<u>Title</u>

A targeted boost-and-sort immunization strategy using *Escherichia coli* BamA identifies rare growth inhibitory antibodies

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Supplemental Figure 1. Schematic of LPS structures on *waaD* and K12 strains. Diagram of LPS structure for both the K12 and waaD strains highlighting lack of elongated LPS in waaD (blue circle: heptose sugar; red triangle: glucose sugar; grey square: Kdo sugar).



Supplemental Figure 2. Western blots against purified BamA with pAbs from K12 immunizations. Western blot results with 10 ug/mL of purified pAbs from bleed 1 are shown. Lane 1 contains 100 ng of an irrelevant protein and lane 2 contains 100 ng of recombinant BamA. All samples were run on a single gel and transferred to a blot. The blot was divided into five parts and each parts was stained with a different purified pAb sample. Images for the full blot are shown.



Supplementary Figure 3. MFI values for purified pAbs from whole cell immunization. MFI values on either $\Delta waaD$ (black box) or K12 (white box) with 40 ug/mL purified pAbs for each of the six rats.

Format	Advantage	Disadvantage
Purified protein	High purity	Detergent-specific
(detergent)		conformation
		Production
Purified protein	High purity	Lipid-specific conformation
(matrix)	Modular choice of lipids	Production
Peptide	Ease of production	Hard to mimic native
		conformation
Membrane	Ease of production	High level of non-target Abs
fraction	Native format	
Membrane	Ease of production	• High level of non-target Abs
vesicle	Native format	Low target amount
Whole cell	Ease of production	• High level of non-target Abs
	Native format	Low target amount
DNA	Ease of production	Low immune response
	Native format	Low expression level

Supplementary Table 1. Comparison of different antigen formats for membrane proteins. Overview of advantages and disadvantages in using different antigen formats for antibody discovery.