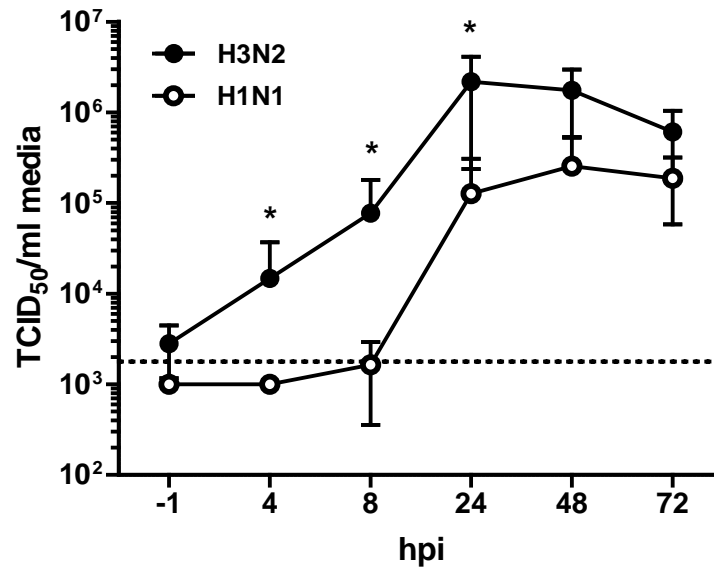


**FIG S1** Replication rates of *S. suis* strain 10, 10Δsly, and 10cpsΔEF on mono- and virus pre-infected porcine PCLS.

Growth kinetics of streptococci determined by counting of colony forming units (CFU) in the supernatant of infected PCLS after replica plating on blood agar plates. (A) PCLS were infected with approximately  $1 \times 10^7$  CFU of the different *S. suis* strains and washed thoroughly after 4 hours (indicated by arrowhead) to remove non-adherent bacteria. Slices were incubated for an additional time period of 20 hours. Supernatant of infected cells were plated at indicated time points. (B) PCLS were pre-infected with  $1 \times 10^5$  TCID<sub>50</sub>/ml of either H1N1 or H3N2 for 1 hour, washed and subsequently infected with *S. suis* strain 10 or 10cpsΔEF as described for bacterial mono-infection. Slices mono-infected with 10 or 10cpsΔEF only served as control. Growth of *S. suis* was recorded over a period of 24 hours.

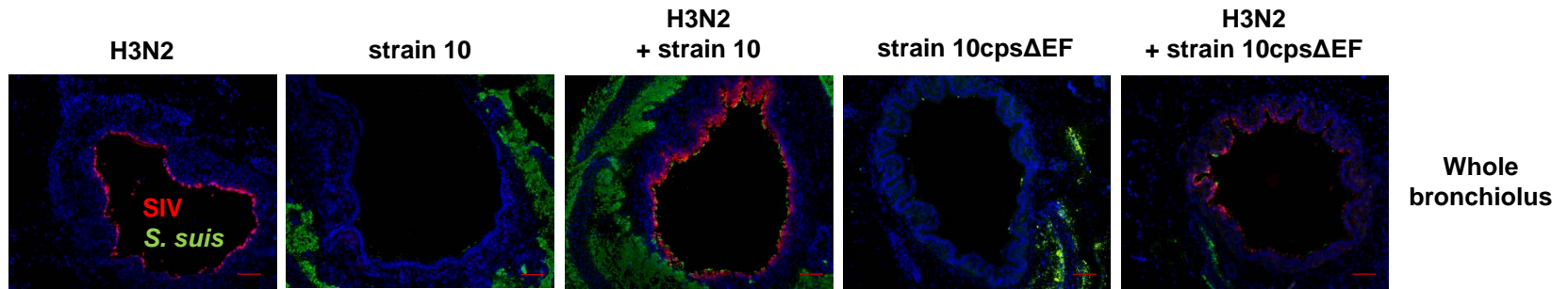


**FIG S2 Infectivity kinetics in supernatants of virus-infected porcine PCLS.** PCLS were infected with  $1 \times 10^5$  TCID<sub>50</sub>/ml of either H3N2 or H1N1 for one hour, washed and incubated for 72 hours (hpi). Infectious virus release into the supernatant was titrated by endpoint dilution titration (TCID<sub>50</sub>/ml media; 50% tissue culture infection dose/ml). Results are expressed as mean with SD. Significant differences are indicated by \* ( $P$ -value < 0.05), Mann-Whitney U-test.

**TABLE S1 Ciliary activity of SIV pre-infected porcine PCLS super-infected with *S. suis*.**

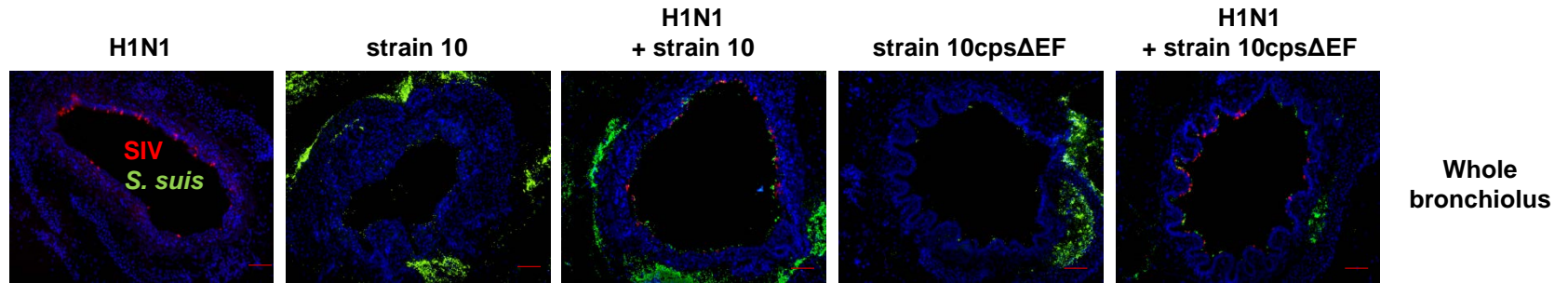
Treatment	ciliary activity [%] <sup>a</sup>					
	0 hpi	4 hpi	8 hpi	24 hpi	48 hpi	72 hpi
Ctr	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	97 ± 2.5	97 ± 2.5
H1N1	100 ± 0.0	100 ± 0.0	100 ± 0.0	99 ± 0.8	97 ± 0.7 <sup>††</sup>	97 ± 1.7 <sup>†††</sup>
H3N2	100 ± 0.0	100 ± 0.0	100 ± 0.0	91 ± 5.7	36 ± 24.2 <sup>***</sup>	16 ± 17.6 <sup>***</sup>
10	100 ± 0.0	100 ± 0.0	100 ± 0.0	99 ± 2.0	91 ± 5.6 <sup>††</sup>	87 ± 4.4 <sup>†††</sup>
H1N1 + 10	100 ± 0.0	100 ± 0.0	97 ± 3.1	93 ± 3.5	86 ± 7.4	78 ± 7.1
H3N2 + 10	100 ± 0.0	100 ± 0.0	99 ± 0.9	90 ± 5.8	39 ± 22.3 <sup>***</sup>	17 ± 19.2 <sup>***</sup>
10cpsΔEF	100 ± 0.0	100 ± 0.0	100 ± 0.0	96 ± 2.5	92 ± 2.9 <sup>#</sup>	86 ± 2.5 <sup>###</sup>
H1N1 +10cpsΔEF	100 ± 0.0	100 ± 0.0	100 ± 0.0	95 ± 3.5	90 ± 5.5	79 ± 6.6
H3N2 +10cpsΔEF	100 ± 0.0	100 ± 0.0	99 ± 1.0	93 ± 5.0	44 ± 33.0 <sup>**</sup>	24 ± 21.3 <sup>***</sup>

<sup>a</sup> Ciliary activity was estimated by analyzing ciliary beating of either H3N2 or H1N1 pre-infected and *S. suis* super-infected PCLS in comparison to uninfected slices (Ctr) under the light microscope up to 72 hours post infection (hpi). Results are expressed as mean with SD. Significant differences are indicated by \*\* (*P*-value < 0.01) and \*\*\* (*P*-value < 0.001) for Ctr versus infected slices, ++ (*P*-value < 0.01) and +++ (*P*-value < 0.001) for 10 versus H3N2 + 10, # (*P*-value < 0.05) and ### (*P*-value < 0.001) for 10cpsΔEF versus H3N2 + 10cpsΔEF and †† (*P*-value < 0.01) and ††† (*P*-value < 0.001) for H1N1 versus H3N2, respectively, one-way-ANOVA followed by a Tukey *post-hoc* test.



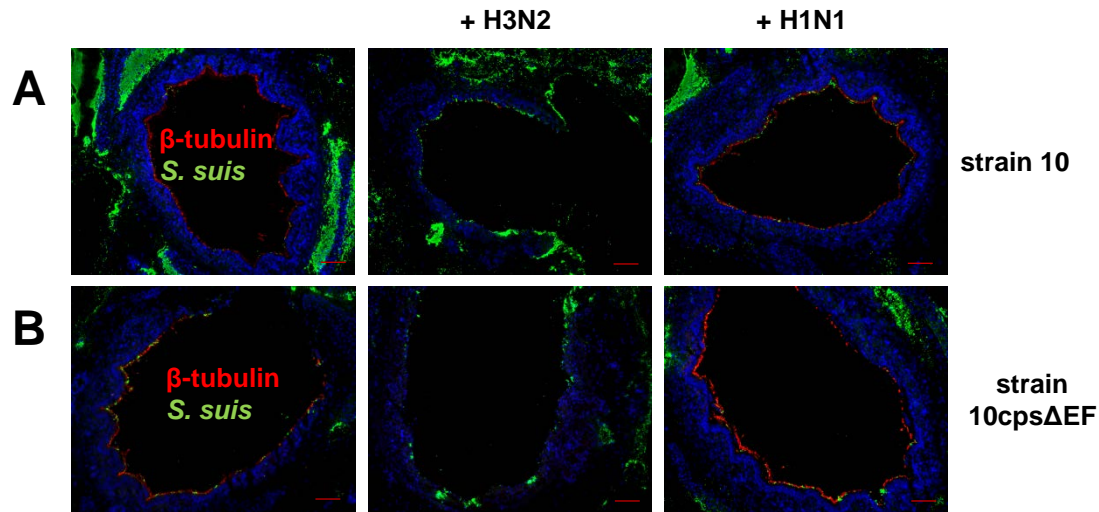
**FIG S3 Adherence and colonization of *S. suis* on H3N2 pre-infected porcine PCLS.**

Porcine PCLS were pre-infected with  $1 \times 10^5$  TCID<sub>50</sub>/ml H3N2 for 1 hour, washed, and subsequently infected with *S. suis* strain 10 or 10cpsΔEF as described for bacterial mono-infection. Slices mono-infected with strain 10, 10cpsΔEF, and H3N2 served as controls. Cryosection of slices followed by immunostaining was performed 24 hours post bacterial infection. Streptococci are labeled in green, nucleoproteins of SIV are stained in red and nuclei are shown in blue (DAPI). Overview images showing whole bronchioli of mono- and co-infected porcine PCLS used for quantification of adherent bacteria (cf. Fig. 4). Bars represent 100 μm.



**FIG S4 Adherence and colonization of *S. suis* on H1N1 pre-infected porcine PCLS.**

Porcine PCLS were pre-infected with  $1 \times 10^5$  TCID<sub>50</sub>/ml H1N1 for 1 hour, washed, and subsequently infected with *S. suis* wild-type strain 10 or 10cpsΔEF as described for bacterial mono-infection. Slices mono-infected with strain 10, 10cpsΔEF, and H1N1 served as controls. Cryosection of slices followed by immunostaining was performed 48 hours post bacterial infection. Streptococci are labeled in green, nucleoproteins of SIV are stained in red and nuclei are shown in blue (DAPI). Overview images showing whole bronchioli of mono- and co-infected porcine PCLS used for quantification of adherent bacteria (cf. Fig. 4). Bars represent 100 μm.



**FIG S5 Adherence and invasion of the bronchiolar epithelium of *S. suis* on porcine PCLS pre-infected with SIV at late stage of infection.**

Porcine PCLS were pre-infected with  $1 \times 10^5$  TCID<sub>50</sub>/ml H3N2 or H1N1 for 1 hour, washed and subsequently infected with *S. suis* strain 10 (A) or 10cpsΔEF (B) as described for bacterial mono-infection. Slices mono-infected with strain 10 and 10cpsΔEF only served as control. Cryosection of slices followed by immunostaining was performed 72 hours post bacterial infection. Streptococci are shown in green, ciliated cells were stained using anti-β-tubulin antibody (red) and nuclei were labeled by DAPI (blue). Overview images showing whole bronchioli of mono- and co-infected porcine PCLS used for quantification of adherent bacteria (cf. Fig. 4). Bars represent 100 μm.