Supplemental information

Assessment of PEEP-ventilation and the time point of parallel-conductance determination for pressure-volume analysis under β-adrenergic stimulation in mice.

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**Supplemental Figures**

**Figure S1. Surgical preparation for open-chest pressure-volume recordings.** (A) Intra-operative picture of the femoral vein depicting the blunted vessel before (left panel) and after (right panel) cannulation with a polyethylene tube. (B) First steps of the thoracotomy showing the incision lines through abdominal muscles (left panel) and the ribs (right panel). (C) Intra-operative view on the dorsal thorax after thoracotomy depicting the occlusion suture beneath the inferior cava vein (ICV) before (left panel) and after (right panel) catheter insertion. Abbreviations: RV, Right ventricle and LV, Left ventricle.
Figure S2. Significance of end-expirational ventilation stops. Representative (A) end-expirational and (B) end-inspirational ventilation stops from the same animal within 10s showing a typical decrease in stroke volume (SV) during an end-inspirational ventilation-stop. Upper traces show the airway pressure and the lower traces depict the intra-ventricular volume.
Figure S3. Representative pressure-volume analysis. (A) Representative traces of intraventricular pressure (upper traces), volume (middle traces) and airway pressure (lower traces). Dashed lines in (A) delimit the analysis of basal PV recordings and those during preload reduction evoked by caval vein occlusion. For the analysis of preload independent parameters, strictly the first five consecutive loops with decreased preload were selected. (B and C) Pressure-volume recordings and corresponding parameters during basal measurement (B) or preload-reduction (C) derived from areas labelled in (A). Abbreviations: dP/dt_{min}: Minimum dP/dt, dP/dt_{max}: Maximum dP/dt, V_{es}: End-systolic Volume, V_{ed}: End-diastolic Volume, ESPVR: End-systolic pressure-volume relationship, PRSW: Preload Recruitable Stroke Work, EDPVR: End-diastolic pressure-volume relationship.
Figure S4. Detection of ectopic beats in pressure-volume recordings. (A) Ectopic beat with reduced ejection and (B) ectopic beat without ejection. Note that each ectopic beat is followed by a compensatory increased ejection (highlighted blue PV loop), a phenomenon called post-extrasystolic potentiation. Red traces: Intra-ventricular pressure; Blue traces: Intra-ventricular volume; Green traces: Electrocardiogram (ECG). Abbreviations: LV: Left ventricular, PV: pressure-volume.
**Supplemental Tables**

*Table S1: General parameters and calibration settings from mice used to analyse the effect of 2cm H₂O positive end-expiratory pressure.*

|                      | No PEEP (n=10) | PEEP (n=8) | p-value |
|----------------------|----------------|------------|---------|
| **Body Weight (g)**  | 33.8 ± 1.1     | 33.7 ± 2.4 | 0.9286  |
| **Heart Weight (mg)**| 149.5 ± 6.5    | 145.9 ± 4.8| 0.1990  |
| **HW/TL (mg/mm)**    | 7.87 ± 0.21    | 7.83 ± 0.24| 0.7421  |
| **Age (w)**          | 20.3 ± 0.1     | 21.0 ± 0.9 | 0.0780  |
| **Body Temperature (°C)** | 36.9 ± 0.2     | 36.9 ± 0.2 | 0.7948  |
| **Gp (RVU)**         | 19.1 ± 3.3     | 18.2 ± 1.9 | 0.4784  |
| **CF (μl/RVU)**      | 1.87 ± 0.09    | 1.83 ± 0.16| 0.5753  |

Abbreviations: PEEP: Positive end-expiratory pressure, HW: Heart Weight, TL: Tibia length, Gp: Parallel-Conductance, RVU: Relative Volume Units, CF: Conversion Factor for conductance to volume conversion; n: number of mice. P-values in an unpaired t-test between groups.

*Table S2: General parameters and calibration settings from mice used to analyse the effect of early hypertonic saline.*

|                      | Late Saline (n=23) | Early Saline (n=15) | p-value |
|----------------------|--------------------|---------------------|---------|
| **Body Weight (g)**  | 30.3 ± 3.4         | 32.0 ± 3.1          | 0.1179  |
| **Heart Weight (mg)**| 141.5 ± 7.3        | 142.1 ± 12.5        | 0.8505  |
| **HW/TL (mg/mm)**    | 7.69 ± 0.34        | 7.61 ± 0.6          | 0.6218  |
| **Age (w)**          | 18.8 ± 1.8         | 19.8 ± 0.8          | 0.0186  |
| **Body Temperature (°C)** | 37.0 ± 0.2        | 37.0 ± 0.2          | 0.7792  |
| **Gp (RVU)**         | 17.0 ± 2.0         | 19.6 ± 2.2          | 0.0008  |
| **CF (μl/RVU)**      | 1.75 ± 0.13        | 1.77 ± 0.16         | 0.6813  |

Abbreviations: Early saline: Hypertonic Saline prior to PV recordings, Late saline: Hypertonic Saline after PV recordings, HW: Heart Weight, TL: Tibia length, Gp: Parallel-Conductance estimated by hypertonic saline injection, RVU: Relative Volume Units, CF: Conversion factor for conductance to volume conversion; n: number of mice. Saline bolus: 10μl bolus of 15% sodium-chloride. Bold digits indicate significant p-values in an unpaired t-test (< 0.05).
Table S3: Extended analysis of pressure-volume parameters from late and early saline groups.

| ISO (ng/min) | Late saline (n=23) | Early saline (n=15) | p-value | Late saline (n=23) | Early saline (n=15) | p-value |
|-------------|--------------------|---------------------|---------|--------------------|---------------------|---------|
|             | Heart rate (bpm)   |                     |         | End-diastolic Volume (µl) |                     |         |
| 0           | 476 ± 27           | 482 ± 33            | 0.5566  | 26.8 ± 3.4         | 37.6 ± 6.9         | < 0.0001 |
| 0.2475      | 496 ± 27           | 489 ± 36            | 0.5222  | 25.8 ± 3.5         | 34.2 ± 6.6         | 0.0002  |
| 0.825       | 541 ± 20           | 517 ± 36            | 0.0331  | 23.8 ± 3.2         | 30.1 ± 5.3         | 0.0004  |
| 2.475       | 604 ± 21           | 580 ± 30            | 0.0127  | 23 ± 3.1           | 26.5 ± 5           | 0.0257  |
| 8.25        | 629 ± 19           | 614 ± 21            | 0.0420  | 24.5 ± 2.7         | 25.7 ± 5           | 0.4179  |
|             | Stroke Volume (µl) |                     |         | End-systolic Volume (µl) |                     |         |
| 0           | 17.6 ± 2.7         | 17.2 ± 3.7          | 0.7163  | 10.85 ± 4.19       | 22.99 ± 6.88       | 0.0001  |
| 0.2475      | 18.5 ± 2.3         | 18.1 ± 3.8          | 0.6869  | 8.6 ± 4.1          | 18.42 ± 6.59       | 0.0007  |
| 0.825       | 20.5 ± 2.7         | 20.2 ± 4.2          | 0.7929  | 4.23 ± 2.5         | 11.67 ± 5.27       | 0.0005  |
| 2.475       | 22.4 ± 2.1         | 24.2 ± 4.2          | 0.1421  | 1.57 ± 1.8         | 3.54 ± 5.04        | 0.0337  |
| 8.25        | 24.1 ± 2.3         | 25.7 ± 4.5          | 0.2139  | 1.3 ± 1.52         | 1.1 ± 4.99         | 0.6385  |
|             | Cardiac Output (µl/min) |                 |         | End-diastolic Pressure (mmHg) |                     |         |
| 0           | 8376 ± 1483        | 8327 ± 2147         | 0.9386  | 5.23 ± 0.94        | 7.63 ± 2.14        | 0.0007  |
| 0.2475      | 9199 ± 1410        | 8912 ± 2322         | 0.6712  | 5.49 ± 0.95        | 7.23 ± 2.15        | 0.0085  |
| 0.825       | 11097 ± 1639       | 10533 ± 2693        | 0.4749  | 5.49 ± 1.36        | 6.69 ± 2.11        | 0.0642  |
| 2.475       | 13515 ± 1366       | 14075 ± 2843        | 0.4858  | 5.25 ± 0.96        | 6.38 ± 2.03        | 0.0592  |
| 8.25        | 15137 ± 1564       | 15817 ± 2984        | 0.4263  | 5.52 ± 0.95        | 6.43 ± 1.95        | 0.1114  |
|             | Maximum Pressure (mmHg) |                 |         | EDPVR (α) |                     |         |
| 0           | 81.2 ± 7.0         | 81.5 ± 9.4          | 0.9254  | 1.05 ± 0.79        | 1.66 ± 1.48        | 0.1564  |
| 0.2475      | 84.8 ± 7.0         | 83.7 ± 10.2         | 0.7293  | 1.2 ± 0.66         | 1.81 ± 1.55        | 0.1662  |
| 0.825       | 86.2 ± 6.0         | 82.5 ± 8.2          | 0.1462  | 1.47 ± 0.74        | 2.32 ± 1.53        | 0.0589  |
| 2.475       | 87.8 ± 6.1         | 84.6 ± 5.7          | 0.1010  | 1.92 ± 0.78        | 2.86 ± 1.56        | 0.0444  |
| 8.25        | 88.7 ± 5.3         | 86.7 ± 5.5          | 0.2960  | 1.82 ± 0.82        | 3 ± 1.56           | 0.0146  |
|             | EDPVR (α) |                     |         | Minimum dP/dt (mmHg/s) |                     |         |
| 0           | 71.5 ± 8.6         | 59.5 ± 14.2         | 0.0081  | -7936 ± 1260       | -7307 ± 2177       | 0.3224  |
| 0.2475      | 80 ± 12.1          | 64 ± 20.5           | 0.0130  | -8682 ± 1207       | -8073 ± 2303       | 0.3569  |
| 0.825       | 97 ± 14.1          | 75.5 ± 15.6         | 0.0002  | -9050 ± 1169       | -8354 ± 1887       | 0.2154  |
| 2.475       | 110.8 ± 17.1       | 90.9 ± 14           | 0.0004  | -8694 ± 1154       | -8693 ± 1215       | 0.9976  |
| 8.25        | 115.6 ± 14.9       | 100.5 ± 10.6        | 0.0008  | -8679 ± 1284       | -8196 ± 1610       | 0.3379  |
|             | Maximum dP/dt (mmHg/s) |                 |         | Tau (Weiss-Equation) |                     |         |
| 0           | 7017 ± 1544        | 6077 ± 2102         | 0.1495  | 6.07 ± 0.55        | 7.1 ± 1.26         | 0.0080  |
| 0.2475      | 8017 ± 1767        | 6775 ± 2128         | 0.0716  | 5.69 ± 0.44        | 6.46 ± 1.1         | 0.0189  |
| 0.825       | 9480 ± 1850        | 7625 ± 2154         | 0.0108  | 5.14 ± 0.51        | 5.75 ± 0.87        | 0.0246  |
| 2.475       | 12144 ± 1572       | 10415 ± 2185        | 0.0142  | 5.01 ± 0.59        | 5.15 ± 0.72        | 0.5418  |
| 8.25        | 13658 ± 1160       | 12458 ± 1394        | 0.0103  | 5.12 ± 0.67        | 5.42 ± 0.84        | 0.2529  |

Application of 10µl 15% sodium-chloride to correct for parallel-conductance either after (Late saline) or prior to (Early saline) PV-Loop recordings. Abbreviations: ISO: Isoprenaline; PRSW: Preload Recruitable Stroke Work; EDPVR: End-diastolic Pressure-Volume Relationship (alpha coefficient); n: number of mice; p-values between Late saline and Early saline: Bold digits indicate significant p-values in an unpaired t-test (< 0.05).
Table S4: General parameters and calibration settings from mice used to analyse the effects of end-systolic pressure-spikes.

| Parameter                        | ESPS (n=6)       | No ESPS (n=17)   | p-value  |
|----------------------------------|------------------|-----------------|----------|
| Body Weight (g)                  | 29.2 ± 3.1       | 30.6 ± 3.5      | 0.3896   |
| Heart Weight (mg)                | 142.1 ± 7.2      | 141.2 ± 7.5     | 0.8050   |
| HW/TL (mg/mm)                    | 7.71 ± 0.31      | 7.69 ± 0.36     | 0.9025   |
| Age (w)                          | 18.6 ± 1.5       | 18.8 ± 1.9      | 0.7475   |
| Body Temperature (°C)            | 37.1 ± 1.5       | 37.0 ± 0.2      | 0.7593   |
| Gp (RVU)                         | 15.8 ± 1.3       | 17.4 ± 2.1      | 0.0511   |
| CF (µl/RVU)                      | 1.79 ± 0.14      | 1.74 ± 0.12     | 0.4232   |

Abbreviations: ESPS: End-systolic pressure-spike, HW: Heart weight, TL: Tibia length, Gp: Parallel-Conductance estimated by hypertonic saline injection, RVU: Relative Volume Units, CF: Conversion Factor for conductance to volume conversion; n: number of mice. P-values in an unpaired t-test between groups.
Table S5: Extended analysis of pressure-volume parameters from mice presenting end-systolic pressure-spike.

| ISO (ng/min) | Systolic Parameters | Diastolic Parameters |
|-------------|---------------------|----------------------|
|             | ESPS (n=6)          | No ESPS (n=17)       | p-value | ESPS (n=6) | No ESPS (n=17) | p-value |
|             | Heart rate (bpm)    | End-diastolic Volume (µl) |
| 0           | 469 ± 15            | 479 ± 31             | 0.4746  | 26.9 ± 1.8 | 26.8 ± 3.9    | 0.8278  |
| 0.2475      | 489 ± 16            | 499 ± 30             | 0.4514  | 25.7 ± 1.9 | 25.8 ± 4.0    | 0.5933  |
| 0.825       | 529 ± 16            | 545 ± 20             | 0.0877  | 23.3 ± 1.3 | 24.0 ± 3.6    | 0.5163  |
| 2.475       | 601 ± 27            | 605 ± 20             | 0.6962  | 21.5 ± 1.3 | 23.5 ± 3.4    | 0.0601  |
| 8.25        | 631 ± 10            | 628 ± 20             | 0.6771  | 23.0 ± 1.4 | 25.0 ± 2.9    | 0.0407  |
|             | Stroke Volume (µl)  | End-systolic Volume (µl) |
| 0           | 16.3 ± 2.9          | 18.0 ± 2.5           | 0.2160  | 12.22 ± 2.16 | 10.37 ± 3.87  | 0.3570  |
| 0.2475      | 17.1 ± 2.5          | 19.0 ± 2.1           | 0.1019  | 9.97 ± 2.24 | 8.12 ± 4.03   | 0.3660  |
| 0.825       | 19.0 ± 2.1          | 21.0 ± 2.7           | 0.0834  | 5.24 ± 2.23 | 3.87 ± 3.62   | 0.2395  |
| 2.475       | 20.6 ± 1.0          | 23.0 ± 2.1           | 0.0005  | 1.54 ± 1.69 | 1.58 ± 3.44   | 0.9629  |
| 8.25        | 22.4 ± 1.6          | 24.7 ± 2.2           | 0.0192  | 1.20 ± 1.17 | 1.33 ± 2.89   | 0.8351  |
|             | Cardiac Output (µl/min) | End-diastolic Pressure (mmHg) |
| 0           | 7663 ± 1393         | 8628 ± 1469          | 0.1744  | 5.05 ± 0.86 | 5.30 ± 0.99   | 0.8622  |
| 0.2475      | 8355 ± 1317         | 9497 ± 1352          | 0.0898  | 5.24 ± 1.22 | 5.58 ± 0.86   | 0.5928  |
| 0.825       | 10057 ± 1263        | 11464 ± 1627         | 0.0524  | 5.20 ± 1.44 | 5.60 ± 1.36   | 0.5716  |
| 2.475       | 12391 ± 885         | 13911 ± 1297         | 0.0073  | 5.18 ± 1.46 | 5.27 ± 0.78   | 0.8858  |
| 8.25        | 14114 ± 1045        | 15498 ± 1578         | 0.0305  | 5.35 ± 1.45 | 5.58 ± 0.75   | 0.7168  |
|             | Maximum Pressure (mmHg) | EDPVR (α) |
| 0           | 79.3 ± 6.6          | 81.9 ± 7.2           | 0.4255  | 0.71 ± 0.31 | 1.17 ± 0.87   | 0.1616  |
| 0.2475      | 81.1 ± 6.1          | 86.1 ± 7.0           | 0.1075  | 0.95 ± 0.40 | 1.28 ± 0.72   | 0.2589  |
| 0.825       | 83.1 ± 5.7          | 87.3 ± 5.9           | 0.1561  | 1.26 ± 0.62 | 1.54 ± 0.78   | 0.3961  |
| 2.475       | 90.0* ± 7.3         | 88.6 ± 6.3           | 0.7453  | 1.91 ± 0.98 | 1.93 ± 0.73   | 0.9623  |
| 8.25        | 99.8* ± 8.8         | 89.5 ± 5.6           | 0.0347  | 2.04 ± 1.01 | 1.75 ± 0.76   | 0.5406  |
|             | PRSW (mmHg)         | Minimum dP/dt (mmHg/s) |
| 0           | 76.2 ± 8.3          | 69.8 ± 8.3           | 0.0897  | -7606 ± 1013 | -8053 ± 1345  | 0.3563  |
| 0.2475      | 79.6 ± 8.6          | 80.1 ± 13.3          | 0.9710  | -7885 ± 796 | -8964 ± 1218  | 0.0221  |
| 0.825       | 97.0 ± 11.3         | 97.0 ± 15.3          | 0.9929  | -8181 ± 834 | -9357 ± 1130  | 0.0197  |
| 2.475       | 107.3 ± 11.8        | 112.0 ± 18.8         | 0.4861  | -8928 ± 696 | -8612 ± 1286  | 0.4650  |
| 8.25        | 116.4 ± 9.7         | 115.4 ± 16.7         | 0.8533  | -9807 ± 1410 | -8281 ± 1001  | 0.0453  |
|             | Maximum dP/dt (mmHg/s) | Tau (Weiss-Equation) |
| 0           | 6541 ± 1320         | 7185 ± 1618          | 0.3275  | 6.18 ± 0.46 | 6.03 ± 0.58   | 0.3783  |
| 0.2475      | 7160 ± 1458         | 8319 ± 1804          | 0.1209  | 5.92 ± 0.56 | 5.61 ± 0.39   | 0.2306  |
| 0.825       | 8522 ± 1868         | 9817 ± 1774          | 0.1756  | 5.39 ± 0.74 | 5.05 ± 0.40   | 0.3225  |
| 2.475       | 11517 ± 1515        | 12365 ± 1575         | 0.2727  | 5.26 ± 0.52 | 4.93 ± 0.60   | 0.2182  |
| 8.25        | 13394 ± 831         | 13751 ± 1264         | 0.4485  | 5.52 ± 0.45 | 4.97 ± 0.69   | 0.0445  |

Abbreviations: ISO: Isoprenaline; PRSW: Preload Recruitable Stroke Work; EDPVR: End-diastolic Pressure-Volume Relationship (alpha coefficient); ESPS: end-systolic pressure-spike; n: number of mice; p-values in an unpaired t-test between recordings with and without ESPS; Bold digits indicate significant p-values (< 0.05). *Before manual correction as described in Figure 5.