Expanded View Figures

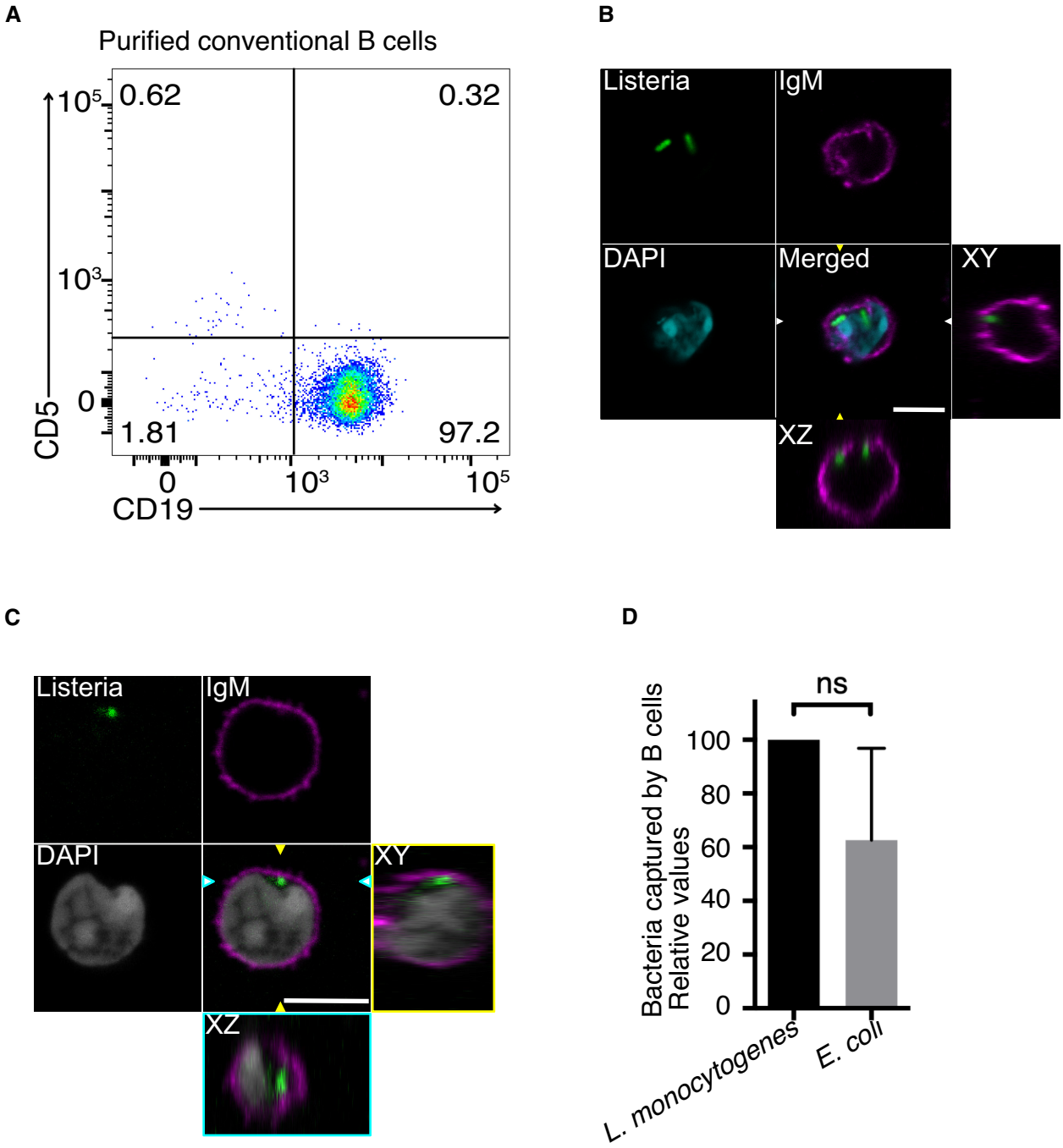

Figure EV1. B cells capture bacteria by trans-phagocytosis.

A Analysis by flow cytometry of CD5 expression (negative in conventional B cells, positive in B1 cells) in B cells isolated from spleen.

B, C Confocal images of B cells from WT mice that have captured *L. monocytogenes* (green), after 4 h of infected DC/B cell contacts. B cell surface was detected using an anti IgM antibody (magenta) the B cell nucleus was stained with DAPI (cyan in (B) and gray in (C)). The orthogonal views show intracellular bacteria. Scale bar represents 5 μm. Arrowheads indicate the XZ and YZ axes.

D Gentamicin survival assay showing transphacosytosis of *E. coli* captured by B cells, in values relative to *L. monocytogenes* capture. The column bars represent the mean of at least three independent experiments. Non-significant differences are marked as (ns) according to Student *t*-test.

Source data are available online for this figure.

Figure EV2. Flow cytometry gating strategy.

A Purity of *Listeria*-WT or *Listeria*-OVA BacB cells after cell sorting. Size and complexity are shown by plotting side (SSC) versus forward-scattered (FSC) light.

B Analysis of DC viability during B cells instruction. DC population was gated by size and complexity after 24 infected-DC/B cells conjugates formation. Cell viability of DC population was further analyzed using Live/Dead Fixable Viability Near-IR staining. More that 90% of the DC died during the instruction protocol.

C Gating strategy for flow cytometry analysis of $CD8^+$ T cells. All cells, excluding debris, were taken by size and complexity. Then, excluded doblets and dead cells. $CD8^+$ T cells were analyzed by antibody staining for IgM and CD8. $CD8^+$ (IgM^-) T cells population were further analyzed for CD25 expression and CellTrace Violet staining (proliferation).

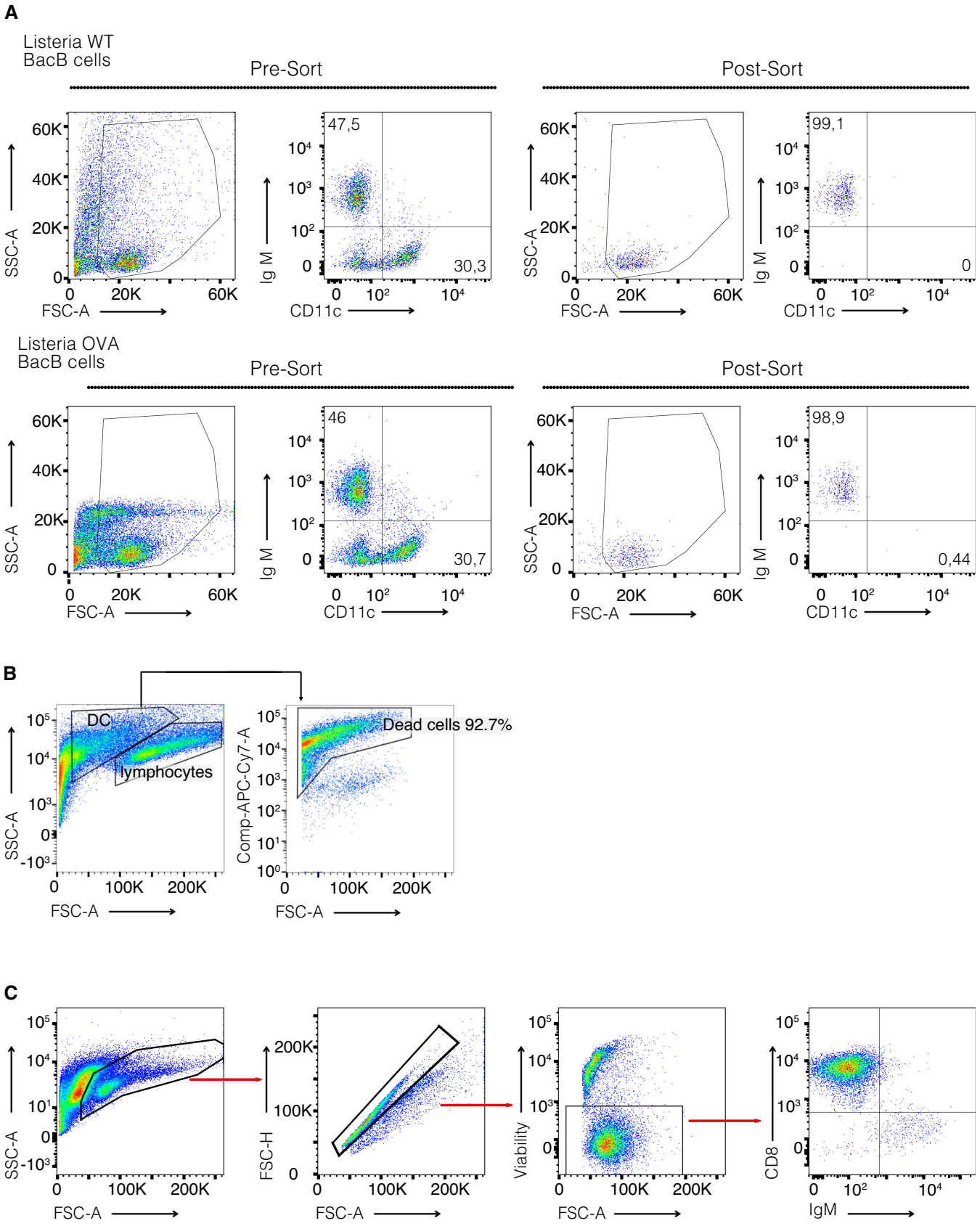
A
Listeria WT
BacB cells
Pre-Sort
Post-Sort
SSC-A
FSC-A
Ig M
CD11c
47,5
30,3
99,1
0
Listeria OVA
BacB cells
46
30,7
98,9
0,44
B
DC
lymphocytes
Comp-APC-Cy7-A
Dead cells 92.7%
C
FSC-H
Viability
CD8
IgM

Figure EV2.

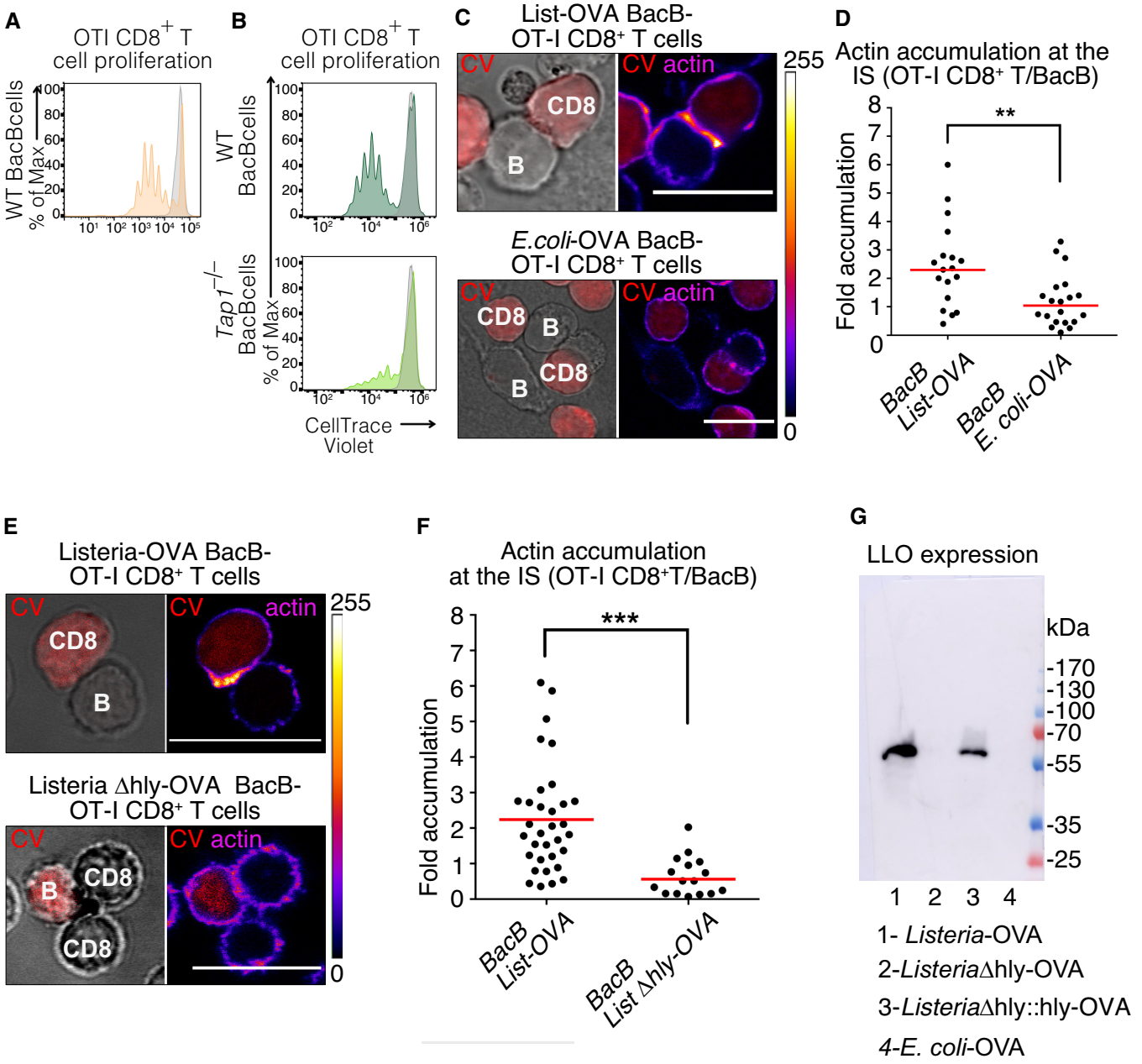

Figure EV3. BacB-mediated cross-presentation.

A Representative experiment showing proliferation of CellTrace Violet-stained OTI CD8$^+$ T cells activated by WT BacB cells (orange). Non-activated naïve CD8$^+$ T cells are shown (gray).

B Representative experiment showing proliferation of CellTrace Violet-stained OTI CD8$^+$ T cells activated by WT upper panel or Tap1$^{-/-}$ BacB cells (lower panel). Non-activated naïve CD8$^+$ T cells are shown (gray).

C Represenatative confocal images showing actin accumulation (fire scale) at the IS in naïve OT-I CD8$^+$ T cells (stained with CellTrace Violet, red) contacting either *Listeria*-OVA (upper panles) or *E. coli*-OVA (lower panles) BacB cells. Scale bars = 10 μm.

D Cuantification of actin accumulation at the IS using *Synapse measures*. Each dot corresponds to one IS analaysis, data were collected from three independent experiments.

E Represenatative confocal images showing actin accumulation (fire scale) at the IS in naïve OT-I CD8$^+$ T cells (stained with CellTrace Violet, red) contacting either *Listeria*-OVA (upper panels) or *Listeria* Δhly-OVA (lower panels) BacB cells. Scale bars = 10 μm.

F cuantification of actin accumulation at the IS using *Synapse measures*. Each dot corresponds to one IS analaysis, data were collected from three independent experiments.

G Expression of LLO in the indicated bacterial strains, measured by Western blotting.

Data information: Bars in the figure represent the mean. Statistical significant differences, analyzed by *t*-test are represented by asterisk; **$P < 0.005$, ***$P < 0.0005$, non-significant differences are marked as (ns).

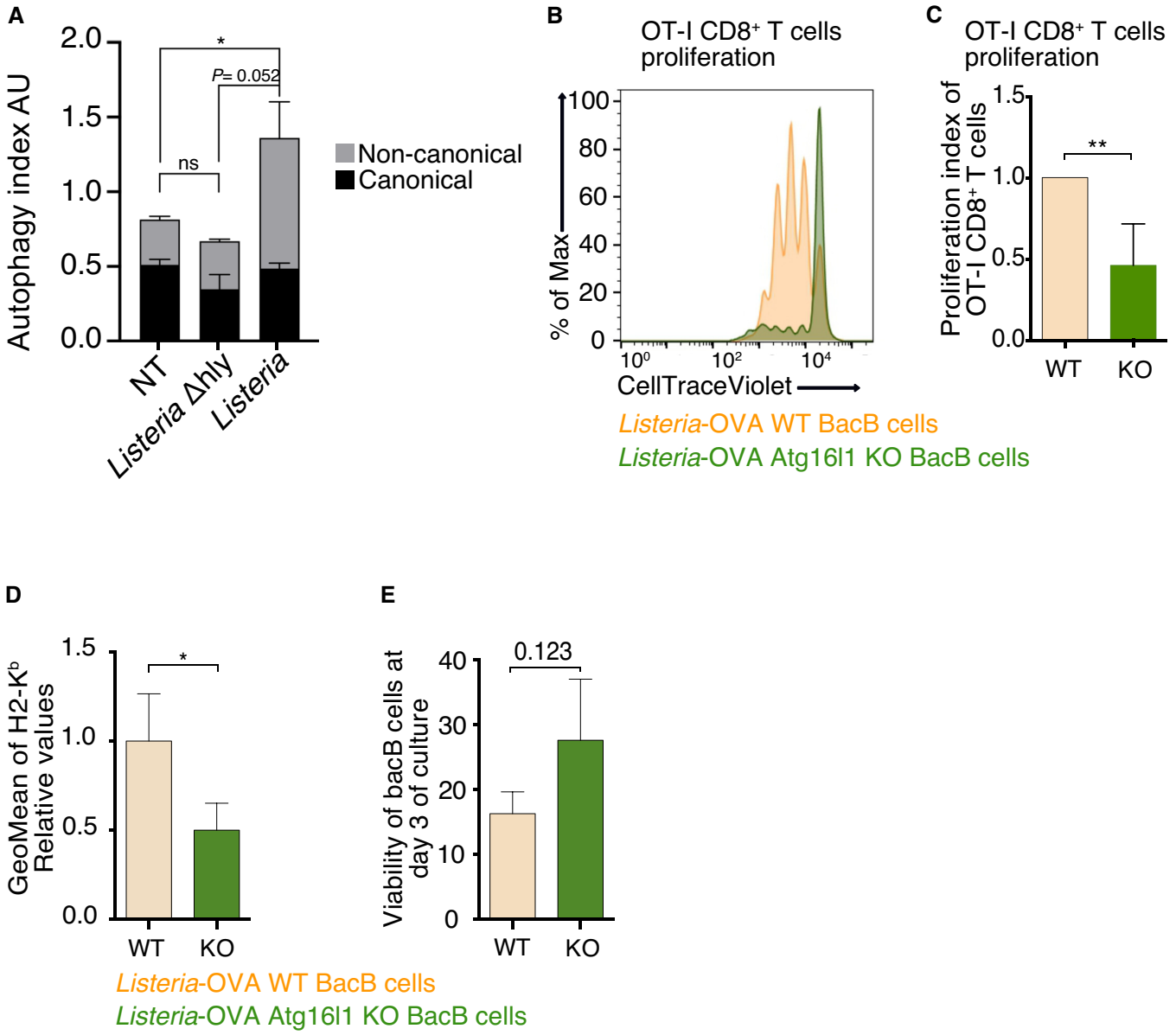

Figure EV4. Role of non-canonical autophagy in BacB-mediated antigen cross-presentation.

A Autophagy index (AI) measuring LC3 by flow cytometry. The total autophagy flux is defined as the mean fluorescence intensity (MFI) after chloroquine treatment, the canonical autophagy flux as the MFI after BafA1 treatment, and the noncanonical autophagy flux as the difference between the two normalized by the MFI of LC3 before inhibitor addition. The Bar charts show the mean of the Autophagic Index in non-instructed B cells (B cells contacting non-infected DC), and BacB cells instructed with either *L. monocytogenes* or *L. monocytogenes* Δhly, from three independent experiments. No statistical differences between the groups were noticed in canonical autophagy. The statistical differences between groups related to non-canonical autophagy are shown; $*P < 0.05$. No statistical differences is marked as "ns". AU, arbitrary units. Error bars indicate SEM.

B, C OTI naïve $CD8^+$ T cells proliferation 3 days after activation with either Listeria-OVA $Atg16l1^{f/f}$ (WT) (orange) or Listeria-OVA $Atg16l1^{f/f}$ KO (Atg16l1 KO) (green) BacB cells. OT-I naïve $CD8^+$ T cells proliferation was measured by CellTraceViolet stain dilution. (B) Representative data and (C) mean from at least three independent experiments.

D Surface expression of H-$2K^b$ at the cell surface of Listeria-OVA $Atg16l1^{f/f}$ (WT) (orange) or Listeria-OVA $Atg16l1^{f/f}$ KO (Atg16l1 KO) (green) BacB cells. Column bars shown the mean of three independent experiments.

E Viability of Listeria-OVA $Atg16l1^{f/f}$ (WT) (orange) or $Atg16l1^{f/f}$ KO (Atg16l1 KO) (green) BacB cells measured by flow cytometry. Column bars shown the mean of three independent experiments.

Data information: (B–E), Bars in the figure represent the mean and error bars the SD. Statistical significant differences, analyzed by *t*-test are represented by asterisk; $*P < 0.05$, $**P < 0.005$, non-significant differences are marked as (ns).
Source data are available online for this figure.