The inhibitory effect on the DNA double strand repair kinetics by a DNA ligase IV inhibitor

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Purpose

In the present study, we elucidate the repair kinetics of DNA double strand breaks (DSBs) in neural stem/progenitor cells (NSPCs).

We investigate the inhibitory effect on the DSB repair kinetics using a DNA ligase IV inhibitor or mutant NSPCs derived from DNA ligase IV deficient mice.

Background

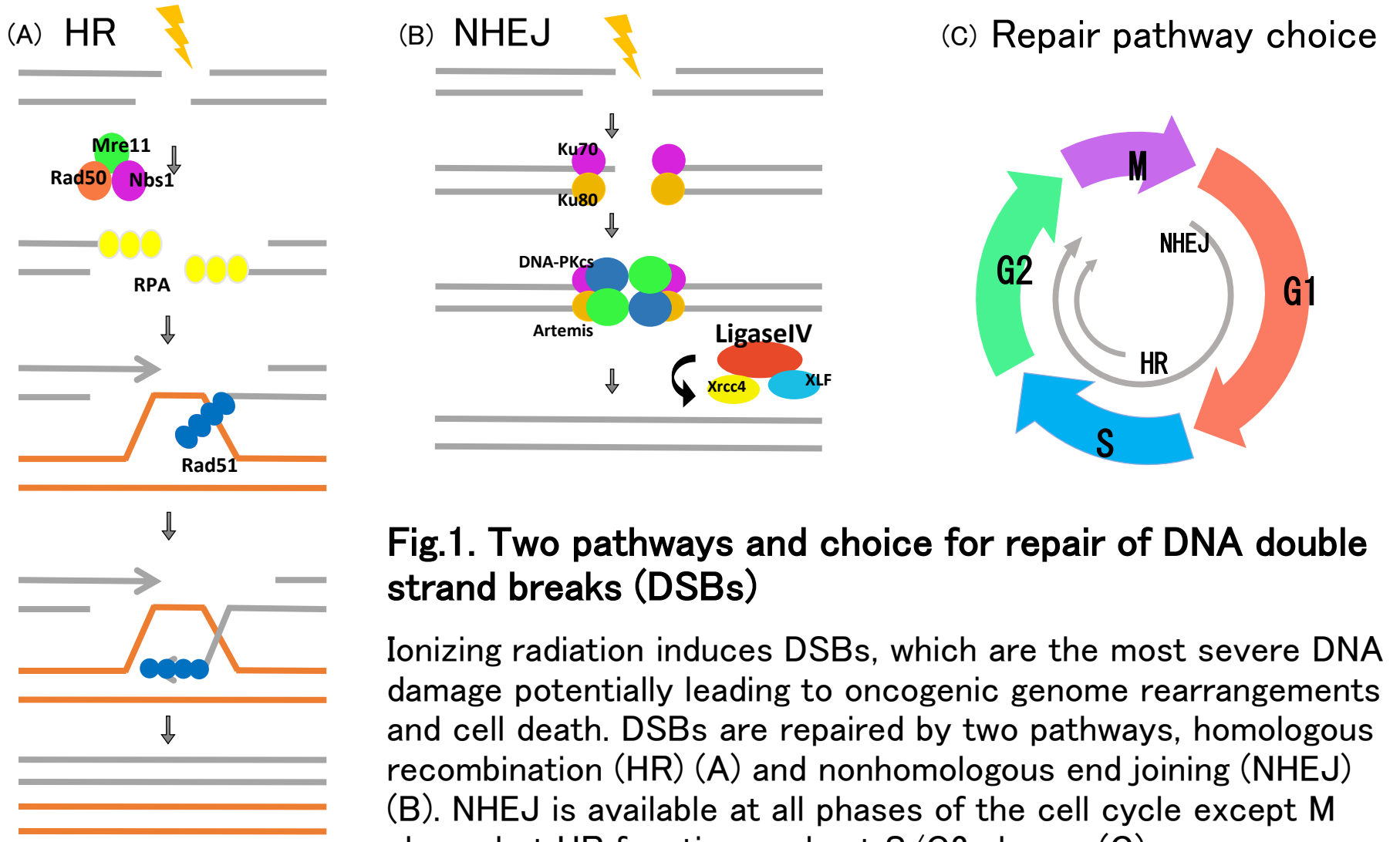


Fig.1. Two pathways and choice for repair of DNA double strand breaks (DSBs)

Ionizing radiation induces DSBs, which are the most severe DNA damage potentially leading to oncogenic genome rearrangements and cell death. DSBs are repaired by two pathways, homologous recombination (HR) (A) and nonhomologous end joining (NHEJ) (B). NHEJ is available at all phases of the cell cycle except M phase, but HR functions only at S/G2 phases (C).

Background

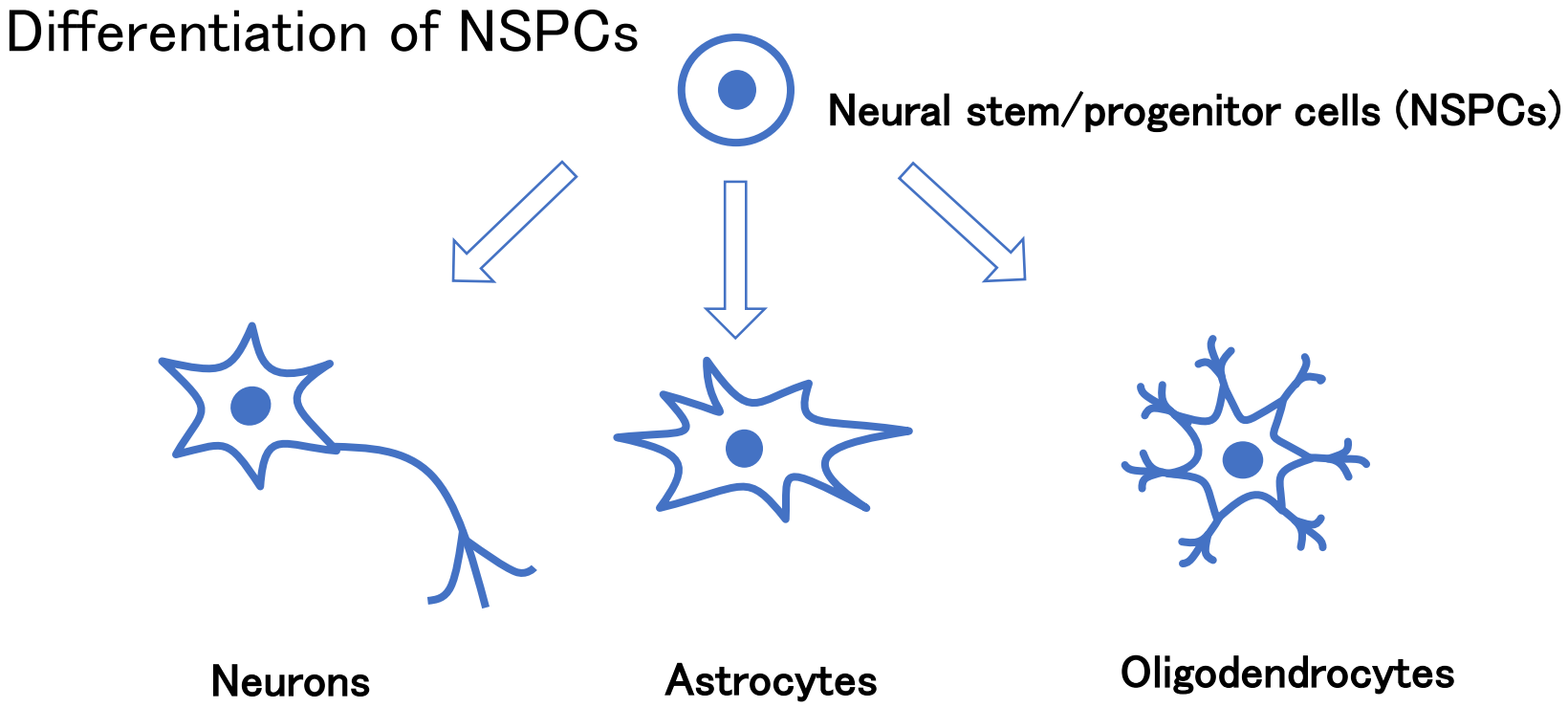
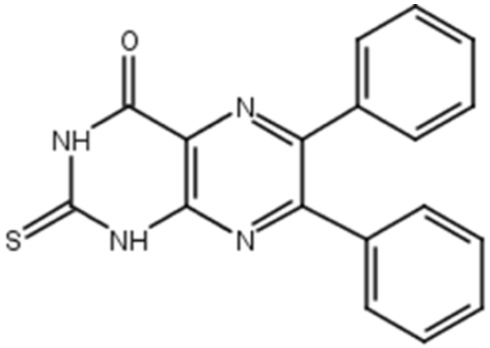


Fig. 2. Differentiation of NSPCs
Neural stem/progenitor cells (NSPCs) can devide in self renewal and differentiate into neurons, astrocytes, and oligodendrocytes.

Background

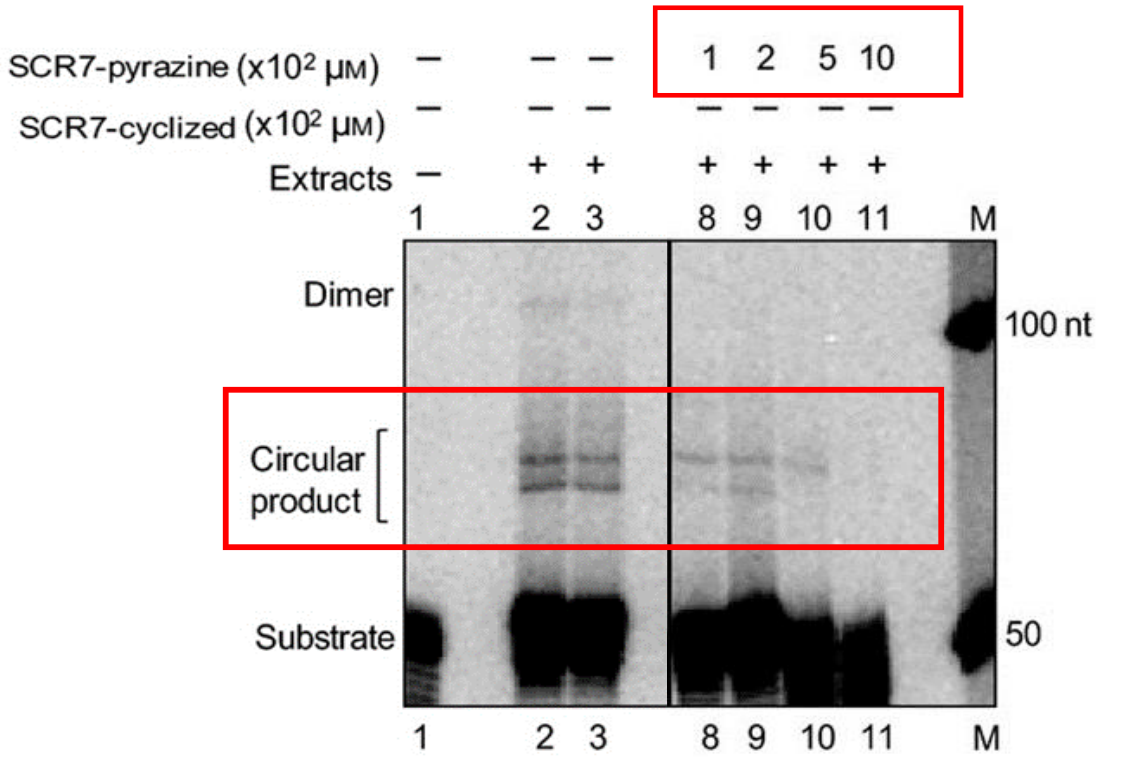
① DNA ligase Ⅳ inhibitor

(A)



SCR7-pyrazine

(B)



(Supriya V, et al., FEBS Journal 285, 2018, 3959–3976)

Fig. 3. Structure of SCR7–pyrazine and its inhibitory effect on DNA ligase Ⅳ

The structure of SCR7–pyrazine, an oxidized form of SCR7, is shown (A). Cell free repair assay derived from rat testicular extract was used for examining the effect of inhibitors on NHEJ. SCR7–pyrazine inhibited end–joining depending on the concentration. (B)

Experimental design

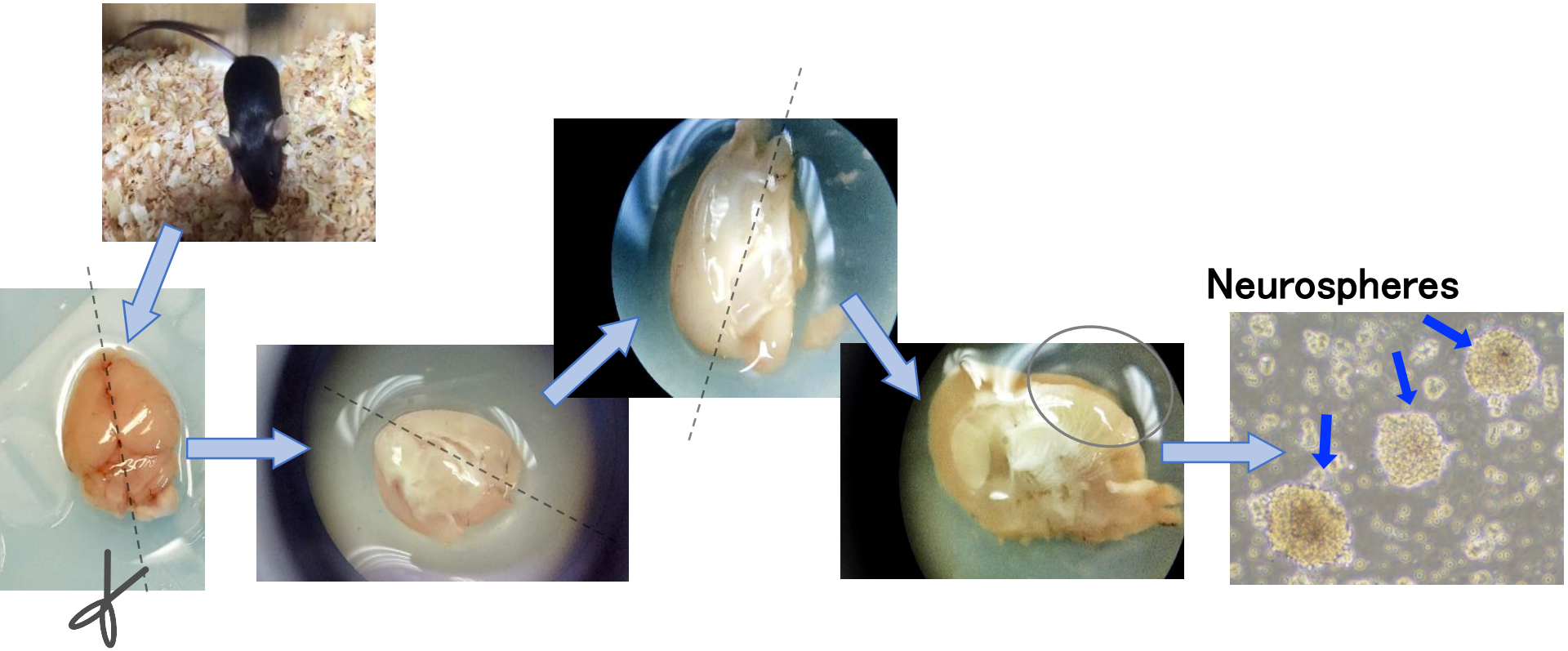


Fig. 4. Isolation and culture of NSPCs

Neural stem/progenitor cells (NSPCs) were isolated from subventricular zone of C57BL/6N mice (6 weeks old). The cells were cultured in DMEM/Ham's F−12 medium supplemented with growth factors and antibiotics as floating neurospheres at 37°C under humidified atmosphere with 5% CO_2.

Experimental design

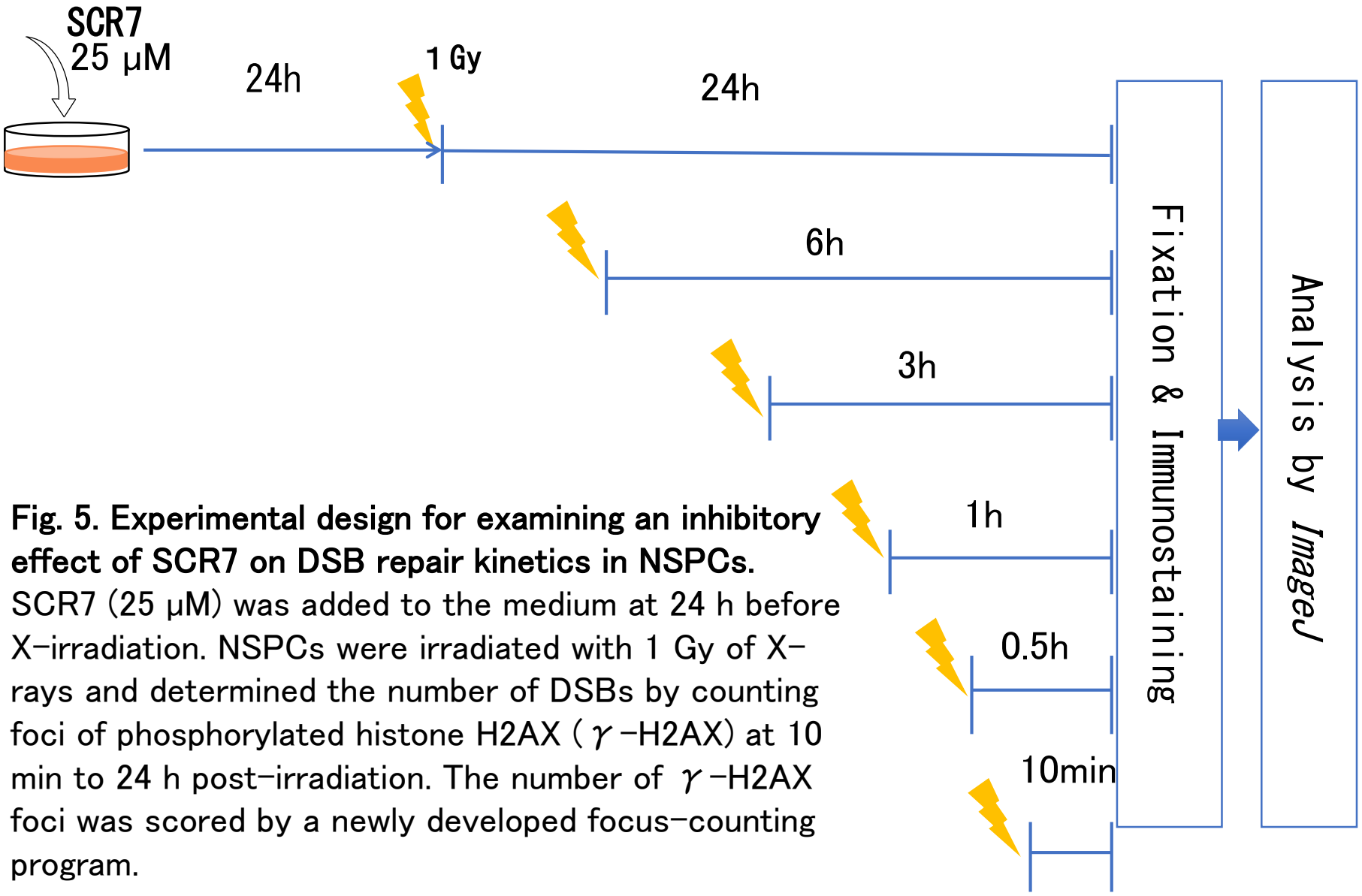
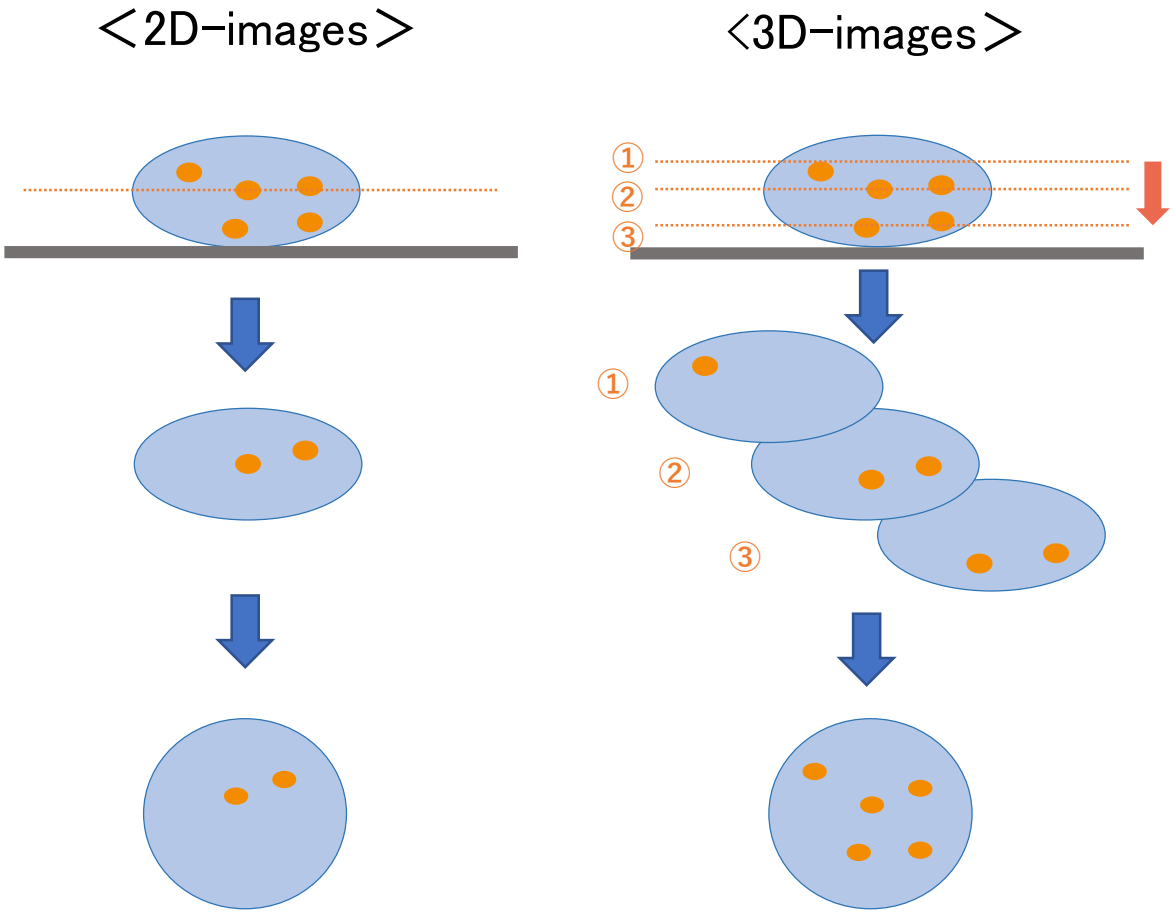


Fig. 5. Experimental design for examining an inhibitory effect of SCR7 on DSB repair kinetics in NSPCs.
SCR7 (25 μM) was added to the medium at 24 h before X-irradiation. NSPCs were irradiated with 1 Gy of X-rays and determined the number of DSBs by counting foci of phosphorylated histone H2AX (γ-H2AX) at 10 min to 24 h post-irradiation. The number of γ-H2AX foci was scored by a newly developed focus-counting program.

Experimental design

Development of an Image-J based computer program counting γ-H2AX foci



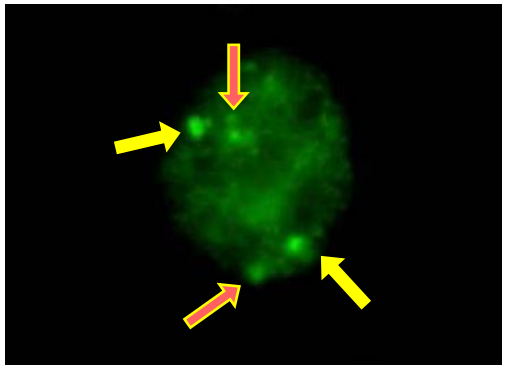


Fig. 6. A new program for counting γ-H2AX foci
Instead of 2D-images of the foci, 3D-images were acquired in a movie format by moving the microscope stage. The 3D-images consisted of twenty-one 2D-images that were captured from the top to the bottom of a cell for 3 s in AVI format.

Results

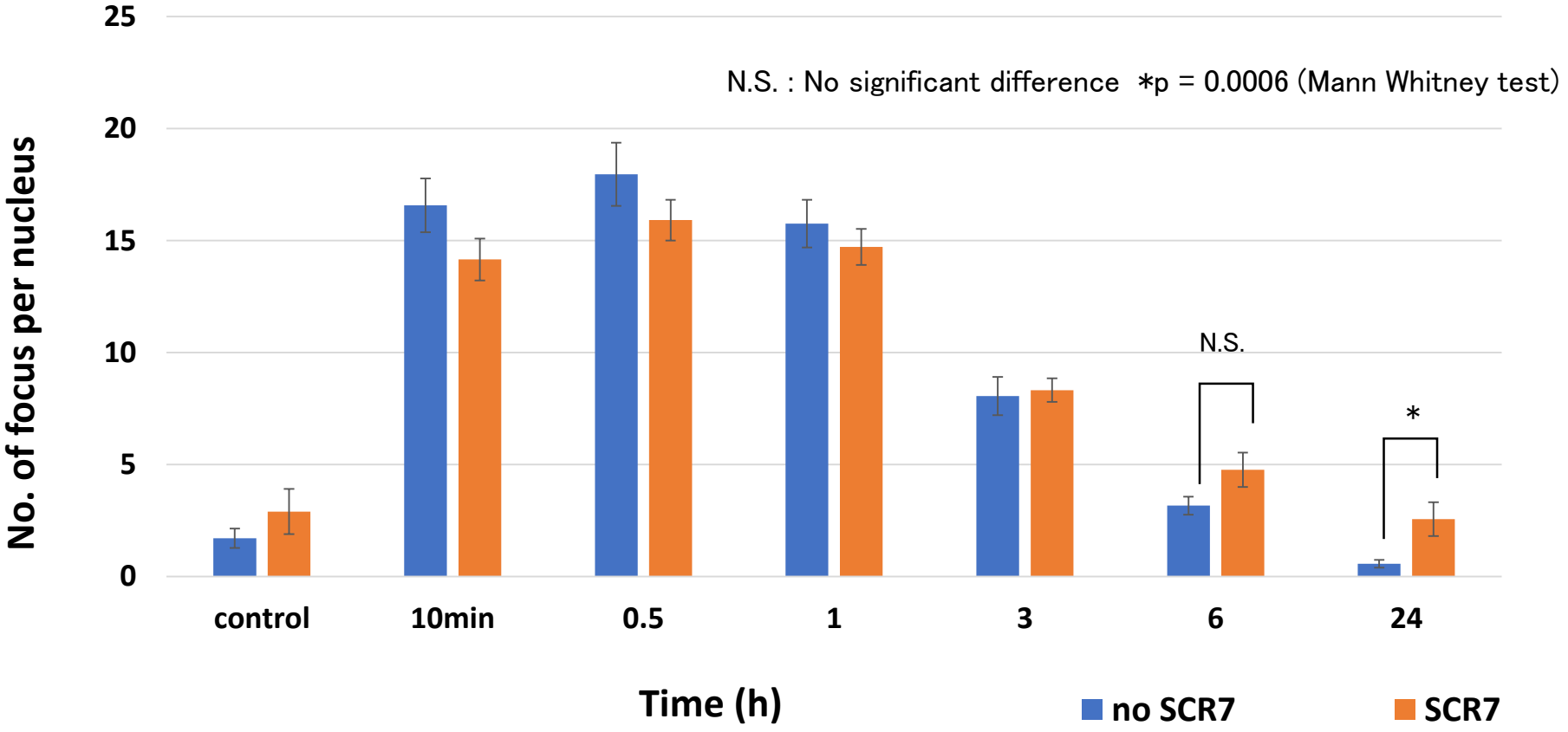


Fig. 7. Repair kinetics of DSBs in NSPCs derived from C57BL/6N mice.
Treatment with 25 μM SCR7 pyrazine induced a delay in DSB repair at 6 h and 24 h post-irradiation in NSPCs, indicating that the inhibitory effect on Lig4 appeared at later stage (6–24 h post-irradiation), but not earlier stage (0.5–3 h post-irradiation), of NHEJ.

Results

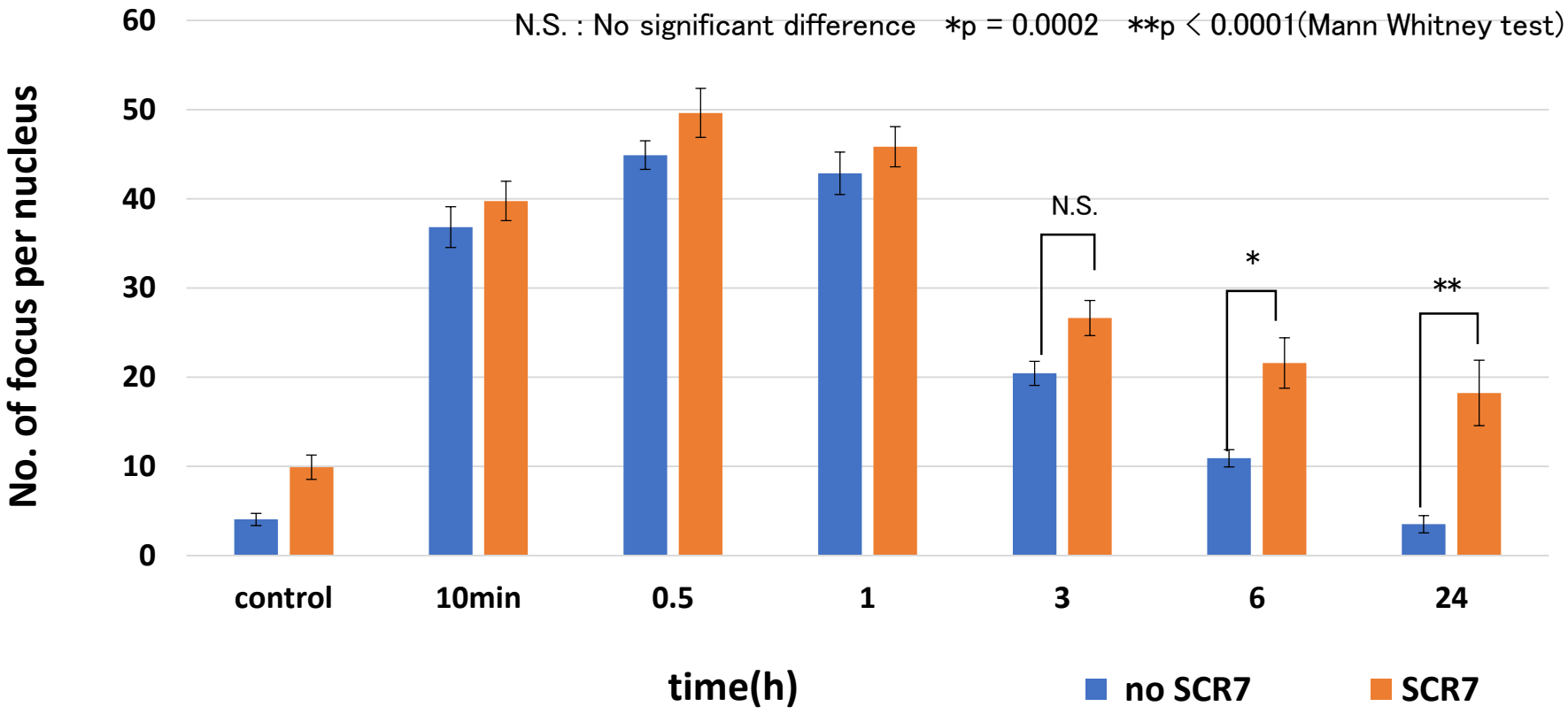


Fig. 8. Repair kinetics of DSBs in embryonic fibroblasts derived from C57BL/6N mice

Treatment with 25 μM SCR7 pyrazine induced a delay in DSB repair at 3 h, 6 h, and 24 h post-irradiation in embryonic fibroblasts, indicating that the inhibitory effect on Lig4 appeared at later stage (3–24 h post-irradiation) of NHEJ.

Discussion

The inhibitory effect on Lig4 appeared at later stage of DSB repair in NSPCs and embryonic fibroblasts.

Suggestions:

(1) Possibility 1:

The inhibitory effect of SCR7 may be partial due to the limitation of its concentration in use because the concentration of SCR7 is determined by considering under the condition for low cytotoxicity.

(2) Possibility 2:

An unknown ligase, but not Lig4, may play a role in an early stage of DSB repair.

Lig4 mutant mice may give us a clue to answer the questions.

Background

② DNA ligase IV (Lig4) deficient mice

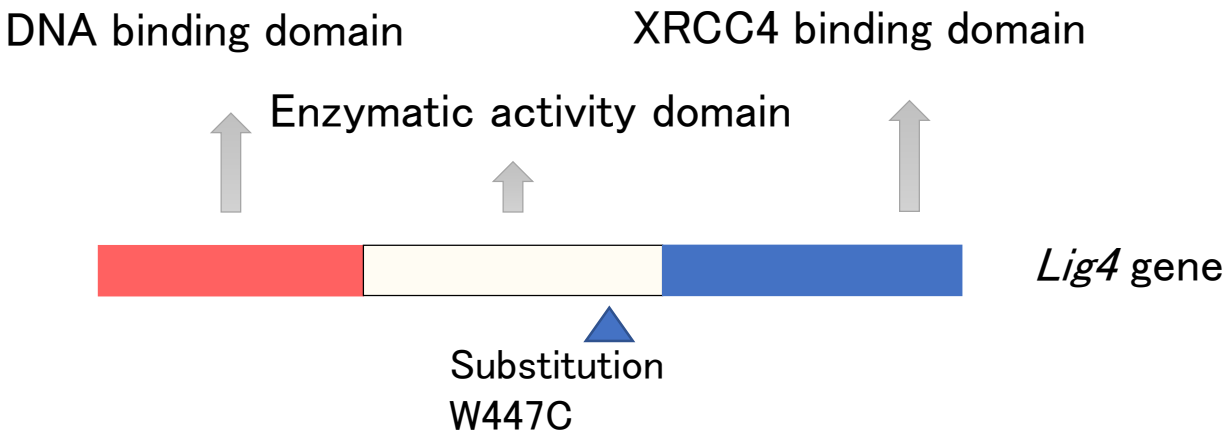


Fig. 9. Genotype of LIG4 mutant mice
DNA ligase IV (Lig4) plays an essential role in a final step of NHEJ where two DNA ends are joined together. The Lig4 deficient mutant mouse has a base substitution in enzymatic activity domain of both allele of *Lig4* gene.

Experimental design

$Lig4^{+/W447C}$ $Lig4^{+/W447C}$

$Lig4^{+/+}$

$Lig4^{+/W447C}$

$Lig4^{W447C/W447C}$

Fig. 10. Production of Lig4 mutant mice.
The mice retaining a homozygous mutation in *Lig4* gene (*Lig4*$^{W447C/W447C}$) were produced by mating with *Lig4* heterozygous mutant mice (*Lig4*$^{+/W447C}$) . Genotype of mice was examined by PCR-based genotyping.

Polymerase used can distinguish the difference of a single base substitution.

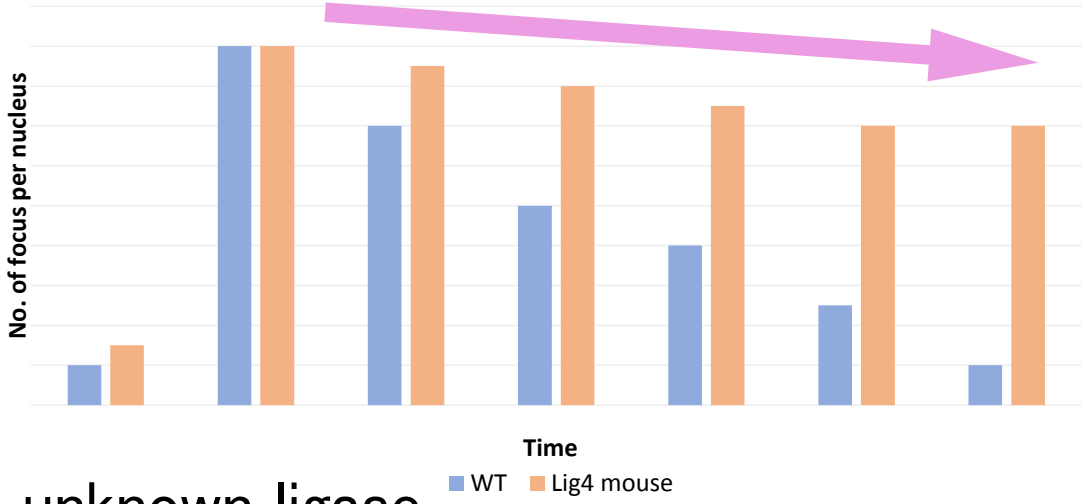
- 2 X AmpDirect buffer
- WT-F or Mut-F primer
- R-primer
- HiDi polymerase
- Tempate DNA
- Pure water

Electrophoresis

Expected results

(1) Possibility 1: a partial inhibitory effect of SCR7

The different result may be expected. The inhibitory effect in mutant cells is appeared even at early stage of DSB repair.



(2) Possibility 2: a role of an unknown ligase

The similar result may be expected. The inhibitory effect in mutant cells is appeared only at later stage of DSB repair.