Supplementary Fig. S8. Blocking SMAD2-ETV4 binding inhibited TGFβ3-mediated SMCs transdifferentiation in MIBC. (**a**) IB analysis of SQOR expression in the indicated cells treated with L-chicoric acid. GAPDH serve as a loading control. (**b**) ChIP assay analysis of ETV4, p-SMAD2 and CARM1 enrichment on the SQOR promoter in the indicated cells. (**c**) ChIP assay analysis of H3R17me2a, H2BK120ub and H3K4me3 enrichment on the *SQOR* promoter in the indicated cells. (**d**) CCK-8 assay analysis of proliferation rate of the indicated cells. (**e**) *In vitro* tube formation/IF staining assays analysis of PBSMCs exposed to different SFC from indicated cells following treatment with vehicle, L-chicoric acid. Scale bars, 20 μm. (**f**) *In vivo* Matrigel plug assays were performed in PBSMCs treated with the indicated SFC as (**e**). Scale bars, 20 μm. n = 3 mice per group. (**g**) Representative immunofluorescence images (left) and quantification of intramuscular CD31$^+$ or α-SMA$^+$/CD31$^+$ vessels (right) in mice following intradetrusor injections of PBSMCs pre-educated with different SFC from indicated cells accompanied with L-chicoric acid or vehicle control treatment, respectively. Scale bars, 20 μm. n = 6 mice per group. Each error bar in b, c, d, e and f represents the mean ± SD of three independent experiments, error bar in g represents as the mean ± SD of n = 6 mice per group. Statistical analysis was performed using Two-way ANOVA with Šídák's multiple comparisons test for (b, c, d, e, f and g). **P < 0.01; ***P < 0.001; ns, not significant.

Supplementary Fig. S8

a

L-chicoric acid (μM)

0 1 2 4 6 8 kDa

SQOR — −40

GAPDH — −35

b

Vehicle / L-chicoric acid

Relative enrichment of ETV4 in SQOR promoter — RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***

Relative enrichment of p-SMAD2 in SQOR promoter — RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***

Relative enrichment of CARM1 in SQOR promoter — RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***

c

Relative enrichment of H3R17me2a in SQOR promoter — RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***

Relative enrichment of H2B-ub in SQOR promoter — RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***

Relative enrichment of H3K4me3 in SQOR promoter — RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***

d

CKK-8 absorption intensity ratio (%) — RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ns

e

Non-treated / SFC-UMUC3/Vehicle / SFC-UMUC3/L-chicoric acid / SFC-MI#1/Vehicle / SFC-MI#1/L-chicoric acid

Bright Field / CD31/DAPI — 20μm

Non-treated / SFC-RT4/TGFβ3/Vehicle / SFC-RT4/TGFβ3/L-chicoric acid / SFC-NMI#3/TGFβ3/Vehicle / SFC-NMI#3/TGFβ3/L-chicoric acid

Bright Field / CD31/DAPI — 20μm

Non-treated / Vehicle / L-chicoric acid

PBSMCs — Tot. length (×10⁴ pixels) — SFC: RT4/TGFβ3, NMI#3/TGFβ3 — ***

PBSMCs — CD31 particles (%) — RT4/TGFβ3, NMI#3/TGFβ3 — ***

PBSMCs — Tot. length (×10⁴ pixels) — SFC: UMUC3, MI#1 — ***

PBSMCs — CD31 particles (%) — UMUC3, MI#1 — ***

f

PBSMCs pre-treated with SFC

Vehicle / L-chicoric acid — CD31/DAPI

RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI-PDBCs#1 — 20μm

Vehicle / L-chicoric acid

PBSMCs — No. microvessel of plug/field — RT4/TGFβ3, NMI#3/TGFβ3 — ***

PBSMCs — No. microvessel of plug/field — UMUC3, MI#1 — **

g

Intramuscular injection PBSMCs pre-educated with SFC from

RT4/TGFβ3 / NMI#3/TGFβ3 / UMUC3 / MI#1

Vehicle / L-chicoric acid — 20μm

α-SMA & CD31

Vehicle / L-chicoric acid

No. intramuscular vessels per field — SFC: RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***

No. intramuscular α-SMA⁺CD31⁺ vessels per field — SFC: RT4/TGFβ3, NMI#3/TGFβ3, UMUC3, MI#1 — ***