Supplementary Methods 1

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1. Methods of the Verification Experiment

Human endotoxin model. Six healthy male and female subjects between 18 and 40 years of age (1 female, 5 males) provided written informed consent. Subjects were intravenously administered the same dose of endotoxin. Arterial blood samples were collected before endotoxin infusion (0 hours) and at post infusion times of 2, and 6 hours. Same protocols were used in endotoxin administration, blood sampling, and leukocyte RNA isolation, as summarized in the Methods of the manuscript.

cRNA synthesis and Chip hybridization. cRNA synthesis was performed with 4 μg of total cellular RNA, and hybridized onto the Human Genome U133 Plus 2.0 Array (Affymetrix) and processed based on an updated protocol[1] outlined by Affymetrix Inc. (Santa Clara, CA).

Microarray data analysis. 54,675 probe sets on the U133 Plus 2.0 Array were analyzed, including complete coverage of the HU133A and HU133B set plus 6,500 additional genes. Because of the platform and protocol differences between the U133 Plus 2.0 Array and the U133 (A, B) Set, it is not feasible to directly compare the signal level of a gene in the verification experiment (U133 Plus 2.0) with that in the initial study (U133 Set)[2]. Besides, the initial time course study was conducted at 6 time points (0, 2, 4, 6, 9 and 24 hours), among which 3 time points (0, 2, and 6 hours) were selected in the verification study. Therefore data of the verification experiment were analyzed separately. Normalization was performed as described in the Methods of the manuscript. Probe sets significantly perturbed at 2 hours or 6 hours after LPS administration were

identified using SAM (two class), with an estimated false discovery rate < 1% based on 1,000 permutations of the dataset.

References

1. GeneChip® Expression Analysis Technical Manual, Rev.5, Affymetrix Inc. (2004)
2. GeneChip® Expression Platform: Comparison, Evolution, and Performance, Affymetrix Inc. (2004) (http://www.affymetrix.com/support/technical/technotes/expression_comparison_technote.pdf)

2. Supplementary Results

Verification of the significant probe sets in the time course study. 5093 probe sets significantly perturbed after LPS administration were identified in the initial time course study. The performance of these probe sets were further investigated at post LPS infusion times of 2, and 6 hours. 4504 probe sets (~88%) were also identified as significant at either 2 or 6 hours with the same directions of change (higher or lower than baseline level), 577 probe sets were not significant, and 12 probe sets revealed changes that might be in opposite directions (significant change in the verification experiment and >1.25 fold change in opposite direction at 2 hours or 6 hours in the time course study). Expression data of the 5093 probe sets and the comparisons are available at the supplementary website at http://www.gluegrant.org/.

The 4504 confirmed probe sets were mapped to 3364 Entrez GeneIDs (account for ~90% of the significant genes identified in the initial study). See Section 5 of Supplementary Information S2, on examining online these genes in the network results presented in the manuscript. The genes list is available at the supplementary website.

Figure 2. Pathway analysis of representative 292 genes involved in innate immunity. 143 of the 147 perturbed genes are confirmed. 4 genes not yet confirmed are COL4A1, IKBKB, MAP2K2 and NCOA4.

Figure 3. Network representation of the complex biological process underlining the temporal response of blood leukocytes to in vivo endotoxin administration. 1104 of the 1214 significant genes are confirmed in the global network representation of the

inflammatory response to endotoxin. See Section 5 of Supplementary Information S2, on examining online these genes in the context of network results (Figure 4a).

Among genes discussed in the manuscript (shown in the 9 sub-network regions Figure 4b), 5 genes are not yet confirmed, NDUFS1, NDUFV2, DLAT, COPS5 and PSMC4.