**Supplemental Table 1**

|  |  |  |  |
| --- | --- | --- | --- |
| **Primers of genetic knock-in mice identification** | | | |
| Names | Sequences (5'-3') | Products (bp) | Notes |
| Screening | F: TGTGTGTCTCCATCTGGGTG | 307/217/90 | (1) |
| R: CTTTGTCCAGCTGCCTAGGA |
| Genotyping | WT\_F: GTCCAGAGCGGCCACGTT | 152 | (2) |
| KI\_F: TCCAGAGCGGCCGCGAG |
| R: CTCCCTCCTGGGGACAGTCT |

Notes: (1) After *NotI* digestion, the Wt mice will show one 307 bp band, Het will have three bands: 307, 217 and 90bp, Hom will have two bands: 217 and 90 bp.

(2) Two PCR reactions will be conducted: Reaction A (WT\_F+R) and Reaction B (KI\_F+R), respectively. Results: Wt mouse: only "A" reaction is positive; Het: "A" and "B" are positive; Hom mice: only "B" reaction is positive.

**Supplemental Table 2**

|  |  |  |
| --- | --- | --- |
| **qPCR primers** | | |
| Names | Sequences (5'-3') | Products (bp) |
| *Scn4b* | F: GGCAGATACACCTGCTTCGT | 112 |
| R: GAGTCACCGTGTTGTCCACT |
| *Ankrd1* | F: ATAAACGGACGGCACTCCAC | 147 |
| R: CATCTGCGTTTCCTCCACGA |
| *Kcne1* | F: CTGCCCAATTCCACGACTGTT | 102 |
| R: GAGCTGAGACTTACGAGCCA |
| *Kcnq1* | F: CTACGACGTGCGAGATGTC | 154 |
| R: GTTACTGCCACGGTCTTTGC |
| *Slc40a1* | F: ACCAAGGCAAGAGATCAAACC | 138 |
| R: AGACACTGCAAAGTGCCACAT |
| *Tbx20* | F: TCTCGAGCCAATGCCTTCTC | 126 |
| R: TGGCTGGGCACATGATGATT |
| *Tecrl* | F: CCCTTCTCGAATTGGCCTAC | 136 |
| R: TGTAGTCCACCCGACTTGC |
| *Mmp2* | F: CCGGTTTATTTGGCGGACAG | 109 |
| R: GGCCTCATACACAGCGTCAA |
| *Fah* | F: ACGGATTGGTGTAGCCATCG | 138 |
| R: GCTTGACCCAGACCCATGAA |
| *Nppb* | F: GAGGTCACTCCTATCCTCTGG | 100 |
| R: GCCATTTCCTCCGACTTTTCTC |
| *Rbm20* | F: TCCAAGACATCCATTCCCAGA | 250 |
| R: TGTGCCCAAACATCCAACC |
| *Gapdh* | F: GGTGGACCTCATGGCCTACA | 82 |
| R: CTCTCTTGCTCAGTGTCCTTGCT |

**Supplemental Table 3**

|  |  |  |
| --- | --- | --- |
| **Splicing RT-PCR primers** | | |
| Names | Sequences (5'-3') | Products (bp) |
| *Camk2d* | F: CGAGAAATTTTTCAGCAGCC | 197, 155, 128, 95 |
| R: GTCTTCATCCTCAATGGTGGTG |
| *RyR2* | F: GTTGTCACGATGAAGAAGACGATG | 177, 153 |
| R: CTTTGCTGGCACTGATAGTCTG |
| *Abcd3* | F: TGCGGTTATGCTGGTATCTCG | 101, 172 |
| R: TGAAATCTTTGCTGCTGCGAC |
| *Tpm2* | F: AGAGCCGAGGTGGCTGA | 154, 230 |
| R: TCAGCTTCTCCTCCAGAAGT |
| *Gapdh* | F: GGTGGACCTCATGGCCTACA | 82 |
| R: CTCTCTTGCTCAGTGTCCTTGCT |

**Supplemental Table 4**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sex** | **Male** | | | | | | |  | **Female** | | | | | | |
| **Genotype** | **WT** | **HT** | **HM** | **ANOVA P value** | **Tukey's multiple**  **comparisons test** | | |  | **WT** | **HT** | **HM** | **ANOVA P value** | **Tukey's multiple**  **comparisons test** | | |
| **N** | **7** | **6** | **8** | **Adjusted *P* Value** | | |  | **10** | **7** | **7** | **Adjusted *P* Value** | | |
| **Left Venticle (LV)**  **(M-Mode)** | **Mean**  **± SEM** | **Mean**  **± SEM** | **Mean**  **± SEM** | **WT vs. HT** | **WT vs. HM** | **HT vs. HM** |  | **Mean**  **± SEM** | **Mean**  **± SEM** | **Mean**  **± SEM** | **WT vs. HT** | **WT vs. HM** | **HT vs. HM** |
| Heart Rate  (average Doppler  & M-mode & EKV)  (BPM) | 466.20  ±24.42 | 473.89  ±16.42 | 454.46  ±24.12 | 0.8330 | 0.972 | 0.926 | 0.824 |  | 474.18  ±16.29 | 460.57  ±15.73 | 459.21  ±27.26 | 0.8268 | 0.875 | 0.851 | 0.999 |
| LVDs: Diameter;s (mm) \*\*\* | 3.60  ±0.10 | 4.40  ±0.12 | 4.54  ±0.16 | 0.0002 | 0.003 | 3.00E-04 | 0.781 |  | 3.32  ±0.08 | 3.82  ±0.07 | 4.21  ±0.23 | 0.0004 | 0.038 | 3.00E-04 | 0.162 |
| LVDd: Diameter;d (mm) \*\*\* | 4.43  ±0.05 | 4.92  ±0.10 | 5.06  ±0.14 | 0.0021 | 0.022 | 0.002 | 0.673 |  | 4.09  ±0.06 | 4.47  ±0.05 | 4.76  ±0.19 | 0.0008 | 0.047 | 0.001 | 0.207 |
| LVVs: Volume;s (uL) \* | 54.98  ±3.99 | 88.27  ±5.22 | 96.31  ±8.70 | 0.0008 | 0.009 | 0.001 | 0.687 |  | 45.55  ±3.19 | 63.27  ±3.22 | 81.78  ±9.99 | 0.0008 | 0.092 | 0.001 | 0.108 |
| LVVd: Volume;d (uL) \* | 89.58  ±2.89 | 114.29  ±4.86 | 123.49  ±9.37 | <0.0001 | <0.0001 | 0.001 | 0.256 |  | 74.13  ±3.33 | 91.51  ±3.49 | 107.82  ±10.11 | 0.0019 | 0.110 | 0.001 | 0.183 |
| Stroke Volume (uL) | 34.64  ±2.03 | 26.09  ±1.94 | 27.09  ±2.12 | 0.0183 | 0.030 | 0.039 | 0.939 |  | 28.55  ±2.11 | 28.05  ±1.78 | 25.46  ±2.48 | 0.5795 | 0.985 | 0.572 | 0.715 |
| Ejection Fraction (%) | 39.06  ±2.88 | 22.98  ±1.81 | 22.65  ±2.24 | 0.0001 | 0.001 | 3.00E-04 | 0.995 |  | 38.74  ±2.67 | 30.9  ±2.13 | 25.37  ±3.52 | 0.0091 | 0.141 | 0.008 | 0.416 |
| Fractional Shortening (%) | 18.94  ±1.62 | 10.58  ±0.89 | 10.47  ±1.12 | 0.0002 | 0.001 | 3.00E-04 | 0.998 |  | 18.67  ±1.46 | 14.51  ±1.09 | 11.81  ±1.73 | 0.0017 | 0.043 | 0.002 | 0.402 |
| Cardiac Output (mL/min) \* | 16.51  ±1.51 | 12.21  ±1.28 | 13.18  ±1.26 | 0.0989 | 0.109 | 0.203 | 0.874 |  | 13.84  ±1.17 | 13.12  ±1.12 | 12.35  ±1.65 | 0.7205 | 0.920 | 0.699 | 0.919 |
| LV Mass (mg) \* | 93.80  ±4.50 | 108.1  ±2.81 | 101.34  ±6.88 | 0.2284 | 0.203 | 0.579 | 0.666 |  | 73.20  ±3.19 | 88.57  ±3.01 | 107.78  ±7.85 | 0.0002 | 0.079 | 1.00E-04 | 0.039 |
| LVAW;s (mm) \*\*\* | 0.72  ±0.02 | 0.69  ±0.02 | 0.63  ±0.03 | 0.0513 | 0.649 | 0.044 | 0.280 |  | 0.67  ±0.03 | 0.67  ±0.03 | 0.68  ±0.02 | 0.9219 | 0.995 | 0.917 | 0.958 |
| LVAW;d (mm) \*\*\* | 0.59  ±0.03 | 0.59  ±0.01 | 0.51  ±0.02 | 0.0237 | 0.968 | 0.054 | 0.040 |  | 0.53  ±0.02 | 0.57  ±0.02 | 0.57  ±0.02 | 0.1573 | 0.233 | 0.232 | >0.9999 |
| LVPW;s (mm) \*\*\* | 0.69  ±0.02 | 0.60  ±0.04 | 0.59  ±0.02 | 0.0191 | 0.058 | 0.024 | 0.968 |  | 0.66  ±0.03 | 0.62  ±0.03 | 0.68  ±0.02 | 0.4067 | 0.613 | 0.871 | 0.388 |
| LVPW;d (mm) \*\*\* | 0.58  ±0.02 | 0.54  ±0.03 | 0.51  ±0.01 | 0.0614 | 0.353 | 0.050 | 0.596 |  | 0.55  ±0.03 | 0.53  ±0.02 | 0.60  ±0.02 | 0.2003 | 0.890 | 0.332 | 0.205 |
| Relative wall thickness  (2\*LVPW(d)/LVDd) (mm) | 0.26  ±0.01 | 0.22  ±0.02 | 0.20  ±0.01 | 0.0016 | 0.031 | 0.001 | 0.466 |  | 0.27  ±0.01 | 0.24  ±0.01 | 0.26  ±0.02 | 0.3421 | 0.3111 | 0.7647 | 0.746 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Left Atria (LA)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| LA Depth (mm) \*\*\* | 2.74  ±0.09 | 3.04  ±0.07 | 3.05  ±0.14 | 0.2315 | 0.297 | 0.243 | 0.998 |  | 2.90  ±0.09 | 2.89  ±0.15 | 3.09  ±0.17 | 0.5611 | 0.998 | 0.590 | 0.607 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Right Ventricle (RV)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| RV Free Wall (mm) \*\*\* | 0.36  ±0.03 | 0.35  ±0.03 | 0.33  ±0.02 | 0.6689 | 0.956 | 0.654 | 0.845 |  | 0.33  ±0.02 | 0.34  ±0.02 | 0.34  ±0.03 | 0.9582 | 0.957 | 0.999 | 0.974 |
| IVS;d (mm) \*\*\* | 0.54  ±0.05 | 0.56  ±0.02 | 0.54  ±0.02 | 0.8515 | 0.884 | 0.999 | 0.860 |  | 0.50  ±0.02 | 0.50  ±0.02 | 0.46  ±0.02 | 0.3375 | 0.997 | 0.388 | 0.404 |
| RVID;d (mm) \*\*\* | 1.90  ±0.15 | 1.70  ±0.28 | 2.22  ±0.21 | 0.2571 | 0.803 | 0.547 | 0.242 |  | 1.91  ±0.09 | 1.54  ±0.23 | 1.70  ±0.28 | 0.2405 | 0.221 | 0.593 | 0.779 |
| RVID;s (mm) \*\*\* | 1.34  ±0.13 | 1.52  ±0.19 | 1.81  ±0.20 | 0.1669 | 0.777 | 0.151 | 0.481 |  | 1.35  ±0.09 | 1.13  ±0.19 | 1.52  ±0.19 | 0.5129 | 0.482 | 0.855 | 0.828 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Aorta** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Measurement** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Aorta Ejection Time (ms) | 46.94  ±1.63 | 43.19  ±1.20 | 40.12  ±1.50 | 0.0131 | 0.230 | 0.010 | 0.343 |  | 44.49  ±1.47 | 46.39  ±1.89 | 43.51  ±4.35 | 0.7343 | 0.837 | 0.957 | 0.727 |
| AoV Diam Length (mm) \*\*\* | 1.54  ±0.05 | 1.53  ±0.06 | 1.50  ±0.05 | 0.8368 | 0.989 | 0.834 | 0.914 |  | 1.41  ±0.05 | 1.49  ±0.09 | 1.43  ±0.03 | 0.6393 | 0.622 | 0.977 | 0.798 |
| AoV VTI (mm) \*\*\* | 37.90  ±1.80 | 34.13  ±4.47 | 28.18  ±5.01 | 0.2460 | 0.811 | 0.225 | 0.580 |  | 28.93  ±2.33 | 30.46  ±3.49 | 23.22  ±3.27 | 0.2640 | 0.923 | 0.379 | 0.268 |
| AoV VTI Mean Vel (mm/s) \*\*\* | 749.35  ±40.67 | 699.98  ±82.23 | 634.34  ±88.2 | 0.5411 | 0.896 | 0.514 | 0.815 |  | 586.41  ±35.64 | 597.71  ±51.05 | 495.39  ±35.7 | 0.2327 | 0.978 | 0.294 | 0.266 |
| AoV VTI Mean Grad (mmHg) \*\*\* | 2.29  ±0.26 | 2.11  ±0.52 | 1.83  ±0.61 | 0.7925 | 0.965 | 0.778 | 0.920 |  | 1.42  ±0.16 | 1.50  ±0.24 | 1.02  ±0.16 | 0.2389 | 0.954 | 0.323 | 0.257 |
| AoV VTI Peak Vel (mm/s) \*\*\* | 1208.73  ±63.33 | 1109.48  ±129.41 | 986.76  ±129.91 | 0.3760 | 0.824 | 0.347 | 0.733 |  | 944.59  ±61.12 | 938.43  ±84.16 | 804.71  ±58.36 | 0.3400 | 0.998 | 0.354 | 0.437 |
| AoV VTI Peak Grad (mmHg) \*\*\* | 5.95  ±0.62 | 5.28  ±1.28 | 4.37  ±1.37 | 0.6165 | 0.919 | 0.592 | 0.850 |  | 3.71  ±0.46 | 3.71  ±0.65 | 2.68  ±0.43 | 0.3452 | >0.9999 | 0.371 | 0.423 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Pulmonary** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| PA Ejection Time | 57.00  ±2.70 | 48.79  ±2.30 | 47.13  ±1.81 | 0.0130 | 0.060 | 0.014 | 0.866 |  | 55.97  ±1.48 | 54.60  ±1.09 | 50.12  ±3.32 | 0.1400 | 0.883 | 0.127 | 0.338 |
| PA VTI (mm) \*\*\* | 24.97  ±1.79 | 24.89  ±3.77 | 16.72  ±1.57 | 0.0298 | 1.000 | 0.049 | 0.064 |  | 21.12  ±1.44 | 22.60  ±1.59 | 17.18  ±1.47 | 0.0686 | 0.765 | 0.172 | 0.068 |
| PA VTI Mean Vel (mm/s) \*\*\* | -407.81  ±37.58 | -475.86  ±66.11 | -344.56  ±38.37 | 0.1702 | 0.590 | 0.591 | 0.147 |  | -349.05  ±24.54 | -381.40  ±21.52 | -348.10  ±22.30 | 0.5628 | 0.598 | 1.000 | 0.629 |
| PA VTI Mean Grad (mmHg) \*\*\* | 0.70  ±0.13 | 1.02  ±0.33 | 0.52  ±0.12 | 0.2260 | 0.533 | 0.765 | 0.200 |  | 0.51  ±0.07 | 0.60  ±0.07 | 0.50  ±0.07 | 0.5669 | 0.633 | 0.990 | 0.602 |
| PA VTI Peak Vel (mm/s) \*\*\* | -663.78  ±72.03 | -767.14  ±110.74 | -536.43  ±58.93 | 0.1456 | 0.652 | 0.479 | 0.129 |  | -541.71  ±37.08 | -609.88  ±33.14 | -580.46  ±33.51 | 0.4016 | 0.379 | 0.723 | 0.852 |
| PA VTI Peak Grad (mmHg) \*\*\* | 1.90  ±0.39 | 2.58  ±0.85 | 1.25  ±0.28 | 0.2170 | 0.645 | 0.630 | 0.191 |  | 1.23  ±0.16 | 1.50  ±0.17 | 1.38  ±0.17 | 0.5063 | 0.482 | 0.799 | 0.877 |
| PA dia Length (mm) \*\*\* | 1.73  ±0.10 | 1.70  ±0.05 | 1.73  ±0.06 | 0.9684 | 0.979 | 0.999 | 0.967 |  | 1.65  ±0.05 | 1.68  ±0.07 | 1.58  ±0.05 | 0.5203 | 0.922 | 0.696 | 0.505 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Mitral** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| IVRT Time (ms) | 19.29  ±1.44 | 22.18  ±0.96 | 20.45  ±1.80 | 0.4533 | 0.423 | 0.845 | 0.715 |  | 21.78  ±1.28 | 21.94  ±1.13 | 23.85  ±1.19 | 0.4588 | 0.995 | 0.469 | 0.577 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Tricuspid** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| TAPSE Depth (mm) \*\*\* | 0.86  ±0.04 | 0.66  ±0.09 | 0.49  ±0.07 | 0.0018 | 0.123 | 0.001 | 0.204 |  | 0.71  ±0.05 | 0.60  ±0.05 | 0.51  ±0.06 | 0.0379 | 0.311 | 0.032 | 0.499 |

Notes: (1) \* means normalized by liner weight; \*\*\* means normalized by power of 1/3 of weight; (2) red values mean there are significant differences.