Fluid phase endocytosis and lysosomal degradation of bovine lactoferrin in lung cells

Supplementary Materials

Table S1: Expression in A549 cells of receptors implicated in bLF uptake.

Receptor	Ligand and/or Competitor	Gene Code	mRNA expression			Ref
			RNAseq value	% Rank compared to lung Cells	% Rank compared to all cells	
LDLR	LDL	LRP1	1.78	44.5%	36.9%	[1,2]
LRP2/Megalin	Insulin/albumin/ haemoglobin	LRP2	-7.70	33.5%	37.3%	[2]
Intelectin-1	Arabinogalactan/Lactoferrin	ITLN1	-13.00	0.0%	0.0%	[3]
TLR4	LPS and others	TLR4	-7.41	15.1%	11.2%	[4]
CXCR4	SDF1	CXCR4	-2.96	31.9%	28.9%	[5]
CD14	LPS	CD14	-0.63	58.1%	70.9%	[6]

RNAseq data obtained from CCLE [7] showing receptors and how A549 cells rank in respective receptor expression relative to cells designated as lung or relative to all cell lines in the database.

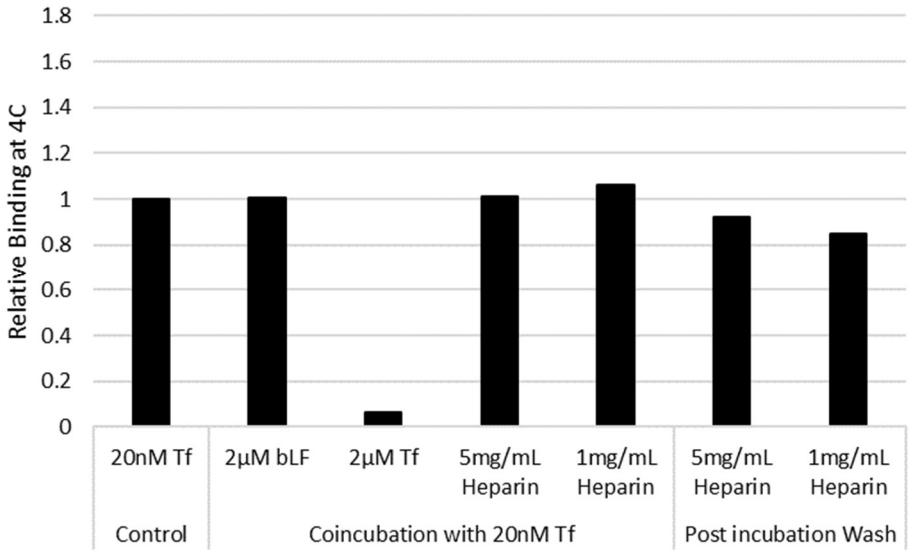

Figure S1: Binding of transferrin to A549 cells.
20 nM Tf488 was incubated with A549 cells for 1 h in the presence of 100X molar excess of unlabelled bLF or Tf, Samples were also co-incubated with 5 or 1 mg/mL heparin or washed after the 1 h incubation. Values are calculated relative to Tf488 uptake alone by flow cytometry, N=1 in duplicate.

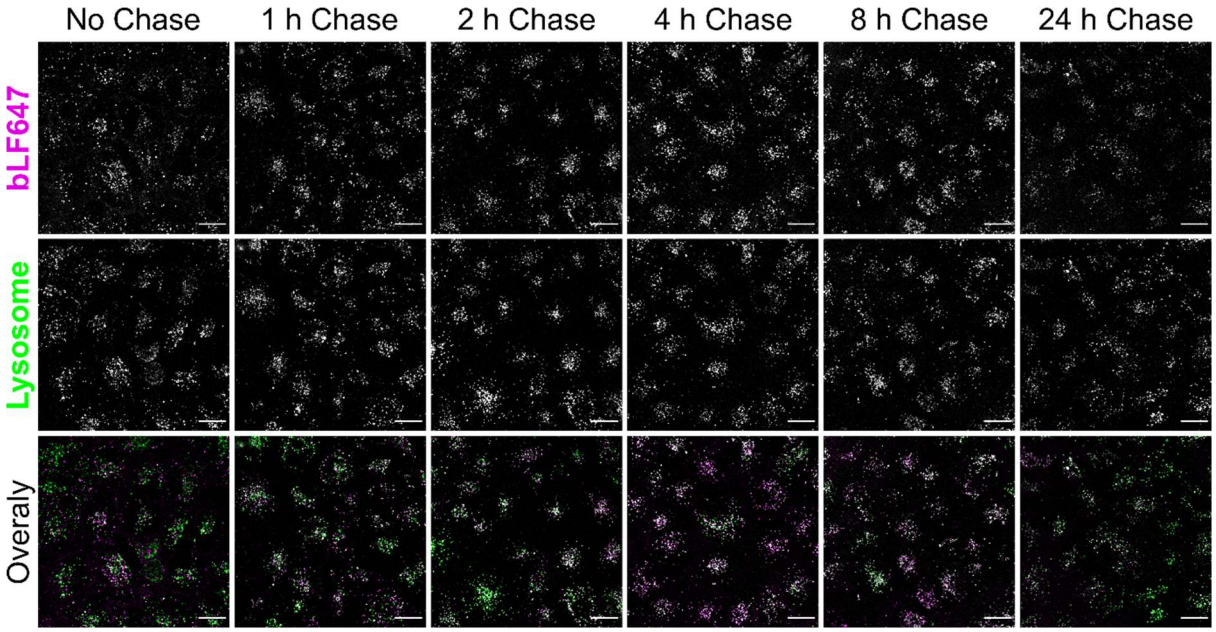

Figure S2: Trafficking of bLF647 to lysosomes by confocal microscopy.
Cells were incubated with 500 nM bLF647 (magenta, top row) for 1 h in SFM, washed in serum containing medium and incubated for the chase period before being washed and imaged by confocal microscopy. Lysosomes (green, middle row) were pre-labelled using a pulse-chase protocol. Scale bar = 20 µm, representative images from three independent experiments quantified in Figure 5.

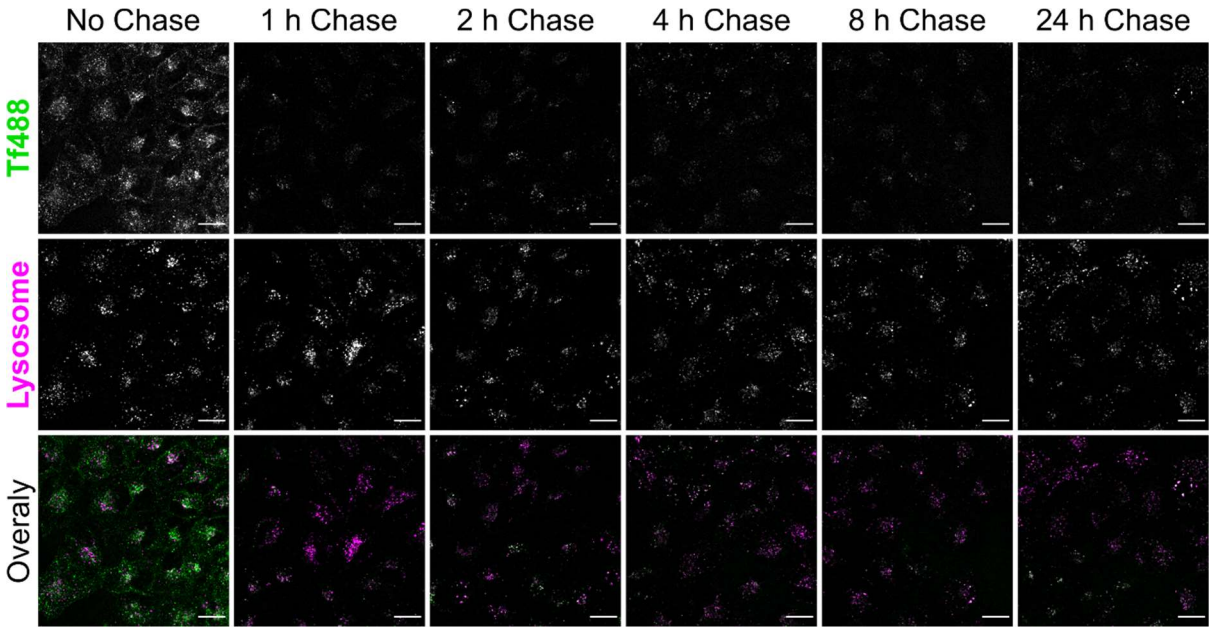

Figure S3: Trafficking of Tf488 to lysosomes by confocal microscopy.
Cells were incubated with 20 nM Tf488 (magenta, top row) for 1 h in SFM, washed in serum containing medium and incubated for the chase period before being washed and imaged by confocal microscopy. Lysosomes (green, middle row) were pre-labelled using a pulse-chase protocol. Scale bar = 20 µm, representative images from three independent experiments quantified in Figure 5.

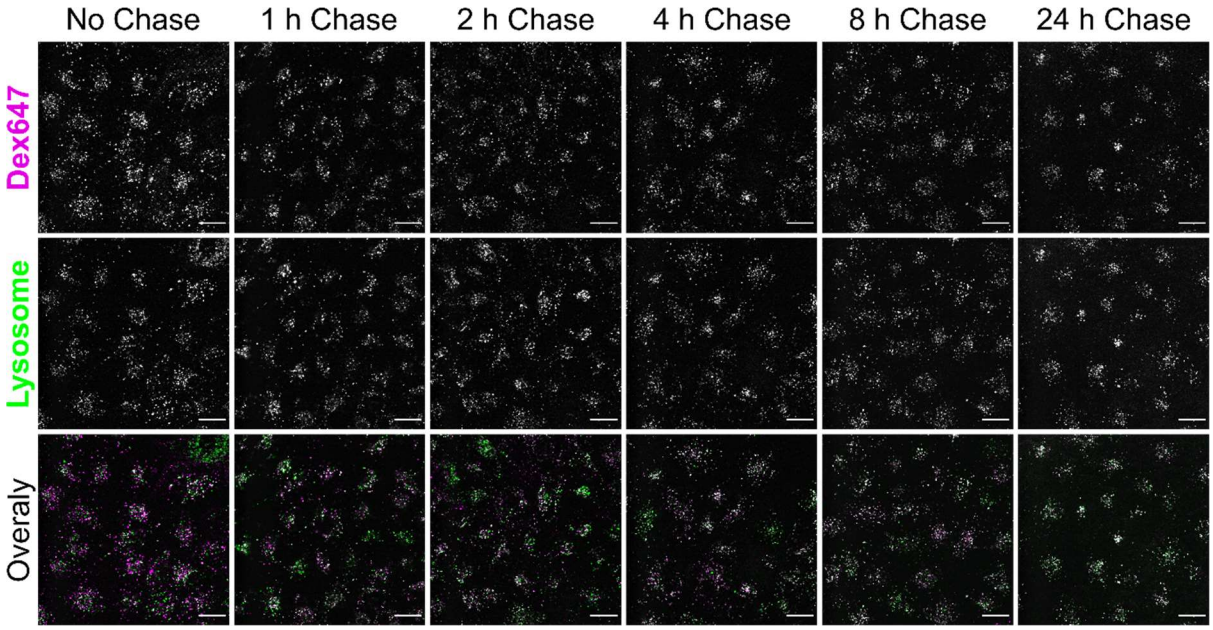

Figure S4: Trafficking of Dex647 to lysosomes by confocal microscopy.
Cells were incubated with 50 µg/mL Dex647 (magenta, top row) for 1 h in SFM, washed in serum containing medium and incubated for the chase period before being washed and imaged by confocal microscopy. Lysosomes (green, middle row) were pre-labelled using a pulse-chase protocol. Scale bar = 20 µm, representative images from three independent experiments quantified in Figure 5.

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