Supplemental Materials

Molecular Biology of the Cell

Leitman et al.

Herp coordinates compartmentalization and recruitment of HRD1 and misfolded proteins for ERAD

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Supplemental Information

Supplemental Bioinformatics:

The evolutionary conservation profile (ConSurf (Ashkenazy *et al.*, 2010)) and algorithms for TM segment identification suggested two putative TM regions; one approximately between residues 263-309 and one at 361-391 (Fig. S6A). Secondary structure prediction suggested that the long first putative TM domain would form two TM helices joined by a short loop (residues Y283 to S288). Monte Carlo simulations of the interaction of this segment (residues 261-313), with an ER-like membrane showed preservation of the initial helical-hairpin fold (Fig. S6B). This is in agreement with one of two alternative models proposed previously (Kokame *et al.*, 2000), except that the hairpin traverses entirely the membrane. Kokame and co-workers had shown that both the N- and C-termini of Herp face the cytosol. They proposed that attachment to the membrane is either through a long hydrophobic segment, which does not traverse the membrane (residues ~ 263-309) or through this region traversing fully the membrane, followed by a luminal loop and a second hydrophobic stop-transfer segment (residues ~361-391) (Kokame *et al.*, 2000). Our simulations discard the latter model because the C-terminal

segment 361-391 does not form a stable structure in the membrane, yielding a positive (unfavourable) free energy value upon membrane-association (ΔG). In contrast, ΔG was -2.74±1.9 kT for association of the 263-309 hairpin. This relatively small negative free energy, due to the short and amphipathic TM helices, suggests a not very stable interaction of Herp with the membrane by itself. For comparison, the membrane-association ΔG of the 23-residue second TM of acetylcholine receptor δ (M2δ) is -10.2±5.6 kT (Kessel *et al.*, 2003). The predicted weak membrane interaction of Herp, when analyzed alone, could provide a method for shuttling of the protein from other subcellular locations (Tuvia *et al.*, 2007) to the ERQC, where it would be stabilized by interaction with HRD1 and by the assembly of a functional ERAD complex. The short loop that connects the TM helices obtained in the simulations (residues 284-286) (Fig. S6B and Fig. S6C), agrees with the predictions of PsiPred and MEMSAT (Fig. S6A). R289 is in the second TM helix N-terminal turn, enabling the charged side chain to "snorkel" into the hydrophilic environment of the lipid polar heads (Killian and von Heijne, 2000).

The FFAS03 fold recognition server (Jaroszewski *et al.*, 2005) predicted that the 3D structure of Herp shows high similarity only to that of hHR23A (PDB ID 1OQY), with a significant score of -38.3. According to the FFAS03 benchmark, scores lower than -9.5 exhibit less than 3% of false positives (Jaroszewski *et al.*, 2005). This similarity is along the entire length of the protein and not just in the ubiquitin-like (UBL) domain, in spite of the fact that the proteins are only remotely homologous. Several other proteins showed

structural similarity only in the UBL domain. Interestingly, hHR23A is a soluble protein, not membrane-bound.

Supplemental figure legends

Fig. S1. Microtubule-dependent ERAD substrate accumulation in the ERQC. Similar to Fig.1A-C, except that cells were treated with Lac plus Noc (20μM). Bar=10μm.

Fig. S2. Herp knockdown and its effect on ERAD substrate accumulation in the ERQC. A, B) Similar to Fig. 2A, B, except that cells were treated with Lac and endogenous Herp was detected with rabbit anti-Herp and Cy2-conjugated goat anti-rabbit IgG (image exposures were identical, to be able to compare Herp levels in control and Herp shRNA expressing cells). **C)** The cell lines used in Fig. 3A, B were treated with Dox in the presence or absence of Tun for 24 hr and subjected to immunoblot analysis as indicated, showing an efficiency of the knockdown of about 50%.

Fig. S3. Endogenous Herp localizes to the ERQC. A-B) Cells expressing H2a-RFP were treated with Tun (A) or Lac (B) for 3h and stained as in Fig. 4A. Bars=10μm.

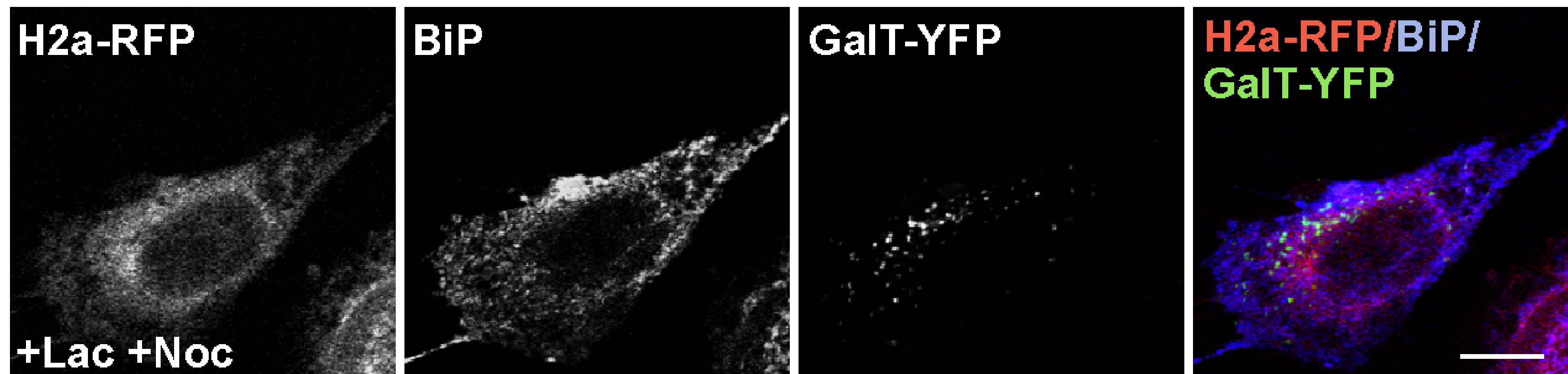
Fig. S4. OS-9 is constitutively localized at the ERQC and its localization is not affected by Herp knockdown. A, B) Similar to the experiment in Fig. 5A-E, but with cells left untreated. Bar=10μm. **C)** Similar to the experiment in Fig. 4D, but with cells expressing HRD1-Myc and S-tagged OS-9.1/2, incubated without (left panel) or with

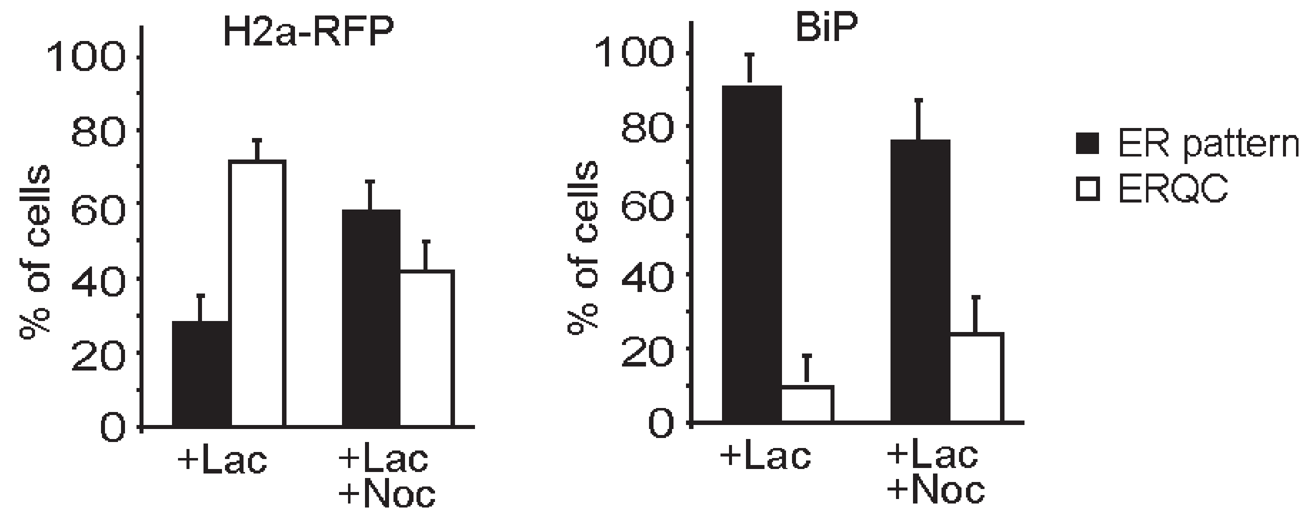
MG-132 (40 μM) for 3h. A representative experiment out of 3 repeat experiments is shown.

Fig. S5. H2aΔGly also accumulates in the ERQC upon proteasomal inhibition. Cells expressing H2aRFP or H2aΔGly were treated without or with Lac for 3h and stained as in Fig. 7A. Bar=10μm.

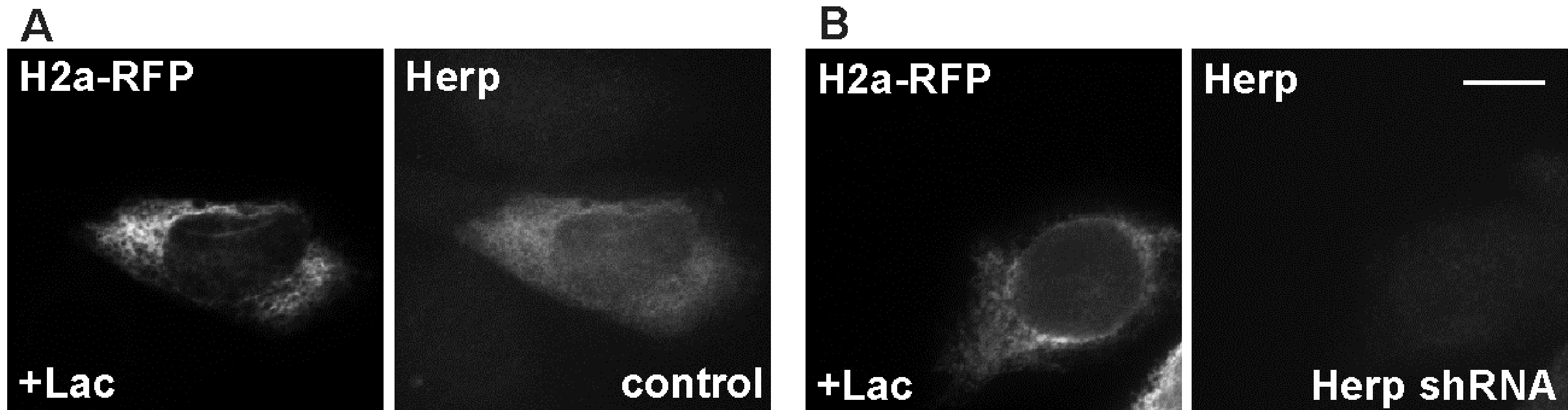
Fig. S6. Sequence analysis and Monte Carlo simulations point to a transmembrane helical hairpin for association of Herp with the membrane. A) Predicted TM helices coincide with evolutionarily conserved segments of Herp. The residues in the C-terminal half of Herp are coloured according to their ConSurf conservation grades using the color-coding bar (Ashkenazy *et al.*, 2010). The helix locations predicted by PsiPred, MEMSAT, HMMTOP, SPLIT4 and Octopus are marked with different colours according to the legend. TM helices were identified only within the 251-391 fragment, shown here. **B)** The average orientation of a peptide, corresponding to Herp residues 261-313, upon interaction with an ER-like model membrane containing 22% charged lipids. Calculated free energy of membrane-association is shown. The horizontal dashed lines are approximate borders of the membrane hydrophobic core. Charged residues (R, K, D, E) are coloured blue; polar residues (M, C, T, S, P, W, Y, H, Q, N) green and hydrophobic residues (G, A, V, L, I, F) orange. The short loop outside the membrane core corresponds to Y284, S285, and S286. **C)** The calculated average helical content of the peptide in (B) upon membrane interaction. Two (TM) helices, between residues 261-283 and 287-313, are connected by a short non-helical stretch, consisting of Y284, S285, and S286.

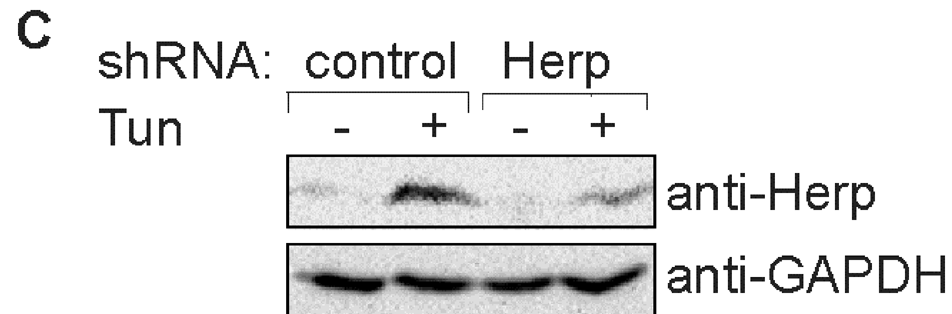
Leitman et al - Fig.S1



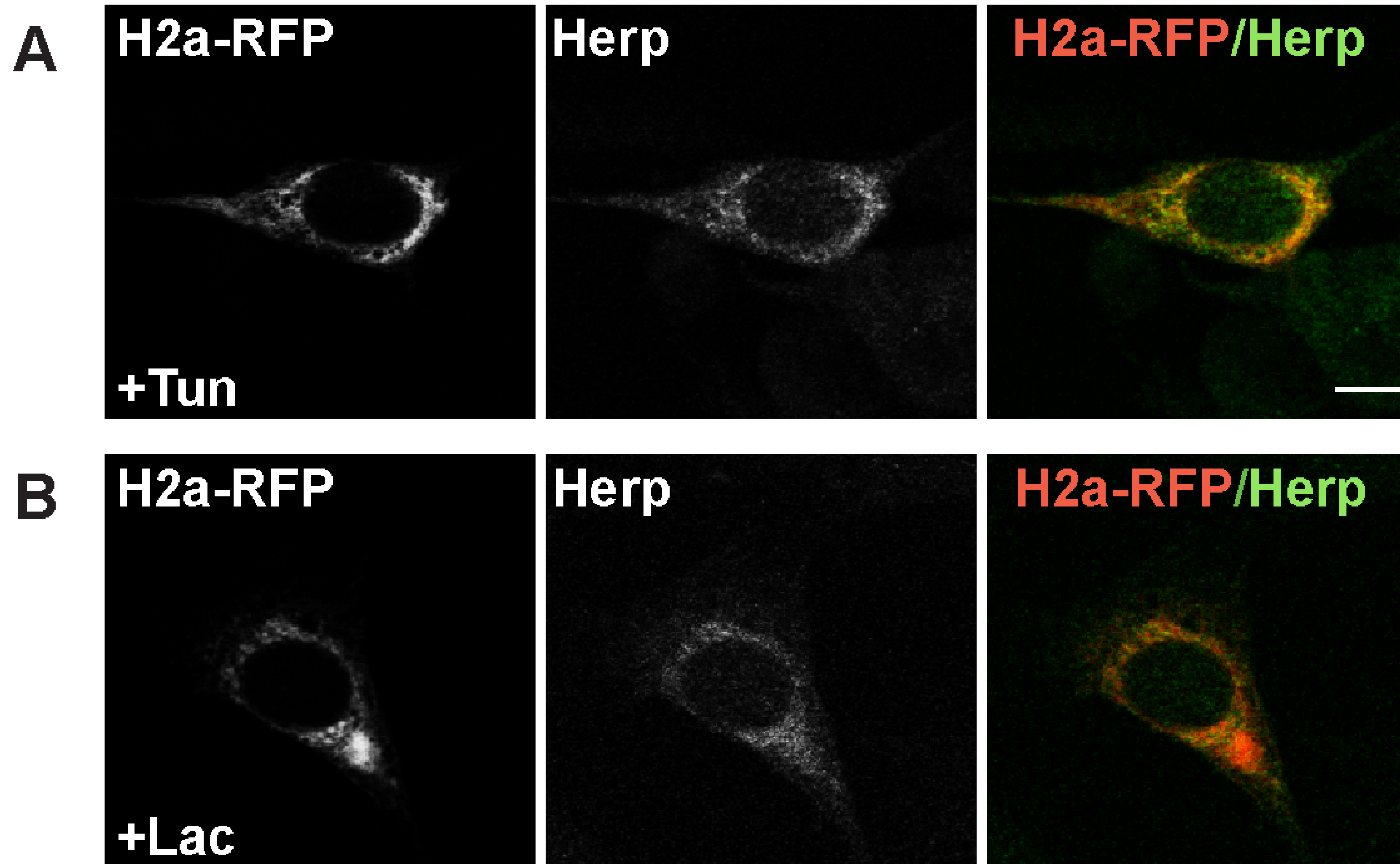


Leitman et al - Fig.S2





Leitman et al - Fig.S3



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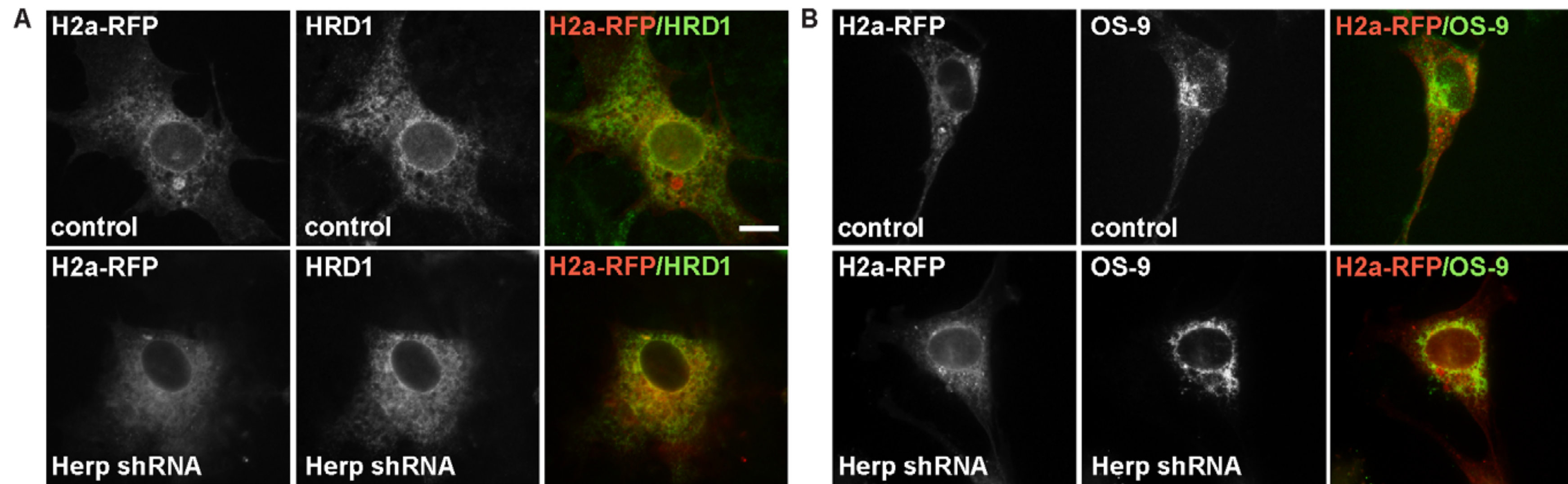
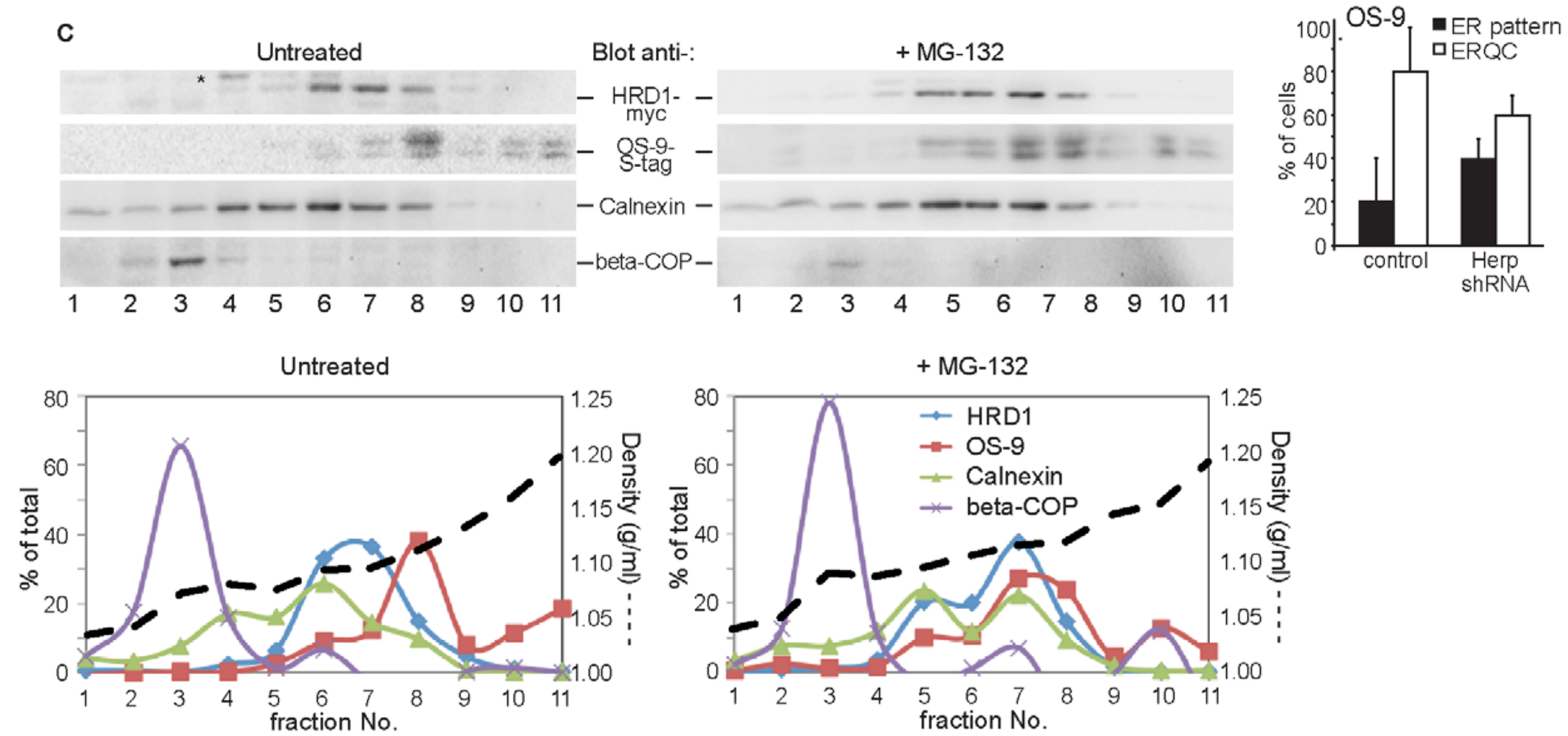
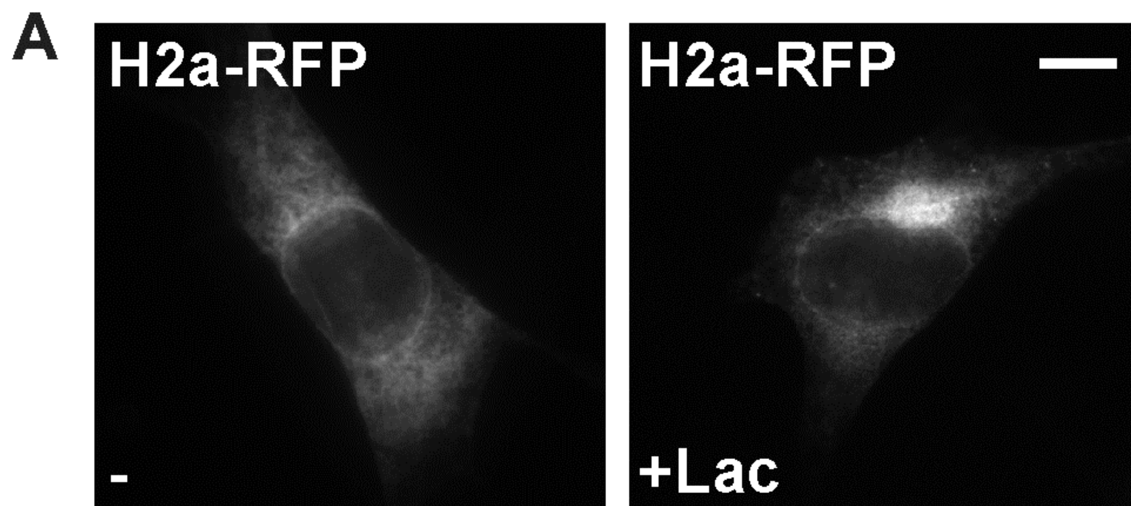
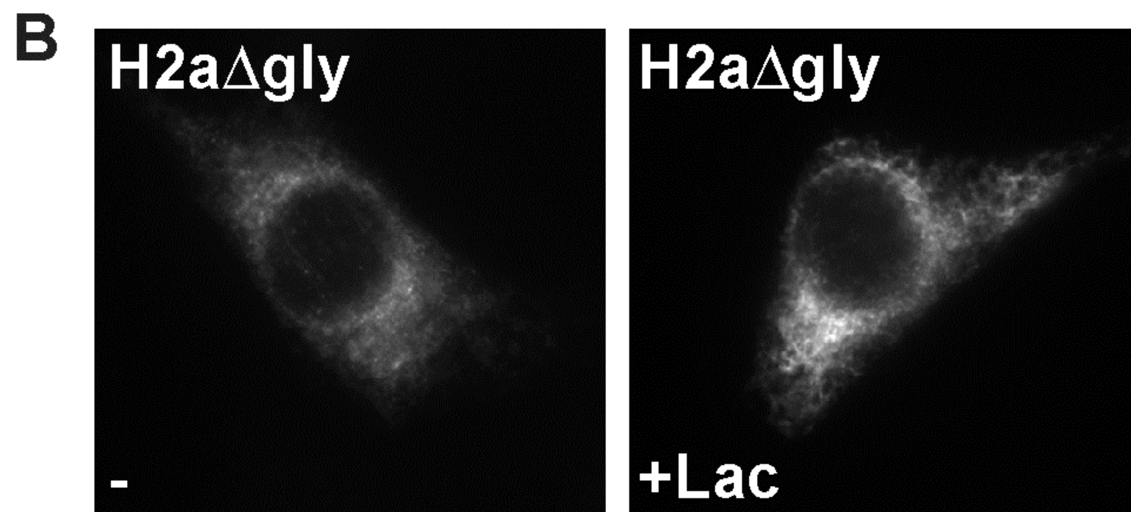




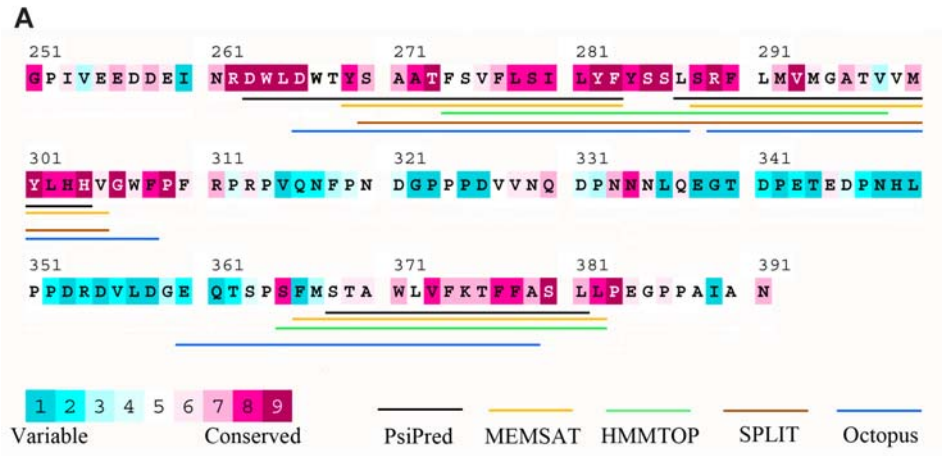
Leitman et al - Fig. S5



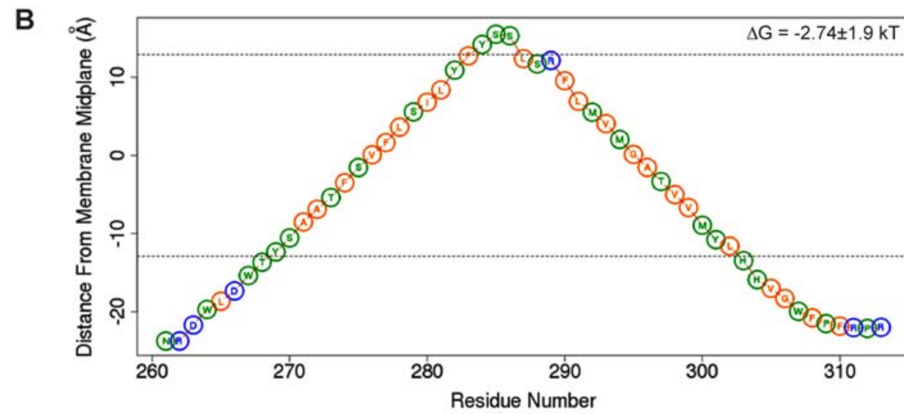


Leitman et al - Fig. S6

A



B



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