Figure S1. Total Pol II levels are not significantly changed in the carotid arteries after vascular injury. (A) Representative images of immunofluorescence staining of Pol II in the carotid arteries at the indicated times after injury. Scale bar, 50 $\mu$m; n=6 animals/group. (B) Quantitative analysis of the relative Pol II intensity. Data are presented as the mean ± SEM. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. ns, not significant; Pol II, RNA polymerase II.

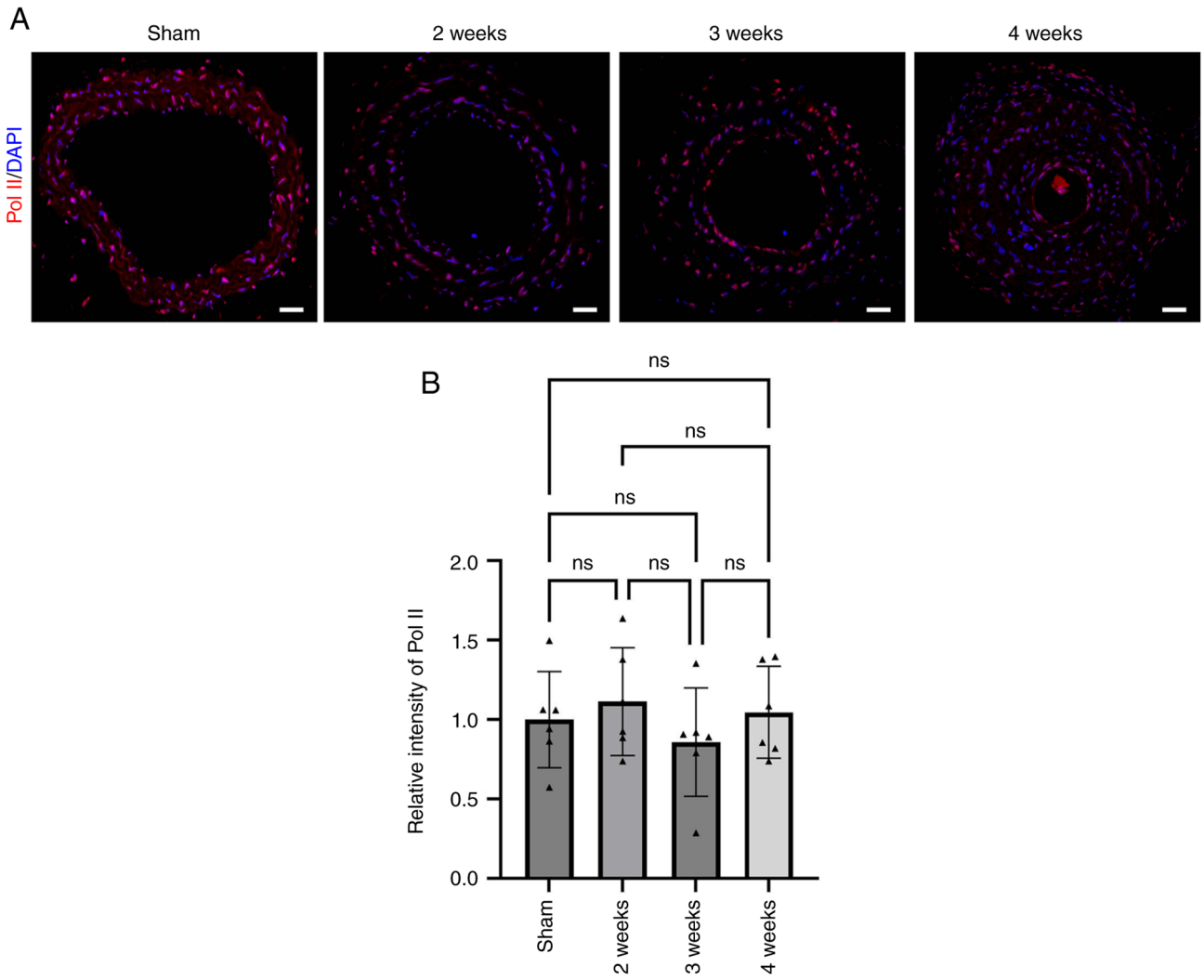


Figure S2. CDK9 levels after PDGF-BB or rapamycin treatment in rat VSMCs. (A) Western blot analysis of CDK9, p-Ser2, α-SMA and Pol II levels in rat VSMCs after PDGF-BB or rapamycin treatment for 24 h. (B) mRNA levels of CDK9 were determined by reverse transcription-quantitative PCR in rat VSMCs after PDGF-BB or rapamycin treatment for 24 h. GAPDH was used as an internal control for normalization. Data are presented as the mean ± SEM. $^{***}$P<0.001 and $^{****}$P<0.0001. Statistical significance was assessed by one-way ANOVA with Tukey's post hoc test. All data are from three biological replicates. α-SMA, α-smooth muscle actin; CDK9, cyclin-dependent kinase 9; p-Ser2, phosphorylated-Pol II (Ser2); PDGF-BB, platelet-derived growth factor-BB; Pol II, RNA polymerase II; VSMC, vascular smooth muscle cell.

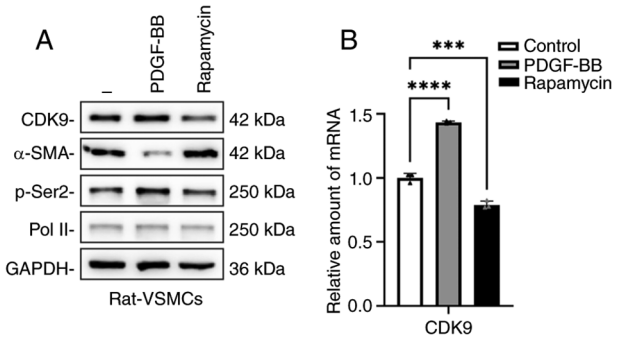


Figure S3. iCDK9 treatment does not significantly change the total Pol II level in the carotid arteries after vascular injury. (A) Representative immunofluorescence staining of the relative intensity of Pol II in carotid arteries on day 21 post-injury with or without iCDK9 treatment. Scale bar, 50 $\mu$m; n=6 animals/group. (B) Quantification of relative intensity of Pol II. Data are presented as the mean ± SEM. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. iCDK9, CDK9 inhibitor; ns, not significant; Pol II, RNA polymerase II.

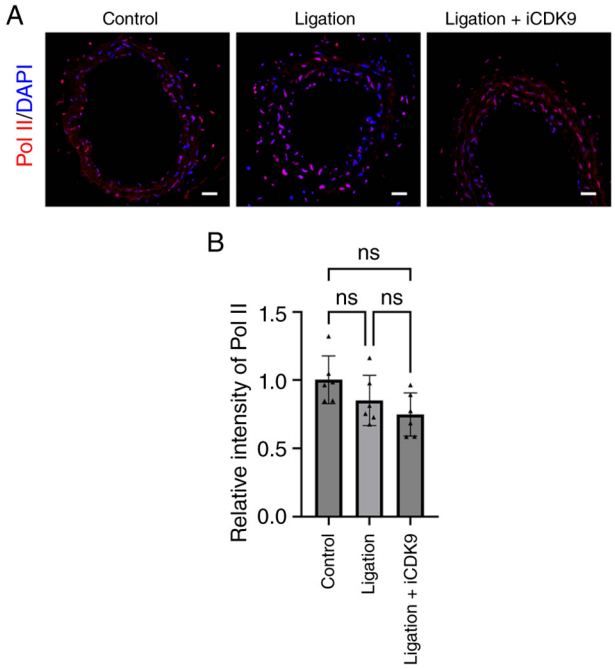


Figure S4. SMC-specific CDK9 overexpression exerts no marked influence on uninjured blood vessels. (A) Validation of SMC-specific CDK9 overexpression efficiency via western blotting 4 weeks after AAV tail vein injection. (B) Representative images of hematoxylin and eosin-stained uninjured carotid arteries in the AAV2-SM22α-NC and AAV2-SM22α-CDK9 groups. (C) Quantitative analysis of the intima/media ratio. Scale bar, 50 $\mu$m; n=7 animals/group. Data are presented as the mean ± SEM. Statistical significance was assessed by unpaired Student's t-test. All data are from three biological replicates. CDK9, cyclin-dependent kinase 9; NC, negative control; ns, not significant; SMC, smooth muscle cell.

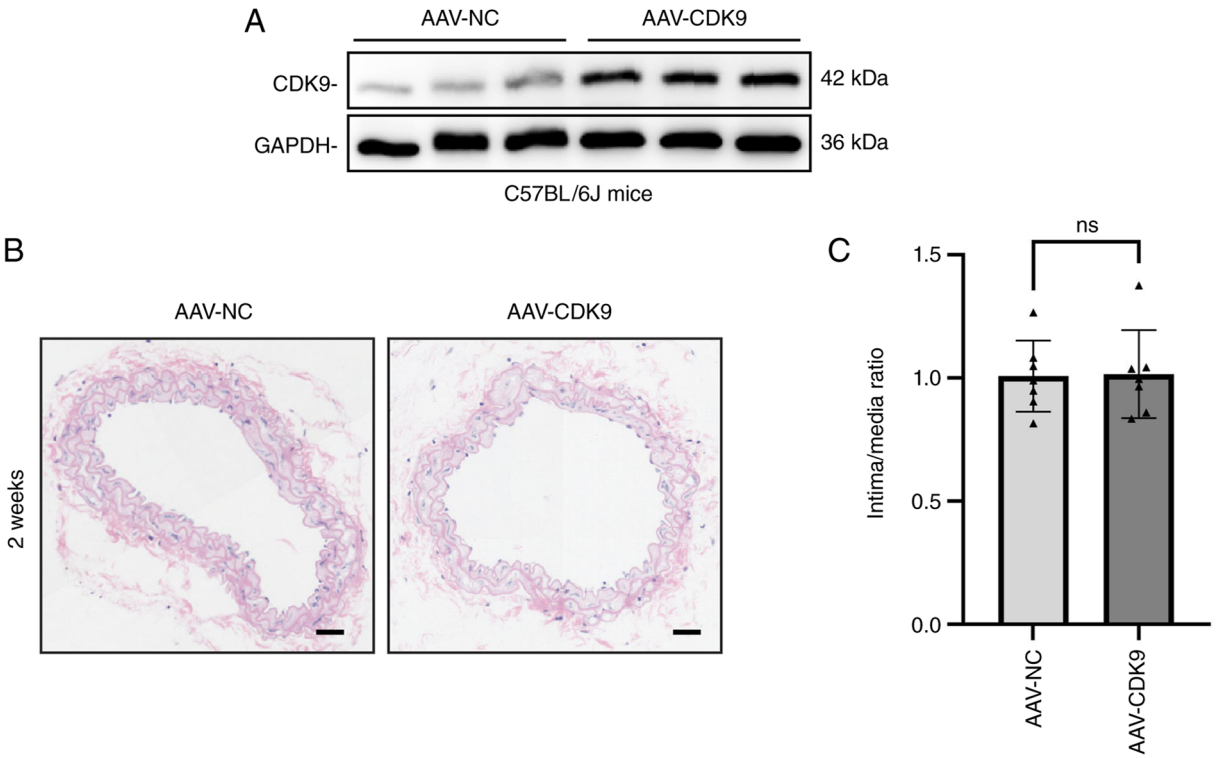


Figure S5. CDK9 inhibition reduces p-Ser2 in a concentration-dependent manner and siCDK9 knockdown is validated. (A) Western blot analysis of p-Ser2 in VSMCs treated with iCDK9 at different concentrations for 24 h. (B) Western blot analysis of p-Ser2 in VSMCs treated with the CDK9 degrader Thal at different concentrations for 24 h. (C) VSMCs were treated with PDGF-BB in the presence or absence of iCDK9 for 24 h, and then subjected to western blot analysis to detect p-Ser2. (D) p-Ser2 was detected by western blot analysis after VSMCs were treated with PDGF-BB in the presence or absence of Thal for 24 h. (E) Knockdown efficacy of siCDK9. VSMCs were transfected with siNC, siCDK9-1# or siCDK9-2#, and the expression of CDK9 was detected by western blotting. CDK9, cyclin-dependent kinase 9; iCDK9, CDK9 inhibitor; NC, negative control; p-Ser2, phosphorylated-Pol II (Ser2); PDGF-BB, platelet-derived growth factor-BB; Pol II, RNA polymerase II; si, small interfering; Thal, Thal-SNS-032; VSMC, vascular smooth muscle cell.

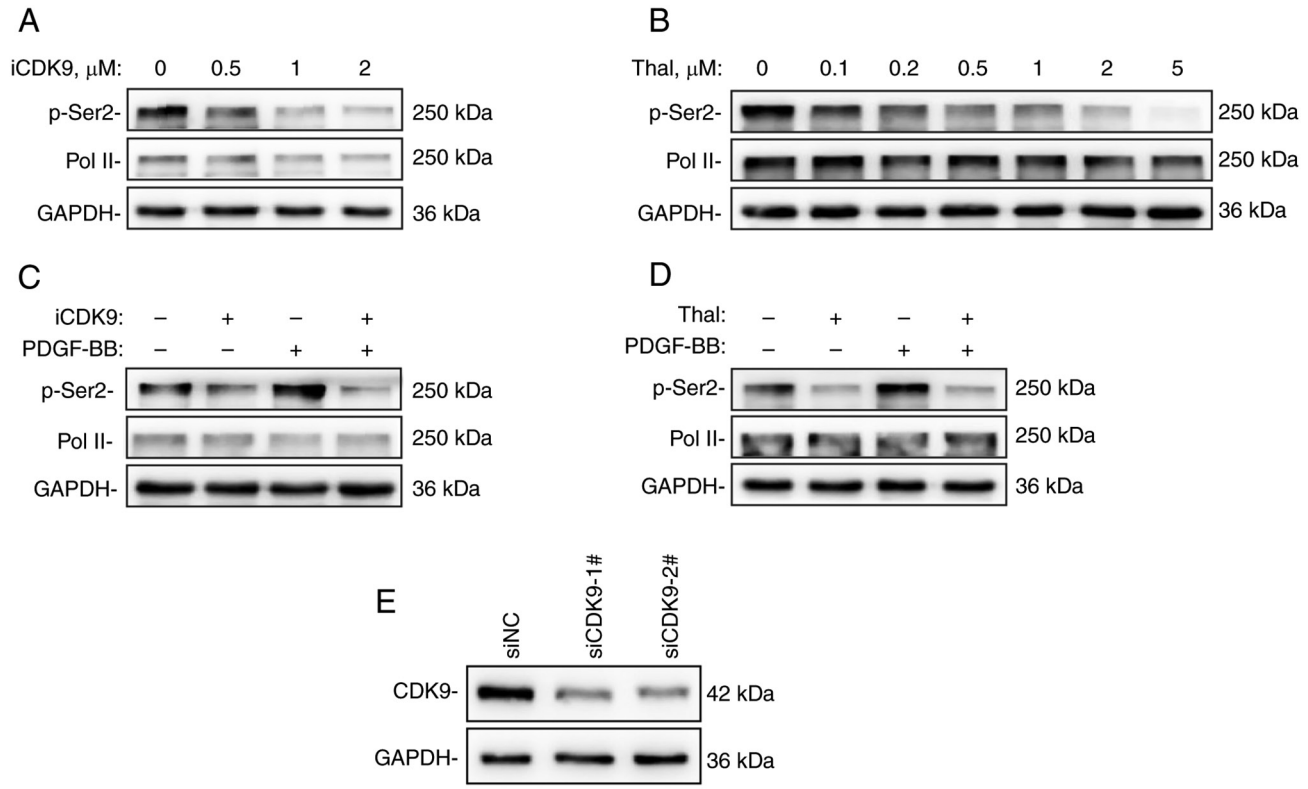


Figure S6. Knockdown efficacy of Twist1 siRNAs. Vascular smooth muscle cells were transfected with siNC or siTwist1 as indicated. The expression of Twist1 was detected by western blotting. siTwist1-5# is shown in red, as this construct was selected for the experiments presented in Fig. 6. NC, negative control; si, small interfering.

