Nuclear Export of Smads by RanBP3L Regulates BMP Signaling and Mesenchymal Stem Cell Differentiation

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Running title: RanBP3L is a nuclear export factor for Smad1/5/8

SUPPLEMENTAL MATERIAL

FIG S1 RanBP3L inhibits BMP-induced transcriptional responses.
(A) RanBP3L inhibits BMP2-induced SBE-OC-luc reporter activity in C3H10T1/2 cells. C3H10T1/2 cells were transfected with empty vector, RanBP3L, PPM1A or RanBP3, together with SBE-OC-luc reporter plasmid. BMP2 treatment and luciferase assays were done as described in "Materials and Methods".
(B) Stable expression of RanBP3L inhibits BMP-induced Id1-luc reporter activity in C2C12 cells. C2C12-RanBP3L-OE and control cells were transfected with luciferase reporter plasmids as described in Fig. 1D.
(C) HaCaT cells were transfected with indicated siRNAs. Cells were treated with or without TGF-β for 12h. Cell lysates were measured by Western blotting analysis with indicated antibodies. GAPDH serves as a loading control.

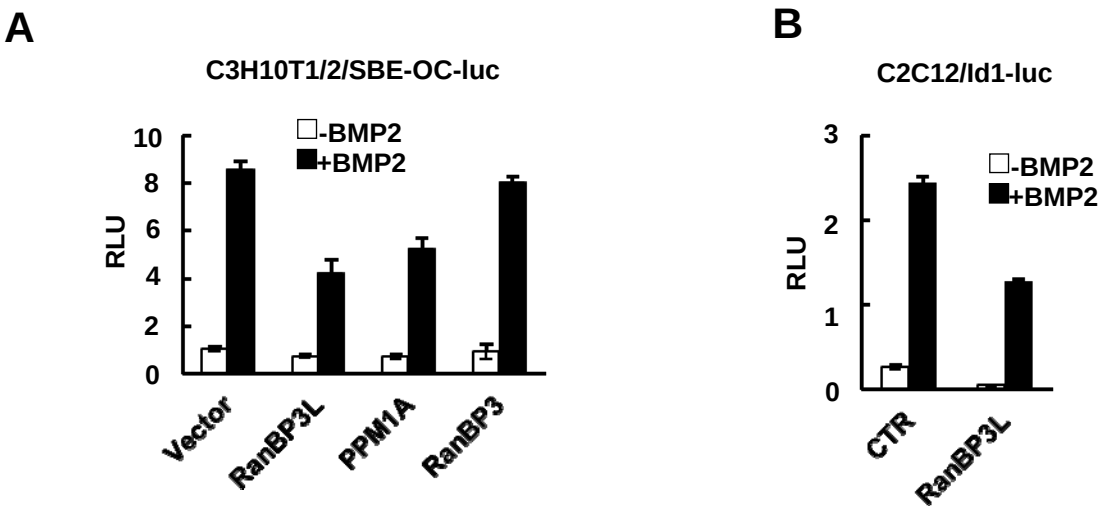
FIG S2 RanBP3L blocks BMP-induced osteogenesis in BMSCs.
(A) Lentivirus infection efficacy in mouse BMSCs.
(B) Alizarin Red staining of mouse BMSCs. BMSCs were cultured in osteogenic differentiation medium, and stained with Alizarin Red at 24 d after differentiation induction.
(C) RanBP3L knockdown efficacy in BMSCs. Relative RanBP3L mRNA levels at the 5th day after siRNA transfection were detected by qRT-PCR.

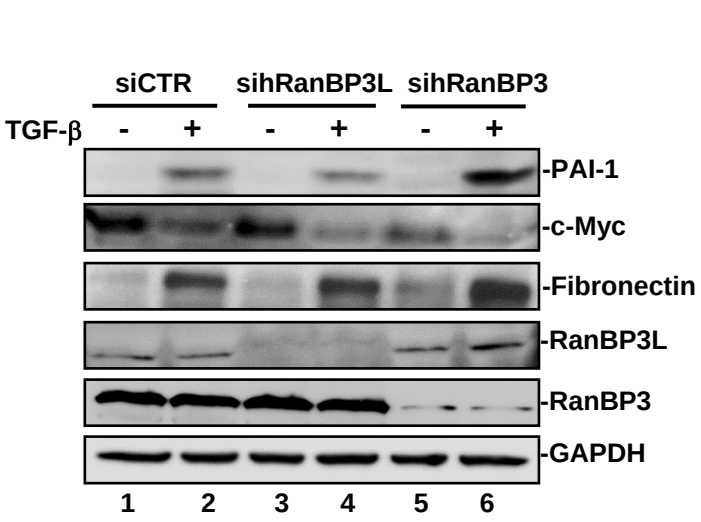
FIG S3 RanBP3L binds to Smad1/5/8.
(A) RanBP3L binds to Smad1, 5, 8 *in vivo*. Co-immunoprecipitation of HA-RanBP3L and FLAG-Smads was done in HEK293T. Levels of these proteins in IP products and whole cell lysates were analyzed by Western blotting.
(B) RanBP3L binds to the MH1 and MH2 domains of Smad1. HEK293T were transfected with HA-RanBP3L and indicated FLAG-Smad1 truncation mutants. Experiments were performed as described in Fig. S4A.
(C) PPM1A enhances the interaction between RanBP3L and Smad5. HEK293T cells were transfected with HA-RanBP3L, FLAG-Smad5 and Myc-PPM1A. Cell lysates were immunoprecipitated with HA antibody. IP products and whole cell lysates were analyzed by Western blotting.
(D) PPM1A enhances the interaction between RanBP3L and Smad8. HEK293T cells were transfected with indicated plasmids. Levels of these proteins in IP products and whole cell

lysates were analyzed by Western blotting.

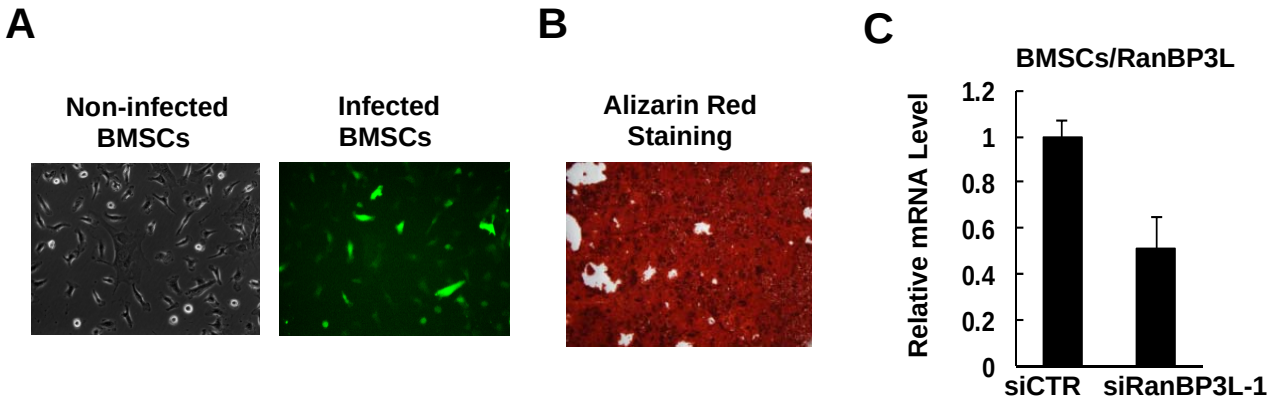
FIG S4 RanBP3L promotes Smad1 nuclear export.
(A) Overexpression of RanBP3L in C3H10T1/2 cells enhances Smad1 nuclear export. C3H10T1/2 cells were transfected with RanBP3L and treated with BMP2 (50 ng/ml) for 4h. Cells were fixed and immune-stained with anti-Smad1 and anti-FLAG antibodies. DNA was stained with DAPI.
(B) A schema of *in vitro* export assay (Fig. 6E).
(C) RanBP3L has no effects on Smad2/3 nuclear export in HaCaT cells. HaCaT cells were first transfected with siRNA target RanBP3, then transfected with HA-RanBP3L and treated with TGF-β (2 ng/ml) for 2 h. Cells were fixed and immune-stained with anti-Smad2/3 and anti-HA antibodies. DNA was stained with DAPI.
(D) Knockdown of RanBP3L increases the level of nuclear Smad1. C2C12 cells were transfected with indicated siRNA. Nuclear-cytosol fractions were isolated and detected as described in “Materials and Methods”. GAPDH serves as a cytosol marker, and Lamin A/C serves as nuclear markers.
(E) HaCaT cells were transfected with siRNAs targeting RanBP3L and RanBP3. Cells were treated with or without TGF-βfor 2h. Cells were washed 3 times with PBS to remove cytokines and then treated with SB431542 for 30 min before fixation. Smad2/3 wereinmmuno-stained with antibodies and images were acquired by confocal microscope.

Supplemental Data
Figure 1

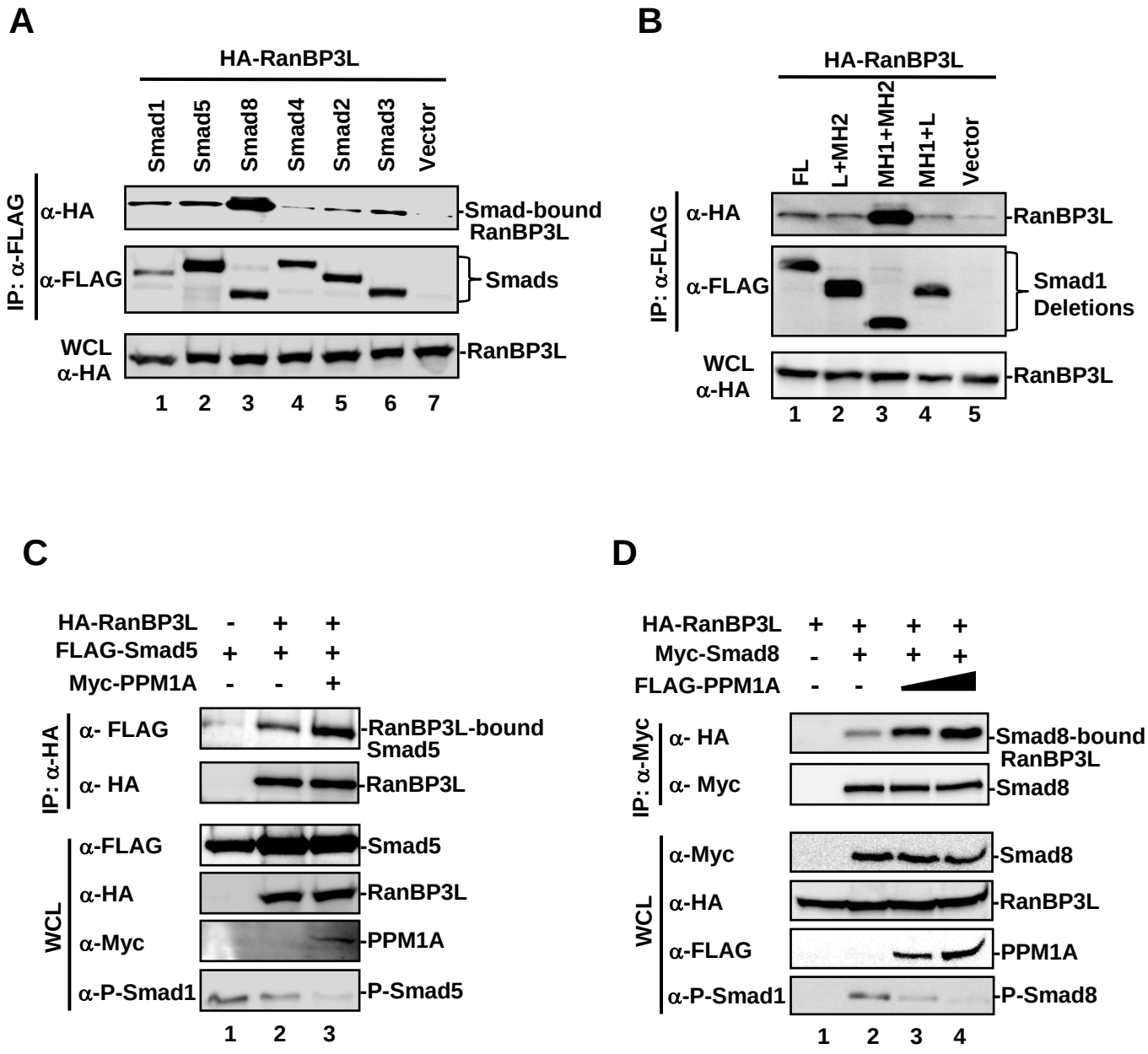




Supplemental Data
Figure 2



Supplemental Data
Figure 3



Supplemental Data
Figure 4