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Supplementary file 1

Supplementary material S1: Production of Abmb

Escherichia coli BL21 (DE3) carrying the Abmb gene was grown for 12–14 hours at 37°C in LB medium containing ampicillin. Abmb expression was induced with 0.05 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were collected by centrifugation at 4°C, 6,000 *g*, for 15 minutes, and subsequently washed with phosphate buffer saline (PBS; 137 mM NaCl, 2.7 mM KCl in 10 mM phosphate buffer, pH 7.4). The cells were resuspended in low-salt PBS (27.5 mM NaCl, 0.5 mM KCl in 10 mM phosphate buffer, pH 7.4) and then sonicated on ice (Misonix, QSonica, Newtown, CT-USA). The crude extract was clarified by centrifugation at 10,000 *g*, 4°C for 15 minutes and then loaded onto a Ni-NTA affinity column (Roche, Singapore). The pure His_6-tagged Abmb was recovered from the affinity column by elution with 200 mM imidazole in the low-salt PBS buffer. Finally, Abmb was cleaned on an Enrich™ SEC70 size exclusion column (BioRad, Singapore) using PBS buffer as the eluent. Purification steps were done with a NGC Scout-Plus chromatography system (BioRad, Singapore). Pure Abmb was stored at -20°C. Prior to use, the protein was thawed, centrifuged at 12,000 *g*, 4°C for 15 minutes, and sterilized over a 0.22 µm membrane filter. The protein concentration was estimated from its absorbance at 280 nm on a nanodrop 2000c spectrophotometer (ThermoFischer Scientific, Singapore).

The purification steps starting from the size exclusion column and protein stock preparation (extensive buffer exchange over several membrane filters) were done in water-for-injection (WFI) based PBS, which had been tested for endotoxin-free conditions. Prior to use, Abmb concentration was adjusted to 58.8 µM (~1 mg/ml, MW ~17 kDa) by dilution with the endotoxin free PBS.

The endotoxin level in Abmb stock solution was less than ~8 ng/mL or ~0.08 nM as measured with a Gel-clot assay (AccuMedi Solution, Zhanjiang, China). As the control of experiment, 0.01 µM LPS (or ~1 µg/mL, assuming MW ~ 100 kDa).

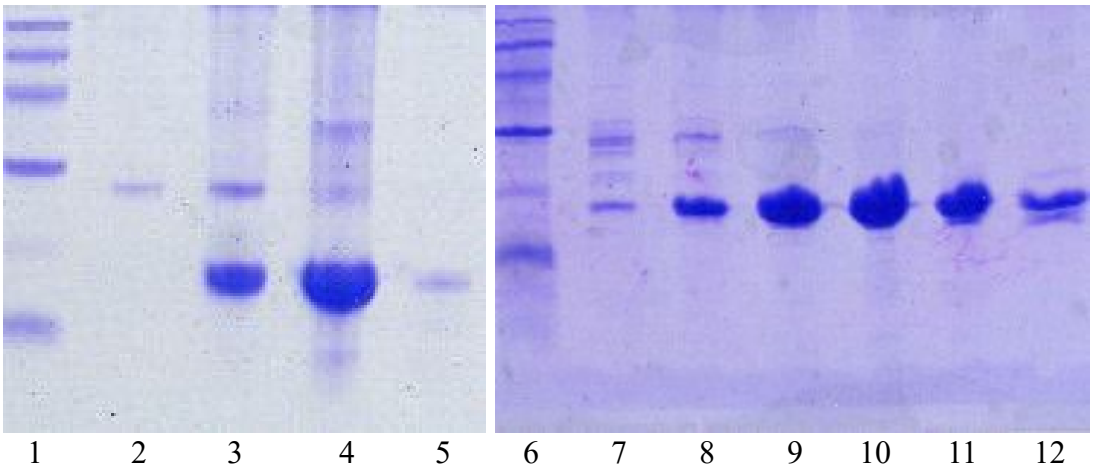

Figure S1. Purification of Abmb. Lane (1) and (6) are the molecular weight marker. The lanes (2), (3), (4) and (5) are the content of the eluted fractions from the Ni-NTA column during washing, early, main, and end of elution, respectively. The lane (7) is content of the fraction eluted in the dead volume of the SEC column, while (8-9) and (10-12) are of the early and main elution, respectively. Only fractions 10-12 were pooled, concentrated, and buffer exchanged for used in the experiments.

Table S1. List of primers

Gene	Forward	Reverse
TNF-α	5'-AGC-CCA-CGT-CGT-AGC-AAA-3'	5'-GGC-AGA-GAG-GAG-GTT-GAC-TT-3'

IL-6	5’-AGT-TGC-CTT-CTT-GGG-ACT-GA-3’	5’-TCC-ACG-ATT-TCC-CAG-AGA-AC-3’
IL-10	5’-AAG-GGT-TAC-TTG-GGT-TGC-CA-3’	5’-TTC-AGC-TCC-TCA-CCC-AGG-GA-3’
actin	5’-ACC-CAC-ACT-GTG-CCC-ATC-TA-3’	5’-CGA-AAC-CGC-TAC-TTG-CC-3’