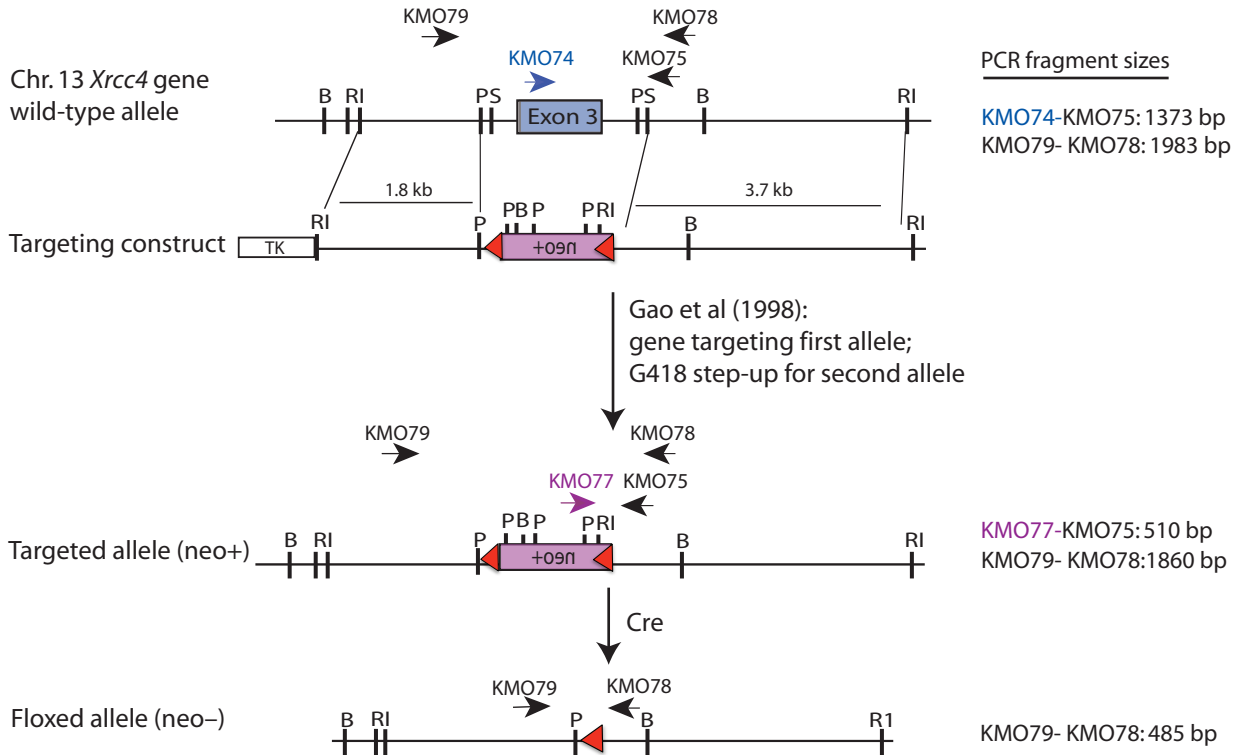


Alternative end-joining is suppressed by the canonical NHEJ component
Xrcc4/ligase IV during chromosomal translocation formation
 Deniz Simsek and Maria Jasin

Supplementary Figure 1

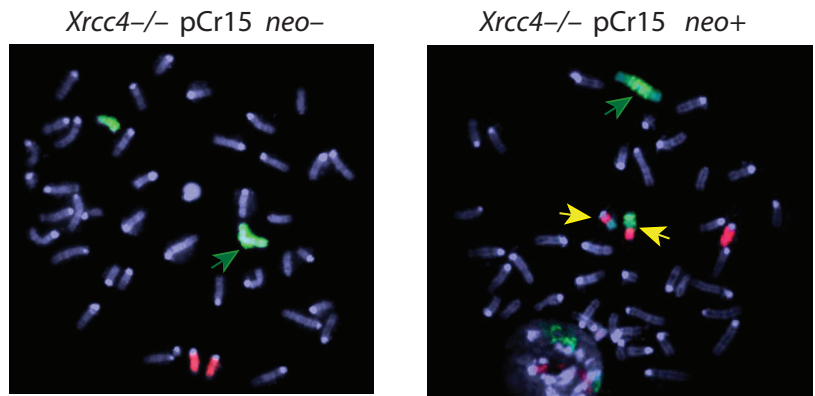
a *Xrcc4* allelic series (chr. 13)



b *Xrcc4* allelic series primers

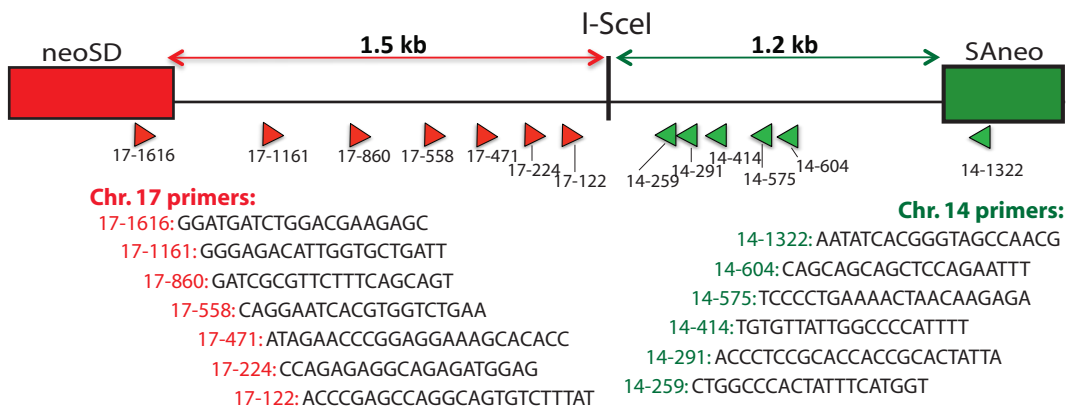
KMO74: GGAGATTCCCAAGAAGCTGATGAC
 KMO75: TCCAGCTAACCAGCATCAATA
 KMO77: CTGCTAAAGCGCATGCTCCAGACTGC
 KMO78: ATATCTGGAACGCAGCCACT
 KMO79: TCCTGCATGGAGCATTATCA

c Chromosome FISH



chr. 14, green; chr. 17, red

d Der(17) break point junction primer positions



Supplementary Figure 1

- (a) Derivation of *Xrcc4*^{-/-} mouse ES cells used in this study. Gene targeting was previously performed to delete exon 3 of *Xrcc4*, which is predicted to result in expression of the first 46 amino acids of the *Xrcc4* protein, followed by a frameshift and termination in exon 4²⁶. Gene targeted clones were then subjected to increased G418 concentrations to identify cells in which the *neo*+ gene replaced exon 3 of both *Xrcc4* alleles²⁶. To delete the *neo*+ gene from both alleles at the *loxP* sites (red triangles), Cre recombinase was expressed and *neo*- clones were identified.
- RI, EcoRI; B, BamHI; P, PstI; S, SacI; H, HindIII.
- (b) Primer sequences used for *Xrcc4*^{-/-} genotyping.
- (c) Fluorescence *in situ* hybridization (FISH) of parental *Xrcc4*^{-/-} pCr15 cells and a *neo*+ translocation clone derived from them. The translocation clone has the expected derivative chromosomes (yellow arrows) derived from chrs. 17 and 14. Parental cells and all *neo*+ clones derived from them also contain a pre-existing Robertsonian translocation involving chr.14 (green arrow) that is not involved in I-SceI induced translocations since the *SAneo* gene (Fig. 1) is integrated into the normal chr.14 in the cells.
- (d) Der(17) primers used to PCR amplify breakpoint junctions of *neo*+ translocation clones. Each primer is named for the chromosome of origin, followed by the distance to the I-SceI site (e.g., 17-1616 means that the primer is 1,616 bp away from the I-SceI site on chr.17).

Supplementary Figure 2

a WILD-TYPE

Chr. 17

CCGAGTTC AAGGCCAGCC **TAGGGATAA**
GGCTCAAGTTC CGGTCGGATCCC

Chr. 14

CCCTAACAGGGTAATGCATGCAA
AATAGGGATTGTCCCATTACGTACGTT

TRANSLOCATION CLONES	TRANSLOCATION JUNCTIONS	
E13P10	CCGAGTTC AAGGCCAGCC TAGGGAT TATCCCTAACAGGGTAATGCATGCAA
E14P8	CCGAGTTC AAGGCCAGCC TAGGGATAA	+126bp .. TCCCTAACAGGGTAATGCATGCAA
E13P18, E14P3	CCGAGTTC AAGGCCAGCC TAGGGATA TCCCTAACAGGGTAATGCATGCAA
E13P24	CCGAGTTC AAGGCCAGCC TAGGGAT .. TA CCCTAACAGGGTAATGCATGCAA
E13P6	CCGAGTTC AAGGCCAGCC TAGGGATA TACAGGGTAATGCATGCAA
E13P7	CCGAGTTC AAGGCCAGCC TA CCCTAACAGGGTAATGCATGCAA
E14P7	CCGAGTTC AAGGCCAGCC TAGGGA ACAGGGTAATGCATGCAA
E14P10	CCGAGTTC AAGGCCAGCC TAGGGATAA T GGTAATGCATGCAA
E13P13	CCGAGTTC AAGGCCAGCC ..	+47bp .. TCCCTAACAGGGTAATGCATGCAA
E13P4	CCGAGTTC AAGGCCAGCC TA ACAGGGTAATGCATGCAA
E13P14	CCGAGTTC AAGGCCAGC TACAGGGTAATGCATGCAA
E14P5, E14P18	CCGAGTTC AAGGCCAGCC TAGGG GTAATGCATGCAA
E14P6, E13P11	CCGAGTTC AAGGCCAGCC TAGGGATAA TGCATGCAA
E13P1, E14P4	CCGAGTTC AAGGCCAGCC TAGGG TAATGCATGCAA
E13P19	CCGAGTTC AAGGCCAGCC TAG TAATGCATGCAA
E13P5	CCGAGTTC AAGGCCAGCC TAGGGAT GCATGCAA
E13P20	CCGAGTTC AAGGCCAGCC TA TAATGCATGCAA
E14P19	CCGAGTTC AAGGCCAG GGTAATGCATGCAA
E14P24	CCGAGTTC A AATGCATGCAA
E14P14	CCGAGTTC AAGGCCAGC AAGC
E13P3	CCGAGTTC AAGGCCAGCC TAGG -33del CTC
E14P22	CCGAGTTC A -40del ATG
E13P8	TAG -35del -28del CTT
E14P18	TAG -43del	TAACTGGTAATGCATGCTTT -32del GCT
E13P23	GCC -136 del CTAACAGGGTAATGCATGCAA
E14P17	CCGAGTTC AAGGCCAGCC TA ..	+184bp -177del AAT
E14P13	GCT -155del -35del CCG
E13P12	CCGAGTTC AAGGCCAGCC TAGGGATA -190del CAT
E13P21	TTT -62del -230del AAG
E14P15	TAGGGT -282del AATGCATGCAA
E14P16	AAT -99del -214del TAA
E14P20	TGG -353del	GGCCTCTA .. GGTAATGCATGCAA
E14P9	CCGAGTTC AAGGCCAGCC TA ..	+376bp -362del ACC
E13P17	AGA -219del -179del ATA
E13P15	CGA -125del -485del AGC
E14P21	CCGAGTTC AAGGCCAGCC TAGGGA -838del GTC
E13P16	CCGAGTTC AAGGCCAGCC TAG ..	A -994del TTG

Supplementary Figure 2, cont'

b *Xrcc4*^{-/-} cells

Chr. 17

CCGAGTTC AAGGCCAGCC TAGGGATAA
GGCTCAAGTTC CGGTCGGATCCC

Chr. 14

CCCTAACAGGGTAATGCATGCAA
AATAGGATTTGCCATTACGTACGTT

TRANSLOCATION CLONES	TRANSLOCATION JUNCTIONS	
E13U25	CCGAGTTC AAGGCCAGCC TAGGGATAA	CAGGG .TATCCCTAACAGGGTAATGCATGCAA
E13U6	CCGAGTTC AAGGCCAGCC TAGGGATAA	.TATCCCTAACAGGGTAATGCATGCAA
E13U28, E13U38	CCGAGTTC AAGGCCAGCC TAGGGATAA	..TCCCTAACAGGGTAATGCATGCAA
E13U31	CCGAGTTC AAGGCCAGCC TAGGGAT..	.TATCCCTAACAGGGTAATGCATGCAA
E14U3	CCGAGTTC AAGGCCAGCC TAGGGATAA	..TCCCTAACAGGGTAATGCATGCAA
E14U23	CCGAGTTC AAGGCCAGCC TAGGG....	.TATCCCTAACAGGGTAATGCATGCAA
E14U17	CCGAGTTC AAGGCCAGCC TAGGGA....	..TCCCTAACAGGGTAATGCATGCAA
E14U31	CCGAGTTC AAGGCCAGCC TAGG.....	.ATCCCTAACAGGGTAATGCATGCAA
E14U41	CCGAGTTC AAGGCCAGCC..... ATTGTA	TTATCCCTAACAGGGTAATGCATGCAA
E13U18, E13U35	CCGAGTTC AAGGCCAGCC TAGGGATAACAGGGTAATGCATGCAA
E14U24	CCGAGTTC AAGGCCAGCC TAGGG.... GCTCTAACAGGGTAATGCATGCAA
E13U40	CCGAGTTC AAGGCC..... TAGGGGATAT	TTATCCCTAACAGGGTAATGCATGCAA
E14U43	CCGAGTTC AAGGCCAGCC TAGGGATAA	+87bpGGTAATGCATGCAA
E14U44, E13U12	CCGAGTTC AAGGCC.....TAACAGGGTAATGCATGCAA
E13U10, E13U17, E13U26, E13U	CCGAGTTC AAGGCCAGCC TA.....ACAGGGTAATGCATGCAA
E14U13	CCGAGTTC AAGGCCAGCC TA.....CAGGGTAATGCATGCAA
E13U34	CCGAGTTC AAGGCCAGCC T.....CAGGGTAATGCATGCAA
E13U11, E13U36	CCGAGTTC AAGGCCAGCC TAGGGATAATGATGCATGCAA
E13U15, E13U30, E14U1, E14U19,	CCGAGTTC AAGGCCAGCC TAGGG....TAATGCATGCAA
E13U41	CCGAGTTC AAGGCCAGCC TAGGGATAACATGCAA
E13U29, E13U43	CCGAGTTC AAGGCCAGCC TAGGGA....ATGCATGCAA
E14U14, E14U36	CCGAGTTC AAGGCCAGCC.....AGGGTAATGCATGCAA
E13U23	CCGAGTTC AAGGCCAGCC TAGGGAT..GCATGCAA
E14U25	CCGAGTTC AAGGCCAGCC TAGG.....TAATGCATGCAA
E13U38	CCGAGTTC AAGGCCAGCC TA..... TGGTAATGCATGCAA
E14U2	CCGAGTTC AAGGCCAGCC TAG.....TAATGCATGCAA
E13U32	CCGAGTTC AAGGCCAGCC TAGGGAT..GCAA
E14U39	CCGAGTTC AAGGC..... TGGTAATGCATGCAA
E13U45	CCGAGTTC AAGG.....AATGCATGCAA
E14U21	CCGAGTTC AAGGCC.....TAATGCATGCAA
E14U12	CCGAGTTC AAGG.....TAATGCATGCAA
E13U4	CCGAGTTC AAGGCCAGC.....ATGCAA
E14U11	CCGAGTTC AAGGCCAGC.....AA
E13U5	CCGAGTTC AAGGCCAGCC TAGGGA....	-39del AAT
E13U21	TCC.....TGATGCAA
E14U15	GGCA -32delTGCAA
E13U1	GGCA -32delAGC
E14U32	CCGAGTTC AAGGCCAGCC TAGGGATAA	+231bp -56del AAC
E14U38	AGG -38delCATGCAA
E14U33	AGG -44del	G -35del CCG
E13U16	AAAC -90delAGGGTAATGCATGCAA
E13U33	CCGAGTTC AAGGCCAGCC TA.....	+207bp -95del CAA
E13U9	CCGAGTTC AA.....	+118bp -104del AGA
E14U26	CCGAGTTC AAGGCCAGCC.....	CTTGGGAGG -113del AGC
E14U29	CCGAGTTC AAGGCCAGC.....	-116del TAA
E14U28	GAG -124delTAACAGGGTAATGCATGCAA
E13U27	CCGAGTTC AAGGC.....	+105bp -132del ACT
E14U30	GCCT -165delAACAGGGTAATGCATGCAA
E13U42	GCAG -220delGGTAATGCATGCAA
E13U20	CCGAGTTC AAGGCCAGCC TAGGGATAA	CAAAAT -280del GGA
E13U46	TTAA -147bp	-137del GTA
E14U42	AAGGG -297delTAATGCATGCAA
E14U27	ATT -321delCCTAACAGGGTAATGCATGCAA
E13U22	CAC -134del	-201del GAG
E13U47	CCGAGTTC AAGGCCAGC.....	-319del GAG
E13U3	GGA -151del	-209del GAA
E13U44	CCGAGTTC AAG.....	-463del AAG
E14U37	CCC -52del	-410del GGG
E14U9	CCGAGTTC AAGGCCAGCC TAGGGATAA	T -548del AGT
E14U34	TTT -596delAGGGTAATGCATGCAA
E13U37	TTA -61del	-612del CTG
E14U18	GCC -122del	-576del TCT
E13U8	TCATT -251del	-504del CTT
E14U26	ACA -785del	+135bpCCTAACAGGGTAATGCATGCAA
E13U7	CCGAGT.....	-840del CAT
E14U20	CCGAGTTC AAGGCCA.....	-914del ACT
E14U35	GGG -505del	-455del CTA
E14U40	CAC -457del	+124bp -512del TAT
E13U13	TTC -333del	-659del CAC
E14U16	AGT -115del	ATAC -888del CTC
E14U8	CCGAGTTC AAGGCCAGCC TAGGGATAA	-1061del CCT
E14U7	CCGAGTTC AAGGCCAGCC TAGGGATAA	-1076del CGT
E13U2	CCGAGTTC AAG.....	-1050del CCG
E14U6	GAG -503del	+69bp -1032del TCC
E14U22	ATT -924del	-1065del TGA

Supplementary Figure 2, con't

Chr. 17 Chr. 14
C *Xrcc4*^{-/-} +*Xrcc4* CCGAGTTC AAGGCCAGCC TAGGGATAA CCCTAACAGGGTAATGCATGCAA
 GGCTCAAGTTC CGGTCCGGATCC C AATAGGGATTGTC CCACTTACGTACGTT

TRANSLOCATION CLONES	TRANSLOCATION JUNCTIONS
E13X5, E13X9, E14X37	CCGAGTTC AAGGCCAGCC TAGGGAT TATCCCTAACAGGGTAATGCATGCAA
E13X6, E14X17	CCGAGTTC AAGGCCAGCC TAGGGATAA TCCTAACAGGGTAATGCATGCAA
E14X8	CCGAGTTC AAGGCCAGCC TAGGGATA TCCTAACAGGGTAATGCATGCAA
E13X38	CCGAGTTC AAGGCCAGCC TAGGGATAA TA CCCTAACAGGGTAATGCATGCAA
E14X1	CCGAGTTC AAGGCCAGCC TAGGGATAA CAGGGTAATG CCTAACAGGGTAATGCATGCAA
E14X12	CCGAGTTC AAGGCCAGCC TAGGGATA TAACAGGGTAATGCATGCAA
E13X20	CCGAGTTC AAGGCCAGCC TAGGGAT TAACAGGGTAATGCATGCAA
E14X22	CCGAGTTC AAGGCCAGCC TAGGGATAA ACAGGGTAATGCATGCAA
E14X15	CCGAGTTC AAGGCCAGCC TA TCCTAACAGGGTAATGCATGCAA
E13X26, E14X41	CCGAGTTC AAGGCCAGCC TAGGGATAA CAGGGTAATGCATGCAA
E14X18, E14X32, E14X43	CCGAGTTC AAGGCCAGCC TAGGG TAACAGGGTAATGCATGCAA
E14X42	CCGAGTTC AAGGCCAGCC T CCCTAACAGGGTAATGCATGCAA
E14X3	CCGAGTTC AAGGCCAGCC TAGGGA ACAGGGTAATGCATGCAA
E13X16	CCGAGTTC AAGGCCAGCC CTACAGGGTAATGCATGCAA
E14X14	CCGAGTTC AAGGCCAGCC T AACAGGGTAATGCATGCAA
E13X45, E14X39	CCGAGTTC AAGGCCAGCC TA ACAGGGTAATGCATGCAA
E13X21	CCGAGTTC AA TTATCCCTAACAGGGTAATGCATGCAA
E13X28, E14X34	CCGAGTTC AAGGCCAGCC TAGGGATAA TGCATGCAA
E14X33	CCGAGTTC TTATCCCTAACAGGGTAATGCATGCAA
E13X1, E13X19, E14X9, E14X38, E	CCGAGTTC AAGGCCAGCC TAGGG TAATGCATGCAA
E14X45	CCGAGTTC AAGGCCAGCC TAGG TAATGCATGCAA
E13X32, E13X48, E14X40	CCGAGTTC AAGGCCAGCC TAG TAATGCATGCAA
E13X4	CCGAGTTC AAGGCCAGCC TAGGGAT GCATGCAA
E13X39	CCGAGTTC AAGGCCAGCC TA TAATGCATGCAA
E13X35	CCGAGT ATCCCTAACAGGGTAATGCATGCAA
E13X17	CCGAGTTC AAGGCC CAGGGTAATGCATGCAA
E13X8	CCGAGTTC AAGGCCAGC TAATGCATGCAA
E14X47	CCGAGTTC AAGGCCAGCC AATGCATGCAA
E13X42	CCGAGTTC AAGGCCAG GGTAATGCATGCAA
E13X23	CCGAGTTC AAGGCCAGCC TAGGGAT GCAA
E13X46	CCG TCCTAACAGGGTAATGCATGCAA
E13X22	CCGAGTTC AA +134bp AGGGTAATGCATGCAA
E13X10	CCGAGTTC AAGGCCAGCC TAGGGA +33bp A
E13X47	CCGAGTTC AAGGCCAGCC GCAA
E13X34	CCGAGTTC AAGGCCAGC ATGCAA
E14X44	CCGAGTTC AAGGCCA TGCAA
E14X19	TCC TAACAGGGTAATGCATGCAA
E14X11	CCG GGTAATGCATGCAA
E14X7	GGC -33del CCTAACAGGGTAATGCATGCAA
E14X29	GTA -36del ACAGGGTAATGCATGCAA
E14X6	CCGAGTTC AAGGCCAGCC AGAATGAA -37del GAA
E14X30	GTA -42del ACAGGGTAATGCATGCAA
E14X26	CCGAGTTC AAGGCCAGCC TAGGG CACT -45del TTT
E14X27	CCGAGTTC AAGGCCAGCC TAGGGA CACTT -48del TGT
E13X27	AGGTA -42del ATGCATGCAA
E13X44	TAA -60del CCTAACAGGGTAATGCATGCAA
E13X24	CCGAGTTC AAGGCCAGCC TAGGGA -114del GCT
E14X5	GCAG -116del GGTAATGCATGCAA
E14X21	TAGAG -39del -88del AAA
E13X37	CCGAGTTC AAGGCCAGCC TAGGG +92bp -127del AGC
E13X11	CCGAGTTC AAGGCCAGC -112del GAG
E13X12	CCGAGTTC AAGGCCAGC T -128del GCC
E14X48	ACCC -127del TAACAGGGTAATGCATGCAA
E14X4	CCGAGTTC AAGGCCAGCC TA -133del CTA
E13X14	GGCA -117del -26del AGC
E14X31	CCGAGTTC AAGGCCAGCC TAGGG +54bp -171del CTC
E13X7	CAT -245del AACAGGGTAATGCATGCAA
E13X25	GAT -29del +51bp -232del ACA
E14X24	CCAG -269del GGTAATGCATGCAA
E14X36	CAA -181del +263bp -184del CAC
E14X28	CCCG -418del -38del AAA
E14X20	AAAAA -202del -318del TGA
E13X40	ACC -536del CTACAGGGTAATGCATGCAA
E14X46	GAA -92del -459del TAA
E13X33	TGT -530del TGCATGCAA
E13X36	AGG -514del -33del CTC
E14X2	CCGAGTTC AAGGCCAGCC TAGG -621del ATG
E13X43	CCC -634del AAT TAACAGGGTAATGCATGCAA
E14X10	GCC -389del -333del CTG
E14X25	CCT -51del +38bp -768del TCT
E14X16	CCGAGTTC AAGGCCAGCC TAGG -836del AGT
E13X15	TGC -480del AA -508del GTG
E13X3	GCA -221del -908del AAC
E14X23	GGCTG -713del -534del GGT
E13X2	TTA -1275del +137bp CCTAACAGGGTAATGCATGCAA
E14X35	TGG -965del +396bp -892del AAC
E14X13	GGTTC -1003del -1154del CTT

Supplementary Figure 2, con't

d *Ku70*^{-/-} cells

Chr. 17 CCGAGTTCAAGGCCAGCC**TAGGGATAA**
GGCTCAAGTTCGGTCGG**ATCCC**

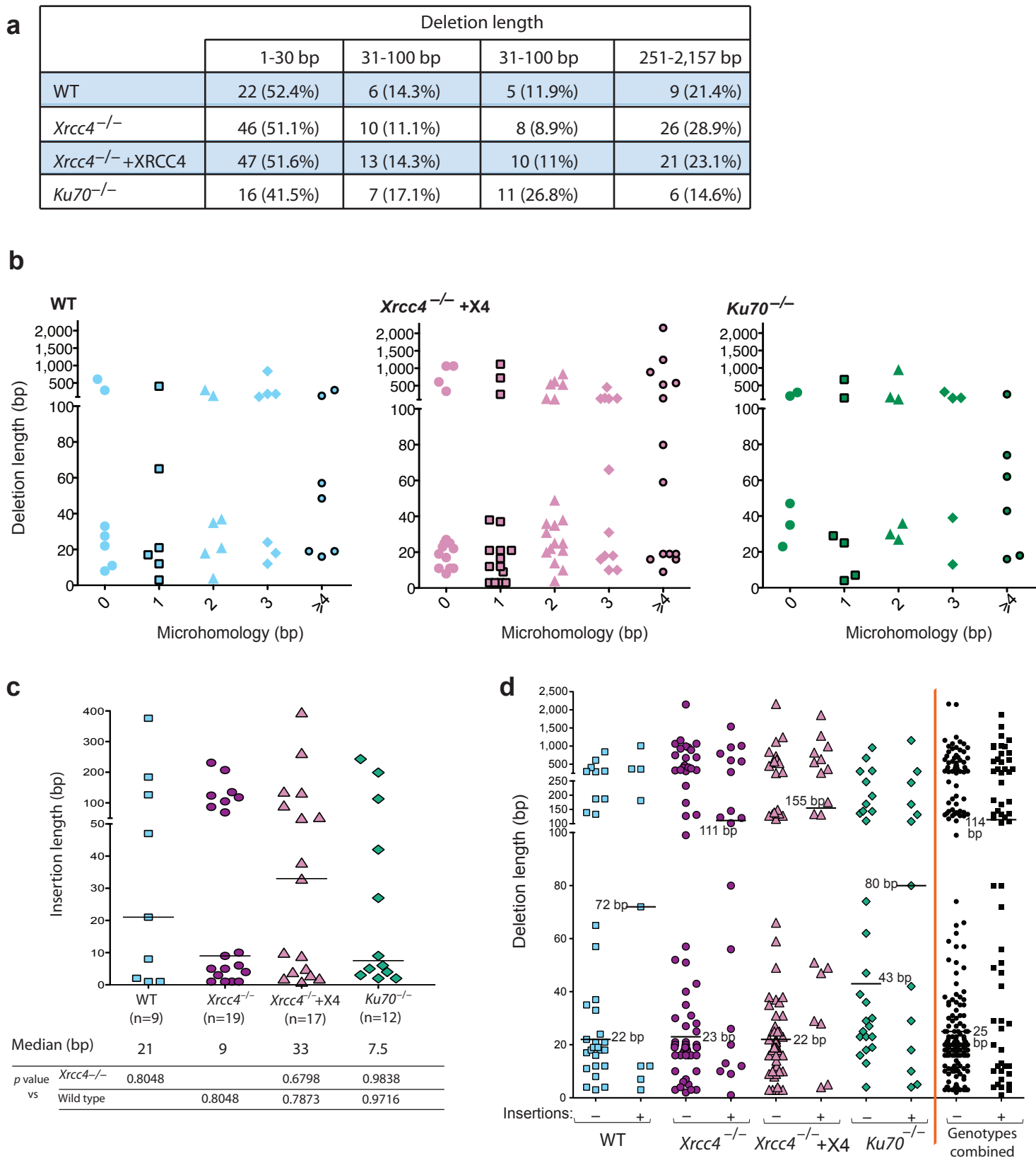
Chr. 14 CCCTAACAGGGTAATGCATGCAA
AATAGGGATTGTCCATTACGTACGTT

TRANSLOCATION CLONES	TRANSLOCATION JUNCTIONS	
E13K24	CCGAGTTCAAGGCCAGCC TAGGGAT ATCCCTAACAGGGTAATGCATGCAA
E14K13	CCGAGTTCAAGGCCAGCC TAGGGATAA +42bp CCCTAACAGGGTAATGCATGCAA
E13K6	CCGAGTTCAAGGCCAGCC TAGGGATAA	GGCGCGCCA CCCTAACAGGGTAATGCATGCAA
E13K23	CCGAGTTCAAGGCCAGCC TA	CAGAG TCCCTAACAGGGTAATGCATGCAA
E14K6	CCGAGTTCAAGGCCAGCC TAGGGATAA CAGGGTAATGCATGCAA
E14K3	CCGAGTTCAAGGCCAGCC TA ACAGGGTAATGCATGCAA
E13K22	CCGAGTTCAAGGCCAGCC TAGGG	+27bp GTAATGCATGCAA
E14K1, E14K20	CCGAGTTCAAGGCCAGCC TAGGG TAATGCATGCAA
E13K20	CCGAGTTCAAGGCCAGCC T TAATGCATGCAA
E13K19	CCGAGTTCAAGGCCAGCC TA AATGCATGCAA
E13K21	CCGAGTTCAAGGCCAGCC TAGGGAT TGCAA
E13K17	CCGAGTTCAAGGCCAGC GTAATGCATGCAA
E13K7	CCGAGTTCAAGGCCAGCC TAGGG	+113bp CAA
E14K17	CCGAGTTCAAGG GTAATGCATGCAA
E14K10	CCGAGTTCAAGGCCAGCC TAG -28del CTT
E14K18	CCGAGTTCAA TGCATGCAA
E14K19	CCGAGT AATGCATGCAA
E13K11	CCGAGTTCAAGGCCAGC -29del TTG
E14K5	GAT -29del	TTC GGGTAATGCATGCAA
E13K12	CCGAGTTCAAGGCCA -38del AAA
E13K8	TCTCCGA -39del AAT
E14K7	CCGAGTTCAAGGCCAGCC TAGGG -71del GAC
E14K2	TCT -69del	CT AGGGTAATGCATGCAA
E13K4	CCGAGTTCAAGGCCAGCC TA	TCCCTA -102del TGA
E14K11	ATA -98del GGGTAATGCATGCAA
E13K3	CCGAGTTCAAGGCCAGCC TAGGGATAA	+199bp -130del CTA
E14K21	ACCC -127del TAACAGGGTAATGCATGCAA
E13K9	CAA -131del GGGTAATGCATGCAA
E14K15	GGCA -117del -26del AGC
E13K1	CCT -51del -117del AAG
E13K15	GGA -151del	+243bp GCATGCAA
E14K4	CCGAGTTCAAGGCCAGCC TAGG -197del CTG
E14K16	CCGAGTTCAAGGCCAGCC T -239del TGA
E13K5	CCA -269del	AGGG -29del TTG
E13K18	CCGAGTTCAAGGCCAGCC TAGGGATAA -297del GCG
E14K12	CCGAGTTCAAGGCCAGCC TAGGG -312del GCA
E13K2	TGG -214del -453del GCT
E14K22	TCT -942del AACAGGGTAATGCATGCAA
E14K14	CCGAGTTCAAGGCCAGCC TAG	CT -1137del CAG
E14K24	TTGTGGG -1089del -313del CAA

Supplementary Figure 2

Der(17) translocation junction sequences obtained from pCr15 wild-type (**a**), *Xrcc4*^{-/-} (**b**), *Xrcc4*-complemented *Xrcc4*^{-/-} (**c**), and *Ku70*^{-/-} (**d**) *neo*⁺ clones. DNA ends generated by I-SceI on chrs.17 and 14 are indicated in red and blue, respectively. Sequences are annotated as follows: del, deletion length from the DNA end after DSB formation; underline, microhomology; middle black letters, sequence of short insertion; +, length of long insertion. If the deletion extends beyond the chromosome sequence indicated at the top of the figure, a few bp flanking the deletion are indicated, with microhomology underlined.

Supplementary Figure 3

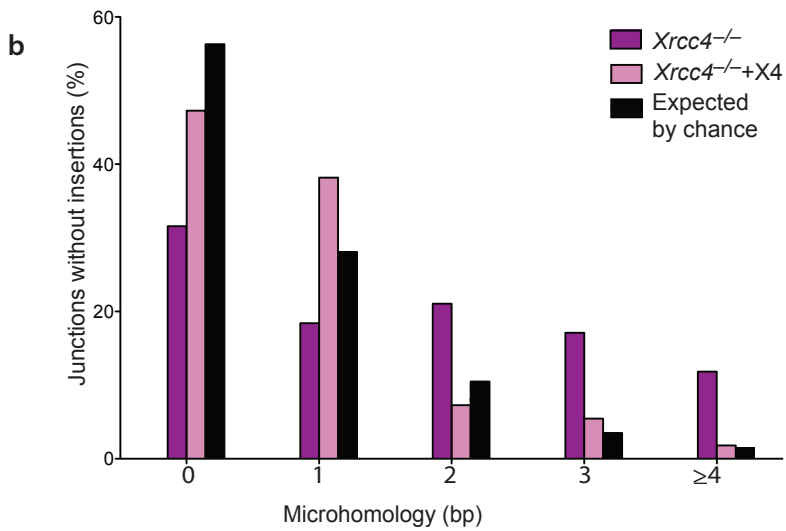
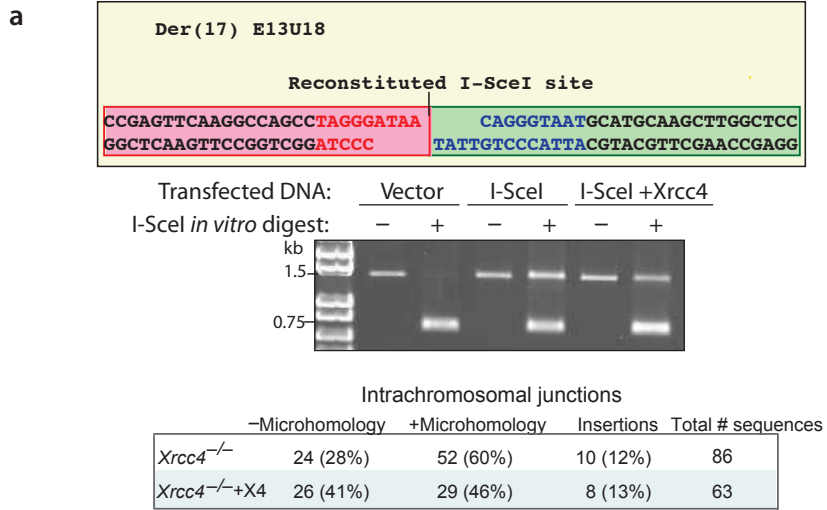


Supplementary Figure 3

Analysis of der(17) translocation junction sequences.

- (a)** Distribution of deletion lengths for der(17) breakpoint junctions derived from the indicated genotypes. Each value represents the combined deletion from chrs. 17 and 14.
- (b)** Lack of correlation between deletion length and microhomology use for the indicated genotypes.
- (c)** Scatter plot of insertion lengths in der(17) junctions for the indicated genotypes. p values are derived from a two-tailed Mann-Whitney test.
- (d)** Distribution of deletion lengths for der(17) breakpoint junctions without and with insertions for the indicated genotypes. Each value represents the total deletion from chrs. 17 and 14. The median deletion length is indicated at the bar on the graph.

Supplementary Figure 4



Supplementary Figure 4, con't

c i) E13U18, *Xrcc4*-/-

CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		ATAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		ATAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>	TA	ATAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>	+46bp	ATAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		TAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		TAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>	TGCATG	TAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		AACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGAT</u>		TAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		ACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGG</u>	GGCG	ATAACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>	GGAGAA	CAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGA</u>		AACAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATA</u>		CAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATA</u>		CAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGAT</u>		CAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATA</u>		CAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		GGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGATA</u>		GGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGA</u>		CAGGGTAATGCATGCAAGCTTGGCTCC
CCGAGTTCAAGCCAGCC <u>TAGGGA</u>		AGGGTAATGCATGCAAGCTTGGCTCC
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CCGAGTTCAAGCC		TTGGCTCC
CCGAGTTCAAGCCAGCC <u>T</u>		CC
CCGAGTTCAAG		CTTGGCTCC
CCGAGTTCAAG		CTTGGCTCC
CTCCGA		-39del AAT
TTG -49del		CAAGCTTGGCTCC
CCGAGTTCAAGG		-60del GGA
CAGC -54del		TTGGCTCC
CCGAGTTCAAGCCAGCC <u>T</u>	+318bp	-77del CAG
GTGG -73del		CTCC
GAG -39del		-65del TGT
GCCCT -51del		-62del ATT
CCGAGTTCAAGCCAGCC <u>TA</u>		-118del CTA
CCGAGTTCAAGCCAGCC <u>TAGGGATAA</u>		-127del GTA
TGA -93del		-107del AAG
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CCGAGTTCAAGCCAGCC <u>TAGGGATA</u>		-418del CTT
CCGAGTTCAAGCCAGCC		-481del TCA

Supplementary Figure 4, con't
 c ii) E13U18, *Xrcc4*^{-/-} +*Xrcc4*

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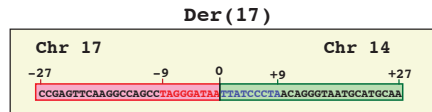
Supplementary Figure 4

Comparison of intrachromosomal repair of a single I-SceI DSB in complemented and uncomplemented *Xrcc4*^{-/-} cells.

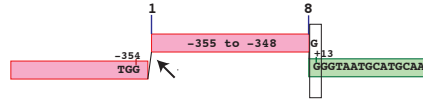
- (a) To maintain a similar sequence context to that of translocation formation, we used a translocation clone, E13U18, derived from *Xrcc4*^{-/-} cells that fortuitously reconstituted an I-SceI site (red and blue sequences) on der(17). The chr. 17 sequence (red shading) is the same in both the intrachromosomal and translocation assays, whereas the chr. 14 sequence is deleted for 10 bp compared with the translocation assays. After PCR with primers 17-754 and 14-726, a 1.5 kb band is amplified from E13U18 genomic DNA which is cleavable by I-SceI *in vitro* to 0.75 kb fragments. Prior expression of I-SceI in cells with or without coexpression of *Xrcc4* leads to an I-SceI resistant population due to loss of the I-SceI site. Cloning and sequencing of the I-SceI resistant population led to the identification of intrachromosomal junctions, which were characterized for whether or not microhomology or insertions were present at the junctions.
- (b) Distribution of microhomology lengths for intrachromosomal junctions after I-SceI expression. Only junctions with simple deletions (i.e., without an insertion) are included.
- (c) Intrachromosomal junction sequences obtained from E13U18 *Xrcc4*^{-/-} (i) and *Xrcc4*-complemented *Xrcc4*^{-/-} (ii) cells.

Supplementary Figure 5

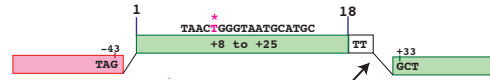
a Wild type



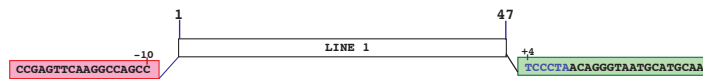
E14P20
 Interstitial duplication of 2 bp
 (chr 17: -355 to -354)



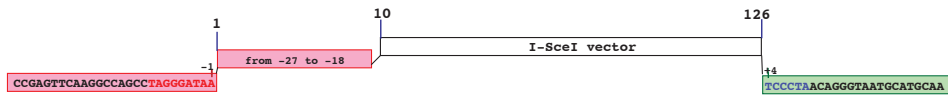
E14P18
 Interstitial deletion of 7 bp
 (chr 14: +26 to +32)
 plus 2bp insertion



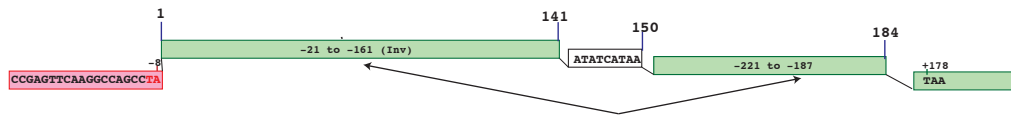
E13P13



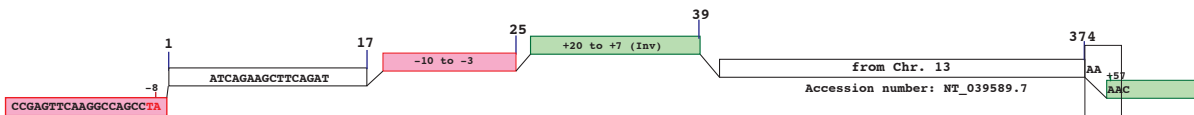
E14P8



E14P17
 Chr 14 inserts are
 nearly contiguous
 but in opposite orientation.

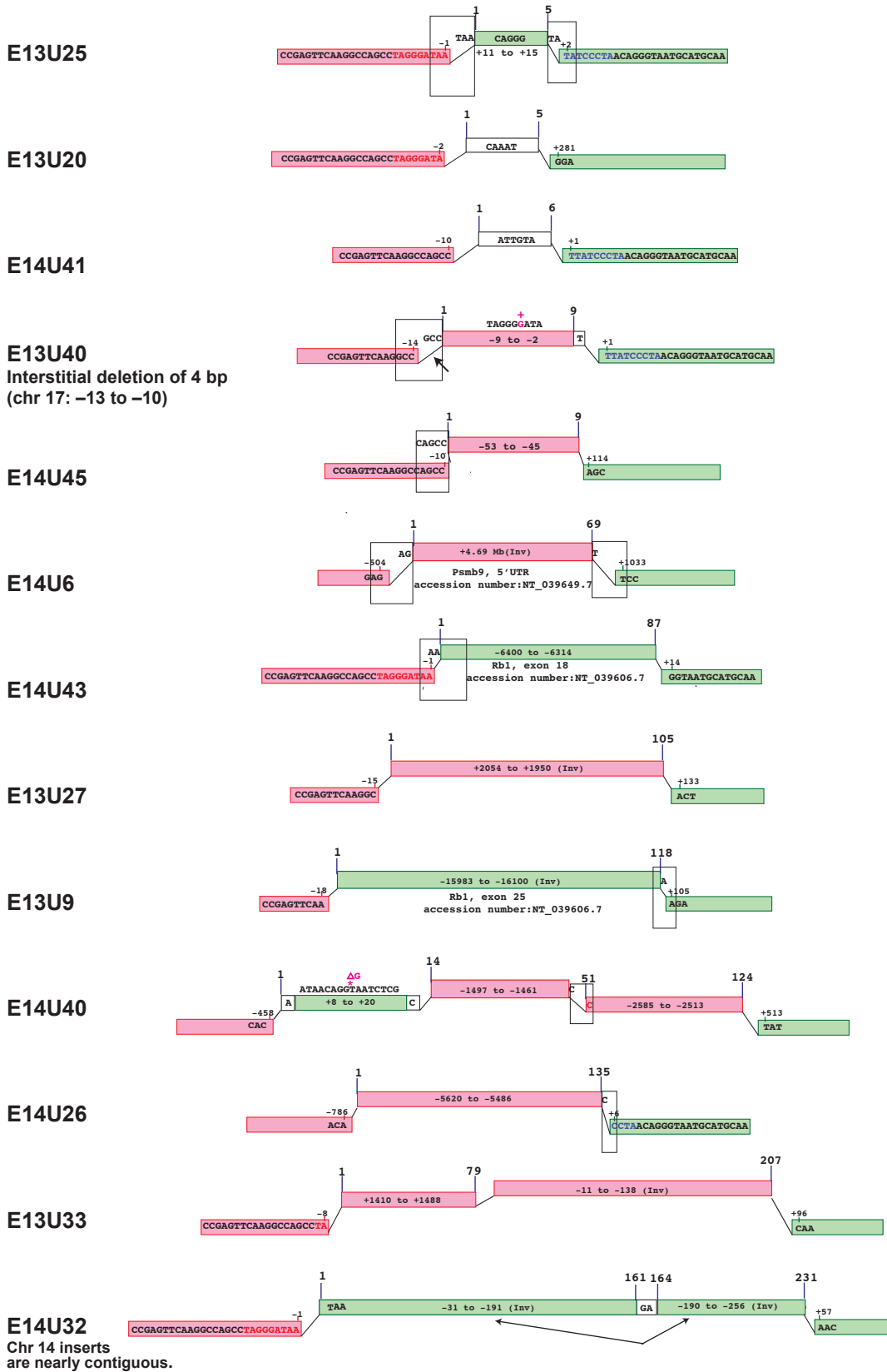
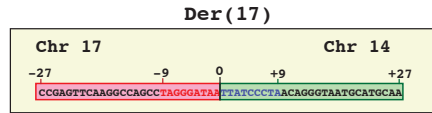


E14P9



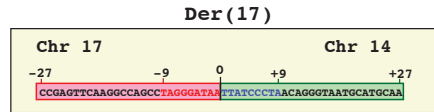
Supplementary Figure 5, con't

b *Xrcc4*^{-/-}

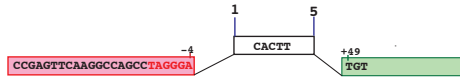


Supplementary Figure 5, con't

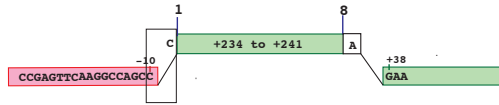
C *Xrcc4*^{-/-} + *Xrcc4*



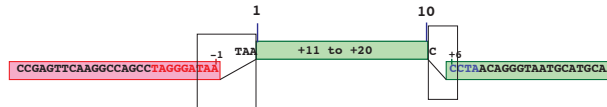
E14X27



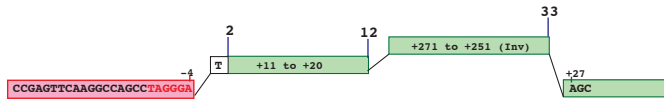
E14X6



E14X1

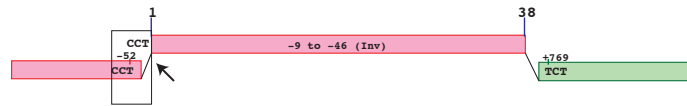


E13X10



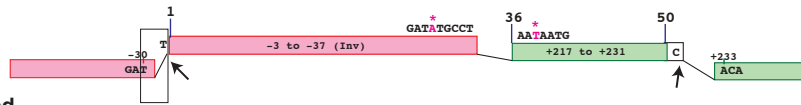
E14X25

Interstitial deletion of 5 bp
(chr 17: -51 to -47) associated
with inversion

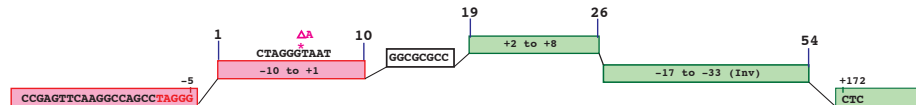


E13X25

Interstitial 8 bp duplication
(chr 17: -30 to -37) associated
with inversion and 1 bp substitution
(chr 14: "C" at +232)



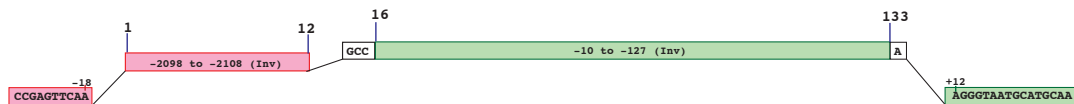
E14X31



E13X37

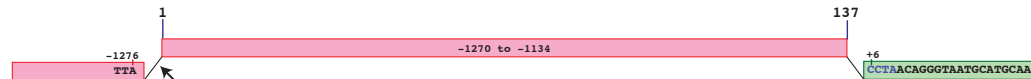


E13X22

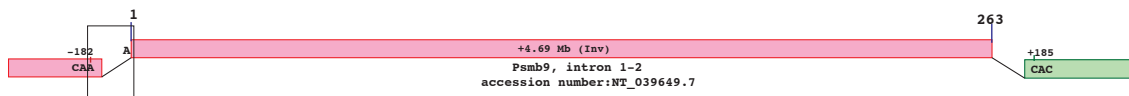


E13X2

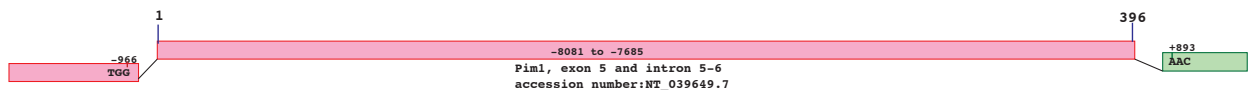
Interstitial deletion of
5 bp (chr 17: -1275 to -1271)



E14X36

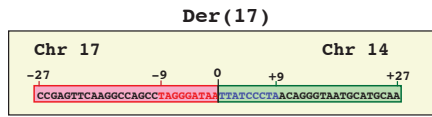


E14X35

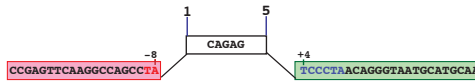


Supplementary Figure 5, con't

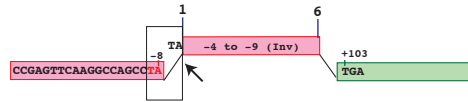
d *Ku70*^{-/-}



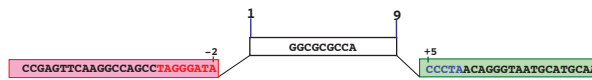
E13K23



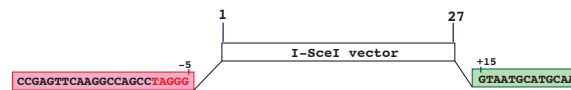
E13K4
Interstitial 2 bp duplication
associated with inversion
(chr 17: -4 to -7)



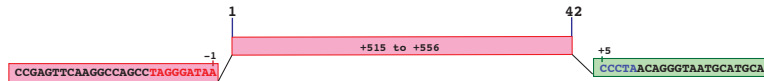
E13K6



E13K22



E14K13



E13K7



E13K3



E13K15



Supplementary Figure 5

Derivation of insertions found in wild-type (a), *Xrcc4*^{-/-} (b), *Xrcc4*-complemented *Xrcc4*^{-/-} (c), and *Ku70*^{-/-} (d) der(17) breakpoint junctions. For each translocation breakpoint junction, deletion lengths from the chrs.17 and 14 ends are indicated in red and green boxes on the far left and far right, respectively, with the inserted segments represented as elevated boxes connected to each end. Inserts derived from chrs. 17 and 14 are indicated in the elevated red and green boxes, respectively, while those derived from sources are in white boxes. Included in this analysis are all inserted sequences >4 bp; for 1-4 bp insertions see Supplementary Fig. 2. Cases in which the inserted sequences are not a perfect match are indicated in magenta with an asterisk (point mutation), Δ (deletion), or + (addition). Accession numbers are provided for inserts derived from distant sequences. Microhomologies are boxed. Inv, inverted.