

SUPPORTING INFORMATION

Distinct roles of Argonaute in the green alga *Chlamydomonas* reveal evolutionary conserved mode of miRNA-mediated gene expression.

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Supplementary Figure 1: Structural features of *Chlamydomonas Argonautes*

- (a) Cr-AGO protein sequences were extracted from Phytozome V5. GR-rich domains are highlighted in pink, N-terminal domains in cyan, L1 in orange, PAZ in purple, L2 in yellow, MID in green and PIWI in grey. All domains are defined by threading using the crystalised Human AGO2 as template – PDB:4W5T³².
- (b) Modelling of Cr-AGO MID domain structure to At-AGO1 MID domain. Homology model of the MID domain of all Cr-AGOs, superimposed in the crystal structure of the At-AGO1 MID domain complexed with UMP. Only the modelled UMP is shown for the Cr-AGO model for clarity. The protein side chain corresponding to residue N687 in At-AGO1 is shown as sticks in magenta and the two surrounding residues are coloured in red. The highly conserved 5' phosphate recognition residues are shown as sticks in yellow. The At-AGO1 structures were derived from PDB:4G0P, where the UMP was modelled into crystallized At-AGO2 and At-AGO5 structures (PDB:4G0M and 4G0O respectively).
- (c) Tandem tryptophan-binding pockets in the PIWI domain. Closed-up view of surface representation showing the tryptophan-binding pockets in human AGO2, *Arabidopsis* AGO1, 4, 6 and 9 and *Chlamydomonas* AGO 1, 2 and 3. Residues forming the binding pockets, that are identical or have conservative changes relative to human AGO2, are highlighted in blue. Residues showing non-conservative changes are highlighted in magenta. All structures are models based on the human AGO2 crystal structure (PDB:4W5O). Bottom panel is the thirty degree anti-clockwise rotated view for clearer representation of pocket 1.
- (d) Homology modelling of Cr-AGO2 complexed with miRNA:grRNA duplex (Phyre 2). The α -carbon for non-conservative and conservative substitutions are indicated with red and yellow spheres respectively. Many of the non-conservative differences are located near loop regions, except within the MID domain.
- (e) Alignment of Cr-AGO2 and Cr-AGO3. The GR-rich domains are highlighted in pink, N-terminal domain in cyan, L1 in orange, PAZ in purple, L2 in yellow, MID in green and PIWI in grey. The alignment was performed using ClustalOmega.
- (f) Figure 1f in higher resolution.
- (g) Annotation of GR-N extensions for *Arabidopsis* Argonautes. At-AGO protein sequences were extracted from TAIR 10. Glycine and arginine residues are highlighted in yellow and red, respectively.
- (h) As in panel (b) except that only species containing germline-specific PIWI proteins are shown. Species with or without N-terminal GR-N domain are highlighted in blue and green, respectively.
- (i) Length distribution of AGO and PIWI G/AR-rich domains.

Supplementary Figure 2:

- (a) Mapping of their corresponding insertional mutagens by RESDA-PCR localized the insertions into Cre16.g689647 (Ago3)²⁶.
- (b) *ago3-25* complemented with Ago3 genomic DNA display the light-dependent lethal phenotype under nitrate induction of artificial miRNA against the PSY gene²⁶.
- (c) Enlarged version of Figure 2b with corresponding Ponceau stain.

(d) Immunoprecipitated Cr-AGO2/3 with α -2/3-PAZ (i.e. in the parental background) in a gradient of NaCl concentrations (0.15M, 0.3M, 0.5M and 0.8M). The western blot was probed with the antibody α -2/3-PIWI and the resulting purified protein at ~110kDA was used for LC-MS-MS.

(e) Peptides detected by mass spectrometry confirming the presence of Cr-AGO2 in the α -2/3-PAZ IP product. Sequences in red are peptide matches that correspond to Cr-AGO2 protein sequence.

(f) Peptides detected by mass spectrometry confirming the presence of Cr-AGO3 in the α -2/3-PAZ IP product. Sequences in red are peptide matches that correspond to Cr-AGO3 protein sequence.

(g) Independent repeat for differential localisation of Cr-AGO2 and 3. Total protein isolated from cytoplasmic and nuclear fractions are indicated as C and N, respectively. Extracts from either Parental or *ago3-25* strain are labelled P and M, respectively. Protein bands corresponding to AGO2/3 are indicated.

(h) Enrichment of Cr-AGO3 in purified polysome fraction from either *ago3-25* or respective parental strain. Anti-histone3 antibody (Agrisera) and Anti-ribosomal protein S3 (Abcam) were utilised to validate nuclear or cytoplasmic extraction.

(h) Figure 3a with corresponding Ponceau stain to reflect total protein loading.

(i) Histogram of 5' end positions of normalized RPF (colored, left-axis) and RNA-Seq (grey, right-axis) 27-nt reads mapped to Cr-AGO2 and 3 transcripts, respectively, in all biological replicates. The red horizontal line indicates the CDS. Data derived from pervious work ¹

Supplementary Figure 3:

(a) Histone 3 Western blot of either total protein (T) or protein extracted from the supernatant (S) used for Cr-AGO2/3-IP.

(b) Small RNA northern blot for miR-C89 and miR-1157 in the wild-type cc-1883, two independent Parental lines, *ago3-25*, two independent lines of *ago3-25* complements and *dcl3-1*. U6 was utilised as loading control.

(c) Small RNA Northern blot for miR-1162, 1157, 9897 in two independent *ago3-25* complement lines, biological replicates of *ago3-25* and the parental line. U6 was utilised as loading control.

(d) Volcano plot for all differentially expressed mRNAs between parental and *ago3-25* in duplicates. *y-axis* represents significance of differential expression using DE-Seq2. All grey dots represents a single mRNA while AGO3 is highlighted in blue, genes containing miRNA precursor within 3'UTR exons or introns in black and green, respectively and Cre05.g239950, the mRNA that also codes for miR-C89 in red. Horizontal dotted-line indicates significance of 0.01.

Supplementary Table 1:

Normalised siRNA abundance.

Supplementary Table 2:

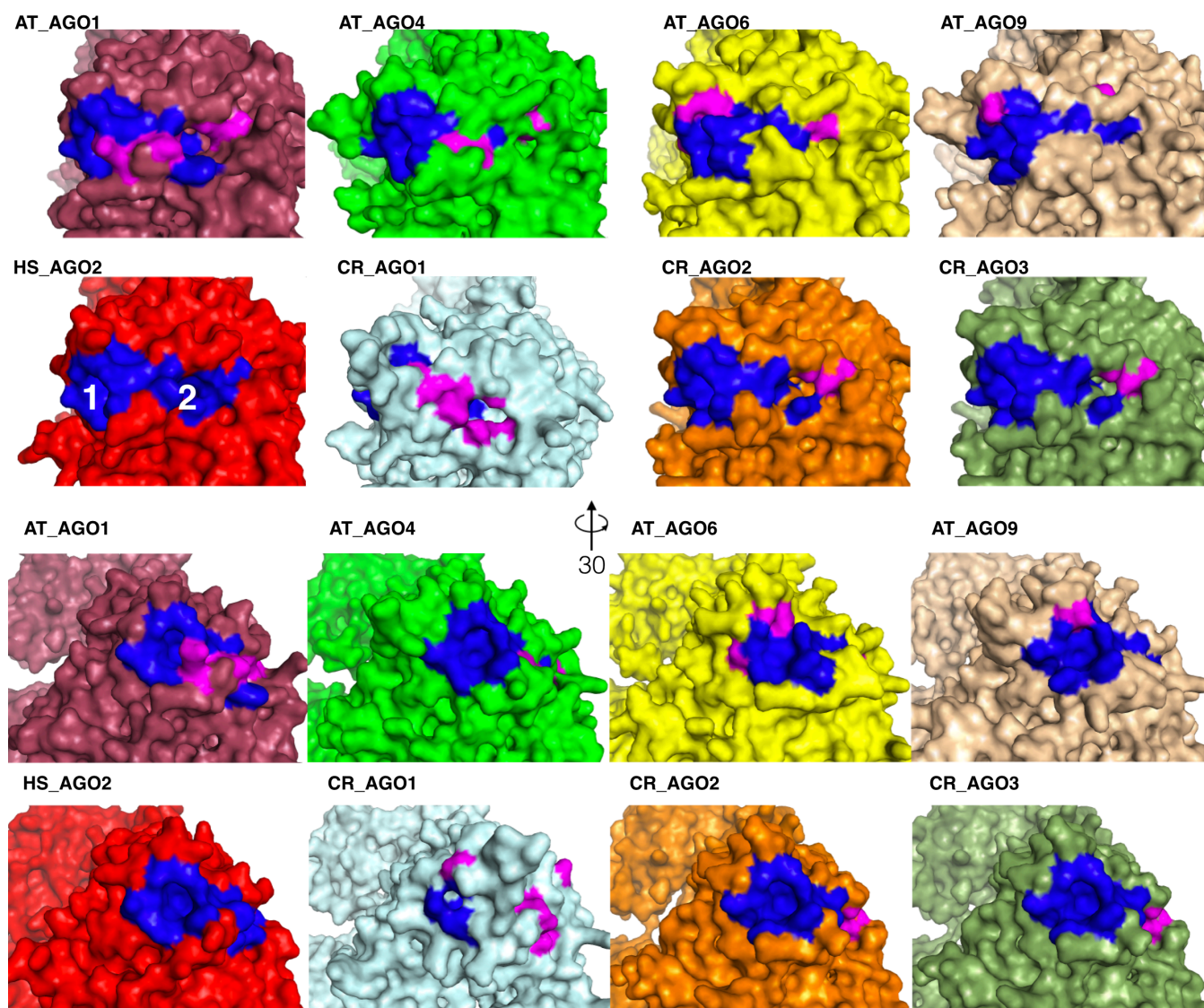
Normalised miRNA abundance (Total RNA).

Supplementary Table 3:

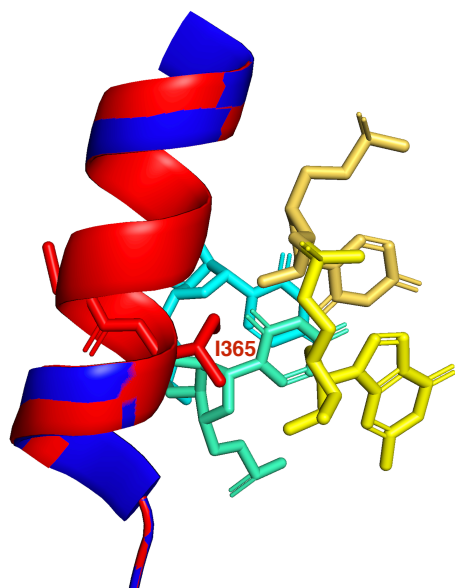
Normalised miRNA abundance (AGO-IP).

Cr-AGO3

(c)



(d)

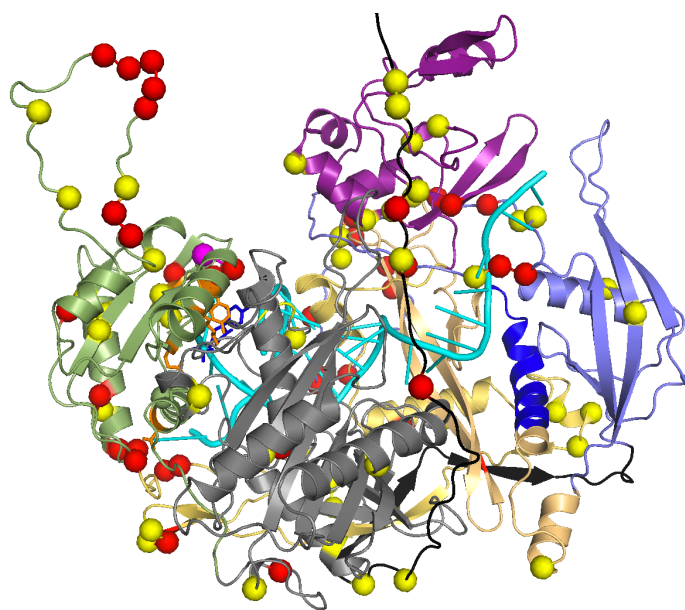


Cr-AGO2 / 3
HS-AGO2

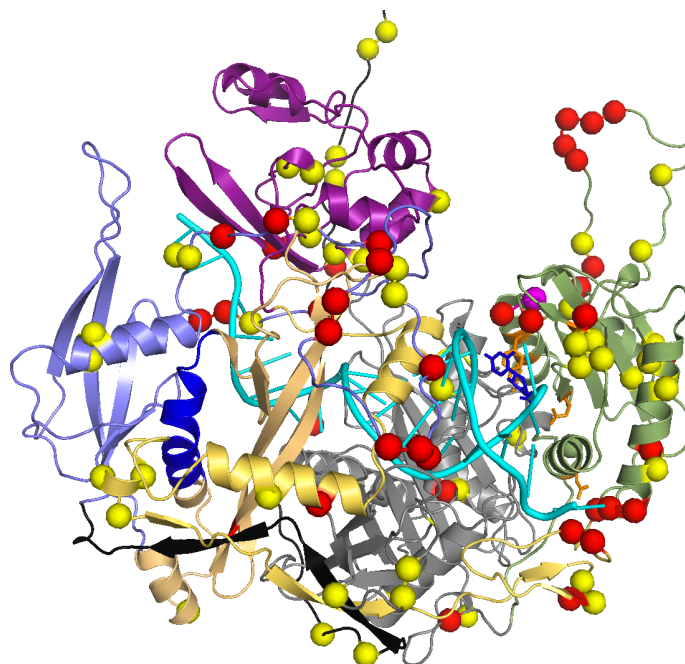
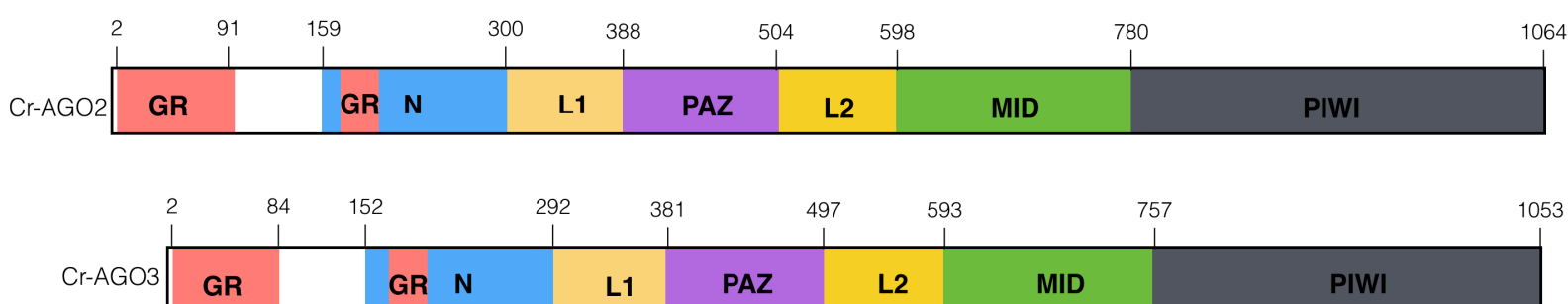
DATQSAGMITAAKQ
TDNQTSTMIRATAR
. * : : * * * : :
I365

(e)

 conservative mutation
 non-conservative mutation




180



(f)

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CR_AG02  MSGRGRGGGGGYGGG---YGGGGGGSRGGGGGGSGGGDRGYGGGGG--GRGGGGYGGGSAG
CR_AG03  MSGRGRGGGGGYGGG---YGGGGGGSRGGGGGGDRGG---GYYGGGGRGGGGGGYGGG---
*****
*****

CR_AG02  GGGYGGGGGRGGGGGGGYGGGG--GGGGGGGGGGVVSPQAAATLDVVLKSARTLTAKV
CR_AG03  ---GGGGGR--GGGGGGGYGGGGGGGGGGGGGGVVSPQAAASLDAILKSAKSLTAPV
*****
*****

CR_AG02  NVETTPDGRPLGITRRPNAGTVGKAVALLANYPALATTPAFPGQAYHYDVEIRSVEEAAG
CR_AG03  KVETTPDGRPLGITRRPSAGSVGKAVALLANYPALATTPAFPGQAYHYDVEIRSVETPGG
:****
*****

CR_AG02  GGGGGRGGRGGGGGGGGGGGGRRDLAPASATGDV-PGGAAAAAAEDLPPRLAHRVMA
CR_AG03  G-GGGRGGRGGGGGGGGGGGGRRDLAPASATGDV-PGGAAAAAAEDLPPRLAHRVMA
*
*****

CR_AG02  AAAAAHGWPAGAWRFDGRKNLFLPGELLPREVREWPVTLKPREGDKSER-KGFVVATKWA
CR_AG03  SAAAAHGWPAGAWRFDGRKNLFLPGELLPREVREWPVTLKPREGDKSER-KGFVVTTKWA
:
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CR_AG02  ACVGLTQLQDYLAQRQQTAPRDAMQVLDIVIRHAFIDPRCTVVGRGFYYGGEGVMPLGG
CR_AG03  ACVGLTQLQDYLAQRQQTAPRDAMQVLDIVIRHAFIDPRCTVVGRGFYYGGEGVMPLGG
*****
*****

CR_AG02  GAEVWSGFQQSFKAVQAGLMLNLDSSFAA-MSARPLPELLAEGAGVRGGPAQLAAADPRR
CR_AG03  GAEVWSGFQQSFKAVQAGLMLNLDSSFAA-MSARPLPELLAEGAGVRGGPAQLAAADPHR
*****
*****

CR_AG02  LRAAARSLVGFKVEFPMPPGGRARRKMLTGLSEQGADRTMFMNEKEGREMSVAEYFRSTGR
CR_AG03  LRAAARSLVGFKVEFPMPPGGRARRKMLTGLSEQGADRTMFMNEKEGREMSVAEYFRSTGR
*****
*****

CR_AG02  PLRHPGLPCANVGDRRAVYIPLELCTVVAGQRRMKLDATQSAGMITAAKQDPAVKAEAC
CR_AG03  PLRHPGLPCANVGDRRAVYIPLELCTVVAGQRRMKLDATQSAGMITAAKQDPAVKKEAC
*****
*****

CR_AG02  NKQAKRVAEALAAGGTERSGLKLTGMLPVQGRVLPNPVLQYGNRQDFDAGPLGSWNTL
CR_AG03  DKQAKRVAEALAAGGTERSGLKLTGMLPVQGRVLPNPVLQYGNRQDFDAGPLGSWNL
:
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CR_AG02  NVKFVDARALDSWAVAVMMNQADVDFDGDNSIWKFLEDLCSAMITRGMRVASPVTRGSND
CR_AG03  NVKFVDARALDSWAVAVMMNQADVDFDGDNSIWKFLEDLCSAMITRGMRVASPVTRGSND
*****
*****

CR_AG02  SPPVEYGGAAAGGGAAGRGGGGARGVEATLRAAADAAAARYKKPAQLVLVVLVPKPSDEYR
CR_AG03  SPPVEYGGAAAGGGAAGRGGGGARGVEATLRAAADAAAARYKKPAQLVLVVLVPKPSDEYR
*****
*****

CR_AG02  EVKRVSDIELGIPSQVVVGKARVGYREQRGGGPQYCANVAMKINNKLGGVNVQLSGGLR
CR_AG03  EVKRVSDIELGIPSQVVVGKARVGYREQRGGGPQYCANVAMKINNKLGGVNVQLSGGLR
*****
*****

CR_AG02  YMPVLGGAGSVPFMVLGADVTHPTGAAARADSRDPSVAADVGSLDASLGRWASRVLLQAG
CR_AG03  YMPVLGGAGSVPFMVLGADVTHPTGAAARADSRDPSVAADVGSLDASLGRWASRVLLQAG
*****
*****

CR_AG02  RQEVITGMCGATKELLEFYRANKQVKPQRLVMYRDGVSEGQFEQVLAEEFTALRRACRE
CR_AG03  RQEVITGMCGATKELLEFYRANKQVKPQRLVMYRDGVSEGQFEQVLAEEFTALRRACRE
*****
*****

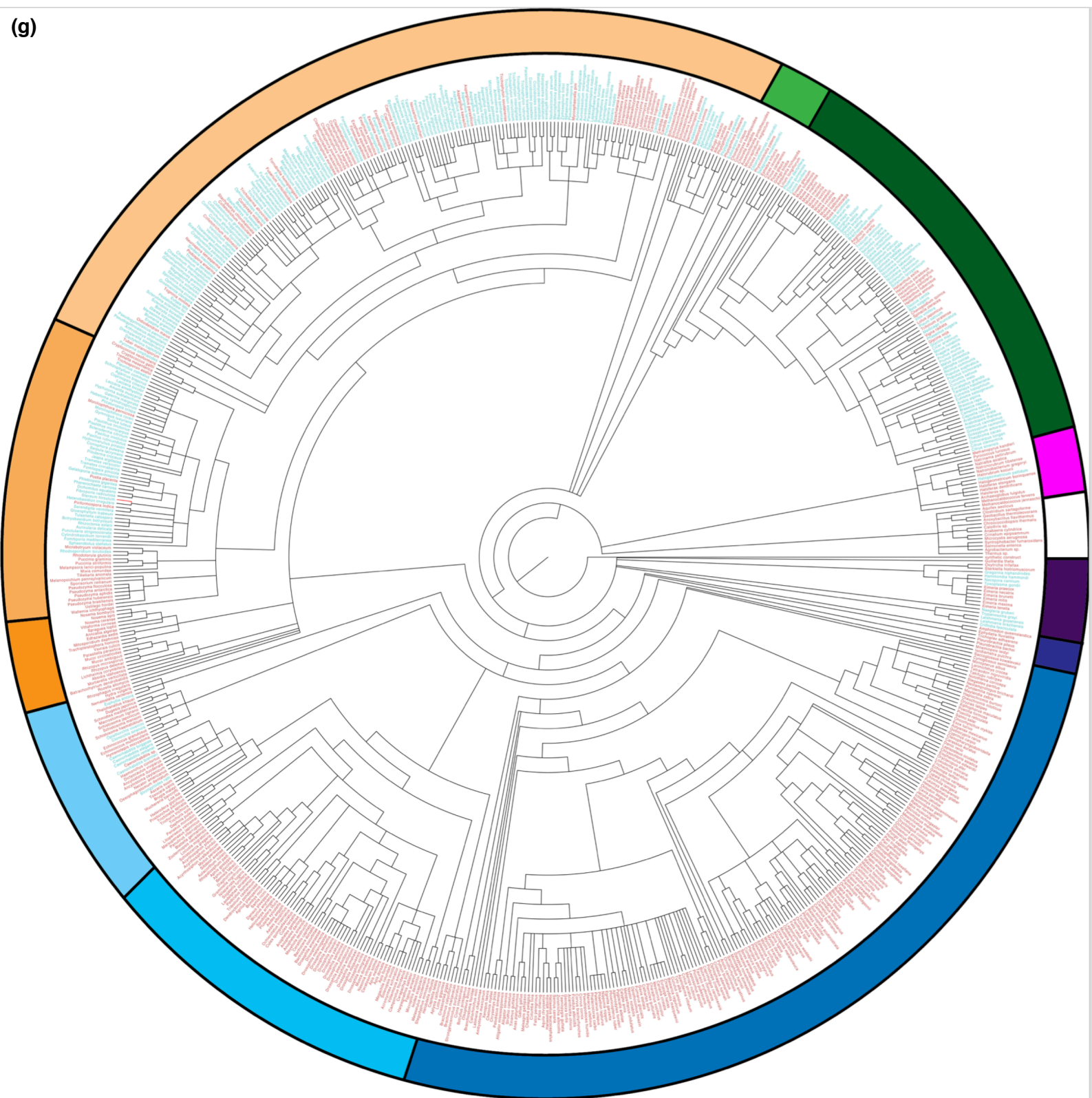
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CR_AG03  LEEGYRPAITFVVVQKRHNTRLLPNDRASADPKGNVVPGTVVDSGITAPDGFDFYLNSHA
*****
*****

CR_AG02  GLQGTNKPAPHYHVLVDEIGFGADGMQLLTYWLCYLYQRTTKSVSYCPAYYADRAAFRGR
CR_AG03  GLQGTNKPAPHYHVLVDEIGFGADGMQLLTYWLCYLYQRTTKSVSYCPAYYADRAAFRGR
*****
*****

CR_AG02  TLLAASSSASDSASESGSRAGRGAGAAEGGASAPPTFAGIHRNLSNVLYFM
CR_AG03  TLLAASSSASDSASESGSRAGRGAGAAEGGASAPPTFAGIHRNLTNVLYFM
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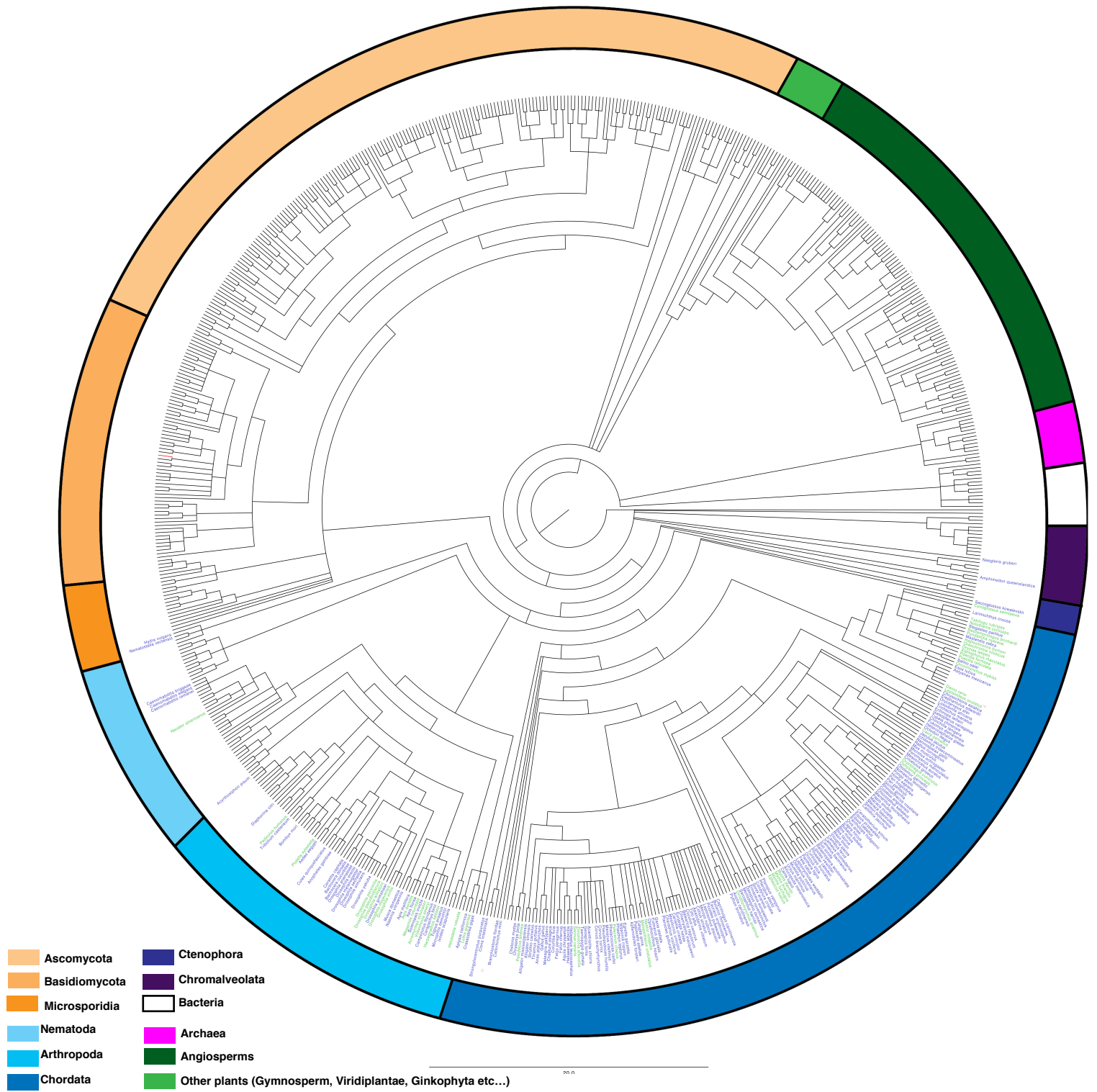
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(g)

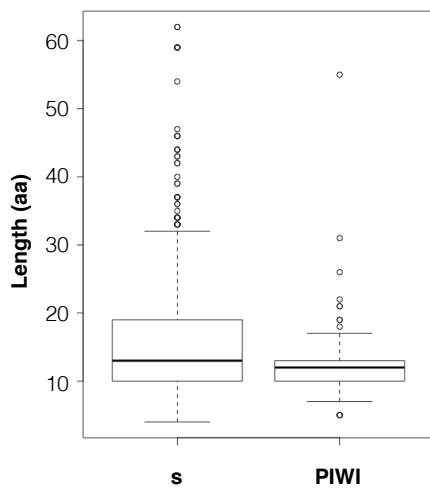


- | | | | |
|---------------|----------------|------------|---|
| Ascomycota | Ctenophora | Nematoda | Archaea |
| Basidiomycota | Chromalveolata | Arthropoda | Angiosperms |
| Microsporidia | Bacteria | Chordata | Other plants (Gymnosperm, Viridiplantae, Ginkophyta etc...) |

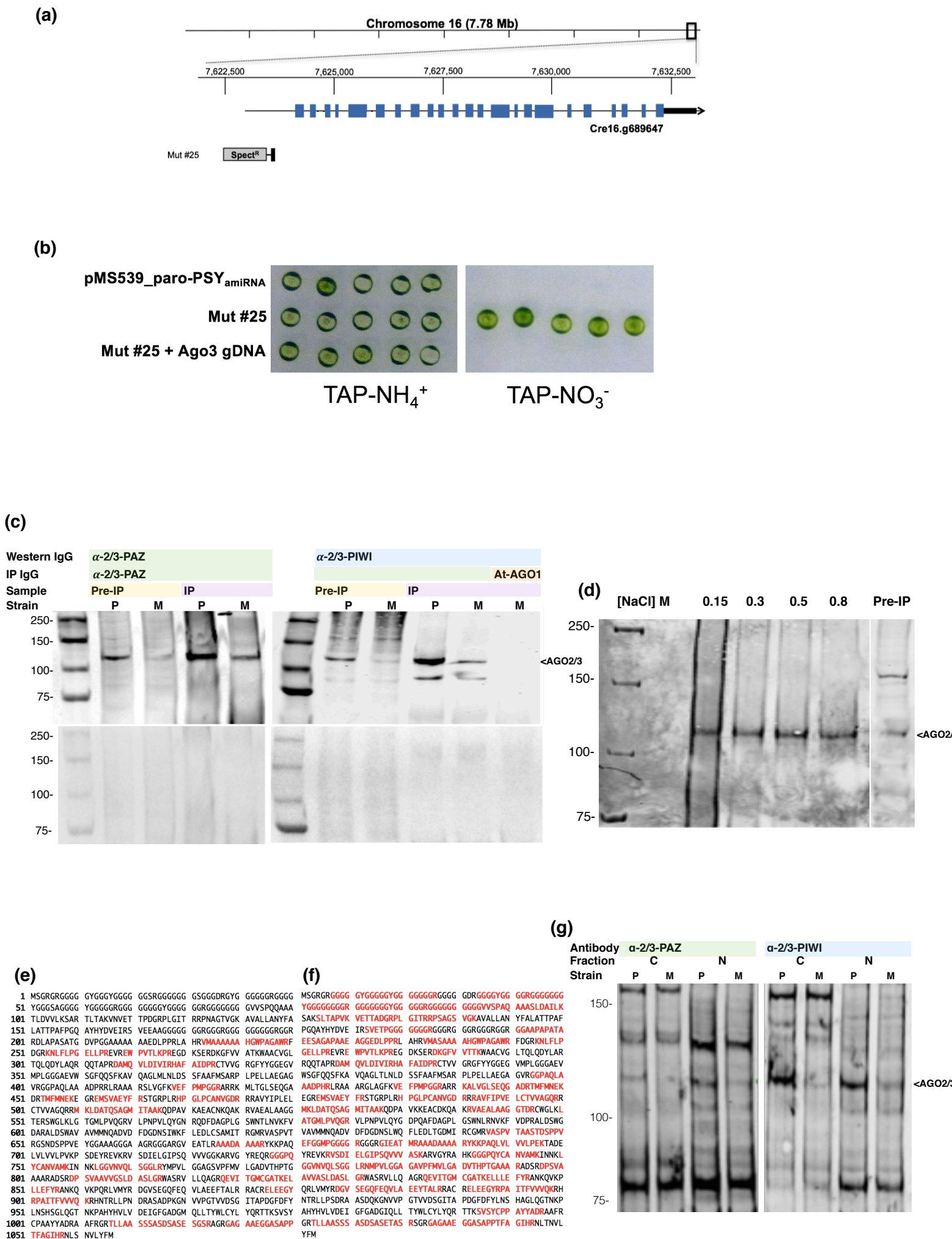
(i)



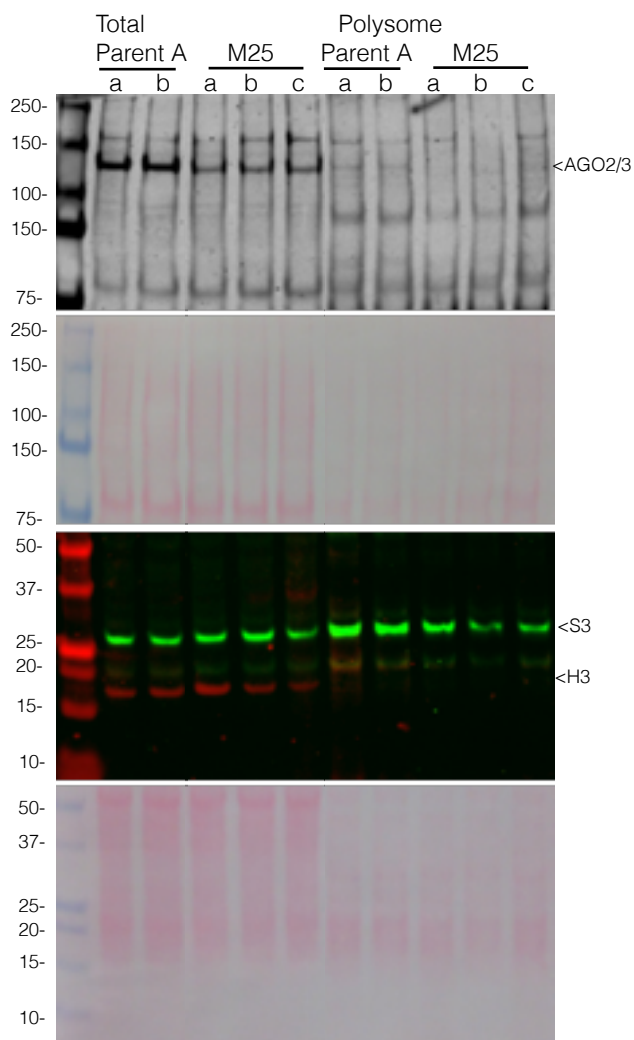
(j)



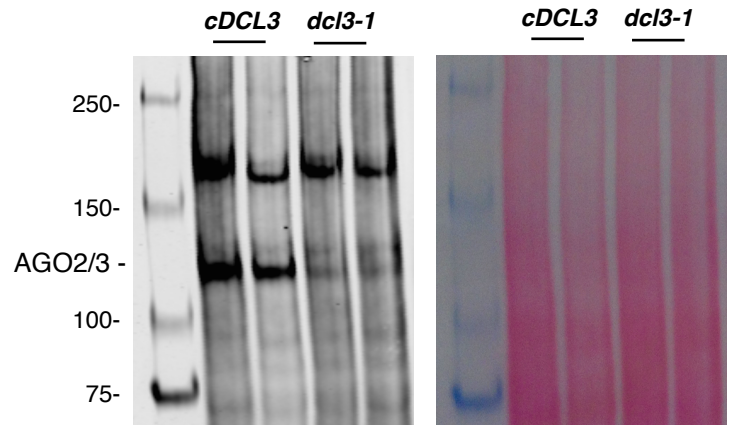
Supplementary Figure 2



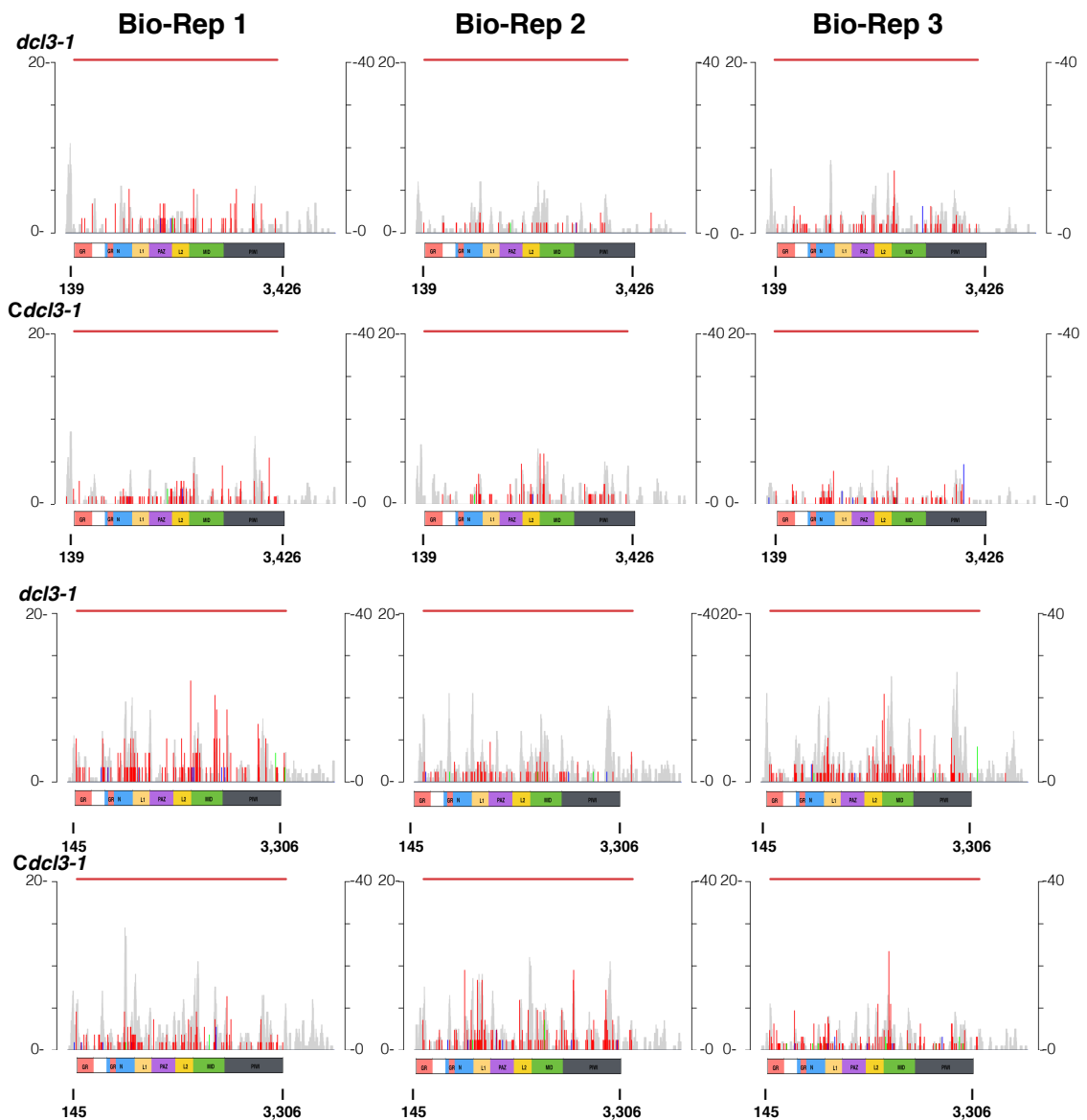
(h)



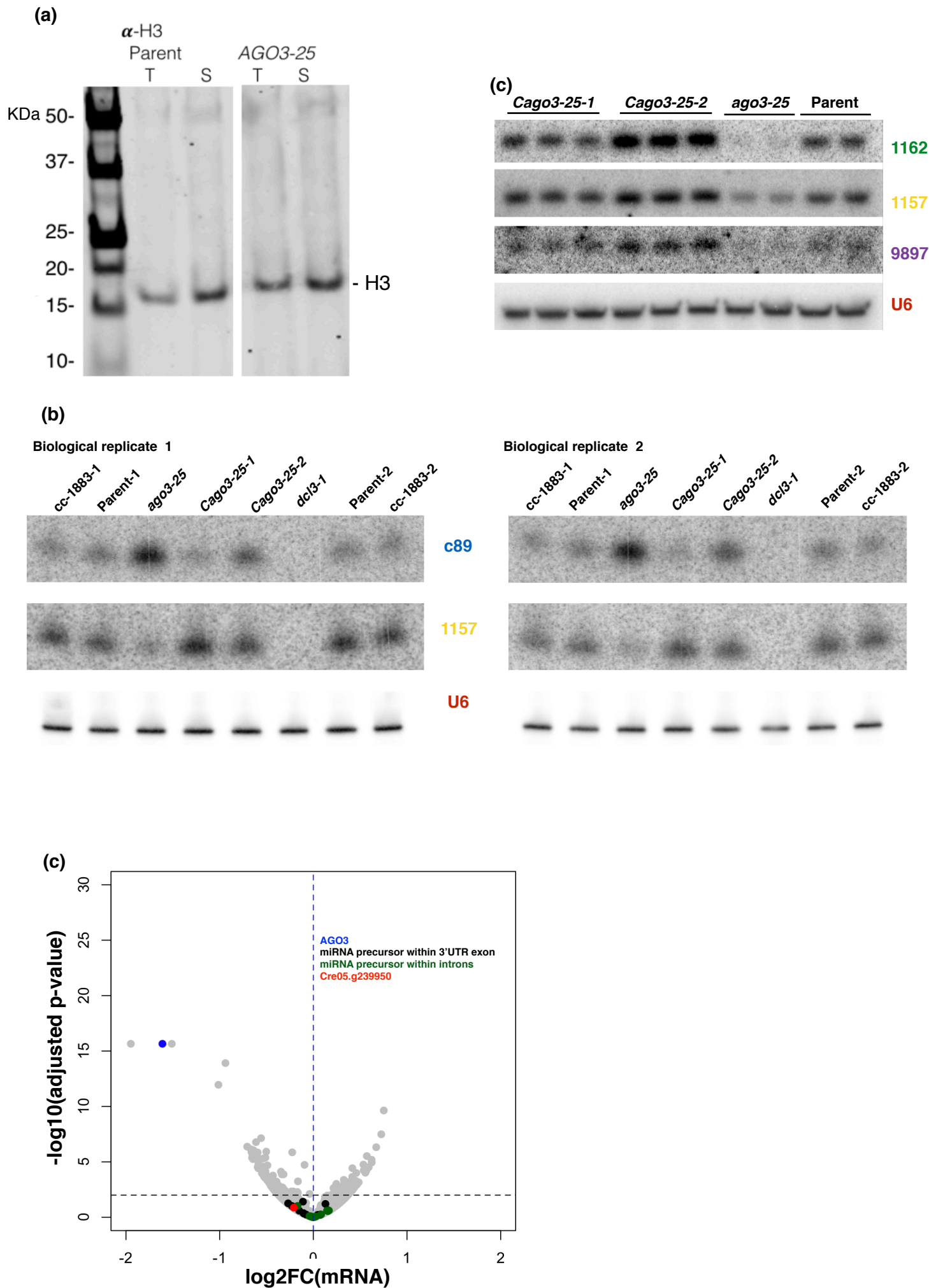
(i)



(j) Cr-AGO2



Supplementary Figure 3:



Supplementary table 1: Normalised siRNA abundance

	Parent-1	Parent-2	Parent-3	AGO3-25-1	AGO3-25-2	AGO-3-25-3
chromosome_4_741459_742332	0.225254615	0.385155893	0.357669483	0.113589486	0.091178661	0.058826061
chromosome_16_6192827_6193080	0.08787003	0.050387576	0.049798413	0.069023079	0.059133533	0.061418595
chromosome_13_4823025_4823624	0.088036272	0.058830454	0.056438984	0.02991756	0.059852016	0.115443414
chromosome_7_2881984_2882520	0.07019275	0.043011013	0.03956098	0.028001714	0.012474047	0.02965377
chromosome_17_3465102_3465976	0.033949784	0.026081158	0.021870839	0.024462116	0.024714487	0.02570224
chromosome_8_347555_348048	0.008299288	0.014461389	0.016135801	0.023423156	0.079165775	0.022127078
chromosome_1_2125447_2125697	0.026821677	0.018962975	0.02513608	0.022597673	0.018240576	0.028326648
chromosome_17_5152761_5152938	0.020906978	0.02243839	0.017035468	0.017913888	0.015943854	0.0186455
chromosome_5_910267_910743	0.032750808	0.024551229	0.022863	0.014792777	0.01049267	0.01616418
chromosome_13_791487_791911	0.030753227	0.026548165	0.021174207	0.014154766	0.018693664	0.017470281
chromosome_12_6166769_6167265	0.017742493	0.014539977	0.01194653	0.013537317	0.014355523	0.017041695
chromosome_14_1191493_1191841	0.016124971	0.012332719	0.009841742	0.012331446	0.010614267	0.01776595
chromosome_3_6440818_6442758	0.017448496	0.012697391	0.010178779	0.010953827	0.010073892	0.014071436
chromosome_13_2348229_2348355	0.017415889	0.012691172	0.010940834	0.010699832	0.010854939	0.012806702
scaffold_21_122657_123081	0.02680564	0.019513094	0.017529969	0.00992031	0.019663413	0.013061683
chromosome_3_8398694_8400260	0.001768262	0.00251087	0.002894816	0.009542946	0.002299245	0.004003747
chromosome_11_2205569_2205928	0.008104714	0.005369451	0.005207601	0.008776124	0.00949517	0.012134664
chromosome_15_112650_114047	0.023366402	0.011217216	0.010259542	0.008407832	0.006647982	0.008756163
chromosome_17_195871_195937	0.016894711	0.018972021	0.015232976	0.00778494	0.009807993	0.010142284
chromosome_17_194526_194857	0.014641619	0.010025387	0.007875921	0.007564811	0.00809756	0.009877809
chromosome_3_6838785_6839658	0.007270783	0.012318584	0.010044325	0.007498893	0.018547848	0.007391742
chromosome_11_1337240_1337592	0.006121566	0.004089422	0.004011504	0.00673449	0.005540489	0.009338008
chromosome_9_893836_894970	0.00748037	0.006768776	0.006121254	0.006491985	0.00568025	0.007174737
chromosome_4_619389_619451	0.003793639	0.001856155	0.002986407	0.006442396	0.004068205	0.008076665
chromosome_5_935248_935504	0.012154399	0.00962453	0.008412833	0.006355312	0.005388114	0.008634097
scaffold_21_46903_47707	0.006196402	0.010474302	0.008220627	0.005608446	0.013351968	0.005642815
chromosome_8_448814_450085	0.007377204	0.004277694	0.003543623	0.005486287	0.004015732	0.005905934
chromosome_3_4522839_4523210	0.008409403	0.006799872	0.005589305	0.005288534	0.00480132	0.006388093
chromosome_4_3389815_3390660	0.00339113	0.003251523	0.002389938	0.004616657	0.010065314	0.006801081
chromosome_17_7004995_7005721	0.004782006	0.004313314	0.003764253	0.003915148	0.003837625	0.004635775
chromosome_4_457766_457962	0.00294853	0.001291902	0.001608933	0.003626683	0.003470815	0.004390966
chromosome_11_427007_427373	0.007438676	0.005614827	0.005307764	0.003595236	0.005403251	0.00326932
chromosome_1_1695652_1695774	0.008485842	0.00678913	0.005294228	0.003545646	0.006720637	0.003355444
chromosome_5_2294486_2295191	0.000126686	0.000149827	7.35435E-05	0.00351178	0.012746001	0.000111893
chromosome_11_1857326_1857429	0.008978689	0.008433266	0.006478143	0.003376921	0.00310451	0.003328318
chromosome_7_3850572_3850929	0.009181815	0.007827174	0.006020188	0.003372688	0.003210466	0.003347306
chromosome_13_2301408_2301719	0.013716863	0.017046324	0.014358576	0.002851394	0.003075751	0.006004943
chromosome_14_1950078_1950361	0.006959193	0.007002845	0.005650215	0.002823575	0.004836639	0.004779541
chromosome_2_9207643_9207901	0.002606423	0.004505544	0.00310552	0.00237727	0.001410728	0.002451481
chromosome_11_422391_422757	0.003780276	0.002453201	0.002446787	0.001777359	0.001704882	0.001620758
scaffold_19_167877_168751	0.000545232	0.001031826	0.000887485	0.001724141	0.004582344	0.001501405
chromosome_11_433372_433738	0.002969377	0.001970363	0.002100276	0.001696927	0.001436965	0.00150683
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chromosome_6_4030753_4031503	0.00343122	0.002458289	0.001875584	0.001601377	0.001643327	0.002151743
chromosome_6_4087982_4088721	0.003264978	0.002386485	0.001865207	0.001516712	0.001578744	0.002125295
chromosome_5_2986270_2986667	0.003025503	0.002513131	0.002249167	0.001504617	0.001889548	0.001750961
chromosome_10_778202_778490	5.55923E-05	4.63615E-05	3.02295E-05	0.001465913	0.004545512	4.54355E-05
chromosome_9_6365908_6366037	0.002865141	0.002125278	0.002013648	0.001396971	0.002416301	0.001915072
chromosome_3_6582826_6583191	0.00214458	0.001791702	0.001581862	0.001387295	0.000898608	0.001310169
chromosome_2_476786_476830	0.001816906	0.002244574	0.001449213	0.001314725	0.001649382	0.001514968
chromosome_7_4783768_4794684	0.000559665	0.000977549	0.000752129	0.001269369	0.001051487	0.003404948
chromosome_10_2431974_2432405	0.002729902	0.002094182	0.001729851	0.001250017	0.001241199	0.001542772
chromosome_16_1558354_1558399	0.001331542	0.00091253	0.000879363	0.001150233	0.000682155	0.001087061
chromosome_9_3000600_3006834	0.00036723	0.000539942	0.000413287	0.001048031	0.000804761	0.002389092
chromosome_10_2432800_2433146	0.00153253	0.001083841	0.001013817	0.000961552	0.000810311	0.001219298
chromosome_16_7701450_7707096	0.000504607	0.000919314	0.000628504	0.000940385	0.000748756	0.002903124
chromosome_8_186488_192134	0.000470396	0.000892176	0.000630308	0.000931919	0.000784074	0.002899055
chromosome_4_2915643_2921289	0.00049552	0.000842422	0.000624894	0.000910148	0.000782056	0.002789196
chromosome_9_6156833_6158503	0.001972992	0.001355225	0.001264226	0.000908334	0.001043919	0.001415959
chromosome_6_2556368_2562014	0.000489105	0.000875214	0.000606847	0.000891401	0.000786093	0.002805471
chromosome_1_6561109_6566755	0.000482691	0.000885957	0.000620833	0.000884748	0.000800725	0.00290448
chromosome_4_585019_585290	0.000656417	0.000408773	0.000543229	0.000878096	0.000768433	0.001137921
chromosome_9_6518825_6519075	0.005775184	0.004490279	0.003245839	0.00086842	0.001334541	0.001150806
chromosome_7_4352836_4361019	0.000359746	0.00061118	0.000465174	0.000829112	0.000645827	0.002333485
chromosome_2_1328804_1334369	0.000458636	0.000793799	0.000566691	0.000823064	0.000668532	0.00266713
chromosome_9_5020215_5020525	0.00040732	0.001520883	0.002842478	0.000814598	0.001851203	0.001280331
chromosome_1_3243522_3249183	0.000440997	0.000830549	0.000580227	0.000803107	0.000713437	0.002494204
chromosome_7_860854_865923	0.000452222	0.000775141	0.000571654	0.00079706	0.000684173	0.002704428
chromosome_1_1124614_1125494	0.00182332	0.002329947	0.001468614	0.000790408	0.001632731	0.002151743

Supplementary table 1: cont.

	Parent-1	Parent-2	Parent-3	AGO3-25-1	AGO3-25-2	AGO-3-25-3
chromosome_11_1367777_1373430	0.000437255	0.000800018	0.000579324	0.000762589	0.000742196	0.002149708
chromosome_7_4518519_4524172	0.000464516	0.000783622	0.000571203	0.000737794	0.000711923	0.002189041
chromosome_12_2358008_2363661	0.000438858	0.000726518	0.000594664	0.000728723	0.000734628	0.002144283
chromosome_4_3690316_3690707	0.000930636	0.00090631	0.000787773	0.000726304	0.000477811	0.000725611
chromosome_15_109180_110407	0.002479202	0.001192395	0.000936664	0.000717838	0.000973786	0.000994155
chromosome_5_1883558_1889064	0.000380059	0.000661499	0.000499013	0.000699695	0.000567621	0.002093423
chromosome_2_8282593_8282661	0.000929033	0.000785318	0.00066505	0.000698486	0.000700823	0.000726968
chromosome_12_6402227_6402312	0.00111238	0.00088426	0.000717387	0.000679738	0.001057542	0.000752059
chromosome_9_6821854_6827511	0.000289721	0.000461353	0.000397947	0.000675505	0.000492443	0.001980173
scaffold_42_5629_10706	0.000393422	0.000683549	0.000488636	0.000668248	0.000669541	0.002399265
chromosome_6_6776067_6776481	0.000957898	0.000915922	0.000729118	0.000667643	0.000730592	0.000825976
chromosome_16_6024629_6029942	0.00029079	0.000470399	0.000360498	0.000656153	0.000479325	0.002028999
chromosome_3_4443661_4449329	0.000273685	0.00048623	0.000333427	0.000647082	0.000505561	0.001951691
chromosome_6_4364533_4370186	0.000323397	0.000544465	0.000397045	0.000642849	0.000599913	0.001616011
chromosome_6_4989009_4994432	0.000245889	0.000429692	0.000320794	0.00064043	0.000433915	0.001877096
chromosome_1_6258205_6264270	0.000284376	0.000460222	0.000342451	0.00063922	0.000487902	0.002009333
chromosome_12_9137554_9143184	0.000271547	0.000477749	0.000347414	0.000631963	0.000510102	0.002014758
chromosome_10_1135214_1140860	0.000273151	0.000472096	0.000363657	0.000631358	0.000488407	0.002022896
chromosome_9_7026197_7031843	0.000265132	0.000451742	0.000377192	0.000628335	0.000478316	0.002037815
chromosome_2_8969911_8975557	0.000280634	0.000491319	0.00034967	0.00062652	0.000496984	0.002009333
chromosome_7_74484_75443	0.001097413	0.000735564	0.000618577	0.000623497	0.000709905	0.000556754
chromosome_1_6436501_6442155	0.00029079	0.000438738	0.000311319	0.000588421	0.000454097	0.001751639
chromosome_5_1111605_1117062	0.000307896	0.000500365	0.000379448	0.000584188	0.000533816	0.001234217
chromosome_12_2335855_2340967	0.000231456	0.00042743	0.000300039	0.000581164	0.000433915	0.001776731
chromosome_10_5335959_5336343	0.000684213	0.000529765	0.000404264	0.000571488	0.001113042	0.000588627
chromosome_11_3085875_3090959	0.000281169	0.000457961	0.000390277	0.000569674	0.000533816	0.001578035
chromosome_16_580543_580860	0.000389146	0.000436476	0.000397496	0.000567255	0.001322432	0.000658475
chromosome_5_1535968_1541622	0.000257649	0.000450046	0.000319892	0.000562417	0.000461161	0.001807925
chromosome_13_5036438_5036905	0.001258845	0.001012603	0.001112627	0.000558184	0.000488407	0.000918204
chromosome_10_5682660_5683283	0.001375375	0.001087233	0.001175793	0.000553951	0.000540376	0.000991443
chromosome_4_667365_672440	0.000199384	0.000320573	0.000254018	0.000546694	0.000450061	0.000968386
chromosome_7_75746_77085	0.000807692	0.00055238	0.000457504	0.000536413	0.00061959	0.000515388
chromosome_2_4103879_4109497	0.000288118	0.000452307	0.000371778	0.000533389	0.000495975	0.001209804
chromosome_1_6965122_6965957	0.001178663	0.000896699	0.000773335	0.000527946	0.000645322	0.000844964
chromosome_2_3660917_3665372	0.000307361	0.000566515	0.000413738	0.000525527	0.000487902	0.001808603
chromosome_3_6909023_6909784	0.001148729	0.000881433	0.000739044	0.000503756	0.000591335	0.000783253
chromosome_2_3688285_3688612	0.000343175	0.000358453	0.00037629	0.000492871	0.000358737	0.000720186
chromosome_7_1805184_1806219	0.001069617	0.000868995	0.00071468	0.000491661	0.000505057	0.000661866
chromosome_14_3593890_3596553	0.000310568	0.000394638	0.000332525	0.000483195	0.000452583	0.000754093
chromosome_12_6158446_6158635	0.000817314	0.000837334	0.000591055	0.000423325	0.000520698	0.00047063
chromosome_11_448833_449199	0.000625413	0.00046418	0.000395691	0.000406392	0.000809302	0.000357381
chromosome_12_6177015_6177204	0.000817314	0.000870691	0.000549997	0.000405787	0.000504048	0.000458424
chromosome_11_442682_443048	0.000595479	0.000481142	0.000397496	0.000400344	0.000767929	0.000400782
chromosome_14_187995_188469	0.000859008	0.000654149	0.000602786	0.000394297	0.000449052	0.000608293
chromosome_14_189484_190319	0.000624879	0.000424603	0.000390728	0.000391878	0.000353187	0.000516744
chromosome_12_6184630_6184819	0.000694904	0.000369761	0.000351475	0.000390063	0.000409696	0.000487584
chromosome_17_1036775_1037166	0.000562872	0.000584607	0.000476002	0.000384016	0.00024824	0.000366196
chromosome_3_7137470_7137553	0.000678867	0.000550119	0.00039524	0.000377364	0.000514139	0.000404172
chromosome_17_2281456_2281644	0.000672453	0.000590826	0.000457955	0.000377364	0.000596885	0.000351955
chromosome_3_6910803_6911638	0.000607773	0.000451742	0.00041058	0.000376154	0.000361259	0.000562179
chromosome_5_2985467_2985777	0.000561268	0.000452307	0.000438554	0.000344102	0.000527762	0.00042316
chromosome_12_3262538_3263099	0.000745685	0.000660934	0.000715131	0.000328379	0.000312822	0.000606937
chromosome_1_6966974_6967735	0.000578374	0.000415557	0.000369522	0.00032475	0.00032695	0.000497078
chromosome_17_1966528_1966835	0.00047788	0.000390115	0.000362754	0.000273347	0.00022503	0.000311945
chromosome_3_6916915_6917412	0.000378455	0.000311527	0.000271163	0.000244319	0.000228562	0.000343818
chromosome_7_5559507_5559571	0.000160362	0.000123819	0.000115053	0.00024069	0.000108479	0.000153938
chromosome_3_3760235_3760258	4.59705E-05	6.78461E-05	6.58733E-05	0.000234038	0.000277504	0.000178351
chromosome_12_8402766_8403646	0.000540421	0.000299088	0.000253567	0.000219524	0.00026489	0.000322117
chromosome_9_7770371_7770651	0.000205264	0.000200711	0.000165586	0.000214081	0.000134211	0.000217005
chromosome_8_436979_438264	0.001158351	0.000445522	0.00044487	0.000208034	0.00015742	0.00022989
chromosome_12_3577642_3577772	0.000117599	0.000195623	0.000152501	0.00020622	0.000361764	0.000354668
chromosome_5_3227660_3227758	0.000110115	0.000139084	0.000138063	0.000198358	0.000250763	0.000292279
chromosome_7_1807237_1808012	0.000365092	0.000231807	0.000227398	0.000191706	0.000199298	0.000298382
chromosome_7_692621_693635	0.000197246	0.000163396	0.000151599	0.000189287	0.000150861	0.000271935
chromosome_11_1347622_1350738	6.30759E-05	0.0001617	0.000119113	0.00018203	0.000159943	0.001006362
chromosome_2_9173275_9173436	0.000297739	0.000277038	0.000225142	0.000165097	0.000149852	0.000190558
chromosome_10_3237925_3237980	0.000219696	0.000339796	0.00017867	0.00014514	0.000217967	0.000172926
chromosome_4_591752_592023	0.000233595	0.000240288	0.000175512	0.000133045	0.000320391	0.000229212

Supplementary table 1: cont.

	Parent-1	Parent-2	Parent-3	AGO3-25-1	AGO3-25-2	AGO-3-25-3
chromosome_7_3068787_3069348	7.26976E-05	9.78114E-05	7.26411E-05	0.000131835	0.000105451	0.000159363
chromosome_17_4288631_4288910	0.000187624	0.000145304	0.000177768	0.000121555	7.97193E-05	0.000203442
chromosome_2_8349154_8349307	0.000167311	0.000160004	0.000189047	0.00011974	7.87102E-05	0.000221752
chromosome_10_3399871_3400004	0.000179071	0.000201277	0.000147989	0.000118531	0.000256313	0.000126134
chromosome_12_9155586_9156147	5.77305E-05	9.95076E-05	0.000084372	0.000114902	0.000105956	0.000168179
chromosome_3_7129085_7129168	0.000104236	8.81999E-05	7.08364E-05	0.000113088	7.11419E-05	9.76524E-05
chromosome_9_1108955_1109026	0.000218627	0.000219934	0.000110541	0.000106436	9.18285E-05	0.000160041
chromosome_4_572391_572455	0.000120806	8.19807E-05	9.79076E-05	9.49457E-05	0.000172557	0.00014241
chromosome_16_4565554_4566037	0.000279565	0.000152088	0.000153404	8.46649E-05	8.77921E-05	0.000105112
chromosome_13_2306295_2307879	0.000019778	5.25807E-05	3.47414E-05	8.46649E-05	5.95372E-05	0.000148513
chromosome_1_3120583_3120625	0.000154482	6.44538E-05	9.47493E-05	7.37794E-05	5.19689E-05	8.34114E-05
chromosome_12_6167927_6169267	0.000127221	8.31114E-05	7.26411E-05	7.19652E-05	9.93968E-05	0.000100365
chromosome_2_403293_403346	0.000159828	0.000121558	9.20422E-05	6.83367E-05	5.85281E-05	6.91704E-05
chromosome_10_4584909_4585470	2.24507E-05	2.99653E-05	1.98522E-05	5.44275E-05	5.70144E-05	6.30672E-05
chromosome_2_9129503_9129616	0.000626482	0.000226719	0.000192657	0.00004838	4.64188E-05	2.91601E-05
chromosome_14_1912136_1912559	7.69739E-05	0.000048623	0.000041058	4.47515E-05	6.81146E-05	8.88365E-05
chromosome_12_3078920_3079187	0.000032607	3.90115E-05	2.30105E-05	3.74945E-05	3.43096E-05	3.45852E-05
chromosome_9_7449955_7450222	4.32978E-05	4.24038E-05	3.33878E-05	3.50755E-05	3.27959E-05	4.06885E-05
chromosome_1_8028918_8029209	3.63488E-05	5.99307E-05	3.15831E-05	0.000033866	4.03642E-05	3.05164E-05
chromosome_4_588926_588995	9.24756E-05	4.07076E-05	5.00818E-05	3.26565E-05	4.03642E-05	6.78141E-05
chromosome_13_3152367_3152569	0.000112788	0.000123819	8.84327E-05	3.08422E-05	3.73369E-05	0.000057642
chromosome_10_6414325_6414592	3.58143E-05	3.73153E-05	0.00002662	3.08422E-05	3.22913E-05	2.98382E-05
chromosome_1_7553839_7554178	4.43669E-05	3.50538E-05	2.43641E-05	3.02375E-05	5.34825E-05	4.06885E-05
scaffold_25_9158_9426	4.11597E-05	8.81999E-05	3.56438E-05	0.000029028	5.04552E-05	0.000048148
chromosome_14_1262217_1262484	4.06251E-05	4.07076E-05	2.30105E-05	2.78185E-05	0.000052978	3.25508E-05
chromosome_4_599513_599553	0.00012241	4.18384E-05	5.63984E-05	0.000026609	4.18778E-05	4.47573E-05
chromosome_16_3391455_3391723	3.10034E-05	8.81999E-05	4.01556E-05	0.000026609	6.20599E-05	5.35732E-05
chromosome_4_210461_210966	1.76399E-05	1.75269E-05	1.26332E-05	0.000026609	1.76593E-05	0.000062389
chromosome_13_2954208_2954463	3.52797E-05	4.80576E-05	2.57177E-05	2.60042E-05	5.04552E-05	4.40792E-05
chromosome_12_4271406_4271681	3.10034E-05	3.10961E-05	1.84987E-05	2.47947E-05	4.89416E-05	3.59415E-05
chromosome_12_1932826_1933105	5.34541E-05	3.84461E-05	2.30105E-05	2.29805E-05	5.34825E-05	3.52634E-05
chromosome_3_5171964_5171988	7.48358E-06	7.34999E-06	4.51187E-06	0.000021771	4.59143E-05	2.23787E-05
chromosome_1_7531927_7532218	1.22944E-05	1.92231E-05	8.57255E-06	0.000016933	2.52276E-05	3.32289E-05
chromosome_13_2001068_2001202	4.16942E-05	0.000026573	2.12058E-05	1.26997E-05	1.11002E-05	1.28847E-05
chromosome_13_1865813_1866090	2.67271E-06	5.65384E-07	2.25594E-06	0.000012095	4.03642E-06	1.35628E-05
chromosome_17_4707939_4708088	1.06908E-06	1.69615E-06	4.51187E-07	1.14902E-05	7.56828E-06	1.62754E-05
chromosome_11_1535091_1535115	1.76399E-05	0.0000147	1.35356E-05	9.07124E-06	0.000010091	0.000018988
chromosome_4_471570_471639	9.62174E-06	2.26154E-06	3.6095E-06	7.25699E-06	4.03642E-06	1.28847E-05
chromosome_4_586361_586389	1.38981E-05	3.3923E-06	5.86543E-06	6.65224E-06	8.57739E-06	7.45956E-06
chromosome_12_1223802_1223826	2.13817E-06	5.65384E-07	9.02374E-07	0.000004838	4.03642E-06	3.39071E-06
chromosome_8_2488474_2488557	5.34541E-07	3.95769E-06	1.80475E-06	4.23325E-06	2.52276E-06	1.35628E-06
chromosome_4_3100623_3100751	4.27633E-05	0.000033923	3.33878E-05	3.6285E-06	1.0091E-06	8.1377E-06
chromosome_12_9189876_9190041	4.27633E-06	5.08845E-06	4.96306E-06	3.02375E-06	1.21093E-05	5.42513E-06
chromosome_13_4029649_4029686	5.34541E-07	0	0	3.02375E-06	2.01821E-06	4.74699E-06
chromosome_10_1462445_1462480	0	5.65384E-07	0	3.02375E-06	5.04552E-07	6.78141E-07
chromosome_11_2733167_2733188	0	0	4.51187E-07	3.02375E-06	4.03642E-06	8.1377E-06
chromosome_1_7070566_7070651	3.74179E-06	1.13077E-06	3.6095E-06	0.000002419	7.06373E-06	2.71257E-06
chromosome_5_700892_700912	1.60362E-06	5.65384E-07	0	0.000002419	1.51366E-06	4.06885E-06
chromosome_12_2578489_2578514	5.34541E-07	2.26154E-06	2.70712E-06	0.000002419	1.66502E-05	4.74699E-06
chromosome_17_1116273_1116293	5.34541E-07	0	4.51187E-07	0.000002419	1.0091E-06	5.42513E-06
chromosome_16_7263002_7263091	1.60362E-06	2.26154E-06	4.51187E-07	1.81425E-06	0	1.35628E-06
chromosome_6_3278336_3278363	0	0	0	1.81425E-06	2.01821E-06	1.35628E-06
chromosome_10_6166171_6166271	3.20725E-06	2.82692E-06	9.02374E-07	1.2095E-06	4.54097E-06	4.74699E-06
chromosome_2_7331851_7331871	1.06908E-06	5.65384E-07	0	1.2095E-06	5.04552E-06	0
chromosome_3_4155052_4155072	5.34541E-07	0	0	1.2095E-06	0	6.78141E-07
chromosome_8_4479431_4479453	5.34541E-07	0	0	1.2095E-06	0	0
chromosome_12_4529745_4529821	5.34541E-07	5.65384E-07	0	1.2095E-06	0	2.71257E-06
chromosome_7_3451388_3451654	1.60362E-06	1.13077E-06	0	6.04749E-07	0	6.78141E-07
chromosome_1_7911753_7911773	5.34541E-07	0	0	6.04749E-07	0	1.35628E-06
chromosome_7_32808_32831	0	0	0	6.04749E-07	0	0
chromosome_14_2636093_2636118	0	1.69615E-06	9.02374E-07	6.04749E-07	1.51366E-06	3.39071E-06
chromosome_7_4614400_4614433	3.20725E-06	0	4.51187E-07	0	3.02731E-06	2.71257E-06
chromosome_1_781444_781469	1.60362E-06	1.13077E-06	4.51187E-07	0	1.51366E-06	3.39071E-06
chromosome_10_4788545_4788612	1.06908E-06	5.65384E-07	0	0	2.52276E-06	6.78141E-07
chromosome_6_6841140_6841160	5.34541E-07	0	0	0	1.51366E-06	0
chromosome_6_3067367_3067455	0	0	0	0	0	0
chromosome_7_4386254_4386279	0	0	0	0	0	0
chromosome_7_4969004_4969026	0	0	4.51187E-07	0	0	0
chromosome_8_1168464_1168491	0	0	0	0	0	6.78141E-07
chromosome_11_3677466_3677486	0	0	0	0	0	0
chromosome_13_249543_249565	0	0	0	0	0	0
chromosome_14_1045259_1045280	0	0	0	0	5.04552E-07	6.78141E-07
chromosome_16_4708166_4708186	0	5.65384E-07	0	0	0	0
chromosome_16_5830783_5830805	0	0	0	0	0	0
chromosome_17_544133_544153	0	0	4.51187E-07	0	5.04552E-07	0

Supplementary table 2: Normalised miRNA abundance (total RNA)

	Parent-1	Parent-2	Parent-3	AGO3-25-1	AGO3-25-2	AGO3-25-3
chromosome_5_3227666_3227753_+	0.039868668	0.061289854	0.049587667	0.149174395	0.229784273	0.30839322
chromosome_10_3399870_3399999_	0.423775702	0.350977394	0.323608037	0.127655484	0.090263553	0.140683464
chromosome_12_6402226_6402307_	0.142005207	0.093417354	0.088144075	0.030168132	0.037247696	0.0275766
chromosome_6_6776108_6776193_+	0.116322702	0.091328506	0.062143571	0.013900142	0.033012295	0.024568244
chromosome_9_6365928_6366014_-	0.033072328	0.020796773	0.019119217	0.013319142	0.014704727	0.016900604
chromosome_17_6144120_6144204_	0.030564383	0.026299104	0.020880305	0.011060917	0.011276068	0.011006182
chromosome_16_185088_185174_-	0.077315542	0.037874379	0.032474133	0.007322788	0.004565433	0.006200149
chromosome_4_3694793_3694875_-	0.013200214	0.008263686	0.006286105	0.005218034	0.008269117	0.009159589
chromosome_5_1790702_1790791_+	0.013449094	0.010281207	0.007933048	0.00509745	0.00330948	0.002996127
chromosome_3_6573899_6573980_-	0.014320175	0.005206835	0.003212355	0.003814865	0.000476712	0.000599225
chromosome_2_2217872_2218043_-	0.008270475	0.005869153	0.005209885	0.003310602	0.003419491	0.003460833
chromosome_14_2347075_2347211_	0.011706934	0.012227402	0.008438546	0.002751527	0.005693039	0.003876622
chromosome_13_3152367_3152452_	0.003063139	0.003362536	0.001687709	0.002630942	0.004134558	0.004952782
chromosome_1_7070552_7070605_-	0.012338707	0.003494999	0.004957136	0.002323999	0.002365224	0.003473062
chromosome_13_2001067_2001197_	0.009735038	0.004401865	0.004932676	0.002181489	0.001356795	0.002445818
chromosome_13_2127530_2127632_	0.001876173	0.004136938	0.00181816	0.001545678	0.002841936	0.000819349
chromosome_14_3218783_3218866_	0.00252709	0.013429764	0.001573565	0.001479905	0.009259211	0.002213465
chromosome_1_5106349_5106475_+	0.010539112	0.007081704	0.005796914	0.001414131	0.000852581	0.001332971
chromosome_4_3100624_3100751_+	0.011706934	0.005390247	0.004484251	0.001315471	0.001164277	0.001919967
chromosome_8_121858_121942_-	0.00501589	0.003046661	0.002755776	0.001172962	0.00119178	0.00107616
chromosome_6_7063792_7063881_-	0.002077191	0.001314446	0.00110068	0.001085263	0.001274287	0.001687615
chromosome_7_4631481_4631544_-	0.000277597	0.000438149	0.000481038	0.000515226	0.000577555	0.000856036
chromosome_2_9129508_9129593_-	0.001876173	0.000957813	0.000945769	0.000460415	0.000476712	0.000513622
chromosome_2_9173306_9173389_+	0.010634836	0.005257783	0.005845833	0.00043849	0.001145942	0.000745975
chromosome_1_3724965_3725113_+	0.000114868	0.000132464	0.000195676	0.000416566	0.000265859	0.000611455
chromosome_7_5632709_5632783_-	0.002316499	0.001192172	0.000823472	0.00033983	0.000476712	0.000256811
chromosome_2_739310_739389_+	0.000526477	0.000427959	0.000407659	0.000317905	0.000403372	0.000635913
chromosome_7_2372822_2372907_-	0.0000765785	0.003107798	0.000244596	0.000306943	0.002970281	0.000207895
chromosome_10_6282484_6282565_	0.00049776	0.000142653	0.00020383	0.000153472	0.0000550052	0.0000978327
chromosome_4_457829_457914_-	0.000469043	0.00021398	0.000130451	0.000131547	0.000256691	0.000293498
chromosome_7_2371057_2371142_-	0.0000191446	0.001100466	0.0000978382	0.0000986603	0.000907586	0.000122291
chromosome_2_8349161_8349264_+	0.000315886	0.000142653	0.000220136	0.0000328868	0.000119178	0.0000611455
chromosome_7_5926395_5926482_+	0	0.000132464	0	0.0000109623	0.0000825078	0
chromosome_16_828236_828325_-	0.00012444	0.000101895	0.0000570723	0	0.0000183351	0.0000244582
chromosome_16_4056196_4056281_	0	0	0	0	0	0
chromosome_17_5149811_5150023_	0	0	0	0	0	0
chromosome_2_9101133_9101252_+	0	0	0	0	0	0
chromosome_4_3820425_3820510_+	0	0	0	0	0	0
chromosome_6_3067368_3067456_+	0	0	0	0	0	0
chromosome_7_4386252_4386309_-	0	0	0	0	0	0
chromosome_8_126807_126910_-	0	0	0	0	0	0
scaffold_24_82182_82317_+	0	0	0	0	0	0

Supplementary table 3: Normalised miRNA abundance (AGO-IP)

IP-Antibody	α2/3-PAZ					α-At-AGO1	
	Parent-1	Parent-2	Parent-3	ago3-25-1	ago3-25-2	Parent-1	ago3-25-2
chromosome_5_3227666_3227753_+	0.335874934	0.090488075	0.983544607	0	0	0	0.000044817
chromosome_3_6573899_6573980_-	0.000491548	0	0	0	0	0	0
chromosome_13_3152367_3152452_	0.000196619	0	0.000574025	0.000305099	0.0000885772	0.002945416	0.001523779
chromosome_13_2001067_2001197_	0.0000491548	0	0.000287013	0.000381374	0.0000885772	0.001453582	0.000313719
chromosome_1_3724965_3725113_+	0	0	0	0	0	0	0
chromosome_1_5106349_5106475_+	0	0	0	0	0.0000885772	0.0000382522	0.000044817
chromosome_1_7070552_7070605_-	0	0	0	0	0	0	0
chromosome_10_3399870_3399999_	0	0	0.0000478354	0	0	0	0.000044817
chromosome_10_6282484_6282565_	0	0	0	0	0	0	0
chromosome_12_6402226_6402307_	0	0	0	0	0	0.000344269	0.000179268
chromosome_13_2127530_2127632_	0	0	0	0	0	0	0
chromosome_14_2347075_2347211_	0	0	0.008993064	0	0	0.000153009	0.000044817
chromosome_14_3218783_3218866_	0	0	0.006314279	0	0	0	0.0000896341
chromosome_16_185088_185174_-	0	0.081766913	0	0	0.0000885772	0	0
chromosome_16_828236_828325_-	0	0	0	0	0	0	0
chromosome_16_4056196_4056281_	0	0	0	0	0	0	0
chromosome_17_5149811_5150023_	0	0	0	0	0	0	0
chromosome_17_6144120_6144204_	0	0	0	0	0	0.0000382522	0.000044817
chromosome_2_739310_739389_+	0	0	0	0	0	0	0
chromosome_2_2217872_2218043_-	0	0	0	0	0	0.0000382522	0
chromosome_2_8349161_8349264_+	0	0	0	0	0	0	0
chromosome_2_9101133_9101252_+	0	0	0	0	0	0	0
chromosome_2_9129508_9129593_-	0	0	0	0	0	0	0
chromosome_2_9173306_9173389_+	0	0	0	0	0	0	0
chromosome_4_457829_457914_-	0	0	0	0	0	0	0
chromosome_4_3100624_3100751_+	0	0	0.0000478354	0	0	0.0000382522	0
chromosome_4_3694793_3694875_-	0	0	0	0	0	0	0
chromosome_4_3820425_3820510_+	0	0	0	0	0	0	0
chromosome_5_1790702_1790791_+	0	0	0.0000478354	0	0	0.0000382522	0
chromosome_6_6776108_6776193_+	0	0	0.0000956709	0.0000762747	0	0.000267765	0.000044817
chromosome_6_7063792_7063881_-	0	0	0	0	0	0	0
chromosome_6_3067368_3067456_+	0	0	0	0	0	0	0
chromosome_7_2371057_2371142_-	0	0	0	0	0	0	0
chromosome_7_2372822_2372907_-	0	0	0	0	0	0	0
chromosome_7_4386252_4386309_-	0	0	0	0	0	0	0
chromosome_7_4631481_4631544_-	0	0	0	0	0	0	0
chromosome_7_5632709_5632783_-	0	0	0	0	0	0	0
chromosome_7_5926395_5926482_+	0	0	0	0	0	0	0
chromosome_8_121858_121942_-	0	0	0	0	0	0	0
chromosome_8_126807_126910_-	0	0	0	0	0	0	0
chromosome_9_6365928_6366014_-	0	0	0.0000478354	0.0000762747	0	0.0000765043	0.000044817
scaffold_24_82182_82317_+	0	0	0	0	0	0	0