Figure S1

A)

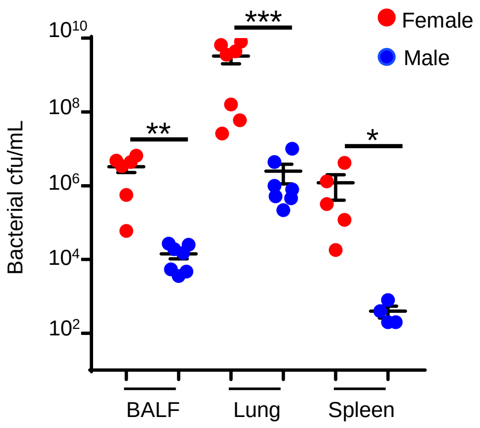


Figure S1. Female mice are more susceptible to *A. baumannii* infection. Male and female age-matched C57BL/6J mice were intranasally infected with 10^7 *A. baumannii* 5075 for 24 h. Bacterial counts were assessed in BALF, lung and spleen. n=7. Each point represents a mouse. Data is from two independent experiments. Graphs show means with standard error. ***P<0.001, **P<0.01 and *P<0.05. A nonparametric Mann-Whitney test was used to assess differences between groups.

Figure S2

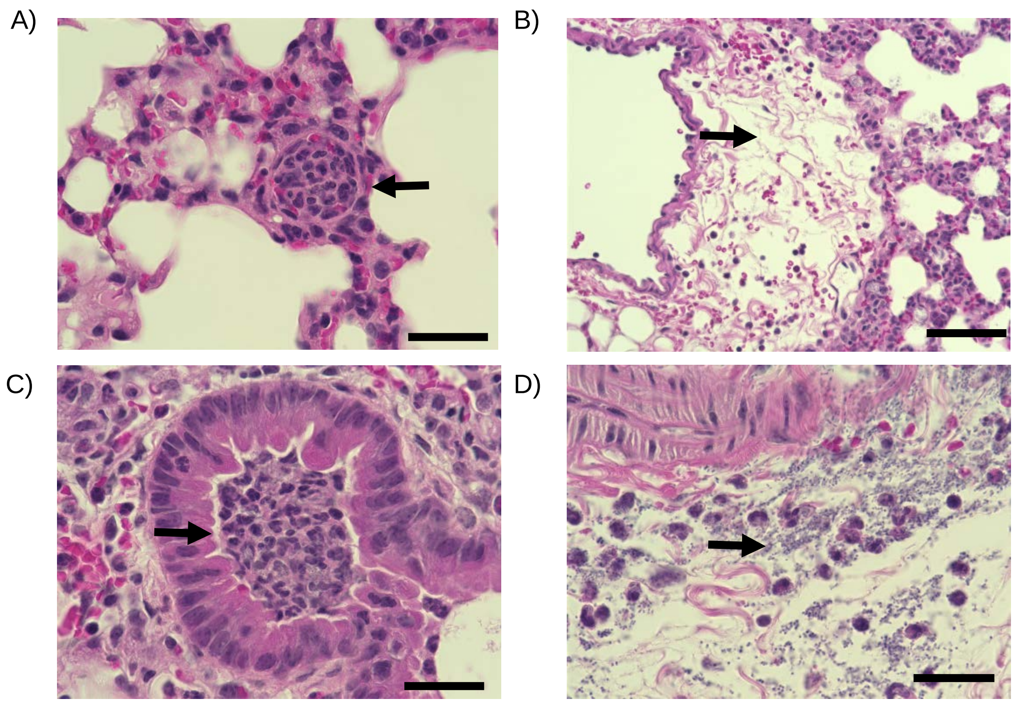


Figure S2. Pathological features of *A. baumannii* infection. Female were intranasally infected with 10^7 cfu of *A. baumannii* for 24 h. H&E sections are shown. A) Microabscesses composed of neutrophils within the lung parenchyma. B) Pulmonary edema. C) Neutrophils within airspaces and D) bacteria evident within pulmonary edema. Scale bars 25 µm except for 100 µm in B). Arrows indicate features.

Figure S3

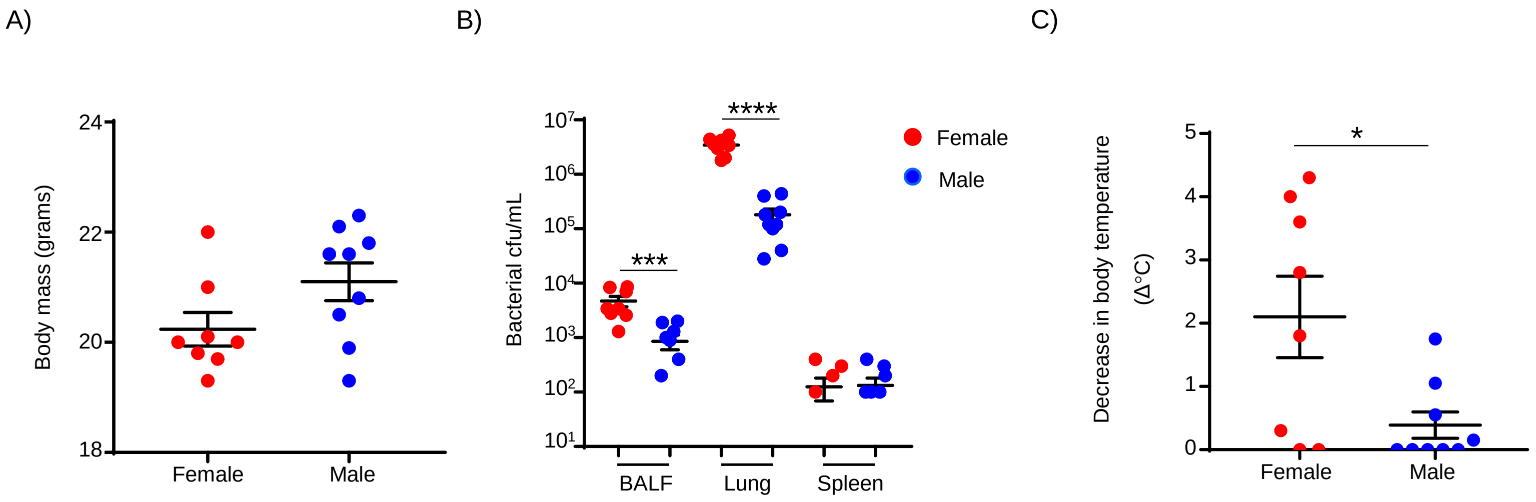
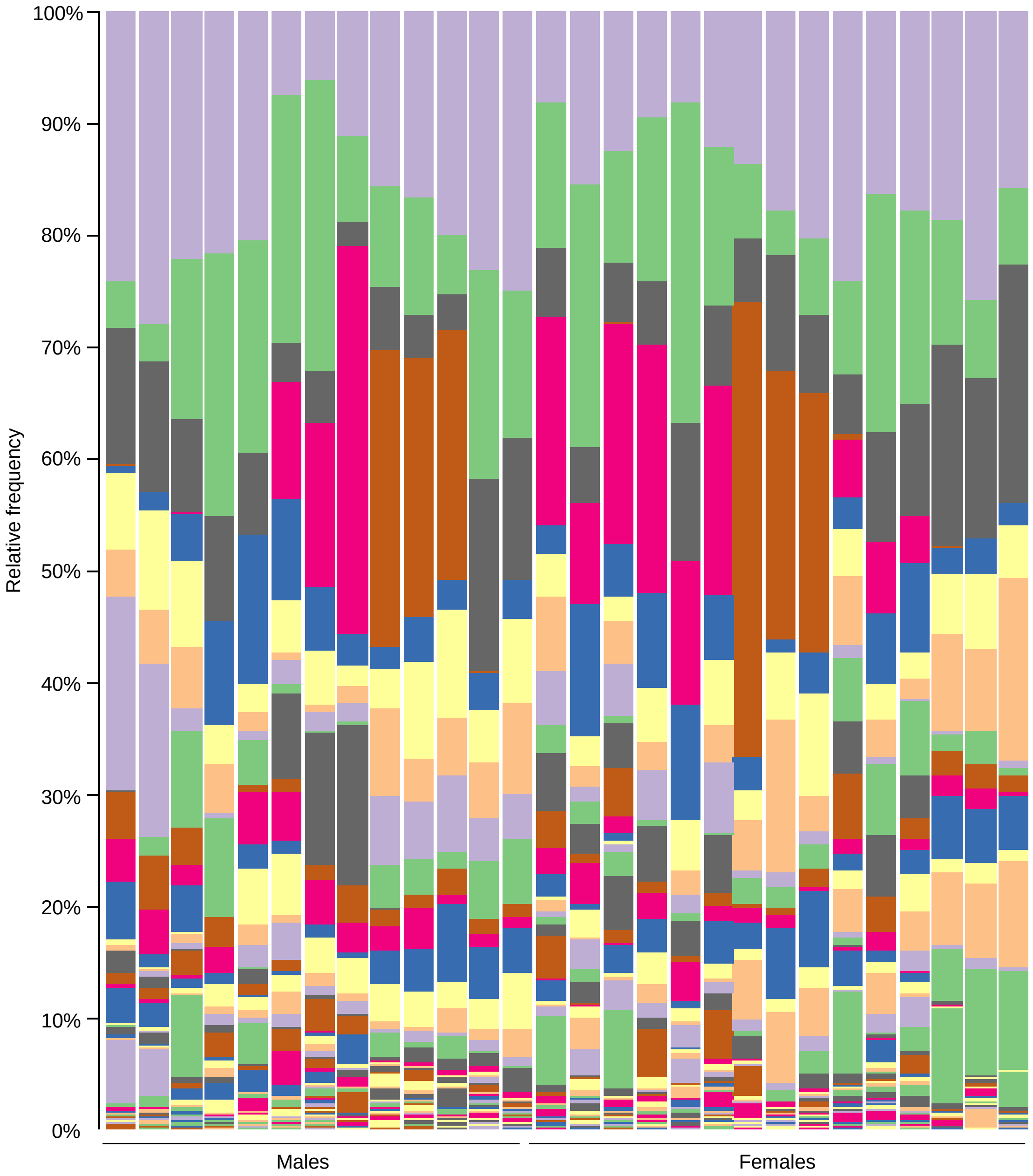


Figure S3. Female mice display enhanced susceptibility to intranasal infection with *A. baumannii* 19606. A) Weight of female (n=8) and male (n=9) mice before infection with *A. baumannii* 19606. B) BALF, lung and spleen homogenate were enumerated for bacterial counts 24 h after infection. C) Change in external temperature of female and male mice 24 h after infection. Each point represents a mouse. Graphs display means with standard error. Data is from two independent experiments. ****P<0.0001, ***P<0.001 and *P<0.05. A nonparametric Mann-Whitney test was used to assess differences between groups.

Figure S4



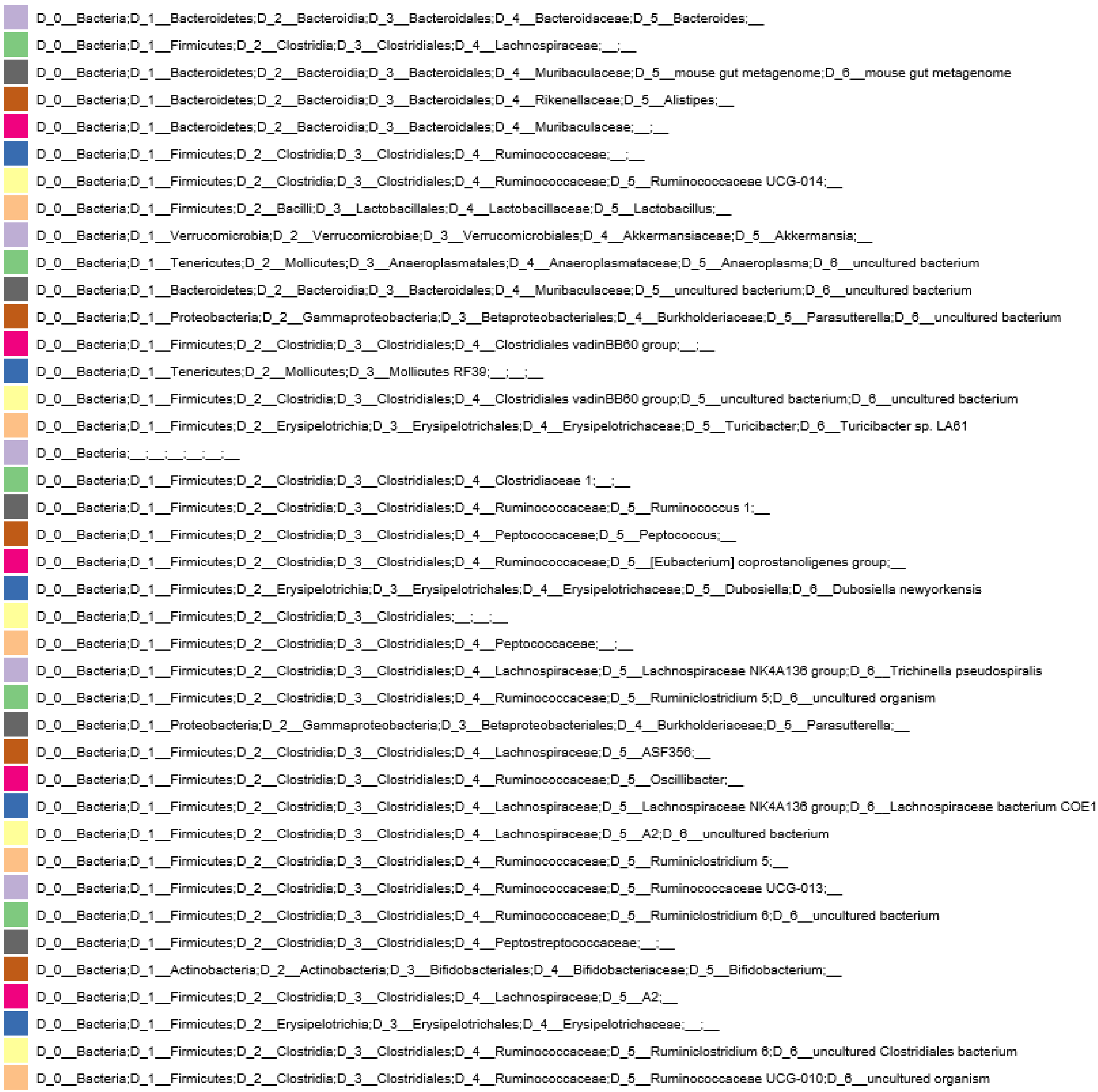


Figure S4. Microbiome analysis of female and male mice demonstrates the presence of equivalent taxa. 16S rRNA analysis was performed on DNA isolated from fecal samples prior to infection. Legend represents the most prevalent taxa identified. n=13 for males and 15 for females.

Figure S5

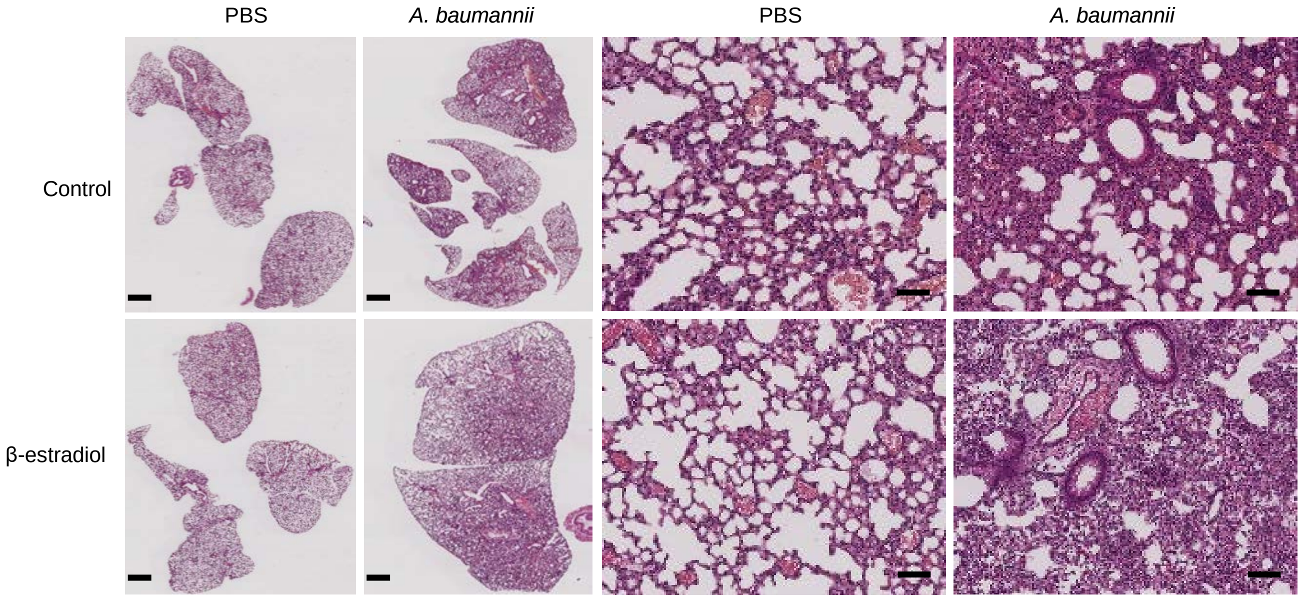
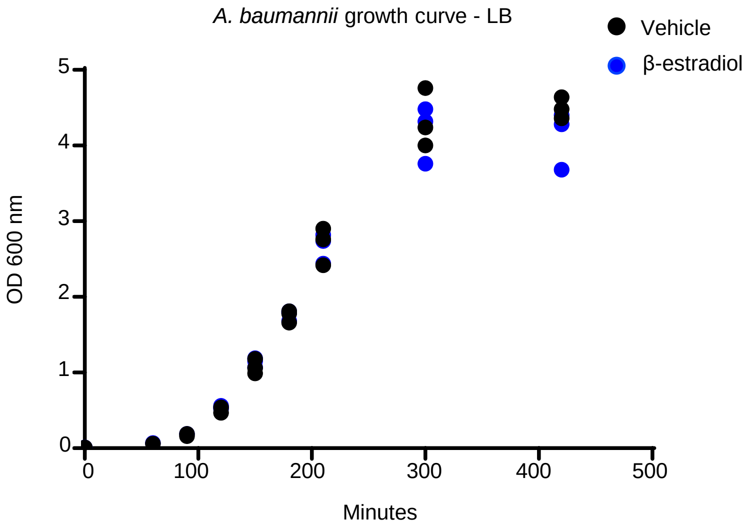


Figure S5. Pathological examination of estrogen treated male mice. Male mice were treated with 17 β-estradiol or vehicle control for five days prior to intranasal infection with 10^7 cfu of *A. baumannii* 5075. Representative hematoxylin and eosin-stained lung sections of mice at 24 h post infection (scale bar, 1 mm – left panel lung lobes; 100 µm – right panel airways).

Figure S6

A)



B)

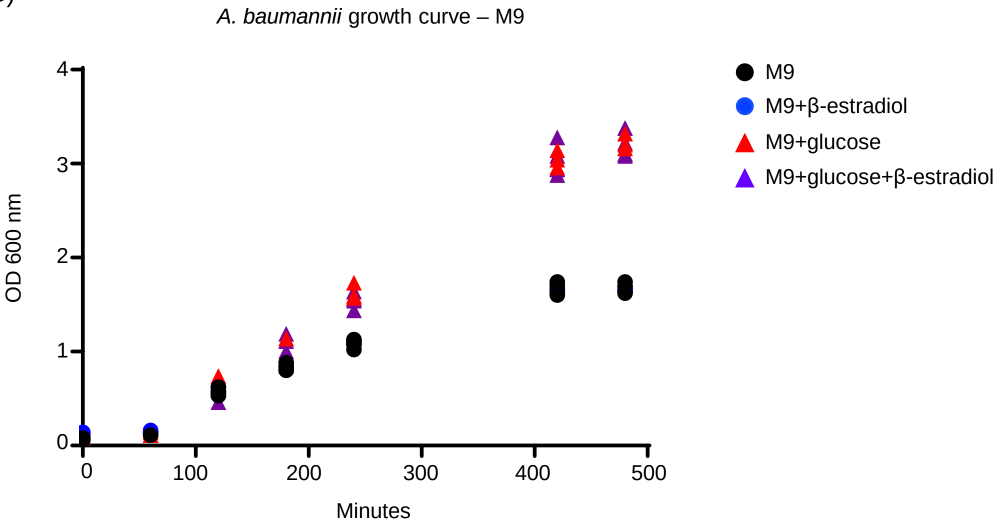


Figure S6. Effect of estrogen on bacterial growth. A) Growth of *A. baumannii* 5075 was assessed for 420 min in LB media containing either β-estradiol or vehicle control. n=3. B) Growth of *A. baumannii* 5075 was assessed for 480 min in M9 minimal media or M9 supplemented with glucose containing either β-estradiol or vehicle control. n=4 except glucose control with n=3.

Figure S7

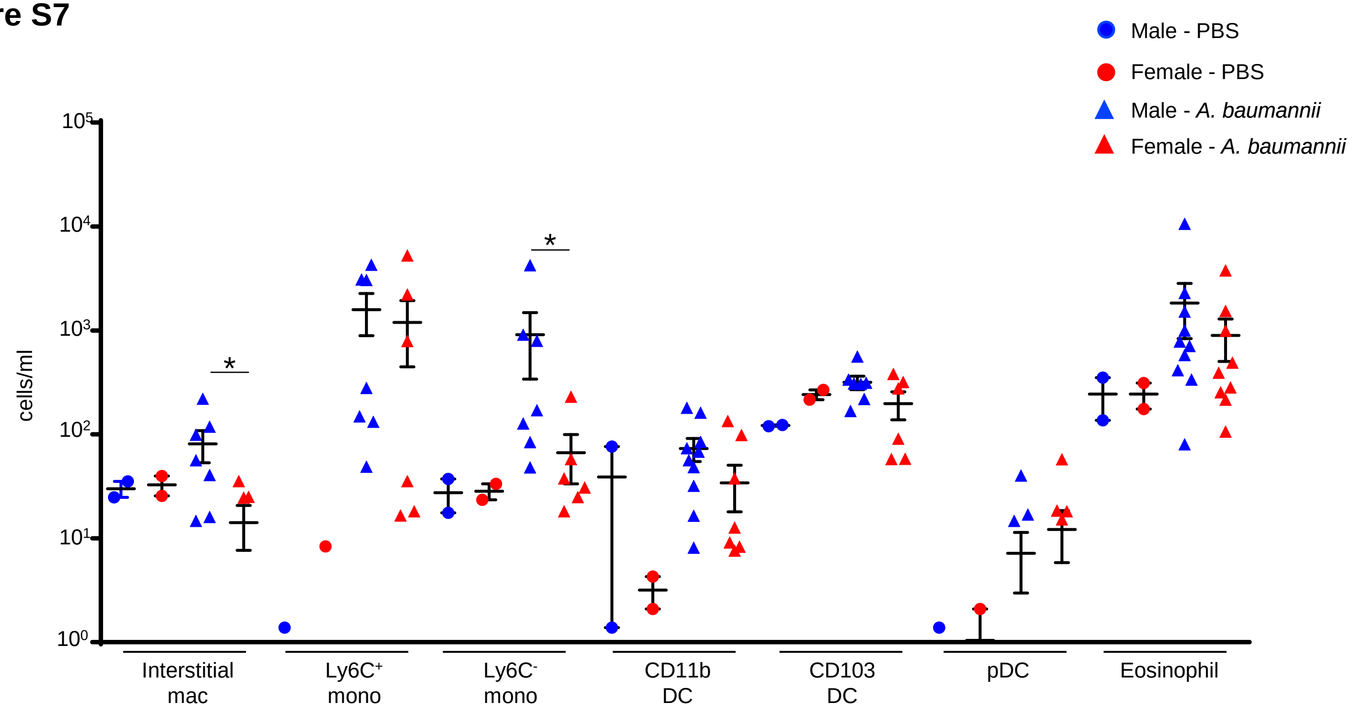


Figure S7. Flow cytometry analysis of cells in BALF from male and female mice.
Male and female weight matched mice were infected with 10^7 cfu of *A. baumannii* 5075 for 24 h. Flow cytometry analysis of cells in BALF. Infected mice n= female-8; male-10; n=2 for uninfected. Graphs show mean with standard error. *P<0.05. A nonparametric Mann-Whitney test was used to assess differences between groups.

Figure S8

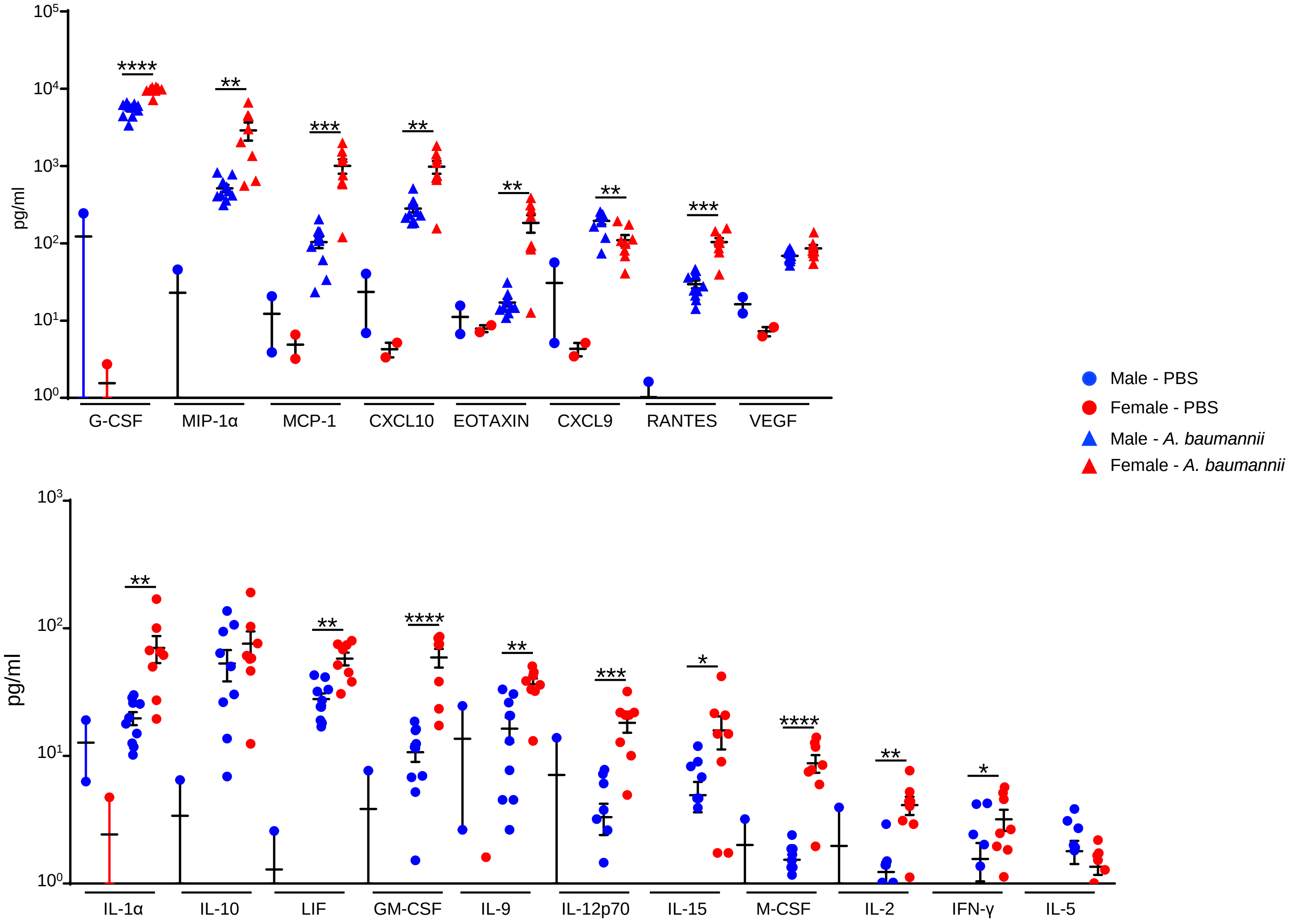


Figure S8. Female mice have an increased inflammatory response to *A. baumannii* infection. Weight matched male and female C57BL/6J mice were infected with 107 cfu of *A. baumannii* 5075 for 24 h. Cytokines were quantified by multiplex from BALF. n=8 for female, 10 for male and 2 for uninfected. Graphs show mean with standard error. Data is from at least two independent experiments. ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05. A nonparametric Mann-Whitney test was used to assess differences between groups.

Figure S9

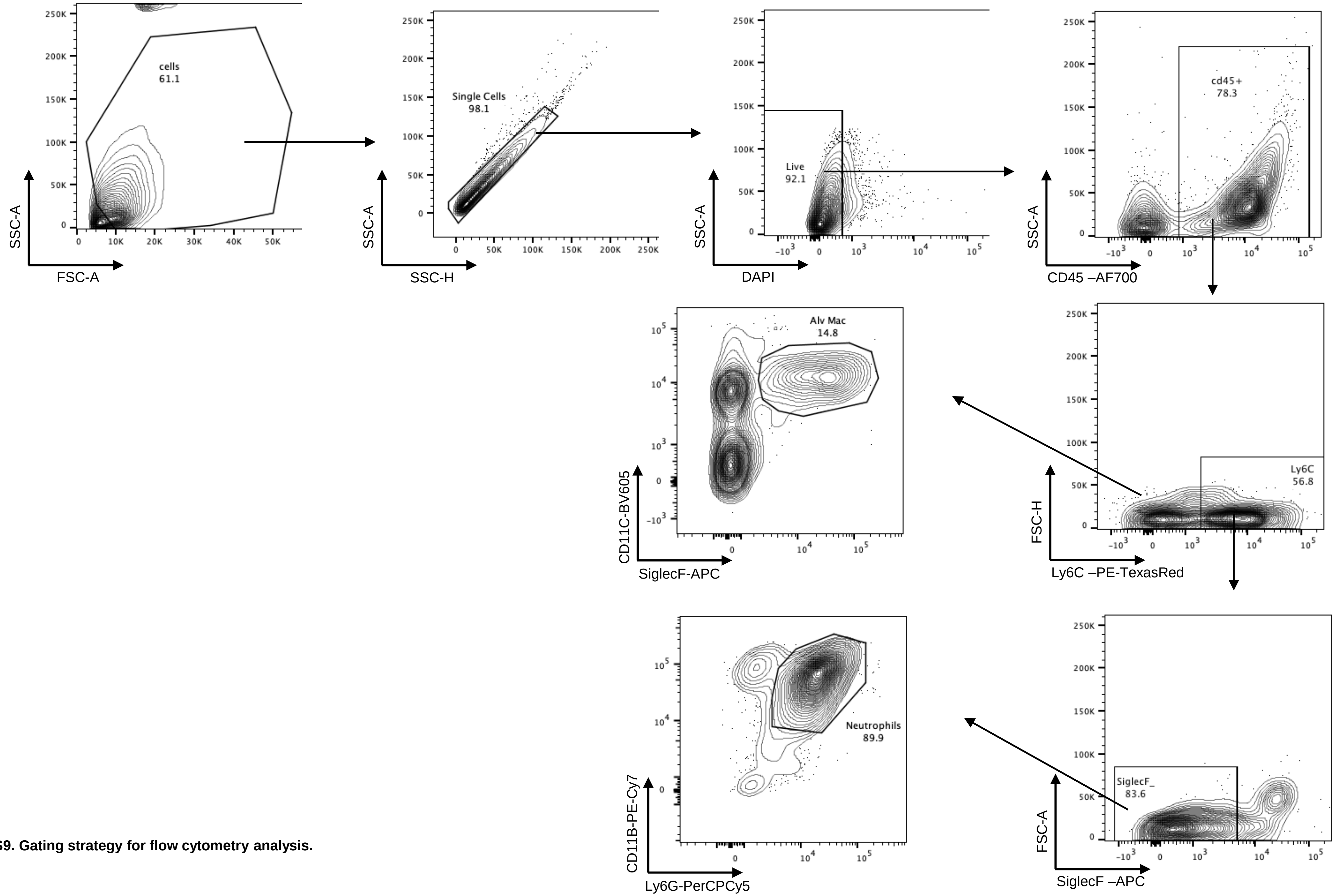


Figure S9. Gating strategy for flow cytometry analysis.

Figure S10

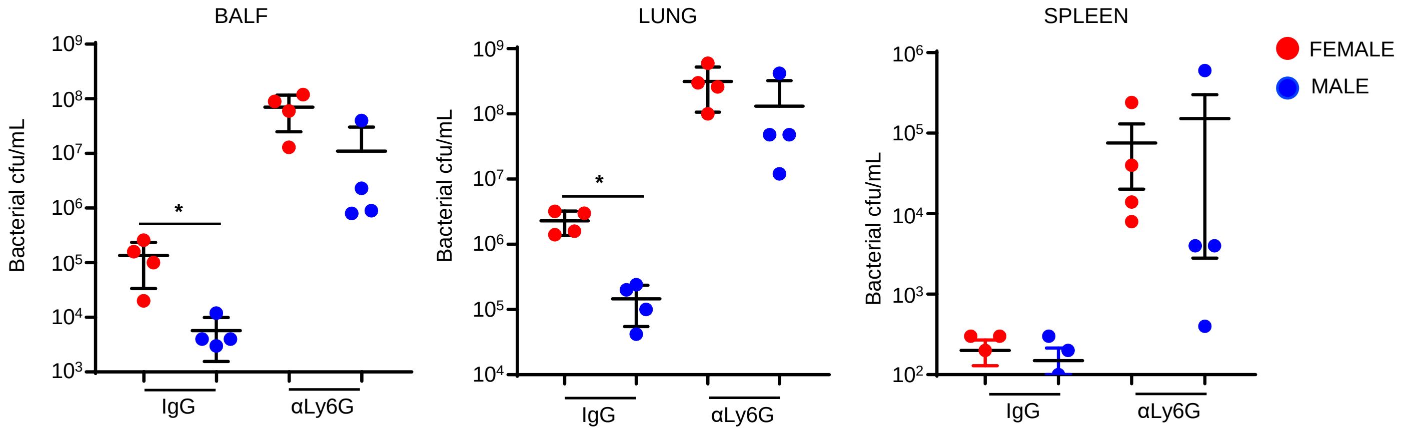


Figure S10. Neutrophil depletion reduces the sex difference in bacterial clearance. Mice were treated with either α-IgG control or α-Ly6G and then infected intranasally with 10^7 cfu of *A. baumannii* for 24 h. Bacteria were enumerated from BALF and lung and spleen homogenates. Representative data is shown. n=4. Graphs show mean with standard error. *P<0.05. A nonparametric Mann-Whitney test was used to assess differences between groups.

Figure S11

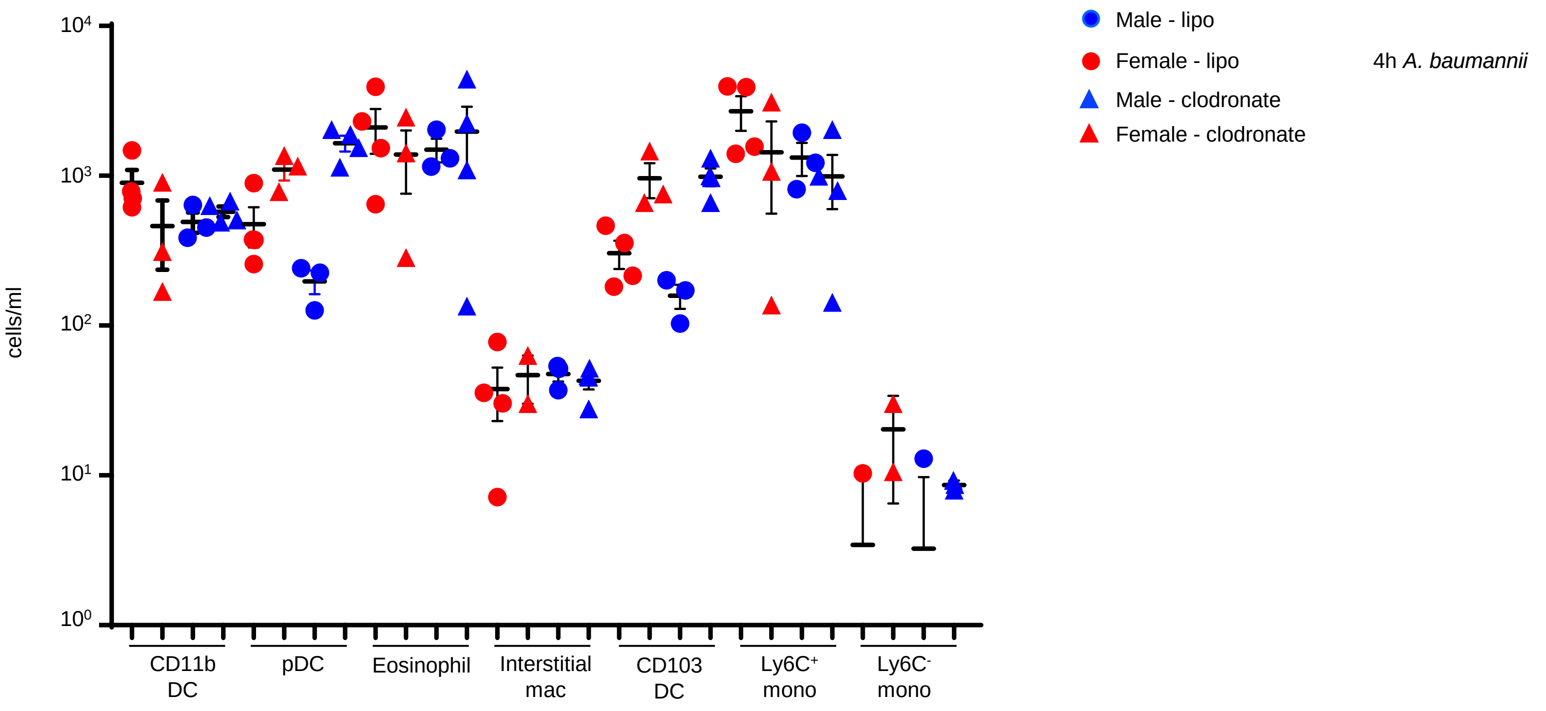


Figure S11. Flow cytometry analysis of cells from clodronate depleted mice. Cell counts in BALF from male and female mice treated with either liposome only control or clodronate loaded liposomes 24 h after intranasal infection with 10^7 cfu of *A. baumannii*.

Figure S12

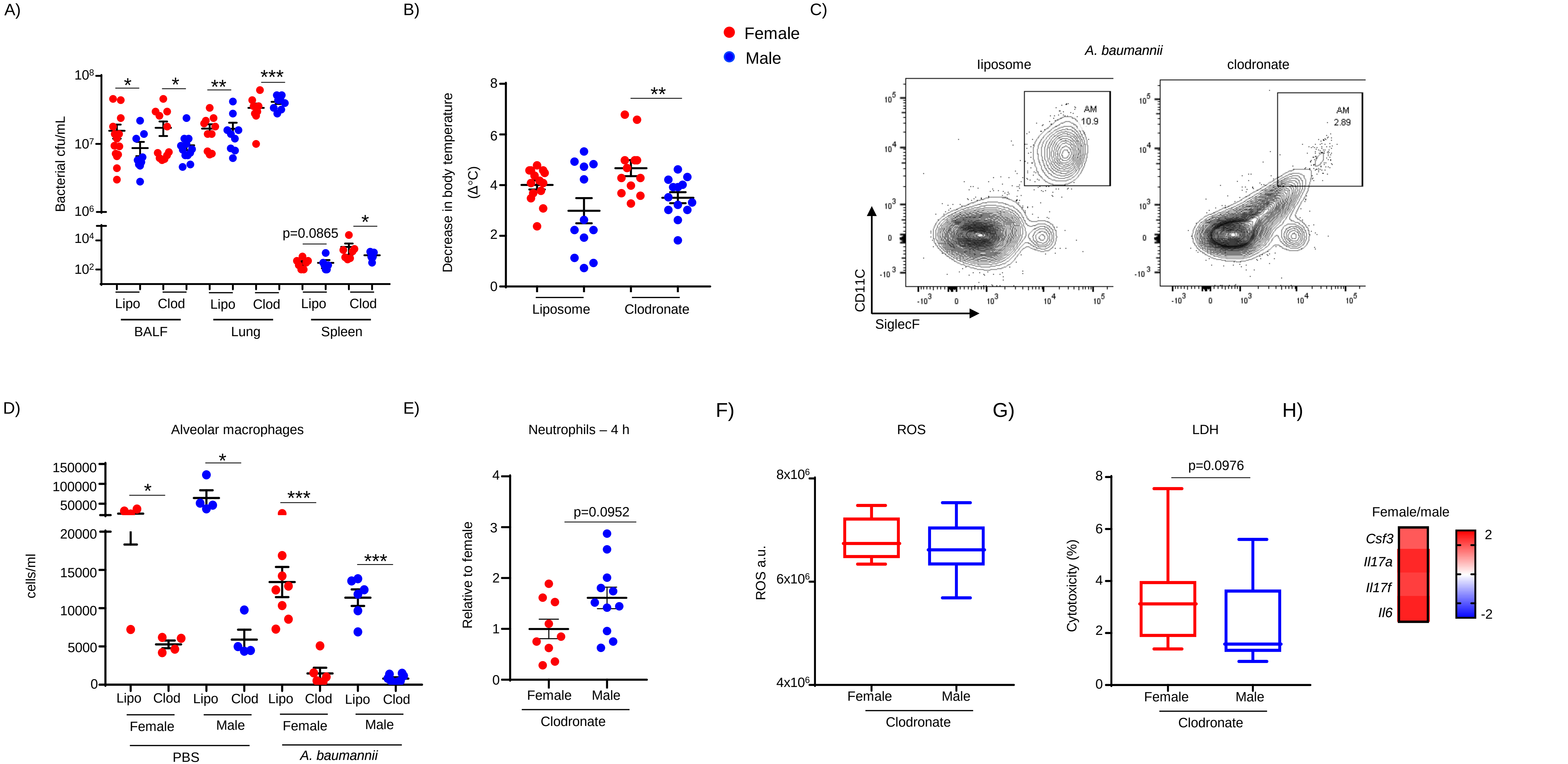


Figure S12. Clearance of *A. baumannii* 4 h after intranasal infection of alveolar macrophage depleted mice. A) Bacterial counts from BALF, lung and spleen homogenates of female and male mice treated with either clodronate loaded liposomes or liposome controls 4 h after intranasal *A. baumannii* infection. B) Change in external temperature of female and male mice. Female liposomes n=14, 10, 8 and 14 for BALF, lung, spleen and temperature respectively. Male liposomes n=12, 9, 8 and 12 for BALF, lung, spleen and temperature respectively. Female clodronate n=12, 8, 9 and 12 for BALF, lung, spleen and temperature respectively. Male clodronate n=13, 9, 8 and 13 for BALF, lung, spleen and temperature respectively. C) Alveolar macrophages were identified by flow cytometry in BALF cells from mice that had been treated with either clodronate loaded liposomes or liposome only controls then a 4 h infection with *A. baumannii* 5075 using the following gating strategy: live CD45$^+$, Ly6C$^-$, CD11c$^+$ and SiglecF$^+$. D) Alveolar macrophage cell numbers in BALF of both uninfected and infected female and male mice treated either with clodronate loaded liposomes or liposome only controls. n=4 for uninfected, n=6 for female clodronate and male liposomes and n=8 for female liposomes and male clodronate. E) Neutrophil numbers in BALF of infected female and male mice treated with clodronate loaded liposomes. n=9 for females and 11 for males. F&G) ROS and LDH levels, respectively, in BALF following a 4 h infection in female and male depleted of alveolar macrophages. n=4. H) Heat map showing the ratio between females and male of transcription of *Csf3*, *Il17a*, *Il17f* and *Il6*. ***P<0.001, **P<0.01, *P<0.05.

Figure S13

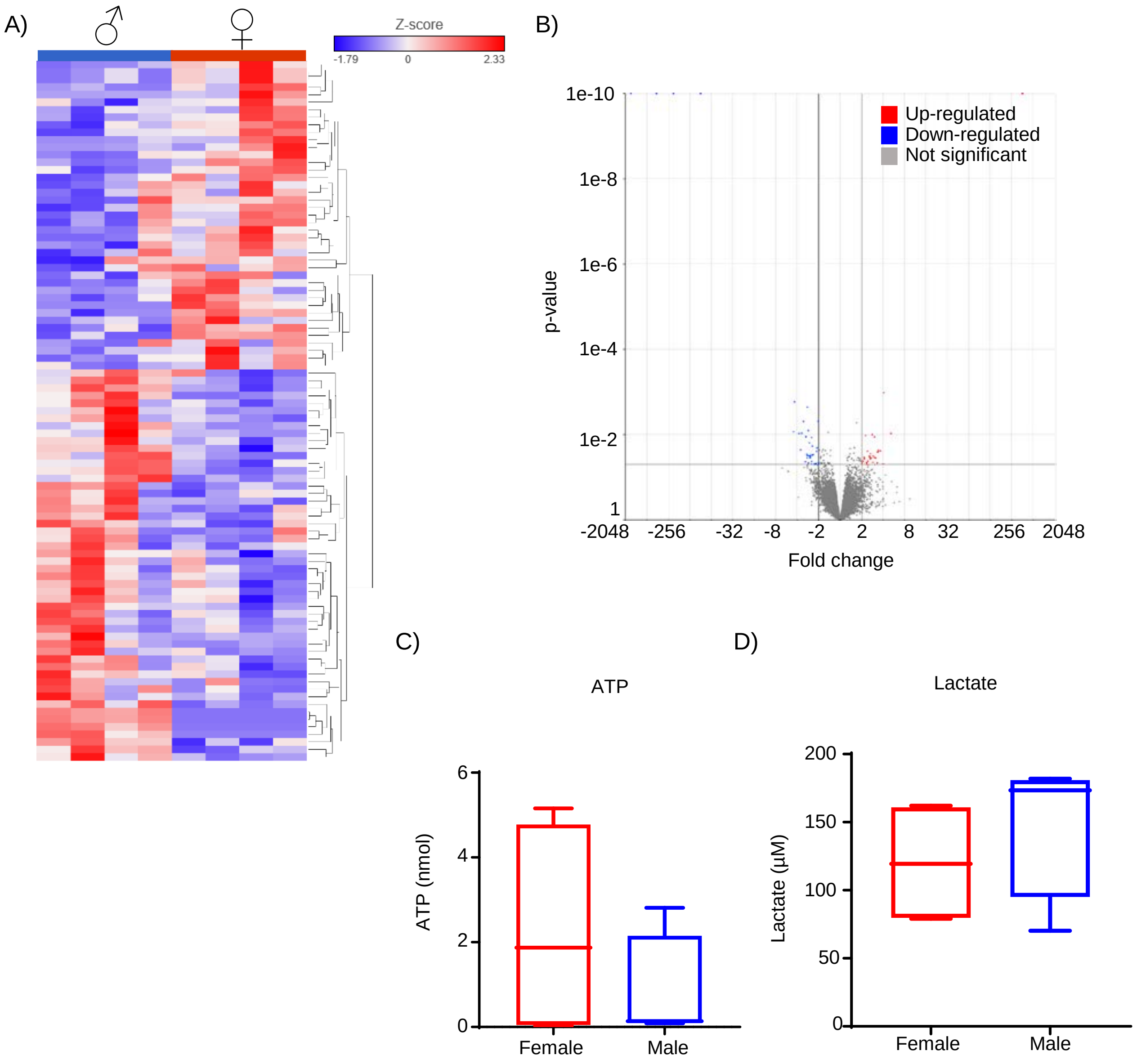


Figure S13. RNA-seq analysis from control male and female mice after a 4 h *A. baumannii* infection. A) Heat map of RNA-seq analysis of the comparison between female and male mice, liposome treated controls, following a 4 h intranasal infection with *A. baumannii* 5075. Each column represents an individual mouse. n=4. B) Volcano plot of differentially expressed genes. C&D) ATP and lactate levels, respectively, in BALF following a 4 h infection in female and male treated with liposome controls. Box and whisker plots show mean with upper and lower quartiles, with whiskers at minimum and maximum values. Data is from two independent experiments.