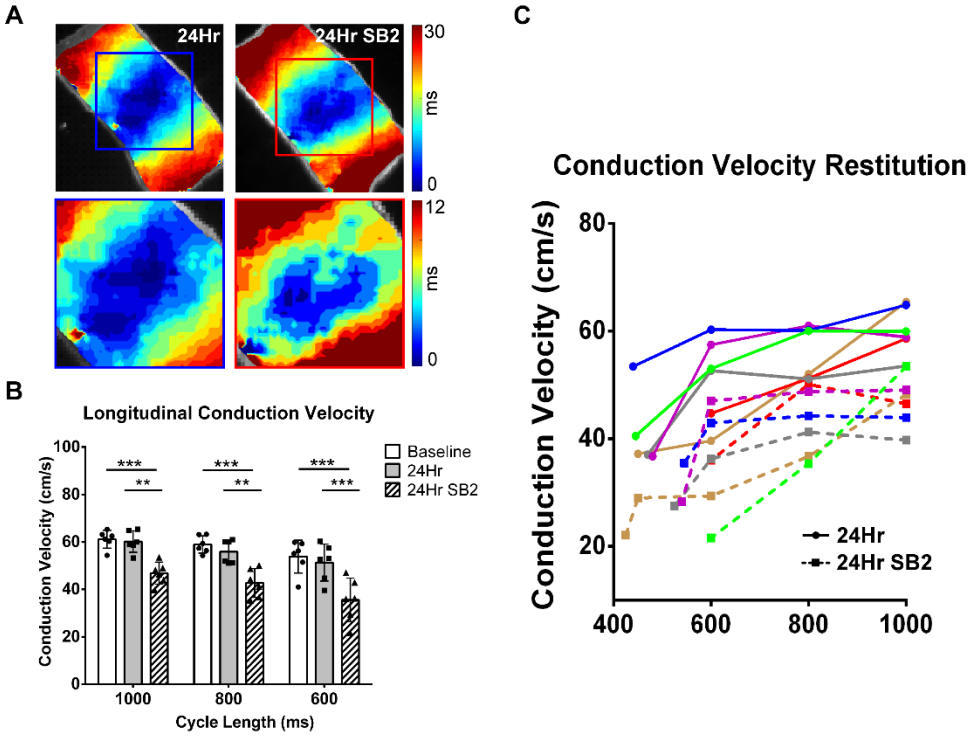
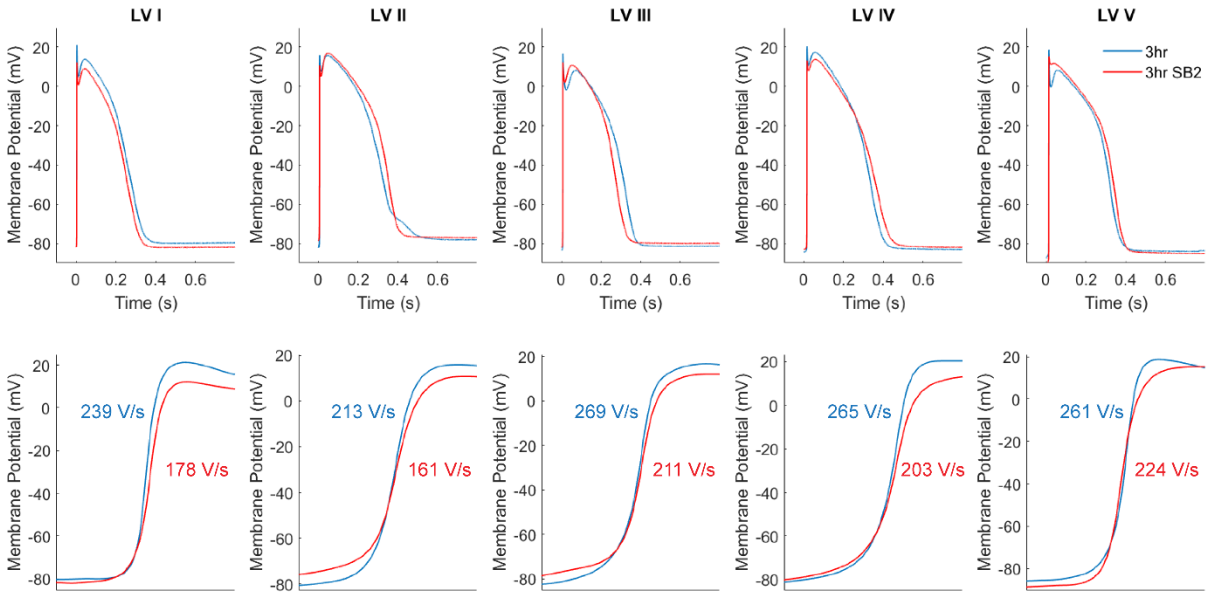
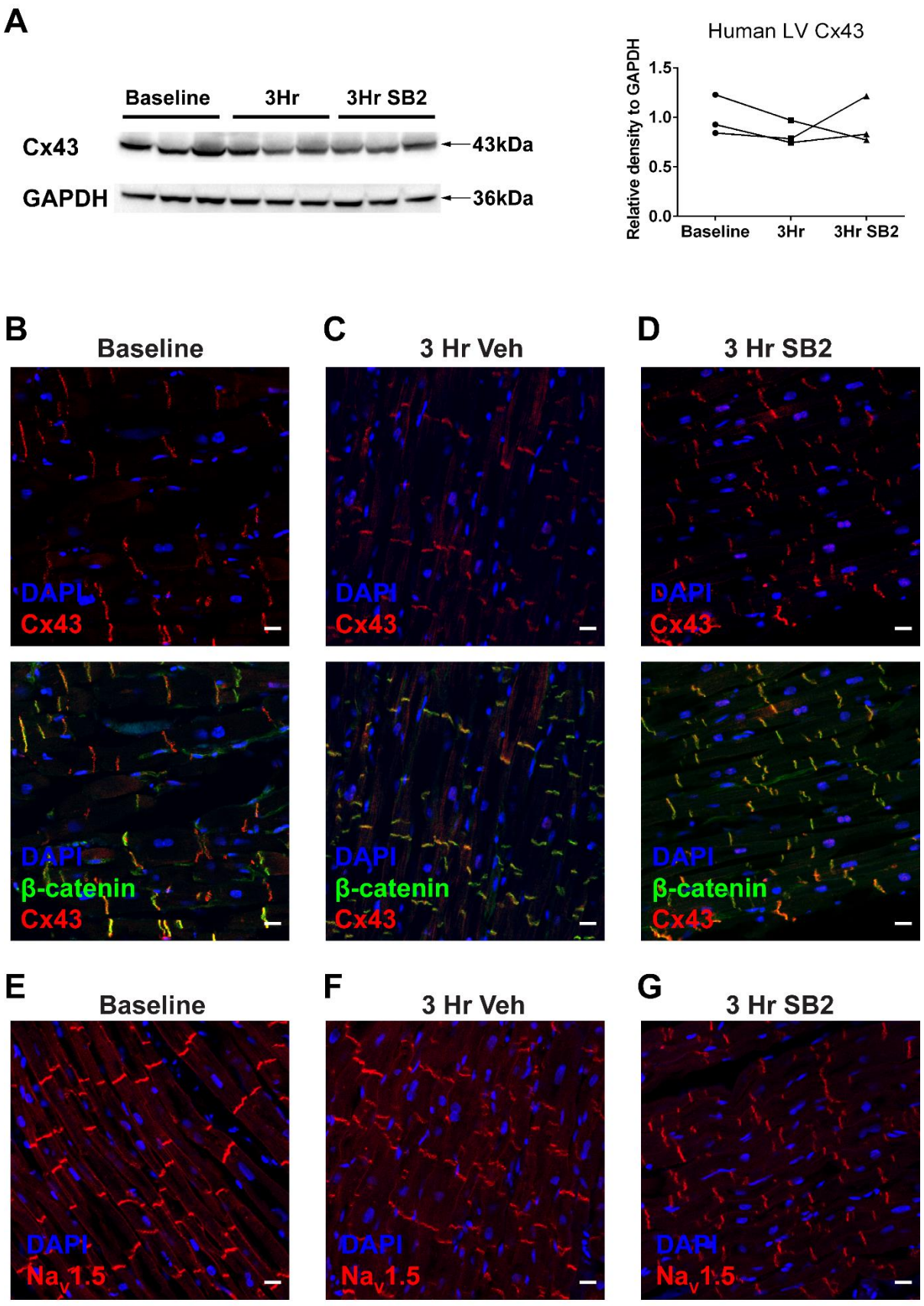
SUPPLEMENTAL APPENDIX



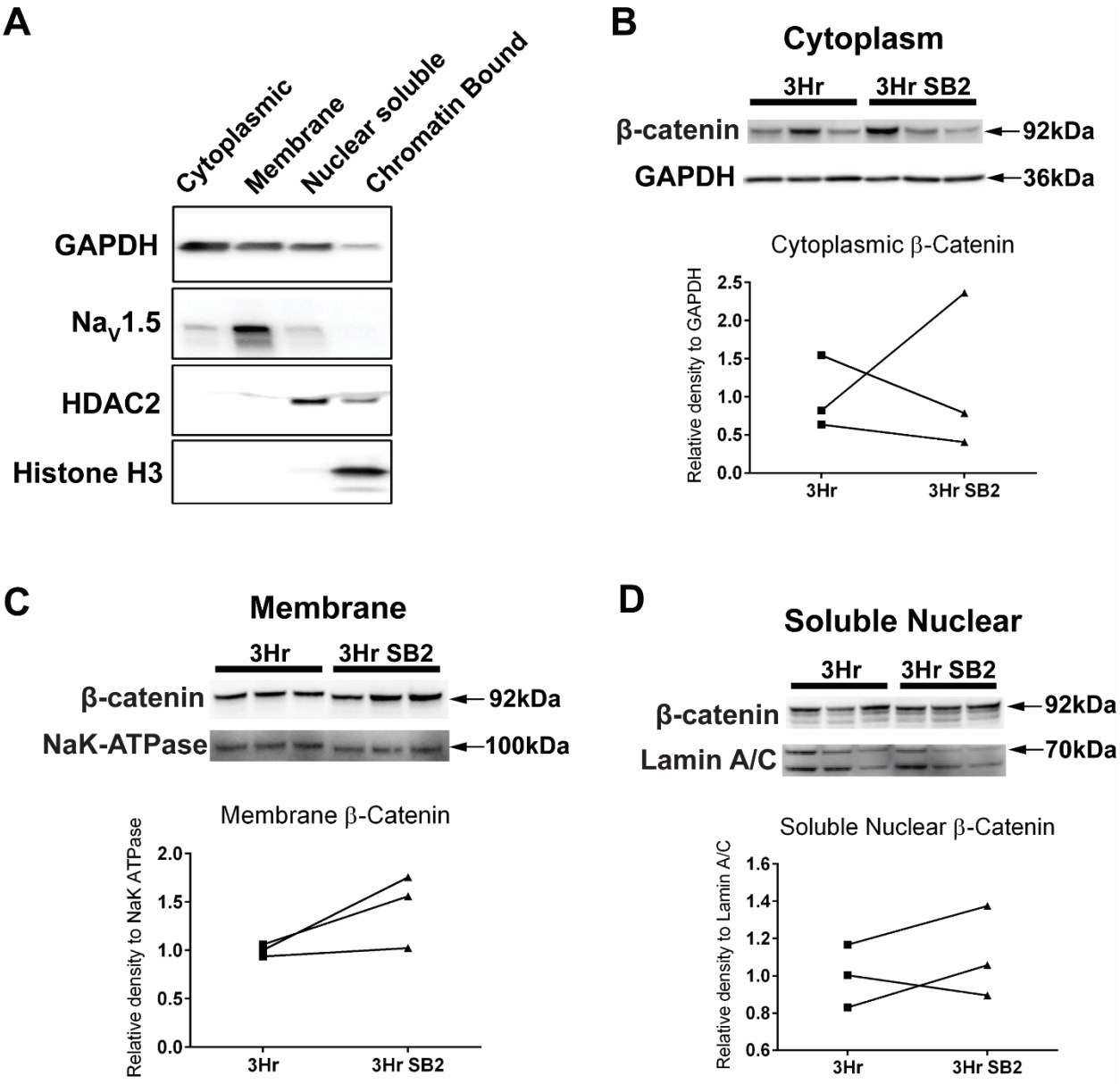
Supplemental Figure 1. Conduction velocity of human LV slices is reduced after 24 hours of SB2 treatment. **A.** Representative activation maps of a control slice (left) compared to a SB2-treated slice (right) from the same heart. Squares in the top panels indicate regions of the activation maps zoomed-in (bottom) to further demonstrate slower activation in the SB2-treated slice as seen by the greater number of isochrones to reach the same distance. **B.** Conduction velocity quantified from activation maps show no difference between baseline and 24-hour control, but ~23% reduced conduction velocity in 24-hour SB2-treated slices at pacing cycle lengths of 1000 ms, 800 ms, and 600 ms. **C.** Conduction velocity restitution of adjacent slices from each individual heart, indicated by different colors, shows reduced conduction velocity at all stimulation cycle lengths but no change in restitution slope in SB2-treated slices. Statistics were performed using repeated measures ANOVA followed by Tukey's post-hoc test for multiple pairwise comparisons. **p<0.01, ***p<0.001.



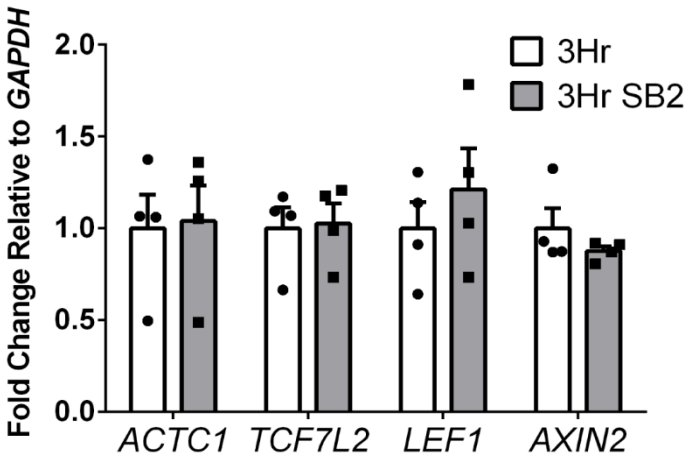
Supplemental Figure 2. SB2 treatment decreases maximum action potential upstroke velocity at 3 hours in culture. Averaged action potentials from slices paced at a cycle length of 1000 ms shows a decrease in dV_m/dt_{max} in each individual heart when cultured with SB2. Each column represents an individual heart, with the top panels showing averaged action potentials of 3-hour control (blue) and 3-hour SB2 (red), and the bottom panels showing zoomed-in action potential upstrokes with dV_m/dt_{max} values.


A
Baseline
3Hr
3Hr SB2
Cx43
43kDa
GAPDH
36kDa
Human LV Cx43
Relative density to GAPDH
1.5
1.0
0.5
0.0
Baseline
3Hr
3Hr SB2
B
Baseline
C
3 Hr Veh
D
3 Hr SB2
DAPI
Cx43
DAPI
β-catenin
Cx43
E
Baseline
F
3 Hr Veh
G
3 Hr SB2
DAPI
Na$_v$1.5

Supplemental Figure 3. Neither Cx43 levels and localization nor $Na_V1.5$ localization is altered by GSK-3 inhibition via SB2. **A.** Western blot for Cx43 and GAPDH shows no significant changes between Cx43 relative to GAPDH for all conditions. **B-D.** Immunostaining for β-catenin (green) and Cx43 (red) at baseline (**B**), 3 hours in culture (**C**), and 3 hours in culture with SB2 (**D**) shows no gross differences or Cx43 lateralization. **E-F.** Immunostaining for $Na_V1.5$ (red) at baseline (**D**), 3 hours in culture (**E**), and 3 hours in culture with SB2 (**F**) shows no differences in general structure or $Na_V1.5$ localization. All slides have nuclei stained with DAPI (blue). Scale = 20 μm. Statistics were performed using repeated measures ANOVA followed by Tukey's post-hoc test for multiple pairwise comparisons.



Supplemental Figure 4. Subcellular fractionation of human slices treated with SB2 shows increase in chromatin-bound β-catenin compared to vehicle control. **A.** Western blot of human heart lysate for each fraction to evaluate fraction purity. GAPDH expression is highest in the cytoplasmic fraction as expected, with relatively lower expression in membrane and nuclear soluble fractions, and minimal expression detected in the chromatin bound fraction. Na$_V$1.5 is detected mostly in the membrane fraction, HDAC2 is highest in the nuclear soluble fraction with lower expression in the chromatin bound fraction as expected, while the chromatin bound Histone H3 is exclusively seen in the chromatin bound fraction. **B.** Cytoplasmic β-catenin is unchanged between SB2 treated heart slices compared to control. **C.** Membrane-bound β-catenin is unchanged between SB2 and control conditions. **D.** β-catenin from the soluble nuclear fraction is unchanged between SB2 and control conditions. Statistics were performed using repeated measures ANOVA followed by Tukey's post-hoc test for multiple pairwise comparisons.



Supplemental Figure 5. Nuclear active β-catenin does not activate Wnt/β-catenin target genes. Transcription of Wnt/β-catenin target genes (*TCF7L2*, *LEF1*, and *AXIN2*) and negative control (*ACTC1*) are unchanged by SB2 at 3 hours relative to the housekeeping gene GAPDH. Statistics were performed using a Student's t-test.