Supplemental Information

MAOA-Mediated Reprogramming of Stromal Fibroblasts Promotes Prostate Tumorigenesis and Cancer Stemness

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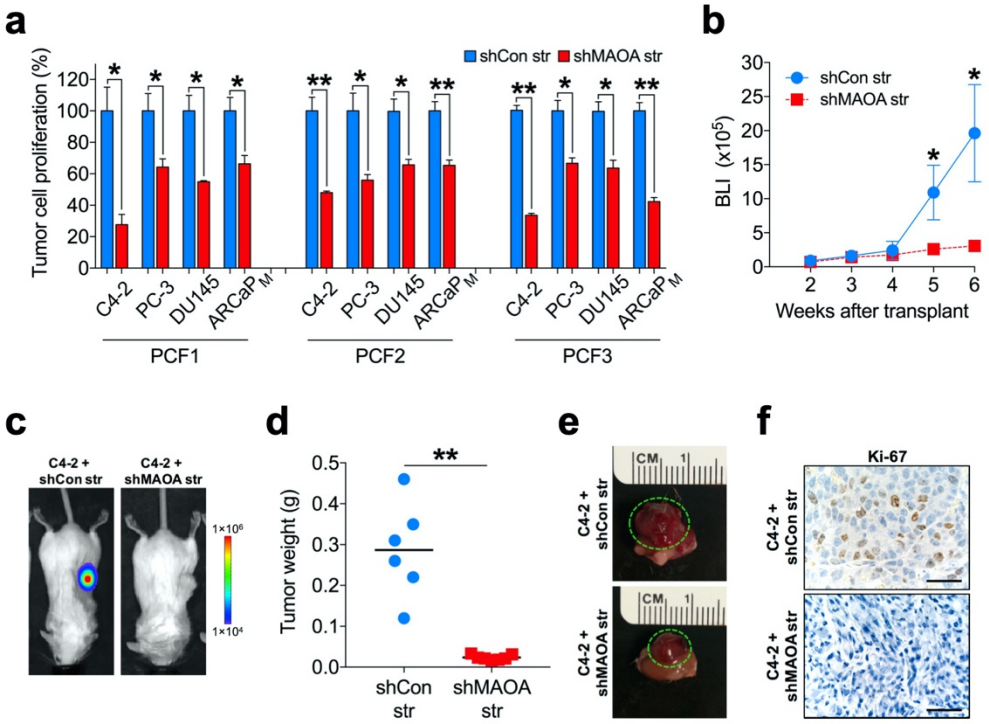
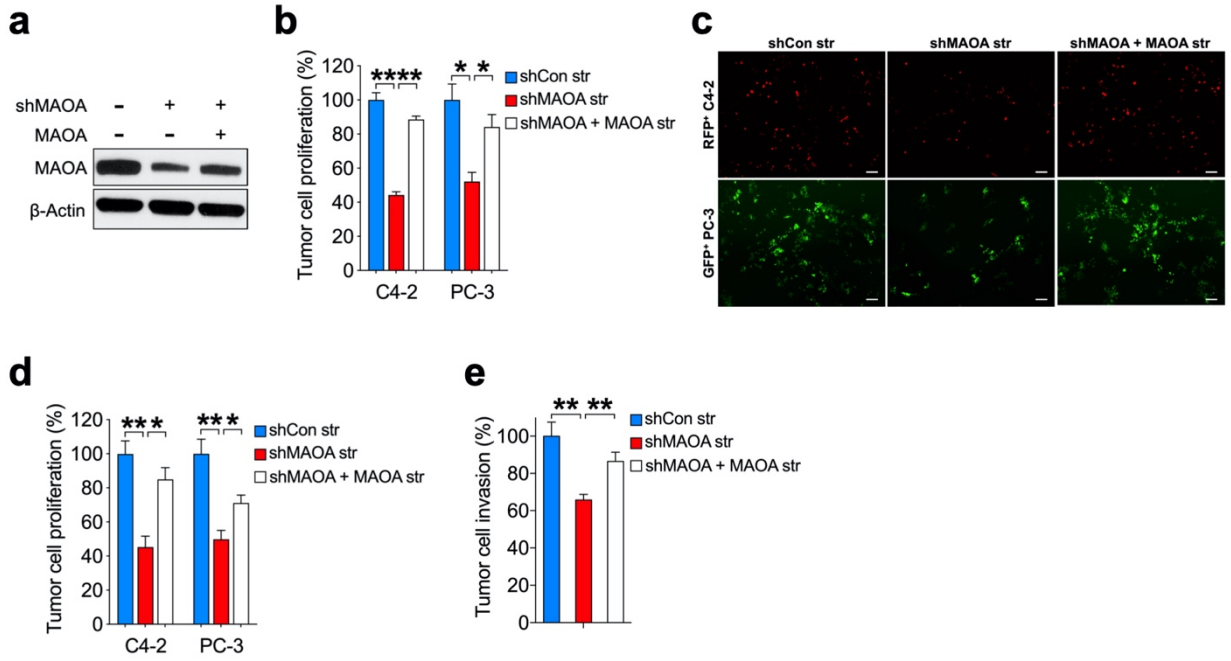
Supplemental Data

Supplemental Fig. 1



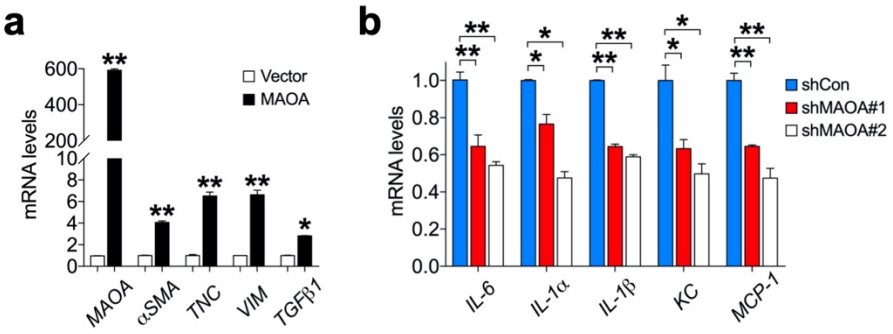
Supplemental Fig. 1 Tumor-promoting activity of stromal MAOA in additional PC models, related to Fig. 2. a Quantification of luciferase-tagged PC cells co-cultured with control or MAOA-KD PCF stromal (str) cells for 5 days by luciferase assays (*n*=3). **b** BLI growth curves of subrenal capsule xenografts comprised of luciferase-tagged C4-2 cells in combination with control (*n*=6) or MAOA-KD (*n*=7) PrSC cells in SCID mice. **c** Representative BLI images from each group in (b). **d** Tumor weights from each group in (b) measured at the experimental endpoint. **e** Representative anatomical images of tumor-grown mouse kidneys from each group in (b) at the experimental endpoint. Green dashed circles denote size of tumor outgrowth from renal capsules. **f** Representative IHC images of tumor Ki-67 staining from each group in (b). Scale bars: 20 μm. Data represent the mean ± SEM. **p*<0.05, ***p*<0.01.

Supplemental Fig. 2



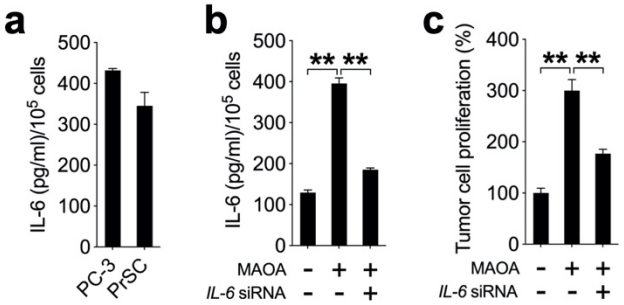
Supplemental Fig. 2 Reintroduction of MAOA rescued PC cell proliferation and invasion in co-cultures with MAOA-KD stromal cells, related to Fig. 2. a Western blot analysis of MAOA expression in prostate stromal fibroblast PrSC cells expressing a scrambled shRNA (shCon), a *MAOA*-targeting shRNA (shMAOA), or a *MAOA*-targeting shRNA further with transient infection with MAOA-expressing lentiviruses (shMAOA + MAOA). **b** Quantification of luciferase-tagged PC cells co-cultured with control, MAOA-KD or MAOA-KD/-overexpressing (OE) PrSC stromal (str) cells for 5 days by luciferase assays (n=3). **c** Representative images of RFP-tagged C4-2 or GFP-tagged PC-3 cells co-cultured with control, MAOA-KD or MAOA-KD/-OE for 5 days. Scale bars: 200 μm. **d** Quantification of PC cell fluorescence signals in the experiment described in (c) (n=3). **e** Quantification of PC-3 cells invading to the lower surface of inserts in transwells stained by crystal violet in co-cultures with control or MAOA-KD PrSC cells grown in the lower chambers (refer to Figs. 2g and 2h) (n=3). Data represent the mean ± SEM. *p<0.05, **p<0.01.

Supplemental Fig. 3



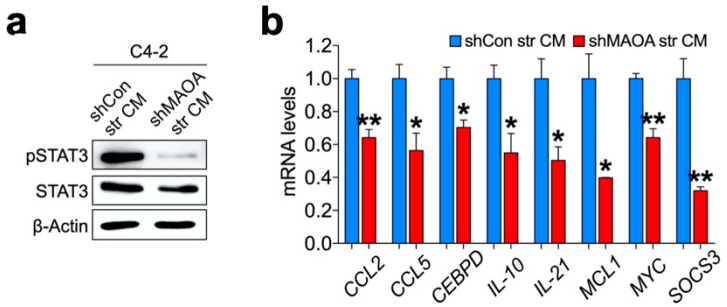
Supplemental Fig. 3 MAOA activates reactive stromal marker and pro-inflammatory cytokine/chemokine gene expression in prostate stromal cells, related to Fig. 3. a Select reactive stromal marker mRNA levels in control and MAOA-OE PrSC cells by RT-qPCR (*n*=3). **b** Select pro-inflammatory cytokine/chemokine mRNA levels in control and MAOA-KD PrSC cells by RT-qPCR (*n*=3). Data represent the mean ± SEM. **p*<0.05, ***p*<0.01.

Supplemental Fig. 4



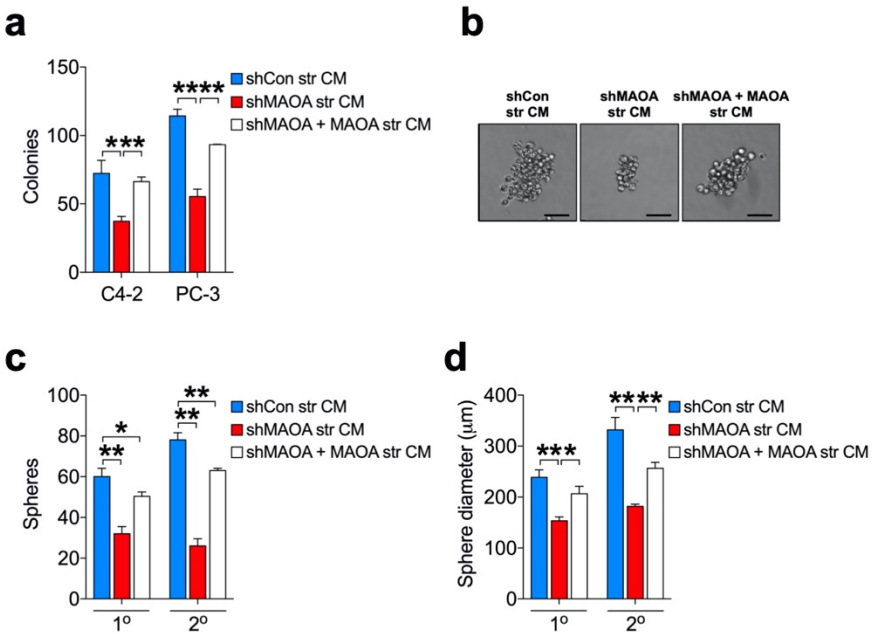
Supplemental Fig. 4 MAOA in prostate stromal cells promotes autocrine IL-6-expressing PC-3 cell proliferation through activation of paracrine IL-6 signaling, related to Fig. 3. a Quantification of IL-6 protein secretion in PC-3 and PrSC cells by ELISA (*n*=3). **b** Quantification of IL-6 protein secretion in control, MAOA-OE, and MAOA-OE/siRNA-mediated IL-6-KD PrSC cells by ELISA (*n*=3). **c** Quantification of luciferase-tagged PC-3 cells treated with conditioned media from respective PrSC cells as described in (b) for 3 days by luciferase assays (*n*=3). Data represent the mean ± SEM. ***p*<0.01.

Supplemental Fig. 5



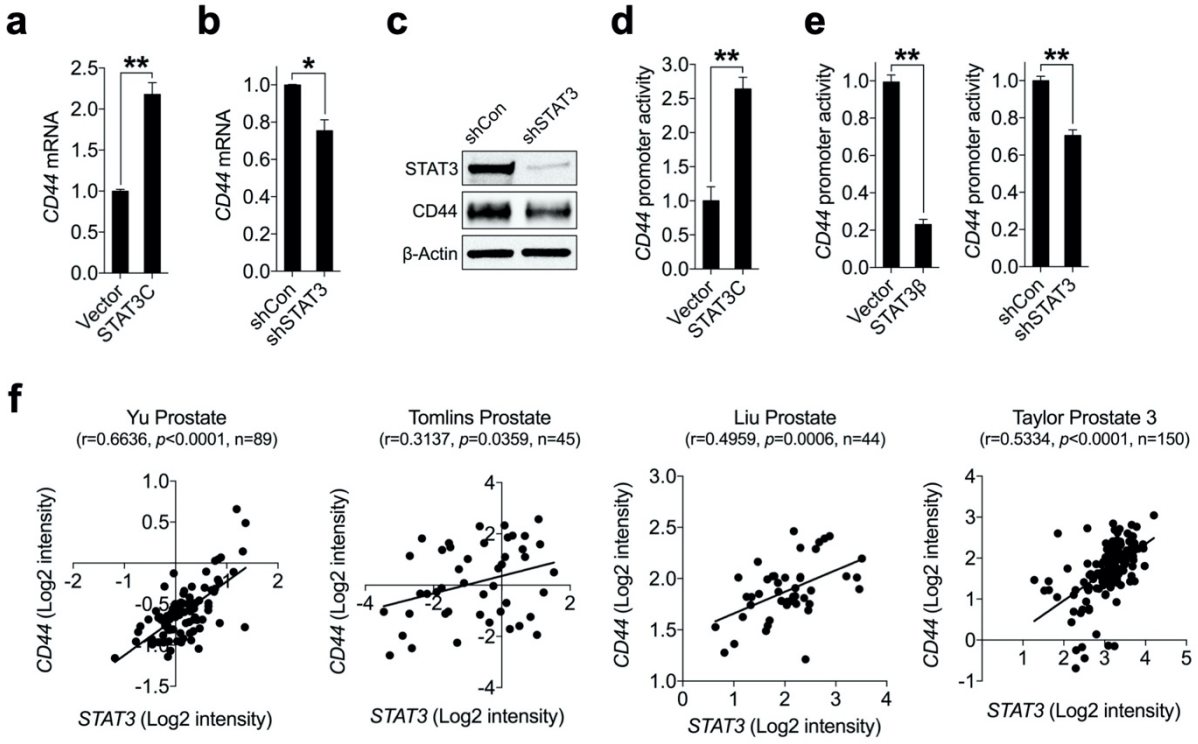
Supplemental Fig. 5 MAOA silencing in stromal cells suppressed STAT3 signaling in adjacent PC, related to Fig. 5. a Western blot analysis of pSTAT3 and total STAT3 protein expression in C4-2 cells treated with conditioned media (CM) from control (shCon) or MAOA-KD (shMAOA) PrSC stromal cells for 1 hr. b Select STAT3 target gene mRNA levels in PC-3 cells treated with CM from control or MAOA-KD PrSC cells for 3 days by RT-qPCR (*n*=3). Data represent the mean ± SEM. **p*<0.05, ***p*<0.01.

Supplemental Fig. 6



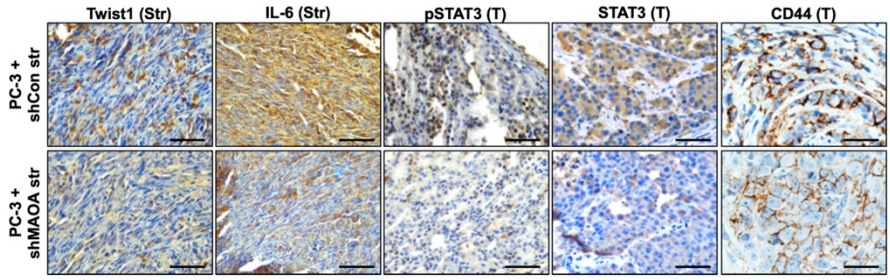
Supplemental Fig. 6 Re-expression of MAOA rescued PC stemness in co-cultures with MAOA-KD stromal cells, related to Fig. 7. a Clonogenic assays of PC cells plated in Matrigel, exposed to control (shCon), MAOA-KD (shMAOA) or MAOA-KD/OE (shMAOA + MAOA) PrSC CM (refer to Supplemental Fig. 2A for MAOA expression levels in these cells), and enumerated on day 9 (*n*=3). **b-c** Sphere-formation assays of PC-3 cells exposed to control, MAOA-KD or MAOA-KD/-OE PrSC CM for 6 days and enumerated for primary and secondary spheres formed on day 6 (*n*=3). Representative primary spheres formed on day 6 are shown. Scale bars: 100 µm. **d** Quantification of sphere diameter in (b and c). Data represent the mean ± SEM. **p*<0.05, ***p*<0.01.

Supplemental Fig. 7



Supplemental Fig. 7 STAT3 activates *CD44* gene expression in PC, related to Fig. 7. a *CD44* mRNA levels in C4-2 cells stably expressing a constitutively active form of *STAT3* (STAT3C) by RT-qPCR (*n*=3). **b** *CD44* mRNA levels in control and STAT3-KD PC-3 cells by RT-qPCR (*n*=3). **c** Western blot analysis of STAT3 and CD44 protein expression in control and STAT3-KD DU145 cells. **d** Determination of STAT3's effect on *CD44* promoter activity in STAT3C-OE PC-3 cells (*n*=3). **e** Determination of STAT3's effect on *CD44* promoter activity in PC-3 cells stably expressing either a dominant negative form of *STAT3* (STAT3β, left) or a *STAT3* shRNA (right) (*n*=3). **f** Positive co-expression correlation between *STAT3* and *CD44* mRNA levels in 4 human PC data sets (Yu, Tomlins, Liu and Taylor 3) deposited in the Oncomine database. Data represent the mean ± SEM. *p<0.05, **p<0.01.

Supplemental Fig. 8



Supplemental Fig. 8 Stromal MAOA silencing downregulated Twist1/IL-6-STAT3/CD44 paracrine signaling mediating MAOA-induced stromal-epithelial interaction in xenograft prostate tumor samples, related to Fig. 7. Representative IHC images of Twist1 and IL-6 staining in stroma (Str) and pSTAT3, STAT3 and CD44 staining in tumor (T) from subrenal xenograft tumors grown from PC-3 cells co-inoculated with control (shCon) or MAOA-KD (shMAOA) PrSC cells in SCID mice (refer to Fig. 2 for details of the animal experiment). Scale bars: 20 μm.

Supplemental Table 1

Sample number	MAOA (Str)	Twist1 (Str)	IL-6 (Str)	pSTAT3 (T)	STAT3 (T)	MAOA (T)	STAT3 (Str)
1	0.020	0.066	0.038	0.055	0.405	0.058	0.069
2	0.017	0.028	0.041	0.057	0.079	0.176	0.044
3	0.012	0.041	0.041	0.061	0.169	0.037	0.043
4	0.013	0.029	0.044	0.053	0.060	0.059	0.027
5	0.020	0.092	0.061	0.054	0.333	0.043	0.053
6	0.010	0.025	0.049	0.044	0.335	0.035	0.029
7	0.015	0.046	0.040	0.060	0.366	0.029	0.097
8	0.016	0.027	0.037	0.044	0.034	0.040	0.031
9	0.019	0.059	0.044	0.058	0.542	0.043	0.081
10	0.010	0.033	0.041	0.067	0.160	0.083	0.023
11	0.024	0.085	0.037	0.049	0.257	0.086	0.080
12	0.015	0.045	0.062	0.044	0.277	0.049	0.047
13	0.013	0.062	0.052	0.059	0.515	0.117	0.030
14	0.012	0.018	0.010	0.057	0.397	0.049	0.025
15	0.013	0.025	0.048	0.053	0.169	0.058	0.029
16	0.018	0.066	0.047	0.062	0.386	0.020	0.052
17	0.021	0.074	0.048	0.075	0.400	0.015	0.049
18	0.021	0.057	0.089	0.050	0.257	0.060	0.017
19	0.019	0.062	0.056	0.059	0.160	0.027	0.075
20	0.016	0.069	0.044	0.047	0.483	0.025	0.050
21	0.020	0.064	0.055	0.049	0.388	0.053	0.045
22	0.014	0.094	0.050	0.077	0.469	0.036	0.064
23	0.011	0.086	0.044	0.059	0.590	0.017	0.058
24	0.014	0.050	0.070	0.066	0.085	0.089	0.050
25	0.016	0.108	0.055	0.082	0.521	0.042	0.050
26	0.013	0.081	0.052	0.068	0.270	0.028	0.079
27	0.017	0.036	0.049	0.057	0.276	0.043	0.072
28	0.003	0.025	0.044	0.053	0.424	0.020	0.021
29	0.005	0.042	0.042	0.050	0.294	0.055	0.020
30	0.003	0.039	0.010	0.057	0.530	0.043	0.064
31	0.033	0.072	0.124	0.057	0.459	0.046	0.071
32	0.039	0.094	0.113	0.076	0.690	0.058	0.089
33	0.007	0.040	0.053	0.044	0.422	0.072	0.052
34	0.003	0.037	0.010	0.060	0.101	0.014	0.022
35	0.048	0.106	0.129	0.090	0.611	0.043	0.061
36	0.001	0.040	0.011	0.053	0.154	0.082	0.048
37	0.001	0.004	0.001	0.057	0.268	0.061	0.008
38	0.004	0.036	0.051	0.062	0.141	0.037	0.077
39	0.005	0.036	0.056	0.060	0.224	0.065	0.041
40	0.002	0.039	0.008	0.050	0.188	0.043	0.029

Supplemental Table 1 Quantification of IHC staining of MAOA (tumor and stroma), Twist1 (stroma), IL-6 (stroma), pSTAT3 (tumor and stroma), and STAT3 (tumor) in serial sections of PC TMAs, related to Fig. 6. Average cell-based staining intensity counts for each individual protein in either tumor or stromal compartments analyzed by HALO software are shown.

Supplemental Materials and Methods

Quantitative real-time PCR

qPCR was conducted using SYBR Green PCR Master Mix and run with the Applied Biosystems QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). PCR conditions included an initial denaturation step of 3 min at 95°C, followed by 40 cycles of PCR consisting of 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C. The PCR data were analyzed by $2^{-\Delta\Delta CT}$ method [1].

Gene	Forward	Reverse
MAOA	CTGATCGACTTGCTAAGCTAC	ATGCACTGGATGTAAAGCTTC
αSMA	ACCCACAATGTCCCCATCTA	GAAGGAATAGCCACGCTCAG
TNC	ACAGTAGAGGCAGCCCAGAA	AGAGAGAGGGGTTGTGCTGA
VIM	GAGAACTTTGCCGTTGAAGC	GCTTCCTGTAGGTGGCAATC
TGFβ1	CCCAGCATCTGCAAAGCTC	GTCAATGTACAGCTGCCGCA
IL-6	ACAGCCACTCACCTCTTC	AAGTCTCCTCATTGAATCCAG
Twist1	GGAGTCCGCAGTCTTACGAG	TCTGGAGGACCTGGTAGAGG
CCL2	CCCCAGTCACCTGCTGTTAT	AGATCTCCTTGGCCACAATG
CCL5	CGCTGTCATCCTCATTGCTA	GCACTTGCCACTGGTGTAGA
CEBPD	AGAAGTTGGTGGAGCTGTCG	TTACCGGCAGTCTGCTGTC
IL-10	TTACCTGGAGGAGGTGATGC	GGCCTTGCTCTTGTTTTCAC
IL-21	TGGTCAGCTTTTTCCTGCTT	TTGTGGAAGGTGGTTTCCTC
MCL1	TAAGGACAAAACGGGACTGG	ACCAGCTCCTACTCCAGCAA
MYC	TCAAGAGGCGAACACACAAC	GGCCTTTTCATTGTTTTCCA
SOCS3	GCCACCTACTGAACCCTCCT	AACACCAGGGGGATCTTCTC
CD44	AGAAGGTGTGGGCAGAAGAA	AAATGCACCATTTCCTGAGA
ALDH1A1	AGTGCCCCTTTGGTGGATTC	AAGAGCTTCTCTCCACTCTTG
ABCG2	TGAAACCTGGTCTCAACGCCATCC	CGTCAGAGTGCCCATCACAACAT
BMI1	TGGAGAAGGAATGGTCCACTTC	GTGAGGAAACTGTGGATGAGGA
β-Actin	TTGTTACAGGAAGTCCCTTGCC	ATGCTATCACCTCCCCTGTGTG

Supplemental References

1 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.