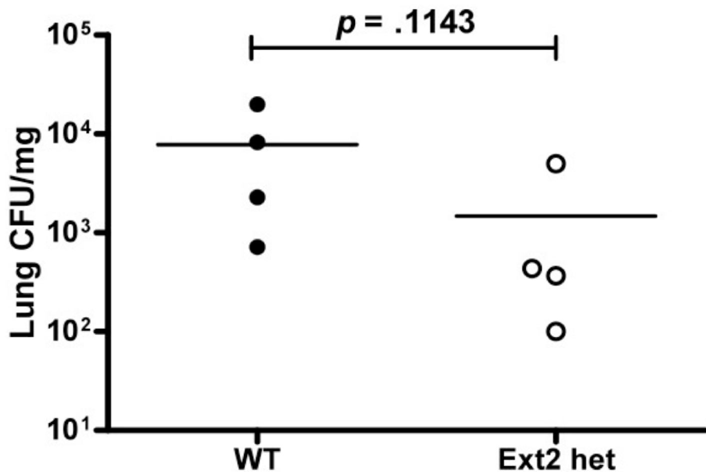
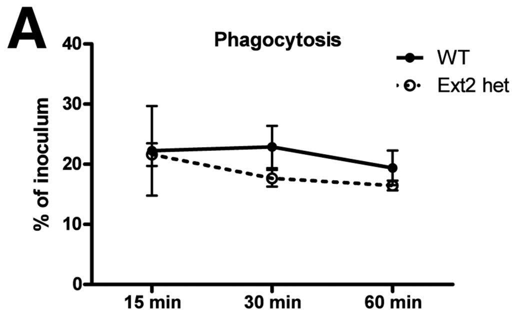
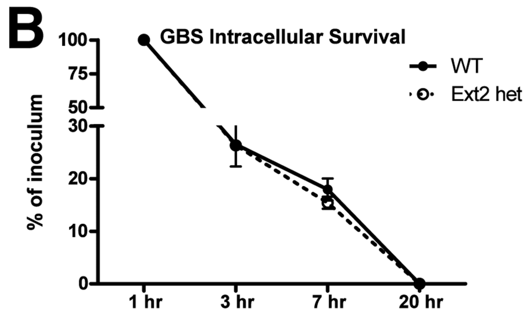
Supplemental Methods

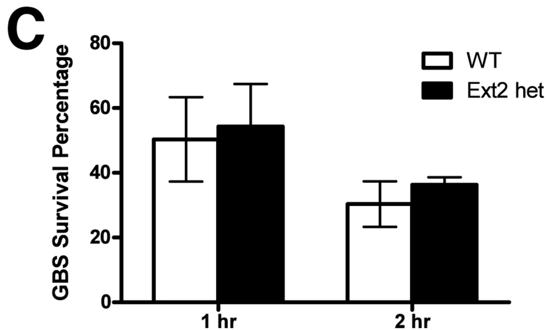
Measurement of heparan sulfate in primary mouse lung microvascular endothelial cells.

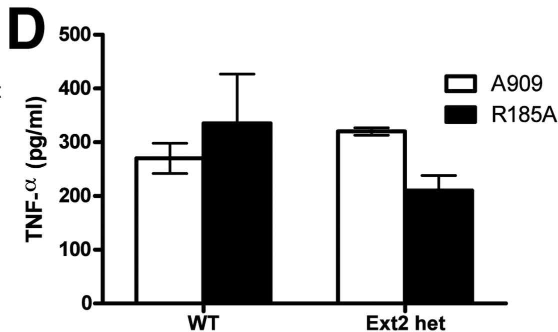
Cells were labeled for 24 h with 100 μCi/ml $Na^{35}SO_4$ in F12 nutrient mixture. Radiolabeled GAG chains were solubilized with 0.1 M NaOH for 30 min, an aliquot was removed for protein determination using the BCA protein assay (Pierce). The remaining material was adjusted to pH 7 with 10 M acetic acid and digested with a protease solution containing 1 mg/ml Pronase (Boehringer Mannheim). After overnight incubation, the reaction mixture was diluted 5-fold with water to reduce the salt concentration to 0.1 M. The solution was applied to 0.2 ml column of DEAE-Sephacel prepared in a disposable polypropylene column. The column was washed with 20 mM sodium acetate buffer (pH 6.0) containing 0.25 M NaCl. Bound GAGs were eluted with 1 M NaCl in 20 mM sodium acetate (pH 6.0) then dialyzed into 50 mM of Ammonium Biocarbonate. The material was dried and dissolved with 200 μl of H_2O. Chondroitin sulfate was removed by treating a sample overnight at 37 °C with 20 milliunits of chondroitinase ABC. The digested material was then put back onto a DEAE-Sephacel column and washed and eluted as described above. The [^{35}S] counts in the eluent were used as a measurement of heparan sulfate in the sample.


p = .1143
Lung CFU/mg
10^5
10^4
10^3
10^2
10^1
WT
Ext2 het


A
Phagocytosis
WT
Ext2 het
% of inoculum
0
10
20
30
40
15 min
30 min
60 min


B
GBS Intracellular Survival
WT
Ext2 het
% of inoculum
100
75
50
30
20
10
0
1 hr
3 hr
7 hr
20 hr


C
WT
Ext2 het
GBS Survival Percentage
0
20
40
60
80
1 hr
2 hr


D
A909
R185A
TNF-α (pg/ml)
0
100
200
300
400
500
WT
Ext2 het