Supporting Information for

Blocking the proteolytic activity of zymogen matriptase with antibody-based inhibitors

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Table S1. Summary of kinetic constants determined from surface plasmon resonance binding data shown in figures S1 and S2

	zSPD			acSPD		
	K_D [nM]	k_{on} [10^6 $M^{-1}s^{-1}$]	k_{off} [$10^{-3}s^{-1}$]	K_D [nM]	k_{on} [10^6 $M^{-1}s^{-1}$]	k_{off} [$10^{-3}s^{-1}$]
aZ-mAb-1	2.2 ±1.1	2.0 ±0.6	3.8 ±0.7	2.2 ±1.8	2.5 ±0.2	3.3 ±0.3
aZ-mAb-2	2.6 ±0.6	1.6 ±0.2	4.1 ±0.3	2.7 ±1.0	1.9 ±0.4	4.1 ±0.4
aZ-mAb-3	1.8 ±0.5	2.0 ±0.4	3.6 ±0.2	1.5 ±0.4	2.5 ±0.3	3.1 ±0.3
aZ-mAb-4	6.1 ±4.9	1.4 ±0.6	12 ±1.0	8.6 ±1.5	1.2 ±0.1	9.1 ±2.0
aZ-mAb-5	5.4 ±1.2	1.8 ±0.1	9.8 ±2.7	4.6 ±0.9	1.7 ±0.3	8.1 ±0.5
aZ-mAb-6	12 ±5.0	4.9 ±1.0	58 ±14	37 ±8.5	1.9 ±0.5	68 ±8
aZ-mAb-7	17 ±2.2	2.7 ±0.2	47 ±2.1	32 ±12	2.6 ±1.0	77 ±8
aZ-mAb-9	5.0 ±1.1	1.6 ±0.3	7.7 ±0.7	2.8 ±0.9	2.3 ±0.6	6.6 ±0.01
aZ-mAb-10	4.3 ±2.5	3.5 ±2.3	12 ±0.6	5.0 ±1.5	2.4 ±0.4	13 ±1

All data presented in the table is an average of at least 3 independent measurements.

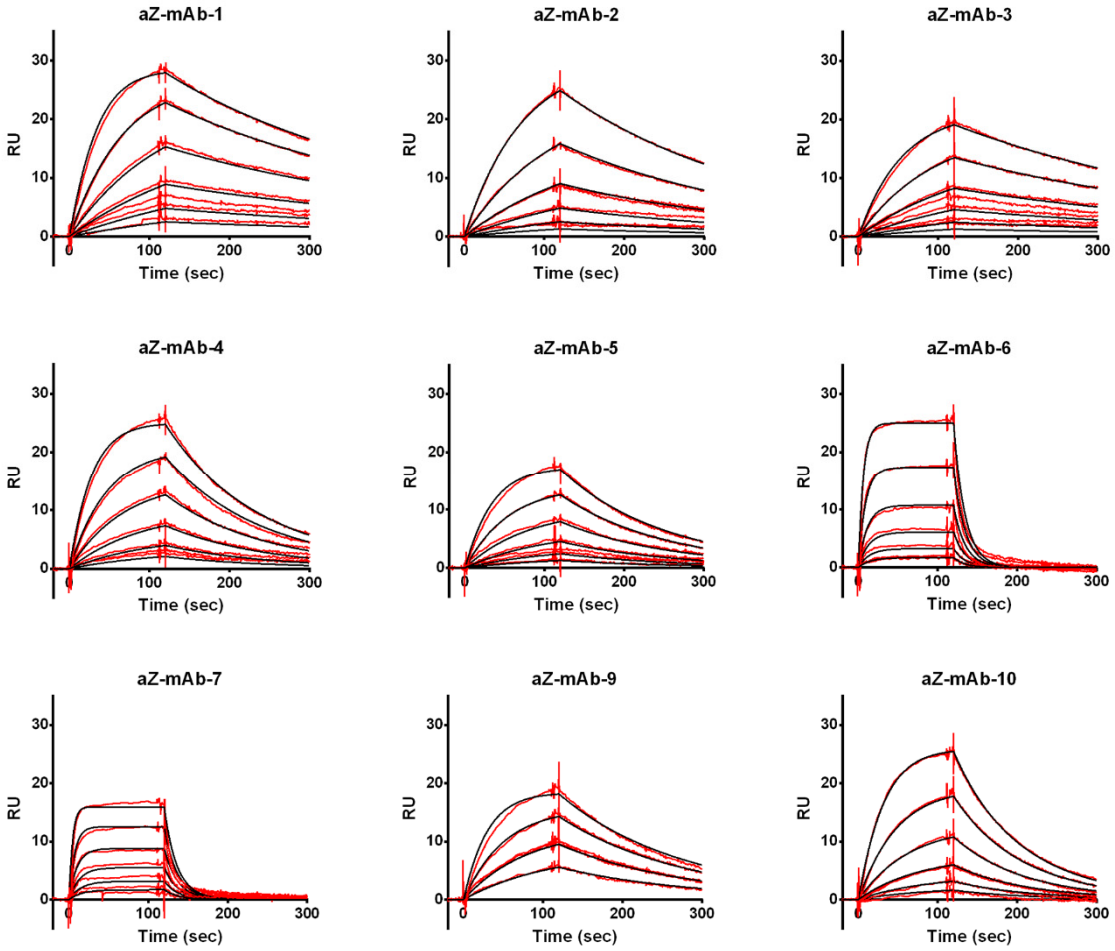

Figure S1. Surface plasmon resonance experiments to determine equilibrium constants for acSPD and the nine monoclonal antibodies. The red lines represent the binding curves and the black lines the 1:1 fit performed by the Biacore evaluation software. The individual monoclonal antibodies noted above the graphs were captured on an anti-mouse IgG surface before acSPD was injected. The concentration ranges of acSPD were 1.56-50 nM for aZ-mAb-6 and 7; 0.78-25 nM for aZ-mAb-4; 0.39-12.5 nM for aZ-mAb-1, 5, 8 and 10; and 0.2-6.25 nM for aZ-mAb-2 and 3. The results are compiled in Table S1. Each graph represents one representative experiment. The data for aZ-mAb-4, 6 and 7, shown in Figure 3, are included along with the data from the other monoclonal antibodies to facilitate direct comparison.

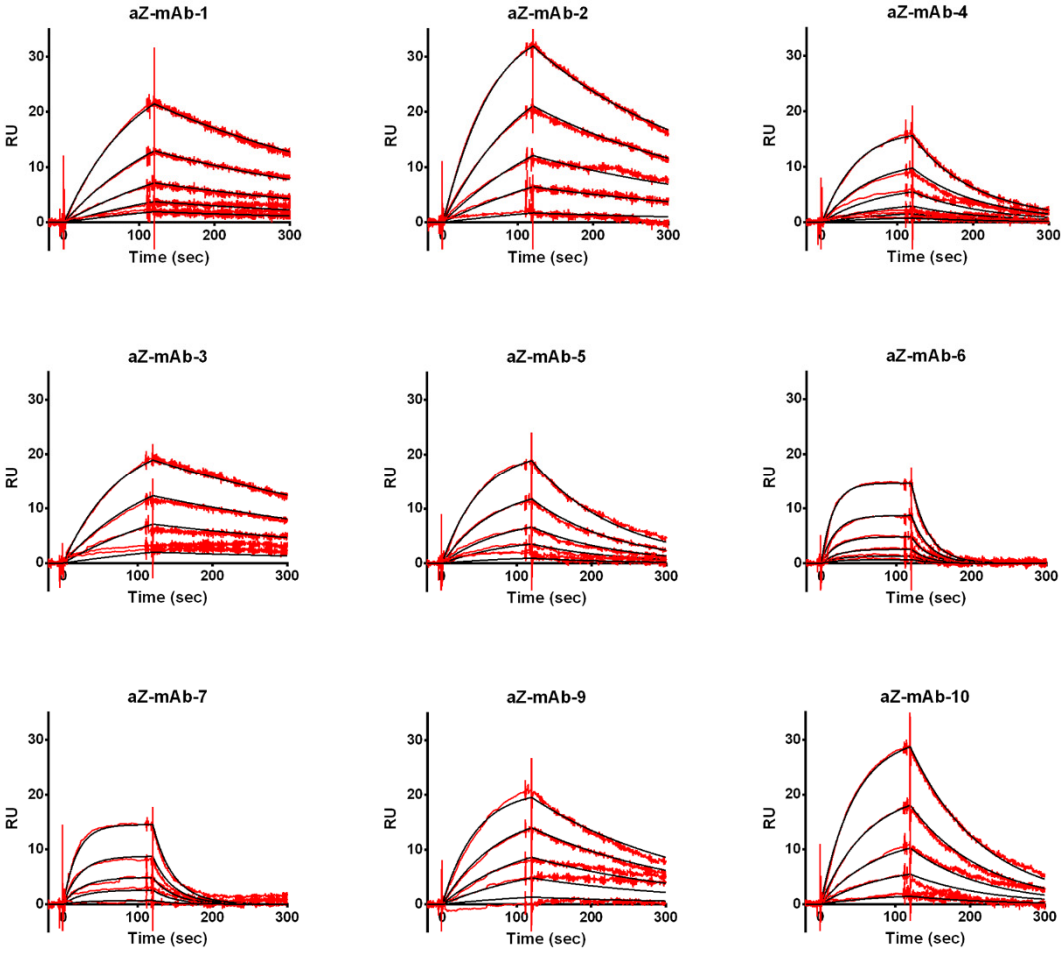

Figure S2. Surface plasmon resonance experiments to determine equilibrium constants for zSPD and the nine monoclonal antibodies. The red lines represent the binding curves and the black lines the 1:1 fit performed by the Biacore evaluation software. The individual monoclonal antibodies noted above the graphs were captured on an anti-mouse IgG surface before zSPD was injected. The concentration ranges of zymogen matriptase were 0.94-1.5 nM for aZ-mAb-1 and 3; and 0.94-30 nM for aZ-mAb-2, 4, 5, 6, 7, 9 and 10. The results are compiled in Table S1. Each graph represents one representative experiment. The data for aZ-mAb-4, 6 and 7, shown in Figure 3, are included along with the data from the other monoclonal antibodies to facilitate direct comparison.

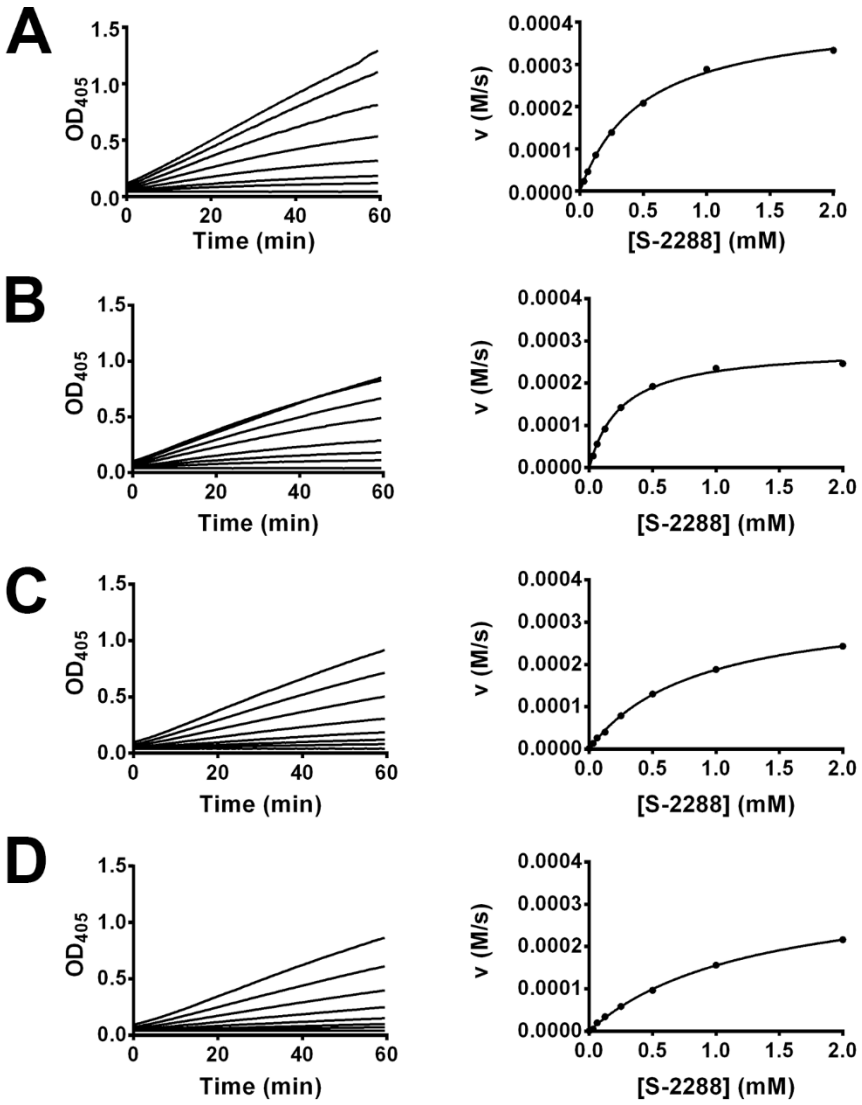

Figure S3. Representative data for the determination of kinetic data for acSPD in the presence or absence of aZ-mAb-4, aZ-mAb-6 or aZ-mAb-7 by chromogenic assay. Active matriptase alone (100 pM, A) or in the presence of 20 nM aZ-mAb-4 (B), 60nM aZ-mAb-6 (C) or aZ-mAb-7 (D) was incubated for one hour before the addition of substrate. Left column of data: the increase in absorbance at 405 nm over 60 minutes where every line of data represents a different concentration of the chromogenic substrate S-2288. Right column of data: the corresponding Michaelis-Menten fit from which the K_M and V_{max} were determined. Data is presented in Table 2.

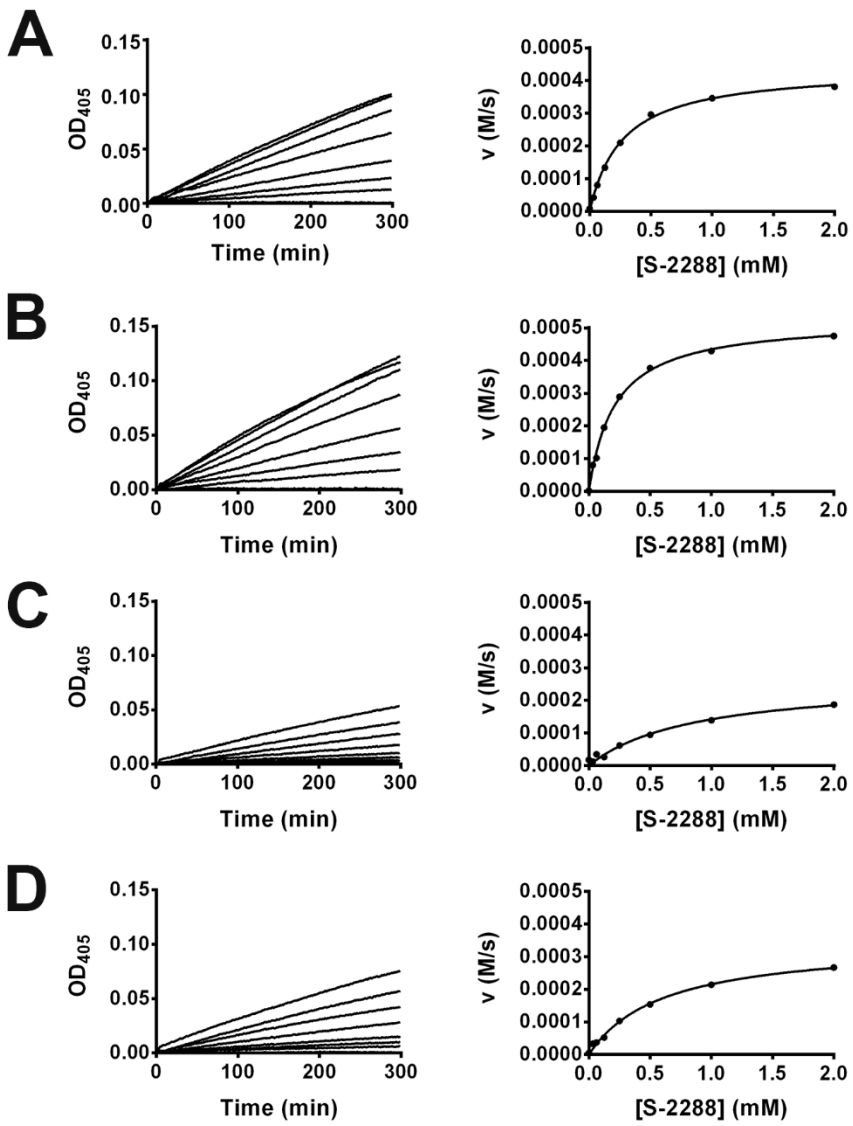

Figure S4: Representative data for the determination of kinetic data for zSPD in the presence or absence of aZ-mAb-4, aZ-mAb-6 or aZ-mAb-7 by chromogenic assay. zSPD alone (30 nM, A) or in the presence of 20 nM aZ-mAb-4 (B), 60nM aZ-mAb-6 (C) or aZ-mAb-7 (D) was incubated for one hour before the addition of substrate. Left column of data: the increase in absorbance at 405 nm over 60 minutes where every line of data represents a different concentration of the chromogenic substrate S-2288. Right column of data: the corresponding Michaelis-Menten fit from which the K_M and V_{max} were determined. Data is presented in Table 2.