SUPPLEMENTAL MATERIAL

Figure S1 KS levels in the plasma and air pouch exudates after GAS infection. At 12 h after hydrodynamic administration of the KS plasmid, control plasmid or saline, GAS was inoculated in the air pouch. Mice were sacrificed 24 and 48 h after infection and the plasma (**a**) and air pouch exudates (**b**) were collected for KS levels using ELISA. ND: not detectable at both 24 and 48 h.

Figure S2 KS has no direct effect on GAS growth rate. A fresh colony of GAS was inoculated in TSBY broth overnight and then transferred to fresh TSBY broth by 1:50 dilution with or without KS. At various time points, bacterial counts were determined by measuring the absorbance at 600 nm (**a**) and by plating (**b**).

Figure S3 KS increases the viability and reduces the apoptosis of infiltrating cells in the air pouch of GAS-infected mice. At 12 h after hydrodynamic administration of the KS plasmid, control plasmid or saline, GAS was inoculated in the air pouch, and the air pouch exudates were collected 12 h after infection ($n = 4$). The infiltrating cell viability was measured by trypan blue staining (**a**) and cell apoptosis was determined by PI staining followed by flow cytometry analysis (**b**). Experiments were repeated twice.*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

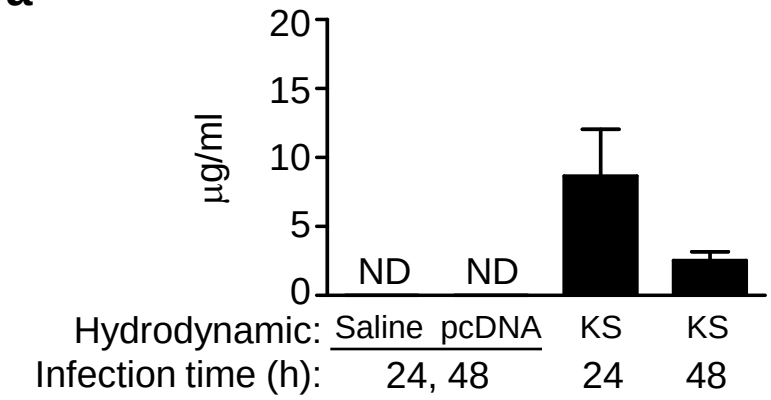
Figure S4 KS has no effect on SpeB activity, whereas SpeB cleaves KS. SpeB mutant protein C192S (**a**) and azocasein (**b**) were used as SpeB substrates. SpeB and its substrates were incubated with or without KS for 1 h at 37^{o}C. The cleavage form of the C192S was detected by SDS-PAGE followed by coomassie blue staining. The cleavage of Azo-dye was detected by measuring the absorbance at 405 nm. (**c**) Normal human serum was added with SpeB protein (full-length form of 42 kDa), and incubated for 1 h at 37^{o}C. SpeB auto-cleaves itself and an active form (28 kDa) can be

observed. KS was detected by specific antibody and at least three cleavage forms of KS were observed. E64 was used as a cysteine protease inhibitor.

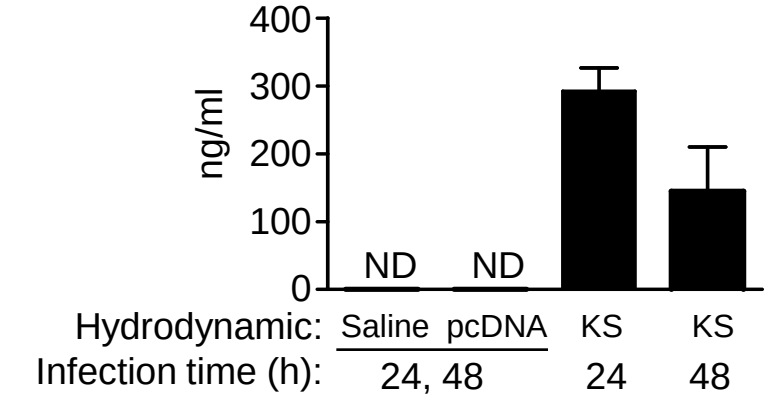
Figure S5 KS protects mice from SpeB-deficient GAS infection. (**a-d**) At 12 h after hydrodynamic injection, mice were infected with SpeB-deficient NZ131, SW574, (2.6×10^8 cfu/mouse) using an air pouch model. One mouse in pcDNA-treated group died on day 3 after infection and one in saline control group died on day 5 after infection. The diapedetic areas were photographed and measured on day 7 after infection. The skin lesions of the dead mice were determined while mice died. (**e**) At 12 h after hydrodynamic injection, mice were subcutaneously injected with SpeB-deficient A20 strain SW507 (3.64×10^8 cfu/mouse) and the survival rate was determined. N=10/group. * $P < 0.05$

Figure S6 KS protects mice when administrated 12 h after GAS infection. Mice were infected with GAS (NZ131; 1×10^8 cfu/mouse) using an air pouch model and hydrodynamic injection was performed at 12 h after infection. The survival rate was determined. N=18/group. * $P < 0.05$

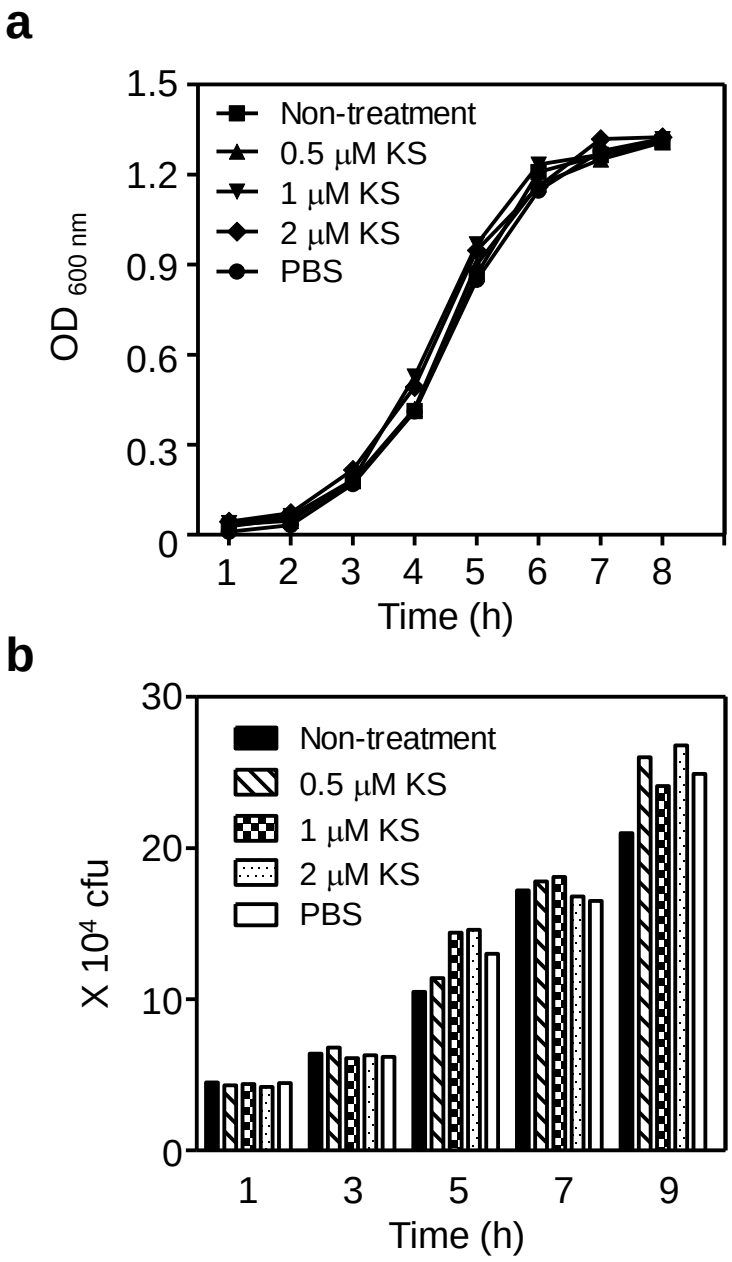
a



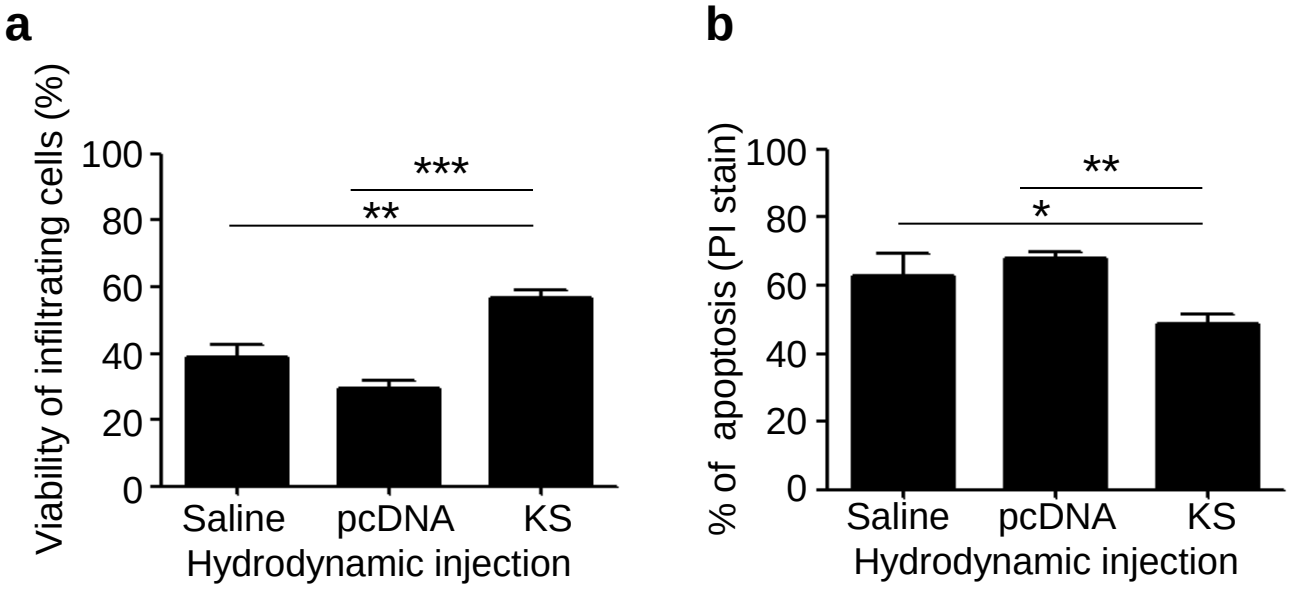
b



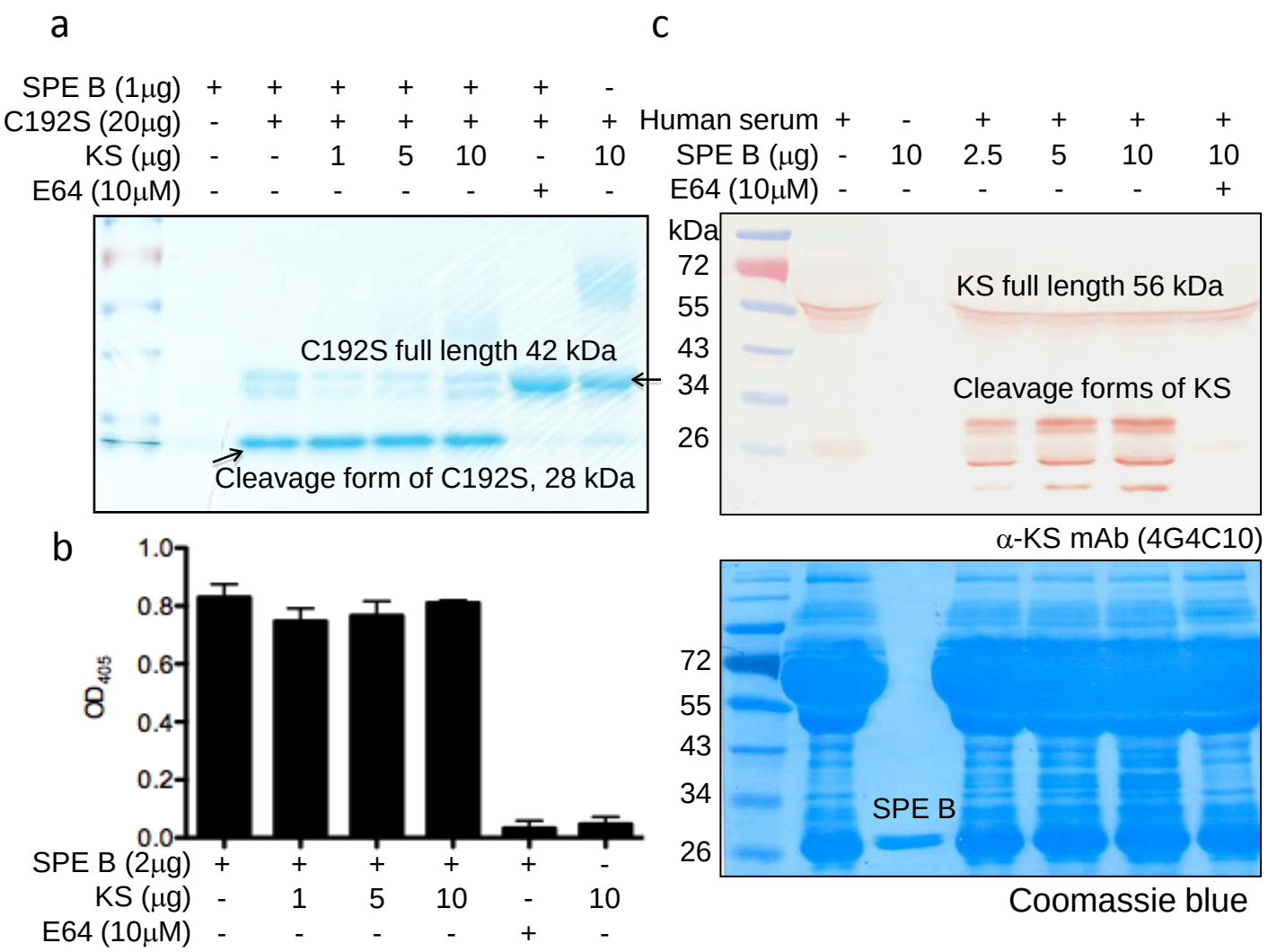
Supplementary Figure S1



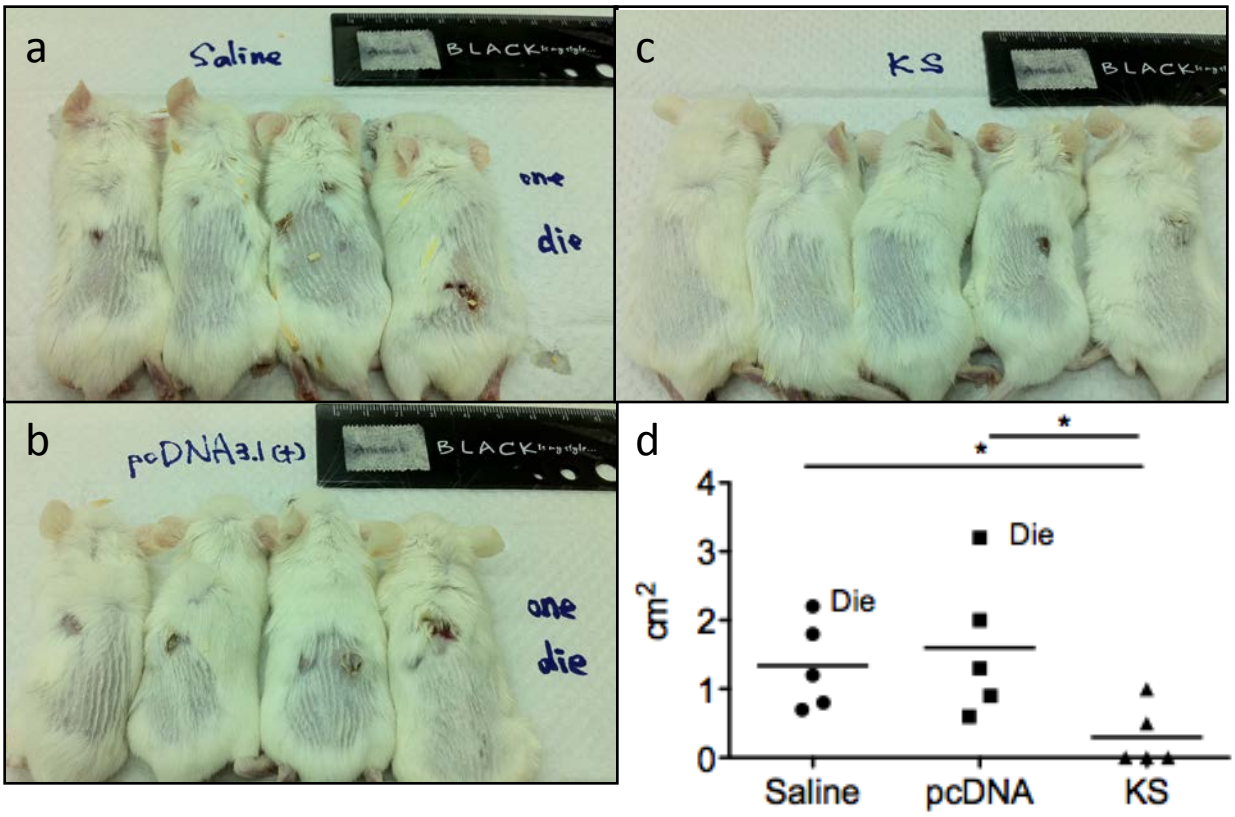
Supplementary Figure S2



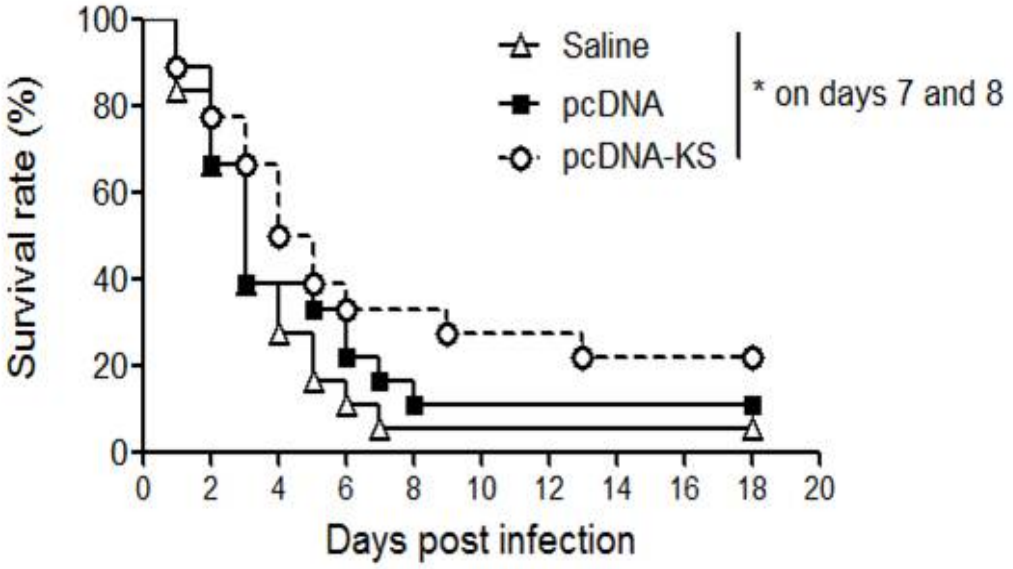
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6