Supplementary Materials

Antigen Production and Conjugation

Nef-biotin was produced using BL21DE3 *E. coli* co-transformed with Nef-AviTag-6His and BirA-cm via heat shock. Transformed cells were grown in a 5 mL 2YT medium (16 g Tryptone, 10 g Yeast Extract, 5 g NaCl with 1 L MilliQ water) supplemented with 2 % v/v glucose, 100 µg/mL ampicillin and 50 µg/mL chloramphenicol and incubated at 37 °C shaking at 220 rpm for 5 hours. An appropriate volume from this starter was transferred into 100 mL of a similar media but without the 2 % v/v glucose to have a starting OD_{600nm} = 0.1. The culture was grown until an OD_{600nm} = 0.5-0.8 was reached and induced by adding 100 µM Isopropyl β-D-1-thiogalactopyranoside (IPTG) and 10 µM biotin and incubated overnight at 30 °C. Afterwards the cells were lysed using a mixture of BugBuster with Benzonase and Lysozine purified using cobalt resin (TALON superflow, GE Healthcare).

Nef-cmyc was produced using BL21DE3 *E. coli* transformed with Nef-cmyc-6His via heat shock. Transformed cells were grown in a 5 mL 2YT medium supplemented with 2 % v/v glucose and 100 µg/mL ampicillin and incubated at 37 °C shaking at 220 rpm for 5 hours. An appropriate volume from this starter was transferred into 100 mL of a similar media but without the 2 % v/v glucose to have a starting OD_{600nm} = 0.1. The culture was grown until an OD_{600nm} = 0.5-0.8 was reached and induced by adding 100 µM Isopropyl β-D-1-thiogalactopyranoside (IPTG) and incubated overnight at 30 °C. Afterwards the cells were lysed and purified as previously described.

CD16a-cmyc was produced using Expi293F cells (A14635, Gibco™) transformed with CD16a-cmyc-6His using Expifectamine DNA lipid complex as described in the product notes. After 1 week of growth, the supernatant was recovered and underwent overnight dialysis using Spectra/Por® 4 RC Dialysis Membrane Tubing at 2 mL/cm with a MWCO of 12,000-14,000 kD in PBS 1x. Purification was also done using cobalt resin.

Nef-cmyc and a portion of CD16a-cmyc underwent labelling with ATTO 647N using the bacterial transglutaminase (L107, TGase Q Protein Labeling Kit, Zedira) as described in the product notes. A portion of the CD16a-cmyc underwent conjugation with biotin using bacterial transglutaminase (L101) as described in the product notes.

CFSE Staining of Negative Yeast

CFSE (CellTrace, C34554A) staining of yeast was adapted from the supplier provided notes and from this staining protocol (41). Stock CFSE was reconstituted with 18 µL DMSO to create a starting concentration of 5 mM. The staining was done on induced yeasts washed and prepared to have an OD_{600nm} = 1 in 1x PBS by adding CFSE stock at a ratio of 1:1000 to reach a working concentration of 5 µM. This was incubated at room temperature on a Stuart tube rotator at 40 rpm for 30 min. Afterwards the yeast was washed twice; this was done by centrifuging the sample at 3500 x g for 1 min at room temperature and resuspending in PBS 1x with 2 % BSA. Staining was checked using cytometry and microscopy. The staining was generally performed on model enrichment trials with non-binding yeasts stained with CFSE, excluding the 1:1 model mixture.

Estimation of Antigen Concentration on the channel surface

In a previous publication (17), it was shown in a microfluidic chamber with glass bottom and PDMS channel, a 7 nM incubation concentration yielded a surface concentration of 30 molecules/µm^2, a conversion factor of 4. Applying this to the concentration used for enrichment, the 45 nM incubation concentration will have 180 molecules/µm^2. For the serial dilution in the optimization of the incubation concentration to be used in the microfluidic channel, the 135, 45, 15, 5, 1.67 and 0.56 nM converts to 540, 180, 60, 20, 7 and 2 molecules/µm^2, respectively.

Derivation of Eq. (1) and (2) from the main text

E^+ = fraction of Expressor Positive (Binding) Cells

E^- = fraction of Expressor Negative (Non-binding) Cells

NE^+ = fraction of Non-Expressor Positive Cells (contains the plasmid but not expressing the Nb)

NE^- = fraction of Non-Expressor Negative Cells

$f^+ = E^+/(E^+ + NE^+)$ = fraction of Expressing Positive Cells

$f^- = E^-/(E^- + NE^-)$ = fraction of Expressing Negative Cells

a^+= captured fraction of Positive Cells after flow

a^-= captured fraction of Negative Cells after flow

The sum of E^+, NE^+, E^- & NE^- equals 1. We assume x as the ratio of positive cells (bearing the positive plasmid) before flow where $x = E^+_{pre} + NE^+_{pre}$. Therefore, the fractions can be expressed as in Table 1.

Table 1. Fractions PreFlow and PostFlow

PreFlow	$E^+pre = xf^+$	$E^-pre = (1-x)f^-$
	$NE^+pre = x(1-f^+)$	$NE^-pre = (1-x)(1-f^-)$
PostFlow	$E^+post = a^+xf^+/S$	$E^-post = a^-(1-x)f^-/S$
	$NE^+post = a^-x(1-f^+)/S$	$NE^-post = a^-(1-x)(1-f^-)/S$

A sum S was applied to normalize the data and ensure a 1 for the sum of all fractions PostFlow. Additionally, we assume that non-expressing positive cells (NE^+) adhere like negative ones (a^-).

$$S = E^+_{post} + E^-_{post} + NE^+_{post} + NE^-_{post}$$

$$S = a^+xf^+ + a^-(1-x)f^- + a^-x(1-f^+) + a^-(1-x)(1-f^-) \qquad \text{(Eq. S1)}$$

$$S = a^- + (a^+ - a^-)xf^+$$

A theoretical enrichment (ε) in positive cells is defined as:

$$\varepsilon = \frac{E^+_{post} + NE^+_{post}}{E^+_{pre} + NE^+_{pre}}$$

Adding the mix ratio x and the equations from Table 1, we get the following:

$$\varepsilon = \frac{[a^+xf^+ + a^-x(1-f^+)]}{Sx} = \frac{a^+xf^+ + a^-x(1-f^+)}{[a^- + (a^+ - a^-)xf^+]x}$$

leading to Eq. 1 and 2 in the main text.

Measurement of *y* using pure yeast population

The fraction of cells adhered after flow for a pure yeast population with the irrelevant Nb is: $(N_{post}/N_{pre})^- = S(x=0) = a^-$

$$S = a^- = \left(\frac{N_{post}}{N_{pre}}\right)^-$$

On the other hand, the fraction of cells adhered for a pure yeast population with the cognate Nb is:

$$\left(\frac{N_{post}}{N_{pre}}\right)^+ = S(x=1) = a^+f^+ + a^-(1-f^+)$$

When we replace these quantities from the previous equation of *y* we get:

$$y = f^+\left(\frac{a^+}{a^-} - 1\right) = \frac{(N_{post}/N_{pre})^+}{(N_{post}/N_{pre})^-} - 1 \qquad \text{(Eq. S2)}$$

Interestingly, this equation shows that the measured capture efficiencies using the monoclonal control populations driven along a surface with an antigen of interest may be used to predict the theoretical enrichment ε.

Measurement of *y* using fluorescent negative yeasts in the mixture

Alternatively, the data from the *in-situ* fluorescence microscopy can be used to measure the adhesion. The number of positive cells corresponds to the total number of cells observed in bright field (BF) minus the number of fluorescent ones (Fluo). One defines therefore the negative and positive capture efficiencies respectively as:

$$\left(\frac{N_{post}}{N_{pre}}\right)^- = \left(\frac{{N_{post}}^{Fluo}}{{N_{pre}}^{Fluo}}\right)$$

$$\left(\frac{N_{post}}{N_{pre}}\right)^+ = \left(\frac{{N_{post}}^{BF} - {N_{post}}^{Fluo}}{{N_{pre}}^{BF} - {N_{pre}}^{Fluo}}\right)$$

which are used in Eq. S2 to determine *y*.

Cytometry Correction for Enrichment Prediction

In an ideal situation, Q1 would not contain any signal. So, if we assume $f^+ = f$, and Q1 = 0, we can calculate E^+ = Q2, E^- = Q3, NE^+ = Q2Q4/(Q2+Q3) and NE^- = Q3Q4/(Q2+Q3). In this case, we can calculate the mix ratio *x* as well as the enrichment ε as a function of Q2, Q3 and Q4. However, cytometry data show signals for both Q1 and Q2 even on a negative control. Using control yeasts in cytometry, we computed for autofluorescence (f_{AF}) and obtained a corrected f^+ as shown in the table below.

Table 2. Autofluoresence f_{AF} and expression fraction f^+ established using Controls

Control	parameter	Definition	Nef19	CD16.21
Negative	fAF	Q1-/(Q1-+Q4-)	0.003 ± 0.003	0.086 ± 0.022
Positive	f+	Q2-fAFQ3	0.26 ± 0.14	0.28 ± 0.09

The estimate for enrichment in the in-text Fig 5 was through Q2/ (Q2+Q3) for PreFlow and PostFlow which represents the fraction of binders over expressors and considering the ratio PostFlow/PreFlow. We can calculate those quantities using fractions, Q2 = E^+ and Q3 = E^- and assume that $f^+ = f^- = f$.

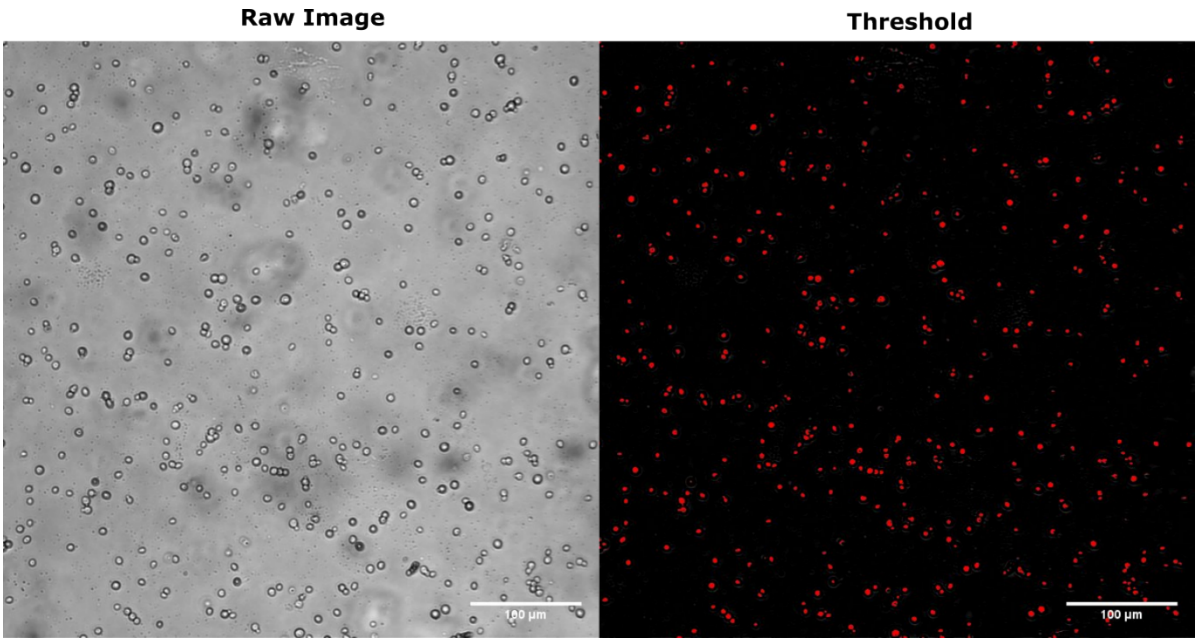
$$\left[\frac{Q2}{Q2+Q2}\right]^{pre} = \frac{E^+_{pre}}{f} = \frac{xf}{f} = x$$

$$\left[\frac{Q2}{Q2+Q2}\right]^{post} = \frac{a^+xf}{a^+xf + a^-(1-x)f} \qquad \text{(Eq. S3)}$$

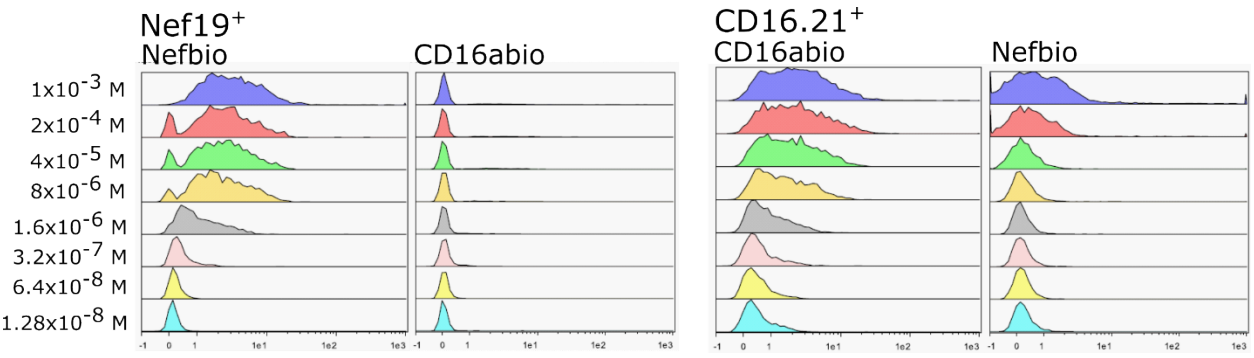
$$\frac{\left[\frac{Q2}{Q2+Q3}\right]^{post}}{\left[\frac{Q2}{Q2+Q3}\right]^{pre}} = \frac{a}{(a-1)x+1} = \varepsilon(f=1)$$

This is only applicable in a condition where f = 1, a condition that is not fulfilled as seen in Table 2. Thus, the ratio Q2/ (Q2+Q3) roughly underestimated the real enrichment factor if cytometry would be performed immediately after the selection. In practice, the cytometry measurements PostFlow is performed after 48-72h of cell culture to allow for cell expansion and induction of expression, a time sufficient to recover the initial fraction of expressors f^+. We thus considered the enrichment estimated from Q2/ (Q2+Q3) to be valid. The predicted enrichment $\varepsilon(f=1)$ can also be calculated as the following:
The factor $y(f^+)$ which corresponds to the real expression fraction f^+ is obtained using the adhesion measurements. We deduced the value of y corresponding to f = 1: $y(f=1) = y(f^+)/f^+$ therefore:

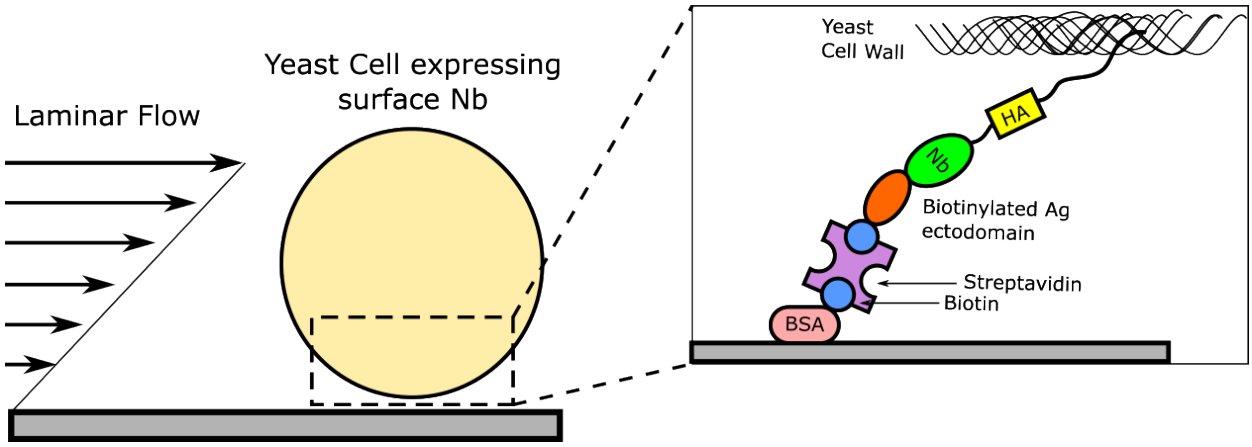
$$\varepsilon(f=1) = \frac{1+y(f=1)}{1+xy(f=1)} = \frac{f^+ + y(f^+)}{f^+ + xy(f^+)}$$



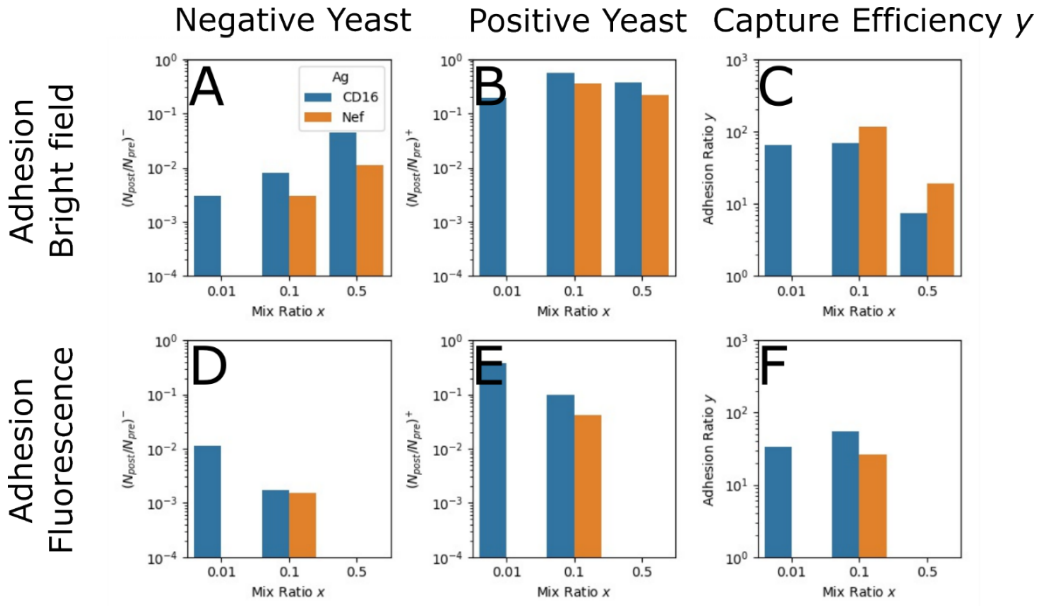
Supplementary Material Figure 1. Detection of Yeast Cells. Detection and counting of yeast cells was done using the MorphoLibJ plugin function Gray Scale Attribute Filtering with Top Hat in FIJI v1.53t. Here we show an example of an image in BF microscopy and the corresponding image after thresholding. The red dots on black background show the yeast cells detected and counted. White Scale bar is at 100 µm.



Supplementary Material Figure 2. Cytometry Histograms to estimate apparent affinity. The apparent affinity of the Nb on the yeast surface was estimated. Nef19^{+} & CD16.21^{+} were incubated with either their cognate or irrelevant antigen and differing concentrations. Each concentrations used are indicated on the corresponding row. Values used in main text Fig 1C were taken here gated using the lowest concentration on the irrelevant antigen.



Supplementary Material Figure 3. Yeast driven along the channel surface. A schematic showing the expected interaction between the flowing yeast cell on the channel surface and the antigen functionalized on the channel surface (image not to scale).



***Supplementary Material Figure 4. Bright Field (BF), Fluorescence (Fluo) and Capture Efficiency* ɣ.** *(A) The fraction of cell count PostFlow to PreFlow of the pure non-binding yeast (negative) used as control during enrichment experiments imaged through BF Microscopy. (B) The fraction of cell count PostFlow to PreFlow of the pure binding yeast (positive) used as control during enrichment experiments imaged through BF Microscopy. (C) The calculated capture efficiency* ɣ *using the BF data using Eq 2 of the main text. (D) The fraction of cell count PostFlow to PreFlow of the pure non-binding & fluorescent yeasts (negative) in the mixture during enrichment experiments imaged through Fluo Microscopy. (E) The fraction of cell count PostFlow to PreFlow of the pure non-binding & non-fluorescent yeast (positive) in the mixture during enrichment experiments imaged through Fluo Microscopy. (F) The calculated capture efficiency* ɣ *using the Fluo data u*