

SUPPLEMENTAL DATA FOR

Increased Ca²⁺ influx through Cav1.2 drives aortic valve calcification

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Supplemental Table S1. Collagen genes up-regulated more than two-fold after infection with adenovirus expressing *Cre* recombinase.

<i>Gene</i>	Log₂Fold Change	P_{adj}
<i>Col1a1</i>	1.18±0.10	4.40E-247
<i>Col4a4</i>	1.93±0.05	5.40E-11
<i>Col5a2</i>	2.72±0.04	3.37E-166
<i>Col5a3</i>	1.01±0.03	5.24E-120
<i>Col6a3</i>	2.29±0.14	5.27E-247
<i>Col7a1</i>	2.16±1.33	1.80E-49
<i>Col8a2</i>	2.26±0.08	1.37E-21
<i>Col9a3</i>	2.56±0.14	1.01E-34
<i>Col11a2</i>	2.11±0.20	1.17E-110
<i>Col12a1</i>	1.36±0.05	<1.0E-300
<i>Col13a1</i>	1.96±0.08	1.09E-15
<i>Col16a1</i>	2.58±0.08	3.16E-149
<i>Col18a1</i>	2.30±0.06	<1.0E-300
<i>Col20a1</i>	3.71±0.24	2.42E-74
<i>Col26a1</i>	1.42±0.17	1.77E-03
<i>Col27a1</i>	1.18±0.10	<1.0E-300
<i>Col28a1</i>	1.26±0.04	1.84E-24

Supplemental Table S2. Expression of altered RAAS signaling components after infection with adenovirus expressing *Cre* recombinase.

Gene	Log₂Fold Change	P_{adj}
<i>Agt</i>	8.99±0.17	<1.0E-300
<i>Ace2</i>	4.32±0.16	2.3E-155
<i>Atp6ap2</i>	2.05±0.04	<1.0E-300
<i>Agtrap</i>	0.46±0.06	1.1E-11
<i>Renbp</i>	0.33±0.05	7.74E-11
<i>Agtr1a</i>	-2.3±0.22	1.19E-26
<i>Agtr1b</i>	-2.5±0.85	9.42E-05

Supplemental Table S3. Ca²⁺ sensitive genes (from: http://software.broadinstitute.org/gsea/msigdb/cards/KEGG_CALCIIUM_SIGNALING_PATHWAY).

Gene	Log₂Fold Change	P_{adj}
<i>Nfatc2</i>	6.08±0.40	2.28E-54
<i>Atp2a3</i>	5.52±0.19	7.19E-187
<i>P2rx1</i>	4.96±1.11	1.70E-08
<i>Atp2a1</i>	4.84±0.62	3.05E-17
<i>Grpr</i>	4.43±0.38	1.83E-32
<i>Mylk</i>	3.52±0.11	1.86E-222
<i>Cacna1s</i>	3.09±1.25	8.11E-05
<i>P2rx5</i>	3.02±0.14	4.58E-98
<i>Gna14</i>	2.91±0.84	8.81E-06
<i>Rcan1</i>	2.73±0.07	2.48E-296
<i>Adecy4</i>	2.42±0.34	1.56E-13
<i>Gna15</i>	2.40±0.24	2.56E-24
<i>Tnnc2</i>	2.19±1.43	1.21E-03
<i>Ptger1</i>	2.13±0.16	2.70E-41
<i>Cacna1c</i>	2.09±0.03	0.00E+00
<i>P2rx3</i>	2.05±0.12	1.35E-62
<i>Camk2a</i>	2.05±0.11	3.81E-67
<i>Nos2</i>	1.99±1.37	1.87E-03
<i>Nfatc1</i>	1.85±0.05	3.85E-211
<i>Rcan2</i>	1.80±0.09	1.02E-89
<i>Bst1</i>	1.75±0.24	2.21E-14
<i>Mylk2</i>	1.65±0.64	2.09E-04
<i>Cacna1g</i>	1.47±0.04	1.39E-209
<i>Camkk1</i>	1.45±0.08	1.13E-70
<i>Tbxa2r</i>	1.42±0.13	7.06E-29
<i>P2rx6</i>	1.40±0.17	5.11E-17
<i>Cacna1b</i>	1.38±0.40	1.39E-05
<i>Prkcg</i>	1.37±0.19	8.86E-15
<i>Adrb2</i>	1.32±0.15	2.16E-19
<i>Calr</i>	1.20±0.04	1.65E-160
<i>Phkg1</i>	1.16±0.22	5.91E-09
<i>Gna11</i>	1.16±0.03	3.24E-223
<i>Nfatc4</i>	1.11±0.03	5.71E-187
<i>Atp2b3</i>	1.07±1.09	1.94E-02
<i>Adra1b</i>	-1.2±0.18	3.14E-14
<i>Mylk3</i>	-1.3±0.36	2.04E-06
<i>Adora2b</i>	-1.4±0.11	7.25E-41
<i>Adrb1</i>	-1.4±0.47	3.54E-05
<i>Oxtr</i>	-1.7±0.75	4.00E-04
<i>Htr2a</i>	-1.9±0.10	5.80E-82
<i>Adecy8</i>	-1.9±0.18	7.57E-29
<i>Plcb1</i>	-2.1±0.13	3.83E-61
<i>Grin2a</i>	-2.2±1.28	7.91E-04
<i>Htr2b</i>	-2.4±0.43	1.50E-09
<i>Adora2a</i>	-2.6±0.27	5.93E-22

Supplemental Table S4. Primers used for quantification of mRNA expression by qRT-PCR.

Gene name	Forward primer (5' 3')	Reverse primer (5' 3')	Primerbank ID (66, 67)
<i>Asma (acta2)</i>	GTCCCAGACATCAGG GAGTAA	TCGGATACTTCAGCG TCAGGA	-
<i>Runx2</i>	TTCAACGATCTGAGA TTTGTGGG	GGATGAGGAATGCG CCCTA	-
<i>Vwf</i>	CTTCTGTACGCCTCA GCTATG	GCCGTTGTAATTCCC ACACAAG	33186908a1
<i>Pecam1 (cd31)</i>	ACGCTGGTGCTCTAT GCAAG	TCAGTTGCTGCCCAT TCATCA	26332198a1
<i>P4hal</i>	AGCCACCATTTCAAA CCCAGT	GCCAAGCACTTTTGC TAATTCTG	33859596a1
<i>Cacna1c</i>	GCTGACGGTGTCCA GTGTA	CCAGGTAGCCTTTGA GATCTTCT	-
<i>Gapdh</i>	TGTCAGCAATGCATC CTGCA	CCGTTTCAGCTCTGGG ATGAC	-

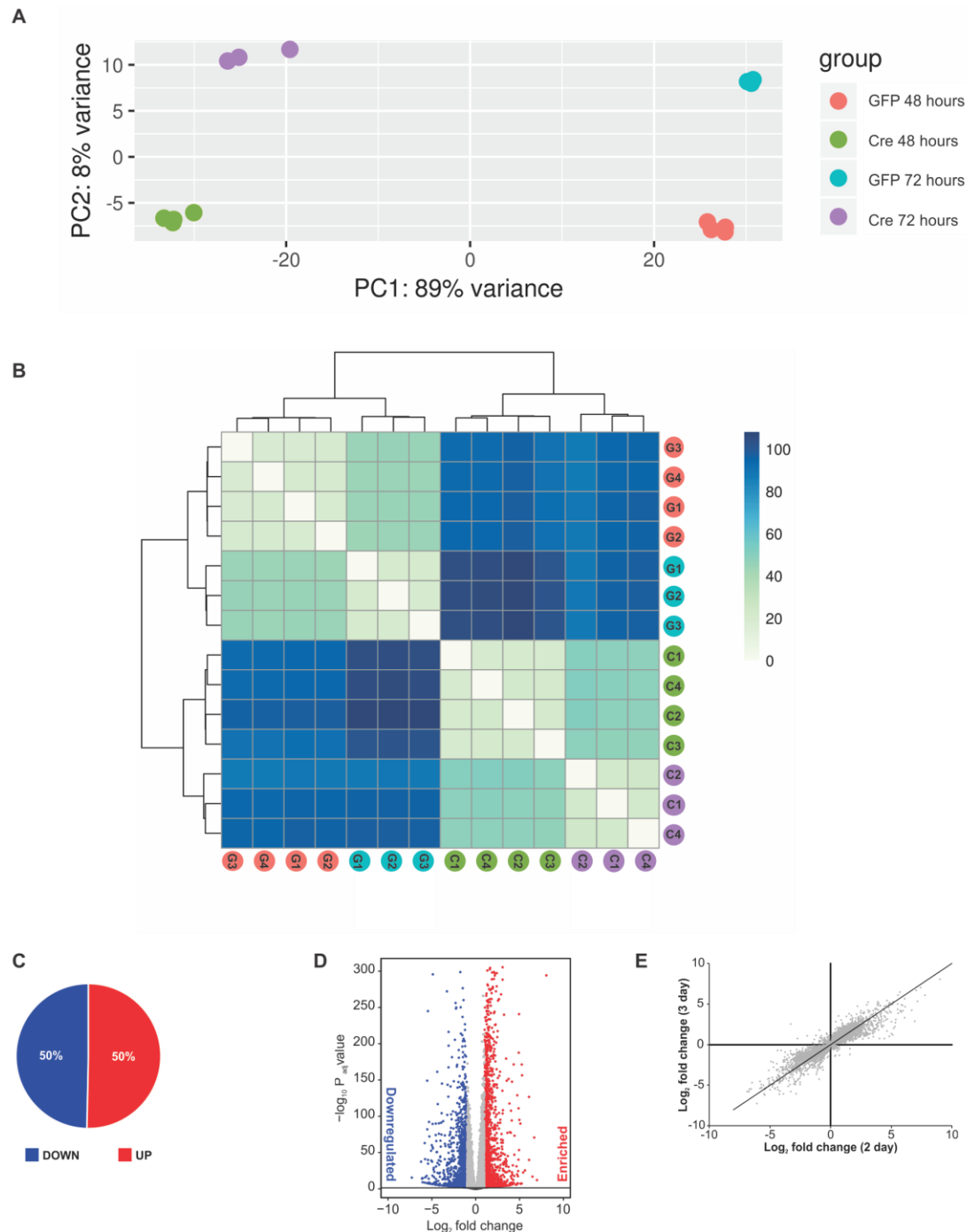


Figure S1: RNA-Seq of cultured valve interstitial cells. **A**, PCA of RNA transcripts isolated from VICs infected with adenovirus expressing Cre recombinase or GFP either 48h ($N=4$ each) or 72h ($N=3$ each) post infection. **B**, Heatmap of sample-to-sample distances. The analysis separates groups by the treatment (GFP ["G"] or Cre ["C"]) and the duration of treatment (48h or 72h). **C**, Distribution of up-regulated and down-regulated differentially expressed genes 72h after viral infection. **D**, Volcano plot of differentially expressed genes 72 h after infection. Gray indicates genes differentially expressed ($P_{adj} < 0.05$) and $\text{Log}_2\text{fold} < 1.0$ or > 1.0 . **E**, Comparison of Log_2fold changes in 48h (2 day) and 72 h (3 day) datasets.