



Additional File 2: Optimization of antibodies against p-Akt (S473) and p-ERK1/2 (T202/Y204) for immunohistochemistry. The antibodies for p-Akt (S473) and p-ERK1/2 (T202/Y204) were optimized for IHC using MCF-7 cells. For p-Akt antibody optimization, cells were starved and treated with 200nM BEZ235 for 1 hour or stimulated with serum and 100ng/ml heregulin for 20 minutes. For the p-ERK1/2 antibody, cells were starved of serum overnight and then treated with either 10uM of the MEK1/2 inhibitor UO126 for 1 hour or with media containing 100ng/ml EGF for 10 minutes. p-Akt (S473) and p-ERK1/2(T202/Y204) signal was detected by IHC staining (**A** and **C**) and confirmed by Western blotting (**B** and **D**). Scale bar 30 um (A and C).