Supplemental Information for

The Making of a Slicer: Activation of Human Argonaute-1

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Figure S1, related to Figure 1: Interactions between hAgo1 and let-7 miRNA and endoRNA

(A) Summary of interactions between hAgo1 and the bases, phosphate backbone and 2'-OH of the let-7 miRNA. Amino acid residues are colored according to the domain color scheme from Figure 1A. (B) Hydrogen bonds (red dash) and ion pair (blue dashes) interactions between hAgo1 and the 5'-U of let-7. (C) 5'-end binding pocket in the hAgo1-endoRNA complex. F_O-F_C difference map prior to inclusion of RNA contoured at 2.5 σ is shown in blue. 5'-A1 modeled in the syn conformation is shown as orange sticks. All of the RNA interactions in the 5'-binding pocket are as indicated in panel B except a new interaction is observed between the side-chain of Y813 and the adenine base. (D) Small RNAs bound to hAgo1-3 were 5'-^{32}P radiolabeled and separated by denaturing 15% PAGE. (E) Size profile of small RNAs bound to purified hAgo1-3 or present in SF9 cells expressing hAgo1 (input) were analyzed by Illumina sequencing. The range of two biological replicates is indicated. (F) First nucleotide preference of 20nt small RNAs bound to hAgo1-3 or present in input extracts. The average of two biological replicates (P-values <0.05) is depicted.

Figure S2, related to Figure 2: Overall structure of hAgo1 with endoRNA and let-7 miRNA along with Domain Comparison with hAgo2

(A) Structure of hAgo1-let-7 complex with the domains colored as in Figure 1A. The F_O-F_C difference map prior to inclusion of RNA in the model contoured at 2.5 σ is shown in blue. Nucleotides 1-10 and 21-22 are modeled as sticks. (B) Structure of hAgo1-endoRNA complex with an identical layout as in panel A. Nucleotides 1-9 and 19-20 are shown as sticks. (C)-(H) Superposition of each isolated domain of hAgo1 onto hAgo2. Domains are labeled and colored as shown in Figure 1A.

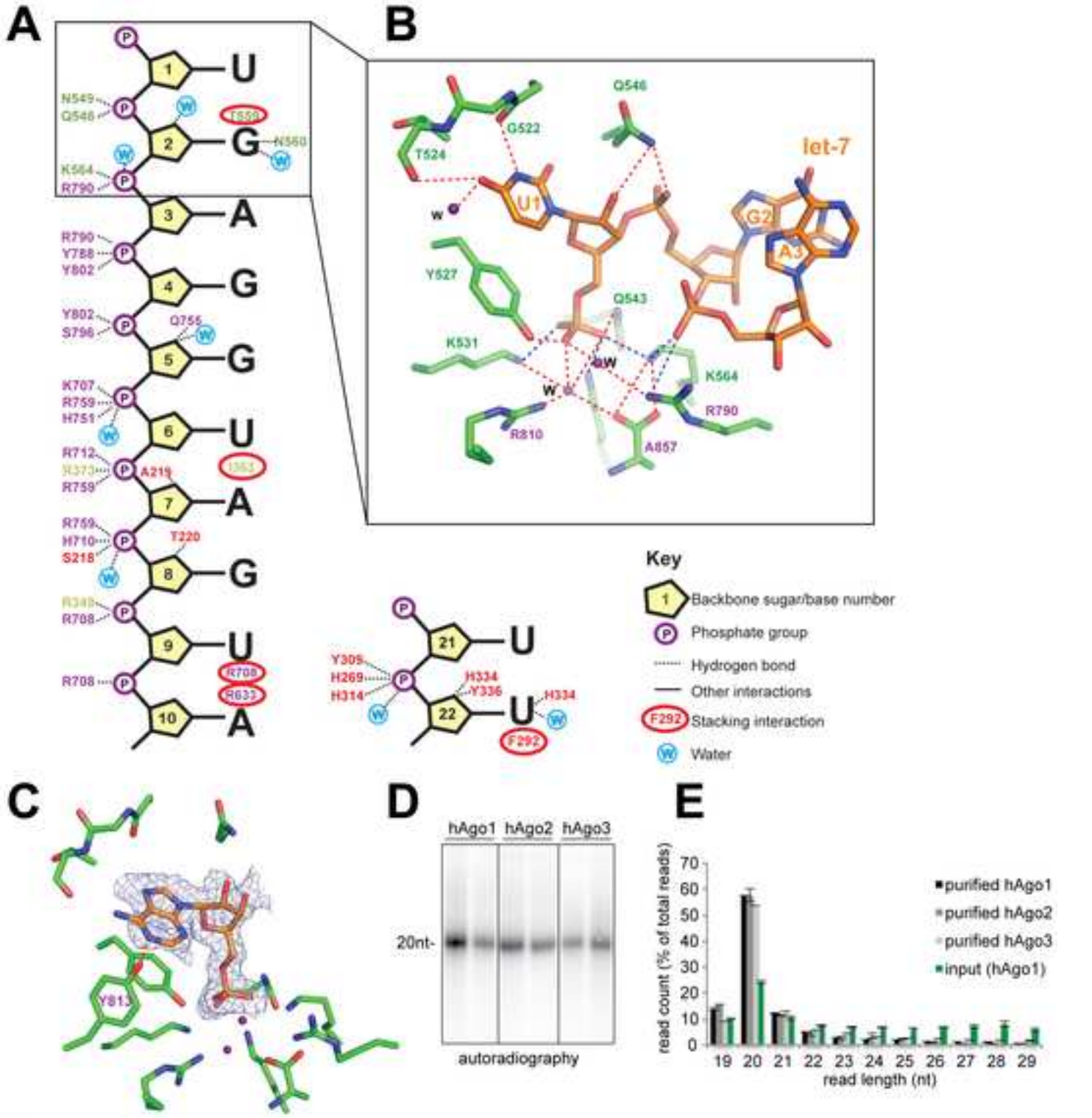
Figure S3, related to Figure 3: Guide RNA binding assay and SDS-PAGE analysis of Argonaute mutants

(A) The indicated Argonaute construct was loaded with 5'-^{32}P radio-labeled miR20 guide RNA using the same loading conditions as described in the Experimental Procedures. To assay for guide RNA loading efficiency the proteins were immobilized on nitrocellulose membranes with a slot-blot apparatus (Schleicher and Schuell). Guide RNA alone failed to bind the nitrocellulose membrane, but was retained in all of the Argonaute mutants described in this study. (B) SDS-PAGE analysis of purified Argonaute mutants.

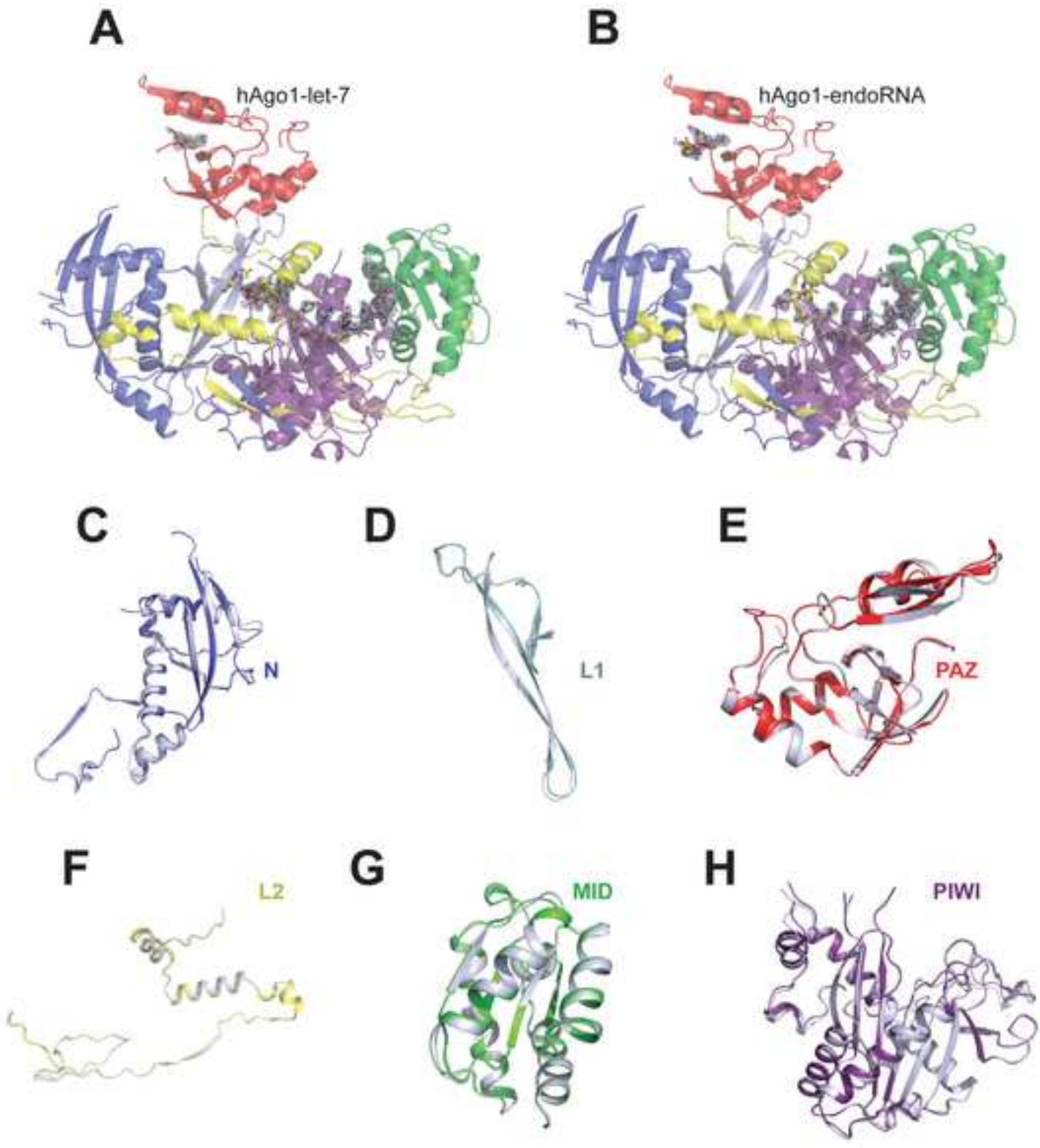
Figure S4, related to Figure 4: Sequence Alignment of hAgo1, hAgo2 and hAgo3

Secondary structure elements extracted from the hAgo1 structure are shown above the sequences and colored according to the domains of hAgo1 as shown in Figure 1A. Conserved amino acids are in black and non-conserved amino acids are shaded blue. The location of the PL1, PL2 and PL3 loops are labeled. Residues that make up the catalytic tetrad are indicated with a red star below. The two point mutations made in hAgo1 that activate slicing are shaded green.

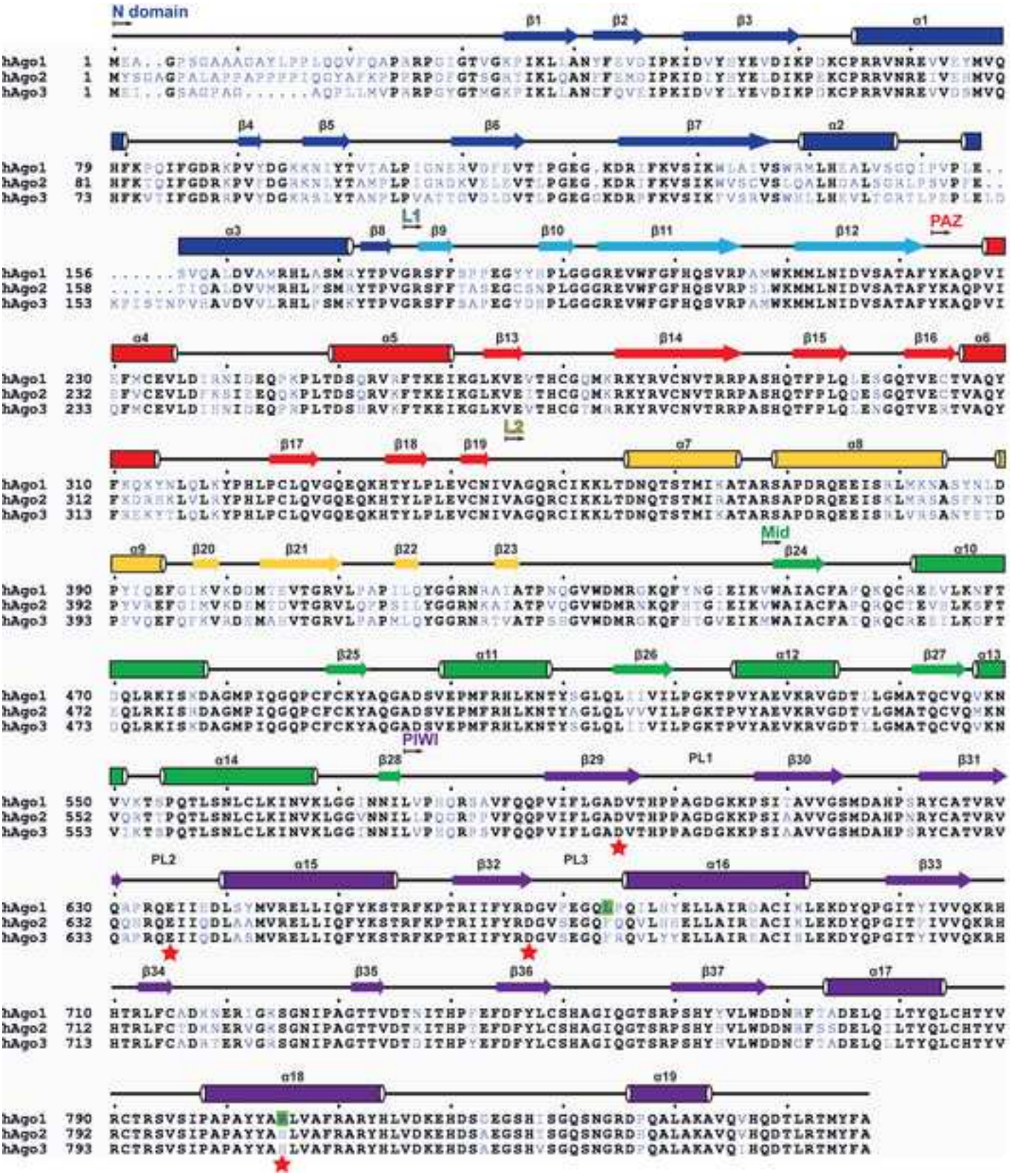
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

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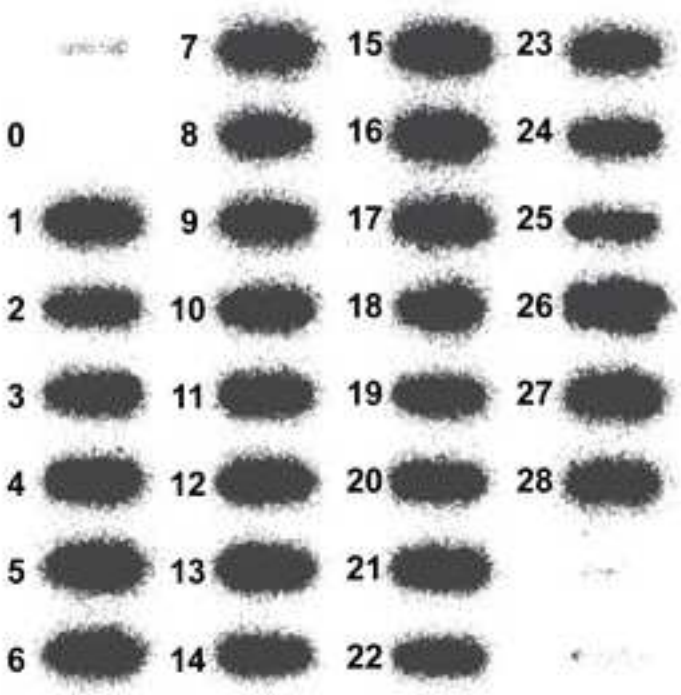

Key:

0- guide RNA
1- hAgo2
2- hAgo1
3- hAgo3
4- hAgo2 H807R
5- hAgo1 R805H
6- hAgo2, hAgo1 N
7- hAgo2, HAgo1 PAZ
8- hAgo2, hAgo1 Mid
9- hAgo2, hAgo1 PIWI R805H
10- $hAgo1_H$, hAgo2 N
11- $hAgo1_H$, hAgo2 PAZ
12- $hAgo1_H$, hAgo2 Mid
13- $hAgo1_H$, hAgo2 PIWI
14- $hAgo1_H$, hAgo2 PIWI,N
15- $hAgo1_H$, hAgo2 PIWI, PAZ
16- $hAgo1_H$, hAgo2 PIWI, Mid
17- $hAgo1_H$, hAgo2 PIWI,
18- $hAgo1_H$ L674F
19- $hAgo1_H$ L674F, hAgo2 N
20- hAgo2 F676L
21- hAgo2 F676L, Q677P
22- hAgo2 F676Y
23- hAgo2 F676A
24- hAgo2 F676I
25- hAgo2 F676V
26- hAgo2 F676M
27- hAgo2 F676W
28- hAgo2 F676R

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