Expanded View Figures

Figure EV1. Schematic model of cell-based functional screen and knockdown of TruB1 repressed expression of relative miRNAs in various human cells.

A Repressors of maturation such as Lin28 lead to high luciferase activity, and activators lead to low luciferase activity. This cell-based screen is assaying for a function directly on endogenous let-7 maturation, rather than affinity/binding. The gain-of-function screen also enables to identify regulatory factors that may cause lethality under loss of function conditions, such as with Crispr-Cas9 or RNAi-KD screens.

B qRT–PCR analysis for TruB1 mRNA expression normalized to GAPDH with TruB1 KD or ctrl (siRNA). TruB1 KD by siRNA significantly repressed the expression of TruB1 mRNA in HEK-293FT cells. Error bars show SD; $n = 3$. Significance was assessed using 2-tailed Student's *t*-test, < 0.05*.

C qRT–PCR analysis for let-7 families levels in A549 cells with TruB1 KD or ctrl (siRNA). TruB1 KD by siRNA significantly repressed the expression of the let-7 family. Error bars show SD; $n = 3$. Significance was assessed using 2-tailed Student's *t*-test, < 0.05*.

Source data are available online for this figure.

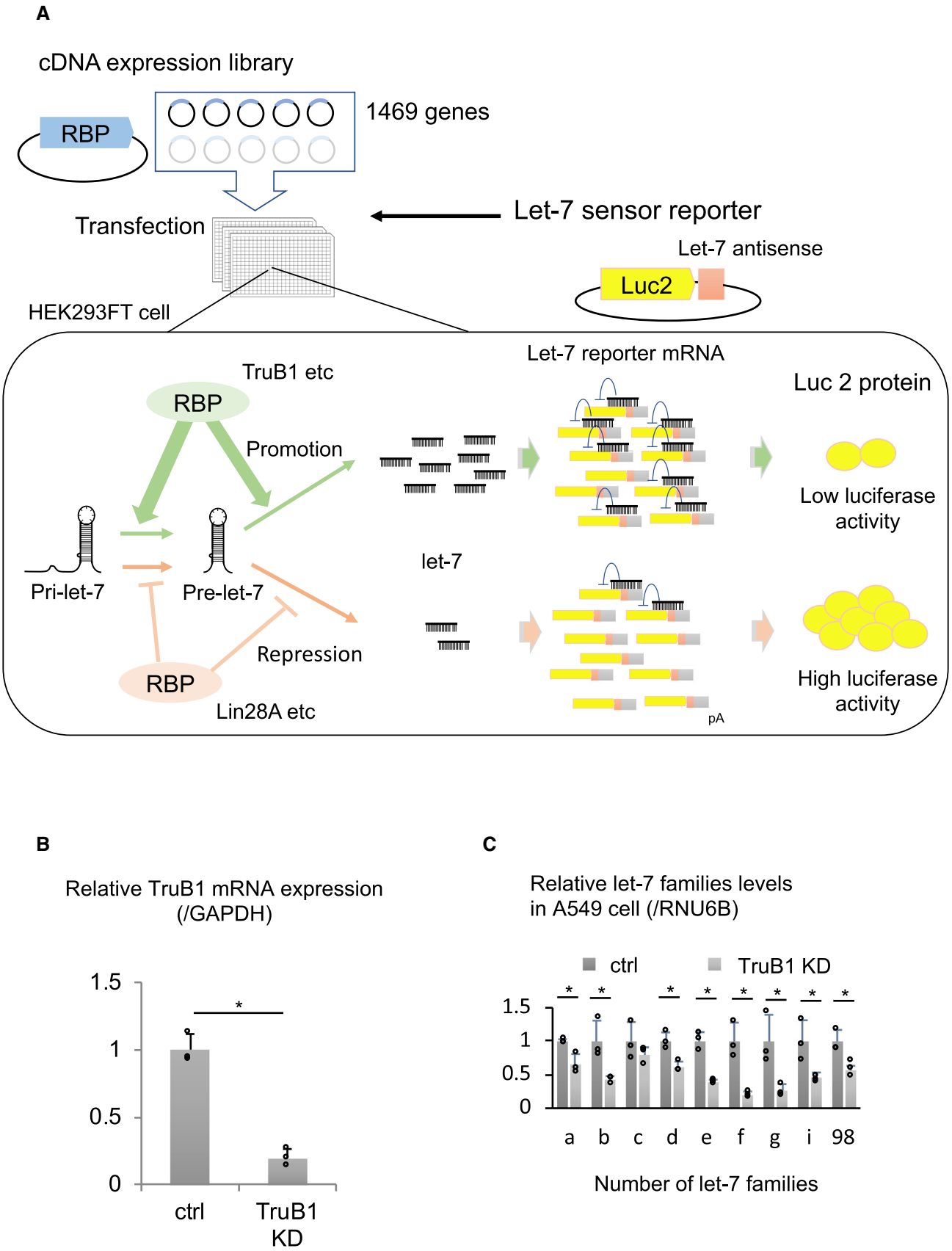
A
cDNA expression library
RBP
1469 genes
Transfection
Let-7 sensor reporter
Let-7 antisense
Luc2
HEK293FT cell
TruB1 etc
RBP
Promotion
Let-7 reporter mRNA
Luc 2 protein
Low luciferase activity
let-7
Pri-let-7
Pre-let-7
Repression
RBP
Lin28A etc
pA
High luciferase activity
B
Relative TruB1 mRNA expression (/GAPDH)
1.5
1
0.5
0
*
ctrl
TruB1 KD
C
Relative let-7 families levels in A549 cell (/RNU6B)
ctrl
TruB1 KD
1.5
1
0.5
0
a b c d e f g i 98
Number of let-7 families

Figure EV1.

Figure EV2. Impact of pseudouridylation enzyme activity on microprocessing of let-7.

A EMSA of 32p-ATP-labeled $tRNA^{Phe}$ mixed with recombinant TruB1, mt1, or mt2 at several doses. RNP: Ribonucleoprotein complexes.

B Protein expression of Flag-tagged TruB1 and its mutants. Protein expression was evaluated by Western blotting. Protein was isolated from HEK-293FT cells infected with tetracycline-inducible lentiviruses expressing TruB1, mt1, or, mt2, 5 days after doxycycline treatment.

C Location of pseudouridine sites were detected by CMC primer extension. Total RNA purified from HEK-293FT cells was treated with CMC. CMC-treated RNA were reverse-transcribed with RI-labeled specific primers for pri-let-7a1. ddATP was used as a sequence control.

D, E *In vitro* processing analysis for pri-let-7 with pseudouridine. 32p-ATP-labeled pri-let-7a1 was synthesized using UTP: pseudouridine at a ratio of 1:1 or 1:0. This labeled RNA was treated with whole cell lysate from TruB1 expressing HEK293FT cells transfected with pcDNA3.1-TruB1. Autoradiographed image (D) and the relative processing rate (E) are shown. Error bars show SD; $n = 3$. Significance was assessed using 2-tailed Student's *t*-test.

Source data are available online for this figure.

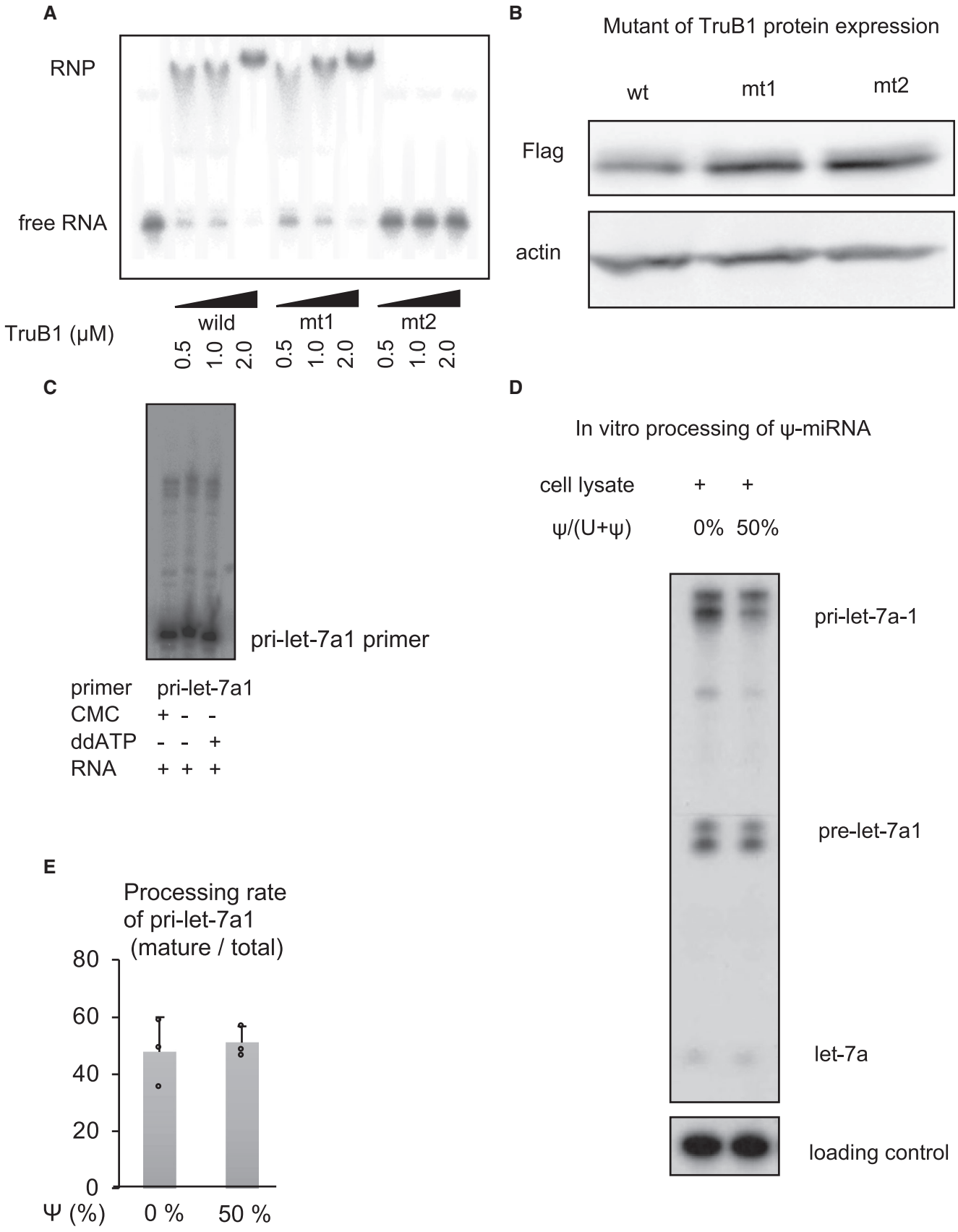
A
RNP
free RNA
TruB1 (μM)
wild
mt1
mt2
0.5
1.0
2.0
0.5
1.0
2.0
0.5
1.0
2.0
B
Mutant of TruB1 protein expression
wt
mt1
mt2
Flag
actin
C
pri-let-7a1 primer
primer pri-let-7a1
CMC + - -
ddATP - - +
RNA + + +
D
In vitro processing of ψ-miRNA
cell lysate + +
ψ/(U+ψ) 0% 50%
pri-let-7a-1
pre-let-7a1
let-7a
loading control
E
Processing rate
of pri-let-7a1
(mature / total)
80
60
40
20
0
Ψ (%)
0 %
50 %

Figure EV2.

Figure EV3. Loop mutant of pri-let7a1 and HITS-CLIP for TruB1.

A Loop sequence of mutant RNA in which the loop structure of pri-let-7a1 was modified (loop mt).
B Protein expression of 3 x Flag-tagged TruB1 in genome edited cells. Flag-tagged TruB1 was most highly expressed in ED-9 cells.
C Western blotting for input and IP samples in HITS-CLIP.
D Autoradiography of RI-labeled RNA in a complex with TruB1 (black frame).
E Agilent data of cDNA library show smeared band at 150–250 bp.
F Mapped reads of sequenced data to human genome.

Source data are available online for this figure.

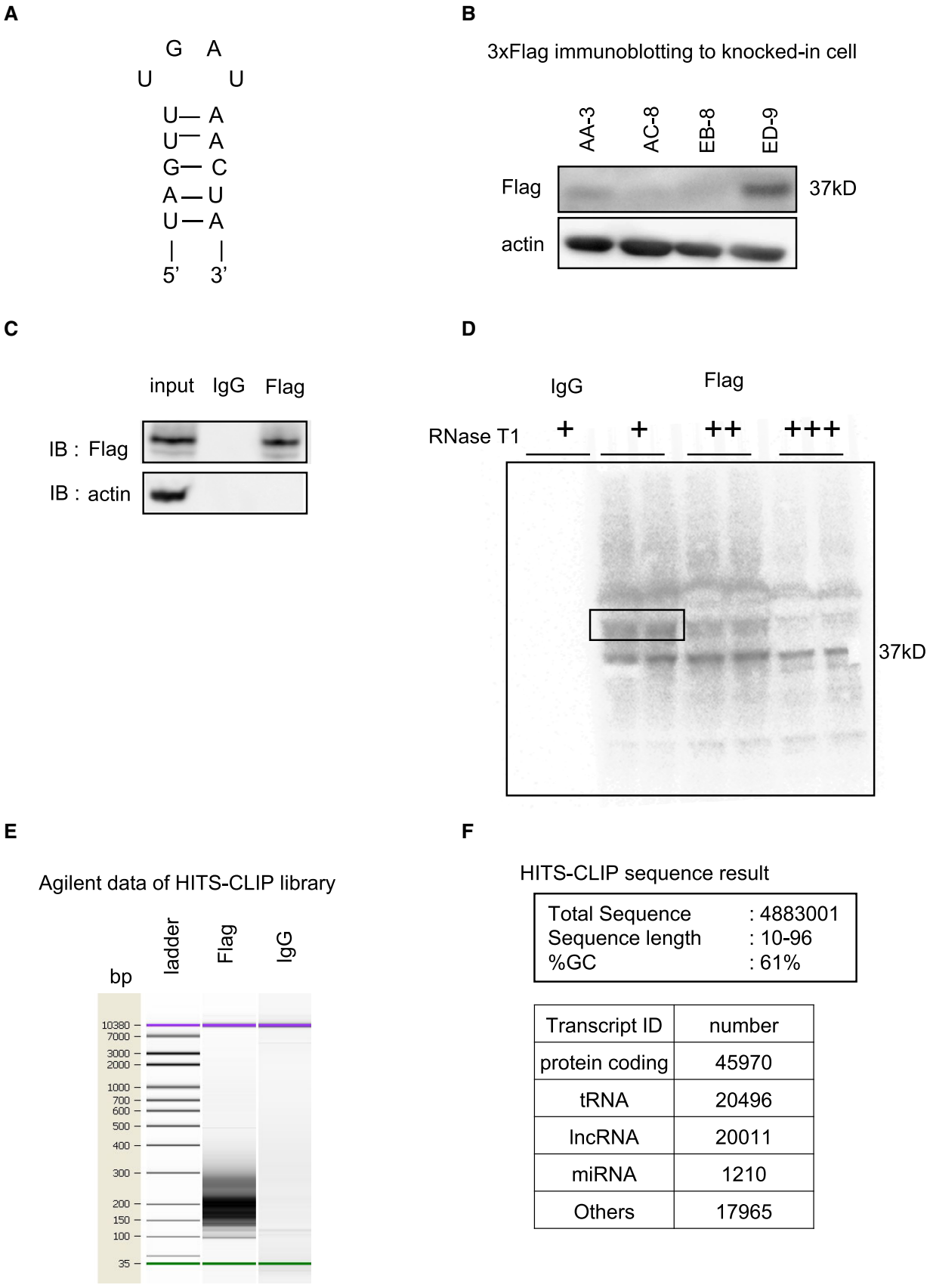

Figure EV3.

Figure EV4. TruB1 selectively enhances binding of DGCR8 to primary let-7.

A RIP assay of pri-microRNAs and DGCR8 from HEK-293FT cells with TruB1 KD or ctrl (siRNA). RNA was extracted from IP material and analyzed by qRT–PCR. Significance was assessed using 2-tailed Student's *t*-test, < 0.05*.

B Immunoprecipitation of Flag-tagged TruB1 and DGCR8 in HEK-293FT cells. Western blotting for input or IP material using anti-Flag antibody, anti-DGCR8 antibody, and anti-actin antibody are shown.

C Relative pri-let-7a1 expression in HEK-293 cells with knockdown of Lin28B or ctrl (siRNA). qRT–PCR analysis for pri-let-7a1 expression normalized to GAPDH. Lin28B KD by siRNA tended to increase the expression of pri-let-7a1, but this was not statistically significant.

Data information: Error bar shows SD; $n = 3$.
Source data are available online for this figure.

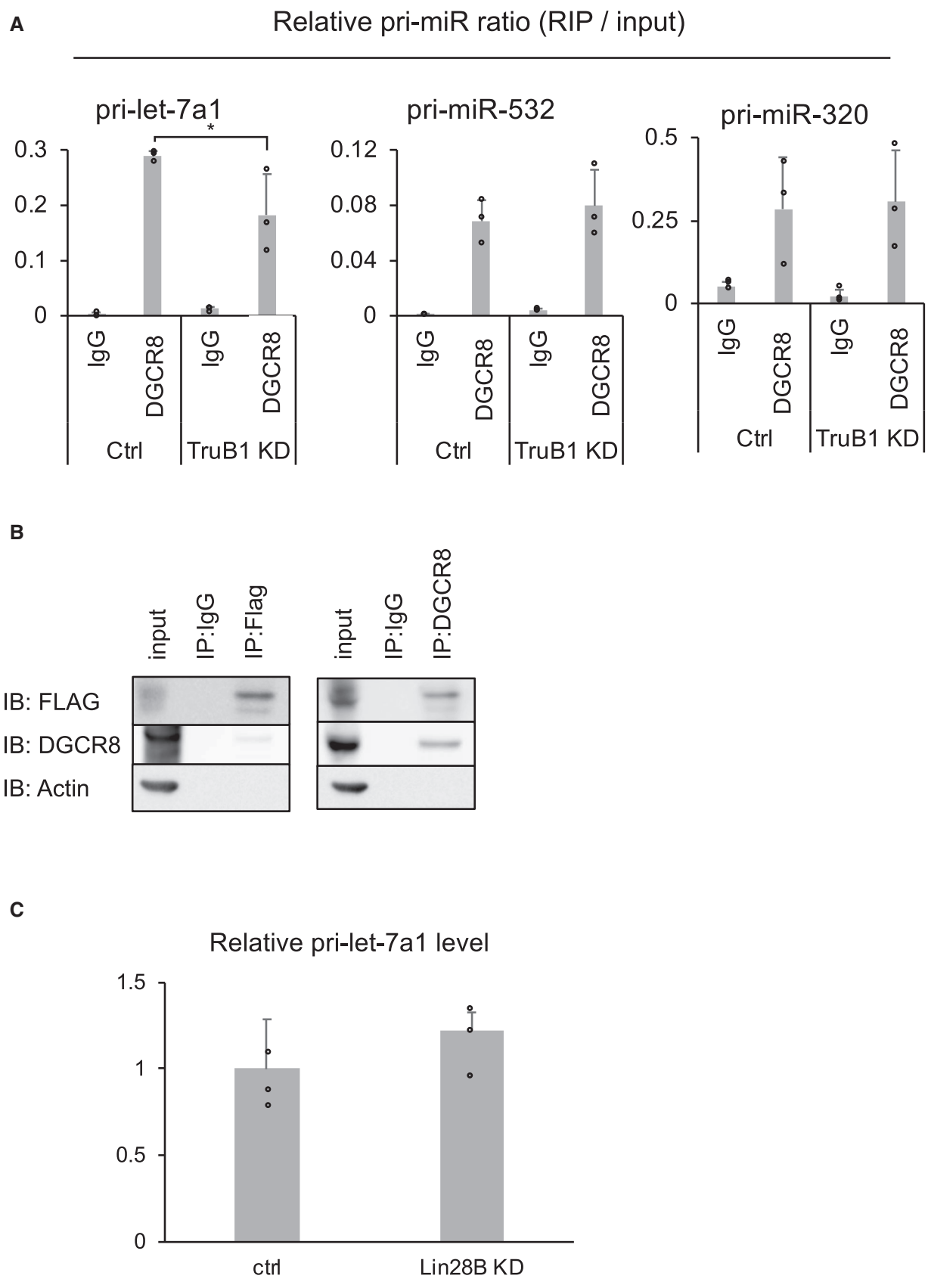
A
Relative pri-miR ratio (RIP / input)
pri-let-7a1
pri-miR-532
pri-miR-320
*
0.3
0.2
0.1
0
0.12
0.08
0.04
0.5
0.25
IgG
DGCR8
Ctrl
TruB1 KD
B
input
IP:IgG
IP:Flag
IP:DGCR8
IB: FLAG
IB: DGCR8
IB: Actin
C
Relative pri-let-7a1 level
1.5
1
0.5
ctrl
Lin28B KD

Figure EV4.

Figure EV5. TruB1 suppressed tumor progression.

A TruB1 and mt1 suppressed cell proliferation. Real-time glo assay for HEK-293 cells infected with tetracycline-inducible lentiviruses expressing TruB1, mt1, or GFP, 5 days after doxycycline treatment. TruB1 and mt1 suppressed cell proliferation. Error bars show SD; $n = 3$. Significance was assessed using 2-tailed Student's t-test, < 0.05*.

B–D Relationship between TruB1, let-7, and tumor progression in various cancers. (B) Published microarray and microRNA sequencing in prostate biopsy samples from patients with prostate cancer (data accessible at NCBI GEO database (Edgar *et al*, 2002), accession GSE64333, Wang *et al*, 2015a; Data ref: Wang *et al*, 2015b). In these data, there were correlations between the expression levels of TruB1 and both let-7b and let-7i. Regarding let-7a, a statistically significant correlation was not found, but the same tendency was observed. Significance was assessed using Pearson product-moment correlation coefficient. Regression lines were showed by red lines (C, D). TruB1 inversely correlates with tumor progression or oncogenesis in prostate cancer (C) and in pancreatic cancer (D). LD: local disease. Data from Varambally *et al*, 2005a; Data ref: Varambally *et al*, 2005b; Pei *et al*, 2009a; Data ref: Pei *et al*, 2009b. Error bar shows SD. Significance was assessed using 2-tailed Student's t-test, < 0.05*. The numbers of cases (C) were as follows: benign ($n = 6$), LD ($n = 7$), and ED ($n = 6$). The numbers of cases (D) were as follows: Cancer ($n = 36$) and normal ($n = 16$).

Source data are available online for this figure.

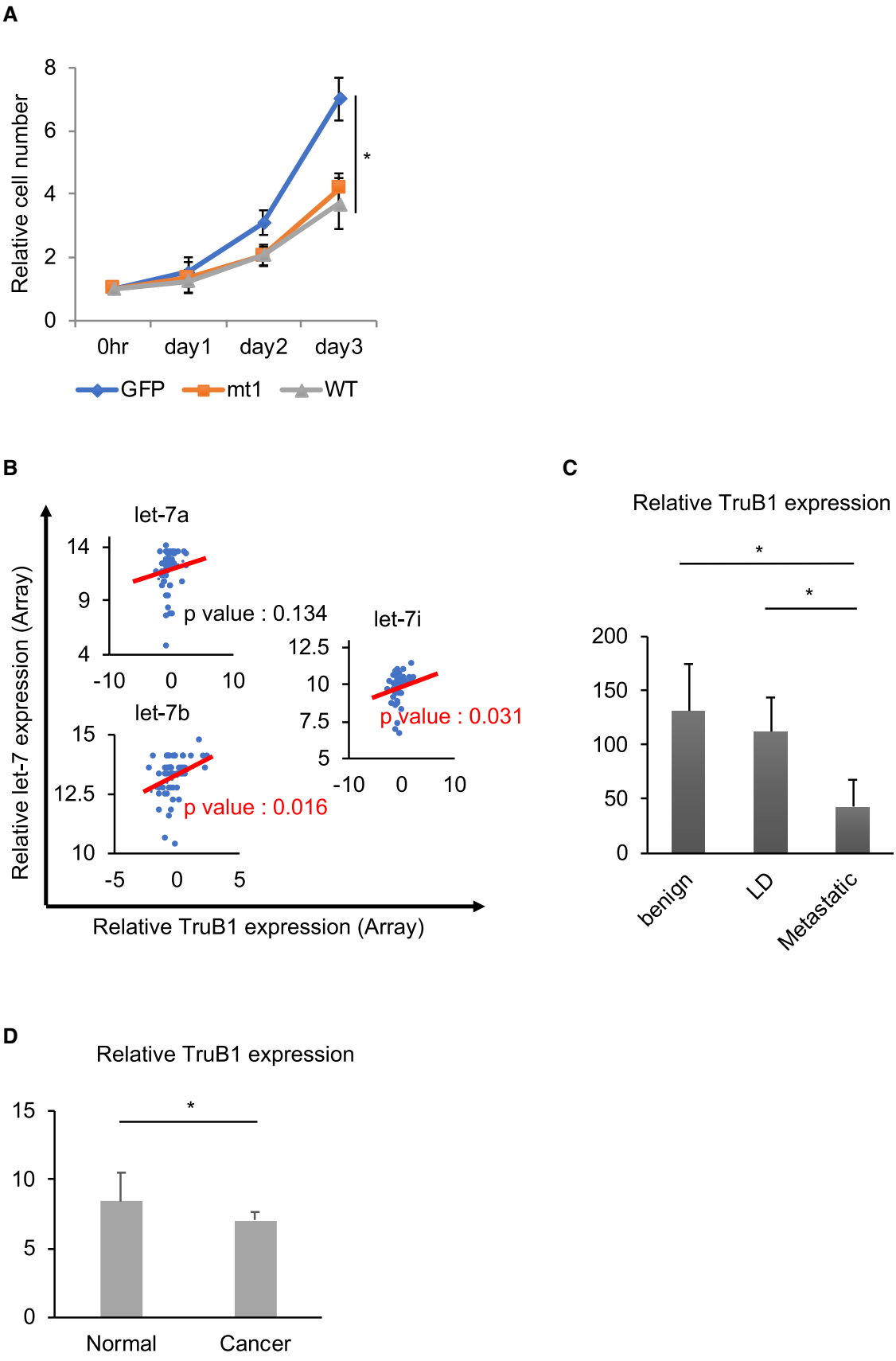
A
Relative cell number
8
6
4
2
0
0hr
day1
day2
day3
GFP
mt1
WT
*
B
Relative let-7 expression (Array)
Relative TruB1 expression (Array)
let-7a
p value : 0.134
let-7i
p value : 0.031
let-7b
p value : 0.016
C
Relative TruB1 expression
200
150
100
50
0
benign
LD
Metastatic
*
*
D
Relative TruB1 expression
15
10
5
0
Normal
Cancer
*

Figure EV5.