Supplementary data

Phosphorylated and Unphosphorylated Serine 13 of CDC37 Stabilize Distinct Interactions Between its Client and HSP90 Binding Domains.

Wenjun Liu and Ralf Landgraf

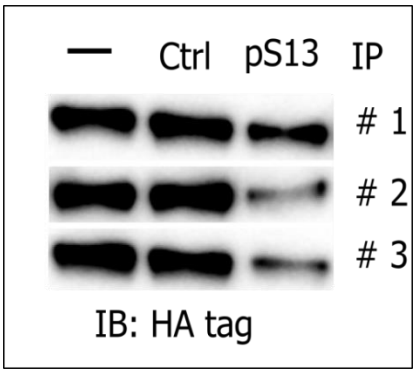

Figure S1: Estimate of phosphorylation efficiency of HA-tagged CDC37, expressed in MCF7 cells. Triple immunodepletion with pSer13/ CDC37 antibody removes approximately 90% from cell lysate.

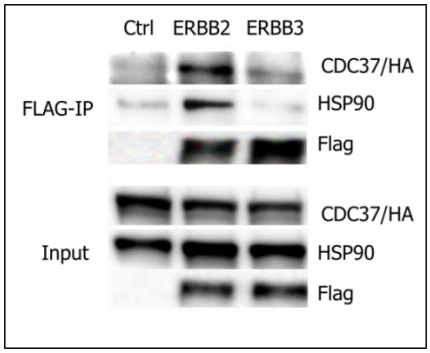

Figure S2: For HSP90, the association with clients in the nascent and mature state has different characteristics. This is a special concern when clients are recombinantly overexpressed at high levels and may include a higher fraction of misfolded protein. To evaluate whether this is a relevant factor for the comparison of CDC37 binding to ERBB2 and ERBB3, we supplemented whole lysate pull down studies with measurements that were limited to cell surface, and therefore mature, receptors. The ERBB2 and ERBB3 receptor tyrosine kinases were expressed recombinant in MCF7 cells, carrying N-terminal (extracellular) FLAG epitope tags. Anti-Flag antibodies were incubated with transfected cells on ice to prevent internalization. Free antibodies were washed away by multiple washes with ice cold PBS. (These conditions are validated for background removal based on previous microscopy studies [(1)]). Cells were lysed and proteins associated with cell surface localized ERBB receptors were precipitated with protein A/G. The IP shows HSP90 and CDC37 stably associated with ERBB2 but not ERBB3

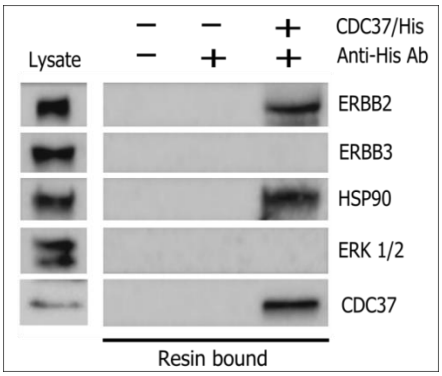

Figure S3: Recombinant, *E.coli* expressed CDC37 was in vitro phosphorylated by CK2 and used as immobilized bait (anti-His antibody) against BT474 cell lysate. This IP recovers ERBB2 and HSP90 but not ERBB3 or the non-client kinase ERK1/2. Note that, except for CDC37, qualitative antibody detection controls for all components in the cell lysate are shown at reduced exposure. The transfection with His-tagged CDC37- and samples that included anti-His antibody are indicated above the lanes. CDC37 was detected with the anti-CDC37 antibody. Therefore the lysate lane shows endogenous CDC37 while lane three shows only the tagged and immunoprecipitated, recombinant CDC37 that was added post lysis. This experiment complements figure two in the main manuscript in that it focuses on client at endogenous expression levels. The elevated levels of ERBB2 and ERBB3 in this case are the consequence of oncogenic gene amplification of ERBB2 and subsequent ERBB3 upregulation in BT474, a well-established model system for ERBB2 overexpressing breast cancer.

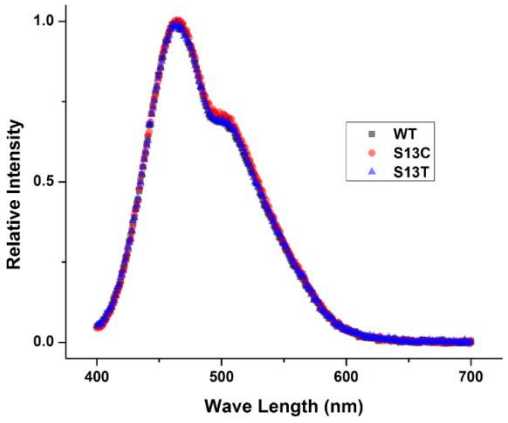

Figure S4: Both S13C and S13T mutations show ANS binding and fluorescence that mirrors wild type CDC37. S13C is shown in figure 5. S13T was omitted in figure 5 as it is less conclusive in isolation. Given the “limited capabilities” of pan-pSer/pThr detection systems and our reliance on an anti-pSer13 antibody, any phosphorylation at Thr13 that may occur upon CK2 treatment may not be detected. At least, a lack of signal cannot be considered conclusive. Hence S13T is inert towards CK2 treatment and shows WT like ANS fluorescence, but this result is only considered to be supportive to the more conclusive S13C replacement.

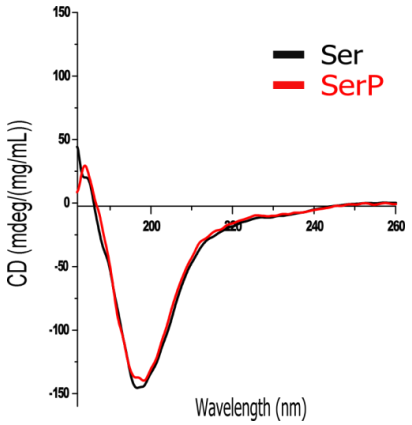

Figure S5: CD spectrum of synthetic peptides representing the first 19 amino acids of CDC37 plus an addition cysteine for immobilization applications in position 20. Despite the addition of trifluoroethnol up to 50% and reduction in temperature (data not shown), no helix formation could be induced for either the phosporylated (pSer) or unphosphorylated (Ser) peptide and both samples display a random coil characteristic spectrum

1. Zhang, Q., Park, E., Kani, K., and Landgraf, R. (2012) Functional isolation of activated and unilaterally phosphorylated heterodimers of ERBB2 and ERBB3 as scaffolds in ligand-dependent signaling, *Proceedings of the National Academy of Sciences of the United States of America 109*, 13237-13242.