**Supplementary Information**

# Isolation of full-length IgG antibodies from combinatorial libraries expressed in the cytoplasm of *Escherichia coli*

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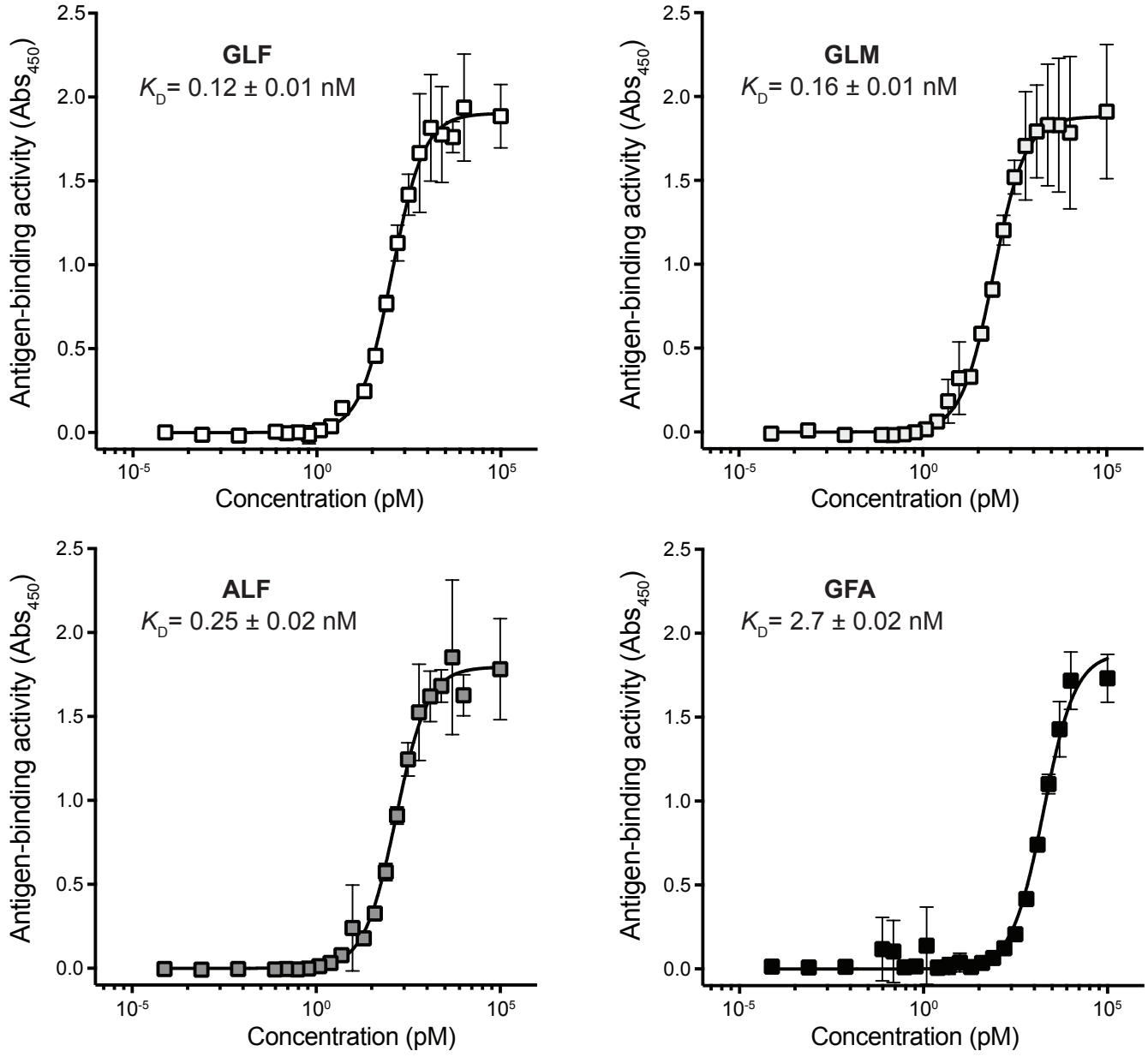



**Supplementary Figure 1. Affinity determination of cyclonal antibodies.** Binding affinity of cyclonals GLF, GLM, ALF, and GFA determined by ELISA with purified GST-Gcn4-PP as immobilized antigen. Absorbance was measured at 450 nm for each cyclonal variant and the resulting measurements were analyzed using GraphPad Prism 9 to determine $K_D$. Data are the average of biological replicates ($n$ = 3) and error bars represent the standard deviation. Source data are provided as a Source Data file.

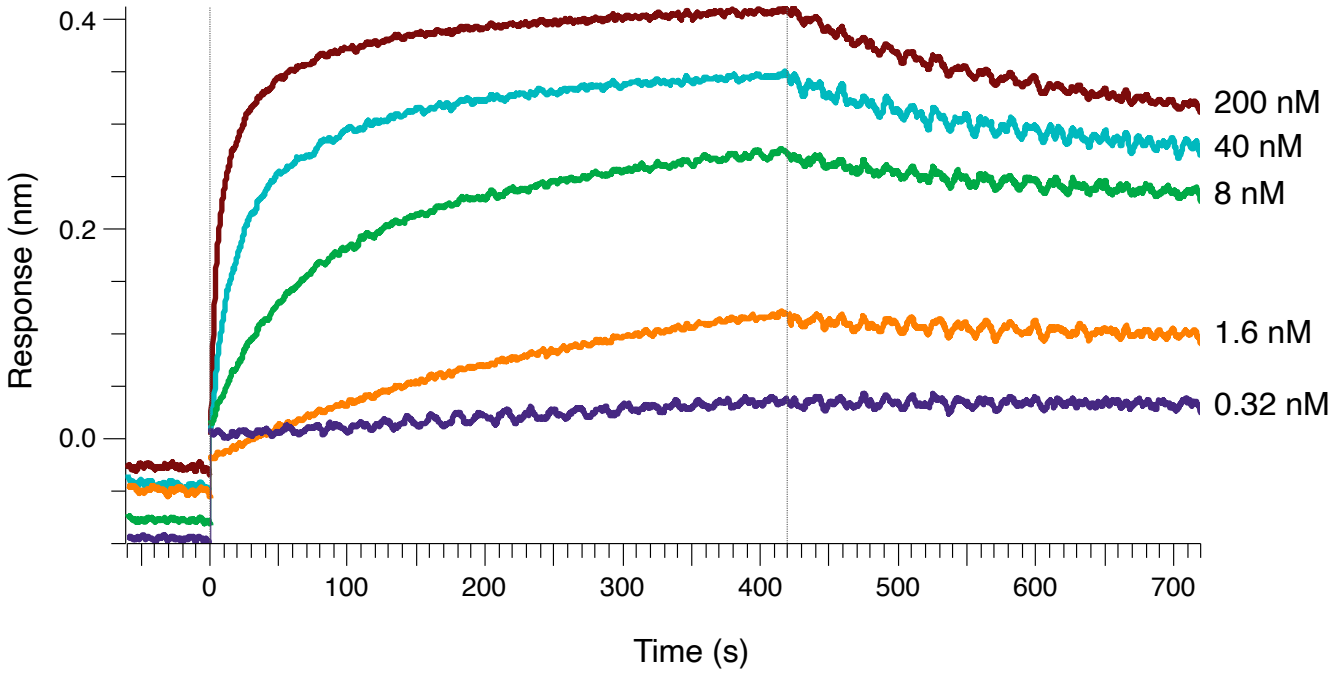

**Supplementary Figure 2. Genetic selection of lead cyclonal IgG candidates against HAG peptide.** Selective spot plating of SHuffle T7 Express cells carrying a plasmid encoding spTorA-CAT-HAG along with a second plasmid encoding a cyclonal IgG candidate that was selected for binding to the HAG peptide antigen. A total of 5 µl of 10-fold serial diluted cells was plated on LB-agar supplemented with 20 µg/ml chloramphenicol (Cm) as well as 0.4 % arabinose and 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) to induce chimeric antigen and cyclonal expression, respectively. Cross-pairing the non-cognate Gcn4 cyclonal with HAG antigen or the HAG cyclonal with export-defective spTorA(KK)-CAT-HAG served as negative controls. Spot plating results are representative of at least three biological replicates.

**a**

| Cyclonal | Light chain V | Light chain J | Heavy chain V | Heavy chain D | Heavy chain J |
|---|---|---|---|---|---|
| 20.2 | IGKV4-1*01 | IGKJ1*01 | IGHV3-72*01 | IGHD1-7*01 | IGHJ2*01 |
| 75.1 | IGKV4-1*01 | IGKJ1*02 | IGHV3-23*04 | IGHD6-13*01 | IGHJ1*01 |

**b**


**Supplementary Figure 3. Immunoglobulin variable domain sequence analysis by IgBLAST of cyclonal hits.** (a) Germline gene usage and (b) assignment of 20-2 and 75-1 putative germline sequences using NCBI IgBLAST (Ye et al., 2013 *Nucleic Acids Research*).




$K_D$ = 7.457 +/- 0.065 x $10^{-10}$ (M)
$k_a$ = 9.029 +/- 0.045 x $10^5$ (1/Ms)
$k_d$ = 6.734 +/- 0.048 x $10^{-4}$ (1/s)
$R^2$ = 0.9901

**Supplementary Figure 4. Binding analysis of anti-HAG cyclonal.** Biolayer interferometry (BLI) affinity measurements for the anti-HAG cyclonal against GST-HAG. The cyclonal IgG was purified by protein A-based affinity capture. Response data are representative of replicate (*n* = 2) BLI experiments.