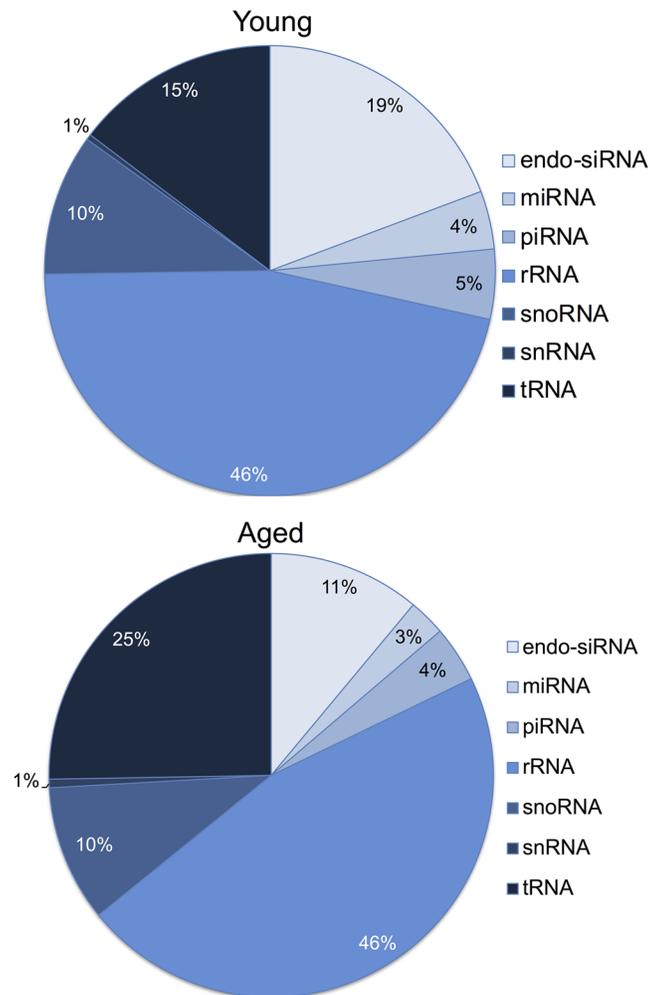


Supplementary Figures



Supplementary Figure S1. The small RNA signatures of young and aged oocytes. Following filtering and normalisation, snRNA reads were mapped to known 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

KIFC1	-----ATTCAAAAGTGGCGGGTGTGGCTGATTGGCCT	32
KIFC5B	GAAGTGCCTTACTGCGCGGAGGTTGAAATTCAAAGTACTGGTGTGGCGGATTGGCTT	60
	***** * ***** *	
KIFC1	GGCTCCTGGA-----CAGCCTGGCTTCGGCGTTTTGCGGCGGAGCCCTTGAGAG	82
KIFC5B	GGCCGATTGGCCTGGCGAATTGGCCTGGCTCCGACGTTTTGCGGCGGAGCCCTTGAGAG	120
	*** * * ***** ** *****	
KIFC1	GACGCGGGTCTCATCGTCCTGCCTGCTGCCTTTGCACC-CTTCTGTGCGGACCACCATG	141
KIFC5B	GACGCGGGTCTCGTCACCCTGCCTGCTGCCTTTGCATCCCTTCTGTGCGGACCACCATG	180
	***** * * ***** * * * *****	
KIFC1	GACGTGCAGGCGCAGAGGCCACCTTTGTTGGAAGTGAAGAGGAACGTAGAAGTGAAGGCA	201
KIFC5B	GACGTGCAGGCGCAGAGAAAAG---GGAAGGGAAGGGAAGAGGAACGTAGAAGTGAAGGCG	237
	***** *****	
KIFC1	GCCCTGGTGAAGTCCTCCTCCCGAGTGCCCTGTGTCAGCAAGCAGGCTCAAGAGGGTCCCT	261
KIFC5B	GCCCTGGTCAAGTCCTCCTCCCGACTGCCCTGTGTCAGCAAGCAGCCTCAAGAGGGTCCCT	297
	***** ***** ***** ***** *****	
KIFC1	GACCAGATGGAGGATGCCTTGGAGCCTGCAAAGAAACGGACACGAGTCATGGGTGCAGTG	321
KIFC5B	GACCAGATGGAGGATGCCTTGGAGCCTGCAAAGAAACGGACACGAGTCATGGGTGCAGTG	357

KIFC1	ACCAAAGTTGACACATCCCGTCCCAGAGGACCACTCCTCAGCACAGTGTACAGACCCAG	381
KIFC5B	ACCAAAGTTGACACATCCCGTCCCAGAGGACCACTCCTCAGCACAGTGTACAGACCCAG	417

KIFC1	GGCCACACTGCAGCTCAGAAAGGCCCTAAGAAGACAGGACCTCGTGGGTGCTCTGCTATT	441
KIFC5B	GGCCACAATGCAGCTCAGAAAGGCCCTAAGAAGACAGGACCTGGTGGGTGCTCTGCTGTT	477
	***** ***** ***** ***** *	
KIFC1	GGTACAGTGTGAGGAGCCAGAAGCCAGTTCCTCCGCTGCTCCTGCCAGAAGCCTGGCACA	501
KIFC5B	GGTACAGTGTGAGGAGCCAGAAGCCAGTTCCTCCGCTGCTCCTGC--GAAGCCTGGCACA	534
	***** ***** ***** ***** *****	
KIFC1	TCCACTGCTCCTGTGGTGGTAGGGAAGAGAGCTGGCAAACGCCCTGCCTGGGACCTGAAG	561
KIFC5B	TCCACTGCTCCTGTGGTGGTAGGGAAGAGAGCTGGCAAACGCCCTGCCTGGGACCTGAAG	594
	***** ***** ***** ***** *****	
KIFC1	GGCCAGTTGTGTGACCTCAATGAAGAGTTGAAACGCTATCGGGAGAAGACTCAAACGCTG	621
KIFC5B	GGCCAGTTGTGTGACCTCAATGAAGAGTTGAAACGCTATCGGGAGAAGACTGAAACGCTG	654
	***** *****	
KIFC1	GAAGTGGAGAACCGGGTCTTCGGGAGCAACTCAGAGAGGTCCAGGAGCAGGCCACGACC	681
KIFC5B	GAAGTGGAGAACCGGGTCTTCGGGAGCAACTCAGAGAGGTCCAGGAGCAGGCCACGACC	714
	***** *****	
KIFC1	CTGGGGACAGAGCGGAACACCCTGGAAGGGGAGCTGGCCAGTGTACGCAGCCGAGCTGAG	741
KIFC5B	CTGGGGACAGAGCGGAACACCCTGGAAGGGGAGCTGGCCAGTGTACGCAGCCGAGCTGAG	774
	***** *****	
KIFC1	CAGGACCAGCAGAGGCTGGAGACGCTGAGTGCCCGTGTCTTGAGCTGGAGGAATGTCTG	801
KIFC5B	CAGGACCAGCAGAGGCTGGAGACGCTGAGTGCCCGTGTCTTGAGCTGGAGGAATGTCTG	834
	***** ***** ***** ***** *****	
KIFC1	GGTACCAGGGAAGGCTGCTTCAGGAGCTTCAGGGAGAGCGGCTGCAATTGCAGGAGGAG	861
KIFC5B	GGTACCAGGGAAGGCTGCTTCAGGAGCTTCAGGGAGAGCGGCTGCAATTGCAGGAGGAG	894
	***** ***** ***** ***** *****	
KIFC1	CGGAGCACACTGAGCACCCAGCTGGAGGAGCAGGAGAGGAGTTTCAGGCCACAGAAGCA	921
KIFC5B	CGGAGCACACTGAGCACCCAAGTGGAGGAGCAGGAGAGGAGTTTCAGGCCACAGAAGCA	954
	***** ***** ***** ***** *****	
KIFC1	GCTCTGTCAAGCAGCCAAGAAGAGGTGTTGTGTTCTTCGGCAGAAGACTGAAGCCCAGGTG	981
KIFC5B	GCTCTGTCAAGCAGCCAAGAAGAGGTGTTGTGTTCTTCGGCAGAAGACTGAAGCCCAGGTG	1014
	***** ***** ***** ***** *****	
KIFC1	ACCTTACTGGCTGAGCAAGGAGACCGGCTCTATGGGTTAGAGATGGAGCGGCGA CGACTC	1041
KIFC5B	ACCTTACTGGCTGAGCAAGGAGACCGGCTCTATGGGTTAGAGATGGAGCGGCGG CGACTC	1074

Supplementary Figure S2 (Part 1). mRNA sequence alignment of Kifc1 and Kifc5b. Location of siRNA targeting sites, 5' and 3' untranslated regions are as marked.

RNA9867

KIFC1 **CACAACCAGCTGCA**AGGAACTGAAGGGCAATATCCGGGTGTTCTGCCGCGTGCGCCCTGTC 1101
KIFC5B **CACAACCAGCTGCA**AGGAACTGAAGGGCAATATCCGGGTGTTCTGCCGCGTGCGCCCTGTC 1134

KIFC1 CTCGAAGGGGAATCCACTCCATCTCCTGGCTTCCTCGTGTTCCTCCTGGCCCTGCTGGA 1161
KIFC5B CTCGAAGGGGAATCCACTCCATCTCCTGGCTTCCTCGTGTTCCTCCTGGCCCTGCTGGA 1194

KIFC1 CCCTCTGATCCCCGACGGGCCTTAGCCTCTCACGATCTGATGATCGGCGCTCCACCCTG 1221
KIFC5B CCCTCTGATCGCCGACGGGCCTTAGCCTCTCACGATCTGATGATCGGCGCTCCACCCTG 1254

KIFC1 ACTGGGGCCCCGGCCCCACTGTCCGCCATGATTTCTCCTTTGATCGGGTGTTCGCCGCCG 1281
KIFC5B ACTGGGGCCCCGGCACCCACTGTCCGCCATGATTTCTCCTTTGATCGGGTGTTCGCCGCCG 1314

KIFC1 GGAAGCAAGCAGGAGGAAGTGTGAGGAGATCGCCATGCTTGTCCAGTCAGCACTGGAT 1341
KIFC5B GGAAGCAAGCAGGAGGAAGTGTGAGGAGATCGCCATGCTTGTCCAGTCAGCACTAGAT 1374

KIFC1 GGCTACCTGTGTGCATTTTTGCCTATGGACAGACAGGCAGTGGCAAGACCTTCACTATG 1401
KIFC5B GGCTACCTGTGTGCATTTTTGCCTATGGACAGACAGGCAGTGGCAAGACCTTCACTATG 1434

KIFC1 GAAGGAGGGCCTAGGGGAGACCCCAATTGGAAGGGCTGATCCCTCGGGCCATGCGGCAT 1461
KIFC5B GAAGGAGGGCCTAGGGGAGACCCCAATTGGCAGGGCTGATCCCTCGGGCCATGCGGCAT 1494

KIFC1 CTGTTCTCTGTGGCCAGGAGATGAGCGGCCAGGCTGGACATACAGTTTTGTGGCGAGT 1521
KIFC5B CTGTTCTCTGTGGCCAGGAGATGAGCGGCCAGGCTGGACATACAGTTTTGTGGCGAGT 1554

KIFC1 TACGTAGAGATCTACAATGAGACCGTTCGAGAC**CTGCTAGCTACTGGGCCCGCAA**GGGA 1581 **RNA9878**
KIFC5B TACGTAGAGATCTACAATGAGACCGTTCGAGAC**CTGCTAGCTACTGGGCCCGCAA**GGGA 1614 **RNA9879**

KIFC1 CAAGGGGGCGAGTGTGAGATCCGTCGGGCAAGCCAGGAAGTGAGGAGCTTACTGTACC 1641
KIFC5B CAAGGGGGCGAGTGTGAGATCCGTCGGGCAAGCCAGGGAGTGAGGAGCTTACTGTACC 1674

KIFC1 AATGCCCGCTATGTCCCTGTTTCCTGTGAGAAAGAGGTGGAGGCCCTGCTCCATTTGGCT 1701
KIFC5B AATGCCCGCTATGTCCCTGTTTCCTGTGAGAAAGAGGTGGAGGCCCTGCTCCATTTGGCT 1734

KIFC1 CATCAGAACCGGGCTGTGGCCACACTGCCAAAATAAGAGATCATCACGCAGTCATAGT 1761
KIFC5B CACCAGAACCGGGCTGTGGCCACACTGCCAGAATAAGAGATCATCACGCAGTCATAGT 1794
** *****
KIFC1 GTGTTCCAGTCGAGATTTCTGGAGAGCATGCAGCTCGGGCCTGCAGTGTGGCGCTCCG 1821
KIFC5B GTGTTCCAGTCGAGATTTCTGGAGAGCATGCAGCCGGGGCCTGCAGTGTGGCGCTCC 1854

KIFC1 CTCAACCTTGTGGACCTAGCTGGGAGTGAGCGGCTAGACCCTGGCTTACACCTAGGCCCT 1881
KIFC5B CTCAACCTTGTGGACCTAGCTGGGAGTGAGCGGCTAGACCCTGGCTTACCCCTAGGCCCT 1914

KIFC1 GGGGAGCGTGATCGTCTTCGGGAGACACAGGCCATTAACAGCAGTCTGTCTACACTGGGA 1941
KIFC5B GGGGAGCGTGATCGTCTTCGGGAGACACAGGCCATTAACAGCAGTCTGTCTACATTGGGA 1974

KIFC1 CTGGTCATAATGGCCCTGAGCAATAAGGAGTCCCACGTGCCTTACCGAAACAGCAAGCTC 2001
KIFC5B CTGGTCATAATGGCCCTGAGCAATAAGGAGTCCCACGTGCCTTACCGAAACAGCAAGCTC 2034

KIFC1 ACCTACTTGCTGCAGAACTCTCTGGGTGGCAGTGCCAAGATGCTTATGTTTGTGAATATT 2061
KIFC5B ACCTACTTGCTGCAGAACTCTCTGGGTGGCAGTGCCAAGATGCTTATGTTTGTGAATATT 2094

KIFC1 TCTCCTCTGGAAGAGAATGTCTCCGAGTCTCTGAATTCACCTACGCTTTGCTTCCAAGGTG 2121
KIFC5B TCTCCTCTGGAAGAGAATGTCTCCGAGTCTCTGAATTCACCTACGCTTTGCTTCCAAGGTG 2154

Supplementary Figure S2 (Part 2). mRNA sequence alignment of Kifc1 and Kifc5b. Location of siRNA targeting sites, 5' and 3' untranslated regions are as marked.

```

KIFC1  AACCAGTGTGTCATTGGTACTGCTCAGGCTAATAAGAAGTGAAGTCCGGATCCAGAGCCTG 2181
KIFC5B  AACCAGTGTGTCATTGGTACTGCTCAGGCTAATAAGAAGTGAAGTCCGGATCCAGAGC--- 2211
*****

KIFC1  ATTC CCTT GCAAGCCAGT GCGCAT GCGTCTCTG TTTTAGTGTGTA----TTCGGTGGGGG 2237
KIFC5B  ----CCTT GCAAGCCAGT GCGCAT GCGTCTCTG TTTTAGTGTGTAATTTATTCGGTGGGGG 2267
*****

KIFC1  TGGGTGGGAGTTGAGACATGATTTTATGGGTGAACAATATTTATTATGTAATCAACTAT 2297
KIFC5B  TGGGTGGGAGTTGAGACATGATATTACTGGG-----TATTTATTATGTAATAAAGAAT 2320
*****

KIFC1  AAAT----AAAGAATAATCTGTTGGTTAAAA-----AAAAA----- 2330
KIFC5B  AATCTTTTGTATGTTAATCCTTTTCTTAATCTTAGATTTTAAAAAATTGTGTGCAGTTGT 2380
**      *      ***** **      *****

KIFC1  ----- 2330
KIFC5B  TTTGCCTGAGTGTATATGCATGCACCATGTGCATGTCTCCTGCTCCAGTGAGTTCAGAAC 2440

KIFC1  ----- 2330
KIFC5B  AGGGCTTCAGGTCCTTCAAGAACTGGGATTATGGAATACATGTGGGTCCCTCGCAAAATC 2500

KIFC1  ----- 2330
KIFC5B  AGTCAGTGCTCTTAACCGCCAGCCATCTTCCCAGCCGCCCCTTGTACAGCTCTAAAC 2560

KIFC1  ----- 2330
KIFC5B  GGTTTTATTAACCTAACGGTTTTAGCTGCCAAAGGAAGGTATTGTAAATAAATACTGTAA 2620

KIFC1  ----- 2330
KIFC5B  GCACGTA 2627

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3' UTR

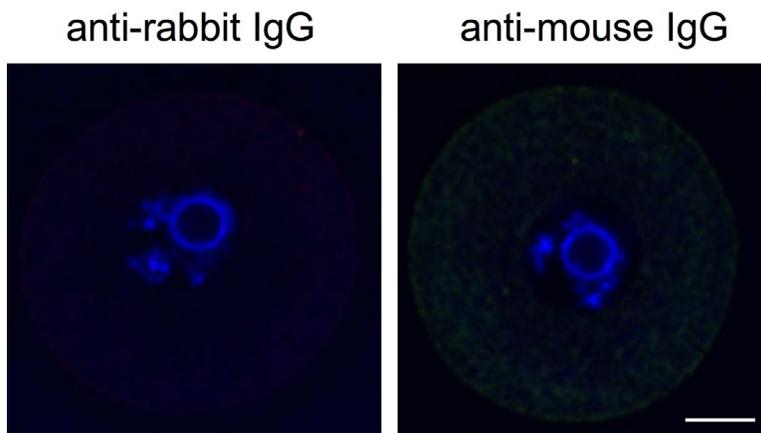
Supplementary Figure S2 (Part 3). mRNA sequence alignment of Kifc1 and Kifc5b. Location of siRNA targeting sites, 5' and 3' untranslated regions are as marked.

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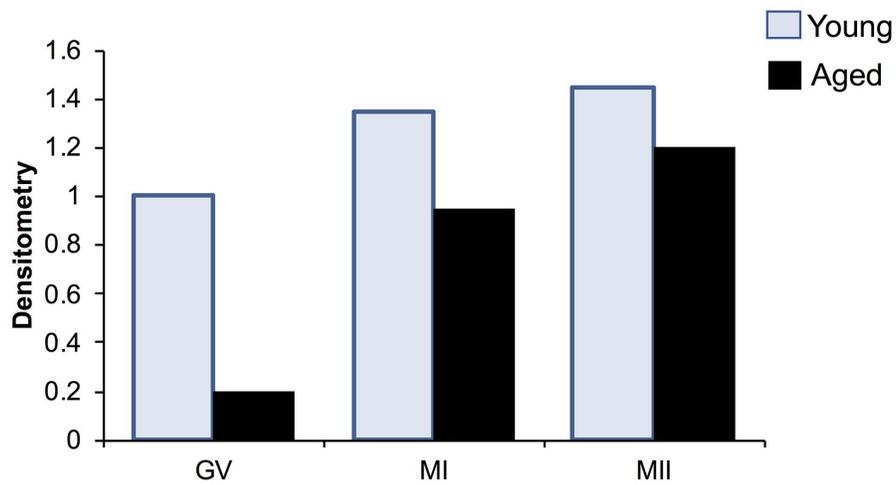
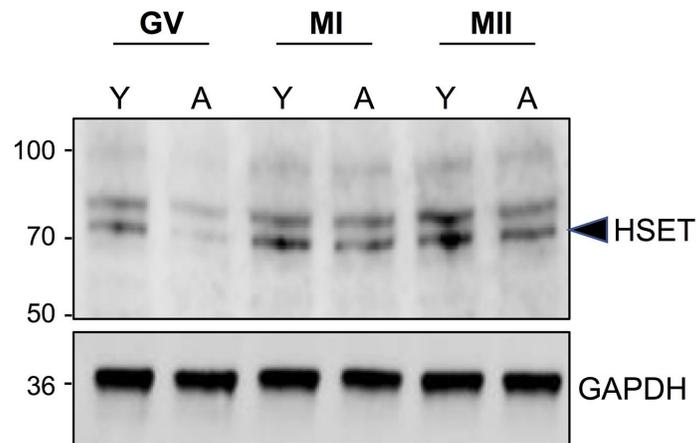
KIFC1 MDVQAQRPPLEVKRNVELKAALVKSSSRVPLSASRLKRGPDQMEDALEPAKKRTRVMGA 60
KIFC5B MDVQAQRKG-REGKRNVELKAALVKSSSRLPLSASSLKRGPDPQMEDALEPAKKRTRVMGA 59
***** * *****:***** *****
KIFC1 VTKVDTSRPRGPLLSTVSQTQGHTAAQKGPCKTGPARGCSAIGTVLRSQKPVAAPAQKPG 120
KIFC5B VTKVDTSRPRGPLLSTVSQTQGHNAAQKGPCKTGGGCSAVGTVLRSQKPAPAAPA-KPG 118
***** *****:***** *****
KIFC1 TSTAPVVVGKRAKRPADLKGQLCDLNEELKRYREKTQTLELENRGLREQLREVQEAT 180
KIFC5B TSTAPVVVGKRAKRPADLKGQLCDLNEELKRYREKTETLELENRGLREQLREVQEAT 178
*****:*****
KIFC1 TLGTERNTLEGELASVRSRAEQDQORLETLSARVLELEECLGTRELLQELQGERLQLQE 240
KIFC5B TLGTERNTLEGELASVRSRAEQDQORLETLSARVLELEECLGTRELLQELQGERLQLQE 238
*****
KIFC1 ERSTLSTQLEEQERRFQATEAALSSSQEEVCLRQKTEAQVTLLEAEQGDRLYGLEMERRR 300
KIFC5B ERSTLSTQLEEQERRFQATEAALSSSQEEVCLRQKTEAQVTLLEAEQGDRLYGLEMERRR 298
*****
KIFC1 LHNQLQELKGNIRVFCRVRPVLEGESTPSPGFLVFPFPGPAGPSDPPTGLSLRSDDRRST 360
KIFC5B LHNQLQELKGNIRVFCRVRPVLEGESTPSPGFLVFPFPGPAGPSDRPTGLSLRSDDRRST 358
*****
KIFC1 LTGAPAPTVRHDFSFDRVFPFPGSKQEEVFEEIAMLVQSALDGYPVCIFAYGQTGSGKTFT 420
KIFC5B LTGAPAPTVRHDFSFDRVFPFPGSKQEEVFEEIAMLVQSALDGYPVCIFAYGQTGSGKTFT 418
*****
KIFC1 MEGGPRGDPQLEGLIPRAMRHLSVAQEMSGQGWYTSFVASYVEIYNETVRDLLATGPRK 480
KIFC5B MEGGPRGDPQLAGLIPRAMRHLSVAQEMSGQGWYTSFVASYVEIYNETVRDLLATGPRK 478
*****
KIFC1 GQGGECEIRRASPGSEELTVTNARYVPVSCKEKEVEALLHLAHQNRAVAHTAQNKRSSRSH 540
KIFC5B GQGGECEIRRASPGSEELTVTNARYVPVSCKEKEVEALLHLAHQNRAVAHTAQNKRSSRSH 538
*****
KIFC1 SVFQLQISGEHAARGLQCGAPLNLVDLAGSERLDPGLHLGPGERDRLRETQAINSSLSTL 600
KIFC5B SVFQLQISGEHAARGLQCGAPLNLVDLAGSERLDPGLPLGPGERDRLRETQAINSSLSTL 598
*****
KIFC1 GLVIMALSNKESHVPYRNSKLTLYLLQNSLGGSAKMLMFVNISPLEENVSESLNSLRFASK 660
KIFC5B GLVIMALSNKESHVPYRNSKLTLYLLQNSLGGSAKMLMFVNISPLEENVSESLNSLRFASK 658
*****
KIFC1 VNQCIVIGTAQANKK 674
KIFC5B VNQCIVIGTAQANKK 672
*****

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Supplementary Figure S3. Protein sequence alignment of Kifc1 and Kif5b.



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 μ m.



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 μ g protein / lane).