Supplementary Information

Loss of Caveolin-1 and caveolae leads to increased cardiac cell stiffness and functional decline of the adult zebrafish heart

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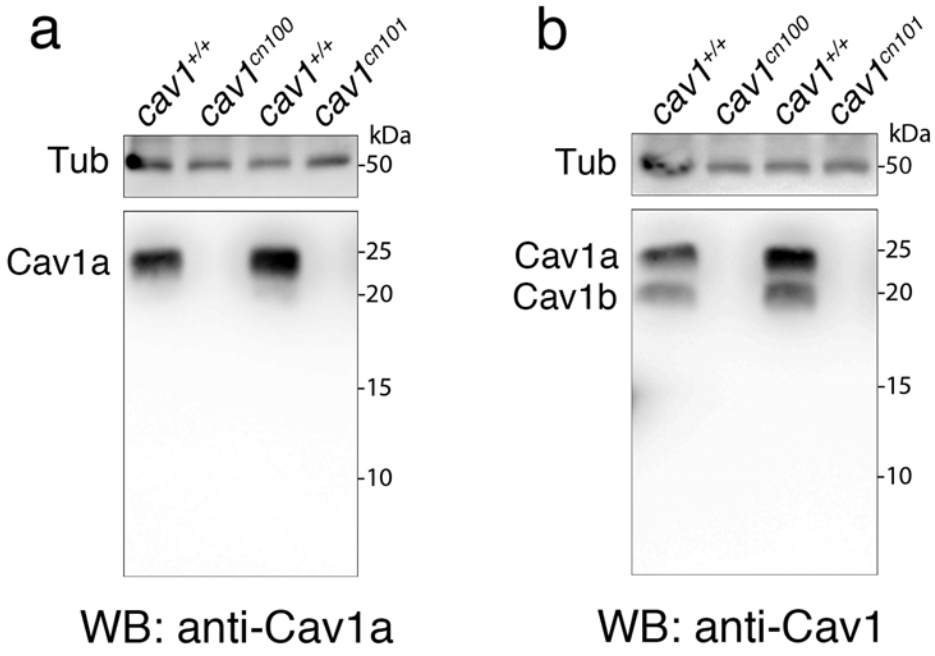
Supplementary Figure Legends

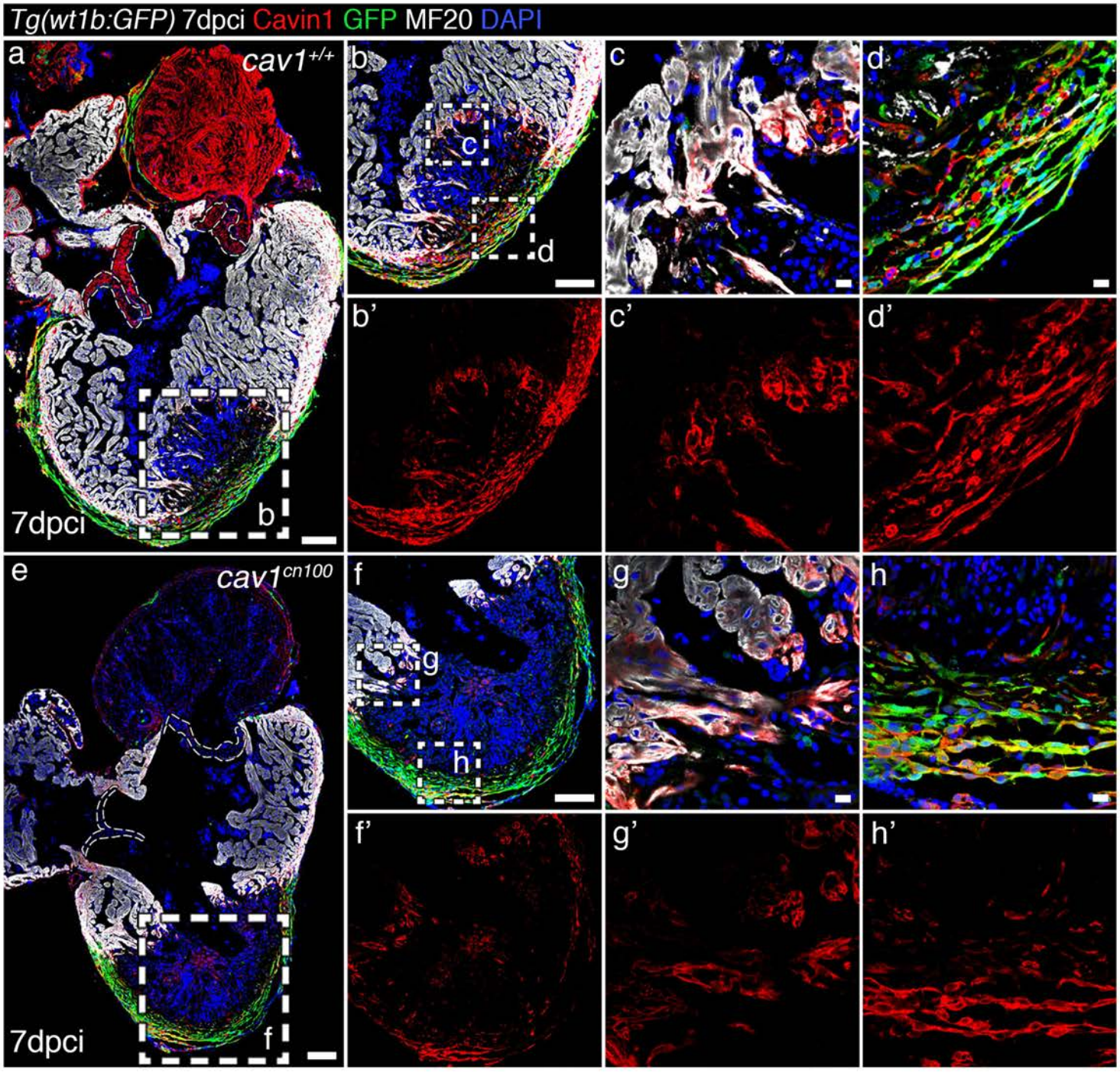
Supplementary Figure S1. Western blot analysis of Cav1a and Cav1 (Cav1a and Cav1b) protein expression in *cav1*^{cn100} and *cav1*^{cn101} mutants.

(a) Samples from *cav1*$^{+/+}$, *cav1*^{cn100} and *cav1*^{cn101} caudal fins were analysed by Western blot using an antibody against Cav1a (Cell Signalling Technology, catalogue #D46G3). Only the higher molecular weight band was detected in *cav1*$^{+/+}$, indicating the specificity of the antibody against Cav1a. In contrast, Cav1a was lost in *cav1*^{cn100} and *cav1*^{cn101}. Tub, alpha-Tubulin; kDa, kilodalton. (b) WB using an antibody against Cav1 (Cav1a and Cav1b, BD Transduction Laboratories, #610059). Two bands were detected in *cav1*$^{+/+}$, indicating that the antibody recognises both Cav1a and Cav1b, in contrast to *cav1*^{cn100} and *cav1*^{cn101} that both Cav1a and Cav1b were lost.


a
cav1+/+
cav1cn100
cav1+/+
cav1cn101
kDa
Tub
50
Cav1a
25
20
15
10
WB: anti-Cav1a
b
cav1+/+
cav1cn100
cav1+/+
cav1cn101
kDa
Tub
50
Cav1a
Cav1b
25
20
15
10
WB: anti-Cav1

Supplementary Figure S2. Cavin1b expression in *cav1*^{cn100} hearts

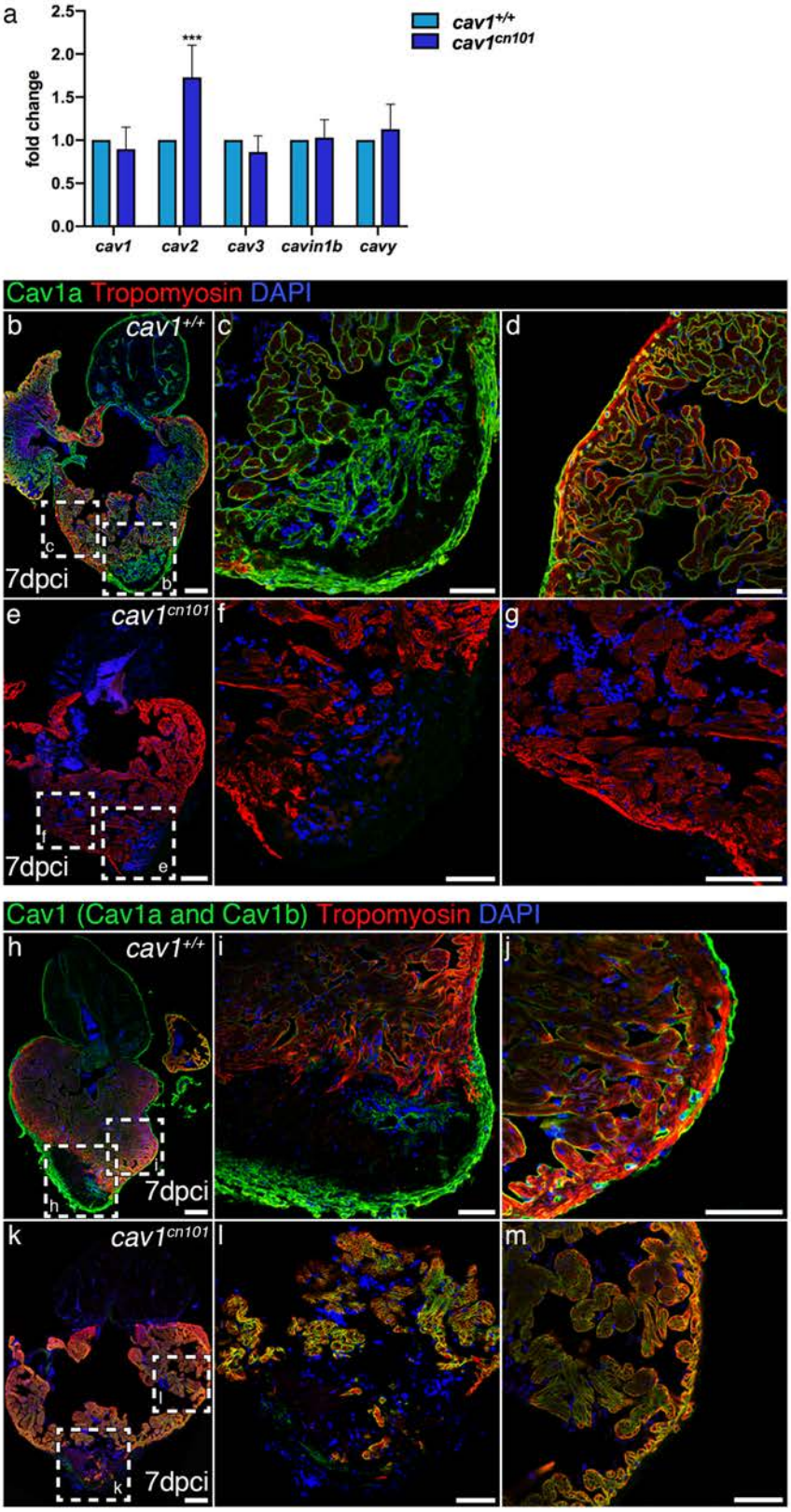
Immunostaining of Cavin1 in 7 dpci *cav1*$^{+/+}$ (a-d´) and *cav1*^{cn100} (e-h´) *Tg(wt1b:GFP)* hearts. (b, b´) Magnification of selected area in a. (c-d´) Magnifications of the dashed areas in b. (f, f´) Magnification of selected area in e. (g-h´) Magnifications of the dashed areas in f. Dashed lines in a and e mark the valves. Scale bars: 100 μm in a, b, e, f; 50 μm in other panels.


Tg(wt1b:GFP) 7dpci Cavin1 GFP MF20 DAPI
a
cav1+/+
7dpci
b
b
c
d
b'
c'
d'
e
cav1cn100
7dpci
f
f
g
h
f'
g'
h'

Supplementary Figure S3. qPCR and Cav1 expression analysis in *cav1*^{cn101} mutants

(a) Relative expression of caveolae-related genes by qPCR in *cav1*^{cn101} embryos. mean±s.d. t-test, ***P<0.001.

(b-m) Immunostaining of 7 dpci *cav1*$^{+/+}$ and *cav1*^{cn101} hearts with an antibody against Cav1a (b-g) or Cav1 (Cav1a and Cav1b; h-m). Scale bars: 100 μm in b, e, h, k; 50 μm in other panels.

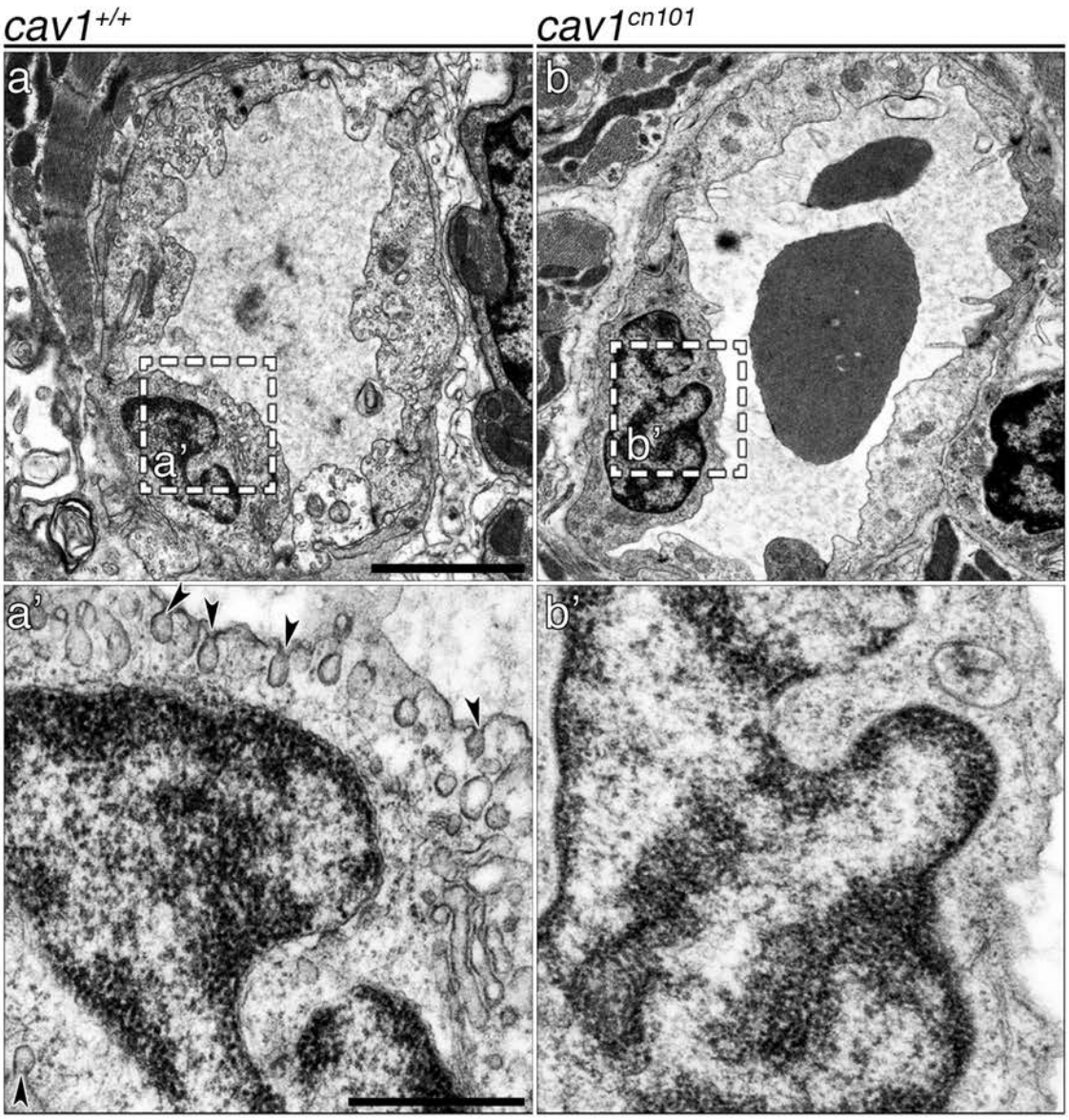

a
2.5
2.0
1.5
1.0
0.5
0.0
fold change
***
cav1+/+
cav1cn101
cav1
cav2
cav3
cavin1b
cavy
Cav1a Tropomyosin DAPI
b
cav1+/+
c
d
7dpci
e
cav1cn101
f
g
7dpci
Cav1 (Cav1a and Cav1b) Tropomyosin DAPI
h
cav1+/+
i
j
7dpci
k
cav1cn101
l
m
7dpci

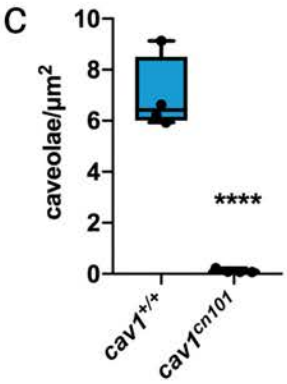
Supplementary Figure S4. Caveolae loss in *cav1*^{cn101} hearts

(a-b′) TEM images of coronary vasculature of the cortical layer in *cav1*$^{+/+}$ and *cav1*^{cn101} hearts.

(a′, b′) higher magnifications of the dashed areas in a and b; arrowheads indicate membrane-bound caveolae. Scale bars: 1 μm in a, b, and 0.5 nm in a′ and b′.

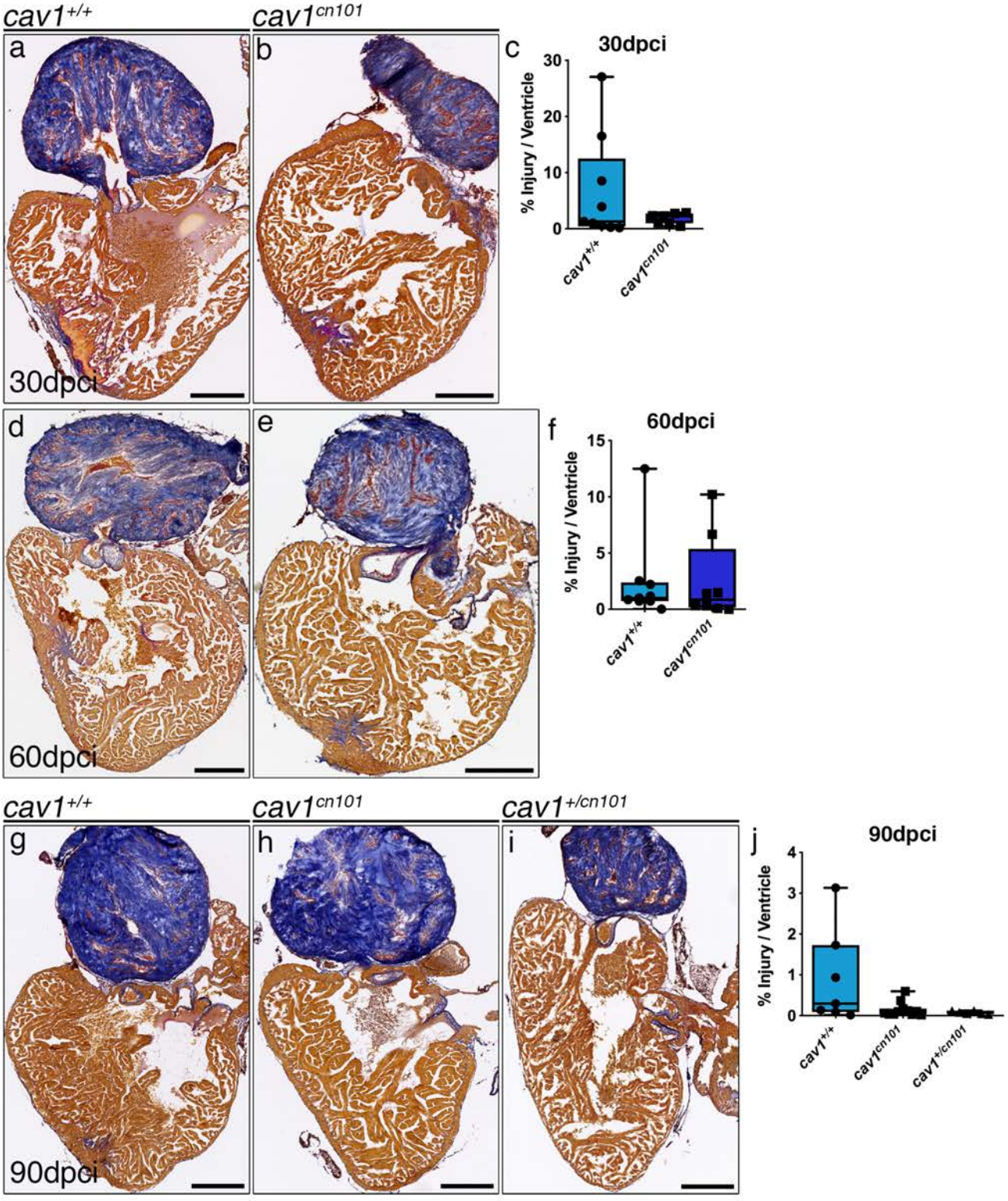
(c) Quantification of caveolae number per μm^2 of coronary endothelium. $n_{WT} = n_{cn101} = 4$, mean±s.d., t-test, ****P<0.0001.


cav1+/+
cav1cn101
a
b
a'
b'
a'
b'


C
caveolae/µm2
10
8
6
4
2
0
****
cav1+/+
cav1cn101

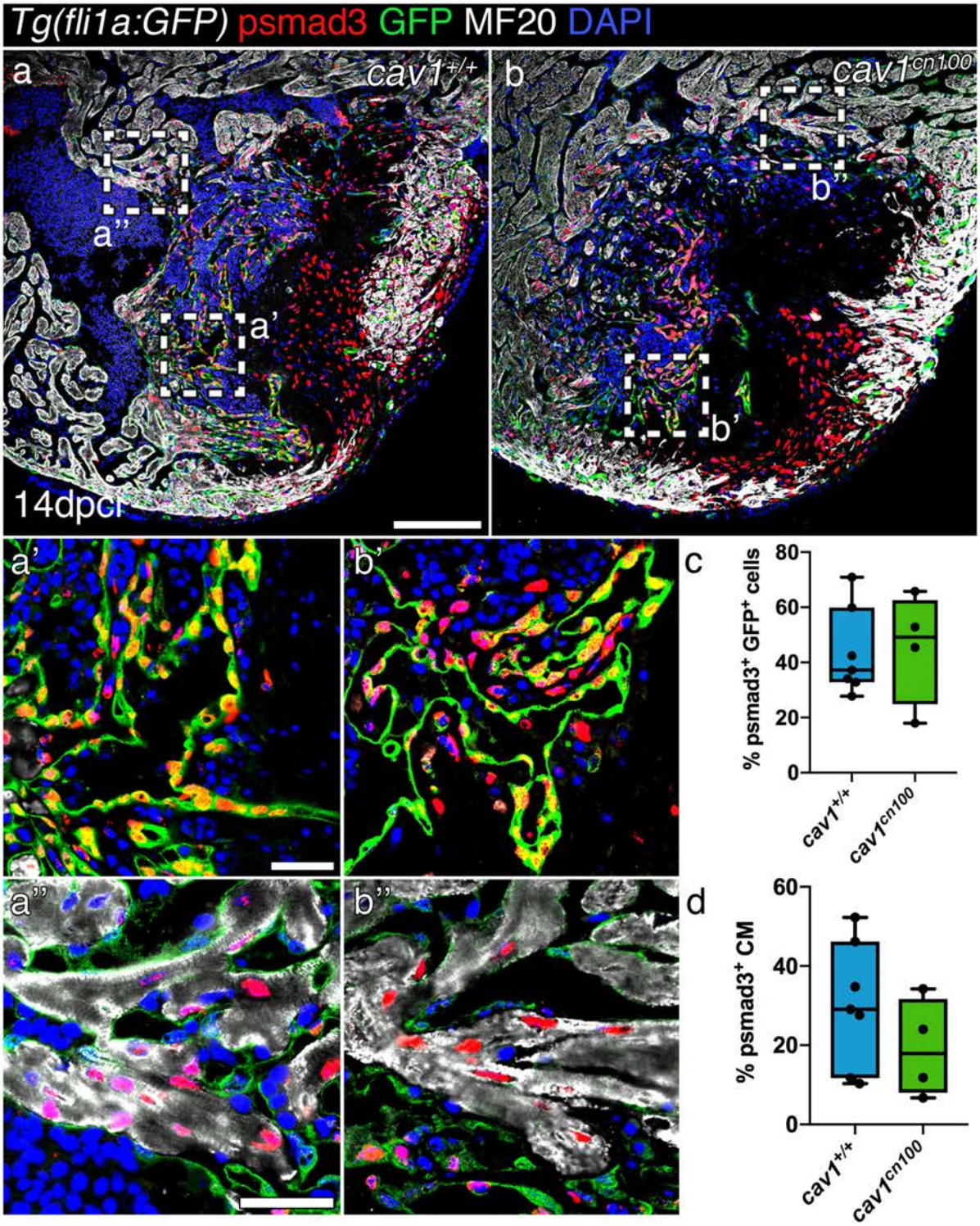
Supplementary Figure S5. Heart regeneration is unaffected in *cav1*^{cn101} mutants

(a-j) AFOG staining of *cav1*$^{+/+}$ and *cav1*^{cn101} hearts after 30 (a, b), 60 (d, e) and 90 (g-i) dpci. Collagen in blue, fibrin in red and healthy myocardium in brown. (c, f, j) The damaged area was quantified as the percentage of the collagen/fibrin area to the total ventricular area. 30 dpci n_{WT}=9, n_{cn100}=7; 60 dpci n_{WT}=8, n_{cn100}=7; 90 dpci n_{WT}=7, n_{cn100}=11. mean±s.d., t-test. Scale bars 250 μm.


cav1+/+
cav1cn101
a
b
c
30dpci
% Injury / Ventricle
30
20
10
0
cav1+/+
cav1cn101
30dpci
d
e
f
60dpci
% Injury / Ventricle
15
10
5
0
cav1+/+
cav1cn101
60dpci
cav1+/+
cav1cn101
cav1+/cn101
g
h
i
j
90dpci
% Injury / Ventricle
4
3
2
1
0
cav1+/+
cav1cn101
cav1+/cn101
90dpci

Supplementary Figure S6. TGFβ signalling is unaffected in regenerating *cav1*^{cn100} hearts

(a-d) 14dpci *cav1*$^{+/+}$ and *cav1*^{cn100} *Tg(fli1a:GFP)* hearts labelled for psmad3, GFP and MF20. (a′, b′) Magnification of GFP^{+} endocardial cells marked in a, b. (a′′, b′′) Higher magnification of cardiomyocytes marked in a, b. (c) Quantification of psmad3^{+}/GFP^{+} in the injured area. t-test. n_{WT} = 7, n_{cn100} = 4. (d) Percentage of cardiomyocytes with psmad3^{+} nuclei in a 100 μm area surrounding the damaged tissue. CM, cardiomyocytes. t-test, n_{WT} = 7, n_{cn100} = 4. Scale bars 100 μm in a and b; 25 μm in other panels.


Tg(fli1a:GFP) psmad3 GFP MF20 DAPI
a
cav1+/+
b
cav1cn100
a''
a'
b''
b'
14dpcr
a'
b'
a''
b''
c
% psmad3+ GFP+ cells
80
60
40
20
0
cav1+/+
cav1cn100
d
% psmad3+ CM
60
40
20
0
cav1+/+
cav1cn100

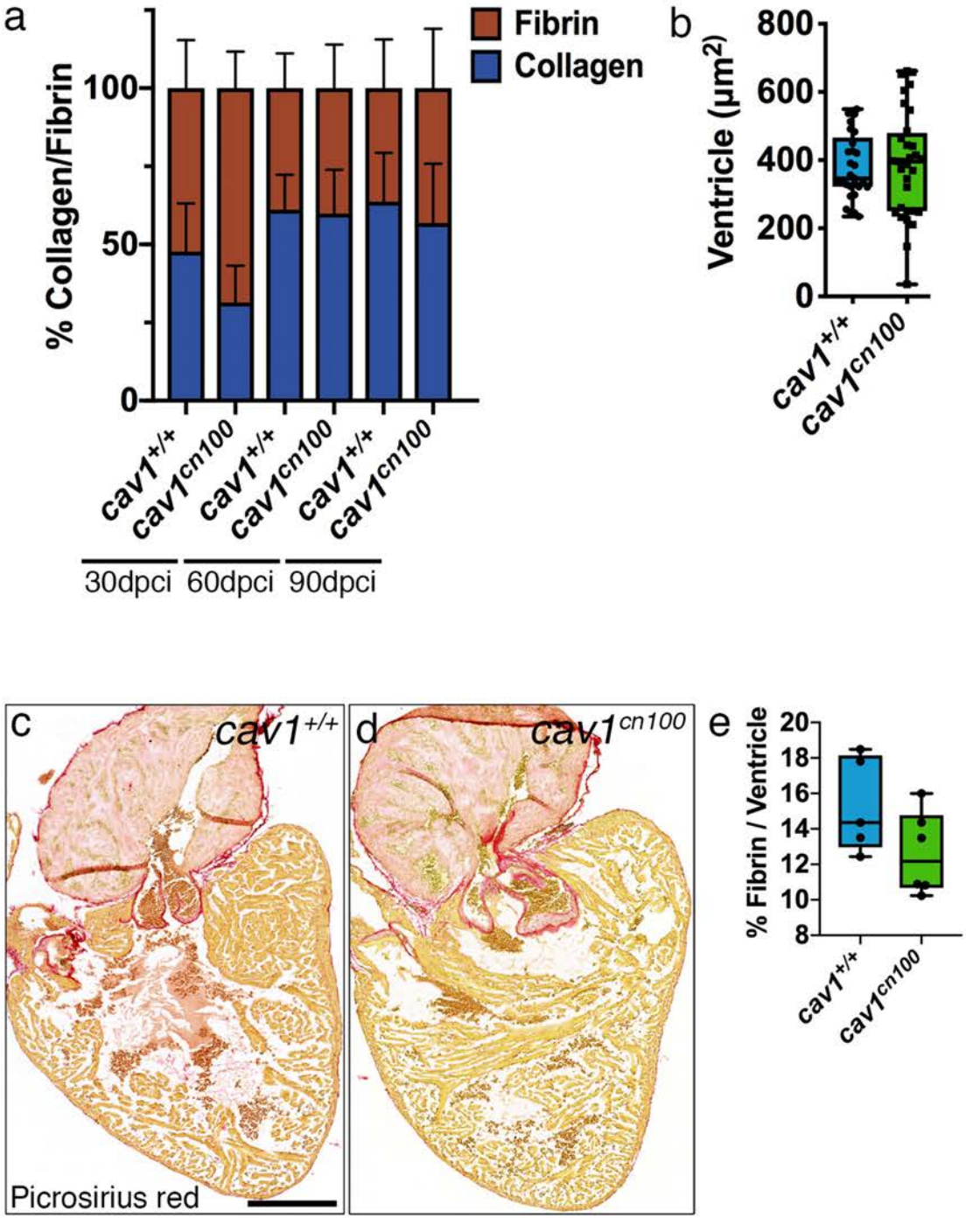
Supplementary Figure S7. Analysis of collagen and fibrin in the injured area, ventricular size and interstitial fibrosis of *cav1*^{cn100} hearts

(a) Percentages of collagen and fibrin within the injury zone 30, 60 and 90 dpci in *cav1*$^{+/+}$ and *cav1*^{cn100} hearts. Two-way ANOVA. n_{WT} 30, 60, 90 dpci = 10, 9, 9; n_{cn100} 30, 60, 90 dpci = 10, 10, 12.

(b) Ventricular size of all hearts analysed by AFOG staining. t-test. $n_{WT} = 29$; $n_{cn100} = 32$.

(c, d) Picrosirius Red staining in intact *cav1*$^{+/+}$ and *cav1*^{cn100} hearts. Scale bar 250 μm.

(e) Quantification of the red-labelled fibres in the ventricle. t-test, $n_{WT} = 5$, $n_{cn100} = 6$.


a
Fibrin
Collagen
% Collagen/Fibrin
100
50
0
cav1+/+
cav1cn100
cav1+/+
cav1cn100
cav1+/+
cav1cn100
30dpci
60dpci
90dpci
b
Ventricle (µm2)
800
600
400
200
0
cav1+/+
cav1cn100
c
cav1+/+
Picrosirius red
d
cav1cn100
e
% Fibrin / Ventricle
20
18
16
14
12
10
8
cav1+/+
cav1cn100

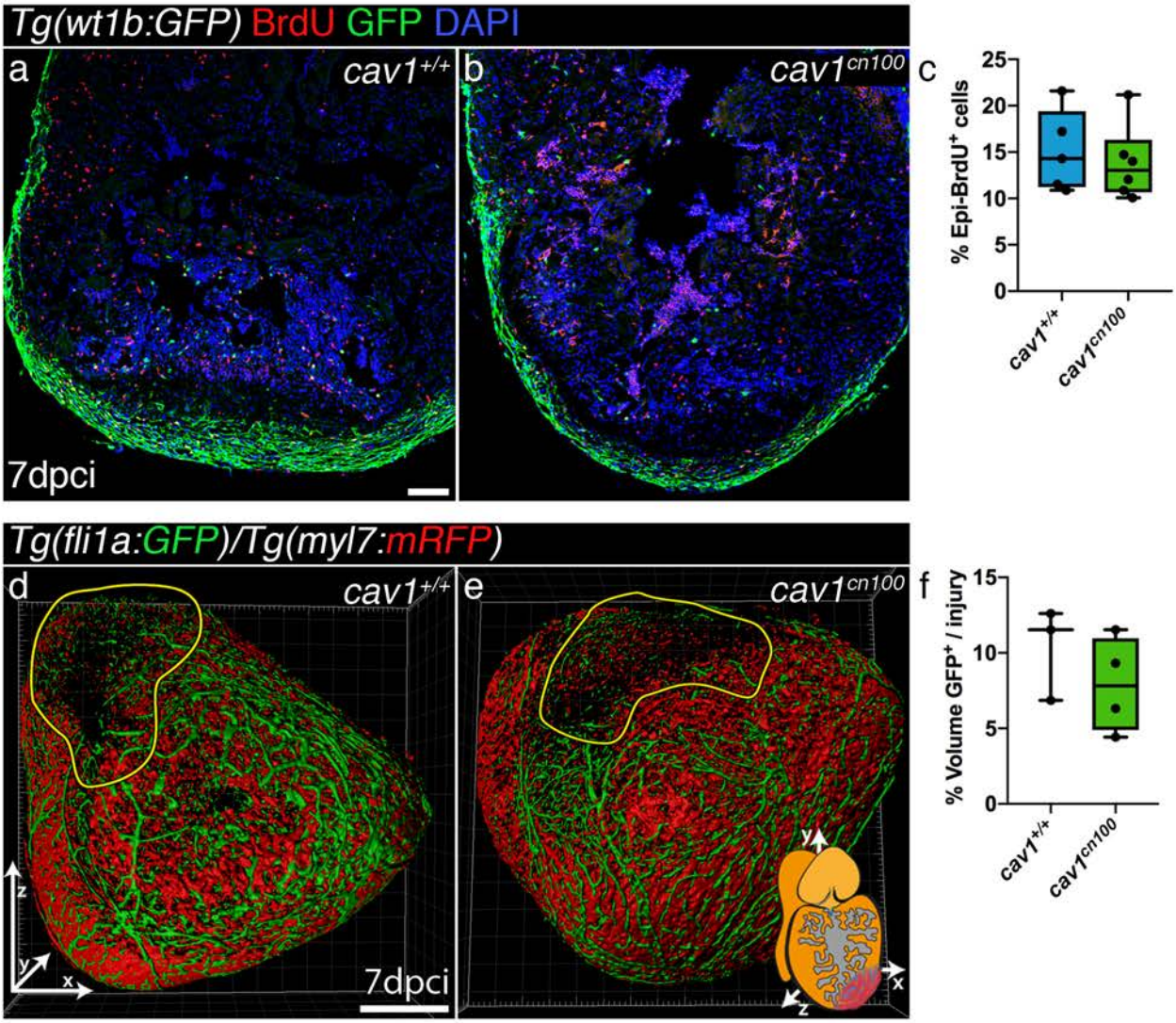
Supplementary Figure S8. Epicardial proliferation and endocardial cell function in *cav1*^{cn100} hearts after injury

(a, b) Immunostaining of 7 dpci *Tg(wt1b:GFP)* heart sections labelled for BrdU and GFP.

(c) Percentage of proliferating epicardial GFP^{+} cells. t-test, $n_{WT} = 5$, $n_{cn100} = 6$. Scale bar 100 μm.

(d, e) 3D volume rendering of the apical injured site of 7dpci *Tg(fli1a:GFP)/Tg(myl7:mRFP)* hearts. Yellow lines indicate the injured area and heart cartoon the x/y/z axes.

(f) Quantification of the volume of GFP^{+} cells inside the RFP^{-} area. t-test, $n_{WT} = 3$, $n_{cn100} = 4$. Scale bar 300 μm.


Tg(wt1b:GFP) BrdU GFP DAPI
a
cav1+/+
b
cav1cn100
7dpci
c
% Epi-BrdU+ cells
25
20
15
10
5
0
cav1+/+
cav1cn100
Tg(fli1a:GFP)/Tg(myl7:mRFP)
d
cav1+/+
e
cav1cn100
z
y
x
7dpci
y
x
z
f
% Volume GFP+ / injury
15
10
5
0
cav1+/+
cav1cn100

Supplementary Figure S9. *cav1*^{cn101} cardiomyocyte proliferation upon injury

(a, b) Immunolabelling of 7 dpci *cav1*$^{+/+}$ and *cav1*^{cn101} hearts for BrdU, MEF2 and MF20. (a′, b′) Higher magnifications of the dashed areas in a and b. Scale bars: 100 μm in a, b; 10 μm in a′, b′.

(c) Percentage of the BrdU^{+} cardiomyocytes to the total number of the cardiomyocytes in a 100 μm area surrounding the damaged tissue. CM, cardiomyocytes. $n_{WT} = 4$, $n_{cn100} = 3$. t-test, **P*<0.05.

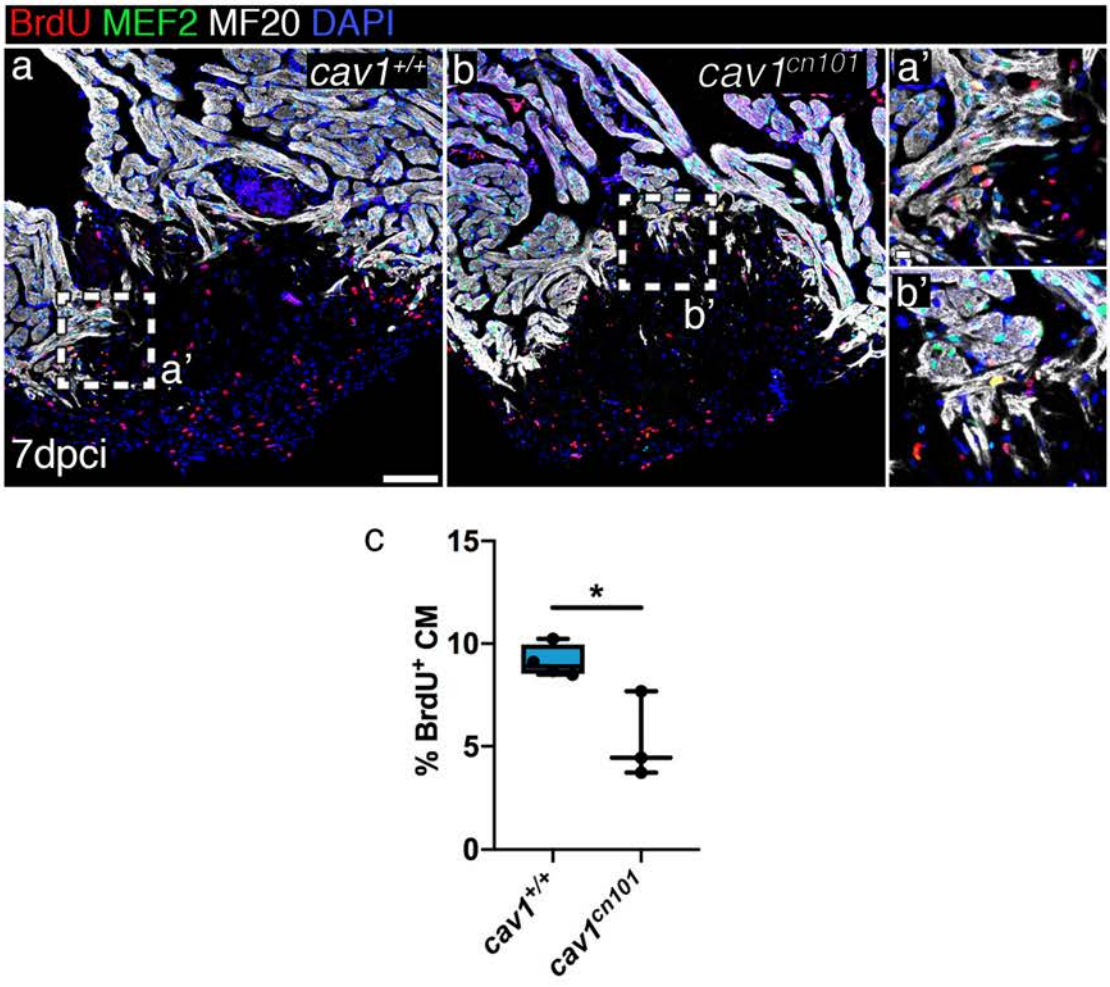


Figure S9_Grivas et al.