

SUPPLEMENTARY MATERIAL

Inhaled Ozone (O₃)-Induces Changes in Serum Metabolomic and Liver Transcriptomic Profiles in Rats

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SI Figure 1: Heatmaps of liver gene expression for cytokine network in rats exposed to O₃ (A: immediately after 1-day 6-hr air or O₃, 0-hr time point; B. immediately after 2-day 6-hr air or O₃, 2-day, 0-hr) and C. 18-hr after 2-day 6-hr air or O₃; 2-day, 18-hr) exposure.

Figure 1A

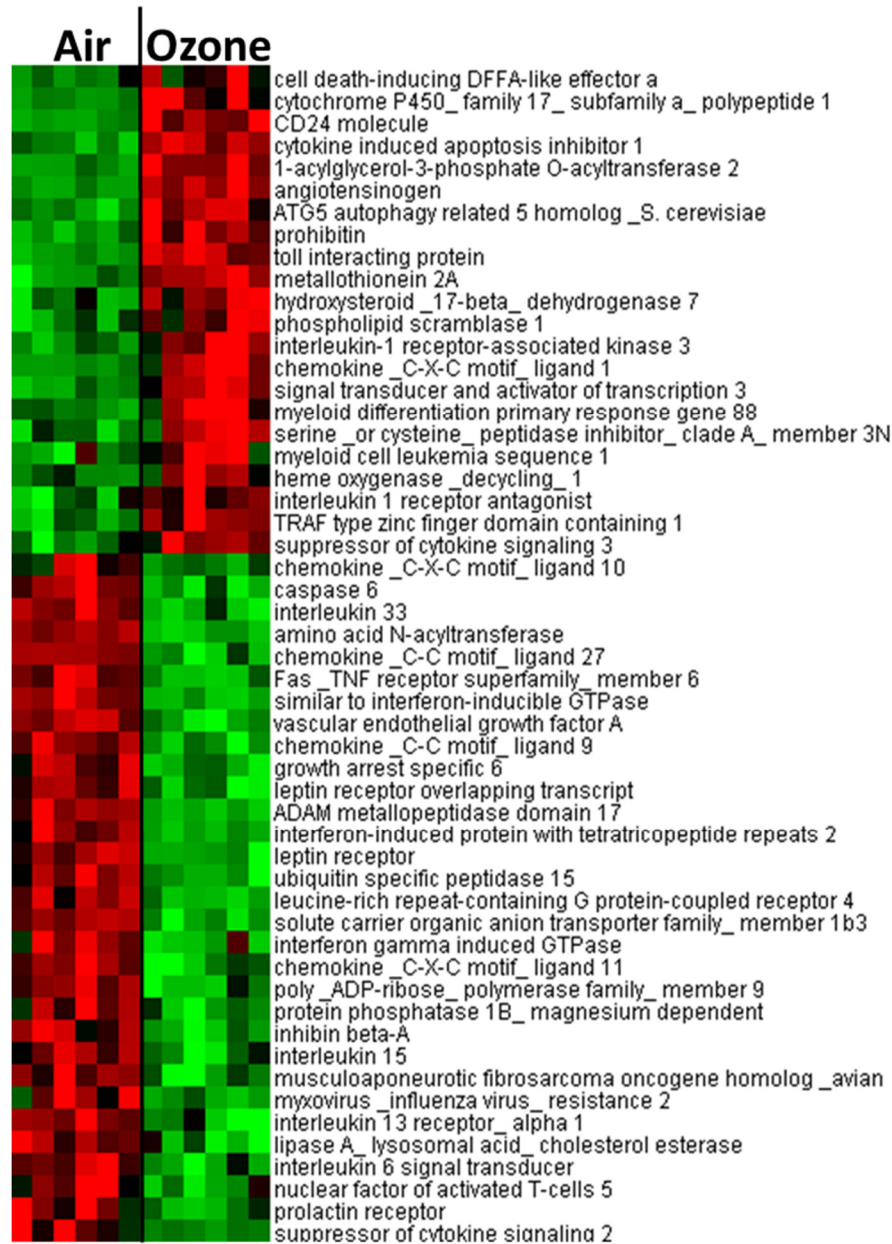


Figure 1B

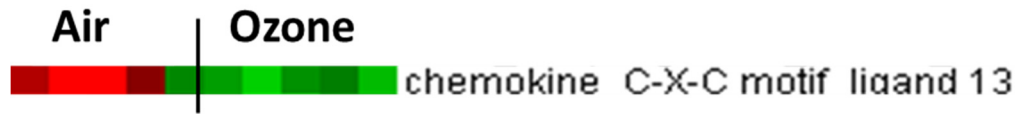
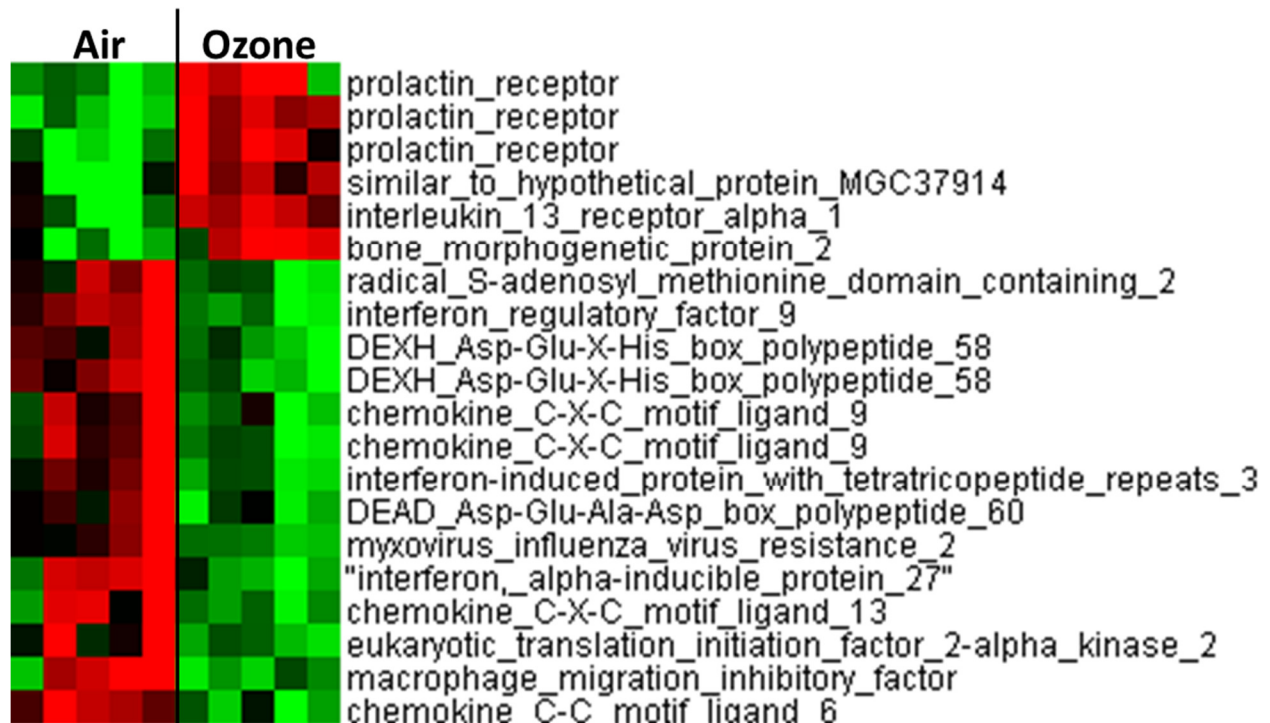


Figure 1C



Functional gene lists were generated by NetAffx queries at the Affymetrix website (www.affymetrix.com) and identified from differentially expressed genes (DEGs) list for each time point (air vs O₃) based on the query term, "cytokines". Genes were then median centered with average linkage, hierarchically clustered using Cluster 3.0 and displayed through Java Treeview. Heat map of DEGs with significant O₃ effect were identified separately at each time point. Red indicates genes that have high expression values across both groups, green indicates genes that have low expression values across both groups, and black indicates median expression.

SI Figure 2: Heatmaps of liver gene expression showing differentially expressed genes (air versus O₃) for apoptosis network in rats exposed to O₃ (A-D, exposed for 6hr/day; immediately after 1-day; E, immediately after 2-day, F, 18-hr after 2-day time point).

Figure 2A

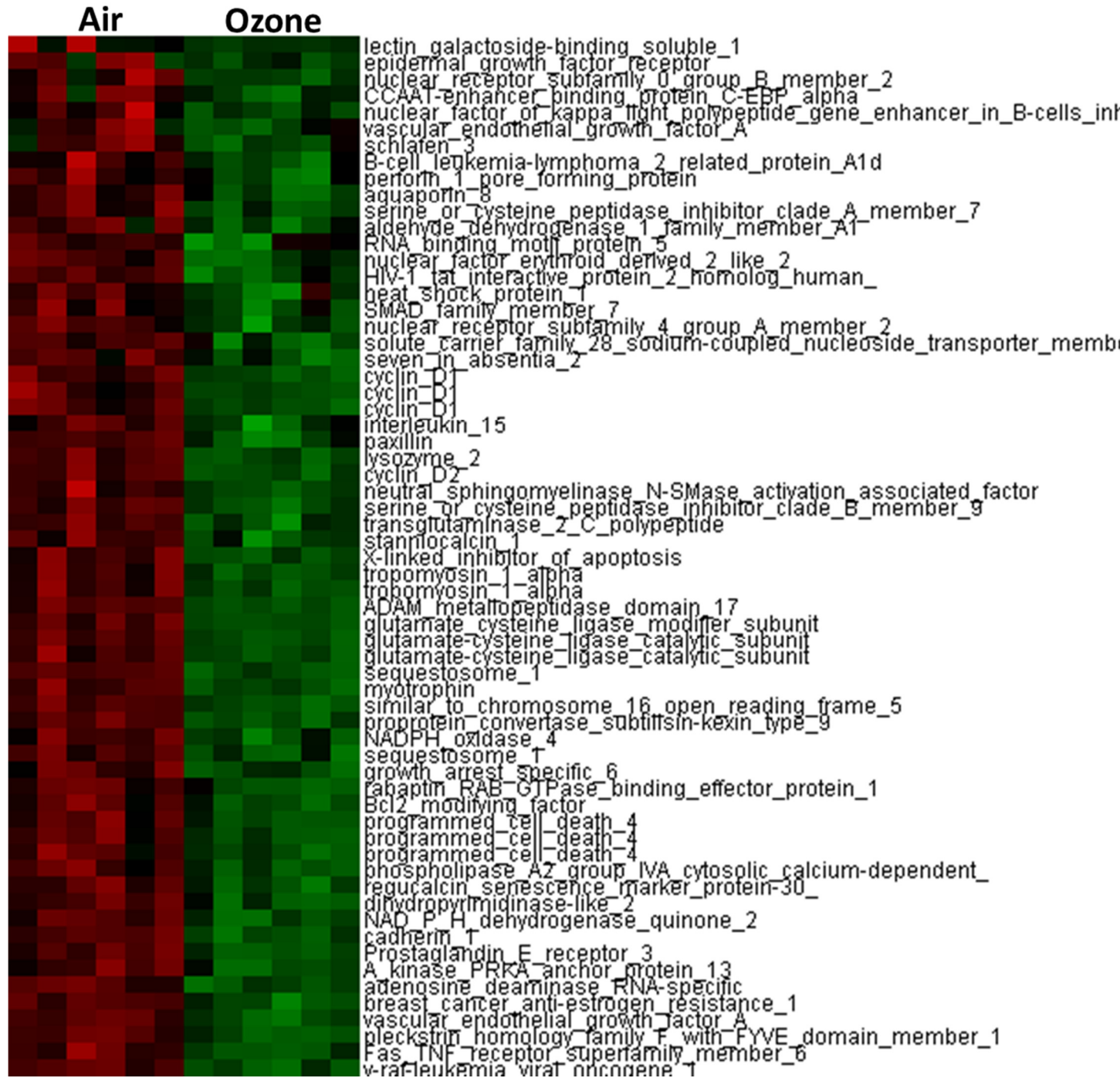


Figure 2B

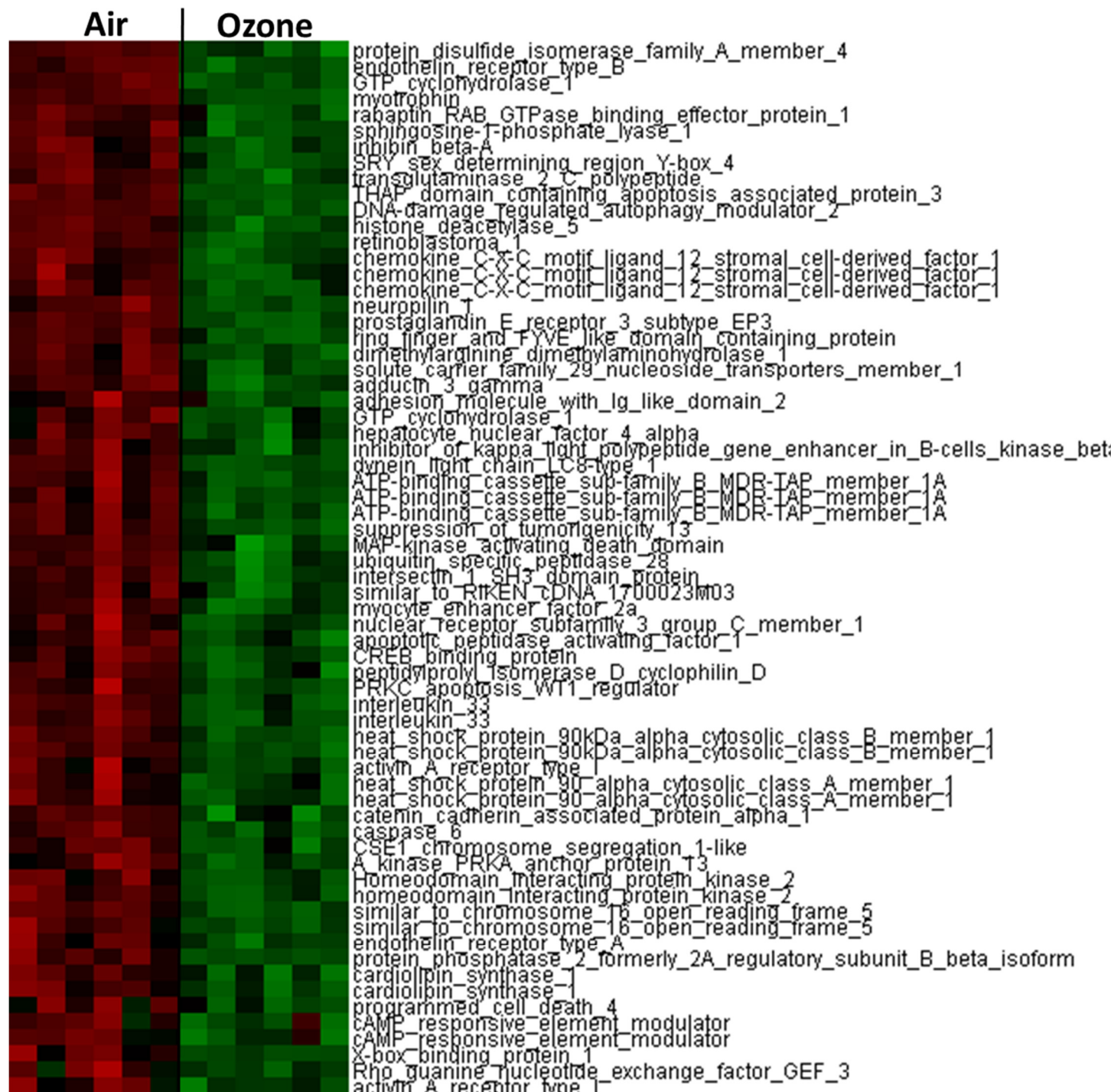


Figure 2C

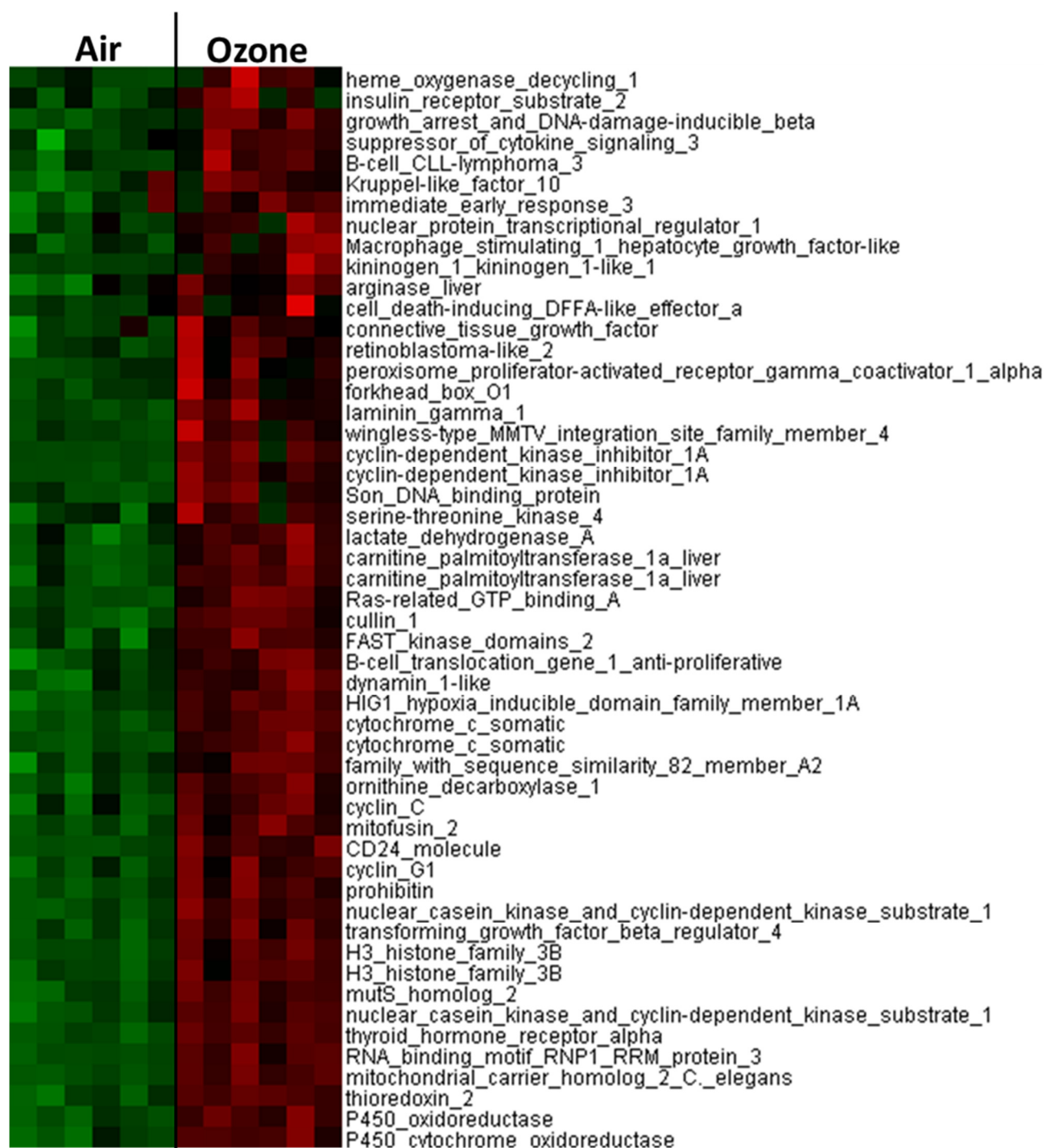


Figure 2D

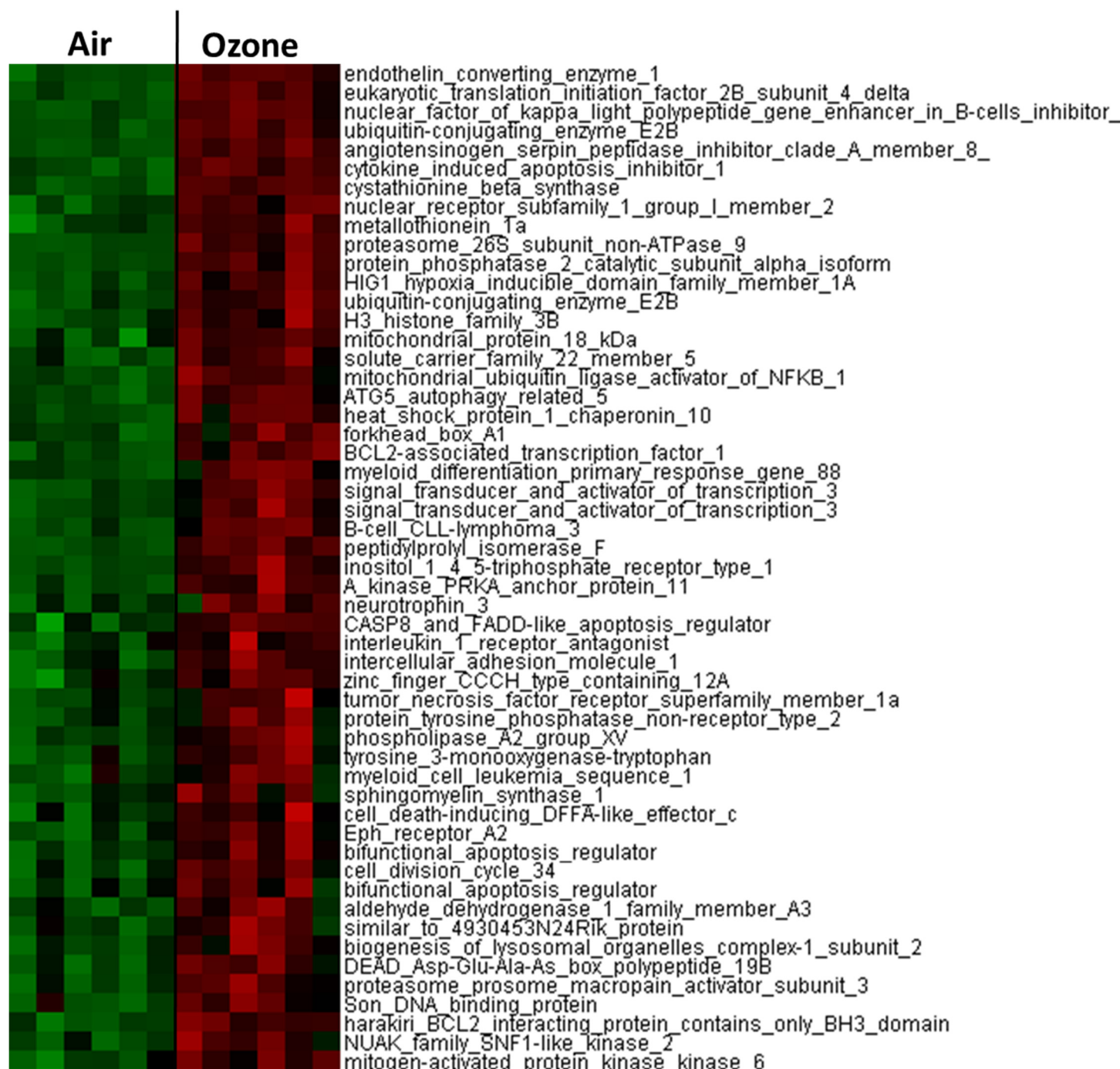


Figure 2E

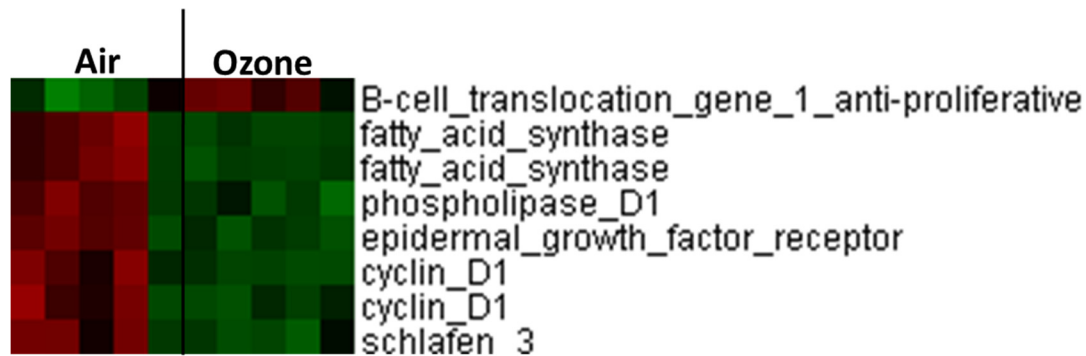
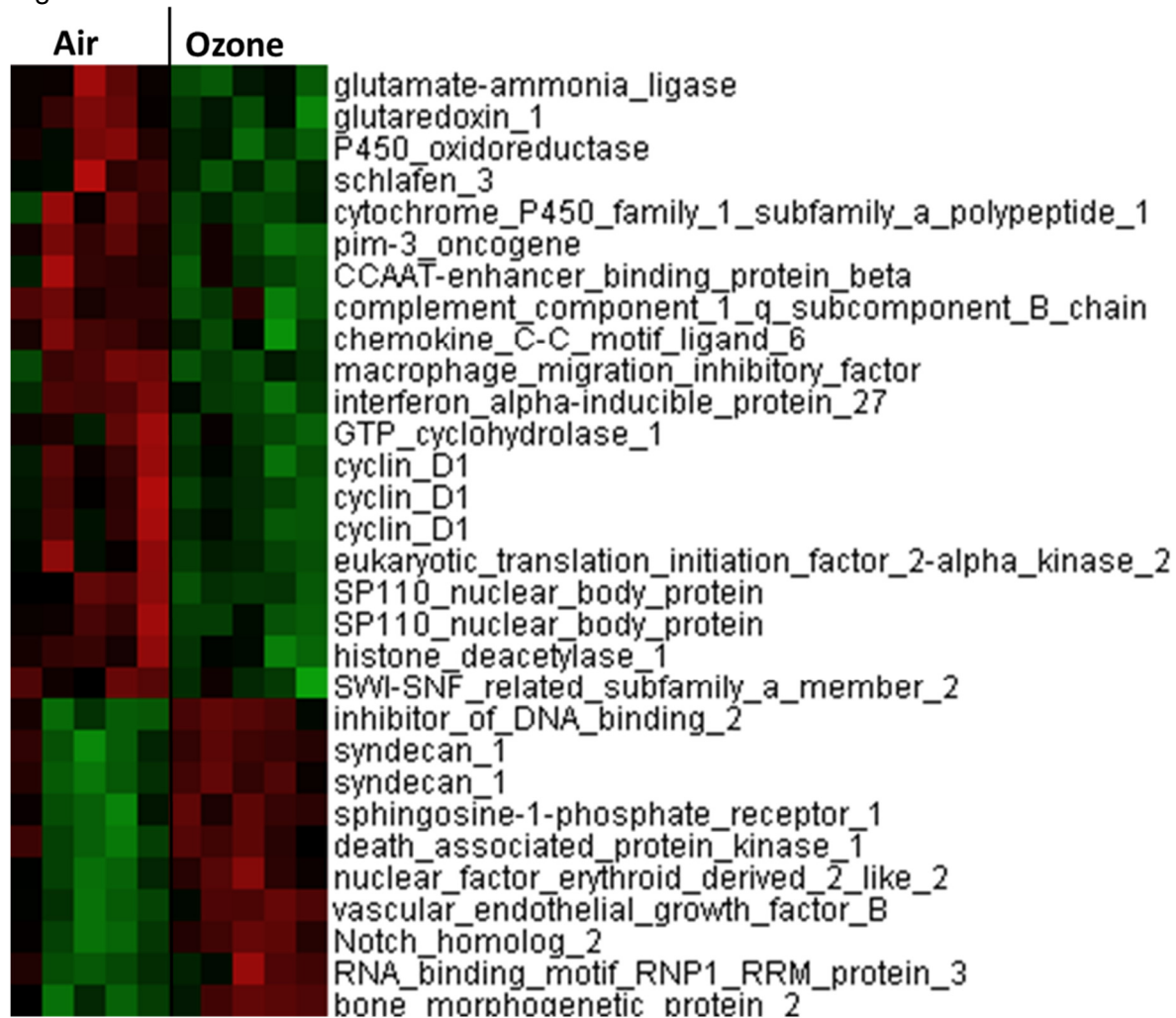
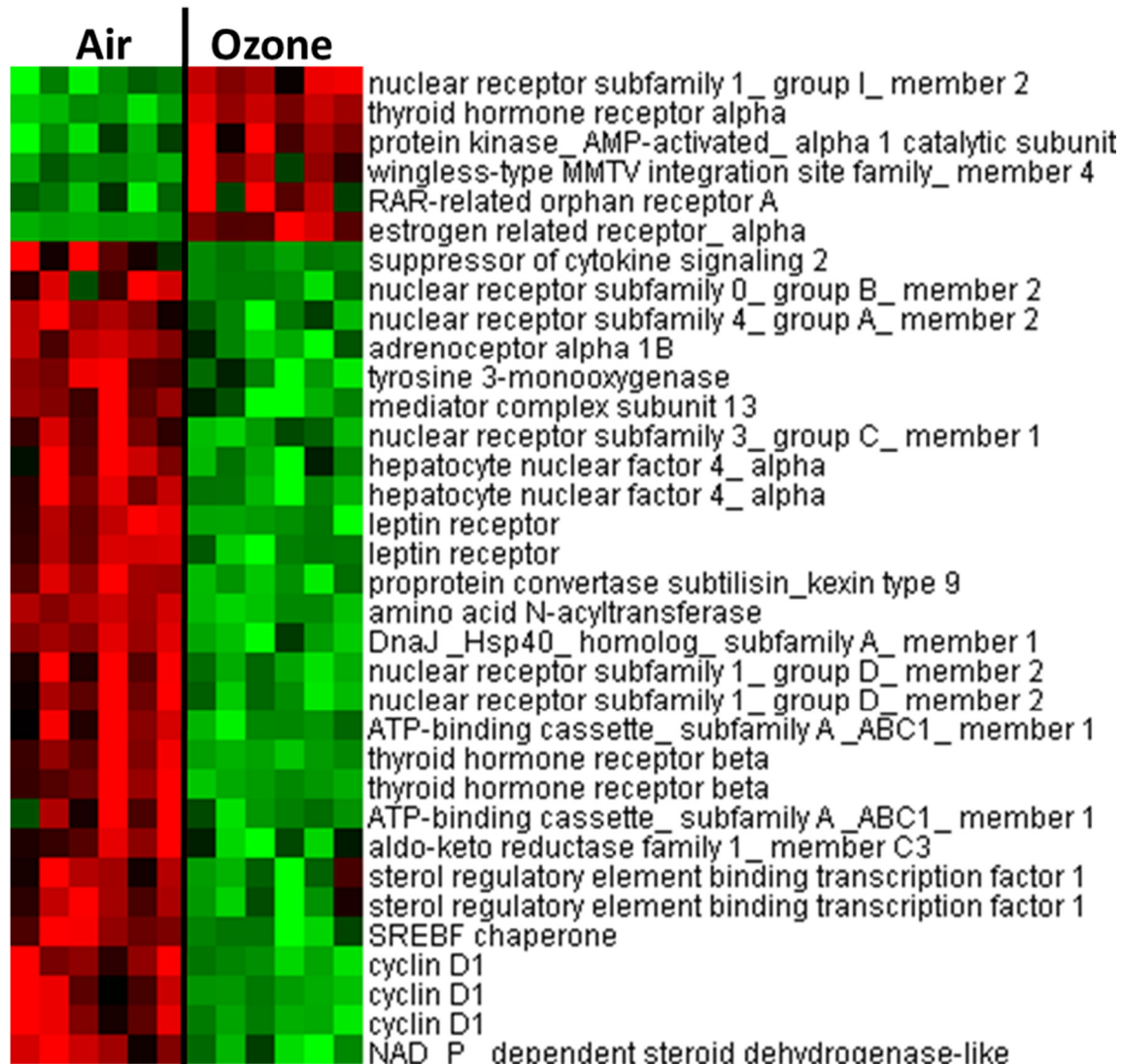


Figure 2F



Functional gene lists were generated by NetAffx queries at the Affymetrix website (www.affymetrix.com) and identified from differentially expressed genes (DEGs) list based on the query term, "apoptosis". Genes were then median centered with average linkage, hierarchically clustered using Cluster 3.0 and displayed through Java Treeview. Heat map of DEGs with significant O₃ effect were identified separately at each time point. Red indicates genes that have high expression values across both groups, green indicates genes that have low expression values across both groups, and black indicates median expression.

SI Figure 3: Heatmap of liver gene expression showing differentially expressed genes (air versus O₃) for Steroid, insulin and fatty acid receptor signaling network in rats exposed to O₃ for 6hr/day for 1-day (immediately after exposure).



Functional gene lists were generated by NetAffx queries at the Affymetrix website (www.affymetrix.com) and identified from differentially expressed genes (DEGs) list based on the query term, "steroid, insulin and fatty acid signaling". Genes were then median centered with average linkage, the normalized expression values for those probesets present on both lists were converted to z values and hierarchically clustered using Cluster 3.0 with average linkage and displayed with Treeview. Red indicates genes that have high expression values across both groups, green indicates genes that have low expression values across both groups, and black indicates median expression.

SI Figure 4. Ingenuity pathway analysis showing ozone exposure-induced changes in liver steroid receptor (A), insulin receptor (B) and fatty acid signaling (C).

Figure 4A

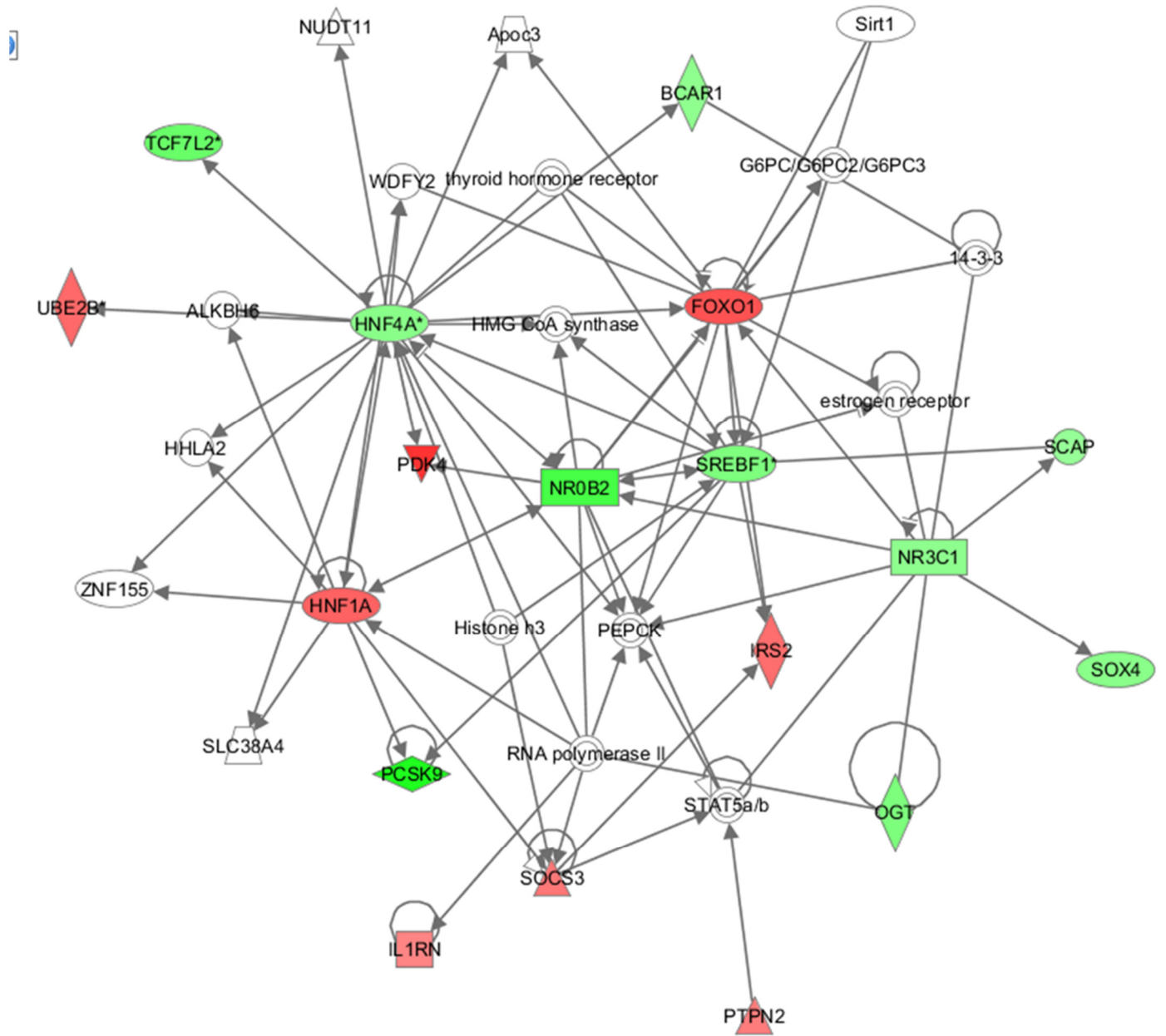


Figure 4B

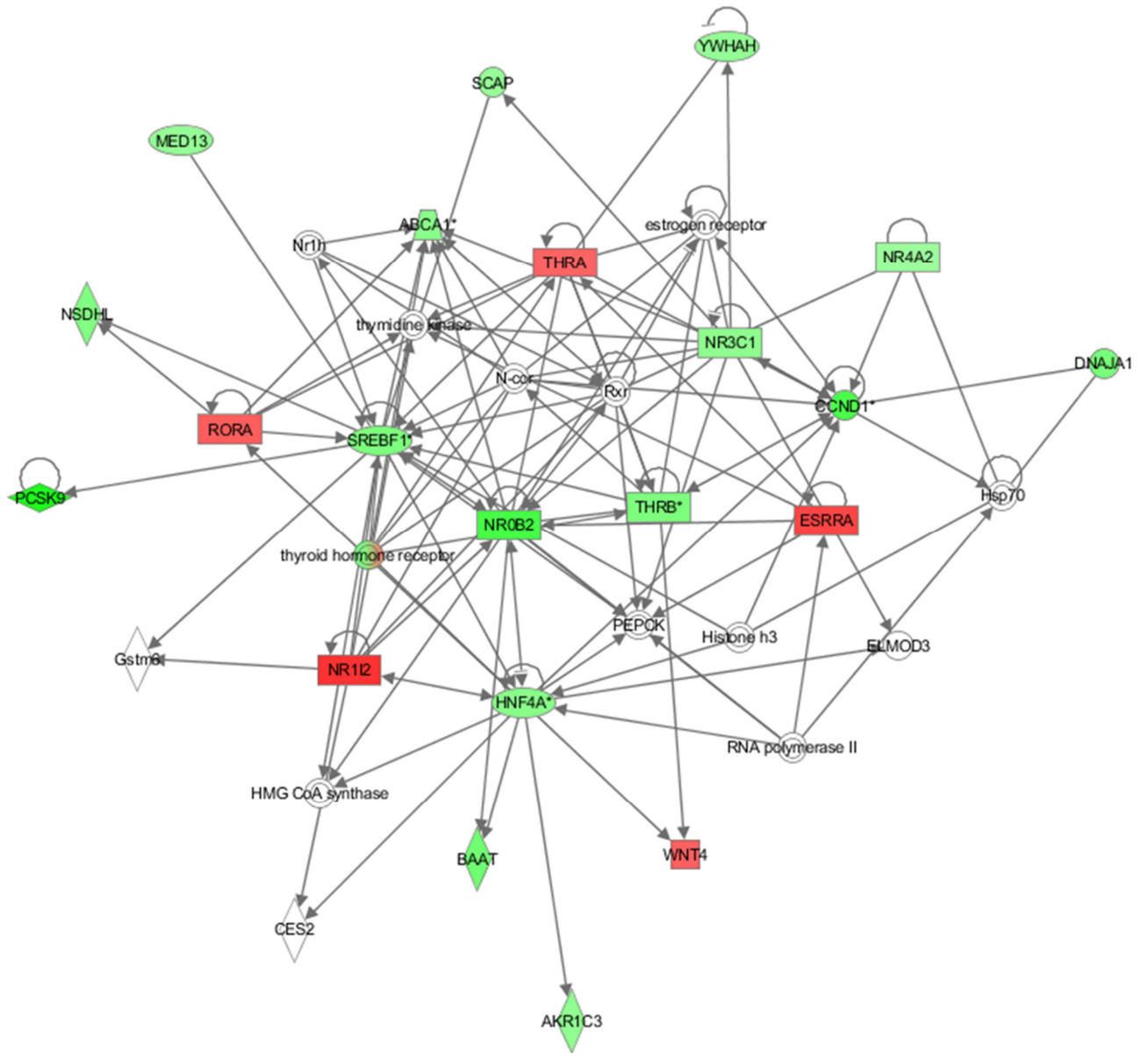
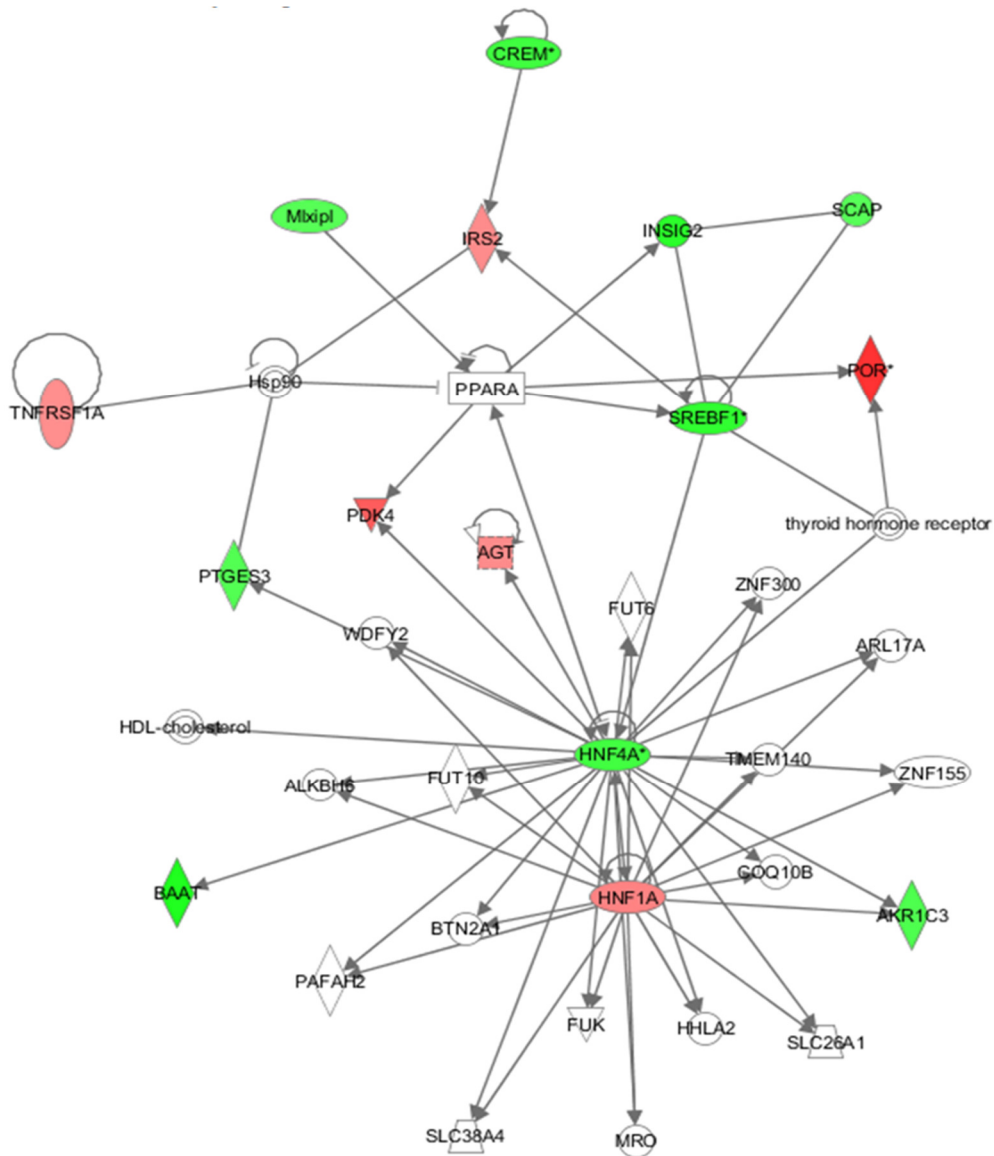


Figure 4C



Three functional probeset lists based on queries of “steroid receptor”, “insulin receptor” or “fatty acid” were obtained from NetAffx. Each of these lists was compared to the list of differentially expressed genes (DEGs) for the one day zero hour time point to identify DEGs in each of the three functional categories. Each functional list was processed separately by Ingenuity Pathway Analysis (IPA) to produce a direct relationship graph. The graph constructed from the Ingenuity knowledgebase depicts some of the biological relationships among the probesets on the list. The shape of a gene-labeled object indicates its molecular function and its color indicates whether it is upregulated (red) or downregulated (green). The features of the connecting lines convey the nature of the molecular interaction. For a detailed legend of these IPA graphs see the ingenuity.com website.

SI Table 1: Effect of acute ozone exposure on the expression of probe sets belonging to different biological processes in the liver.

Process	Number of probes on array	Number of DEGS from Master List	Per Cent Master List DEGs on Functional List
Amino acid biosynthesis	4650	507	11
Apoptosis	2345	259	11
Diabetes	743	117	16
Gluconeogenesis	80	21	26
Glycolysis	114	20	18
Mitochondria	671	111	17
Steroid Metabolism	416	77	19
Tricarboxylic	29	10	34
Unfolded protein	154	26	17

Significantly different probes for specific biological processes were identified from a master differentially expressed genes list (DEGs) for air versus O₃ exposure which included genes for all three time points (Immediately after 1-day 6-hr, immediately after 2-day, 6-hr and 18 hours after 2-day, 6-hr).