



**Figure S2: Formation of cell-cell contacts in BCa organoids.** Analysis of CL4 and ZO-1 immunoreactivity in BCa cells. (a) Representative images of BCa Orgs immunostained for CL4 and ZO-1 (brown); cell nuclei (blue). CL4 was mainly observed in the urothelial cell layers. ZO-1-IR was not detected. Scale bar: 100  $\mu$ m. (b) Quantification of CL4 immunoreactivity (IHC OD score; immunohistochemistry optical density score) in the BCa cell layer; CL4-IR was significantly higher in RT-112, T-24 and CAL-29 cells than in RT-4 cells. (c) Quantification of ZO-1 immunoreactivity in the BCa cell layer; all BCa Orgs showed low ZO-1 immunoreactivity; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\* $p < 0.0001$ ; One-way ANOVA; mean + SD;  $n=8$ .