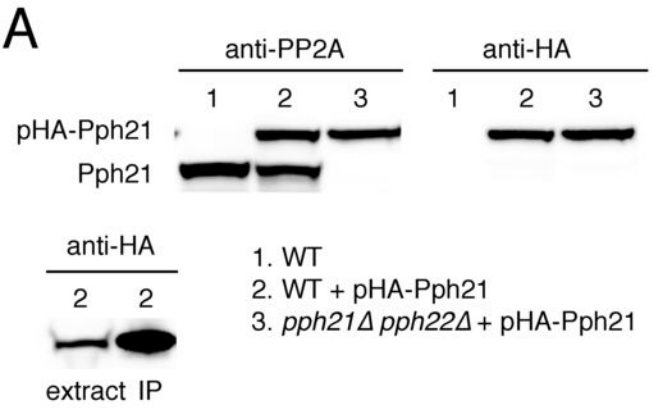
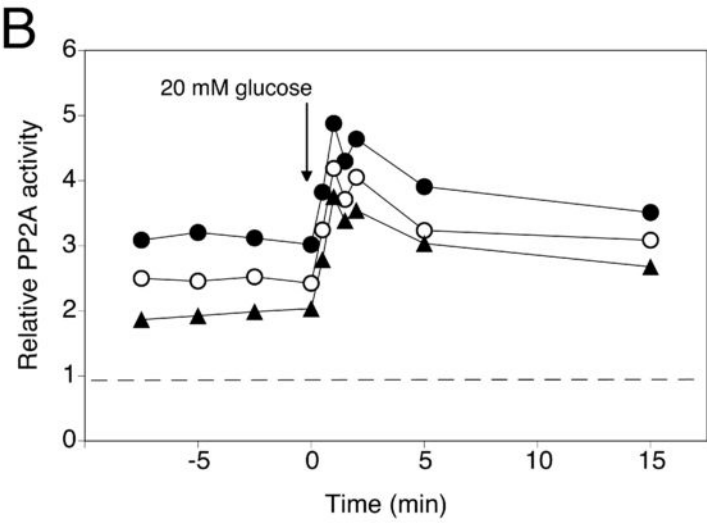

A
anti-PP2A
anti-HA
1 2 3
1 2 3
pHA-Pph21
Pph21
anti-HA
2 2
extract IP
1. WT
2. WT + pHA-Pph21
3. pph21Δ pph22Δ + pHA-Pph21


B
20 mM glucose
Relative PP2A activity
0
1
2
3
4
5
6
-5
0
5
10
15
Time (min)

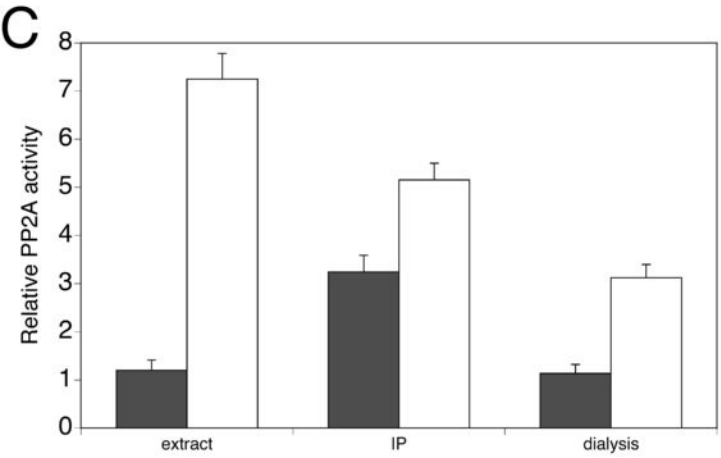

C
Relative PP2A activity
0
1
2
3
4
5
6
7
8
extract
IP
dialysis

Figure S3 High basal level of immunopurified Pph21 is most likely due to the high concentration of this catalytic subunit, and not to the loss of any interacting molecule/protein during the immunopurification. (A) Cells were grown to exponential phase in glucose-containing medium. Cell extracts (directly, upper panel, or after immunopurification with anti-HA, lower panel) were used for immunodetection of Pph21, either encoded by the genomic *PPH21* gene (Pph21) or from a plasmid (pHA-Pph21). Strains and antibodies used are indicated. **(B)** At time zero 20 mM glucose was added to a wild type strain and activity was measured with HA-Pph21 immunopurified from cell extracts (anti-HA) to measure specific PP2A phosphatase activity. Different concentrations of cell extract were used for the immunopurification: 400 µg (●), 300 µg (○), 150 µg (▲). The dashed line indicates the PP2A activity measured in the same extracts without immunopurification step. **(C)** Wild type cells were grown to exponential phase in glucose-deprived conditions. Cell extracts were used to measure specific PP2A activity in derepressed conditions (basal level, grey bars) and in repressed conditions (2 minutes after glucose addition, white bars) after different treatments of the extracts: no treatment (extract), after immunopurification (IP) or after dialysis against the lysis buffer used for extraction (dialysis).