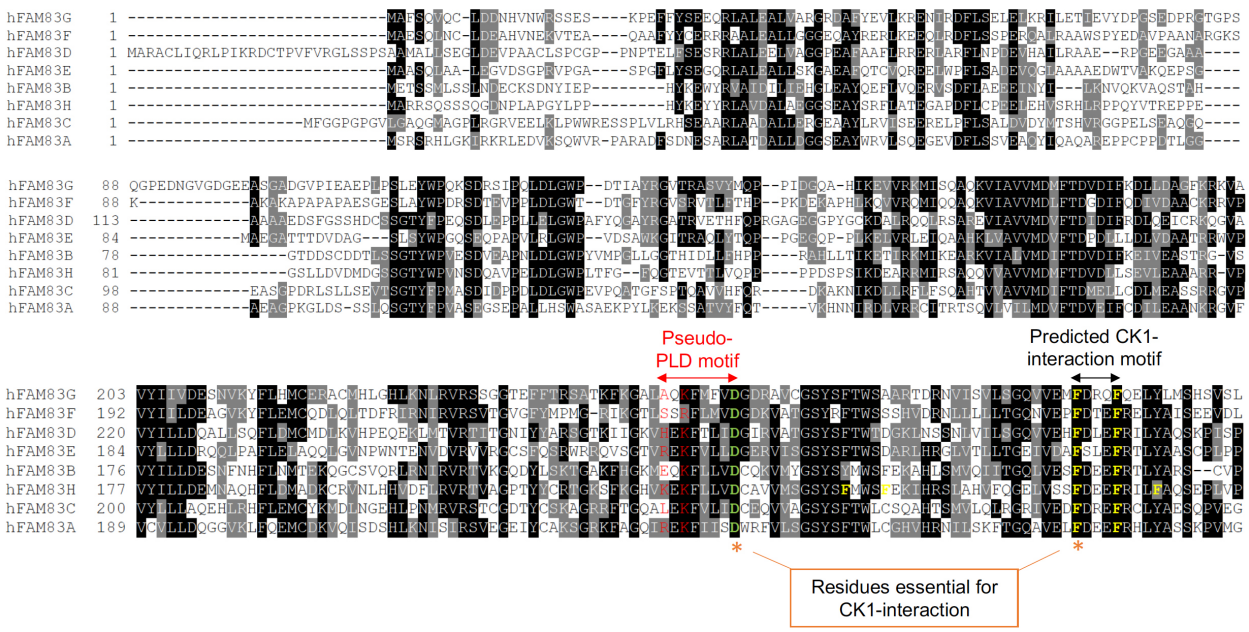

Figure S1. Sequence alignment of the DUF1669 domain of the FAM83 proteins.

Full sequence alignment of the DUF1669 domain indicating the putative pseudo-PLD catalytic motif, the location of predicted CK1-interaction FXXXF motifs (F in yellow), and the location of two residues essential for CK1-interaction (asterisks).

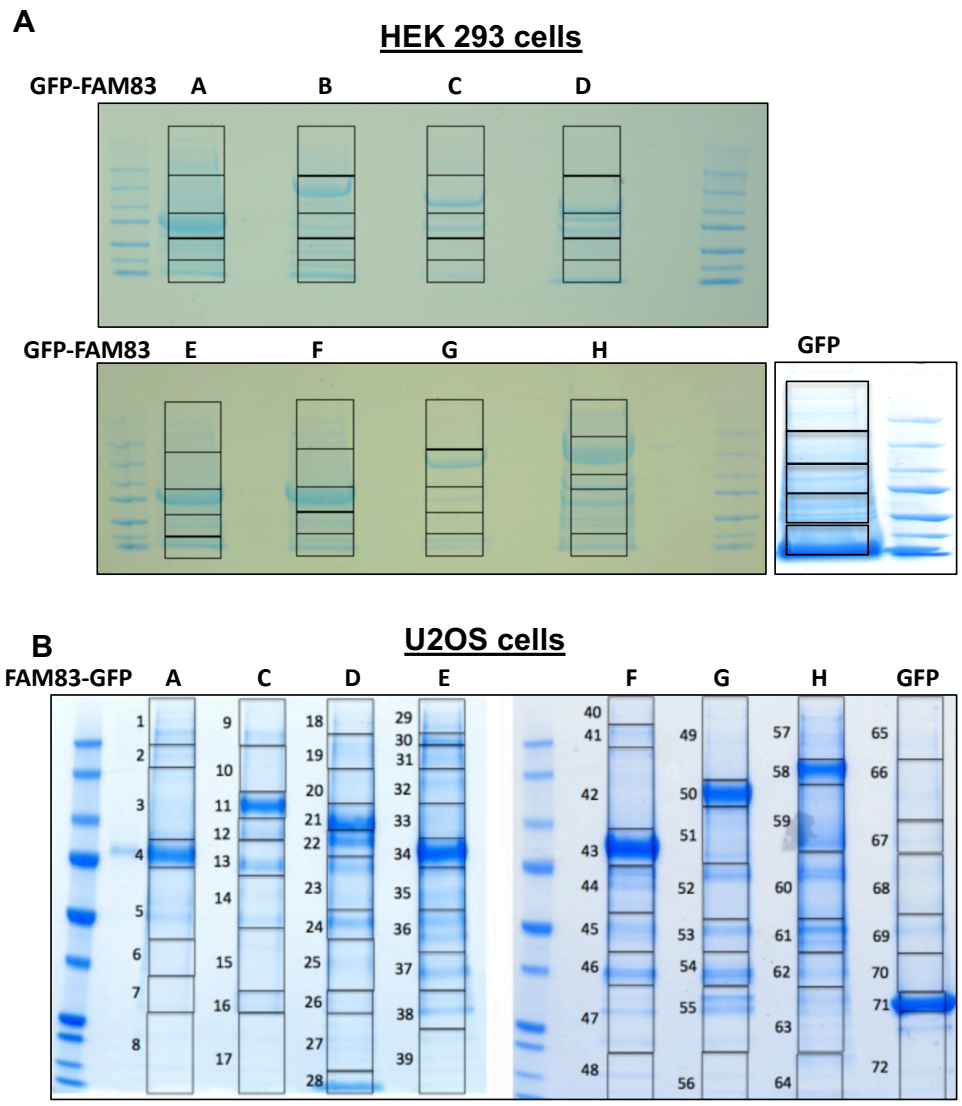

Figure S2. Coomassie images of GFP-TRAP immunoprecipitations of FAM83A-H proteins used to identify interacting partners by mass spectrometry.

(**A**) Extracts from doxycycline-treated (24 h) HEK 293 cells expressing N-terminally GFP-tagged FAM83A–H or GFP alone under the control of a Tet-inducible promoter were subjected to GFP trap immunoprecipitation and resolved by SDS-PAGE. The gels were Coomassie stained and imaged. The boxed regions in each lane represent the approximate excisions made in order to perform in-gel trypsin digestion and process the samples for protein identification by mass spectrometry. n=1. (**B**) As in A, except that C-terminally GFP-tagged FAM83A, FAM83C–H or GFP alone were expressed in U2OS cells, n=1.

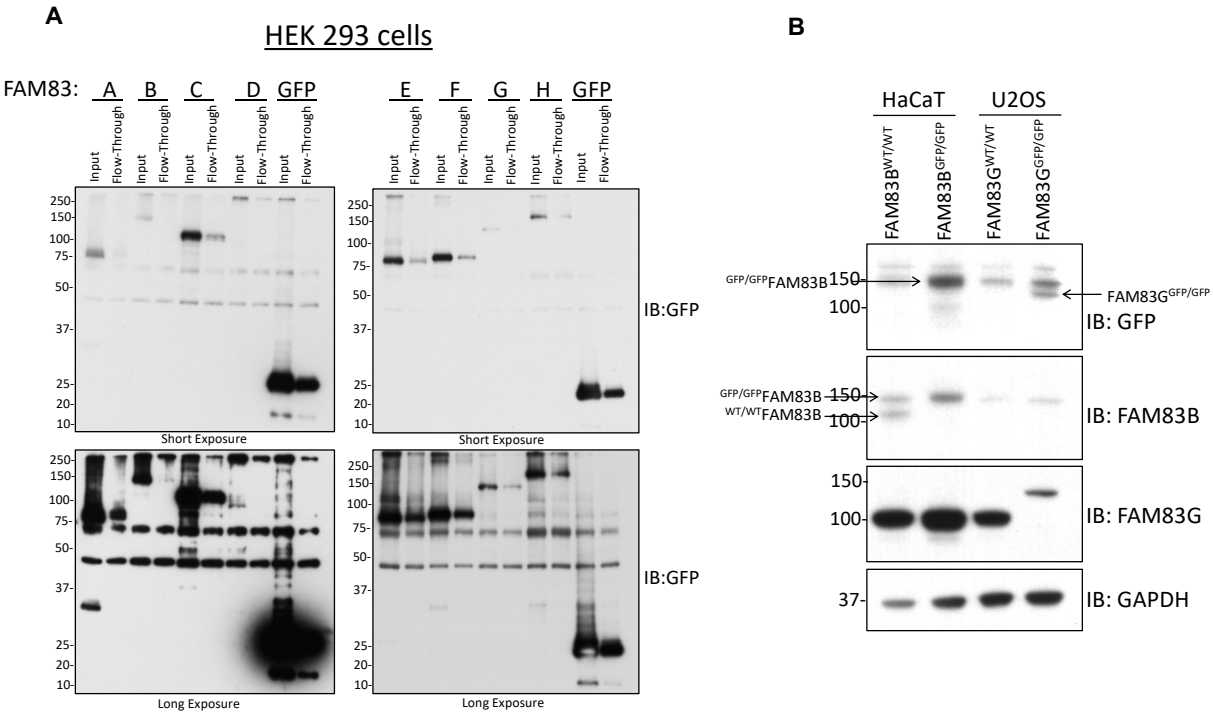

Figure S3. Immunoblots of controls for Figure 2.

(**A**) Cell extracts (Input) and flow-through following GFP immunoprecipitation were resolved by SDS-PAGE and immunoblotted with GFP antibodies. Short and long exposure images are shown. This blot is representative of 3 independent experiments. (**B**) Endogenous *FAM83B* and *FAM83G* genes were modified using CRISPR/Cas9 genome editing to insert GFP tags at the N terminus ($^{GFP/GFP}$FAM83B) or C terminus (FAM83G$^{GFP/GFP}$) of the gene. Cell extracts (Input) were resolved by SDS-PAGE and subjected to immunoblotting (IB) using the indicated antibodies. Cell extracts from unmodified cell lines (FAM83G$^{WT/WT}$) are included. GAPDH is a loading control. This blot is representative of 3 independent experiments.

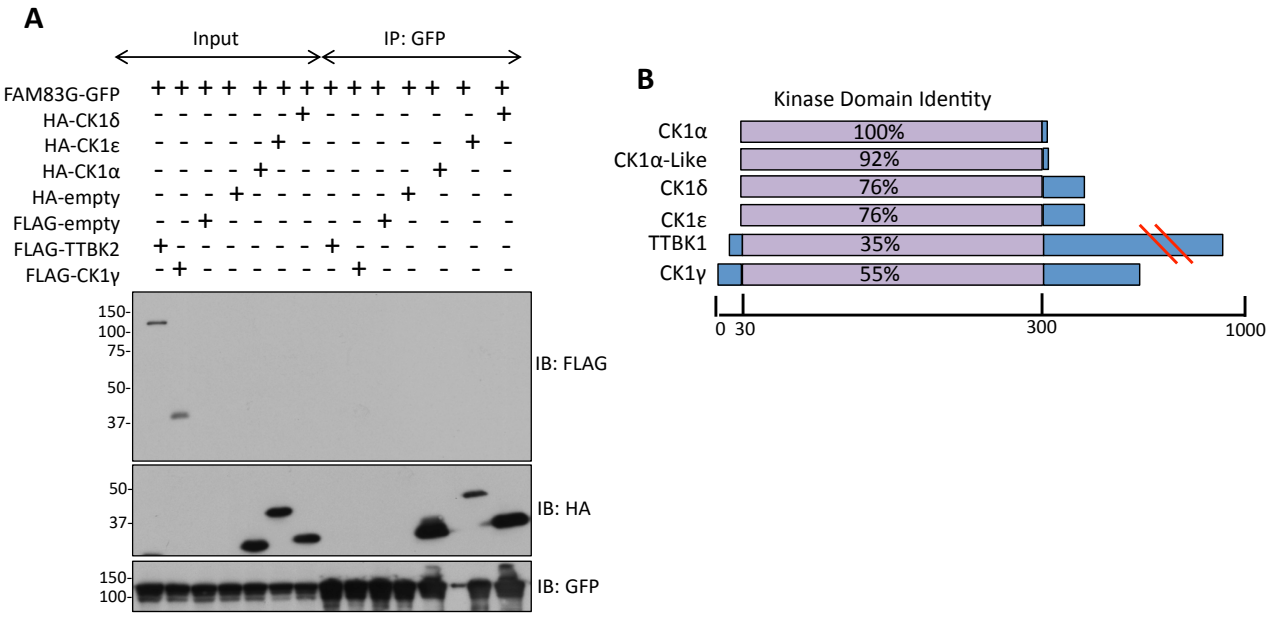

Figure S4. FAM83G interacts with CK1α, but not CK1γ or TTBK1.

(**A**) The indicated FLAG- or HA-tagged CK1 isoforms and the related kinase TTBK2 were co-expressed with FAM83G-GFP in U2OS cells. Cell extracts and GFP immunoprecipitations (IP) were resolved by SDS-PAGE and subjected to immunoblotting using the indicated antibodies. This blot is representative of 2 independent experiments. (**B**) Schematic highlighting the similarity of the kinase domains of various kinases of the CK1 kinase family. The percentage amino acid identity within the kinase domains of each kinase to that of CK1α kinase domain (aa 10-302) is indicated.

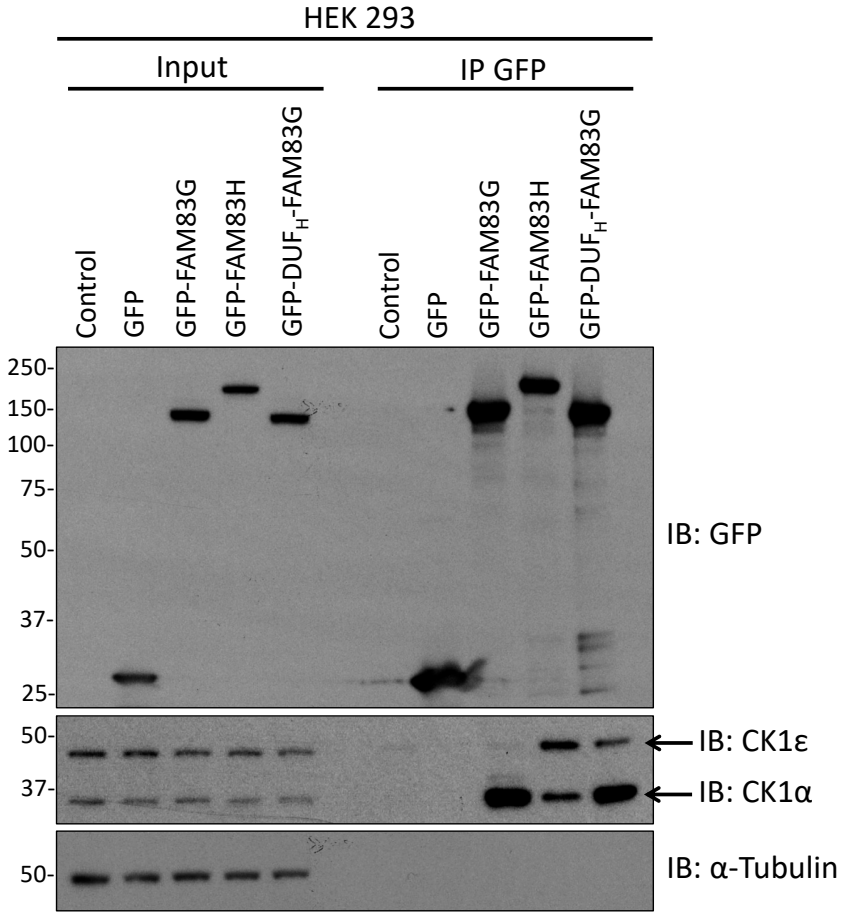

Figure S5. CK1-specificity switch with DUF1669 chimera.

The DUF1669 domain of FAM83G, which interacts with CK1α only, was replaced with the DUF1669 domain of GFP-FAM83H, which interacts with both CK1α and CK1ε. U20S cells were transfected with a construct encoding this chimeric protein (GFP-DUF$_H$-FAM83G) or a construct encoding wild-type GFP-FAM83G or GFP-FAM83H. Cell extracts (Input) and GFP immunoprecipitations (IP) were resolved by SDS-PAGE and subjected to immunoblotting using the indicated antibodies. This blot is representative of 3 independent experiments.

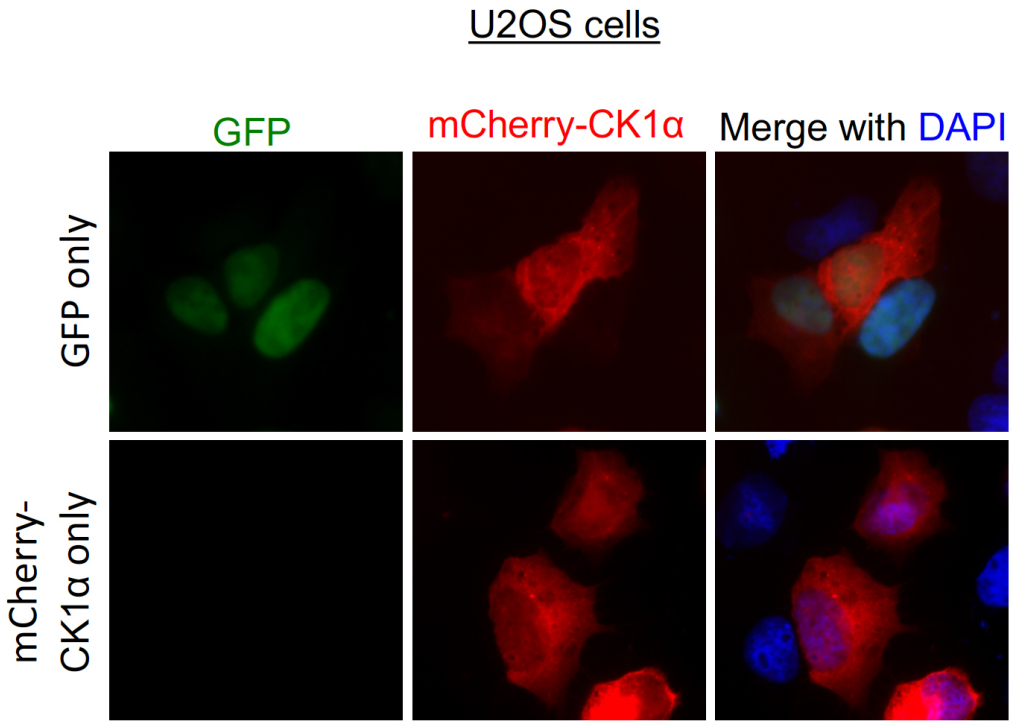

Figure S6. Fluorescence images of GFP and mCherry-CK1α controls.

U2OS cells stably integrated with Tet-inducible expression of GFP were transfected with mCherry-CK1α. Wild-type U2OS cells were transfected with mCherry-CK1α as a negative control. GFP expression was induced with doxycycline for 24 h prior to processing cells for fluorescence microscopy. DNA was stained with DAPI. Representative images from one field of view from 3 independent experiments are shown.

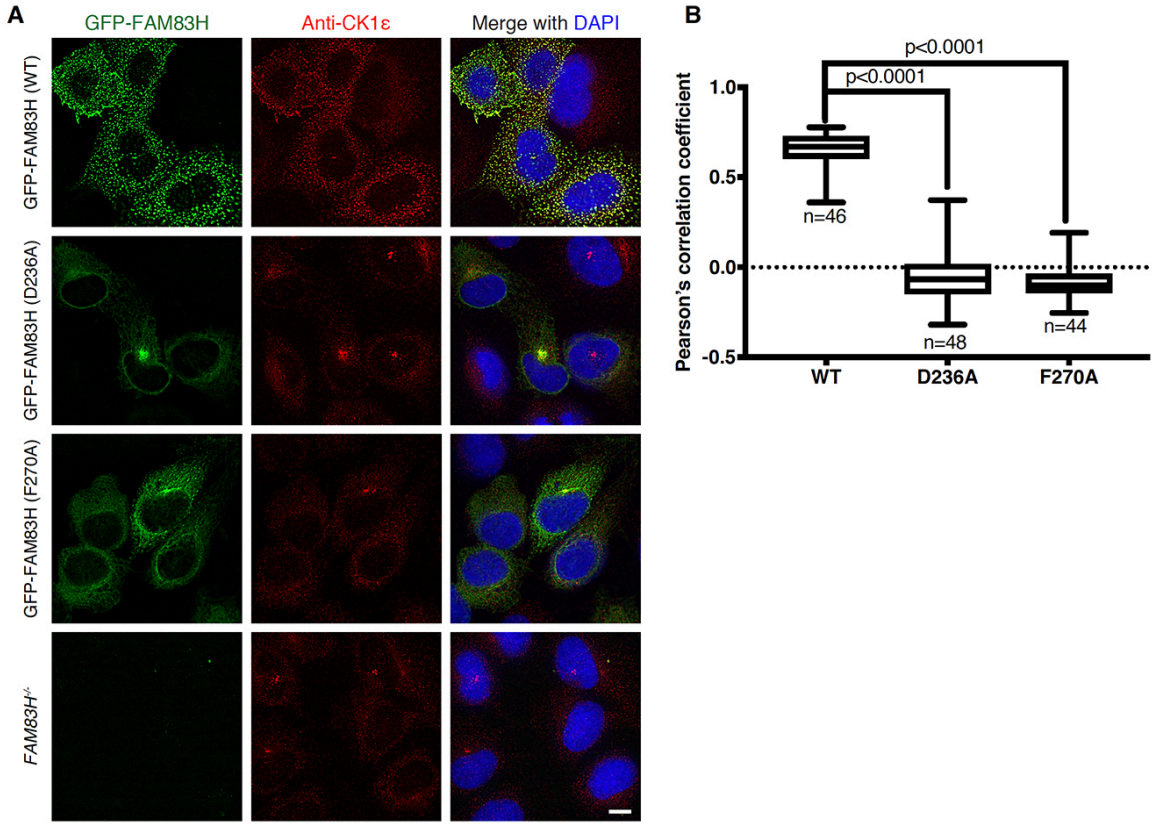

Figure S7. FAM83H co-localizes with and contributes to the subcellular localization of endogenous CK1ε.

(**A**) *FAM83H*$^{-/-}$ U2OS cells were transfected with vectors encoding GFP-FAM83H, GFP-FAM83H (D236A), or GFP-FAM83H (F270A). Untransfected cells are included as a control. Cells were processed for fluorescence microscopy with an antibody recognizing CK1ε. DNA was stained with DAPI. Representative images from one field of view from 3 independent experiments are shown. Scale bar, 10 µm. (**B**) The boxplot shows the range, mean, and lower and upper quartiles of the Pearson's correlation coefficients of GFP-FAM83H and endogenous CK1ε intensities within above-background pixels in the cytoplasm.

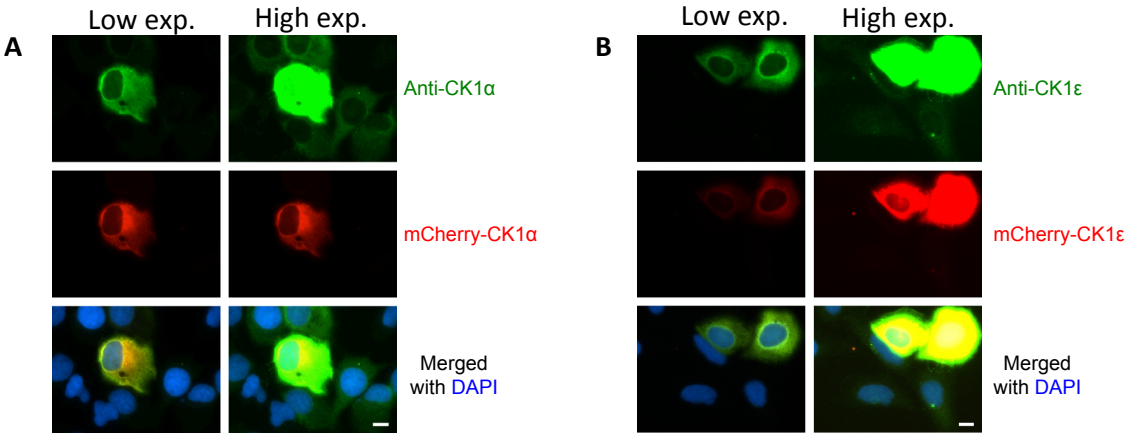

Figure S8. Validation of antibodies recognizing CK1α and CK1ε for immunofluorescence applications.

(**A**) U2OS cells were transfected with mCherry-CK1α. Cells were processed for fluorescence microscopy with the CK1α antibody. DNA was stained with DAPI. Representative low and high exposure (exp.) images from one field of view from 1 experiment are included. (**B**) As in A, except that U2OS cells were transfected with mCherry-CK1ε and stained with the CK1ε antibody.