Supplementary Information

Discovery of an uncovered region in fibrin clots and its clinical significance

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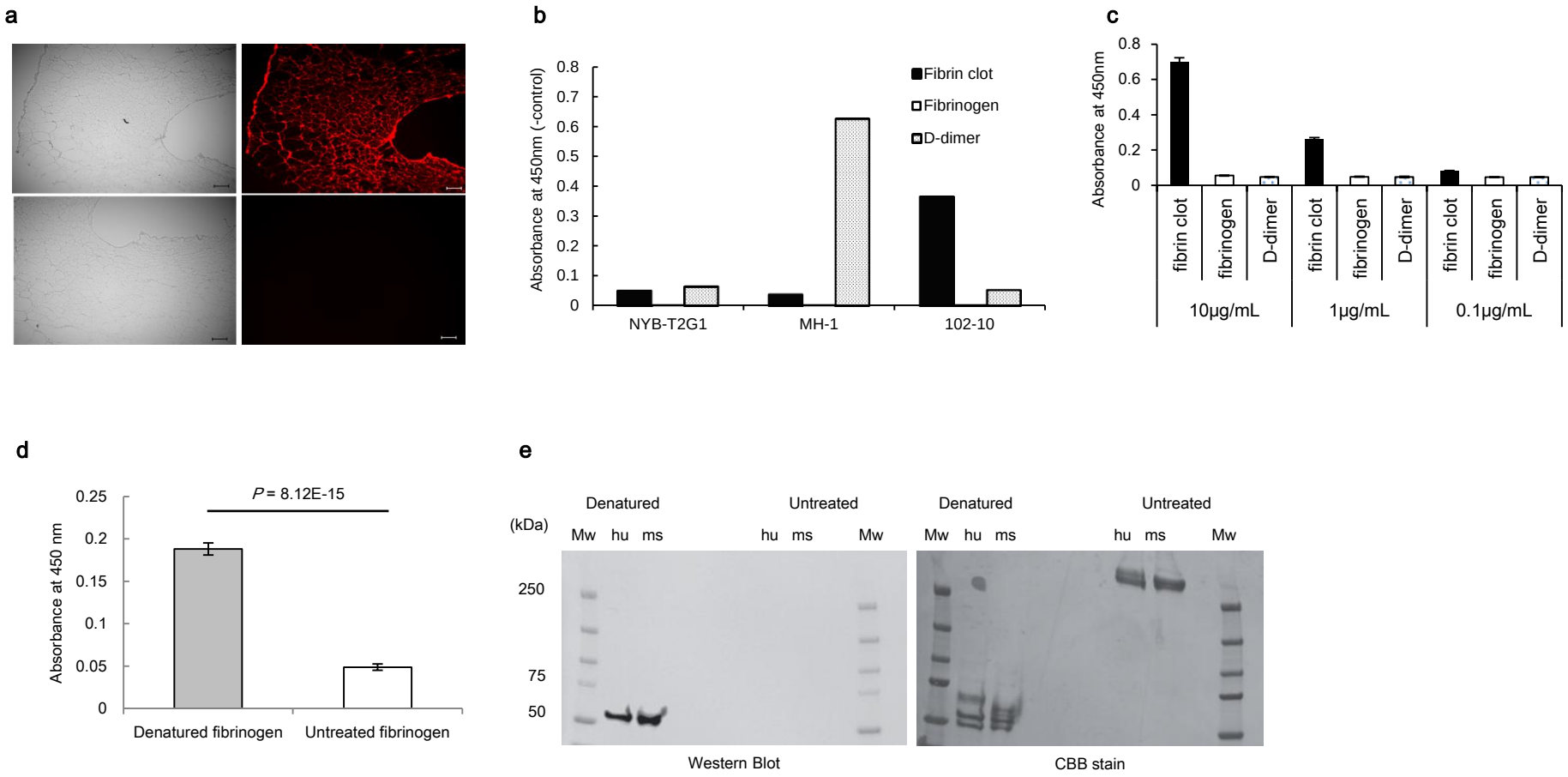
Materials and Methods

Immunofluorescence staining of fibrin clots

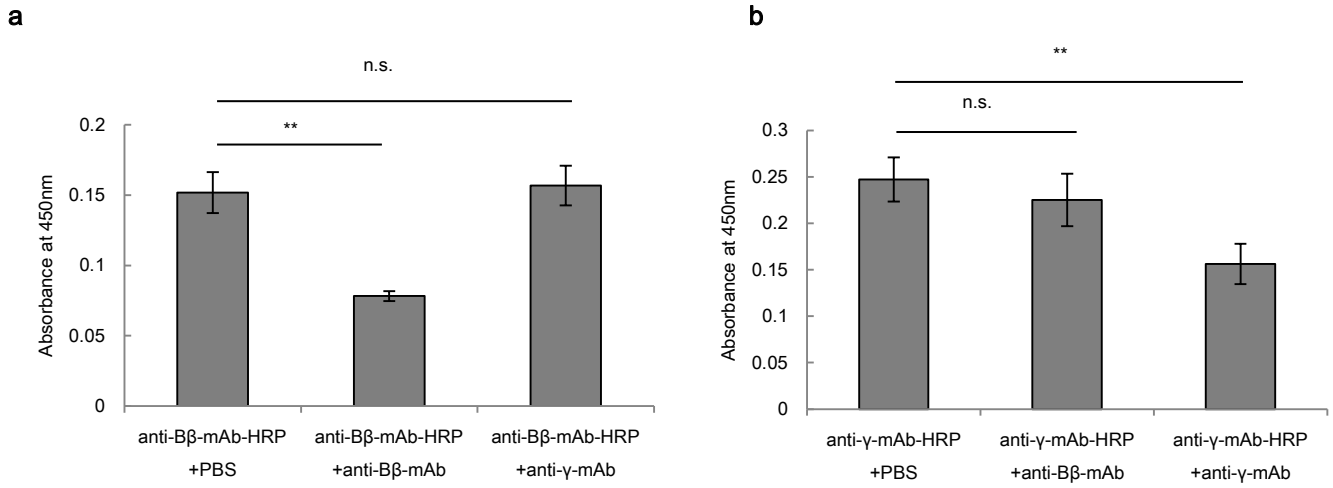
Fibrin clots were generated in vitro. In brief, fibrinogen (20 mg/mL) was mixed with 0.1 M $CaCl_2$ and thrombin (200 NIH units/mL) in TBS for 1 hour at 37° C. The fibrin clot was then filled with OCT compound (Sakura Finetek) and frozen with dry ice. The frozen fibrin clot was then cut into 6-μm-thick sections using a Tissue-Tek Cryo3 (Sakura Finetek). The sections were washed with PBS for 5 minutes and blocked with 4% Difco skim milk in PBS for 30 minutes at room temperature. After washing, Alexa Fluor 647-labelled mAb was added at a dilution of 1:100, followed by incubation for 30 minutes at room temperature. The sections were then washed three times with PBS. Finally, the sections were mounted using Fluoromount-G (Southern Biotech, Birmingham, AL, USA) for observation with a BZ-9000 (Keyence, Osaka, Japan).

Competition ELISA for anti-Bβ-mAb and anti-γ-mAb

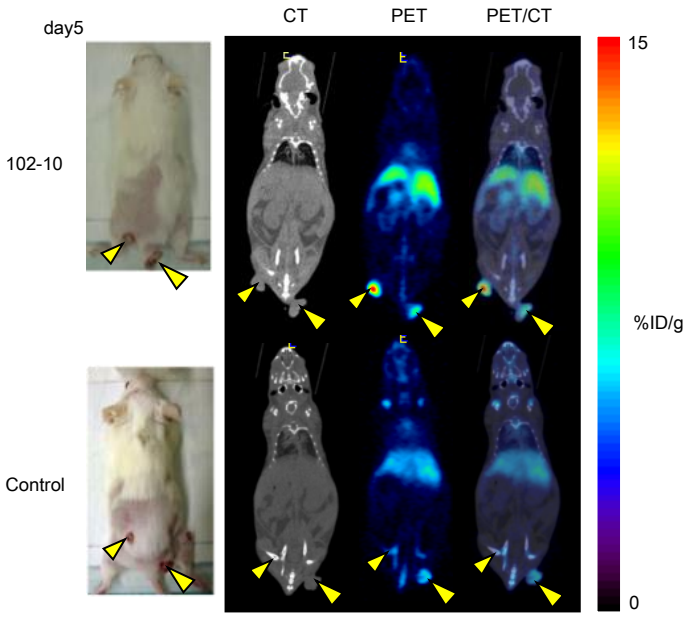
For the ELISA, 10 μg/mL peroxidase-conjugated anti-Bβ mAb was mixed with an equal volume of PBS, 100 μg/mL of anti-Bβ mAb, and 100 μg/mL of anti-γ mAb. Next, 100 μL of each mixture was applied to fibrin-coated 96-well plates. For 1 μg/mL peroxidase-conjugated anti-γ mAb, 10 μg/mL of anti-Bβ mAb and 10 μg/mL of anti-γ mAb were used. All incubations were performed for 1 hour. Subsequently, the wells were washed with TBS-T. Finally, the antibodies bound to the wells were visualised using the 1-Step Slow TMB ELISA as a substrate for 30 minutes.



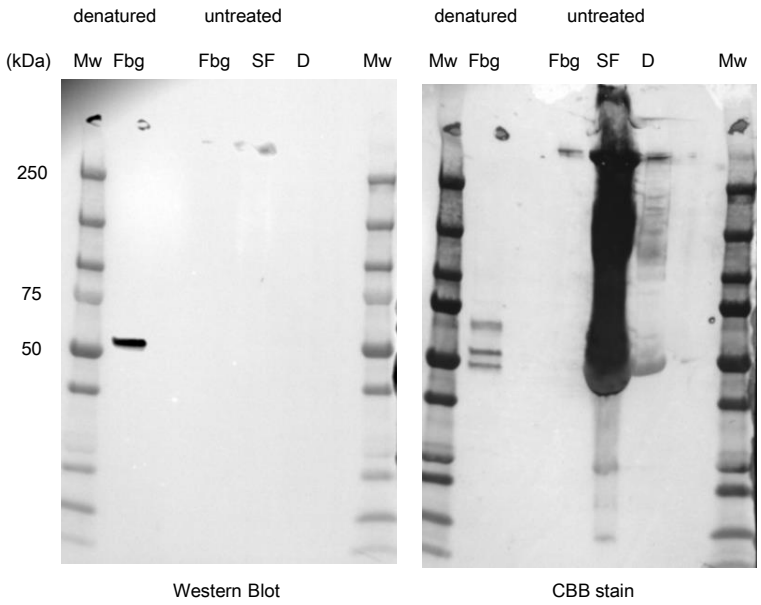
Supplementary Figure 1 Characterization of 102-10 mAb. (**a**) Immunohistochemistry was performed on a fibrin clot using Alexa Fluor 647-conjugated 102-10 mAb (upper) and control IgG (Cetuximab, lower). Only the 102-10 mAb reacted with the fibrin clot. Scale bar, 100 μm. (**b**) A comparison of the 102-10 mAb was performed with previously studied anti-fibrin antibodies. (**c**) To clarify the specificity of the 102-10 mAb, we prepared a fibrinogen plate, a D-dimer plate, and a fibrin clot plate. The peroxidase-conjugated 102-10 mAb (0.1, 1.0, and 10.0 μg/mL) was incubated in each plate (n = 8). The results are presented as the means ± s.d. (**d**) The reactivity of 102-10 mAb against fibrinogen under reducing, heat-shocked (denatured), and untreated conditions was examined (n = 7). The results are presented as the means ± s.d. (**e**) The 102-10 mAb was reactive to human (hu) and mouse (ms) fibrinogen under denatured conditions but not to fibrinogen under untreated conditions.



Supplementary Figure 2 Search of the area of the unique hole in a fibrin clot by competition ELISA. (**a**) The binding of peroxidase-conjugated anti-Bβ mAb (anti-Bβ mAb-HRP) to the fibrin clot was blocked by the cold anti-Bβ mAb but not the cold anti-γ mAb. (**b**) The binding of peroxidase-conjugated anti-γ mAb (anti-γ mAb-HRP) to the fibrin clot was blocked by the cold anti-γ mAb but not the cold anti-Bβ mAb (n = 8). The results are presented as the means ± s.d., ** $P < 0.01$.



Supplementary Figure 3 PET/CT with ^{89}Zr-labelled 102-10 in whole body of spontaneous tumour models. Compared with the ^{89}Zr-labelled control IgG (cetuximab), the ^{89}Zr-labelled 102-10 mAb PET probe accumulated more specifically in tumour. The yellow arrows indicated tumours.



Supplementary Figure 4 Comparing CBB staining with western blot. The 102-10 mAb reacted with the Bβ-chain of denatured fibrinogen (Fbg) but did not recognize untreated fibrinogen, soluble fibrin (SF), and D-dimer (D). In the CBB, SF showed smear pattern indicating several sizes of self assembly of soluble fibrin. D-dimer consists of several sizes of degradation products of fibrin clots.

Supplementary Table 1 Summary of the appearance of fibrin in malignant and non-malignant diseases

Appearance of fibrin in malignant diseases*

	Gliobla-stoma (GBM)	Lung cancer (LC)	Pancreatic cancer (PC)	Ovarian cancer (OC)	Gastric Cancer (GC)	Malignant lymphoma (ML)
No. positive	15	4	6	3	10	0
No. negative	0	0	1	0	0	5

*Itemization of each cancer:
GBM: Glioblastoma in 15 patients (pts); LC: Adenocarcinoma in 4 pts; PC: Well-differentiated adenocarcinoma in 7 pts; OC: Mucinous type in 2 pts and clear cell type in 1 pts; GC: Intestinal type in 2 pts and diffuse type in 8 pts; ML: Hodgkin lymphoma in 1 pts and diffuse large B-cell lymphoma in 4 pts.

Appearance of fibrin in non-malignant diseases

	Acute myocardial infarction	Old myocardial infarction	Acute cerebral infarction	Old cerebral infarction	Acute pancreatitis	Chronic pancreatitis
No. positive	2	0	4	0	4	0
No. negative	0	2	0	2	0	3

Positive: Fibrin deposition was found in more than 30% of the microscopic field.
Negative: No fibrin deposition was found.