Supporting information

Activation mechanism of the *Bacteroides fragilis* cysteine peptidase, Fragipain

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Table S1: primers

Primer	Sequence	Comment
R147A-UP R147A-LO	GCAAAGACA**GCT**TGGTTTGGT ACCAAACCA**AGC**TGTCTTTGC	Primers used to mutate residue R147 present in the cleavage site to alanine.
R147K-UP R147K-LO	GCAAAGACA**AAG**TGGTTTGGT ACCAAACCA**CTT**TGTCTTTGC	Primers used to mutate residue R147 present in the cleavage site to lysine.
LΔ5-UP LΔ5-LO	GAGGGATGGATACCTCGTTGGTTTGGTCAG CTGACCAAACCAACGAGGTATCCATCCCTC	Primers used to delete 5 residues (ΔS142-T146) in the cleavable loop.
LΔ7-UP LΔ7-LO	CACGGTGAGGGATGGCGTTGGTTTGGTCAG CTGACCAAACCAACGCCATCCCTCACCGTG	Primers used to delete 7 residues (ΔI140-T146) in the cleavable loop.
LΔ7+R147A-UP LΔ7+R147A-LO	CACGGTGAGGGATGG**GCA**TGGTTTGGTCAG CTGACCAAACCA**TGC**CCATCCCTCACCGTG	Primers used to delete 7 residues (ΔI140-T146) in the cleavable loop and to mutate residue R147 to alanine.

Table S2: strains

Strain #	Organism	Genetic background	Plasmid	Comment	Reference
FC2470	*E. coli Rosetta (DE3) PlysS*	-	pET28-*fpn*	Strains used to express wild-type Fpn.	Choi V. *et al.* (2016). Nature Medicine.
FC2471	*E. coli Rosetta (DE3) PlysS*	-	pET28-*fpn(H135A+C180A)*	Strains used to express Fpn (H135A+C180A) active site mutant.	
FC2472	*E. coli Rosetta (DE3) PlysS*	-	pET28-*fpn(R147A)*	Strains used to express Fpn (R147A) cleavage site mutant.	This work.
FC2473	*E. coli Rosetta (DE3) PlysS*	-	pET28-*fpn(R147K)*	Strains used to express Fpn (R147K) cleavage site mutant.	
FC2474	*E. coli Rosetta (DE3) PlysS*	-	pET28-*fpn(LΔ5)*	Strains used to express Fpn (LΔ5) cleavable loop mutant.	
FC2475	*E. coli Rosetta (DE3) PlysS*	-	pET28-*fpn(LΔ7)*	Strains used to express Fpn (LΔ7) cleavable loop mutant.	
FC2476	*E. coli Rosetta (DE3) PlysS*	-	pET28-*fpn(LΔ7+R147A)*	Strains used to express Fpn (LΔ7+R147A) cleavable loop mutant.	
FC2477	*E. coli Rosetta (DE3) PlysS*	-	pET28-*bft*	Strains used to express BFT.	Choi V. *et al.* (2016). Nature Medicine.
-	*B fragilis*	Δ*fpn*	-	Strain used for western-blot.	
-	*B. fragilis*	Δ*fpn*	pAH2-*fpn*	Strain used for western-blot.	This work. See [Choi V. *et al.* (2016). Nature Medicine and Hecht A *et al.* (2016; in press). EMBO reports] for primer and method information.
-	*B. fragilis*	Δ*fpn*	pAH2-*fpn(H135A+C180A)*	Strain used for western-blot.	
-	*B. fragilis*	Δ*fpn*	pAH2-*fpn(LΔ7)*	Strain used for western-blot.	

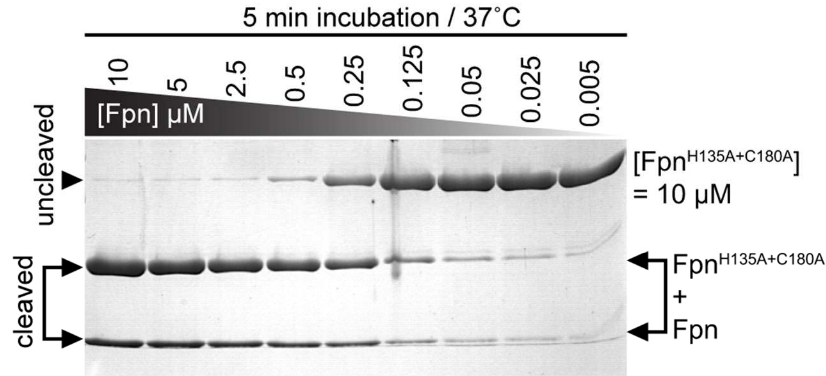

Figure S1: Concentration dependence of Fpn cleavage in *trans*. 10-µl samples containing 10 µM of inactive $Fpn^{H135A+C180A}$ and decreasing concentrations of wild-type Fpn (final concentrations tested: 10, 5, 2.5, 0.5, 0.25, 0.125, 0.05, 0.025 and 0.005 µM) were incubated at 37˚C for 5 minutes and resolved by SDS-PAGE.

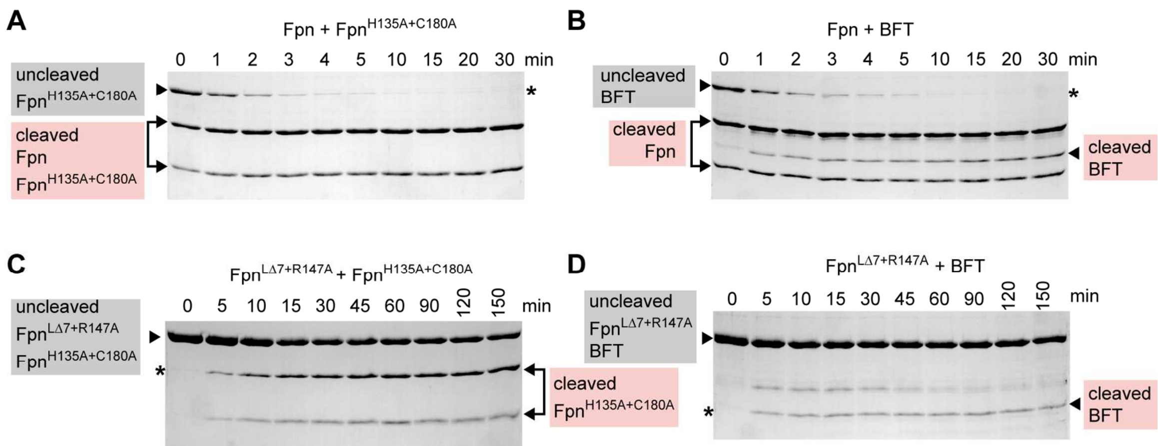

Figure S2: Time course of BFT or $Fpn^{H135A+H180}$ cleavage by wild-type Fpn or the loopless pro form Fpn mutant ($Fpn^{L\Delta7+R147A}$). A) Digestion of Fpn active site mutant ($Fpn^{H135A+H180}$) (5 µM) by wild-type Fpn (5 µM). B) Digestion of BFT (5 µM) by wild-type Fpn (5 µM). C) Digestion of $Fpn^{H135A+H180}$ (5 µM) by loopless $Fpn^{L\Delta7+R147A}$ (5 µM). D) Digestion of BFT (5 µM) by $Fpn^{L\Delta7+R147A}$ (5 µM). Bands quantified with imageJ are marked with a star.

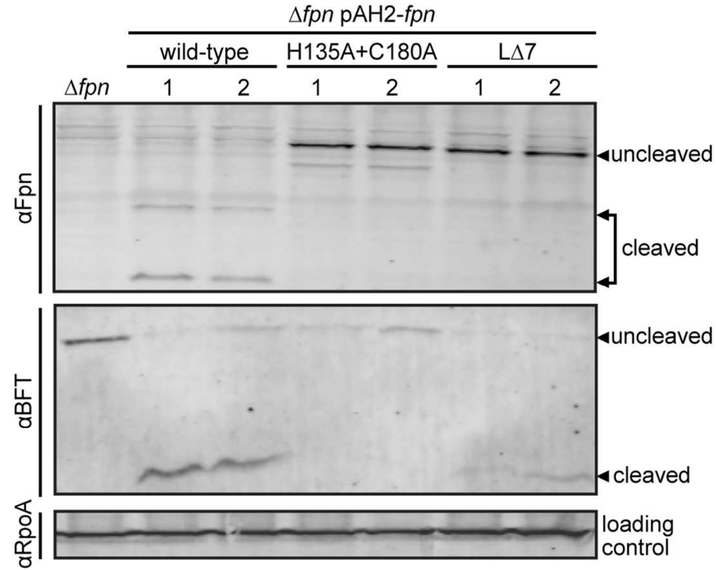

Figure S3: Western blot of *B. fragilis Δfpn* lysate ectopically expressing wild-type Fpn, $Fpn^{H135A+C180A}$ and $Fpn^{L\Delta7}$ proteins. Cell lysate supernatants were used to evaluate the ability of these proteins to auto-cleave (upper blot) and to process BFT (middle blot). RpoA protein (lower blot) was used as a loading control.