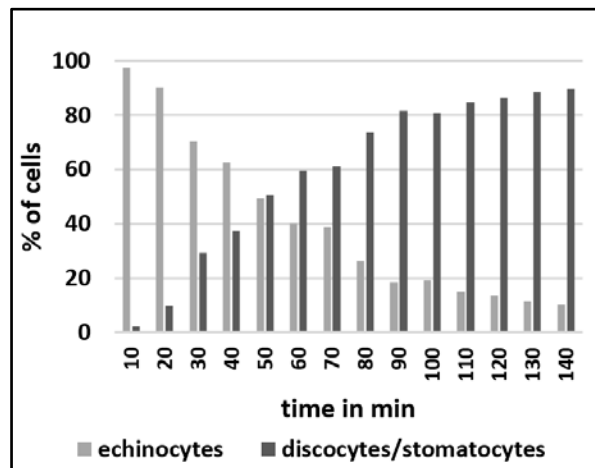
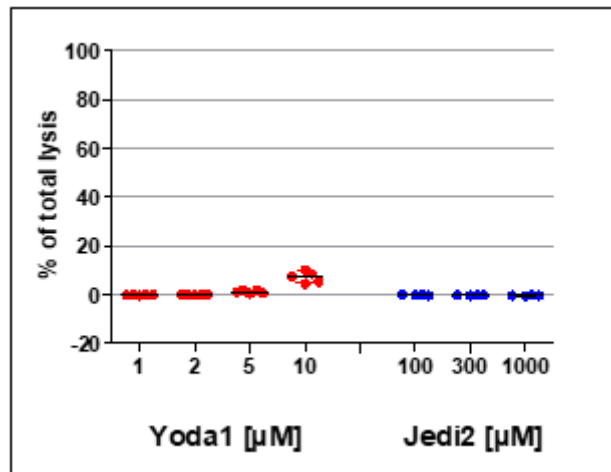


## Supplemental Data

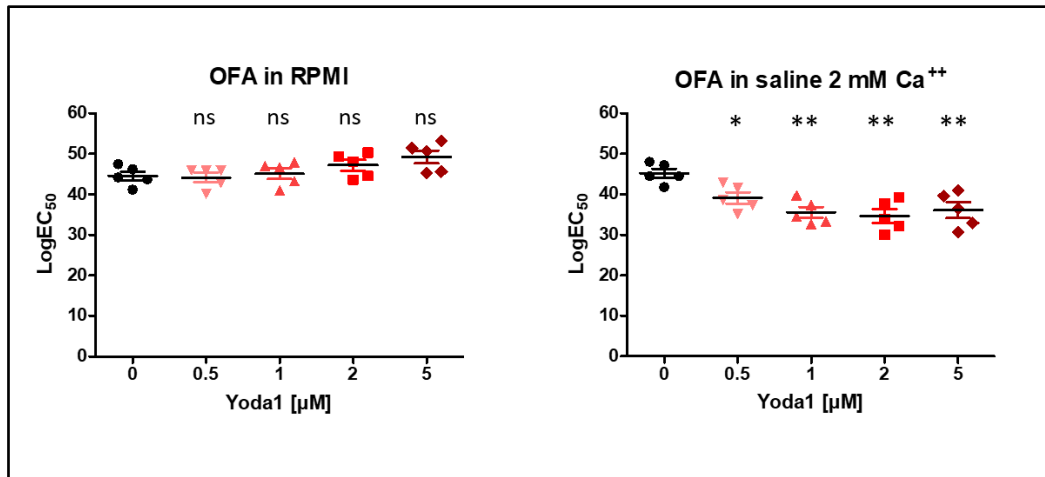
### Figures



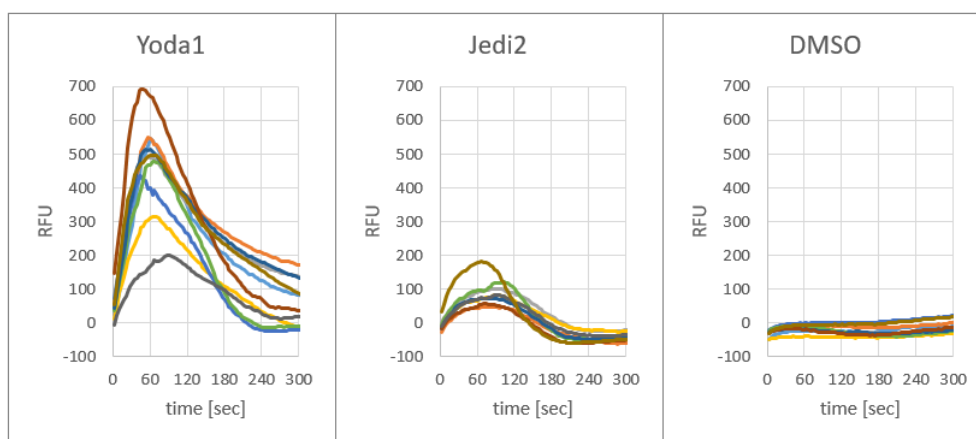
**Supplemental Fig. 1:** RBC shape changes over prolonged application (2½ hours) of Yoda1. Graphical representation of cell shape changes over time (data extracted from supplemental Video 4).



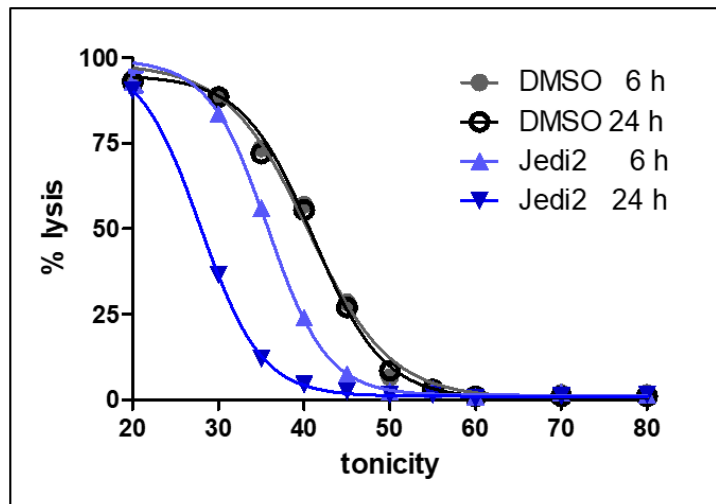
**Supplemental Fig. 2:** Haemolytic activities of the PIEZO1 activators Yoda1 and Jedi2. Blood of different donors was cultured for 24 hours at 2.5% haematocrit in standard culture conditions (RPMI without phenol red and with 0.5% albumax) in the presence of the indicated concentrations of compounds. Haemoglobin released into the culture supernatant was quantified by measuring the absorbance at 540 nm in a TECAN spectrophotometer and expressed as percentage of total lysis induced by the addition of 0.15% saponin. Shown are mean and SD,  $n = 5$  for Yoda1 and  $n = 4$  for Jedi2, all performed in triplicates.



**Supplemental Fig. 3:** Osmotic fragility assays after incubation in RPMI medium (left panel) and saline solution containing 2 mM CaCl<sub>2</sub> (right panel). Blood of five different donors was incubated with the indicated concentrations of Yoda1 for 30 minutes either in saline solution (149 mM NaCl, 4 mM KCl, 2 mM CaCl<sub>2</sub>, 2 mM HEPES, pH 7.4) or in RPMI medium before determining their osmotic fragility after 5 min in varying iso- and hypo-osmotic solutions. Shown are the mean and SEM, n = 5, all performed in duplicates. \* = p < 0.05, \*\* = p < 0.01, ns = not significant (Mann-Whitney test).



**Supplemental Fig. 4:** Fluorescence readings of Fluo4-AM loaded RBCs by video microscopy. Each line represents the reading of an individual cell over time. Time = 0 corresponds to the addition of complete medium RPMI/albumax containing 5  $\mu$ M Yoda1, 1 mM Jedi2 or DMSO vehicle and followed over 5 min.



**Supplemental Fig. 5:** Osmotic fragility curves of human RBCs after prolonged incubations with 1 mM Jedi2 or DMSO solvent in RPMI/albumax medium. Shown is one representative example done in duplicates (n=3).

## Videos

### Video 1

Absence of changes of the RBC cell shape upon the addition of DMSO vehicle. Human RBCs were loaded in complete medium RPMI/albumax in poly-L-lysine treated 15  $\mu$ -Slide VI (ibidi) and imaged using a 63 $\times$  Apochromat objective (NA 1.4) on an inverted brightfield microscope (Axioobserver, Zeiss), equipped with an incubation chamber set at 37°C and 5% CO<sub>2</sub> and a CoolSNAP HQ2 CCD camera (Photometrics). Time-lapse acquisition over 20 minutes at 1 image every 10 seconds was started prior to the addition of DMSO vehicle (defined as time = 0) in complete medium.

### Video 2

Changes of the RBC cell shape upon the addition of 1  $\mu$ M Yoda1. Human RBCs were loaded in complete medium RPMI/albumax in poly-L-lysine treated 15  $\mu$ -Slide VI (ibidi) and imaged using 63 $\times$  magnification. Time-lapse acquisition over 20 minutes (1 image every 10 seconds) was started prior to the addition of 1  $\mu$ M Yoda1 (defined as time = 0) in complete medium.

### Video 3

Changes of the RBC cell shape upon the addition of 5  $\mu$ M Yoda1. Human RBCs were loaded in complete medium RPMI/albumax in poly-L-lysine treated 15  $\mu$ -Slide VI (ibidi) and imaged using 63 $\times$  magnification. Time-lapse acquisition (1 image every 2 seconds) was started prior to the addition of 5  $\mu$ M Yoda1 in complete medium (defined as time = 0). Note the reduced duration of the recording (70 sec) compared to 20 min for 1  $\mu$ M Yoda1 and DMSO control.

### Video 4

To observe the recovery of RBC cell shape, RBCs in complete medium were treated with 10  $\mu$ M Yoda1 (at this concentration, all RBCs become strong echinocytes), directly placed in glass bottom dishes (MatTek), and time-lapse video microscopy recorded at 37°C and 5% CO<sub>2</sub> with 63  $\times$  magnifications over 2 ½ h. Time indicated in min. Scale bar 10  $\mu$ M. A graphical representation of cell shape changes over time is presented in Supplemental Fig. 1.

### Video 5

Representative video of invasion events in complete medium with mock-treated RBCs. RBCs that become invaded are labelled 1 and 2. Selected images of RBC 1 are shown in Figure 4B. Scale bar 5  $\mu$ M.

### Video 6

Representative video of the reaction of RBCs pre-treated with 5  $\mu$ M Yoda1 to contact with *P. falciparum* merozoites and absence of invasion. Scale bar 2  $\mu$ M.