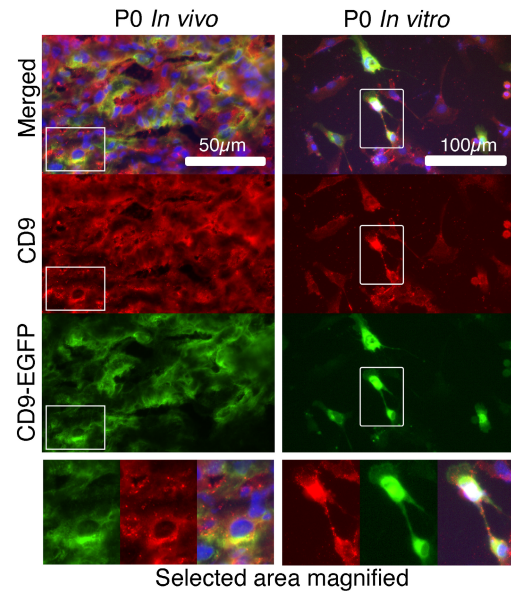
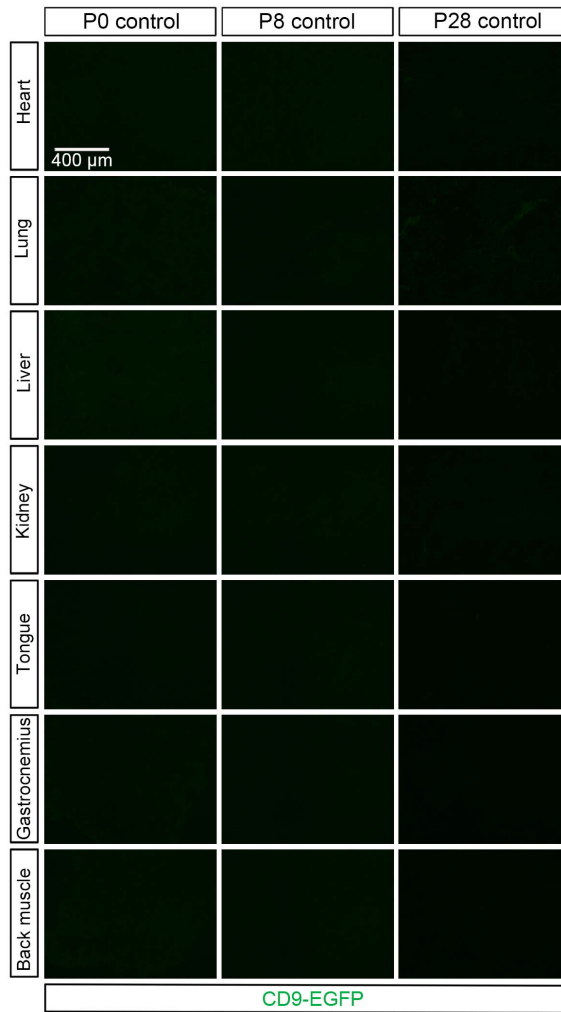


## **Supplemental Information**

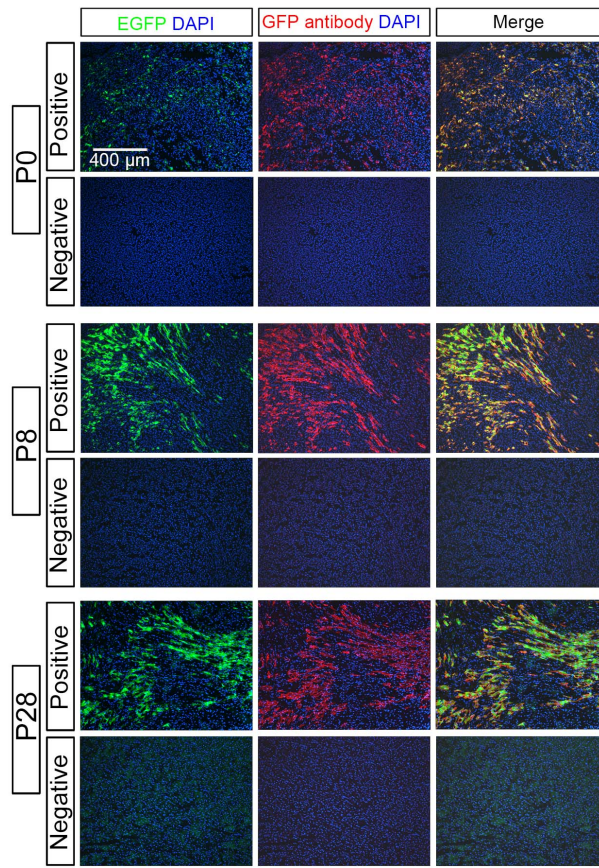
Transfer of cardiomyocyte-derived extracellular vesicles to neighboring cardiac cells  
requires tunneling nanotubes during heart development

Ting Chen et al.



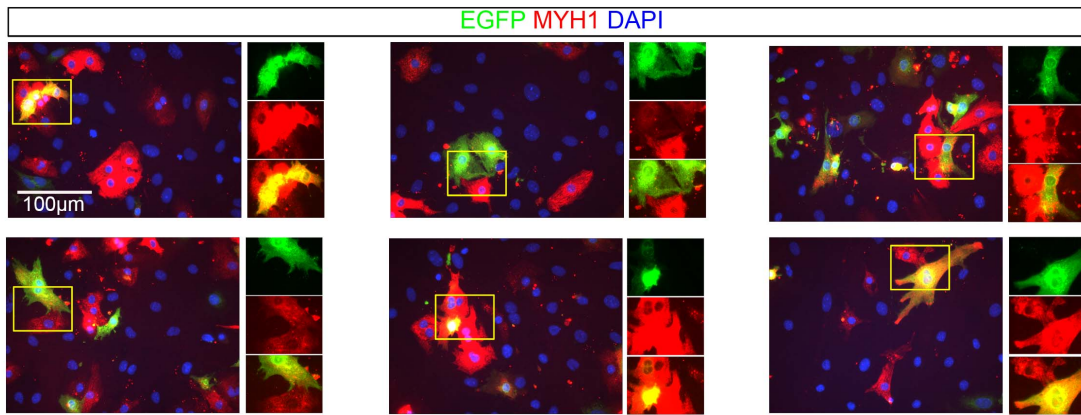
**Figure S1. Direct fluorescence of different organs sections from P0, P8, and P28 *Tnnt2-Cre*; double-floxed inverted *CD9/EGFP*<sup>-</sup> mice and double staining with CD9 and EGFP both *in vitro* and *in vivo*.**

No EGFP signal was detected in different organs sections from P0, P8, and P28 *Tnnt2-Cre*; double-floxed inverted *CD9/EGFP*<sup>-</sup> mice. Double staining with CD9 and EGFP show co-localization both *in vitro* and *in vivo* in *CD9/EGFP*<sup>+</sup> mice.



**Figure S2. GFP antibody was used to amplify EGFP signals.**

Immunofluorescence staining of heart sections from P0, P8, and P28 *Tnnt2-Cre*; double-floxed inverted *CD9/EGFP*<sup>+</sup> and <sup>-</sup> mice. The GFP antibody signals (red) were co-localized with the EGFP signals (green). Positive: *Tnnt2-Cre*; double-floxed inverted *CD9/EGFP*<sup>+</sup> mice, Negative: *Tnnt2-Cre*; double-floxed inverted *CD9/EGFP*<sup>-</sup> mice.



**Figure S3. EGFP-labeled EVs were present in a fraction of cardiomyocytes.**

Immunofluorescence staining of EGFP<sup>+</sup> cardiac cultures for EGFP (green), MYH1 (red), and DAPI (blue) (n = 4).

**Table S1. The pregnant  *$\alpha$ MHC-MerCreMer* mouse exhibited poor outcomes after tamoxifen administration.**

Mouse ID	Methods	Outcome
1	Injecting tamoxifen 50 mg/kg body weight/day to pregnant	Stillbirth
2	mice for 2 consecutive days.	Dystocia
3	Injecting tamoxifen 20 mg/kg body weight/day to pregnant	Dystocia
4	mice for 4 consecutive days from E14-E17.	Dystocia
5		Dystocia

**Table S2. List of genotyping primers used in qPCR-HRM curve analysis.**

Gene	Primer	Sequence
<i>Cre</i>	Cre Trangene F	GCGGTCTGGCAGTAAAAACTATC
	Cre Trangene R	GTGAAACAGCATTGCTGTCACCTT
<i>CD9-EGFP</i>	EGFP F	AAGCTGACCCTGAAGTTCATCTGC
	EGFP R	CTTGTAGTTGCCGTCGTCCTTGAA
<i>Interleukin-2</i>	Internal Ctrl F	CTAGGCCACAGAATTGAAAGATCT
	Internal Ctrl R	GTAGGTGGAAATTCTAGCATCATCC

**Table S3. List of qRT-PCR primers used in this study.**

Gene	Primer	Sequence
<i>CD9-EGFP</i>	PSV 0504	GCAACGTGCTGGTTATTGTG
	PSV 0506	GTCCAATAGCAAGCACTGCA
<i>Beta-actin</i>	Beta-actin F	GCTGTATTCCCCTCCATCGTG
	Beta-actin R	CACGGTTGGCCTTAGGGTTCAG
<i>RPL13A</i>	RPL13A F	CGAAGATGGCGGAGGGGCAG
	RPL13A R	ACCACCACCTCCGGCCCAG

**Table S4. List of antibodies used in this study.**

Antibody	Manufacturer, catalog #	Host species	Dilution
GFP	Abcam (Cat. no.: ab290)	Rabbit	1:500 (IHC, ICC, Flow)
GFP	Abcam (Cat. no.: ab13970)	Chicken	1:500 (IHC)
MYH1	DSHB (Clone MF20)	Mouse	1:200 (IHC, ICC), 1:300 (Flow)
GFP	GeneTex(Cat. no.: GTX113617)	Rabbit	1:1000 (WB)
CD31	BD Pharmingen (Cat. no.: 553370)	Rat	1:50 (IHC)
Vimentin	Abcam (Cat. no.: ab45939)	Rabbit	1:500 (IHC)
Phalloidin	Invitrogen (Cat. no.: A30107)	Amanita phalloides	1:800 (IHC)
Connexin 43	Sigma-Aldrich (Cat. no.: SAB4200730)	Mouse	1:250 (IHC)
Donkey anti-Rabbit IgG, Alexa Fluor 488	Invitrogen (Cat. no.: A-21206)	Donkey	1:200 (IHC, ICC, Flow)
Goat anti-Chicken IgY, Alexa Fluor 488	Invitrogen (Cat. no.: A-11039)	Goat	1:200 (IHC)
Donkey anti-Rabbit IgG, Alexa Fluor 555	Invitrogen (Cat. no.: A-31572)	Donkey	1:200 (IHC)
Donkey anti-Mouse IgG, Alexa Fluor 555	Invitrogen (Cat. no.: A-31570)	Donkey	1:200 (IHC, ICC, Flow)
Goat anti-Rat IgG, Alexa Fluor 555	Invitrogen (Cat. no.: A-21434)	Goat	1:200 (IHC)
Donkey anti-Mouse IgG, Alexa Fluor 647	Invitrogen (Cat. no.: A-31571)	Donkey	1:200 (Flow)
Goat Anti-Rabbit Immunoglobulins/HRP	Dako (Cat. no.: P0448)	Goat	1:2000 (WB)



**Movie S1. Time-lapse microscopic imaging of tunneling nanotubes linking EGFP<sup>+</sup> cardiomyocyte with a recipient cell of EGFP<sup>-</sup> origin.**

EGFP signals appeared along the nanotube and at the junction of the recipient cell.