3 experiments. Molecular weights (kDa) are as indicated to the left. **D**, Representative confocal micrographs to show co-localisation of GFP-CHC22-FL or GFP-CHC17-FL (green) with α-adaptin detected with AP.6/A546 (red). Cells were depleted of endogenous CHC17 by RNAi, DAPI/DNA is blue in the merged image. Scale bar = 10 µm.

Fig 4 Association between CHC22 and clathrin light chains

A-B, IPs were performed using either anti-myc (M) or anti-clathrin light chain (C) from lysates of HEK293 cells transfected as indicated. Samples of the lysates (**A**) or the IP (**B**) were analysed by blotting for GFP. Long (above) and short (below) exposures are shown to visualise the signal for GFP-CHC22-FL, these blots are typical of three experiments. **C**, Representative confocal micrographs of cells expressing GFP-CHC17-FL or GFP-CHC22-FL (green) in the presence (no RNAi) or near absence (CHC17 RNAi) of endogenous CHC17. Cells were stained for endogenous clathrin light chains using CON.1/A546 (CLC, red). **D**, Representative confocal micrographs of cells expressing GFP-CHC22-FL (left, green in merge) and mCherry-LCa (middle, red in merge) in the presence (no RNAi) or near absence (CHC17 RNAi) of endogenous CHC17. Scale bar = 10 µm.

Supplementary Fig S1 Predicted structure of ∆Exon9 clathrins

A, Primary sequence comparison to illustrate the missing residues in CHC22∆Exon9. **B**, Molecular model of CHC17 (Fotin et al., 2004). N-terminal domain (blue), linker (grey), CHCR0 (pink), CHCR1 (green), CHCR2 (brown) are shown. Exon 9 codes for residues 457-507 in CHCs, corresponding to helices *e-h* (dark pink) in CHCR0. **C**, A model of CHCs lacking exon 9. Deletion of residues 457-507 and realignment of CHCR0 helices *c* and *d* from ∆Exon9 with helices *g* and *h* from FL resulted in a ~22Å translation and ~21° rotation of the terminal domain relative to the axis between 'foot' and 'ankle'.

Supplementary Fig S2 Full-length CHC22 transcripts contain exon 9 whereas the ∆Exon9 form is only present as a truncated transcript.

A, Schematic diagram of the full-length CHC22 transcript (FL) and the putative CHC∆Exon9 transcript (∆Exon9); predicted PCR products are shown below. **B**, PCRs using skeletal muscle cDNA and primers to amplify exon 7-32 (1), exon 3 to 3'UTR (2), exon 3-30 (3) and exon 3-28 (4). The products were as expected for CHC22-FL. **C**, Reaction products 1, 3 and 4 in B were gel extracted and tested by restriction digest. EcoRI digests gave two bands of 2.1 kb and 1.8 kb (1); 2.5 and 1.9 kb (3); 2.5 and 1.6 kb (4) as predicted for CHC22-FL. If exon 9 were spliced in the full-length product we would expect the 1.8 kb band (1) and the 2.5 kb bands (3 & 4) marked with asterisks to shift downwards by 153 bp. The ApaLI and EcoRI digests also confirm that our PCRs did not amplify any CHC17. The weak band at 1.9 kb (1) probably

represents the splicing of exon 29 that has been described previously. **D**, Internal PCR (5) using primers flanking exon 9 and the gel extracted bands (3 or 4 from A) as template. Only a 666 bp product was amplified (corresponding to exon 9-containing CHC22). **E**, Examples of typical PCRs directly from cDNA from various human sources using primers flanking CHC22 exon 9 (5) show presence of exon 9-containing (666 bp upper band) and exon 9-absent (513 bp lower band) forms. **F**, No splicing of exon 9 in CHC17 was found in short PCRs flanking exon 9 (5') or targeting exon 9 (6'). PCRs used either HeLa or HEK293 cDNA or CHC17-FL plasmid DNA.

Supplementary Table 1 Details of oligonucleotide primers used for cloning experiments.