Hippo Pathway Effectors Control Cardiac Progenitor Cell Fate by Acting as Dynamic Sensors of Substrate Mechanics and Nanostructure

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Supporting Information

Supplementary Figures 1-6 provide graphical data supporting the observed results, as mentioned throughout the text in the Results section;

Supplementary Videos 1-2 consist on animations of material preparation and characterization.

**Supplementary figure 1:** YAP expression is hardly detected in the remote area of infarcted myocardium (a), while its activation is triggered in cells at the infarction (MI) border zone within 3, 5 days and persists at least until day 36 after left coronary artery ligation (b). The images are
representative of at least 12 fields. Scale bar: 50 µm. YAP expression is sensitive to cell-to-cell contact in human resident cardiac progenitor cells: hCMPCs show nuclear expression when sparsely grown, while cytoplasmic localization prevails in confluent cells. Cytochalasin D treatment triggers YAP shuttling to the cytoplasm in sparse cells (c). Similar results were obtained with TAZ staining (data not shown). YAP/TAZ expression is independent of substrate coating, as demonstrated by their expression on collagen-coated poly-acrylamide gels displaying different stiffness values (d). The values are consistent with the ones reported for YAP/TAZ expression on fibronectin (see Figure 1d). Poly-ε-caprolactone (PCL) films display thermo-responsive properties. The contact angles of the PCL layers were determined by a sessile drop method 30 s after a water drop was placed on the surface at 25 or 45°C, yielding similar results independently of the temperature (d, left panel). The dependency of substrate stiffness from temperature is shown in the right panel. The yellow area is a guide for the eyes highlighting the slope of the stiffness curve encountered in the temperature range used in the study, between 32 and 37°C (e). Myosin II and Rho/ROCK pathway inhibitors blebbistatin (blebb) and Y27632 determine a modification in human cardiac progenitor cell cytoskeleton assembly, as demonstrated by F-actin staining in bent cells (red, f). This modification affects YAP nuclear localization and can be reverted by removing the inhibitors from the culture medium. The percentage of cells expressing nuclear YAP is enhanced after 6 hours washout (g). *: P < 0.01 between samples treated with the inhibitors as compared to non treated or the washout samples.
**Supplementary figure 2:** Murine cardiac progenitor cells (mCPCs, stained for Stem Cell Antigen 1 (Sca-1) marker in green, a) and neonatal cardiomyocytes (identified by cardiac Troponin T expression, green, b) display YAP/TAZ nuclear expression. In murine CPCs, the expression of YAP and TAZ can co-localize with early markers of commitment, like GATA-4 and TBX-5 (a). The re-localization of YAP and TAZ starts as early as the cells get in contact with the substrate, within 3 and 5 hours, as shown in human cardiac progenitor cells (hCMPCs, c).
Supplementary figure 3: YAP/TAZ intracellular localization in human cardiac progenitors (hCMPCs) is determined as early as the cells get in contact with the substrate (3-5 hours) on fibronectin-coated micropatterned islands (a). Similarly to what happens with human cells, TAZ nuclear localization in murine cardiac progenitor cells (mCPCs) is regulated by cell confinement (F-actin stained in red, b), being mostly expressed in the cytoplasm of cells grown onto fibronectin-coated islands not allowing cell spreading (300, 1024 μm²). Scale bar: 40 μm. Panel (c) shows the quantification of nuclear YAP/TAZ expression in human and murine cardiac
progenitor cells. *: P<0.05, between 300 μm² and other islands; **: P<0.05, between 1024 μm² and other islands. n=3. No specific effect on hCMPC cardiac differentiation could be ascribed to cell confinement, as demonstrated by the absence of sarcomeres in hCMPCs with diffuse basal expression of sarcomeric actinin (d). Similar results were obtained by using a Collagen coating.
**Supplementary figure 4:** Transient silencing of YAP (siYAP) and TAZ (siTAZ) expression can be achieved in human cells. Similar results can be obtained in murine cardiac progenitors (mCPCs) by stable silencing (shYAP) technique (a). The effectiveness of YAP/TAZ silencing is confirmed by immunofluorescence (b) and by the significant reduction in connective tissue growth factor (CTGF) expression, an early effector of YAP and TAZ activation (b). Scale bar: 25 µm. Panel (c) shows an example of a photo-activated patterned surface for single cell
migration before UV light irradiation. 2nd Ab: immunofluorescence control performed only with secondary antibody.

**Supplementary figure 5:** YAP silencing enhances the expression of ki67 proliferation marker in human cardiac progenitor cells, as shown in the representative immunofluorescence picture (a). When grown onto fibronectin-coated poly-acrylamide gels having cardiac-like stiffness (10 kPa), hCMPCs in which Green Fluorescent Protein (GFP) is cloned under the cardiac specific
promoter Troponin T (cTnT-GFP) show a selective activation of cardiac-specific Troponin T (cTnT) gene. Clear differences in cell morphology (i.e. 10 kPa vs 0.5 kPa) can be noticed after 12, 24, 48, 72 h (b), with cells grown onto soft (E = 0.5 kPa) substrates being rounded while acquiring elongated morphology on stiffer substrates (E = 10, 40 kPa).

**Supplementary figure 6:** YAP expression is exclusively detected in the cytoplasm of hCMPCs branching in 3D Matrigel™ (E < 1 kPa). A typical tubular structure formed by the cells
is shown in (a). Nuclear transport inhibitor Leptomycin triggers an increase in YAP/TAZ nuclear expression in confluent cardiac progenitor cells (b). *P<0.05 as compared to 0.2, 2 and 20 nM Leptomycin.

**Supplementary video 1:** Photo-activated micropatterned surfaces reveal the involvement of YAP/TAZ in 2D cell migration. 900 µm² rounded spots allow hCMPC adhesion but not spreading. When the surfaces are exposed to UV irradiation (UV flood exposure), the cells are induced to migrate out of the islands. Significant differences in cell migration capacity can be noticed between controls and YAP silenced (YAP-/-) cells.

**Supplementary video 2:** Thermo-responsive PCL cross-linked surfaces display shape-memory effect. AFM time-lapse reconstruction of substrate nanotopography modification as a function of temperature, between 25 and 37°C.