Supplementary Information

Molecular Cloning. The DNA sequence corresponding to the UBZ domain of human DNA Y-polymerase η (residues 628-662) was synthesized using three primers 5'-GGG AGC CCA TAT GGC TGC TGA AGA CCA GGT TCC GTG CGA AAA ATG CGG TTC TCT GGT T-3', 5'-GAA AAA TGC GGT TCT CTG GTT CCG GTT TGG GAC ATG CCG GAA CAC ATG GAC TAC CAC TTC-3', 5'-GGA GCC GGA TCC TTA AGA TTT CTG CAG TTC CAG AGC GAA GTG GTA GTC CAT GTG TTC-3'. PCR-amplified DNA was digested and ligated into the pET15b vector (EMD Biosciences, Inc., Madison, WI) between the NdeI and BamHI restriction sites to produce an N-His_6 fused UBZ domain construct. The DNA sequence of human ubiquitin was PCR-amplified from a human cDNA library and cloned into the pET15b vector. Single-point mutants of the pol η UBZ domain (A656F, Q660A) and ubiquitin (I44A, V70A) were prepared using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA).

A modified pol η UBZ domain construct was made to introduce a C-terminal free cysteine (as SSC) for the site-directed spin-labeling experiment using the wild-type UBZ domain construct as the template. The PCR-amplified DNA fragment was cloned into the pET15b vector between NdeI and BamHI restriction sites.

The presence of the correct inserts of these constructs was confirmed by DNA sequencing.

Protein Purification. The UBZ domain of pol η was overexpressed in *Escherichia coli* BL21(DE3) STAR cells (Invitrogen, Carlsbad, CA). Bacterial cells were induced at 20 ºC with 0.5 mM IPTG and 50 μM Zn^{2+} for 18 hours. The UBZ domain of pol η was initially purified by a Ni^{2+}-NTA column, followed by thrombin digestion to remove the N-His_6 tag. Thrombin was removed with a benzamidine column and the N-His_6 tag by a second Ni^{2+}-NTA column. The resulting fragment, which contains the N-terminal GSHM sequence and residues 628-662 of pol η, was further purified using size-exclusion chromatography (Superdex 75, GE Healthcare, Piscataway, NJ). Human ubiquitin, the modified pol η UBZ domain containing the free cysteine at the C-terminus, and various UBZ domain and ubiquitin point-mutants were expressed and purified by identical purification procedures described above.

Mass Spectrometry. Mass spectrometry was performed on the native and denatured states of the pol η UBZ domain using a quadruple time-of-flight mass spectrometer (Q-TOF 2) (Waters Corporation, Milford, MA) equipped with a Z-spray source and run in the positive ion mode. The mass of the native state was determined by infusing the pol η UBZ domain, diluted in a buffer containing 25 mM NH_4HCO_3, pH 7.0, directly into the ionization source at a flow rate of 5 μL/min using a syringe pump. The denatured protein was prepared by diluting the UBZ domain in a solution containing 50% acetonitrile and 0.1% formic acid. This denatured sample was infused at a flow rate of 30 μL/min using Waters CapLC. Data were processed using the MaxEnt1 algorithm in MassLynx 4.0 to yield the average mass of the proteins.

The mass of the native domain was determined to be 4480.32 Da (4480.38 Da, expected); under denaturing condition, the mass was reduced to 4417.07 Da (4416.99 Da,

expected) (Fig S1B). The difference of 63.25 Da in molecular mass corresponds to the expected value of one zinc ion after the subtraction of two protons (63.39 Da).

NMR. Backbone resonances were assigned by standard 3D triple-resonance experiments, and sidechain resonances were assigned using 3D HCCH-TOCSY and 2D homonuclear TOCSY and NOESY experiments (Clore and Gronenborn, 1993; Ferentz and Wagner, 2000). NMR data were processed by NMRPIPE (Delaglio *et al*, 1995) and analyzed with XEASY/CARA (Bartels *et al*, 1995). Three-dimensional ^{15}N- and ^{13}C-separated NOESY-HSQC experiments were used to generate distance constraints, calibrated using the CALIBRATION module in CYANA (Güntert, 2004; Herrmann *et al*, 2002). Three-bond coupling constants ($^3J_{HN\text{-}H\alpha}$) were obtained from a two-dimensional HMQC experiment (Kay and Bax, 1990). Dihedral angles were derived from the combined input of TALOS analysis of chemical shift information, $^3J_{HNH\alpha}$ couplings, and analysis of local NOE patterns (Cornilescu *et al*, 1999; Wang and Bax, 1996). Stereo-specific assignment of valine and leucine methyl groups was obtained via a high-resolution ^{1}H-^{13}C HSQC spectrum of a 10% ^{13}C-labeled sample (Szyperski *et al*, 1992). Initial structures were generated with CYANA (Güntert, 2004; Herrmann *et al*, 2002).

Residual dipolar couplings ($^1D_{HN}$, $^1D_{H\alpha C\alpha}$) were determined from the difference in couplings between an isotropic and a liquid crystalline Pf1 phage sample (~17 mg/mL) in 25 mM sodium phosphate, 400 mM KCl, 20% D_2O (pH=7.0). A 2D ^{1}H-^{15}N IPAP experiment (Ottiger *et al*, 1998) and a modified, $J_{H\alpha C\alpha}$-coupled (HACACO)NH experiment (Boucher *et al*, 1992) were used to measure the $^1D_{HN}$ and $^1D_{H\alpha C\alpha}$ couplings, respectively. These residual dipolar couplings were used for structure refinement using XPLOR-NIH with a water-refinement protocol (Linge *et al*, 2003; Nabuurs *et al*, 2004; Schwieters *et al*, 2003). A zinc ion was included during the final stages of structural refinement.

Binding Affinity Measurement by NMR. The K_d value for the pol η UBZ domain-ubiquitin complex was calculated based on NMR titration data. Unlabeled ubiquitin was titrated with increasing molar ratios into a 0.5 mM sample of the ^{15}N-labeled pol η UBZ domain. A series of ^{1}H-^{15}N HSQC spectra of the UBZ domain were obtained and normalized chemical shift changes (Δδ) measured for each molar ratio (M). The dissociation constant (K_d) and value of maximum chemical shift perturbation ($\Delta\delta_{max}$) were extracted from perturbation data of six nonoverlapping residues, E649, D652, H654, F655, L659, and sidechain of Q660 using Equation S1.

$$\frac{\Delta\delta}{\Delta\delta_{\max}} = 0.5\left[(M+1+\frac{K_d}{[Ub]_T}) - \sqrt{\left(\left(M+1+\frac{K_d}{[Ub]_T}\right)^2 - 4M\right)}\right] \qquad \text{[Eq. S1]}$$

Isothermal Titration Calorimetry. Wild-type or mutant human ubiquitin (4.6 mM) was titrated into a solution of the pol η UBZ domain (0.31-0.51 mM) in a buffer containing 25 mM sodium phosphate, 100 mM KCl, pH 7.0. 40 injections of 7 μl each were performed at 25 ºC using a VP-ITC Microcalorimeter (MicroCal, Northampton, MA), and data were analyzed using the Origin software assuming one-site binding (Origin Lab).

Table S1: Structural statistics for the UBZ domain of pol η (15 structures)[a]

NOE distance restraints	767
Intraresidue	377
Sequential (|*i*-*j*|=1)	152
Medium-range (|*i*-*j*|≤4)	115
i, *i*+2	27
i, *i*+3	63
i, *i*+4	25
Long-range (|*i*-*j*|≥5)	93
Hydrogen bonds	30
Dihedral angle constraints	74
Residual dipolar couplings ($^1D_{NH}$)	24
Residual dipolar couplings ($^1D_{CH}$)	21
Dipolar coupling *R* factor of $^1D_{NH}$, % [b]	1.7 ± 0.2
Dipolar coupling *R* factor of $^1D_{CH}$, % [b]	5.1 ± 0.7
Ramachandran Plot [c]	
Favored Region (%)	95.0
Allowed region (>99.8%)	100.0
Deviations from idealized geometry	
Bonds, Å	0.0104 ± 0.0004
Angles, º	1.43 ± 0.04
Impropers, º	1.29 ± 0.12
Mean pairwise rmsd	
Backbone (residues 631-659), Å	0.37 ± 0.07
Heavy Atoms (residues 631-659), Å	0.92 ± 0.12

[a] None of these structures exhibit distance violations greater than 0.4 Å or dihedral angle violations greater than 4°.

[b] *R*-factor for residual dipolar coupling is defined as the ratio of the r.m.s deviation between observed and calculated values to the expected r.m.s deviation if the vectors were randomly distributed (Clore and Garrett, 1999).

[c] MOLPROBITY was used to assess the quality of the structures (Davis *et al*, 2004; Lovell *et al*, 2003).

Table S2: Binding affinities of the pol η UBZ domain-ubiquitin complexes

pol η UBZ domain	ubiquitin	K_d (μM)	method
WT	WT	73 ± 15	NMR
WT	WT	81	ITC
A656F	WT	No detectable binding	ITC
Q660A	WT	142	ITC
WT	I44A	No detectable binding	ITC
WT	V70A	130	ITC

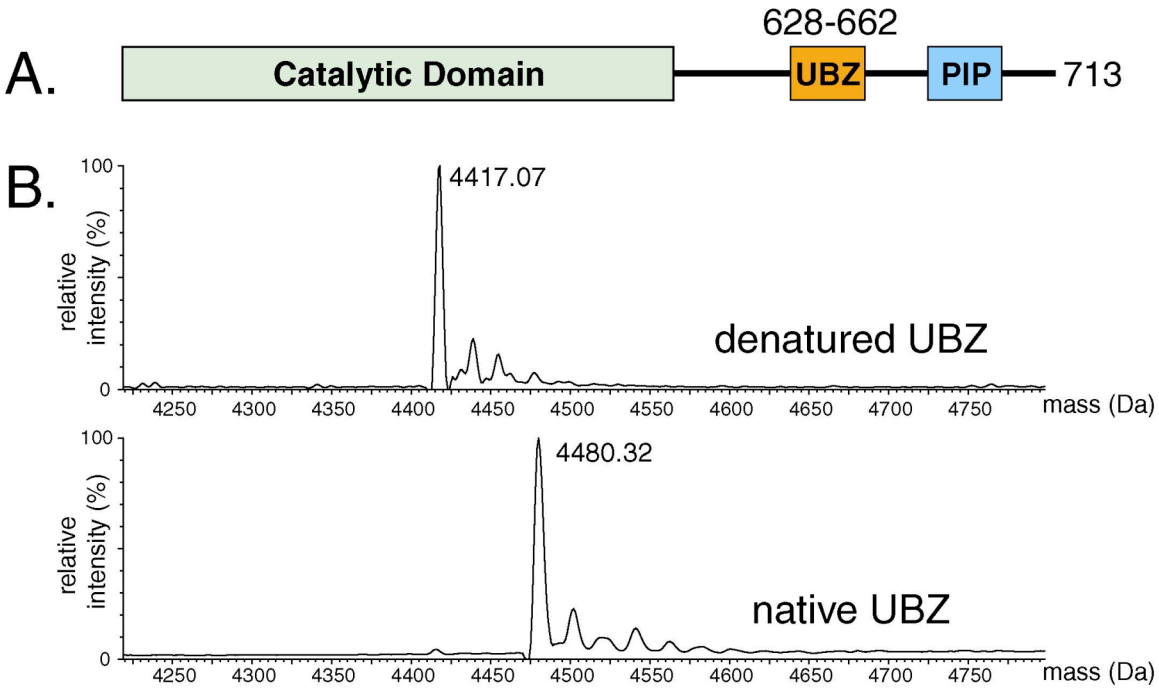

Fig S1 | Pol η UBZ domain contains a single zinc ion. (**A**) A schematic representation of the domain structure of human pol η. The catalytic domain is shown in pale green, the ubiquitin-binding zinc finger (UBZ) in orange, and the PCNA-interacting peptide (PIP box) in blue. (**B**) Mass spectrometric analysis of the native and denatured UBZ domain confirms that a single zinc ion is present in the native structure.

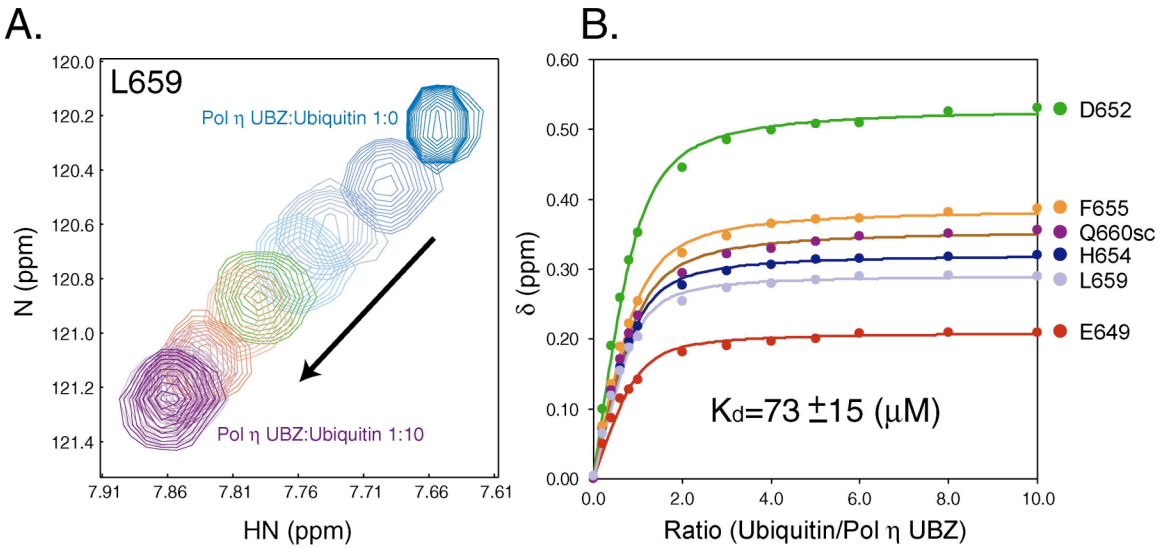

Fig S2 | Determination of the binding affinity between the pol η UBZ domain and ubiquitin by NMR titration. (**A**) A section of the ^{1}H-^{15}N HSQC spectra depicting changes of the amide resonance for a representative residue, $L659_{UBZ}$ as the molar ratio of ubiquitin to the UBZ domain increases (blue to purple). (**B**) Determination of the dissociation constant (K_d) by NMR. The chemical shift perturbations for the backbone amides of five residues (E649, D652, H654, F655 and L659) and the sidechain of Q660 in the UBZ domain were used to determine the binding affinity of the UBZ domain toward ubiquitin according to equation S1.

Supplementary References

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