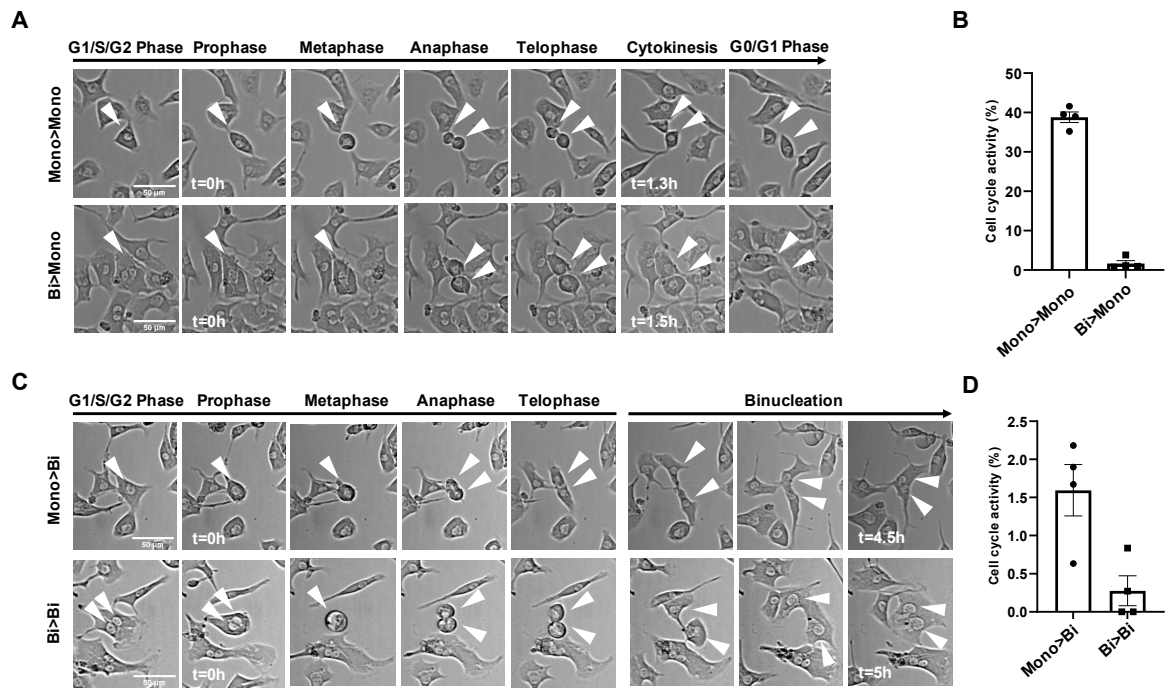
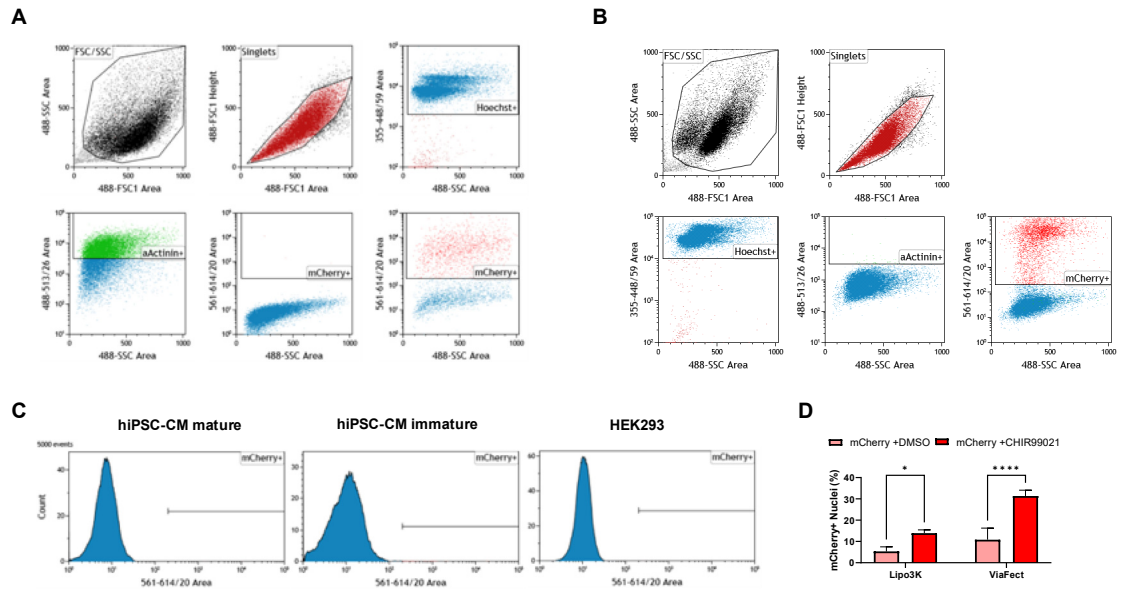


**Figure S1:** Immunofluorescence channels in mitotic hiPSC-CMs. The 594-channel (red) represents Ki67, the 488nm channel (green) represents cardiac troponin T (cTnT) and the 350nm channel (blue) represents all nuclei (DAPI).



**Figure S2:** Time lapse analysis of cytokinesis and binucleation in hiPSC-CMs. (A) Mononuclear (Mono>Mono) or binuclear (Bi>Mono) hiPSC-CMs undergoing the cytokinesis after chromosomal segregation (rolled-up morphology). Time is indicated in hours (h). Scale bar indicates 20  $\mu$ m. (B) Average relative number of mitotic mononuclear (Mono>Mono) or binuclear (Bi>Mono) hiPSC-CMs undergoing cytokinesis. Average consists of 4 replicates including in total 564 mitotic cells of 1456 counted cells for the SCVI-111 hiPSC line. Error bars indicate standard deviation (C) Bright field time-lapse images showing binucleation and chromosomal segregation of preexistent mononuclear (Mono>Bi) or binuclear (Bi>Bi) hiPSC-CMs. Time is indicated in hours (h). Scale bar indicates 10  $\mu$ m. (D) Relative number of mitotic mononuclear (Mono>Bi) or binuclear (Bi>Bi) hiPSC-CMs undergoing binucleation. Average consists of 4 replicates including in total 564 mitotic cells of 1456 counted cells for the SCVI-111 hiPSC line. Error bars indicate standard deviation.



**Figure S3:** Flowcytometry strategy and transfection efficiency between different reagents. **(A-B)** Gating strategy to select nuclei positive for Hoechst and/or mCherry and/or alpha-actinin-488 conjugated fluorescence. **(C)** Non-transfected hiPSC-CMs and HEK293 cells for negative control. **(D)** Side-to-side comparison of ViaFect and Lipofectamin 3000 (Lipo3k) transfection reagents in hiPSC-CMs transfected with mCherry plasmid in the presence or absence of 2uM CHIR99021.

**Video S1:** Mononuclear cell division of hiPSC-CMs (Mono>Mono).

**Video S2:** Binuclear self-duplication of hiPSC-CMs (Bi>Mono).

**Video S3:** Binucleation from mononucleated hiPSC-CMs (Mono>Bi).

**Video S4:** Binucleation from binucleated hiPSC-CMs (B>Bi).