SUPPLEMENTARY FIGURE LEGENDS

Supplemental Fig. 1. The DRH/RIG-I/Dicer family of RNA helicases. ClustalW scoring and sequence alignment of helicase motifs from worm (Ce, Cb), mouse (Mm), human (Hs), platypus (Oa), chicken (Gg), fish (Dr), fly (Dm), fungi (Nc), and yeast (Sc) Dicer/DRH/RIG-I orthologs. The conserved helicase motifs (I through VI) are highlighted in red. Motifs specific to Dicer family are highlighted in blue. Divergent sequences are highlighted in green. The long linker region between motifs III and IV is not shown.

Supplemental Fig. 2. DRH-3 expression construct and protein preparation. pET-sumo-DRH-3 construct was digested with a single cutter (Xba1) or double cutter (EcoRI) and resolved on 1 % agarose gel (left). Sypro Orange (Invitrogen)-stained 4-20 % gradient gel showing protein preparation of 130 kD full-length DRH-3 (right).

Supplemental Fig. 3. DRH-3 binds to and is activated by RNA containing either hydroxyl or triphosphates at their 5′ termini. DRH-3 binds untreated (red) and CIP-treated (blue) 18 nt-long transcripts with equal binding constants of ~140 nM (top). A short RNA (18 nt) was chosen to emphasize the effect of the 5′ termini on DRH-3 binding. We attribute the observed decrease in affinity for ssRNA to the length of the RNA substrate. Both untreated (solid) and CIP-treated (dashed) blunt duplex stimulate the ATP hydrolysis by DRH-3 with equal efficiency.

Supplemental Fig. 4. DRH-3 has a general RNA-stimulted NTPase activity, but does not have unstimulated ATPase activity. (*Top*) TLC showing ATPase reactions containing 100, 500, and 1000 nM DRH-3 and units of RNAseV1. ATPase activity was monitored over 30 min. Spontaneous hydrolysis (no protein) and positive (100 nM DRH-3 + 100 nM ds25 RNA) controls are shown. (*Bottom*) TLC showing the general NTPase activity of DRH-3. DRH-3 hydrolyzed all four NTPs, although its ATPase activity was more efficient. (ø) No protein; (-) no RNA; (+) 100 nM ds25 RNA.

Supplemental Fig. 5. DRH-3 ATPase activity is stimulated by double-stranded RNA. (A) Thin layer chromatography showing end point ATP hydrolysis (60 min) by 100 nM DRH-3 in the presence of single stranded 25 nt long RNA (left) and 25 bp RNA (right). (B) Analysis of TLC experiment (in A) showing end point ATP hydrolysis (60 min) as a function of RNA concentration. In the presence of ss25 RNA, DRH-3 displayed low levels of ATPase activity (8.7 pmol ATP/min/pmol DRH-3 at $0.5\,\mu$M RNA). Detectable ss25-activated ATPase activity required RNA concentrations exceeding $1\,\mu$M, despite the fact that DRH-3 binds ssRNA at nanomolar concentrations. By contrast, robust DRH-3 ATPase activity was observed in the presence of duplex RNA of the same sequence (ds25 RNA, 141 pmol ATP/min/pmol DRH-3 at $0.5\,\mu$M RNA), even at low RNA concentrations (<100 nM of dsRNA, 39.5 pmol ATP/min/pmol DRH-3 at 50nM RNA). (C) End point ATPase (60 min) assay showing that 34 bp RNA/RNA (red) and RNA/DNA (black) duplexes stimulate DRH-3 (100 nM) but not DNA/DNA duplex (blue). Data fit best to exponentials with maximum amplitudes of 0.99454 ± 0.131 mM ATP for RNA/RNA duplex, 0.87847 ± 0.111 mM ATP for RNA/DNA duplex, and 0.001361 ± 0.000473 mM ATP for DNA/DNA duplex. Error for each based on fit of the ATPase data.

Supplemental Fig. 6. DRH-3 does not unwind RNA duplexes *in vitro*. A 5′-^{32}P-radiolabeled RNA duplexes with 18 nt long single-stranded overhangs were incubated for 90 min with DRH-3, increasing concentrations of poly-ribo(I:C), 4 mM ATP, and excess cold 12 mer RNA. The reactions were resolved through a 3 M Urea/0.5X TBE semi-native gel. No unwinding activity

was seen for 12 bp or longer 18 bp RNA duplexes in the presence or absence of dsRNA *in trans*. HCV NS3 was used as a positive control for unwinding (Fig. 4).

Supplemental Fig. 7. Analytical ultracentrifugation results showing DRH-3 as a monomer in solution. Sedimentation velocity ultracentrifugation profile of 5 µM DRH-3 based on Sedfit (NIH) analysis. The major peak (99%) shows a sedimentation coefficient of 4.5 S, a molecular weight of ~120 kD.

Supplemental Fig. 8. High dsRNA concentration inhibits ATP hydrolysis by DRH-3. NADH-coupled ATPase assay confirming results (Figure 5B) that high concentrations of ds25 RNA (>50 nM) inhibits DRH-3 ATPase function.

Supplemental Table 1: RNA and DNA oligomers used in our experiments

1) DNA templates for *T7* RNA transcription (T7 binding site underlined; transcript in bold, upon converting T to U in the product)

```
25mer top strand:
5´-GCGCGCGGTAATACGACTCACTATAGGGTATAGACTGACTAGGATCTCCC-3´

5´-GGGAGATCCTAGTCAGTCTATACCCTATAGTGAGTCGTATTACCGCGCGC-3´

25mer bottom strand:
5´-GCGCGCGGTAATACGACTCACTATAGGGAGATCCTAGTCAGTCTATACCC-3´

5´-GGGTATAGACTGACTAGGATCTCCCTATAGTGAGTCGTATTACCGCGCGC-3´

80mer top strand:
5´-GCGCGCGGTAATACGACTCACTATAGGGAGCACTACGTTCGGACTAGTGTACTCT
GCCTTGCGACTGCTGGCCTCCAGGTCTCCCGAACAGTTCCTCACACTCCC-3´

5´-GGGAGTGTGAGGAACTGTTCGGGAGACCTGGAGGCCAGCAGTCGCAAGGCAGAGT
ACACTAGTCCGAACGTAGTGCTCCCTATAGTGAGTCGTATTACCGCGCGC-3´

80mer bottom strand:
5´-GCGCGCGGTAATACGACTCACTATAGGGAGTGTGAGGAACTGTTCGGGAGACCTG
GAGGCCAGCAGTCGCAAGGCAGAGTACACTAGTCCGAACGTAGTGCTCCC-3´

5´-GGGAGCACTACGTTCGGACTAGTGTACTCTGCCTTGCGACTGCTGGCCTCCAGGT
CTCCCGAACAGTTCCTCACACTCCCTATAGTGAGTCGTATTACCGCGCGC-3´
```

2) Synthetic RNA and DNA sequences:

```
34 nt (top strand) RNA:
5'-GGAGUGCAUCUCCUUCCCUCCUUUCCUUCUGGUC-3´

34 nt (top strand) DNA:
5'-GGAGTGCATCTCCTTCCCTCCTTTCCTTCTGGTC-3´
```

54 nt (bottom strand) RNA:
5'–GACCAGAAGGAAAGGAGGGAAGGAGAUGCACUCCACUGACUAACACGUACU
AAC–3´

54 nt (bottom strand) DNA:
5'–GACCAGAAGGAAAGGAGGGAAGGAGATGCACTCCACTGACTAACACGTACTAAC–3´

Figure S1

```
                        Motif I                                    Motif Ia                                              Motif Ib
CeDRH-3 QEELVQPALEGKNCVIVAPTGSGKTEVAIYAALKHIEERTSQ----GKPSRVVLLVPKIPLVGQQK-DRFLKYCNGMYEVNGFHGSESSVSGTGRRDEVIATHVSVMTPQILINMLQSVR 115
CbDRH-3 QEELVQPALEGKNCVVVAPTGAGKTEVAIYAALNHIKERHNQ----EKAARVVLLVPKIPLVSQQK-ERFLKYCAGQYHVCGIHGSEKSDTGEGRRDDVLQAHIVVMTPQILINMLQSVR 115
CbDRH-1 QLELCQVALRGENTIVTAPTGSGKTVIAANIIKEHFETRDRN----GQRFKALFMTPNSMILKQQS-DSISSYLDHSYQVQIVQGAD--NLPVR--NAVQTKDLIVATPQMIVNLCNEHR 111
CeDRH-1 QEELCQVALQGKNTIVTAPTGSGKTVIAANIIKEHFESRSSE----GKRFKALFMTPNSMILNQQA-ASISSYLDHVYHTQIIQGSD--NVPTR--NVIQSKDLIVATPQMIVNLCNEHR 111
MmRIG-I QLELALPAKKGKNTIICAPTGCGKTFVSLLICEHHLKKFPC-----GQKGKVVFFANQIPVYEQQATVFSRYFERLGYNIASISGATSDSVSVQ--HIIEDNDIIILTPQILVNNLNNGA 113
HsRIG-I QLELALPAMKGKNTIICAPTGCGKTFVSLLICEHHLKKFPQ-----GQKGKVVFFANQIPVYEQQKSVFSKYFERHGYRVTGISGATAENVPVE--QIVENNDIIILTPQILVNNLKKGT 113
OaRIG-I QMELAKPALVGKNTIICAPTGCGKTFVAIMICKNHLQNMPN-----GQRGKVVFLVTKVPVYEQQKAVFIEQFERESYTVAGISGETAGNTPVE--HVIKNSDIIVLTPQILVNSLKNGT 113
MmMDA-5 QMEVAQPALDGKNIIICLPTGSGKTRVAVYITKDHLDKKKQA----SESGKIVILVNKVMLAEQLFRKEFNPYLKKWYRIIGLSGDTQLKISFP--EVVKSYDVIISTAQILENSLLNLE 114
HsMDA-5 QMEVAQPALEGKNIIICLPTGSGKTRVAVYIAKDHLDKKKKA----SEPGKIVILVNKVLLVEQLFRKEFQPFLKKWYRIIGLSGDTQLKISFP--EVVKSCDIIISTAQILENSLLNLE 114
GgMDA-5 QMEVAKPALNGENIIICLPTGSGKTRVAVYITKDHLDKKKRA----SEQGKIVILVNKVPLVEQHLRKEFNPFLKRWYQVIGLSGDSELKISFP--EVVKRYDVIICTAQILENSLLNAT 114
DrMDA-5 QMEVARPALEEKNIIVCLPTGSGKTRVAVFITKEHLERKQRM----GQKGKVVVLVNKVPLVEQHYKAEFGRFLKHQYSVERVSGASQLKISFP--QIIEKNDIIICTAQILENSLAKAK 114
MmLGP2  QWEVILPALEGKNIIIWLPTGAGKTRAAAFVAKRHLET-------VDRGKVVVLVNRVHLVSQHA-EEFRRMLDKHWTVTTLSGDMGSRAGFG--LMARSHDLLICTAELLQLALNSSE 109
HsLGP2  QWEVIMPALEGKNIIIWLPTGAGKTRAAAYVAKRHLET-------VDGAKVVVLVNRVHLVTQHG-EEFRRMLDGRWTVTTLSGDMGPRAGFG--HLARCHDLLICTAELLQMALTSPE 109
MmDCR-1 QVELLEAALD-HNTIVCLNTGSGKTFIAVLLTKELAHQIRGDLN--PHAKRTVFLVNSANQVAQQVSAVRTHSDLKVGEYSDLE-VNASWTKERWSQEFTKHQVLIMTCYVALTVLKNGY 116
HsDCR-1 QVELLEAALD-HNTIVCLNTGSGKTFIAVLLTKELSYQIRGDFS--RNGKRTVFLVNSANQVAQQVSAVRTHSDLKVGEYSNLE-VNASWTKERWNQEFTKHQVLIMTCYVALNVLKNGY 116
DrDCR-1 QVELLEAALE-HNTIVCLNTGSGKTFIAVLLIKELSHQIRGE-----NGKRTVFLVNAASSVAQQASTVRTHSDLQVGDYMSED-MTS-WPEEMWNREMIENQVLVMTCHIFLHVLKNGV 112
DmDcr-1 QVELLATAYE-RNTIICLGHRSSKEFIALKLLQELSRRARRH------GRVSVYLSCEVGTSTEPCSIYTMLTHLTDLRVWQEQ-PDMQIPFDHC---WTDYHVSILRPEGFLYLLETRE 109
CeDCR-1 QVELLDKATK-KNTIVQLGTGSGKTFIAVLLLKEYGVQLFAPLD--QGGKRAFFVVEKVNLVEQQQAIHIEVHTSFKVGQVHGQTSSGLWDSKEQCDQFMKRHHVVVITAQCLLDLIRHAY 117
NcDCL1  QVELFERAKQ-QNTIAVLDTGSGKTLIAAMLLRWVITGELEDREKGLPRRIAFFLVDKVALVFQQHSFLTKNLDFPMEKLCGEMVEGVESKAFWK-EALEQNEVVVCTAEILSTALHHSW 118
DmDcr-2 QLRLVDHLTK-SNGIVYLPTGSGKTFVAILVLKRFSQDFDKPIE--SGGKRALFMCNTVELARQQAMAVRRCTNFKVGFYVGEQGVDDWTRGMWS-DEIKKNQVLVGTAQVFLDMVTQTY 116
ScMPH1  QYTIVHKSLF-QNTLCAIPTGMGKTFIASTVMLNYFRWTKKA--------KIIFTAPTRPLVAQQIKACLGITGIP----SDQTAILLDKSRKNREEIWANKRVFFATPQVVENDLKRGV 107

                  Motif II                                    Motif III
CeDRH-3 QNE---------RLYVSDFSMMIFDEVHKAA-KNHPYVLINQMVQEWKYEK------------PQIIGLTASLSVKVDGQKDENQMLNDIYNMLALINAPHLSTITRQSSIDELNEHVG 212
CbDRH-3 RNE---------RLYVSDFSMMVFDEVHKTS-GNHPYVLINRLVQEWDYEK------------PQIIGLTASLNVNAKVHTETSAMLGNIYTMLALLNTPCLSSITHQASIDELNEHVT 212
CbDRH-1 DVLKTENEIGIEQFFLSTFTIIFFDECHNTM-KNTPYANIMREYHTLKNMGN-----MPDGHHLPQIVGLTASLGTGDG--KNVLGVKEHIANLCAMMDVKELSTVR--ENTEELQNYSP 221
CeDRH-1 NSLDDESRL--DQFFLSTFTIIFFDECHNTV-KNSPYSNIMREYHYLKNMGN-----MPEGHSLPQIIGLTASLGTGDK--NDCLQVRNYIAGLCASMDVKDLSIVK--DNLEELRGYSP 219
MmRIG-I IP-----------SLSVFTLMIFDECHNTS-KNHPYNQIMFRYLDHKLGE--------SRDPLPQVVGLTASVGVGDA--KTAEEEAMQHICKLCAALDASVIATVR--DNVAELEQVVY 208
HsRIG-I IP-----------SLSIFTLMIFDECHNTS-KQHPYNMIMFNYLDQKLGG--------SSGPLPQVIGLTASVGVGDA--KNTDEALDYICKLCASLDASVIATVK--HNLEELEQVVY 208
OaRIG-I VP-----------SLSMFTLMIFDECHNTD-KSHPYNKIMINYLDNKLGT--------SACQLPQIVGLTASVGVGDA--KNKNEAIQHICKLCACLDAPIISTVR--ENLDDLEKIVF 208
MmMDA-5 SGD-------DDGVQLSDFSLIIIDECHHTN-KEAVYNNIMRRYLKQKLRNNDLKKQNKPAIPLPQILGLTASPGVGAA--KKQSEAEKHILNICANLDAFTIKTVK--ENLGQLKHQIK 222
HsMDA-5 NGE-------DAGVQLSDFSLIIIDECHHTN-KEAVYNNIMHLMQKLKNNRLKKENKPVIPLPQILGLTASPGVGGA--TKQAKAEEHILKICANLDAFTIKTVK--ENLDQLKNQIQ 222
GgMDA-5 E-E-------DESVRLSDFSLIIIDECHHTQ-KEGVYNNIMRRYLKEKIKNRKQAKENKPLIPQPQILGLTASPGVGGA--RSNSKAEEHILKICANLDACRIMTVK--EHASQLKNQVK 221
DrMDA-5 NGD-------EDGIELSQFTLMVIDECHHTK-KGGVYNHIMIRYLKQKNRNQLLKKQDKTLVPIPQILGLTASPGVGGA--VSQQMAEEHILQICANLDAFTIKTKT--FEE----EEAK 218
MmLGP2  E---------DEHVELREFSLIVVDECHHTH-KDTVYNTILSRYLEQKLKK---------AEPLPQVLGLTASPGTGGA--TKLQGAIDHILQLCANLDTCHIMSPK--NCYSQLLMHNP 206
HsLGP2  E---------EEHVELTVFSLIVVDECHHTH-KDTVYNVIMSQYLELKLQR---------AQPLPQVLGLTASPGTGGA--SKLDGAINHVLQLCANLDTWCIMSPQ--NCCPQLQEHSQ 206
MmDCR-1 LS-------------LSDINLLVFDECHLAI-LDHPYREIMK---LCESCP----------SCPRILGLTASILNGKCDPEELEEKIQKLERILRSDAETATDLVVLDRYTSQPCEIVV 208
HsDCR-1 LS-------------LSDINLLVFDECHLAI-LDHPYREIMK---LCENCP----------SCPRILGLTASILNGKCDPEELEEKIQKLERILKSNAETATDLVVLDRYTSQPCEIVV 208
DrDCR-1 LP-------------LSKINLLVFDECHLAI-TGHPYREIMK---ICEGCP----------SCPRILGLTASILNGKCDPCDLEEKIQNLEKILQSNAETATDLVVLDRYASQPREEVL 204
DmDcr-1 LL-------------LSSVELIVLEDCHDSA-VYQRIRPLFENHIMPAPPA----------DRPRILGLAGPLHSAGCELQQLSAMLATLEQSVLCQIETASDIVTVLRYCSRPHEYIV 204
CeDCR-1 LK-------------IEDMCVLIFDECHHALGSQHPYRSIMVDYKLLKKDK----------PVPRVLGLTASLIKAKVAPEKLMEQLKKLESAMDSVIETASDLVSLSKYGAKPYEVVI 213
NcDCL1  IR-------------MDQINLLIFDEAHHTK-KDHPYARIIKNFYIDEQLE---------RRPRILGLTASPVDAKVDPRRAAAELEALLHSQIATAADPAALQH--TICKPKTELVV 211
DmDcr-2 VA-------------LSSLSVVIIDECHHGT-GHHPFREFMRLFTIANQTK-----------LPRVVGLTGVLIKGNE-ITNVATKLKELEITYRGNIITVSDTKEMENVMLYATKPTE 209
ScMPH1  LDP-------------KDIVCLVIDEAHRAT-GSSAYTNVVKFIDRFNSSY-------------RLLALTATPASDLEGVQEVVNNLDISKIEIRTEESMDIVKYMKKRKKEKIEVPLL 198

                               Motif IV                                                Motif V
CeDRH-3 RLKNTLTNQFHVAPES-------------------RVIIFVTQRSTAQRVSDFLNESK-VLDQFGNYG---EQMVGYVLGTNKQGAVQQTSQEQQLTLDKFNNGRLKVIVATSVVEEG- 466
CbDRH-3 KLKDTLITQFATIPDS-------------------RVIIFVVQRSTAQRVSDFLNRSEE VMKQLSSSGDKREDMIGYVLGTNKQGAVQQSPDEQKTVLGRFNTGKLKVIVATSVAEEG- 468
CbDRH-1 KVEQFIVDQNEQRGDS-------------------RSIIFVRTRYEATILNEILNKRE----TLERLGIKSEWISGLNKSTSSSADISASKQKQMEKLRKFATGEIRVLVATSVAEEG- 489
CeDRH-1 KTVQYIVEQNLQRADS-------------------RTIIFVRTRYEATILNKVLNSNE----ELLMLGIKSEWMSGLNKSTASSADISASKQKQMEKLKMFADGEIRILVSTSVAEEG- 483
MmRIG-I DLYLVLQEEYHLKPET-------------------KTILFVKTRALVDALKKWIEENP----ALSFL--KPGILTGRGRTNRATGMTLPAQKCVLEAFRAS--GDNNILIATSVADEG- 457
HsRIG-I DLCFILQEEYHLNPET-------------------ITILFVKTRALVDALKNWIEGNP----KLSFL--KPGILTGRGKTNQNTGMTLPAQKCILDAFKAS--GDHNILIATSVADEG- 457
OaRIG-I ELCFLLEEEYHLNPEN-------------------RTILFVKTRALANALKRWIEENP----KLDYL--KSDVLMGRGKRNQATGMTLPLQKDVLETFKAS--GETKVLIATSVADEG- 457
MmMDA-5 KLRNTILEQFTRSEESS------------------RGIIFTKTRQSTYALSQWIMENA----KFAEVGVKAHHLIGAGHSSEVKPMTQTEQKEVISKFRT---GEINLLIATTVAEEG- 483
HsMDA-5 KLRNTIMEQYTRTEESA------------------RGIIFTKTRQSAYALSQWITENE----KFAEVGVKAHHLIGAGHSSEFKPMTQNEQKEVISKFRT---GKINLLIATTVAEEG- 484
GgMDA-5 KLRNTLMEEFTKTEEP-------------------RGIIFTKTRQSALALYHWIMDNP----KFEEVGIKAHFLIGAGHNSETKPMTQNEQREVIDKFRG---GSINLLIATTVAEEG- 465
DrMDA-5 QLKTIILKEFS-TREKA------------------RGIIFTQTRLSAIALCQWIEENP----KFDEVGVRASYLIGGGDQSVVKPMTAAEQKDVLKRFRT---GEINLLIATTVAEEG- 458
MmLGP2  MLERILLKQFG-SPGHT------------------RGIIFTRTRQTASSLLLWLRQQP----CLQTVGIKPQMLIGAGNTSQSTHMTQKDQQEVIQEFRD---GILSLLVATSVAEEG- 438
HsLGP2  MLEKILQRQFS-SSNSP------------------RGIIFTRTRQSAHSLLLWLQQQQ----GLQTVDIRAQLLIGAGNSSQSTHMTQRDQQEVIQKFQD---GTLNLLVATSVAEEG- 438
MmDCR-1 EKEKPE--TNFPSPFTN------------------ILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFITGHGIGKNQPRSKQMEAEFRKQEEVLRKFRAHETNLLIATSVVEEG 478
HsDCR-1 EKEKPE--TNFPSPFTN------------------ILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFITGHGIGKNQPRNKQMEAEFRKQEEVLRKFRAHETNLLIATSIVEEG 478
DrDCR-1 AKEKTE--ANFPSPFTN------------------ILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFITGHGIGKNQPRNKQMEVEFRKQEEVLRKFRAHETNLLIATSIVEEG 474
DmDcr-1 AKPKPSSGANTAQPRTRRRVYTRRHHRDHNDGSDTLCALIYCNQNHTARVLFELLAEISR RDPDLKFLRCQYTTDR-VADPTTEPKEAELEHRRQEEVLKRFRMHDCNVLIGTSVLEEG- 555
CeDCR-1 KAEHLS-----------------------------AIIFVDQRYIAYSLLLMMRHIKS WEPKFKFVNPDYVVGASGRNLAS--SDSQGLHKRQTEVLRRFHRNEINCLIATSVLEEG- 450
NcDCL1  MLVRILRDQFERGVGAQ------------------RCIIFVRQRNTAMLLADLLQQPE---IKSHIPSIAAEVLVGGGTTGSSYVNAKINFQQQNRIIRKFKLGEINCLFATSVAEEG- 422
DmDcr-2 RFLMSLKVSFADKDPKD------------------ICCLVFVERRYTCKCIYGLLLNYIQS-TPELRNVLTPQFMVGRNNISPDFESVLERKWQKSAIQQFRDGNANLMICSSVLEEG- 456
ScMPH1  PHIFIGQARAKEGFDEVKYT---------------RKHAPKGRKKVERLHRQEQEKFLEAERTKRAANDKLERSARRTGSSEEAQISGMNQKMQQKEVIHNFKKGEYNVLVCTSIGEEG- 490

                     Motif VI
CeDRH-3 -LDVTACNLIIKYNCSSGSAIQLVQQRGRARAK--- 498
CbDRH-3 -LDVAACNLIIKYNCSSGSAIQLIQQRGRARAK--- 500
CbDRH-1 -LDVAECNLVIKYNYATN-EIAHVQRGRGRAM--- 520
CeDRH-1 -LDVPECSLVIKYNYATN-EIAHVQRGRGRAL--- 514
MmRIG-I -IDIAECNLVILYEYVGN-VIKMIQTRGRGRAR--- 488
HsRIG-I -IDIAQCNLVILYEYVGN-VIKMIQTRGRGRAR--- 488
OaRIG-I -IDIAQCNVVILYEYIGN-VIKMIQTRGRGRAK--- 488
MmMDA-5 -LDIKECNIVIRYGLVTN-EIAMVQARGRARAD--- 514
HsMDA-5 -LDIKECNIVIRYGLVTN-EIAMVQARGRARAD--- 515
GgMDA-5 -LDIKECNIVIRYGLVTN-EIAMVQARGRARAD--- 496
DrMDA-5 -LDIAECNVVIRYCLVTN-EVAMIQARGRARAE--- 489
MmLGP2  -LDIAQCNVVVRYGLLTN-EISMVQARGRARAG--- 469
HsLGP2  -LDIPHCNVVVRYGLLTN-EISMVQARGRARAD--- 469
MmDCR-1 -VDIPKCNLVVRFDLPTE-YRSYVQSKGRARAP--- 509
HsDCR-1 -VDIPKCNLVVRFDLPTE-YRSYVQSKGRARAP--- 509
DrDCR-1 -VDIPKCNLVVRFDLPTE-YRSYVQSKGRARAPV-- 506
DmDcr-1 -IDVPKCNLVVRWDPPTT-YRSYVQCKGRARAA--- 586
CeDCR-1 -VDVKQCNLVIKFDRPLD-MRSYVQSKGRARRAG-- 482
NcDCL1  -LDIPDCNIVIRFDLYDT-LIQCIQSRGRARRP--- 453
DmDcr-2 -IDVQACNHVFILDPVKT-FNMYVQSKGRARTTEAK 490
ScMPH1  -LDIGEVDLIICYDTTSS-PIKNIQRMGRTGRK--- 521
```
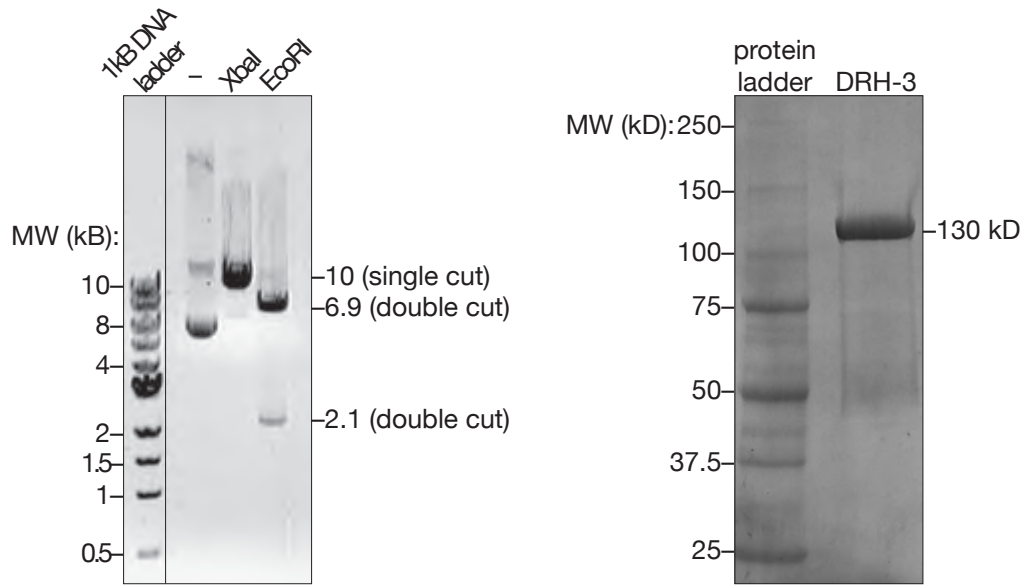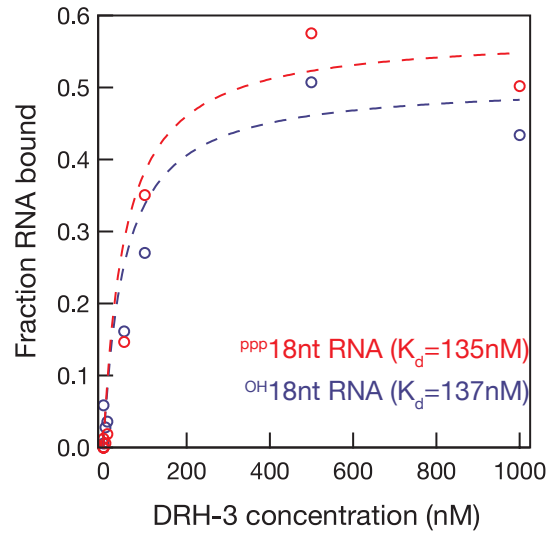
20

Figure S2

RNA binding (Double Filter Method):

Plot: Fraction RNA bound vs DRH-3 concentration (nM)

^{ppp}18nt RNA (K_d=135nM)

^{OH}18nt RNA (K_d=137nM)

ATPase activity (TLC):

Plot: nmol ATP hydrolyzed min^{-1} DRH-3^{-1} vs ds25 RNA concentration (nM)

○ − ^{OH}25/^{OH}25 RNA
● − ^{ppp}25/^{OH}25 RNA

Unstimulated ATPase Activity:

time (min) 2 5 10 20 30 2 5 10 20 30 2 5 10 20 30 2 5 10 20 30 30 30

ADP–
ATP–

100 nM DRH-3   500 nM DRH-3   1 M DRH-3   no DRH-3   100 nM
DRH-3
(-/+ds25)

NTPase activity:

ATPase	CTPase	GTPase	UTPase

ADP–
ATP–
Ø – +
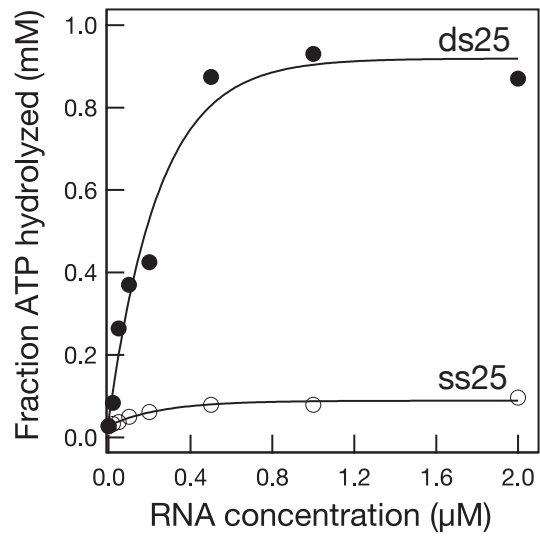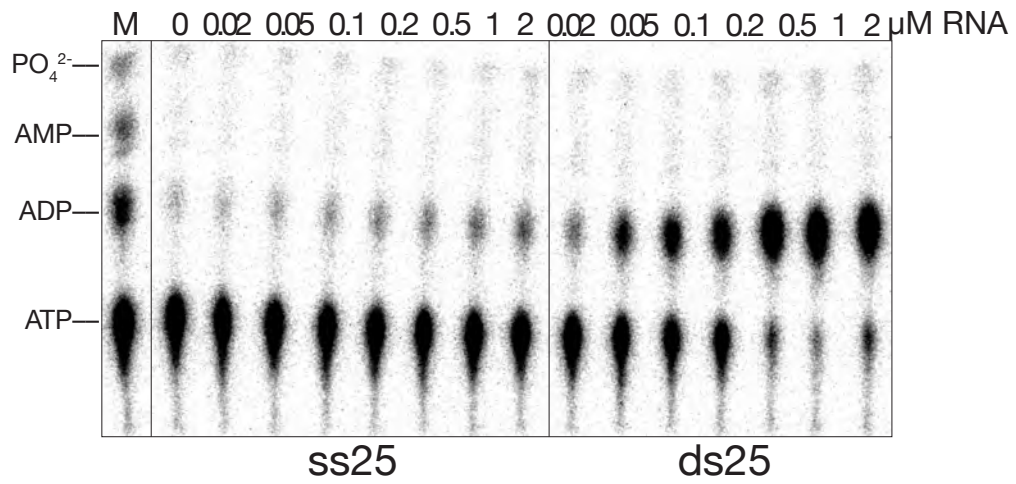
CDP–
CTP–
Ø – +

GDP–
GTP–
Ø – +

UDP–
UTP–
Ø – +

23
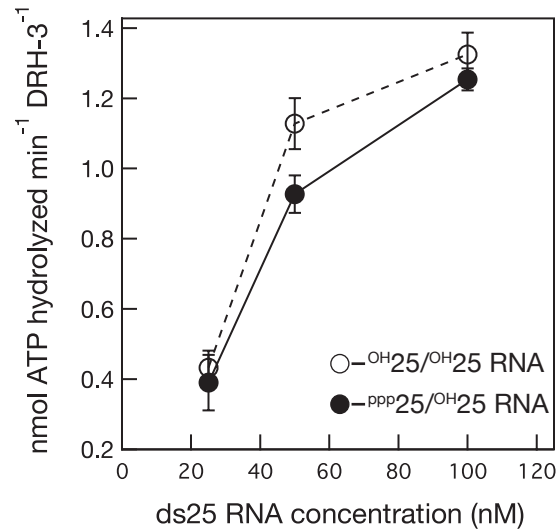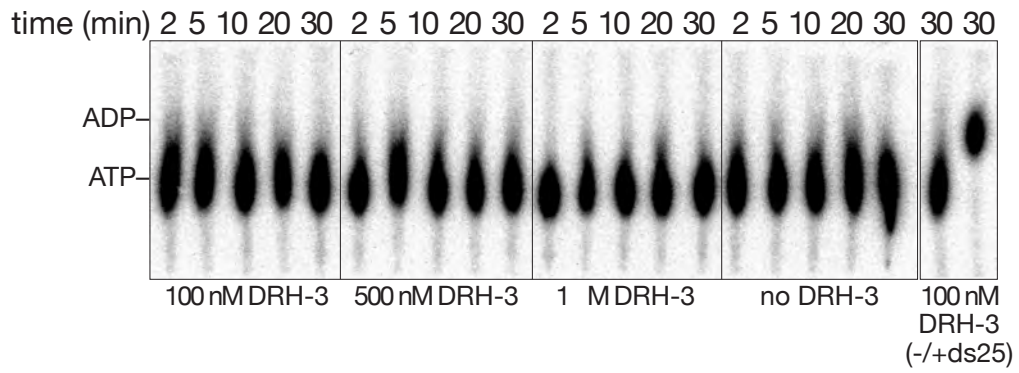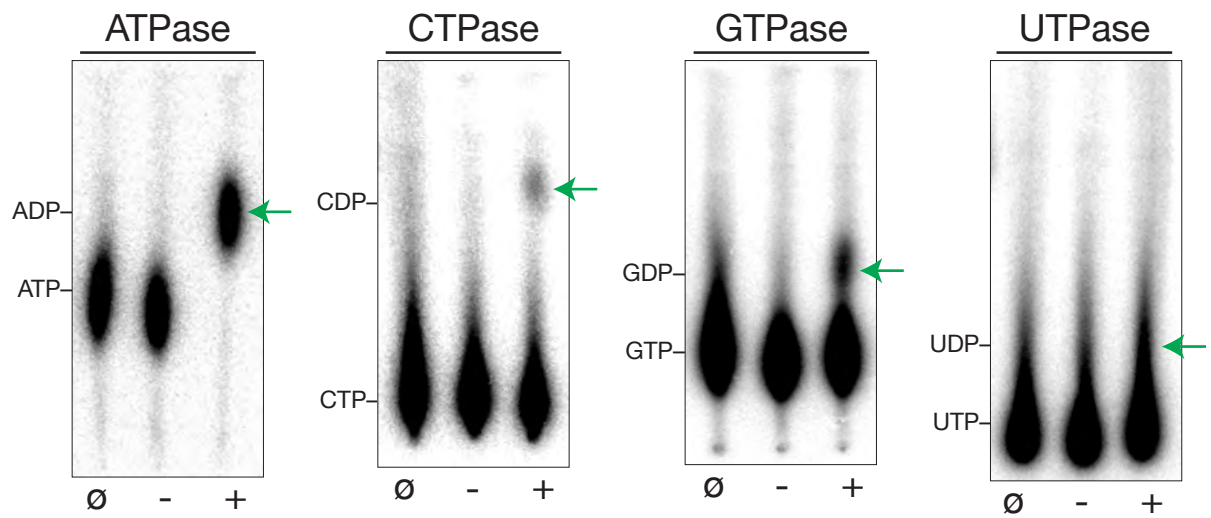
Figure S5

ss25   ds25
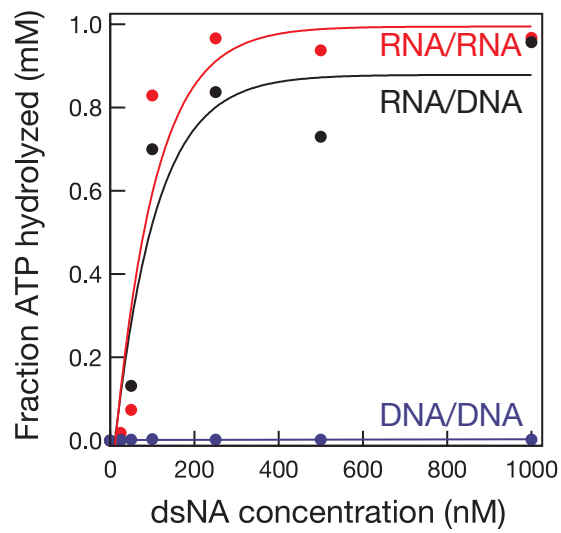
Figure S6

Figure S7

Figure S8