Supplementary Information

Ccn2a/Ctgfa is an injury-induced matricellular factor that promotes cardiac regeneration in zebrafish

Fig. S1

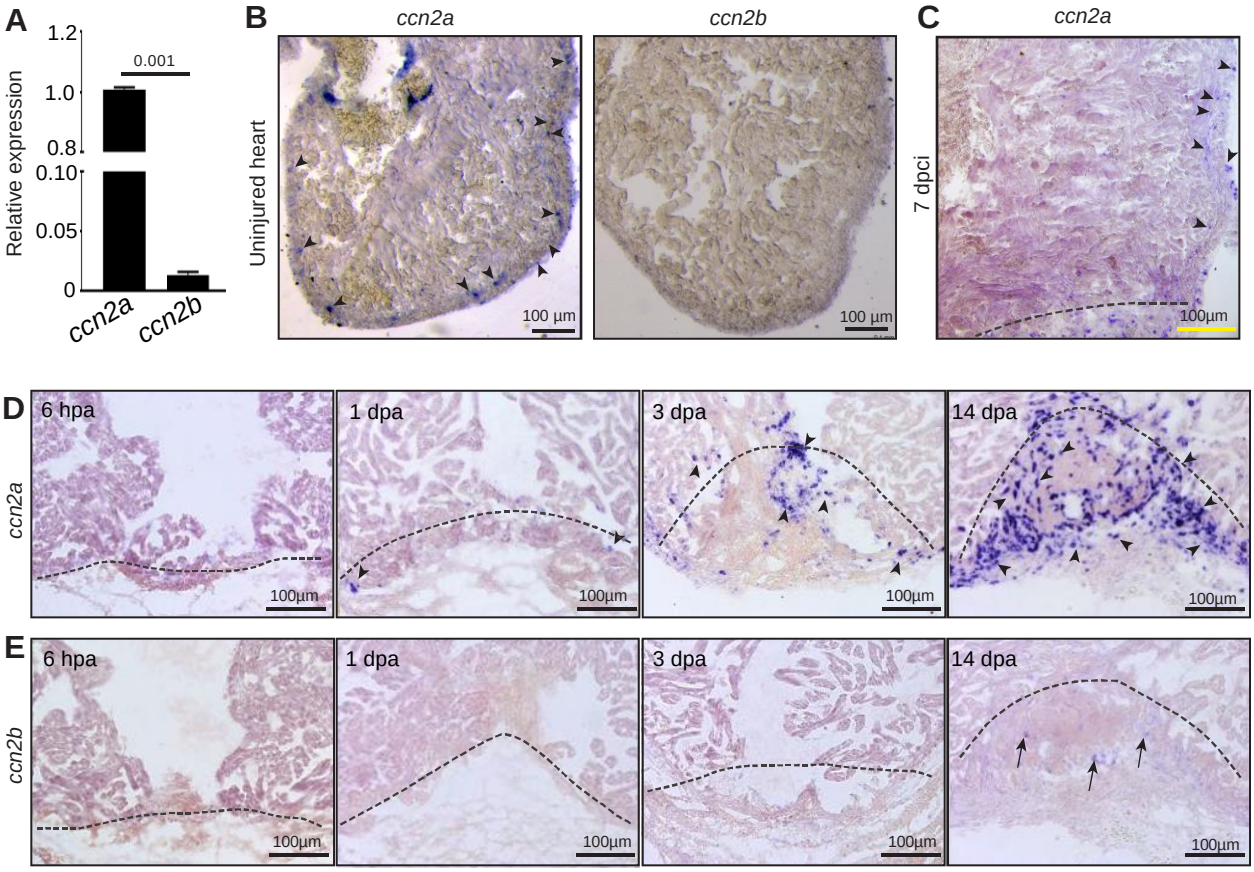

Fig. S1: *ccn2a* expression is induced upon myocardial injury. (**A**) Quantification of *ccn2a* and *ccn2b* expression in 4 dpci hearts (n=3, each sample represents a pool of 6 hearts). Error bars indicate the mean ± s.d.. The mean *ccn2a* expression value was set to 1. The statistical significance of differences was evaluated by a two-tailed Student's t-test (GraphPad Prism). Mean Ct values for this figure are provided in Supplementary Table 4. (**B**) Sections of an uninjured heart showing the expression pattern of *ccn2a* and *ccn2b*. *ccn2a* is expressed in the primordial layer and cortical myocardium (arrowheads). *ccn2b* is not detected in uninjured heart. (**C**) Sagittal section of a 7 dpci heart showing *ccn2a* is expressed in the primordial layer and cortical myocardium at the remote tissue (arrowheads). Dotted lines mark the injury border. (**D**) Images showing the time course of *ccn2a* expression in the heart post resection-injury. Expression is induced in the wound as early as 1 dpa (arrowheads), and expression levels are seen to increase through the regenerative stages (arrowheads). (**E**) Spatio-temporal expression pattern of *ccn2b* in injured heart tissue. Arrows indicate *ccn2b*- expressing cells. hpa: hours post amputation, dpa: days post amputation. Dotted lines mark the wound edge.

Fig. S2

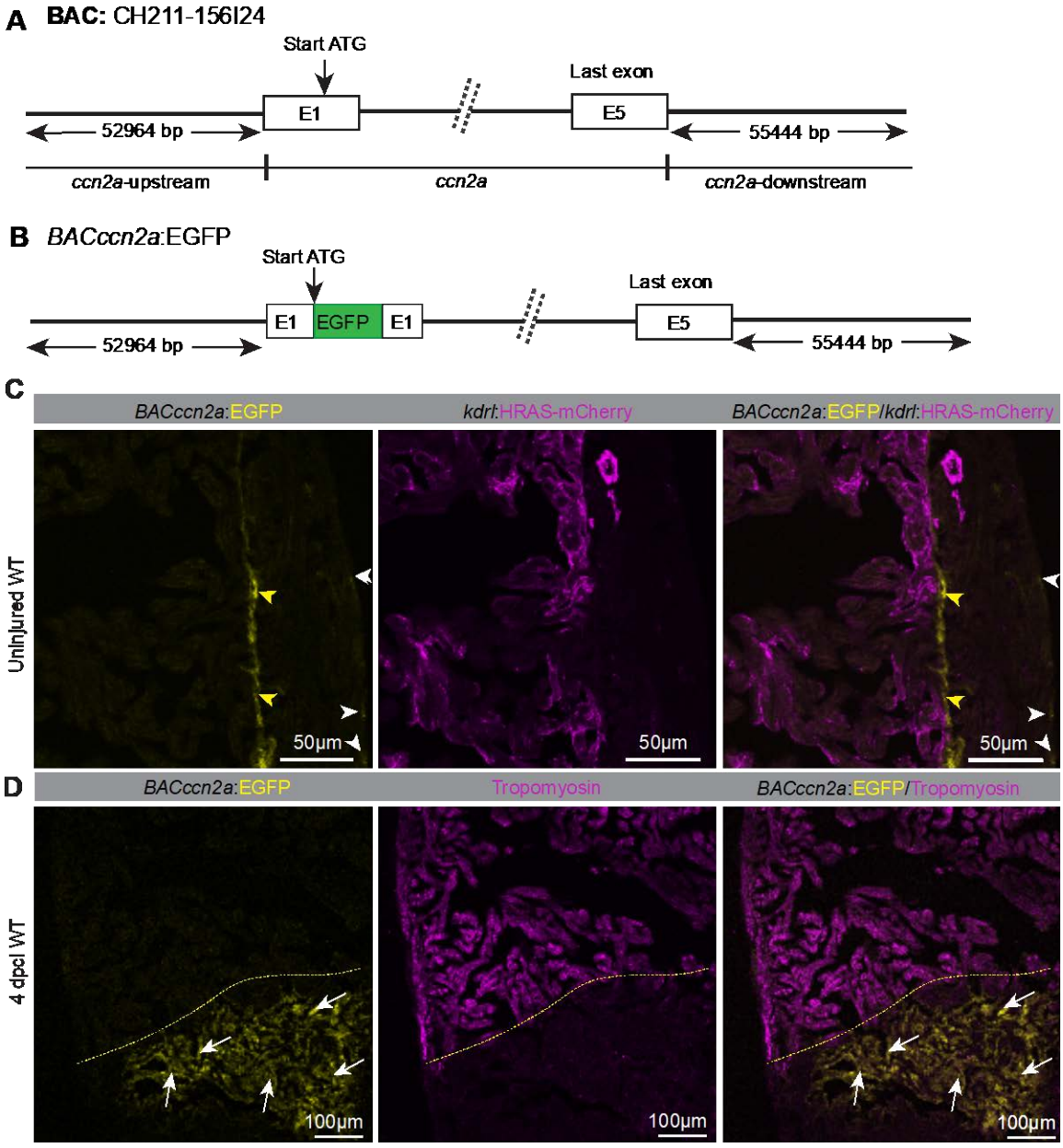

Fig. S2: *BACccn2a*:EGFP reporter expression is seen in the injured heart tissue. (**A**) Schematic representation of a BAC vector used to create *ccn2a* reporter line. (**B**) Schematic representation of a recombineered BAC vector to generate *BACccn2a*:*EGFP* transgenic reporter line. The EGFP cassette was sub-cloned at the ATG start site of the *ccn2a* gene in the BAC construct. (**C**) *BACccn2a*:EGFP expression is seen in the primordial layer (yellow arrowheads), and scattered expression in the cortical myocardium (white arrowheads) is seen in the sagittal section of an uninjured heart. (**D**) Sagittal section of a 4dpci *BACccn2a*:EGFP expressing (yellow) heart immunostained for Tropomyosin (magenta; marks CMs). Arrows point to EGFP- expressing cells in the wound. CMs (Magenta) are devoid of *BACccn2a*:EGFP expression. Dotted lines mark the injury border.

Fig. S3

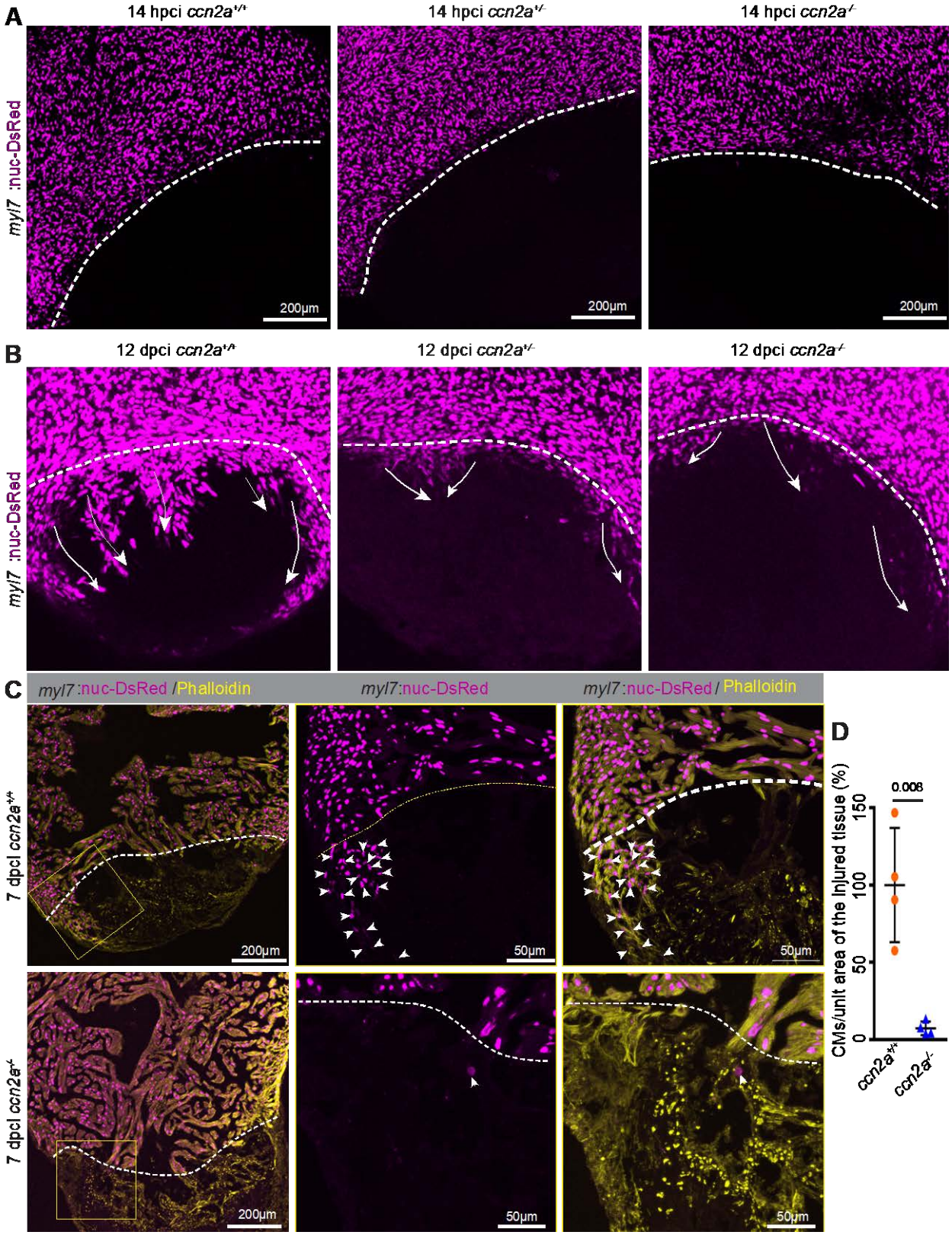

Fig. S3: CM infiltration into injured cardiac tissue is impaired in *ccn2a*$^{-/-}$. (**A**) Maximum intensity projection (MIP) of confocal sections taken of whole-mount heart at 14 hpci (hours post cryoinjury). DsRed^{+} CMs are not visible in the injured tissue. (**B**) MIP of confocal images taken of whole-mount heart at 12 dpci. DsRed (magenta) marking CM nuclei and arrows indicate the direction of CMs infiltration. (**C**) MIP of confocal images taken of a 10-µm sagittal cryosection of a 7 dpci heart, expressing DsRed in CM nuclei (magenta), and stained with phalloidin (yellow; marks F-actin). Arrowheads indicate CMs in the injured tissue. (**D**) Quantification of the total number of CMs in the injured cardiac tissue of *ccn2a*$^{+/+}$, and *ccn2a*$^{-/-}$ hearts at 7 dpci (n=4 each). At least two sections of each heart were used for quantification. The mean of the wild-type control value was set to 100%. Error bars indicate the mean ± s.d.. A two-tailed Student's t-test evaluated the statistical significance of differences (GraphPad Prism). Dotted lines mark the injury border.

Fig. S4

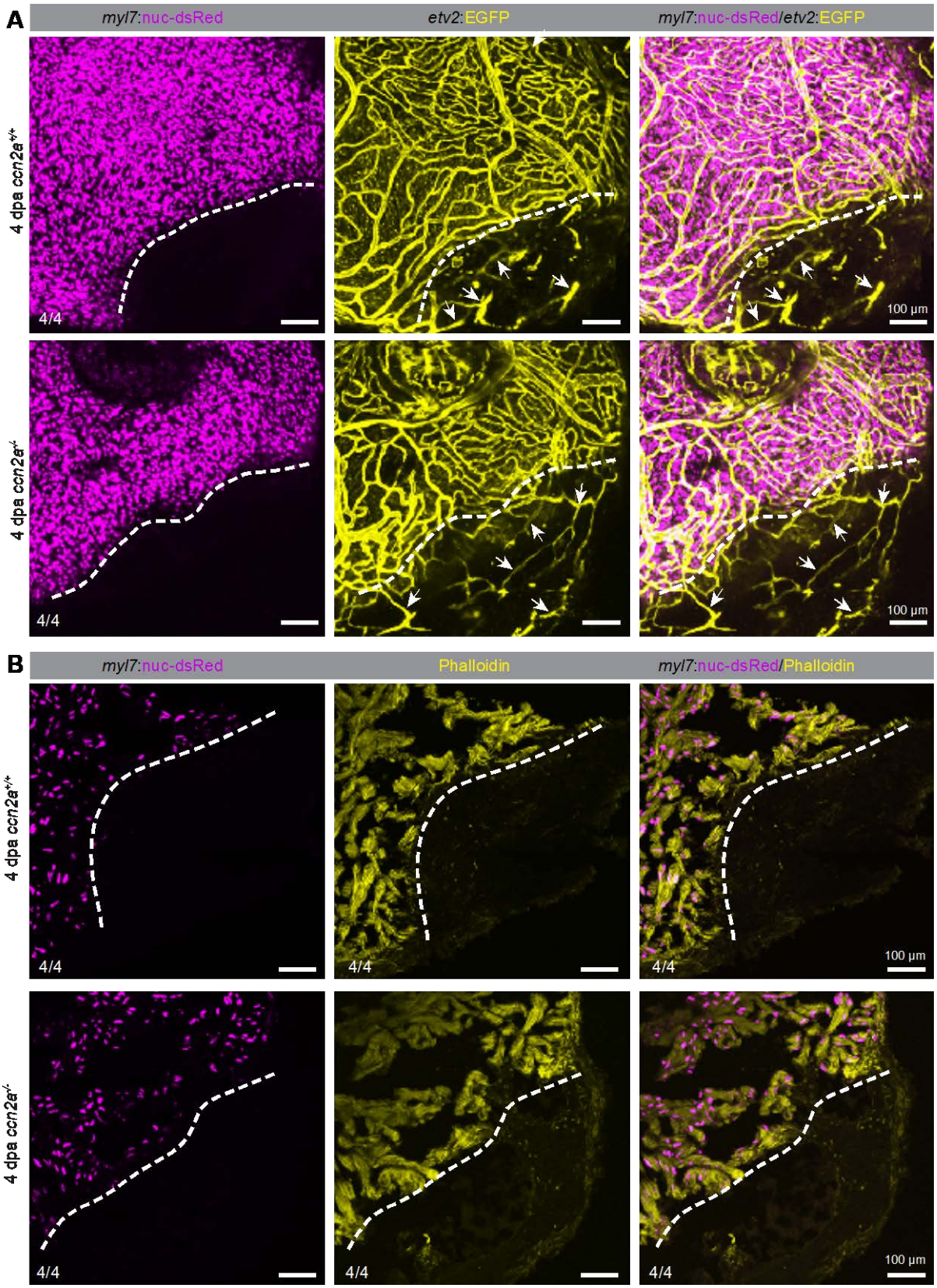

Fig. S4: CMs are not visible in the cardiac wound at 4 dpa. (**A**) MIP of optical sections of freshly isolated whole-mount heart at 4 dpa. DsRed marks CM nuclei (magenta) and EGFP marks coronary vessels (yellow). Arrows point to coronary vessels in the wound. (**B**) MIP of optical sections of a 10-µm ventricular sagittal cryosection stained with phalloidin (yellow; marks F-actin). DsRed marks CM nuclei (magenta). Dotted lines mark injury edge. dpa: days post amputation.

Fig. S5

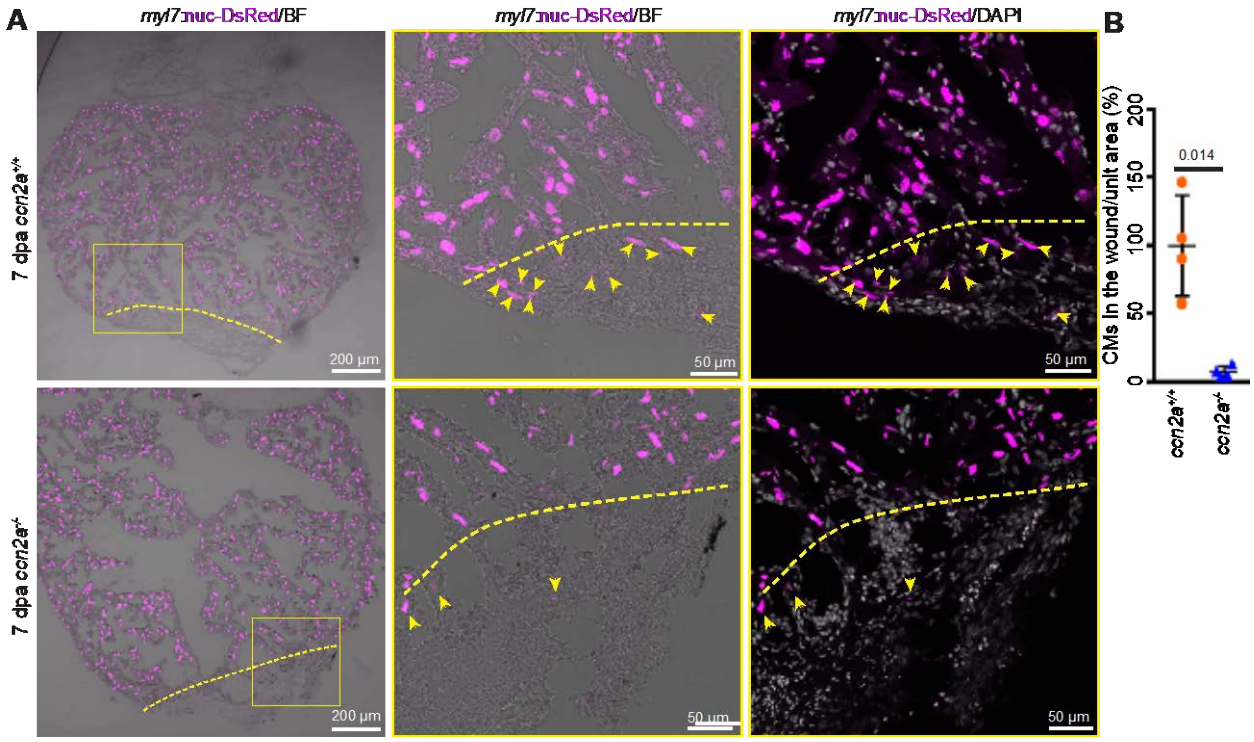

Fig. S5: Reduced CM infiltration into the apical wound of amputated *ccn2a*$^{-/-}$ hearts. (**A**) Representative MIP of optical sections of 10-µm thick ventricular sagittal cryosections of a 7 dpa hearts expressing dsRed in the CM nuclei (magenta), stained with DAPI (white; marks all nuclei). Dotted lines mark the wound border and arrowheads indicate CMs in the wound. (**B**) Quantification of the number of CMs found in the wound tissue in *ccn2a*$^{+/+}$, and *ccn2a*$^{-/-}$ hearts at 7 dpa (n=4 each). Two sections of each heart were analyzed for quantification. The mean of the wild-type control value was set to 100%. Error bars indicate the mean ± s.d.. The statistical significance of differences was evaluated by a two-tailed Student's t-test (GraphPad Prism). dpa: days post amputation.

Fig. S6

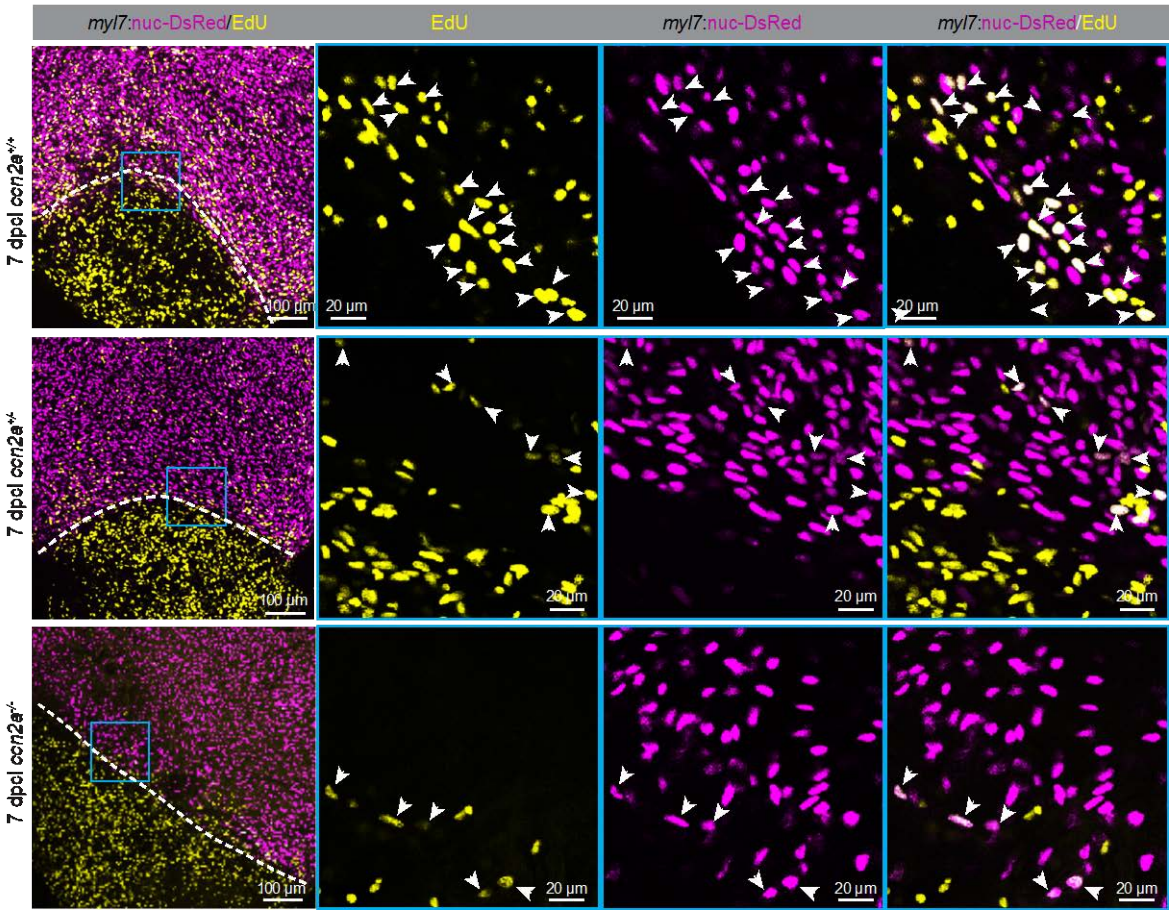

Fig. S6: *ccn2a* mutants show reduced CM proliferation at 7 dpci. (**A**) Panels in column 1 show maximum intensity projections of confocal images of 7 dpci whole-mount hearts stained for EdU (yellow; marks proliferating cells). DsRed (magenta) mark CM nuclei. The corresponding high magnification, single-plane optical sections are shown in panels of column 2-4. Arrowheads point to EdU^{+}/$DsRed^{+}$ cells. Dotted lines mark the injury border.

Fig. S7

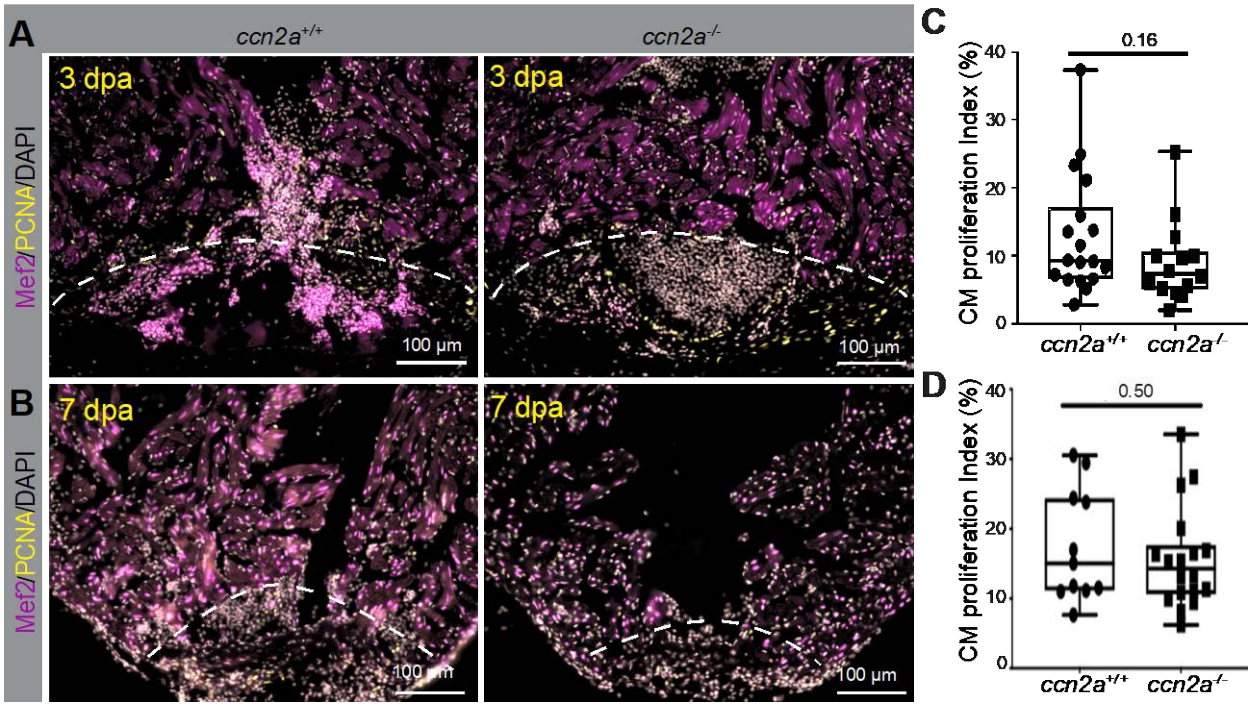

Fig. S7: CM proliferation is not affected in *ccn2a* mutants in cardiac amputation model. (**A**,**B**) Representative images of 10 µm thick ventricular sagittal cryosections at 3 and 7 dpa, immunostained for Mef2 (magenta; marks CMs nuclei), PCNA (yellow; marks proliferating cells), and stained with DAPI (white; labels all nuclei). (**C**,**D**) Quantitative analysis of CM proliferation at 3 and 7 dpa (C and D, respectively). The graphs represent the ratio of $Mef2^+/PCNA^+$ CMs to the total number of $Mef2^+$ CMs at the border zone (up to 100 µm away from the injury edge). 18 wild-type, and 15 *ccn2a*$^{-/-}$ at 3 dpa and 11 wild type, and 17 *ccn2a*$^{-/-}$ hearts at 7 dpa were analyzed from two independent experiments. Statistical significance of the differences was evaluated by Mann–Whitney nonparametric tests (GraphPad Prism).

Fig. S8

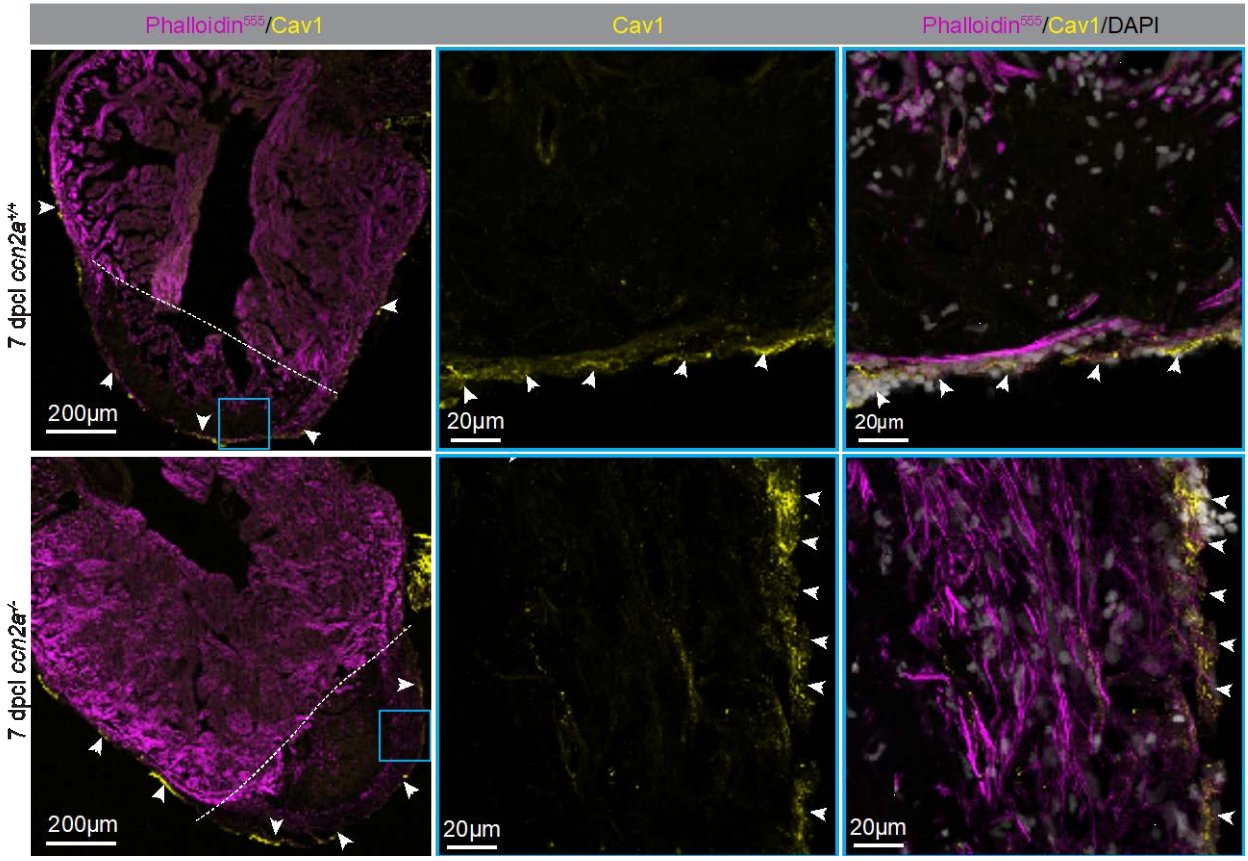

Fig. S8: Epicardial cell migration is not affected in injured *ccn2a* mutant heart. MIP of confocal images of sagittal cryosection of a 7 dpci heart immunostained for Cav1 (yellow; marks epicardial cells), stained with phalloidin (magenta; marks F-actin), and DAPI (white; marks all nuclei). Panels in column 2 and 3 show high-magnification images of the boxed region in column 1. Arrowheads point to Cav1 positive epicardial cells. Dotted lines mark the injury edge.

Fig. S9

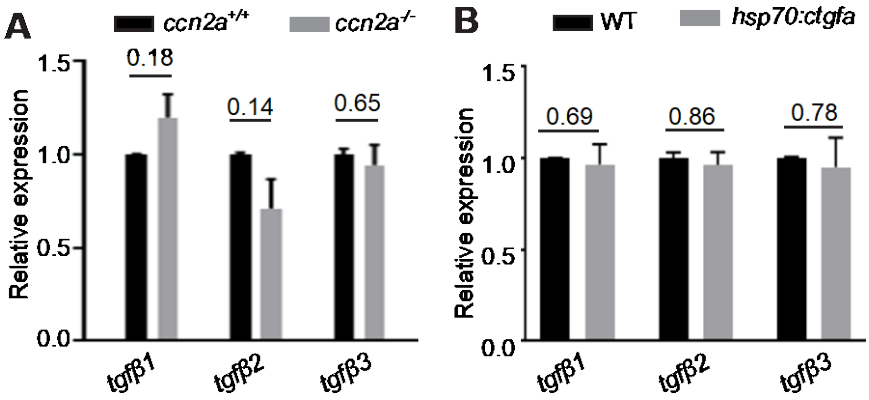

Fig. S9: Ccn2a does not regulate expression of *tgfβ* transcripts. (**A**,**B**) A comparison of level of *tgfβ* transcripts expression in wild-type and *ccn2a*$^{-/-}$ hearts (A), and in wild-type and *ccn2a* overexpressing (*hsp70:ctgfa*) hearts (B) at 4 dpci (n=3, each sample represents a pool of 6 hearts). The mean value for each gene in the control was set to 1. Error bars indicate the mean ± s.d. The statistical significance of differences was evaluated by a two-tailed Student's t-test (GraphPad Prism). ns ≥ 0.05. Mean Ct values for this Fig. are provided in Supplementary Table 4.

Fig. S10

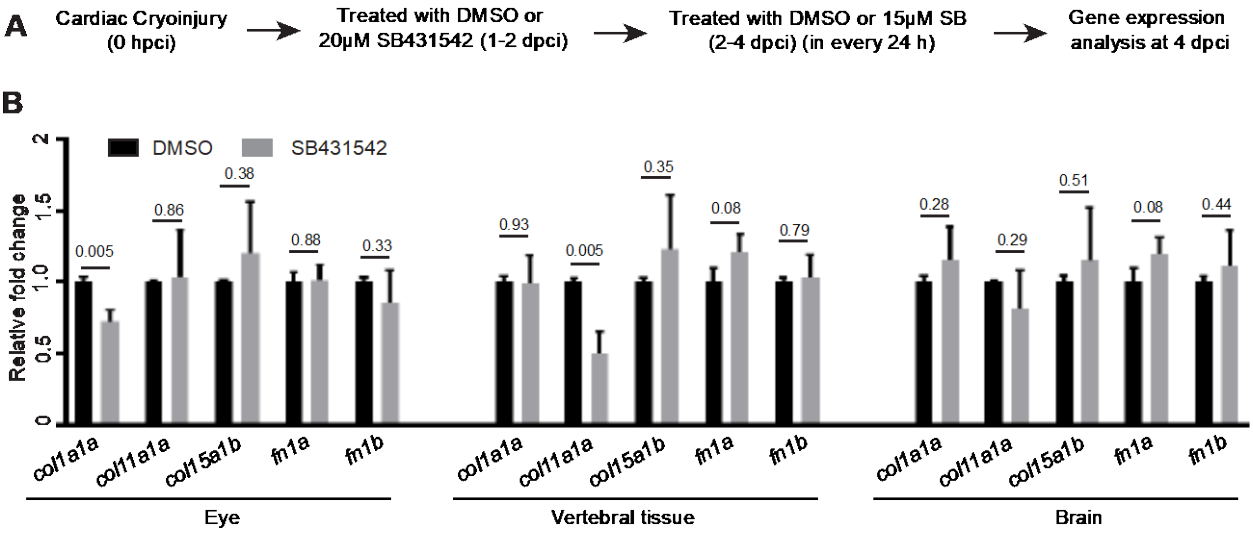

Fig. S10: SB431542 treatment mostly does not affect collagen and fibronectin gene expression in non-cardiac healthy tissues. (**A**) Schematic of the experimental procedures. (**B**) A comparison of level of collagen and fibronectin transcripts expression in DMSO or SB431542 treated animals at 4 dpci (n=3). The mean value for each gene in the control was set to 1. Error bars indicate the mean ± s.d.. The statistical significance of differences was evaluated by a two-tailed Student's t-test (GraphPad Prism).

Supplementary Tables

Table S1: List of secretory molecule encoding genes which were tested for differential expression between sham and 4 days cryo-injured cardiac ventricles.

Corresponding gene accession number with fold changes are listed

Sl#	Gene name	Accession #	Mean fold change
1	*paxillin a (pxna)*	NM_201588.1	1.272212
2	*paxillin b (pxnb)*	XM_021478354.1	1.191006
3	*periostin a (postna)*	XM_002663548.5	4.150509
4	*periostin b (postnb)*	NM_001077786.1	4.780527
5	*nephronectin (npnt)*	NM_001145580.1	1.028286
6	*fibrillin 2b (fbn2b)*	NM_001135790.1	1.97104
7	*fibulin 1 (fbln1)*	NM_131042.2	1.348248
8	*laminin alpha 1 (lama1)*	NM_001034986.1	5.254547
9	*laminin alpha 5 (lama5)*	NM_001039171.1	2.852084
10	*nidogen 1a (nid1a)*	XM_686064.8	1.125959
11	*nidogen 1b (nid1b)*	Gene ID: 562429 NR_023340.1	1.414458
12	*cellular communication network factor 2a (ccn2a)*	NM_001015041.2	3.775421
13	*cellular communication network factor 2b (ccn2b)*	NM_001102573.1	1.684559
14	*secreted phosphoprotein 1 (spp1)*	NM_001002308.1	13.99058
15	*tenascin C (tnc)*	NM_001312916.1	2.507331
16	*vitronectin a (vtna)*	NM_001020672.1	0.718661
17	*vitronectin b (vtnb)*	NM_001139461.1	1.93415
18	*collagen, type XI, alpha 2 (col11a2)*	NM_001079992.2	1.667507
19	*vimentin (vim)*	NM_131872.2	1.047533

Table S2: List of genes that are differentially expressed in injured *ccn2a*$^{-/-}$ hearts.

Corresponding gene accession number, base mean value with log2 fold changes and P values are listed

Sl. Nr.	**Ensembl gene id**	**Ensembl gene name**	**UniProt proteins**	**base Mean Wild-type 4dpci**	**base Mean *ctgfa*$^{-/-}$ 4dpci**	**log2FoldChange *ctgfa*$^{-/-}$ 4dpci /Wild-type 4dpci**	**P value**
1	ENSDARG00000054753	*col10a1a*	Collagen. type X. alpha 1a	509.0653338	2.6950739	-7.108935024	2.70E-06
2	ENSDARG00000043396	*fndc4a*	Fibronectin type III domain-containing 4a	128.9091458	0.777316967	-6.191658198	1.30E-14
3	ENSDARG00000089362	*grn1*	Granulin 1,Zgc:136318 protein	3228.185894	208.6745437	-3.944946874	5.15E-14
4	ENSDARG00000071662	*si:rp71-36a1.3*	Si:rp71-36a1.3	650.1344933	81.45900754	-2.981206564	2.28E-06
5	ENSDARG00000045453	*f13a1a.1*	Coagulation factor XIII. A1 polypeptide a. tandem duplicate 1	7685.820072	1146.738126	-2.743593392	2.11E-13
6	ENSDARG00000090428	*ctrb1*	Chymotrypsinogen B1	352.6346127	61.74822359	-2.494612966	1.88E-07
7	ENSDARG00000007358	*cxcr3.1*	Chemokine (C-X-C motif) receptor 3. tandem duplicate 1	113.2313003	423.4286932	1.893564177	2.34E-06

Table S3: List of oligos used for quantitative RT-PCR analysis.

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Table S4 : Mean ct values of quantitative RT-PCR analyses.

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