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.

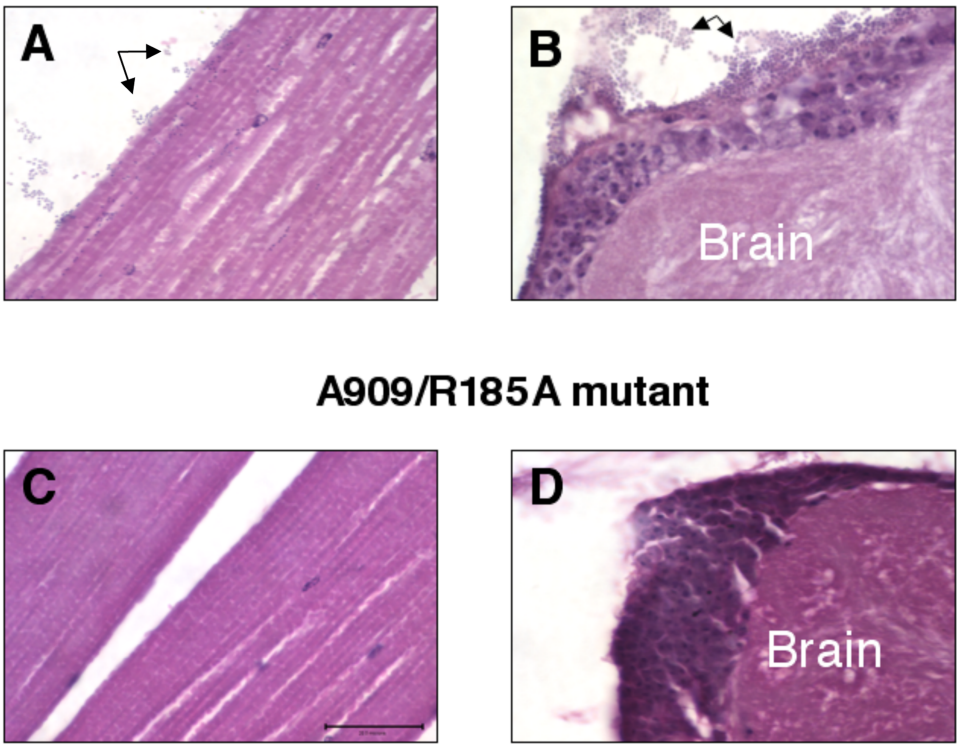
TABLE S1

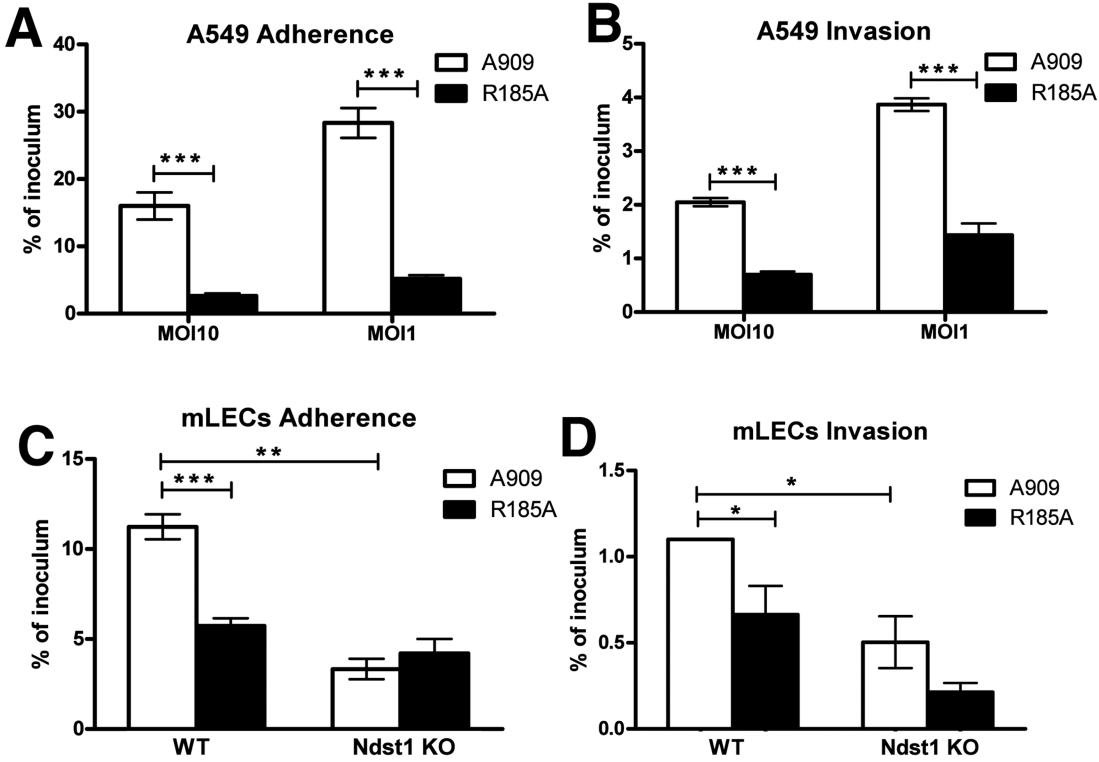
GBS strain	A909	A909			R185A	R185A		
fly strain	yw	yw			yw	yw		
	head cfu/group of 10 flies	body cfu/group of 10 flies	head/body	head/whole fly	head cfu/group of 10 flies	body cfu/group of 10 flies	head/body	head/whole fly
	62000	499000	0.124248	0.110517	1000	17800	0.05618	0.053191
	560000	3160000	0.177215	0.150538	250	8000	0.03125	0.030303
	342500	3470000	0.098703	0.089836	2800	44300	0.063205	0.059448
	1215000	6140000	0.197883	0.165194	200	18900	0.010582	0.010471
	705000	4590000	0.153595	0.133144	425	11100	0.038288	0.036876
	765000	5020000	0.15239	0.132239	50	8150	0.006135	0.006098
	30250	341000	0.08871	0.081481	11000	164500	0.066869	0.062678
	152750	940000	0.1625	0.139785	66250	325000	0.203846	0.169329
	95500	1110000	0.086036	0.07922	19500	237500	0.082105	0.075875
	615000	1580000	0.389241	0.280182	1000	45000	0.022222	0.021739
	200000	890000	0.224719	0.183486	0	20000	0	0
	575000	3100000	0.185484	0.156463	1500	38500	0.038961	0.0375
	620000	3440000	0.180233	0.152709	1500	14000	0.107143	0.096774
	1090000	5160000	0.21124	0.1744				

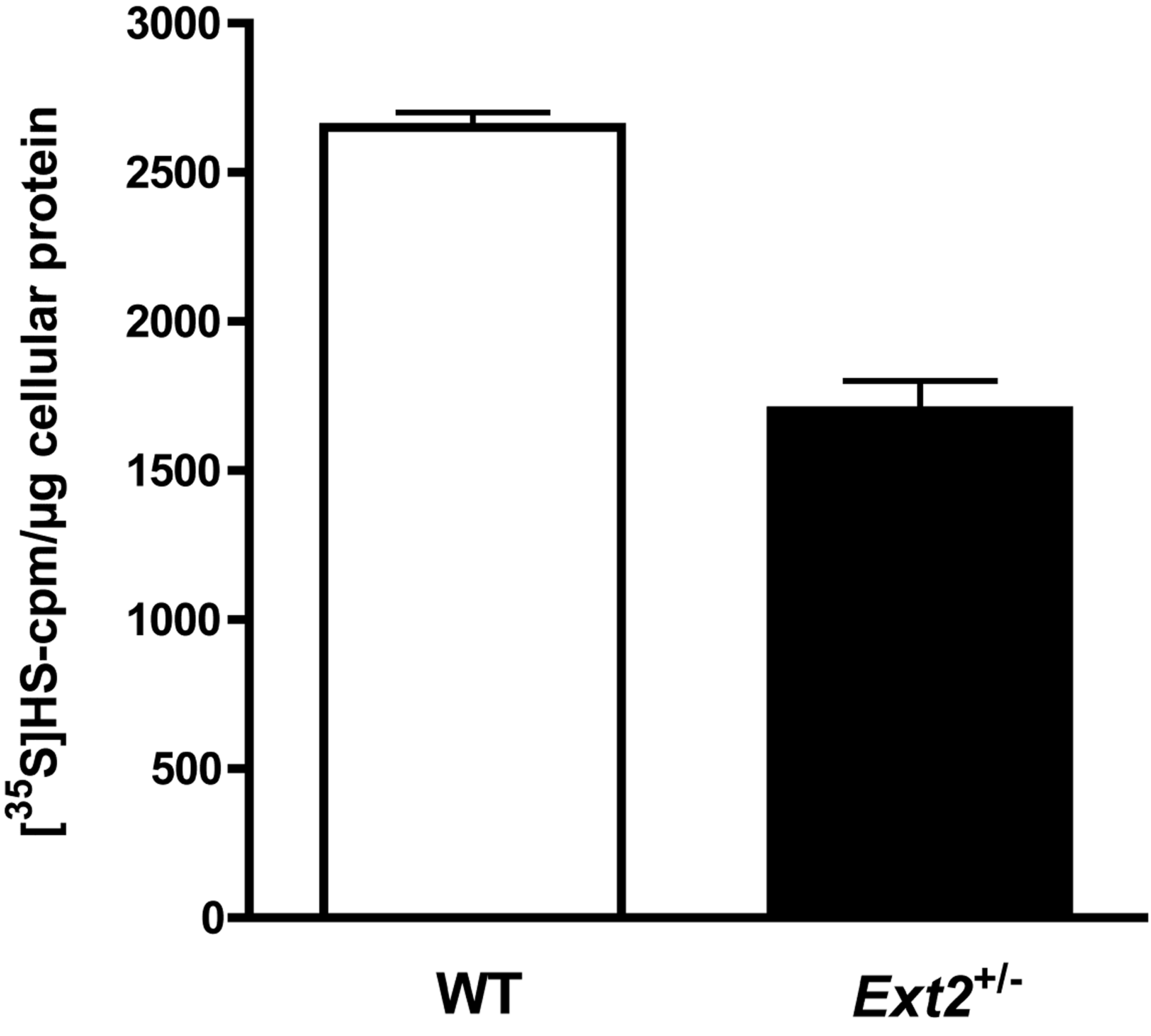
Data for Figure 1 *B*

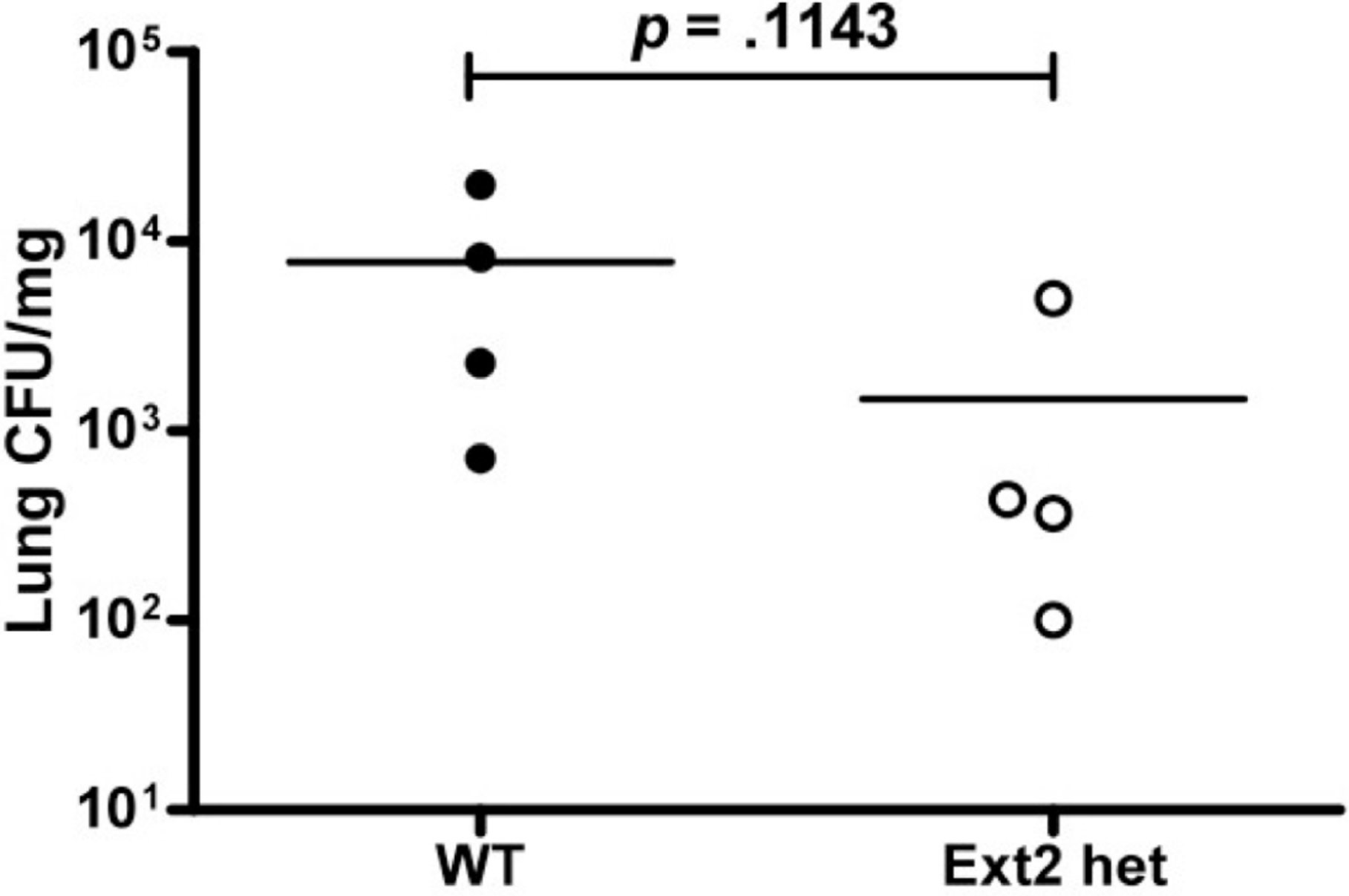
GBS strain	A909	A909			A909	A909		
fly strain	yw	yw			dally+dlp+sdc	dally+dlp+sdc		
	head cfu/group of 10 flies	body cfu/group of 10 flies	head/body	head/whole fly	head cfu/group of 10 flies	body cfu/group of 10 flies	head/body	head/whole fly
	560000	3160000	0.177215	0.150538	193000	1000000	0.193	0.161777
	342500	3470000	0.098703	0.089836	8000	151500	0.052805	0.050157
	1215000	6140000	0.197883	0.165194	284500	2160000	0.131713	0.116384
	705000	4590000	0.153595	0.133144	51500	650000	0.079231	0.073414
	765000	5020000	0.15239	0.132239	258000	1660000	0.155422	0.134515
	30250	341000	0.08871	0.081481	105500	464000	0.227371	0.18525
	152750	940000	0.1625	0.139785	308000	1160000	0.265517	0.209809
	95500	1110000	0.086036	0.07922	3500	29500	0.118644	0.106061
	615000	1580000	0.389241	0.280182	10000	188000	0.053191	0.050505
	200000	890000	0.224719	0.183486	13500	161000	0.083851	0.077364
	575000	3100000	0.185484	0.156463	680000	4120000	0.165049	0.141667
	750000	5000000	0.15	0.130435	36900	165000	0.223636	0.182764
	500000	2050000	0.243902	0.196078	1000	23000	0.043478	0.041667
	141250	504000	0.280258	0.218907	6400	37750	0.169536	0.14496
	435000	1650000	0.263636	0.208633	100	1100	0.090909	0.083333
	265500	1250000	0.2124	0.17519				
	162500	1320000	0.123106	0.109612				
	920000	3400000	0.270588	0.212963				
	1595000	9030000	0.176633	0.150118				
	116000	394000	0.294416	0.227451				
	620000	3440000	0.180233	0.152709				
	1090000	5160000	0.21124	0.1744				

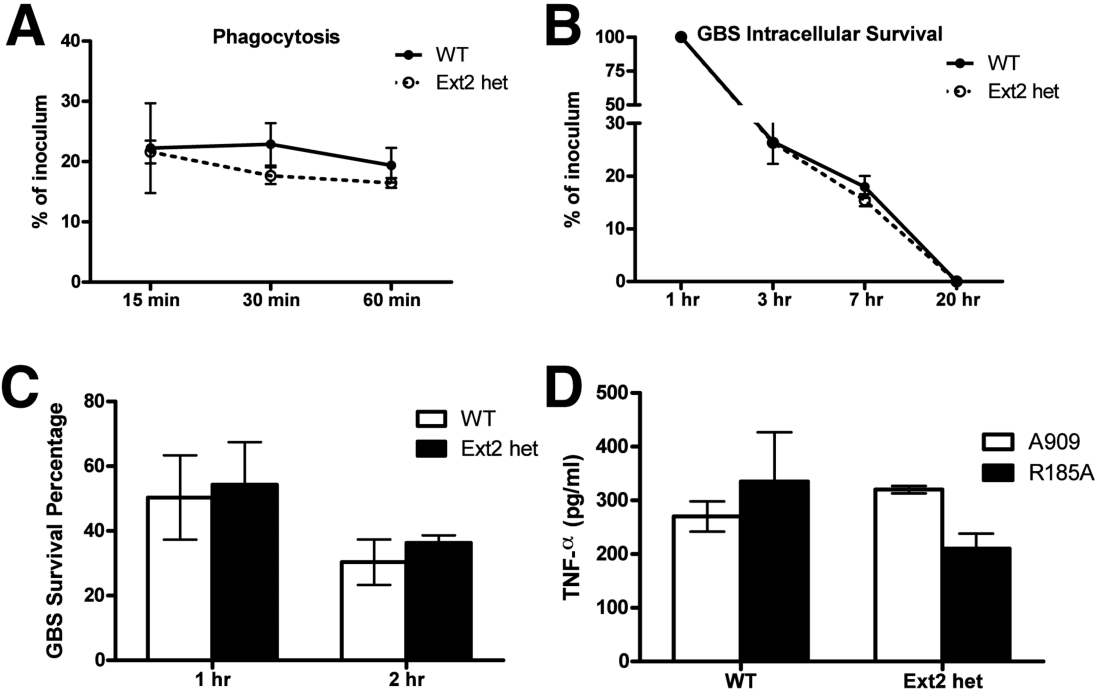
A909			A909	A909		
ttv+sotv			sfl	sfl		
body cfu/group of 10 flies	head/body	head/whole fly	head cfu/group of 10 flies	body cfu/group of 10 flies	head/body	head/whole fly
3760000	0.11383	0.102197	91500	990000	0.092424	0.084605
260000	0.070385	0.065756	2700	35000	0.077143	0.071618
7000	0.214286	0.176471	121000	746500	0.16209	0.139481
13000	0.038462	0.037037	13925	194500	0.071594	0.066811
2030000	0.134729	0.118732	3850	53000	0.072642	0.067722
2750000	0.088	0.080882	0	35000	0	0
2770000	0.17148	0.146379	365000	2860000	0.127622	0.113178
1750000	0.014857	0.01464	201250	2100000	0.095833	0.087452
1840000	0.009103	0.009021	123500	1480000	0.083446	0.077019
2760000	0.127174	0.112825	770000	4300000	0.17907	0.151874
2640000	0.284091	0.221239				
16150	0.111455	0.100279				


A909 (WT GBS)
A
B
Brain
A909/R185A mutant
C
D
Brain


A
A549 Adherence
% of inoculum
40
30
20
10
0
***
***
A909
R185A
MOI10
MOI1
B
A549 Invasion
% of inoculum
5
4
3
2
1
0
***
***
A909
R185A
MOI10
MOI1
C
mLECs Adherence
% of inoculum
15
10
5
0
**
***
A909
R185A
WT
Ndst1 KO
D
mLECs Invasion
% of inoculum
1.5
1.0
0.5
0.0
*
*
A909
R185A
WT
Ndst1 KO


3000
2500
2000
1500
1000
500
0
[35S]HS-cpm/µg cellular protein
WT
Ext2+/-


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
40
30
20
10
0
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
GBS Survival Percentage
WT
Ext2 het
80
60
40
20
0
1 hr
2 hr
D
TNF-α (pg/ml)
A909
R185A
500
400
300
200
100
0
WT
Ext2 het