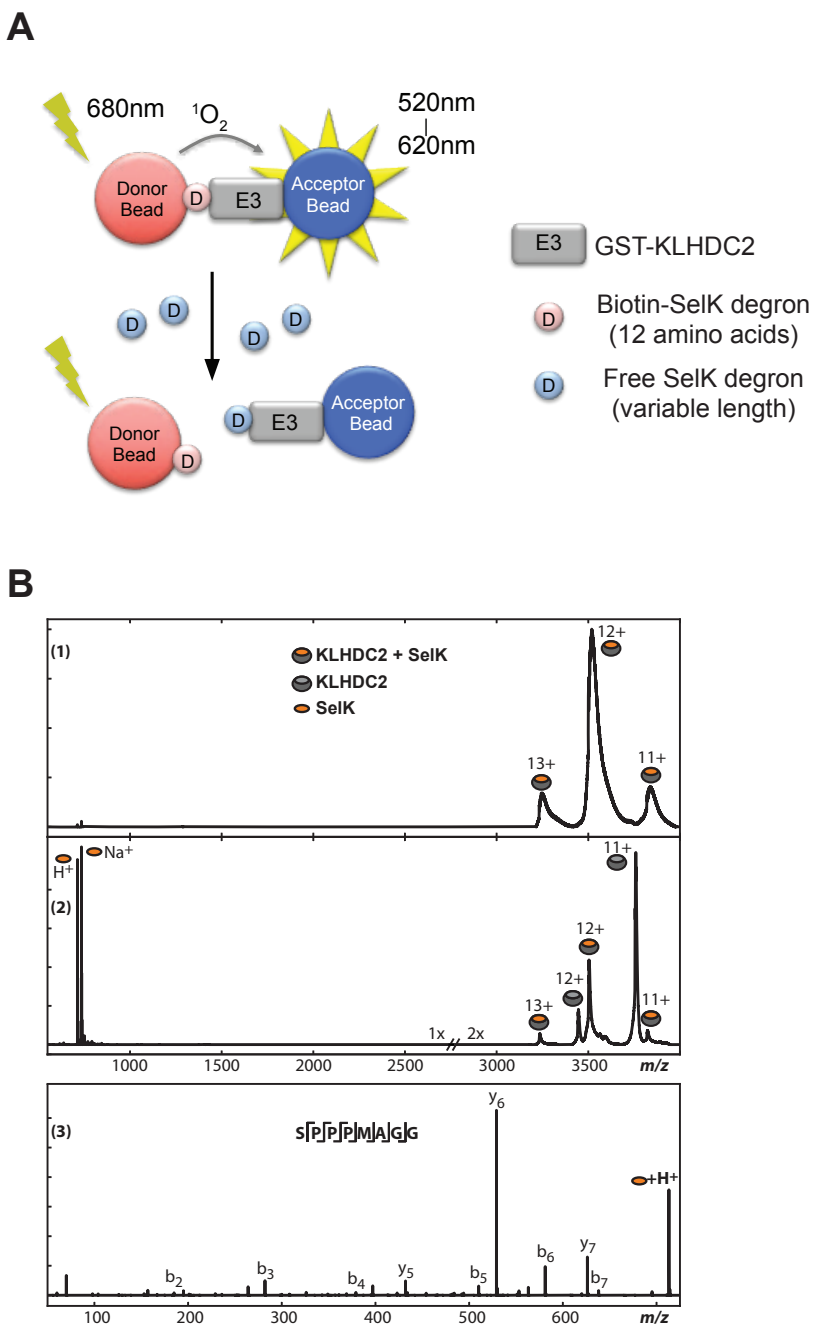

Figure S1, related to Figure 1. Quantitative and mass spectrometry analysis of SelK diglycine C-end degron binding to KLHDC2. (A) The AlphaScreen-based competition assay designed for assessing the affinity of SelK C-end degron peptides with KLHDC2. (B) (1) Native mass spectrum of the KLHDC2-SelK complex, which exhibits no evidence for apo KLHDC2. (2) Mass spectrum obtained using conditions similar to that in (1), but with in-source activation. Under these conditions, $[SelK+H]^+$ or $[SelK+Na]^+$ are released from some of the KLHDC2-SelK complexes. (3) $[SelK+H]^+$ released from the complex was quadrupole selected and subjected to collision-induced dissociation. This resulted in an information-rich fragmentation spectrum, confirming the assignment of the released peptide cation. Additional details of these experiments are discussed in the Methods section.

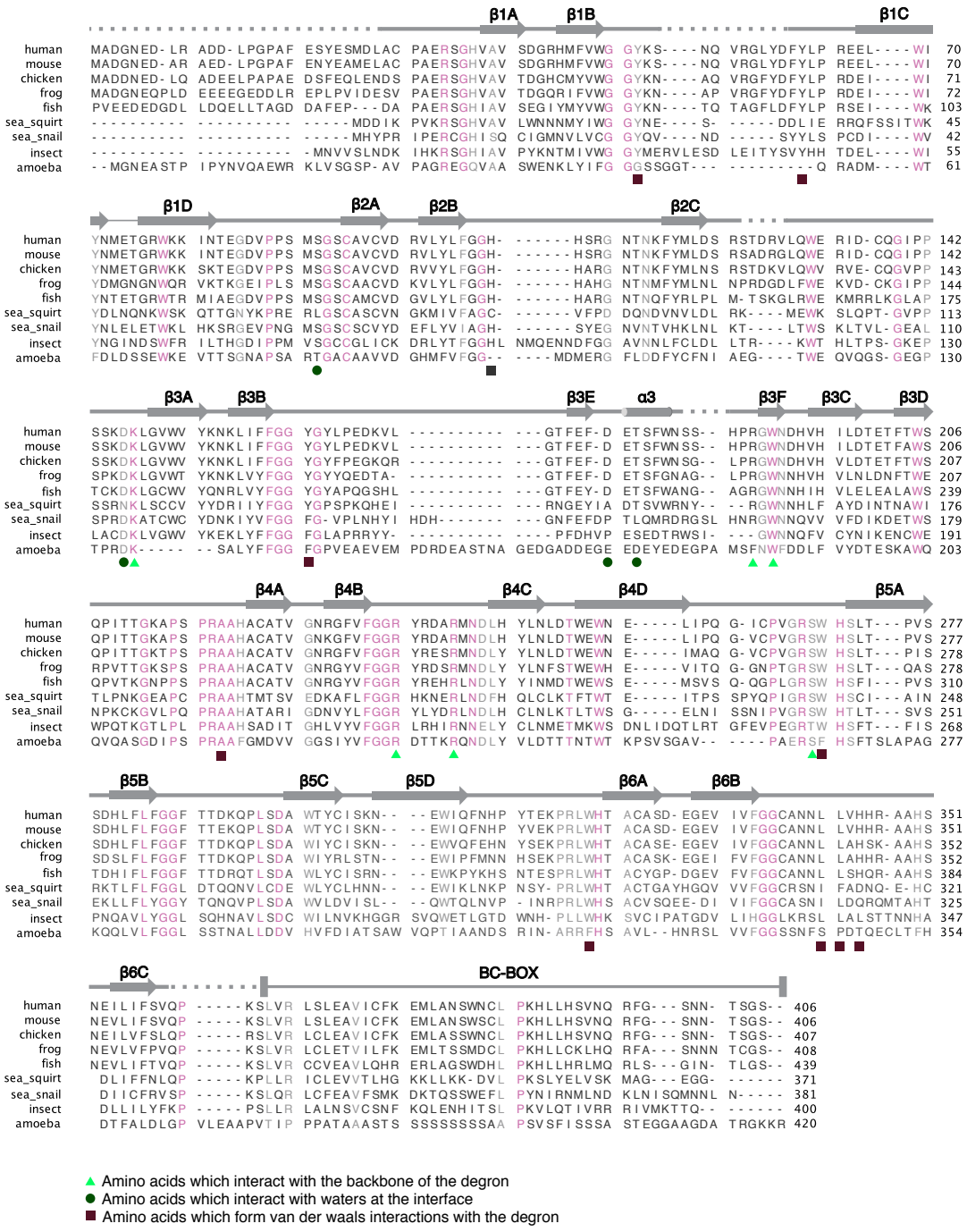

Figure S2, related to Figure 2 and Figure 3. Sequence alignment of KLHDC2 orthologs from different species, including human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), frog (*Xenopus lavis*), fish (*Scleropages formosus*), sea squirt (*Ciona Intestinalis*), sea snail (*Lottia gigantea*), insect (*Clastoptera arizonana*), and amoeba (*Acanthamoeba castellanii str. Neff*). The N-terminal sequence of fish KLHDC2 and the C-terminal sequence of sea squirt and amoeba KLHDC2 orthologs are omitted for clarity. Strictly conserved residues (100%) are colored in magenta. Highly conserved residues (80-100%) are colored in light grey. Second structure elements including α-helices and β-strands are indicated by cylinders and arrows, respectively.

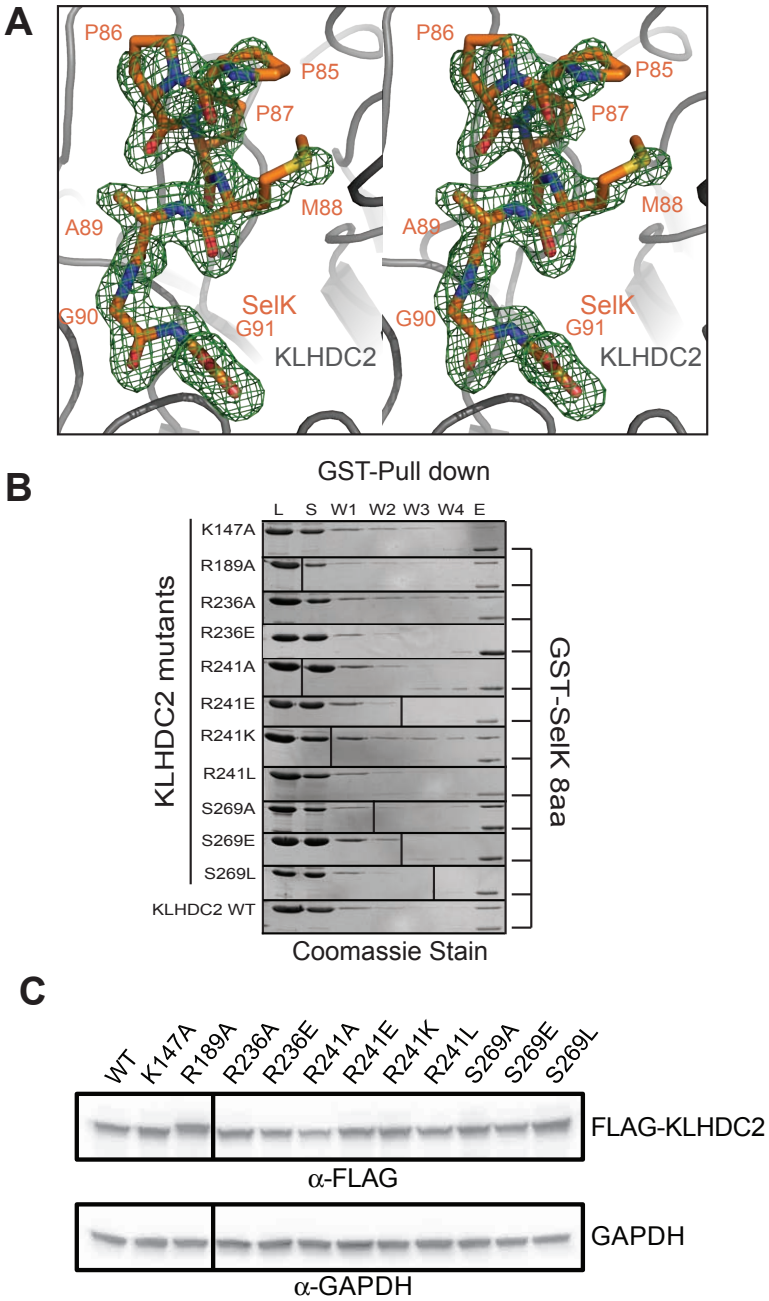

Figure S3, related to Figure 2 and Figure 3. Binding mode of the SelK diglycine C-end degron to KLHDC2 and schematic diagram of the global protein stability assay. (A) Stereo view of the KLHDC2 kelch repeat domain pocket with a SelK C-end degron bound. KLHDC2 (gray) is shown in cartoon. SelK C-end degron (orange) is shown in sticks together with its positive F_o-F_c electron density (forest green) calculated and contoured at 3σ before it was built into the complex model. (B) GST pull down assays assessing the binding of KLHDC2 mutants with 8 aa SelK degron fused to GST. Loading control (L), Supernatant (S, i.e. unbound), four washes (W1–W4), and elution (E) fractions were analyzed by SDS-PAGE with Coomassie stain. Vertical lines indicate discontinuity of the lanes in SDS-PAGE gels due to removal of molecular weight marker lanes. (C) Western blot analysis on the expression of exogenous wild type (WT) and KLHDC2 mutants from HEK293T cells with endogenous KLHDC2 knocked down in the GPS assay.

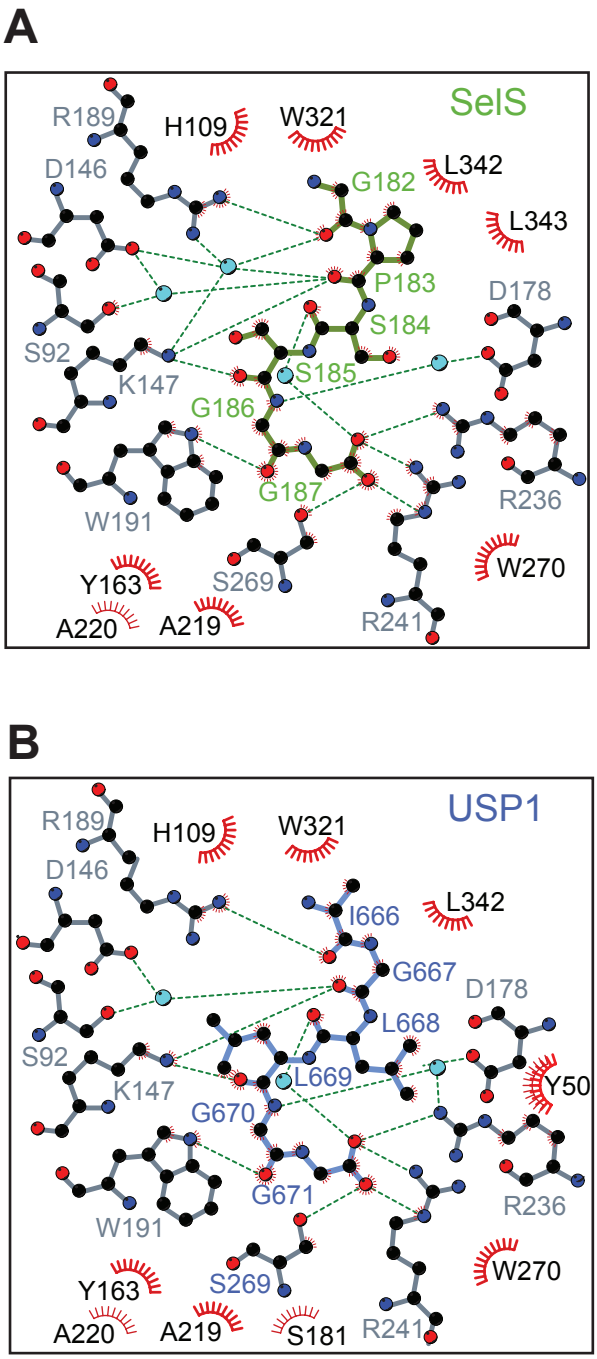

Figure S4, related to Figure 4. LIGPLOT diagrams showing interactions between KLHDC2 and SelS C-end degron and between KLHDC2 and USP1 C-end degron