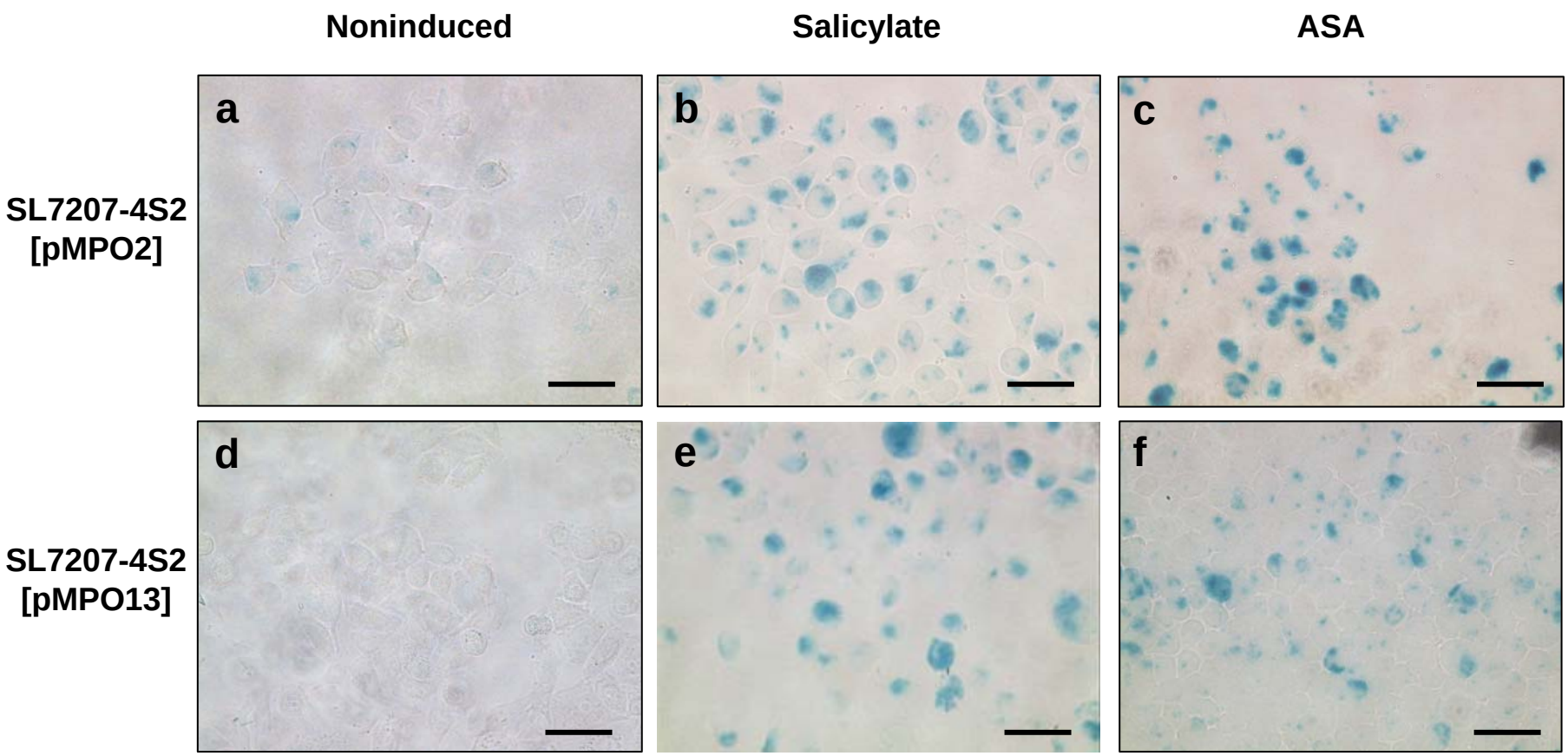
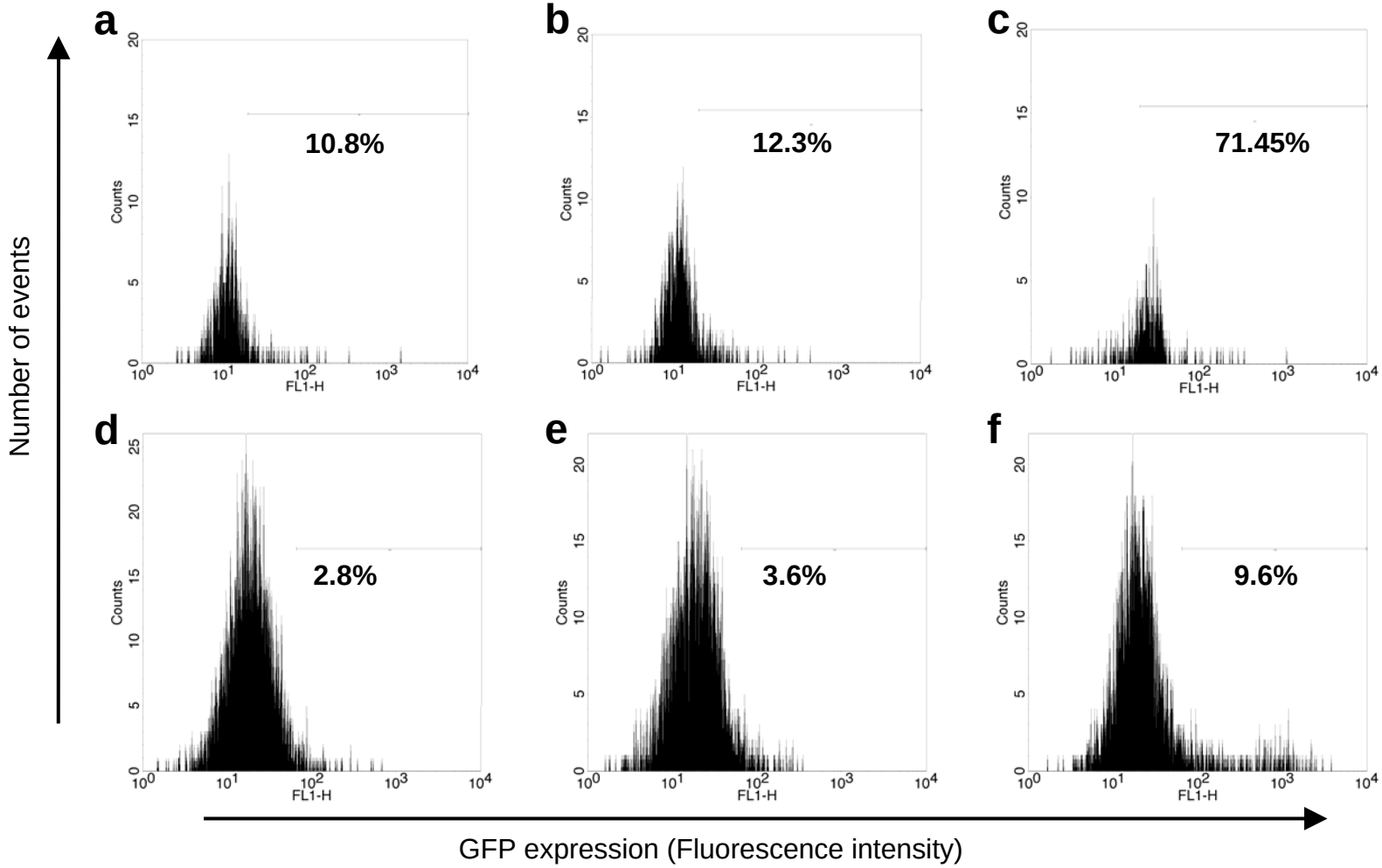
Supplementary Figure 1. Microscopic analysis of HeLa cells infected with recombinant *Salmonella* carrying the *lacZ* gene under the control of the regulatory module *nahR/Psal::xylS2* after induction.



HeLa cells infected with SL7207-4S2 carrying the high copy number vector pMPO2 (**a**, **b** and **c**) or the low copy number vector pMPO13 (**d**, **e** and **f**) after induction with salicylate or ASA (2 mM). The analysis is representative of 3 independent experiments. Scale bar, 50 µm.

Supplementary Figure 2. Flow cytometric analysis of cells from spleen and mesenteric lymph nodes from mice infected with recombinant *Salmonella* carrying the *gfp* gene under the control of the regulatory module *nahR/Psal::xylS2* after induction.



Cells were obtained from spleen (**a** to **c**) and mesenteric lymph nodes (**d** to **f**) from mice infected with SL7207-4S2 carrying either pWSK29 (**a** and **d**) or pMPO15 (**b**, **c**, **e** and **f**). Some mice received 150 ml of salicylate 100 mM by intraperitoneal route 4 h before sampling (**a**, **c**, **d** and **f**). Plots represent cell counts versus the fluorescence intensity in the green channel, which is derived from the GFP.

Supplementary Table 1: Strains, plasmids and cell lines used in this work

	Relevant genotype or characteristics	Reference or source
Strains		
E. coli DH5α	*deoR*, *endA1*, *gyrA96*, *recA1*, *supE44*.	1
E. coli S17-1 (λpir)	F^- *recA*, *hsdR*, RP4-2 (Tc::Mu)(Km::Tn7) lysogenized with λpir phage.	2
S. enterica aroA SL7207	*Salmonella typhimurium* 2337-65 (=WRAY) derivative, *his*G46 DEL407 [*aroA*::Tn10 {Tc^s}].	B. Stocker
S. enterica SL7207-4S2	Rif^R, Km^R; SL7207 derivative with a miniTn5 bearing a *nahR/Psal::xylS2* fusion inserted in the chromosome.	This study
S. enterica SL7207-4S2-MPO27	Rif^R, Km^R, Cm^R; SL7207-4S2 derivative carrying the Pm-*codA* expression module integrated into the *aroC* locus.	This study
S. enterica SL7207-4S2-MPO28	Rif^R, Km^R, Cm^R; SL7207-4S2 derivative carrying the Pm control expression module integrated into the *aroC* locus.	This study
Plasmids		
pCAS	Ap^R; expression vector with rrnBT1-Pm::MCS, ColE1 replication origin.	Active Motif, CA.
pCNB4-S2	Ap^R, Km^R; miniTn5 vector with the *nahR/Pasl::xylS2* fusion cloned between the I and O sites.	3
pASK-IBA43	Ap^R, pUC18-derivative with the tetR/tetO expression system.	IBA GmbH, Göttingen
pMPO2	Ap^R; pCAS derivative with a *trp′::′lacZ* fusion downstream of Pm.	This study
pMPO13	Ap^R; pWSK29 derivative with the Pm-*trp′::′lacZ* fusion of pMPO2.	This study
pMPO15	Ap^R; pWSK29 derivative with a Pm-*gfp* fusion replacing the Pm-*trp′::′lacZ* fusion of pMPO13.	This study
pMPO16	Ap^R, pCAS derivative with a Pm-*codA* fusion.	This study
pMPO17	Ap^R, pASK-IBA43 derivative with a Ptet-*codA* fusion.	This study
pWSK29	Ap^R; low-copy number vector for cloning and expression, pSC101 replication origin.	4
Cell Lines		
HeLa	Cell line derived from a human cervix adenocarcimoma.	ATCC CCL-2
J774.A1	Macrophage-like cell line derived from BALB/cN mice.	ATCC TIB-67
F1.A11	Cell line derived from a spontaneous murine fibrosarcoma from BALB/c mice.	5

References

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3. Cebolla, A., Sousa, C. & de Lorenzo, V. Rational design of a bacterial transcriptional cascade for amplifying gene expression capacity. *Nucleic Acids Res* **29**, 759-766 (2001).
4. Wang, R.F. & Kushner, S.R. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* **100**, 195-199 (1991).
5. Paglia, P., Medina, E., Arioli, I., Guzman, C.A. & Colombo, M.P. Gene transfer in dendritic cells, induced by oral DNA vaccination with *Salmonella typhimurium*, results in protective immunity against a murine fibrosarcoma. *Blood* **92**, 3172-3176 (1998).

Supplementary Methods

Integration of the regulatory module *nahR/Psal::xylS2* into the chromosome of *Salmonella*

To integrate the regulatory module *nahR/Psal::xylS2* (Fig. 1a), we conjugated a spontaneous rifampicin-resistant mutant of the *S. enterica aroA* serovar Typhimurium SL7207 strain with an *E. coli* S17-1(λ*pir*) strain carrying pCNB4-S2, which contains a minitransposon encompassing the regulatory cassette, in 1% sodium citrate at 30°C for 3 h. We selected transconjugants on LB agar supplemented with rifampicin and kanamycin. To evaluate the usefulness of salicylate/ASA as *in vivo* inducer, we electroporated a transconjugant clone (SL7207-4S2) with plasmids encoding *lacZ* (pMPO13 and pMPO2) or *gfp* (pMPO15). In this clone, the regulatory module was integrated between positions 7,351 and 7,352 of the *siiE* gene. We also generated clones harboring the empty vectors as controls for the characterization studies.

Integration of the cytosine-deaminase expression module into SL7207-4S2

We amplified the gene coding for chloramphenicol resistance from pKD3[1] using the primers Sac-P1 (5'-tatagagctctgtaggctggagctgcttc-3') and Sac-P2 (5'-tatagagctcatatgaatatcctccttag-3') and cloned into pMPO16 digested with *Hind*III and filled-in with Klenow polymerase, thereby generating pMPO65, which includes the FRTs sequences downstream of *codA* in the opposite orientation. We generated an additional pMPO16 derivative, pMPO66, in which we cloned the same PCR product into the vector digested with *Eco*RI/*Hind*III and end-filled with Klenow (without *codA*). We used these plasmids as templates for a PCR with the primers aroC-Pm (5'-agcgcaatcgcggttttttttcatttcttaccagcgtggaatctctgtcttaggctcagtcgaaagactgg-3') and aroC-P2 (5'-caaacgacaacaacgataacggagccgtgatggcaggaaacacaattggacatatgaatatcctccttag-3'). We purified the resulting PCR products digested with *Dpn*I, and electroporated them into a SL7207-4S2 strain containing pKD46 to integrate the expression module in the *aroC* locus[1]. We verified integration using primers aroC-E1 (5'-gttgaccaaagcgcagttgc-3') and aroC-E2 (5'-gcgctactgacaaaccatgc-3'). The resulting strains were SL7207-4S2-MPO27 (CD expression module) and SL7207-4S2-MPO28 (control expression module). If required, the chloramphenicol resistance gene can be subsequently deleted using pCP20[1].

Reference

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