**Figure S1**

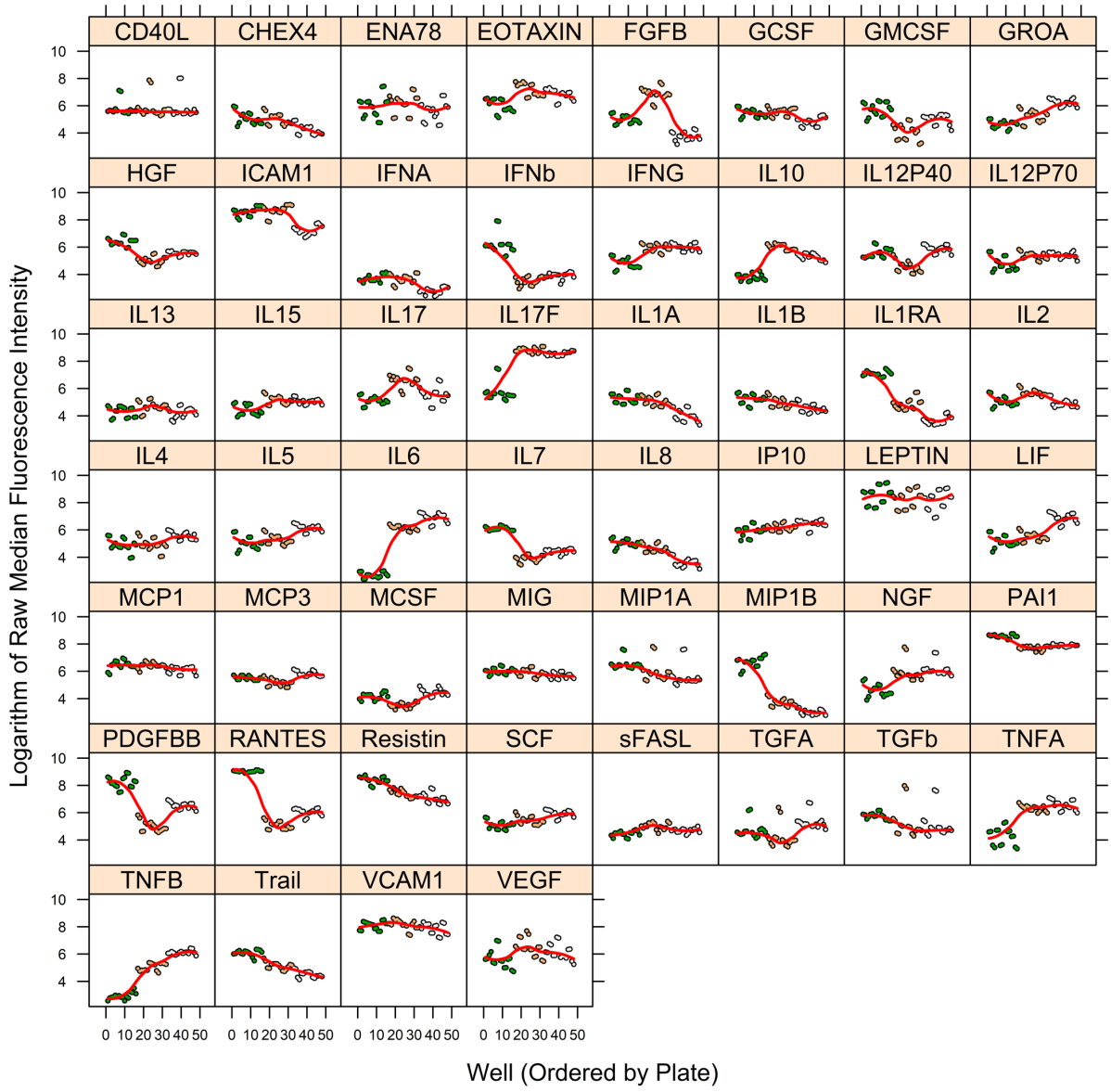



**Figure S1. MFI per well by cytokine *before* removal of lot and nonspecific binding artifacts, for lot experiment**. The vertical axis is MFI before removal of lot and nonspecific bindings artifacts and the horizontal axis is the ordering of wells (across lots). A separate figure is shown for each cytokine and for the NC microbeads (labeled "CHEX4"). Color coding of circles distinguishes wells from different lots. Red line within each figure is a spline smooth of trend in mean. Figure was prepared in R package `lattice` (18).

**Figure S2.**

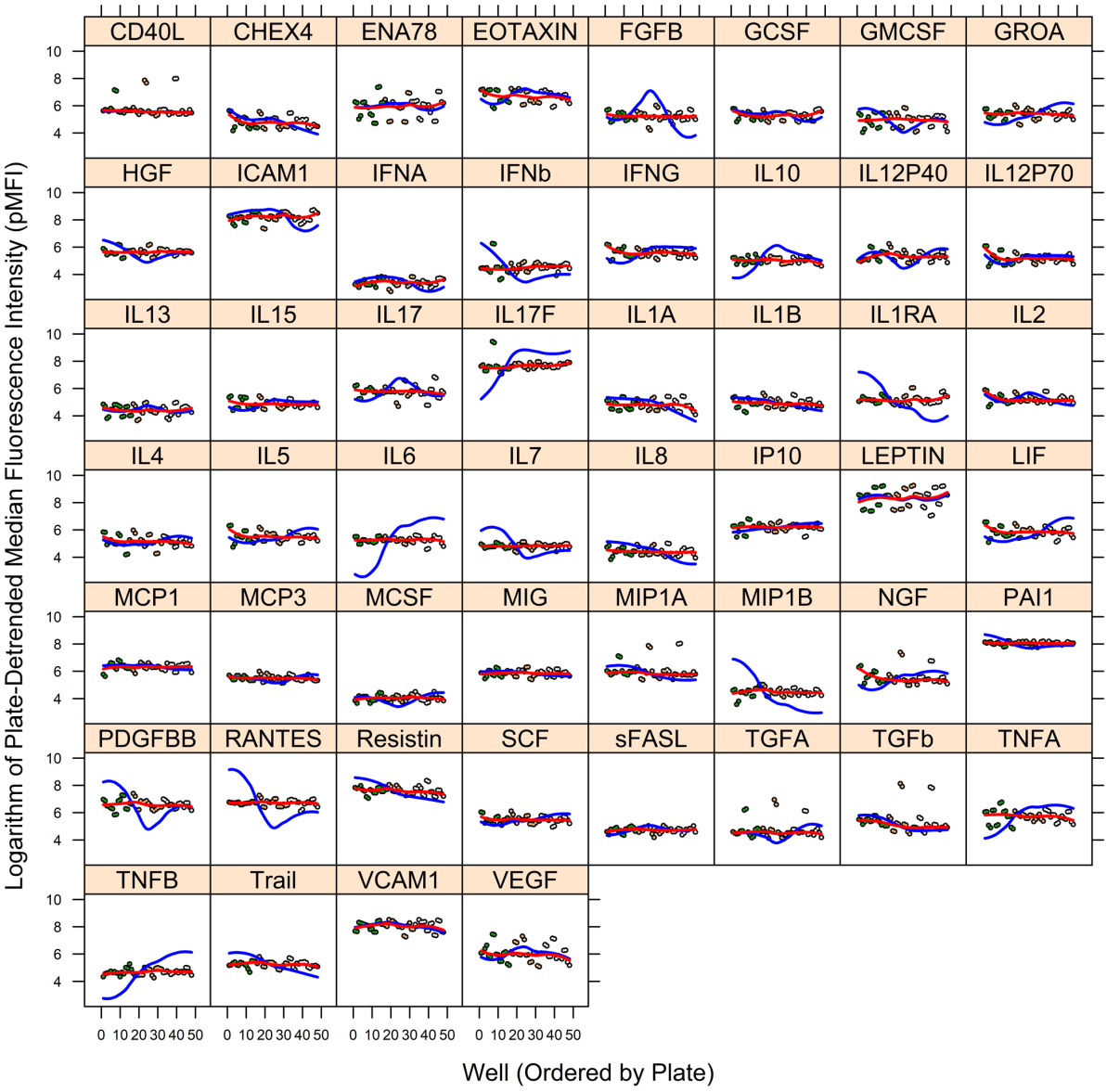



**Figure S2. MFI per well by cytokine *after* removal of lot artifacts (pMFI), for lot experiment.** The vertical axis is MFI after removal of lot artifacts (pMFI) and the horizontal axis is the ordering of wells (across lots). A separate figure is provided for each of the 51 cytokines plus the NC microbeads (labeled "CHEX4"). Color coding of circles distinguishes wells from different lots. Blue and red lines within each figure are spline smooths of trends in mean before and after removal of lot artifacts, respectively. Correction for lot artifact is substantial. Compare to the unprocessed data of Figure S1. Figure was prepared in R package `lattice` (18).

**Figure S3.**

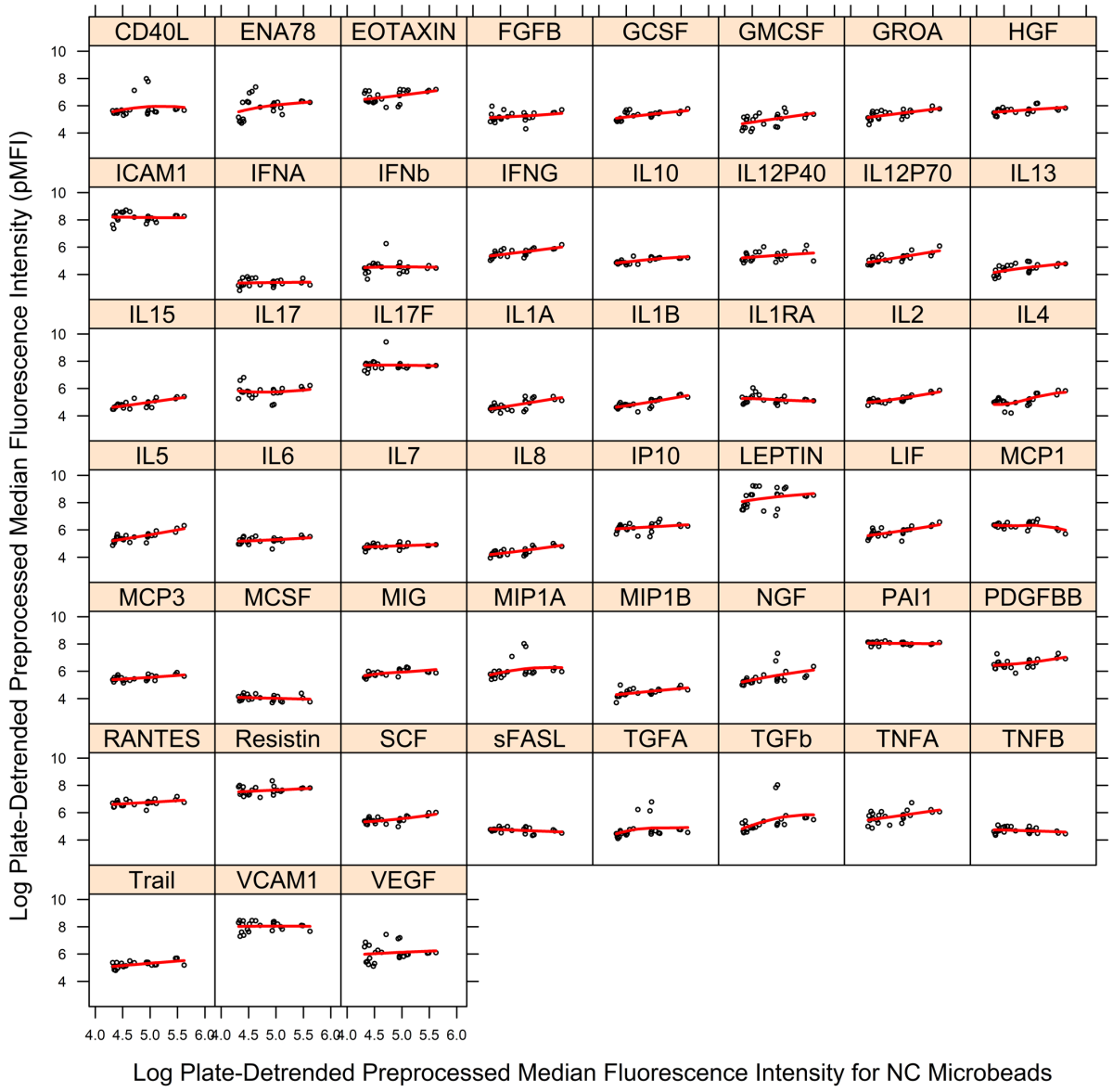



**Figure S3. Fit of regression model to pMFI data by cytokine for removal of nonspecific binding artifact, for lot experiment.** Vertical axis is pMFI for cytokine and horizontal axis is pMFI for NC microbeads (nonspecific binding). Black circles are observed pMFI data and red line is estimated local, error-in-variables regression curve. A separate figure is provided for each of the 51 cytokines. The *vertical* departures of observed pMFI values (black) from the regression curve (red) are estimates of pMFI corrected for nonspecific binding (dpMFI), where dpMFI is shown in Figure S4. Figure was prepared in R package `lattice` (18).

**Figure S4.**

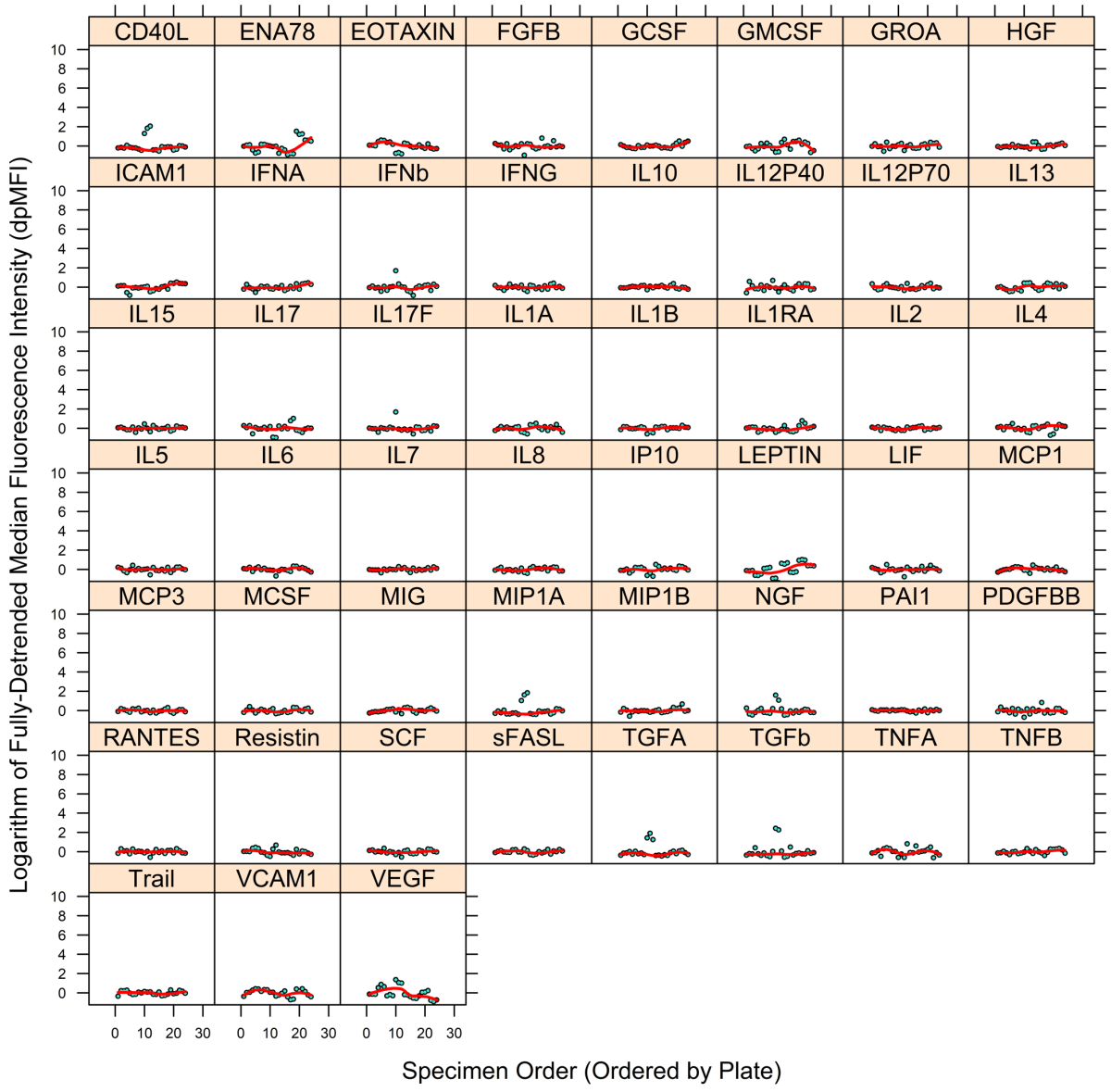



**Figure S4. Final output dataset for lot experiment.** Vertical axis is MFI after removal of lot and nonspecific-binding artifacts (dpMFI) for each cytokine and horizontal axis is the ordering of specimens (across lots). A separate figure is provided for each of the 51 cytokines. Red line within each figure is a spline smooth of trend in mean. Compare to the data (pMFI) prior to removal of nonspecific binding artifact (Figure S2). Figure was prepared in R package `lattice` (18).

**Figure S5.**

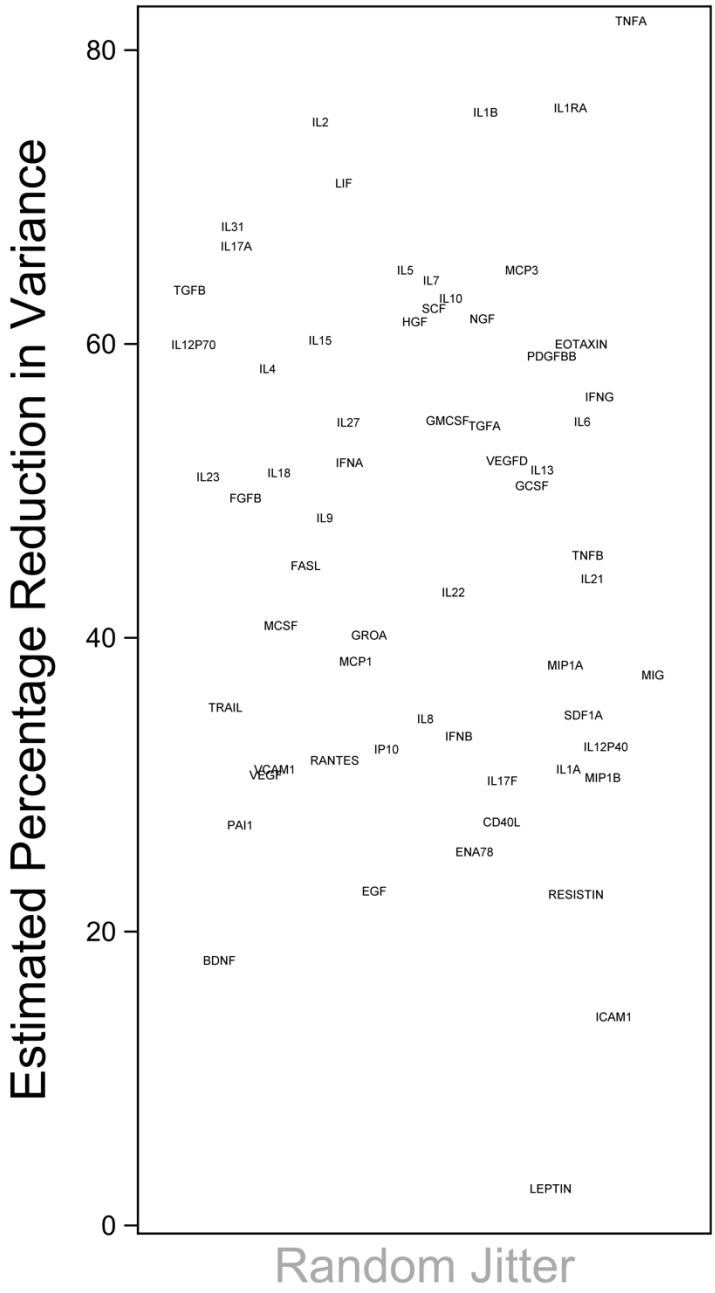
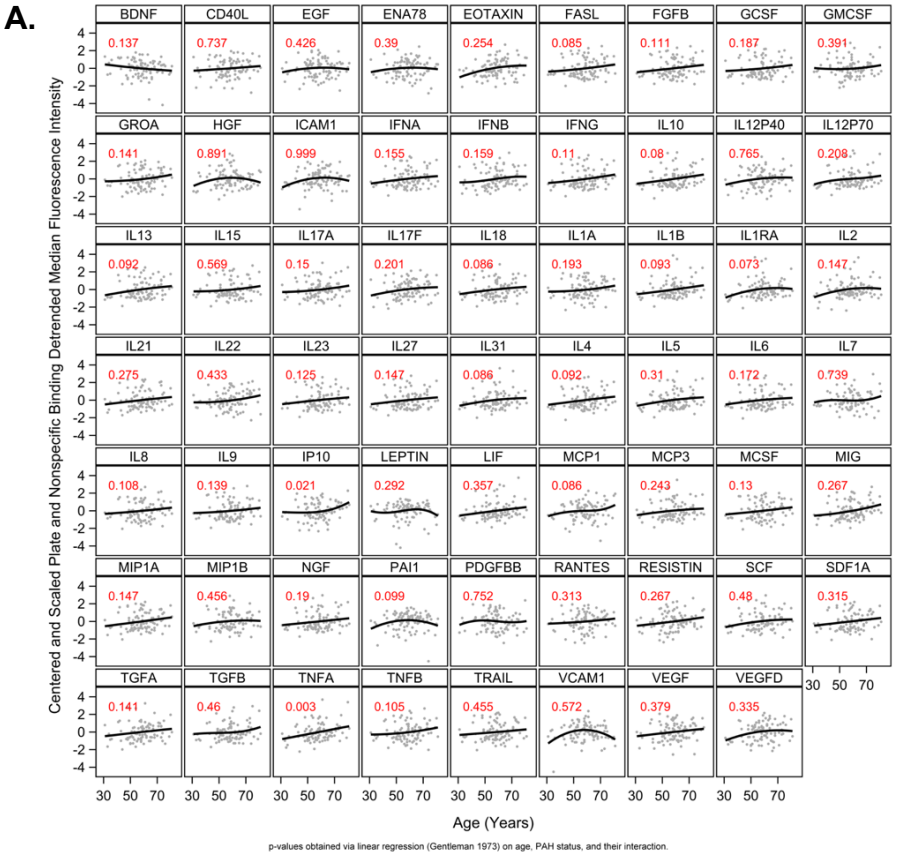



**Figure S5. Estimated percentage reduction in variance among specimens with correction for nonspecific binding, by cytokine, for PHAROS study.** Percentage variance reduction varies greatly among cytokines, due to differences in the relative proportion of signal contributed by nonspecific binding artifact. Figure was generated in SAS® ODS Graphics (SAS® Institute, Cary, North Carolina, USA).

# Figure S6.


A.
BDNF 0.137
CD40L 0.737
EGF 0.426
ENA78 0.39
EOTAXIN 0.254
FASL 0.085
FGFB 0.111
GCSF 0.187
GMCSF 0.391
GROA 0.141
HGF 0.891
ICAM1 0.999
IFNA 0.155
IFNB 0.159
IFNG 0.11
IL10 0.08
IL12P40 0.765
IL12P70 0.208
IL13 0.092
IL15 0.569
IL17A 0.15
IL17F 0.201
IL18 0.086
IL1A 0.193
IL1B 0.093
IL1RA 0.073
IL2 0.147
IL21 0.275
IL22 0.433
IL23 0.125
IL27 0.147
IL31 0.086
IL4 0.092
IL5 0.31
IL6 0.172
IL7 0.739
IL8 0.108
IL9 0.139
IP10 0.021
LEPTIN 0.292
LIF 0.357
MCP1 0.086
MCP3 0.243
MCSF 0.13
MIG 0.267
MIP1A 0.147
MIP1B 0.456
NGF 0.19
PAI1 0.099
PDGFBB 0.752
RANTES 0.313
RESISTIN 0.267
SCF 0.48
SDF1A 0.315
TGFA 0.141
TGFB 0.46
TNFA 0.003
TNFB 0.105
TRAIL 0.455
VCAM1 0.572
VEGF 0.379
VEGFD 0.335
Centered and Scaled Plate and Nonspecific Binding Detrended Median Fluorescence Intensity
Age (Years)
p-values obtained via linear regression (Gentleman 1973) on age, PAH status, and their interaction.

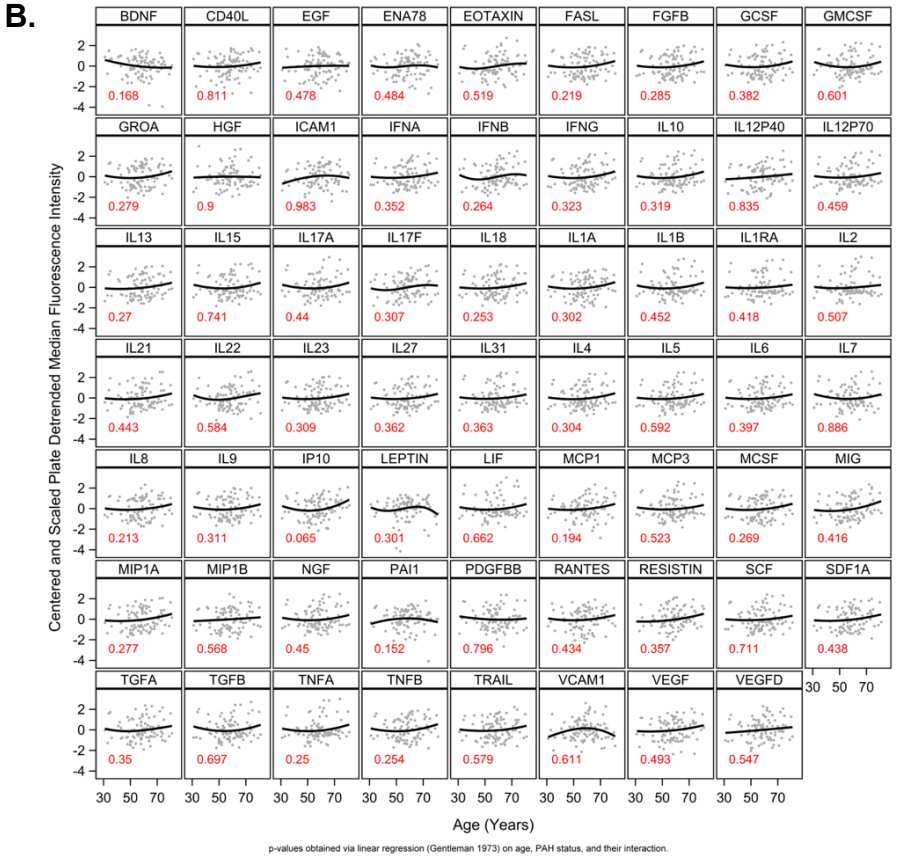

B.
BDNF 0.168
CD40L 0.811
EGF 0.478
ENA78 0.484
EOTAXIN 0.519
FASL 0.219
FGFB 0.285
GCSF 0.382
GMCSF 0.601
GROA 0.279
HGF 0.9
ICAM1 0.983
IFNA 0.352
IFNB 0.264
IFNG 0.323
IL10 0.319
IL12P40 0.835
IL12P70 0.459
IL13 0.27
IL15 0.741
IL17A 0.44
IL17F 0.307
IL18 0.253
IL1A 0.302
IL1B 0.452
IL1RA 0.418
IL2 0.507
IL21 0.443
IL22 0.584
IL23 0.309
IL27 0.362
IL31 0.363
IL4 0.304
IL5 0.592
IL6 0.397
IL7 0.886
IL8 0.213
IL9 0.311
IP10 0.065
LEPTIN 0.301
LIF 0.662
MCP1 0.194
MCP3 0.523
MCSF 0.269
MIG 0.416
MIP1A 0.277
MIP1B 0.568
NGF 0.45
PAI1 0.152
PDGFBB 0.796
RANTES 0.434
RESISTIN 0.357
SCF 0.711
SDF1A 0.438
TGFA 0.35
TGFB 0.697
TNFA 0.25
TNFB 0.254
TRAIL 0.579
VCAM1 0.611
VEGF 0.493
VEGFD 0.547
Centered and Scaled Plate Detrended Median Fluorescence Intensity
Age (Years)
p-values obtained via linear regression (Gentleman 1973) on age, PAH status, and their interaction.

**Figure S6. Association between age and cytokine median fluorescence intensity with removal of plate artifact only versus removal of plate + nonspecific binding artifacts, for PHAROS study.** Panel A shows associations between age and cytokine MFI after plate artifact correction, while panel B shows associations between age and cytokine MFI after plate and nonspecific binding corrections. No cytokines show statistically significant associations with age after plate artifact correction alone; whereas two cytokines (IP-10 and TNFA) show statistically significant associations with age after plate + nonspecific binding corrections. Grey dots are observed data. Black line is a penalized b-spline smooth curve fit to these observed data. Red numbers are p-values from linear regression of variable on vertical axis on age, PAH status (yes/no), and their interaction, using the `orthoreg` procedure in SAS®/STAT (SAS® Institute, Cary, North Carolina, USA) applying the method of Gentleman, W.M. (1973). Least Squares Computations by Givens Transformations without Square Roots. Journal of the Institute of Mathematics and Its Applications 12:329-336. Figure, including smooth curves, was generated in SAS® ODS Graphics (SAS® Institute, Cary, North Carolina, USA).