Supporting Information

Yeast biopanning against site-specific phosphorylations in tau

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METHODS

scFv Production and Purification

Following a previously established protocol (D. Li et al., 2018), a BJα-strain yeast colony transformed with pRS316 expression vector was selected and grown in 3 mL SD-CAA + Trp medium at 30°C and 250 rpm. After several hours, the culture was expanded to 20 mL by adding 17 mL of fresh SD-CAA + Trp and allowed to grow overnight. The following day, 10 mL of the overnight culture was added to 500 mL fresh SD-CAA + Trp and grown for 24 hours at 30°C and 250 rpm to achieve an OD_{600} value between 4-5. When the culture reached sufficient density, cells were pelleted by centrifugation at 4000 rpm and resuspended in 500 mL SG-CAA + Trp + 1.0 g/L BSA induction medium. The culture was induced for 3 days at 20°C. After induction, cells were pelleted and the supernatant containing secreted scFv was collected and vacuum filtered (Nalgene, 0.22 μm) to remove residual cellular debris. The 500 mL of filtered secretion media was then concentrated to 50 mL by ultrafiltration with a 10 kDa membrane (Ultracel) under 50-55 psi nitrogen pressure and dialyzed overnight in 7 L of 1x PBS pH 8.0. Purification was carried out following manufacturer instructions using 3 mL of a 50% slurry of Marvelgent Ni-Penta Agarose beads. The resulting fractions containing purified scFv were assessed by SDS-PAGE in 1x Tris-glycine running buffer with an 8% polyacrylamide gel stained with 0.1% Coomassie Brilliant Blue R-250 dye.

ELISA with Peptide Immobilization

To carry out enzyme-linked immunosorbent assay (ELISA) with peptide ligands, necessary wells in a neutravidin-coated 96-well plate (Thermo Scientific Cat. No. 15123) were first washed twice with 200 μL of Tris-buffered saline wash buffer (TBSw, 25 mM Tris, 150 mM NaCl, 0.1% (w/v) BSA, and 0.05% Tween-20 (v/v), pH 7.2). The wells were incubated with the appropriate concentration of biotinylated p-tau peptide diluted in TBSw at a volume of 100 μL per well for 2 hours at room temperature. After this, the wells were washed three times with 200 μL TBSw. Then, the wells were incubated with purified scFv 3.24 (~5 μg/mL) diluted in TBSw to achieve a final volume of 100 μL per well for 30 minutes on ice. The wells were washed three times with 200 μL TBSw. The wells were then incubated with a secondary antibody (HIS.H8, 1:500 dilution, ThermoFisher Cat. No. MA1-21315) in 100 μL TBSw for 30 minutes on ice. After incubation, the wells were washed three times with 200 μL TBSw. The wells were then incubated with a tertiary antibody (anti-mouse IgG HRP, 1:2,000 dilution, RRID: AB_2534739) in 100 μL TBSw for 30 minutes on ice. After incubation, the wells were washed three times with 200 μL TBSw. The wells were then incubated with 100 μL TMB (3,3',5,5'-Tetramethylbenzidine) substrate prepared according to the manufacturer's protocol (BD Biosciences Cat. No. 555214) for 10 minutes at room temperature. Then, 50 μL of stop solution (0.5 M H_2SO_4) was added and the absorbance at 450 nm was read using a plate reader (BioTek Synergy HT).

Yeast Biopanning Against Tau Peptide Ligands on Neutravidin-Coated Plates

To test yeast biopanning, we used the same neutravidin-coated plate used for ELISA. Necessary wells in a neutravidin plate were first washed twice with 200 μL of TBSw. The wells were then incubated with of biotinylated peptide antigens (0.1 μM) diluted in TBSw to a final volume of 100 μL per well for 2 hours at room temperature. The wells were then washed three times with 200 μL TBSw. Yeast cells expressing the pBEVY-pT231 scFv 3.24-GFP plasmid were applied at a concentration of 10^6 cells per well. These cells were washed three times with 500 μL of ice-cold PBSA and resuspended in PBSA at a volume of 50 μL per 10^6 yeast cells. The plate wells were then incubated with 50 μL of yeast cells for 30 minutes at room temperature. After incubation, the wells were washed then imaged in accordance with the methods for 96-well biopanning described in the main text.

RESULTS

Peptide Immobilization and Yeast Biopanning Using Neutravidin Plates

We initially attempted yeast biopanning using 96-well plates coated with neutravidin. To ensure that our biotinylated peptide antigens were successfully immobilized to the surface of a 96-well neutravidin-coated plate and accessible for antibody binding, we designed an ELISA using purified pT231 scFv 3.24 protein (**Fig. S1a**). We tested a range of pT231 tau peptide concentrations from 1-1000 nM and repeated this ELISA on two separate days (**Fig. S1b**). After measuring the absorbance at 450 nm and subtracting background signal, we observed clear binding of pT231 scFv 3.24 in wells with immobilized peptide (**Fig. S1b**), indicating successful immobilization of the biotinylated p-tau peptide accessible for scFv binding. The absorbance signal reached saturation for a p-tau peptide concentration of 100 nM (**Fig. S1b**), which is the concentration of peptide used in subsequent yeast biopanning experiments on neutravidin plates.

We then carried out yeast biopanning using the biotinylated p-tau peptide immobilized to the neutravidin plate surface. We prepared wells with immobilized p-tau peptide as well as wells with non-phosphorylated peptide and wells with no peptide (**Fig. S1c**). These biopanning experiments were repeated across two days with duplicates on each day. After the biopanning protocol and image analysis was carried out, we saw that there was no significant difference between the cell counts of the yeast cells present in the p-tau wells when compared with the control wells (**Fig. S1d**). These results indicate that the neutravidin plate surface is insufficient for yeast biopanning. Therefore, we cultured a layer of mammalian cells and biotinylated the cell surface for peptide immobilization as described in the main text.

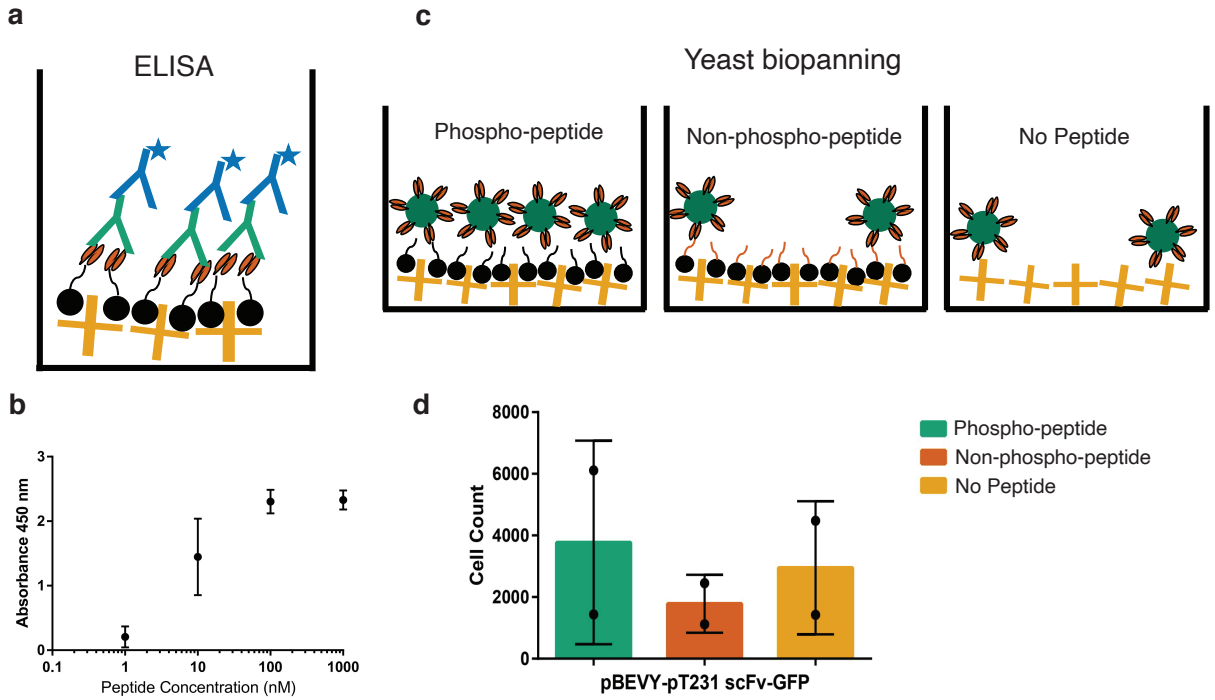

Figure S1. Testing peptide immobilization and yeast biopanning using 96-well plates with immobilized neutravidin. **a**, schematic representation of ELISA using purified pT231 scFv 3.24. Wells in a neutravidin plate are first incubated with biotinylated peptides. To detect peptide immobilization, wells were then incubated with the purified pT231 scFv 3.24, a secondary antibody (green), and finally, a tertiary antibody conjugated with HRP (blue). **b**, absorbance readings at 450 nm after TMB substrate addition with a range of peptide concentrations. **c**, schematic representation of yeast biopanning using various immobilized peptides on a neutravidin-coated plate. Wells were immobilized with target peptide (left), non-target peptide (middle), and without peptide (right). After washing, wells were incubated with yeast cells expressing scFv on their surface and GFP intracellularly. **d**, quantification of yeast cells expressing pT231 scFv 3.24 and GFP present in wells after washing.

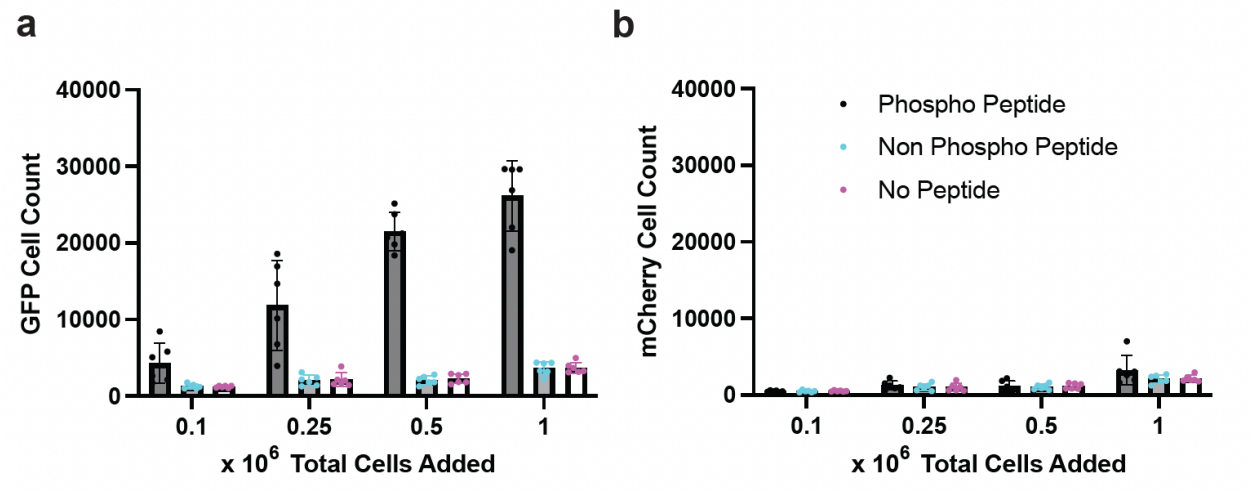

Figure S2. Cell count data corresponding to **Fig. 3C**. Each datapoint indicates a ratio measured from a biological replicate. Each condition was repeated in two separate experiments with three biological replicates. Error bars indicate the standard deviation of the datapoints.