## CD40 and CD80/86 act synergistically to regulate inflammation and

## mortality in polymicrobial sepsis

Anna Nolan, MD, Michael Weiden MD Ann Kelly BS, Yoshihiko Hoshino MD, Satomi Hoshino BS, Nehal Mehta MD, Jeffrey A. Gold, MD

### **Online Data Supplement**

#### Figure Legends for Supplementary Figures

Supplementary Figure E1- CD40/80/86<sup>-/-</sup> mice have attenuated cytokine production after CLP. WT CD80/86<sup>-/-</sup> and CD40/80/86<sup>-/-</sup> mice underwent 19ga CLP and harvested 6hrs post CLP. Plasma and BALF collected and analyzed for IL-6 (panel A and B), plasma IL-12 (Panel C) and plasma IL-10 (Panel D) by commercially available ELISA. Panels E and F- Peritoneal lavage IL-6(E) and IL-10(F) 18hrs post CLP. 4-5 mice/group. Data analyzed by non-parametric ANOVA, p-values at bottom of each graph. Statistically significant group comparisons are presented with brackets.

Supplementary Figure E2- Monocyte costimulatory molecule expression returns to normal in septic individuals with improvement in severity of illness. Panel A. Representative Dot plot for CD40 on circulating monocytes in a healthy control and septic subject. Monocytes were initially identified by FSC/SSC characteristics and subsequently gated on CD14. Gates were determined by cells stained with Isotype mAB. Panels B-D 14 septic subjects had blood drawn for FACS analysis for CD40 (Panel B), CD80 (Panel C) or CD86 (Panel D) on Days 1,3-5, 7 and 14 or until hospital discharge. At each point severity of illness was assessed by APACHE II score. Data is presented with costimulatory molecules expression of left Y-axis and APACHE II on right. For CD40 and CD80 normalization in costimulatory molecule expression paralleled improvement in APACHE II while an inverse relation was observed for CD86.

# **Supplementary Figure E1**



D. Plasma IL-12-6hrs post CLP E. PL IL-6-18hrs post CLP





# Supplementary Figure E2

