Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary Information

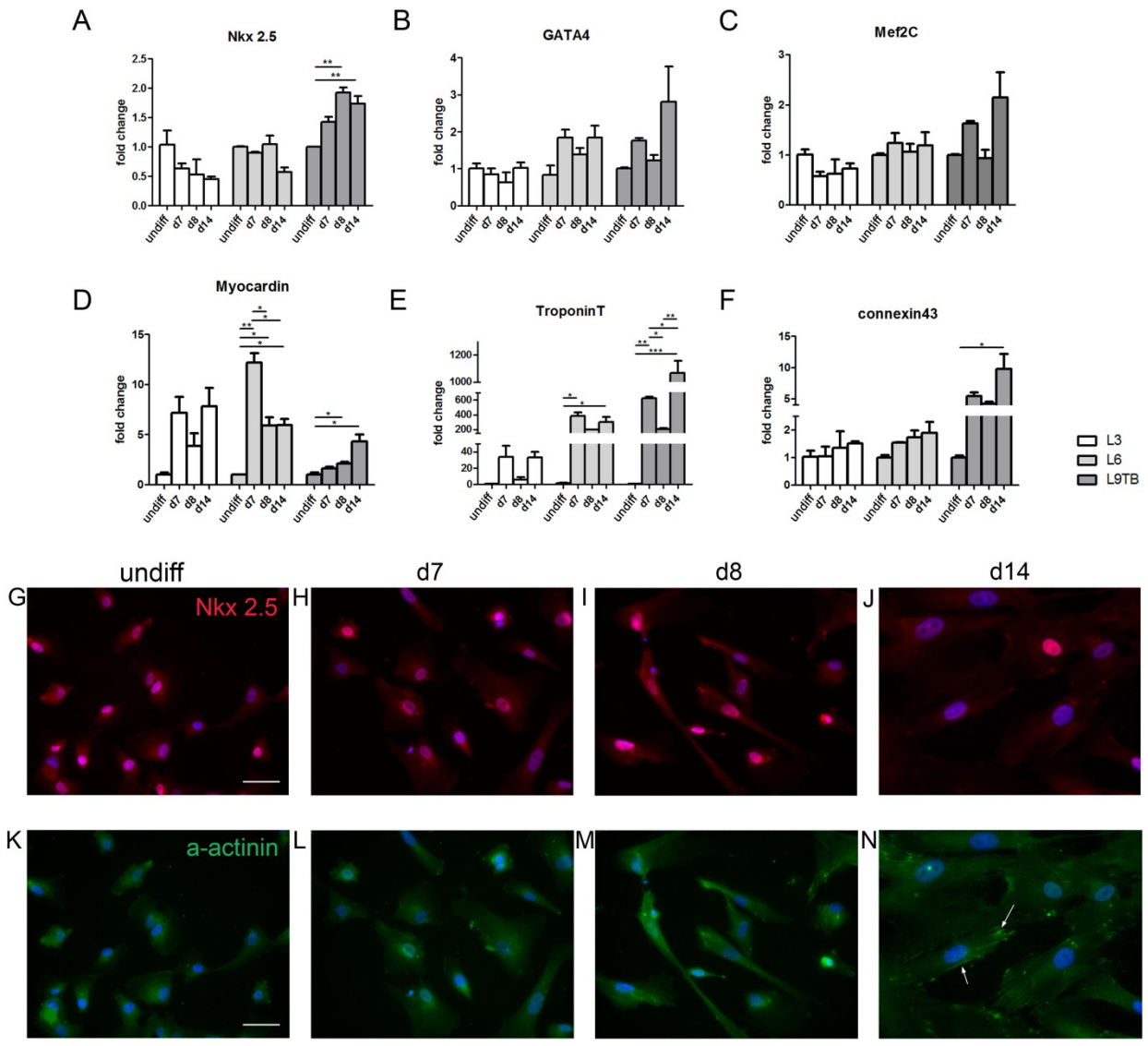
To verify the commitment of CMPCs to the cardiac lineage and their differentiation potential prior to and during straining, gene expression was verified(SI Figure 1).

Differentiation status before strain

The cardiomyogenic potential of three CMPC isolations (L3, L6, L9TB) was assessed by qPCR and immunofluorescent stainings for undifferentiated CMPCs (undiff), and during the early differentiation process on day 7 (just before TGFβ-1 stimulation), day 8 (24h after TGFβ-1 treatment) and one week after starting TGFβ-1 stimulation, on day 14.

Undifferentiated CMPCs showed gene expression of the cardiac transcription factors Nkx2.5, GATA4, Mef2c, and myocardin (SI Figure 1 A-D), as well as the cardiac markers connexin43 and troponinT (SI Figure 1 E, F). At a protein level, Nkx2.5 was also expressed in undifferentiated cells (SI Figure 1 G), and a diffuse cytoplasmic staining of the sarcomeric component α-actinin was observed (SI Figure 1 K).

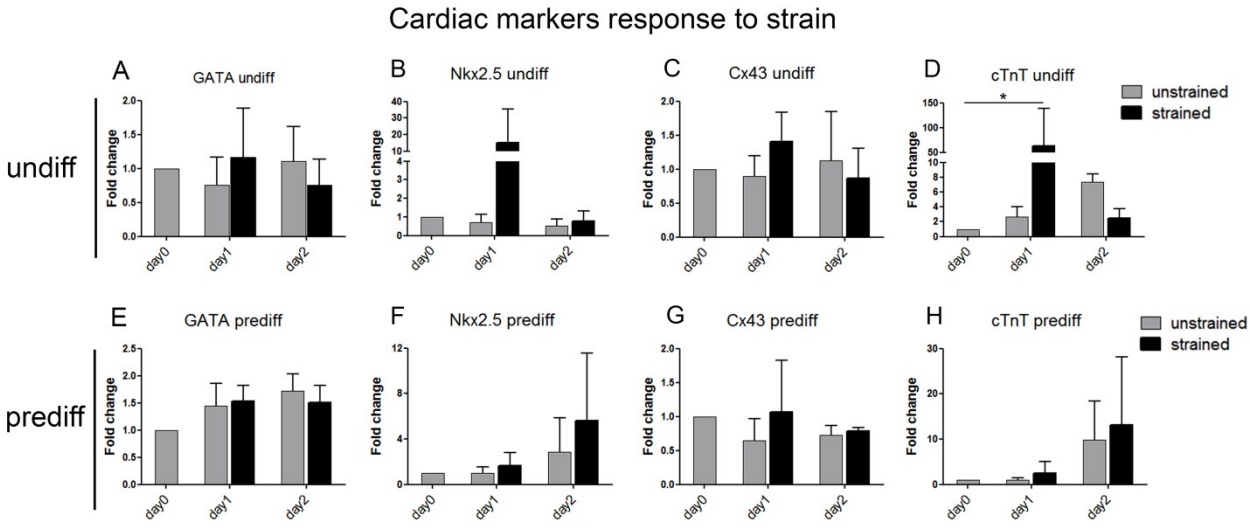
During the first 14 days of the differentiation protocol, increased gene expression of all cardiac markers, and in particular of myocardin, connexin43, and troponinT was observed (SI Figure 1 A-F). On protein level, all three CMPC isolations expressed Nkx2.5 on day 7, day 8 and day 14 (SI Figure 1 G-J). Additionally, the sarcomeric component α-actinin was present although no striations could be observed (SI Figure 1 K-N). On day 14, clusters of α-actinin close to the cell membrane indicate early signs of sarcomeric organization (SI Figure 1 N).



SI Figure 1: Predifferentiated CMPCs are in an early differentiated state. A-F) Expression of cardiac transcription factors Nkx 2.5 (A), GATA4 (B), Mef2c (C), Myocardin (D), cardiac troponin T (E), and connexin 43 (F) in L3, L6, and L9TB CMPCs during the first two weeks of cardiomyogenic differentiation, as determined by qPCR. Data are presented as mean fold change ± SEM, n=2 per cell isolation per time point. *= P<0.05; **= P<0.01; ***= P<0.001. G-N) Immunofluorescent images of the cardiac transcription factor Nkx 2.5 (G-J, red) and the sarcomeric component α-actinin (K-N, green) in L9TB CMPCs during the first two weeks of differentiation. At day14 of differentiation, early signs of sarcomeric organization were observed (N). Nuclei are stained in blue (DAPI). Images are representative for all three cell isolations. Scale bar is 50µm.

Differentiation status after strain

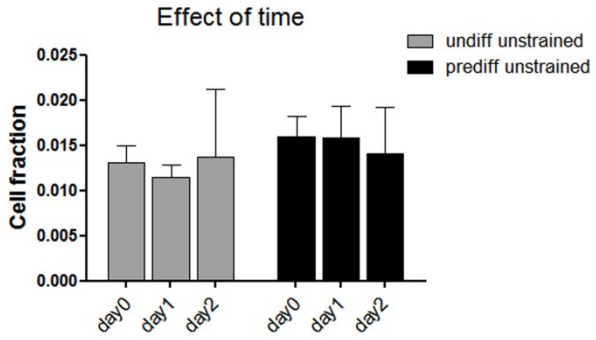
During the straining procedure, the gene expression of cardiac markers appeared stable over time, with no significant difference between strained and unstrained samples in both cell types (SI Figure 2), except for an upregulation of Nkx2.5 and troponinT in strained samples at day1 (SI Figure 2 B, D). The induction of cardiac markers might be attributed to cells that spontaneously start to differentiate, especially in areas characterized by high cell density. These cells might therefore become sensitive to the applied strain, and change their alignment with the stretch. Moreover, due to the very low expression of troponinT in undifferentiated CMPCs, the large increase in the expression of this gene at day1 of strain can be seen as an on/off response, for which the spontaneously differentiated cells might partially be responsible.



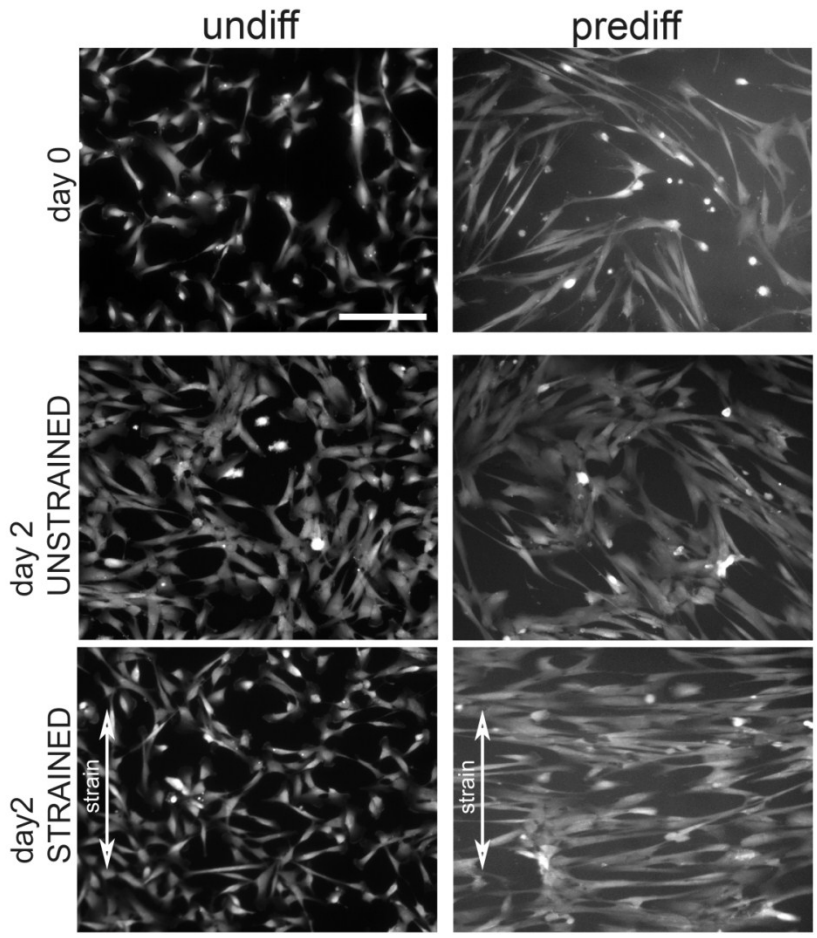
SI Figure 2: Cyclic strain does not affect the cardiac lineage commitment of undifferentiated and predifferentiated CMPCs. A-H) Gene expression of cardiac markers is not affected by the strain; at day1 upregulation of the cardiac transcription factor Nkx2.5 (C, n.s.) and of the cardiomyocyte marker cTnT (G, $P<0.05$) was observed in strained undifferentiated CMPCs (n=3 independent experiments).

Cell reorientation

For the statistical analysis on the cell orientation, only the cell fraction oriented at exactly 90° was considered, and therefore only a small part of the sample is represented. The fraction of unstrained cells oriented at 90° showed similar values at day0, day1 and day2 for both undifferentiated and predifferentiated CMPCs, indicating that the cell alignment does not occur over time in the absence of strain (SI Figure 3).



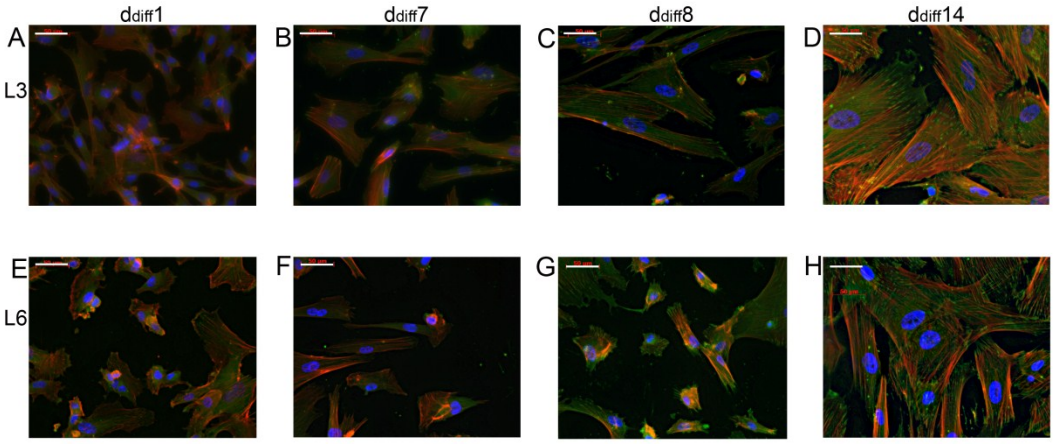
SI Figure 3: The mechanoresponse of CMPCs is not induced over time or by the cyclic strain per se, unless cardiomyogenic differentiation occurs. A) No significant differences are present among unstrained undifferentiated or predifferentiated CMPCs, nor among B) strained undifferentiated or predifferentiated CMPCs over time, indicating that time or cyclic strain alone cannot induce mechanosensitivity in CMPCs. N=16-23 from 3 independent experiments.



SI Figure 4: Fluorescent images (calcein) of undifferentiated (undiff) and predifferentiated (prediff) CMPCs at day0 (before strain) and day2 of cyclic strain. After 2 days of strain, undifferentiated CMPCs show a random orientation, whereas predifferentiated cells dispjay a clear strain avoidance response, by reorienting (nearly) perpendicularly to the strain direction (represented by the arrow). Scale bar is 200 μm.

Mechanosensitivity

The mechanosome development observed in L9TB CMPCs during early cardiac differentiation, was confirmed in not-immortalized cell lines L6 and L9. Immunofluorescent stainings show maturation of FAs and SF production with CMPC differentiation (SI Figure 4).



SI figure 5: The mechanosome is developed with differentiation in L3 and L6 CMPCs. Immunofluoresce stainings of L3 and L6 CMPCs during cardiomyogenic differentiation show absence of vinculin (green) and actin stress fibers (red) in undifferentiated cells ($d_{diff}1$, A, E), and progressive maturation of the mechanosome at $d_{diff}7$ (B, F), $d_{diff}8$ (C, G) and $d_{diff}14$ (D, H). Scale bar 50 µm.