SUPPLEMENTAL MATERIAL

Xiao et al., http://www.jem.org/cgi/content/full/jem.20111354/DC1

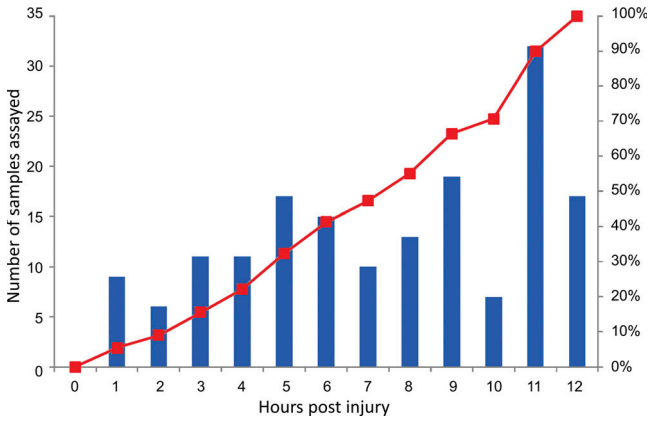

Figure S1. Distribution of 167 samples assayed within the first 12 h of injury. The bar graph shows the number of samples collected within each hour after injury, and the line graph represents the cumulative frequency distribution. On average, 14 samples were assayed each hour between 1 and 12 h after injury.

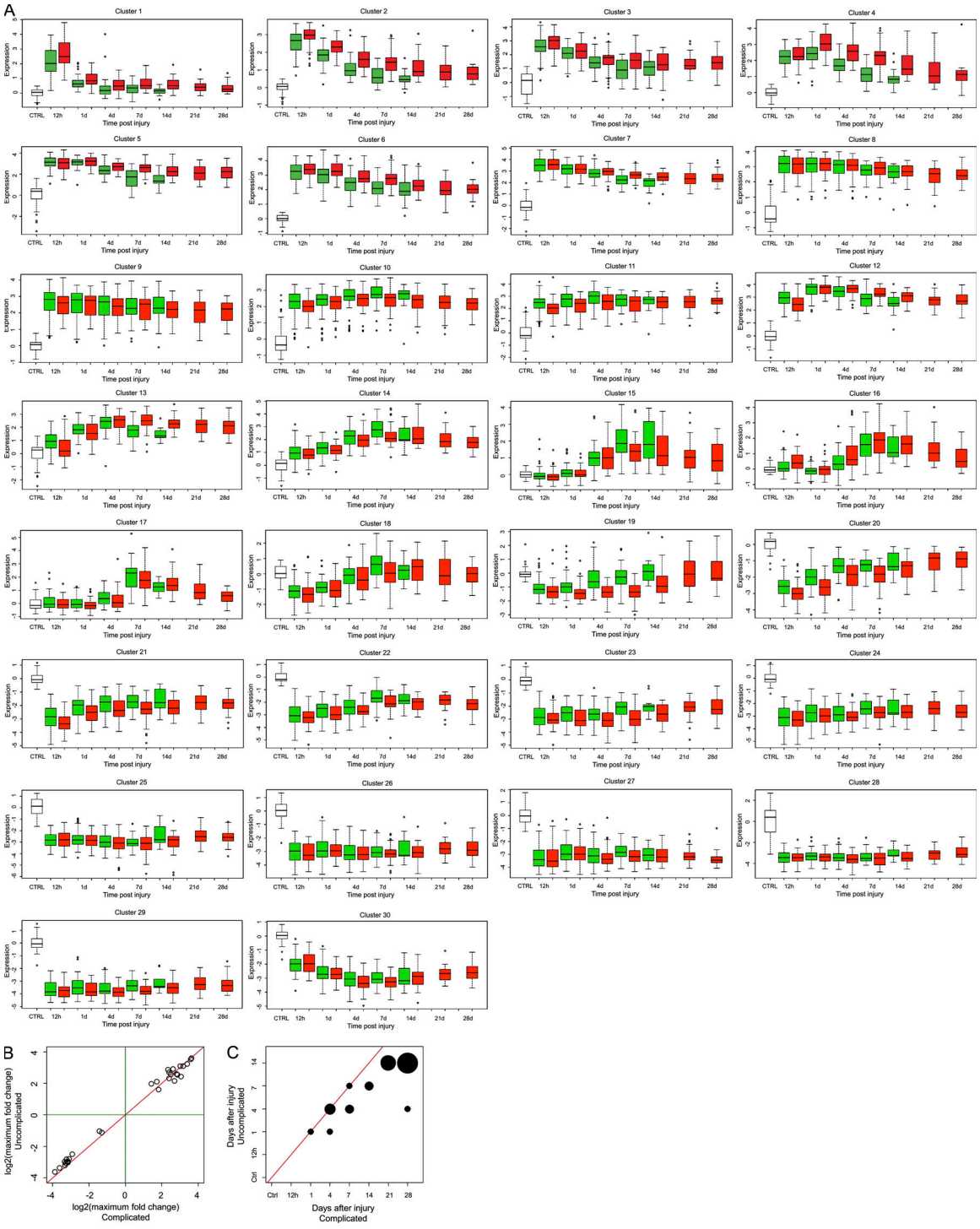

Figure S2. Comparison of gene expression patterns of the 5,136 genes with a more than twofold change from control subjects for patients with a complicated (n = 55) and uncomplicated (n = 41) clinical recovery. (A) Expression over time from injury (12 h to 28 d after injury) of genes in each of the 30 clusters from k-mean clustering of the 5,136 genes in Fig. 1 A for the patients in the two clinical cohorts (green, uncomplicated; red, complicated). The expression patterns of each gene between the outcome groups can be further examined online (Massachusetts General Hospital, 2011). (B) Scatter plot showing the direction and magnitude of the peak perturbation of each of the 30 clusters between the complicated (x axis) and uncomplicated (y axis) recovery. Because the direction and magnitude of the response falls on a line of unity between complicated and uncomplicated outcomes, the transcriptional evidence indicates that the direction and magnitude of response are not different between the clinical outcomes. (C) Bubble chart of the resolution time (half-time of recovery) from peak perturbation for each of the 30 clusters between the complicated (x axis) and uncomplicated (y axis) recovery. At an x–y coordinate, the area of a circle is proportional to the number of clusters that have the specific pair of resolution time in the two cohorts. The dramatic shift to the right indicates that in complicated outcomes, the half-time to recovery is markedly prolonged. The primary differences are associations with resolution of the changes in gene expression.

Cytokine	p-value (Time)
IL6	**5.34E-22**
IL1RA	**1.84E-07**
IL8	**1.18E-05**
MCP1	**2.50E-05**
MIP1A	0.0367588
IL1B	0.0542563
IL6R	0.0666458
IL10	0.070006
GROA	0.118252
IL4	0.124059
MIP1B	0.17878
IL12P70	0.276304
TNFA	0.328675
IFNG	0.548104
IL1R2	0.636043
TNFR2	0.787875
TNFR1	0.809762

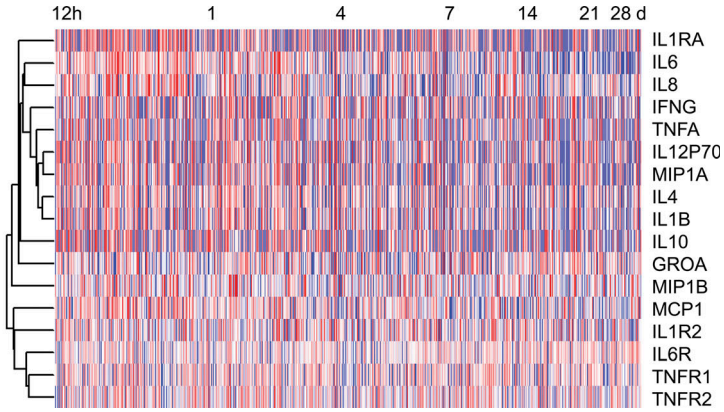

Figure S3. Plasma cytokine/chemokine levels after severe blunt injury. Concentrations of 17 cytokines in plasma were measured from study patients at 0, 1, 4, 7, 14, 21, and 28 d after injury. The heat map shows the normalized concentration values by hierarchical clustering (red, higher than the mean; blue, lower than the mean). The brackets to the left of the heat map indicate the relationship of the cytokines in the hierarchical clustering. Levels of IL-6, IL-1ra, IL-8, and MCP1 were significantly decreased over time after injury ($P < 0.001$).

A

	12h	1d	4d
ISS	0	0	0
Base Deficit	8	0	0
Transfusion	28	207	0

B

Blood Transfusion	12h	1d	4d
Up	12	50	0
Down	235	169	0
Total	247	219	0

C

FDR <0.001	Transfusion	
	12h	1d
2391 outcome genes	247 genes	219 genes
	94 (70 same direction)	115 (107 same direction)

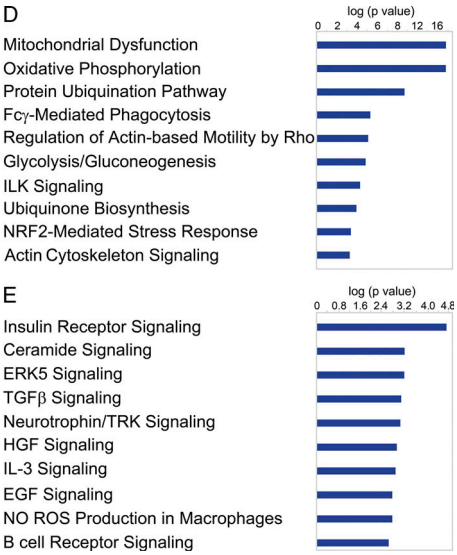

Figure S4. Effect of transfusion, ISS, and base deficit on early patterns of gene expression. (A) Univariate analyses (linear regression analyses) were performed to identify the number of genes whose expression varied with variations in the quantity of blood transfused, the magnitude of the injury severity (ISS), and the degree of physiological derangements (base deficit). Significance was set at an FDR <0.001. (B) Genes whose expression can be attributed to blood transfusion, based on a propensity score developed from the confounding variables using logistic regression. (C) Genes identified as being significant between the two outcomes and affected by transfusion, as determined by the propensity score. (D and E) The 10 canonical pathways most affected by genes whose expression is altered by transfusion (D, up-regulated; E, down-regulated) are identified. The data suggest that blood transfusion only minimally affects gene expression in the early injury response, and the patterns show only overlap with injury-affected genes, reflecting canonical pathways very different from those seen in the injury response.

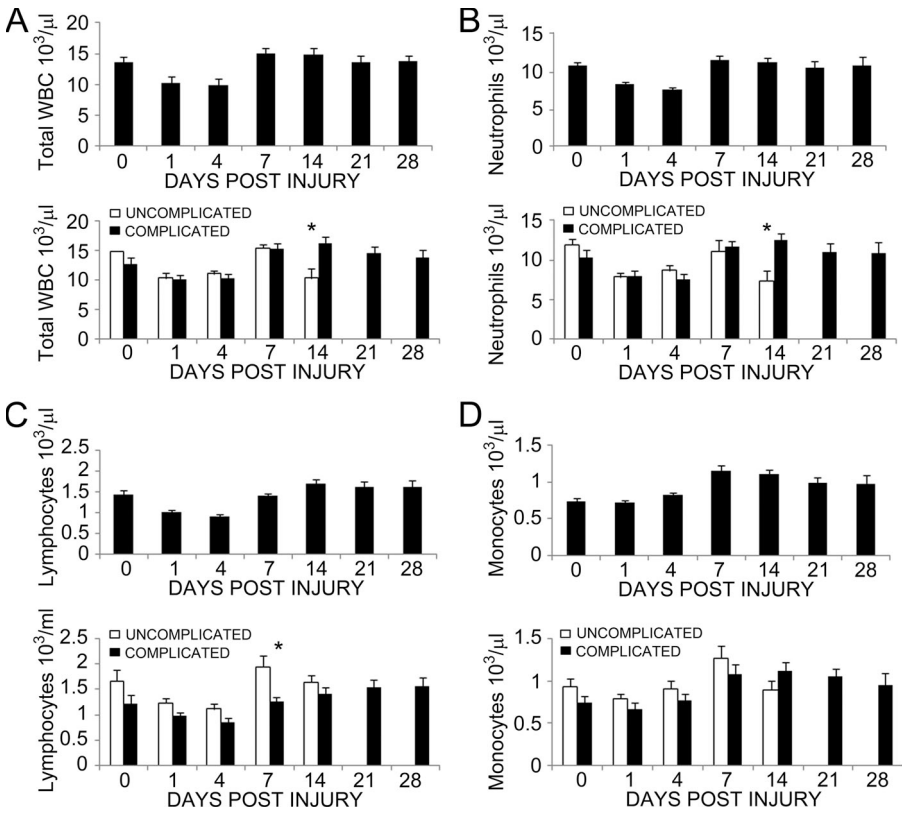

Figure S5. Blood leukocyte populations in the trauma patients. (A–D) Blood leukocyte populations were evaluated at each time point that genomic analyses were performed. Values are presented for each of the two cohorts at the specified time intervals. Data were analyzed by analysis of variance and Tukey's multiple range test. An asterisk (*) indicates that the concentrations of leukocyte populations at that time point differ between the two cohorts, at $P < 0.05$. (A) Total leukocytes (WBC). (B) Neutrophils. (C) Lymphocytes. (D) Monocytes. Error bars indicate the standard deviation in each group of patients at each time point.

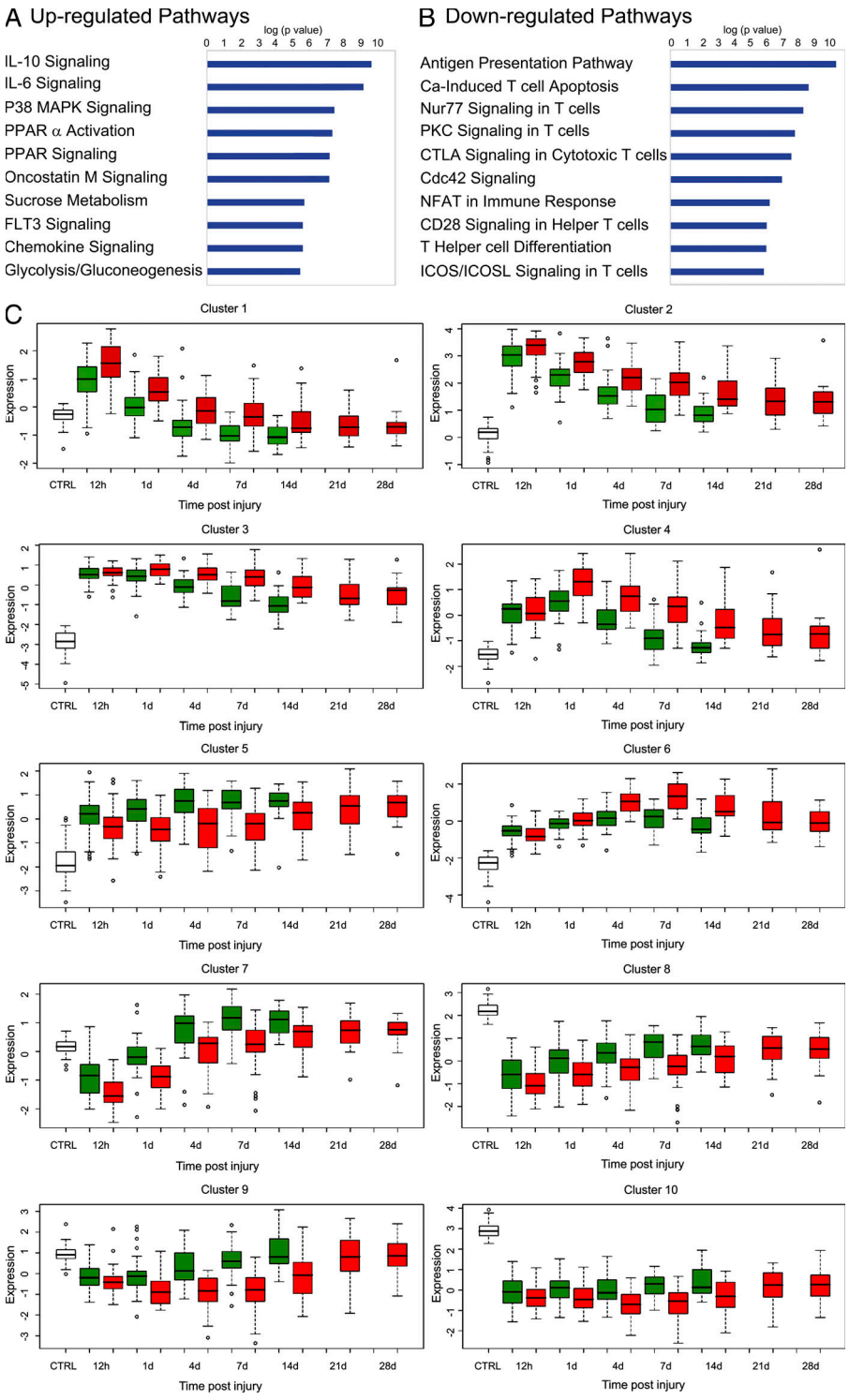

Figure S6. Differences in gene expression patterns between patients with a complicated and uncomplicated recovery. 1,201 genes with FDR <0.01 between outcomes and at least twofold changes over the time course were analyzed. (A and B) Summary of the canonical pathways. (C) Comparison of the box plots over time for each of the 10 clusters as shown in Fig. 3 for uncomplicated and complicated outcomes. Expression patterns of each gene between outcomes can be further examined online (Massachusetts General Hospital, 2011).

REFERENCE

Massachusetts General Hospital. 2011. The Human Genomic Response to Severe Traumatic Injury: An Interactive Website for Exploring Gene Expression and Clinical Outcomes. Available at: http://web.mgh.harvard.edu/TRT/.