JACBTS 331 Supplementary Material:

Suppl.Fig.1: Acute alcohol intoxication-induced cardiac dysfunction recovers by 12 h after alcohol exposure measured by ultrasound.

(A) Representative ultrasound M-mode images of heart left ventricles acquired in mice before and after EtOH binge. (B) Indices of LV function (heart rate, stroke volume, cardiac output, ejection fraction and fractional shortening) in mice before (baseline), and after alcohol gavage (EtOH 3h and EtOH 12h respectively) Data are expressed as mean±SEM, n=8; *p<0.05 *vs.* baseline.

Suppl.Fig.2: Acute alcohol intoxication-induced cardiovascular dysfunction recovers by 12h after alcohol exposure measured by P-V approach.

(A) Systolic indices of LV performance [maximal slope of pressure increment (dP/dtmax), end-systolic pressure, stroke work] in control and alcohol-binged (EtOH 12h) mice 12h after maltodextrin or alcohol gavage respectively. (B) Load-independent indices of LV performance: Ees, the dP/dtmax -end-diastolic volume (EDV) relation and preload recruitable stroke work (PRSW). (C) Mean arterial blood pressure and total peripheral resistance measured in control and alcohol-binged (EtOH 12h) mice 12h after maltodextrin or alcohol ingestion respectively. Data are expressed as mean±SEM, n=4-6, except for PRSW in panel B, where data are shown as median ± 25th, 75th percentiles.

Suppl.Fig.3: Binge alcohol drinking induces left ventricular and vascular dysfunction in both genders.

(A) Systolic indices of LV performance [maximal slope of pressure increment (dP/dtmax), end-systolic pressure, stroke work, cardiac output and ejection fraction] in control and alcohol-binged male and female mice 3h after maltodextrin or alcohol gavage. (B) Load-independent indices of LV performance: Ees, the dP/dt_{max} -end-diastolic volume (EDV) relation and preload recruitable stroke work (PRSW). (C) Binge alcohol-induced changes of mean arterial blood pressure and total peripheral resistance measured in control and alcohol-binged male and female mice. Data are expressed as mean±SEM, n=5-8; *p<0.05 *vs.* corresponding control group, except for dP/dt_{max} in panel A, where data are shown as median ± 25th, 75th percentiles.

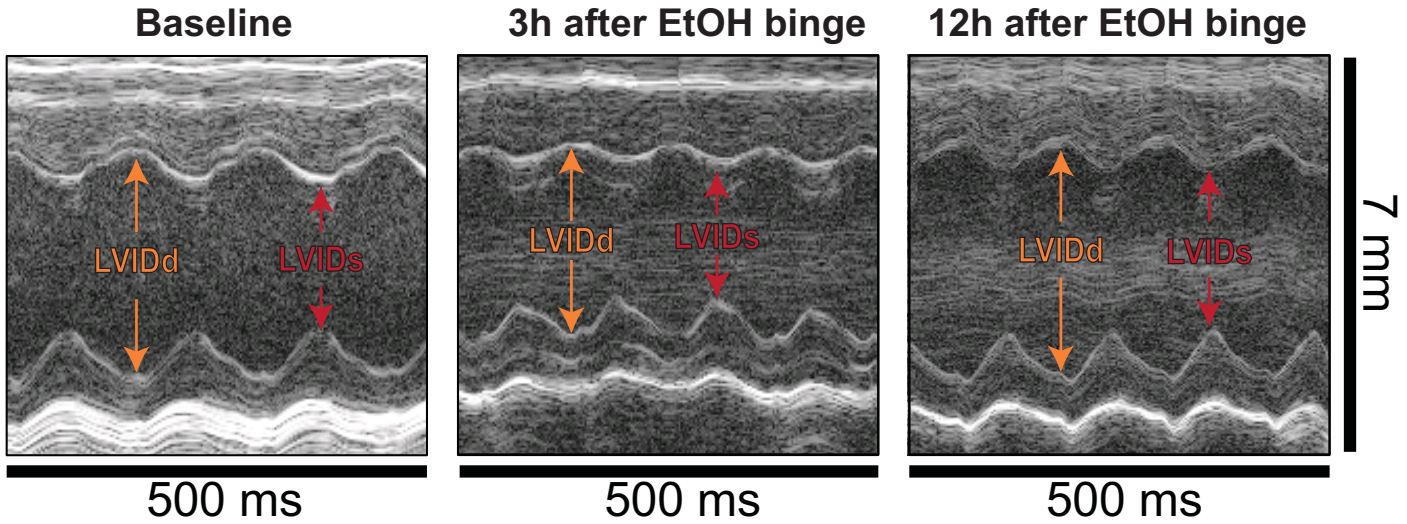
Suppl.Fig.4: Intravenous vehicle administration has no effect on cardiovascular function.

(A) Representative pressure-volume (P-V) loops of maltodextrin and alcohol-binged mice before (red and dark blue respectively) and 15min after intravenous vehicle (grey and orange loops respectively) injection. (B) Systolic indices of LV performance [maximal slope of pressure increment (dP/dtmax), end-systolic pressure, stroke work] measured after 3h in control or in alcohol-binged mice before (ctrl base and EtOH base respectively) and 15min after intravenous vehicle (ctlr+veh and EtOH+veh respectively) administration. (C) Mean arterial blood pressure and total peripheral resistance measured after 3h in control or in alcohol-binged mice before (ctrl base and EtOH base respectively) and 15min after intravenous vehicle (ctlr+veh and EtOH+veh respectively) administration. Data are expressed as mean±SEM, n=5-7; *p<0.05 *vs.* corresponding control group, except for Total peripheral resistance in panel C, where data are shown as median ± 25th, 75th percentiles.

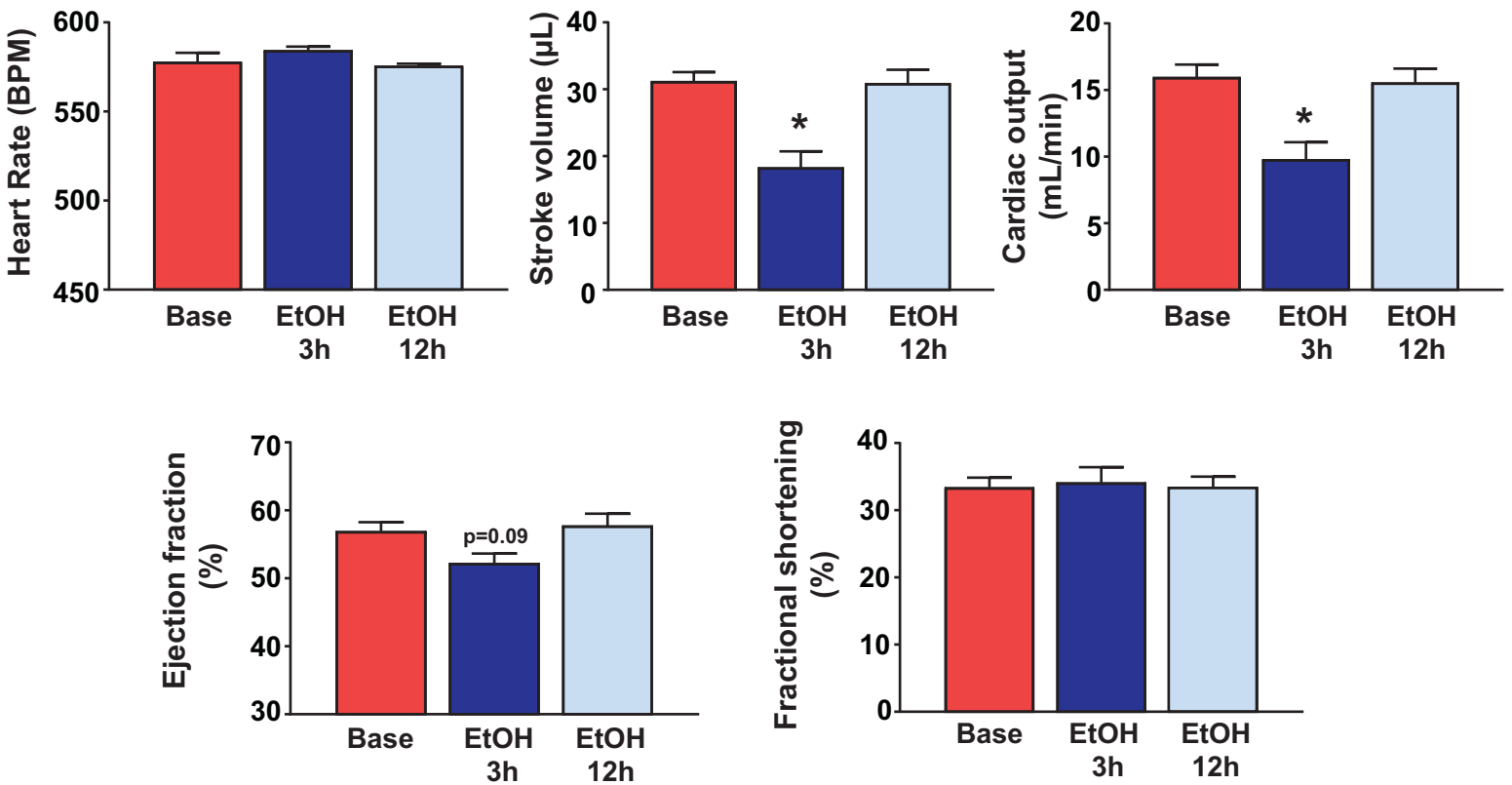
Suppl.Fig.5: Attenuation binge alcohol-induced vascular effects in CB1-R knockout mice. (A) Systolic indices of LV performance [maximal slope of pressure increment (dP/dtmax), end-systolic pressure, stroke work] in control and alcohol-binged $CB1^{+/+}$ and $CB1^{-/-}$ mice 3h after maltodextrin or alcohol gavage. (C) Binge alcohol-induced changes of mean arterial blood pressure and total peripheral resistance measured in control and alcohol-binged $CB1^{+/+}$ and $CB1^{-/-}$ mice. Data are expressed as mean±SEM, n=4-5; *p<0.05 *vs.* corresponding control group; $^{\#}$p<0.05 *vs.* alcohol-binged $CB1^{+/+}$ group.

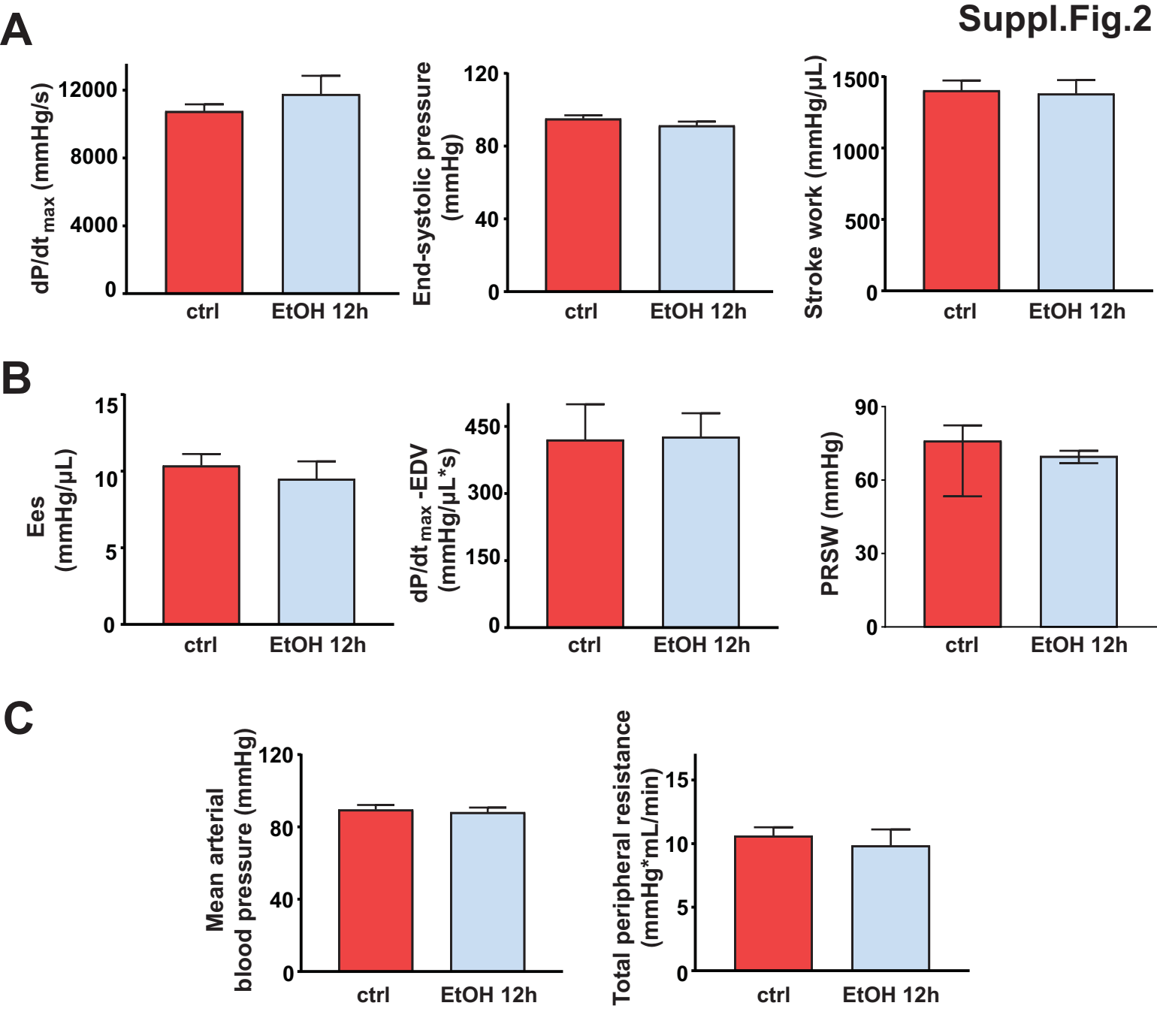
Suppl.Fig.6: Binge alcohol-induced cardiovascular effects are unaltered in CB2-R knockout mice. (A) Representative P-V loops of alcohol-binged $CB2^{+/+}$ and $CB2^{-/-}$ mice after gradual preload reduction obtained by vena cava occlusion. Red lines indicate the slope of end-systolic PV relationship, whereas green lines depict the slope of end-diastolic P-V relationship (B) Systolic indices of LV performance [maximal slope of pressure increment (dP/dtmax), end-systolic pressure, stroke work] in control and in alcohol-binged $CB2^{+/+}$ and $CB2^{-/-}$ mice 3h after maltodextrin or alcohol gavage. (C) Load-independent indices of LV performance [end-systolic elastance (Ees) the dP/dtmax -end-diastolic volume (EDV) relation and preload recruitable stroke work (PRSW)] in control and in alcohol-binged $CB2^{+/+}$ and $CB2^{-/-}$ mice. (D) Binge alcohol-induced changes of mean arterial blood pressure and total peripheral resistance measured in $CB2^{+/+}$ and $CB2^{-/-}$ mice. Data are expressed as mean ± SEM, n=4-5; *p<0.05 *vs.* corresponding control group.

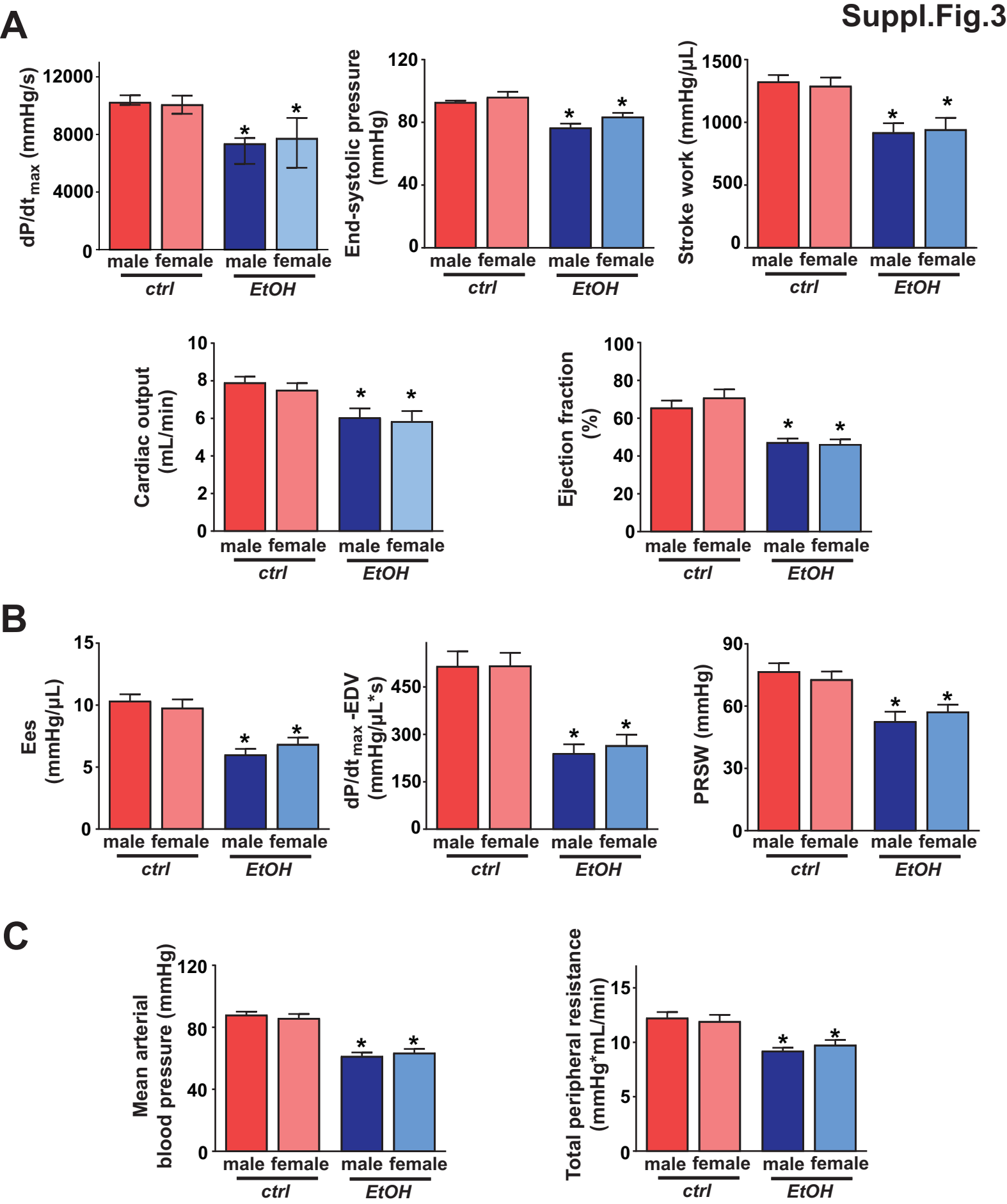
A


Baseline
3h after EtOH binge
12h after EtOH binge
LVIDd
LVIDs
LVIDd
LVIDs
LVIDd
LVIDs
7 mm
500 ms
500 ms
500 ms

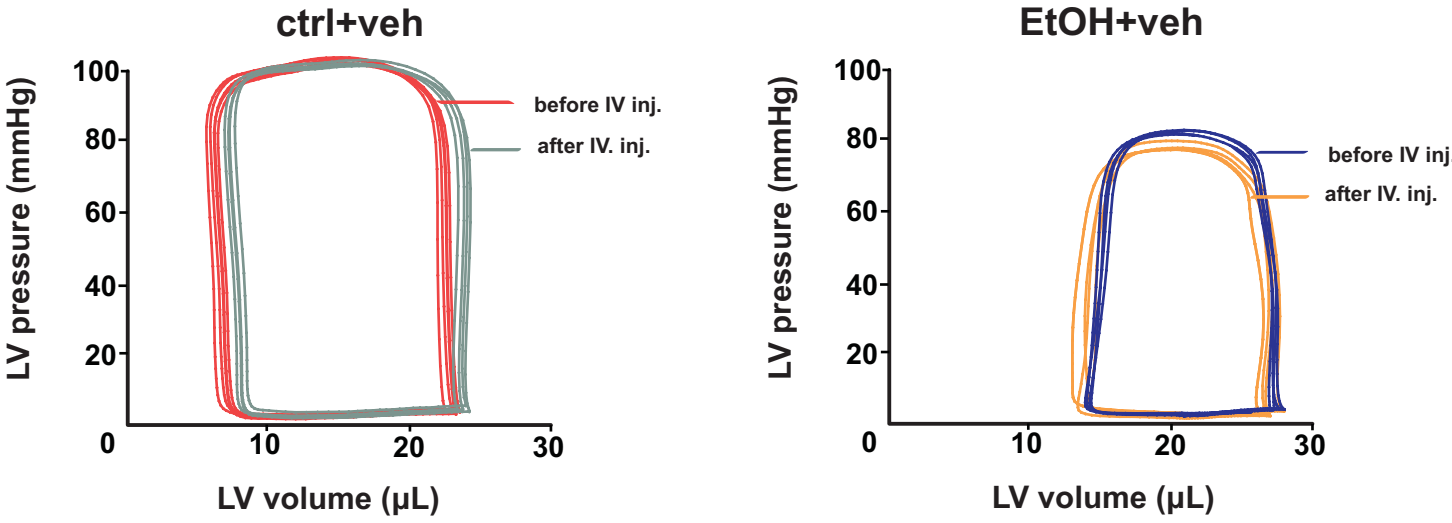
B


Heart Rate (BPM)
600
550
500
450
Base
EtOH 3h
EtOH 12h
Stroke volume (µL)
40
30
20
10
0
*
Base
EtOH 3h
EtOH 12h
Cardiac output (mL/min)
20
15
10
5
0
*
Base
EtOH 3h
EtOH 12h
Ejection fraction (%)
70
60
50
40
30
p=0.09
Base
EtOH 3h
EtOH 12h
Fractional shortening (%)
40
30
20
10
0
Base
EtOH 3h
EtOH 12h

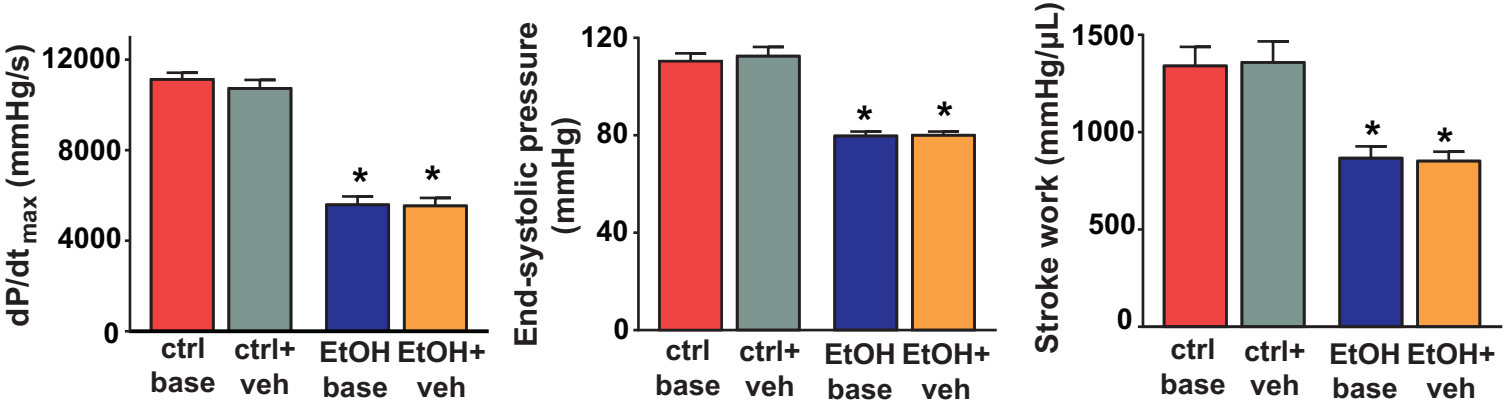

A
dP/dt max (mmHg/s)
12000
8000
4000
0
ctrl
EtOH 12h
End-systolic pressure (mmHg)
120
80
40
0
ctrl
EtOH 12h
Stroke work (mmHg/µL)
1500
1000
500
0
ctrl
EtOH 12h
B
Ees (mmHg/µL)
15
10
5
0
ctrl
EtOH 12h
dP/dt max -EDV (mmHg/µL*s)
450
300
150
0
ctrl
EtOH 12h
PRSW (mmHg)
90
60
30
0
ctrl
EtOH 12h
C
Mean arterial blood pressure (mmHg)
120
80
40
0
ctrl
EtOH 12h
Total peripheral resistance (mmHg*mL/min)
15
10
5
0
ctrl
EtOH 12h


A
dP/dt$_{max}$ (mmHg/s)
12000
8000
4000
0
End-systolic pressure (mmHg)
120
80
40
0
Stroke work (mmHg/µL)
1500
1000
500
0
Cardiac output (mL/min)
10
8
6
4
2
0
Ejection fraction (%)
100
80
60
40
20
0
male female male female
ctrl EtOH
B
Ees (mmHg/µL)
15
10
5
0
dP/dt$_{max}$-EDV (mmHg/µL*s)
450
300
150
0
PRSW (mmHg)
90
60
30
0
C
Mean arterial blood pressure (mmHg)
120
80
40
0
Total peripheral resistance (mmHg*mL/min)
15
10
5
0

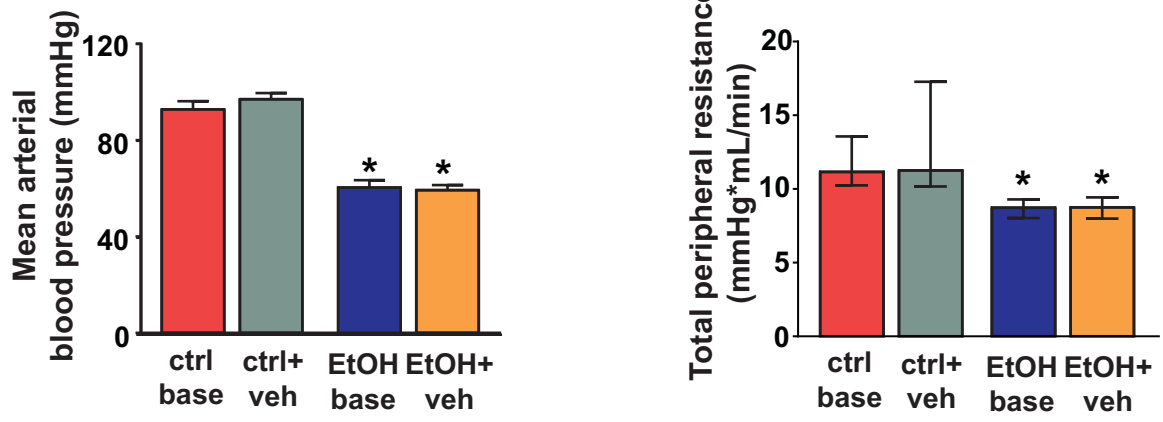
A

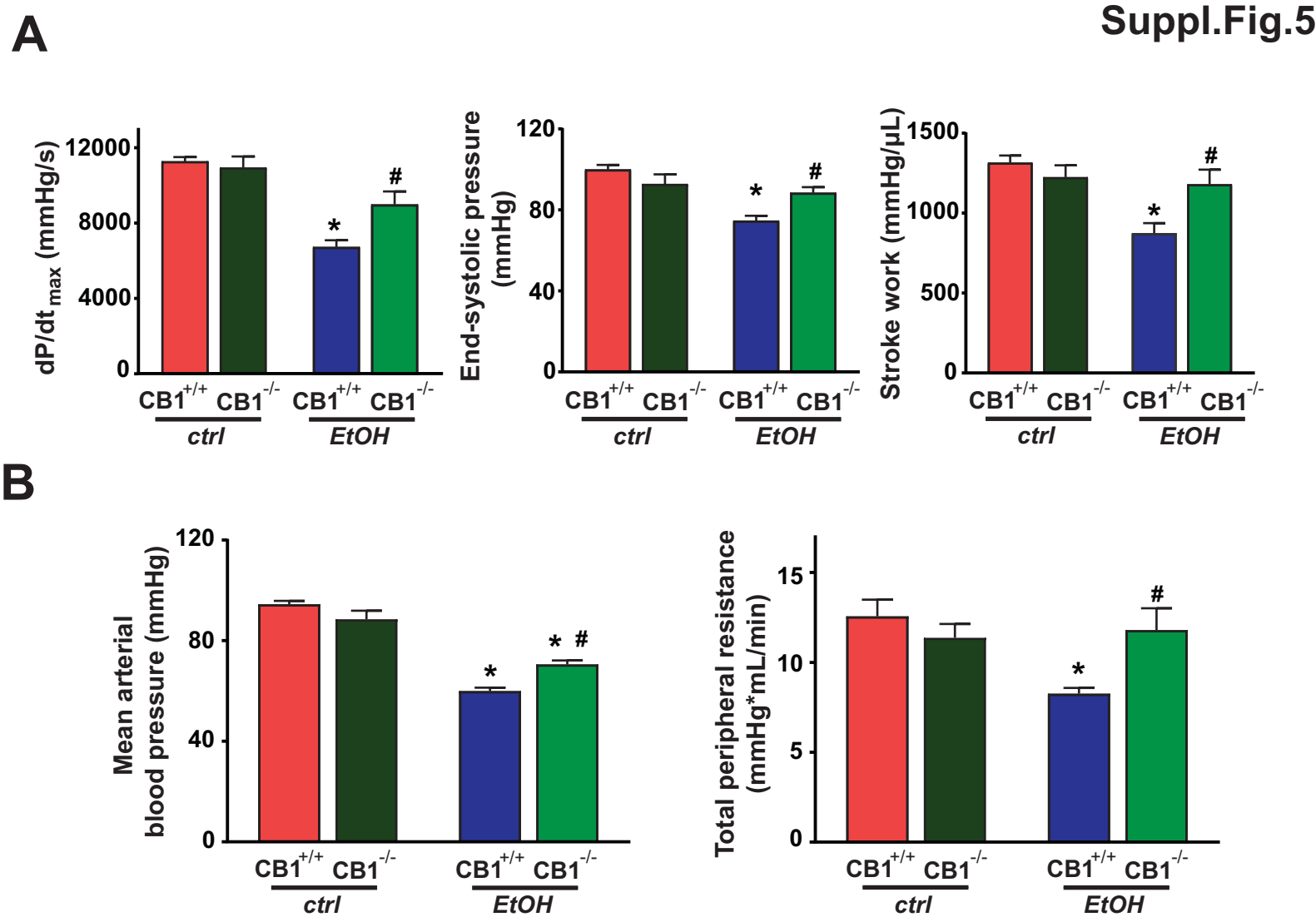

ctrl+veh
EtOH+veh
LV pressure (mmHg)
LV volume (µL)
before IV inj.
after IV. inj.
0
20
40
60
80
100
10
20
30

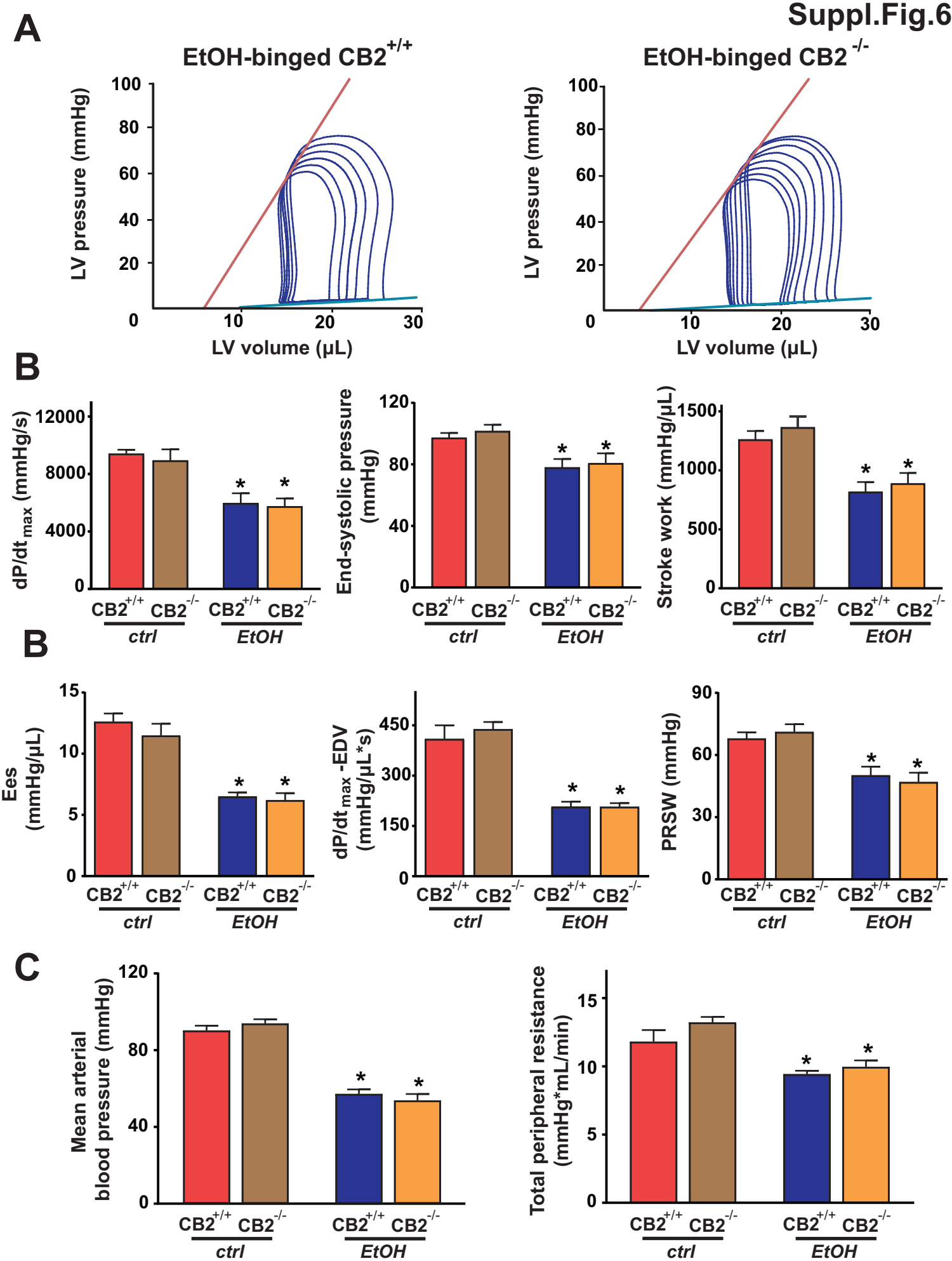
B


dP/dt max (mmHg/s)
End-systolic pressure (mmHg)
Stroke work (mmHg/µL)
ctrl base
ctrl+ veh
EtOH base
EtOH+ veh
0
4000
8000
12000
40
80
120
500
1000
1500
*

C


Mean arterial blood pressure (mmHg)
Total peripheral resistance (mmHg*mL/min)
ctrl base
ctrl+ veh
EtOH base
EtOH+ veh
0
40
80
120
5
10
15
20
*


A
dP/dt max (mmHg/s)
12000
8000
4000
0
End-systolic pressure (mmHg)
120
80
40
0
Stroke work (mmHg/µL)
1500
1000
500
0
CB1+/+ CB1-/-
ctrl
EtOH
B
Mean arterial blood pressure (mmHg)
120
80
40
0
Total peripheral resistance (mmHg*mL/min)
15
10
5
0


A
EtOH-binged CB2+/+
EtOH-binged CB2 -/-
LV pressure (mmHg)
LV volume (µL)
B
dP/dtmax (mmHg/s)
End-systolic pressure (mmHg)
Stroke work (mmHg/µL)
B
Ees (mmHg/µL)
dP/dtmax -EDV (mmHg/µL*s)
PRSW (mmHg)
C
Mean arterial blood pressure (mmHg)
Total peripheral resistance (mmHg*mL/min)
CB2+/+ CB2-/-
ctrl
EtOH