

in depth Proteomics of Hair Follicles of whiskers from *W* mutant mice clarifies KIT restriction on hematopoiesis and melanogenesis

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Overview

MALDI-IMS, Proteomics, *W* mutant, KIT, Hematopoiesis, Melanogenesis
Introduction

The proto-oncogene *c-kit* mapped to the white-spotting (*W*) locus of mice encodes the receptor tyrosine kinase (KIT). The characteristic phenotype of *W* mutants, which includes anemia and white coat color, can be attributed to the failure of stem cell populations to migrate and/or proliferate effectively during development. Here we established a novel strategy to study effects of KIT on hematopoiesis and melanogenesis in whisker follicles which have melanocytes (pigment cells) and cavernous sinus through *in situ* proteomic analysis.

W mutant mice and the receptor tyrosine kinase, KIT¹⁾

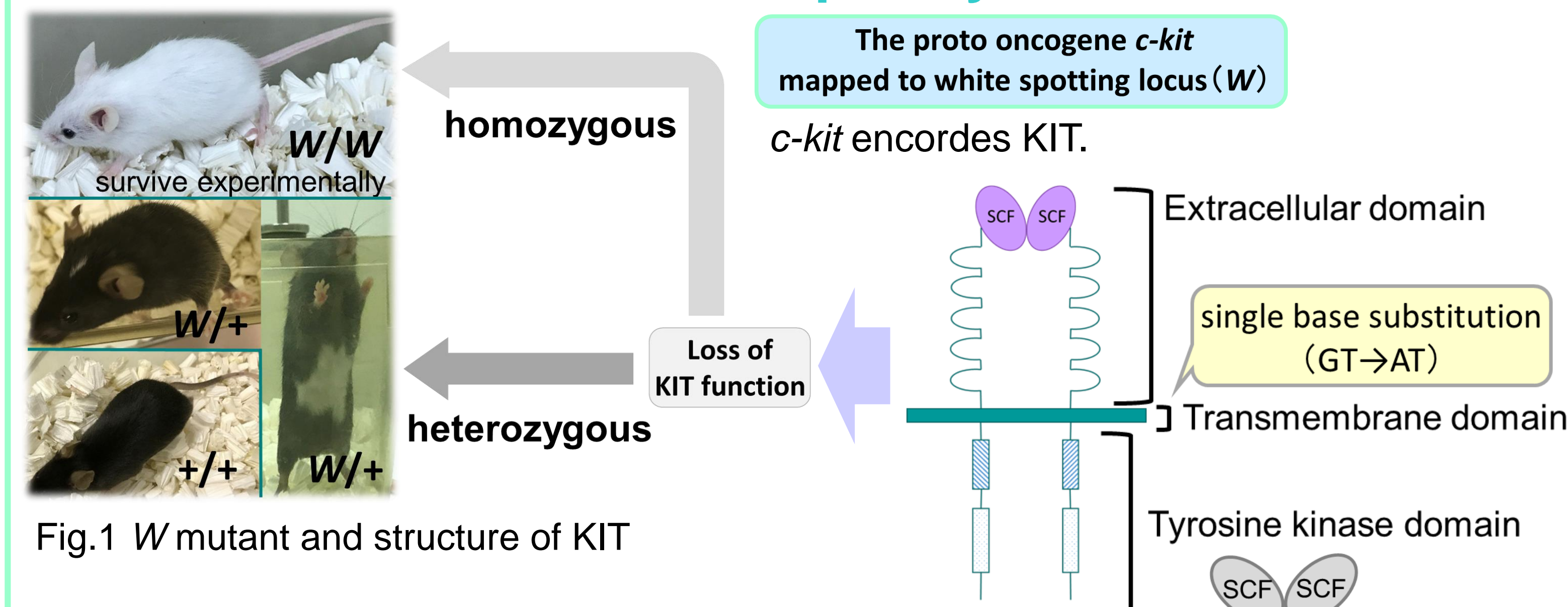
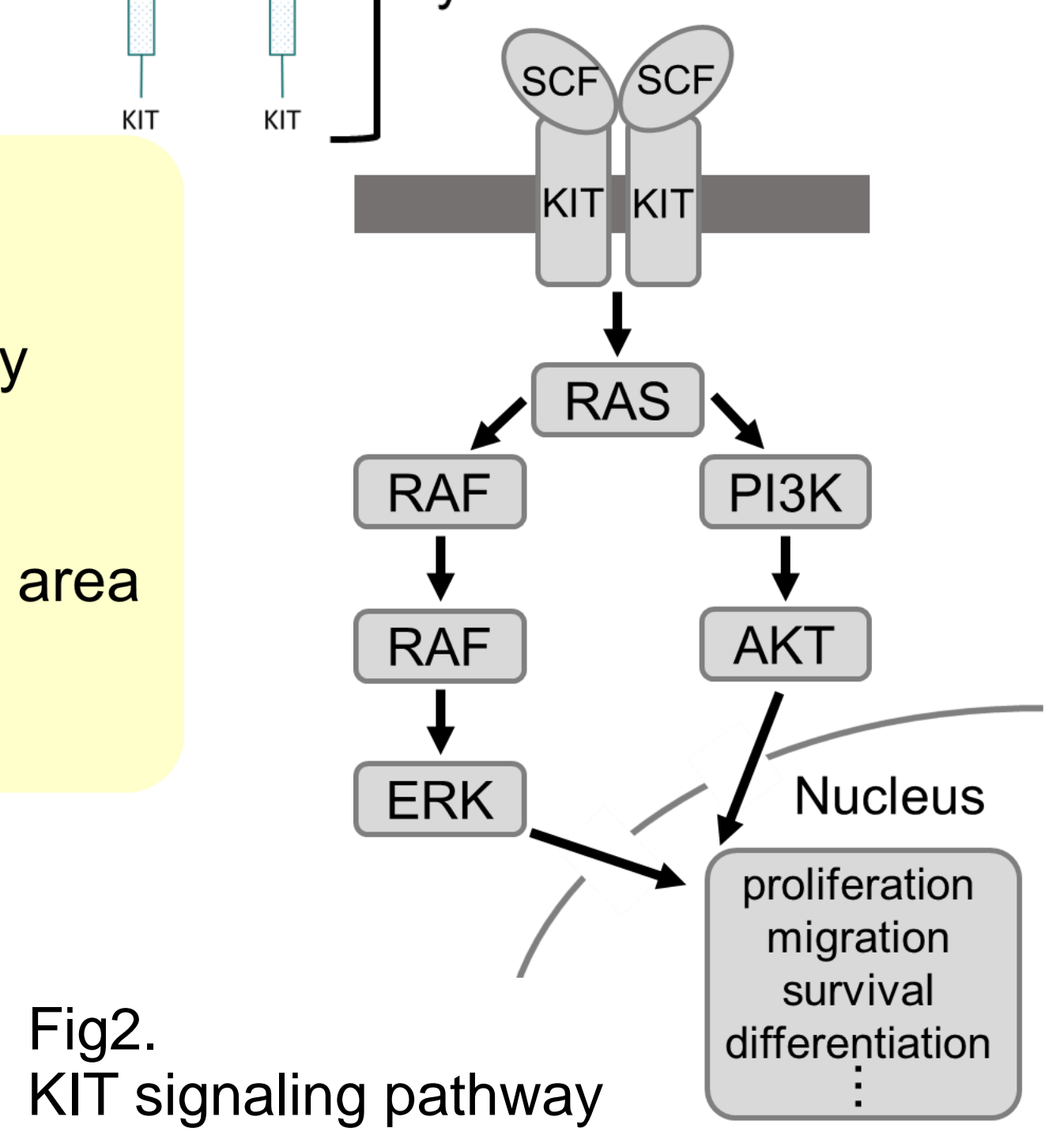


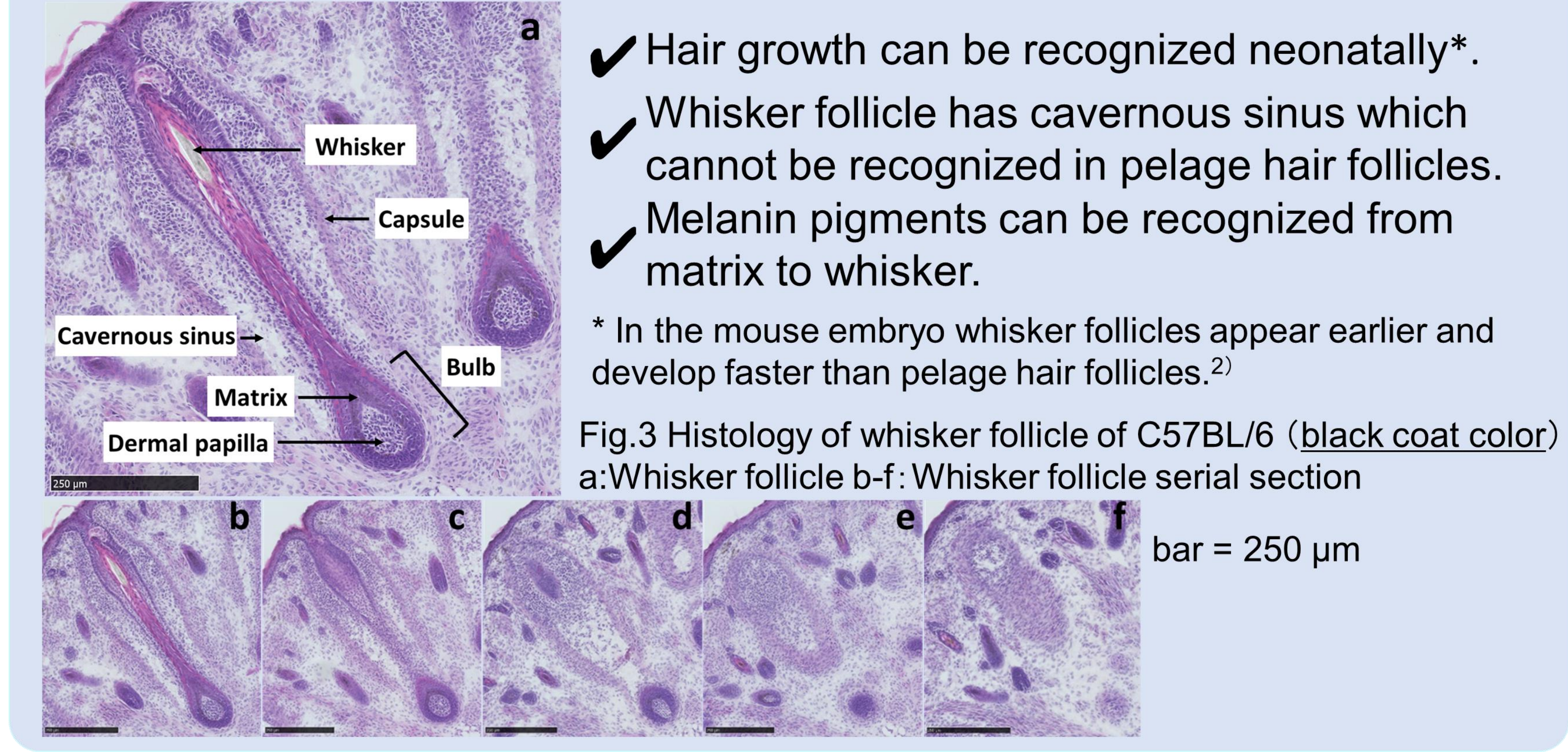
Fig.1 *W* mutant and structure of KIT

«Homozygous mice: *W/W*»
 • White coat color and black eye color
 • macrocytic anemia, sterility, mast cell deficiency
 → death is within 10 days after birth
«Heterozygous: *W/+*»
 • white coat color only in forelock and abdominal area
«Wild type: *+/+*»
 • black coat color

Ligand : stem cell factor; SCF)
 The SCF/KIT pathway plays critical role in cell differentiation and proliferation via activation of the MAPK and PI3K pathways.



Histology of the whisker follicle of neonatal mouse



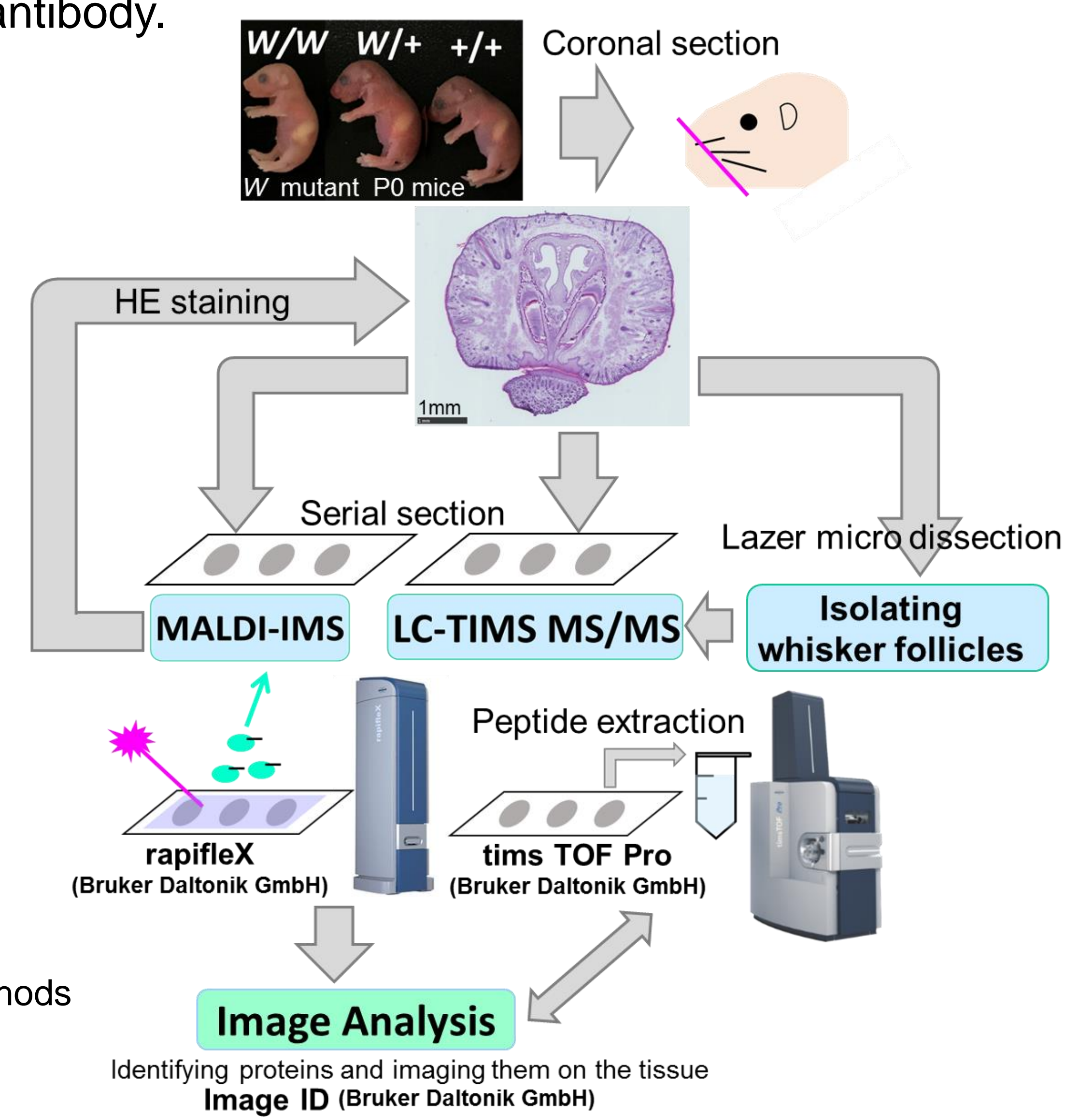
Methods

Animals

W mutant mice (gift from Dr. Kazuo. K) at postnatal day (P0) were sacrificed and snap-frozen in liquid nitrogen.

Histopathological and immunohistochemical analysis

10 μm coronal sections through tips of nose were stained with Hematoxylin & Eosin (HE), Fontana - Masson Stain. Immunostaining were performed with anti - MelanA antibody.



MALDI-IMS

- The sections were transferred to conductive Indium-Tin-Oxide coated glass slides.
- After washing the sections, on tissue digestion with trypsin was performed with TM-Sprayer (HTX imaging).
- α-cyano-4-hydroxycinnamic acid (CHCA, 10mg/ml in 70% acetonitrile, 1% trifluoroacetic acid) as a matrix was uniformly deposited on the slides by using TM-Sprayer.
- The peptides measurements were carried out by using rapifleX (Bruker Daltonics) with the spatial resolution of 50 μm. The mass range : m/z 800-4,000

Shotgun proteomics with the timsTOF Pro

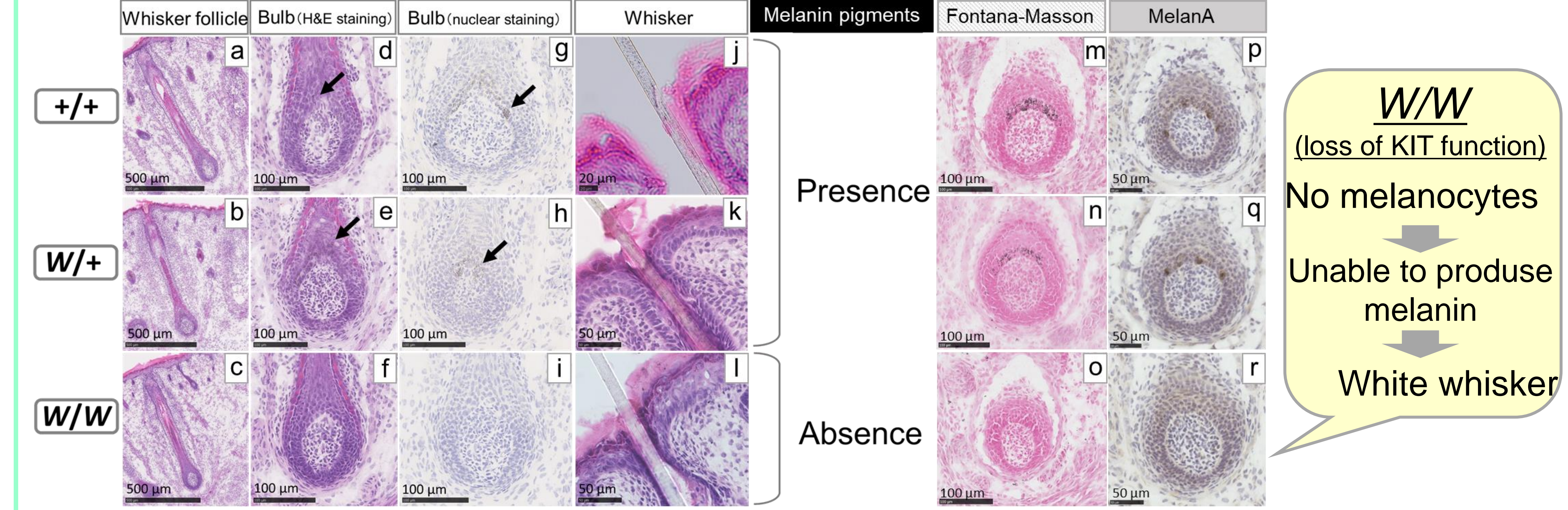
By using timsTOF Pro with nanoElute (Bruker Daltonics) shotgun proteomics was performed with the same tissue sample as IMS and whisker follicles isolated by laser micro dissection. Column used was 25 cm x 75 μm 1.6 μm C18 column. Number of MS/MS ramps was 10PASEF scan.

Data analysis

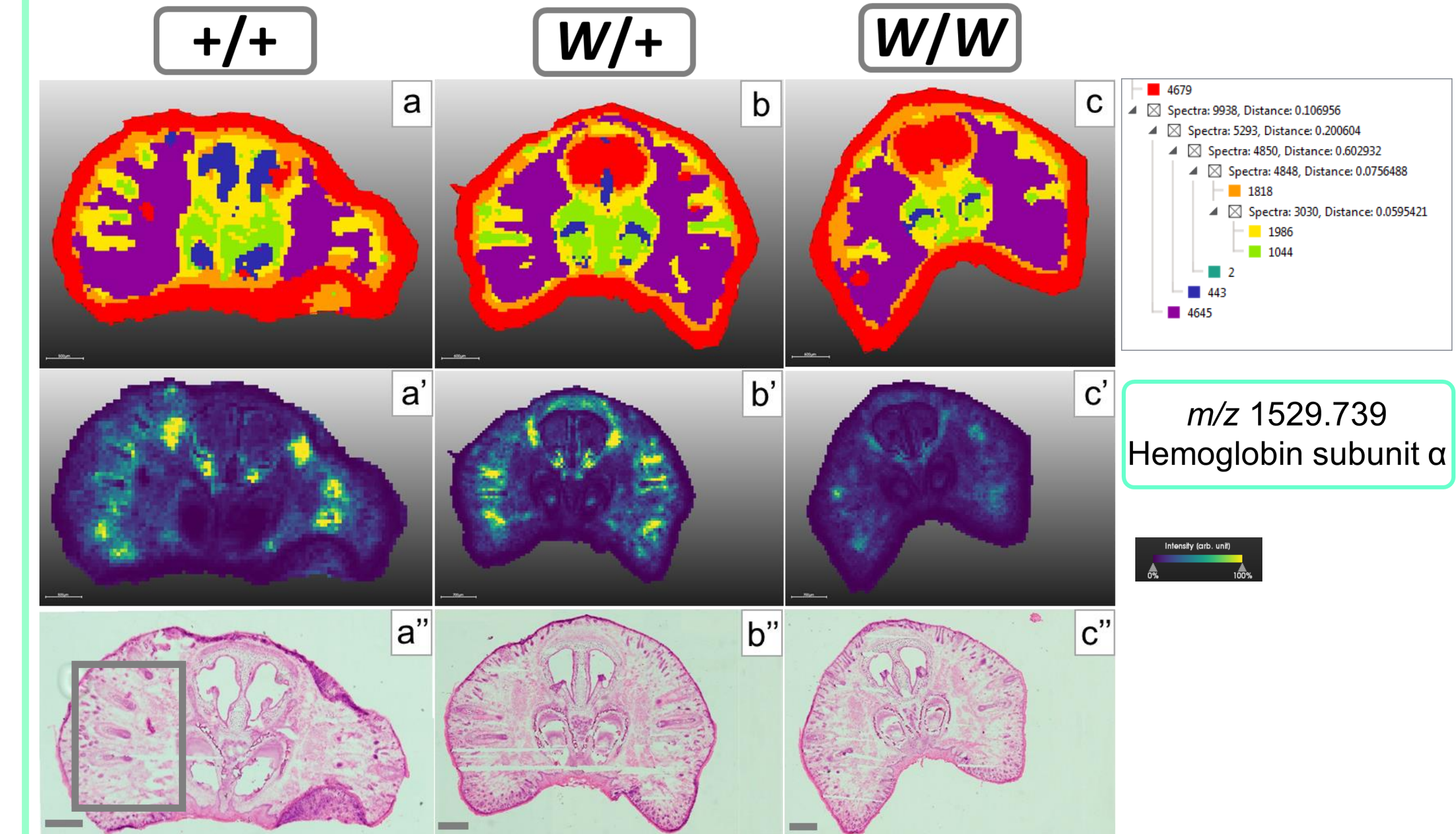
Obtained mass spectra imaging were visualized with flexImaging 5.0, SCiLS Lab 2019b software. About 2,000 proteins were successfully annotated from coronal sections with Proteinscape 4.0, and database was Swiss-prot. Merged spatial information from MALDI-IMS and identified peptide / proteins information by using ImageID (Bruker Daltonics).

Results

1. Histopathological analysis



2. MALDI - IMS



W/W has different proteomic pattern from *+/+* and *W/+*.

Single peak analysis
 Receiver Operating Characteristic (ROC)
 • Comparing *+/+* with *W/W*
 • Finding the discriminative single *m/z*
***m/z* 1529.739** is specifically distributed in cavernous sinus of *+/+* whisker follicle.
 MS/MS ion search
m/z = 1529.739 was identified **Hemoglobin subunit α (HBA)**.

3. Proteomics of whisker follicle

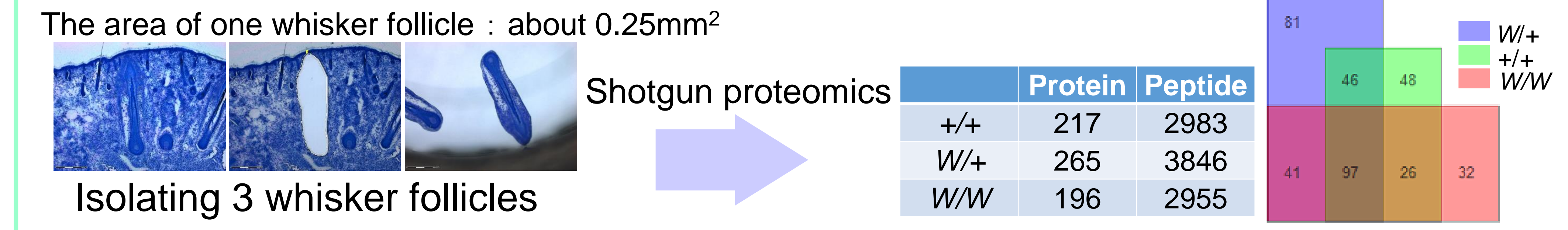


Fig.8 Isolation of whisker follicles from tissue slides using laser micro dissection and LC – tims MS/MS

Conclusions

- ✓ Anemia caused by hematopoietic defect, the phenotype of *W/W* mice was visualized as low intensity of HBA in cavernous sinus by *In situ* proteomics with MALDI-IMS.
- ✓ Proteomics of isolated whisker follicle tissue samples by timsTOF Pro is now ongoing to
- ✓ Identify key proteins of melanogenesis under KIT restriction in whisker follicles.

References

1) Nocka K, Majumder S, Benoit C, et al. Expression of *c-kit* gene products in known cellular targets of *W* mutations in normal and *W* mutant mice—evidence for an impaired *c-kit* kinase in mutant mice. *Genes & Development*. 1989, 3 (6), 816-826
 2) Davidson P and Hardy MH. The Development of Mouse Vibrissae *in vivo* and *in vitro*. *Journal of Anatomy*. 1952, 86(Pt 4), 342-356