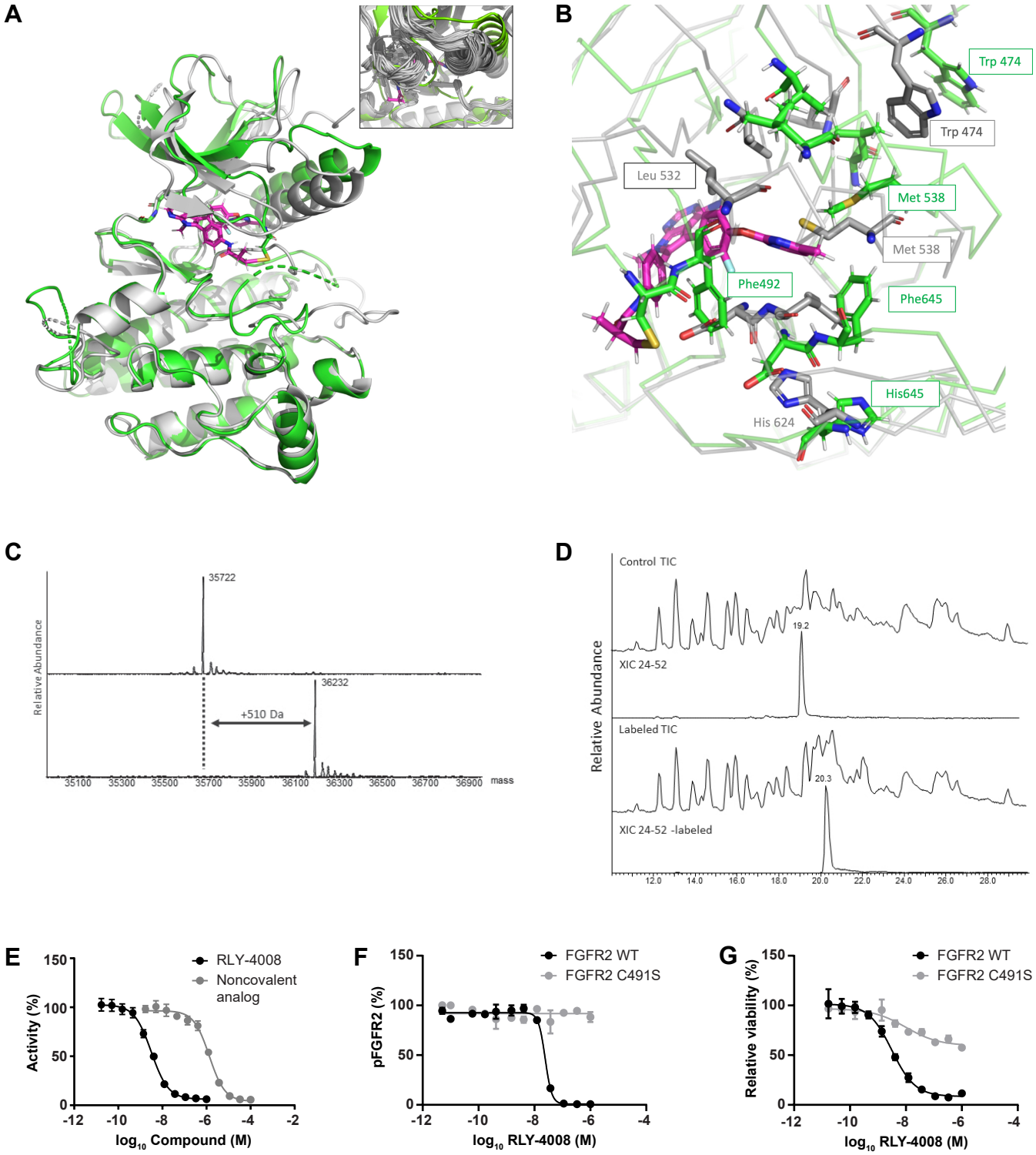
Supplementary Figure S1



Supplementary Figure S1. RLY-4008 is an irreversible inhibitor of FGFR2. **A** and **B,** Conformational modulation of FGFR2 by RLY-4008. **A,** Crystal structures of FGFR2 crystallized with an ATP analog (PDB ID: 2PVF) shown in gray and FGFR2:RLY-4008 shown in green. Structures are aligned with respect to the C-lobe. Inset shows a representative alignment of all FGFR2 kinase domain structures (colored gray) in the PDB. The overall superposition of the RLY-4008 structure shows a novel FGFR2 conformation. **B,** Changes along the FGFR2 hydrophobic spine. As reference point, equivalent residues from structure PDB ID: 2PVF are shown in gray. **C,** Deconvoluted intact mass spectra of FGFR2 (top) and RLY-4008-labeled FGFR2 (bottom). The spectra shown were obtained with 1 μg of protein. The molecular weight shift corresponds to a single addition of RLY-4008. **D,** Peptide maps of labeled and unlabeled FGFR2. Total ion current (TIC) traces FGFR2 and RLY-4008-labeled FGFR2 are shown along with the extracted ion traces (XIC) of the labeled and unlabeled peptide containing Cys491. The mass shift of the labeled peptide corresponds to the addition of a single molecule of RLY-4008, which was confirmed by exact mass and tandem MS measurements. **E,** Biochemical inhibition of FGFR2 by RLY-4008 and a noncovalent analogue of RLY-4008 with a reduced acrylamide warhead. Enzymatic activity of FGFR2 was determined using a fluorescently labelled peptide substrate and Caliper technology (PerkinElmer) following a 30 min preincubation of FGFR2 and compounds. **F,** Inhibition of FGFR2 in *FGFR2* fusion-positive intrahepatic cholangiocarcinoma (ICC) cells (ICC13-7; FGFR2 WT) and ICC13-7 cells in which $FGFR2^{C491S}$ is overexpressed (ICC13-7-$FGFR2^{C491S}$; FGFR2 C491S). Cells were incubated with RLY-4008 for 2 h prior to lysis and analysis via pFGFR2 (Y653/654) HTRF (PerkinElmer). **G,** Inhibition of proliferation by RLY-4008 in ICC13-7 (FGFR2 WT) and ICC13-7-$FGFR2^{C491S}$ (FGFR2 C491S) cells. Cells were treated for 96 h and cellular viability was assayed using CellTiter-Glo (Promega).