Figure S1

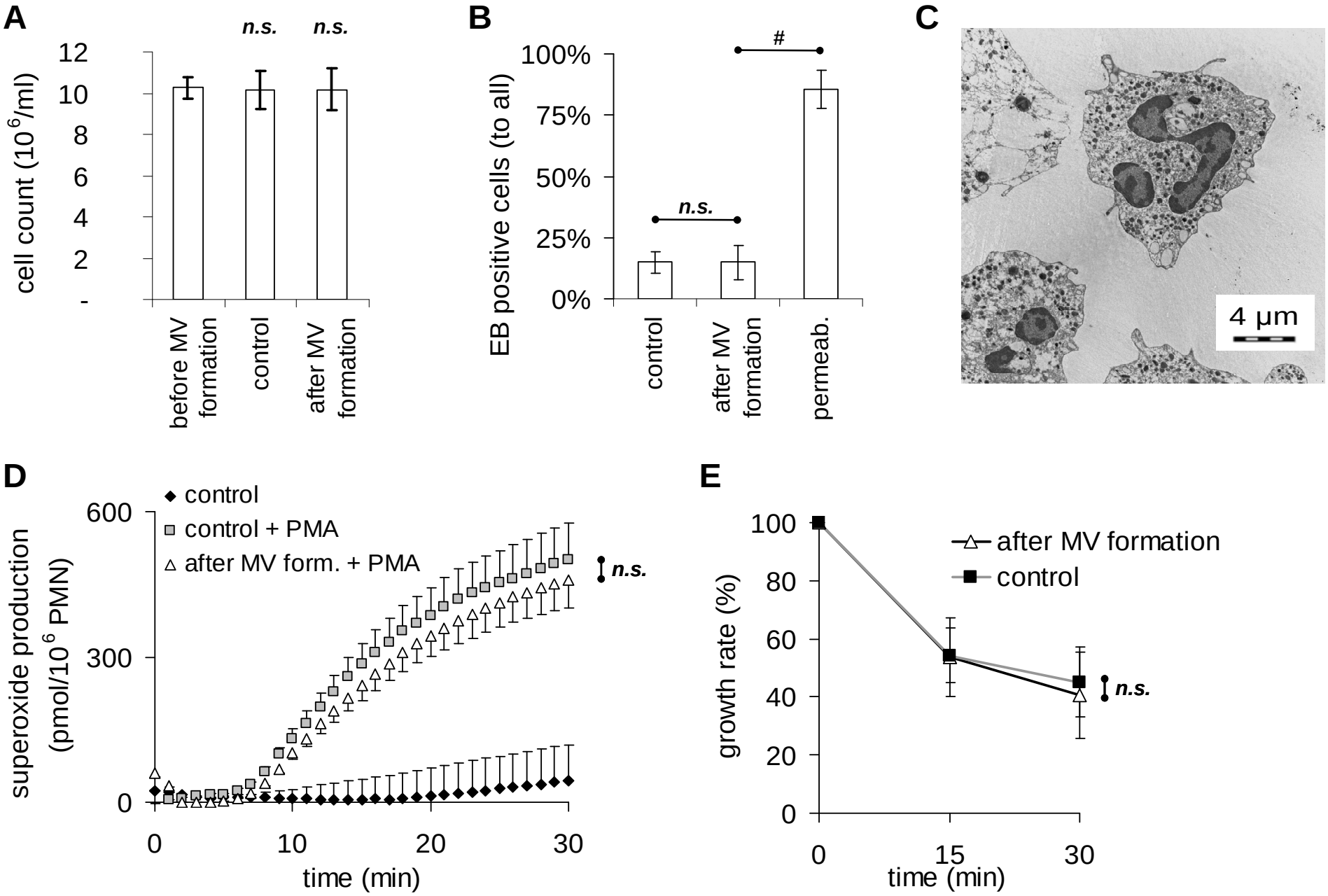
A
n.s.
n.s.
cell count (10^6/ml)
12
10
8
6
4
2
-
before MV formation
control
after MV formation
B
#
100%
75%
50%
25%
0%
EB positive cells (to all)
n.s.
control
after MV formation
permeab.
C
4 µm
D
control
control + PMA
after MV form. + PMA
n.s.
superoxide production (pmol/10^6 PMN)
600
300
0
0
10
20
30
time (min)
E
after MV formation
control
n.s.
growth rate (%)
100
80
60
40
20
0
0
15
30
time (min)

Figure S1. Characterization of PMN after b-MV formation. To estimate changes in total cell counts (**A**) during b-MV production, aliquots of non activated (control) and activated samples (before and after separation of MVs) were measured with Beckman Coulter flow cytometer for the same time. Cells were identified on the basis of forward and side scatter characteristics. To investigate the effect of b-MV production on viability of PMNs, we used vital stain (**B**), electron microscopy (**C**) and functional tests (**D,E**). To determine membrane integrity we used Erthyrosin B dye exclusion (**B**), following the manufacturer's instructions. As negative control, we used not stimulated cells, as positive control we used digitonin-treated (100 μg/mL for 30 minutes in HBSS, at room temperature) PMN. Staining of PMN was followed with a flow cytometer (Beckman Coulter Cell Lab Quanta SC flow cytometer, Beckman Coulter, Brea, CA, USA). As functional tests, superoxide production stimulated by 100 nM PMA (**D**) and bacterial killing assays (**E**) were carried out. Superoxide production was measured with the superoxide dismutase–inhibitable cytochrome c reduction assay, as described by Rada et al., 2004. Mean ± SEM of 4 independent experiments is shown in A,B,D and E. In C one representative image of 200 simular one, from 4 independent experiments is shown.

Figure S2

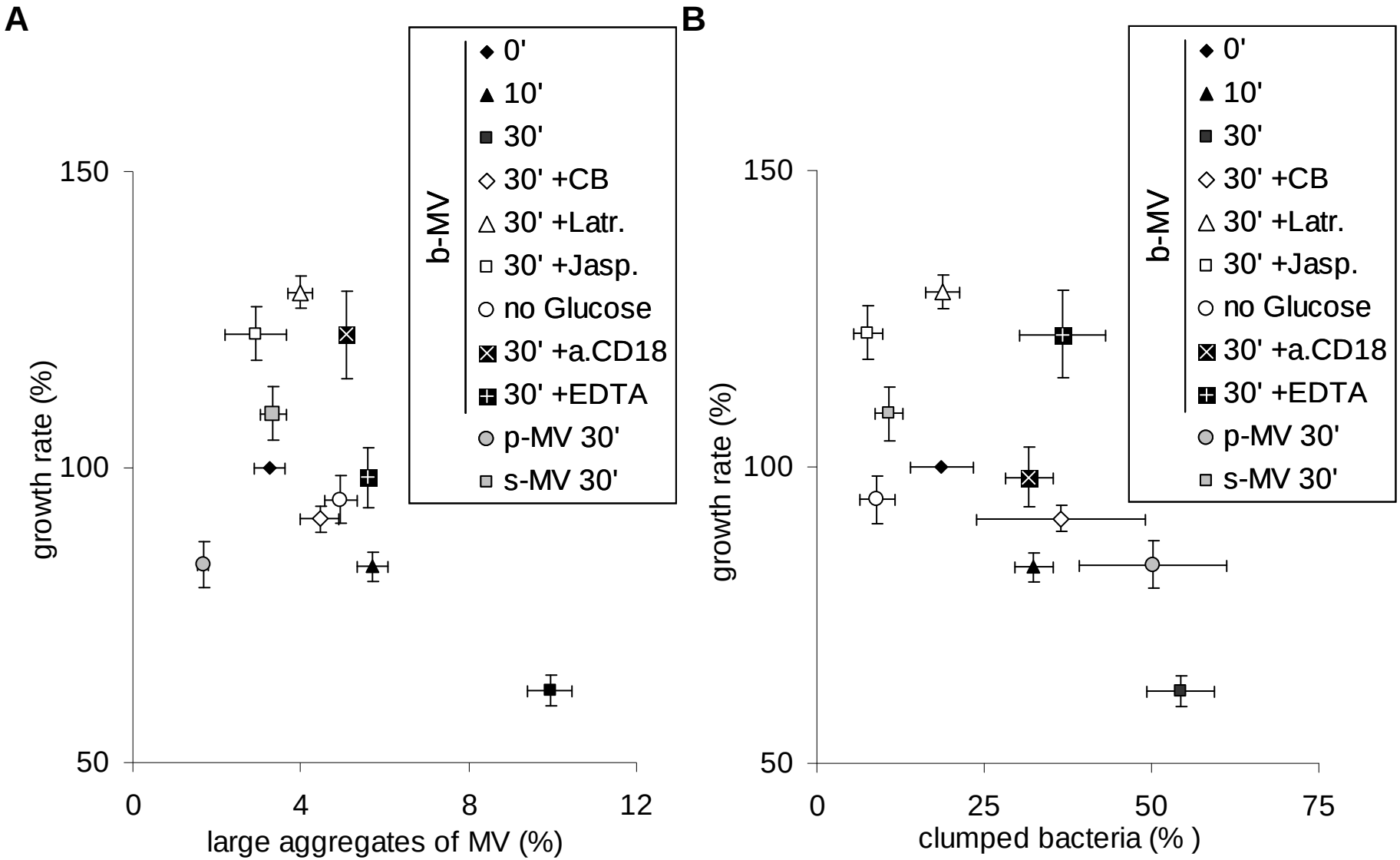
A
B
b-MV
0'
10'
30'
30' +CB
30' +Latr.
30' +Jasp.
no Glucose
30' +a.CD18
30' +EDTA
p-MV 30'
s-MV 30'
growth rate (%)
150
100
50
0
4
8
12
large aggregates of MV (%)
25
50
75
clumped bacteria (%)

Figure S2. (A) Relation between ability of producing large, bacteria positive aggregates and bacterial growth. The data presented in Fig. 5E are plotted on the x axis, whereas data of Figs 3C, and 4A are on the y axis. Concentration of different inhibitors is given in the legend to Fig. 3. (**B**) Relation of clumped bacteria to all bacteria. A more complete version of Fig. 5F of the main text.

Table S1. Result of proteomic analysis of b-MV, s-MV and p-MV preparations. All proteins that were identified by 95% confidence are listed.

Table S2. Proteins with differential expression in b-MV and s-MV preparations. Those proteins are listed where the difference between b-MV and s-MV exceeded 40%.

Table S3. Summary of the most important data of bacteremic patients and control donors.

Supplemental video:

Changes after formation of clumps were followed for 45 minutes in video microscopic experiments. To prevent unwanted adhesions, slips were coated as described in Material and Methods. All samples were in LB medium at 37 °C. In “A” clumps, made of b-MV (stained red with anti-CD11b) and GFP-expressing *S.aureus* were observed (representative video out of 11 similars). In “B” GFP expressing *S.aureus* without MV were incubated, prepared and followed under same conditions as in “A”.