

Figure S6

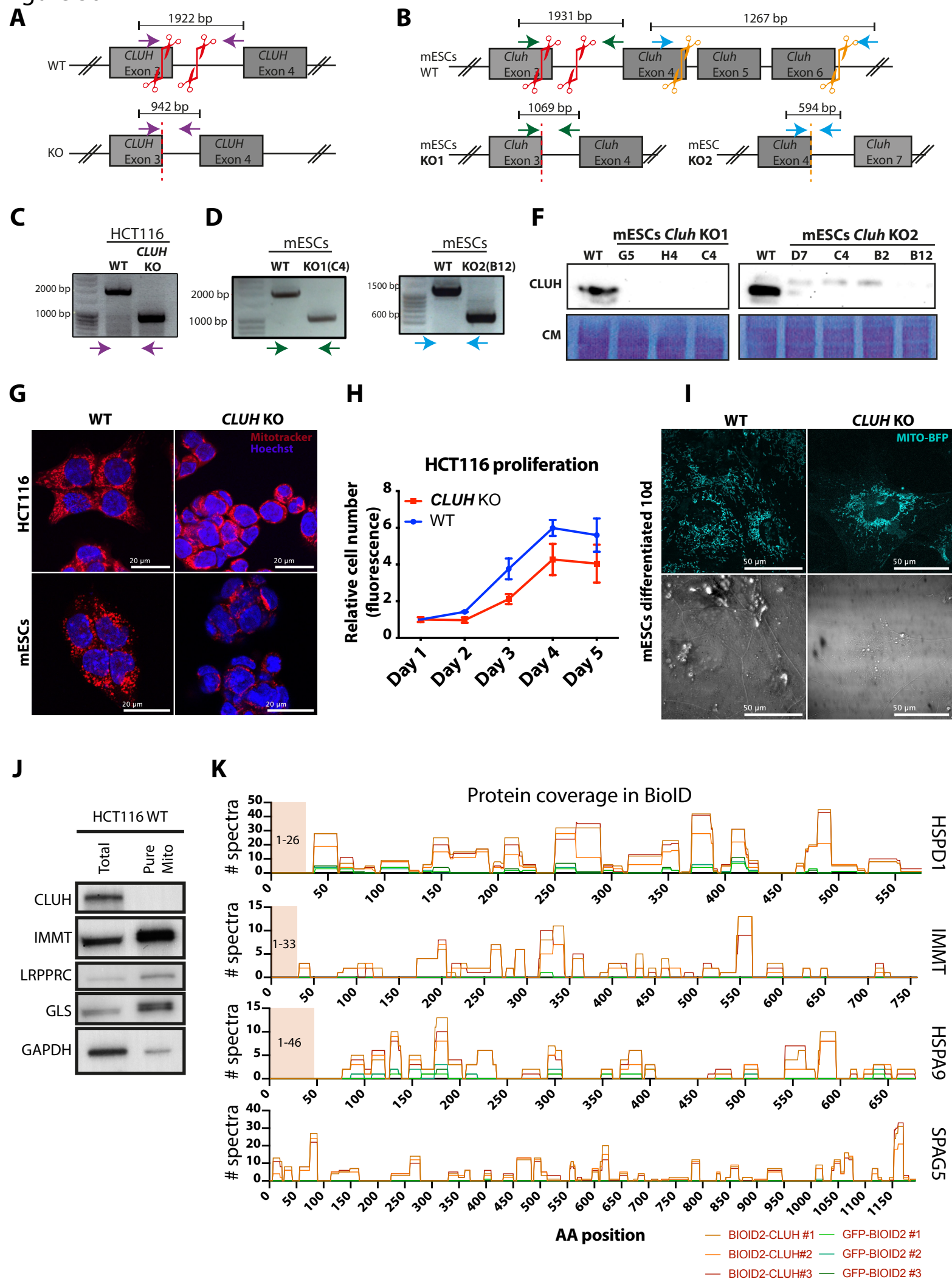


Figure S6: Generation of CRISPR/Cas9 knockout cells for *CLUH* and BioID protein coverage.

(A-B) Schematic representation of the CRISPR/Cas9 mediated knock-out of *CLUH* in HCT116 **(A)** and mESCs **(B)**. A paired sgRNA strategy is used to delete DNA fragments leading to gene inactivation. Red scissors indicate the cleavage site of each sgRNA and colored arrows show the location of PCR genotyping primers. PCR amplification products size are indicated for both wild-type cells and each mutant. **(C-D)** Agarose gel showing the genotyping PCR results of WT and mutant HCT116 **(C)** and mESCs **(D)**. The used PCR primers match the color code indicated in **(A, B)**. **(F)** Western blot showing the expression of *Cluh* in selected mESC *Cluh* KO clones. *CLUH* is detected using specific antibodies. Coomassie staining of the membrane (CM) is used as loading control. **(G)** Confocal microscopy images of mESC and HCT116 *CLUH* KO cells. Parental wildtype cells are also shown. The mESC *Cluh* KO1 (C4) clone is shown. Mitochondria (red) are labeled using MitoTracker™ Red CMXRos. Nuclei (blue) are stained with Hoechst. The scale bar is indicated in white. **(H)** Proliferation assay performed on HCT116 *CLUH* KO cells and WT cells over 5 days. The relative cell number is measured compared to day1, using a fluorescence assay (CellTiter-Fluor™, Promega). The error bars correspond to the standard deviation of three biological replicate experiments. **(I)** Confocal microscopy images of both WT and *Cluh* KO (C4) mESCs stably expressing mitochondrial BFP protein (fusion with Cox8a mitochondrial targeting sequence). Mitochondria are shown in light blue (upper panels) and transmitted light images are shown in gray. The scale bar is indicated in white. **(J)** Western blot on total and “pure” mitochondrial fractions from wild-type HCT116 cells. Indicated proteins are revealed using specific antibodies. **(K)** Plot showing the protein coverage from LC MS/MS identification of the most abundant proteins from *CLUH* BioID experiment on HCT116 cells (see Figure 4). The x-axis corresponds to the amino acid (aa) position for each protein and the y-axis show specific spectral counts for both BioID2-*CLUH* and GFP-BioID2 samples. All replicate samples are indicated with a color scale. The N-terminal region of the proteins, containing the mitochondrial targeting peptide, according to Uniprot annotations, is highlighted in orange shadow.