

Supplementary Material

Cardiomyocyte proliferation and heart regeneration in adult *Xenopus tropicalis* evidenced by a transgenic reporter line

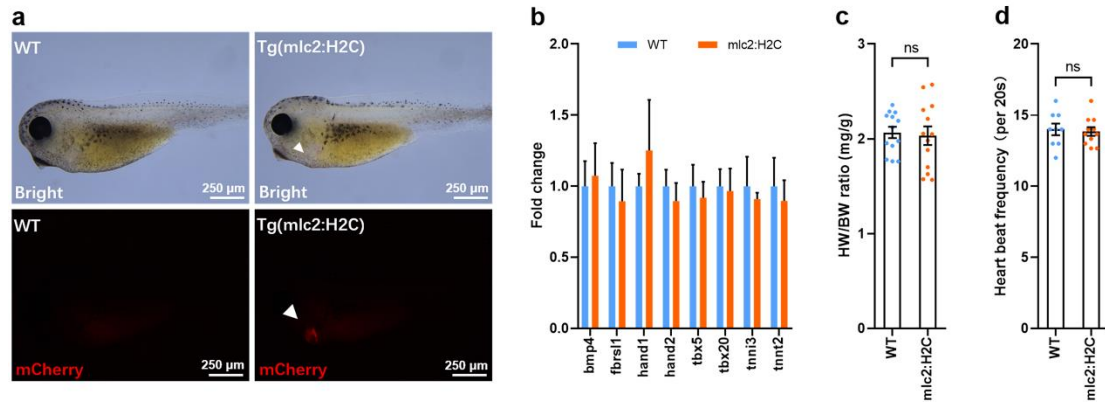
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Supplementary figures 1-4

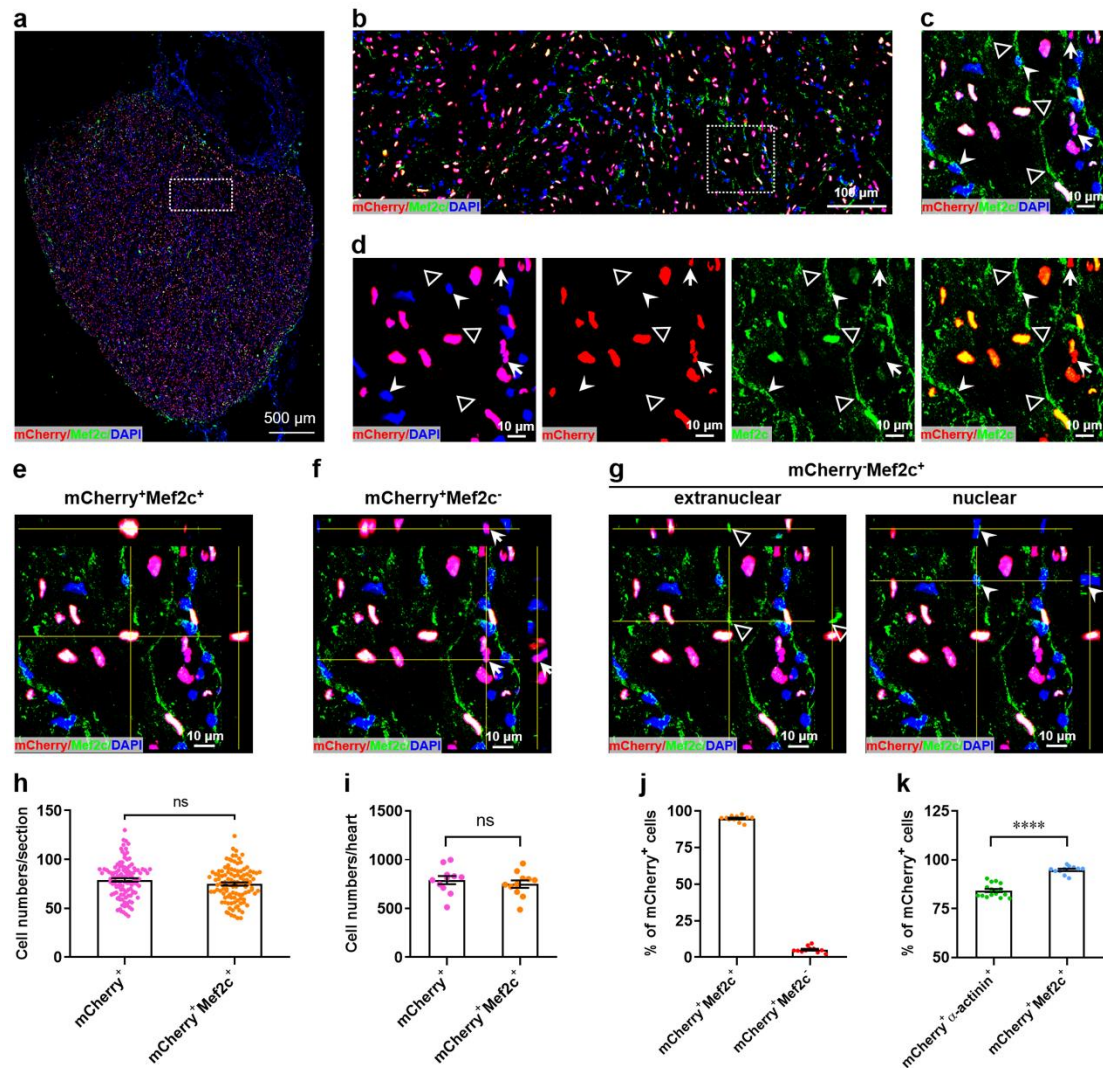
Supplementary table 1

Supplementary video legends 1-2

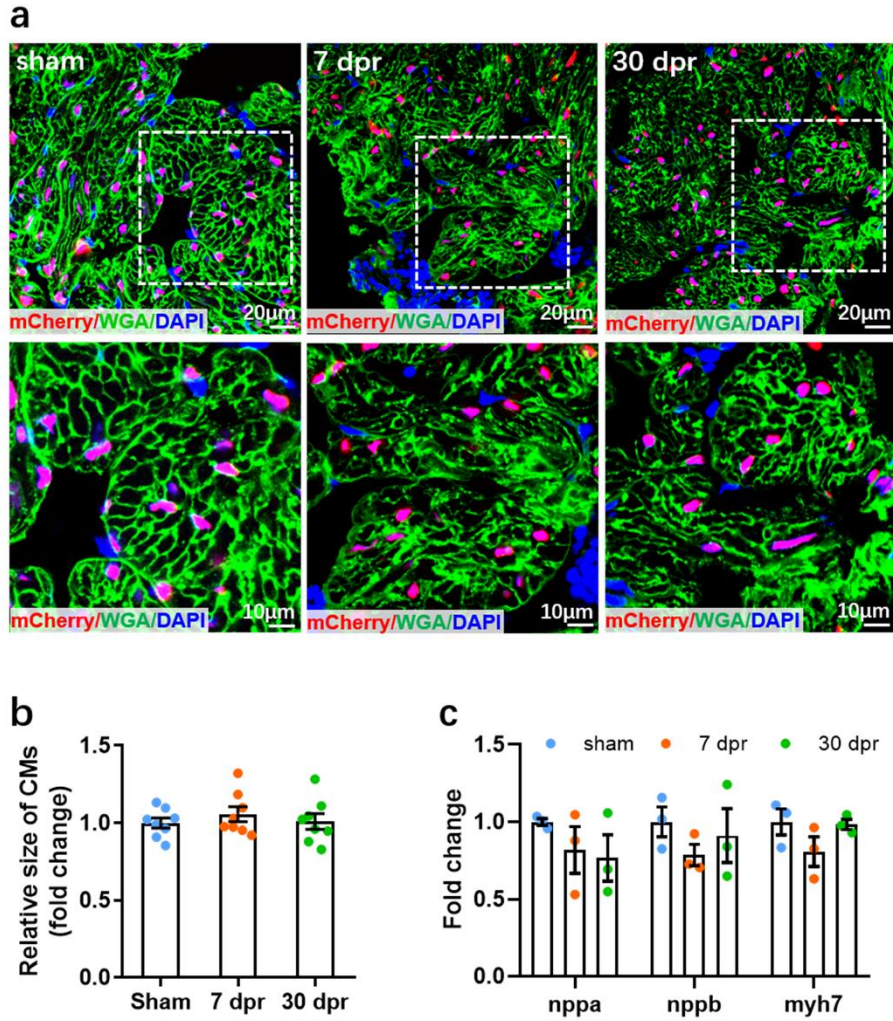
Supplementary figure and legend



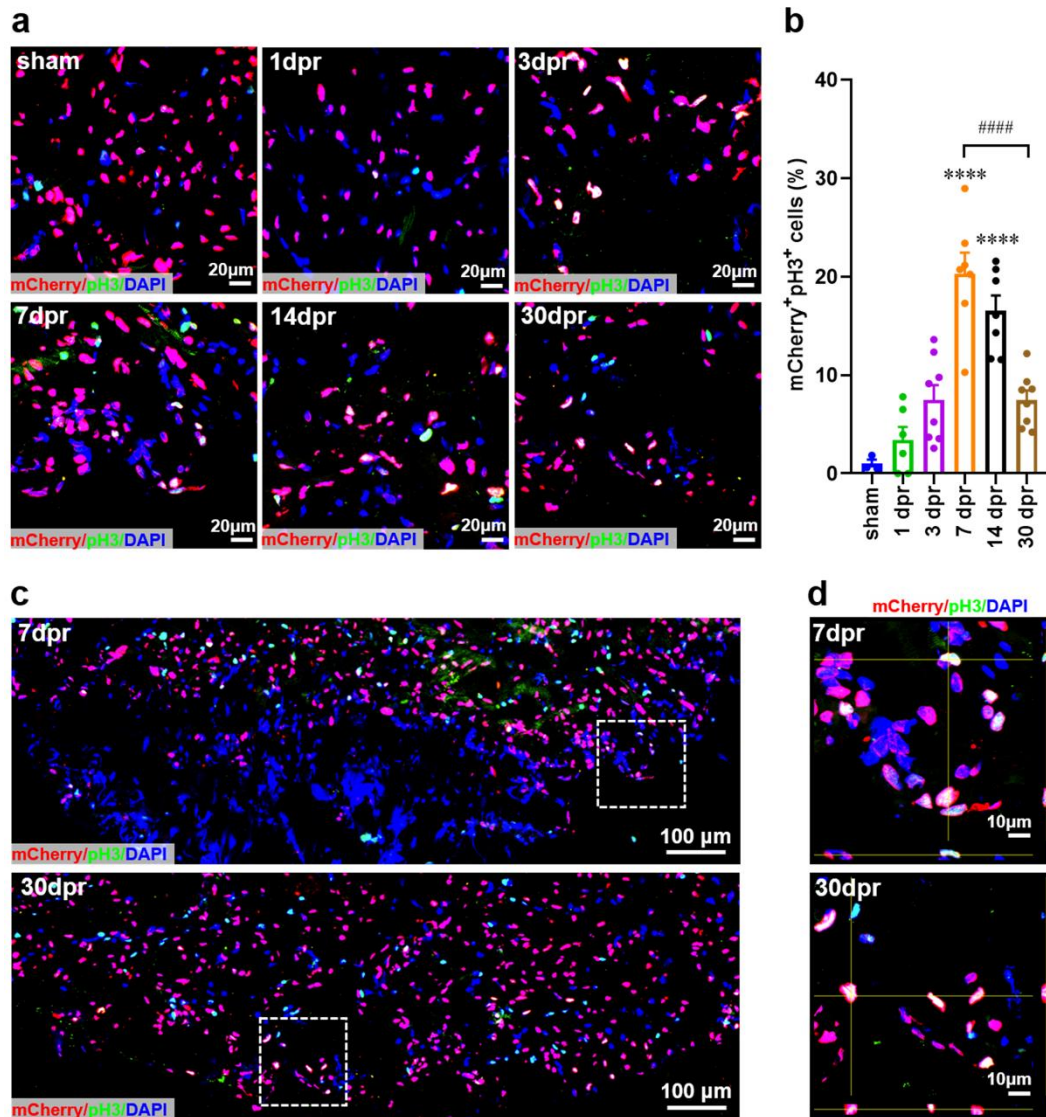
Supplementary Figure 1. Effects of reporter gene on heart development and function in transgenic reporter line *Tg(mlc2:H2C)*. **a** Whole-mount bright-field (upper) and epifluorescence (lower) images showing mCherry expression in Wild-type (WT, left) and F1 *Tg(mlc2:H2C)* (right) tadpoles. Arrows indicate heart expressing mCherry. **b** qPCR validation of heart development-related genes in WT and *Tg(mlc2:H2C)* tadpoles. Data are presented as mean \pm SEM ($n = 4$ independent experiments). Ns, no significant difference (one-way ANOVA test). **c** Quantification of HW/BW ratio in adult frogs. Data are presented as mean \pm SEM ($n = 13$ frogs). Ns, no significant difference (Student's *t* test). **d** Quantification of cardiac systolic function in adult frogs. Data are presented as mean \pm SEM ($n = 9 \sim 11$ frogs). Ns, no significant difference (Student's *t* test). This figure was created by Photoshop Image 12 software using our own data in the work.



Supplementary Figure 2. Accuracy evaluation of nuclear staining of cardiomyocytes in adult *X. tropicalis*. **a** Immunostaining for mCherry (red) and Mef2c (green) expression in a whole cardiac section from the adult heart of F1 *Tg(mlc2:H2C)* frog. DAPI was used as a nuclear stain (blue). **b** Magnified immunostaining image of the non-apical region white boxed in the whole cardiac section. **c** Magnified immunostaining image of the white boxed region in figure B showing that most mCherry-positive nuclei are co-labeled by Mef2c (mCherry⁺Mef2c⁺). Arrow denotes the mCherry⁺Mef2c⁻ cells. Arrowhead denotes mCherry⁻Mef2c⁺ cells with intranuclear expression of Mef2c. Triangle denotes mCherry⁻Mef2c⁺ cells with extranuclear expression of Mef2c. **d** Single- and double-channel fluorescence images of figure c for mCherry/DAPI (pink), mCherry (red), Mef2c (green), and mCherry/Mef2c (yellow) expression. **e-g** Representative Z-stack confocal images of mCherry⁺Mef2c⁺ (e), mCherry⁺Mef2c⁻ (f), and mCherry⁻Mef2c⁺ (g) cells in figure c. **h** and **i** Quantification of mCherry⁺ and mCherry⁺Mef2c⁺ cell numbers in adult hearts of F1 *Tg(mlc2:H2C)* frogs. Data are presented as mean ± SEM ($n = \sim 150$ sections from 11 hearts for h, $n = 11$ hearts for i). Ns, no significant difference (Student's *t* test). **j** Quantification of mCherry⁺Mef2c⁺ and mCherry⁺Mef2c⁻ cells percentages in adult hearts of F1 *Tg(mlc2:H2C)* frogs. Data are presented as mean ± SEM ($n = 11$ hearts). **k** Quantification and comparison of mCherry⁺ α -actinin⁺ and mCherry⁺Mef2c⁺ cells percentages in adult hearts of F1 *Tg(mlc2:H2C)* frogs. Data are presented as mean ± SEM ($n = 16$ and 11 hearts, respectively). **** $p < 0.0001$ (Student's *t* test).



Supplementary Figure 3. Evaluation of cardiomyocyte hypertrophy in adult reporter line during heart regeneration. **a** and **b** Representative WGA staining images (**a**) and quantification (**b**) of cardiomyocyte size located in the ventricular apex at the indicated time points. Data are presented as mean \pm SEM ($n = 8$ hearts, one-way ANOVA test). **c** qPCR validation of hypertrophic markers including *nppa*, *nppb*, and *myh7* in ventricles at the indicated time points. Data are presented as mean \pm SEM ($n = 3$ hearts, two-way ANOVA test).



Supplementary Figure 4. Determination of cardiomyocyte proliferation in adult *Tg(mlc2:H2C)* transgenic *X. tropicalis* line using antibody against pH3 (CST, #3377). **a** Immunostaining for mCherry (red) and pH3 (green, antibody from CST, #3377) expression in the apex of adult hearts from F1 *Tg(mlc2:H2C)* frogs at the indicated time points after resection. DAPI was used as a nuclear stain (blue). **b** Quantification of mCherry⁺pH3⁺ cells in ventricular apex during heart regeneration within 30 days. Data are presented as mean ± SEM ($n = 3$ hearts for sham, 5 ~ 8 hearts for injured groups). **** $p < 0.0001$ versus sham, ##### $p < 0.0001$ (one-way ANOVA test). **c** Representative images of mCherry⁺pH3⁺ cells in the whole apical regions at 7 and 30 dpr. **d** Magnified Z-stack confocal images of mCherry⁺pH3⁺ cells indicated by dashed line in Figure c are shown.

Supplementary Table 1. Primer sequences for real-time PCR analysis in *Xenopus tropicalis*.

Genes	Primer sequence (5'-3')	
	Forward primer	Reverse primer
<i>nppa</i>	CAGTCCTGCATACAGCTC	AAGCATCGGCAACATCA
<i>nppb</i>	CAGCCCACCCAGTTATGGA	ATTTGTAGCATCTGCGTCC
<i>myh7</i>	TTCATTGACTTCGGCATGGA	AATGAAGTGTCTGTTGCCTT
<i>tbx5</i>	GGGTCAGTAGCACCTCC	TCCAGAACGATAGTAAGGGT
<i>tbx20</i>	GCTATGGGACAAATTCCATG	TTTTCCAGCCACCAACC
<i>tnni3</i>	TGTCCGGCTTGTCCCTA	ACTTTAGCCTCCATGTCTG
<i>tnnt2</i>	AAAAGATCTTACTGAACTGC	G TTCAGCTCGTCTCTTC
<i>hand1</i>	AAGACCCTAAGACTAGCCAC	CCGCACCCCAATATCCTTC
<i>hand2</i>	ACCTGCTGGCCAAGGAC	CCAGACATGTTGCGGCCAA
<i>bmp4</i>	TAACACCGTGAGGAGCTTC	ATAGAGTCTTAGTTCTGCT
<i>fbrsl1</i>	AAAGAAGGACCTTTTGCT	CAATGTCTTTGGAGCGAT
<i>Odc</i>	AGGCCACACTGGCAACTCA	TGCGCTCAGTTCTGGTACTTCA

Supplementary video legends

Supplementary Video 1. Characterization of cardiac expression of mCherry in *Tg(mlc2:H2C)* tadpole. Beating heart of *Tg(mlc2:H2C)* tadpole was captured by Leica M205FA stereo fluorescence microscope in bright- and epifluorescence-field vision. Red fluorescence indicates mCherry expression in heart. Scale bar, 250 μm .

Supplementary Video 2. Characterization of nuclear expression of mCherry in cardiomyocytes of *Tg(mlc2:H2C)* tadpole. Beating heart of *Tg(mlc2:H2C)* tadpole was captured by the SpinSR10 spinning disk confocal super resolution microscope (Olympus). Red fluorescence indicates nuclear expression of mCherry in cardiomyocytes. Scale bar, 100 μm .