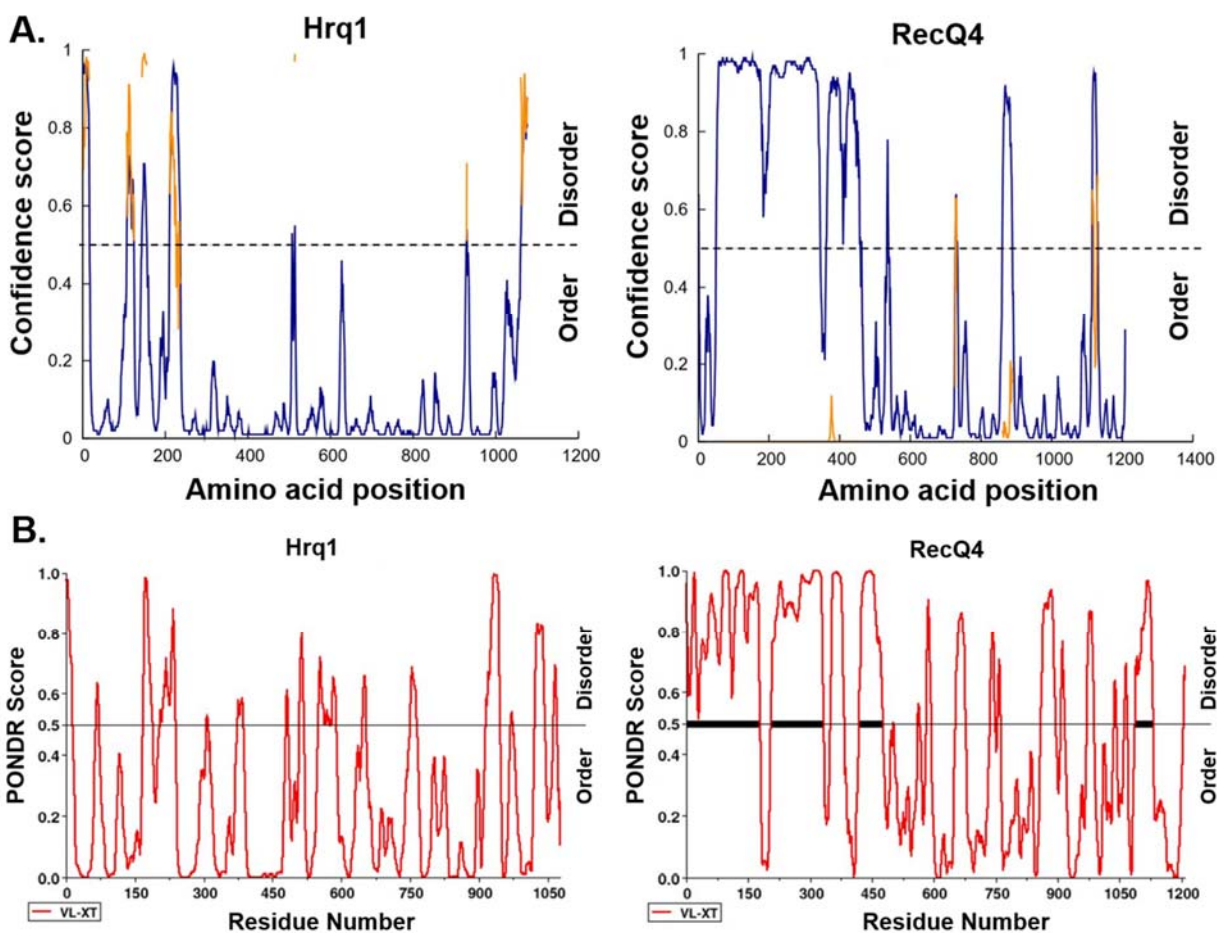
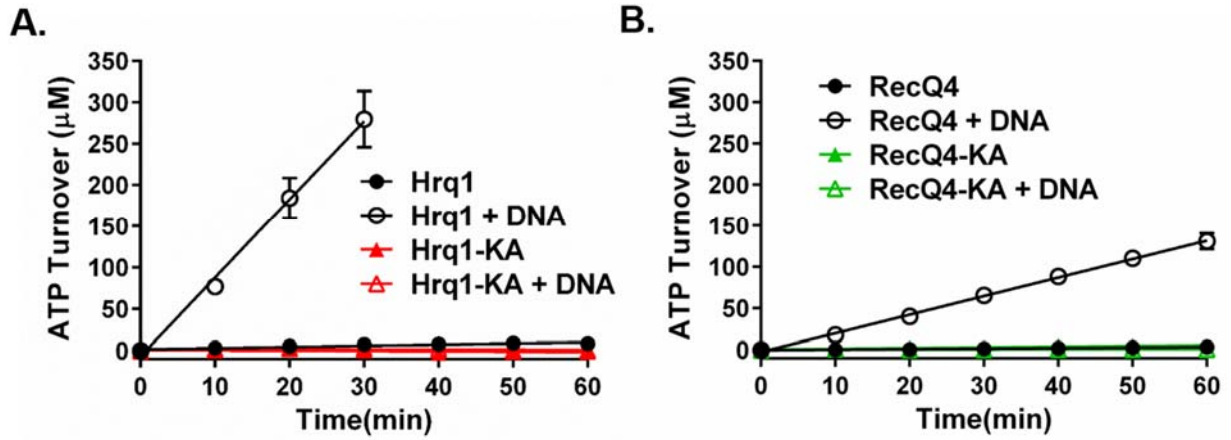


## Supplemental Materials

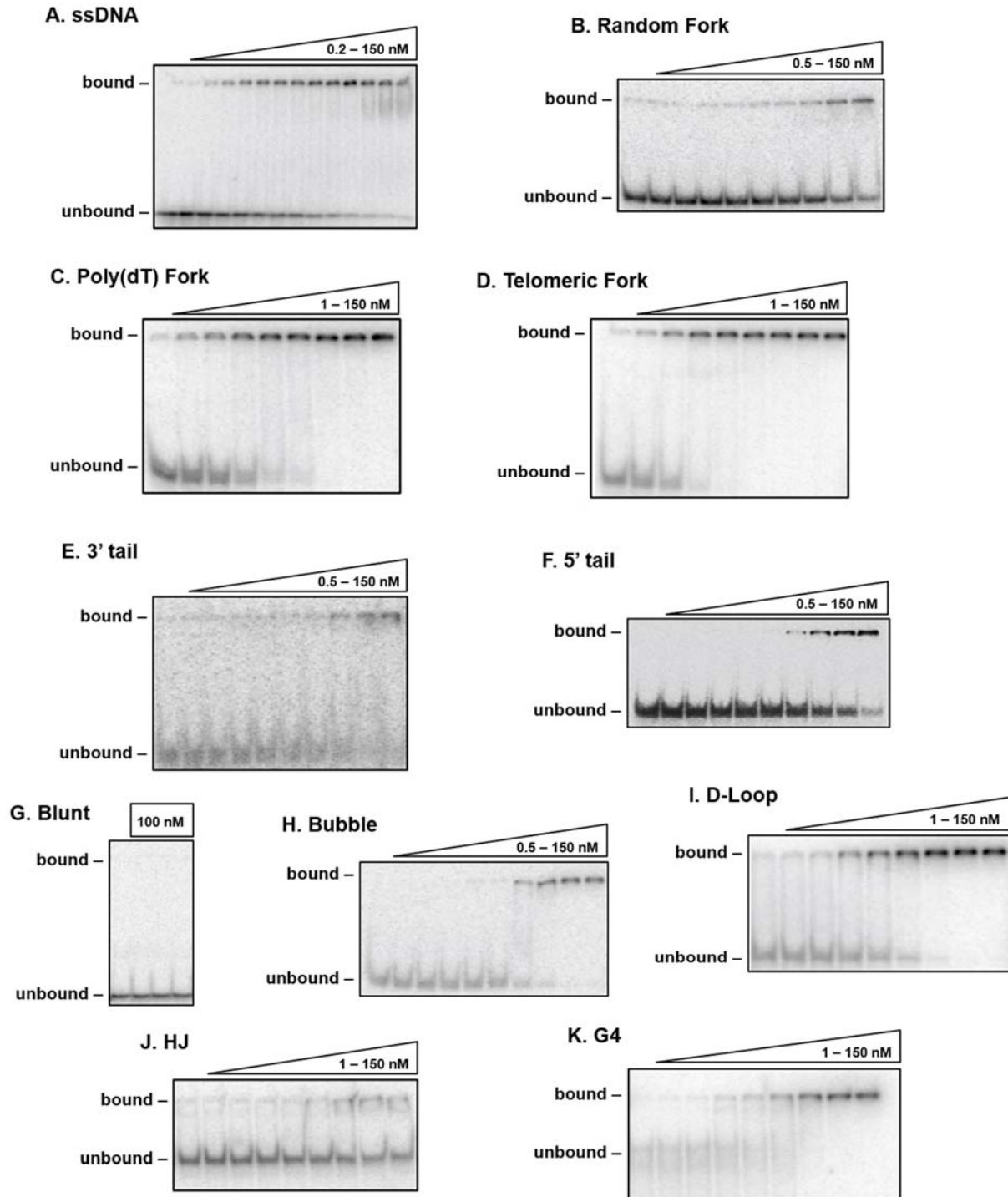
### SUPPLEMENTAL FIGURES AND LEGENDS



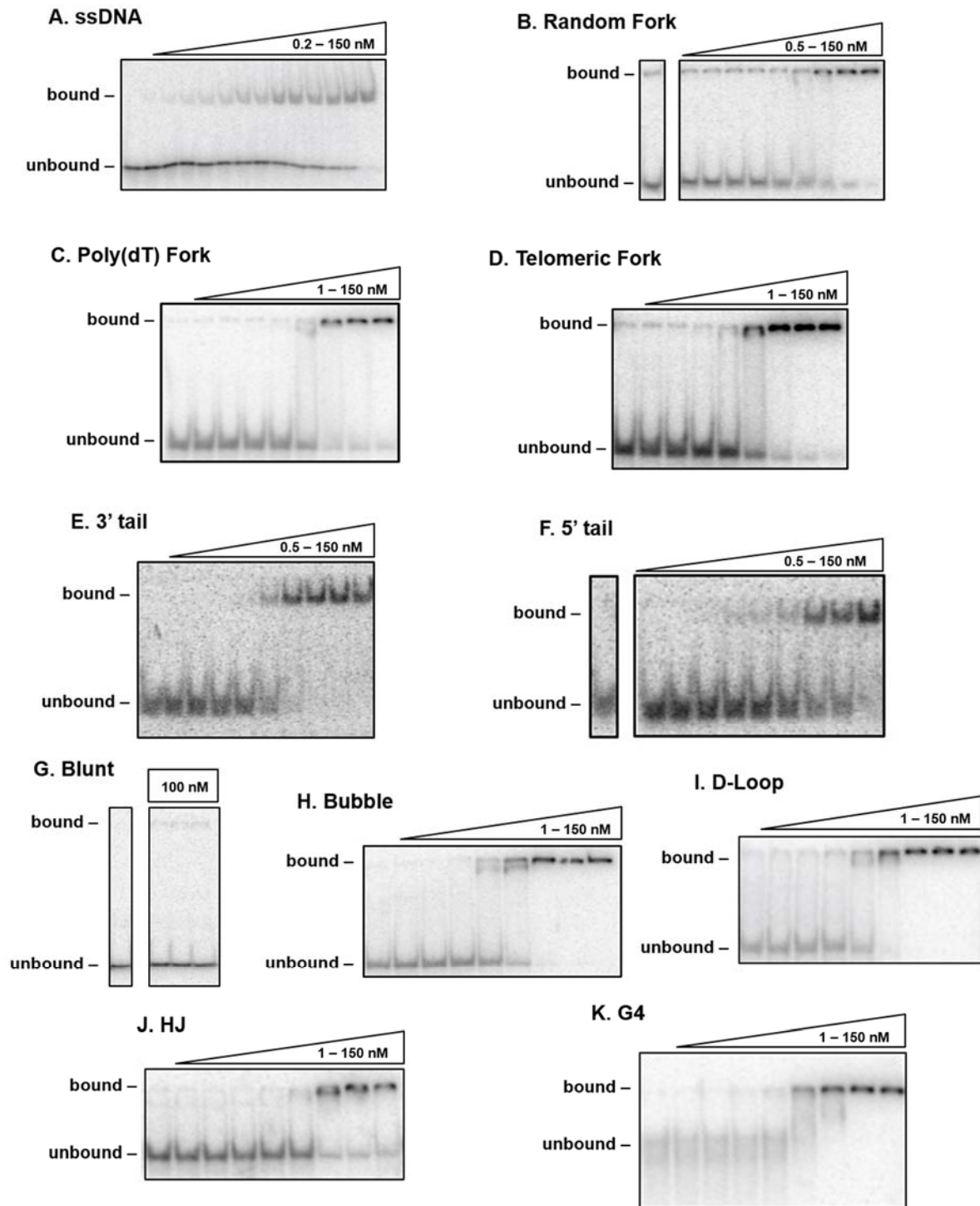
**Supplemental Figure 1. Disorder prediction for Hrq1 and RecQ4.** A) DISOPRED3 analysis from the UCL Department of Computer Science web server (<http://bioinf.cs.ucl.ac.uk/psipred/?disopred=1>) was performed on Hrq1 (left) and RecQ4 (right) to predict intrinsically disordered regions. A confidence score above the dashed line represents likely unstructured regions. Both helicases are predicted to contain disordered regions in the N-terminus, with RecQ4 likely having very few structured regions within the first ~500 residues. B) Predictor of Naturally Disordered Regions (PONDNR) plots for Hrq1 (left) and RecQ4 (right). Protein sequences were analysed using the VL-XT algorithm with default settings (<http://www.pondr.com/>). PONDNR scores > 0.5 represent potentially disordered regions. Analysis again demonstrates that Hrq1 and RecQ4 likely have disordered N-termini, with the amount of predicted disorder greater for RecQ4.



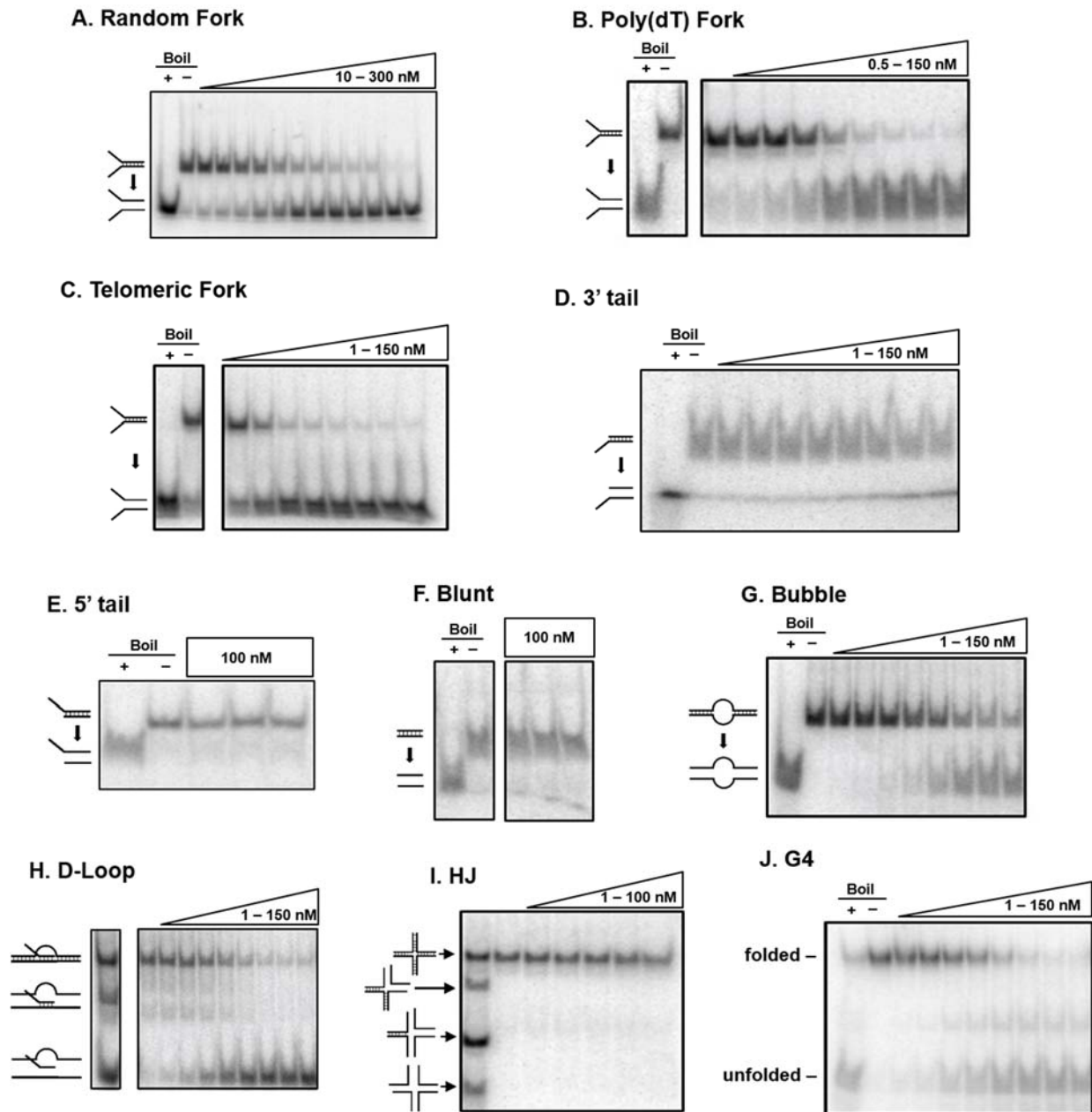
**Supplemental Figure 2. Analysis of catalytically inactive Hrq1-K318A and RecQ4-K508A mutant proteins demonstrates that the observed ATPase activities of the wild type proteins are not due to contaminating enzymes from insect cell culture. A) and B) Hrq1-K318A and RecQ4-K508A are ATPase-dead. The rate of ATP hydrolysis by Hrq1-K318A and RecQ4-K508A was not significantly above background in the presence or absence of poly(dT) ssDNA.**



**Supplemental Figure 3. Representative gel images of Hrq1 binding to each substrate tested.** The first lane in each gel is a no protein control. The concentration ranges indicated do not necessarily reflect the values used for the plotted binding curves in the main text.

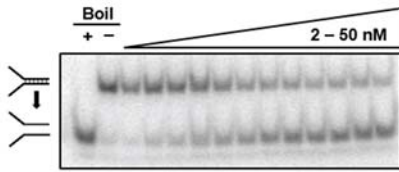


**Supplemental Figure 4. Representative gel images of RecQ4 binding to each substrate tested. The first lane in each gel is a no protein control. The concentration ranges indicated do not necessarily reflect the values used for the plotted binding curves in the main text.**

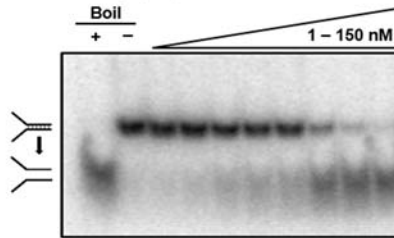


**Supplemental Figure 5. Representative gel images of Hrq1 helicase assays.** The indicated boiled and unboiled lanes were used as a reference for unwound product and as a no protein control, respectively. For substrates containing >2 oligonucleotides (HJ and D-Loop), lane 1 instead contains full-length, intermediate, and fully unwound products for reference. The no protein control is in lane 2 of these gels.

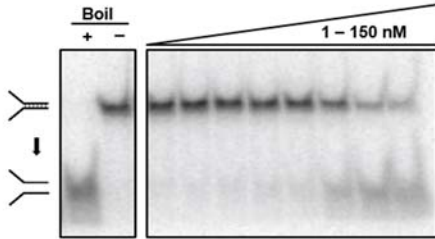
### A. Random Fork



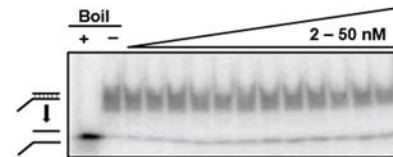
### B. Poly(dT) Fork



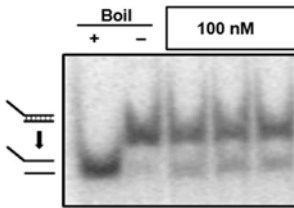
### C. Telomeric Fork



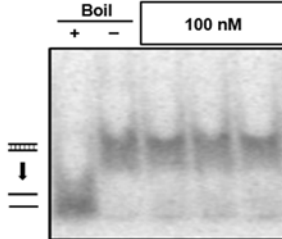
### D. 3' tail



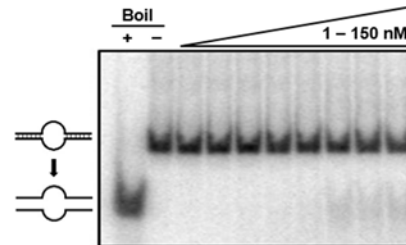
### E. 5' tail



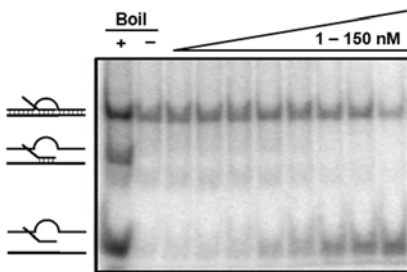
### F. Blunt



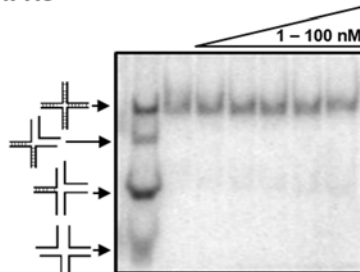
### G. Bubble



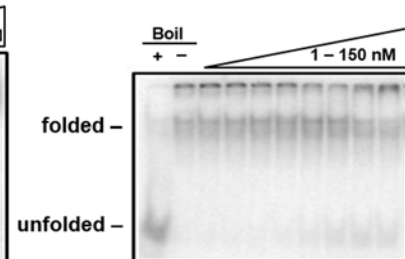
### H. D-Loop



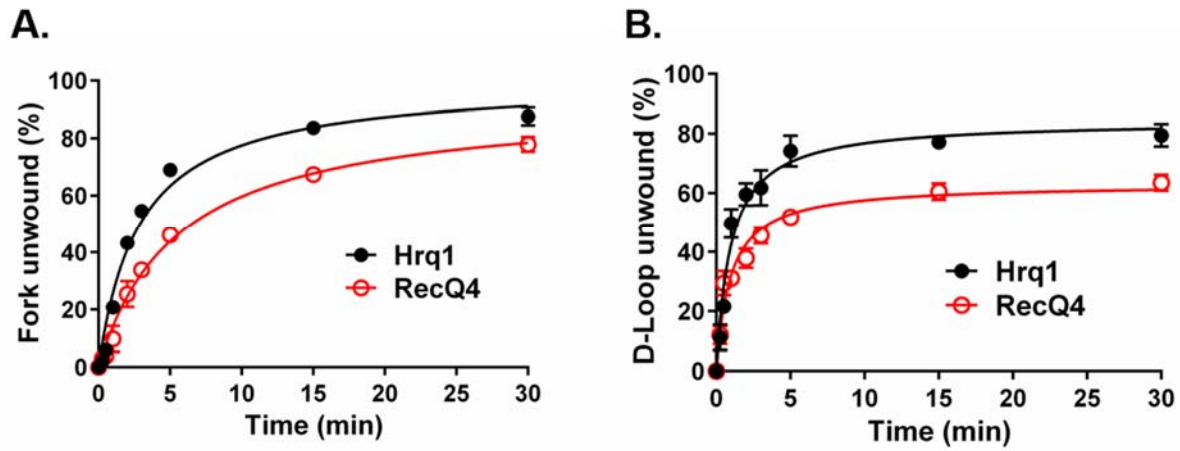
### I. HJ



### J. G4

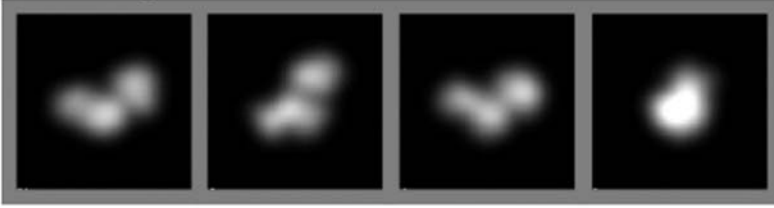


**Supplemental Figure 6. Representative gel images of RecQ4 helicase assays.** The indicated boiled and unboiled lanes were used as a reference for unwound product and as a no protein control, respectively. For substrates containing >2 oligonucleotides (HJ and D-Loop), lane 1 instead contains full-length, intermediate, and fully unwound products for reference. The no protein control is in lane 2 of these gels.

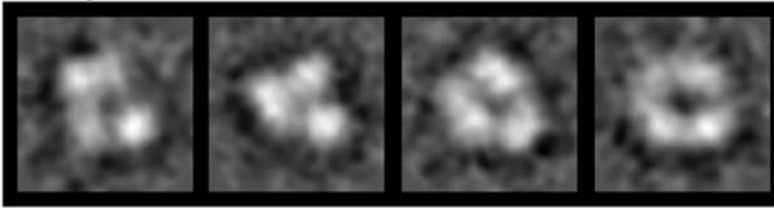


**Supplemental Figure 7. Hrql (black) and RecQ4 (red) helicase activity as a function of time for the fork (A) and D-loop (B) substrates.** At a concentration of 100 nM, both helicases display similar unwinding kinetics on the individual substrates, with the fastest unwinding for the D-loop.

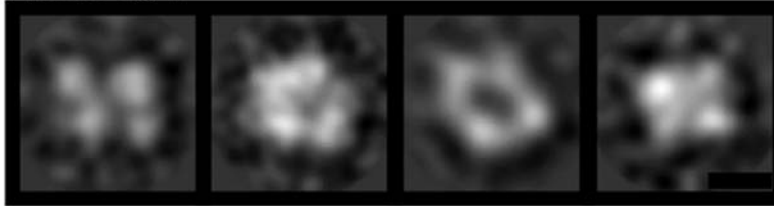
RecQ1



Hrq1

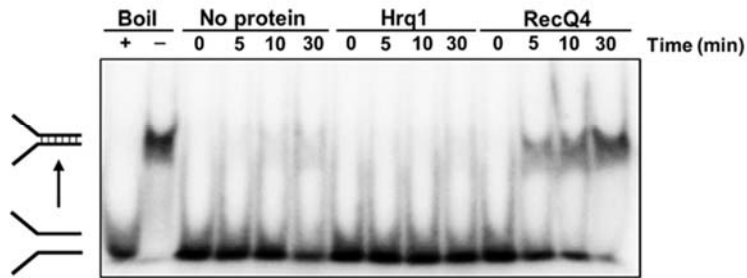
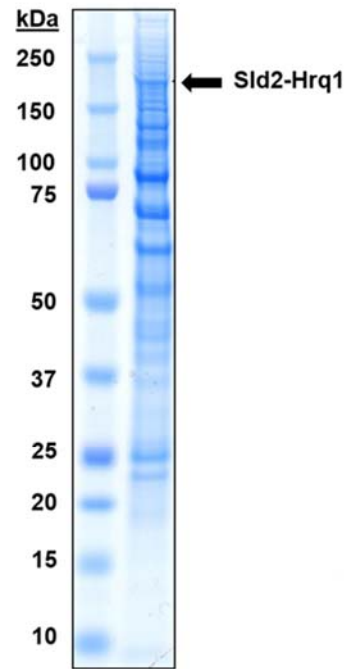


RecQ4



**Supplemental Figure 8. Projections of RecQ1 and structural comparisons.** (Upper panel) A gallery of model projection images of RecQ1 low-pass filtered to 30 Å (PDB 2V1X). (Lower panel) Enlarged views of selected projection images of RecQ1, selected 2D class averages of Hrq1, and selected 2D class averages of RecQ4. Scale bar, 5 nm. The crystal structure of RecQ1 used here has 591 residues, which is approximately half the size of Hrq1 and RecQ4 (13,69).



**A.****B.**

**Supplemental Figure 9. Hrq1 does not anneal DNA under a variety of conditions.** A) Annealing assays were performed with 100 nM Hrq1 or RecQ4 for up to 30 min at 37°C. Hrq1 was unable to anneal DNA above background levels. B) Partial purification of 10xHis-Sld2-Hrq1 by Ni-affinity chromatography. The fusion protein is marked with an arrow.

**SUPPLEMENTAL TABLES**

**Supplemental Table 1.** Names and sequences of the oligonucleotides used to generate all of the substrates used in the biochemical assays.

Name	Sequence	Substrate	Ref.
MB547	GACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGC	Random (ATPase)	(1)
MB548	AA	Poly(dA) (ATPase)	-
MB549	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCTCCCCCCCCCCCCCCCCCCC	Poly(dC) (ATPase)	-
MB550	GGGGGGGGGGGGGGGGGGGGGAGGGGGGGGGGGGGGGGGGGGGGAGGGGGGGG	Poly(dG) (ATPase)	-
MB551	TTT	Poly(dT) (ATPase)	-
MB583	GACGCTGCCGAATTCTGGCTTGTAGGACATCTTTGCCACGTTGACCC	HJ	-
MB584	TGGGTCAACGTGGGCAAAGATGTCCTAGCAATGTAATCGTCTATGACGTT	HJ	-
MB585	CAACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGA	HJ	-
MB586	ATCGATAGTCTCTAGACAGCATGTCCTAGCAAGCCAGAATTCGGCAGCGT	HJ	-
MB733	ACCGTTGTGCAACTGAGTGGACAACGTGTCACTCACATAGCGTTC	25-nt Random Fork, 5'-tail	-
MB734	GAACGCTATGTGAGTGACACCAACAGGTGAGTCAACGTGTTGCCA	25-nt Random Fork, 3'tail	-
MB808	GGTGAACCTGCAGGTGGGCGGCTGCTCATCGTAGGTTAGTTGGTAGAATTCGGCAGCGTC	Bubble	(2)
MB811	GACGCTGCCGAATTCTAGGTTGATTGGATGCTACTCGTCGGCGCCACCTGCAGGTTCCACC	Bubble	(2)
MB819	AAAGGGGGAGCTGGGGTAGATGGGAATGTGAGGGCAACAGGTGAGTCAACGTGTTGCCA	G4	(3)
MB820	GAACGCTATGTGAGTGACAC	5'-tail, Blunt	-
MB821	GTGCTCACTCACATAGCGTTC	3'-tail, Blunt	-

MB1057	CTA CTC TAA CTC CGA CCG CTT GCA TGC CTG CAG G	Strand Annealing/Exchange Fork	(4)
MB1058	CCT GCA GGC ATG CAA GCG GTC GGA GTT AGA GTA G	Strand Exchange Blunt	(4)
MB1059	ATC CTC TCT AGA GTC AAG GTC GGA GTT AGA GTA G	Strand Annealing/Exchange Fork	(4)
MB1113	ACCGTTGTGCAACTGAGTGGACAACACCGTTGTGCAACTGAGTGGACAACGTGTCACTCACATAGCGTTC	50-nt Random Fork	-
MB1114	GAACGCTATGTGAGTGACACCAACAGGTGAGTCAACGTGTTGCCACAACAGGTGAGTCAACGTGTTGCCA	50-nt Random Fork	-
MB1115	ACCGTTGTGCAACTGAGTGGACAACACCGTTGTGCAACTGAGTGGACAACACCGTTGTGCAACTGAGTGGACAACGTGTCACTCACATAGCGTTC	75-nt Random Fork	-
MB1116	GAACGCTATGTGAGTGACACCAACAGGTGAGTCAACGTGTTGCCACAACAGGTGAGTCAACGTGTTGCCACAACAAGTGTGAGTCAACGTGTTGCCA	75-nt Random Fork	-
MB1117	GTG TGG GTG TGG TGT GGG TGT GGT GTG GGT GTG TGG GTG TGG TGT GGG TG	Yeast telomeric repeat G-strand (ATPase)	-
MB1118	CAC CCA CAC CAC ACC CAC ACA CCC ACA CCA CAC CCA CAC CAC ACC CAC AC	Yeast telomeric repeat C-strand (ATPase)	-
MB1119	TTT AGG GTT TAG GGT TTA GGG TTT AGG GTT TAG GGT TTA GGG TTT AGG GTT TAG GG	Human telomeric repeat G-strand (ATPase)	-
MB1120	CCC TAA ACC CTA AAC CCT AAA CCC TAA ACC CTA AAC CCT AAA CCC TAA ACC CTA AA	Human telomeric repeat C-strand (ATPase)	-

MB1162	GGGTGAACCTGCAGGTGGGCGGCTGCTCATCGTAGGTTAGTGGTAGAATTCGGCAGCGTC	D-Loop	(5)
MB1163	GACGCTGCCGAATTCTACCATGCCTTGCTAGGACATCTTTGCCACCTGCAGGTTACCC	D-Loop	(5)
MB1165	TAAGAGCAAGATGTTCTATAAAAGATGTCCTAGCAAGGCA	D-Loop	(5)
MB1167	TTTTTTTTTTTTTTTTTTTTTTTTTTTTGTGTCACACTACATAGCGTTC	Poly(dT) Fork	-
MB1168	GAACGCTATGTGAGTGACACTTTTTTTTTTTTTTTTTTTTTTTTT	Poly(dT) Fork	-
MB1169	GGTGTGGTGTGGGTGTGGTGTGGGTGTGTCACACTACATAGCGTTC	Poly(TG <sub>1-3</sub> ) Fork	-
MB1170	GAACGCTATGTGAGTGACACTGGGTGTGGTGTGGGTGTGGTGTGG	Poly(TG <sub>1-3</sub> ) Fork	-
MB1171	TTAGGGTTAGGGTTAGGGTTAGGGTGTGTCACACTACATAGCGTTC	Poly(TTAGGG) Fork	-
MB1172	GAACGCTATGTGAGTGACACTTAGGGTTAGGGTTAGGGTTAGGGT	Poly(TTAGGG) Fork	-
MB1185	GGT GAA CCT GCA GGT GG	Bubble Trap	-
MB1186	TAG AAT TCG GCA GCG TC	Bubble Trap	-

**Supplemental Table 2.** Full peptide sequence list observed by mass spectrometry analysis of the recombinant Hrq1 and RecQ4 preparations.

Hrq1			RecQ4		
Protein	Peptide	E value	Protein	Peptide	E value
Hrq1	IEECPCSDGCPDCVAASFCK	1.90E-09	RecQ4	YPPQEAQQLSHQAAPGPR	2.60E-10
Hrq1	DMFGINEVTLIHEDGSPTGAK	1.70E-08	RecQ4	SPGDLTAEKDKQICDFLYGR	6.90E-09
Hrq1	VETVIPVSEHVNFSDDFK	2.60E-08	RecQ4	VVVATVAFGMGLDRPDVR	7.00E-08
Hrq1	TSFTLYDGGIFIHQGYPLVK	3.50E-08	RecQ4	RPSQDDVEAAPEETR	7.80E-08
Hrq1	IIDAIETHNPPVIINSK	5.60E-08	RecQ4	AVLHLGLPPSFESYVQAVGR	7.90E-08
Hrq1	QLNVAGAIHGAQHAIMGMLPR	6.50E-08	RecQ4	AGKAEGTAPLHIFPR	1.80E-07
Hrq1	RIEEPCSDGCPDCVAASFCK	8.00E-08	RecQ4	YFEEEEGQEPGGMEDAQGPEPGQAR	1.90E-07
Hrq1	NIFIETGREDLVTEVMSYR	1.30E-07	RecQ4	LNCPPGPAQLQALHR	2.50E-07
Hrq1	STQLLIFEFDGTMQR	1.70E-07	RecQ4	ANLKGTLQAGPALGR	5.40E-07
Hrq1	NAVVDTYDGDTEPEER	1.90E-07	RecQ4	IFHGIGSPCYPAQVYQDR	6.70E-07
Hrq1	VDVDWVTNQRDFTDVDPQEILIR	2.30E-07	RecQ4	DGQPAHCHLFLQPQGEDLR	8.10E-07
Hrq1	QLNVAGAIHGAQHAIMGMLPR	2.50E-07	RecQ4	TFQAFHSVAFSPCGPCLEQQDEER	1.10E-06
Hrq1	GGEEDQFAVVDITNGR	3.40E-07	RecQ4	APKTTAEAYHAGMCSR	1.10E-06
Hrq1	FIVAGVDEIQTECKAPEK	3.50E-07	RecQ4	FQNLDSIIICNR	1.30E-06
Hrq1	DMFGINEVTLIHEDGSPTGAK	4.70E-07	RecQ4	RGTGVLVEFSELAFLHR	1.30E-06
Hrq1	AFEHIDDIIESSLR	5.10E-07	RecQ4	ILSGISTLLVLPTGAGK	1.30E-06
Hrq1	ERDYEDPIEAMMK	5.20E-07	RecQ4	SLCYQLPALLYSR	2.80E-06
Hrq1	ANLDPFSELTNLAQK	5.70E-07	RecQ4	RTFQAFHSVAFSPCGPCLEQQDEER	3.40E-06
Hrq1	HLVVWNPPILPQHER	6.00E-07	RecQ4	GTGVLVEFSELAFLHR	4.80E-06
Hrq1	FIVAGVDEIQTECK	1.00E-06	RecQ4	SSESLPAAAEAEPEPR	5.10E-06
Hrq1	TVTKEDLAMVMALMPR	1.50E-06	RecQ4	TTAEAYHAGMCSR	6.50E-06
Hrq1	TVTKEDLAMVMALMPR	2.40E-06	RecQ4	VSDEPPQLPEPQPRPGR	8.10E-06
Hrq1	QLNVAGAIHGAQHAIMGMLPR	3.90E-06	RecQ4	VGSPQPSSSGGEKR	1.70E-05
Hrq1	QLNVAGAIHGAQHAIMGMLPR	4.50E-06	RecQ4	YFEEEEGQEPGGMEDAQGPEPGQAR	2.10E-05
Hrq1	YIDENQIYTETK	8.50E-06	RecQ4	LQHLQASLSQR	2.60E-05
Hrq1	ENDIFELKDVDDQNQTQK	9.40E-06	RecQ4	CHSEVPDFLGAPK	2.80E-05
Hrq1	VQRVDVDWVTNQR	9.40E-06	RecQ4	ALCQLQWDHEPR	3.30E-05

Hrq1	EKANLDPFSELTNLAQK	9.40E-06	RecQ4	MGVHCFGLTATATR	4.80E-05
Hrq1	ILVQLILNNVR	1.10E-05	RecQ4	GRRPSQDDVEAAPEETR	5.40E-05
Hrq1	TTIIVFGFFK	1.60E-05	RecQ4	TCLHAAWVPGSGGR	6.20E-05
Hrq1	ERDYEDPIEAMMK	1.90E-05	RecQ4	ASTPKPPGTGPVPSFAEK	6.60E-05
Hrq1	TLCGPIETALKR	2.00E-05	RecQ4	GECFGGGGATVTTK	7.00E-05
Hrq1	DFTDVPQEIILIR	2.00E-05	RecQ4	HVHADSTDFLAVKR	7.60E-05
Hrq1	LHHNQDGYHASNR	2.50E-05	RecQ4	HVHADSTDFLAVK	7.60E-05
Hrq1	DPESTFMYIFPTK	3.10E-05	RecQ4	SRQGSVPDYGQR	8.40E-05
Hrq1	NSDVPVYFGK	4.80E-05	RecQ4	KGECFGGGGATVTTK	9.00E-05
Hrq1	IPELKNAVVDTYDGDTEPEER	5.60E-05	RecQ4	AEGTAPLHIFPR	1.30E-04
Hrq1	TVTKEDLAMVMALMPR	5.70E-05	RecQ4	TTGQAGGGLRSSESLPAAAEAEPEPR	1.40E-04
Hrq1	LNTLYTFLICR	6.00E-05	RecQ4	QGSVPDYGQR	1.40E-04
Hrq1	SWSASDRFSQIK	6.90E-05	RecQ4	RPSQDDVEAAPEETR	1.70E-04
Hrq1	NIIIEIEASR	7.20E-05	RecQ4	LGSLDPGWLQR	1.90E-04
Hrq1	QYFTESHLR	7.90E-05	RecQ4	DGQPAHCHLFLQPQGEDLREL	2.10E-04
Hrq1	IPTYTTEEMKK	9.00E-05	RecQ4	TTAEAYHAGMCSR	2.30E-04
Hrq1	STEFYASQIK	9.10E-05	RecQ4	AATKSPQPTPGR	2.40E-04
Hrq1	QESNEMSIPNYSNNSVITTIPQMIEK	1.10E-04	RecQ4	GRRPSQDDVEAAPEETR	2.40E-04
Hrq1	IFDFNNGGFQQK	1.10E-04	RecQ4	AACIHSGMTR	2.50E-04
Hrq1	YGSGLCVK	1.10E-04	RecQ4	LVDSMGWELASVR	2.60E-04
Hrq1	DKQYFTESHLR	1.10E-04	RecQ4	TTGQAGGGLRSSESLPAAAEAEPEPR	2.60E-04
Hrq1	QYFTESHLR	1.90E-04	RecQ4	AVMRILSGISTLLVLPTGAGK	2.90E-04
Hrq1	KQLNVAGAIHGAQHAIMGMLPR	2.10E-04	RecQ4	QFLSLRPEEKFSR	3.30E-04
Hrq1	LKSTEFYASQIK	2.20E-04	RecQ4	AGRDGQPAHCHLFLQPQGEDLR	3.60E-04
Hrq1	IPTYTTEEMKK	2.90E-04	RecQ4	SPGDLTAEK	3.80E-04
Hrq1	APEKEFAER	2.90E-04	RecQ4	YPPQEAELSHQAAPGPRR	4.30E-04
Hrq1	IFDFNNGGFQQKENDIFELK	3.30E-04	RecQ4	AATKSPQPTPGR	6.20E-04
Hrq1	VETVIPVSEHVNFSDDFKIIDVR	3.60E-04	RecQ4	IFHGIGSPCYPAQVYGQDRR	7.80E-04
Hrq1	STQLLIFEFIDGTMQR	3.90E-04	RecQ4	VVVATVAFGMGLDRPDVR	0.0022
Hrq1	AFEHIDDIIESSLRR	5.60E-04	RecQ4	GTLQAGPALGR	0.0024

Hrq1	DDTHTNEIHK	6.10E-04	RecQ4	SPCLTLVVSPLLSLMDDQVSGLPCLK	0.003
Hrq1	LCHCFYENSGLQFISCSATLK	7.40E-04	RecQ4	MGVHCFLGLTATATRR	0.0031
Hrq1	YALEICQKK	7.90E-04	RecQ4	VGSPQPSSSGGEK	0.0032
Hrq1	DYEDPIEAMMK	0.0011	RecQ4	YHGLSPSSQAR	0.0032
Hrq1	IPTYTTEEMK	0.0011	RecQ4	AACIHSGMTR	0.0034
Hrq1	TLCGPIETALK	0.0013	RecQ4	YHGLSPSSQAR	0.0045
Hrq1	VIFTNPDMIHTSILPNHANWR	0.0016	RecQ4	QFLSLRPEEK	0.0053
Hrq1	GGKYGSGLCVK	0.002	RecQ4	SLCYQLPALLYSRR	0.0056
Hrq1	VDVDWVTNQR	0.0025	RecQ4	LQDWEDQVR	0.0057
Hrq1	DYEDPIEAMMK	0.0028	RecQ4	AFMQGQLR	0.0063
Hrq1	ERDYEDPIEAMMK	0.003	RecQ4	MGVHCFLGLTATATRR	0.0063
Hrq1	YALEICQK	0.0033	RecQ4	LQDWEDQVRC DIR	0.0068
Hrq1	ATKDDTHTNEIK	0.0034	RecQ4	LVDSMGWELASVRR	0.0071
Hrq1	HCFTIPSR	0.0041	RecQ4	ASSKASTPKPPGTGPVPSFAEK	0.0078
Hrq1	HVVPTFK	0.0059	RecQ4	RALCQLQWDHEPR	0.01
Hrq1	EMFHGNLK	0.0067	RecQ4	AFMQGQLR	0.013
Hrq1	DGPEPNMPEIK	0.0067	RecQ4	ESVLQKIR	0.013
Hrq1	SWSASDRFSQIK	0.0078	RecQ4	DTDQALLTLLQ GK	0.023
Hrq1	KQLNVAGAIHGAQHAIMGMLPR	0.0081	RecQ4	AACIHSGMTRK	0.024
Hrq1	DPESTFMYIFPTK	0.013	RecQ4	QFLSLRPEEK	0.038
Hrq1	RIIDAIETHNPPVIINSK	0.013	RecQ4	EDTERIAALLR	0.039
Hrq1	NFEQFFFF	0.016	CG6833, isoform A	LQASMER	0.0081
Hrq1	KHVVPFK	0.02			
Hrq1	DDTHTNEIHK	0.02			
Hrq1	LREFILEK	0.021			
Hrq1	SAGQGS GKTDAFR	0.022			
Hrq1	LIFYDSK	0.028			
Hrq1	DYEDPIEAMMK	0.03			
Hrq1	ENDIFELK	0.047			
Heat shock 70 kDa protein cognate 4	IINEPTAAAIAYGLDK	1.40E-06			

Heat shock 70 kDa protein cognate 4	STAGDTHLGGEDFDNR	6.30E-06			
Heat shock 70 kDa protein cognate 4	TTPSYVAFTDTER	1.40E-05			
Heat shock 70 kDa protein cognate 4	MKETAEAYLGK	5.10E-05			
Heat shock 70 kDa protein cognate 4	APAVGIDLGTTYSCVGVFQHGK	2.00E-04			
Heat shock 70 kDa protein cognate 4	VEIANDQGNR	0.0045			
Heat shock 70 kDa protein cognate 4	ITITNDKGR	0.05			
Tubulin alpha-1 chain	VGINYQPPTVVPGGDLAK	1.30E-04			
Tubulin alpha-1 chain	DVNAAIATIK	0.019			



## SUPPLEMENTAL REFERENCES

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