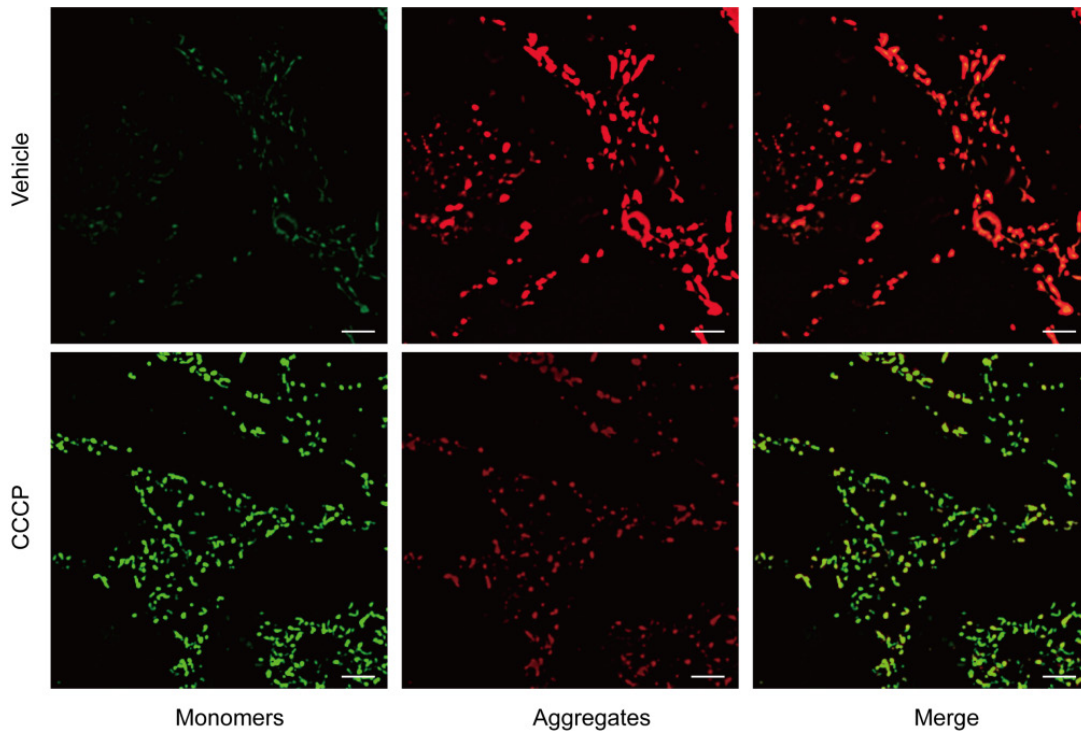


**Figure S1** The viability of cells was measured by using the CCK-8 assay. Primary cardiomyocytes exposed to different concentrations of tirzepatide for 24 h. Results of the CCK-8 assay (n=6 per group). Data are mean  $\pm$  SEM. \*\*,  $P < 0.01$ ; ns, not significant ( $P > 0.05$ ). Data were analyzed using one-way ANOVA followed by Dunnett's *post-hoc* test. ANOVA, analysis of variance; CCK-8, cell counting kit-8; DOX, doxorubicin; SEM, standard error of the mean; TIRZ, tirzepatide.



**Figure S2** In JC-1 assays employing CCCP as a positive control, treatment with this mitochondrial uncoupler induces depolarization of the MMP ( $\Delta\Psi_m$ ). Consequently, JC-1 is unable to accumulate as J-aggregates within the inner mitochondrial membrane. This results in a marked reduction in red fluorescence intensity, with a concomitant significant increase in green fluorescence. Scale bar = 10  $\mu\text{m}$ . CCCP, carbonyl cyanide 3-chlorophenylhydrazone; MMP, mitochondrial membrane potential.