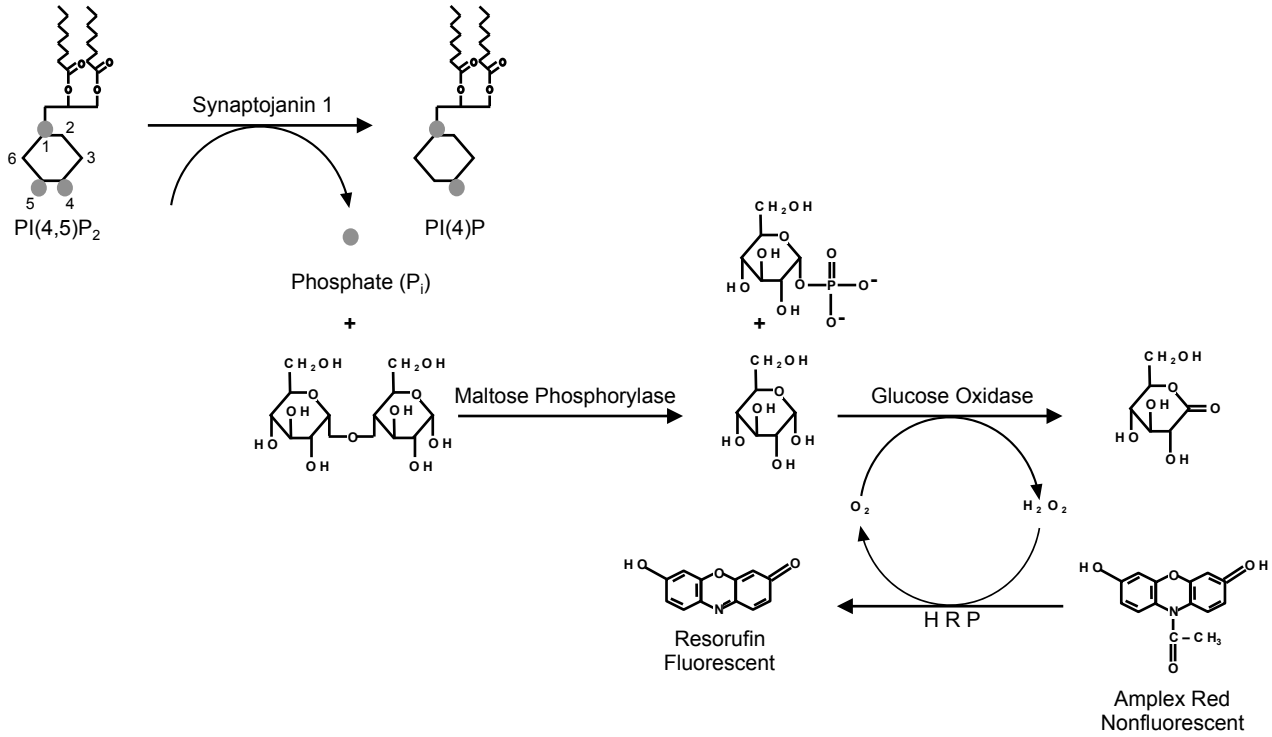
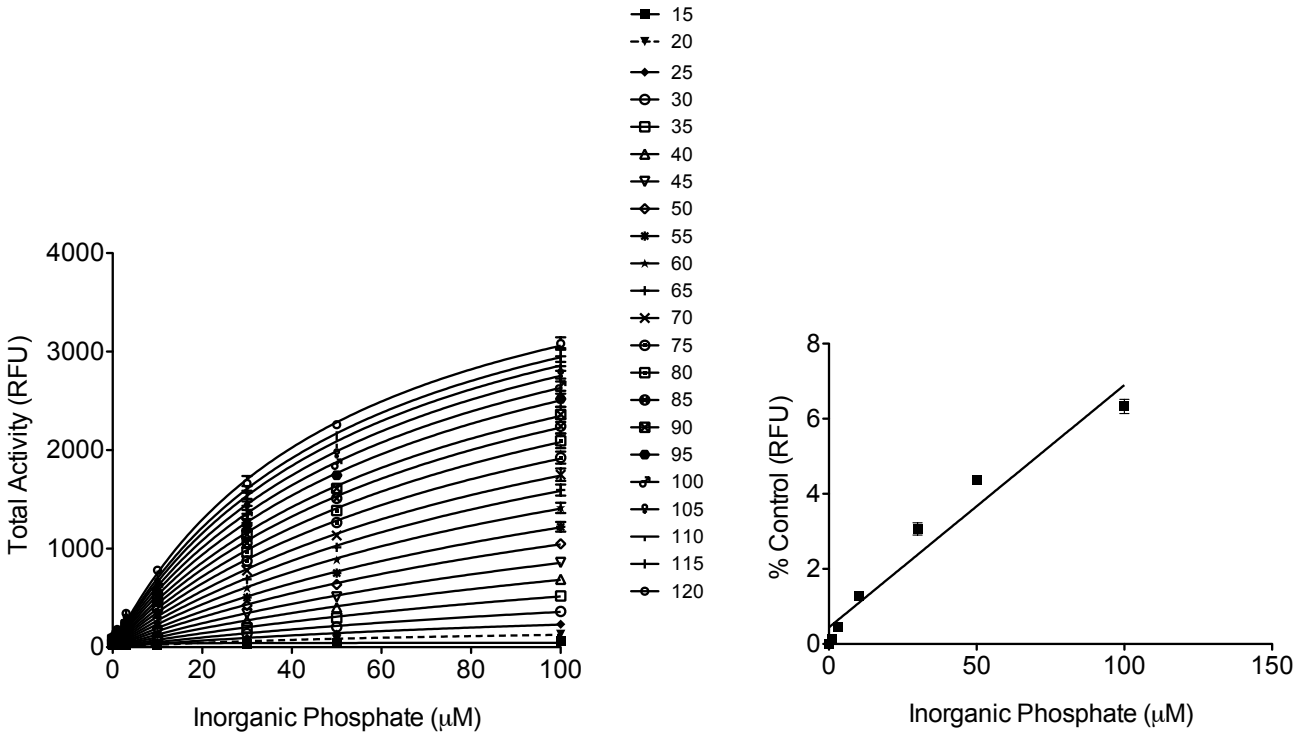
Supplemental Fig. 1



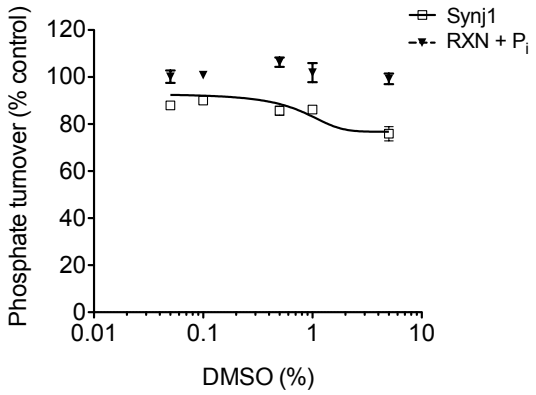
Supplemental Fig 1. Schematic representation of Synj1 phosphatase activity and fluorescent detection of liberated inorganic phosphate. The liberation of inorganic phosphate (P_i) from the substrate di-C8-phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] by Synj1 drives the formation of the fluorescent compound resorufin. Horseradish peroxidase (HRP) is a catalyst for the formation of resorufin.

Supplemental Fig. 2



Supplemental Fig 2. Standard curve generation. (A) Free phosphate detection from standards of known concentration of potassium phosphate shown as total fluorescent signal in relative fluorescent units (RFU) at time points 15 – 120 minutes after assay start. **(B)** Background fluorescence was subtracted from total fluorescence to determine Specific RFU 100 minutes after reaction start, which is displayed as % total fluorescent control determined by H_2O_2 driven resorufin fluorescence. Error bars represent standard deviation.

Supplemental Fig. 3



Supplemental Fig 3. DMSO tolerance. Free phosphate detection in presence of increasing concentrations of DMSO incubated with Synj1 and100μM PI(4,5)P_2 (Synj1) or with assay detection enzymes and 100μM potassium phosphate (RXN + P_i). Phosphate turnover is displayed as % control. Error bars represent standard deviation.