

Supplementary Materials

# Novel Nile Blue Analogue Stains Yeast Vacuolar Membrane, Endoplasmic Reticulum and Lipid Droplets, Inducing Cell Death through Vacuole Membrane Permeabilization

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## Captions:

**Figure S1 Effect of BaP1 on mitochondrial membrane potential.**

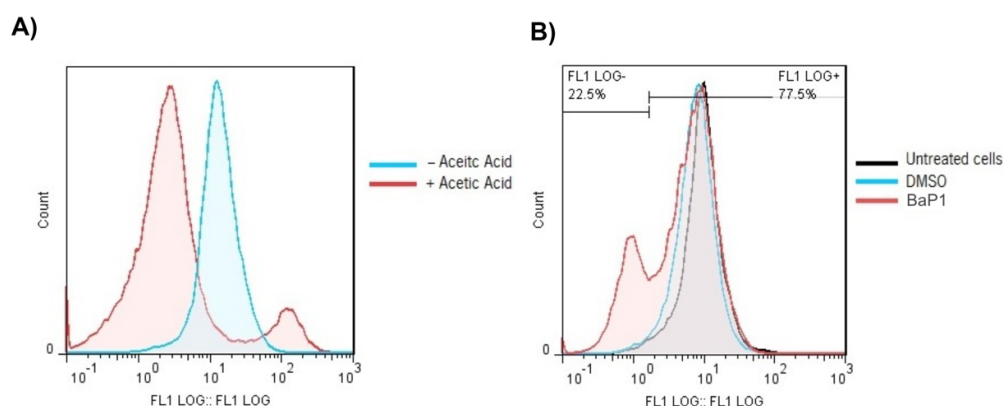
**Figure S2 Effect of BaP1 on ROS production.**

**Figure S3 Effect of BaP1 on intracellular calcium accumulation.**

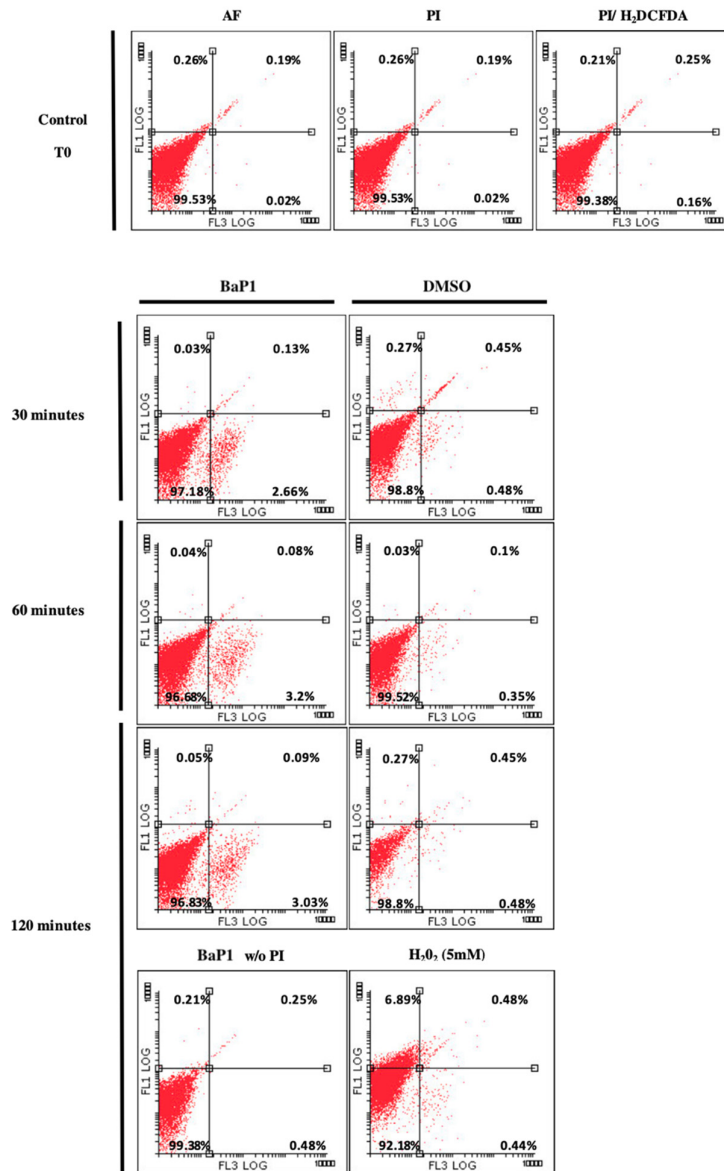
**Figure S4 A and B Evaluation of DNA fragmentation and DNA cell content.**

**Figure S5 Effect of BaP1 on plasma membrane integrity.**

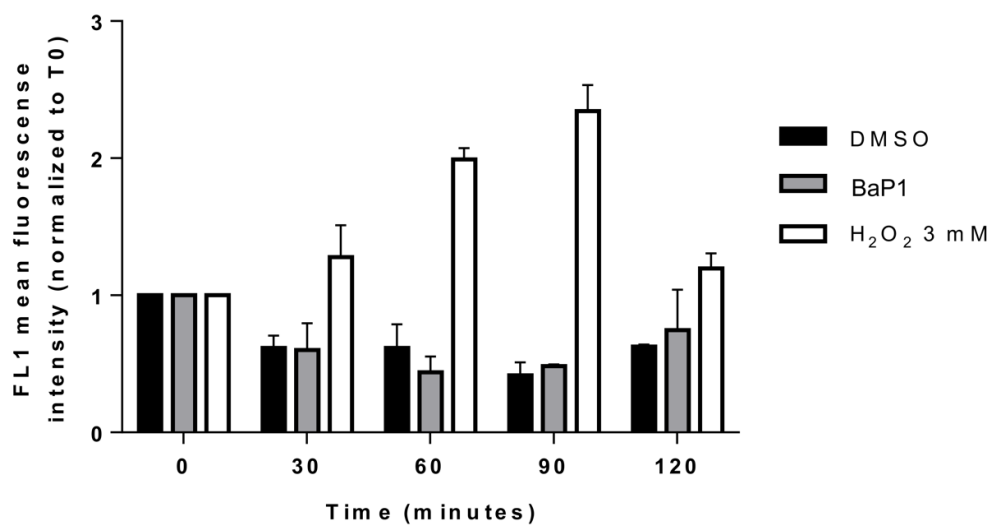
**Figure S6 BaP1 intracellular distribution in lipid droplets mutants.**



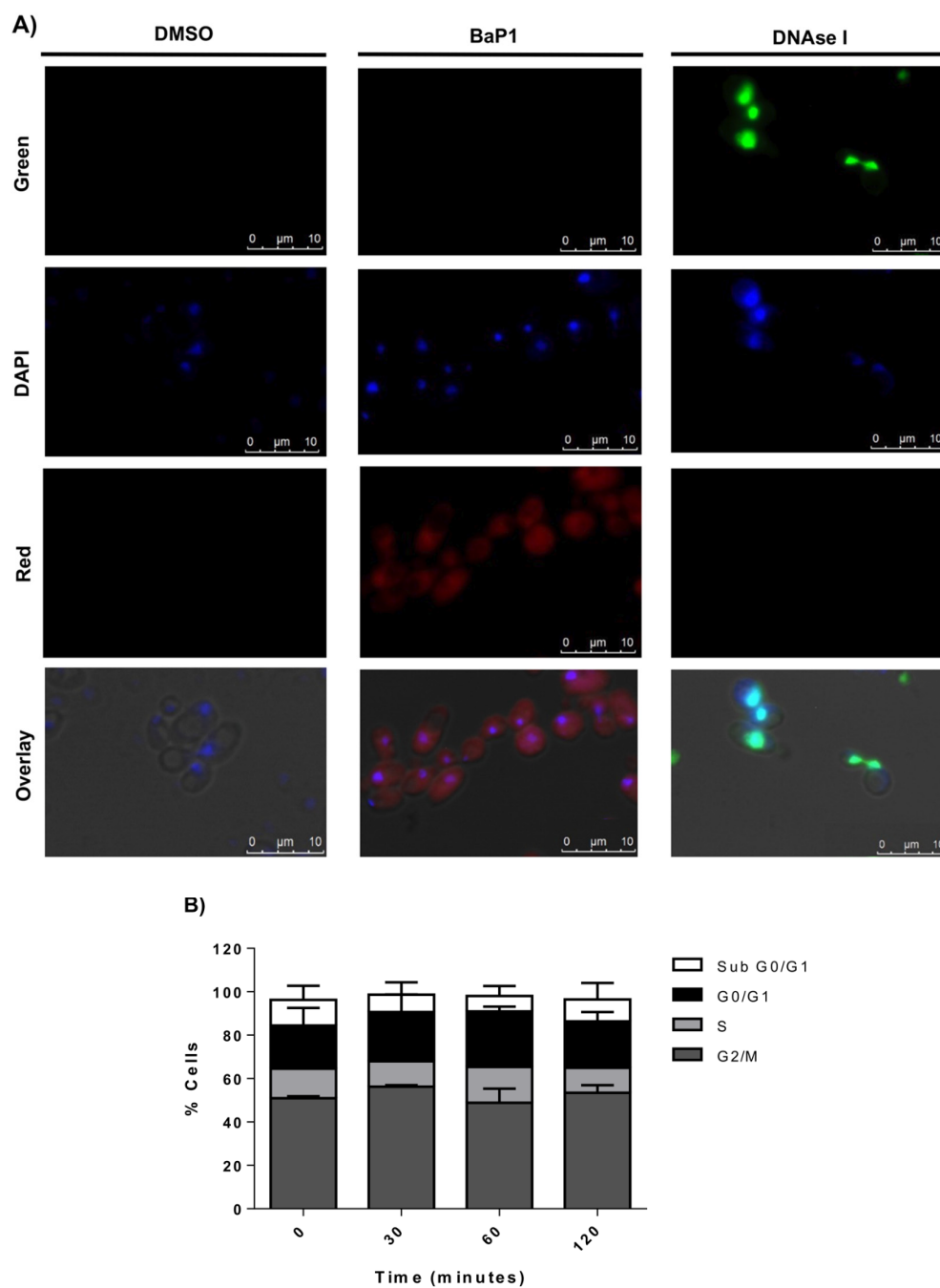
**Figure S1.** Effect of BaP1 on mitochondrial membrane potential. A) Effect of acetic acid (150 mM at pH 3.0 (positive control)) on *S. cerevisiae* BY4741 mitochondrial membrane potential. For flow cytometry analysis, the cells were collected before (- acetic acid) and after 60 minutes of acetic acid treatment (+ acetic acid) and stained with 0.24  $\mu$ M of Dioc<sub>6</sub>(3). B) Effect of BaP1(300  $\mu$ M) and DMSO (0.35%) on BY4741 mitochondrial membrane potential. The represented results correspond to the 60 minutes time point. Results are expressed as a mono-parametric histogram of Dioc<sub>6</sub>(3) fluorescence representing one of three independent experiments. Gate defined by auto-fluorescence sample.



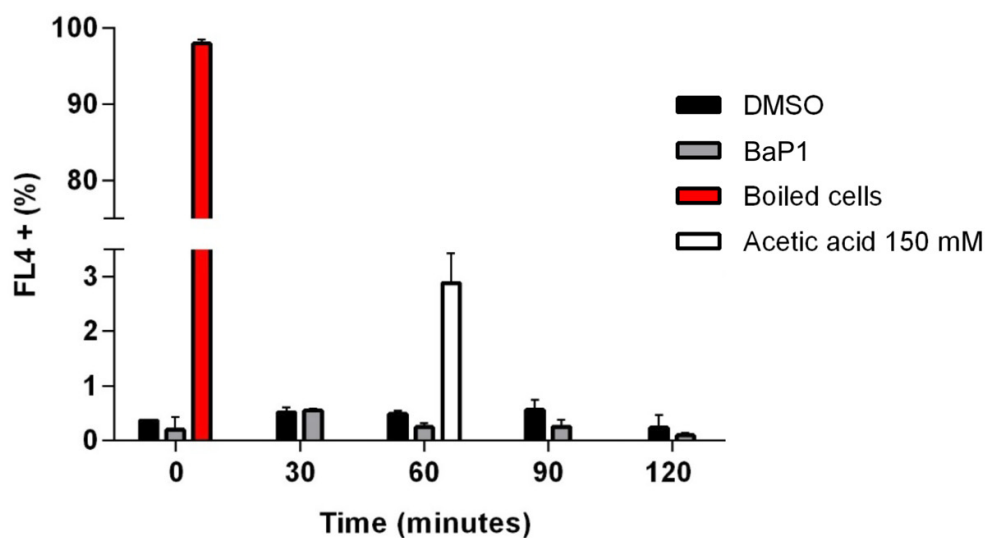
**Figure S2.** Effect of BaP1 on ROS production. Representative experiment of the effect of BaP1(300  $\mu$ M) on *S. cerevisiae* W303-1A ROS production. For flow cytometry analysis the cells were collected at different time points and stained with 40 $\mu$ g/mL H<sub>2</sub>DCFDA and 2  $\mu$ g/mL of PI. 5mM Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was used as positive control.



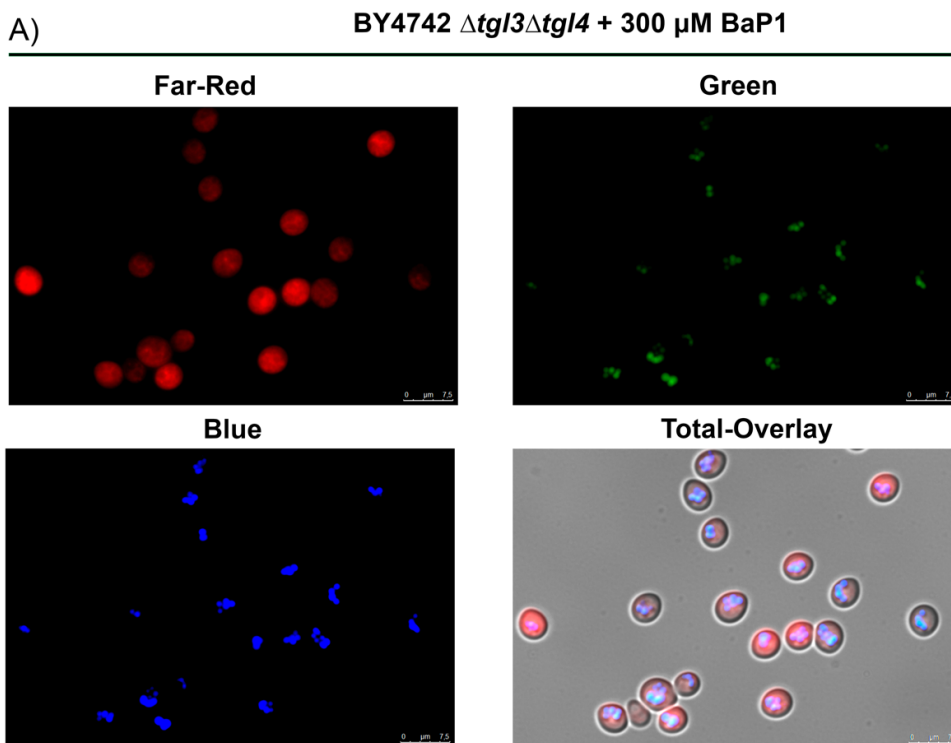
**Figure S3.** Effect of BaP1 on intracellular calcium accumulation. Effect of BaP1 (300  $\mu$ M), DMSO (0.35%) and H<sub>2</sub>O<sub>2</sub> (3 mM at pH 3.0 (positive control)) on *S. cerevisiae* BY4741 intracellular calcium accumulation. For flow cytometry analysis the cells were collected at different time points and stained with 5  $\mu$ M FLuo4-AM. Results are expressed as ratio values estimated by dividing the FL1 mean fluorescence intensity of each sample by the FL1 mean of the time 0 sample. Statistical analysis: BaP1: (T0 vs T30) ns, (T0 vs T60) ns, (T0 vs T90) ns and (T0 vs T120) ns; DMSO: (T0 vs T30) ns, (T0 vs T60) ns, (T0 vs T90) ns and (T0 vs T120) ns; H<sub>2</sub>O<sub>2</sub>: (T0 vs T30) ns, (T0 vs T60)\*\*, (T0 vs T90)\*\*\* and (T0 vs T120) ns. Values are means with SD (n $\geq$ 2). Statistical analysis was performed by two-way ANOVA. ns non-significant, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001.

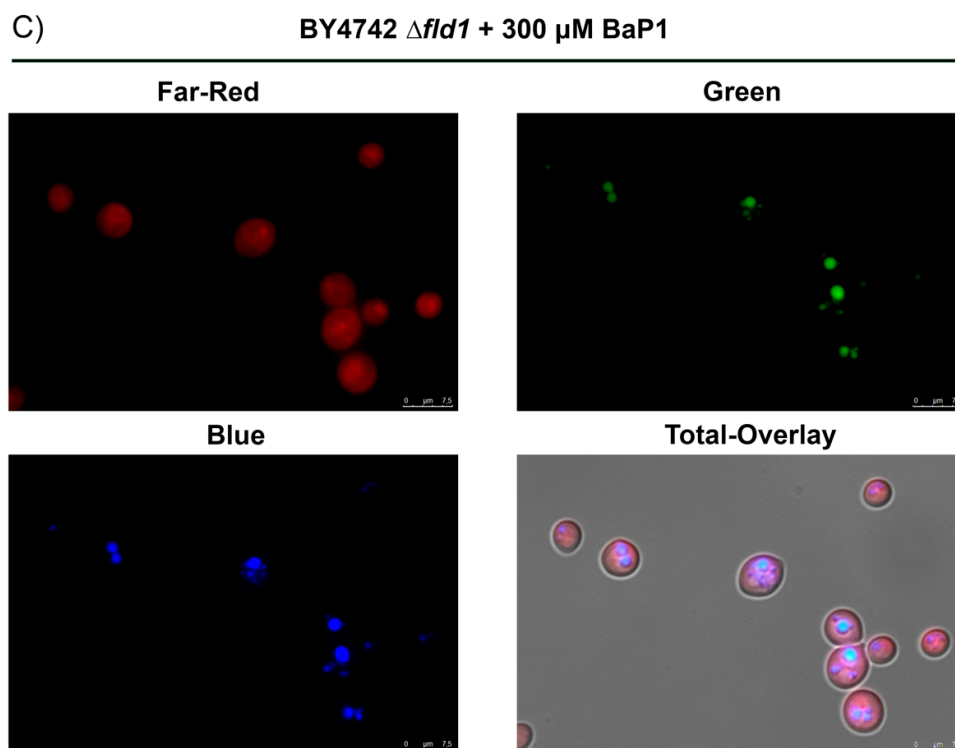
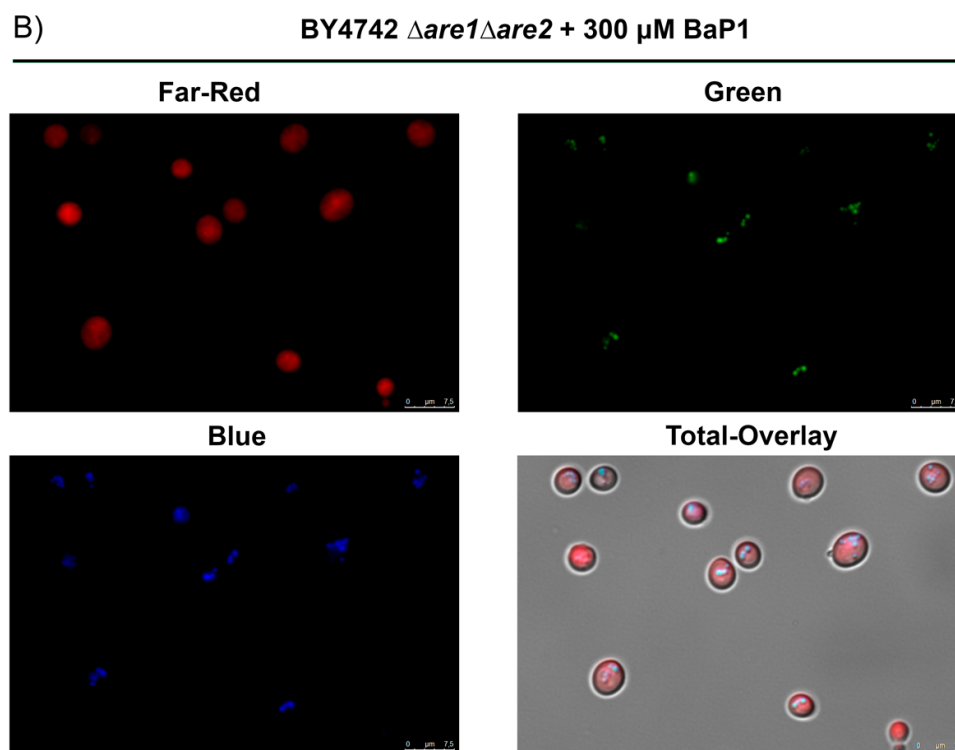


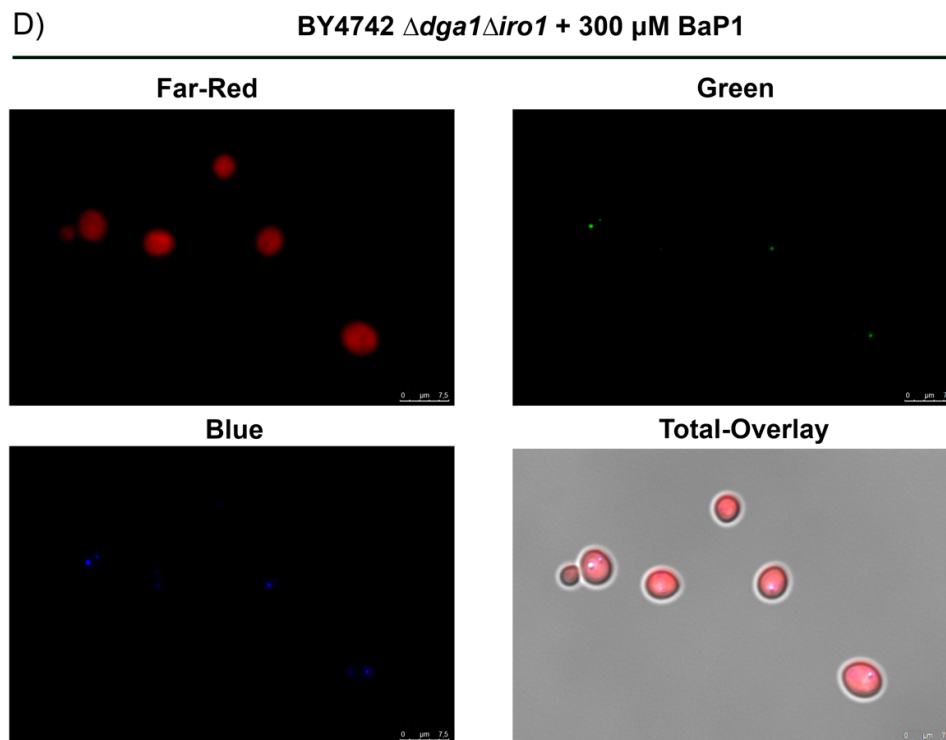
**Figure S4.** Evaluation of DNA fragmentation and DNA cell content A) Florescence microscopy images of TUNEL assay of *S. cerevisiae* BY4741 after 30 minutes treatment with BaP1 (300  $\mu$ M) and DMSO(0.35%). Samples were collected at different time points and subjected to TUNEL protocol. B) Effect of BaP1 (300  $\mu$ M) and DMSO (0.35%) in cell cycle of *S. cerevisiae* W303-1A. Cells were stained with SYTOX Green and analyzed by flow cytometry. Values are means with SD ( $n \geq 2$ ). Scale bar = 10  $\mu$ m.



**Figure S5.** Effect of BaP1 on plasma membrane integrity. Effect of BaP1 (300  $\mu$ M), DMSO (0.35%) and acetic acid (150mM at pH 3.0) on propidium iodide (PI) staining of *S. cerevisiae* BY474. Boiled cells were used as positive control for PI staining. For flow cytometry analysis the cells were collected at different time points and stained with 2 $\mu$ g/mL of PI. Results are expressed as percentage of FL4 positive population (FL4 LOG +). Statistical analysis: BaP1: (t0 vs t30)ns, (t0 vs t60)ns, (t0 vs t90) ns, (t0 vs t120) ns; DMSO: (t0 vs t30) ns, (t0 vs t60) ns, (t0 vs t90) ns, (t0 vs t120) ns; Acetic acid: (t0 vs t60)\*\*\*. Values are means with SD (n $\geq$ 2). Statistical analysis was performed by two-way ANOVA. ns non-significant, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001.







**Figure S6.** BaP1 intracellular distribution in lipid droplets mutants. A) Florescence microscopy images of BY4742  $\Delta tgl3\Delta tgl4$  cells after incubation with BaP1 (300  $\mu$ M). B) Florescence microscopy images of BY4742  $\Delta are1\Delta are2$  cells after incubation with BaP1 (300  $\mu$ M). C) Florescence microscopy images of BY4742  $\Delta fld1$  cells after incubation with BaP1 (300  $\mu$ M). D) Florescence microscopy images of BY4742  $\Delta dga1\Delta iro1$  cells after incubation with BaP1 (300  $\mu$ M). BY4742  $\Delta tgl3\Delta tgl4$  (lacking the major TAG lipases, which exhibit higher LD accumulation), BY4742  $\Delta are1\Delta are2$  (lacking the steryl ester synthases and thus harbors LDs that contain triacylglycerols only), BY4742  $\Delta fld1$  (lacking the yeast ortholog of seipin, resulting in a fewer and larger LDs) and BY4742  $\Delta dga1\Delta iro1$  (mutant lacking acyl-CoA and phospholipid dependent diacylglycerol (DAG) acyltransferases and thus harbors LDs that contain steryl esters only). Samples were stained in YPD at room temperature and visualized by epifluorescence microscopy after 5 minutes with a 100x oil immersion objective.