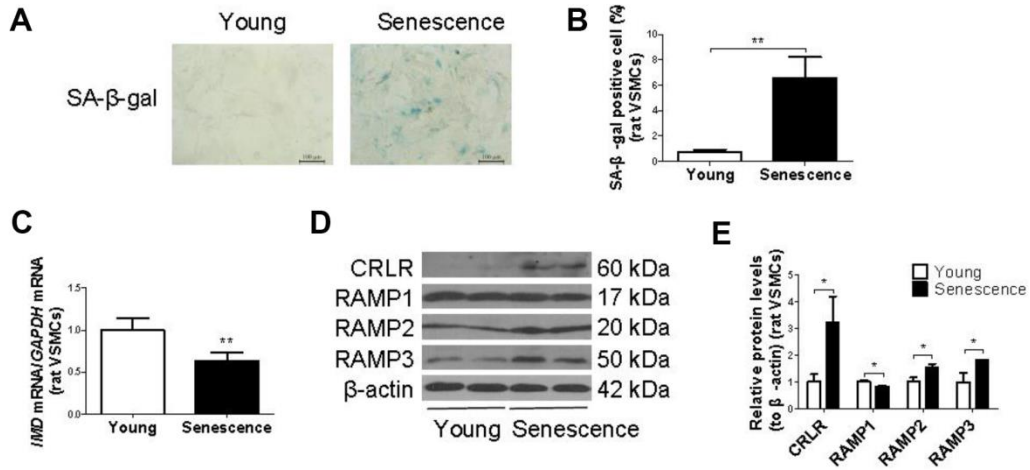
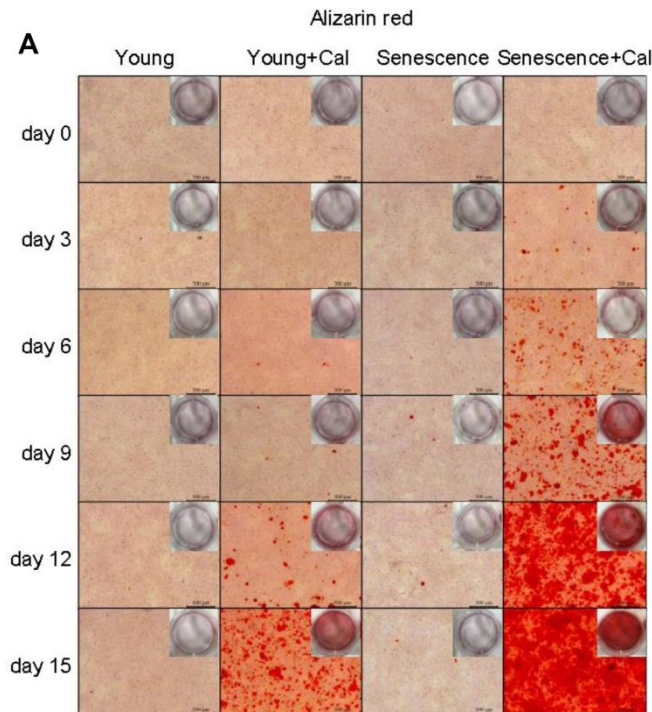


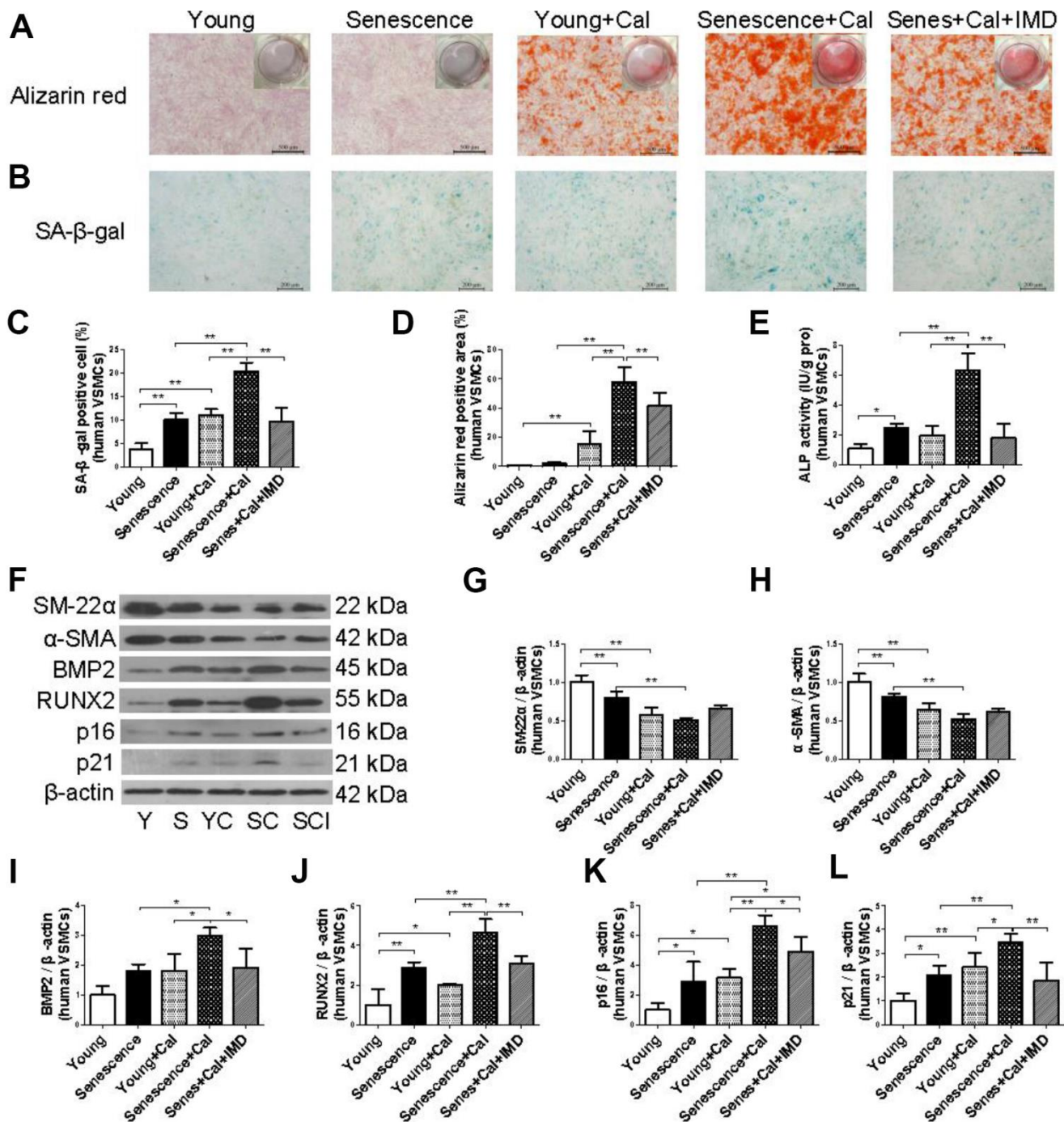
**SUPPLEMENTARY FIGURES**



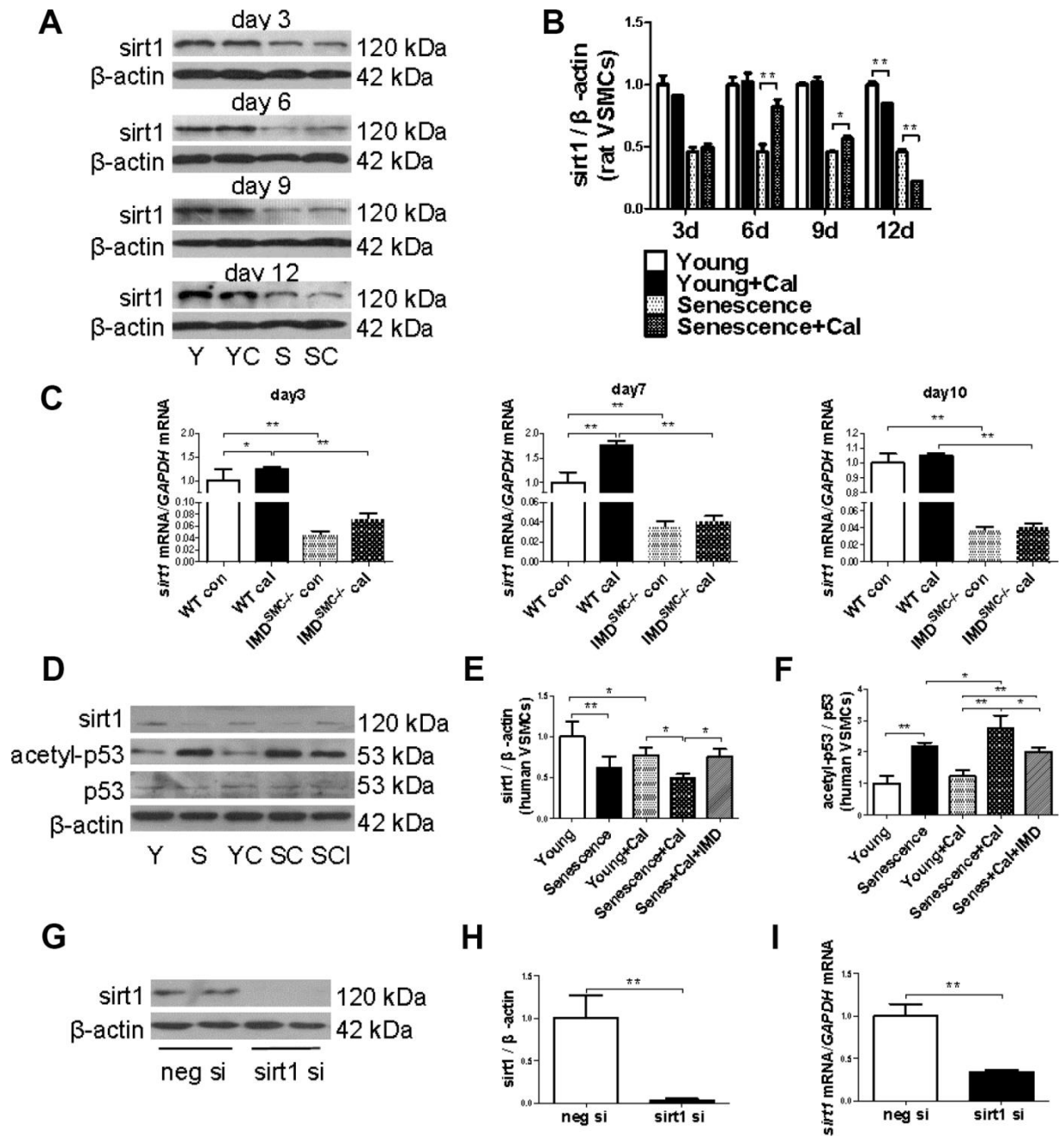
**Supplementary Figure 1. The level of *IMD* and its receptors in rat young (passage 4-6) and senescent (passage 14-18) VSMCs.** (A) SA-β-gal staining (Scale bar=100 μm) and (B) quantification of β-galactosidase-positive staining (blue) in rat young and senescent VSMCs (n=6). (C) RT-PCR analysis of mRNA level of *IMD* in rat VSMCs (n=3). (D) Western blot analysis of protein levels of calcitonin receptor-like receptor (CRLR), receptor activity-modifying protein 1 (RAMP1), 2 and 3 in rat VSMCs, and (E) quantification (n=3). Data are mean ± SD. \**P*<0.05, \*\**P*<0.01.



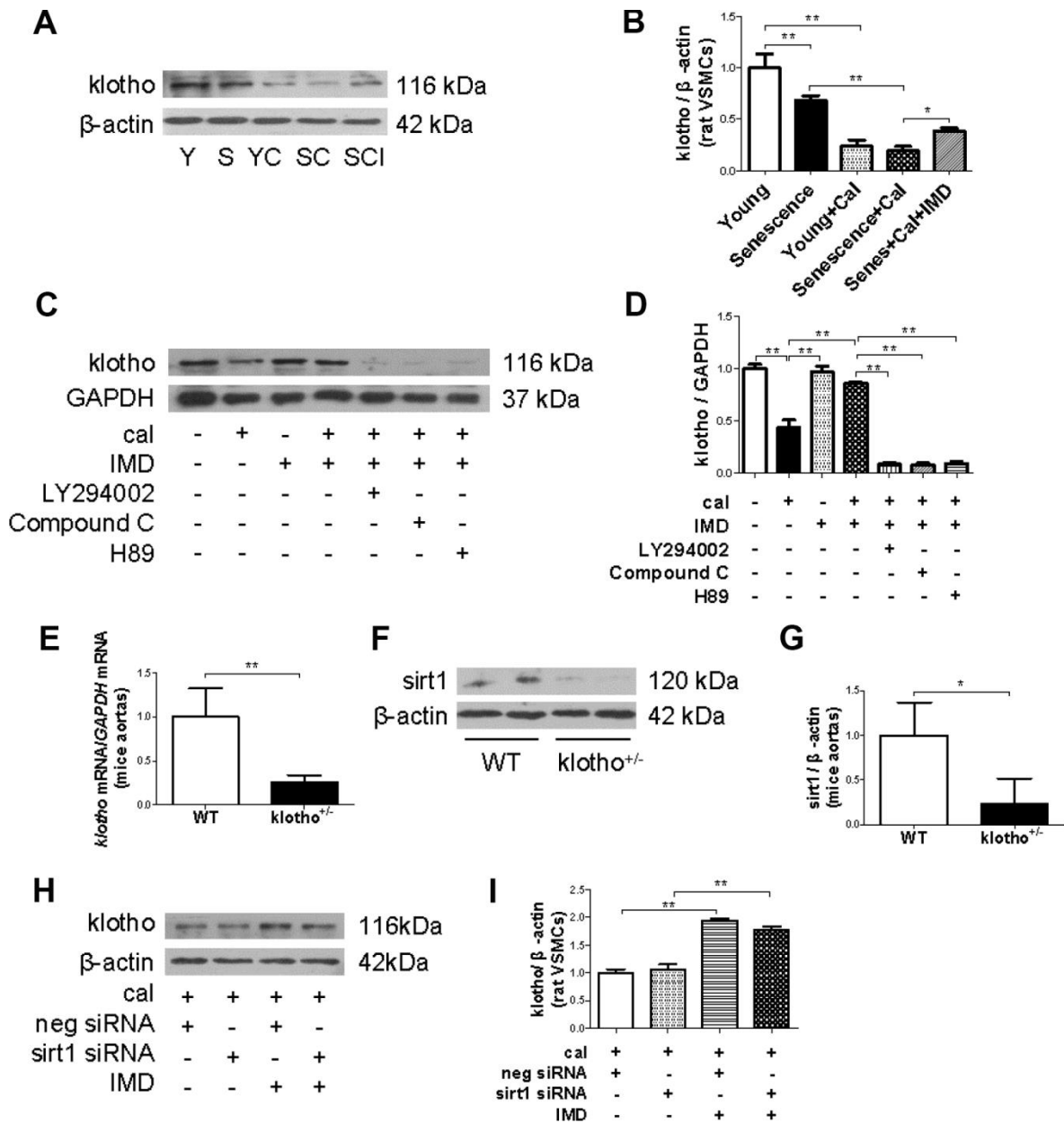
**Supplementary Figure 2. Calcification in rat young (passage 4-6) and senescent (passage 14-18) VSMCs.** (A) Alizarin red staining for calcium deposition (positive staining: red) with calcification time in rat VSMCs (Scale bar=500 μm).



**Supplementary Figure 3. Exogenous IMD<sub>1-53</sub> attenuated senescence-associated calcification in human VSMCs.** (A) Alizarin red staining for human VSMCs (positive staining: red) (Scale bar=500 μm). (B) SA-β-gal staining (blue) (Scale bar=100 μm), and (C) quantification of β-galactosidase-positive staining (n=6) and (D) calcium deposition-positive staining (n=6) in human VSMCs. (E) ALP activity assay (n=6) in human VSMCs. (F) Western blot analysis of protein levels of smooth muscle 22 alpha (SM-22α), alpha smooth muscle actin (α-SMA), bone morphogenetic protein 2 (BMP2), runt-related transcription factor 2 (RUNX2), and cyclin-dependent kinase inhibitors p16 and p21 in human VSMCs, and (G–L) quantification (n=3). Y=young. S=senescence. YC=young+calcification. SC=senescence+calcification. SCI=senescence+calcification+IMD<sub>1-53</sub>. Data are mean ± SD. \*P<0.05, \*\*P<0.01.



**Supplementary Figure 4. *IMD<sub>1-53</sub>* inhibited aging-associated vascular calcification by increasing sirt1 expression and deacetylase activity.** (A) Western blot analysis of protein level of sirt1 with calcification time in rat VSMCs, and (B) quantification (n=3). (C) RT-PCR analysis of mRNA level of *sirt1* with calcification time in WT and *IMD<sup>SMC-/-</sup>* mouse VSMCs (passage 5-6) (n=3). (D) Western blot analysis of protein levels of sirt1, acetylation p53 (acetyl-p53), and total p53 (p53) in human VSMCs, and (E-F) quantification (n=3). (G-I) Western blot and quantitative RT-PCR analysis of protein and mRNA levels of sirt1 after 72 h and 36 h, respectively, with siRNA addition (n=3). WT=wild type. *IMD<sup>SMC-/-</sup>*=VSMC-specific *IMD*-deficient. Con=control. Cal=calcification. Y=young. S=senescence. YC=young+calcification. SC=senescence+calcification. SCI=senescence+calcification+*IMD<sub>1-53</sub>*. neg si=negative siRNA. sirt1 si=sirt1 siRNA. Data are mean ± SD. \**P*<0.05, \*\**P*<0.01.



**Supplementary Figure 5. The kloθο-sirt1 axis in IMD<sub>1-53</sub> attenuating senescence-associated VSMC calcification.** (A) Western blot analysis of protein level of kloθο in rat VSMCs, and (B) quantification (n=3). (C) Western blot analysis of protein level of kloθο in rat senescent VSMCs preincubation with or without PI3K inhibitor LY294002, AMPK inhibitor Compound C or PKA inhibitor H89 (all 10 μmol/L) before IMD<sub>1-53</sub> administration and calcification induction, and (D) quantification (n=3). (E) RT-PCR analysis of mRNA level of *kloθο* in aortas from WT and *kloθο*<sup>+/-</sup> mouse aortas (n=3). (F) Western blot analysis of protein level of sirt1 in WT and *kloθο*<sup>+/-</sup> mice, and (G) quantification (n=4). (H) Western blot analysis of protein level of kloθο in calcified-rat senescent VSMCs treated with IMD<sub>1-53</sub> plus sirt1 siRNA or negative siRNA, and (I) quantification (n=3). WT=wild type. *kloθο*<sup>+/-</sup>= heterozygous *kloθο*-deficient. Cal=calcification. Y=young. S=senescence. YC=young+calcification. SC=senescence+calcification. SCI=senescence+calcification+IMD<sub>1-53</sub>. neg si=negative siRNA. sirt1 si=sirt1 siRNA. Data are mean ± SD. \*P<0.05, \*\*P<0.01.