Supplementary Information

Activation-induced cytidine deaminase, an antibody diversification enzyme, interacts with chromatin modifier UBN1 in B-cells

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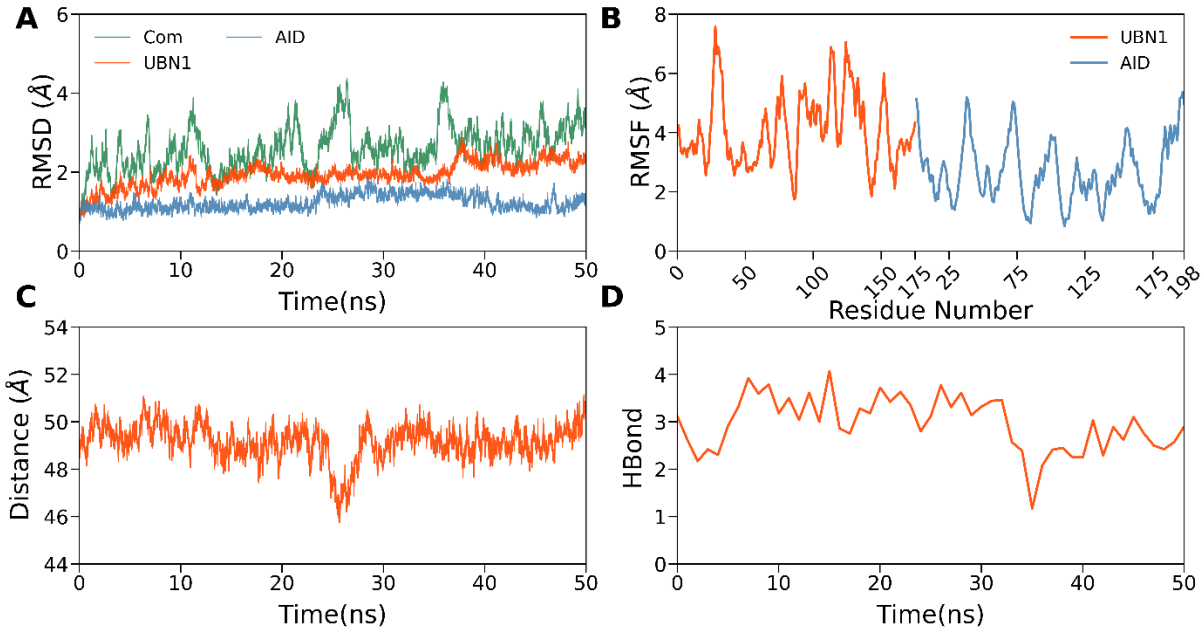

Fig. S1. (A) Time evolution of root mean squared deviation (RMSD) of complex and its components, (B) Root mean squared fluctuation (RMSF) of UBN1 and AID, (C) Time evolution of protein-protein center of mass distance, (D) Time evolution of hydrogen bonds between UBN1 and AID.

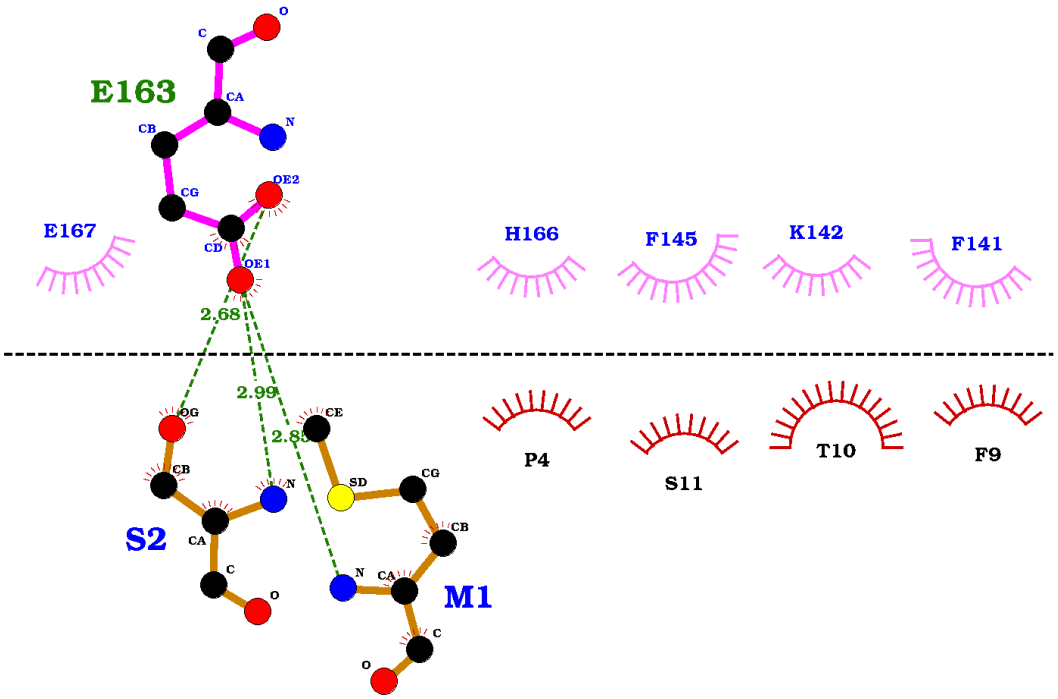

Fig. S2. The protein-protein interaction profile between UBN1 and AID was constructed using Ligplot^{+}. Hydrogen bonds are shown in the green dotted line. Semicircles show the residues involving hydrophobic contacts.

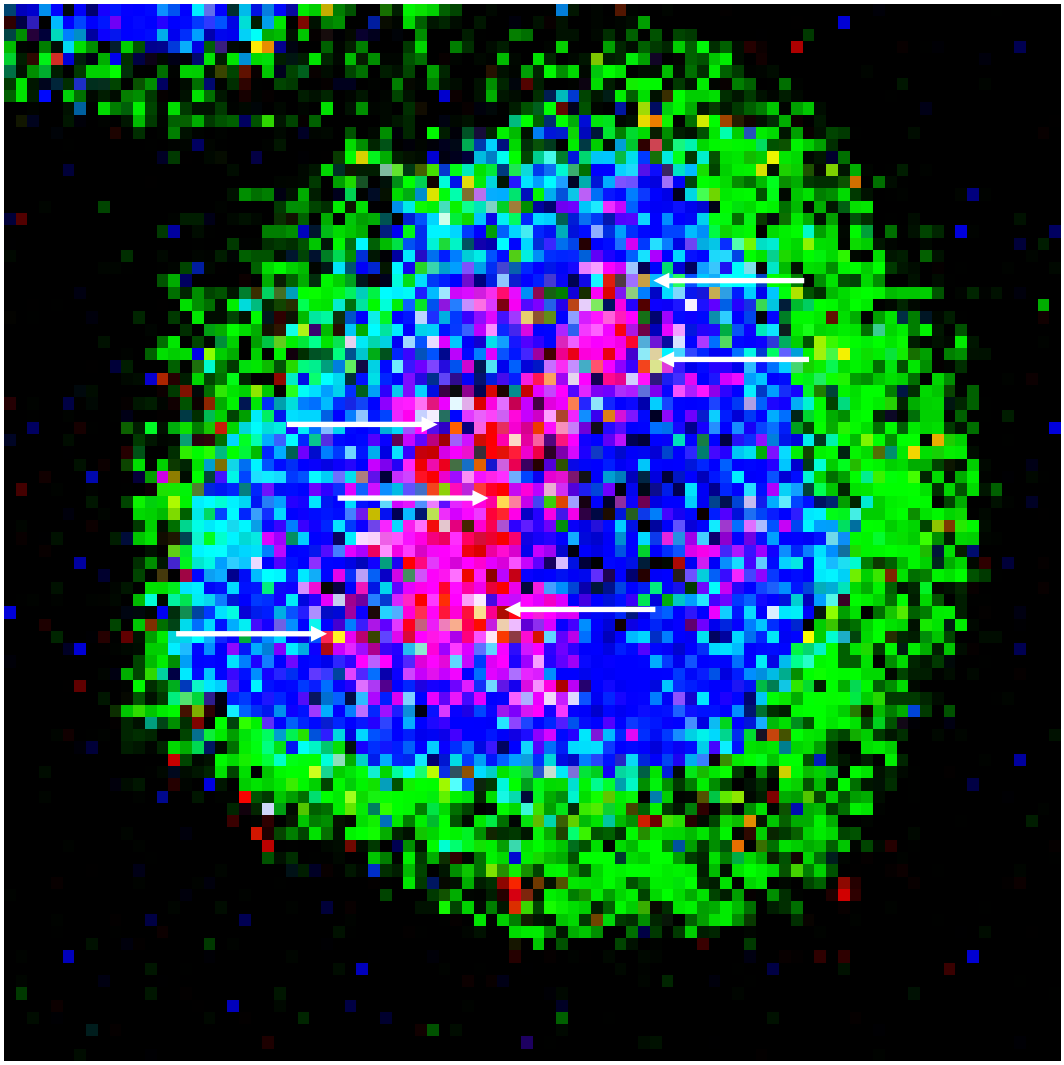

Fig. S3. Zoomed image of co-localization of UBN1 and AID in DT40ψV KO cells. Cells were stained with polyclonal anti-UBN1 (red stained) and monoclonal anti-AID (green stained). Yellow signals that merge with UBN1 and AID co-localized are highlighted with arrows.

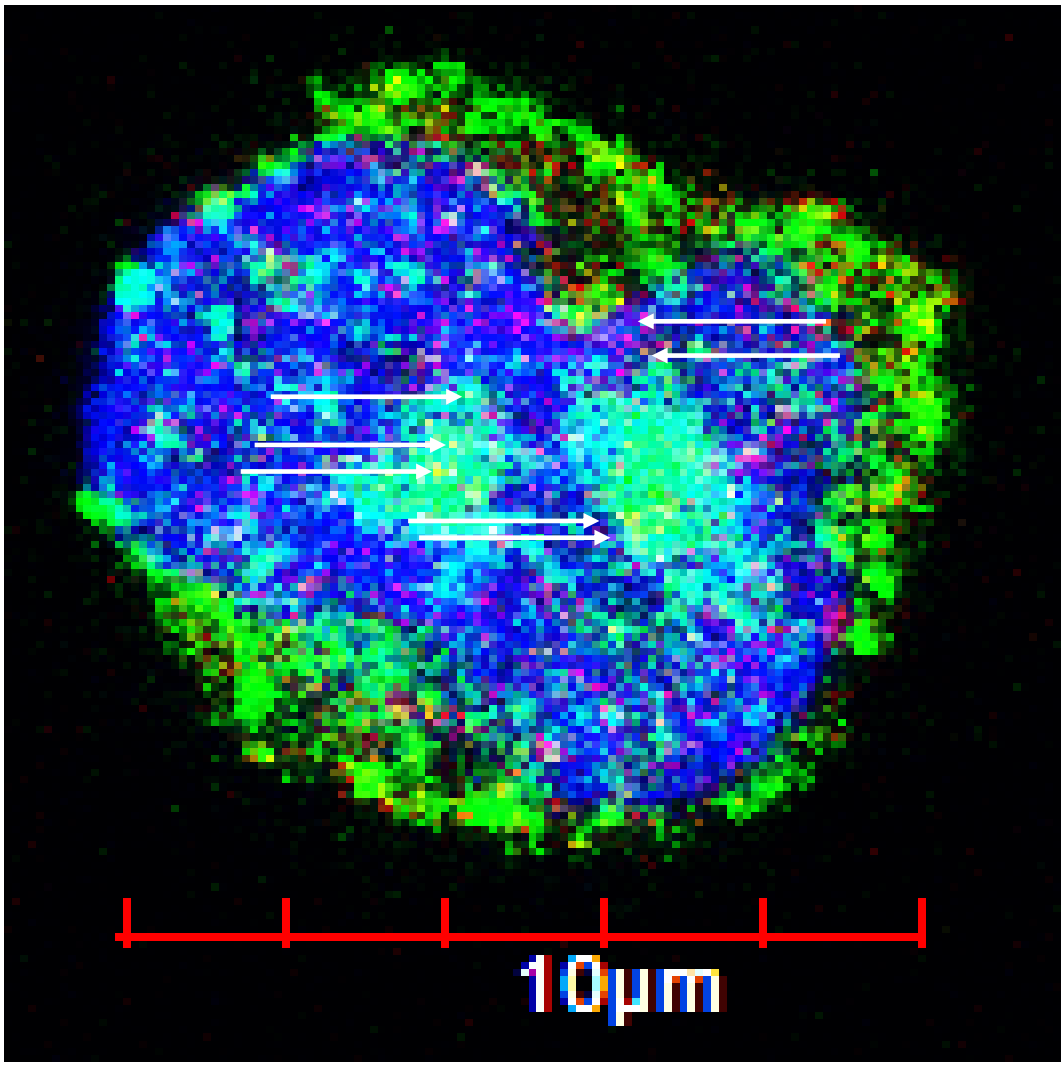

Fig. S4. Zoomed image of co-localization of UBN1 and AID in Raji cells. Cells were stained with polyclonal anti-UBN1 (red-stained) and monoclonal anti-AID (green stained). Yellow signals that merge with UBN1 and AID co-localized are highlighted with arrows.

Fig. S5

DT40 AID KO Cells

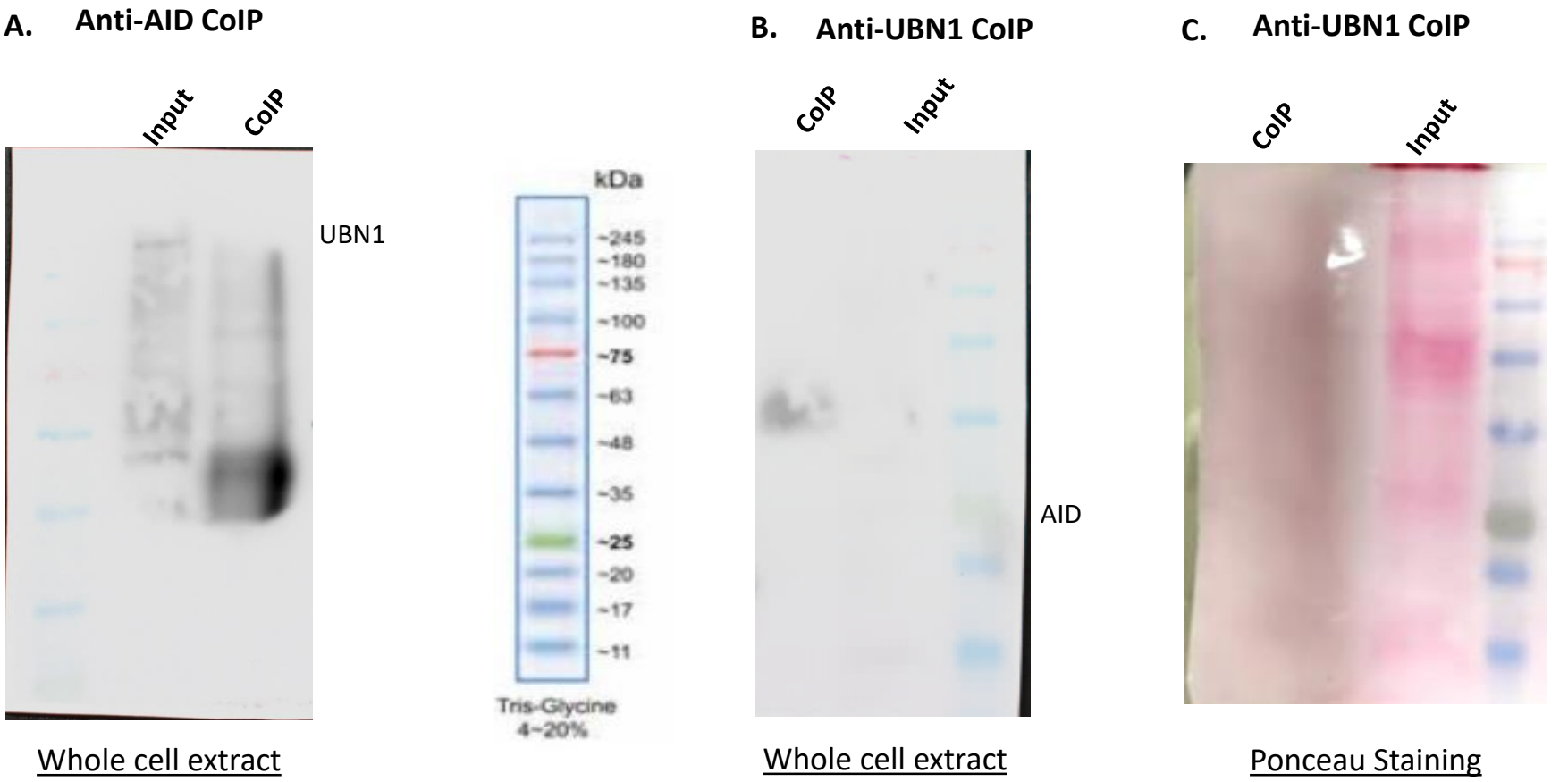

Fig. S5. Co-immunoprecepitation of AID andUBN1 in DT40 AID KO cells. A. Co-immunoprecepitation of AID from whole cell lysate was performed on DT40 AID KO cells using anti-AID Ab and protein A/G beads, detection via WB using anti-UBN1 Ab. B. Co-immunoprecepitation of UBN1 from whole cell lysate was performed on DT40 AID KO cells using anti-UBN1 Ab and protein A/G beads, detection vai WB using anti-AID Ab. C. Blot in Fig. S1B. was stained with ponceau before adding Ab.

Fig. S6

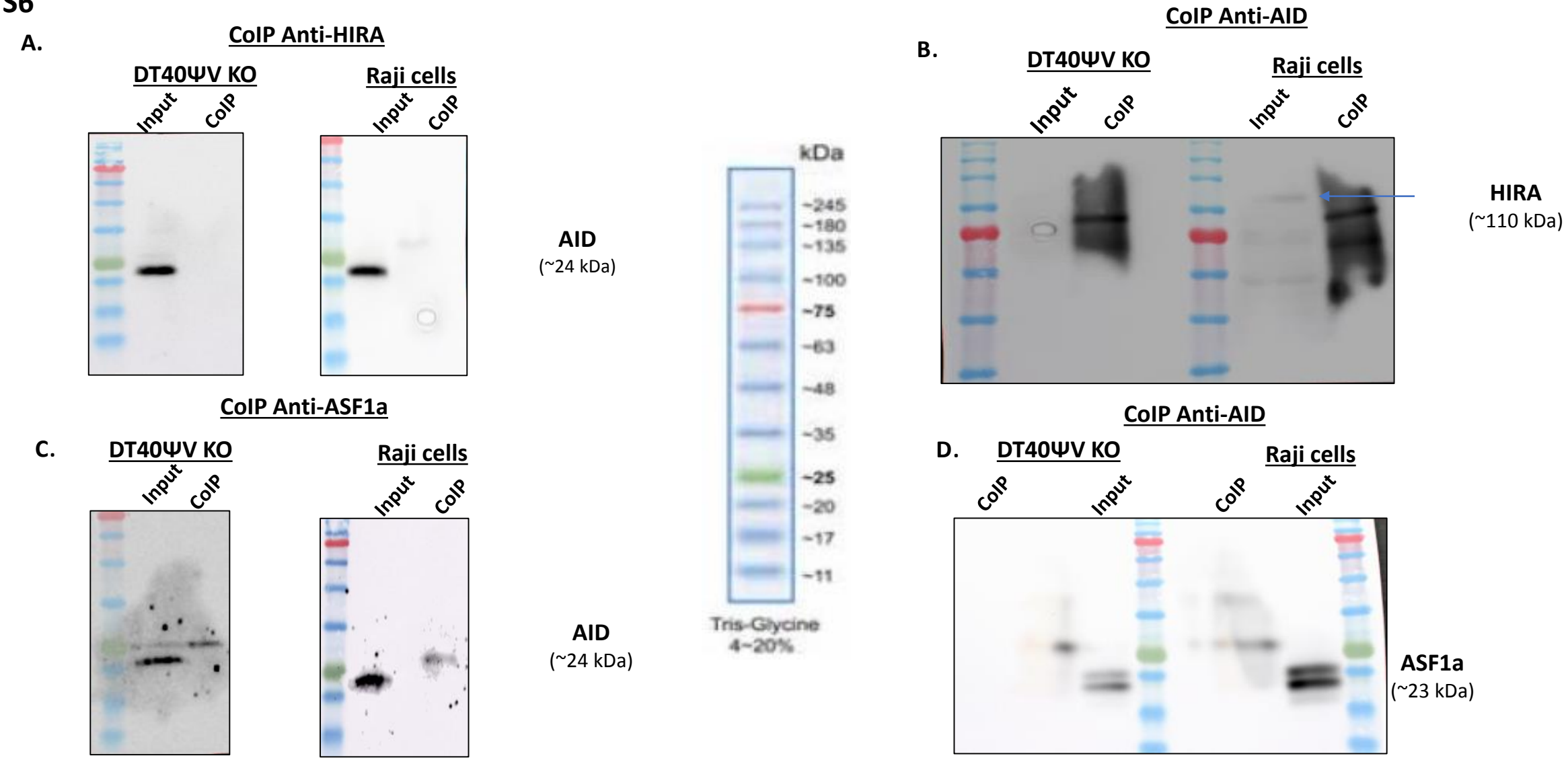

Fig. S6. AID interaction with HIRA and ASF1a in DT40ΨV KO and Raji cell lines. **A**. Co-immunoprecepitation of HIRA from whole cell lysate was performed on DT40ΨV KO and Raji cell lines using anti-HIRA Ab and protein A/G beads, detection via WB using anti-AID Ab. **B**. Co-immunoprecepitation of AID from whole cell lysate was performed on DT40ΨV KO and Raji cell lines using anti-AID Ab and protein A/G beads, detection via WB using anti-HIRA Ab. **C**. Co-immunoprecepitation of ASF1a from whole cell lysate was performed on DT40ΨV KO and Raji cell lines using anti-ASF1a Ab and protein A/G beads, detection via WB using anti-AID Ab. **D**. Co-immunoprecepitation of AID from whole cell lysate was performed on DT40ΨV KO and Raji cell lines using anti-AID Ab and protein A/G beads, detection via WB using anti-ASF1a Ab.

Fig. S7

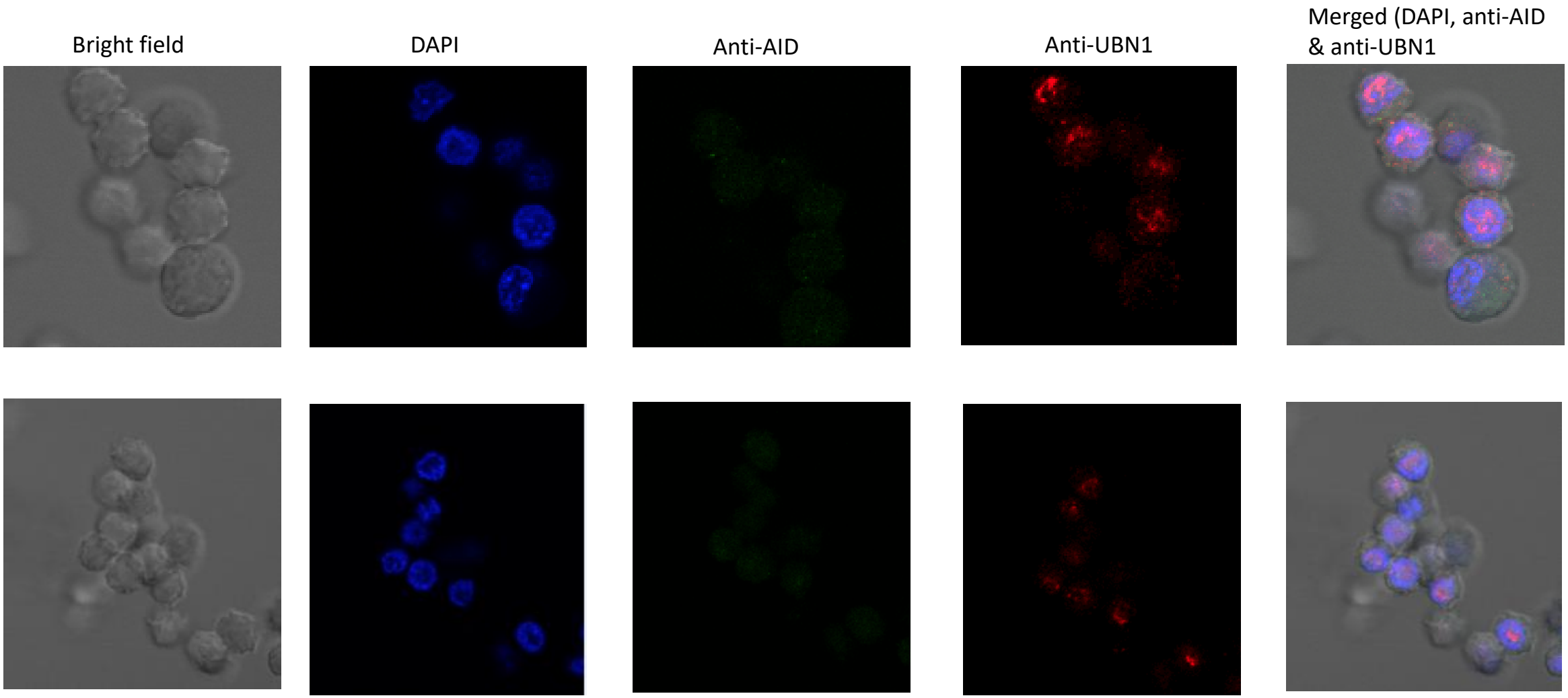

Fig. S7. Co-localization of AID with UBN1 in DT40 AID KO cells. Cells were fixed and double stained with monoclonal anti-AID (green) and polyclonal anti-UBN1 (red) antibodies. AID is predominantly located in the cytoplasm whereas UBN1 is mostly inside the nucleus.

Fig. S8

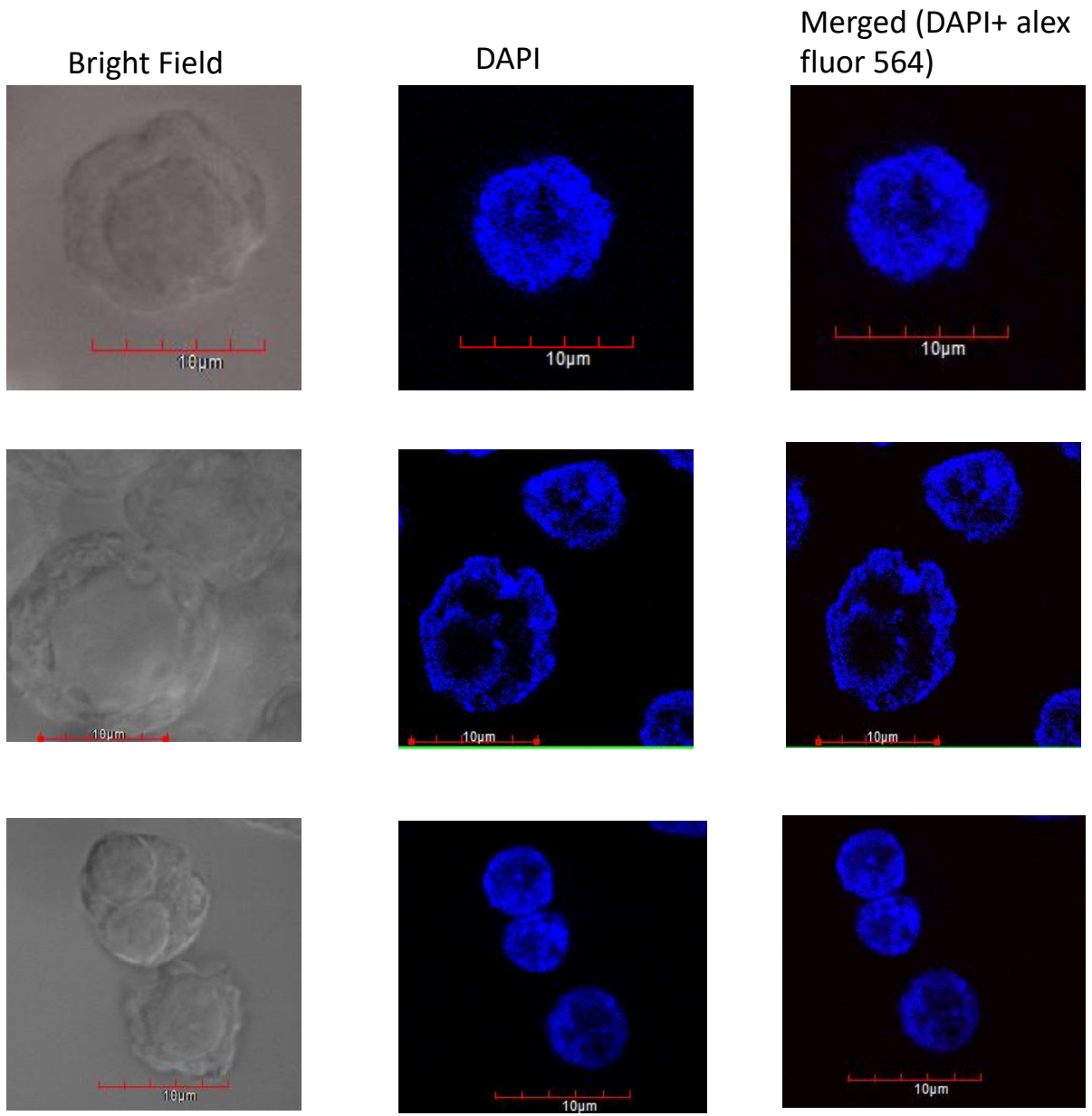

Fig. S8. Proximity ligation assay for AID and UBN1. Images demonstrate the PLA signals in DT40ΨV KO cells and the proximity between two proteins, AID and UBN1. Nuclei were stained with DAPI. Anti-AID and anti-UBN1 primary antibodies were incubated, followed by incubation with secondary antibodies conjugated to oligonucleotides (PLA Probes anti-mouse MINUS and PLA Probes anti-rabbit PLUS).

Fig. S9

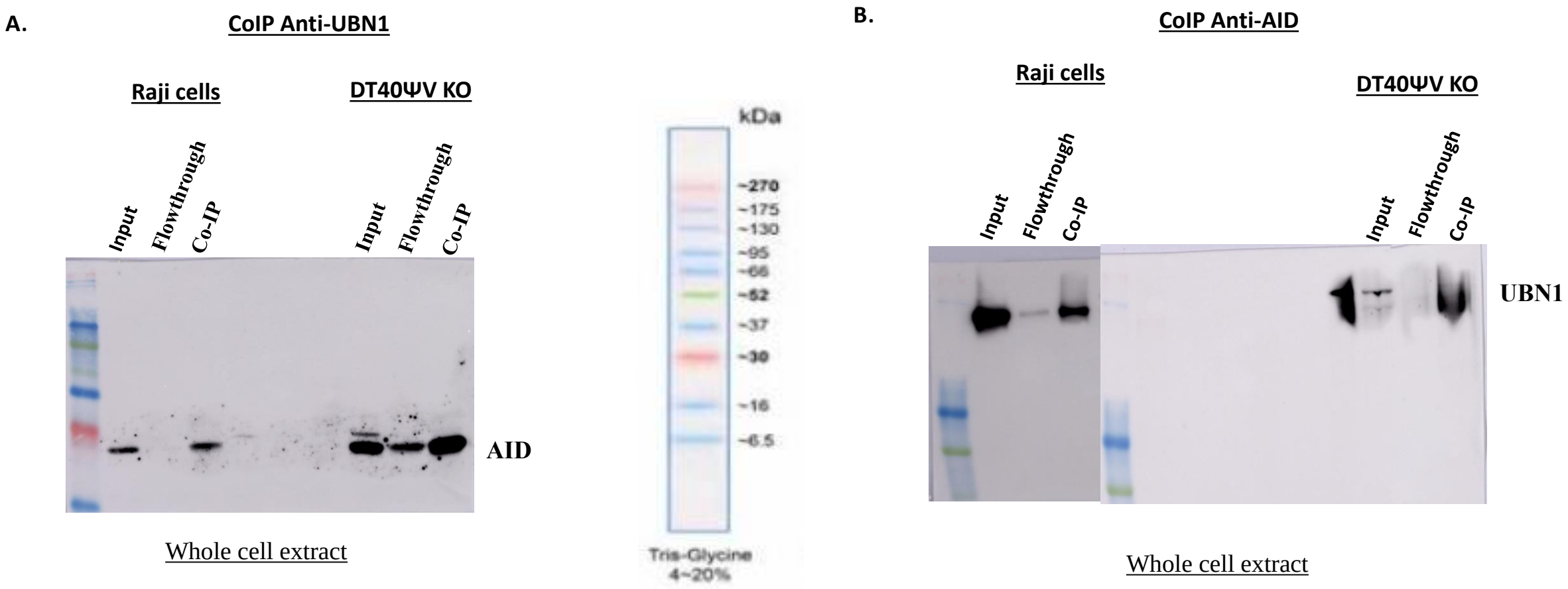

Fig. S9. The co-immunoprecipitation original image used to create fig. 3. AID interaction with UBN1 in Raji and DT40ψV KO cells. A. Co-immunoprecipitation of UBN1 from whole cell lysate was performed of Raji & DT40 ψV KO cells using anti-UBN1 pAb and proteinA/G beads, detection via WB using anti-AID mAb. B. Co-immunoprecipitation of AID from whole cell lysate of Raji & DT40 ψV KO cells using anti-AID mAb and protein A/G beads, detection via WB using anti-UBN1 mAb.

Table. S1. Binding free energy components (kcal/mol) for the binding of the UBN1-AID complex. **ΔE_{vdW}:** van der Waals energy; **ΔE_{elec}:** electrostatics energy in the gas phase; **ΔG_{pol}:** polar solvation energy; **ΔG_{np}:** nonpolar solvation energy; **ΔG_{bind}:** total binding free energy.

	ΔE_{vdW}	ΔE_{elec}	ΔG_{pol}	ΔG_{np}	ΔE_{MM}[a]	ΔG_{solv}[b]	ΔG_{bind}[c]
UBN1-Aid complex	-29.90 (0.07)	-167.05 (0.59)	169.88 (0.54)	-4.13 (0.01)	-196.94 (0.58)	165.76 (0.54)	-31.18 (0.11)

a $=\Delta E_{vdW} + \Delta E_{elec}$

b $=\Delta G_{pol} + \Delta G_{np}$

c $=\Delta E_{vdW} + \Delta E_{elec} + \Delta G_{pol} + \Delta G_{np}$