

Supplemental Figures



FIGURE S1. Expression of BMPER in mouse carotid artery. Mouse carotid artery section was doubly stained with the anti-BMPER pAb and the α SMA mAb. The nuclei were stained with DAPI.

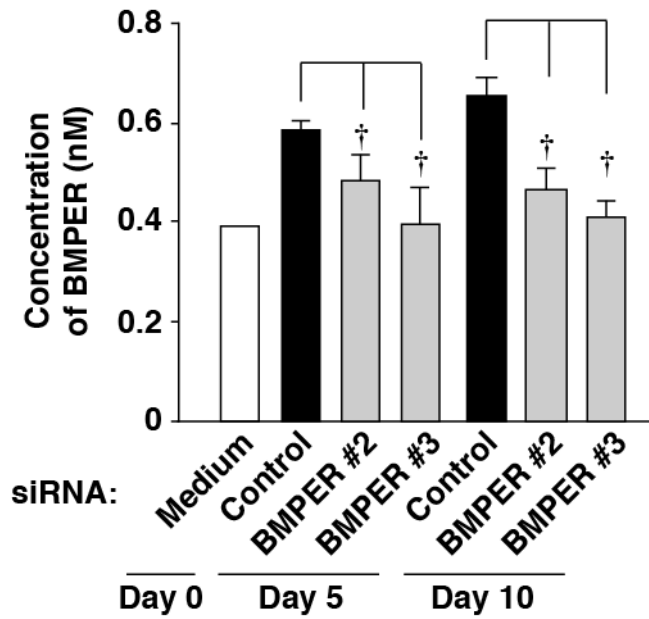


FIGURE S2. Persistent suppression of BMPER release from HCASMCs into the culture media by knockdown of BMPER. Forty-eight hours after transfection of control or BMPER siRNAs, culture media were replaced to new media containing 15% FBS and then HCASMCs were cultured for up to 10 days ($n=4$). The BMPER concentration in the culture media was measured by sandwich ELISA using the anti-BMPER pAb (capture) and the anti-BMPER mAb (detection). Human recombinant BMPER was used as a standard.

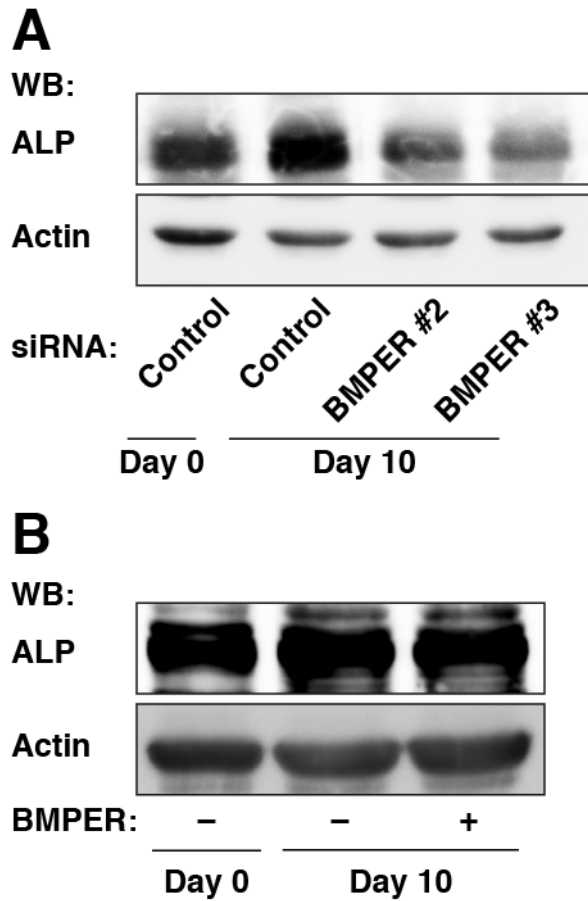


FIGURE S3. Expression of ALP in HCASMCs. **A**, Inhibition of ALP expression by knockdown of BMPER. Forty-eight hours after transfection of control or BMPER siRNAs, culture media were replaced to new media containing 15% FBS and then HCASMCs were cultured for up to 10 days ($n=4$). Cell lysates were subjected to Western blotting with the anti-ALP pAb or the anti-actin mAb. Representative results are shown. **B**, Enhancement of ALP expression by recombinant BMPER. HCASMCs were cultured for 10 days ($n=4$). Cell lysates were subjected to Western blotting with the anti-ALP pAb or the anti-actin mAb. Representative results are shown.

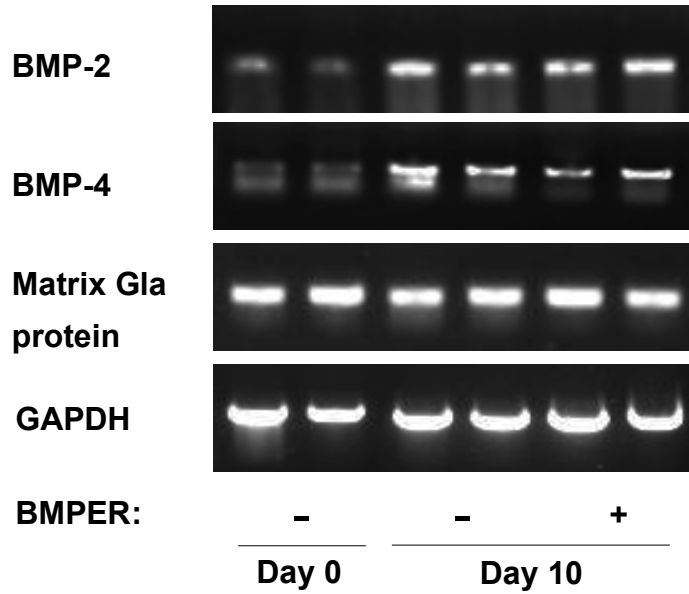


FIGURE S4. mRNA Expression of BMPs and matrix Gla protein in HCASMCs. HCASMCs were cultured for 10 days and total RNAs were extracted ($n=3$). Representative results of RT-PCR for BMP-2, BMP-4, matrix Gla protein and GAPDH are shown.

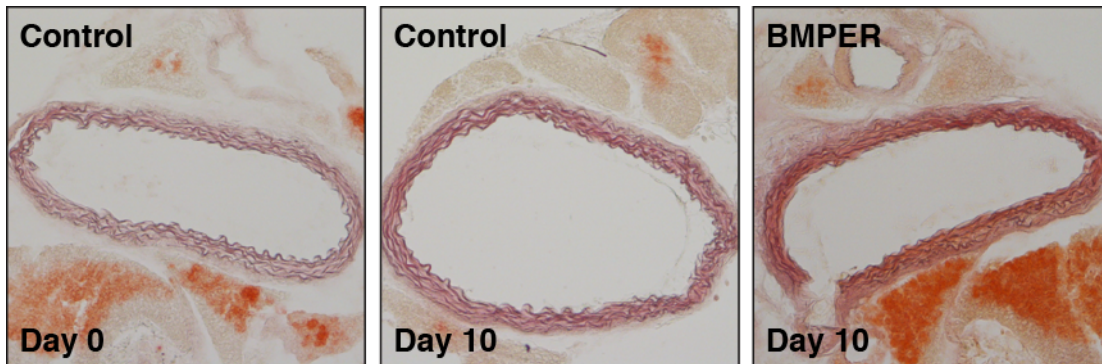


FIGURE S5. Alizarin red staining of mouse aortic rings incubated with or without mouse recombinant BMPER. Mouse aortic rings were prepared and incubated with or without mouse recombinant BMPER (50 nM, final concentration) in the mineralization medium. Representative results of alizarin red staining are shown.

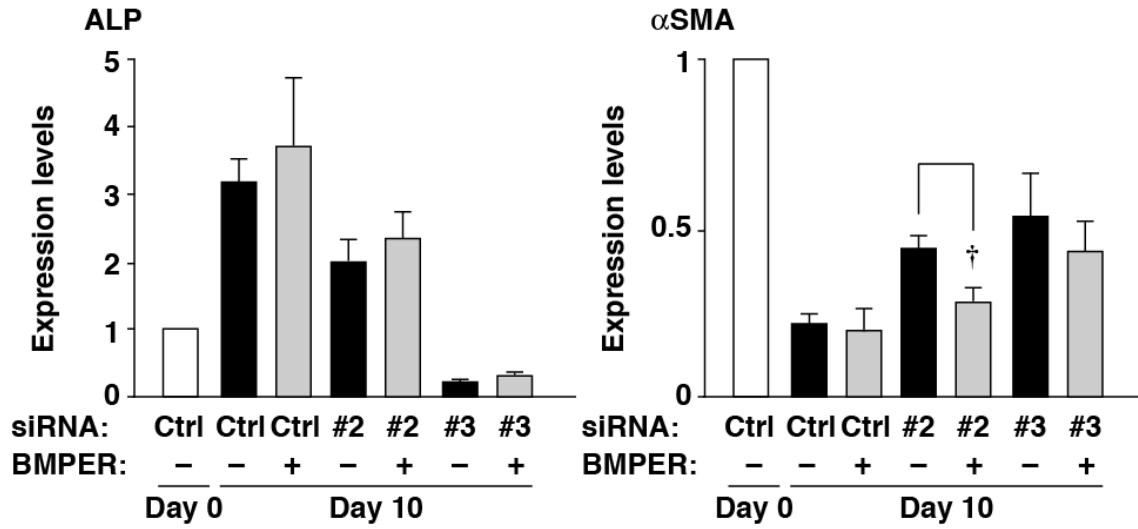


FIGURE S6. mRNA expression of ALP and α SMA analyzed by real-time PCR ($n=4$). Forty-eight hours after transfection of each siRNA, culture media were replaced to new media containing 15% FBS with or without recombinant BMPER (50 nM) and then HCASMCs were cultured for 10 days. Each value was standardized by the GAPDH mRNA expression and is presented as the fold increase over that in the control at day 0.

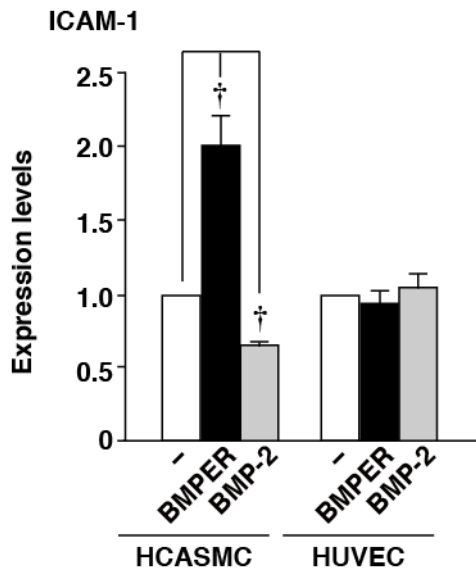


FIGURE S7. mRNA expression of ICAM-1 analyzed by real-time PCR ($n=3$). HCASMCs or HUVECs were cultured for 24 hours in media containing 15% FBS in the absence or presence of recombinant BMPER (50 nM) or BMP-2 (300 ng/ml). Each value was standardized by the GAPDH mRNA expression and is presented as the fold increase over that in the control (-).