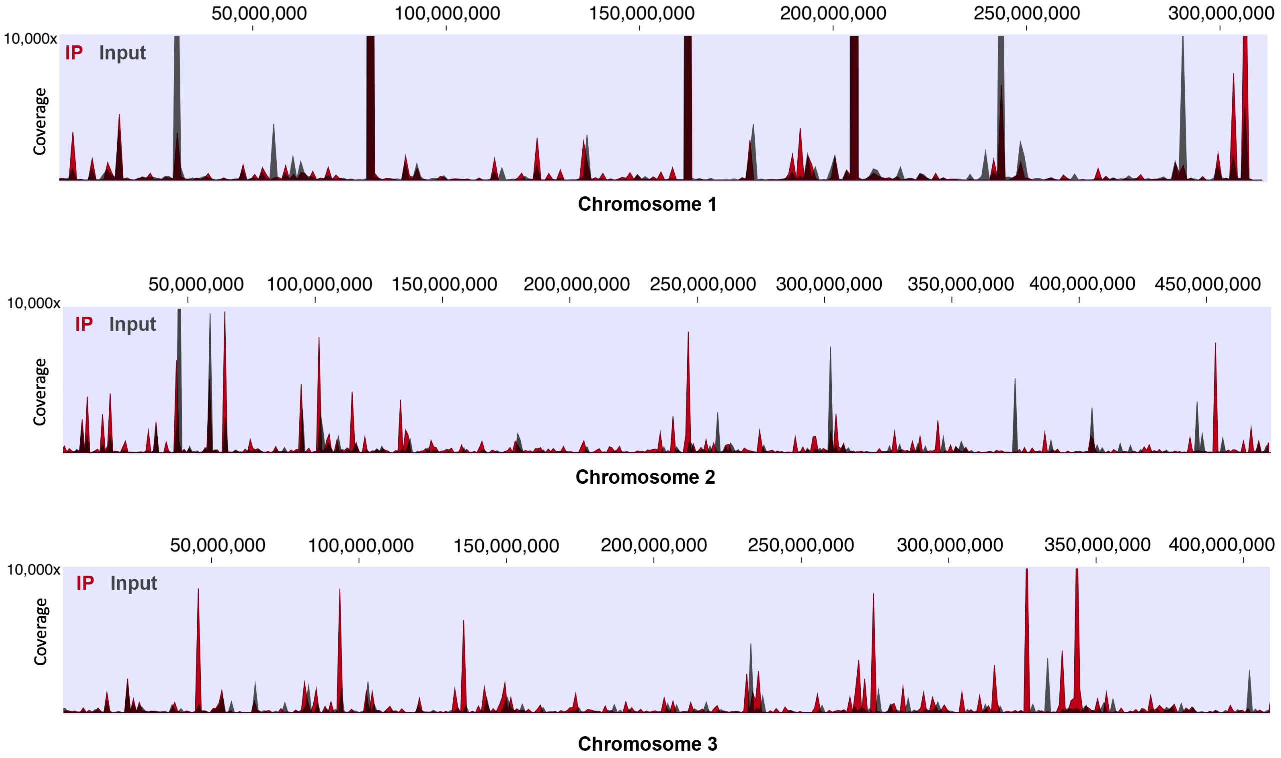
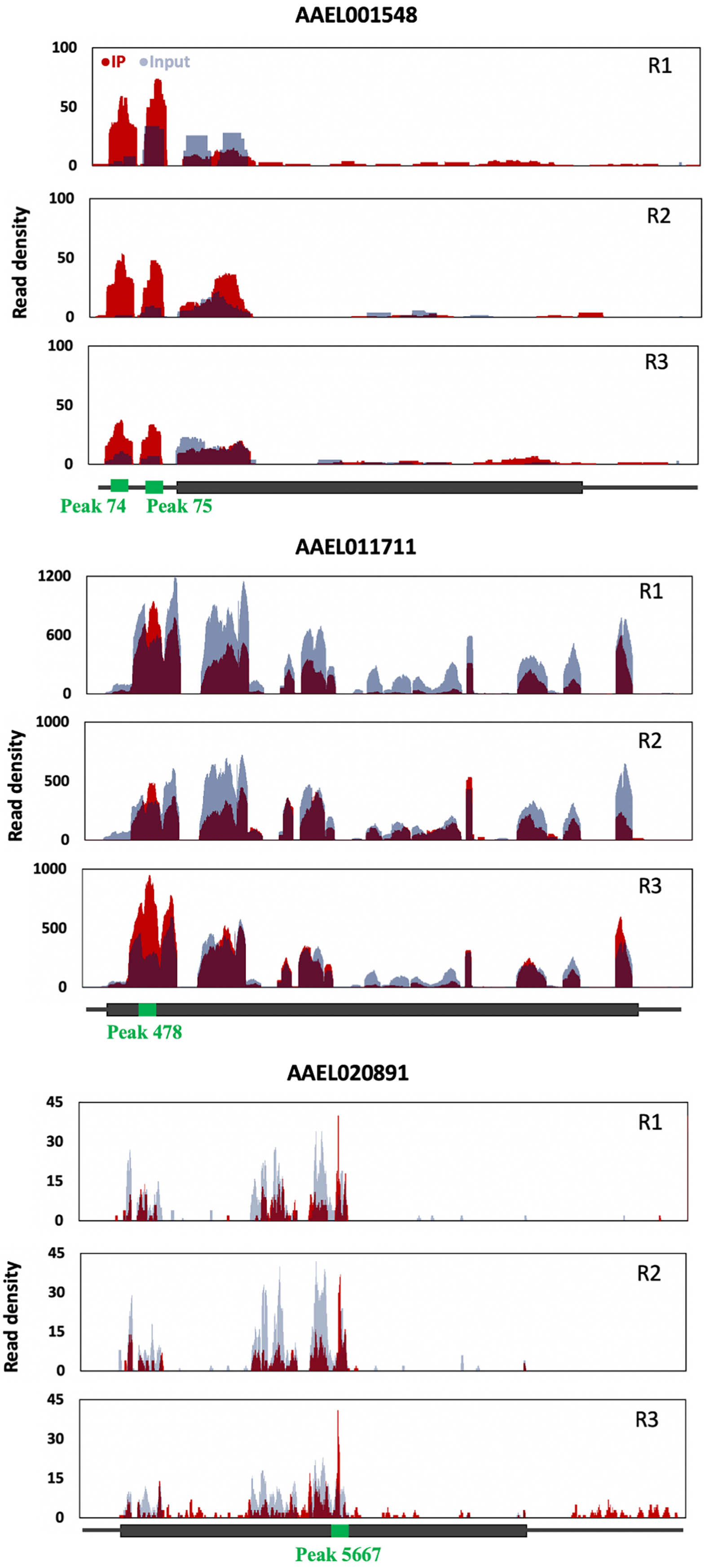
# $N^6$-methyladenosine modification of the *Aedes aegypti* transcriptome and its alteration upon dengue virus infection in Aag2 cell line

Zhenkai Dai, Kayvan Etebari and Sassan Asgari*

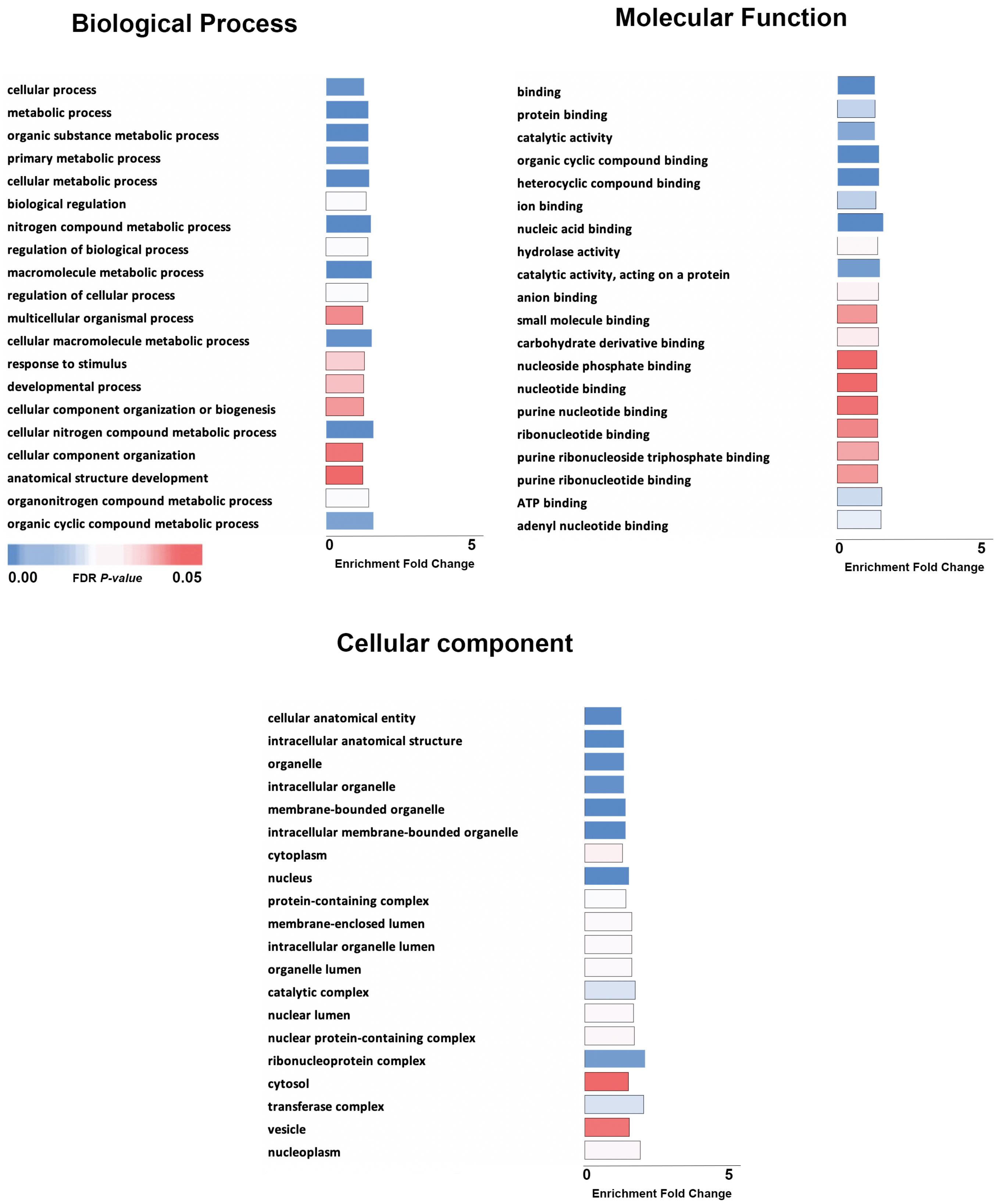
## Supplementary information



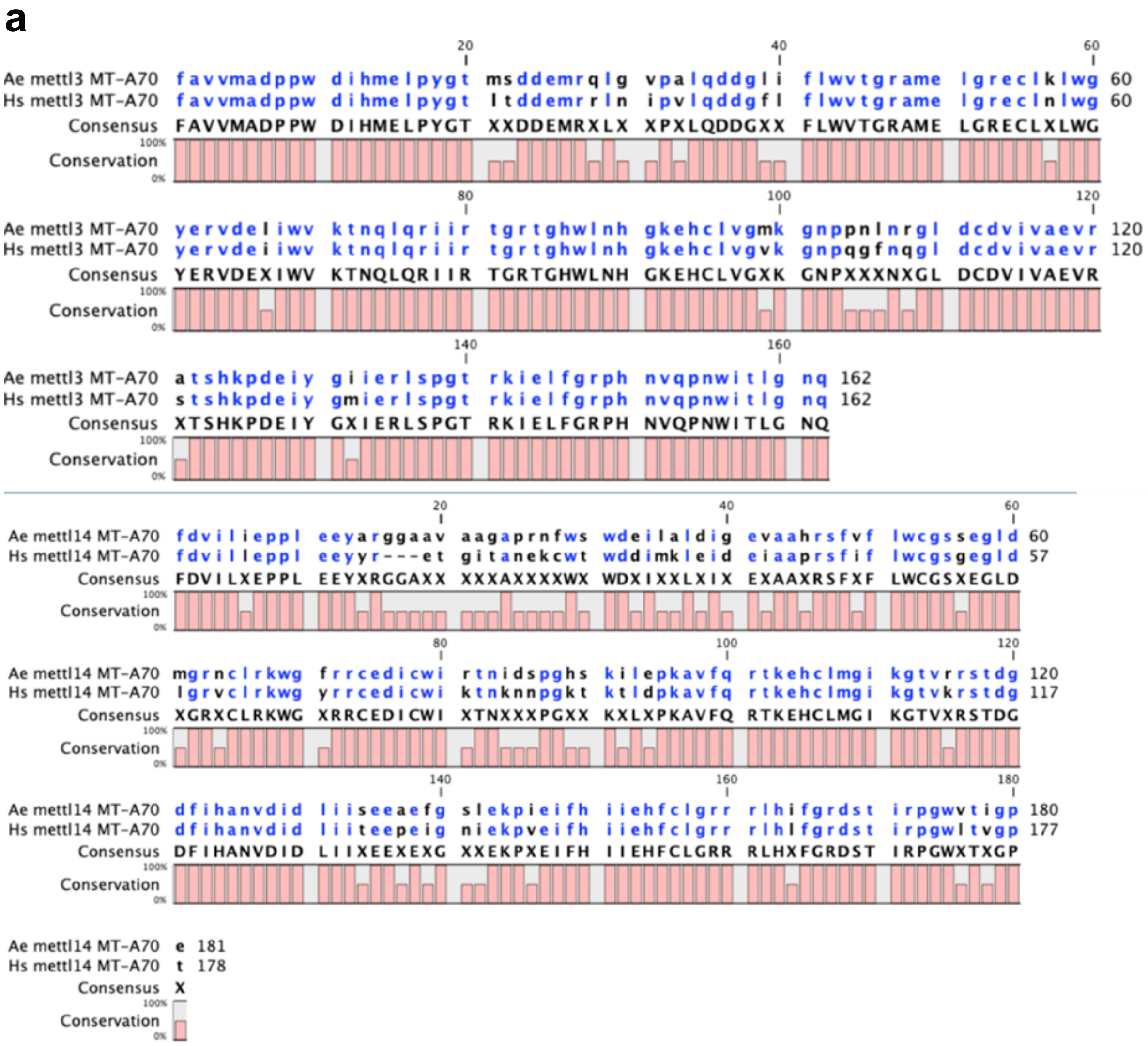
**Supplementary Fig. 1:** The reads distribution patterns from Input and IP groups at the *Aedes aegypti* chromosomal level. The read density chart determined by mapping MeRIP-Seq data of RNA isolated from Aag2 cells against AaegL5.0 genome reference.



**Supplementary Fig. 2:** Distribution of m$^6$A peaks in Input and IP groups in three replicates for three representative *Aedes aegypti* genes with identified m$^6$A peaks determined by MeRIP-Seq of RNA isolated from Aag2 cells.



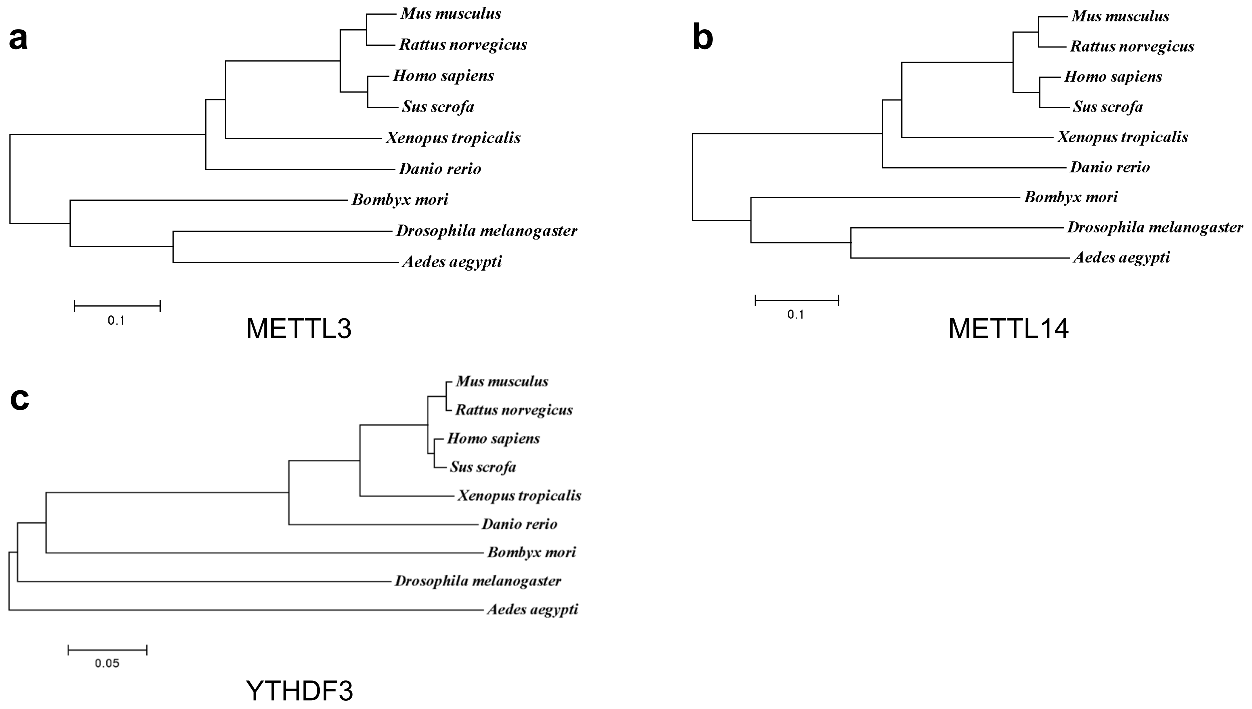
**Supplementary Fig. 3: Gene ontology term enrichment analysis of *Ae. aegypti* transcripts with m$^6$A modification.** Genes were classified into three categories of biological process, molecular function, and cellular component.


a
20
40
60
Ae mettl3 MT-A70 favvmadppw dihmelpygt msddemrqlg vpalqddgli flwvtgrame lgreclklwg 60
Hs mettl3 MT-A70 favvmadppw dihmelpygt ltddemrrln ipvlqddgfl flwvtgrame lgreclnlwg 60
Consensus FAVVMADPPW DIHMELPYGT XXDDEMRXLX XPXLQDDGXX FLWVTGRAME LGRECLXLWG
100%
Conservation
0%
80
100
120
Ae mettl3 MT-A70 yervdeliwv ktnqlqriir tgrtghwlnh gkehclvgmk gnppnlnrgl dcdvivaevr 120
Hs mettl3 MT-A70 yervdeiiwv ktnqlqriir tgrtghwlnh gkehclvgvk gnpqgfnqgl dcdvivaevr 120
Consensus YERVDEXIWV KTNQLQRIIR TGRTGHWLNH GKEHCLVGXK GNPXXXNXGL DCDVIVAEVR
100%
Conservation
0%
140
160
Ae mettl3 MT-A70 atshkpdeiy giierlspgt rkielfgrph nvqpnwitlg nq 162
Hs mettl3 MT-A70 stshkpdeiy gmierlspgt rkielfgrph nvqpnwitlg nq 162
Consensus XTSHKPDEIY GXIERLSPGT RKIELFGRPH NVQPNWITLG NQ
100%
Conservation
0%
20
40
60
Ae mettl14 MT-A70 fdvilieppl eeyarggaav aagaprnfws wdeilaldig evaahrsfvf lwcgssegld 60
Hs mettl14 MT-A70 fdvilleppl eeyyr---et gitanekcwt wddimkleid eiaaprsfif lwcgsgegld 57
Consensus FDVILXEPPL EEYXRGGAXX XXXAXXXXWX WDXIXXLXIX EXAAXRSFXF LWCGSXEGLD
100%
Conservation
0%
80
100
120
Ae mettl14 MT-A70 mgrnclrkwg frrcedicwi rtnidspghs kilepkavfq rtkehclmgi kgtvrrstdg 120
Hs mettl14 MT-A70 lgrvclrkwg yrrcedicwi ktnknnpgkt ktldpkavfq rtkehclmgi kgtvkrstdg 117
Consensus XGRXCLRKWG XRRCEDICWI XTNXXXPGXX KXLXPKAVFQ RTKEHCLMGI KGTVXRSTDG
100%
Conservation
0%
140
160
180
Ae mettl14 MT-A70 dfihanvdid liiseeaefg slekpieifh iiehfclgrr rlhifgrdst irpgwvtigp 180
Hs mettl14 MT-A70 dfihanvdid liiteepeig niekpveifh iiehfclgrr rlhlfgrdst irpgwltvgp 177
Consensus DFIHANVDID LIIXEEXEXG XXEKPXEIFH IIEHFCLGRR RLHXFGRDST IRPGWXTXGP
100%
Conservation
0%
Ae mettl14 MT-A70 e 181
Hs mettl14 MT-A70 t 178
Consensus X
100%
Conservation
0%

**b**

```
                                              20                     40                     60                     80                    100
YTHDF 3 [Homo sapiens]   MSATSVDQRP KGQGN----- ---------- ---------- -KVSVQNGSI HQKD------ ---------AV NDDDFEPY-- ---------- 38
YTHDF 3 [Aedes aegypti]  MSTVS-DQRM KGQGNHGYGY GSQQTTSGAQ FQYPPFSGVG ETTTAWSNGT ESIAIIGGND YNNSWGTQQV NHGHAGAGAG GQSQRKPYDD YYHRNQGAYH 99
             Consensus   MSXXSVDQRX KGQGNHGYGY GSQQTTSGAQ FQYPPFSGVG ETTTAWSNGT EXXXXXXGXX XXXXWGTQQV NHGHAGAGAX XXXXXXPYDD YYHRNQGAYH
          Conservation

                                             120                    140                    160                    180                    200
YTHDF 3 [Homo sapiens]   ---------- ---------- ------LSSQ TNQSNS---- ---------- ---------- ---------- ---------Y PPMSDPYMPS YYAPSIGFPY 69
YTHDF 3 [Aedes aegypti]  DGIKNVEQGM QGLGLGSMRH NRDGNHHSSH GNSSLSKSDQ HHQQQKEAPK KMTWASIASQ PAKPQVNTTS TTVKKKGPGM PP--PPMVPG KHNMDIG--- 194
             Consensus   DGIKNVEQGM QGLGLGSMRH NRDGNHXSSX XNXSXSKSDQ HHQQQKEAPK KMTWASIASQ PAKPQVNTTS TTVKKKGPGX PPMSXPXXPX XXXXXIGFPY
          Conservation

                                             220                    240                    260                    280                    300
YTHDF 3 [Homo sapiens]   SLGEAAWSTA GDQPMPYLTT YGQMSNGEHH YIPDGV---- ----FSQPGA LGNTPPFLGQ HGFNFFPGNA DFSTWGTSGS QGQS------ -TQSSAYSSS 154
YTHDF 3 [Aedes aegypti]  -----TW--- -DSPS----- ----KNGPNA MVPTPTPPPI VPPPVIEPSP LAE-PPAGGK GG----PNNA P--TMGGGGH EGGSGRHHPH HQQYQQQQQN 269
             Consensus   SLGEAXWSTA GDXPXPYLTT YGQMXNGXXX XXPXXXPPPI VPPPXXXPXX LXXTPPXXGX XGFNFFPXNA XFSTXGXXGX XGXSGRHHPH HXQXXXXXXX
          Conservation

                                             320                    340                    360                    380                    400
YTHDF 3 [Homo sapiens]   YGYPPS-SLG RAITDGQAGF GNDTLSKVPG ISSIEQGMTG L----KIGGD LTAAVTKTVG TALSSSGMTS IATNSVPPVS SAAPKPTSWA AIARKPA-KP 248
YTHDF 3 [Aedes aegypti]  MGPPPGVQNS RWPTPGQA-- -QNPLPQQPP QQQQPQSQHP QHQQNQSGGG MMSQQHHAQR PDHRQYHQDR PQHNNRGNYS GPPPSMNQQG GYHQPPPYHQ 366
             Consensus   XGXPPXVXXX RXXTXGQAGF GXXXLXXXPX XXXXXQXXXX XHQQNXXGGX XXXXXXXXXX XXXXXXXXXX XXXNXXXXXS XXXPXXXXXX XXXXXPXYXX
          Conservation

                                             420                    440                    460                    480                    500
YTHDF 3 [Homo sapiens]   QPKLKPKGNV GIGGSAVPPP PIKHNMNIGT WDEKGSVVKA PPTQPVLPPQ ---------- ------TIIQ QPQPLIQPPP LVQSQLPQQQ PQPPQPQQQQ 332
YTHDF 3 [Aedes aegypti]  QPPAMQHHHS RQNQNHHEPP PAHHQQQHPQ SNERGSY-QQ PPPQHRMPQE DNRRGPSNGP NNYNQDNQVS QPQPTVAPP- -------QQQ PQPPQQQQQQ 457
             Consensus   QPXXXXXXXX XXXXXXXXPP PXXHXXXXXX XXEXGSXVXX PPXQXXXPXX DNRRGPSNGP NNYNQDXXXX QPQPXXXPPP LVQSQLPQQQ PQPPQXQQQQ
          Conservation

                                             520                    540                    560                    580                    600
YTHDF 3 [Homo sapiens]   GPQPQAQPHQ V-----QPQQ QQLQNRWVAP RNRGAGFNQN NGAGSENFGL GVVPVSASPS SVEV---HP- --VLEKLKAI NNYNPKDFDW --NLKNGRVF 419
YTHDF 3 [Aedes aegypti]  QP-PQPQPPV QPQPAVQPQQ PPMQQPPVHP ---------- ---------- ---PLSPTPS SGETAAANPP AAILNQLQNK NNYNPTNLDM LETAHLARFF 533
             Consensus   XPQPQXQPXX XPQPAVQPQQ XXXQXXXVXP RNRGAGFNQN NGAGSENFGL GVVPXSXXPS SXEXAAAXPP AAXLXXLXXX NNYNPXXXDX LEXXXXXRXF
          Conservation

                                             620                    640                    660                    680                    700
YTHDF 3 [Homo sapiens]   I KSYSEDDI HRSIKYSIWC STEHGNKRLD AAYRSLNGKG PL-YLLFSVN GSGHFCGVAE MKSVVDYNAY AGVWSQDKWK GKFEVKWIFV KDVPNNQLRH 518
YTHDF 3 [Aedes aegypti]  V KSYSEDDI HRSIKYEIWC STEHGNQRLD QAYREREEKG GMVYLFFSVN GSGHFCGIAQ MMTAVDYNSV STVWSQDKWK GTFKVRWIYV KDVPNAQLRQ 633
             Consensus   X KSYSEDDI HRSIKYXIWC STEHGNXRLD XAYRXXXXKG XXVYLXFSVN GSGHFCGXAX MXXXVDYNXX XXVWSQDKWK GXFXVXWIXV KDVPNXQLRX
          Conservation

YTHDF 3 [Homo sapiens]   IRLENNDNKP VTNSRDTQEV PLEKAKQVLK IIATFKHTTS IFDDFAHYEK RQEEEEAMRR E--------- ---------- ---------- ---------- 579
YTHDF 3 [Aedes aegypti]  VRLENNENKP ITNSRDTQEV PNAKGIQALK IIHSFKHTMS IFDDFIHYEQ RQLEENTKKH DVGHQQPSYR PQQYGGGYDG PSKYHNSYNK YNDRDGGSDG 733
             Consensus   XRLENNXNKP XTNSRDTQEV PXXKXXQXLK IIXXFKHTXS IFDDFXHYEX RQXEEXXXXX XVGHQQPSYR PQQYGGGYDG PSKYHNSYNK YNDRDGGSDG
          Conservation

                                             820                    840                    860                    880                    900
YTHDF 3 [Homo sapiens]   ---------- ---------- RNRNK----- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 584
YTHDF 3 [Aedes aegypti]  YSRGGYGRDY QGGYNKSYGG YNRNQYNQDG GRGGYQSYDR RNNNTSGNGS NSGDDRDGGS NYSRDGQDGG GYQRSYGRPN RDYYGRGDDN RGGYRDRDGG 833
             Consensus   YSRGGYGRDY QGGYNKSYGG XNRNXYNQDG GRGGYQSYDR RNNNTSGNGS NSGDDRDGGS NYSRDGQDGG GYQRSYGRPN RDYYGRGDDN RGGYRDRDGG
          Conservation

                                             920                    940
YTHDF 3 [Homo sapiens]   ---------- ---------- ---------- ---------- ------Q 585
YTHDF 3 [Aedes aegypti]  FRSRDGMDRG GSRMDADGGS RDGVDNNGGG GGGGYRGDSR DHYRVGN 880
             Consensus   FRSRDGMDRG GSRMDADGGS RDGVDNNGGG GGGGYRGDSR DHYRVGX
          Conservation
```

**Supplementary Fig. 4. Sequence alignment of three m$^6$A related protein. a** Amino acid sequence alignment of the MTA-70 domain of METTL3 and METTL14 proteins from *Homo sapiens* (Hs) and *Ae. aegypti* (Ae). **b** Amino acid sequence alignment of the entire YTHDF3 protein from *H. sapiens* and *Ae. aegypti*. The YTH domain is indicated by a green rectangle. All the alignments were generated using Clustal W algorithm of CLC Genomics Workbench software.



**Supplementary Fig. 5. Phylogenetic trees of m$^6$A-modification-related genes**. **(a)** METTL3, **(b)** METTL14, and **(c)** YTHDF3.

**Supplementary Table 1.** DENV-2 m$^6$A peaks and their positions in the virus genome.

| | Start | End | Length | Coding region |
|---|---|---|---|---|
| Peak 1 | 540 | 789 | 249 | pM |
| Peak 2 | 3460 | 3766 | 306 | NS1-NS2A |
| Peak 3 | 7121 | 7868 | 747 | NS4B-NS5 |
| Peak 4 | 8778 | 8933 | 155 | NS5 |
| Peak 5 | 8957 | 9117 | 160 | NS5 |

**Supplementary Table 2.** Primers used in this study.

| qPCR Primers | Gene names | Primer sequences |
|---|---|---|
| AAEL026586F | uncharacterized protein LOC110676643 isoform X1 | GGTAGAGGACGAGCGAATGG |
| AAEL026586R | | ACCGGTTACTTCCGCATTGT |
| AAEL011112F | sorbitol dehydrogenase-like | CGGTGACTTCGTGGTTCAGA |
| AAEL011112R | | CAAAAGTCGCAAACTCGGCA |
| AAEL020023F | histone H2B-like | GGAAAGGCCGCGAAGAAATC |
| AAEL020023R | | CCAGTGTCGGGATGGACTTG |
| AAEL025779F | SUMO-conjugating enzyme UBC9-B | CCACTGTTCCACCCGAATGT |
| AAEL025779R | | CAGCAGATCCTGAATGCCGA |
| MEETL3F | METTL3 | ACATGGAGCTTCCTTACGGA |
| MEETL3R | | GGCCAGTAACCCAAAGGAAT |
| METTL14F | METTL14 | CTACGGACGGAGATTTCATC |
| METTL14R | | ACGTCTTCCCAAGCAGAAAT |
| YTHDF-qF | YTHDF | GCAACAAGTGAACCACGGTC |
| YTHDF-qR | | CCCTGCATTCCTTGCTCAAC |
| DENV-qF | DENV-2 NS1 | GGTATGGTGGGCGCTACTA |
| DENV-qR | | CAAGGCTAACGCATCAGTCA |
| RPS17-qF | Ribosomal protein subunit 17 | CACTCCGAGGTCCGTGGTAT |
| RPS17-qR | | GGACACTTCGGGCACGTAGT |

| RNAi primers | Primer sequences |
|---|---|
| AAEL026586RNAiF | TAATACGACTCACTATAGGGAGTAAGAAGCCGACGAGTGCTG |
| AAEL026586RNAiR | TAATACGACTCACTATAGGGAGCAGCATGCGGTCCATTTCAG |
| AAEL011112RNAiF | TAATACGACTCACTATAGGGAGCGGTCCGATTGGATTGGTGA |
| AAEL011112RNAiR | TAATACGACTCACTATAGGGAGCGTCGATTTTTCCACTCGCC |
| AAEL020023RNAiF | TAATACGACTCACTATAGGGAGCTGGCGTTTCTTCAAAGGCT |
| AAEL020023RNAiR | TAATACGACTCACTATAGGGAGTTCGCGGGAAGTGATTGTCG |
| AAEL025779RNAiF | TAATACGACTCACTATAGGGAGGCGTGCTAATCGGGGTAGTT |
| AAEL025779RNAiR | TAATACGACTCACTATAGGGAGAGGCGAGTTGGTTGTGAGAA |
| MEETL3_RNAi-F | TAATACGACTCACTATAGGGAGAAAAGCAAGATTCTCCTCACGGC |
| MEETL3_RNAi-R | TAATACGACTCACTATAGGGAGTTGGCTTCGTCAGCAGTTCCAGA |
| METTL14_RNAi-F | TAATACGACTCACTATAGGGAGCTACGTGAAAAGTCGCAGAAGCG |
| METTL14_RNAi-R | TAATACGACTCACTATAGGGAGGAGCGGCCACTTCACCAATATC |

Sequences in red represent the T7 promoter sequences.