SUPPLEMENTARY ONLINE DATA Differential functions of phospholipid binding and palmitoylation of tumour suppressor EWI2/PGRL

Bo HE*1, Yanhui H. ZHANG*1, Mekel M. RICHARDSON*, Julian S. ZHANG†, Eric RUBINSTEIN‡, and Xin A. ZHANG*2

*Vascular Biology Center, Center for Cancer Research, and Departments of Medicine and Molecular Science, University of Tennessee Health Science Center, Memphis, TN 38163, U.S.A., †White Station High School, Memphis, TN 38117, U.S.A., and ‡Inserm, U1004, 14 Av Paul Vaillant Couturier, 94807, Villejuif, France and Univ. Paris Sud, Institut André Lwoff, 94807, Villejuif, France

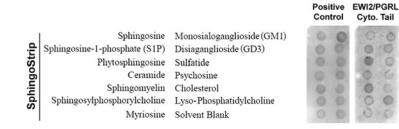


Figure S1 The binding of the EWI2/PGRL cytoplasmic domain to sphingolipids

The peptide with the EWI2/PGRL cytoplasmic domain sequence and the positive control were overlaid on the Sphingo strip following the manufacturer's protocol, as described in the Materials and methods section of the main text. Because the peptide was labelled with biotin at the N-terminus, the PIP strips were blotted with HRP-conjugated avidin and the bindings were visualized with chemiluminescence. The names of lipids on the left indicate the corresponding position of these lipids in the Sphingo strip. All lipids immobilized on the strip were at the same molar concentrations. The positive control for the Sphingo strip was Chloral toxin B subunit.

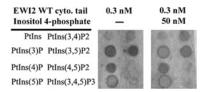


Figure S2 The role of the acidic moiety of PIPs in the binding to the $\ensuremath{\mathsf{EW12/PGRL}}$ cytoplasmic domain

The EWI2/PGRL peptide–PIP overlay assay was performed as described above, with or without Ins4P in the solution.

Received 27 August 2010/13 April 2011; accepted 25 May 2011 Published as BJ Immediate Publication 25 May 2011, doi:10.1042/BJ20101381

¹ These authors contributed equally to this work.

² To whom correspondence should be addressed (email xzhang@uthsc.edu).