Supplemental Materials

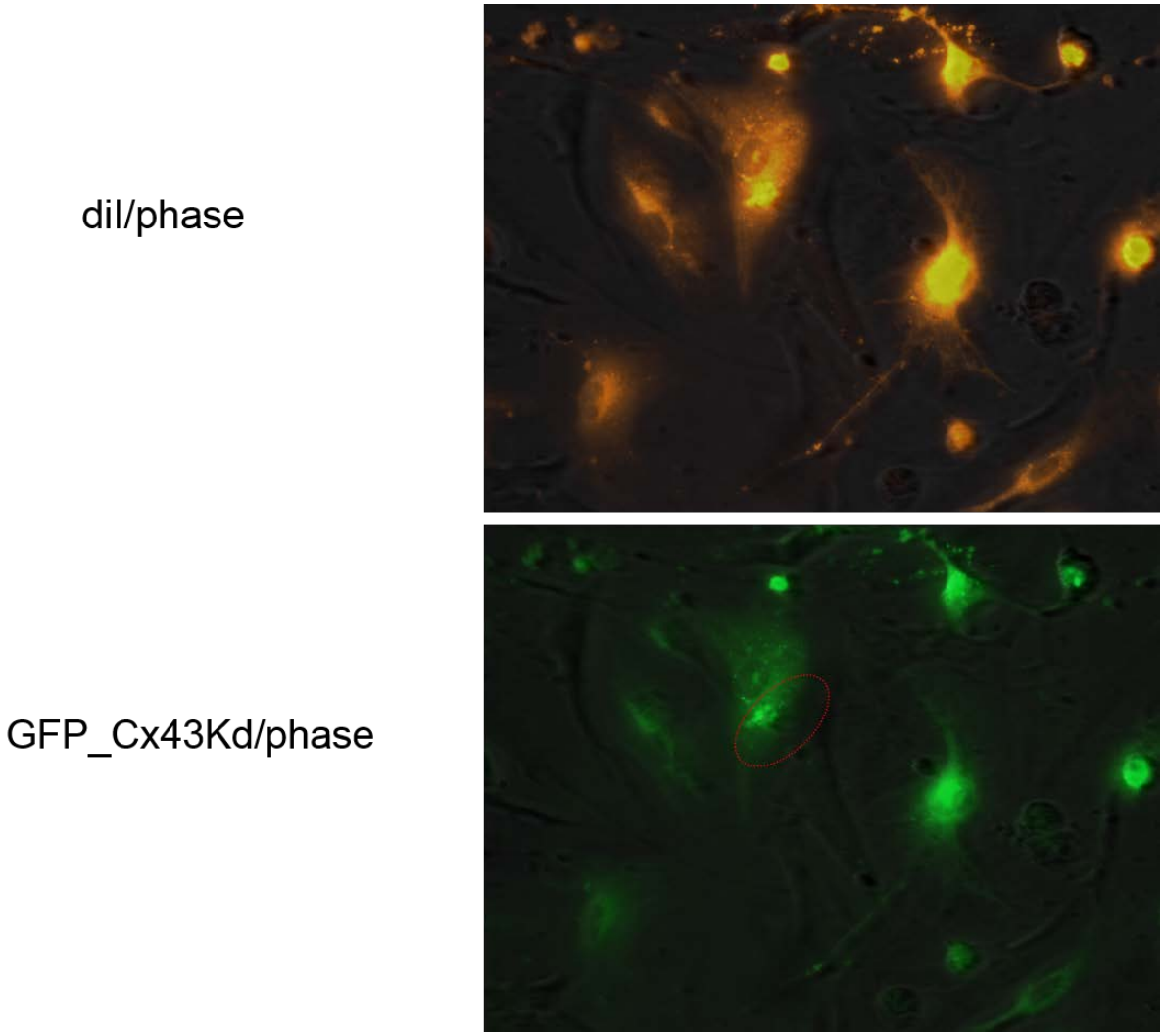
Supplemental Videos

Supplemental Video 1. Representative video showing the MFB–MFB dynamism. Control SICM scans between MFB–MFB. *n* = 20 images, 1.5-h recording.

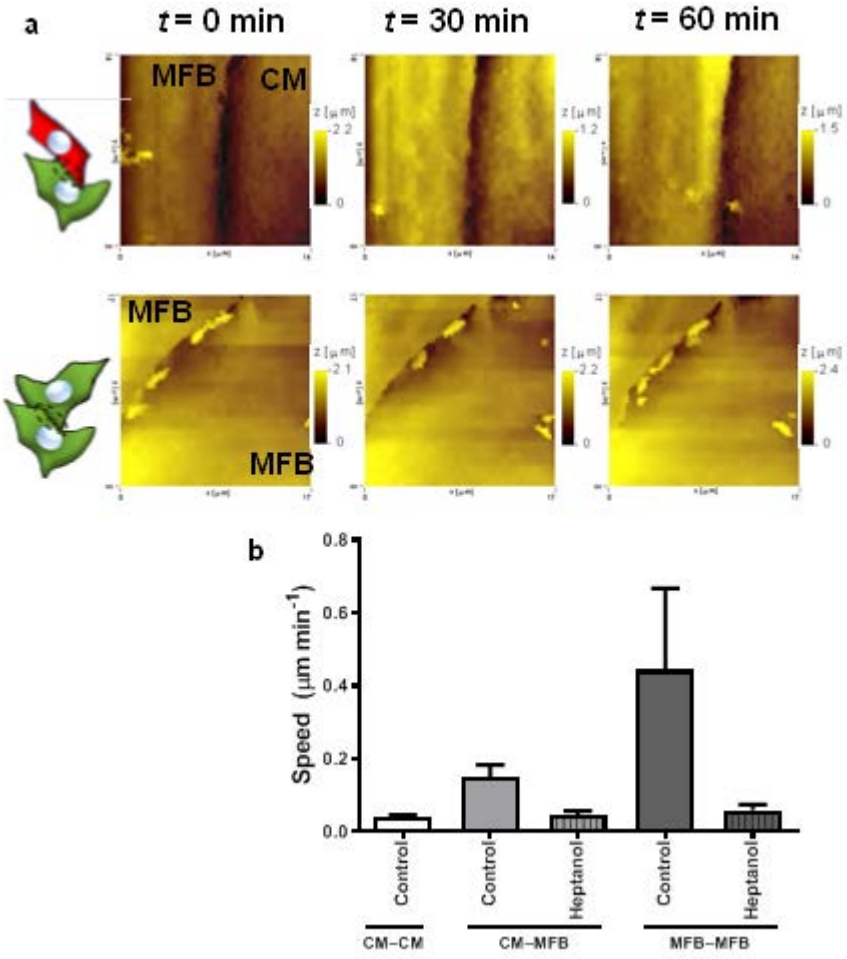
Supplemental Video 2. Representative video showing the CM–MFB dynamism. Control SICM scans between CM–MFB. The cardiomyocyte is the bottom cell (brighter yellow); the myofibroblast is the cell at the top. *n* = 9 images, 1-h recording.

Supplemental Figures

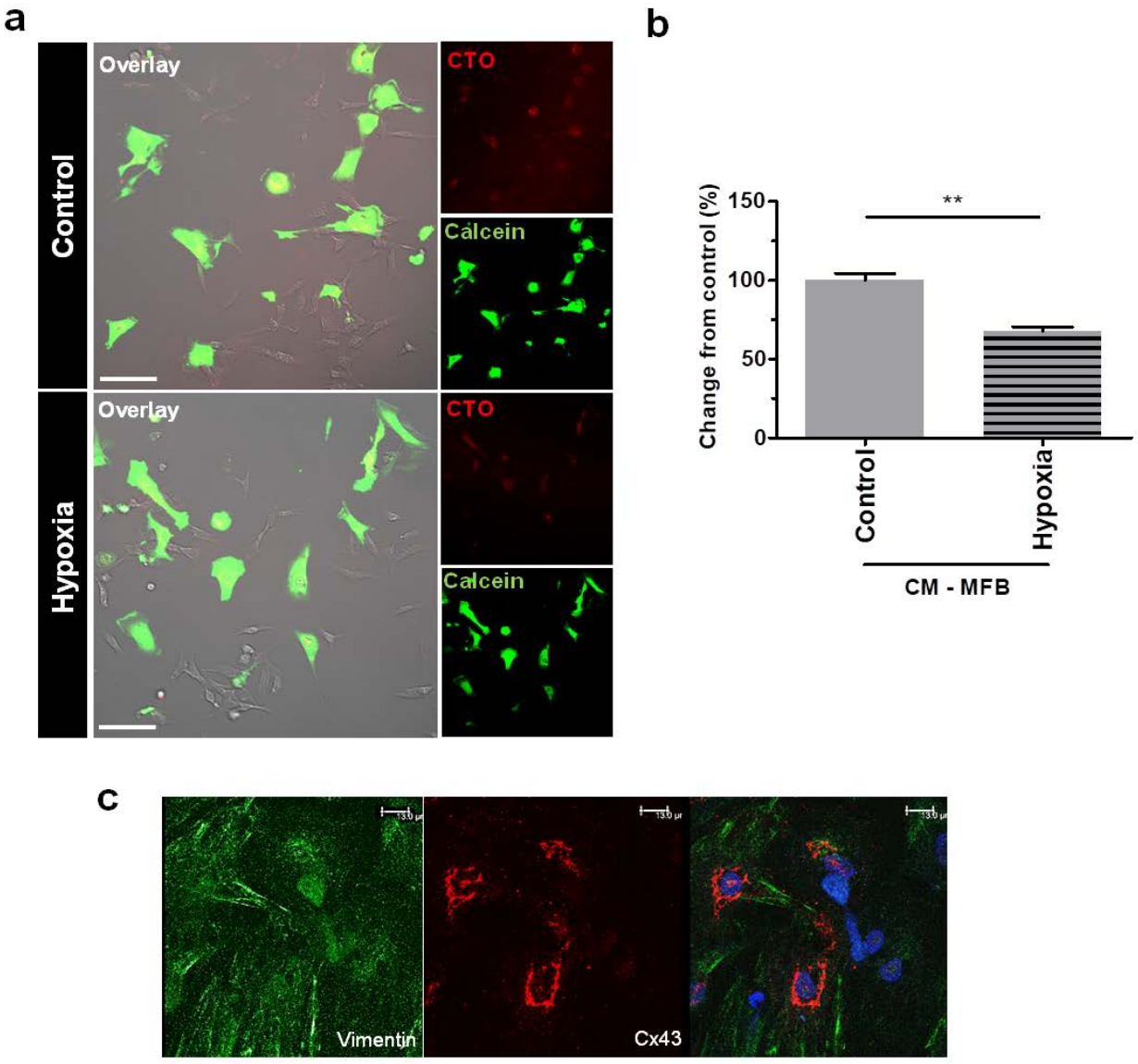
Supplemental Figure 1. GFP–Cx43 knockdown in myofibroblasts. Representative images of myofibroblasts labelled with a lipophilic membrane dye (DiI) and GFP-positive cells. Scale bar, 100 μm.



Supplemental Figure 2. Dynamism in control and heptanol-treated CM–CM, CM–MFB and MFB–MFB contacts. a, Representative set of scans for CM–MFB and MFB–MFB heptanol-treated samples. **b**, Movement speed of the contacts.



Supplemental Figure 3. Hypoxia internalizes Cx43 in myofibroblasts and the effect of hypoxia pre-treatment on neonatal CM–MFB co-cultures. a, Representative live-cell confocal images of parachute assays between neonatal CM–MFB co-cultures under normoxia (control) and after 24-h hypoxia treatment. Scale bars, 10 mm. **b**, Amount of calcein transferred represented as the percentage change from control normoxic conditions for normoxia-treated co-cultures ($n = 32$ images, $n = 3$ isolations) and hypoxia-treated co-cultures ($n = 35$ images, $n = 3$ isolations). $^{**}P < 0.001$; Student's t-test. **c**, Representative images of immunostaining of myofibroblasts. Green, vimentin; red, Cx43; blue, Hoechst. Scale bars, 13 μm.



Supplemental Figure 4. Effect of latrunculin-B treatment on adult rat MFB cultures. a, Representative SICM dynamism scans of control and latrunculin-B-treated MFB–MFB cell pairs from sham-operated adult rats. **b**, Mean speed of contact movement from untreated control (n = 15 scan sets, n = 3 isolations) and latrunculin-B-treated (n = 18 scan sets, n = 3 isolations) MFB–MFB cultures from sham-operated adult rats. Results from myocardial infarction assays are shown for clarity (as shown in Fig. 6g). *P < 0.05; **P < 0.01; Student's t-test. Significant effects of both condition (sham versus myocardial infarction (P = 0.0006)) and treatment (control versus latrunculin-B (P = 0.0364)) on contact dynamism was found (two-way ANOVA), but the interaction between treatment and condition was not significant. **c**, Representative live-cell confocal images of parachute assays between MFB–MFB cultures from sham that were untreated (control) or treated with latrunculin-B for 24 h. Scale bars, 10 mm. **d**, Amount of calcein transferred represented as a percentage of control for untreated cultures (n = 26 images, n = 3 isolations) and latrunculin-B-treated cultures (n = 29 images, n = 3 isolations). ***P < 0.0001; Student's t-test.