

Electronic supplementary information

Single Molecule Array (Simoa) Assay with Optimal Antibody Pairs for Cytokine Detection in Human Serum Samples

Danlu Wu,^{a †} Milena Dumont Milutinovic,^{a †} David R. Walt ^{*a}

^a Department of Chemistry, Tufts University, 62 Talbot Ave, Medford MA 02155 USA

* To whom all correspondence should be addressed. Phone/fax: 617-627-3470 /617-627-3443.

Email: david.walt@tufts.edu

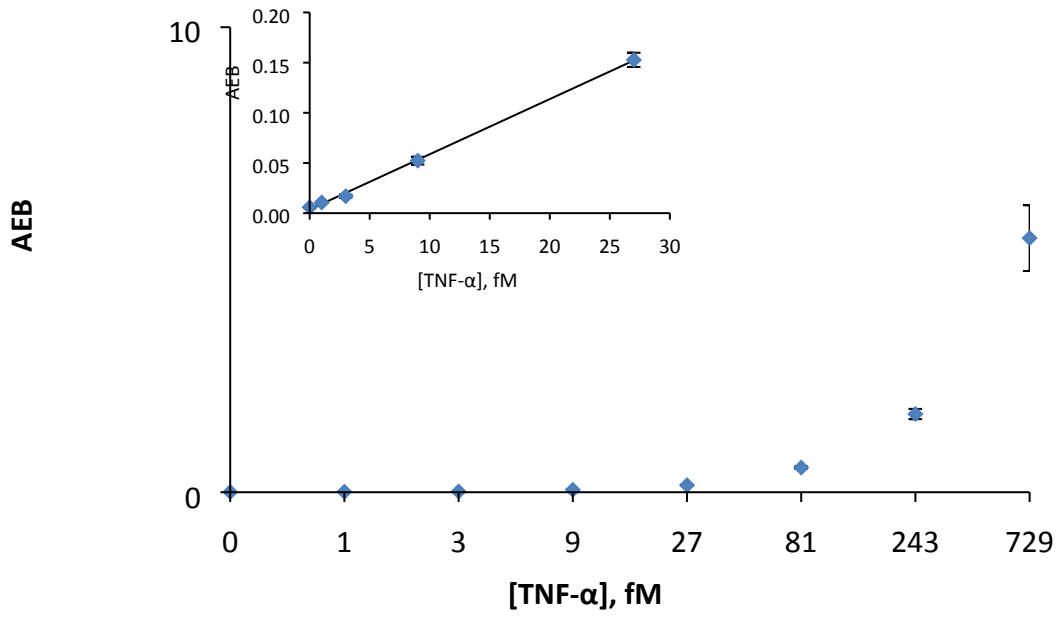
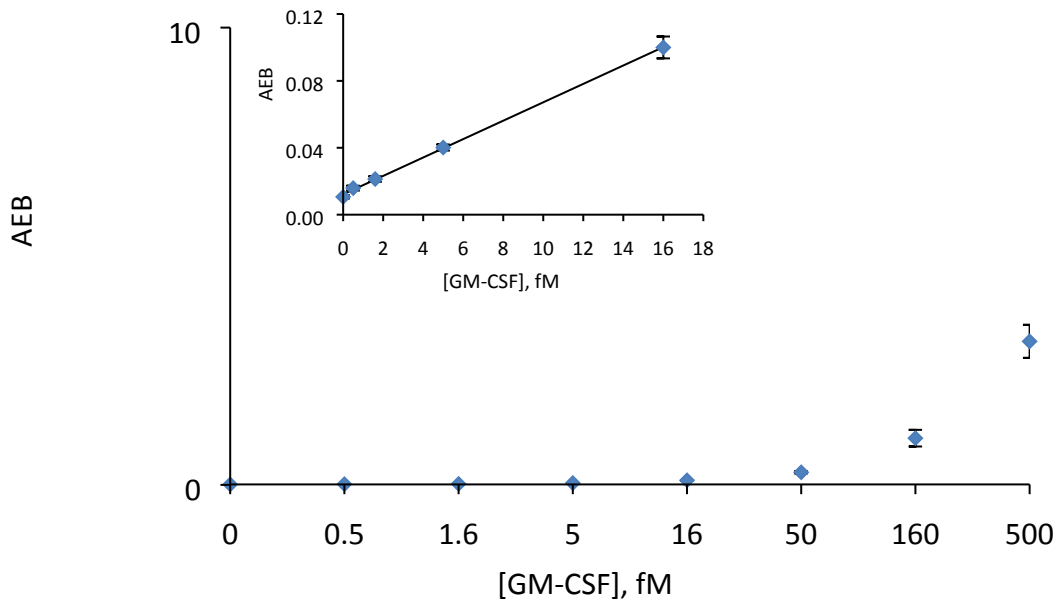
[†] These authors contributed equally to the work

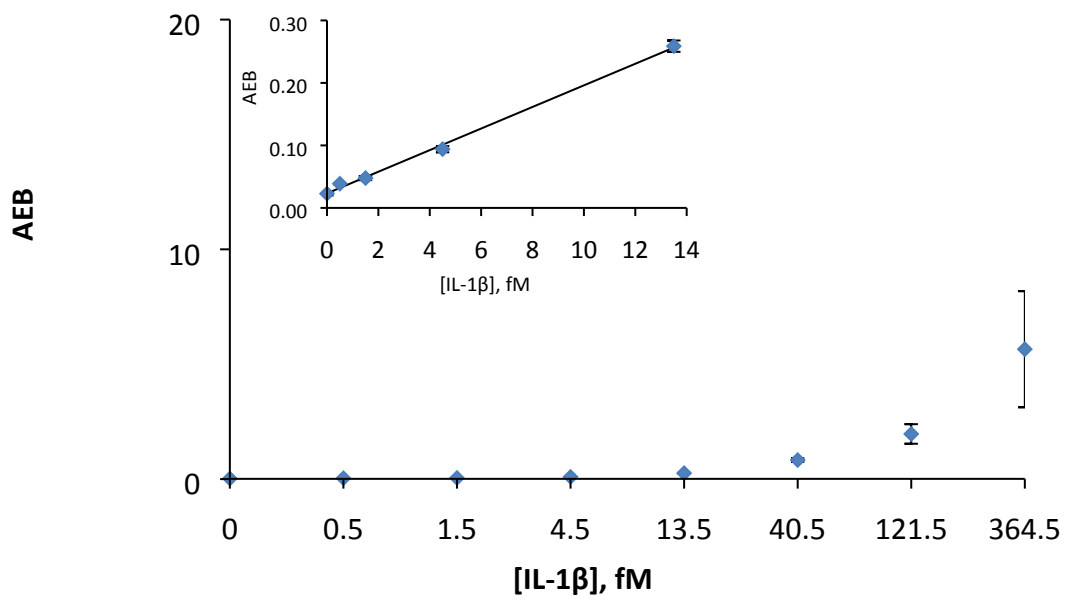
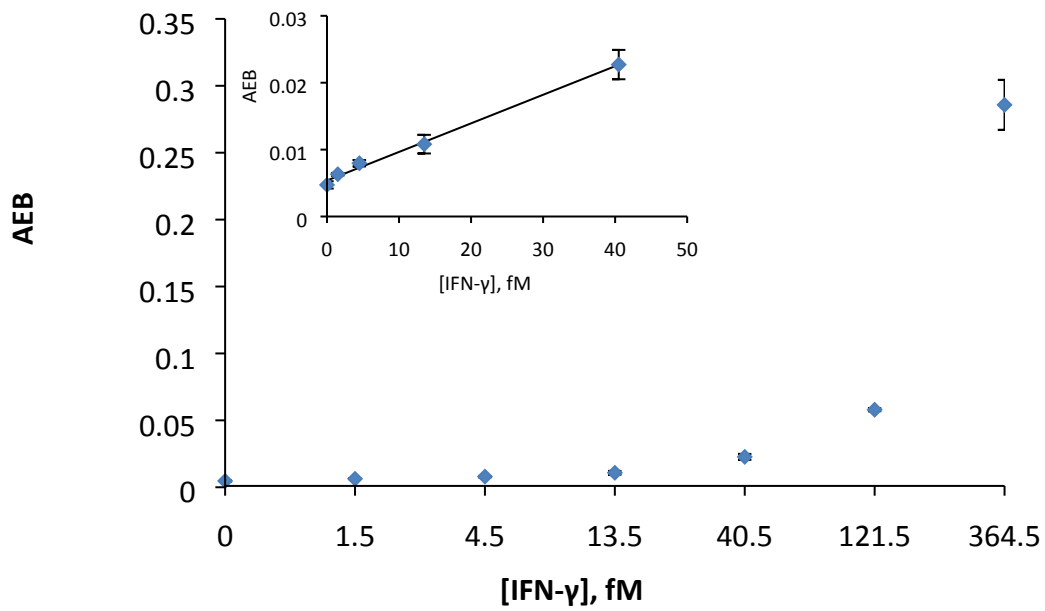
Table S1 List of tested antibody pairs (R&D = R&D Systems; BL = Biolegend). The optimal pairs are shown in bold.

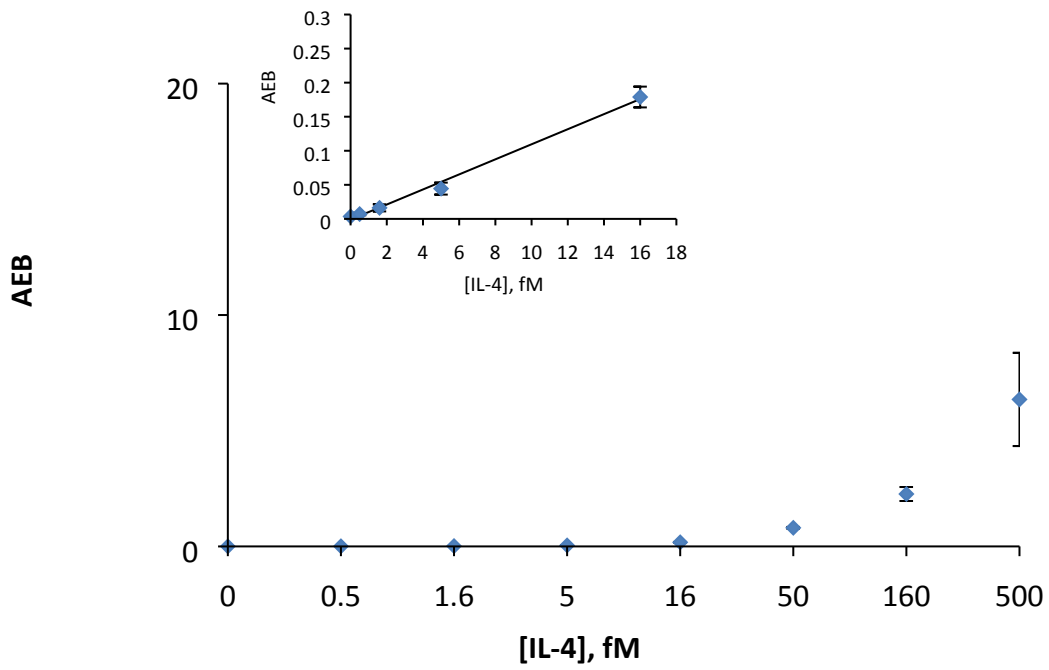
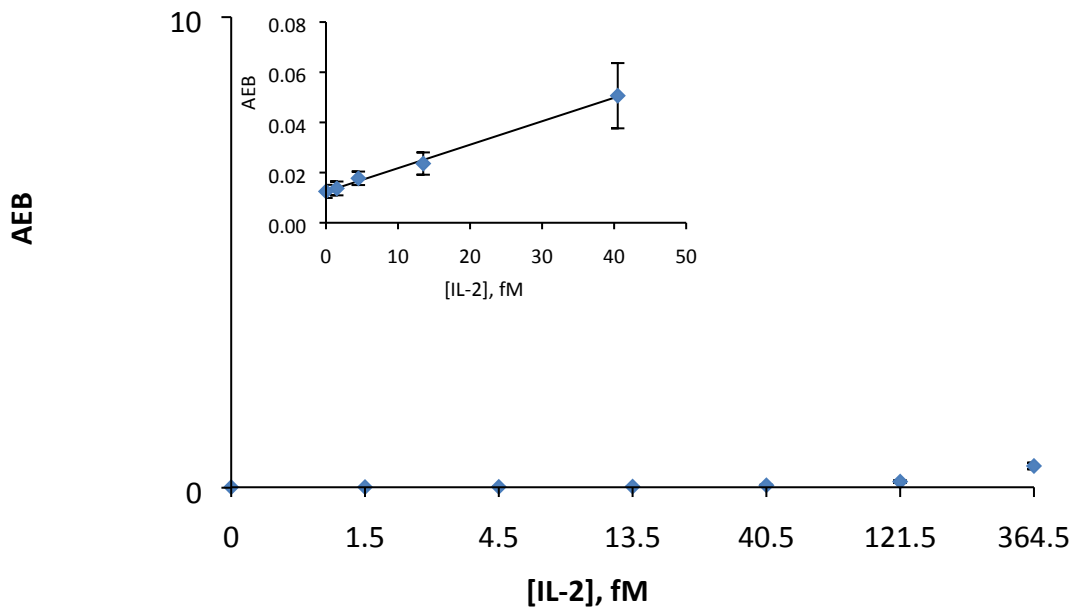
Cytokine	Capture Antibody	Detection Antibody	Results
TNF- α	MAB610(R&D)	BAF210(R&D)	High background
	MAB610(R&D)	MAB210(R&D, biotinylation in house)	Relative low response
	MAB610(R&D)	502903(BL)	Low response
	MAB610(R&D)	ab9635 (Abcam, biotinylation in house)	Good
	502803(BL)	502903(BL)	OK
	502803(BL)	ab9635 (Abcam, biotinylation in house)	OK
	502803(BL)	MAB210(R&D, biotinylation in house)	Low response
	502803(BL)	BAF210(R&D)	Low response
	ab8348(Abcam)	BAF210(R&D)	Low response
	ab8348(Abcam)	502903(BL)	Low response
	ab8348(Abcam)	MAB210(R&D, biotinylation in house)	Low response
	ab8348(Abcam)	ab9635 (Abcam, biotinylation in house)	Low response
	GM-CSF	MAB615(R&D)	MAB215(R&D, biotinylation in house)

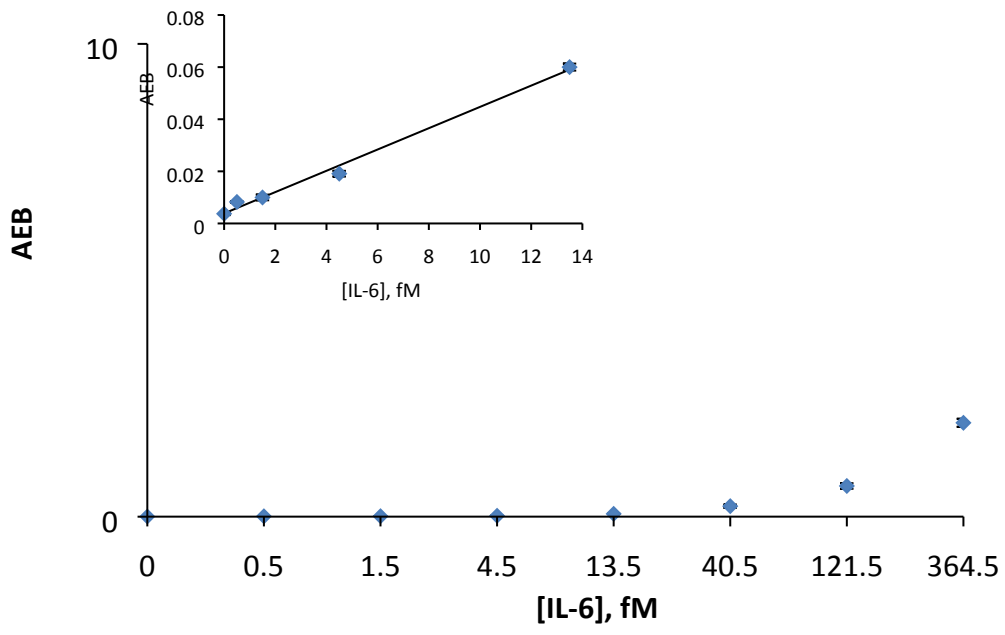
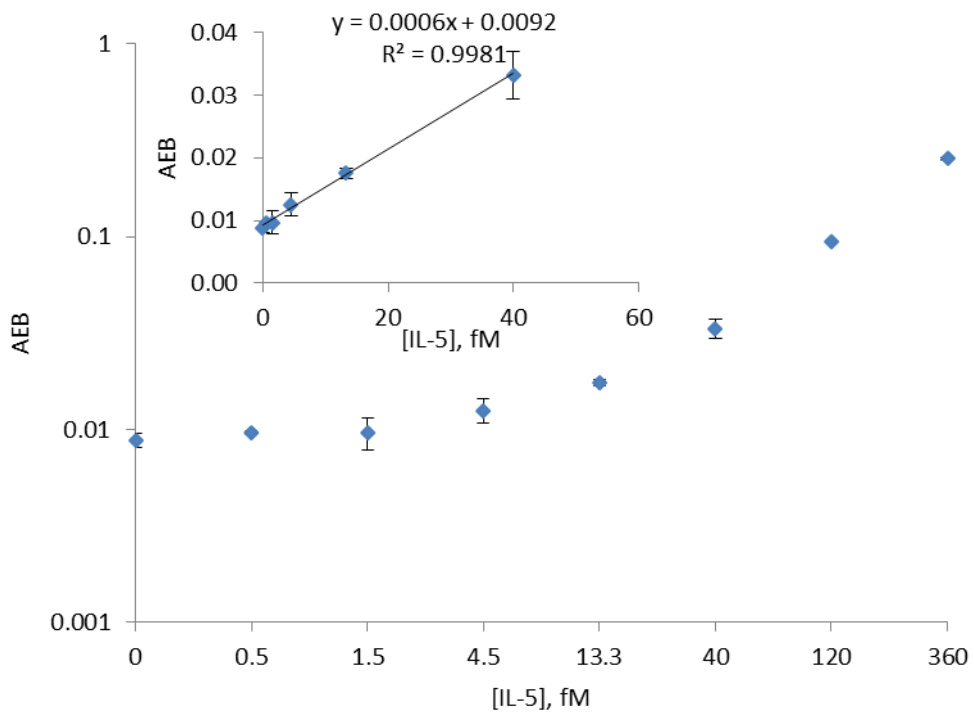
IFN- γ	MAB2852(R&D)	MAB285(R&D, biotinylation in house)	High background
	MAB2852(R&D)	BAF285(R&D)	Low response
	502401 (BL)	502503(BL, 4S.B3)	No response
	507501(BL)	502503(BL)	No response
	MAB2852(R&D)	502503(BL)	No response high background
	507501(BL)	MAB285(R&D, biotinylation in house)	Good
	507501(BL)	BAF285(R&D)	No response
	502401 (BL)	BAF285(R&D)	High background
	502401 (BL)	MAB285(R&D, biotinylation in house)	Low response
	IL-1 β	MAB601(R&D)	AF-201-NA(R&D, biotinylation in house)
MAB601(R&D)		MAB201(R&D, biotinylation in house)	No response
MAB601(R&D)		508301(BL)	Relatively low response
MAB201(R&D)		508301(BL)	Good
508201(BL)		508301(BL)	Relative low response
508201(BL)		AF-201-NA(R&D, biotinylation in house)	Low
IL-2		MAB602(R&D)	BAF202(R&D)
	MAB602(R&D)	MAB202(R&D, biotinylation in house)	OK
	MAB602(R&D)	555040(BD)	Relatively low response
	MAB602(R&D)	ab38151(Abcam)	No response
	MAB602(R&D)	517601(BL)	High background

	555051(BD)	555040(BD)	No response
	555051(BD)	517601(BL)	No response
	555051(BD)	ab38151(Abcam)	No response
	555051(BD)	MAB202(R&D, biotinylation in house)	Relatively low response
	ab10090(Abcam)	ab38151(Abcam)	No response
	ab10090(Abcam)	555040(BD)	No response
	ab10090(Abcam)	517601(BL)	No response
	ab10090(Abcam)	MAB202(R&D, biotinylation in house)	Low response
	500301(BL)	517601(BL)	High background low S/N
	500301(BL)	ab38151(Abcam)	No response
	500301(BL)	555040(BD)	No response
	500301(BL)	MAB202(R&D, biotinylation in house)	High background relatively low S/N
IL-4	MAB604(R&D)	BAF204(R&D)	No response
	MAB604(R&D)	MAB204(R&D, biotinylation in house)	No response
	500701(BL)	500803(BL)	Good
IL-5	MAB405(R&D)	BAM6051(R&D)	Low response
	500902(BL)	Ab84248 (Abcam)	OK
	500902(BL)	501002(BL)	OK
IL-6	MAB206(R&D)	BAF206(R&D)	Good
IL-7	501302(BL)	506601(BL)	Good
	MAB207(R&D)	BAF207(R&D)	Low response
	MAB207(R&D)	506601(BL)	No response
	501302(BL)	BAF207(R&D)	OK
IL-10	MAB2172(R&D)	BAF217(R&D)	No response
	506801(BL)	501501(BL)	Good









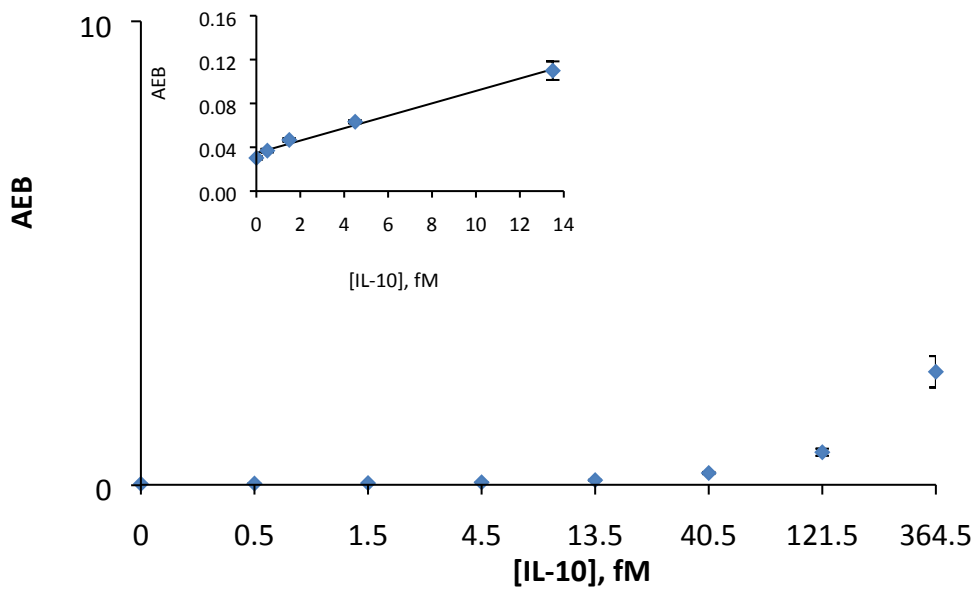
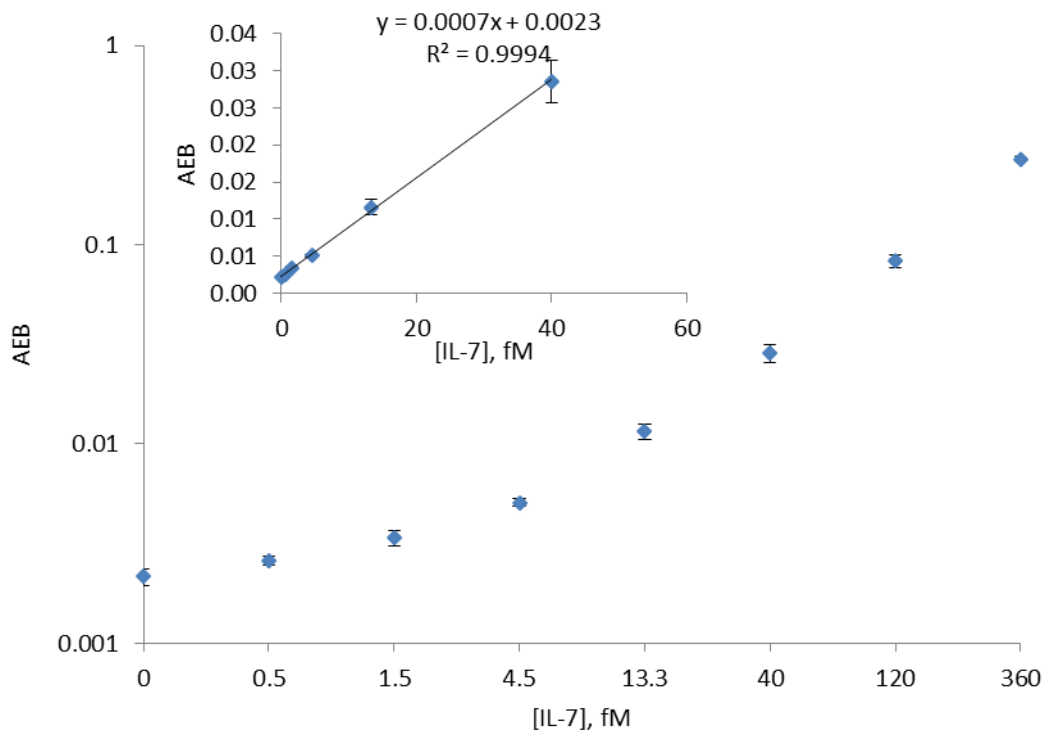


Fig. S1 Calibration plots of various cytokine assays. Normal plots in insets are drawn from the first five points presented in the log-log plots. Error bars are shown for three replicate measurements.

Table S2 Patient characteristics

Sample	Gender:	Age:	Race:
1	Male	45	Black
2	Male	24	Black
3	Male	49	Caucasian
4	Male	48	Black
5	Male	27	Black
6	Male	29	Caucasian
7	Male	26	Black
8	Male	62	Caucasian
9	Female	45	Hispanic
10	Female	25	Black
11	Female	31	Black
12	Female	30	Hispanic
13	Female	32	Hispanic
14	Female	48	Black
15	Female	51	Black

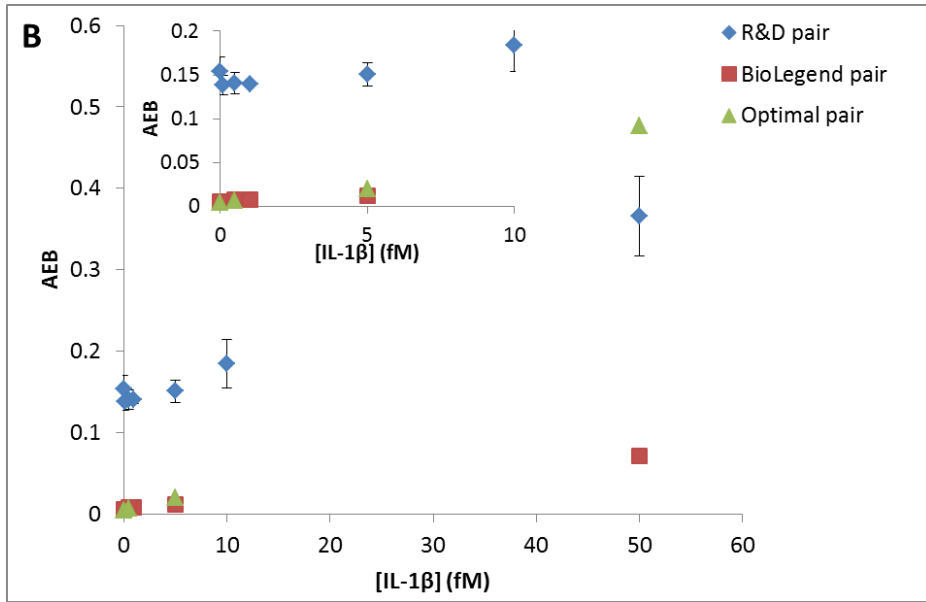
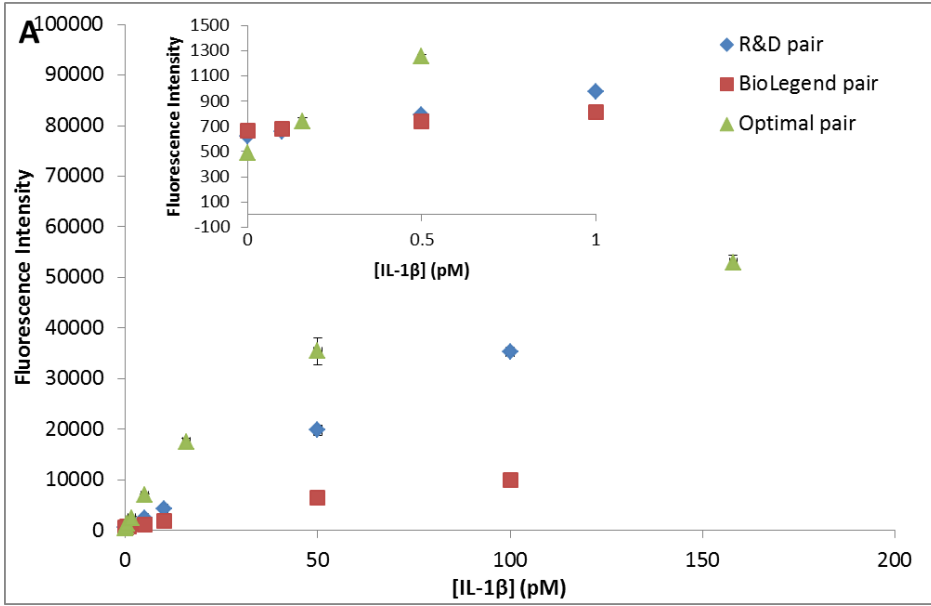


Fig. S2 Results of A) bead-based bulk assay B) Simoa assay for IL-1 β using antibody pairs from R&D Systems (blue diamond), BioLegend (red square), and optimal pair (green triangle, details are in Table S1). Error bars are shown for three replicate measurements. Insets are the enlargements of the plots in low concentration range.

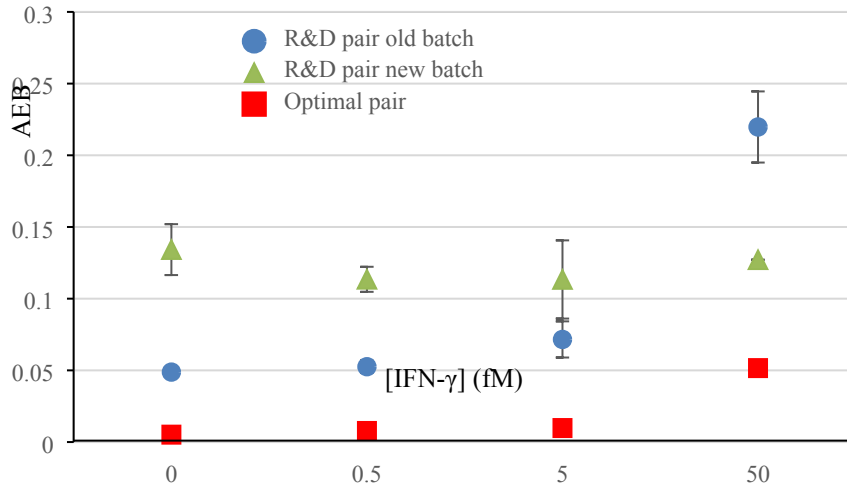
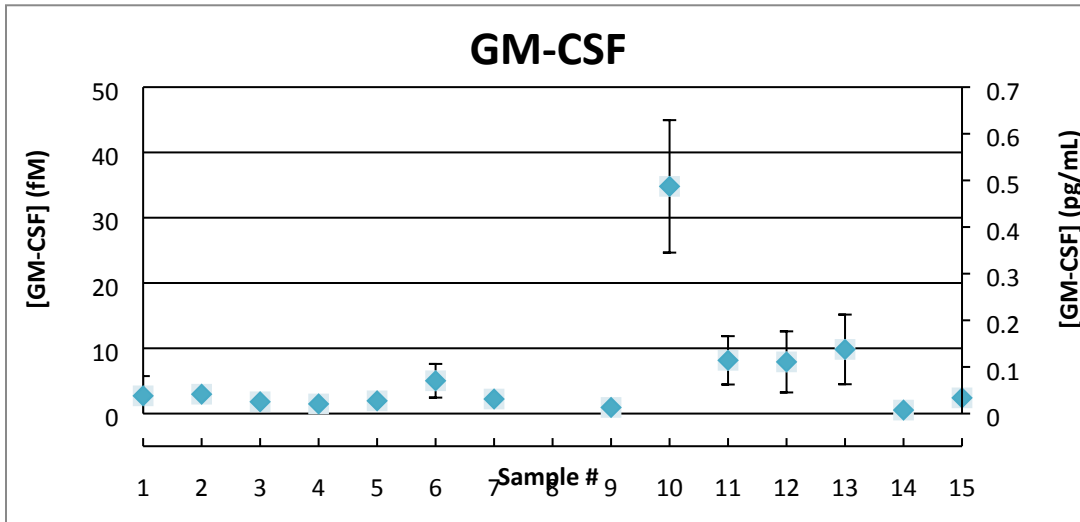
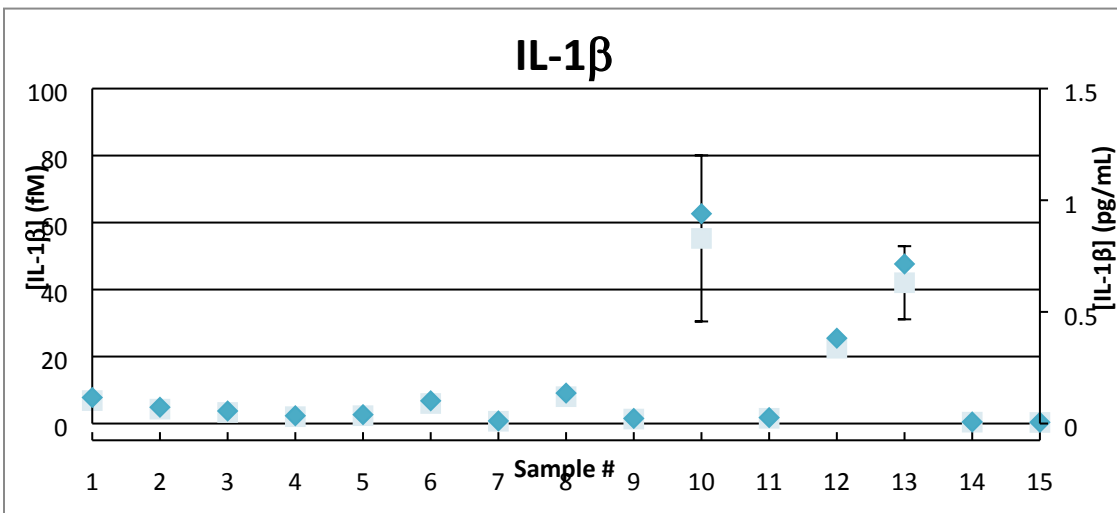
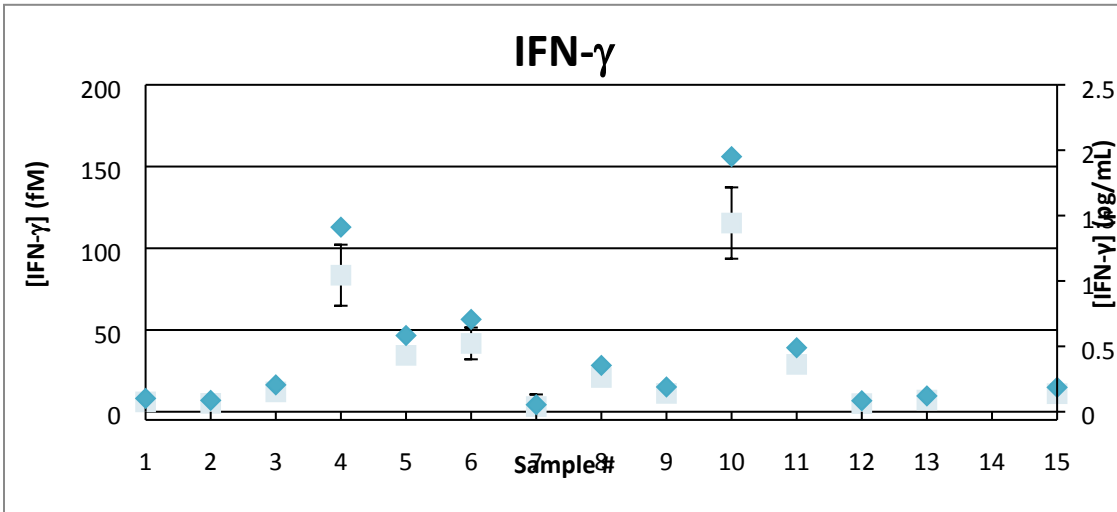
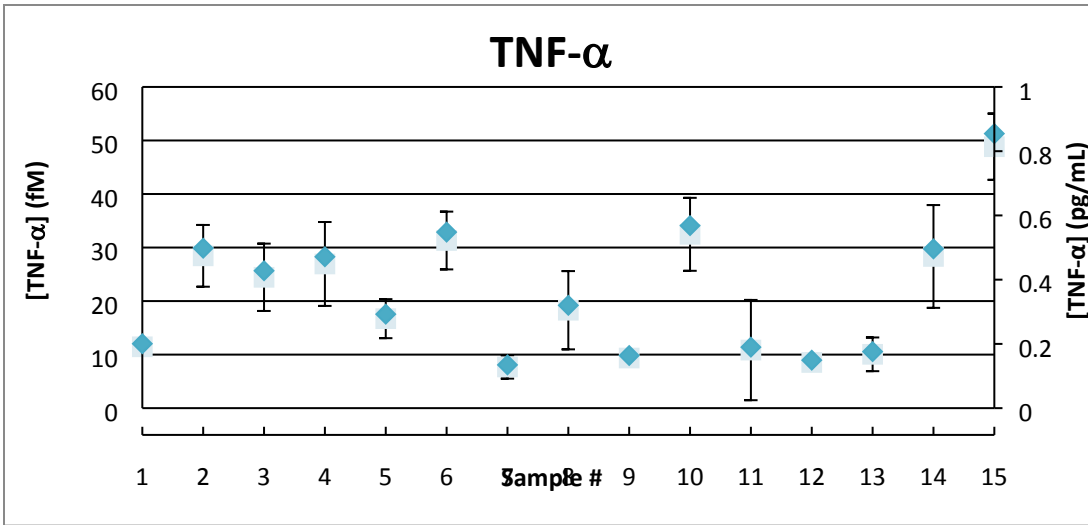
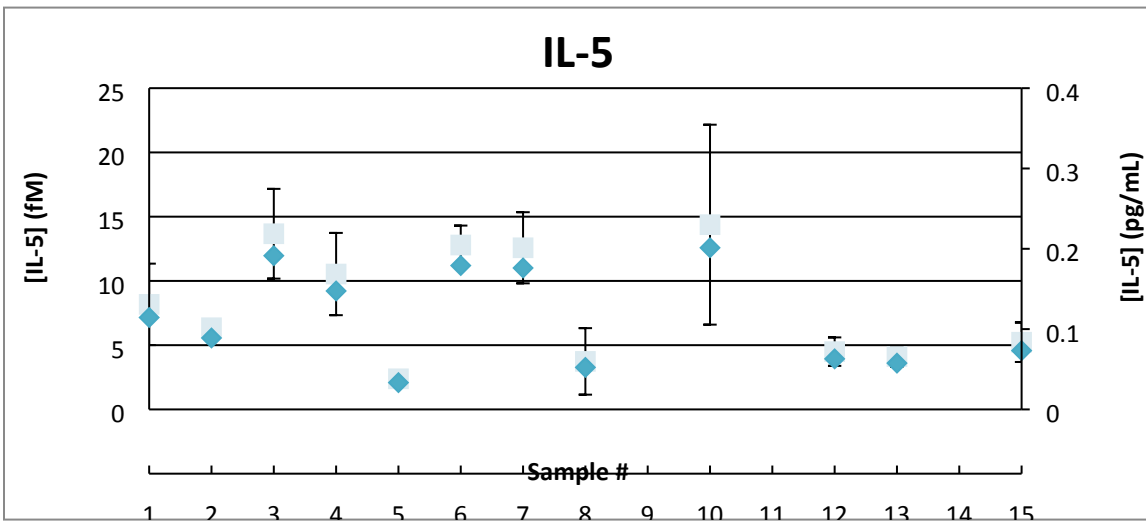
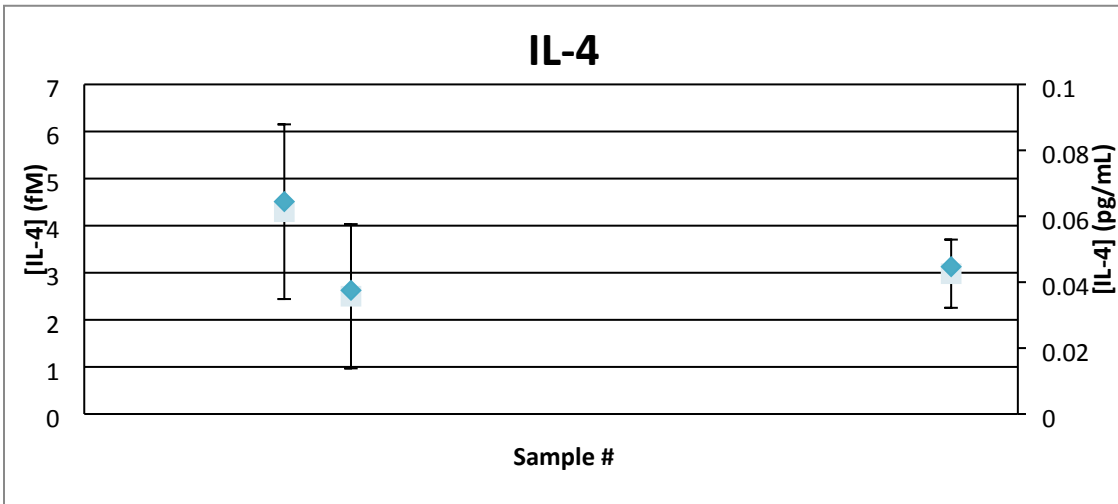
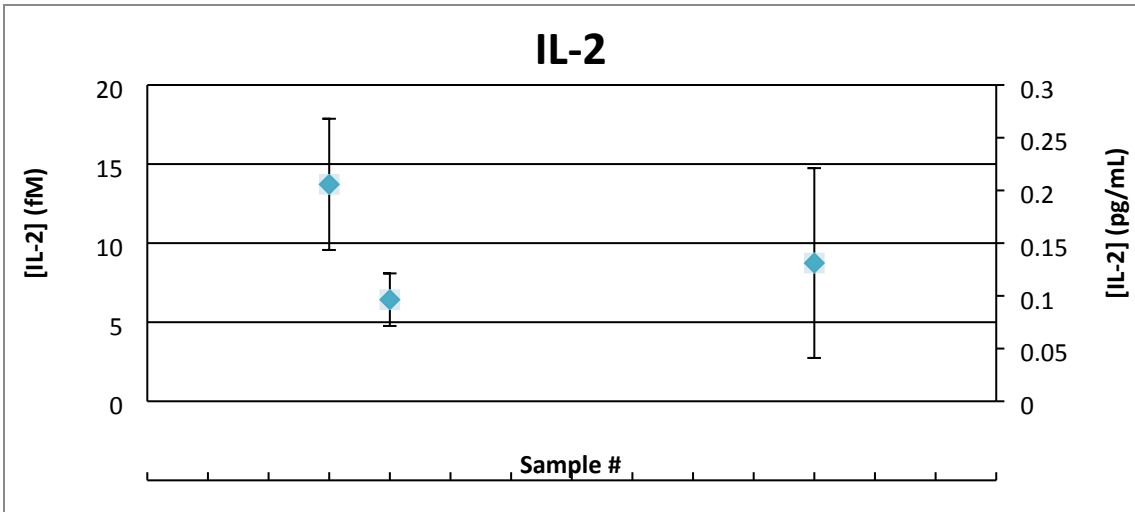


Fig. S3 Results of Simoa assay for IFN- γ using antibody pairs from R&D Systems (BAF285, old batch: blue round, new batch: green triangle) and optimal pair (red square, details are in Table S1). Error bars are shown for three replicate measurements.







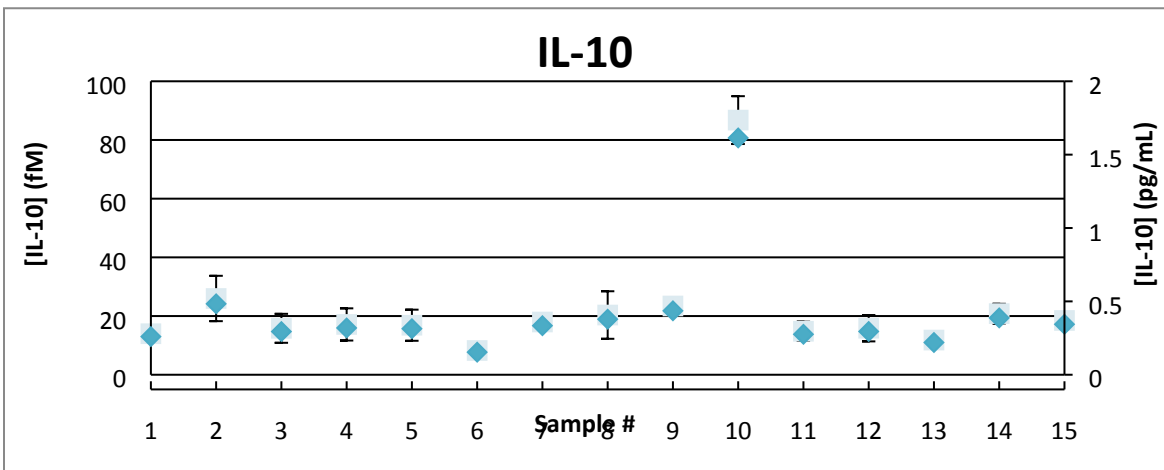
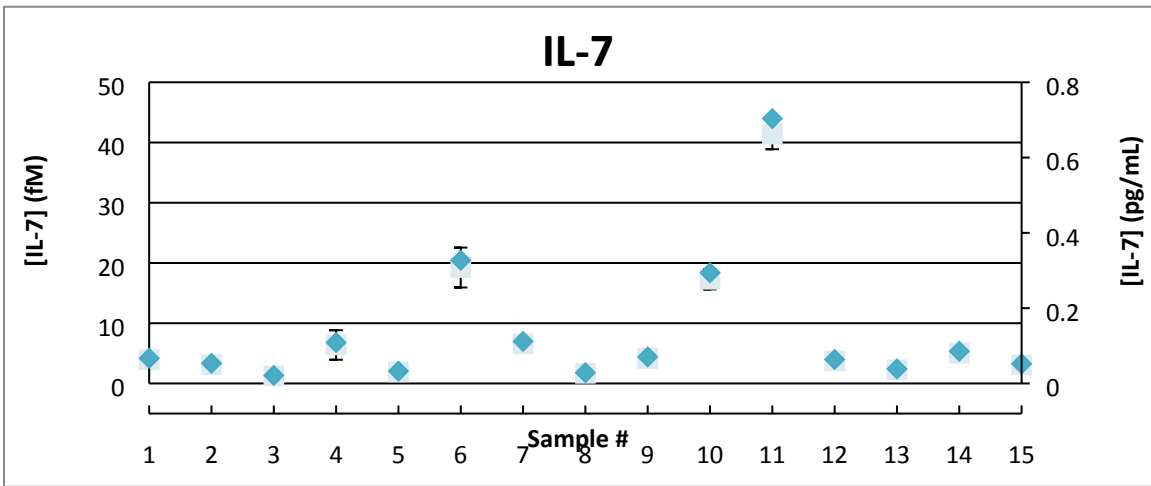
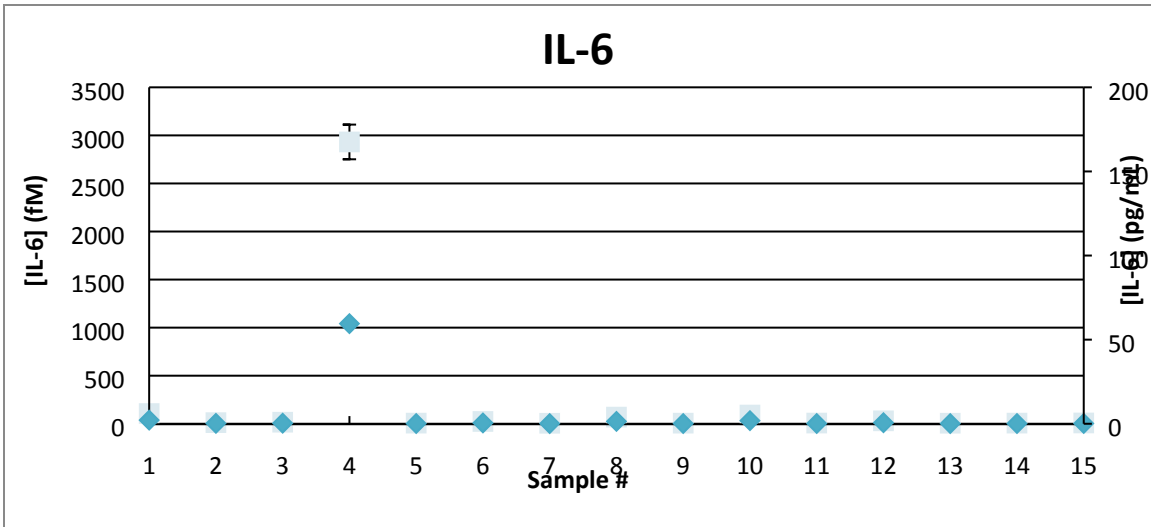


Fig. S4 Measured concentrations (after 4-fold dilution) of ten cytokines in 15 samples shown in both fM and pg/mL units. Error bars are shown for three replicate measurements.