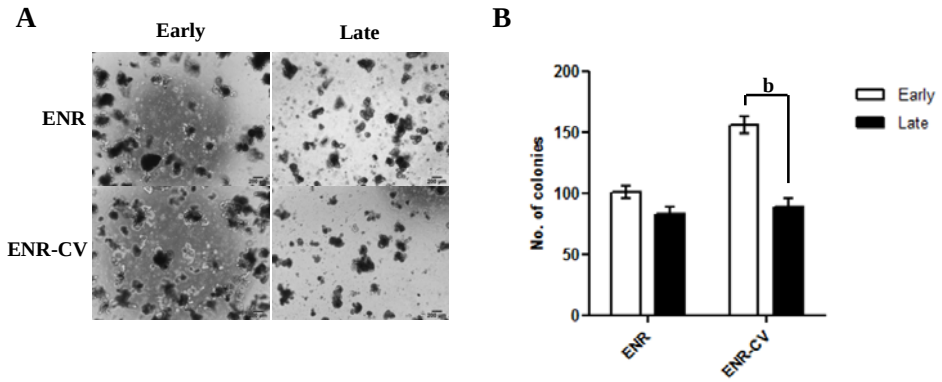
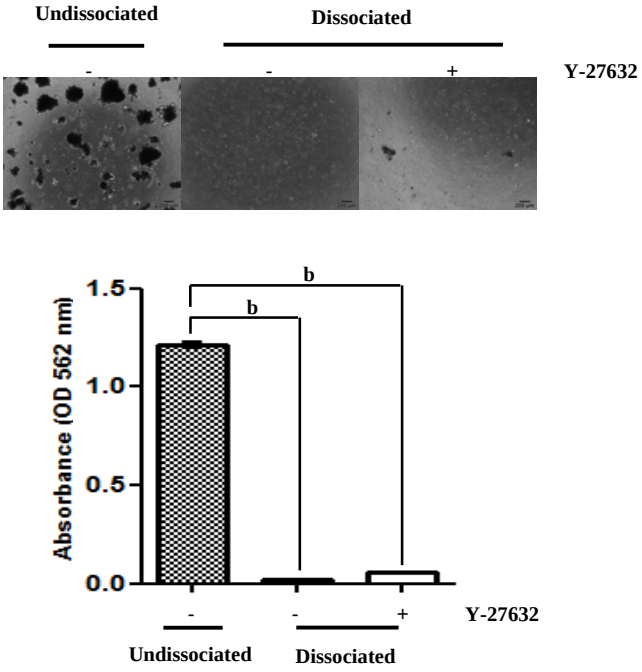
Supplementary Materials



Supplementary Figure 1 Morphology (low magnification) and number of colonies for long-term passaged organoids cultured under ENR or ENR-CV conditions. Organoids were continually passaged up to 12 times with splitting (1:4) once per week. A: Representative low-magnification images of organoids at passage 3 (P3) and passage 10 (P10) cultured under ENR or ENR-CV conditions. Scale bars: 200 μm. B: Numbers of organoids grown in ENR or ENR-CV medium for 7 days. Organoids exhibiting at least two budding structures in each group were counted. The data are shown as means ± SDs of triplicate experiments ($^{b}P < 0.01$, two-way ANOVA with Dunnett's T3 tests). P3 and P10 were used to represent early and late passages, respectively.



Supplementary Figure 2 Quantification of recovery after thawing of cryopreserved organoids with dissociation and Y-27632. Undissociated and dissociated organoids (P3) were cryopreserved in the presence of 10% DMSO and ROCK inhibitor (10 μmol/L). After 1 month, organoids were cultured under ENR conditions for 7 days. Quantification of recovery from cryopreserved organoids was determined by MTT assays. The data are shown as means ± SDs of triplicate experiments ($^{b}P < 0.01$, two-way ANOVA with Dunnett's T3 tests).