

ONLINE SUPPLEMENTAL MATERIAL

Section 1: Analysis of IL-13-dependent STAT6 responses using a random effects model

In Figure 2, a conventional two-tailed paired t-test was used to compare STAT6 responses to each concentration of WT IL-13 or IL-13 R130Q. However, this method does not incorporate the correlated nature of the data for an individual donor over the concentration range. Therefore we analyzed the dose-response curves for STAT6 induction in response to the IL-13 variants using a random effects model (43) which can adjust for intra-subject correlations which unavoidably come into play whenever dose-response curves are generated from measurements taken in the same individuals. Such a model may therefore represent an interesting complementary approach to analyze the data generated in experiments like ours.

A random effect model estimates a fixed effect of IL-13 R130Q in reference to WT IL-13 on STAT6 induction over a concentration range chosen to reflect the results of the paired t-test analysis. Using this approach, as compared to WT IL-13, IL-13 R130Q was associated with a $10.1 \pm 2.6\%$ (SEM) increase in the percentage of STAT6+ monocytes over the 10-90 pg/ml concentration range ($p=0.001$). For example, at the 90 pg/ml dose, the predicted value for WT IL-13 would be 56% and the value for IL-13 R130Q would be 66.1%. Thus the increased biological potency of IL-13 R130Q relative to WT IL-13 was confirmed by two independent statistical approaches

Section 2: Analysis of IL-13-dependent CD23 responses using a random effects model

Analysis of CD23 responses using a random effects model demonstrated that, compared

with WT IL-13, IL-13 R130Q was associated with a $10.9 \pm 1.8\%$ (SEM) increase in the CD23 response ($p < 0.001$) over the 10-250 pg/ml concentration range.

Legend to Figure S.1.: Comparison of dose-response curves for WT IL-13, IL-13R130Q (corrected values) and IL-13 R130Q (uncorrected values) The Figure shows the induction of STAT6 phosphorylation (top panel) and CD23 expression (bottom panel) in normal human monocytes stimulated with increasing concentrations of IL-13. The solid lines represent the dose-response curves for WT IL-13 (closed circles) and IL-13 R130Q (values adjusted using the correction factor: closed triangles) as shown in Figures 2 and 3. The dashed lines represent the dose-response curves for IL-13 R130Q values *not* adjusted using the correction factor (open triangles).

Legend to Figure S.2.: Induction of AID expression by IL-13 variants cDNA was prepared from normal human PBMC stimulated with HC (1 μ M) and WT IL-13, IL-13 R130Q or IL-4 (all at 90 pg/ml) for 72 hours. AID expression was assessed by RT-PCR followed by densitometric analysis of the amplicons, and was normalized to the expression of the housekeeping gene GAPDH. The Figure shows the mean \pm SE of the results obtained in 2 experiments.

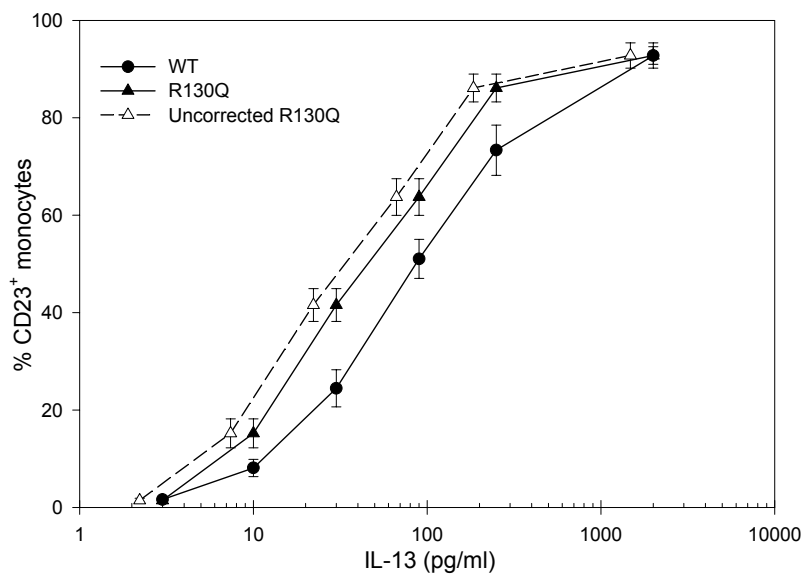
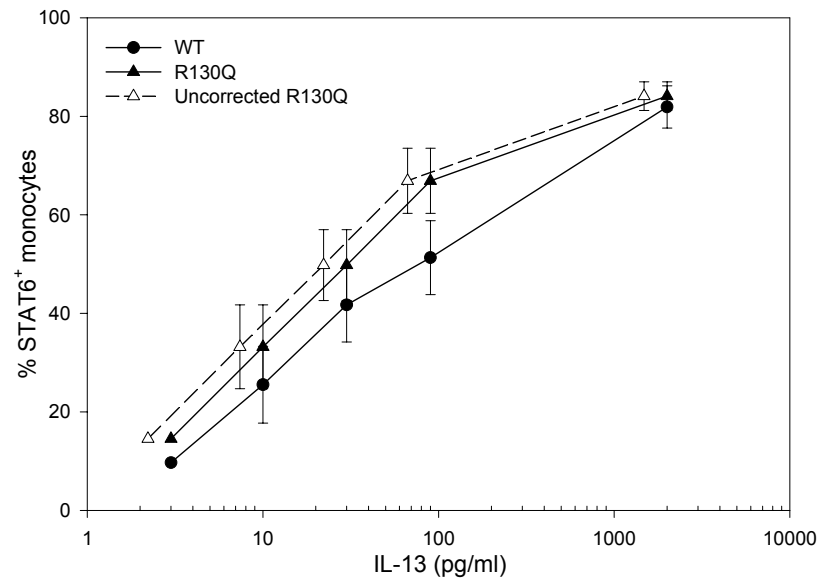


Figure S.1 (Vladich et al.)

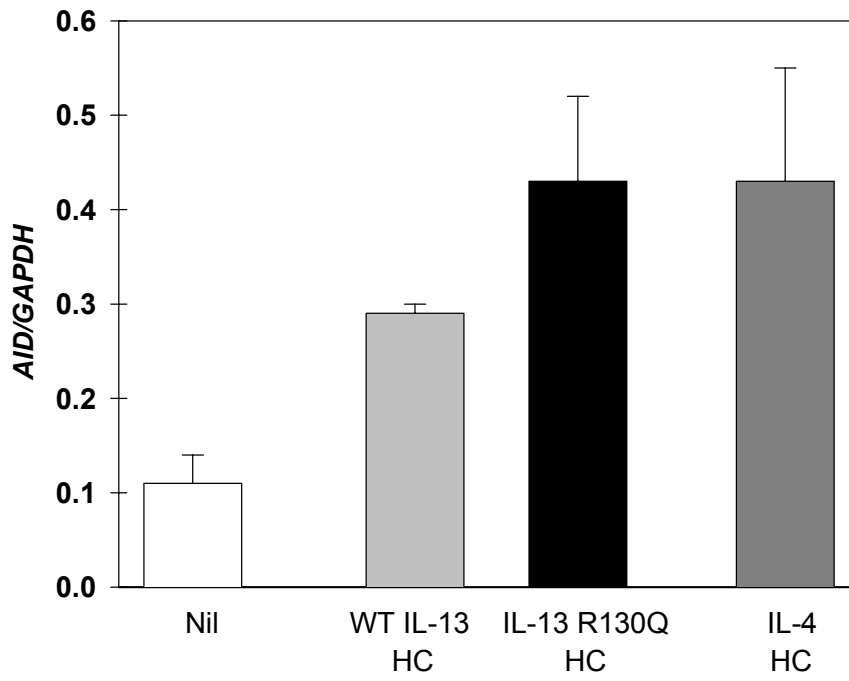


Figure S.2 (Vladich et al.)

Table S.1 *IL-13-dependent induction of STAT6 phosphorylation* The Table shows the anti-log EC₅₀ values for WT IL-13, IL-13 R130Q (corrected values) and IL-13 R130Q (uncorrected values: UC-R130Q) for individual experiments.

Experiment	IL-13 EC ₅₀ (pg/ml)		
	WT	R130Q	UC-R130Q
1	0.46	0.005	0.004
2	7.3	0.41	0.29
3	32.0	35.3	26.1
4	78.8	20.6	15.3
5	205	17.0	12.6
6	294	163	120
7	549	376	278
Geometric mean (95%CI)	43 (4.4-426)	7.7 (0.2-279)[†]	5.7 (0.2-206)[‡]

[†] p=0.03 compared to WT

[‡] p=0.02 compared to WT

Table S.2 *IL-13-dependent induction of CD23 expression* The Table shows the anti-log EC₅₀ values for WT IL-13, IL-13 R130Q (corrected values) and IL-13 R130Q (uncorrected values: UC-R130Q) for individual experiments.

Experiment	IL-13 EC ₅₀ (pg/ml)		
	WT	R130Q	UC-R130Q
1	42.0	30.1	22.3
2	62.5	31.0	23.0
3	64.3	20.8	22.8
4	75.4	38.6	28.6
5	95.7	85.6	63.4
6	118	46.8	34.6
7	117	36.0	26.7
8	147	25.6	18.9
9	163	34.9	25.9
10	210	130	96.0
11	251	104.8	77.5
Geometric mean (95%CI)	107 (74-155)	46 (32-67)[†]	34 (23-50)[‡]