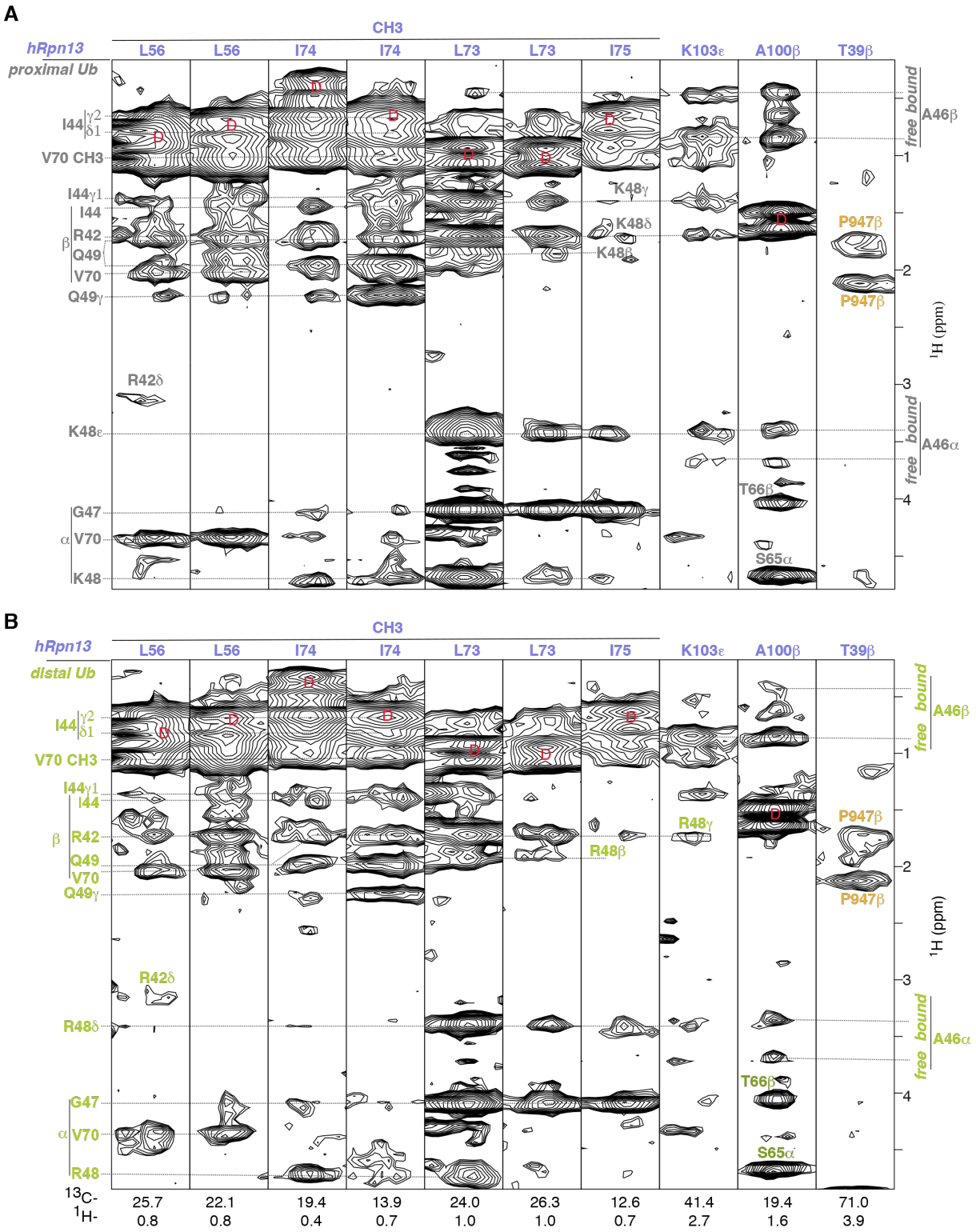

Figure S1. Intermolecular NOEs from hRpn13 to hRpn2 (orange) and ubiquitin of K48-diubiquitin. Related to Figures 1 and 3.

(A and B) Selected regions of ^{13}C-half-filtered NOESY experiments (100 ms mixing time) recorded on Ternary-^{13}C-D (A) or Ternary-^{13}C-P (B) samples, as indicated in Figure 1A. Diagonal breakthrough signals are labeled by ‘D’ (red) and assignments for hRpn13 Pru (purple), proximal (gray) or distal (green) ubiquitin, and hRpn2 (orange) are included. Ubiquitin atoms with two sets of chemical shift signals are labeled according to overlap with free ubiquitin (free) or shifting by hRpn13 binding (bound).

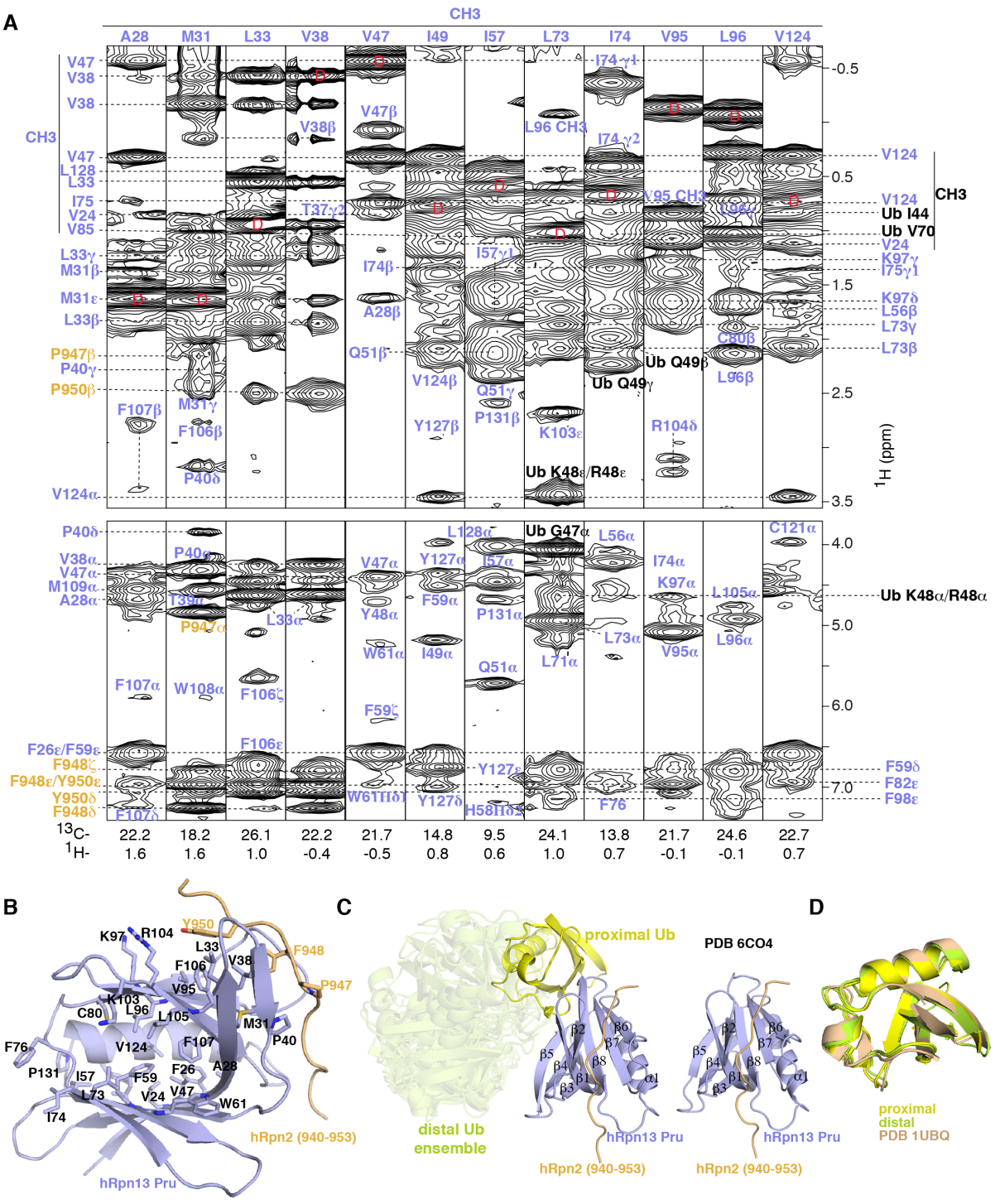

Figure S2. hRpn13 and its interaction with hRpn2 as well as the ubiquitin structure are maintained in the hRpn13:hRpn2:K48-diubiquitin complex. Related to Figure 1.

(A) Selected regions from a ^{13}C-edited NOESY experiment (100 ms mixing time) acquired on Ternary-^{13}C-P (Figure 1A, left panel) to highlight intramolecular interactions within hRpn13 and intermolecular interactions between hRpn13 and hRpn2 that are maintained with K48-diubiquitin

present. Diagonal breakthrough signals are labeled ‘D’ (red) and assignments for hRpn13 Pru (purple), hRpn2 (orange), and proximal or distal ubiquitin (black) are included.

(B) Residues with NOE data shown in (A) are displayed on a ribbon diagram of structure PDB-6CO4 of hRpn2 (orange):hRpn13 (purple).

(C) Side-by-side structural comparison of Ternary-P (left panel, displayed as in Figure 1E) and structure PDB-6CO4 of hRpn13 Pru:hRpn2 (940-953) (right panel, colored as in B).

(D) Superposition of proximal (yellow) and distal (green) ubiquitin from our Ternary-P structure with free monoubiquitin from crystal structure PDB-1UBQ (wheat).

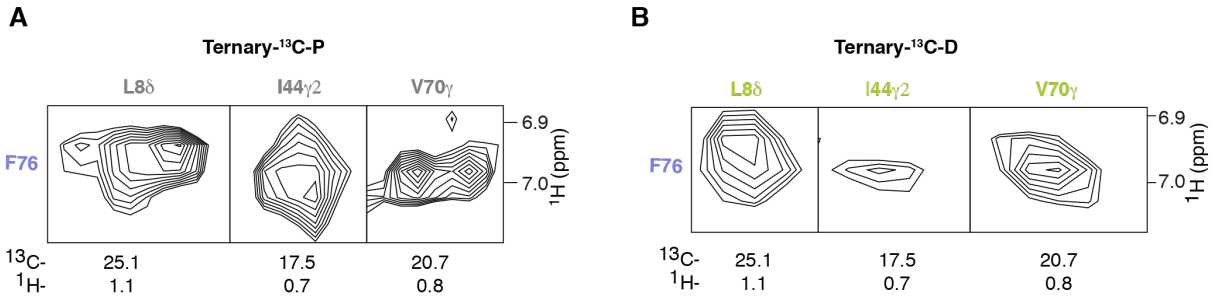

Figure S3. Intermolecular NOE data between hRpn13 F76 and ubiquitin of K48-diubiquitin. Related to Figures 3 and 6.

(A and B) Selected regions of a ^{13}C-edited NOESY experiment (100 ms mixing time) recorded on Ternary-^{13}C-P (A) or Ternary-^{13}C-D (B) as indicated in Figure 1A to highlight intermolecular interactions displayed in Figure 3B between hRpn13 F76 (purple) and proximal (A, gray) or distal (B, green) ubiquitin L8, I44 and V70.

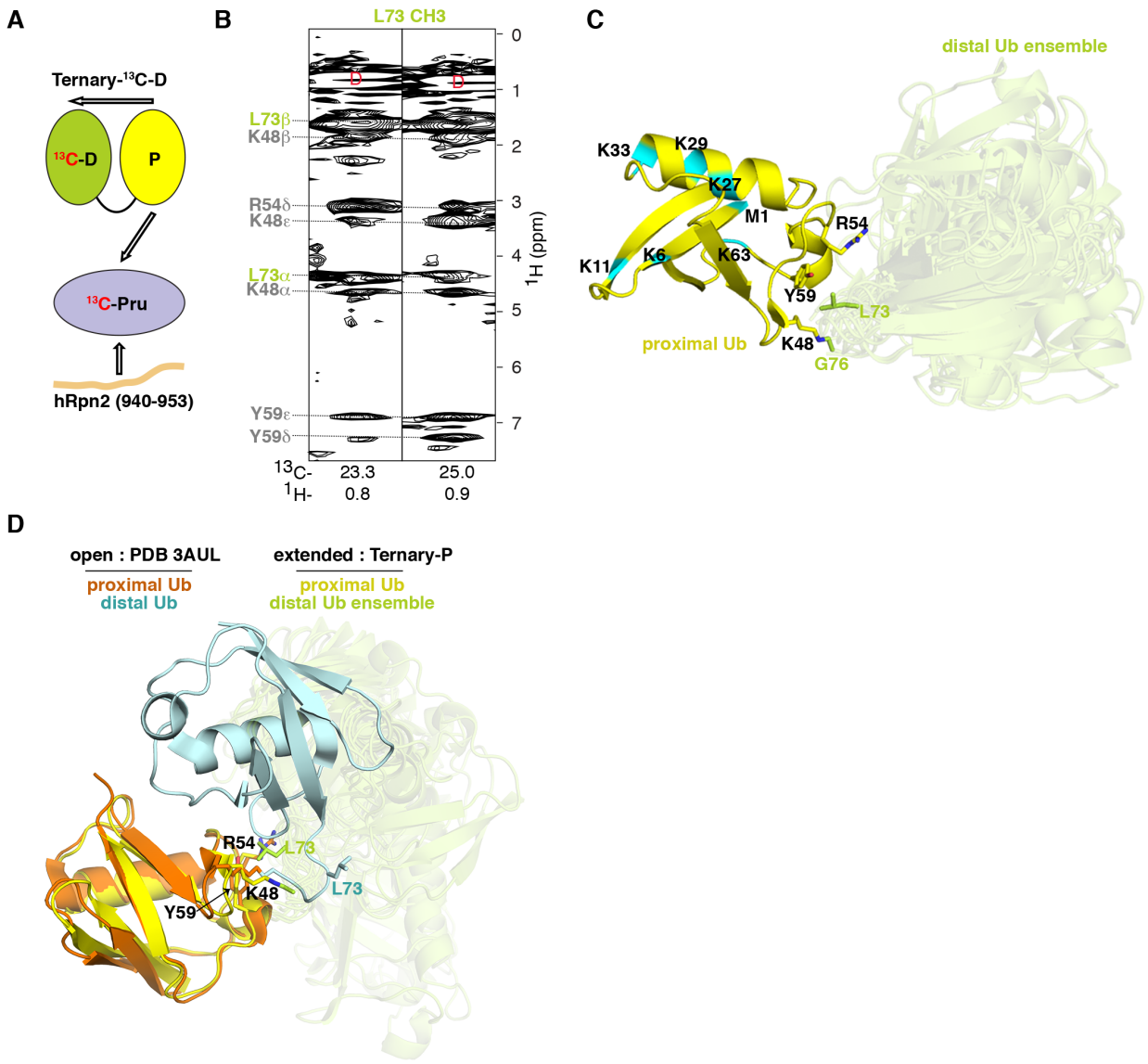

Figure S4. Interactions between the two ubiquitin moieties of K48-diubiquitin. Related to Figures 4 and 5.

(A) Schematic representation of interactions in Ternary-^{13}C-D detectable by a ^{13}C-half-filtered NOESY experiment (indicated by arrows, colored as in Figure 1A). ^{13}C-labeled constituents are indicated by '^{13}C' (red).

(B) Selected regions of a ^{13}C-half-filtered NOESY experiment (100 ms mixing time) recorded on sample Ternary-^{13}C-D (A) highlighting interactions between the two ubiquitin moieties. Diagonal breakthrough signals are labeled by 'D' (red) and assignments for proximal (gray) and distal (green) ubiquitin included.

(C) Ribbon diagram of K48-diubiquitin from the Ternary-P structure displaying and labeling distal ubiquitin L73 (green) as well as proximal ubiquitin (yellow) K48, R54, Y59 and all other ubiquitin residues that can form chains by isopeptide bonds (cyan). Sidechain nitrogen and oxygen atoms are colored in blue and red, respectively. The full conformational ensemble is displayed for distal ubiquitin, as in Figure 1E.

(D) Superposition of proximal ubiquitin in the ‘extended’ conformation of Ternary-P to proximal ubiquitin in the ‘open’ conformation of the free crystal structure of K48-diubiquitin (PDB 3AUL), with proximal ubiquitin K48, R54, Y59 and distal ubiquitin L73 displayed (presented as in Figure 1E and Figure 5B).