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                                27
Ago1 M---EAGPSGAAAGAYLPPLQQVFQAPRRPGIGTVGKPIKLLANYFEVDIPKIDVYHYEV
Ago2 M-YSGAGPALAPPAPPPPIQGYAFKPPRPDFGTSGRTIKLOANFFEMDIPKIDIYHYEL
Ago3 MEIGSAGPAGAQP-----LLMVPRRPGYGTMGKPIKLLANCFQVEIPKIDVYLYEV
*   ***: * .           : * *. ** *:.*** ** *::*****:* **:

                                64                                100
Ago1 DIKPDKCPRRVNREVVEYMVQHFKPQIFGDRKPVYDGKKNIYVTALPIGNERVDFEVTI
Ago2 DIKPEKCPRRVNREIVEHMQHFKTQIFGDRKPVFDGRKNLYTAMPLPIGRDKVELEVTL
Ago3 DIKPDKCPRRVNREVVDSMVQHFKVTIFGDRRPVYDGKRSLYTANPLPVATTGVDLDVTL
****:*****:*. ***** *****:***:***:***. .**:. *::**:.

                                137                                151                                160
Ago1 PGE-GKDRIFKVSIKWLAIVSWRMLHEALVSGQIPVPLE-----SVQALDVAMRHLA 168
Ago2 PGE-GKDRIFKVSIKWVSCVSLQALHDALSGRLPSVPFE-----TIQALDVVMRHLA 170
Ago3 PEGGGKDRPFKVSIKFVSRVSWHLLHEVLTGRTLPEPLELDKPISTNPVHAVDVVLRHLA 171
*** *** *****: : * * : **:* . . *:* . . :*:**:*:****.

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Supplementary Figure 1: Sequence alignment of the human Ago1, Ago2 and Ago3 N-termini (amino acids 1-170). Positions of chimeric borders are indicated by amino acid numbering according to Ago2. Critical residues of cleavage-competent Ago3 (Mut5) are underlined.

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      ▼                                     ▼
      cluster 1
Ago1 588 PVIFLGADVTHPPAGDGKKPSITAVVGSMDAHPNRYCATVRVQ RPRQEIIEDLSY MVREL
Ago2 590 PVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVRVQ QHRQEIIQDLAAMVREL
Ago3 591 PVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVRVQ RPRQEIIQDLASMVREL
      *****:*****:*****: *****:**: *****

      ▼                                     ▼
      cluster 2
Ago1     LIQFYKSTRFKPTRIIFYRDGV PEGQLPQILHY ELLAIRDACIKLEKDYQPGITYIVVQK 707
Ago2     LIQFYKSTRFKPTRIIFYRDGV SEGQFQQVLHHELLAIREACIKLEKDYQPGITFIVVQK 709
Ago3     LIQFYKSTRFKPTRIIFYRDGV SEGQFRQVLYYELLAIREACISLEKDYQPGITYIVVQK 710
      *****:**: *:*:*:*****:**:*****:*****:*****

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Supplementary Figure 2: Alignment of partial PIWI domains of human Ago1, Ago2 and Ago3. Catalytic residues are indicated by black triangles. The two Ago1-specific clusters are boxed.

Supplementary Table 1: Primer sequences. Bold letters indicate Ago2 sequences.

construct	orientation	sequence
Ago1	fwd	5'-TAAT GCGGCCGC ATGGAAGCGGGACCCTCGGGA-3'
	rev	5'-CGTA GAATTC TCAAGCGAAGTACATGGTGCGC-3'
Ago2	fwd	5'-TAAT GCGGCCGC ATGTA CTCGGGAGCCGGCCC-3'
	rev	5'-CGTA GAATTC TCAAGCAA AGTACATGGTGCGCA-3'
Ago3	fwd	5'-TAAT GCGGCCGC ATGGAAATCGGCTCCGCAGGAC-3'
	rev	5'-CGTA GAATTC TTAAGCGAAGTACATTGTGCGTAAGG-3'
Ago3-2 PIWI	fwd/rev	5'- ACCTTCTGTGTTCCAGCAACCAGT CATCTTTCTGGGAGCAGACGTCA -3'
Ago2-3 PIWI	fwd/rev	5'- AGGCCGCCGGTGTTC CAGCAG CCAGTGATCTTTTTGGGAGCCGAT-3'
MutA	rev/fwd	5'-CCTCGCATGTCCATACTCCATG GACAGGGGT CGCAATAGCTTTATTC-3'
MutB	rev/fwd	5'-GTGGAAGTCTGATTGTCTGTTAGCTT TTAATA CATCTTTIGTCCTGCCACAATG-3'
MutC	rev/fwd	5'-CTTCACACATGAAGTGAATTACAGGTTG TGCCTTG AAAAACGCTGTTGCTG-3'
MutD	rev/fwd	5'-CAGGTGTGTATTCATGGAGGGCAG GTGCCT CATGACCACGTCCAGG-3'
Mut1	fwd	5'-AGACCTGGCTATGGCACCATGG-3'
	rev	5'- AGGTGGAGGCTGA AGGCATATCC-3'
Mut2	fwd	5'-CCTGTCCATGCCGTTGATGTGGT-3'
	rev	5'-TTCCAGTGGCTCAGGCAAGGTC-3'
Mut3	fwd	5'- CTGCC AGCGTCCCTTTTGAGACGATCCAG GCCGTTGATGTGGTGCTACGAC-3'
	rev	5'-CCGTCCTGTCAGTACTTCATGCAG-3'
Mut4	fwd	5'- CTGCC AGCGTCCCTTTTGAGACGATCCAG GCCGTTGATGTGGTGCTACGAC-3'
	rev	5'- CCGCCCTGAAAGTGCATCGTG TAACGCCTGCAAGCTCACGCA AGAGACAAATTTGATTGACACCTTGAAAGGTC-3'
Mut5	rev/fwd	5'-CCCTGTTCACTCTCCTAGGACACTT CTCTGGCTTGATATCCAATTCATAATGATAG -3'
Mut6	rev/fwd	5'-CACAGGAAGTGGATTGGCGGTGTA TAGATTCTTCTGCCGTCAAACACG -3'
Ago1 DEDH	fwd	5'-CCTGCCTACTATGCCACCTGGTGGC-3' and reverse complement
Ago2-1 PIWI	fwd/rev	5'- AGGCCGCCGGTGTTC CAGCAG CCAGTGATATTCCTGGGAGCAGAT-3'
Ago2-1 PIWI DEDH c11	fwd	5'- ATTCAA AGACTTGGCCGCC ATGGTGCCTGAGCTCCTCATCC-3'
	rev	5'- GATCTCTT GCCGATGTTG CTGTACCCGCACAGTAGCACAG-3'
Ago(2-)1 PIWI DEDH c12	fwd	5'-GTGTCTGAAGGCCAG TTCCAGCAGG TACTCCACCAT GAGCTACTGGCCATTC-3' and reverse complement
Ago1 N	rev/fwd	5'-GGGGTGTACCTCATGGATGCCA GGTGCCT CATGACCACGTCCAGG-3'
Ago1 N(1-64)	rev/fwd	5'-CCGTTGACTCTACGGGGACACTT CTCTGGCTTGATATCCAATTCATAATGATAG -3'