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Defined composition of culture media promotes rodent neonatal cardiomyocyte maturation and enables functional neuro-cardiac co-culture

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Supplementary information

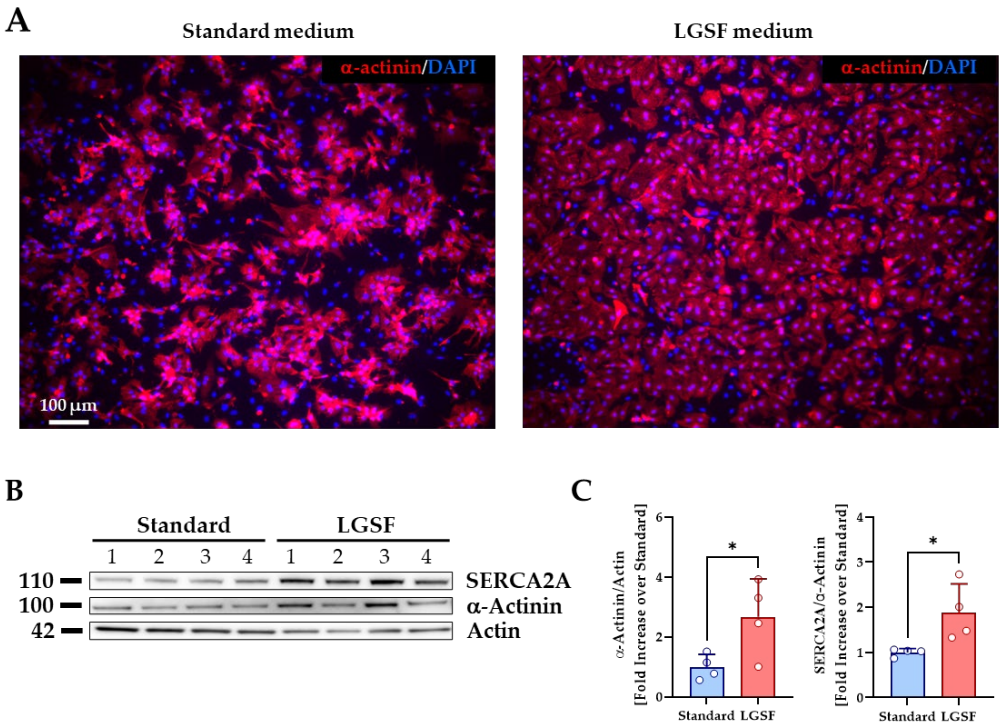
-Supplementary Figure Legends 1-3

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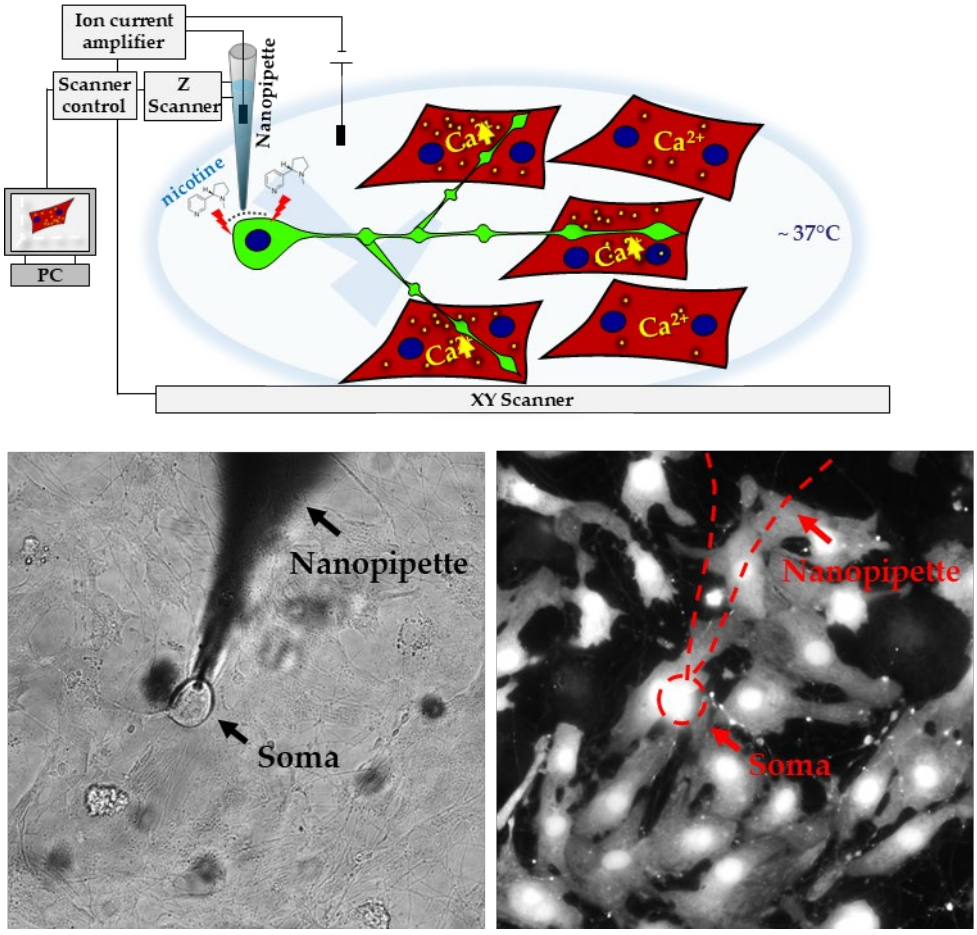
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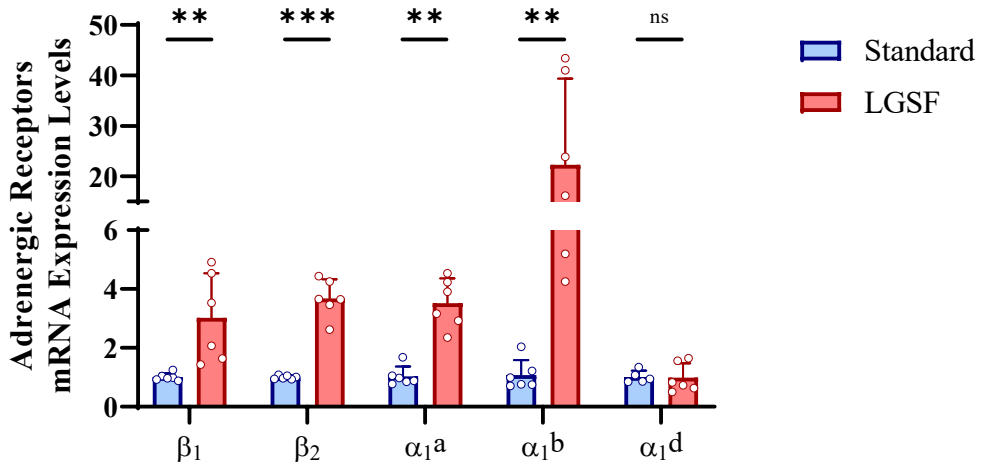
Supplementary Figure S1. LGSF medium increases culture purity of neonatal rat ventricular cardiomyocytes.

(A) Immunofluorescence staining of CM cultured in Standard (left) or Low-Glucose Serum-Free (LGSF) medium (right), showing α-actinin$^+$ CM (red) and 4',6-diamidino-2-phenylindole (DAPI) -stained nuclei (blue). Reduced fibroblast contamination is observed under LGSF conditions. **(B)** Western blot analysis of α-actinin and SERCA2A expression in protein lysates from CM cultured in Standard or LGSF medium. Actin was used as a loading control. **(C)** Densitometric quantification of α-actinin expression relative to Actin, and SERCA2A expression relative to α-actinin. Statistical analysis: unpaired t-test; *, $p \leq 0.05$; $n = 4$ independent samples per group.



Supplementary Figure S2. Scanning Ion Conductance Microscopy (SICM) setup for local neuronal stimulation.

Schematic overview of the SICM-based stimulation protocol. After identifying the neuronal soma, a glass nanopipette (30 MΩ) is positioned above the cell. Nicotine is locally delivered via combined pressure and voltage pulses, while Ca^{2+} transients are simultaneously recorded in Fluo-4–loaded CM paced at 0.5 Hz within the same field of view.



Supplementary Figure S3. LGSF medium modulates β-adrenergic receptor expression in neonatal cardiomyocytes.

RT-qPCR analysis of adrenergic receptor mRNA levels in CM cultured under Standard

(blue) or LGSF (red) conditions. Data are expressed as mean ± SD from n = 6 independent samples per group. Statistical analysis: unpaired t-test (with Welch's correction when required) or Mann–Whitney test; **, $p \leq 0.01$; ***, $p \leq 0.001$.