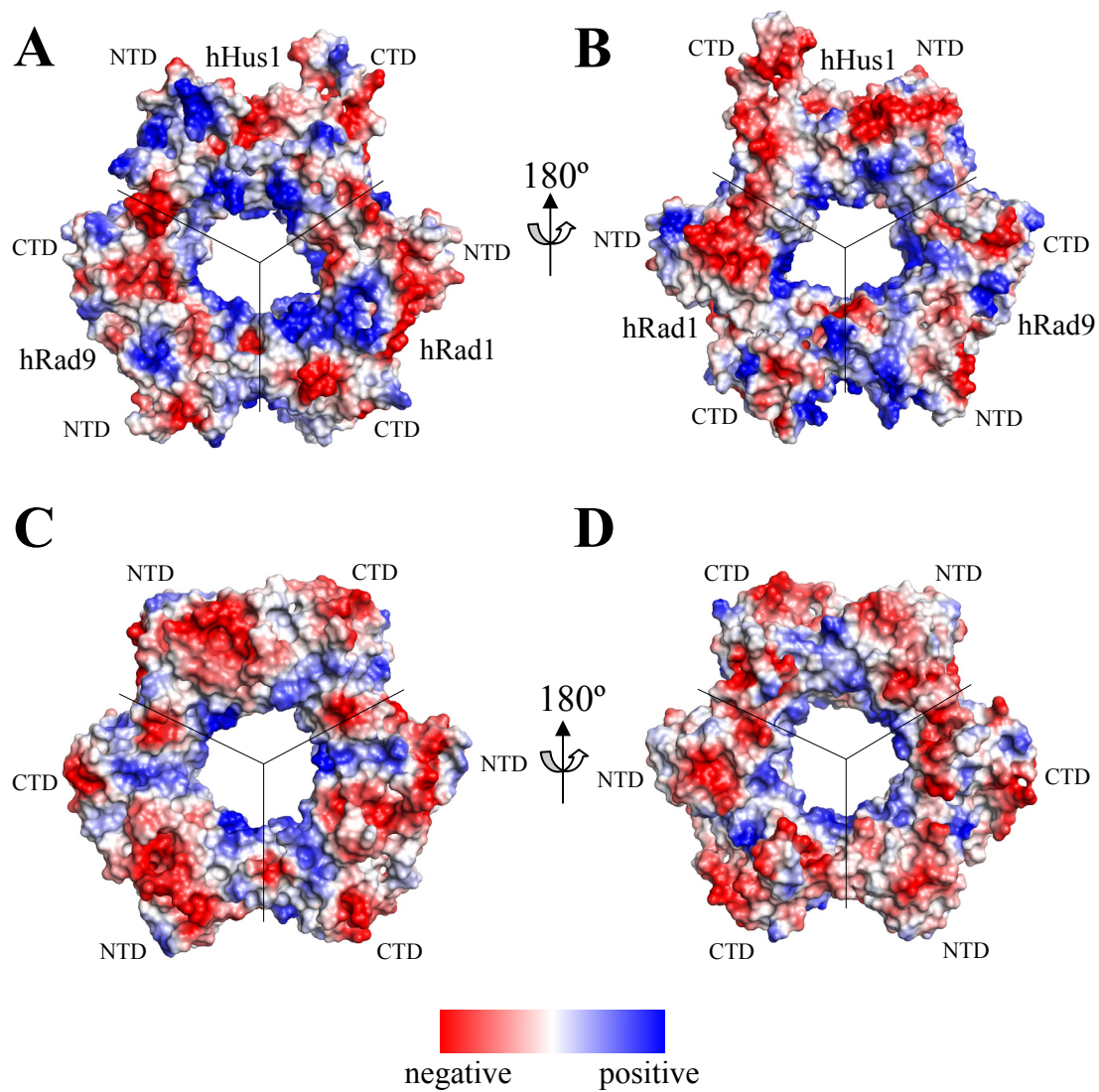


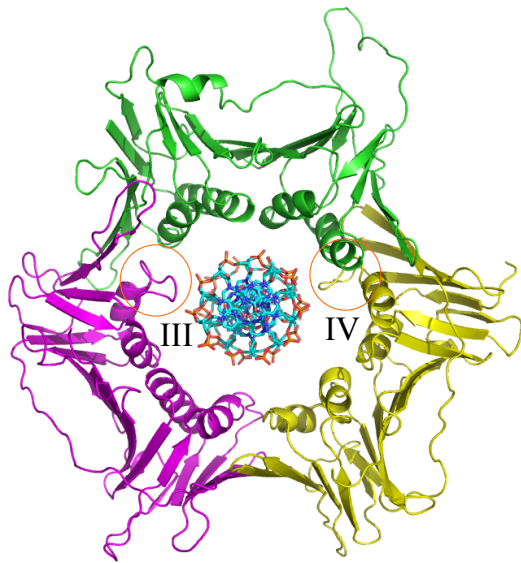
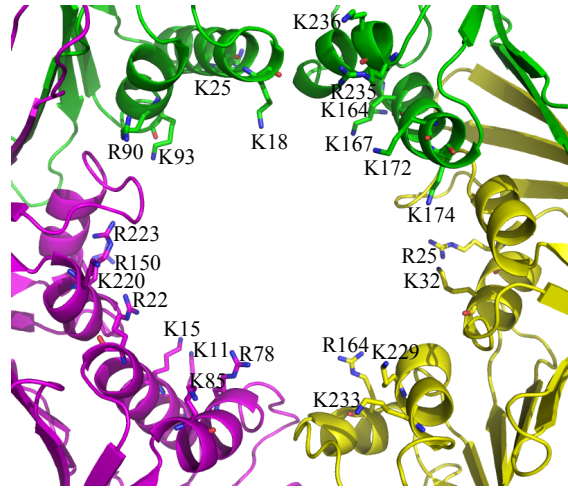
## Supplemental Figures



**Figure S1. The zipper-like series of hydrogen bonds between each two components of the human 9-1-1 complex.** hRad9 is shown in magenta, hHus1 in green, and hRad1 in yellow. The zipper-like series of hydrogen bonds is formed between  $\beta$ II4 of C-terminal domain of one molecule and  $\beta$ I9 of N-terminal domain of the next molecule.

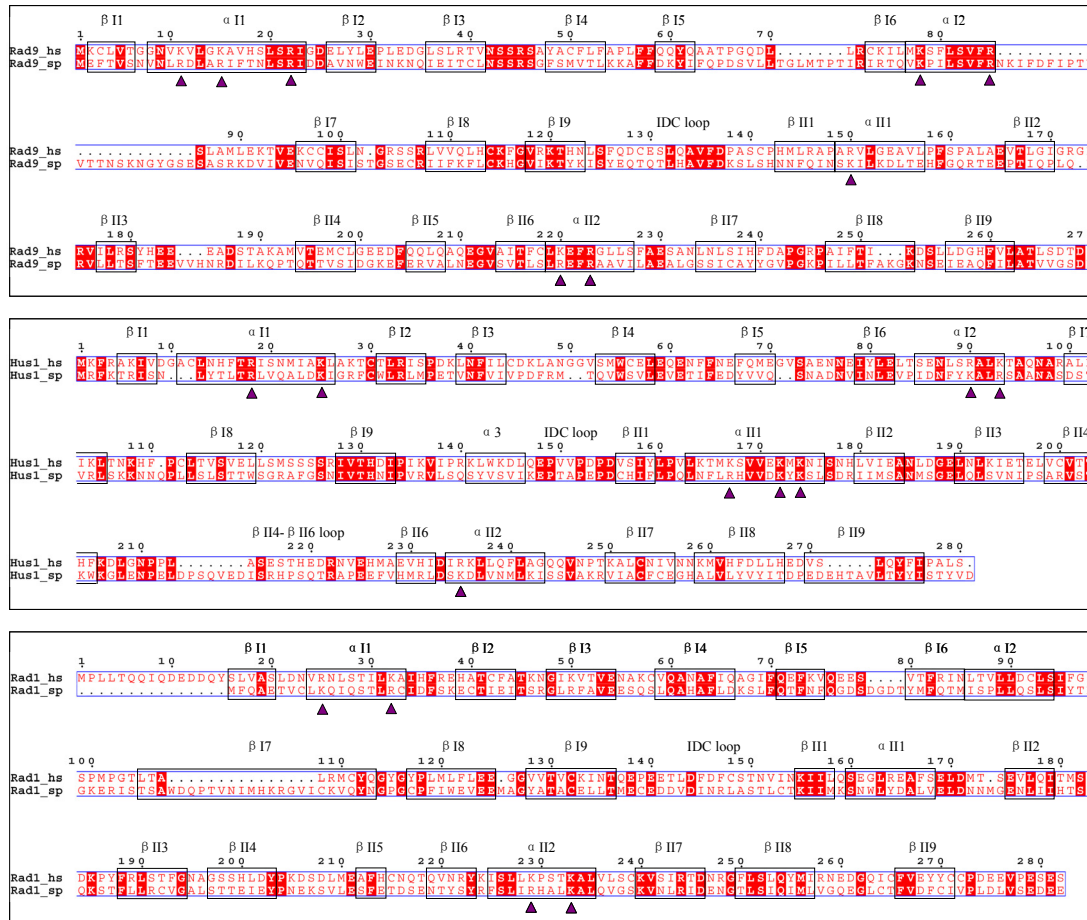


**Figure S2. The net charge distribution on the surface of the human 9-1-1 complex and PCNA. A, B,** The net charge distribution on the surface of the human 9-1-1 complex. **C, D,** The net charge distribution on the surface of the human PCNA (PDB code: 1vym).

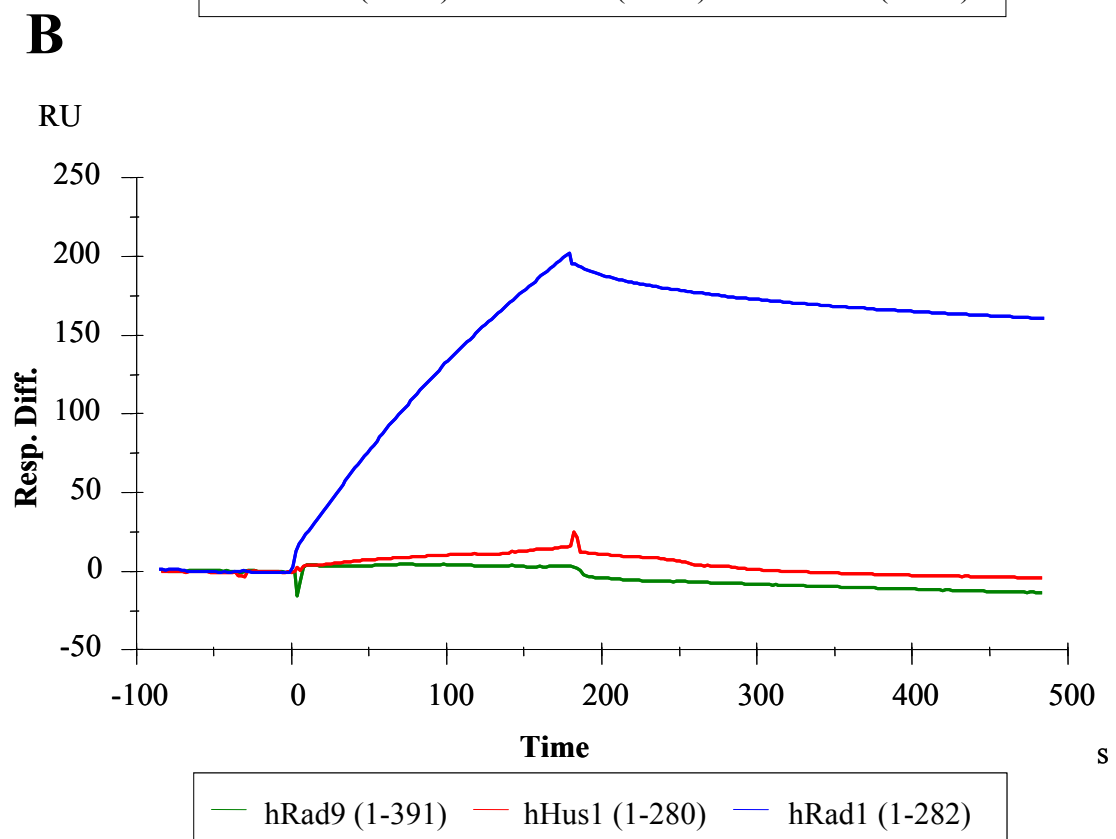
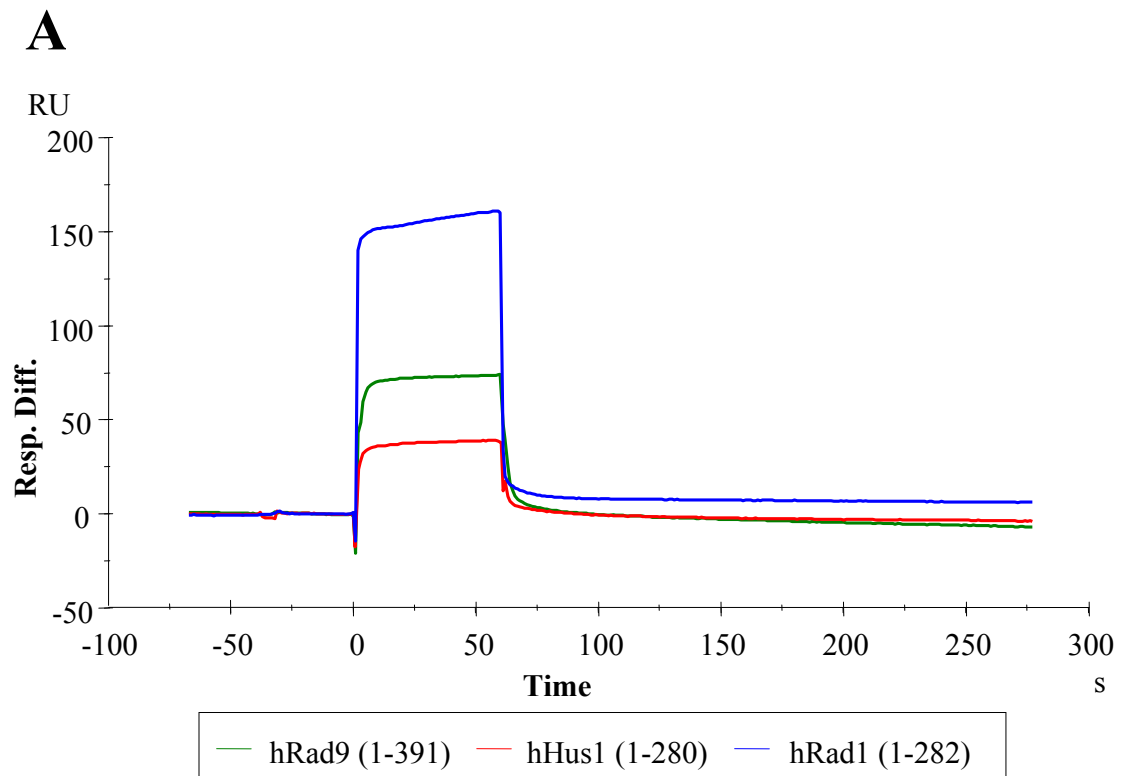
**A****B**

**Figure S3. Potential interactions between the human 9-1-1 complex and dsDNA.**

hRad9 is shown in magenta, hHus1 in green, and hRad1 in yellow. **A**, An interaction model of the 9-1-1 complex and duplex B-form DNA. III indicates the  $\alpha$ II1- $\beta$ II2 loop of hRad9 near the channel in the 9-1-1 complex; IV indicates the  $\alpha$ I2- $\beta$ I7 loop of hRad1 near the channel in the 9-1-1 complex. **B**, Lysine and arginine residues in the 12  $\alpha$  helices extend into the channel in the 9-1-1 ring.

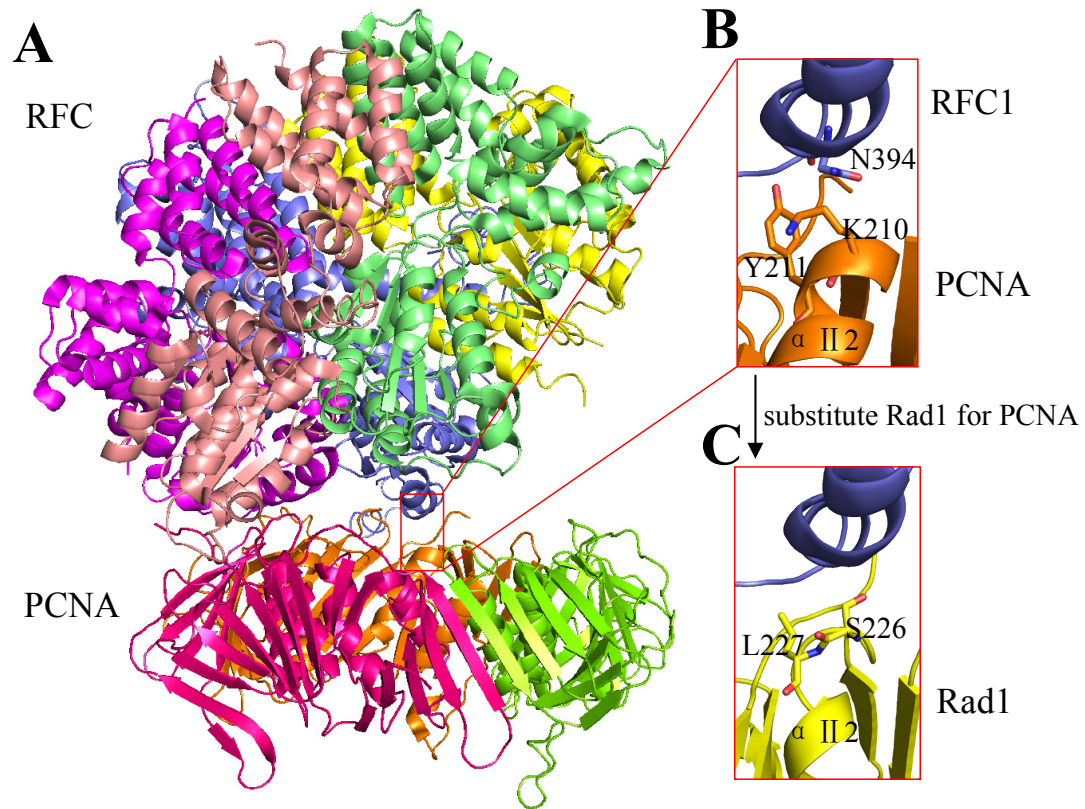


**Figure S4. Sequence alignments between human and fission yeast Rad9, Hus1, and Rad1.** Purple triangles indicate conserved lysine and arginine residues in the  $\alpha$  helices of these proteins which extend into the channel of the human 9-1-1 complex. Red shadow shows the conserved residues of each protein pair by sequence alignment from human and fission yeast sources. hs represents *human*, sp represents *Saccharomyces pombe*. The sequence alignments between *human* and *Saccharomyces pombe* sources were generated by ESPript (1).

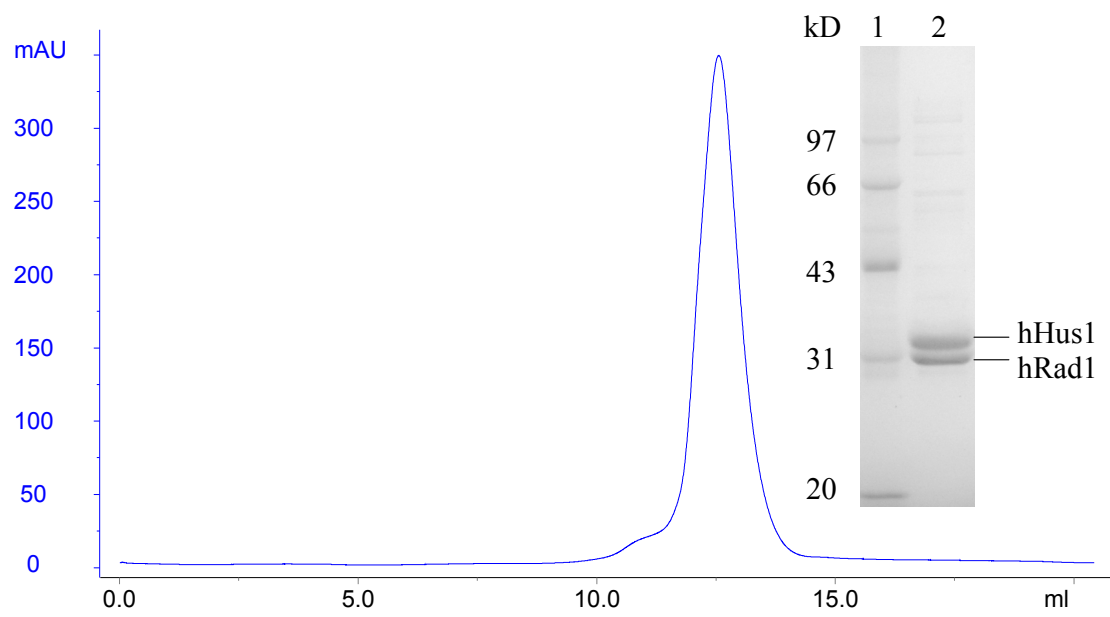


**Figure S5. Biacore analysis.** **A**, Biacore analysis of the hFen1 peptide (residues 335-364) binding to immobilized hRad9, hHus1, and hRad1. hRad1 has the highest binding affinity, hRad9 has the intermediate, and hHus1 has the lowest. **B**, Biacore

analysis of truncated hRad17 (residues 78-337) binding to immobilized hRad9, hHus1, and hRad1. Truncated hRad17 (residues 78-337) interacts with hRad1, but not with hHus1 or hRad9.



**Figure S6. *Saccharomyces cerevisiae* PCNA/RFC complex.** **A**, Stereoview of the *Saccharomyces cerevisiae* PCNA/RFC complex. PDB code: 1SXJ. **B**, The interface between RFC1 and the N-terminal residues of PCNA  $\alpha$ II2 helix. The interacting residues in the interface are indicated. **C**, Substitution of hRad1 for PCNA. The residues 226-227 in  $\alpha$ II2 helix of hRad1 corresponding to that of PCNA are indicated.



**Figure S7. Size-exclusion column chromatography and SDS-PAGE of human Hus1-Rad1 complex.** Line 1 shows the Protein markers. Line 2 shows the purified proteins obtained via size-exclusion column chromatography.



## Supplemental tables

**Table S1. Data Collection and Refinement Statistics**

	Native	Hg Derivative
<b>Data collection</b>		
X-ray source	BL41XU at Spring-8 (Japan)	PX at SLS (Switzerland)
Wavelength (Å)	1.0716	1.0063
Resolution (Å)	15-3.2 (3.31-3.2)	15-3.2 (3.31-3.2)
Space group	P2 <sub>1</sub>	P2 <sub>1</sub>
Unit cell (Å)	a=71.03, b=67.17, c=83.41, β=97.58	a=70.54, b=67.38, c=82.97, β=97.85
Unique reflections	13,001(1272)	12,762 (1879)
Average redundancy	6.5	5.7
Anomalous redundancy		2.9
I/σ (I)	17.22(4.5)	24.6 (4.1)
Completeness (%)	99.8 (99.5)	99 (100)
Anomalous Completeness (%)		98.2 (99.7)
R <sub>merge</sub> (%) <sup>a</sup>	8.0 (43.2)	8.7 (29.0)
<b>Refinement</b>		
Resolution (Å)	15-3.2	
R <sub>working</sub> (%) <sup>b</sup>	28.9	

$R_{\text{free}}(\%)^c$	30.6
Average B factor (Å)	67.8
rmsd bounds (Å)	0.011
rmsd angles (°)	1.728

Numbers in parentheses represent statistics for the highest resolution shell.

<sup>a</sup> $R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$ , where  $I$  is the measured intensity for reflections with indices  $hkl$ .

<sup>b</sup> $R_{\text{working}} = \sum ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum |F_{\text{obs}}|$ .

<sup>c</sup> $R_{\text{free}}$  = R factor for a selected subset (10%) of the reflections that were not included in prior refinement calculations.

### Table S2. Interface parameters of the human 9-1-1 complex.

Calculated using the CCP4 PISA server (2) and the CCP4 SC program (3).

Interface	Complexation Significance Score (CSS)*	Shape Complementarity (SC)**
hRad9-hHus1	1.0	0.609
hHus1-hRad1	1.0	0.649
hRad1-hRad9	0.24	0.540

\* Complexation Significance Score (CSS) ranges from 0 to 1 as the interface relevant to complexation increases.

\*\* Shape Complementarity (SC) is in the range 0–1 (1 = best fit).

## Supplemental References

1. Gouet, P., Courcelle, E., Stuart, D. I., and Metoz, F. (1999) *Bioinformatics* **15**, 305-308
2. Krissinel, E., and Henrick, K. (2007) *J. Mol. Biol.* **372**, 774-797
3. Lawrence, M. C., and Colman, P. M. (1993) *J. Mol. Biol.* **234**, 946-950