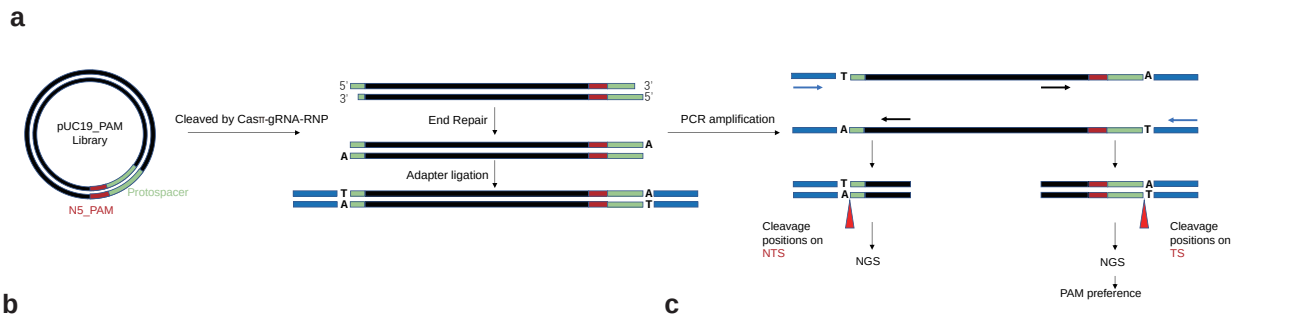
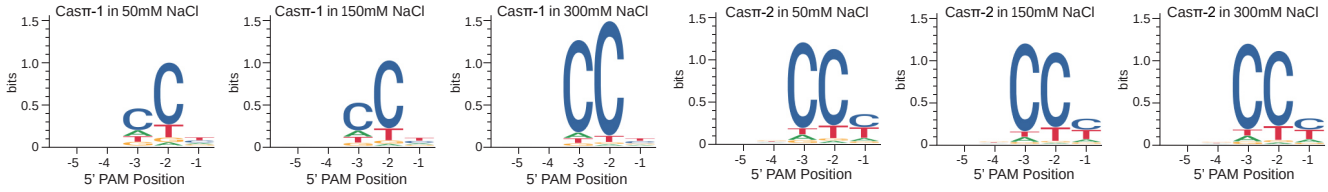
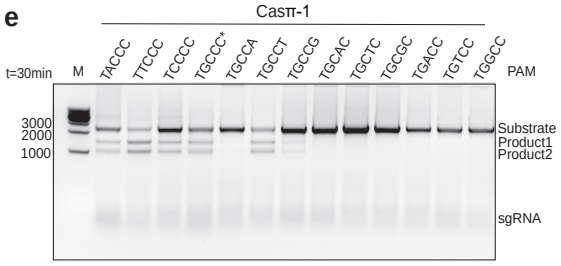
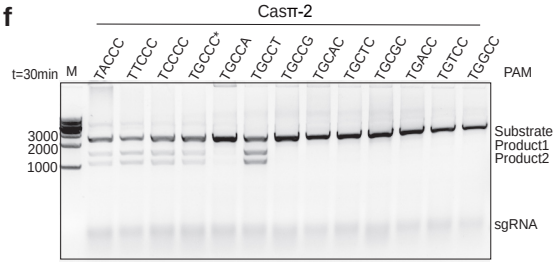


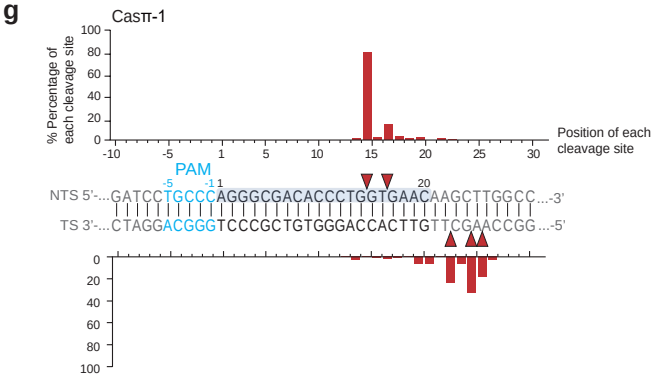


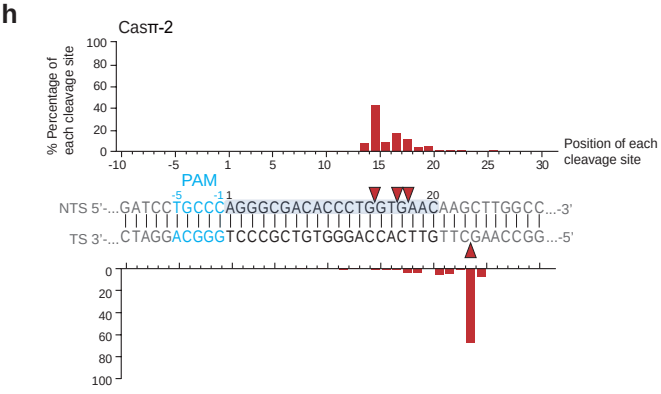
d

Subtype	Protein	Length(aa)	PAM	tracrRNA/scoutRNA
	Casπ	854-867	CCN	tracrRNA
TypeV-A	Cas12a	1100-1300	TTTV	No
TypeV-B	Cas12b	1100-1500	TTN	tracrRNA
TypeV-C	Cas12c	1200-1300	TN	scoutRNA
TypeV-D	Cas12d (CasY)	1200-1300	TR	No
TypeV-E	Cas12e (CasX)	986	TTCN	tracrRNA
TypeV-F	Cas12f (Cas14)	500-800	TTTN	tracrRNA
TypeV-G	Cas12g	800-900	No	tracrRNA
TypeV-H	Cas12h	900-1000	RTR	No
TypeV-I	Cas12i	~1100	TTN	No
TypeV-J	Cas12j (CasΦ)	700-800	GTN	No
TypeV-K	Cas12k	~700	TBN	tracrRNA









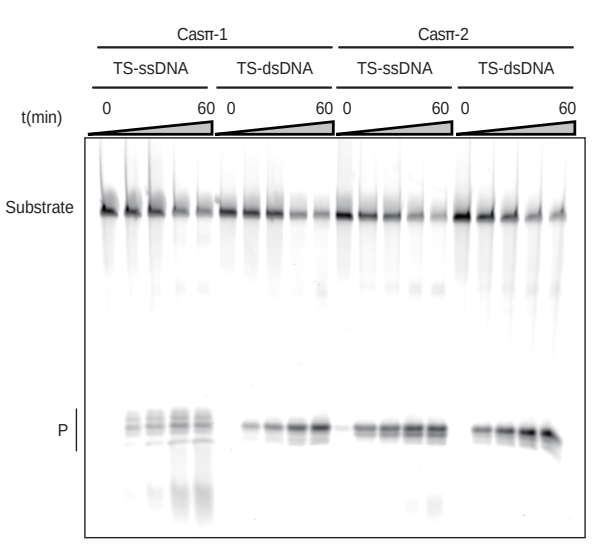


Fig. S2 Casπ recognizes protospacer with 5' C-rich PAM and creates a staggered end with 5' overhang.

(**a**) Pipeline of N5-PAM screening assay.

(**b**) PAM preference of Casπ-1 effector in buffers with different salt concentrations.

(**c**) PAM preference of Casπ-2 effector in buffers with different salt concentrations.

(**d**) Comparison of the Cas nuclease size, PAM preference and gRNA composition among type V Casπ and Cas12a-k systems.

(**e** and **f**) PAM preference validation for Casπ-1 (**e**) and Casπ-2 effectors (**f**) with dsDNA substrate. The PAM sequence designed in each dsDNA target is shown on top of each lane. M means DNA marker.

(**g** and **h**) Percentage distribution of Casπ-1 (**g**) and Casπ-2 effectors (**h**) cleavage sites analyzed by NGS data. (Percentage value was calculated by the number of reads belonging to each cleavage site divided by the number of total reads for all sites, X axis indicates the position of each nucleotide).

(**i**) Comparison of ssDNA and dsDNA cleavage by Casπ effectors. The ssDNA target containing complementary sequence to sgRNA spacer was labeled as TS-ssDNA. The dsDNA target containing complementary sequence to sgRNA spacer was labeled as TS-dsDNA.