No.	K25-3165	
Project Title	Harnessing CRISPR-Cas9 and DNA Repari Pathways (COMBI-CRISPR) for Temporally Inducible IFNAR1 Mouse Models to Advance Vaccine Research for Severe Viral Diseases	
Principal Investigator	Silvia Marina Vidal (McGill University・Professor)	
Project Member(s)	**IMSUT Host Researcher**	Tomoji Mashimo (The Institute of Medical Science, The University of Tokyo・Professor)
	Project Member (s)	Anavaj Sakuntabhai (Institut Pasteur du Japon・Professor)

(Form 2)

Principal Investigator
Silvia Marina Vidal

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

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Report

1. Communication and Collaborative Discussions
We conducted a series of discussions with Dr. Silvia Vidal (McGill University; total of 8 exchanges), primarily focusing on the design strategy for mouse models (DC-SIGN and IFNAR1) and preparation for applications to the IMSUT Joint Research Program.

Key milestones include:
May 15, 2024: Scheduling coordination and Zoom meeting setup
May 23, 2024: Receipt of publications and technical materials on hDC-SIGN mouse models
November 26-30, 2024: Proposal and discussions on IFNAR suppression models related to dengue vaccine research, including a shift from a doxycycline-inducible system to the AID2 system

In addition, we held technical consultations with Dr. Masato Kanemaki (National Institute of Genetics; total of 6 exchanges until September 2025), focusing on implementation of the AID2 system and access to mouse resources. These discussions provided detailed technical guidance, including:

Recommendation of the AID2 system
Availability of OsTIR1-expressing mouse lines from NIG/RIKEN BRC
Mechanistic insights into degradation of membrane proteins such as IFNAR1

2. Design Strategy and Internal Coordination
Based on the above discussions, a detailed technical design was shared with Prof. Mashimo on November 20, 2025, and the following strategy was agreed upon:

System: AID2 (~250 bp; Addgene #140532)
Knock-in design: Fusion of mAID2 tag to the C-terminal cytoplasmic region of IFNAR1
Resources: Introduction of OsTIR1-expressing mice from NIG or RIKEN BRC
Induction method: Administration of synthetic auxin (5-Ph-IAA) via intraperitoneal injection or drinking water

3. Project Summary and Current Status

This project aims to generate a novel IFNAR1 knock-in mouse model using the auxin-inducible degron (AID2) system, which enables rapid and precise protein degradation at the organismal level.

Discussions initiated in May 2024 with Dr. Silvia Vidal on interferon receptor regulation led to a transition from a doxycycline-inducible system to the AID2-based post-translational control system, which offers superior responsiveness and reversibility. In September 2025, intensive consultations with Dr. Masato Kanemaki further refined the system through the adoption of mAID2 and 5-Ph-IAA, improving inducibility while minimizing background degradation in vivo.

Based on these developments, the genome editing design was finalized to fuse the mAID2 tag to the C-terminal cytoplasmic region of IFNAR1. This system is expected to enable rapid and reversible depletion of IFNAR1 in specific tissues or developmental stages, thereby providing a powerful platform for dynamic functional analysis.

At present, all preparatory steps prior to mouse generation have been completed, including gene sequence optimization, donor DNA design, and arrangements for obtaining OsTIR1-expressing mice. The next phase will involve electroporation into fertilized embryos using the COMBI-CRISPR technology developed in the Mashimo laboratory, with the goal of establishing the mouse line efficiently.

The successful implementation of this approach is expected to provide a broadly applicable in vivo

platform for studying membrane proteins.