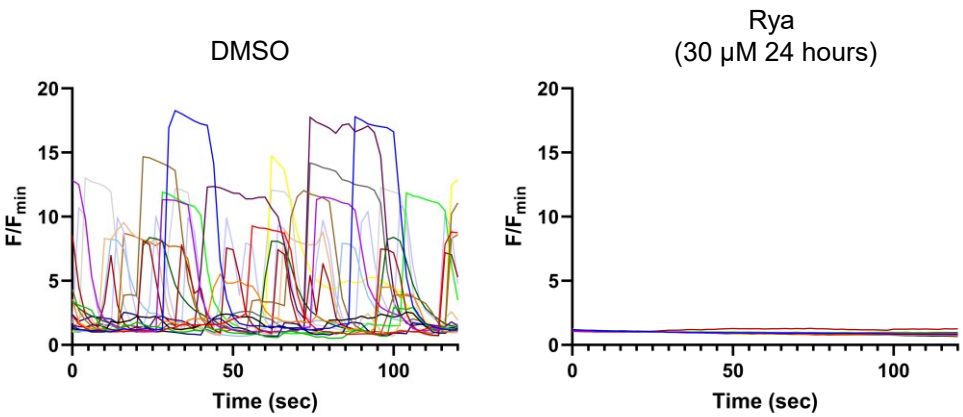
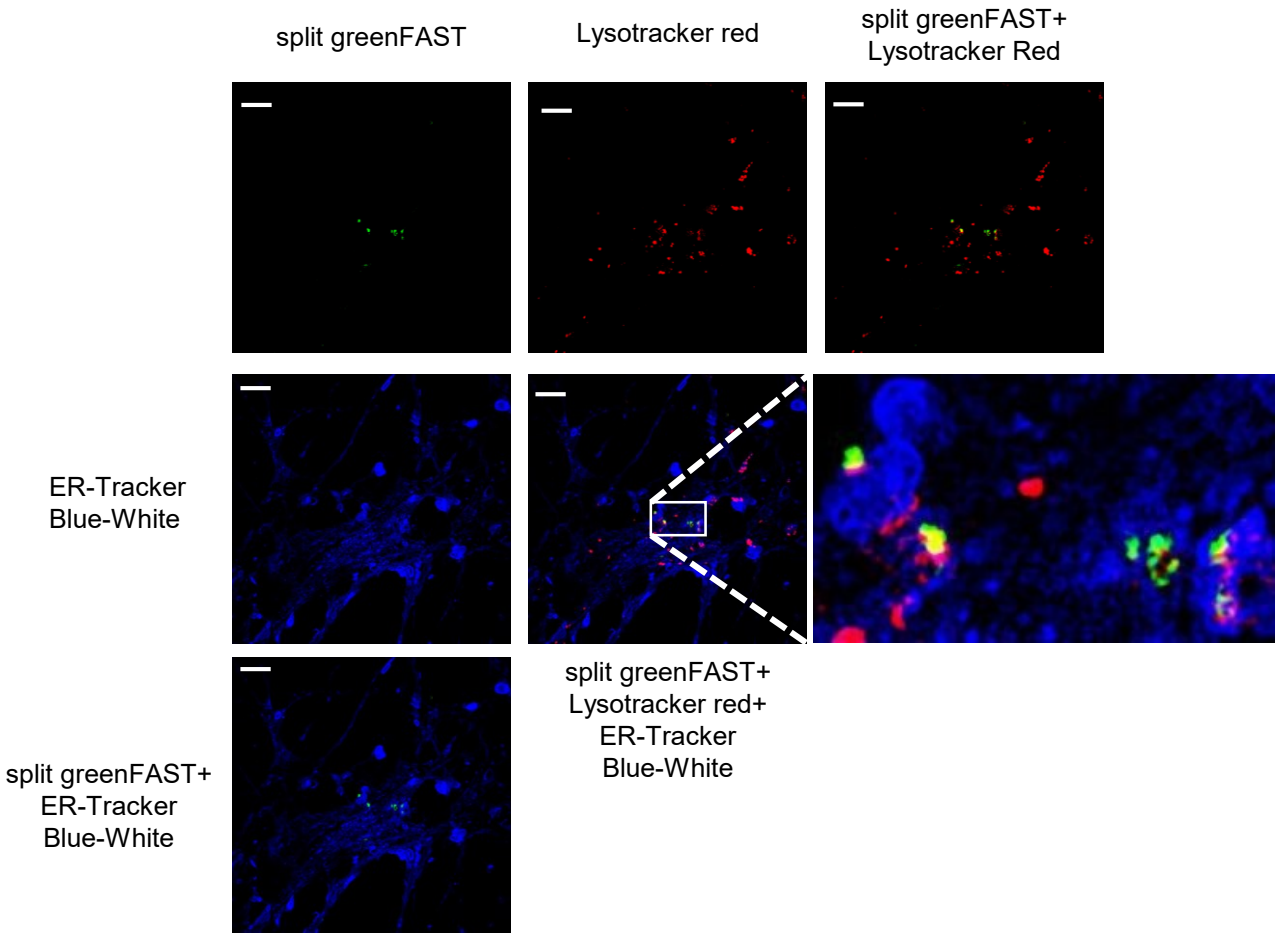


Supplemental figure 1: Validation of ryanodine treatment in T-Rex RyR2 cells.
Representative traces of single-cell Ca^{2+} measurements performed in T-Rex RyR2 cells treated for 48 h with tetracycline to induced RyR2 overexpression. LAMP1-GCaMP6s was transiently introduced in these cells to measure changes in peri-lysosomal Ca^{2+}. Each trace represents a single cell within the same experiment. Traces are depicted as F/F_{min} where F_{min} is taken as minimum value of the first 15 seconds. Both 24 h treatments with DMSO or Ryanodine (Rya) were performed in phenol red-free medium allowing to directly measure spontaneous Ca^{2+} releases without medium changes.



Supplemental figure 2: Validation of split greenFAST approach. Z-stack 3D SIM imaging of fixed hIPSC-derived cortical neurons transfected with the split greenFAST probe to detect ER-lysosomal contact sites. Lysotracker® Red DND-99 (red) and ER-Tracker™ Blue-White DPX (blue) were loaded in the cells 30 min prior to fixation. ^{TF}LIME (green) dye was loaded during the last 15 min wash step before mounting and was also included in the mounting medium. The images depict one plane (200 nm) of an obtained Z-stack. White bar indicates 5 µM.