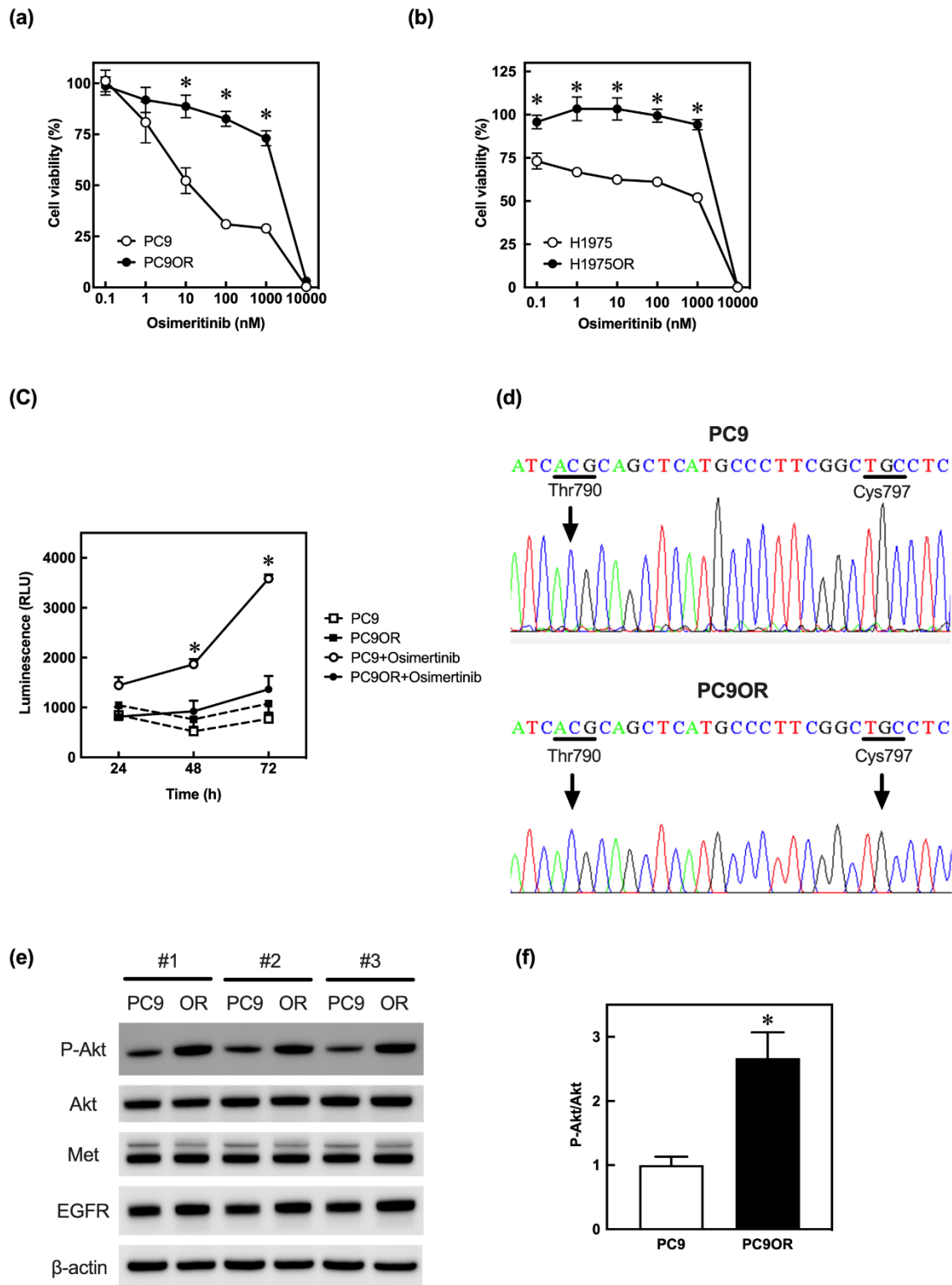


Supplementary Information

MicroRNA-130a-3p regulates osimertinib resistance by targeting runt-related transcription factor 3 in lung adenocarcinoma

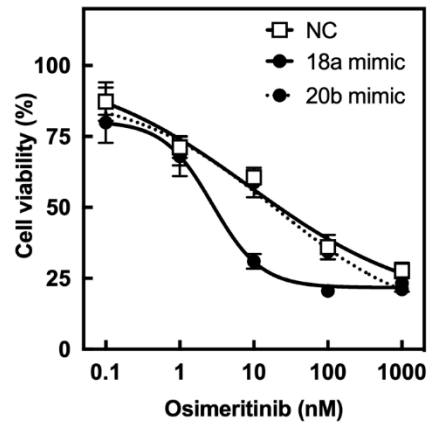
Takuya Shintani, Yu-Ting Shun, Yuji Toyozumi, Kenji Ikemura, Takayuki Shiroyama, Izumi Nagatomo, Kentaro Jingushi, Yoshito Takeda, Atsushi Kumanogoh, Masahiro Okuda



Supplementary Figure S1. Establishment of osimertinib-resistant cell lines

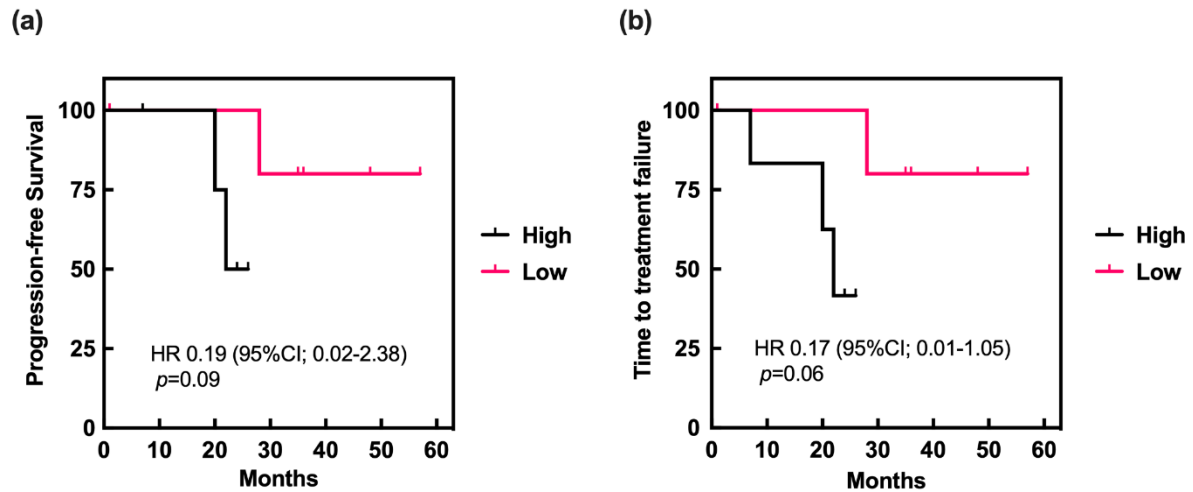
(a, b) Viability of PC9, PC9OR, H1975, and H1975OR cells after exposure to various concentrations of osimertinib for 72 h. * $P < 0.05$, compared with PC9 or H1975 at each concentration of osimertinib. **(c)** Apoptotic rates in PC9 and PC9OR cells in the absence or

presence of 1 μ M osimertinib for 24, 48, and 72 h. * $P < 0.05$, compared with PC9OR+osimertinib at each time point. **(d)** Sequences of *EGFR* exon 20 in PC9 and PC9OR cells. **(e)** Protein concentrations of EGFR signaling in PC9 and PC9OR (OR) cells normalized with β -actin. #1-3 represent sample numbers. **(f)** Protein concentrations of P-Akt/Akt in PC9 and PC9OR cells. * $P < 0.05$, compared with PC9. P-values were determined using ANOVA with Tukey–Kramer **(a–c)** or Student’s t-test **(f)**. Data are presented as mean $\pm$ SEM (n=3–10). RLU, relative luminescence units; EGFR, epidermal growth factor receptor



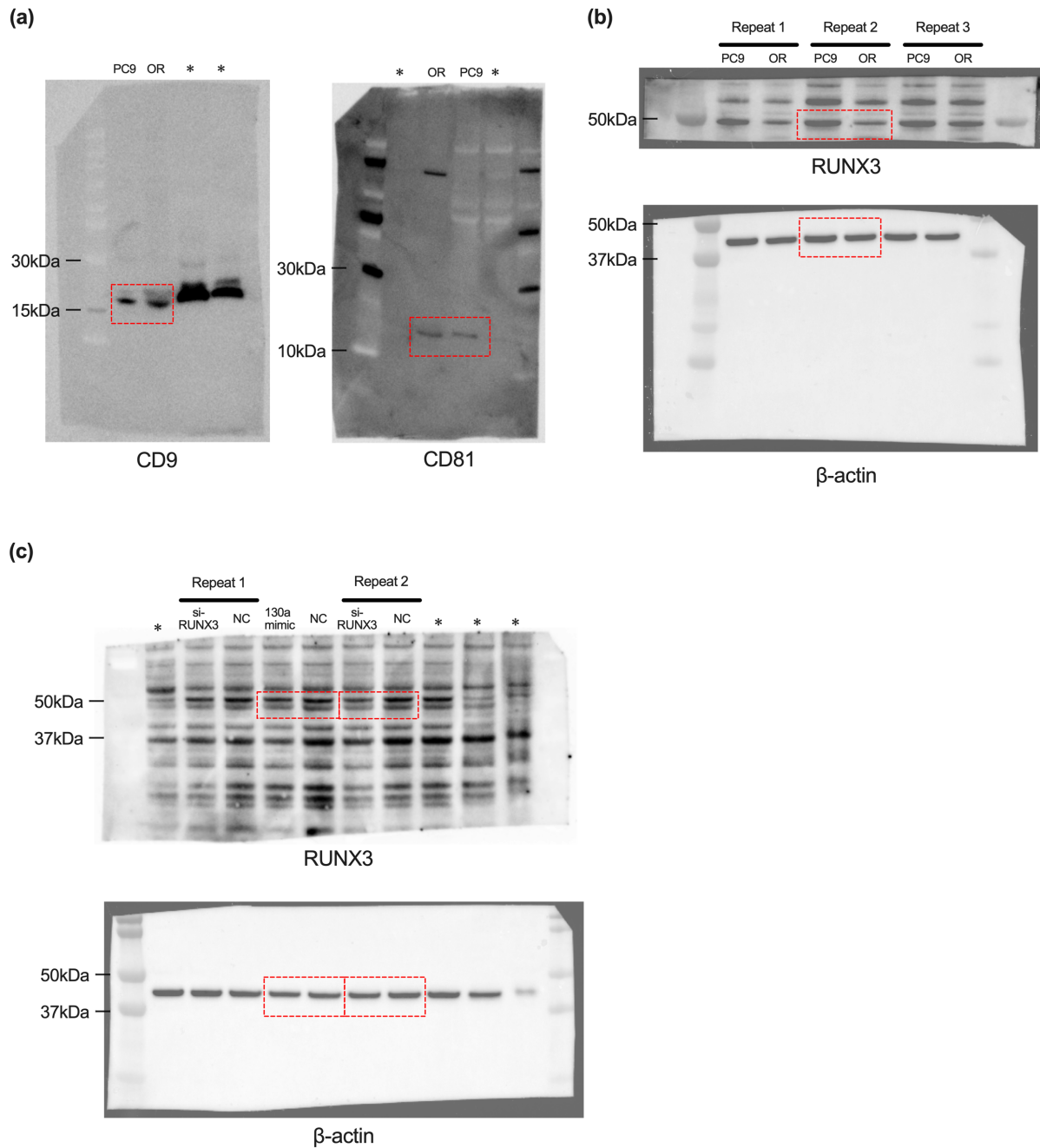
Supplementary Figure S2. Effects of miR-18a-5p and miR-20b-5p on osimertinib response in PC9 cells

Viability of PC9 cells transfected with the negative control (NC), miR-18a-5p (18a) mimic, and miR- 20b-5p (20b) mimic after exposure to various concentrations of osimertinib for 72 h. Data are presented as mean $\pm$ SEM (n=3–9).



Supplementary Figure S3. Progression-free survival and time to treatment failure plotted against serum miR-130a-3p concentrations in patients with NSCLC treated with osimertinib

Kaplan–Meier survival curves for **(a)** progression-free survival and **(b)** time to treatment failure in patients with NSCLC treated with first-line osimertinib. The patients were divided into two groups (high and low expression) based on the median miR-130a-3p expression level (cutoff value = 1.04). The mean miR-130a-3p concentration in the high expression group (n=6) was 1.65 ± 0.41 , whereas that in the low expression group (n=6) was 0.55 ± 0.33 . P-values were calculated using the log-rank test. HR, hazard ratio; CI, confidence interval; NSCLC, non-small-cell lung cancer



Supplementary Figure S4. The original blots of Immunoblotting

(a) Detection of CD9 and CD81 protein in isolated EVs from PC9 and PC9OR (OR) cells, related to Figure 1b. **(b)** Detection of RUNX3 and β-actin protein in PC9 and PC9OR cells, related to Figure 5b. **(c)** Detection of RUNX3 and β-actin protein in PC9 cells transfected with the negative control (NC), miR-130a-3p (130a) mimic, and si-RUNX3, related to Figures 5d and g. * samples from different experiments. EVs, extracellular vesicles

Supplementary Table S1. mRNA primer sequences

Name	Notation
RUNX3-Forward	5'- GACAGCCCCCAACTTCCTCT -3'
RUNX3-Reverse	5'- CACAGTCACCACCGTACCA -3'
GAPDH-Forward	5'- GCACCGTCAAGGCTGAGAAC -3'
GAPDH-Reverse	5'- TGGTGAAGACGCCAGTGGA -3'

Supplementary Table S2. microRNA primer sequences

Name	Notation
hsa-miR-4800-5p	5' - AGTGGACCGAGGAAGGAAGGA -3'
hsa-miR-203a-5p	5' - AGTGGTTCTTAACAGTTCAACAGTT -3'
hsa-miR-378a-3p	5' - ACTGGACTTGGAGTCAGAAGGC -3'
hsa-miR-130a-3p	5' - CAGTGCAATGTAAAAGGGCAT -3'
hsa-miR-424-3p	5' - CAAAACGTGAGGCGCTGCTAT -3'
hsa-miR-18a-5p	5' - TAAGGTGCATCTAGTGCAGATAG -3'
hsa-miR-126-3p	5' - TCGTACCGTGAGTAATAATGCG -3'
hsa-miR-378f	5' - ACTGGACTTGGAGCCAGAAG -3'
hsa-miR-503-5p	5' - TAGCAGCGGGAACAGTTCTGCAG -3'
hsa-miR-20b-5p	5' - CAAAGTGCTCATAGTGCAGGTAG -3'
hsa-miR-193a-5p	5' - TGGGTCTTTGCGGGCGAGATGA -3'
hsa-miR-335-5p	5' - TCAAGAGCAATAACGAAAAATGT -3'

Supplementary Table S3. DNA primer sequence

Name	Notation
<i>EGFR</i> -exon 18-Forward	5' - CAAATGAGCTGGCAAGTGCCGTGTC -3'
<i>EGFR</i> -exon 18-Reverse	5' - GAGTTTCCCAAACACTCAGTGAAAC -3'
<i>EGFR</i> -exon 19-Forward	5' - GCAATATCAGCC TTAGG TGC GGCTC -3'
<i>EGFR</i> -exon 19-Reverse	5' - CATAGAAAGTGAACATTTAGGATGTG -3'
<i>EGFR</i> -exon 20-Forward	5' - CCATGAGTACGTATTTTGAAACTC -3'
<i>EGFR</i> -exon 20-Reverse	5' - CATATCC CCATGGC AA ACTCTTGC -3'
<i>EGFR</i> -exon 21-Forward	5' - CTAACGTTCGCCAG CCATAAGTCC -3'
<i>EGFR</i> -exon 21-Reverse	5' - GCTGCGAGCTCACCCAGAATGTCTGG -3'