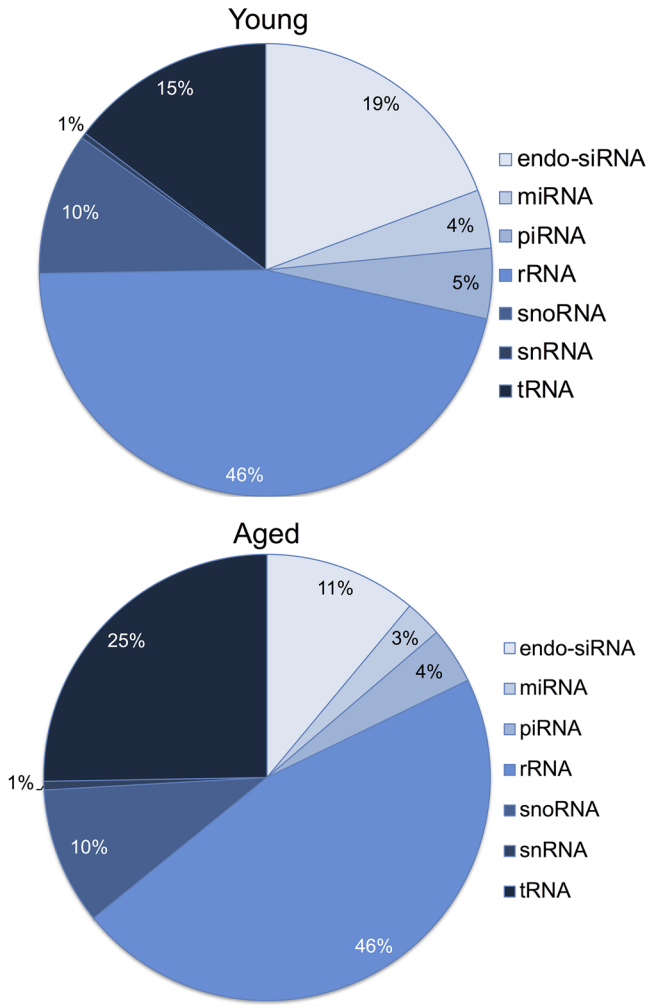
Supplementary Figures



Supplementary Figure S1. The small RNA signatures of young and aged oocytes. Following filtering and normalisation, sncRNA reads were mapped to known and miRNA, endo-siRNA, piRNA, rRNA, snoRNA, snRNA, and tRNA fragments from RNAcentral. Pie chart was generated to illustrate the number of total sequences aligning to each small RNA category.

5' UTR

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KIFC1   ----------------------------ATTCAAAAGTGGCGGGTGTGGCTGATTGGCCT 32
KIFC5B  GAAGTGCGTTGACTGCGCGGAGGTTGAAATTCAAAAGTGACTGGTGTGGCGGATTGGCTT 60
                                    *********** * ******** ******* *

KIFC1   GGCTCCTGGA----------CAGCCTGGCTTCGGCGTTTTGCGGCGCGAGCCCTTGAGAG 82
KIFC5B  GGCCGATTGGCCTGGCGAATTGGCCTGGCTCCGACGTTTTGCGGCGCGAGCCCTTGAGAG 120
        ***   * *              ******** ** **************************

KIFC1   GACGCGGGTCTCATCGTCCTGCCTGCTGCCTTTGCACC-CTTTCTGTGCGGACCACCATG 141
KIFC5B  GACGCGGGTCTCGTCACCCTGCCTGCTGCCTTTGCATCCCTTCCTGTGCGGACCACCATG 180
        ************ **  ****************** * *** ******************

KIFC1   GACGTGCAGGCGCAGAGGCCACCTTTGTTGGAAGTGAAGAGGAACGTAGAACTGAAGGCA 201
KIFC5B  GACGTGCAGGCGCAGAGAAAG---GGAAGGGAAGGGAAGAGGAACGTAGAACTGAAGGCG 237
        *****************            ***** ************************

KIFC1   GCCCTGGTGAAGTCCTCCTCCCGAGTGCCCCTGTCAGCAAGCAGGCTCAAGAGGGGTCCT 261
KIFC5B  GCCCTGGTCAAGTCCTCCTCCCGACTGCCCCTGTCAGCAAGCAGCCTCAAGAGGGGTCCT 297
        ******** *************** ******************** ***************

KIFC1   GACCAGATGGAGGATGCCTTGGAGCCTGCAAAGAAACGGACACGAGTCATGGGTGCAGTG 321
KIFC5B  GACCAGATGGAGGATGCCTTGGAGCCTGCAAAGAAACGGACACGAGTCATGGGTGCAGTG 357
        ************************************************************

KIFC1   ACCAAAGTTGACACATCCCGTCCCAGAGGACCACTCCTCAGCACAGTGTCACAGACCCAG 381
KIFC5B  ACCAAAGTTGACACATCCCGTCCCAGAGGACCACTCCTCAGCACAGTGTCACAGACCCAG 417
        ************************************************************

KIFC1   GGCCACACTGCAGCTCAGAAAGGCCCTAAGAAGACAGGACCTCGTGGGTGCTCTGCTATT 441
KIFC5B  GGCCACAATGCAGCTCAGAAAGGCCCTAAGAAGACAGGACCTGGTGGGTGCTCTGCTGTT 477
        ******* ********************************** ************** **

KIFC1   GGTACAGTGCTGAGGAGCCAGAAGCCAGTTCCCGCTGCTCCTGCCCAGAAGCCTGGCACA 501
KIFC5B  GGTACAGTGCTGAGGAGCCAGAAGCCAGCTCCCGCTGCTCCTGC---GAAGCCTGGCACA 534
        **************************** ***************   **************

KIFC1   TCCACTGCTCCTGTGGTGGTAGGGAAGAGAGCTGGCAAACGCCCTGCCTGGGACCTGAAG 561
KIFC5B  TCCACTGCTCCTGTGGTGGTAGGGAAGAGAGCTGGCAAACGCCCTGCCTGGGACCTGAAG 594
        ************************************************************

KIFC1   GGCCAGTTGTGTGACCTCAATGAAGAGTTGAAACGCTATCGGGAGAAGACTCAAACGCTG 621
KIFC5B  GGCCAGTTGTGTGACCTCAATGAAGAGTTGAAACGCTATCGGGAGAAGACTGAAACGCTG 654
        ************************************************* ********

KIFC1   GAACTGGAGAACCGGGGTCTTCGGGAGCAACTCAGAGAGGTCCAGGAGCAGGCCACGACC 681
KIFC5B  GAACTGGAGAACCGGGGTCTTCGGGAGCAACTCAGAGAGGTCCAGGAGCAGGCCACGACC 714
        ************************************************************

KIFC1   CTGGGGACAGAGCGGAACACCCTGGAAGGGGAGCTGGCCAGTGTACGCAGCCGAGCTGAG 741
KIFC5B  CTGGGGACAGAGCGGAACACCCTGGAAGGGGAGCTGGCCAGTGTACGCAGCCGAGCTGAG 774
        ************************************************************

KIFC1   CAGGACCAGCAGAGGCTGGAGACGCTGAGTGCCCGTGTCTTGGAGCTGGAGGAATGTCTG 801
KIFC5B  CAGGACCAGCAGAGGCTGGAGACGCTGAGTGCCCGTGTCTTGGAGCTGGAGGAATGTCTG 834
        ************************************************************

KIFC1   GGTACCAGGGAAAGGCTGCTTCAGGAGCTTCAGGGAGAGCGGCTGCAATTGCAGGAGGAG 861
KIFC5B  GGTACCAGGGAAAGGCTGCTTCAGGAGCTTCAGGGAGAGCGGCTGCAATTGCAGGAGGAG 894
        ************************************************************

KIFC1   CGGAGCACACTGAGCACCCAGCTGGAGGAGCAGGAGAGGAGGTTTCAGGCCACAGAAGCA 921
KIFC5B  CGGAGCACACTGAGCACCCAACTGGAGGAGCAGGAGAGGAGGTTTCAGGCCACAGAAGCA 954
        ******************* ************************************

KIFC1   GCTCTGTCAAGCAGCCAAGAAGAGGTGGTGTGTCTTCGGCAGAAGACTGAAGCCCAGGTG 981
KIFC5B  GCTCTGTCAAGCAGCCAAGAAGAGGTGGTGTGTCTTCGGCAGAAGACTGAAGCCCAGGTG 1014
        ************************************************************

KIFC1   ACCTTACTGGCTGAGCAAGGAGACCGGCTCTATGGGTTAGAGATGGAGCGGCGACGACTC 1041
KIFC5B  ACCTTACTGGCTGAGCAAGGAGACCGGCTCTATGGGTTAGAGATGGAGCGGCGGCGACTC 1074
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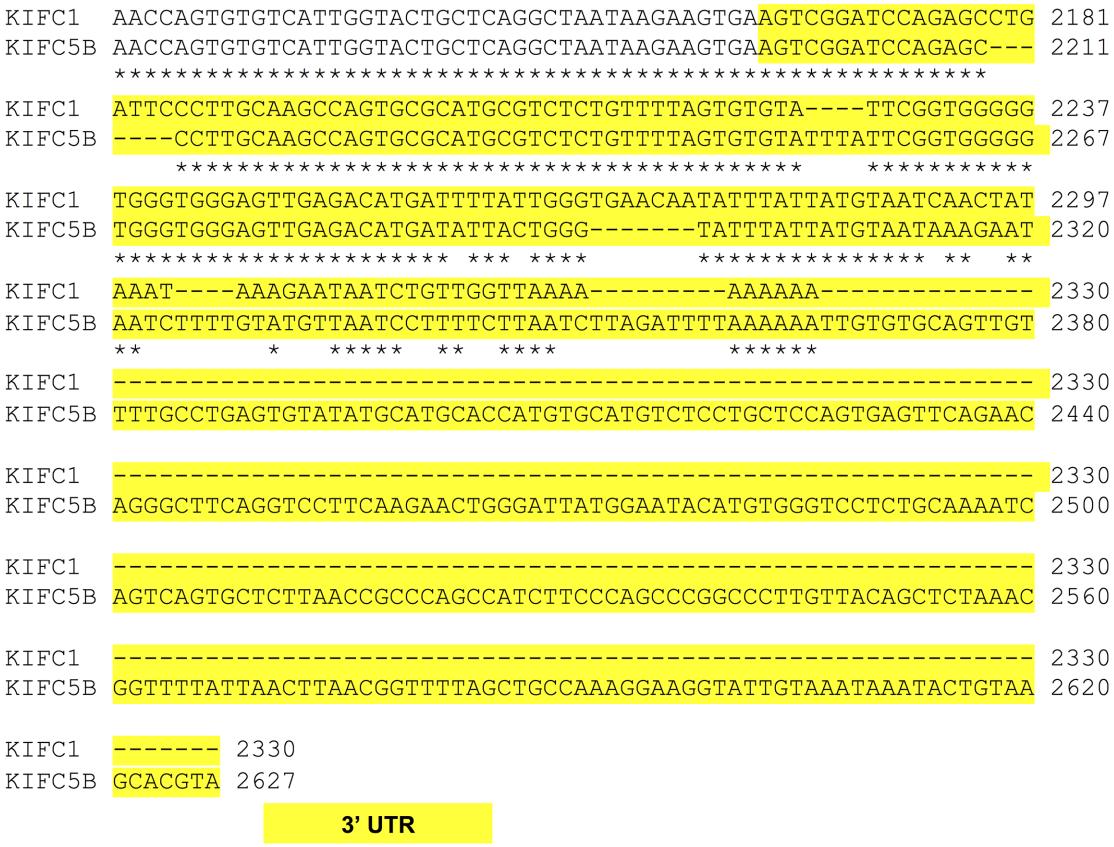
Supplementary Figure S2 (Part 1). mRNA sequence alignment of Kifc1 and Kicf5b. Location of siRNA targeting sites, 5' and 3' untranslated regions are as marked.

Supplementary Figure S2 (Part 2). mRNA sequence alignment of Kifc1 and Kicf5b. Location of siRNA targeting sites, 5' and 3' untranslated regions are as marked.



Supplementary Figure S2 (Part 3). mRNA sequence alignment of Kifc1 and Kicf5b. Location of siRNA targeting sites, 5' and 3' untranslated regions are as marked.

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KIFC1  MDVQAQRPPLLEVKRNVELKAALVKSSSRVPLSASRLKRGPDQMEDALEPAKKRTRVMGA 60
KIFC5B MDVQAQRKG-REGKRNVELKAALVKSSSRLPLSASSLKRGPDQMEDALEPAKKRTRVMGA 59
       *******    * ****************:***** ************************

KIFC1  VTKVDTSRPRGPLLSTVSQTQGHTAAQKGPKKTGPRGCSAIGTVLRSQKPVPAAPAQKPG 120
KIFC5B VTKVDTSRPRGPLLSTVSQTQGHNAAQKGPKKTGPGGCSAVGTVLRSQKPAPAAPA-KPG 118
       ************************.*********** ****:*********.***** ***

KIFC1  TSTAPVVVGKRAGKRPAWDLKGQLCDLNEELKRYREKTQTLELENRGLREQLREVQEQAT 180
KIFC5B TSTAPVVVGKRAGKRPAWDLKGQLCDLNEELKRYREKTETLELENRGLREQLREVQEQAT 178
       ************************************:*********************

KIFC1  TLGTERNTLEGELASVRSRAEQDQQRLETLSARVLELEECLGTRERLLQELQGERLQLQE 240
KIFC5B TLGTERNTLEGELASVRSRAEQDQQRLETLSARVLELEECLGTRERLLQELQGERLQLQE 238
       ************************************************************

KIFC1  ERSTLSTQLEEQERRFQATEAALSSSQEEVVCLRQKTEAQVTLLAEQGDRLYGLEMERRR 300
KIFC5B ERSTLSTQLEEQERRFQATEAALSSSQEEVVCLRQKTEAQVTLLAEQGDRLYGLEMERRR 298
       ************************************************************

KIFC1  LHNQLQELKGNIRVFCRVRPVLEGESTPSPGFLVFPPGPAGPSDPPTGLSLSRSDDRRST 360
KIFC5B LHNQLQELKGNIRVFCRVRPVLEGESTPSPGFLVFPPGPAGPSDRPTGLSLSRSDDRRST 358
       ******************************************* ***************

KIFC1  LTGAPAPTVRHDFSFDRVFPPGSKQEEVFEEIAMLVQSALDGYPVCIFAYGQTGSGKTFT 420
KIFC5B LTGAPAPTVRHDFSFDRVFPPGSKQEEVFEEIAMLVQSALDGYPVCIFAYGQTGSGKTFT 418
       ************************************************************

KIFC1  MEGGPRGDPQLEGLIPRAMRHLFSVAQEMSGQGWTYSFVASYVEIYNETVRDLLATGPRK 480
KIFC5B MEGGPRGDPQLAGLIPRAMRHLFSVAQEMSGQGWTYSFVASYVEIYNETVRDLLATGPRK 478
       *********** ********************************************

KIFC1  GQGGECEIRRASPGSEELTVTNARYVPVSCEKEVEALLHLAHQNRAVAHTAQNKRSSRSH 540
KIFC5B GQGGECEIRRASPGSEELTVTNARYVPVSCEKEVEALLHLAHQNRAVAHTAQNKRSSRSH 538
       ************************************************************

KIFC1  SVFQLQISGEHAARGLQCGAPLNLVDLAGSERLDPGLHLGPGERDRLRETQAINSSLSTL 600
KIFC5B SVFQLQISGEHAARGLQCGAPLNLVDLAGSERLDPGLPLGPGERDRLRETQAINSSLSTL 598
       ********************************** **********************

KIFC1  GLVIMALSNKESHVPYRNSKLTYLLQNSLGGSAKMLMFVNISPLEENVSESLNSLRFASK 660
KIFC5B GLVIMALSNKESHVPYRNSKLTYLLQNSLGGSAKMLMFVNISPLEENVSESLNSLRFASK 658
       ************************************************************

KIFC1  VNQCVIGTAQANKK 674
KIFC5B VNQCVIGTAQANKK 672
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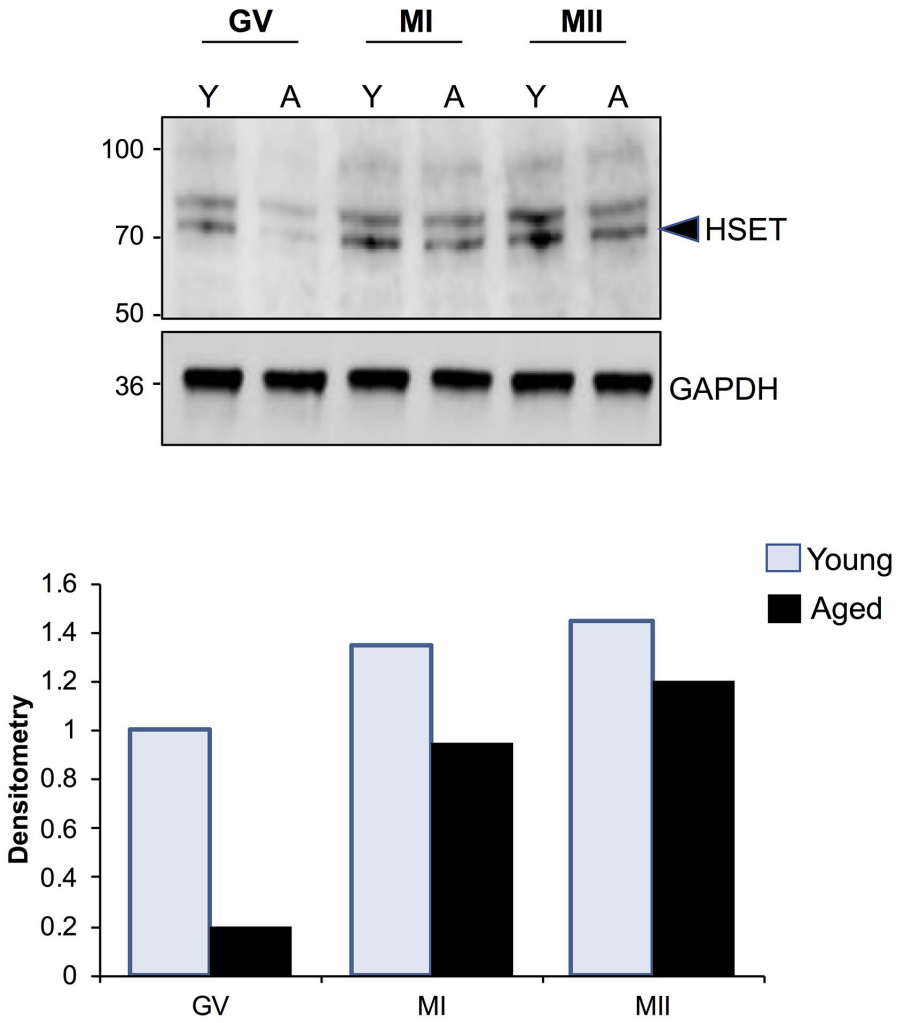
Supplementary Figure S3. Protein sequence alignment of Kifc1 and Kicf5b.

Supplementary Figure S4. Antibody specificity. Primary antibodies were substituted with the appropriate concentration of either anti-rabbit (red) or anti-mouse (green) IgG in negative control groups for immunocytochemistry. Oocytes were counterstained with the nuclear stain Hoechst 33342 (blue) and viewed using confocal microscopy. Scale bar = 20 µm.



Supplementary Figure S5. Anti-HSET immunoblot in young and aged oocytes. Immunoblotting with anti-HSET antibodies revealed a predominant band at the appropriate molecular weight of approximately 74 kDa in cell lysates prepared from isolated GV, MI and MII stage oocytes of young (Y) and aged (A) animals. Densitometric analysis of the labeling intensity of the HSET band relative to that of the GAPDH loading control, revealed an age-dependent decrease in the relative abundance of HSET in lysates of aged GV, MI and, to a lesser extent, MII stage oocytes. This experiment was repeated once with each lane being loaded with total protein lysates prepared from the equivalent of 100 oocytes (i.e. ~ 2 µg protein / lane).