Supplementary Information

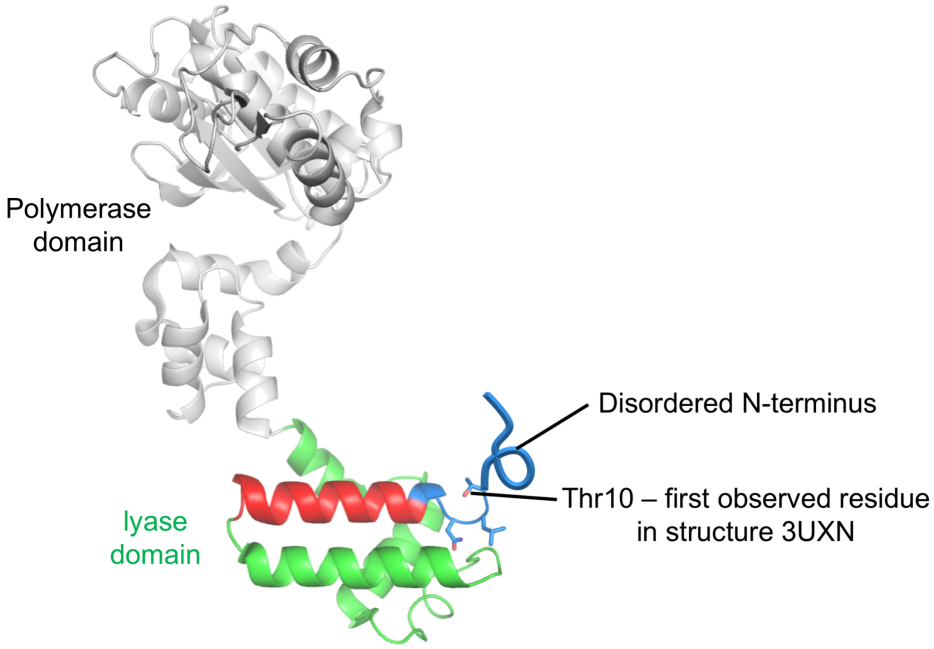
Supplementary Table S1. Quantitation of pol β in mouse fibroblast cells by immunoblotting

Cell line	Relative pol β
pol β+/+	0.53
pol β-/-	0
pol β WT 96	0.48
pol β(R4S,K5S) 18	0.53

Wild-type, pol β null (-/-), pol β WT 96, and pol β(R4S,K5S) 18 cells were seeded in 100 mm dishes at 10^6 cells/dish and harvested when cells were 90% confluent. Cells were washed in phosphate buffered saline (PBS) and collected by scraping, suspended in two volumes of lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.2% Triton X-100, and 0.3% NP-40) containing 1 x Halt protease and phosphatase inhibitors (ThermoFisher) and incubated on ice for 30 min. After agitating the tubes briefly, the lysates were centrifuged at 20,800 × g for 15 min at 4° C, and the supernatant fraction was removed. Protein concentrations were determined using the Bio-Rad protein assay with bovine serum albumin (BSA) as standard.

60 µg of prepared cell lysates were separated by 4-20% Tris-Glycine SDS-PAGE. The proteins were then transferred onto a nitrocellulose membrane with a Trans-Blot Turbo transfer system using the standard 30 min setting for mini blots. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) containing 0.1% (v/v) Tween-20 (TBS-T) and probed with the anti-pol β [18S] monoclonal antibody (ab3181, 1:500 dilution; Abcam). Detection was by ECL following incubation with secondary antibody conjugated to HRP. Membrane was imaged using the ChemiDoc MP (Bio-Rad) imager. The blot was stripped with Gentle Review Stripping buffer (VWR) for 30 min at 37°C, then washed twice for 30 min with room temperature TBS-T. After stripping, the membrane was then probed with anti-Tubulin (1:10,000; Sigma-Aldrich) as a loading control.

Quantification of pol β protein bands was conducted by densitometry using the Band Analysis tools of ImageLab software version 4.1 (Bio-Rad). Density of pol β bands was divided by the density of the loading control for each cell line of interest.

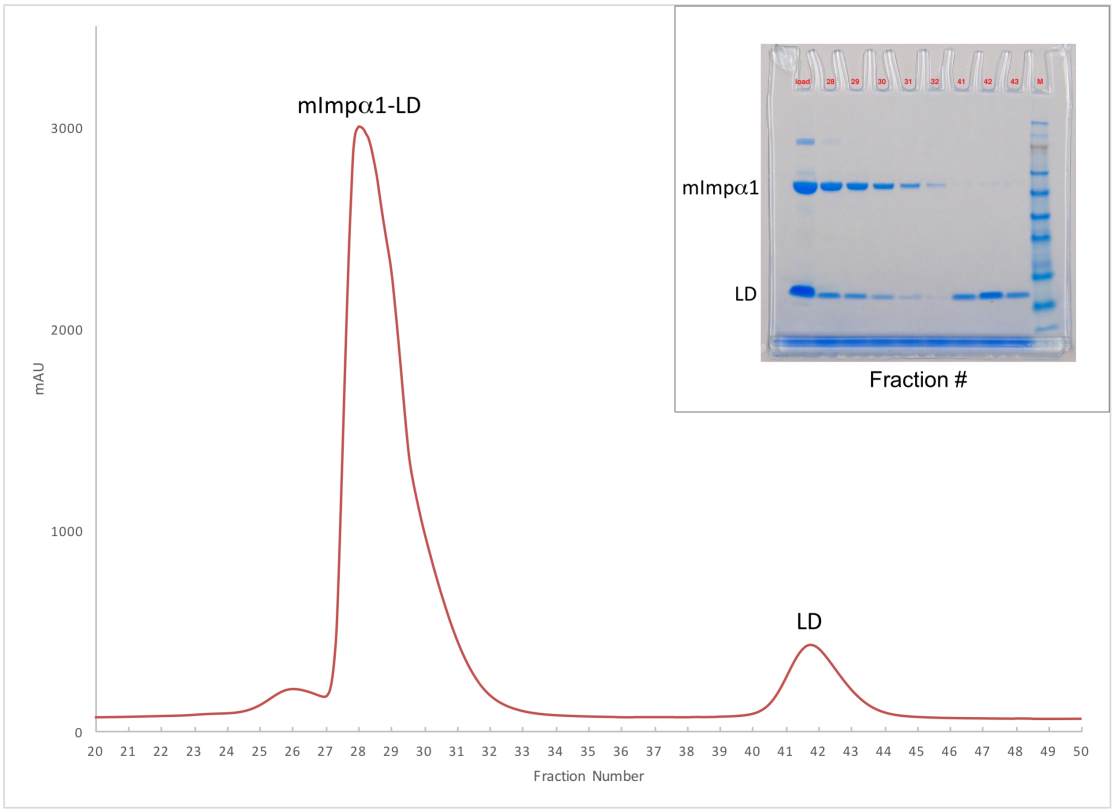


NLS Mapper-identified bipartite sequence: KRKAPQETLNGGITDMLTELANFEKNVS

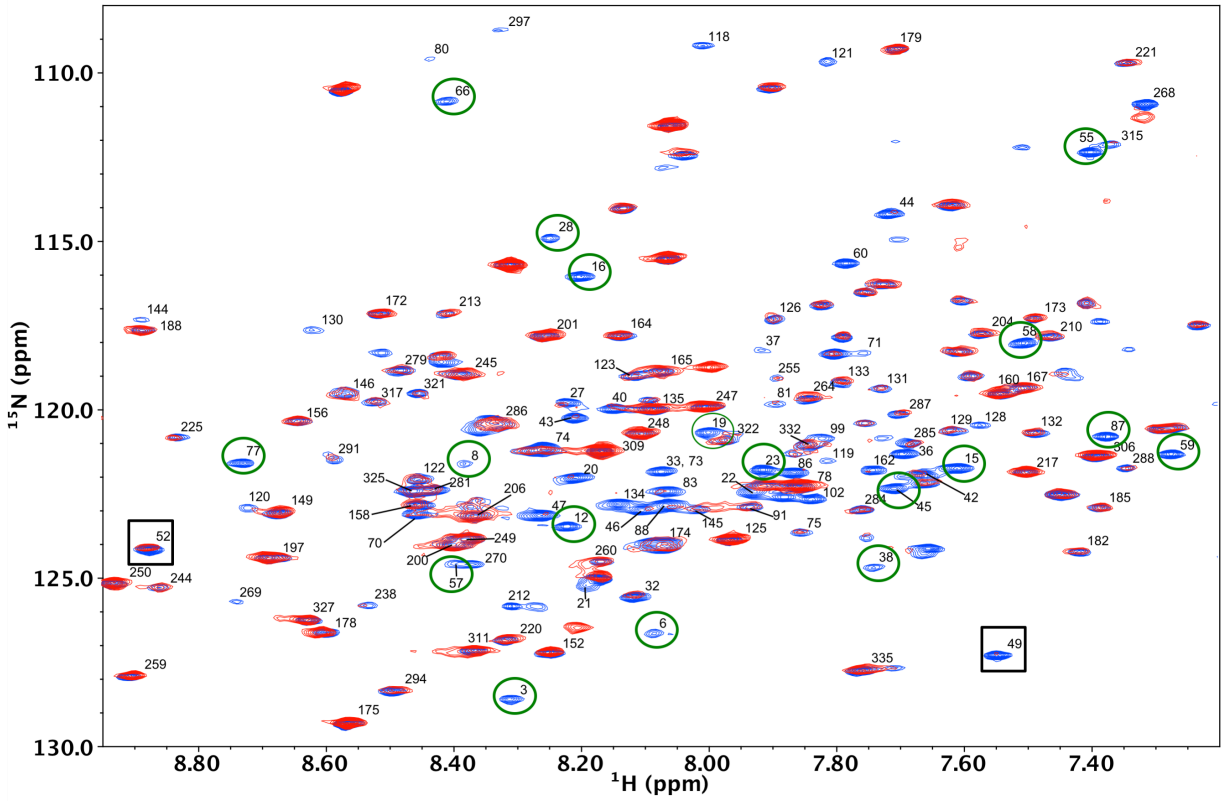
NLS Sequence studied in this paper: SKRKAPQETLNGG

Supplementary Figure S1. Ribbon diagram of unliganded pol β showing the polymerase (gray) and lyase (green) domains. The N-terminal bipartite sequence identified by NLS Mapper (red + blue residues) includes the entire N-terminal helix (helix A). Interaction with the nuclear transport proteins would require complete unfolding of helix A and, in turn, the entire N-terminal lyase domain. Alternatively, the N-terminal sequence shown in blue includes several basic residues and is mostly disordered making it available for interaction with Impα.

Supplementary Figure S2. Interaction of pol β lyase domain with mImpα1ΔIBB. A sample of mImpα1ΔIBB was mixed with 2.8-fold excess of pol β lyase domain and the sample was eluted on a HiLoad 26/60 Superdex 200 column (GE Healthcare) with a buffer containing 20 mM Tris-HCL, pH 7.8, 125 mM NaCl, 2 mM DTT, 1 mM EDTA. SDS PAGE shows that the complex elutes in fractions 28-32 which contain both proteins while the excess pol β lyase domain elutes in fractions 41-43.

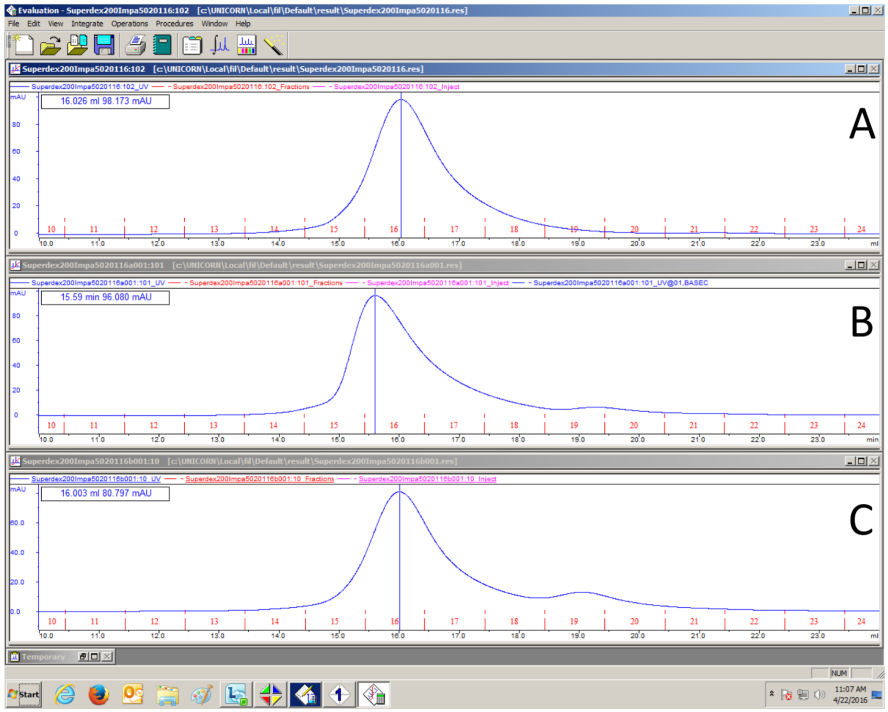


Supplementary Figure S3. Expanded region of the ^{1}H-^{15}N HSQC NMR spectrum of the pol β-mImpα1ΔIBB complex. The expanded spectral region corresponds to the sample shown in Figure 1A. Blue spectrum: U-[^{2}H,^{15}N]pol β; red spectrum: U-[^{2}H,^{15}N]pol β + mImpα1ΔIBB. Circled peaks correspond to lyase domain resonances that become broadened in the mImpa1ΔIBB complex, while a few resonances (such as Lys52 indicated by a square) resist most of the broadening, probably due to greater internal motional freedom.



Supplementary Figure S4. Interaction of the pol β lyase domain with Impα5. Analytical gel filtration experiments performed on mixtures of hImpα5ΔIBB and either wild-type or pol β(R4S,K5S) lyase domain. For these experiments, hImpα5ΔIBB was mixed with 2-fold excess of wild-type or the NLS variant pol β(R4S,K5S) lyase domain and the samples were eluted at a flow rate of 1 ml/min on a Superdex 200 10/300 GL column (GE Healthcare) with a buffer containing 20 mM HEPES, 125 mM NaCl, 5mM DTT, 1mM EDTA, pH 7.4.

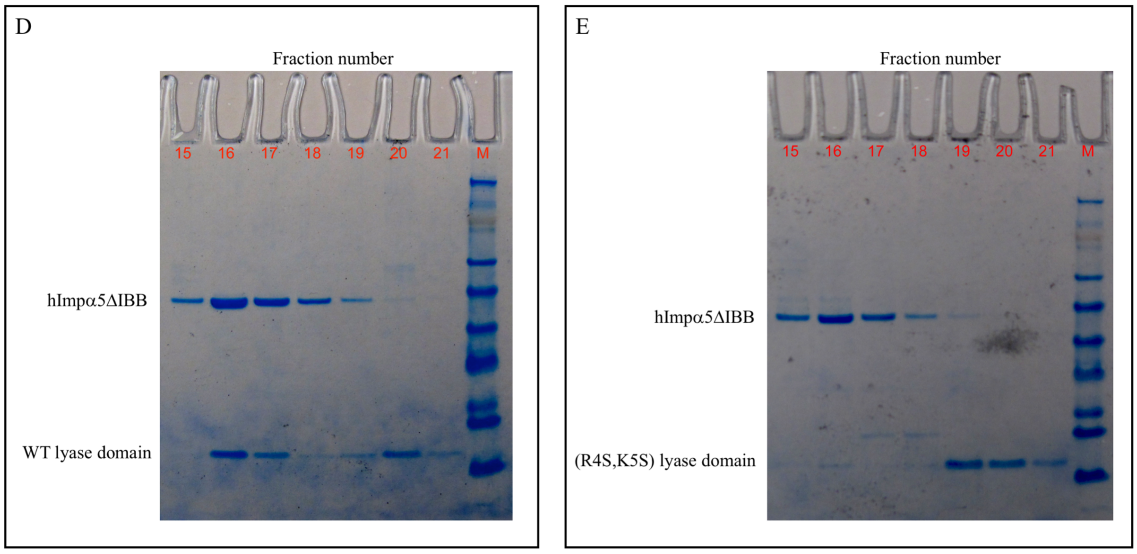
A) Impα5ΔIBB elutes as a single entity; B) a complex of Impα5ΔIBB with pol β lyase domain elutes earlier than Impα5ΔIBB alone; C) a mixture of Impα5ΔIBB with pol β(R4S,K5S) lyase domain elutes as two separate proteins—no complex is formed; the vertical line in each chromatogram shows the elution position of the Impα5ΔIBB peak (panels A and C) or the Impα5ΔIBB/pol β lyase domain (panel B); D) SDS PAGE confirms the coelution of Impα5ΔIBB and wild-type pol β lyase domain; E) SDS PAGE confirms that Impα5ΔIBB and pol β(R4S,K5S) lyase domain do not form a complex.



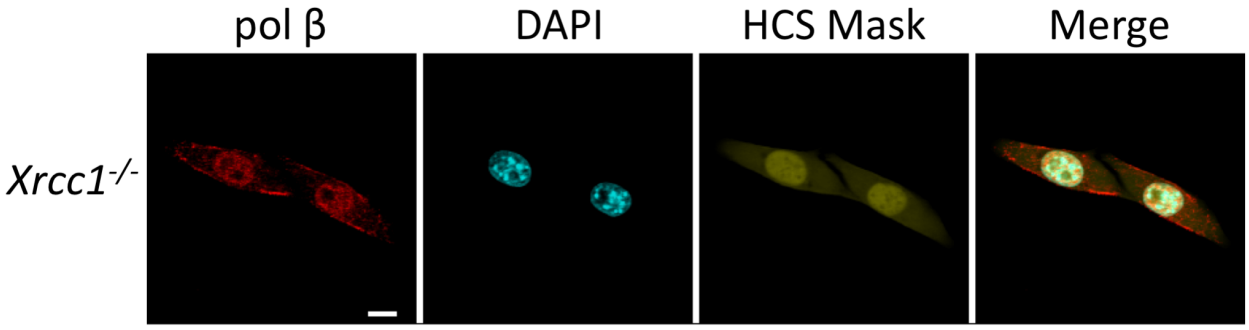
Impα5ΔIBB alone

Impα5ΔIBB
+ pol β lyase domain

Impα5ΔIBB
+ pol β (R4S,K5S) lyase domain


D
Fraction number
15
16
17
18
19
20
21
M
hImpα5ΔIBB
WT lyase domain
E
Fraction number
15
16
17
18
19
20
21
M
hImpα5ΔIBB
(R4S,K5S) lyase domain

Supplementary Figure S5. Localization of pol β in *Xrcc1-/-* cells. The nuclear/cytoplasmic pol β ratio obtained in the *Xrcc1*-/- cell line, β_N/β_C =3.4 ± 0.13 (n=74) is similar to the value obtained in the *Xrcc1*-/- cells transfected with XRCC1, β_N/β_C =3.77 ± 0.28 (n = 50), where n is the number of cells evaluated and results are given as the ratio ± standard error. Scale bar is 10 μm. Cotransport behavior (or its absence) is not expected to vary with the R4S,K5S mutations; N-terminal pol β mutations will not affect the interaction with XRCC1, since this interaction involves only the C-terminal nucleotide-binding sudomain of pol β.



Supplementary Figure S6. Sequence alignment of pol β N-terminal residues. A comparison of the N-terminal sequences of pol β from multiple species shows that the NLS sequence is highly conserved among mammals, fish, birds, amphibians, reptiles, and insects. The amino acid abbreviations in red show the instances of variation from the sequence of pol β of rat.