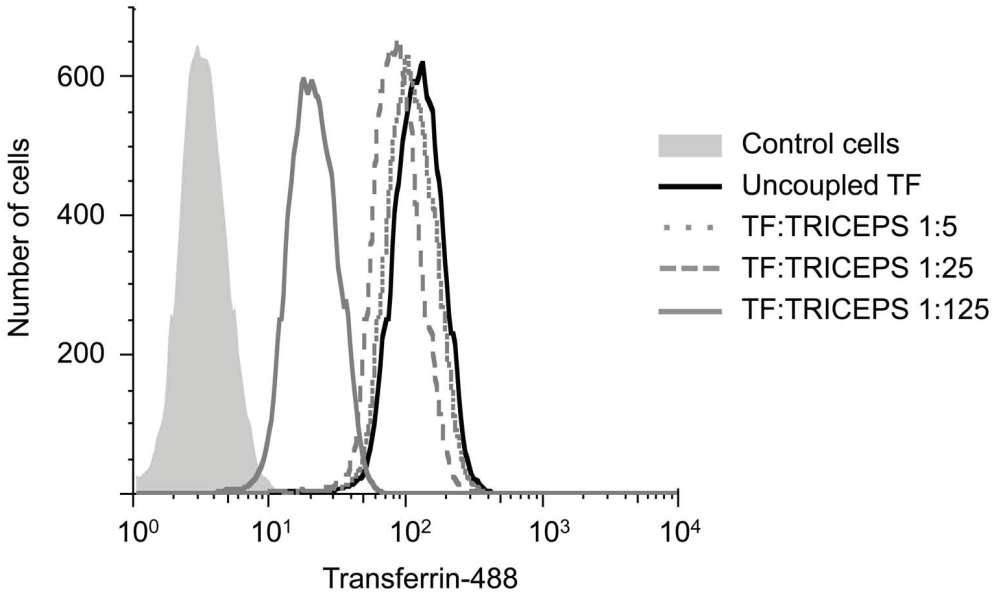

Supplementary Figure 1: Uptake of fluorescently labeled transferrin-488 (TF) into HeLa cells after coupling to TRICEPS. Coupling reactions were set up with the indicated ratios of TF:TRICEPS. Notably, the actual coupling ratios (average number of TRICPES attached to a TF protein) are substantially lower since hydrolysis of the NHS ester is a major competing reaction of the coupling reaction in solution. In every LRC experiment, a fraction of ligands is compromised by the attachment of TRICEPS to particular lysines that are sterically or otherwise important for the respective ligand-receptor interaction. Since the coupling of TRICEPS to lysine side chains is unspecific and several lysines are typically present in protein ligands, only high TRICEPS:protein ratios lead to an impairment of the majority of ligands.

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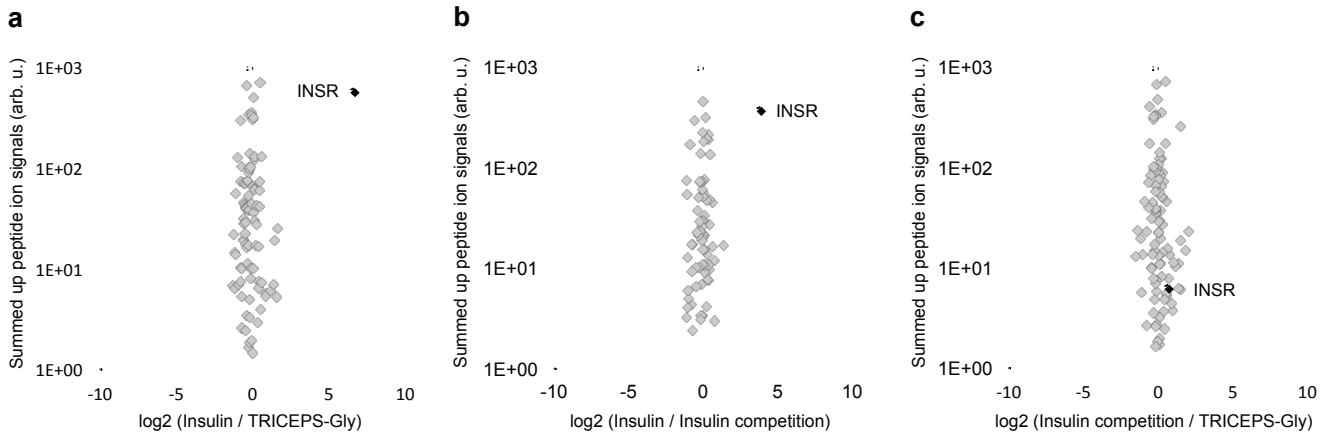
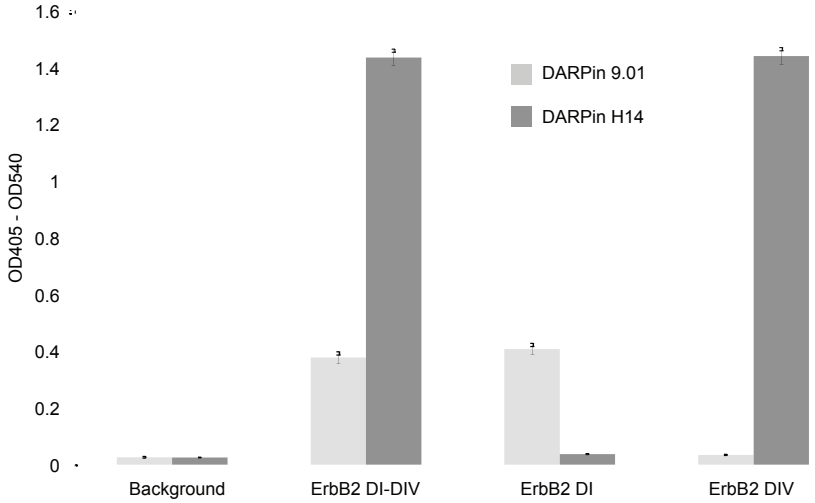


Supplementary Figure 2: LRC competition experiment with insulin. (**a**) Jurkat T lymphocytes were incubated with TRICEPS-coupled insulin or Glycine-quenched TRICEPS, respectively. (**b** and **c**) For the insulin competition sample, cells were incubated with a 10-fold molar excess of uncoupled insulin prior to the addition of TRICEPS-coupled insulin. Data are shown on the protein level.



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ErbB2 DIV
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ACQP

ErbB2 DI-DIV
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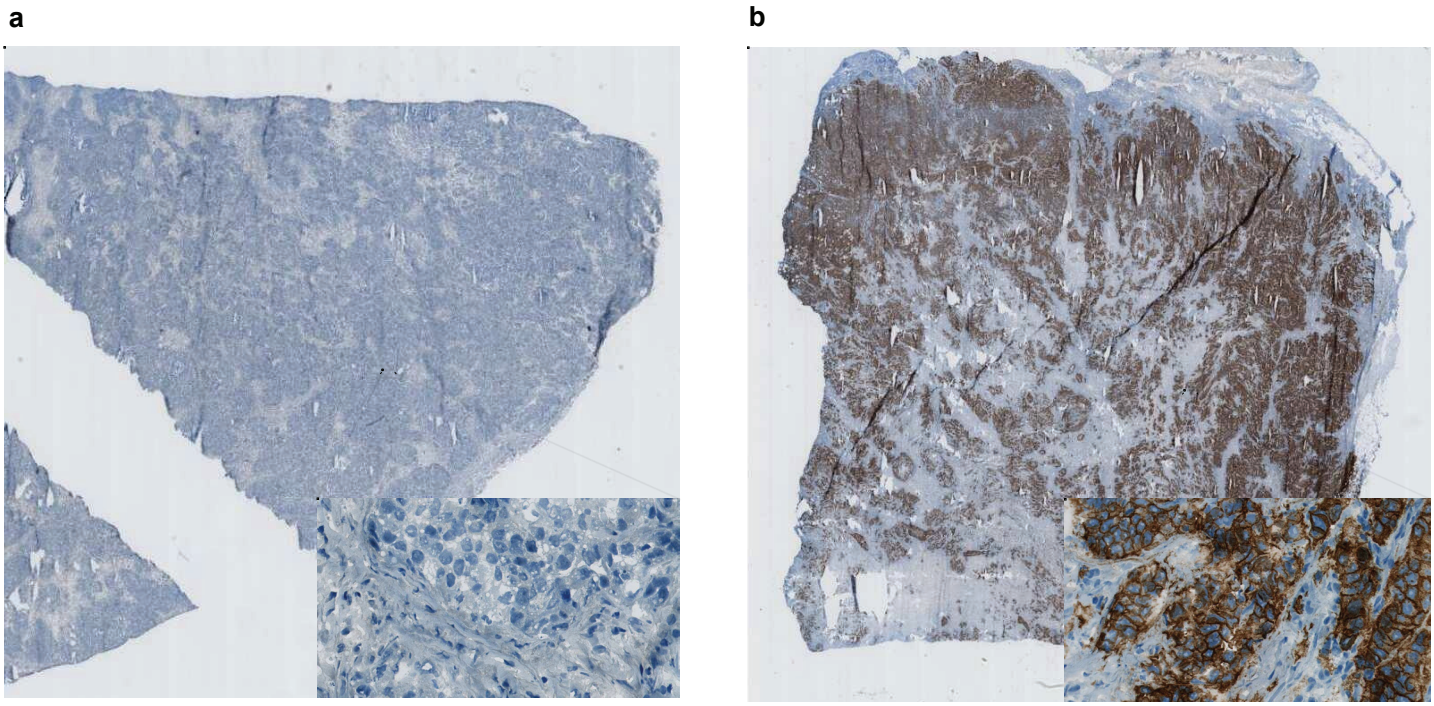
Supplementary Figure 3: ELISA of the ErbB2 binding DARPins 9.01 and H14. The respective DARPins were tested for binding to the immobilized complete extracellular domain of ErbB2 (ErbB2 DI-DIV), extracellular domain 1 (ErbB2 DI), and extracellular domain 4 (ErbB2 DIV), respectively. Amino acid sequences of the recombinant ErbB2 domains are shown on the right. Higher signals for DARPin H14 (K_D = 200 pM) than for 9.01 ($K_D \approx$ 100 nM) can be explained by the difference in the affinities for ErbB2 as determined by surface plasmon resonance and competition ELISA (data not shown). Error bars, s.d. (n=3)



Supplementary Figure 4: ErbB2 immunohistochemical staining of two ductal breast carcinomas. (**a**) ErbB2-negative tissue section of the carcinoma used in the trastuzumab LRC experiment. (**b**) ErbB2-positive tissue section with comparatively high ErbB2 expression levels.

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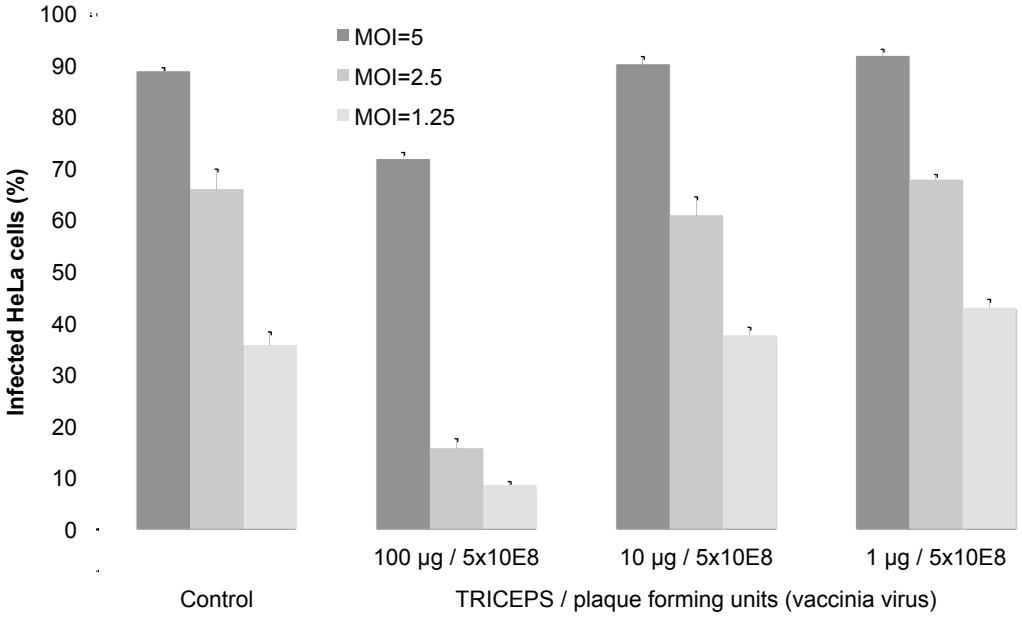
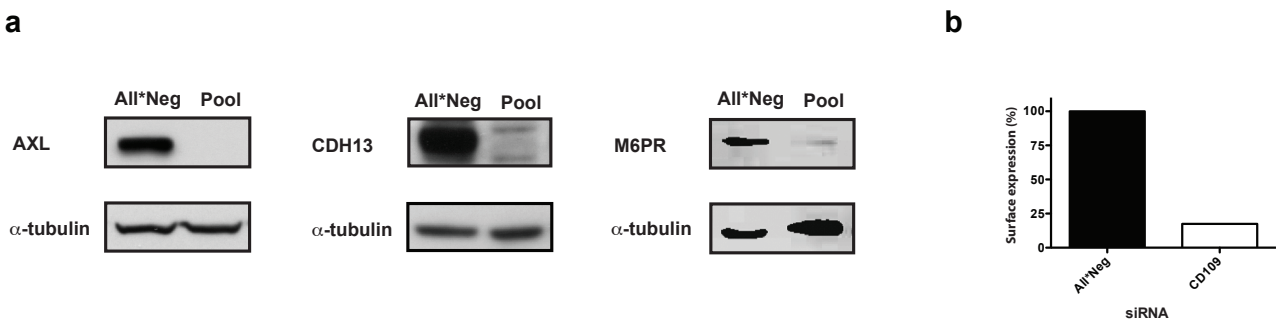


Supplementary Figure 5: Infection assay using vaccinia virus after coupling to TRICEPS at different ratios. Vaccinia virus (strain Western Reserve) Early-EGFP expression in HeLa cells was measured by flow cytometry. Multiplicities of infection (MOI) are indicated. Error bars, s.d. (n=3, technical triplicate)

Nature Biotechnology: doi:10.1038/nbt.2354

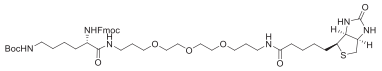


Supplementary Figure 6: Confirmation of siRNA-mediated protein depletion. (**a**) Western blots in total HeLa cell extracts after transfection of cells with targeting siRNAs against AXL, CDH13, and M6PR (Pool) or with non-targeting siRNAs (All*Neg). (**b**) Cell surface expression of CD109 as measured by flow cytometry after transfection of cells with targeting siRNAs against CD109 or with non-targeting siRNAs.

Supplementary Note 1: Chemical synthesis description for TRICEPS.

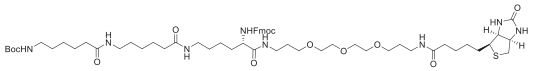
Abbreviations: Boc, butoxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DIPEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMSO, dimethylsulfoxide; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Et_2O, diethylether; EtOH, ethanol; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, *N,N,N',N'*-tetramethyl-O-(1H-benzotriazol-1-yl) uronium hexafluoro-phosphate; Hex, hexane; *i*-PrOH, isopropanol; NHS, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride.

Synthesis of compound 1:



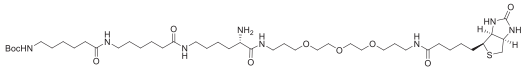
Fmoc-*N*-ε-Boc-L-Lysine (1.7 g, 3.7 mmol) was dissolved in DMF (20 ml) and mixed with DIPEA (0.63 ml, 3.7 mmol) and HBTU (1.7 g, 4.4 mmol). After 10 min, a solution of 1-*N*-biotinyl-4,7,10-trioxatridecane-1,13-diamine (1.8 g, 4.1 mmol) in DMF (5 ml) was added and stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography ($CHCl_3$:MeOH:H_2O = 10:6:1) providing the desired compound as a white viscous foam (2.6 g, 78%).

Synthesis of compound 2:



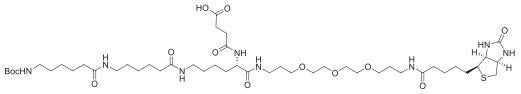
Compound 1 (2.6 g, 2.9 mmol) was stirred in a mixture of CH_2Cl_2:TFA (1:1, 20 ml). Subsequently, the reaction mixture was subjected to concentration and drying under high vacuum. The crude product (2.4 g, 3.0 mmol) and 2,5-dioxopyrrolidin-1-yl 6-(6-{[(*tert*-butoxy)carbonyl]amino}hexanamido)hexanoate (1.5 g, 3.5 mmol; synthesized as described previously[1]) were dissolved in MeOH (6 ml). TEA was added (0.83 ml, 5.9 mmol) and the mixture was stirred at room temperature for 20 min. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography (CH_2Cl_2:MeOH = 9:1 to $CHCl_3$:MeOH:H_2O = 85:15:1) providing the desired compound as a white viscous foam (2.8 g, 84%).

Synthesis of compound 3:



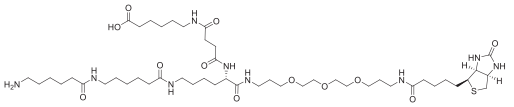
Compound 2 (0.39 g, 0.35 mmol) was dissolved in DMF (3 ml) and mixed with piperidine (0.2 ml) at room temperature for 5 min followed by the addition of Et_2O (100 ml). The ether layer was decanted and the oil crude mixture was dissolved in MeOH. The product was concentrated under reduced pressure and purified by flash chromatography ($CHCl_3$:MeOH:H_2O = 65:25:4 to 10:6:1) providing the desired compound as a white viscous foam (0.21 g, 67%).

Synthesis of compound 4:



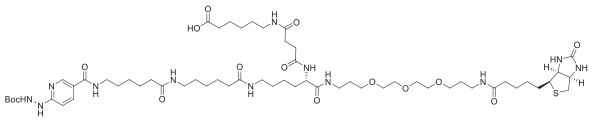
Compound 3 (2.1 g, 2.3 mmol) was dissolved in DMF (1 ml) followed by the addition of DIPEA (0.48 ml, 2.8 mmol) and succinic anhydride (0.28 g, 2.8 mmol) at room temperature for 1.5 h. The solvent was evaporated under reduced pressure and purified by flash chromatography (CH_2Cl_2:MeOH = 10:1 to $CHCl_3$:MeOH:H_2O = 65:25:4 to 10:6:1) providing the desired compound as a white viscous foam (1.4 g, 61%).

Synthesis of compound 5:



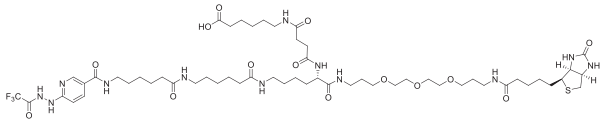
Compound 4 (1.3 g, 1.3 mmol), DIPEA (0.27 ml, 1.6 mmol) and HBTU (0.61 g, 1.6 mmol) were dissolved in DMF (15 ml). 6-aminocaproic acid (0.21 g, 1.6 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography ($CHCl_3$:MeOH:H_2O = 85:15:1 to 65:25:4) providing the compound as a white viscous foam (1.5 g, quant.). Subsequently, the product (1.5 g, 1.3 mmol) was dissolved in a mixture of CH_2Cl_2:TFA (1:1, 20 ml) and stirred at 0°C for 10 min. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography (CH_2Cl_2:MeOH = 10:1 to $CHCl_3$:MeOH:H_2O = 10:6:1) providing the desired compound as a white viscous foam (1.4 g, 61%).

Synthesis of compound 6:



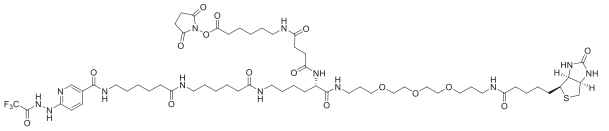
6-({[(*tert*-Butoxy)carbonyl]amino}amino)pyridine-3-carboxylic acid (0.43 g, 1.7 mmol, synthesized as described previously[2]), HBTU (0.51 g, 1.4 mmol), and DIPEA (0.23 ml, 1.4 mmol) were dissolved in DMF (10 ml). Compound 5 (1.1 g, 1.1 mmol) was added as a solution in DMF (10 ml) and the mixture was stirred at room temperature for 40 min. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography (CH_2Cl_2:MeOH = 10:1 to $CHCl_3$:MeOH:H_2O = 65:25:4) providing the desired compound as a slightly yellow viscous foam (0.96 g, 68%).

Synthesis of compound 7:



Compound 6 (0.16 g, 0.14 mmol) was dissolved in TFA (4 ml) and stirred at room temperature for 30 min. The solvent was evaporated and the product was purified on sephadex LH-20 resin and dried under vacuum. The resulting purple amorphous solid was dissolved in DMF (1.5 ml). TFAA (21 µL, 0.15 mmol) was added to the solution and the mixture was stirred at room temperature for 30 min. The crude mixture was evaporated under reduced pressure and purified on sephadex LH-20 ($CHCl_3$:MeOH = 95:5) providing the desired compound as a slightly yellow viscous foam (0.14 g, 90%).

Synthesis of compound 8 (TRICEPS):



Compound 7 (28 mg, 23 µmol), NHS (3.1 mg, 27 µmol), and EDCI (5.6 mg, 27 µmol) were dissolved in DMF (0.3 ml) and the reaction mixture was stirred overnight. The crude mixture was concentrated under reduced pressure and purified on sephadex LH-20 ($CHCl_3$:MeOH = 95:5) providing the desired compound as a slightly green viscous foam (19 mg, 62%).

Supplementary References

1 Srinivasan, B. & Huang, X. Functionalization of magnetic nanoparticles with organic molecules: loading level determination and evaluation of linker length effect on immobilization. *Chirality* **20**, 265–277 (2008).

2 Abrams, M. *et al.* Technetium-99m-Human Polyclonal Igg Radiolabeled via the Hydrazino Nicotinamide Derivative for Imaging Focal Sites of Infection in Rats. *J Nucl Med* **31**, 2022–2028 (1990).