SUPPLEMENTAL DATA

Supplemental Table 1 - Interactome of FBXO25. List of proteins associated an FBXO25 identified in MS analyses.

Supplemental Figure 1 - Purified proteins were visualized by Coomassie Blue staining. The bands taken for mass spectrometry analysis are indicated.

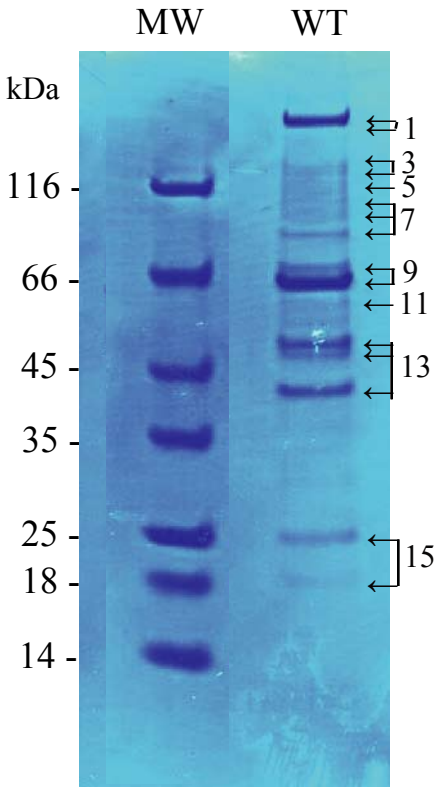
Supplemental Figure 2 - HEK293T cells were transient transfected with Skp1, CUL1, Roc1 and FBXO25. Cell nuclear extracts were generated by standard cell fractionation [10]. Nuclear fractions were subjected to immunoprecipitation with anti-β-actin antibodies, and then the FBXO25 protein was detected by using anti-FBXO25 antibodies. The membrane was stripped and reprobed with anti-actin antibodies.

Supplemental Figure 3 - Confocal microscopy of labeled FBXO25 in untreated and latrunculin-A-treated (0,5 μg/ml for 2 h) HeLa cells are shown. Without latrunculin-A treatment, FANDs were found in the nucleoplasm (Ai–Aiii). In the presence of latrunculin -A, the majority of endogenous (Bi–Biii) FANDs disappeared (B). (C) Proportion of cells containing FANDs was estimated. After treatment with latrunculin-A, the proportion of cells containing FANDs (red) significantly differs from the control population. Four separate experiments were performed, and around 100 cells were analyzed for latrunculin-A treatment.

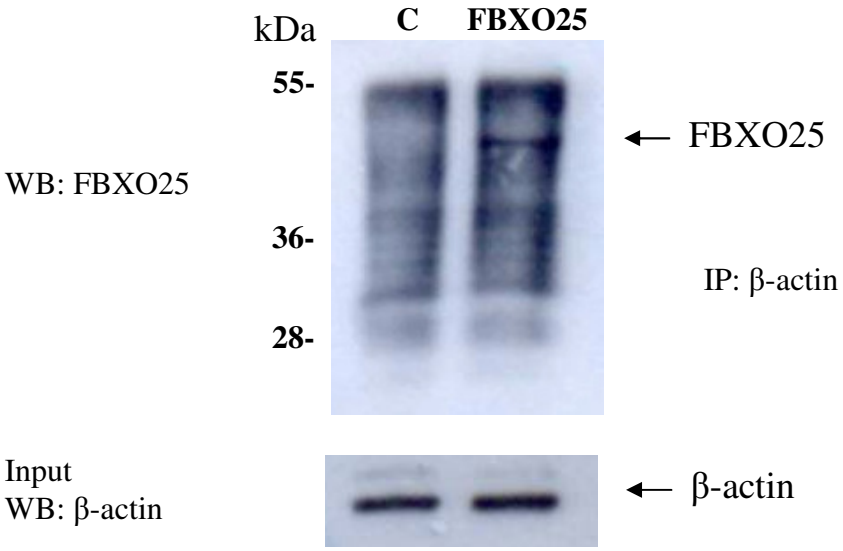
Supplemental Figure 4 - Confocal microscopy of labeled PML (green) in untreated and cytochalasin-D-treated (5 μg/ml for 24 h) HeLa cells are shown. After treatment with cytochalasin-D, the pattern of PML not differs from the control population.

Supplemental Figure 5 - Analysis of nuclear β-actin degradation in the presence of FBXO25. HEK293T cells were transient transfected with Skp1, CUL1, Roc1 in combination with full-length wild-type (WT) or mutant version of FBXO25 (ΔF), and incubated in the presence of DMSO (-) or 10 μM of MG132 for 12 hs (+) before harvesting. Cell nuclear extracts were generated by standard cell fractionation [10]. (A) Fifty micrograms of nuclear proteins were subjected to western blotting with anti-β-actin antibodies. (B) Western blotting (WB) analysis of nuclear proteins immunoprecipitated (IP) using antibodies to β-actin. Note that FBXO25 does not affect the level of endogenous β-actin.

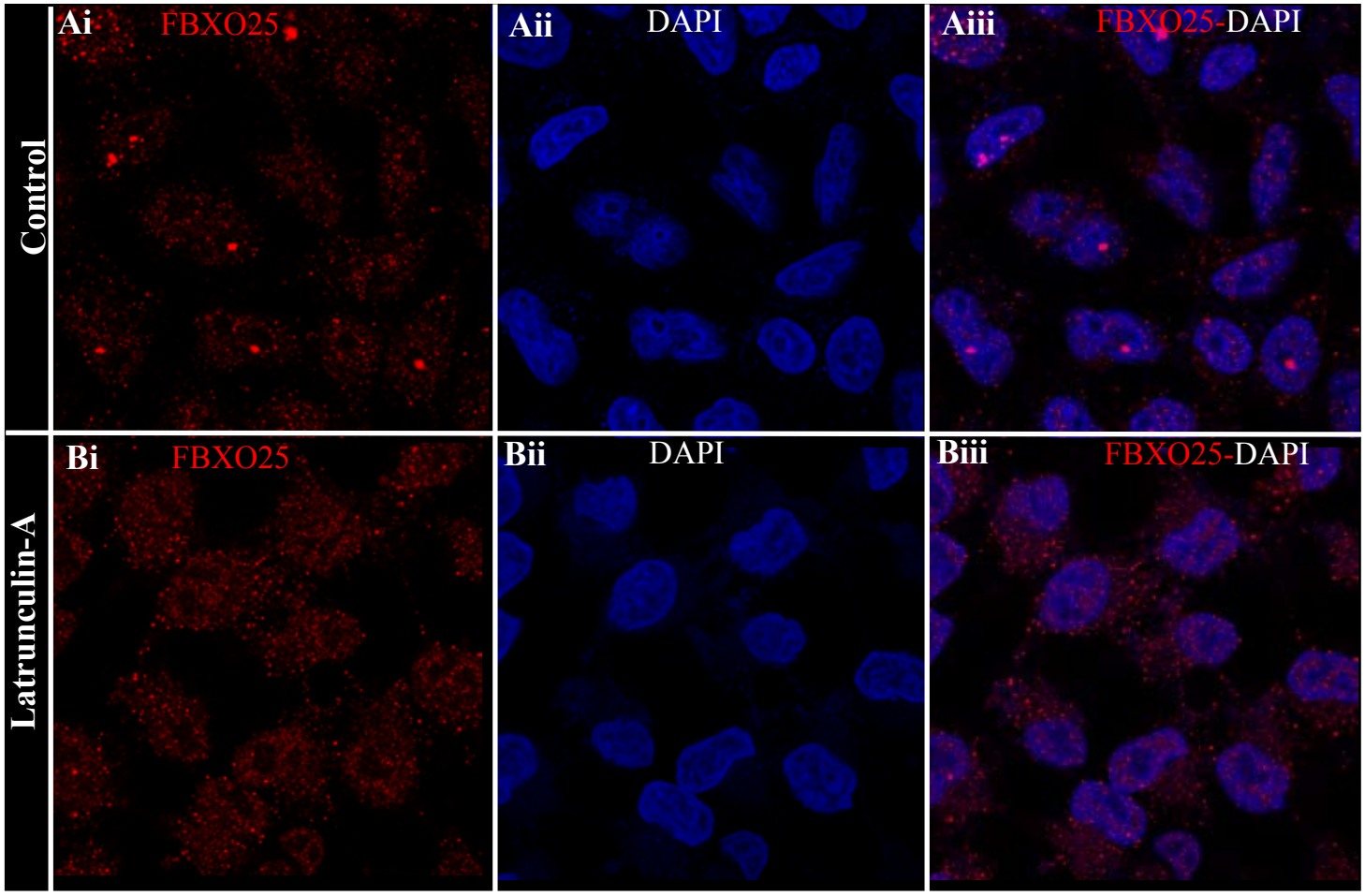
Supplemental Figure 1

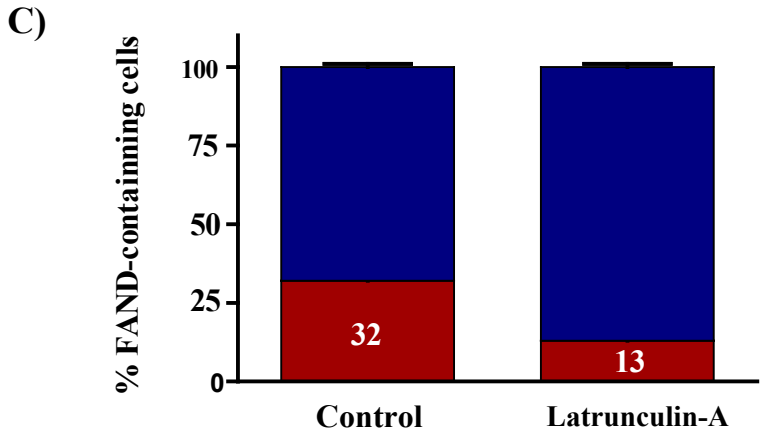


Supplemental Figure 2

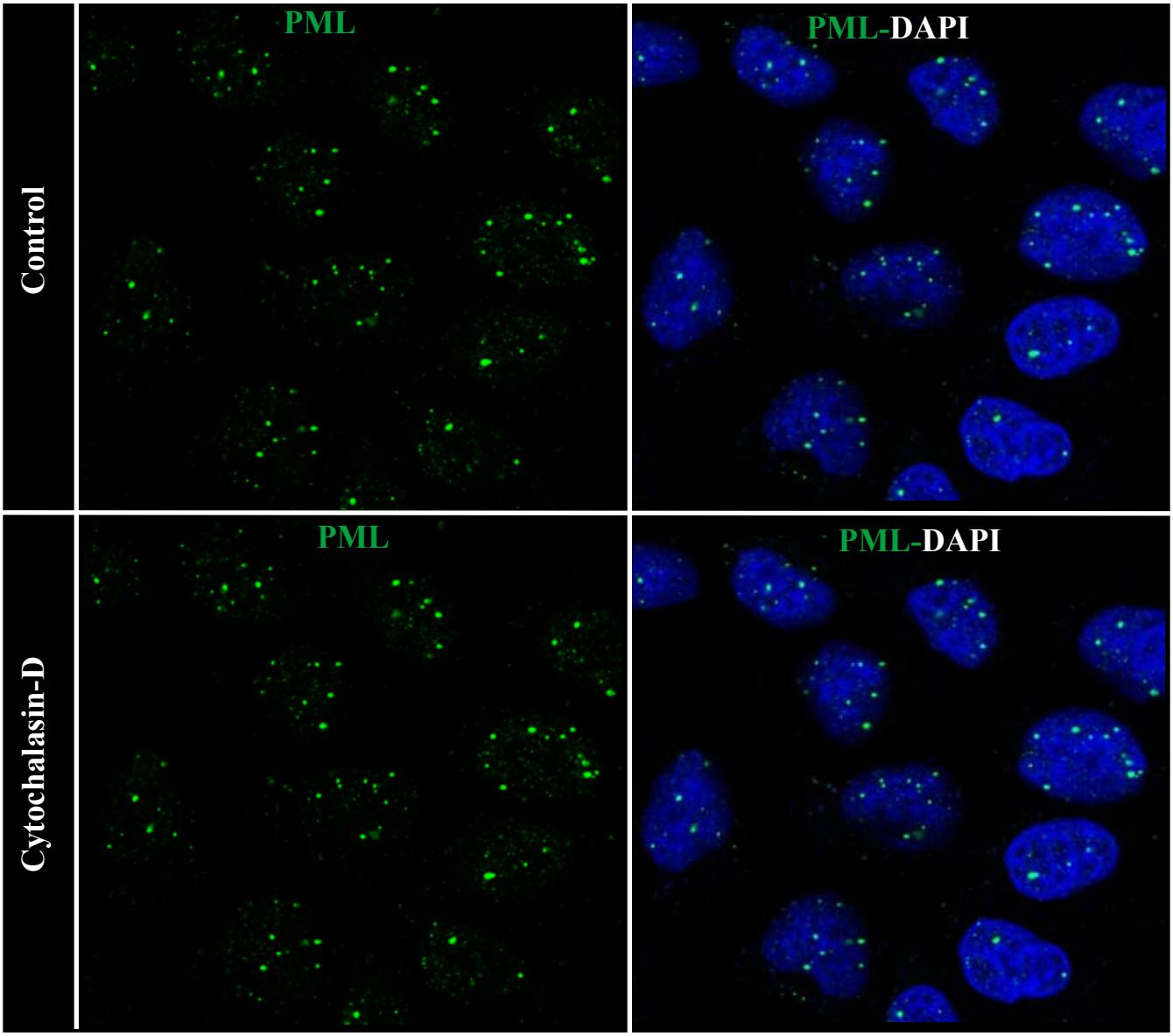


Supplemental Figure 3





Supplemental Figure 4



Supplemental Figure 5