Soluble SLAMF7 is generated by alternative splicing in multiple myeloma cells

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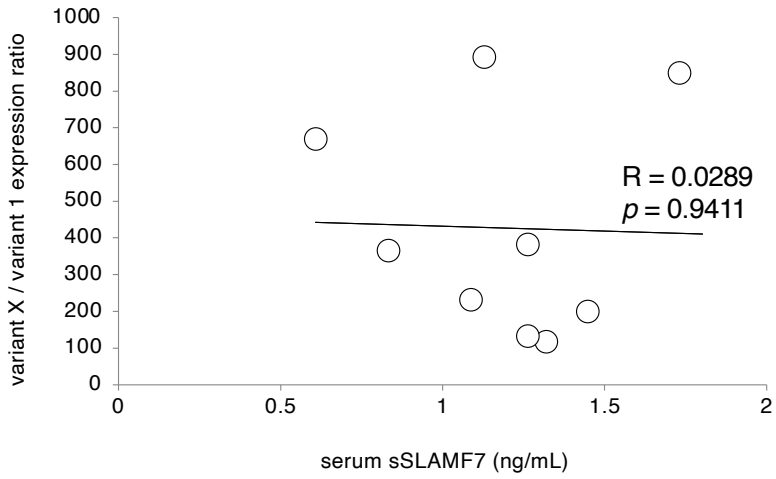
Figure S1

A


variant X / variant 1 expression ratio
1000
900
800
700
600
500
400
300
200
100
0
0
0.5
1
1.5
2
serum sSLAMF7 (ng/mL)
R = 0.0289
p = 0.9411

B

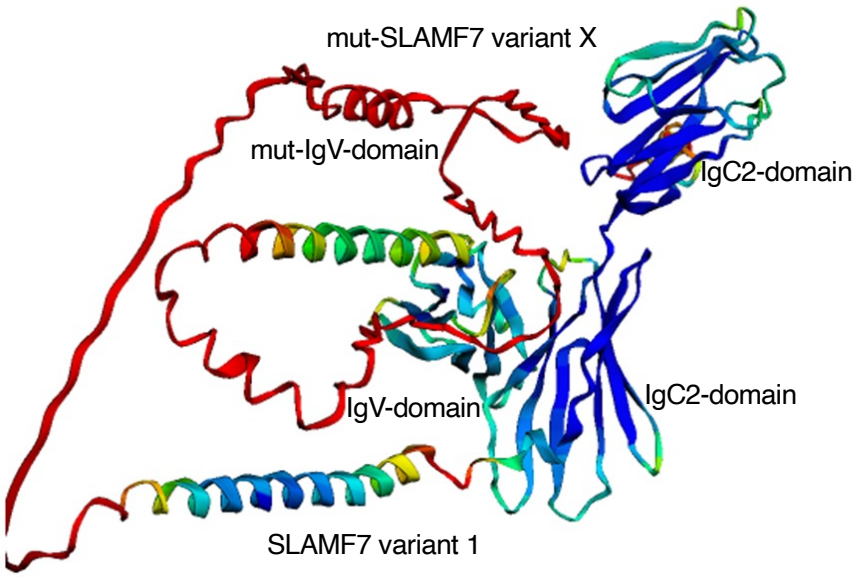

mut-SLAMF7 variant X
mut-IgV-domain
IgC2-domain
IgV-domain
IgC2-domain
SLAMF7 variant 1

Figure S1. (a) The expression levels of *SLAMF7* variant 1 in 10 MM patients were determined by Q-PCR, normalized to that of *GAPDH*, and quantified using the $2^{-\Delta\Delta Ct}$ method with the values of BM-MNC set at 1.0. Serum levels of soluble SLAMF7 (sSLAMF7) were also measured using the enzyme-linked immunosorbent assay (ELISA). The correlation between *SLAMF7* variant X/variant 1 expression ratio and sSLAMF7 was evaluated by calculating Pearson's correlation coefficient. (b) Structure-based prediction of SLAMF7 variant 1-mutant SLAMF7 variant X (mut-SLAMF7 variant X) interactions was performed using the AlphaFold2 program. The mutant SLAMF7 variant X was designed by randomly rearranging the amino acid sequence of the IgV domain.

Figure S2

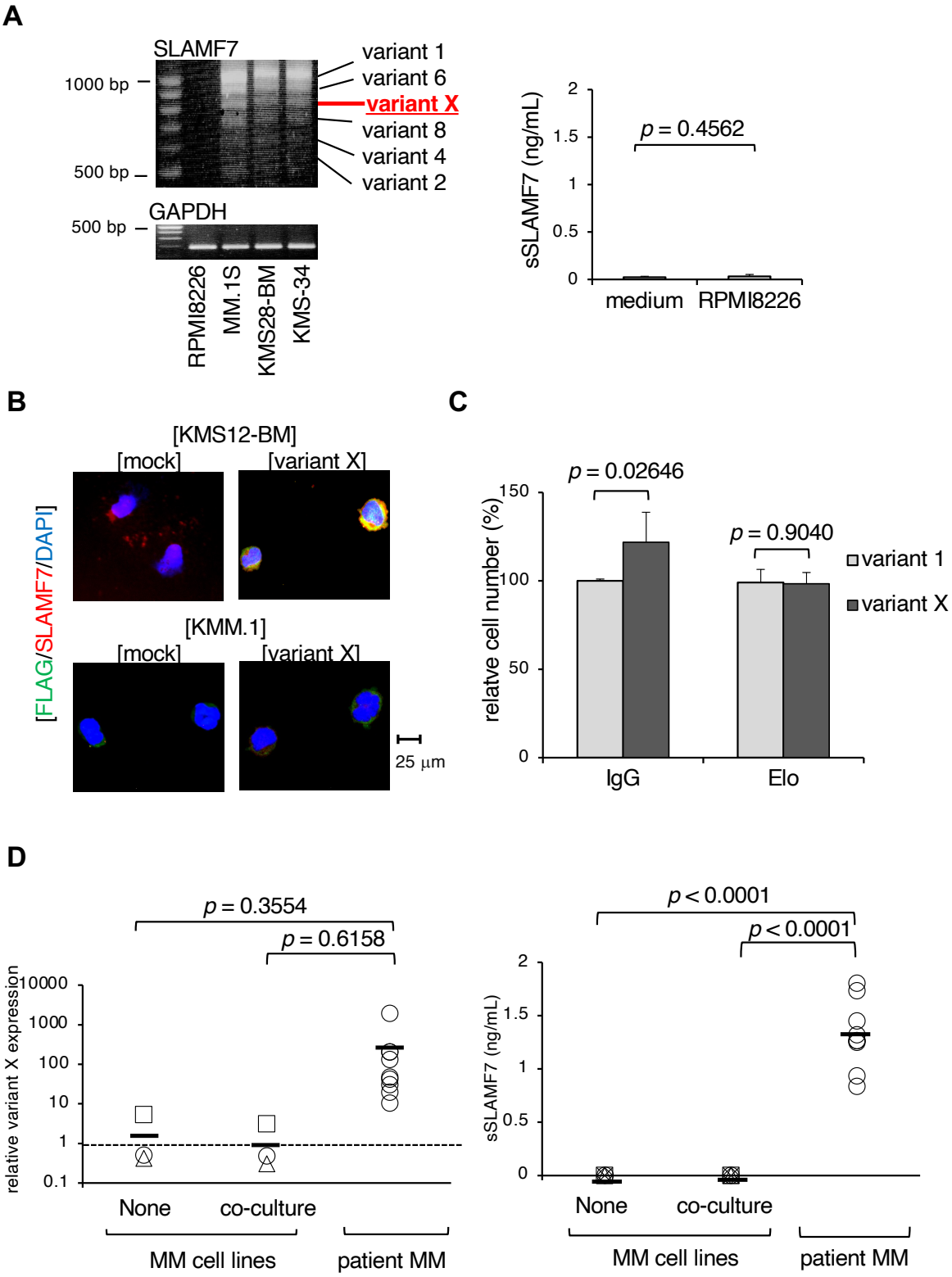

A
SLAMF7
1000 bp
500 bp
variant 1
variant 6
variant X
variant 8
variant 4
variant 2
GAPDH
500 bp
RPMI8226
MM.1S
KMS28-BM
KMS-34
sSLAMF7 (ng/mL)
p = 0.4562
medium
RPMI8226
B
[KMS12-BM]
[mock]
[variant X]
[KMM.1]
[mock]
[variant X]
[FLAG/SLAMF7/DAPI]
25 μm
C
p = 0.02646
p = 0.9040
relative cell number (%)
variant 1
variant X
IgG
Elo
D
p = 0.3554
p = 0.6158
relative variant X expression
None
co-culture
MM cell lines
patient MM
p < 0.0001
p < 0.0001
sSLAMF7 (ng/mL)
None
co-culture
MM cell lines
patient MM

Figure S2. (a) Left panel: The full length *SLAMF7* or *GAPDH* (internal control) was amplified from the cDNA of RPMI8226, MM.1S, KMS28-BM, and KMS-34 cells. PCR was conducted for 40 cycles and the products were analyzed using agarose gel electrophoresis and ethidium bromide staining. Right panel: The supernatants were prepared from $1x10^5$ cells of RPMI8226 cells and the concentrations of sSLAMF7 were measured using the enzyme-linked immunosorbent assay (ELISA) (right panel). Bars indicate the means of three independent experiments. *P* value was calculated using a paired Student's *t* test. (b) Cells were mounted onto glass slides using a Cytospin device (Shandon Scientific, Cheshire, England). Immunostaining was performed using PE-conjugated anti-SLAMF7 (Thermo Fisher Scientific) and Alexa Fluor 488-conjugated anti-FLAG (MBL, Tokyo, Japan) antibodies at 1:200 dilution. The nuclei were counterstained with DAPI. Only merged images are shown. Data shown are representative of multiple independent experiments. (c) MM.1S cells were cultured with 5ug/mL of human IgG (IgG) or elotuzumab (Elo) in the supernatant derived from 1 x 10^5 RPMI8226 cells transduced with *SLAMF7* variant 1 or *SLAMF7* variant X after 24 h of culture. Cell proliferation was assessed after 72 h using the MTT reduction assay and is shown relative to the variant 1 group. Bars indicate the mean of three independent experiments. *P* value was calculated using a paired Student's *t* test. (d) Left panel: KMS12-BM, KMS26, and MM.1S cells were cultured with or without UBE6T-7 cells for 24 hours, respectively. The expression levels of *SLAMF7* variant X in KMS12-BM (△), KMS26 (◇), and MM.1S (□), and patient MM cells (○) were determined by Q-PCR, normalized to that of *GAPDH*, and quantified using the $2^{-\Delta\Delta Ct}$ method with the values of BM-MNC set at 1.0. Right panel: The supernatants were prepared from $1x10^5$ cells of KMS12-BM (△), KMS26 (◇), and MM.1S (□) cells co-cultured with or without UBE6T-7 cells for 24 hours, respectively, and the concentrations of sSLAMF7 were measured using ELISA. Serum levels of sSLAMF7 in MM patients (○) were also measured using the ELISA. *P* value was determined by one-way ANOVA with Tukey's multiple comparison test.

Supplementary Table S1. PCR primers used in this study

gene	sequence (location)	product size
SLAMF7 variant 1 (full length)	forward: 5'- agggaagtggcttcatttcagtg -3'(exon 1) reverse: 5'- tctcataggcaaatagccttggt -3' (exon 7)	1100 bp
SLAMF7 variant 1	forward: 5'- acagctcatcactccagcagcc-3'(exon 2) reverse: 5'- agtgtgagggattgtgtcgtac-3' (exon 5)	547 bp
SLAMF7 variant X	forward: 5'- aagctctgtgaagagaacaatcc -3' (exon 3/6) reverse: 5'- gcagagacttaggggagtgc -3' (exon 7)	162 bp
GAPDH	forward: 5'-gagtcaacggatttggtcgt-3' reverse: 5'-gacaagcttcccgttctcag-3'	185 bp