

Supplementary Information

Molecular dissection of human Argonaute proteins using DNA shuffling

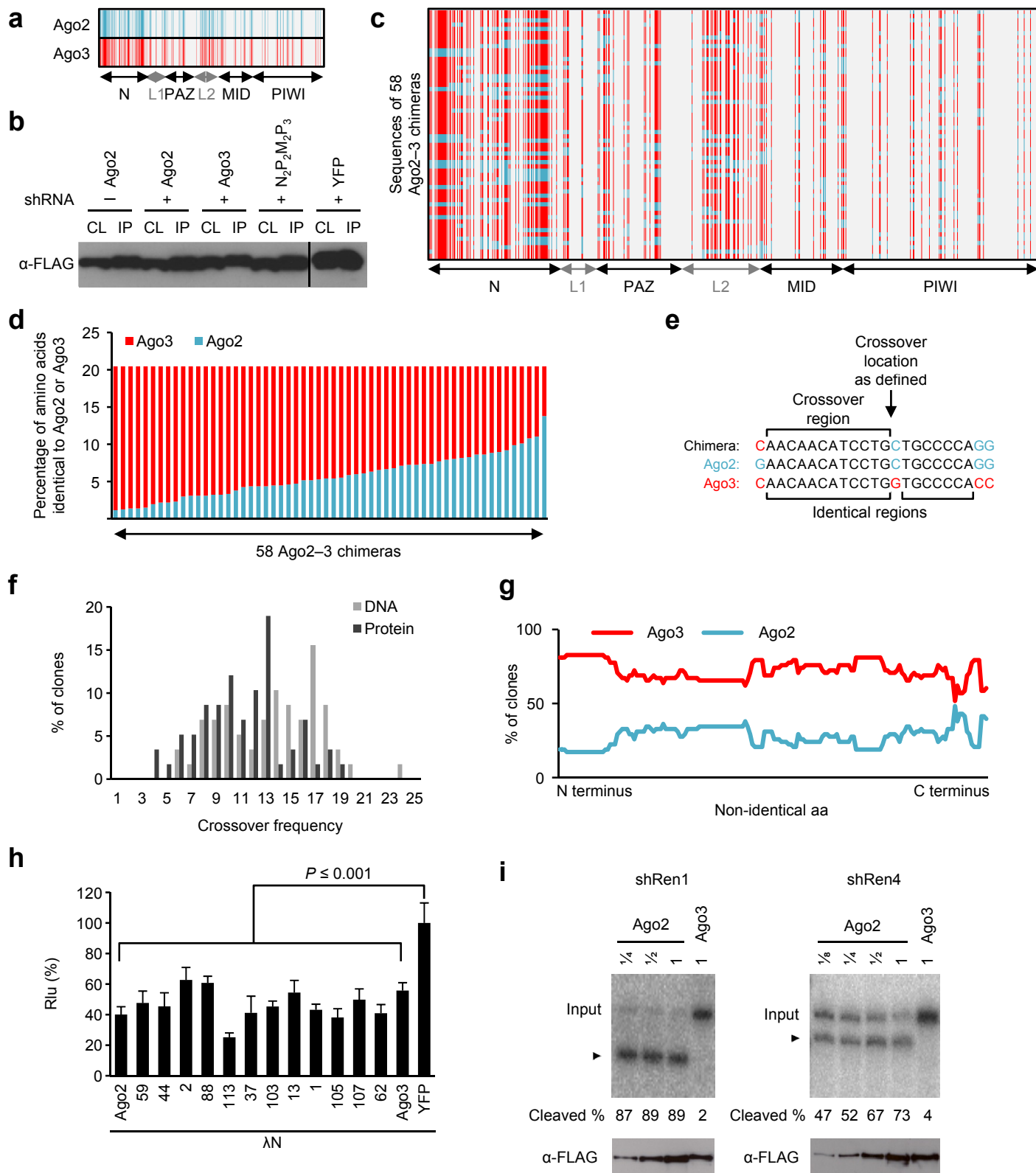
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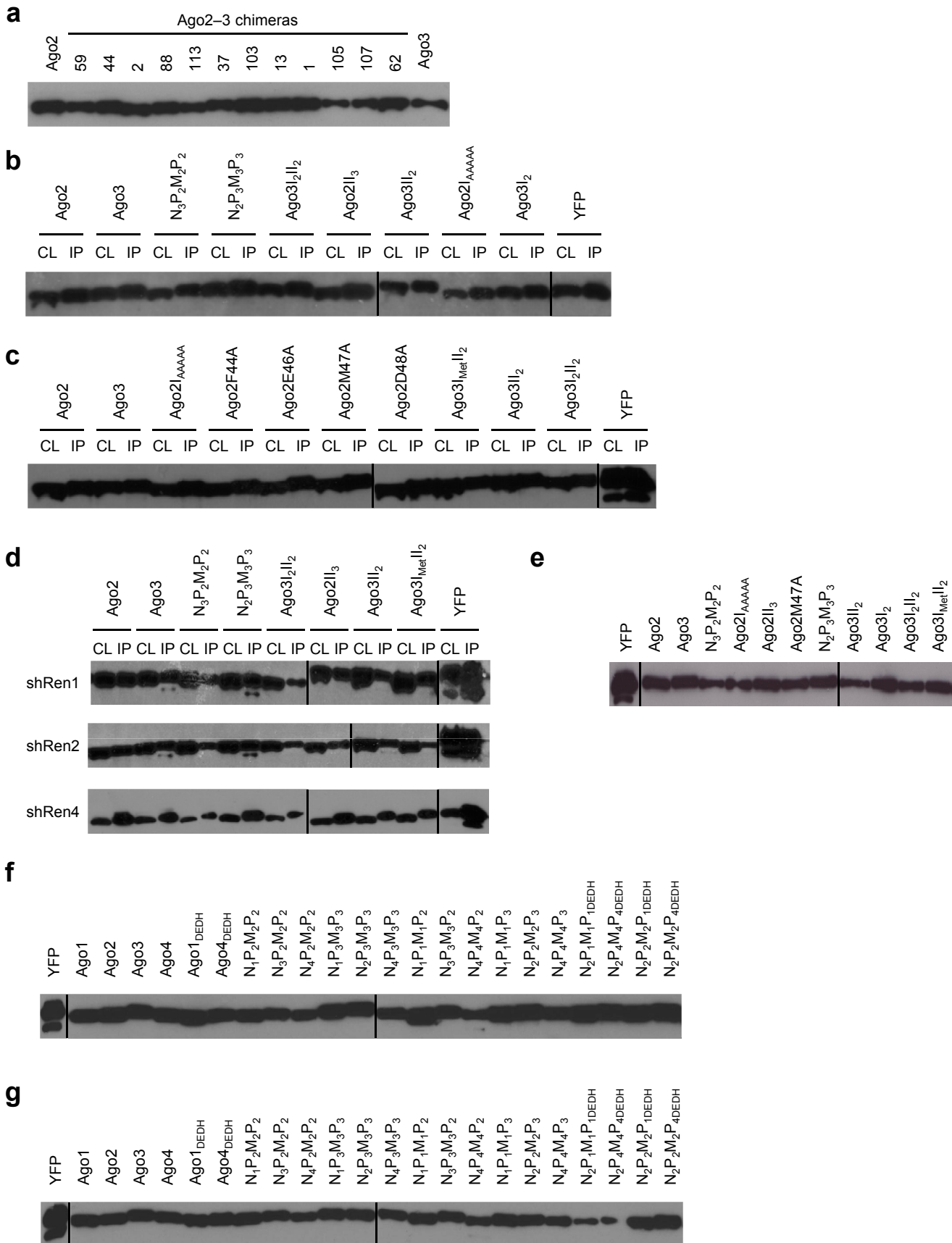
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Supplementary Fig. 1 Additional characterization and validation of Ago2-3 constructs.

(a) Schematic alignment of human Ago2 and Ago3 proteins. Blue regions are unique to Ago2, and red to Ago3; gray are identical in both. (b) Western blot analysis of immunoprecipitated Ago proteins used for the RISC cleavage assay in Fig. 1a. Samples were taken before (cell lysate, CL) or after immunoprecipitation (IP). (c) Alignment (type assignment generated via Salanto) of 58 randomly chosen clones (horizontal bars, one each) after Ago2-3 DFS. Color code as in a. (d) Percentage of Ago2- or Ago3-derived amino acids in 58 sequenced Ago2-3 chimeras (calculated by the Salanto software). For better visualization, only residues unique to one of the two parents (21.2% of the total proteins) were included. (e) Representative Ago2-3 crossover event, defined as the first nucleotide distinct from the preceding reference sequence in chimera alignments to the two parents. (f) Salanto-based analysis of crossover frequencies in the 58 chimeric Ago2-3 clones from c on DNA and protein level. Note that Salanto follows a parsimonious rationale according to which it tries to minimize the number of crossovers in the chimeric sequence. (g) Frequency of Ago2- or Ago3-derived amino acids in the 58 chimeric Ago2-3 clones from c at every position where the two parental proteins differ from each other. (h) Tethering assay of λ N-fused Ago constructs (see Fig. 4 for experimental details). Rlu, relative light units. P values were determined by Student's t -test ($n=3$). (i) Pilot experiment to assess the robustness of the RISC cleavage assays, using various amounts of Ago-IP input levels ($1/8$, $1/4$, $1/2$ or entire IP) and two different shRens. Western blots visualizing the range of Ago protein input levels in the corresponding samples are shown below. As indicated by the numbers, even an 8-fold variation in Ago input only translated into minor quantitative effects (26% at most) on target mRNA slicing. Notably, in all RISC cleavage assays in this paper, the experimental variation of Ago input levels was less than 8-fold.

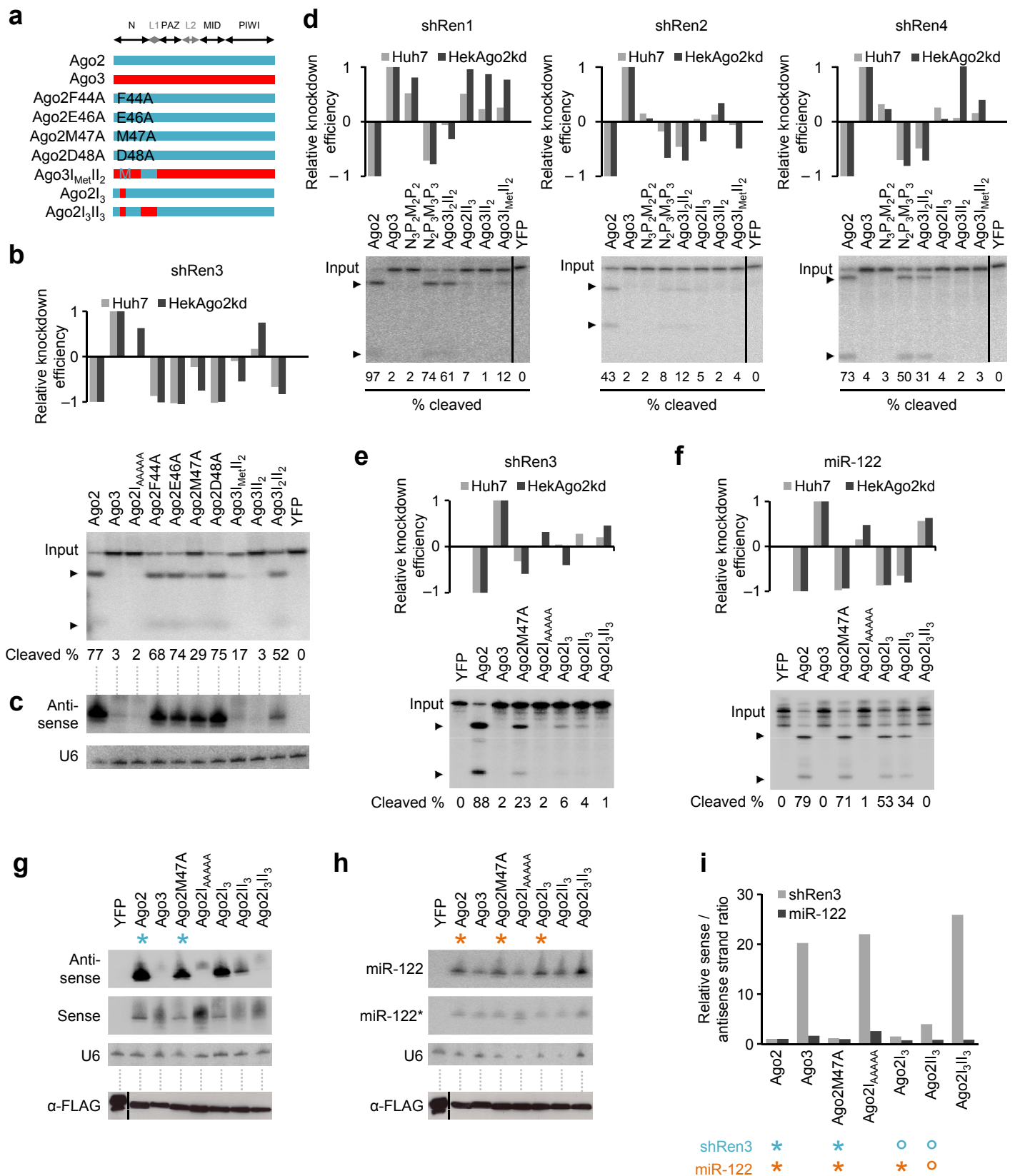
Supplementary Figure 1 Grimm



Supplementary Fig. 2 Western blot analyses of immunoprecipitated Ago proteins used for RISC cleavage assays and Northern blotting (loading controls).

Samples were taken before (cell lysate, CL) or after immunoprecipitation (IP). (a) IP samples from RISC cleavage assay in Fig. 1e. (b) IP and CL samples from RISC cleavage assay in Fig. 2b and Northern blot in Fig. 2c. (c) IP and CL samples from RISC cleavage assay in Supplementary Fig. 3b and Northern blot in Supplementary Fig. 3c. (d) IP and CL samples from RISC cleavage assay in Supplementary Fig. 3d and Northern blot in Fig. 2d. (e) IP samples from RISC cleavage assay in Fig. 2f and Northern blot in Fig. 2g. (f) IP samples from RISC cleavage assay in Fig. 4c and Northern blot in Supplementary Fig. 6a. (g) IP samples from RISC cleavage assay in Fig. 4d and Northern blot in Supplementary Fig. 6b.

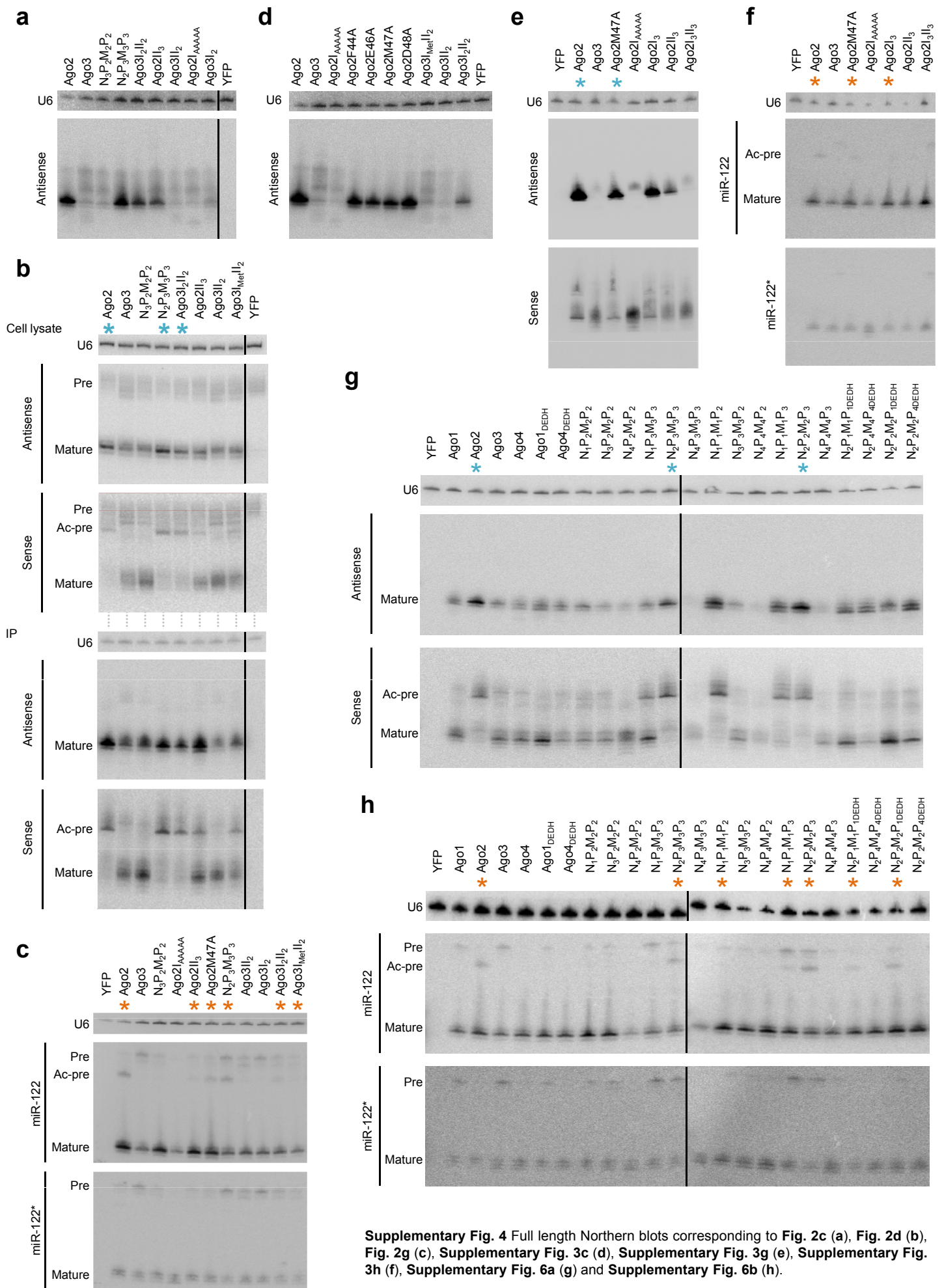
Supplementary Figure 2 Grimm



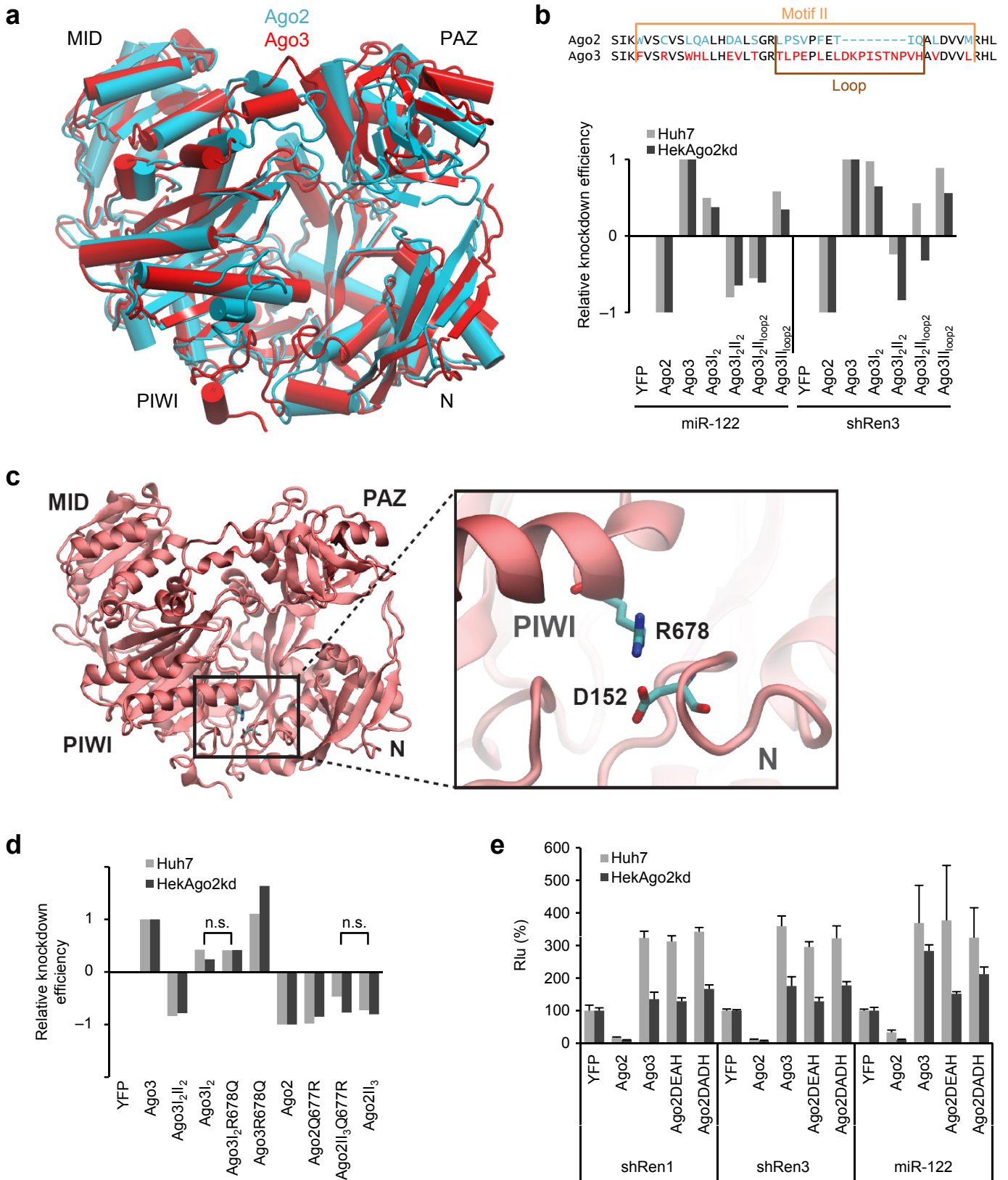
Supplementary Fig. 3 Cooperative action of motifs I and II for small RNA duplex activation and target cleavage.

(a) Schemes depicting Ago2–3 mutants (color-coded according to wildtypes on top). (b) The shown mutants were tested in luciferase knockdown experiments (top) and RISC cleavage assays (bottom) using shRen3 as RNAi trigger. (c) Co-immunoprecipitated small RNAs (same samples as in b (bottom)) were analyzed by Northern blotting using a probe against the shRen3 antisense strand. (d–f) The indicated mutants were analyzed akin to b, using the shown different shRNAs or miR-122, respectively. (g,h) Northern blot analysis of small RNAs in IP samples (same as in e,f) using probes against the shRen3 antisense or sense strand (g), or against miR-122 or miR-122* (h). Comparable protein input levels for e–h were validated by Western blotting (bottom). U6 small nuclear RNA signals (from naive RNA spiked in before RNA extraction) served as loading control in all Northern blots. Blue (shRen3) or orange (miR-122) asterisks: high slicer activity; blue (shRen3) or orange (miR-122) circles: intermediate slicer activity. (i) Northern blot signals from g,h were quantified and ratios between sense / antisense strand or miR-122* / miR-122 were calculated relative to Ago2 (set to 1). See g,h for symbols.

Supplementary Figure 3 Grimm



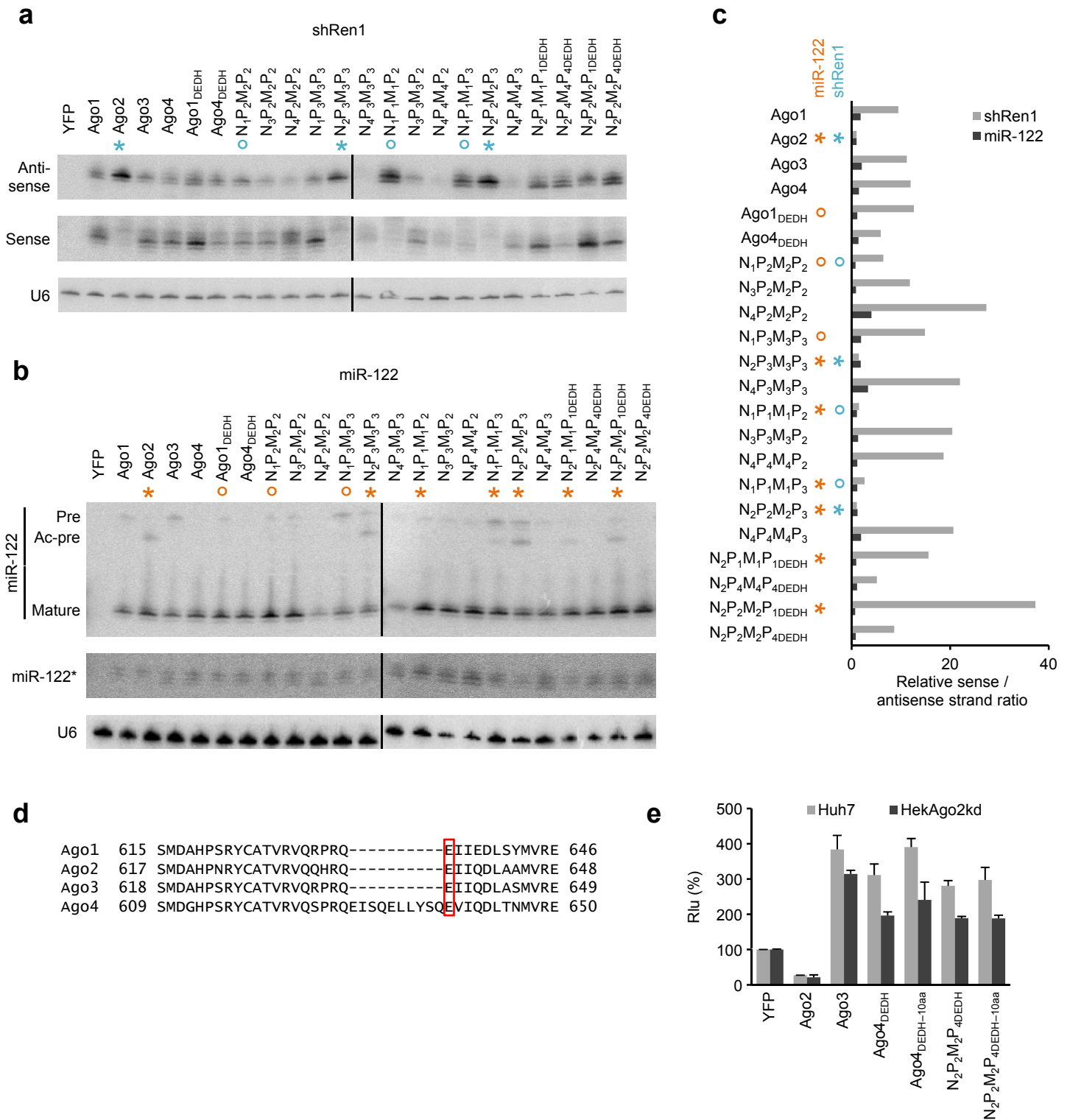
Supplementary Figure 4 Grimm



Supplementary Fig. 5 Additional theoretical and experimental dissection of critical elements in Ago2–3.

(a) Superposition of Ago2–3 homology models. (b) Analysis of Ago3 mutants harboring complete motif II or only the loop structure from Ago2 (see alignment on top) in luciferase assays with either miR-122 or shRen3 (see Fig. 1 for experimental details). Note that motif II in Ago2 contains two helices linked by a short loop, whereas Ago3 has to accommodate 8 additional amino acids within this region (see also Fig. 3 and Supplementary Fig. 7b), which, according to our Ago3 model, partly occupy and obstruct the RNA channel. (c) Salt bridge potentially connecting Ago3 N and PIWI domains, which cannot occur in Ago2 according to our homology models. Also note that R678 is in the vicinity of D670 (not shown), which belongs to the catalytic tetrad. Interaction of R678 with D152 might therefore prevent D670 from efficiently joining the catalytic site in Ago3. Interestingly, R678 is only present in Ago3, potentially explaining the residual activity of mutant Ago2I₃. (d) miR-122-based luciferase knockdown experiments with Ago2–3 mutants in which we either aimed to destroy (Ago3I₂R678Q) or reconstitute (Ago2I₃Q677R) the potential salt bridge (Ago3R678Q, Ago2Q677R served as controls). No significant changes relative to Ago3I₂ (for Ago3I₂R678Q) or Ago2I₃ (Ago2I₃Q677R) were observed. However, the correct formation of this putative salt bridge might depend on adjacent residues and structures that are not captured by these mutants. n.s., non-significant. (e) Analysis of the performance of the Ago2E637A mutant with two perfectly (shRen1 and shRen3) and one imperfectly duplexed small RNA triggers (miR-122) in luciferase knockdown experiments (data were normalized to the YFP control).

Supplementary Figure 5 Grimm



Supplementary Fig. 6 Functional analysis of Ago1–4 hybrids.

(a,b) Immunoprecipitated Ago complexes from Hek293T cells co-transfected with YFP or Ago and shRen1 (a) or miR-122 (b) were analyzed by Northern blotting (same samples as used in the RISC cleavage assays in Fig. 4 c,d). See Fig. 2 for experimental details. (c) Northern blot signals from a,b were quantified and ratios between sense and antisense strands, or miR-122* / miR-122, were calculated (relative to Ago2, set to 1). Blue (shRen3) or orange (miR-122) asterisks: high slicer activity; blue (shRen3) or orange (miR-122) circles: intermediate slicer activity. (d) Partial alignment of human Ago1–4 PIWI domains around the Ago2 E637 residue (red frame) which is fully conserved in all four human Agos. Evident is an N-terminal 10 aa insertion in Ago4 which we deleted to test whether it interferes with the function of the glutamate (see e for results). (e) Knockdown assays (co-transfection of miR-122-tagged *Renilla* luciferase, miR-122 expression plasmid as well as the indicated Ago constructs) showed that deletion of the 10 aa insertion did not reconstitute slicing ability in Ago4, neither in the wildtype context (Ago4_{DEDH-10aa}) nor after fusion of the altered Ago4 PIWI domain with the slicing-compatible N to MID domains from Ago2 (N₂P₂M₂P₄_{DEDH-10aa}). This suggests that other, as-of-yet unknown sequence/structure elements in Ago4 PIWI cause the distinctive slicing deficiency of this Ago family member.

Supplementary Figure 6 Grimm

a

Ago2	MYSG-AGPALAPPAPPPP IQGYAFKPPPRDFGTSGRTIKLANFFEMD IPKID IYHYELD IKPEK CPRRVNREIVEH MV	79
59	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71
44	MYSG-AGPALAPPAPPPP IQGYAFKPPPRDFGTSGRTIKLANFFEMD IPKID IYHYELD IKPKDCPRRVNREVDSMV	79
2	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71
88	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71
113	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREIVEH MV	71
37	MEIGSAGPAGAQP-----LLMVP RRP DFGTSGRTIKLANFFEMD IPKID IYHYELD IKPEK CPRRVNREVDSMV	71
103	MYSG-AGPALAPPAPPPP IQGYAFKPPPRDFGTSGRTIKLANFFEMD IPKID IYHYELD IKPEK CPRRVNREIVEH MV	79
13	MYSG-AGPALAPPAPPPP IQGYAFKPPPRDFGTSGRTIKLANFFEMD IPKID IYHYELD IKPEK CPRRVNREVDSMV	79
1	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71
105	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71
107	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71
62	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71
Ago3	MEIGSAGPAGAQP-----LLMVP RRP GYGTMGKPIKLLANCFQVE IPKIDVYLYEVD IKPKDCPRRVNREVDSMV	71

Motif I

N

Ago2	QHFKTQIFGDRKPVFDGRKNLYTAMPLP IGRDKVELEVTLPGEG-KDRIFKVS IKWVSCVSLQALHDALS GRLPSVFPFET	158
59	QHFKVTIFGDRKPVFDGRKNLYTAMPLP IGRDKVELEVTLPGEG-KDRIFKVS IKWVSCVSLQALHDALS GRLPSVFPFET	150
44	QHFKTQIFGDRKPVFDGRKNLYTAMPLP IGRDKVELEVTLPGEG-KDRIFKVS IKWVSCVSLQALHDALS GRLPSVFPFET	158
2	QHFKVTIFGDRRPPVYDGKRSLYTAMPLP IGRDKVELEVTLPGEG-KDRIFKVS IKWVSCVSLQALHDALS GRLPSVFPFET	150
88	QHFKVTIFGDRRPPVYDGKRSLYTAMPLP IGRDKVELEVTLPGEG-KDRIFKVS IKWVSCVSLQALHDALS GRLPSVFPFET	150
113	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	151
37	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	151
103	QHFKTQIFGDRKPVFDGRKNLYTAMPLP VATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	159
13	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	159
1	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	151
105	QHFKTQIFGDRRPPVYDGKRSLYTANPLPVATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	151
107	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	151
62	QHFKTQIFGDRKPVFDGRKNLYTAMPLP VATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	151
Ago3	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVDL DVTLPGEGGKDRPFKVS IKFVSRVSWHLLHEVLTGR TLPEPEL	151

Motif II

L1

Ago2	-----IQALDVVMRHLPSMRYTPVGRSFF TASEGCSNPLGGGREVWFGFHQSVRP SLWKMLNIDVSATAFYKAQPV	230
59	-----IQALDVVMRHLPSMRYTPVGRSFF TAP EGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	222
44	-----IQALDVVMRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	230
2	-----IQALDVVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	222
88	-----IQALDVVMRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	222
113	-----IQALDVVMRHLPSMRYTPVGRSFF TASEGCSNPLGGGREVWFGFHQSVRP SLWKMLNIDVSATAFYKAQPV	222
37	DKP ISTNPNVHADV D VVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP SLWKMLNIDVSATAFYKAQPV	231
103	DKP ISTNPNVHALDVVMRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	239
13	DKP ISTNPNVHADV D VVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	239
1	DKP ISTNPNVHADV D VVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	231
105	DKP ISTNPNVHADV D VVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	231
107	DKP ISTNPNVHADV D VVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	231
62	DKP ISTNPNVHADV D VVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	231
Ago3	DKP ISTNPNVHADV D VVLRHLPSMRYTPVGRSFF SAPEGYDHP LGGGREVWFGFHQSVRP AMWKMLNIDVSATAFYKAQPV	231

PAZ

Ago2	IEFVCEVLD FKSIEEQKPLTDSQRVKFTKEIKGLKVEITHCGQMRRKYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	310
59	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	302
44	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	310
2	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	302
88	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	302
113	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	302
37	IEFVCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	311
103	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	319
13	IQFMCEVLDIHNIDEQPRPLTDSQRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	319
1	IEFVCEVLD FKSIEEQKPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	311
105	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	311
107	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	311
62	IQFMCEVLDIHNIDEQPRPLTDSQRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	311
Ago3	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEVTHCGTMRKRYRVCN VTRRPASHQTFFLQLENGQTVECTVAQ	311

L2

Ago2	YFKDRHKLVLRYPHLPCLOVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISKLMSASFNT	390
59	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	382
44	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	390
2	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	382
88	YFKDRHKLVLRYPHLPCLOVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSASFNT	382
113	YFKDRHKLVLRYPHLPCLOVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	382
37	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	391
103	YFKDRHKLVLRYPHLPCLOVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	399
13	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	399
1	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	391
105	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISKLMSASFNT	391
107	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	391
62	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	391
Ago3	YFREKYTLQLKYPHLPCLQVGGQE QKHTYLPLEVCNIVAGQRCIKKLDNQTSTMIRATARSAPDRQEEISRLVRSANYET	391

Supplementary Figure 7 Grimm

L2

Ago2	DPYVREFGIMVKDEMTDVTGRVLQPPSILYGGRNKAIATPVQGVWDMRNKQFHTGIEIKVWAIACFAPQRQCTEVHLKSF	470
59	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCREEILKGF	462
44	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCREEILKGF	470
2	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGIEIKVWAIACFAPQRQCTEVHLKGF	462
88	DPYVREFGIMVKDEMTDVTGRVLQPPSILYGGRNKAIATPVQGVWDMRNKQFHTGVEIKMWAIAACFATORQCREEILKGF	462
113	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCTEVHLKGF	462
37	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCREEILKGF	471
103	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCTEVHLKGF	479
13	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPVQGVWDMRNKQFHTGIEIKVWAIACFAPQRQCTEVHLKSF	479
1	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKVWAIACFAPQRQCTEVHLKSF	471
105	DPYVREFGIMVKDEMTDVTGRVLQPPSILYGGRNKAIATPVQGVWDMRNKQFHTGIEIKVWAIACFATORQCREEILKSF	471
107	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCREEILKGF	471
62	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCREEILKGF	471
Ago3	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIAACFATORQCREEILKGF	471

MID

Ago2	TEQLRKISRDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLVIVILPGKTPVYAEVKRVGDTVLGMAEQCVQMK	550
59	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	542
44	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTVLGMAEQCVQMK	550
2	TDQLRKISRDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLVIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	542
88	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	542
113	TDQLRKISRDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	542
37	TDQLRKISRDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	551
103	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	559
13	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	559
1	TEQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLVIVILPGKTPVYAEVKRVGDTVLGMAEQCVQMK	551
105	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	551
107	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLVIVILPGKTPVYAEVKRVGDTVLGMAEQCVQMK	551
62	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLVIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	551
Ago3	TDQLRKISKDAGMPIQQQPCFCKYAQGADSVEPMFRHLKNTYAGLQLIIVILPGKTPVYAEVKRVGDTLLGMAEQCVQMK	551

Ago2	NVQRTTPQTLNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	630
59	NVQRTTPQTLNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	622
44	NVQRTTPQTLNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	630
2	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	622
88	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	622
113	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	622
37	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	631
103	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	639
13	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	639
1	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	631
105	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	631
107	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	631
62	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	631
Ago3	NVIKTSPTLSNCLKINVKLGGVNNILVPHQRPSVFQPPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	631

PIWI

Ago2	VQQRQREI IQDLAAMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLHHELLAIREACIKLEKDYQPGITFIVVQKR	710
59	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACIKLEKDYQPGITFIVVQKR	702
44	VQQRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	710
2	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLHHELLAIREACIKLEKDYQPGITFIVVQKR	702
88	VQRPRQREI IQDLAAMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	702
113	VQQRQREI IQDLAAMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITFIVVQKR	702
37	VQQRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	711
103	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	719
13	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	719
1	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	711
105	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITFIVVQKR	711
107	VQRPRQREI IQDLAAMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLHHELLAIREACIKLEKDYQPGITYIVVQKR	711
62	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	711
Ago3	VQRPRQREI IQDLASMVRELLIQFYKSTRFKPTRIIFYRQGVSEGFQVQLYELLAIREACISLEKDYQPGITYIVVQKR	711

Ago2	HHTRLFCADTKNERVGRSGNIPAGTTVDTKITHPTEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	790
59	HHTRLFCADRTERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	782
44	HHTRLFCADRTERVGRSGNIPAGTTVDTKITHPTEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	790
2	HHTRLFCADTKNERVGRSGNIPAGTTVDTKITHPTEFDFYLCSHAGIQGTSRPSHYHVLWDDNCFTELQILTYQLCHTY	782
88	HHTRLFCADRTERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNCFTELQILTYQLCHTY	782
113	HHTRLFCADTKNERVGRSGNIPAGTTVDTKITHPTEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	782
37	HHTRLFCADTKNERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNCFTELQILTYQLCHTY	791
103	HHTRLFCADRTERVGRSGNIPAGTTVDTKITHPTEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	799
13	HHTRLFCADRTERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	799
105	HHTRLFCADTKNERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	791
107	HHTRLFCADRTERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTY	791
62	HHTRLFCADRTERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNCFTELQILTYQLCHTY	791
Ago3	HHTRLFCADRTERVGRSGNIPAGTTVDTDITHPYEFDFYLCSHAGIQGTSRPSHYHVLWDDNCFTELQILTYQLCHTY	791

Supplementary Figure 7 Grimm - continued

	PIWI	
Ago2	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA	859
59	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHVSGQSNGRDPQALAKAVQIHQDTLRTMYFA	851
44	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA	859
2	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHVSGQSNGRDPQALAKAVQIHQDTLRTMYFA	851
88	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA	851
113	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHVSGQSNGRDPQALAKAVQIHQDTLRTMYFA	851
37	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA	860
103	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHVSGQSNGRDPQALAKAVQIHQDTLRTMYFA	868
13	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA	868
1	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA	860
105	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA	860
107	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHVSGQSNGRDPQALAKAVQIHQDTLRTMYFA	860
62	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHVSGQSNGRDPQALAKAVQIHQDTLRTMYFA	860
Ago3	VRCTRSVSIAPAYYAHLVAFRARYHLVDKEHDSAEGSHVSGQSNGRDPQALAKAVQIHQDTLRTMYFA	860

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b

Ago2	MYSG-AGPALAPPAPPPPIQGYAFKPPRPDFGTSGRITKQLQANFFEMDIPKIDIVHYELDIKPEKCPRRVNREIVEHVM	79
59	MEIGSAGPAGAQP-----LLMVRRPGYGTSGRTIKLQANFFEMDIPKIDIVHYELDIKPEKCPRRVNREIVEHVM	71
110	MEIGSAGPAGAQP-----LLMVRRPGYGTSGRTIKLQANFFEMDIPKIDIVHYEVDIKPEKCPRRVNREIVEHVM	71
44	MYSG-AGPALAPPAPPPPIQGYAFKPPRPDFGTSGRITKQLQANFFEMDIPKIDIVHYELDIKPKDKCPRRVNREIVVDSMV	79
78	MEIGSAGPAGAQP-----LLMVRRPGYGTSGRTIKLQANFFEMDIPKIDIVHYELDIKPEKCPRRVNREIVEHVM	71
129	MYSG-AGPALAPPAPPPPIQGYAFKPPRPDFGTSGRITKQLQANFFEMDIPKIDVLYEVDIKPKDKCPRRVNREIVVDSMV	79
74	MYSG-AGPALAPPAPPPPIQGYAFKPPRPDFGTSGRITKQLQANFFEMDIPKIDIVHYELDIKPEKCPRRVNREIVVDSMV	79
2	MEIGSAGPAGAQP-----LLMVRRPGYGTSGRTIKLQANFFEMDIPKIDVLYEVDIKPKDKCPRRVNREIVVDSMV	71
82	MYSG-AGPALAPPAPPPPIQGYAFKPPRPDFGTSGRITKQLQANFFEMDIPKIDIVHYELDIKPEKCPRRVNREIVEHVM	79
Ago3	MEIGSAGPAGAQP-----LLMVRRPGYGTSGRTIKLQANFFEMDIPKIDVLYEVDIKPKDKCPRRVNREIVVDSMV	71

Motif I

N		
Ago2	QHFKTQIFGDRKPVFDGRKNLYTAMPLPIGRDKVELEVTLPPEG-KDRIFKVSIKWVSCVSLQALHDALSGRLPSVPFET	158
59	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVLDLVTLPPEGGKDRPFKVSIKWVSCVSLQALHDALSGRLPSVPFET	150
110	QHFKTQIFGDRRPPVYDGKRSLYTANPLPVATTGVLDLVTLPPEGGKDRPFKVSIKWVSCVSLQALHDALSGRLPSVPFET	151
44	QHFKTQIFGDRKPVFDGRKNLYTAMPLPIGRDKVELEVTLPPEG-KDRIFKVSIKWVSCVSLQALHDALSGRLPSVPFET	158
78	QHFKTQIFGDRKPVFDGRKNLYTAMPLPIGRDKVELEVTLPPEG-KDRIFKVSIKWVSCVSLQALHDALSGRLPSVPFET	150
129	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVLDLVTLPPEGGKDRPFKVSIKWVSCVSLQALHDALSGRLPSVPFET	159
74	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVLDLVTLPPEGGKDRPFKVSIKWVSCVSLQALHDALSGRLPSVPFET	159
2	QHFKVTIFGDRRPPVYDGKRSLYTAMPLPIGRDKVELEVTLPPEG-KDRIFKVSIKWVSCVSLQALHDALSGRLPSVPFET	150
82	QHFKTQIFGDRKPVFDGRKNLYTAMPLPIGRDKVELEVTLPPEG-KDRIFKVSIKWVSCVSLQALHDALSGRLPSVPFET	158
Ago3	QHFKVTIFGDRRPPVYDGKRSLYTANPLPVATTGVLDLVTLPPEGGKDRPFKVSIKWVSCVSLQALHDALSGRLPSVPFET	151

Motif II

L1		
Ago2	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	230
59	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	222
110	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	223
44	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	230
78	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	222
129	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	231
74	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	231
2	-----IQALDVVLRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	222
82	-----IQALDVVMRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	230
Ago3	DKP1STNPVHAVDVVLRHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYKAQPV	231

PAZ

Ago2	IEFVCEVLDFKSIEEQKPLTDSQRVKFTKEIKGLKVEITHCGQMKRKYRVCNVRTRRPASHQTFPLQESGQTVECTVAQ	310
59	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQLENGQTVERTVAQ	302
110	IEFVCEVLDFKSIEEQKPLTDSQRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQLENGQTVERTVAQ	303
44	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQLENGQTVERTVAQ	310
78	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQLENGQTVERTVAQ	302
129	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQESGQTVECTVAQ	311
74	IQFVCEVLDFKSIEEQKPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQLENGQTVERTVAQ	311
2	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQLENGQTVECTVAQ	302
82	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGQMKRKYRVCNVRTRRPASHQTFPLQESGQTVECTVAQ	310
Ago3	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTKEIKGLKVEITHCGTMRKRYRVCNVRTRRPASHQTFPLQLENGQTVERTVAQ	311

Ago2	YFKDRHKLIVLRYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIRATARSAPDRQEEISKLMRSASFNT	390
59	YFREKYTLQLKYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIRATARSAPDRQEEISRLVRSANYET	382
110	YFKDRHKLIVLRYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIKATARSAPDRQEEISRLVRSANYET	383
44	YFREKYTLQLKYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIRATARSAPDRQEEISRLVRSANYET	390
78	YFREKYTLQLKYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIRATARSAPDRQEEISRLVRSANYET	382
129	YFKDRHKLIVLRYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIKATARSAPDRQEEISRLVRSANYET	391
74	YFREKYTLQLKYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIKATARSAPDRQEEISRLVRSANYET	391
2	YFREKYTLQLKYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIRATARSAPDRQEEISRLVRSANYET	382
82	YFKDRHKLIVLRYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIKATARSAPDRQEEISRLVRSANYET	390
Ago3	YFREKYTLQLKYPHLPCLQVQEQKHTYLPLEVCNIVAGQRCIKKLTNDQSTSMIKATARSAPDRQEEISRLVRSANYET	391

L2

MID

Ago2	DPFVREFGIMVKDEM TDVTGRVLPQPSILYGGRNKAIATPVQGVWDMRNRKQFHTGIEIKVWAIACFAPQRQCTEVHLKSF	470
59	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILKGF	462
110	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILKGF	463
44	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILKGF	470
78	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKVWAIACFAPQRQCTEVHLKSF	462
129	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILKGF	471
74	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCTEVHLKSF	471
2	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGIEIKVWAIACFAPQRQCTEVHLKGF	462
82	DPFVQEFQFKVRDEMAHVTGRVLPQPSILYGGRNKAIATPVQGVWDMRNRKQFHTGIEIKVWAIACFAPQRQCTEVHLKSF	470
Ago3	DPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGNRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILKGF	471

MID		
Ago2	TEQLRKISR DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLV VVILPGKTPVYAEVKRVGDTVLGMA TQCVQMK	550
59	TDQLRKISK DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLI IIVILPGKTPVYAEVKRVGDTLLGMA TQCVQMK	542
110	TDQLRKISK DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLI IIVILPGKTPVYAEVKRVGDTVLGMA TQCVQVK	543
44	TDQLRKISK DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLI IIVILPGKTPVYAEVKRVGDTVLGMA TQCVQMK	550
78	TEQLRKISR DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLV VVILPGKTPVYAEVKRVGDTLLGMA TQCVQVK	542
129	TDQLRKISK DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLV VVILPGKTPVYAEVKRVGDTLLGMA TQCVQVK	551
74	TEQLRKISK DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLV VVILPGKTPVYAEVKRVGDTLLGMA TQCVQMK	551
2	TDQLRKISR DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLV VVILPGKTPVYAEVKRVGDTLLGMA TQCVQVK	542
82	TEQLRKISK DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLI IIVILPGKTPVYAEVKRVGDTLLGMA TQCVQVK	550
Ago3	TDQLRKISK DAGMPIQGQPCFCKYAQGADSV EPMFRHLKNTYAGLQLI IIVILPGKTPVYAEVKRVGDTLLGMA TQCVQVK	551

Ago2	NVQRTTPQ TLSNLCLKINVKLGGVNNILLPQGR PVPVFPQVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	630
59	NVQRTTPQ TLSNLCLKINVKLGGINNILVPHQR PVSFQQPVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHP SRYCATVR	622
110	NVIKTS PQLSNLCLKINVKLGGINNILVPHQR PVSFQQPVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	623
44	NVQRTTPQ TLSNLCLKINVKLGGVNNILLPQGR PVPVFPQVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	630
78	NVIKTS PQLSNLCLKINVKLGGINNILVPHQR PVSFQQPVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHP SRYCATVR	622
129	NVIKTS PQLSNLCLKINVKLGGINNILVPHQR PVSFQQPVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHP SRYCATVR	631
74	NVQRTTPQ TLSNLCLKINVKLGGVNNILLPQGR PVPVFPQVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHP SRYCATVR	631
2	NVIKTS PQLSNLCLKINVKLGGINNILVPHQR PVSFQQPVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	622
82	NVIKTS PQLSNLCLKINVKLGGINNILVPHQR PVSFQQPVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVR	630
Ago3	NVIKTS PQLSNLCLKINVKLGGINNILVPHQR PVSFQQPVIFL GADVTHPPAGDGKKPSIAAVVGSMDAHP SRYCATVR	631

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PIWI		
Ago2	VQQHROE I IQDLAAMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFQQV LHHELLAIREACIKLEKDYQPGITFIVVQKR	710
59	VQRPROE I IQDLASMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFRQV LLYELLAIREACIKLEKDYQPGITFIVVQKR	702
110	VQRPROE I IQDLASMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFQQV LHHELLAIREACISLEKDYQPGITFIVVQKR	703
44	VQQHROE I IQDLAAMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFRQV LLYELLAIREACISLEKDYQPGITFIVVQKR	710
78	VQQHROE I IQDLAAMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFRQV LLYELLAIREACISLEKDYQPGITFIVVQKR	702
129	VQRPROE I IQDLAAMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFQQV LHHELLAIREACISLEKDYQPGITFIVVQKR	711
74	VQRPROE I IQDLAAMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFQQV LHHELLAIREACIKLEKDYQPGITFIVVQKR	711
2	VQRPROE I IQDLASMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFRQV LLYELLAIREACIKLEKDYQPGITFIVVQKR	702
82	VQQHROE I IQDLASMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFRQV LLYELLAIREACISLEKDYQPGITFIVVQKR	710
Ago3	VQRPROE I IQDLASMVRELLIQFYKSTRFKP TRIIFYR DGVSEGGFRQV LLYELLAIREACISLEKDYQPGITFIVVQKR	711

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Ago2	HHTRLFCAD RTERVGRSGNIPAGTTVDTKI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	790
59	HHTRLFCAD RTERVGRSGNIPAGTTVDTDI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	782
110	HHTRLFCAD RTERVGRSGNIPAGTTVDTDI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	783
44	HHTRLFCAD RTERVGRSGNIPAGTTVDTKI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	790
78	HHTRLFCAD RTERVGRSGNIPAGTTVDTDI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	782
129	HHTRLFCAD RTERVGRSGNIPAGTTVDTKI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	791
74	HHTRLFCAD RTERVGRSGNIPAGTTVDTDI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	791
2	HHTRLFCAD RTERVGRSGNIPAGTTVDTKI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	782
82	HHTRLFCAD RTERVGRSGNIPAGTTVDTDI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	790
Ago3	HHTRLFCAD RTERVGRSGNIPAGTTVDTDI THPEYFD FYLCSHAGIQGTSRPSHYHVLWDDNCFSSDELQILTYQLCHTY	791

Ago2	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHTSGQSNGRDHQALAKAVQVHQD TLRMTMYFA	859
59	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHVSGQSNGRDPQALAKAVQIHQD TLRMTMYFA	851
110	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHTSGQSNGRDHQALAKAVQVHQD TLRMTMYFA	852
44	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHTSGQSNGRDHQALAKAVQVHQD TLRMTMYFA	859
78	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHTSGQSNGRDPQALAKAVQIHQD TLRMTMYFA	851
129	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHTSGQSNGRDPQALAKAVQIHQD TLRMTMYFA	860
74	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHTSGQSNGRDHQALAKAVQIHQD TLRMTMYFA	860
2	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHVSGQSNGRDPQALAKAVQIHQD TLRMTMYFA	851
82	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHTSGQSNGRDPQALAKAVQIHQD TLRMTMYFA	859
Ago3	VRCTRSVSI PAPAYYAHLVAFRARYHLVDKEHDS AEGSHVSGQSNGRDPQALAKAVQIHQD TLRMTMYFA	860

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C

Ago1	MEAGPSGAAAGAYLPP---LQQVFQAPRRPGIGTVGKPIKLLANYFEVDIPKIDVYHYEVDIKPDKCPRRVNREVVEYMV	77
Ago2	-MYSGAGPALAPPAPPPP IQGYAFKPPPRDFGTSGRITIKLQANFFEMDIPKID IYHYELDIKPEKCPRRVNREIVEHVM	79
Ago3	MEIGSAGPAG-----AQLLMVPRRP GYGTMGKPIKLLANCFQVEIPKIDVYLYEVDIKPDKCPRRVNREVVDSTMV	71
Ago4	--MEALGPGP-----PASLFQPPRRPGLGTGKPIRLLANHFQVQIPKIDVYHYDVIDIKPEKRRRVNREVVDTMV	69
#1	--MEALGPGP-----PASLFQPPRRPGLGTGKPIRLLANHFQVQIPKIDVYHYDVIDIKPEKRRRVNREVVDTMV	69
#3	-MYSGAGPALAPPAPPPP IQGYAFKPPPRDFGTSGRITIKLQANFFEMDIPKID IYHYELDIKPEKCPRRVNREIVEHVM	79
#5	MEAGPSGAAAGAYLPP---LQQVFQAPRRPGIGTVGKPIKLLANYFEVDIPKIDVYHYEVDIKPDKCPRRVNREVVEYMV	77
#7	MEIGSAGPAG-----AQLLMVPRRP GYGTMGKPIKLLANCFQVEIPKIDVYLYEVDIKPDKCPRRVNREVVDSTMV	71
#14	MEIGSAGPAG-----AQLLMVPRRP GYGTSGRITIKLQANFFEMDIPKID IYHYELDIKPEKCPRRVNREIVEHVM	71
#31	-MYSGAGPALAPPAPPPP IQGYAFKPPPRDFGTSGRITIKLQANFFEMDIPKID IYHYELDIKPEKCPRRVNREIVEHVM	79
#32	-MYSGAGPALAPPAPPPP IQGYAFKPPPRDFGTSGRITIKLQANFFEMDIPKID IYHYELDIKPEKCPRRVNREIVEHVM	79
#33	MEAGPSGAAAGAYLPP---LQQVFQAPRRPGIGTVGKPIKLLANYFEVDIPKIDVYHYEVDIKPDKCPRRVNREVVDSTMV	77
#34	--MEALGPGP-----PASLFQPPRRPGLGTGKPIRLLANHFQVQIPKIDVYHYDVIDIKPEKRRRVNREVVDTMV	69
#38	MEAGPSGAAAGAYLPP---LQQVFQAPRRPGIGTVGKPIKLLANYFEVDIPKIDVYHYEVDIKPDKCPRRVNREVVEYMV	77

Motif I

N		
Ago1	QHFKPQIFGDRKPVYDGRKNLYTAMPLP IGRDVELEVTIPGEG-KDRIFKVISIKWLAIVSWRMLHEALVSGQIP----	151
Ago2	QHFKTQIFGDRKPVDFGRKNLYTAMPLP IGRDKVELEVTIPGEG-KDRIFKVISIKWVSCVSLQALHDALSGRLPS----	153
Ago3	QHFKVTIFGDRRPFVYDGRKSLYTANPLP VATTGVDLDVTLPGEGGKDRPFKVISIKFVSRVSWHLLHEVLTGRTLPEPLEL	151
Ago4	RHFQMIFGDRQPGYDGRKNMYTAHPLP IGRDRVMEVTLPGEG-KDQTFKVSQVWVSVVSLQLLLEALAGHLN----	142
#1	RHFQMIFGDRQPGYDGRKNMYTAHPLP IGRDRVMEVTLPGEG-KDQTFKVSQVWVSVVSLQLLLEALAGHLN----	142
#3	QHFKTQIFGDRKPVDFGRKNLYTAMPLP IGRDKVELEVTIPGEG-KDRIFKVISIKWVSCVSLQALHDALSGRLPS----	153
#5	QHFKPQIFGDRKPVYDGRKNLYTAMPLP ICNERVDFEVTIPGEG-KDRIFKVISIKWLAIVSWRMLHEALVSGQIP----	151
#7	QHFKVTIFGDRRPFVYDGRKSLYTANPLP VATTGVDLDVTLPGEGGKDRPFKVISIKFVSRVSWHLLHEVLTGRTLPEPLEL	151
#14	QHFKTQIFGDRKPVDFGRKNLYTAMPLP IGRDKVELEVTIPGEG-KDRIFKVISIKWLAIVSWRMLHEALVSGQIP----	145
#31	QHFKTQIFGDRKPVDFGRKNLYTAMPLP IGRDKVELEVTIPGEG-KDRIFKVISIKWVSCVSLQALHDALSGRLPS----	153
#32	QHFKTQIFGDRKPVDFGRKNLYTAMPLP IGRDKVELEVTIPGEG-KDRIFKVISIKWVSCVSLQALHDALSGRLPS----	153
#33	QHFKVTIFGDRRPFVYDGRKNLYTAMPLP IGRDRVMEVTLPGEG-KDQTFKVSQVWVSVVSLQLLLEALAGHLN----	151
#34	RHFQMIFGDRQPGYDGRKNMYTAHPLP IGRDRVMEVTLPGEG-KDQTFKVSQVWVSVVSLQLLLEALAGHLN----	142
#38	QHFKPQIFGDRKPVDFGRKNLYTAMPLP IGRDKVELEVTIPGEG-KDRIFKVISIKWVSCVSLQALHDALSGRLPS----	151

Motif II

L1		
Ago1	---VPLESVQALDVAMRHLASMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWKMLNIDVSAFAYKAQPV	228
Ago2	---VPFETIQALDVVMRHLPSMRYPVGRSFFTASEGCSNP LGGGREVWVGFHQSVRPSLWKMLNIDVSAFAYKAQPV	230
Ago3	DKPISTNPVHAVDVVLRHLPSMKYTPVGRSFFSAPEGYDHP LGGGREVWVGFHQSVRPAMWKMLNIDVSAFAYKAQPV	231
Ago4	--EVPDSDVQALDVITRHLPSMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWNMLNIDVSAFAYRAQPI	220
#1	--EVPDSDVQALDVITRHLPSMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWNMLNIDVSAFAYRAQPI	220
#3	---VPFETIQALDVVMRHLPSMRYPVGRSFFTASEGCSNP LGGGREVWVGFHQSVRPSLWKMLNIDVSAFAYKAQPV	230
#5	---VPLESVQALDVAMRHLASMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWKMLNIDVSAFAYKAQPV	228
#7	DKPISTNPVHAVDVVLRHLPSMKYTPVGRSFFSAPEGYDHP LGGGREVWVGFHQSVRPAMWKMLNIDVSAFAYKAQPV	231
#14	---VPLESVQALDVAMRHLASMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWKMLNIDVSAFAYKAQPV	222
#31	---VPFETIQALDVVMRHLPSMRYPVGRSFFTASEGCSNP LGGGREVWVGFHQSVRPSLWKMLNIDVSAFAYKAQPV	230
#32	---VPFETIQALDVVMRHLPSMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPSLWKMLNIDVSAFAYKAQPV	230
#33	---VPLGVSQALDVAMRHLASMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWKMLNIDVSAFAYKAQPV	228
#34	--EVPDSDVQALDVITRHLPSMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWNMLNIDVSAFAYRAQPI	220
#38	---VPFETIQALDVAMRHLASMRYPVGRSFFSPPEGYHPLGGGREVWVGFHQSVRPAMWKMLNIDVSAFAYKAQPV	228

PAZ

Ago1	IEFMCEVLDIRNIDEQPKPLTDSQRVRFKTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLESQQTVECTVAQ	308
Ago2	IEFVCEVLDFKSIEEQKPLTDSQRVKFTEIKGLKVEITHCGQMKRKYRVCNTRRPASHQTFPLQQESQQTVECTVAQ	310
Ago3	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLENGQTVERTVAQ	311
Ago4	IEFMCEVLDIQNINEQTKPLTDSQRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLENGQAMECTVAQ	300
#1	IEFMCEVLDIQNINEQTKPLTDSQRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLENGQAMECTVAQ	300
#3	IEFMCEVLDIRNIDEQPKPLTDSQRVRFKTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQQESQQTVECTVAQ	310
#5	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLENGQTVERTVAQ	308
#7	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLENGQTVERTVAQ	311
#14	IEFVCEVLDFKSIEEQKPLTDSQRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQQENGQAMECTVAQ	302
#31	IEFVCEVLDFKSIEEQKPLTDSQRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQQESQQTVECTVAQ	310
#32	IEFVCEVLDFKSIEEQKPLTDSQRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQQESQQTVECTVAQ	310
#33	IQFMCEVLDIHNIDEQPRPLTDSHRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLENGQTVERTVAQ	308
#34	IEFMCEVLDIQNINEQTKPLTDSQRVKFTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQLENGQAVECTVAQ	300
#38	IEFMCEVLDIRNIDEQPKPLTDSQRVRFKTEIKGLKVEVTHCGQMKRKYRVCNTRRPASHQTFPLQQESQQTVECTVAQ	308

L2

Ago1	YFKQKYNLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILMKNASYN-	387
Ago2	YFKDRHKLVLRYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILMRSASFN-	389
Ago3	YFREKYLTLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVRSANYE-	390
Ago4	YFKQKYSLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVKSNSMVG	380
#1	YFKQKYSLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVKSNSMVG	380
#3	YFKDRHKLVLRYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILMRSASFN-	389
#5	YFKQKYNLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILMKNASYN-	387
#7	YFKDRHKLVLRYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILMKNASYN-	390
#14	YFKQKYSLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVKSNSMVG	382
#31	YFKQKYSLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVKSNSMVG	390
#32	YFKQKYNLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVRSANYE-	389
#33	YFREKYLTLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVRSANYE-	387
#34	YFKDRHKLVLRYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILMRSASFN-	379
#38	YFKQKYSLQKYPHLPCLQVQGEQKHTYLP LEVCNIVAGQRCIKKLTNDQSTMIKATARSAPDRQEEISRILVRSANYE-	387

Supplementary Figure 7 Grimm - continued

L2

Ago1	-LDPYIQEFGIKVKDDMTEVTGRVLPAPILQYGGRRNRAIATPNQGVWDMRGKQFYNGIEIKVWAIACFAPQKQCREEVLK	466
Ago2	-TDPYVREFGIMVKDEMTDVTGRVLPQPPSILYGGRRNKAIAITPVQGVWDMRNKQFHTGIEIKVWAIACFAPQRQCTEVHLK	468
Ago3	-TDPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGRRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILK	469
Ago4	GPDPYLKEFGIVVHNEMTELTGRVLPAPMLQYGGRRNRTVATPNQGVWDMRGKQFYAGIEIKVWAVACFAPQKQCREDDLK	460
#1	GPDPYLKEFGIVVHNEMTELTGRVLPAPMLQYGGRRNRTVATPNQGVWDMRGKQFYAGIEIKVWAVACFATQRQCREEILK	460
#3	-TDPYVREFGIMVKDEMTDVTGRVLPQPPSILYGGRRNKAIAITPVQGVWDMRNKQFHTGIEIKVWAIACFAPQKQCREEVLK	468
#5	-LDPYIQEFGIKVKDDMTEVTGRVLPAPILQYGGRRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILK	466
#7	-LDPYIQEFGIKVKDDMTEVTGRVLPAPILQYGGRRNRAIATPNQGVWDMRGKQFYNGIEIKVWAIACFAPQKQCREEVLK	469
#14	GPDPYLKEFGIVVHNEMTELTGRVLPAPMLQYGGRRNRTVATPNQGVWDMRGKQFYAGIEIKVWAVACFAPQKQCREDDLK	462
#31	GPDPYLKEFGIVVHNEMTELTGRVLPAPMLQYGGRRNRTVATPNQGVWDMRGKQFYAGIEIKVWAVACFAPQKQCREDDLK	470
#32	-TDPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGRRNRAIATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILK	468
#33	-TDPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGRRNRTVATPSHGVDWDMRGKQFHTGVEIKMWAIACFATQRQCREEILK	466
#34	-TDPYVREFGIMVKDEMTDVTGRVLPQPPSILYGGRRNKAIAITPVQGVWDMRNKQFHTGIEIKVWAIACFAPQRQCTEVHLK	458
#38	-TDPFVQEFQFKVRDEMAHVTGRVLPAPMLQYGGRRNRTVATPNQGVWDMRGKQFHTGVEIKMWAIACFATQRQCREEILK	466

MID

Ago1	NFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	546
Ago2	SFTEQLRKISRDA GMP IQGQPCFKYAQGADSVPEPMFRHLKNTYAGLQLVVILPGKTPVYAEVKRVGDTVLGMATQCVQ	548
Ago3	GFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	549
Ago4	SFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYVGLQLIIVVILPGKTPVYAEVKRVGDTLLGMATQCVQ	540
#1	GFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	540
#3	NFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	548
#5	GFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	546
#7	NFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVVILPGKTPVYAEVKRVGDTLLGMATQCVQ	549
#14	SFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYVGLQLIIVVILPGKTPVYAEVKRVGDTLLGMATQCVQ	542
#31	SFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	550
#32	GFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYAGLQLVVILPGKTPVYAEVKRVGDTVLGMATQCVQ	548
#33	GFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	546
#34	SFTEQLRKISRDA GMP IQGQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	538
#38	GFTDQLRKISKDAGMPIQQQPCFKYAQGADSVPEPMFRHLKNTYSGLQLIIVILPGKTPVYAEVKRVGDTLLGMATQCVQ	546

Ago1	VKNVVKTSPTLSNLCLKINVKLGGINNILVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	626
Ago2	MKNVQRTTPTLSNLCLKINVKLGGVNNILLPQGRPPVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	628
Ago3	VKNVVKTSPTLSNLCLKINVKLGGINNILVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	629
Ago4	VKNVVKTSPTLSNLCLKINAKLGGINNVLVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDGHPNRYCAT	620
#1	VKNVVKTSPTLSNLCLKINVKLGGINNILVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	620
#3	VKNVVKTSPTLSNLCLKINVKLGGINNILLPQGRPPVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	628
#5	MKNVQRTTPTLSNLCLKINVKLGGINNILVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	626
#7	VKNVVKTSPTLSNLCLKINAKLGGINNVLVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	622
#14	MKNVQRTTPTLSNLCLKINVKLGGVNNILLPQGRPPVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	629
#31	VKNVVKTSPTLSNLCLKINAKLGGINNVLVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	630
#32	MKNVQRTTPTLSNLCLKINVKLGGIYNILVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	628
#33	VKNVVKTSPTLSNLCLKINVKLGGINNILVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	626
#34	VKNVVKTSPTLSNLCLKINAKLGGINNVLVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDGHPNRYCAT	618
#38	VKNVVKTSPTLSNLCLKINVKLGGINNILVPHQRSVVFQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCAT	626

PIWI

Ago1	VRVQRPRQ-----EIIEDLSYMVRELLIQFYKSTRFKPTRIIFYRDGVPEGQLPQILHYELLAIRDACIKLEKDY	696
Ago2	VRVQQHRQ-----EIIQDLAMVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFQVLLHHELLAIREACIKLEKDY	698
Ago3	VRVQRPRQ-----EIIQDLASVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFRQVLYHELLAIREACIKLEKDY	699
Ago4	VRVQTSRQEIISQELLYSCEVIQDLTNMVRELLIQFYKSTRFKPTRIIFYRGGVSEGQMKQVAWPELIAIRKACISLEEDY	700
#1	VRVQRPRQ-----EIIQDLASVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFRQVLYHELLAIRDACIKLEKDY	690
#3	VRVQRPRQ-----EIIQDLAMVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFQVLLHHELLAIREACIKLEKDY	698
#5	VRVQQHRQ-----EIIQDLAMVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFQVLLHHELLAIREACIKLEKDY	696
#7	VRVQRPRQ-----EIIQDLASVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFRQVLYHELLAIREACISLEKDY	699
#14	VRVQQHRQ-----EIIQDLAMVRELLIQFYKSTRFKPTRIIFYRDGVPEGQLPQILHYELLAIRDACIKLEKDY	692
#31	VRVQRPRQ-----EIIEDLSYMVRELLIQFYKSTRFKPTRIIFYRDGVPEGQLPQILHYELLAIRDACIKLEKDY	700
#32	VRVQRPRQ-----EIIEDLSYMVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFRQVLYHELLAIRDACISLEKDY	698
#33	VRVQRPRQ-----EIIEDLSYMVRELLIQFYKSTRFKPTRIIFYRDGVPEGQLPQILHYELLAIREACIKLEKDY	696
#34	VRVQTSRQEIISQELLYSCEVIQDLTNMVRELLIQFYKSTRFKPTRIIFYRGGVSEGQMKQVAWPELIAIRKACISLEEDY	698
#38	VRVQRPRQ-----EIIQDLASVRELLIQFYKSTRFKPTRIIFYRDGVSEGQFRQVLYHELLAIREACISLEKDY	696

Ago1	QPGITYIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTNITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNRFSTADE	776
Ago2	QPGITFIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTKITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNRFSSDE	778
Ago3	QPGITYIVVQKRHHTRLFCADRTERVGRSGNIPAGTTVDTDITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	779
Ago4	RPGITYIVVQKRHHTRLFCADKTERVGRSGNIPAGTTVDSTITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	780
#1	QPGITFIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTNITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNRFSSDE	770
#3	QPGITFIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTNITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNRFSTADE	778
#5	QPGITFIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTKITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNRFSSDE	776
#7	QPGITYIVVQKRHHTRLFCADRTERVGRSGNIPAGTTVDTDITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	779
#14	QPGITYIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTNITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNRFSTADE	772
#31	QPGITYIVVQKRHHTRLFCADKTERVGRSGNIPAGTTVDSTITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	780
#32	QPGITYIVVQKRHHTRLFCADRTERVGRSGNIPAGTTVDTDITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	778
#33	QPGITFIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTKITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	776
#34	QPGITFIVVQKRHHTRLFCADKNERIGKSGNIPAGTTVDTKITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	778
#38	QPGITYIVVQKRHHTRLFCADRTERVGRSGNIPAGTTVDTDITHPEFDFYLCSHAGIQGTSRPSHYVWLWDDNCFSTADE	776

Supplementary Figure 7 Grimm - continued

	PIWI		
Ago1	LQILTYQLCHTYVRC	TRSVSIPAPAYYA	RLVAFRARYHLVDKEHDSGEGSHISGQSNRDPQALAKAVQVHQDTLRRTMYF 856
Ago2	LQILTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSAEGSHISGQSNRDPQALAKAVQVHQDTLRRTMYF 858
Ago3	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSAEGSHVSGQSNRDPQALAKAVQIHQDTLRRTMYF 859
Ago4	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	RLVAFRARYHLVDKDHDSAEGSHVSGQSNRDPQALAKAVQIHQDTLRRTMYF 860
#1	LQILTYQLCHTYVRC	TRSVSIPAPAYYA	RLVAFRARYHLVDKEHDSGEGSHISGQSNRDPQALAKAVQVHQDTLRRTMYF 850
#3	LQILTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSAEGSHVSGQSNRDPQALAKAVRQVHQDTLRRTMYF 858
#5	LQILTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSAEGSHVSGQSNRDPQALAKAVQIHQDTLRRTMYF 856
#7	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKDHDSAEGSHVSGQSNRDPQALAKAVQIHQDTLRRTMYF 859
#14	LQILTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSGEGSHISGQSNRDPQALAKAVQVHQDTLRRTMYF 852
#31	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	RLVAFRARYHLVDKEHDSGEGSHISGQSNRDPQALAKAVRQVHQDTLRRTMYF 860
#32	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSAEGSHISGQSNRDPQALAKAVQVHQDTLRRTMYF 858
#33	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	RLVAFRARYHLVDKDHDSAEGSHVSGQSNRDPQALAKAVQIHQDTLRRTMYF 856
#34	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSAEGSHVSGQSNRDPQALAKAVQIHQDTLRRTMYF 858
#38	LQLLTYQLCHTYVRC	TRSVSIPAPAYYA	HLVAFRARYHLVDKEHDSAEGSHVSGQSNRDPQALAKAVQIHQDTLRRTMYF 856

Ago1	A	857
Ago2	A	859
Ago3	A	860
Ago4	A	861
#1	A	851
#3	A	859
#5	A	857
#7	A	860
#14	A	853
#31	A	861
#32	A	859
#33	A	857
#34	A	859
#38	A	857

Supplementary Fig. 7 Alignments (generated using ClustalX2) of parental Ago and selected chimeric sequences.

(a) Chimeras tested in the luciferase and slicer assays in **Fig. 1e** and the tethering assay in **Supplementary Fig. 1h**. (b) The eight Ago2–Ago3 chimeras with the strongest Ago2-like phenotype (see **Fig. 1d,f**). (c) Ago1–4 chimeras tested in the luciferase assays in **Fig. 5c**. Regions identical in all sequences are marked in gray and unique positions in white. Residues constituting the catalytic tetrad are highlighted by red frames and an asterisk. Sequences encompassing motif I and motif II are framed in orange. The Ago domain structure is depicted above the alignment.

Supplementary Table 1 Template and primer combinations used to generate fragments for OE-PCRs

mutant	PCR fragment	template	forward primer	reverse primer
N ₃ P ₂ M ₂ P ₂	5'	Ago3	#459	#505
	3'	Ago2	#506	#517
N ₂ P ₃ M ₃ P ₃	5'	Ago2	#459	#505
	3'	Ago3	#506	#517
Ago3I ₂ II ₂	5'	Ago3II ₂	#459	#521
	3'	Ago3II ₂	#520	#517
Ago2II ₃	5'	Ago2	#459	#513
	middle	Ago3	#514	#505
	3'	Ago2	#506	#517
Ago3II ₂	5'	Ago3	#459	#515
	middle	Ago2	#516	#505
	3'	Ago3	#506	#517
Ago2I _{AAAAA}	5'	Ago2	#459	#523
	3'	Ago2	#522	#517
Ago2F44A	5'	Ago2	#459	#539
	3'	Ago2	#538	#517
Ago2E46A	5'	Ago2	#459	#541
	3'	Ago2	#540	#517
Ago2M47A	5'	Ago2	#459	#543
	3'	Ago2	#542	#517
Ago2D48A	5'	Ago2	#459	#545
	3'	Ago2	#544	#517
Ago3I ₂	5'	Ago3	#459	#521
	3'	Ago3	#520	#517
Ago3I _{Met} II ₂	5'	Ago3II ₂	#459	#581
	3'	Ago3II ₂	#580	#517
N ₁ P ₂ M ₂ P ₂	5'	Ago1	#459	#505
	3'	Ago2	#506	#517
N ₃ P ₂ M ₂ P ₂	5'	Ago3	#459	#505
	3'	Ago2	#506	#517
N ₄ P ₂ M ₂ P ₂	5'	Ago4	#459	#505
	3'	Ago2	#506	#517
N ₁ P ₃ M ₃ P ₃	5'	Ago1	#459	#505
	3'	Ago3	#506	#517
N ₂ P ₃ M ₃ P ₃	5'	Ago2	#459	#505
	3'	Ago3	#506	#517
N ₄ P ₃ M ₃ P ₃	5'	Ago4	#459	#505
	3'	Ago3	#506	#517
N ₁ P ₁ M ₁ P ₂	5'	Ago1	#459	#525

	3'	Ago2	#524	#517
N ₃ P ₃ M ₃ P ₂	5'	Ago3	#459	#525
	3'	Ago2	#524	#517
N ₄ P ₄ M ₄ P ₂	5'	Ago4	#459	#525
	3'	Ago2	#524	#517
N ₁ P ₁ M ₁ P ₃	5'	Ago1	#459	#525
	3'	Ago3	#524	#517
N ₂ P ₂ M ₂ P ₃	5'	Ago2	#459	#525
	3'	Ago3	#524	#517
N ₄ P ₄ M ₄ P ₃	5'	Ago4	#459	#525
	3'	Ago3	#524	#517
Ago1 _{DEDH}	5'	Ago1	#459	#500
	3'	Ago1	#499	#517
Ago4 _{DEDH}	5'	Ago4	#459	#502
	middle	Ago4	#501	#504
	3'	Ago4	#503	#517
N ₂ P ₁ M ₁ P ₁ _{DEDH}	5'	Ago2	#459	#505
	3'	Ago1 _{DEDH}	#506	#517
N ₂ P ₄ M ₄ P ₄ _{DEDH}	5'	Ago2	#459	#505
	3'	Ago4 _{DEDH}	#506	#517
N ₂ P ₂ M ₂ P ₁ _{DEDH}	5'	Ago2	#459	#525
	3'	Ago1 _{DEDH}	#524	#517
N ₂ P ₂ M ₂ P ₄ _{DEDH}	5'	Ago2	#459	#525
	3'	Ago4 _{DEDH}	#524	#517
Ago3I ₂ II _{loop2}	5'	Ago3I ₂	#459	#772
	3'	Ago3I ₂	#771	#517
Ago3II _{loop2}	5'	Ago3	#459	#772
	3'	Ago3	#771	#517
Ago2 _{DADH}	5'	Ago2	#459	#855
	3'	Ago2	#854	#517
Ago4 _{DEDH-10AA}	5'	Ago4 _{DEDH}	#459	#801
	3'	Ago4 _{DEDH}	#800	#517
N ₂ P ₂ M ₂ P ₄ _{DEDH-10AA}	5'	N ₂ P ₂ M ₂ P ₄ _{DEDH}	#459	#801
	3'	Ago4 _{DEDH}	#800	#517

Supplementary Table 2 Primers for cloning and shuffling reactions

#102	5' - TCGAGACAAACACCATTGTCACACTCCATCTAGAGC - 3'
#103	5' - GGCCGCTCTAGATGGAGTGTGACAATGGTGTTTGTC - 3'
#189	5' - CACCGCCTGACATCGAGGAGGATATTCAAGAGATATCCTCCTCGATGTCAGGC - 3'
#190	5' - AAAAGCCTGACATCGAGGAGGATATCTCTTGAATATCCTCCTCGATGTCAGGC - 3'
#459	5' - GACTACAAGGACGACGATGACAAG - 3'
#460	5' - CACTGAATTCTCATCAGGCGAAG - 3'
#499	5' - CCTGCCTACTACGCCATCTGGTGGCCTTCCGG - 3'
#500	5' - CCGGAAGGCCACCAGATGGGCGTAGTAGGCAGG - 3'
#501	5' - CATCTACTACAGAGACGGCGTGAGCGAGG - 3'
#502	5' - CCTCGCTCACGCCGTCTCTGTAGTAGATG - 3'
#503	5' - CCTGCCTACTACGCCATCTGGTGGCCTTCCGG - 3'
#504	5' - CCGGAAGGCCACCAGATGGGCGTAGTAGGCAGG - 3'
#505	5' - CATGCTGGGCAGGTGCCG - 3'
#506	5' - CGGCACCTGCCAGCATG - 3'
#513	5' - GCTCACGAACCTTGATGGACACCTTGAAGAT - 3'
#514	5' - AAGGTGTCCATCAAGTTCGTGAGC - 3'
#515	5' - CACCCACTTGATGGACACCTTAAAGG - 3'
#516	5' - TCAAGGTGTCCATCAAGTGGGTG - 3'
#517	5' - CAATCTTAGCGCAGAAGTCATGC - 3'
#520	5' - AACTTCTTCGAGATGGACATCCCCAAGATCGACGTG - 3'
#521	5' - GTCCATCTCGAAGAAGTTGGCCAGCAGCTTGATGG - 3'
#522	5' - GCCAACGCCGCCGCTGCCGCCATCCCCAAGATCGACATC - 3'
#523	5' - GATGGCGGCAGCGGCGGCGTTGGCCTGCAGCTTGAT - 3'
#524	5' - CAACCTGTGCCTGAAGATCAAC - 3'
#525	5' - GTTGATCTTCAGGCACAGGTTG - 3'
#538	5' - CAGGCCAACGCCTTCGAGATGGACATCCCCAAGATC - 3'
#539	5' - CATCTCGAAGGCGTTGGCCTGCAGCTTGATGGTCCG - 3'
#540	5' - AACTTCTTCGCCATGGACATCCCCAAGATCGACATC - 3'
#541	5' - GATGTCCATGGCGAAGAAGTTGGCCTGCAGCTTGAT - 3'
#542	5' - TTCTTCGAGGCCGACATCCCCAAGATCGACATCTAC - 3'
#543	5' - GGGGATGTGCGCCTCGAAGAAGTTGGCCTGCAGCTT - 3'
#544	5' - TTCGAGATGGCCATCCCCAAGATCGACATCTACCAC - 3'
#545	5' - CTTGGGGATGGCCATCTCGAAGAAGTTGGCCTGCAG - 3'
#580	5' - CTGGCCAACTGCTTCCAGATGGAGATCCCCAAG - 3'
#581	5' - CTT GGG GAT CTC CAT CTG GAA GCA GTT GGC CAG - 3'
#602	5' - GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGACTA CAAGGACGACGATGACAAG - 3'
#603	5' - GGGGACCACTTTGTACAAGAAAGCTGGTCCACTGAATTCTCATCAGGCGAAG - 3'
#614	5' - AATTTAGCGGCCGACGCGTGGTACCTCTAGGATATCGAATTCA - 3'
#615	5' - GGCCGCAATCAGGATCCATTACGG - 3'
#616	5' - GGCCGCAATCAGGATCCATTACGG - 3'
#617	5' - AATCCGTAATGGATCCTGATTGC - 3'

#631	5' - GGCCGCATCTTATAATGTGGCGCGCCATTTATATAG - 3'
#632	5' - AATTCTATATAAATGGCGCGCCACATTATAAGATGC - 3'
#771	5' - CCAGCGTGCCCTTCGAGACCATCCAG GCCGTGGACGTGGTGCTGC - 3'
#772	5' - ATGGTCTCGAAGGGCACGCTGGGCAG CCTGCCGGTCAGCACCTCGTGC - 3'
#800	5' - CAGACCAGCCGGCAGGAAGTGATCCAGGACCTGACC - 3'
#801	5' - GTCCTG GATCACTTCCTGCCGGCTGGTCTGCACC - 3'
#854	5' - GTGCAGCAGCACCGGCAGGCAATCATTAGGATCTGGCTG - 3'
#855	5' - CAGCCAGATCCTGAATGATTGCCTGCCGGTGCTGCTGCAC - 3'
#872	5' - TCGACTCGAGTGCTGAAGAACGAGCAGTAATTC - 3'
#873	5' - TCAATGTATCTTATCATGTCTGCTCG - 3'

Supplementary Table 3 Template and primer pairs used in PCRs for generation of DNA templates for *in vitro* transcription

<i>in vitro</i> transcript for	template (plasmid)	5' primer	3' primer
shRen1 and shRen2	psiCheck2	5' - TAATACGACTCACTATAG GCTTCCAAGGTGTACGAC - 3'	5' - TCACGGCGTTCTCGGCGTG - 3'
shRen3	psiCheck2	5' - TAATACGACTCACTATAG GGCTCATATCGCCTCCTGGAT C - 3'	5' - AACTCTCAGCATGGACG - 3'
shRen4	psiCheck2	5' - TAATACGACTCACTATAG GACGTGATCGAGTCCTGGGA C - 3'	5' - CTCGCCCTTCTCCTTGAATG - 3'
miR-122	psi2-miR-122perf	5' - GAAATTAATACGACTCAC TATAGGCCTCCACTTCAGCCA - 3'	5' - CCAACACACAGATGTAATG - 3'

Supplementary Table 4 DNA oligonucleotides used as probes

probe for	sequence
shRen1 antisense	5' - GCAACGCAAACGCATGATCAC - 3'
shRen1 sense	5' - GTGATCATGCGTTTGCCTTGC - 3'
shRen3 antisense	5' - GGCCTTTCACTACTCCTACGA - 3'
shRen3 sense	5' - TCGTAGGAGTAGTGAAAGGCC - 3'
shRen4 antisense	5' - GCCTGACATCGAGGAGGATAT - 3'
shRen4 sense	5' - ATATCCTCCTCGATGTCAGGC - 3'
miR-122	5' - CAAACACCATTGTCACACTCCA - 3'
miR-122*	5' - TATTTAGTGTGATAATGGCGTT - 3'
RNU6-1	5' - TGTGCTGCCGAAGCGAGCAC - 3'