Cell Reports, Volume *43*

Supplemental information

Independent compartmentalization of functional, metabolic, and transcriptional maturation of hiPSC-derived cardiomyocytes

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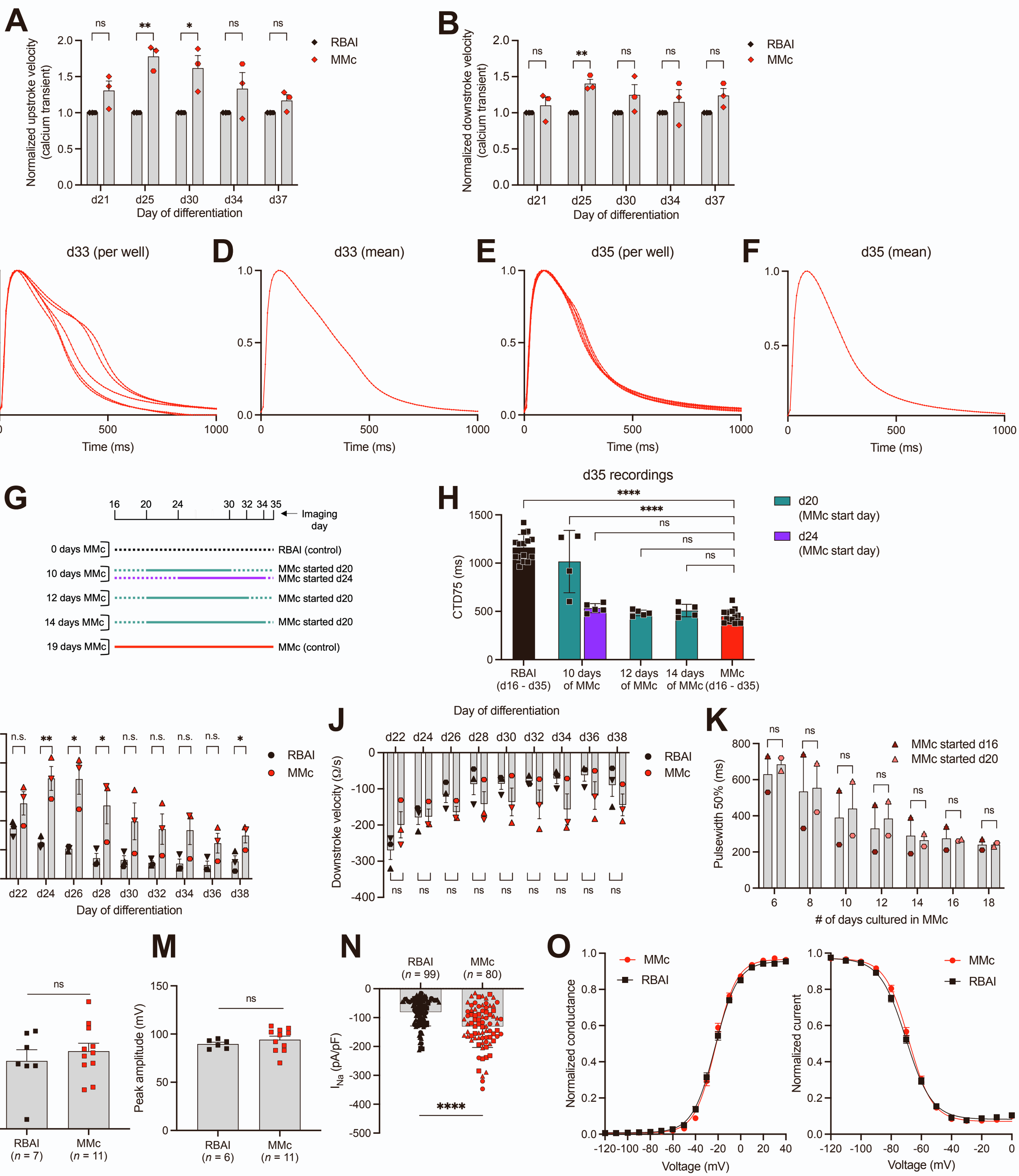

Figure S1: High-throughput assays detect changes in maturation status of hiPSC-CMs. (A) Maximum upstroke velocity and (B) downstroke velocity of high-throughput Ca^{2+} transient recordings (n = 3). (C-F) Representative spontaneous Ca^{2+} transient recordings of hiPSC-CMs in MMc. Each trace represents the average trace from a single well from one experiment on d33 (C) or d35 (E) or the average of all the wells in one experiment on d33 (D) or d35 (F). (G) Schematic of experimental design. Dotted lines indicate RBAI cultured cells and solid lines indicate MMc cultured cells. RBAI control (black) and MMc control (red) is d16- d35 in RBAI or MMc, respectively. Cells cultured in MMc for either 10, 12, or 14 days starting at d20 (green) or d24 (purple). Ca^{2+} transients for all conditions recorded on d35. (H) CTD75 of Ca^{2+} transients recorded on d35 (n = 1). (I) Maximum upstroke velocity and (J) downstroke velocity of high-throughput impedance recordings (n = 3). (K). Pulsewidth 50% of hiPSC-CMs cultured in MMc starting at d16 or d20. (L) Action potential duration at 90% (APD_{90}) and (M) peak amplitude measured in hiPSC-CMs paced at 1 Hz using manual patch clamp (n > 3). (N) Peak I_{Nav} density at 0 mV. (O) I_{Nav} voltage-dependance of activation and inactivation. n = experimental replicates, unpaired Student's T-test, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.005$, ****$P \leq 0.0001$, ns = not significant.

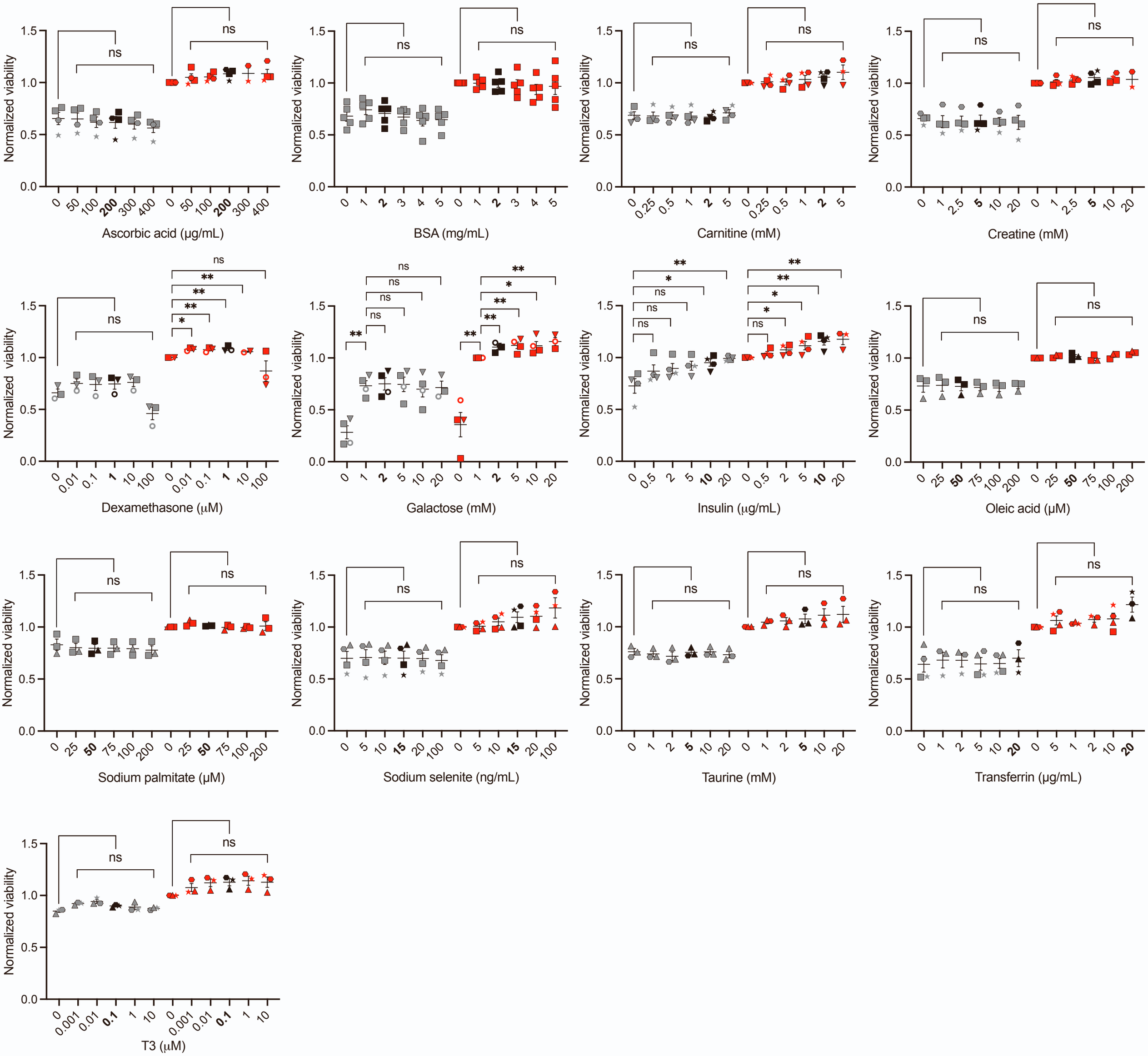

Figure S2: Concentration optimization of MMc components. a, Concentration optimization of each MMc component (*n* ≥ 3). A range of concentrations was tested and measured by viability. Each component was either added to MMb (gray circles) or added to MMc (red circles) at various concentrations. Black circles indicate final concentrations used in MMc formulation. Viability normalized to MMc without (concentration = 0) the component being tested except where viability was compromised (galactose; normalized to lowest dose tested in MMc). *n* = experimental replicates, ANOVA, **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.005, *****P* ≤ 0.0001, ns = not significant.

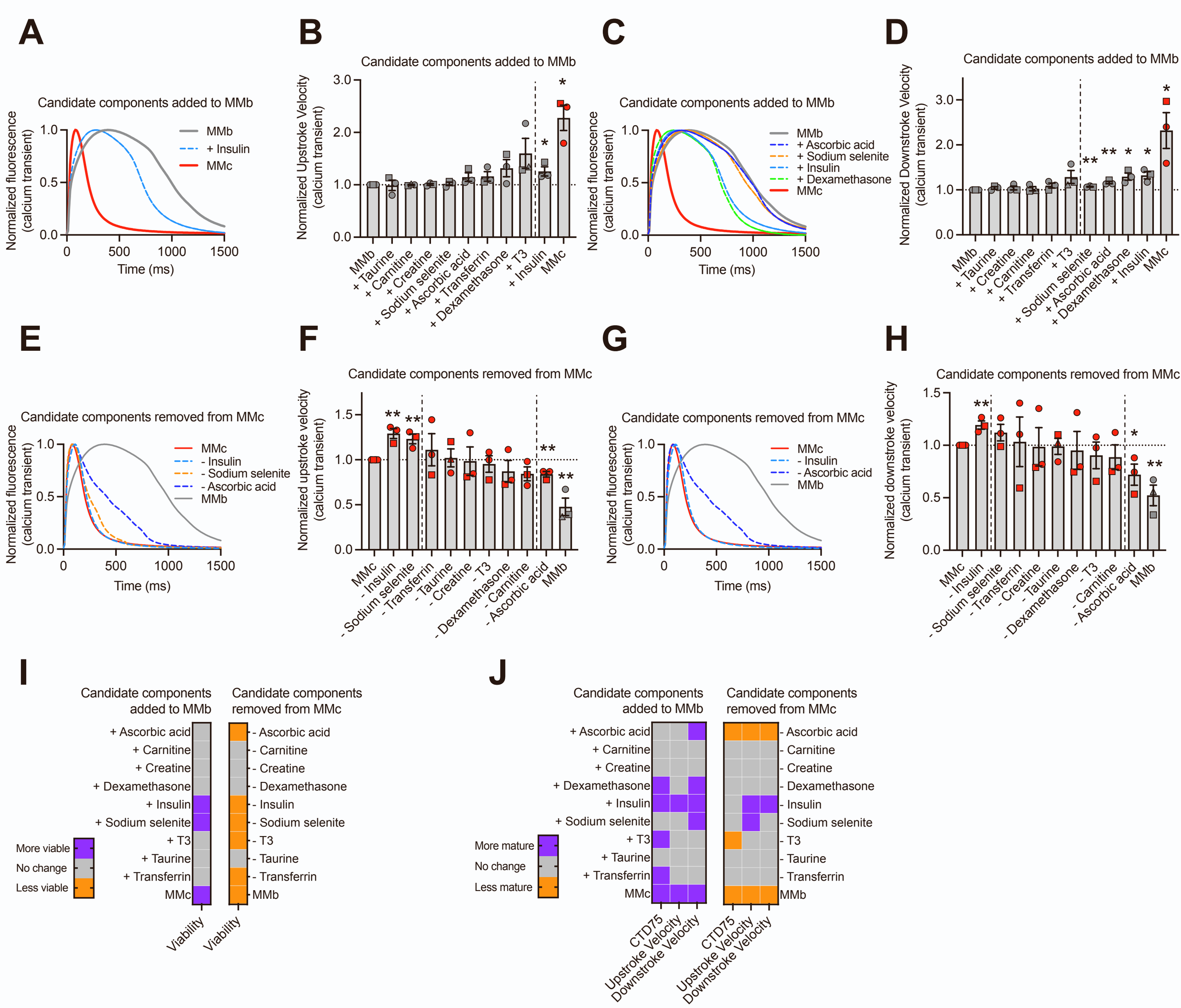

Figure S3: Concentration-optimized candidate component contribution to Ca^{2+} dynamics. (A-D) Representative spontaneous hiPSC-CM Ca^{2+} transient recordings and measurements of candidate components that significantly increased maximum (A-B) upstroke velocity and (C-D) downstroke velocity when added one at time to MMb (n = 3). (E-H) Representative spontaneous hiPSC-CM Ca^{2+} transient recordings and measurements of candidate components that significantly decreased maximum (E-F) upstroke velocity and (G-H) maximum downstroke velocity when removed one at time from MMc (n = 3). (I) Heatmap indicating changes to viability when candidate components are added to MMb (normalized and compared to MMb) or removed from MMc (normalized and compared to MMc). (J) Heatmap indicating changes to Ca^{2+} parameters (CTD75, maximum upstroke and downstroke velocity) when candidate components are removed from MMc (normalized and compared to MMc). Purple and orange boxes indicate candidate components that have $P \leq 0.05$. compared to control. n = experimental replicates, unpaired Student's T-test, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.005$, ****$P \leq 0.0001$, ns = not significant. All components to the right of the dashed line have $P \leq 0.05$. When two dashed lines are present, all values to the left of the dashed line on the left and to the right of the dashed line on the right have $P \leq 0.05$.

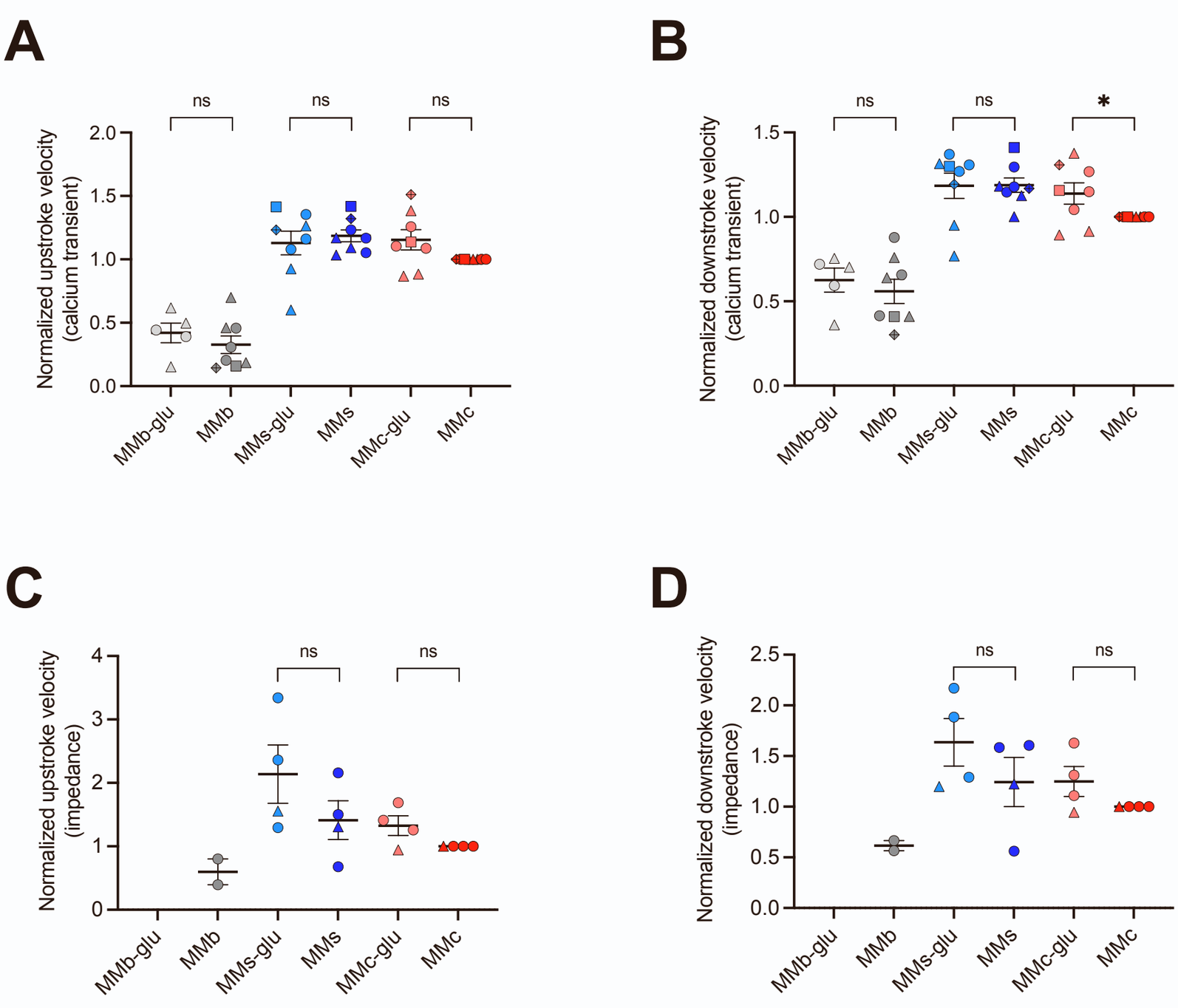

Figure S4: Replacing galactose with glucose does not change Ca^{2+} transient, impedance, and automated Nav current recordings. (A) Maximum upstroke velocity and (B) downstroke velocity of spontaneous Ca^{2+} transient recordings (*n* = 8). (C) Maximum upstroke velocity and (D) downstroke velocity of paced (0.8 Hz) impedance recordings (*n* = 4). (E) Representative recordings of I_{Nav} normalized to cell capacitance. (F) I_{Nav} current-voltage plot from automated patch clamp and peak current density at 0 mV (*n* ≥ 3). (G) I_{Nav} voltage-dependance of activation and inactivation (*n* ≥ 3). *n* = biological replicates, unpaired Student's T-test, **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.005, *****P* ≤ 0.0001, ns = not significant

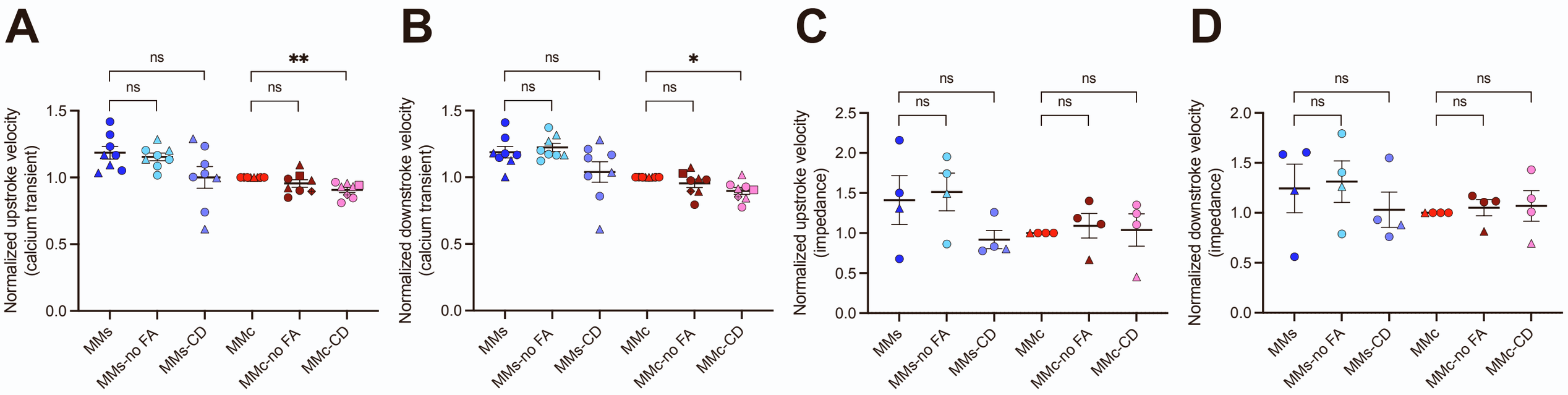

Figure S5: Removing fatty acids does not change Ca^{2+} transient, impedance, and automated Nav current recordings. (A) Maximum upstroke velocity and (B) downstroke velocity of spontaneous Ca^{2+} transient recordings (*n* = 8). (C) Maximum upstroke velocity and (D) downstroke velocity of paced (0.8 Hz) impedance recordings (*n* = 3). (E) Representative automated patch clamp recordings of I_{Nav} normalized to cell capacitance. (F) I_{Nav} current-voltage plot and peak current density at 0 mV of MMc conditions (*n* = 7). (G) Voltage-dependance of activation and inactivation of MMc conditions. (H) I_{Nav} current-voltage plot and peak current density at 0 mV of MMs conditions (*n* = 7). (I) Voltage-dependance of activation and inactivation of MMs conditions. *n* = experimental replicates, ANOVA, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.005$, $****P \leq 0.0001$, ns = not significant.

Table S1: Maturation media formulations.

Component	Base (MMb)	Complete (MMc)	Simple (MMs)	MMc- no fatty acids (MMc-no FA)	MMc-chemically defined (MMc-CD)	MMs- no fatty acids (MMs-no FA)	MMs-chemically defined (MMs-CD)
RPMI	500 mL	500 mL	500 mL	500 mL	500 mL	500 mL	500 mL
Galactose	2 mM	2 mM	2 mM	2 mM	2 mM	2 mM	2 mM
Bovine serum albumin	2 mg/mL	2 mg/mL	2 mg/mL	2 mg/mL	-	2 mg/mL	-
Sodium palmitate	50 µM	50 µM	50 µM	-	-	-	-
Oleic acid	50 µM	50 µM	50 µM	-	-	-	-
Insulin	-	10 µg/mL	10 µg/mL	10 µg/mL	10 µg/mL	10 µg/mL	10 µg/mL
Ascorbic acid	-	200 µg/mL	200 µg/mL	200 µg/mL	200 µg/mL	200 µg/mL	200 µg/mL
Dexamethasone	-	1 µM	1 µM	1 µM	1 µM	1 µM	1 µM
T3	-	100 nM	100 nM	100 nM	100 nM	100 nM	100 nM
Transferrin	-	20 µg/mL	20 µg/mL	20 µg/mL	20 µg/mL	20 µg/mL	20 µg/mL
Sodium selenite	-	15 ng/mL	15 ng/mL	15 ng/mL	15 ng/mL	15 ng/mL	15 ng/mL
Carnitine	-	2 mM	-	2 mM	2 mM	-	-
Creatine	-	5 mM	-	5 mM	5 mM	-	-
Taurine	-	5 mM	-	5 mM	5 mM	-	-

Table S2: Voltage-gated sodium current parameters.

Media	Cell capacitance (pF)	Peak current density at 0 mV (pF/pA)	$V_{1/2}$ of activation (mV)	k of activation	$V_{1/2}$ of inactivation (mV)	k of inactivation
RBAI	19.01 ± 0.49	-80.73 ± 4.83	-22.48 ±1.05	7.42 ± 0.25	-70.30 ± 0.89	-7.11 ± 0.15
MMc	19.02 ± 0.44	-130.6 ± 8.17	-22.14 ± 1.02	6.80 ± 0.40	-67.96 ± 0.75	-6.41 ± 0.16
MMc-glu	21.45 ± 0.61	-127.8 ± 7.82	-22.38 ± 0.94	6.75 ± 0.30	-69.41 ± 0.79	-6.58 ± 0.14
MMs	19.01 ± 0.65	-128.4 ± 9.08	-23.57 ± 1.52	6.87 ± 0.33	-71.12 ± 1.08	-6.36 ± 0.14
MMs-glu	20.67 ± 0.88	-139.7 ± 14.17	-23.94 ± 1.72	6.35 ± 0.35	-71.57 ± 1.60	-6.57 ± 0.18
MMc-no FA	19.72 ± 0.82	-150.8 ± 15.1	-18.57 ± 1.82	6.65 ± 0.48	-66.32 ± 1.55	-6.13 ± 0.26
MMc-CD	20.45 ± 0.76	-137.1 ± 21.2	-17.86 ± 2.67	6.66 ± 0.58	-68.09 ± 1.80	-6.62 ± 0.21
MMs-no FA	20.13 ± 1.38	-66.99 ± 10.96	-20.15 ± 2.04	6.65 ± 0.48	-65.28 ± 1.65	-6.76 ± 0.31
MMs-Cd	23.32 ± 1.68	-127.95 ± 14.66	-16.60 ± 1.83	6.66 ± 0.58	-60.69 ± 1.34	-6.33 ± 0.31