The role of CDK6 in tumor microenvironment



Figure S1. The CDK6 expression was further evaluated by the UALCAN database according to different subtypes. The number in parentheses indicates sample size in each group. *P < 0.05, as compared between each group.



Figure S2. Expression of CDK6 in normal human mammary epithelial cell (M10) and two TNBC cell lines (MDA-MB-231 and MDA-MB-468). Cell lysates were immunoblotted by anti-CDK6. Actin was used as a loading control. Experiments were performed independently at least three times.



Figure S3. The migration of fibroblast was performed in co-culture with 4T1 and CDK6-deficient 4T1 cells. 40,000 RMF-EG cells were seeded onto 24-well plates in 500 μ L Dulbecco's modified Eagle's medium (DMEM). Additionally, 40,000 4T1 or 4T1 sh-CDK6 pooling cells were seeded onto 0.2 μ m Transwell inserts with 100 μ L DMEM and co-cultured with RMF-EG cells for 48 h. Migration of RMF-EG cells into the scratch wound after 24 h was analyzed. Original magnification: 40 ×, scale bar: 500 μ m.



Figure S4. The migration of 4T1 was performed in co-culture with CAF cells. 40,000 4T1 or 4T1 sh-CDK6 pooling cells were seeded onto 24-well plates in 500 μ L DMEM. Additionally, 40,000 cancer-associated fibroblasts were seeded onto 0.2 μ m Transwell inserts in 100 μ L DMEM and co-cultured with RMF-EG cells for 48 h. Migration of 4T1 or 4T1 sh-CDK6 pooling cells into the scratch wound after 8 h and 24 h was analyzed. Original magnification: 40 ×, scale bar: 500 μ m.



Figure S5. Protein expression levels of CDK6, c-Jun, Sp1, MMP-2, and MMP-9 in 4T1 and 4T1 sh-CDK6 pooling cells were determined by western blotting.

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Figure S6. Immunohistochemistry confirmed nuclear translocation of c-Jun and Sp1 in 4T1 and 4T1 sh-CDK6 pooling cells.