Table 1. Body weight, cardiac weight, cardiac BNP mRNA, and lung weight in DS rats.

		High Na			
	Low Na (n=5)	Veh (n=9)	Hyd (n=7)	Tem (n=7)	Irb (n=8)
BW (g)	400±10 #	376±6	384±4	405±6*	391±3 #
LV/BW(mg/g)	2.21±0.07 #	3.42±0.04	3.25±0.03*	3.03±0.06 #§	3.08±0.03*§
BNP mRNA expression	1.26±0.22 #	3.69±0.30	3.51±0.32	3.08±0.50*	2.65±0.48*
Lung/BW(mg/g)	3.42±0.04 #	4.31±0.08	4.29±0.07	3.94±0.07 #	3.89±0.05 #

Low Na indicates low salt fed DS rats. High salt fed DS rats were treated with vehicle (Veh), hydralazine (Hyd), tempol (Tem), or irbesartan (Irb) for 4 weeks. Abbreviations used: BW, body weight; LV, left ventricular weight; BNP, brain natriuretic peptide. Values are means±SEM . * $P<0.05$, # $P<0.01$ vs Veh. § $P<0.01$ vs Hyd.

Table 2. Echocardiographic data in each group of DS rats.

		High Na			
	Low Na (n=9)	Veh (n=12)	Hyd (n=9)	Tem (n=11)	Irb (n=13)
LVAw (mm)	1.38±0.04#	2.16±0.03	1.95±0.04#	1.76±0.04#§	1.77±0.03#§
LVPw (mm)	1.38±0.04#	2.14±0.02	1.95±0.03#	1.78±0.04#§	1.77±0.03#§
LVDd (mm)	8.67±0.12	8.46±0.10	8.46±0.26	8.90±0.13	8.79±0.20
LVDd/BW (mm/100g BW)	2.23±0.05	2.44±0.11	2.33±0.03	2.47±0.11	2.45±0.06
LVDs (mm)	5.56±0.15	5.08±0.26	5.15±0.44	5.72±0.19	5.38±0.25
LVDs/BW (mm/100g BW)	1.43±0.05	1.44±0.06	1.40±0.09	1.58±0.06	1.51±0.05
EF (%)	69.8±2.46	74.6±2.67	73.7±3.99	71.1±2.0	73.7±2.46
FS (%)	35.7±2.06	40.2±2.67	39.9±3.95	35.9±1.46	39.0±2.14

The abbreviations used are the same as in Table 1. LVAw, left ventricular anterior wall thickness; LVPw, left ventricular posterior wall thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; EF, ejection fraction; FS, fractional shortening. Values are means±SEM . # $P<0.01$ vs Veh. § $P<0.01$ vs Hyd.

Figure 1

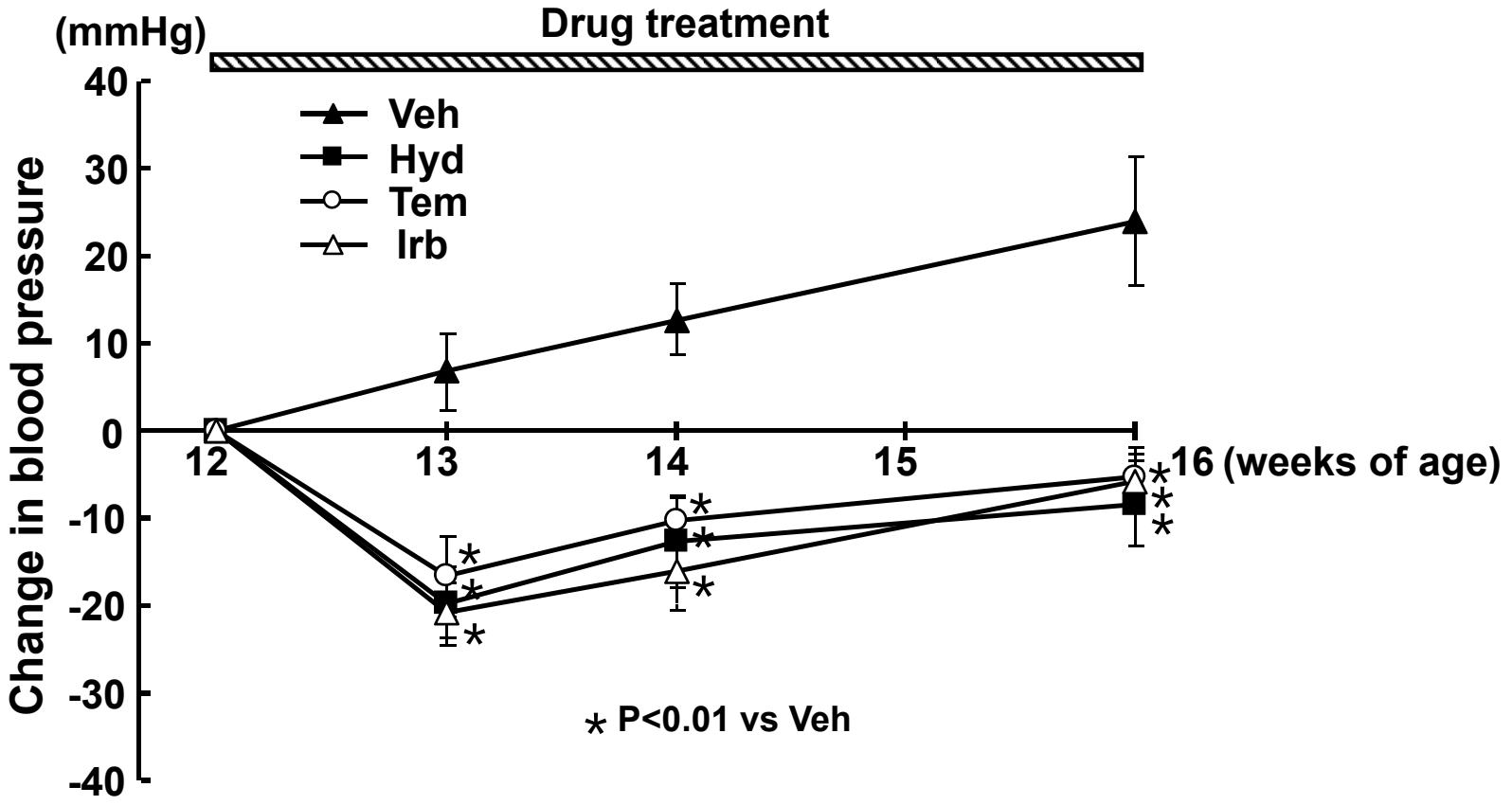

(mmHg)
Drug treatment
Change in blood pressure
40
30
20
10
0
-10
-20
-30
-40
Veh
Hyd
Tem
Irb
12
13
14
15
16 (weeks of age)
* P<0.01 vs Veh

Figure 2

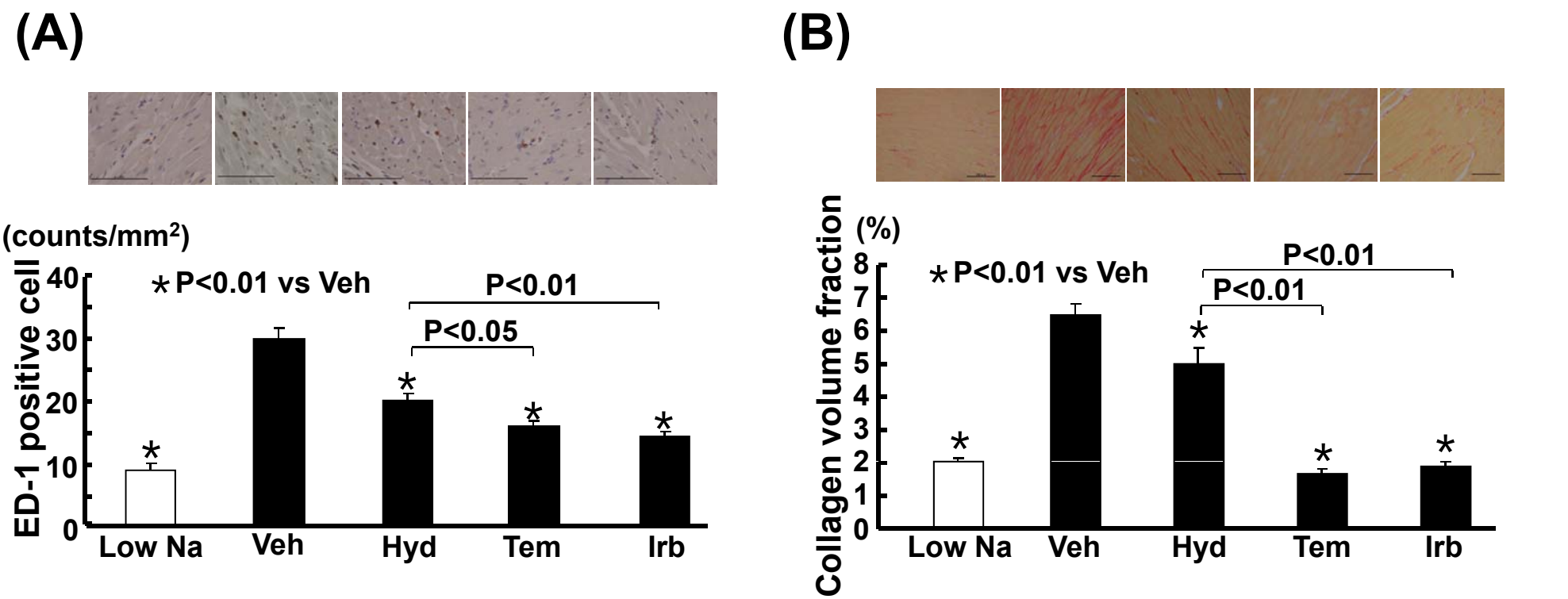
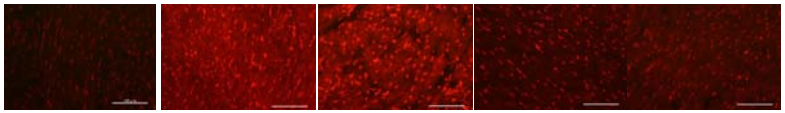
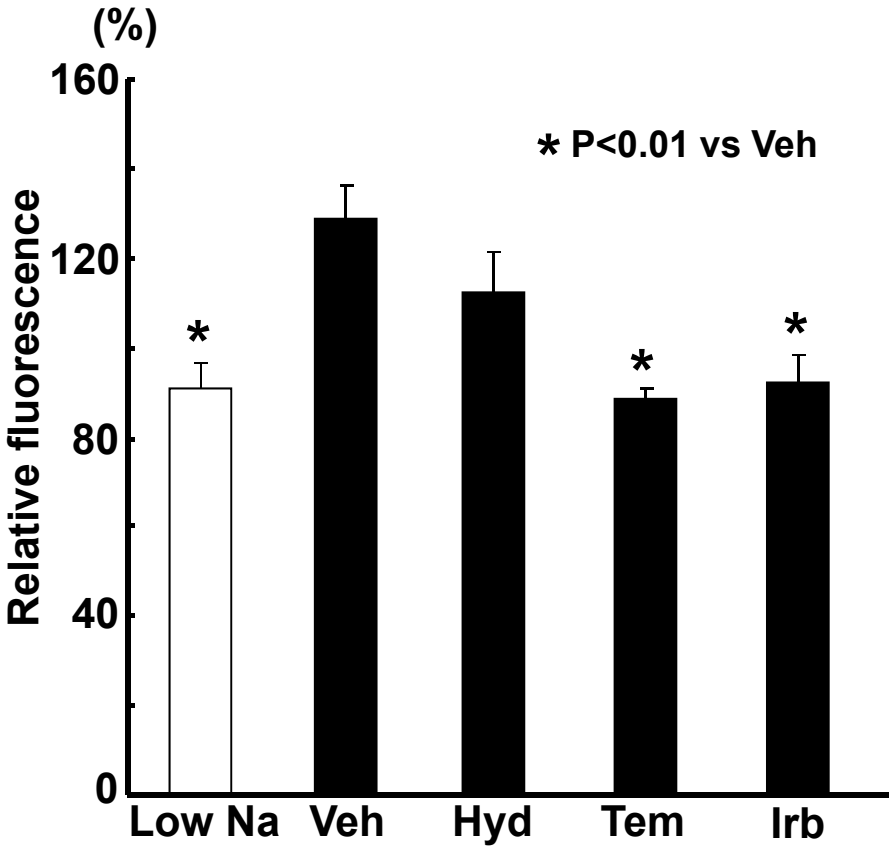

(A)
(counts/mm2)
ED-1 positive cell
0
10
20
30
40
*P<0.01 vs Veh
P<0.01
P<0.05
*
Low Na
Veh
Hyd
Tem
Irb
(B)
(%)
Collagen volume fraction
0
1
2
3
4
5
6
7
8
*P<0.01 vs Veh
P<0.01
P<0.01
*
Low Na
Veh
Hyd
Tem
Irb

Figure 3

(A)




(%)
160
120
80
40
0
Relative fluorescence
* P<0.01 vs Veh
*
*
*
Low Na
Veh
Hyd
Tem
Irb

(B)

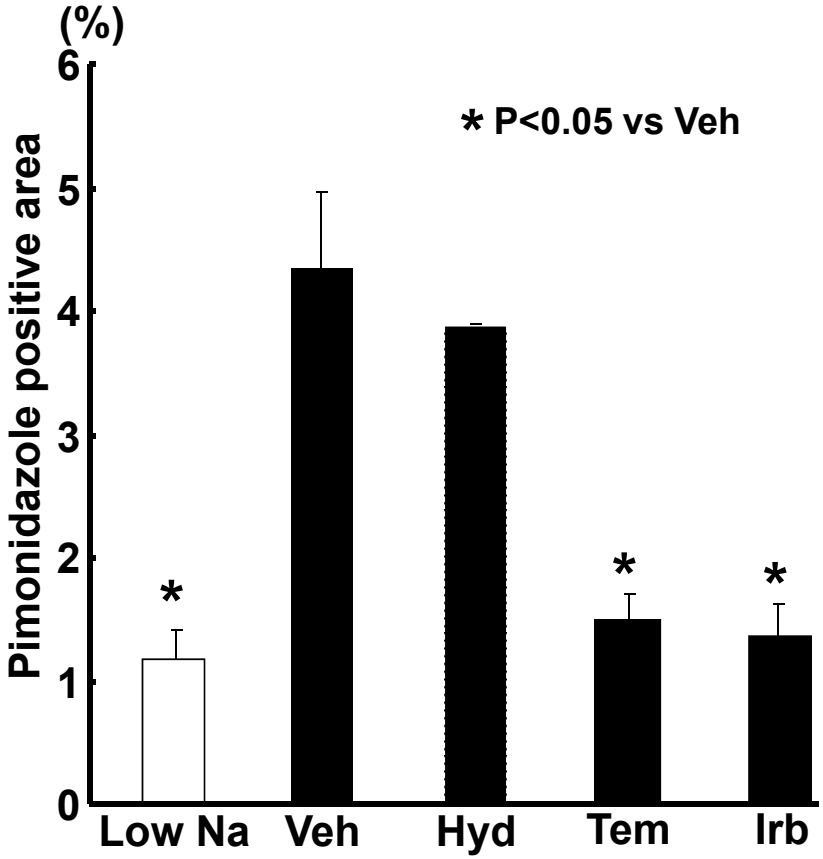

(%)
6
5
4
3
2
1
0
Pimonidazole positive area
* P<0.05 vs Veh
*
*
*
Low Na
Veh
Hyd
Tem
Irb

Figure 4

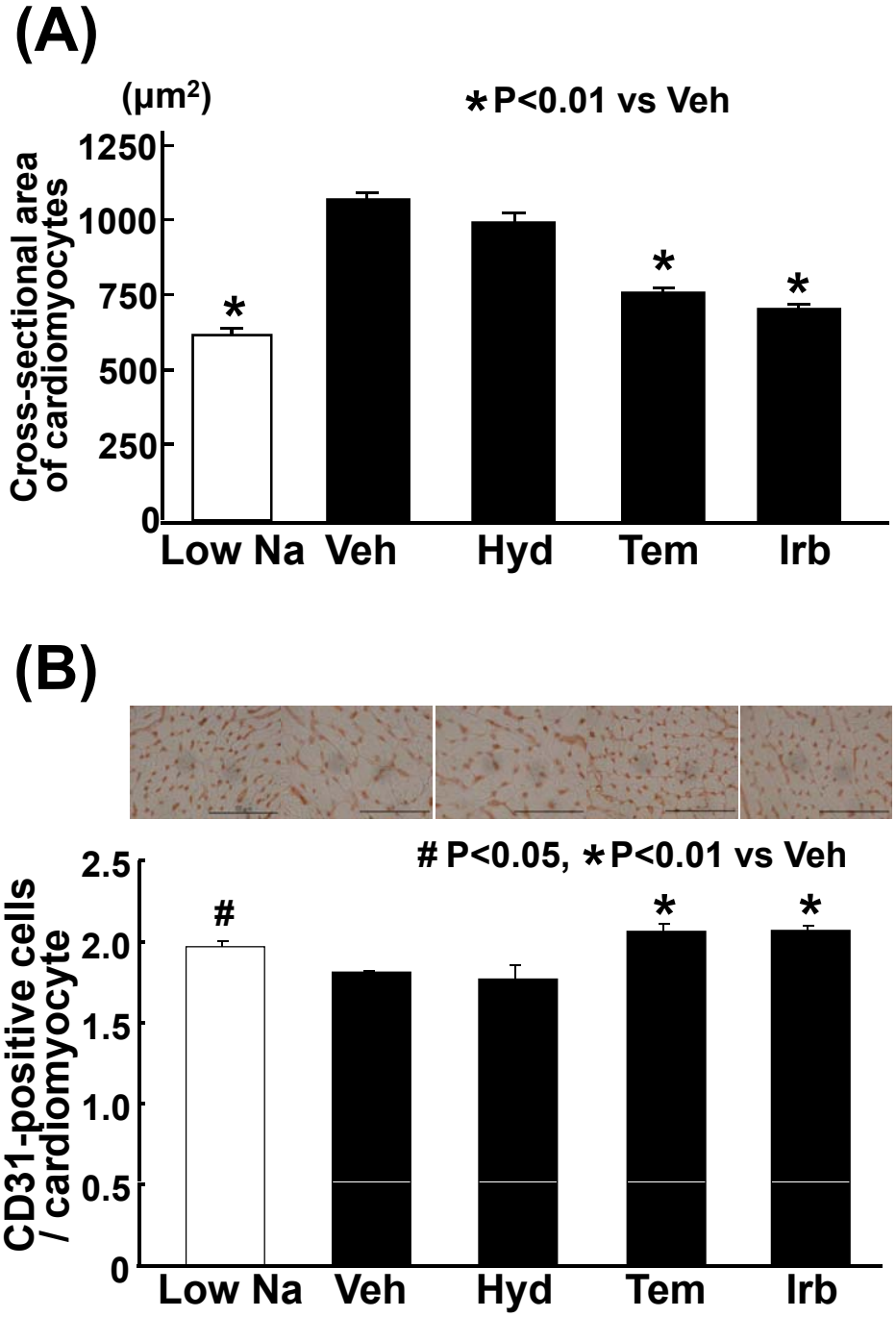
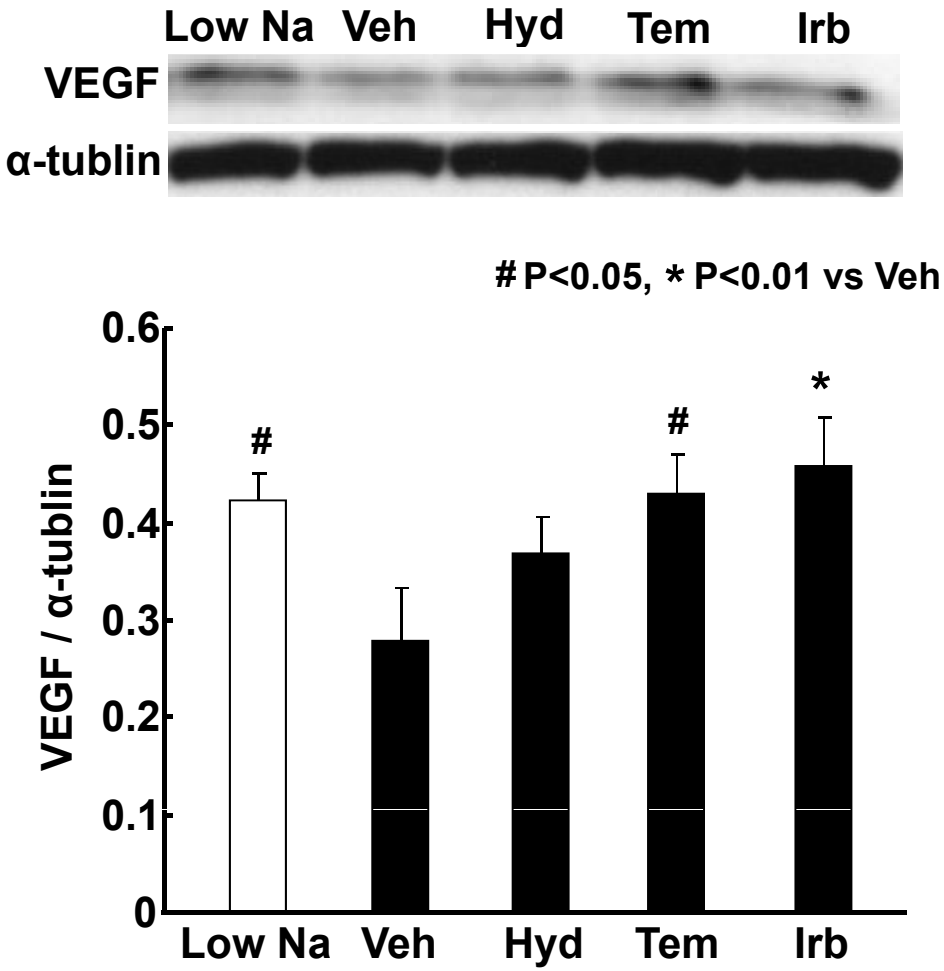

(A)
(μm²)
＊P<0.01 vs Veh
Cross-sectional area of cardiomyocytes
1250
1000
750
500
250
0
Low Na
Veh
Hyd
Tem
Irb
(B)
# P<0.05, ＊P<0.01 vs Veh
CD31-positive cells / cardiomyocyte
2.5
2.0
1.5
1.0
0.5
0
Low Na
Veh
Hyd
Tem
Irb
(C)
CD31
TUNEL
merge
(%)
# P<0.05, ＊P<0.01 vs Veh
Tunel-positive / CD31-positive cell
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
P<0.01
P<0.05
Low Na
Veh
Hyd
Tem
Irb

Figure 5


(A)
Low Na
Veh
Hyd
Tem
Irb
VEGF
α-tublin
# P<0.05, * P<0.01 vs Veh
VEGF / α-tublin
0.6
0.5
0.4
0.3
0.2
0.1
0
#
#
*
Low Na
Veh
Hyd
Tem
Irb

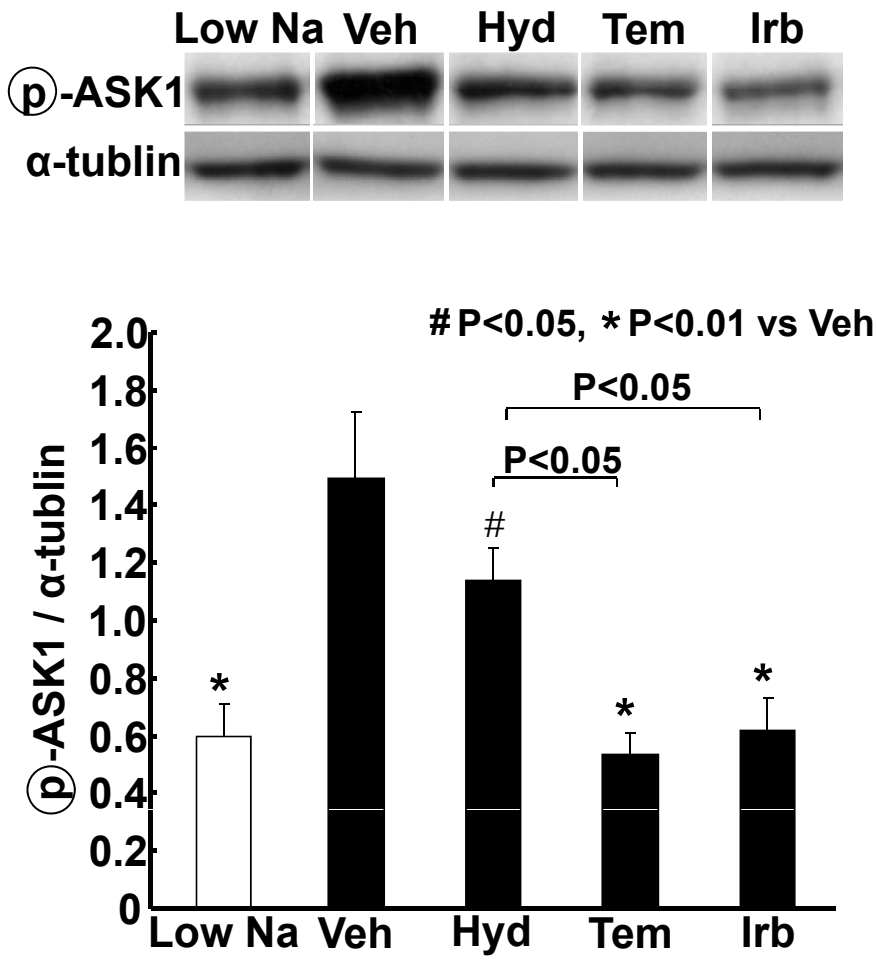

(B)
Low Na
Veh
Hyd
Tem
Irb
ⓟ-ASK1
α-tublin
# P<0.05, * P<0.01 vs Veh
P<0.05
P<0.05
ⓟ-ASK1 / α-tublin
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
*
#
*
*
Low Na
Veh
Hyd
Tem
Irb

Figure 6

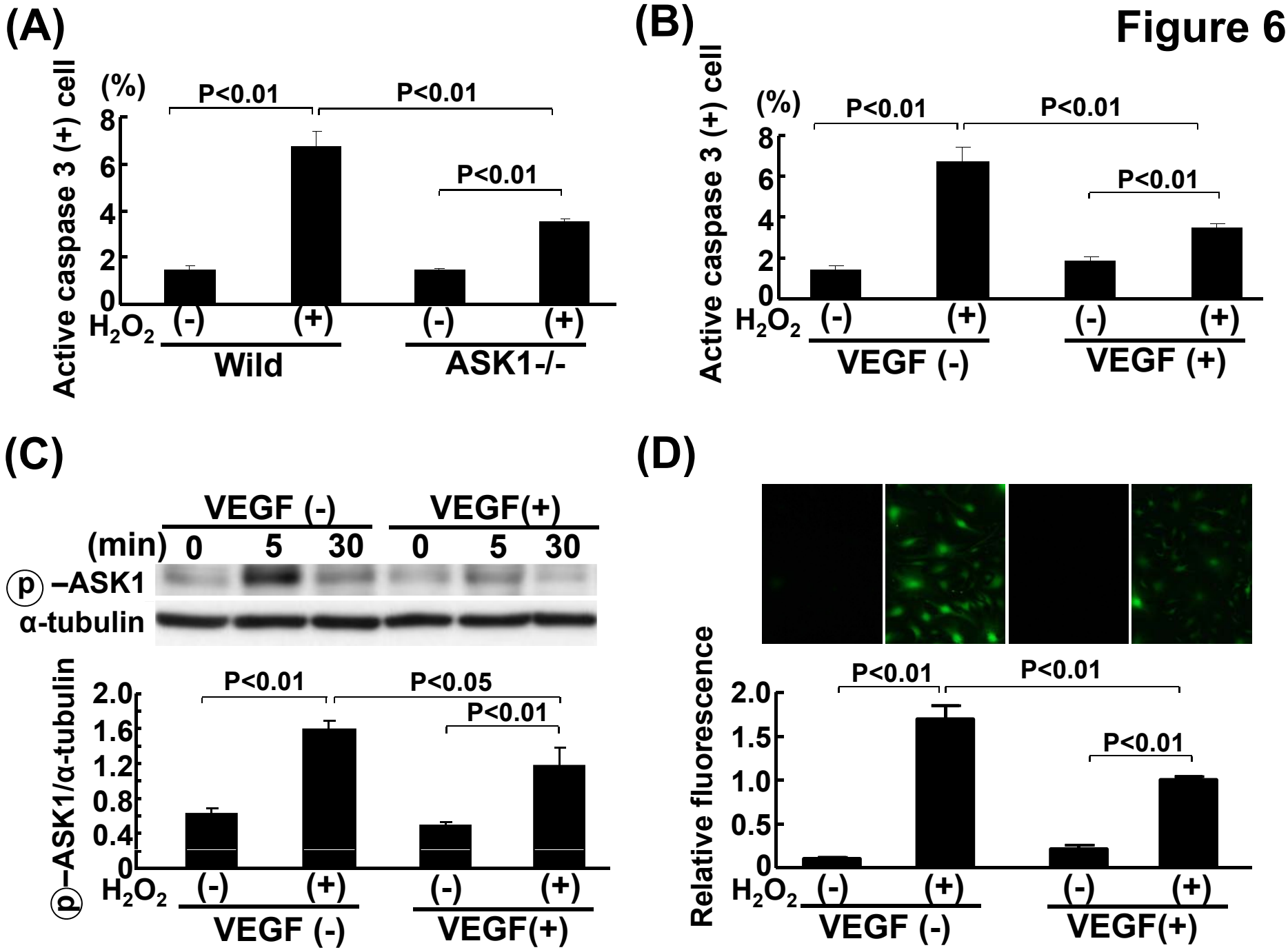

(A)
Active caspase 3 (+) cell
(%)
P<0.01
P<0.01
P<0.01
H2O2
(-)
(+)
(-)
(+)
Wild
ASK1-/-
(B)
Active caspase 3 (+) cell
(%)
P<0.01
P<0.01
P<0.01
H2O2
(-)
(+)
(-)
(+)
VEGF (-)
VEGF (+)
(C)
VEGF (-)
VEGF(+)
(min) 0 5 30 0 5 30
ⓟ –ASK1
α-tubulin
ⓟ–ASK1/α-tubulin
P<0.01
P<0.05
P<0.01
H2O2
(-)
(+)
(-)
(+)
VEGF (-)
VEGF(+)
(D)
Relative fluorescence
P<0.01
P<0.01
P<0.01
H2O2
(-)
(+)
(-)
(+)
VEGF (-)
VEGF(+)

Figure legends

Fig.1 Effect of hydralazine, tempol, and irbesartan on blood pressure of DS rats

Abbreviations used: Veh, vehicle-treated DS rats; Hyd, hydralazine-treated DS rats; Tem, tempol-treated DS rats; Irb, irbesartan-treated DS rats. Each value represents mean±SEM (n=9 in Veh, n=7 in Hyd, n=7 in Tem, n=8 in Irb).

Fig.2 Effect of hydralazine, tempol, and irbesartan on cardiac inflammation (A) and cardiac interstitial fibrosis (B) of DS rats

Upper panels in (A) and (B) indicate representative photomicrographs stained with ED1 and Sirius red, respectively. Low Na indicates low salt fed DS rats. High salt fed DS rats were orally given hydralazine (Hyd), tempol (Tem), or irbesartan (Irb) for 4 weeks. Values are means±SEM (n=5 in Low Na, n=9 in Veh, n=7 in Hyd, n=7 in Tem, n=8 in Irb). Bar=100 μm.

Fig.3 Effect of hydralazine, tempol, and irbesartan on cardiac superoxide (A) and cardiac ischemia (B) of DS rats

Cardiac superoxide was detected with DHE staining as described in Materials and Methods. Upper panels in (A) and (B) indicate representative photomicrographs stained with DHE and pimonidazole, respectively. Abbreviations used are the same as in Fig.1. Values are means±SEM (n=5 in Low Na, n=9 in Veh, n=7 in Hyd, n=7 in Tem, n=8 in Irb). Bar=100 μm.

Fig.4 Effect of hydralazine, tempol, and irbesartan on cross-sectional area of cardiomyocytes (A), cardiac capillary density (B), and capillary endothelial apoptosis (C) of DS rats

Upper panels in (B) and (C) indicate representative photomicrographs immunostained with CD31 antibody and double stained with CD31 and TUNEL, respectively. Abbreviations used are the same as in Fig.1. Each value represents mean±SEM (n=5 in Low Na, n=9 in Veh, n=7 in Hyd, n=7 in Tem, n=8 in Irb). Bar=100 μm.

Fig.5 Effect of hydralazine, tempol, and irbesartan on cardiacVEGF (A) and phospho-ASK1 (B) of DS rats

Upper panels in (A) and (B) indicate representative Western blots of VEGF and phospho-ASK1, respectively. Abbreviations used are the same as in Fig.1. VEGF or phospho-ASK1 density in individual samples was corrected for α-tubulin density. Each value represents mean±SEM (n=5 in Low Na, n=9 in Veh, n=7 in Hyd, n=7 in Tem, n=8 in Irb).

Fig.6 Role of ASK1 (A) and VEGF (B) in H_2O_2-induced endothelial apoptosis, and effect of VEGF on endothelial ASK1 activation (C), and endothelial oxidative stress (D)

(A) indicates comparison between H_2O_2-induced endothelial apoptosis from wild type and ASK1-/- mice. (B) indicates the effect of VEGF on H_2O_2-induced endothelial apoptosis from wild type mice. (C) indicates the effect of VEGF on H_2O_2-induced endothelial ASK1 activation from wild type mice. (D) Upper panels indicate representative photomicrographs of fluorescent images in each group. Each value represents mean±SEM (n=5 in each group).