Supporting Information

MATERIALS AND METHODS

ELISA protocol for three different control assays to validate each step of scFv Fc sensors shown in Scheme 1

Control assay A In control assay A, 50 µl of 1: 2,500 diluted goat anti-rabbit IgG HRP conjugate in PBS-T were added to macrophages/rabbit IgG coated wells and then incubated at 37°C for 1 hour. ABTS substrate was added to each well followed by washing plate 4 times and then incubated for another 30 minutes.

Control assay B (Anti E-tag monoclonal antibody react with macrophage assay) Serial diluted A10B RG3 at the concentration of 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL were added to designed blocked and non-blocked macrophage-coated wells, then incubated for 1 hour at 37 C for reacting with macrophage. 50 µL of 1:5,000 diluted anti E-tag HRP conjugate monoclonal antibody was added to each well followed by washing and then incubated for 1 hour at 37 °C. After washing with WB four times, substrate ABTS was added to each well incubated for another 30 minute.

Control assay C (A10B scFv-RG3 reacts with rabbit IgG) ELISA plate was coated with rabbit IgG at 5 µg/mL in ELISA coating buffer pH 9.6; incubated the plate at room temperature for 2 hours and then transferred to 4 °C for overnight. The plate was washed with WB three times next morning and then blocked the plate with PBS containing 0.1% Tween 20 for 20 minutes. Serial diluted A10B ScFv RG3 in PBS from 5 µg/mL then 1:2 dilution to 0.019 µg/mL were added to designed wells and incubated in a humid chamber at room temperature for 1 hour. The plate was washed three times once again and 1: 5,000 diluted anti E-tag monoclonal antibody HRP conjugate was added to the wells,

incubated at same condition for another hour. After wash four times with WB, substrate ABTS was added to wells, and then OD was read at 405 nm by an ELISA reader.

RESULTS AND DISCUSSION

ELISA Characterization of purified protein A using scFv Fc sensor

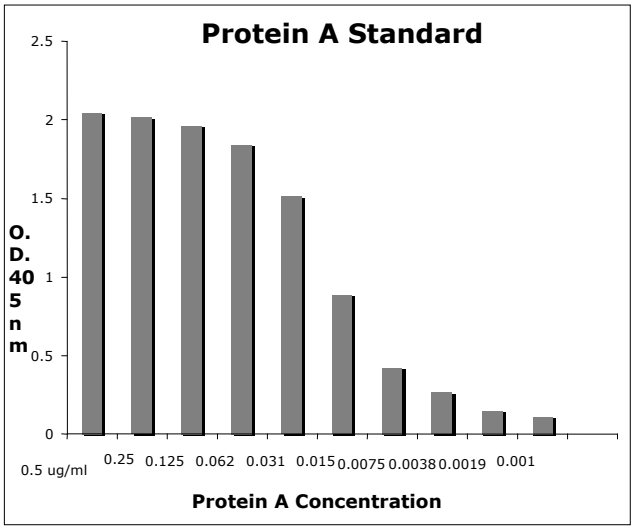

Figure S1 standard protein A samples detected by mouse anti-protein A biotin conjugate and streptavidin-HRP on microtiter plate by ELISA.

Table S1 Controls of ELISA Detection of Fc receptors on macrophage

ANTIBODIES (μg/ml)	BLOCKERS					
	BSA		NFDM		Gelatin	
*Rabbit IgG	5%	Non	5%	Non	5%	Non
5	0.371	0.374	0.352	0.361	0.357	0.335
2.5	0.355	0.325	0.361	0.353	0.337	0.317
1.25	0.321	0.297	0.305	0.309	0.304	0.294
**A10B RG3	5%	Non	5%	Non	5%	Non
5	0.244	0.285	0.251	0.279	0.261	0.254
2.5	0.247	0.214	0.212	0.261	0.232	0.241
1.25	0.193	0.149	0.194	0.244	0.199	0.212

* Fc receptors on macrophage react with rabbit IgG detected by anti-rabbit IgG-HRP

**Fc receptors on macrophage react with ScFv(A10BRG3) detected by anti E-tag HRP.

ABTS substrate are used in bothe assays and readiding in 405nm.

Damping resistance changes for the detection of *S. aureus* and macrophages.

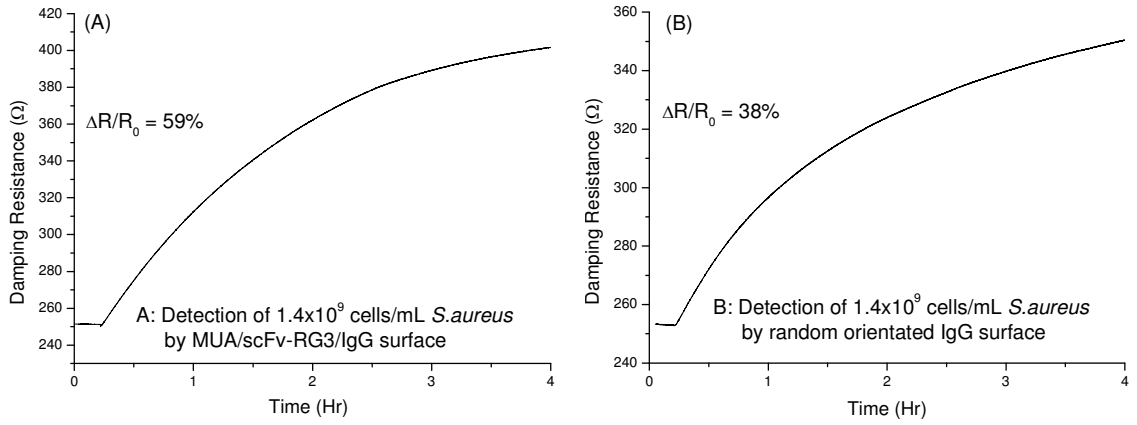

Figure S4 Damping resistance changes for the detection of *S. aureus. (*A) Acid treated *S. aureus* were added to MUA/scFv-RG3/IgG surface; (B) Acid treated *S. aureus* were added to random oriented IgG surface

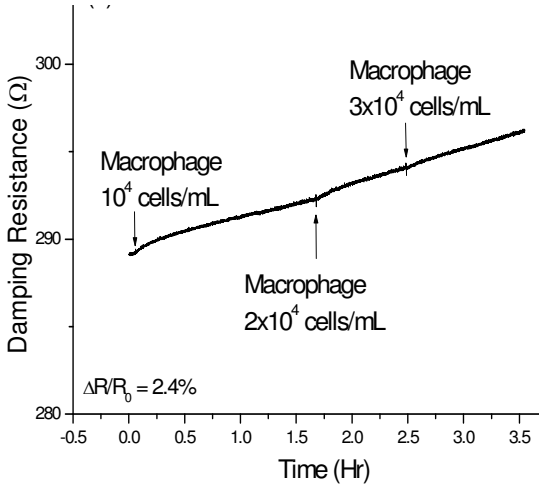

Figure S5 Damping resistance change for the detection of macrophage by MUA/scFv-RG3/Rabbit IgG modified QCM sensors.

The A10B scFv recognizes the CH1 domain on rabbit IgG.

Our studies have not been able to show that it binds to IgG from any other species of animal. The evidence that demonstrates that A10B recognizes the CH1 domain on rabbit IgG is follows.

(a) When rabbit IgG is boiled in SDS and beta mercaptoethanol, and electrophoresed on a polyacrylamide gel, two bands are seen. One of the bands is the rabbit IgG light chain that migrates at around 25,000 molecular weight. The other band is the IgG heavy chain that migrates at around 55,000 molecular weight. When the protein bands on the polyacrylamide gel are transferred to nitrocellulose and probed with A10B, the heavy chain 55,000 molecular weight band is stained.

(b) When rabbit IgG is digested with the enzyme papain, it is broken into 3 parts. One part is the rabbit IgG Fc (bottom of the Y on an antibody) region, and the other 2 parts are the rabbit IgG Fab fragments. The Fab fragments contain the variable regions of the antibody heavy and light chains. The Fab fragments contain the light chain constant region and the heavy chain (CH1) region. When the papain-digested rabbit IgG is passed over protein A on beads, the Fc region becomes bound to the protein A. The Fab fragments remain in solution, and can be separated from the bound Fc on protein A beads.

(c) When the Fab fragments are boiled in SDS and mercaptoethanol, and electrophoresed on polyacrylamide gels, two bands are obtained. One band is the heavy chain variable region linked to the CH1 region and the other band is the light chain linked to the light chain constant region. Both the light and heavy chain bands run at about the same location on the polyacrylamide gel. When the protein bands are transferred to the nitrocellulose and probed with A10B, A10B stains one of the bands. Since the Fc region (which contains CH2 and CH3, but not CH1) has been removed, and since A10B stains only the heavy chain (55,000 mw) as addressed at above (a), then it can be assumed that A10B recognizes the CH1 on rabbit IgG. Since A10B has bound to every different antigen-specific rabbit IgG from multiple diverse and sources in ~~every~~ all assays, ~~ed~~, and since every different molecule of antigen-specific rabbit IgG has a differ of amino acid sequence within it's variable region, it is logical that A10B binds to the CH1 region, and not the variable region of the rabbit IgG heavy chain. It is possible that A10B binds to one of the frameworks near the CH1 region on rabbit IgG variable regions, but we do not have any evidence that it does so. However, as far as the paper is concerned, we do know for a fact that A10B does not bind rabbit IgG Fc regions making those regions freely available for binding to Fc receptors on cells. We do know that A10B binds the rabbit IgG heavy chain portion of the Fab fragment.