

Fig. S1

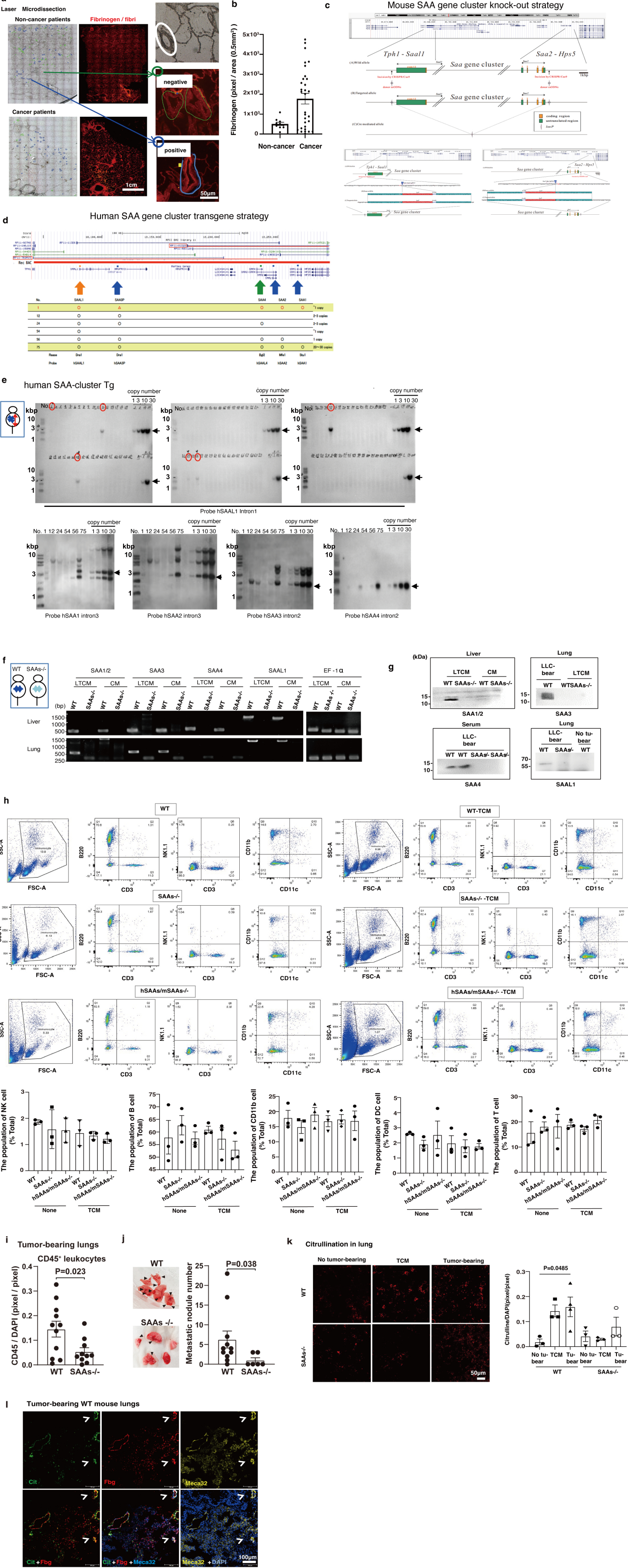


Fig. S1

Replacement of mouse *Saa* cluster genes with human *SAA* cluster genes *in vivo*.

a) Non-stained (upper), Fbg-positive (blue circle), and negative (green circle) pulmonary small vessels derived from non-cancer and cancer patients. Scale bars, 1 cm (right), and 50 μ m (left). **b)** Quantification of Fbg signals in lung autopsy samples shown in Fig. 1a. (n = 10 non-cancer patients, n = 31 cancer patients) **c)** Strategy to delete 70 kb of mouse *Saa* cluster genes. **d)** Transgene strategy to integrate ~200 kb of human *SAA* cluster genes. In three mouse lines, the presence of a full-length human *SAA*s region was confirmed by southern blot for each *SAA* probe (arrow). **e)** Genomic southern blot analysis for human *SAA* genes in human *SAA* cluster transgenic (Tg) mice. The signals of 1–30 copy numbers by the hSAAL1 probe were shown to validate the copy number of the human transgene in each mouse (red circle, upper panel). Mice possessing positive hSAAL1 signals were confirmed by the other probes for hSAA1, hSAA2, hSAA3, and hSAA4. Arrows and arrowheads indicate copy number control (lower). **f)** mRNA expression of *SAA* cluster genes in LTCM-stimulated and LLC-bearing mouse lungs and liver using RT-PCR analysis. *SAA*1 PCR amplified *SAA*1 and 2 due to a high homology of their sequences. CM and LTCM were prepared from the conditioned medium devoid of tumor cells or LLC cells, respectively. EF-1 α is an endogenous control. Three independent experiments. **g)** Induction of *SAA*s in LTCM-stimulated and LLC-bearing mice detected by western blot. **h)** Flow cytometric analysis of DC, NK, CD11b⁺, B and T cells in PBMC derived from normal and TCM-stimulating condition (n = 3). Gating strategy (upper) and each cell population (lower). **i)** Increased CD45⁺ leukocytes in tumor-bearing-lungs from wild-type compared with those from *SAA*s^{-/-} mice (n = 11 WT, n = 10 *SAA*s^{-/-}). Student's two-sided t-test. **j)** Representative photo of spontaneous metastasis of 3LL tumor, a spontaneous metastatic cell line in Lewis lung carcinoma (LLC), to the lungs (left, arrow indicates metastatic nodules) and lung metastasis number (right) (n = 11 WT, n = 6 *SAA*s^{-/-} mice). Student's two-sided t-test. **k)** IHC photo of citrullination in no tumor-bearing-, TCM-stimulated-, and tumor-bearing- wild-type and *SAA*s^{-/-} mouse lungs (left). Quantification of the citrullination signals (right). (n = 3 WT; n = 3 *SAA*s^{-/-}). One-way ANOVA with Bonferroni correction. **l)** Representative staining of citrullination, Fbg, and endothelial marker, MECA32, in a low magnified image related to Fig. 1i. Arrowheads indicate merged signals in small vessels/capillaries in tumor-bearing wild-type mouse lungs. Scale bars, 100 μ m. Five independent experiments. In all graphs, data are mean $\pm$ SEM.

Fig. S2

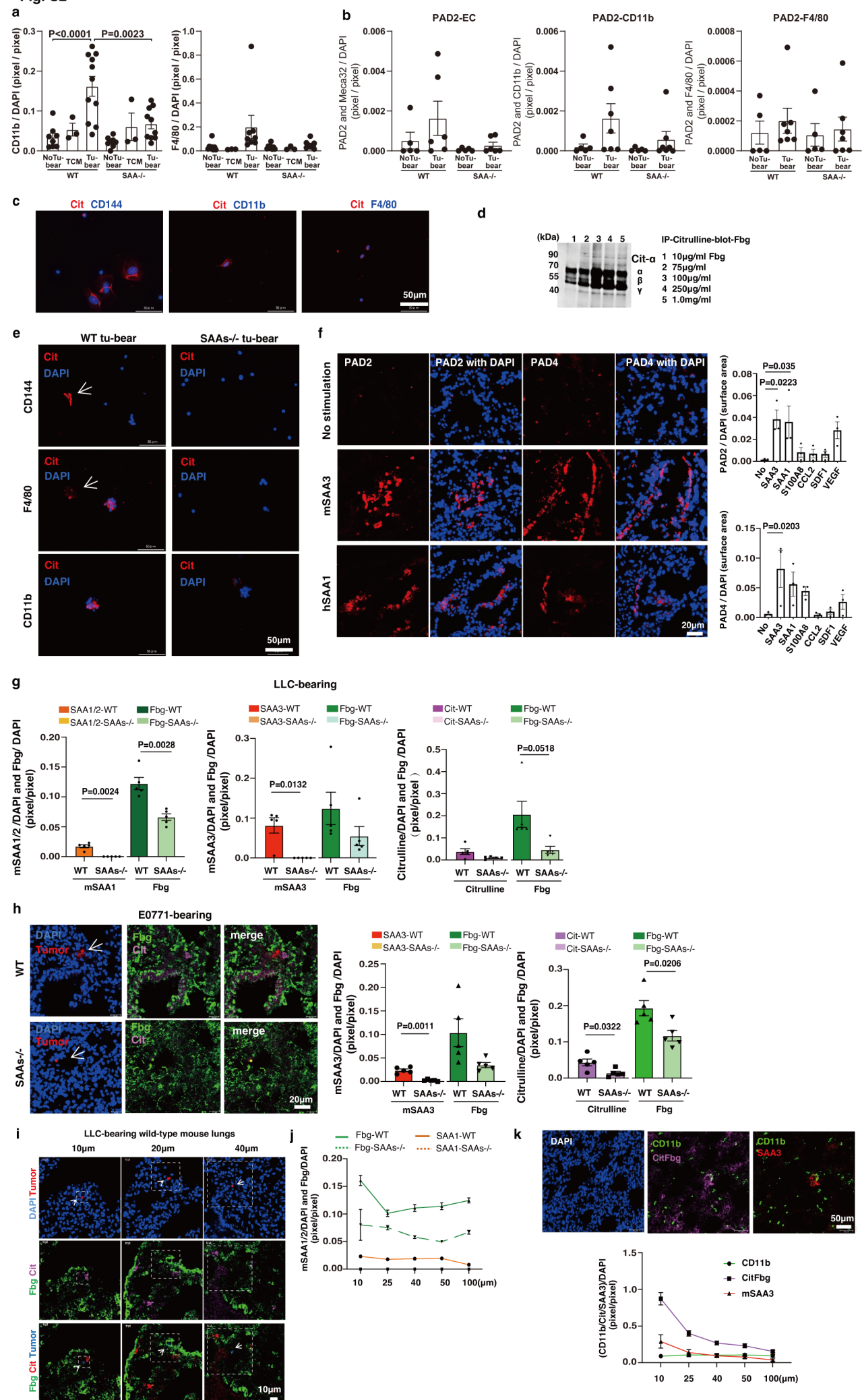


Fig. S2

Endothelial cells (EC)-mediated SAAs-CitFbg around metastatic tumor cells.

a) The number of CD11b⁺ cells (n = 8 WT-no tumor; n = 3 WT-TCM; n = 11 WT- LLC tumor, n = 8 SAAs^{-/-}-no tumor; n = 3 SAAs^{-/-} TCM; n = 10 SAAs^{-/-}-LLC tumor) and F4/80⁺ cells (n = 8 WT-no tumor; n = 3 WT-TCM; n = 8 WT- LLC tumor, n = 8 SAAs^{-/-}-no tumor; n = 3 SAAs^{-/-} TCM; n = 8 SAAs^{-/-}-LLC tumor) in tumor-bearing lung tissues in IHC analysis. One-way ANOVA with Bonferroni correction. **b)** Quantifications of PAD2 in EC (PAD2-EC) (n = 5 WT-no tumor; n = 6 WT-LLC tumor; n = 5 SAAs^{-/-}-no tumor; n = 6 SAAs^{-/-}-LLC tumor), CD11b⁺ cells (PAD2-CD11b) (n = 5 WT-no tumor; n = 7 WT-LLC tumor; n = 5 SAAs^{-/-}-no tumor; n = 7 SAAs^{-/-}-LLC tumor) and F4/80⁺ cells (PAD2-F4/80) (n = 5 WT-no tumor; n = 7 WT-LLC tumor; n = 5 SAAs^{-/-}-no tumor; n = 7 SAAs^{-/-}-LLC tumor) in no tumor-bearing and LLC-bearing lung tissues in IHC analysis. **c)** Citrullination signals on CD144⁺ ECs, CD11b⁺ cells, and F4/80⁺ cells derived from LLC-bearing mouse lungs. Scale bars, 50 μ m. Four independent experiments. **d)** Measurement of enzymatic activity of citrullination for various concentrations of Fbg. The citrullinated Fbg was detected after applying lung cells derived from tumor-bearing mice in western blot. Two independent experiments. **e)** Lung-derived cells citrullinate pre-coated Fbg. Three independent experiments. Arrows indicate CitFbg. Scale bar, 50 μ m. **f)** PADs in mouse lung organ culture after application of mSAA3 and hSAA1 (left). Quantification of PAD signals 72 h after stimulation by various factors (right). Scale bars, 20 μ m. **g)** Quantitative IHC signals of SAA1/2, SAA3, and citrulline under increased Fbg in the post-metastatic phase using LLC-bearing wild-type and SAAs^{-/-} mouse lungs (n = 5 WT; n = 5 SAAs^{-/-}). Student's two-sided t-test. **h)** Fbg and citrulline around metastatic tumor cells (arrow) in E0771-bearing wild-type and SAAs^{-/-} mouse lungs (left). Scale bars, 20 μ m. Quantitative signals of SAA3 and citrulline with Fbg staining in E0771-bearing wild-type and SAAs^{-/-} lungs (right) (n = 5 WT; n = 5 SAAs^{-/-}). Student's two-sided t-test. **i)** Calculation methods of IHC signals. Labeled metastatic cell was located at the central portion (arrowheads). Scale bars, 10 μ m. **j)** Distance kinetics between tumor cells and proteins, such as SAA1/2 and Fbg, in LLC-bearing wild-type and SAAs^{-/-} mouse lungs (n = 5 WT; n = 5 SAAs^{-/-}). **k)** Representative IHC image of CD11b, CitFbg and SAA3 in lungs from LLC-bearing mice. Distance between SAA3-CitFbg and CD11b signals (lower) (n = 5). Scale bars, 50 μ m. In all graphs, data are mean $\pm$ SEM.

Fig. S3

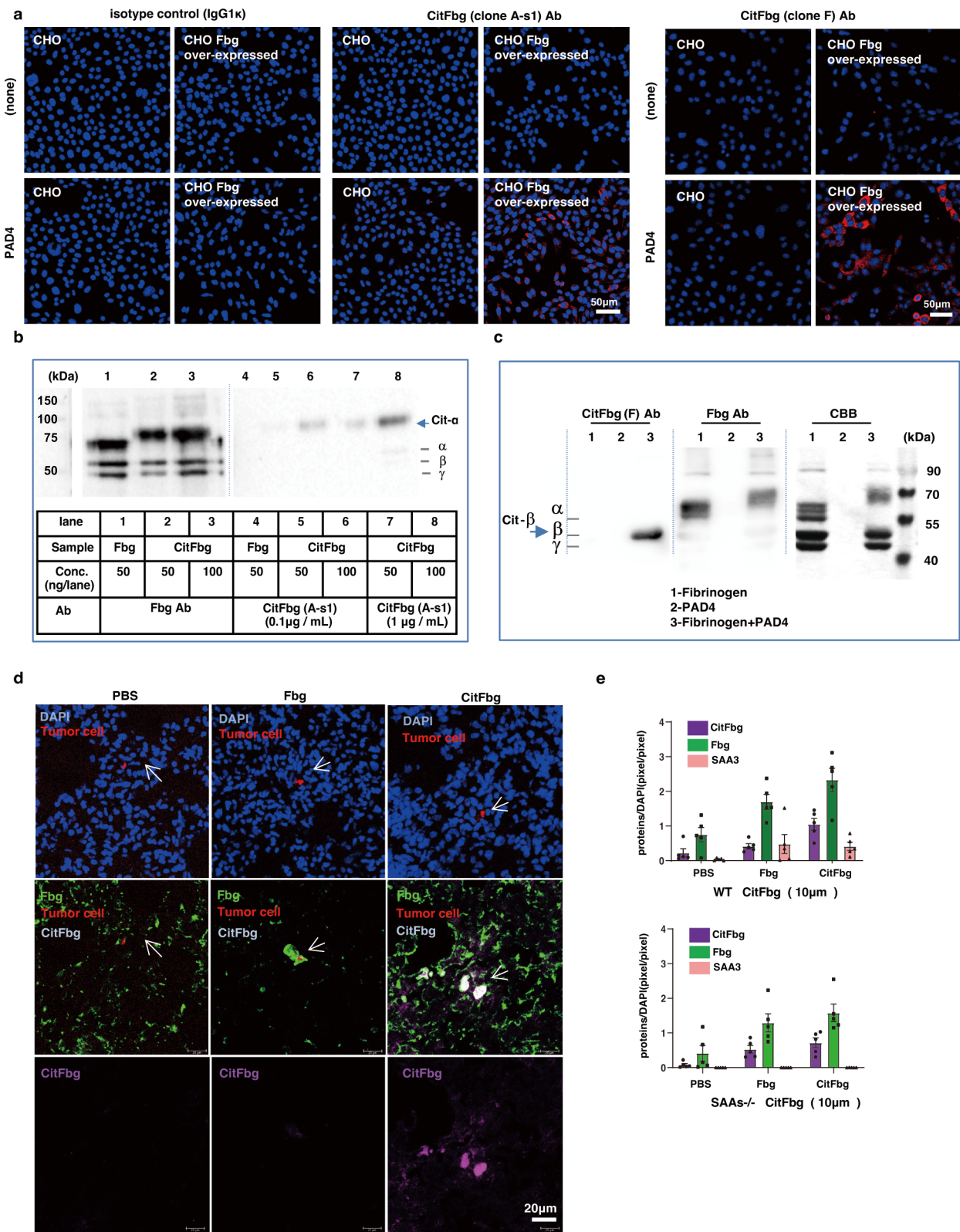
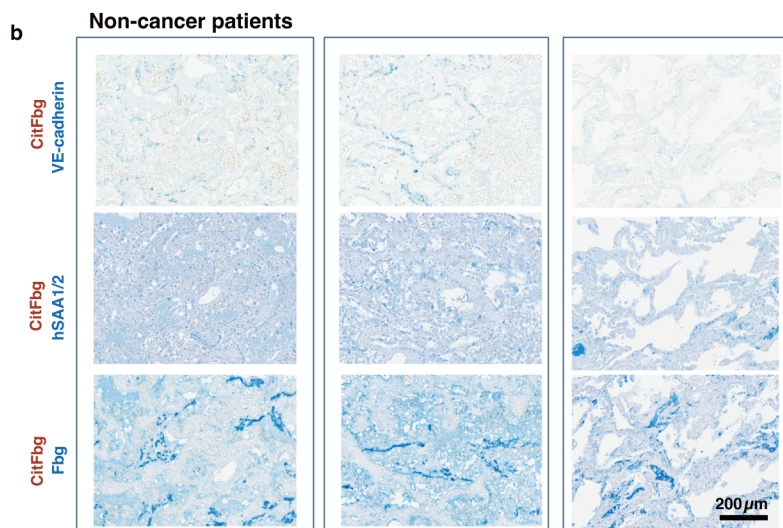
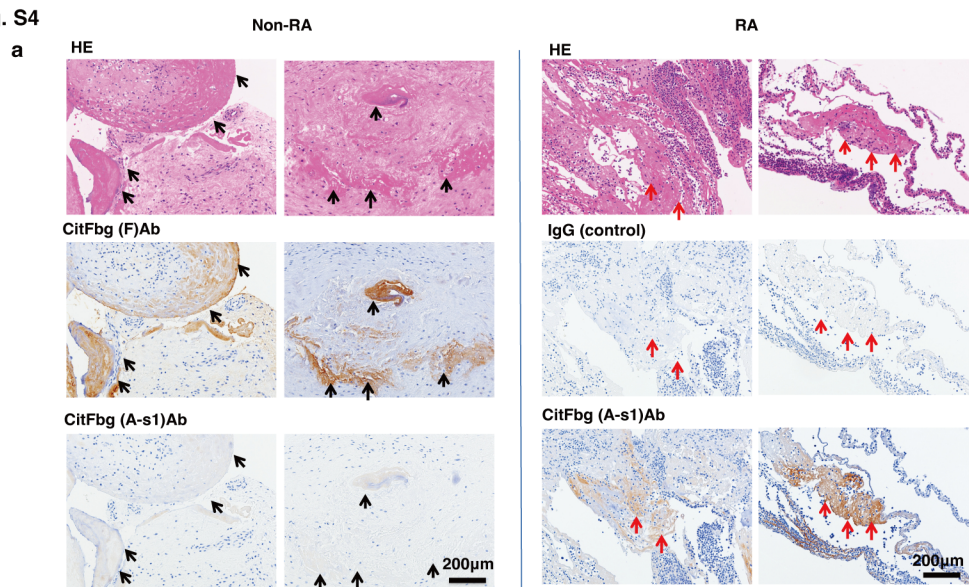


Fig. S3

Validation of anti-hCitFbg antibody and measurement of distance between CitFbg and tumor cells by the antibody.

a) Evaluation of antibodies for hCitFbg using hFbg-overexpressed CHO cells. The cells were citrullinated by PAD4 before IHC analysis. Scale bars, 50 μm . Two independent experiments. **b)** and **c)** specificity of antibodies for hCitFbg protein in western blot analysis. The bands of α and β chains of hCitFbg were detected by clone A-s1 (**b**) and clone F (**c**), respectively. Various combinations were demonstrated to determine the specificity and sensitivity of the A-s1 antibody. Two independent experiments. **d)** Immunohistochemical detection of injected hCitFbg with colocalization of tumor cells (arrow) using an anti-hCitFbg antibody (clone A-s1). Scale bars, 20 μm . Four independent experiments. **e)** Measurement of several protein signals around metastatic tumor cells in the pre-inoculation of PBS, Fbg, and CitFbg into wild-type mice. The calculation area was within 10 μm of tumor cells ($n = 5$ WT; $n = 5$ SAAs^{-/-}). One-way ANOVA with Bonferroni correction. Data are mean $\pm$ SEM.

Fig. S4

cancer patients

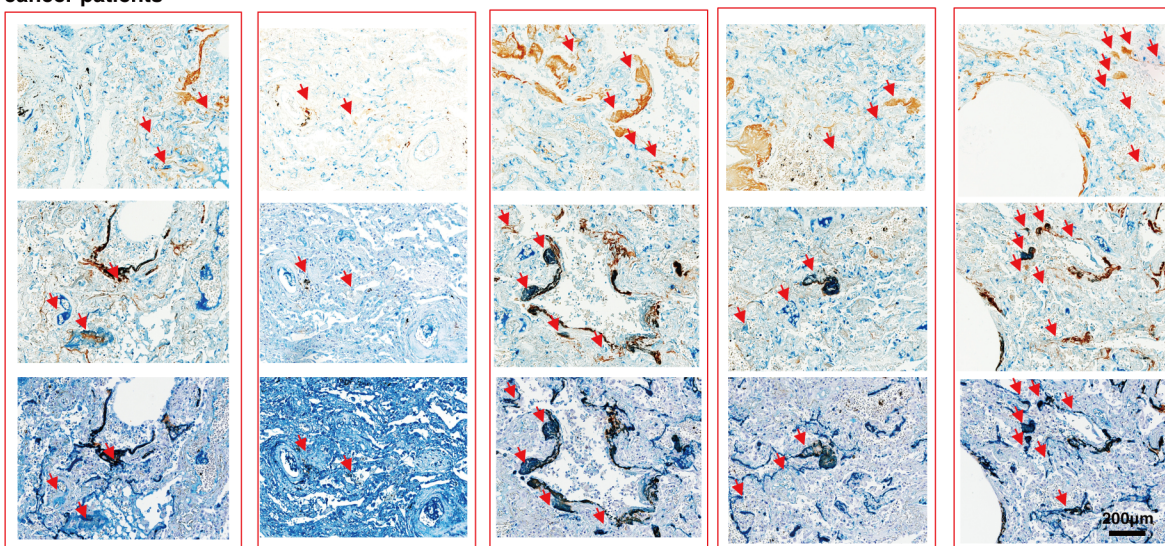


Fig. S4

The deposition of SAA1/2-CitFbg in pulmonary vessels in the lungs of patients.

a) IHC analysis for Fbg in joint tissues derived from RA and non-RA patients using clone A-s1 and clone F antibodies. An anti-CitFbg Clone A-s1 antibody reacted to CitFbg (red arrow) in RA but not Fbg (black arrow) of non-RA, specifically IHC detection for CitFbg. Scale bars, 200 μm . Four independent experiments. **b)** Representative images with double staining of CitFbg/VE-cadherin and CitFbg/SAA1/2 and CitFbg/Fbg in lung specimens derived from non-cancer patients ($n = 3$) and cancer patients ($n = 3$) (data related to Fig. 4c). Arrows indicate the same position in continuous sections. Scale bars, 200 μm .

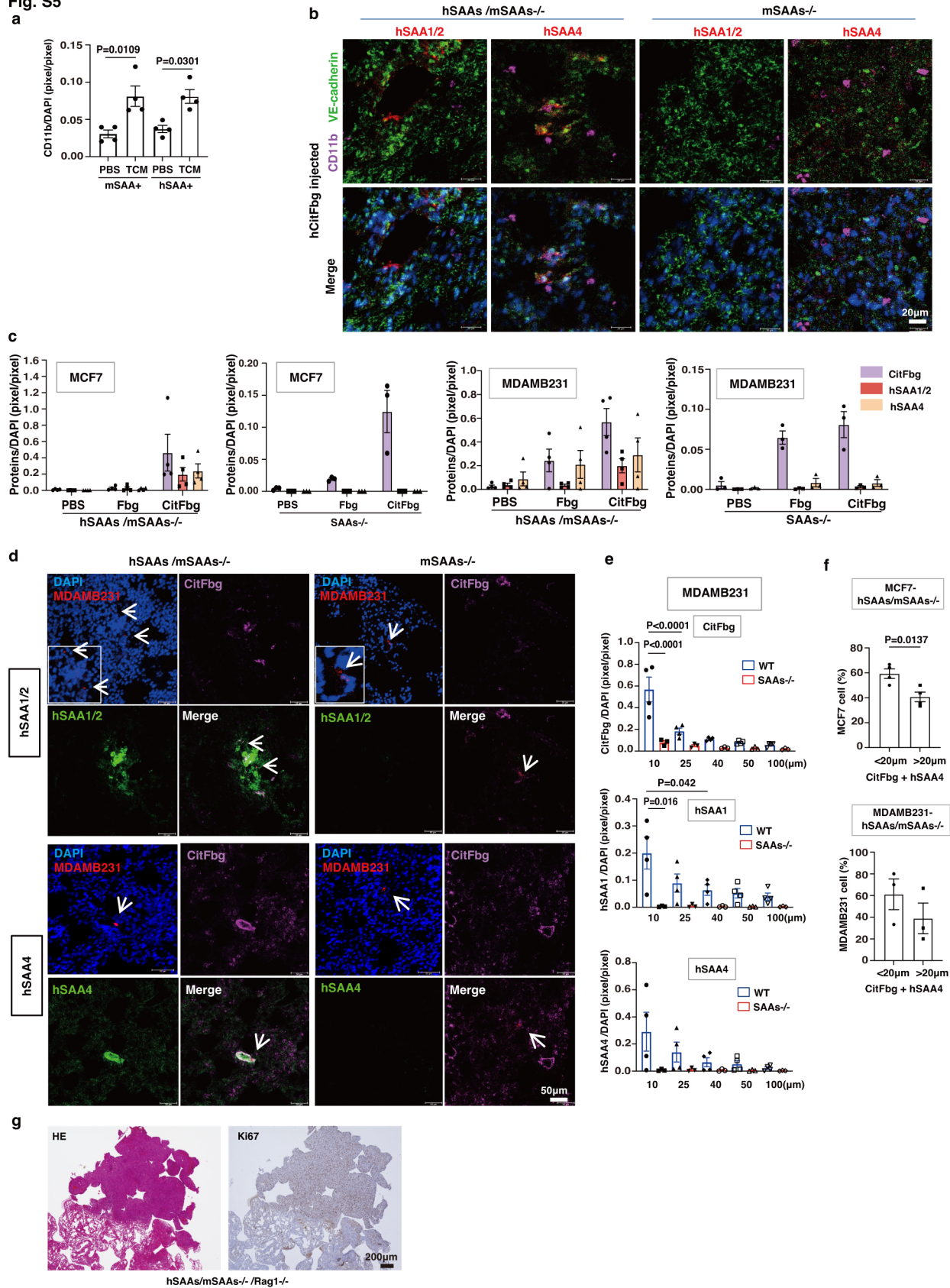
Fig. S5

Fig. S5

The induction of the protein complex of SAA-CitFbg is an initiator for cancer metastasis in SAA-humanized mice.

a) Quantifications of CD11b⁺ cells in the lungs from WT (mSAA⁺) or hSAAs/mSAAs^{-/-} (hSAA⁺) mice with or without TCM-injection (n = 4). One-way ANOVA with Bonferroni correction. **b)** Representative photos of the induction of hSAA1/2 and hSAA4 by injecting hCitFbg to hSAAs/mSAAs^{-/-} mice. Scale bars, 20 μ m. **c)** Measurement of signals of the hSAA1/2, hSAA4, and hCitFbg after injecting PBS, hFbg, and hCitFbg into hSAAs/mSAAs^{-/-} and mSAAs^{-/-} mice that were followed by tumor cell injection. The CitFbg signals were kept in both genotypes (n = 4 hSAAs/mSAAs^{-/-}; n = 3 mSAAs^{-/-}) One-way ANOVA with Bonferroni correction. **d)** Representative photos of metastatic MDAMB231 cells (arrows, magnified lower left) trapped by the hSAAs-hCitFbg complex. Scale bars, 50 μ m. **e)** Distances among hCitFbg, hSAA1, hSAA4, and MDAMB231 cells shown in **(d)** were analyzed. **d** and **e** are related to Fig. 5d, e) (n = 4 hSAAs/mSAAs^{-/-}; n = 3 mSAAs^{-/-}). One-way ANOVA with Bonferroni correction. **f)** The percentage of MCF7 and MDAMB231 cells located within 20 μ m from hSAA4-hCitFbg (n = 4 hSAAs/mSAAs^{-/-} for MCF7; n = 4 hSAAs/mSAAs^{-/-} for MDAMB231). Student's two-sided t-test. **g)** (Related to Fig. 5g) Representative HE and Ki67 stainings of lungs 3 weeks after injection of tumor cells in hSAAs/mSAAs^{-/-}/Rag1^{-/-} and mSAAs^{-/-}/Rag1^{-/-} mice. Scale bars, 200 μ m. In all graphs, data are mean $\pm$ SEM.

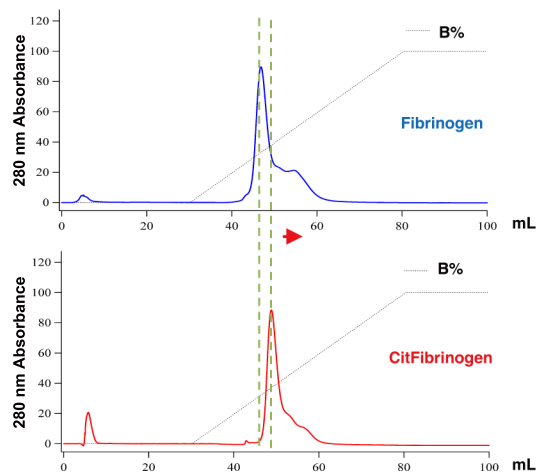
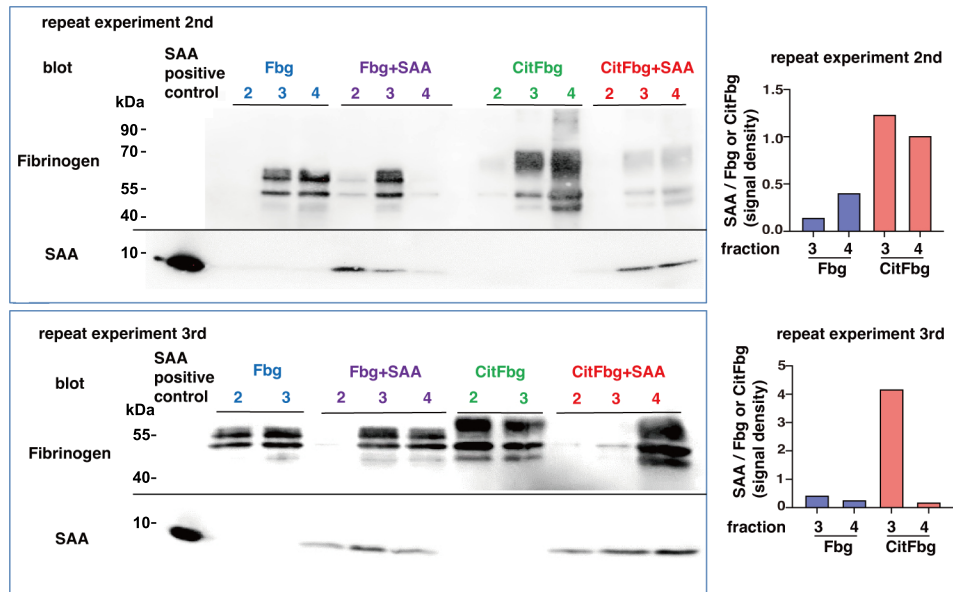
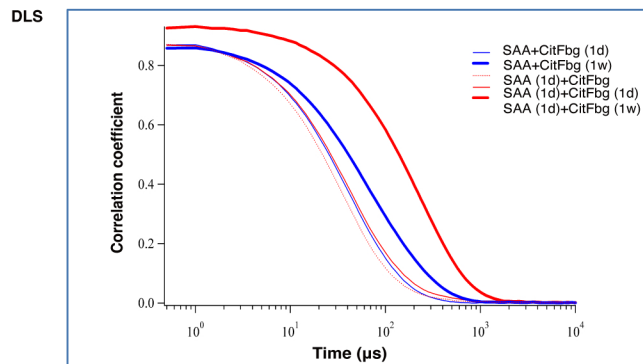
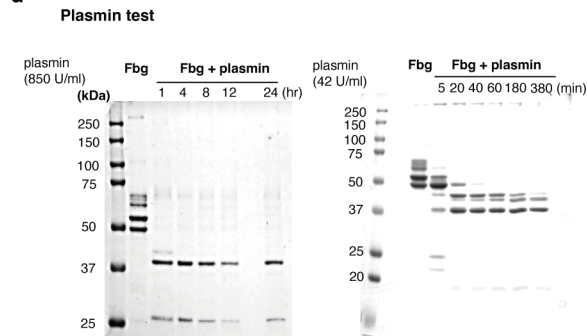
Fig. S6**a Anion exchange****b****c****d**

Fig. S6

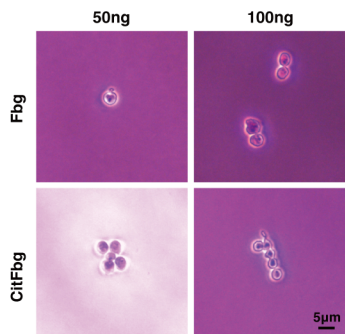
Characterization of hFbg and hCitFbg in the presence and absence of SAA.

a) hFbg and hCitFbg were analyzed using anion exchange chromatography. The elution of CitFbg occurred at a higher NaCl concentration than that of Fbg, consistent with converting the positively charged guanidino group to the neutral ureido group by citrullination. Three independent experiments. **b)** Examination of SAA1 associated with Fbg and CitFbg based on gel filtration chromatography (Superdex 200 Increase) and western blotting analysis. The experiments shown in Fig. 6b were repeated an additional two times. CitFbg bound more SAA1 than Fbg. **c)** DLS correlation curves. (1) SAA1 and CitFbg co-incubation for 1 day (blue), (2) SAA1 and CitFbg co-incubation for 1 week (blue solid line), (3) SAA1 1 day incubation and addition of CitFbg (red dotted line), (4) SAA1 1 day incubation followed by co-incubation with CitFbg for additional 1 day (red line), and (5) SAA1 1 day incubation followed by co-incubation with CitFbg for 1 week (red solid line) were depicted. In the data acquisition, SAA1 (0.2 mg/mL) and CitFbg (1 mg/mL) were dissolved in 50 mM Hepes, pH 7.7 for sample (3) or in 50 mM Hepes, pH 7.7, 10 mM MgCl₂ for samples (1), (2), (4), and (5). All samples were incubated at 37°C. **d)** (related to Fig. 6e) Degradation assay of Fbg at two concentrations of plasmin. The gel was stained with CBB.

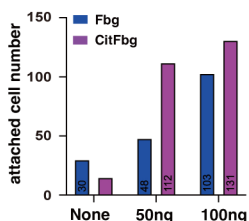
Fig. S7

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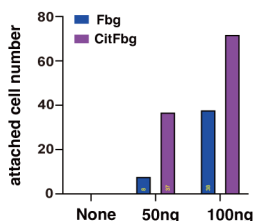
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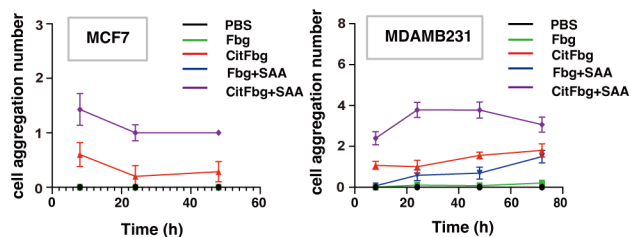
MCF7



MDAMB231

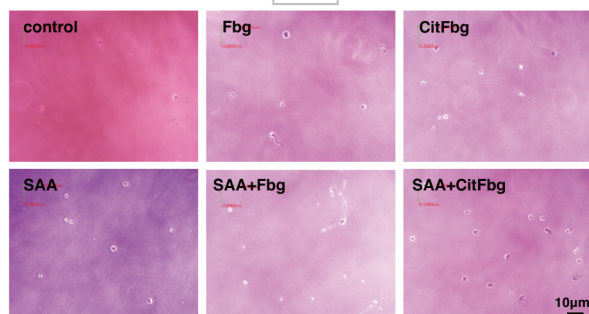


c

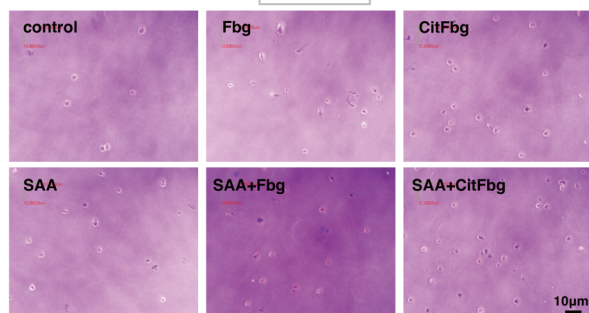


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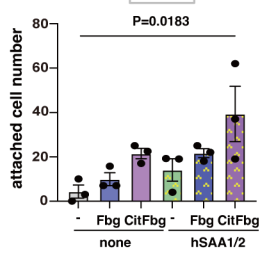
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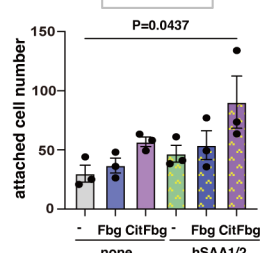
MDAMB231



MCF7



MDAMB231



d Model of citrullination-mediated metastasis

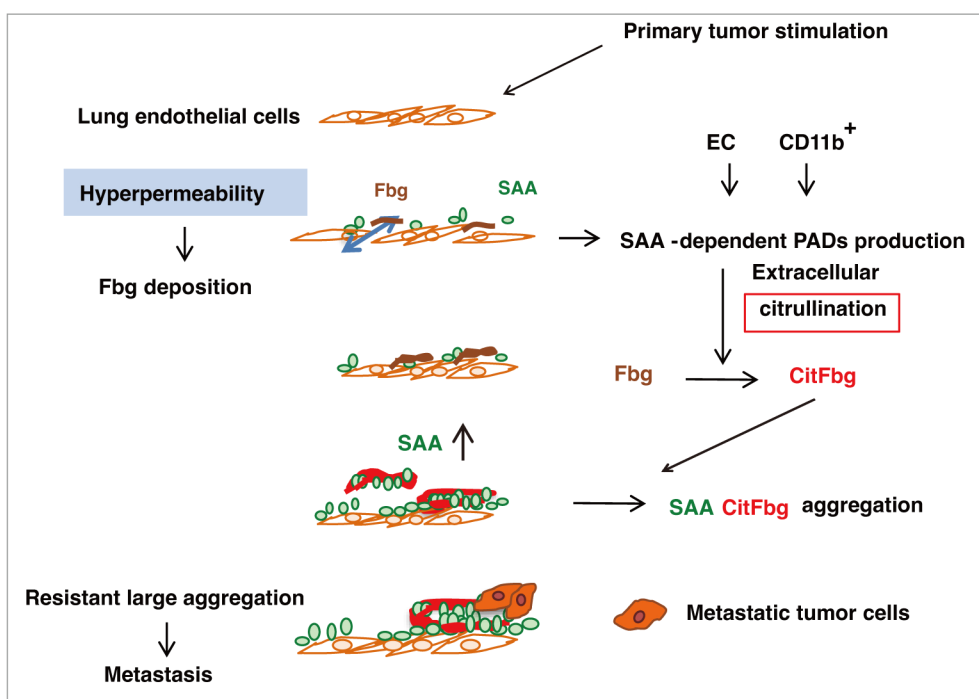


Fig. S7

Aggregation of SAA-CitFbg protein complex and tumor cells

a and **b**) Human tumor cell attachment assay to Fbg-, CitFbg-, and SAA1-CitFbg- coated plates 1 h after cell seeding. Representative photo (left in **a**). The number of MCF7 and MDAMB231 cells exposed to various concentrations of Fbg and CitFbg-coating plates (right in **a** Scale bars, 5 μ m). Attached are MCF7 and MDAMB231 cells to SAA1-CitFbg-coating plates (upper in **b** Scale bars, 10 μ m) and the cell numbers of attached tumor cells (lower in **b**) (n = 3). One-way ANOVA with Bonferroni correction. **(c)** Time course of cell aggregation of MCF7 and MDAMB231 cells (5 or more cells in aggregation) cultured on various protein complex-coating plates. (n = 3 for MCF7 and n = 4 for MDAMB231 cells). Data are mean $\pm$ SEM. **(d)** Model of citrullination-mediated metastasis. SAAs induced extracellular citrullination of Fbg in hyperpermeable spots. The citrullination on the pulmonary ECs was catalyzed by PADs. Finally, SAAs-CitFbg aggregation recruits circulating tumor cells.

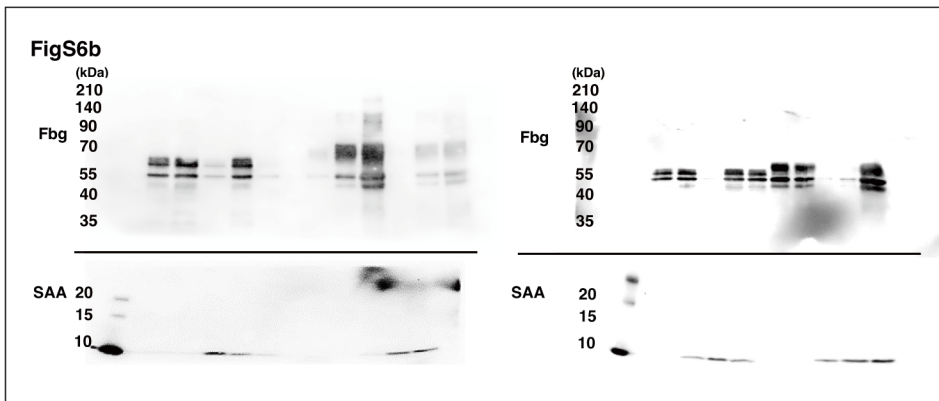
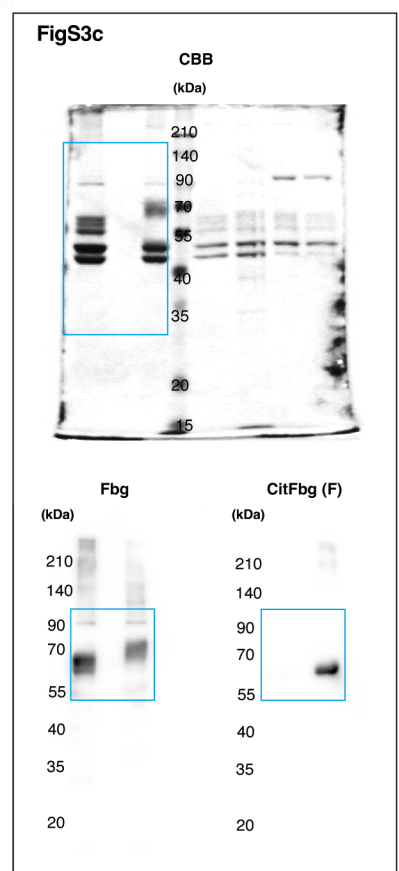
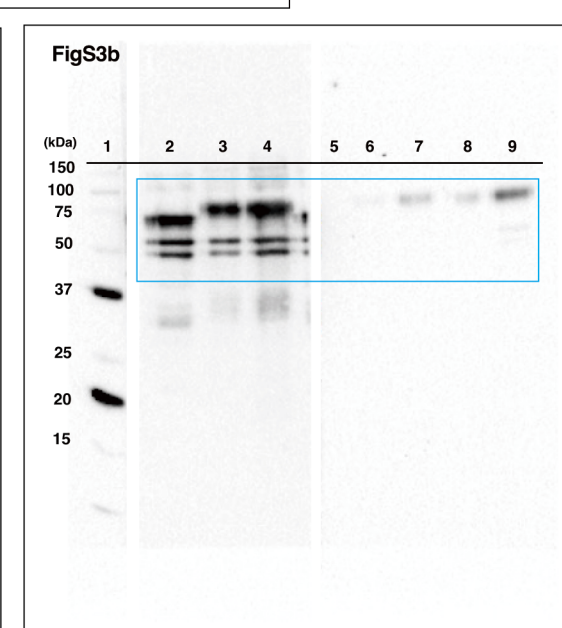
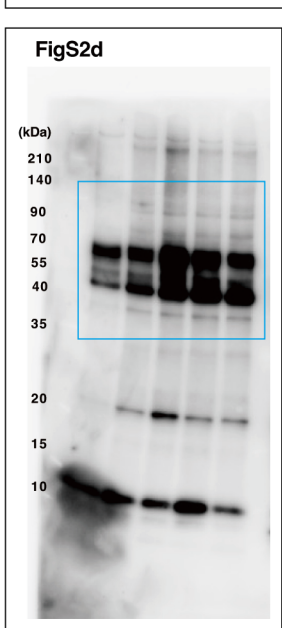
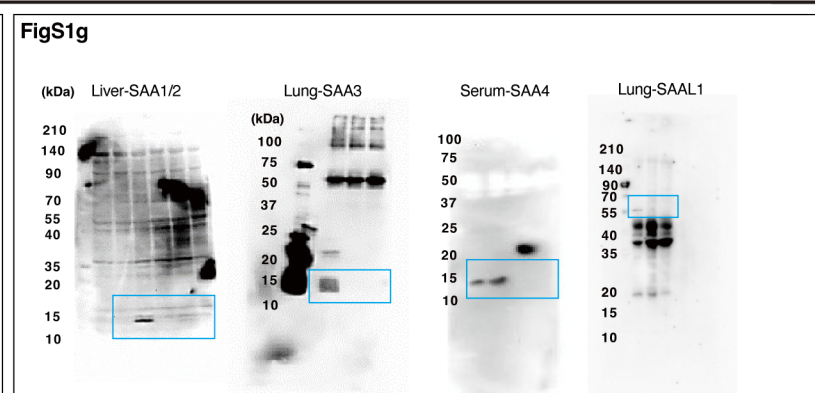
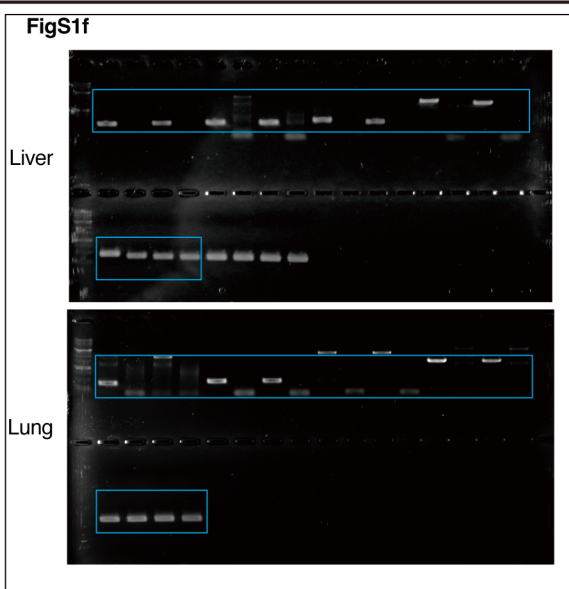
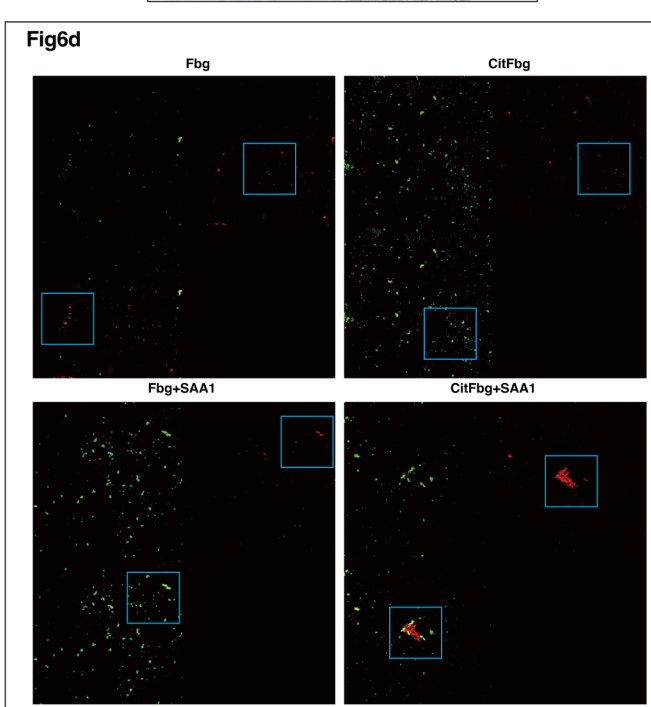
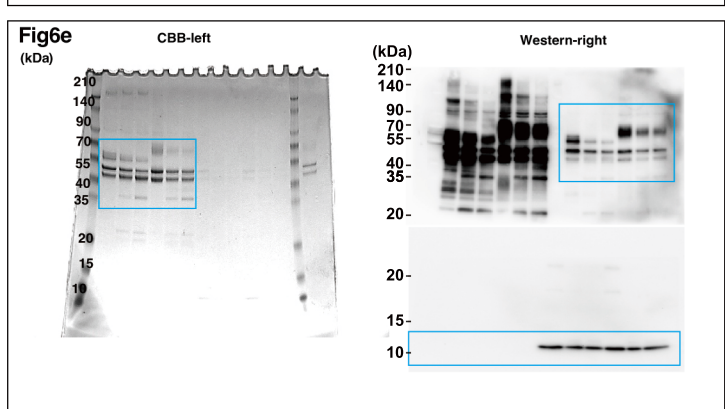
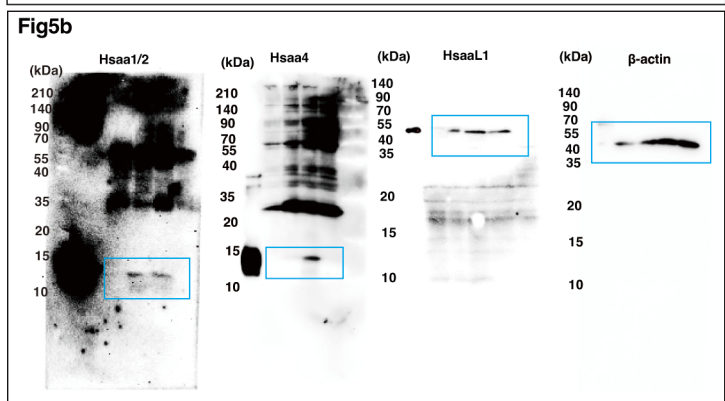
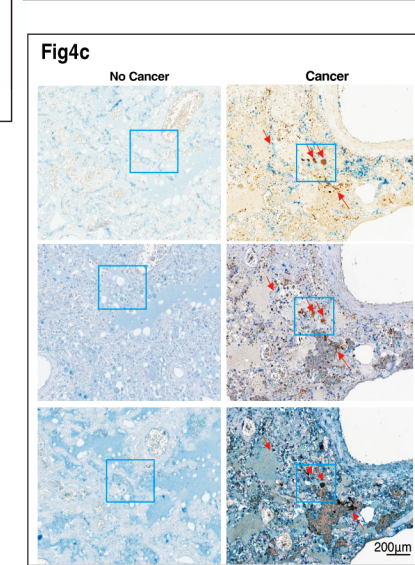
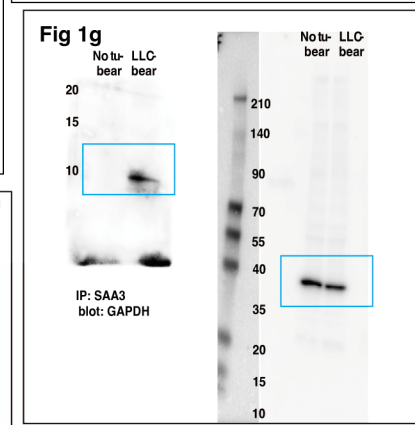
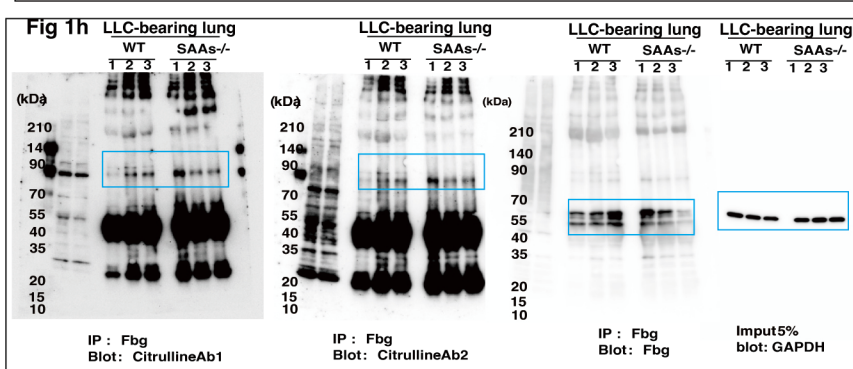
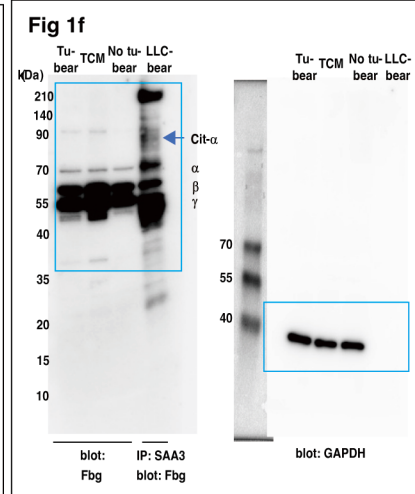
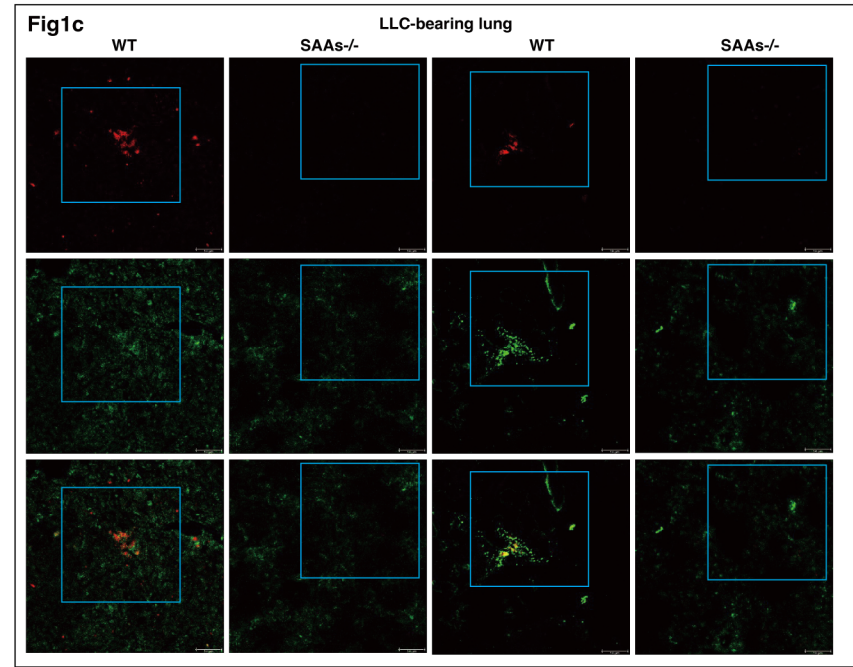


Fig. S8

Full gels for all Western blot images used for data presentation in Figs. 1f, 1g, 1h, 2c, 5b, 6e, S1g, S2d, S3b, S3c, and S6b, and original pictures used for data presentation in Figs. 1c, 4c, and 6d.

Supplementary Table 1

Gene Symbol	Gene Title	Non-cancer patients (Raw)	Cancer Patients (Raw)	Fold change	RefSeq Transcript ID
RBMS1	RNA binding motif, single stranded interacting protein 1	5.5950274	433.03644	5.755966	NM_002897///NM_016836///NM_016839
RPL6	ribosomal protein L6	4.358773	328.1133	5.715895	NM_000970///NM_001024662
RPL10A	ribosomal protein L10a	5.5631275	324.13657	5.346328	NM_007104
MORF4L1	mortality factor 4 like 1	4.7582364	264.61035	5.279065	NM_001265603///NM_001265604///NM_001265605///NM_006791///NM_206839
RPS4X	ribosomal protein S4, X-linked	6.559085	355.10107	5.240358	NM_001007
SAA1///SAA2///SAA2-SAA4	serum amyloid A1///serum amyloid A2///SAA2-SAA4 readthrough	31.830032	1678.5461	5.202446	NM_000331///NM_001127380///NM_001178006///NM_001199744///NM_030754///NM_199161
GPX3	glutathione peroxidase 3 (plasma)	7.483164	361.4908	5.075933	NM_002084
RPS10	ribosomal protein S10	10.147761	415.12775	4.836088	NM_001014///NM_001203245///NM_001204091
RPL23A///SNORD42A	ribosomal protein L23a///small nucleolar RNA, C/D box 42A	6.979831	259.7433	4.699517	NM_000984///NR_000014
HUWE1	HECT, UBA and WWE domain containing 1, E3 ubiquitin protein ligase	10.953302	396.56732	4.659895	NM_031407
NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	9.6121855	343.58865	4.641440	NM_001037738///NM_002520///NM_199185
RPL24	ribosomal protein L24	19.095425	682.21045	4.640684	NM_000986
RPS3A///SNORD73A	ribosomal protein S3A///small nucleolar RNA, C/D box 73A	6.628325	212.60031	4.485122	NM_001006///NM_001267699///NM_000007
ACTG1	actin, gamma 1	4.8529983	152.97282	4.460021	NM_001199954///NM_001614///NM_037688
MAP2K5	mitogen-activated protein kinase kinase 5	44.27736	1391.8768	4.456085	NM_001206804///NM_002757///NM_145160///NM_145161///NM_145162
LDHB	lactate dehydrogenase B	5.4338665	169.75435	4.447092	NM_001174097///NM_002300
RPS17///RPS17L	ribosomal protein S17///ribosomal protein S17-like	5.9380016	175.185	4.364524	NM_001021///NM_001199057
RPS11///SNORD35B	ribosomal protein S11///small nucleolar RNA, C/D box 35B	19.39767	535.12146	4.267677	NM_001015///NR_001285
RPL21///RPL21P28///SNORA27///SNORD102	ribosomal protein L21///ribosomal protein L21-pseudogene 28///small nucleolar RNA, H/ACA box 27///small nucleolar RNA, C/D box 102	11.757041	309.21115	4.198762	NM_000982///NR_002574///NR_002575///NR_026911
KCMF1	potassium channel modulatory factor 1	20.280977	504.82816	4.119360	NM_020122
HMGB1	high mobility group box 1	10.717781	261.31653	4.089487	NM_002128
RPL38	ribosomal protein L38	13.629604	328.38217	4.072327	NM_000999///NM_001035258

Supplementary Table 1

The gene list related to the pre-metastatic phase using Fbg-deposited pulmonary vessels from noncancer and cancer patients. Microarray data up-regulated over 4-fold in cancer patients (No. 4) as compared to noncancer patients (No. 2) are shown. Related to Fig. 1a and b.

Supplementary Table 2

patients	non-cancer	number	cancer	number
	neurodegenerative disease	5	hepatocellular carcinoma	18
	aneurysm	4	pancreatic cancer	11
	infarction	4	gastric cancer	7
	cerebral hemorrhage	2	cholangiocarcinoma	5
	cardiomyopathy	2	esophageal cancer	5
	primary biliary cholangitis	2	ovarian cancer	4
	arrhythmia	2	colon cancer	3
	liver failure	2	melanoma	2
	liver cirrhosis	1	bladder cancer	2
	heart failure	1	gallbladder cancer	2
	respiratory failure	1	prostatic cancer	2
			malignant lymphoma	1
			adrenal cancer	1
			breast cancer	1
			duodenal papilla cancer	1
			pharyngeal cancer	1
			kidney cancer	1

Supplementary Table 2

A list for the samples derived from non-cancer patients and cancer patients. These were analyzed in Figs. 1, 4, and S4.