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Supplementary Materials for

Viperin catalyzes methionine oxidation to promote protein expression and function of helicases

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Supplementary Materials

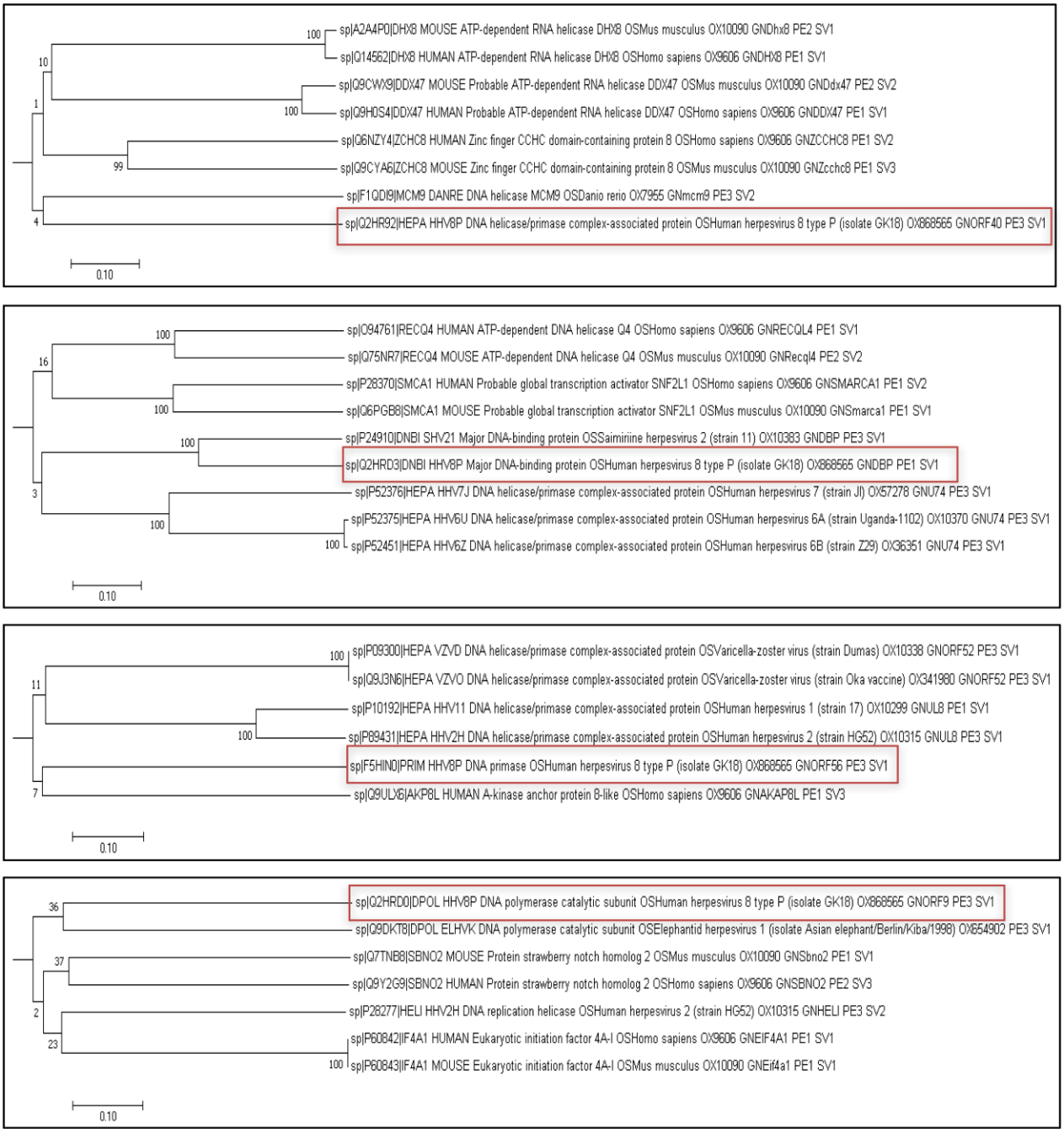

Fig. S1. KSHV helicase and cellular DNA helicase MCM7 are evolutionarily conserved. The evolutionary relationships of KSHV helicase and KSHV helicase-associated proteins of taxa included in the evolutionary tree. The data represent a subset of phylogenetic analysis of helicases and helicase-associated proteins covering human, mice, yeast, zebrafish and herpesviruses.

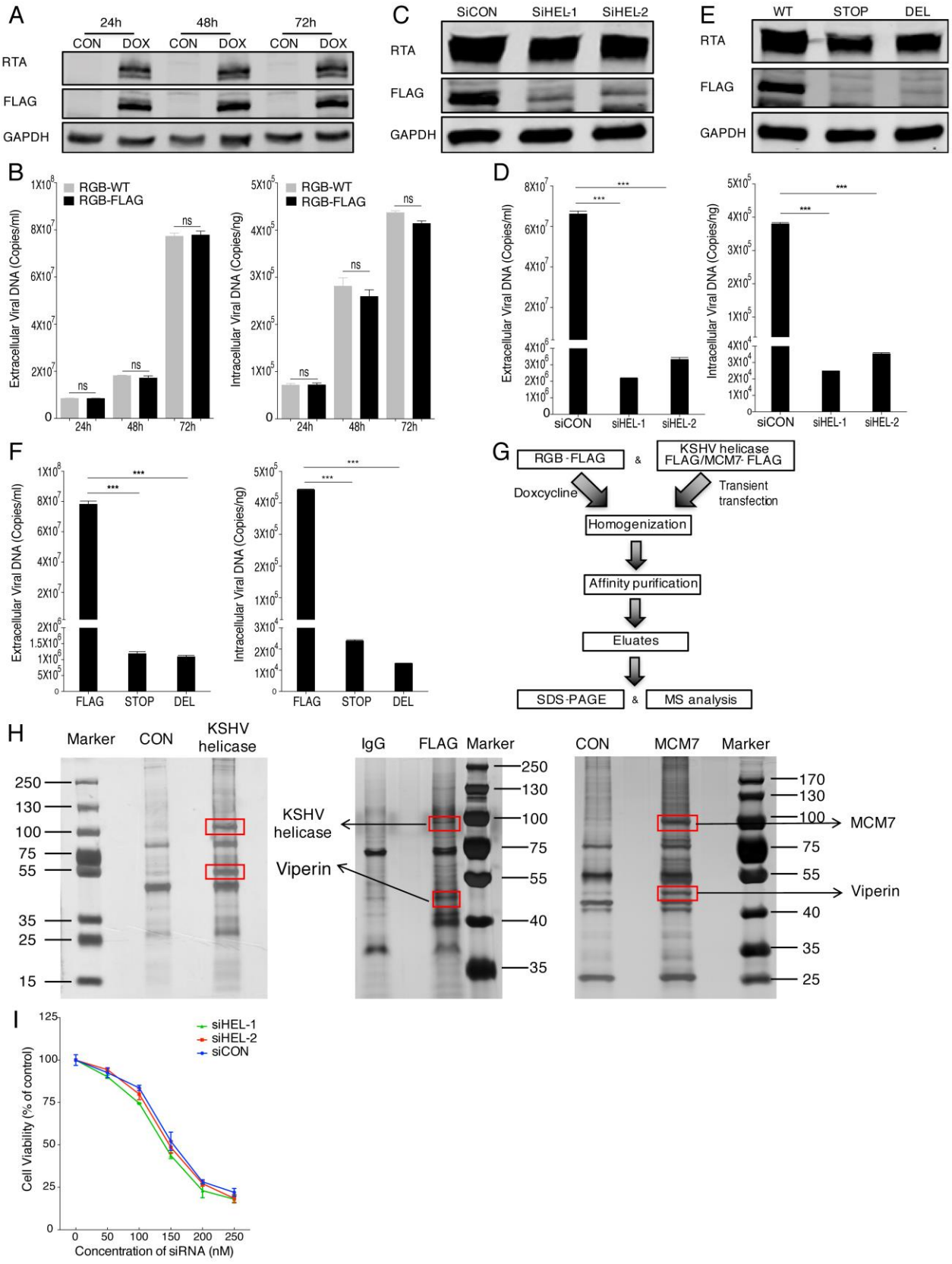

Fig. S2. KSHV helicase is critical for viral DNA replication. (**A**) RGB-FLAG cells were induced with or without doxycycline. Whole cell lysates (WCLs) were analyzed by immunoblotting. (**B**) RGB-WT and RGB-FLAG cells were induced with or without

doxycycline. Viral DNA was extracted and analyzed by qPCR. (**C** and **D**) RGB-FLAG cells were transfected with siRNA as indicated. At 6 h post-transfection, cells were induced with doxycycline for 72 hours. (C) WCLs were then analyzed by immunoblotting; (D) viral DNA was extracted and analyzed by qPCR. (**E** and **F**) RGB-FLAG, RGB-STOP and RGB-DEL cells were induced with doxycycline for 72 hours. (E) WCLs were then analyzed by immunoblotting; (F) viral DNA was extracted and analyzed by qPCR. (**G**) Flow chart of the immunoprecipitation experiment. (**H**) 293T cells were transfected with plasmids containing indicated genes. RGB-FLAG cells were induced with doxycycline. At 48 h post-transfection and 72 h post-induction, WCLs were precipitated with anti-FLAG. The precipitated proteins were analyzed by silver staining and bands in red box were identified by tandem mass spectrometry. (**I**) iSLK-BAC16 KSHV cells were transfected with siRNA of different concentration as indicated. At 6 h post-transfection, cells were induced with doxycycline for 72 hours. Cell viability was measured by CCK8 assay. The concentration of doxycycline is 2 μg/ml. For **B**, **D**, **F** and **I** the data are expressed as the mean ± s.e.m.; n=3; ***p <0.001; ****p <0.0001; n.s. indicates not significant.

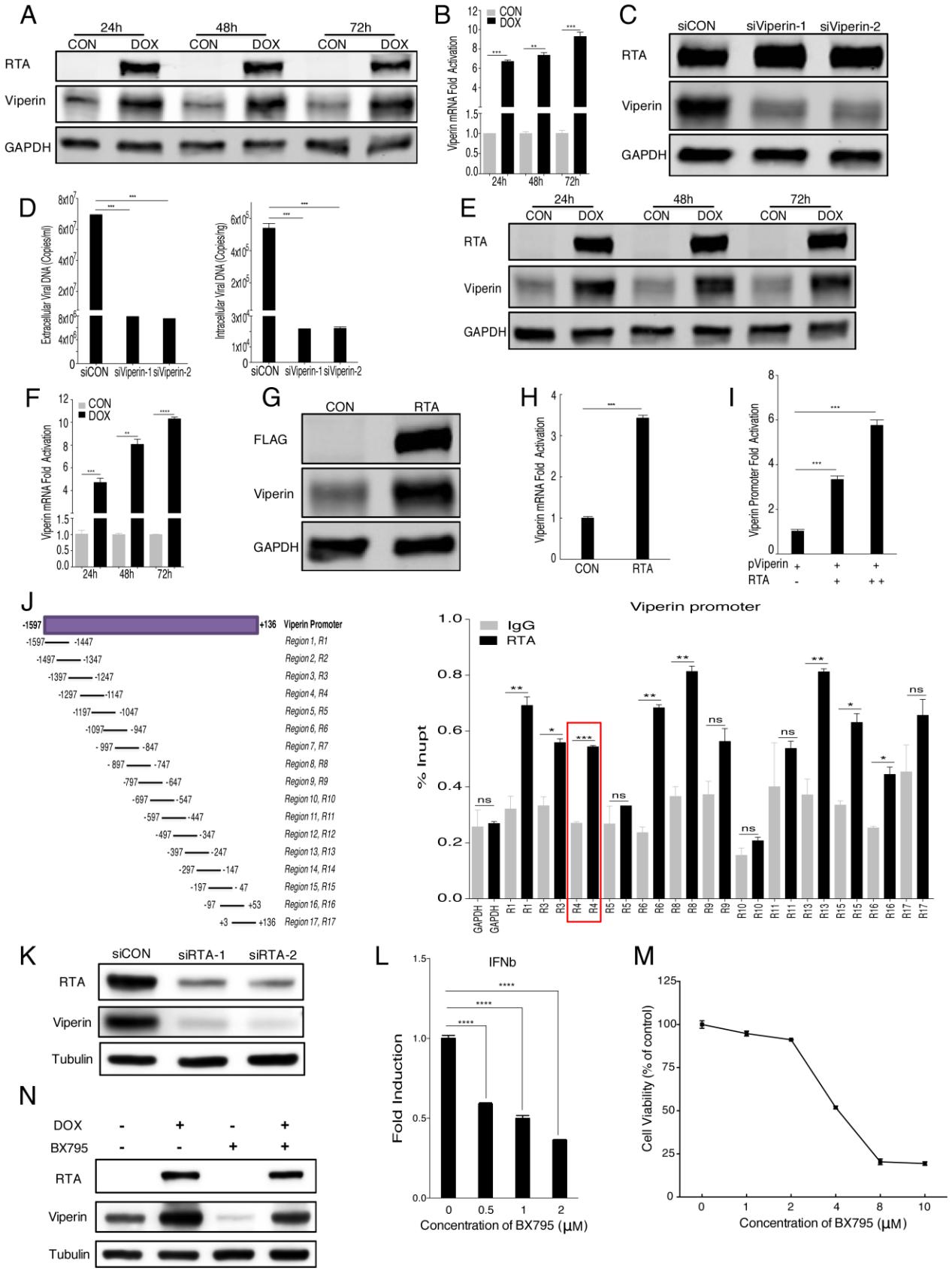

Fig. S3. Viperin is critical for viral DNA replication and KSHV-encoded RTA binds to viperin promoter to up-regulate viperin expression. (**A** and **B**) RGB-FLAG cells were treated with or without doxycycline. (A) Whole cell lysates (WCLs) were analyzed by

immunoblotting; (B) RNA was extracted and cDNA was prepared to determine viperin mRNA by qPCR analysis. (**C** and **D**) RGB-FLAG cells were transfected with siRNA as indicated. At 6 h post-transfection, cells were induced with doxycycline for 72 hours. (C) WCLs were then analyzed by immunoblotting; (D) viral DNA was extracted and analyzed by qPCR. (**E** and **F**) iSLK-Puro cells were treated with or without doxycycline. (E) WCLs were analyzed by immunoblotting; (F) RNA was extracted and cDNA was prepared to determine viperin mRNA by qPCR analysis. (**G** and **H**) 293T cells were transfected with plasmids containing indicated genes. (G) At 48 h post-transfection, WCLs were analyzed by immunoblotting; (H) RNA was extracted and cDNA was prepared to determine viperin mRNA by qPCR analysis. (**I**) 293T cells were transfected with plasmids containing indicated genes. Fold induction of the viperin promoter was determined by luciferase assay. (**J**) Schematic illustration of the primers designed to encompass the viperin promoter (Left). 293T cells were transfected with empty vector or a plasmid containing RTA. At 48 h post-transfection, cells were harvested, crosslinked and utilized for ChIP assay to determine relative binding of RTA at viperin promoter. (**K**) RGB-FLAG cells were transfected with siRNA as indicated. At 6 h post-transfection, cells were induced with doxycycline for 72 hours. WCLs were then analyzed by immunoblotting. (**L**) RGB-FLAG cells were induced with doxycycline for 72 hours after adding different concentration of BX795 for 6 hours as indicated. RNA was extracted and cDNA was prepared to determine IFN β mRNA by qPCR analysis. (**M**) iSLK-BAC16 KSHV cells were induced with doxycycline for 72 hours after adding different concentration of BX795 for 6 hours as indicated. Cell viability was measured by CCK8 assay. (**N**) RGB-FLAG cells were induced with doxycycline for 72 hours after adding 1 μM BX795 for 6 hours as indicated. WCLs were analyzed by immunoblotting. The concentration of doxycycline is 2 μg/ml. For **B**, **D**, **F**, **H-J, L** and **M**, the data are expressed as the mean ± s.e.m.; n=3; *p <0.05; **p <0.01; ***p <0.001; ****p <0.0001; n.s. indicates not significant.

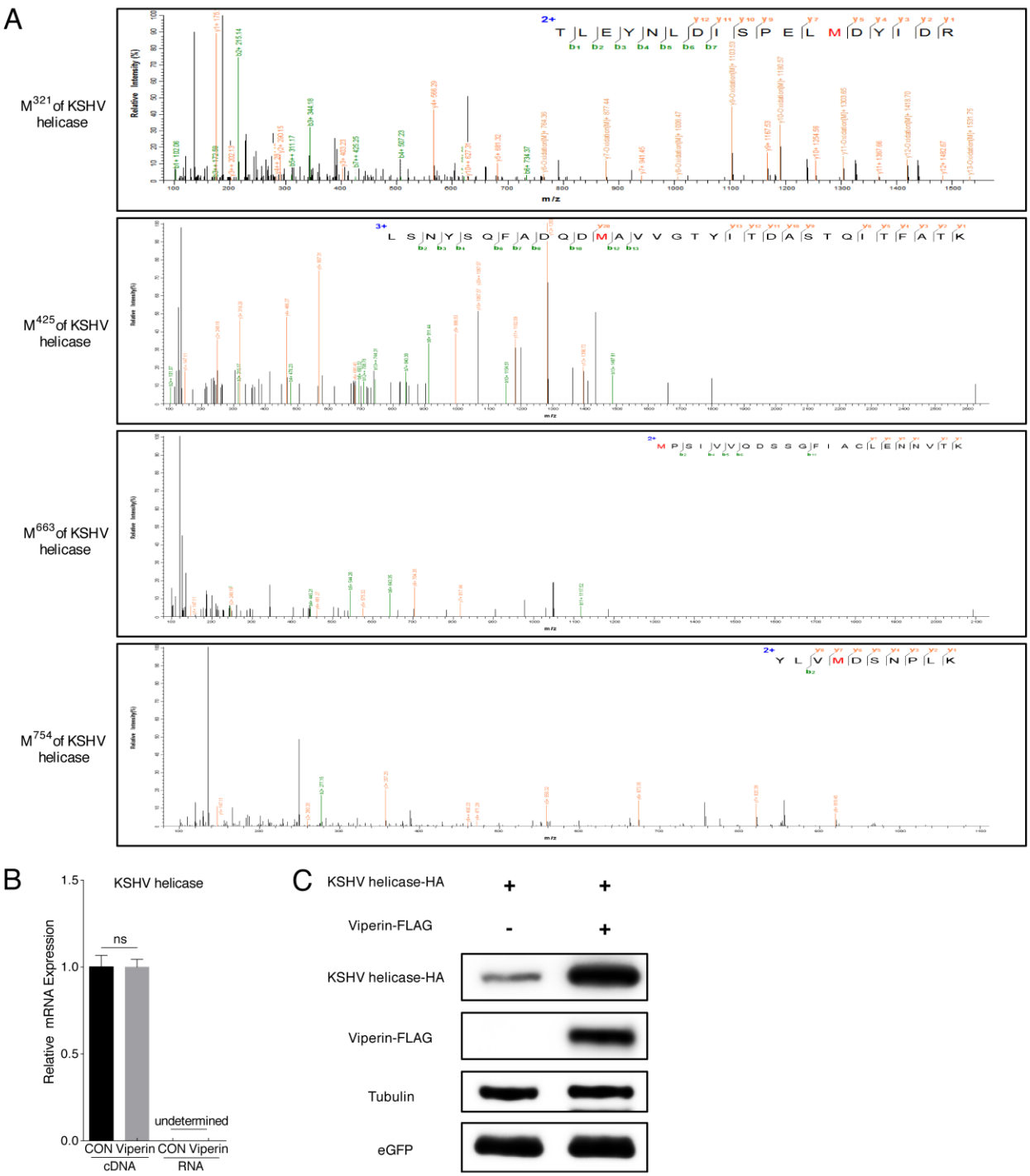

Fig. S4. Viperin promotes methionine oxidation of KSHV helicase. (**A**) Mass spectrometry analysis of methionine oxidation in 293T cells transfected with KSHV helicase with or without viperin expression. The M^{321}, M^{425}, M^{663} and M^{754} sites were oxidized (in red) under viperin overexpression. (**B**) 293T cells were transfected with plasmids containing indicated genes. At 48 h post-transfection, RNA was extracted and cDNA was prepared to determine KSHV helicase mRNA by qPCR analysis. RNA group was treated with DNase without transcription to cDNA. The data are expressed as the mean ± s.e.m.; n=3; n.s. indicates not significant. (**C**) 293T cells were transfected with plasmids containing indicated genes. At 48 h post-transfection, whole cell lysates were analyzed by immunoblotting.

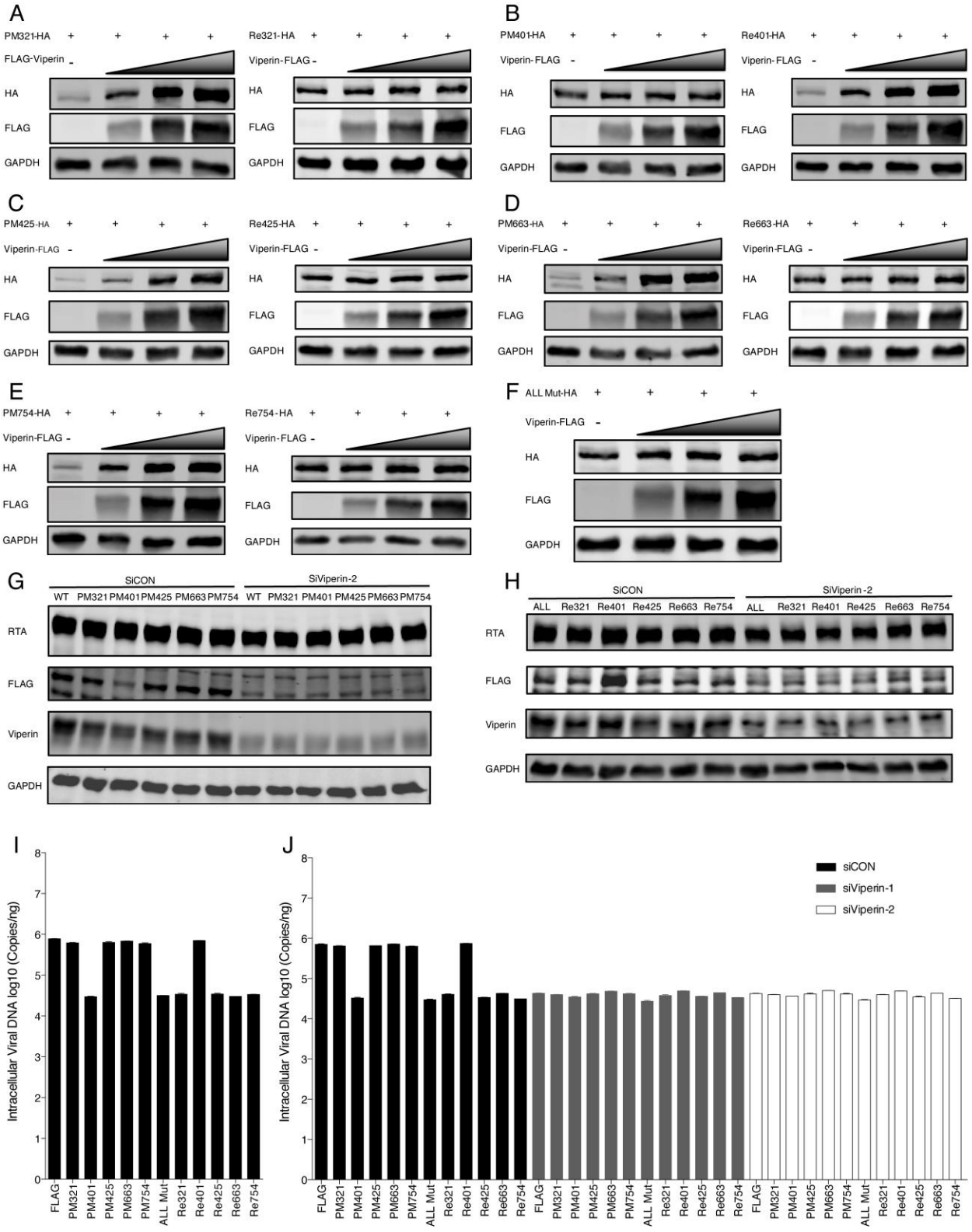

Fig. S5. Viperin-induced methionine-401 oxidation enhances the stability and function of KSHV helicase. (**A** to **F**) 293T cells were transfected with plasmids containing indicated genes. At 48 h post-transfection, whole cell lysates (WCLs) were analyzed by immunoblotting. (**G** and **H**) RGB-FLAG and RGB-mutated FLAG cells were transfected with siRNA as indicated. At 6 h post-transfection, cells were induced with doxycycline for 72 hours. WCLs were then analyzed by immunoblotting. (**I**) RGB-FLAG and RGB-mutated

FLAG cells were induced with doxycycline for 72 hours. Intracellular viral DNA was extracted and analyzed by qPCR. (**J**) RGB-FLAG and RGB-mutated FLAG cells were transfected with siRNA as indicated. At 6 h post-transfection, cells were induced with doxycycline for 72 hours. Intracellular viral DNA was extracted and analyzed by qPCR. The concentration of doxycycline is 2 μg/ml. For **I** and **J**, the data are expressed as the mean ± s.e.m.; n=3.

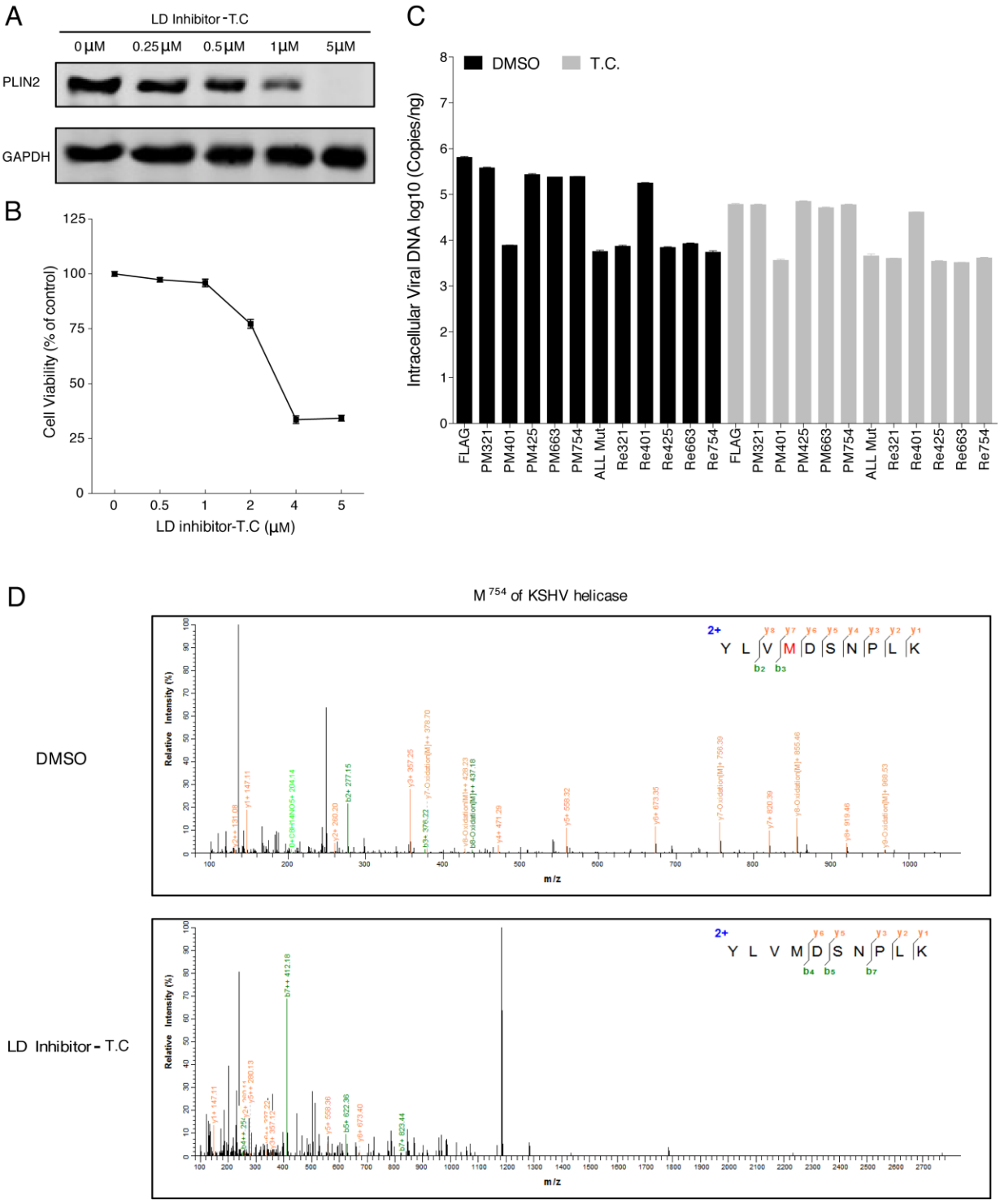

Fig. S6. Viperin associates with lipid droplets that are required to induce the methionine oxidation of KSHV helicase. (**A**) RGB-FLAG cells were treated with different concentration of T.C as indicated for 3 hours and then cells were induced with doxycycline for 72 hours. Whole cell lysates (WCLs) were analyzed by immunoblotting. (**B**) iSLK-BAC16 KSHV cells were induced with doxycycline for 72 hours after adding different concentration of T.C for 3 hours as indicated. Cell viability was measured by CCK8 assay. (**C**) RGB-FLAG and RGB-mutated FLAG cells were treated with 1 μM T.C or DMSO as a negative control for 3 hours and then cells were induced with doxycycline for 72 hours. Intracellular viral DNA was

extracted and analyzed by qPCR. (**D**) Mass spectrometry analysis of methionine oxidation in RGB-FLAG cells induced with doxycycline for 72 h after treatment with 1 μM T.C or DMSO as a negative control for 3 h. The KSHV helicase M^{754} site was deprived of oxidative modification (in black) and the KSHV helicase M^{321}, M^{401}, M^{425} and M^{663} sites were undetectable under T.C treatment. The concentration of doxycycline is 2 μg/ml. For **B** and **C**, the data are expressed as the mean ± s.e.m.; n=3.

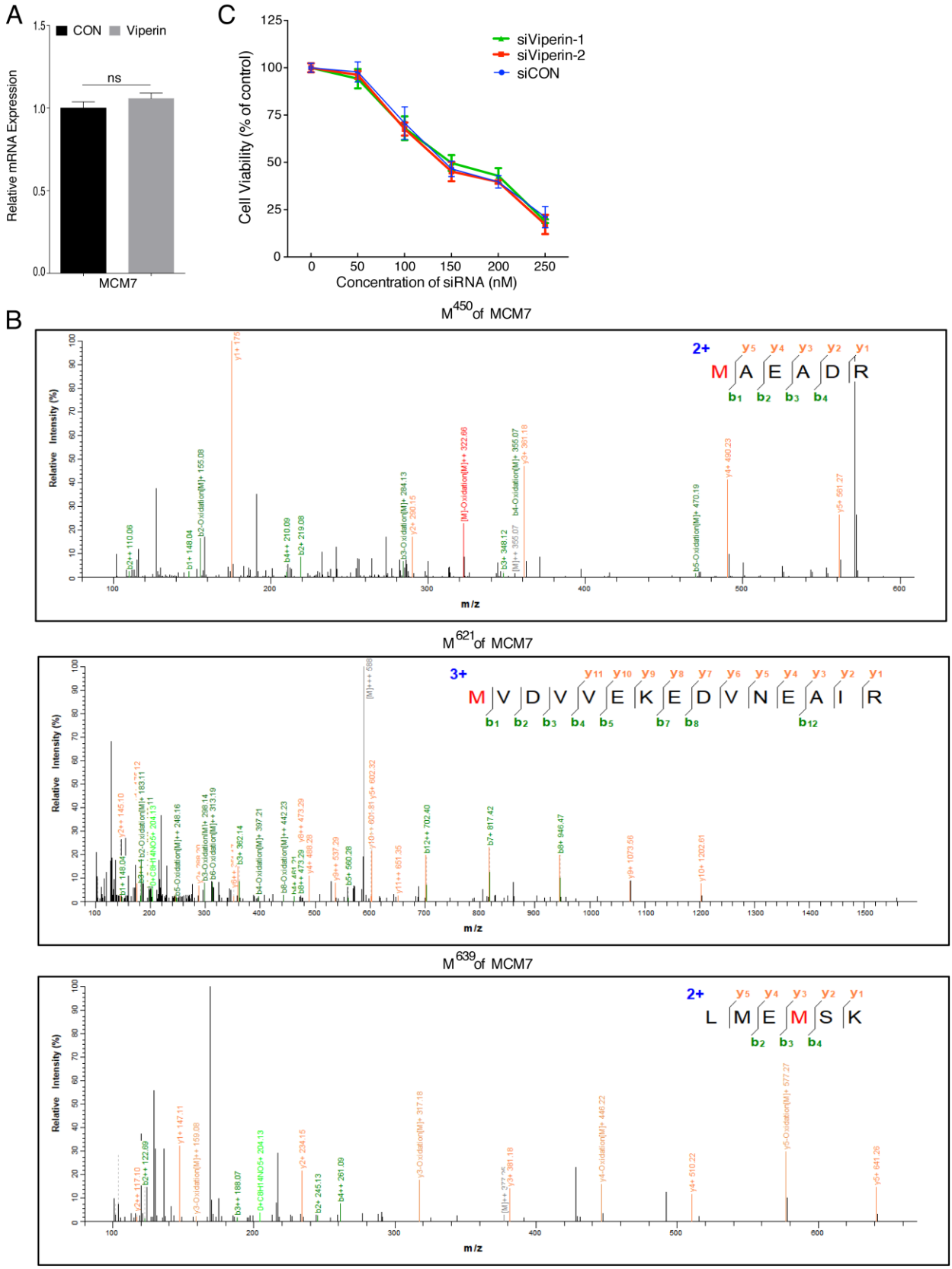

Fig. S7. Viperin-induced methionine oxidation stabilizes MCM7 and promotes DNA replication. (**A**) 293T cells were transfected with plasmids containing indicated genes. At 48 h post-transfection, RNA was extracted and cDNA was prepared to determine MCM7 mRNA

by qPCR analysis. (**B**) Mass spectrometry analysis of MCM7 methionine oxidation with viperin overexpression in 293T cells. The MCM7 M^{450}, M^{621} and M^{639} sites were oxidized (in red) under viperin overexpression. (**C**) 293T cells were transfected with siRNA of different concentration as indicated. At 48 h post-transfection, cell viability was measured by CCK8 assay. For **A** and **C**, the data are expressed as the mean ± s.e.m.; n=3; n.s. indicates not significant.

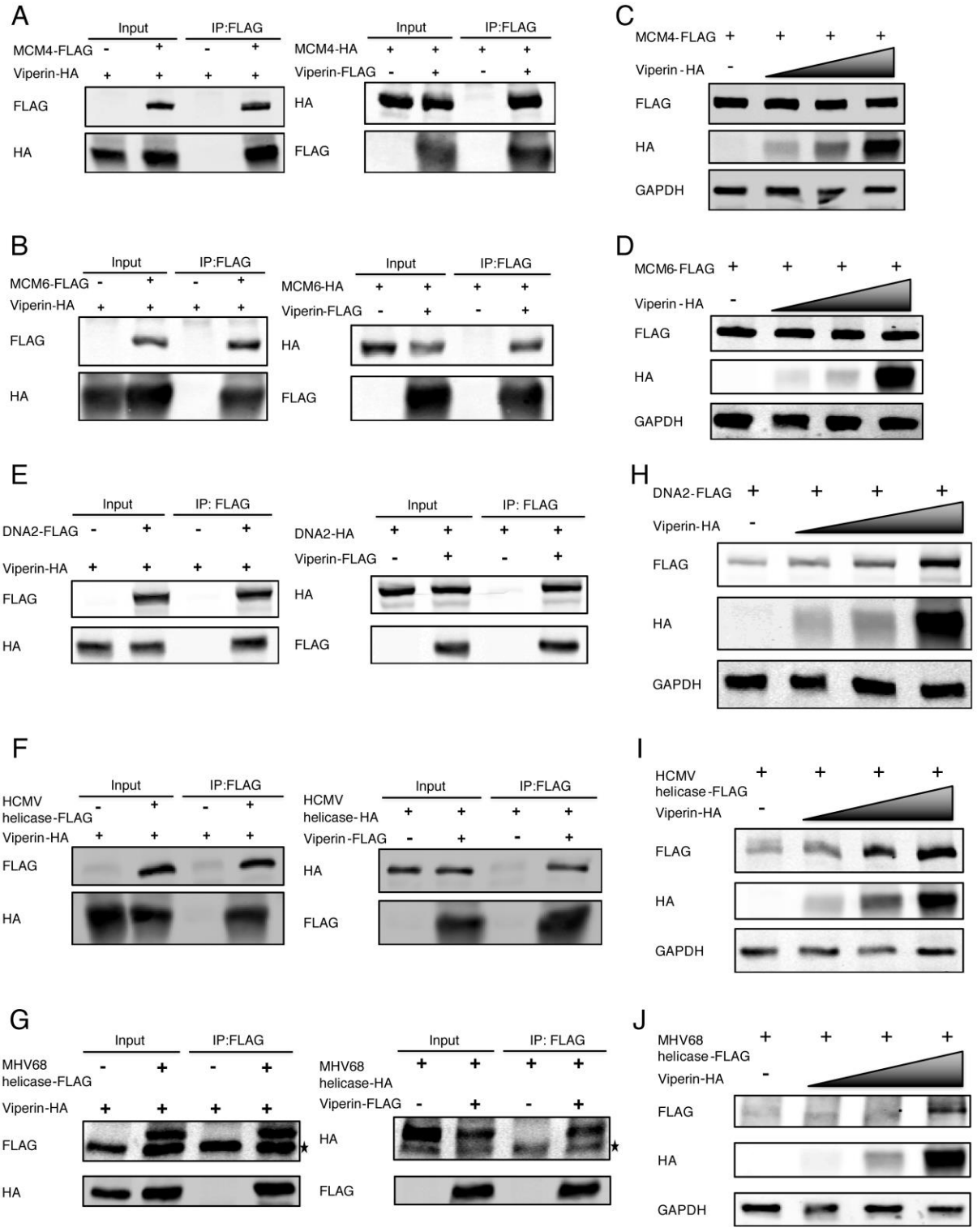

Fig. S8. Viperin interacts with several DNA helicases. (**A** and **B**) Reciprocal co-immunoprecipitation assays to examine physical interactions between viperin and MCM4 (A) or MCM6 (B). (**C** and **D**) 293T cells were transfected with plasmids containing indicated genes. At 48 h post-transfection, whole cell lysates (WCLs) were analyzed by immunoblotting. (**E** to **G**) Reciprocal co-immunoprecipitation assays to examine physical interactions between viperin and DNA2 (E) or HCMV helicase (F) or MHV68 helicase (G). The asterisk denotes an unspecific band. (**H** to **J**) 293T cells were transfected with plasmids

containing indicated genes. At 48 h post-transfection, WCLs were analyzed by immunoblotting.

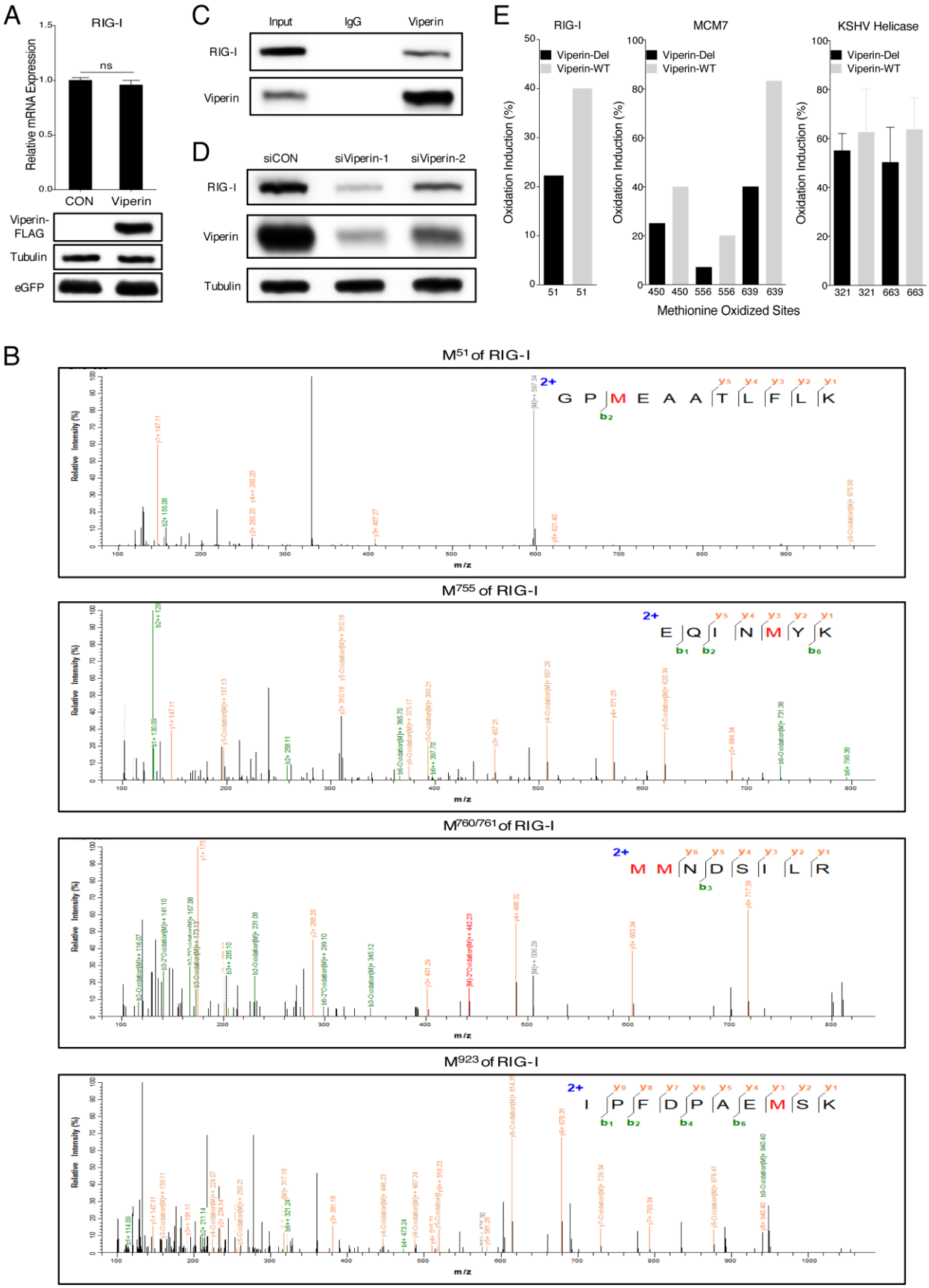

Fig. S9. Methionine oxidation catalyzed by viperin increases the stability and function of RNA helicase RIG-I. (**A**) 293T cells were transfected with plasmids containing indicated

genes. At 48 h post-transfection, RNA was extracted and cDNA was prepared to determine RIG-I mRNA by qPCR analysis and whole cell lysates (WCLs) were analyzed by immunoblotting. The data for mRNA are expressed as the mean ± s.e.m.; n=3; n.s. indicates not significant. (**B**) Mass spectrometry analysis of RIG-I methionine oxidation with viperin overexpression in 293T cells. The RIG-I M^{51}, M^{755}, M^{760}, M^{761} and M^{923} sites were oxidized (in red) under viperin overexpression. (**C**) *In vivo* co-immunoprecipitation assays to detect the interaction between RIG-I and viperin. The WCLs of 293T were precipitated with anti-Viperin. Precipitated proteins and WCLs were analyzed by immunoblotting. (**D**) 293T cells were transfected with siRNA as indicated. At 48 h post-transfection, WCLs were then analyzed by immunoblotting. (**E**) Viperin-WT, viperin-Del, RIG-I, MCM7 and KSHV helicase were purified separately from 293T cells. Oxidative reaction was performed *in vitro* and methionine oxidized peptides were quantitatively determined by mass spectrometry analysis. Data on M^{51} of RIG-I, M^{450}, M^{556} and M^{639} of MCM7, and M^{321} and M^{663} of KSHV helicase represents one or two independent experiments.

Table S1. Amino acid sequence alignment for each human DNA helicase with KSHV helicase.

Description	Max Score	Total score	Query cover	E value	Ident	Accession
sp|P33993|MCM7 HUMAN DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4	20.4	128	13%	0.32	30%	Query_76819
sp|Q8NG08|HELB HUMAN DNA helicase B OS=Homo sapiens GN=HELB PE=1 SV=2	24.3	101	26%	0.037	46%	Query_75769
sp|P51530|DNA2 HUMAN DNA replication ATP-dependent helicase/nuclease DNA2 OS=Homo sapiens GN=DNA2 PE=1 SV=3	21.2	92.0	23%	0.30	29%	Query_199423
sp|O94761|RECQ4 HUMAN ATP-dependent DNA helicase Q4 OS=Homo sapiens GN=RECQL4 PE=1 SV=1	22.7	91.2	29%	0.14	27%	Query_217033
sp|O94762|RECQ5 HUMAN ATP-dependent DNA helicase Q5 OS=Homo sapiens GN=RECQL5 PE=1 SV=2	20.4	88.9	24%	0.48	25%	Query_215355
sp|Q9H611|PIF1 HUMAN ATP-dependent DNA helicase PIF1 OS=Homo sapiens GN=PIF1 PE=1 SV=2	30.4	88.5	17%	3e-04	40%	Query_150863
sp|Q9Y265|RUVB1 HUMAN RuvB-like 1 OS=Homo sapiens GN=RUVBL1 PE=1 SV=1	20.4	87.8	14%	0.21	38%	Query_207489
sp|Q8NFZ0|FBH1 HUMAN F-box DNA helicase 1 OS=Homo sapiens GN=FBXO18 PE=1 SV=2	19.2	70.8	10%	1.3	36%	Query_219595
sp|Q14191|WRN HUMAN Werner syndrome ATP-dependent helicase OS=Homo sapiens GN=WRN PE=1 SV=2	19.6	57.4	17%	1.3	43%	Query_237731
sp|Q9ULG1|INO80 HUMAN DNA helicase INO80 OS=Homo sapiens GN=INO80 PE=1 SV=2	19.2	56.2	14%	1.8	23%	Query_152073
sp|Q13472|TOP3A HUMAN DNA topoisomerase 3-alpha OS=Homo sapiens GN=TOP3A PE=1 SV=1	16.9	49.6	13%	5.3	41%	Query_147093
sp|O14646|CHD1 HUMAN Chromodomain-helicase-DNA-binding protein 1 OS=Homo sapiens GN=CHD1 PE=1 SV=2	18.5	36.2	4%	3.8	36%	Query_30209
sp|Q8TDG4|HELQ HUMAN Helicase POLQ-like OS=Homo sapiens GN=HELQ PE=1 SV=2	17.7	68.1	11%	4.0	35%	Query_35459