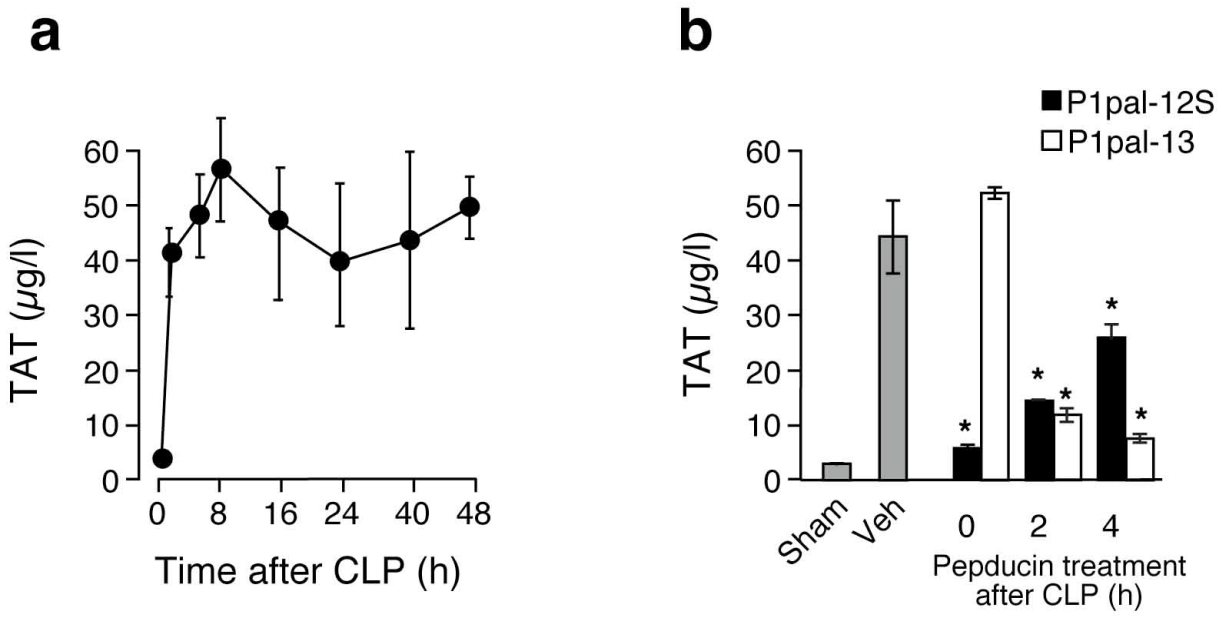
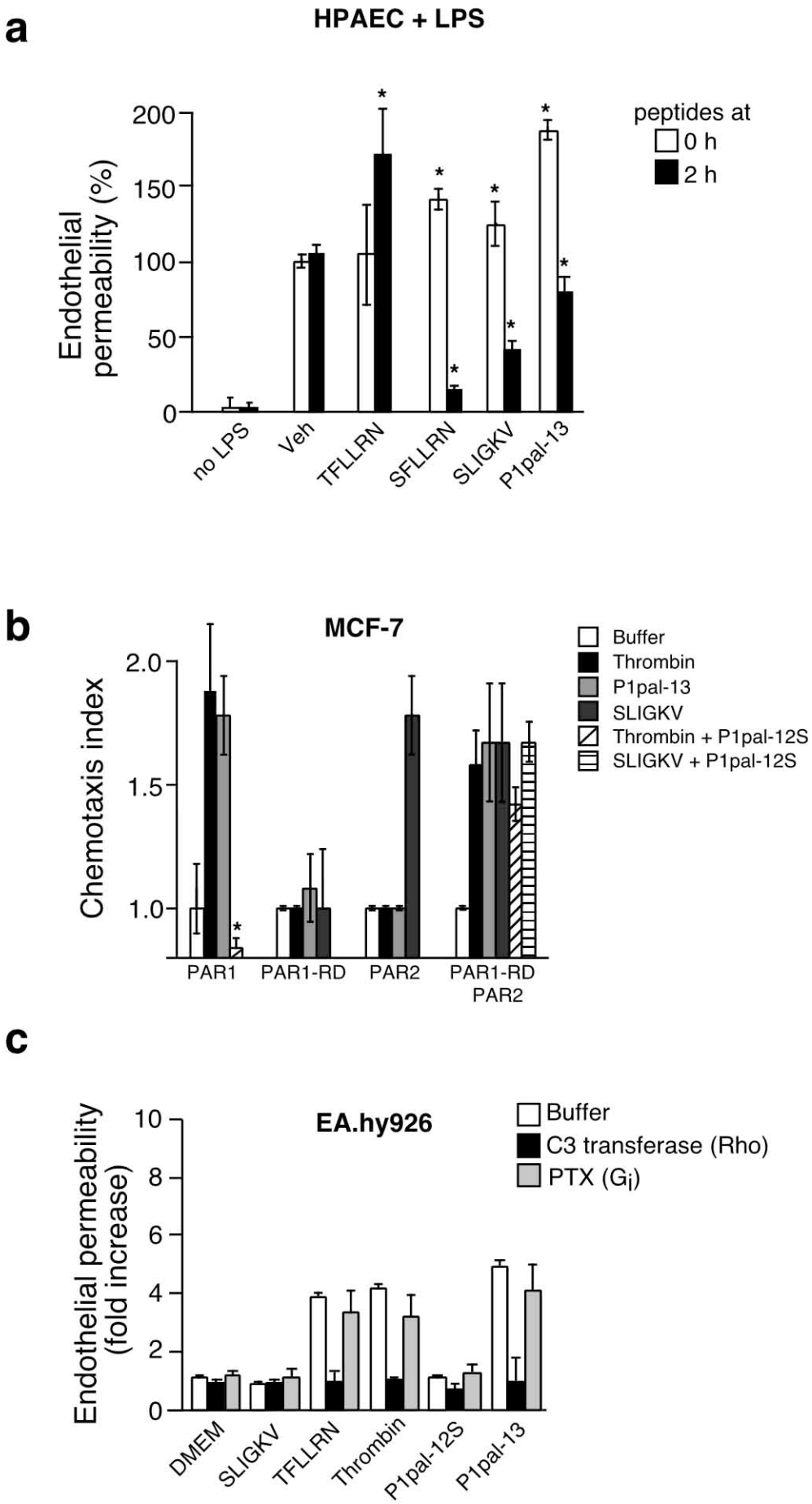
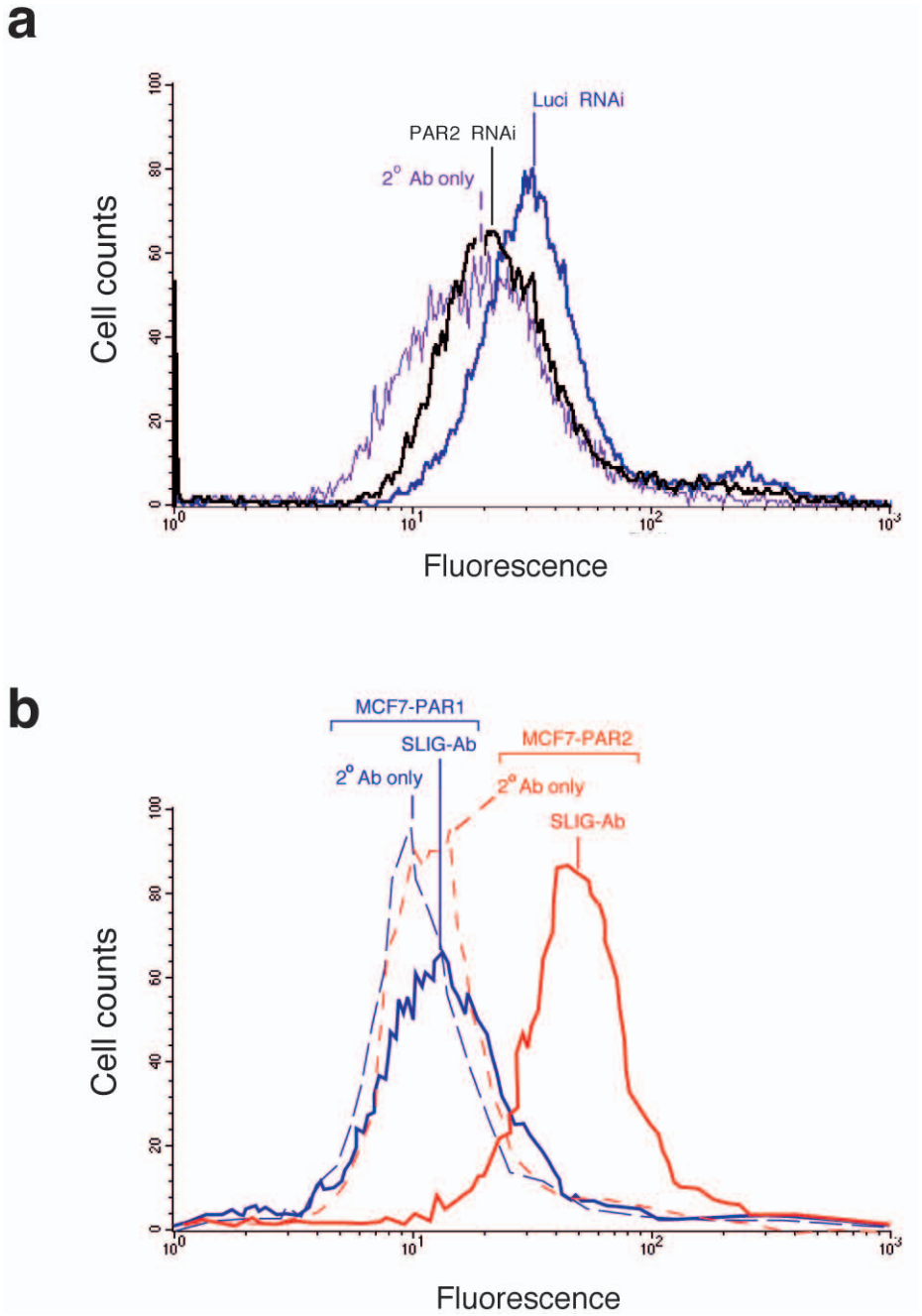

a
TAT (μg/l)
60
50
40
30
20
10
0
0
8
16
24
40
48
Time after CLP (h)
b
P1pal-12S
P1pal-13
TAT (μg/l)
60
50
40
30
20
10
0
*
*
*
*
*
Sham
Veh
0
2
4
Pepducin treatment
after CLP (h)

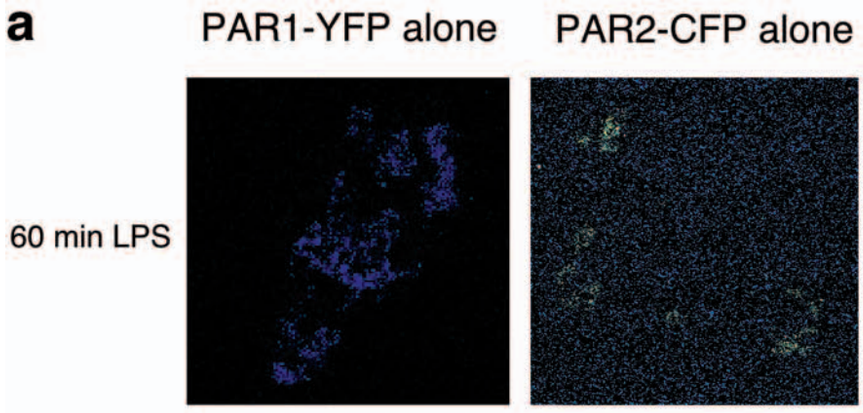
Supplementary Figure 1. Treatment of Septic Mice with PAR1 Pepducins Inhibits Formation of Thrombin-Antithrombin (TAT) Complexes. (**a**) TAT complexes increase soon after CLP. Mice were subjected to CLP and blood was drawn by terminal cardiac puncture at 0-48 h after the procedure. TAT concentrations were measured by ELISA. $n = 6$. (**b**) Early treatment with the P1pal-12S antagonist pepducin or delayed treatment with the P1pal-13 agonist pepducin reduces TAT concentrations. Mice were subjected to CLP, then P1pal-12S (2.5 mg/kg) or P1pal-13 (2.5 mg/kg) were injected s.c. either immediately, 2 or 4 h after the procedure as indicated. Mice were sacrificed 48 h after CLP and blood was drawn by cardiac puncture. TAT concentrations (48 h) were measured by ELISA. $n = 6$, *, $P < 0.05$

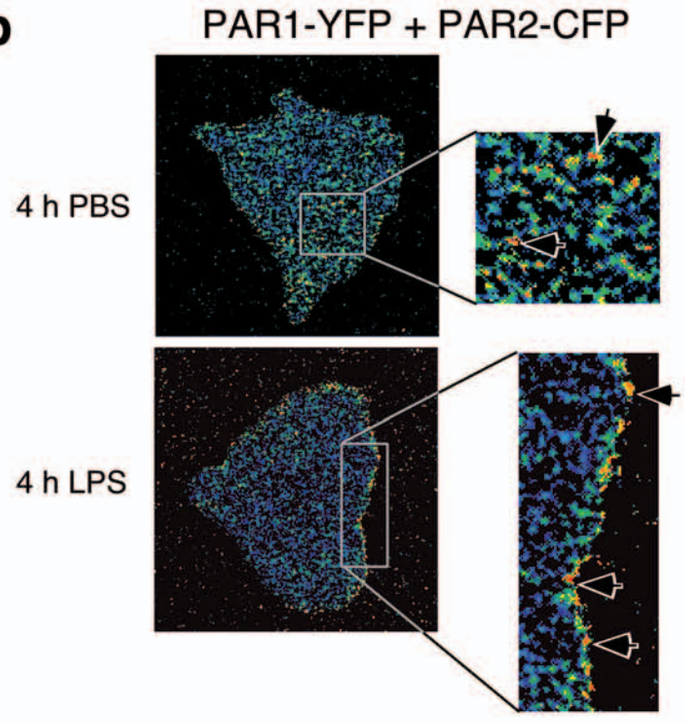

a
HPAEC + LPS
Endothelial permeability (%)
200
150
100
50
0
peptides at
0 h
2 h
no LPS
Veh
TFLLRN
SFLLRN
SLIGKV
P1pal-13
b
MCF-7
Chemotaxis index
2.0
1.5
1.0
Buffer
Thrombin
P1pal-13
SLIGKV
Thrombin + P1pal-12S
SLIGKV + P1pal-12S
PAR1
PAR1-RD
PAR2
PAR1-RD PAR2
c
EA.hy926
Endothelial permeability (fold increase)
10
8
6
4
2
0
Buffer
C3 transferase (Rho)
PTX (Gi)
DMEM
SLIGKV
TFLLRN
Thrombin
P1pal-12S
P1pal-13

Supplementary Figure 2. Effects of PAR Agonists on Barrier Function and PAR1-PAR2 dependent Chemotaxis. (**a**) Reversal of human pulmonary artery endothelial cell (HPAEC) barrier leakage is induced by delayed treatment with PAR1 and PAR2 agonists. Confluent HPAEC monolayers were stimulated with LPS (1 μg/ml) and then peptide agonists (same concentrations as in **Fig. 4**) or 0.2% DMSO vehicle were added either immediately after LPS challenge (0 h) or 2 h later. Endothelial permeability was measured at the 4 h time point. $n = 3$, *, $P < 0.05$ (**b**) MCF7 cells, transiently transfected with PAR1 (wild-type), PAR1-RD ($D_{199}R_{200}\rightarrow$RD), and/or PAR2 (wild-type) were allowed to migrate for 24 h toward chemotactic gradients of thrombin (0.3 nM), P1pal-13 (1 M), SLIGKV (10 M), P1pal-12S (3 M), or buffer (RPMI) using a 48-blindwell microchemotaxis chamber (Neuroprobe) equipped with 8 μm pores. Data are expressed as chemotaxis index (mean ± 2se), which is the ratio between the distance of migration toward chemoattractants versus RPMI medium alone. ($n = 4$) * $P < 0.01$ versus RPMI alone. (**c**) PAR1 agonists increase permeability of quiescent endothelium via Rho but not G_i. Confluent EA.hy926 cells were treated with DMEM containing buffer (PBS), C3 transferase (100 μg/ml), or pertussis toxin (PTX; 100 ng/ml) overnight. After washing, cells were exposed to SLIGKV (100 μM), TFLLRN (10 μM), thrombin (0.3 nM), P1pal-12S (0.3 μM) or P1pal-13 (0.3 μM). Endothelial permeability at 4 h was determined by Evans blue leakage into the lower wells. $n = 3$.


a
Luci RNAi
PAR2 RNAi
2° Ab only
Cell counts
Fluorescence
b
MCF7-PAR1
SLIG-Ab
2° Ab only
MCF7-PAR2
2° Ab only
SLIG-Ab
Cell counts
Fluorescence

Supplementary Figure 3. Silencing of Surface-expressed PAR2 with RNAi. (**a**) EA.hy926 endothelial cells were transfected with RNAi targeted against *Par2* or luciferase control as in **Fig. 4f,g** and FACs analysis was conducted using SLIG (PAR2-polyclonal) primary antibody (9.3 g/ml) and an anti-rabbit-FITC secondary antibody from goat (10 g/ml). These data are representative of 3 independent experiments. (**b**) As a control for the specificity of the SLIG-Ab for PAR2 relative to PAR1, MCF7 cells were transiently transfected with pcDEF3-PAR1 (blue) or pcDEF3-PAR2 (red) and FACs analysis conducted as in **a.**

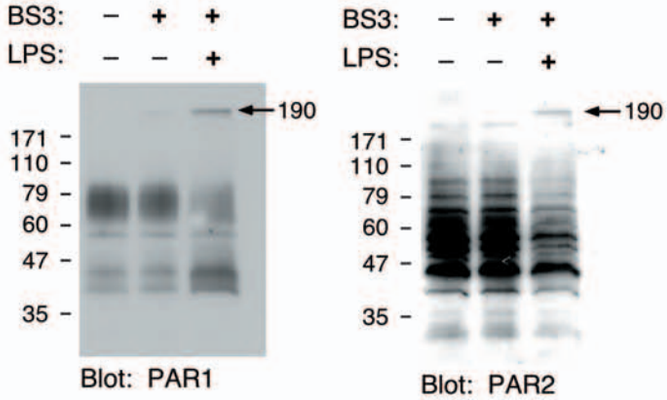




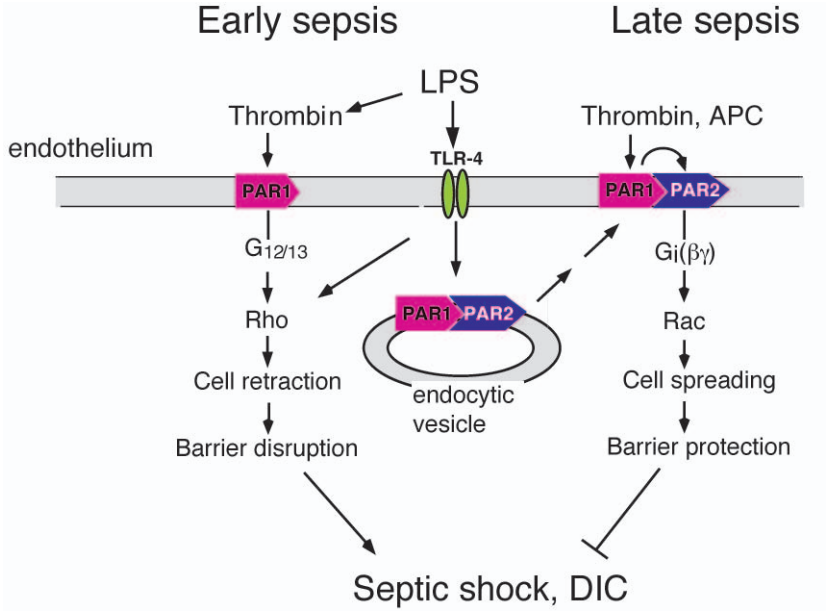
c

Ratio of FRET Signal in Membrane / Cytosol

Treatment	Ave ± se	Cells (*n*)	*P* value
buffer	0.30 ± 0.03	16	–
1 μg/mL LPS	11.3 ± 4.25	10	0.003



Supplementary Figure 4. Detection of Complexes of PAR1 and PAR2 by FRET Microscopy and Chemical Crosslinking of LPS-stimulated Endothelial Cells. (**a,b**) EA.hy926 endothelial cells were transfected with PAR1-YFP and/or PAR2-CFP and stimulated with either LPS or PBS buffer for the indicated times andconfocal photobleaching FRET microscopy was performed as in **Fig. 7f** and are representative of 10-15 cells for each condition(**c**) The ratio of the membrane/cytosol FRET signal (highest 5–10% intensity) emanating from Ea.hy926 endothelial cells (n=10-16) co-transfected with PAR1-YFP and PAR2-CFP treated with buffer or LPS as in **Fig. 7f.** (**d**) Cross-linking of PAR1-PAR2 complexes from LPS-stimulated EA.hy926 endothelial cells with non-cell permeant bis(sulfosuccinimidyl)suberate (BS3). EA.hy926 cells were treated with 1 g/ml LPS or PBS (-) for 4 h, crosslinked with 1 mM BS3 for 30 min, then quenched with 10 mM ethanolamine plus 1 M Tris-HCl. Cell lysates were separated by 12% SDS-PAGE, and immunoblot analysis was conducted with ATAP2 (PAR1-monoclonal) and SLIGK (PAR2-polyclonal) antibodies. This experiment was conducted 3 times and gave similar results.


Early sepsis
Late sepsis
LPS
Thrombin
Thrombin, APC
endothelium
TLR-4
PAR1
PAR1
PAR2
G12/13
Gi(βγ)
Rho
PAR1
PAR2
Rac
Cell retraction
endocytic
vesicle
Cell spreading
Barrier disruption
Barrier protection
Septic shock, DIC

Supplementary Figure 5. Proposed Model of the PAR1-PAR2 Transactivation Switch Mechanism in Endothelial Cells During Sepsis