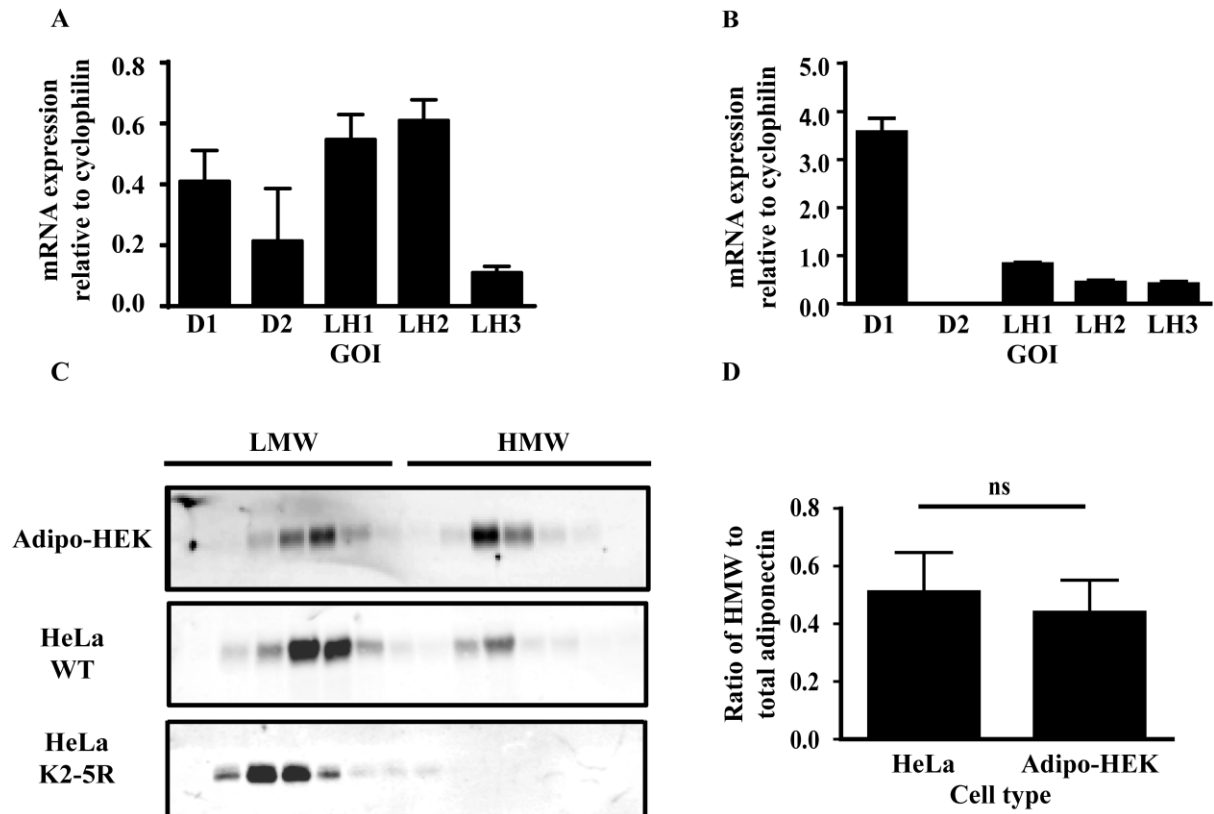


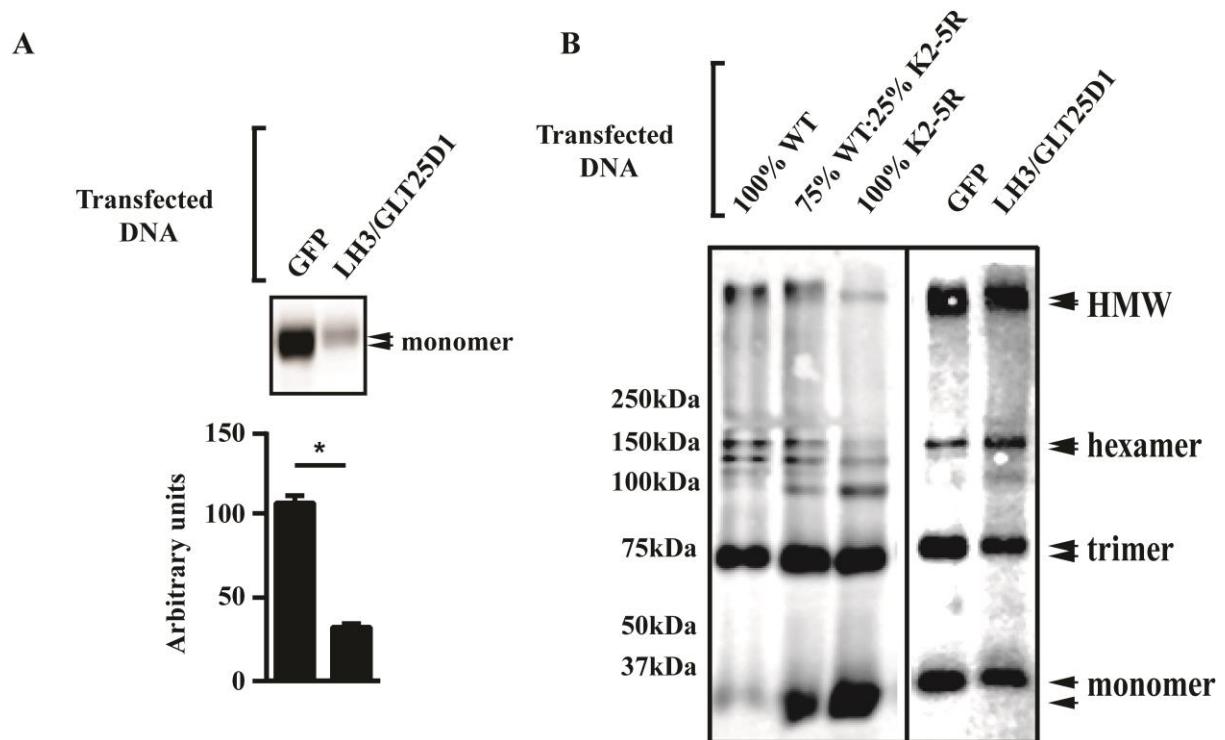
Supplementary Figure 1



Supplementary Figure 1. GLT25D2 is not essential for HMW adiponectin secretion

Wild type and the K2-5R mutant adiponectin were transiently expressed in HeLa cells. (A) Secreted adiponectin was analysed using sucrose gradients and western blot to determine whether HMW adiponectin was formed. Wild type adiponectin secreted from both HEK and HeLa cells formed HMW adiponectin. K2-5R adiponectin did not form HMW adiponectin. (B) The ratio of HMW to total adiponectin was not significantly different between the two cell types (t-test, $n=3$, $P>0.05$).

Supplementary Figure 2



Supplementary Figure 2. Analysis of adiponectin after transient expression

Adiponectin, LH3 and GLT25D1 were transiently expressed in HEK cells from 24-48hrs. GFP was used as filler DNA to ensure equal amounts of DNA were transfected. The K2-5R mutant adiponectin and wild type adiponectin were titrated against each other to confirm post-translational modifications could be detected. (A) Overexpression of LH3/GLT25D1 significantly inhibited adiponectin secretion (t-test, n=6, * $P < 0.0001$). (B) Equal amounts of adiponectin were loaded per lane. Mobility shifts demonstrate that differences in post-translational modifications could be detected. The amount of HMW adiponectin secreted was not increased, however small increases in PTM were present.

Supplementary Figure 3

CLUSTAL O(1.2.1) multiple sequence alignment

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GLT25D1_MAAAPRAGRRRGQPLLALLLLLLLAPLPFGAPP-----GADAYFPEERWSPESPLQAPRV 54
GLT25D2_MAAPPAAT-----LAWSLLLLSSALLREGCRARFVAERDSEDDGEEPVVFPESPLQSPTV 55
      * * *****      * * * * *****      * *

GLT25D1_LIALLARNAAHALPTTLGALERLRHPRERTALWVATDHNMDNTSTVLREWLVAVKSLYHS 114
GLT25D2_LVAVLARNAAHTLPHFLGCLERLDYPKSRMAIWAATDHNVDNTEIFREWLKNVQRLYHY 115
      * *      * * * *      * * * * * * *      * * * * *

GLT25D1_VEWRPAAEPRSYPDDEGPKHWSDSRYEYHVMKLRQAALKSARDMWADYILFVDADNLILNP 174
GLT25D2_VEW RPMDEPESYPDEIGPKHWPTS RFAHVMKLRQAALRTAREKWSYILFIDVDNFLTNP 175
      * *      *      * * * *      * * * *      * * * *

GLT25D1_DTLSLLIAENKTIVAPMLDSRAAYSNFWCGMTSQGYKRTPAYIPIRKRRRGCFVAPMV 234
GLT25D2_QTLNLLIAENKTIVAPMLDSRGLYSNFWCGITPKGYKRTPDYVQIREWKRTGCFVPMV 235
      * *      *      * *      * * * *      * * * * *

GLT25D1_HSTFLIDLRKAASRNLAIFYPPHPDYTWSFDDIIVFAFSCQAEVQMYVCNKEEYGFPLPVP 294
GLT25D2_HSTFLIDLRKEASDKLTIFYPPHQDYTWTFDDIIVFAFSSRQAGIQMYLCNREHYGYLPIP 295
      * * * *      *      *      * * * *      * * * * *

GLT25D1_LRAHSTLQDEAESFMHVQLEVMVKHPPAEPSRFISAPTKTPDKMGFDEVFMINLRRRQDR 354
GLT25D2_LKPHQTLQEDIENLIHVQIEAMIDRPPMEPSQYVSVVPKYPDKMGFDEIFMINLKRKDR 355
      * * * * * * * * * * * * * * * *      *      *

GLT25D1_RERMLRALQAQIEICRLVEAVDVGKAMNTSQVEALGIQMLPGYRDPYHGRPLTKGELGCFL 414
GLT25D2_RDRMLRTLQEIEIEVKIVEAVDVGKALNTSQLKALNIEMLPGYRDPYSSRPLTRGEIGCFL 415
      *      * * * * *      *      * * * *      * * * *

GLT25D1_SHYNIWKEVVDRLQKSLVFEDDLRFEIFFKRRMLNLMRDVEREGLDWDLIYVGRKRMQV 474
GLT25D2_SHYSVWKEVIDRELEKTLVIEDDVRFHQFKKKLMKLMNDIDQAQLDWELIYIGRKMV 475
      * *      * * * * *      *      * * * *      * * * * *

GLT25D1_EHPEKAVPRVRNLVEADYSYWTLAYVISLQGAR KLLAAEPLSKMLPVDEFPLPVMFDKHPV 534
GLT25D2_KEPEKAVPNVANLVEADYSYWTLYVISLEGAQKLVGANPFGKMLPVDEFPLPVMYNKHPV 535
      * *      * *      *      * * * * * * *      * *

GLT25D1_SEYKAHFSRLNLHAFSVEPLLIYPHTYTGDDGYVSDTETSVVWNNEHVKTWDWRAKSQKM 594
GLT25D2_AEYKEYYESRDLKAFSAEPLLIYPHTYTGQPGYLSDTETSTIWDNETVATDWDRTWAKS 595
      *      * * * * *      *      * *      * * * * * * *

GLT25D1_REQQALSREAKNSDVLQSPLDSA---ARDEL 622
GLT25D2_RKQSRIYSNAKNTEALPPPTS LDTVPSRDEL 626
      * * * * *      * * * * * * * * *
```

Supplementary Figure 3. DNA alignment of GLT25D1 and GLT25D2 showing mass spectrometry peptides

Peptides detected by mass spectrometry are highlighted yellow. * indicate amino acids that are not conserved between GLT25D1 and GLT25D2. All peptides detected have at least one non-conserved amino acid. All peptides detected were only present in GLT25D1.

Supplementary Table 1. RT-qPCR Primer Sequences

Gene	5' to 3'
<i>Homo sapiens Adiponectin-F</i>	GAC CAG GAA ACC ACG ACT CA
<i>Homo sapiens Adiponectin-R</i>	CGA TGT CTC CCT TAG GAC CA
<i>Homo sapiens PLOD3-F</i>	ACC TGC AGA CCC TGC GTA TC
<i>Homo sapiens PLOD3-R</i>	TGC ACC AGC TCC ACG TAG TC
<i>Homo sapiens GLT25D1-F</i>	GAT GCT GCC TGT GGA CGA GTT C
<i>Homo sapiens GLT25D1-R</i>	CTC ACA TAG CCA TCG TCT CCT G
<i>Homo sapiens GLT25D2-F</i>	CAG AGA GCA CTA TGG CTA CCT G
<i>Homo sapiens GLT25D2-R</i>	CAT ACT GGG AGG GTT CCA TTG G
<i>Homo sapiens ATGL-F</i>	CCC ACT TCA ACT CCA AGG ACG A
<i>Homo sapiens ATGL-R</i>	GCA GGT TGT CTG AAA TGC CAC C
<i>Homo sapiens PPARγ -F</i>	GAA ACT TCA AGA GTA CCA AAG TGC AA
<i>Homo sapiens PPARγ -R</i>	AGG CTT ATT GTA GAG CTG AGT CTT CTC