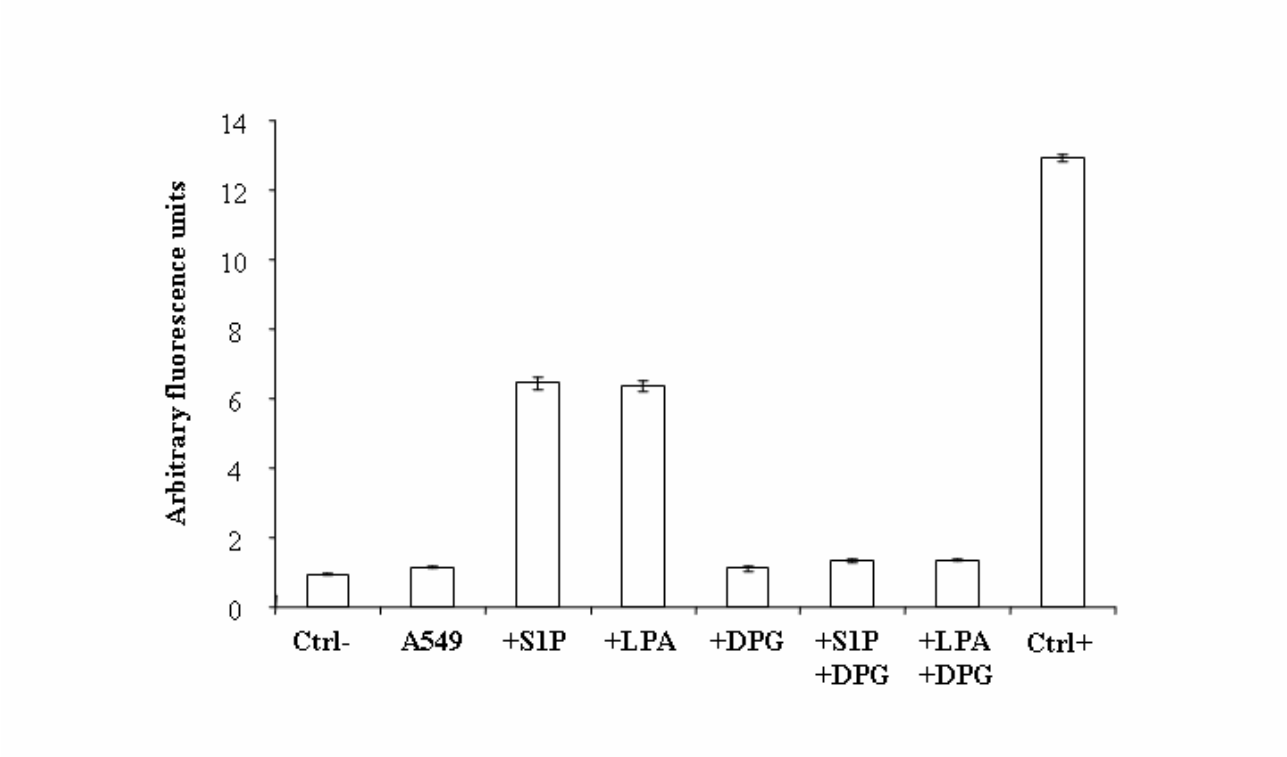
Supplementary material

Additional figure 1. Analysis of phospholipase D (PLD) activity was performed on A549 cells stimulated for 18 hours with 0.5 µM of either LPA or S1P. Cells were harvested and tested for PLD activity using Amplex Red Phospholipase D assay kit according to the manufacturer's instructions (Molecular Probes, Italy). Finally, the specificity of PLD assay has been assessed by incubating the cells for 18 hours at 37 °C in the presence of 2 mM 2,3-Diphosphoglycerate (DPG, Sigma) which is a direct competitive inhibitor of PLD (Kanaho Y., Nakai Y., Katoh M., Nozawa Y. The phosphatase inhibitor 2,3-diphosphoglycerate interferes with phospholipase D activation in rabbit neutrophils. J. *Biol. Chem.* 1993: 268:12492-12497).



Additional figure 1

Results, expressed in additional figure 1, show that the addition of 2,3-DPG almost completely inhibits LPA or S1P induced PLD activity assessed by "Amplex Red PLD Assay Kit", demonstrating the specificity of the assay in the present experimental model. Values are shown as means ± S.D of the triplicate values.