



4 Supplementary Figure 1 | ETs trap extracellular particles. (a) Image gallery of 5 Supplementary Movie 2. The disaggregated slug cells were incubated with LPS, 1 µm green 6 fluorescent latex beads and PI. The dashed line outlines the migration zone being explored 7 by cells and clusters of disaggregated slugs during the time of the experiment. The green and 8 red channels correspond to the latex beads and PI-stained extracellular DNA, respectively. 9 (b) Quantification of the fluorescence intensity of green and red channel inside and outside 10 the outlined region over time for Supplementary Movie 2, as a representative result from 11 more than 5 similar movies.

- 12
- 13



2

Supplementary Figure 2 | ETs kill extracellular bacteria. The overall quantification of Supplementary Movie 3 shows a general decrease of green fluorescence (Kp-GFP) and increase of red fluorescence (PI staining both ETs and dead bacteria) over time. A single bacterium, marked by an arrowhead in the movie, exhibits dramatic GFP-fluorescence loss and PI-fluorescence gain at around 200-min as it dies. This quantification for Supplementary Movie 3 is a representative result from more than 4 similar movies.



b

Parameter	mean ± s.e.m.
Pearson's coefficient in dataset volume	0.80 ± 0.01
Pearson's coefficient in ROI volume	0.52 ± 0.03
Pearson's coefficient in colocalized volume	0.48 ± 0.03

2

1

3

4 Supplementary Figure 3 | in situ colocalization of S cells and ROS. AX4 slugs developed 5 on K.p. bacteria lawn were sprayed with DHE and AO, and 8 independent slugs were imaged 6 in confocal microscopy as described in the methods section, n=8. (a) A representative 2D 7 histogram of the green and red channels was shown. Regions of interest (ROI) and thresholds 8 were manually adjusted, and pixel intensity spatial correlation analyses were automatically 9 performed on each of the samples using the Imaris software. (b) The summary of resulting Pearson's coefficients is listed, indicating a significant correlation between AO staining (S 10 11 cells) and DHE staining (ROS). Correlation coefficient: 1 = total positive correlation, 0 = no12 correlation, -1 = total negative correlation.

13





5 Supplementary Figure 4 | Generation of the triple NoxABC-KO strain. (a) The NoxA 6 single KO strain was generated by transfecting pLoxP-NoxA plasmid into AX2 wild type 7 cells, followed by clone isolation on a K.p. lawn. Insertion of the Bsr cassette increased the 8 size of the NoxA PCR product. The NoxA single knockout (NoxA-KO) was then transfected 9 with pTX-NLS-Cre plasmid to excise the Bsr cassette, resulting in a PCR product differring 10 from the one in wild type AX2 only by the remaining LoxP site. (b) The NoxA-KO without 11 the Bsr cassette was used to generate the NoxA-NoxB double knockout (NoxAB-KO) following the same strategy by transfecting pLoxP-NoxB and pTX-NLS-Cre plasmids 12 sequentially. The size shift of NoxB PCR products was used to identify positive clones. (c) 13 14 The NoxAB-KO double knockout without Bsr cassette was transfected with pLoxP-NoxC to 15 generate NoxA, NoxB and NoxC triple knockout (NoxABC-KO). The clones were confirmed 16 by the increased size of the NoxC PCR product due to Bsr cassette insertion.



- 1 2
- 3

Supplementary Figure 5 | S cells visualization in slugs from wild type and mutant strains. Slugs were developed on agar plates containing Lucifer Yellow, and the S cells were visualized *in situ* by confocal microscopy. Representative examples show that TirA-KO and NoxABC-KO produce S cells. However, the fluorescence intensity from TirA-KO and NoxABC-KO S cells is less than that from wild types, indicating somehow compromised S cell functionality. Scale bar: 100 μm

10

Primer	Sequence
rnl-F	TGATCCAATAGTTCTGTGTGGA
rnl-R	CCGAACCACATAACAGATATGA
H3a-F	GGTTCTAAACAAGCCCATAAACA
H3a-R	CTCTAAGAGCGACAGTAC
act-F	TGATATGGAAAAAATCTGG
act-R	GCTTTTGGATTTAATGG
NoxA-F	AATAGATTATTATAATAGTCCAGCATTTGAAG
NoxA-R	CGGTAGTTCTAAGTTTTCAAAGTTTTC
NoxB-F	ATCAATGTGGAGAATTAGAAGACC
NoxB-R	TGTAAATTTGTAATGTGACATTTTTGAGC
NoxC-F	AATTTGCAAATCCATTGTCTTTTCC
NoxC-R	GGTTATTTTTGTTCTTCACTACCAAC

3 Supplementary Table 1 | Primers used in this study