

Supplemental Figure S1. Generation of functional cyclin E and Cdk2 protein

complementation fusion proteins. (A) Shown is a schematic of the orientation and predicted size of the IFP fusion proteins. We subcloned FLAG-tagged EL cDNA and truncated EL sequences representing the elastase-generated LMW-E isoforms (termed T1 and T2) into the IFPN expression vector. Cdk2 cDNA was subcloned into the complimentary IFPC expression vector. (B) To determine if the protein complementation constructs could generate fusion proteins, we used *in vitro* transcription and translation (TnT). TnT products were analyzed on western blots probed with GFP antibodies, which recognize the IFP fragments, and cyclin E or Cdk2 antibodies. T2-FLAG protein served as a control for mobility and antibody specificity. T2-FLAG is 40.4 KDa and was recognized by the cyclin E antibody but not the by GFP antibody. Both the cyclin E and GFP antibodies recognized the EL-, T1-, and T2-IFPN fusion proteins, which migrate at 66, 61.3, and 58.4 KDa, respectively. The Cdk2 and GFP antibodies recognized the 42 KDa IFPC-Cdk2 fusion protein. (C) We performed an *in vitro* histone H1 kinase assay to assess fusion protein functionality using whole cell lysates from 293T cells co-expressing cyclin E-IFPN with IFPC-Cdk2. Non-transfected cells (“Non-trans”) and expression of IFPC-Cdk2 alone were used as negative controls.

Supplemental Figure S2: Cyclin E-IFPN/IFPC-Cdk2 localization is specific to

cyclin E/Cdk2 interaction. Shown are representative images of 293T cells co-transfected with EL-IFPN/IFPC-Cdk2, IFPN-AKT1/PDK1-IFPC, EL-IFPN/PDK1-IFPC, or IFPN/IFPC cloning vectors. Nuclei are DAPI-stained blue. Images were taken using a 20X objective and merged. Scale bar = 10 μ m.

Supplemental Figure S3: Cytoplasmic cyclin E-IFPN/IFPC-Cdk2 localization may render the LMW-E isoforms less susceptible to Fbw7-mediated degradation. The bar graph shows the percentage of green fluorescent 293T cells that show cyclin E-IFPN/IFPC-Cdk2 signal in the cytoplasm/perinuclear membrane, the nucleus, or in both subcellular compartments. A total of $386 \leq n \leq 767$ green fluorescent cells over 5-10 microscopic fields were counted for each condition. Error bars represent the standard deviation of three biological replicates. The images shown represent cells expressing IFP in the cytoplasm/perinuclear membrane, nucleus, or both subcellular compartments. Nuclei are DAPI-stained blue. Images were taking using a 20X objective and merged. Scale bar = 5 μ m.