1 **Supplementary methods**

2 **OSAS-AD mouse model**

3 The animal procedures conform to the guidelines from Directive

4 2010/63/EU of the European Parliament on the protection of animals used

5 for scientific purposes and approved by the Institutional Animal Research

6 Committee of Tongji Medical College. ApoE^{-/-} mice purchased from

7 Beijing Vital River Laboratory Animal Technology Co., Ltd. ApoE^{-/-} mice

8 (C57BL/6 background) were housed at the animal care facility of Tongji

9 Medical College under specific pathogen-free conditions and fed a normal

10 diet. 8-week-old male ApoE^{-/-} mice were given β-aminopropionitrile

11 (BAPN) at a concentration of 0.1 % for 3 weeks[1, 2] and infused *via* osmotic

12 mini pumps (Alzet, Cupertino, CA) with either saline or 2,500 ng/min/kg

13 angiotensin II (Ang II) (Sigma-Aldrich, St. Louis, MO) for 14 days. To

14 evaluate the effect of HIF-1α inhibitor KC7F2[3] treatment on AD initiation

15 and progression, treatment was initiated 14 days before and terminated 14

16 days after Ang II infusion. KC7F2 was freshly prepared in PBS and

17 administered to mice at a dose of 10 mg/kg every other day through

18 intraperitoneal injection. The IH paradigm consisted of alternating cycles

19 of 20.9% O2/8% O2 FiO2 (30 episodes per hour) with 20 sec at the nadir

20 FiO2 during the 12-h light phase (07:00 a.m.–07:00 p.m.), as

21 deoxygenation-reoxygenation episodes occur in moderate to severe OSAS

22 patients. After 4 weeks of IH exposure, including 2 weeks before and

23   sustained 2 weeks during Ang II infusion, mice were transferred to room

24   air, cardiac function was measured by echocardiography, and then

25   humanely euthanized by anesthetic overdose (pentobarbital) for organ

26   collection (Figure S1). Blood pressure was measured using the tail-cuff

27   method described previously and after implantation, and prior to sacrifice.

28

29   **Histology and immunohistochemistry**

30   Sacrificed mice were perfused with ice-cold PBS and then with 4%

31   buffered paraformaldehyde. Tissues were further fixed in 4% buffered

32   paraformaldehyde for 2 days at 4°C, embedded in paraffin and processed

33   for sectioning, 4 μm cross-sections were then obtained. Aortic morphology

34   was evaluated using hematoxylin and eosin stained histological sections[4].

35   Images were captured using an LEICA DM4000B Microscope (LEICA,

36   Beijing, China). At least 10 random images per mouse around the

37   dissection position were taken, and the maximal diameter was picked for

38   further analysis, at least 12 mice per group were included into each group.

39   van Gieson Stain was performed according to the manufacture's

40   description (Boster, Wuhan, China)[4]. Aortic sections were stained with

41   Weigert Solution (Fuchsin basic, resorcinol, water, and hydrochloric acid)

42   for 6 hours, directly immersed into Differentiation Solution (1%

43   hydrochloric acid alcohol), and then flushed with water. Van Gieson Dye

44   Solution (1% Fuchsinacid aqueous solution and saturated aqueous picric

45   acid solution) was used to restain the sections for 1-2 minutes. A

46   microscope (LEICA DM4000B) was used to observe images. At least 5

47   independent samples in each group (for each sample 3 sections were

48   obtained) were observed.

49   Immunohistochemical analyses of HIF-1α, VEGF, MMP2, MMP9 and

50   GP91 was conducted essentially as previously described[5]. Paraffin

51   embedded tissue sections were deparaffinised and rehydrated, incubated

52   with a specific primary antibody (1 h, at room temperature), washed 3

53   times with PBS, and incubated with an appropriate, horseradish

54   peroxidase-conjugated secondary antibody. Peroxidase activity was

55   detected using a DAB substrate (3,3'-diaminobenzidine) and slides were

56   counterstained with haematoxylin. Control images were obtained

57   following incubation with a non-specific primary antibody and were used

58   for background correction. All histological analyses were done by two

59   independent blinded investigators. Images were obtained using an LEICA

60   DM4000B Microscope (LEICA, Beijing, China) at 20x or 40x

61   magnification.

62

63   **Cell culture and *in vitro* IH model**

64   Vascular smooth muscle cells (VSMCs) (ATCC, Manassas, VA) were

65   cultured in 10% fetal bovine serum (Gibco, Grand Island, NY) containing

66   Dulbecco's modification of Eagle's medium (Gibco, Grand Island, NY)

67 under 37 ℃ and 5% CO_2 conditions[6]. In the IH group, cells were

68 maintained at 37 ℃ at 5% CO2 in a chamber (Oxycycler model A42,

69 Biospherix) in which O2 levels were alternated between 21% for 5 min and

70 1% for 10 min, for a total of 64 cycles (18 h). After confluence, VSMCs

71 were incubated with 10 μM Ang II (Sigma-Aldrich, St. Louis, MO) for 24

72 h. For intervention study, 40 μM KC7F2 (Sigma-Aldrich, St. Louis, MO)

73 were added 1 h before Ang II treatment. Cells were exposed to

74 deferoxamine (DFO) (0, 30, 60, 120 μM) for 24h. Cells were cultured in

75 media containing 0.1% FBS for 20 h, followed by the addition of PI3K

76 inhibitor LY294002 (1 μM), AKT inhibitor MK-2206 (5 μM) or FRAP

77 inhibitor rapamycin (1 nM) 1 h before Ang II and IH treatment. Cells were

78 harvested at various time-points or interventions after IH for RNA and

79 protein isolation.

80

81 **Western Blot Analysis**

82 Proteins were isolated using RIPA buffer and the concentration was

83 determined using the BCA protein assay (Thermo Fisher Scientific,

84 Waltham, MA, USA). Aorta and cell extracts were separated by

85 SDS/PAGE and transferred to PVDF membranes. Membranes were

86 blocked in Tris-buffered saline with 0.1% Tween 20 with 5% non-fat dry

87 milk or bovine serum albumin. Membranes were incubated with

88 appropriate primary antibodies overnight at 4 ℃. After washing 5 times

89   with 1 x TBS-T membranes were incubated with an appropriate secondary

90   peroxidase-conjugated antibody, and immunoreactive proteins were

91   visualized using an enhanced chemiluminescence system (Tanon,Shanghai,

92   China). The following antibodies were applied: HIF-1α, VEGF, MMP2,

93   MMP9, and GP91 were from Epitomic (Burlingame, CA, USA); MMP2

94   and GAPDH (Santa Cruz Biotechnologies, CA, USA). GAPDH was used

95   for calibration of total protein or cytosolic protein determination. Bands

96   were quantified by densitometry using Quantity One software (Bio-Rad,

97   Hercules, CA).

98

99   **RT-PCR**

100   Total RNA was extracted from vascular smooth muscle cells (VSMCs)

101   with Trizol reagent (TaKaRa, Japan). Then, 750 ng RNA was added to a

102   20 μL reaction volume for cDNA reverse transcription using the Prime

103   Script™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan), and

104   quantitative real-time polymerase chain reaction (qRT-PCR) was

105   performed using the SYBR R Premix Ex Taq™ (TaKaRa, Japan) in a Step

106   One Plus real-time PCR system (Applied Biosystems, USA). The HIF-1α,

107   VEGF, MMP2, MMP9 and GAPDH genes were analyzed. Primer sets for

108   selected genes were designed by TianYi Huiyuan (Wuhan, China) and their

109   sequences are listed in Supplementary Table S1. Gene expression was

110   quantified according to the $2^{-\triangle\triangle Ct}$ method. Message RNA levels in vascular

111   smooth muscle cells were expressed as fold changes relative to their

112   respective controls.

113

114   **Statistical Analysis**

115   All data analysis was performed with the use of SPSS 13.0 statistical

116   software. Data are reported as mean $\pm$ SEM. Depending on the nature of

117   the data, Kaplan–Meier survival analysis, one-sample *t*-test, or two-way

118   analysis of variance followed by the Newman-Keuls post hoc correction

119   was used to determine significance between groups. Log-rank test,

120   ANOVA or the Student's *t* test was used to determine statistical

121   significance with $p<0.05$. Each experiment was done at least in triplicate.

122

123 **Supplementary Table S1:** Sequences of human primers used within the

124 current study.

Gene	Forward	Reverse
HIF-1α	5′ -GAACGTCGAAAAGAAAAGTCTCG-3′	5′ -CCTTATCAAGATGCGAACTCACA-3′
VEGF	5′ -AGGGCAGAATCATCACGAAGT-3′	5′ -AGGGTCTCGATTGGATGGCA-3′
MMP2	5′ -GATACCCCTTTGACGGTAAGGA-3′	5′ -CCTTCTCCCAAGGTCCATAGC-3′
MMP9	5′ -GGGACGCAGACATCGTCATC-3′	5′ -TCGTCATCGTCGAAATGGGC-3′
GAPDH	5′ -GTTCAACGGCACAGTCAAGG-3′	5′ -GTGGTGAAGACGCCAGTAGA-3′

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Reference

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Obstructive sleep apnea syndrome promotes the progression of aortic dissection *via* a ROS- HIF-1α- MMPs associated pathway

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Supplementary Figure 1
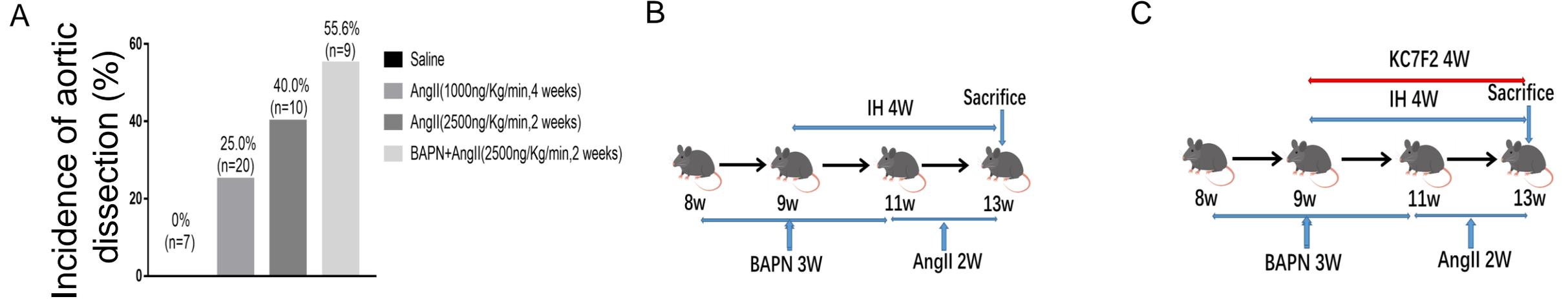
Figure S1. Schematic illustration of the OSAS-AD experimental mouse model. 8 week-old male ApoE$^{-/-}$ mice were fed on normal chow diet and infused via osmotic mini pumps with either saline or 1,000 ng/min/kg Ang II for 4 weeks , 2,500 ng/min/kg Ang II for 2 weeks; and were given BAPN at a concentration of 0.1 % for 3 weeks in the drinking water and then infused via osmotic mini pumps with either saline or 2,500 ng/min/kg Ang II for 2 weeks(A). 8 week-old male ApoE$^{-/-}$mice were fed on normal chow diet and were given BAPN at a concentration of 0.1 % for 3 weeks in the drinking water and then infused via osmotic mini pumps with either saline or 2,500 ng/min/kg Ang II for 2 weeks. The mice were exposed to IH condition from the second week of BAPN treatment and last for total 4 weeks until the Ang II treatment was finished. Finally the mice were sacrificed for further analysis at the end of Ang II treatment (B). For interventional study KC7F2 was freshly prepared in PBS and administered to mice at a dose of 10 mg/kg every other day through intraperitoneal injection during the IH period, thereafter the mice were sacrificed and analyzed(C). BAPN: β-aminopropionitrile; IH: intermittent hypoxia; Ang II: angiotensin II; KC7F2: a HIF-1α inhibitor.

Supplementary Figure 2
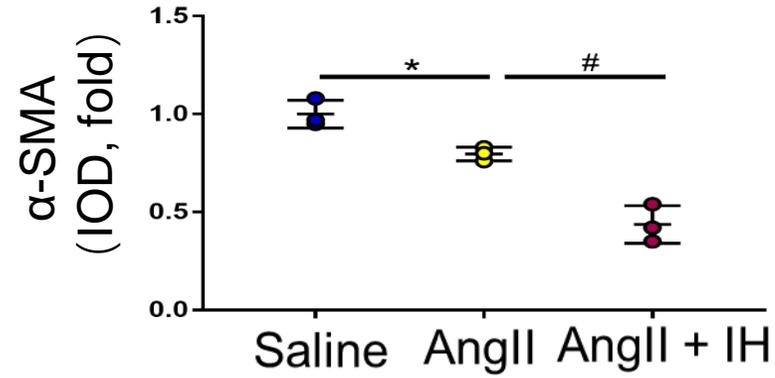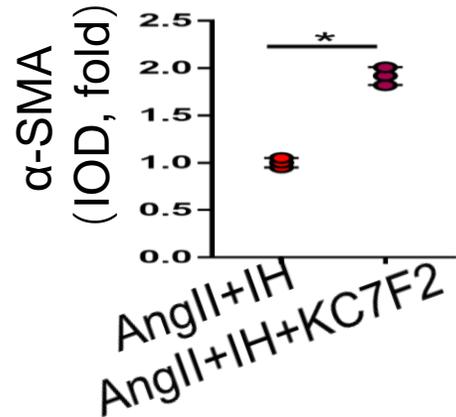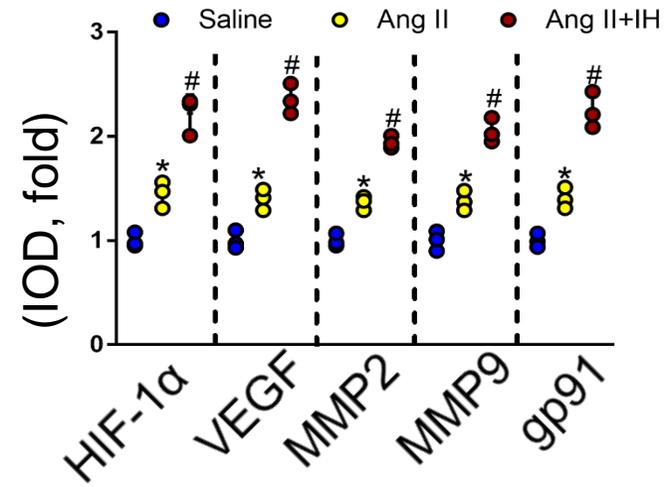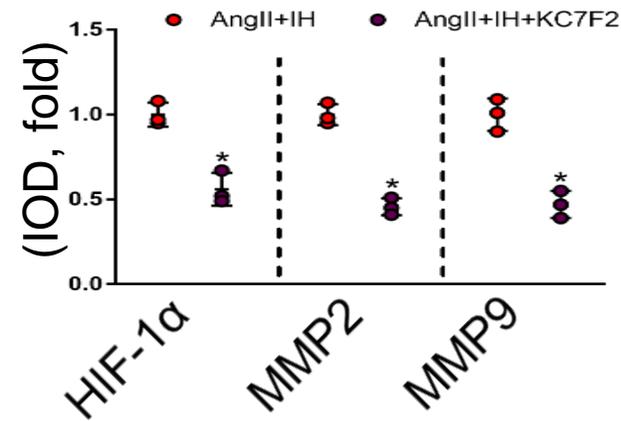
Figure S6. (A)Quantitative analysis of α-SMA.(B) quantitative analysis of HIF-1α, VEGF, MMP2, MMP9 and the subunits of NAD (P) H gp-91 expression (*p<0.05 vs. saline; #p <0.05 vs. Ang II, one-way ANOVA). (C)Quantitative analysis of α-SMA.(D) quantitative analysis of HIF-1α, MMP2, MMP9 expression (*p<0.05, Ang II+IH group *vs.* the Ang II+IH+KC7F2 group, *t*-test).Scatter plot summarized the results. All data represent the means ± SEM.

Supplementary Figure 3
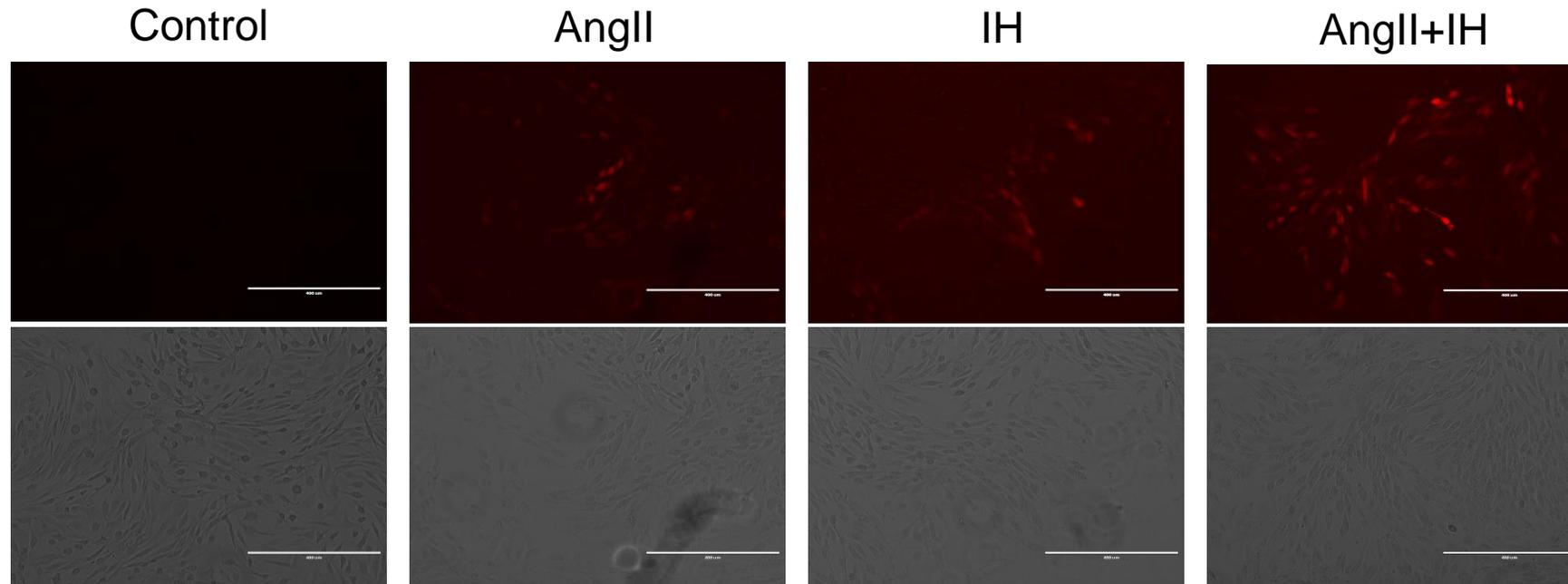
Figure S3. Ang II and IH can promote the ROS production in cultured VSMCs *in vitro*, IH on top can further increase the ROS production. Dihydroethidium staining of VSMCs were pretreated with Ang II (10 µM) or IH and both of Ang II and IH for 24 h (upper panel) , and representative light microcopy pictures for each group respectively (lower panel). Scale Bar: 400µm.

Supplementary Figure 4
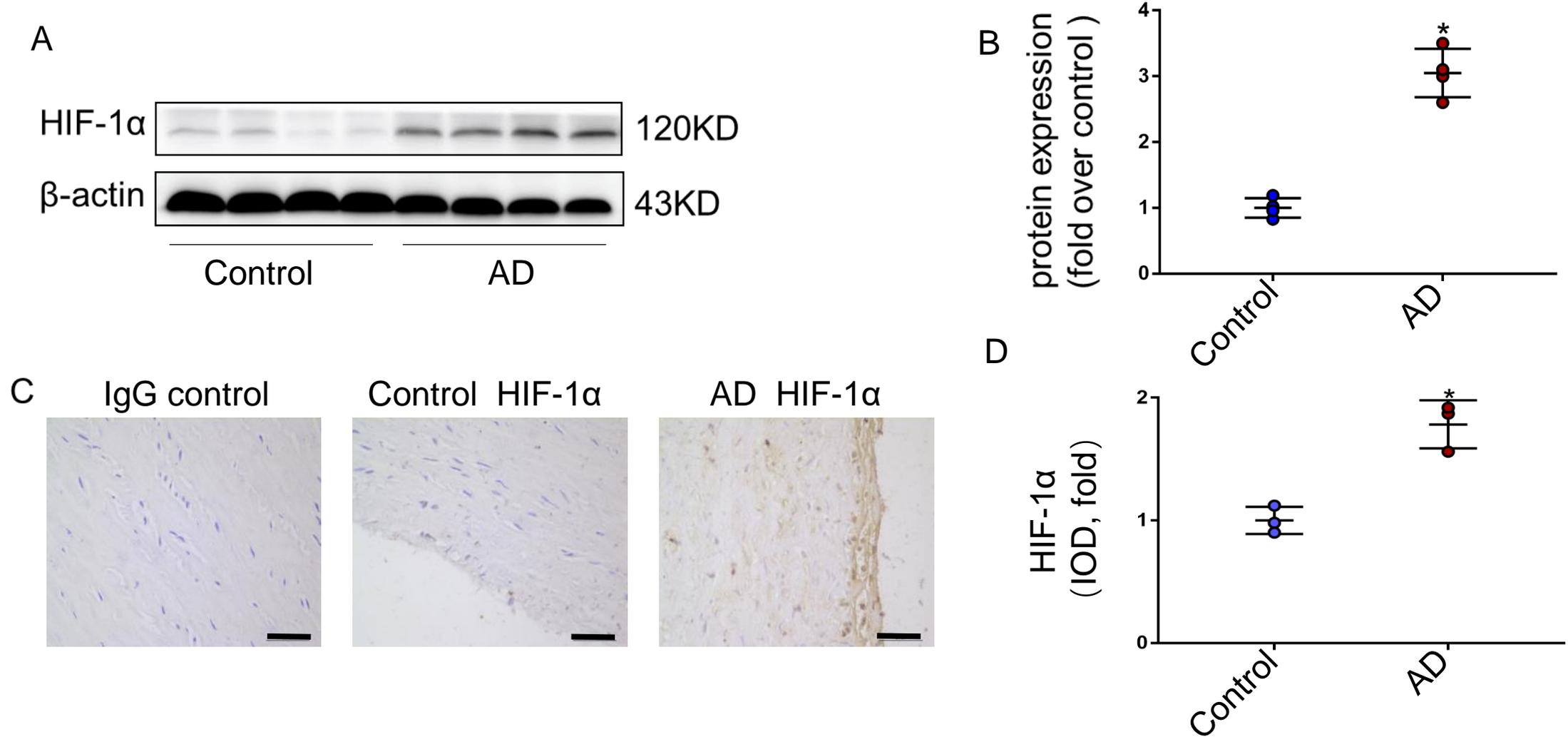
Figure S4. HIF-1α expression was upregulated in human AD samples. Western blotting shows the expression of HIF-1α in human AD tissues compared with respective control aortae(A) and Scatter plot summarized the results(B). Immunohistochemistry staining show the expression of HIF-1α in human AD tissues compared with respective control aortae (C) and Scatter plot summarized the results(C). All data represent the means ± SEM; * p < 0.05 *vs.* control, Scale Bar 50μm.

Supplementary Figure 5
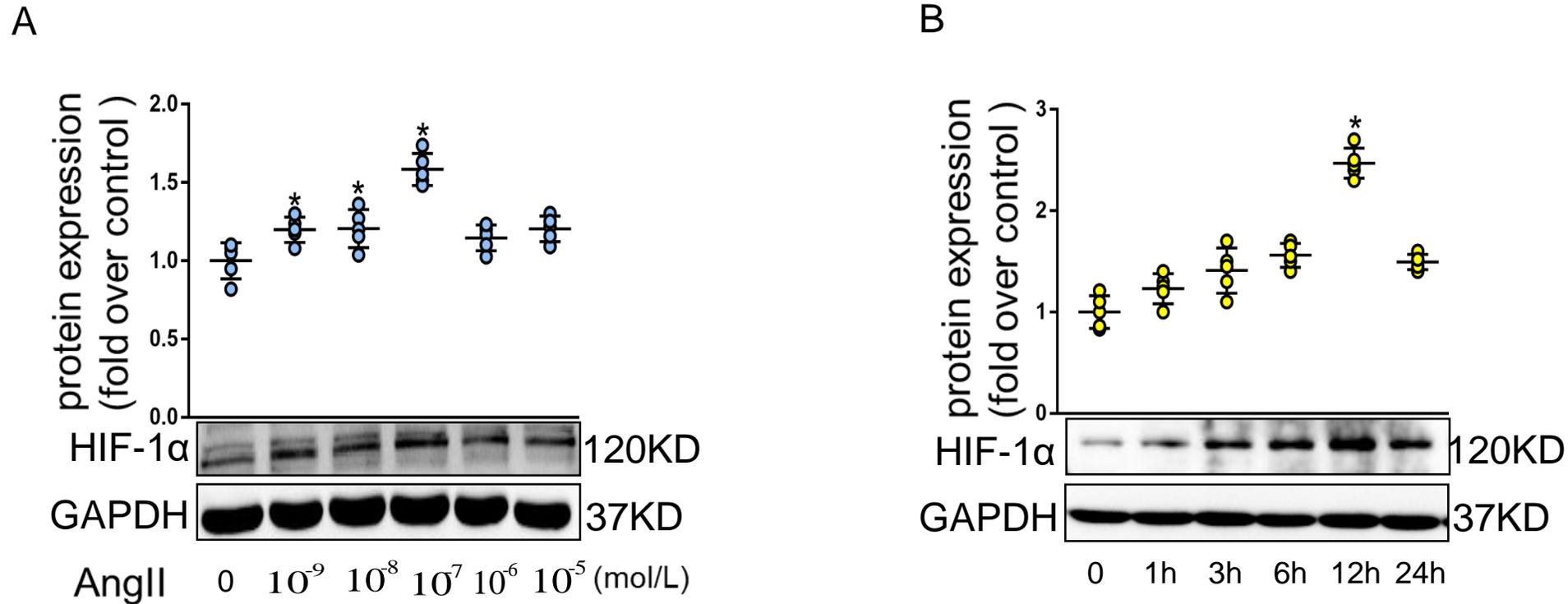
Figure S5. **HIF-1α expression can be induced by Ang II treatment in cultured VSMCs *in vitro*.** Western blotting shows the induction of HIF-1α by AngII in a concentration dependent (A) and time dependent (B) manner, Scatter plot summarized the results. All data represent the means ± SEM; * $p < 0.05$ *vs.* control.

Supplementary Figure 6
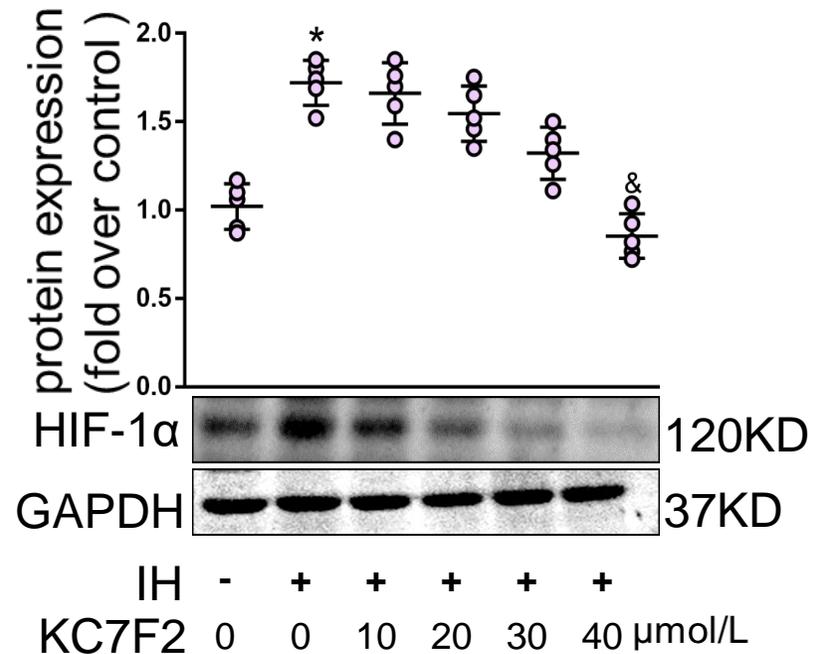
Figure S6. KC7F2 can dose dependently block the IH induced HIF-1α expression in cultured VSMCs *in vitro*. Western blotting shows the expression of HIF-1α in VSMCs can be significantly induced by IH exposure. The HIF-1α inhibitor KC7F2 can suppress the IH induced HIF-1α in a concentration dependent manner. Scatter plot summarized the results. All data represent the means ± SEM; * $p < 0.05$ vs. control, & $p < 0.05$ *vs.* IH alone.