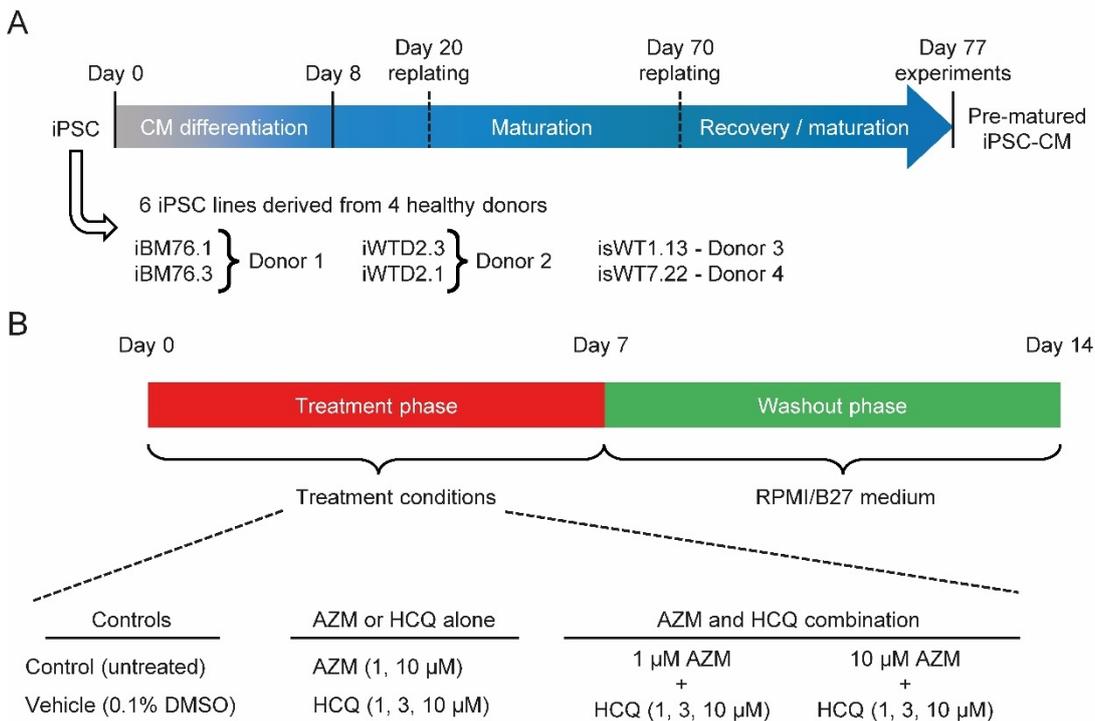


## Supplementary information

**Video S1.** Contractile behavior of iPSC-CM after 3 days of treatment.

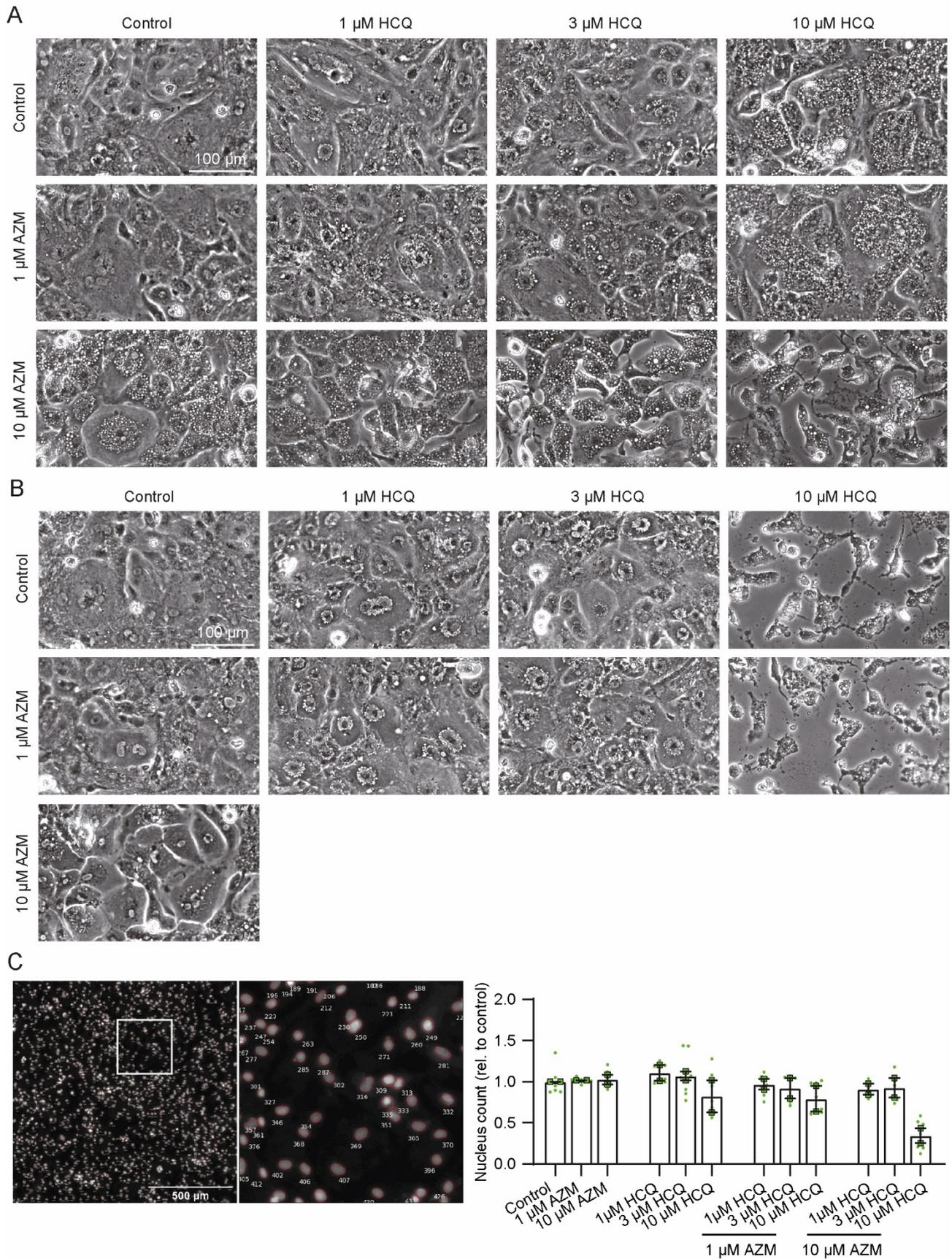
**Video S2.** Contractile behavior of iPSC-CM after 7 days of treatment.

Figure S1



**Figure S1.** Experimental scheme. **(A)** Timeline of iPSC-CM differentiation and maturation under long-term cultivation for 77 days before the drug treatment and functional analysis. iPSC lines derived from 4 healthy donors were used in the experiments to consider different genetic background. **(B)** Scheme of all conditions used in the study with drug treatment and washout phases.

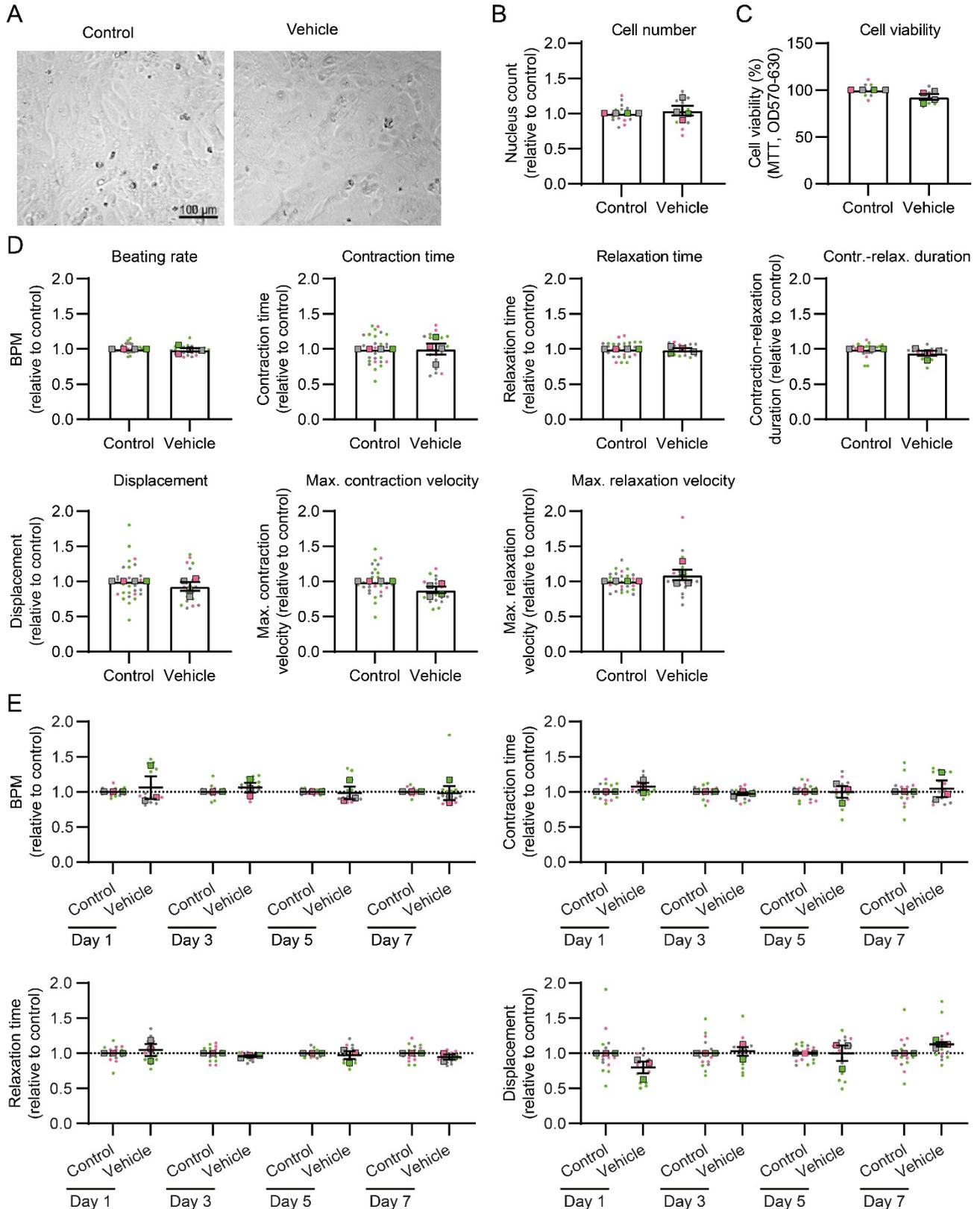
Figure S2



**Figure S2.** Morphological changes induced by treatment with HCQ and AZM, and quantification of

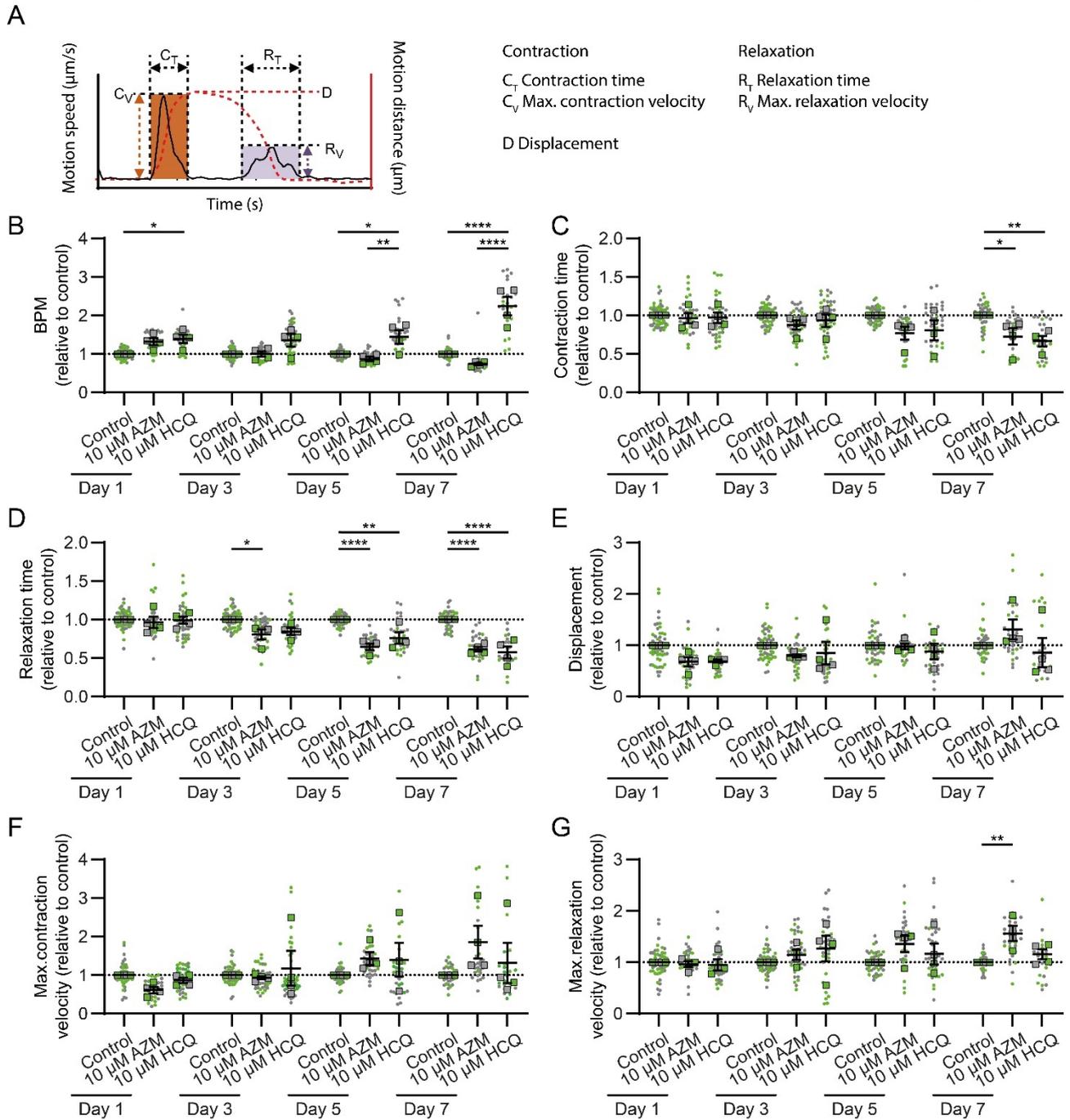
nucleus number after 7-day treatment with the drugs. **(A, B)** Representative brightfield images depicting morphology of iPSC-CMs after 7-day **(A)** treatments with HCQ and AZM in different concentrations and after subsequent 7-day washout **(B)**. Scale bar: 100  $\mu$ m. **(C)** Representative images of iPSC-CMs stained with Hoechst33342 and nucleus count using CellProfiler software. Nucleus counts after the 7-day drug treatment relative to the control group (n = 7-8 images from 2 independent experiments). Data represent mean and SEM of n = 2 different iPSC-CM differentiations from healthy donor iBM76.

Figure S3



**Figure S3.** Cell viability and contractility in vehicle (0.1% DMSO) treated iPSC-CMs. **(A)** Brightfield images of iPSC-CMs after 7-day culture with RPMI/B27 medium (control) or in the presence of 0.1% DMSO vehicle showing that vehicle treatment did not affect CM morphology. **(B)** Cell density was

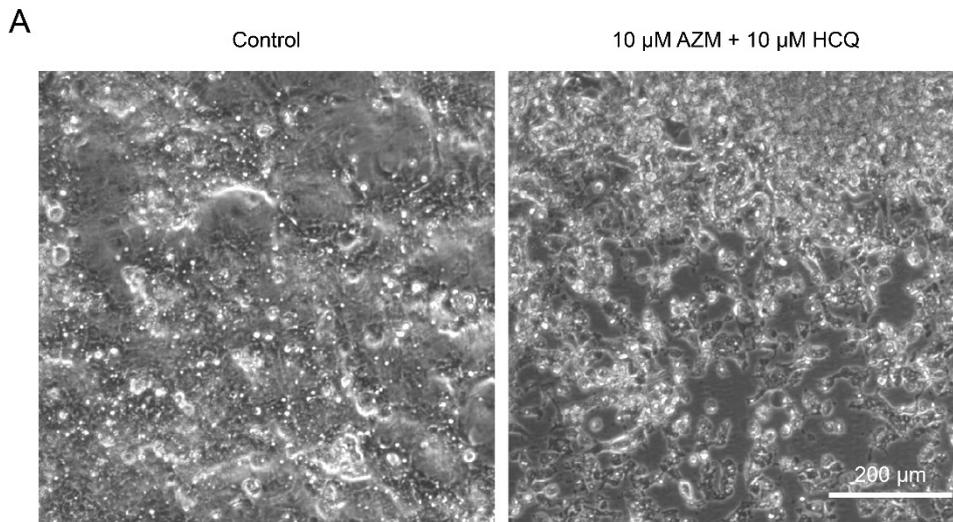
investigated based on the quantification of cell nucleus counts using Hoechst33342 staining and fluorescence imaging. **(C)** Cell viability examined by MTT assay. **(D)** Quantification of contractile function using video-based motion vector analysis. Data represent mean and SEM of  $n = 4$  different iPSC-CM differentiations from 3 healthy donors indicated by different colors (iBM76.3 in green, iWTD2.1 in grey, isWT7.22 in pink). Points show technical replicates and squares represent mean values. **(E)** Changes in beating parameters over time. Pooled individual replicates from  $n = 3$  different iPSC-CM differentiations (iBM76.3 in green, iWTD2.1 in grey, isWT7.22 in pink).



**Figure S4.** Effects of AZM and HCQ treatments on contraction parameters. **(A)** Scheme of beating trace and calculated parameters using Maia motion analysis software. **(B-G)** Effects of 10  $\mu$ M AZM and 10  $\mu$ M HCQ during the treatment period of 7 days. Shown are data of the beating rate **(B)**, contraction time **(C)**, relaxation time **(D)**, displacement **(E)**, max. contraction velocity **(F)** and max. relaxation velocity **(G)**, which were normalized to the control. Points represent technical replicates and squares depict the means of individual experiments using iPSC-CM from 3 healthy donors indicated by different colors (iBM76.3 in green, iWTD2.1 in grey, isWT7.22 in pink). Lines indicate overall mean.  $n = 26-54$  videos from 4-5 experiments were analyzed per group. Statistical analysis

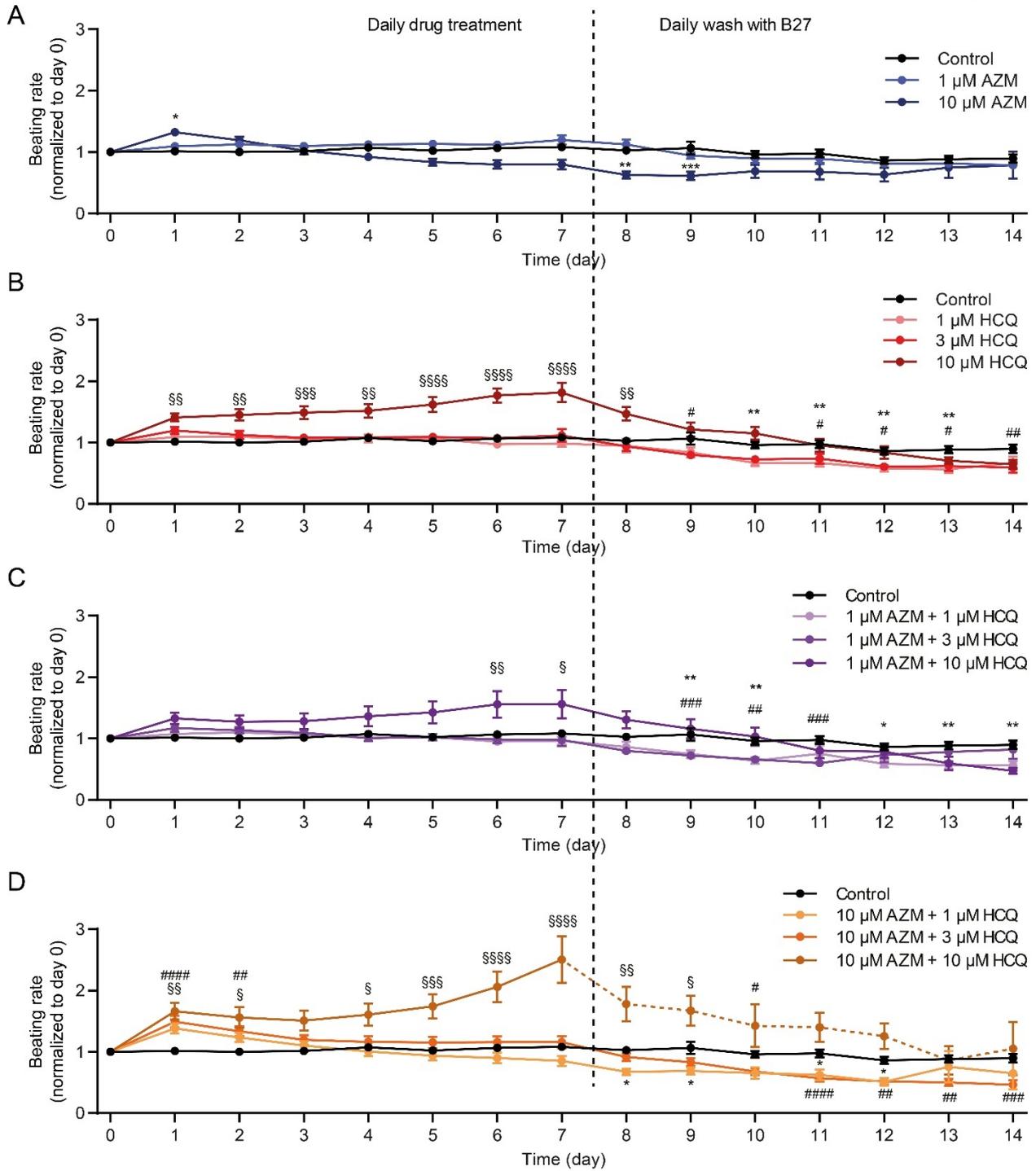
was performed based on the mean values of the individual experiments using two-way ANOVA and Tukey's multiple comparison test. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ .

Figure S5



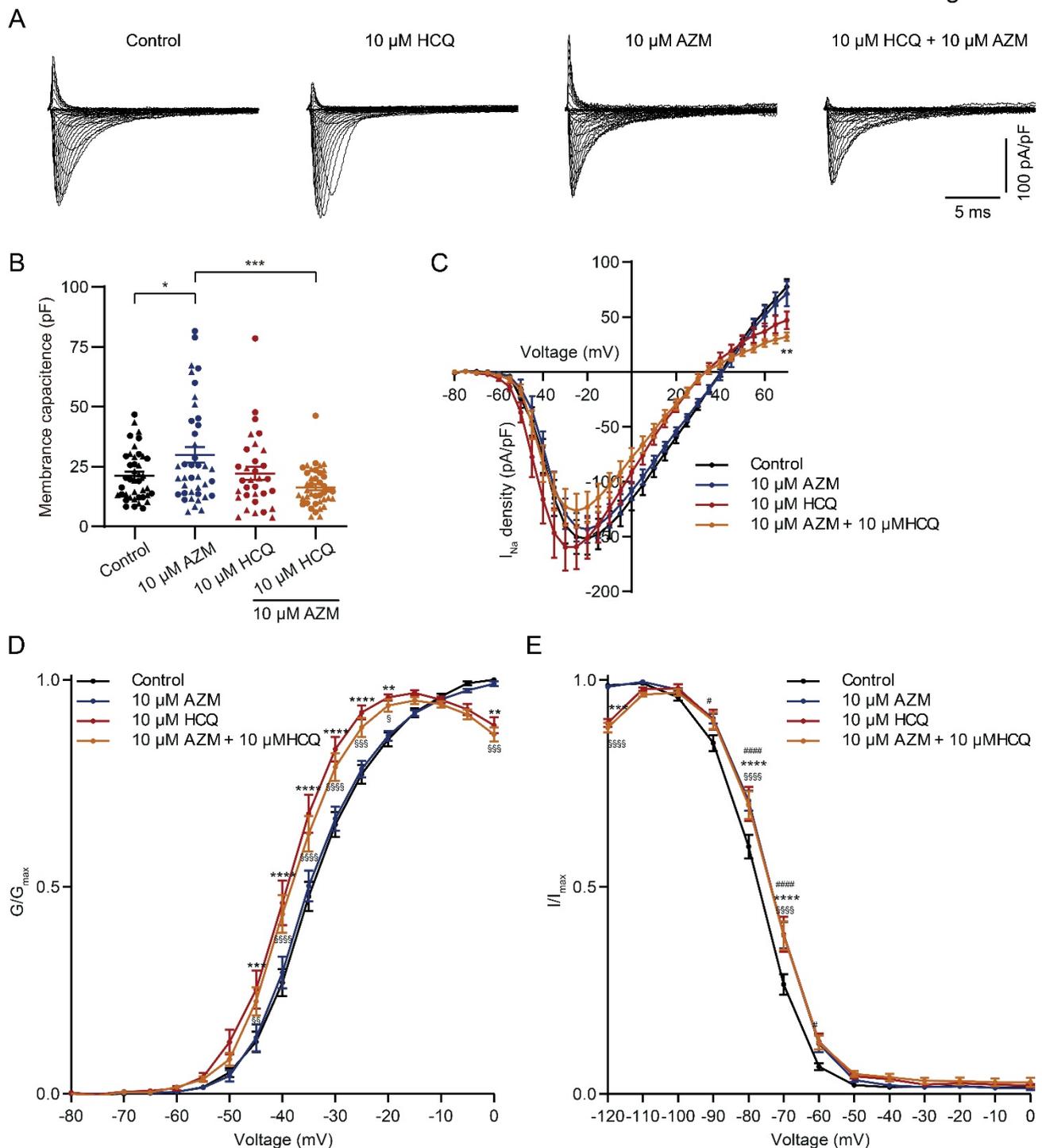
**Figure S5.** Representative images of iPSC-CMs on MEA plate. (A, B) Morphology of iPSC-CMs in the vehicle treated (A) and 10  $\mu$ M AZM and 10  $\mu$ M HCQ treated (B) groups at day 8. Combination treatment with 10  $\mu$ M AZM and 10  $\mu$ M HCQ increased the number of dead cells.

Figure S6



**Figure S6.** Effects of HCQ and AZM treatments on the beating rates of iPSC-CMs. (**A**, \*: 10  $\mu$ M AZM vs. control) Effect of AZM treatment (1 and 10  $\mu$ M) on spontaneously beating rates (normalized to day 0) during 7-day drug treatment (left) and following 7-day washout (right). (**B**, \*: 1  $\mu$ M HCQ vs. control, #: 3  $\mu$ M HCQ vs. control, §: 10  $\mu$ M HCQ vs. control) Effect of HCQ treatment (1, 3 and 10  $\mu$ M) on spontaneously beating rates during 7-day drug treatment (left) and following 7-day washout (right). (**C**, \*: 1  $\mu$ M AZM + 1  $\mu$ M HCQ vs. control, #: 1  $\mu$ M AZM + 3  $\mu$ M HCQ vs. control, §: 1  $\mu$ M AZM + 10  $\mu$ M HCQ vs. control) Effect of HCQ (1, 3, and 10  $\mu$ M) combined with 1  $\mu$ M AZM on spontaneous

beating rates during 7-day drug treatment (left) and following 7-day washout (right). (**D**, \*: 10  $\mu$ M AZM +1  $\mu$ M HCQ vs. control, #: 10  $\mu$ M AZM + 3  $\mu$ M HCQ vs. control, §: 10  $\mu$ M AZM + 10  $\mu$ M HCQ vs. control) Effect of HCQ (1, 3, and 10  $\mu$ M) combined with 10  $\mu$ M AZM on spontaneously beating rates during 7-day drug treatment (left) and following 7-day washout (right). iPSC-CMs derived from four donors were used for MEA recording. For the initial recording (day 0),  $10 \leq n \leq 13$  for all conditions. Numbers of beating iPSC-CM cultures used for the analysis are listed in Supplementary Table 1. Two-way ANOVA with Bonferroni post-hoc test (\*, #, §  $p < 0.05$ , \*\*, ##, §§  $p < 0.01$ , \*\*\*, ###, §§§  $p < 0.001$ , and ####, §§§§  $p < 0.0001$ ).



**Figure S7.** Effects of HCQ and AZM on the sodium channel. **(A)** Representative  $I_{Na}$  traces of iPSC-CMs in the control and drug-treated groups (10  $\mu$ M AZM, 10  $\mu$ M HCQ, and their combination). **(B)** Statistical analysis of membrane capacitance of iPSC-CMs in the control and drug-treated groups. Different shapes of symbols indicate different differentiations. **(C, \***: 10  $\mu$ M AZM + 10  $\mu$ M HCQ vs. control) Statistical analysis of  $I_{Na}$  in control and 7-day drug-treated groups. **(D, E, #**: 10  $\mu$ M AZM vs. control, \*: 10  $\mu$ M HCQ vs. control, §: 10  $\mu$ M AZM + 10  $\mu$ M HCQ vs. control) Steady-state activation **(D)** and inactivation **(E)** in control and 7-day drug treated groups.  $I_{Na}$  steady-state kinetics are altered

by AZM and HCQ. n=41, 40, 30, and 41 cells for the control, 10  $\mu$ M AZM-treated, 10  $\mu$ M HCQ-treated, and AZM and HCQ combination groups, respectively, were analyzed; shown are mean and SEM from 6 independent differentiations (**B-E**). One-way ANOVA with Tukey's multiple comparison test (**B**) and two-way ANOVA with Bonferroni post-hoc test (**C-E**) were used for statistical analysis (\*, #, \$  $p < 0.05$ , \*\*, \$\$  $p < 0.01$ , \*\*\*, \$\$\$  $p < 0.001$ , and \*\*\*\*, ####, \$\$\$\$  $p < 0.001$ ).

**Table S1. Spontaneously beating status of iPSC-CM culture during 15 days of the recording**

Number of beating culture	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Basal	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
1 $\mu$ M AZM	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
10 $\mu$ M AZM	11	11	11	11	11	11	11	11	11	11	9	9	9	9	9
1 $\mu$ M HCQ	10	10	10	10	10	10	10	10	10	10	9	8	8	8	8
3 $\mu$ M HCQ	10	10	10	10	10	10	10	10	10	10	10	10	9	9	8
10 $\mu$ M HCQ	12	12	12	12	12	12	12	12	12	12	11	11	10	8	6
1 $\mu$ M AZM & 1 $\mu$ M HCQ	10	10	10	10	10	10	10	10	10	10	10	10	9	9	9
1 $\mu$ M AZM & 3 $\mu$ M HCQ	11	11	11	11	11	11	11	11	11	11	10	9	8	8	8
1 $\mu$ M AZM & 10 $\mu$ M HCQ	11	11	11	11	11	11	11	11	11	11	11	10	8	6	6
10 $\mu$ M AZM & 1 $\mu$ M HCQ	11	11	11	11	11	11	11	11	11	11	10	9	9	9	9
10 $\mu$ M AZM & 3 $\mu$ M HCQ	11	11	11	11	11	11	11	11	11	11	11	11	8	8	6
10 $\mu$ M AZM & 10 $\mu$ M HCQ	11	11	11	11	11	11	11	8	5	4	4	3	2	2	2