

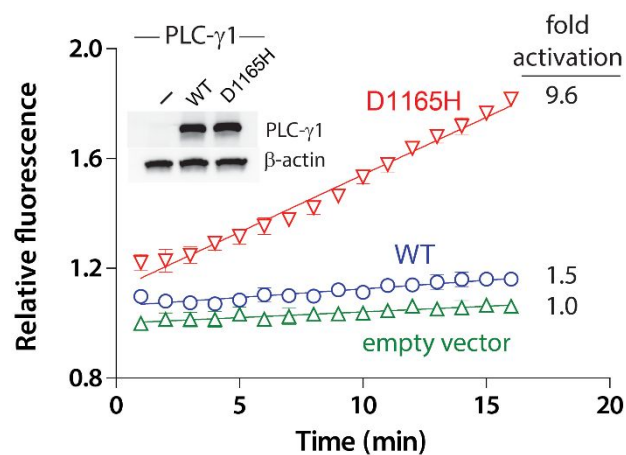
## Supporting information

### **A high-throughput assay to identify allosteric inhibitors of the PLC- $\gamma$ isozymes operating at membranes**

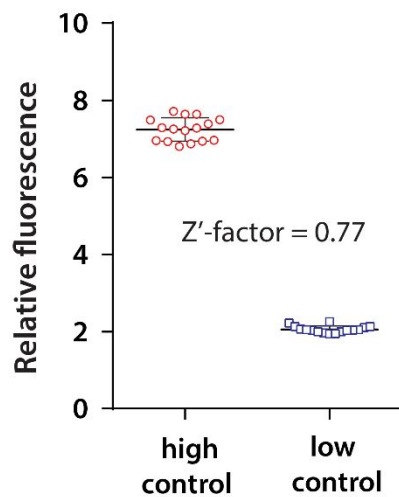
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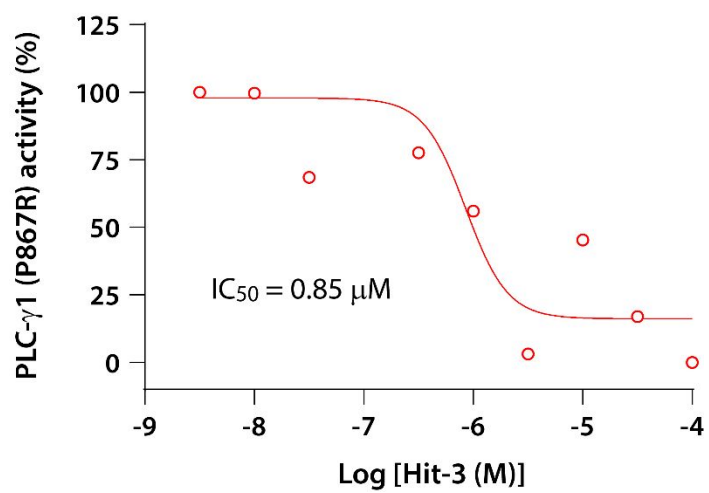
This information contains **Figures S1-S4** (5 pages in total)



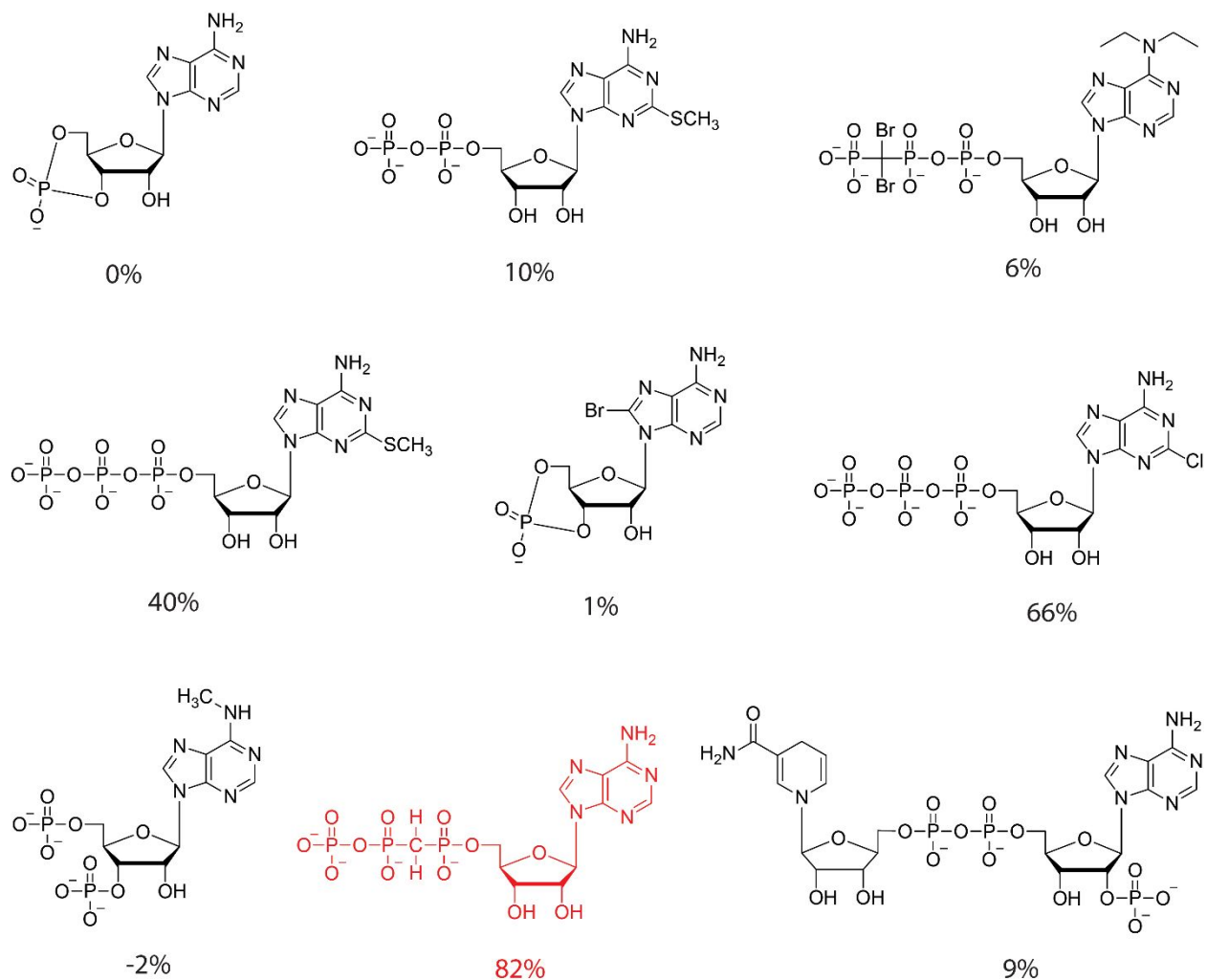
**Figure S1. XY-69 measures phospholipase activity with cellular lysates.** HEK293 cells were transfected to express the indicated forms of PLC- $\gamma$ 1 prior to lysis, addition of XY-69 and monitoring of fluorescence ( $\lambda_{\text{ex/em}} = 485/520$  nm). Fold activation is relative to the rate of fluorescence change for cells transfected with empty vector (no PLC- $\gamma$ 1) calculated from the mean of four replicates. Inset: western blots against PLC- $\gamma$ 1 and  $\beta$ -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- $\gamma$ 1 (D1165H) after 1 h is used as the high control while substituting PLC- $\gamma$ 1 (D1165H) for BSA as the low control. Each condition was measured 16 times.



**Figure S3. Inhibition of PLC- $\gamma$ 1 (P867R) by Hit-3.**



**Figure S4. Chemical structures of ribonucleotides and derivatives in the LOPAC<sub>1280</sub> library.** Hit-3 is in red and the percent inhibition of the phospholipase C activity of PLC- $\gamma$ 1 (D1165H) by each compound is listed under its chemical structure.