A Novel Fluorescent Reporter System Identifies Laminin-511/521 as Potent

Regulators of Cardiomyocyte Maturation

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- 1 **Supplementary Figure S1.** Localizations of Myom2 and cTnT in sarcomere
- 2 structure. (a) Representative fluorescence images of RFP⁺ cardiomyocyte. Yellow
- 3 lines indicate line-scan regions shown in (b). The regions around the lines are shown
- 4 in inset. Myom2-RFP (red); cTnT (green); Nuclei (blue). Scale bar, 20 μm. (b)
- 5 Corresponding line scans for Myom2-RFP and cTnT localizations.



6 Supplementary Figure S2. Comparison of sarcomere alignment between RFP⁻ and RFP⁺ cardiomyocytes. (a) The color-coded representations of the α -actinin 7 8 orientation in both RFP⁻ and RFP⁺ cardiomyocytes. Scale bar, 20 µm. (b) The 9 corresponding distributions of orientation shown in (a). (c) From the orientation distribution, sarcomere alignment index was calculated by sum of frequencies within 10 20° from peak angles at -90° and 90° (n, blue area) against total distribution 11 frequency $(n+\omega)$. Then, this ratio was normalized to that of an ideally random 12 frequency distribution $(\eta_{IR}/(\eta_{IR} + \omega_{IR}))$, ~0.22. (d) RFP⁺ cardiomyocytes showed 13 higher sarcomere alignment index than RFP⁻ cardiomyocytes (n > 65, from three 14 different cardiac differentiation runs). Violin plots are explained in Fig. 2. Student's t 15 test; † *P* < 0.0001. 16



17 Supplementary Figure S3. A heatmap of ECM expressions during heart

- 19 transcripts per million reads (TPM) divided by the highest expression level
- 20 (TPM/max: high expression, red; low expression, blue).

¹⁸ development (E11 to P56). Colors are coded on the red-to-blue scale of normalized



Supplementary Figure S4. ECMs increased RFP intensity as a dose-dependent manner. (a) Percentages of Myom2-RFP⁺ cardiomyocyte and (b) RFP intensity at day 17, 24, and 38. ECM concentration are shown at 0.125, 0.5, and 1.0 μ g/cm². Data are presented as means ± SD (*n* = 3). Fluorescence intensities are presented as arbitrary units (a.u.).