

Top2b Cas9-CKO Strategy

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Overview

Target Gene Name

- *Top2b*

Project Type

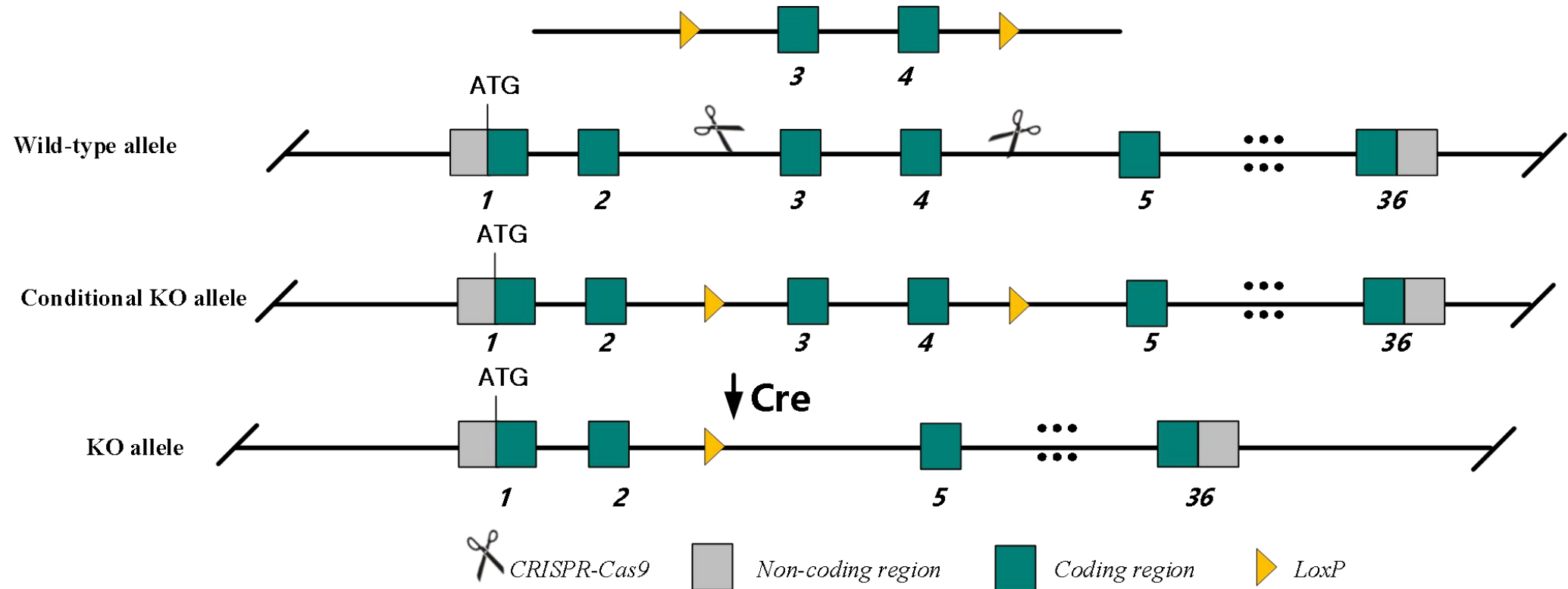
- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

Donor and CRISPR-Cas9 System



Schematic representation of CRISPR-Cas9 engineering used to edit the *Top2b* gene.

Technical Information

- The *Top2b* gene has 8 transcripts. According to the structure of *Top2b* gene, exon3-4 of *Top2b*-201 (ENSMUST00000017629.12) transcript is recommended as the knockout region. The region contains 155 bp of coding sequences. Knocking out the region will result in deletion the most coding region of *Top2b*, which may disrupt the function of *Top2b*.
- In this project we use CRISPR-Cas9 technology to modify *Top2b* gene. The brief process is as follows: CRISPR-Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Gene Information

Top2b topoisomerase (DNA) II beta [*Mus musculus* (house mouse)]

[Download Datasets](#)

Gene ID: 21974, updated on 7-Sep-2023

Summary

Official Symbol	Top2b provided by MGI
Official Full Name	topoisomerase (DNA) II beta provided by MGI
Primary source	MGI:MGI:98791
See related	Ensembl:ENSMUSG00000017485 AllianceGenome:MGI:98791
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Top-2; D230016L12Rik
Summary	Predicted to enable several functions, including enzyme binding activity; protein C-terminus binding activity; and protein heterodimerization activity. Acts upstream of or within axonogenesis; forebrain development; and neuron migration. Located in nucleus. Is expressed in central nervous system; genitourinary system; and retina. Orthologous to human TOP2B (DNA topoisomerase II beta). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Broad expression in CNS E11.5 (RPKM 40.7), CNS E14 (RPKM 36.8) and 22 other tissues See more
Orthologs	human all
NEW	Try the new Gene table Try the new Transcript table

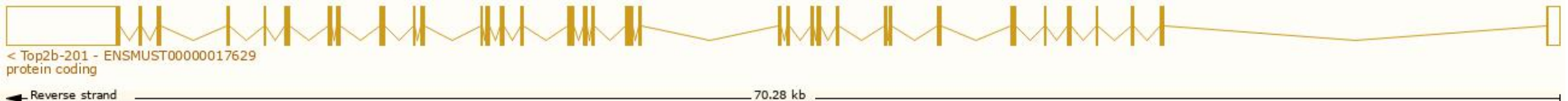
Source: <https://www.ncbi.nlm.nih.gov/>

Transcript Information

The gene has 8 transcripts, all transcripts are shown below:

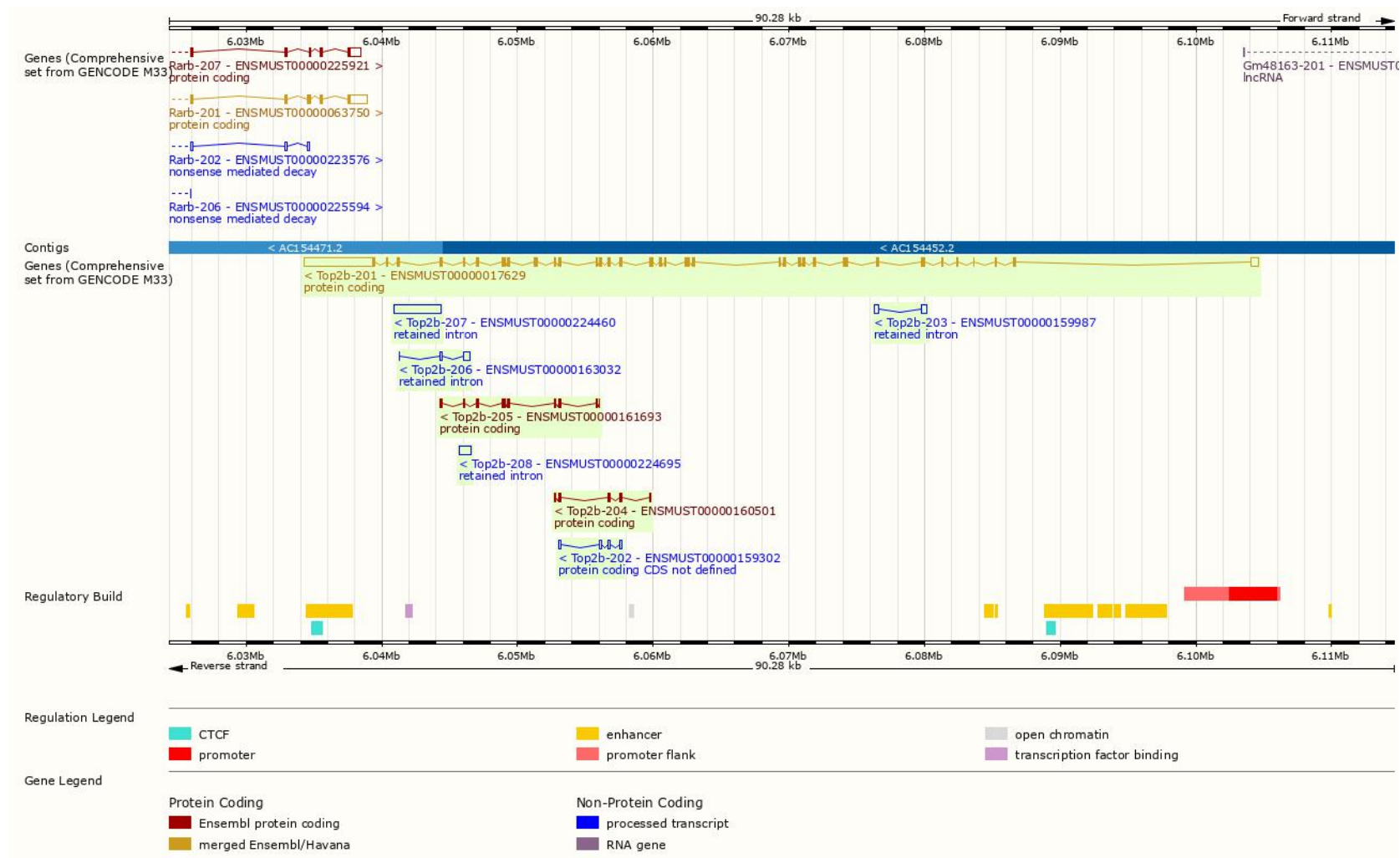
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000224695.2	Top2b-208	837	No protein	Retained intron		-	-
ENSMUST00000224460.2	Top2b-207	3401	No protein	Retained intron		-	-
ENSMUST00000163032.2	Top2b-206	634	No protein	Retained intron		-	TSL:3
ENSMUST00000161693.2	Top2b-205	1044	348aa	Protein coding		F6XX57	TSL:5 CDS 5' and 3' incomplete
ENSMUST00000160501.8	Top2b-204	689	230aa	Protein coding		F6U5K2	TSL:3 CDS 5' and 3' incomplete
ENSMUST00000159987.2	Top2b-203	671	No protein	Retained intron		-	TSL:2
ENSMUST00000159302.2	Top2b-202	658	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST0000017629.12	Top2b-201	10335	1612aa	Protein coding	CCDS26833	Q64511	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1

The strategy is based on the design of *Top2b-201* transcript, the transcription is shown below:

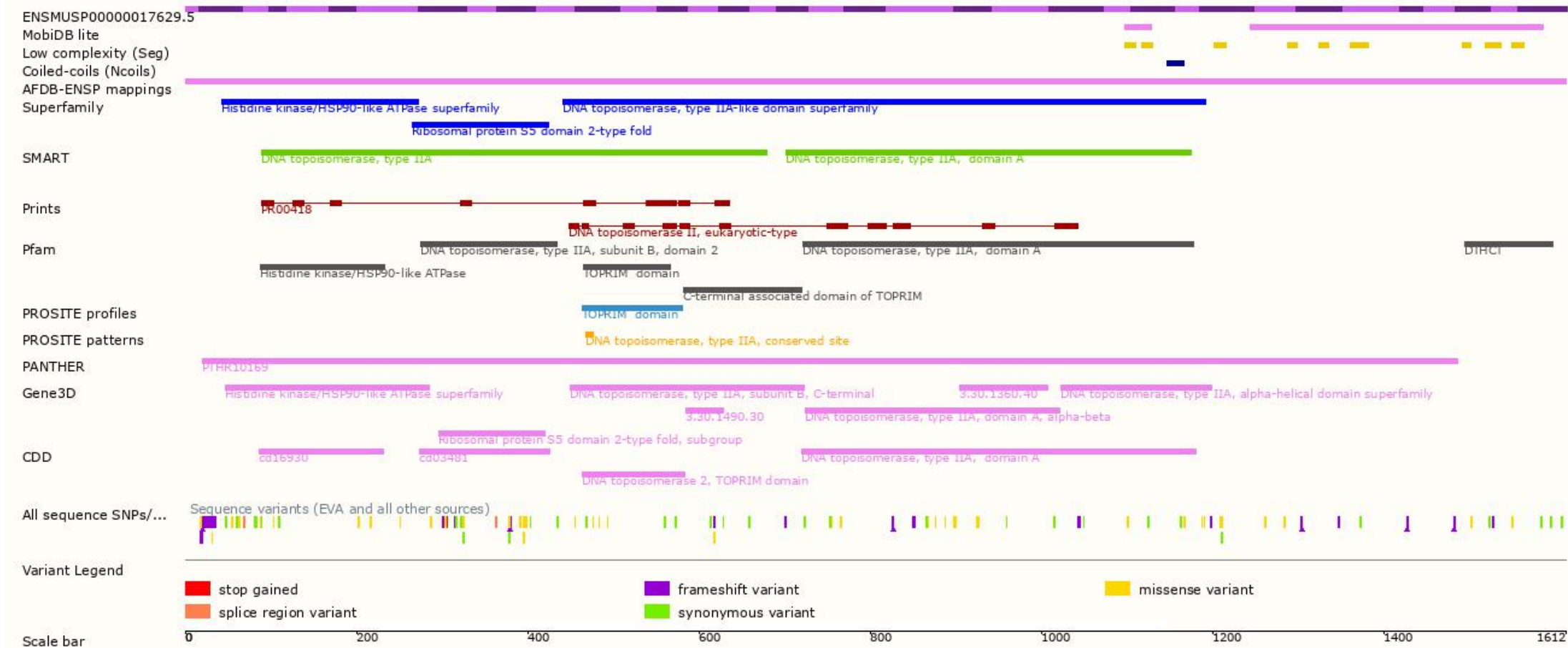


Source: <https://www.ensembl.org>

Genomic Information



Protein Information



Important Information

- **Homozygous null mice exhibit abnormal innervation. Offspring die shortly after birth due to respiratory failure. Conditional KO in the retina results in postnatal retinal degeneration.**
- The 5' of transcription *Top2b*-204, 205, the effect is unknown.
- This strategy is not affect the *Top2b*-202, 204, 206, 207 and 208, the risk is unknown.
- *Top2b* is located on Chr14. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Reference

[1] Yang, X. DNA Topoisomerase II β and Neural Development [J]. Science, 2000, 287(5450):131-134. DOI:10.1126/science.287.5450.131.

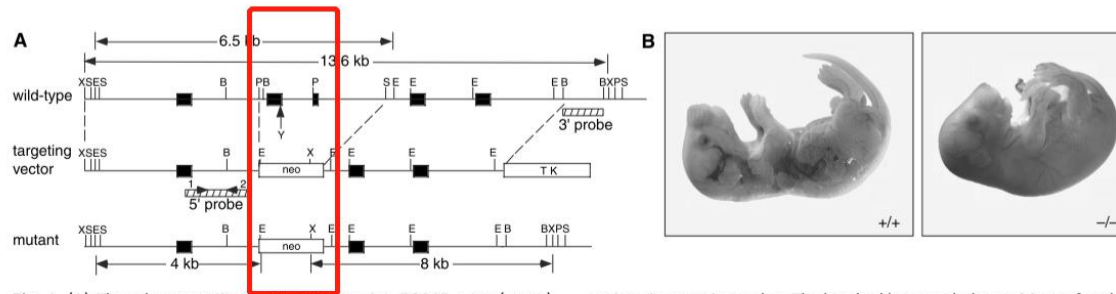


Fig. 1. (A) The relevant regions in the WT murine *TOP2β* gene (upper), the neo/TK targeting vector (middle), and the mutated *top2β* allele (lower). Coding stretches are represented by filled boxes; B, E, P, S, and X denote restriction sites of Bam HI, Eco RI, Pst I, Sac I, and Xba I, respectively, and neo and TK denote the neomycin-resistance and thymidine kinase markers. The letter Y (upper) marks the position of the

active-site tyrosine codon. The hatched bars mark the positions of probes used in genotyping; the "5' probe" was prepared by polymerase chain reaction with primers represented by the arrows 1 and 2 [see supplementary Web material (6) for details on the construction of the targeting vector and examples of genotyping and mRNA blot-hybridization results]. (B) Images of E17.5 WT (left) and *top2β*^{-/-} (right) embryos.

by distinct genes (1). The II α rather than the II β isoform appears to unlink DNA during chromosome segregation. Cell lines expressing II α but not II β have been identified, indicating that II β is dispensable in cellular processes (4). To determine the role of II β in vivo, we disrupted the murine *TOP2β* gene according to standard procedures (5). Two adjacent exons in one copy of *TOP2β* in embryonic stem cells, one of which contains the active-site tyrosine codon, were replaced by the neomycin-resistance marker (Fig. 1A) [see supplementary Web material (6) for details on targeting vector construction]. Germ line chimeras from blastocysts injected with the mutated cells were then used to obtain heterozygous *top2β*^{+/-} mice (5).

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Uniprot:

The screenshot shows the Uniprot entry for Q64511. The protein is a DNA topoisomerase II beta. The active site is highlighted in yellow and labeled as "Active site" with position 814. The description for the active site is "Manual assertion inferred from sequence similarity".

TYPE	ID	POSITION(S)	DESCRIPTION
Site	671		Interaction with DNA
Site	732		Interaction with DNA
Site	766		Interaction with DNA
Site	813		Transition state stabilizer
Active site	814		O ² -(5'-phospho-DNA)-tyrosine intermediate
Site	865		Important for DNA bending; intercalates between base pairs of target DNA
Site	940		Interaction with DNA

<https://www.uniprot.org/uniprotkb/Q64511/entry>