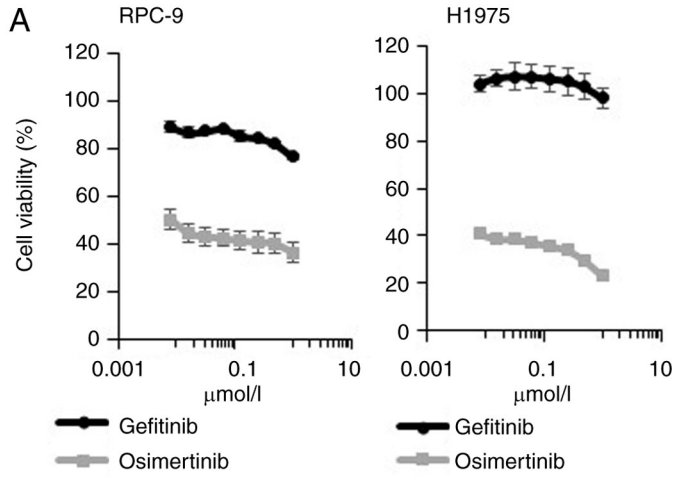
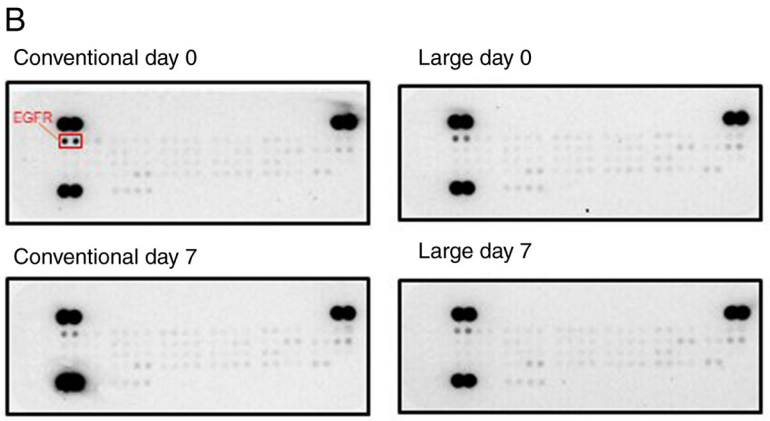
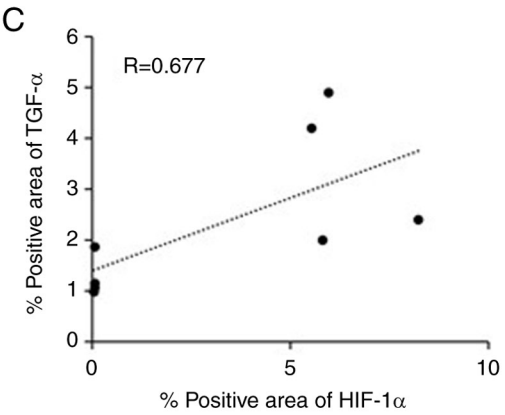
Figure S1. HIF-1α/TGF-α expression attenuates sensitivity to osimertinib in RPC-9 cells harboring EGFR mutations. (A) Effect of gefitinib or osimertinib on RPC-9 or H1975 cell viability 50% inhibitory concentration: RPC-9 (gefitinib, not reached; osimertinib, 17.46 nM); H1975 (gefitinib, not reached; osimertinib: 7.81 nM). Data are presented as the mean ± SEM. (B) Phospho-RTK array. Effect of osimertinib (5 mg/kg/day, day 7) on receptor tyrosine kinase phosphorylation in RPC-9 cell xenograft tumors from the conventional or large models. (C) Correlation between HIF-1α and TGF-α expression levels. (D) Crystal violet assay. Effect of osimertinib (72 h) on the viability of RPC-9 cells pre-incubated under hypoxic or normoxic conditions. HIF-1α, hypoxia-inducible factor-1α; TGF-α, transforming growth factor-α; Osi, osimertinib.







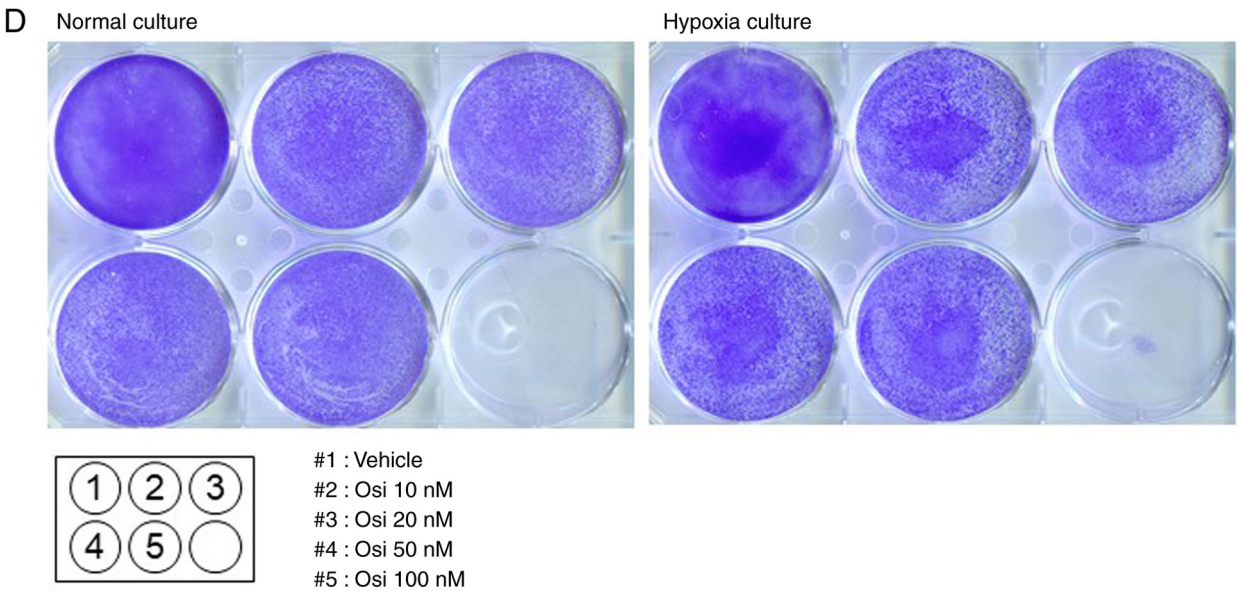


Figure S2. Inhibitory effect of cetuximab, osimertinib or a combination of osimertinib and cetuximab (72 h) on the viability of RPC-9 cells pre-incubated under hypoxic conditions for 48 h. Crystal violet assay data were quantified using ImageJ software. Data are presented as the mean ± SEM. Comb., combination of osimertinib and cetuximab.

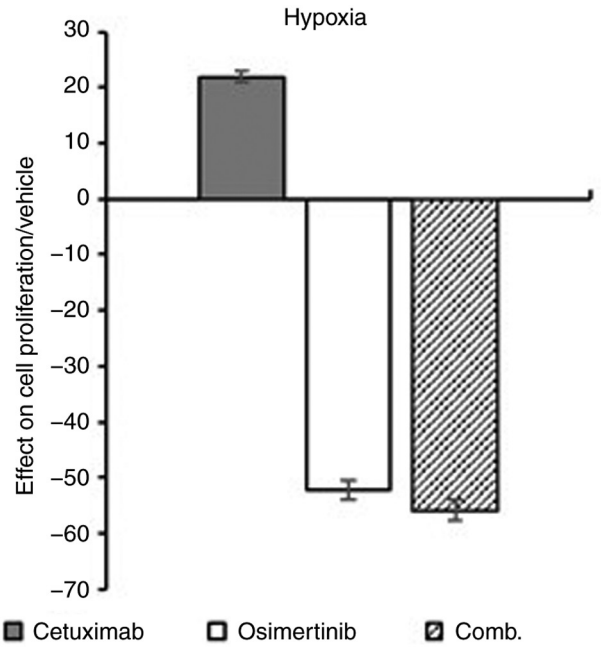


Figure S3. Effect of bevacizumab, cetuximab or a triple therapy with osimertinib, bevacizumab and cetuximab in xenograft tumors with HIF-1α/TGF-α expression. (A) Effect of bevacizumab (5 mg/kg, twice/week) and cetuximab (1 mg/body, twice/week) for 28 days on RPC-9 cell xenograft tumors from the large model (starting tumor volume, 500 mm^3; n=8), with a 28-day observation period. (B) Effect of osimertinib monotherapy (5 mg/kg, 5 times/week), its combination with cetuximab (1 mg/body, twice/week) or triple therapy with bevacizumab (5 mg/kg, twice/week) and cetuximab (1 mg/body, twice/week) for 28 days on RPC-9 cell xenograft tumors from the large model (starting tumor volume, 500 mm^3; n=6), with a 28-day observation period. Data are presented as the mean ± SEM. $^{**}P<0.01$. n.s., not significant.

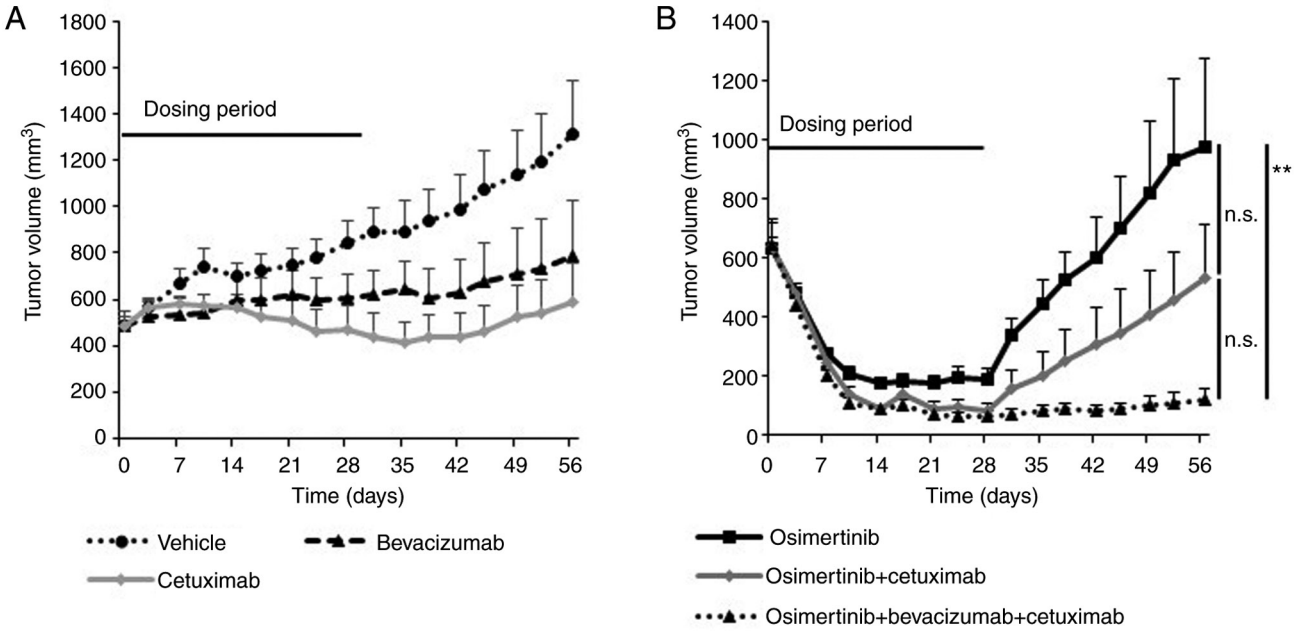


Figure S4. Mechanism of triple therapy with osimertinib, bevacizumab and cetuximab in tumors with HIF-1α/TGF-α expression. (A-a) EGFR-mutant lung cancer with low HIF-1α/TGF-α expression. (b) Osimertinib plus bevacizumab inhibits EGFR signaling and angiogenesis. (B-a) EGFR-mutant lung cancer with high HIF-1α/TGF-α expression. (b) Osimertinib plus bevacizumab does not inhibit the activation loop of the HIF-1α/TGF-α axis. (C) EGFR-mutant lung cancer with high HIF-1α/TGF-α expression. Triple therapy with osimertinib, bevacizumab and cetuximab inhibits EGFR signaling, angiogenesis and the activation loop of the HIF-1α /TGF-α axis. HIF-1α, hypoxia-inducible factor-1α; TGF-α, transforming growth factor-α; EGFR, epidermal growth factor receptor; Osi, osimertinib; Bev, bevacizumab; Cetu, cetuximab.