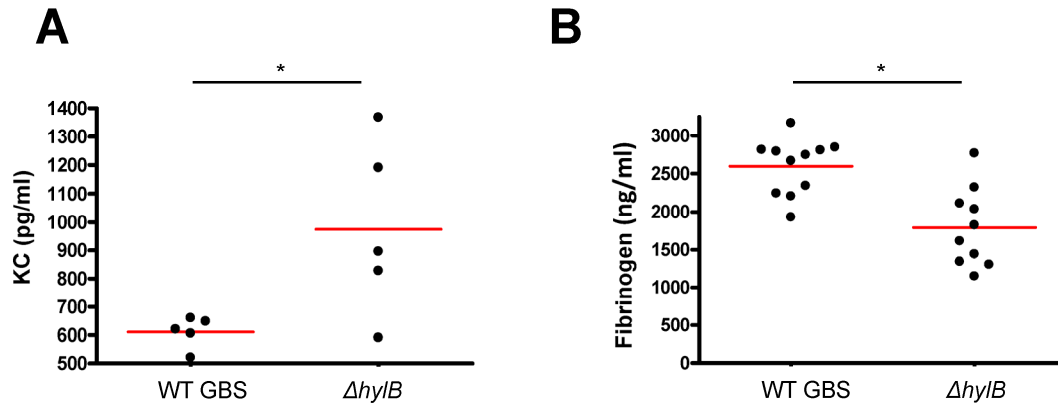


**Figure S1, related to Figure 1. IL-6 production and bacterial burden after infection of CD1 mice with WT or  $\Delta$ *hylB* GBS.**

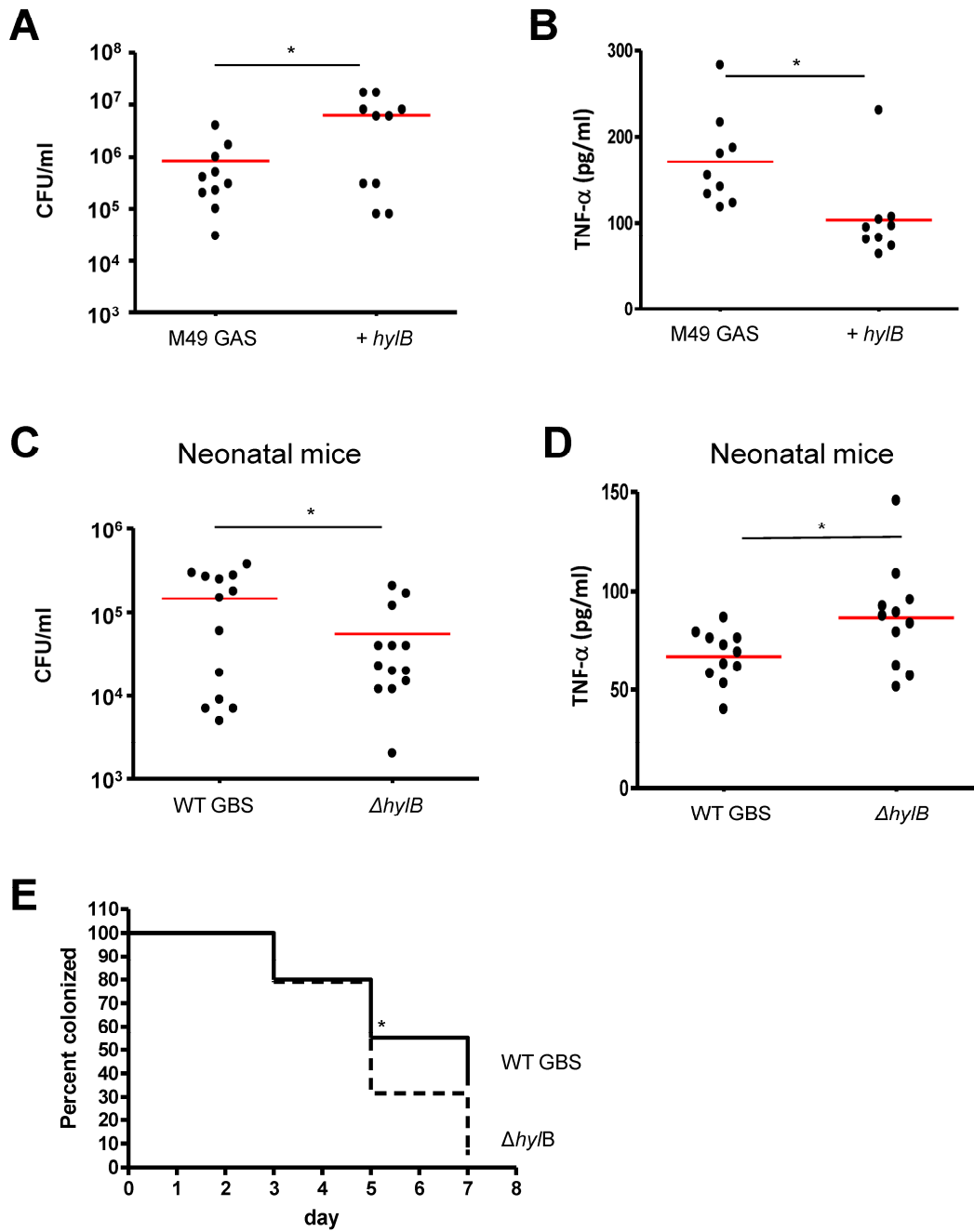
(A) Overnight bacterial culture supernatants from WT and  $\Delta$ *hylB* GBS were added to a hyaluronan agar plate. The plate was visualized after 24 h for a zone of clearance as an indication of the presence of hyaluronidase activity. (B-C) CD1 mice (n = 12 per group) were infected i.p. with WT,  $\Delta$ *hylB*, or the complemented  $\Delta$ *hylB* GBS strain. (B) IL-6 in the spleen and (C) CFU in the blood and spleen were determined after 24 h. (D) CD1 mice were infected i.p. with either WT or  $\Delta$ *hylB* and CFU in the blood determined

after 48 hours. Data analysis was performed using ANOVA for (B) and (C) and unpaired two-tailed *t*-test for (D). \**P* < 0.05 and \*\**P* < 0.01.



**Figure S2, related to Figure 1. Immunopathologic findings in C57BL/6 mice 48 h after infection with WT or  $\Delta hyIB$  GBS.**

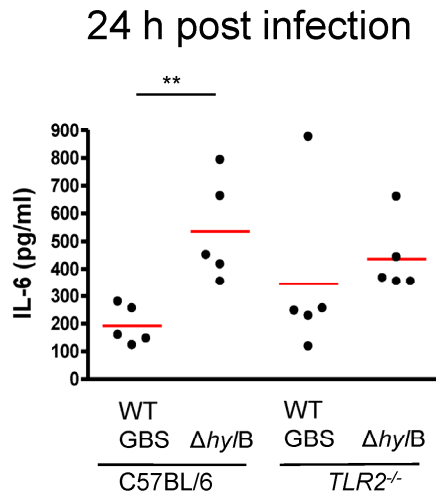
Mice were injected i.p. with either WT or  $\Delta hyIB$  GBS and spleens were harvested after 48 h. Homogenized spleens were assayed for (A) KC (n=5 per group) or (B) fibrinogen by ELISA ( $P=0.06$ ; n=10 per group). Each data point represents an individual mouse. Data analysis was performed using Mann-Whitney U for (A) and unpaired two-tailed  $t$ -test for (B). \* $P < 0.05$ .



**Figure S3, related to Figure 1. GBS hyaluronidase reduces inflammation in GAS and GBS murine models.**

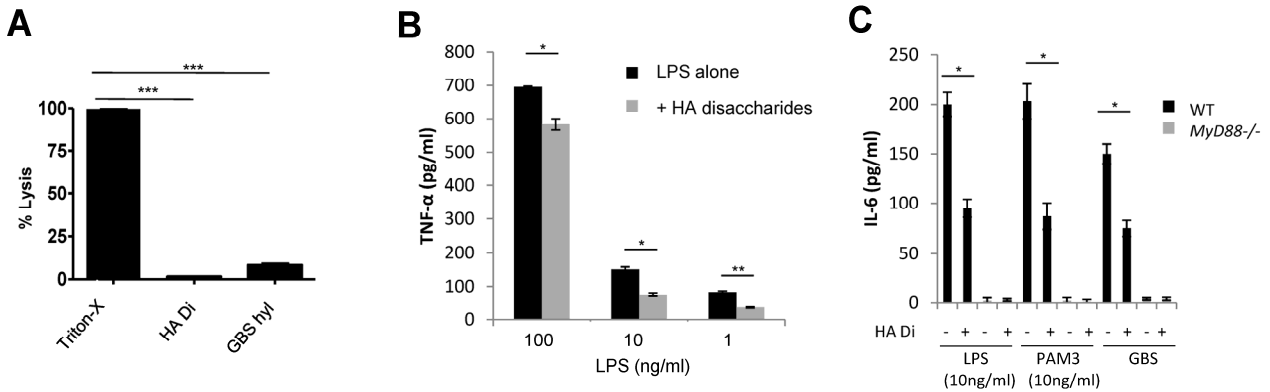
(A-B) C57BL/6 mice were injected i.p. with either WT GAS (no hyaluronidase) or GAS expressing hyaluronidase from GBS. (A) CFU and (B) TNF- $\alpha$  from the spleen were

determined after 48 h. (C-D) Infection of neonatal mice. Four day old C57BL/6 pups were injected i.p. with WT or  $\Delta hyIB$  GBS. After 48 h, kidneys were homogenized and (C) bacterial loads and (D) TNF- $\alpha$  levels were enumerated. (E) Mice were administered WT or  $\Delta hyIB$  GBS into the vaginal lumen as described in Figure 1H. The vaginal cavities were swabbed every other day and percentage of mice remaining colonized analyzed. Each data point represents an individual mouse. Data analysis was performed by Mann-Whitney U for (A) and (B), by unpaired two-tailed  $t$ -test for (C) and (D) and Fisher exact test for (E). \* $P < 0.05$ .



**Figure S4, related to Figure 2. Bacterial burden, cytokine production, and host survival in WT C57BL/6 and TLR2<sup>-/-</sup> after infection with WT or  $\Delta hyIB$  GBS.**

(A) WT C57BL/6 and TLR2<sup>-/-</sup> mice were injected i.p. with either WT or  $\Delta hyIB$  GBS. IL-6 from the spleen was determined at 24 h. Analysis was performed using Mann-Whitney U. *P*-value: \*\**P* < 0.01.



**Figure S5, related to Figure 3. Toxicity of HA disaccharides and effect of HA disaccharides on LPS-, PAM<sub>3</sub>CSK<sub>4</sub>-, and CpG- induced TNF- $\alpha$  release.**

(A) Toxicity of HA disaccharides. BMDM were incubated for 6 h with HA disaccharides (10  $\mu$ g/ml), GBS hyaluronidase (0.5mg/ml), or Triton X-100. An LDH assay was performed to quantify cell death. (B-C) Murine BMDM (WT or *MyD88*<sup>-/-</sup>) were stimulated with LPS, Pam<sub>3</sub>CSK<sub>4</sub> or GBS in the presence or absence of HA disaccharides. Supernatants were collected after 4 h for TNF- $\alpha$  or IL-6 determination. (B) Effect of varying LPS dose with HA disaccharides maintained at 10  $\mu$ g/ml. (C) Effect of HA disaccharides (10  $\mu$ g/ml) on stimulation of WT and *MyD88*<sup>-/-</sup> macrophages by various agonists. Data are shown as mean  $\pm$  SD, where \**P* < 0.05. \*\*\**P* < 0.001, and results from (A) to (C) each are representative of three experiments. Data analysis was performed using ANOVA for (A) and (C), and unpaired two-tailed *t*-test for (B).

**Table S1, related Figures 1 and 5. Strains, plasmids, and primers used in this study**

Strain, plasmid, or primer	Genotype or description	Reference or source
<b>Bacterial Strains</b>		
<i>E. coli</i> DH5 $\alpha$	$\phi$ 80 $\Delta(lacZ)$ M15 $\Delta(argF-lac)$ U169 <i>endA1</i> <i>recA1</i> <i>hsdR17</i> (r-K m-K) <i>deoR</i> <i>thi-1</i> <i>supE44</i> <i>gyrA96</i> <i>relA1</i>	Lab stocks
<i>E. coli</i> MC1061	F <sup>-</sup> <i>araD139</i> $\Delta(ara-leu)$ 7696 <i>galE15</i> <i>galK16</i> $\Delta(lac)$ X74 <i>rpsL</i> (Str <sup>R</sup> ) <i>hsdR2</i> ( <i>rK</i> <sup>-</sup> <i>mK</i> <sup>+</sup> ) <i>mcrA</i> <i>mcrB1</i>	Lab stocks
GAS M49	Wild-type clinical isolate	Lab stocks
GBS A909	Wild-type clinical isolate	Lab stocks
<i>S. aureus</i> Newman	Wild-type lab strain	Lab stocks
$\Delta$ <i>hylB</i>	GBS A909 <i>hylB</i> mutant	This study
$\Delta$ <i>hylB</i> + <i>phylB</i>	GBS A909 <i>hylB</i> mutant complement with <i>hylB</i>	This study
$\Delta$ <i>hylB</i> + <i>S. aureus</i> hyl	GBS A909 <i>hylB</i> mutant complement with <i>hysA</i> hyl gene from <i>S. aureus</i> Newman	This study
$\Delta$ <i>hylB</i> + <i>S. coelicolor</i> hyl	GBS A909 <i>hylB</i> mutant complement with hyl gene from <i>S. coelicolor</i>	This study
GAS M49 + <i>phylB</i>	GAS M49 complement with <i>phylB</i>	This study
<b>Plasmids</b>		
pDCerm	Shuttle vector encoding erythromycin resistance cassette	Jeng et al. 2003
pKODestErm	Created for Gateway cloning	Locke et al. 2007
pUC19	Amp shuttle vector	Invitrogen
<b>Primers</b>		
	5'-3' orientation	
<u><i>GBS</i> <math>\Delta</math><i>hylB</i></u>		
<i>hylB</i> upst fwd	ATC CCGCGG CGAGCTGTTCTCTAACCCTA	This study
<i>hylB</i> dwnst rev	ATC GGATCC ACATACCTTATTGGTGCCGACGGT	This study
<i>hylB</i> upstr/cat rev	ATATCCAGTGATTTTTTCTCCAT GTGTGCTACTTTGATAGTAAATTGCC	This study
<i>hylB</i> dwnstr/cat fwd	GCGATGAGTGGCAGGGCGGGGCGTAAACTTGGGCTGTTA TCAAACACGA	This study
cat fwd	ATGGAGAAAAAATCACTGGATATACCACC	This study
cat rev	ATGGAGAAAAAATCACTGGATATACCACC	This study
<u>Complement</u>		
<i>hylB</i> compl fwd	AGGCAATTTACTATCAAAGTAGCAC	This study
<i>hylB</i> compl rev	TGCTGTAGAAGCCAAGTATG	This study
<u><math>\Delta</math><i>hylB</i> + <i>S. aureus</i> hyl (<i>hysA</i>)</u>		
<i>hysA</i> fwd	GTTTGGTCGTCTAAAGTAATGTTGC	This study
<i>hysA</i> rev	CAAAGGGGATGTGGTCTGCG	This study