Supplementary Figures

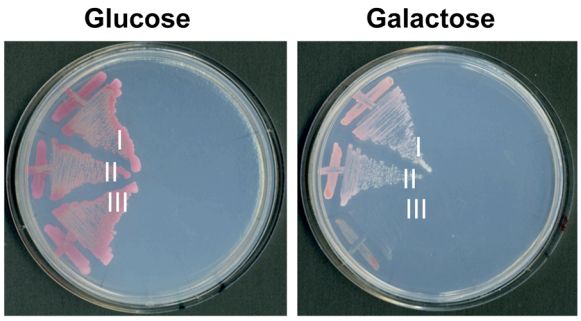

Fig. S1 SidD is unable to suppress the toxicity of AnkX. Plasmids that direct the expression of SidD were introduced into yeast strains expressing AnkX or SidM from a galactose-dependent promoter. Yeast cells grown on glucose plates were streaked onto plates containing glucose (left) or galactose (right). The growth of yeast cells was documented after incubation at 30ºC for 3 days. Strains: I, vector/vector; II, pSidM/pSidD; III, pAnkX/pSidD.

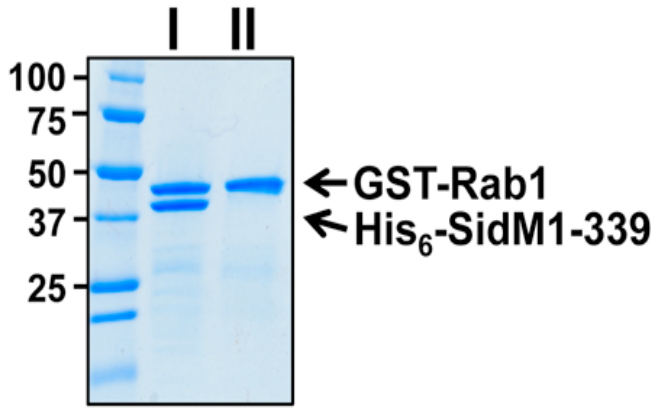

Fig. S2 SidM(1-339) can be removed from the AMPylation reaction. GST-Rab1associated with glutathione beads was incubated with His_6-SidM1-339 and ATP for 30 min at 35ºC to allow for AMPylation of Rab1. The beads were split into samples and one (II) was subjected to be washed with a regime described in Materials and Methods. The retained proteins were resolved by SDS-PAGE and visualized by Commassie brilliant blue staining. Note that even an excessive amount of His_6-SidM1-339 was used, the level of this protein was beyond detection after washing.

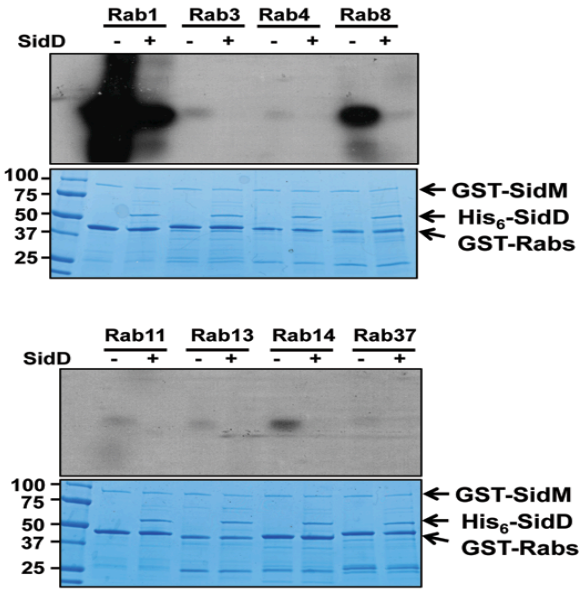

Fig. S3 SidD deAMPylates other Rab proteins modified by SidM. Indicated GST-Rab proteins purified from *E. coli* were incubated with GST-SidM and of ^{32}P-α-ATP for 30 min at 35ºC. Each sample was split into two reactions and His_6-SidD was added to one of the reactions. After further incubation for 30 min, the proteins were separated by SDS-PAGE and AMPylated GST-Rabs were detected by autoradiograph (upper panels) and the proteins in the gels were visualized by commassie brilliant blue (lower panels). Note that the efficiency of AMPylation toward other Rab proteins by SidM is significantly lower.

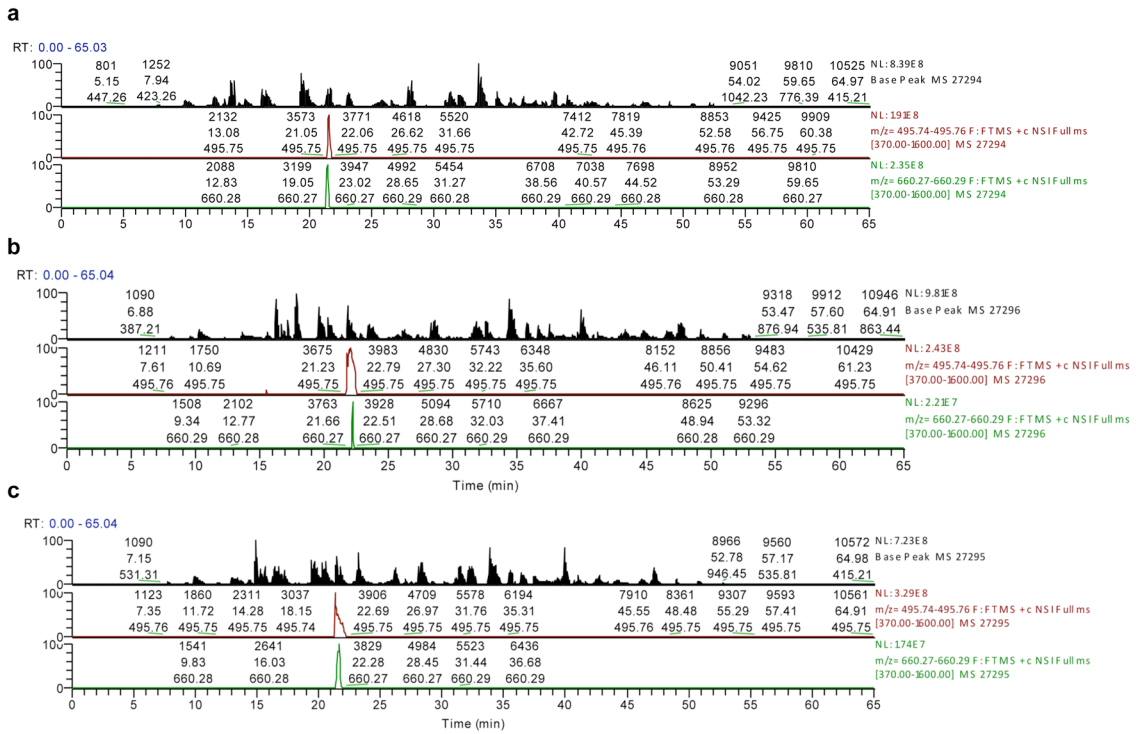

Fig. S4 Mass spectrometric analysis of deAMPylation of Rab1 by SidD. GST-Rab1 was incubated with GST-SidM in the presence of ATP for 30 min. A sample was withdrawn (**a**) before adding His_6-SidD, identical samples were further withdrawn after incubation for 10 (**b**) and 30 (**c**) min, respectively. After resolved by SDS-PAGE, protein bands corresponding to GST-Rab1 was excised, digested with trypsin and analyzed by mass spectrometry. On each chromatogram, data of the relative intensity ratio for modified Rab1 (green) and unmodified Rab1 (red) were shown. The ratio of unmodified Rab1 increased about 10-fold when 4 μg His_6-SidD was added to the reaction for 10 min and further increased to 20-fold when the incubation was extended for 30 min (Fig. S4 **b-c**).

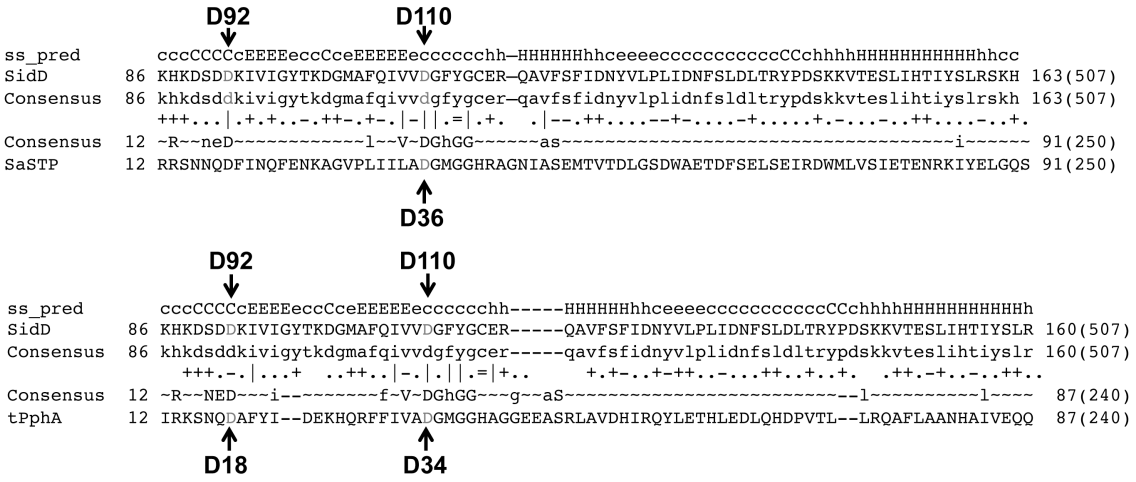

Fig. S5 Sequence alignment between SidD and two protein phosphatase generated by HHpred. Alignment was generated with online bioinformatics service (http://toolkit.tuebingen.mpg.de/hhpred). The Asp36 in SaSTP of *Streptococcus agalactiae* (**a**), which is conserved with Asp110 of SidD, is critical for its enzymatic activity [14]. Similarly, Asp18 and Asp34 in tPphA of *Thermosynechococcus elongates* important for binding the metal ion critical for catalysis (**b**)[15], are conserved with Asp92 and Asp110 in SidD, respectively.

Table S1 Sequences of primers used in this study

Name	Sequence	note
SidD-5'	ATAGGATCCTTGGTATATTATGAGATC	*Bam*HI for SidD cloning
SidD-3'	ATAGTCGACTTAAATAGTAAGACTCG	*Sal*I for SidD cloning
SidM-5'	ATAGGATCCATGAGCATAATGGGGAG	*Bam*HI for SidM cloning
SidM-3'	ATAGTCGACTTATTTTATCTTAATGG	SalI for SidM cloning
SidM 1-339-3'	ATAGTCGACTTAACGTTGAACACCCAGCTC	*Sal*I for SidM AMPylation domain cloning
Rab1-5'	ATAGGATCCATGTCCAGCATGAATCC	*Bam*HI for Rab1 cloning
Rab1-3'	ATAGTCGACTTAGCAGCAACCTCCAC	*Sal*I for Rab1 cloning
SidD D60A up	AAGCTATCAATCTGGTTCCAATGCTTTAGAT AAGGGTAATAGTGAAA	SidD mutation D61A
SidD D60A down	TTTCACTATTACCCTTATCTAAAGCATTGGAA CCAGATTGATAGCTT	SidD mutation D61A
SidD D92A up	TATAAAGCACAAGGATTCTGACGCTAAAATA GTCATTGGATATACCA	SidD mutation D92A
SidD D92A down	TGGTATATCCAATGACTATTTTAGCGTCAGA ATCCTTGTGCTTTATA	SidD mutation D92A
SidD D110A up	GCTTTTCAAATAGTGGTGGCTGGCTTTTATGG ATGCGAA	SidD mutation D110A
SidD D110A down	TTCGCATCCATAAAAGCCAGCCACCACTATT TGAAAAGC	SidD mutation D110A
SidD Knockin 1 5'	ATAGTCGACATCTTTTATGAATTGCATTATAC C	*Sal*I for SidD upstream
SidD Knockin 2 3'	ATAGGATCCATGATATACTTAATTACAAGTA GAG	*Bam*HI for SidD upstream
SidD Knock In3	ATA CCATGG TTAAATAGTAAGACTCGAG	*Nco*I for SidD downstream
SidD Knock In4	ATA GCGGCCGC TTTAGGATTTCAATCAG	*Not*I for SidD downstream
SidD FLAG NT	ATA GGATCC ATG GACTACAAAGACGATGACGACAAG	*Bam*HI for N-terminal FLAG- SidD
SidD 3' *Nco*I	ATACCATGGGTATTATAAGTTCATTATG	*Nco*I for SidD
Rab3-5' *Bam*HI	ATAGGATCCATGGCATCCGCCACAGACTCGC	
Rab3-3' *Sal*I	ATAGTCGACTCAGCAGGCGCAGTCCTGGTGC G	
Rab4-5'*Bgl*II	ATAAGATCTATGGCTGAGACCTACGACTTCC	
Rab4-3' *Sal*I	ATAGTCGACTCAGCAGCCACACGGCTGAGGG	
Rab8-5' *Bam*HI	ATAGGATCCATGGCGAAGACCTACGATTACC	
Rab8-3' *Sal*I	ATAGTCGACTCACAGAAGAACACATCGGAA AAAGC	
Rab11-5' *Bam*HI	ATAGGATCCATGGGCACCCGCGACGACGAG	
Rab11-3'*Sal*I	ATAGTCGACTTAGATGTTCTGACAGCACTGC	

Rab13-5'*Bam*HI	ATAGGATCCATGGCCAAAGCCTACGACCAC
Rab13-3'*Sal*I	ATAGTCGACTCAGCCCAGGGAGCACTTGTTGG
Rab14-5'*Bam*HI	ATAGGATCCATGGCAACTGCACCATACAACTAC
Rab14-3'*Sal*I	ATAGTCGACCTAGCAGCCACAGCCTTCTCTC
Rab37-5'*Bam*HI	ATAGGATCCATGACGGGCACGCCAGGCGCCG
Rab37-3'*Sal*I	ATAGTCGACTCACATGAAGGAGCAGCAGCTG

Note: Sequences for restriction sites used for subcloning or for site-directed mutagenesis were underlined.