Supplementary Figures Legends

Figure S1. Validation of C-NHEJ ligation in the end-joining assay.

(A) End-joining assay catalyzed with EcoRI linearized pBluescript-KS-II(-)plasmid and HeLa extracts under standard reaction conditions in the presence of anti-XRCC4 preimmune serum (lane 2), DNA-PK specific inhibitor NU7026 (lane 1) or anti-XRCC4 serum (lane 3). DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining.

(B) End-joining assay catalyzed under standard reaction conditions with the indicated plasmids restricted as specified and HeLa extracts, in the presence or not DNA-PK specific inhibitor NU7026. DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining. pBS, Eco Bam, Hind stand for pBluescript-KS-II(-), EcoRI, BamHI and HindIII, respectively.

Figure S2. Effect of a non telomeric sequence on end-joining and DNA-PK activities at 3'telomeric ends.

(A) End-joining assay catalyzed under standard reaction conditions with unbiotinylated (pT) or biotinylated (biopT) plasmids as indicated and HeLa extracts. DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining.

(B) DNA-PK assay catalyzed under standard conditions with the indicated DNA fragments and HeLa extracts, in the presence of streptavidin or DNA-PK specific inhibitor NU7026. DNA-PK peptide substrate was isolated by polyacrylamide denaturing gel electrophoresis followed by autoradiography of the gel.

Figure S3. Codepletion of TR2/RAP1 with TRF1.

Western blotting analysis of HeLa protein extracts after immuno-depletion as indicated. Protein samples were denatured and separated on 8% SDS-PAGE gel followed by electrotransfer on membrane and blotting with the antibodies as indicated.

Figure S4. Characterization of end-joining activity in the absence of LIG4.

(A) Western blotting analysis of Nalm6 (*LIG4*$^{+}$) and N114P2 (*LIG4*$^{-}$) protein extracts after immuno-depletion as indicated. Protein samples were denatured and separated on 8% SDS-PAGE gel followed by electrotransfer on membrane and blotting with the antibodies as indicated.

(B) End-joining assay catalyzed under standard reaction conditions with the indicated plasmids and Nalm6 (*LIG4*$^{+}$) or N114P2 (*LIG4*$^{-}$) extracts depleted as specified. DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining.

(C) End-joining assay catalyzed under standard reaction conditions with biopT3' plasmid and KU-depleted N114P2 *LIG4*$^{-}$ extracts in the presence NU7026 or βNAD as indicated. DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining.
(D) End-joining assay catalyzed under standard reaction conditions with biopT3' plasmid and Nalm6 *LIG4*+ or N114P2 *LIG4*$^{-}$ extracts depleted as specified, in the presence anti-XRCC4 preimmune (PI) or immune (I) serum. DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining.

Figure S5. Joining of compatible or non compatible DNA ends under C- or B-NHEJ conditions.
End-joining assay catalyzed with low concentration of pBS plasmid bearing non compatible ends (HindIII/ BamHI, nonC) or compatible ends (HindIII, C) and *LIG4*$^{+}$ IgG-depleted extracts (C-NHEJ conditions) or *LIG4*$^{-}$ KU-depleted extracts (B-NHEJ conditions).
DNA ligation products were separated by agarose gel electrophoresis. Migration was without ethidium bromide (BET) intercalating dye and then staining with SYBR-Green (left gel) or in the presence of BET (right gel). Plasmids species are dimer (D), multimers (M), relaxed circle (RC), linear monomer (LM), supercoiled circles (SC).

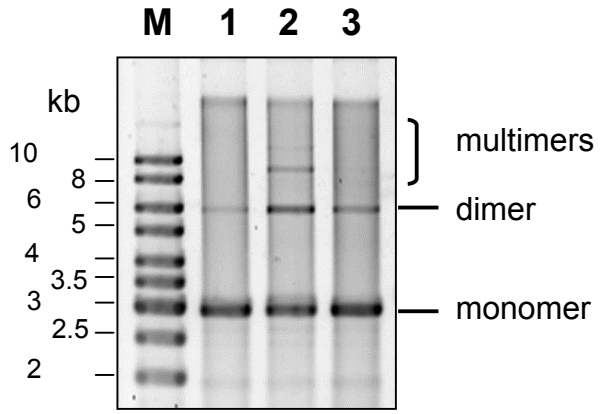
Figure S6. Effect of KU or DNA-PK-depletion on end-joining activity in the absence of LIG4.
End-joining assay catalyzed under standard reaction conditions with the indicated plasmids and N114P2 *LIG4*$^{-}$ extracts depleted as specified. DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining. For each plasmid substrate, relative ligation efficiency was calculated as the % of ligation obtained in each case related to that obtained with IgG-depleted control extracts.

Figure S7. Analysis of the amount of endogenous KU protein.
Western blotting analysis of N114P2 (*LIG4*$^{-}$) protein extracts (WCE, 40 μg) and increasing amount of purified KU protein as indicated. Protein samples were denatured and separated on 8% SDS-PAGE gel followed by electrotransfer on membrane and blotting with the antibodies as indicated.

Figure S8. Effect of TRF2/RAP1 antibodies on B-NHEJ activity
End-joining assay catalyzed under standard reaction conditions with plasmids as indicated and KU-depleted N114P2 *LIG4*$^{-}$ extracts in the presence or not of TRF2/RAP1 antibodies. DNA ligation products were separated by agarose gel electrophoresis followed by SYBR-Green staining.

Figure S1

A


M
1
2
3
kb
10
8
6
5
4
3.5
3
2.5
2
multimers
dimer
monomer

B

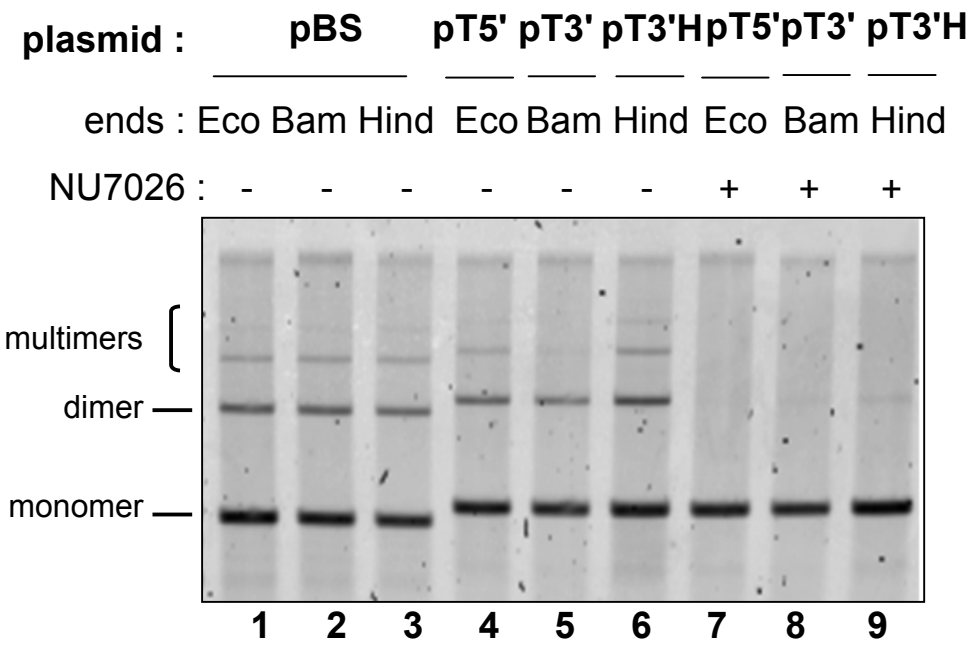
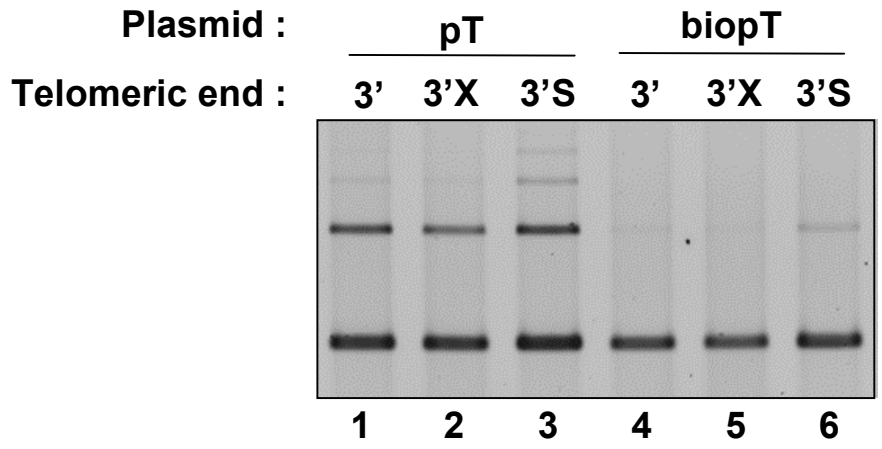

plasmid :
pBS
pT5'
pT3'
pT3'H
pT5'
pT3'
pT3'H
ends : Eco Bam Hind Eco Bam Hind Eco Bam Hind
NU7026 : - - - - - - + + +
multimers
dimer
monomer
1 2 3 4 5 6 7 8 9

Figure S2

A


Plasmid :
pT
biopT
Telomeric end :
3'
3'X
3'S
3'
3'X
3'S
1
2
3
4
5
6

B

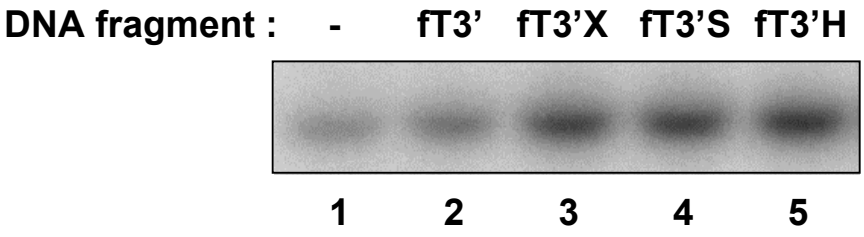

DNA fragment :
-
fT3'
fT3'X
fT3'S
fT3'H
1
2
3
4
5

Figure S3

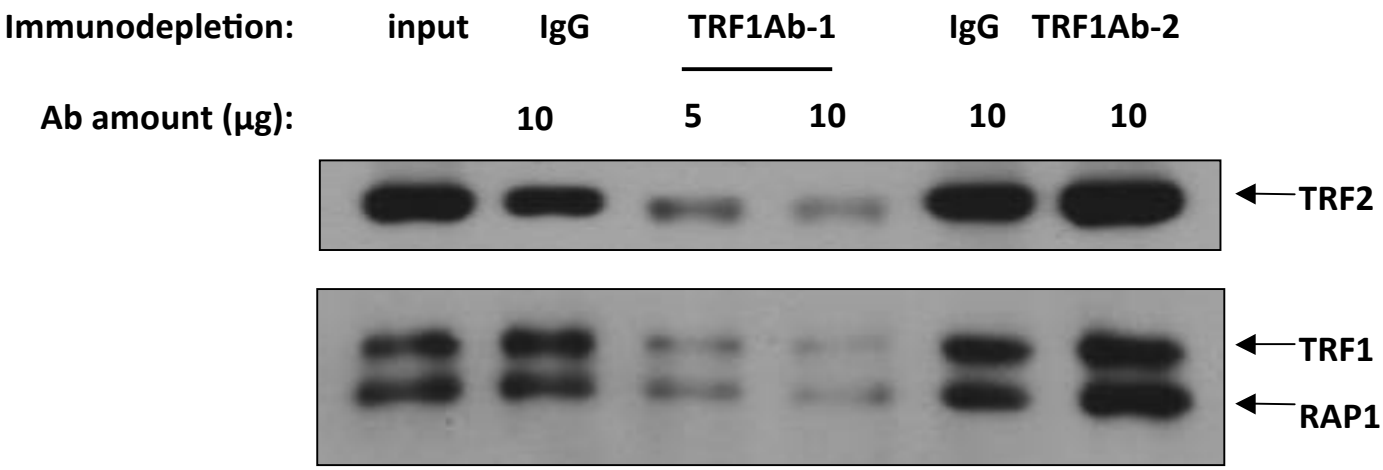

Immunodepletion:
input
IgG
TRF1Ab-1
IgG
TRF1Ab-2
Ab amount (μg):
10
5
10
10
10
TRF2
TRF1
RAP1

Figure S4

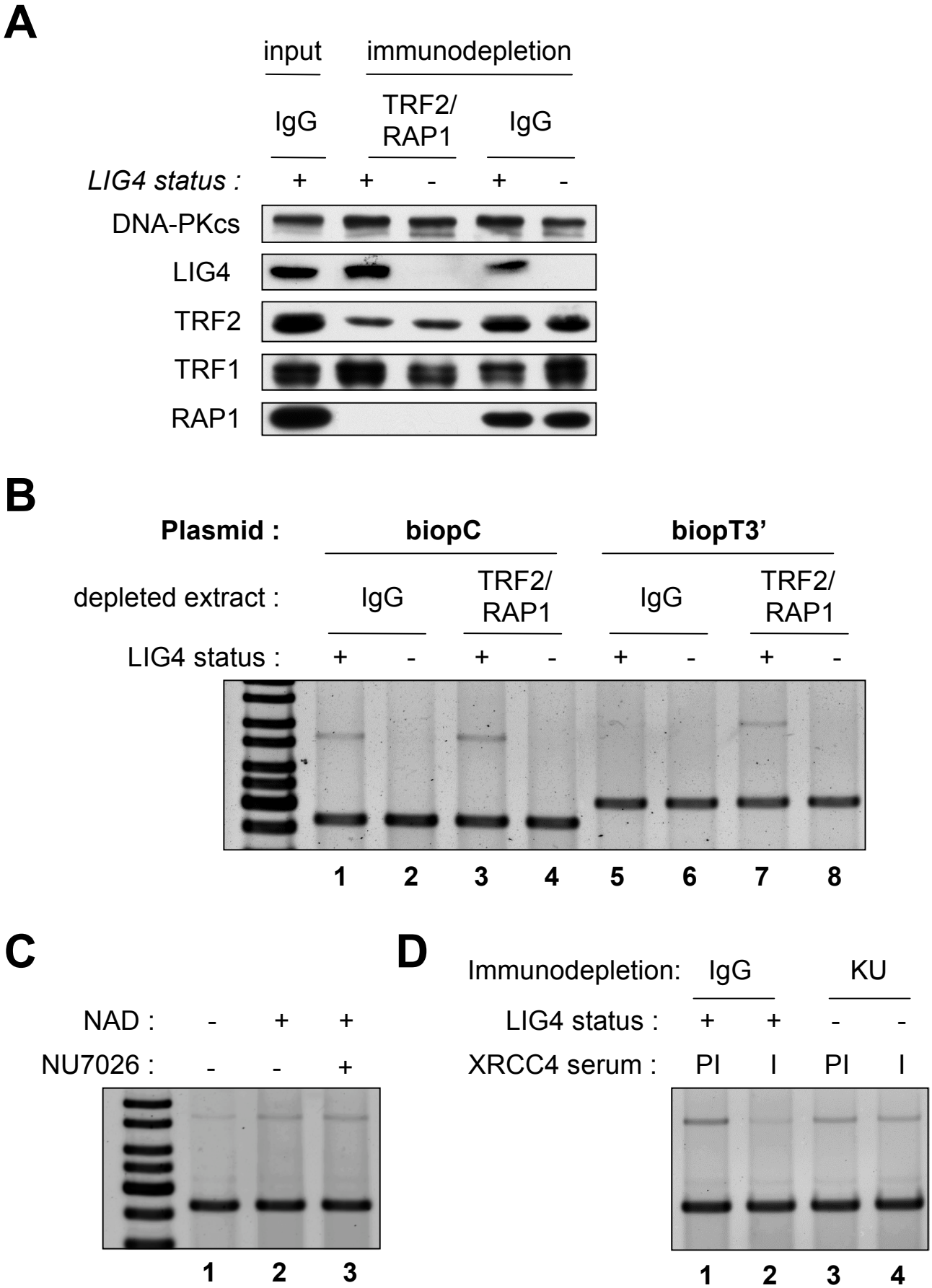

A
input
immunodepletion
IgG
TRF2/ RAP1
IgG
LIG4 status :
+ + - + -
DNA-PKcs
LIG4
TRF2
TRF1
RAP1
B
Plasmid :
biopC
biopT3'
depleted extract :
IgG
TRF2/ RAP1
IgG
TRF2/ RAP1
LIG4 status :
+ - + - + - + -
1 2 3 4 5 6 7 8
C
NAD : - + +
NU7026 : - - +
1 2 3
D
Immunodepletion: IgG KU
LIG4 status : + + - -
XRCC4 serum : PI I PI I
1 2 3 4

Figure S5

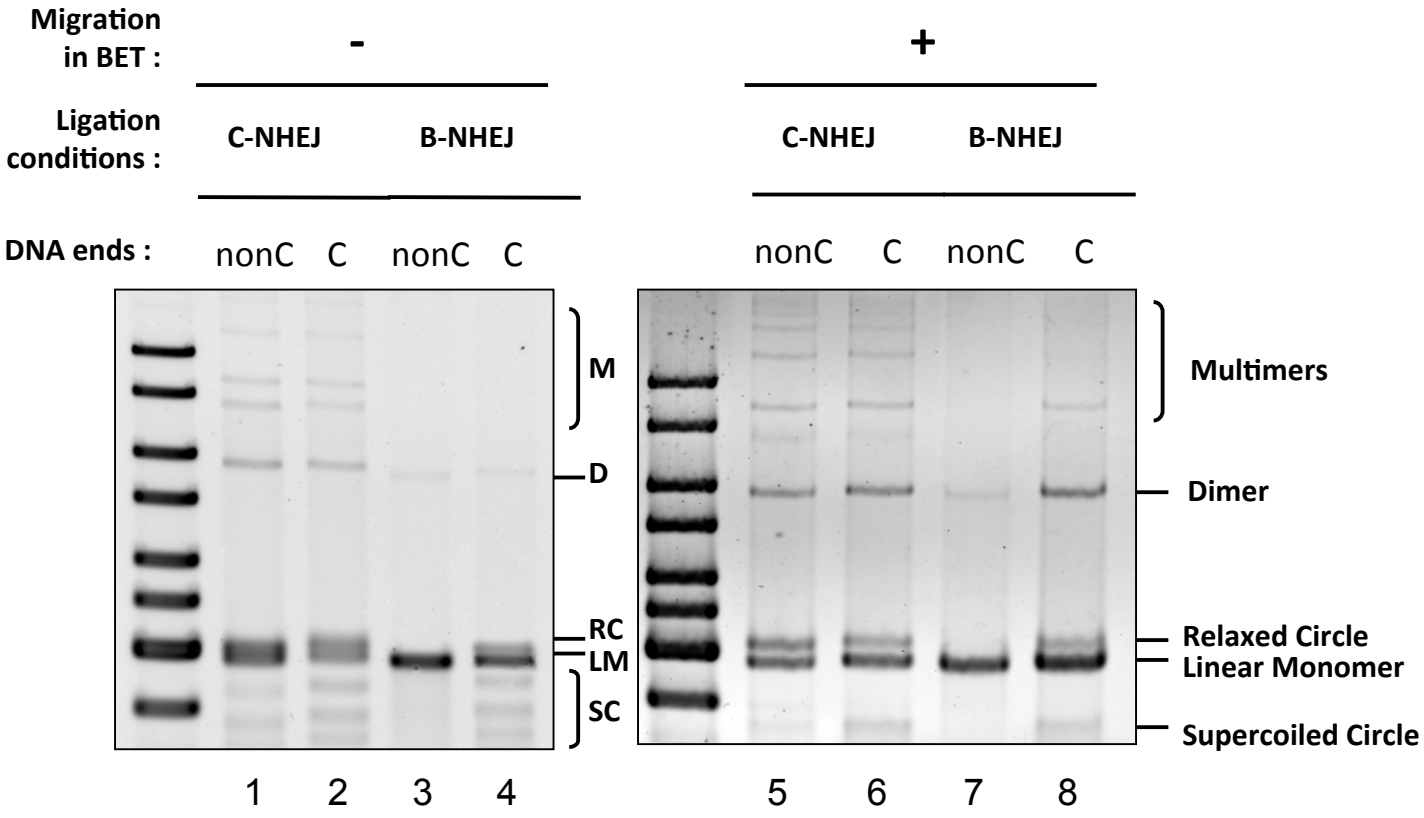

Migration in BET :
-
+
Ligation conditions :
C-NHEJ
B-NHEJ
C-NHEJ
B-NHEJ
DNA ends :
nonC
C
nonC
C
nonC
C
nonC
C
M
D
RC
LM
SC
Multimers
Dimer
Relaxed Circle
Linear Monomer
Supercoiled Circle
1
2
3
4
5
6
7
8

Figure S6

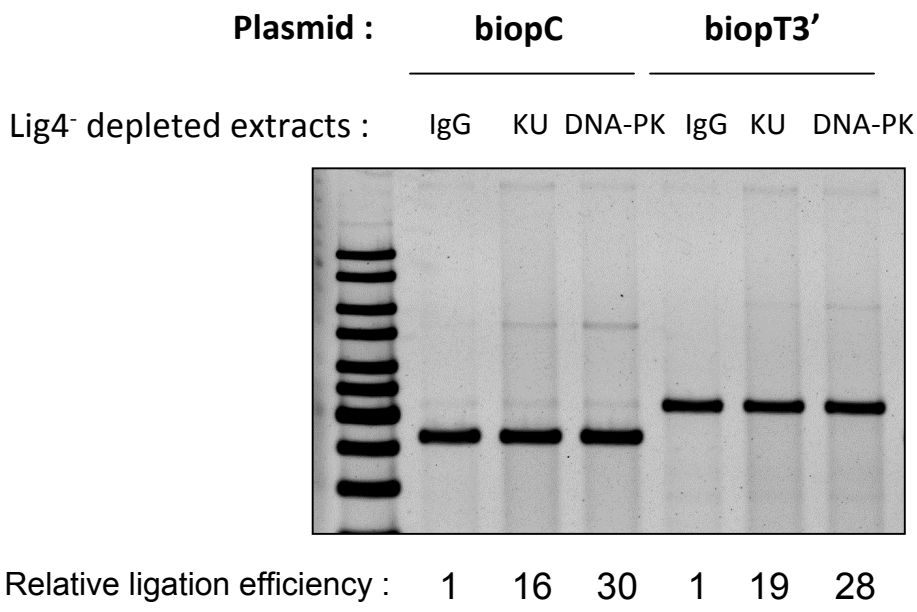

Plasmid :
biopC
biopT3'
Lig4- depleted extracts :
IgG
KU
DNA-PK
IgG
KU
DNA-PK
Relative ligation efficiency :
1
16
30
1
19
28

Figure S7

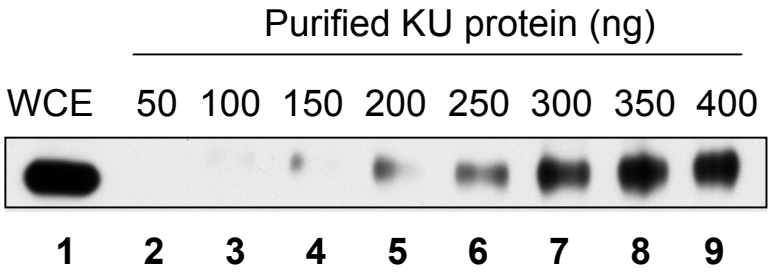

Purified KU protein (ng)
WCE
50
100
150
200
250
300
350
400
1
2
3
4
5
6
7
8
9

Figure S8