

Supplementary Information

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

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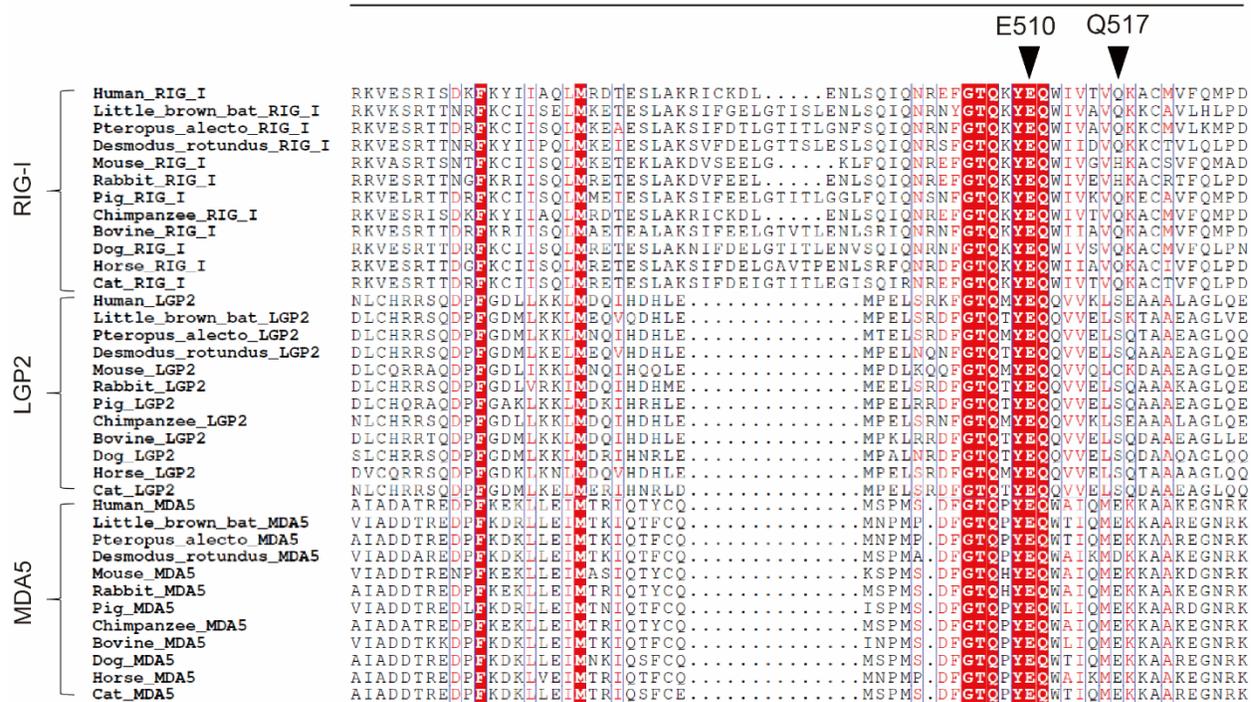
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HEL2i

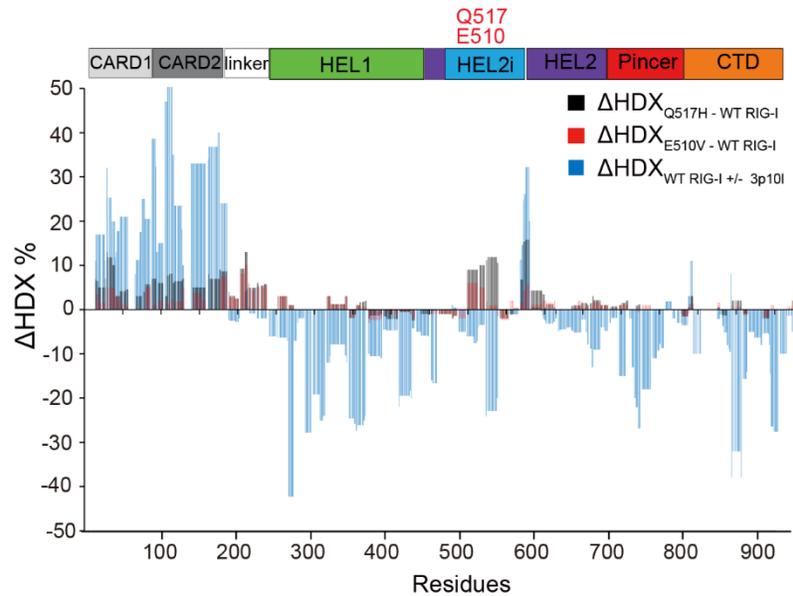


CARD2 Latch region

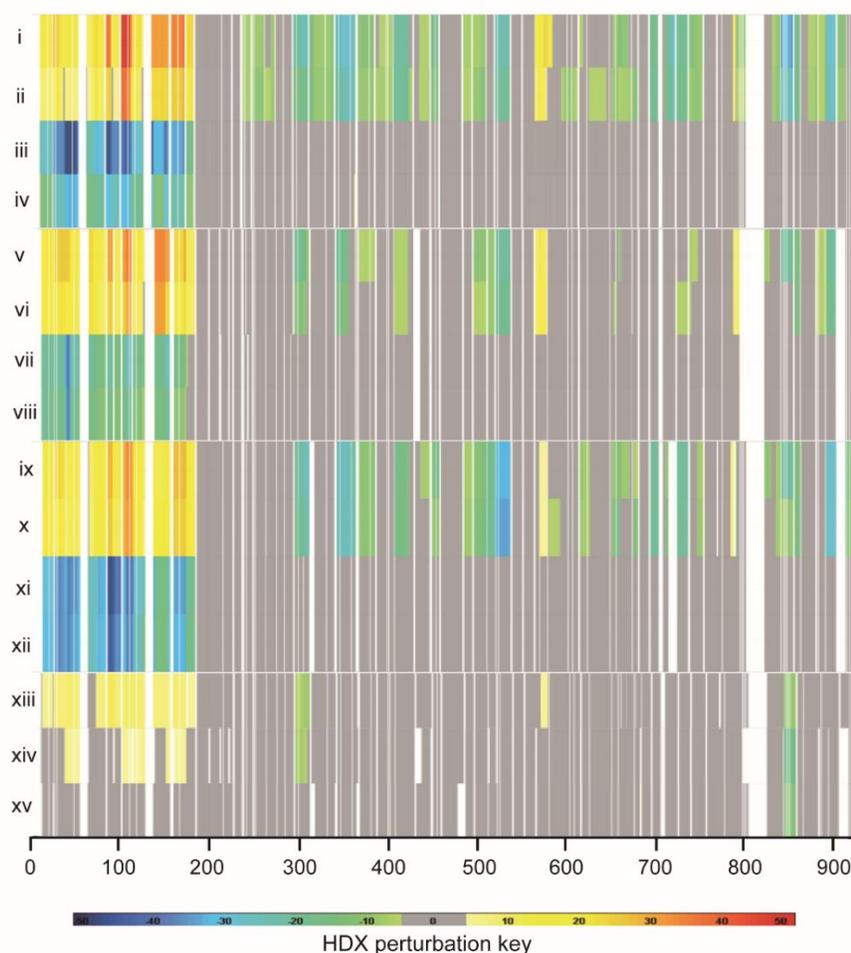


Supplementary Figure S1. Sequence alignments of RLR HEL2i gate motif and RIG-I CARD2 latch interface.

Supplementary Figure S2. HDX profiles of RIG-I, RIG-I_{E510V} and RIG-I_{Q517H} 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 ΔHDX (%) 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.



- i: $\Delta\text{HDX}=\text{RIG-I}\pm 3\text{p10I}$
- ii: $\Delta\text{HDX}=\text{RIG-I}\pm\text{Cap2-10I}$
- iii: $\Delta\text{HDX}=\text{RIG-I}\&3\text{p10I}\pm\text{K63polyUb}_9$
- iv: $\Delta\text{HDX}=\text{RIG-I}\&\text{Cap2-10I}\pm\text{K63polyUb}_9$
- v: $\Delta\text{HDX}=\text{RIG-I}_{\text{E510V}}\pm 3\text{p10I}$
- vi: $\Delta\text{HDX}=\text{RIG-I}_{\text{E510V}}\pm\text{Cap2-10I}$
- vii: $\Delta\text{HDX}=\text{RIG-I}_{\text{E510V}}\&3\text{p10I}\pm\text{K63polyUb}_9$
- viii: $\Delta\text{HDX}=\text{RIG-I}_{\text{E510V}}\&\text{Cap2-10I}\pm\text{K63polyUb}_9$
- ix: $\Delta\text{HDX}=\text{RIG-I}_{\text{Q517H}}\pm 3\text{p10I}$
- x: $\Delta\text{HDX}=\text{RIG-I}_{\text{Q517H}}\pm\text{Cap2-10I}$
- xi: $\Delta\text{HDX}=\text{RIG-I}_{\text{Q517H}}\&3\text{p10I}\pm\text{K63polyUb}_9$
- xii: $\Delta\text{HDX}=\text{RIG-I}_{\text{Q517H}}\&\text{Cap2-10I}\pm\text{K63polyUb}_9$
- xiii: $\Delta\Delta\text{HDX}=(\Delta\text{HDX}=\text{RIG-I}\pm 3\text{p10I}) - (\Delta\text{HDX}=\text{RIG-I}\pm\text{Cap2-10I})$
- xiv: $\Delta\Delta\text{HDX}=(\Delta\text{HDX}=\text{RIG-I}_{\text{E510V}}\pm 3\text{p10I}) - (\Delta\text{HDX}=\text{RIG-I}_{\text{E510V}}\pm\text{Cap2-10I})$
- xv: $\Delta\Delta\text{HDX}=(\Delta\text{HDX}=\text{RIG-I}_{\text{Q517H}}\pm 3\text{p10I}) - (\Delta\text{HDX}=\text{RIG-I}_{\text{Q517H}}\pm\text{Cap2-10I})$

Supplementary Figure S4. Single residue ΔHDX and $\Delta\Delta\text{HDX}$ 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)
EGVIRKLEPTFLS	3	12	27	CARD1	11 (2)	7 (4)	18 (2)	7 (4)
GVNWRTPFTYL	3	12	26		10 (4)	16 (4)	16 (4)	30 (4)
SYVAIAKRFRE	2	27	38		30 (2)	27 (2)	-27 (2)	-25 (2)
YRANVYRREVG	2	28	39		27 (2)	14 (2)	-27 (2)	-27 (2)
YRANVYRREVG	3	38	46		11 (2)	9 (4)	-24 (2)	-22 (2)
VQVDAENRQNEEATL	3	38	56		10 (2)	10 (2)	-21 (2)	-18 (4)
YGLAKNQKQKQEA	3	40	53		20 (2)	11 (4)	-25 (2)	-23 (4)
ENRQKRFKAAFL	3	46	56		23 (2)	14 (4)	-47 (2)	-34 (2)
LSREYRPSGL	2	44	74		7 (4)	6 (4)	11 (2)	19 (4)
DFQWVRFRL	2	65	74		13 (2)	6 (2)	36 (2)	18 (2)
FQKFLA	2	70	76		20 (2)	16 (2)	-20 (2)	-17 (2)
DALLRPAVYSGL	2	79	85		22 (4)	16 (4)	-38 (2)	-19 (4)
LWAGVYGL	2	77	85		17 (4)	9 (4)	-28 (4)	-19 (4)
YEASWD	1	86	90		27 (2)	18 (4)	-49 (2)	-26 (4)
YEASWD	2	86	90		11 (2)	10 (2)	-37 (2)	-25 (2)
YEASWD	3	86	90	10 (2)	9 (4)	-38 (2)	-26 (2)	
YKLLKLDLRF	3	303	311	45 (2)	36 (2)	-41 (2)	-30 (2)	
YKLLKLDLRF	3	303	311	20 (2)	11 (4)	-43 (2)	-30 (2)	
YKLLKLDLRF	3	303	311	15 (2)	9 (4)	-31 (2)	-28 (2)	
YKLLKLDLRF	3	303	311	10 (2)	9 (4)	-31 (2)	-28 (2)	
YKLLKLDLRF	3	303	311	5 (2)	4 (4)	-24 (2)	-17 (2)	
YKLLKLDLRF	3	303	311	0 (2)	0 (4)	-17 (2)	-10 (2)	
YKLLKLDLRF	3	303	311	-5 (2)	-4 (4)	-10 (2)	-3 (2)	
YKLLKLDLRF	3	303	311	-10 (2)	-9 (4)	-3 (2)	4 (2)	
YKLLKLDLRF	3	303	311	-15 (2)	-14 (4)	4 (2)	11 (2)	
YKLLKLDLRF	3	303	311	-20 (2)	-19 (4)	11 (2)	18 (2)	
YKLLKLDLRF	3	303	311	-25 (2)	-24 (4)	18 (2)	25 (2)	
YKLLKLDLRF	3	303	311	-30 (2)	-29 (4)	25 (2)	32 (2)	
YKLLKLDLRF	3	303	311	-35 (2)	-34 (4)	32 (2)	39 (2)	
YKLLKLDLRF	3	303	311	-40 (2)	-39 (4)	39 (2)	46 (2)	
YKLLKLDLRF	3	303	311	-45 (2)	-44 (4)	46 (2)	53 (2)	
YKLLKLDLRF	3	303	311	-50 (2)	-49 (4)	53 (2)	60 (2)	
YKLLKLDLRF	3	303	311	-55 (2)	-54 (4)	60 (2)	67 (2)	
YKLLKLDLRF	3	303	311	-60 (2)	-59 (4)	67 (2)	74 (2)	
YKLLKLDLRF	3	303	311	-65 (2)	-64 (4)	74 (2)	81 (2)	
YKLLKLDLRF	3	303	311	-70 (2)	-69 (4)	81 (2)	88 (2)	
YKLLKLDLRF	3	303	311	-75 (2)	-74 (4)	88 (2)	95 (2)	
YKLLKLDLRF	3	303	311	-80 (2)	-79 (4)	95 (2)	102 (2)	
YKLLKLDLRF	3	303	311	-85 (2)	-84 (4)	102 (2)	109 (2)	
YKLLKLDLRF	3	303	311	-90 (2)	-89 (4)	109 (2)	116 (2)	
YKLLKLDLRF	3	303	311	-95 (2)	-94 (4)	116 (2)	123 (2)	
YKLLKLDLRF	3	303	311	-100 (2)	-99 (4)	123 (2)	130 (2)	
YKLLKLDLRF	3	303	311	-105 (2)	-104 (4)	130 (2)	137 (2)	
YKLLKLDLRF	3	303	311	-110 (2)	-109 (4)	137 (2)	144 (2)	
YKLLKLDLRF	3	303	311	-115 (2)	-114 (4)	144 (2)	151 (2)	
YKLLKLDLRF	3	303	311	-120 (2)	-119 (4)	151 (2)	158 (2)	
YKLLKLDLRF	3	303	311	-125 (2)	-124 (4)	158 (2)	165 (2)	
YKLLKLDLRF	3	303	311	-130 (2)	-129 (4)	165 (2)	172 (2)	
YKLLKLDLRF	3	303	311	-135 (2)	-134 (4)	172 (2)	179 (2)	
YKLLKLDLRF	3	303	311	-140 (2)	-139 (4)	179 (2)	186 (2)	
YKLLKLDLRF	3	303	311	-145 (2)	-144 (4)	186 (2)	193 (2)	
YKLLKLDLRF	3	303	311	-150 (2)	-149 (4)	193 (2)	200 (2)	
YKLLKLDLRF	3	303	311	-155 (2)	-154 (4)	200 (2)	207 (2)	
YKLLKLDLRF	3	303	311	-160 (2)	-159 (4)	207 (2)	214 (2)	
YKLLKLDLRF	3	303	311	-165 (2)	-164 (4)	214 (2)	221 (2)	
YKLLKLDLRF	3	303	311	-170 (2)	-169 (4)	221 (2)	228 (2)	
YKLLKLDLRF	3	303	311	-175 (2)	-174 (4)	228 (2)	235 (2)	
YKLLKLDLRF	3	303	311	-180 (2)	-179 (4)	235 (2)	242 (2)	
YKLLKLDLRF	3	303	311	-185 (2)	-184 (4)	242 (2)	249 (2)	
YKLLKLDLRF	3	303	311	-190 (2)	-189 (4)	249 (2)	256 (2)	
YKLLKLDLRF	3	303	311	-195 (2)	-194 (4)	256 (2)	263 (2)	
YKLLKLDLRF	3	303	311	-200 (2)	-199 (4)	263 (2)	270 (2)	
YKLLKLDLRF	3	303	311	-205 (2)	-204 (4)	270 (2)	277 (2)	
YKLLKLDLRF	3	303	311	-210 (2)	-209 (4)	277 (2)	284 (2)	
YKLLKLDLRF	3	303	311	-215 (2)	-214 (4)	284 (2)	291 (2)	
YKLLKLDLRF	3	303	311	-220 (2)	-219 (4)	291 (2)	298 (2)	
YKLLKLDLRF	3	303	311	-225 (2)	-224 (4)	298 (2)	305 (2)	
YKLLKLDLRF	3	303	311	-230 (2)	-229 (4)	305 (2)	312 (2)	
YKLLKLDLRF	3	303	311	-235 (2)	-234 (4)	312 (2)	319 (2)	
YKLLKLDLRF	3	303	311	-240 (2)	-239 (4)	319 (2)	326 (2)	
YKLLKLDLRF	3	303	311	-245 (2)	-244 (4)	326 (2)	333 (2)	
YKLLKLDLRF	3	303	311	-250 (2)	-249 (4)	333 (2)	340 (2)	
YKLLKLDLRF	3	303	311	-255 (2)	-254 (4)	340 (2)	347 (2)	
YKLLKLDLRF	3	303	311	-260 (2)	-259 (4)	347 (2)	354 (2)	
YKLLKLDLRF	3	303	311	-265 (2)	-264 (4)	354 (2)	361 (2)	
YKLLKLDLRF	3	303	311	-270 (2)	-269 (4)	361 (2)	368 (2)	
YKLLKLDLRF	3	303	311	-275 (2)	-274 (4)	368 (2)	375 (2)	
YKLLKLDLRF	3	303	311	-280 (2)	-279 (4)	375 (2)	382 (2)	
YKLLKLDLRF	3	303	311	-285 (2)	-284 (4)	382 (2)	389 (2)	
YKLLKLDLRF	3	303	311	-290 (2)	-289 (4)	389 (2)	396 (2)	
YKLLKLDLRF	3	303	311	-295 (2)	-294 (4)	396 (2)	403 (2)	
YKLLKLDLRF	3	303	311	-300 (2)	-299 (4)	403 (2)	410 (2)	
YKLLKLDLRF	3	303	311	-305 (2)	-304 (4)	410 (2)	417 (2)	
YKLLKLDLRF	3	303	311	-310 (2)	-309 (4)	417 (2)	424 (2)	
YKLLKLDLRF	3	303	311	-315 (2)	-314 (4)	424 (2)	431 (2)	
YKLLKLDLRF	3	303	311	-320 (2)	-319 (4)	431 (2)	438 (2)	
YKLLKLDLRF	3	303	311	-325 (2)	-324 (4)	438 (2)	445 (2)	
YKLLKLDLRF	3	303	311	-330 (2)	-329 (4)	445 (2)	452 (2)	
YKLLKLDLRF	3	303	311	-335 (2)	-334 (4)	452 (2)	459 (2)	
YKLLKLDLRF	3	303	311	-340 (2)	-339 (4)	459 (2)	466 (2)	
YKLLKLDLRF	3	303	311	-345 (2)	-344 (4)	466 (2)	473 (2)	
YKLLKLDLRF	3	303	311	-350 (2)	-349 (4)	473 (2)	480 (2)	
YKLLKLDLRF	3	303	311	-355 (2)	-354 (4)	480 (2)	487 (2)	
YKLLKLDLRF	3	303	311	-360 (2)	-359 (4)	487 (2)	494 (2)	
YKLLKLDLRF	3	303	311	-365 (2)	-364 (4)	494 (2)	501 (2)	
YKLLKLDLRF	3	303	311	-370 (2)	-369 (4)	501 (2)	508 (2)	
YKLLKLDLRF	3	303	311	-375 (2)	-374 (4)	508 (2)	515 (2)	
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YKLLKLDLRF	3	303	311	-390 (2)	-389 (4)	529 (2)	536 (2)	
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YKLLKLDLRF	3	303	311	-415 (2)	-414 (4)	564 (2)	571 (2)	
YKLLKLDLRF	3	303	311	-420 (2)	-419 (4)	571 (2)	578 (2)	
YKLLKLDLRF	3	303	311	-425 (2)	-424 (4)	578 (2)	585 (2)	
YKLLKLDLRF	3	303	311	-430 (2)	-429 (4)	585 (2)	592 (2)	
YKLLKLDLRF	3	303	311	-435 (2)	-434 (4)	592 (2)	599 (2)	
YKLLKLDLRF	3	303	311	-440 (2)	-439 (4)	599 (2)	606 (2)	
YKLLKLDLRF	3	303	311	-445 (2)	-444 (4)	606 (2)	613 (2)	
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YKLLKLDLRF	3	303	311	-455 (2)	-454 (4)	620 (2)	627 (2)	
YKLLKLDLRF	3	303	311	-460 (2)	-459 (4)	627 (2)	634 (2)	
YKLLKLDLRF	3	303	311	-465 (2)	-464 (4)	634 (2)	641 (2)	
YKLLKLDLRF	3	303	311	-470 (2)	-469 (4)	641 (2)	648 (2)	
YKLLKLDLRF	3	303	311	-475 (2)	-474 (4)	648 (2)	655 (2)	
YKLLKLDLRF	3	303	311	-480 (2)	-479 (4)	655 (2)	662 (2)	
YKLLKLDLRF	3	303	311	-485 (2)	-484 (4)	662 (2)	669 (2)	
YKLLKLDLRF	3	303	311	-490 (2)	-489 (4)	669 (2)	676 (2)	
YKLLKLDLRF	3	303	311	-495 (2)	-494 (4)	676 (2)	683 (2)	
YKLLKLDLRF	3	303	311	-500 (2)	-499 (4)	683 (2)	690 (2)	
YKLLKLDLRF	3	303	311	-505 (2)	-504 (4)	690 (2)	697 (2)	
YKLLKLDLRF	3	303	311	-510 (2)	-509 (4)	697 (2)	704 (2)	
YKLLKLDLRF	3	303	311	-515 (2)	-514 (4)	704 (2)	711 (2)	
YKLLKLDLRF	3	303	311	-520 (2)	-519 (4)	711 (2)	718 (2)	
YKLLKLDLRF	3	303	311	-525 (2)	-524 (4)	718 (2)	725 (2)	
YKLLKLDLRF	3	303	311	-530 (2)	-529 (4)	725 (2)	732 (2)	
YKLLKLDLRF	3	303	311	-535 (2)	-534 (4)	732 (2)	739 (2)	
YKLLKLDLRF	3	303	311	-540 (2)	-539 (4)	739 (2)	746 (2)	
YKLLKLDLRF	3	303	311	-545 (2)	-544 (4)	746 (2)	753 (2)	
YKLLKLDLRF	3	303	311	-550 (2)	-549 (4)	753 (2)	760 (2)	
YKLLKLDLRF	3	303	311	-555 (2)	-554 (4)	760 (2)	767 (2)	
YKLLKLDLRF	3	303	311	-560 (2)	-559 (4)	767 (2)	774 (2)	
YKLLKLDLRF	3	303	311	-565 (2)	-564 (4)	774 (2)	781 (2)	
YKLLKLDLRF	3	303	311	-570 (2)	-569 (4)	781 (2)	788 (2)	
YKLLKLDLRF	3	303	311	-575 (2)	-574 (4)	788 (2)	795 (2)	
YKLLKLDLRF	3	303	311	-580 (2)	-579 (4)	795 (2)	802 (2)	
YKLLKLDLRF	3	303	311	-585 (2)	-584 (4)	802 (2)	809 (2)	
YKLLKLDLRF	3	303	311	-590 (2)	-589 (4)	809 (2)	816 (2)	
YKLLKLDLRF								

Supplementary Figure S5. Differential HDX view of WT RIG-I and indicated complexes upon binding to RNA and K63-polyUb₉ 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.

