

**Figure I Suppression of Csn5 in HEK 293 human cells. (A)** Expression of Csn5-specific shRNA suppresses endogenous Csn5 mRNA levels. Cells induced with doxycycline for 8 days were harvested and analyzed by RT-PCR using oligos specific for Csn5. **(B)** Expression of Csn5-specific shRNA suppresses endogenous Csn5 protein levels. Cells induced with doxycycline for 8 days were harvested and lysed. Lysates were analyzed by western blot using antibodies specific toward human Csn5. **(C)** Silencing of Csn5 does not appreciably affect the levels of other CSN subunits. Cells induced with doxycycline for 8 days were harvested, and cell lysates were analyzed by western with the antibodies indicated. **(D)** Silencing of Csn5 results in altered cullin neddylation. Cells were induced with doxycycline for 8 days and harvested. Cells were lysed and analyzed by western blot using the antibodies indicated.