
Supplementary information

piRNA processing by a trimeric Schlafen-domain nuclease

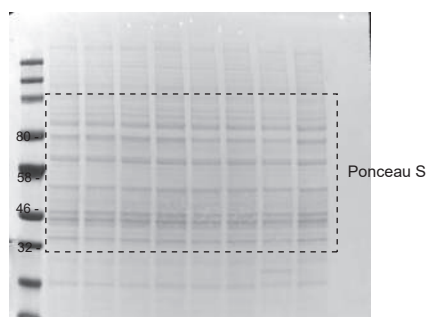
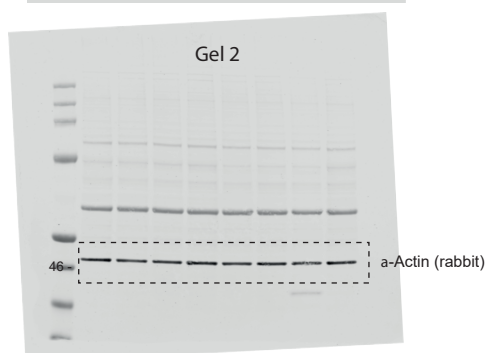
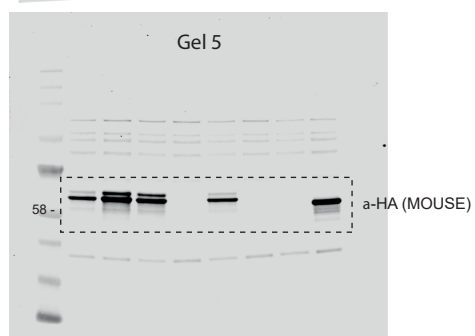
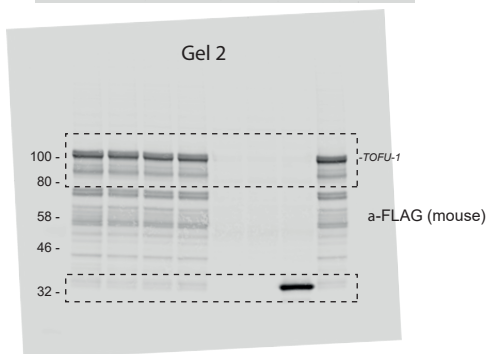
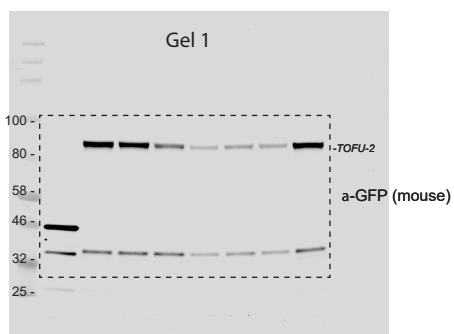
In the format provided by the
authors and unedited

Figure 2A_Raw image files

HA-SLFL-4:	-	-	-	-	-	-	-	+
HA-SLFL-3:	+	+	+	-	+	-	-	-
3xFLAG-mCherry:	-	-	-	-	-	-	+	-
TOFU-1-3xFLAG-mCherry:	+	+	+	+	-	-	-	+
eGFP-TOFU-2-E216A:	-	+	-	-	+	-	-	-
eGFP-TOFU-2-WT:	-	-	+	+	+	+	+	+
3xMyc-eGFP:	+	-	-	-	-	-	-	-

HA-SLFL-4:	-	-	-	-	-	-	-	+
HA-SLFL-3:	+	+	+	-	+	-	-	-
3xFLAG-mCherry:	-	-	-	-	-	-	+	-
TOFU-1-3xFLAG-mCherry:	+	+	+	+	-	-	-	+
eGFP-TOFU-2-E216A:	-	+	-	-	+	-	-	-
eGFP-TOFU-2-WT:	-	-	+	+	+	+	+	+
3xMyc-eGFP:	+	-	-	-	-	-	-	-

Input (1 %)



GFP-IP

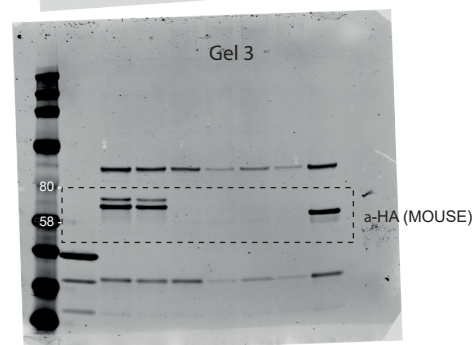
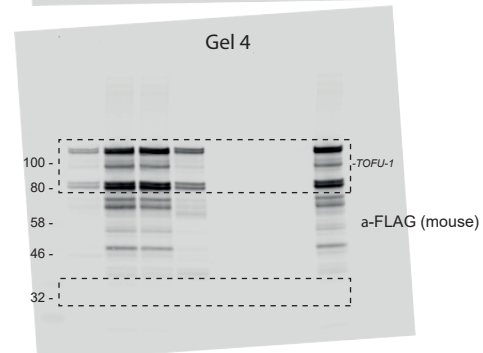
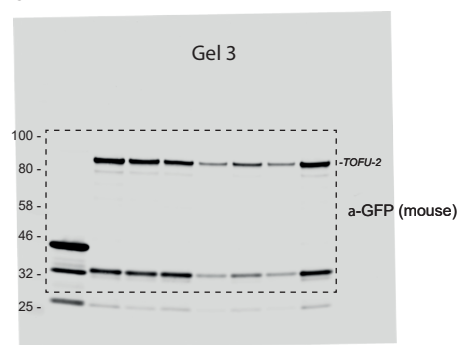


Figure 3b_Raw image files

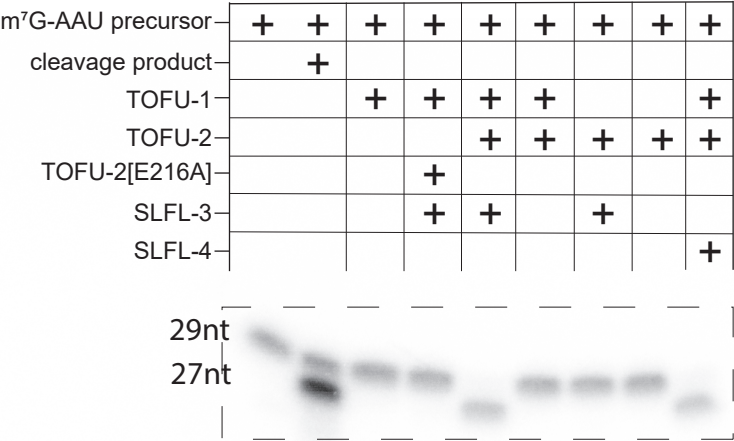


Figure 3c_Raw image files

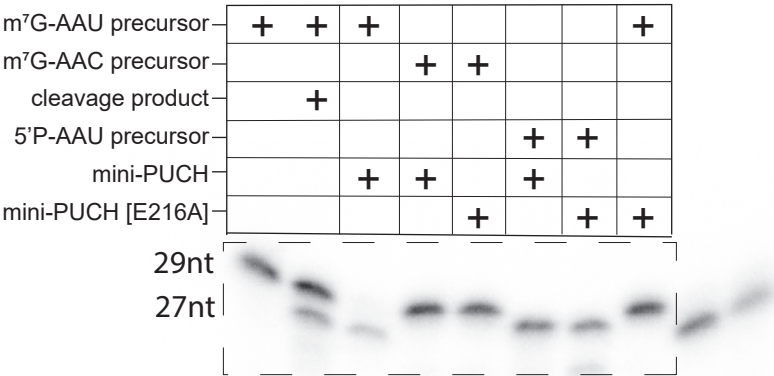


Figure 3d_Raw image files

m ⁷ G-AAU precursor	+	+	+	+
cleavage product	+			
10-nt RNA oligo			+	+
mini-PUCH			+	
mini-PUCH [E216A]				+



Figure 3e_Raw image files

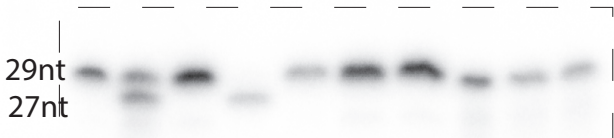


Figure 3f_Raw image files

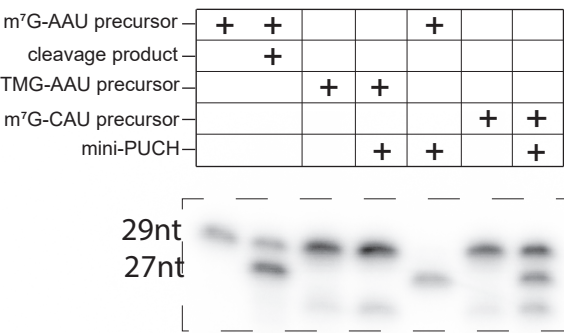
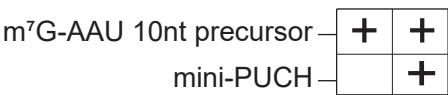
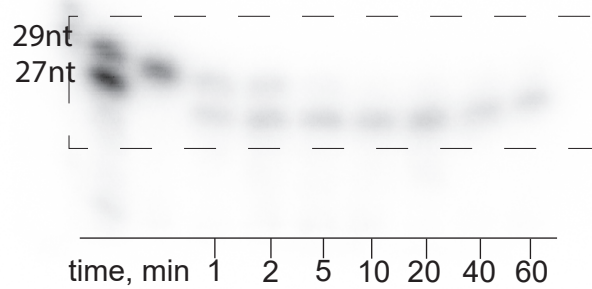


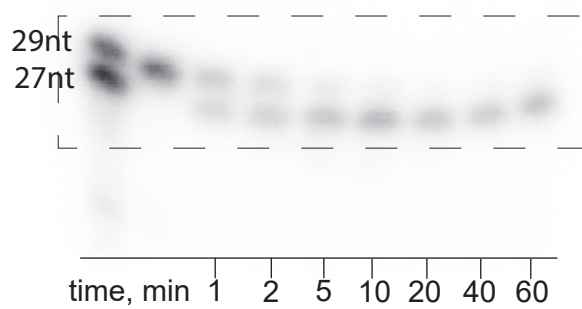
Figure 3g_Raw image files



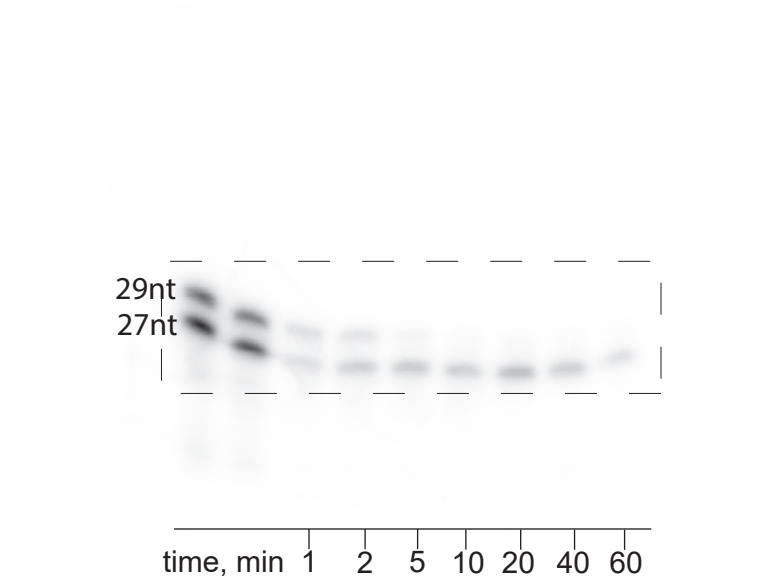
With PETISCO



With PETISCO



Without PETISCO



Without PETISCO

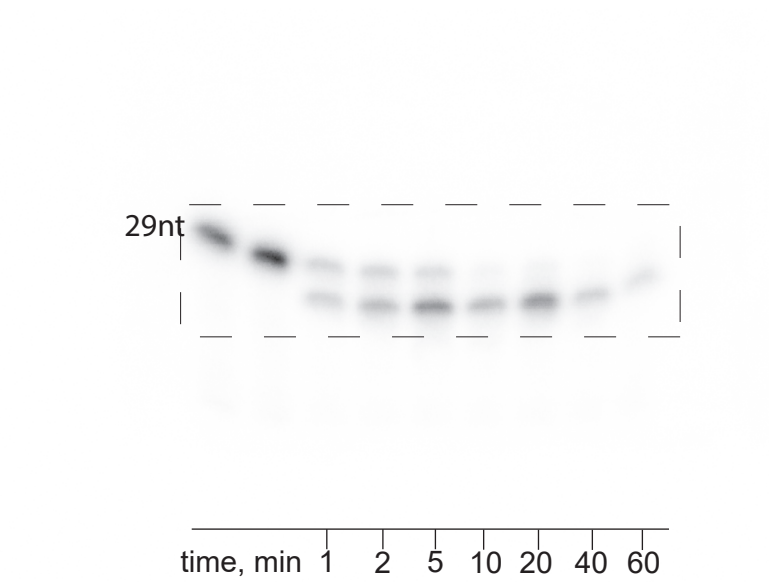
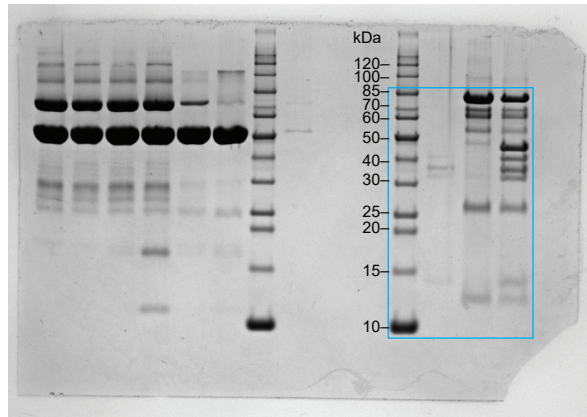
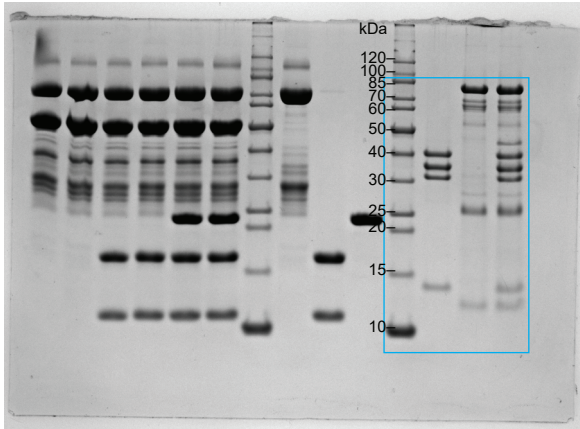
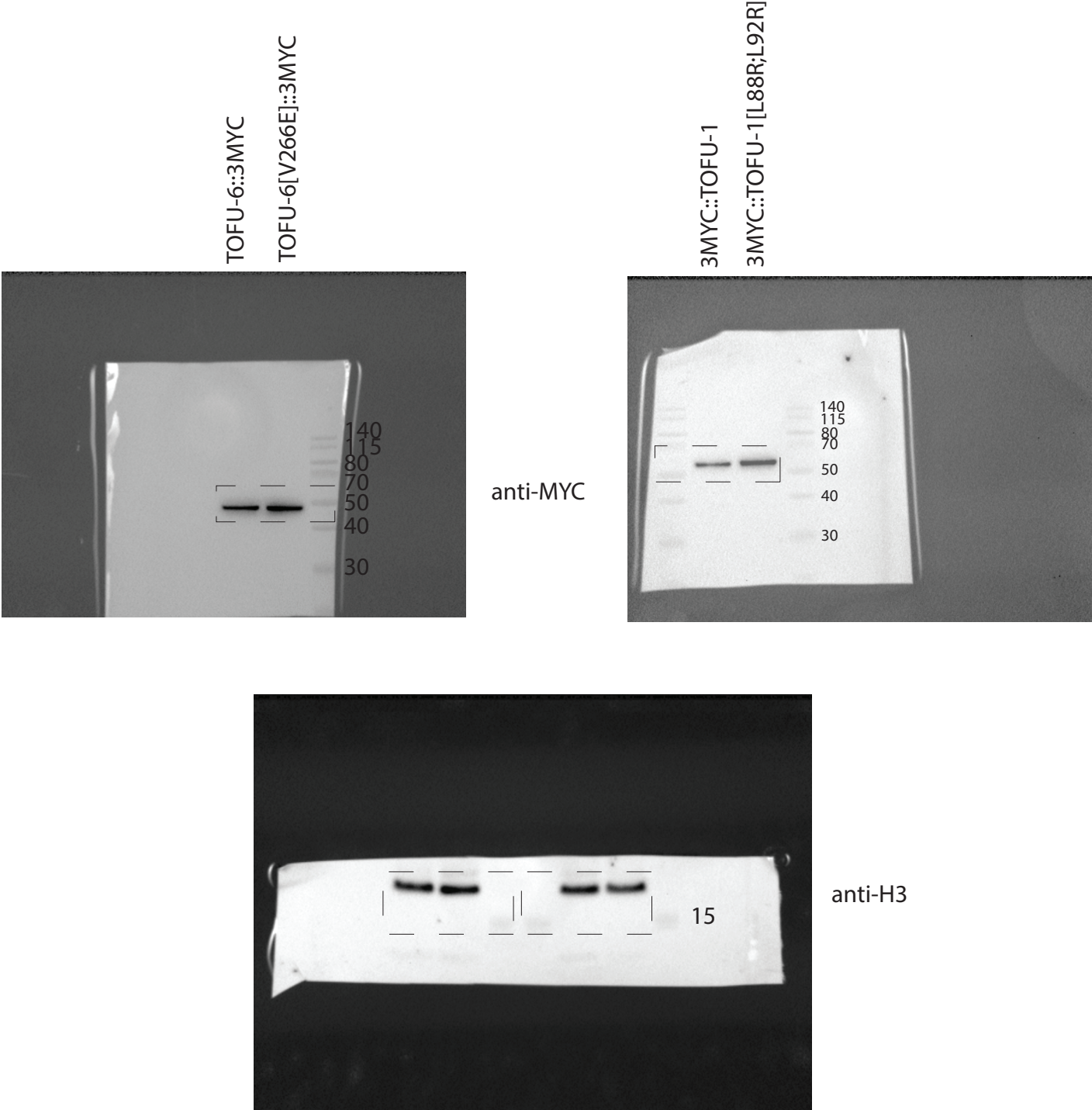


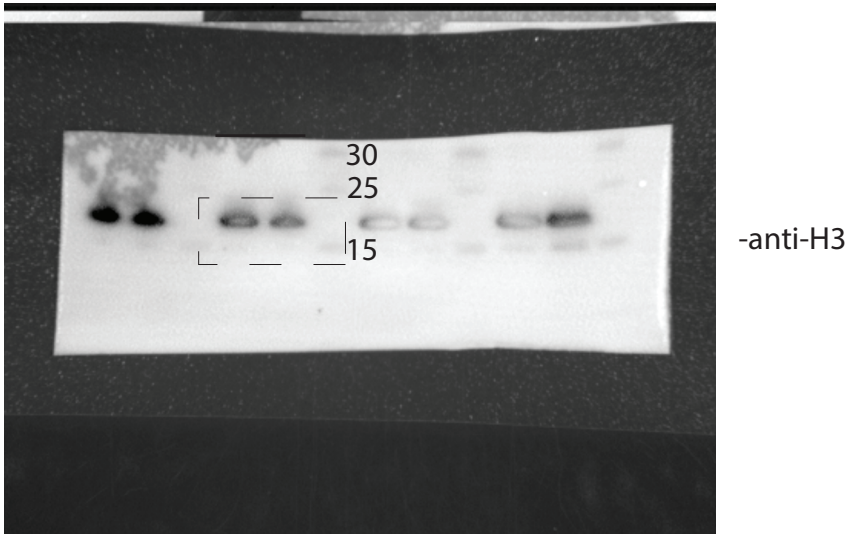
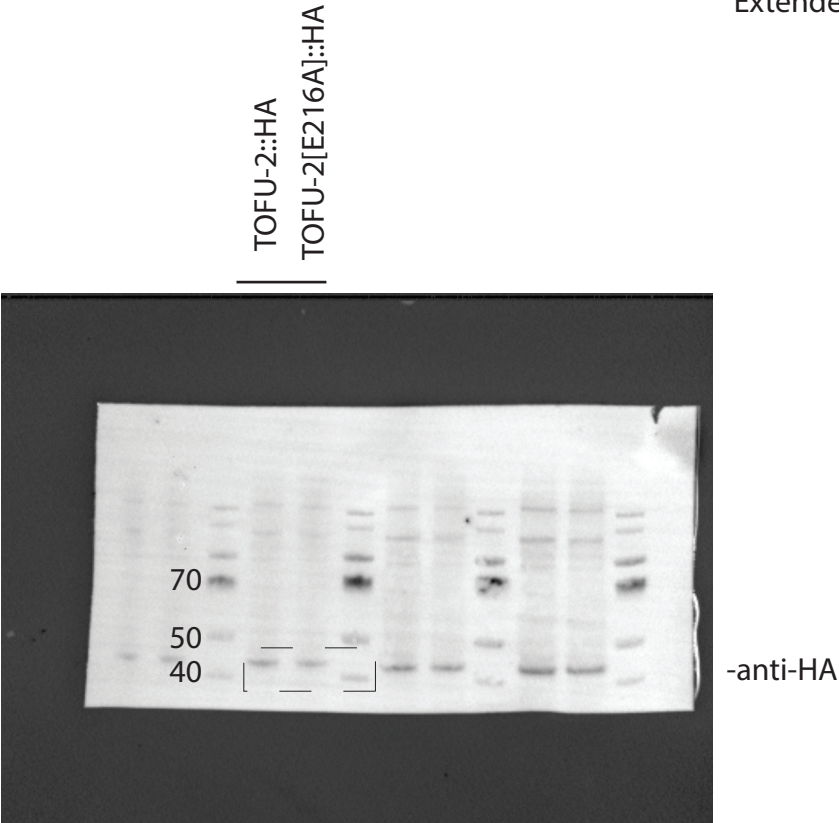
Figure 5a raw data



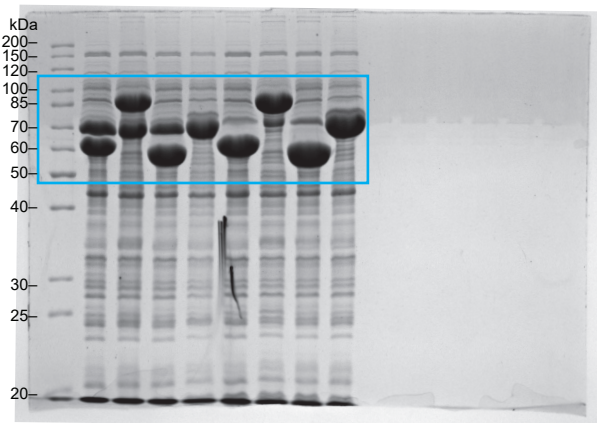
Everything had been run on the same gel, transferred together, membrane was cut to incubate with different primary antibodies, TOFU-6 and TOFU-1 membranes were separated before image

Figure5d_Raw data

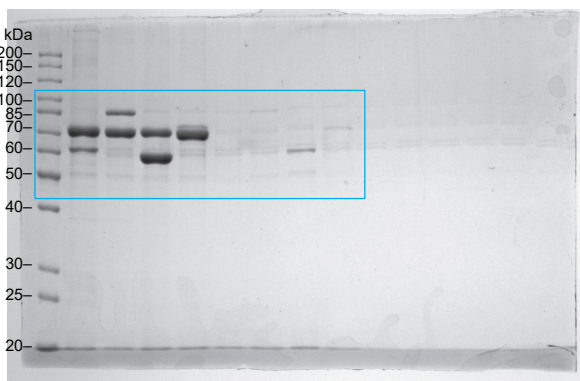
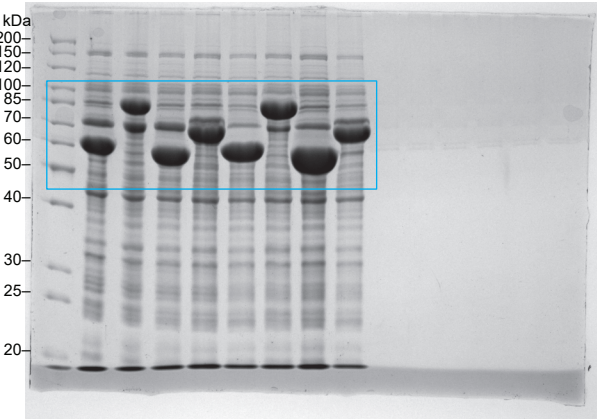




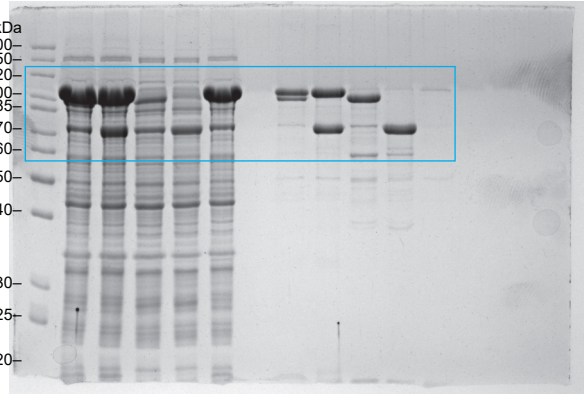
Extended data Figure 5b



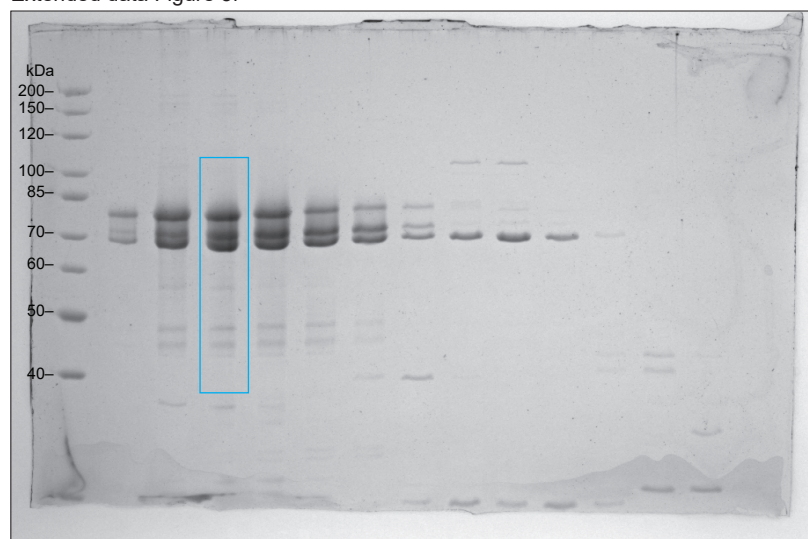
Extended data Figure 5c



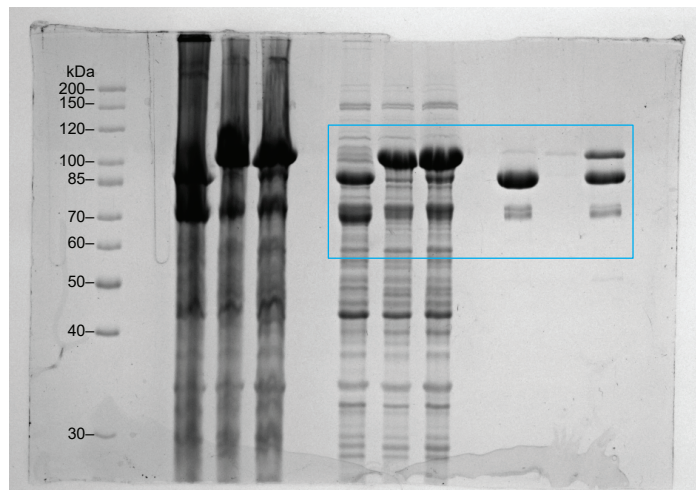
Extended data Figure 5d



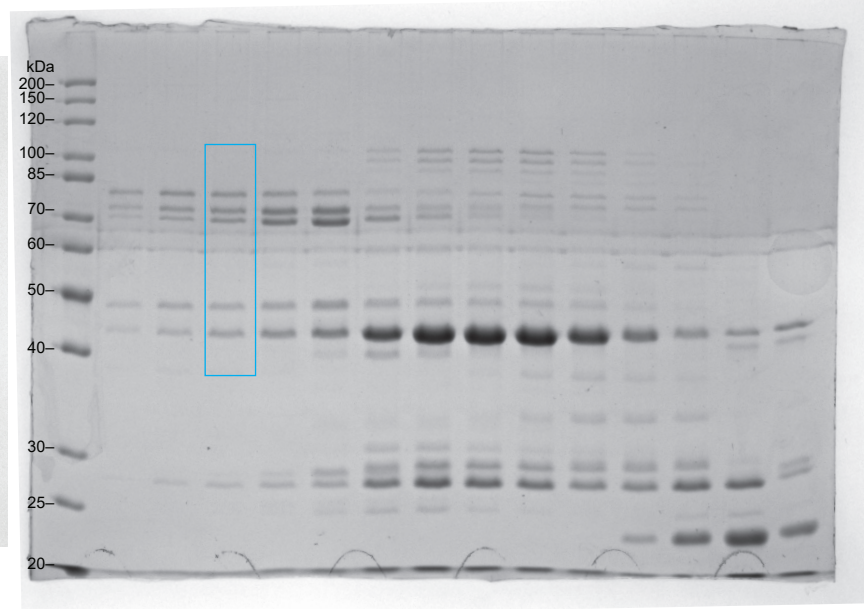
Extended data Figure 5f



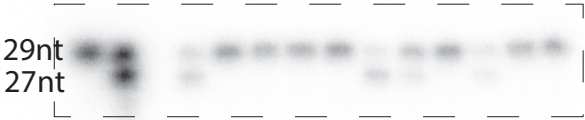
Extended data Figure 5e



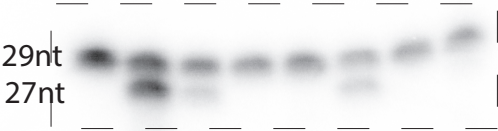
Extended data Figure 5g



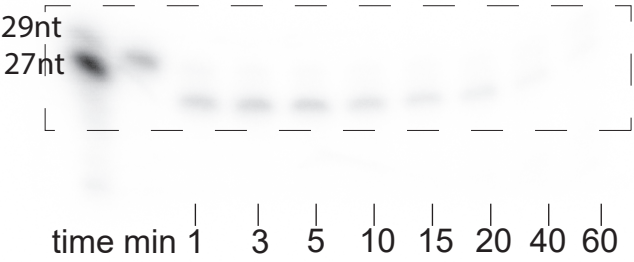
cap-AAU precursor	+	+		+	+	+	+	+	+	+	+	+	+
cleavage product	+												
PUCH E216A												+	+
PUCH			+	+	+	+	+	+	+	+			
		Mg ²⁺		Ca ²⁺		Zn ²⁺		Mn ²⁺		–	Mg ²⁺		
	mM	4		1 4		1 4		1 4			11 4 11		



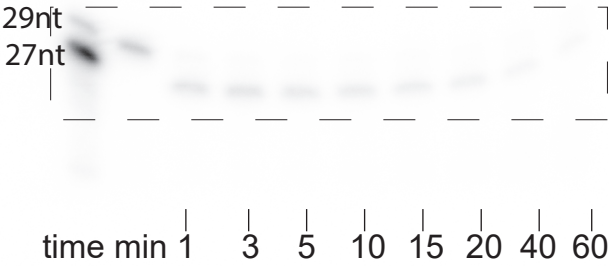
cap-AAU precursor	+	+	+	+	+	+	+	+
cleavage product		+						
PUCH E216A								+
PUCH			+	+	+	+	+	
	11 mM	Ca ²⁺ Mn ²⁺		Zn ²⁺ Mg ²⁺		–	Mg ²⁺	



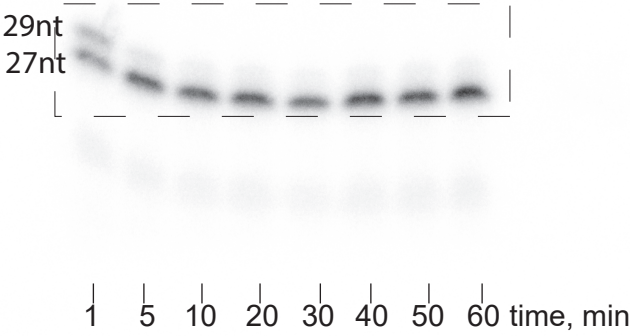
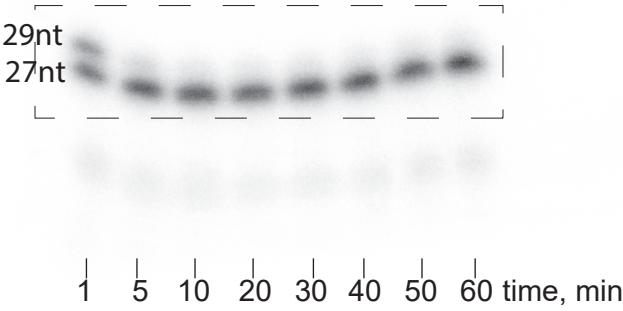
AAU substrate

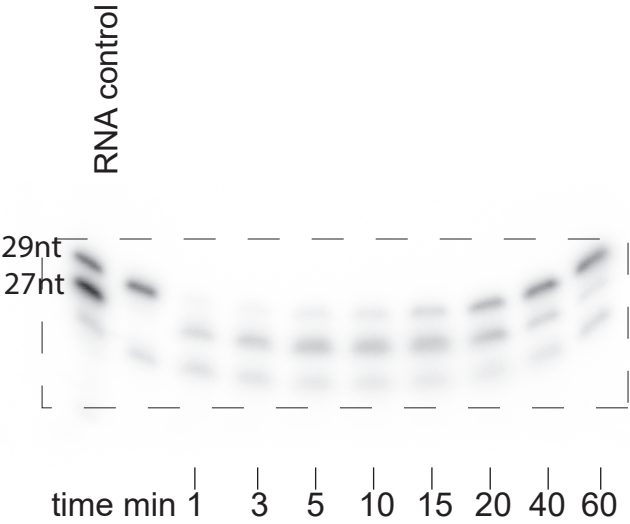
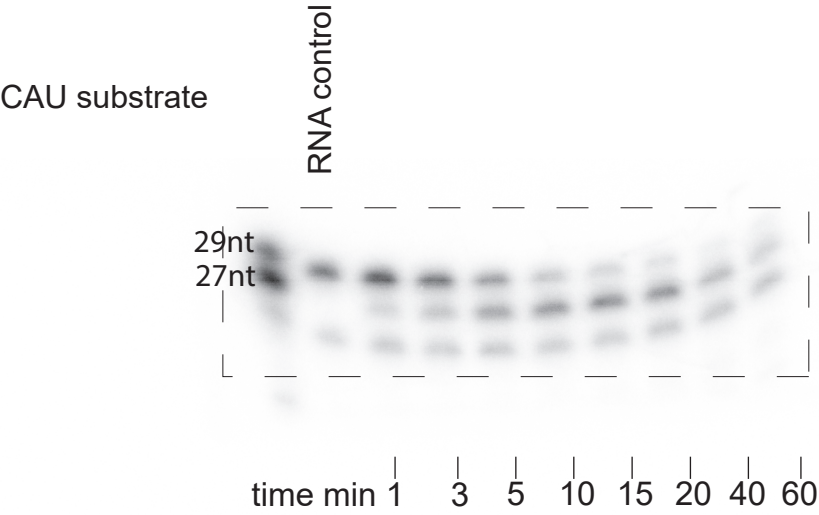


RNA control

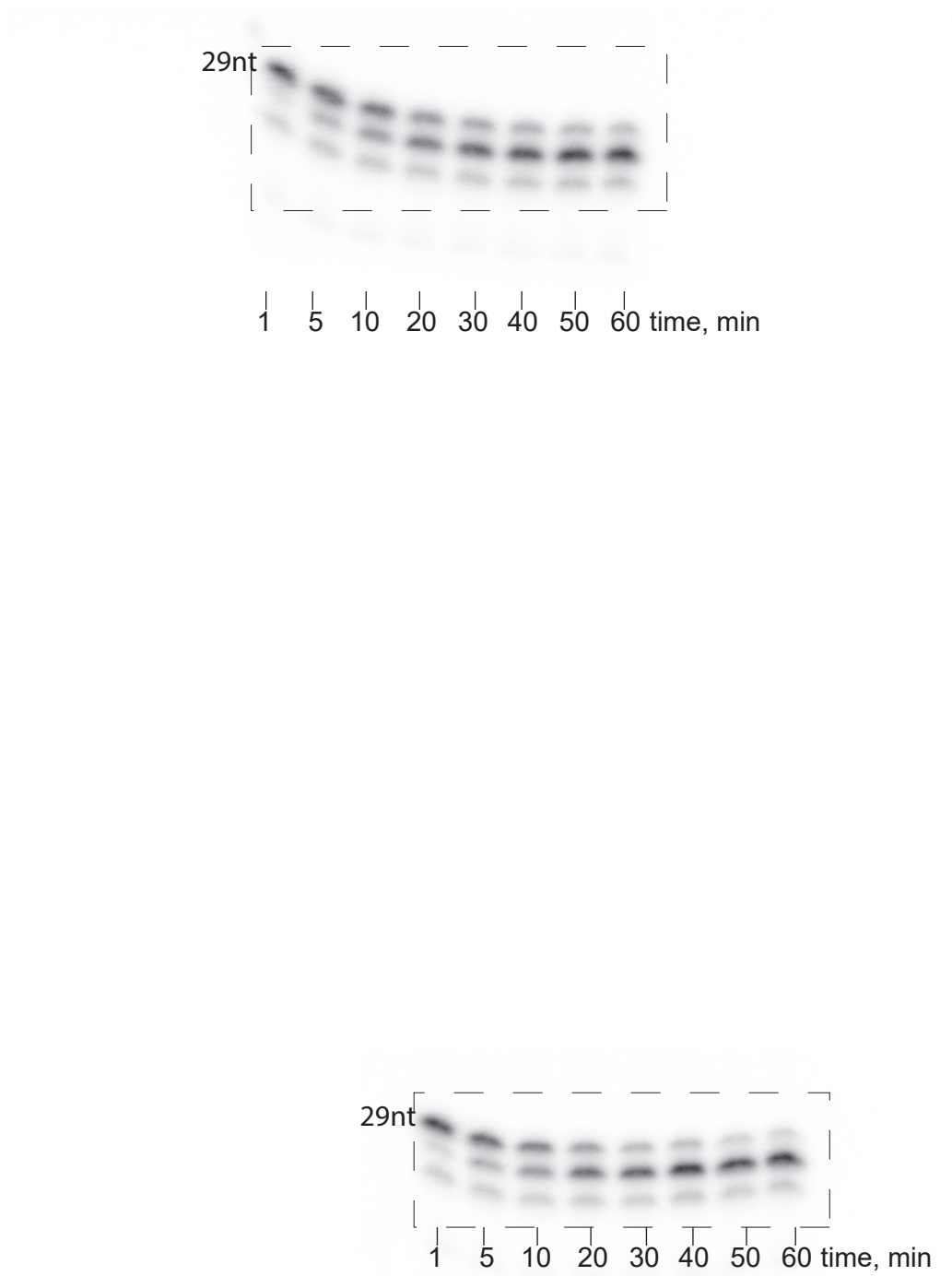


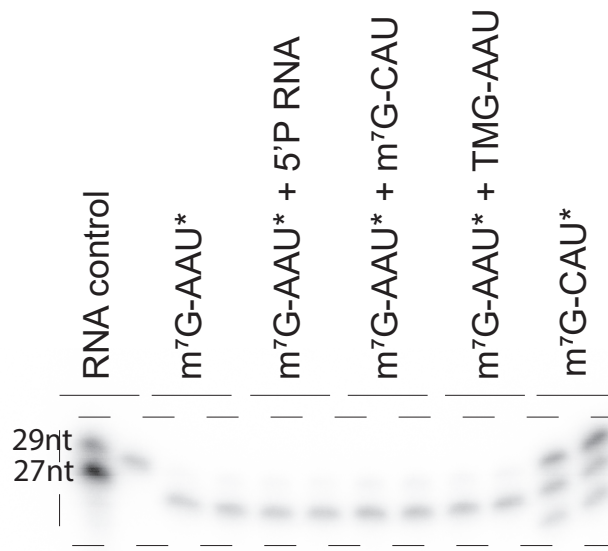
AAU

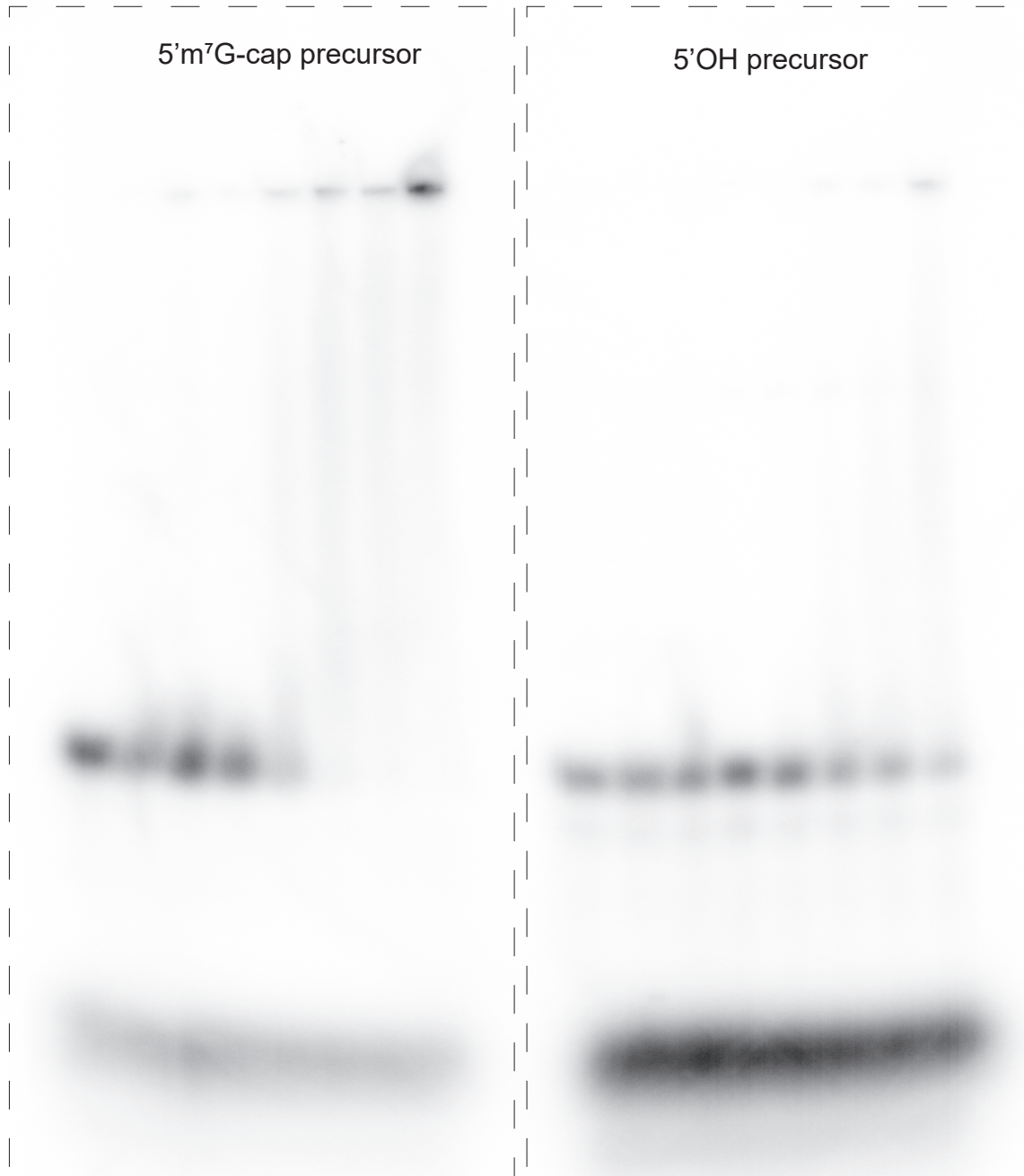




CAU-substrate

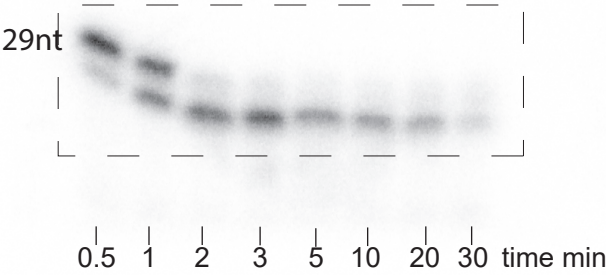
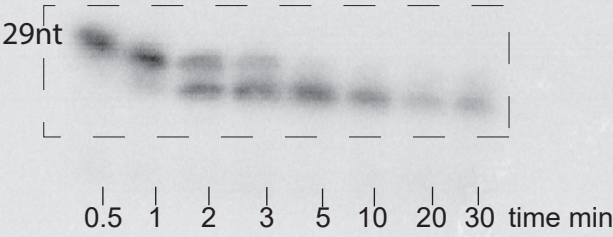




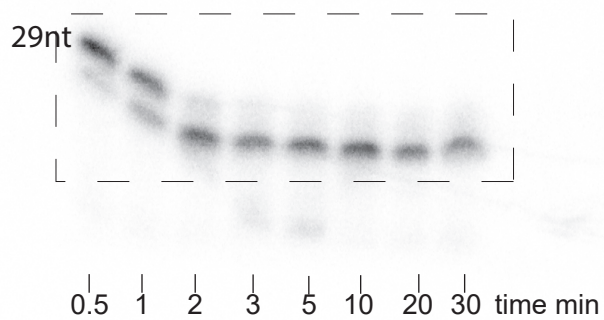
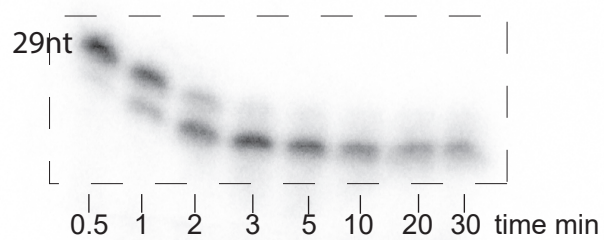




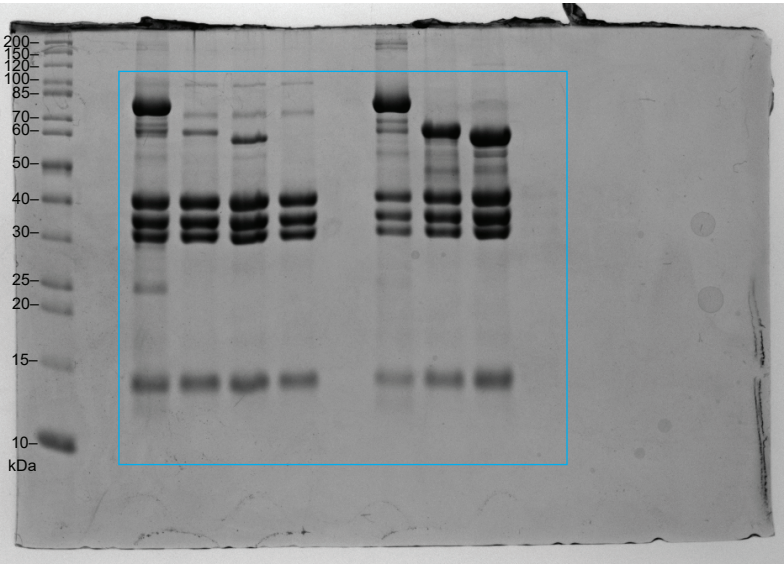
With PETISCO



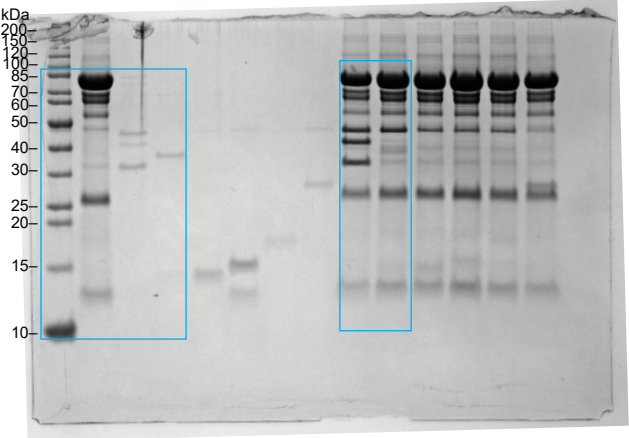
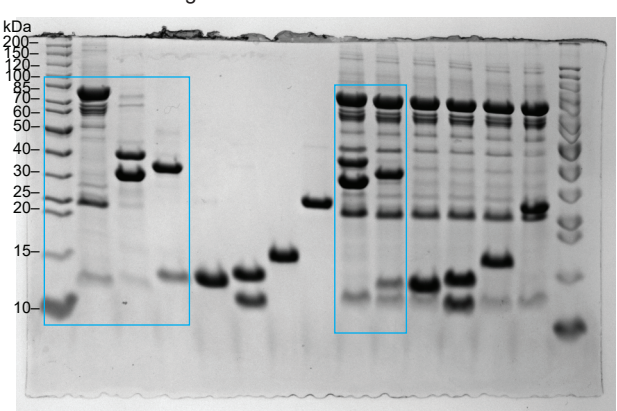
Without PETISCO



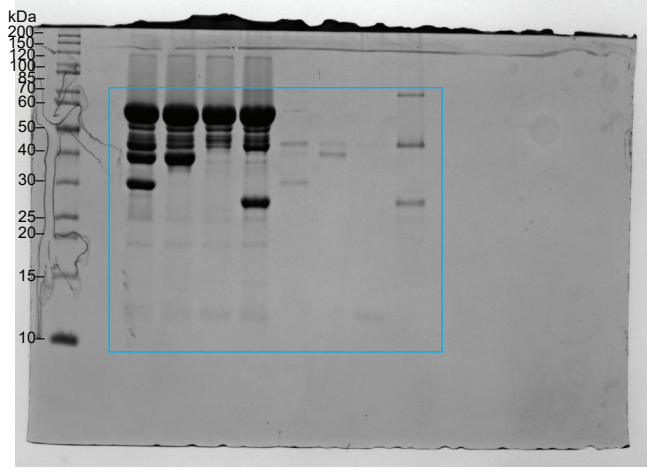
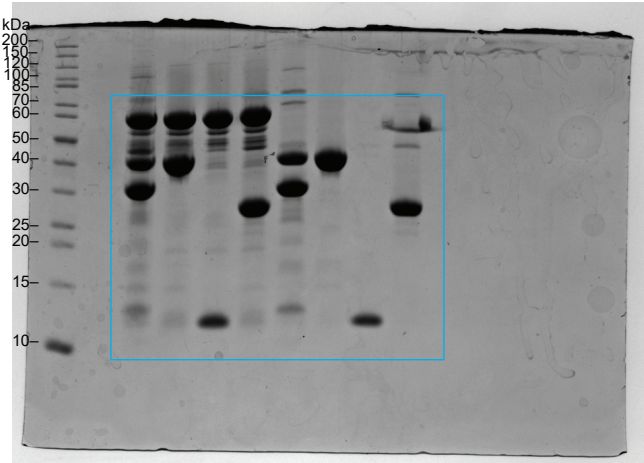
Extended data 7a



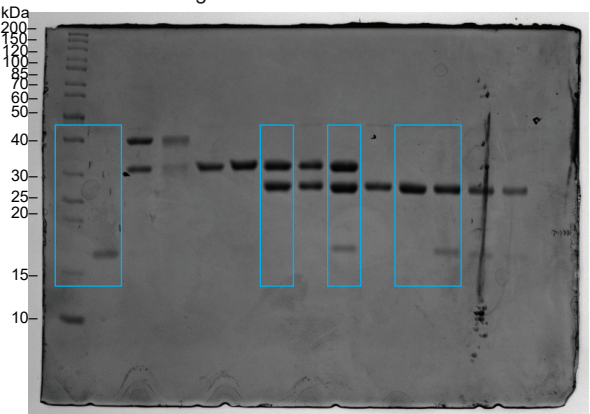
Extended data Figure 7b



Extended data Figure 7c



Extended data Figure 7d/e



Extended data Figure 9a

