Supplementary Information for "Normalization and Statistical Analysis of Multiplexed Bead-Based Immunoassay Data Using Mixed-Effects Modeling"

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Glossary of Statistical Terms

Effect
The deviation from an overall mean caused by the level of a factor. In the case of interaction terms, the deviation is from a lower-level interaction or a main effect.

Experimental Unit
The basic entity being studied in the experiment. In our case, the experimental unit is the group of HepG2 cells contained within a single well of the 24-well tissue culture plate.

Factors
Variables that contribute variance to the experiment. Factors are classified as categorical, which feature levels that are discrete entities, e.g., the analytes, or continuous, in which the levels are values sampled from a continuous range, e.g., the concentration of a ligand.

Fixed-effect term
A term that represents a factor whose levels are of specific interest and the inferences about the factor are restricted to those levels. Each fixed effect is estimated by a unique parameter, the best linear unbiased estimator (BLUE). For categorical factors, each level is associated with a model term and is therefore associated with a parameter. For continuous factors, the terms describing the function (e.g., line, polynomial) each have a parameter.

Levels
The specific categories or values of the factors applied to the experimental units.

Model terms
The predictor variables and their associated parameters.

Observation
A unique measurement or data point in the dataset.

Random-effect terms
A term that represents a factor whose levels can be considered a random sample from a large population. The inferences made apply to the population such that a single parameter, the variance, is estimated for each factor irrespective of the number of levels. Nevertheless, the effects for each level are "predicted" by the best linear unbiased predictor (BLUP), which is used in the normalization calculations. We note a potential source of confusion in our accounting of the number of random-effect "terms" in the model (see

Methods, subsection on “Statistical modeling” and Results, subsection on “Model building and performance”). We consider the number of random-effect terms equal to the number of random *factors* because this corresponds to the number of parameters estimated; the model, however, features a term for each *level* of a random factor. For this reason, we list the number of levels associated with each random factor in the main text.

Supplementary Figure Legends

Supplementary Figure 1. Assessment of the random effect distributions. A and C. Histograms of the distributions of the Well effects (A) and Day× Kit interaction effects (C). B and D. Normal probability plots of the Well effects (B) and Day × Kit interaction effects (D). Both effects approximately conformed to Gaussian distribution. (Similar plots for the Day, Day × IL-1 and Day × TGF-α random effects were not done because each effect featured too few levels to be usefully plotted.)

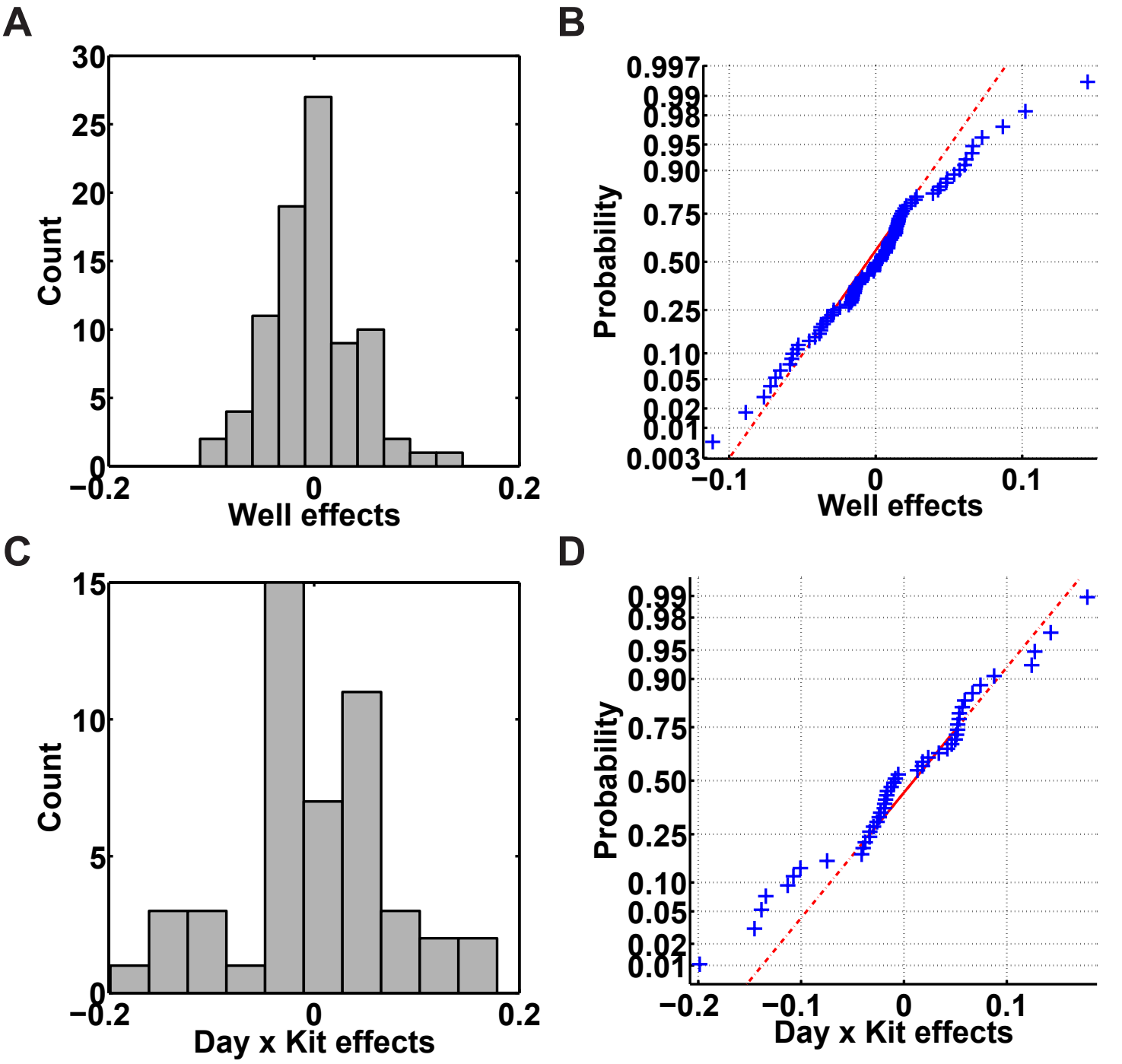
Supplementary Figure 2. Dexamethasone, IL-6 and IL-1α synergized in promoting acute phase protein secretion. The accumulation in the cell culture media of four acute phase proteins, C-reactive protein (CRP), haptoglobin, fibrinogen and serum amyloid A (SAA), was measured by Bio-Plex assay in response to treating HepG2 cells with varying concentrations of IL-6 and IL-1α (5, 10, and 200 ng/mL, proportionally represented in the figure by the density of grey) in the presence or absence of pretreatment with dexamethasone (1 μM).

Supplementary Figure 3. Additional plots associated with constrained fuzzy logic modeling of phospho-protein data. A. The prior knowledge network (PKN) employed in the cFL algorithm. The prior knowledge network features the edges to be tested for removal. We based our PKN on one from a previous study in which we fit a model to data from a similar experimental system (1). In this case, we constructed a simpler PKN compared to the one in reference 1 because we were specifically interested in determining whether pathway activity was consistent with our data. To address this question, we inserted edges between dexamethasone and each of the measured phospho-proteins to address whether dexamethasone influenced their levels. Green nodes represent the ligands used to treat the cells, blue nodes represent measured phospho-proteins and white nodes represent molecules that were neither measured nor perturbed but whose inclusion in the network is necessary for logical consistency. B and C. CFL fits of the raw data (B) and normalized back-transformed data (C). For both panels, the measured phospho-proteins are listed on the left and the experimental conditions listed at the top. Within each panel, the experimental data is indicated by a black line. The first point of each black line was zero (indicating no relative signal prior to stimulation) and the second point was the relative fold change of the average MFI of the phospho-proteins in response to stimulation relative to the average vehicle- or DMSO-treated conditions (the DMSO condition was used as the basis of comparison for any treatment that included dexamethasone). The average cFL model fit was indicated by a blue dashed line, with pink lines indicating individual model fits. Panels colored green indicate a good fit between the model and data whereas those colored white

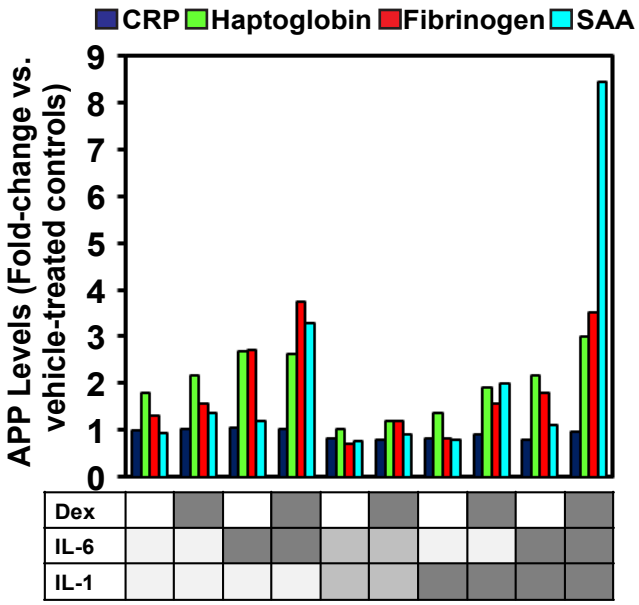
and pink indicate discrepancy between the model and data, with the degree of discrepancy proportional to the shade of pink.

References

1. Morris, M. K., Saez-Rodriguez, J., Clarke, D. C., Sorger, P. K., and Lauffenburger, D. A. (2011) Training signaling pathway maps to biochemical data with constrained fuzzy logic: quantitative analysis of liver cell responses to inflammatory stimuli. *PLoS Comput Biol* 7, e1001099.

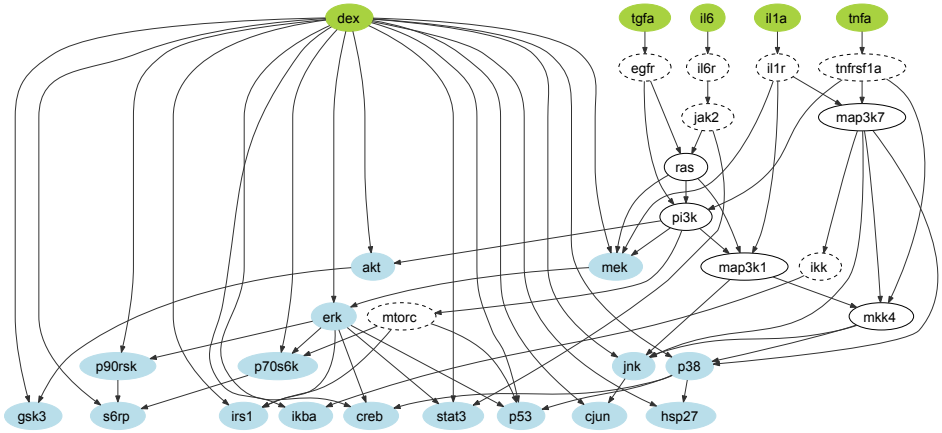


Supplementary Figure 1



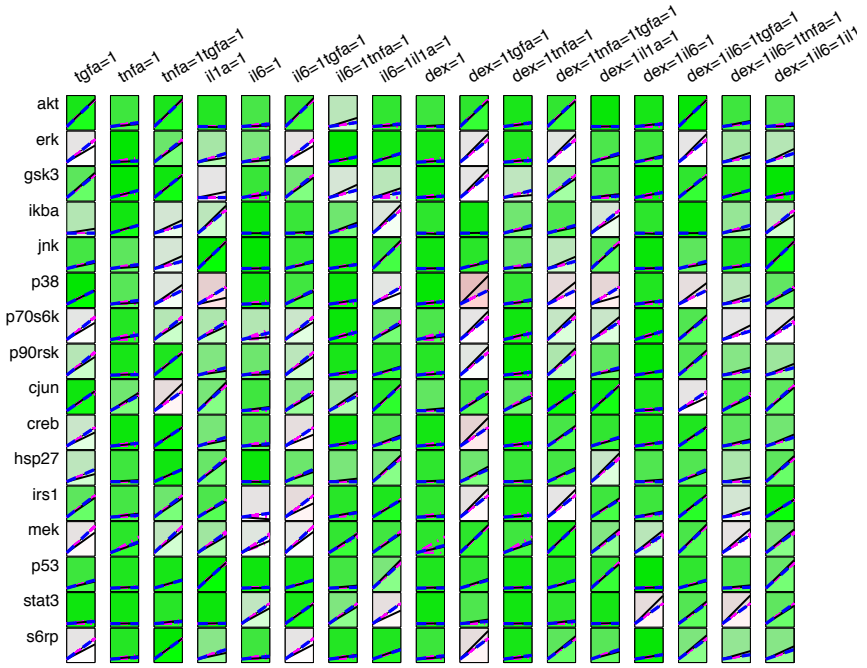
Supplementary Figure 2

A



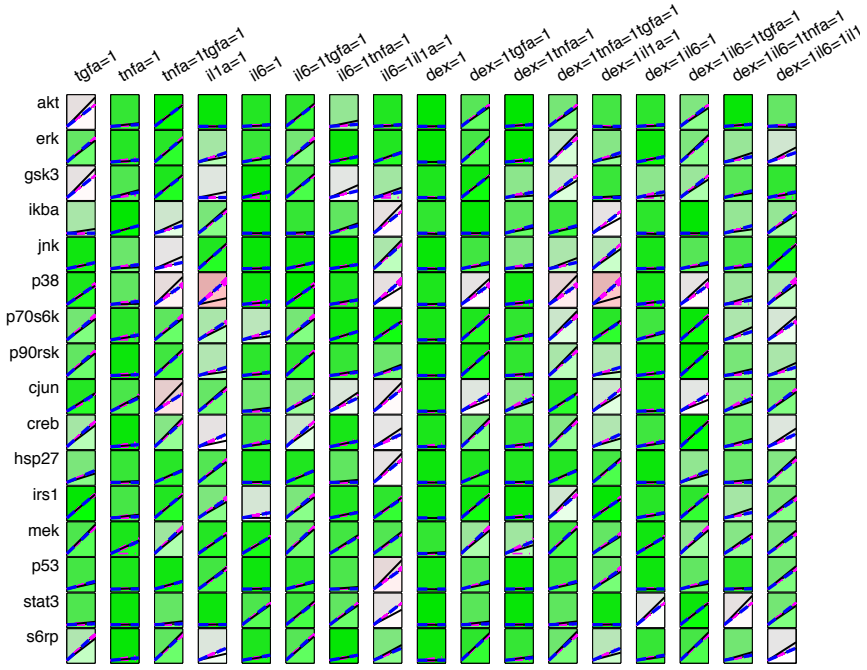
Prior knowledge network

B



Data and fits for raw data

C



Data and fits for normalized back-transformed data