SUPPORTING INFORMATION

Structure and catalytic regulatory function of ubiquitin specific protease 11 N-terminal and ubiquitin-like domains

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Figure S1

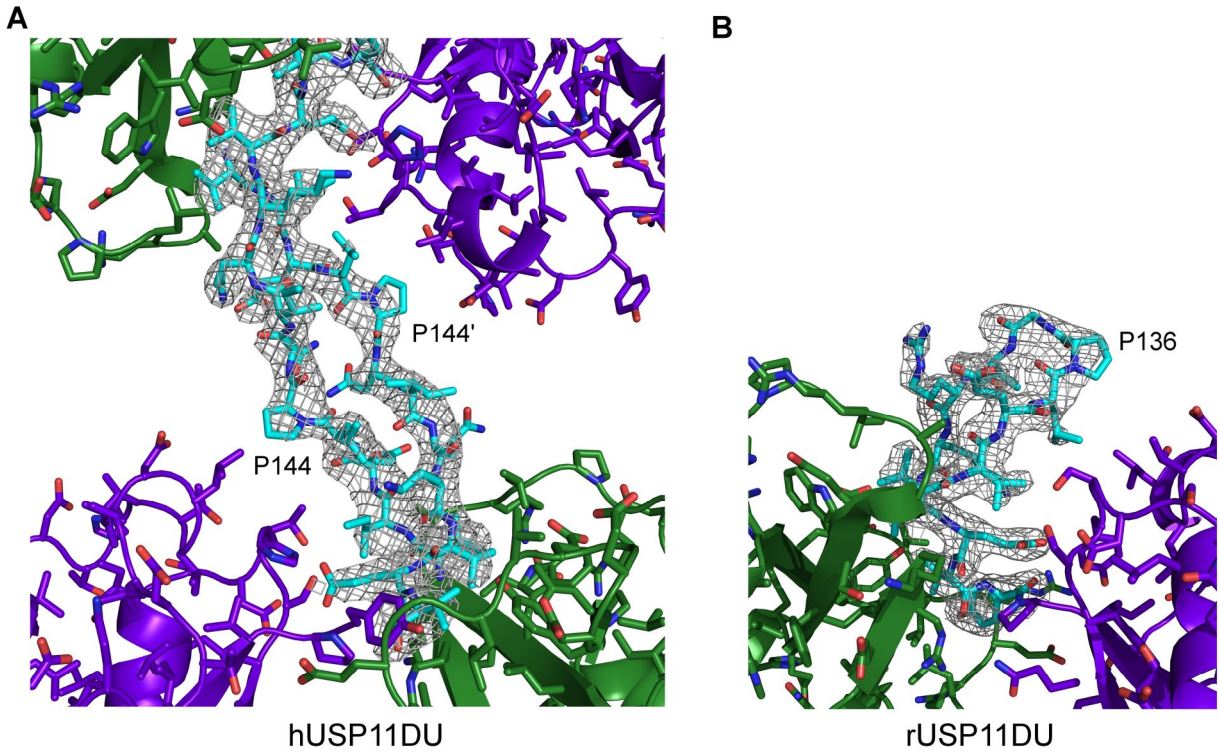

Figure S1: Electron density of the linker region and DU finger. (A) Cartoon representation depicting the extended conformation of the hUSP11DU linker region. The DUSP domains from each chain are shown in green, the UBL domains in purple and the linker region, residues 141-152, is shown in cyan. Side chains are displayed as sticks and the sigmaA weighted $2F_{obs} - F_{calc}$ electron density map is contoured at 1.2σ. (B) Cartoon depicting monomeric rat USP11DU in the same colour scheme. The DUSP domain stacks against the UBL domain from the same chain; This arrangement is mediated by the linker region, residues 133-144, which forms a β-hairpin structure denoted the DU finger with sigmaA weighted $2F_{obs} - F_{calc}$ electron density map contoured at 1.2σ.

Figure S2

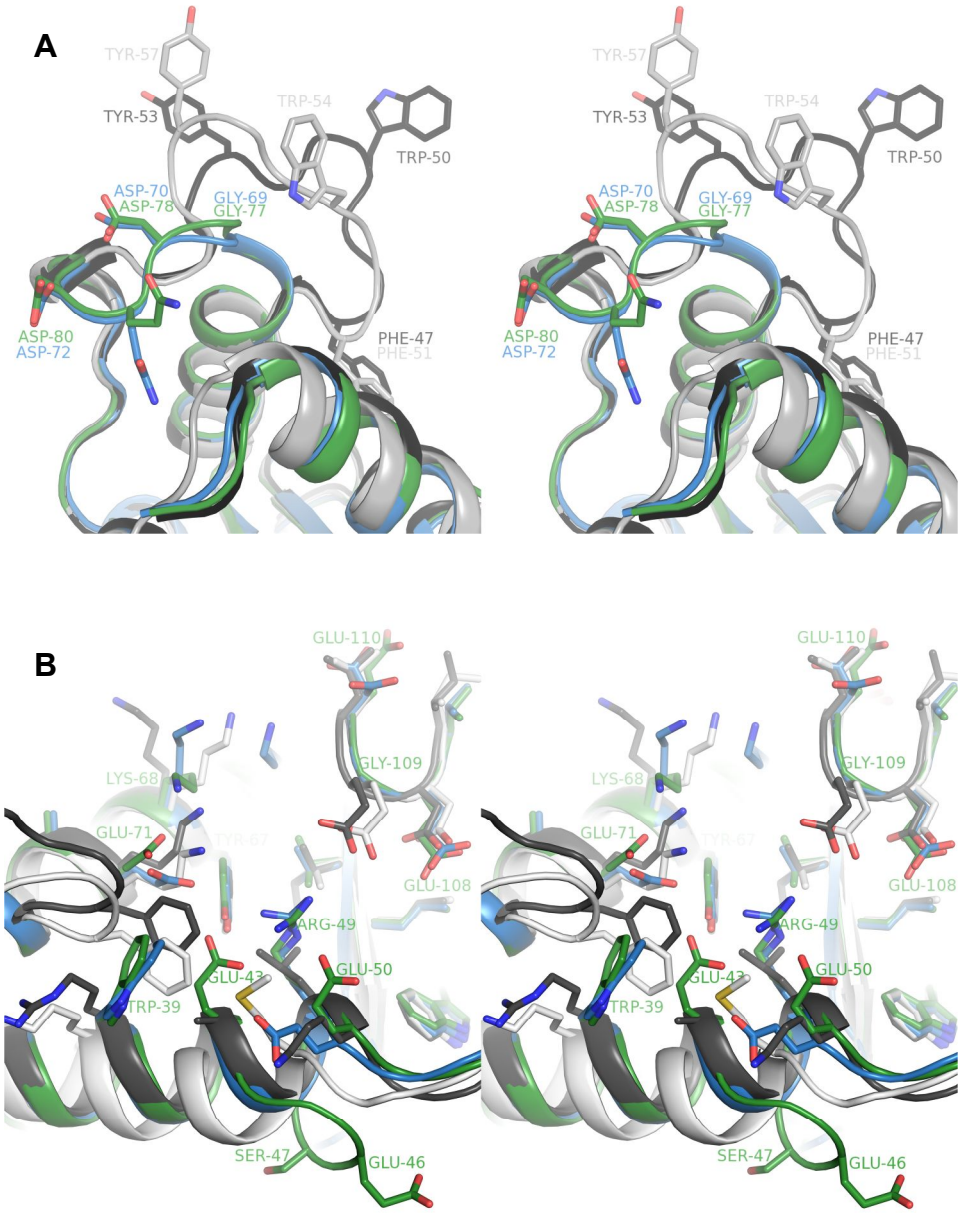

Figure S2: Differences in the DUSP domain of USP11, USP4, and USP15**.** (A) Stereo representation as in Figure 3B focusing on the differences in loop 2 in the DUSP domains with key residues labelled (B) Stereo representation as in Figure 3D highlighting the differences in helix 1 and the cleft feature of the DUSP domain with key hUSP11 residues labelled. The colour code for the structures and labels is as follows: hUSP15 (dark grey), mUSP4 (light grey), rUSP11 (blue) and hUSP11 (green).

Figure S3

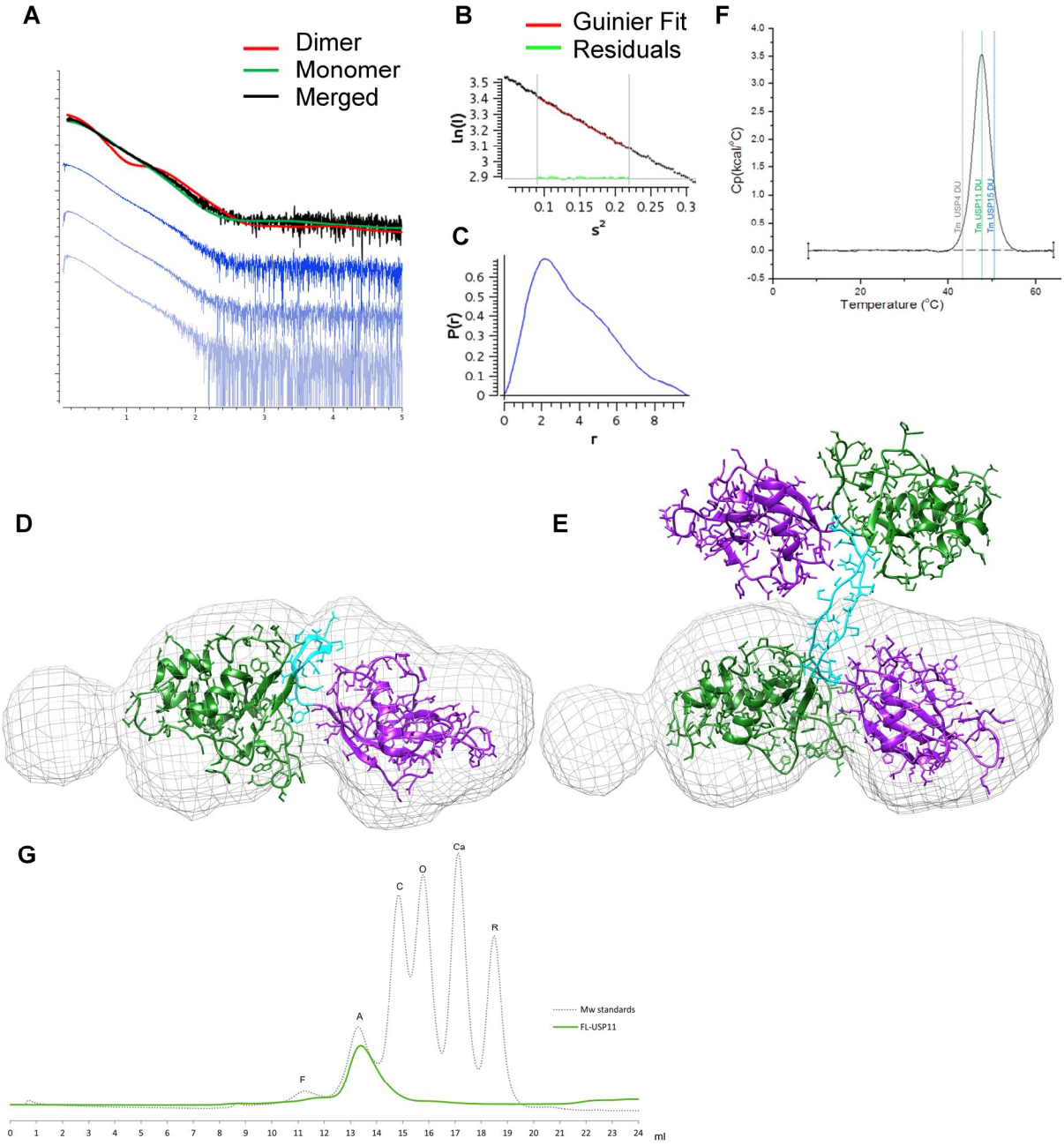

Figure S3: Solution studies of human USP11DU and FL-USP11. (A) Small angle X-ray scattering curves showing intensity as a function of the scattering vector for hUSP11DU at 7.6, 3.8 and 1.9 mg ml^{-1} (in shades of blue). The top curve depicts the merged data in comparison with the theoretical scattering curves computed using CRYSOL from the domain swapped hUSP11 dimer as observed in the crystal (red, chi^2=10.32) and a hUSP11 monomer (green, chi^2=2.30). (B) Guinier plot region and (C) P(r) function from which a D_{max} of ~97.2 Å was derived. (D) hUSP11DU monomer modelled into the resulting molecular envelope. (E) The hUSP11DU domain swapped dimer is too big to fit into the molecular envelope. This shows that this arrangement is not present in solution to any significant extent (F) DSC thermogram of hUSP11DU illustrating the presence of a single peak. The melting temperatures Tm associated with a single phase transition as seen with hUSP4DU and hUSP15DU are also indicated. (G) Gel filtration trace of full-length hUSP11 (green) using a Superdex 200 10/300 GL column with the molecular weight standards (GE Healthcare) shown as a dotted line. The standards

are annotated as follows: Ferritin (F) 440 kDa; Aldolase (A) 158 kDa; Conalbumin (C) 75 kDa; Ovalbumin (O) 43 kDa; Carbonic anhydrase (Ca) 29 kDa; Ribonuclease A (R) 13.7 kDa.

Figure S4

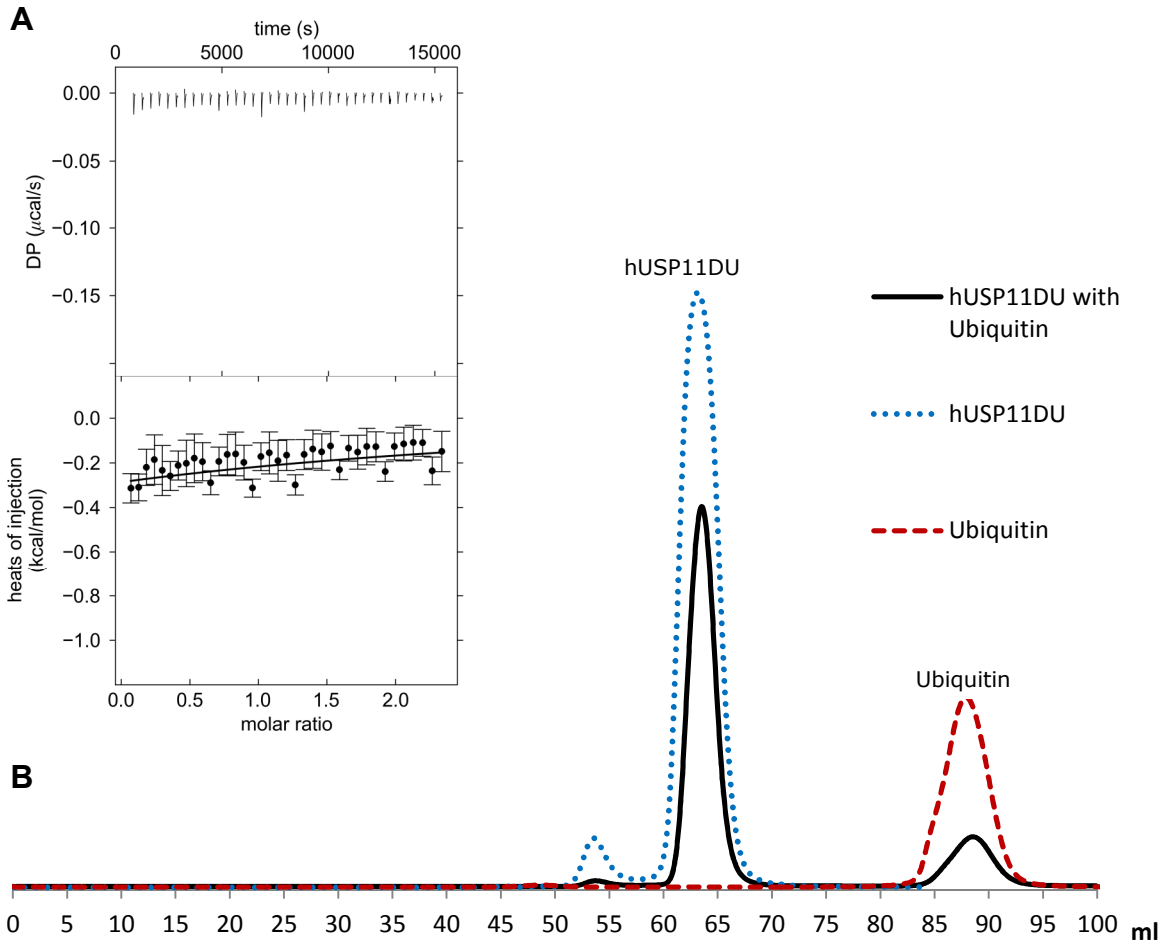

Figure S4: hUSP11DU does not detectably associate with ubiquitin in solution. (A) Isothermal titration calorimetry experiment injecting 8 µl aliquots of a solution of 200 µM ubiquitin into a 20 µM solution of hUSP11DU. Raw measured heat changes as a function of time are shown on top and the integrated measured heats of injection are shown below. These correspond to heats of dilution with no binding detectable. Trace analysis and figure generation was carried out using NITPIC, SEDFIT and GUSSI [(1)]. (B) Superimposed gel filtration traces of hUSP11DU (blue dotted line), ubiquitin (red dashed line) and hUSP11DU incubated with ubiquitin (solid black line) in a buffer of 50 mM Tris-Cl, pH 8, 50 mM NaCl using a Superdex 75 16/60 (GE Healthcare). No complex formation between USP11DU and ubiquitin is observed.

Reference:

1. Keller, S., Vargas, C., Zhao, H., Piszczek, G., Brautigam, C. A., and Schuck, P. (2012) High-precision isothermal titration calorimetry with automated peak-shape analysis, *Analytical chemistry 84*, 5066-5073.

Figure S5

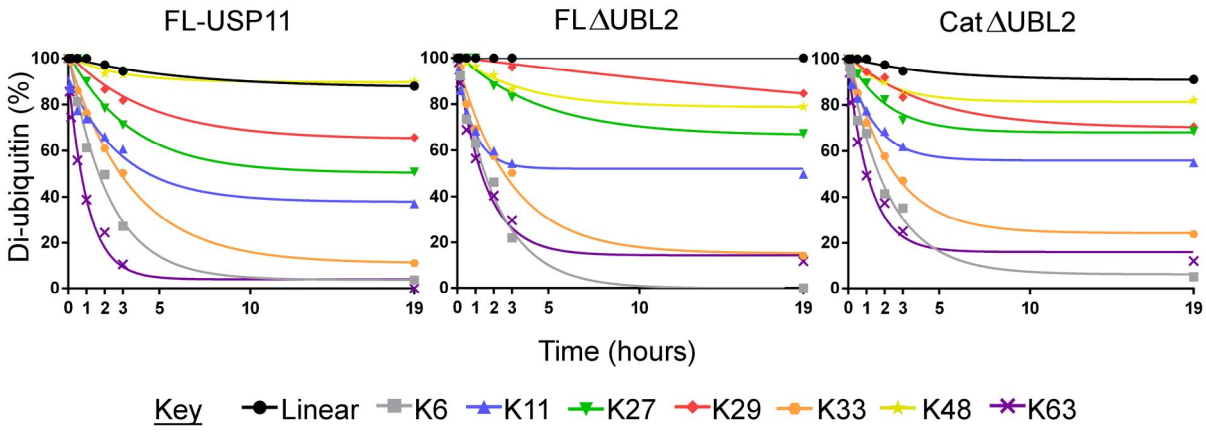

Figure S5: Densitometric analysis of gels probing ubiquitin chain selectivity with the overnight time point included. XY scatter plots with di-ubiquitin percentage plotted against time in hours for each of the three hUSP11 constructs (as labelled) with each of the eight di-ubiquitin linkage types (colours and symbols indicated in key). Di-ubiquitin percentage was calculated from ImageJ analyses of SDS-PAGE gels (Figure 5) by quantifying the amount of di-ubiquitin and mono-ubiquitin in each lane. The mean was calculated from duplicate assays and non-linear regression analyses were performed on time points between 0 and 19 hours.