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SUPPLEMENTARY ONLINE DATA

New insights into the catalytic mechanism of histidine phosphatases revealed by a functionally essential arginine residue within the active site of the Sts phosphatases

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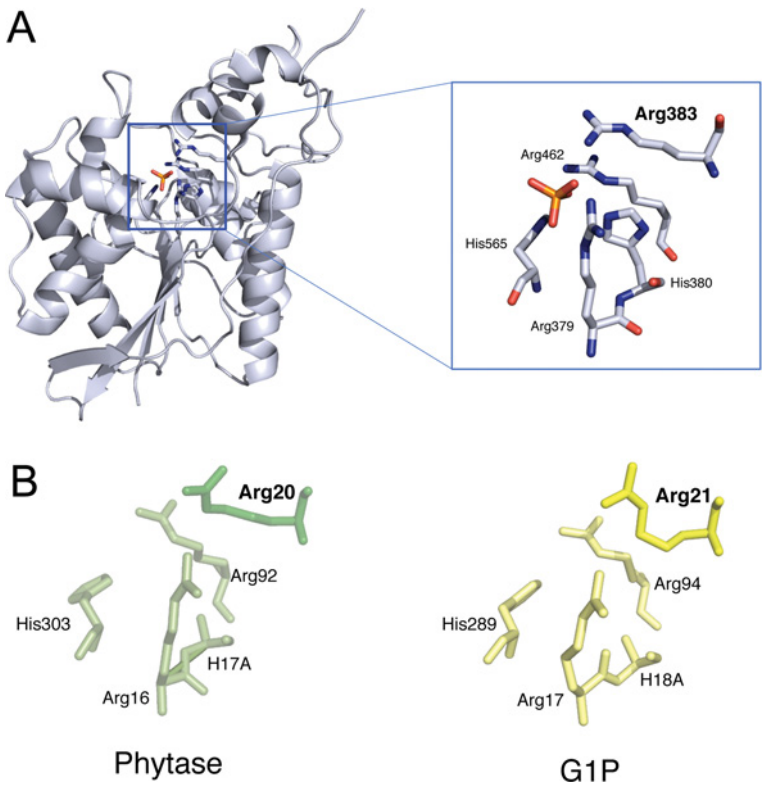

Figure S1 Similar orientation of Sts-1 Arg^{383} equivalents in other HP enzymes

Comparison of active-site residues in murine Sts-1_{PGM} (**A**) and *A. fumigatus* phytase (**B**, left-hand panel) and *E. coli* glucose-1-phosphatase (G1P; **B**, right-hand panel), highlighting the orientation of Arg^{383} in Sts-1_{PGM} and its equivalents in other HP enzymes. Stick representations were created in PyMOL using the following crystal structures: PDB code 2H0Q (Sts-1_{PGM}), PDB code 1NT4 (*E. coli* glucose-1-phosphatase) and PDB code 1DKL (*E. coli* phytase).

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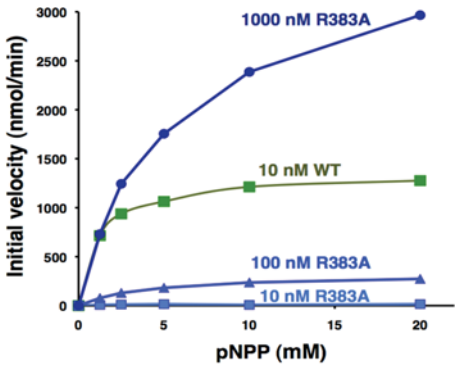

	WT	R383A
Specific activity (nmol/min/mg)	18,583	387
Km (mM)	1.107	3.303

Figure S2 Requirement for Arg^{383} in the Sts-1$_{PGM}$ catalytic reaction towards pNPP

Comparison of the initial velocities of phosphatase reactions containing either wild-type Sts-1$_{PGM}$ (WT; 10 nM, green) or Sts-1$_{PGM}$ R383A (10, 100 or 1000 nM, blue) against various concentrations of pNPP (1.25–20 mM). Michaelis–Menten parameters (lower panel) were calculated from the saturation curves using GraphPad Prism.

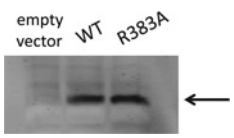

Figure S3 Equivalent levels of expression for wild-type Sts-1 (WT) and Sts-1 R383A in reconstituted primary T-cells

GFP$^+$ *Sts-1/2*$^{-/-}$ reconstituted cells were sorted by flow cytometry, lysed in Laemmli sample buffer and the levels of Sts-1 proteins (arrow) were assessed by Western blot analysis using an anti-FLAG antibody.

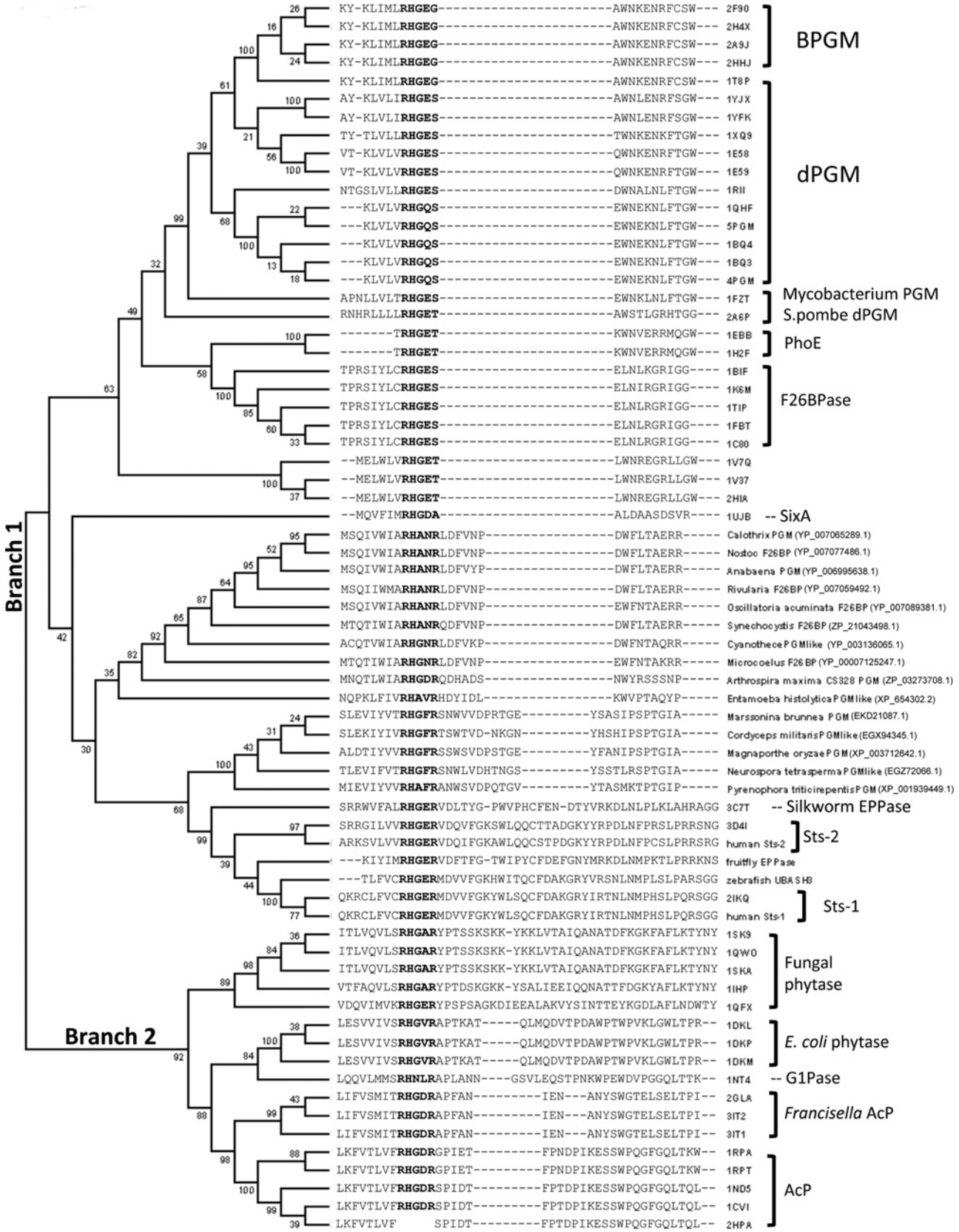

Figure S4 Alignment of individual members of Branch 1 and 2 HPs, highlighting the presence of an arginine residue in the active sites of both Branch 2 AcPs and a small number of Branch 1 enzymes

The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model [1,2]. The evolutionary analysis was conducted in MEGA5 [3] and involved 68 amino acid sequences.

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