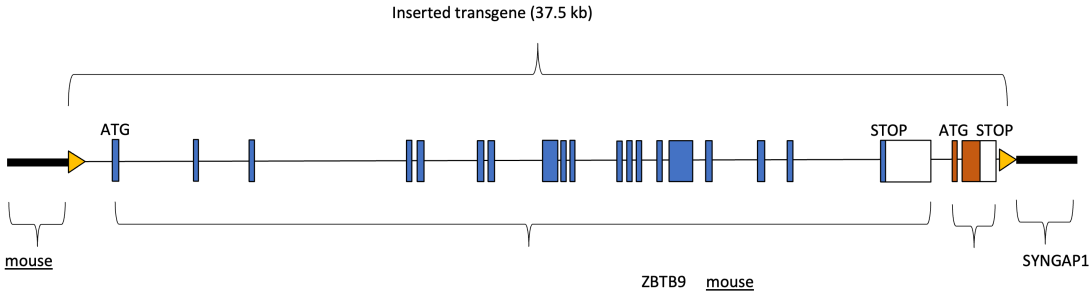
Introducing OzBIG technology

Example 1: Standard genomic replacement
Syngap1 – full genomic humanization

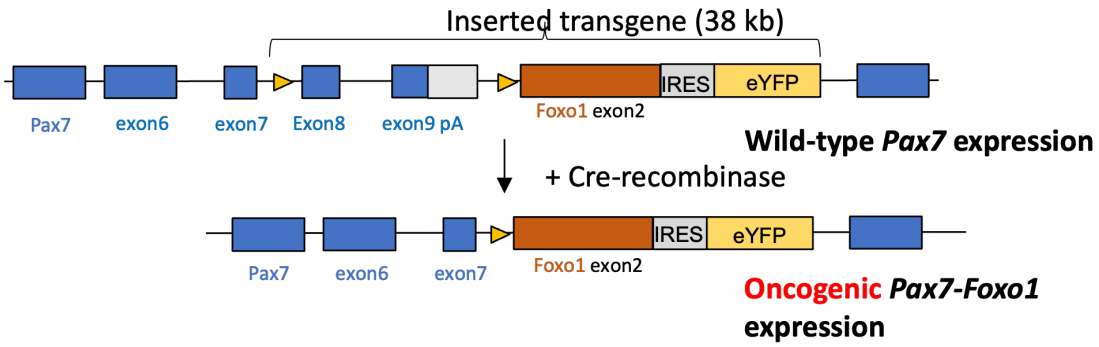
The Synaptic Ras GTPase-activating protein 1 (encoded by SYNGAP1) is essential for normal synapse function and development of cognition. Sporadic mutations in SYNGAP1 are responsible for rare, dominant disorders of intellectual disability, epilepsy autism and sensory processing. The SynGAP Research Fund (https://www.syngapresearchfund.org/) sponsored the development of a humanized model as the first step towards an animal model enabling the study and therapeutic development for these rare diseases.



Transgene design
A Floxed genomic fragment of 37.5 kb carrying the entire human SYNGAP1 gene, including 2 kb of promoter sequence, and the ZBTB9 gene to the Stop codon replaced the mouse 34.7 kb orthologous genomic region.

Example 2: Inducible rare disease & reporter gene
Pax7-Foxo1 Transgene

The *Pax7-Foxo1* chimeric oncogene drives the childhood muscle cancer alveolar rhabdomyosarcoma. To better understand this sarcoma, a mouse model is being generated containing a conditional knock-in of the *Pax7-Foxo1* fusion.



Transgene design

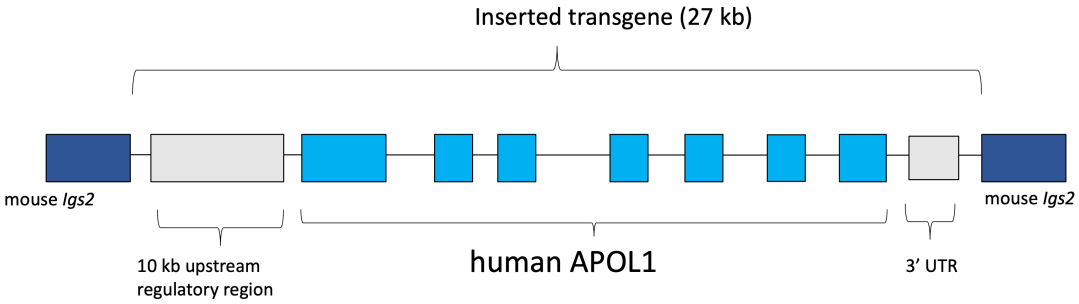
To create a conditional *Pax7-Foxo1* fusion, the mouse *Pax7* gene was modified such that exons 8 and 9 were flanked with loxP sites, and a downstream cassette containing exon2 of *Foxo1* followed by an IRES and eYFP coding sequence was inserted.

In the resulting transgenic mouse, wild-type *Pax7* will be expressed by default. Induction of Cre-recombinase will cause genomic rearrangement to yield a model of the oncogenic genotype with a fluorescent reporter of expression. Cre expression triggers excision of the 3' terminal *Pax7* sequence, resulting in fusion with the downstream *Foxo1* exon. The IRES-eYFP sequence allows expression of the oncogene to be inferred via expression of the fluorescent marker.

Example 3: Non-orthologous humanization

Human APOL1 Transgene

Apolipoprotein L1 (encoded by *APOL1*) shows clinical relevance in chronic kidney disease (CKD). Two polymorphisms (G1 and G2) strongly correlate with an increased risk for developing CKD, which suggests a promising opportunity for treatment. However, *APOL1* is lacking in rodents, which greatly hinders drug discovery efforts. We have generated a mouse model for expression of human *APOL1* to support these studies.



Transgene design

A targeting construct was created to insert the human gene *APOL1* into the murine safe harbor locus, *Igs2*. To optimize for expression in relevant tissues and at relevant levels, 10 kb of upstream sequence was included in the transgene to capture the endogenous human regulation of expression. All *APOL1* exons were sequenced to confirm the presence of the desired *APOL1* allele, G1.