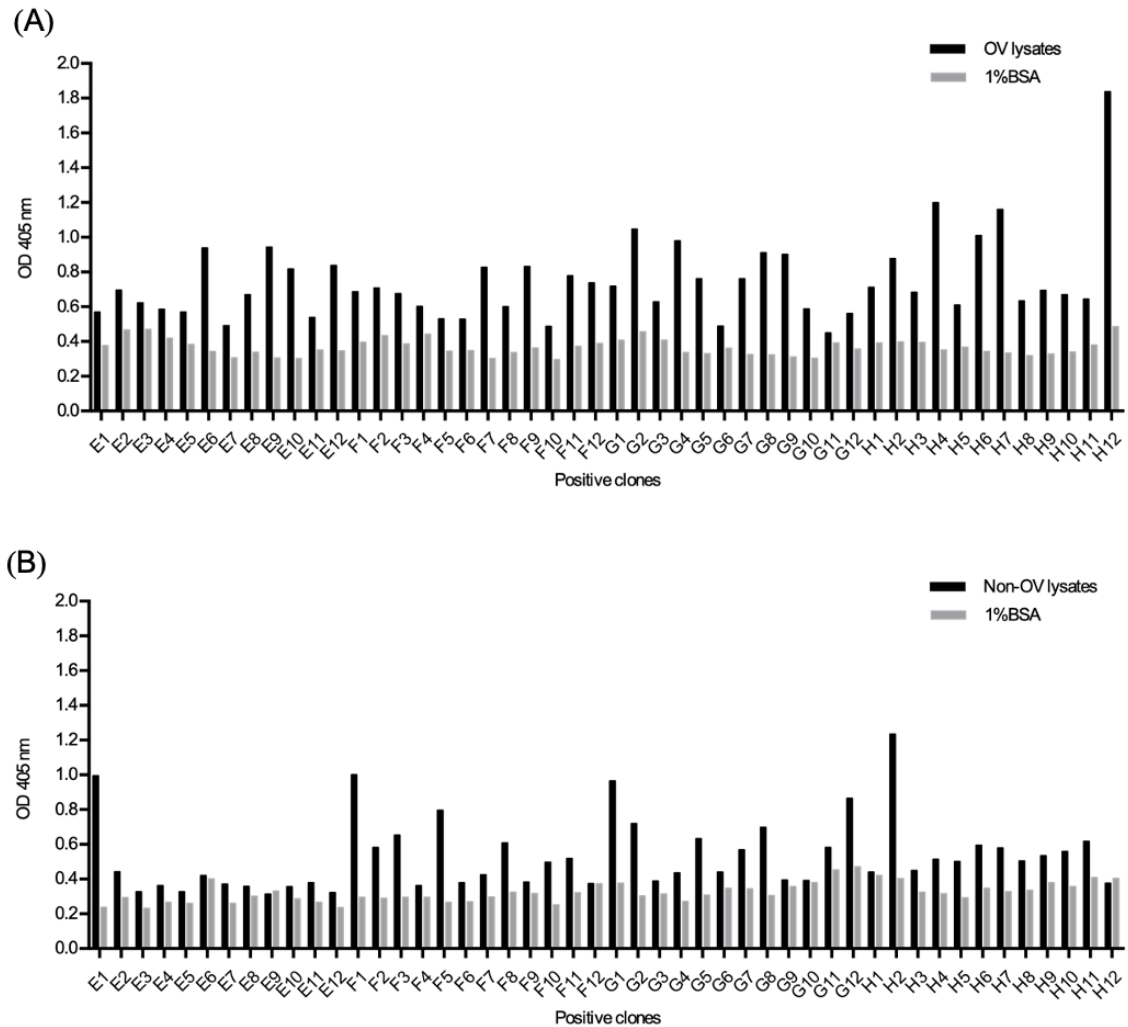
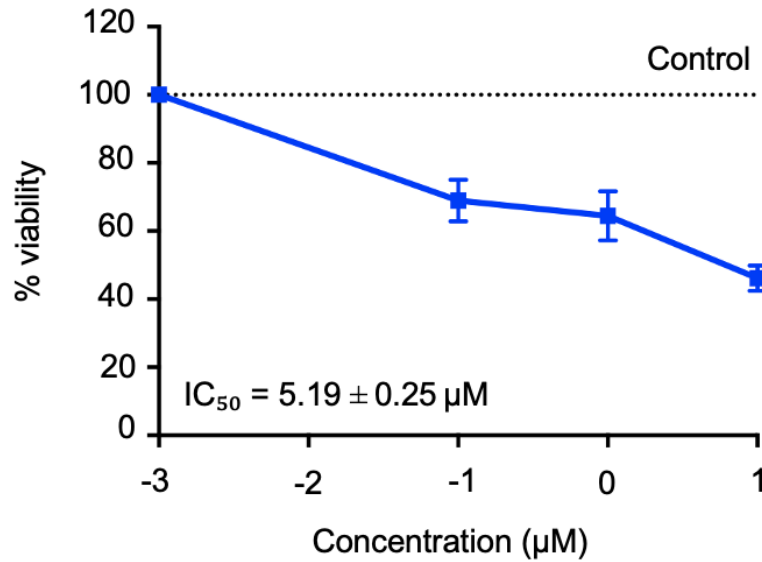


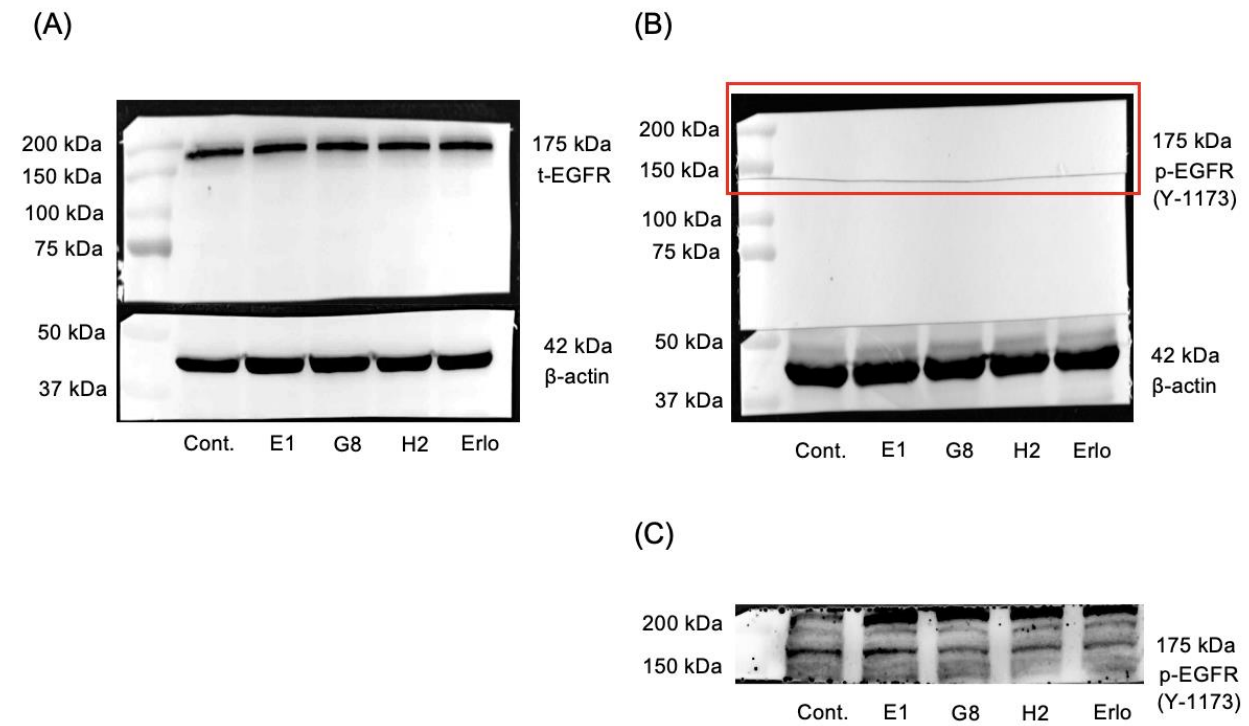


Supplementary Fig. S1. Identification of positive scFv phage clones from the YAMO I library. Indirect ELISA showing binding of scFv phage clones to protein lysates from (A) OV-CCA and (B) non-OV CCA. Binding is shown as OD405 compared to 1% BSA control.

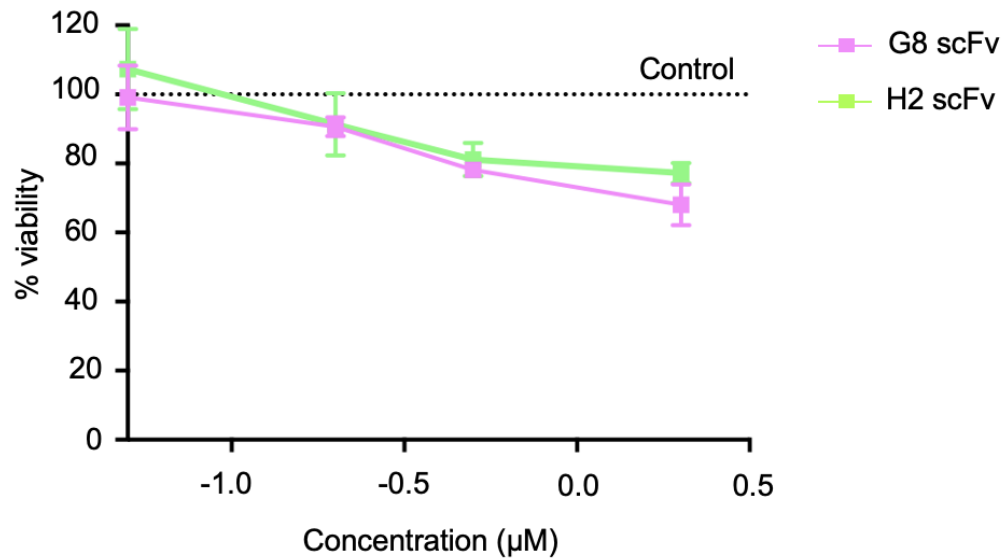


Supplementary Fig. S2. Effect of erlotinib on the viability of HuCCA-1 cells.

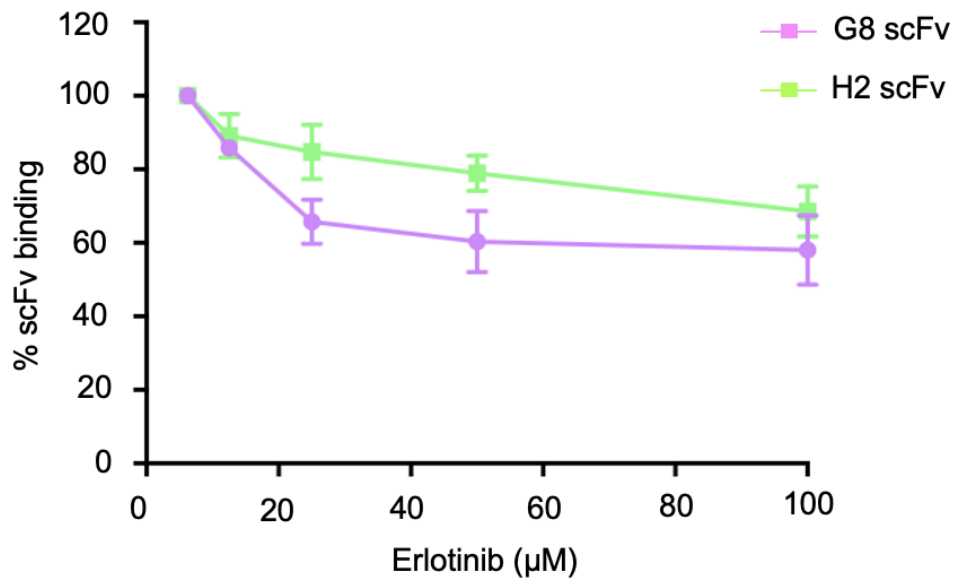
HuCCA-1 cells were treated with increasing concentrations of erlotinib, an EGFR inhibitor, for 48 h. Cell viability, measured by MTT assay, decreased in a dose-dependent manner, with an IC₅₀ value of 5.19 ± 0.25 μM. Data represent the mean ± SEM of three independent experiments.



Supplementary Fig. S3. (A) and (B) show the original western blot membranes probed for total EGFR (t-EGFR) and phosphorylated EGFR (p-EGFR), respectively, in HuCCA-1 cells treated with scFv antibodies (E1, G8, and H2) or erlotinib. (C) presents the cropped region of the p-EGFR blot (outlined in red in Fig. B), showing the chemiluminescent signal used for data analysis and figure presentation.



Supplementary Fig. S4. Effect of scFv antibodies G8 and H2 on the viability of KKKU-M213 cells. KKKU-M213 cells were treated with increasing concentrations (0.05, 0.2, 0.5, and 2.0 μM) of G8 or H2 for 48 h. Cell viability, measured by MTT assay, showed a modest reduction, with approximately 70–80% of cells remaining viable. Data represent the mean ± SEM of three independent experiments.



Supplementary Fig. S5. Competitive binding assay of G8 and H2 scFv with erlotinib.

Increasing concentrations of erlotinib progressively reduced the binding of both scFvs, indicating that G8 and H2 compete with erlotinib for EGFR-TK engagement. Recombinant EGFR-TK protein (5 μg/well) was immobilized on ELISA plates. Twofold increasing concentrations of erlotinib (6.25-100 nM) were pre-incubated with the coated EGFR-TK, followed by washing to remove unbound drug. Subsequently, G8 or H2 scFvs (1 μM) were added. A dose-dependent decrease in scFv binding was observed in the presence of erlotinib. Data represent the mean $\pm$ SEM of three independent experiments.