Supplemental Information for:

DNA polymerase ι interacts with the TRAF-like and UBL1-2 domains of USP7

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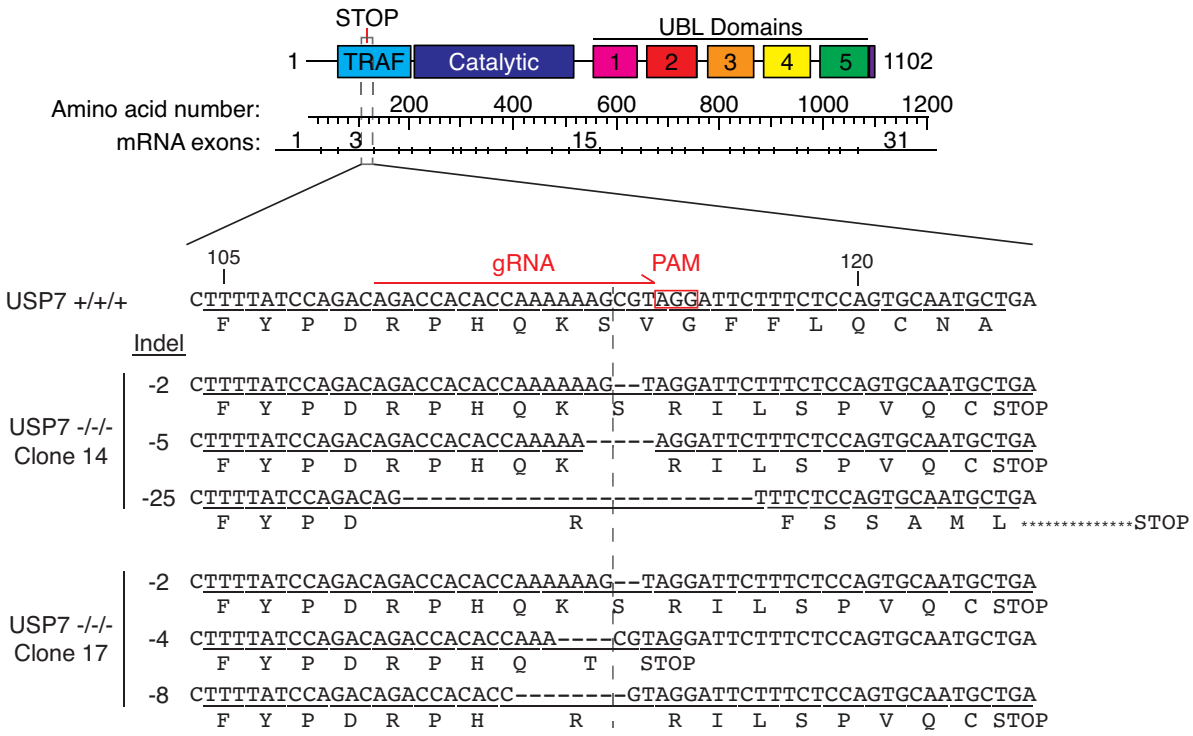
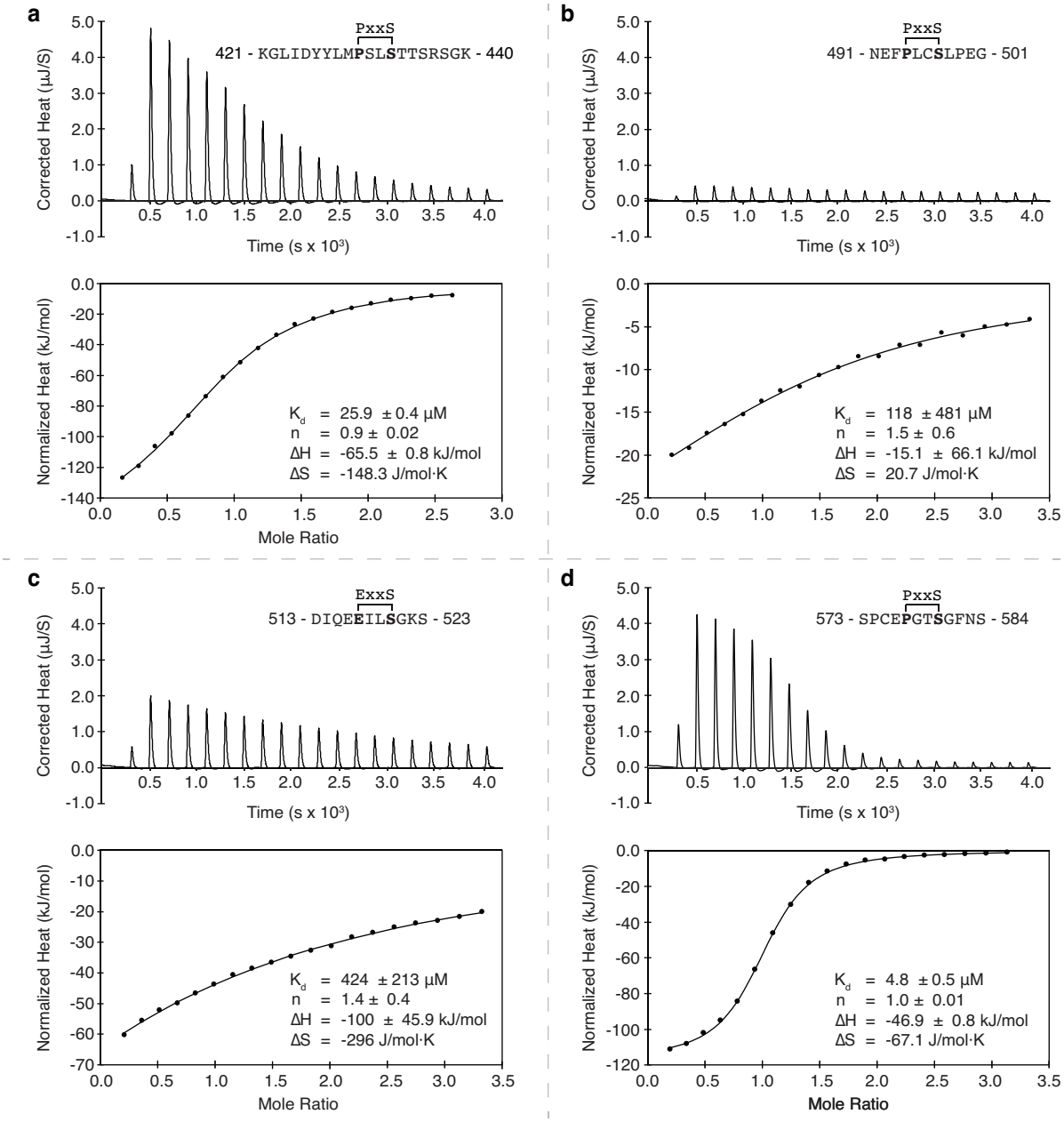
This document includes:

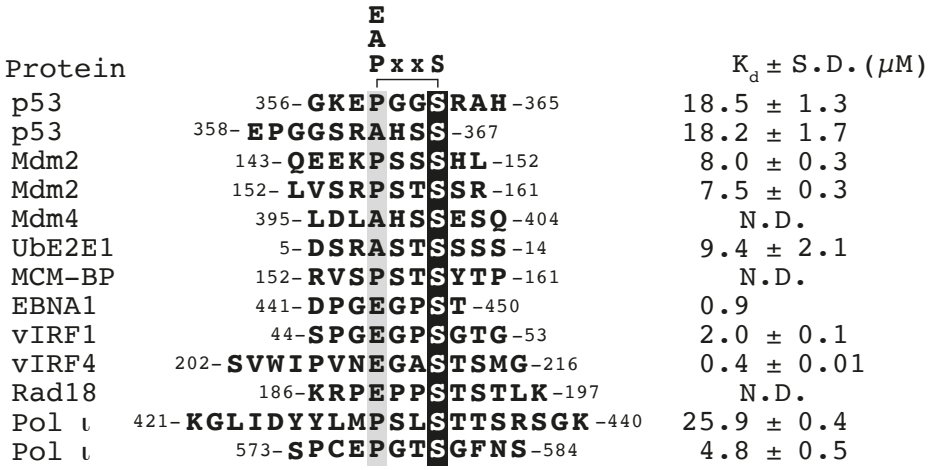
Supplemental Figures



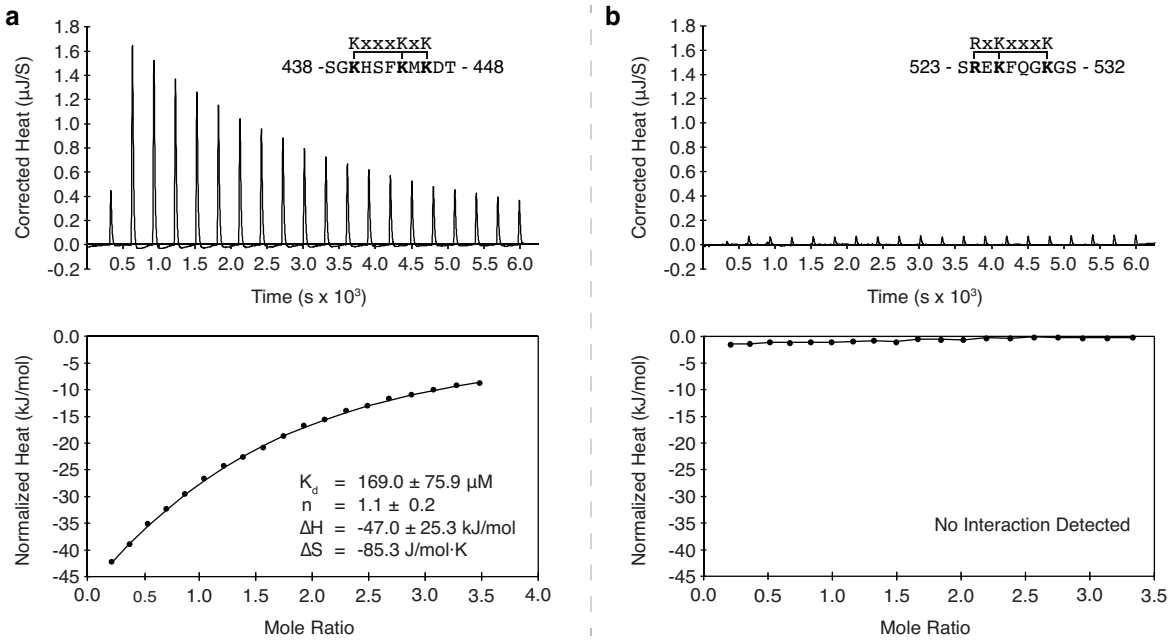
Supplemental Figure 1: Indel analysis of 293T USP7 knock-out cells. USP7 is encoded on chromosome 16; 293T cells are hypotriploid and contain three copies of this chromosome [1]. Exon 3 of the *USP7* gene was targeted using the Cas9-expressing construct px330-U6-Chimeric_BB-Cbh-hSpCas9 and the indicated guide RNA. The vertical dashed line indicates the site of the induced double-strand break. The numbers above the nucleotide residues indicate the corresponding USP7 amino acid number, and the letters below indicate the encoded amino acids. Horizontal dashes indicate lost nucleotides. Asterisks indicate additional encoded amino acids not shown here.



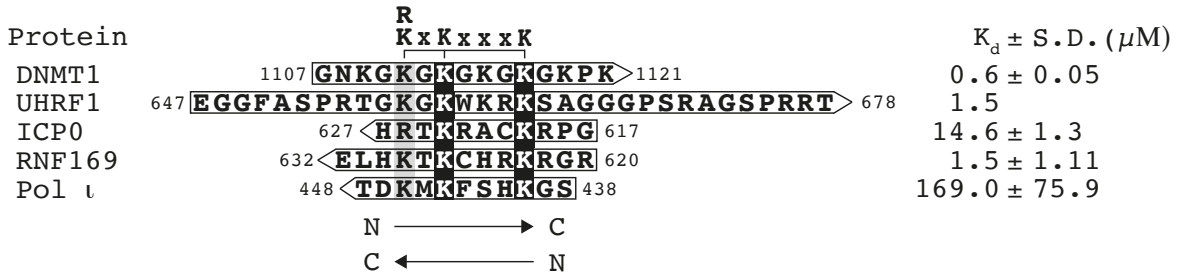
Supplemental Figure 2: ITC profiles of binding between the USP7 TRAF domain and Pol ι peptides. (**a - d)** The top panels show heat change upon the addition of the indicated peptide; the bottom panels show an integrated ITC isotherm and its best fit to an independent site model. (d) is also shown in Figure 4c.



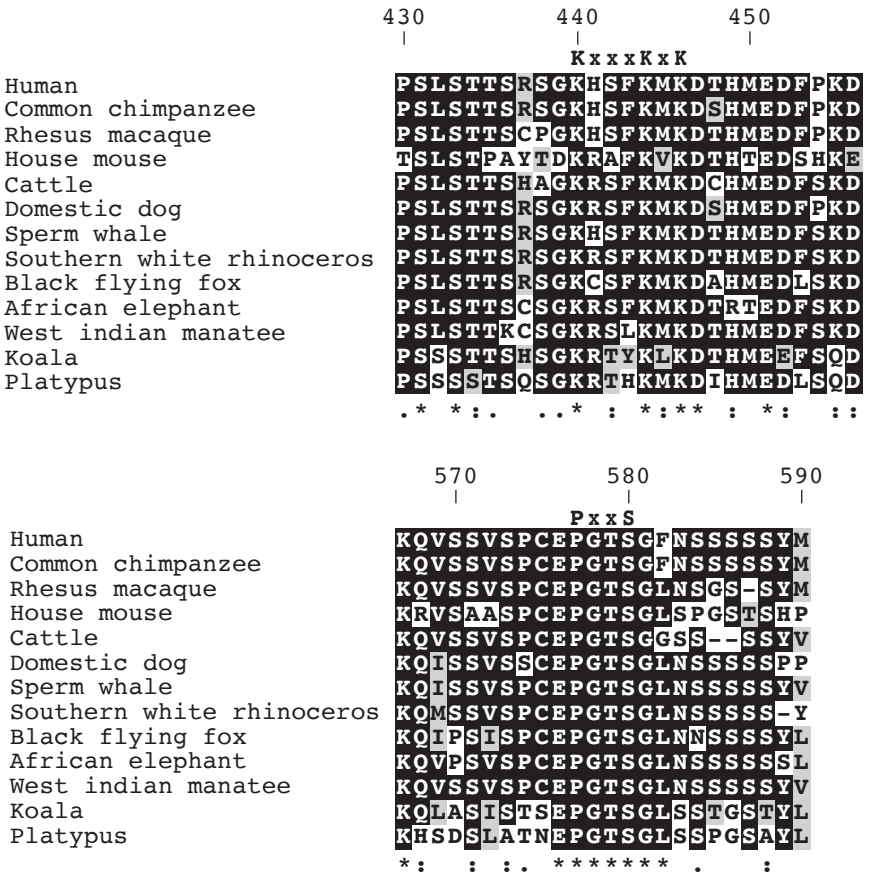
Supplemental Figure 3: Alignment of TRAF binding peptides from USP7 substrate proteins. The location of the TRAF-binding motif (P/A/ExxS) is indicated above the alignment. Shading indicates: black = conserved residue, gray = partially conserved residue. Peptide sequences and K_d values are listed for p53 [2], Mdm2 [2], Mdm4 [3], UbE2E1 [4], MCM-BP [5], EBNA1 [6], vIRF1 [7], vIRF4 [8], Rad18 [9] and Pol ι. K_d values are from ITC (vIRF4, Pol ι), fluorescence polarization (vIRF1) or tryptophan fluorescence (p53, Mdm2, UbE2E1, EBNA1) measurements of the indicated peptides binding to recombinant TRAF (p53, Mdm2, UbE2E1, vIRF1, vIRF4, Pol ι) or full length USP7 (EBNA1).



Supplemental Figure 4: ITC profiles for UBL1-2 of USP7 binding to Pol ι peptides. (**a and b**) The top panels show heat change upon the addition of the indicated peptide; the bottom panels show an integrated ITC isotherm and its best fit to an independent site model. (a) is also shown in Figure 6b.



Supplemental Figure 5. Alignment of UBL1-2 binding peptides from USP7 substrate proteins. The arrows indicate the orientation of each peptide when bound to USP7 UBL1-2. Shading indicates: black = conserved residue, gray = conservative mutation. Peptide sequences and K_d values are listed for DNMT1 [10], UHRF1 [11], ICP0 [12], RNF169 [13] and Pol ι. K_d values were derived from ITC measurements of the indicated peptides binding to recombinant UBL1-2 (for UHRF1, ICP0, and Pol ι) or UBL1-3 (for RNF169). This is with the exception of DNMT1, where binding was measured between a large DNMT1 fragment (residues 600-1600) and the USP7 C-terminus (residues 560-1102).



Supplemental Figure 6. Phylogenetic alignment of human Pol ι amino acids 430 – 456 and 567 – 590 with corresponding amino acids from homologous proteins encoded by other select mammals. The numbers above the alignment indicate the amino acid number for human Pol ι. Shading indicates: black = conserved residue, gray = conservative mutation, white = divergence. Symbols below the alignment indicate: * = single, fully conserved residue, : = conservation between residues of strongly similar properties, : = conservation between residues of weakly similar properties

References for Supplemental Material

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Dataset 1 (separate file)

A dataset of Pol ι-associating proteins identified by GeLC-MS/MS. 293T cells were transfected with WT FLAG Pol ι, or with an empty vector, 20 hours prior to cell lysis. M2 magnetic anti-FLAG beads were used to immunoprecipitate proteins from 3 mg of whole cell lysate. Eluents were electrophoresed 1 cm into a 1 mm 4-12% Bis-Tris NuPage precast gel and then excised as a single gel slice. Proteins were digested with trypsin and identified by GeLC-MS/MS.

Movie 1 (separate file)

A model of the USP7 TRAF-like domain in complex with Pol ι peptide 573-584. The USP7 TRAF domain is represented as a combined ribbon and space-filling model. Pol ι peptide 573-584 is in pink; numbers represent the residue location within the full-length protein.

Movie 2 (separate file)

A model of the UBL1-2 domains in complex with Pol ι peptide 438-448. USP7 UBL1-2 is represented as a combined ribbon and space-filling model. Pol ι peptide 438-448 is in purple; numbers represent the residue location within the full-length protein.

Movie 3 (separate file)

A model of full-length USP7 in an inactive, open conformation, in complex with Pol ι peptides 573-584 (TRAF-binding) and 438-448 (UBL1-2-binding). A space-filling model of full-length USP7. The location of each domain is indicated. The TRAF substrate-binding interface is indicated in blue. The UBL1-2 substrate-binding site is indicated in red. Pol ι peptides 438-448 and 573-584 are modelled in blue.