



**Fig. S1. Northern blot analysis of the excision of TAR miRNA.** Flp-In 293 cells with control FLAG-tag alone, FLAG-tagged TRBP-WT, TRBP-dsRBD $\Delta$ 3, or TRBP-L326A/Y358A were transfected with pGL2-TAR-Luciferase, and IP was performed with anti-FLAG antibody. Northern blotting was performed using purified total RNA from input samples and IP samples with a probe for detecting the 5' stem region of TAR RNA (red). A band of approximately 60 bases corresponding to the cleaved stem-loop-structured TAR RNA was observed in the IP sample of TRBP-WT, but not in those of TRBP-dsRBD $\Delta$ 3 or TRBPL326A/Y358A.

**Table S1**

PCR primers used for generating mutant TRBP expression plasmids.

| Primer name   | Sequence (5' to 3')             |
|---------------|---------------------------------|
| L326A-F       | cgcagtggaaactgtccacccagccggcca  |
| L326A-R       | cactggcagagtccactcaggctcag      |
| V336A_H338A-F | gtgcaggctctgcaaccaccaggaggcag   |
| V336A_H338A-R | atgcagtggccggctgggtggacagttccac |
| Y358A-F       | ctcaagatcatggcaggcagcaagtg      |
| Y358A-R       | tgcctgcagggcacggcgcccagcctc     |

F indicates forward primer. R, reverse primer.

**Table S2**

siRNA sequences expected to be cleaved out from shRNAs used for dual luciferase reporter assay.

| shRNA        | Guide strand sequence (5' to 3') | Passenger strand sequence (5' to 3') |
|--------------|----------------------------------|--------------------------------------|
| pSUPER GY441 | gccacaacgucuauaucaagg            | augauauagacguuguggcug                |
| pSUPER FL774 | auuaagacgacucgaaaucca            | gauuucgagucgucuuuaugu                |

**Table S3**

PCR primers used for generating pGL2-ΔTAR-luciferase plasmid.

| Primer name | Sequence (5' to 3')         |
|-------------|-----------------------------|
| 3ΔTAR-F     | actgcttaaggcctcaataaagcttgg |
| 3ΔTAR-R     | agtacaggcaaaaaggcagctgcttat |

F indicates forward primer. R, reverse primer.

**Table S4**

PCR primers used for qRT-PCR.

| Primer name   | Sequence (5' to 3')         |
|---------------|-----------------------------|
| hGAPDH-F      | tgcaccaccaactgcttag         |
| hGAPDH-R      | agaggcaggatgtatgttc         |
| pGL2-luc-F    | gaaccgctggagacaactg         |
| pGL2-luc-R    | tagttctgccaaccgaacg         |
| hTubulinB-F   | ctggcacccatggactctg         |
| hTubulinB-R   | tcggctccctctgtgttag         |
| GIT2-F        | aacaccccactccatgttgc        |
| GIT2-R        | tgccttccttgccctgcataa       |
| IER3-F        | acttcggagccctcgacta         |
| <u>IER3-R</u> | <u>gagtgcggggagtcacagtt</u> |

F indicates forward primer. R, reverse primer.

**Table S5**

DNA probe used for northern blot.

| Probe name | Sequence (5' to 3')      |
|------------|--------------------------|
| TAR_RNA    | gggttccctagtttagccagagac |