

The stress specific impact of ALKBH1 on tRNA cleavage and tiRNA generation

Sherif Rashad, MD, PhD^{1,2*}. Xiaobo Han, PhD¹. Kanako Sato, MD¹. Eikan Mishima, MD, PhD³. Takaaki Abe, MD, PhD⁴. Kuniyasu Niizuma, MD, PhD^{1,2,6}. Teiji Tominaga, MD, PhD¹

1- Department of Neurosurgery, Tohoku University Graduate school of Medicine, Japan.

2- Department of Neurosurgical Engineering and Translational Neuroscience, Tohoku University Graduate school of Medicine, Japan.

3- Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

4- Department of Clinical Biology and Hormonal Regulation, Tohoku University Graduate School of Medicine, Sendai, Japan

5- Department of Medical Science, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan

6- Department of Neurosurgical Engineering and Translational Neuroscience, Graduate school of Biomedical Engineering, Tohoku University, Japan.

Corresponding author: Sherif Rashad, MD, PhD

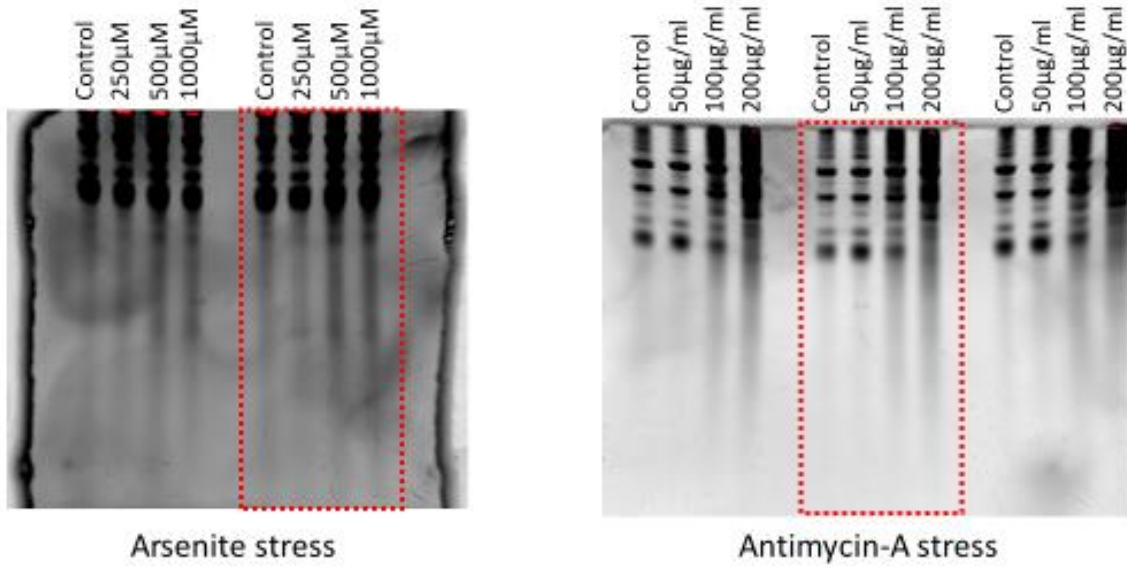
2-1 Seriyomachi, Aoba-ku, Sendai, Japan.

Postal code: 980-8575

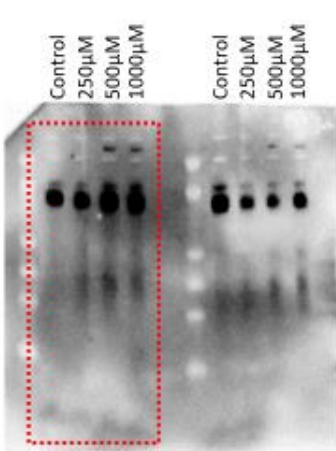
Email: sherif@nsg.med.tohoku.ac.jp

Supplementary Figures

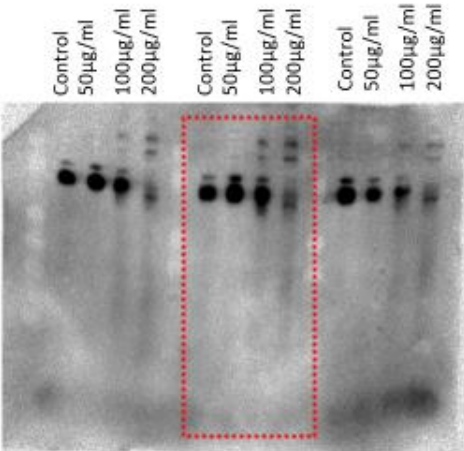
Supplementary Figure 1: Cropped gels in figure 1A



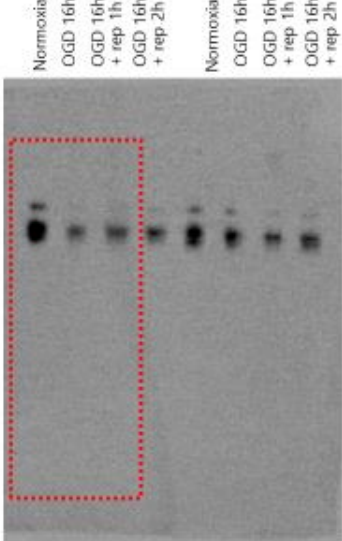
Supplementary Figure 2: Cropped gels in Figure 1B



Arsenite stress

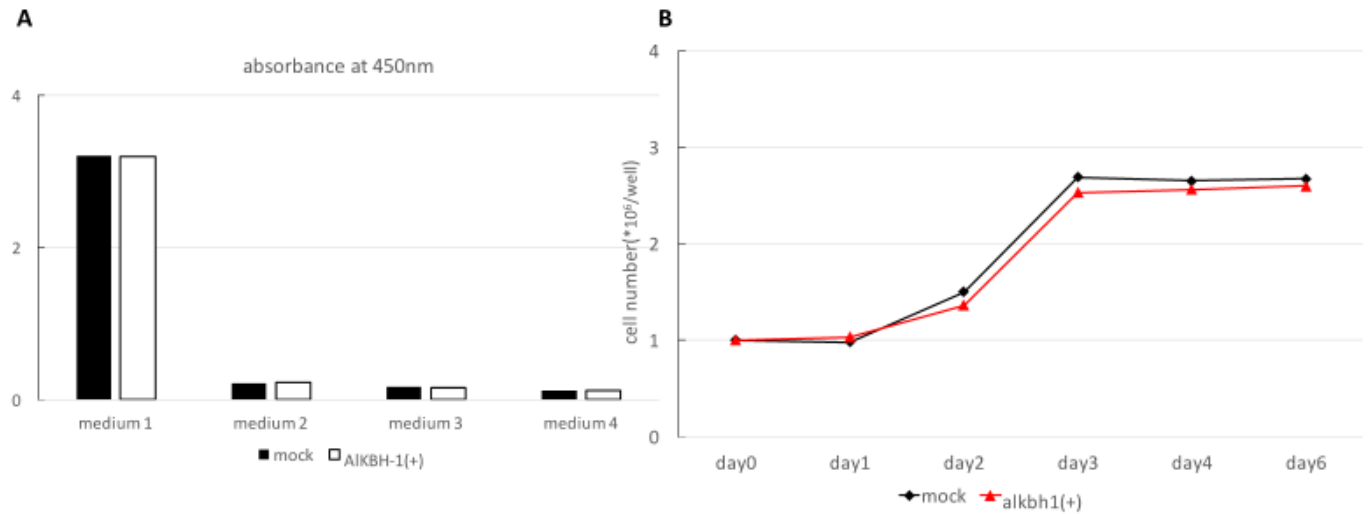


Antimycin-A stress

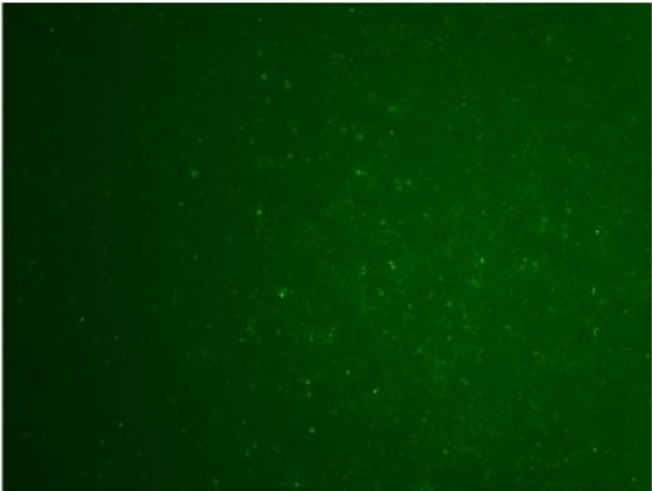


OGD-R stress

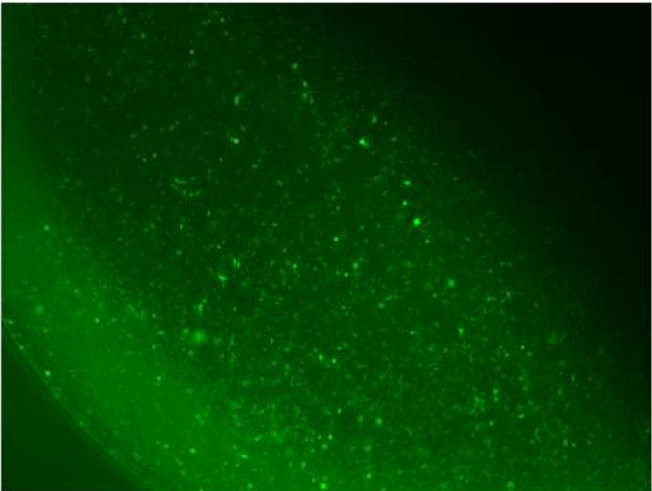
Supplementary Figure 3: Analysis of cell growth after Alkbh1 stable overexpression. **A:** WST-8 assay 1 week after seeding 5000 cells in 96 well plate and growth using different media (Medium 1: Full growth medium, Medium 2: Serum free medium, Medium 3: glucose free medium, Medium 4: serum/glucose free medium). **B:** Daily cell counting after plating 1×10^6 in 6 well plate showing equal growth in $B35^{\text{Mock}}$ and $B35^{\text{ALK}}$ cells.



Supplementary Figure 4: GFP signal analysis 48 hours after transfection of B35 cells with Lentiviral particles encoding Alkbh1 or Mock sequence showing > 90% transfection efficiency

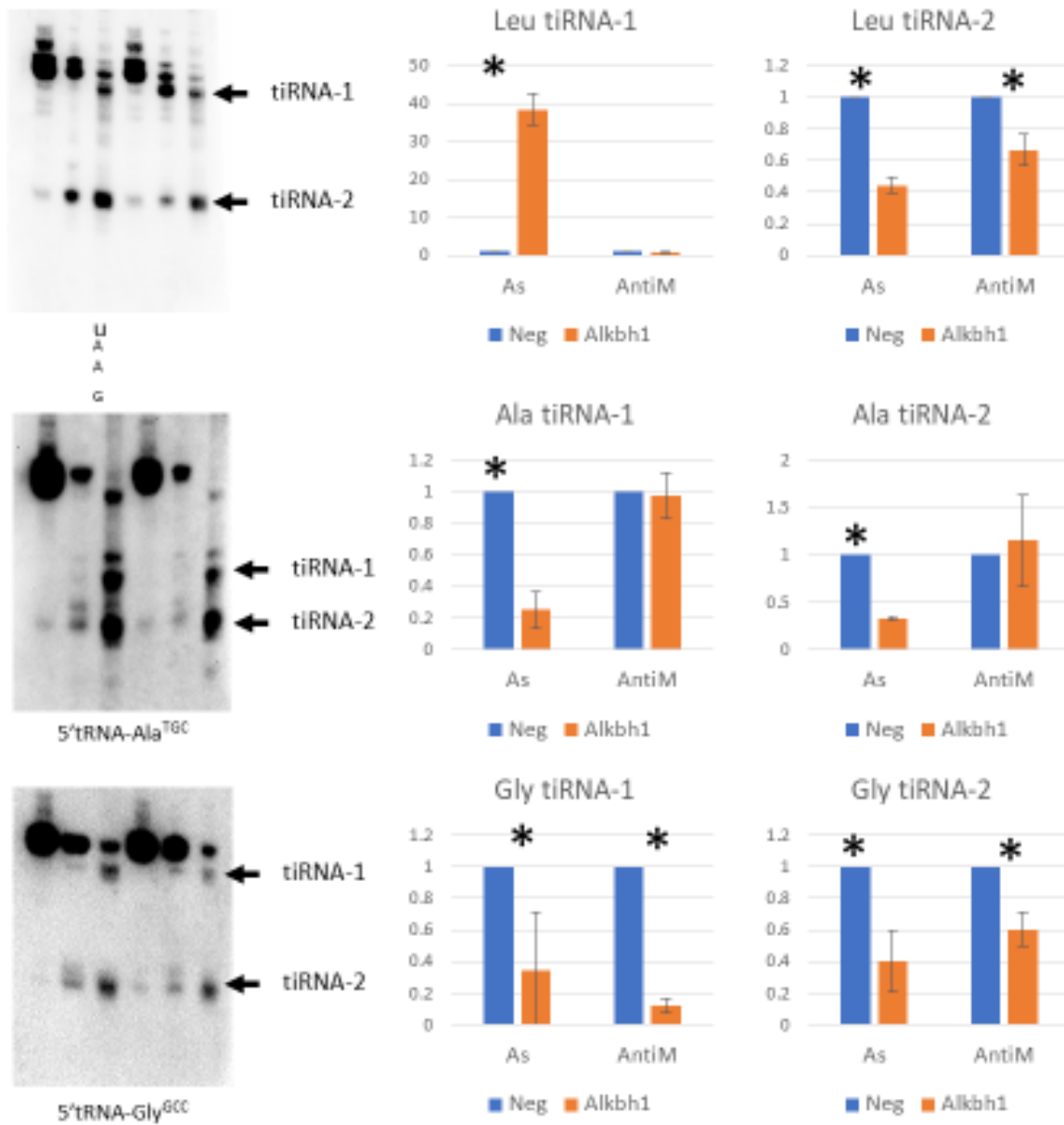


Lenti-ALKBH1

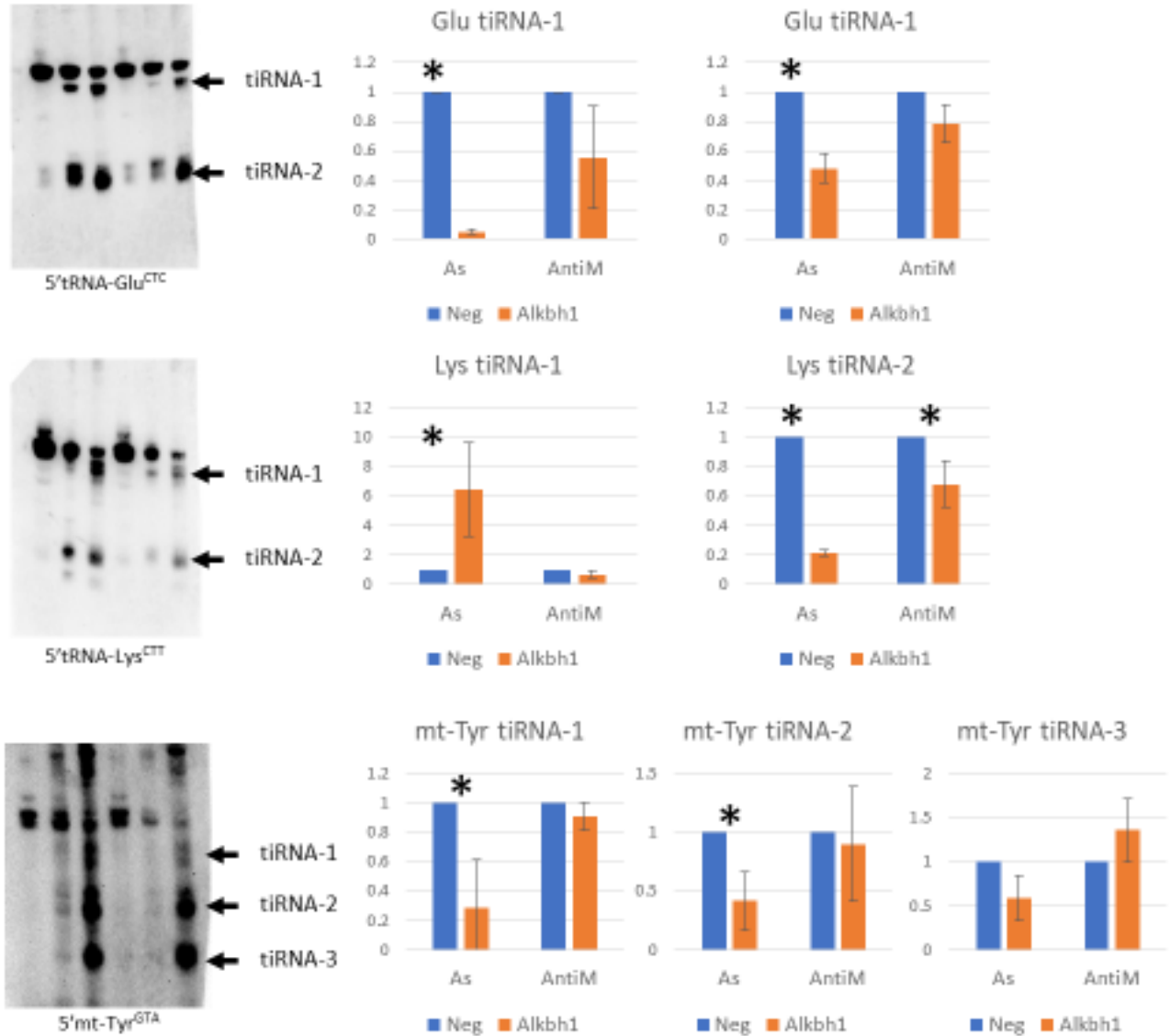


Lenti-Mock

Supplementary Figure 5: Quantification of tRNA fragments after Alkbh1 siRNA and stress. Asterisk: Statistically significant ($p < 0.05$, Fold change > 1.5). N = 3 per group



Supplementary Figure 6: Quantification of tRNA fragments after Alkbh1 siRNA and stress (Continued). Asterisk: Statistically significant ($p < 0.05$, Fold change > 1.5). N = 3 per group



Supplementary Figure 7: Quantification of tRNA fragments after Alkbh1 Lentiviral overexpression and stress. Asterisk: Statistically significant ($p < 0.05$, Fold change > 1.5). N = 3 per group. Note: mt-Tyr tRNAs were only calculated with Antimycin A stress as cleavage after arsenite was not sufficient for quantification.

