

Rig-I regulates NF- κ B activity through binding to *Nf- κ b1* 3'-UTR mRNA

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Retinoic acid inducible gene I (RIG-I) senses viral RNAs and triggers innate antiviral responses through induction of type I IFNs and inflammatory cytokines. However, whether RIG-I interacts with host cellular RNA remains undetermined. Here we report that Rig-I interacts with multiple cellular mRNAs, especially *Nf- κ b1*. Rig-I is required for NF- κ B activity via regulating *Nf- κ b1* expression at posttranscriptional levels. It interacts with the multiple binding sites within 3'-UTR of *Nf- κ b1* mRNA. Further analyses reveal that three distinct tandem motifs enriched in the 3'-UTR fragments can be recognized by Rig-I. The 3'-UTR binding with Rig-I plays a critical role in normal translation of *Nf- κ b1* by recruiting the ribosomal proteins [ribosomal protein L13 (Rpl13) and Rpl8] and rRNAs (18S and 28S). Down-regulation of Rig-I or Rpl13 significantly reduces *Nf- κ b1* and 3'-UTR-mediated luciferase expression levels. These findings indicate that Rig-I functions as a positive regulator for NF- κ B signaling and is involved in multiple biological processes in addition to host antiviral immunity.

Retinoic acid inducible gene I (RIG-I) is a cytosolic pattern recognition receptor that senses viral RNAs and triggers innate antiviral responses through induction of type I IFNs and inflammatory cytokines. However, whether RIG-I interacts with host cellular RNA remains undetermined. Here we report that Rig-I interacts with multiple cellular mRNAs, especially *Nf- κ b1*. Rig-I is required for NF- κ B activity via regulating *Nf- κ b1* expression at posttranscriptional levels. It interacts with the multiple binding sites within 3'-UTR of *Nf- κ b1* mRNA. Further analyses reveal that three distinct tandem motifs enriched in the 3'-UTR fragments can be recognized by Rig-I. The 3'-UTR binding with Rig-I plays a critical role in normal translation of *Nf- κ b1* by recruiting the ribosomal proteins [ribosomal protein L13 (Rpl13) and Rpl8] and rRNAs (18S and 28S). Down-regulation of Rig-I or Rpl13 significantly reduces *Nf- κ b1* and 3'-UTR-mediated luciferase expression levels. These findings indicate that Rig-I functions as a positive regulator for NF- κ B signaling and is involved in multiple biological processes in addition to host antiviral immunity.

... n h u in n infl u u r i u li (22). h r -
i l r r h i - f i n u l l i -
l i h n u u r r i n n i l h - i 2
(α 2) r i n n - l l i i n (23), r r i r n -
l i i h r i n i n r r n n n n n
i n i n r i n (l) r i n n l i i n (24,
25), n i n r i l i i n i n i h *Escherichia coli*
u u r r - i n h i i r i (26).
h r r , I - I u u r l i l i n l i n u l i l
i l i l r i n l i n r n i n n n l i r l N .

Results

Rig-I Interacts with Multiple Cellular mRNAs. ...
n i l i n r i n i - l i h h l l r u , N , r -
r u N i u u n r i i n (I) i n n n i - i
n i r u h l l l h u r i n l n i - l l
l i n (1 . 4 . 6) , h i h l r i - r i - i - l .
l N r r u h l i - r i i u -
l i f i i n r l l (i . 1 A) , n n l i n n i n -
014868 h l n u i r r . I n u r i n N
n n n i - n i - i - i - r i i ,
n h h i n l i n n i i 298 r u r h n -
r l h i n r l (l r i > 2) . n i n l , h i h
h i h l r i (5 . 9) i n i f i *Nf- κ b1/p105* n l r
r r r r i n 105 , h i h n r u - u i r -
i n n r h u r N - κ 1 n i 50 (i . 1 B n
l 1) . u r r i n n , r h u N i h l r i
> 2 , i n l i n *Nf- κ b1/p105*, *Inhibitor of kappaB kinase epsilon*
(*Ikk ϵ*), *Irf3*, *Rous sarcoma oncogene (Src)*, *kinesin family member*
24 (*Kif24*), etc., r r i f i - i n h u r r i i
(i . 1 C) n i n n i - i - r i i r u h l l -
f i r l () (i . 1 D) . r h r u r , h i n r i n
i - l i h *Nf- κ b1* n f r u i n n i - i - r i i r u
h i n i r r i n n f i r u i n i - l r i n i h
l N r r u l n (i . 1 A) . i
n i n r h N - κ - i i n i n l i n (27,
28) . l h i l N - κ i i n n h
i n r i n h u l l i n i . 1 C r r i h
I , n n - h N - κ i i n
h n n h i n r i n i - l i h *Nf- κ b1* u N (i .
1 B) . h h i - l i n r i h u l i l h
l l r N , i l l *Nf- κ b1/p105* . h r u N u l l

Author contributions: Z.C. and Z.-G.W. designed research; H.-X.Z., Z.-X.L., Y.-P.S., J.Z., S.-Y.L., X.-S.L., Q.-H.H., Y.-Y.X., H.-B.Z., S.-Y.D., H.-F.C., G.-Y.Z., and Y.K. performed research; H.-X.Z., Y.-X.L., J.F., S.-J.C., and Z.-G.W. analyzed data; and Z.C. and Z.-G.W. wrote the paper.

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GENETICS

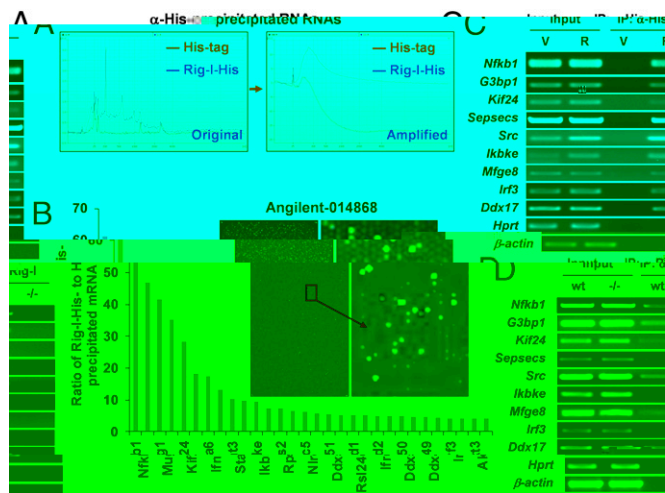


Fig. 1. Multiple cellular RNAs are immunoprecipitated with Rig-I. (A) Quantitative or qualitative evaluation for the total RNA extracted from the pooled anti-His precipitates from total cell lysates of 1.B4.B6 cell line stably expressing His-tag or His-tagged Rig-I. The graphs shown are of RNA abundance against molecular size before or after two-round amplification. (B) Equal amounts of labeled cRNA samples were analyzed by using Agilent 44k Whole Mouse Genome Microarray (Agilent; 014868). Some of the positive mRNAs were shown in the histogram. The indicated mRNAs were further detected by RT-PCR in either anti-His precipitates from the cell lysates of the 1.B4.B6 cell line stably expressing His-tagged Rig-I (C) or anti-Rig-I precipitates from the cell lysates of WT but not from that of *Rig-I*^{-/-} MEFs (D). X-box binding protein 1 (*Xbp1*), hypoxanthine-guanine phosphoribosyl-transferase (*Hprt*), and β -actin are shown as negative controls.

Interferon alpha-6 precursor (Ifna6), *Interferon gamma receptor 2 (Ifngr2)*, *Interferon alpha B (Ifnab)*, *NLR family, CARD domain containing 5 (Nlr5)*, *Src*, *Stat3* n n l i n n h N - i n i n r i n l n i n r l n i l r l i n n r i n i r l i n h r n l i n N , n u r n r i n l n u h N i i n n i i n (29). h , h f i n i n u i l l h i - r r u i n i r n i n b r h u l l u h n i u .

Rig-I Interacts with *Nf- κ b1* 3'-UTR mRNA and Positively Regulates *Nf- κ b1* Expression.

Nf- κ b1 mRNA fragments as indicated in IgG or anti-Rig-I precipitates from the reactions of Rig-I protein with 5' or 3' mRNA fragments are detected by RT-PCR using the primers located within overlapping regions between two fragments. (*f estM()*)TJETETBT/F11Tf1(1h55me)(nt1(s)-(0)T

Rig-I^{-/-} MEFs. (i. 2C). r n i n *Rig-I*^{-/-} i h i - I - r i n r n 3 r *Luc-p105* 3' - , n h *Luc-105* 3' - - n l i r i i l l r i n n n n n r u r i h l l n r l (i . 2D). i u l r r n *p105* 3' - i - i n l i r i i l r i n N L 3 3 l l (i . 2B). h h i n i n h i - i r i r r N - κ 1 r i n h r h i n i n 3' - r h r f i n h i n i n i - i i h i n *p105* 3' - , i r n r n r i r u *p105* 3' - i l l - r i n i . 2C u r i n r n r u h l i r i n r i n i n 3 , r i l . n i n i r i n i n i n r u i n n i - i n i r n r i u N r - u n n i n l i r n *p105* 3' - n i n i n i n i n i . 2D r l h l l r u n d r i i i n h r i i l i n h i - i n r i h l r u n a n c . h r n l l n h *p105* 3' -

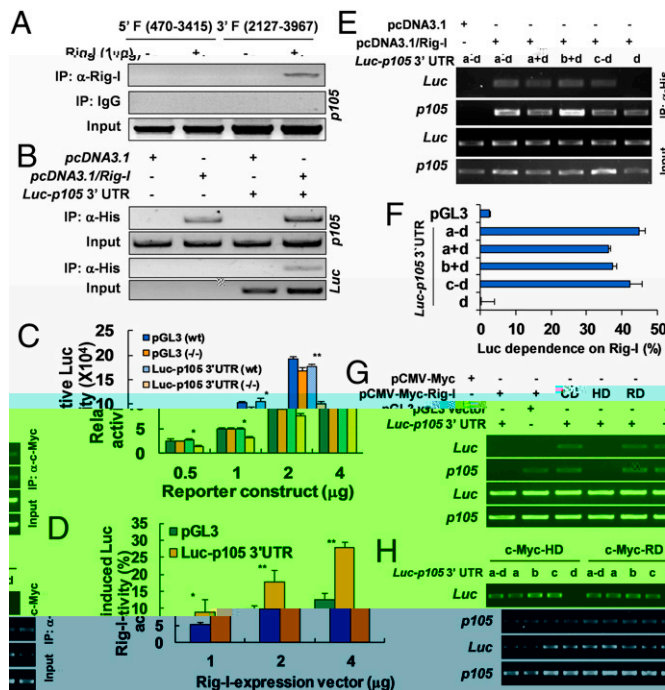


Fig. 2. Rig-I interacts with *Nf- κ b1* 3'-UTR mRNA and positively regulates *Nf- κ b1* expression. (A) *Nf- κ b1/p105* mRNA fragments as indicated in IgG or anti-Rig-I precipitates from the reactions of Rig-I protein with 5' or 3' mRNA fragments are detected by RT-PCR using the primers located within overlapping regions between two fragments. (*f estM()*)TJETETBT/F11Tf1(1h55me)(nt1(s)-(0)T

Rig-I Can Recognize Three Distinct Tandem Motifs Enriched in *Nf- κ b1* 3-UTR mRNA.

(a-d, ii nr1) n h in i i l r u n a, b, c
n d (n i nr1) in h r i i i h i i l
r h r n f i r u n i n i in- l l r u n (i.
2E). r h r r u n a n b i h r u n
d n in i l () n i n r h u i n h u i i n
in 3, r i l. r n i n n l r i. 2B
h h l l r u n d 3', l l n n
p105 r r i i i h i i l (i. 2E). h f i n i n i n
i h h r r l b r i i i n i n i h r i n
p105 3' u N h i n i h h r h i n i n r
n i n l i n r l i n l i r i i. r h i
i n r n r r R i g - I ^{-/-} i h 3 r L u c - p 1 0 5
3' n r h r n i r n 3' r u n
h n i n i. 2F. h r i l i r i i i n R i g - I ^{-/-} r
h i n i n h h L u c - p 1 0 5 3' n r i h
h i n r i n n i n 3' r i n i i l r u n (a, b, n
c) r n i l l l i r i i i n R i g - I ^{-/-} r
h n i n l l, u n i n u r n n n i i l i n r -
l i n l i r i i. r, 3 n r l r r 3
i h i n r i n r u n d i l n i r n i n l i r
i i n n (i. 2F). h
i n i n i i h i f i 3' n p 1 0 5 u N
l n i n i n l r l i n r i n r n l i n r l i n
h i n u l i l i n i n i r i l i n b r -
n i l u N r u n r h i n p 1 0 5 3' h
i r n r u n r i n i r n u n i n
i i l h b r r u n h r n n i u l i i.
i h h i n u i n, N I 3 3 l l r r n i h L u c -
p 1 0 5 3' n h r n i n i n, i n, r
r n n i i l, r i l. I r l h l i r n
n n p 1 0 5 r r i i i h h / h i
u i n () n h - r u i n l r l r u i n ()
n h N - r u i n l r r i u n u i n () i l
(i. 2G), i n i n h i n r i n i i h p 1 0 5 3'
u N i u i b r h h n u i n. I n h i
u i n r i n r i h h r r r n r h r i n
i r n r u n p 1 0 5 3' r i n l, u N r -
u n a, b, n c p 1 0 5 3' r r i i i h h
(i. 2H), i n h h i n r i n i u r l i
n n n h r u r n - n r r r r.
l n h f i n i n, h h r p 1 0 5 3'
u N r u n h i n i h i l r l i i n i i l
i n l n r l N n N i h 5' - n h h n r r.
h i n, r R i g - I ^{-/-} r r n i h 3 r
L u c - p 1 0 5 3' n r h r n i n p 1 0 5 3' r u n. I
n h I N - β r i n i n l r r n n r n
h n i n h r n p 1 0 5 3' r u n, h r n r u l
r n n l l (i. 2F). r h r u r, n i h r n r R i g - I ^{-/-}
, h h r i r l r n i h p 1 0 5 3' u N
r u n, h I N - β r n p 1 0 5 3' u N r -
u n, h r i l r u i i r i n i n i n.
I N - β r i n i n R i g - I ^{-/-} (i. 2G). h
i r n r u n n n l i r l N. I i u r l i h i l
l r l i n r l i n r i n r n l i n i i n i n l l r
u N r h r h n i n i n I I N r i n.

i i i n l r r l n u n n n h u
i n i f i n r u n d, h i h n l i n i i b r h
i n i n. i n r i h l r u r (i.
3A). h i f i n i u l r r u h n i i -
i n r u i n l n h n i l u i r, 3. N
r r r i n n l i n n l i i l N
r (30) r l h b r l n u u i (27-45) r
l f i n l l n h r u u r l h i r -
r h n n r l N (45, 70%, 50% l i n) (i.
34). h n, b r n u u i n n r l N r
i h I - I i n i r i 2.5, n h r r l
i n r u i n I - I - N u l r r u r h n
r i n n (: 2) (17). h n r
u l r r u l l r n u i u l i n i n h
11 u l i n (31, 32). I n h r u
N u i r n 2 n 5 r h u l
I - I n b r n u u i ; h r n r l N i n h
u l, h u h h r r u l (~13) (i. 3B). i
n l l, h r r l i u l i n i l l - u h i n i n
i n r n I - I n n u N u i n n r l
N h i n i n - r n r l l i n h l l r
h n i / i n - l u n n r r u h. I
h n h u h l r i n i n - r n r n r
r u i n h u l n I - I n h l - n u
u i i n u n i h h u l n I - I n n
r l N, i n h h u l i h b r u i r
u r l h h h i h N n r l (i. 3C). l l h
i n h h i h h n u N u i u h
u i h i n i n I - I i h p 1 0 5 3' u N
r h i i i i, i n r l - r n l - n u u i
(46-66) r n r l n (60) n l i r i n
r i n n r u n d i n L u c - p 1 0 5 3' n r h
u r u n l i n i n h r i i u n (i.
2E), h l i r u N, r r u h L u c - p 1 0 5 3'

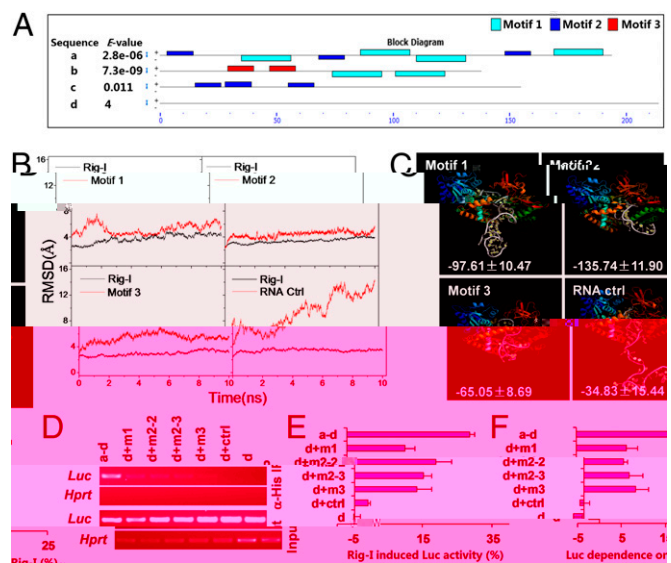


Fig. 3. Structural features of *p105* 3'-UTR mRNA for Rig-I binding. (A) The distribution of the three enriched motifs in *p105* 3'-UTR mRNA fragments. (B) The C5' and C α atom rmsds for dual-tandem motifs or control RNA and Rig-I protein are shown. (C) The binding model between Rig-I and dual-tandem motifs or control RNA. The binding-free energy data are labeled in each

nr nlin 3', u i 1,2, n 3, n ih
 nrl n rd ln, in i-i r i-
 i (i. 3D). r iu rnl, h l i r ii
 r Luc-p105 3', n r ih h in rin
 u i 1,2, n 3 finl in i-i r r in
 r, hr r r r r ih nrl n in r
 rd ln h n r n i-i (i. 3E). r, r, h u
 riu n r i. 2F r ru in h n r
 hr an h u i. h r l i u l r h h nin i. 2F
 ini h Luc-p105 3', n r ih h n u
 u i r n n n h r n i-l in h r i n
 l i r ii u r ih h in nrl n
 in r r r u n d ln (i. 3F). rhr nfr u h
 in in i h n u u i ih i-i, h u i
 r h r r u in i n r ih l
 r u r in h ih riu n l i n r i-i in
 (i. 1C n D). h h h u u N
 Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase
 (Sepsecs) h r r n n r u i r r in h u - r
 h r n u (i. 3B), h r n n r n l n u i i n
 fi in h riu n ll u n r n in r ih i-
 I (i. 3C). h fin in r h i-i in r in ih h
 ll r u N n r h r r n h n h r l h u i in
 n u in u i in i-i in in. n h r, u n l
 l h h n u u i in p105 3', u N r r r
 nil r i-i in p105 3', u N h in in
 r n in ll r i r r in r n l i n r l in.

Rig-I Regulates Nf-kb1 Translation via Interacting with Ribosomal Components.

r h r n r n h i l i l i n i f i n
 i-i in in Nf-kb1 3', u N, fir h h r
 h in r in h u i n N-k1 u N n
 l, ni h r u i- (i. 4A) n r N r h r n l
 (i. 4B) r Nf-kb1/p105 r n i in l n n
 i u l in h i n i f i n i r n in p105 ll p65
 u N l l n n n Rig-I^{-/-} ll n r h u
 n i i n, p105 u N l l r u in n h n in h l. 4. 6
 ll in r in Rig-I i N (i. 4C). r h r u r, h
 r u r l n r l. 4. 6 ll l r in i-i r
 r ih 10 μ/u in u in (), ll
 r u in l i h r in h n i-i (i. 4D) r n
 n i-i r in (i. 4E) u r ih h i r n
 r l. h n, n l h r in l l 50 n i r r r
 105 n r u r l n n l in r n i n l, h
 105 n 50 r in r i n i f i n l r in Rig-I^{-/-} ll
 i h r ih i u l l i n u r ih h in ll,
 h r h r in l l 65 in h u r u
 r l (i. 4A). i u l r r l r r in i-i- i n ll
 (i. 5A). In r in l, n i-i r in in h u
 ll in n n h n r n 105/ 50 r in
 i u l l i n (i. 4B). h fin in h in i h i-i
 r i l r n r u l r in 105/ 50 r in r
 h h r i-i in l in 105/ 50 r in r, h
 n Rig-I^{-/-} l n r r ih 10 μ/u l l
 h i u i (), ll i u l l i n r n l n l
 i r l h h 105 n 50 l i n u r l r
 n n, l i r l i l l r l l 105/ 50
 in Rig-I^{-/-} l n (i. 5B). r h r N-k1/ 50
 n l r r n l i n r i n n i u l l i n n
 h u r ll, 105 n 50 r i n i f i n l r
 in l ll n u r in Rig-I^{-/-} ll i h r
 ih i u l l i n r i n l, N-k1/ 50 in n l r r in
 Rig-I^{-/-} ll n l n r i u l l i n (i.
 4C). i u l r r l r r ih i u u n f l r n
 in n r 105/ 50 (i. 4D). n i n ih h fin in, N-
 κ i i n i n i f i n l r n d r n
 i u l l i n in Rig-I^{-/-} (i. 4E). I l l ll
 r i-i in n n u n n r (i. 4F). i u l r
 h n in h r in l l 105/ 50 n N-κ i i r
 r h r r in ih r l (i.) r u n

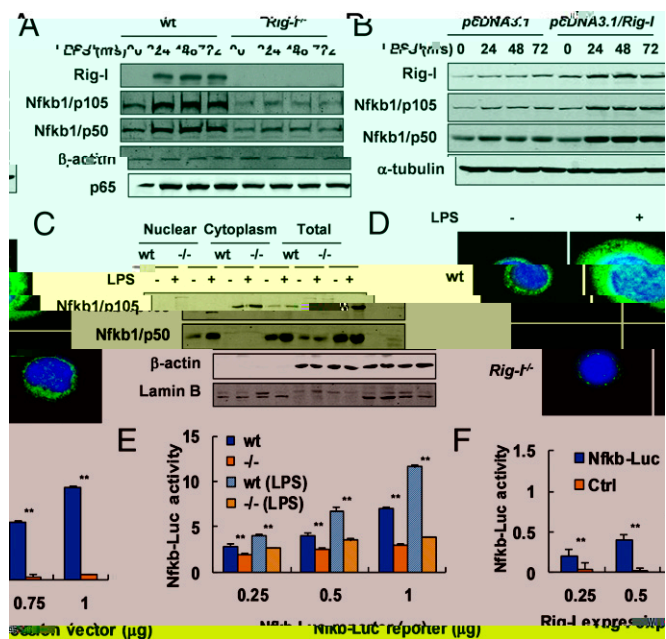


Fig. 4. Rig-I is a positive regulator for NF-κB activity. Western blots for the indicated proteins in splenocytes (A) and the 1.B4.B6 cell line (B) stably expressing Rig-I. (C) Immunoblots of Nf-kb1/p105/p50 in total cell lysate, cytoplasmic and nuclear fractions from splenocytes. (D) Confocal images for Nf-kb1/p105/p50 (green) distribution in splenic B lymphocytes with an original magnification of 400x. (E) Nf-kb1-Luc activity in wt and Rig-I^{-/-} cells treated with LPS. (F) Nf-kb1-Luc activity in wt and Rig-I^{-/-} cells transfected with Nf-kb1-Luc reporter.

i r n i u in (i. 6). n h fin in, li
 h i-i r i l r N-κ i i i r l in N-κ l
 r in r n i n l, i l l r in r n l in
 r, h i n h r u in r r ih
 i-i r l 105 r n l i n i in in i 3'- u N
 n h i l n r l l h i-i r r i r n
 l i n l u h i n r in h i l i n p105 3'-
 i i r in r n l i n. h i n, r l i n l
 n i f i n u r u r (i.) r
 r u ih h l i u u n r i i ih n i- i n i
 r u h ll 1. 4. 6 ll l r in i-
 i-i. u i n ih h u ll r in i
 l n, r l l i n i 28 r in (l i n r n f i
 n in r l > 80%) n i l l in r in ih i-i (l
 2). u n h u i l n i n i r i u l r
 in h i u n n h l r 60 n i r u,
 ll3 (60 r u l r in 13). ll in h i l, r i
 N r r in i u u n r i i n in n i
 i in ll3, i- n i-i, r i l l ll
 l 1. 4. 6 ll l r in i- (h n in f i.
 5A) r i- i-i (h n in f i. 5A) r
 i u u n r i i in ih u n l n l n i ll3.
 r u n n n n i-i r r r
 i- i-i n in h r i i. n h n
 r r, n n ll3, ll ll8, n h r u n n
 h 60 n i n. i u u n r i i r u h u
 l ih i- i-i in n n i- i n i
 n i n r l n, l i- i, ll3, ll8, r-β- in r
 n in h l r i i r u h u l (i. 5A).
 i n i- r l, h r h r h i l
 in r in i-i ih ll3 n ll8, h l n in h 60
 n i r u n n i l l in r in ih h h r
 (i. u. i. n. / - i). l n h i fin in,

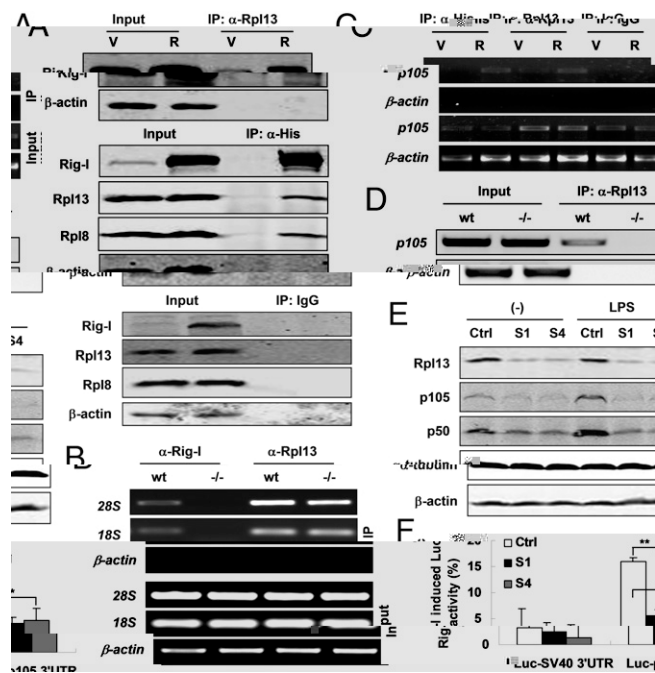


Fig. 5. Rig-I regulates Nf- κ B1 translation via interacting with ribosomal components. (A) Immunoblots for the indicated proteins in IgG, anti-His, and anti-Rpl13 precipitates from the lysates of 1.B4.B6 cell line stably transfected with empty vector (V) or His-tagged Rig-I expression vector (R). (B) RT-PCR for 28S, 18S rRNAs in anti-Rig-I, or anti-Rpl13 precipitates from the lysates of WT or *Rig-I*^{-/-} MEFs. (C) *p105* mRNA by RT-PCR in the precipitates under the same experimental settings as in A. (D) RT-PCR for *p105* mRNA in anti-Rpl13 precipitates as in B. (E) Western blots for the indicated proteins in NIH 3T3 cells stably expressing *Rpl13* siRNA (S1 and S4) and control siRNA (Ctrl). (F) Luc activity in the same cell lines as in E but cotransfected with Rig-I expression and Luc-reporter constructs with *p105* or SV40 3'-UTR is expressed as a percentage relative to that of Rig-I empty vector. One of three independent experiments is shown as mean \pm SD ($n = 3$, * $P < 0.05$; ** $P < 0.01$).

rnl in hr, h h in r in. n p105 3': n
i-I h h [13 in.

Discussion

r i i r l h I-I l i l i n r i h r i n
N u l l i h i f i r r l r . i n h r l
r l r r n l n I-I i n i h r l i n - r r
N - n n , i u u r l i h r i u r . n -
n n r i r n f i r i n N l i n l r -
i r r I-I r n i n . I n h i r u n , i l
h r l h i l i h I-I r n i r r
i h h l l r N i h r i n r r l r r
n i l i n i h i-I l r l N- κ 1 r n l i n
h r h i n r i n i h l l r Nf- κ B1 u. N . l i i h
r r l i i n p105 3': h n n i i-I i n i n ,
h n i i i n r u i n l n p105 3': r u n
h i n r i h i-I r r r u . I n r i n l , f i n h :
(i) h r i i n u i r n i h i n i-I i n i n r u n
(a, b, n c) . n i n r u n d, h i h h n i n r i n i h
i-I. h h r u i n n i n 11-21, r r r l r
u n h r u i n u i i n h u r r h r n u
u n b r i-I i n i n r u n . (ii) b r - i u n i n l N
r r r i n n l i n n l r l h b r u i i n
h u n u i h (48, 24, n 29 i n l n h, r -
i l) n f i n l n r u u r l h l i r r
h n r l N (44 .) . (iii) l r r n u i u l l i n
i l h l l - u h i n i n r n I-I n
l - n u u i , n u r l I-I u l i h b r
l - n u u i , n i h N n r l n . r u . (iv)
h i n i n - r n r n r u h u l i u h
l r h h h i n r l . (v) b r u i i n h u l r l
n u (24-51) i n u i h i n i n I-I i h p105
3' . (vi) i n l l , h r n r n n l i r
i i , r n I-I, h n i n l i u r n h
I-I i n u i i n r l i n N- κ 1 / 105 r i n n
h r, h u n r h I-I i n r i h 3' .
Nf- κ B1/p105 u. N b r h h r i n n r u i
i n l h l i r r , i u l i n h r i u r . n -
n n r i r n f i r i n N l i n i u r r i l
r i-I i n i n .
I n r i l l , r i n n h i i u l r h
n i r u u r : i i i i n l i n i n r u i n i n
n u r l i n . l l h i n l i n r i n n h i
n h r r r r l i n (33) . i-I i h n
i l r l N- κ 1 / 105 r n l i n h i r i n i n
i 3' . u. N , n h n i l b i i n i n
n r i n r n l i n . I n , 3' . u. N l r i l r l
i n r n n i n l r l i n n r i n u l i n
u. N l l i n , i i , n r n l i n (34, 35) . r r u
h i n i n i r u i N , 3' . h r r r i n u i h
i n r i h i f i N - i n i n r i n (36) . r n l i n l
r l u h n i u . n l i n r i n . n h 3' - r 5' -
u. N n N - i n i n r i n . n h u h u u
r i r i r l i n i n h u. N u l l i n l i n r i n -
r i n r i n . n h u. N r u i n i (37) .
n 3' . i n i n r i n , i-I i l i n r
i h h r r i n h i l i n 3' . i l l h r i n
i n l i n r i n r n l i n . 3' . i l l h r i n
n h r i i i h n i - i n i r u h l l l
h l i - l l i n l r i n i - i-I, n -
n i u u r r i i n n r n l i n , r l h i -
I i l i n r i h u l r i n 13 (113) , l l
n u l r i n 8 (18) . h r 60 n u n n .
13 . l n h 13 u l n u l r i n . I h n
h n h i u l r i n u h . r r u l i n i n
r r u i u n r i n i n h i (38) . 18 l n
h 2 u l n u l r i n . I i l i n h l u . I n
r , h r i n i i h h 5.8 r N , r l i l r i i
i n h i n u i n l - N , n i n i n h l n -
i n r 2 - i n i n i h u u l . n i n r . (h : //
u u . r / n r / 604177) . r r , 18 i r i n i l l

h i n h h r i-I i n r i h h u. l l 40 n i
n i u r i . h n r i i u. l l 28
(60 n i) n 18 (40 n i) r N r l
i u u n r i i r u h l l r i g - I - / -
i n n n i - l l 3 n i n l r u h h n
i n u n l n l n i i-I (i . 5 B) , i u l i n h i-I
i n r i h n n l h l r l u l l u l n i
h r i i i n 105 r n l i n . I h n h n h i-I
n i n i h p105 u. N l l i n i h p105
u. N , l i i i-I r i n ? l l 3 i n i h
r r u . n i i n l i u u n r i i i n i n
I (n i n r l) r n i i i n l l 3 n i -
(i i n r l) r i n . I n h p105 u. N
r i i i h n n . l l 3 r u h l l i h r
i h r r i n i - I . r h u n r
i i p105 u. N r r l i-I (i . 5 C) ,
i n h l l 3 i n r i h p105 u. N b r h i-I
r i n . u h i n l r r , i u l r r u n
r r u i n n R i g - I - / - . I h n h p105
u. N i n h r i i i h l l 3 n l r u h
l l (i . 5 D) . l h
l l 3 n 105 r i n , h N i l n i n r u n i n l
3 3 l l r r u . i n i N l i i n l l 3 .
h n i n i . 5 E , 105 / 50 r i n l l r i n i f i n l
r n h r n n u l l i n r l i h
i h n r l i n l l 3 . r h r u r , p105 3' -
u i l i r i i f i n l i n r n -
i-I - r i n r u r i h u . r ,
h i n i n i n i f i n l i u r n l l 3 i l n i n
(i . 5 F) . h i n i h l l 3 i r r 105

in r ihini in r I 5 (. u . i . n . . /
 - i /), n u - n n h u i i l -
 u n i i i n r r u h 40 n u l n i n 48
 i i i n u l n i n 40 n 60 n i (39). h ,
 u r h I J r i i i n h r r n u
 u l , u N i r l r i n , r r i u r l i n . I n r
 h i n i n , 18 n 28 r N , h u n n 40 n 60
 n u l n i , r h n i n r i h i - I . r h r i -
 n u r r u Rpl13 u n i n r i u n . h h h
 N - k 1 / 105 r i n l i n i n i n l r i h h i
 n r l i n 113 . n i n i h h i f i n i n , p105 3'-
 - u i l i r i i i r i n N I 3 3 u l
 r i n Rpl13 i N , n i n , u n r i n r i l r l
 h 3' : i n r l i n N - k 1 / 105 r n l i n .
 I n n l i n , r u n r h i - J n i n
 i i r l r N - k i n l i n . h r l r
 i - J i n n n h i n r i n i h h u l i l i
 i h i n 3' : Nf- k b 1 u N h r h i / h l i
 u i n n - r u i n l r l r u i n h i l h l i
 r r r u i h h n u u i n r i h i h i n p105
 3' : i r i l r i - J i n i n r i n l i n . I n
 i i n , i - J i r i n l i n i u u -
 l , u N i r l r i n , r r i u r l i n r r i n

n i u l u n n h i l i n i n p105 3' : u N .
 i n h i u r n r l i - J i n h n i r r n ,
 r l i n N - k i i , n r i n r n l i n , l l
 h u l i n r i n n r , h i h h u r i n h i
 , r h r i n n i i n h i " i n " u l l r
 n i n r . I i i t i h h r i l l r l
 n f i l r r n r n i n i l i l r n
 r l u n r r h .

Materials and Methods

All of the experiments were processed according to the standard protocols. The detailed materials and methods are provided as *SI Materials and Methods*. Primers used in this study are listed in *Table S3*.

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