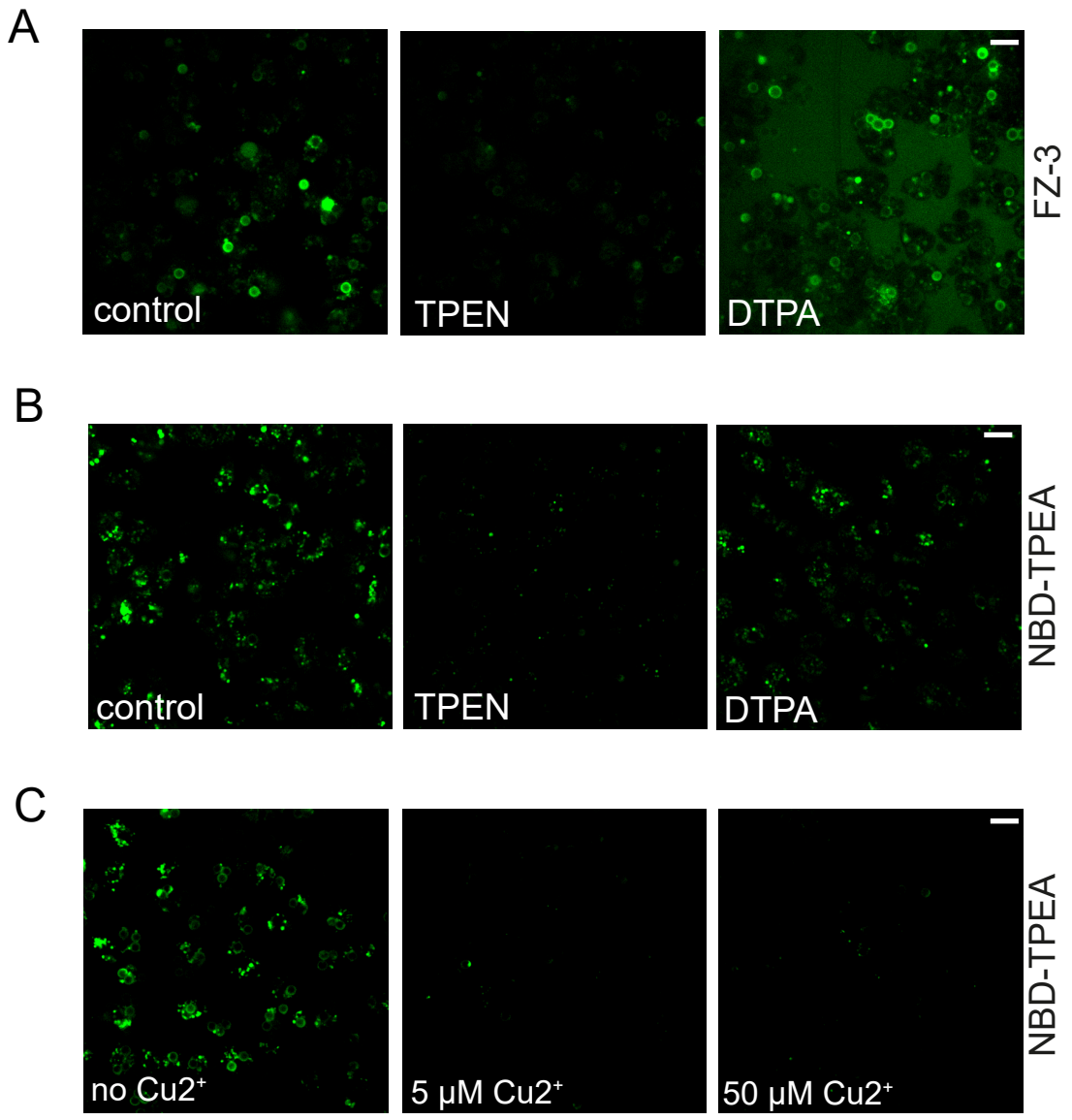


Fig. S1. Control experiments showing the specificity of FZ-3 and NBD-TPEA for Zn and the quenching of NBD-TPEA by Cu. A.-C. Cells were incubated with the different chelators or $CuSO_4$ at the indicated concentrations for 3 hrs before the staining with FZ-3 and NBD-TPEA was performed. Afterwards cells were fed with 3 µm and images were taken by live-microscopy. Importantly, the chelators and $CuSO_4$ were present throughout the experiment.

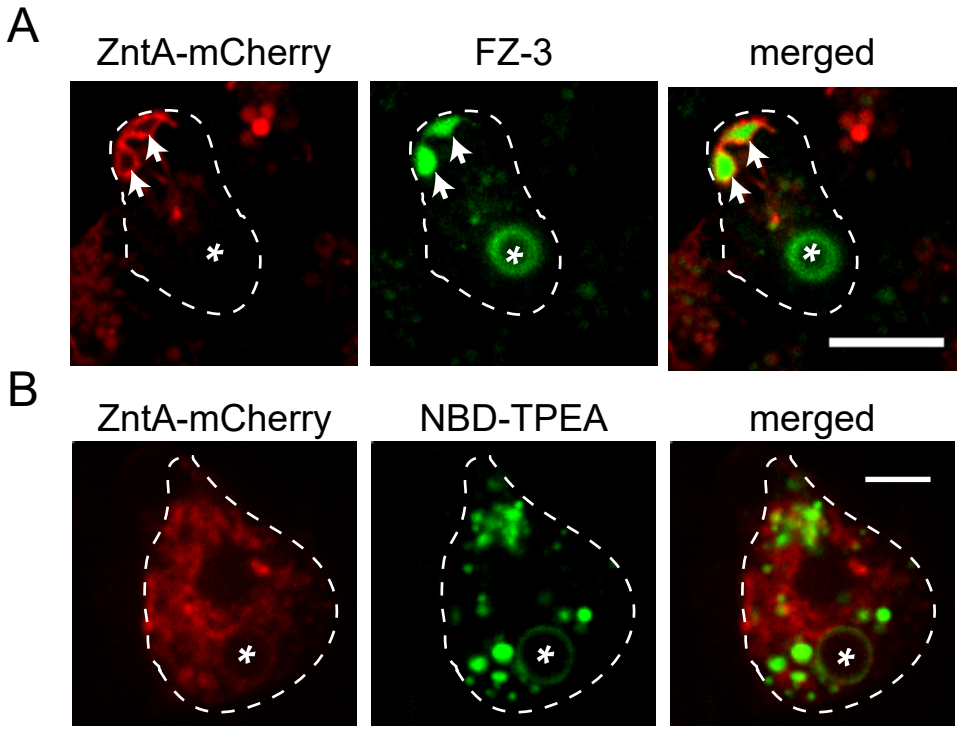


Fig. S2. ZntA-mCherry is not present on BCPs. A. and **B.** Cells expressing ZntA-mCherry were stained with FZ-3 and NBD-TPEA and fed with 3 µm latex beads. Images were taken live. Asterisks label BCPs, arrows point to FZ-3-positive CV-bladders. Scale bars 10 µm (A), 5 µm (B).

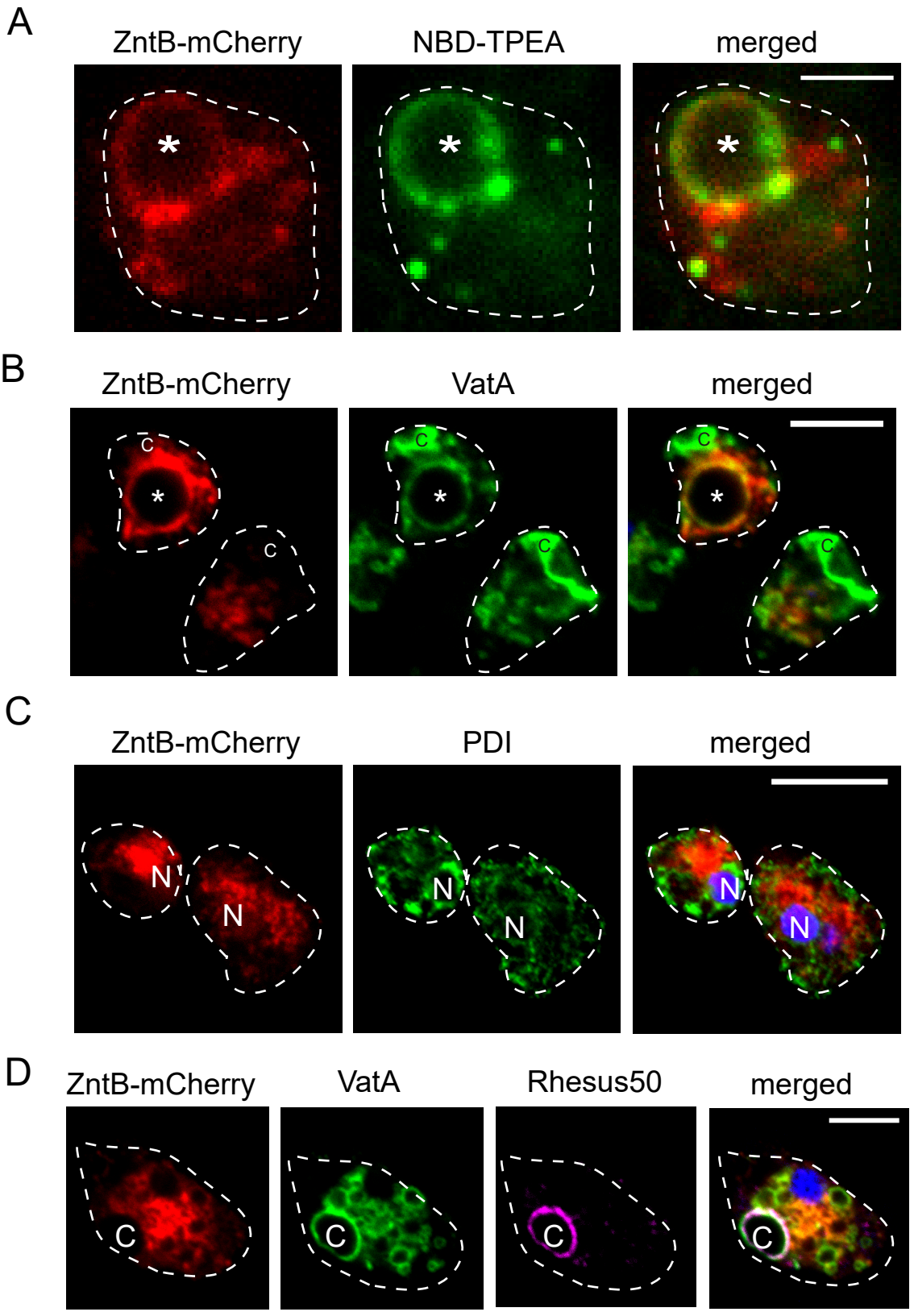
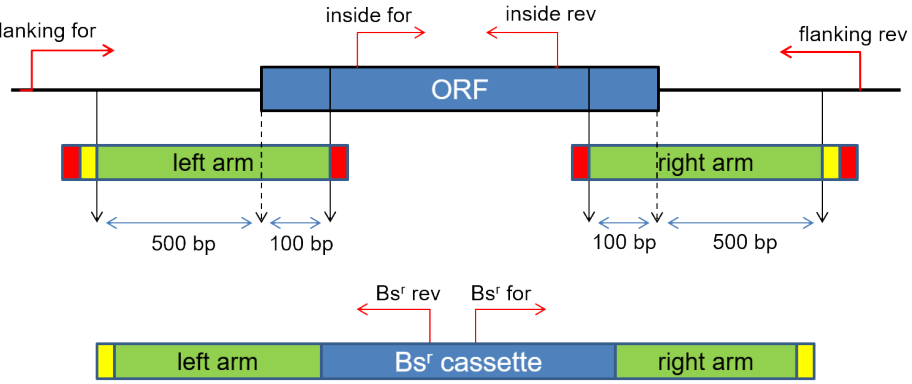


Fig. S3. ZntB-mCherry decorates BCPs and does not co-localize with ER- and CV-markers. A. ZntB is present at BCPs at the lysosomal maturation stage. Cells expressing ZntB-mCherry were stained with NBD-TPEA and fed with 3 µm latex beads. Images were taken live. Scale bar, 5 µm. **B.** ZntB-mCherry co-localizes with the vATPase at BCPs. Scale bar, 10 µm. **C. and D.** ZntB-mCherry does not localize at the ER and the at the CV. ZntB-mCherry expressing cells were fixed and stained with antibodies against PDI (C) and Rhesus50 (D). Nuclei were stained with DAPI. Scale bars, 10 µm (C) and 5 µm (D). Asterisks label a BCPs, C: tubular network of the CV, N: nucleus.

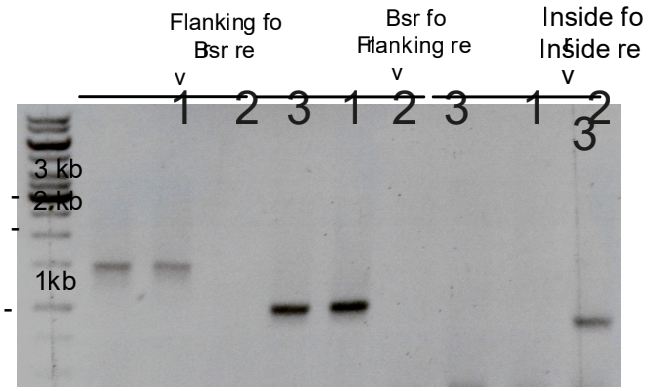
A



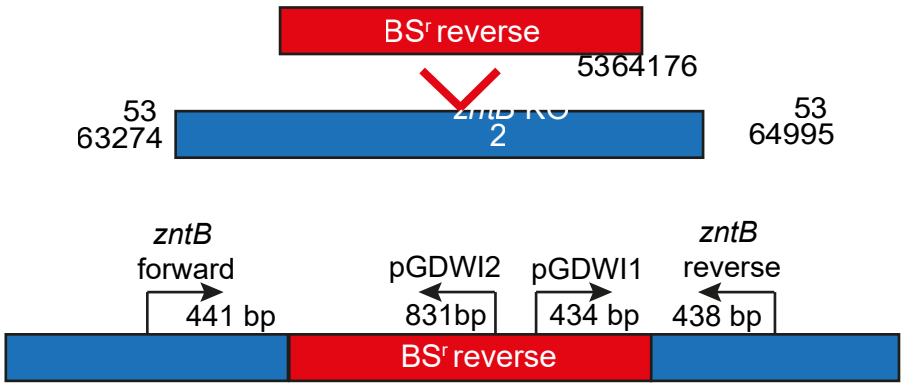
Recombination site and StarCombinase™ recognition area

Unique restriction site

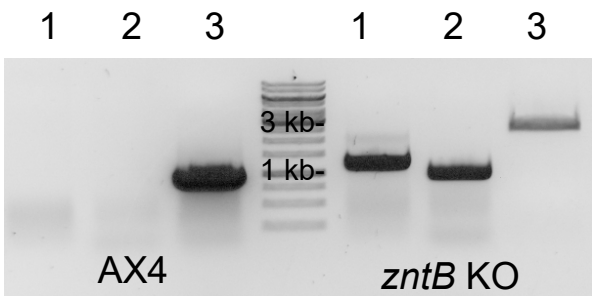
B



C



D



1: *zntB* forward / pGWDI2
2: pGWDI1 / *zntB* reverse
3: *zntB* forward & reverse

Fig. S4. Generation of a *zntA* KO by homologous recombination and localization of the insertion in the *zntB* KO. A. Schematic drawing of the *zntA*-encoding gene locus (ORF, blue) flanked by non-coding segments. For gene disruption, the resistance cassette (BSr, green) was integrated removing a segment in the middle of the gene (between the inside forward/inside reverse primers) using the StarCombinase and the StarGate cloning kit. The red arrows indicate primers that were used to monitor correct integration. **B.** PCR-analysis of two *zntA* mutants (#1 and #2) and wild type (#3). Using the flanking forward/BSR reverse or the flanking reverse/BSR forward primer combinations small products were obtained in both mutants, but not in the wild type. The inside forward/inside reverse primer combination yielded a small product in the wild type, but not in the mutants. Experiments were performed using mutant #1. **C.** The restriction-mediated insertion of the *zntB* KO interrupts the gene approximately in the middle at chromosomal position 5364176 (Chromosome 3). **D.** A diagnostic PCR was performed to confirm the insertion into *zntB* using the primers indicated in C.

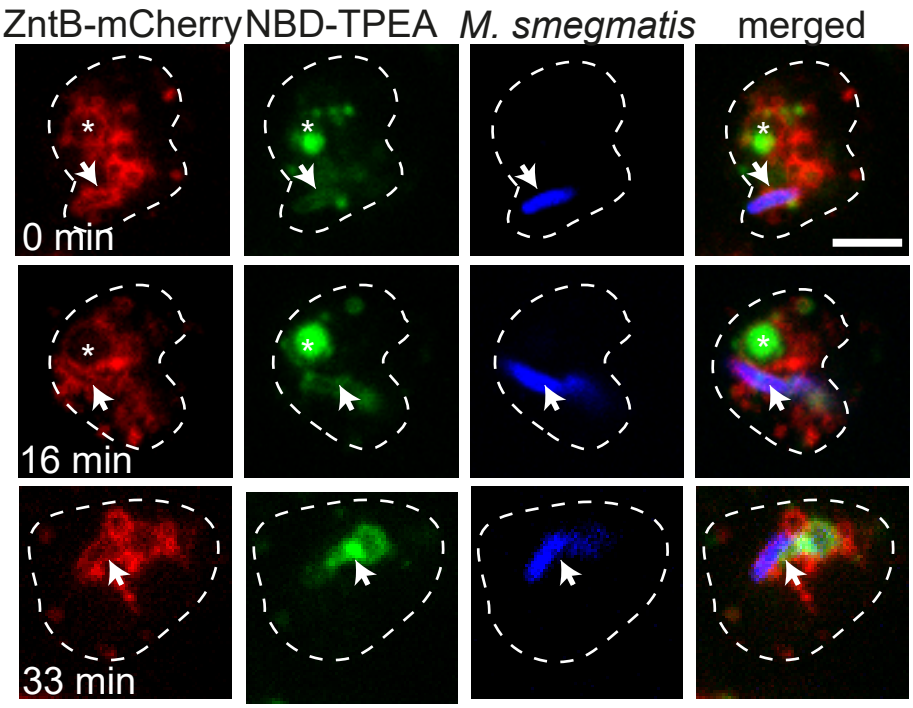


Fig. S5. ZntB-mCherry is localized at the *M. smegmatis*-containing phagosome. Cells expressing ZntB-mCherry were stained with NBD-TPEA and fed with *M. smegmatis*. Images were recorded live. Scale bar, 5 µm. Arrows label phagosomes, asterisks point to zincosomes.

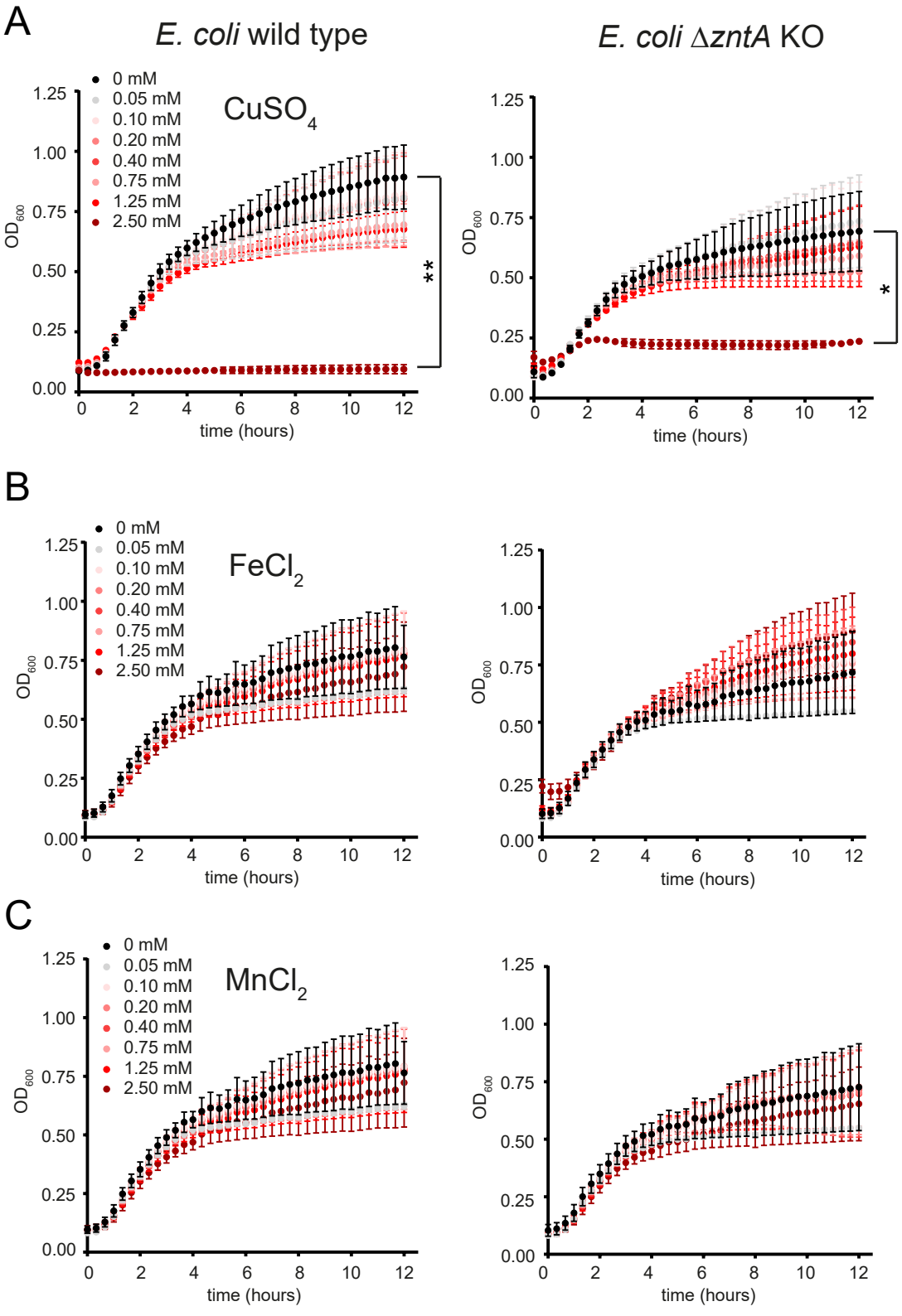


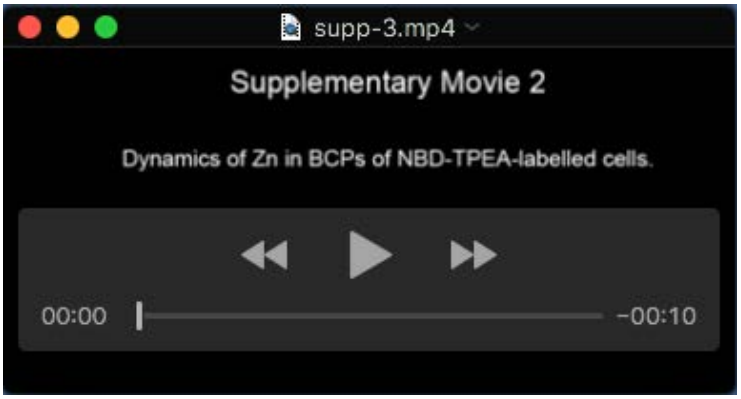
Fig. S6. The *zntA E. coli* KO is not susceptible to increasing concentrations of $CuSO_4$, $FeCl_2$, $MnCl_2$. *E. coli* strains were incubated in LB. Metals were added as indicated. The OD_{600} was measured with the help of a 96-well plate reader (SpectraMax i3, Molecular Devices). Statistical differences were calculated with a Bonferroni post hoc test after two-way ANOVA. Significantly different values were indicated by an asterisk (* $P < 0.5$, ** $P < 0.01$).

Table S1. *Dictyostelium* material used for this study.

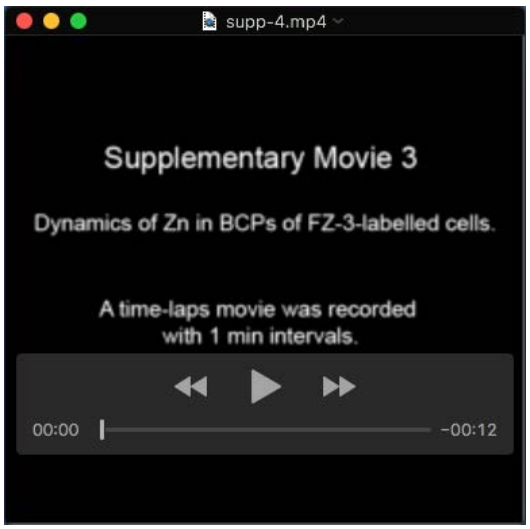
***Dictyostelium* strains**	**Plasmids used for transformation**	**Reference**
Ax2(Ka) ZntA-mCherry	ZntA-mCherry (see below)	This study
Ax2(Ka) ZntB-mCherry	ZntB-mCherry (see below)	This study
Ax2(Ka) ZntC-mCherry	ZntC-mCherry (see below)	This study
Ax2(Ka) ZntD-mCherry	ZntD-mCherry (see below)	This study
Ax2(Ka) *zntA* KO	See below	This study
Ax2(Ka) *zntA* KO mCherry-ZntA	mCherry-ZntA (see below)	This study
AX4 *zntB* KO	-	REMI library Prof. Christopher Thompson
AX4 *zntB* KO ZntB-mCherry	ZntB-mCherry (see below)	This study
Ax2(Ka) AmtA-mCherry		(Barisch et al., 2015)
Ax2(Ka) VatB-RFP		(Carnell et al., 2011)
Ax2(Ka) RFP-VacA		This study
AX2 *wshA* KO		(Carnell et al., 2011)
AX2 *pikfyve* KO		(Buckley et al., 2018)

Plasmids generated for this work	**Insert**	**Plasmid &Reference**
ZntA-mCherry	*zntA* gDNA(*DDB_G0283629*)	pDM1044 (Veltman et al., 2009)
ZntB-mCherry	*zntB* gDNA(*DDB_G0282067*)	pDM1044 (Veltman et al., 2009)
ZntC-mCherry	*zntC* cDNA(*DDB_G0269332*)	pDM1044 (Veltman et al., 2009)
ZntD-mCherry	*zntD* cDNA(*DDB_G0291141*)	pDM1044 (Veltman et al., 2009)
mCherry-ZntA	*zntA* amplified from ZntA-mCherry	pDM1042 (Veltman et al., 2009)
ZntA KO plasmid	pKOSG-IBA-ZntA (please see Materials and Methods)	StarGate® Acceptor Vector pKOS-IBA-Dicty1 (Wiegand et al., 2011)
RFP-VacA	vacA was amplified from pDM323 GFP-VacA (lab plasmid)	pDM 324 (Veltman et al., 2009)

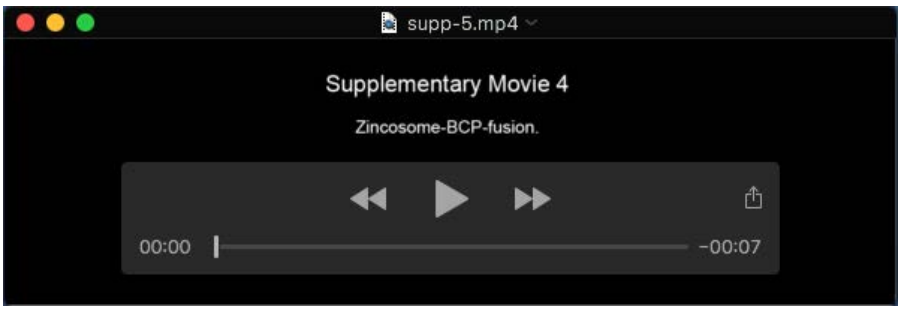
Movie 1. Zn is expelled from the cells when the CV discharges. For more information, see Fig. 1C.



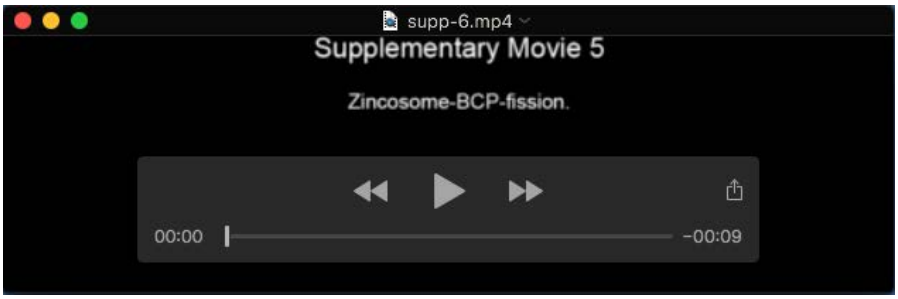
Movie 2. Dynamics of Zn in BCPs of NBD-TPEA-labelled cells. For more information, see Fig. 2A.



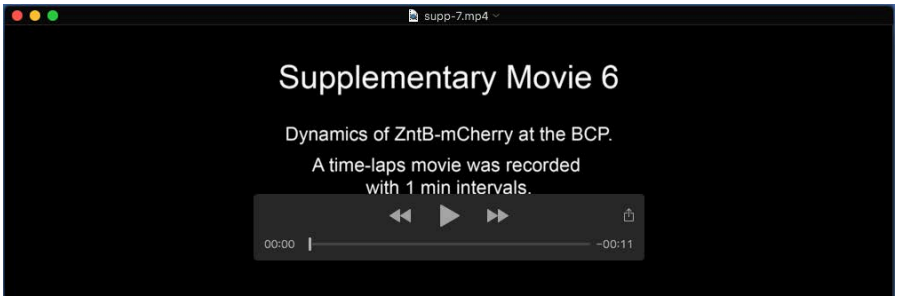
Movie 3. Dynamics of Zn in BCPs of FZ-3-labelled cells. For more information, see Fig. 2C.



Movie 4. Zincosome-BCP-fusion. For more information, see Fig. 3A.



Movie 5. Zincosome-BCP-fission. For more information, see Fig. 3C.



Movie 6. Dynamics of ZntB-mCherry at the BCP. For more information, see Fig. 4D.