Tarloxotinib (pro-drug)   **Tarloxotinib-E (active form)**
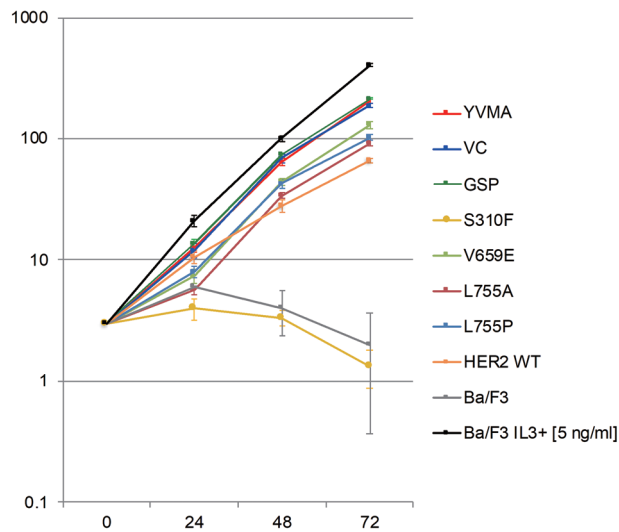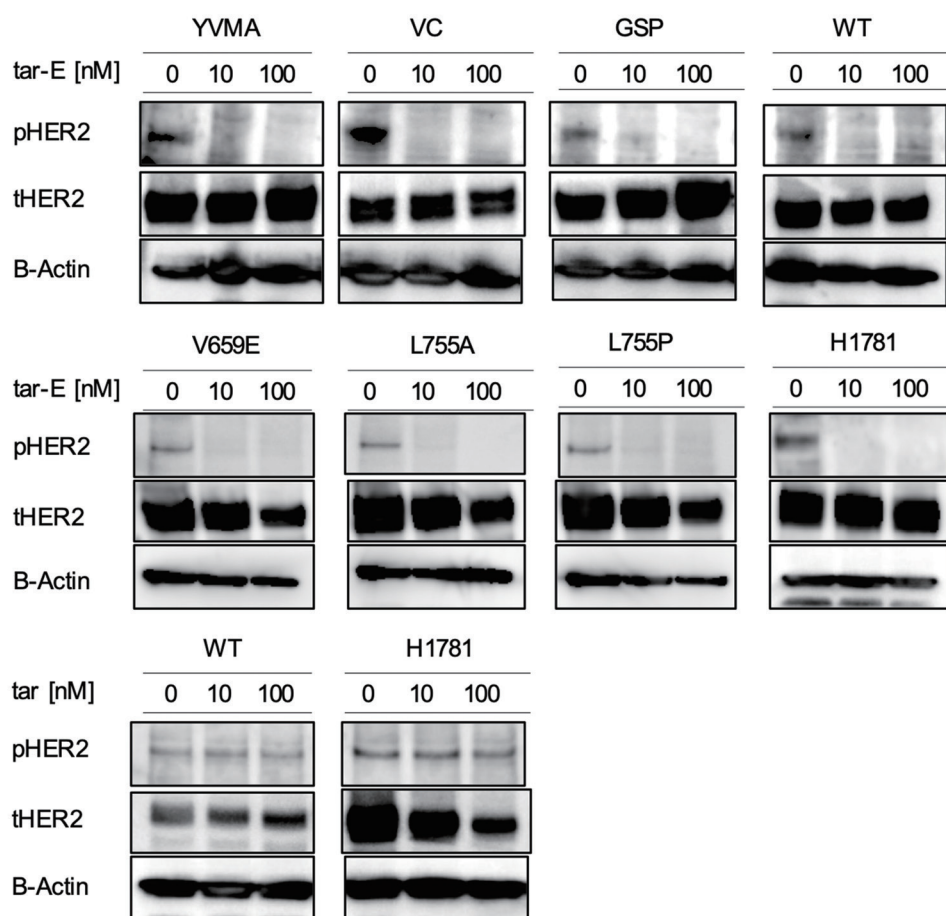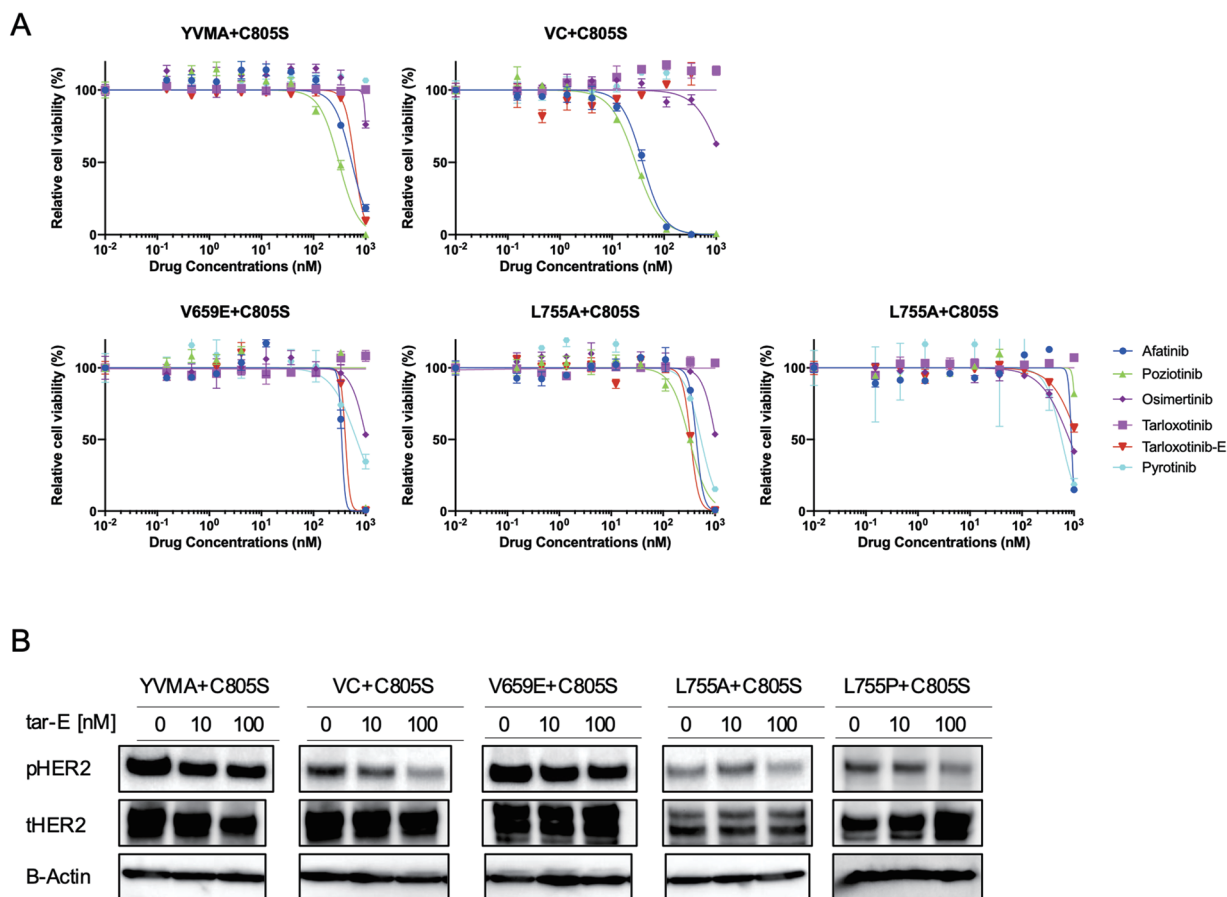

Figure S1 The structures of tarloxotinib (prodrug) and tarloxotinib-E (active drug). Tarloxotinib conversions to active form tarloxotinib-E, an irreversible EGFR/*HER2* inhibitor, under hypoxic condition in tumor microenvironment.

Figure S2 Growth of transfected Ba/F3 cells with *HER2* point mutations. Ba/F3 cells transfected with *HER2* V659E, L755A or L755P proliferated in the absence of IL-3. On the other hand, Ba/F3 cells with the S310F mutation were not viable under IL-3-independent growth conditions. YVMA, A775_G776insYVMA; VC, G776_delinsVC; GSP, P780_Y781insGSP.

Figure S3 Western blotting of pHER2, tHER2 and β-actin extracted from Ba/F3 cells transfected with WT *HER2*, *HER2* with exon 20 insertions, and *HER2* with point mutations that were treated with the indicated concentrations of tarloxotinib-E or tarloxotinib. YVMA, A775_G776insYVMA; VC, G776_delinsVC; GSP, P780_Y781insGSP; tar-E, tarloxotinib-E; pHER2, phospho-HER2; tHER2, total-HER2.

Figure S4 Growth inhibition curves and western blotting of tarloxotinib-E resistant clones. (A) Growth inhibition curves of Ba/F3 cells with a secondary C805S mutation generated through ENU mutagenesis show the resistance of these cells to all *HER2* TKIs. (B) Western blotting of p-HER2, total *HER2* and β-actin extracted from the TR clones treated with each *HER2* TKI. Phosphorylation of *HER2* in C805S clones was not decreased by tarloxotinib-E. YVMA, A775_G776insYVMA; VC, G776_delinsVC; GSP, P780_Y781insGSP; tar-E, tarloxotinib-E; pHER2, phospho-HER2; tHER2, total-HER2.

Table S1 Designed primers for HER2 mutation mutagenesis

HER2 mutation		Sequence
S310F	Forward	5'- TGGGATTCTGCACCCTCGTCTGCCCC -3'
	Reverse	5'- GGGTGCAGAATCCCACGTCCGTAGAAAGGTAG -3'
V659E	Forward	5'- CGGTGGAAGGCATTCTGCTGGTCGTGGTCTTG -3'
	Reverse	5'- GAATGCCTTCCACCGCAGAGATGATGGACGTC -3'
L755A	Forward	5'- TCAAAGTGGCGAGGGAAAACACATCCCCC -3'
	Reverse	5'- CCCTCGCCACTTTGATGGCCACTGGAA -3'
L755P	Forward	5'- AGTGCCGAGGGAAAACACATCCCCCAAAG -3'
	Reverse	5'- TTTTCCCTCGGCACTTTGATGGCCACTG -3'

Table S2 Designed primers for HER3 copy number analysis

	Sequence
Forward	5'- TGGGTTATGAGTACATGGATGTG -3'
Reverse	5'- CTTCATCTGGAGTTGTGCCTG -3'