Generation and characterization of Affibody affinity probes to Ephrin B3 in non-small cell lung cancer

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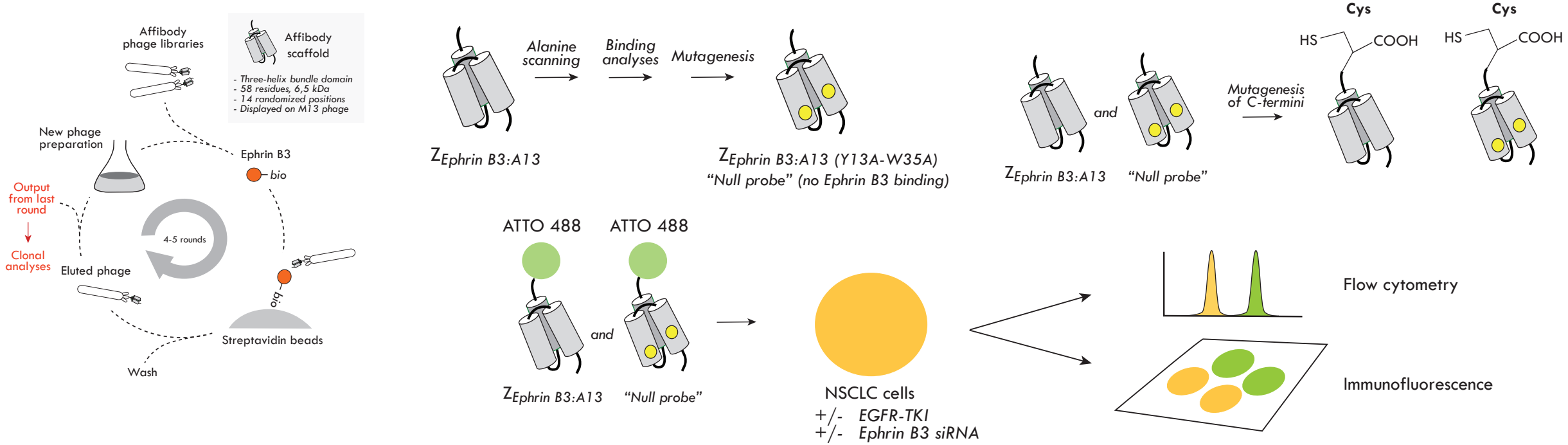

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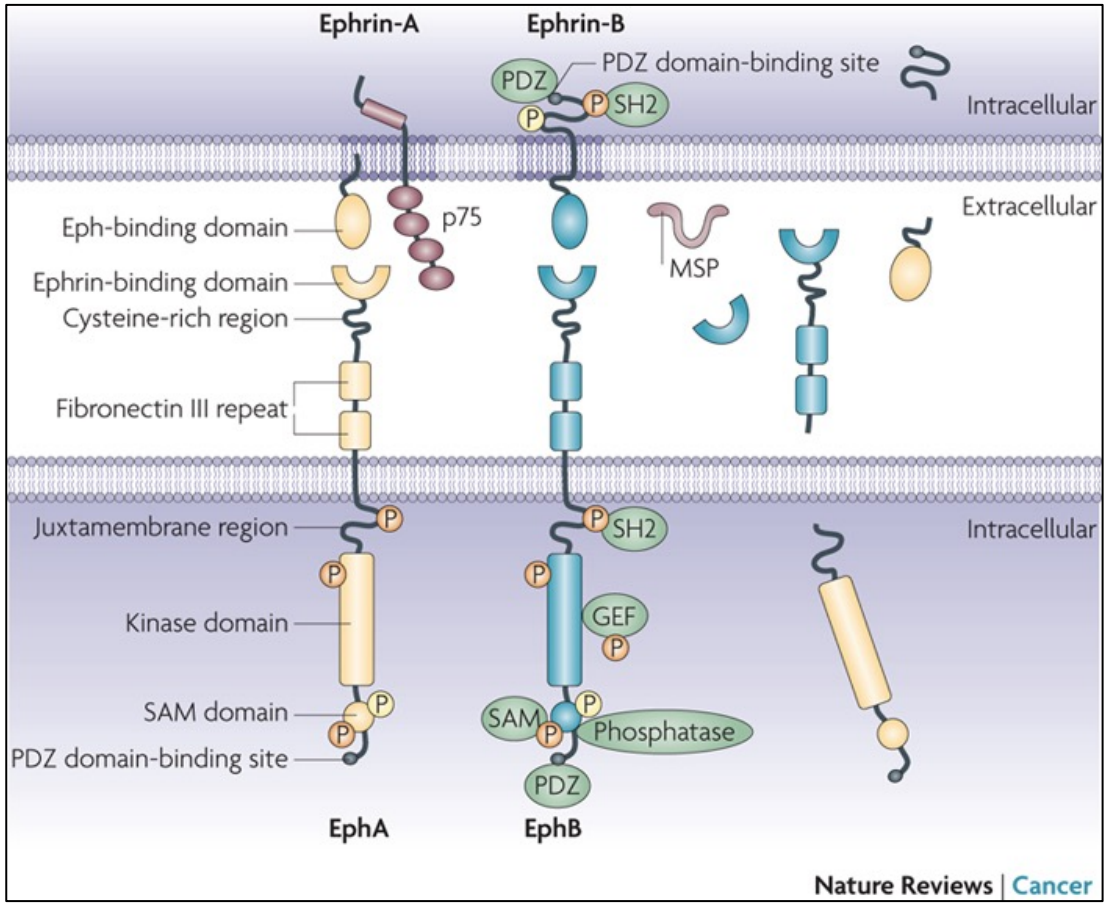

There are nine EphA- and five EphB receptors that promiscuously interact with five Ephrin-A and three Ephrin-B ligands

Ephrin B3

The Eph:Ephrin receptor system is the largest subfamily of receptor tyrosine kinases. Ephrin B3 is a membrane-bound ligand that interacts with Eph receptors to promote both forward and reverse signaling. Ephrin B3 is hypothesized to be overexpressed in certain lung cancers

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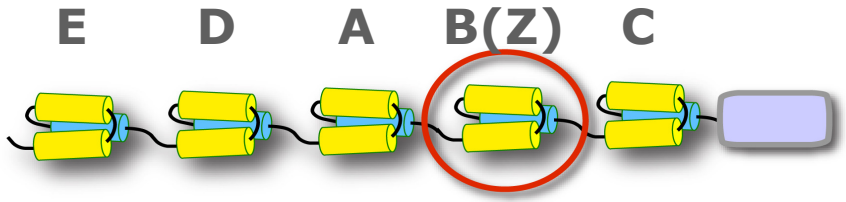
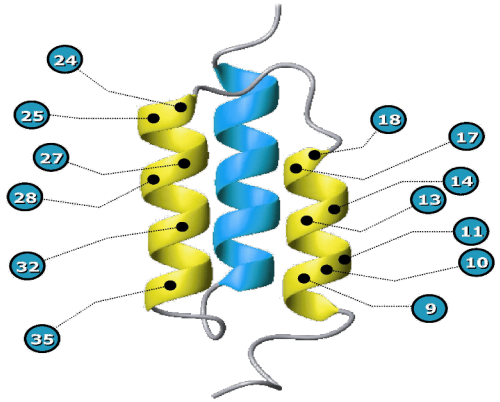
Affibody molecules

Affibodies are a group of non-immunoglobulin affinity proteins that can be engineered to bind target proteins. They are based on a 6.5 kDa domain derived from Staphylococcal Protein A

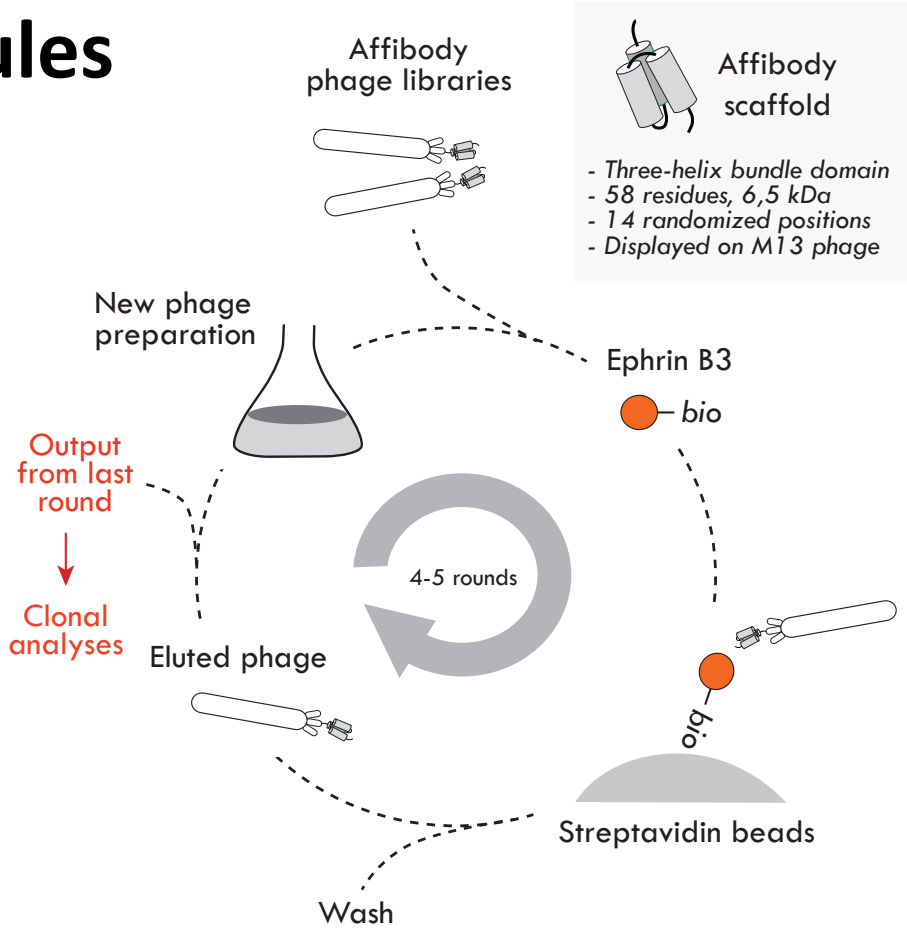
Protein A



Affibody library



A binding surface is produced via randomization of 13-14 residues on two helices and binders to target proteins of interest can be selected by e.g. phage display



Phage display was used to identify a panel of Ephrin B3-binding affibody molecules. Clone $Z_{\text{Ephrin B3:A13}}$ is a lead candidate

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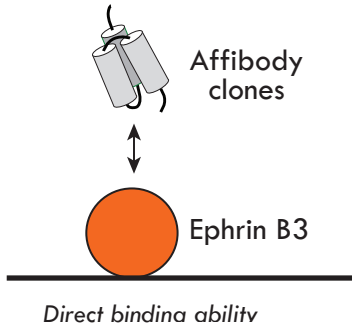
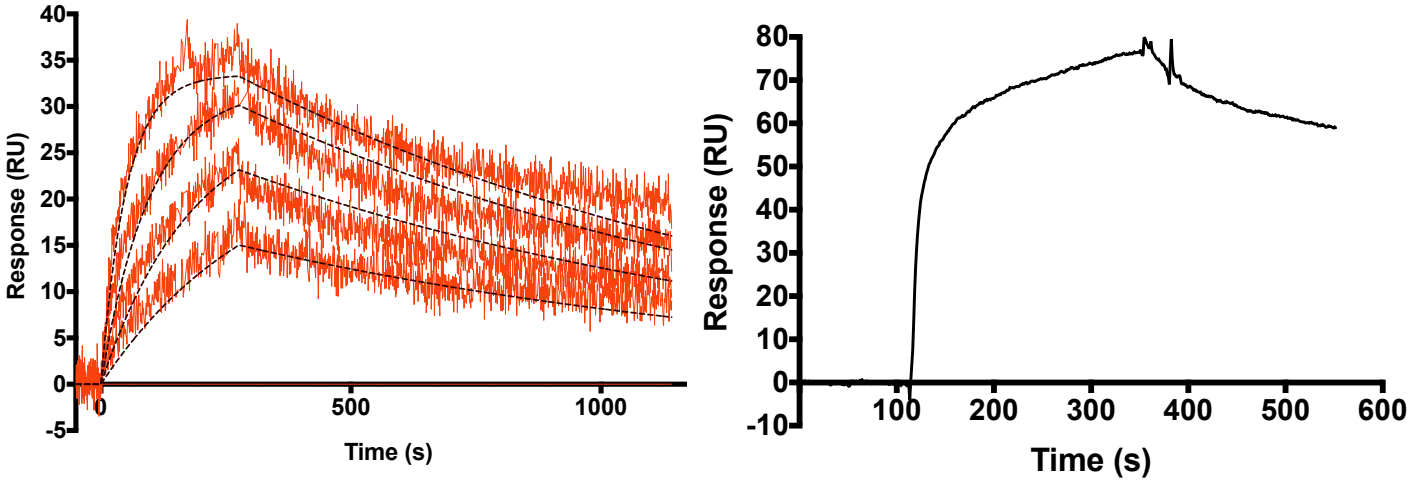
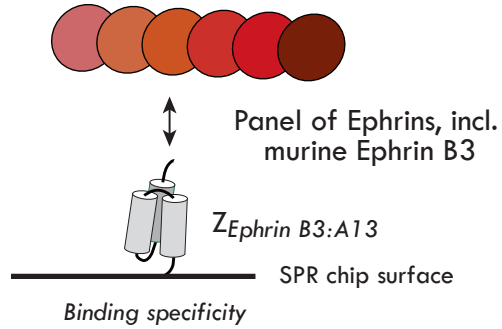
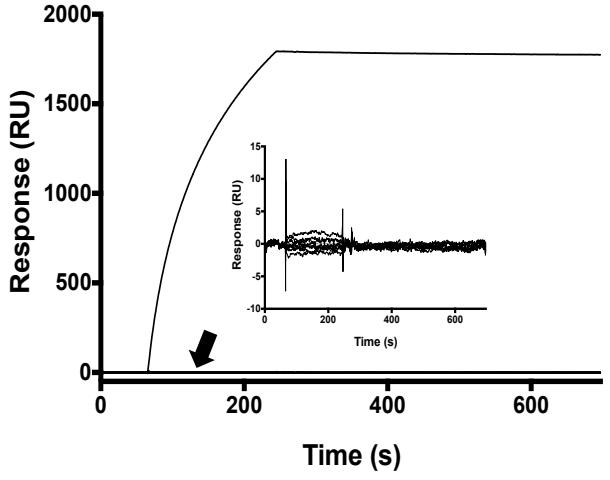
In vitro binding assays





Clone $Z_{Ephrin\ B3:A13}$ binds to human Ephrin B3 with high affinity (left) and also cross-reacts with the murine ortholog (right)





Clone $Z_{Ephrin\ B3:A13}$ specifically binds to human Ephrin B3 without any observed cross-reactivity to any other Ephrin ligand

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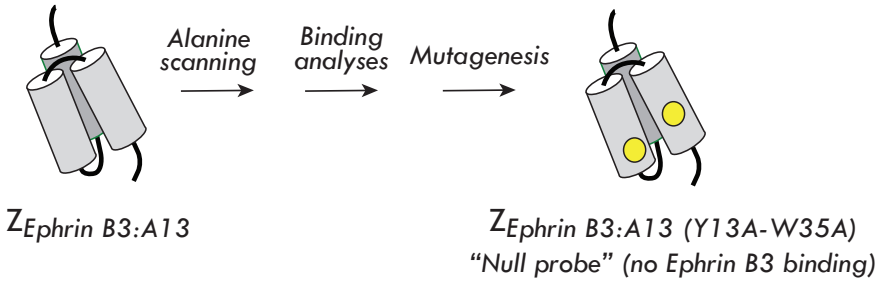
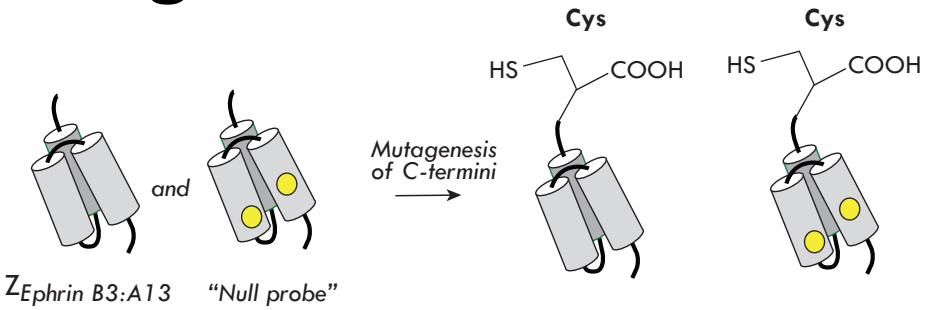
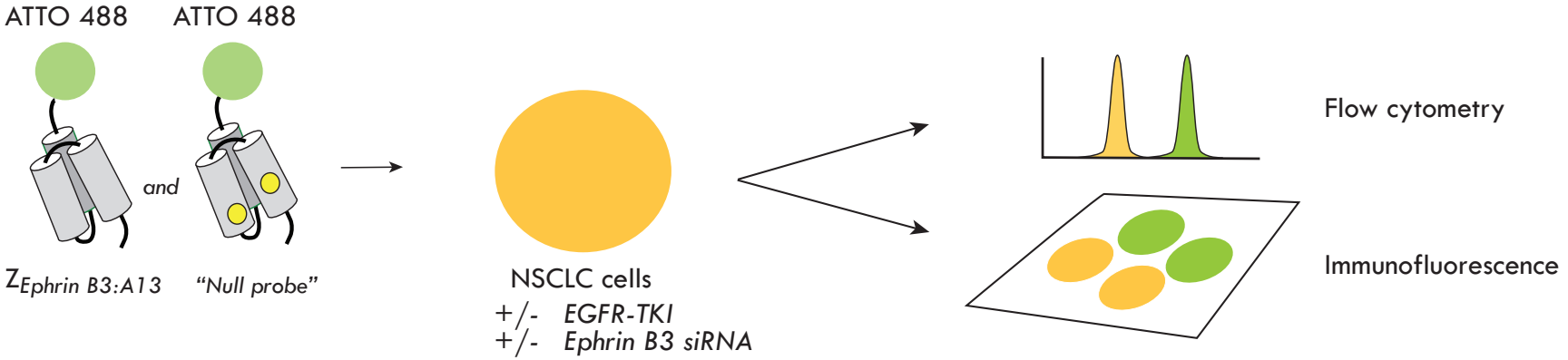
Null probe and cell binding studies





An ala-scan of the binding interface was performed to design a non-binding derivative of $Z_{Ephrin\ B3:A13}$. All alanine mutants retained secondary structure and stability. A few substitutions resulted in significant loss of binding to Ephrin B3 and when combined the resulting null-affibody did not show any binding (not shown). $Z_{Ephrin\ B3:A13}$ and null-probe were equipped with unique C-terminal cysteine residues for site-specific fluorophore labeling to enable more detailed characterization



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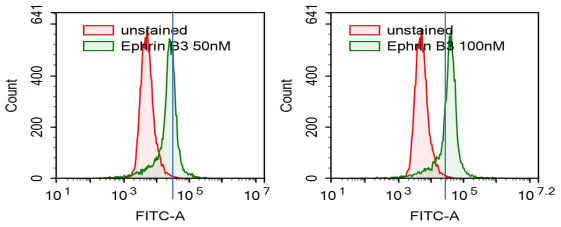
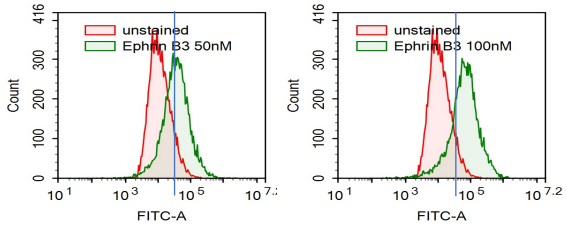
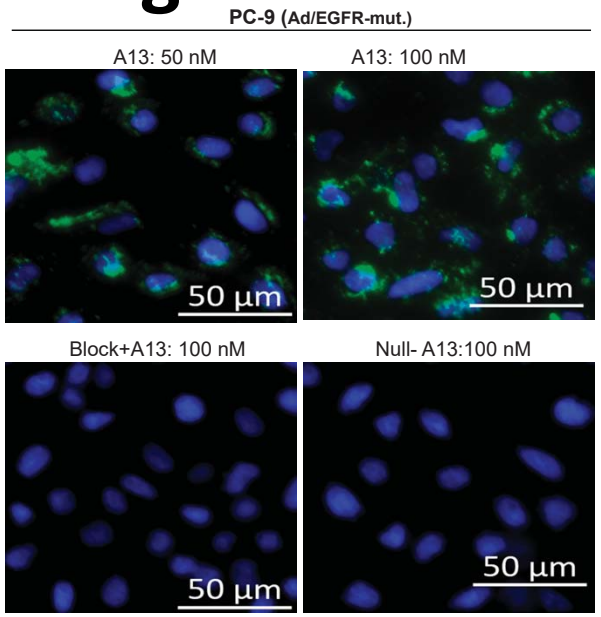
Cell binding

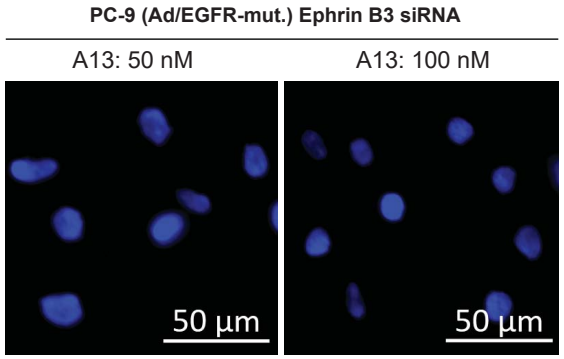
U-1810 – NSCLC



H460 – NSCLC

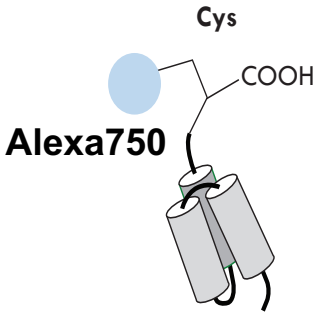




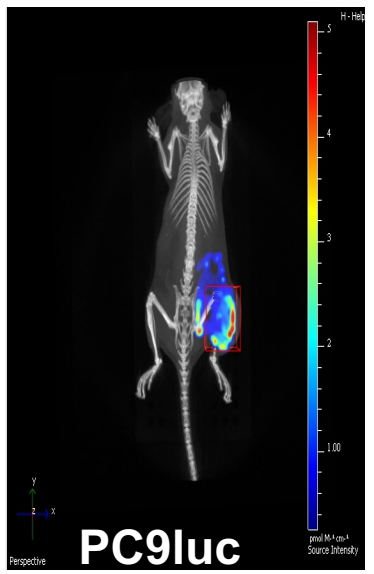


Fluorescently labeled affibody binds NSCLC cells in flow cytometry (top left) and immunofluorescence (IF) microscopy (top and bottom right). In IF the null probe shows no binding and siRNA-treated cells are blank. Signal can be blocked by excess non-labeled affibody

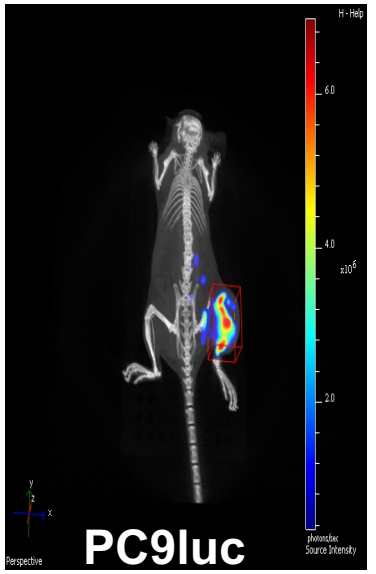
In vivo tumor targeting



$Z_{EphrinB3:A13}$-Alexa750



Luciferin/luciferase



Preliminary evaluation for tumor visualization *in vivo*