Supplemental to 'c-Abl regulates YAP^{Y357} phosphorylation to activate endothelial atherogenic responses to disturbed flow'

Bochuan Li[*], Jinlong He[*], Huizhen Lv, Yajin Liu, Xue Lv, Chenghu Zhang, Yi Zhu [#], Ding Ai [#]

Tianjin Key Laboratory of Metabolic Diseases; Key Laboratory of Immune Microenvironment and Disease (Ministry of Education); Collaborative Innovation Center of Tianjin for Medical Epigenetics and Department of Physiology and Pathophysiology, Tianjin Medical University, Tianjin, 300070, China;

[*]These two authors contributed equally to this study.

[#]To whom correspondence may be addressed:

Yi Zhu

Department of Physiology and Pathophysiology, Tianjin Medical University, 22 Qixiangtai Rd, Tianjin, China, 300070.

Tel: +86-22-83336665

E-mail: zhuyi@tmu.edu.cn

or

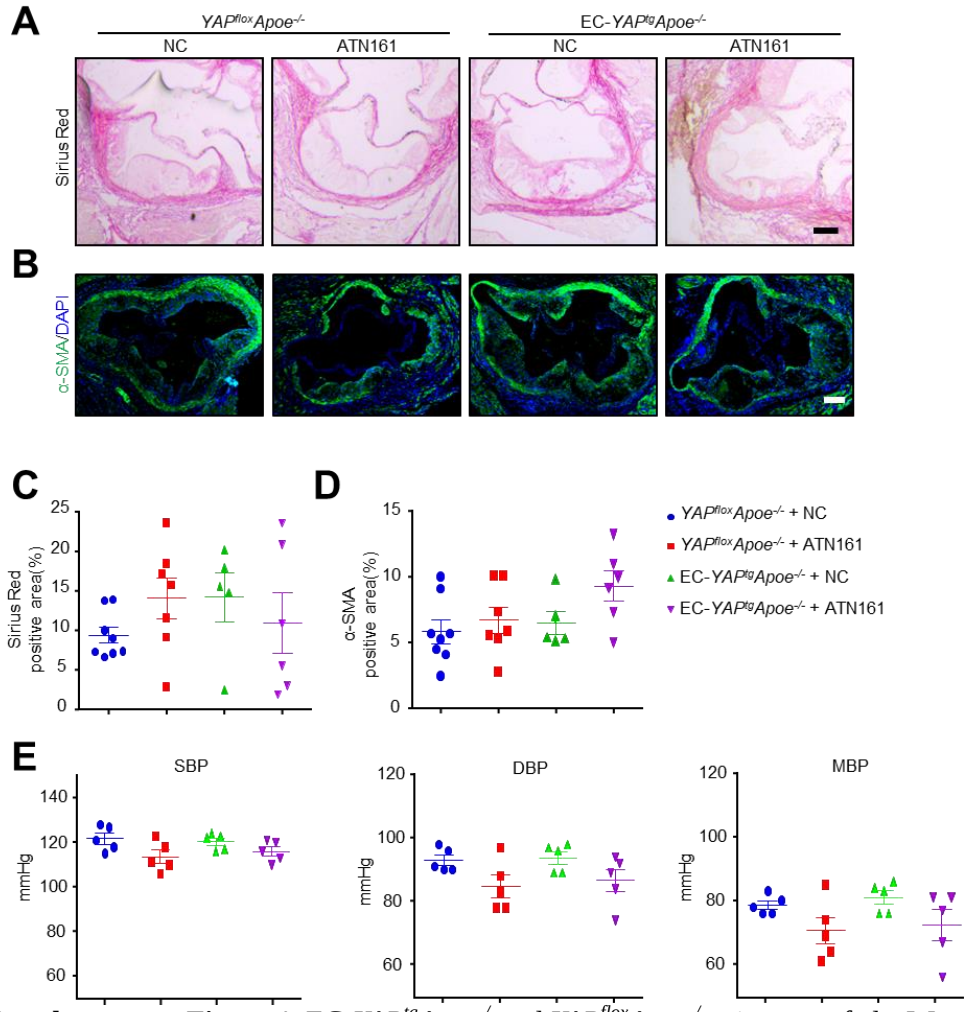
Ding Ai

Department of Physiology and Pathophysiology, Tianjin Medical University, 22 Qixiangtai Rd, Tianjin, China, 300070.

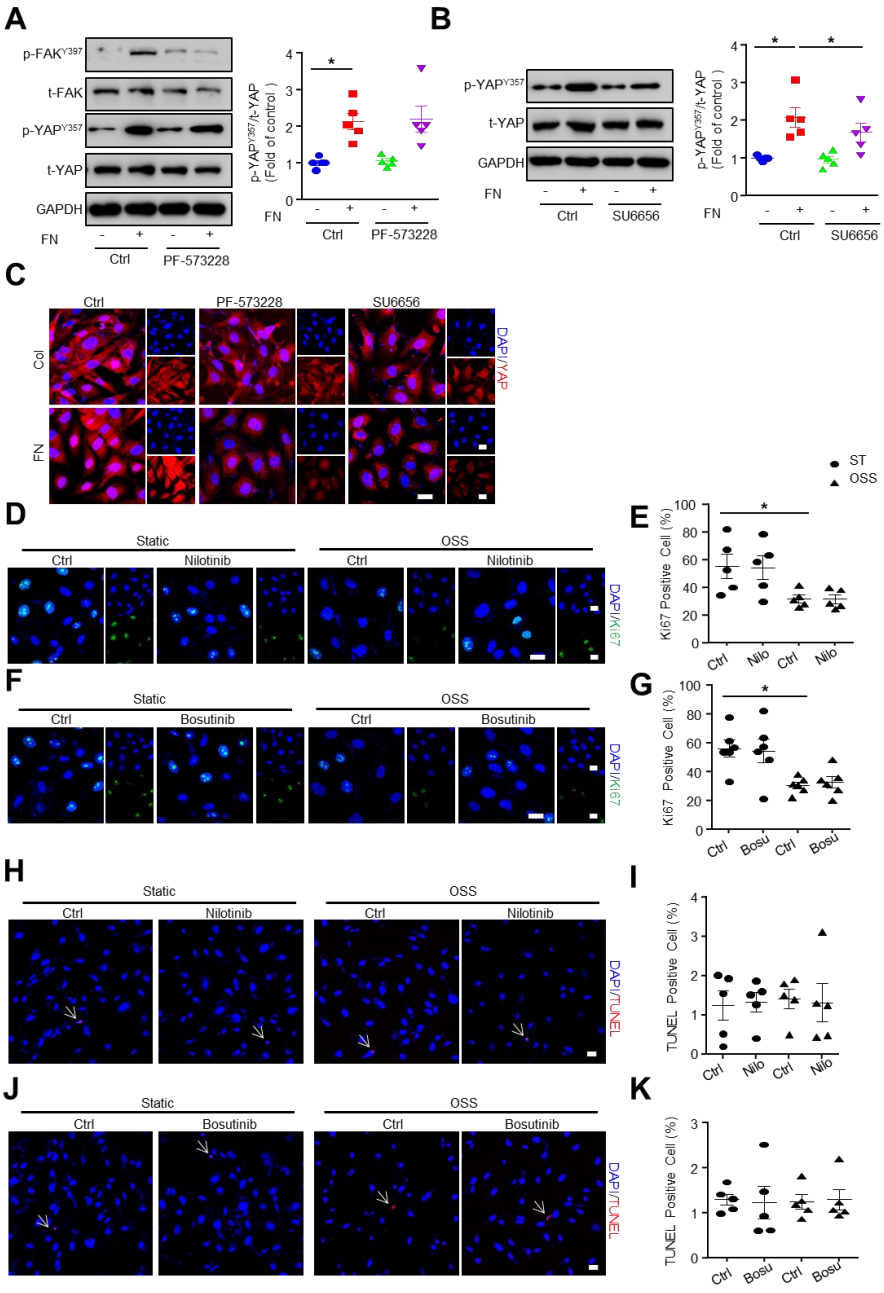
Tel: +86-22-83336591

E-mail: edin2000cn@gmail.com

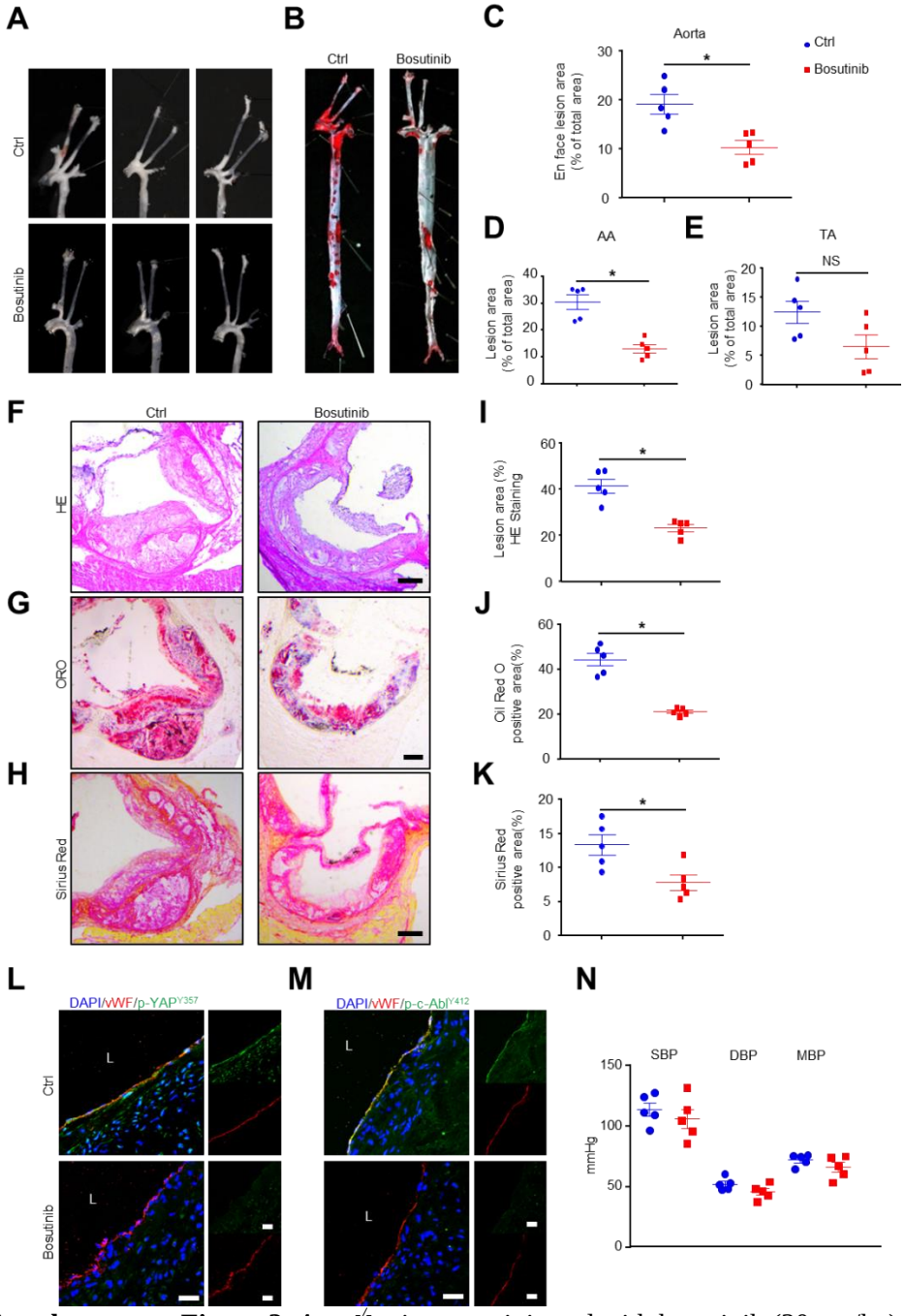
Supplementary Figures and Figure Legends



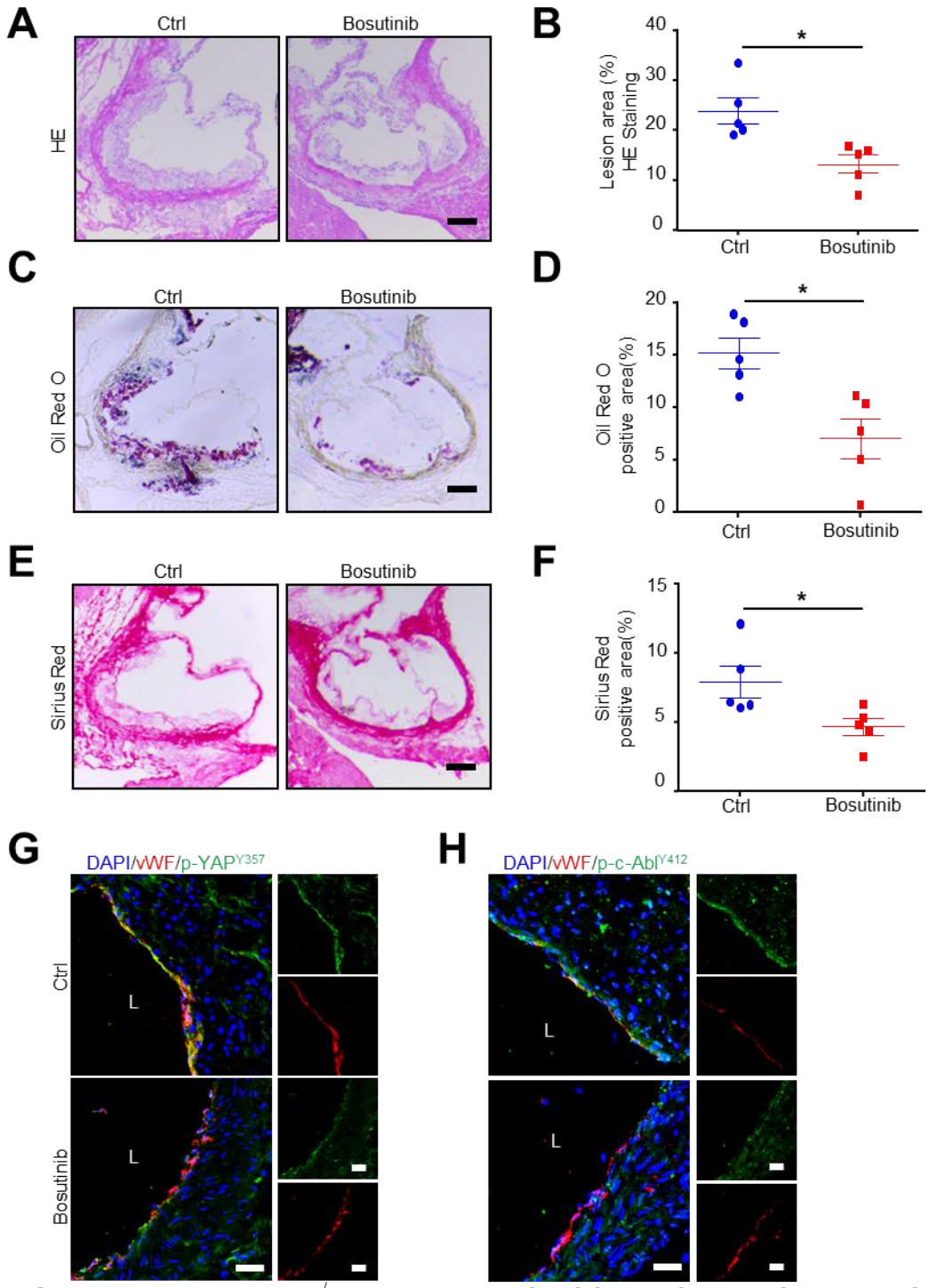
Supplementary Figure 1. EC-*YAP*^{tg}*Apoe*$^{-/-}$ and *YAP*^{flox}*Apoe*$^{-/-}$ mice were fed a Western diet for 4 weeks, during which mice were intraperitoneally injected with scramble peptide (NC) or ATN161 (100 mg/kg) every 3 days. Aortic roots of mice were stained with Sirius red (**A**) and underwent immunofluorescence staining for SMA (smooth muscle cell marker, green) (**B**). (**C-D**) Quantification of collagen fiber (Sirius red) and α-SMA–positive area staining in plaque. Data are mean ± SEM. *YAP*^{flox}*Apoe*$^{-/-}$ +NC (n=8), *YAP*^{flox}*Apoe*$^{-/-}$ +ATN161 (n=7), EC-*YAP*^{tg}*Apoe*$^{-/-}$ +NC (n=5), EC-*YAP*^{tg}*Apoe*$^{-/-}$ +ATN161 (n=6). Scale bar, 100 μm. (**E**) Noninvasive tail-cuff monitoring of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP) of mice. Data are mean ±SEM. n=5.



Supplementary Figure 2. HUVECs were seeded on dishes coated with fibronectin (FN) or collagen and cultured for 6 hr with or without inhibitors (10 μmol/L), as indicated. **(A-B)** Western blot analysis of expression of indicated proteins and quantification of ratio of p-YAP^{Y357} to t-YAP. Data are mean ± SEM, *p<0.05 (two-way ANOVA with Bonferroni multiple comparison post-test). n=5. (**C**) Immunofluorescence staining of YAP (red) or DAPI (blue). Representative images are shown. n=6. Scale bar, 20 μm. (**D-G**) HUVECs were exposed to oscillatory shear stress (OSS) or static treatment (ST) for 6 hr with or without inhibitors (10 μmol/L) as indicated. Quantification of the percentage of Ki67-positive cells. Data are mean ± SEM, *p<0.05 (two-way ANOVA with Bonferroni multiple comparison post-test). (D-E), n=5. (F-G), n=6. Scale bar, 100 μm. (**H-K**) HUVECs were exposed to OSS or ST for 6 hr with or without inhibitors (10 μmol/L) as indicated. Quantification of the percentage of TUNEL-positive cells. White arrows indicate TUNEL-positive cells. Data are mean ± SEM. n=5. Scale bar, 40 μm.



Supplementary Figure 3. *Apoe*$^{-/-}$ mice were injected with bosutinib (30 mg/kg) every 3 days and fed a Western diet for 12 weeks. (**A-B**) Representative gross and Oil-red O staining of aortas. n=5. (**C-E**) Quantification of lesion area in (B). AA, aortic arch; TA, thoracic aorta; NS, not significant. Data are mean ± SEM, *p<0.05 (Student's *t* test). n=5. Aortic roots were sectioned and underwent HE (**F**), Oil-red O (**G**) and Sirius red (**H**) staining. n=5. Scale bar, 100 μm. (**I-K**) Quantification of lesion area or positive area staining. Data are mean ± SEM, *p<0.05 (Student's *t* test). n=5. (**L-M**) Representative immunofluorescence staining of p-YAP^{Y357} or p-c-Abl^{Y412} in aortic roots. n=5. Scale bar, 30 μm. L, lumen. (**N**) Noninvasive tail-cuff monitoring of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP) of mice. Data are mean±SEM. n=5.



Supplementary Figure 4. *Apoe*$^{-/-}$ mice were injected with bosutinib (30 mg/kg) every 3 days for 5 weeks and were fed a Western diet for 4 weeks from the second week. Aortic roots were sectioned and underwent HE (**A**), Oil-red O (**B**) and Sirius red (**C**) staining. n=5. Scale bar, 100 μm. (**D-F**) Lesion area or positive area staining was quantified. Data are mean ± SEM, *p<0.05 (Student's *t* test). n=5. (**G-H**) Representative immunofluorescence staining of p-YAP^{Y357} or p-c-Abl^{Y412} in aortic roots. n=5. Scale bar, 30 μm. L, lumen.