Growth heterogeneity of *Streptococcus suis* in host-mimicking conditions is independent of clinical origin

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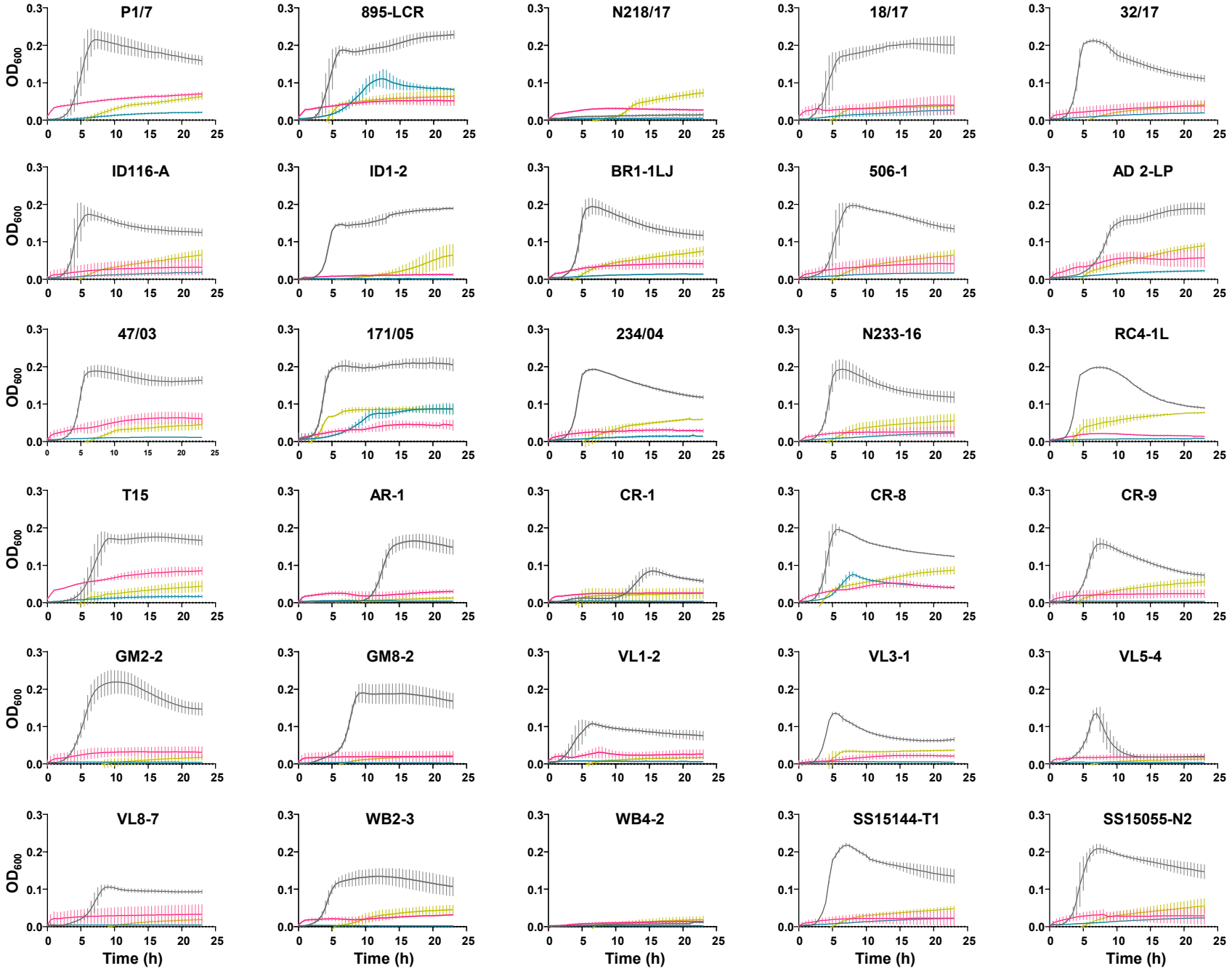
P1/7
895-LCR
N218/17
18/17
32/17
ID116-A
ID1-2
BR1-1LJ
506-1
AD 2-LP
47/03
171/05
234/04
N233-16
RC4-1L
T15
AR-1
CR-1
CR-8
CR-9
GM2-2
GM8-2
VL1-2
VL3-1
VL5-4
VL8-7
WB2-3
WB4-2
SS15144-T1
SS15055-N2
OD600
Time (h)
THY
HPLM
SNM
SNM + 0,5% Mucin

Figure S1. Growth curves of *Streptococcus suis* strains isolates from different clinical origins. Growth kinetics of 28 *S. suis* strains, including the reference strains P1/7 and T15, were assessed in THY (gray line), human plasma-like medium (HLPM; pink line), synthetic nasal medium (SNM; blue line), and SNM supplemented with 0.5% mucin (SNM+M; dark yellow line). The strain collection included isolates obtained from systemic lesions, lung, nasal cavity, and tonsils, as described in Table 1 of the main text. Growth was monitored by measuring the optical density at 600 nm (OD_{600}). Data represent the mean ± standard deviation of at least two independent biological replicates, each including three technical replicates.

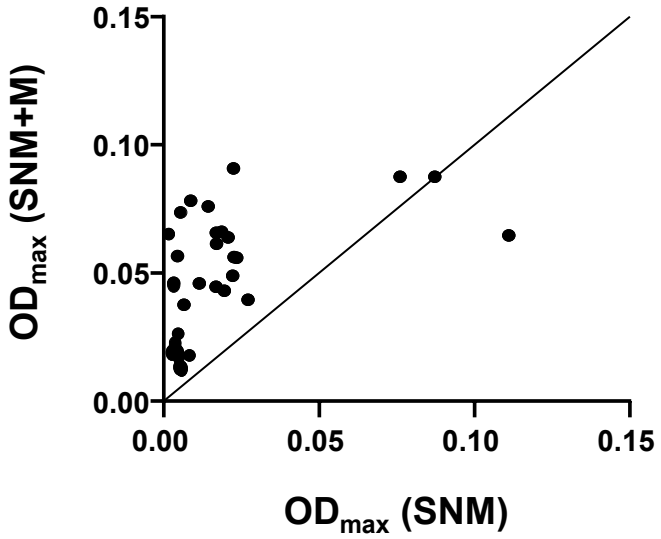

Figure S2. Comparison of *Streptococcus suis* growth in synthetic nasal medium with or without mucin. *S. suis* strains were grown in synthetic nasal medium (SNM) and SNM supplemented with 0.5% mucin (SNM+M) and growth was assessed by measuring the optical density at 600 nm (OD600). Results are expressed as the maximal OD600 (ODmax) after 24h of incubation. The line represents a linear one-to-one correlation.

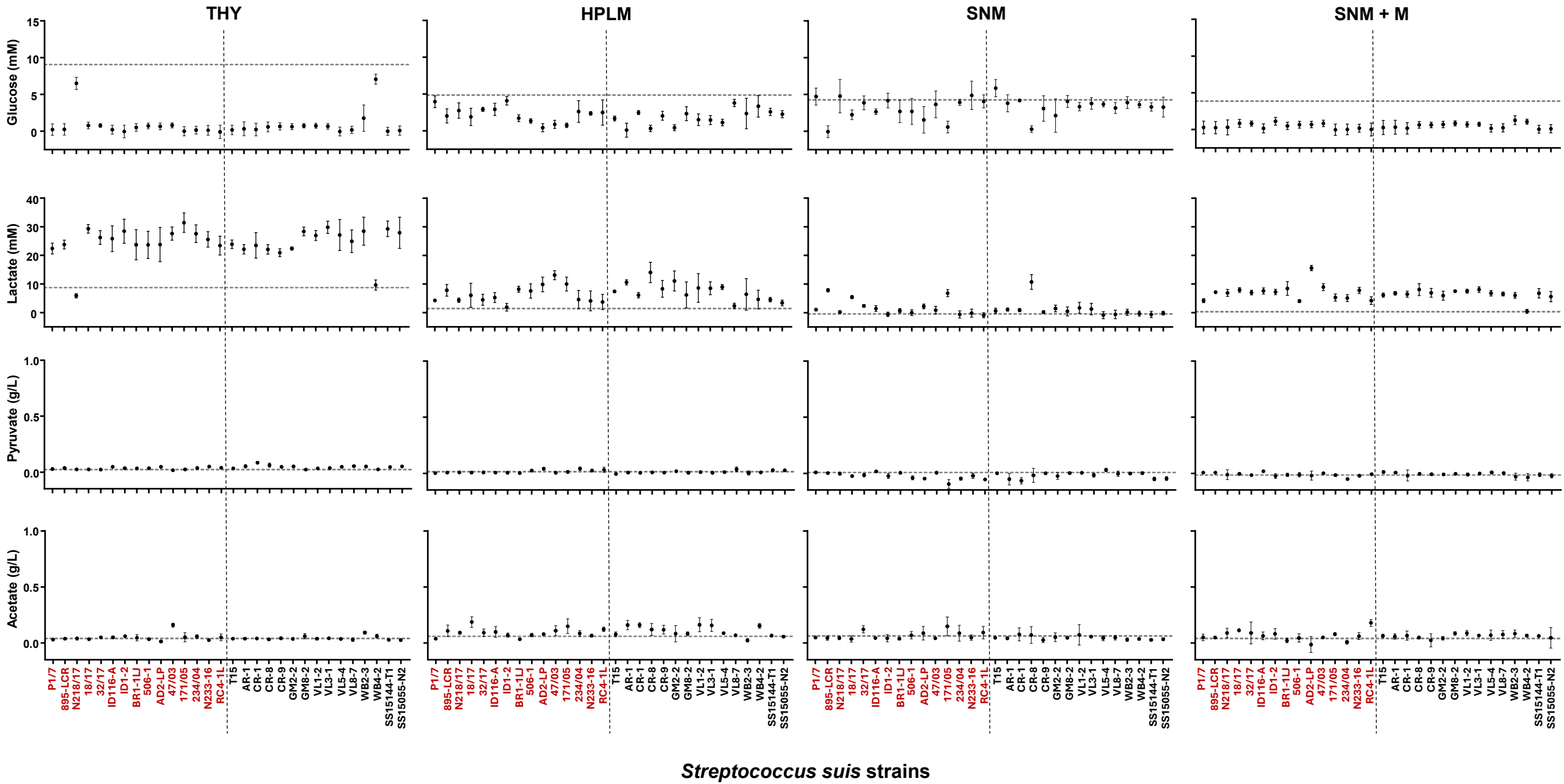
THY
HPLM
SNM
SNM + M
Glucose (mM)
Lactate (mM)
Pyruvate (g/L)
Acetate (g/L)
P1/7
895-LCR
N218/17
18/17
32/17
ID116-A
ID1-2
BR1-1LJ
506-1
AD2-LP
47/03
171/05
234/04
N233-16
RC4-1L
T15
AR-1
CR-1
CR-8
CR-9
GM2-2
GM8-2
VL1-2
VL3-1
VL5-4
VL8-7
WB2-3
WB4-2
SS15144-T1
SS15055-N2
Streptococcus suis strains

Figure S3. Key metabolites from systemic and upper respiratory tract *Streptococcus suis* strains grown in host-like media. *S. suis* strains (x-axis) were grown in THY, human plasma-like medium (HPLM), synthetic nasal medium (SNM), and SNM supplemented with 0.5% mucin (SNM+M). After 24h of incubation, glucose, lactate, pyruvate and acetate were quantified. Results are presented as the mean and standard deviation of two independent experiments, each performed with three technical replicates per condition. Systemic strains are indicated in red, whereas upper respiratory tract commensal strains are shown in black. Dashed gray lines indicated the level of the metabolite in each fresh medium.

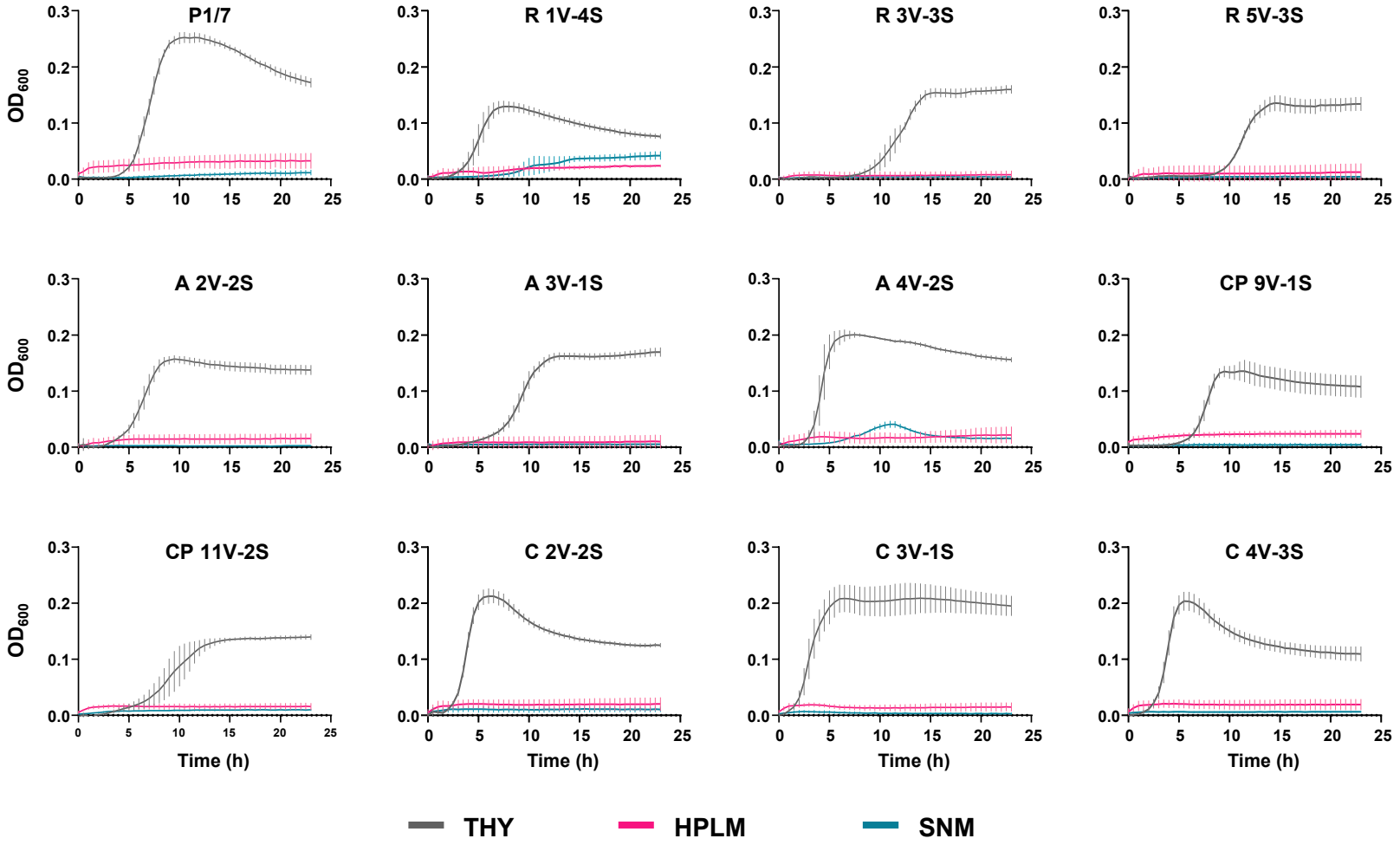

Figure S4. Growth curves of 11 vaginal *Streptococcus suis* strains. Strains isolated from vaginal samples were cultured in THY (gray line), human plasma-like medium (HLPM; pink line) and synthetic nasal medium (SNM; blue line). Growth was monitored by measuring optical density at 600 nm (OD_{600}). Strain P1/7 was used as reference. Data represent the mean ± standard deviation of at least two biological replicates, each including three technical replicates.

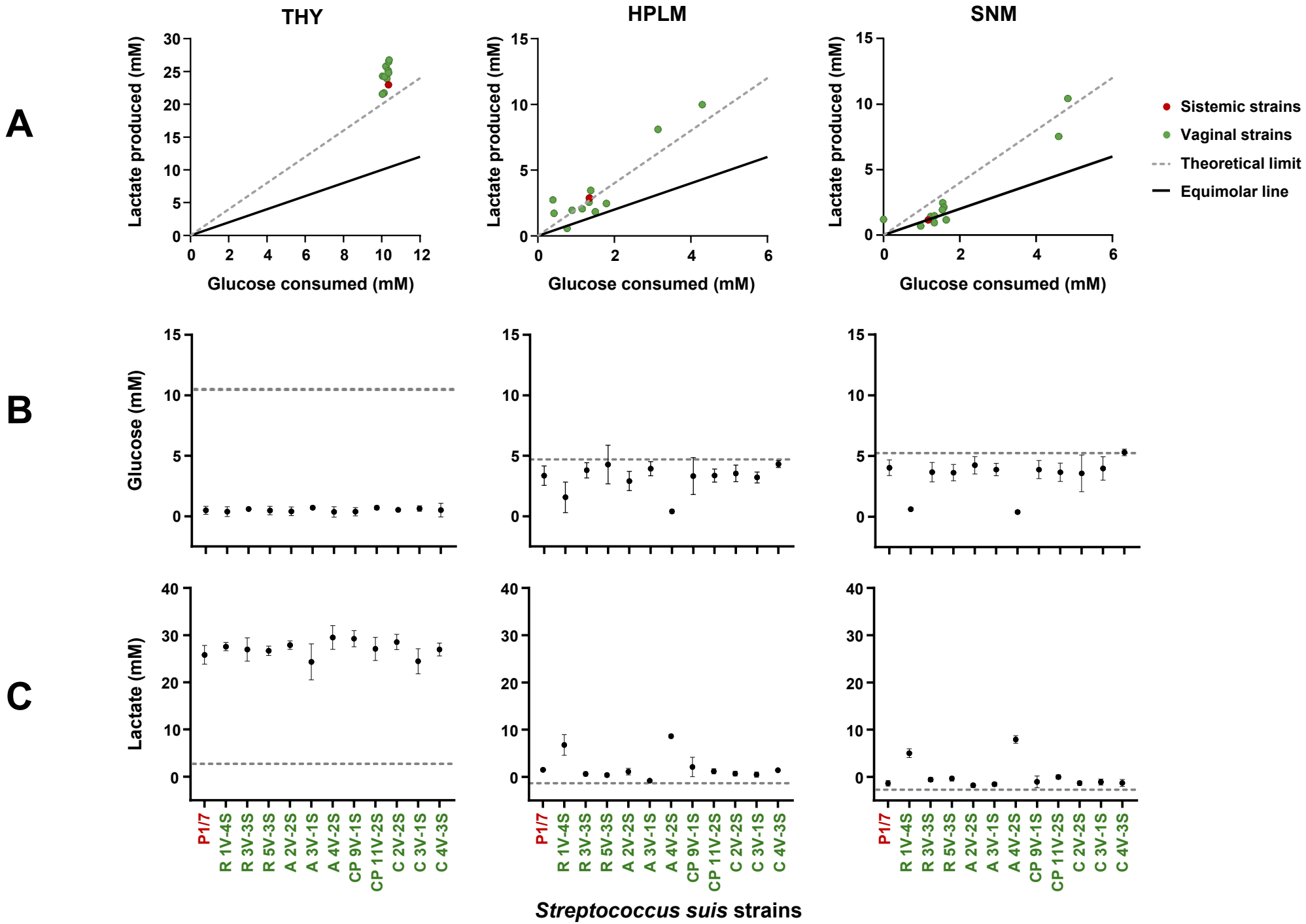
A
B
C
THY
HPLM
SNM
Lactate produced (mM)
Glucose consumed (mM)
Sistemic strains
Vaginal strains
Theoretical limit
Equimolar line
Glucose (mM)
Lactate (mM)
P1/7
R 1V-4S
R 3V-3S
R 5V-3S
A 2V-2S
A 3V-1S
A 4V-2S
CP 9V-1S
CP 11V-2S
C 2V-2S
C 3V-1S
C 4V-3S
Streptococcus suis strains

Figure S5. Glucose consumption and lactate production in vaginal *Streptococcus suis* strains grown in host-like media. Glucose depletion and lactate production (A) (mean, mM) were quantified in cultures of *S. suis* strains grown in THY, human plasma-like medium (HPLM) and synthetic nasal medium (SNM). Continuous lines denote an equimolar relationship of glucose to lactate, and the dashed lines denote the theoretical limit (i.e. 2 mol lactate per mol glucose) if glucose is the sole carbon source. Glucose (B) and lactate (C) concentration (mean and standard deviation) for each *S. suis* strain (x-axis) are also shown. Results were obtained in two independent experiments, each performed with three technical replicates per condition. The systemic strain P1/7 served as reference and it is indicated in red, whereas vaginal strains are shown in green.

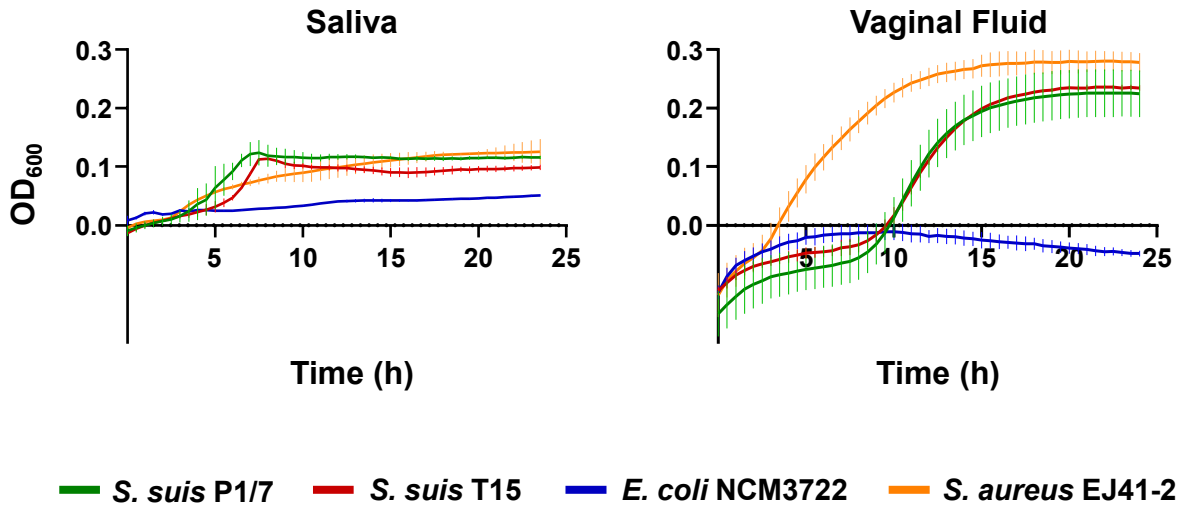

Figure S6. Growth kinetics of *Streptococcus suis* in porcine saliva and vaginal fluid. Growth curves of *S. suis* strains P1/7 (green line) and T15 (red line), *Escherichia coli* NCM3722 (blue line), and *Staphylococcus aureus* EJ41-2 (orange line) cultured in porcine saliva (left panel) or vaginal fluid (right panel). Data represent the mean ± standard deviation of at least two independent biological replicates, each including three technical replicates, based on optical density measurements at 600 nm (OD_{600}) over 24h.

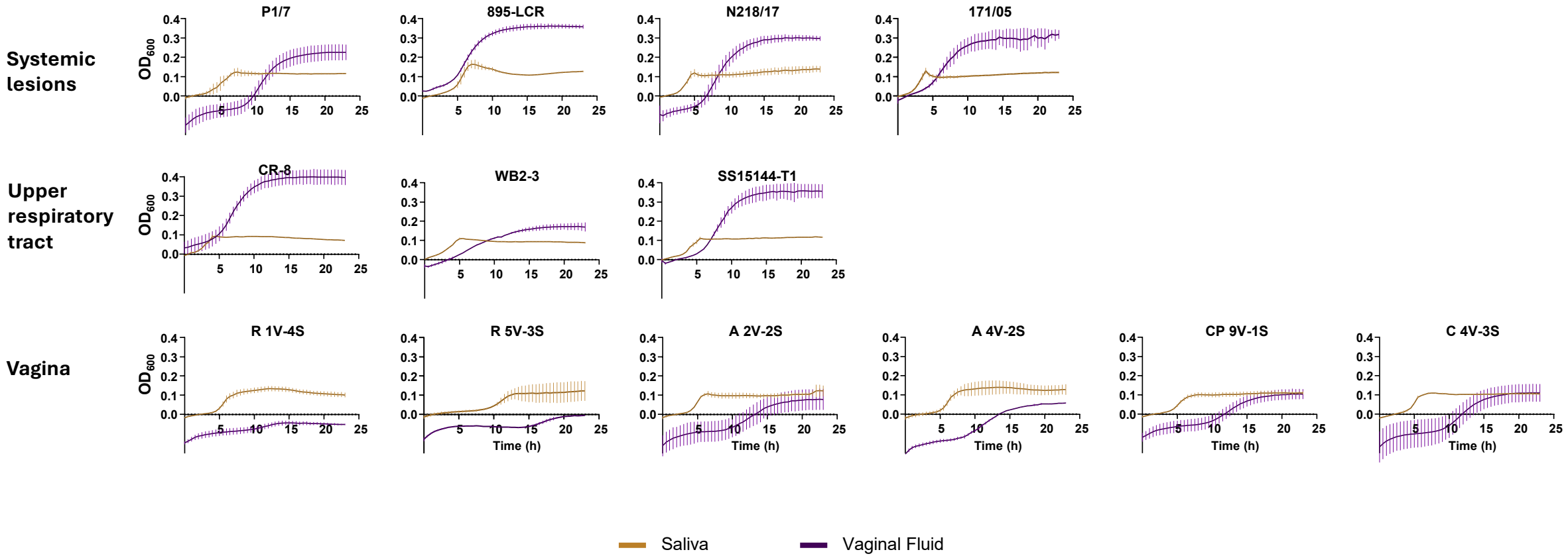

Figure S7. Growth curves of *Streptococcus suis* strains in saliva and vaginal fluid. Strains isolated from systemic lesions (n=3), the nasal cavity (n=2), tonsils (n=1), and vaginal samples (n=6) were grown in saliva (brown line) and vaginal fluid (purple line). Growth was monitored by measuring optical density at 600 nm (OD_{600}). Data represent the mean ± standard deviation of at least two biological replicates, each including three technical replicates.

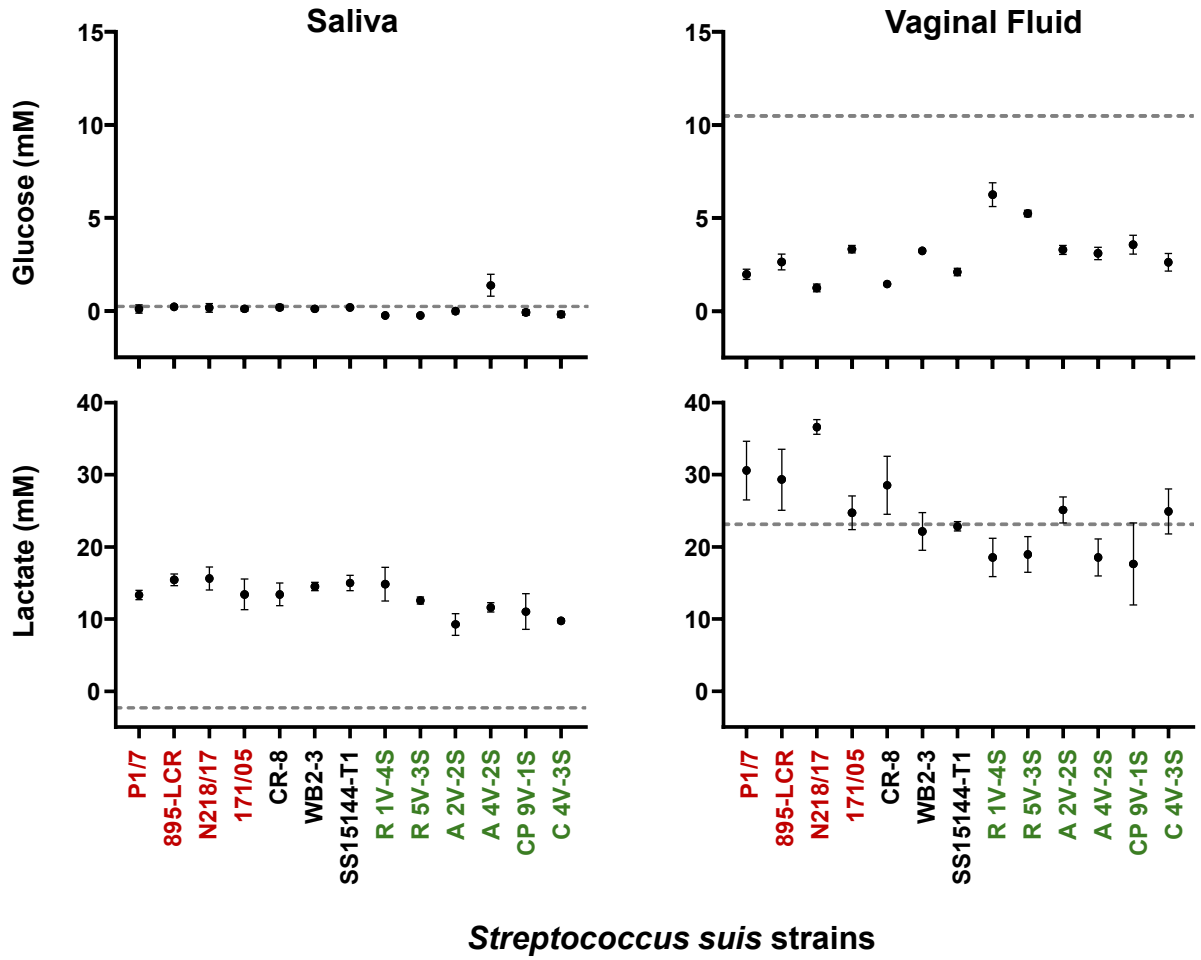

Figure S8. Glucose and lactate in cultures of *S. suis* isolates grown in porcine saliva and vaginal fluid. Glucose and lactate concentration (mM) were quantified in cultures of *S. suis* strains (x-axis) grown in saliva and vaginal fluid. Strains included isolates from systemic lesions (n=3), the nasal cavity (n=2), tonsils (n=1), and vaginal samples (n=6). Data represent the mean ± standard deviation of two independent experiments, each performed with three technical replicates per condition. Strains are color-coded according to their origin: systemic isolates in red, nasal and tonsillar isolates in black, and vaginal isolates in green.

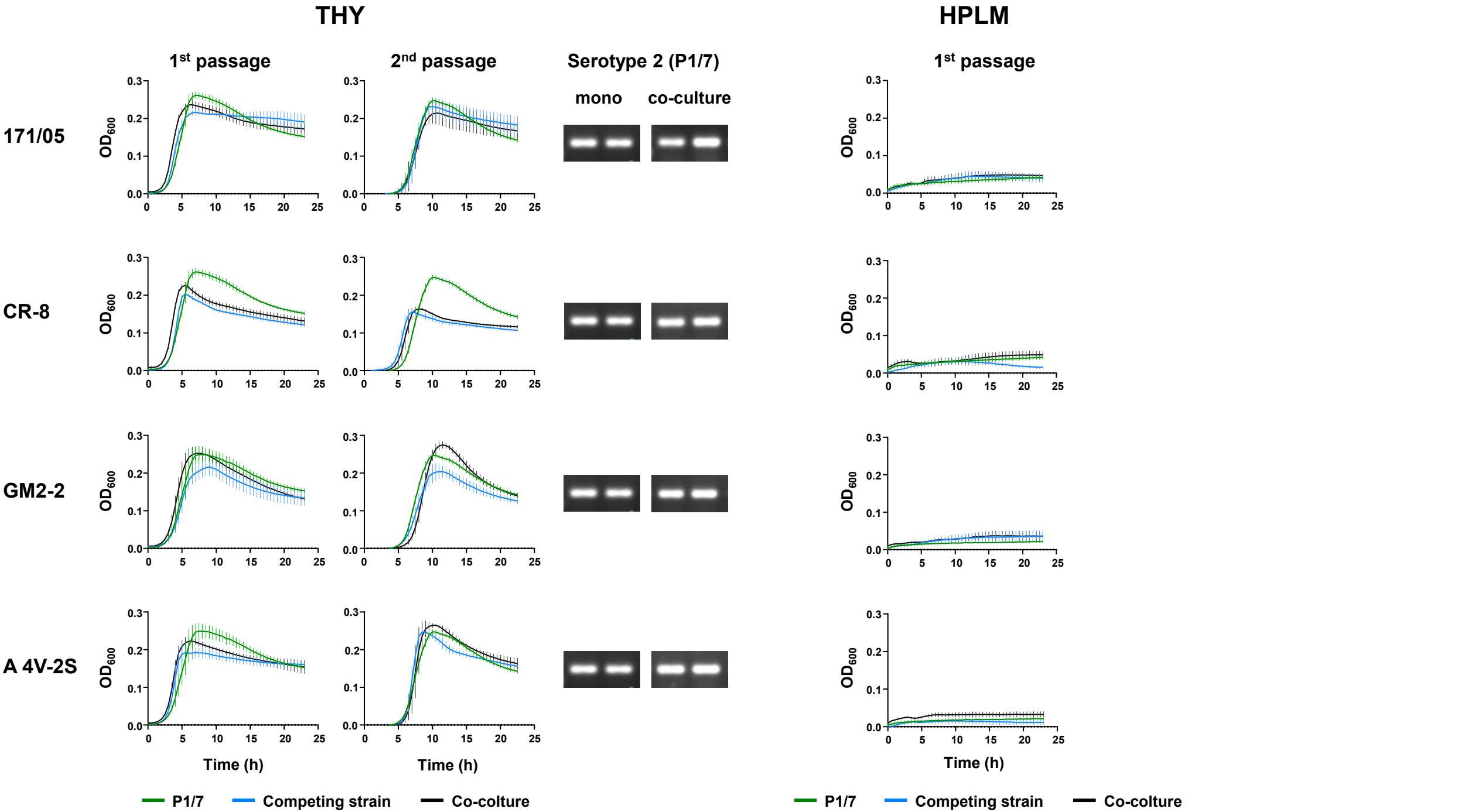
THY
HPLM
1st passage
2nd passage
Serotype 2 (P1/7)
1st passage
mono
co-culture
171/05
CR-8
GM2-2
A 4V-2S
OD600
Time (h)
P1/7
Competing strain
Co-colture

Figure S9. Growth dynamics of *Streptococcus suis* P1/7 co-cultures across sequential passages in THY and human plasma-like media. Co-cultures of *S. suis* P1/7 (serotype 2) with four additional isolates representing different anatomical origins, one systemic (171/05), two nasal (CR-8 and GM2-2), and one vaginal strain (A4V-2S), were performed in THY and human plasma-like medium (HPLM). Growth curves in THY correspond to the initial culture (first passage) and a subsequent subculture (second passage). In HPLM, only the first passage was performed. Growth was monitored by measuring optical density at 600 nm (OD_{600}). Data represent the mean ± standard deviation of at least two independent biological replicates, each including three technical replicates. Detection of P1/7 in mono and co-cultures was achieved using a serotype 2-specific PCR. In the growth curves, P1/7 is shown in green, competing strains are shown in light blue, and co-cultures are represented in black.

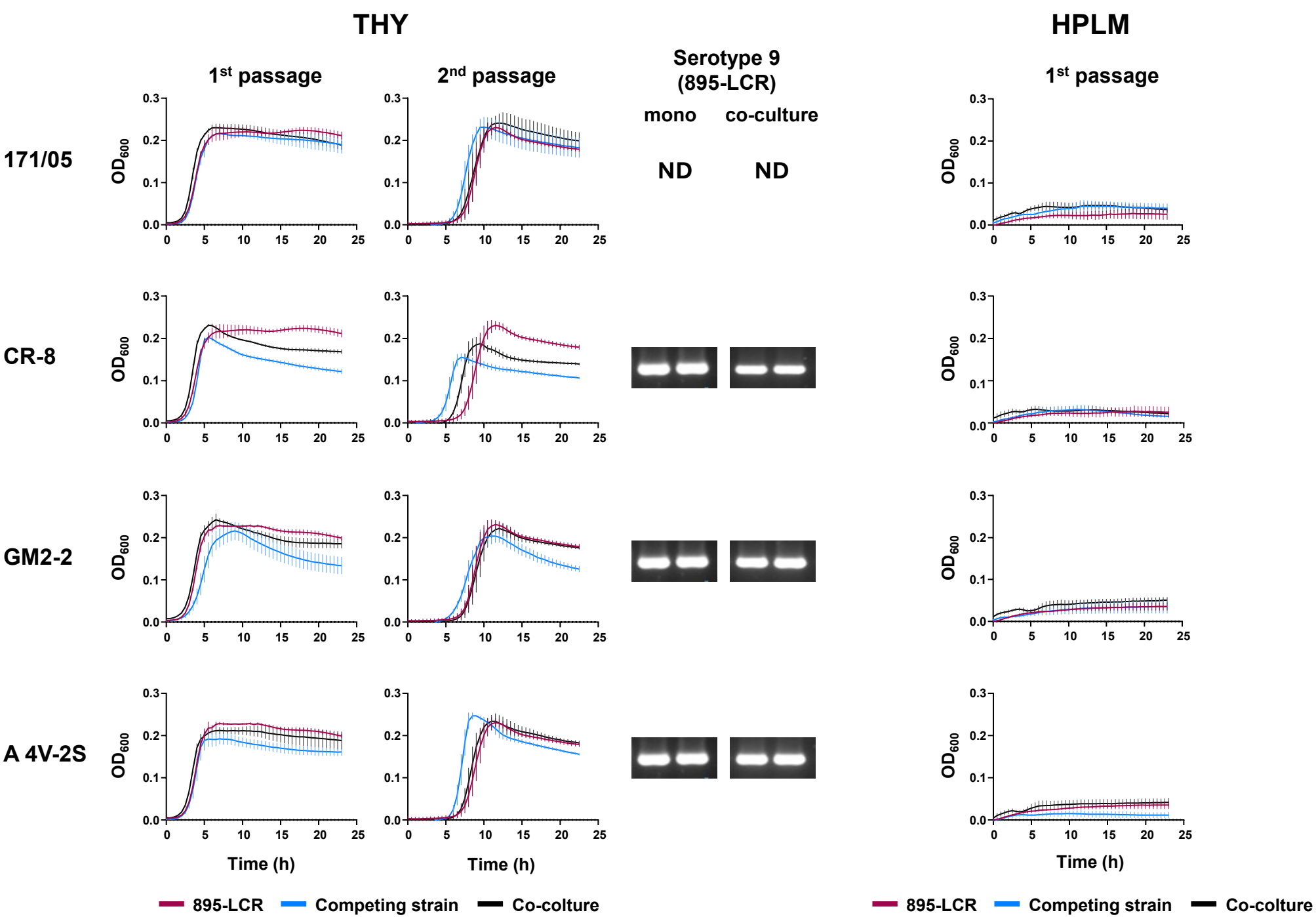
THY
HPLM
1st passage
2nd passage
Serotype 9 (895-LCR)
mono
co-culture
1st passage
171/05
CR-8
GM2-2
A 4V-2S
ND
ND
OD600
Time (h)
895-LCR
Competing strain
Co-colture

Figure S10. Growth dynamics of *Streptococcus suis* 895-LCR co-cultures across sequential passages in THY and human plasma-like media. Co-cultures of *S. suis* 895-LCR (serotype 9) with four additional isolates representing different anatomical origins, one systemic (171/05), two nasal (CR-8 and GM2-2), and one vaginal strain (A4V-2S), were performed in THY and human plasma-like medium (HPLM). Growth curves in THY correspond to the initial culture (first passage) and a subsequent subculture (second passage). In HPLM, only the first passage was performed. Growth was monitored by measuring optical density at 600 nm (OD_{600}). Data represent the mean ± standard deviation of at least two independent biological replicates, each including three technical replicates. Detection of 895-LCR in mono and co-cultures was achieved using a serotype 9-specific PCR, except in co-cultures with 171/05, which is also a serotype 9 strain. In the growth curves, 895-LCR is shown in dark red, competing strains are shown in light blue, and co-cultures are represented in black. ND: not done.

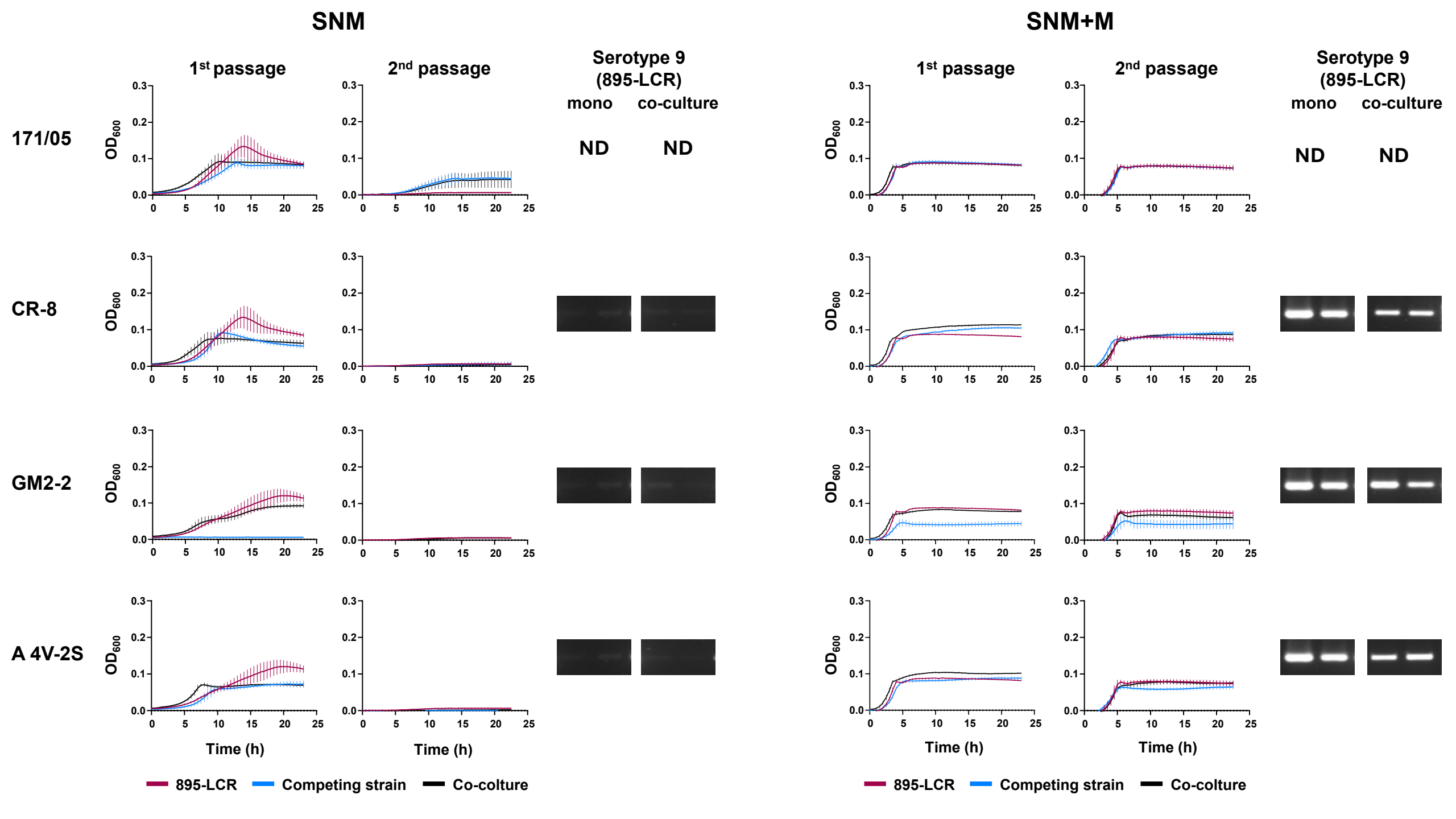
SNM
SNM+M
1st passage
2nd passage
Serotype 9 (895-LCR)
mono
co-culture
ND
171/05
CR-8
GM2-2
A 4V-2S
OD600
Time (h)
895-LCR
Competing strain
Co-colture

Figure S11. Growth dynamics of *S. suis* 895-LCR co-cultures across sequential passages in synthetic nasal media. Co-cultures of *S. suis* 895-LCR (serotype 9) with four additional isolates representing different clinical origins, one systemic (171/05), two nasal (CR-8 and GM2-2), and one vaginal strain (A4V-2S), were performed in synthetic nasal medium (SNM) and SNM supplemented with 0.5% mucin (SNM+M). Growth curves correspond to the initial culture (first passage) and a subsequent subculture (second passage). Growth was monitored by measuring optical density at 600 nm (OD_{600}). Data represent the mean ± standard deviation of at least two independent biological replicates, each including three technical replicates. Detection of 895-LCR in mono and co-cultures was achieved using a serotype 9-specific PCR, except in co-cultures with 171/05, which is also a serotype 9 strain. In the growth curves, 895-LCR is shown in dark red, competing strains are shown in light blue, and co-cultures are represented in black. ND: not done.