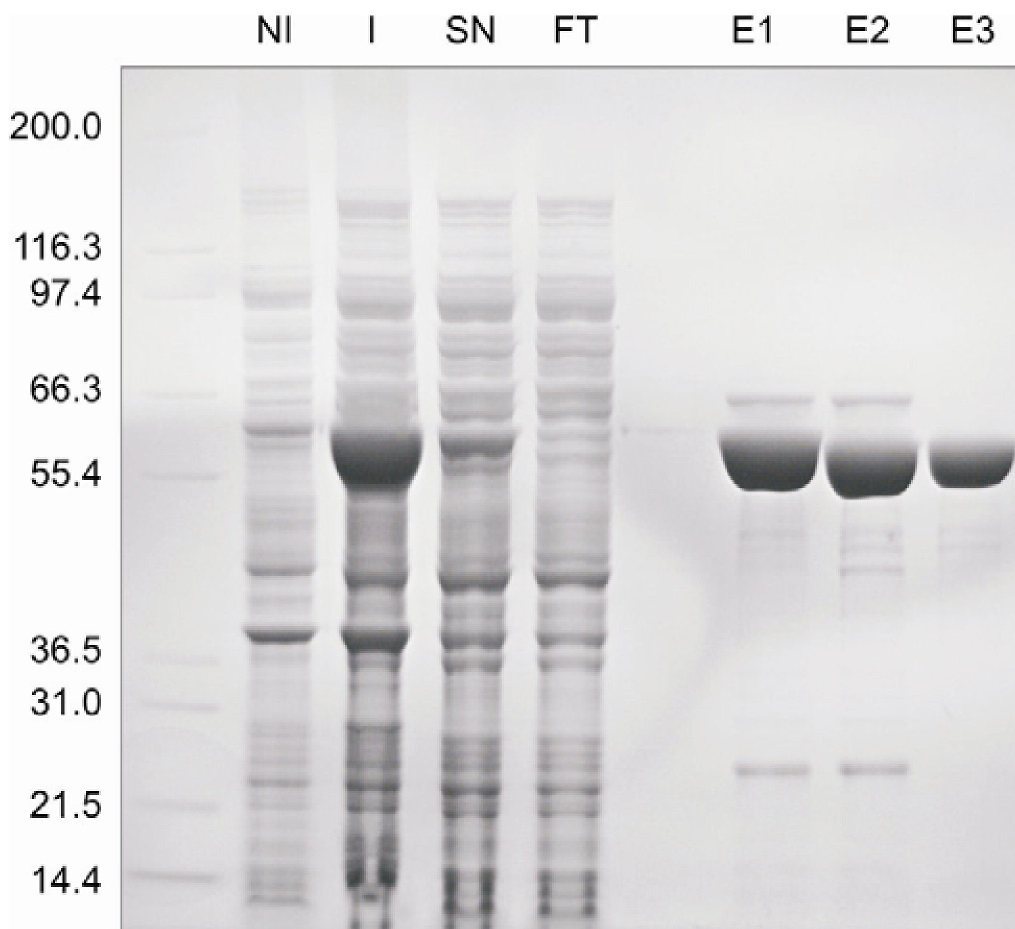
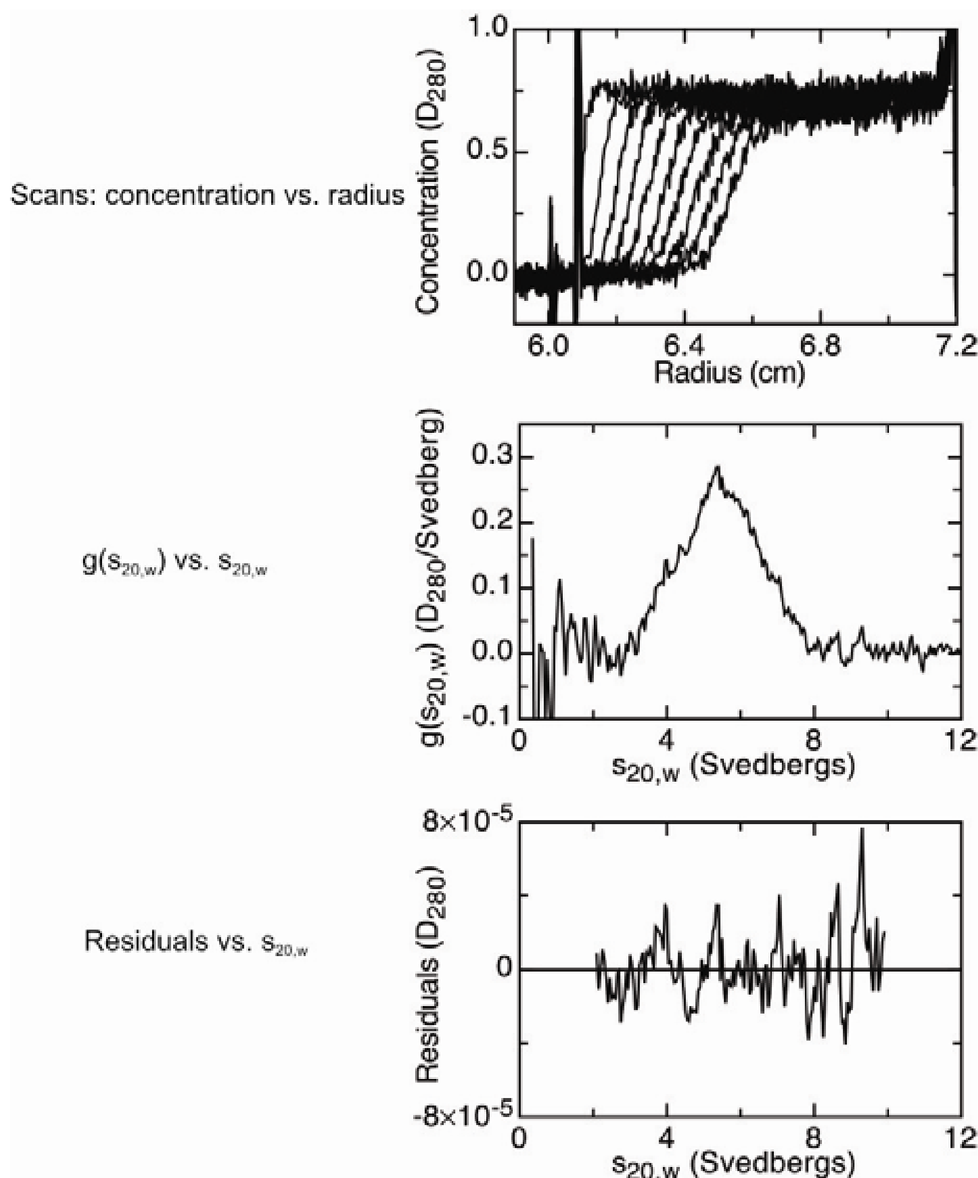
Contents of Supplementary Figures

Supplementary Figure 1: Purification of mouse EHD2.
Supplementary Figure 2: Analytical ultracentrifugation analysis.
Supplementary Figure 3: EM analysis of liposome deformation.
Supplementary Figure 4: Liposome-dependence of the stimulated ATPase reaction.
Supplementary Figure 5: Alignment with secondary structure assignment.
Supplementary Figure 6: Conservation plot of the dimerisation interface.
Supplementary Figure 7: Interactions of the EH-domain.
Supplementary Figure 8: Nucleotide specificity in comparison to Ras.
Supplementary Figure 9: Curvature-dependence of liposome binding.
Supplementary Figure 10: The phosphate cap.
Supplementary Figure 11: Characterisation of mutations in the oligomerisation interface.
Supplementary Figure 12: Model of the oligomeric ring.
Supplementary Table 1: Data collection statistics.
Supplementary Notes: References of Supplementary Material.

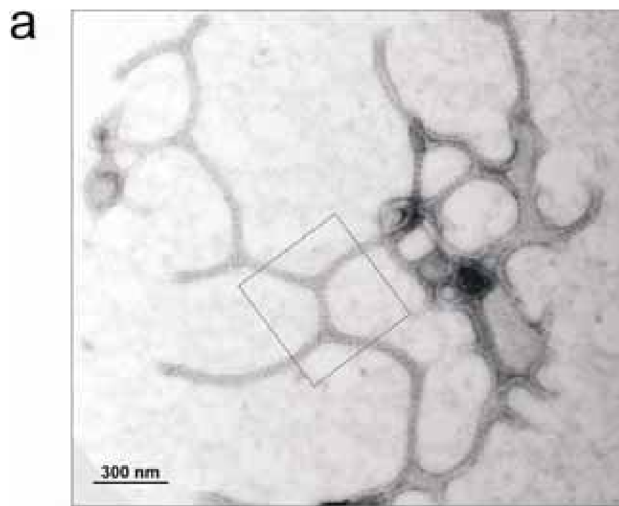


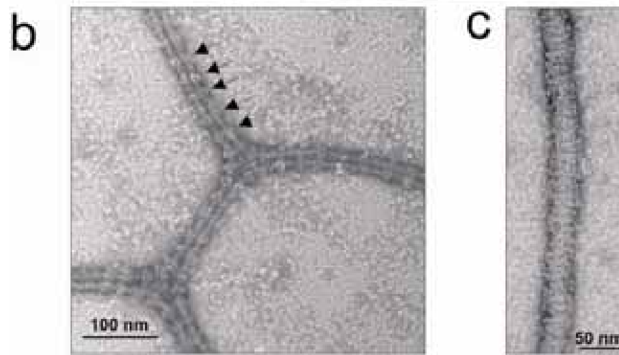
Supplementary Fig. 1: Expression and purification of mouse EHD2. Mouse EHD2 was expressed in *Escherichia coli* as a His-fusion protein as described in Materials and Methods. NI - Non-induced culture. I - Induced culture. SN - Soluble extract. FT – Soluble extract after application to NiNTA Sepharose. E1- EHD2 after elution from NiNTA-Sepharose. E2 – EHD2 after dialysis and thrombin cleavage. E3 – EHD2 after re-application and elution from the NiNTA column. This protein was further purified by size-exclusion chromatography using a Sephadex S200 column (data not shown).



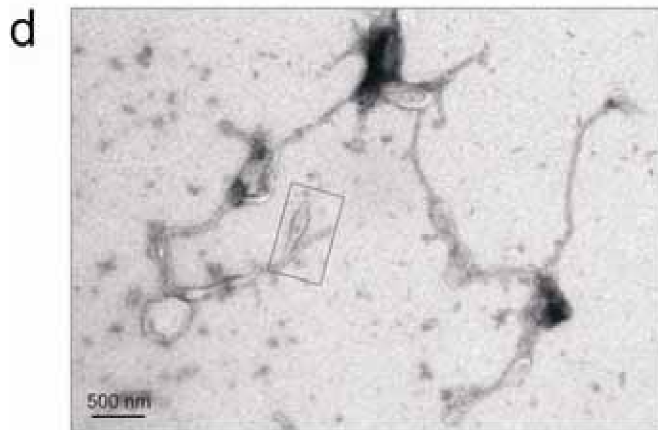
Supplementary Figure 2: Ultracentrifugation analysis indicates that EHD2 is a dimeric protein. Sedimentation velocity experiments were performed as described in methods at 300 mM NaCl. Selected scans (at equal, ~15 min intervals), and of $g(s_{20,w})$ (the amount of material sedimenting between $s_{20,w}$ and ($s_{20,w}+\delta s$)) and also the residuals for fitting the data with DCDT+, were plotted with the program profit v.5.6.7 (Quantum soft, Switzerland). The fitted value is 113 $\pm$ 4 kDa which corresponds well with the calculated mass of the dimer of 124 kDa.

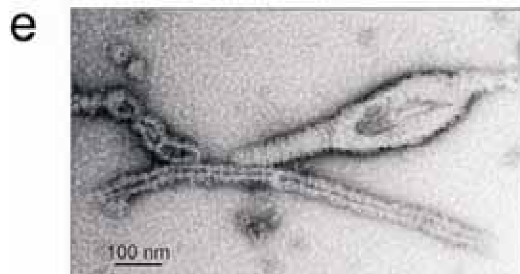
We also observed an EHD2 dimer by size-exclusion chromatography and by dynamic light scattering (data not shown). At 50 μM protein concentration, the hydrodynamic radius did not change in the presence or absence of nucleotides (or in 150 mM versus 300 mM NaCl), as judged by dynamic light scattering experiments (data not shown).





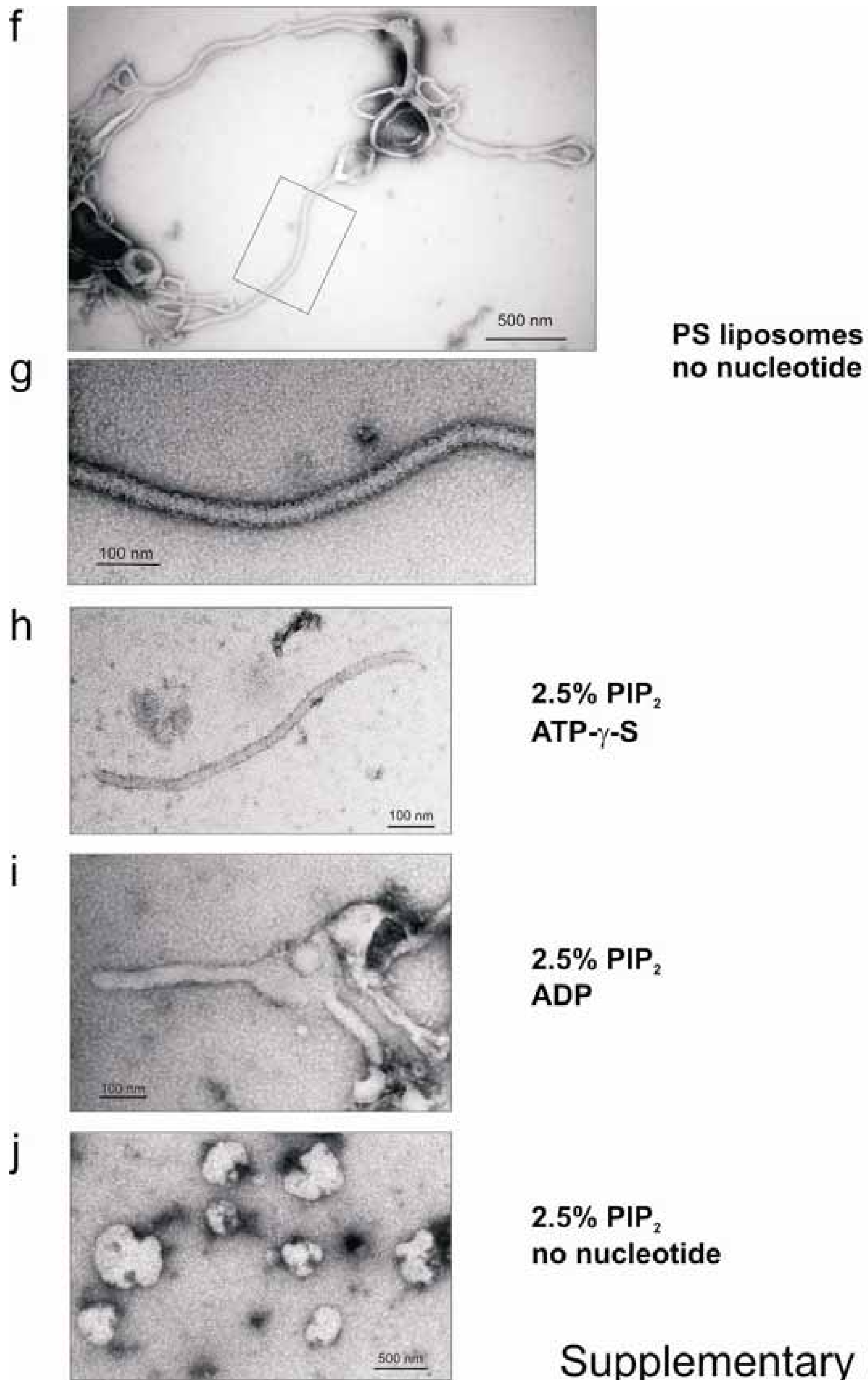
PS liposomes
ATP-γ-S





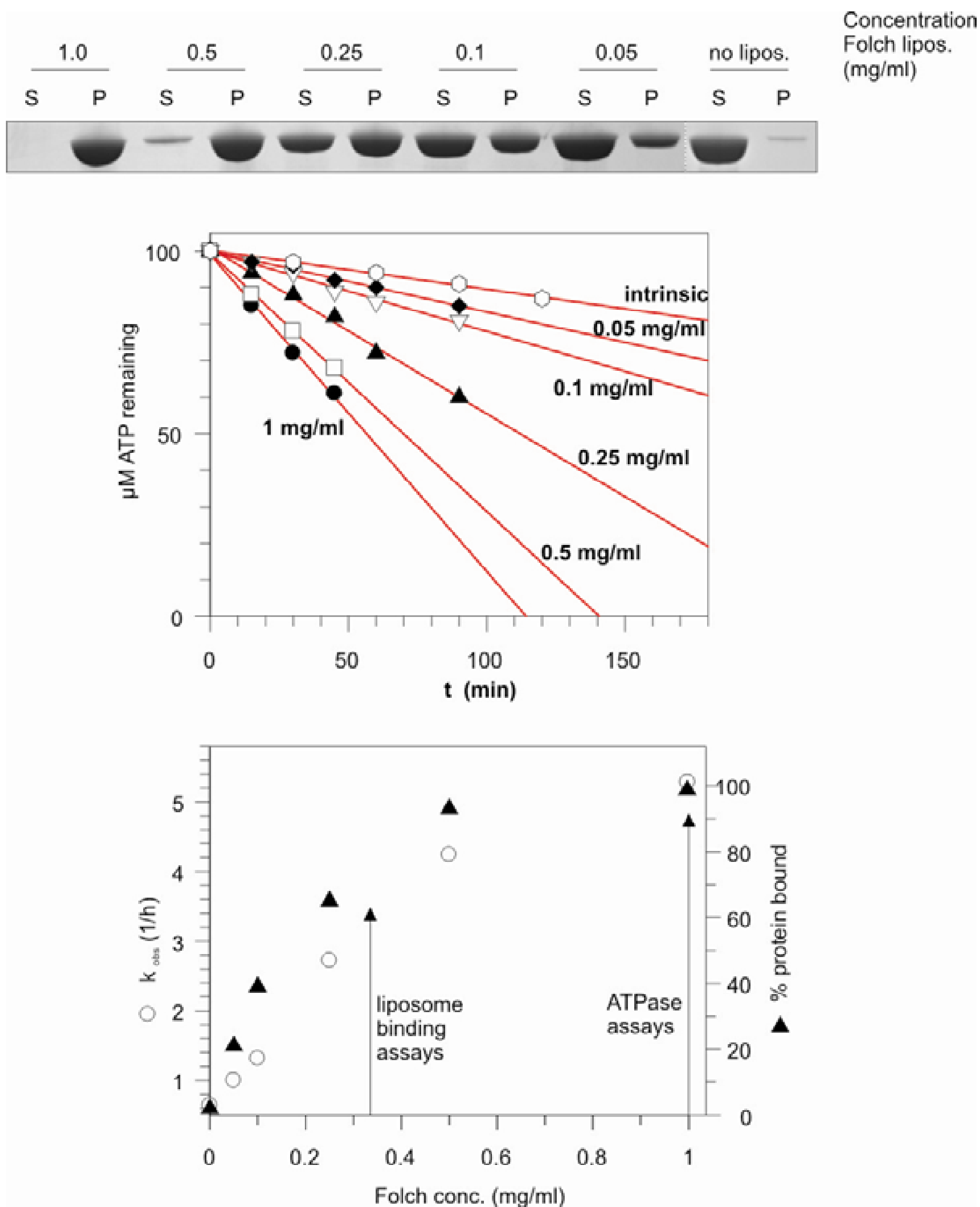
PS liposomes
ADP

Supplementary Fig. 3



Supplementary Fig. 3

Supplementary Figure 3: EHD2 tubulation of liposomes. EHD2 was incubated with the indicated liposomes in the presence and absence of nucleotides and analysed by EM as described in Methods. **a**, EHD2 deformed PS liposomes into tubular networks, here in the presence of ATP-γ-S. **b**, Enlarged views of the indicated area in a. Note the presence of regularly spaced EHD2 rings (some are indicated with arrows). This even spacing may be due to the curvature stress generated by an EHD2 ring along the axis of the lipid tubule which might disfavour binding of the next ring in the direct vicinity (see below). **c**, Some of the lipid tubules (especially at higher protein concentration) were tightly packed with EHD2 oligomeric rings. **d,e** In the presence of ADP and in the absence of nucleotide (**f,g**), EHD2 also tubulated PS liposomes and formed ring-like structures around the tubules. **e,g** are enlarged views of the indicated areas in d and f, respectively. We did not observe a noticeable change of size of the tubules with the different nucleotide conditions. **h,i,j**, Under less favourable lipid binding conditions (synthetic liposomes with 87.5% phosphatidyl-choline, 10% cholesterol and only 2.5% PIP_2), EHD2 tubulated liposomes in the presence of ATP-γ-S (h) and ADP (i), but we did not observe tubulation in the absence of nucleotides (j).



Supplementary Figure 4: a, Membrane binding of 10 μM EHD2 to 0.8 μm filtered Folch liposomes of the indicated concentration in the presence of 1 mM $MgCl_2$ and 1 mM ATP-γ-S. **b,** ATP hydrolysis, as measured by HPLC analysis, under the same conditions as in a. **c,** Observed rate and % liposome-bound EHD protein (measured by densitometry). The rate increased with increasing EHD2 bound to liposomes. In all further ATPase experiments 1 mg/ml Folch was used to determine the maximal rate. For liposome binding experiments we used 0.33 mg/ml Folch (unless otherwise indicated) to be in the range where binding differences could be better observed.

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mmEHD2   : ---------------------------------------------------------------------------------------------------- :   -
hsEHD1   : ---------------------------------------------------------------------------------------------------- :   -
hsEHD3   : ---------------------------------------------------------------------------------------------------- :   -
hsEHD4   : ---------------------------------------------------------------------------------------------------- :   -
drEHD    : ---------------------------------------------------------------------------------------------------- :   -
xlEHD1   : ---------------------------------------------------------------------------------------------------- :   -
xlEHD4   : ---------------------------------------------------------------------------------------------------- :   -
dmPAST1  : ---------------------------------------------------------------------------------------------------- :   -
ceRME1   : ---------------------------------------------------------------------------------------------------- :   -
sjEHD    : ---------------------------------------------------------------------------------------------------- :   -
ddEHD    : ---------------------------------------------------------------------------------------------------- :   -
pfEHD    : ---------------------------------------------------------------------------------------------------- :   -
ehEHD    : ---------------------------------------------------------------------------------------------------- :   -
tcEHD    : -------------------------------------------------------------MVFGDVVLSLFIIIFFHFLSLHFLQVLFVCCIPRAVPLG :  39
lmEHD    : ---------------------------------------------------------------------------------------------------- :   -
atEHD    : METSSTISIGSCLKEHQKIYKEWFNIADSDGDGRVSGNDATKFFAMSKLSRQELKQVWAVADSKRQGFLGLSEFITAMKLVSLAQEGHEITSDLLKGSID : 100
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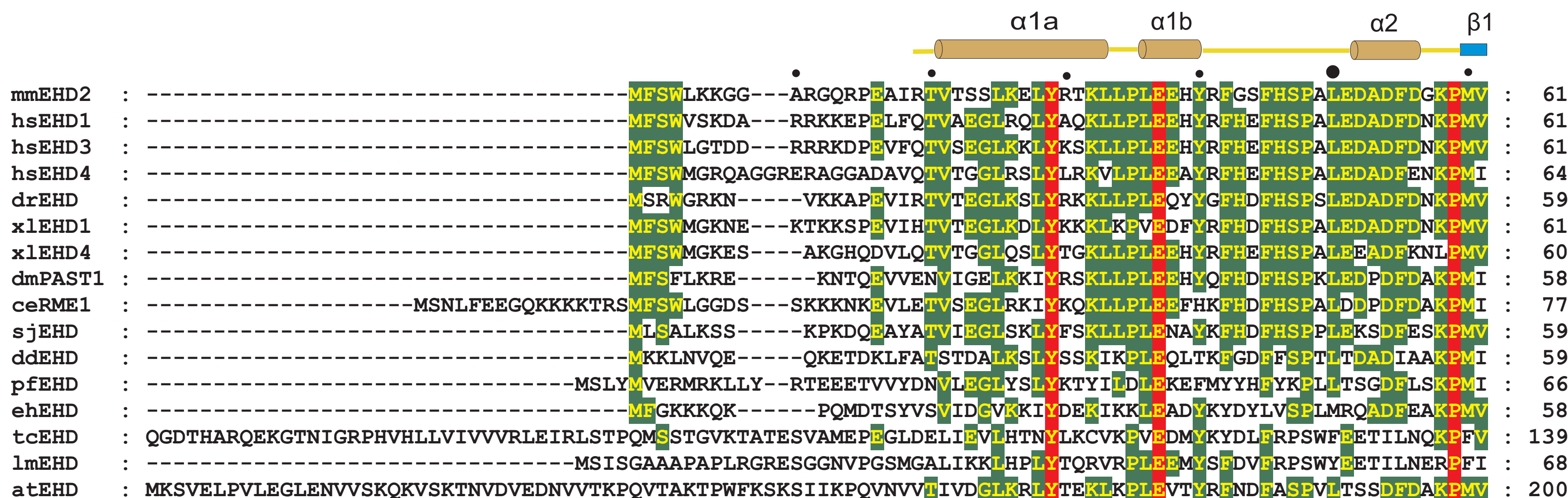

Supplementary Fig.5