SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. Examination of the RAD51AP1 C-terminal region for physical and functional intercations with RAD51.
A, SDS-PAGE analysis of the purified, tagged RAD51AP1 species. *B*, Full-length (FL) RAD51AP1 and the F3 fragment were tested for DNA binding with the D-loop substrate. *C,* Quantification of the DNA binding data in *B*. Shown are the mean ± SD from at least three independent experiments. *D,* MBP-tagged FL RAD51AP1 and fragments were tested for RAD51 interaction in the amylose pulldown assay. The supernatant (S), wash (W), and eluate (E) fractions of the reactions were analyzed by SDS-PAGE. MBP alone did not pull any RAD51 down (data not shown). *E, S*chematic of the D-loop assay is shown in the boxed region. RAD51AP1 FL protein and fragments (0.5 or 1 μM) were tested with RAD51 in the D-loop reaction with or without ATP. The results were quantified and plotted; error bars represent mean ± SD from at least three independent experiments.

SUPPLEMENTAL FIGURE 2. Fusion of the C-terminal RAD51 binding domain to F1 allows for RAD51 interaction.
A, SDS-PAGE analysis of the purified, tagged RAD51AP1 F1-C60 and C60 polypeptides. *B,* MBP-tagged F1-C60 and C60 were tested for RAD51 interaction in the amylose pulldown assay. *C,* Quantification of binding of the D-loop, dsDNA, and ssDNA substrates by F1 and F1-C60 where the data are the mean ± SD from at least three independent experiments. The data points for F1 are taken from Fig. 1C.

SUPPLEMENTAL FIGURE 3. Residues in F1 critical for DNA binding and RAD51 enhancement.
A, Truncation mutants of F1-C60 used in this study. *B,* SDS-PAGE analysis of the purified F1-C60 truncation mutants. *C,* MBP-tagged F1-C60 truncation mutants were tested for RAD51 interaction in the amylose pulldown assay. *D,* Quantification of F1-C60 truncation mutants bound to D-loop, dsDNA, ssDNA, where the data are the mean ± SD from at least three independent experiments. *E,* F1-C60 truncation mutants (0.5, 0.75, 1 μM) were tested with RAD51 in the D-loop reaction. The results were quantified and plotted; error bars are mean ± SD from at least three independent experiments.

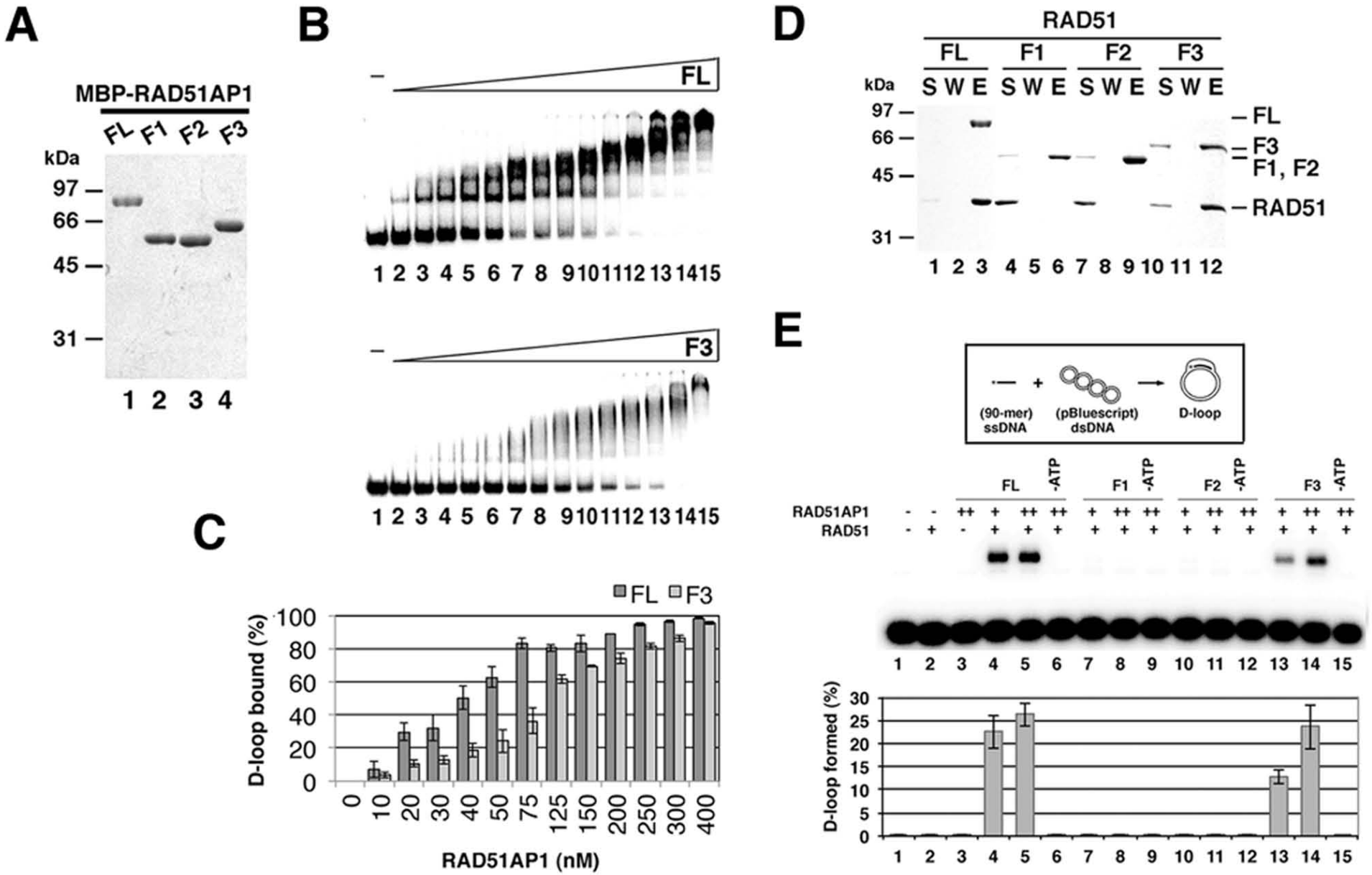
SUPPLEMENTAL FIGURE 4. Sequence alignment of the RAD51AP1 N-terminal and C-terminal portions that lie within the DNA binding domains.
A, Alignment of the N-terminal region of human RAD51AP1 encompassing residues 30-49 against the equivalent region in orthologs. *B,* Alignment of the C-terminal regions encompassing residues 226-240 and 267-290 against the equivalent regions in orthologs.

SUPPLEMENTAL FIGURE 5. DNA binding point mutants of F1-C60, F3, and full-length RAD51AP1 can still associate with RAD51.
A, SDS-PAGE analysis of the purified wild type and mutant F1-C60 polypeptides. *B,* The wild type and mutant F1-C60 polypeptides were tested for RAD51 interaction in the amylose pulldown assay. *C,* SDS-PAGE analysis of the purified wild type and mutant F3 fragments. *D,* The wild type and mutant F3 fragments were tested for RAD51 interaction in the amylose pulldown assay. *E,* SDS-PAGE analysis of the purified full length (FL) wild type and mutant RAD51AP1 proteins. *F,* The FL RAD51AP1 wild type and mutant proteins were tested for RAD51 interaction in the amylose pulldown assay.

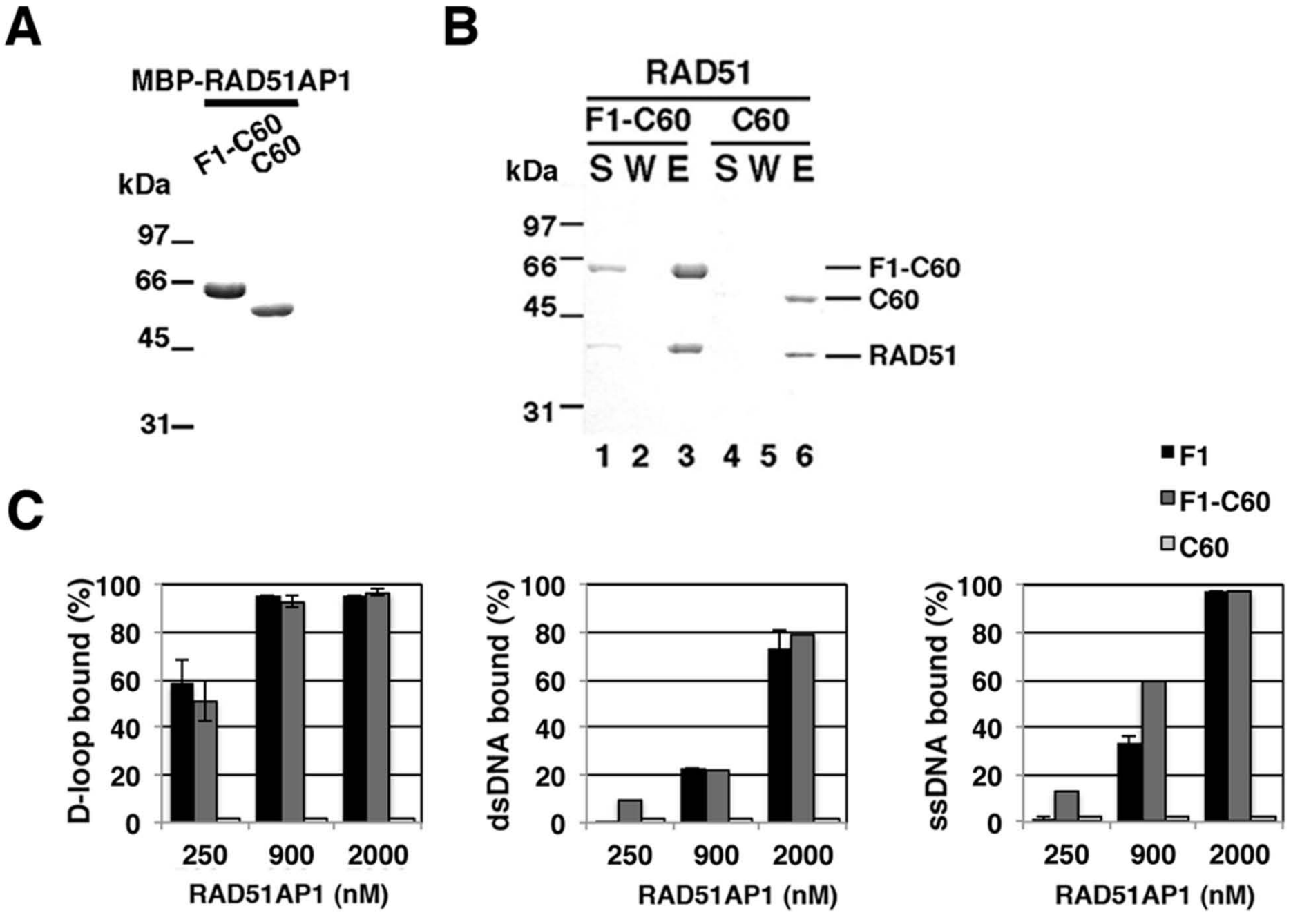
SUPPLEMENTAL TABLE 1. Oligonucleotides used for cloning or site-directed mutagenesis of RAD51AP1

Name	Comments	Sequence
C60 (for)		5'-CACGGAATTCCGCAGTCCTTCAGCTGAAAGC- 3'
C60 (rev)		5'-CACGGGATCCTCAGGTGCTAGTGGCATTTGG-3'
F1-C60 (for)	Deletion of residues 95-187 in FL RAD51AP1	5'-CACTAGCTTTATCAGTGAAGGAACTTCCACGCAGTCCTTCAGCTGAAAGCAAGAAACC-3'
F1-C60 (rev)		5'-GGTTTCTTGCTTTCAGCTGAAGGACTGCGTGGAAGTTCCTTCACTGATAAAGCTAGTG-3'
69-C60 (for)	Deletion of residues 70-94 in F1-C60	5'-CCCAGTACAAGAGAAAACCCCTAAAAAACGCAGTCCTTCAGCTGAAAGCAAGAAAC-3'
69-C60 (rev)		5'-GTTTCTTGCTTTCAGCTGAAGGACTGCGTTTTTTAGGGGTTTTCTCTTGTACTGGG -3'
49-C60 (for)	Deletion of residues 50-94 in F1-C60	5'-CAAAGGAGTTAAAACAAGATAAACCAAAACGCAGTCCTTCAGCTGAAAGCAAGAAAC- 3'
49-C60 (rev)		5'-GTTTCTTGCTTTCAGCTGAAGGACTGCGTTTTGGTTTATCTTGTTTTAACTCCTTTG-3'
29-C60 (for)	Deletion of residues 30-94 in F1-C60	5'-CAGTGATGATGATTTTGTTTCTGCAACTCGCAGTCCTTCAGCTGAAAGCAAGAAAC -3'
29-C60 (rev)		5'-GTTTCTTGCTTTCAGCTGAAGGACTGCGAGTTGCAGAAACAAAATCATCATCACTG-3'
F1D53-C60 (for)	Deletion of residues 1-53 in F1-C60	5'-GAGCGAGAATCTTTATTTTCAGGGCCATAATCTCCGGAAAGAAGAAATCCCAGTAC-3'
F1D53-C60 (rev)		5'-GTACTGGGATTTCTTCTTTCCGGAGATTATGGCCCTGAAAATAAGATTCTCGCTC -3'
K2RA (for)		5'-GTTTCTGCAACTGTACCTTTAAACGCGGCATCCGCAACAGCACC AAAGGAGTTAAAAC-3'
K2RA (rev)		5'-GTTTTAACTCCTTTGGTGCTGTTGCGGATGCCGCGTTTAAAGGTACAGTTGCAGAAAC -3'
K6RA #1 (for)	Seven mutations were introduced in two steps	5'-CGCGGCATCCGCAACAGCACCAGCGGAGTTAGCACAAGATAAACCAAAACCTAACT-3'
K6RA #1 (rev)		5'-AGTTAGGTTTTGGTTTATCTTGTGCTAACTCCGCTGGTGCTGTTGCGGATGCCGCG-3'
K6RA #2 (for)	Used K6RA#1 first, followed by K6RA#2.	5'-CACCAGCGGAGTTAGCACAAGATGCACCAGCACCTAACTTGAACAATCTCCG-3'
K6RA #2 (rev)		5'CGGAGATTGTTCAAGTTAGGTGCTGGTGCATCTTGTGCTAACTCCGCTGGTG-3'
K4A (for)		5'- CCCCAGTAGAAAAGAAAGAGGCGGCATCTGCATCCGCATG TAATGCTTTGGTGACTTC-3'
K4A (rev)		5'-GAAGTCACCAAAGCATTACATGCGGATGCAGATGCCGCCTC TTTCTTTTCTACTGGGG -3'
K3WA (for)		5'-CGCAGTCCTTCAGCTGAAAGCGCGGCACCTGCAGCGGTCCCACCAGCGGCATCTGGAGG-3'
K3WA (rev)		5'- CCTCCAGATGCCGCTGGTGGGACCGCTGCAGGTGCCGCGCT TTCAGCTGAAGGACTGCG-3'
siRNA*res* (for)	Resistance to siRNA of Modesti et al. (2007)	5'-GAAACAATGAATAAGTCTCCTCATATAAGCAACTGCAGTGTGCCAGTGATTATTTAGA -3'
siRNA*res* (rev)		5'-TCTAAATAATCACTGGCTACACTGCAGTTGCTTATATGAGGAGACTTATTCATTGTTTC -3'

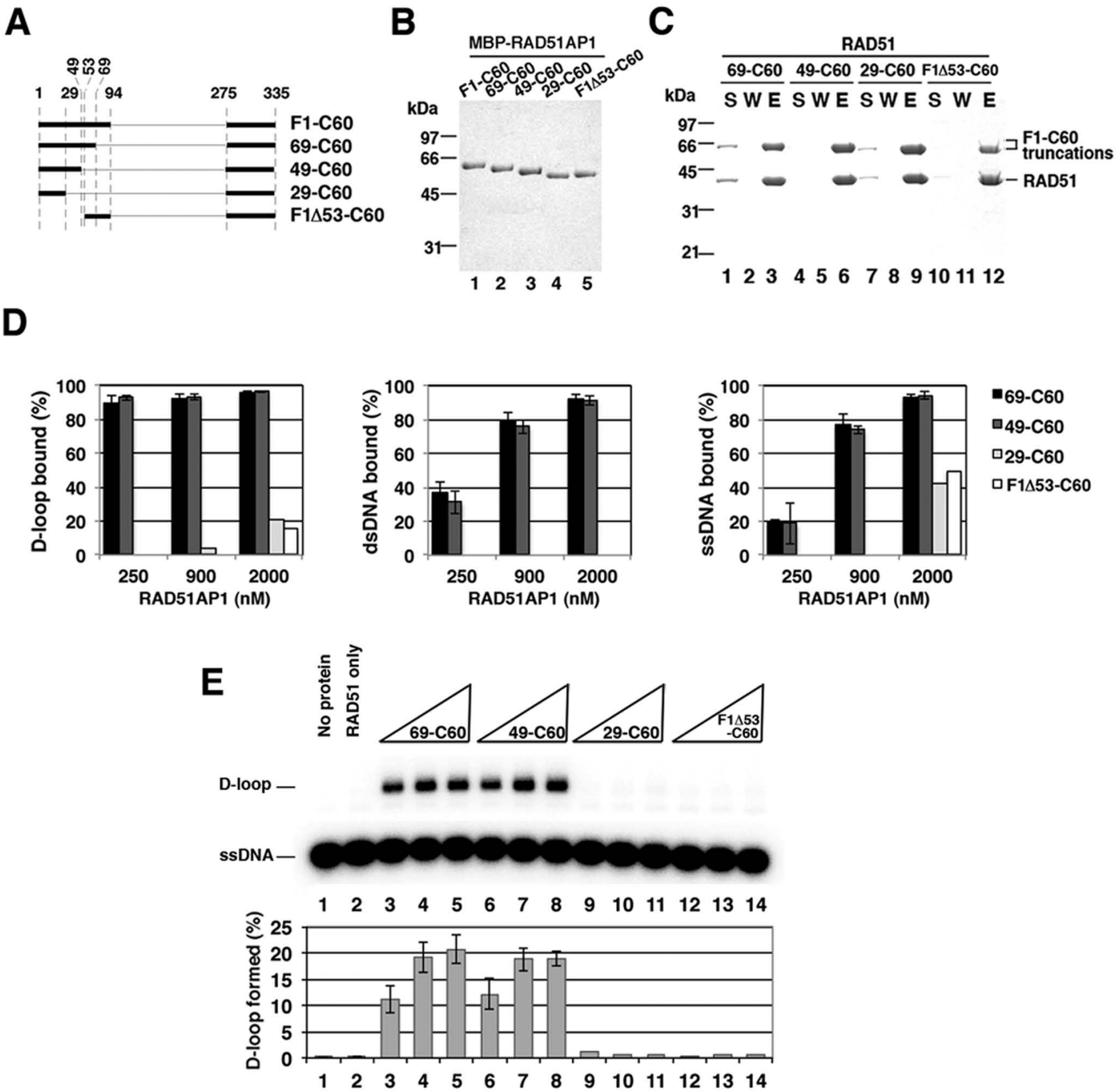
Supplemental Figure 1



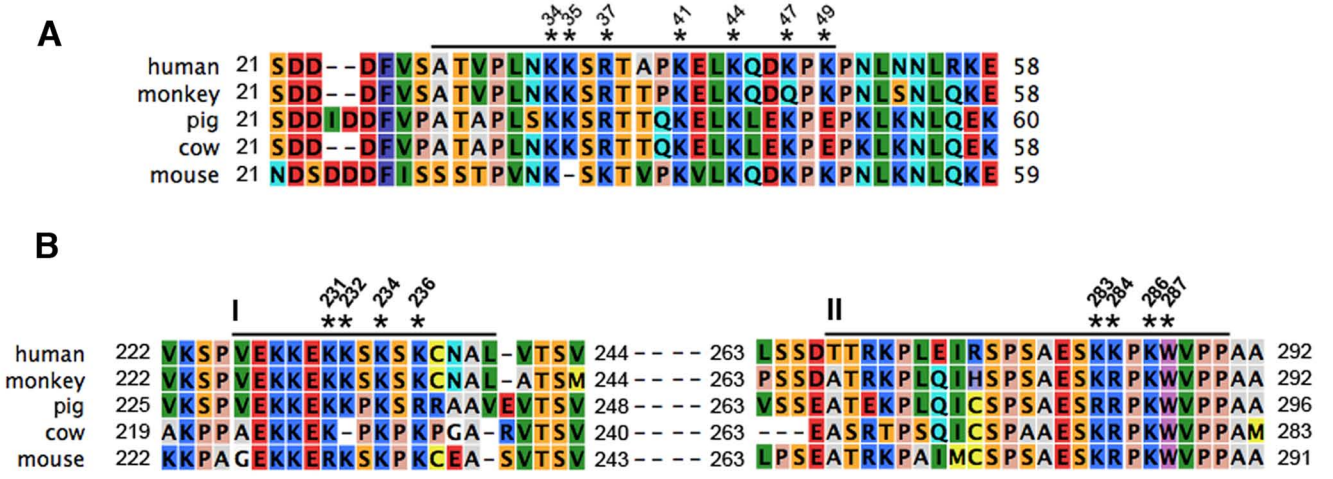
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5