# The inflammation-resolution promoting molecule resolvin-D1 prevents atrial proarrhythmic remodeling in experimental right heart disease

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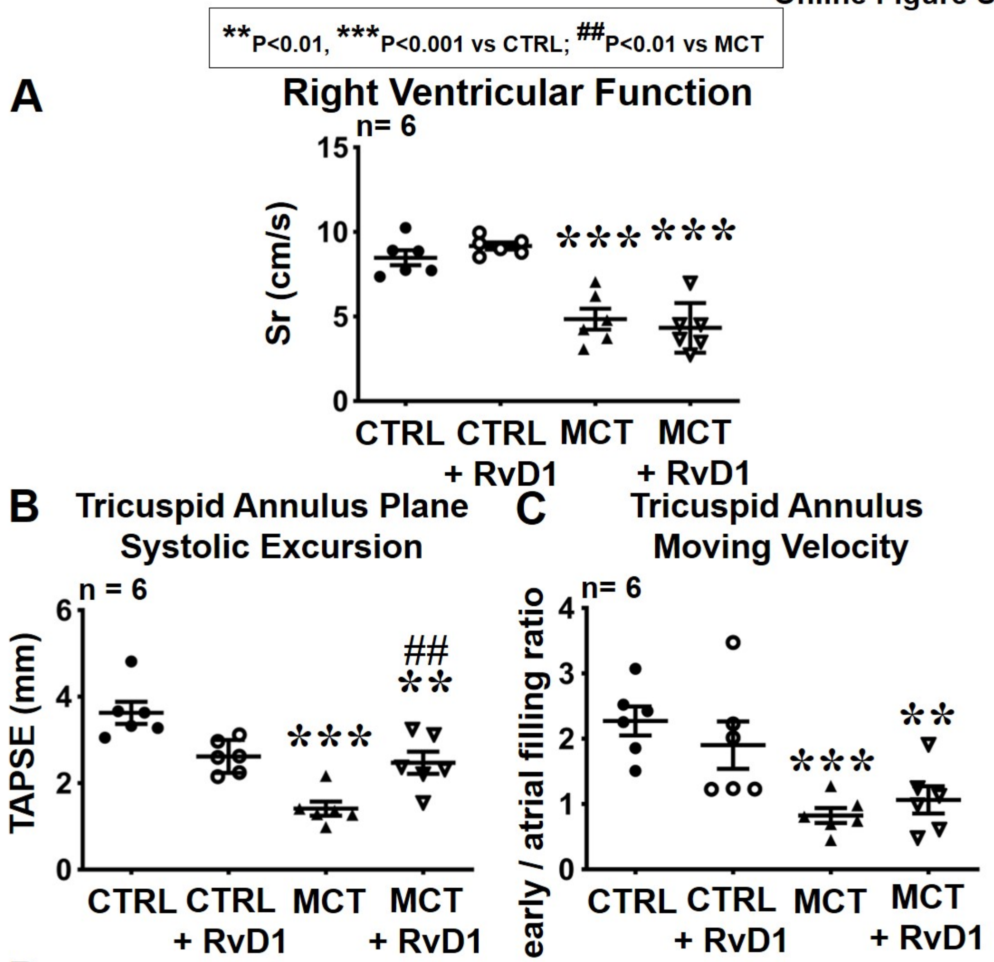
**SUPPLEMENTARY FIGURES and TABLES**

**CVR-2020-0301**

**Online Figure S1. RV Echocardiography. (A)** Right ventricular lateral wall systolic contractility (Sr). (**B**) Tricuspid annulus plane systolic excursion (TAPSE). (**C**) Ratio of tricuspid annulus moving velocity (TAMV) during early filling over TAMV during atrial filling. **(D)** Tricuspid regurgitation (TR) recorded on echocardiography. TR was not observed in control rats. MCT results are regurgitant area in mm$^2$, velocity in cm/s, transvalvular peak gradient ($G_{peak}$) and mean peak ($G_{mean}$) of TR in mmHg. Echocardiographic images show TR from apical 4-chamber view (upper pictures) and systolic gradient cross tricuspid valve (lower pictures) in MCT and MCT+RvD1 rats. *(Statistical analysis: One-way ANOVA followed by Bonferroni correction. Each point represents result from an individual animal. n = 6 rats per group.)*

Online Figure S1

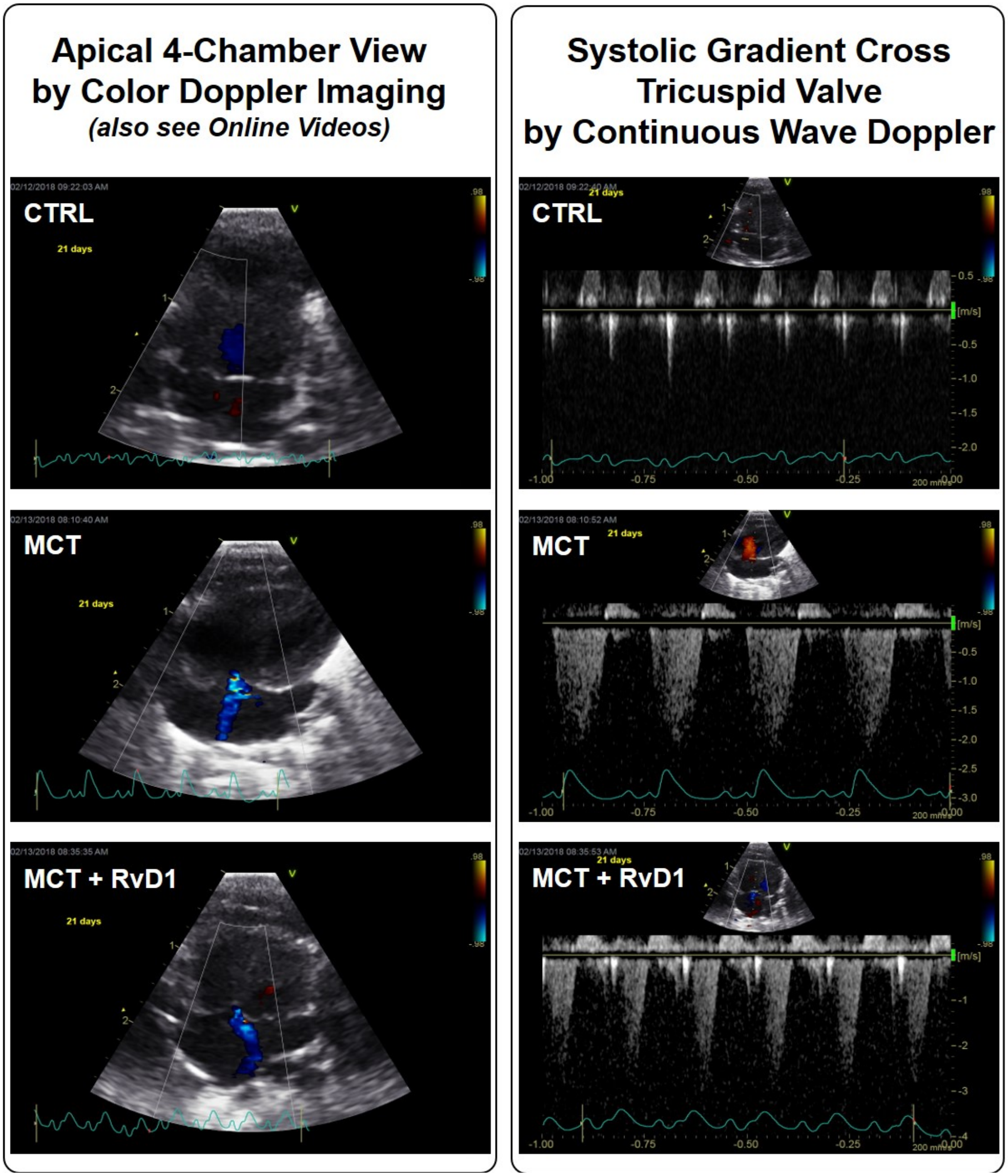


**D** Tricuspid Regurgitation Parameters

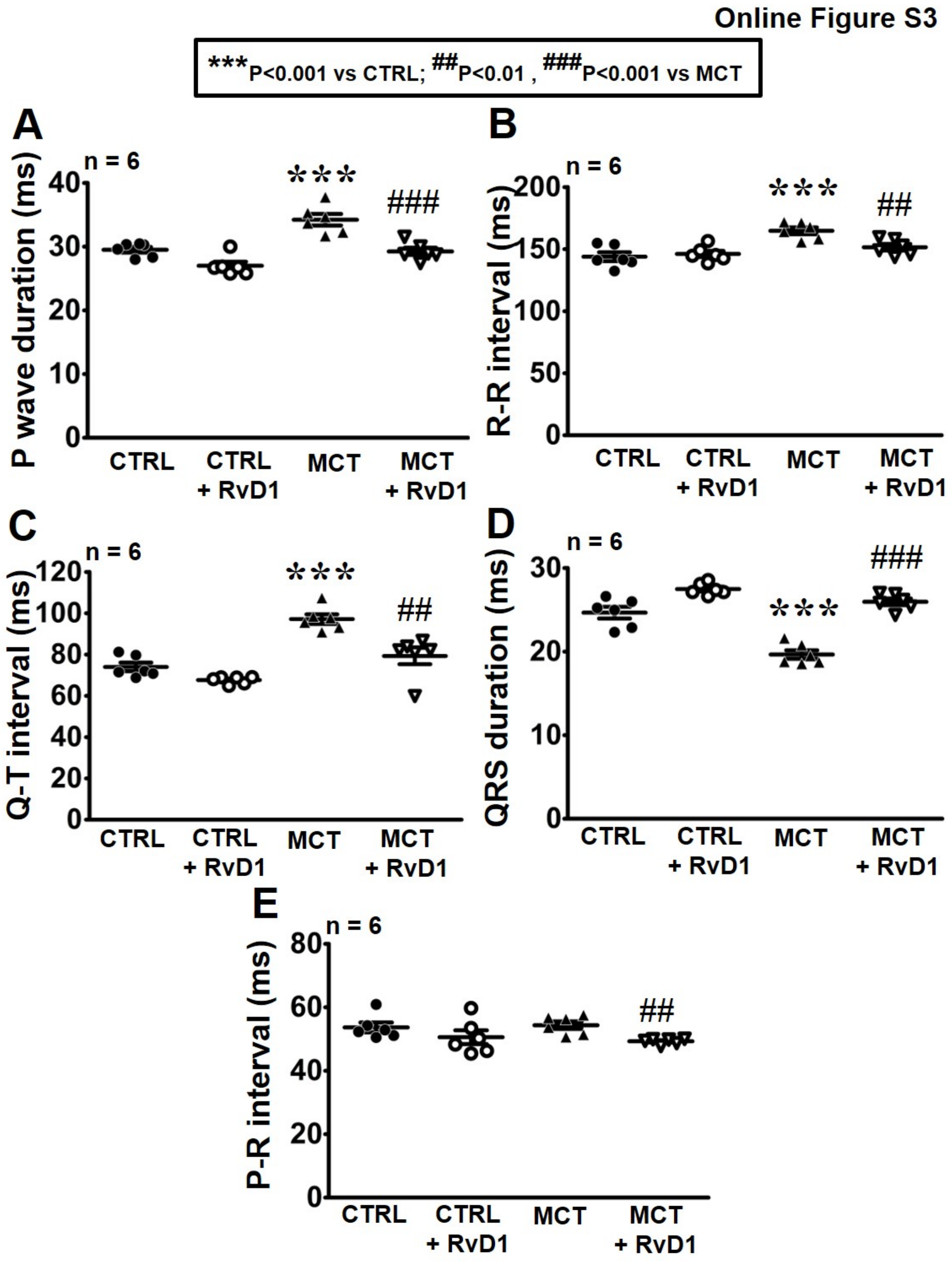
| Tricuspid Reg. | CTRL | CTRL + RvD1 | MCT | MCT + RvD1 |
|---|---|---|---|---|
| Area (mm$^2$) | 1.57 ± 3.85 | 0.9 ± 2.2 | 16.7 ± 9.9 | 14.1 ± 7.3 |
| Velocity (cm/s) | 0 | 0 | 296.2 ± 50 | 280.3 ± 16.8 |
| $G_{peak}$ (mmHg) | 0 | 0 | 35.9 ± 12.4 | 32.1 ± 3.9 |
| $G_{mean}$ (mmHg) | 0 | 0 | 25.4 ± 3.8 | 26.1 ± 8.3 |

**Online Figure S2. Tricuspid Valve Echocardiography.** Ultrasound imaging from apical four-chamber view and systolic gradient showing normal tricuspid valve function in control rats and tricuspid regurgitation in MCT- and MCT+RvD1-treated rats.

Online Figure S2

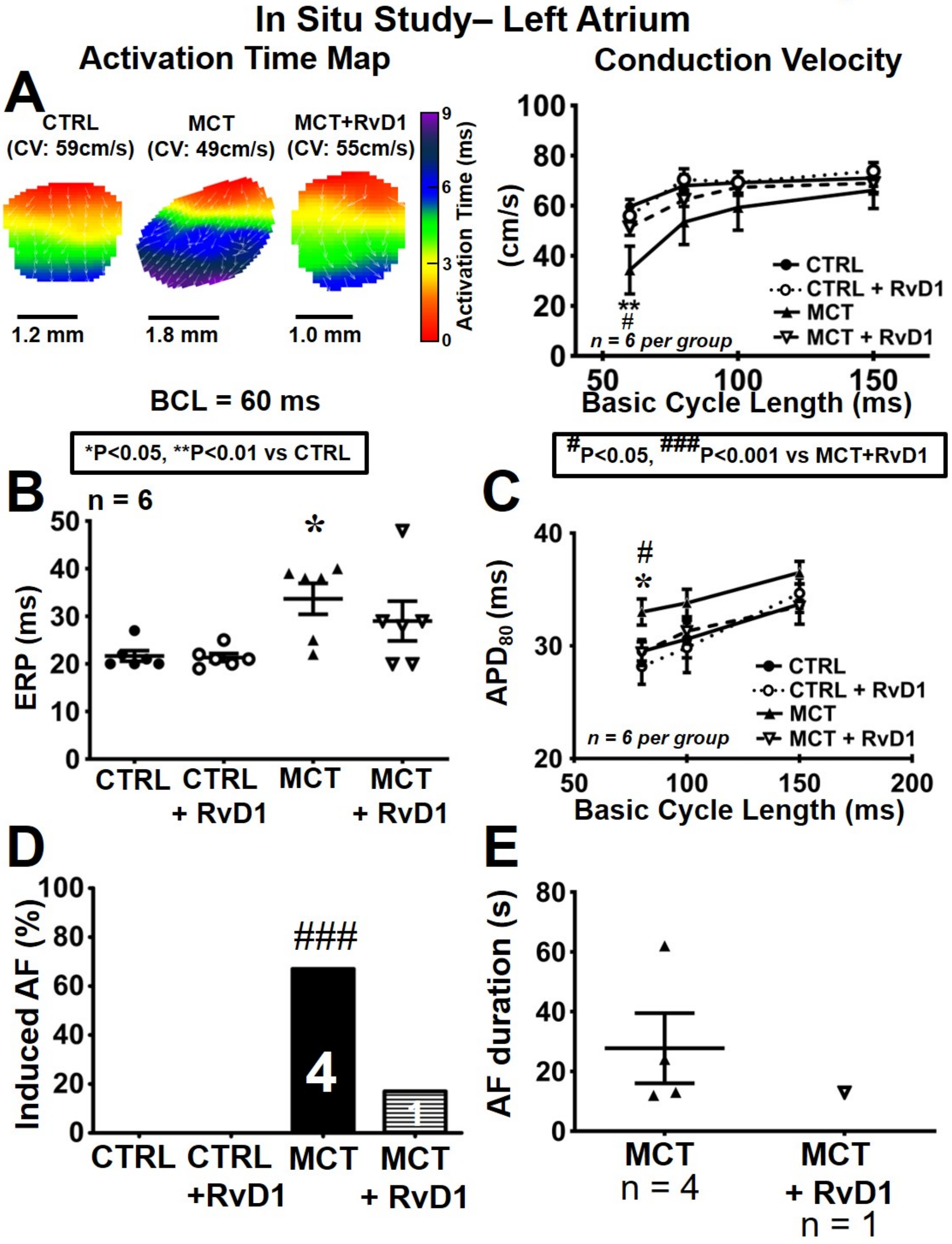


**Online Figure S3. ECG analysis *in vivo*.** ECG analyses for: P wave duration (**A**), R-R interval (**B**), Q-T interval (**C**), QRS duration (**D**) and P-R interval (**E**) in control rats vs. monocrotaline- and RvD1-treated rats *(Statistical analysis: One-way ANOVA followed by Bonferroni correction. Each point represents results from an individual animal. n = 6 rats per group.)*

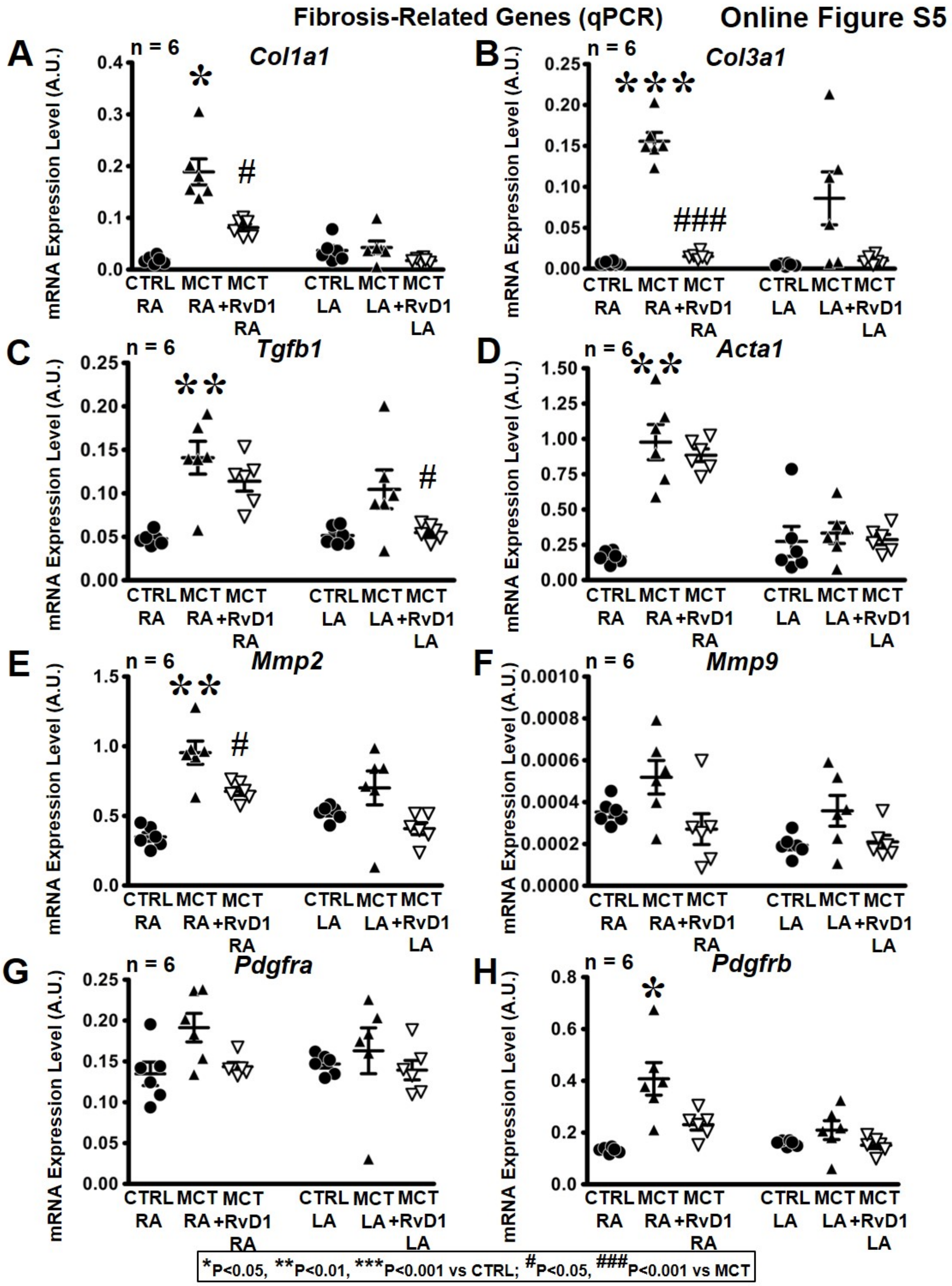


**Online Figure S4**. **Left Atrial (LA) Optical Mapping.** (**A**) Left: LA activation maps at BCL 60 ms. Right: Conduction velocity results (mean±SEM). (**B**) LA effective refractory period (ERP) (mean±SEM). (**C**) Action potential duration at 80% repolarization ($APD_{80}$; mean±SEM). (**D**) Percentage of rats in which AF could be induced AF. The numbers in the bars are the number of rats in which AF was induced. (**E**) Mean duration of induced AF. *(Statistical analysis: A and C: Two-way ANOVA followed by Tukey's test. D: Fisher's exact test. B and E: One-way ANOVA followed by Bonferroni correction. Each point represents results from an individual animal. n = 6 rats per group.)*

Online Figure S4

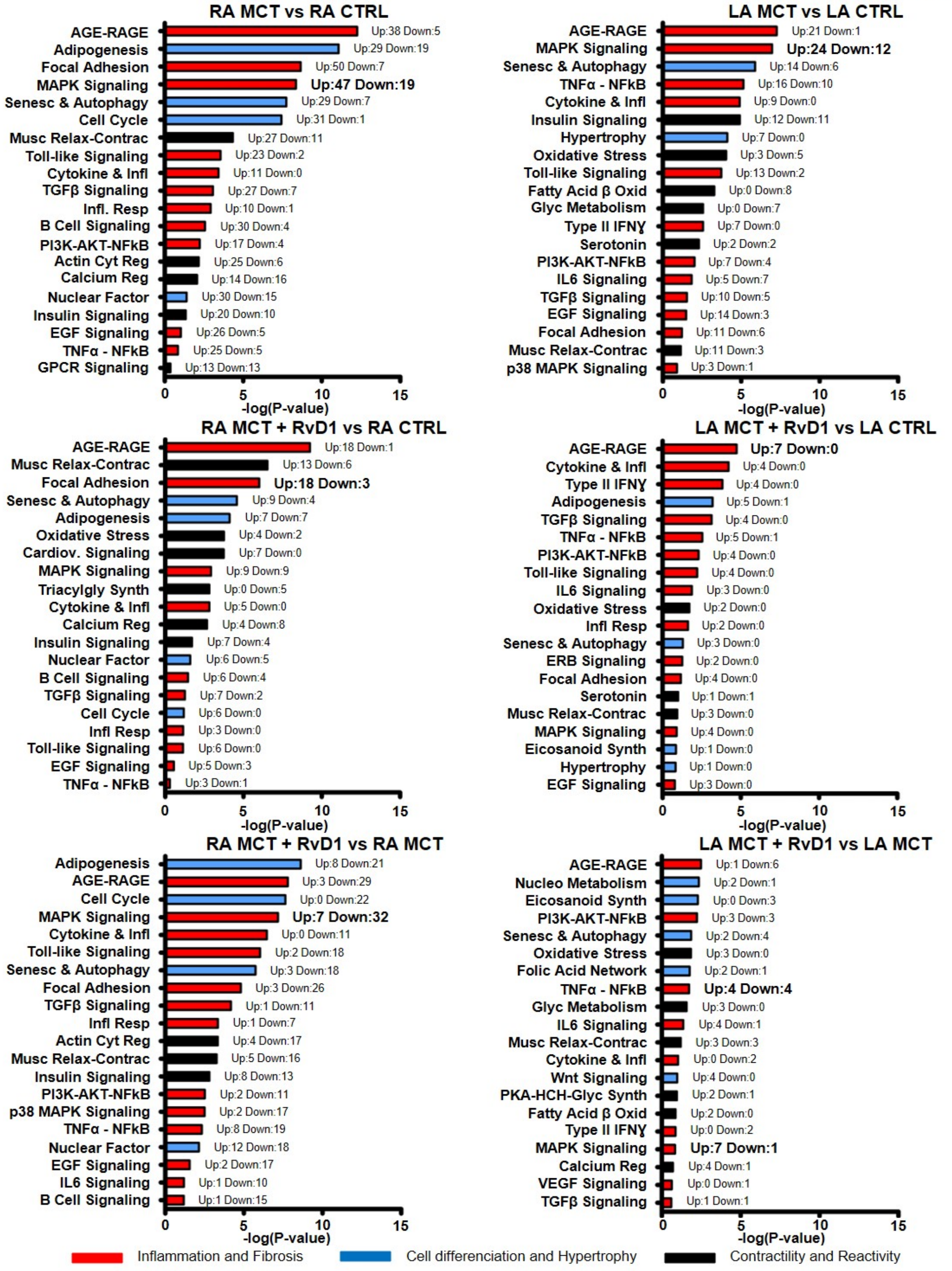


**Online Figure S5. Fibrosis marker and mediator expression.** Messenger RNA expression (qPCR) in arbitrary units (A.U.) in the right (RA) and left (LA) atrium. *(Statistical analysis: One-way ANOVA followed by Bonferroni correction. Each point represents results from an individual animal. n = 6 rats per group.)*



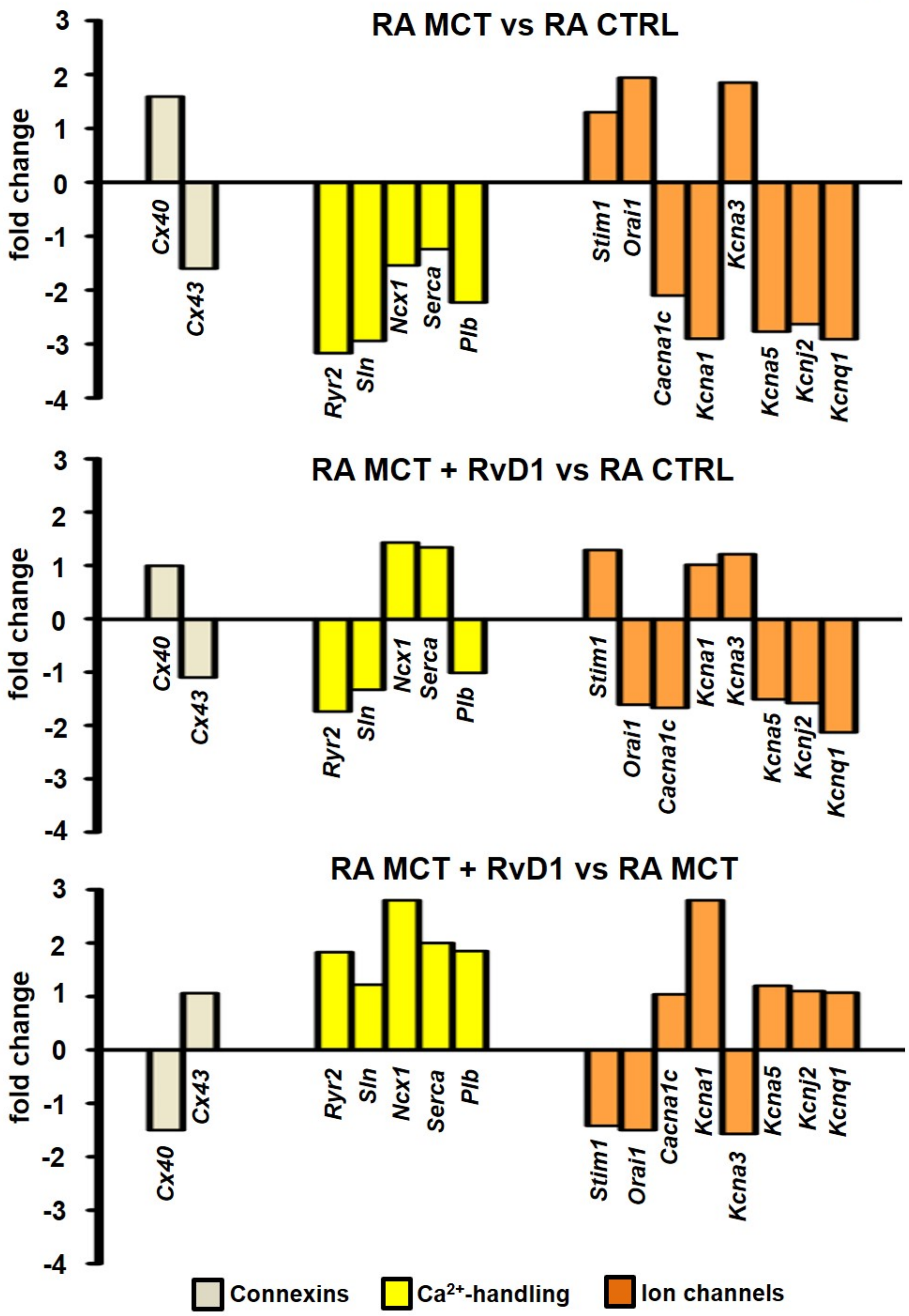
**Online Figure S6. Most Changed Pathways.** Enriched signaling pathways (KOBAS). The top 20 pathways are listed according to log[P]) in RA (left) and LA (right). Bold numbers correspond to the pathway with the most dysregulated genes. Abbreviations: Cardiov = cardiovascular; Contrac = contraction; Cyt = cytoskeleton; Glyc = glycogen; Infl = inflammation; Musc = muscular; Nucleo = nucleotide; Oxid = oxidation; Reg = regulation; Relax = relaxation; Resp = response; Senesc = senescence; Synth = synthesis; Triacylgly = triacylglyceride.

**Online Figure S6**



**Online Figure S7. Gap-junction, Ca$^{2+}$-handling, and Ion-channel Genes.** Expression changes (microarray data) in connexin (*Cx40 and Cx43*), Ca$^{2+}$-handling (*Ryr2, Sln, Ncx1, Serca, Plb*), and ion-channel (*Stim1, Orai1, Cacna1c, Kcna1, Kcna3, Kcna5, Kcnj2 and Kcnq1*) related genes, in the RA from MCT-only rats and MCT+RvD1 rats vs control, and MCT+RvD1 vs MCT-only.

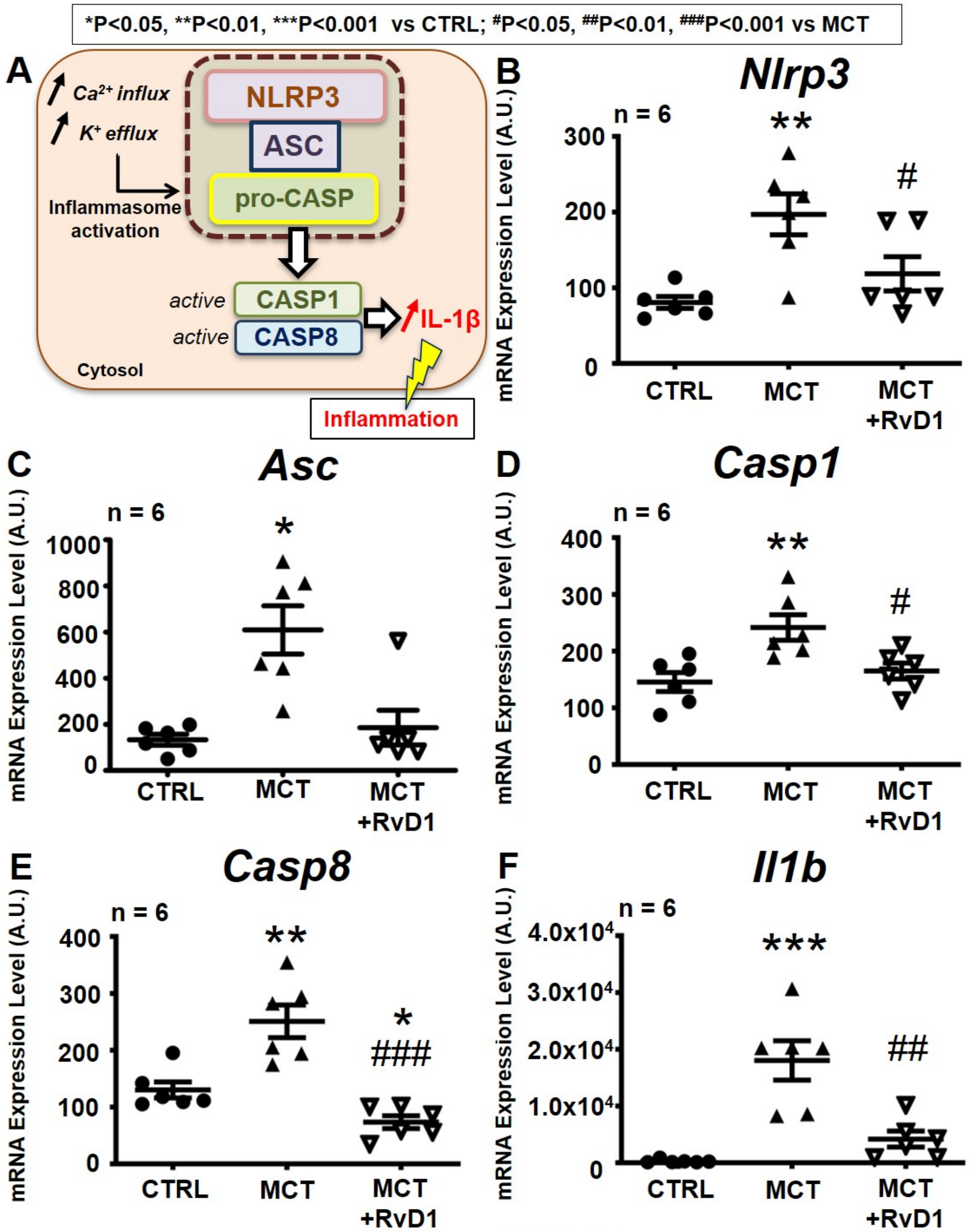
**Online Figure S7**



For LA data, see Online Table 3

**Online Figure S8. Inflammasome Related Genes.** (**A**) Schematic of NLRP3-inflammasome related signaling. Gene expression level, determined by pangenomic transcriptome microarray data, expressed in arbitrary units (A.U.) for inflammasome-related genes: (**B**) *Nlrp3,* (**C**) *Asc,* (**D**) *Casp1,* (**E**) *Casp8 and* (**F**) *Il1b* in the RA from control, MCT and RvD1- treated rats. *(Statistical analysis: One-way ANOVA followed by Bonferroni correction. Each point represents results from an individual animal. n = 6 rats per group.)*

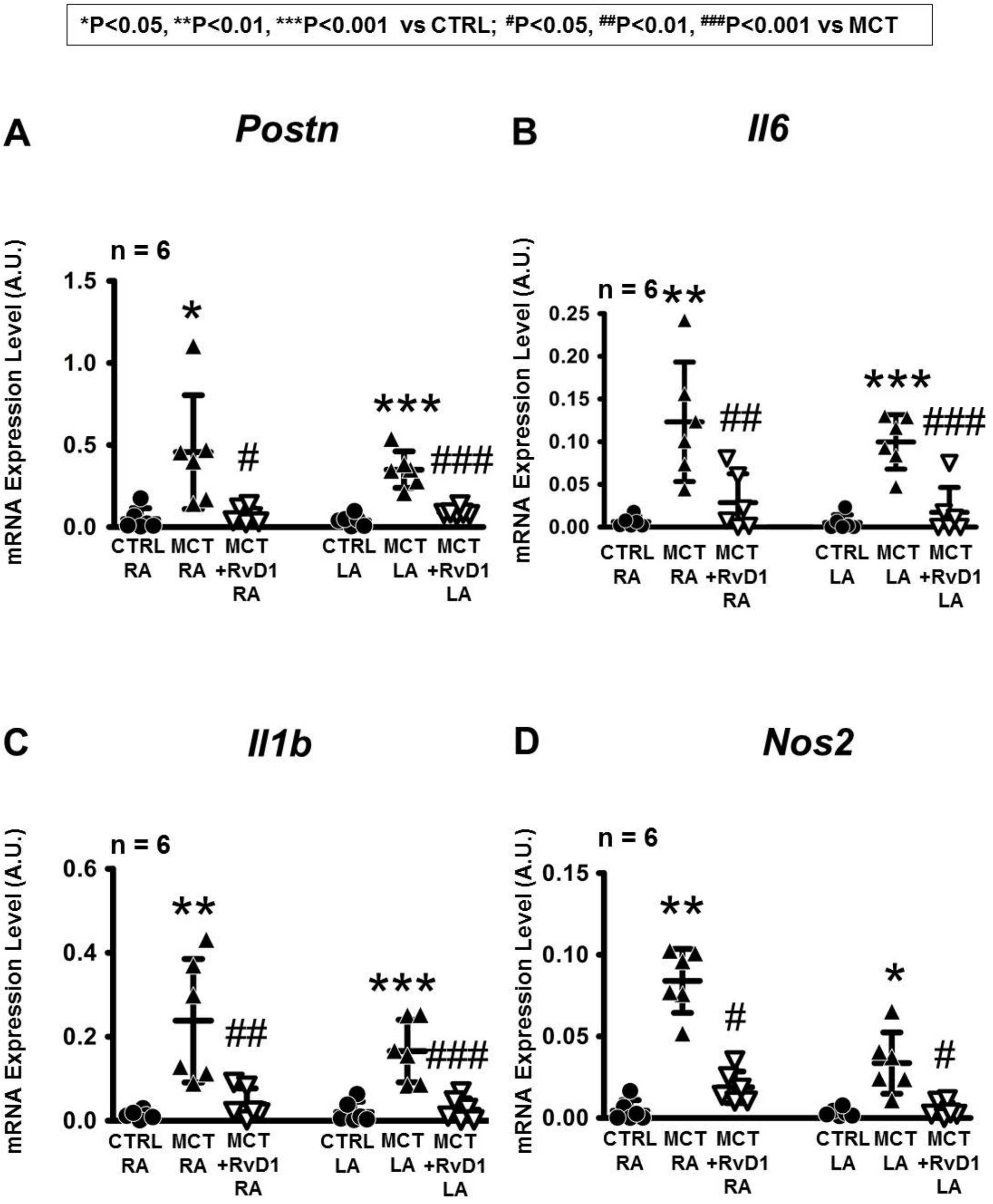
Online Figure S8



For LA data, see Online Table 3

**Online Figure S9. Inflammation markers.** Gene expression level obtained by RT-qPCR, expressed in arbitrary units (A.U.) for genes involved in inflammation: (**A**) *Postn*, (**B**) *Il6*, (**C**) *Il1β* and (**D**) *Nos2* in the RA and LA from control, MCT- and MCT+RvD1- treated rats. *(Statistical analysis: One-way ANOVA followed by Bonferroni correction. Each point represents results from an individual animal. n = 6 rats per group.)*

Online Figure S9



**Online Table S1. SYBR green primers.**

| Gene | Forward Primer | Reverse Primer | Amplicon size (bp) |
|---|---|---|---|
| ***Gapdh*** | GCATCTTCTTGTGCAGTGCC | GAGAAGGCAGCCCTGGTAAC | 105 |
| ***Il1β*** | TAGCAGCTTTCGACAGTGAGG | CCACAGCCACAATGAGTGAC | 180 |
| ***Mmp2*** | AAGAGGCCTGGTTACCCTGT | AAGTAGCACCTGGGAGGGAT | 137 |
| ***Mmp9*** | TCCAGTAGACAATCCTTGCAATGTG | CTCCGTGATTCGAGAACTTCCAATA | 110 |
| ***Nos2*** | AGCCTAGTCAACTACAAGCCC | GGGGTTTTCTCCACGTTGTTG | 160 |
| ***Pdgfrα*** | CACCGGATGGTACACTTGCT | GGCAGGGTATGATGGCAGAA | 170 |
| ***Pdgfrβ*** | GGGGAGACACCGGAGAATAC | TCGTCTCAGTGACATCCGTG | 162 |
| ***Postn*** | CTGCCCCGGCTATATGAGAA | TGTTGAGTGGTCGTGGCTC | 108 |
| ***Tgfβ1*** | CCATGACATGAACCGACCCT | TGCCGTACACAGCAGTTCTT | 141 |

Abbreviations: *Gapdh* = Glyceraldehyde-3-Phosohate Dehydrogenase; *Il1β* =Interleukin 1 beta; *Mmp* = Matrix Metalloproteinase; *Nos2* = inducible nitric oxide synthase, *Pdgfr* = Platelet-derived growth factor receptor; *Postn* = Periostin; *Tgfβ1* = Transforming growth factor beta 1.

**Online Table S2. LV Echocardiographic data.** *(n = 6 rats per group)*

| [redacted] | [redacted] | [redacted] | [redacted] | [redacted] |
|---|---|---|---|---|
| **BP (mmHg)** | **109 ± 1.47** | **106 ± 2.6** | **96.3 ± 2.53** $^{**}$ | **104.5 ± 3.8** $^{\#\#}$ |
| **LVP (mmHg)** | **109 ± 0.82** | **107.5 ± 2.9** | **92.75 ± 4.3** $^{*}$ | **101.75 ± 1.4** |
| **LVAWd (mm)** | **1.77 ± 0.06** | **1.8 ± 0.06** | **1.6 ± 0.03** | **1.9 ± 0.08** $^{\#}$ |
| **LVPWd (mm)** | **1.67 ± 0.04** | **1.75 ± 0.08** | **1.7 ± 0.03** | **1.8 ± 0.08** |
| **LVDd (mm)** | **8.3 ± 0.35** | **8.2 ± 0.45** | **7.14 ± 0.19** $^{*}$ | **7.64 ± 0.28** |
| **LVDs (mm)** | **4.3 ± 0.25** | **4.29 ± 0.48** | **3.1 ± 0.18** $^{**}$ | **3.87 ± 0.21** |
| **LVVd (ml)** | **1.26 ± 0.14** | **1.24 ± 0.21** | **0.83 ± 0.06** $^{*}$ | **0.98 ± 0.1** |
| **LVVs (ml)** | **0.21 ± 0.03** | **0.23 ± 0.1** | **0.08 ± 0.01** $^{**}$ | **0.17 ± 0.01** |
| **LVEF (%)** | **84.06 ± 1.18** | **83.24 ± 3.17** | **92.1 ± 0.45** $^{***}$ | **88.68 ± 2.22** $^{\#\#}$ |
| **LVFS (%)** | **48.28 ± 1.35** | **48.15 ± 3.33** | **57.07 ± 1.8** $^{**}$ | **55.76 ± 2.83** |

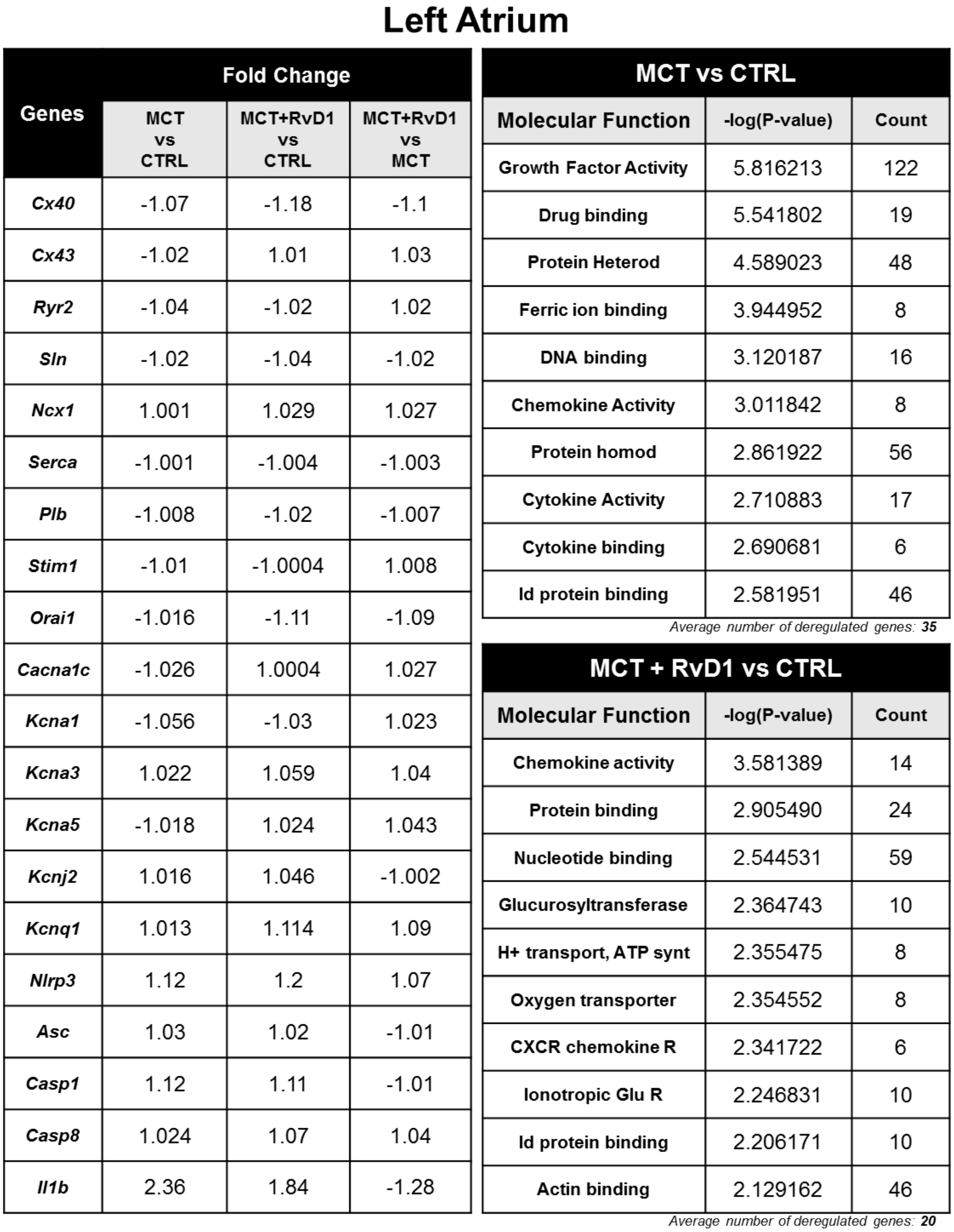
Abbreviations: BP = Aortic blood pressure; LV = left-ventricular; LVP = LV pressure; LVAWd and LVPWd = LV anterior and posterior wall thickness at end of diastole; LVDd and LVDs = LV dimension at end of diastole and systole; LVVd and LVVs = LV ventricular volume at end of diastole and systole; EF = ejection fraction and FS = fraction shortening.
*$P<0.05$, **$P<0.01$, ***$P<0.001$ vs CTRL; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs MCT

**Online Table S3. AF Episodes during In Situ Electrophysiology Study.** Distribution of induced AF following pacing of the atrium (RA and LA) at basic cycle lengths (BCL) of 300, 250, 200, 150, 100, 80 and 60 ms. *(n = 6 rats per group)*

| RA | | Pacing BCL (ms) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ID | | 300 | 250 | 200 | 150 | 100 | 80 | 60 |
| CTRL | 1 | | | | | AF | | |
| | 2 | | | | | | | |
| | 3 | | | | | | | |
| | 4 | | | | | | | |
| | 5 | | | | | | | |
| | 6 | | | | | | | |
| CTRL + RvD1 | 1 | | | | | | | |
| | 2 | | | | | | | |
| | 3 | | | | | | | |
| | 4 | | | | | | | |
| | 5 | | | | | | | |
| | 6 | | | | | | | |
| MCT | 1 | | | | | | | AF |
| | 2 | | | | | | AF | AF |
| | 3 | | | | | | AF | AF |
| | 4 | | | | | | AF | AF |
| | 5 | | | | AF | | AF | AF |
| | 6 | AF | | AF | | AF | AF | AF |
| MCT + RvD1 | 1 | | | | | | | |
| | 2 | | | | AF | AF | AF | AF |
| | 3 | | | | | | | |
| | 4 | | | | | AF | AF | |
| | 5 | | | | | | | |
| | 6 | | | | | | | |

| LA | | Pacing BCL (ms) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ID | | 300 | 250 | 200 | 150 | 100 | 80 | 60 |
| CTRL | 1 | | | | | | | |
| | 2 | | | | | | | |
| | 3 | | | | | | | |
| | 4 | | | | | | | |
| | 5 | | | | | | | |
| | 6 | | | | | | | |
| CTRL + RvD1 | 1 | | | | | | | |
| | 2 | | | | | | | |
| | 3 | | | | | | | |
| | 4 | | | | | | | |
| | 5 | | | | | | | |
| | 6 | | | | | | | |
| MCT | 1 | | | | | | | |
| | 2 | | | | AF | | AF | AF |
| | 3 | | | | | | AF | AF |
| | 4 | | | | | | AF | AF |
| | 5 | | | | | | AF | AF |
| | 6 | | | | | | | |
| MCT + RvD1 | 1 | | | | | | | |
| | 2 | | | | | AF | | |
| | 3 | | | | | | | |
| | 4 | | | | | | | |
| | 5 | | | | | | | |
| | 6 | | | | | | | |

**Online Table S4. LA gene expression changes (pangenomic microarray data).** Left: expression changes in connexin, $Ca^{2+}$-handling, ion-channel and NLRP3 inflammasome related genes. Right: Top 10 dysregulated molecular functions. (Glu = glutamate; Heterod = heterodimerization; Homod = homodimerization; Id = identical; R = receptor; Synt = synthesis).



## Left Atrium

| Genes | Fold Change | | |
|---|---|---|---|
| | MCT vs CTRL | MCT+RvD1 vs CTRL | MCT+RvD1 vs MCT |
| ***Cx40*** | -1.07 | -1.18 | -1.1 |
| ***Cx43*** | -1.02 | 1.01 | 1.03 |
| ***Ryr2*** | -1.04 | -1.02 | 1.02 |
| ***Sln*** | -1.02 | -1.04 | -1.02 |
| ***Ncx1*** | 1.001 | 1.029 | 1.027 |
| ***Serca*** | -1.001 | -1.004 | -1.003 |
| ***Plb*** | -1.008 | -1.02 | -1.007 |
| ***Stim1*** | -1.01 | -1.0004 | 1.008 |
| ***Orai1*** | -1.016 | -1.11 | -1.09 |
| ***Cacna1c*** | -1.026 | 1.0004 | 1.027 |
| ***Kcna1*** | -1.056 | -1.03 | 1.023 |
| ***Kcna3*** | 1.022 | 1.059 | 1.04 |
| ***Kcna5*** | -1.018 | 1.024 | 1.043 |
| ***Kcnj2*** | 1.016 | 1.046 | -1.002 |
| ***Kcnq1*** | 1.013 | 1.114 | 1.09 |
| ***Nlrp3*** | 1.12 | 1.2 | 1.07 |
| ***Asc*** | 1.03 | 1.02 | -1.01 |
| ***Casp1*** | 1.12 | 1.11 | -1.01 |
| ***Casp8*** | 1.024 | 1.07 | 1.04 |
| ***Il1b*** | 2.36 | 1.84 | -1.28 |

| MCT vs CTRL | | |
|---|---|---|
| **Molecular Function** | **-log(P-value)** | **Count** |
| **Growth Factor Activity** | 5.816213 | 122 |
| **Drug binding** | 5.541802 | 19 |
| **Protein Heterod** | 4.589023 | 48 |
| **Ferric ion binding** | 3.944952 | 8 |
| **DNA binding** | 3.120187 | 16 |
| **Chemokine Activity** | 3.011842 | 8 |
| **Protein homod** | 2.861922 | 56 |
| **Cytokine Activity** | 2.710883 | 17 |
| **Cytokine binding** | 2.690681 | 6 |
| **Id protein binding** | 2.581951 | 46 |

*Average number of deregulated genes:* ***35***

| MCT + RvD1 vs CTRL | | |
|---|---|---|
| **Molecular Function** | **-log(P-value)** | **Count** |
| **Chemokine activity** | 3.581389 | 14 |
| **Protein binding** | 2.905490 | 24 |
| **Nucleotide binding** | 2.544531 | 59 |
| **Glucurosyltransferase** | 2.364743 | 10 |
| **H+ transport, ATP synt** | 2.355475 | 8 |
| **Oxygen transporter** | 2.354552 | 8 |
| **CXCR chemokine R** | 2.341722 | 6 |
| **Ionotropic Glu R** | 2.246831 | 10 |
| **Id protein binding** | 2.206171 | 10 |
| **Actin binding** | 2.129162 | 46 |

*Average number of deregulated genes:* ***20***

**Online Table S5. Gene expression of G-protein coupled receptors (GPCRs) for pro-resolution mediators**. Pangenomic transcriptome microarray data for RA and LA expression of GPCRs (*Alx/Fpr2, Ltb4r, Chemr23, Gpr18 and Gpr37*) that are implicated in effects of pro-resolving mediator ligands (RvD1-5, RvE1-2, LXA4, MAR1 and Protectin D1).

## Right Atrium

| Receptors (genes) | Specialized Pro-resolving Mediators | mRNA expression level (A.U.) | | |
|---|---|---|---|---|
| | | **CTRL** | **MCT** | **MCT + RvD1** |
| ***Alx/Fpr2*** | RvD1, LXA4, MAR1 | 3.38 ± 0.13 | 3.8 ± 0.17 | 3.3 ± 0.14 |
| ***Ltb4r*** | RvE1, RvE2 (antagonists) | 3.62 ± 0.17 | 3.72 ± 0.15 | 3.51 ± 0.16 |
| ***Chemr23*** | RvE1, RvE2 | 8.4 ± 0.3 | 8.2 ± 0.5 | 10.4 ± 0.2$^{**}$ |
| ***Gpr18*** | RvD2 | 3.28 ± 0.11 | 3.26 ± 0.09 | 3.34 ± 0.21 |
| ***Gpr37*** | Protectin D1 | 5.63 ± 0.2 | 6.27 ± 0.14 | 6.01 ± 0.11 |

## Left Atrium

| Receptors (genes) | Specialized Pro-resolving Mediators | mRNA expression level (A.U.) | | |
|---|---|---|---|---|
| | | **CTRL** | **MCT** | **MCT + RvD1** |
| ***Alx/Fpr2*** | RvD1, LXA4, MAR1 | 3.5 ± 0.1 | 3.5 ± 0.23 | 3.9 ± 0.2 |
| ***Ltb4r*** | RvE1, RvE2 (antagonists) | 3.54 ± 0.07 | 3.67 ± 0.1 | 3.80 ± 0.14 |
| ***Chemr23*** | RvE1, RvE2 | 9 ± 0.13 | 8.93 ± 0.24 | 9.11 ± 0.26 |
| ***Gpr18*** | RvD2 | 3.26 ± 0.15 | 2.9 ± 0.21 | 3.4 ± 0.11 |
| ***Gpr37*** | Protectin D1 | 5.65 ± 0.14 | 5.8 ± 0.18 | 5.63 ± 0.26 |