Canonical and Non-canonical G-protein Signaling Helps Coordinate Actin Dynamics to Promote Macrophage Phagocytosis of Zymosan

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CONTENTS

SUPPLEMENTARY VIDEOS

Video 1. $G\alpha_{i2}$ proteins are recruited to phagocytic sites. Images collected by live cell confocal microscopy of RAW 264.7 cells transfected with YFP-$G\alpha_{i2}$ (green) and exposed to red fluorescent zymosan. A simultaneous intensity map created from each image is shown below. Beads engulfed during the imaging are indicated by arrowheads. A 4 slice image stack (0.5 μm/slice) was collected approximately every 12 seconds for 15 minutes. The sequence of image stacks was transformed into a volume rendered four-dimensional video using Imaris software.

Video 2. $G\alpha_{i2}$ and F-actin co-localize during phagocytosis. Images collected by live cell confocal microscopy of RAW 264.7 cells transfected with YFP-$G\alpha_{i2}$ (green) and RFP-Lifeact (red) and exposed to zymosan. A 4 slice image stack (0.5 μm/slice) was collected approximately every 12 seconds for 4 plus minutes. The sequence of image stacks was transformed into a volume rendered four-dimensional video using Imaris software. Shown are overlap images (left) and superimposed bright field images (right).

Video 3. Dynamic changes in the localization of $G\alpha_{i2}$ and F-actin following latrunculin B washout. Images were collected by live cell imaging of RAW 264.7 cells 15 minutes following latrunculin washout. A 3 slice image stack (0.5 μm/slice) was collected every minute for 30 minutes. The sequence of image stacks was transformed into a volume rendered four-dimensional video using Imaris software.

Video 4. PTX toxin treatment impairs macrophage zymosan bead engulfment. RAW 264.7 cells expressing YFP-$G\alpha_{i2}$ were treated with PTX toxin for 2 hours and exposed to fluorescent zymosan for 10 minutes prior to imaging. A 3-slice image stack was collected every 30 seconds for 10 plus minutes. The sequence of fluorescent images was transformed into a volume rendered 4-dimensional video using Imaris software.

Video 5. Robust phagocytosis by macrophages expressing a GTP bound $G\alpha_{i2}$. Images collected by live cell confocal microscopy of RAW 264.7 cells transfected with YFP-$G\alpha_{i2}$ Q205L (green) and RFP-Lifeact (red) and exposed to zymosan. A 3 slice image stack (0.5 μm/slice) was collected approximately every 15 seconds for 14 plus minutes. The sequence of image stacks was transformed into a volume rendered four-dimensional video using Imaris software.

Video 6. The loss of $G\alpha_{i3}$ impairs macrophage phagocytosis of zymosan. WT and *Gnai3*$^{-/-}$ bone marrow derived macrophages were exposed to fluorescent (green) zymosan for 10 minutes prior to imaging. A single slice image was collected every 17 or 20 seconds for 30 plus minutes. The sequences of fluorescent images and corresponding bright field images were transformed into three dimensional videos using Imaris software and linked together using Adobe premiere.