

SUPPLEMENTARY INFORMATION

Supplemental Fig. 1

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hAgo 1 --MEAGVPSGA AAGAYLPPFQ QVFQAPRRPG IGTVGKPIKL LANYFEVDIP KIDVYHYEVD IKPDKCPRRV NREVVEXMVQ HFKQPIFGDR KPVYDGKKNL 98
hAgo 2 MYSGAGSALA PPAPPPPIQG YAFKPPPRPD FQTSGRITKL QANFFEMDIP KIDIYHYELD IKPEKCPRRV NREIVHEMVQ HFKQPIFGDR KPVYDGKKNL 100
hAgo 3 --HEIGSACP AG-----AQ PLLMVPRRPG YGAMGKPIKL LANCFOVEIP KIDVYLYEVD IKPDKCPRRV NREVVDSMVQ HFKVTIFGDR RPYVDGKRSL 92
hAgo 4 ----MEALGP GP-----PA SLFQPPRRPG LGTVGKPIRL LANHFQVQIP KIDVYHYDVD IKPEKRRRV NREVVDMVR HFKQPIFGDR QPGYDGKKNM 90

hAgo 1 YTVTALPIGN ERVDFEVTIP GEG-KDRIFK VSIKMLAIVS WRMLHEALVS G-----Q IFVPLESVQA LDVAMRHLS MRYPVGRSF FSPPEGYTHF 189
hAgo 2 YTAMPLPIGR DKVELEVTLG GEG-KDRIFK VSIKMYSCVS LQALHDALSG R-----L PSVPFETIQA LDVVMRHLS MRYPVGRSF FTASEGCSNF 191
hAgo 3 YTAHPLFVAT TGVLDLVTLG GEGKDRPFK VSIKSVSRVS WHLLHEVLG RLLPELELD KPISINDVHA VDVVLRHLS MKYTPVGRSF FSAPEGDHP 192
hAgo 4 YTAHPLPIGR DRVDMEVTLG GEG-KDQTFK VSVQWVSVS LQLLLEALG -----HLN -EVPDSSVQA LDVITRHLS MRYPVGRSF FSPPEGYTHF 181

120
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hAgo 2 LGGGREVWFG FHQSVRPSLW KMLINIDVSA TAFYKAQPMI EFPVCEVDFK SIEEQPKPLT DSQRVRFKE IKGLKVEITH CGQMKRKYR CNVTRRPASH 291
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hAgo 4 LGGGREVWFG FHQSVRPAMW KMLINIDVSA TAFYKAQPMI EFMCEVLDIQ NINEQTKPLT DSQRVRFKE IRGLKVEVTH CGQMKRKYR CNVTRRPASH 281

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hAgo 4 QTFPLQESG QAMECTVAQY FKQKYSLQLK YPHLPCLQVG QBQKHYYLPL EVCNIVAGQR CIKKLTDNQT STMKATARS APDRQEEISR LVKNSMVG 381

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hAgo 2 -DPYVREFGI MVDKEMTDVT GRVLQPPSIL YGGRNKALAT PVQGVWDMRN KQFHTGIEIK VMAIACFAPQ RQCTEVHLKS PTEQLRKISK DAGMPIQQQP 489
hAgo 3 -DPFVQEFQF KVRDEMAHVT GRVLPAPMLQ YGGRNRTVAT PSHGVWDMRG KQFHTGVEIK VMAIACFAPQ RQCREEILKS FTDQLRKISK DAGMPIQQQP 490
hAgo 4 PDPYLKEFGI VVINEMTELT GRVLPAPMLQ YGGRNKTVAT PNQGVWDMRG KQFYAGIEIK VMAIACFAPQ KQCREDLKS FTDQLRKISK DAGMPIQQQP 481

hAgo 1 CFCKYAQGAD SVSEPMFRHLK NTYSGLQLII VILPGKTPVY AEVNRVGDIT LGMATQCVQV KNVVKTSPQT LSNLCLKINV KLGGINNVL PHQRSVVFQ 587
hAgo 2 CFCKYAQGAD SVSEPMFRHLK NTYAGLQLVV VILPGKTPVY AEVNRVGDIT LGMATQCVQM KNVVKTSPQT LSNLCLKINV KLGGINNVL PQRPPVVFQ 589
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hAgo 4 CFCKYAQGAD SVSEPMFRHLK MTYVGLQLIV VILPGKTPVY AEVNRVGDIT LGMATQCVQV KNVVKTSPQT LSNLCLKINA KLGGINNVL PHQRSVVFQ 581

523
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hAgo 2 PVIFLGADVT HPPAGDGKKP SIAAVVGSMD AHPNRYCATV RVQQRHQ--- -----EII QDLAMVREL LIQFYKSTRF KPTRIIIFYRD GVSSEGQFQV 679
hAgo 3 PVIFLGADVT HPPAGDGKKP SIAAVVGSMD AHPSTRYCATV RVQRFRQ--- -----EII QDLAMVREL LIQFYKSTRF KPTRIIIFYRD GVSSEGQFQV 680
hAgo 4 PVIFLGADVT HPPAGDGKKP SIAAVVGSMD GHPSRYCATV RVQSTRQES QELLYSQEVI QDLTMVREL LIQFYKSTRF KPTRIIIFYRD GVSSEGQMKV 681

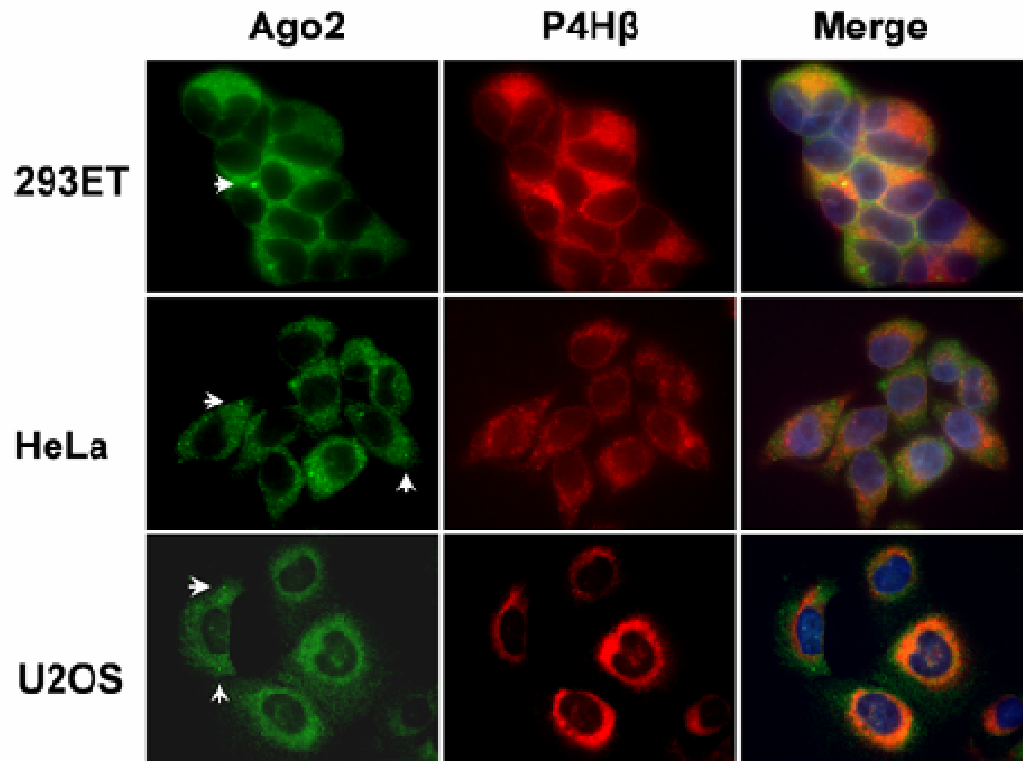
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hAgo 3 LYVELLAIRD ACISLEKDYQ PGITYIVVQK RHHTRLFCAD RTERVGRSGN IPAGTTVDTD ITHPEFDFY LCSHAGIQT SRPSHYVVLV DDNCFTADEL 780
hAgo 4 ANVELLAIRD ACISLEKDYQ PGITYIVVQK RHHTRLFCAD KTERVGRSGN VPAGTTVDST ITHPEFDFY LCSHAGIQT SRPSHYVVLV DDNCFTADEL 781

700
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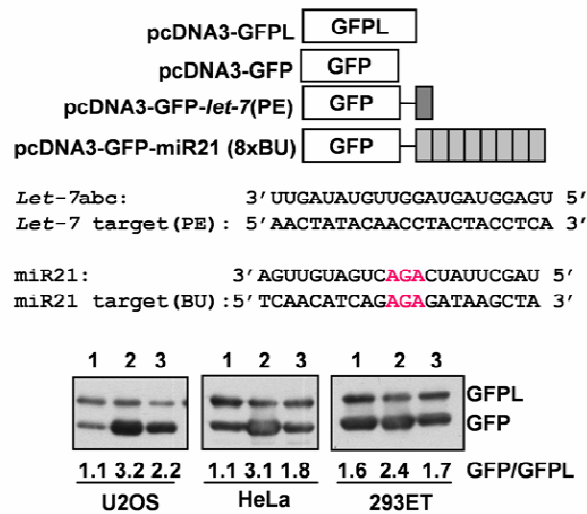
Supplemental Fig. 1 | Potential C-P4H(I) hydroxylation sites in human Argonaute proteins. Human Ago1 to 4 proteins are aligned. The X-P-G (blue) and X-P-A (red) motifs are highlighted. PAZ (blue) and PIWI (red) domains are underlined.

Supplemental Fig. 2



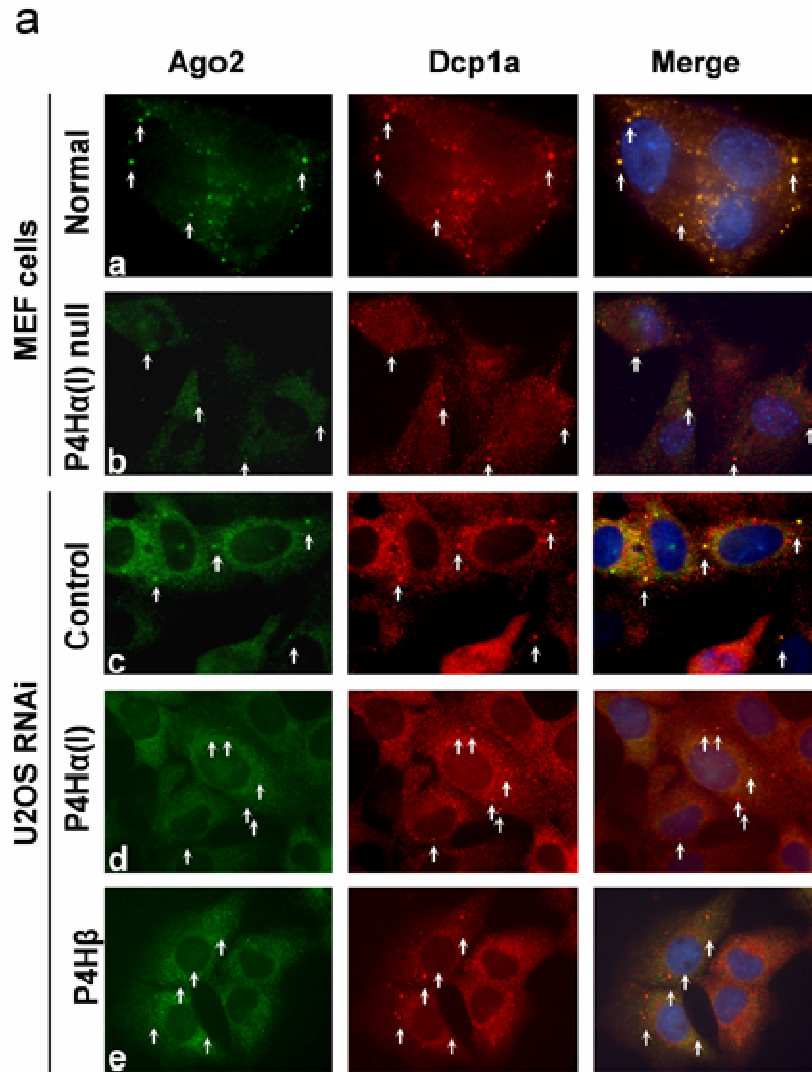
Supplemental Fig. 2 | Ago2 is cytoplasmic and partially co-localized with P4Hβ. Immunofluorescence was performed on 293ET, HeLa and U2OS cells using monoclonal anti-Ago2 (Wako Chemicals) and polyclonal anti-P4Hβ antibodies. Ago2 and P4Hβ were visualized with secondary antibodies again mouse IgG conjugated with Alexa Fluor 488 and rabbit IgG with Alexa 594. Nuclei were stained with Hoechst 33258. Images were digitally merged. P-bodies are indicated with arrows.

Supplemental Fig. 3

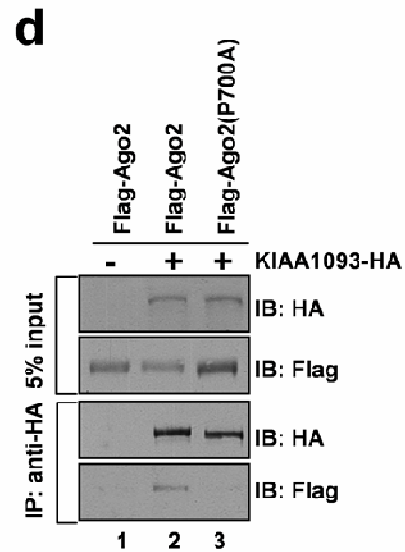
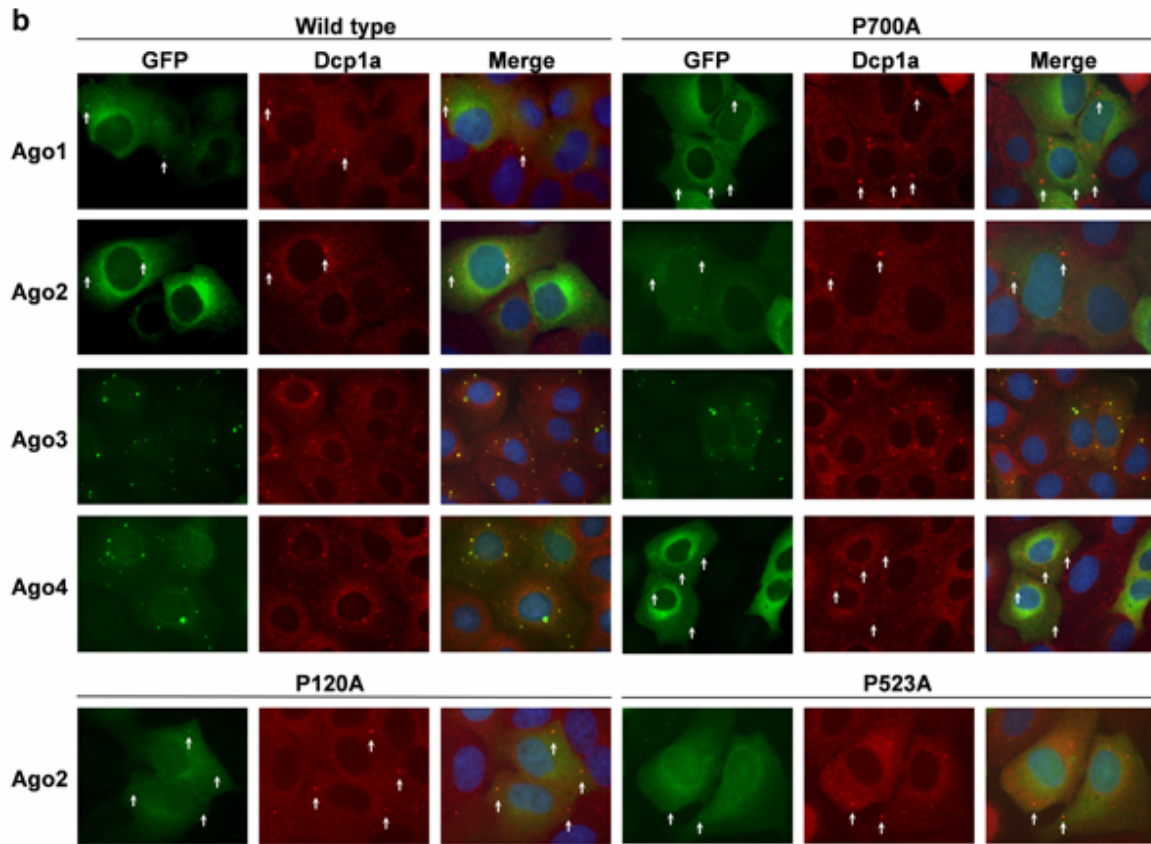


Supplemental Fig. 3 | GFP-*let7*(PE) and GFP-miR21(8×BU) reporters are repressed by endogenous microRNA *let-7* and miR21. pcDNA3-GFP, GFPL (mutant long GFP) and pCDNA3-GFP-*let7*(PE) or miR21(8×BU) containing perfect or imperfect complementary sequences for *let-7* or miR21 are illustrated. Under 1:3 ratio (GFPL/GFP reporter), pcDNA3-GFPL was co-transfected with either GFP-*let-7*(PE) (lower panel, lane 1), or GFP (lower panel, lane 2) or GFP-miR21(8×BU)(lower panel, lane 3) into U2OS, HeLa and 293ET cells. The expression of GFP constructs were detected by western blot and the expression ratios between GPF and GFPL are calculated.

Supplemental Fig. 4a

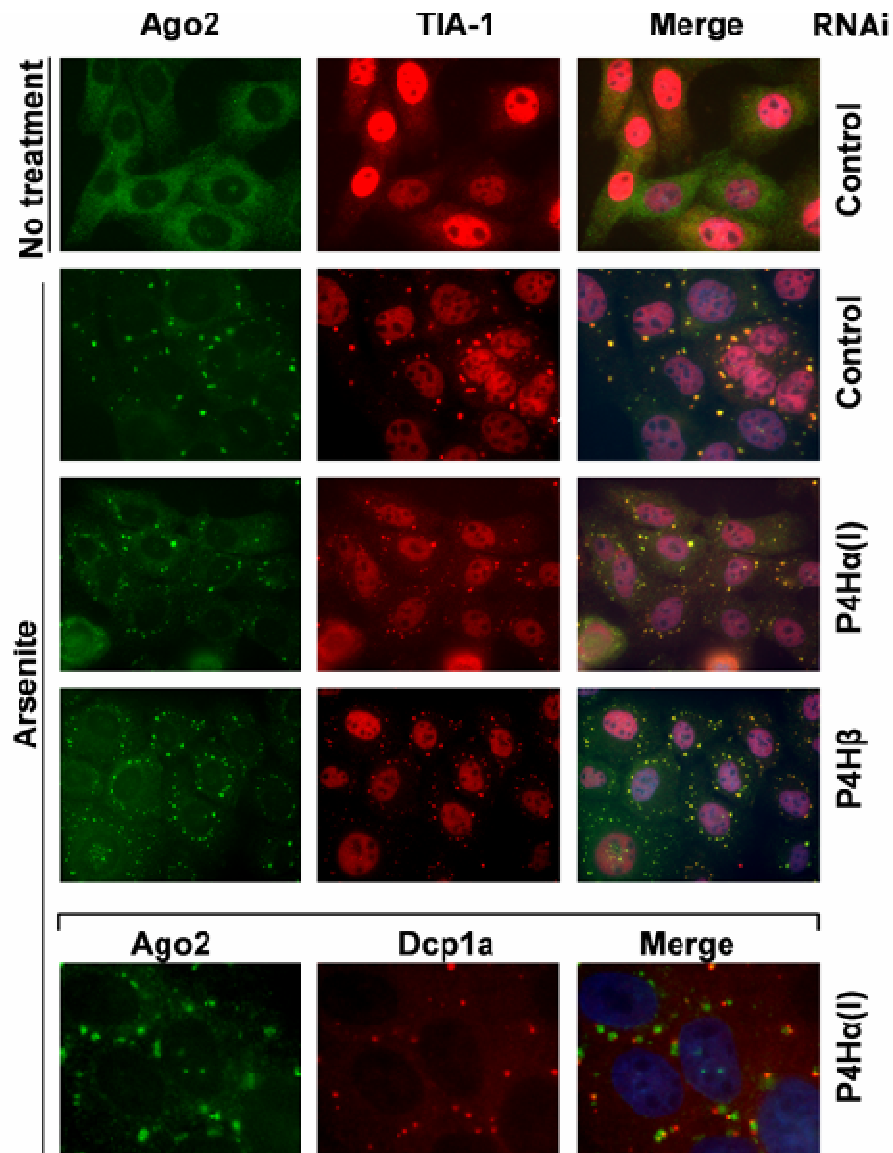


Supplemental Fig. 4b, c, d



Supplemental Fig. 4 | Impaired hydroxylation reduced/abolished endogenous/mutant (P700A) Ago2 P-body localization. **a.** Impaired C-P4H(I) reduced Ago2 P-body localization. Immunofluorescence using polyclonal anti-Dcp1a and monoclonal anti-Ago2 antibodies were performed on normal (a), P4H α (I) null MEF (b) and U2OS cells (c,d,e), which were transfected with indicated shRNA for 24 hours followed by puromycin selection (2 μ g/ml, 36 hours). The P-bodies marked by Dcp1a are indicated by arrows. **b.** Mutation of Proline 700 to Alanine (P700A) abolished Ago1, 2 and reduced Ago4 P-body localization. U2OS cells were transfected with wild type and mutant GFP-Ago constructs as indicated. Immunofluorescence was performed with monoclonal anti-Dcp1a antibody. **c.** TAP purifications of wt-Ago2 and Ago2(P700A) were performed as described in Fig.1a. HA-eluates were immunoblotted with anti-HA, Dicer and Dcp1a antibodies. **d.** KIAA1093-HA was co-transfected with empty vector (lane 1), Flag-Ago2 (lane 2) or Ago2(P700A) (lane 3) into 293ET cells. Immunoprecipitates with anti-HA antibody were probed with anti-HA and anti-Flag antibodies, respectively.

Supplemental Fig. 5



Supplemental Fig. 5 | Oxidative stress (Arsenite) recruited Ago2 to Stress Granule (SG) despite of C-P4H(I). U2OS cells were transfected with P4H α (I) or P4H β shRNA for 36 hours and selected with puromycin (2 μ g/ml) for an additional 36 hours. Selected cells were seeded on coverslips and treated with Arsenite (250 μ M, 1 hour) or heat shock (44°C, 30 min). Ago2 and either TIA-1 or Dcp1a were visualized by immunofluorescence using anti-Ago2, TIA1 or Dcp1a antibodies and secondary anti-mouse IgG conjugated with Alexa Fluor 488, rabbit IgG with Alexa 594 antibodies. Nuclei were stained with Hoechst 33258. Images were digitally merged.