Appendix 1: Methods

qRT-PCR analysis

The SPP1 mRNA expression level was measured by qRT-PCR through utilizing qRT-PCR kit according to manufacture protocol. Primers sequences for SPP1 are listed in Table S1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was employed as the internal reference for qRT-PCR.

Clinical sample analysis

Human BC tissues were fixed by formalin before experiment. The slides were treated with 100 μL 6 μM SPP1 MB for 4 hours at 37 ℃. Subsequently, the slides were washed 3 times with PBS and were examined by laser confocal microscope (Zeiss LSM 710 with a 40× objective).

IHC

The tissue was fixed with 4% paraformaldehyde and embedded in paraffin, which was further cut into 5 μm sections. Subsequently, the slides were incubated with blocking solution for 30 minutes. After that, the slides were subjected to the primary anti-SPP1 antibody at 4 ℃ overnight. The slides were incubated with secondary antibody in the next day for 30 minutes at 25 ℃. Finally, 3,3'-diaminobenzidine (DAB) was added onto the slides and stained with hematoxylin. The images were obtained by microscope (Leica DM IL LED).

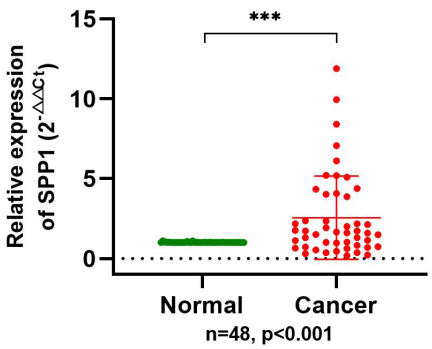

Figure S1 The relative levels of SPP1 by real-time qRT-PCR (in triplicate) analysis. Results showing the different expression of SPP1 in bladder tumor adjacent tissues (n=42) and cancer tissues (n=42) by comparison of fold change ($2^{-\Delta\Delta Ct}$). Paired student's *t*-test was used for the statistical analysis. ***, P<0.001. SPP1, secreted phosphoprotein 1; qRT-PCR, quantitative real-time polymerase chain reaction.

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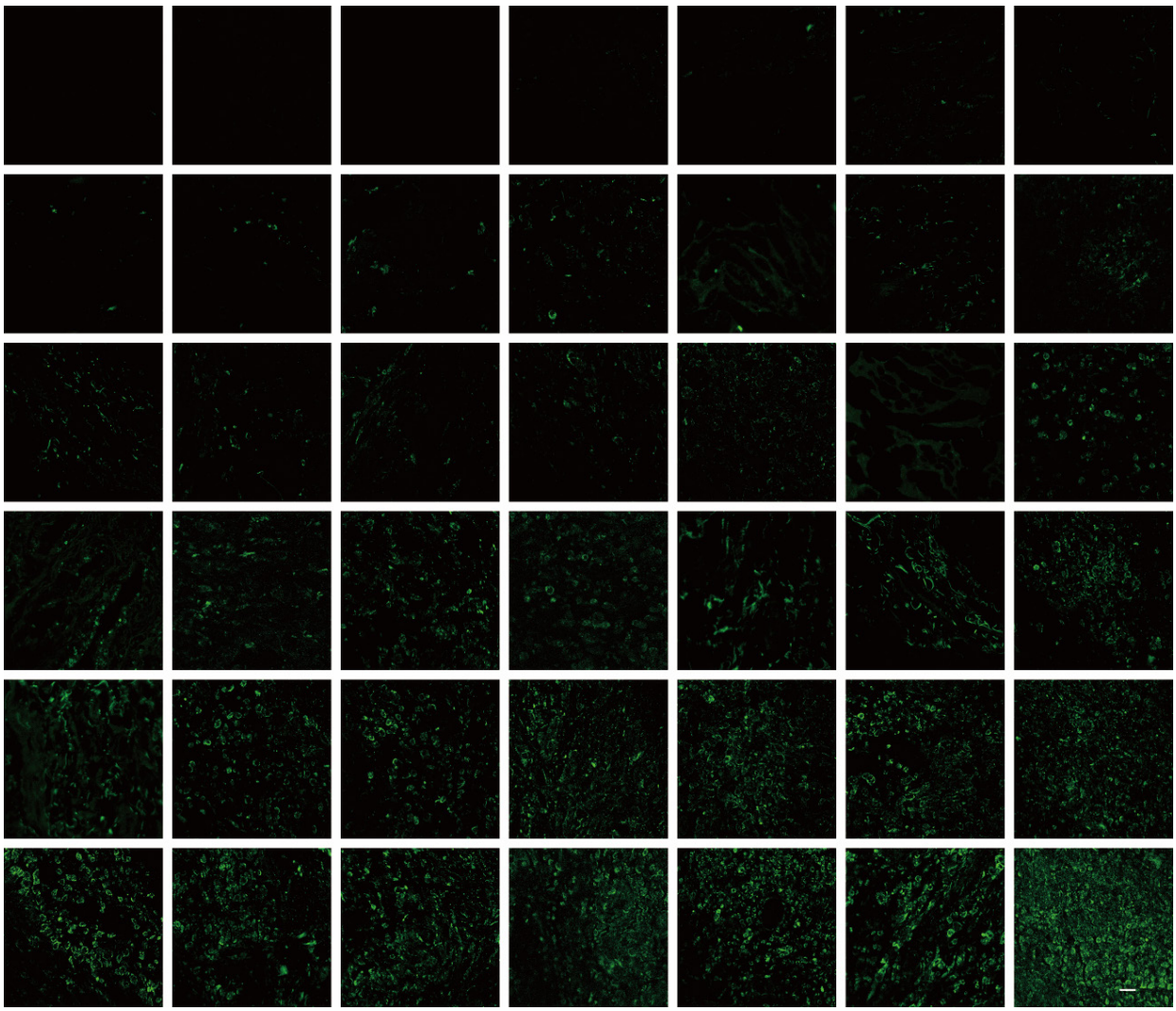

Figure S2 The SPP1 MB application in tumor tissues (n=42). The slides were cultured with 100 μL 6 μM SPP1 MB for 4 h at 37 ℃. The images were obtained by laser confocal microscope (Zeiss LSM 710 with a 40× objective). Scale bar: 20 μm. SPP1, secreted phosphoprotein 1; MB, molecular beacon.

Table S1 DNA sequences designed in this work

Name	Sequence (5' to 3')
MB	FAM-TCGACACTGGTCATGGCTTTCGTTGGAGTGTCGA-Dabcyl
Target	TCCAACGAAAGCCATGACCA
SM	TCCAACTAAAGCCATGACCA
TM	TCCAACTACAGCCATGACCA
Primer-F	CCAACGAAAGCCATGACCAC
Primer-R	TGAAAACTTCGGTTGCTGGC

MB, molecular beacon; SM, single-base mismatched strand; TM, three-base mismatched strand; F, forward; R, reverse.

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