Supplementary Materials

SAA1 is upregulated in gastric cancer-associated fibroblasts possibly by its enhancer activation

Yoshimi Yasukawa[1, 2], Naoko Hattori[1], Naoko Iida[1], Hideyuki Takeshima[1], Masahiro Maeda[1], Tohru Kiyono[3], Shigeki Sekine[4], Yasuyuki Seto[2], and Toshikazu Ushijima[1*]

Supplementary Table 1. Primers for RT-qPCR and ChIP-qPCR

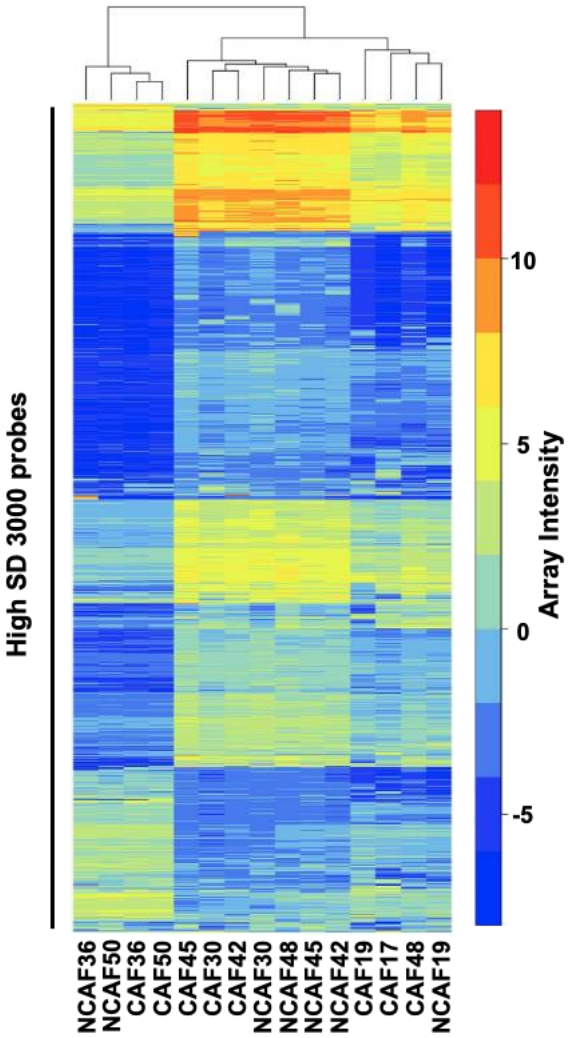
Experiment	Region	Primer sequence	
		Forward	Reverse
RT-qPCR	*SAA1* exon 3-4	TGCCTGGGCTGCAGAAGTGA	TGATCAGCCAGCGAGTCCTC
	GAPDH exon 2-3	AGGTGAAGGTCGGAGTCAACG	AGGGGTCATTGATGGCAACA
ChIP-qPCR	*SAA1* enhancer 1	GCAAAGTGAACGAGCTTCGG	CTCCACCCACTCTCTCAGGA
	SAA1 enhancer 2	GACGACAGTCAGGAGCTCAC	CATGTCCCCTCCCATTCCAG
	SAA1 promoter	CCAGGCACATCTTGTTCCCT	AGTCCCTGCAGGTCATTTCC

Supplementary Table 2. Oligonucleotide sequences for shRNA

	Region	Oligonucleotide sequence	
		Top	Bottom
sh1	*SAA1* exon 3	GATCCAAGCCAATTACATCGGCT CAGACAAACTTCCTGTCAGATT TGTCTGAGCCGATGTAATTGGCT TTTTTTG	AATTCAAAAAAAGCCAATTACA TCGGCTCAGACAAATCTGACAG GAAGTTTGTCTGAGCCGATGTA ATTGGCTTG
sh2	*SAA1* exon 4	GATCCGTGATCAGCGATGCCAG AGAGAATCTTCCTGTCAGAATT CTCTCTGGCATCGCTGATCACTT TTTG	AATTCAAAAAGTGATCAGCGAT GCCAGACACAATTCTGACAGGA AGATTCTCTCTGGCATCGCTGAT CACG

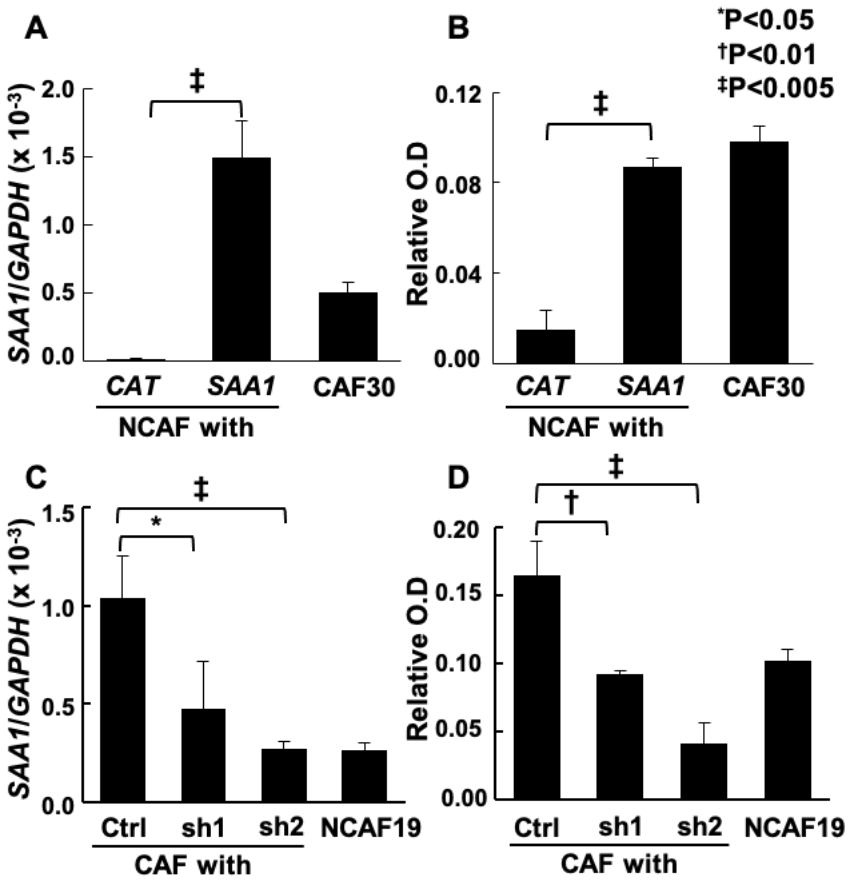
Supplementary Table 3. Deposited Data from ChIP-Atlas

	Source	Identifier
ChIP-seq, human fibroblast, H3K4me1	https://www.ncbi.nlm.nih.gov/	GSM2550214
ChIP-seq, human fibroblast, H3K27ac	https://www.ncbi.nlm.nih.gov/	GSM2214081
ChIP-seq, MSC, MED1	https://www.ncbi.nlm.nih.gov/	GSM2802953
ChIP-seq, Detroit 562, RELA	https://www.ncbi.nlm.nih.gov/	GSM2419817
ChIP-seq, MCF 10A, NFκB1	https://www.ncbi.nlm.nih.gov/	GSM3184663
ChIP-seq, MCF 10A, STAT3	https://www.ncbi.nlm.nih.gov/	GSM3184665



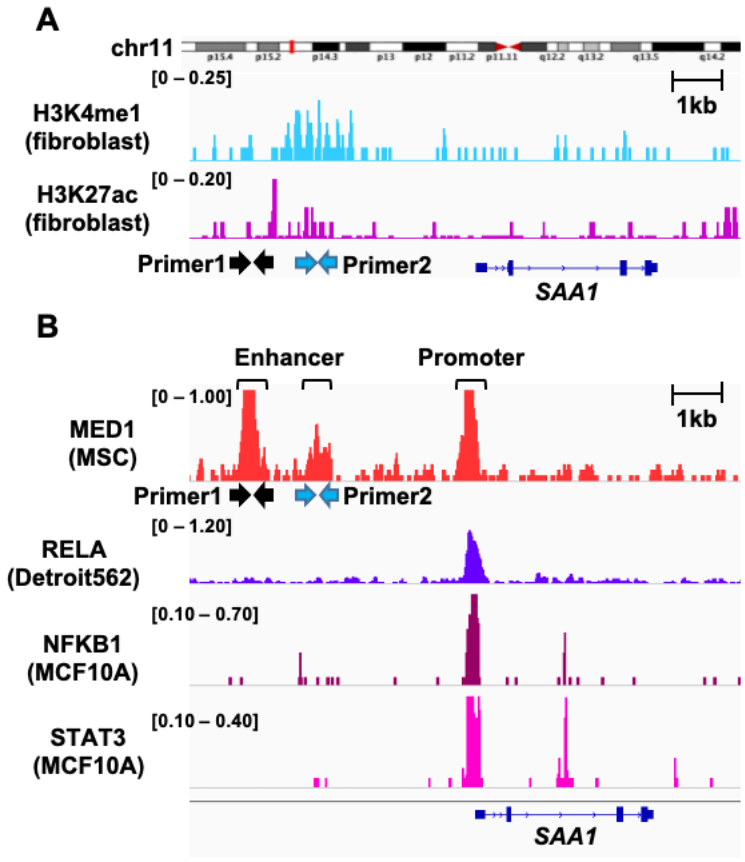
Supplementary Figure 1. Expression analysis using eight CAFs and seven NCAFs from gastric cancer

Unsupervised hierarchical clustering was conducted using the 3000 most variable probes for eight CAFs and seven NCAFs from gastric cancer. CAFs and NCAFs were not classified into distinct clusters by the expression profiles.



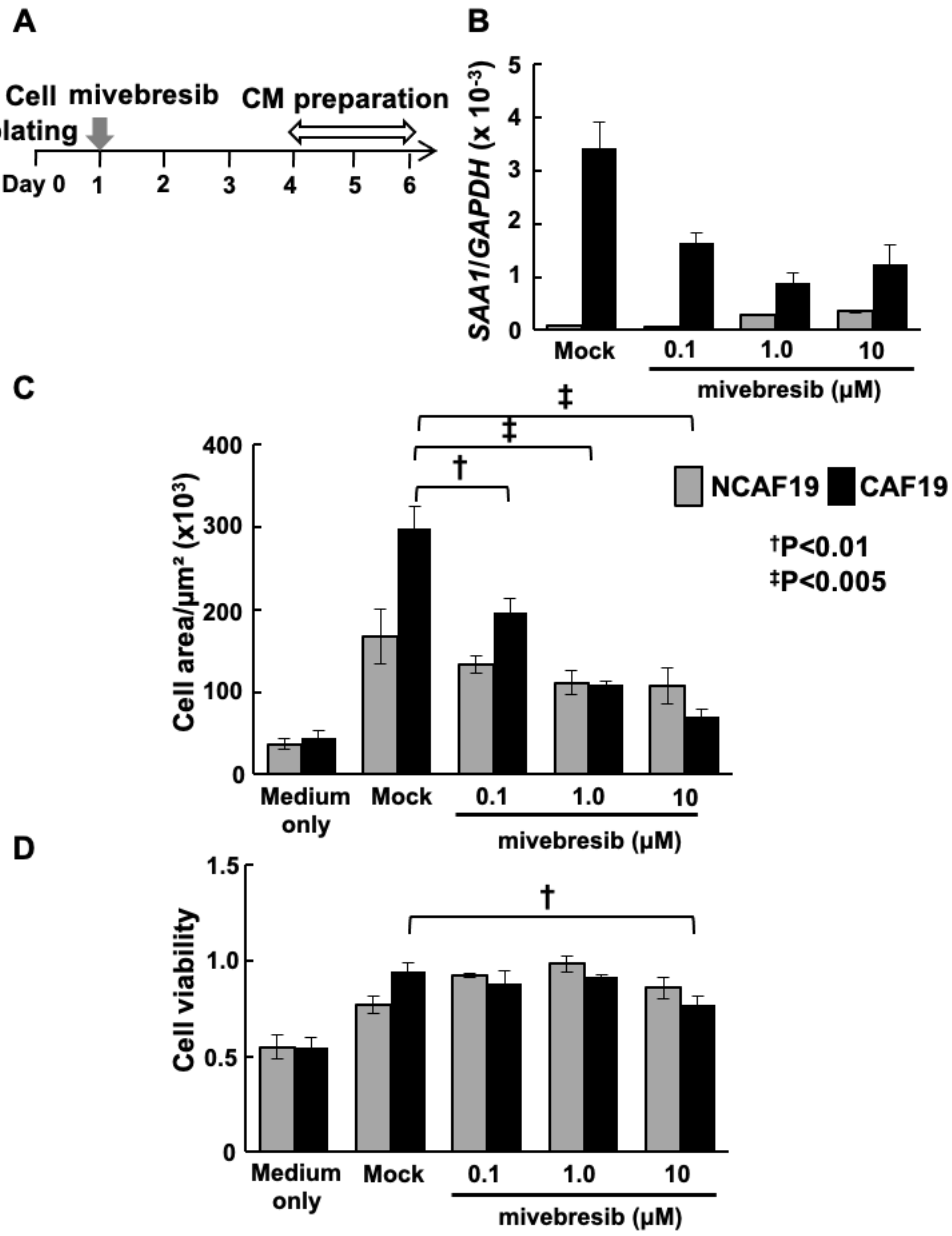
Supplementary Figure 2. Overexpression and knockdown efficiency of *SAA1*

(A) Overexpression efficiency of *SAA1* by RT-qPCR. *SAA1*-overexpressing immortalized NCAF showed higher *SAA1* expression than *CAT*-overexpressing NCAF (control NCAF), and CAF30. (B) Overexpression efficiency of *SAA1* in CM assessed by ELISA assay. Relative optical density of CM derived from *SAA1*-overexpressing NCAF was higher than that from control NCAF, almost at a similar level as CAF30. (C) Knockdown efficiency of *SAA1* by RT-qPCR. *SAA1*-knockdown immortalized CAF showed lower *SAA1* expression than control CAF. (D) Knockdown efficiency of *SAA1* in CM assessed by ELISA assay. Relative optical density of CM derived from *SAA1*-knockdown CAF was lower than that from control CAF, and NCAF.



Supplementary Figure 3. *SAA1* regulation obtained from the ChIP Atlas

(A) ChIP Atlas data upstream of the *SAA1* promoter region. The peaks of both H3K4me1 and H3K27ac were observed 3.3 kbp upstream of *SAA1* TSS in human fibroblasts, indicating that an enhancer is located at this site. (B) ChIP Atlas data in the *SAA1* genomic region. MED1 peaks were observed 4.5 and 3.3 kbp upstream of *SAA1* TSS in MSC, and the peaks of RELA, NF-κB1, and STAT3 were enriched at the promoter of the *SAA1* gene.



Supplementary Figure 4. The effect of mivebresib treatment on CAFs´ tumor-promoting capacity

(A) Preparation protocol of CM from CAF/NCAF with mivebresib treatment. (B) *SAA1* expression in mivebresib-treated CAF/NCAF19 assessed by RT-qPCR. *SAA1* expression was decreased by mivebresib treatment of CAFs. (C) Effect of CM on cancer cell

migration. Migration-promoting effect of the CM from CAFs was markedly suppressed by mivebresib treatment, whereas the same treatment did not show a clear effect for the CM from NCAFs. (D) Effect of CM on cancer cell viability. Increase in the viable number of N87 gastric cancer cells was canceled when they were exposed to a CM derived from CAFs treated with high dose of mivebresib.