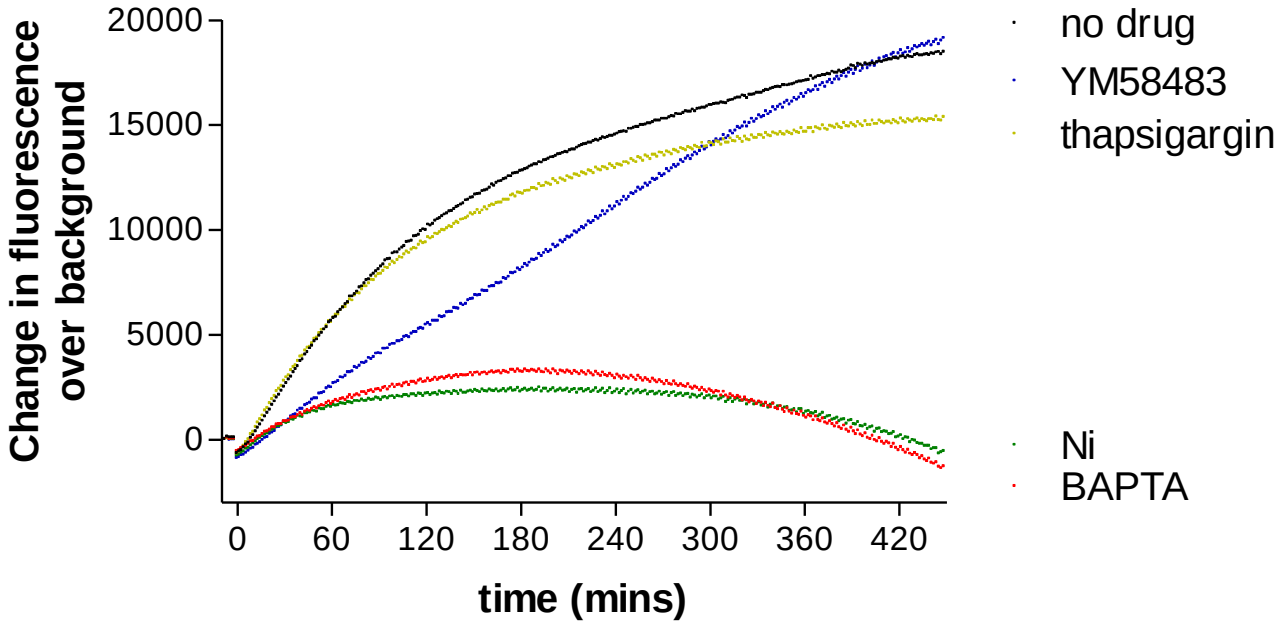
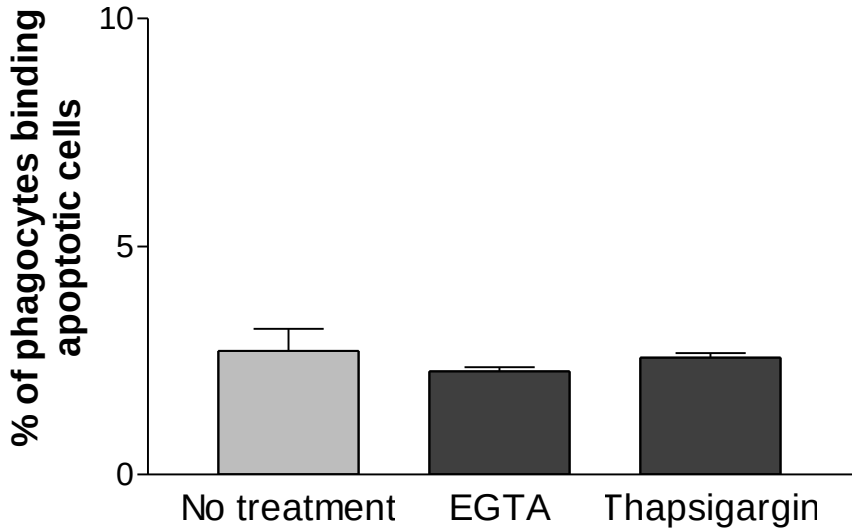


Calcium flux in LR73 cells incubated with the indicated drugs (100 μM YM-58483, 10 μM thapsigargin, 1 mM Nickel or 10 μM BAPTA) followed for 7 hours after the addition of apoptotic thymocytes (added at time 0). Data are shown as change in fluorescence over background (LR73 cells without the addition of apoptotic thymocytes).

We addressed how the drugs we used to block steps within the calcium flux machinery and which in turn inhibited engulfment correlated with calcium flux during engulfment. As above, we measured cellar calcium levels using the fluorimetry based assay that assesses flux in a whole population of cells in a well. Nickel, which blocks extracellular calcium influx, as well as BAPTA, which chelates extracellular calcium, greatly reduced calcium flux. However, thapsigargin, which prevents refilling of the depleted ER calcium stores, had only a slight effect on the flux. Since thapsigargin seems to strongly inhibit the uptake of apoptotic cells in the engulfment assay (Fig. 3a), this suggests that perhaps the plate calcium flux assay we used is not sensitive enough to detect changes in calcium flux due to intracellular store release. Blocking CRAC channels with YM58483 delayed calcium flux, although by 7 hours the levels reached were similar to those in the control. We interpret these data to mean that calcium flux from intracellular stores as well as calcium entry during the early stages of corpse recognition are important for internalization of apoptotic cells by the phagocyte.

Sup. Fig. 1



LR73 phagocytes were incubated with TAMRA labeled apoptotic thymocytes for 2 hours at 4°C, followed by extensive washing. The degree of apoptotic cell binding to phagocytes was obtained by flow cytometry as the percentage of FL2 positive cells.

Sup. Fig. 2

Drug	Concentration used	Mode of action	Refs
Thapsigargin	up to 8 μM	SERCA	(1;2)
YM-58483	up to 10 μM	CRAC channels	(3;4)
Nickel	up to 100 μM	Stabilizes closed channel states, blocks open channels	(5)
Ru360	10 μM	Mitochondrial calcium uptake inhibitor	(6)

References

(1) Lytton J, Westlin M, Hanley MR. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. J Biol Chem 1991; 266(26):17067-17071.

(2) Wictome M, Henderson I, Lee AG, East JM. Mechanism of inhibition of the calcium pump of sarcoplasmic reticulum by thapsigargin. Biochem J 1992; 283 (Pt 2):525-529.

(3) Ishikawa J, Ohga K, Yoshino T, Takezawa R, Ichikawa A, Kubota H et al. A pyrazole derivative, YM-58483, potently inhibits store-operated sustained Ca2+ influx and IL-2 production in T lymphocytes. J Immunol 2003; 170(9):4441-4449.

(4) Singaravelu K, Lohr C, Deitmer JW. Regulation of store-operated calcium entry by calcium-independent phospholipase A2 in rat cerebellar astrocytes. J Neurosci 2006; 26(37):9579-9592.

(5) McFarlane MB, Gilly WF. State-dependent nickel block of a high-voltage-activated neuronal calcium channel. J Neurophysiol 1998; 80(4):1678-1685.

(6) Matlib MA, Zhou Z, Knight S, Ahmed S, Choi KM, Krause-Bauer J et al. Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca2+ uptake into mitochondria in vitro and in situ in single cardiac myocytes. J Biol Chem 1998; 273(17):10223-10231.

Sup. Table 1