Supporting information

A high-throughput assay to identify allosteric inhibitors of the PLC-γ isozymes operating at membranes

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This information contains **Figures S1-S4** (5 pages in total)

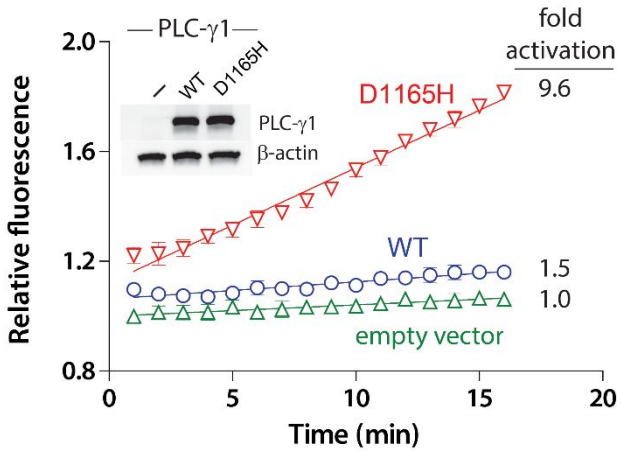

Figure S1. XY-69 measures phospholipase activity with cellular lysates. HEK293 cells were transfected to express the indicated forms of PLC-γ1 prior to lysis, addition of XY-69 and monitoring of fluorescence ($\lambda_{ex/em}$ = 485/520 nm). Fold activation is relative to the rate of fluorescence change for cells transfected with empty vector (no PLC-γ1) calculated from the mean of four replicates. Inset: western blots against PLC-γ1 and β-actin as indicated.

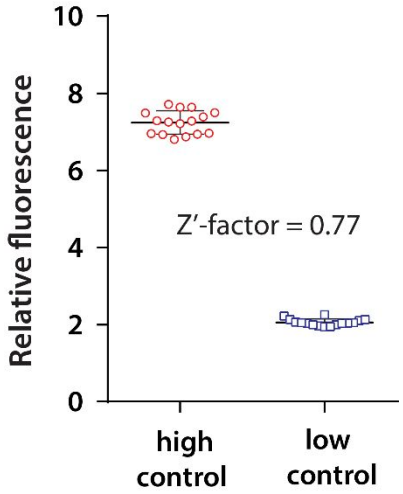

Figure S2. Robust, high-throughput screen using XY-69. The Z'-factor was measured using the optimized conditions described in the text. Hydrolysis of XY-69 by PLC-γ1 (D1165H) after 1 h is used as the high control while substituting PLC-γ1 (D1165H) for BSA as the low control. Each condition was measured 16 times.

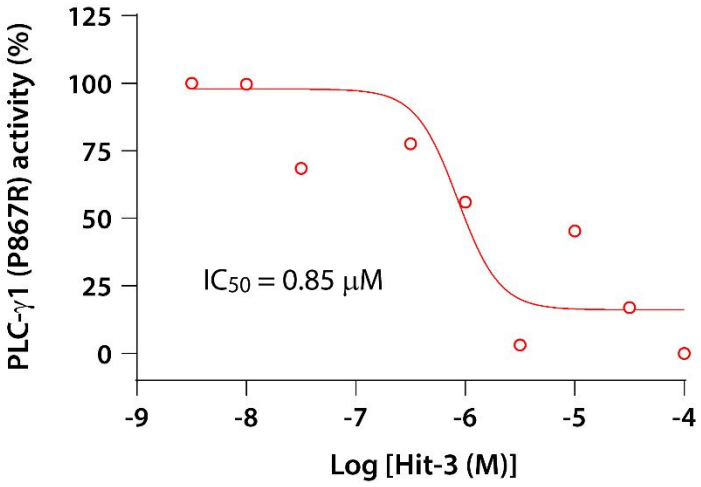

Figure S3. Inhibition of PLC-γ1 (P867R) by Hit-3.

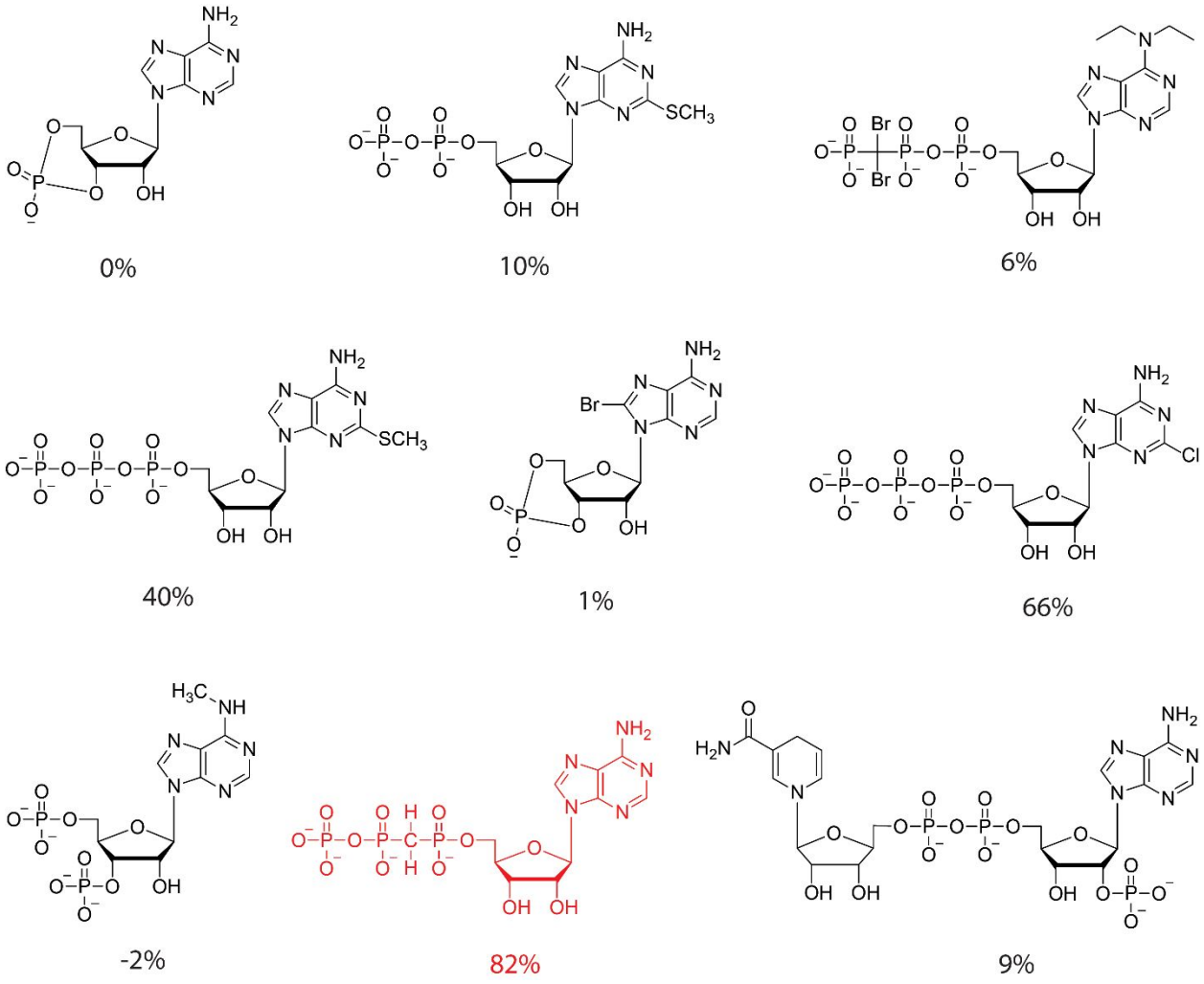

Figure S4. Chemical structures of ribonucleotides and derivatives in the LOPAC$_{1280}$ library. Hit-3 is in red and the percent inhibition of the phospholipase C activity of PLC-γ1 (D1165H) by each compound is listed under its chemical structure.