Supplemental Information

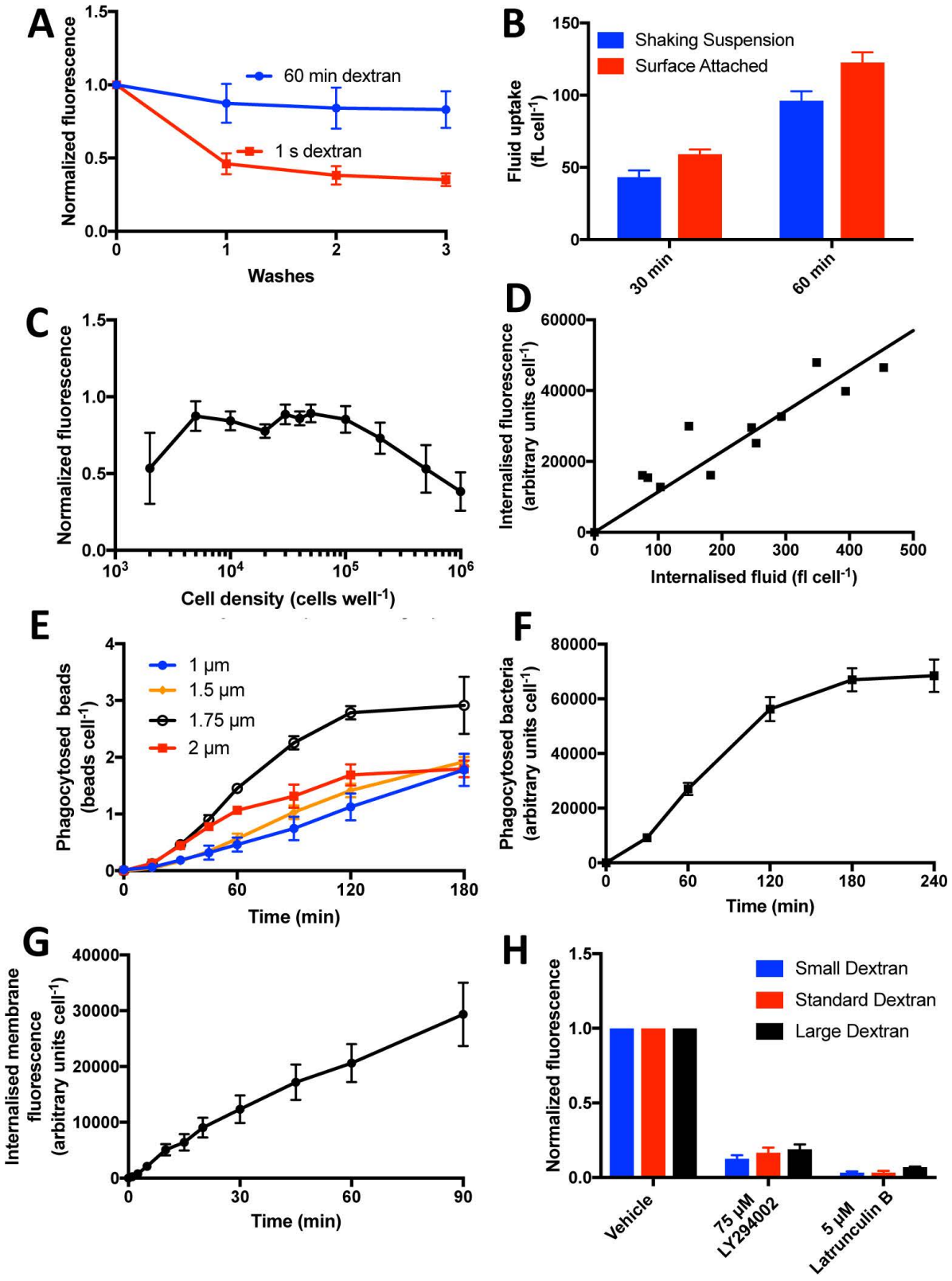

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Figure S1: Fluid and other uptake measurements by flow cytometry

A) One wash is sufficient to remove most TRITC-dextran bound to the surface of cells. Ax2 cells in 96-well plates were loaded with TRITC-dextran for either 60 minutes (average 0-wash fluorescence 11670) or 1 second (average 0-wash fluorescence 695), the media thrown off, the cells washed *in-situ* with ice-cold KK_2MC and cellular fluorescence quantified by flow cytometry (n=4). Fluorescence was normalised to that of unwashed cells from the same condition. **B)** Fluid uptake by Ax2 cells is similar whether they are on a surface, as in the high-throughput assay, or in the more standard shaking suspension. Uptake was measured by flow cytometry. **C)** Cell density affects the rate of fluid uptake. Axenically grown Ax2 cells were spun down by centrifugation and resuspended to various densities in HL5. 50 µl of cells were added to triplicate wells of a 96-well plate and allowed to settle for one hour. TRITC-dextran was added for one hour after which internalised fluorescence was measured and normalised to the cell concentration giving maximum fluid uptake that day; 5000 to 50,000 cells per well gave very similar results. **D)** Calibration curve for converting flow cytometry fluorescence values to fluid uptake volumes. Cells in shaking suspension were fed TRITC-dextran for various times on 4 different days and their internalised fluorescence was determined by both fluorimetry and flow cytometry. Absolute volumes of uptake were determined from the fluorimetric measurements and used to construct a calibration curve for the flow cytometry measurements (since cells measured in both ways have taken up the same volumes). **E)** Phagocytosis of beads by Ax2 cells grown in HL5 measured by flow cytometry using the bead concentrations indicated in the materials and methods. The small lag in uptake after adding beads is likely due to the beads settling onto the cells, which increases their effective concentration. Differences in the rate of settling means that uptake of different sized beads should not be directly compared using this assay. **F)** Uptake of Texas-red *E. coli* bioparticles by axenically growing Ax2 cells measured using high-throughput flow cytometry. The uptake dynamics are similar to those for bead uptake. **G)** Membrane uptake dynamics measured using FM1-43 dye (n=4). These are similar to those

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previously published (Aguado-Velasco and Bretscher, 1999), with the highest rate of membrane uptake within the first 10 minutes. **H)** Fluid uptake is similar with different sized dextrans. Fluid uptake was measured using small (4,400 MW), standard (155,000 MW) and large (500,000 MW) TRITC-dextran as the fluid phase marker. Inhibition of fluid uptake was similar with each dextran when either the PI3K inhibitor LY294002 or the actin inhibitor Latrunculin B were used, indicating that macropinocytosis is responsible for the overwhelming majority of fluid uptake by axenically growing *Dictyostelium*. Error bars show the s.e.m.; n=3 unless otherwise stated.

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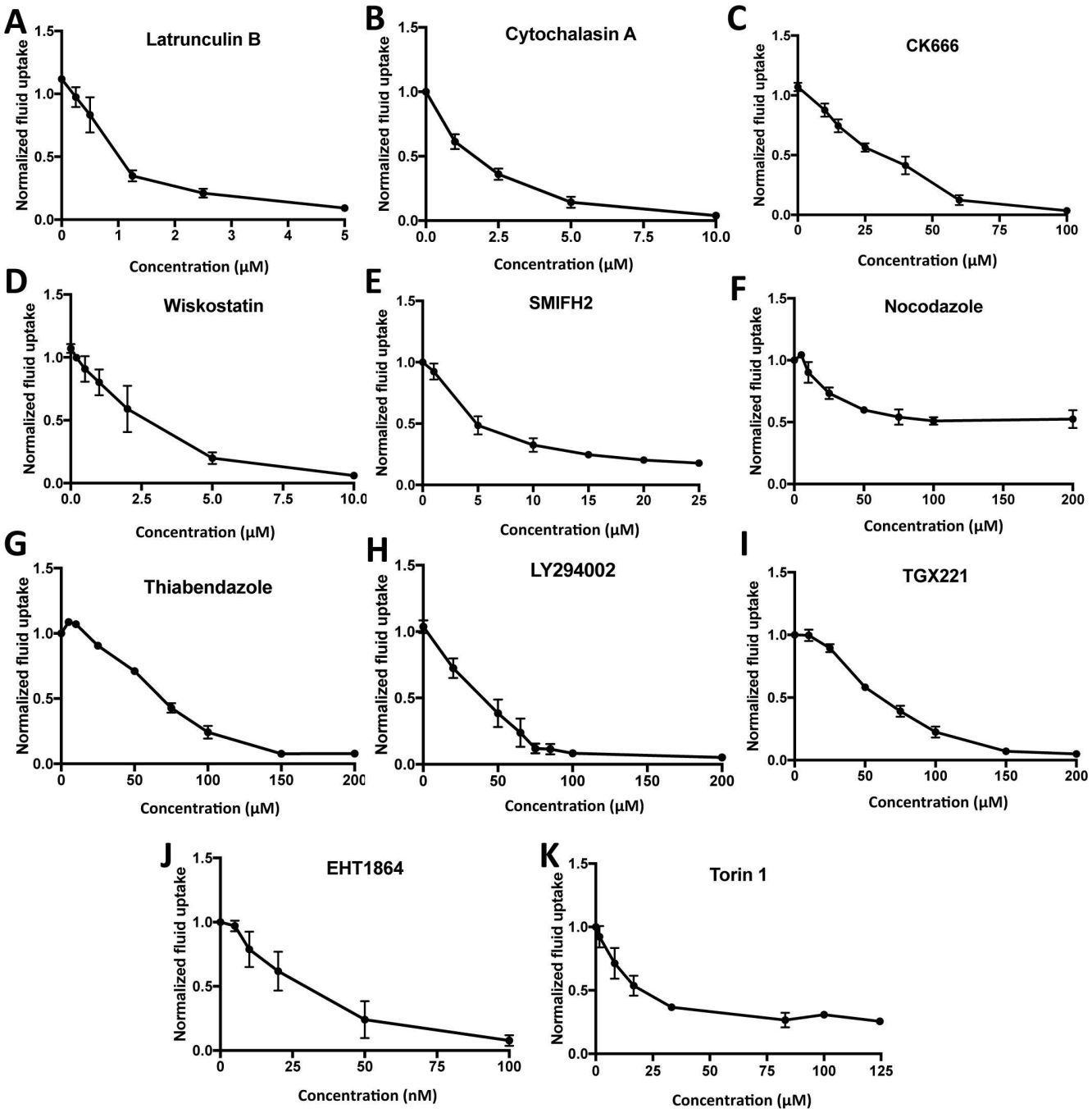

Figure S2: Inhibitor dose response curves

Dose response curves were determined for compounds from table 1 that inhibited macropinocytosis. Fluid uptake by Ax2 cells grown in HL5 was measured for the hour after the addition of each inhibitor, and normalised to treatment with vehicle alone. **A)** Latrunculin B **B)** Cytochalasin A **C)** CK666 **D)** Wiskostatin **E)** SMIFH2 **F)** Nocodazole **G)** Thiabendazole **H)** LY294002 **I)** TGX221 **J)** EHT1864 **K)** Torin 1. The error bars shown are the s.e.m.; n=3.

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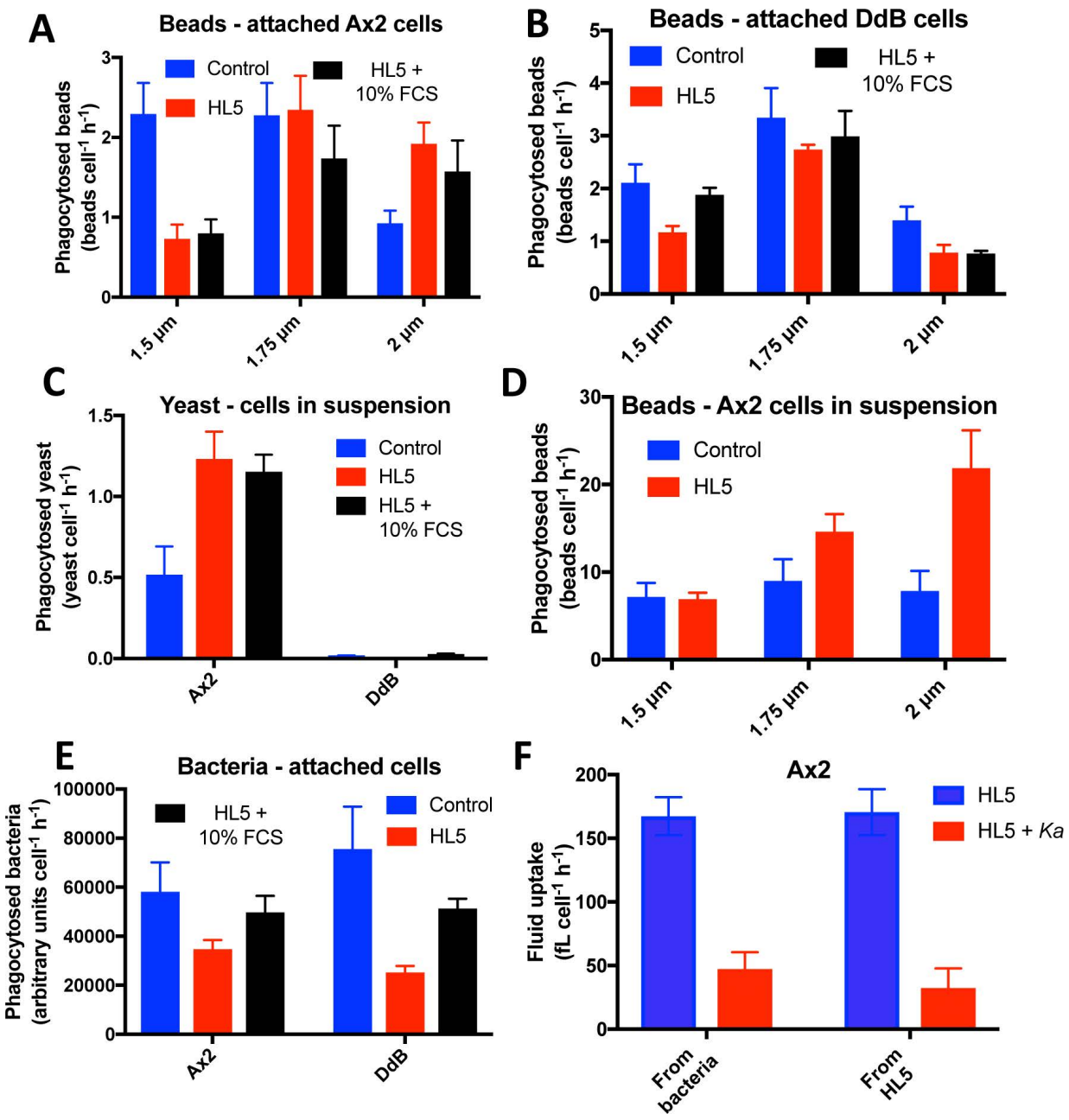

Figure S3: Phagocytosis of large particles correlates with increased size of macropinosomes; and phagocytosis is the preferred feeding mode for cells presented with both bacteria and liquid medium.

A) The increased rates of macropinocytosis and larger macropinosomes (see

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Figure 2C&D) of Ax2 cells grown in liquid media correlates with an increased ability to take up large particles. The uptake of various sizes of beads by Ax2 cells adapted to the indicated conditions is compared (n=3). Cells growing on bacteria take up 1.5 µm beads better than those from HL5 (p=0.02, unpaired t-test), but this is reversed for 2 µm beads (p=0.03, unpaired t-test). **B)** The increased rate of macropinocytosis in DdB cells grown in HL5+10% FCS, which does not result in larger macropinosomes (Figure 2F), does not result in increased uptake of large particles. DdB cells adapted to the indicated conditions are compared. As phagocytosis of beads by cells attached to a surface is limited by the availability of beads, we tested cells in shaking suspension with an excess of particles. **C)** Phagocytosis of yeast by Ax2 is increased when the cells are incubated in growth media beforehand (p=0.04 for control compared to HL5, unpaired t-test), whereas DdB cells do not take up yeast under any of the conditions tested (n=3). **D)** Phagocytosis of larger beads by Ax taken from growth medium was higher than that of Ax2 cells taken from bacteria ($p<0.05$ for 2µm beads, unpaired t-test). Uptake was in shaking suspension with a large excess of particles (n=3). **E)** The uptake of small, bacteria-sized particles is not increased in Ax2 cells with high macropinocytosis rates; if anything the reverse. The uptake of Texas-red *E. coli* bioparticles is compared in cells adapted to the indicated conditions (n=3). **F)** The addition of bacteria to Ax2 cells growing in HL5 medium results in a downregulation of macropinocytosis indicating that phagocytosis is the preferred feeding mode in these conditions (n=6, $p<0.0001$ for cells from bacteria and 0.0006 for cells from HL5 in unpaired t-tests). Control indicates cells grown on bacteria, for the other conditions cells were harvested and incubated for 24 hours in the indicated medium, except in D where the 'HL5' cells were taken from logarithmic growth in HL5 in shaking suspension. Error bars show the s.e.m.

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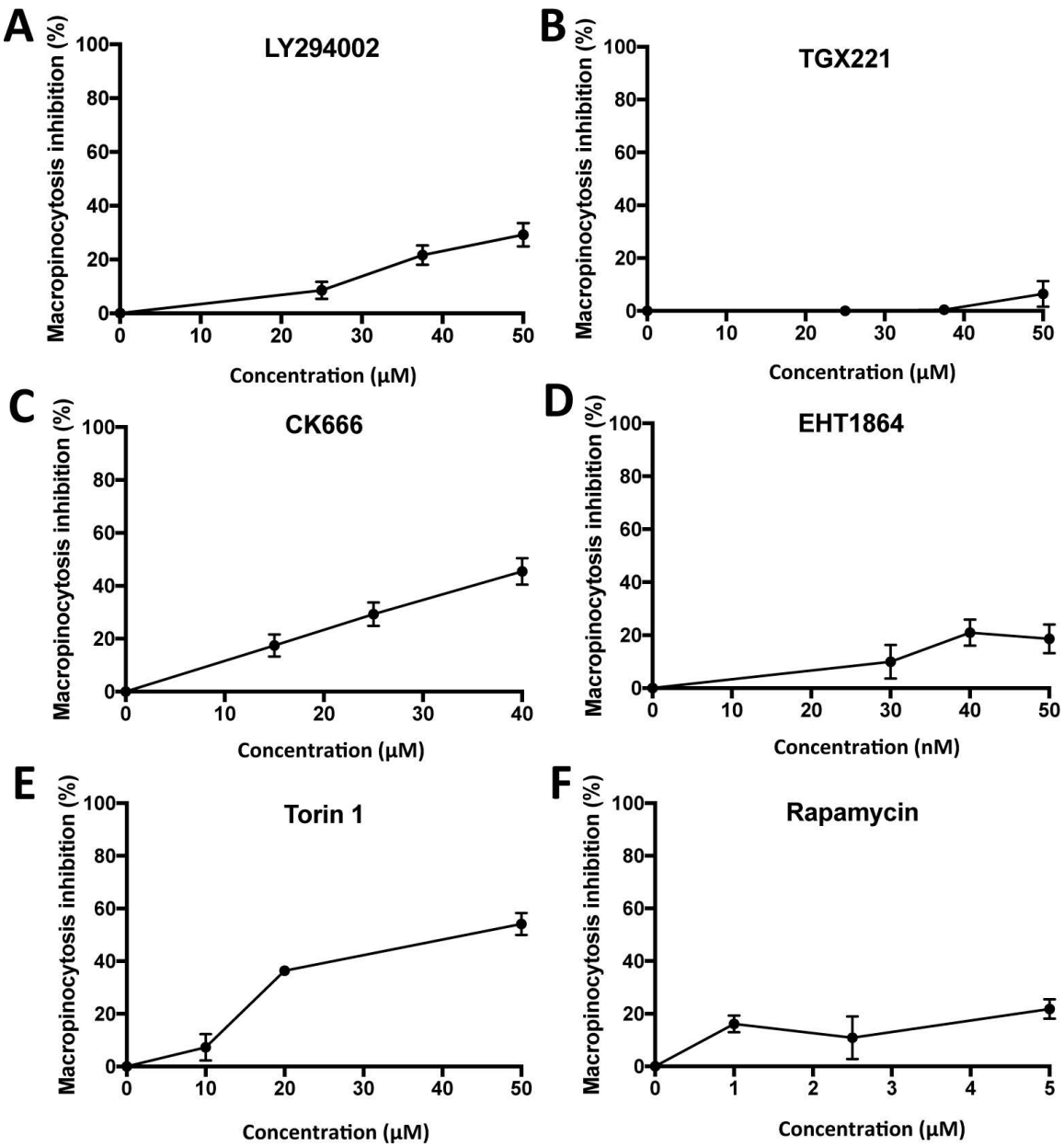

Figure S4: Recovery of cells from long-term inhibitor treatment

To obtain correction factors for Figure 4, Ax2 cells growing in HL5 in 96-well plates were treated with **(A)** LY294002, n=3, **(B)** TGX221, n=4, **(C)** CK666, n=4, **(D)** EHT1864, n=4, **(E)** torin 1, n=3, and **(F)** rapamycin, n=3, for 10 hours. The drugs were washed away by dunk-banging, the cells allowed to recover in HL5 for 10 minutes before fluid uptake was measured over 1 hour using the high-throughput flow cytometry assay. Fluid uptake by treated cells was compared to that by those treated with vehicle to calculate the percentage inhibition of macropinocytosis.

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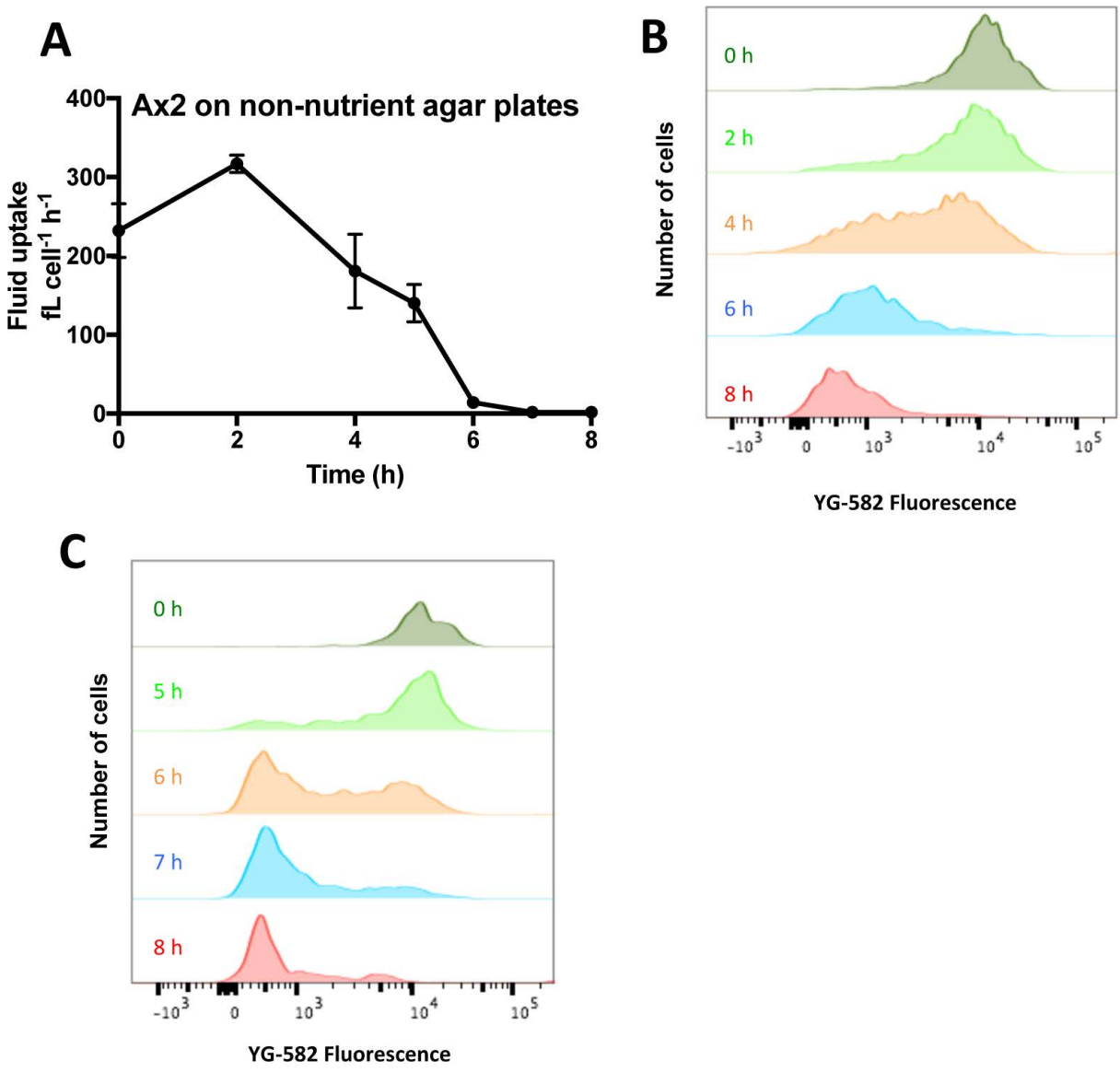

Figure S5: Downregulation of macropinocytosis during development

A) Ax2 cells grown in HL5 (high macropinocytosis) were washed free of nutrients, developed on non-nutrient agar and their fluid uptake in one hour measured over time (n=4). **B)** Representative histograms showing the fluid uptake of cell populations over time during development induced by cyclic-AMP pulsing. **C)** Representative histograms showing the fluid uptake of cell populations over time during development on non-nutrient agar. In both standard development assays, cells switch macropinocytosis off prior to forming tight aggregates.

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Table S1: Induction of macropinocytosis by individual amino acids

Only arginine, glutamate and lysine can individually induce upregulation of macropinocytosis of 2-fold or greater at the concentrations tested. Ax2 cells, harvested from bacterial plates, were incubated for 24 hours with all 20 common L-amino acids in KK_2MC, pH 6.2 at the concentrations indicated, and fluid uptake then measured using the high-throughput flow cytometry assay (N/A indicates that no result was obtained due to solubility problems of the amino acid in this buffer). Results are given relative to the control without amino acids; s.e.m. is shown in brackets; n=3.

	Normalized fluid uptake (s.e.m.)				
Amino acid / Concentration	0 mM	5 mM	10 mM	20 mM	50 mM
Alanine	1	0.74 (0.08)	0.86 (0.11)	1.09 (0.29)	0.97 (0.10)
Arginine	1	0.80 (0.09)	0.83 (0.11)	1.04 (0.11)	2.88 (0.72)
Cysteine	1	1.29 (0.20)	1.26 (0.38)	0.69 (0.24)	N/A
Glycine	1	0.82 (0.26)	0.62 (0.13)	0.65 (0.16)	0.70 (0.15)
Lysine	1	0.94 (0.14)	1.46 (0.38)	2.49 (0.96)	6.66 (2.18)
Proline	1	1.12 (0.30)	0.69 (0.09)	0.83 (0.13)	0.83 (0.08)
Threonine	1	0.95 (0.23)	1.06 (0.20)	0.79 (0.09)	0.75 (0.26)
Serine	1	0.65 (0.19)	0.46 (0.29)	0.68 (0.29)	0.95 (0.49)
Histidine	1	1.16 (0.66)	0.64 (0.20)	0.75 (0.19)	0.62 (0.10)
Valine	1	0.82 (0.30)	0.97 (0.41)	0.82 (0.38)	0.91 (0.41)
Glutamine	1	0.67 (0.28)	0.74 (0.47)	1.10 (0.56)	0.99 (0.37)
Isoleucine	1	0.41 (0.18)	0.53 (0.23)	0.58 (0.26)	0.68 (0.33)
Leucine	1	0.70 (0.07)	0.65 (0.24)	0.71 (0.24)	0.69 (0.21)
Methionine	1	0.66 (0.13)	0.59 (0.19)	0.67 (0.10)	0.73 (0.10)
Asparagine	1	1.08 (0.52)	0.84 (0.15)	0.94 (0.09)	0.90 (0.10)
Aspartate	1	0.76 (0.16)	0.55 (0.09)	0.77 (0.18)	1.00 (0.30)
Glutamate	1	0.76 (0.32)	0.84 (0.30)	1.58 (0.49)	6.88 (2.89)
Phenylalanine	1	0.78 (0.09)	0.73 (0.07)	0.80 (0.12)	0.91 (0.29)
Tryptophan	1	0.73 (0.14)	0.62 (0.08)	0.62 (0.17)	0.51 (0.07)
Tyrosine	1	0.89 (0.24)	N/A	N/A	N/A

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Table S2: Only metabolisable sugars induce macropinocytosis

Only sugars known to be metabolisable by *Dictyostelium* (Ashworth and Watts, 1970, Watts and Ashworth, 1970) induce upregulation of macropinocytosis (glucose, fructose, mannose, maltose all induced a ~2-fold increase or greater compared to the control). This suggests that sugars are sensed by their effect on metabolism, perhaps through downstream sensing based on a common metabolite. Ax2 cells, harvested from bacterial plates, were incubated with the individual sugars for 24 hours, and fluid uptake then measured using the high-throughput flow cytometry assay. Monosaccharides were dissolved at 55 mM (the glucose concentration in SIH) and in addition, sugars with more than one saccharide unit were tested at concentrations to obtain equivalent numbers of saccharides in the media. Each experiment was repeated at least three times, with three replicates a time; s.e.m. is shown in brackets; n=6.

Sugar	**Concentration (mM)**	**Metabolisable by *Dictyostelium*?**	**Fold increase in macropinocytosis over buffer alone (s.e.m.)**
None	0	N/A	1 (0)
Glucose	55	Yes	3.83 (0.38)
Galactose	55	No	1.17 (0.15)
Fructose	55	Poorly	2.34 (0.46)
Sorbitol	55	No	1.16 (0.21)
Mannose	55	Yes	4.09 (0.67)
Maltose	55 / 27.5	Yes	2.22 (0.31) / 4.00 (0.66)
Sucrose	55 / 27.5	No	1.48 (0.13) / 1.54 (0.15)
Raffinose	55 / 18.3	?	2.49 (0.40) / 2.86 (0.62)

Table S3: Developmental signals tested for the ability to induce downregulation of macropinocytosis in non-nutrient conditions

Various compounds involved in *Dictyostelium* development were tested for the ability to induce downregulation of macropinocytosis by low-density starving cells, although none did. Axenically growing Ax2 cells in a 96-well plate were washed twice by dunk-banging in KK_2MC and incubated for 17 hours in KK_2MC containing the compounds at various concentrations (n=3). The effect of the compound on downregulation of Ax2 macropinocytosis was ascertained by comparing the fluid uptake of cells treated with the compound to those in KK_2MC without any compound.

Media addition Tested	**Maximum concentration tested**	**Induced downregulation of macropinocytosis?**
cyclic-AMP	10 mM	No
Non-hydrolysable cyclic-AMP	10 mM	No
Non-hydrolysable ATP	100 µM	No
Adenosine	500 µM	No
DIF-1	100 nM	No
DIF-2	100 nM	No
MPBD	100 nM	No
Polyphosphate	200 µM	No

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Table S4: Strains used in this work

A list of the strains used in this work, and where they were obtained.

Strain	**Parent**	**Source**	**Strain ID**
DdB	NC4	D Welker	N/A
Ax2-Ka	DdB	R R Kay	N/A
fAR1-	Ax2	Pan et al., 2016	DBS0350717
gpbA-	Ax2-MRC	P Devreotes	HM1691
gpaD-	JH8	Hadwiger and Firtel, 1992	JH417
erkB-	Ax2-MRC	Nichols et al., in preparation	HM1735
pkaC-	Ax2	Primpke et al., 2000	HM1037
regA-	Ax2-MRC	Thomason et al., 1998	HM1015
rdeA-	Ax2	Chang et al., 1998	WTC10-H2

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