Supplemental Information for:

Modularized Functions of the Fanconi Anemia Core Complex

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Figure S1

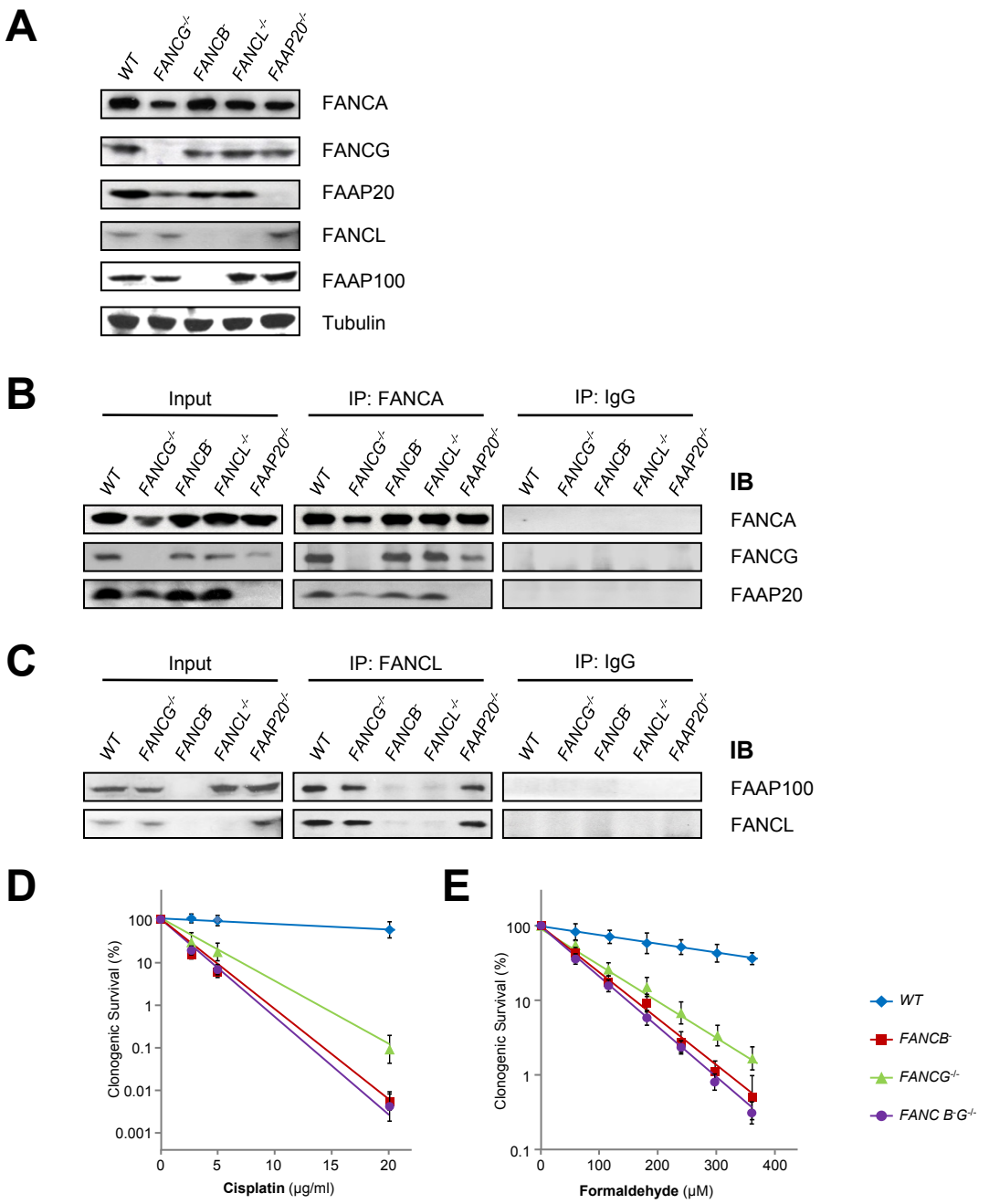

Fig. S1. Sub modules in the FA core complex. Related to Fig. 1A-B.
(**A**) Immunoblots detecting FA core complex protein levels in *FANCG*$^{-/-}$, *FANCB*$^{-}$, *FANCL*$^{-/-}$, and *FAAP20*$^{-/-}$ knockout mutant cells generated in the HCT116 background. Whole cell extracts were prepared from untreated exponentially growing cultures. Protein stability interdependencies are observed in *FANCG*$^{-/-}$, *FANCB*$^{-}$, and *FANCL*$^{-/-}$ cells.
(**B**) Co-immunoprecipitation of FANCA in HCT116 (*WT)*, *FANCG*$^{-/-}$, *FANCB*$^{-}$, *FANCL*$^{-/-}$, and *FAAP20*$^{-/-}$ mutants, showing the presence of an A-G-20 sub module in *WT*, *FANCB*$^{-}$, and *FANCL*$^{-/-}$ cells.
(**C**) Co-immunoprecipitation of FANCL in HCT116 (*WT*), *FANCG*$^{-/-}$, *FANCB*$^{-}$, *FANCL*$^{-/-}$, and *FAAP20*$^{-/-}$ mutants, showing the presence of an L-B-100 sub module in *WT, FANCG*$^{-/-}$*, and FAAP20*$^{-/-}$ cells.
(**D-E**) Clonogenic survival of parental HCT116 (*WT*), *FANCB*$^{-}$, *FANCG*$^{-/-}$, and *FANCB*$^{-}$*G*$^{-/-}$ mutants treated with indicated ICL reagents. Error bars were derived from SDs from four independent experiments with triplicates.

Figure S2

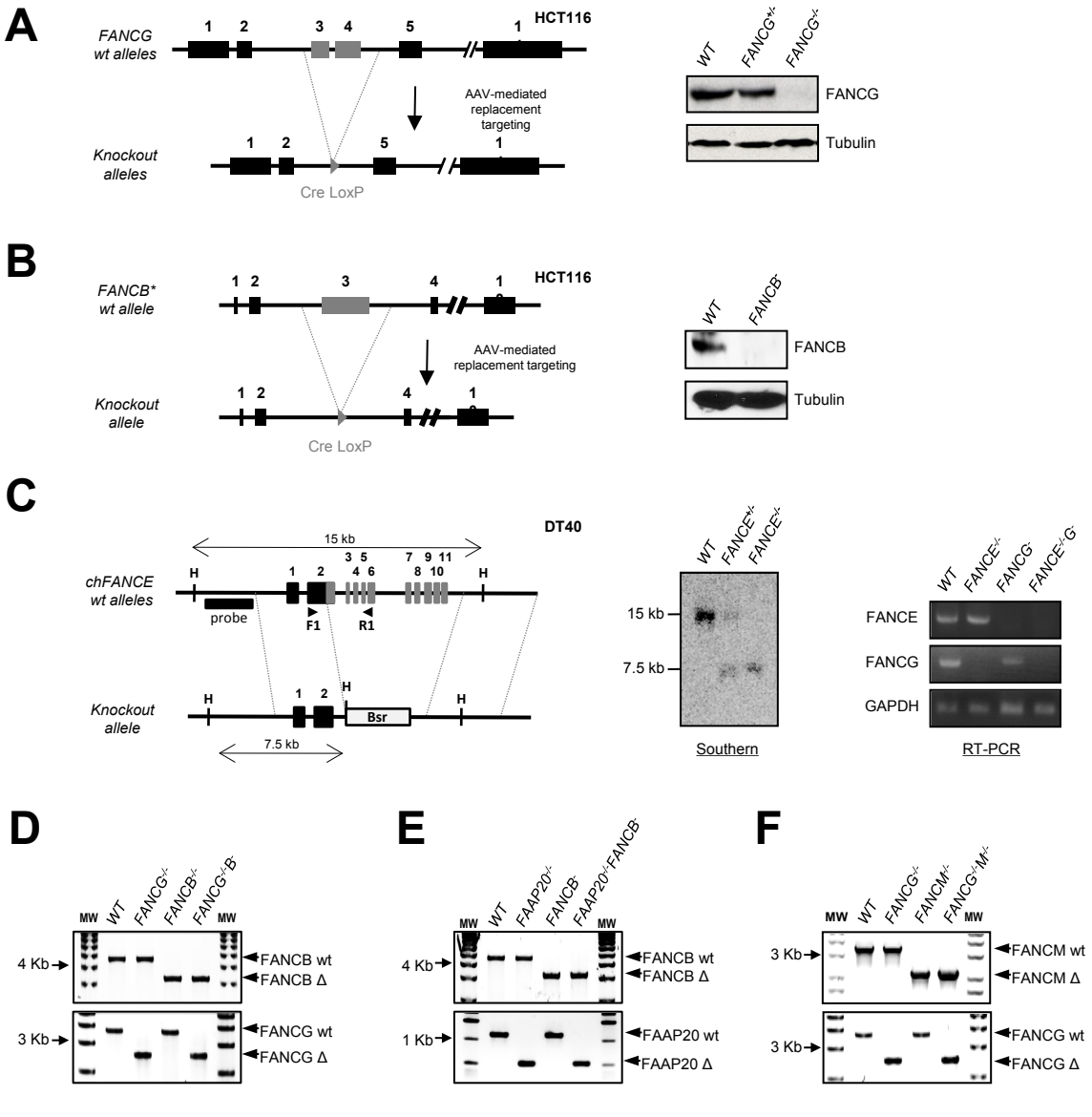

Fig. S2. Construction of Fanconi anemia somatic cellular knockout mutants in human HCT116 and chicken DT40 cells. Related to Fig. 1B-C, 3D, and 4D-F.

(**A**) Targeting design for the *FANCG* loci (left) and Immunoblotting of FANCG in parental HCT116 (*WT*), *FANCG*$^{+/-}$ and *FANCG*$^{-/-}$ mutant cell extracts (right).

(**B**) Targeting design for the *FANCB* locus (left) and IP-immunoblotting of FANCB in parental HCT116 (*WT*) and FANCB^{-} mutant cell extracts (right). * FANCB gene is X-linked and the HCT116 cells is of male origin.

(**C**) Targeting design (Left), Southern genotyping (middle), and expression validation (right) of the chicken FANCE, FANCG, and FANCE-FANCG double mutant. F1 and R1 indicate PCR primer pairs for screening. Bsr: Blasticidin-resistant marker.

(**D**) PCR genotyping of the *FANCG*$^{-/-}$*B*$^{-}$ double knockout mutant.

(**E**) PCR genotyping of the *FAAP20*$^{-/-}$*FANCB*$^{-}$ double knockout mutant.

(**F**) PCR genotyping of the *FANCG*$^{-/-}$*M*$^{-/-}$ double knockout mutant.

Δ, knockout allele. wt, wild-type allele. In all genotyping analyses, flanking PCR primers are selected from outside the homologous arms used in targeting constructs.

Figure S3

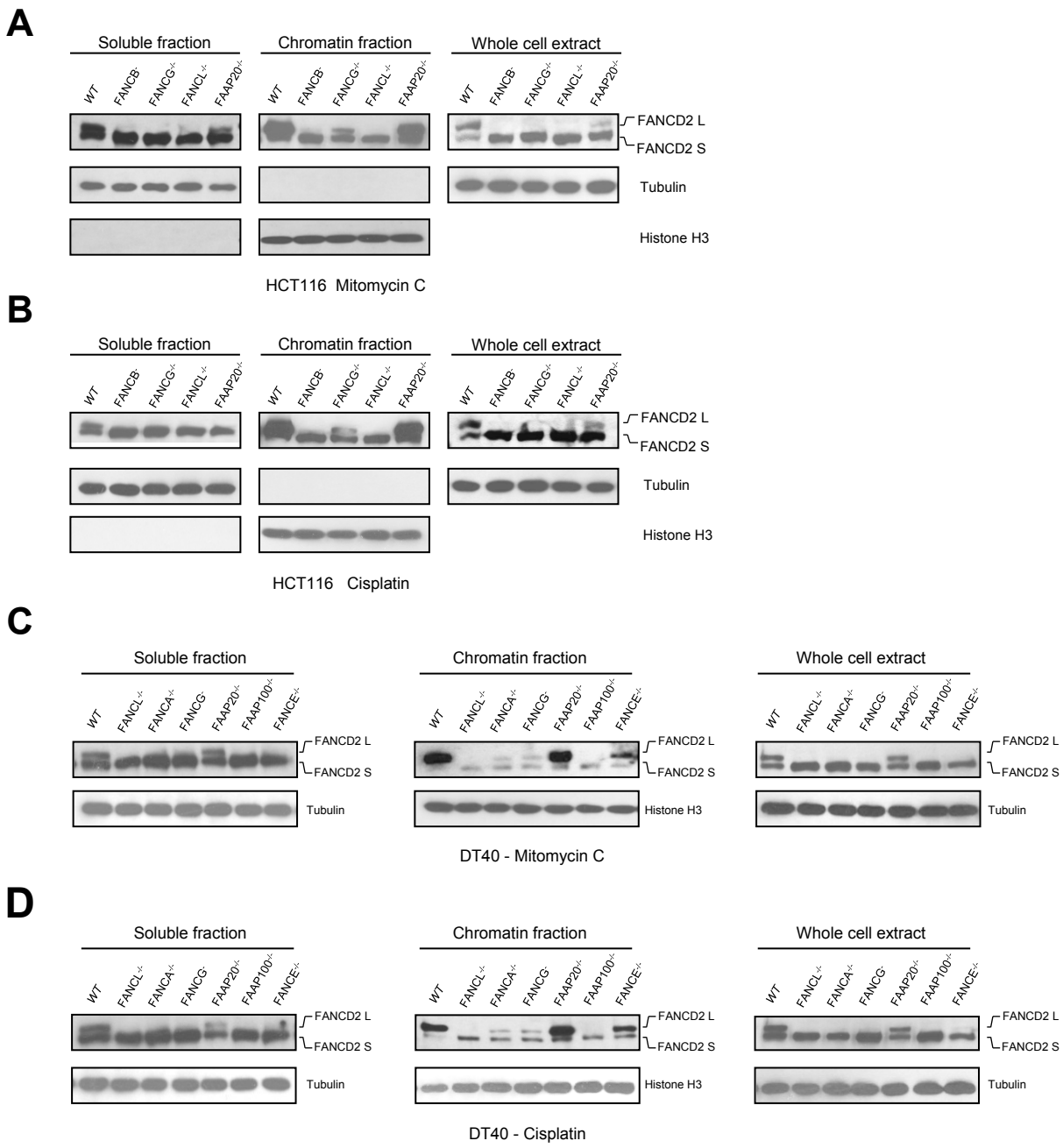

Fig. S3. FANCD2 levels in HCT116 and DT40 knockout mutants. Related to Fig. 1E-F.
(**A**) Immunoblots detecting MMC-induced monoubiquitination of FANCD2 in wild-type (*WT*) and the indicated knockout mutants in human HCT116 background.
(**B**) Immunoblot detecting cisplatin-induced monoubiquitination of FANCD2 in wild-type (*WT*) and indicated knockout mutants in human HCT116 background.
(**C**) Immunoblots detecting MMC-induced monoubiquitination of FANCD2 in wild-type (*WT*) and indicated knockout mutants in chicken DT40 background.
(**D**) Immunoblot detecting cisplatin-induced monoubiquitination of FANCD2 in wild-type (*WT*) and indicated knockout mutants in chicken DT40 background.
Tubulin and Histone H3 are used as loading and extraction controls.

Figure S4

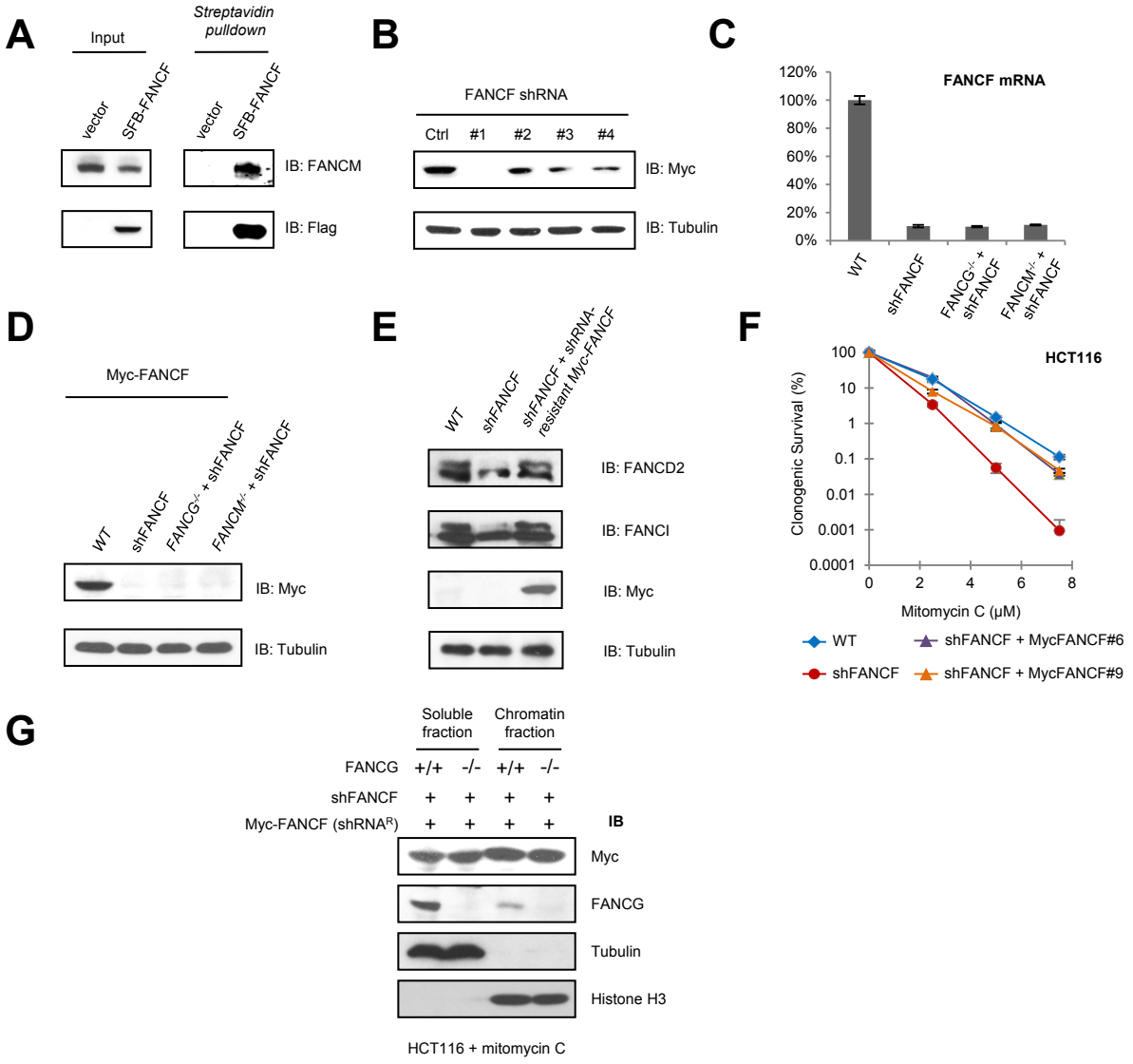

Fig. S4. FANCF-FANCM interaction and FANCF shRNA experiments. Related to Fig. 3A-C and 4A-C.
(**A**) Co-immunoprecipitation between FANCF and FANCM. S-tag-Flag-Biotin (SFB)–tagged FANCF or the empty vector was stably expressed in 293T cells. Protein extracts prepared from both cell lines were subjected to streptavidin beads pulldown and bound proteins were immunoblotted by FANCM or Flag antibodies.
(**B**) Screening of FANCF shRNAs. Four Lentivirus (#1 to #4) shRNA to FANCF were used to infect 293T cells stably expressing Myc-tagged FANCF. The Myc-tagged FANCF stable line was constructed to circumvent the lack of effective FANCF antibody. Immunoblotting with Myc antibody identifies the most effective shRNA (#1), which was used in all subsequent experiment.
(**C**) Reverse transcription/Q-PCR measuring stable FANCF knockdown in the indicated mutant cell lines.
(**D**) Immunoblotting with Myc antibody testing FANCF knockdown efficiency in the indicated stable cell lines transiently transfected with wild-type Myc-FANCF.
(**E**) Immunoblot detecting MMC-induced monoubiquitination of FANCD2 and FANCI in parental HCT116 (*WT*) and indicated *FANCF* knockdown mutants complemented stably with shRNA-resistant Myc-tagged FANCF.
(**F**) Clonogenic survival of wild-type (*WT*), shFANCF stable knockdown cell line, and two complemented cell lines (#6 and #9) against mitomycin C. Error bars for clonogenic survival are derived from SDs from three independent tests with triplications.
(**G**) Immunoblots detecting chromatin-bound Myc-tagged FANCF in mitomycin C-treated FANCG$^{+/+}$ and FANCG$^{-/-}$ cells with stable knockdown of endogenous FANCF and complemented with shRNA-resistant Myc-tagged FANCF.

Supplementary Experimental Procedures

Generation of lentiviral shRNA against FANCF

GIPZ Lentiviral shRNA constructs were purchased from the ShRNA and ORFeome Core, the University of Texas MD Anderson Cancer Center. The mature antisense sequences of the four FANCF shRNAs are ACTTCAAAATCTCCATCCT (#1), TGGGTTCTCTCTATAGCCA (#2), TTCTGAAGGTCATAGTGCA (#3), and TGGAGTGTCTCCTCATCGG (#4) (see **Fig. S5B**). Lentiviral stocks were prepared from HEK293T cells and used to generate stable expression cells via puromycin selection (2ug/ml).

Nuclear extract preparation and gel filtration profiling

To obtain nuclear extracts for gel filtration profiling, cells were incubated in hypotonic buffer (10 mM Tris, pH 7.4, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM Dithiothreitol, and protease inhibitor cocktail (Roche 04693159001)) on ice. After 10 min incubation, NP-40 (0.1% final concentration) was added and nuclei were pelleted by 10-sec centrifugation at 5,500 × g. Upon washing three times with the hypotonic buffer, the nuclei were extracted with extraction buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 1 mM EDTA, 1 mM dithiothreitol, and protease inhibitor cocktail). Subsequently, the freshly prepared nuclear extract (2 mg total protein) was applied directly to a Superose 6 10/300 GL column (GE Healthcare 17-5172-01). Fractions of 0.4 ml were collected and analyzed by western blot.