Supplementary Figures and Legends

The F-Box protein FBXO7 positively regulates bone morphogenetic protein-mediated signaling through Lys-63-specific ubiquitination of neurotrophin receptor-interacting MAGE (NRAGE)

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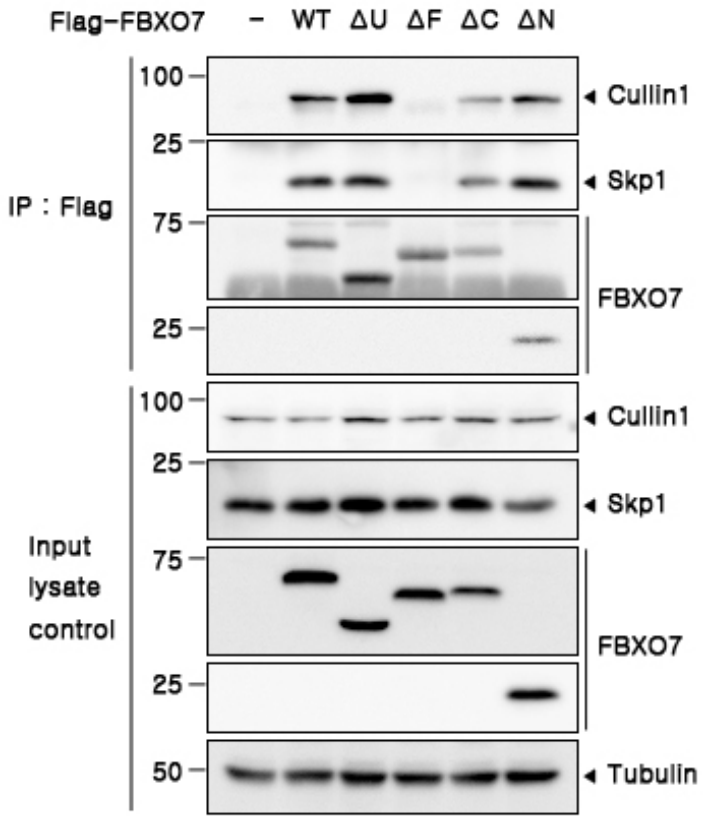
Supplementary Figure S1



Supplementary Figure S1: **Various FBXO7 deletion mutants form the SCF complex except FBXO7-ΔF mutant.** HEK293 cells were transfected with Flag-tagged wild type FBXO7 or one of its deletion mutants, as indicated. Immunoprecipitation of HEK293 cell lysates was performed with anti-Flag antibody, followed by immunoblotting with anti-Skp1 and anti-cullin1 antibodies. Proper expression of various FBXO7 deletion mutants, endogenous Skp1, and endogenous cullin1 in cell extracts was determined by immunoblotting with each antibody. To determine equal protein loading, cell lysates were analyzed by immunoblotting with anti-tubulin antibody.

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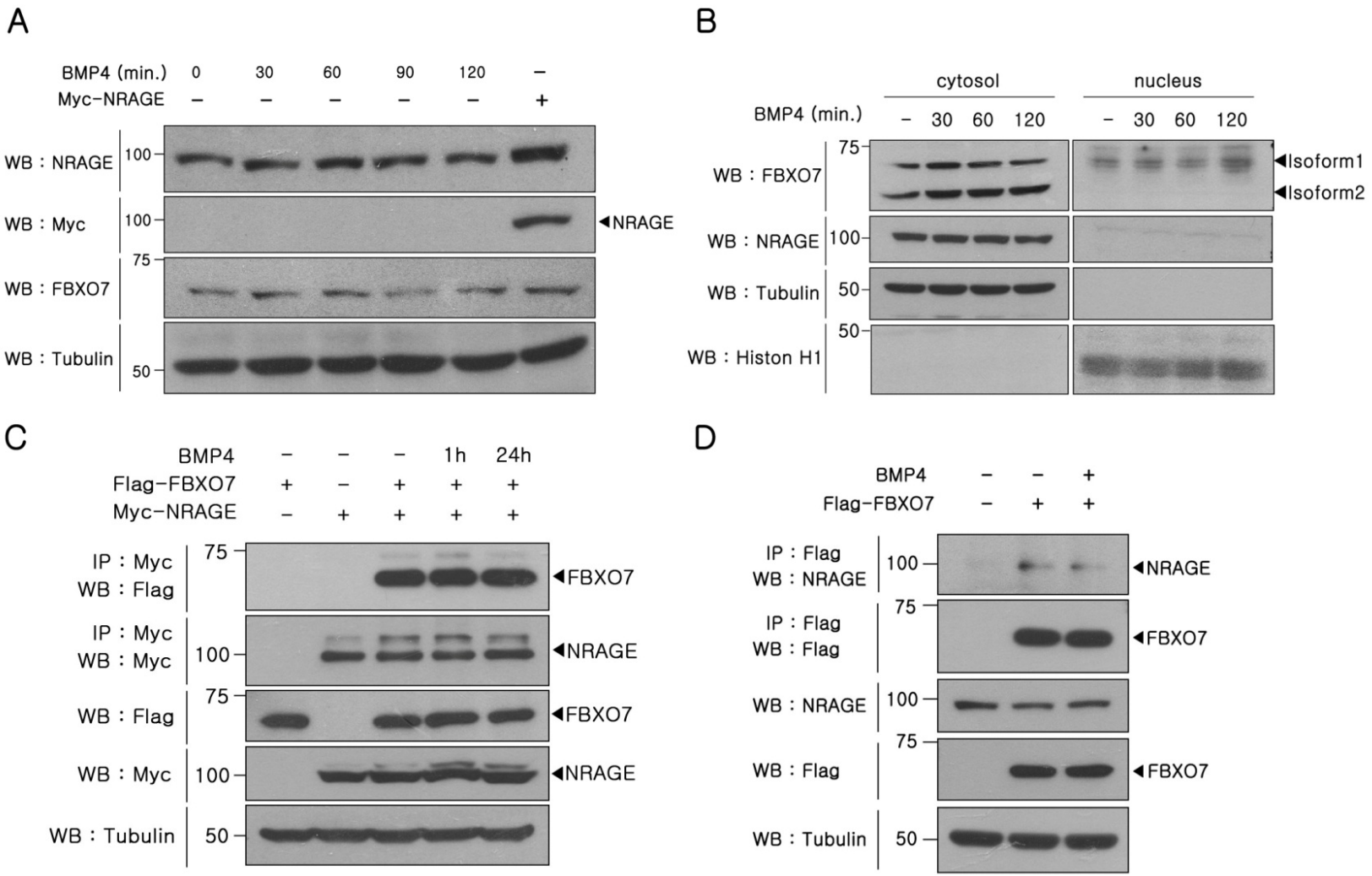
Supplementary Figure S2
(see the legend at the next page)



Supplementary Figure S2: **BMP4 does not affect the stability, subcellular localization, or binding affinity of FBXO7 and NRAGE. (A)** Where indicated, HEK293 cells were transfected for 24 hr with Myc-tagged NRAGE and treated with 10 ng/ml BMP4 for the indicated times. Endogenous levels of NRAGE and FBXO7 in cell lysates were analyzed by immunoblotting with their specific antibodies. Immunoblotting analyses with the anti-Myc antibody were performed to verify NRAGE overexpression in the last control lane. **(B)** HEK293 cells were treated with 10 ng/ml BMP4 for the indicated times, and cell lysates were fractionated into cytosolic and nuclear fractions. Immunoblot assays were performed using anti-NRAGE and anti-FBXO7 antisera. To determine equal protein loading and evaluate the purity of each fraction, samples were immunoblotted with anti-tubulin (cytosolic fraction) and anti-histone H1 (nuclear fraction) IgG, respectively. **(C)** HEK293 cells were transfected for 24 hr with plasmids encoding Flag-tagged FBXO7 and/or Myc-tagged NRAGE, and treated with 10 ng/ml BMP4 for the indicated times. Immunoprecipitation of the cell lysates was performed with anti-Myc IgG, followed by immunoblotting with the anti-Flag antibody. Proper expression of transiently transfected proteins in the cell lysates was examined using their antisera, respectively. **(D)** Where specified, HEK293 cells were mock-transfected or transfected for 24 hr with Flag-tagged FBXO7, and treated with 10 ng/ml BMP4 for additional 24 hr. Immunoprecipitation assays were performed with the Flag antibody, followed by immunoblotting with the NRAGE antibody. To determine equal loading, cell lysates were analyzed by immunoblotting with the anti-tubulin antibody.

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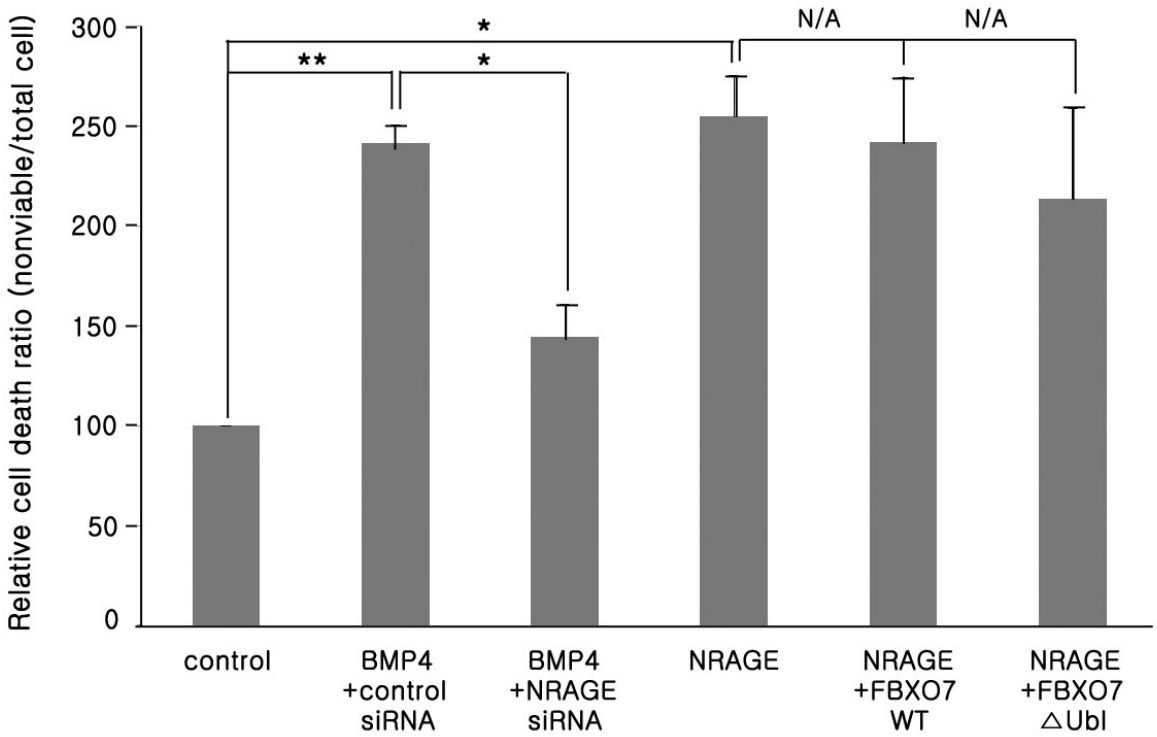
Supplementary Figure S3



Supplementary Figure S3: **FBXO7 does not alter NRAGE-mediated P19 cell apoptosis in response to BMP4.** P19 cells were mock-transfected or transfected with Myc-NRAGE alone or in combination with *NRAGE*-specific siRNAs, control siRNAs, Flag-tagged wild type FBXO7, or FBXO7-ΔU, as indicated. Cells were left unstimulated or treated with 10 ng/ml BMP4 for 24 hr, as indicated. The P19 cell death ratio was measured using trypan blue exclusion assays. Relative cell death ratios were calculated by dividing the number of nonviable cells by total cells. Data are presented as the mean ± SD (n =3, * p <0.05; ** p <0.01). N/A indicates 'not applicable'.