Supplemental data

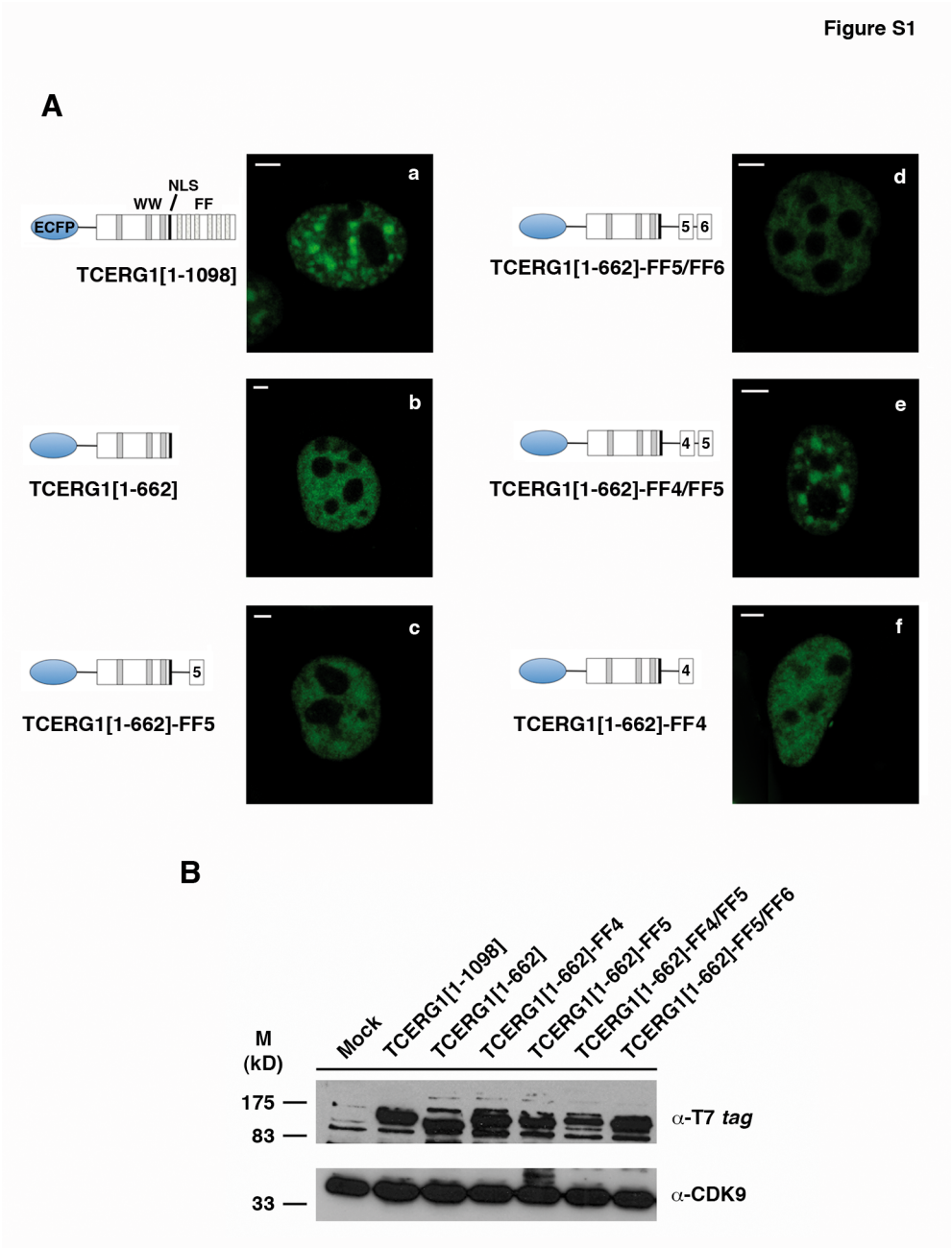

FIGURE S1. **The FF4/FF5 domains are required for efficient targeting of TCERG1 to the speckle compartment.** A, The ECFP-TCERG1 fusion proteins were expressed by transient transfection in HEK293T cells and their nuclear localization was analyzed by confocal microscopy. A diagrammatic representation of the ECFP-

TCERG1 fusion proteins is shown at the left of each panel. The numbers in parentheses represent the TCERG1 amino acids contained in the construct. Shown are the three WW domains, the putative nuclear localization signal (NLS), and the six FF domains. Bars, 3 µm. B, Cell lysates from transfected cells were analyzed by immunoblotting with the indicated antibodies to detect the TCERG1 and CDK9 proteins. Numbers to the left of the gels indicate molecular masses in kDa.

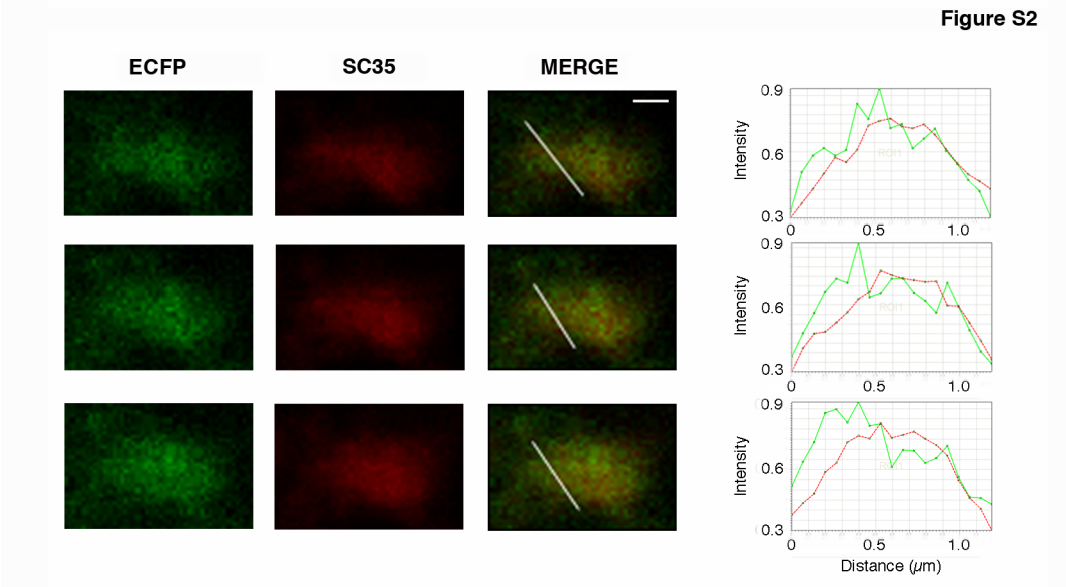

FIGURE S2. **The speckle-associated TCERG1[1-662]-FF4/FF5 localizes mainly to the periphery and outside the core region of speckles.** Seriated optical sections (z-plane step, 0.3 µm) through individual nuclear speckles were analyzed at low intensity. Individual staining (ECFP and SC35) and merge images are shown. Line scans showing local intensity distributions of TCERG1[1-662]-FF4/FF5 in green and SC35 in red are shown to the right of the panels. Bar, 0.3 µm.

Figure S3

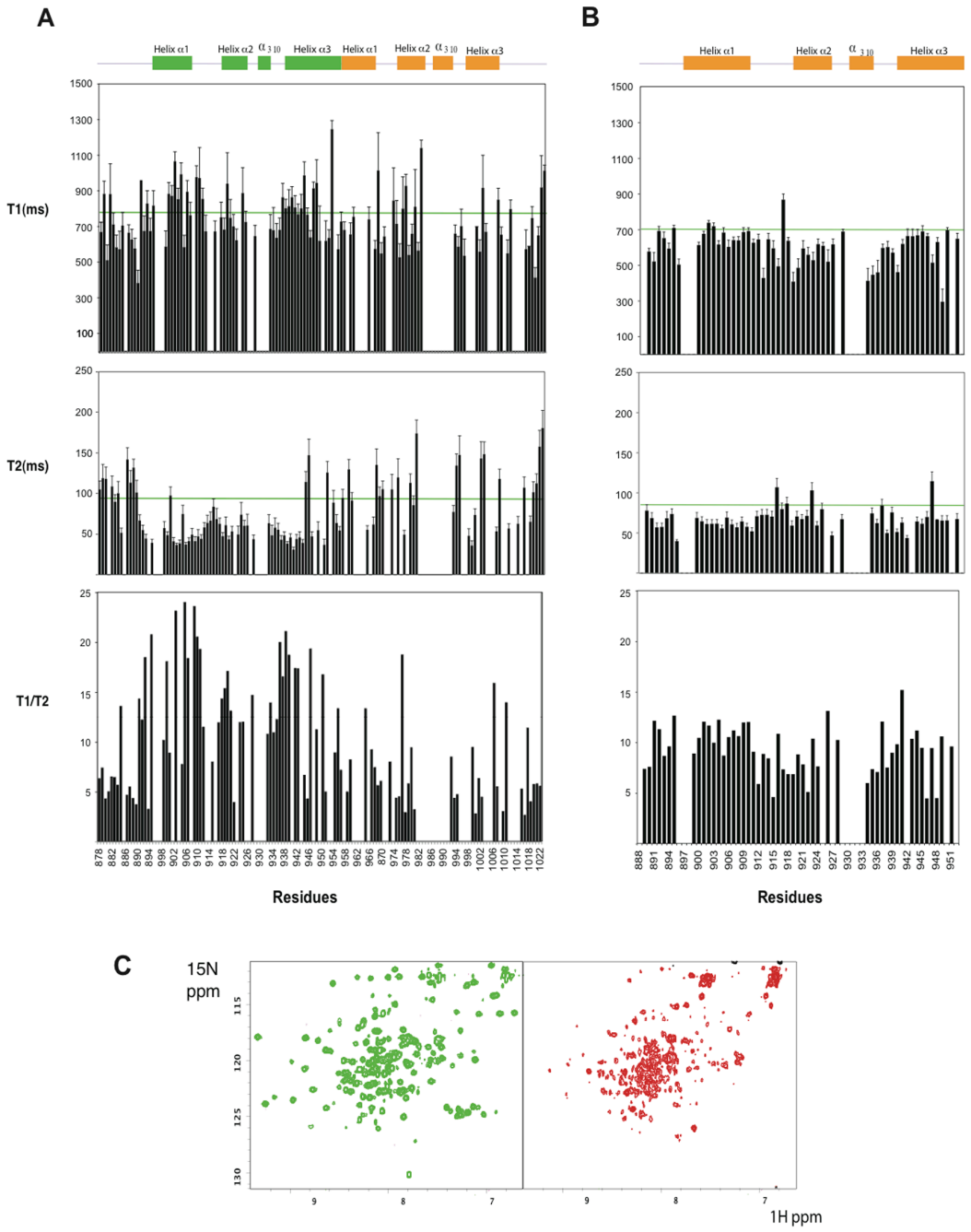

FIGURE S3. **Backbone amide ^{15}N relaxation data.** A, Plots of T_1, T_2 and T_1/T_2 ratio for the FF4-FF5 construct. Unassigned residues, proline residues that lack a proton amide and overlapped peaks were excluded from the analysis. B, Plots of T_1, T_2 and T_1/T_2 ratio for FF4 construct. C, ^{15}N-HSQC spectra at 298K for the FF4-FF5 (green)

and FF5-FF6 (red) pairs. The comparison of the NMR data shows a significant difference in peak dispersion. Data show no structural organization when FF5 is combined with FF6.

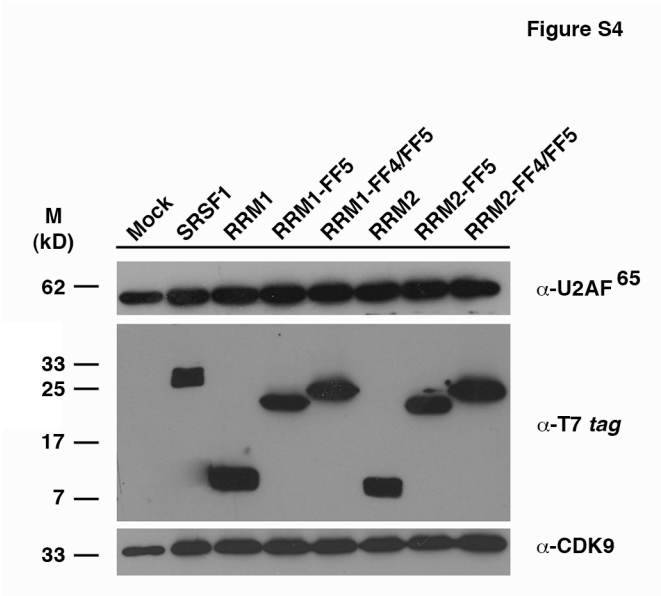

FIGURE S4. **Analysis of the expression levels by immunoblotting.** Cell lysates from transfected cells were analyzed by immunoblotting with the indicated antibodies to detect the TCERG1, $U2AF^{65}$, and CDK9 proteins. Numbers to the left of the gels indicate molecular masses in kDa.

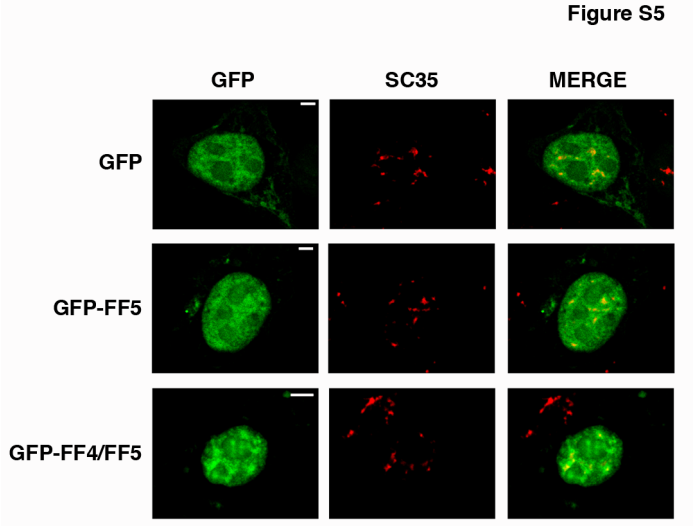

FIGURE S5. **The presence of the FF4/FF5 domains targets GFP to nuclear speckles.** GFP, GFP-FF5, and GFP-FF4/FF5 were expressed by transient transfection in HeLa cells and dually labeled with antibodies directed against the SC35 protein. In all cases, colocalization of expressed proteins with the endogenous marker was assessed by confocal microscopy. Bars, 3 μm.

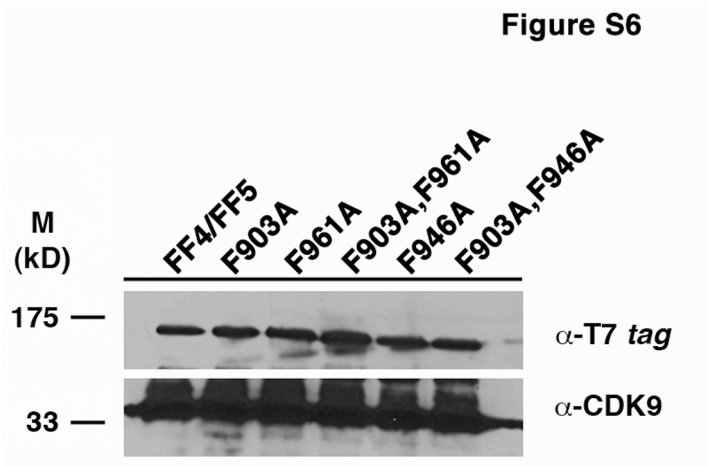

FIGURE S6. **Analysis of the expression levels by immunoblotting.** Cell lysates from transfected cells were analyzed by immunoblotting with antibodies against the T7-tag

and CDK9 to detect the TCERG1[1-662]-FF4/FF5 (FF4/FF5) and the indicated phenylalanine-to-alanine mutant constructs. Numbers to the left of the gels indicate molecular masses in kDa.