Bonnans et al. http://www.jem.org/cgi/content/full/jem.20052143/DC1

SUPPLEMENTAL MATERIALS AND METHODS

LTB₄ was otained from Cayman Chemical; LY294002 was obtained from Cell Signaling Technology; dolichol monophosphate was obtained from American Radiolabeled Chemical, Inc.; o-dianisidine dihydrochloride, cytochrome *c*, and ATP were obtained from Sigma Chemical Co.; human p110 γ PI3K was obtained from Alexis Biochemicals; L- α -phosphatidylinositol was obtained from Avanti Polar Lipids, Inc.; and anti-p110 γ and anti-p85–PI3K antibodies were obtained from Upstate Biotechnology. IHC zinc buffer and mouse anti–LY-6G antibody were obtained from BD Biosciences.

PSDP determination.

The amount of PSDP was determined by densitometry (Scion Image software) after materials were saponified (10% KOH in methanol, 30 min, 37°C), extracted, and separated by TLC. Dolichol monophosphate (2 μ g) was used as an internal control to correct for extraction losses.

P85 and p110y-PI3K gene expression.

Total RNA was extracted from snap-frozen lungs and semi-quantitative gene expression was determined using specific primers for murine p85 (sense primer 5'-ACCCCAGTTTTTGTTGCTTG-3', antisense primer 5'-CCTGCCCAACATT-TAGTCCA-3'), p110 γ PI3K (sense primer 5'-TTCTCGTGTGTCCACCATGT-3', antisense primer 5'-CCTGGGCATCT-CAGTGGTAT-3'), and β -actin (internal control). After electrophoresis, densitometry was performed using Scion Image software.

CMC measurement.

CMC was determined by light scattering (Levy, B.D., N.A. Petasis, and C.N. Serhan. 1997. *Nature*. 389:985–990). In brief, PSDP mimetic, the related PIPPs PSMP and farnesyl diphosphate (FDP), or L- α -phosphatidylinositol (PI) ($10^{-9}-10^{-3}$ M) were added to HBSS containing 1.6 mM CaCl₂. After vortexing (30 s, room temperature) and sonication (30 s × 3, full power), materials were kept at room temperature for 30 min. For each compound, the relationship between absorbance at 762 nm (Abs 762 nm) and concentration was determined and the breakpoint in Abs 762 nm was taken as an estimate of the CMC (see Fig. S1).