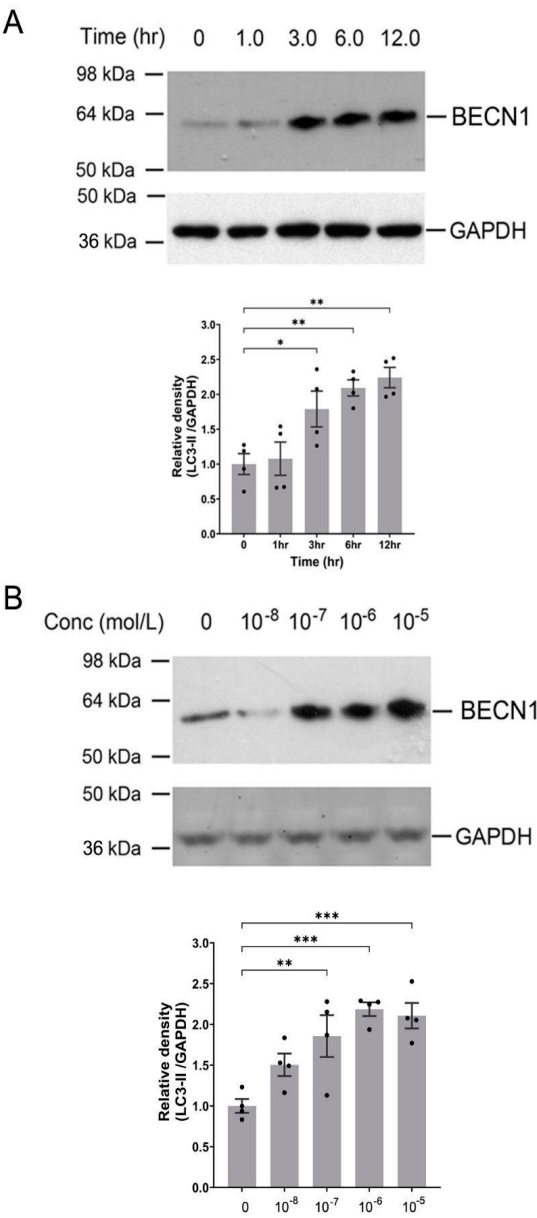


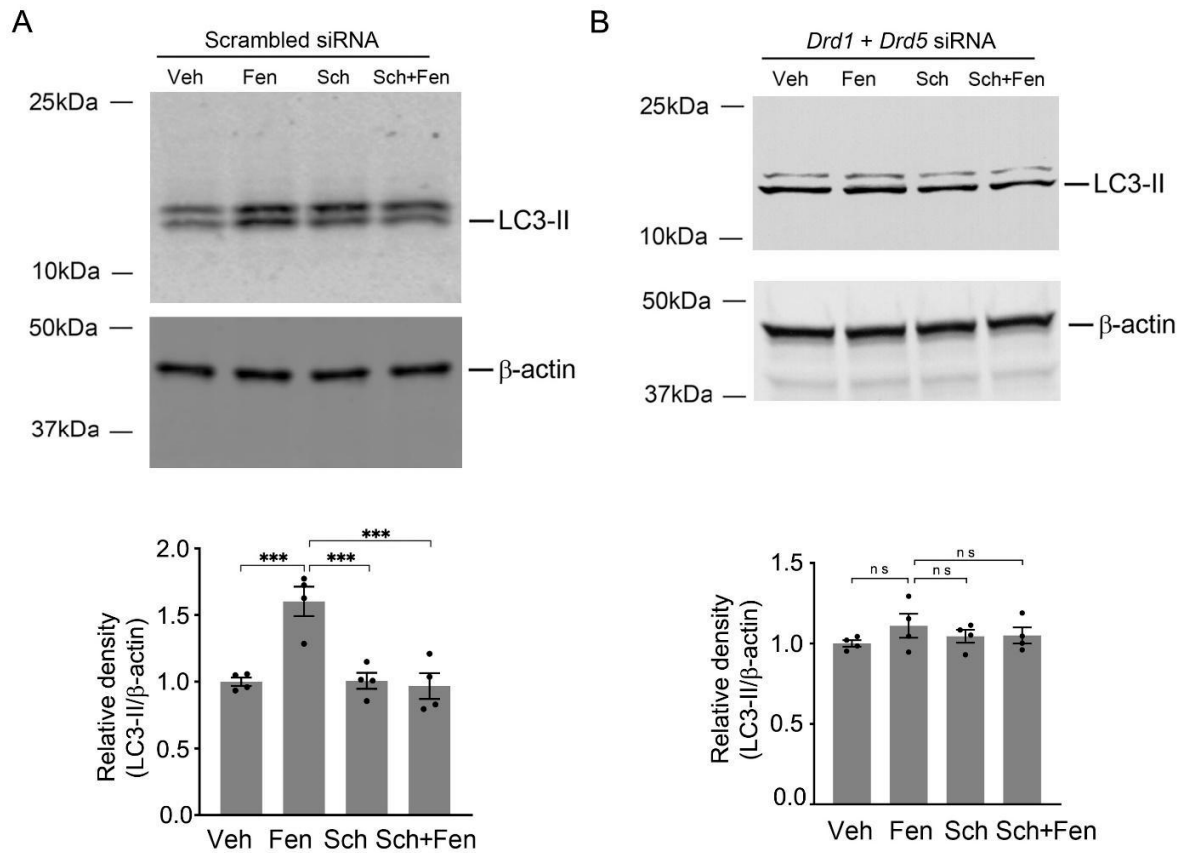
Supplementary file

Supplementary Figures

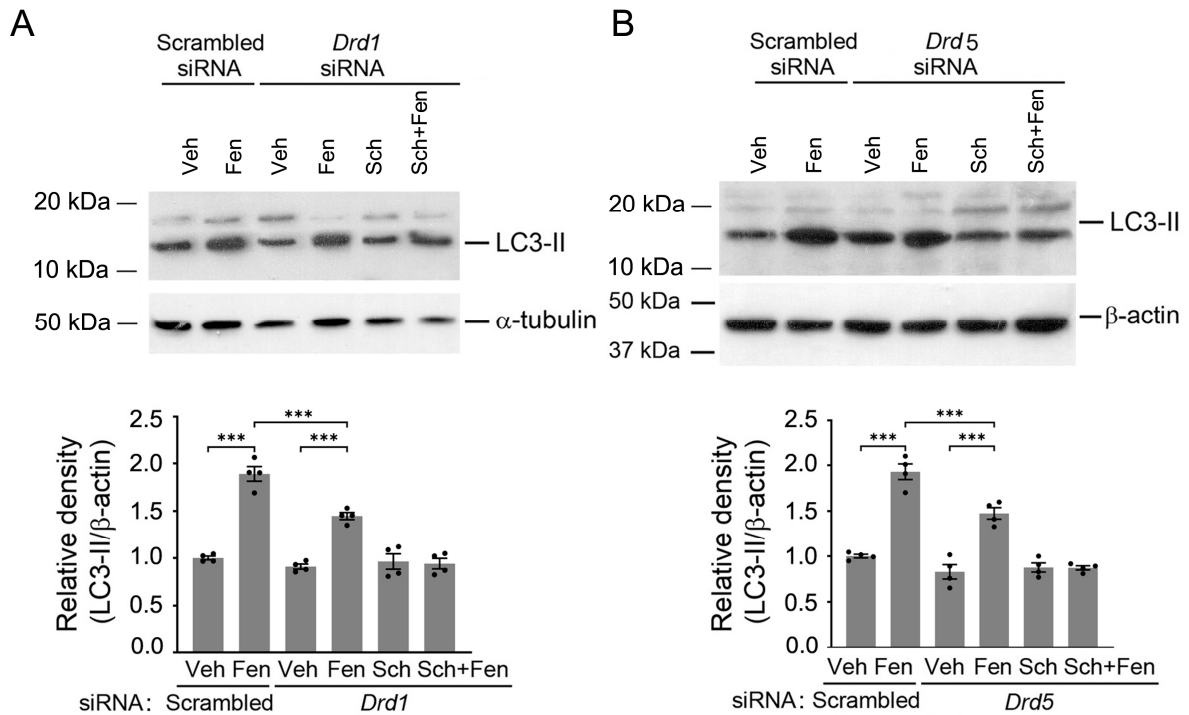


Supplementary Figure S1. Time course and concentration response of the fenoldopam (Fen)-mediated increase in beclin-1 (BECN1) protein expression in VSMCs. (A) VSMCs

were treated with Fen (1 $\mu\text{mol/L}$) at the indicated time. **(B)** VSMCs were treated with Fen for 6 hours by the indicated concentrations. **(A,B)** BECN1 protein expressions were quantified and expressed as the ratio of BECN1 over GAPDH densities, then normalized by their controls (0 hour or 0 mol/L). $n = 4/\text{Group}$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs control (0 hour or 0 mol/L), ns, not significant. One-way ANOVA, Bonferroni multiple comparison test.



Supplementary Figure S2. LC3-II protein expression in VSMCs transfected with scrambled or combined *Drd1* and *Drd5* siRNAs. (A) VSMCs were transfected with scrambled siRNA (48 hours) and treated with vehicle (Veh) or Fen (1.0 μ mol/L) for 6 hours, prior to the end of the 48 hour-transfection, in the absence or presence of the D1-like receptor antagonist, Sch23390 (Sch) (1.0 μ mol/L, added 30 minutes prior to the addition of Fen). $n = 4$ /Group, *** $p < 0.001$. One-way ANOVA, Bonferroni multiple comparison test. (B) VSMCs were transfected with combined *Drd1* and *Drd5* siRNAs (48 hours) and treated with Veh or Fen as in (A). Veh, vehicle; Fen, fenoldopam, Sch, Sch23390. $n = 4$ /Group, ns, not significant. One-way ANOVA, Bonferroni multiple comparison test.

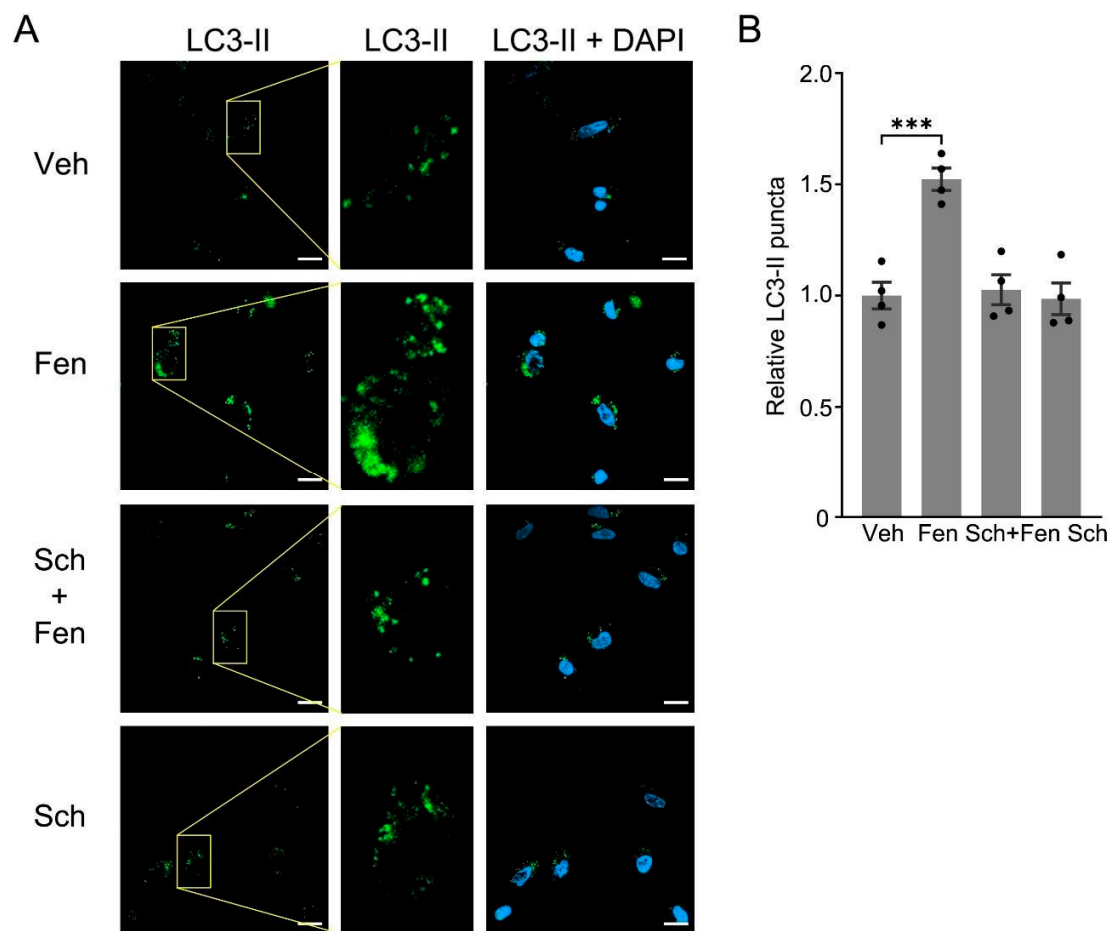


Supplementary Figure S3. LC3-II protein expression in VSMCs transfected with either

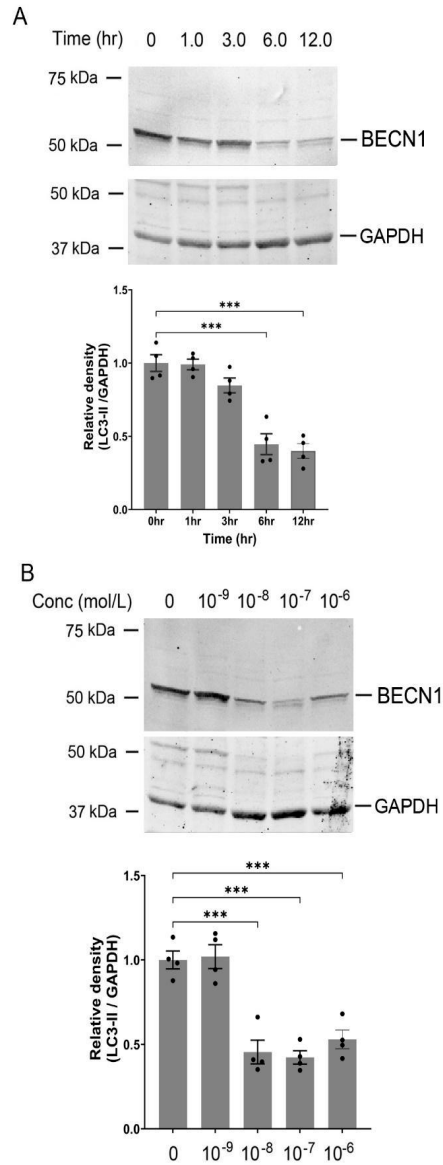
***Drd1* or *Drd5* and their scrambled siRNAs.** (A) VSMCs were transfected with *Drd1* or its scrambled siRNA (48 hours) and treated with vehicle (Veh) or Fen (1.0 $\mu\text{mol/L}$), as indicated, for 6 hours, prior to the end of the 48 hour-transfection, in the absence or presence of the D1-like receptor antagonist, Sch23390 (Sch) (1.0 $\mu\text{mol/L}$, added 30 minutes prior to the addition of Fen).

(B) VSMCs were transfected with combined *Drd5* or its scrambled siRNA (48 hours) and treated with Veh or Fen as in (A). (A,B) Veh, vehicle; Fen, fenoldopam, Sch, Sch23390. $n = 4/\text{Group}$,

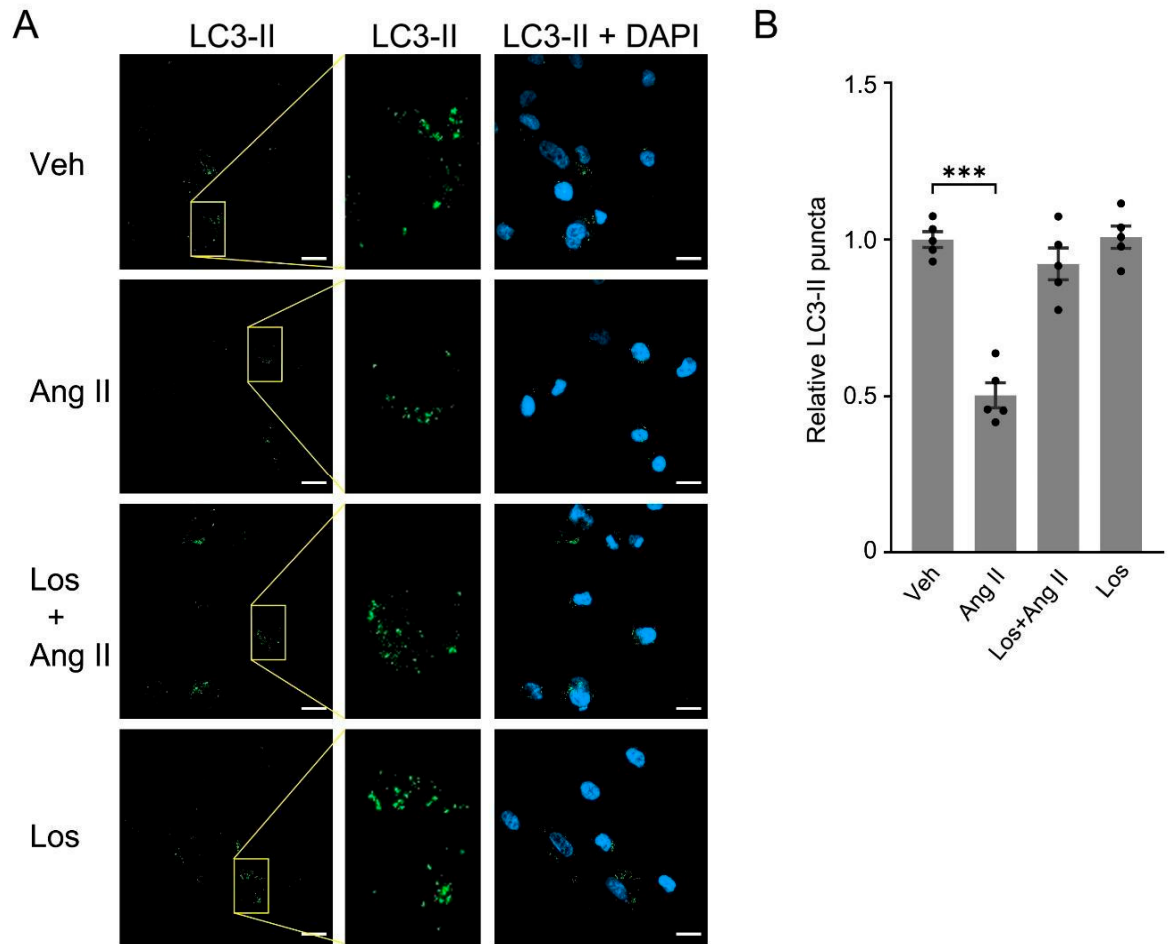
*** $p < 0.001$, ns, not significant. One-way ANOVA, Bonferroni multiple comparison test.



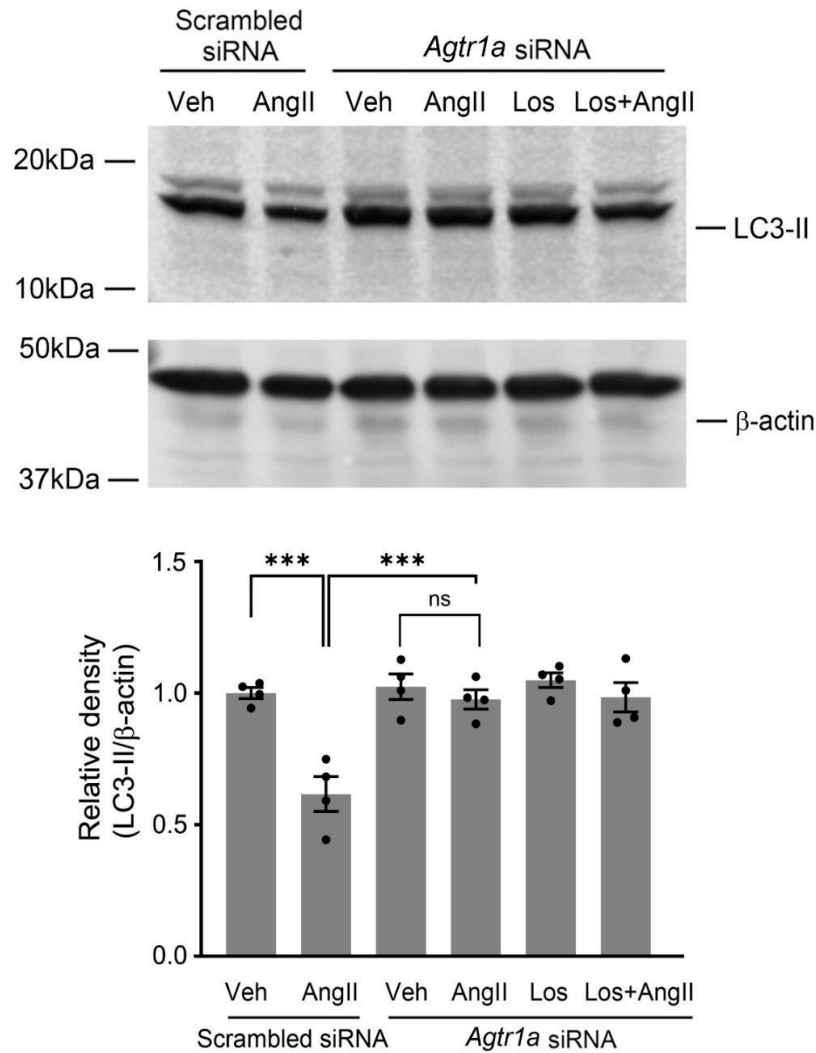
Supplementary Figure S4. Fen increases LC3-II puncta through D1-like receptors in VSMCs. (A) VSMCs were treated with Veh (vehicle), Fen (fenoldopam, 1.0 $\mu\text{mol/L}$), Sch (1 $\mu\text{mol/L}$) or Sch + Fen (combination of Fen and Sch) as indicated for 12 hours. Representative immunofluorescence images show endogenous LC3-II (green). Blue nuclei (DAPI). Scale bars, 10 μm . (B) The number of LC3-II puncta was counted by ImageJ; the number of cells were counted manually by their nuclei (cells on the edges were excluded for analysis). The number of LC3-II puncta was divided by the number of cells; each group was normalized by the average number of the Veh group. $n = 4/\text{Groups}$, *** $P < 0.001$. One-way ANOVA, Bonferroni multiple comparison test.



Supplementary Figure S5. Time course and concentration response of the Ang II-mediated decrease in beclin-1 (BECN1) protein expression in VSMCs. (A) VSMCs were treated with Ang II (100 nmol/L) at the indicated times. (B) VSMCs were treated with Ang II by the indicated concentrations for 6 hours. (A,B) BECN1 protein expression was quantified and expressed as the ratio of BECN1 over GAPDH densities, then normalized by the controls (0 hour or 0 mol/L). $n = 4/\text{Groups}$, *** $P < 0.001$. One-way ANOVA, Bonferroni multiple comparison test.

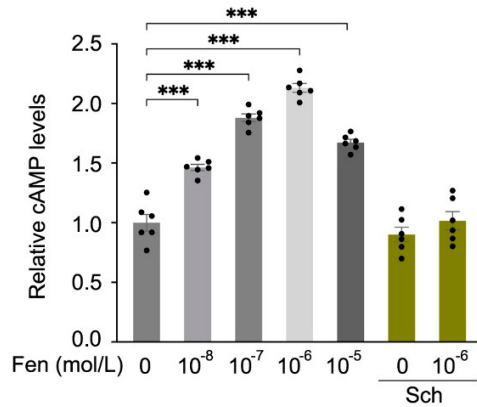


Supplementary Figure S6. Angiotensin II (Ang II) decreases LC3-II puncta through AT₁R in VSMCs. (A) VSMCs were treated with Veh (vehicle), Ang II (100 nmol/L), Los (losartan, 10 μ mol/L), or their combination (Los + Ang II), as indicated for 12 hours. Representative immunofluorescence images show endogenous LC3-II (green). Blue, nuclei (DAPI). Scale bars, 10 μ m. (B) The numbers of LC3-II puncta were counted by ImageJ; the number of cells were counted manually by their nuclei (cells on the edges were excluded for analysis). The number of LC3-II puncta were divided by the number of cells; each group was normalized by the average number of Veh group. $n = 5/\text{Group}$, *** $p < 0.001$. One-way ANOVA, Bonferroni multiple comparison test.

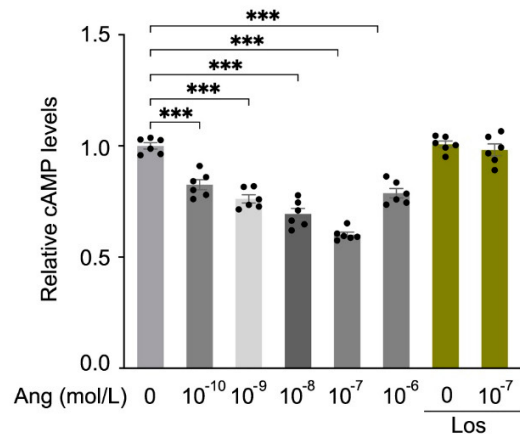


Supplementary Figure S7. LC3-II protein expression in VSMCs transfected with either scrambled or *Agtr1a* siRNA in VSMCs. VSMCs were transfected with siRNAs (48 hours) and then treated with vehicle (Veh) or Ang II (Ang, 100 nmol/L) for 6 hours, prior to the end of 48 hour-transfection in the absence or presence of the AT₁R antagonist, losartan (Los) (10 μmol/L), added 30 minutes prior to the addition of Ang II. LC3-II protein expressions were quantified and expressed as the ratio of LC3-II over β-actin densities, then normalized by the controls (0 hour or 0 mol/L). $n = 4/\text{Group}$, *** $p < 0.001$, ns, not significant. One-way ANOVA, Bonferroni multiple comparison test.

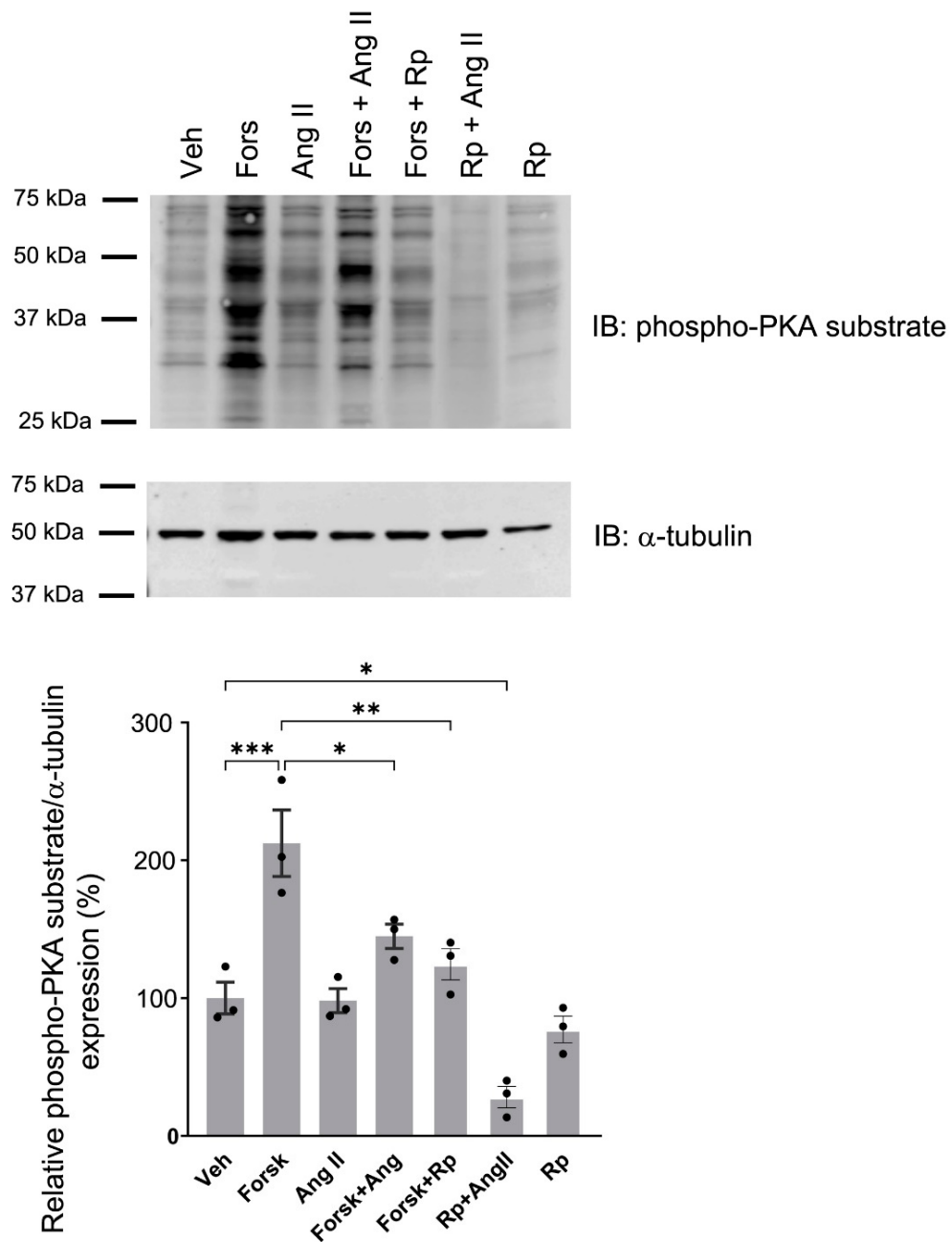
A



B

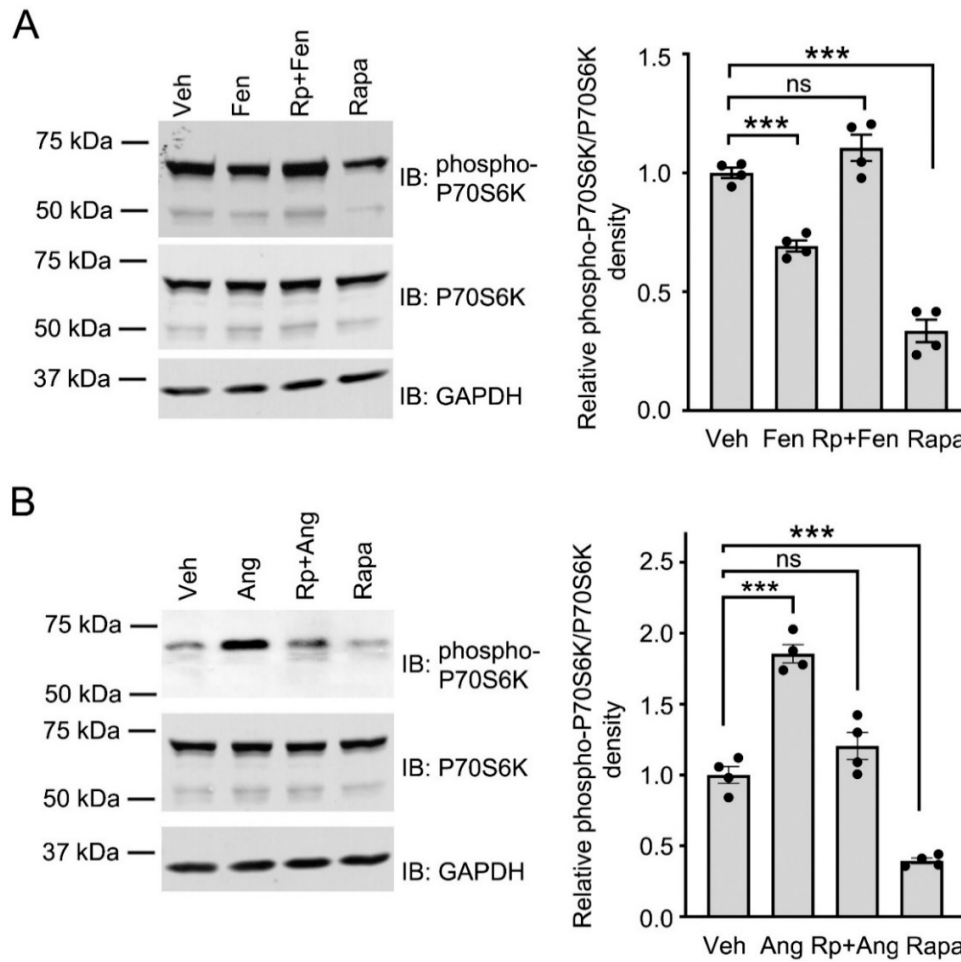


Supplementary Figure S8. Concentration responses of fenoldopam and angiotensin II on cAMP production in VSMCs. (A) VSMCs were treated with fenoldopam (Fen) by the indicated concentrations for 15 minutes. Sch, Sch23390 (1 $\mu\text{mol/L}$). (B) VSMCs were incubated with forskolin for 10 minutes before treatment with Ang II by the indicated concentrations for 15 minutes. Ang, Ang II; Los, losartan (10 $\mu\text{mol/L}$); Veh, vehicle. $n = 6/\text{Group}$, *** $p < 0.001$. One-way ANOVA, Bonferroni multiple comparison test.



Supplementary Figure S9. Protein expression of phospho-PKA substrate in VSMCs treated with Ang II, Rp-cAMPS, and their combinations. VSMC were exposed to Vehicle, forskolin (10 μ M), Ang II (100 nM), Rp-cAMPS (50 μ M) and their combinations as indicated for 15 min. For the combination groups, Rp-cAMPS was added 30 min earlier before Ang II or forskolin was

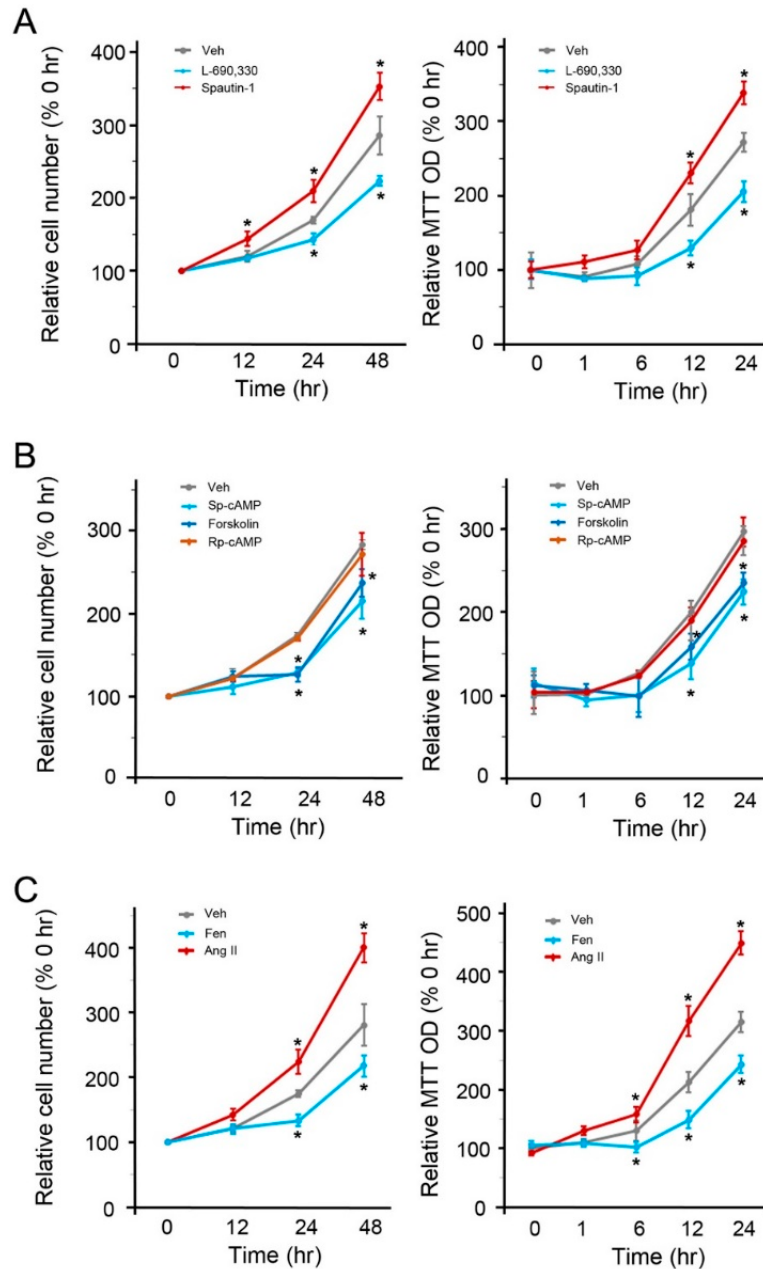
added and then incubated together for 15 min. Ang II, angiotensin II; Fors, forskolin; Rp, Rp-cAMPS. The α -tubulin blot served as the sample loading control. The total band intensities of phospho-PKA substrates between 25 kDa and 75 kDa were quantified over their corresponding α -tubulin band intensities. The average band intensity ratio of phospho-PKA substrate/ α -tubulin of the vehicle group was set at 100%. $n = 3/\text{Group}$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. One-way ANOVA, Bonferroni multiple comparison test.



Supplementary Figure S10. Fen- and Ang II-mediated mTOR regulation is cAMP

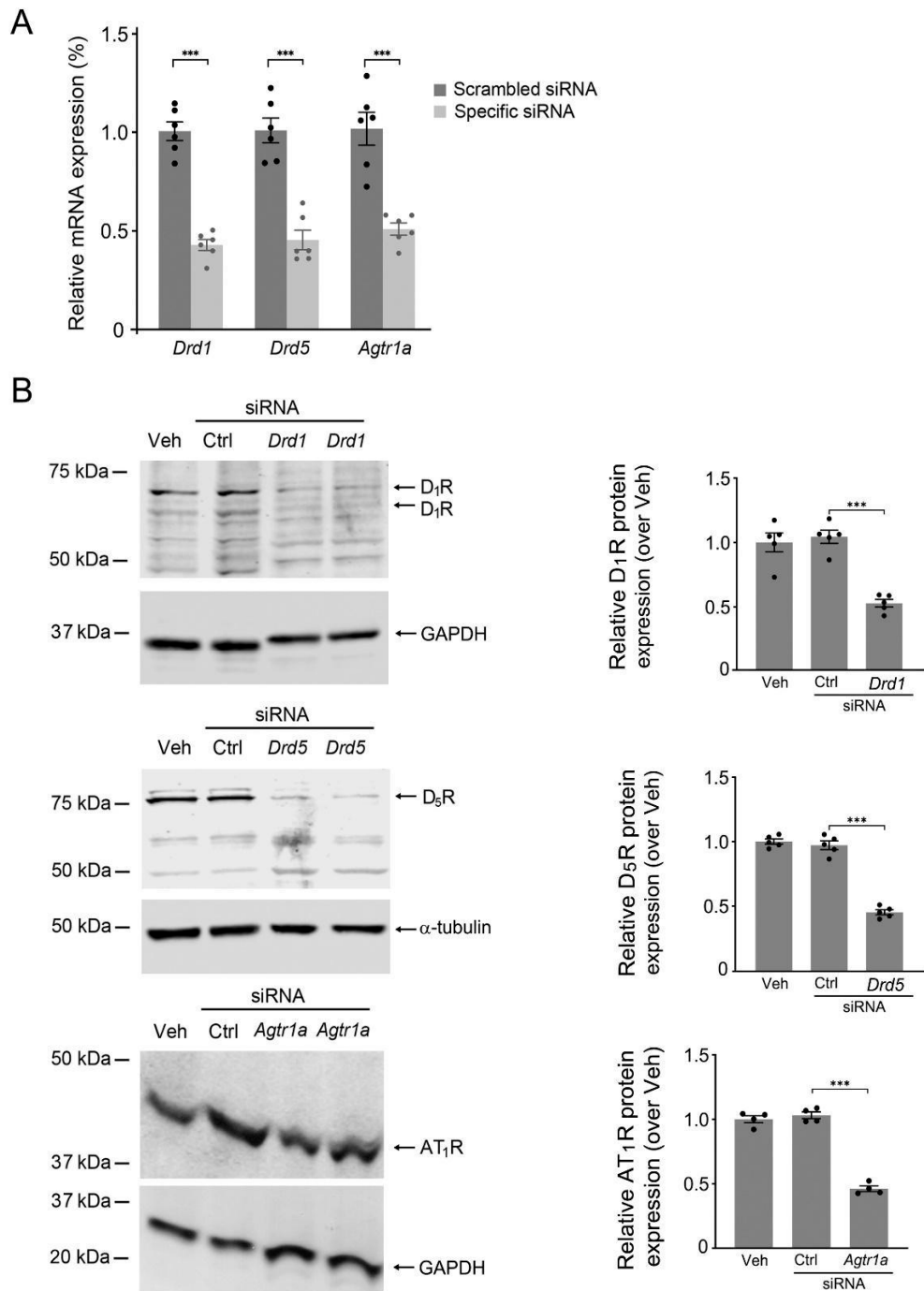
dependent in VSMCs. (A) Fen (1 $\mu\text{mol/L}$, 6 hours) decreased the phosphorylation of P70S6K (a direct substrate of mTOR), which was inhibited by Rp-cAMPS (Rp, 50 $\mu\text{mol/L}$, 6 hours).

Rapamycin (Rapa, 100 nmol/L , 6 hours), a positive control, decreased the phosphorylation of P70S6K. $n = 4/\text{Group}$, *** $P < 0.001$, ns, not significant. One-way ANOVA, Bonferroni multiple comparison test. (B) Ang II (100 nmol/L , 6 hours) increased the phosphorylation of P70S6K, which was inhibited by Rp-cAMPS (Rp, 50 $\mu\text{mol/L}$, 6 hours). Rapamycin (Rapa, 100 nmol/L , 6 hours), a positive control, decreased the phosphorylation of P70S6K. $n = 4/\text{Group}$, *** $p < 0.001$, ns, not significant. One-way ANOVA, Bonferroni multiple comparison test.



Supplementary Figure S11. Analysis of VSMC proliferation by cell counting (left) and MTT assay (right). (A) VSMCs were treated with vehicle (Veh), L-690,330, an autophagy inducer (10 μ mol/L), or spautin-1, an autophagy inhibitor (10 μ mol/L), at the indicated times. (B) VSMCs were treated with vehicle (Veh), Sp-cAMPS, a PKA activator (1.0 μ mol/L), forskolin (10 μ mol/L), an adenylyl cyclase activator, or Rp-cAMPS (50 μ mol/L), a PKA

inhibitor at the indicated times. (C) VSMCs were treated with vehicle (Veh), Fen (1 μ mol/L), or Ang II (100 nmol/L) at the indicated times. Values (100 % at 0 time) are shown, using Veh-treated cells at their initial time point (0 hour) for normalization. (A – C) Data are mean $\pm$ standard deviation, $n = 3$ /Group, * $p < 0.05$ vs Veh. Two-way ANOVA, Bonferroni multiple comparison test.



Supplementary Figure S12. Effects of *Drd1*, *Drd5*, and *Agtr1a* siRNAs transfected into VSMCs. (A) mRNA expression was quantified by qRT-PCR. VSMCs were transfected with *Drd1*, *Drd5*, and *Agtr1a* siRNAs or scrambled siRNA. $n = 6/\text{Group}$, *** $p < 0.001$ vs scrambled

siRNA. One-way ANOVA, Bonferroni multiple comparison test. **(B)** Protein expression was quantified by immunoblotting. VSMCs were transfected with *Drd1*, *Drd5*, *Agtr1a* siRNAs, or scrambled siRNA (Ctrl, control), as in Figure S1A. The cell lysates were electrophoresed (SDS-PAGE) and the proteins, transferred onto nitrocellulose membranes, were immunoblotted with specific anti-D₁R, anti-D₅R, and anti-AT₁R antibodies. Veh, vehicle (RNase-free water); Ctrl, control (scrambled siRNA); IB, immunoblot. One set of blots from four independent experiments are shown. The densities of D₁R, D₅R, or AT₁R immunoblots from Veh, Ctrl (scrambled siRNA), and average of duplicated specific siRNA immunoblots were quantified and expressed as the ratio of D₁R, D₅R, or AT₁R over their individual loading controls and then normalized with Veh group. $n = 4-5/\text{Group}$, *** $p < 0.001$. One-way ANOVA, Bonferroni multiple comparison test.

Supplementary Table S1. Primary antibodies used in this study

Antibody	Source	Cat. No.	Working condition	Note
LC3-II	Cell Signaling Technology	3868	1:500	Monoclonal Rabbit
Beclin-1	Cell Signaling Technology	3495	1:500	Monoclonal Rabbit
P70S6K	Cell Signaling Technology	9202	1:500	Polyclonal Rabbit
Phospho-P70S6K	Cell Signaling Technology	9205	1:500	Polyclonal Rabbit
Phospho-PKA substrate (RRXS*/T*)	Cell Signaling Technology	9624	1:500	Monoclonal Rabbit
D ₁ R	In-house	NA	1:200	Polyclonal Rabbit
D ₅ R	In-house	NA	1:250	Chicken IgY
AT ₁ R	GeneTex	GTX89149	1:200	Polyclonal goat
α -tubulin	Abcam	ab4074	1:2000	Polyclonal Rabbit
GAPDH	Abcam	ab8245	1:2000	Monoclonal mouse
α -actin	Millipore Sigma	CBL171	1:500	Monoclonal mouse
β -actin	Millipore Sigma	A1978	1:5000	Monoclonal mouse