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Supplemental Information

Clonal Evolution and Heterogeneity of Osimertinib Acquired Resistance Mechanisms in EGFR Mutant Lung Cancer

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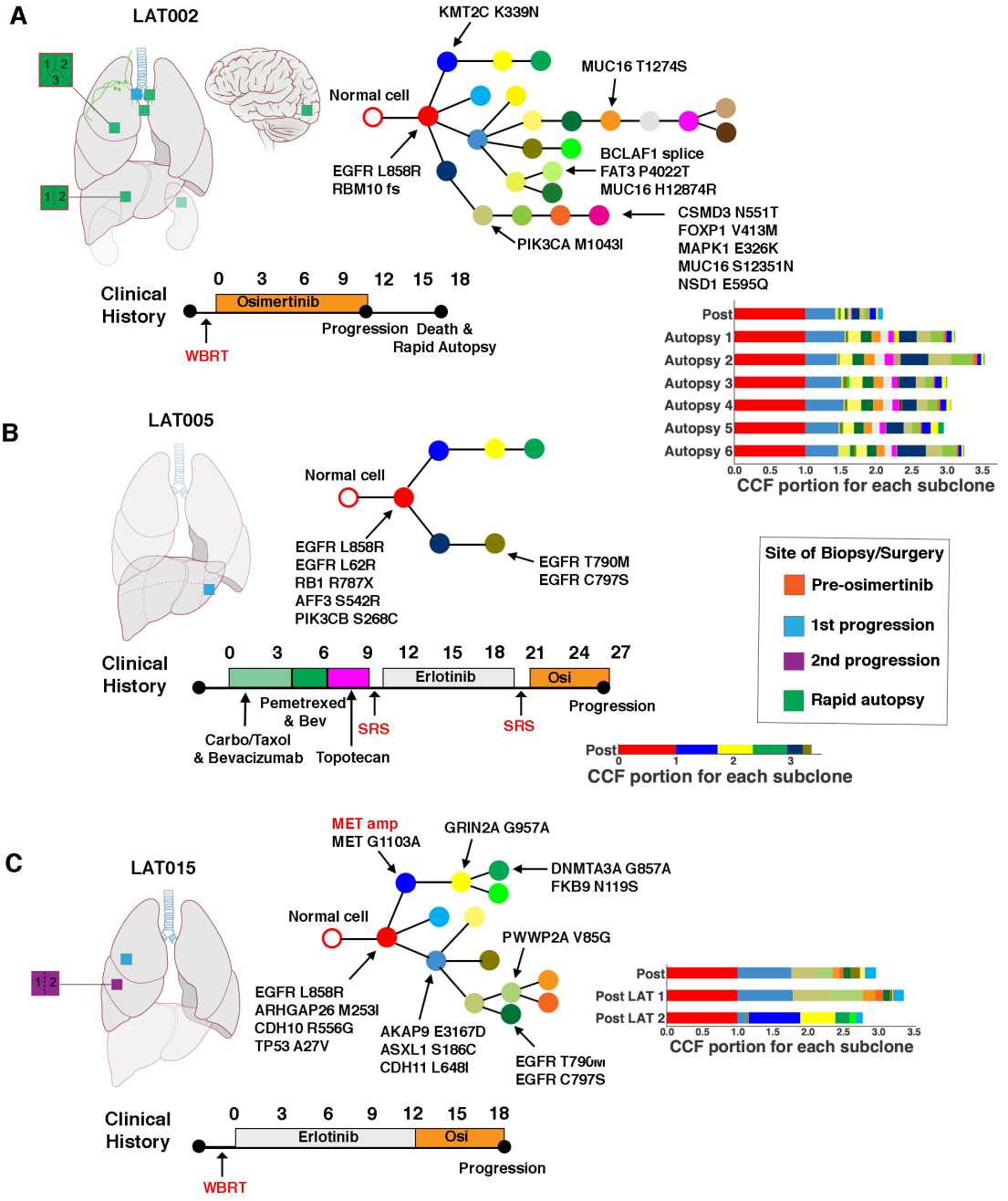


Figure S1. Clonal evolution of post-osimertinib resistant tumors from patients with prior treatment with an EGFR tyrosine kinase inhibitor. Related to Figure 2. Phylogenetic trees were constructed from WES of post-osimertinib resistant tumors of patients **(A)** LAT002, **(B)** LAT005, **(C)** LAT015. Mutations and CNAs in cancer-related genes in each branch are indicated with arrows. Clinical timeline from diagnosis of metastatic disease to progression on osimertinib for each patient is summarized below each phylogenetic tree. Anatomic locations of post-osimertinib resistant tumors are shown for each patient. Color of circles in phylogenetic trees signal relative timepoint in tumor evolution: red (clonal), blue and yellow (early subclonal), green, orange, silver, magenta and brown (late subclonal). WBRT: whole brain radiation therapy; SRS: stereotactic radiosurgery; Osi: osimertinib; amp: amplification. Amplifications are shown in red. Bar plots for each patient indicate the cancer cell fraction (CCF) of subclones for each tumor used to generate the phylogenetic trees.

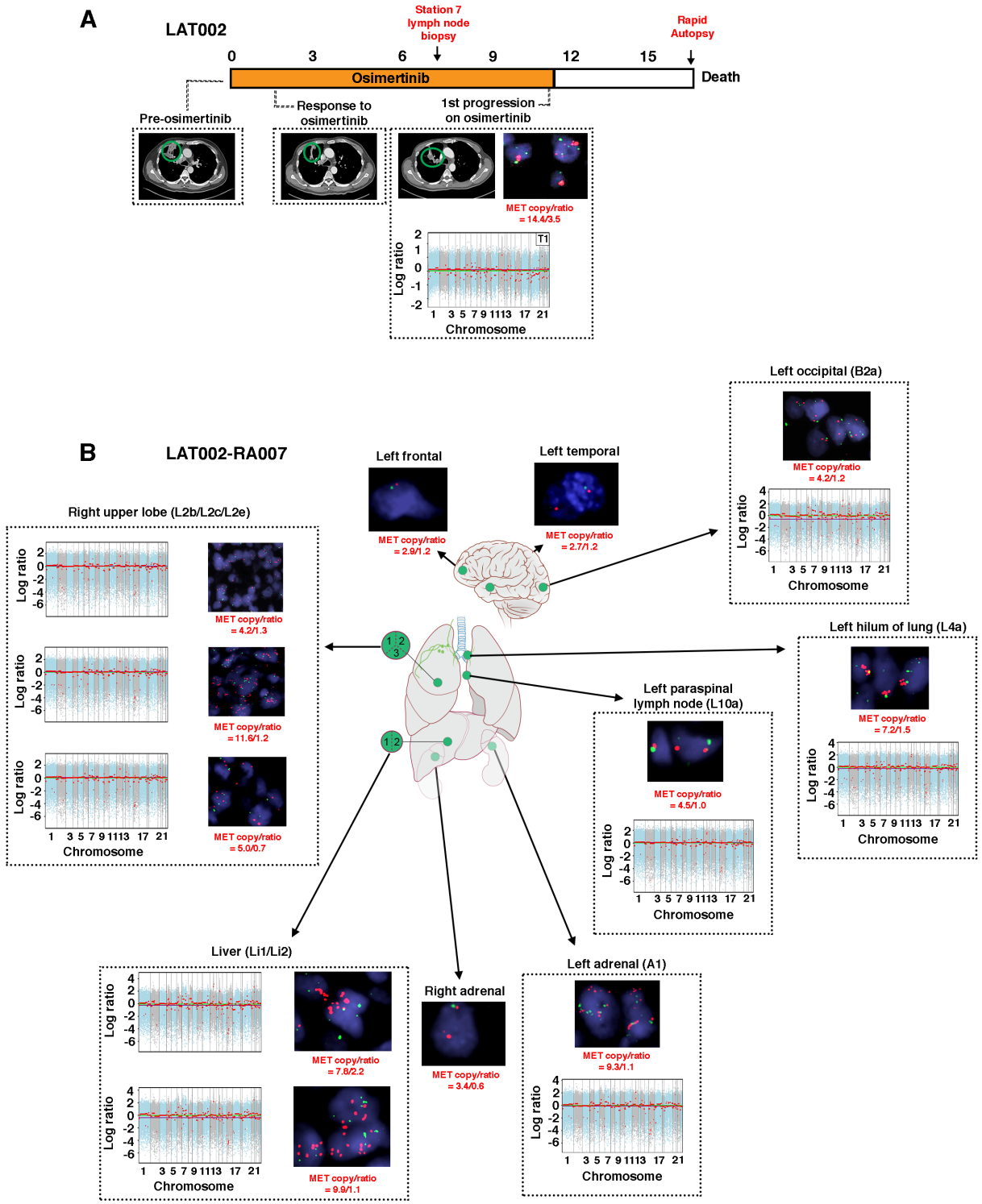


Figure S2. Heterogeneity of *MET* amplification in the development of acquired resistance to osimertinib in patient LAT002 without prior therapy. Related to Figures 5 and 6. (A) Treatment timeline from diagnosis to death for patient LAT002. Patient initiated treatment with osimertinib under this study upon the diagnosis of EGFR mutant metastatic lung adenocarcinoma. Imaging at first restaging showed a response to treatment in the posterior liver (green circle). Upon first progression (green circle), the patient was not eligible for LAT and he opted for no further treatment due to poor performance status. Red text signifies anatomic sites of biopsies. FACETS copy number plots from tumor exome sequencing and FISH for *MET* from site of first progression on osimertinib is shown. **(B)** FACETS copy number plots from tumor exome sequencing and FISH for *MET* for tumors obtained by rapid autopsy for patient LAT002 (RA007).

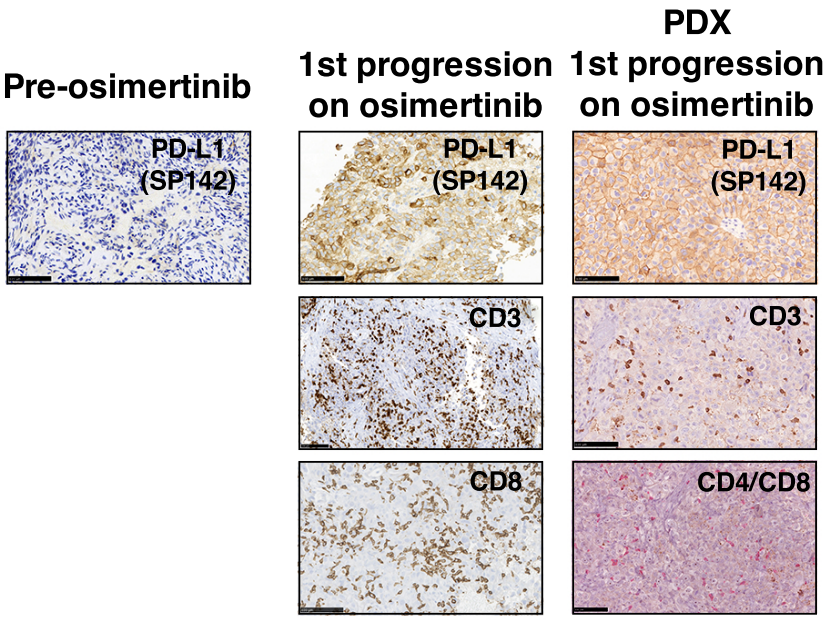


Figure S3. PD-L1 amplification upon acquired resistance to osimertinib in patient LAT021. Related to Figure 5. Hematoxylin and eosin (H&E), PD-L1, CD3, and CD8 immunohistochemical staining of pre- and post-osimertinib resistant tumors in patient LAT021 and LAT021 PDX derived at time of first progression on osimertinib. CD4/CD8 double immunohistochemical stain was used for the PDX. Scale bars, 100 μm.

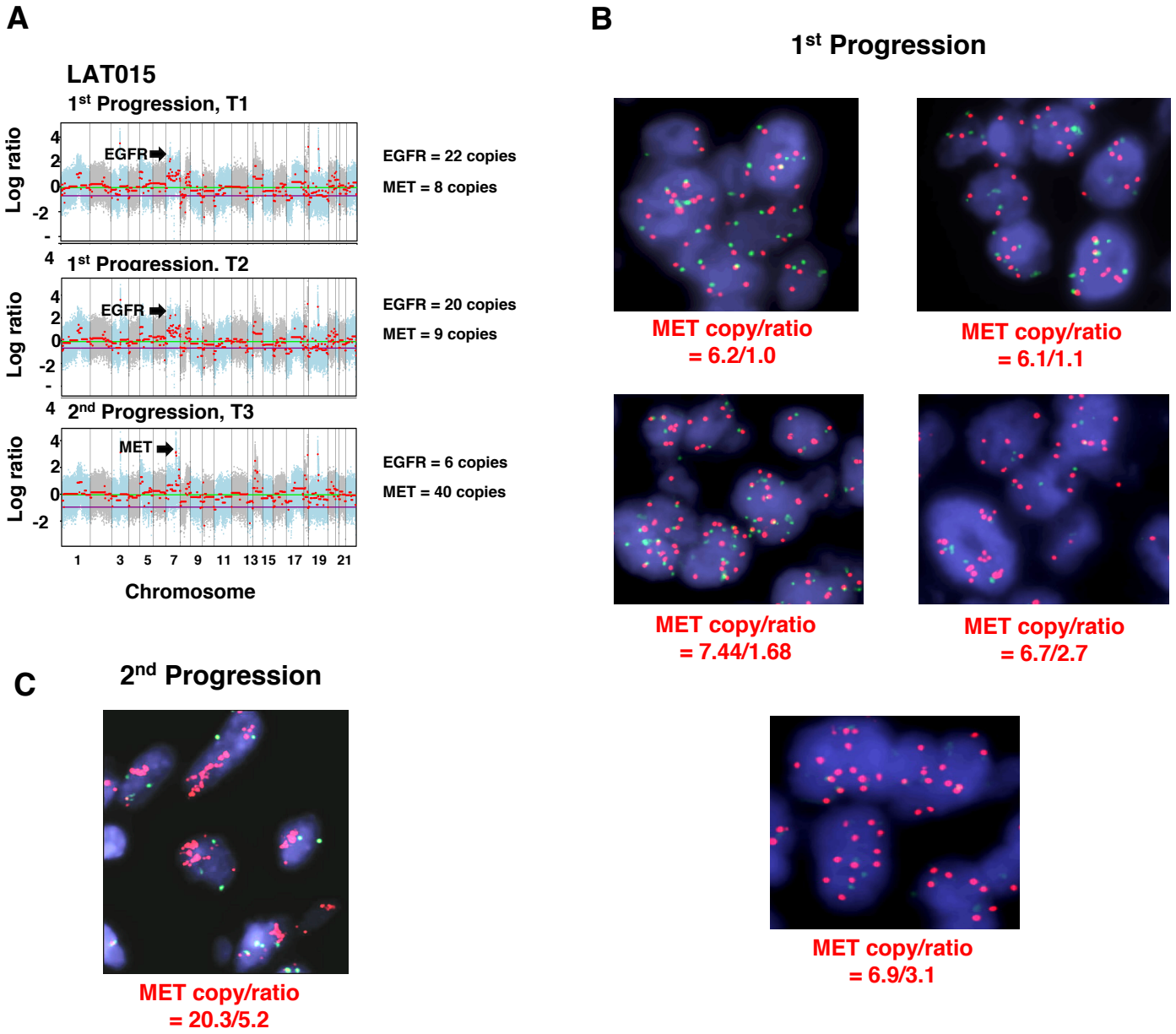


Figure S4. Multi-region heterogeneity of *MET* amplification by *MET* copy number and *MET/CEP7* ratio in the development of acquired resistance to osimertinib in patient LAT015. Related to Figures 5 and 6. (A) FACETS copy number plots from WES are shown at first and second progression on osimertinib. **(B)** FISH for *MET* at first progression and **(C)** at second progression.

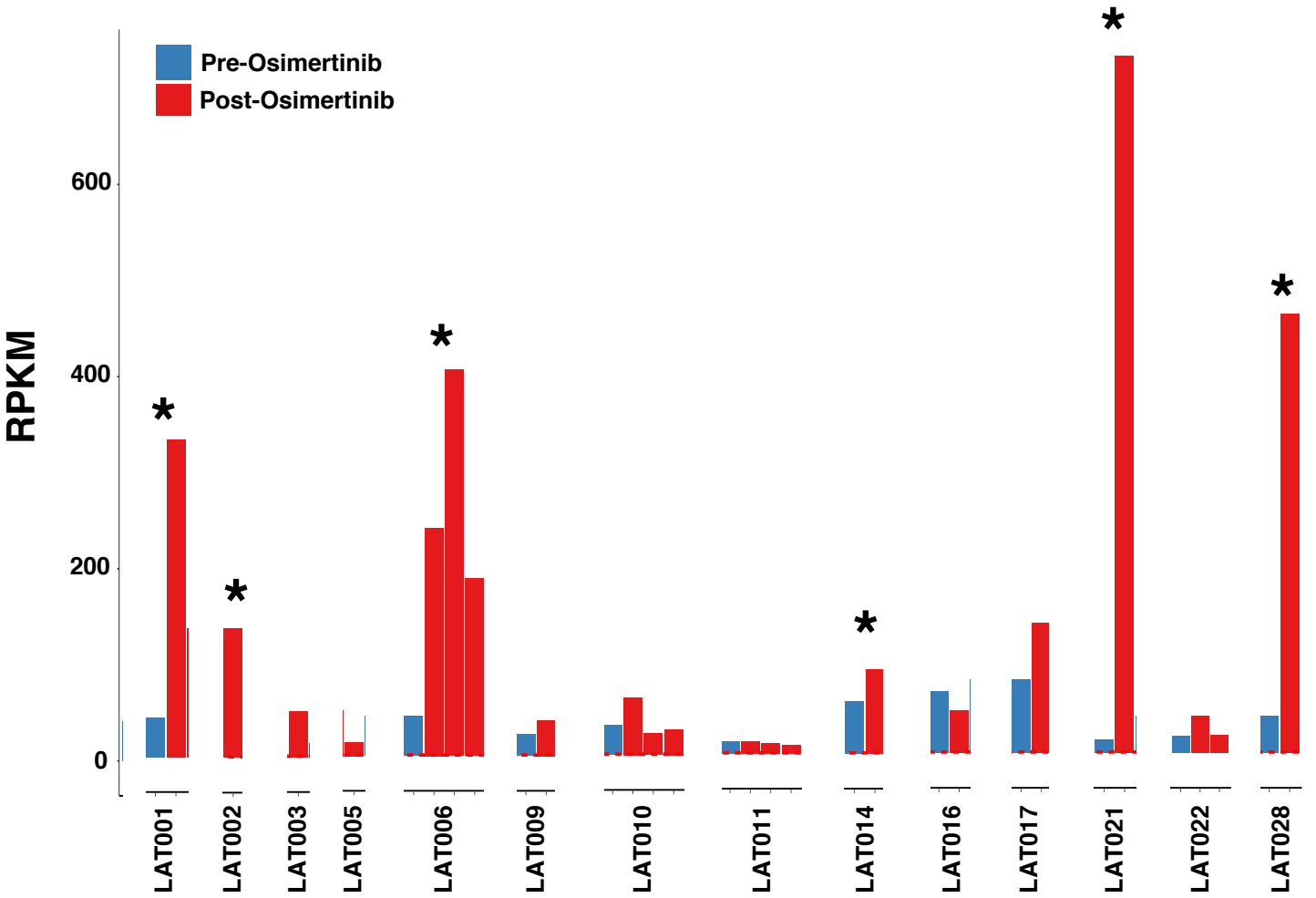
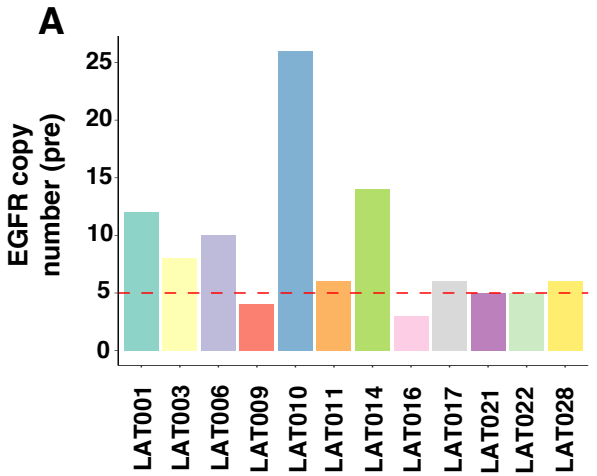
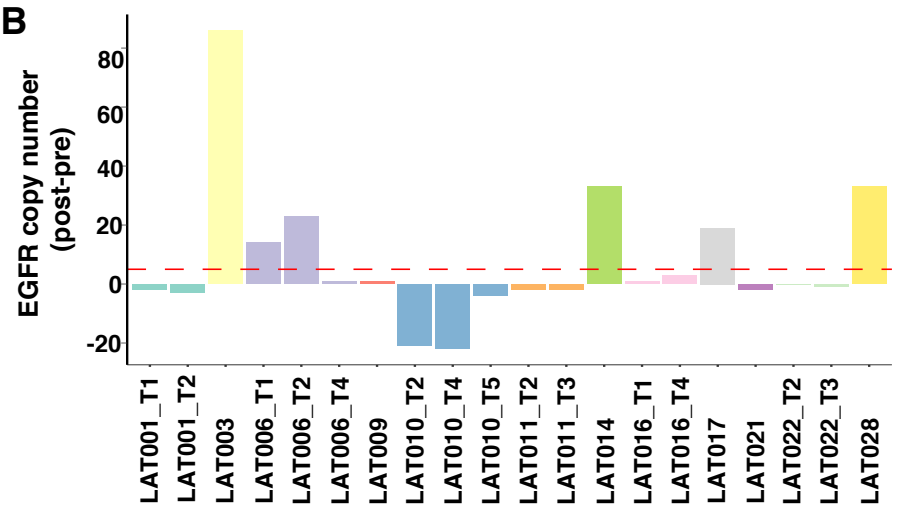
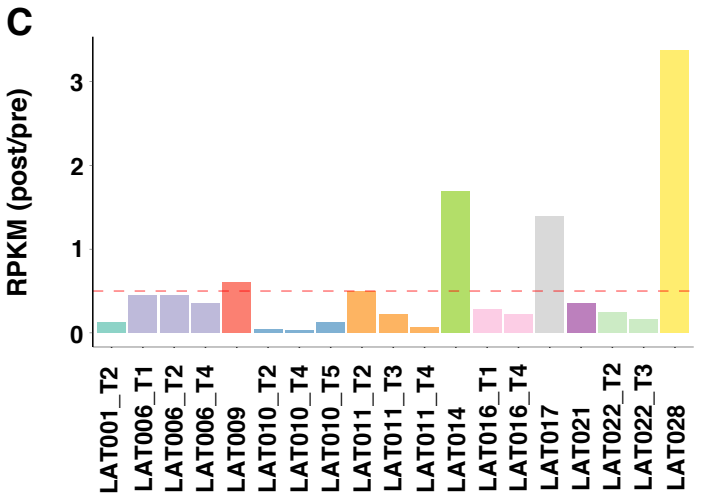
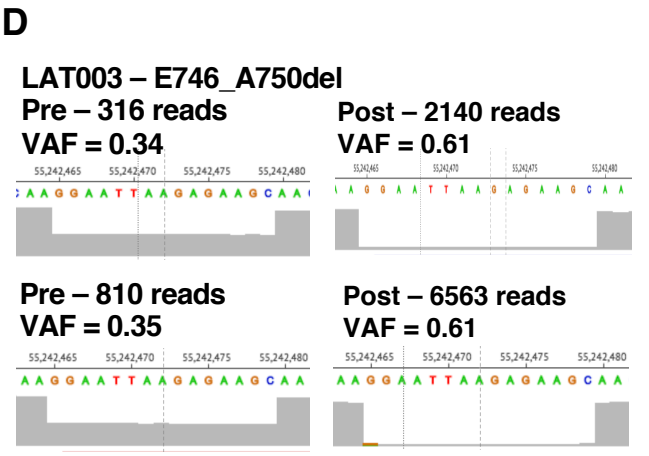


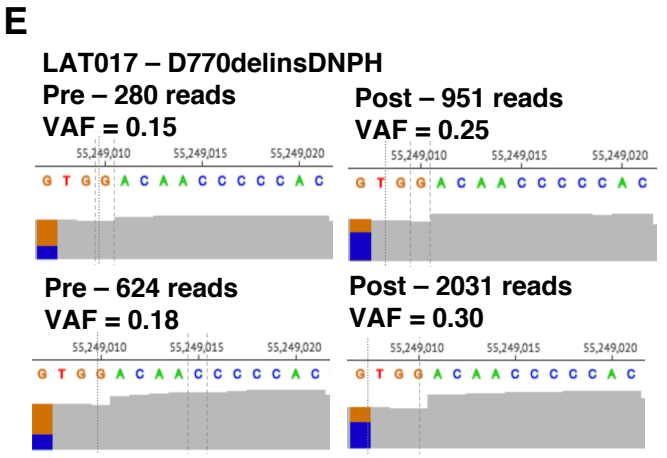
Figure S5. *MET* expression in pre- and post-osimertinib resistant tissues. Related to Figures 5 and 6. Asterisks represent patients with *MET* amplification upon acquired resistance to osimertinib.

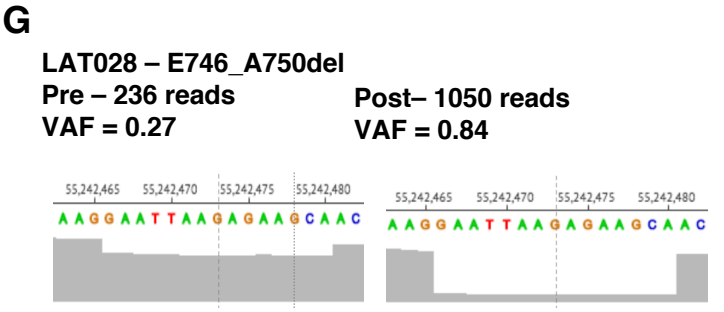












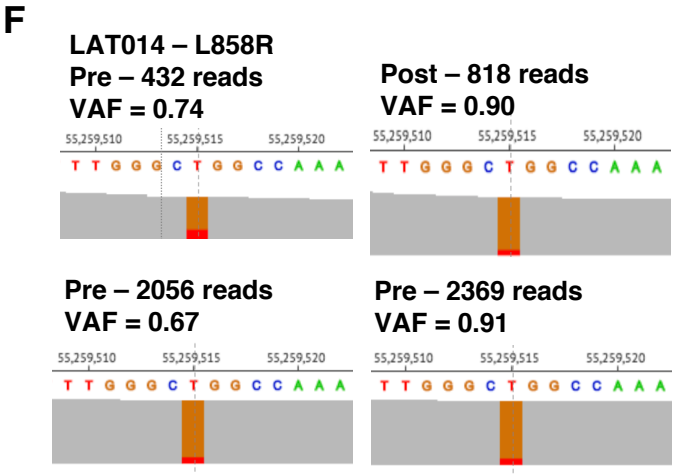


Figure S6. Amplification of *EGFR* mutant allele as a mechanism of acquired resistance to osimertinib. Related to Figures 3 and 4. (A) *EGFR* copy number in pre-osimertinib treated tumors. **(B)** *EGFR* copy number in post-osimertinib treatment minus pre-osimertinib treated tumors. **(C)** Fold change in EGFR RPKM between post-osimertinib and pre-osimertinib treated tumors. IGV of EGFR sensitizing mutations in pre- and post-osimertinib treated tumors in patients **(D)** LAT003, **(E)** LAT017 and **(F)** LAT014 using from both targeted and exome sequencing and **(G)** LAT028 using exome sequencing only.