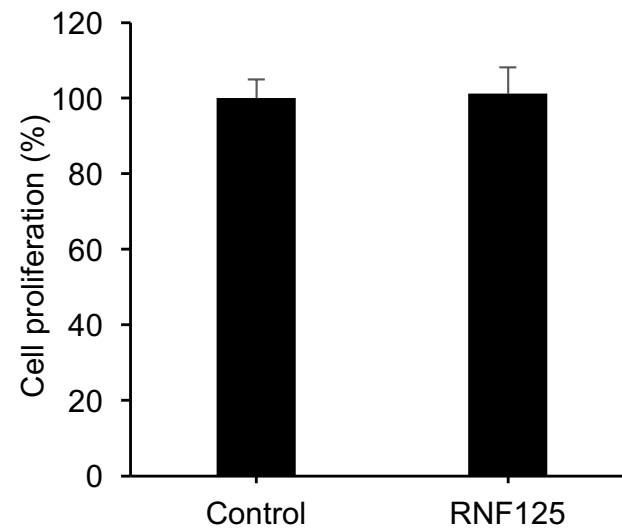


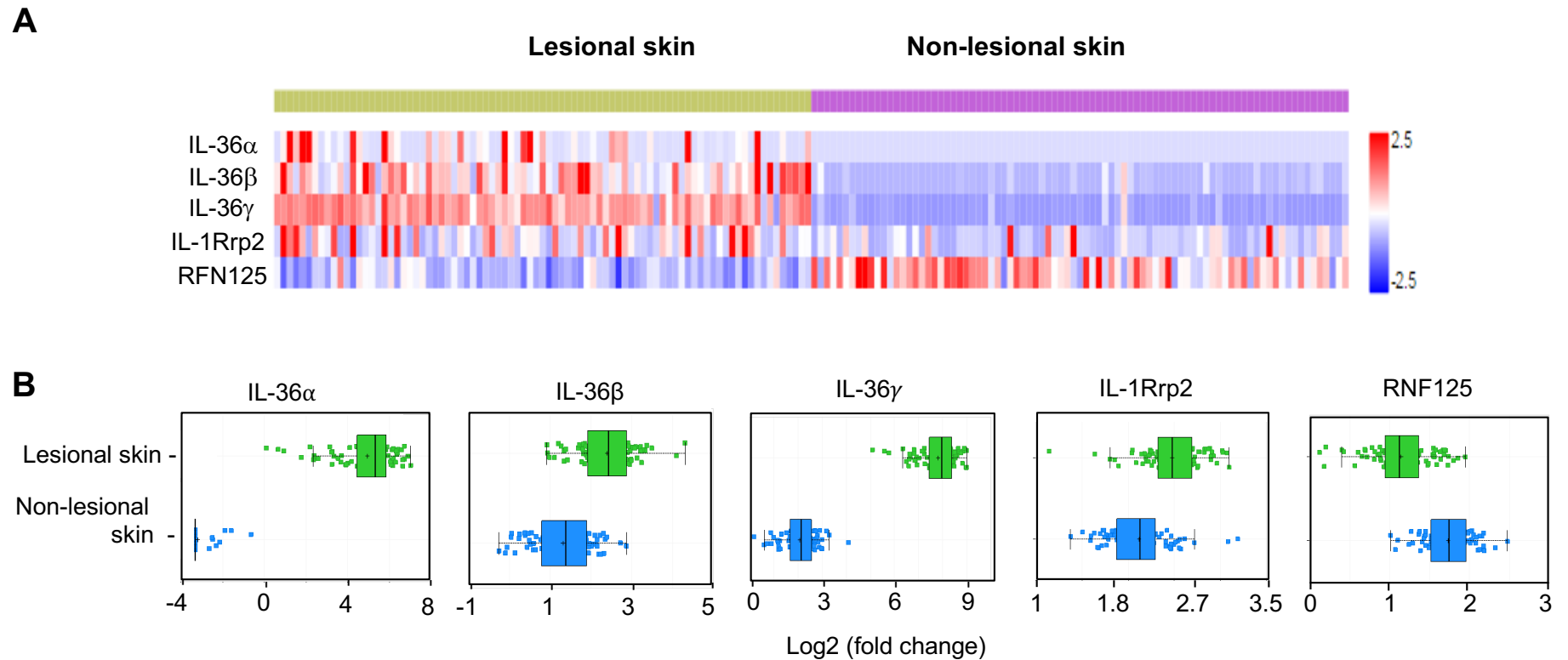
**Supplemental Table 1. Effects of reduction in E3 ubiquitin ligases on signaling by the IL-36R.**

<b>siRNA</b>	<b>IL-6 (fold)</b>	<b>siRNA</b>	<b>IL-6 (fold)</b>
Mock	5.54 ± 0.26*	PHF6	4.04 ± 0.07
Nonspecific	5.79 ± 0.06	PHF5A	8.20 ± 0.23
ANKIB1	6.28 ± 0.23	PHF7	6.05 ± 0.48
BAHD1	3.89 ± 0.32	PHF20	2.57 ± 0.07
BIRC3	3.97 ± 0.21	PHF20L1	3.19 ± 0.21
BMI1	4.98 ± 0.09	PHF21A	3.11 ± 0.22
CBL	6.12 ± 0.24	RBCK1	4.82 ± 0.09
CHD5	3.09 ± 0.02	RFWD2	2.53 ± 0.04
HRC	4.49 ± 0.04	RLIM	3.91 ± 0.10
INTS12	5.45 ± 0.17	RNF5	1.78 ± 0.07
JHDM1D	3.69 ± 0.20	RNF7	5.33 ± 0.17
KDM5B	6.29 ± 0.44	RNF32	15.34 ± 0.33
LOC644006	6.44 ± 0.39	RNF39	3.83 ± 0.10
MDM2	2.09 ± 0.04	RNF114	5.44 ± 0.28
MARCH4	3.46 ± 0.17	RNF122	2.98 ± 0.19
MARCH8	7.41 ± 0.19	RNF123	12.64 ± 0.14
MEX3A	8.85 ± 0.14	RNF125	1.24 ± 0.08
MEX3D	7.28 ± 0.37	RNF133	1.84 ± 0.09
MGRN1	5.36 ± 0.20	RNF135	8.64 ± 0.59
MID2	6.42 ± 0.33	RNF152	2.11 ± 0.20
MLLT6	8.42 ± 0.82	RNF180	1.57 ± 0.03
MIB2	5.06 ± 0.10	RNF185	5.26 ± 0.52
MYCBP2	3.74 ± 0.32	TRAF6	1.03 ± 0.31
PCGF1	7.07 ± 0.49	TRIM39	6.07 ± 0.12
PDZRN3	5.00 ± 0.39	TRIM60	6.27 ± 0.56
PRPF19	5.65 ± 0.38	ZFAND6	2.07 ± 0.05

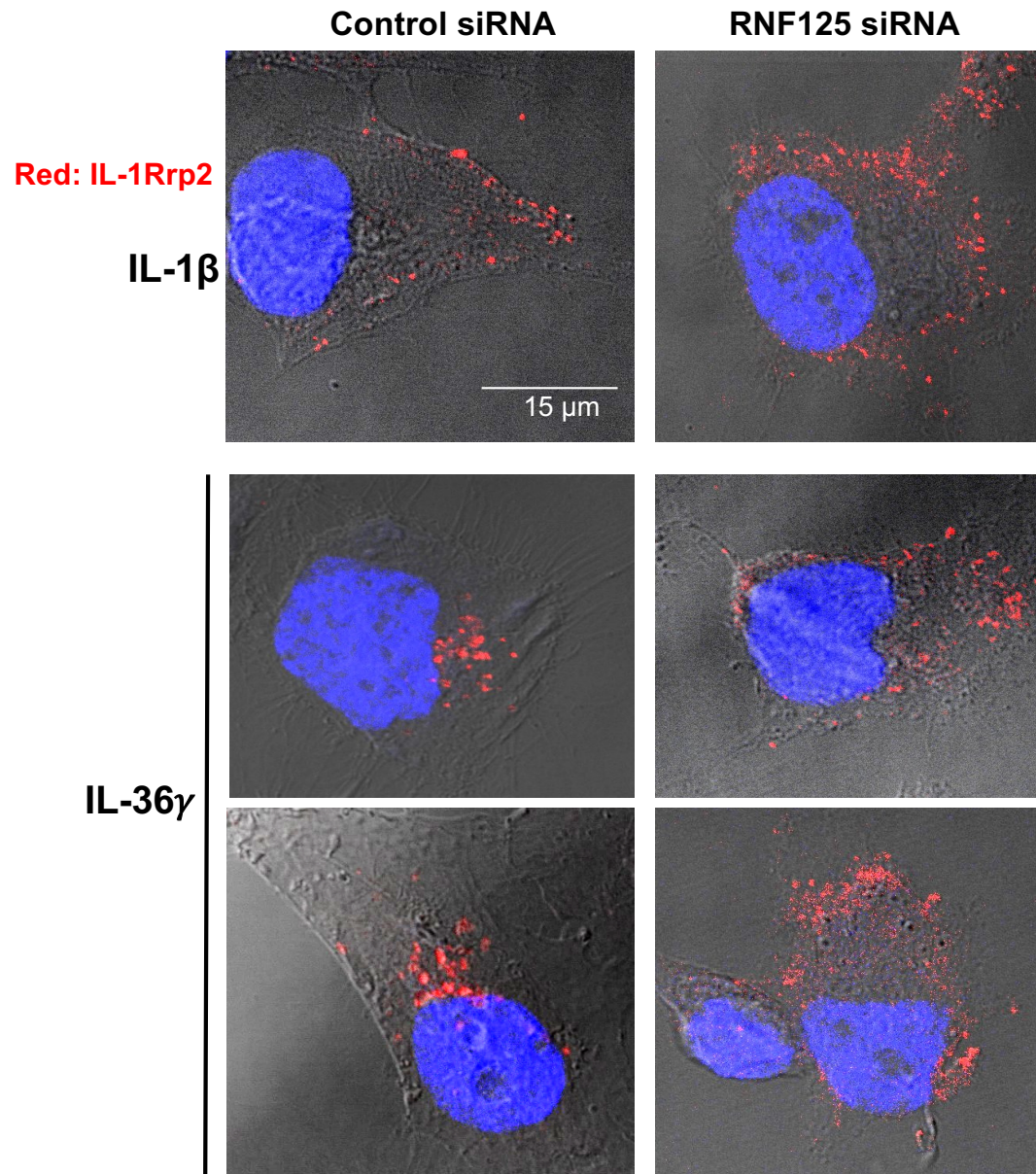
\*Fold change was calculated by dividing the IL-6 concentration of IL-6 produced in cells induced with IL-36 $\gamma$  from mock-treated cells. Each result represents mean and one standard error from three independent assays.



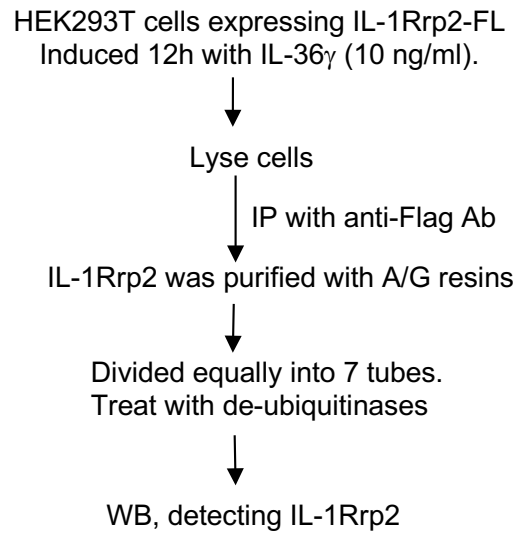
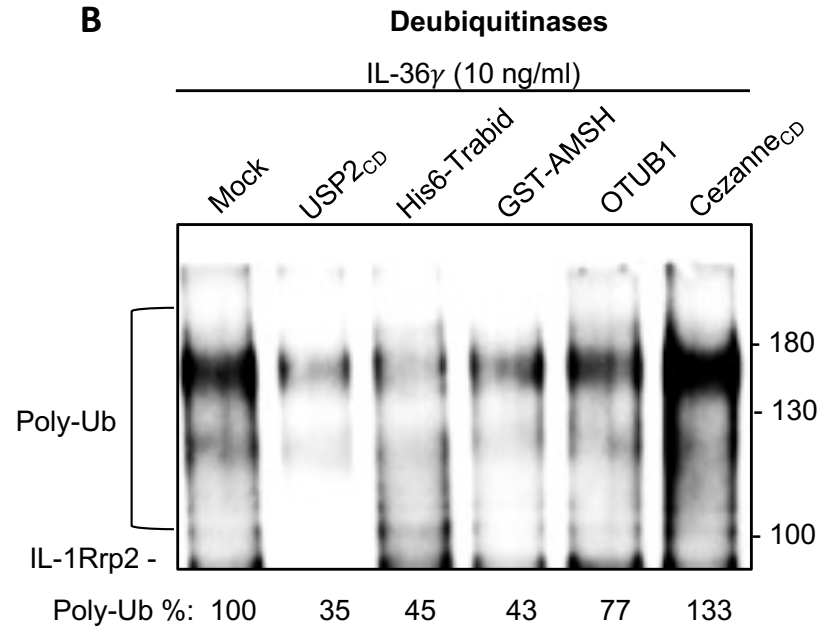
**Supplementary Fig. 1.** WST-1 assay was performed to measure the cell proliferation following siRNA transfection in NCI cells. WST-1 reagent (1:10 dilution) was added to the cells after 48 h of siRNA transfection and incubated for additional 4 h before the absorbance was measured.



**Supplementary Fig. 2.** Ubiquitin ligase RNF125 expression is reduced in disease tissue with elevated expression of IL-36R and its agonist cytokines. **A)** Expression levels of the RNF125 are decreased in disease tissues that overexpress the IL-36R or cytokines that can induce IL-36R signaling. The data was from Boehringer Ingelheim collection of patient samples. **B)** RNA sequencing also reveals that RNF125 is inversely related to the expression of IL-1Rrp2 and the IL-36 cytokines in non-lesional and lesional skin. All differences have p values of < 0.01.



**Supplemental Fig. 3.** Knockdown of RNF125 inhibits the intracellular translocation of agonist-activated IL-1Rrp2. The epinuclear locations of the IL-36R have been previously demonstrated to be LAMP1+ lysosomes. Knockdown of RNF125 used a mixture of 4 siRNAs and reduced the level of RNF125 protein to 30%. DNA has been stained with DAPI. IL-1Rrp2 was stained with a specific antibody and a secondary antibody labeled with Alexa Fluor 594.

**A****B**

**Supplementary Fig. 4.** HEK293T cells expressing IL-36R contains a variety of ubiquitinations. **A)** Schematic of the protocol used to examine the types of ubiquitination on recombinant IL-1Rrp2 proteins. **B)** Western blot of the effects of de-ubiquitinases on polyubiquitinated IL-1Rrp2.