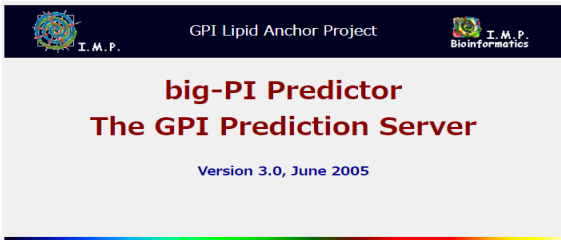


Supplemental Figure S1

Prediction of the GPI ω -site in LOTUS using bioinformatic tools.

- (a) Big-PI Predictor analysis identified Ser617 as the most probable GPI ω -site in LOTUS.
- (b) PredGPI analysis likewise predicted Ser617 as a highly probable GPI ω -site.

(a) Big-PI Predictor analysis



Output of the prediction tool:

~~~~~

Query sequence Query (length 645 amino acids):

|            |            |            |            |            |            |
|------------|------------|------------|------------|------------|------------|
| MAPSADPGMS | RMLPFLLLW  | FLPITEGSQR | AEPMTAVTN  | SVLPPDYDSN | PTQLNYGVAV |
| TDVDHGDFFE | IVVAGYNGPN | LVLKYDRAQK | RLVNIAYDER | SSPYVALRDR | QGNAIGVTAC |
| DIDDGGREEI | YFLNTNNAFS | GVATYTDKLF | KFRNNRWEDI | LSDEVNVARG | VASLFAGRSV |
| ACVDRKGSGR | YSIYIANYAY | GNVGPDALIE | MDPEASDLR  | GILALRDVAA | EAGVSKYTGG |
| RGVSVGPILS | SSASDIFCDN | ENGNFLFHN  | RGDGTFDAA  | ASAGVDDPHQ | HGRGVALADF |
| NRDGGVDIYY | GNWNGPHRLY | LQMSTHGKVR | FRDIASPKFS | MPSPVRTYIT | ADFDNDQELE |
| IFFNNIAYRS | SSANRLFRVI | RREHGDPLIE | ELNPGDALEP | EGRGTGGVYT | DFDGDGMLDL |
| ILSHGESMAQ | PLSVFRGNQG | FNNWLRVVP  | RTRFGAFARG | AKVLYTKKS  | GAHLRIIDGG |
| SGYLCMEPEV | AHFGLGKDEA | SSVEVTWPDG | KMVSERNVAG | EMNSVLEILY | PRDEDTLQDP |
| APLECGGGS  | QQENGHCMDT | NECIGPFVC  | PRDKPVCVNT | YGSYRCRTNK | KCSRGYEPNE |
| DGTACVAQVA | FLGGYSSAAS | RISEPLSRAS | YLSLGLGLCL | QLYAL      |            |

The site with the best score is shown in black.

~~~~~

Prediction of potential C-terminal GPI-Modification Sites

~~~~~

Use of the prediction function for METAZOA

**None** potential GPI-modification site was found.  
Among all positions checked, sequence position 617 had the best score.

**Total Score**.....: **-6.67** (PValue = 4.529494e-03)

Components of the Score Function:

|                                                           |        |
|-----------------------------------------------------------|--------|
| Profile Score                                             | 5.54   |
| Term 0 Contents and Windows of DE in Region [-11..1]      | 0.00   |
| Term 1 Hydrophilicity of N-terminal Region [-11..1]       | -0.00  |
| Term 2 Penalty for low Profile Score in Region [0..2]     | 0.00   |
| Term 3 Volume Limitation [-1..+2]                         | 0.00   |
| Term 4 Volume Compensation (-1, 1, 2)                     | 0.00   |
| Term 5 Volume Compensation (-1, 2)                        | 0.00   |
| Term 6 Backbone Flexibility [-1..2]                       | 0.00   |
| Term 7 Propeptide Length                                  | 0.00   |
| Term 8 Hydrophilicity of Spacer Region [3..8]             | 0.00   |
| Term 9 Volume Limitation [3..8]                           | -0.05  |
| Term 10 Penalty for charged AAs in Spacer Region [3..10]  | 0.00   |
| Term 11 Backbone Flexibility [3..8]                       | 0.00   |
| Term 12 Penalty for low Profile Score in Region [10..end] | -12.00 |
| Term 13 Hydrophobicity of Tail [10..end]                  | -0.16  |
| Term 14 Hydrophobicity of Tail [26..end]                  | 0.00   |
| Term 15 Even Distribution of Hydrophobicity [9..end]      | 0.00   |
| Term 16 Penalty for polar Windows in Region [10..end]     | 0.00   |
| Term 17 Penalty for SGC-Windows in Region [10..end]       | 0.00   |
| Term 18 LVI Contents [10..end]                            | 0.00   |
| Term 19 Penalty for FYHW - Sections in Region [10..end]   | 0.00   |
| Term 20 Penalty for Windows with small Volume [10..end]   | 0.00   |
| Profile independent Score                                 | -0.21  |

(b) PredGPI analysis

PredGPI

GPI-anchor Predictor

Home

Prediction

Datasets

Proteomes

Info

Prediction Results

Prediction accuracy summary:

|                  |   |
|------------------|---|
| Highly probable  | 1 |
| Probable         | 0 |
| Weakly probable  | 0 |
| Not GPI-anchored | 0 |

Proteins predicted

| Protein name | omega-site position | Specificity | Protein sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|--------------|---------------------|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| LOTUS        | 617                 | 100.0%      | MAPSADPGMSRMLPFLLLLWFLPITEGSQRAEPMFTAVTNSVLPDYDSNPTQLNYGVAVTDVDHGDGEIVVAGYNGPNLVLYDRAQKRLVNIAYDERSSPYVALRDRQNAIGVTACDIDGQREEIYFLNTNNAFSGVATYTDKLFKFRNNRWEDILSDEVNVARGVASLFAGRSVACVDRKGSGRYSIYIANYAYGNVGPDALIEMDPEASDLRGI LALRDVAEEAGVSKYTGGRGVSVGPILSSASDIFCDNENGNPNFLFHNRGDGTVDAAASAGVDDPHRHGRGVALLADFNRDGKVDIYYGNWNGPHRLYLQMSHGRVRFDDI ASPKFSMPSPVRTVITADFDNDRELEIFFNNIAYRSSSANRLFRVIRREHGDPLIEELNPGDALEPEGRTGGVYTFDGDGMLDLILSHGESMAQPLSVFRGNQGFNNNLRVVPRTRFGAFARGAKVLYTKKSGAHLRIIDGSGYLCEMEPVVHFGLGKDEASSVEVTWPDGKMYSRNVASGEMNSVLEILYPRDEDTLQDPAPLECGQGSQQENGHCMDTNECIQFPFVCPDKPVVCVNTYGSYRCRTNKKCSRGYEPNE DGTACVAAVAFLLGGYS\$AASRISEPLSRASVLSLGLCLQLYAL |

For further information and bug report please contact: Pier Luigi Martelli or Andrea Pierleoni

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## Supplementary Figure S2

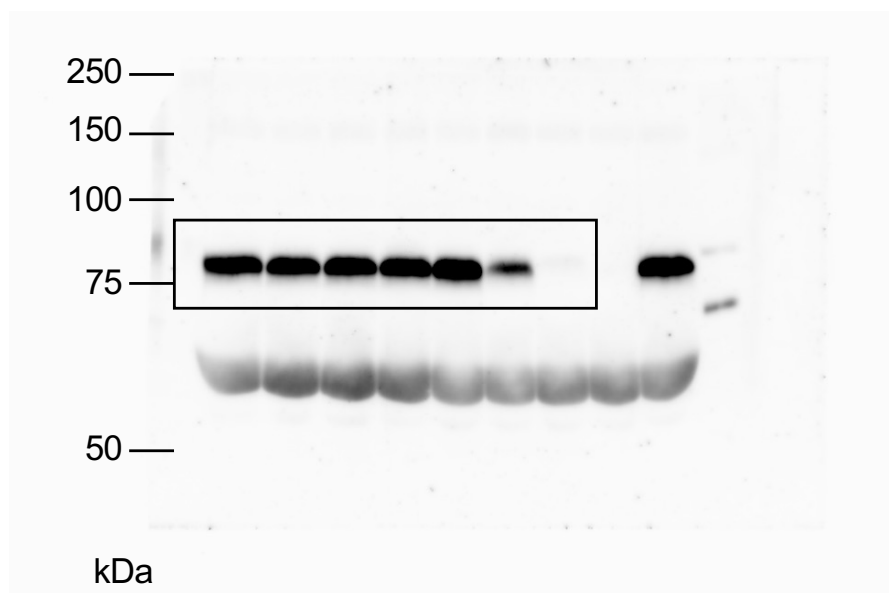
Original uncropped gels and blots corresponding to figures in the main text

Original uncropped blot images are shown in panels (a-g): (a) the original uncropped blot corresponding to Fig. 3b; (b) the upper panel of Fig. 4a; (c) the lower panel of Fig. 4a; (d) the upper panel of Fig. 4b; (e) the lower panel of Fig. 4b; (f) the upper panel of Fig. 4c; and (g) the lower panel of Fig. 4c. Rectangles indicate the regions displayed in the corresponding figure panels in the main text. Because the original uncropped images shown in panels (d) and (e) were dark, brightness was adjusted uniformly across each entire image solely to improve visibility. No brightness or contrast adjustments were applied to panels (a-c), (f), and (g). No bands or other image features were added, removed, repositioned, or selectively altered. Although the edges of the gels are not clearly visible in some images, the images encompass the entire gel area that was transferred to the membrane. Molecular weights were determined using molecular weight markers on the original gels. Although these markers are not visible in the blot images, their corresponding positions are indicated.

**(a)** Original uncropped image for Figure 3b



**(b)** Original uncropped blot for the upper panel of Figure 4a

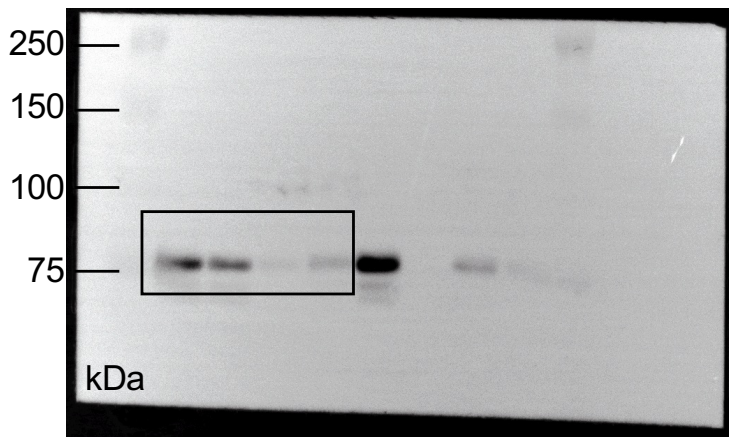


**Supplemental Figure S2**

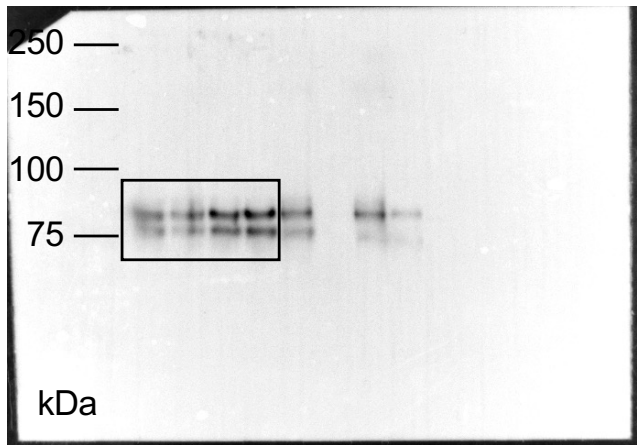
**(c)** Original uncropped blot for the lower panel of Figure 4a



**(d)** Original uncropped blot for the upper panel of Figure 4b



**(e)** Original uncropped blot for the lower panel of Figure 4b



Supplemental Figure S2

(f) Original uncropped blot for the lower panel of Figure 4c



(g) Original uncropped blot for the lower panel of Figure 4c

