Supplementary materials: De Novo T790M Mutation in an L858R Epidermal Growth Factor Receptor Mutant-Associated Lung Adenocarcinoma

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Figure S1. Human epidermal growth factor receptor (EGFR) bacterial artificial chromosome (BAC) constructs. Structure of the human EGFR BAC clone composed of the full-length coding sequence and 5' and 3'-flanking sequences and diagrams of the transgenes construction. The tetracycline-response operon promoter element (TetO) gene was transferred to the BAC clones containing the [L858R]-hEGFR mutant or the [L858R+T790M]-hEGFR mutant (a, b). Sequence analysis of the constructs to confirm the presence of [L858R] mutation or double [L858R+T790M] mutation (c, d).
Figure S2. Mouse Clara cell secretory protein (CCSP) bacterial artificial chromosome (BAC) construct. Structure of the mouse CCSP BAC clone composed of the full-length coding sequence and 5'- and 3'-flanking sequences and diagram of the transgene construction (a). The reverse tetracycline transactivator (rtTA) gene was transferred to the BAC clone downstream of the CCSP promoter. Sequence analysis of the construct to confirm the presence of the rtTA sequence (b).
Figure S3. Polymerase chain reaction performed using tail DNA from wild type mice, [L858R] hEGFR and L858R/T790M hEGFR transgenic mice. DNA was extracted from the tail tissue and polymerase chain reaction (PCR) was carried out using specific primers and experimental conditions as described under materials and methods. hEGFR, human epidermal growth factor receptor; WT, wild type; TG, transgenic.
Fig. 2e

1° Ab: rabbit anti-phospho-AKT
2° Ab: goat anti-rabbit IgG-HRP

|        | (L858R) | (L858R+T790M) | A549 |
|--------|---------|---------------|------|
| WT     | EGFR    | TEGFR         |      |

72 kDa →
52 kDa →
Fig. 2e

1st Ab: rabbit anti-β-actin
2nd Ab: goat anti-rabbit IgG-HRP

|       | WT | EGFR | TEGFR | A549 |
|-------|----|------|-------|------|
| (L858R) |    |      |       |      |
| (L858R+T790M) |    |      |       |      |

72 kDa → β-Actin
52 kDa

Figure S3

T790M mutation specific PCR

L858R mutation specific PCR
Figure S4. Uncropped western blot figures.

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