## TNF-α inhibits SATB2 expression and osteoblast differentiation through NF-κB and MAPK pathways

## SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Adv-BMP2 induces C2C12 cells to differentiate into osteoblasts and promotes SATB2 expression. The C2C12 cells were treated with Adv-BMP2 (150 pfu/cell) or Adv- $\beta$ -Gal (150 pfu/cell) for five days (A) The *Col I*, *Bsp*, and *Ocn* mRNA levels were examined. The ALP activity was normalized to the Adv- $\beta$ -Gal group, and (B) ALP staining was performed, (C) The C2C12 cells were treated with Adv-BMP2 (150 pfu/cell) or Adv- $\beta$ -Gal (150 pfu/cell) for various time points and the *SATB2* gene expressions were assessed via real-time PCR. The fold change was calculated as follows: (relative *Col I*, *Bsp*, *Ocn*, *SATB2* levels in Adv- $\beta$ -Gal-treated sample), the data were presented as mean  $\pm$  S.D. (n = 3; \*p < 0.05; \*p < 0.01).



**Supplementary Figure 2: TNF-α inhibits SATB2 expression by inhibiting the smad1/5/8 signaling pathway.** Uncropped western blot images corresponding to Figure 4(A), Figure 4(C) and Figure 4(E).

Adv-β-Gal		-	-	+	+	-	-	-	-		
Adv-BMP-2		+	+	-	-	+	+	+	+		
TNF-α (ng/m	)	-	-	30	-	30	-	30	30		
U0126(µM)		10	30	-	-	-	-	10	30		
SATB2		-	-	1	-	-		 -		—83 I	KD

**Supplementary Figure 3: TNF-a inhibits SATB2 expression by activating the MAPK signaling pathway.** Uncropped western blot images corresponding to Figure 5C.



Supplementary Figure 4: TNF- $\alpha$  inhibits SATB2 expression by activating NF- $\kappa$ B. Uncropped western blot images corresponding to Figure 6E.



**Supplementary Figure 5: TNF-α induced NF-κB directly interacts with SATB2 gene promoter to inhibit its expression.** Uncropped RT-PCR images corresponding to Figure 7D.