Supplementary information for

Cell-free expression of functional receptor tyrosine kinases

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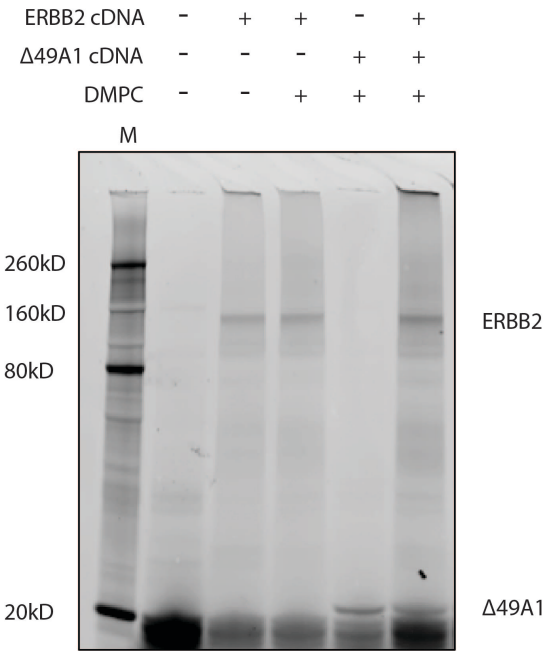
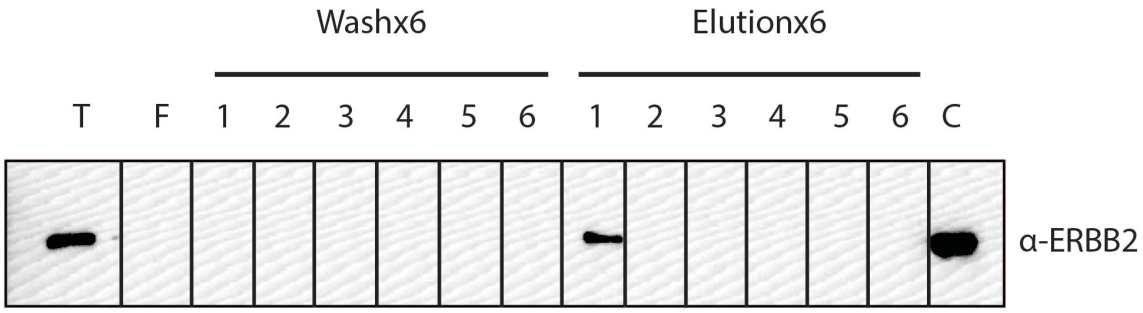
Supplementary Figure 1



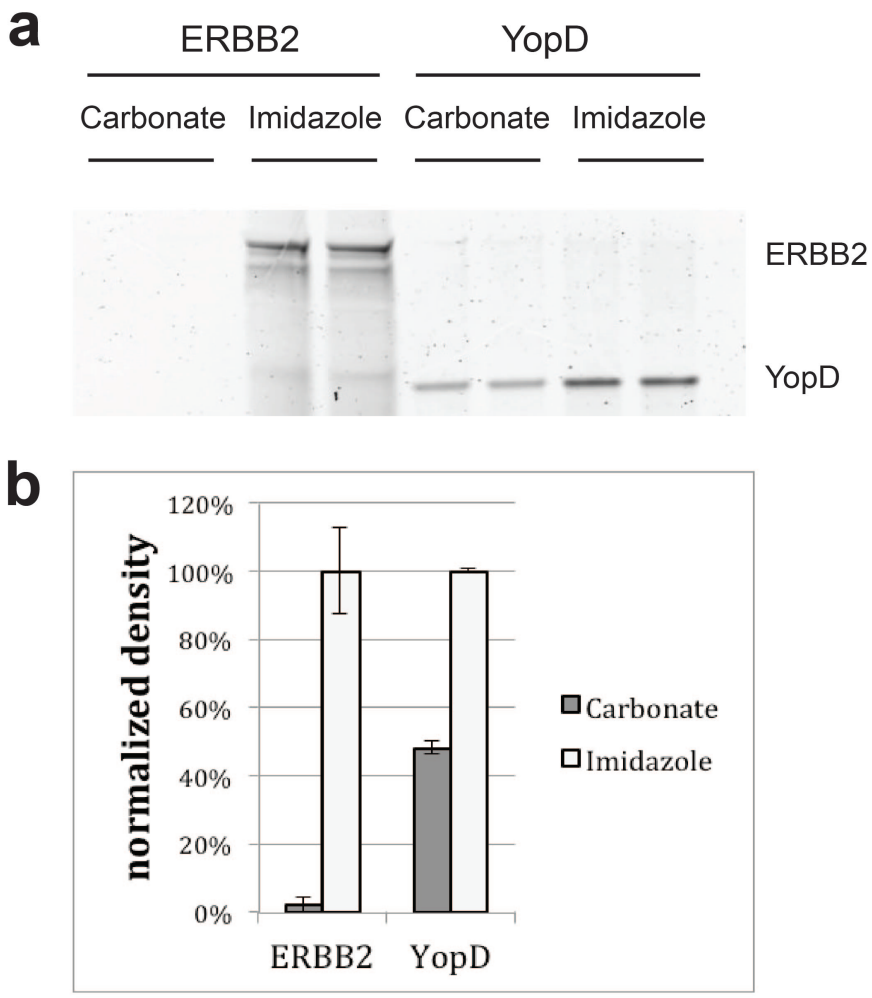
Fluorescent image of denaturing SDS PAGE of cell-free expressed Δ49A1 and ERBB2. Cell-free expressions were set up using the Expressway Maxi Cell-Free *E. coli* Expression System from Life Technologies according to manufacturer's user manual. Reactions were set up with: no DNA, DNA encoding ERBB2 only, DMPC and DNA encoding ERBB2, DMPC and DNA encoding Δ49A1 (empty NLPs), DMPC and DNA encoding both ERBB2 and Δ49A1 (ERBB2-NLPs). FluoroTect™ GreenLys (Promega) was added for visualizing synthesized protein. Reactions were ended after 18 hours by adding LDS sample buffer from Life Technologies. All samples were boiled for 5mins and resolved by 4-12% SDS-PAGE along with a molecular weight standard (M). Gel images were taken using Molecular Dynamics Typhoon 9410 Molecular Imager from GE Healthcare.

Supplementary Figure 2



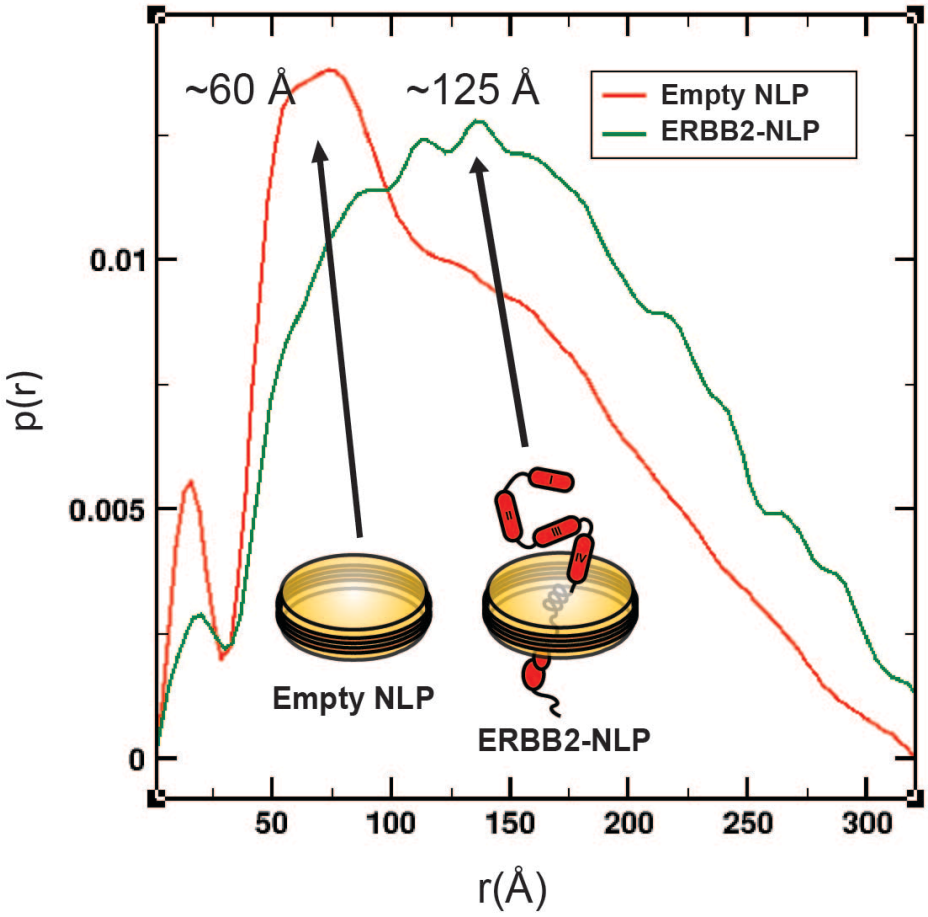
Western blot shows ERBB2-NLP can be Ni purified. Set up 1mL cell-free expression reaction to produce ERBB2-NLP as described in Methods. After 18 hours reaction, a small aliquot of crude was saved as total (T). The rest of the crude mixture was collected and incubated with 0.5 mL Ni-NTA SuperFlow resin (Qiagen) at 4 degree for 2 hours; the unbound crude was collected as flow through (F). Ni resin was then washed with 6mL wash buffer containing increasing concentration (10mM, 20mM, 50mM, two of each, 6 mL total) of imidazole (Wash, 1mLx6). The bound protein was then eluted with 1mL elution buffer containing 400mM imidazole for 6 times (Elution, 1mLx6). Elutions were combined and dialyzed against 1L of TBS, twice; then concentrated with Vivaspin column MWCO=100kDa (C). Final volume is 300uL. Samples were then resolved by SDS-PAGE and western blotting with anti-ERBB2 antibody Ab-3.

Supplementary Figure 3



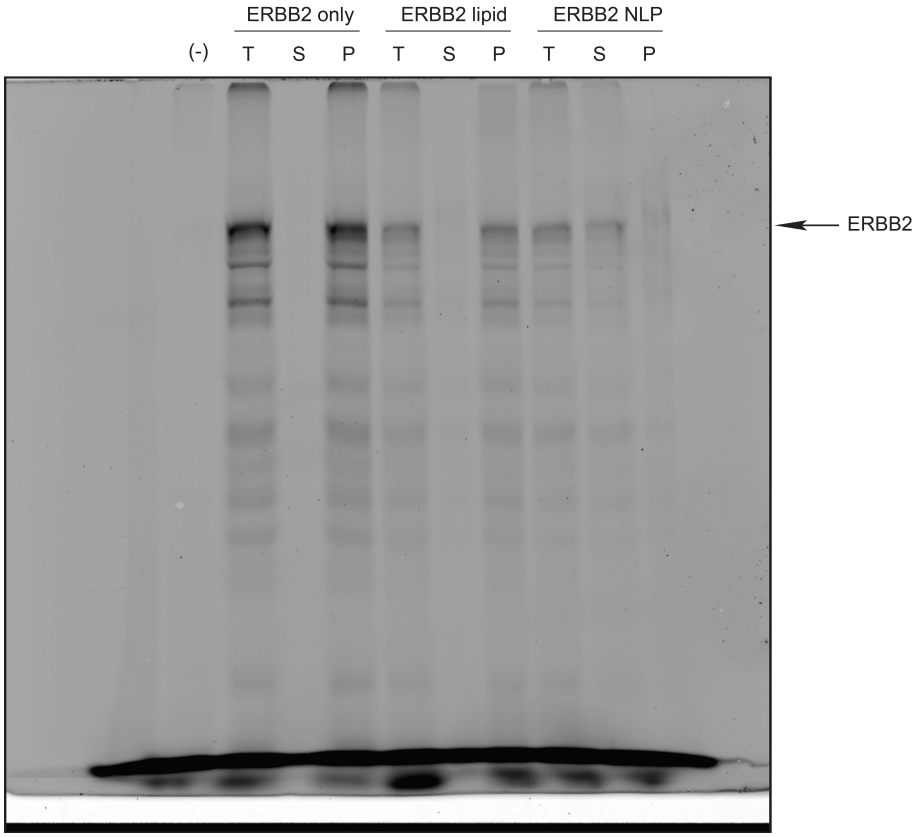
Cell-free expressed ERBB2-NLP and YopD-NLP (Bodipy labeled) were bound to Ni beads through a 6xHis tag located on the scaffold protein Δ49A1. The Ni beads were washed with buffer containing 20mM imidazole, and then eluted with 100 mM sodium carbonate, or 400mM imidazole. The eluents were concentrated and resolved by SDS-PAGE. Pictures of the gel were taken with GE Typhoon 9410 fluorescent imager Ext/Emt: 488 nm/520 nm (a). 48% of YopD protein can be carbonate extracted, whereas only 3% of ERBB2 protein can be carbonate extracted (b).

Supplementary Figure 4



Small angle X-ray scattering (SAXS) data for ERBB2-NLPs that were prepared by cell-free expression comparing with empty NLP. NLP and ERBB2 NLP samples were cell-free produced and Ni purified as described. Each sample consisted of 20 microliters of 0.23 mg/ml of total protein. There was a clear difference in the spectra of the NLP with ERBB2 incorporation indicating a 2-fold or more increase in thickness of the nanoparticles compared to the empty NLPs.

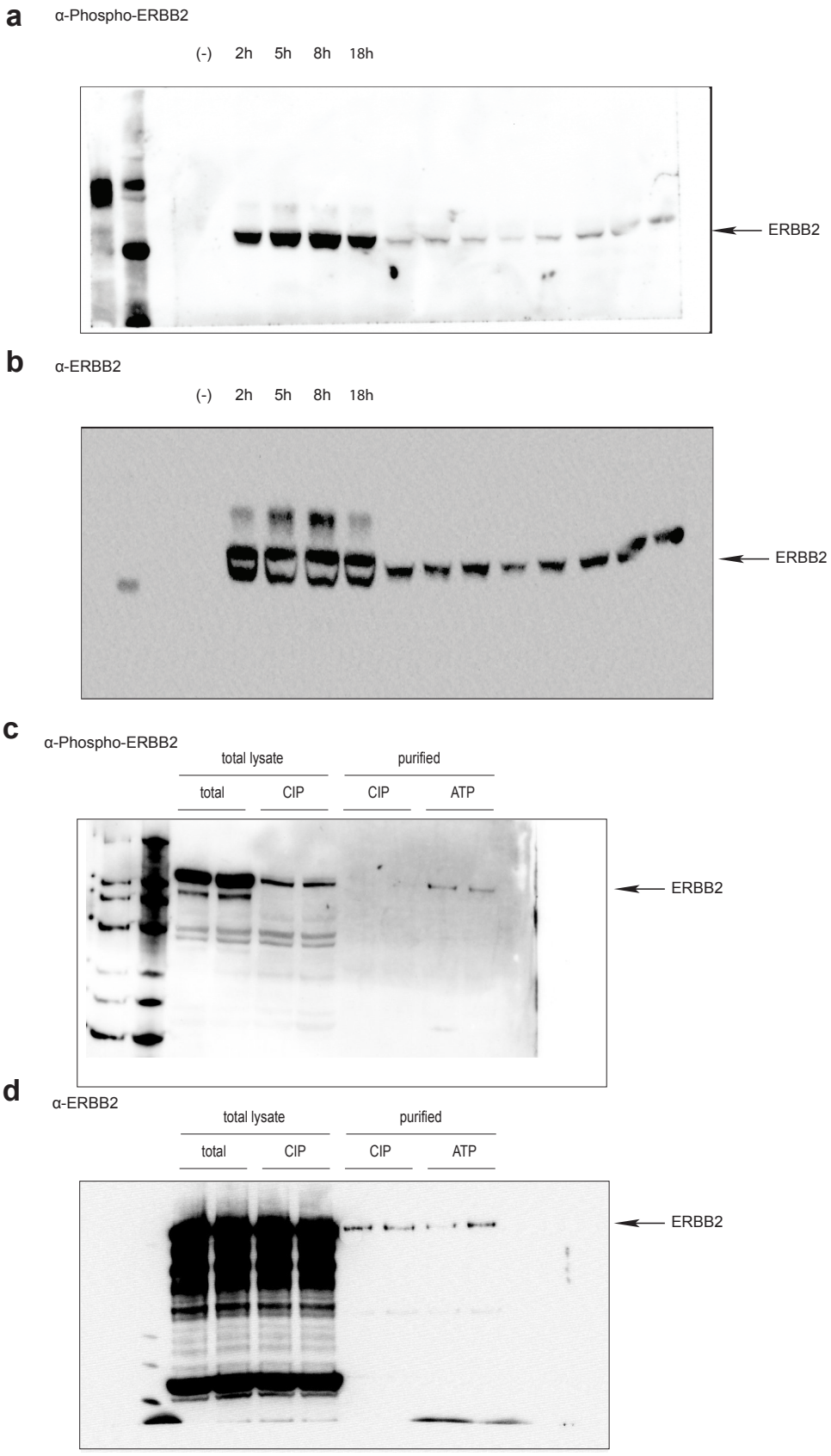
Supplementary Figure 5

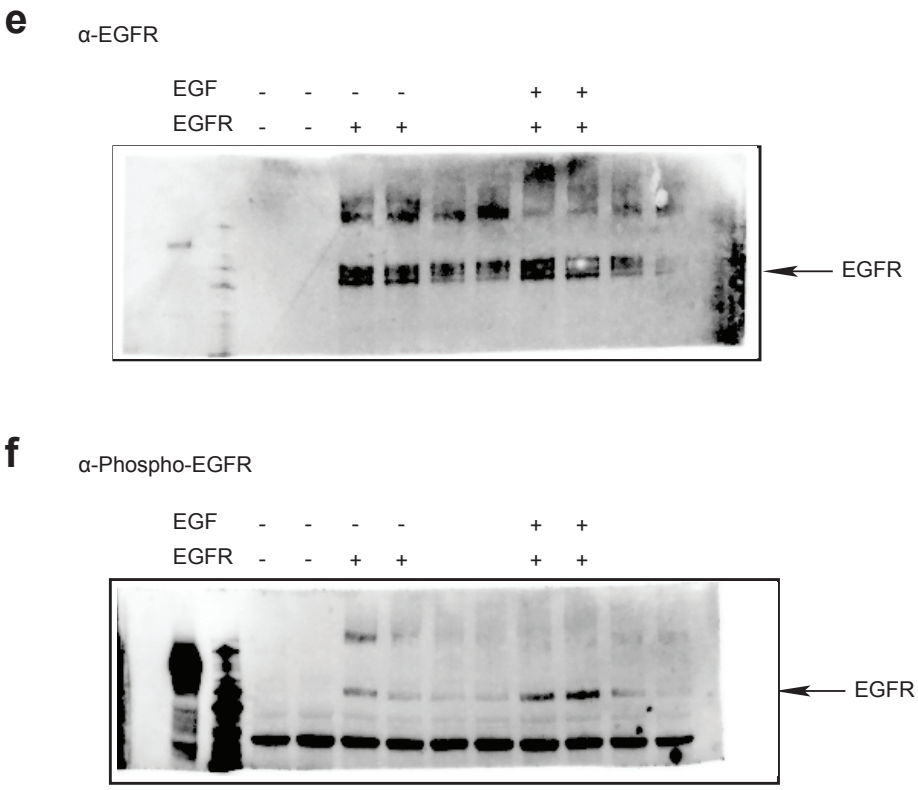


Full-length versions of gel image presented in figure 1

ERBB2 was cell-free produced in the presence and absence of DMPC or with DMPC and co-expressed Δ49A1. FluoroTect™ GreenLys (Promega) was added for visualizing newly synthesized ERBB2 protein. After 4 hours of expression, cell-free reactions were centrifuged at 14,000 rpm for 10 minutes. Small aliquots of sample before centrifuging (total, T), the supernatant (soluble, S) and pellet (P) after centrifuging were collected. All samples were loaded along with a cell-free reaction mixture only (-). Gel images were taken using Molecular Dynamics Typhoon 9410 Molecular Imager from GE Healthcare.

Supplementary Figure 6





Full-length versions of western blots presented in figure 2

NLP associated ERBB2 is tyrosine phosphorylated. Cell-free expressions were set up with and without (-) ERBB2 plasmid. Samples were collected at 2hr, 5hr, 8hr and overnight 18hr and resolved by SDS-PAGE and western blotting with anti-phospho-tyrosine ERBB2 antibody pY1248 (a) and anti-ERBB2 antibody Ab-3 (b) after stripping.

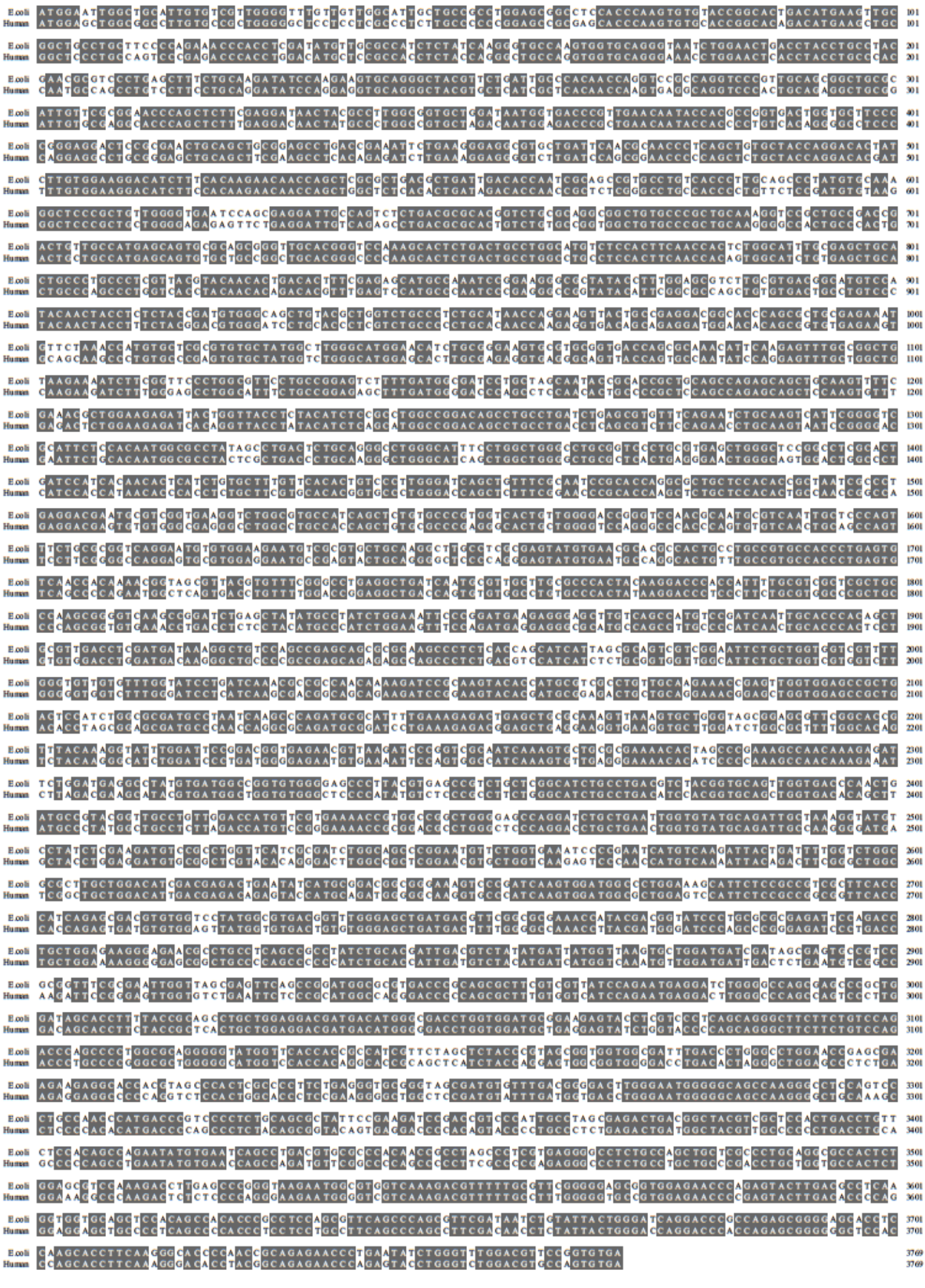
The NLP associated ERBB2 is phosphorylated independent of protein expression. Cell free expressed ERBB2 is treated with calf-intestinal alkaline phosphatase (CIP) and Ni purified. The purified ERBB2-NLPs are then incubated with ATP, Mn^{2+}, Mg^{2+} and buffer to allow for re-phosphorylation. Samples are resolved by SDS_PAGE and western blotting with anti-phospho-tyrosine antibody 4G10 (c) and anti-ERBB2 antibody Ab-3 (d).

NLP associated EGFR is also phosphorylated. Presence of EGF in the cell free reaction increases the level of phosphorylation. EGFR-NLPs showed low level of phosphorylation during cell-free expression. Adding EGF, the natural ligand of EGFR, increases the phosphorylation. Cell-free expressions were set up with and without (-) EGFR plasmid, with and without EGF. After 8hrs reaction, cell-free mixtures were resolved by SDS-PAGE and western blotting with anti-EGFR (e) comparing to western blotting with anti-phospho-tyrosine EGFR antibody pY1110 (f).

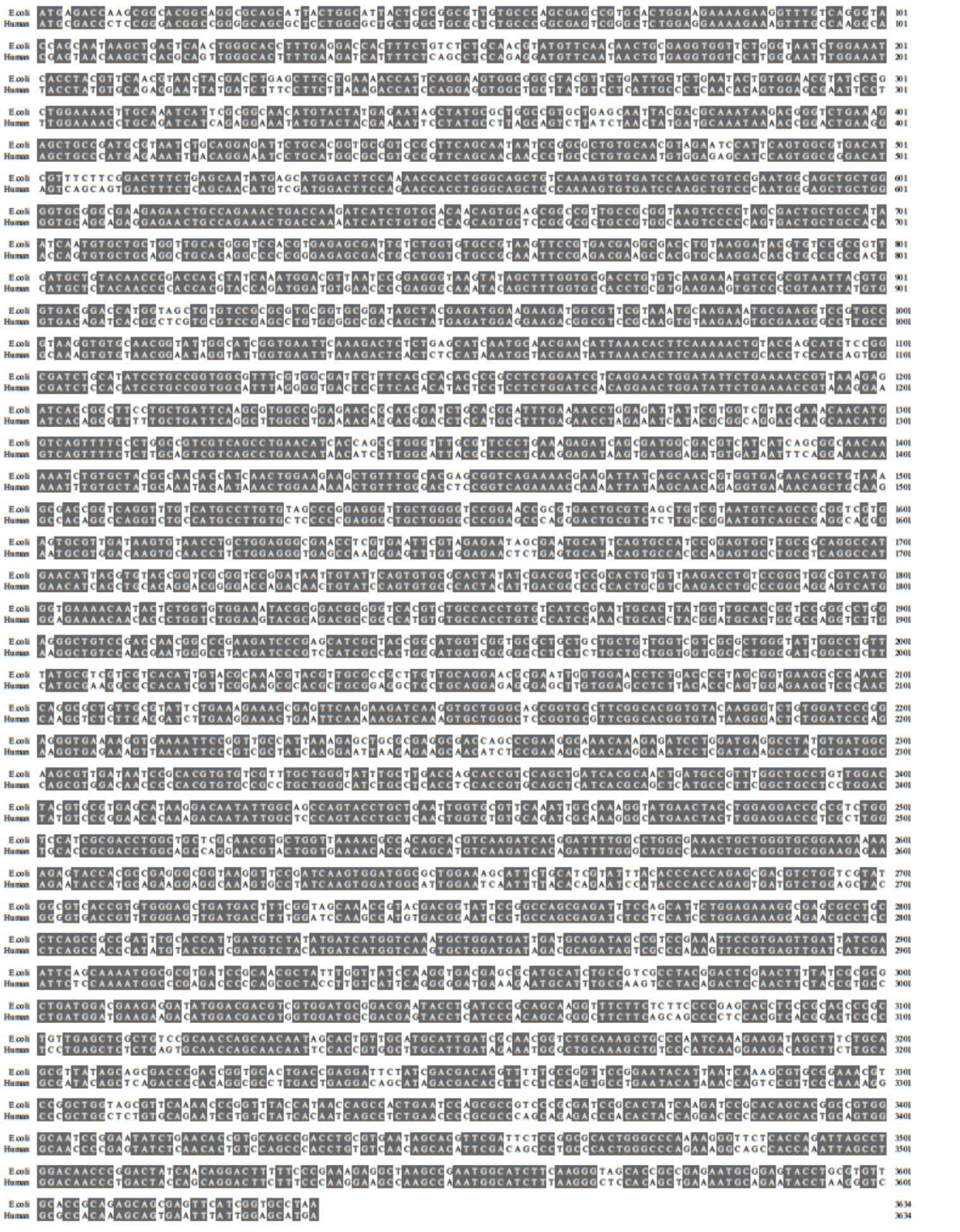
Supplementary Figure 7.

Alignment of the DNA sequences of human ERBB2 and EGFR genes with the DNA sequences that were codon optimized for *E.coli* expression. A dark block represents where the two sequences share the same base.

DNA encoding human ERBB2: comparing E.coli codon optmized sequence to human gene sequence.



DNA encoding human EGFR: comparing E.coli codon optmized sequence to human gene sequence.



Supplementary Tables

Supplementary Table 1. List of genes and plasmids.

Plasmid	Vector	Gene	Tag	Resistance
pJexpress 414- EGFR	pJexpress 414	EGFR *	None	Amp
pJexpress 414- ERBB2	pJexpress 414	ERBB2 *	None	Amp
pIVEX-Δ49A1	pIVEX2.4b	Δ49A1	6xHis	Amp

pJexpress 414 is from DNA2.0; pIVEX2.4b is from Roche.

- Genes are codon optimized for *E.coli* expression. For sequence details see Supplementary Figure 7.

Supplementary Table 2. List of antibodies.

Antibody name	Target	Host	Comp.	Clone	Dilution
Anti-c-ErbB2/c-Neu (Ab-3)	hERBB2, C terminal	Mouse	EMD	mAb	1:1000
p-Tyr Antibody (4G10)	phosphotyrosine	Mouse	EMD	mAb	1:1000
Phospho-ErbB2 (Tyr1248) Antibody	hERBB2, phosphor-tyrosine at 1248	Rabbit	Assay Biotech	pAb	1:1000
Trastuzumab	hERBB2, juxtamembrane	Humanized	Genentech	mAb	N/A
EGFR Antibody (1005)	hEGFR, C terminal	Rabbit	Santa Cruz	pAb	1:1000
Phospho-EGF Receptor (Tyr1110) Antibody	hEGFR, phosphor-tyrosine at 1110	Rabbit	Assay Biotech	pAb	1:1000