851 Figure S1: Sequence alignment of Ybr074 compared to the human ortholog ERMP1

852 and the rat ortholog FXNA. The light grey shaded region represents the M28

853 metalloprotease domain, and conserved metal-binding residues are shown in bold and
854 shaded in dark grey. Segments predicted to be transmembrane helices are underlined
855 for each respective sequence. Please note that the first underlined transmembrane helix
856 refers only to Ybr074 and not to ERMP1 or FXNA. * denotes amino acid identity, :
857 denotes a conserved amino acid substitution, and . denotes a semi-conserved amino
858 acid substitution. Amino acid positions are numbered on the right hand side.

859 Figure S2: Topological analysis of GFP-tagged Ybr074. Crude membrane extracts were
860 isolated from yeast expressing chromosomally integrated, N-terminally GFP-tagged
861 Ybr074 protein. Protein samples were incubated in the presence or absence of
862 proteinase K, as indicated. Note that, in contrast to other proteinase K-treated samples
863 shown in this study, the 0 min time point shown here was collected immediately after
864 addition of proteinase K.

865 Figure S3: FXNA ER-localization does not vary with the position of the HA epitope tag in
866 COS7 cells. FXNA localization was assessed in COS7 cells expressing N-terminally
867 FLAG-tagged FXNA (FLAG-FXNA) or C-terminally FLAG-tagged FXNA (FXNA-FLAG).
868 FXNA localization using indirect immunofluorescence as described in the *Materials and*
869 *Methods* section. LAMP1 was used as a lysosomal marker, and PDI1 was used as an
870 ER marker.

871 Figure S4: Live cell imaging of yeast expressing N-terminally GFP-tagged Ybr074.
872 Yeast were grown in selective medium supplemented with raffinose, and where
873 indicated GFP-Ybr074 expression was induced in the same media supplemented with

874 galactose. The left panel shows a DIC image where the vacuole is visible, while the
875 right panel shows GFP fluorescence.

876 Table S1: Genes whose GFP-tagged protein products were found to accumulate in a
877 *ybr074Δ* strain. The fluorescence signal derived from each query protein was measured
878 in wild type and *ybr074Δ* cells. The ratio of *ybr074Δ* to wild type GFP signal is tabulated,
879 as well as subcellular localization and cellular function as curated by the
880 *Saccharomyces* Genome Database (SGD).

881 Table S2: Genes whose GFP-tagged protein products exhibit diminished levels in a
882 *ybr074Δ* strain. The fluorescence signal derived from each query protein was measured
883 in wild type and *ybr074Δ* cells. The ratio of *ybr074Δ* to wild type GFP signal is tabulated,
884 as well as subcellular localization and cellular function as curated by *Saccharomyces*
885 Genome Database (SGD).

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