

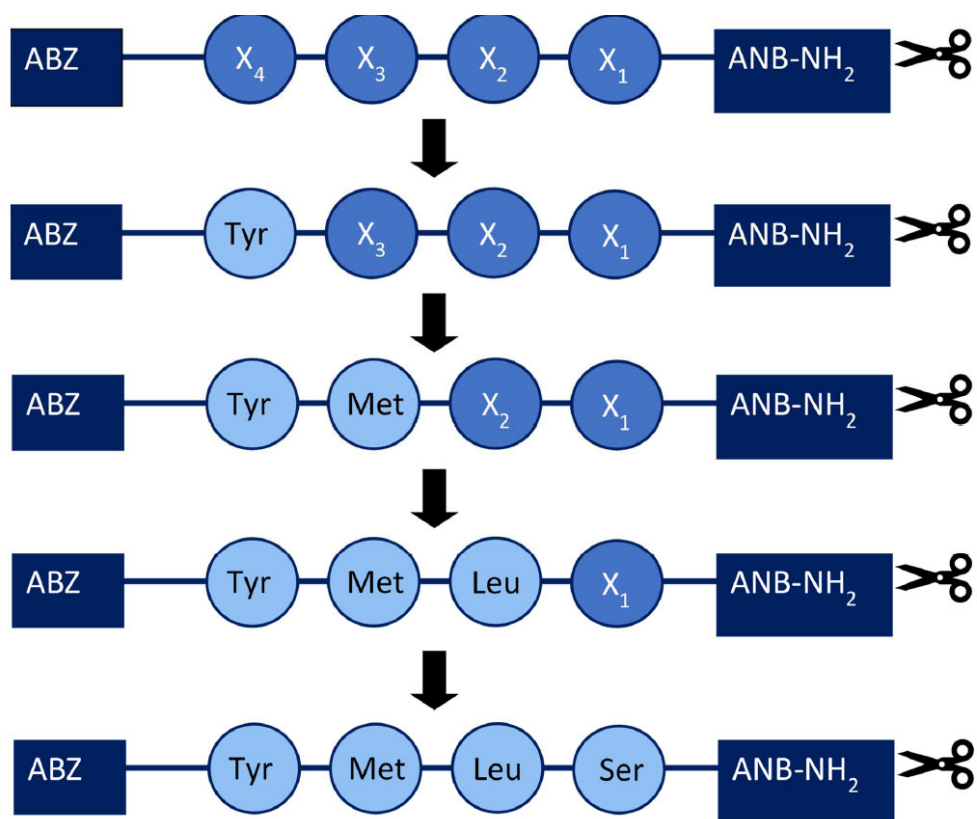


Figure S1. Deconvolution of a chromogenic tetrapeptide library against protease SplF.

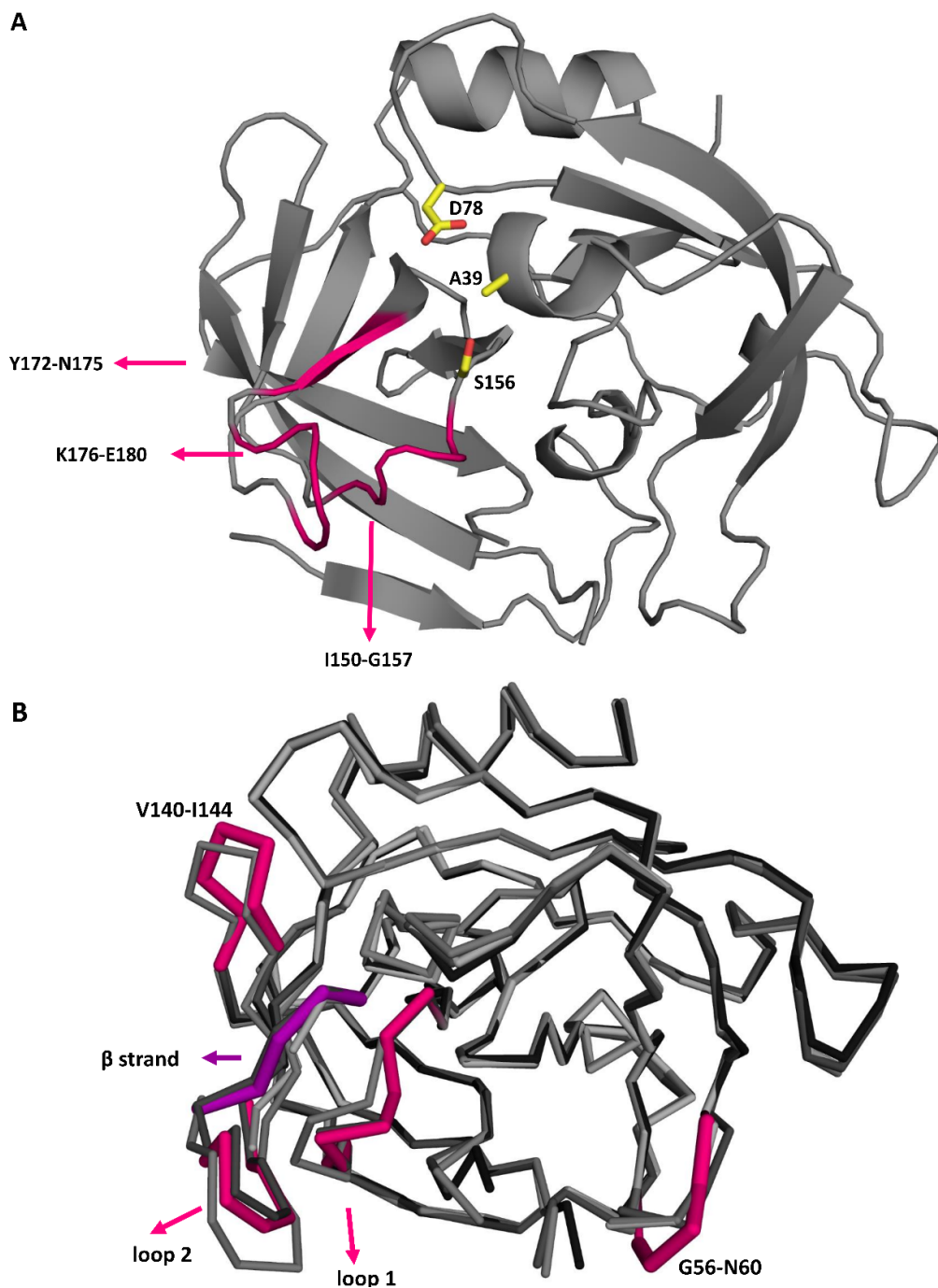


Figure S2. Overall fold of SplF (A) and comparisons of chains contained in the asymmetric unit (B). (A) Overall fold of SplF protease. Primary features described in the text are highlighted. (B) Superposition of chains contained in the unit cell (grey tones). Major differences among chains are observed in loops 1 and 2 constituting the binding pocket and additionally in fragments V140-I144 and G56-N60 (highlighted in colour).

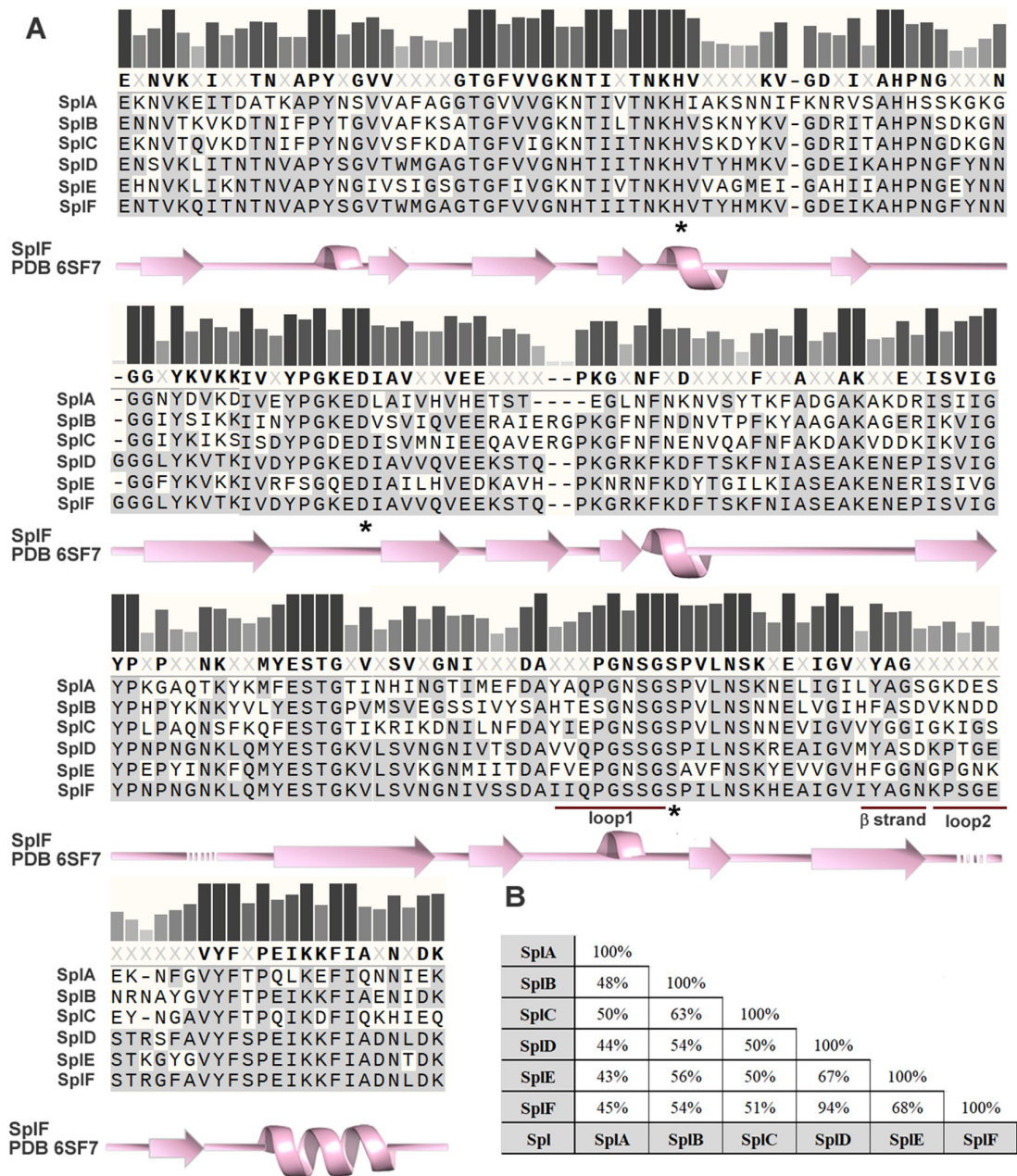


Figure S3. Sequence alignment of Spl proteases.

(A) The sequences of Spl proteases were alignment to the sequence of SplF. Secondary structure elements (SplF) are shown below the sequence. The restudies of the catalytic triad (asterisk) and residues involved in S1 pocket formation (loop1, loop2 and β strand; underlined) are highlighted.

(B) Amino acid sequence identities calculated using paired alignments are shown for particular Spl proteases.

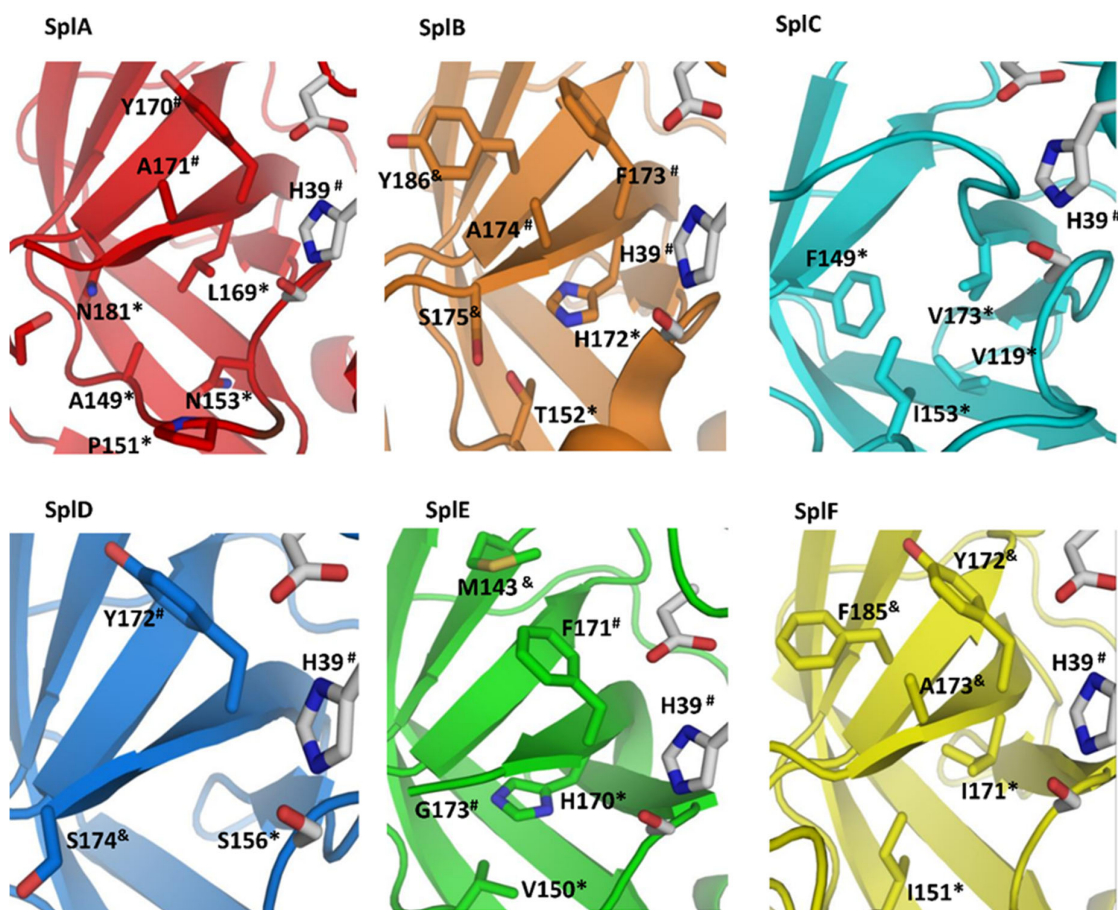


Figure S4. Comparison of substrate binding clefts of Spl proteases.

The residues defining the S1 (*), S2 (#), and S3 (&) pockets of Spl proteases are shown in stick model: SplA (PDB 2W7S, red) [13], SplB (PDB 2VID, orange) [14], SplC (PDB 2AS9, cyan) [17] and SplD (PDB 4INK, blue) [15], SplE (PDB 5MM8, green) [16] and SplF (PDB 6SF7, yellow). Residues constituting the catalytic triad are highlighted grey.

Table S1. Primers (forward and reverse) used to generate expression constructs of SplF protein. Restriction sites introduced for cloning purpose are bolding, the part which is complementary to the gene is underline.

Construct	sequence	Sequence
	5'- forward primer – 3'	5'- reverse primer – 3'
pGEX-5T Gly-Ser-SplF	CGTGGATCCGAAAATACTGTAAAC AAATTA	TACCTCGAGATTATTTATCTAAA TTATCTGCAATGAAT
pGEX-5T Gly-Ser-SplF His39Ala	ATCATTACCAATAAAGCTGTACCTA TCACATG	CATGTGATAGGTAACAGCTTTATTGGTAAT GAT
pETDuet-1 His6-SplF	GCTTCCATGGGCCATCACCATCATCA CCACGAAAATACTGTTA	GCTTGGATCCTTATTTATCTAAA TTATCTGCAATGAAT
pETDuet-1 Met-Gly-SplFHis6	TTAACCATGGGCGAAAATACTGTTA AACAAATTA	TTAAAAGCTTTTAGTGGTGATGATGGTGAT GTTTATCTAAATTATCTGC
pET-20b(+) SplF-His6	CAGCCGGCCATGGAAAATACTGTTA AA	TTAAAAGCTTTTAGTGGTGATGATGGTGAT GTTTATCTAAATTATCTGC