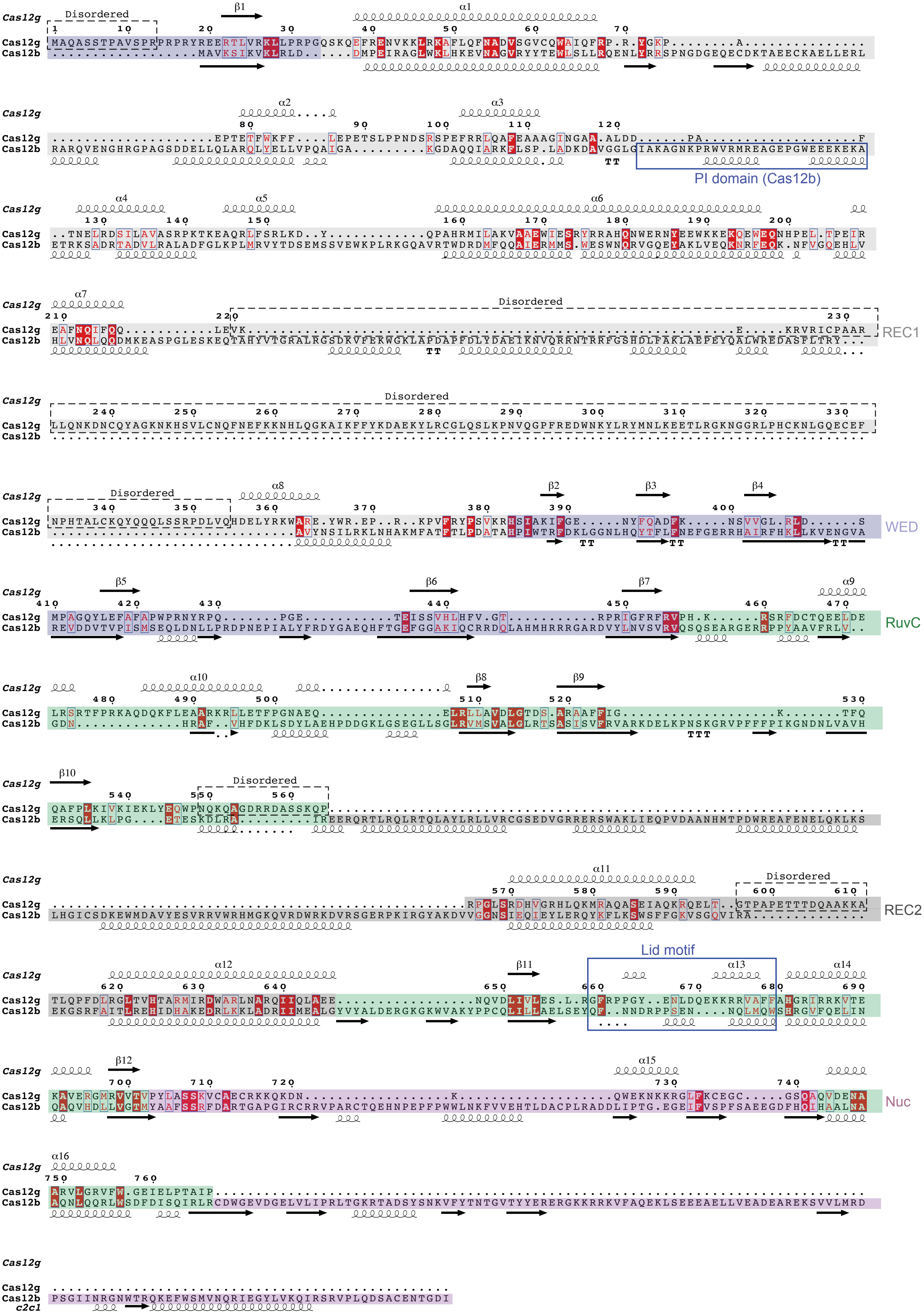
**SUPPLEMENTARY INFORMATION**

# Cryo-EM structure of the RNA-guided ribonuclease Cas12g

Zhuang Li[1,3], Heng Zhang[1,3], Renjian Xiao[1,3], Ruijie Han[1] and Leifu Chang[1,2,*]

[1]Department of Biological Sciences
[2]Purdue University Center for Cancer Research
Purdue University, 915 W. State Street, West Lafayette, IN 47907.
[3]These authors contributed equally to this work.
[*]Correspondence to L.C. (lchang18@purdue.edu)


Cas12g
Cas12b
c2c1
Disordered
β1
β2
β3
β4
β5
β6
β7
β8
β9
β10
β11
β12
α1
α2
α3
α4
α5
α6
α7
α8
α9
α10
α11
α12
α13
α14
α15
α16
PI domain (Cas12b)
REC1
WED
RuvC
REC2
Lid motif
Nuc

**Supplementary Figure 1. Structure-based sequence alignment between Cas12g and Cas12b.** Domains of Cas12g are indicated by background colors according to domain architecture in **Fig. 1a**. Secondary structures of each domain of Cas12g are labeled. Lid motifs and PI domain (Cas12g) are indicated by blue boxes. Disordered regions of Cas12g are indicated by dashed boxes.

**Supplementary Table 1. Statistics of cryo-EM data and structure refinement.**

| | Cas12g-sgRNA (EMD-22257, PDB 6XMF) | Cas12g(E655A)-sgRNA-RNA (EMD-22258, PDB 6XMG) |
|---|---|---|
| **Data collection and processing** | | |
| Magnification | 81,000 | 81,000 |
| Voltage (kV) | 300 | 300 |
| Electron exposure ($e^{-}$/Å$^2$) | 54 | 54 |
| Defocus range (μm) | 1.5 – 2.5 | 1.5 – 2.5 |
| Pixel size (Å) | 1.05 | 1.05 |
| Symmetry imposed | *C1* | *C1* |
| Initial particle images (no.) | 6,435,750 | 462, 268 |
| Final particle images (no.) | 469,157 | 38,238 |
| Map resolution (Å) | 3.0 | 4.8 |
| FSC threshold | 0.143 | 0.143 |
| Map resolution range (Å) | 2.9-5 | 4.6-7 |
| **Refinement** | | |
| Initial model used | PDB 5U30 | PDB 6XMF |
| Model resolution (Å) | 3.3 | 7.8 |
| FSC threshold | 0.5 | 0.5 |
| Model resolution range (Å) | 2.9 - 50 | 7.6 - 50 |
| Map sharpening *B* factor (Å$^2$) | -82 | NA |
| Model composition | | |
| Non-hydrogen atoms | 7490 | 8263 |
| Protein residues | 627 | 638 |
| Ligands | 1 | 1 |
| *B* factors (Å$^2$) | | |
| Protein | 38.40 | 394.34 |
| Ligand | 79.64 | 347.38 |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.002 | 0.002 |
| Bond angles (°) | 0.548 | 0.504 |
| **Validation** | | |
| MolProbity score | 1.92 | 1.98 |
| Clashscore | 8.93 | 10.79 |
| Poor rotamers (%) | 0.00 | 0.00 |
| Ramachandran plot | | |
| Favored (%) | 93.17 | 93.47 |
| Allowed (%) | 6.83 | 6.53 |
| Disallowed (%) | 0.00 | 0.00 |

**Supplementary Table 2. Comparison between Cas12g and other Cas12 and Cas13 nucleases.**

| | **Cas12g (PDB:6XMG)** | **Cas12a (PDB:5XUS)** | **Cas12b (PDB:5U30)** | **Cas12e (PDB:6NY2)** | **Cas12i (PDB:6W5C)** | **Cas13a (PDB:5XWP)** |
|---|---|---|---|---|---|---|
| **Classification** | Class 2, type V | Class 2, type V | Class 2, type V | Class 2, type V | Class 2, type V | Class 2, type VI |
| **Protein length (aa)** | 767 | 1228 (*Lachnospiraceae bacterium)* | 1129 (*Alicyclobacillus acidoterrestris*) | 986 (*Deltaproteobacteria bacterium*) | 1093 | 1159 (*Leptotrichia buccalis*) |
| **tracrRNA** | Yes | No | Yes | Yes | No | No |
| **Target type** | ssRNA | DNA | DNA | DNA | DNA | ssRNA |
| **Collateral cleavage activity** | ssRNA, ssDNA | ssDNA | ssDNA | ssDNA | ssDNA | ssRNA |
| **Pre-crRNA processing activity** | No | Yes | No | No | Yes | Yes |
| **Enzymatic domain** | RuvC | RuvC | RuvC | RuvC | RuvC | HEPN |
| **PI domain** | No | Yes | Yes | Yes | Yes | No |
| **Zinc finger motif** | Yes | No | No | Yes | No | No |
| **REC1 domain** | Partially flexible (REC1$^{220-354}$) | Rigid | Rigid | Rigid | Rigid | - |
| **crRNA seed sequence** | Not reported | PAM-adjacent 5-nt | PAM adjacent 5-nt | Not reported | PAM-adjacent 7-nt | Central seed |
| **crRNA-target duplex length** | 24 bp | 20 bp | 20 bp | 20 bp | 26-28 bp | 28 bp |
| **References** | This study, [20] | [28,31-39] | [40-42] | [24] | [43] | [44] |