**Table W1.** Number of Colonies Obtained in the BaF3 Screen.

| Screen | Concentration | Cells Seeded per Well | Wells Seeded | Wells with Outgrowth | Clones Sequenced | Mutant Clones of Sequenced Colonies | Mutants | Occurrence | Frequency among Clones | Frequency among Mutants |
|---|---|---|---|---|---|---|---|---|---|---|
| FGFR2b screen | 100 nM (5 × $IC_{50}$) | 1 × $10^5$ | 192 | 31 | 24 | 7 of 35 | Parental | 28 | 80 | |
| | | | | | | | M538I | 1 | 2.9 | 14.3 |
| | | | | | | | N550H | 4 | 11.4 | 57.1 |
| | | 4 × $10^5$ | 192 | 42 | 11 | | E566G | 1 | 2.9 | 14.3 |
| | | | | | | | L618M | 1 | 2.9 | 14.3 |
| | 200 nM (10 × $IC_{50}$) | 1 × $10^5$ | 192 | 8 | 8 | 13 of 19 | Parental | 6 | 31.6 | |
| | | | | | | | M536I | 1 | 5.3 | 7.7 |
| | | | | | | | I548V | 1 | 5.3 | 7.7 |
| | | 4 × $10^5$ | 192 | 11 | 11 | | N550H | 10 | 52.6 | 76.9 |
| | | | | | | | V565I | 1 | 5.3 | 7.7 |
| | 300 nM (15 × $IC_{50}$) | 1 × $10^5$ | 192 | 3 | 3 | 6 of 9 | Parental | 3 | 33.3 | |
| | | | | | | | N550H | 3 | 33.3 | 50 |
| | | | | | | | N550K | 1 | 11.1 | 16.7 |
| | | 4 × $10^5$ | 192 | 6 | 6 | | N550S | 1 | 11.1 | 16.7 |
| | | | | | | | Y770IfsX14 | 1 | 11.1 | 16.7 |
| FGFR2b S252W screen | 100 nM (5 × $IC_{50}$) | 1 × $10^5$ | 192 | 148 | | None | | | | |
| | | 4 × $10^5$ | 192 | 147 | | | | | | |
| | 200 nM (10 × $IC_{50}$) | 1 × $10^5$ | 192 | 29 | 20 | 3 of 20 | Parental | 17 | 85 | |
| | | | | | | | N550T | 1 | 5 | 33.3 |
| | | | | | | | E566A | 2 | 10 | 66.7 |
| | | 4 × $10^5$ | 192 | 58 | 0 | | | | | |
| | 300 nM (15 × $IC_{50}$) | 1 × $10^5$ | 192 | 4 | 1 | 1 of 15 | Parental | 14 | 93.3 | |
| | | | | | | | K642N | 1 | 6.7 | 100 |
| | | 4 × $10^5$ | 192 | 18 | 14 | | | | | |

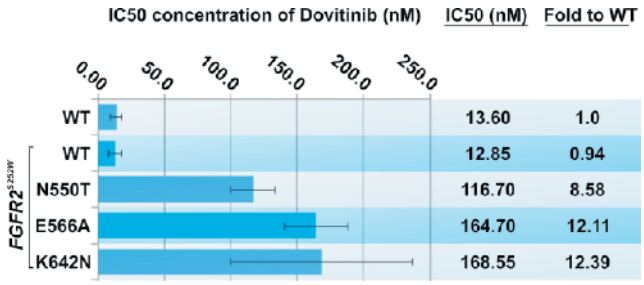


**Figure W1.** Sensitivity of compound $FGFR2^{S252W}$ dovitinib resistance mutations to dovitinib. The $IC_{50}$ values of FGFR2 WT, $FGFR2^{S252W}$ WT, and $FGFR2^{S252W}$ mutant (N550T, E566A, and K642N) BaF3 lines are presented. All three kinase mutations identified conferred significant resistance to dovitinib.

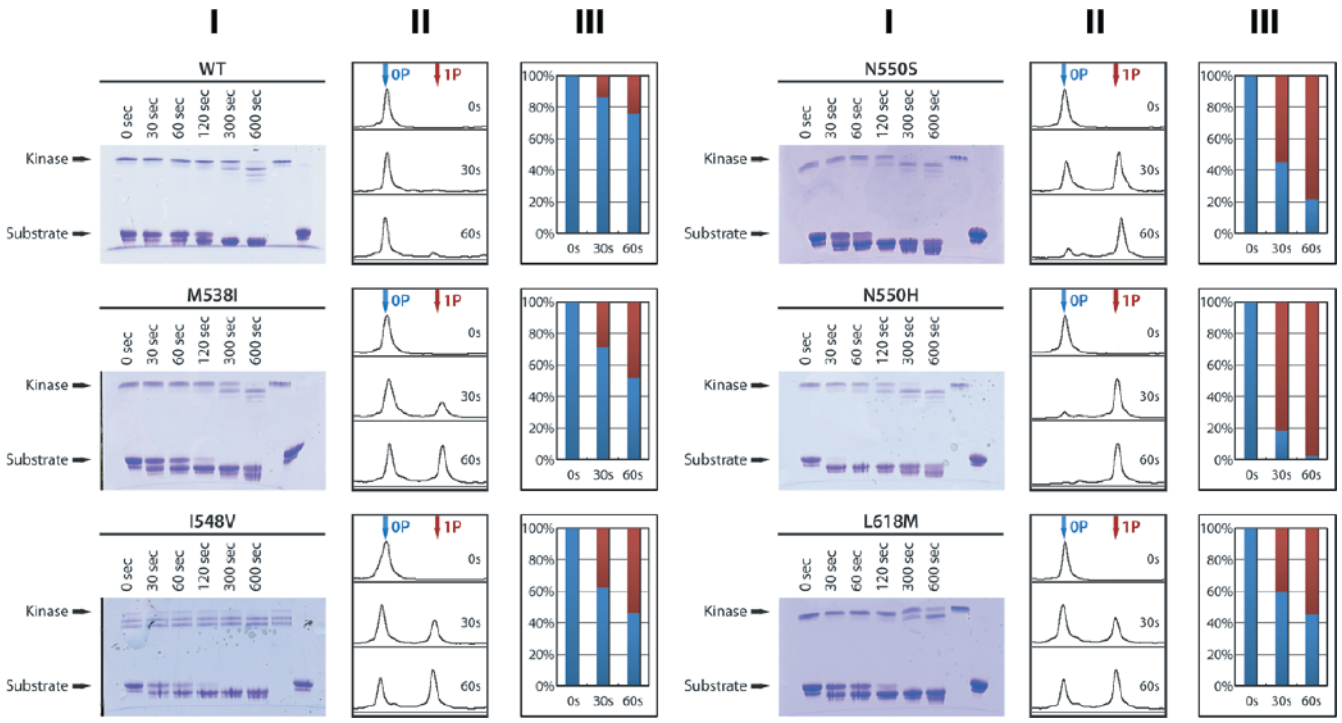


**Figure W2.** Dovitinib-resistant mutations increase the intrinsic kinase activity of FGFR2. The substrate phosphorylation activities of WT and mutated FGFR2 kinase domain harboring the drug-resistant mutations were compared using native PAGE (panel I) coupled with time-resolved mass spectrometry (panels II and III). For accuracy, only the early time point (30- and 60-second) MS data, which are in the linear phase of the kinase assay, were processed. The percentage of at least one site phosphorylation on the substrate (panel III) was estimated by comparing peak intensities generated by mass spectrometry of phosphorylated and nonphosphorylated substrate peptides.

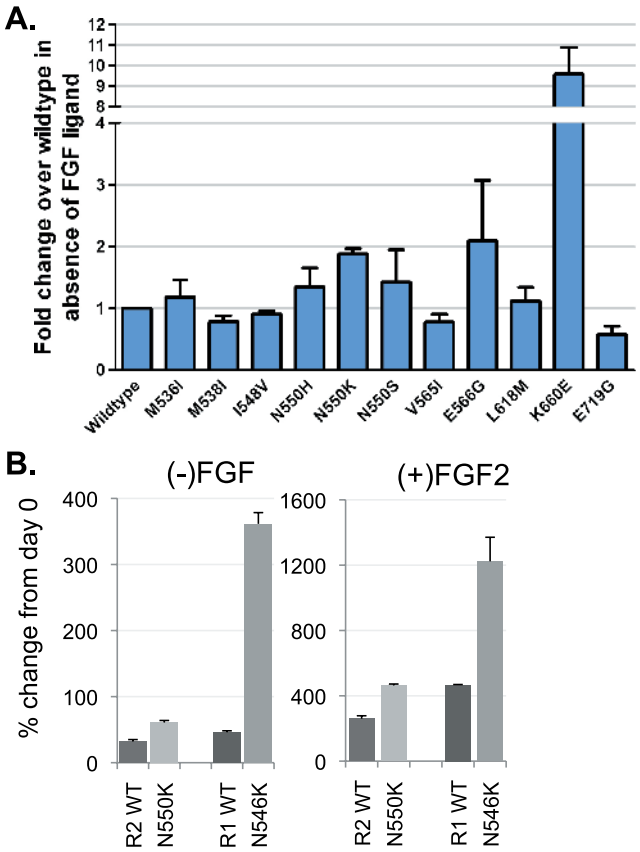


**Figure W3.** Ligand-independent proliferation of stable BAF3-FGFR2 cells. (A) FGFR2 WT and mutants are not sufficient to drive ligand-independent proliferation in BaF3 cells. Stable BaF3.FGFR2 cells were seeded at 10,000 cells/well in 96-well plates in IL-3–free media. The cells were incubated at 37°C for 72 hours and proliferation was measured using the ViaLight proliferation kit. The increase in proliferation compared to FGFR2 WT cells is presented. (B) In both the absence and presence of ligand, the homologous N546K mutation in FGFR1c can drive significantly more BaF3 proliferation than the FGFR2c N549K mutation, indicating the relative weak strength of FGFR2 *in vivo*.