

Supplementary Materials

RNA m6A dynamic modification mediated by nucleus-localized FTO

is involved in follicular reserve

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Fto-wt

ATGAAGCGCGTCCAGACCGCGGAGGAACGAGAGCGGGAAGCTAAGAAACTGAGGCTCCTTGAGGA GCTTGAAGACACTTGGCTTCCTTACCTGACCCCCAAAGATGATGAGTTCTATCAGCAGTGGCAGCTG AAATACCCTAAACTGGTTTTCCGAGAGGCCGGCAGCATACCAGAGGAGCTGCATAAGGAGGTCCCC GAGGCCTTTCTCACACTGCATAAGCATGGCTGCTTGTTTCGGGACGTGGTGAGGATCCAAGGCAAA GATGTGCTCACCCCAGTGTCTCGCATCCTCATCGGGGACCCAGGCTGCACCTACAAGTACTTGAAC ACCAGACTCTTCACGGTGCCCTGGCCCGTGAAGGGCTGCACGGTCAAGTACACAGAGGCTGAGATC GCCGCTGCATGTCAGACCTTCCTAAAGCTCAATGACTACCTCCAGGTGGAGACCATCCAGGCCTTG GAAGAACTGGCTGTCAGAGAGAAGGCCAATGAAGACGCTGTGCCACTGTGCATGGCAGAGTTCCCC AGGGCCGGCGTGGGGCCGTCCTGCGATGATGAAGTGGACCTTAAGAGCAGAGCAGCCTACAACGT GACTTTGCTAAACTTCATGGATCCTCAGAAGATGCCCTACTTGAAAGAGGAGCCCTATTTCGGCATG GGGAAGATGGCGGTGAGCTGGCATCACGATGAGAACCTGGTGGACAGGTCAGCCGTGGCAGTGTA CAGCTATAGCTGCGAAGGCTCTGAGGATGAAAGTGAGGACGAGTCCAGCTTCGAAGGCAGAGATCC TGATACTTGGCATGTTGGTTTTAAGATCTCTTGGGACATCGAGACACCAGGATTAACAATCCCTCTTC ACCAGGGAGACTGCTATTTCATGCTGGATGACCTCAATGCCACCACCAGCACTGTGTTTTGGCTGG CTCACAGCCTCGGTTTAGTTCCACTCACCGTGTGGCAGAGTGCTCAACAGGCACCTTGGATTATATC TTAGAACGCTGTCAGTTGGCGCTGCAGAATGTCCTCAATGACTCAGACGATGGCGACGTCTCGTTGA AATCCTTTGATCCTGCAGTTTTGAAACAAGGAGAGAGAAATCCATAATGAGGTGGAGTTTGAGTGGCT GAGGCAGTTCTGGTTTCAAGGCAATCGATACAAACTTTGCACCGATTGGTGGTGTGAGCCCATGACT CACCTGGAGGGGCTGTGGAAGAAGATGGAGAGCATGACAAATGCGGTGCTCCGTGAAGTTAAAAGA CCAGAACCTGAGGAAGGAGTGGCATGCCAGGTGCCAGTCCCGAGTCGTCCGGACTTTACCAGTACA GCAGAAACCAGACTGCCGGCCATATTGGGAGAAGGATGACCCTTCCATGCCTCTGCCCTTTGACCT CACAGACGTGGTTTCCGAGCTCAGAGGCCAGCTGCTGGAAGCAAGATCC

Fto-∆NLS

ATGAGGCTCCTTGAGGAGCTTGAAGACACTTGGCTTCCTTACCTGACCCCCAAAGATGATGAGTTCT ATCAGCAGTGGCAGCTGAAATACCCTAAACTGGTTTTCCGAGAGGCCGGCAGCATACCAGAGGAGC TGCATAAGGAGGTCCCCGAGGCCTTTCTCACACTGCATAAGCATGGCTGCTTGTTTCGGGACGTGG TGAGGATCCAAGGCAAAGATGTGCTCACCCCAGTGTCTCGCATCCTCATCGGGGACCCAGGCTGCA CCTACAAGTACTTGAACACCAGACTCTTCACGGTGCCCTGGCCCGTGAAGGGCTGCACGGTCAAGT ACACAGAGGCTGAGATCGCCGCTGCATGTCAGACCTTCCTAAAGCTCAATGACTACCTCCAGGTGG GCATGGCAGAGTTCCCCAGGGCCGGCGTGGGGGCCGTCCTGCGATGATGAAGTGGACCTTAAGAGC AGAGCAGCCTACAACGTGACTTTGCTAAACTTCATGGATCCTCAGAAGATGCCCTACTTGAAAGAGG AGCCCTATTTCGGCATGGGGAAGATGGCGGTGAGCTGGCATCACGATGAGAACCTGGTGGACAGGT CAGCCGTGGCAGTGTACAGCTATAGCTGCGAAGGCTCTGAGGATGAAAGTGAGGACGAGTCCAGCT TCGAAGGCAGAGATCCTGATACTTGGCATGTTGGTTTTAAGATCTCTTGGGACATCGAGACACCAGG ATTAACAATCCCTCTTCACCAGGGAGACTGCTATTTCATGCTGGATGACCTCAATGCCACCACCAG CACTGTGTTTTGGCTGGCTCACAGCCTCGGTTTAGTTCCACTCACCGTGTGGCAGAGTGCTCAACAG GCACCTTGGATTATATCTTAGAACGCTGTCAGTTGGCGCTGCAGAATGTCCTCAATGACTCAGACGA GTGGAGTTTGAGTGGCTGAGGCAGTTCTGGTTTCAAGGCAATCGATACAAACTTTGCACCGATTGGT GGTGTGAGCCCATGACTCACCTGGAGGGGCTGTGGAAGAAGATGGAGAGCATGACAAATGCGGTG GTCCCGCTCACCGTGCGCCAGAACCTGAGGAAGGAGTGGCATGCCAGGTGCCAGTCCCGAGTCGT CCGGACTTTACCAGTACAGCAGAAACCAGACTGCCGGCCATATTGGGAGAAGGATGACCCTTCCAT GCCTCTGCCCTTTGACCTCACAGACGTGGTTTCCGAGCTCAGAGGCCAGCTGCTGGAAGCAAGATC C

Supplementary Figure S1 Detailed sequences of full-length *Fto* and *Fto* without NLS for *Fto* expression

Sequence marked in red is NLS.

Cdk5

GAGATTGTGAAGTCATTCCTCTTCCAGCTGCTGAAAGGCC	40
TGGGATTCTGTCACAGCCGCAACGTGCTACATAGGGACCT	80
GAAGCCCCAGAACCTGCTCATAAACAGGAATGGGGAGTTG	120
AAATTGGCTGATTTTGGCCTGGCCCGAGCCTTTGGTATCC	160
CCGTCCGCTGCTACTCTGCTGAGGTGGTCACGCTGTGGTA	200

Cdk5-VS

GAGATTGTGAAGAATGGGGAGTTGAAATTGGCTGATTTTG	40
GCCTGGCCCGAGCCTTTGGTATCCCCGTCCGCTGCTACTC	80
TGCTGAGGTGGTCACGCTGTGGTA	104

Supplementary Figure S2 Detailed sequences of two *Cdk5* (*Cdk5* and *Cdk5-VS*) isoforms (*Cdk5* and *Cdk5-VS*) Highlighted sequence is the sequence of exon 7 that does not exist in *Cdk5-VS*.



Supplementary Figure S3 m6A sites predicted in *Ki67*, *Pcna*, and *Cdk5* mRNA by SRAMP software



Supplementary Figure S4 Sequencing results of two *Cdk5* alternative splicing isoforms (*Cdk5* and *Cdk5-VS*)

Arrows show alternative splicing sites. Sequence in red box is partial sequence of exon 7 that does not exist in Cdk5-VS.



Supplementary Figure S5 *Alkbh5* mRNA expression level during primordial follicle assembly detected by RT-qPCR **: P<0.01.

Gene	Primer sequence (5'-3')
Gapdh	F: TCGGAGTGAACGGATTTGGC
	R: TGCCGTGGGTGGAATCATAC
Fto	F: GGTCCGATCAATCATCTGT
	R: GAAGTAACGAATAGGCATGT
Lhx8	F: ACACGAGCTGCTACATTAAGGA
	R: CCAGTCAGTCGAGTGGATGTG
Figla	F: GCTCTGGTGCCGTTTCTACC
	R: ACACAGCCGAGTATCTGTATGTA
Ki67	F: ATCATTGACCGCTCCTTTAGGT
	R: GCTCGCCTTGATGGTTCCT
Pcna	F: TTGCACGTATATGCCGAGACC
	R: GGTGAACAGGCTCATTCATCTCT
Klf4	F: GGCGAGTCTGACATGGCTG
	R: GCTGGACGCAGTGTCTTCTC
Pck1	F: CTGCATAACGGTCTGGACTTC
	R: GCCTTCCACGAACTTCCTCAC
Ptprn	F: TGTTTGACCGCAGACTTTGTT
	R: GGAGCACACCTTGTAGGCG
Ereg	F: AACTGTACCGCCTTAGTTCAGA
	R: TCACATCGCAGACCAGTGTAG
Alkbh5	F: GCATACGGCCTCAGGACATTA
	R: TTCCAATCGCGGTGCATCTAA

Supplementary Table 1 Primers used for RT-qPCR.