Supplementary information

TRMT1-Mediated tRNA m^2_2G Modification Drives Osimertinib Resistance via the ATXN3/USP25 Axis in Lung Adenocarcinoma

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Supplementary materials

Figure S1. CCK-8 assays measuring the IC_{50} values of Osimertinib-sensitive (HCC827, H1975) and Osimertinib-resistant (HCC827OR, H1975OR) cell lines. n=3.

Figure S2. USP25 promotes Osimertinib resistance and proliferation.

A-F HCC827 and H1975 cells were transfected with the control EV or Myc-USP25. protein level of USP25 were detected by Western Blot **(A, D)**; The sensitivity to Osimertinib and proliferation ability were detected by CCK8 **(B-C, E-F)**. n=3; Mean ± SEM; Student's t test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

Figure S3. Osimertinib treatment upregulates the expression of ATXN3, USP25, and TRMT1.

A Treatment of HCC827 cells with 100 nM Osimertinib for 0, 2, 4, 6, 12, and 24 h, cellular extracts were collected for Western Blot with indicated antibodies. **B-D** Treatment of HCC827 and H1975 cells with different concentrations of Osimertinib for 12 h. cellular extracts were collected for Western Blot with indicated antibodies **(B-C)** and Q-PCR **(D)**. **E** Treatment of H1975 cells with different concentrations of Osimertinib for 12 h, cellular extracts were collected for Western Blot with indicated antibodies. n=3; Mean ± SEM; Analysis of variance (ANOVA) with Tukey's multiple-comparison test..

Figure S4. tRNA mass spectrometry analysis of tRNA modification levels in Osimertinib-sensitive and resistant cells. n=3.

Figure S5. Therapeutic targeting of the ATXN3-USP25-TRMT1 axis overcomes Osimertinib resistance in patient-derived models.

Organoid Model derived from patients were cultured in the control medium or in the presence of Osi and/or AZ1 for 72 h. Representative images were shown and growth inhibition rate were measured of organoid Model treated with DMSO, Osi (5.6 μM), AZ1 (5.6 μM), and Osi (5.6 μM) + AZ1 (5.6 μM). Scale bar: 200 μm. n=3; Mean ± SEM; Analysis of variance (ANOVA) with Tukey's multiple-comparison test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

Figure S6. Patients treated with Osimertinib with low baseline expression of ATXN3, USP25, and TRMT1 had a longer progression-free survival (PFS). A Kaplan–Meier Plot of Progression-Free Survival treated with Osimertinib based on the overall expression levels of baseline ATXN3/USP25/TRMT1. **B** Kaplan–Meier Plot of Progression-Free Survival treated with Osimertinib based on the expression levels of baseline USP25. **C** Kaplan–Meier Plot of Progression-Free Survival treated with Osimertinib based on the expression levels of baseline TRMT1.