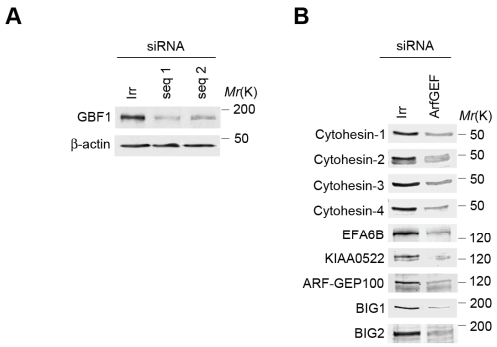
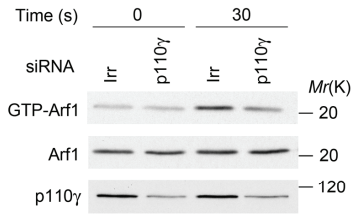
Supplemental material

Supplemental Figure S1



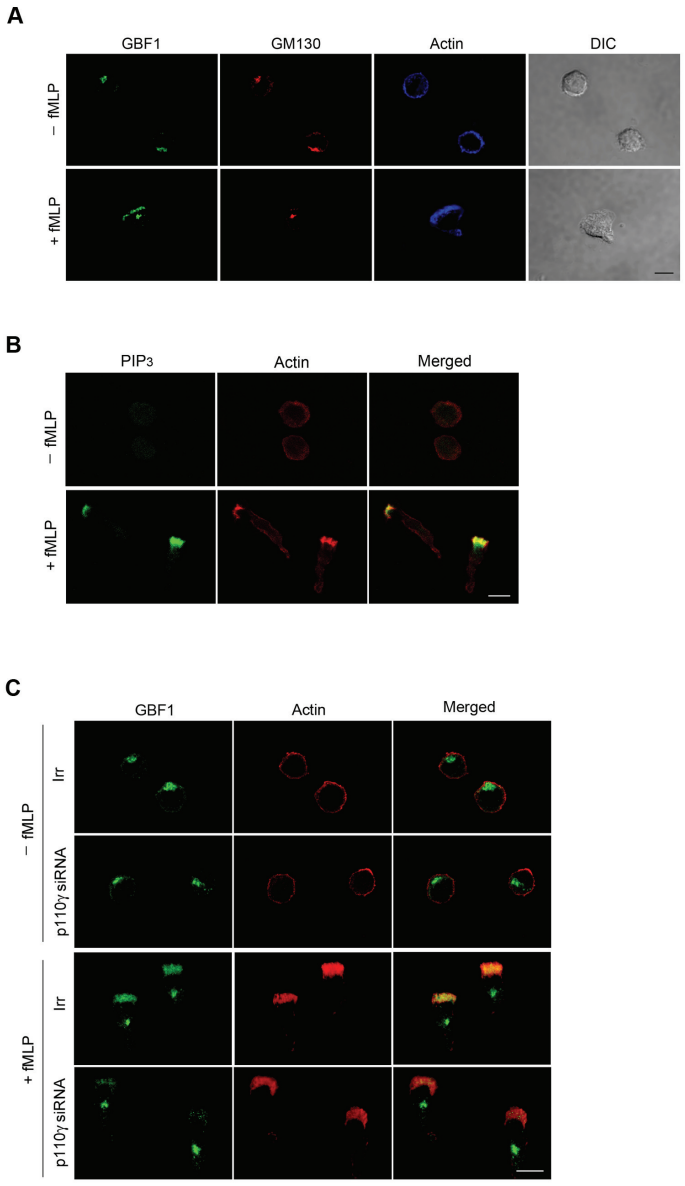
Supplemental Figure S1 (**A**, **B**) Suppression of protein expression of ArfGEFs by their specific siRNAs in differentiated HL-60 cells. Cells transfected with siRNA against ArfGEFs or an irrelevant RNA duplex (Irr) were analyzed for their expression of ArfGEF proteins by immunoblotting of the lysates (20 μg each), coupled with SDS-polyacrylamide gel electrophoresis, by using each corresponding ArfGEF antibody, as indicated. For suppression of GBF1, two different siRNA duplexes were used (**B**). In **A** and **B**, data are representative of three independent experiments.

Supplemental Figure S2



Supplemental Figure S2 Effects of PI3Kγ knockdown on the Arf1 activation. Differentiated HL-60 cells, transfected with siRNA duplexes specific for p110γ or with irrelevant sequences (Irr), were subjected to the measurement of Arf1 activity after incubation with or without *f*MLP for 30 sec.

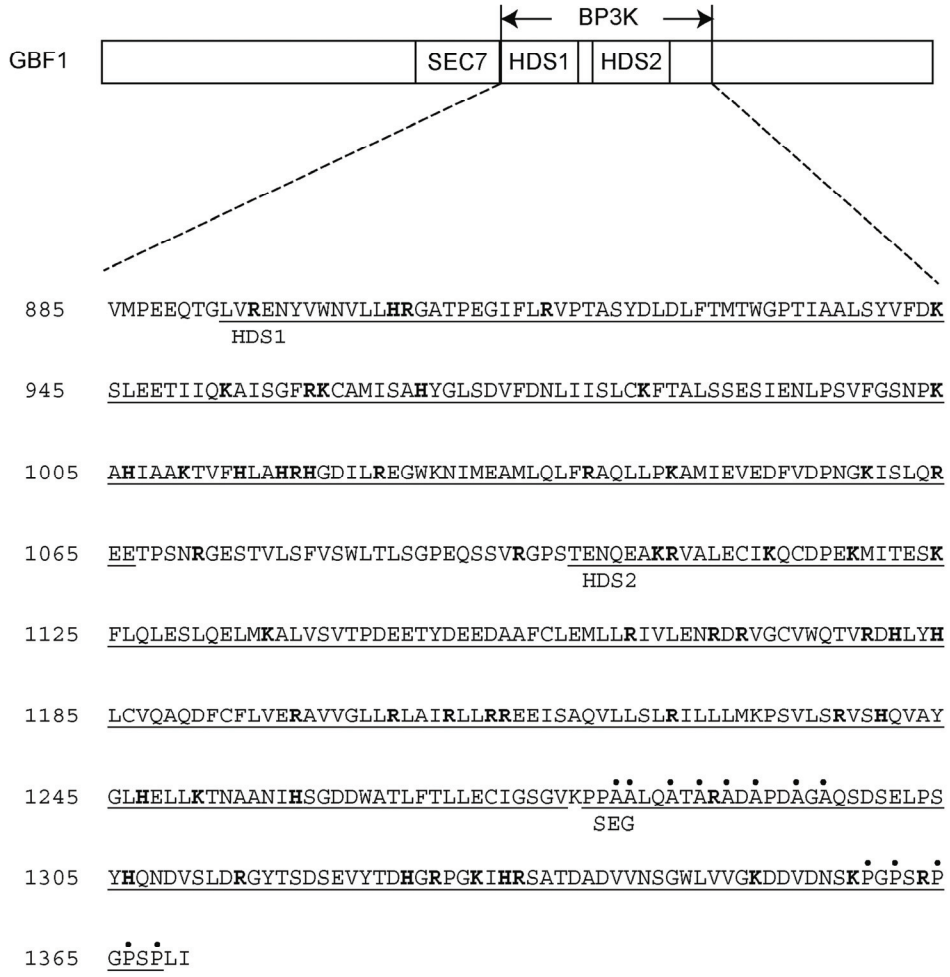
Supplemental Figure S3



Supplemental Figure S3 (**A**) Subcellular localization of GBF1. Differentiated HL-60 cells were incubated with or without *f*MLP for 15 min, and subjected to immunostaining, using antibodies as indicated. F-actin was visualized by phalloidin conjugated with Alexa Fluor 647. DIC, differential interference contrast image (**B**) Subcellular localization of PI[3,4,5]P_3. Differentiated HL-60 cells were incubated

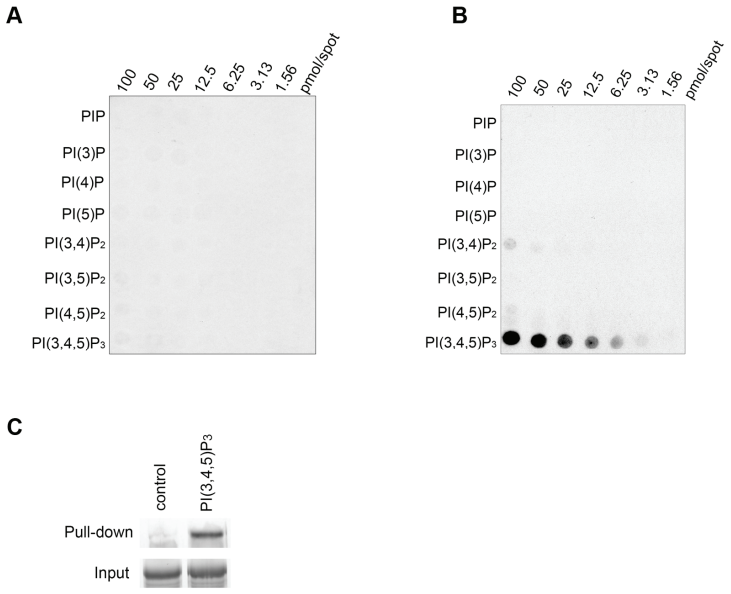
with or without *f*MLP for 15 min, and subjected to anti-PIP_3 immunostaining, after fixation. F-actin was visualized by phalloidin conjugated with Texas Red. (**C**) Effects of PI3Kγ knockdown on the translocation of GBF1. Differentiated HL-60 cells, transfected with siRNA duplexes specific for p110γ or with irrelevant sequences (Irr), were incubated with or without *f*MLP for 15 min, and subjected to an anti-GBF1 immunostaining, after fixation. F-actin was visualized by phalloidin conjugated with Texas Red. Bars, 10 μm.

Supplemental Figure S4



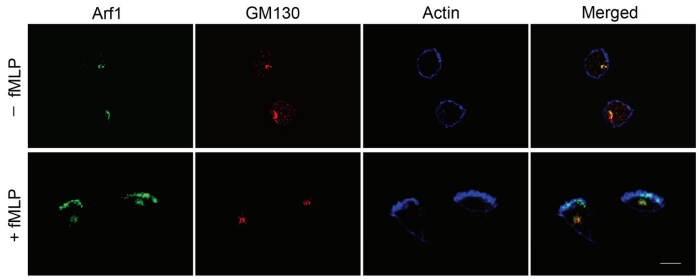
Supplemental Figure S4 The primary amino acid sequence of the BP3K domain of GBF1, which consists of the putative HDS1 and HDS2 domains, and the 'SEG' region. Boldface, basic amino acids. Dot, alanine or proline clusters in the 'SEG' region.

Supplemental Figure S5



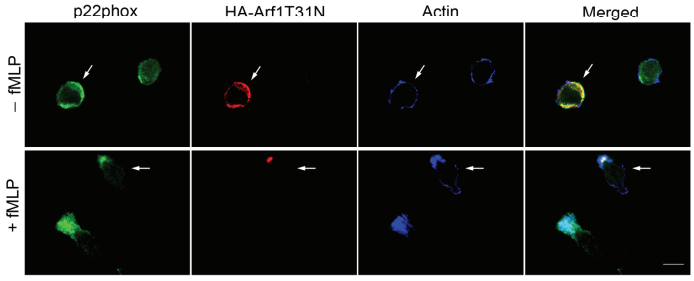
Supplemental Figure S5 In vitro binding of GST (**A**) and the Cytohesin-3 PH domain fused to GST (**B**) with phosphatidyl inositol phosphates. A nitrocellulose membrane, loaded with phosphoinositides at the indicated concentrations, was incubated with 1 μg/ml GST or 0.5 μg/ml GST-cytohesin3 PH, and bound GST proteins were visualized as in **Figure 3A**. (**C**) GBF1 BP3K- PI[3,4,5]P_3 bead binding assay. GST-GBF1 BP3K were incubated with PI[3,4,5]P_3 beads or control beads. input, 5% of GST fusion protein that was incubated with the beads.

Supplemental Figure S6



Supplemental Figure S6 Subcellular localization of Arf1. Differentiated HL-60 cells were incubated with or without *f*MLP for 15 min, and subjected to immunostaining as indicated, after fixation. F-actin was visualized by phalloidin conjugated with Alexa Fluor 647. Data are representative of three independent experiments. Bar, 10 μm.

Supplemental Figure S7



Supplemental Figure S7 Arf1T31N does not block the *f*MLP-induced translocation of p22phox to the leading edge. Differentiated HL-60 cells, transfected with Arf1T31N-HA cDNA, were incubated with or without *f*MLP for 15 min, as indicated, and subjected to anti-p22phox and anti-HA immunostaining. F-actin was visualized by phalloidin conjugated with Alexa Fluor 647. Arrows indicate cells expressing Arf1T31N-HA. Data are representative of three independent experiments. Bar, 10 μm. $p<0.01$.

Supplemental Movie S1 Chemotaxis of differentiated HL-60 cells, transfected with irrelevant sequences, toward *f*MLP in a micropipette. Lines indicate trajectories of cells migration.

Supplemental Movie S2 Chemotaxis of differentiated HL-60 cells, transfected with siRNA against GBF1, toward *f*MLP in a micropipette. Lines indicate trajectories of cells migration.