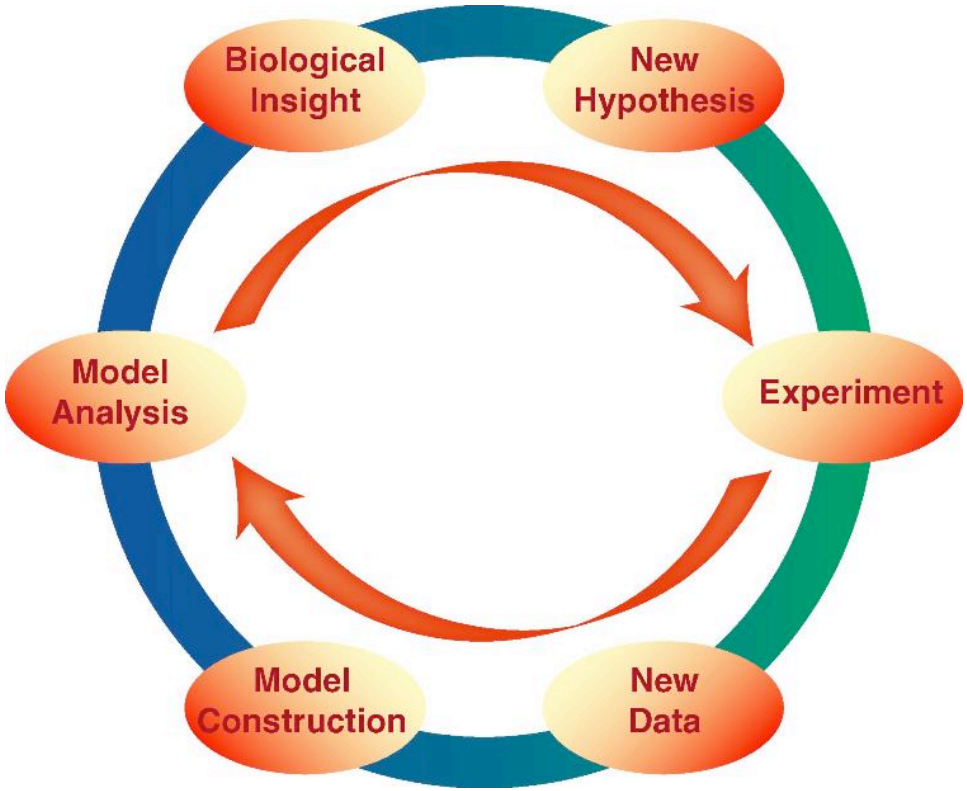
31-10-2007
CISBIC subproject meeting

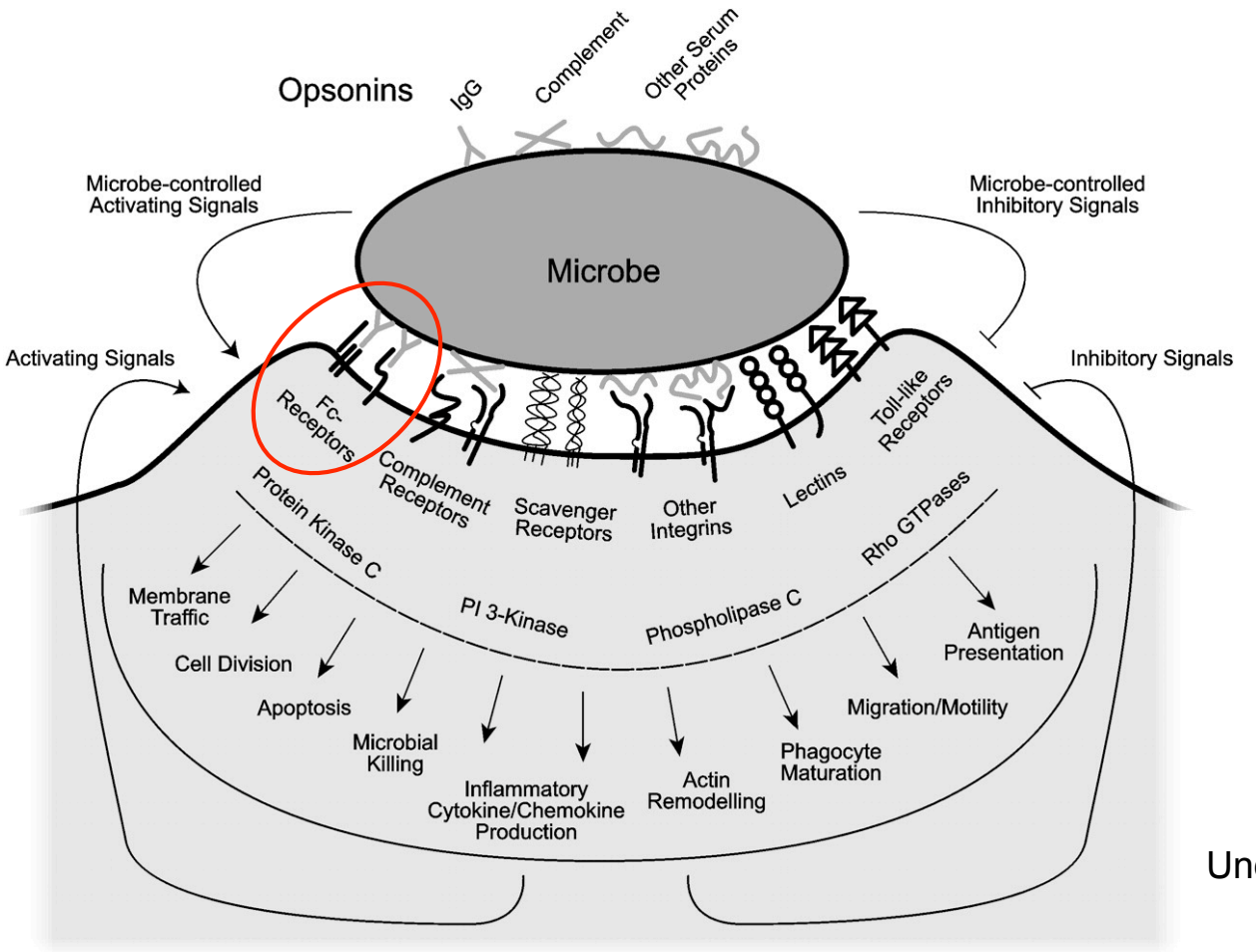
Sub-project 2



Understanding phagocytic signalling

George Tzircotis

Analysis of early signaling following phagocytic receptor engagement



Underhill &Ozinsky 2002

Aims - Biological sub-project 2

Experimental work divided into two parts:

A - Fc receptor dynamics during early stages of phagocytosis -

Generation of Fc receptor mutants

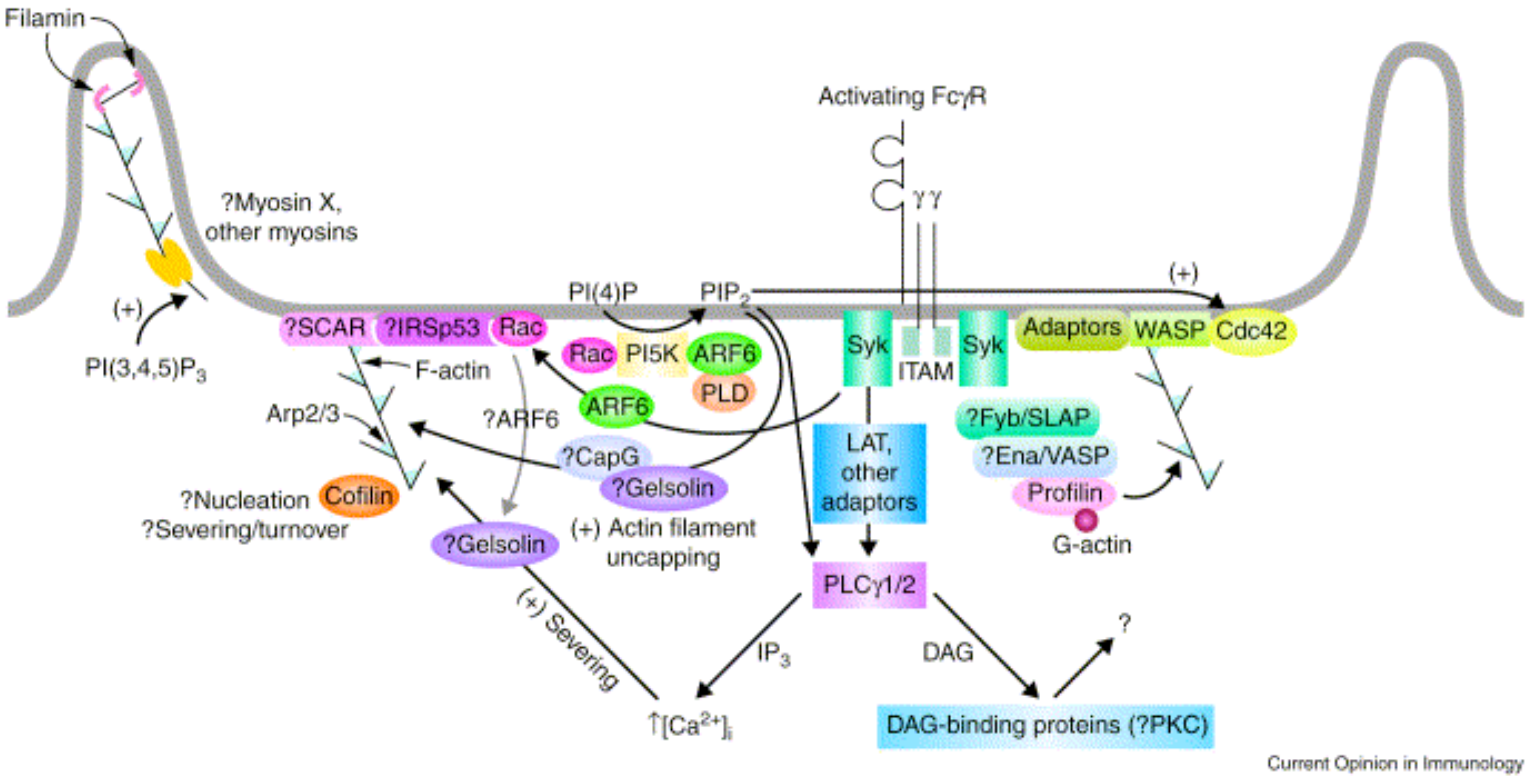
Confocal microscopy of live/fixed cells undergoing phagocytosis under various conditions

B - Identification of molecules involved in phagocytosis -

Screen of siRNA library

Molecules involved in phagocytosis

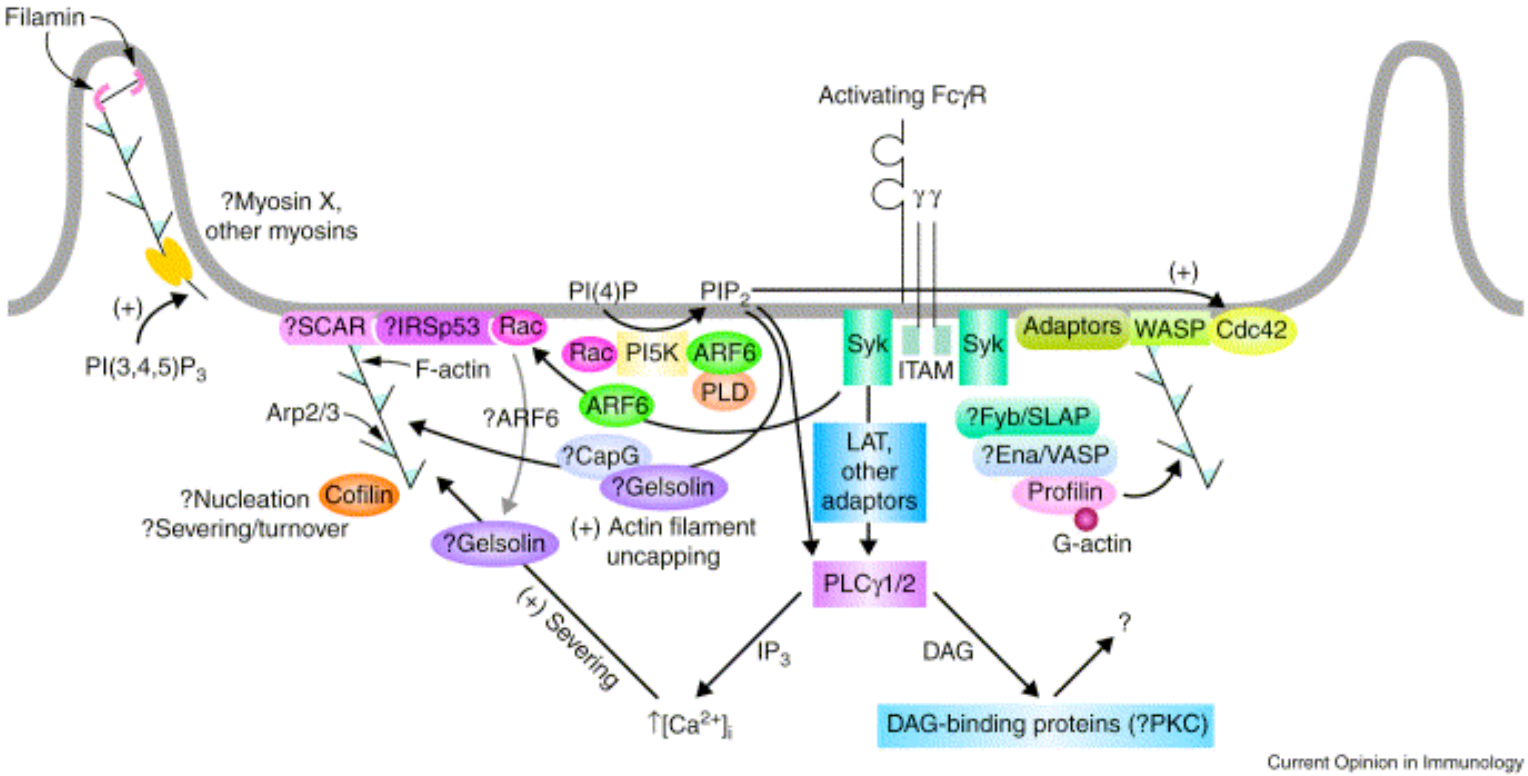
Protrusive force for generation of phagocytic cup is provided by actin polymerisation



Molecules involved in phagocytosis

Protrusive force for generation of phagocytic cup is provided by actin polymerisation

- Human RNAi library of actin binding proteins, Rho GTPases and Rho GTPase regulators and effectors



Screening the siRNA library

Need for a suitable cell system (physiological + amenable to RNAi)

+ a robust, high-throughput phagocytosis assay

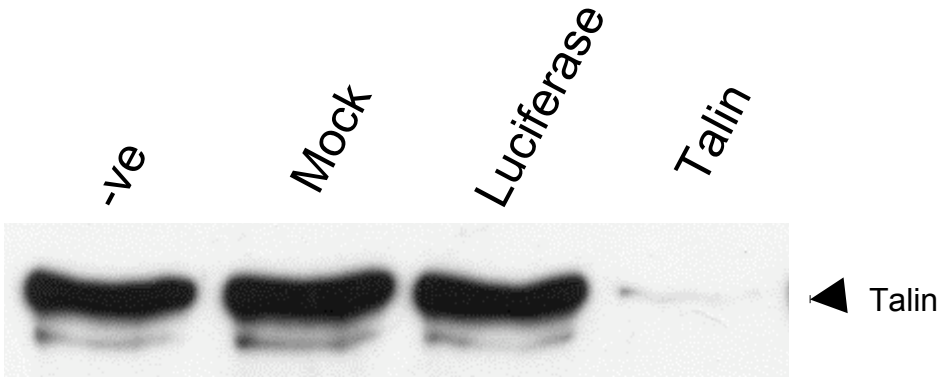
A. Macrophages: complex

B. Cell line transfected with FcγRIIA: simplified system

A. Human macrophages

THP-1 cells: (monocytes)

Lipid trasfection methods don't work - electroporation using Amaxa nucleofector



0.5μg oligo for $1.5x10^6$ cells

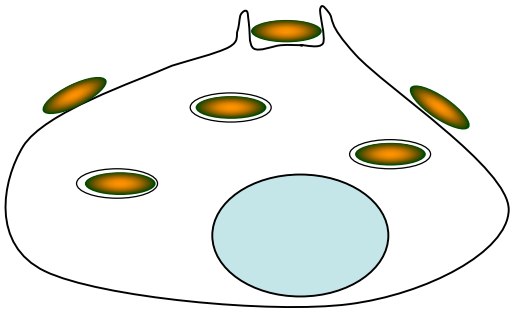
Cells differentiated for 96h following transfection

Survival is 20%

Screening method

Tested several methods, best is “pre-post” staining

Phagocytosis assay conducted using red blood cells opsonised with IgG

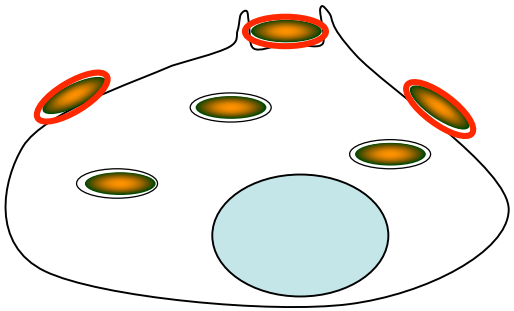


Screening using “Pre-post staining”

Tested several methods, best is “pre-post” staining

Phagocytosis assay conducted using red blood cells opsonised with IgG

Red anti-IgG antibody applied to label external RBC, cells fixed and permeabilised



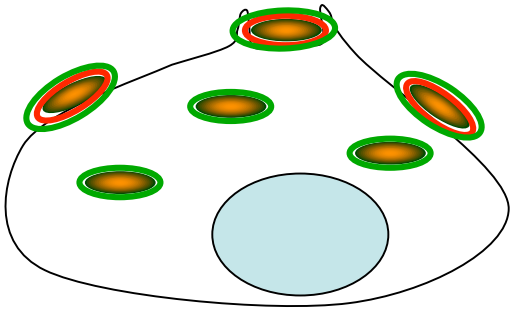
Screening using “Pre-post staining”

Tested several methods, best is “pre-post” staining

Phagocytosis assay conducted using red blood cells opsonised with IgG

Red anti-IgG antibody applied to label external RBC, cells fixed and permeabilised

Green anti-IgG antibody applied to label all, internal and external RBC

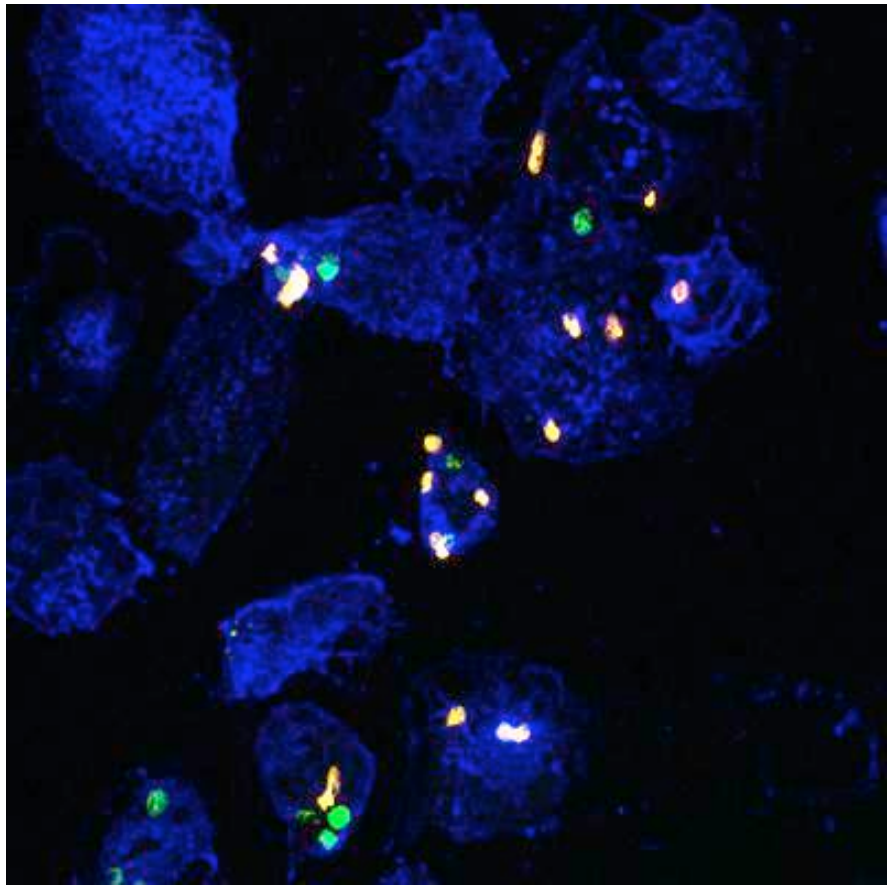


Counting can be done manually/automatically by simple counting of external/total red blood cells

A. Human macrophages

THP-1 cells: (monocytes)

Lipid trasfection methods don’t work - electroporation using Amaxa nucleofector



Blue - Actin
Red - External RBC
Green - All RBC

Waiting for Amaxa 96-well attachment to enable high-throughput (and use less oligo)

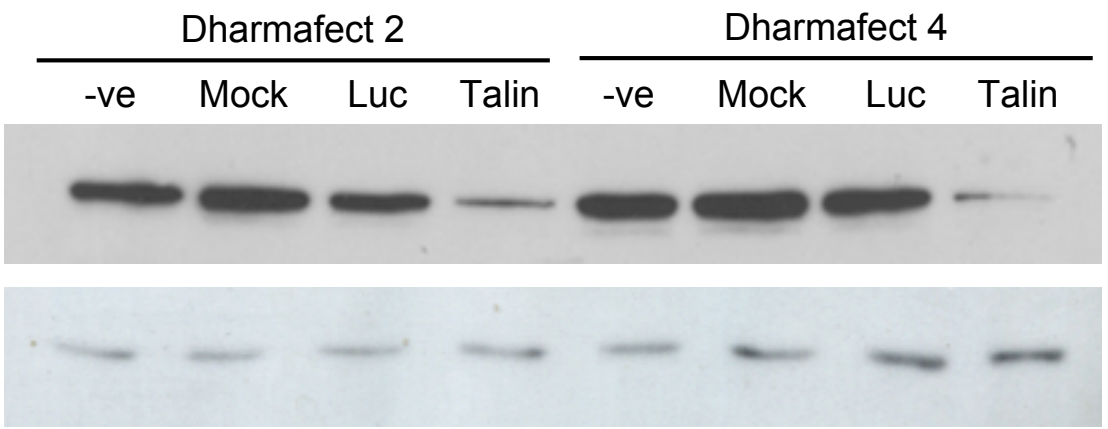
B. Cell line transfected with FcγRIIA

HT1080 cells:

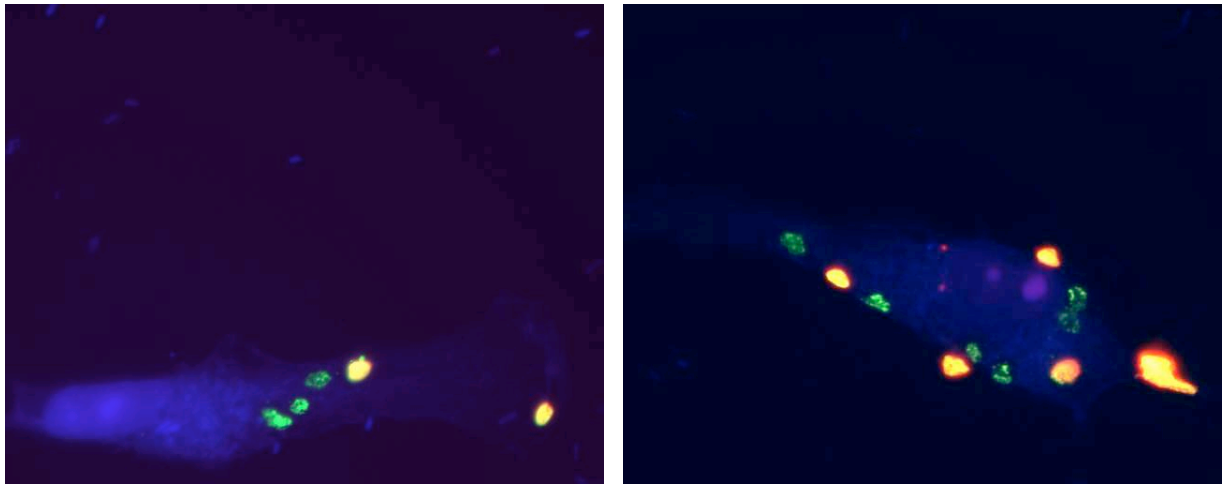
- Human Fibrosarcoma
- RNAi using lipid transfection reagent from Dharmafect

Dharmafect

100nM each oligo



Phagocytosis assay

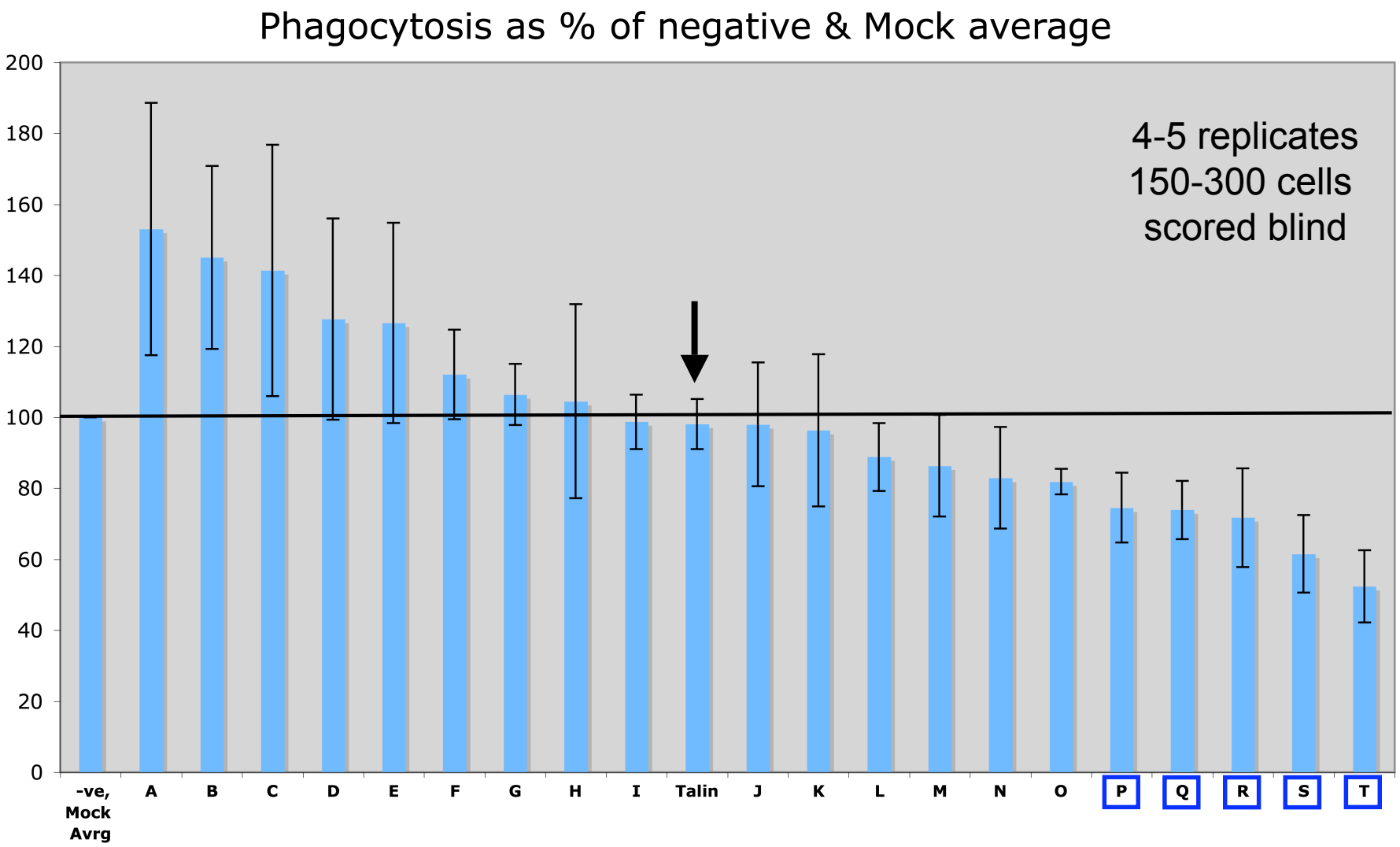


Meanwhile…

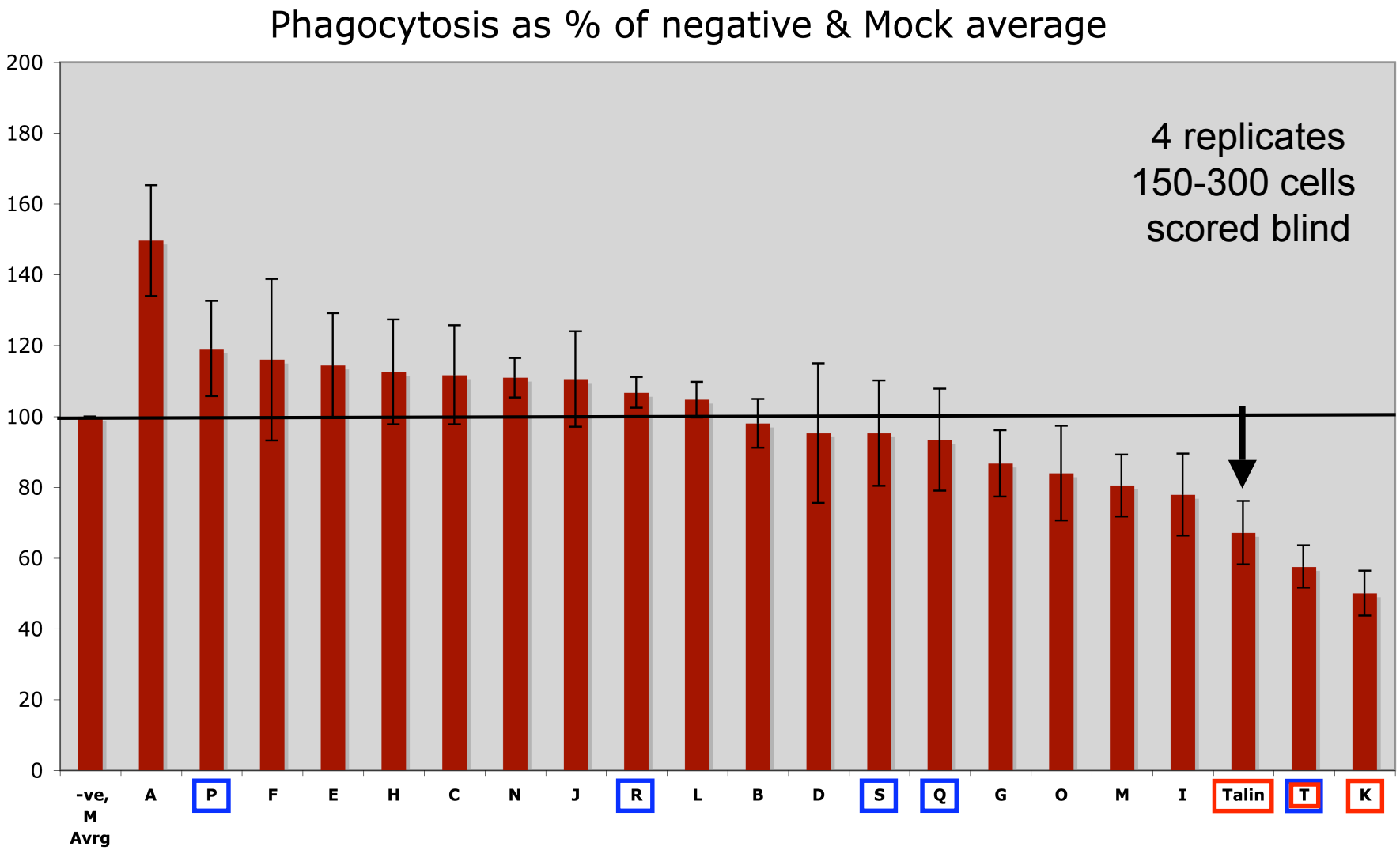
Can perform phagocytic assays in J774 mouse macrophages

Purchased mini-library of 20 mouse GTPases and screened these for involvement in FcγRIIa mediated phagocytosis

Screen of GTPase involvement in FcγRIIa phagocytosis



Screen of GTPase involvement in CR3 phagocytosis



The future:

Mini screen

Verify efficacy of RNAi by antibody / qPCR

GFP-tagged constructs of candidates - localisation to phagocytic cups?

Mechanisms?

Large screen

Optimise phagocytosis conditions for FcγRIIa transfected HT1080 cells

Optimise 96-well amaxa transfection and phagocytosis conditions

Screen