Fig. S1

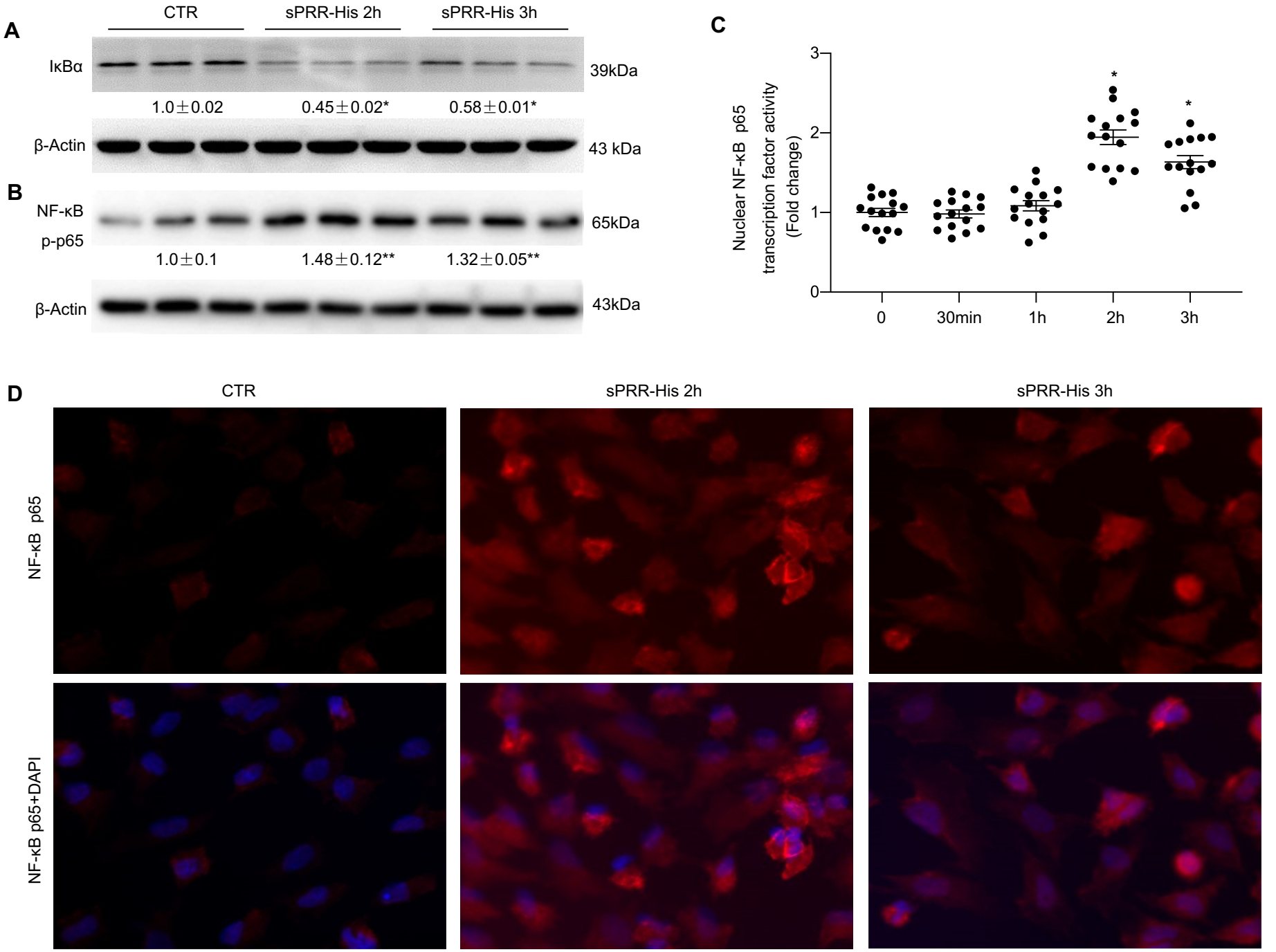

A
CTR
sPRR-His 2h
sPRR-His 3h
IκBα
39kDa
1.0±0.02
0.45±0.02*
0.58±0.01*
β-Actin
43 kDa
B
NF-κB
p-p65
65kDa
1.0±0.1
1.48±0.12**
1.32±0.05**
β-Actin
43kDa
C
Nuclear NF-κB p65
transcription factor activity
(Fold change)
0
1
2
3
*
*
0
30min
1h
2h
3h
D
CTR
sPRR-His 2h
sPRR-His 3h
NF-κB p65
NF-κB p65+DAPI

Fig. S1. sPRR-His activates NF-κB in HUVECs. HUVECs were treated with blank buffer or 50nM sPRR-His for the indicated durations. Relative protein expression levels of IκBα (**A**) and NF-κB p-p65 (**B**) were measured by Western blotting. The value beneath the image indicates the densitometry of the protein normalized to β-Actin for 3 separate experiments. (**C**) Nuclear proteins were extracted for a NF-κB p65 transcription factor activity assay. Statistical significance was determined by using unpaired Student's t test. *P < 0.01, compared with the CTR; **P < 0.05, compared with the CTR. (**D**) Cells were processed for immunofluorescence staining to assess the activation of NF-κB p65. N=9-15 (N represents the number of samples in each group, and the repetitions of separate experiments are 3-5). DAPI indicates 4′,6-diamidino-2-phenylindole. Data are mean ± SEM.

Fig. S2

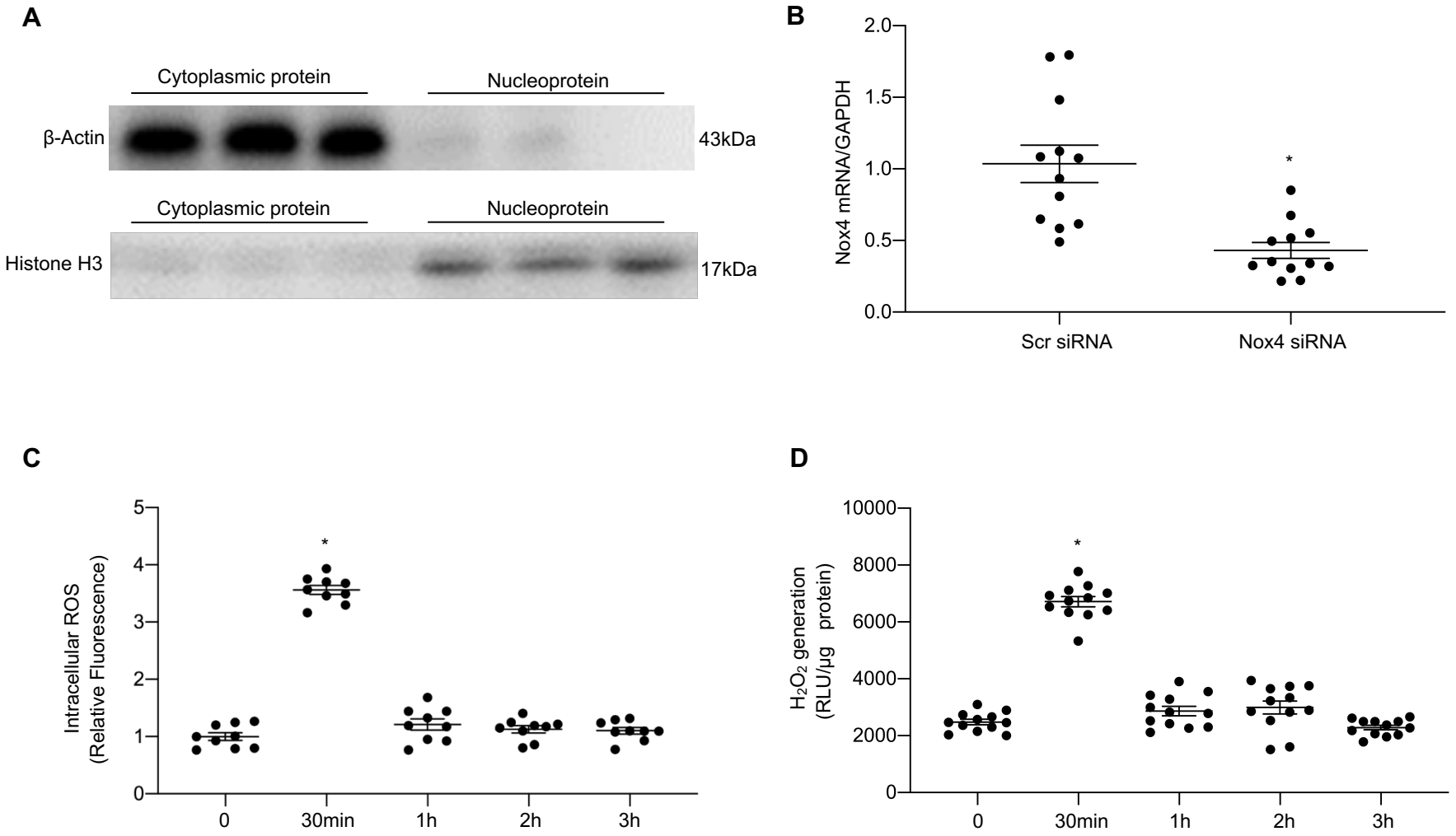

A
Cytoplasmic protein
Nucleoprotein
β-Actin
43kDa
Cytoplasmic protein
Nucleoprotein
Histone H3
17kDa
B
Nox4 mRNA/GAPDH
2.0
1.5
1.0
0.5
0.0
*
Scr siRNA
Nox4 siRNA
C
Intracellular ROS
(Relative Fluorescence)
5
4
3
2
1
0
*
0
30min
1h
2h
3h
D
H2O2 generation
(RLU/μg protein)
10000
8000
6000
4000
2000
0
*
0
30min
1h
2h
3h

Fig. S2. sPRR-His increases intracellular H_2O_2. (**A**) Successful separation of cytoplasmic and nuclear fractions was verified using immunoblotting procedures. Relative protein expression levels of β-Actin in cytoplasmic protein and nucleoprotein were measured by Western blotting. Relative protein expression levels of Histone H3 in cytoplasmic protein and nucleoprotein were measured by Western blotting. (**B**) Validation of Nox4 knockdown by qRT-PCR. (**C**-**D**) HUVECs were treated with blank buffer or 50nM sPRR-His for 30min, 1h, 2h and 3h. (**C**) The formation of intracellular ROS was measured via monitoring the increasing fluorescence of 2'7'-dichlorofluorescein (DCF), and results are given as fold of changes over untreated controls. (**D**) Time course of H_2O_2 generation after 50nM sPRR-His treatment. RLU, relative light units. N=9-12 (N represents the number of samples in each group, and the repetitions of separate experiments are 3-4). Statistical significance was determined by using unpaired Student's t test. *P<0.01, compared with the CTR or Scr siRNA. Data are mean ± SEM.

Fig. S3

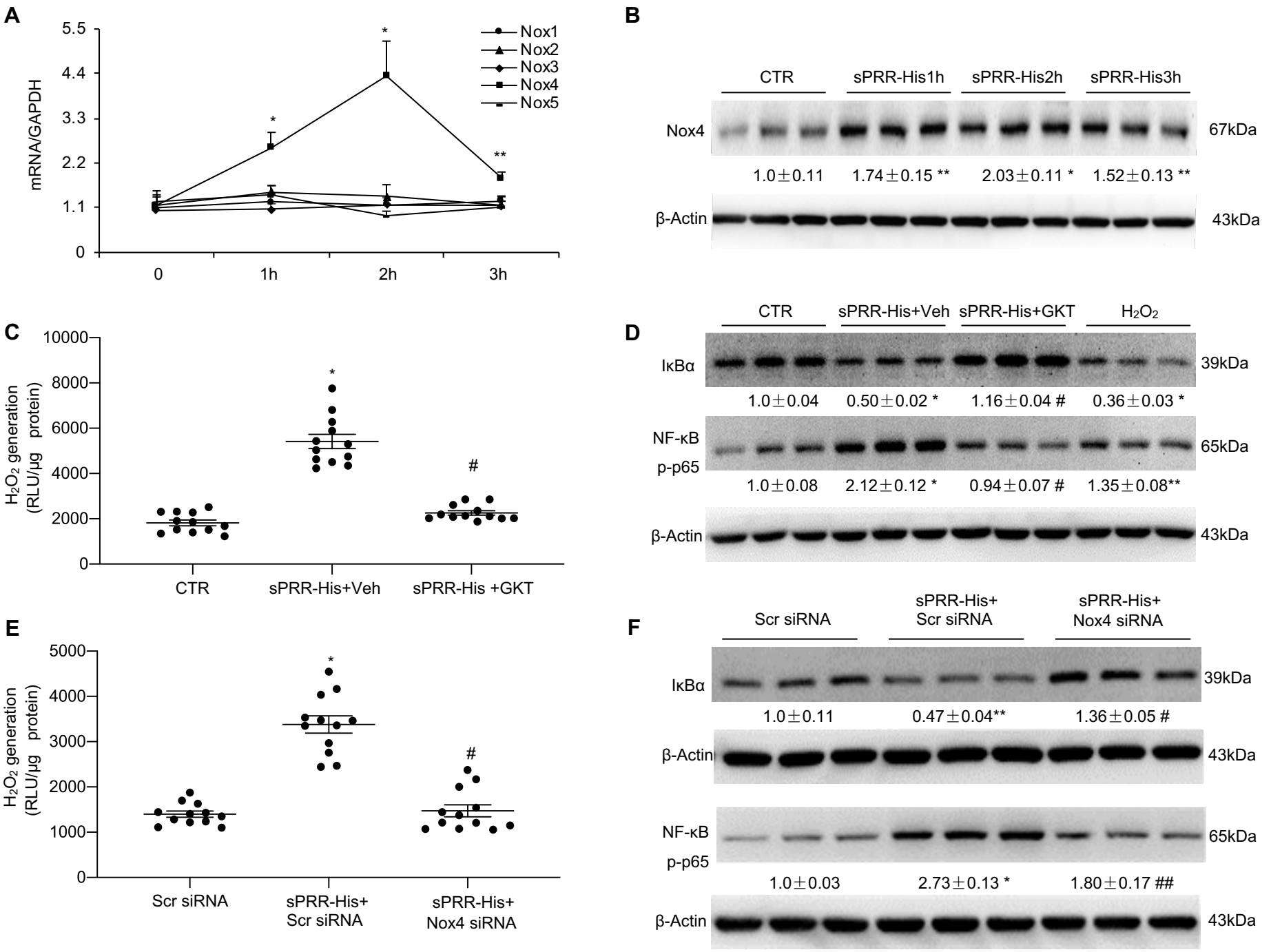

A
mRNA/GAPDH
5.5
4.4
3.3
2.2
1.1
0
0
1h
2h
3h
Nox1
Nox2
Nox3
Nox4
Nox5
B
CTR
sPRR-His1h
sPRR-His2h
sPRR-His3h
Nox4
67kDa
1.0±0.11
1.74±0.15 **
2.03±0.11 *
1.52±0.13 **
β-Actin
43kDa
C
H2O2 generation (RLU/μg protein)
10000
8000
6000
4000
2000
0
CTR
sPRR-His+Veh
sPRR-His +GKT
D
CTR
sPRR-His+Veh
sPRR-His+GKT
H2O2
IκBα
39kDa
1.0±0.04
0.50±0.02 *
1.16±0.04 #
0.36±0.03 *
NF-κB p-p65
65kDa
1.0±0.08
2.12±0.12 *
0.94±0.07 #
1.35±0.08**
β-Actin
43kDa
E
H2O2 generation (RLU/μg protein)
5000
4000
3000
2000
1000
0
Scr siRNA
sPRR-His+ Scr siRNA
sPRR-His+ Nox4 siRNA
F
Scr siRNA
sPRR-His+ Scr siRNA
sPRR-His+ Nox4 siRNA
IκBα
39kDa
1.0±0.11
0.47±0.04**
1.36±0.05 #
β-Actin
43kDa
NF-κB p-p65
65kDa
1.0±0.03
2.73±0.13 *
1.80±0.17 ##
β-Actin
43kDa

Fig. S3. sPRR-His activates NF-κB secondary to increasing intracellular Nox4-derived H_2O_2. (**A**) Relative mRNA expression of Nox1, Nox2, Nox3, Nox4 and Nox5 in response to 50nM sPRR-His for different time. (**B**) Relative protein expression of Nox4 in response to 50nM sPRR-His. (**C**) HUVECs were pretreated with 10μM Nox1/4 inhibitor GKT137892 (GKT) or vehicle (Veh) for 1h followed by exposure to sPRR-His for 30min. Effect of GKT on sPRR-His-induced H_2O_2 generation. (**D**) HUVECs were pretreated with the 10μM GKT or vehicle (Veh) for 1h then followed by exposure to sPRR-His for 2h. Effect of GKT on sPRR-His-induced NF-κB activation. Effect of the administration of 200μM exogenous H_2O_2 on NF-κB activation. (**E**) HUVECs were transfected with scrambled small interfering RNA (Scr siRNA) or Nox4 siRNA, followed by exposure to sPRR-His for 30min. Effect of Nox4 siRNA knockdown on sPRR-His-induced H_2O_2 generation. (**F**) HUVECs were transfected with Scr siRNA or Nox4 siRNA, followed by exposure to sPRR-His for 2h. Effect of Nox4 siRNA knockdown on sPRR-His-induced NF-κB activation. The value beneath the image indicates the densitometry of the protein normalized to β-Actin for 3 separate experiments. N=9-12 (N represents the number of samples in each group, and the repetitions of separate experiments are 3-4). Statistical analysis was performed by using one-way ANOVA with the Bonferroni test for multiple comparisons or by using unpaired Student's t test for two comparisons. *P<0.01, compared with the CTR or Scr siRNA; **P<0.05, compared with the CTR or Scr siRNA; #P<0.01, compared with the sPRR-His or sPRR-His+Scr siRNA; ##P<0.05, compared with the sPRR-His or sPRR-His +Scr siRNA. Data are mean ± SEM.

Fig. S4

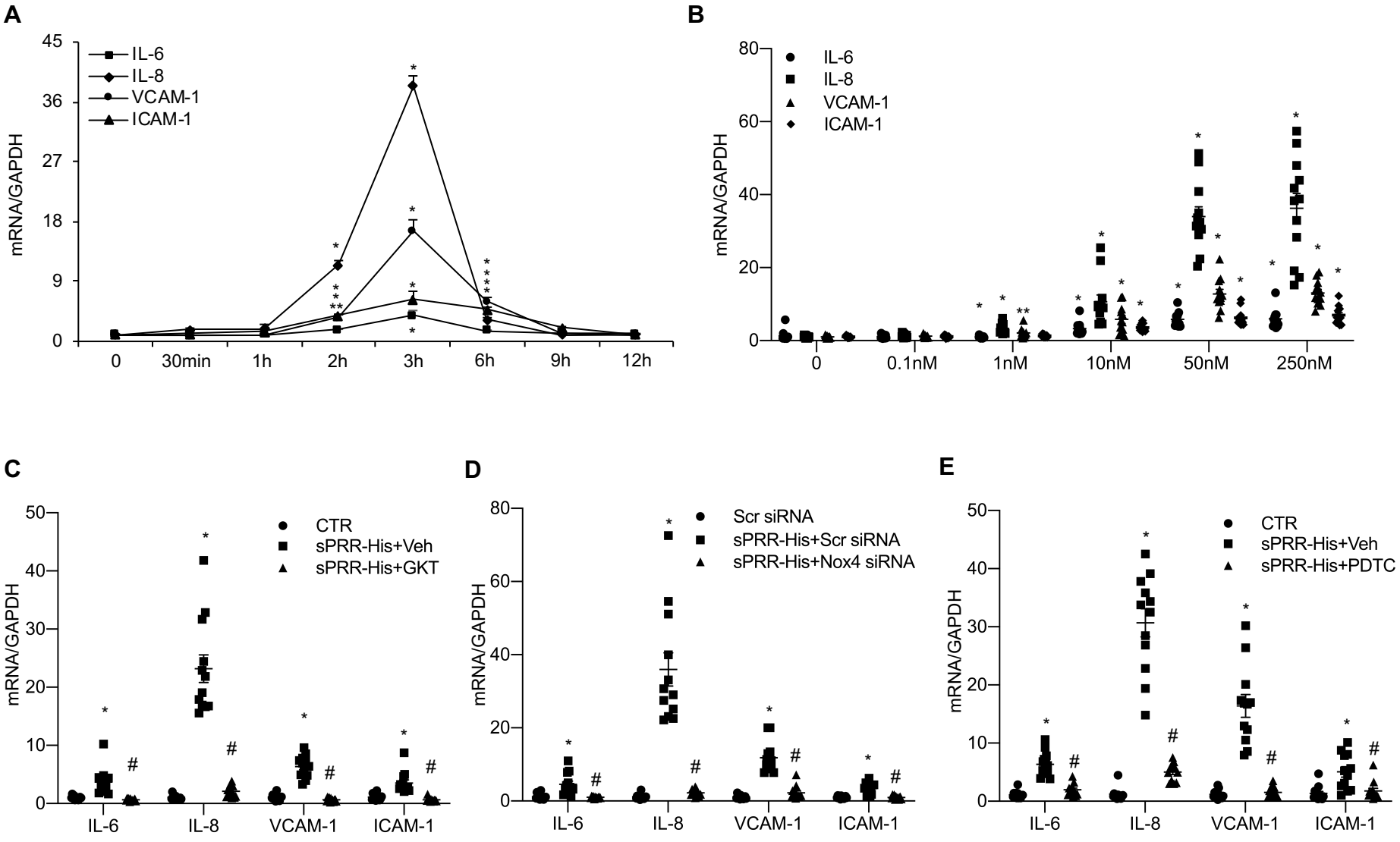

A
IL-6
IL-8
VCAM-1
ICAM-1
mRNA/GAPDH
0
9
18
27
36
45
0
30min
1h
2h
3h
6h
9h
12h
B
IL-6
IL-8
VCAM-1
ICAM-1
mRNA/GAPDH
0
20
40
60
80
0
0.1nM
1nM
10nM
50nM
250nM
C
CTR
sPRR-His+Veh
sPRR-His+GKT
mRNA/GAPDH
0
10
20
30
40
50
IL-6
IL-8
VCAM-1
ICAM-1
D
Scr siRNA
sPRR-His+Scr siRNA
sPRR-His+Nox4 siRNA
mRNA/GAPDH
0
20
40
60
80
IL-6
IL-8
VCAM-1
ICAM-1
E
CTR
sPRR-His+Veh
sPRR-His+PDTC
mRNA/GAPDH
0
10
20
30
40
50
IL-6
IL-8
VCAM-1
ICAM-1

Fig. S4. sPRR-His evokes inflammation. Relative mRNA expression levels of IL-6, IL-8, VCAM-1 and ICAM-1 were measured by qRT-PCR. (**A**) HUVECs were treated with blank buffer or 50nM sPRR-His for 30min, 1h, 2h, 3h, 6h, 9h and 12h. (**B**) HUVECs were treated with blank buffer or 0.1nM, 1nM, 10nM, 50nM, 250nM sPRR-His for 3h. Effects of 10μM GKT (**C**), Nox4 siRNA (**D**) or 50μM PDTC (**E**) on mRNA expression of IL-6, IL-8, VCAM-1 and ICAM-1 after sPRR-His 3-h treatment. N=12 (N represents the number of samples in each group, and the repetitions of separate experiments are 3). Statistical analysis was performed by using one-way ANOVA with the Bonferroni test for multiple comparisons or by using unpaired Student's t test for two comparisons. *P<0.01, compared with the CTR or Scr siRNA; **P<0.05, compared with the CTR or Scr siRNA; #P<0.01, compared with the sPRR-His or sPRR-His+Scr siRNA. Data are mean ± SEM.

Fig. S5

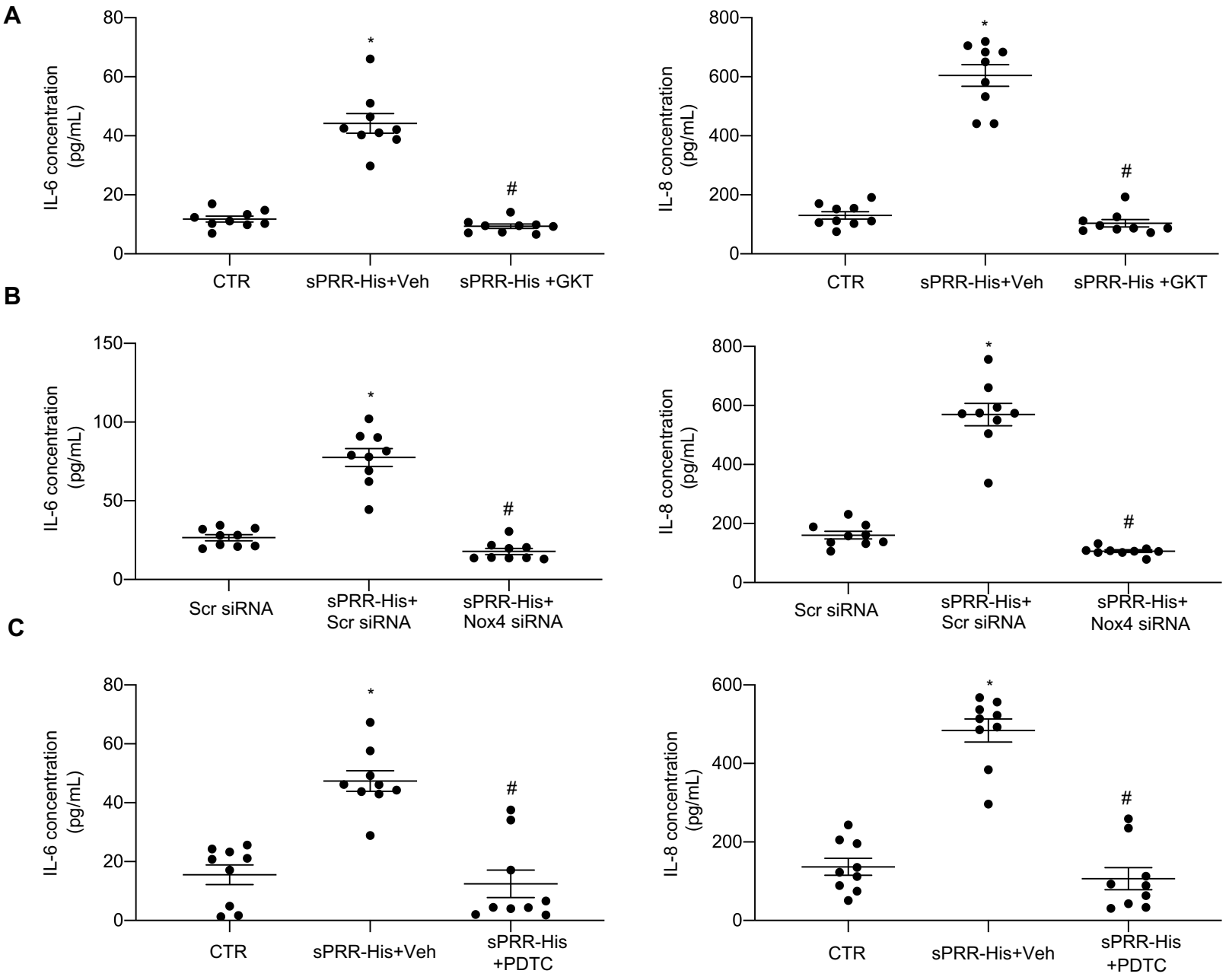
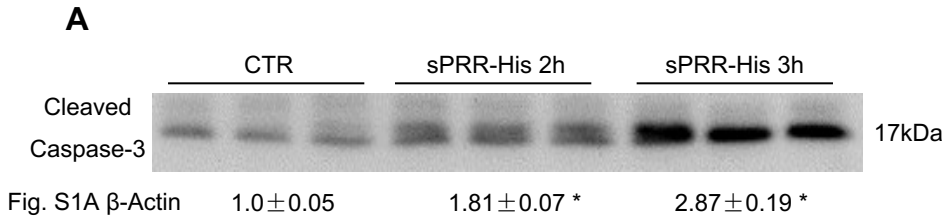
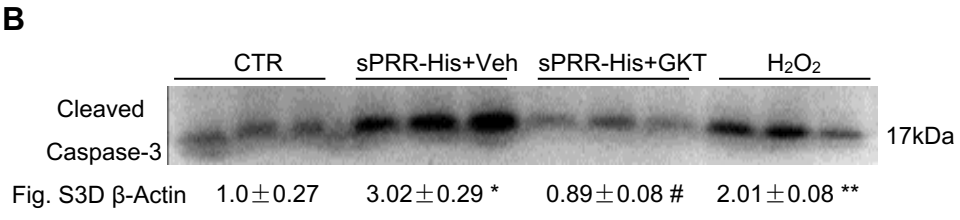
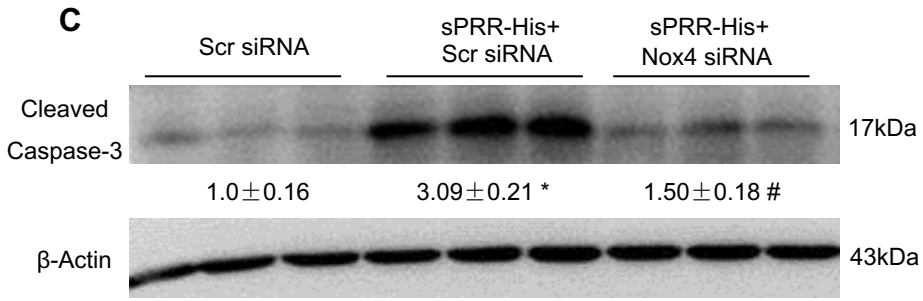

A
IL-6 concentration (pg/mL)
80
60
40
20
0
*
#
CTR
sPRR-His+Veh
sPRR-His +GKT
IL-8 concentration (pg/mL)
800
600
400
200
0
*
#
CTR
sPRR-His+Veh
sPRR-His +GKT
B
IL-6 concentration (pg/mL)
150
100
50
0
*
#
Scr siRNA
sPRR-His+ Scr siRNA
sPRR-His+ Nox4 siRNA
IL-8 concentration (pg/mL)
800
600
400
200
0
*
#
Scr siRNA
sPRR-His+ Scr siRNA
sPRR-His+ Nox4 siRNA
C
IL-6 concentration (pg/mL)
80
60
40
20
0
*
#
CTR
sPRR-His+Veh
sPRR-His +PDTC
IL-8 concentration (pg/mL)
600
400
200
0
*
#
CTR
sPRR-His+Veh
sPRR-His +PDTC

Fig. S5. sPRR-His evokes inflammation. The protein levels of the IL-6 and IL-8 in the cell medium supernatant were measured by ELISA. Effect of 10μM GKT (**A**), Nox4 siRNA (**B**) or 50μM PDTC (**C**) on protein levels of IL-6 and IL-8 in the medium of HUVECs after 50nM sPRR-His treatment. N=9 (N represents the number of samples in each group, and the repetitions of separate experiments are 3). Statistical significance was determined by using one-way ANOVA with the Bonferroni test for multiple comparisons. *P<0.01, compared with the CTR or Scr siRNA; #P<0.01, compared with the sPRR-His or sPRR-His+Scr siRNA. Data are mean ± SEM.

Fig. S6


A
CTR
sPRR-His 2h
sPRR-His 3h
Cleaved
Caspase-3
17kDa
Fig. S1A β-Actin
1.0±0.05
1.81±0.07 *
2.87±0.19 *


B
CTR
sPRR-His+Veh
sPRR-His+GKT
H2O2
Cleaved
Caspase-3
17kDa
Fig. S3D β-Actin
1.0±0.27
3.02±0.29 *
0.89±0.08 #
2.01±0.08 **


C
Scr siRNA
sPRR-His+
Scr siRNA
sPRR-His+
Nox4 siRNA
Cleaved
Caspase-3
17kDa
1.0±0.16
3.09±0.21 *
1.50±0.18 #
β-Actin
43kDa

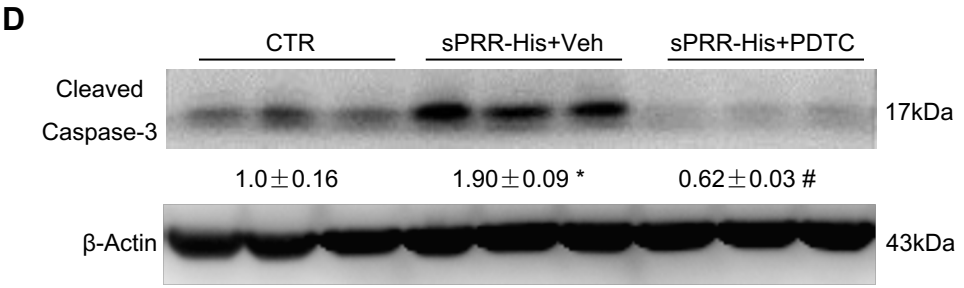

D
CTR
sPRR-His+Veh
sPRR-His+PDTC
Cleaved
Caspase-3
17kDa
1.0±0.16
1.90±0.09 *
0.62±0.03 #
β-Actin
43kDa

Fig. S6. sPRR-His increases apoptosis. Relative protein expression levels of cleaved caspase-3 were measured by Western blotting. (**A**) Relative protein expression levels of cleaved caspase-3 after 50nM sPRR-His 2-h and 3-h treatment. Effects of 10μM GKT (**B**), Nox4 siRNA (**C**) or 50μM PDTC (**D**) on relative protein expression levels of cleaved caspase-3 after sPRR-His 3-h treatment. Effect of the administration of 200μM exogenous H_2O_2 (**B**) on relative protein expression levels of cleaved caspase-3. Fig. S6**A** and Fig. S1**A** are from the same western blotting membrane, the β-Actin between them are same. Fig. S6**B** and Fig. S3**D** are from the same western blotting membrane, the β-Actin between them are same. The value beneath the image indicates the densitometry of the protein normalized to β-Actin for 3 separate experiments. N=9. Statistical analysis was performed by using one-way ANOVA with the Bonferroni test for multiple comparisons or by using unpaired Student's t test for two comparisons. *P<0.01, compared with the CTR or Scr siRNA; **P<0.05, compared with the CTR or Scr siRNA; #P<0.01, compared with the sPRR-His or sPRR-His+Scr siRNA. Data are mean ± SEM.

Fig. S7

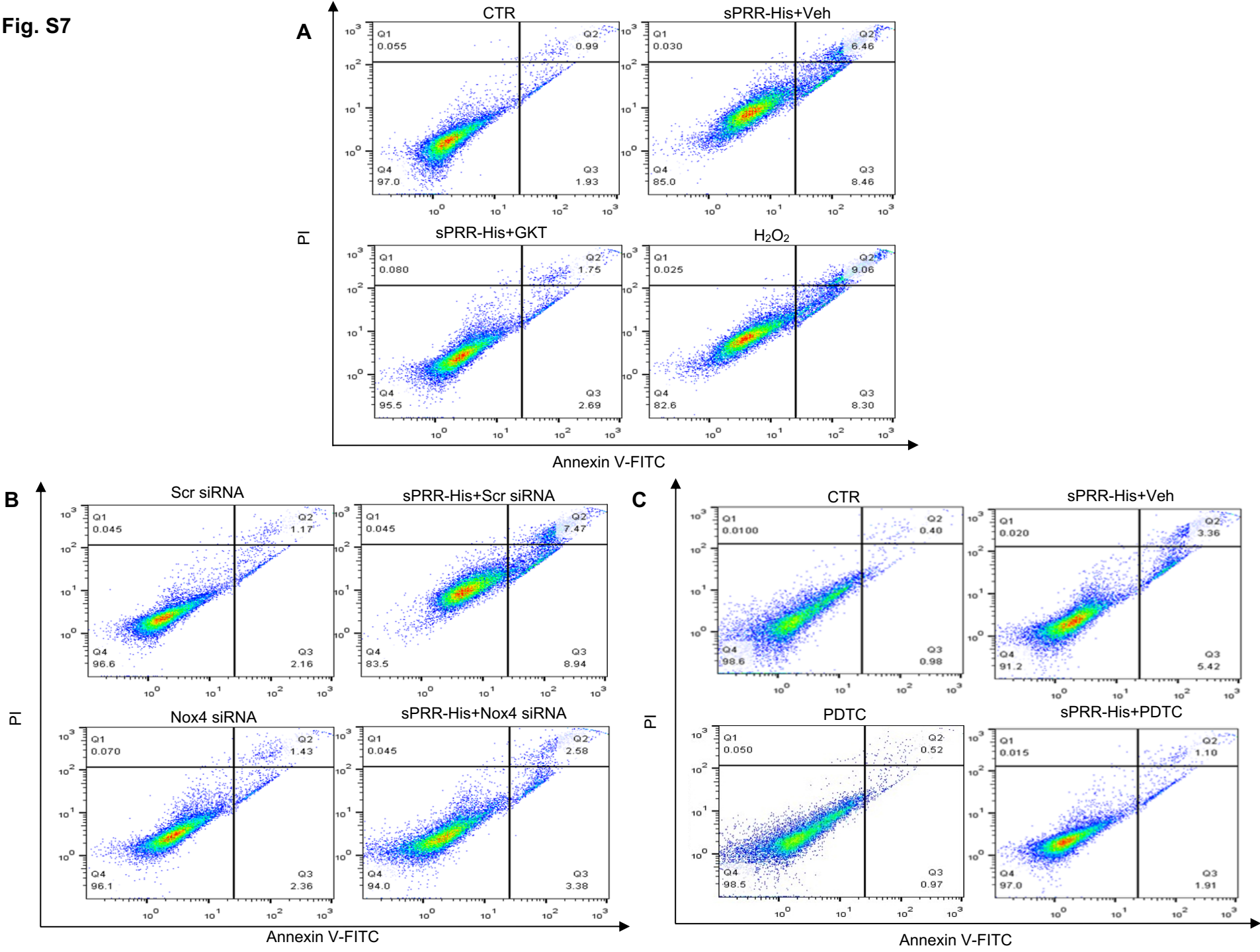

A
CTR
sPRR-His+Veh
sPRR-His+GKT
H2O2
PI
Annexin V-FITC
Q1 0.055
Q2 0.99
Q4 97.0
Q3 1.93
Q1 0.030
Q2 6.46
Q4 85.0
Q3 8.46
Q1 0.080
Q2 1.75
Q4 95.5
Q3 2.69
Q1 0.025
Q2 9.06
Q4 82.6
Q3 8.30
B
Scr siRNA
sPRR-His+Scr siRNA
Nox4 siRNA
sPRR-His+Nox4 siRNA
PI
Annexin V-FITC
Q1 0.045
Q2 1.17
Q4 96.6
Q3 2.16
Q1 0.045
Q2 7.47
Q4 83.5
Q3 8.94
Q1 0.070
Q2 1.43
Q4 96.1
Q3 2.36
Q1 0.045
Q2 2.58
Q4 94.0
Q3 3.38
C
CTR
sPRR-His+Veh
PDTC
sPRR-His+PDTC
PI
Annexin V-FITC
Q1 0.0100
Q2 0.40
Q4 98.6
Q3 0.98
Q1 0.020
Q2 3.36
Q4 91.2
Q3 5.42
Q1 0.050
Q2 0.52
Q4 98.5
Q3 0.97
Q1 0.015
Q2 1.10
Q4 97.0
Q3 1.91

Fig. S7. sPRR-His increases apoptosis. The apoptotic populations were determined by Annexin V-FITC/PI flow cytometry analysis. The right lower quadrant (Q3) represents the early apoptotic cells and the right upper quadrant (Q2) represents the late apoptotic cells. The left lower quadrant (Q4) represents the viable cells and the left upper quadrant (Q1) represents the necrotic cells. Effects of 10μM GKT (**A**), Nox4 siRNA (**B**) or 50μM PDTC (**C**) on sPRR-His-induced apoptosis. Effect of the administration of 200μM exogenous H_2O_2 (**A**) on apoptosis.

Fig. S8

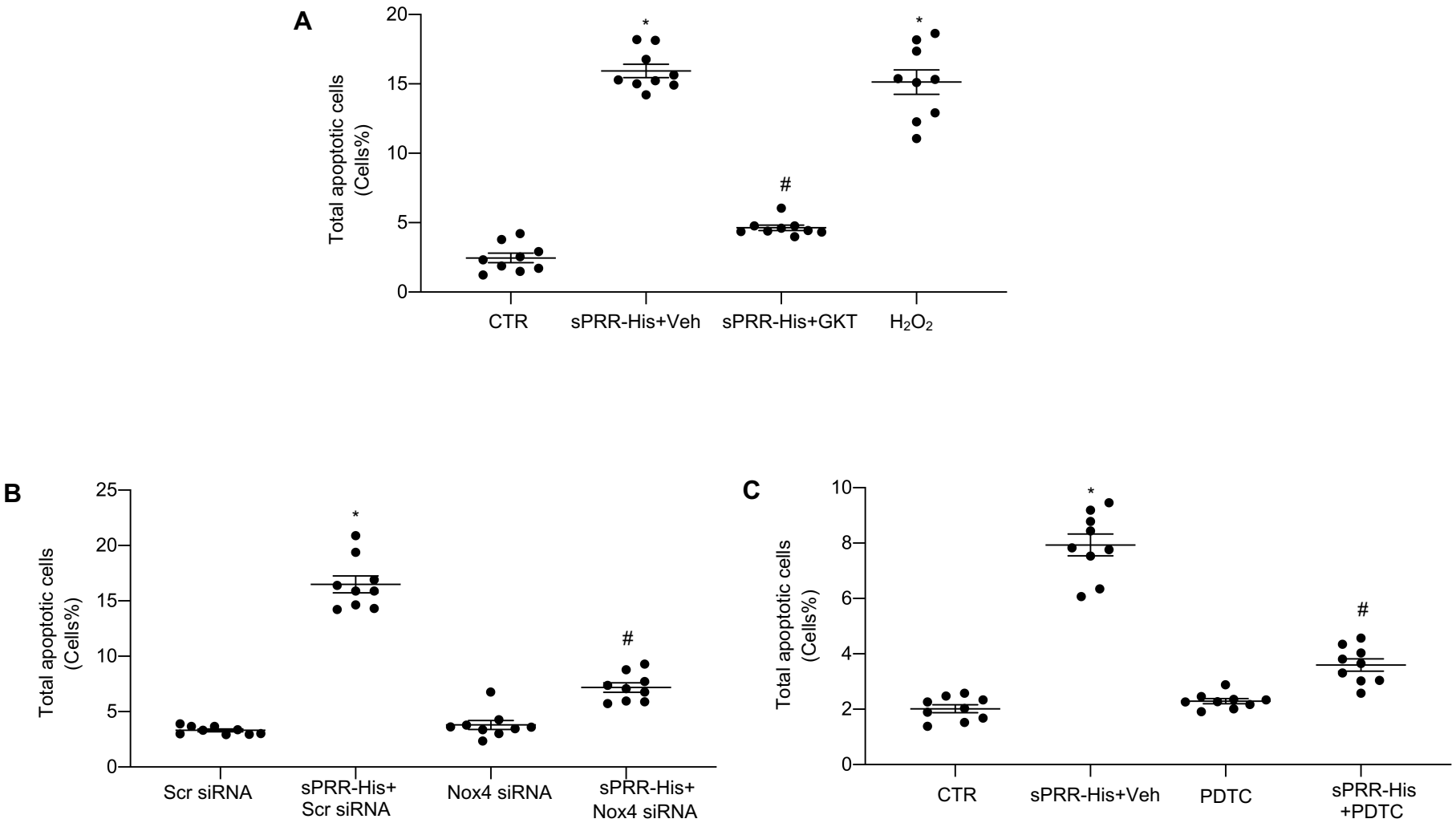
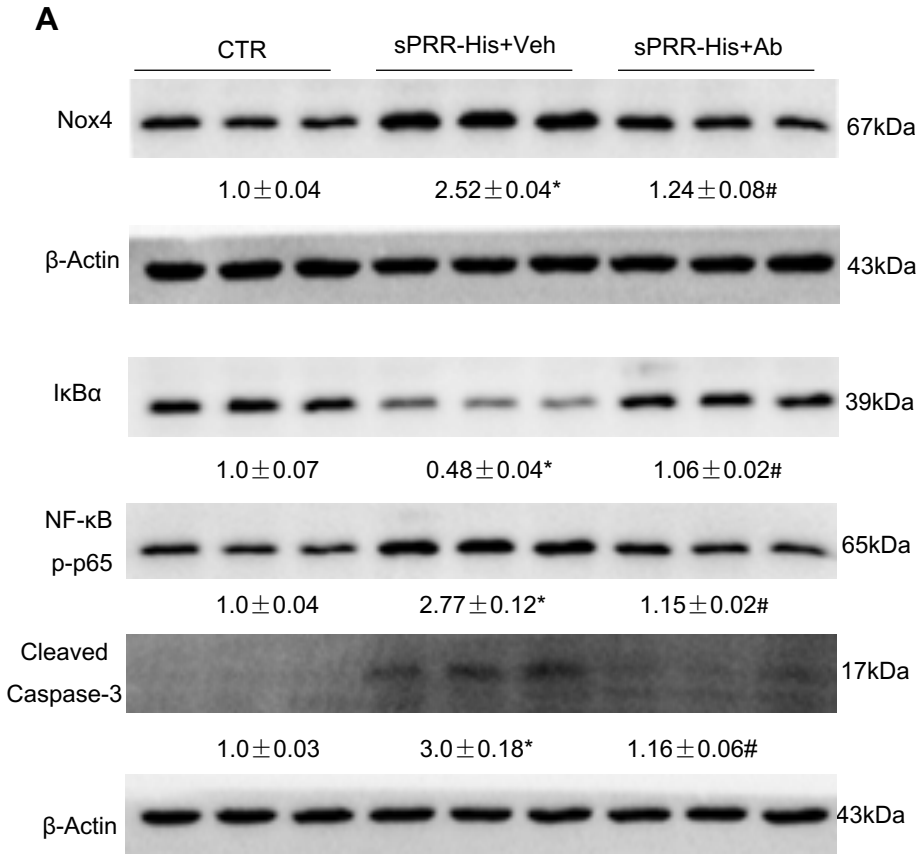

A
Total apoptotic cells (Cells%)
20
15
10
5
0
*
*
#
CTR
sPRR-His+Veh
sPRR-His+GKT
H2O2
B
Total apoptotic cells (Cells%)
25
20
15
10
5
0
*
#
Scr siRNA
sPRR-His+ Scr siRNA
Nox4 siRNA
sPRR-His+ Nox4 siRNA
C
Total apoptotic cells (Cells%)
10
8
6
4
2
0
*
#
CTR
sPRR-His+Veh
PDTC
sPRR-His +PDTC

Fig. S8. Quantitative analysis of the percentage of total apoptotic cells. The early apoptotic cells percentage added to the late apoptotic cells percentage equals the total apoptotic cells percentage. Effects of 10μM GKT (**A**), Nox4 siRNA (**B**) or 50μM PDTC (**C**) on sPRR-His-induced apoptosis. Effect of the administration of 200μM exogenous H_2O_2 (**A**) on apoptosis. N=9 (N represents the number of samples in each group, and the repetitions of separate experiments are 3). Statistical significance was determined by using one-way ANOVA with the Bonferroni test for multiple comparisons. *P<0.01, compared with the CTR or Scr siRNA; #P<0.01, compared with the sPRR-His or sPRR-His+Scr siRNA. Data are mean ± SEM.

Fig. S9


A
CTR
sPRR-His+Veh
sPRR-His+Ab
Nox4
67kDa
1.0±0.04
2.52±0.04*
1.24±0.08#
β-Actin
43kDa
IκBα
39kDa
1.0±0.07
0.48±0.04*
1.06±0.02#
NF-κB
p-p65
65kDa
1.0±0.04
2.77±0.12*
1.15±0.02#
Cleaved
Caspase-3
17kDa
1.0±0.03
3.0±0.18*
1.16±0.06#
β-Actin
43kDa

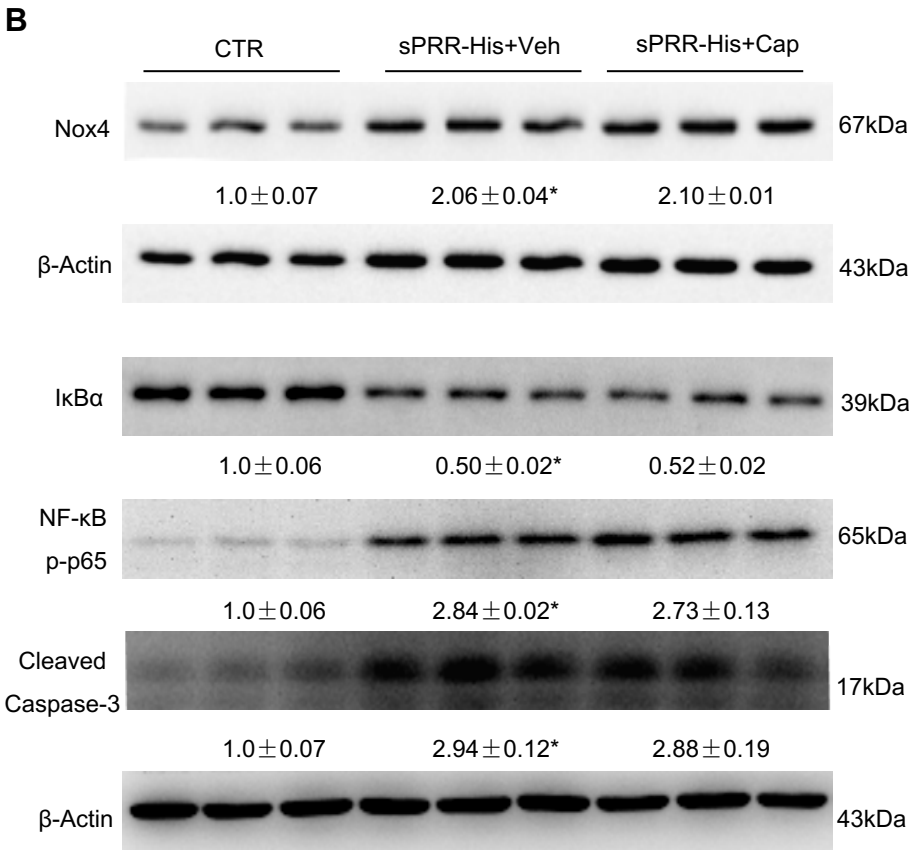

B
CTR
sPRR-His+Veh
sPRR-His+Cap
Nox4
67kDa
1.0±0.07
2.06±0.04*
2.10±0.01
β-Actin
43kDa
IκBα
39kDa
1.0±0.06
0.50±0.02*
0.52±0.02
NF-κB
p-p65
65kDa
1.0±0.06
2.84±0.02*
2.73±0.13
Cleaved
Caspase-3
17kDa
1.0±0.07
2.94±0.12*
2.88±0.19
β-Actin
43kDa

Fig. S9. The effect of sPRR neutralizing antibody or ACEi on the functions of sPRR-His in HUVECs. (**A**) HUVECs were pretreated with 1.5 μg/ml sPRR neutralizing antibody (Ab) or vehicle (Veh) for 1h followed by exposure to 50nM sPRR-His. Effect of sPRR-His on relative protein expression levels of Nox4, IκBα, NF-κB p-p65 and cleaved caspase-3 in the presence or absence of Ab. (**B**) HUVECs were pretreated with 10μM ACEi Captopril (Cap) or vehicle (Veh) for 1h followed by exposure to 50nM sPRR-His. Effect of sPRR-His on protein expression of Nox4, IκBα, NF-κB p-p65 and cleaved caspase-3 in the presence or absence of 10μM Cap. The value beneath the image indicates the densitometry of the protein normalized to β-actin for 3 separate experiments, N=9. Statistical significance was determined by using one-way ANOVA with the Bonferroni test for multiple comparisons. *P<0.01, compared with the CTR; #P<0.01, compared with the sPRR-His. Data are mean ± SEM.

Fig. S10

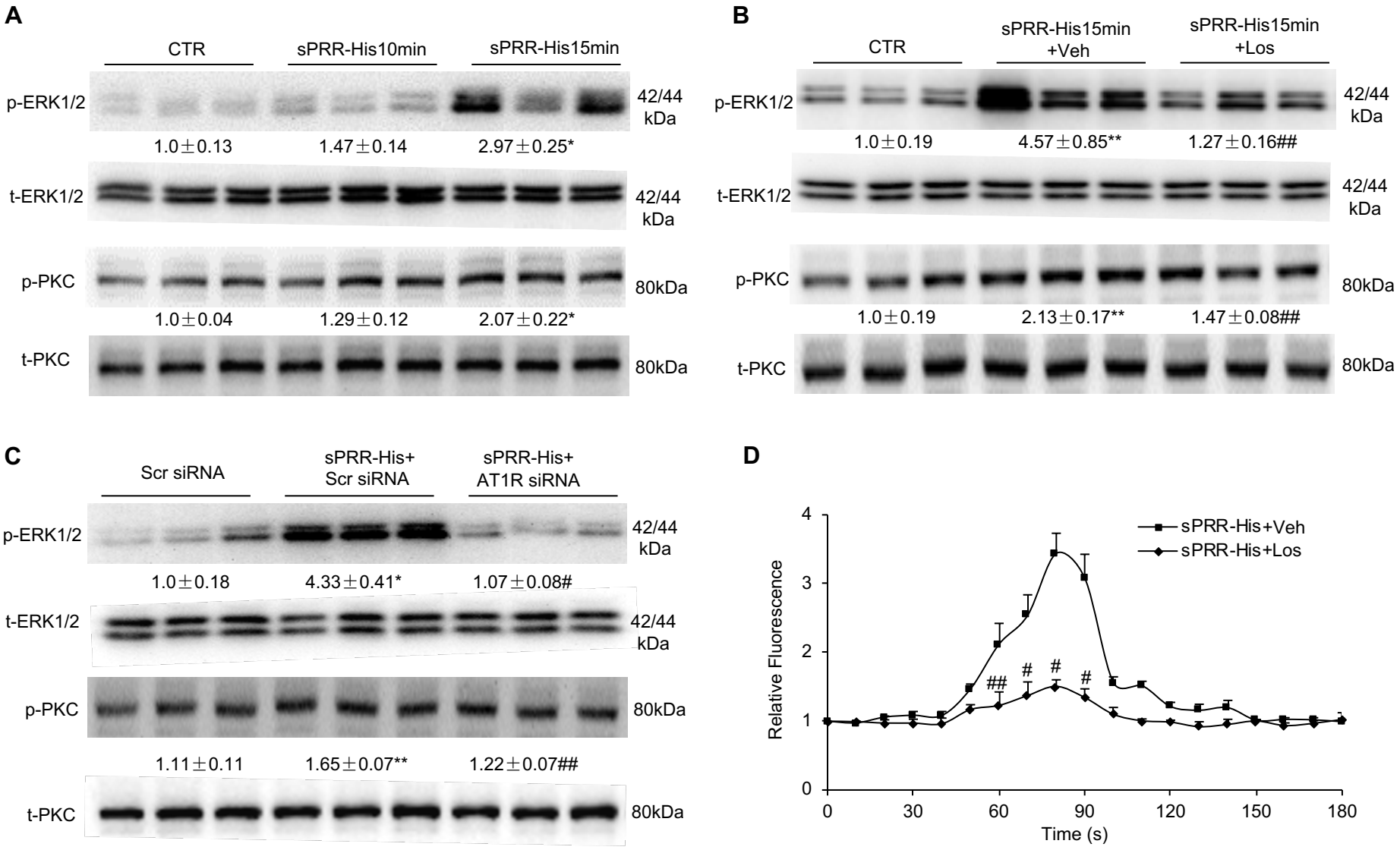

A
CTR
sPRR-His10min
sPRR-His15min
p-ERK1/2
42/44 kDa
1.0±0.13
1.47±0.14
2.97±0.25*
t-ERK1/2
42/44 kDa
p-PKC
80kDa
1.0±0.04
1.29±0.12
2.07±0.22*
t-PKC
80kDa
B
CTR
sPRR-His15min +Veh
sPRR-His15min +Los
p-ERK1/2
42/44 kDa
1.0±0.19
4.57±0.85**
1.27±0.16##
t-ERK1/2
42/44 kDa
p-PKC
80kDa
1.0±0.19
2.13±0.17**
1.47±0.08##
t-PKC
80kDa
C
Scr siRNA
sPRR-His+ Scr siRNA
sPRR-His+ AT1R siRNA
p-ERK1/2
42/44 kDa
1.0±0.18
4.33±0.41*
1.07±0.08#
t-ERK1/2
42/44 kDa
p-PKC
80kDa
1.11±0.11
1.65±0.07**
1.22±0.07##
t-PKC
80kDa
D
sPRR-His+Veh
sPRR-His+Los
Relative Fluorescence
Time (s)
0
1
2
3
4
0
30
60
90
120
150
180
##
#
#
#

Fig. S10. sPRR-His directly activates AT1R-Gq signaling. (**A**-**B**) Representative western blots and quantification of phosphorylated and total ERK1/2 and PKC in 50nM sPRR-His treated HUVECs with or without 10μM Losartan (Los) pretreatment. (**C**) Effect of 50nM sPRR-His on the phosphorylation of ERK1/2 and PKC in the presence or absence of AT1R siRNA knockdown. The value beneath the image indicates the densitometry of the protein normalized to total ERK1/2 or total PKC for 3 separate experiments. (**D**) Ca^{2+} signaling in HUVECs stimulated by 50nM sPRR-His with or without 10μM Los pretreatment, detected using Fluo 3-AM. N=9 (N represents the number of samples in each group, and the repetitions of separate experiments are 3). Statistical analysis was performed by using one-way ANOVA with the Bonferroni test for multiple comparisons or by using unpaired Student's t test for two comparisons. *P<0.01, compared with the CTR or Scr siRNA; **P<0.05, compared with the CTR or Scr siRNA; #P<0.01, compared with the sPRR-His or sPRR-His+Scr siRNA; ##P<0.05, compared with the sPRR-His or sPRR-His +Scr siRNA. Data are mean ± SEM.

Fig. S11

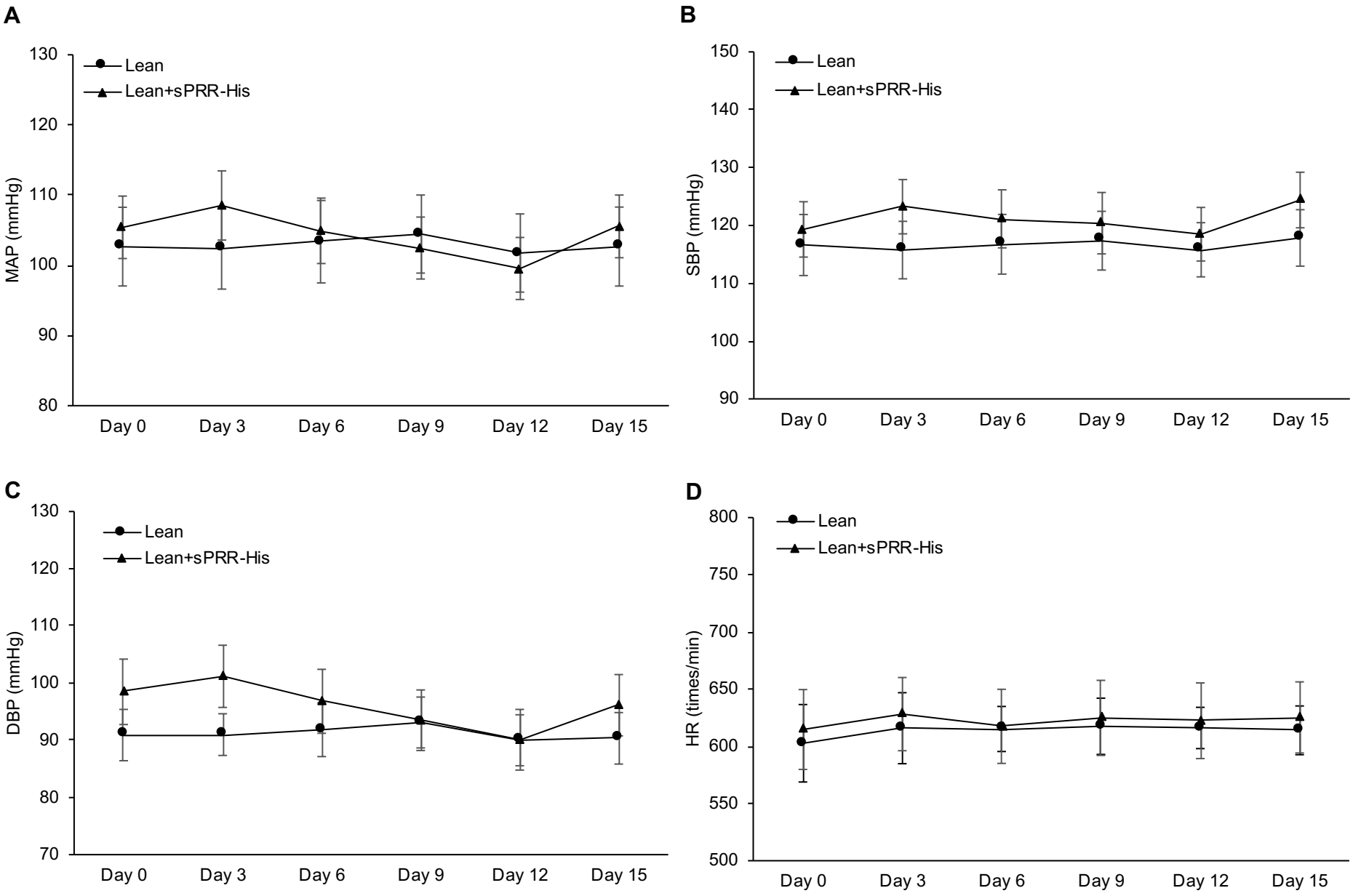

A
MAP (mmHg)
130
120
110
100
90
80
Lean
Lean+sPRR-His
Day 0
Day 3
Day 6
Day 9
Day 12
Day 15
B
SBP (mmHg)
150
140
130
120
110
100
90
Lean
Lean+sPRR-His
Day 0
Day 3
Day 6
Day 9
Day 12
Day 15
C
DBP (mmHg)
130
120
110
100
90
80
70
Lean
Lean+sPRR-His
Day 0
Day 3
Day 6
Day 9
Day 12
Day 15
D
HR (times/min)
800
750
700
650
600
550
500
Lean
Lean+sPRR-His
Day 0
Day 3
Day 6
Day 9
Day 12
Day 15

Fig. S11. The effects of sPRR-His on blood pressure and heart rate in Lean mice. Radiotelemetry was performed to record (**A**) mean arterial pressure (MAP), (**B**) systolic blood pressure (SBP), (**C**) diastolic blood pressure (DBP) and (**D**) heart rate (HR). N=4 per group. Statistical significance was determined by using unpaired Student's t test. Data are mean ± SEM.

Fig. S12

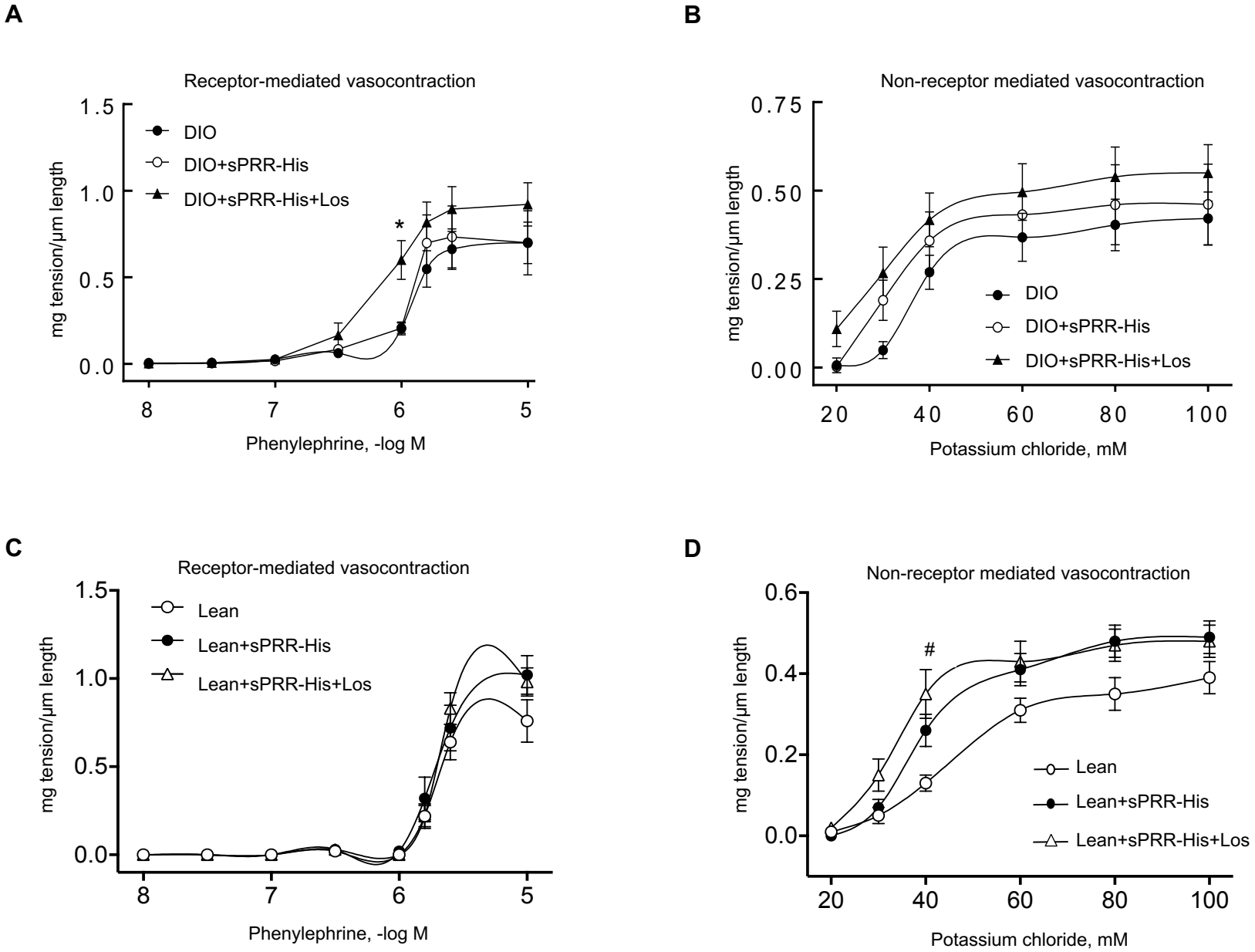

A
Receptor-mediated vasocontraction
mg tension/μm length
1.5
1.0
0.5
0.0
DIO
DIO+sPRR-His
DIO+sPRR-His+Los
*
8
7
6
5
Phenylephrine, -log M
B
Non-receptor mediated vasocontraction
mg tension/μm length
0.75
0.50
0.25
0.00
DIO
DIO+sPRR-His
DIO+sPRR-His+Los
20
40
60
80
100
Potassium chloride, mM
C
Receptor-mediated vasocontraction
mg tension/μm length
1.5
1.0
0.5
0.0
Lean
Lean+sPRR-His
Lean+sPRR-His+Los
8
7
6
5
Phenylephrine, -log M
D
Non-receptor mediated vasocontraction
mg tension/μm length
0.6
0.4
0.2
0.0
#
Lean
Lean+sPRR-His
Lean+sPRR-His+Los
20
40
60
80
100
Potassium chloride, mM

Fig. S12. The effects of sPRR-His and Losartan on vasocontraction function in DIO mice or lean mice. Vasocontraction to (**A**&**C**) phenylephrine and (**B**&**D**) potassium chloride were measured by wire myograph. For **A** and **B**, N=6–8 per group. For **C** and **D**, N=7-8 per group. Comparison of one time point among groups was made using one-way ANOVA. Comparison of multiple time points among groups was made using one-way or two-way repeated-measures ANOVA. Tukey post hoc tests were performed when significant main effects were obtained. *P<0.05, compared with DIO+ sPRR-His; #P < 0.05, compared with the Lean. Data are mean ± SEM.

Fig. S13

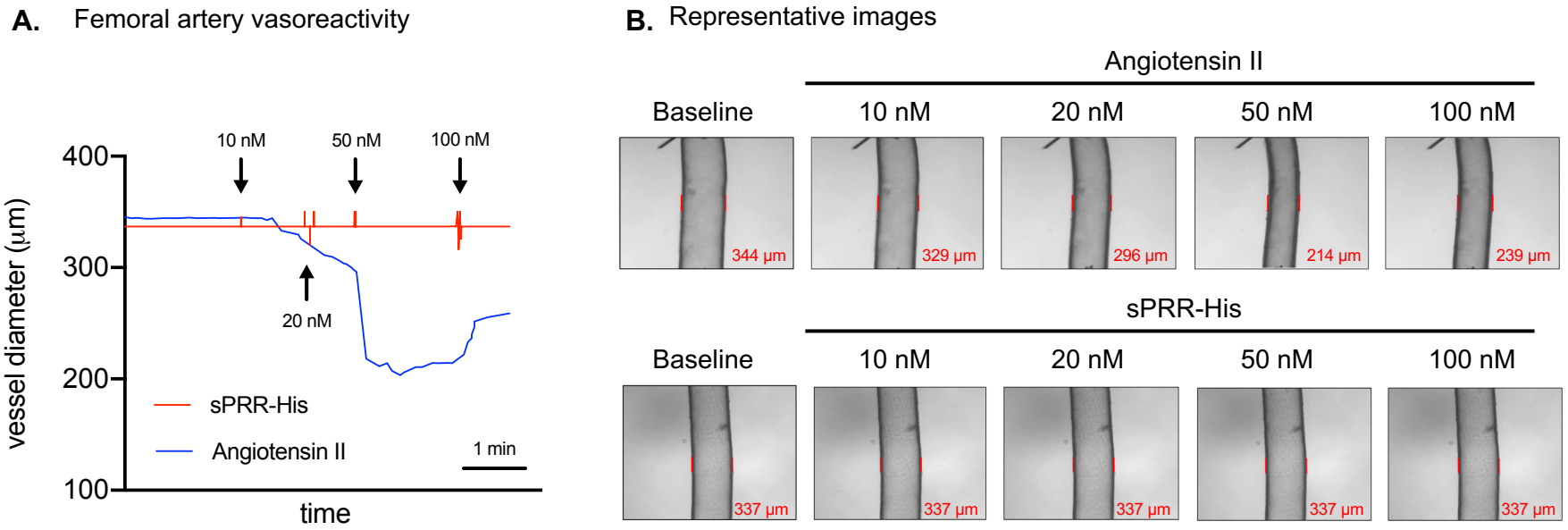


Fig. S13. Mesenteric artery reactivity to angiotensin II (Ang II) and sPRR-His. Ang II evokes vasoconstriction of murine mesenteric artery at 10, 20, and 50 nM, but relative vasodilation at 100 nM. sPRR-His (10-100 nM) does not influence vasoreactivity of mesenteric arteries. Vessels were mounted on glass micropipettes and placed in a myograph chamber. The chamber was warmed from room temperature to 36.5℃ as vessels pressurized from 0 mmHg to 60 mmHg over 60-min. After noting that responses to 100 mM KCl were similar in both arteries (~ 60% vasoconstriction from baseline), the chambers were rinsed twice over a 30-min period. Next, one artery was treated with 10, 20, 50, and 100 nM of Ang II, while another artery was treated with equivalent doses of sPRR-His (1 min per dose). **A**. Using edge-detection tracker software (Myoview; DMT, Aarhus, Denmark), the outer diameter of each artery was recorded. Ang II constricted the mesenteric artery at 10, 20, and 50 nM (blue line indicates vessel diameter in A). The maximal response was 37% vasoconstriction from baseline. However, 100 nM caused relative vasodilation from maximal constriction. On the other hand, 10-100 nM sPRR-His did not influence mesenteric artery vasoreactivity (red line indicates vessel diameter in A). **B**. Representative images of mesenteric arteries in response to 10-100 nM Ang II or sPRR-His. The values in red text in each image indicate the outer diameter of the artery.