Supplementary Fig. S2. MIBC-derived succinate induces the lineage characteristics of endothelial on SMCs. (**a-b**) Representative immunofluorescence (IF) staining images (**a**) and protein expression (**b**) of endothelial signature in PBSMCs co-cultured with the indicated CM/PDBCs or CM/PDBCs. Scale bars, 20 μm. GAPDH served as a loading control. (**c**) *In vitro* tube formation assay was performed with PBSMCs pre-educated with CM from NMI/PDBCs or MI/PDBCs, followed by CD31-staining in the tube forming cells (Scale bars, 50 μm); and the angiogenesis capability of PBSMCs were quantified by in *vivo* Matrigel plug assay (Scale bars, 20 μm). (**d**) NMI/PDBCs-CM and MI/PDBCs-CM were fractionated into SFC with micromolecular (< 3 kDa) and LFC with macromolecules (> 3 kDa). IB analysis of expression of endothelial specific marker in PBSMCs co-cultured with NMIBC- or MIBC-derived SFC or LFC. GAPDH served as a loading control. (**e**) Real-time PCR analysis of the mRNA expression of *CD31, CD144*, and *vWF* in PBSMCs under the indicated treatment. *GAPDH* serves as the internal control. (**f-g**) Succinate levels in the condition medium of bladder cancer cell lines (**e**) and NMI-PDBC (n = 8) or MI-PDBC cells (n = 7) (**f**). (**g**) Real-time PCR analysis of the mRNA expression of *CD31, CD144*, and *vWF* in PBSMCs treated with the indicated concentrations of succinate. (**h**) The morphology changes of PBSMCs with the indicated treatments. Scale bars, 50 μm. (**i**) AUROC curve analysis of the discriminative power of serum succinate in NMIBC and MIBC patients ($P = 0.001$; SE = 0.026; AUC = 0.796). Each error bar in a, c, d, e, f and h represents the mean ± SD of three independent experiments. Statistical analysis was performed using Two-way ANOVA with Šídák's multiple comparisons test for (a, e and h), and unpaired two-tailed t tests for (c and g). $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

Supplementary Fig. S2