# **Supplementary Information**

# A loosened gating mechanism of RIG-I leads to autoimmune disorders

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**Supplementary Figure S1**. Sequence alignments of RLR HEL2i gate motif and RIG-I CARD2 latch interface.

### WT RIG-I

### 910 930 VQTLTSEVEDFHFEEIPFDPABISE

## RIG-I

\_\_\_\_\_\_ 10 \_\_\_ 20 \_\_\_\_ 20 \_\_\_ 40 \_\_\_ 20 \_\_\_ 40 \_\_\_ 10 \_\_\_\_ 10 \_\_\_ 90 \_\_\_ 90 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_ 10 \_\_\_\_\_ 10 \_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_\_ 10 \_\_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_ 10 \_\_\_\_\_\_\_ 10 \_\_\_\_\_\_\_ 10 \_\_\_\_\_\_\_ 10 \_\_\_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_\_\_10 \_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 0 \_\_\_\_\_10 \_\_\_\_\_10 \_\_\_\_\_10 0 \_\_\_\_\_10 0 \_\_\_\_\_10 0 \_\_\_\_\_10 \_\_\_\_\_10 0 \_\_\_\_\_10 0 0 \_\_\_\_\_10 0 

910 920 VQTLTSEVEDFHFEEIPFDPABISE

### WT RIG-I



### RIG-I

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N T T E Q R R S L Q A	PODTIRETLI	PTTILSTN	A P <b>V F</b> R E E E V Q	LICVERNEC	PNEAATLPLE	FLLELQEE6	FRGFLDALI	HAGTSGLTEA	IESTOPEKIEKI	2
	H	40(n (+1)	51(0 (+2)	-	40(0) (+3)	H-	39(2)(-2)	35(2) (+2)	36(1) (+1)	
	- H	30(2) (+3)	55(4) (+2)	-	25(0)(+3) 27(0)(+2)		-	42(2) (+2)		
110	13	,	130	140	25(3) (+3)	160	170	180	190	
BETRLLLERLQ	PEFETRIIPI	TDIISDLSB	CLINGBOBBI	LQICSTEGEN	AGABELVECL	LESDEENTPI	TLELALEE	RHEFSELVIV	REGIEDVETEDI	5
(-3) - s	000 (+30	49(0 (+3)	F		20(1) (+3)		2000 (+2)	48(2) (+3)	87(0)(+3	6
19(0)(+2) 33	001-20	55(3) (+2) 37(5) (+4)	H		15(4) (+2)		37(3) (+4)	51(2) (+36	53() (+3) 87() (+3	
- 3	(6) (+2)	55(3) (*3)						51(2) (+4)		
210	20	)	230	240	250	260	270	200	290	200
EDENETSDIQI	FTQEDPECQI	ILSENSCPP	SEVSDTHLYS	PFEPRNYQLE	LALPANEGEN	TIICAPTOC		BHHLEEFPQG	QEGEVYPPANQ:	Ľ
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				- 40(	10(+1)	45(3) (+7) 48(3) (+3)	-	18(1) (+3)	-	1
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PLPQVIGLTAS	VOVODAINTI	BALDVICE	LCASLDASVI	ATVENUEL	ROVVIEPOEP	FREVESRIS	TPETIAGI	RDTESLATE	ICEDLENLSQI	2
3400 (+2)		54(4) (+2)	H-	47(3) (+2)		-	23(1) (*3	703 (+2)	52 ga (+3)	
33(7) (+7)		29(3) (+7) 5(2) (+	<b>1</b>		7353 (+7)		H	47(0) (+2)		
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HREFGTORYO	AIALAST	VYQNPDID	RESRICEALF	LYTSHLRKYN	DALIISEHAR	REDALDYLES	FFSHVRAA	PDBIEQDLTQ	RFERELQELES	1
96(7) (*	(0) (000) (+3		41(7) (+4)		3(1) (+3)	27(8) (+3)	12(1) 12(1)	-1	22(1) (+3)	
		1 H	350 (+0	-	3(1) (+2)	22(2) (+3)	sap)	+7)	25(1) (+3)	2
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SRDPSHENPEL	RDLCFILQEN	THL WP BTI	TILFVETRAL	VDALENVIEG	NPELSFLEPG	ILTEREETH	HTGHTLPA	ECILDAFEAS	GDHHILIATSVI	4
51(2) (+3)	⊢	-	38(7) (+7)	H-	41(7	0.02		75(2) (+3)	53(3) (+2)	£.
	39(2) (+3)				27(2) (+3) 35(1)	10-20	1163 (+18		24(2) (+3)	1
710	14+38				35(2) (+2)					
	. 79		790	740	159	169	179	799	799	999 P
DEGIDIAQCEL	72 VILTETVON	VIXWIQTRG	730 RGRARGSECF	740 LLTSNAGVIN	TEO INNYKEK	TGO NNHDSILRL	170 . 	790 EILHIQTHEE	790	200 7
75(0)(+2)	720 (***	) /IXNIQTRG	730 R G R A R G S E C F 5300 (+4)	740 L L T S N A G V I N	3600 (+2)	700 N N N D S I L R L 1 19(0 (+7)	170 2 T V D B A V F R I 36(0 (+2)	700 EXILHIQTHEE 10(1)(***	799 FIRDSQEEPEP	200 7
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DEGIDIACCNL 7908(62) 1008620 PDEENEELLCR 910 VQTLTSEEEP		2 VIENIQTRG 100(1-2) 10	120 R G R A R G S E C F 20(0) (+4)	100 49(2)(-2 800 8 C F V S K P N P N N (401 10) 402	100 100 100 100 100	100 110 HD SILELU 110 (10) 110 (1	770 27 T D B A V F R I 36(0 (-70) 670 670 570 570 570 570 570 570	790 KILNIQTHEEL 101140 1011	796         1           FIRDSQEEPEF         3           30001-02         3           40001-02         3           990         1           12001001         1           12001001         1	200 7
DEGIDIACCNL 7100(07) 900(07) 910 900(07) 910 910 910 910 910 910 910 910	12 13 14 14 14 14 14 14 14 14 14 14	2 7 I I N I Q T R G 100 020 100 000 100 000	130 R G R A R G S X C F 33(0; i-4) 830 N Y T V L G D A F K 33(0; -3) 1(-3) 1(-3) 1(-3) 1(-3) 1(-3)	100 49(2)(-2 800 8 C F V S K P N P N N 640 101 640	100 1 K E Q I N J Y K E K 3 400 (-2) 100 (-	100 110 HD SILELU 110 (10) 110 (1	179 27 T D E A V P R 36(1) (-2) 10(1) (-1) 10(1) (-1) (		790           7 IR D S OF REPEP           30           31           32           45(2)           45(2)           690           1 IE S F V V E D IA T (110)           1 10(2)           32(3)           32(4)	200

# RIG-I<sub>Q517H</sub>

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	45(3) (+3)	63(4) (42)	5610 (*20	36(0)(*3)		42(8)(*2)	40(2) (+2	42(4) (+1)	
F	44(0) (+3)		E	33(2) (+2) 29(3) (+3)					
110	120	130	140	150	160	170 .	190	190	200
************	RIIPTDIISD	LSECLINQEO	BEILQICS	TEGNNAGABELV	ECLLRSDEENT	PETLELAL	E K E R H E F S E L	TIVEEGIEDVE	TEDL
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21(2) (+2) 37(3) (+2)	60 (H	2)		25(3) (+2)		43(4) (+4)	6707 ( - T	5440.043	
42(4) (+2)	61(R) (*	n					54(2) (+4		
210	220	130	240	150	260	270	290	294	300
EDENETSDIGIFTOED	PECQHLSENS	CPPSEVSDI	LISPERPRI	RTGLELALPARE	GENTIICAPIO	COLTIVEL	LICBERLEEP	POSOLOIVIPP	ANGI
62(2) (+2)	78(8(-2)	#2(0) #7(5) (	(+2)	63(3) (*3) 63(3) (*3)	28(4)(*2)	77(1) (+2)	-	23(1) (+3)	_
		12(4)	(-1)						
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			- i-i	33(1) (+2)		•			
. 410 .	420 .	430	<b>\$4</b> 0	. 450 .	<b>4</b> 60 .	470 <u>.</u>	<b>4</b> 90	490	<u>500</u>
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34(0) (+2)	F479-4-79								
2000	angle of	100.00	-	u(7) (+7) 🖂 👘	1(+) (qt	23	70.62	540) (*1	e
35(2) (+2) 43(2) (+2)	59(2) (+2)	6(1) (*1) 6(3) (*7)	-	46(7) (+7)	3(7) (+7) 4(7) (+2) 4(7) (+7)	23	47(3) (+2	54D) (*3)	e
35(2) (+2) 43(2) (+7) 510	520 (*2)	6(1) (+1) 6(2) (+2) 5(30	540	550	5(2) (*3) 4(3) (*2) 4(3) (*3) 560	570	590 - 590 -	540) (+3 590	600
9509 (+2) 4309 (+2) 510 NREPGTQXYEQVIVIV	520 HIACNYFQJP	500 (*10 500 (*20 500 DIDEESRICE	540 EALPLYTSH	550	501 (*3) 403 (*2) 403 (*3) 560 HARMEDALDYI	20 570 . LEDFFSNVR	00 (+3) 700 (+2 47 (8) (+2 500 A A G F D E I E Q I	000 LTQRPBELQE	000 185V
35(2) (*2) 42(2) (*2) 	500 23(1) (*2) HIACNVFQNF 23(1) (*2)	500 (+3) 500 (+3) 500 DIDBESRICE	540 EALPLYTSH: \$340 (~40	550 550 LRETEDALTISE	200 (~7) 401 (~7) 401 (~7) 560 E A R X E D A L D Y I 2409 (~3)	20 570 LED <b>7</b> PSN7R	500 - 200 -	500	600 LESV 2014-31
95(9)(+2) 41(9)(+2) 	500 500 EIACNVFQNP 2000(+2)	500 500 510 (*2) 520 510 2 2 5 2 1 C 2 510 1	540 (ALPLYTSH) (3)(0 (-4) (-3) (7)(0 (-4)	550 550 LRETNDALIISE 801(43) 100(44) 100(42)	201 (-21) 401 (-21) 560 E A R II E D A L D Y I 2400 (-21) 2400 (-21) 	23 570 LEDFFSHTR 25(1)(+2)	200 (+20)	500 1 T Q R F B B B L Q B 2001 2001 2001 2001	600 LESV 701(-31)-
3309(-03) 3109(-03) 510 1107(-03) 1107(-03) 4309(-03)	500 500 HIACNYFQNF 200	500 500 500 500 500 500 500 500 500 500	540 E A L P L Y T S H I (3) (3) (4) (3) (4) (4) (4) (4) (4) (4) (4) (4	500 500 1 R I T N D A L I I S E 100 (-1) 701 (-1) 100 (-2)	200 (-20) 400 (-20) 500 E A B B E D A L D T 1 2400 (-3) 2200 (-3) 2000 (-3)	23 570 LEDFFSHTR 35(1)(-3)	500-030 P20 P20 500 A A G F D E I E Q I 44(4) (~7) 72(7) (~7)	500	600 LESV 2014-31
100 (-0) 100 (-0) 510 1 R 2 F G T Q X T 2 Q X I V T V 400 (-0) 410	500 500 2200 (-2) 2200 (-2) 2200 (-2) 500 500 500 500 500 500 500 50	400 (-41) 500 500 D I D B B S R I C B 500 500 500 500	540 1 A L P L Y T S H : (3) (3) (4) (40) (40)	550	300 (-3) 	23 570 LEDFFSHTR 23(1) (-2) 	500-03 700 F2 500 A A G F D E I E G I 40(0) (-2) 520(0) (-2) 600 600	500 500 10 T Q R P B B L Q B 200 200 200 200 	000 LESV 201(-3) 
539 (20) 530 (20) 510 10 (20) (20) 10 (20)	500 500 2200 (-2) 2200 (-2) 200 (-	4(1) (4) 4(1) (4) 500 D L D Z E S R I C E 34(1) 52(1) 6(30 E T I T I L F V I I	540 EALPLYTSH (-3) (-3) (-3) (-3) (-3) (-3) (-3) (-3) (-40) (		300 (-3) 500 HARBEDALDTI 2400 (-3) 2400 (-3) 2200 (-3) 2200 (-3) 200 (-3) 500 100 (-3) 100 (-3)	570 	500-00 - 2700 (+2 500 A A G F D E I E G I 44(4) (-3) 22(0) (-2) 600 P A G I C I L D A F	500 500 500 500 500 500 500 500	606 LESV 2014-30 
Signeral         Sio	900103 500 ELACW7FQMP 2000103 4200 11LQEETHLMP	500 500 500 500 500 500 500 500 51111LFVE1 3000 500	540 CALFLYTSH: SNG (-4) (-7	4609(+-3) - 550 - 550 - 550 - 11 S B - 10 (+3) - 1	320 (c7) 420 (c7) 420 (c7) 2400 (c7) 2400 (c7) 2400 (c7) 2400 (c7) 2400 (c7) 2400 (c7) 2400 (c7) 4200 (c7) 4200 (c7) 4200 (c7)	23 570 LED FFSHTR 29(11(47) 54(2)(47) (70 114 CHTGNTL 4200 (23)	00	000 (700 (700 (700 (700 (700 (700 (700	600 LESV 2014-31 
Signedition           Signedition           NR BP 6 T 0 T T B 0 T 1 T T 0 T 1 T T T           exp(ca)           410           SR DP S N B NP T L E D L C F           Sign(ca)           Sign(ca)	500 100 500 HIACWYPQHP 200 100 110 HETHLWP	ACTIVATI SCO SCO SCO SCO SCO SCO SCO SCO	540 EALPLITSH: 500 (-0) 501 (-0) 501 (-0) 501 (-0) 502 (-0) 503 (-0)	4600 (+-3)  - 4 4 4 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	220 (-7)	23 570 LEDFFSHYR 29(11(42) 570 (70 1140HT6HTL 42(1)(42)	001-3         2001-12           2001-2         2001-12           2001-2         2001-12           2001-2         2001-2           2001-2         2001-2           2001-2         2001-2           2001-2         2001-2           2001-2         2001-2           2001-2         2001-2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2001-2         2           2011-2         2           2011-2         2           2011-2         2           2011-2         2           2         2           2         2           2<	500 500 500 500 500 500 500 500	000 LESV 200 (*3) 
000,000 000,000 100	200 r0 200 r0 500 EIACBY76BP 2200 r0 11 L G E ET BL WP 11 L G E ET BL WP 720	ann	540 EALPLITSH: 500(-00) 501 501 501 502 502 502 502 502 502 502 502	400 (-3)         -         4           2         -         2           500         -         2           700 (-0)         -         2           700 (-0)         -         2           100 (-0)         -         2           100 (-0)         -         2           200 (-0)         -         2           200 (-0)         -         3           200 (-0)         -         -           700         -         -	30(30) (30) (30) (30) 80 × 10 × 10 × 10 × 10 × 10 × 10 × 10 ×	23 570 LEDFFSHYR 29(11(42) 570 1140 HIGHI 470 1140 HIGHI 470 170	000-3         2000-2           200         2000-2           200         2000-2           200         2000-2           200         2000-2           200         2000-2           2000-200-2         2000-2           2000-200-2         2000-2           2000-200-2         2000-2           2000-200-2         2000-2           2000-2	500 500 500 500 500 500 500 500	600 LESV 2010-0-0
100,000 - 500 0,00,000 - 500 HREPOTOLISOURCE 011VIV 0,00,000 - 500,000 - 500,000 - 500,000 - 500,000	\$200 color \$200 c	T R & K & G & A & R & C & C & C & C & C & C & C & C & C	540 CALFLYTSH: SN()(-0) (-3	4400 (-3)   - 4 - 500	30 ( 30) 500 500 500 500 500 500 500 5	27 570 . LEDFFSNTR 2300 (07) 500 (07) 500 (07) 100 UTONTL 4200 (03) 170 . RLQTTDEAY	00	500 (-7 500 (-7 500 (-7 500 (-7 500 (-7 500 (-7 500 (-7) 500 (-7 500 (-7) 500	000 LESV 700
30           30           30           31           32           33           34           35           36           37           38           38           38           39	500 co 500 co 500 co 81 L C B V 7 Q B P 200 co 400 co 1 L G E E T BL NP 700 co 1 V G B V I I B I G 700 co 1 V G B V I I B I G	troop of the second sec		100         100 <td>30 ( 20) 500 500 500 500 500 500 500 5</td> <td>27 570 LEDFFSNTR 2010(07) →401(01) 670 10001000 1000100 1000000 1000000 1000000 1000000 1000000 1000000 1000000 1000000 10000000 1000000 1000000 1000000 1000000 1000000 1000000 10000000 10000000 100000000</td> <td></td> <td>500 - 510 -</td> <td>000 LESV 700</td>	30 ( 20) 500 500 500 500 500 500 500 5	27 570 LEDFFSNTR 2010(07) →401(01) 670 10001000 1000100 1000000 1000000 1000000 1000000 1000000 1000000 1000000 1000000 10000000 1000000 1000000 1000000 1000000 1000000 1000000 10000000 10000000 100000000		500 - 510 -	000 LESV 700
300,000         510           0,000,000         510           M.R.P.P.T.C.R.P.G.R.P.T.V.P.V         900,000	\$200 \$200	1000 CONTRACTOR CONTRA		4000 (-03) ↓ 500 ↓ 100 ↓	30 ( 47) 470 ( 47) 500 10 ( 47) 10 ( 47)	23 570 LEDFFSNYR 29(11(-27) (70 (70 11 H C H T C H T L 420(-23) 170 	200	500 (-7) 500 (	000 LESV 700-00 
300,000         100           100,000<	500 500 500 500 500 500 500 500	SOD SOD SOD SOD SOD SOD SOD SOD	540 EALFLTSH SNG C-0 C-0 C-0 C-0 C-0 C-0 C-0 C-0	KCO (+3)	190 (10) (5)) (5	27 570 2500 2500 2500 570 3000 570 1100 HT 6 MT L 4000 670 1100 HT 6 MT L 4000 670 1100 HT 6 MT L 4000 100 100 100 100 100 100 10	200         200 <td>500</td> <td>000 LESV 7004-01 </td>	500	000 LESV 7004-01 
Dany vol   Dany v	200 00 200 00	100 per se		400         400 <td>190 (20) 190 (2</td> <td>23 570 LEDYPSNYR </td> <td>200 1-2     2</td> <td>500 (-7 500 - 500 - 500 -</td> <td>000 L B S V 701</td>	190 (20) 190 (2	23 570 LEDYPSNYR 	200 1-2     2	500 (-7 500 - 500 -	000 L B S V 701
Bayon       Bayon <t< td=""><td>200 F 200 F</td><td>500</td><td>Ş40           Ç40           Q14 LF LT SH           Q16 Q14           Q1</td><td>4400 (+3) 500 190 0 +10 190 0</td><td>130 (131) (50) (</td><td>270 270 270 270 270 270 270 270</td><td>(00     (0)     (</td><td></td><td>000 L B S V 701</td></t<>	200 F	500	Ş40           Ç40           Q14 LF LT SH           Q16 Q14           Q1	4400 (+3) 500 190 0 +10 190 0	130 (131) (50) (	270 270 270 270 270 270 270 270	(00     (0)     (		000 L B S V 701
Banging	500 500 500 500 500 500 500 500	10000 100000 100000 10000 10000 10000 10000 10000 10000 10000		400 (-0)	30 (13)     (13	270 570 ↓ EDFFSHYR ↓ EDFFSHYR ↓ Syntest 570 1100HTGHTGHT 170 170 170 170 170 170 170 170	00000000000000000000000000000000000000	500 (2) 500	006 LBSV 707474 1006 1006 1006 000 000 000 000
00000	300 (100) 500 500 500 500 500 500 500	500	540 540 CALPLUTSE CONTON C		190 - 03 (60 (60 (60 (60 (60 (60 (60 (60	270 570 E B J F S M Y R → H C M T G M T G 1000 H T G M T G M T G 1000 H T G M T G 1000 H T G M T G 1000 H C M D F G I 670 000 C M C M D F G I 000 C M D F G I	(00         (0)         (	500         500           500         700           200         200           200         200           200         200           200         200           200         200           200         200           200         200           200         200           200         200           200         200           200         200           200         200           200         200           100         200           100         200           100         200           100         200           100         200           100         200           100         200           100         200           100         200           100         200	000 LBSV 100 100 100 000 000 000 000 00
Bayes     B	300 (co) 500 100 (co) 400 (co) 500 400 (co) 500 500 500 500 (co) 500 (co) 5	00000000000000000000000000000000000000	540 540 540 540 540 540 540 540	4400149 149 149 149 149 149 149 149 149 149	00 - 0	20 20 20 20 20 20 20 20 20 20	(00)     (00)	۲۵۵ (۲۹۹ - ۲۹۹ (۲۹۹ (۲۹۹ (۲۹۹ (۲۹۹ (۲۹۹ (۲۹	000 LESV 700-731- 53- 700 TSTA 900 PEPV 990 - IATG
30000	200 (cd) (c) (c) (c) (c) (c) (c) (c) (c	500 500 500 500 500 500 500 500		2000 GIO	00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000           00000         00000	27 570 570 570 570 570 570 570 57	10000000000000000000000000000000000000		996 L B S V SI

### RIG-I<sub>Q517H</sub>

410 600 600 600 77 SRDPSHIPFLIDLCFILGESTHLFFETITILFFETERLFDALEFTIGEFELSFLEFGILTGEGTERBIGETLFAGECLLAATEAGGDEBILLAATEAGGDEBILLAATEAG 910 900 VQTLTSEVEDFHFEKIPFDPARKSE

**Supplementary Figure S2**. HDX profiles of RIG-I, RIG-I<sub>E510V</sub> and RIG-I<sub>Q517H</sub> at their *apo* states. The values listed under each HDX experiments demonstrate the averaged percentage of deuterium incorporation of that corresponding peptide derived from each state across all exchange time points (i.e., 0s, 10s, 60s, 300s, 900s). HDX Workbench colors each peptide according to the smooth color gradient HDX perturbation key (D%) shown in each indicated figure. On the right panel, the deuterium incorporation data from all overlapping peptides were consolidated to individual amino acid values using a residue averaging approach.



**Supplementary Figure S3**. Comparative analysis of single amide consolidated HDX-MS profiling. The y- and x-axes illustrate the  $\Delta$ HDX (%) and domain arrangement of RIG-I, respectively. A positive or negative value on the y-axis (% $\Delta$ D) represents deprotection (involved in solvent exposure) or protection (involved in binding events or become more stabilized) against deuterium exchange in the corresponding region depicted along the x-axis of indicated receptors or complexes.



**Supplementary Figure S4**. Single residue  $\Delta$ HDX and  $\Delta\Delta$ HDX consolidation view of indicated protein or protein complexes. The x-axis represents the RIG-I sequence from N-terminal to C-terminals. Percentages of deuterium differences are color-coded according to the HDX perturbation key (below). Grey, no statistically significant changes between compared states.

peptide sequence	charge	start	end	domain	(i)	(ii)	(iii)	(iv)
FODVIRKTLOPTVILS ODVIRKTLOPTVIL	3	12 13	27 26		11 (3) 22 (4)	7 (4) 15 (4)	-18 (3) -32 (3)	-7 (4) -20 (4)
SYMAPWERE YMAPWEREEVQ	2	27 28	35 39		32 (5) 27 (6)	23 (7) 15 (7)	-37 (3) -37 (3)	-25 (6) -21 (5)
YMARWPREEEVQY VQYIQAEKINKGPWEAATL	2 3 3	28	40 56	CARD1	11 (3) 12 (6)	6 (6) 2 (4)*	-38 (2) -39 (5)	-27 (8) -19 (4)
E KNNKGPMEAAT). LGEGWFRGFL	3	44 64	56 74		23 (6) 7 (5)	14 (6) 4 (4)*	-46 (5) -47 (4)	-34 (5) -10 (4)
OEEGWIERGEL FRGELDA	2	65 70	74		12 (6) 25 (8)	8 (5) 18 (5)	-26 (6) -28 (5)	-10 (6) -17 (5)
DALDHAGYSGL LDHAGYSGL YEARSW	2	75 77 86	85 85 92		23 (4) 17 (4) 45 (5)	16 (4) 9 (4) 32 (4)	-32 (3) -28 (4) -48 (5)	-19 (4) -15 (4) -12 (4)
YEAESWD YEAESWDFKKEKLEE	1	86 86	93 102		40 (3) 11 (6)	28 (4) 1 (6)*	-48 (3) -38 (5)	-28 (4) -28 (5)
PKKEKLEE VRLLLKRLGREF	3	94 303	102 114		15 (3) 40 (4)	9 (4) 38 (2)	-38 (2) -40 (4)	-26 (5) -30 (3)
LUKILOPEF	3	106 106	114	Latch region	58 (4) 25 (4)	40 (4) 26 (5)	-49 (3) -36 (3)	-31 (5) -25 (4)
KTRIFTOISOL KTRIFTOISOL	2	115	120		23 (6) 8 (5)	14 (6) 2 /5/*	-28 (3) -28 (5) -22 (5)	-10 (6)
EEILQICSTKOMMAQAEKL ILQICSTKOMMAQAEKLVE	3	137	188 157		33 (4) 31 (5)	24 (5) 25 (5)	-46 (4) -36 (3)	-20 (4) -27 (4)
LLRSDKENWPKTLKLAL	4	159	173	CANDZ	55 (4) 59 (4)	24 (4) 29 (4)	-28 (3) -35 (3)	-16 (3) -22 (4)
RSCREINVERTURL RSCREINVERTURLAL ERFEINVERFEI	4	161 161	175		25 (4) 29 (5) 24 (3)	25 (4) 31 (4) 18 (5)	-32 (4) -37 (5) -17 (5)	-22 (4) -28 (4) -10 (3)
WWEKGKOVE WVEKGKOVETEDL	3	386 385	196		-3 (6)* -3 (2)*	-4 (5)* -5 (2)*	0 (4)* -3 (2)*	-1 (5)* -2 (5)*
WVEKGKOVETEDLEDKMETSD IVEKGKOVETEDL	3	186 187	208		-2 (2)' -4 (5)*	-5 (2)* -5 (4)*	4 (2)' 1 (3)'	-2 (3)* 0 (5)*
EDIMIETSDIGP EDIMIETSDIGP IDEVOTSPECONI SE	2	201	212 224	linker	-3 (4)* 3 (4)* 5 (2)*	-4 (5)* 2 (4)* 0 /5)*	-4 (3)* -3 (3)*	-3 (6)* -2 (8)* -3 (7)*
FY GEDPECONL YGEDPECONL	2 2	212 213	222 222		-1 (5)* -3 (4)*	-5 (4)* -5 (4)*	-2 (4)* -5 (3)*	-1 (5)" -4 (2)"
SENSCPPSEVSDTNL VSDTNI,	2	223 232	237 237		-3 (3)* -1 (4)*	-5 (4)* -3 (3)*	-3 (2)* -2 (4)*	-2 (4)* -2 (4)*
YSPERPRIVOLE LALPANKOKITIC LALPANKOKITICAPTOCOKTEVSLL	2 4	238 250 250	249 263 276	HEL1	-7 (5) -7 (5) _2( (6)	-7 (5) -10 (4) _0 (4)	2 (3)* 0 (4)*	0 (3)* 4 (3)* 0 (6)*
APTGCGKTF LICEHHLKKFP030K0KVVF	2 3	264 276	272 295		-1 (4)* -3 (2)*	-4 (5)* -3 (2)*	-4 (4)* 0 (2)*	-2 (8)* -1 (2)*
ICEHHLKKFPQGQKGKVVF PQGQKGKVVF	3	277	295 295		-2 (3)* -2 (4)*	-3 (3)* -5 (5)*	0 (2)* 1 (2)*	0 (3)* 0 (5)*
FANGPVYEDD FANGPVYEDDKSVF FANGPVYEDDKSVFSKY	2 2 2	256 256 264	306 310 347	motif la	-12 (4) -26 (3) -27 (5)	-9 (4) -18 (3) -15 (3)	-2 (4)* -4 (3)* -2 /4*	1 (4)* -3 (3)* -2 (7)*
FERHGYRVTGISGATAENVPVEGMENND GYRVTGISGATAENVPVEGMENN	3	314	342 341		-13 (4) -3 (3)	-14 (5) -7 (3)	-5 (4)* -5 (2)*	-1 (5)* -1 (4)*
YRVTOISOATAEN/PVEQ/VE	3 2	319 343	339		-4 (3)* -21 (3)	-6 (2) -15 (2)	-1 (3)* -1 (2)*	-2 (4)* -2 (2)*
ILLINGLAN ILLINGLANN ILLINGLANN	2 2 2	343 343 343	353 354 355		-21 (3) -29 (3) -29 (3)	-16 (2) -23 (3) -23 (3)	-1 (2)* 0 (3)* -1 (2)*	-3 (2)* -4 (3)* -4 (2)*
ILTPOLVINI, KOTPSL ILTPOLVINI, KKOTPSL	3	343 343	363	motif Ic	-28 (5) -28 (5) -28 (4)	-22 (3) -24 (4) -23 (4)	-1 (2)* -3 (4)* -3 (4)*	-1 (2)* -2 (4)* -2 (4)*
ILTPOLVNNLKKGTIPSLSF LMF0ECENTSK0HPYNM	3	343 368	366 385	motifilia	-24 (4) -11 (2)	-23 (5) -8 (2)	-3 (3)* 0 (1)*	3 (4)* -1 (1)*
PDECHNTSKOHPYIMM FNYLDOKLGOSSGRLPOVIG Fund Dami openogi oglugi	2 2	370	387 407		-10 (2) -5 (4)'	-8 (2) -7 (4)	0 (1)* -1 (2)*	-1 (2)* 0 (3)*
DQKLGGSSGPLPQVIGL DQKLGGSSGPLPQVIGL LTASVCVDDAKITDEAL	2 2 2	392 408	100 408 424		-4 (4)* -5 (3)* -22 (6)	-0 (3) -7 (4) -19 (5)	-1 (2)* 0 (2)* -3 (5)*	0 (2)* 1 (3)* -1 (5)*
TASVGVGDAKNTDEAL TASVGVGDAKNTDEALD	2 2	409 409	424 425		-20 (5) -19 (5)	-19 (4) -17 (3)	-2 (5)* -2 (4)*	-1 (5)* -1 (4)*
TASVOVODAKNTDEALDYICKI, DYICKI,	3	909 425	430		-20 (2) -2 (3)*	-21 (3) -3 (3)*	-2 (2)* 1 (0)*	0 (3)* 2 (1)*
CASLDASVIATVKINLEE CASLDASVIATVKINLEEL SVIATVKINLEE	2 2 2	431 437	419 448		-4 (3)* -7 (3) -8 (6)	-3 (2)* -6 (3) -7 (5)	-1 (2)* -5 (2)* -2 (4)*	0 (3)* -4 (3)* 0 (3)*
EQV/YKPOKF VVYKPOKF	3	450 452	459 459		-15 (7) -18 (8)	-11 (6) -13 (8)	-8 (6)* -4 (6)*	0 (7)* 0 (5)*
FRKVESRISOKFKYIAQL	3	460	478		-1 (2)* 0 (4)*	-3 (2)* -1 (1)*	0 (1)* -2 (4)*	0 (3)* 0 (1)*
AKPICKDLENL SOUMEFERTO	3	486 497	-105 -196 507	HEL2i	-7 (5) -4 (5)*	-6 (4) -4 (4)*	-1 (5)* 9 (3)*	0 (5)*
SQIONREPGTOKYEQ SQIONREPGTOKYEQW	3	497 497	511 512		-9 (3) -3 (3)	-12 (4) -11 (4)	-1 (3)* -1 (2)*	-1 (5)* 2 (5)*
WINTVOKACM INTVOKACM	2	512	521 521		-3 (1)* -3 (2)*	-3 (1)* -3 (2)*	0 (1)* 0 (0)*	2 (2)* 0 (1)*
VFONPORDEESRICKALF FONPORDEESRICKALF	4	522 523	539 537	CTD:HEL2i	-28 (3) -21 (3) -25 (3)	-21 (3) -17 (3) -21 (3)	0 (3)* 0 (2)* -1 (3)*	-2 (3)" -2 (3)" -2 (3)"
FOMPORDEESRICKALF	4	523 540	539 552		-18 (3) 0 (2)*	-15 (3) -1 (2)*	0 (2) <sup>4</sup> 9 (1) <sup>4</sup>	-1 (3)* 0 (1)*
YTSHLRKYNDAL ISEHARMKDALD	2 3	541 553	552 565		1 (3)* -2 (2)*	1 (3)* -3 (1)*	2 (1)* -1 (2)*	0 (3)* 0 (2)*
HARNKDALD VI KOFSNY	3	557	565		-2 (2)* 12 (2)	-4 (2)* -4 (2)* -8 (2)	-4 (5)* -1 (2)* -2 (2)*	-3 (4)* -1 (2)* 0 (3)*
YLKDIFSNVRAAGI FFSNV	3 1	566 570	579 574	Gate motif	19 (4) 28 (6)	14 (4) 23 (4)	0 (5)* -4 (5)*	2 (3)* 3 (3)*
FFSNVRAAGF FFSNVRAAGFD	2	570	579		1 (2)* 20 (4)	-1 (2)* 14 (5)	1 (2)* -4 (3)*	1 (2)* 2 (4)*
DEECOLTORFEENLOE DEECOLTORFEENLOE	3	571 NBD 580	509 507		0 (3)* -1 (2)*	-1 (2)*	0 (3)*	-5 (3)* 0 (2)* -1 (2)*
DEEGDLTORFEEKL	3	580 591	509 504		-1 (3)* 2 (3)*	-2 (2)* -2 (3)*	-2 (3)*	-1 (2)* 1 (3)*
GELESVSRDPSNENRLED E SVISROPSNENRLEDLC	3	595 598	613 615		-4 (4) <sup>1</sup> -3 (3) <sup>1</sup>	-6 (3) -8 (2)	-3 (4)* 0 (3)*	0 (3)* 1 (3)*
VSRUPSREINKLED FLOBEYKLINPET	2	600	613		-4 (0)* -4 (4)* -5 (2)	-0 (*) -8 (3) -4 (2)*	-2 (5)* -2 (2)*	0 (5)* 0 (4)* 0 (3)*
IL GEEVHLINPETITIL EEVHLINPETITILEVIKTR	2 Z	617 520	632 637	HEL2	-4 (2)* -4 (4)*	-6 (4)	-2 (2)* -2 (2)*	0 (2)* -1 (3)*
FV/RTRALVDAL/RIV/EGNPKL FV/RTRALVDAL/RIV/EGNPKLSF EV/RTRALVDAL/RIV/EGNPKLSF	3	633	653		-5 (2)* -5 (2)	-5 (2)* -8 (3)	-1 (1)* -2 (1)*	-1 (2)* 0 (3)*
WEGNPRL WEGNPRLSFLKPGIL	2	646 646	653	motif IV	-5 (5)* -5 (3)*	-1 (4)* -7 (4)	-3 (2)* -2 (4)* -3 (3)*	-5 (2) 0 (4)* 1 (3)*
SFLKPGIL KPGILTGROKTNONTG	2 3	654 657	661 672		-11 (4) -13 (2)	-11 (3) -6 (2)	-1 (3)* 0 (2)*	-1 (3)* 0 (3)*
TORGETNONTOWTLPACKOL PACKOL	3	662 676	682 682		-4 (4)* -5 (4)	-7 (4) -13 (4)	-1 (4)* -3 (3)*	0 (5)* -2 (5)*
FKASGOHNE ATSVAFCD	3	685	694 705		-2 (1)* -1 (2)* +15 (4)	-+ (2)* -1 (3)*	1 (2)* -5 (4)*	0 (5)* 3 (4)* -2 /81#
CNLVLYEYVORV VLYEYVORVKM	4 2	709 712	721		-2 (1)* -24 (4)	-3 (1)* -24 (5)	-1 (1)* -5 (2)*	0 (2)*
YEYVGIVIKI	2	715	724		-30 (7) -27 (5)	-25 (4) -23 (5)	-3 (6)* -4 (3)*	-3 (5)* -4 (6)*
FLLTSNAGVE FLLTSNAGVE FLLTSNAGVERSONM	2	725 739 739	738 749 755	Pincer	-18 (3) -13 (3) _8 (3)	-16 (3) -9 (4) _8 (3)	0 (3)* 3 (1)* -3 (1)*	-2 (3)* 4 (3)* -3 (2)*
LTSNAGVEREQNM	2 2	741 745	755		-11 (5) -9 (6)	-10 (5) -10 (5)	-2 (3)* -1 (6)*	-1 (3)* 1 (5)*
YKEKMINDSIL YKEKMINDSILRI	3	756 756	766 768		4 (3)* -2 (4)*	0 (3)* -1 (4)*	-4 (3)* -3 (2)*	-5 (3)* 0 (6)*
NDEAVE FRENCHIGTNEN	1	264 771 776	770 776 797		-2 (3)* -3 (2)* -4 (4)*	-2 (2)* -3 (3)* -4 (4)*	-1 (2)* -1 (2)* 1 (1)*	0 (2)* 0 (2)* 0 (1)*
REKLHKOTHEKF THEKFIRDS	3 2	777 785	799		-4 (4)* 11 (3)	-4 (4)* 8 (3)	0 (1)* +2 (3)*	0 (1)* 1 (3)*
RDSGEKPKPVPDK VRVEE	2 2	791 823	803		-9 (8) -3 (2)*	-6 (8) -3 (2)*	-4 (5)* -2 (3)*	1 (7)* 1 (3)*
VRVELOTY VRVEECHYTVLGDA CHYTVLGDA	3	823 823 879	831 837 837	CTD	1 (2)* -5 (1)* -3 (3)*	-3 (2)* -4 (3)* -4 (7)*	1 (3)* -3 (1)* 0 (3)*	-1 (2)* 0 (3)* 0 (3)*
TVLGDAFKEOF FRECF	2	832	842		-9 (5) -10 (8)	-10 (4) -0 (8)	-3 (3)* -1 (2)*	0 (3)* -1 (2)*
FVSRPHPKPKQFSS VSRPHPKPKQFSSF SVBARE	4	842	855	Capping loop	-23 (3) -22 (2)	-10 (4) -10 (3)	0 (3)* 0 (2)*	-1 (5)* -2 (8)*
ERRAKEC GARONGSHOWSHVXVKT	2 4	857 857	863 864 881		-16 (4) -14 (5) -5 (7)	-17 (4) -18 (5) -5 (2)	1 (3)* -1 (4)* 0 (**	0 (2)* -1 (4)* 0 (2)*
DWGHVKVKTHEPVIKESHVV FEIPVIKES	4	872	894 891		-3 (3) -4 (4)*	-10 (5) -5 (4)*	-4 (3)* 0 (1)*	0 (6)* 0 (1)*
FEIPVIKESF	2	882 883	892 892		-4 (3)* -4 (4)*	•3 (3)* •5 (4)*	0 (2)* 0 (1)*	+2 (3)" 0 (1)*
VVEDIATGVOTL MTGVOTL	2 2 2	892 893 897	904 904 914	RNA binding	-19 (6) -34 (6) -26 (8)	-19 (5) -31 (5) -28 (6)	0 (6)* -4 (4)* -1 /8**	-4 (3)* 2 (5)* -1 (5)*
YSKWKDFHFEKIPFDPAE EKIPFDPAENISK	3	905	922 925	- eBioli	-11 (6) -5 (3)	-Q (6) -8 (3)	1 (6) <sup>4</sup> -5 (4) <sup>4</sup>	3 (5) <sup>4</sup> 0 (3) <sup>4</sup>

Supplementary Figure S5. Please see next page for caption

**Supplementary Figure S5.** Differential HDX view of WT RIG-I and indicated complexes upon binding to RNA and K63-polyUb<sub>9</sub> chain. The values listed under each HDX experiments demonstrate the averaged difference in percentage of deuterium incorporation of that corresponding peptide derived from two different states across all exchange time points (i.e., 0s, 10s, 60s, 300s, 900s). When comparing the two samples, the perturbation %D is determined by calculating the difference between the two samples. HDX Workbench colors each peptide according to the smooth color gradient HDX perturbation key (D%) shown in each indicated figure. Differences in %D between -5% to 5% are considered nonsignificant and are colored gray according to the HDX perturbation key. A negative value represents decreased deuterium incorporation or stabilization while a positive value represents increased deuterium incorporation or destabilization in the corresponding region of the receptor when a binding event takes place. Blank region represents undetected peptide for corresponding experiment.

peptide sequence	charge	start	end	domain	(v)	(vi)	(vii)	(viii)
FODVIRKTLDPTYILS	3	12	27		17 (3)	13 (3)	-14 (3) -24 (2)	-11 (2)
RKTLDPTYIL SYMAPWERE	3	15 27	26 35		25 (3) 26 (4)	21 (3) 22 (4)	-19 (2) -20 (2)	-16 (3) -17 (3)
YWAPWFREEEVQ VQYIQAEKNNKGPI/EAATL	2 3	28 38	39 56	CARD1	27 (2) 26 (4)	22 (2) 20 (3)	-20 (3) -31 (5)	-16 (3) -25 (3)
EKINIKOPMEAATL QEEGWFRGFL	2	44 65	56 74 76		18 (1) 14 (2)	12 (2) 14 (2)	-17 (1) -20 (4)	-13 (2) -15 (3)
DALDHAGYSGL LDHAGYSGL	2 2 2	75	85		18 (2) 11 (2)	18 (2) 14 (2) 9 (3)	-17 (2) -15 (2) -13 (2)	-13 (3) -13 (2) -8 (3)
VEAIESWD	1 3	85 94	93 102		31 (3) 12 (2)	25 (3) 12 (2)	-20 (3) -13 (3)	-15 (2) -12 (3)
YRLLL YRLLLKRLQPEF	2 3	103 103	107		31 (2) 34 (2)	26 (3) 28 (2)	-18 (3) -18 (2)	-14 (4) -13 (2)
LL KRL OPEF KRL OPEF	2	106 108	114	Latch region	39 (4) 40 (2)	33 (4) 34 (1)	-72 (3) -29 (3)	-17 (2) -23 (3)
KTRIPTDISDL KTRIPTDISDL KTRIPTDISDL SE	3 Z 3	115	126		16 (2) 22 (2) 7 (3)	18 (2) 19 (2) 2 (3)4	-13 (3) -14 (3) -12 (4)	-10 (2) -11 (2) -5 (3)
LOICSTROMMAGA	2	139 139	152	CARD2	39 (3) 31 (1)	40 (3) 23 (2)	-10 (3) -26 (1)	-9 (3) -18 (2)
RSDKENWPKTLKL RSDKENWPKTLKLAL	3	161 161	173 175		19 (4) 31 (2)	14 (3) 25 (2)	-17 (2) -14 (2)	-11 (2) -7 (2)
ALEKERNKPSEL	4	174	185		15 (2) 13 (2)	14 (2) 12 (2)	-5 (3) -5 (2)	-5 (2)* -5 (2)*
WVEKGKOVE WVEKGKOVETED	3	185	196		1 (3)*	3 (4)*	2 (2)*	-5 (2)* -1 (4)* 0 (3)*
MEKGKOVETEDL EDKWETSD	3	187 201	200 208	linker	0 (3)* -3 (4)*	1 (3)* -1 (5)*	1 (2)* 4 (2)*	-1 (3)* 4 (4)*
EDKWETSDIGF SENSCPPSEVSDTNL	2	201 223	212 237		3 (4)* -3 (8)*	4 (5)* 1 (5)*	3 (2)* 3 (4)*	1 (4)* -2 (3)*
VSDTNL VSPEKPRNYOLE	2	232	237 249		2 (4)* 3 (2)*	3 (4)* 5 (3)*	0 (2)* -1 (2)*	-1 (3)* -4 (4)*
LALPANKGKNTICAPTGCGKTFVSLL LICEUULKKEROGOKGKOVE	4	250	276	HEL1	-2 (1)* 0 (1)*	4 (d)* -1 (1)* 0 (1)*	-1 (2)* 2 (0)* -2 (2)*	-4 (4)* -1 (1)* -3 (3)*
ICEHILIOK/POGOKOKVV/ FANO/PV/EQQ	3	277	295 306		0 (1)*	-1 (1)* -14 (3)	-3 (1)* -2 (1)*	-4 (1)* -1 (4)*
FANGPVYEQCKSVF FANGPVYEQCKSVFSKY	2	296 296	310 313	motif la	-21 (2) -8 (3)	-14 (2) -4 (4)*	0 (2)* -3 (3)*	0 (3) <sup>x</sup> -3 (3) <sup>*</sup>
FERHGYRVTGISGATAENVPVEQIVENND YRVTGISGATAENVPVEQIVE	4	314 319	342 339		-2 (5)* 1 (3)*	-1 (5)* 2 (4)*	-2 (3)* 3 (2)*	-2 (3)* 1 (3)*
ILTPOL ILTPOL	1 2	343 343	351	motifia	-20 (1) -16 (1)	-14 (2) -12 (2)	-2 (2)* -1 (2)*	-4 (3)* -3 (2)*
ILTPOLVNI ILTPOLVNINLKKGTIPSL II TROLVINI KKGTIPSI SI	3	343	363	mounic	-18 (3)	-12 (4)	-3 (2)*	-5 (3)* 2 (3)*
LMIFDECHNTSKOHPYNM IFDECHNTSKOHPYNMM	3	368 370	385 387	motif Ila	-8 (1) -6 (1)	-5 (1) -5 (1)	0 (1)* 0 (1)*	0 (2)*
FNYLDOKLOGSSGPLPOVIG FNYLDOKLOGSSGPLPOVIGL	2	388 388	407 408		0 (2)* -1 (1)*	1 (3)* -1 (2)*	-1 (2)* -1 (1)*	2 (2)* -1 (2)*
DOKLOGSSOPLPOVIOL LTASVGVGDAKNTDEAL	2	392 408	408		-1 (1)* -8 (3)	-1 (2)* -8 (3)	1 (1)* -1 (4)*	0 (2)* -4 (4)*
TASVGVGDAKNTDEAL TASVGVGDAKNTDEALD TASVGVGDAKNTDEALD	2	100	425		-11 (3) -7 (2)	-6 (3) -6 (2)	0 (2)*	-3 (4)* -3 (3)* 2 (3)*
DYICKL	1	425	430		-2 (1) <sup>4</sup> -2 (1) <sup>4</sup>	-3 (1)*	0(1)*	2 (6) <sup>4</sup>
SVIATVKHNLEE EQVVYKPQKF	2 3	437 450	448 459		-1 (2)* 0 (3)*	1 (3)* 3 (1)*	-2 (1)* -4 (3)*	-4 (4) <sup>x</sup> -4 (2) <sup>x</sup>
VVYKPOKF VVYKPOKF	2 3	452 452	459		-1 (4)* -3 (3)*	3 (5)* 2 (4)*	-4 (3)* -3 (3)*	-4 (5)* -4 (4)*
FREVESRISDINFRYINGL QUMRDTESL	2	460 477 470	4/8	HEL2i	0 (1) <sup>4</sup> 1 (2) <sup>4</sup>	-1 (1)* 2 (1)*	-2 (1) <sup>4</sup> 0 (2) <sup>4</sup>	-3 (1) <sup>1</sup> -2 (2) <sup>1</sup>
AKRICKOLENL SQIQNREFGTOKYVQ	3	405	496		-2 (2)* -5 (2) -10 (4)	-3 (2)* -3 (2)* -6 (5)	2 (3)* 1 (2)* 4 (2)*	-1 (2)* -1 (5)*
SQIQNREFIGTORY/VQW IVT/QKACM	3	497 513	512 521		-9 (2) -9 (1)	-6 (3) -9 (1)	1 (1)* 3 (1)*	-1 (3)* 0 (1)*
VFOMPDKDEESRICKA VFOMPDKDEESRICKALF	3	522 522	537 539	CTD:HEL2i	-18 (1) -20 (2)	-15 (2) -17 (2)	3 (3)* 1 (1)*	0 (2)* -2 (2)*
FOMPOKDEESRICKA FOMPOKDEESRICKALF	1	523	537		-24 (4) -17 (2)	-23 (5) -13 (3)	-1 (2)* 2 (1)*	-2 (4)* -2 (3)*
LYTSHLRKYNDAL	4	540	552 552		-2 (1)* 0 (1)*	-2 (1)*	1(1)*	1 (1)*
ISEHARIIKDALD HARMKDALD	3 2	553 557	565 565		-1 (1) <sup>4</sup> -3 (1) <sup>4</sup>	1 (1)* -2 (1)*	2 (2) <sup>4</sup> 3 (1) <sup>4</sup>	0 (1)* 0 (1)*
HARMKDALD YLKDFFSNV	3 2	557 566	565 574		-1 (1)* 8 (1)	-2 (1) <sup>x</sup> 8 (1)	0 (1)* -1 (1)*	0 (1) <sup>x</sup> 0 (1) <sup>s</sup>
YLKDFFSNVRAAGF FFSNV	1	570	579	Gate motif	14 (3) 18 (3)	18 (3) 17 (3)	3 (4)* 1 (2)*	-4 (3)* 0 (2)*
FFSNVRAAGPD FSNVRAAGP	2	570	580 579	oute moti	8 (5) 11 (2)	10 (5) 13 (3)	3 (4)* 4 (2)*	2 (4)*
DEEQDLTORFEEKLQE	3	580 580	596 597		1 (1)* 2 (1)*	0 (1)* 2 (2)*	1 (1)* 0 (1)*	1 (2)* -2 (2)*
DEEQDLTGRFEEKLGELES EIEQDLTGRFEEKL	3	580 581	599 504		2 (1)* 0 (2)*	2 (2)* -2 (3)*	0 (2)* 1 (2)*	-2 (2)* -4 (3)*
ESVSRDPSNENPKLEDLC VSRDPSNENPKL	3	598 600	615 611		0 (2)* 0 (2)*	-1 (2)* 0 (4)*	1 (1)* 4 (3)*	0 (3)* -3 (5)*
VSHOPSNENPKLED FILGEEVHLINPET	2	616	613	HEL2	0 (2)* -2 (1)*	-4 (3)* -1 (2)*	5 (4)* -1 (2)*	-2 (3)* -1 (2)*
EEVHLNPETITLEVKTR	2 2	620 633	637 642		-1 (1)* 0 (1)*	-1 (2)*	-1 (1)*	-2 (2)* -4 (2)*
FVKTRALVDALKNIVEGNPKL FVKTRALVDALKNIVEGNPKLSF	3	633 633	653 655		-2 (1)* 0 (2)*	-1 (2)* 0 (2)*	0 (1) <sup>4</sup> -3 (1) <sup>4</sup>	-2 (2)* -3 (2)*
WEGNPRI, SFLKPGIL	2	646 654	653 661	motif IV	1 (1) <sup>x</sup> -2 (2)*	1 (2)× -6 (2)	1 (2) <sup>4</sup> 1 (2) <sup>4</sup>	0 (2)* -5 (1)*
KHGELIGRGKTNONTG TGRGKTNONTGHTLPAQKCE DARKASCOHNI	3 3	657 662 689	672		-9 (2) 1 (3)*	-1 (2)* 2 (4)*	1 (1)* 3 (2)*	0 (2)* 0 (3) <sup>4</sup>
PKASGDHNL IATSVADEGD	3	685	694 705		0 (1)* -3 (2)*	0 (1)* -3 (3)*	2 (1)* 4 (2)*	1 (1)* -3 (3)*
CREVEYEYVGNV	4	709 712	721 724		-2 (1)* 2 (2)*	-1 (1)* 4 (5)*	1 (1)* 0 (2)*	1 (1)* -4 (8)*
YEYVGNVKII YVGNVKII	2	715	724 724		-4 (3)* 0 (4)*	-4 (3)* -3 (4)*	-1 (4)* -1 (3)*	-4 (2)* -5 (5)*
RETRIGRIGHARGISKC FLETSNAGVE	2	725 739 739	7.38 749 200	Pincer	-8 (2) -9 (2) 2 (2)	-5 (2) -8 (2)	1 (2)* 1 (2)*	0 (2)* 1 (3)*
LTSNAGVEREONM VKERMINDSL	2	741 756	755	i meer	-2 (3)* -5 (2)* 2 (2)*	-1 (a)* -4 (2)* 1 (2)*	0 (3)*	-2 (3)* -3 (3)* -2 (2)*
YKEKMINDSIL SURLOT	3 2	755 764	766 770		3 (2)* -1 (3)*	1 (3)* 0 (4)*	-1 (2)* 0 (3)*	-2 (3)* 0 (3)*
RLQTWDEAVF WDEAVF	2	767 771	776		-2 (1)* -1 (1)*	0 (1) <sup>4</sup> 0 (2)*	2 (2) <sup>4</sup> -1 (1) <sup>4</sup>	0 (2) <sup>4</sup> -2 (3) <sup>7</sup>
FREKLHIQTHEKF	4	776	789		-3 (1)* -2 (1)*	-2 (2)* -3 (1)*	-1 (2)* -3 (1)*	0 (2)* -1 (1)*
EKFROSQEK VRVIEE	2 2 2	785 787 823	793 756 818		9 (1) 10 (2) _2 (1)	8 (1) 10 (2) -3 (1)	-1 (1) <sup>4</sup> -2 (2) <sup>4</sup> 4 (1) <sup>4</sup>	-1 (1) <sup>4</sup> -3 (3) <sup>4</sup> 3 (1) <sup>4</sup>
VRVIEECHY VRVIEECHYTVLGDA	3	823 823	831 837	CTD	-9 (2) -2 (1)*	-4 (1)* 0 (2)*	2 (1)* 0 (2)*	4 (1)* -2 (1)*
CHYTVLGDA TVLGDAFKECF	2 2	829 832	837 842		1 (1)* -2 (3)*	2 (2)* -2 (3)*	0 (1)* 0 (3)*	-2 (2)* -2 (3)*
FKECF FKECF	1	838 838	842 842		-3 (4)* -8 (2)	-3 (4)* -7 (3)	0 (2)* 2 (1)*	1 (3)* 1 (4)*
VSRPHPKPKQFSS VSRPHPKPKQFSSF VSRPHPKPKQFSSF	4	842 843	855	Capping loop	-17 (3) -18 (2)	-2 (3)* -4 (2)*	4 (2)* 3 (2)*	0 (3)* 0 (3)*
EKRAKIF	2	857 857	856 863 864	· · · · · ·	-18 (2) -12 (1) -46 (2)	-4 (2) -13 (2) -14 (4)	3 (2)* 5 (2)*	1 (2)* 3 (3)*
EKRAKIFC CARONCSHDWGHVKYKT	3	857 364	861		-10 (1) -3 (1)*	-12 (2) -3 (1)*	0 (2)* -3 (1)*	0 (2)* -2 (1)*
FEFVKES FEFVKESF	2	882 882	891 892		-8 (2) -5 (1)	-5 (2) -5 (1)*	1 (1) <sup>4</sup> 3 (1) <sup>4</sup>	1 (0) <sup>4</sup> 0 (0) <sup>x</sup>
EIPVIKESF FVVEDIATGVQTL	2	883 892	892 904	DNA bindir -	-8 (2) -12 (4)	-5 (2) -10 (4)	1 (1)* -1 (2)*	1 (0)* -5 (2)*
VVEDATGVQTL IATGVQTL EKIPEDAE	2 2 2	893 897 014	904 904	region	-21 (2) -26 (3)	-17 (3) -22 (4) 3 (2)	0 (1) <sup>4</sup> 0 (2) <sup>4</sup>	-4 (2)* -4 (3)* -4 (2)*
EKIPPDPAEMSK EKIPPDPAEMSK	2	914 914	925 925		-1 (4)* 0 (3)*	-2 (4)* 1 (3)*	0 (2)* -1 (1)*	0 (3)* -2 (3)*

**Supplementary Figure S6.** Differential HDX view of RIG-I<sub>E510V</sub> and indicated complexes upon binding to RNA and K63-polyUb<sub>9</sub> chain. Data interpretation is similar as **Supplementary Figure S5**.

peptide sequence	charge	start	end	domain	(ix)	(x)	(xi)	(xii)
ODYRKTLOPTVIL SYMAPWFRE	3	13 27	26 35		16 (4) 26 (6)	16 (3) 24 (3)	-29 (5) -37 (8)	-31 (3) -37 (5)
YWAPAFREEVQ VQYDAEKNIKGPIIEAATIL	2 3	28 38	39 56		22 (5) 7 (2)	20 (3) 6 (3)	-35 (5) -30 (3)	-35 (3) -32 (4)
YIQAEKNIKGPIIEA EKNIKGPIIEAATL	2	40 44	53	CARD1	17 (4) 18 (4)	15 (4) 17 (5)	-42 (4) -32 (5)	-32 (3) -29 (6)
CLEEGWINNEL OFEGWINNEL FROT DA	2	65 70	24 76		5 (5) 15 (4) 18 (6)	5 (4) 13 (5) 21 (4)	-20 (4) -26 (7)	-17 (3) -29 (5) -28 (4)
DALDHAGYSGL LDHAGYSGL	2	75 77	85 85		21 (4) 16 (4)	20 (3) 13 (5)	-33 (4) -29 (4)	-34 (3) -26 (5)
YEAESW YEAESWD	1	85 86	92 93		34 (5) 29 (5)	31 (4) 27 (4)	-47 (5) -46 (5)	-44 (4) -44 (4)
YEAESWORKERLEE	3	86 94	102		15 (6) 13 (6)	13 (6) 12 (6)	-44 (5) -36 (5)	-40 (5) -34 (6)
THELENALUPER LUKALOPER	2	105	114	Latch region	24 (5) 35 (8) 27 (8)	29 (4) 37 (4) 28 (8)	-30 (4) -43 (5) -19 (5)	-23 (4) -40 (5)
KTUPEF KTUPTDISD	2	108 115	114		39 (6) 18 (5)	35 (6) 15 (4)	-39 (5) -25 (5)	-10 (5) -22 (4)
KTRIPTDISOL KTRIPTDISOL	2 3	115	127 127		18 (5) 21 (5)	15 (4) 19 (5)	-24 (6) -28 (6)	-22 (5) -26 (6)
KTRIPTDIBOLSE EDLOI: STKOMAGAEKL	3	115	129	CARD2	11 (5) 18 (4)	7 (4) 18 (3)	-20 (5) -34 (4)	-14 (4) -34 (2)
L GLOST ROMMAGAENLAVE LRSDKENWERTIKLAL BSDKENWERTIKL	2	160	175		20 (6)	21 (3) 28 (3) 25 (4)	-32 (b) -32 (f)	-23 (3) -32 (3) -28 (4)
RSDRENWWRTHCAL ALEKERKFSEL	4	161 174	175		30 (6) 20 (4)	28 (5) 18 (4)	-36 (5) -18 (5)	-34 (5) -16 (5)
EKERNKFSEL WIVEKOKOVE	3	176	185		18 (4) D (4)*	18 (3) -3 (4)*	-16 (4) -2 (6)*	-16 (4) 1 (4)*
W/VEKGKD/ETEDLED	2	186 186	200 202 209		-2 (6)* 2 (4)*	-4 (4)* 1 (3)*	0 (6)* 3 (5)*	0 (3)* 2 (4)*
VERSIKOVETED. EDKNEVETED.	3	187	200		-2 (2)* -2 (5)* -4 (7)*	-2 (4)* -3 (3)* -2 (3)*	0 (G)* 4 (Z)*	-4 (4)* -1 (3)*
EDWAETSDIGIF	2	201 209	212 224	l linker	4 (3)* 4 (4)*	3 (3)* 2 (4)*	0 (5)*	0 (3)* -5 (4)*
FYGEDRECONL YGEDRECONL	2	212 213	222		0 (4)* -2 (4)*	-3 (2)* -3 (2)*	-1 (6)* 0 (5)*	0 (2)* -1 (2)*
SENSCIPSEVSDTNL NSCIPSEVSDTNL	2	225	237		-2 (4)*	-4 (3)* -4 (3)*	-1 (5)* 2 (5)*	0 (2)* 2 (2)*
YSPHRANYOLE YSPHRANYOLE	2	238	249 249		-1 (4)*	-3 (3)* -5 (3)* -4 (2)*	-1 (5)* 0 (5)* -1 (5)*	0 (4)*
LALPANKORITEC LALPANKORITECAPTOCORTEVELL	2 4	250 250	253 276	HEL1	-2 (1)* -2 (3)*	-3 (2)* -5 (3)*	0 (1)* 0 (2)*	1 (2)* 3 (2)*
LICEHHLIKKFPQGQIKQKVVF ICEHHLIKKFPQGQIKQKVVF	3	276 277	295 295		-1 (1)* 0 (3)*	-2 (2)* -1 (3)*	-1 (2)* -1 (5)*	0 (2)* 1 (5)*
FANGPVYEQDKSVF FANGPVYEQDKSVFSKY	2	296	310	motif la	-25 (3) -23 (3)	-23 (2) -22 (3)	-2 (3)* -4 (2)*	-4 (3)* -3 (3)*
GYNYTOBGATAENVPVEDVEN YRYTGISGATAENVPVEDVE III TEOI	3	319 343	339		-5 (2)* -2 (4)* -27 (2)	-4 (4)* -5 (3) -25 (2)	-5 (4)* 0 (5)* -2 (2)*	-5 (4)* -1 (2)* -2 (3)*
ILTFOL ILTFOL	2	343 343	351 353		-21 (1) -22 (1)	-20 (2) -20 (2)	-1 (1)* -1 (1)*	-2 (2)* -2 (2)*
ILTFOLVIN ILTFOLVINL	2	943 343	354 355	motif Ic	-31 (2) -23 (3)	-29 (2) -24 (3)	-2 (2)* 3 (2)*	-4 (2)* 0 (3)*
ILTFOLVINLOGTPSL ILTFOLVINLOGTPSL	2	343	363		-26 (4) -28 (3)	-25 (3) -25 (4)	-2 (3)* -2 (4)*	-3 (2)* -3 (5)*
IL FRALVINIENA PSISI ENFOECHITSKOHPYNM FEDERATSKOHPYNM	3	368 370	385 387	motif IIa	-10 (1) -10 (1)	-18 (4) -9 (1)	-5 (3)* -1 (1)* -1 (1)*	-4 (4)* 0 (1)* 0 (1)*
PDECHNTSKGHP/NMM FNYLDORLOGSSGPLPQV/G	3	370	387 407		-10 (1) -2 (2)*	-9(1) -4(2)*	-1 (1)* 0 (2)*	0 (1)* 0 (2)*
FNYLDOXLCOSSGFLPQVIGL DQKLGGSSGFLPQVIGL	2	388 392	408 408		-2 (1)* -2 (2)*	-3 (1)* -3 (2)*	-1 (2) <sup>x</sup> -1 (2) <sup>x</sup>	0 (1)* 0 (2)*
LTASVGVGDAXNTDEAL TASVGVGDAXNTDEAL	2	408	424		-19 (4) -10 (4)	-18 (5) -19 (5)	-1 (6)* -1 (6)*	-2 (8)* -1 (6)*
TASVOVODANITOEALDVICKL DVICKL	3	409	430		- 16 (3) 1 (2)* 0 (1)*	-16 (+) 0 (2)* -2 (1)*	-1 (5)* -3 (2)* -1 (1)*	-1 (5)* -4 (2)* 1 (1)*
DYICKL GASLDASVATVINILEE	2	425 431	430		0 (1)* -3 (2)*	-1 (1)* -2 (2)*	0 (1)* 0 (3)*	0 (1)* -3 (2)*
SVMTVKINLEE EQVVYKPOKF	2	450	918 459		.8 (4) -12 (6)	-6 (3) -9 (4)	3 (5)* 1 (7)*	1 (4)* -3 (6)*
VVVKPQKP FRKVESRSDKFKVBAQL	2	452	459 470		-16 (4) 1 (2)*	-11 (4) -1 (2)*	3 (4)* -1 (3)*	-3 (8)* -1 (2)*
UDARUTES. MROTES. AKRECKI FN	2	79	485	HEL2i	0 (1)* 0 (1)* -7 (4)	0 (1)* 0 (1)* -7 (4)	-1 (1)*	0 (3)* 0 (1)* -1 (4)*
SCIONREFGTO SCIONREFGTOKYED	2	497 497	507 511		-7 (3) -13 (3)	-7 (2) -15 (3)	-1 (4)* -1 (4)*	-4 (3)* -1 (3)*
SQIQNREFCTQKYEQW	2	497 512	512 521		-12 (3) -17 (3)	-14 (2) -17 (3)	-1 (4)* -1 (1)*	0 (2)* 0 (1)*
I/TVHKACMV I/TVHKACMV	2	513 513 522	521 522 537		-13 (4) -10 (4) 95 (2)	-13 (4) -23 (4) 27 (2)	0 (1)* -4 (1)*	0 (1)* 1 (1)*
VFGMPDRCEESRCKALF FOMPDRCEESRCKA	4	522 523	539	CTD:HEL2i	-32 (3) -32 (3)	-34 (3) -35 (3)	-1 (3)*	-1 (3)* -2 (3)*
FOMPDROEESRICKALF LYTSHLRKYNDAL	4	523 540	539 552		-27 (3) 0 (1)*	-23 (3) -1 (1)*	-1 (2)* 0 (1)*	-1 (2)* 0 (1)*
LVTSHLRKVNDAL YTSHLRKVNDAL	2	540 541	552 552		0 (1)* 0 (1)*	-1 (1)* -1 (2)*	-1 (1)' -2 (2)*	0 (1)* 0 (2)*
	2	557 557	565 565		-1 (2)* -2 (2)* -3 (2)*	-3 (2)* -4 (2)* -4 (3)*	-1 (2)* -1 (3)* 1 (2)*	-1 (3)*
YLKOFFSNV YLKOFFSNVRAAGF	2	565	\$74 579		2 (3)* 1 (5)*	3 (2)* 4 (4)*	-1 (4)* 3 (5)*	-2 (2)* 4 (4)*
FPSNV FPSNVRAAGF	1 2	570 570	574 579	Gate motif	5 (3) 6 (5)	9 (4) 6 (2)	2 (3)* -2 (7)*	-4 (4)* -2 (3)*
PPSN/RAAGPD DEEODLTGRFEEKLOE	2	570 580	500 596		8 (3) -4 (2)*	6 (4) -5 (3)*	4 (3)* 0 (2)*	4 (4)* 0 (2)*
DEBODLTORFEEKLOEL DEBODLTORFEEKLOELES FEODLTORFEEKLOELES	3	580 580	597 599 514		-3 (3)* -3 (3)* -4 (4)*	-4 (2)* -5 (3)*	3 (3)* 0 (3)* -2 (4)*	1 (2)* 0 (3)* 2 (3)*
GELESVSRDPSNENPKLED ESVSRDPSNENPKLEDLC	3	595 598	613		-2 (4)* 0 (2)*	-5 (2)* -4 (3)*	-1 (5)* -1 (4)*	-3 (3)* -1 (4)*
VSRDPSNENPKLED	2	600	611 613		-2 (3)* -2 (3)*	-5 (3) -5 (1)*	0 (7)* -1 (5)*	-3 (4)* -3 (3)*
FLOREYHLNPET LOREYHLNPETTL	2	616 617 620	628 632	HEL2	-11 (2) -3 (1)*	-11 (3) -3 (2)*	-1 (2)* -1 (2)*	-1 (3)* 0 (2)*
FVIKTRALNDA FVIKTRALNDALKVIMEGNIPKL	2	635 633	612		-4 (5) <sup>4</sup> -5 (1) <sup>4</sup>	-6 (2) -5 (1)*	-1 (3) <sup>1</sup> -1 (2) <sup>4</sup>	-2 (2)/ -1 (1)*
FVKTRALVDALKVMEGNFKLSF WEGNFKL	3 2	633 646	655 653		-5 (2)* -1 (3)*	-5 (1)* -2 (4)*	-2 (2)* -1 (5)*	-5 (2)* -1 (4)*
WEONINLSPLKPGE SFLKFGE	2	646	661 661	motif IV	-7 (4) -12 (3)	-10 (4) -17 (3)	2 (4)* 0 (4)*	1 (4)* -1 (4)*
KPGLIGHERINDERS TGROKTNGETGETLPAGKOL PAGKOL	3	662 676	672 682 682		-11 (3) -2 (4)* -5 (4)	-5 (2) -5 (2) -13 (3)	-2 (3)* 0 (5)* -1 (6)*	-1 (2)* -1 (2)* -2 (4)*
DAFKASGOHNL DAFKASGOHNL	2 3	683 683	694 694		-2 (2)* -2 (2)*	-4 (2)* -5 (1)*	0 (2)* 0 (3)*	0 (2)* -1 (1)*
INTEVADEDD CNLVLYEYVGNV	2 4	695 209	705 721		-19 (2) -2 (1)*	-19 (2) -3 (1)*	0 (5)* -1 (1)*	-4 (4)* 0 (1)*
VEYEVGNVKN YEVGNVKN	2	712 715 715	721		-14 (6) -28 (5)	-19 (5) -23 (5)	-1 (5)* -1 (7)*	-3 (2)* -3 (6)*
T YURYONI IDTRORGRARDSKC FLTSNAKOVEKEDIM	1	725	738	Pincer	-29 (4) -20 (4) -5 (3)	-20 (5) -19 (3) -15 (3)	3 (5)* 3 (5)* -2 (7)*	-3 (7)* -2 (4)* -1 (2)*
LTSNAGVEREQINN YKEKININDSL	2 2	741 756	755		-8 (3) 1 (2)*	-9 (4) 0 (2)*	-1 (3)* 0 (2)*	-1 (4)* -1 (2)*
SILRLOT WDEAVF	2	764	770 776		-2 (2)* 2 (4)*	-2 (3)* 2 (3)*	© (2)* -1 (4)×	0 (3)* -2 (3)*
FHENLFIGTHERF REKLINGTHERF REGERER/PVPCK	3	776 777 791	789 789 879	$\mid$	-2 (1)* -3 (1)* -3 (4)*	-2(1)* -3(1)* -4.49	0 (1)* -1 (1)* -1 (4)*	0 (1)* 0 (1)* 5 (3)*
VRVEECHY VRVEECHY	23	823 823	603 831 831		-8 (3) -12 (3)		2 (4)* -1 (4)*	-5 (3)* -1 (2)*
VRVEEOHYTVLGDA CHYTVLGDA	3	823 029	837 837	CTD	-6 (3) -1 (2)*	-2 (3)* -2 (2)*	1 (4)* -1 (3)*	0 (4)* 0 (2)*
TVLGDAFREOF FREOF	2	832 838	842 842		-6 (4) -10 (5)	-7 (3) -8 (4)	0 (4)* 0 (5)*	-1 (4)* -3 (4)*
FNSRPHPKRKQFS5 VISIDHDKDKDFS5	4	0.58 242 0.41	842 855	Canning loop	-6 (3) -19 (4)	-5 (3)* -8 (3) -8 (3)	-2 (4) <sup>4</sup> -3 (5) <sup>4</sup> -3 (57	-2 (4) <sup>4</sup> -2 (3) <sup>4</sup> -3 (2) <sup>4</sup>
VSRPHPKPKQFSSF	4	843 857	856 856		-20 (4) -15 (2)	-8 (2) -15 (2)	-3 (5)* -3 (5)* 0 (5)*	-2 (3) <sup>4</sup> -1 (2) <sup>4</sup>
EKRAKIFC EKRAKIFC	2 3	857 857	854 851		-10 (4) -12 (2)	-12 (4) -13 (2)	-4 (6)* 0 (3)*	-2 (5)* -2 (3)*
CARONCSHOWOHVKYKT	4	864 882	80 L 89 1		-4 (1)* -5 (1)*	-3 (1)* -3 (2)*	-1 (2)* 0 (1)*	0 (2)* 0 (1)*
EPVICESE EPVICESE ENABLISTICATE	2	882 883	802 892		-4 (4)* -3 (1)*	-3 (4)* -3 (2)*	-1 (2)* 0 (1)*	-1 (2)* 0 (1)*
VVEDATGVDTL VVEDATGVDTL	1	875	904 904 904	RNA binding	-20 (5) -31 (5) -28 (4)	-27 (5) -30 (6) -28 (3)	-2 (6)* -2 (6)*	-3 (6)* -4 (4)*
EKPFDPAENSK EKPFDPAENSK	2	914 914	925 925	- oBioli	-5 (4) -5 (3)*	-8 (2) -5 (2)	-2 (5)* -2 (5)*	0 (3)* -2 (2)*

**Supplementary Figure S7.** Differential HDX view of RIG-I<sub>Q517H</sub> and indicated complexes upon binding to RNA and K63-polyUb<sub>9</sub> chain. Data interpretation is similar as **Supplementary Figure S5**.

peptide sequence	charge	start	end	domain	(xiii)	(xiv)	(xv)
QOYIRKTLOPTYIL	3	13	26		7 (5)	3 (2)*	0 (5)*
YMAPWFREEEVO	2	28	39		10 (4) 12 (5)	3 (3)* 4 (2)*	2 (5)*
VOYIDAEKINKOPMEAATI. EKNNKOPMEAATI	3	36 44	56		11 (4) 9 (2)	5 (2) 6 (1)	0 (3)* 2 (3)*
QEEGWERGEL	2	65	74	CARD1	5 (3)	1 (2)*	1 (3)*
FRGELDA DALDHAGYSOL	2	70 75	85		6 (3) 8 (4)	3 (2)* 3 (3)*	-4 (4)* 1 (4)*
LDHAGYSGL VEARSWD	2	77	85		9 (5)	2(2)*	3 (5)*
FKKEKLEE	3	94	102		6 (4)	0 (2)*	1 (4)*
VALLIKALOPEF	3	105	114		9 (4) 15 (5)	5 (2) 5 (2)	0 (5)* -1 (4)*
KTRIFTDISD	3	115	126		13 (3)	3 (2)*	3 (5)*
KTRIPTOISOLSE	3	115	129	CARD2	8 (5)	5 (3)	5 (5)*
ILQCSTKGINACAEKLVE RSDKENWPKTLKL	3	139	157	C, III D L	7 (5) 9 (5)	6 (2) 5 (2)	-4 (4)* 3 (5)*
RSDKENWPKTEREAL	1	161	175		8(4)	5 (2)	2 (3)*
EKERNKPSEL WIVENGKDVE	3	186	196		5 (3) 2 (3)'	0 (2)* -1 (2)*	0 (4)* 3 (5)*
WIVERGIRDVETEDL MERGIRDVETEDL	3	186	200		2 (2)*	0 (2)*	3 (1)*
EDKNETSD	2	201	208		1 (4)*	-2 (3)*	2 (3)*
YGEDPECANL	2	213	212		2 (3)* 2 (3)*	-1 (2)* 1 (3)*	1 (3)* 1 (4)*
SENSCRPSEVSUTNL VEDTW	2	223	237		2 (2)*	-4 (3)*	2 (4)* 2 (4)*
YSPFKPRNYQLE	3	238	249		0 (2)*	-2 (3)*	0 (4)*
LALPANKGKITICAPTGCGKTFVSLL LICEHHLKKFPGGQKGKVVF	4	250 276	276 295		2 (4)* 1 (1)*	-1 (1)* 0 (1)*	3 (2)* 0 (2)*
ICENHLIKKEPOGOKOKVVE	3	277	295		2 (3)*	1 (1)*	1 (4)*
FANGEVECKSVF	2	296	310		-0 (4)	-7 (3)	-1 (J) -5 (J)*
FANDEVYEQDRSVESKY	2	296	313 339	motifle	-8 (5) 2 (3)	-4 (2)* -1 (3)*	-2 (3)* 3 (3)*
ILTPOL	2	343	351	mounte	-5 (2)	-4 (2) <sup>x</sup>	-1 (2)*
ILTPOLVN ILTPOLVNLKKGTIPSL	3	343	363		-5 (3) -3 (4)*	-4 (2)* -3 (3)*	-1 (2)* -1 (5)*
ILTPOLVMILKKGTIPSLSI	3	343	365		-2 (4)*	-3 (3)*	0 (5)*
FOECHNTSKOHPYNM FOECHNTSKOHPYNMM	2	370	387		-2 (2)* -2 (1)*	-1 (1)*	0 (1)*
FIVEDOKLOGSSGPLPOVIG EIVEDOKLOGSSGPLPOVIG	2	388	407		2 (2)*	0 (2)*	1 (2)*
DUKLOGSSGPLPUVIGL	2	392	408		3 (2)*	0 (2)*	1 (2)*
LTASVGVGDAKNTDEAL TASVGVGDAKNTDEAL	2 2	408	424 424		-3 (4)* 0 (5)*	-2 (3)* -2 (3)*	-3 (3)* -1 (3)*
TASVGVGDAKNTDEALD	2	409	425		-1 (4)*	-2 (3)*	0 (5)*
DYICKL	1	425	430		2 (0)*	1 (1)*	2 (1)*
SVIATVKHNLEE	2	437	448		0 (4)* -4 (3)*	-2 (3)* -3 (2)*	-2 (4)* -4 (2)*
VVYKPQKF	2	452	459		-4 (5)*	-5 (4)*	-5 (2)*
RKVESRISUKFKYIAQL	2	460	478		2 (2)* 1 (4)*	2 (1)* 0 (2)*	2 (3)* 0 (1)*
MADTESL AVERAGE AVE	2	479	485		0 (1)1	0 (1)*	0 (1)4
SQIANREFQTAKYEQ	3	497	511		3 (3)*	-2 (2)* -4 (2)*	2 (4)*
SOIDNREFGTOKYEOW	2	197 513	512		3 (3)*	-2 (2)* 0 (1)*	2 (3)*
VFOMPOKDEESRICKA	3	522	537		-4 (3)*	-3 (2)*	2 (3)*
VFOMPOKDEESRICKALF FOMPOKDEESRICKA	4	522 523	539		-4 (3)* -4 (3)*	-3 (2)* -1 (3)*	2 (3)* 1 (3)*
FGMPOKDEESPICKALF	4	523 540	539		-3 (2)*	-3 (2) <sup>4</sup>	1 (2)*
YTSHLRKYNDAL	2	541	552		-1 (3)*	1 (1)*	1 (2)*
ISEHARMKDALD HARMKDALD	3	553	565 565		2 (2)* 2 (2)*	-1 (1)* 0 (1)*	2 (2)* 1 (3)*
YLKDFFSNV	2	566	574		3 (2)*	1 (1)*	0 (3)*
FFSNV	1	570	574	Gate motif	6 (5)	-4 (3)* 1 (2)*	-3 (5)* -3 (3)*
FFSNVRAAGFD DEEDDLTORFFERLOF	2	570	580	Gute motif	6 (4) 1 (2)7	-2 (3)*	1 (4)* 1 Cox
DEEQDLTGRFEEKLGEL	3	580	597		1 (2)*	1 (2)*	1 (3)*
DEEQDLTGRFEEKLGELES	3	580	599		2 (2)' 4 (4)'	0 (2)* 2 (3)*	1 (3)* 3 (5)*
GELESVSRDPSNENPKLED	3	595	613		5 (3)*	-5 (2)*	2 (4)*
VSRDPSNENPKL	š	600	611		a (a)* 5 (3)*	0 (4)*	3 (3)*
VSRDPSNENPKLED	2	600 616	613 628		5 (3)* -1 (3)*	4 (2)" B (1)"	3 (3)*
LOEEYHUNPETTTU	2	617	632		1 (1) <sup>1</sup>	0 (1) <sup>z</sup>	0 (S)x
EVELOCITIE VKIR FVKTRALVDALKIWIEGNPKL	2 3	633	653		2 (2) <sup>1</sup> 0 (1) <sup>4</sup>	0 (2)* -1 (1)*	1 (2)' 0 (1)4
PVKTRALVDALKNWEGNPKLSP WEGNPKL	3	633 646	655		1 (2)*	0 (1)*	0 (2)* 1 (5)*
SFLKPGL	2	654	661		1 (2)*	4 (1)*	5 (4)*
DAFKASODHNE	2	683	694		4 (5)* 2 (2)*	-1 (J)* B (1)*	3 (3)* 2 (2)*
IATSVADEGD CNLVILYEYVONV	2	695 709	705		3 (4)*	0 (2)*	1 (2)*
VILYEYVONVIKM	2	712	724		-1 (3)*	-2 (6)*	5 (4)*
YEYVGNVKM	2	715 717	724		-5 (3)* -4 (4)*	0 (3)* 3 (4)*	-2 (3)*
IOTRGRGRARGSKC	4	725	738		-1 (3)*	0 (1)*	-1 (5)*
FLLTSNAGVEKEDINM	2	739	755		0 (2)*	-1 (2)*	1 (2)*
LTENAGVIEKEDINM	2	741 756	755		0 (3)* 5 (3)*	-1 (3)* 1 (2)*	1 (3)*
SLRLQT	2	764	770		0 (3)*	-1 (3)*	0 (2)*
WDEAVF FRENLHIGTHENF	4	771 776	776		0 (2) <sup>1</sup> 0 (1) <sup>1</sup>	-1 (2)* 0 (2)*	Q (4)* Q (1)*
REKLHIGTHEKF	3	777 785	789		0 (1) <sup>1</sup> 3 (2 <sup>34</sup>	1 (1)*	0 (1)*
VRVEECHY	2	823	831		3 (2)*	-5 (1)*	-3 (3)*
VRVEECH/TVLGDA CH/TVLGDA	3	823 829	837 837		-1 (3)* 2 (1)*	-2 (1)* -1 (2)*	-3 (4)* 1 (2)*
TVLGDAFKECF	2	832	842	1	2 (4)*	0 (2)*	1 (4)*
PNECE EVSRPHPKPKOESS	4	638 842	842 855 (	apping loop	-1 (3)* -12 (4)	-1 (2)* -15 (2)	-1 (3)* -12 (4)
VSRPHPKPKQFSSF FRUNKIE	4	843	856	sepping loop	-13 (3)	-15 (2)	-11 (4)
EKRAKIFC	2	857	864		2 (4)*	-2 (3)*	2 (4)*
CARONCSHDWGHVKYKT FEIPVIRES	4	864 882	881 891		0 (2)*	0(1)*	0 (2)*
FEPVINESF	2	882	892		-1 (3)*	-1 (1)*	0 (3)*
EVVEDIATGV0TL	2	892	892 904		0 (3)*	= (1)* -2 (2)*	0 (1)* 0 (4)*
VVEDIATGVQTL	2	893 897	904		-3 (4)* -1 ( <sup>3)#</sup>	-4 (3)*	-4 (4)* -2 (4)*
EKIPEDPAE	2	914	922		5 (4)'	0 (3)*	-4 (5)*
EKIPFDPAENSK	3	914	925		3 (3)*	0 (2)*	1 (3)*

**Supplementary Figure S8.** ΔΔHDX view of indicated RIG-I upon RNA discrimination. Data interpretation is similar as **Supplementary Figure S5**.