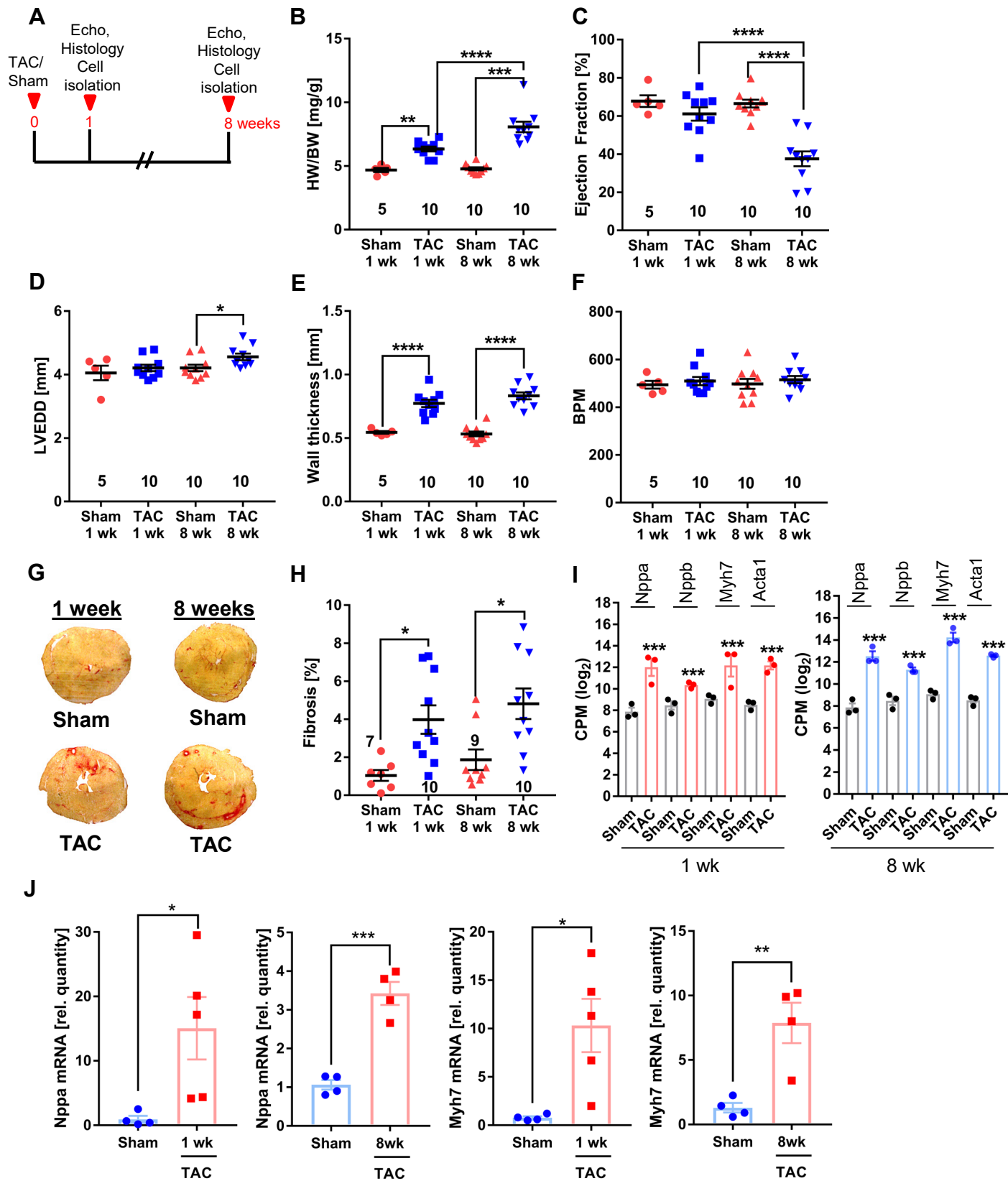


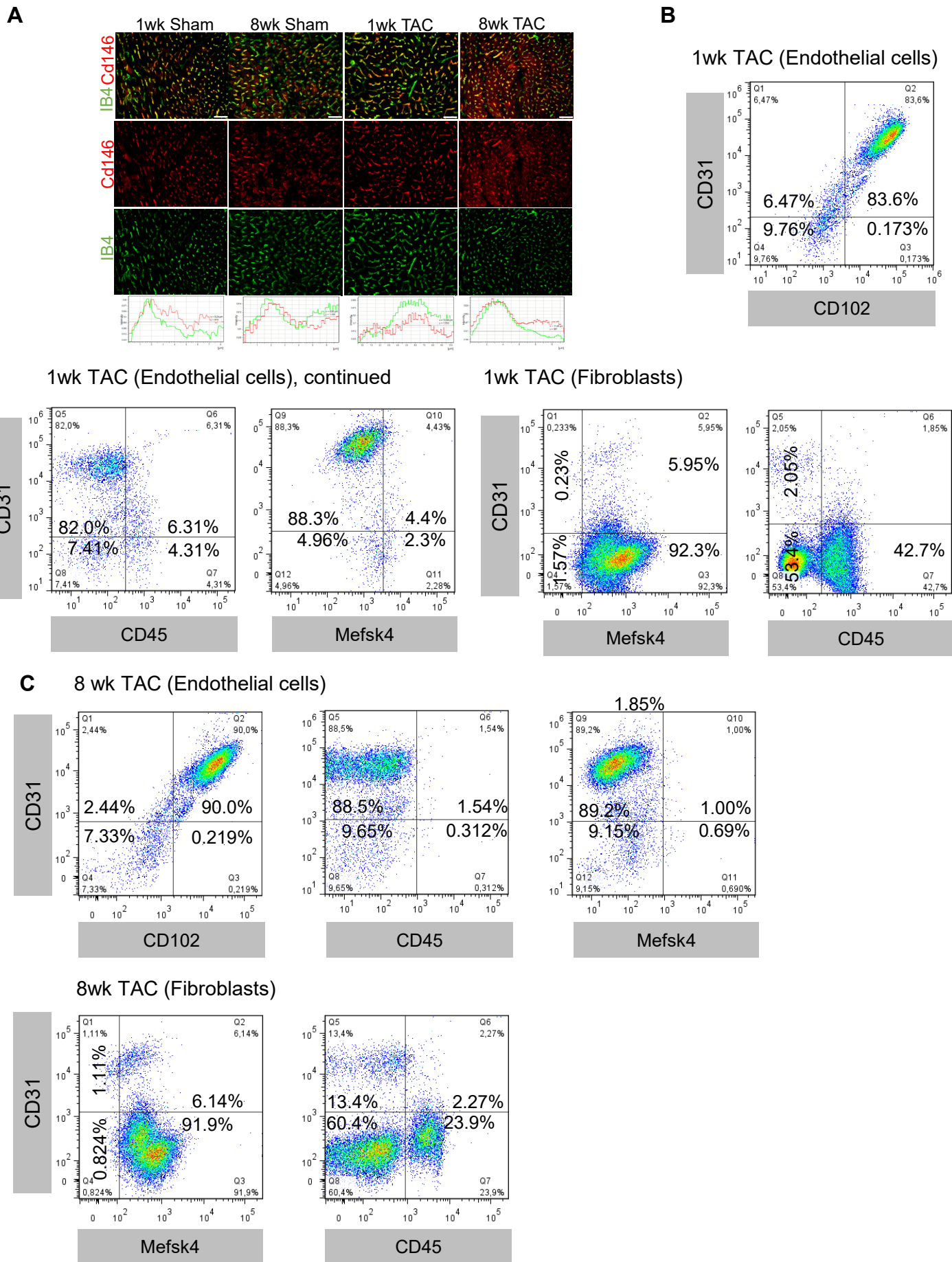
Supplemental information

Analysis of myocardial cellular gene expression during pressure overload reveals matrix based functional intercellular communication

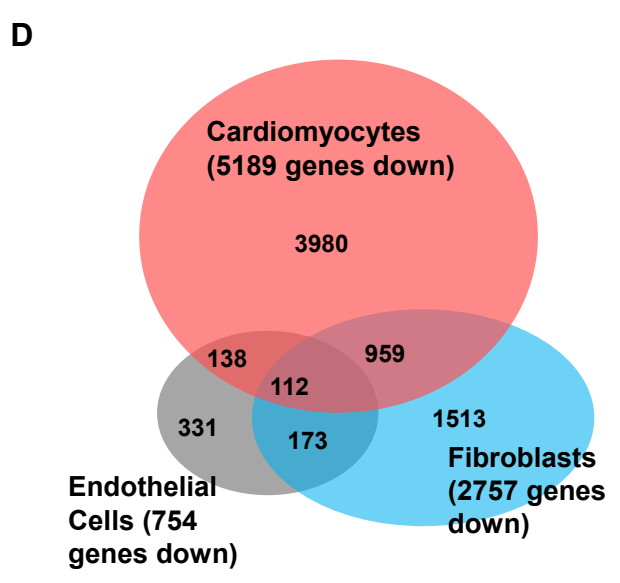
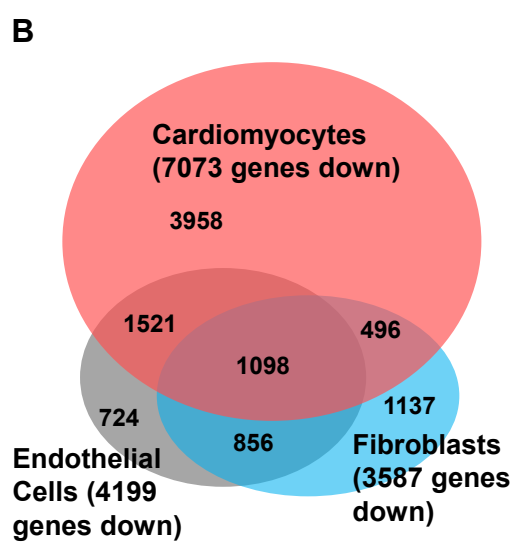
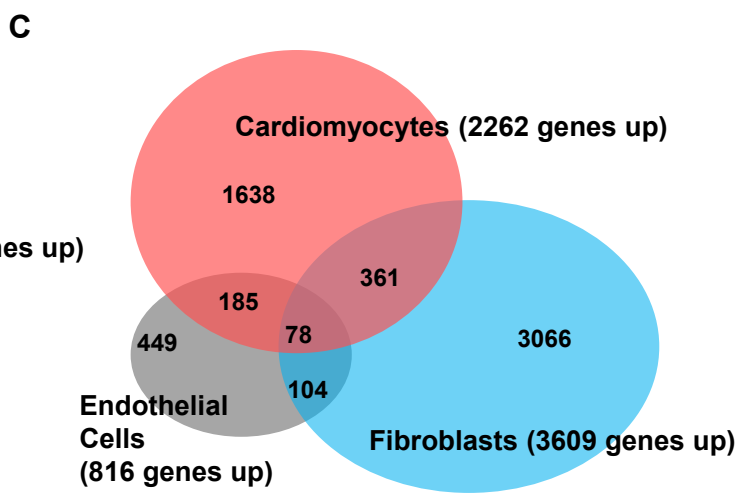
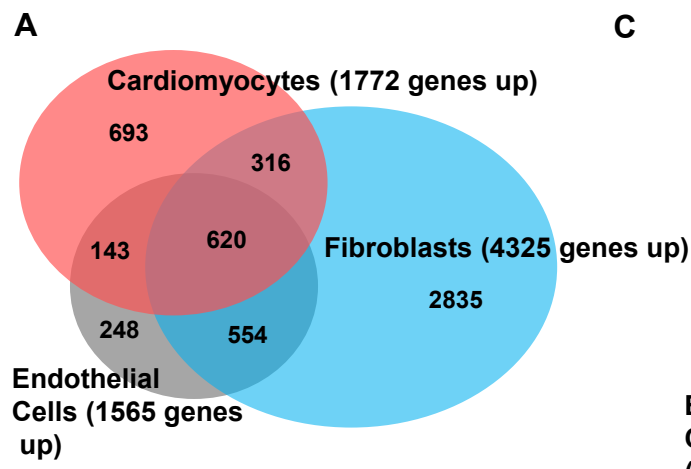
Natali Froese, Julio Cordero, Aya Abouissa, Felix A. Trogisch, Steve Grein, Malgorzata Szaroszyk, Yong Wang, Anna Gigina, Mortimer Korf-Klingebiel, Berislav Bosnjak, Colin F. Davenport, Lutz Wiehlmann, Robert Geffers, Eva Riechert, Lonny Jürgensen, Etienne Boileau, Yanzhu Lin, Christoph Dieterich, Reinhold Förster, Johann Bauersachs, Roxana Ola, Gergana Dobрева, Mirko Völkers, and Joerg Heineke



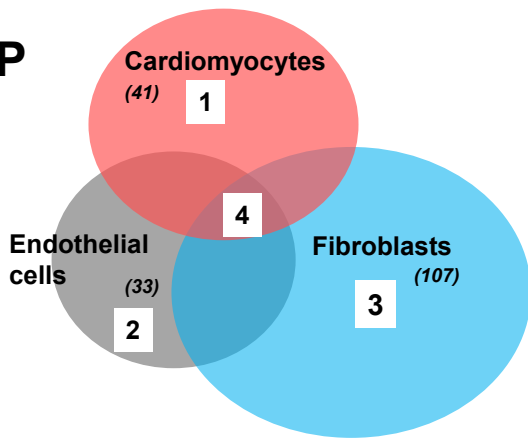
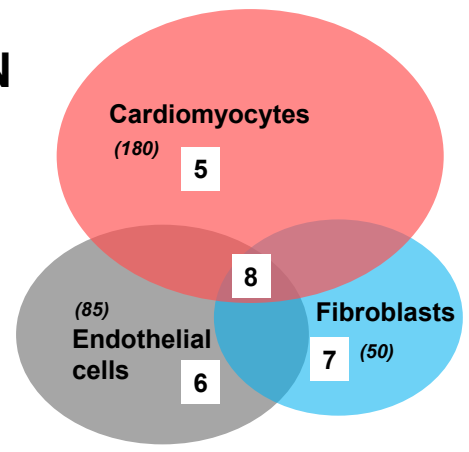
Supp. Figure 1. Effects of subacute and chronic pressure overload. Related to Figure 1. Adult mice were subjected to sham or TAC (transverse aortic constriction) surgery. **(A)** Scheme of the experimental time line. **(B)** Heart weight/body weight (HW/BW), as well as echocardiographic ejection fraction **(C)**, left ventricular enddiastolic diameter (LVEDD) **(D)**, wall thickness **(E)** and heart rate (in BPM, beats per minute). **(G)** Sirius red staining of hearts after sham and TAC surgery with the quantification of the extent of fibrosis shown in **(H)**. **(I)** Expression of the indicated genes after sham surgery versus 1 week and 8 weeks after TAC surgery in isolated cardiomyocytes (determined by RNA-sequencing). **B-F, H:** the number of animals analyzed per group is indicated in the graph. **I:** $n=3$ /group. **(J)** Relative mRNA levels of the indicated genes measured by qPCR in isolated cardiomyocytes from mice treated as indicated. Quantitative data are shown as mean \pm SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ as determined by as determined by two-tailed unpaired t-test (panels I and J) or one-way ANOVA with Sidak's multiple comparisons test (other panels).



Supp. Figure 2. Verification of cell purity after TAC by flow cytometry. Related to Figure 1. (A) Immunofluorescence staining for the indicated markers from mouse heart sections after treatment as indicated. Scale bar: 50µm. Below the images, a graph shows overlap between the red (Cd146) and green (IB4) channels. (**B**) Cardiac endothelial cell and fibroblasts were isolated from mouse hearts after 1 week (wk) and 8 wks (**C**) after TAC and stained for the endothelial markers CD31 and CD102, for the leukocyte marker CD45, and the fibroblast marker Mefsk4. Subsequently, flow cytometric analyses were performed and representative results are shown here. The numbers indicated in each quadrant indicates the percentage of cells localized in that particular quadrant.



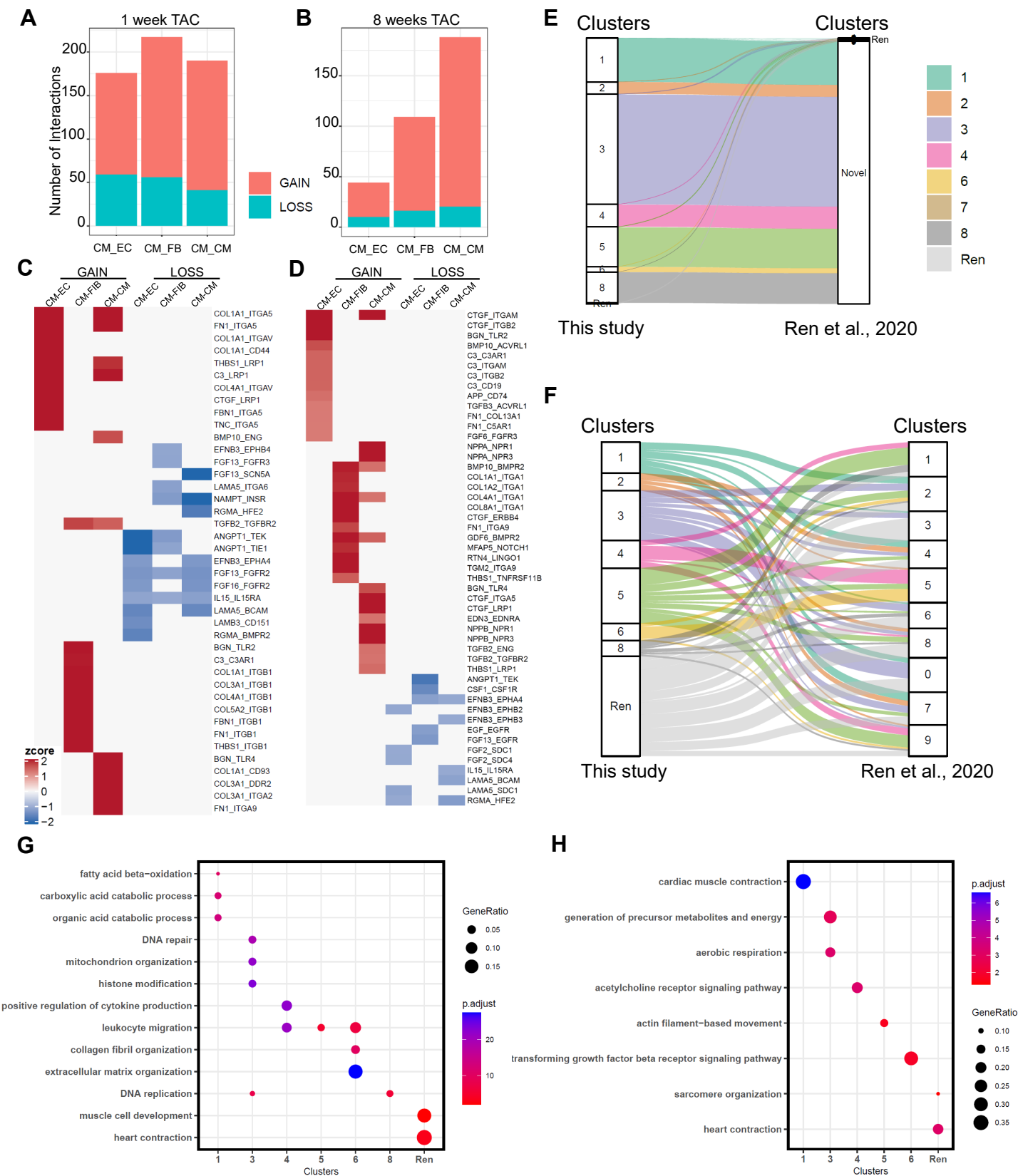
Supp. Figure 3. Overview of significantly regulated coding genes in the different cell types. Related to Figure 1. Venn diagrams of significantly up- (**A**) and downregulated genes (**B**) in cardiac endothelial cells, fibroblasts and cardiomyocytes one week after TAC. (**C**) Venn diagrams of significantly up- and downregulated genes (**D**) in cardiac endothelial cells, fibroblasts and cardiomyocytes 8 weeks after TAC. The numbers in the different sections indicate the number of genes (co-)regulated in the indicated sections of the diagram.

UP**DOWN**

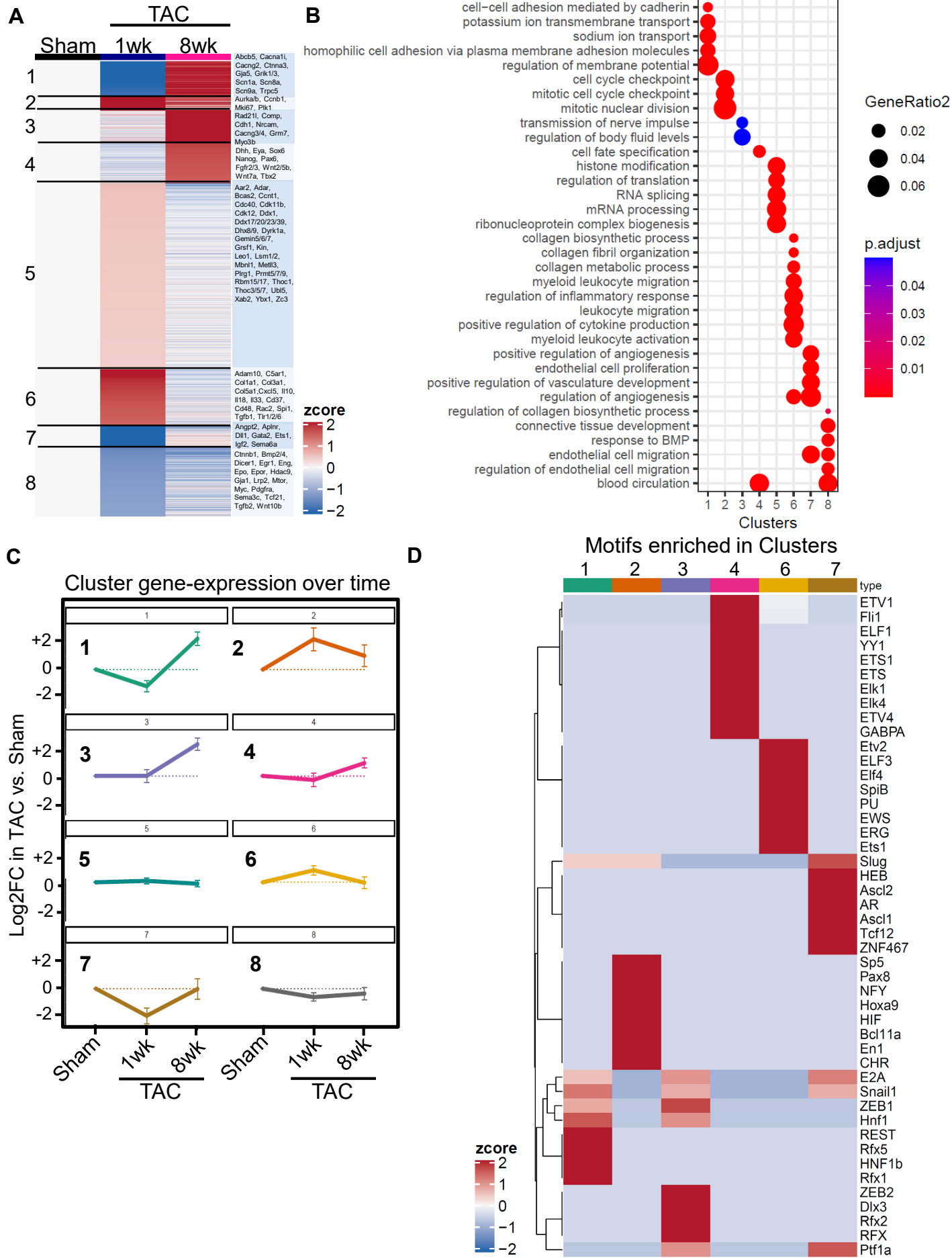
#	UP NC RNA (official symbol)
1	Gm13054 Gm16192 Gm16033 Med9os Gm36569 Gm13601 Gm12092 Gm15418 Gm13481 Ighm Gm16091 Gm16897 Gm12295 1500017E21Rik Ppp1r18os Gm15328 Gm20659 Gm15533 Gm15581 Snhg15 Gm2415 Sorbs2os Gm17041 Dubr Gm11747 Gm37335 Gm14005 R74862 Gm15598 Gm17036
2	Prr33 Gm15728 Gm12426 2900009J06Rik Gm32569 Gm26902 BC037039 Gm6277 2610203C20Rik
3	Gm13749 H19 Bach2os Gm13391 Apol7d Gm15987 Gm27008 Gm28727 Gm26771 Ptgs2os2 Gm2245 Gm12764 Gm15832 Gm37168 Al662270 Gm10451 Gm26947 Gm17586 1700040F17Rik Al427809 Gm11769 Gm13986 Gm26637 Gm27177 Gm15867 Plxna4os1 5930403N24Rik Ptpv Gm13710 Dhx58os D130020L05Rik 6330403L08Rik 2600014E21Rik 2500004C02Rik Gm20528 1700016P03Rik Gm26522 BC039771 4833418N02Rik 5830432E09Rik A530013C23Rik F630028O10Rik Gm26756 4933433G15Rik Map2k3os 1110002J07Rik A930006K02Rik 4930469K13Rik Igf1os 8030453O22Rik Gm26740 Gm26735 8430429K09Rik 1500002C15Rik Gm13372 Il1bos 1810058I24Rik Gm26716 F730043M19Rik Gm26527 Mir155hg Gm17233 Gm16845 4930520O04Rik Gm13091 Gm26760 Gm12868 Gm26917 Cct6a Gpr137b-ps 9230114K14Rik Snhg17 1600010M07Rik BE692007 Zeb2os Gm7160 2310009A05Rik 4921531C22Rik Tug1 2610020C07Rik 1110002L01Rik 4931403E22Rik Tmem134 4933421O10Rik Ptpmt1 C330006A16Rik
4	Lockd, Dnm3os, Gm15270, Gm15283

#	DOWN NC RNA (official symbol)
5	Uckl1os Gm12224 Gm28979 Gm12100 Gm3235 BB218582 B430212C06Rik Gm12319 Gm28651 4632428C04Rik Gm27252 1700123M08Rik Gm11642 Gm12167 2310039L15Rik T2 A730020M07Rik Gm32200 Gm20712 Mir133a-1hg 4930412C18Rik Gm15956 Gm19461 Gm15788 Gm26794 Gm14493 Gm11264 Tug1 Gm14097 Gm13111 Gm26862 Gm12519 Gm17473 Gm20619 2310040G07Rik Gm28638 Gm16793 Cct6a D930015M05Rik Gm10435 Gm15337 1810021B22Rik Gm28376 Gm28609 A530013C23Rik Gm15506 4921504A21Rik Prr33 A330032B11Rik 2310020H05Rik Foxo6os Gm14261 Banf2os Gm16268 Proscos A330069E16Rik Hopxos Gm15408 Gm13594 2810414N06Rik Gm12381 A430046D13Rik 9330136K24Rik Gm10603 1600010M07Rik Gm9899 D830032E09Rik 2310010J17Rik Gm15860 BC037032 6330403L08Rik 5033430115Rik Gm13375 2410022M11Rik 4932441J04Rik 1700048M11Rik Gm15964 A930016O22Rik Spata5l1 4930458D05Rik 2310015D24Rik Gm15417 4933421O10Rik Mccc1os Gm20655 Gm11228 Gm16316 H3f3aos Gm16090 Gm13938 1700093J21Rik Gm26752 9430037G07Rik Gm16093 Gm16172 5031425E22Rik B230312C02Rik 6030443J06Rik D130017N08Rik Gm15492 0610040F04Rik Gm15991 Gm17690 Gm28379 Gm15943 C030014I23Rik Gm26760 C920021L13Rik Gm15543 Gm16537 Zbtb11os1 4930480K23Rik B230369F24Rik BC065397 Gm26534 Gm20544 B130046B21Rik E030042O20Rik 9530052E02Rik Gm5860 2610306M01Rik Gm15787 2310047D07Rik Gm11899 4921531C22Rik Gm26672 Gm16576 Gm26745 Cep83os Gm10501 2810403D21Rik Gm26795 E330017L17Rik Gm10602 Gm16163 5430405H02Rik 9330158H04Rik Gm20522 D830039M14Rik B230110C06Rik Gm16599 Gm11613 Gm12655 Gm20468 4833418N02Rik Gm26722 Gm26668 9230114K14Rik Gm10010 Gm10516 A330094K24Rik 2900076A07Rik 1010001N08Rik Gm16861 Gm26694 Gm42418 Gm20463 Fcnaos 9330159M07Rik 2310009A05Rik Gm15976 Gm15491 1810034E14Rik 4632404H12Rik Gm11266 Lsmem2 D930016D06Rik 4631405J19Rik Snhg6 2010320M18Rik 9430091E24Rik Ptpmt1 C330011M18Rik Gm15816 Map2k3os 4732491K20Rik Gm11973 1700052K11Rik Sp3os 1110002L01Rik
6	Gm11730 Gm16565 Gm13861 Gm12472 Gm15984 Tbx3os1 AU020206 4932435O22Rik Gm14965 Ighd A830082K12Rik B230206H07Rik Gm26776 2310002F09Rik Gm38048 Gm28933 Gm26660 Gm27151 Malat1 Gm29536 B130024G19Rik Hotairm1 Gm26759 Gm16070 Gm11476 2610027K06Rik Gm12703 Mannr B630019A10Rik Gm26510 Gm26723 Gm28153 Gm28529 Gm28610 Gm26719 A430035B10Rik Kcnq1ot1 Gm12454 Gm16933 Gm20417 Gm26821 Gm29007 1700061E17Rik C630043F03Rik Gm8098 Ighm 4930511M06Rik 9530027J09Rik Gm13066 Gm17035 4833422C13Rik C230034O21Rik C030034L19Rik B230217O12Rik BC065403 Gm10371 Rsf1os2 1500015A07Rik 5830432E09Rik 1700016P03Rik Gm26781 A530072M11Rik Gm13056 Gm10785 Gm16156 Gm17344 A730081D07Rik Dleu2 F420014N23Rik Gm13563 Gm28039 4930470G03Rik Gm26590 Gm12992 Gm26582 Gm20457 Gm26778 Gm26716 Gpr137b-ps Gas5 D030028A08Rik4930599N23Rik Gm26561 5530601H04Rik Dubr
7	D830026I12Rik Gm28836 Gm15587 Gm15581 6430562O15Rik 2610307P16Rik Gm26703 Gm11747 Gm4793 9530082P21Rik F730311O21Rik Gm9917 Gm15851 Gm11816 C430049B03Rik C230037L18Rik Gm26624 4930512B01Rik Gm12963 Gm12473 Gm26906 Gm15598 9930014A18Rik Prss23os Gm26848 Gm12426 4930590L20Rik Kdm6bos Gm10125 C430014B12Rik Zfp335os Gm16567 Gm28496 Gm9947 Gm32569 Gm16008 2610203C22Rik 6030408B16Rik Gm21781 Gm6225 4933431E20Rik 5430416N02Rik Ftx Gm16343 Gm12758 Gm16897 BC037039 Gm17494 9930104L06Rik Gm1976
8	Gm26870 Gm11175 9530026P05Rik 4930481A15Rik Junos D230017M19Rik Gm15879 9130221H12Rik Gm11944 2810001G20Rik Gm13610 BC002059 1110018N20Rik2310015A10Rik Snhg12 Firre Trmt61b 1300002E11Rik Snhg14 Zfas1

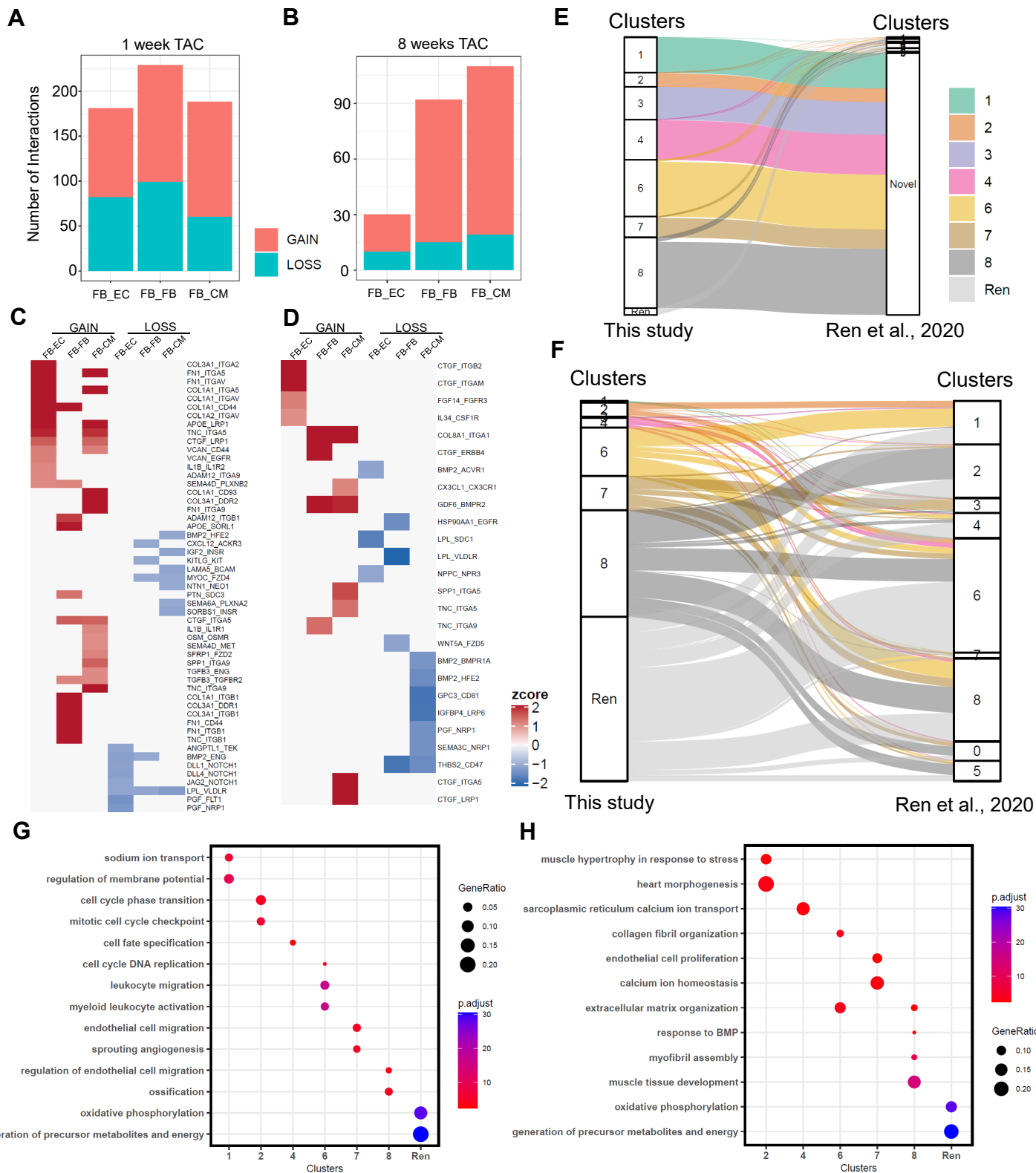
Supp. Figure 4. Venn diagrams and tables of significantly regulated non-coding RNAs. Related to Figure 1. Only RNAs expressed at a level ≥ 2 CPMs were considered. Numbers in italics: *total number of genes upregulated in the respective cell type*. [1], [2], [3] list non-coding RNAs (mostly lncRNAs) that are selectively upregulated in cardiomyocytes, endothelial cells or fibroblasts, respectively. [4] lists (l)ncRNAs upregulated in all three cell populations. [5], [6], [7] list non-coding RNAs (mostly lncRNAs) that are selectively downregulated in cardiomyocytes, endothelial cells or fibroblasts, respectively. [8] lists (l)ncRNAs downregulated in all three cell populations.



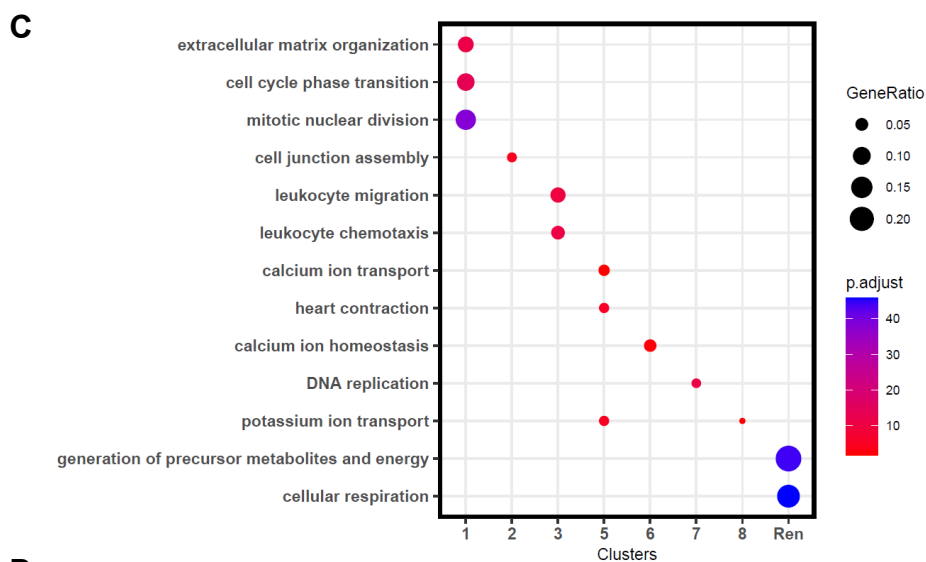
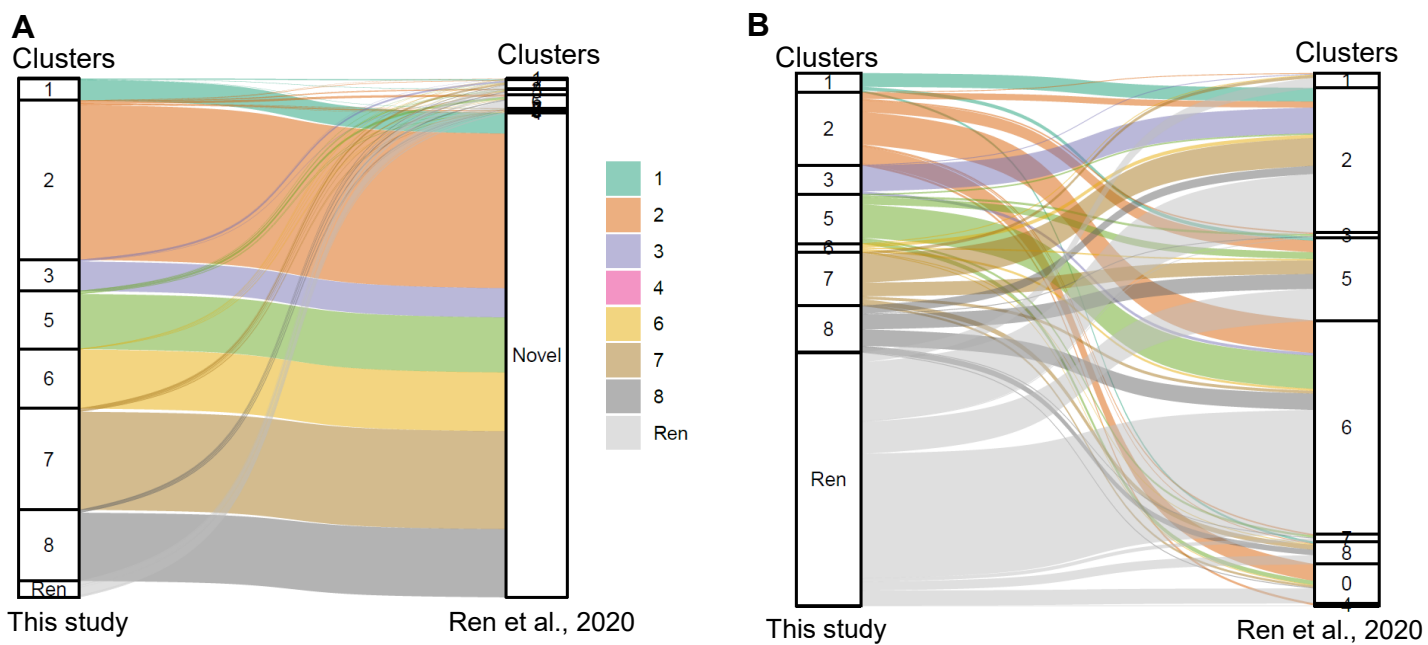
Supp. Figure 5. Interaction of cardiomyocyte derived ligands during pressure overload. Related to Figure 2. (A) Number of interactions between upregulated (gain) or downregulated (loss) cardiomyocyte (CM) derived ligands after 1 week (A) and 8 weeks (B) of TAC with receptors expressed in endothelial cells (EC), fibroblasts (FB) and CM. Example interactions gained or lost after 1 week (C) or 8 weeks (D) of TAC are shown. (E) Comparison of cardiomyocyte gene-expression found in the clusters in our study (left column) and the one by Ren et al., 2020. Genes only found in our study are termed „novel“. Genes found in both studies are connected by lines between both sides. Genes only found in our study connect from the left side to „novel“ on the right side. (F) Comparison of gene-expression found in the clusters in our study (left column) and the one by Ren et al., 2020 after removal of the „novel“ genes only found in our study. (G) Gene ontology terms (GO, biological process) of genes detected only in clusters of our study or only by Ren et al.. (H) Gene ontology terms (GO, biological process) of genes detected in clusters of our study and in Ren et al., as well as only in Ren et al..



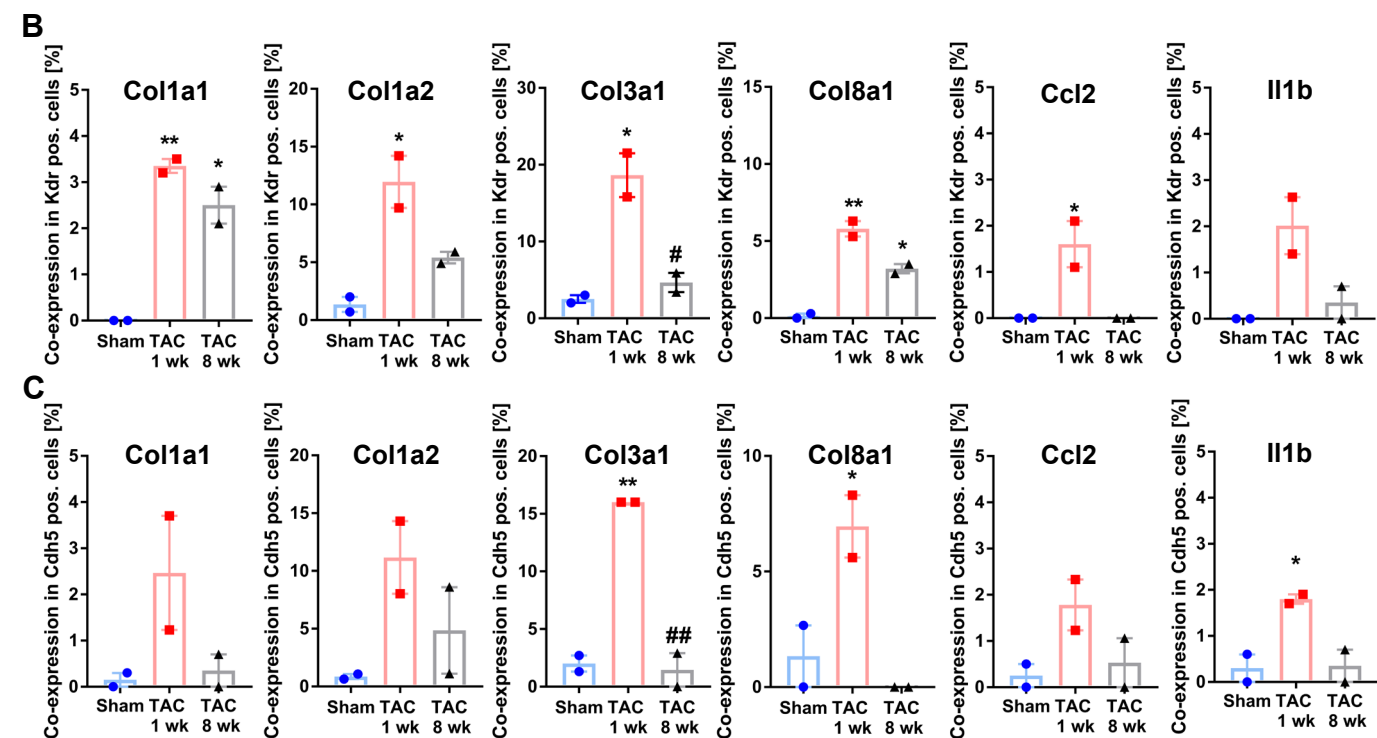
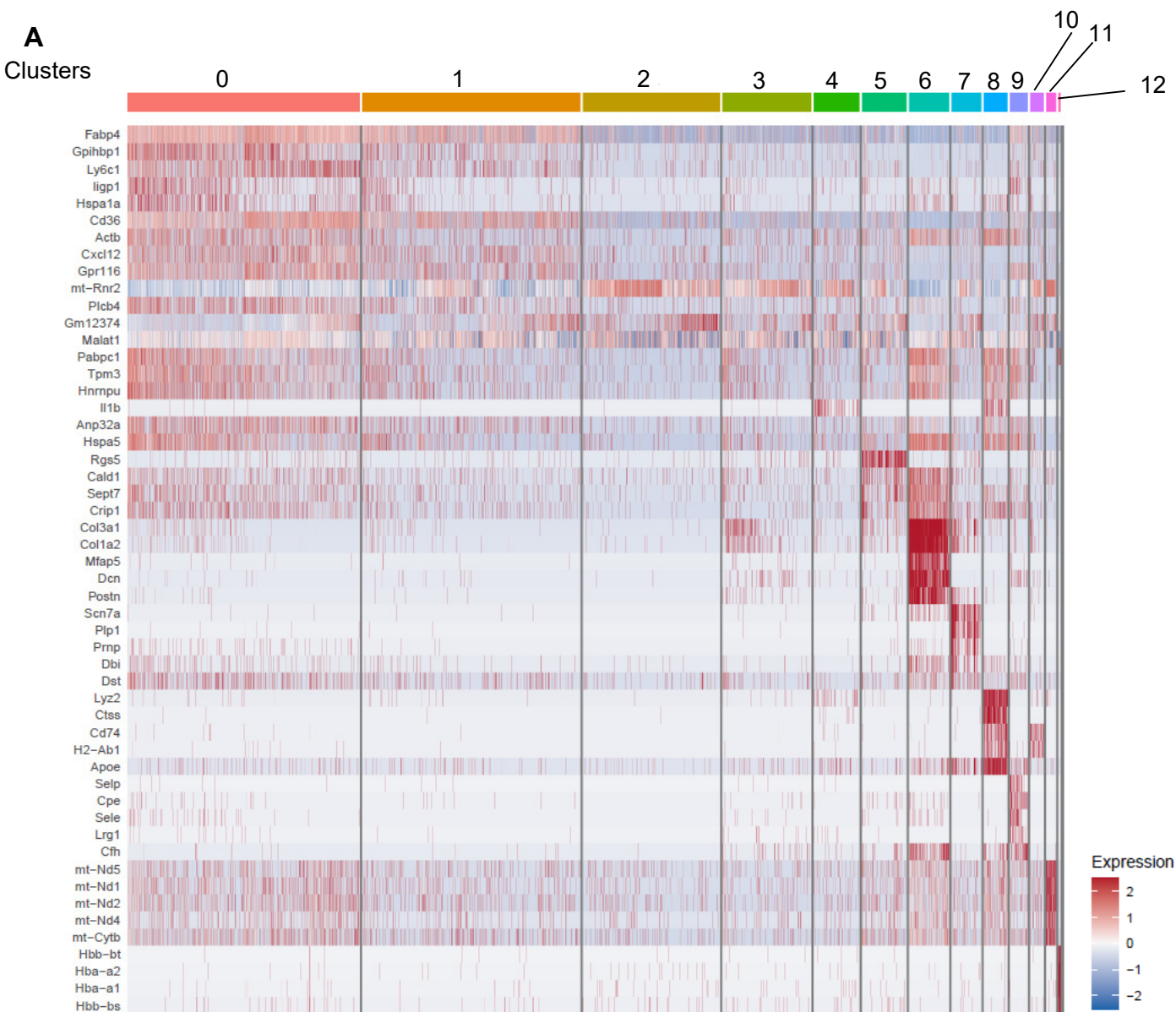
Supp. Figure 6. Fibroblast gene expression after TAC. Related to Figure 2. The fibroblast gene expression was analyzed with RNA sequencing. **(A)** A heatmap of differentially expressed genes in fibroblasts in sham vs. 1 wk and vs. 8 wk of TAC is shown. 8 different clusters were observed that each had a characteristic expression pattern. Example genes are indicated on the right. **(B)** Gene ontology terms (GO, biological process) related to the 8 clusters are shown. **(C)** Visualization of cluster gene regulation over time under the indicated conditions. Data are shown as mean \pm SEM. **(D)** Enrichment of top ten transcription factor binding motifs in the promoter region of regulated genes in the indicated clusters.



Supp. Figure 7. Interaction of fibroblast derived ligands in response to pressure overload. Related to Figure 2. (A) Number of interactions between upregulated (gain) or downregulated (loss) fibroblast (FB) derived ligands after 1 week (A) and 8 weeks (B) of TAC with receptors expressed in endothelial cells (EC), FB, and cardiomyocytes (CM). Example interactions gained or lost after 1 week (C) or 8 weeks (D) of TAC are shown. (E) Comparison of gene-expression found in the clusters in fibroblasts in our study (left column) and the one by Ren et al., 2020. Genes only found in our study are termed „novel“. Genes found in both studies are connected by lines between both sides. Genes only found in our study connect from the left side to „novel“ on the right side. (F) Comparison of gene-expression found in the clusters in our study (left column) and the one by Ren et al., 2020 after removal of the „novel“ genes only found in our study. (G) Gene ontology terms (GO, biological process) of genes detected only in clusters of our study or only in Ren et al.. (H) Gene ontology terms (GO, biological process) of genes detected in clusters of our study and in Ren et al., as well as only in Ren et al..



Supp. Figure 8. Comparison of endothelial gene expression between our study and the one by Ren et al., 2020. Related to Figure 3. (A) Comparison of gene-expression found in the clusters in endothelial cells in our study (left column) and the one by Ren et al., 2020. Genes only found in our study are termed „novel“. Genes found in both studies are connected by lines between both sides. Genes only found in our study connect from the left side to „novel“ on the right side. **(B)** Comparison of gene-expression found in the clusters in our study (left column) and the one by Ren et al., 2020 after removal of the „novel“ genes only found in our study. **(C)** Gene ontology terms (GO, biological process) of genes detected only in clusters of our study or only by Ren et al.. **(D)** Gene ontology terms (GO, biological process) of genes detected in clusters of our study and in Ren et al., as well as only in Ren et al..



Supp. Figure 9. Endothelial single cell sequencing. Related to Figure 5. (A) Heatmap of enriched genes in different clusters of endothelial cells in single cell sequencing shown in Figure 5. **(B)** Quantification of the expression of the indicated genes in sham and 1 and 8 weeks after TAC in Kdr positive endothelial cells or in Cdh5 positive endothelial cells (C), N=2 samples/condition. **(B)** and **(C)**, data are shown as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$ vs. sham; # $p < 0.05$, ## $p < 0.01$ vs. TAC 1wk as determined by one-way ANOVA with Sidak's multiple comparisons test.

Functional class	Gene	CM Sham	CM TAC 1 wk	CM TAC 8 wk	Fib Sham	Fib TAC 1wk	Fib TAC 8wk	EC Sham	EC TAC 1wk	EC TAC 8 wk
Cell cycle	E2f1	-0.7±0.1	2.0±0.1	0.6±0.4	2.1±0.1	3.7±0.1*	2.2±0.1#	2.1±0.1	3.8±0.1*	1.6±0.1#
	Ccna2	2.1±0.2	4.6±0.3*	2.3±0.1#	2.6±0.1	5.3±0.2*	2.6±0.2#	3.7±0.2	6.2±0.2*	2.9±0.1##
	Aurkb	-0.2±0.3	1.9±0.4*	-0.7±0.3#	0.8±0.2	3.1±0.2*	0.8±0.1#	1.1±0	4±0.1*	0.7±0.1#
	Mki67	4.4±0.3	6.7±0.5*	4.12±0.2#	5.6±0.4	7.8±0.3*	5.5±0.3#	6.2±0.3	8.6±0.2*	5.4±0.1##
Cell adhesion	Acan	-2.6±0.5	1.5±0.1*	-0.9±0.1#	-0.5±0.2	1±0.5	0.6±0.5	1.3±0.1	3.7±0*	2.1±0.1##
	Thbs4	3.2±0.1	5.9±0.2*	7.3±0.1##	5.6±0.2	9.6±0.3*	9.1±0.3*	2.3±0.1	7.3±0.4*	4.9±0.2##
	Fn1	5.3±0.2	8.6±0.2*	7.3±0.1##	8.6±0.1	12.2±0.2*	9.0±0.3#	9.5±0.2	11±0.1*	9.7±0.1#
	Thbs1	2.3±0.3	5.9±0.4*	6.5±0.1##	11.9±0.35	11.95±0.4	11.8±0.5	8.9±0.1	9.9±0.1*	8.8±0.2#
Collagen fibrils	Col1a1	4.3±0.01	8.4±0.15*	7±0.1##	10.4±0.1	12.6±0.1*	10.1±0.1#	6.2±0.2	10.5±0.1*	5.9±0.1#
	Col3a1	5.7±0.01	9.2±0.1*	7.8±0.1##	11.3±0.1	13.6±0.1*	10.8±0.1#	7.7±0.1	11.2±0.1*	7.0±0.1##
	Lox	0.5±0.2	5.5±0.2*	5.4±0.2*	6.8±0.1	9.7±0.2*	7±0.3#	5.3±0.1	7.8±0.3*	4.9±0.1#
	Col11a1	-1.8±0.9	2.2±1.1*	-0.1±0.5#	1.8±0.4	6.0±1*	2.5±0.5#	-0.7±0.1	4.6±0.9*	-0.5±0.1#
Inflammatory response	Il1b	-2.0±0.6	0.1±0.3*	1.9±0.1##	7.8±0.2	9±0.1*	7.1±0.2#	2.6±0.3	5.3±0.4*	3.9±0.2##
	Ccl2	-0.3±0.0	0.6±0.2*	0.04±0.4	7.5±0	7.4±0.3	6.6±0.3	4.3±0.2	5.4±0.1*	3.8±0.1##
	Il6	-3.2±0.1	-0.7±0.5*	0.1±0.3*	9.1±0	8.6±0.4	8.4±0.2	7.4±0	8.0±0*	7.1±0.1#
	Ccl4	-2±0.4	-0.7±1.1	-1±0.96	4.9±0.3	5.3±0.2	4.3±0.4	-1±0.5	0.6±0.2*	0.3±0.5
Growth factors	Igf1	3.5±0.2	5±0*	4.7±0.07*	7.9±0	8.8±0.1*	7.3±0.2#	4.6±0.1	6.4±0.1*	4.1±0.1##
	Tgfb1	4.1±0.1	4.3±0	4.4±0.03	6.5±0.1	7.1±0.1*	7.0±0.1#	7.3±0.1	7.3±0	1.8±0.1##
	Ctgf	6.8±0.5	8.5±0.5*	10.7±0.5##	8.1±0.1	10.2±0.5*	10.5±0.1#	6.7±0.3	8.3±0.4*	7.2±0.1
	Tgfb2	4.3±0.2	6.5±0.5*	7.7±0.3##	6.1±0.1	5.8±0.1	5.5±0.3#	5.3±0.2	4.9±0*	5.3±0.2

Supp. Table 1. Expression of selected genes in the different cell types after TAC. Related to Figure 1. Gene expression (in counts per million reads, log2), determined by RNA-Seq in different cardiac cell types under the conditions as indicated. Data are shown as mean ± SD. *p<0.0001 vs. sham; #p<0.0001 vs. TAC 1 wk as determined by the Wald test.