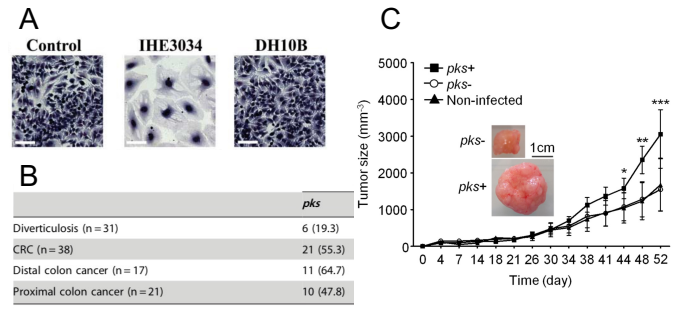
Figure 1

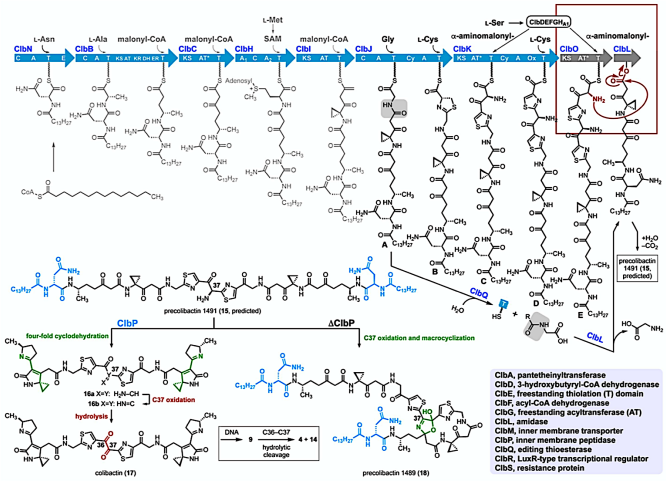


B

	pks
Diverticulosis (n = 31)	6 (19.3)
CRC (n = 38)	21 (55.3)
Distal colon cancer (n = 17)	11 (64.7)
Proximal colon cancer (n = 21)	10 (47.8)

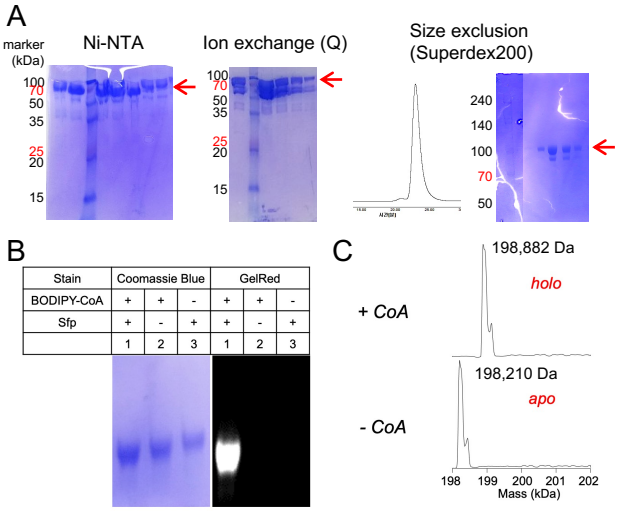
The effect of colibactin (A) HeLa cells infected with *pks*-cluster harboring *E.coli* (IHE3034), and control *E.coli* (DH10B) (Nougayre`de, *Science*, 2006). (B) The number of people who has *pks* cluster in their microbiome. Numbers in the parentheses indicate the percentage of the population (Buc, *PLoS ONE*, 2013). (C) The growth of tumors injected into mice. The tumor cells were infected either control or *pks* cluster-containing *E.coli* before injection (Cougnoux, *Gut*, 2014).

Figure 2



Proposed biosynthesis of (pre)colibactin with biosynthetic enzymes (Xue and Kim, *Science*, 2019)

Figure 3

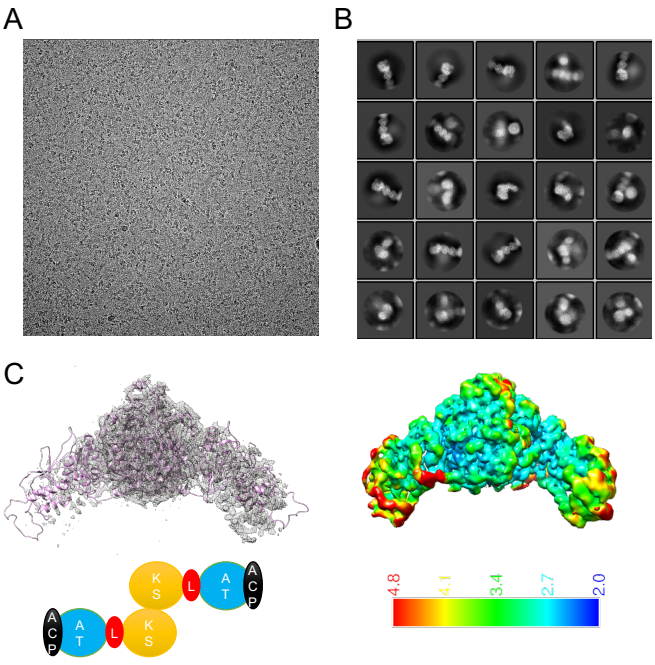


B

Stain	Coomassie Blue			GelRed		
BODIPY-CoA	+	+	-	+	+	-
Sfp	+	-	+	+	-	+
	1	2	3	1	2	3

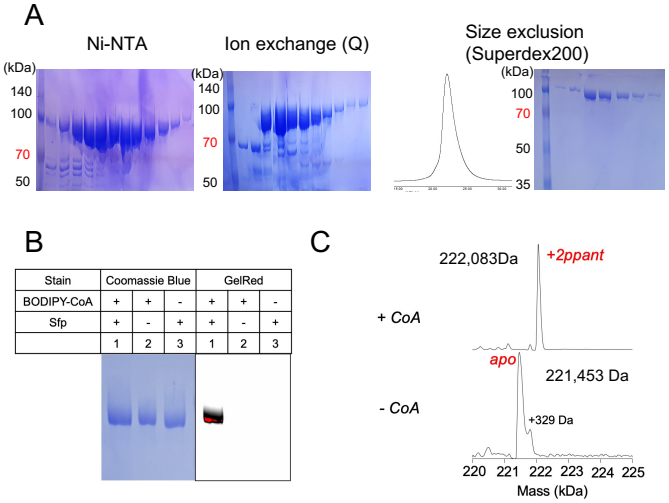
Purification and activity test of ClbC (A) ClbC was purified in Ni-NTA, ion exchange, and size exclusion chromatography. (B) The purified ClbC was phosphopantetheinylated (ppantylated) with a fluorescent dye (BODIPY)-labelled CoA. (C) Native mass spectrometry conformed that ClbC is 100% ppantylated.

Figure 4



CryoEM data of apo ClbC (A) Motion-corrected micrograph image. (B) Representative 2D averages. (C) (left) 2.95 Å cryo-EM map superimposed with the homology model of ClbC. Schematic domain arrangement in the model is shown in below. (right) Local resolution electron density map

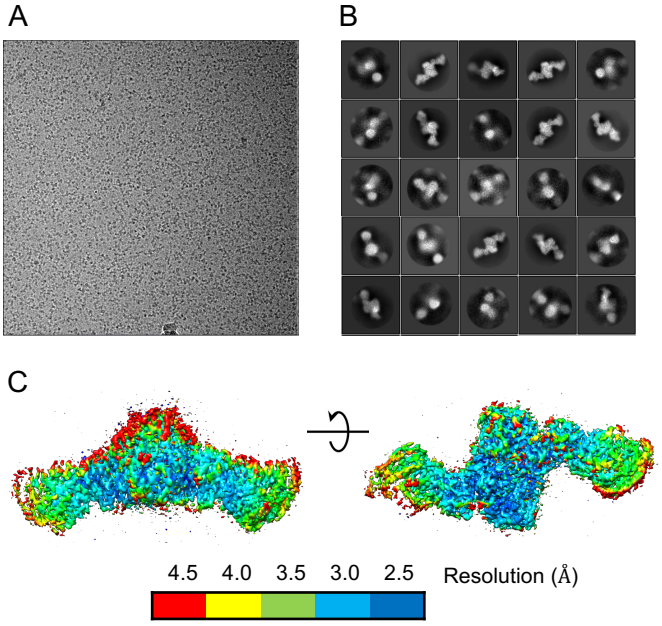
Figure 5



Stain	Coomassie Blue			GelRed		
BODIPY-CoA	+	+	-	+	+	-
Sfp	+	-	+	+	-	+
	1	2	3	1	2	3

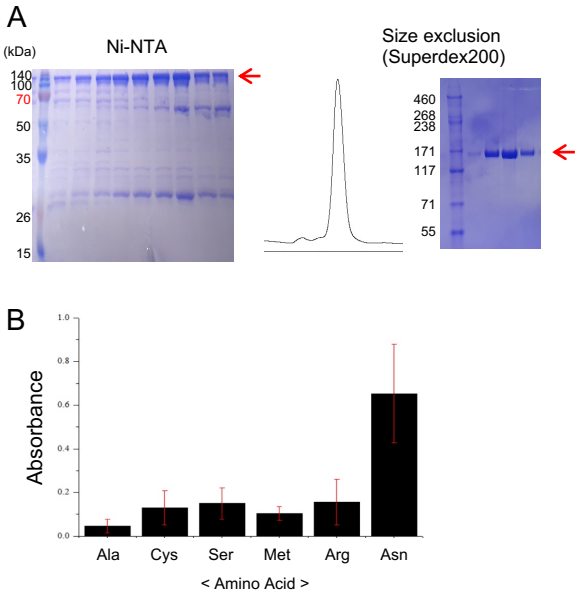
Purification and activity test of ClbI (A) ClbI was purified in Ni-NTA, ion exchange, and size exclusion chromatography. (B) The purified ClbI was ppantylated with a BODIPY-labelled CoA. (C) Native mass spectrometry conformed that ClbI is 100% ppantylated.

Figure 6



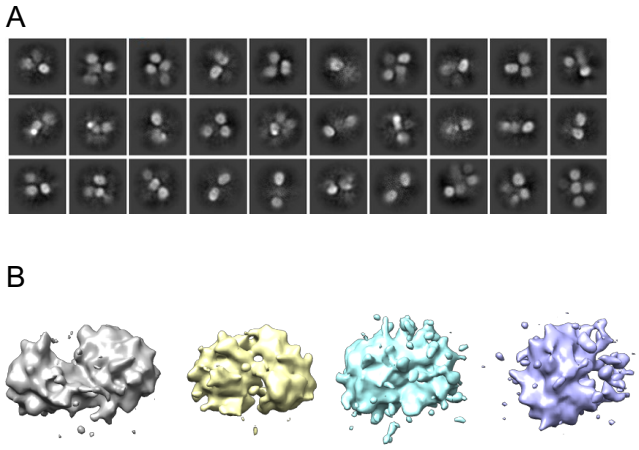
CryoEM data of apo ClbI (A) Motion-corrected micrograph image. (B) Representative 2D averages. (C) (left) 3.14 Å cryo-EM map colored by local resolution value. (right) 90 º rotated map

Figure 7



Purification and activity test of ClbN (A) ClbN was purified in Ni-NTA and size exclusion chromatography. (B) The purified ClbN was tested by colorimetric assay with various amino acids. Asn had larger absorbance than any other amino acids, indicating that Asn is the genuine substrate of ClbN

Figure 8



Cryo-EM data analysis of ClbN (A) 2D averages showed multiple blobs in diverse orientation. (B) 3D reconstructions show two main blobs interacting in different distances, but further refinement was failed.