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online data

SUPPLEMENTARY ONLINE DATA

High-resolution NMR studies of structure and dynamics of human ERp27 indicate extensive interdomain flexibility

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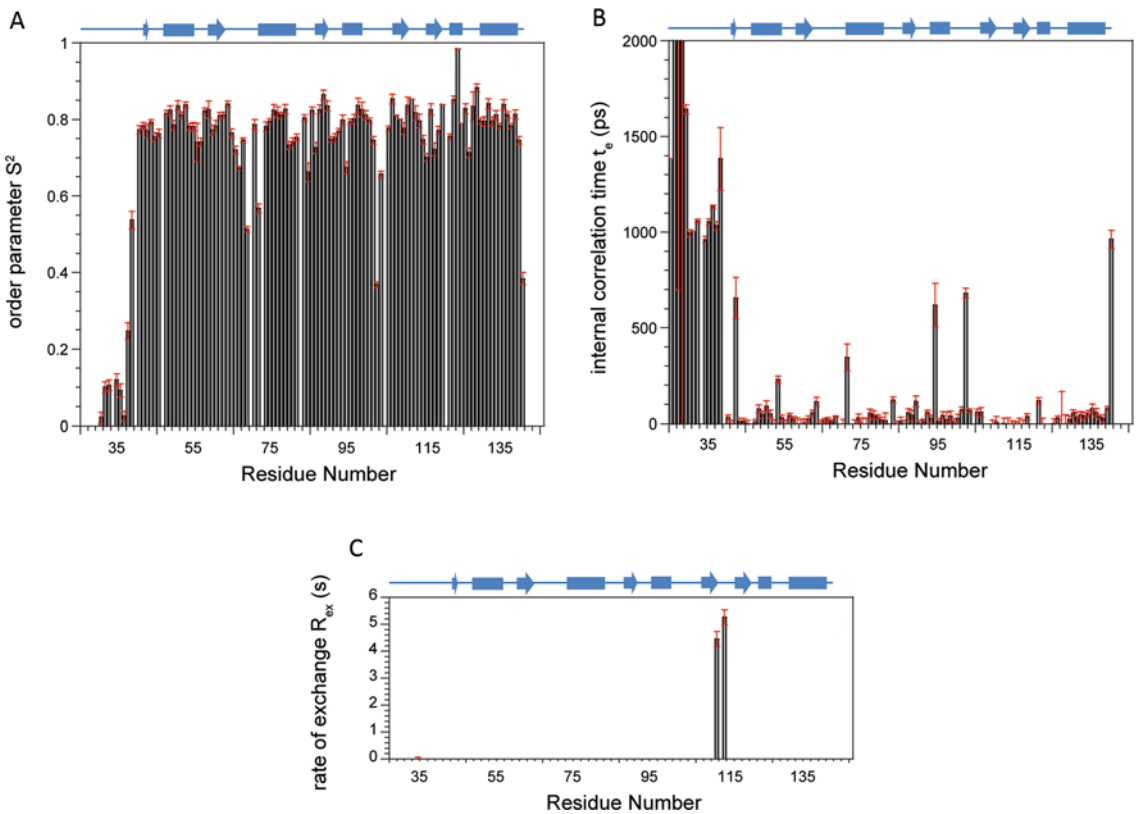

Figure S1 ModelFree analysis of ^{15}N-NMR relaxation data of the ERp27 b domain

Data were collected at 25 °C and 14.1 T. Histograms illustrate the changes across the sequence of (**A**) order parameter S^2, (**B**) internal correlation time τ_e and (**C**) rate of chemical exchange broadening R_{ex}. A schematic diagram of the domain secondary structure as solved by NMR is shown above each plot. Cylinders represent α-halices and arrows represent β-strands.

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The structural co-ordinates for the **b** domain of human ERp27 reported will appear in the PDB under accession code 2L4C.

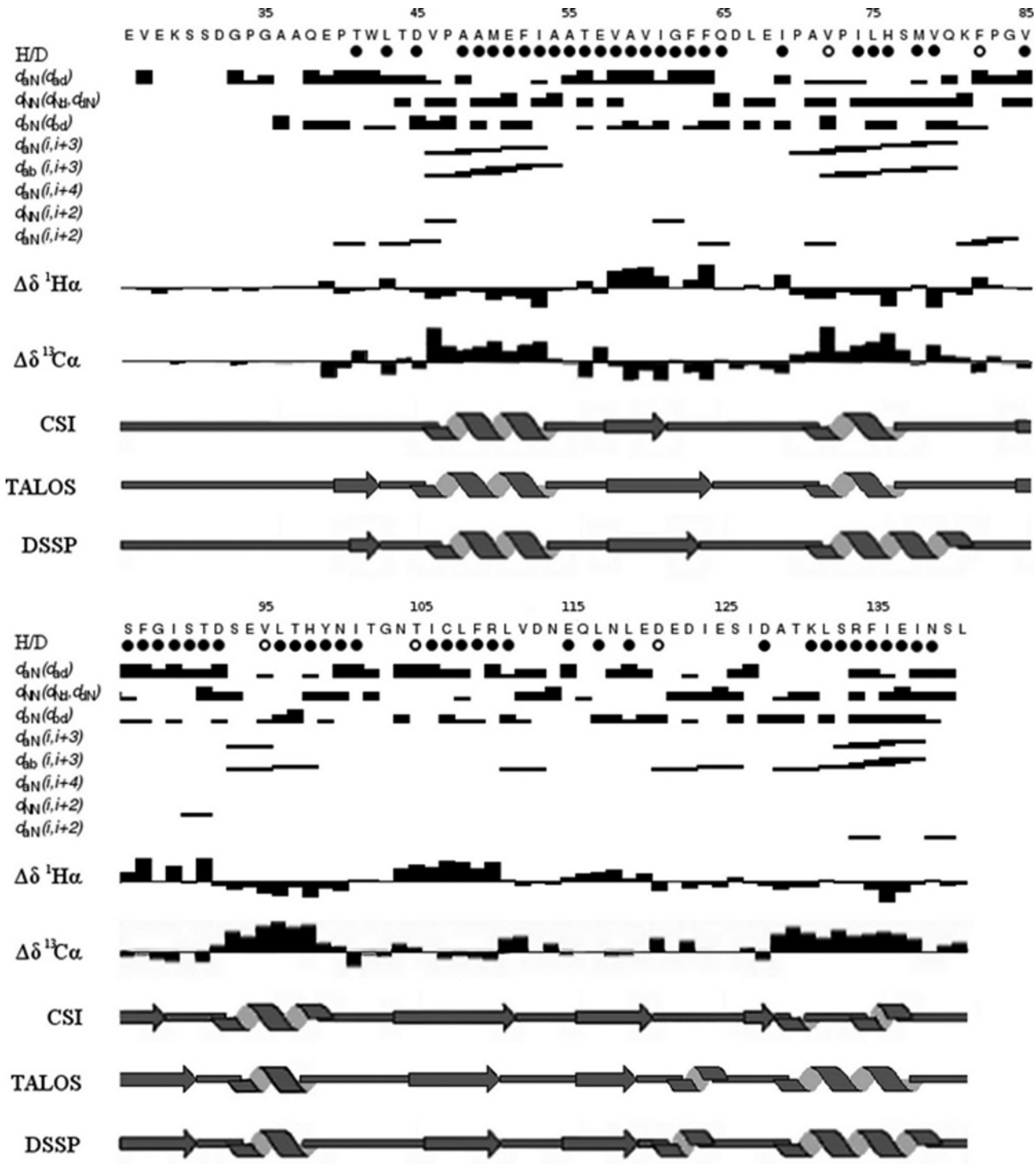

Figure S2 Chemical-shift data supporting the identification of the secondary structure in the ERp27 b domain

A closed circle represents a residue with an amide proton which is still present in the 2D ^{15}N-^{1}H spectrum after incubation in $^{2}H_2O$ for 300 s. An open circle represents an amide proton still present in the 2D ^{15}N-^{1}H spectrum after exchange to $^{2}H_2O$ for which there is ambiguity over the assignment. The sequential proton–proton NOE connectivities ($d_{\alpha N}$, d_{NN} and $d_{\beta N}$) are represented by thick and thin bars. These correspond to strong and weak NOE intensities respectively. The medium-range proton–proton NOE connectivities [$d_{\alpha N}(i,i+3)$, $d_{\alpha\beta}(i,i+3)$, $d_{\alpha N}(i,i+4)$, $d_{NN}(i,i+2)$ and $d_{\alpha N}(i,i+2)$] are represented by lines connecting the residues whose protons are correlated. $^{1}H\alpha$ and $^{13}C\alpha$ chemical-shift deviations from random coil values, as determined by CSI are plotted and are labelled as $\Delta\delta$ $^{1}H\alpha$ and $\Delta\delta$ $^{13}C\alpha$ respectively, with units on the *y*-axis of p.p.m. Positive values represent shifts to lower field. The CSI and TALOS secondary structure predictions and the DSSP-CONT secondary structure assignment are represented by arrows and ribbons. Arrows represent β-sheets and ribbons represent α-helices. The Figure was generated using CCPN Analysis.

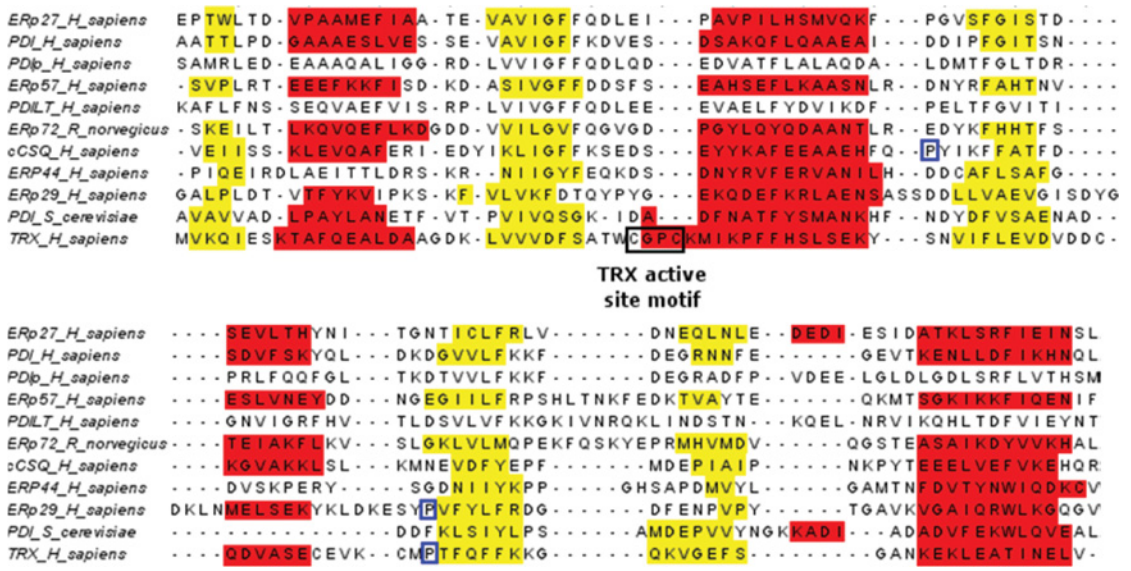

Figure S3 Multiple sequence alignment between ERp27 b and selected homologous proteins

Sequences of human Trx and various **b** domains of the PDI family are shown. The alignment was generated using ClustalW and modified to take into account known secondary structure. Secondary structure elements were defined using DSSP. Strands are shown in yellow and helices are shown in red. The conserved *cis*-proline residues in the *cis*-proline loop of Trx and ERp29 are boxed in blue. The conserved *cis*-proline in the loop between $\alpha 2$ to $\beta 3$ of calsequestrin is also boxed in blue. The active site CGPC motif of Trx is boxed in black. *H_sapiens*, *Homo sapiens*; *R_norvegicus*, *Rattus norvegicus*; *S_cerevisiae*, *Saccharomyces cerevisiae*.

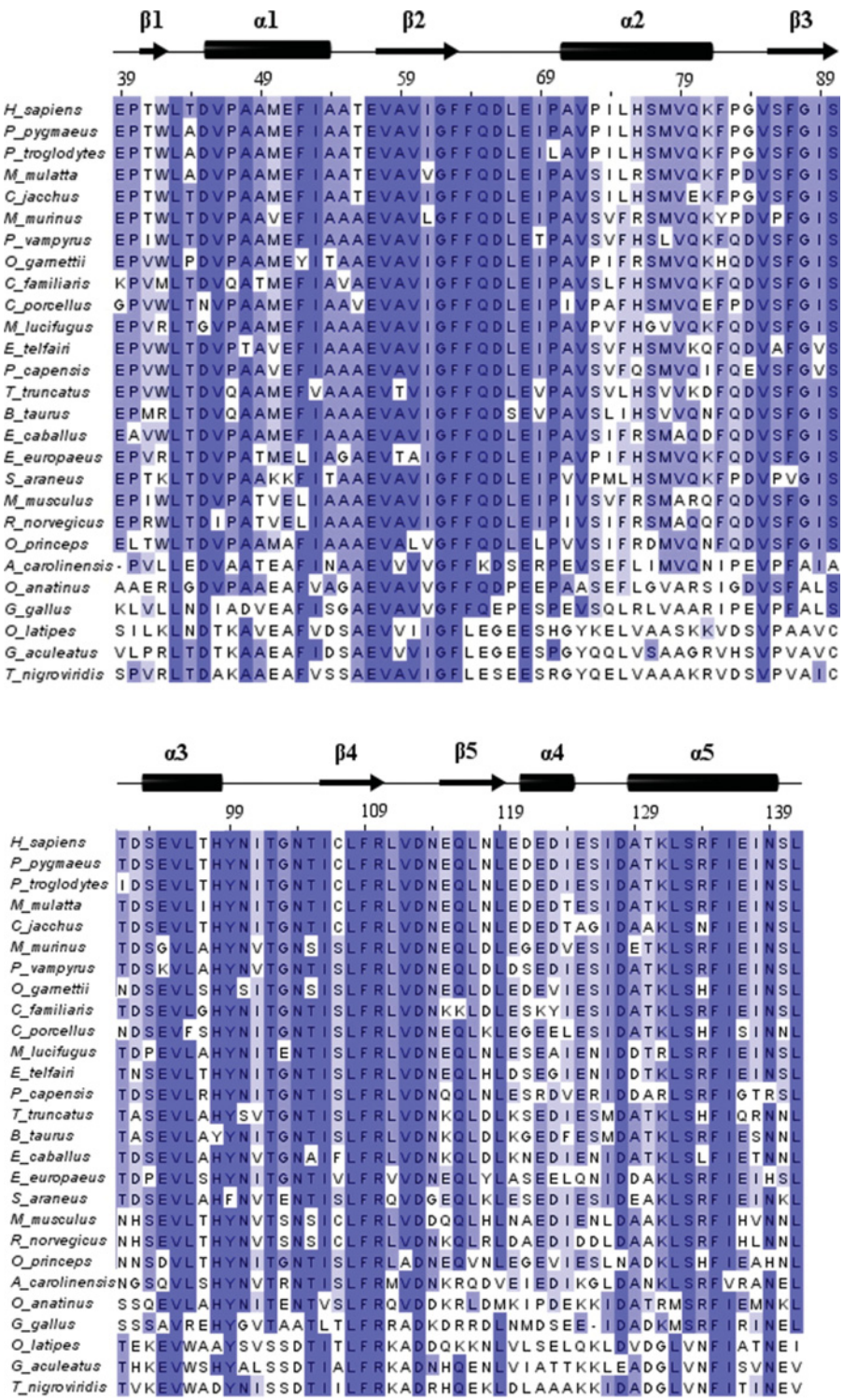

Figure S4 Sequence conservation between species in the b domain of ERp27

ERp27 **b** sequences from 27 different species were aligned using ClustalW. Secondary structure is indicated by cylinders for α-helices and arrows for β-strands. Sequence conservation is denoted by colour coding: light blue for >40 % identity, medium blue for >60 % identity and dark blue for >80 % identity. Residue numbering above the alignment is for human ERp27. *A_carolinensis*, *Anolis carolinensis*; *B_taurus*, *Bos taurus*; *C_familiaris*, *Canis familiaris*; *C_jacchus*, *Callithrix jacchus*; *C_porcellus*, *Cavia porcellus*; *E_caballus*, *Equus caballus*; *E_europaeus*, *Euonymus europaeus*; *E_telfairi*, *Echinops telfairi*; *G_aculeatus*, *Gasterosteus aculeatus*; *G_gallus*, *Gallus gallus*; *H_sapiens*, *Homo sapiens*; *M_lucifugus*, *Myotis lucifugus*; *M_mulatta*, *Macaca mulatta*; *M_murinus*, *Microcebus murinus*; *M_musculus*, *Mus musculus*; *O_anatinus*, *Ornithorhynchus anatinus*; *O_gamettii*, *Otolemur gamettii*; *O_latipes*, *Orizyas latipes*; *O_princeps*, *Ochotona princeps*; *P_capensis*, *Procavia capensis*; *P_pygmaeus*, *Pongo pygmaeus*; *P_troglodytes*, *Pan troglodytes*; *P_vampyrus*, *Pteropus vampyrus*; *R_norvegicus*, *Rattus norvegicus*; *S_araneus*, *Sorex araneus*; *T_nigroviridis*, *Tetraodon nigroviridis*; *T_truncatus*, *Tupsiops truncatus*.

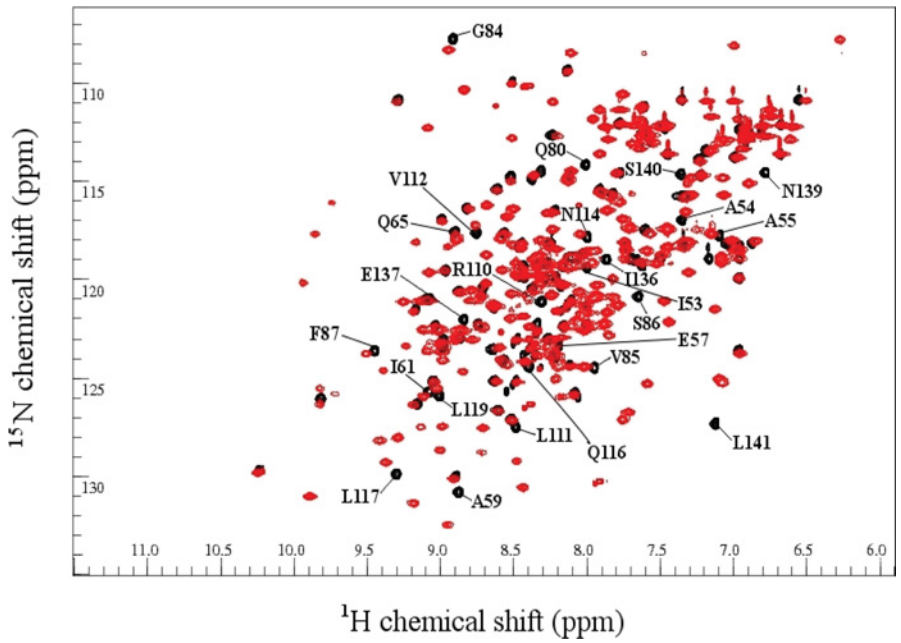

Figure S5 Comparison of spectra of b domain and full-length ERp27

Overlay of the ^{15}N-^{1}H HSQC spectra for the **b** domain of ERp27 (black) and full-length ERp27 (red). Residues with minimal shifts greater than 1 S.D. from the mean for residues 39–135 (0.0333 + 0.0292 p.p.m.) are labelled.

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