Additional File 1 for:

The *C. elegans* 3'-UTRome V2: an updated genomic resource to study 3'-UTR biology

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This PDF includes:

- **Figures S1-S14**
- **Supplemental Materials and Methods**

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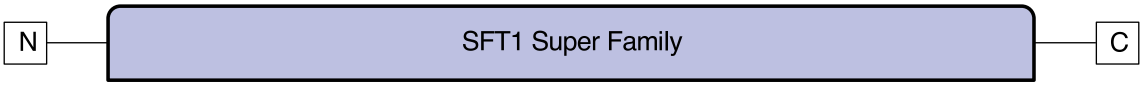
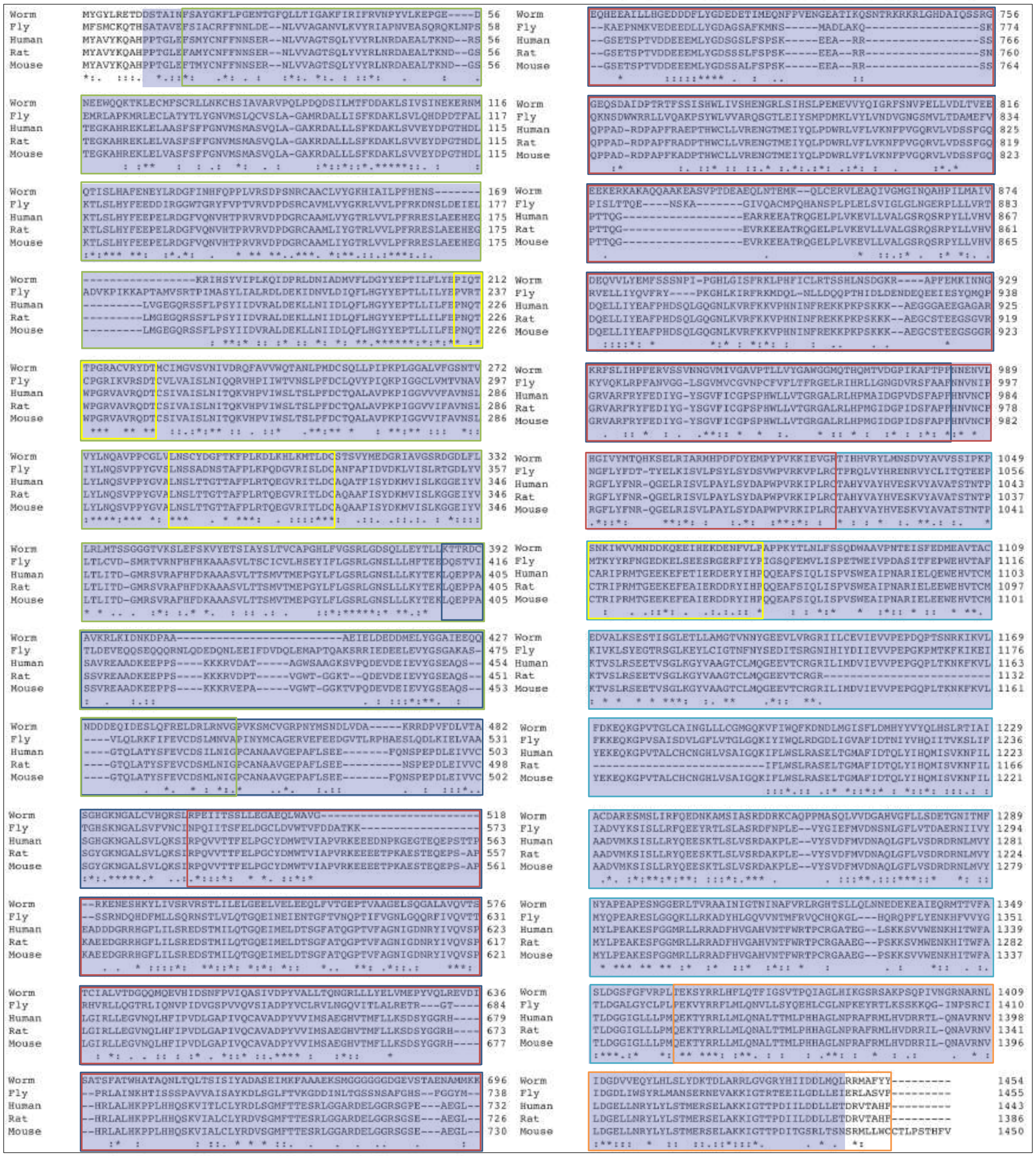
Additional File 1

CPSF-1 (CPSF1)





▭ Residues 400 to 973 interact with HAT-C domain of CstF-77 (Bai et al., 2007)

▭ bP1 domain made of WD40 repeats (Clerici et al., 2017, Sun et al., 2018)

▭ bP2 domain made of WD40 repeats (Clerici et al., 2017, Sun et al., 2018)

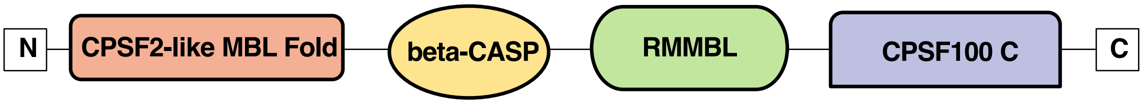
▭ bP3 domain made of WD40 repeats interacts with CPSF30 (Clerici et al., 2017, Sun et al., 2018)

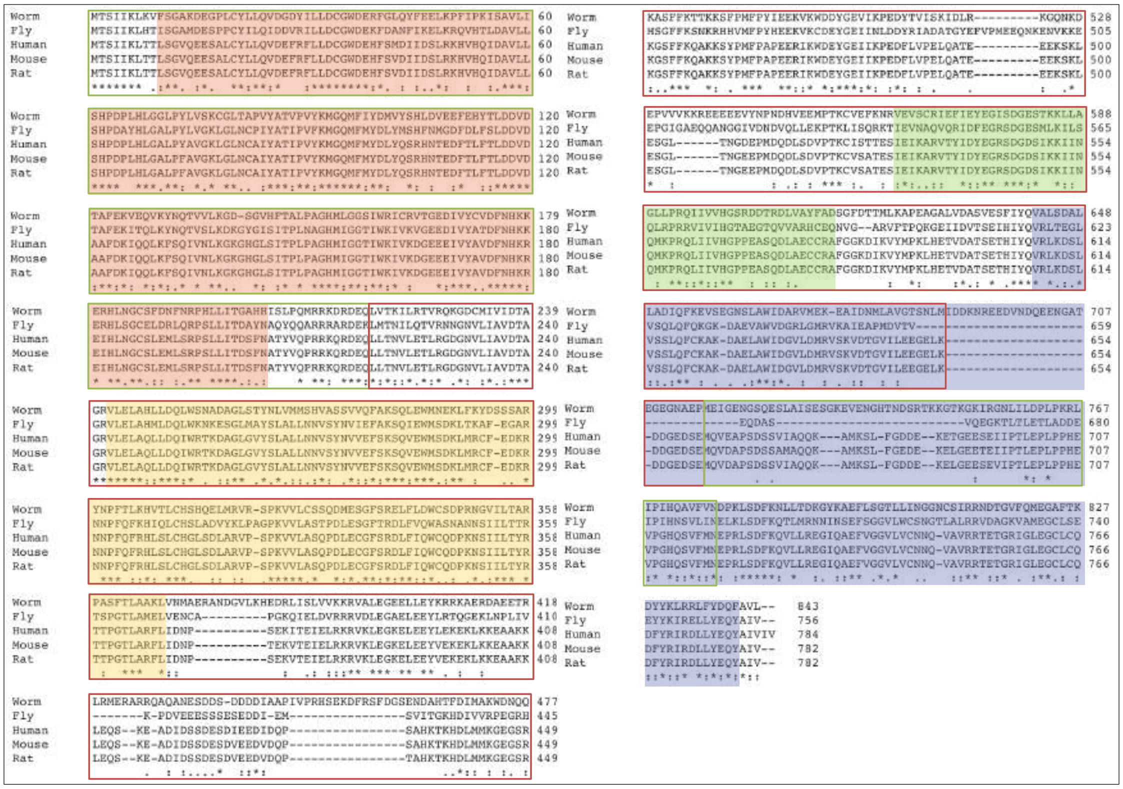
▭ C-terminal domain (Clerici et al., 2017, Sun et al., 2018)

▭ Elongated loops 1-3 stabilize interaction with Wdr33 NTD (Clerici et al., 2017, Sun et al., 2018)

Supplemental Figure S1: Protein alignment of members of the CPC complex in five organisms. Amino acid sequence alignment for the members of the CPC from five organisms produced with Clustal Omega Multiple Sequence Alignment. Conserved domains produced with the Batch Conserved Domain Search on NCBI are represented by the highlighted regions of each figure. Known domains determined from previously published literature are outlined.

CPSF-2 (CPSF2)

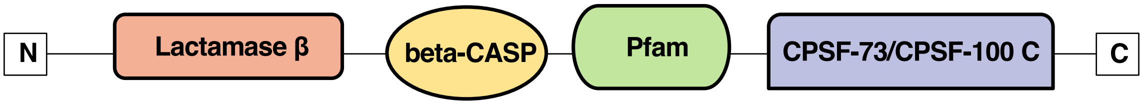


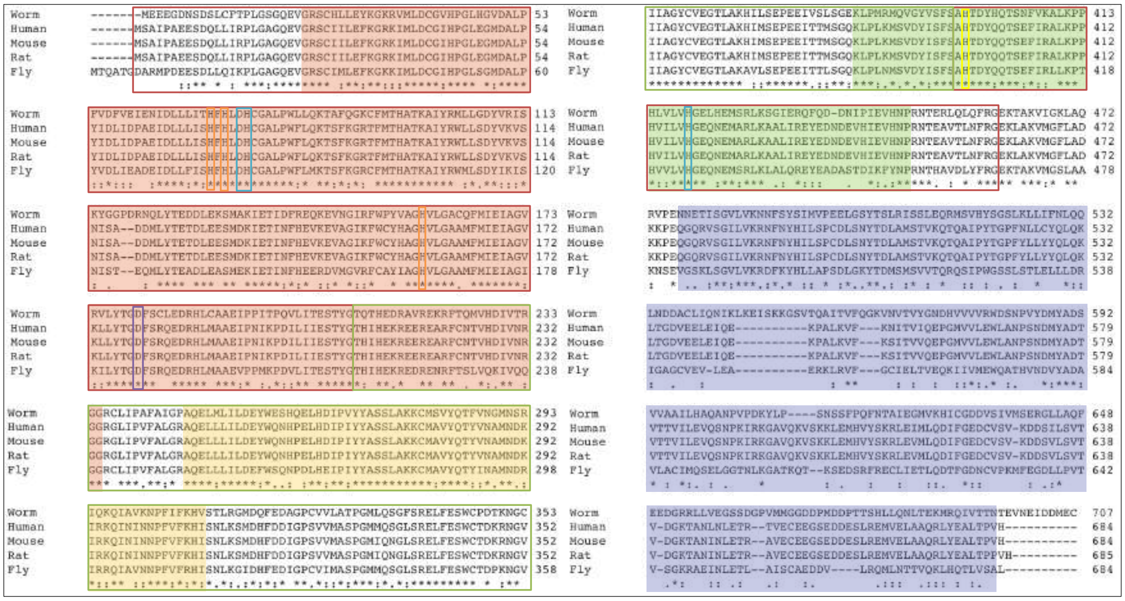


🟩 Metallo-beta-lactamase domain (Mandel et al., 2006)

🟥 Beta-CASP domain (Mandel et al., 2006)

CPSF-3 (CPSF3)





🟥 Metallo-beta-lactamase domain (Mandel et al., 2006)

🟩 Beta-CASP domain (Mandel et al., 2006)

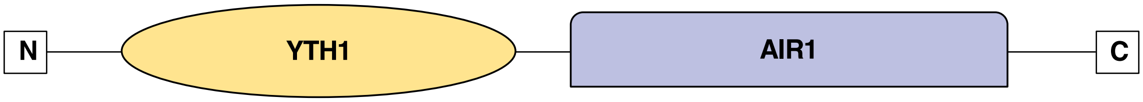
🟪 Bridging ligand to Zinc atoms (Mandel et al., 2006)

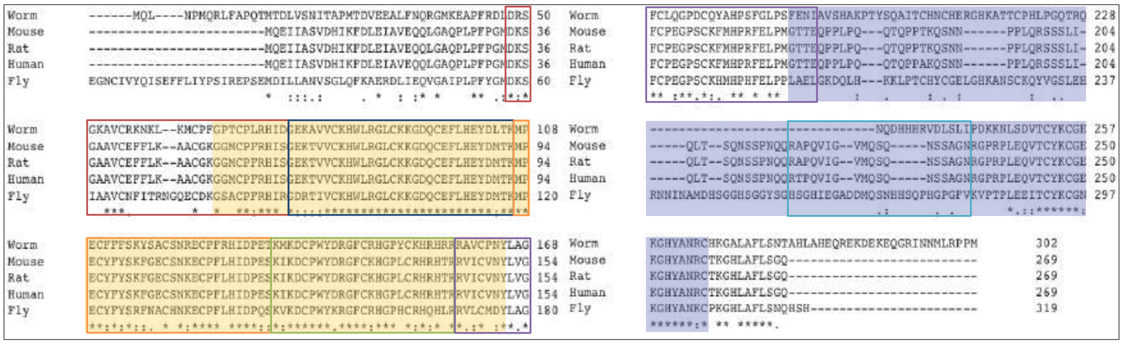
🟧 Ligands to Zn1 (Mandel et al., 2006)

🟦 Ligands to Zn2 (Mandel et al., 2006)

🟨 General acid for catalysis (Mandel et al., 2006)

CPSF-4 (CPSF4)





🟥 Zinc Finger 1 (Clerici et al., 2017)

🟦 Zinc Finger 2, contacts A1 and A2 of PAS (Clerici et al., 2017, Sjun et al., 2018)

🟧 Zinc Finger 3, contacts A4 and A5 of PAS (Clerici et al., 2017, Sjun et al., 2018)

Contact Wdr33 and CPSF160

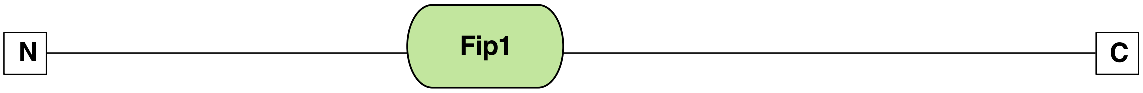
🟩 Zinc Finger 4 (Clerici et al., 2017)

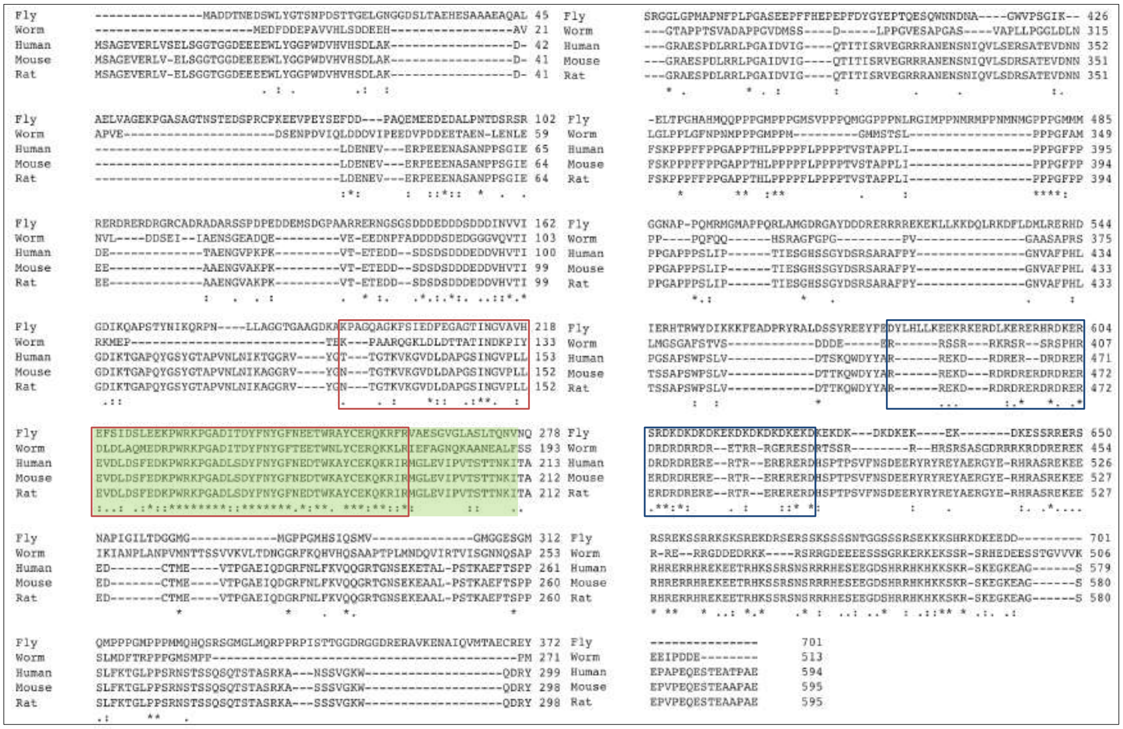
🟪 Zinc Finger 5 (Clerici et al., 2017)

Contact Fip1

🟦 Zinc Knuckle (Clerici et al., 2017)

FIPP-1 (FIP1L1)

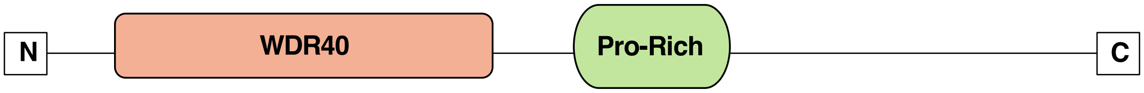


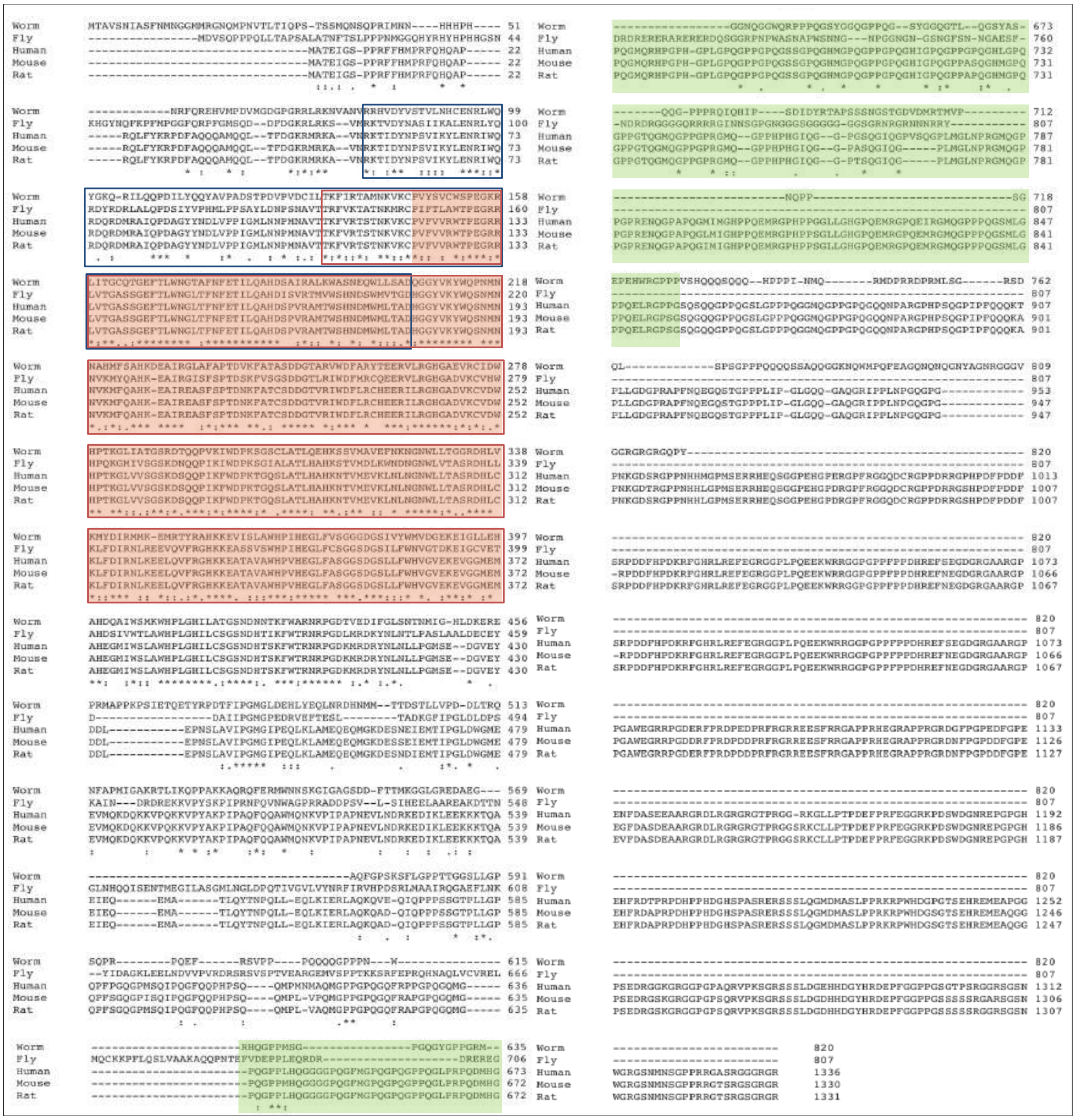


🟥 Conserved Domain contacts CPSF30 (Clerici et al., 2017)

🟦 RE/D region interacts with CFIm68/59 (Zhu et al., 2018)

PFS-2 (WDR33)

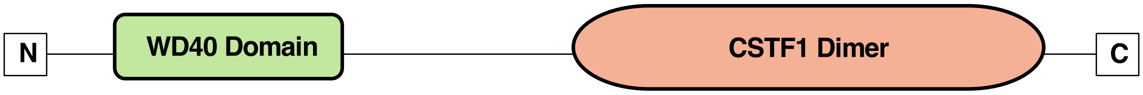


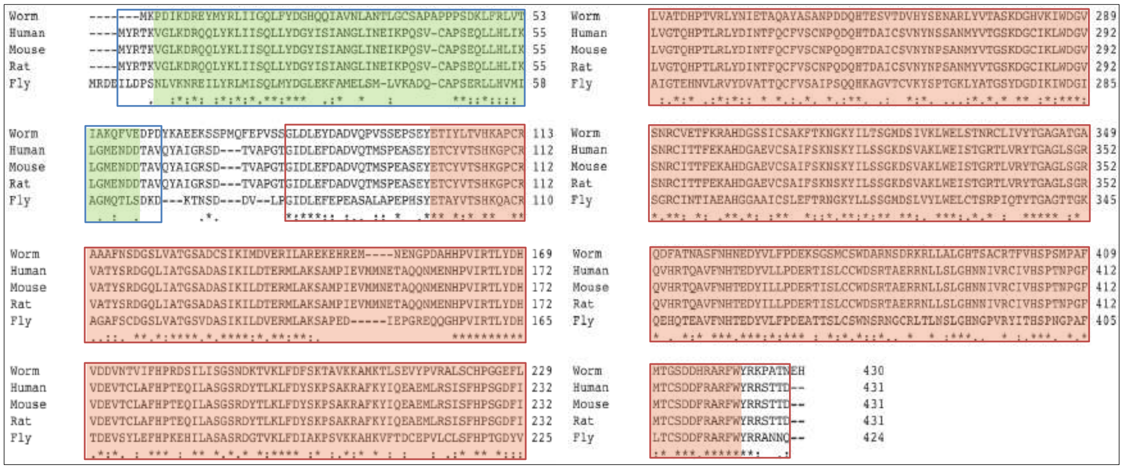


🟦 N-terminal domain, contacts CPSF160 and CPSF30 (Clerici et al., 2017)

🟥 WD40 repeats, contact U3 and A6 of PAS (Clerici et al., 2017)

CPF-1 (CST1)

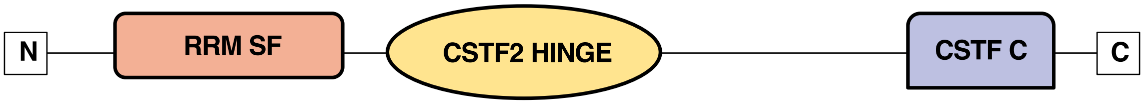


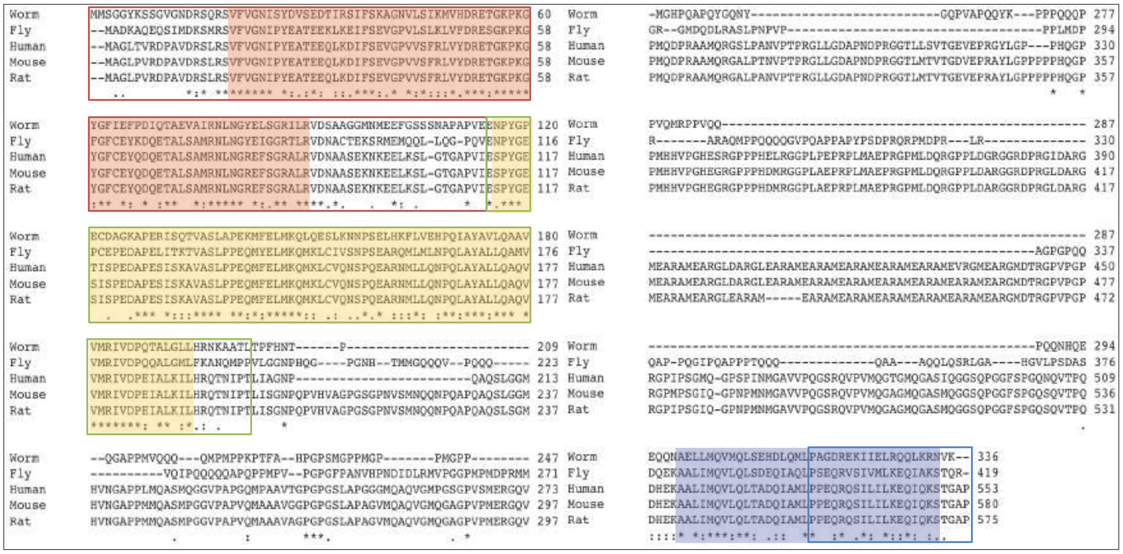


🟥 WD40 Repeats (Yang et al., 2018)

🟦 N-terminal domain (Yang et al., 2018)

CPF-2 (CSTF2)





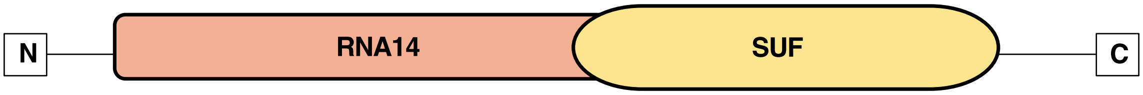
🟥 RRM domain (Yang et al., 2018)

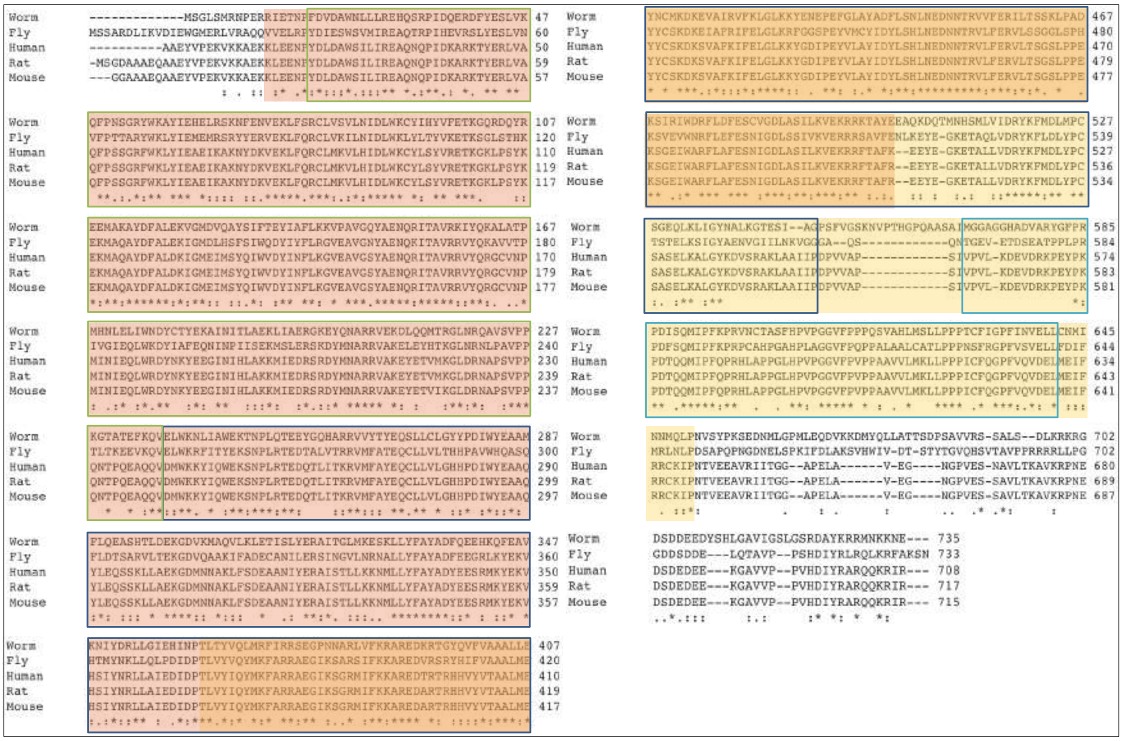
🟨 HINGE domain (Yang et al., 2018)

🟦 C-terminal domain (Yang et al., 2018)

CstF Complex

SUF-1 (CSTF3)





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Worm     --------------MSGLSMRNPERRIETNPFDVDAWNLLLREHQSRPIDQERDFYESLVK 47   Worm     YNCMKDKEVAIRVFKLGLKKYENEPEFGLAYADFLSNLNEDNNTRVVFERILTSSKLPAD 467
Fly      MSSARDLIKVDIEWGMERLVRAQQVVELRPYDIESWSVMIREAQTRPIHEVRSLYESLVN 60   Fly      YYCSKDKEIAFRIFELGLKRFGGSPEYVMCYIDYLSHLNEDNNTRVLFERVLSSGGLSPH 480
Human    ----------AAEYVPEKVKKAEKKLEENPYDLDAWSILIREAQNQPIDKARKTYERLVA 50   Human    YYCSKDKSVAFKIFELGLKKYGDIPEYVLAYIDYLSHLNEDNNTRVLFERVLTSGSLPPE 470
Rat      -MSGDAAAEQAAEYVPEKVKKAEKKLEENPYDLDAWSILIREAQNQPIDKARKTYERLVA 59   Rat      YYCSKDKSVAFKIFELGLKKYGDIPEYVLAYIDYLSHLNEDNNTRVLFERVLTSGSLPPE 479
Mouse    ---GGAAAEQAAEYVPEKVKKAEKKLEENPYDLDAWSILIREAQNQPIDKARKTYERLVA 57   Mouse    YYCSKDKSVAFKIFELGLKKYGDIPEYVLAYIDYLSHLNEDNNTRVLFERVLTSGSLPPE 477
                                : . :: :* .*:*:::*.:::** *.:**.: *, ** **              * * ***.:*:::*:****:: . **: :.* *:**:*********:***:*:*. *  .

Worm     QFPNSGRYWKAYIEHELRSKNFENVEKLFSRCLVSVLNIDLWKCYIHYVFETKGQRDQYR 107  Worm     KSIRIWDRFLDFESCVGDLASILKVEKRRKTAYEEAQKDQTMNHSMLVIDRYKFMDLMPC 527
Fly      VFPTTARYWKLYIEMEMRSRYYERVEKLFQRCLVKILNIDLWKLYLTYVKETKSGLSTHK 120  Fly      KSVEVWNRFLEFESNIGDLSSIVKVERRRSAVFENLKEYE-GKETAQLVDRYKFLDLYPC 539
Human    QFPSSGRFWKLYIEAEIKAKNYDKVEKLFQRCLMKVLHIDLWKCYLSYVRETKGKLPSYK 110  Human    KSGEIWARFLAFESNIGDLASILKVEKRRFTAFK--EEYE-GKETALLVDRYKFMDLYPC 527
Rat      QFPSSGRFWKLYIEAEIKAKNYDKVEKLFQRCLMKVLHIDLWKCYLSYVRETKGKLPSYK 119  Rat      KSGEIWARFLAFESNIGDLASILKVEKRRFTAFR--EEYE-GKETALLVDRYKFMDLYPC 536
Mouse    QFPSSGRFWKLYIEAEIKAKNYDKVEKLFQRCLMKVLHIDLWKCYLSYVRETKGKLPSYK 117  Mouse    KSGEIWARFLAFESNIGDLASILKVEKRRFTAFR--EEYE-GKETALLVDRYKFMDLYPC 534
          **.:.*:** *** *:::: ::.*****.***:.:*:***** *: ** ***.     ::              ** .:* *** *** :***:**:***:** :.:.  :: :  :.:  ::*****:** **

Worm     EEMAKAYDFALEKVGMDVQAYSIFTEYIAFLKKVPAVGQYAENQRITAVRKIYQKALATP 167  Worm     SGEQLKLIGYNALKGTESI--AGPSFVGSKNVPTHGPQAASAIMGGAGGHADVARYGFPR 585
Fly      EKMAQAYDFALEKIGMDLHSFSIWQDYIYFLRGVEAVGNYAENQKITAVRRVYQKAVVTP 180  Fly      TSTELKSIGYAENVGIILNKVGGGA--QS-------------QNTGEV-ETDSEATPPLPR 584
Human    EKMAQAYDFALDKIGMEIMSYQIWVDYINFLKGVEAVGSYAENQRITAVRRVYQRGCVNP 170  Human    SASELKALGYKDVSRAKLAAIIPDPVVAP-------------SIVPVL-KDEVDRKPEYPK 574
Rat      EKMAQAYDFALDKIGMEIMSYQIWVDYINFLKGVEAVGSYAENQRITAVRRVYQRGCVNP 179  Rat      SASELKALGYKDVSRAKLAAIIPDPVVAP-------------SIVPVL-KDEVDRKPEYPK 583
Mouse    EKMAQAYDFALDKIGMEIMSYQIWVDYINFLKGVEAVGSYAENQRITAVRRVYQRGCVNP 177  Mouse    SASELKALGYKDVSRAKLAAIIPDPVVAP-------------SIVPVL-KDEVDRKPEYPK 581
         *:**:******:*:**:: ::.*: :** **: * ***.*****:*****::**:. ..*               :. :** :**                                              *:

Worm     MHNLELIWNDYCTYEKAINITLAEKLIAERGKEYQNARRVEKDLQQMTRGLNRQAVSVPP 227  Worm     PDISQMIPFKPRVNCTASFHPVPGGVFPPPQSVAHLMSLLPPPTCFIGPFINVELLCNMI 645
Fly      IVGIEQLWKDYIAFEQNINPIISEKMSLERSKDYMNARRVAKELEYHTKGLNRNLPAVPP 240  Fly      PDFSQMIPFKPRPCAHPGAHPLAGGVFPQPPALAALCATLPPPNSFRGPFVSVELLFDIF 644
Human    MINIEQLWRDYNKYEEGINIHLAKKMIEDRSRDYMNARRVAKEYETVMKGLDRNAPSVPP 230  Human    PDTQQMIPFQPRHLAPPGLHPVPGGVFPVPPAAVVLMKLLPPPICFQGPFVQVDELMEIF 634
Rat      MINIEQLWRDYNKYEEGINIHLAKKMIEDRSRDYMNARRVAKEYETVMKGLDRNAPSVPP 239  Rat      PDTQQMIPFQPRHLAPPGLHPVPGGVFPVPPAAVVLMKLLPPPICFQGPFVQVDELMEIF 643
Mouse    MINIEQLWRDYNKYEEGINIHLAKKMIEDRSRDYMNARRVAKEYETVIKGLDRNAPSVPP 237  Mouse    PDTQQMIPFQPRHLAPPGLHPVPGGVFPVPPAAVVLMKLLPPPICFQGPFVQVDELMEIF 641
         : .:* :*.**  :*: **  :::*:  :*.::* ***** *: :   :**:*:  :***              ** .*****:**  .  . **: ***** * : . *   **** .* ***:.*: * :::

Worm     KGTATEFKQVELWKNLIAWEKTNPLQTEEYGQHARRVVYTYEQSLLCLGYYPDIWYEAAM 287  Worm     NNMQLPNVSYPKSEDNMLGPMLEQDVKKDMYQLLATTSDPSAVVRS-SALS--DLKRKRG 702
Fly      TLTKEEVKQVELWKRFITYEKSNPLRTEDTALVTRRVMFATEQCLLVLTHHPAVWHQASQ 300  Fly      MRLNLPDSAPQPNGDNELSPKIFDLAKSVHWIV-DT-STYTGVQHSVTAVPPRRRRLLPG 702
Human    QNTPQEAQQVDMWKKYIQWEKSNPLRTEDQTLITKRVMFAYEQCLLVLGHHPDIWYEAAQ 290  Human    RRCKIPNTVEEAVRIITGG--APELA------V-EG----NGPVES-NAVLTKAVKRPNE 680
Rat      QNTPQEAQQVDMWKKYIQWEKSNPLRTEDQTLITKRVMFAYEQCLLVLGHHPDIWYEAAQ 299  Rat      RRCKIPNTVEEAVRIITGG--APELA------V-EG----NGPVES-SAVLTKAVKRPNE 689
Mouse    QNTPQEAQQVDMWKKYIQWEKSNPLRTEDQTLITKRVMFAYEQCLLVLGHHPDIWYEAAQ 297  Mouse    RRCKIPNTVEEAVRIITGG--APELA------V-EG----NGPVES-SAVLTKAVKRPNE 687
           *   * :**::**. * :**:***:**:     ::**::: **.** * ::* :*::*:               . ::*:          .    : .        :         ..  .* .*:       :

Worm     FLQEASHTLDEKGDVKMAQVLKLETISLYERAITGLMKESKLLYFAYADFQEEHKQFEAV 347  Worm     DSDDEEDYSHLGAVIGSLGSRDAYKRRMNKKNE--- 735
Fly      FLDTSARVLTEKGDVQAAKIFADECANILERSINGVLNRNALLYFAYADFEEGRLKYEKV 360  Fly      GDDSDDE---LQTAVP--PSHDIYRLRQLKRFAKSN 733
Human    YLEQSSKLLAEKGDMNNAKLFSDEAANIYERAISTLLKKNMLLYFAYADYEESRMKYEKV 350  Human    DSDEDEE---KGAVVP--PVHDIYRARQQKRIR--- 708
Rat      YLEQSSKLLAEKGDMNNAKLFSDEAANIYERAISTLLKKNMLLYFAYADYEESRMKYEKV 359  Rat      DSDEDEE---KGAVVP--PVHDIYRARQQKRIR--- 717
Mouse    YLEQSSKLLAEKGDMNNAKLFSDEAANIYERAISTLLKKNMLLYFAYADYEESRMKYEKV 357  Mouse    DSDEDEE---KGAVVP--PVHDIYRARQQKRIR--- 715
         :*: ::: * ****:: *::: *  .: **:*. :::.. ********::* : ::* *              ..*.:::     :.:     :* *: *  *:

Worm     KNIYDRLLGIEHINPTLTYVQLMRFIRRSEGPNNARLVFKRAREDKRTGYQVFVAAALLE 407
Fly      HTMYNKLLQLPDIDPTLVYVQYMKFARRAEGIKSARSIFKKAREDVRSRYHIFVAAALME 420
Human    HSIYNRLLAIEDIDPTLVYIQYMKFARRAEGIKSGRMIFKKAREDTRTRHHVYVTAALME 410
Rat      HSIYNRLLAIEDIDPTLVYIQYMKFARRAEGIKSGRMIFKKAREDARTRHHVYVTAALME 419
Mouse    HSIYNRLLAIEDIDPTLVYIQYMKFARRAEGIKSGRMIFKKAREDARTRHHVYVTAALME 417
         :.:*::** : .*:***.*:* *:* **:** :.:* :*:******:* : :::::*:*:*
```

□ Proline-rich segment that facilitates interactions with CstF-64 and CstF-55 (Bai et al., 2007)

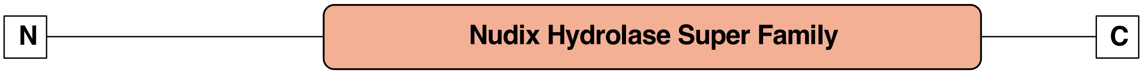
□ HAT-N Domain (Bai et al., 2007)

□ HAT-C Domain that interacts with CPSF160 (Bai et al., 2007)

CFIm Complex

Supplemental Fig. S1

CFIM-1 (NUDT21)



```
Worm    ---------MEDIWPTIERTTISA----SVPEAPANFDEKPPFNRTINVYPLTNYTFGTK 47
Human   -MSVVPPNRSQTGWPRGVTQFGNK---------YIQQTKPLTLERTINLYPLTNYTFGTK 50
Mouse   -MSVVPPNRSQTGWPRGVNQFGNK---------YIQQTKPLTLERTINLYPLTNYTFGTK 50
Rat     -MSVVPPNRSQTGWPRGVNQFGNK---------YIQQTKPLTLERTINLYPLTNYTFGTK 50
Fly     MASSQVSNKSGSGWPRRGSQGQADAASSNNNGTQKYTNQALTINRTINLYPLTNYTFGTK 60
                     **                   :   ::****:***********

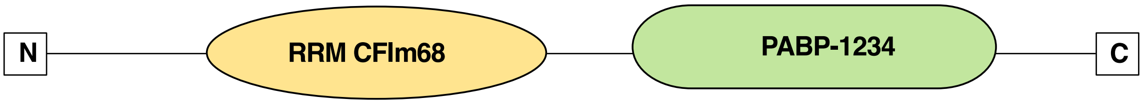
Worm    DAQAEKDKSVPERFKRMKDEYEVMGMRRSVEAVLIVHEHSLPHILLLQIGTTFYKLPGGE 107
Human   EPLYEKDSSVAARFQRMREEFDKIGMRRTVEGVLIVHEHRLPHVLLLQLGTTFFKLPGGE 110
Mouse   EPLYEKDSSVAARFQRMREEFDKIGMRRTVEGVLIVHEHRLPHVLLLQLGTTFFKLPGGE 110
Rat     EPLYEKDSSVAARFQRMREEFDKIGMRRTVEGVLIVHEHRLPHVLLLQLGTTFFKLPGGE 110
Fly     EPLFEKDPSVPSRFQRMREEFDRIGMRRSVEGVLLVHEHGLPHVLLLQLGTTFFKLPGGE 120
        :    *** **   **:**::*::  :****:**.**:****  ***:****:****:******

Worm    LELGEDEISGVTRLLNETLGRTDGETNEWTIEDEIGNWWRPNFDPPRYPYIPAHVTKPKE 167
Human   LNPGEDEVEGLKRLMTEILGRQDGVLQDWVIDDCIGNWWRPNFEPPQYPYIPAHITKPKE 170
Mouse   LNPGEDEVEGLKRLMTEILGRQDGVLQDWVIDDCIGNWWRPNFEPPQYPYIPAHITKPKE 170
Rat     LNPGEDEVEGLKRLMTEILGRQDGVLQDWVIDDCIGNWWRPNFEPPQYPYIPAHITKPKE 170
Fly     LNAGEDEVEGLKRLLSETLGRQDGVKQEWIVEDTIGNWWRPNFEPPQYPYIPPHITKPKE 180
        *:  ****:.*:.**:.*  ***  **    ::*  ::*  *********:**:*****  *:*****

Worm    HTKLLLVQLPSKSTFCVPKNFKLVAAPLFELYDNAAAYGPLISSLPTTLSRFNFIFNDSN 227
Human   HKKLFLVQLQEKALFAVPKNYKLVAAPLFELYDNAPGYGPIISSLPQLLSRFNFIYN--- 227
Mouse   HKKLFLVQLQEKALFAVPKNYKLVAAPLFELYDNAPGYGPIISSLPQLLSRFNFIYN--- 227
Rat     HKKLFLVQLQEKALFAVPKNYKLVAAPLFELYDNAPGYGPIISSLPQLLSRFNFIYN--- 227
Fly     HKRLFLVQLHEKALFAVPKNYKLVAAPLFELYDNSQGYGPIISSLPQALCRFNFIYM--- 237
        *.:*:****  .*:  *.****:************:  .***:*****   *.*****:
```

☐ Nudix domain (Yang et al., 2011) ☐ CFIm68 and CFIm59 tethering site (Zhu et al.,2017)

CFIM-2 (CPSF6)



```
Worm    MAELD-EAALLGDGNEQHDGPIDENALLDGKELKEEDIDDLYDEAIAPTNSTESAKPVS- 58
Fly     MADVV--LDLYAE-------DLDKDFAGQAQDEFGGDGVDLYDDIGGPTESAASGGGGGG 51
Mouse   ----------------------------EAEYGGHDQIDLYDDVISPSANNGDAPEDR- 30
Human   MADGVDHIDIYAD--------VGEEFN-QEAEYGGHDQIDLYDDVISPSANNGDAPEDR- 50
Rat     MADGVDHIDIYAD--------VGEEFN-QEAEYGGHDQIDLYDDVISPSANNGDAPEDR- 50
                                  :    *  ****:  .*: .  ..

Worm    -PTITTVTAPTA-----GIG----------AKPATSSEGRKYCCYVGNLLWYTTDADLLK 102
Fly     TPSADGAAGPGSGEPGERNSGGPNGVYHQSSGSLTPTMNRRYQLYVGNLTWWTTDQDIAN 111
Mouse   -DYMD-TLPPTVGDD-VGKGAAPNVV--------YTYTGKRIALYIGNLTWWTTDEDLTE 79
Human   -DYMD-TLPPTVGDD-VGKGAAPNVV--------YTYTGKRIALYIGNLTWWTTDEDLTE 99
Rat     -DYMD-TLPPTVGDD-VGKGAAPNVV--------YTYTGKRIALYIGNLTWWTTDEDLTE 99
             .  *         .                  .::    *:*** *:*** *: :

Worm    ALQSTGLARSQFADMKFFENRTNGQSKGYALLVLNSDAAVKQTMEILPTKTIHGQSPTVL 162
Fly     SLRDIGV--SDLQEVKFFENRANGQSKGFSVISLGSESSLRAVLDQLPKKEMHGQAPVVT 169
Mouse   AVHSLGV--NDILEIKFFENRANGQSKGFALVGVGSEASSKKLMDLLPKRELHGQSPVVT 137
Human   AVHSLGV--NDILEIKFFENRANGQSKGFALVGVGSEASSKKLMDLLPKRELHGQNPVVT 157
Rat     AVHSLGV--NDILEIKFFENRANGQSKGFALVGVGSEASSKKLMDLLPKRELHGQNPVVT 157
        :::. *:  .:: ::******:******::::  :.*::: :  :: **.: :*** *.*

Worm    SYNKTNQAKLEDAQAKNQTRPDVKKK---G---------FEEGCVNMGTIRIGAGGQTGR 210
Fly     YPSKQALTQFESLQKTRPVPP----PQQNGPPRGPAPPSM-----GGGPMPTGH--PGGP 218
Mouse   PCNKQFLSQFEMQSRKTTQSGQMSGEGKAGPPGGGSRAAFPQGGRGRGRFPGAV--PGGD 195
Human   PCNKQFLSQFEMQSRKTTQSGQMSGEGKAGPPGGSSRAAFPQGGRGRGRFPGAV--PGGD 215
Rat     PCNKQFLSQFEMQSRKTTQSGQMSGEGKAGPPGGGSRAAFPQGGRGRGRFPGAV--PGGD 215
         .*   :::*  . .              *        :    . * :  .     *

Worm    TGTSVSGRSGPPPLMMQQV-----RPTP-----LMSQP--------------TSLPSNLN 246
Fly     QGGGPP--GHPPRGMNSIMQPGQYRPQHMSQVPQVGGPNSGPPRMQPPMHPQGGLMGNQQ 276
Mouse   RFPGPAGPGGPPPP----FPAGQTPP-----R---------------PPLGPPGP-PGPPG 231
Human   RFPGPAGPGGPPPP----FPAGQTPP-----R---------------PPLGPPGP-PGPPG 251
Rat     RFPGPAGPGGPPPP----FPAGQTPP-----R---------------PPLGPPGP-PGPPG 251
          .   . **     .      *

Worm    QAPQ---MRLQINGQQVPLM-----NR-APVPQQTMLGNGPLGGMNQQIQQQPQMMMGQQ 297
Fly     PPPRYPSAQGQWPGQRPGGPRPGPP---NGPPQRPMFQGGPMGMPVRGP-------AGPD 326
Mouse   PPP----------PGQVLPPPLAGPPNRGDRPPPPVLFPGQPFGQPPLGP-------LPPG 275
Human   PPP----------PGQVLPPPLAGPPNRGDRPPPPVLFPGQPFGQPPLGP-------LPPG 295
Rat     PPP----------PGQVLPPPLAGPPNRGDRPPPPVLFPGQPFGQPPLGP-------LPPG 295
           *          **                *    :: .  *:*

Worm    VRPMMQTSMGIQP----MMGMNAPPPMNNQFQNRPPQ------------------------ 330
Fly     W----------------------RRPPMHGGFPPQ-----GPPRGLPP---APGPGGPHGA 357
Mouse   PPP---PVPGYGPPPGPPPPQQGPPPPPGPFPPRPPGPLGPPLTLAPPPHLPGPPPGAPP 332
Human   PPP---PVPGYGPPPGPPPPQQGPPPPPGPFPPRPPGPLGPPLTLAPPPHLPGPPPGAPP 352
Rat     PPP---PVPGYGPPPGPPPPQQGPPPPPGPFPPRPPGPLGPPLTLAPPPHLPGPPPGAPP 352
                              **  . *  :

Worm    --------------------------LGSMGVQPLMQMNTA--M-RPPINGLPPV----- 356
Fly     PAPHVNPAFFNQPGGPAQHPGMGGPPHGAPGPQPGMNMPPQQGMNMTPQHGPPPQFAQHG 417
Mouse   PAPHVNPAFFPPPTN--------------------------SGMPTSDSRGPPPTDPY-G 365
Human   PAPHVNPAFFPPPTN--------------------------SGMPTSDSRGPPPTDPY-G 385
Rat     PAPHVNPAFFPPPTN--------------------------SGMPTSDSRGPPPTDPY-G 385
                                                  *     .* **

Worm    ---------HVNPQ--MFPGLQGTVLSDAEFEDVMTRNQTVSSSAIARAITDASVGDIKG 405
Fly     PRGPWPPPQGKPPGPPPDPQQMGPQLTEVEFEEVMSRNRTVSSSAIARAVSDAAAGEYSS 477
Mouse   RPPPYDRGDYGPPG--REMDTARTPLSEAEFEEIMNRNRAISSSAISRAVSDASAGDYGS 423
Human   RPPPYDRGDYGPPG--REMDTARTPLSEAEFEEIMNRNRAISSSAISRAVSDASAGDYGS 443
Rat     RPPPYDRGDYGPPG--REMDTARTPLSEAEFEEIMNRNRAISSSAISRAVSDASAGDYGS 443
                  *           *::.***::*.**:::*****:**::**:.*:  .

Worm    ASETILTAIQLIKNSRIGHDERCRQLVYGLEHTLKGLESKGYSSRSKSHRDRSRSRERDR 465
Fly     AIETLVTAISLIKQSKVAHDERCKILISSLQDTLHGIEAKSYNRRERS-RSRERSHRS-R 535
Mouse   AIETLVTAISLIKQSKVSADDRCKVLISSLQDCLHGIESKSYGSGSRRERSRERDHSRSR 483
Human   AIETLVTAISLIKQSKVSADDRCKVLISSLQDCLHGIESKSYGSGSRRERSRERDHSRSR 503
Rat     AIETLVTAISLIKQSKVSADDRCKVLISSLQDCLHGIESKSYGSGSRRERSRERDHSRSR 503
        * **::***.***:*::. *:*:. *:.*: .*:. *:*:*.*.   .:  *.*.*.:   *

Worm    KRRR----RSRTRSRSYSRSPSPRRRRY-------------------------------- 489
Fly     --------QRRERSTSRYRERSRERERDRDRERERDGGSYRERSRSRERERQAPDHYRDD 587
Mouse   EKSRRHKSRSRDRHDDYYRERSRERERHRDRDRDRDRE---------------------- 521
Human   EKSRRHKSRSRDRHDDYYRERSRERERHRDRDRDRDRE---------------------- 541
Rat     EKSRRHKSRSRDRHDDYYRERSRERERHRDRDRDRDRE---------------------- 541
                : * *  .  *. *  .*.*

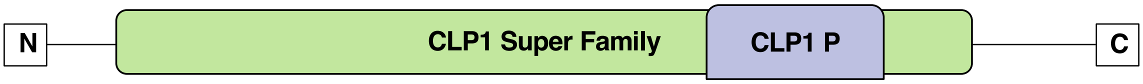
Worm    ------------------------------------------------------------ 489
Fly     SRSVRPRKSPEPVVAEAAEAPSSKRYYEDRERYRSSDRERRDRDRDRDRERERDRDRREE 647
Mouse   ----------------------------------------------------RDREREYR 529
Human   ----------------------------------------------------RDREREYR 549
Rat     ----------------------------------------------------RDREREYR 549

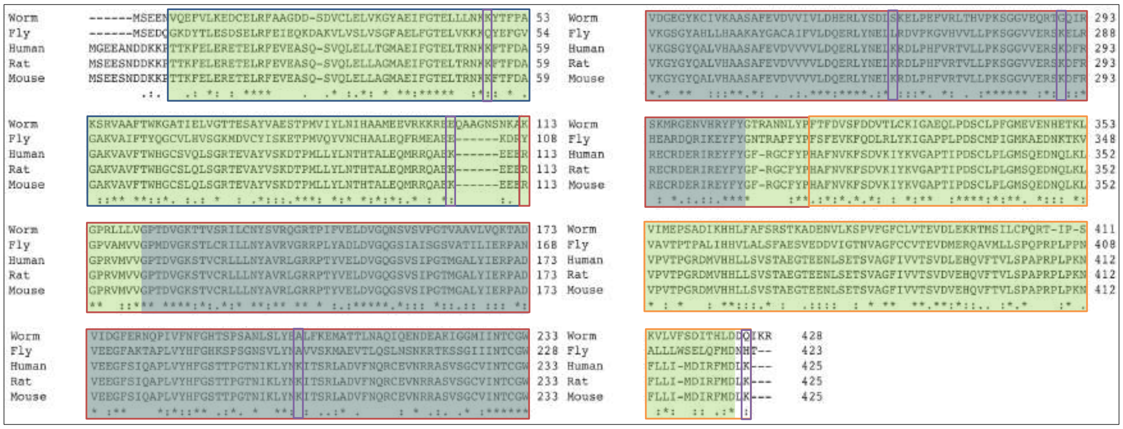
Worm    -----      489
Fly     HRSRH      652
Mouse   HR---      531
Human   HR---      551
Rat     HR---      551
```

☐ RRM domain (Yang et al., 2011, Martin et al., 2010) ☐ RS/RD/RE region (Yang et al., 2011)

☐ Pro/Gly-rich region (Yang et al., 2011)

CLPF-1 (CLP1)





```
Worm    ------MSEENVQEFVLKEDCELRFAAGDD-SDVCLELVKGYAEIFGTELLLNKKYTFPA 53
Fly     ------MSEDQGKDYTLESDSELRFEIEQKDAKVLVSLVSGFAELFGTELVKKKQYEFGV 54
Human   MGEEANDDKKPTTKFELERETELRFEVEASQ-SVQLELLTGMAEIFGTELTRNKKFTFDA 59
Rat     MSEESNDDKKPTTKFELERETELRFEVEASQ-SVQLELLAGMAEIFGTELTRNKKFTFDA 59
Mouse   MSEESNDDKKPTTKFELERETELRFEVEASQ-SVQLELLAGMAEIFGTELTRNKKFTFDA 59
                .:.   .: *: : ****     .   .* :.*: * **:*****  :*:: * .

Worm    KSRVAAFTWKGATIELVGTTESAYVAESTPMVIYLNIHAAMEEVRKKREEQAAGNSNKAK 113
Fly     GAKVAIFTYQGCVLHVSGKMDVCYISKETPMVQYVNCHAALEQFRMEAEE------KDRY 108
Human   GAKVAVFTWHGCSVQLSGRTEVAYVSKDTPMLLYLNTHTALEQMRRQAEK------EEER 113
Rat     GAKVAVFTWHGCSLQLSGRTEVAYVSKDTPMLLYLNTHTALEQMRRQAEK------EEER 113
Mouse   GAKVAVFTWHGCSLQLSGRTEVAYVSKDTPMLLYLNTHTALEQMRRQAEK------EEER 113
         ::** **::*. :.: *  : .*:::.***: *:* *:*:*:.* : *:       :.

Worm    GPRLLLVGPTDVGKTTVSRILCNYSVRQGRTPIFVELDVGQNSVSVPGTVAAVLVQKTAD 173
Fly     GPVAMVVGPMDVGKSTLCRILLNYAVRVGRRPLYADLDVGQGSIAISGSVATILIERPAN 168
Human   GPRVMVVGPTDVGKSTVCRLLLNYAVRLGRRPTYVELDVGQGSVSIPGTMGALYIERPAD 173
Rat     GPRVMVVGPTDVGKSTVCRLLLNYAVRLGRRPTYVELDVGQGSVSIPGTMGALYIERPAD 173
Mouse   GPRVMVVGPTDVGKSTVCRLLLNYAVRLGRRPTYVELDVGQGSVSIPGTMGALYIERPAD 173
        **  ::*** ****:*:.*:* **:** ** *  :.:*****.*::: *::.:: ::: *:

Worm    VIDGFERNQPIVFNFGHTSPSANLSLYEALFKEMATTLNAQIQENDEAKIGGMIINTCGW 233
Fly     VEEGFAKTAPLVYHFGHKSPSGNSVLYNAVVSKMAEVTLQSLNSNKRTKSSGIIINTCGW 228
Human   VEEGFSIQAPLVYHFGSTTPGTNIKLYNKITSRLADVFNQRCEVNRRASVSGCVINTCGW 233
Rat     VEEGFSIQAPLVYHFGSTTPGTNIKLYNKITSRLADVFNQRCEVNRRASVSGCVINTCGW 233
Mouse   VEEGFSIQAPLVYHFGSTTPGTNIKLYNKITSRLADVFNQRCEVNRRASVSGCVINTCGW 233
        * :**    *:*::** .:*. *  **: :  ..:* .      : * .:. .* :******

Worm    VDGEGYKCIVKAASAFEVDVVIVLDHERLYSDLSKELPEFVRLTHVPKSGGVEQRTGQIR 293
Fly     VKGSGYAHLLHAAKAYGACAIFVLDQERLYNELLRDVPKGVHVVLLPKSGGVVERSKELR 288
Human   VKGSGYQALVHAASAFEVDVVVVLDQERLYNELKRDLPHFVRTVLLPKSGGVVERSKDFR 293
Rat     VKGYGYQALVHAASAFEVDVVVVLDQERLYNELKRDLPHFVRTVLLPKSGGVVERSKDFR 293
Mouse   VKGYGYQALVHAASAFEVDVVVVLDQERLYNELKRDLPHFVRTVLLPKSGGVVERSKDFR 293
        *.* **  :::**.*:  .:.:***.****.:* ::.*: *:.. :******:*: .::*

Worm    SKMRGENVHRYFYGTRANNLYPFTFDVSFDDVTLCKIGAEQLPDSCLPFGMEVENHETKL 353
Fly     HEARDQRIKEYFYGNTRAPFYPFSFEVKFQDLRLYKIGAPPLPDSCMPIGMKAEDNKTKV 348
Human   RECRDERIREYFYGF-RGCFYPHAFNVKFSDVKIYKVGAPTIPDSCLPLGMSQEDNQLKL 352
Rat     RECRDERIREYFYGF-RGCFYPHAFNVKFSDVKIYKVGAPTIPDSCLPLGMSQEDNQLKL 352
Mouse   RECRDERIREYFYGF-RGCFYPHAFNVKFSDVKIYKVGAPTIPDSCLPLGMSQEDNQLKL 352
         : *.:.:..****      :**.:*:*.*.*: : *:**  :****:*:**. *::: *:

Worm    VIMEPSADIKHHLFAFSRSTKADENVLKSPVFGFCLVTEVDLEKRTMSILCPQRT-IP-S 411
Fly     VAVTPTPALIHHVLALSFAESVEDDVIGTNVAGFCCVTEVDMERQAVMLLSPQPRPLPPN 408
Human   VPVTPGRDMVHHLLSVSTAEGTEENLSETSVAGFIVVTSVDLEHQVFTVLSPAPRPLPKN 412
Rat     VPVTPGRDMVHHLLSVSTAEGTEENLSETSVAGFIVVTSVDVEHQVFTVLSPAPRPLPKN 412
Mouse   VPVTPGRDMVHHLLSVSTAEGTEENLSETSVAGFIVVTSVDVEHQVFTVLSPAPRPLPKN 412
        * : *   : **:::.* :.  :.:::  . * **  **.**:*:..  :*.*    :* .

Worm    KVLVFSDITHLDDQIKR 428
Fly     ALLLWSELQFMDNHT-- 423
Human   FLLI-MDIRFMDLK--- 425
Rat     FLLI-MDIRFMDLK--- 425
Mouse   FLLI-MDIRFMDLK--- 425
         :*:  :: .:* :
```

🟦 N-terminal domain (Dikfidan et al., 2014)

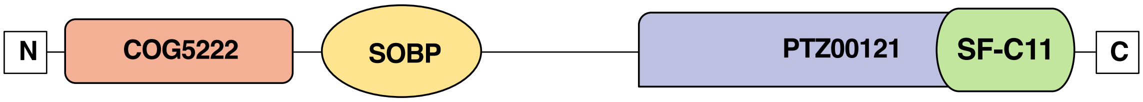
🟥 Polynucleotide kinase domain (Dikfidan et al., 2014)

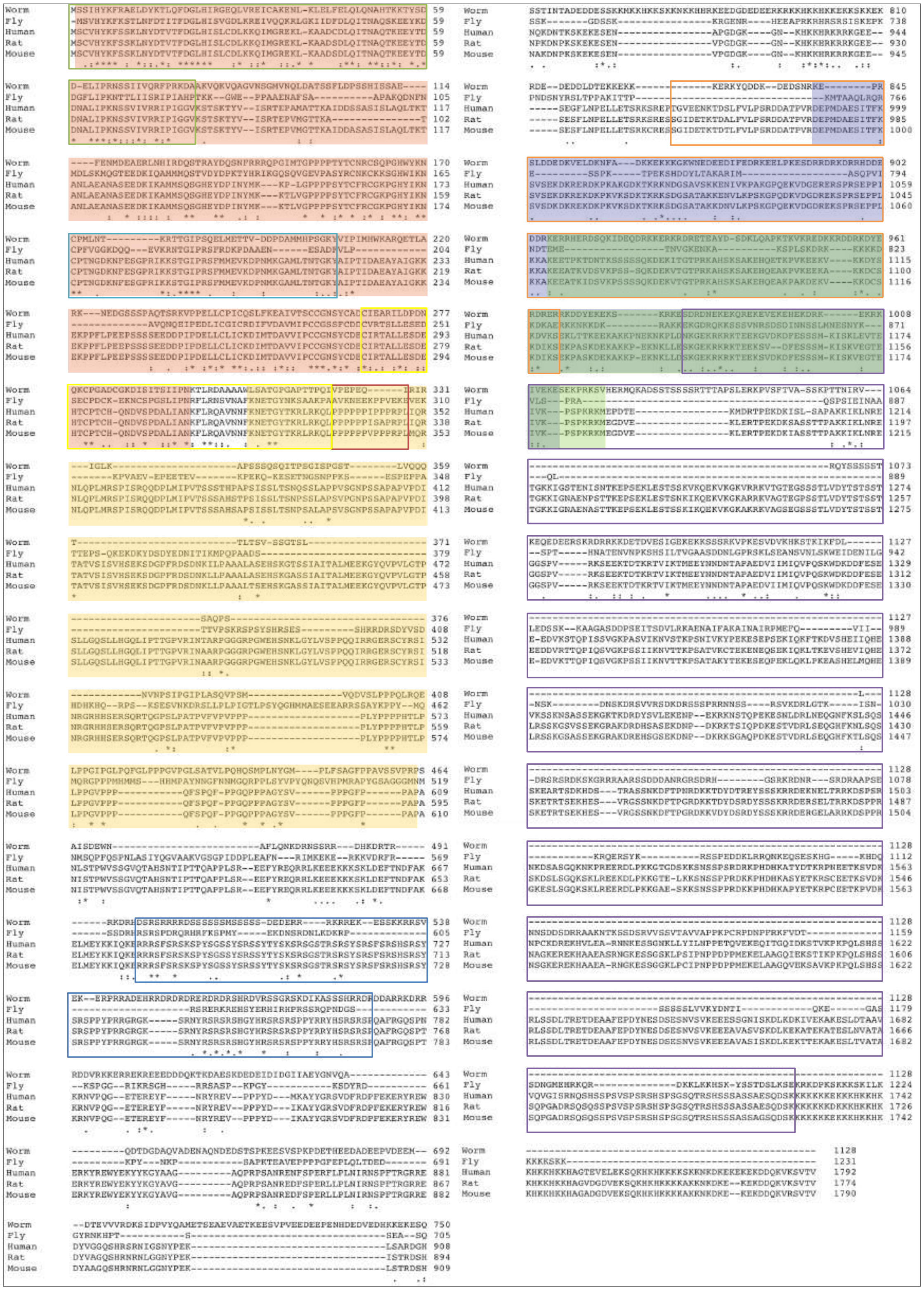
🟧 C-terminal domain (Dikfidan et al., 2014)

🟪 Residues that crosslink to PCF-11 (Schäfer et al., 2018)

Supplemental Fig. S1

RBPL-1 (RBBP6)





□ Domain With No Name (DWNN), ubiquitin-like (Pugh et al., 2006)

□ RING finger domain (Chibi et al., 2008, Lee et al., 2014)

□ Proline-rich domain (Pugh et al., 2006)

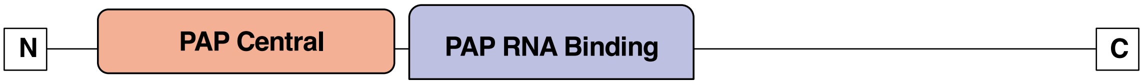
□ SR domain (Pugh et al., 2006)

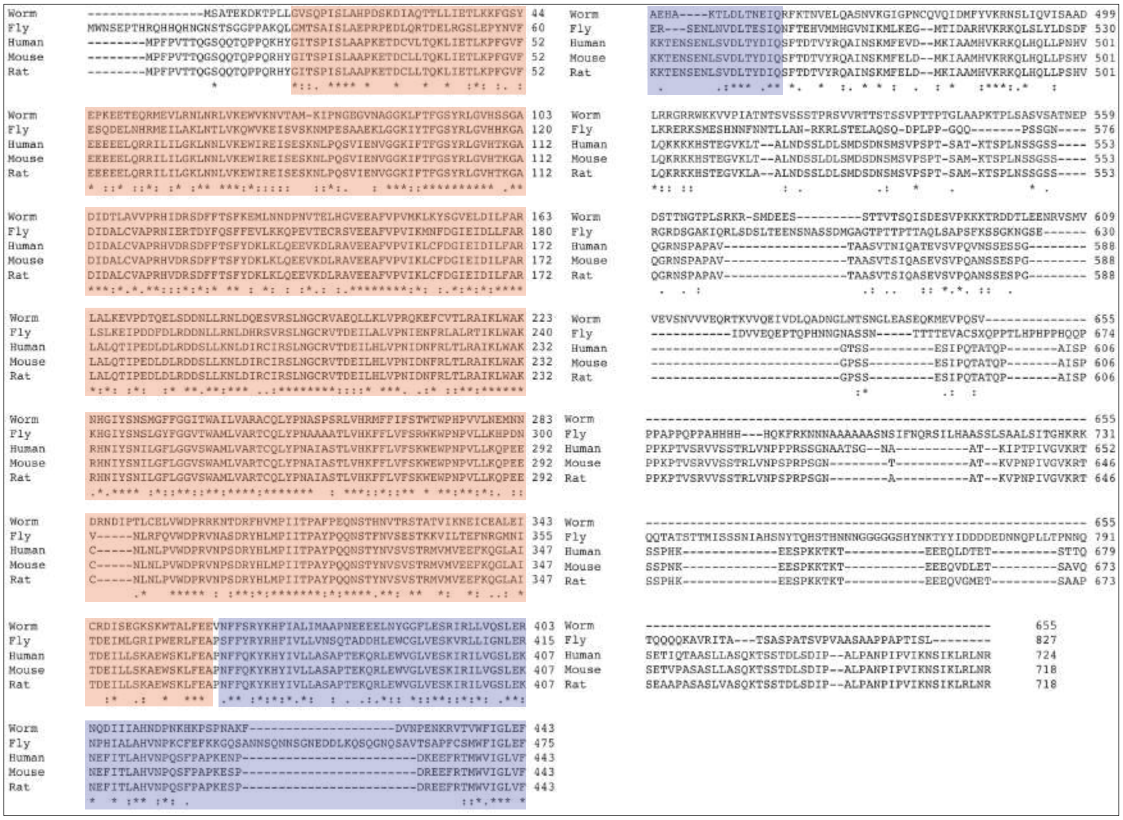
□ Rb-binding domain (Pugh et al., 2006)

□ p53-binding domain (Pugh et al., 2006)

□ Zinc knuckle (Lee et al., 2014)

PAP-1 (PAP1N)





```
Worm      ----------------MSATEKDKTPLLGVSQPISLAHPDSKDIAQTTLLIETLKKFGSY  44
Fly       MWNSEPTHRQHHQHNGNSTSGGPPAKQLGMTSAISLAEPRPEDLQRTDELRGSLEPYNVF  60
Human     --------MPFFPVTTQGSQQTQPPQKHYGITSPISLAAPKETDCVLTQKLIETLKPFGVF  52
Mouse     --------MPFFPVTTQGSQQTQPPQRHYGITSPISLAAPKETDCLLTQKLIETLKPFGVF  52
Rat       --------MPFFPVTTQGSQQTQPPQRHYGITSPISLAAPKETDCLLTQKLIETLKPFGVF  52
                           *          *::. **** *    *   *  *  :*: :. :

Worm      EPKEETEQRMEVLRNLNRLVKEWVKNVTAM-KIPNGEGVNAGGKLFTFGSYRLGVHSSGA  103
Fly       ESQDELNHRMEILAKLNTLVKQWVKEISVSKNMPESAAEKLGGKIYTFGSYRLGVHHKGA  120
Human     EEEEELQRRILILGKLNNLVKEWIREISESKNLPQSVIENVGGKIFTFGSYRLGVHTKGA  112
Mouse     EEEEELQRRILILGKLNNLVKEWIREISESKNLPQSVIENVGGKIFTFGSYRLGVHTKGA  112
Rat       EEEEELQRRILILGKLNNLVKEWIREISESKNLPQSVIENVGGKIFTFGSYRLGVHTKGA  112
          * ::* ::*: :* :** ***:*:::::   ::*:.   : ***::********** .**

Worm      DIDTLAVVPRHIDRSDFFTSFKEMLNNDPNVTELHGVEEAFVPVMKLKYSGVELDILFAR  163
Fly       DIDALCVAPRNIERTDYFQSFFEVLKKQPEVTECRSVEEAFVPVIKMNFDGIEIDLLFAR  180
Human     DIDALCVAPRHVDRSDFFTSFYDKLKLQEEVKDLRAVEEAFVPVIKLCFDGIEIDILFAR  172
Mouse     DIDALCVAPRHVDRSDFFTSFYDKLKLQEEVKDLRAVEEAFVPVIKLCFDGIEIDILFAR  172
Rat       DIDALCVAPRHVDRSDFFTSFYDKLKLQEEVKDLRAVEEAFVPVIKLCFDGIEIDILFAR  172
          ***:*.*.**:::*:*:* ** : *: : :*.: :.********:*: :.*:*:*:****

Worm      LALKEVPDTQELSDDNLLRNLDQESVRSLNGCRVAEQLLKLVPRQKEFCVTLRAIKLWAK  223
Fly       LSLKEIPDDFDLRDDNLLRNLDHRSVRSLNGCRVTDEILALVPNIENFRLALRTIKLWAK  240
Human     LALQTIPEDLDLRDDSLLKNLDIRCIRSLNGCRVTDEILHLVPNIDNFRLTLRAIKLWAK  232
Mouse     LALQTIPEDLDLRDDSLLKNLDIRCIRSLNGCRVTDEILHLVPNIDNFRLTLRAIKLWAK  232
Rat       LALQTIPEDLDLRDDSLLKNLDIRCIRSLNGCRVTDEILHLVPNIDNFRLTLRAIKLWAK  232
          *:*: :*:  :* **.**:*** ..:********::::* ***. .:* ::**:******

Worm      NHGIYSNSMGFFGGITWAILVARACQLYPNASPSRLVHRMFFIFSTWTWPHPVVLNEMNN  283
Fly       KHGIYSNSLGYFGGVTWAMLVARTCQLYPNAAAATLVHKFFLVFSRWKWPNPVLLKHPDN  300
Human     RHNIYSNILGFLGGVSWAMLVARTCQLYPNAIASTLVHKFFLVFSKWEWPNPVLLKQPEE  292
Mouse     RHNIYSNILGFLGGVSWAMLVARTCQLYPNAIASTLVHKFFLVFSKWEWPNPVLLKQPEE  292
Rat       RHNIYSNILGFLGGVSWAMLVARTCQLYPNAIASTLVHKFFLVFSKWEWPNPVLLKQPEE  292
          .*.**** :*::**::**:****:*******  : ***::*::** * **:**:*:. ::

Worm      DRNDIPTLCELVWDPRRKNTDRFHVMPIITPAFPEQNSTHNVTRSTATVIKNEICEALEI  343
Fly       V-----NLRFQVWDPRVNASDRYHLMPIITPAYPQQNSTFNVSESTKKVILTEFNRGMNI  355
Human     C-----NLNLPVWDPRVNPSDRYHLMPIITPAYPQQNSTYNVSVSTRMVMVEEFKQGLAI  347
Mouse     C-----NLNLPVWDPRVNPSDRYHLMPIITPAYPQQNSTYNVSVSTRMVMVEEFKQGLAI  347
Rat       C-----NLNLPVWDPRVNPSDRYHLMPIITPAYPQQNSTYNVSVSTRMVMVEEFKQGLAI  347
                .*   ***** : :**:*:*******:*:****.**: **  *:  *: ..: *

Worm      CRDISEGKSKWTALFEEVNFFSRYKHPIALIMAAPNEEEELNYGGFLESRIRLLVQSLER  403
Fly       TDEIMLGRIPWERLFEAPSFFYRYRHFIVLLVNSQTADDHLEWCGLVESKVRLLIGNLER  415
Human     TDEILLSKAEWSKLFEAPNFFQKYKHYIVLLASAPTEKQRLEWVGLVESKIRILVGSLEK  407
Mouse     TDEILLSKAEWSKLFEAPNFFQKYKHYIVLLASAPTEKQRLEWVGLVESKIRILVGSLEK  407
Rat       TDEILLSKAEWSKLFEAPNFFQKYKHYIVLLASAPTEKQRLEWVGLVESKIRILVGSLEK  407
            :*  .:  *  ***   .** :*:*:*.*:  : . .:.*:: *::**::*:*: .**:

Worm      NQDIIIAHNDPNKHKPSPNAKF--------------------DVNPENKRVTVWFIGLEF  443
Fly       NPHIALAHVNPKCFEFKKGQSANNSQNNSGNEDDLKQSQGNQSAVTSAPFCSMWFIGLEF  475
Human     NEFITLAHVNPQSFPAPKENP------------------------DKEEFRTMWVIGLVF  443
Mouse     NEFITLAHVNPQSFPAPKESP------------------------DREEFRTMWVIGLVF  443
Rat       NEFITLAHVNPQSFPAPKESP------------------------DREEFRTMWVIGLVF  443
          *  * :** :*: .                                   ::*.*** *

Worm      AEHA----KTLDLTNEIQRFKTNVELQASNVKGIGPNCQVQIDMFYVKRNSLIQVISAAD  499
Fly       ER---SENLNVDLTESIQNFTEHVMMHGVNIKMLKEG--MTIDARHVKRKQLSLYLDSDF  530
Human     KKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFEVD--MKIAAMHVKRKQLHQLLPNHV  501
Mouse     KKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFELD--MKIAAMHVKRKQLHQLLPSHV  501
Rat       KKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFELD--MKIAAMHVKRKQLHQLLPSHV  501
           .        .:*** .** *.   *  :. * * :  .  : *   :***:.*   :

Worm      LRRGRRWKKVVPIATNTSVSSSTPRSVVRTTSTSSVPTTPTGLAAPKTPLSASVSATNEP  559
Fly       LKRERKSMESHNNFNNTLLAN-RKRLSTELAQSQ-DPLPP-GQQ-------PSSGN----  576
Human     LQKKKKHSTEGVKLT--ALNDSSLDLSMDSDNSMSVPSPT-SAT-KTSPLNSSGSS----  553
Mouse     LQKRKKHSTEGVKLT--ALNDSSLDLSMDSDNSMSVPSPT-SAM-KTSPLNSSGSS----  553
Rat       LQKRKKHSTEGVKLA--ALNDSSLDLSMDSDNSMSVPSPT-SAM-KTSPLNSSGSS----  553
          *:: ::              : .           .:   *    .           * .

Worm      DSTTNGTPLSRKR-SMDEES---------STTVTSQISDESVPKKKTRDDTLEENRVSMV  609
Fly       RGRDSGAKIQRLSDSLTEENSNASSDMGAGTPTTPTTAQLSAPSFKSSGKNGSE------  630
Human     QGRNSPAPAV-----------------TAASVTNIQATEVSVPQVNSSESSG--------  588
Mouse     QGRNSPAPAV-----------------TAASVTSIQASEVSVPQANSSESPG--------  588
Rat       QGRNSPAPAV-----------------TAASVTSIQASEVSVPQANSSESPG--------  588
           .  . :                            .: ..   :: *.*. ::  .

Worm      VEVSNVVVEQRTKVVQEIVDLQADNGLNTSNGLEASEQKMEVPQSV--------------  655
Fly       -----------IDVVEQEPTQPHNNGNASSN-----TTTTEVACSXQPPTLHPHPPHQQP  674
Human     --------------------------GTSS---------ESIPQTATQP-------AISP  606
Mouse     --------------------------GPSS---------ESIPQTATQP-------AISP  606
Rat       --------------------------GPSS---------ESIPQTATQP-------AISP  606
                                   :*          .:  :

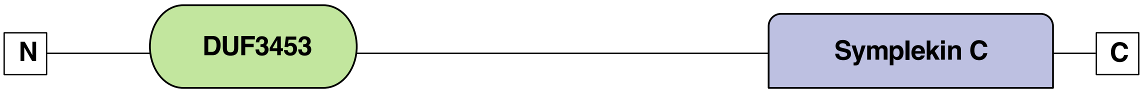
Worm      ------------------------------------------------------------  655
Fly       PPAPPQPPAHHHH---HQKFRKNNNAAAAAASNSIFNQRSILHAASSLSAALSITGHKRK  731
Human     PPKPTVSRVVSSTRLVNPPPRSSGNAATSG--NA----------AT--KIPTPIVGVKRT  652
Mouse     PPKPTVSRVVSSTRLVNPSPRPSGN--------T----------AT--KVPNPIVGVKRT  646
Rat       PPKPTVSRVVSSTRLVNPSPRPSGN--------A----------AT--KVPNPIVGVKRT  646

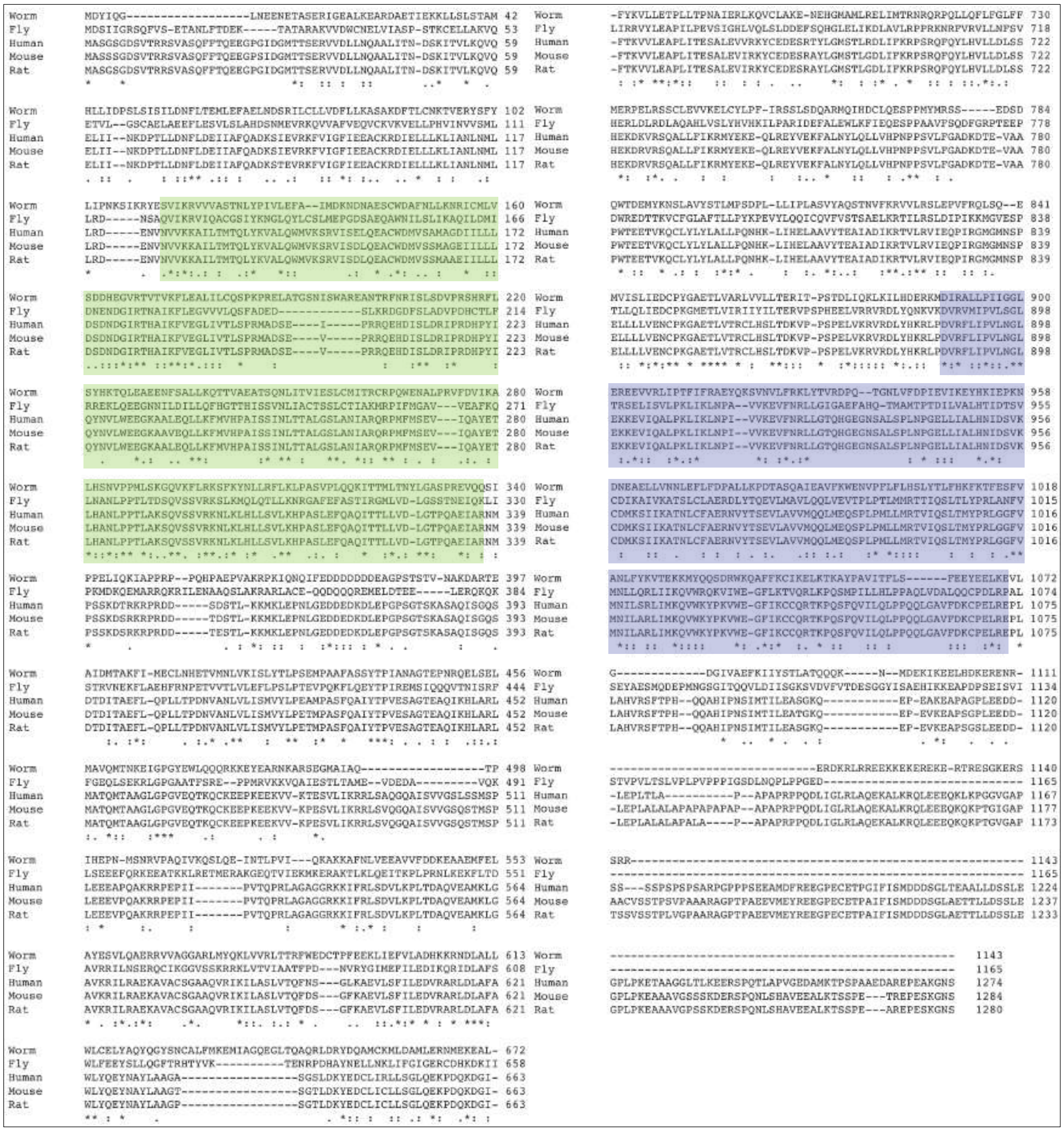
Worm      ------------------------------------------------------------  655
Fly       QQTATSTTMISSSNIAHSNYTQHSTHNNNGGGGGSHYNKTYYIDDDDEDNNQPLLTPNNQ  791
Human     SSPHK-------------EESPKKTKT-----------EEEQLDTET---------STTQ  679
Mouse     SSPNK-------------EESPKKTKT-----------EEEQVDLET---------SAVQ  673
Rat       SSPHK-------------EESPKKTKT-----------EEEQVGMET---------SAAP  673

Worm      ------------------------------------------------      655
Fly       TQQQQKAVRITA---TSASPATSVPVAASAAPPAPTISL--------      827
Human     SETIQTAASLLASQKTSSTDLSDIP--ALPANPIPVIKNSIKLRLNR      724
Mouse     SETVPASASLLASQKTSSTDLSDIP--ALPANPIPVIKNSIKLRLNR      718
Rat       SEAAPASASLVASQKTSSTDLSDIP--ALPANPIPVIKNSIKLRLNR      718
```

Supplemental Fig. S1

SYMK-1 (Symplekin)





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Worm     MDYIQG------------------LNEENETASERIGEALKEARDAETIEKKLLSLSTAM 42
Fly      MDSIIGRSQFVS-ETANLFTDEK-----TATARAKVVDWCNELVIASP-STKCELLAKVQ 53
Human    MASGSGDSVTRRSVASQFFTQEEGPGIDGMTTSERVVDLLNQAALITN-DSKITVLKQVQ 59
Mouse    MASSSGDSVTRRSVASQFFTQEEGPSIDGMTTSERVVDLLNQAALITN-DSKITVLKQVQ 59
Rat      MASGSGDSVTRRSVASQFFTQEEGPGIDGMTTSERVVDLLNQAALITN-DSKITVLKQVQ 59
         *    *                        *:  :: :  ::       ..*   *  .

Worm     HLLIDPSLSISILDNFLTEMLEFAELNDSRILCLLVDFLLKASAKDFTLCNKTVERYSFY 102
Fly      ETVL--GSCAELAEEFLESVLSLAHDSNMEVRKQVVAFVEQVCKVKVELLPHVINVVSML 111
Human    ELII--NKDPTLLDNFLDEIIAFQADKSIEVRKFVIGFIEEACKRDIELLLKLIANLNML 117
Mouse    ELII--NKDPTLLDNFLDEIIAFQADKSIEVRKFVIGFIEEACKRDIELLLKLIANLNML 117
Rat      ELII--NKDPTLLDNFLDEIIAFQADKSTEVRKFVIGFIEEACKRDIELLLKLIANLNML 117
         . ::  .    : ::** .:: :   .. .:   :: *: :..  .. *  : :   .:

Worm     LIPNKSIKRYESVIKRVVVASTNLYPIVLEFA--IMDKNDNAESCWDAFNLLKNRICMLV 160
Fly      LRD-----NSAQVIKRVIQACGSIYKNGLQYLCSLMEPGDSAEQAWNILSLIKAQILDMI 166
Human    LRD-----ENVNVVKKAILTMTQLYKVALQWMVKSRVISELQEACWDMVSAMAGDIILLL 172
Mouse    LRD-----ENVNVVKKAILTMTQLYKVALQWMVKSRVISDLQEACWDMVSSMAGEIILLL 172
Rat      LRD-----ENVNVVKKAILTMTQLYKVALQWMVKSRVISDLQEACWDMVSSMAAEIILLL 172
         *       .  .*:*:.: :  .:*   *::        .:  * .*: .. :    *  ::

Worm     SDDHEGVRTVTVKFLEALILCQSPKPRELATGSNISWAREANTRFNRISLSDVPRSHRFL 220
Fly      DNENDGIRTNAIKFLEGVVVLQSFADED------------SLKRDGDFSLADVPDHCTLF 214
Human    DSDNDGIRTHAIKFVEGLIVTLSPRMADSE----I-----PRRQEHDISLDRIPRDHPYI 223
Mouse    DSDNDGIRTHAIKFVEGLIVTLSPRMADSE----V-----PRRQEHDISLDRIPRDHPYI 223
Rat      DSDNDGIRTHAIKFVEGLIVTLSPRMADSE----V-----PRRQEHDISLDRIPRDHPYI 223
         ..:::*:** ::**:*.:::  *    :                :   :**  :*     :

Worm     SYHKTQLEAEENFSALLKQTTVAEATSQNLITVIESLCMITRCRPQWENALPRVFDVIKA 280
Fly      RREKLQEEGNNILDILLQFHGTTHISSVNLIACTSSLCTIAKMRPIFMGAV---VEAFKQ 271
Human    QYNVLWEEGKAALEQLLKFMVHPAISSINLTTALGSLANIARQRPMFMSEV---IQAYET 280
Mouse    QYNVLWEEGKAAVEQLLKFMVHPAISSINLTTALGSLANIARQRPMFMSEV---IQAYET 280
Rat      QYNVLWEEGKAALEQLLKFMVHPAISSINLTTALGSLANIARQRPMFMSEV---IQAYET 280
           .    *.:  .. **:       :* ** :   **. *:: ** : . :   .:. :

Worm     LHSNVPPMLSKGQVKFLRKSFKYNLLRFLKLPASVPLQQKITTMLTNYLGASPREVQQSI 340
Fly      LNANLPPTLTDSQVSSVRKSLKMQLQTLLKNRGAFEFASTIRGMLVD-LGSSTNEIQKLI 330
Human    LHANLPPTLAKSQVSSVRKNLKLHLLSVLKHPASLEFQAQITTLLVD-LGTPQAEIARNM 339
Mouse    LHANLPPTLAKSQVSSVRKNLKLHLLSVLKHPASLEFQAQITTLLVD-LGTPQAEIARNM 339
Rat      LHANLPPTLAKSQVSSVRKNLKLHLLSVLKHPASLEFQAQITTLLVD-LGTPQAEIARNM 339
         *::*:** *:..**. :**.:* :*  .**   .:. :    *  :*.: **:    *: : :

Worm     PPELIQKIAPPRP--PQHPAEPVAKRPKIQNQIFEDDDDDDDEAGPSTSTV-NAKDARTE 397
Fly      PKMDKQEMARRQKRILENAAQSLAKRARLACE-QQDQQQREMELDTEE-----LERQKQK 384
Human    PSSKDTRKRPRDD-----SDSTL-KKMKLEPNLGEDDEDKDLEPGPSGTSKASAQISGQS 393
Mouse    PSSKDSRKRPRDD-----TDSTL-KKMKLEPNLGEDDEDKDLEPGPSGTSKASAQISGQS 393
Rat      PSSKDSRKRPRDD-----TESTL-KKMKLEPNLGEDDEDKDLEPGPSGTSKASAQISGQS 393
         *     .                 . : *: ::  :  :*::: : * . .       :    .

Worm     AIDMTAKFI-MECLNHETVMNLVKISLYTLPSEMPAAFASSYTPIANAGTEPNRQELSEL 456
Fly      STRVNEKFLAEHFRNPETVVTLVLEFLPSLPTEVPQKFLQEYTPIREMSIQQQVTNISRF 444
Human    DTDITAEFL-QPLLTPDNVANLVLISMVYLPEAMPASFQAIYTPVESAGTEAQIKHLARL 452
Mouse    DTDITAEFL-QPLLTPDNVANLVLISMVYLPETMPASFQAIYTPVESAGTEAQIKHLARL 452
Rat      DTDITAEFL-QPLLTPDNVANLVLISMVYLPETMPASFQAIYTPVESAGTEAQIKHLARL 452
            :. :*:      . :.* .**   :  **   :*  *   ***: . . : :  .::.:

Worm     MAVQMTNKEIGPGYEWLQQQRKKEYEARNKARSEGMAIAQ------------------TP 498
Fly      FGEQLSEKRLGPGAATFSRE--PPMRVKKVQAIESTLTAME--VDEDA---------VQK 491
Human    MATQMTAAGLGPGVEQTKQCKEEPKEEKVV-KTESVLIKRRLSAQGQAISVVGSLSSMSP 511
Mouse    MATQMTAAGLGPGVEQTKQCKEEPKEEKVV-KPESVLIKRRLSVQGQAISVVGSQSTMSP 511
Rat      MATQMTAAGLGPGVEQTKQCKEEPKEEKVV-KPESVLIKRRLSVQGQAISVVGSQSTMSP 511
         :. *::   :***    .:       . :     *.

Worm     IHEPN-MSNRVPAQIVKQSLQE-INTLPVI---QKAKKAFNLVEEAVVFDDKEAAEMFEL 553
Fly      LSEEEFQRKEEATKKLRETMERAKGEQTVIEKMKERAKTLKLQEITKPLPRNLKEKFLTD 551
Human    LEEEAPQAKRRPEPII-------PVTQPRLAGAGGRKKIFRLSDVLKPLTDAQVEAMKLG 564
Mouse    LEEEVPQAKRRPEPII-------PVTQPRLAGAGGRKKIFRLSDVLKPLTDAQVEAMKLG 564
Rat      LEEEVPQAKRRPEPII-------PVTQPRLAGAGGRKKIFRLSDVLKPLTDAQVEAMKLG 564
         : *     :.     :               :        * :.* :    :       :

Worm     AYESVLQAERRVVAGGARLMYQKLVVRLTTRFWEDCTPFEEKLIEFVLADHKKRNDLALL 613
Fly      AVRRILNSERQCIKGGVSSKRRKLVTVIAATFPD---NVRYGIMEFILEDIKQRIDLAFS 608
Human    AVKRILRAEKAVACSGAAQVRIKILASLVTQFNS---GLKAEVLSFILEDVRARLDLAFA 621
Mouse    AVKRILRAEKAVACSGAAQVRIKILASLVTQFDS---GFKAEVLSFILEDVRARLDLAFA 621
Rat      AVKRILRAEKAVACSGAAQVRIKILASLVTQFDS---GFKAEVLSFILEDVRARLDLAFA 621
         * . :*.:*:     .*.     *::. :.: * .     ..  ::.*:* * : * ***:

Worm     WLCELYAQYQGYSNCALFMKEMIAGQEGLTQAQRLDRYDQAMCKMLDAMLERNMEKEAL- 672
Fly      WLFEEYSLLQGFTRHTYVK----------TENRPDHAYNELLNKLIFGIGERCDHKDKII 658
Human    WLYQEYNAYLAAGA-----------------SGSLDKYEDCLIRLLSGLQEKPDQKDGI- 663
Mouse    WLYQEYNAYLAAGT-----------------SGTLDKYEDCLICLLSGLQEKPDQKDGI- 663
Rat      WLYQEYNAYLAAGP-----------------SGTLDKYEDCLICLLSGLQEKPDQKDGI- 663
         ** : *     .                                . *:: :  :: .: *:  .*: :

Worm     -FYKVLLETPLLTPNAIERLKQVCLAKE-NEHGMAMLRELIMTRNRQRPQLLQFLFGLFF 730
Fly      LIRRVYLEAPILPEVSIGHLVQLSLDDEFSQHGLELIKDLAVLRPPRKNRFVRVLLNFSV 718
Human    -FTKVVLEAPLITESALEVVRKYCEDESRTYLGMSTLRDLIFKRPSRQFQYLHVLLDLSS 722
Mouse    -FTKVVLEAPLITESALEVIRKYCEDESRAYLGMSTLGDLIFKRPSRQFQYLHVLLDLSS 722
Rat      -FTKVVLEAPLITESALEVIRKYCEDESRAYLGMSTLGDLIFKRPSRQFQYLHVLLDLSS 722
          : :* **:*::   ::  : : .  ..     *:  : :* . *   :: : ::.*:.:

Worm     MERPELRSSCLEVVKELCYLPF-IRSSLSDQARMQIHDCLQESPPMYMRSS-----EDSD 784
Fly      HERLDLRDLAQAHLVSLYHVHKILPARIDEFALEWLKFIEQESPPAAVFSQDFGRPTEEP 778
Human    HEKDKVRSQALLFIKRMYEKE-QLREYVEKFALNYLQLLVHPNPPSVLFGADKDTE-VAA 780
Mouse    HEKDRVRSQALLFIKRMYEKE-QLREYVEKFALNYLQLLVHPNPPSVLFGADKDTE-VAA 780
Rat      HEKDRVRSQALLFIKRMYEKE-QLREYVEKFALNYLQLLVHPNPPSVLFGADKDTE-VAA 780
          *:  :*. .   :  :       :   :.. *    ::   : .** : .

Worm     QWTDEMYKNSLAVYSTLMPSDPL-LLIPLASVYAQSTNVFKRVVLRSLEPVFRQLSQ--E 841
Fly      DWREDTTKVCFGLAFTLLPYKPEVYLQQICQVFVSTSAELKRTILRSLDIPIKKMGVESP 838
Human    PWTEETVKQCLYLYLALLPQNHK-LIHELAAVYTEAIADIKRTVLRVIEQPIRGMGMNSP 839
Mouse    PWTEETVKQCLYLYLALLPQNHK-LIHELAAVYTEAIADIKRTVLRVIEQPIRGMGMNSP 839
Rat      PWTEETVKQCLYLYLALLPQNHK-LIHELAAVYTEAIADIKRTVLRVIEQPIRGMGMNSP 839
          * ::  * .: :  :*:* .     :  :. *:..:   :**.:** ::  :: :.

Worm     MVISLIEDCPYGAETLVARLVVLLTERIT-PSTDLIQKLKILHDERKMDIRALLPIIGGL 900
Fly      TLLQLIEDCPKGMETLVIRIIYILTERVPSPHEELVRRVRDLYQNKVKDVRVMIPVLSGL 898
Human    ELLLLVENCPKGAETLVTRCLHSLTDKVP-PSPELVKRVRDLYHKRLPDVRFLIPVLNGL 898
Mouse    ELLLLVENCPKGAETLVTRCLHSLTDKVP-PSPELVKRVRDLYHKRLPDVRFLIPVLNGL 898
Rat      ELLLLVENCPKGAETLVTRCLHSLTDKVP-PSPELVKRVRDLYHKRLPDVRFLIPVLNGL 898
          :: *:*:** * **** * :  **:::  *  :*::::: *:.::  *:* ::*::.**

Worm     EREEVVRLIPTFIFRAEYQKSVNVLFRKLYTVRDPQ--TGNLVFDPIEVIKEYHKIEPKN 958
Fly      TRSELISVLPKLIKLNPA--VVKEVFNRLLGIGAEFAHQ-TMAMTPTDILVALHTIDTSV 955
Human    EKKEVIQALPKLIKLNPI--VVKEVFNRLLGTQHGEGNSALSPLNPGELLIALHNIDSVK 956
Mouse    EKKEVIQALPKLIKLNPI--VVKEVFNRLLGTQHGEGNSALSPLNPGELLIALHNIDSVK 956
Rat      EKKEVIQALPKLIKLNPI--VVKEVFNRLLGTQHGEGNSALSPLNPGELLIALHNIDSVK 956
          :.*::  :*.:*          *: :*.:*                  : * :::   *.*:

Worm     DNEAELLVNNLEFLFDPALLKPDTASQAIEAVFKWENVPFLFLHSLYTLFHKFKTFESFV 1018
Fly      CDIKAIVKATSLCLAERDLYTQEVLMAVLQQLVEVTPLPTLMMRTTIQSLTLYPRLANFV 1015
Human    CDMKSIIKATNLCFAERNVYTSEVLAVVMQQLMEQSPLPMLLMRTVIQSLTMYPRLGGFV 1016
Mouse    CDMKSIIKATNLCFAERNVYTSEVLAVVMQQLMEQSPLPMLLMRTVIQSLTMYPRLGGFV 1016
Rat      CDMKSIIKATNLCFAERNVYTSEVLAVVMQQLMEQSPLPMLLMRTVIQSLTMYPRLGGFV 1016
          :   ::  .   : :  : . :.    .:: :.:   :* *::::     :  :  : .**

Worm     ANLFYKVTEKKMYQQSDRWKQAFFKCIKELKTKAYPAVITFLS------FEEYEELKEVL 1072
Fly      MNLLQRLIIKQVWRQKVIWE-GFLKTVQRLKPQSMPILLHLPPAQLVDALQQCPDLRPAL 1074
Human    MNILSRLIMKQVWKYPKVWE-GFIKCCQRTKPQSFQVILQLPPQQLGAVFDKCPELREPL 1075
Mouse    MNILARLIMKQVWKYPKVWE-GFIKCCQRTKPQSFQVILQLPPQQLGAVFDKCPELREPL 1075
Rat      MNILARLIMKQVWKYPKVWE-GFIKCCQRTKPQSFQVILQLPPQQLGAVFDKCPELREPL 1075
          *:: ::  *::::     *: .*:*  :. * ::    :: :           :::  :*:   *

Worm     G-------------DGIVAEFKIIYSTLATQQQK-----N--MDEKIKEELHDKERENR- 1111
Fly      SEYAESMQDEPMNGSGITQQVLDIISGKSVDVFVTDESGGYISAEHIKKEAPDPSEISVI 1134
Human    LAHVRSFTPH--QQAHIPNSIMTILEASGKQ-----------EP-EAKEAPAGPLEEDD- 1120
Mouse    LAHVRSFTPH--QQAHIPNSIMTILEATGKQ-----------EP-EVKEAPSGPLEEDD- 1120
Rat      LAHVRSFTPH--QQAHIPNSIMTILEASGKQ-----------EP-EVKEAPSGSLEEDD- 1120
                          *  ..  * .  . :                 . *:   .  .  .

Worm     ------------------------------ERDKRLRREEKKEKEREKE-RTRESGKERS 1140
Fly      STVPVLTSLVPLPVPPPIGSDLNQPLPPGED----------------------------- 1165
Human    -LEPLTLA----------P--APAPRPPQDLIGLRLAQEKALKRQLEEEQKLKPGGVGAP 1167
Mouse    -LEPLALALAPAPAPAPAP--APAPRPPQDLIGLRLAQEKALKRQLEEEQKQKPTGIGAP 1177
Rat      -LEPLALALAPALA----P--APAPRPPQDLIGLRLAQEKALKRQLEEEQKQKPTGVGAP 1173

Worm     SRR--------------------------------------------------------- 1143
Fly      ------------------------------------------------------------ 1165
Human    SS---SSPSPSPSARPGPPPSEEAMDFREEGPECETPGIFISMDDDSGLTEAALLDSSLE 1224
Mouse    AACVSSTPSVPAAARAGPTPAEEVMEYREEGPECETPAIFISMDDDSGLAETTLLDSSLE 1237
Rat      TSSVSSTPLVGPAARAGPTPAEEVMEYREEGPECETPAIFISMDDDSGLAETTLLDSSLE 1233

Worm     -------------------------------------------------------     1143
Fly      -------------------------------------------------------     1165
Human    GPLPKETAAGGLTLKEERSPQTLAPVGEDAMKTPSPAAEDAREPEAKGNS          1274
Mouse    GPLPKEAAAVGSSSKDERSPQNLSHAVEEALKTSSPE---TREPESKGNS          1284
Rat      GPLPKEAAAVGPSSKDERSPQNLSHAVEEALKTSSPE---AREPESKGNS          1280
```
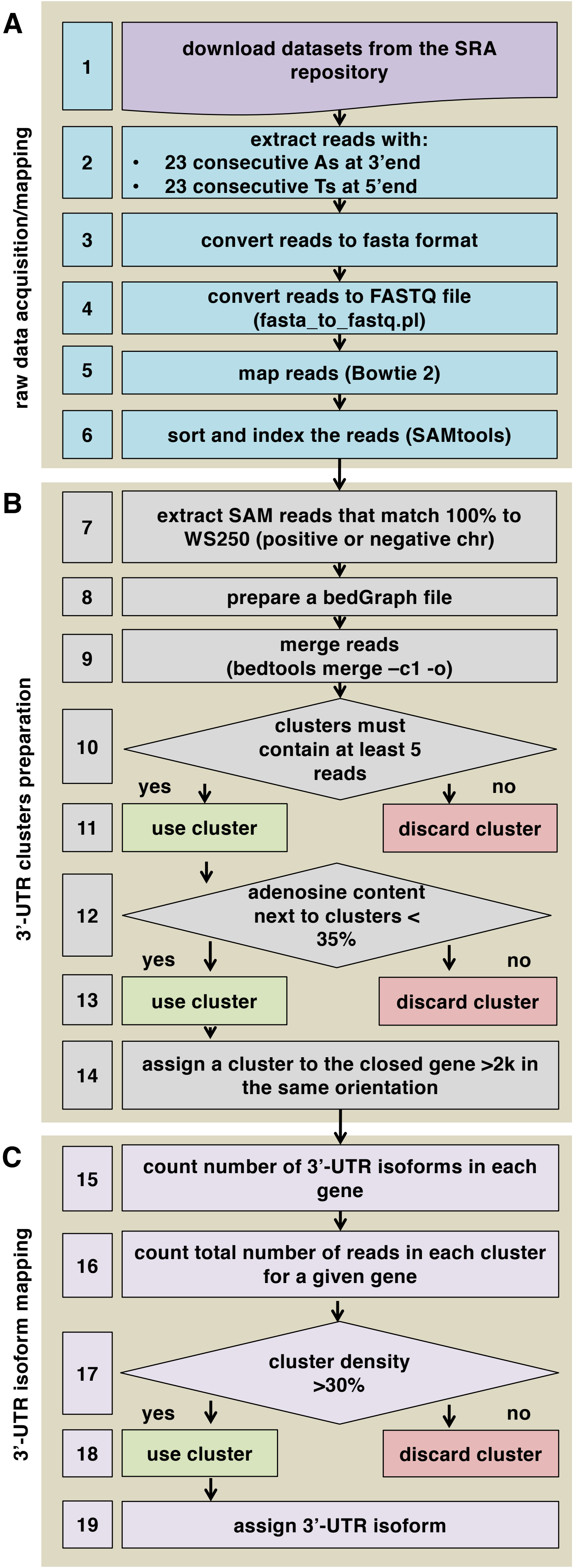

Supplemental Fig. S2

Name	Experiment #	Eggs/Hatched	Lethality (%)
cpsf-1 *(CPSF1)*	1	163/11	**93.7**
	2	238/5	**97.9**
	3	149/1	**99.3**
cpsf-2 *(CPSF2)*	1	68/37	**64.8**
	2	178/28	**86.4**
	3	221/25	**89.8**
cpsf-4 *(CPSF4)*	1	323/5	**98.5**
	2	699/17	**97.6**
	3	204/3	**98.6**
cpf-1 *(CST1)*	1	251/76	**76.8**
	2	200/64	**75.8**
	3	185/25	**88.1**
cpf-2 *(CSTF2)*	1	446/54	**89.2**
	2	138/14	**90.8**
	3	61/3	**95.3**
cfim-1 *(NUDT21)*	1	249/23	**91.5**
	2	196/20	**90.7**
	3	154/2	**98.7**
cfim-2 *(CPSF6)*	1	137/1	**99.3**
	2	282/27	**91.3**
	3	260/32	**89.0**
symk-1 *(Symplekin)*	1	62/4	**93.9**
	2	19/4	**82.6**
	3	114/5	**95.8**
rbpl-1 *(RBBP6)*	1	293/0	**100**
	2	344/0	**100**
	3	116/0	**100**
pcf-11 *(PCF11)*	1	141/5	**96.6**
	2	105/0	**100**
	3	172/5	**97.2**
clpf-1 *(CLP1)*	1	286/23	**92.6**
	2	289/18	**94.1**
	3	208/17	**92.4**
pkc-3 *(negative control)*	1	5/0	**100**
	2	8/0	**100**
	3	18/1	**94.7**
	4	51/1	**98.1**
	5	3/0	**100**
	6	22/0	**100**
	7	53/0	**100**
	8	6/0	**100**
	9	13/0	**100**
	10	19/0	**100**
	11	5/0	**100**
	12	4/0	**100**

Supplemental Figure S2: Results of the RNAi experiments of the *C. elegans* CPC. Twelve genes for the members of the *C. elegans* CPC were knocked-down using RNAi. Clones/rows are color-coded as from Figure 1. The human orthologs of each gene are shown in parenthesis in the first column. For each RNAi experiment we use 15 worms, and the number of eggs unhatched vs hatched at the end of the experiment were counted. The percent lethality was consistently high across all tested clones. *pkc-3* RNAi was used as a negative RNAi control, since it is known to induce strong embryonic lethality.


A
raw data acquisition/mapping
1
download datasets from the SRA repository
2
extract reads with:
• 23 consecutive As at 3'end
• 23 consecutive Ts at 5'end
3
convert reads to fasta format
4
convert reads to FASTQ file (fasta_to_fastq.pl)
5
map reads (Bowtie 2)
6
sort and index the reads (SAMtools)
B
3'-UTR clusters preparation
7
extract SAM reads that match 100% to WS250 (positive or negative chr)
8
prepare a bedGraph file
9
merge reads (bedtools merge –c1 -o)
10
clusters must contain at least 5 reads
yes
no
11
use cluster
discard cluster
12
adenosine content next to clusters < 35%
yes
no
13
use cluster
discard cluster
14
assign a cluster to the closed gene >2k in the same orientation
C
3'-UTR isoform mapping
15
count number of 3'-UTR isoforms in each gene
16
count total number of reads in each cluster for a given gene
17
cluster density >30%
yes
no
18
use cluster
discard cluster
19
assign 3'-UTR isoform

Supplemental Figure S3: Bioinformatic Pipeline used in this study. The pipeline uses raw transcriptome datasets downloaded from the public repository SRA trace archive to extract and map 3'-UTR end clusters to the closest protein-coding genes in the correct orientation.

The pipeline is divided in three large steps: A) Acquisition/Mapping, B) 3'-UTR cluster preparation and C) 3'-UTR isoforms mapping.

In the acquisition/mapping step, we used custom made Perl scripts to extract reads with 23 consecutive As at the 3'-end or 23 consecutive Ts at the 5'-end and then mapped these filtered reads to the WS250 version of the *C. elegans* genome (Bowtie 2). We then sorted and indexed the reads for visualization purposes.

In the 3'-UTR cluster preparation step, we extracted SAM reads with 100% match to the WS250 and used them to prepare a new bedGraph file (BEDTools).

We then merged the reads and discarded the clusters with less than 5 reads.

Restrictive parameters for cluster identification and 3'-UTR end mapping included the discard of clusters with an adenosine content of <35% downstream of its end.

Clusters were assigned to a mapped 3'-UTR end and attached to the closest gene with 2,000 nt in the same orientation.

At the completion of these steps we performed the 3'-UTR isoform mapping step, which consists of the counting and assignment the total number of 3'-UTR isoforms to a given gene.

We discarded clusters with a density of less than 30% of the total number of reads.

Supplemental Fig. S4

A

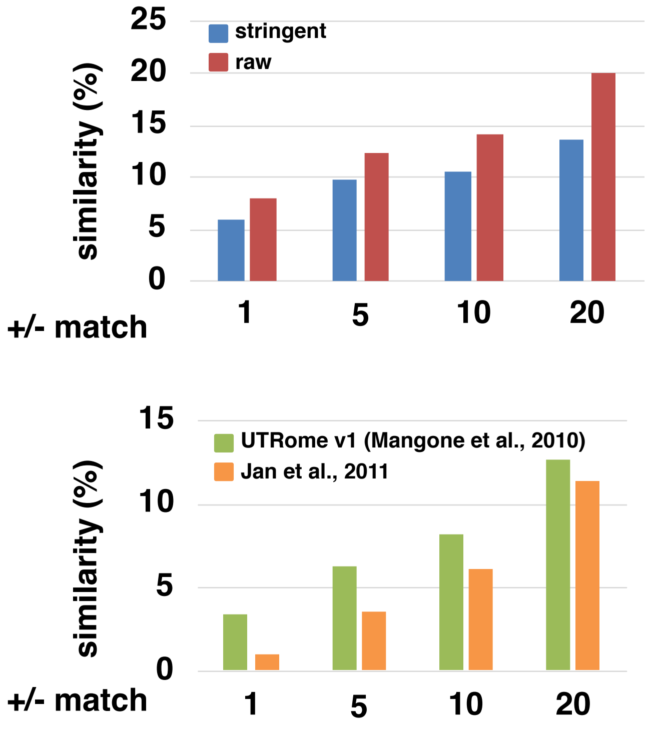
n=27,264

mismatch (+/-)	stringent	raw
1	1,604	2,167
5	2,637	3,373
10	2,898	3,842
20	3,702	5,437

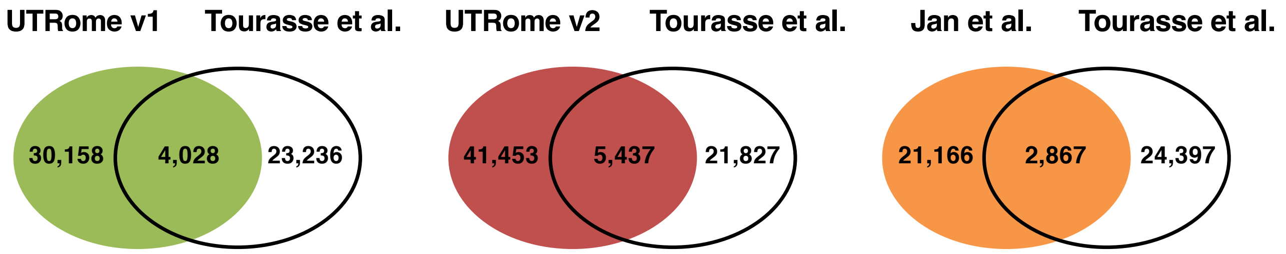
n=27,264

mismatch (+/-)	Mangone et al., 2010	Jan et al., 2011
1	930	285
5	1,717	989
10	2,229	1,664
20	3,436	3,106

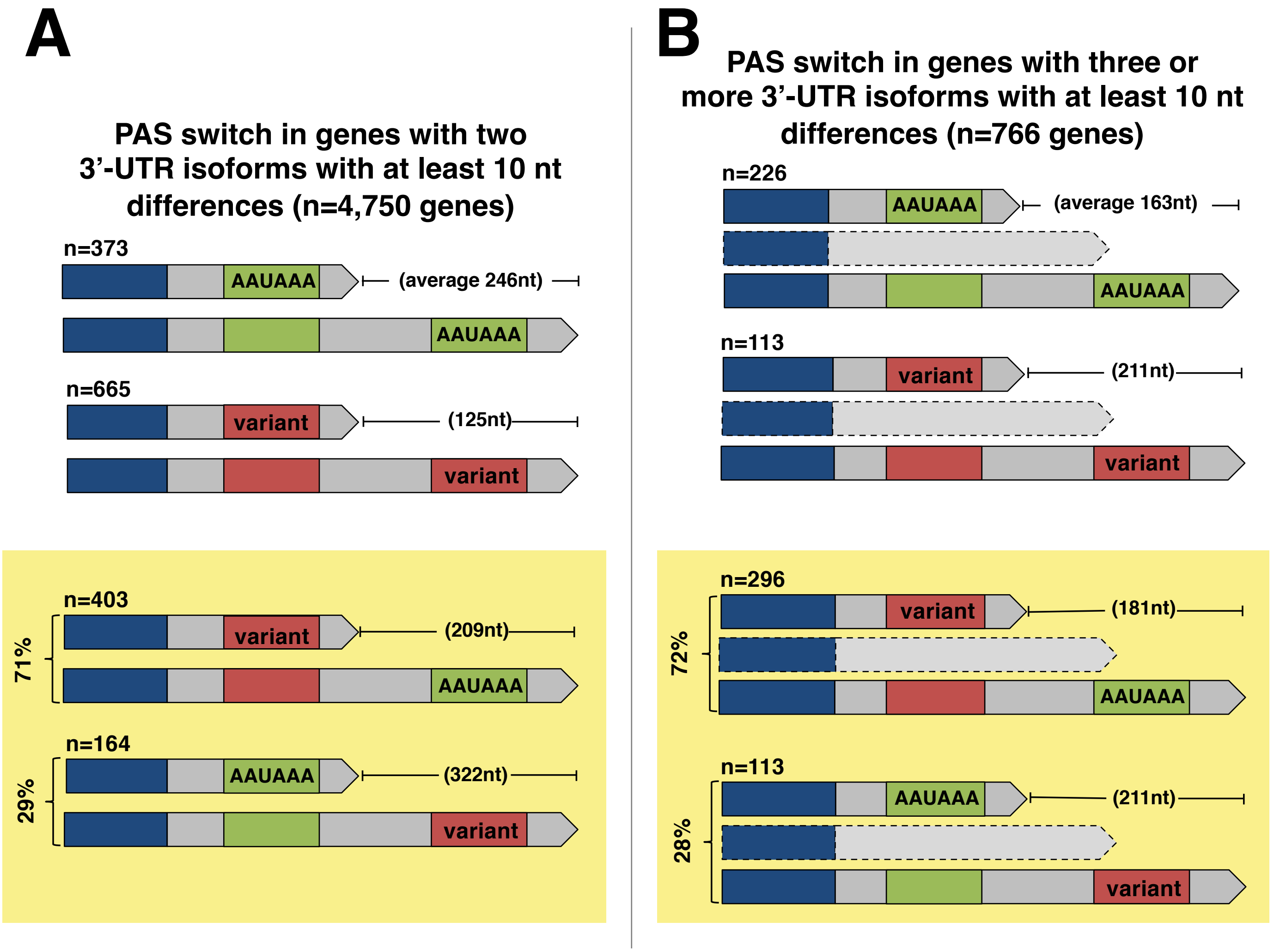
B



C



Supplemental Figure S4: Comparison with Tourasse et al., 2017. We have downloaded the poly(A) sites mapped in Tourasse et al. and performed a comparison with the 3’-UTRs present in our 3’-UTRome v2. A) Top Panel: number of mapped poly(A) sites from Tourasse et al. that match our stringent and raw datasets within +/-1 nt, +/- 5 nt, +/- 10 nt or +/- 20 nt. Bottom Panel: number of poly(A) sites in common between Tourasse et al. and Mangone et al. and between Tourasse et al. and Jan et al. within +/-1nt, +/- 5nt, +/- 10nt or +/- 20nt. B) Bar chart showing the % of similarity of the two datasets in Panel A. C. Venn diagrams comparing the 3’-UTRs shared (+/- 20nt) between Tourasse et al., and the UTRome v1 (Mangone et al., - (green), this study (UTRome v2 - red), and Jan et al. (orange). We have used our unfiltered dataset to compare UTRome v2 with Tourasse et al.



A

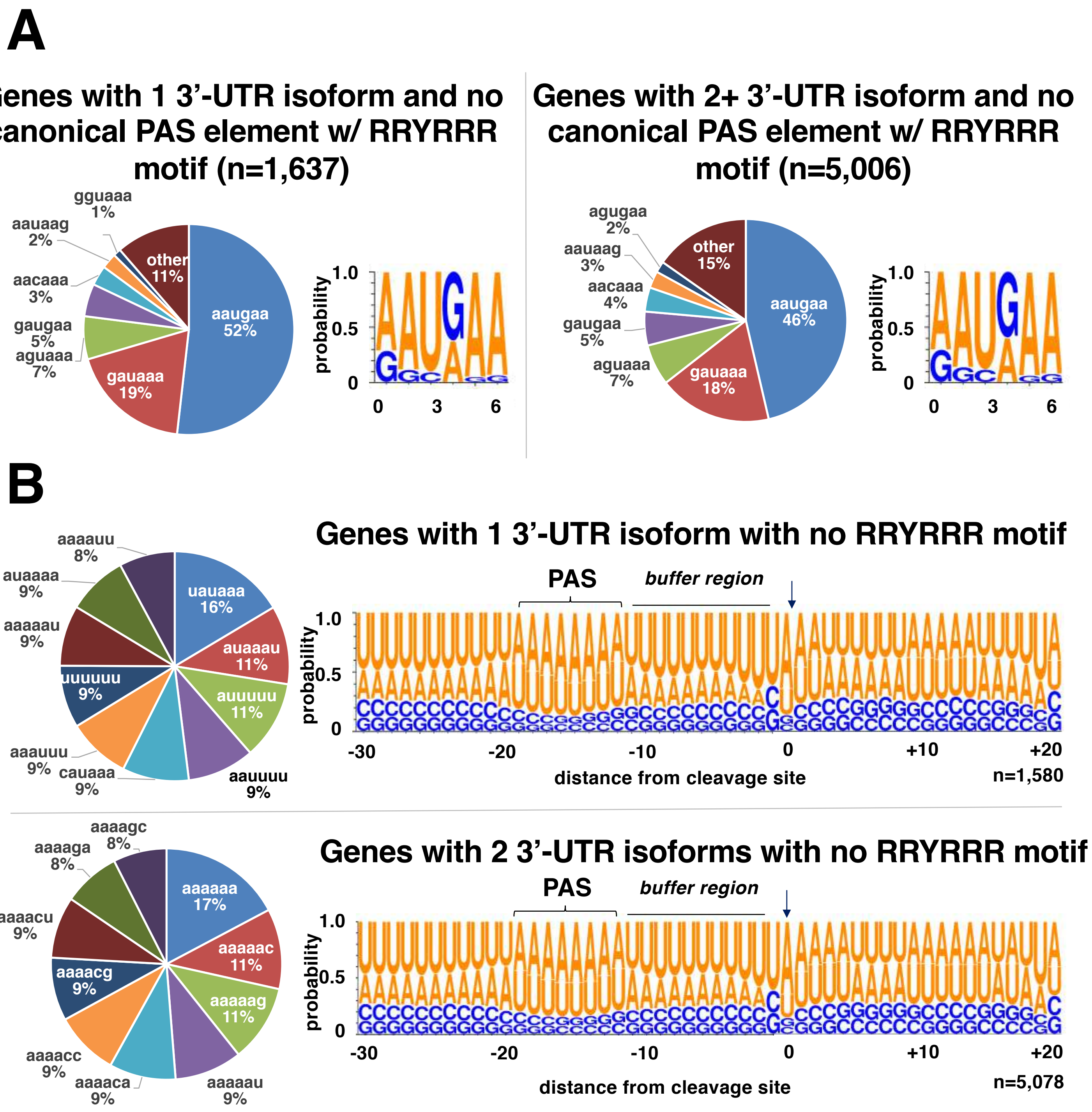
PAS switch in genes with two 3’-UTR isoforms with at least 10 nt differences (n=4,750 genes)

B

PAS switch in genes with three or more 3’-UTR isoforms with at least 10 nt differences (n=766 genes)

Supplemental Figure S5: PAS site usage in genes with multiple 3’-UTR isoforms. A) In genes with only two 3’-UTR isoforms with a difference of at least 10 nt between isoforms, 373 pairs of isoforms had canonical PAS elements in both isoforms with an average of 246 nt difference between isoforms while 665 pairs had variant PAS elements in both isoforms with an average of 125 nt difference between them. In isoform pairs where the type of PAS element switches, 71% have a shorter isoform with a variant PAS element and a longer isoform with a canonical PAS element with an average of 209 nt between them while the remaining 29% have a canonical PAS element on the shorter isoform and a variant PAS element on the longer isoform with an average of 322 nt between them. B) In genes with three or more 3’-UTR isoforms, genes where the longest and the shortest isoform both have canonical PAS elements have an average of 163 nt between them while genes where the longest and the shortest isoforms both have variant PAS elements have an average of 211 nt between them. 72% of genes switch from a variant PAS elements in the short isoform to a canonical PAS element in the long isoform, with an average of 181 nt between them. 28% of genes have canonical PAS elements in the short isoforms and variant PAS elements in the long 3’-UTR isoform, with an average of 211 nt between the two.

Supplemental Fig. S6



Supplemental Figure S6: Detection of the PAS element in genes lacking a canonical AAUAAA hexamer. A) We have searched for the most common RRYRRR motifs located within the last 30 nt in genes with 1 (top) or 2+ (bottom) 3'-UTR isoforms with no canonical PAS and with a detectable RRYRRR motif. The left chart shows the occurrences of the seven most common PAS elements identified in these groups, and the right logo shows the identified PAS motif. Apart from a slight increase in percentage of adenosines and guanosines in 3'-UTRs of genes with 2+ 3'-UTRs, both results are similar. The overwhelming majority of PAS conform with the AAU(G/A)AA with a tolerance of 1 nt purine-purine or pyrimidine-pyrimidine replacement. B) PAS site usage in genes with 1 or 2 3'-UTR isoforms with non canonical PAS and no RRYRRR motif. The pie chart shows the 10 most common hexamers located within 30 nt upstream of the the cleavage site. Right logo plot shows the nucleotide conservation of the intergenic region encompassing the cleavage site from -30 to +20 nt. Arrow marks the cleavage site. The buffer region and the PAS is marked.

Supplemental Fig. S7

Biological Process

1 3’-UTR isoform (n=8,537)

adj.Pval	nGenes	Pathways
5.7e-28	165	phosphorus metabolic process
7.2e-28	220	single-organism metabolic process
2.5e-26	159	phosphate-containing compound metabolic process
8.7e-23	149	macromolecule modification
1.3e-17	98	phosphorylation
1.3e-16	130	cellular protein modification process
1.3e-16	130	protein modification process
4.9e-16	164	cellular protein metabolic process
6.2e-14	75	protein phosphorylation
4.7e-13	90	oxidation-reduction process

2 3’-UTR isoform (n=4,741)

adj.Pval	nGenes	Pathways
3.6e-20	135	single-organism metabolic process
1.4e-13	38	immune response
1.4e-13	38	immune system process
5.0e-13	63	oxidation-reduction process
8.7e-12	35	innate immune response
7.5e-11	82	phosphorus metabolic process
4.0e-10	41	defense response
1.1e-09	78	phosphate-containing compound metabolic process
9.9e-08	41	carbohydrate derivative metabolic process
1.0e-07	51	small molecule metabolic process

3+ 3’-UTR isoform (n=1,530)

adj.Pval	nGenes	Pathways
2.3e-03	19	small molecule metabolic process
2.3e-03	21	organonitrogen compound metabolic process
2.3e-03	8	ribonucleoside monophosphate metabolic process
2.3e-03	8	nucleoside monophosphate metabolic process
2.3e-03	11	innate immune response
2.3e-03	11	immune response

Cellular Component

1 3’-UTR isoform (n=8,537)

adj.Pval	nGenes	Pathways
5.4e-28	122	cytoplasmic part
6.1e-22	48	mitochondrion
9.7e-22	102	organelle part
5.2e-18	89	intracellular organelle part
2.5e-15	73	non-membrane-bounded organelle
2.5e-15	73	intracellular non-membrane-bounded organelle
8.7e-13	29	mitochondrial part
1.0e-12	94	nucleus
6.8e-12	40	membrane-enclosed lumen
4.2e-11	38	intracellular organelle lumen

2 3’-UTR isoform (n=4,741)

adj.Pval	nGenes	Pathways
4.1e-17	73	cytoplasmic part
9.9e-08	20	endoplasmic reticulum
9.9e-08	50	organelle part
4.3e-07	21	mitochondrion
2.9e-06	43	intracellular organelle part
2.9e-06	52	nucleus
3.4e-06	23	extracellular region
4.5e-05	23	endomembrane system
1.9e-04	13	mitochondrial part
1.9e-04	7	membrane raft

3+ 3’-UTR isoform (n=1,530)

adj.Pval	nGenes	Pathways
4.0e-07	25	organelle part
6.0e-07	26	cytoplasmic part
6.0e-07	8	mitochondrial inner membrane
6.0e-07	10	mitochondrial part
6.0e-07	8	organelle inner membrane
6.0e-07	10	organelle envelope
6.0e-07	9	mitochondrial envelope
6.0e-07	10	envelope
3.5e-06	11	mitochondrion
4.5e-06	8	mitochondrial membrane

Molecular Component

1 3’-UTR isoform (n=8,537)

adj.Pval	nGenes	Pathways
7.6e-64	231	transferase activity
4.2e-54	220	hydrolase activity
4.1e-39	204	nucleotide binding
4.1e-39	204	nucleoside phosphate binding
2.6e-38	206	small molecule binding
6.5e-38	195	anion binding
4.4e-36	119	transferase activity, transferring phosphorus-containing groups
1.1e-29	163	ribonucleotide binding
1.7e-29	166	carbohydrate derivative binding
2.1e-29	160	purine nucleoside binding

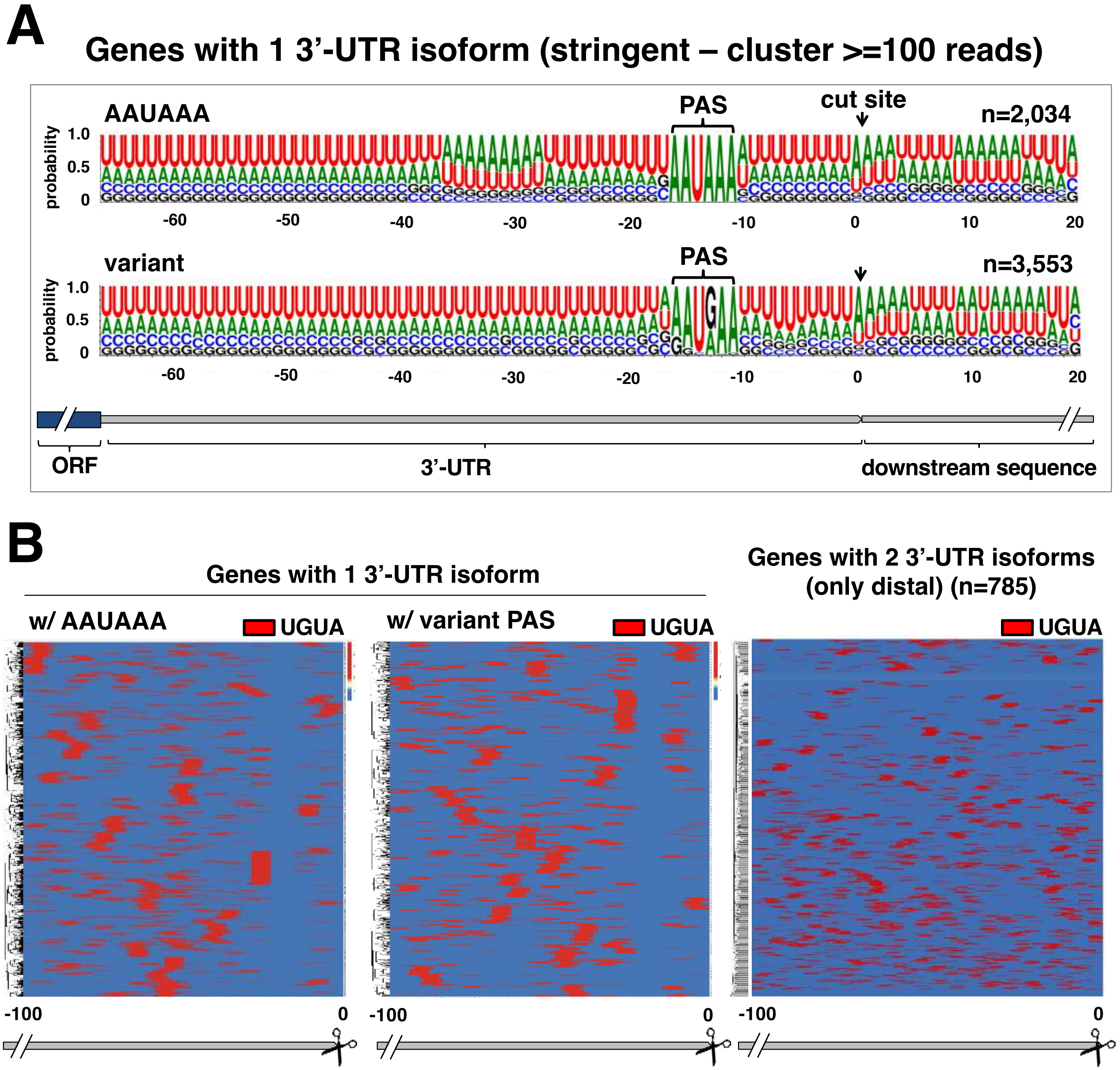
2 3’-UTR isoform (n=4,741)

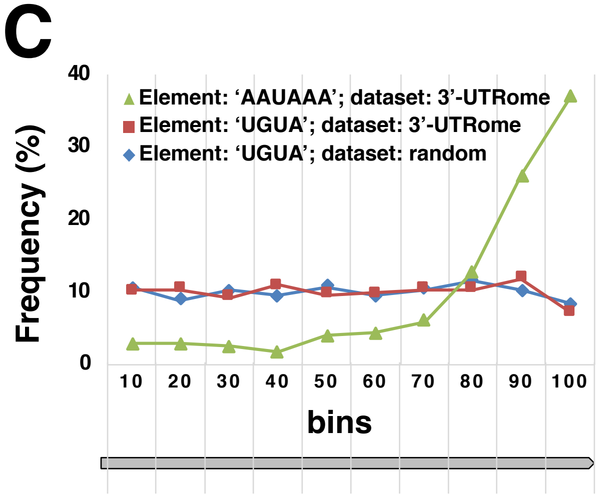
adj.Pval	nGenes	Pathways
2.4e-28	121	hydrolase activity
1.3e-27	117	transferase activity
1.7e-16	56	oxidoreductase activity
2.2e-15	103	small molecule binding
4.6e-15	100	nucleotide binding
4.6e-15	100	nucleoside phosphate binding
5.3e-15	59	transferase activity, transferring phosphorus-containing groups
1.7e-13	70	zinc ion binding
4.5e-12	76	transition metal ion binding
5.7e-11	89	nucleic acid binding

3+ 3’-UTR isoform (n=1,530)

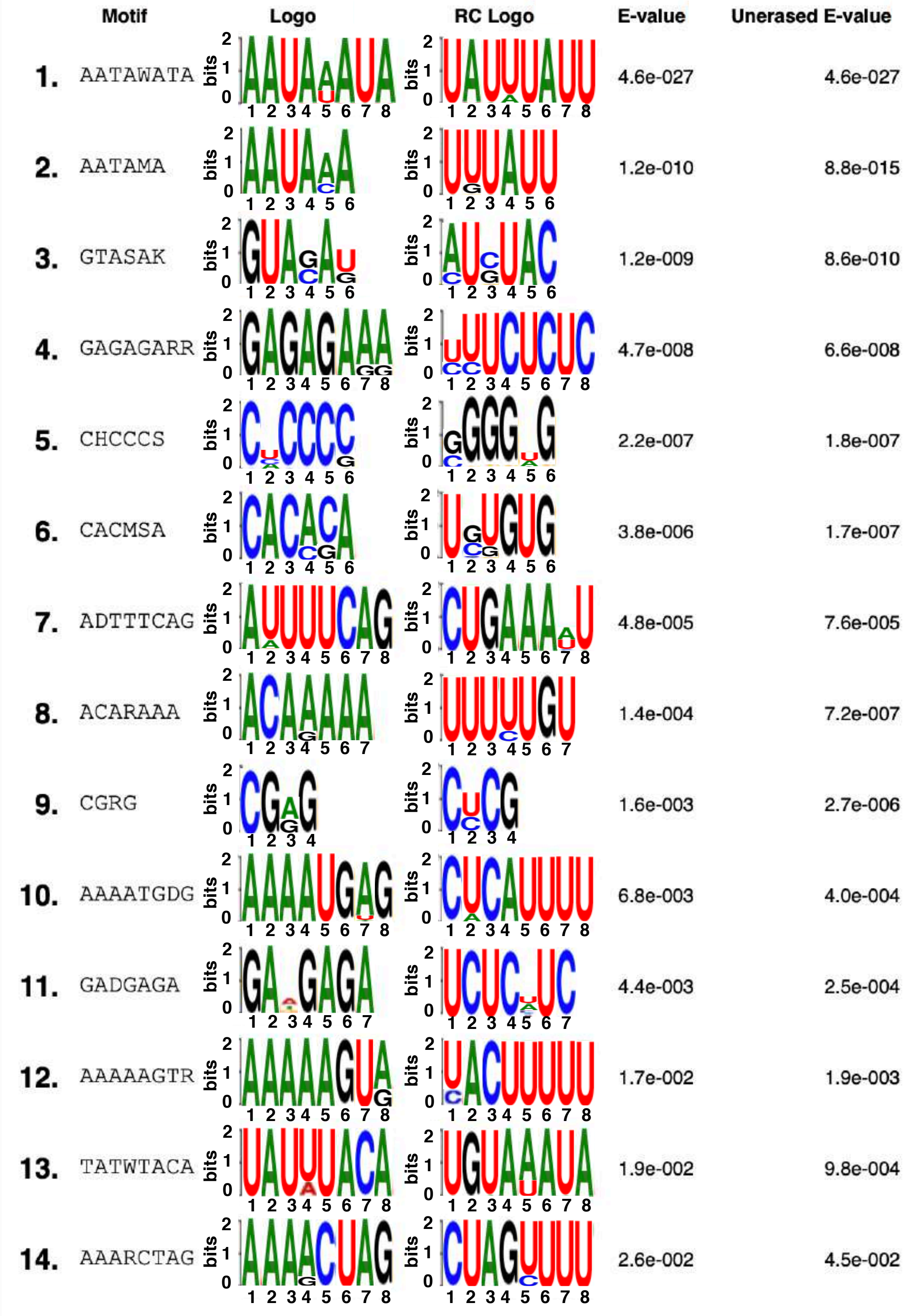
adj.Pval	nGenes	Pathways
2.5e-09	39	nucleic acid binding
3.5e-09	37	hydrolase activity
5.3e-08	37	small molecule binding
2.0e-07	35	nucleotide binding
2.0e-07	35	nucleoside phosphate binding
1.1e-06	15	RNA binding
6.0e-06	31	anion binding
1.8e-04	26	carbohydrate derivative binding
2.2e-04	21	zinc ion binding
3.5e-04	15	oxidoreductase activity

Supplemental Figure S7: GO term analysis for genes with 1, 2 or 3 3’-UTR isoforms.



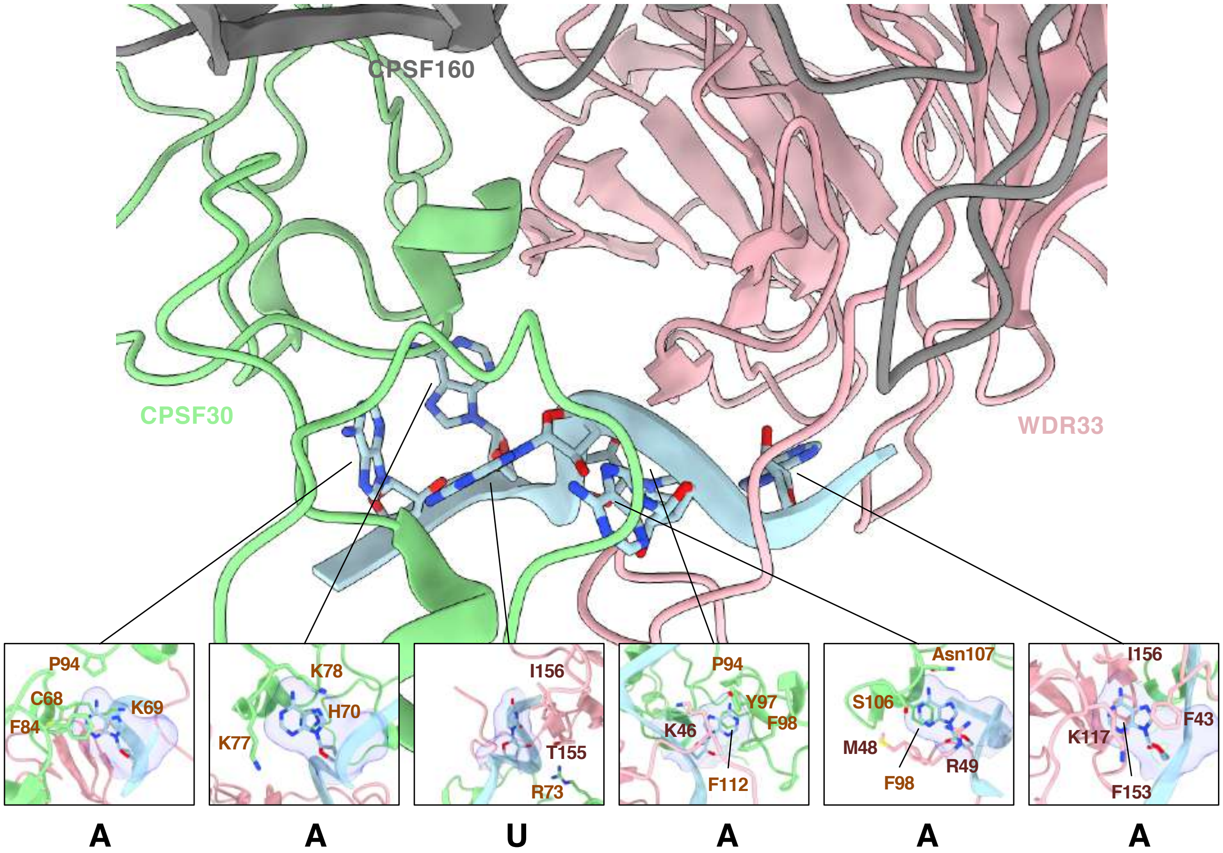


Supplemental Figure S8: Detection of the 'UGUA' element in *C. elegans* 3'-UTRs. A) Logo plot of the transcript's region within the cleavage site in genes with only one 3'-UTR isoform and with a canonical or variant PAS element. B) Identification of the 'UGUA" motif (red) within 100 nt upstream of the cleavage site in genes with one or two 3'-UTR isoforms. C) Binned frequency distribution of the occurrences of the AAUAAA and UGUA elements in distal 3'-UTR isoforms as in the right heatmap in Panel B (green and red) vs. the occurrences of the UGUA motif in a randomly generated 3'-UTR dataset (blue) (n=785).

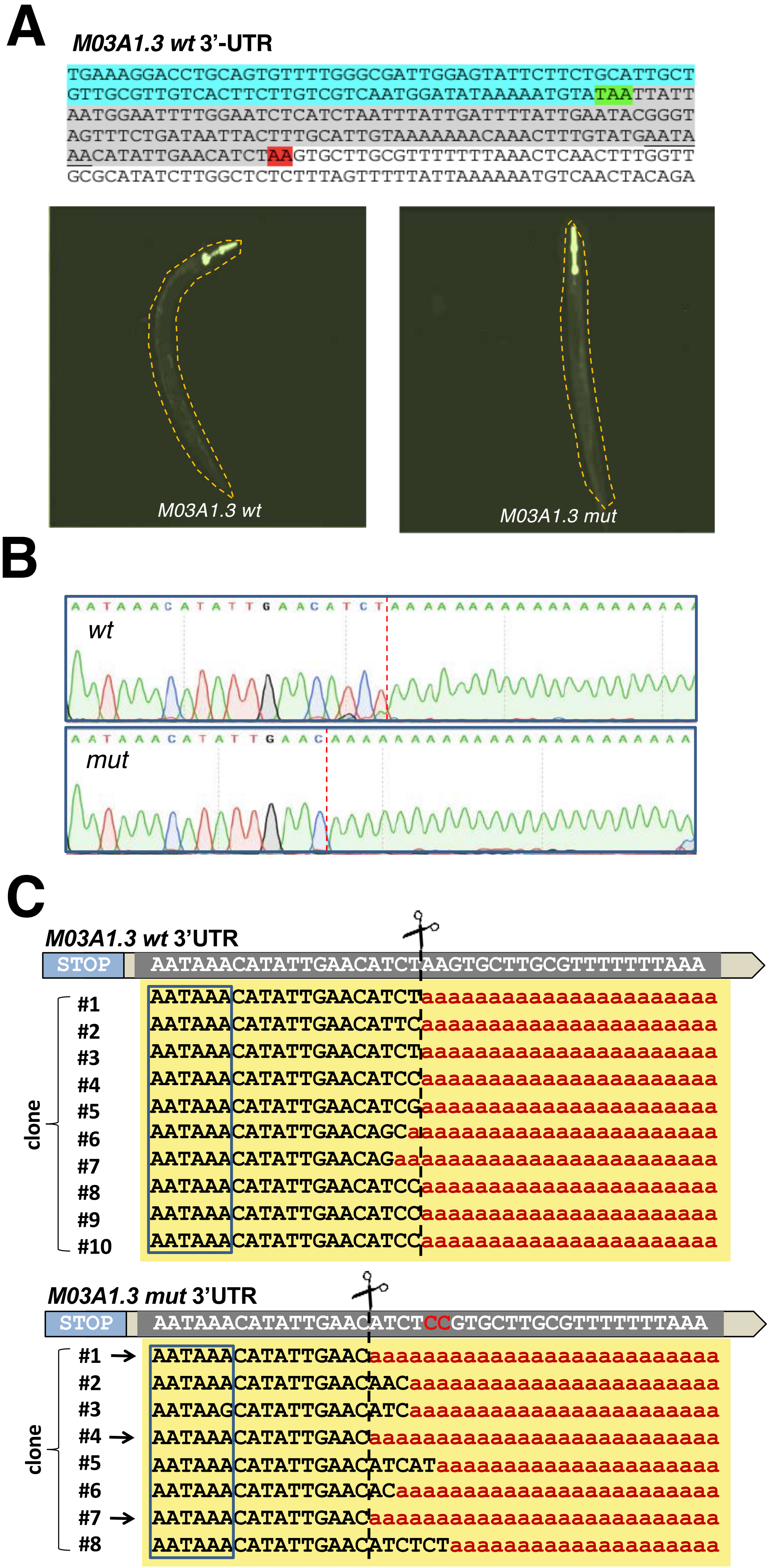


	Motif	Logo	RC Logo	E-value	Unerased E-value
1.	AATAWATA			4.6e-027	4.6e-027
2.	AATAMA			1.2e-010	8.8e-015
3.	GTASAK			1.2e-009	8.6e-010
4.	GAGAGARR			4.7e-008	6.6e-008
5.	CHCCCS			2.2e-007	1.8e-007
6.	CACMSA			3.8e-006	1.7e-007
7.	ADTTTCAG			4.8e-005	7.6e-005
8.	ACARAAA			1.4e-004	7.2e-007
9.	CGRG			1.6e-003	2.7e-006
10.	AAAATGDG			6.8e-003	4.0e-004
11.	GADGAGA			4.4e-003	2.5e-004
12.	AAAAAGTR			1.7e-002	1.9e-003
13.	TATWTACA			1.9e-002	9.8e-004
14.	AAARCTAG			2.6e-002	4.5e-002

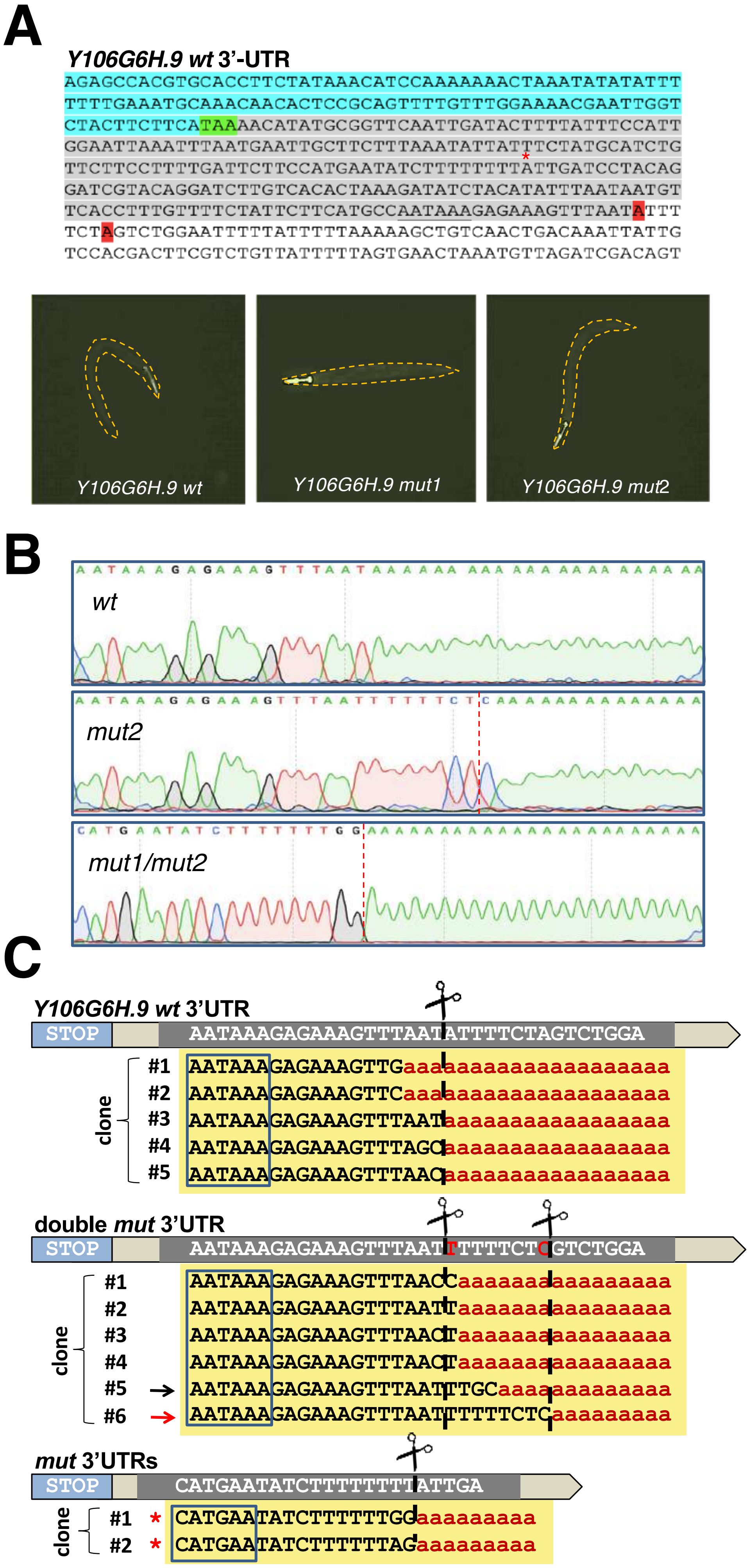
Supplemental Figure S9: Detection of enriched elements in *C. elegans* 3'-UTRs of genes with 2 3'-UTR isoforms (only distal) (n=785). These motifs have been detected using the meme suite (Bailey et al., 2015.)



Supplemental Figure S10: Nucleotide binding site of the human CPSF160-WDR33-CPSF30 complex. Ribbon representation of the cryo-EM structure of human CPSF160-WDR33-CPSF30 complex (PDB code: 6DNF) (Sun et al., 2018). The nucleotides of the bound RNA fragment do not show a specific interaction with either CPSF30 or WDR33. The interactions are mostly established by π-π ring stacking. Color gray shows the CPSF160, pink for WDR33, and light green for CPSF30. Sticks represent the RNA molecules bound with CPSF30 and WDR33. Surfaces in the inlets are for individual nucleotides.


A
M03A1.3 wt 3'-UTR
TGAAAGGACCTGCAGTGTTTTGGGCGATTGGAGTATTCTTCTGCATTGCT
GTTGCGTTGTCACTTCTTGTCGTCAATGGATATAAAAATGTATAATTATT
AATGGAATTTTGGAATCTCATCTAATTTATTGATTTTATTGAATACGGGT
AGTTTCTGATAATTACTTTGCATTGTAAAAAAACAAACTTTGTATGAATA
AACATATTGAACATCTAAGTGCTTGCGTTTTTTTAAACTCAACTTTGGTT
GCGCATATCTTGGCTCTCTTTAGTTTTTATTAAAAAATGTCAACTACAGA
M03A1.3 wt
M03A1.3 mut
B
AATAAACATATTGAACATCTAAAAAAAAAAAAAAAAAAA
wt
AATAAACATATTGAACAAAAAAAAAAAAAAAAAAAAAAA
mut
C
M03A1.3 wt 3'UTR
STOP
AATAAACATATTGAACATCTAAGTGCTTGCGTTTTTTTTAAA
clone
#1 AATAAACATATTGAACATCTaaaaaaaaaaaaaaaaaaaaaa
#2 AATAAACATATTGAACATTCaaaaaaaaaaaaaaaaaaaaaa
#3 AATAAACATATTGAACATCTaaaaaaaaaaaaaaaaaaaaaa
#4 AATAAACATATTGAACATCCaaaaaaaaaaaaaaaaaaaaaa
#5 AATAAACATATTGAACATCGaaaaaaaaaaaaaaaaaaaaaa
#6 AATAAACATATTGAACAGCaaaaaaaaaaaaaaaaaaaaaaa
#7 AATAAACATATTGAACAGaaaaaaaaaaaaaaaaaaaaaaaa
#8 AATAAACATATTGAACATCCaaaaaaaaaaaaaaaaaaaaaa
#9 AATAAACATATTGAACATCCaaaaaaaaaaaaaaaaaaaaaa
#10 AATAAACATATTGAACATCCaaaaaaaaaaaaaaaaaaaaaa
M03A1.3 mut 3'UTR
STOP
AATAAACATATTGAACATCTCCGTGCTTGCGTTTTTTTTAAA
clone
#1 → AATAAACATATTGAACaaaaaaaaaaaaaaaaaaaaaaaaaaa
#2 AATAAACATATTGAACAACaaaaaaaaaaaaaaaaaaaaaaaa
#3 AATAAGCATATTGAACATCaaaaaaaaaaaaaaaaaaaaaaaa
#4 → AATAAACATATTGAACaaaaaaaaaaaaaaaaaaaaaaaaaaa
#5 AATAAACATATTGAACATCATaaaaaaaaaaaaaaaaaaaaaa
#6 AATAAACATATTGAACACaaaaaaaaaaaaaaaaaaaaaaaaa
#7 → AATAAACATATTGAACaaaaaaaaaaaaaaaaaaaaaaaaaaa
#8 AATAAACATATTGAACATCTCTaaaaaaaaaaaaaaaaaaaaa

Supplemental Figure S11: *In vivo* cleavage assay for *M03A1.3*.
A) *M03A1.3* genomic region cloned downstream of the GFP reporter. Blue: terminal portion of the *M03A1.3* ORF. Green: STOP codon. Gray: 3'-UTR. Red: mutated terminal adenosine nucleotides. The transgenic worms expressing the P*myo-3*::GFP::*M03A1.3*_3'-UTR *wt* and mutant cassette are shown below.
B) At the completion of the experiment, we recovered the total RNA and performed RT-PCR experiments using a forward primer annealing within the GFP ORF and a reverse polydT primer with two anchors containing Invitrogen Gateway adapters. The resultant amplicons were then subcloned in gateway vectors and sequenced to detect the cleavage site. An example of resultant trace files is shown.
C) Examples of 10 clones identified in this study for *M03A1.3*. The removal of the terminal genomic adenosine nucleotide induces a cleavage site 3 nt upstream of the canonical cleavage site in three clones (arrows), which also contain a terminal adenosine nucleotide. The PAS element is boxed in blue color.


A
Y106G6H.9 wt 3'-UTR
AGAGCCACGTGCACCTTCTATAAACATCCAAAAAAACTAAATATATATTT
TTTTGAAATGCAAACAACACTCCGCAGTTTTGTTTGGAAAACGAATTGGT
CTACTTCTTCATAAAACATATGCGGTTCAATTGATACTTTTATTTCCATT
GGAATTAAATTTAATGAATTGCTTCTTTAAATATTATTTCTATGCATCTG
TTCTTCCTTTTGATTCTTCCATGAATATCTTTTTTTTATTGATCCTACAG
GATCGTACAGGATCTTGTCACACTAAAGATATCTACATATTTAATAATGT
TCACCTTTGTTTTCTATTCTTCATGCCAATAAAGAGAAAGTTTAATATTT
TCTAGTCTGGAATTTTTATTTTTAAAAAGCTGTCAACTGACAAATTATTG
TCCACGACTTCGTCTGTTATTTTTAGTGAACTAAATGTTAGATCGACAGT
Y106G6H.9 wt
Y106G6H.9 mut1
Y106G6H.9 mut2
B
wt
mut2
mut1/mut2
C
Y106G6H.9 wt 3'UTR
STOP
AATAAAGAGAAAGTTTAATATTTTCTAGTCTGGA
clone
#1 AATAAAGAGAAAGTTGaaaaaaaaaaaaaaaaaaaa
#2 AATAAAGAGAAAGTTCaaaaaaaaaaaaaaaaaaaa
#3 AATAAAGAGAAAGTTTAATaaaaaaaaaaaaaaaaa
#4 AATAAAGAGAAAGTTTAGCaaaaaaaaaaaaaaaaa
#5 AATAAAGAGAAAGTTTAACaaaaaaaaaaaaaaaaa
double mut 3'UTR
STOP
AATAAAGAGAAAGTTTAATTTTTTCTCGTCTGGA
clone
#1 AATAAAGAGAAAGTTTAACCaaaaaaaaaaaaaaaa
#2 AATAAAGAGAAAGTTTAATTaaaaaaaaaaaaaaaa
#3 AATAAAGAGAAAGTTTAACTaaaaaaaaaaaaaaaa
#4 AATAAAGAGAAAGTTTAACTaaaaaaaaaaaaaaaa
#5 AATAAAGAGAAAGTTTAATTTGCaaaaaaaaaaaaa
#6 AATAAAGAGAAAGTTTAATTTTTTCTCaaaaaaaaa
mut 3'UTRs
STOP
CATGAATATCTTTTTTTTATTGA
clone
#1 * CATGAATATCTTTTTTTGGaaaaaaaaa
#2 * CATGAATATCTTTTTTTAGaaaaaaaaa

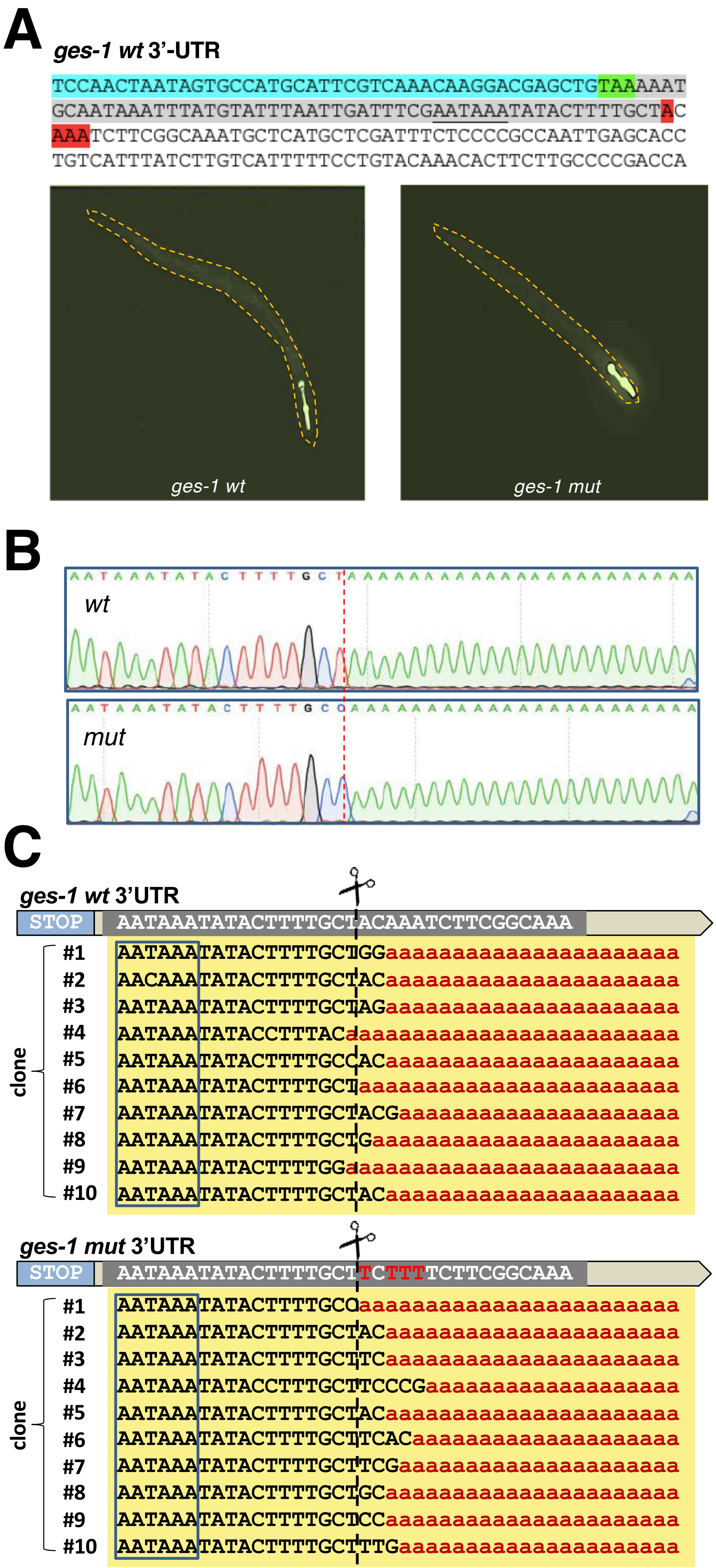
Supplemental Figure S12: *In vivo* cleavage assay for *Y106G6H.9*.

A) *Y106G6H.9* genomic region cloned downstream of the GFP reporter. Blue: terminal portion of the *Y106G6H.9* ORF. Green: STOP codon. Gray: 3'-UTR. Red: mutated terminal adenosine nucleotides. Red Asterisk: position of the cryptic cleavage site (see below). The transgenic worms expressing the P*myo-3*::GFP:: *Y106G6H.9*_3'-UTR *wt* and mutant cassette are shown below.

B) At the completion of the experiment we recovered the total RNA and performed RT-PCR experiments using a forward primer annealing within the GFP ORF and a reverse polydT primer with two anchors containing Invitrogen Gateway adapters. The resultant amplicons were then subcloned in gateway vectors and sequenced to detect the cleavage site. An example of resultant trace files is shown.

C) Examples of several clones identified in this study for *Y106G6H.9*. In the *wt* we were able to detect two classes of cleavage sites, both ending within 4 nt of each other with a terminal adenosine nucleotide. In the double mutant, the removal of the terminal genomic adenosine induces a cleavage skip in two clones (arrows). In one case (red arrow), the cleavage occurs 20 nt downstream of the PAS element. Two of the mutant clones also shown an occurrence of a new cryptic cleavage site 100 nt upstream of the natural site (red asterisks), which also contain a terminal adenosine nucleotide at their 3'end. The PAS element is boxed in blue color.

Supplemental Fig. S13


A
ges-1 wt 3'-UTR
TCCAACTAATAGTGCCATGCATTCGTCAAACAAGGACGAGCTGTAAAAAT
GCAATAAATTTATGTATTTAATTGATTTCGAATAAATATACTTTTGCTAC
AAATCTTCGGCAAATGCTCATGCTCGATTTCTCCCCGCCAATTGAGCACC
TGTCATTTATCTTGTCATTTTTCCTGTACAAACACTTCTTGCCCCGACCA
ges-1 wt
ges-1 mut
B
AATAAATATACTTTTGCTAAAAAAAAAAAAAAAAAAAAAAA
wt
AATAAATATACTTTTGCCAAAAAAAAAAAAAAAAAAAAAAA
mut
C
ges-1 wt 3'UTR
STOP
AATAAATATACTTTTGCTACAAATCTTCGGCAAA
clone
#1 AATAAATATACTTTTGCTGGaaaaaaaaaaaaaaaaaaaaaa
#2 AACAAATATACTTTTGCTACaaaaaaaaaaaaaaaaaaaaaa
#3 AATAAATATACTTTTGCTAGaaaaaaaaaaaaaaaaaaaaaa
#4 AATAAATATACCTTTACaaaaaaaaaaaaaaaaaaaaaaaaa
#5 AATAAATATACTTTTGCCACaaaaaaaaaaaaaaaaaaaaaa
#6 AATAAATATACTTTTGCTaaaaaaaaaaaaaaaaaaaaaaaa
#7 AATAAATATACTTTTGCTACGaaaaaaaaaaaaaaaaaaaaa
#8 AATAAATATACTTTTGCTGaaaaaaaaaaaaaaaaaaaaaaa
#9 AATAAATATACTTTTGGaaaaaaaaaaaaaaaaaaaaaaaaa
#10 AATAAATATACTTTTGCTACaaaaaaaaaaaaaaaaaaaaaa
ges-1 mut 3'UTR
STOP
AATAAATATACTTTTGCTTCTTTTCTTCGGCAAA
clone
#1 AATAAATATACTTTTGCCaaaaaaaaaaaaaaaaaaaaaaaa
#2 AATAAATATACTTTTGCTACaaaaaaaaaaaaaaaaaaaaaa
#3 AATAAATATACTTTTGCTTCaaaaaaaaaaaaaaaaaaaaaa
#4 AATAAATATACCTTTGCTTCCCGaaaaaaaaaaaaaaaaaaa
#5 AATAAATATACTTTTGCTACaaaaaaaaaaaaaaaaaaaaaa
#6 AATAAATATACTTTTGCTTCACaaaaaaaaaaaaaaaaaaaa
#7 AATAAATATACTTTTGCTTCGaaaaaaaaaaaaaaaaaaaaa
#8 AATAAATATACTTTTGCTGCaaaaaaaaaaaaaaaaaaaaaa
#9 AATAAATATACTTTTGCTCCaaaaaaaaaaaaaaaaaaaaaa
#10 AATAAATATACTTTTGCTTTGaaaaaaaaaaaaaaaaaaaaa

Supplemental Figure S13: *In vivo* cleavage assay for *ges-1.*

A) *ges-1* genomic region cloned downstream of the GFP reporter. Blue: terminal portion of the *ges-1* ORF. Green: STOP codon. Gray: 3'UTR. Red: mutated terminal adenosine nucleotides. The transgenic worms expressing the Pmyo-3::GFP::ges-1_3'-UTR *wt* and mutant cassette are shown below.

B) At the completion of the experiment we recovered the total RNA and performed RT-PCR experiments using a forward primer annealing within the GFP ORF and a reverse polydT primer with two anchors containing Invitrogen Gateway adapters. The resultant amplicons were then subcloned in gateway vectors and sequenced to detect the cleavage site. An example of resultant trace files is shown.

C) Examples of 10 clones identified in this study for *ges-1*. The removal of the terminal genomic adenosine nucleotide does not alter the cleavage site but makes it more variable. The PAS element is boxed in blue color.

A

GO Term: Biological Process

adj.Pval	nGenes	Pathways
7.2e-05	7	oxidation-reduction process
5.9e-04	2	deoxyribonucleoside diphosphate metabolic process
1.7e-03	8	single-organism metabolic process

GO Term: Molecular Component

adj.Pval	nGenes	Pathways
1.2e-03	5	oxidoreductase activity

Functional Enrichment: Kegg

adj.Pval	nGenes	Pathways
1.7e-03	1	Pentose and glucuronate interconversions
1.7e-03	1	Fructose and mannose metabolism

B

miRNA	Sequence	Hits
miR-272	uguaggcauggguguuug	7
miR-2217a	cagagugggcagucggugucgauc	6
miR-2217b	cagagcgggcagucggugucaauc	6
miR-5553	ucaauggguagcacguggcaaga	6
miR-265	ugagggaggaaggguggua u	5
miR-34	aggcagugugguuagcugguug	5
miR-44	ugacuagagacacauucagcu	5
miR-4935	gccggcgagagagguggagagcg	5
miR-795	ugagguagauugaucagcgagcuu	5
miR-8190	cgggaaaucgcuuuggaauccagga	5
miR-8194	augcgccuuuaaaaagguacgg	5
miR-1822	aguuucucugggaaagcuaucggc	4
miR-71	ugaaagacaugggguagugagacg	4

Supplemental Figure S14: miRNA target analysis in genes with 2 3'-UTR isoforms which either gain or lose a miRNA binding site. A) GO Term analysis of genes with multiple 3'-UTRs which gain or lose a miRNA target as predicted using our miRanda 'stringent' dataset (n=132). B) Most common miRNAs, their sequences, and number of occurrences.

Additional Materials and Methods

Comparative analysis of *C. elegans* members of the CPC

We have downloaded the protein sequences of each known member of the human CPC and used BLAT algorithm to identify *C. elegans* genes with high homology to their human counterparts. We then performed a protein BLAST analysis using the tools available at the NCBI website to obtain the amino acid sequences for the fly, rat, and mouse orthologs. These amino acid sequences were then aligned using Clustal Omega Multiple Sequence Alignment with standard parameters. At the completion of the analysis, we used the Batch NCBI Conserved Domain Search (Batch CD-Search) against the database CDD- 52910 PSSMs using standard parameters to identify the conserved domains across the aligned protein sequences. We then used these results to populate the location of these elements within the alignment shown in **Supplemental Figure S1**. We were unable to identify the *C. elegans* homolog of the human gene CPSF7.

Plasmid DNA isolation, sequencing and visualization

All plasmids used in this study were prepared from cultures grown overnight in LB using the Wizard Plus SV Minipreps DNA Purification System (Promega) according to the manufacturer's instructions. DNA samples were sequenced with Sanger sequencing performed at the DNASU Sequencing Core Facility (The Biodesign Institute, ASU, Tempe, AZ).

RNAi experiments

RNAi experiments were performed in standard NGM agar containing 1mM IPTG and 50 μg/ml ampicillin. These plates were seeded with 75 μl of RNAi clone bacteria and allowed to induce for a minimum of 16 hours. 5 N2 *C. elegans* at the L1 stage were aliquoted for each RNAi clone tested. Three days after plating, the progeny was scored for embryonic lethality. Each RNAi experiment was performed in triplicate. The total number of hatched and not hatched eggs was the following: *cpsf-1(CPSF160)* n=567*; cpsf-2(CPSF100)* n=557*; cpsf-4(CPSF30)* n=1,251*; cpf-2(CstF64)* n= 716*; cpf-1(CstF50)* n=801*; cfim-1(CFIm25)* n=644*; cfim-2(CFIm68)* n=739*; symk-1(symplekin)* n=208*; tag-214(RBBP6)* n=753*; pcf-11(CPF11)* n=428*; clpf-1(CLP1*) n=841*.*

Mutagenesis of 3'-UTRs cleavage sites

The mutagenesis reactions to remove the adenosine nucleotides near the cleavage sites were carried out using the QuikChange Site-Directed Mutagenesis Kit (Agilent). The mutagenesis DNA primers for the site mutation reactions are available in **Supplemental Table S2**. Each mutagenesis reaction was followed by DNA digestion using Dpn-1 enzyme and transformed in Top10 competent cells (Thermo Fisher Scientific) in agar plates containing 20mg/μL of kanamycin. We validated the nucleotide mutation using Sanger sequencing approach. *Wild type* and mutant 3'-UTRs cloned in pDONR P2RP3 were then shuttled into destination vectors using the Gateway LR Clonase II Plus Enzyme Mix (Invitrogen, Carlsbad, CA). The finalized destination vectors contained the *C. elegans* pharynx promoter (Pmyo-2) in the first position, a GFP sequence with a mutated STOP codon in the second position, and the *wt* or mutant 3'-UTRs used in this study in the third position. The resultant recombined constructs were then transformed in Top10 competent cells (Thermo Fisher Scientific) and plated on 10mg/μL ampicillin plates overnight. The success of the recombination reaction was confirmed using Sanger sequencing with the M13F DNA primer.

Preparation of transgenic worm lines

EG6699 strain worms were kindly provided by Christian Frokjaer-Jensen (Frokjaer-Jensen et al. 2008). These worm strains were maintained at 18°C on nematode growth media (NGM) agar plates and propagated on plates seeded with OP50-1 bacteria. To synchronize worms for injections, EG6699 worms were bleached with bleaching solution (1 M NaOH) four days before injections. Each construct was mixed with an injection master mix containing pCFJ601 (25 ng/μl), pgH8 (10 ng/μl), and pCFJ104 (5 ng/μl) vectors. Injection needles were loaded with the injection mixture and mounted to the Leica DMI300B microscope. The needle was pressurized with 22 psi through the FemtoJet (Eppendorf). Young adult EG6699 worms were picked onto an agarose pad covered with mineral oil on a glass coverslip. Injected worms were rescued onto an NGM plate and rinsed with M9 buffer. Two days post-injections, the F1 progeny were screened with a Leica DMI3000B microscope for both *unc-119* rescues and expression of the red fluorescence produced by the co-injection marker and then isolated onto individual plates. These worms were allowed to lay eggs, and then the F2 progeny was screened for fluorescence. Once 75% of the progeny on a single plate were transgenic, the strains were used for further experimentation.

Worm genotype validation

Populations obtained from single worms from each of the seven strains were lysed using worm lysis buffer (EDTA, 0.1 M Tris, 10% Triton-X, Proteinase K, 20% Tween 20). These worms were subjected to heating in a Bio-Rad T100 Thermal Cycler. To confirm that the mutated cleavage site was present in the injected strains, we used PCR approach using Platinum Taq polymerase (Invitrogen) with a forward DNA primer binding the beginning of the GFP sequence and 3'-UTR-specific reverse DNA primers. The PCR product was then sequenced using Sanger sequencing with a forward DNA primer binding to the GFP sequence present in the injected construct.

Detection of the 3'-UTR cleavage skipping

Total RNA was extracted from transgenic strains using the Direct-zol RNA MiniPrep Plus kit (RPI) according to the manufacturer's instructions. We tested approximately 10 independent *wt* and mutant clones for each 3'-UTR. Approximately 50 μL of worm pellet was used for extraction. cDNA was synthesized using a reverse transcription reaction using Superscript II enzyme (Invitrogen). The first strand reaction was performed using a reverse poly dT DNA primer containing two anchors and the attB Gateway BP recombination element (Invitrogen). The second strand of the cDNA was synthesized using a PCR with HiFi taq polymerase (Thermo Fisher Scientific) and the forward DNA primer containing the pDONR P2RP3 Gateway element (Invitrogen), which binds to GFP and the same reverse poly dT DNA primer used in the first strand reaction. The BP Gateway kit (Invitrogen) was once again used to clone the cDNA which contains the polyA tail into pDONR P2RP3. These constructs were then transfected into Top10 competent cells (Thermo Fisher Scientific) and plated on agar plates containing 20mg/μL of Kanamycin. About 8-10 colonies were then sequenced with Sanger sequencing using the M13F DNA primer to map the location of the cleavage site.

Updated miRanda Predictions

We downloaded a complete list of *C. elegans* miRNAs from miRBase (Griffiths-Jones et al. 2006) and the miRanda algorithm v3.3a (John et al. 2004) from the microrna.org website. We queried the 3'-UTRome v2 with the miRanda algorithm using both standard and stringent parameters. The stringent query used was '-strict -sc -1.2'. The standard query produced 58,330 putative miRNA targets; the stringent query produced 12,136 putative miRNA targets. Both these predictions are included in WormBase (Lee et al. 2018) as individual tracks.

Homology model building

Homology modeling was performed using SWISS_MODEL (Waterhouse et al. 2018) with a matched templated of human CPSF160-WDR33-CPSF30 complex (PDB code: 6DNF) (Sun et al. 2018). The molecular graphics were prepared using the UCSF ChimeraX software (version 0.8) (Goddard et al. 2018).