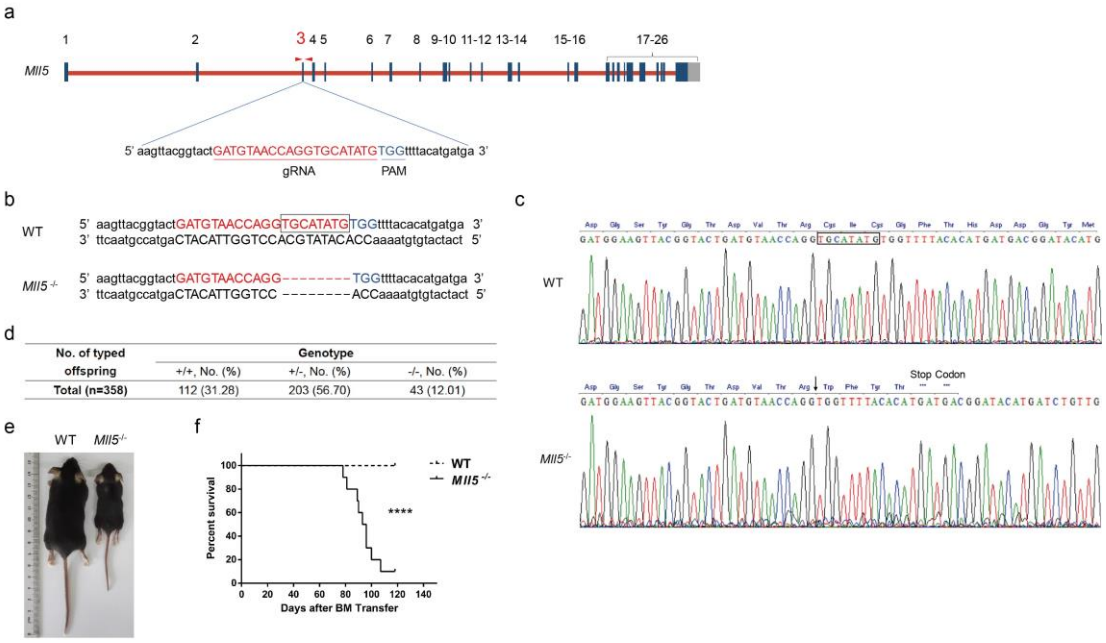
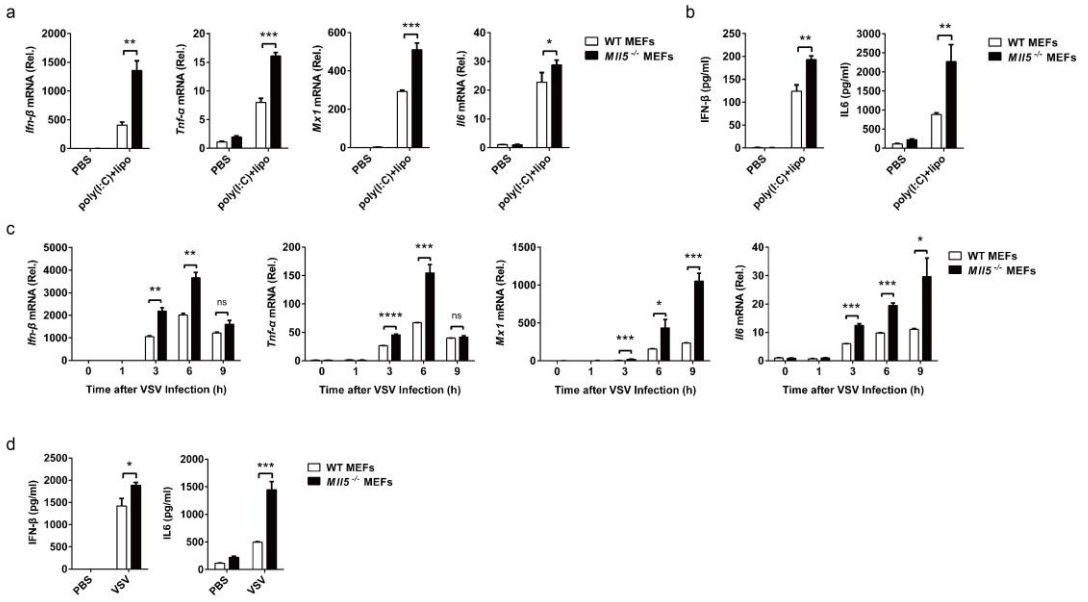
MLL5 suppresses antiviral innate immune response by facilitating STUB1-mediated RIG-I degradation

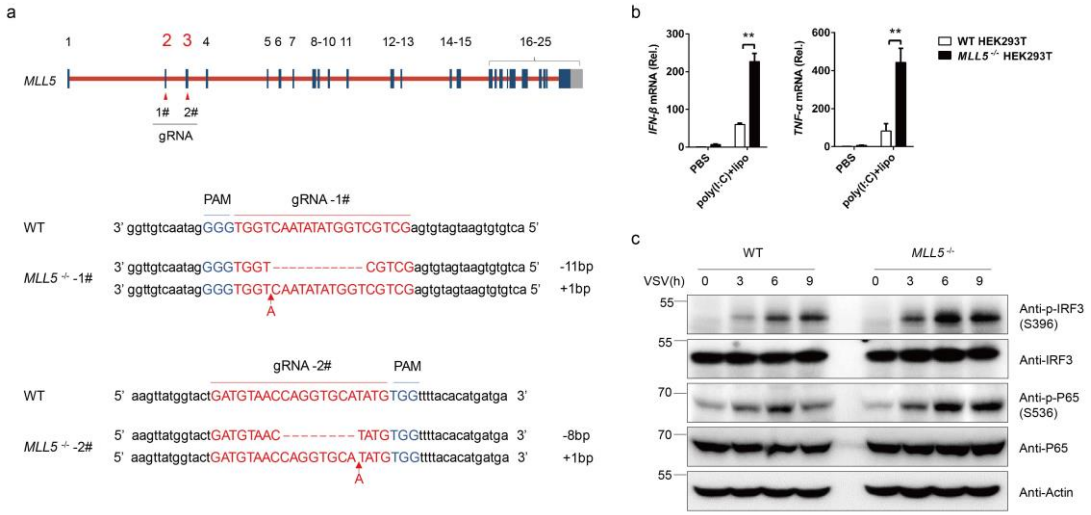
Zhou et al.



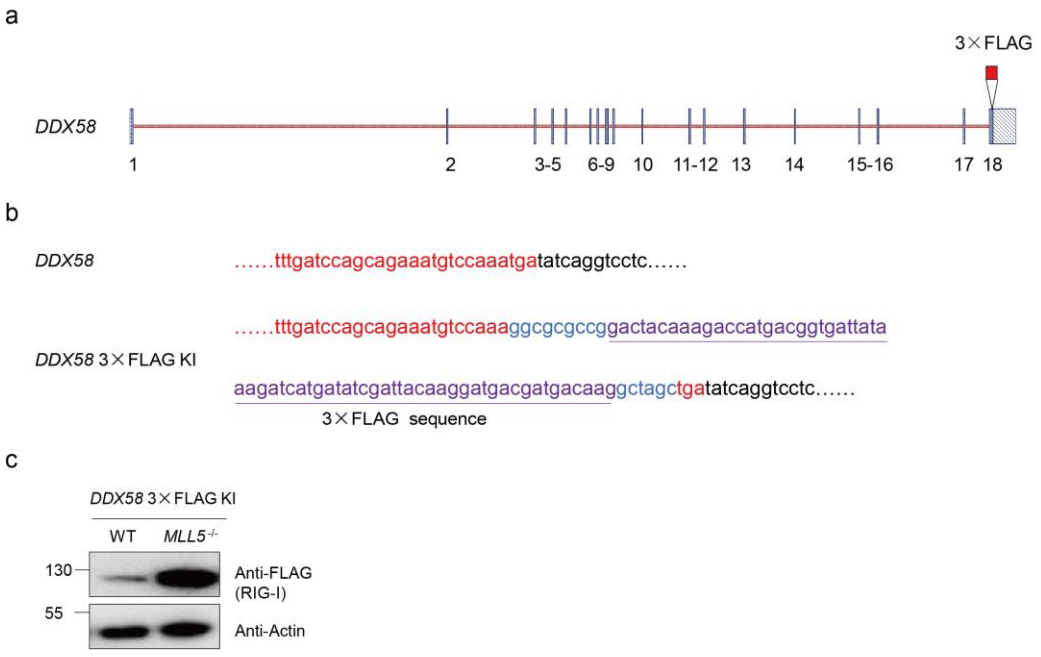
Supplementary Figure 1. Generation of *Mll5*-deficient mice with CRISPR-Cas9 approach. **(a)** Graphic representation of strategy used to generate *Mll5*$^{-/-}$ mice. gRNA (red) and PAM (blue) sequences locus in exon 3 of *Mll5* gene. **(b)** DNA sequence of WT (upper) and *Mll5*$^{-/-}$ mice (bottom). The region of the target sequence is shown in red. The black box indicates the nucleotides lost in *Mll5*$^{-/-}$ mice. **(c)** Sequence analysis of the the Reverse Transcription-PCR band in MEFs from WT (upper) and *Mll5*$^{-/-}$ mice (bottom). The nucleotide sequence confirms the reading frame shift in exon 3 in *Mll5*$^{-/-}$ mice; a premature stop codon (TGA) in exon 3 is induced. **(d)** Number and frequency of WT, *Mll5*$^{+/-}$ and *Mll5*$^{-/-}$ pups by *Mll5*$^{+/-}$ intercrosses at the time of weaning. **(e)** Postnatal growth retardation in *Mll5*-deficient mice; one pair of 6-week-old WT and *Mll5*$^{-/-}$ male littermates. **(f)** Survival curves of lethally irradiated recipients transpanted with WT and *Mll5*$^{-/-}$ BM (n=10). Data were analyzed by log-rank (Mantel-Cox) test. (**** p<0.0001).



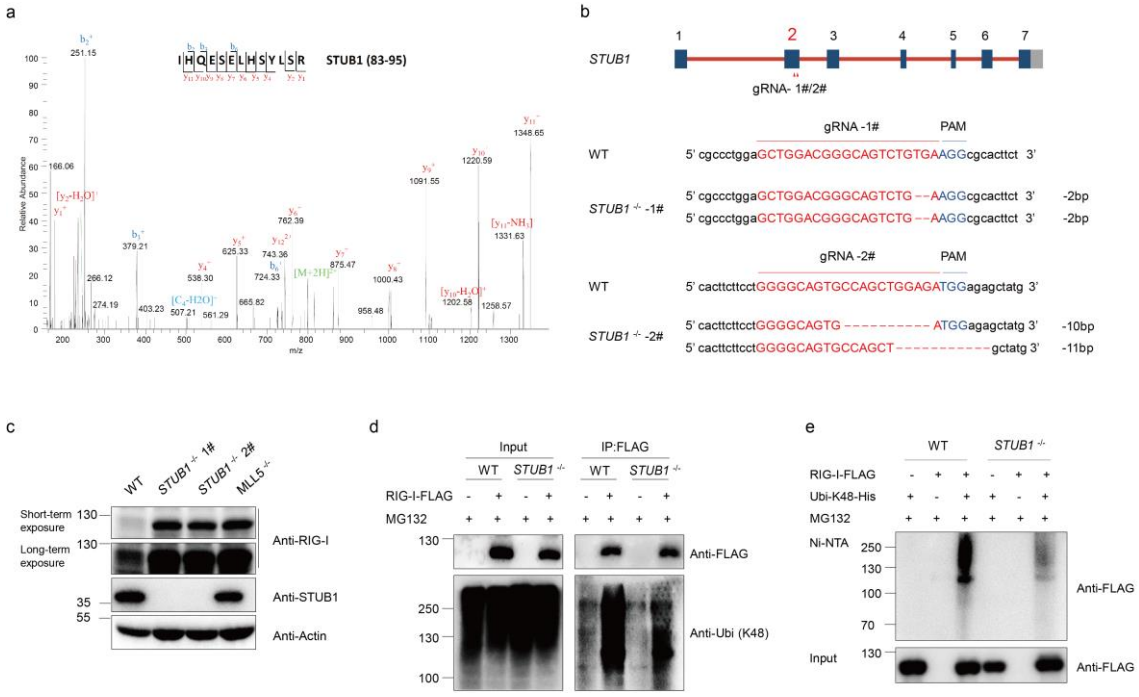
Supplementary Figure 2. MLL5 selectively suppresses RLR-mediated antiviral innate immune response in mouse embroynoic fibroblasts. **(a)** Expression of *Ifn-β*, *Tnf-α, Mx1* and *Il6* mRNA in MEFs from WT or *Mll5*$^{-/-}$ mice stimulated with intracellular poly(I:C) (1 μg/ml) for 6 h. *Gapdh* served as a control. **(b)** ELISA quantification of IFN-β and IL-6 secretion in MEFs treated as in **a**. **(c)** Expression of *Ifn-β*, *Tnf-α, Mx1* and *Il6* mRNA in MEFs from WT or *Mll5*$^{-/-}$ mice infected with VSV-GFP (MOI:1) . *Gapdh* served as a control. **(d)** ELISA quantification of IFN-β and IL-6 secretion in MEFs from WT or *Mll5*$^{-/-}$ mice infected with VSV-GFP (MOI:1) for 6 h. Data were from three independent experiments and were analyzed with the Student's *t*-test (two-tailed) and were presented as mean ± SD. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).



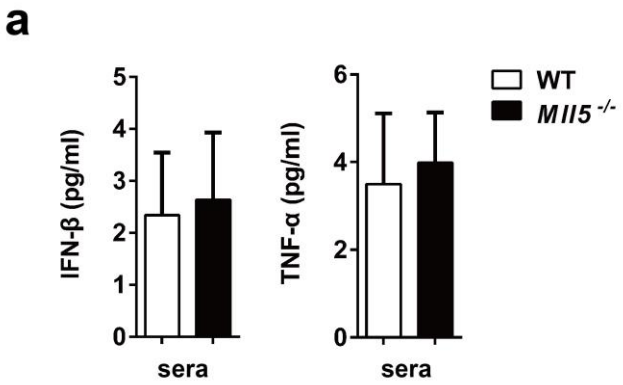
Supplementary Figure 3. MLL5 suppresses RLR-mediated immune response in HEK293T cells. **(a)** Graphic representation of strategy used to generate *MLL5*$^{-/-}$ HEK293T cells (upper) and DNA sequence of *MLL5*$^{-/-}$ HEK293T cells (bottom). gRNA (red) and PAM (blue) sequences locus in exon 2 and 3 of *MLL5* gene. The column on the right indicates the number of inserted or deleted bases. **(b)** Expression of *IFN-β* and *TNF-α* mRNA in WT and *MLL5*$^{-/-}$ (2#) HEK293T cells stimulated with intracellular poly(I:C) (1 μg/ml) for 6 h. *GAPDH* served as a control. **(c)** Immunoblot analysis of p-IRF3 and P65 in WT and *MLL5*$^{-/-}$ (2#) HEK293T cells upon infection with VSV-GFP (MOI:1) for indicated times. Actin served as a loading control. Data were representative of three independent experiments with similar results **(c)** or were from three independent experiments **(b)** and were analyzed with the Student's *t*-test (two-tailed) and were presented as mean ± SD. (** $p<0.01$).



Supplementary Figure 4. Generation of FLAG-tagged endogenous RIG-I protein in HEK293T cells. **(a)** Schematic of 3×FLAG coding sequence insertion in *DDX58* gene in WT and *MLL5*$^{-/-}$ HEK293T cells. **(b)** DNA sequence around the stop codon (tga, red letters) of *DDX58* gene in WT cells (upper) and the 3×FLAG knock-in cells (bottom). Purple indicates the 66-bps 3×FLAG coding sequence. Red indicates the coding sequence of *DDX58*. Blue indicates the restriction enzyme cutting sites. **(c)** Immunoblot analysis of FLAG-tagged RIG-I in the *DDX58* 3×FLAG knock-in WT and *MLL5*$^{-/-}$ HEK293T cells. Actin served as a loading control. Data are representative of three independent experiments with similar results.



Supplementary Figure 5. Identification of STUB1 as the E3 ligase of RIG-I. **(a)** STUB1 in RIG-I-interacting proteins identified by mass spectrometry. **(b)** Graphic representation of strategy used to generate *STUB1*$^{-/-}$ HEK293T cells (upper) and DNA sequence of *STUB1*$^{-/-}$ HEK293T cells (bottom). gRNA (red) and PAM (blue) sequences locus in exon 2 of *STUB1* gene. The column on the right indicates the number of inserted or deleted bases. **(c)** Immunoblot analysis of RIG-I and STUB1 in the WT and *STUB1*$^{-/-}$ HEK293T cells. Actin served as a loading control. **(d)** Co-immunoprecipitation and immunoblot analysis of K48-linked polyubiquitination of RIG-I in WT and *STUB1*$^{-/-}$ (1#) HEK293T cells transfected with RIG-I-FLAG and treated with MG132 (5 μM) for 12 h before. **(e)** His-pull down and immunoblot analysis of K48-linked polyubiquitination of RIG-I in WT and *STUB1*$^{-/-}$ (1#) HEK293T cells cotransfected with RIG-I-FLAG and mutant ubiquitin K48-ubi-His and treated with MG132 (5 μM) for 12 h. Data are representative of three independent experiments with similar results.

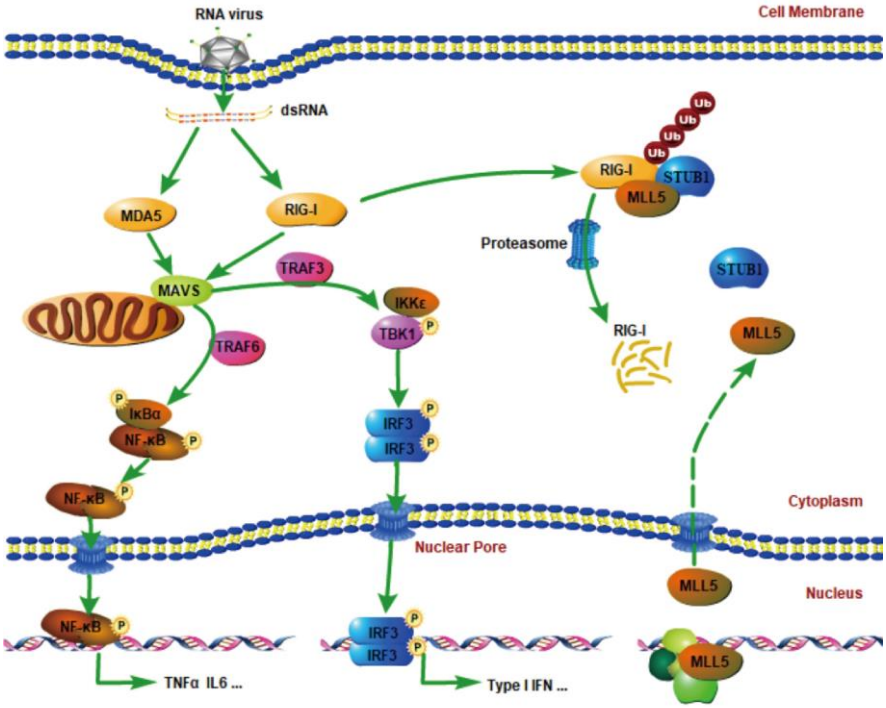


78

79 **Supplementary Figure 6.** ELISA quantification of IFN-β and TNF-α in sera in 6-9

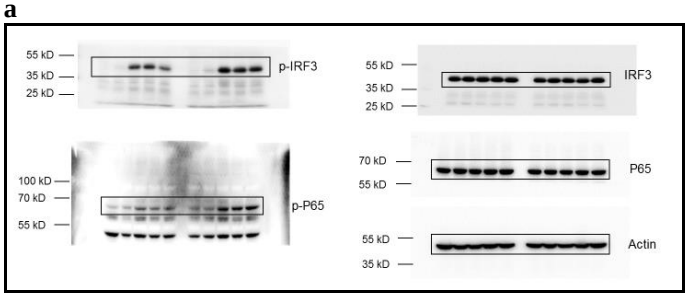
80 weeks old WT or *Mll5*$^{-/-}$ littermates (n=3).

81

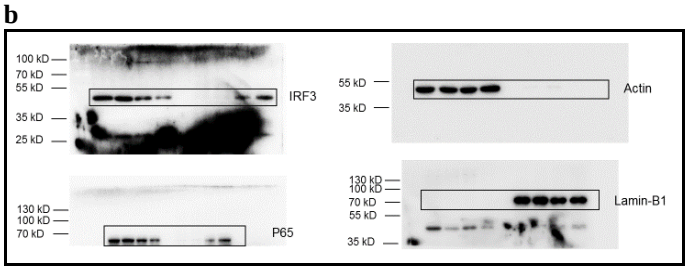


Supplementary Figure 7. Working model. A small fraction of MLL5 proteins located in the cytoplasm facilitated interaction between RIG-I and its E3 ligase STUB1 and promoted K48-linked polyubiquitination and proteasome degradation of RIG-I. MLL5 translocate from the nucleus to the cytoplasm in responses to RNA virus infection, thereby promoting degradation of RIG-I through E3 ubiquitin ligase STUB1.

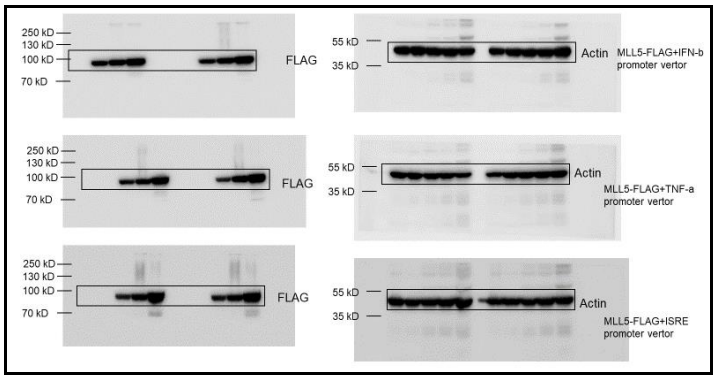
a


55 kD
35 kD
25 kD
p-IRF3
55 kD
35 kD
25 kD
IRF3
100 kD
70 kD
55 kD
p-P65
70 kD
55 kD
P65
55 kD
35 kD
Actin

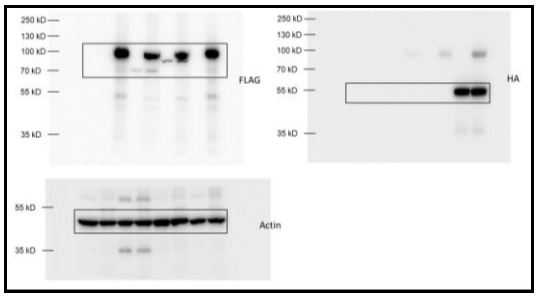
b


100 kD
70 kD
55 kD
IRF3
35 kD
25 kD
55 kD
35 kD
Actin
130 kD
100 kD
70 kD
P65
130 kD
100 kD
70 kD
55 kD
35 kD
Lamin-B1

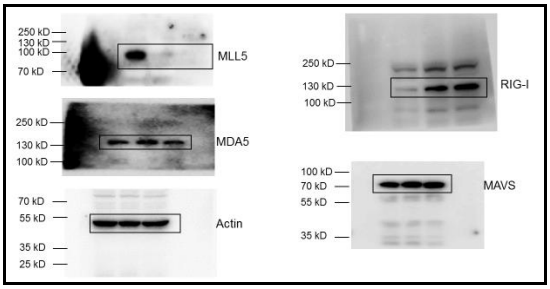
c


250 kD
130 kD
100 kD
70 kD
FLAG
55 kD
35 kD
Actin
MLL5-FLAG+IFN-b promoter vertor
250 kD
130 kD
100 kD
70 kD
FLAG
55 kD
35 kD
Actin
MLL5-FLAG+TNF-a promoter vertor
250 kD
130 kD
100 kD
70 kD
FLAG
55 kD
35 kD
Actin
MLL5-FLAG+ISRE promoter vertor

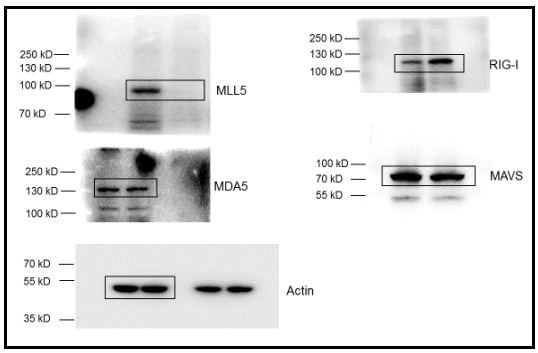
d


250 kD
130 kD
100 kD
70 kD
55 kD
35 kD
FLAG
HA
55 kD
35 kD
Actin

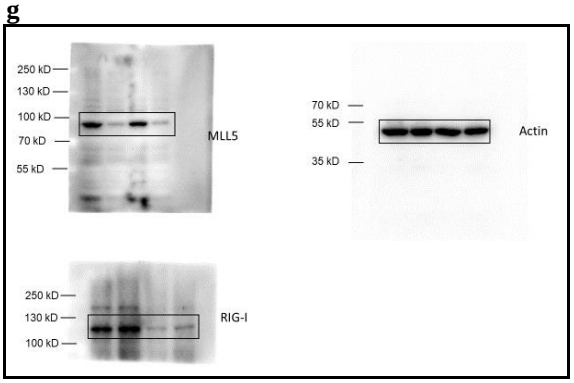
e


250 kD
130 kD
100 kD
70 kD
MLL5
250 kD
130 kD
100 kD
RIG-I
250 kD
130 kD
100 kD
MDA5
100 kD
70 kD
55 kD
35 kD
MAVS
70 kD
55 kD
35 kD
25 kD
Actin

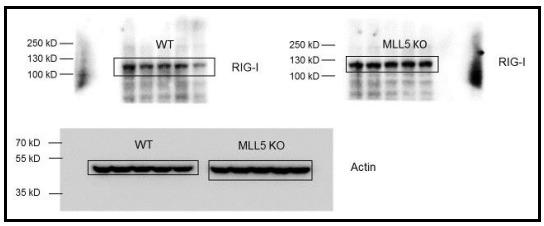
f


250 kD
130 kD
100 kD
70 kD
MLL5
250 kD
130 kD
100 kD
RIG-I
250 kD
130 kD
100 kD
MDA5
100 kD
70 kD
55 kD
MAVS
70 kD
55 kD
35 kD
Actin

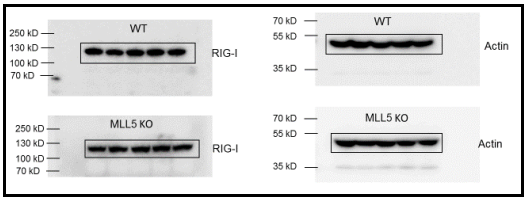
g


250 kD
130 kD
100 kD
70 kD
55 kD
MLL5
70 kD
55 kD
35 kD
Actin
250 kD
130 kD
100 kD
RIG-I

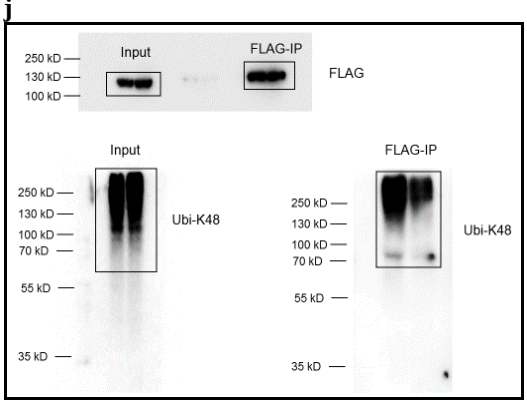
h


WT
MLL5 KO
RIG-I
Actin
250 kD
130 kD
100 kD
70 kD
55 kD
35 kD

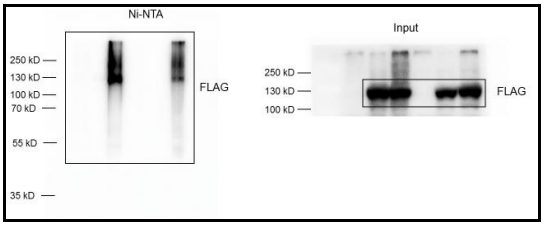
i


WT
MLL5 KO
RIG-I
Actin
250 kD
130 kD
100 kD
70 kD
55 kD
35 kD

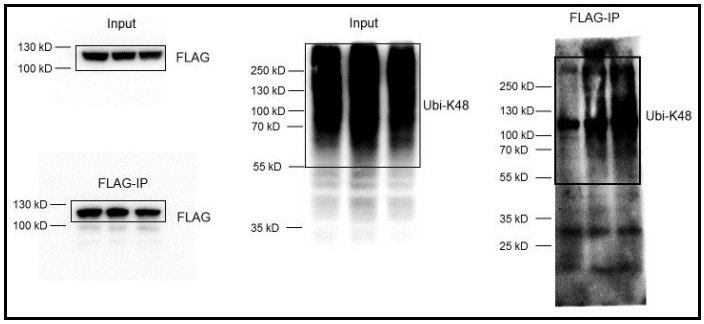
j


Input
FLAG-IP
FLAG
Ubi-K48
250 kD
130 kD
100 kD
70 kD
55 kD
35 kD

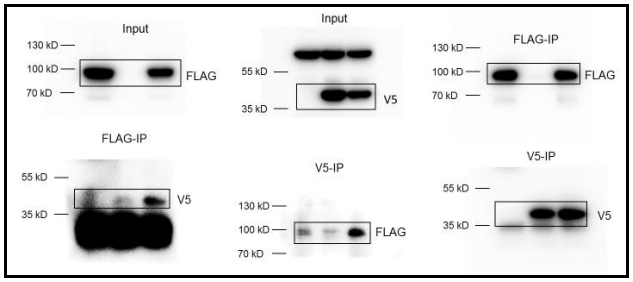
k


Ni-NTA
Input
FLAG
250 kD
130 kD
100 kD
70 kD
55 kD
35 kD

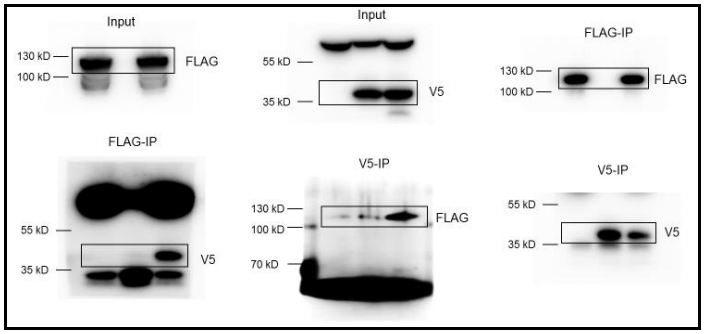
l


Input
FLAG-IP
FLAG
Ubi-K48
130 kD
100 kD
250 kD
70 kD
55 kD
35 kD
25 kD

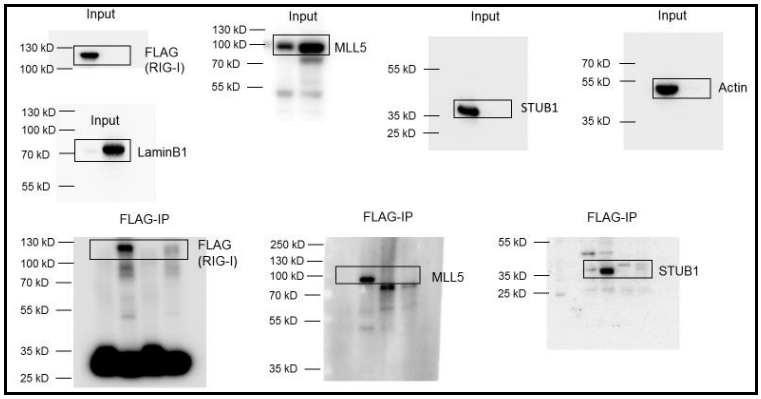
m


Input
FLAG-IP
V5-IP
FLAG
V5
130 kD
100 kD
70 kD
55 kD
35 kD

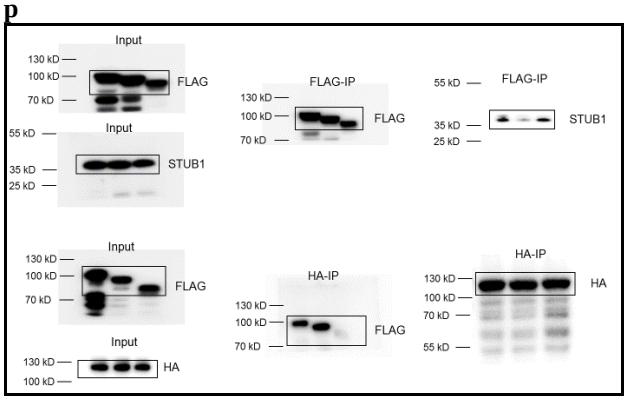
n


Input
FLAG-IP
V5-IP
FLAG
V5
130 kD
100 kD
70 kD
55 kD
35 kD

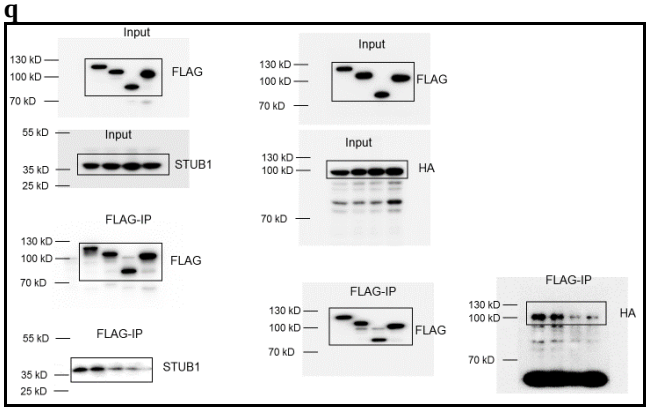
o


Input
130 kD
100 kD
FLAG
(RIG-I)
Input
130 kD
100 kD
70 kD
55 kD
MLL5
Input
55 kD
35 kD
25 kD
STUB1
Input
70 kD
55 kD
Actin
35 kD
130 kD
100 kD
Input
70 kD
LaminB1
55 kD
FLAG-IP
130 kD
100 kD
70 kD
55 kD
35 kD
25 kD
FLAG
(RIG-I)
FLAG-IP
250 kD
130 kD
100 kD
70 kD
55 kD
35 kD
MLL5
FLAG-IP
55 kD
35 kD
25 kD
STUB1

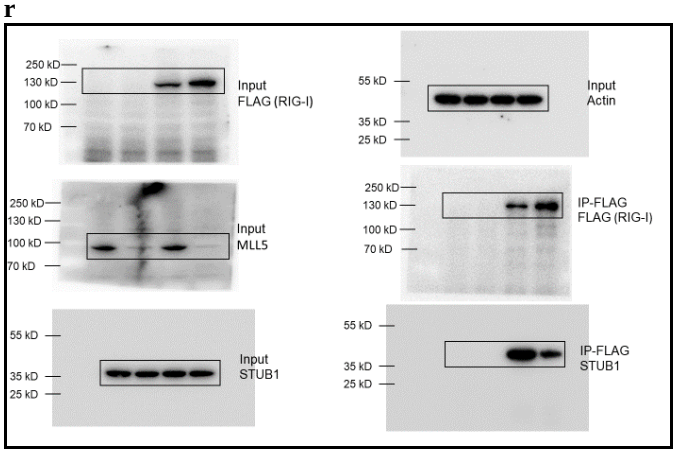
p


Input
130 kD
100 kD
70 kD
FLAG
FLAG-IP
130 kD
100 kD
70 kD
FLAG
55 kD
FLAG-IP
35 kD
25 kD
STUB1
Input
55 kD
35 kD
25 kD
STUB1
Input
130 kD
100 kD
70 kD
FLAG
HA-IP
130 kD
100 kD
70 kD
FLAG
HA-IP
130 kD
100 kD
70 kD
55 kD
HA
Input
130 kD
100 kD
HA

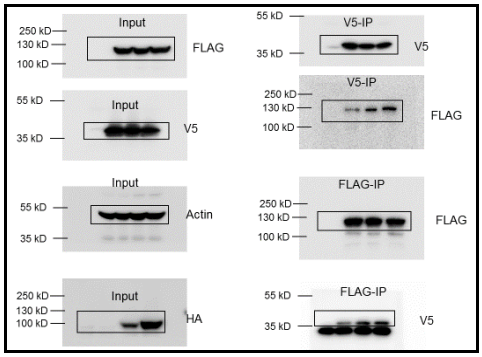
q


Input
130 kD
100 kD
70 kD
FLAG
Input
130 kD
100 kD
70 kD
FLAG
55 kD
Input
35 kD
25 kD
STUB1
Input
130 kD
100 kD
70 kD
HA
FLAG-IP
130 kD
100 kD
70 kD
FLAG
FLAG-IP
130 kD
100 kD
70 kD
FLAG
55 kD
FLAG-IP
35 kD
25 kD
STUB1
FLAG-IP
130 kD
100 kD
70 kD
HA

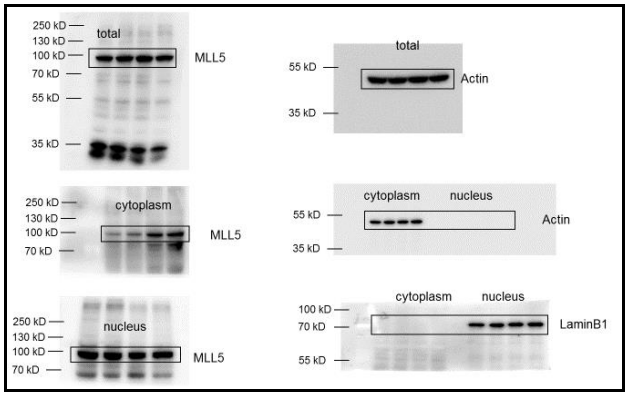
r


250 kD
130 kD
100 kD
70 kD
Input
FLAG (RIG-I)
55 kD
35 kD
25 kD
Input
Actin
250 kD
130 kD
100 kD
70 kD
Input
MLL5
250 kD
130 kD
100 kD
70 kD
IP-FLAG
FLAG (RIG-I)
55 kD
35 kD
25 kD
Input
STUB1
55 kD
35 kD
25 kD
IP-FLAG
STUB1

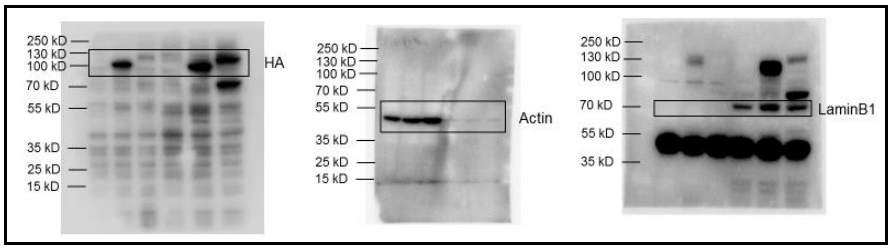
s



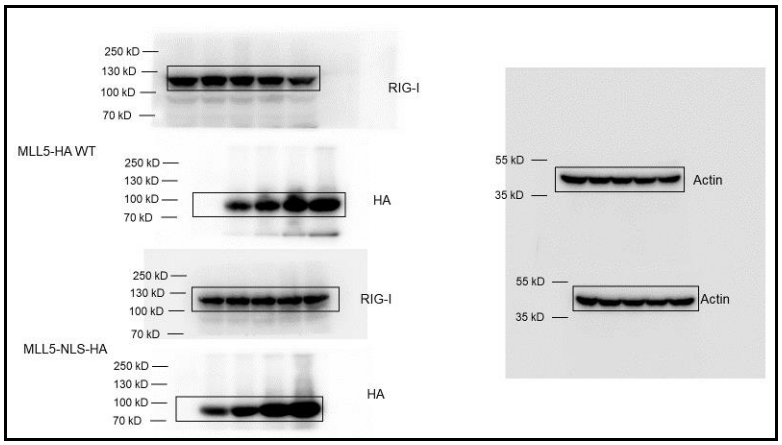
t



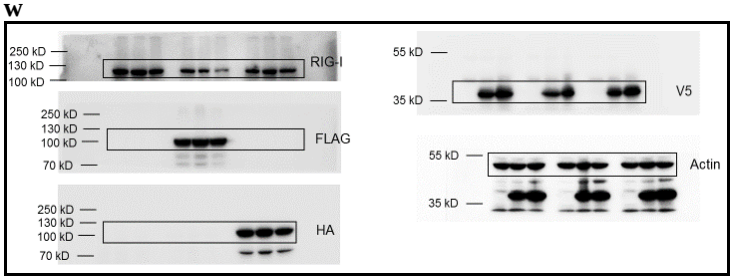
u



v

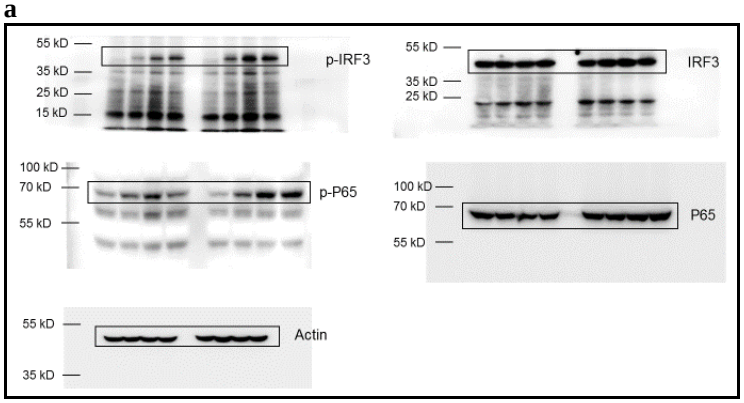


w



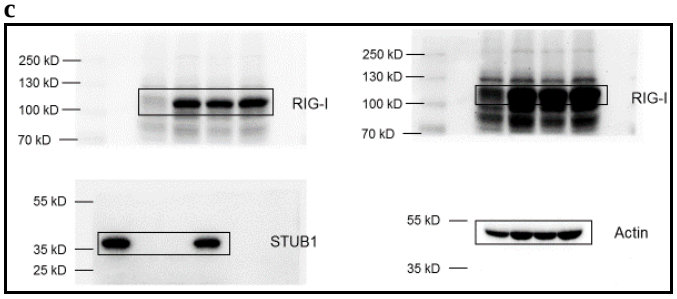
Supplementary Figure 8. Uncropped dcans of the western blots related to main figures. **(a-c)** related to Figure 2 a-c, **(d-g)** related to Figure 4 a-d, **(h-l)** related to Figure 4 g-k, **(m-o)** related to Figure 5 a-c, **(p-q)** related to Figure 5 e and f, **(r-s)** related to Figure 6 a and b, **(t-w)** related to Figure 7 a, c, e and f.

a

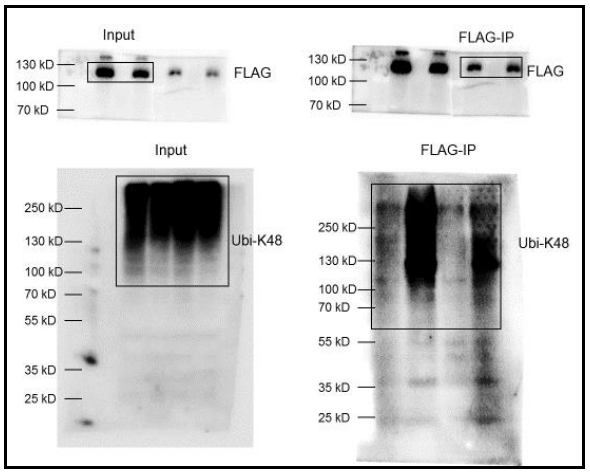


b

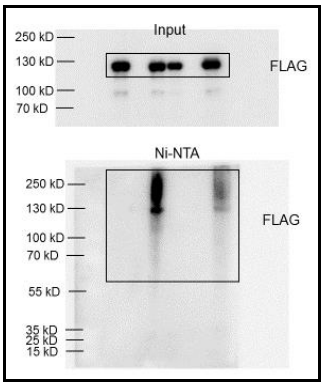
c



d



e



Supplementary Figure 9. Uncropped dcans of the western blots related to Supplementary Figures. **(a)** related to Supplementary Figure 3 c, **(b)** related to Supplementary Figure 4 c, **(c-e)** related to Supplementary Figure 5 d-f.

Supplementary Table 1. Relative band densities indicating RIG-I protein levels in WT and *MLL5*$^{-/-}$ HEK293T cells treated with CHX (100 μg/ml).

Time	0h	15h	20h	25h	30h
WT	1.0	0.90	0.68	0.69	0.60
MLL5$^{-/-}$	1.0	0.98	1.02	1.05	1.0
Time	0h	6h	12h	18h	24h
WT	1.0	0.95	0.68	0.48	0.47
MLL5$^{-/-}$	1.0	1.02	0.94	0.86	0.88

Actin served as a loading control. Band density indicating protein amount was quantified using Image J software.

Supplementary Table 2. Candidates RIG-I-interacting proteins identified by mass spectrometry.

seq count	gene name
703	DDX58
91	HUWE1
34	MLL5
41	TRIM28
40	HECTD1
29	HERC5
25	RNF135
20	TRIM21
13	TRIM25
9	UBR5
7	ECM29
6	MUL1
4	HUWE1
3	RNF138
4	HERC1
4	STUB1
3	MARCH7
3	XIAP
3	UBL4A
3	UFL1
2	KCMF1
2	DTX3L
2	RNF187
2	TRIM26
2	RANBP2
2	CBX4

Supplementary Table 3. Relative band densities indicating MLL5 protein levels in the nuclear and cytoplasmic fractions in WT HEK293T cells infected with VSV-GFP (MOI:1).

Time (VSV-GFP)	0h	5h	10h	15h
Cytoplasm	1.0	1.19	1.65	1.70
Nucleus	1.0	0.96	0.83	0.85
Time (VSV-GFP)	0h	4h	8h	
Cytoplasm	1.0	1.10	1.62	
Nucleus	1.0	0.95	0.87	

Actin served as a cytoplasmic control. Lamin B1 served as a nuclear protein control. Band density indicating protein amount was quantified using Image J software.

Supplementary Table 4. Relative band densities indicating RIG-I protein levels in *MLL5*$^{-/-}$ HEK293T cells transfected with different amount of MLL5-HA or MLL5-NLS-HA plasmids (0, 300, 600, 1000 and 2000 ng).

Amount of Plasmids	0	300ng	600ng	1000ng	2000ng
MLL5-HA	1.0	0.46	0.46	0.49	0.37
	1.0	0.85	0.80	0.76	0.75
MLL5-HA-NLS	1.0	1.22	1.16	1.02	0.93
	1.0	1.04	1.08	1.02	1.0

Actin served as a loading control. Band density indicating protein amount was quantified using Image J software.