

Figure S1. Results of RT-qPCR analysis of miR profiling-indicated miRNAs in patients with BAV and TAV. RT-qPCR analysis on the expression of (A) miR-574, (B) miR-887, (C) miR-3158, (D) miR-1228, (E) miR-215, (F) miR-3149, (G) miR-32, (H) miR-3610, (I) miR-4455, (J) miR-451, (K) miR-5001, (L) miR-6068, (M) miR-6081, (N) miR-6125, and (O) miR-6847 in aortic valve tissues from patients with BAV and TAV (n=15 per group). Student's t-test was performed. Data are presented as the mean \pm standard deviation. miR, microRNA; BAV, bicuspid aortic valve; TAV, tricuspid aortic valve; RT-qPCR, reverse transcription-quantitative PCR.

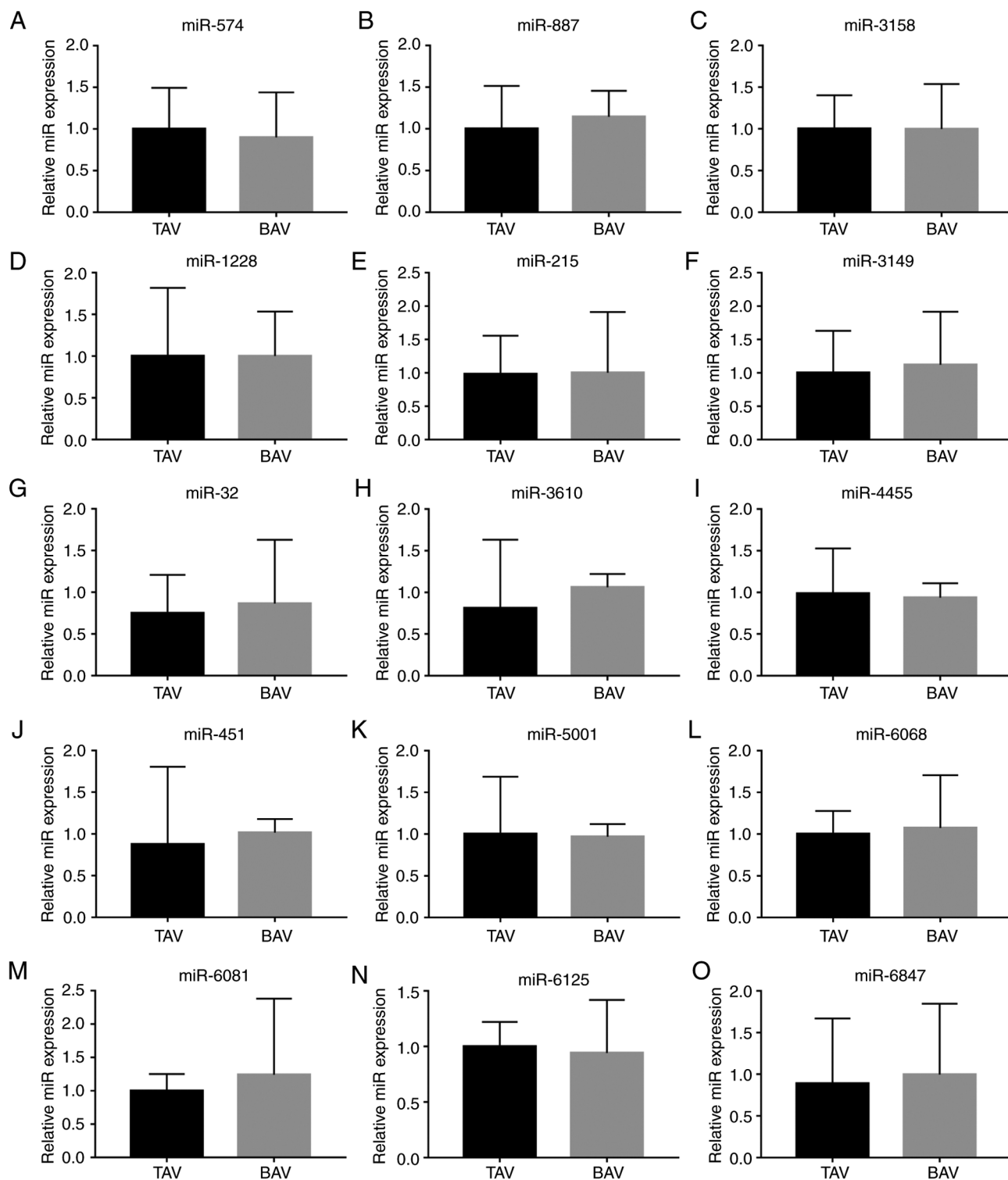


Figure S2. miR-659, miR-663, miR-146 influence on the calcification progress in porcine valve interstitial cells. The mRNA expression of (A) miR-659, (E) miR-663 and (I) miR146 in cultured porcine interstitial cells following transfection with miR-659 mimic, miR-663 mimic, or miR-146 inhibitor. RT-qPCR results of the expression of (B, F, and J) Runx 2, (C, G, and K) BMP2, and (D and H) collagen I following miR transfection in porcine interstitial cells. Student's t-test was performed. Data are presented as the mean \pm standard deviation. *P<0.05 vs. control group. miR, microRNA; BMP2, bone morphogenetic protein 2; Runx 2, Runt-related transcription factor 2.

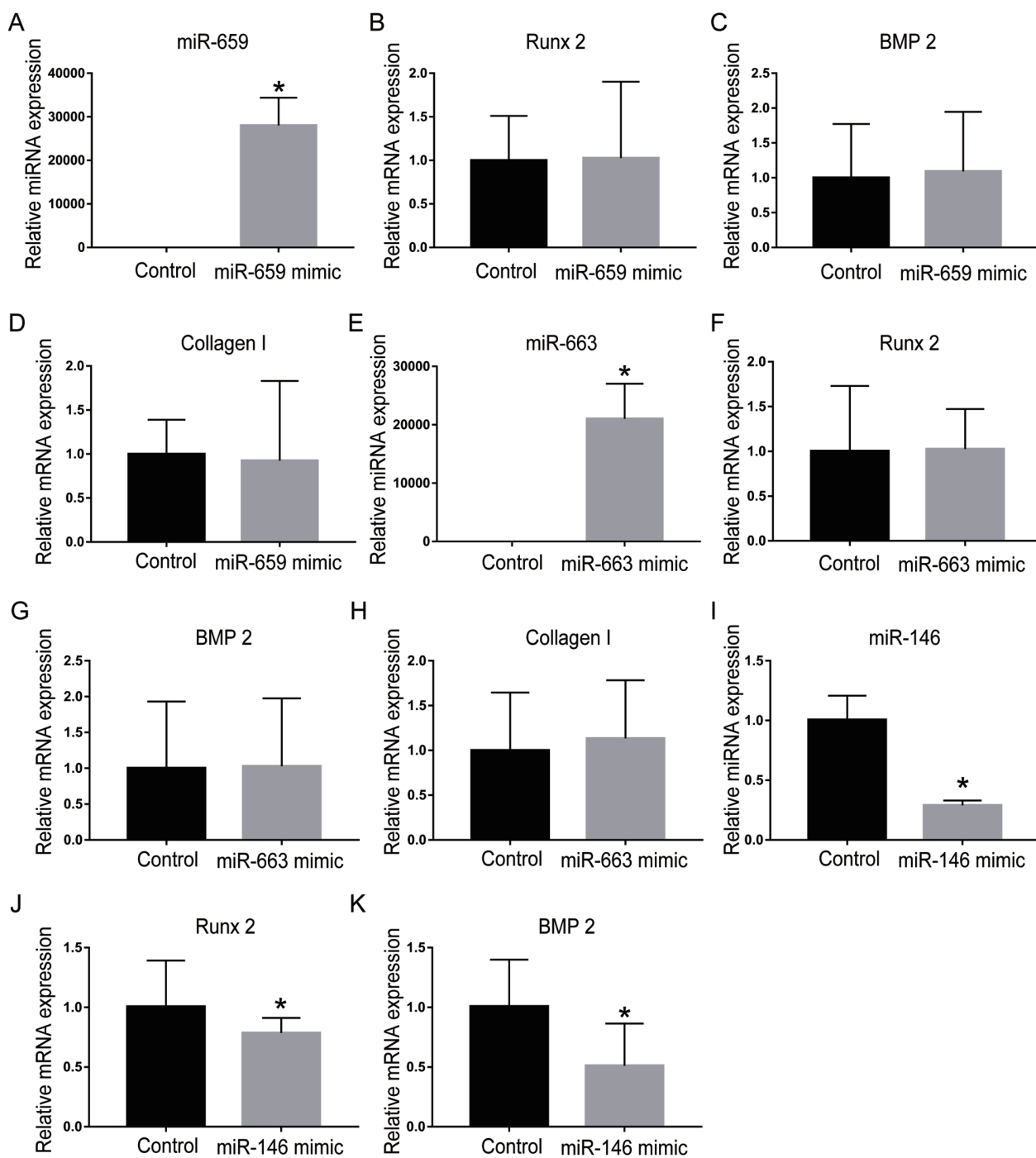


Figure S3. Western blot results from experimental repeats. (A and B) Western blots of collagen I, BMP2, Runx 2, and CREBBP in valve interstitial cells transfected with miR-330-3p mimic or miR-330-3p inhibitor compared with the control groups following pro-calcification culture in experimental repeats (C and D) Western blots of BMP 2 and Runx 2 in valve interstitial cells overexpressing CREBBP by the CRISPRa strategy in the presence of miR-330-3p mimic in experimental repeats. miR, microRNA; BMP2, bone morphogenetic protein 2; Runx 2, Runt-related transcription factor 2; CREBBP, CREB-binding protein; NC, negative control; CRISPR, clustered regularly interspaced short palindromic repeats; CRISPRa, CRISPR activation.

