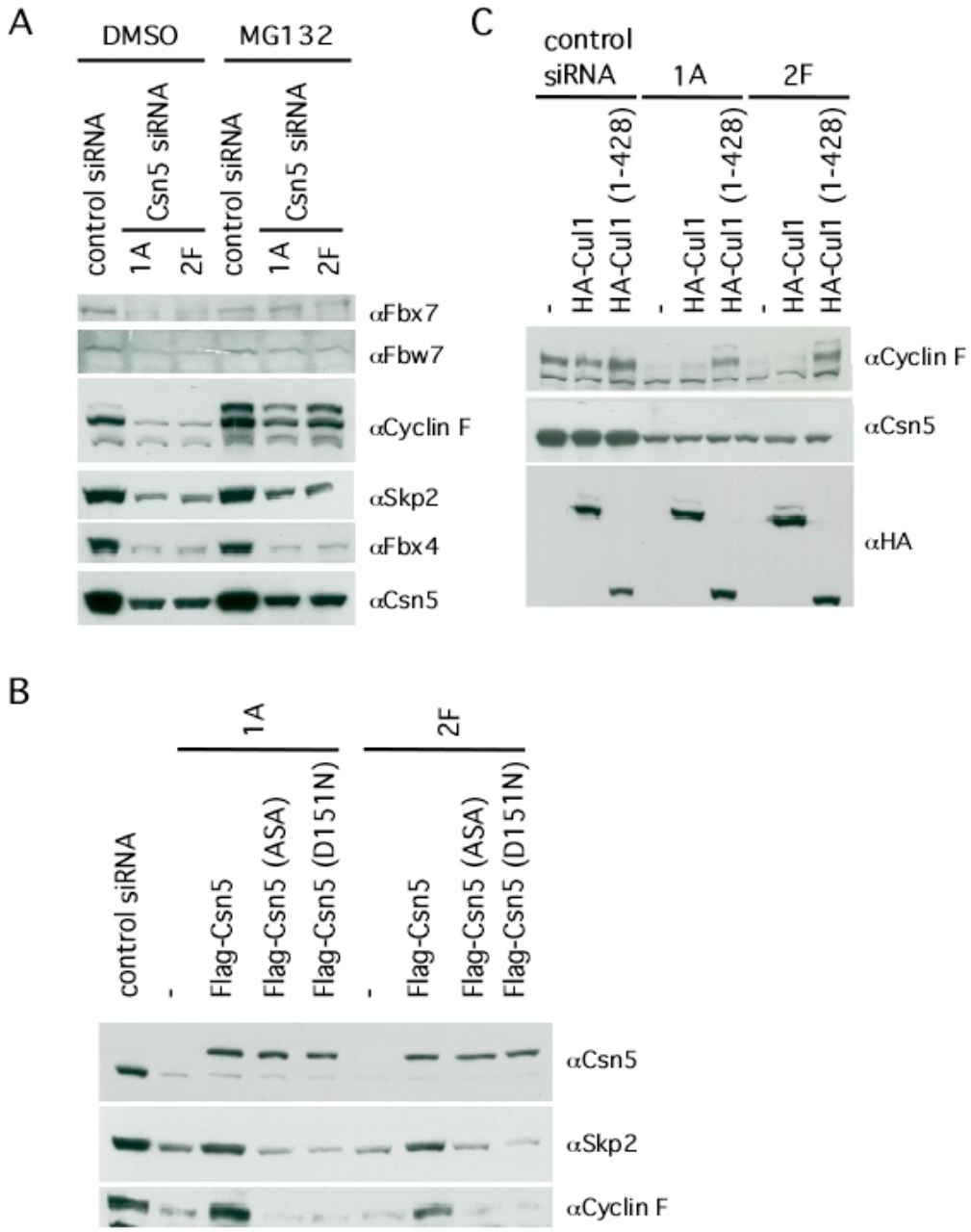

Figure 3

Loss of Csn5 results in proteasome-dependent turnover of F-box proteins. (A) Supplementation of doxycycline-treated cells with MG132 inhibits the turnover of F-box proteins. Cells were treated with doxycycline for eight days followed by treatment with MG132 (25 μM) for eight hours. Cells were lysed in SDS and analyzed by SDS-PAGE/western blot for the indicated proteins. **(B)** Ectopic expression of wild-type, but not JAMM point mutant Csn5, can rescue the decrease in protein levels of Skp2 and cyclin F. Cells were treated with doxycycline for six days, followed by transient transfection with wild-type Flag-Csn5, Flag-Csn5 (ASA), or Flag-Csn5 (D151N). Forty-eight hours post-transfection, cells were lysed in SDS and analyzed by SDS-PAGE/western blot for the indicated proteins. **(C)** Ectopic expression of a dominant-negative Cul1 restores cyclin F protein levels. Control, wild-type, or Cul1 (1–428) expression plasmids were transfected into cells induced with doxycycline for six days. Forty-eight hours post-transfection, cells were lysed in SDS and analyzed by SDS-PAGE/western blot with antibodies against the indicated proteins.