Supplementary Data for:
Local Anesthetic Cardiac Toxicity is Mediated By Cardiomyocyte Calcium Dynamics

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Supplementary Video 1: Beating arrest of iPSC-CM tissues induced by nifedipine co-treatment with bupivacaine, in contrast to ropivacaine. Bright-field microscopy videos 5 hours post drug treatment. BUP = 6 µg/ml bupivacaine; ROP = 6 µg/ml ropivacaine; NIF = 50 nM nifedipine. Scale bar = 50 microns.

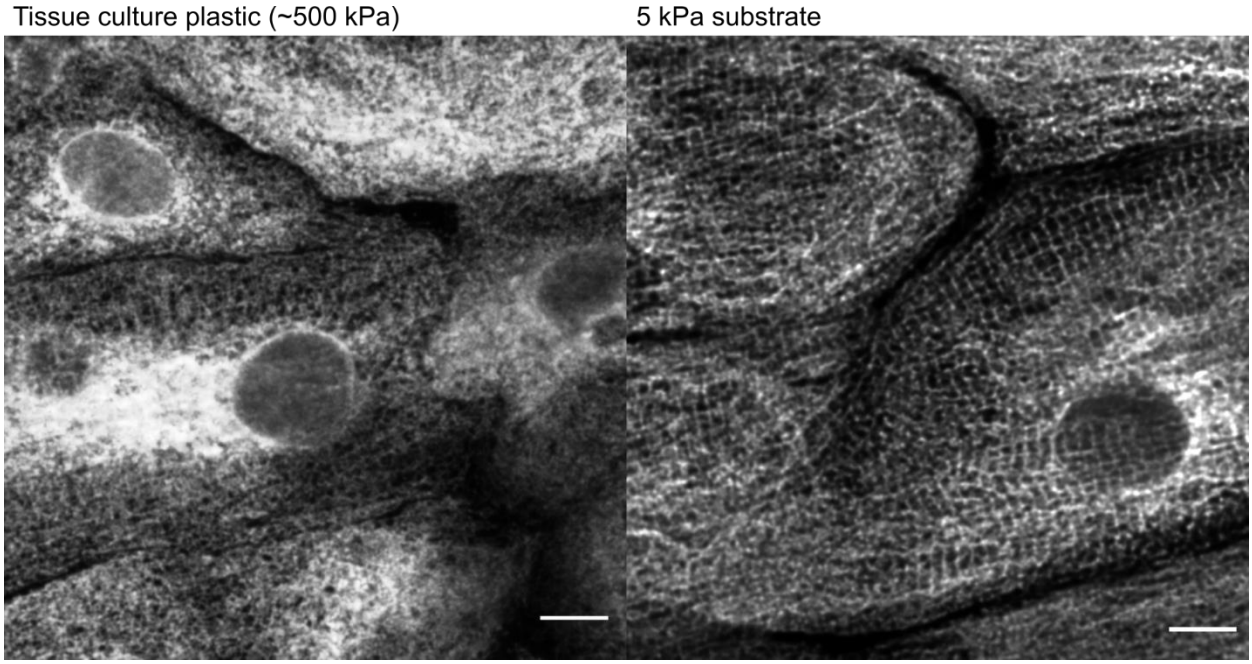

Figure S1: SERCA-2a immunofluorescence shows improved structural maturation of iPSC-CM tissues cultured on physiologic substrates. iPSC-CM tissues were cultured on conventional tissue culture plastic, or silicone substrates of physiolgic myocardial stiffness (5 kPa).[1,2] Given our interest in cardiomyocyte calcium dynamics, we probed for structural maturation of the sarcoplasmic reticulum, marked by SERCA-2a.[3] The striated and aligned pattern of SERCA-2a observed in tissues cultured on the 5 kPa susbtrates indicates sarcoplasmic alignment along sarcomeres, typically present in cardiomyocytes with matured calcium handling.[4] Scale bar = 10 microns.

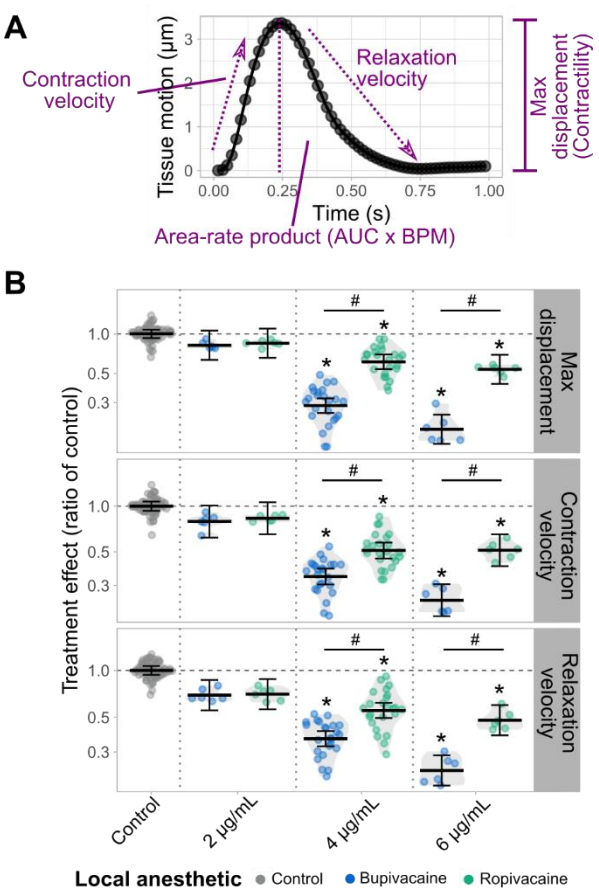

Figure S2: Anesthetic effects on iPSC-CM contractile function cultured on physiologic substrates. **(A)** Example tissue beating profile captured from brightfield videos of beating human iPSC-CM tissues cultured on 5 kPa soft substrates. Tissue contractility parameters were evaluated by tracking tissue displacement over time. **(B)** Select treatment groups of data in Figure S9 highlighting the increasing severity of contractile depression by bupivacaine over ropivacaine as drug dose increases. Data are mixed-effects model estimates and 95% CI. **P*<0.05 versus vehicle control tissues, $^{\#}p$<0.05 bupivacaine versus ropivacaine at each drug dose (multiplicity-adjusted post-hoc Welch's t-tests of model estimates).

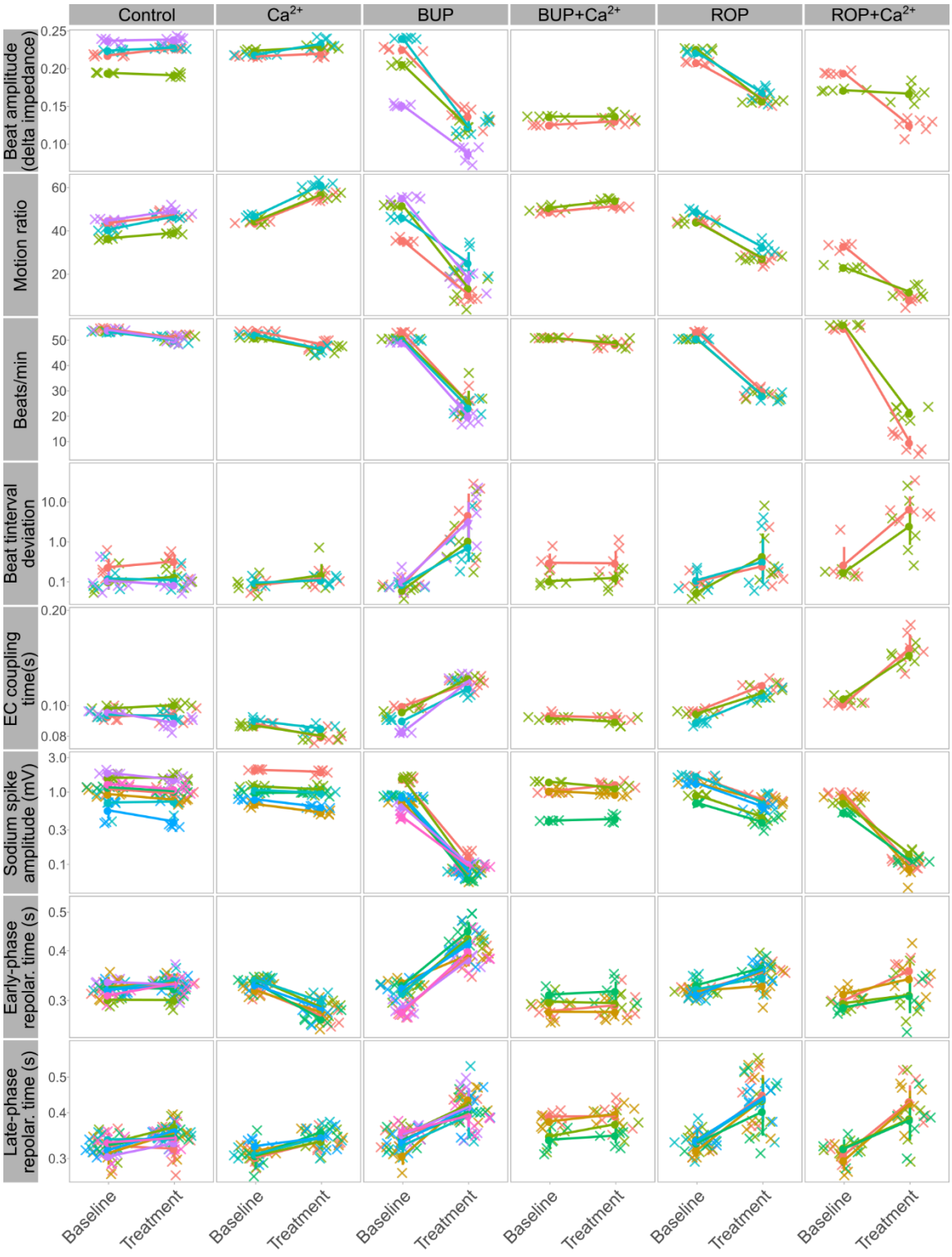

Figure S3: The effects of local anesthetics and calcium supplementation on iPSC-CM tissue contractility and field potential profile. In relation to Figure 3. Raw multielectrode array tissue impedance and field potential data measuring functional changes of human iPSC-CM beating monolayer tissues treated with 6 µg/mL bupivacaine (BUP) or ropivacaine (ROP). Ca^{2+} = final concenration 2.5 mM $CaCl_2$ versus 2 mM in culturing medium (Control). Data is coloured and paired by tissue (lines), with multiple measures per tissue at each timepoint (crosses). Motion ratio is calculated as downwards impedance amplitude divided by the upwards impedance amplitude, representing the contractile profile of the tissue.

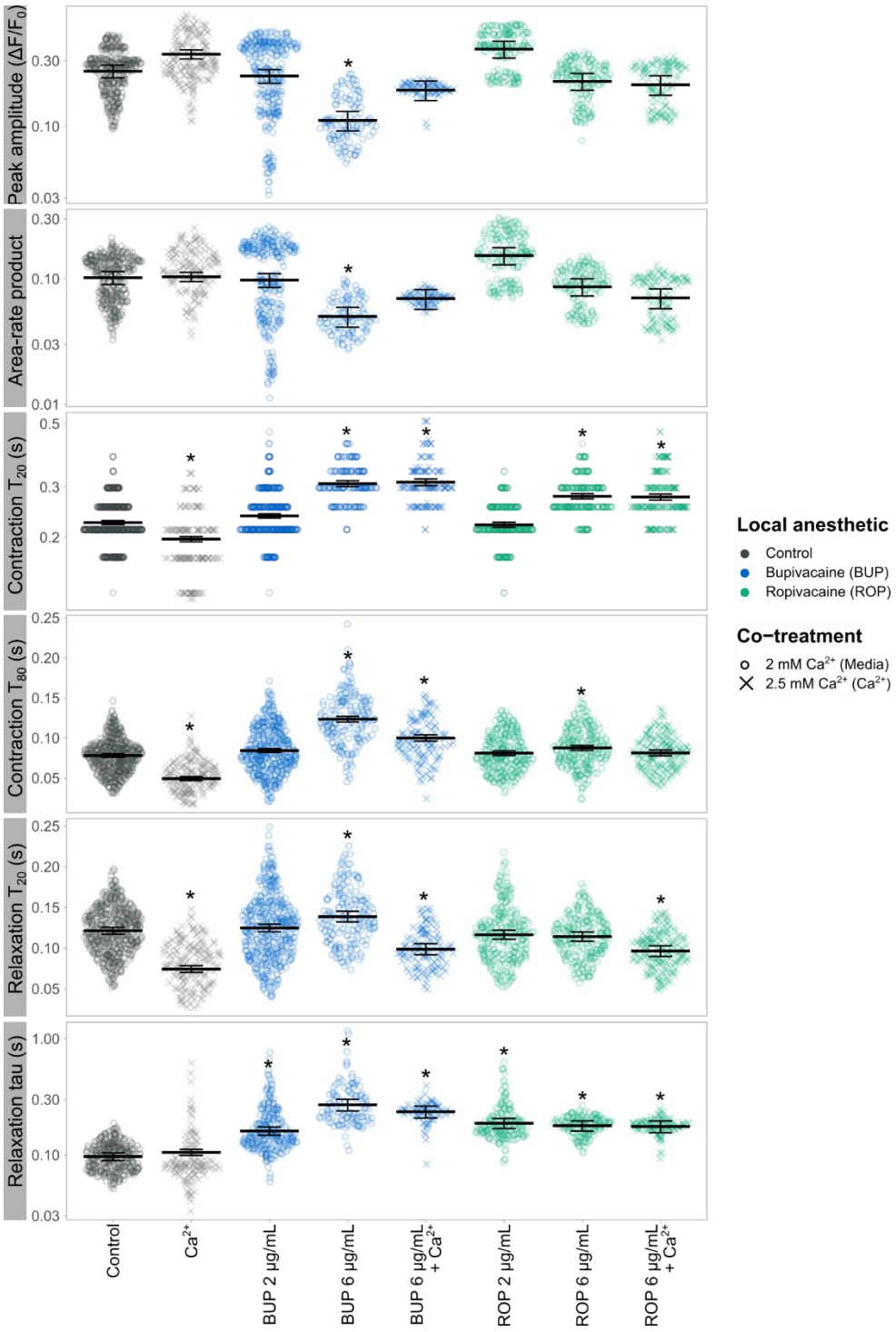

Figure S4: Effects of local anesthetics and calcium supplementation on iPSC-CM tissue intracellular calcium dybamics. In relation to Figure 4. Quantification of fluorescent imaging calcium dynamics (expanded data of Figure 4C). Calcium co-treatment was only evaluated at the toxic anesthetic dose (6 µg/mL). Horizonal bars are mixed-effects model estimates and 95% CI, *p<0.05 (Dunnett adjustment for multiple comparisons to control).

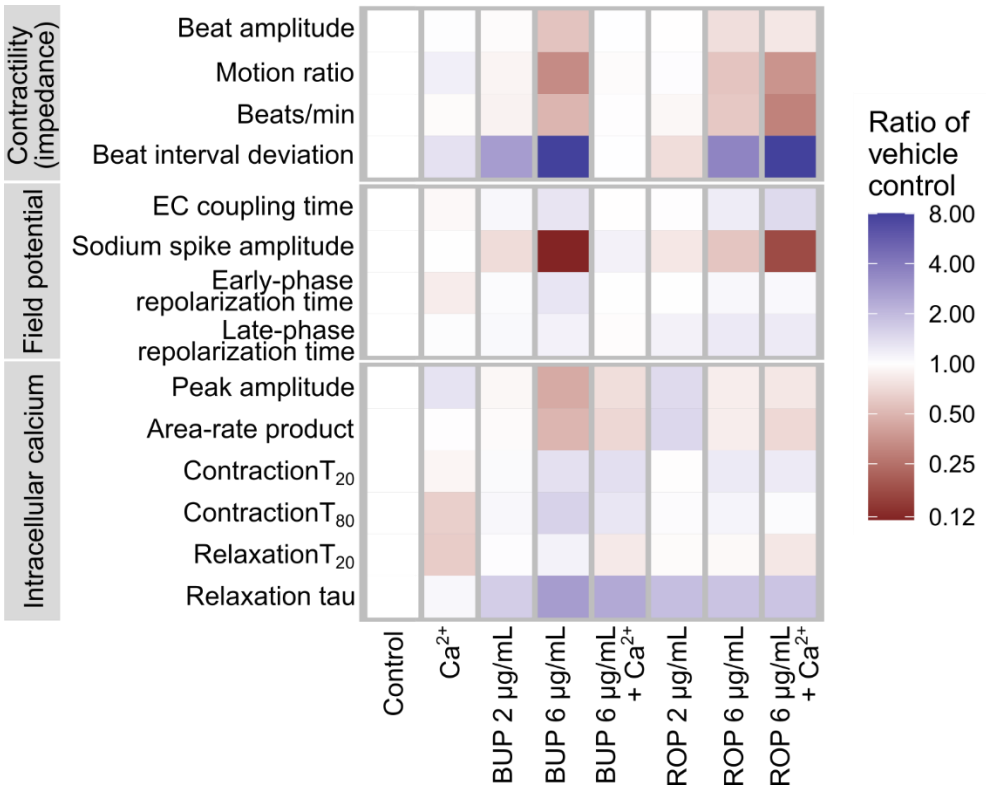

Figure S5: Heatmap illustrating overall changes to bupivacaine- and ropivacaine-induced effects on iPSC-CM tissue contractility, field potential, and intracellular calcium dynamics. Input data are mixed-effects model estimates of the combined data from multielectrode array and intracellular calcium dye video assays. Ca^{2+} = final concentration 2.5 mM $CaCl_2$ versus 2 mM in culturing medium (control); BUP = bupivacaine; ROP = ropivacaine. Calcium co-treatment was only evaluated at the toxic anesthetic dose (6 µg/mL).

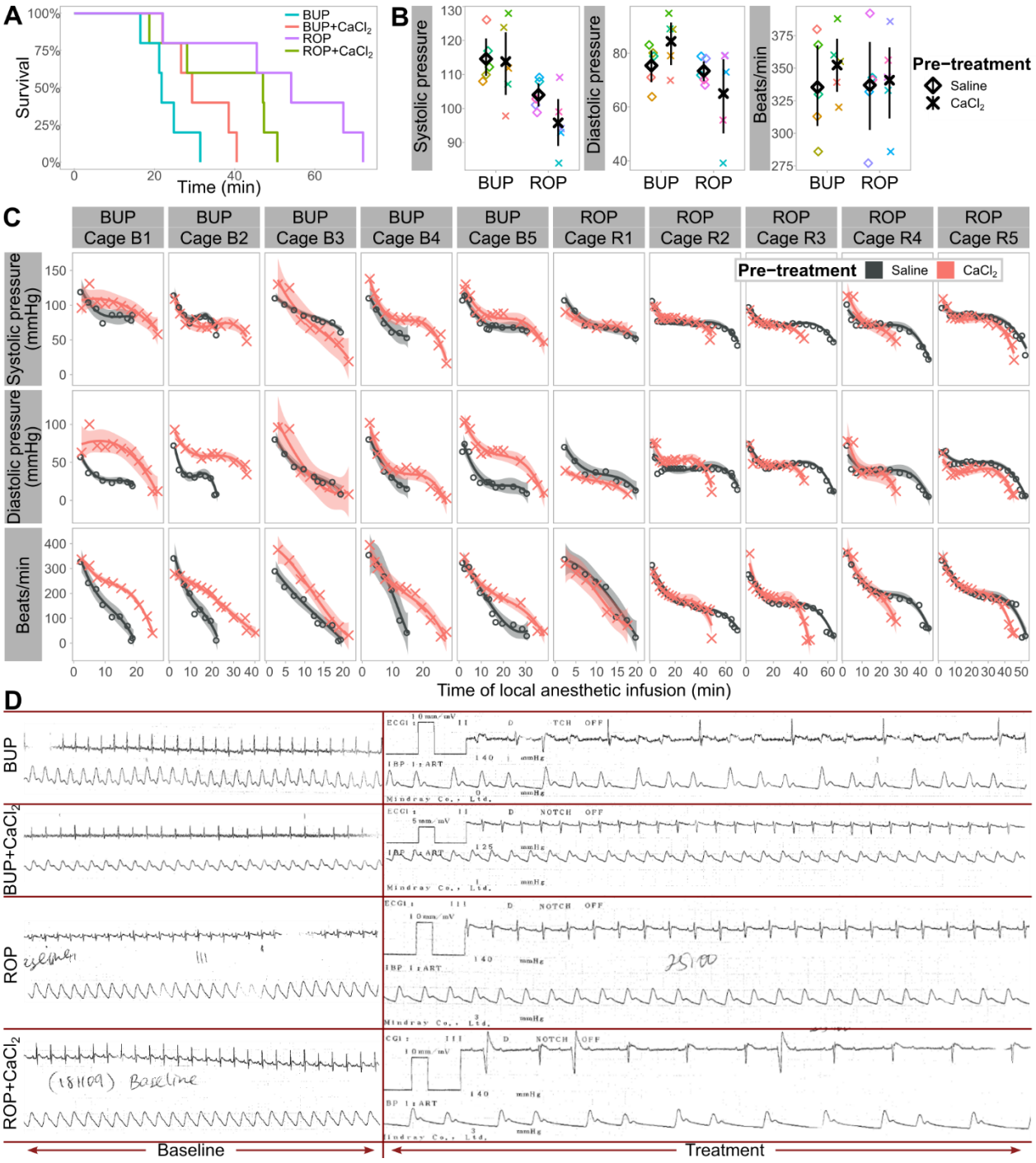

Figure S6: Rat model of anesthetic cardiotoxicity comparing the effects of $CaCl_2$ or saline pre-treatment. In relation to Figure 6. **(A)** Kaplan-Meier survival curve showing divergent effects of calcium between anesthetics. **(B)** Baseline hemodynamic parameters recorded for each animal prior pre-treatment infusion. Individual values are coloured by cage with group means and 95% CI overlaid in black. Although differences among groups were non-significant (p>0.1 Welch's two-sided t-test with Tukey multiple comparisons adjustment), baseline values per animal were included in the statistical model as a covariate term. **(C)** Raw data of statistical model in Figure 6B. Data is shown for each animal, paired by cage. **(D)** Sample ECG and arterial blood pressure traces (treatment time at 10 min post bupivacaine infusion, and 25 min post ropivacaine infusion, the approximate midpoint survival time for each drug) used to quantify beat-to-beat RR-intervals in Figure 6E, showing disturbances to beating rhythm.

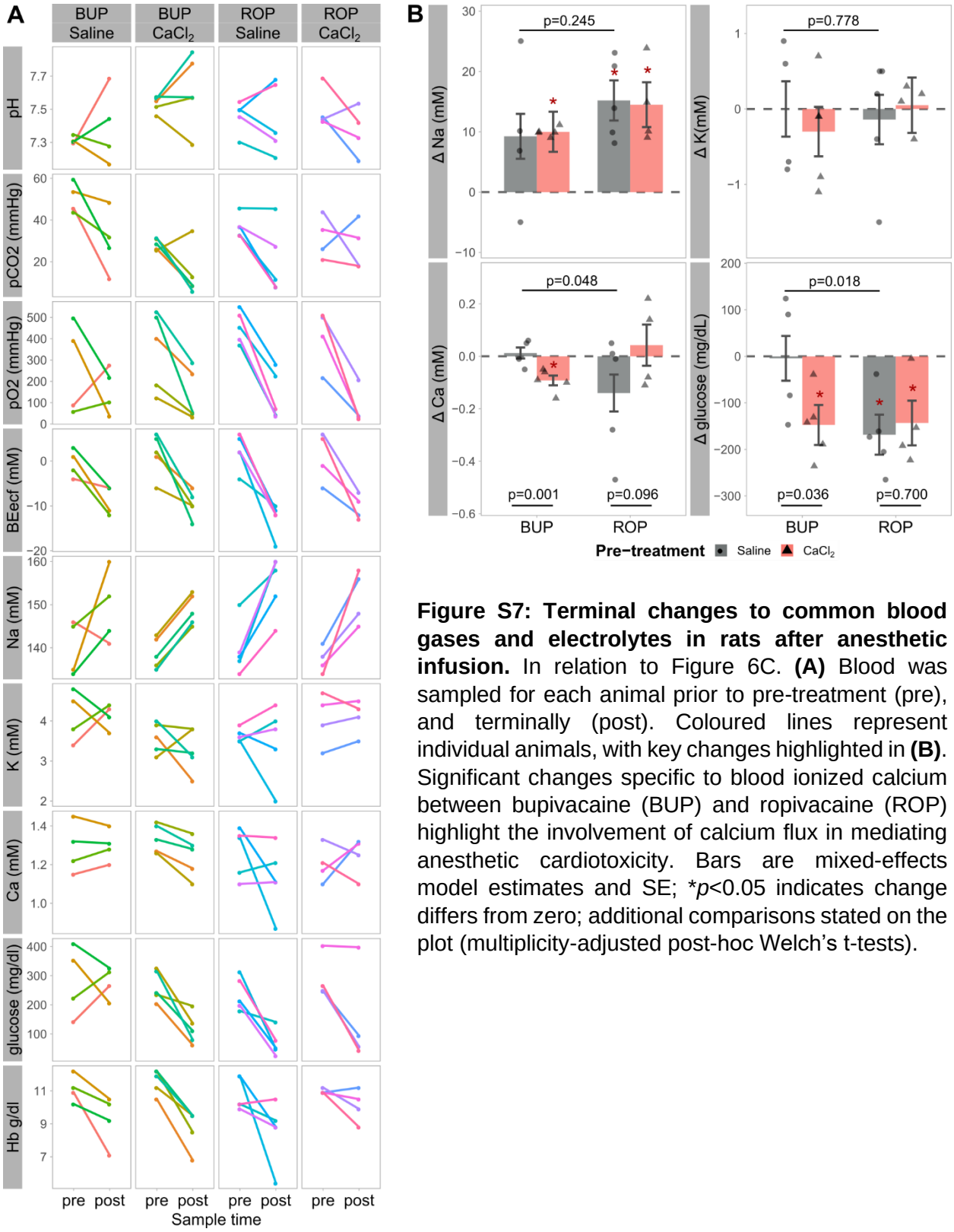

Figure S7: Terminal changes to common blood gases and electrolytes in rats after anesthetic infusion. In relation to Figure 6C. **(A)** Blood was sampled for each animal prior to pre-treatment (pre), and terminally (post). Coloured lines represent individual animals, with key changes highlighted in **(B)**. Significant changes specific to blood ionized calcium between bupivacaine (BUP) and ropivacaine (ROP) highlight the involvement of calcium flux in mediating anesthetic cardiotoxicity. Bars are mixed-effects model estimates and SE; **p*<0.05 indicates change differs from zero; additional comparisons stated on the plot (multiplicity-adjusted post-hoc Welch's t-tests).

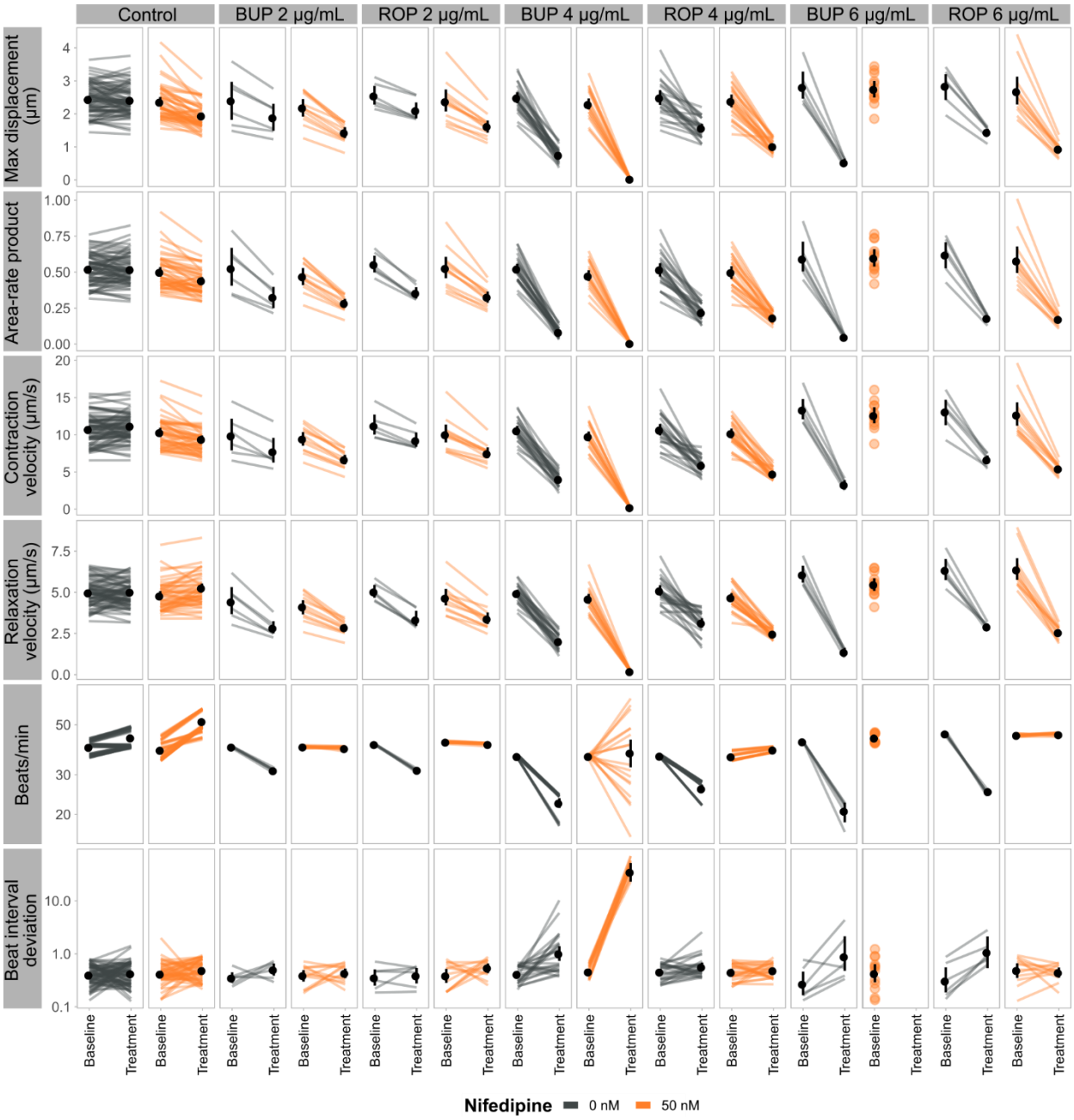

Figure S8: iPSC-CM tissue displacement data for the negative interaction between nifedipine and anesthetics. In relation to Figures 5A-B and S9. BUP = bupivacaine; ROP = ropivacaine. Tissue contractility parameters were evaluated from brightfield microscopy videos, as shown in Figure S2A; beat interval deviation was calculated as in Figure 1A. Data are paired by sample (lines), black dotplot represents mean and 95% bootstrap CI. Data for 6 µg/mL bupivacaine + nifedipine group is missing due to beating arrest.

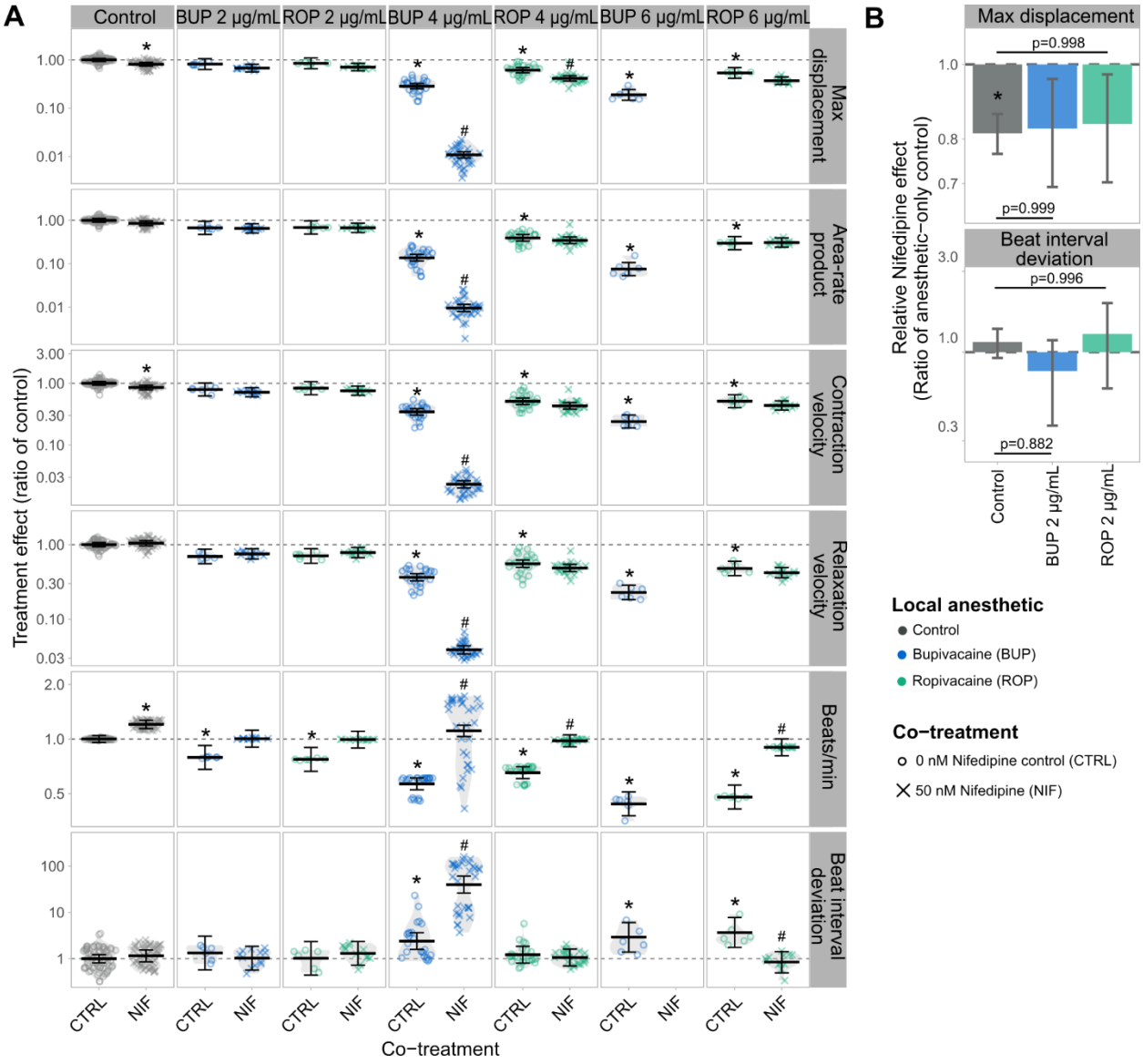

Figure S9: Nifedipine effects on anesthetic-mediated contractile dysfunction. In relation to Figures 5AB and S8. **(A)** Tissue contractility measurements from brightfield microscopy videos. Doubly normalized (to baseline, then control) data of Figure S8; expanded data of Figure 5B. Horizonal bars are mixed-effects model estimates and 95% CI. The lack of significant nifedipine effects on tissue displacement (contractility) and beat interval deviation (rhythm) at the sub-toxic 2 µg/mL anesthetic dose is highlighted in **(B)**, in contrast to the significant nifedipine effects at the toxic 4 µg/mL anesthetic dose shown in Figure 5B (model estimate and SE). **P*<0.05 versus vehicle control tissue (CTRL), $^{\#}p$<0.05 versus non-nifedipine control for each resepctive anesthetic and dose (multiplicity-adjusted post-hoc Welch's t-tests of mixed-effects model estimates).

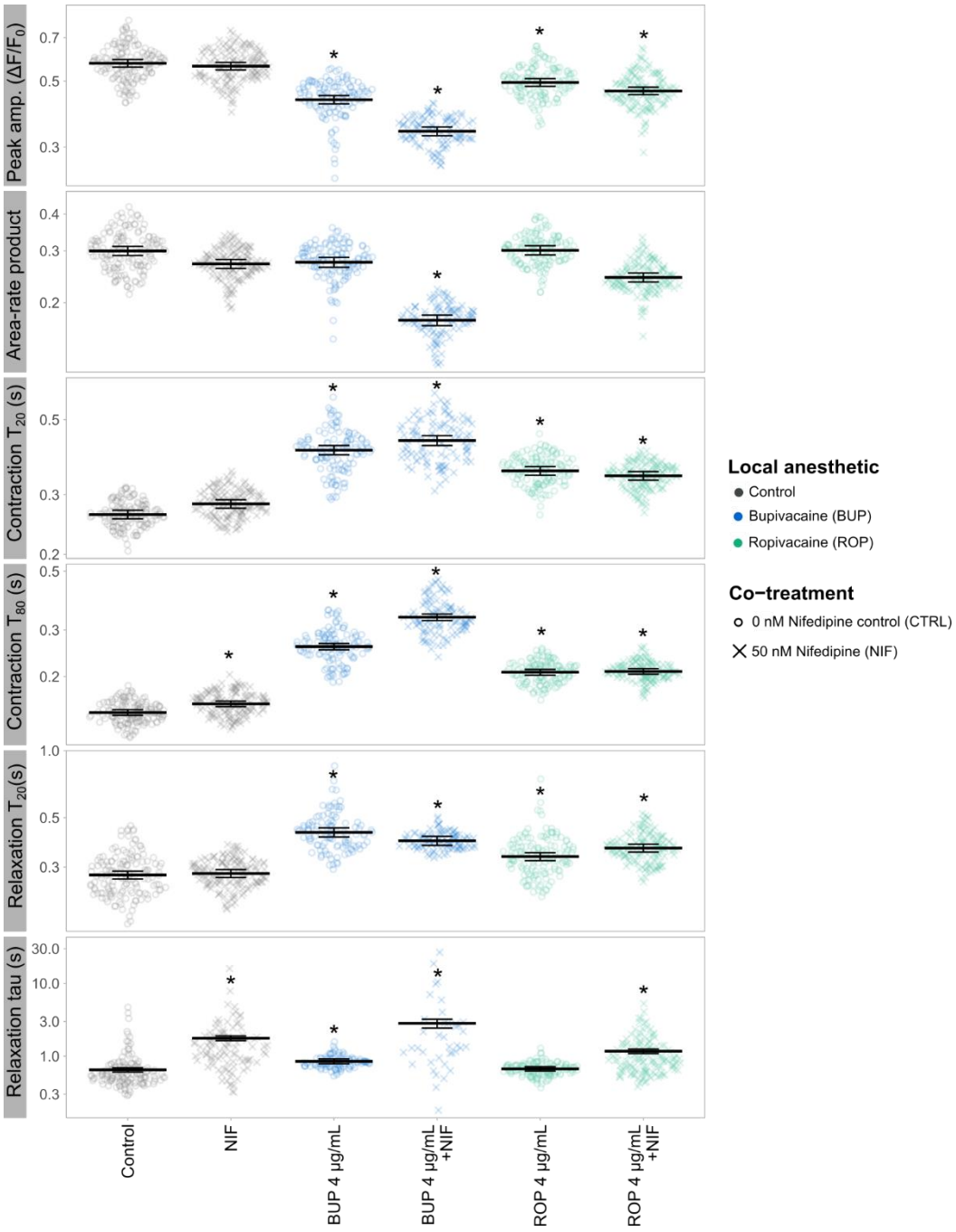

Figure S10: Effects of nifedipine on anesthetic-mediated dysregulation of calcium dynamics. Expanded data of Figure 5C. Data was obtained from FLIPR5-loaded iPSC-CM tissues and quantified as in Figure 4A. Horizonal bars are model estimates and 95% CI, *p<0.05 (Dunnett adjustment for multiple comparisons to control).

Supplementary Methods

iPSC-CM tissue immunostaining for SERCA-2a

Tissues were fixed with 4% parafolmadehyde, permeabilized with 0.15% Triton X-100, blocked with 5% goat serum, then probed with mouse monoclonal anti-SERCA2a antibody (1:200 dilution, Abcam Ab2861), followed by goat anti-mouse alexafluor-647 secondary antibody (1:500 dilution, Cell Signaling Technology #4410S). Images were acquired using a Quorum spinning disk confocal microscope (637 nm laser excitation and 700 nm emission filter) using a 63X 1.4 aperture oil immersion objective.

Supplementary References

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