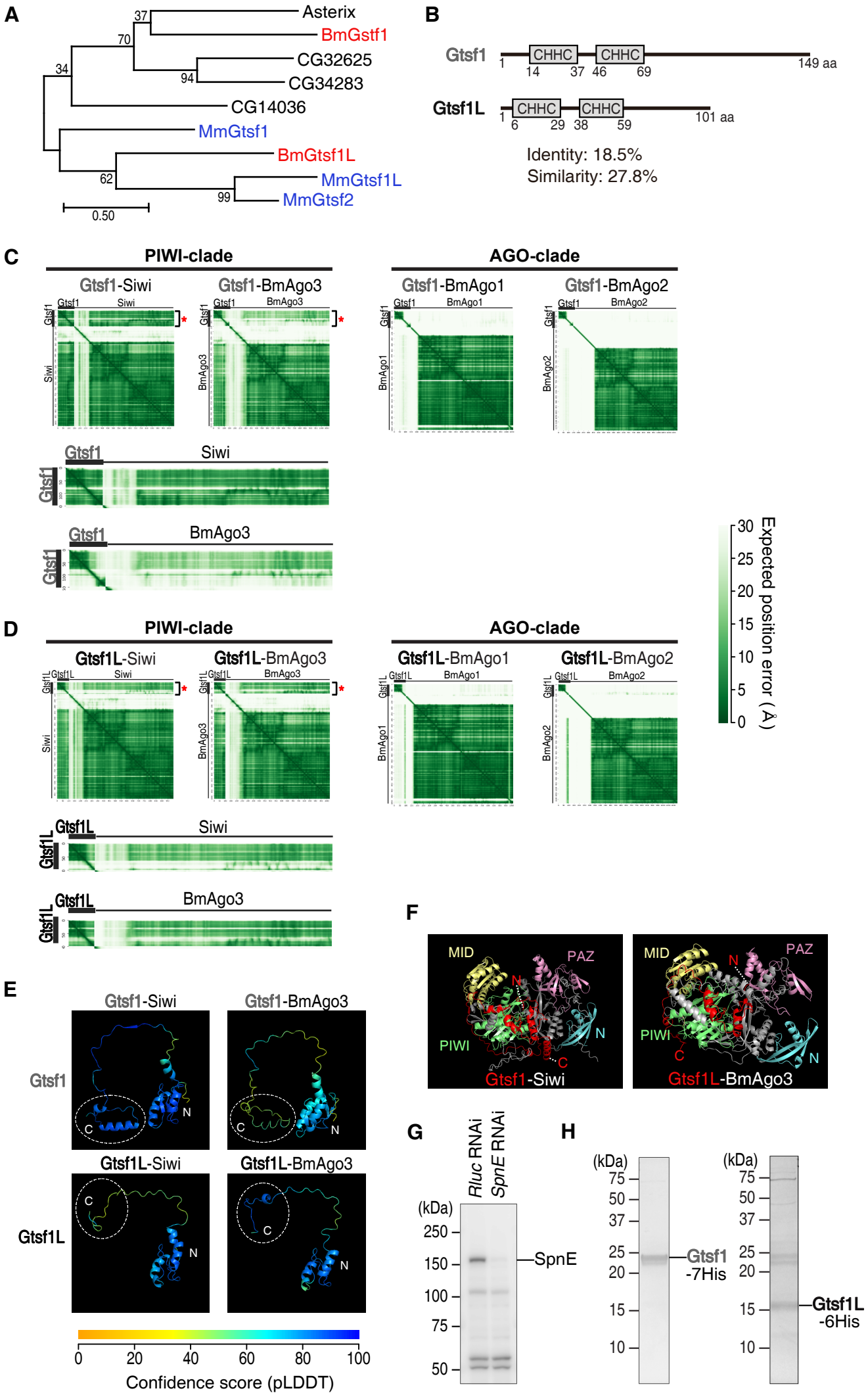
A
Asterix
BmGstf1
CG32625
CG34283
CG14036
MmGtsf1
BmGtsf1L
MmGtsf1L
MmGtsf2
37
70
34
94
62
99
0.50
B
Gtsf1
CHHC
CHHC
1
14
37
46
69
149 aa
Gtsf1L
1
6
29
38
59
101 aa
Identity: 18.5%
Similarity: 27.8%
C
PIWI-clade
AGO-clade
Gtsf1-Siwi
Gtsf1-BmAgo3
Gtsf1-BmAgo1
Gtsf1-BmAgo2
Gtsf1
Siwi
BmAgo3
BmAgo1
BmAgo2
D
PIWI-clade
AGO-clade
Gtsf1L-Siwi
Gtsf1L-BmAgo3
Gtsf1L-BmAgo1
Gtsf1L-BmAgo2
Gtsf1L
Siwi
BmAgo3
BmAgo1
BmAgo2
Expected position error (Å)
30
25
20
15
10
5
0
E
Gtsf1-Siwi
Gtsf1-BmAgo3
Gtsf1
Gtsf1L-Siwi
Gtsf1L-BmAgo3
Gtsf1L
N
C
0
20
40
60
80
100
Confidence score (pLDDT)
F
MID
PAZ
PIWI
N
C
Gtsf1-Siwi
Gtsf1L-BmAgo3
G
Riluc RNAi
SpnE RNAi
(kDa)
250
150
100
75
50
SpnE
H
(kDa)
75
50
37
25
20
15
10
Gtsf1
-7His
Gtsf1L
-6His

Supplemental Figure 1. Prediction of Gtsf-PIWI complexes using AlphaFold-Multimer.

(A) Phylogenetic tree of fly (black), mouse (blue), and silkworm (red) Gtsf homologs. The phylogenetic tree was constructed using the maximum likelihood method with bootstrapping in MEGAX (Tamura et al. 2021). The numbers at the branch point indicate the bootstrap support values.

(B) Schematic presentation of the domain structure of *Bombyx* Gtsf1 and Gtsf1L.

(C, D) Predicted Aligned Error (PAE) plots for the top-ranked model of Gtsf1-PIWI or AGO (C) and Gtsf1L-PIWI or AGO (D) complexes predicted by AlphaFold-Multimer. The regions indicated with asterisks in the Gtsf1/1L-PIWI plots are enlarged below the plots.

(E) AlphaFold-Multimer-predicted structure of Gtsf1 and Gtsf1L in complex with Siwi or BmAgo3. The C-terminal structure (white dotted circles) of Gtsf1 and Gtsf1L is predicted with higher confidence in complex with Siwi and BmAgo3, respectively.

(F) AlphaFold-Multimer-predicted structure of the Gtsf1-Siwi and Gtsf1L-BmAgo3 complexes. The domain definition (the N, PAZ, MID, and PIWI domains) of Siwi follows a previous study (Matsumoto et al. 2016), and the corresponding regions of BmAgo3 based on the sequence alignment with Siwi are colored.

(G) Western blot analysis of whole cell lysate of BmN4 cells treated with dsRNA for *Rluc* (control) or *SpnE*. The anti-SpnE antibody successfully detects endogenous SpnE.

(H) CBB staining of purified Gtsf1 and Gtsf1L proteins used for *in vitro* target cleavage assay.