Supplementary data for:

Pseudomonas aeruginosa lectin LecB inhibits tissue repair processes by triggering β-catenin degradation

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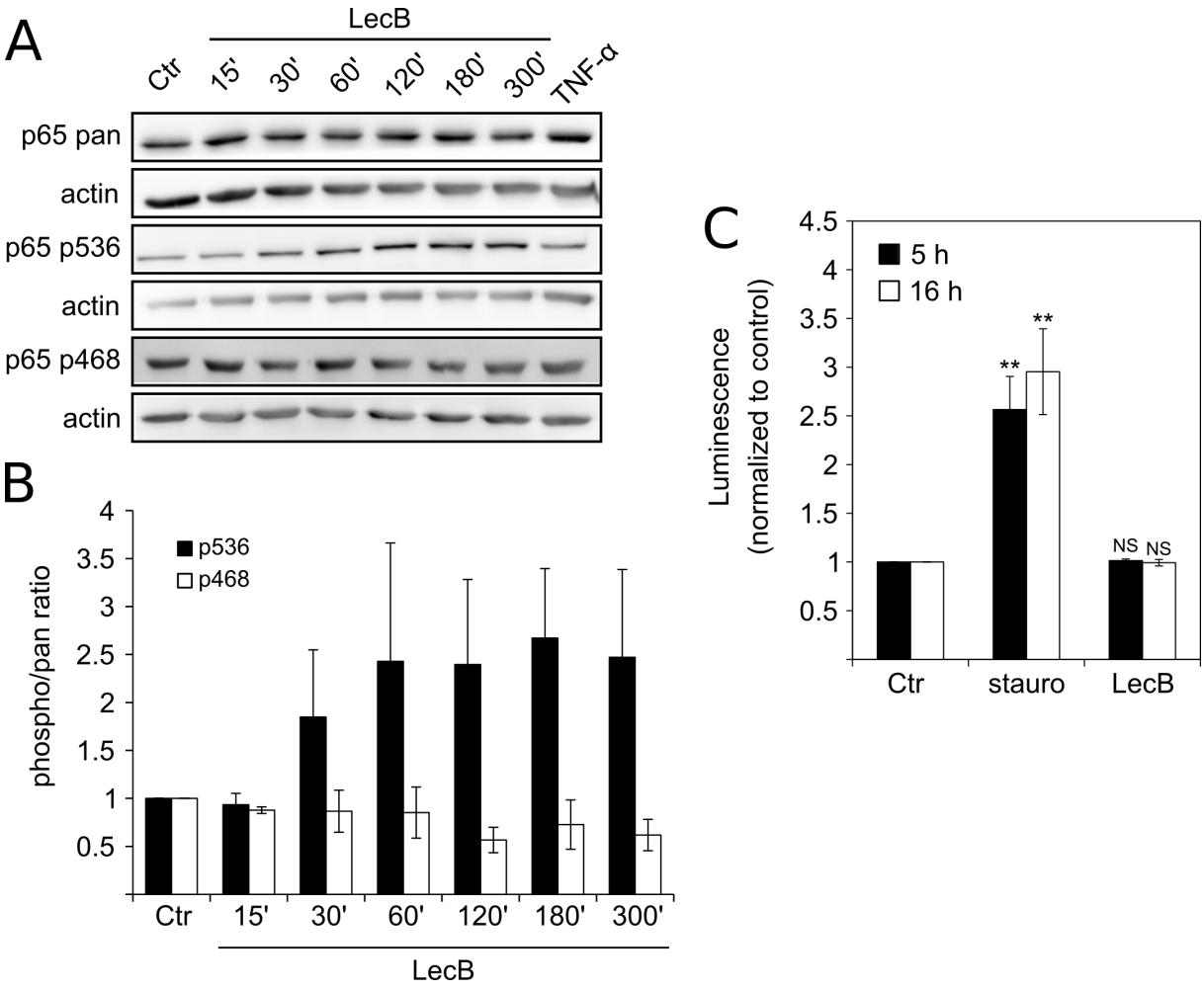
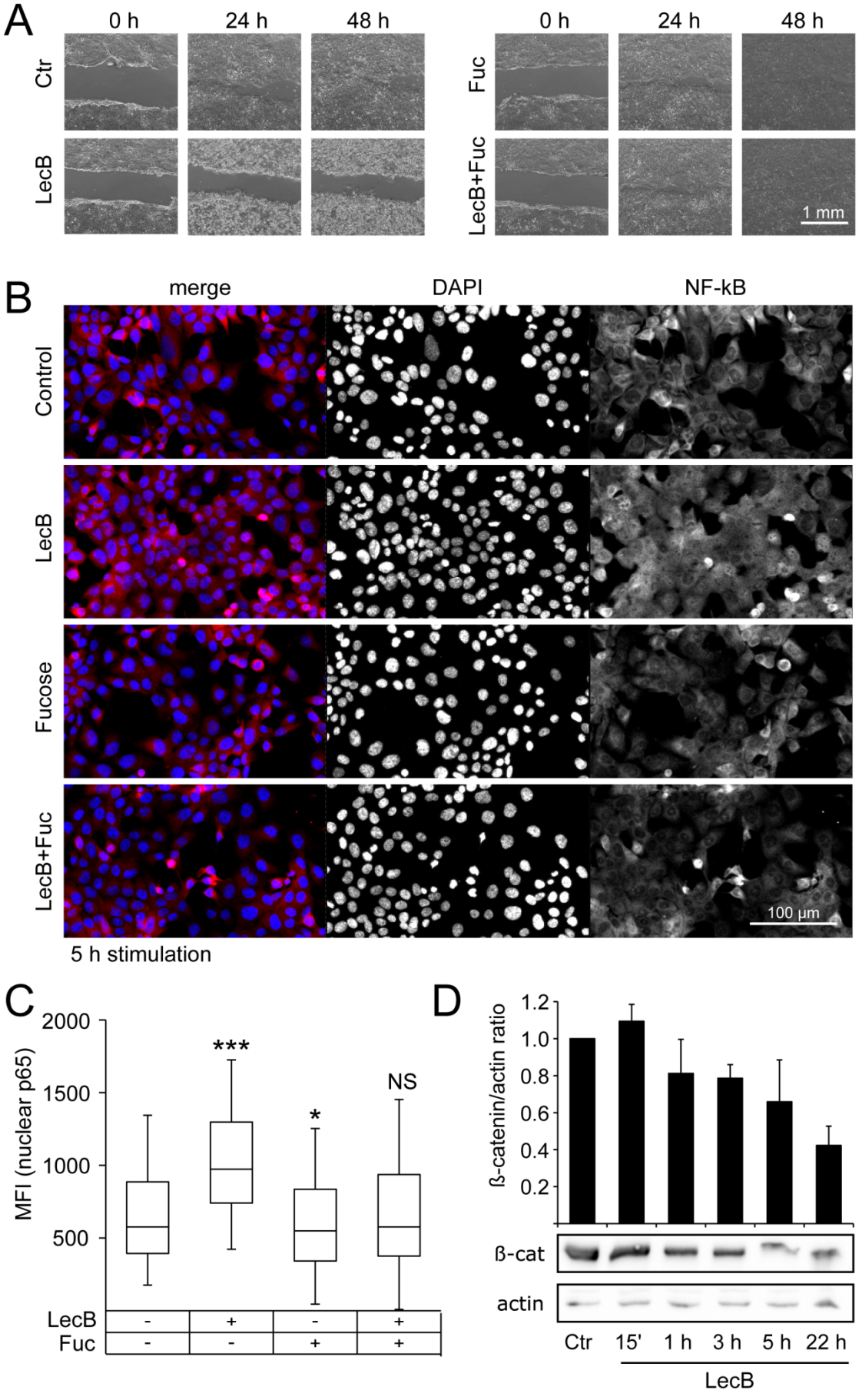
Suppl. Fig. 1



Suppl. Fig. 1: LecB induces NF-kB p65 phosphorylation but no apoptosis

(A) H1299 cells were treated with 4.3 µM LecB for indicated time periods and with 10 ng/ml TNF-α as positive control. Separate Western blots of NF-kB p65 pan and the phosphorylation of NF-kB p65 on Serine 536 and 468 are shown. (B) Densitometric quantification of Western blots using ImageJ from samples treated as described in (A). Phosphorylation levels were normalized to actin, and then to p65 pan. Values represent the mean of at least three independent experiments ± SEM. (C) Cells were left untreated (control) or stimulated for different time periods with 4.3 µM LecB or 1 µM staurosporine as positive control for apoptosis. Afterwards a Caspase-Glo® 3/7 Assay (Promega) was performed according to the instructions of the manufacturer. Graph presents normalized luminescence read outs from three independent experiments run in triplicate ± SEM. Asterisks indicate the statistical significance compared to control samples.

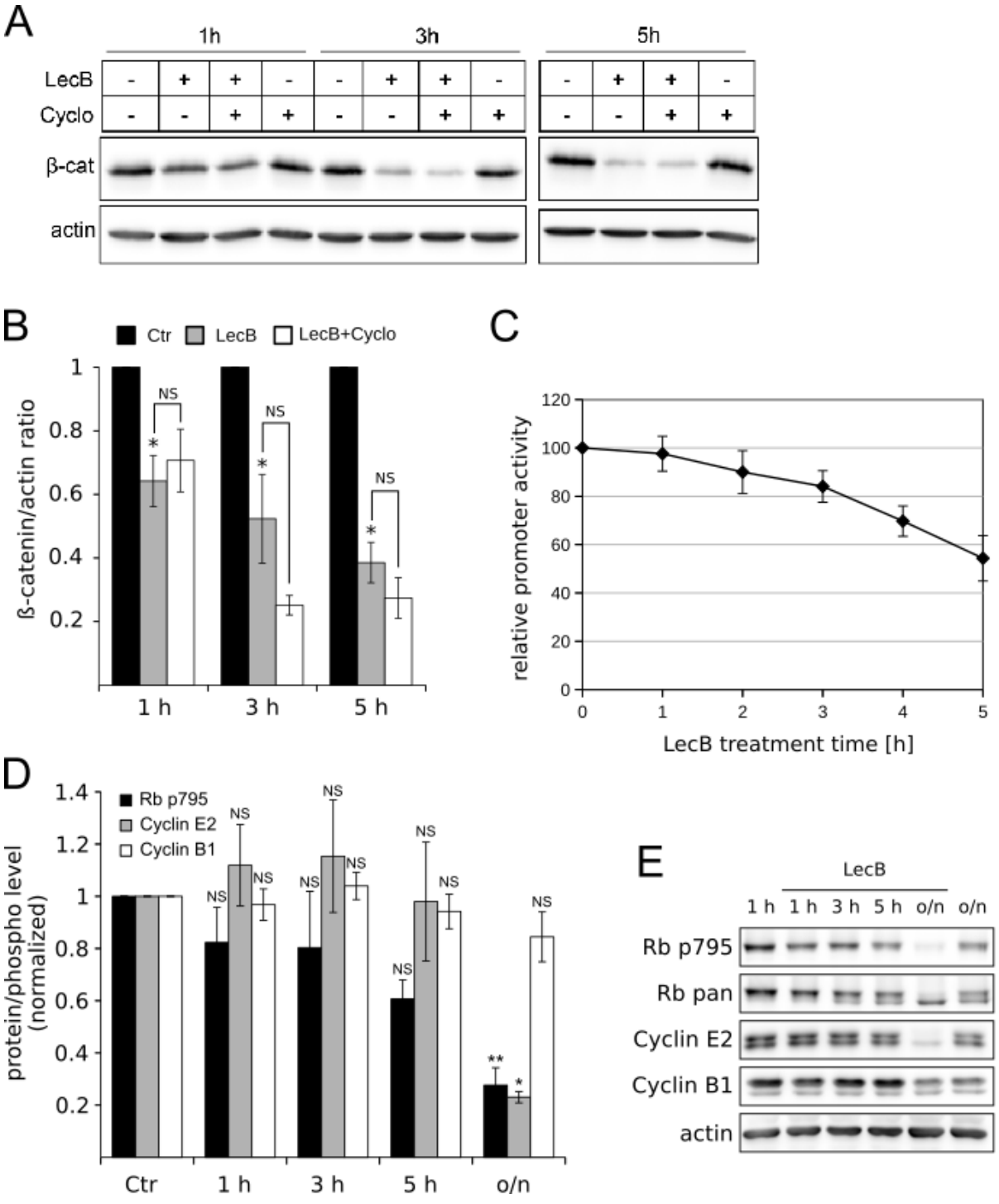
Suppl. Fig. 2


A
0 h
24 h
48 h
Ctr
LecB
Fuc
LecB+Fuc
1 mm
B
merge
DAPI
NF-kB
Control
LecB
Fucose
LecB+Fuc
100 µm
5 h stimulation
C
MFI (nuclear p65)
2000
1500
1000
500
***
*
NS
LecB
Fuc
D
ß-catenin/actin ratio
1.2
1.0
0.8
0.6
0.4
0.2
ß-cat
actin
Ctr
15'
1 h
3 h
5 h
22 h
LecB

Suppl. Fig. 2: LecB attenuates migration and induces NF-kB p65 nuclear translocation and β-catenin degradation in H1975 cells

(A) H1975 cells were allowed to form confluent cell layers. After scratching the cell layers with a pipet tip, closure of the cell gap was monitored in presence or absence of 4.3 µM LecB. As control, LecB binding to cell surface receptors was blocked by addition of 43 mM L-fucose (Fuc). (B) H1975 cells were grown on glass cover slips and were left untreated or treated with 4.3 µM LecB or 43 mM L-fucose or both for 5 hours. Cells were analyzed by confocal fluorescence microscopy after immunostaining for NF-kB p65 (red) and counterstaining for DNA (DAPI, blue). Representative confocal microscopy images are shown. (C) Microscopy samples were prepared as described in (B) and nuclear location of p65 was quantified by estimating the nuclear mean fluorescence intensity (MFI) of p65. Median values of three independent experiments with lower and higher quartile are shown. Error bars represent 1.5 interquartile range (IQR) values (statistical analysis was performed using Kruskal-Wallis test with Dunn´s post-hoc test, asterisks indicate statistical significance compared to the untreated control). (D) H1975 cells were treated with 4.3 µM LecB for the indicated time periods and subjected to Western blot analysis. Representative images of β-catenin and β-actin staining are shown below. The graph shows densitometric quantification of β-catenin level of Western blots using ImageJ. Beta-catenin levels were normalized to actin. Values represent the mean of a two independent experiments ± SEM.

Suppl. Fig. 3



Suppl. Fig. 3: LecB-induced β-catenin degradation is not influenced by cycloheximide, results in less transcriptional activity of β-catenin and regulates cyclin D1-associated proteins

(A) H1299 cells were treated as indicated with 4.3 µM LecB in the presence or absence of 100 µg/ml cycloheximide for different time periods and subjected to Western blot. Representative images of β-catenin and β-actin staining are shown. (B) The graph presents a densitometric quantification of β-catenin protein levels of corresponding blots (A) of three independent experiments ± SEM. The β-catenin/actin ratio of LecB-treated cells was normalized to untreated cells and values of LecB+cycloheximide-treated cells were normalized to cycloheximide-only-treated cells. Asterisks indicate the statistical significance compared to corresponding control samples. (C) H1299 cells were

co-transfected with a firefly luciferase-based Topflash reporter (7TFP) and a renilla luciferase reporter with a CMV-promoter as expression control using Lipofectamine2000®. After 12 hours of expression, cells were treated with LecB for the indicated time periods, lysed, and assayed for firefly and renilla luciferase activity. For each condition, three technical replicates were analysed. The graph shows the firefly luciferase readout normalized to the renilla luciferase values for each time point, relative to the 0 hours value, which was set to 100. (D) H1299 cells were stimulated with 4.3 µM LecB for indicated time periods. Protein levels of cyclin E2 and B1 and phosphorylation levels at Ser795 of the Rb protein were determined by Western blot analysis, densitometric quantification using ImageJ and normalization to actin. Representative blots and quantification data (E) are depicted. Values represent the mean of at least three independent experiments ± SEM. Asterisks indicate the statistical significance compared to the untreated control.