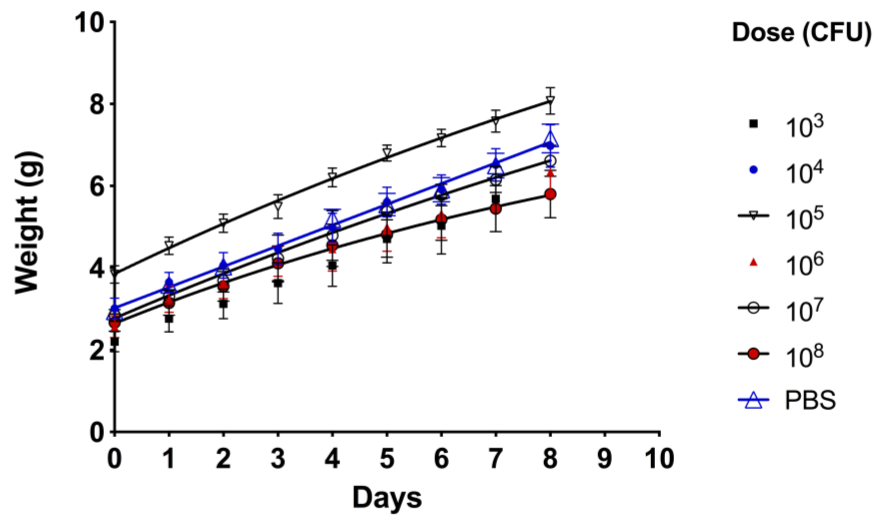

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3 **Figure S1**. *Pup weight unaffected by inocula <10^{8} CFU.* The weight (g) of pups over the course of
4 Day 1-8 post-inoculation in litters inoculated with MGAS10870 and control litters administered
5 PBS. Mean and SD of pup weights in experimental litters is shown. Linear regression of data for
6 selected inocula (10^{5}, 10^{7}, 10^{8}, PBS control) is shown to indicate rate of weight gain. Litters are
7 indicated by inocula (CFU) administered in the legend adjacent to the graphs and days post-
8 inoculation are enumerated on the x-axis.

Figure S2: Shedding and transmission of *emm3* serotype GAS in infant FVB/NJ and C57BL/6 mice is inoculum-dependent.

Figure S2. *Shedding and transmission of* emm3 *serotype GAS in infant FVB/NJ and C57BL/6 mice is inoculum-dependent.* **(a)** Shedding titers (CFU) of MGAS10870 from index mice in FVBN/J and **(b)** C57BL/6 litters inoculated at a 1:1 index-to-contact ratios were detected throughout the monitoring period (Days 1-9 post-inoculation). **(c,d)** The shedding titers of MGAS10870 from contact mice in the same such litters were likewise assessed. The median of shed titers detected daily is shown. Litters are indicated by inocula (CFU) administered in the legend adjacent to the graphs and days post-inoculation are enumerated on the x-axis. **(e)** The intranasal GAS tissue burden from contact and index mice in FVBN/J and **(f)** C57BL/6 litters were likewise assessed at

18 day 11 post-inoculation. The geometric mean of GAS burden (red line) is shown to indicate mean
19 colonization rate and burden. Litters are indicated by inocula (CFU) administered on the x-axis.
20 Limit of detection is indicated by dotted line (shedding titer = 1 CFU; colonization burden = 0.1
21 CFU/mg). Statistically significant differences in mean colonization burden were determined
22 between inocula by Mann-Whitney U-test (*p<0.05, **p<0.01, ***p<0.001, #p<0.0001).

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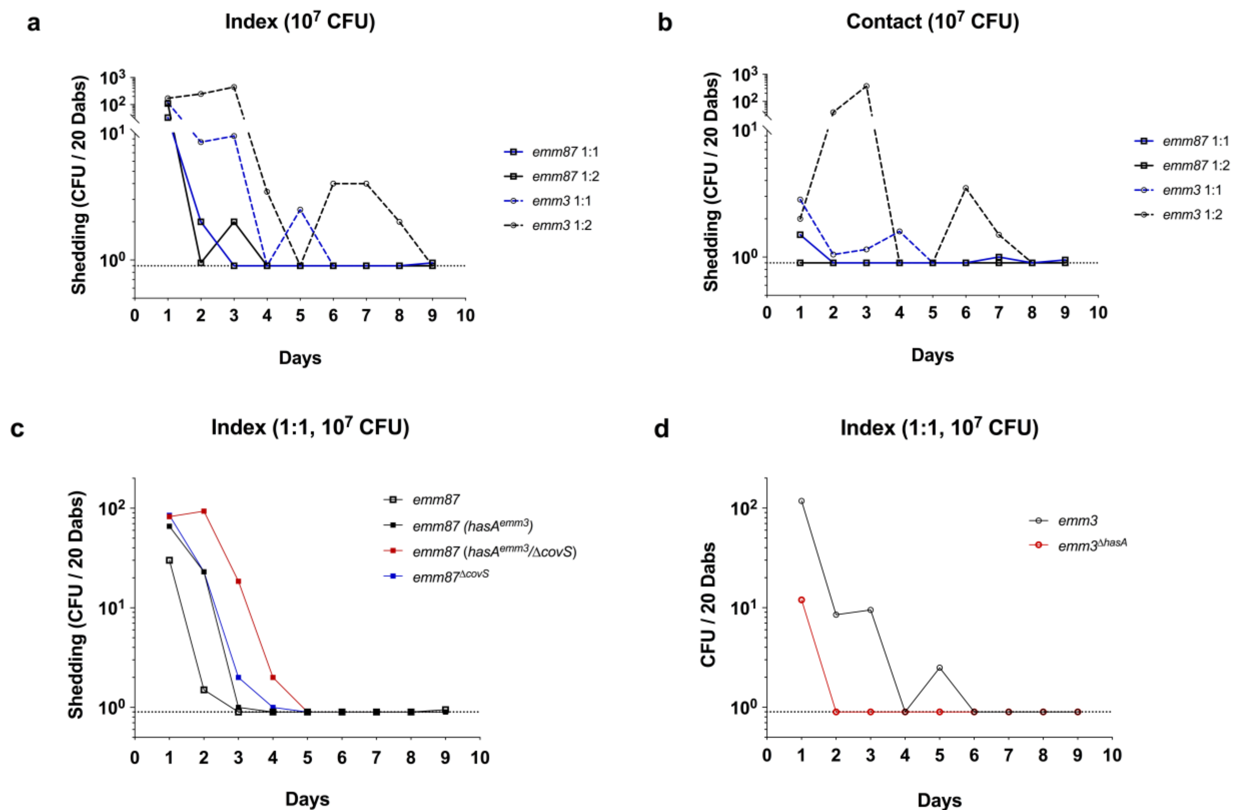

26 **Figure S3.** Production of hyaluronic acid capsule enhances GAS shedding from CD-1 mice

27 **Figure S3.** *Production of hyaluronic acid capsule enhances GAS shedding from CD-1 mice.* **(a)**
28 Shedding titers (CFU) of TSPY55 (*emm87*; solid lines) from index and **(b)** contact mice in CD-1
29 litters inoculated with 10^7 CFU at a 1:1 (blue) and 1:2 (black) index-to-contact ratios were
30 detected throughout the monitoring period (Days 1-9 post-inoculation) and compared to
31 detected shed titers of MGAS10870 (*emm3*; dashed lines). **(c)** The shedding titers (CFU) from
32 index mice in CD-1 litters inoculated at a 1:1 index-to-contact ratio with 10^7 CFU of *emm87* strains

33 mutagenized in *hasA* and *covS* are compared to shedding titers of TSPY55 (*emm87*) throughout
34 the monitoring period. **(d)** The shedding titers from index mice in CD-1 litters inoculated at a 1:1
35 index-to-contact ratio with 10^7 CFU of *emm3*$^{\Delta hasA}$ is compared to shedding titers of MGAS10870
36 (*emm3*) throughout the monitoring period. The median of shed titers detected daily is shown.
37 Shedding titers detected from *emm3* strains shown in Figure 2 are reproduced here for ease of
38 comparison. Litters are indicated by strain name in the legend adjacent to the graphs and days
39 post-inoculation are enumerated on the x-axis.
40
41 **Table S1.** Primers and plasmids used in study.

Primer	Sequence	Reference
MSP114	TGGGTTATTATAATGCATTC	This Study
MSP116	TTTGCAATTAGTTCTGGGCT	This Study
MSP121	GTGCCTATTTTTAAAAAAACTTTAATTGTTTTATCCAATACATGTTATAATAACTATAAC	This Study
MSP122	TTATTTAAAAATAGTGACCTTTTTACGTGTTCCTTATAATTTTTTTAATCTGTTATTTAAATAG	This Study
MSP123	GTTATAGTTATTATAACATGTATTGGATAAAACAATTAAAGTTTTTTTAAAAATAGGCAC	This Study
MSP124	CTATTTAAATAACAGATTAAAAAAATTATAAGGAACACGTAAAAAGGTCACTATTTTTAAATAA	This Study
MSP408	ATATGAGATGGATCCCATAGTTTTC	This Study
MSP409	ACTGTATTAAAGATATCGAATCAAG	This Study
MSP410	CGTTATTCGTGAGAAATAAGTCATATGATTGAACAAGATGGATTGCACG	This Study
MSP411	CGTGCAATCCATCTTGTTCAATCATATGACTTATTTCTCACGAATAACG	This Study
MSP412	CACATTAACAATGCCTTAAGCTACTTCAGAAGAACTCGTCAAGAAGGCG	This Study
MSP413	CGCCTTCTTGACGAGTTCTTCTGAAGTAGCTTAAGGCATTGTTAATGTG	This Study
MSP459	AAACTCGAGAACATCGATCATCCCCAATGC	This Study
MSP460	AAAGGATCCAGATTGATGAACTGCTCTTTTT	This Study

Plasmid	Description	
pJL1055	*E. coli* Gram-positive shuttle vector derived from pWV01 for allelic replacement mutagenesis	(3)
pMAS27	A 1930bp fragment spanning N-terminus of *hasA* and upstream promoter region in pJL1055 vector	This study
pMAS20	Kanamycin cassette (*aph*) between regions of homology flanking *covS* coding sequence in pCR™II vector	This study
pJSF29	Spectinomycin cassette (*aad9*) between regions of homology flanking *hasA* coding sequence in pCR™2.1 vector	This study

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43

44 **Supplemental Materials and Methods**

45

46 *Generation of isogenic mutants in MGAS10870 and TSPY55*. Plasmids and primers used in this
47 study are listed in Table S1. We used a previously described procedure for generating the capsule
48 and *covS* mutants in MGAS10870 (*emm3*) and TSPY55 (*emm87*) strains 3 (1, 2). Briefly, the *hasA*
49 gene in MGAS10870 was replaced with a spectinomycin resistance cassette (*aad9*) using plasmid
50 pJSF29 to generate $emm3^{\Delta hasA}$. Regions flanking *hasA* (*SpyM3_1851*) were amplified using primer
51 pairs MSP114/MSP124 and MSP116/MSP123. The *aad9* cassette was amplified using primers
52 MSP121 and MSP122. The *hasA*-flanking regions and *aad9* cassette were joined by overlap
53 extension PCR using MSP114 and MSP116. The PCR product was given an A overhang with GoTaq
54 and ligated into pCR™2.1-TOPO® using TOPO® TA cloning kit (ThermoFisher, K450001) per the
55 manufacturer's instructions to generate pJSF29.

56 The *emm87*($hasA^{emm3}$) mutant was generated in the TSPY55 background using plasmid
57 pMAS27. A 1930bp fragment spanning the 783 N-terminal nucleotides of *hasA* and the upstream
58 promoter was amplified from MGAS10870 using primers MSP459 and MSP460. The PCR product
59 was inserted into the pJL1055 vector using BamHI and XhoI restriction sites to generate pMAS27.
60 Allelic replacement using pMAS27 repaired the single nucleotide frameshift insertion in the
61 TSPY55 *hasA* coding sequence and replaced the native *hasA* promoter with that of the *emm3*
62 serotype.

63 The *covS* gene was deleted by allelic replacement with a kanamycin resistance cassette
64 (*aph*) using plasmid pMAS20 to generate the *emm87*$^{\Delta covS}$ and *emm87*(*hasA*^{emm3}/*ΔcovS*) strains.
65 Regions flanking *covS* (*SpyM3_0245*) were amplified using primer pairs MSP408/MSP411 and
66 MSP413/MSP409. The *aph* cassette was amplified using primers MSP410 and MSP412. The *covS*-
67 flanking regions and *aph* cassette were joined by overlap extension PCR using MSP408 and
68 MSP409 primers. The PCR product was inserted into a pCR™II-TOPO® vector to generate
69 pMAS20. The pJSF29 and pMAS20 plasmids were linearized by restriction digest with XmnI to
70 disrupt the ampicillin resistance cassette of the pCR™2.1-TOPO® and pCR™II-TOPO® vectors
71 prior to transformation into GAS. The *aph* and *aad9* resistance cassettes were stably integrated
72 in mutagenized strains as confirmed by PCR amplification following growth in the absence of
73 antibiotic selection. PCR amplification was performed using either the Phusion® (NEB, #M0530S)
74 or Q5® (NEB, #M049S) high fidelity polymerases according to manufacturer's specifications.
75
76 *Statistical methods*. For mouse model experimentation, 3 pup litters were used per each GAS
77 strain, inoculum and index-to-contact ratio tested, unless otherwise indicated. A minimum n=12
78 total infant mice were used per test condition for adequate study power (α=0.05; β=0.2). The
79 geometric mean of shed titers and bacterial intranasal tissue burden were calculated to assess
80 streptococcal output, and the rates of transmission and persistence. Differences in transmission
81 frequency between GAS strains and experimental conditions (i.e. index-to-contact ratios and
82 inocula) were tested for statistical significance by comparison of numbers of infected contact
83 mice using 2X2 contingency test (Fisher's exact test, two tailed; p<0.05). An unpaired U-test
84 (Mann-Whitney) was used to assess statistical significance of differences in mean colonization
85 burden and median shedding titers between mouse experimental data sets (p<0.05). For assays
86 measuring hyaluronan capsule production, epithelial cell adherence and survival in whole blood,
87 an unpaired t-test was used to assess statistical significance of differences between data sets
88 (p<0.05).
89

90 **References**

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