The zebrafish as a model organizm for biomedical research

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The zebrafish as a system for biomedical research - advantages

- Small size, produce 100-200 ofspring each week

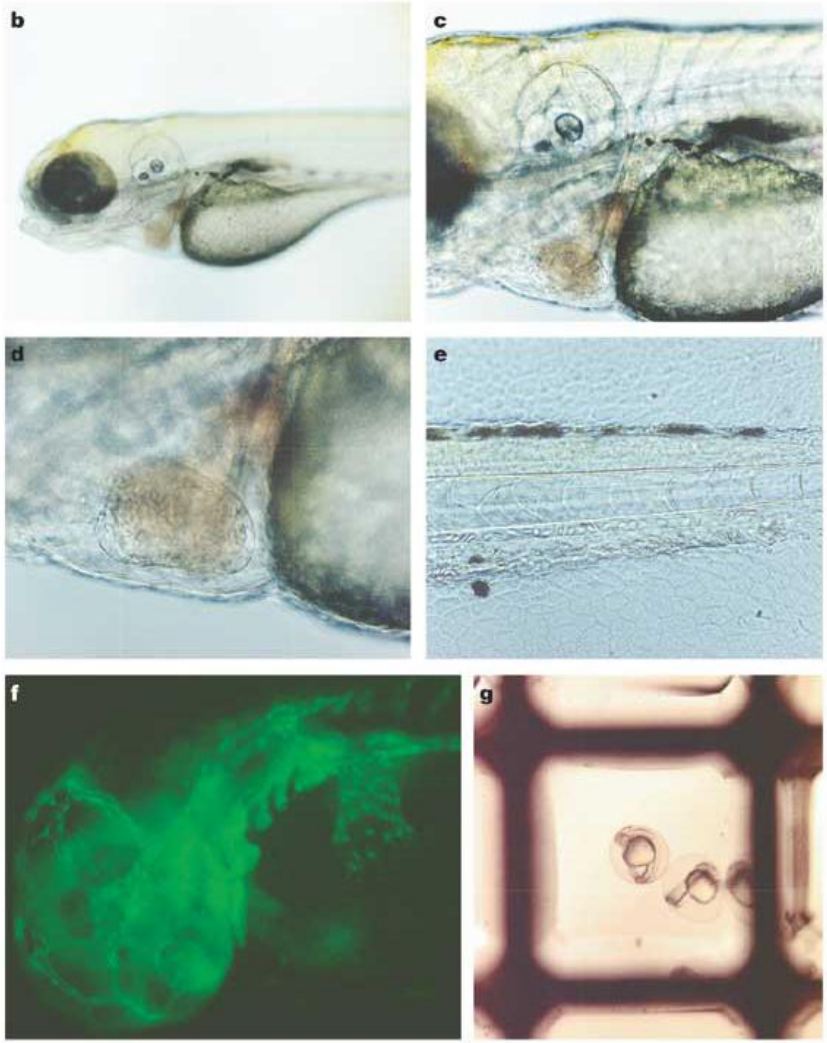
- Develop rapidly ex utero and are transparent much of their life, which allows visualization of functional and morphological changes

- Live in water and easily take up chemicals from their environment, which makes these aquatic animals ideally suited for carrying out toxicological and chemical studies

- Similar to mammals, birds and reptiles, zebrafish have both T and B cells, which allows the study of these lymphoid-cell populations in this model

- Gynogenetic diploid offspring can be produced, which leads to progeny with two sets of maternally inherited chromosomes. The use of this technology, in conjunction with mutagen treatment, has allowed researchers to carry out large-scale genetic screens to identify mutations that disrupt heart, eye, jaw, blood and fin development

The zebrafish as a system for biomedical research



In vivo drug discovery in zebra fish, Nature Reviews Drug Discovery 2005

The zebrafish as a system for biomedical research - disadvantages

- Short-lived when compared with humans, which makes direct comparison of age-related phenotypes limited.

- Organs are typically simpler than mammalian counterparts

- Some mammalian organs are not conserved, including the mammary and prostate glands

- The genome size is approximately one-half the size of the human genome, making comparisons difficult

- The genome underwent a genome duplication event, so many genes have redundant copies, which complicates loss-of-function studies

- Low incidence of spontaneous tumorigenesis, necessitating the use of mutagens and/or transgenic techniques

- Limited range of antibody reagents, making protein-based analysis more difficult

Table 1 | **The zebrafish toolbox**

Technology	Description
Forward genetics	
Chemical mutagenesis	High mutation rates, large-scale screens
Insertional mutagenesis	Efficient cloning of mutations
Reverse genetics	
Morpholinos	Rapid, inexpensive gene knock-downs
TILLING	Directed identification of permanent mutations
Expression profiling	
Gene chip	Zebrafish Affymetrix chip
Spotted microarrays	cDNA and oligonucleotide microarrays
Other tools	
Transgenesis	Rapid production of stable transgenic lines
cDNA collections	Full-length cDNA collections
Mutant collections	Thousands of catalogued mutant lines Hundreds of lines available through public stock centres
Physical and genetic maps	Radiation hybrid and microsatellite genetic linkage maps
Genomic sequence	5.7-fold coverage of the zebrafish genome Substantial genome annotation

TILLING, targeting-induced local lesions in genomes.

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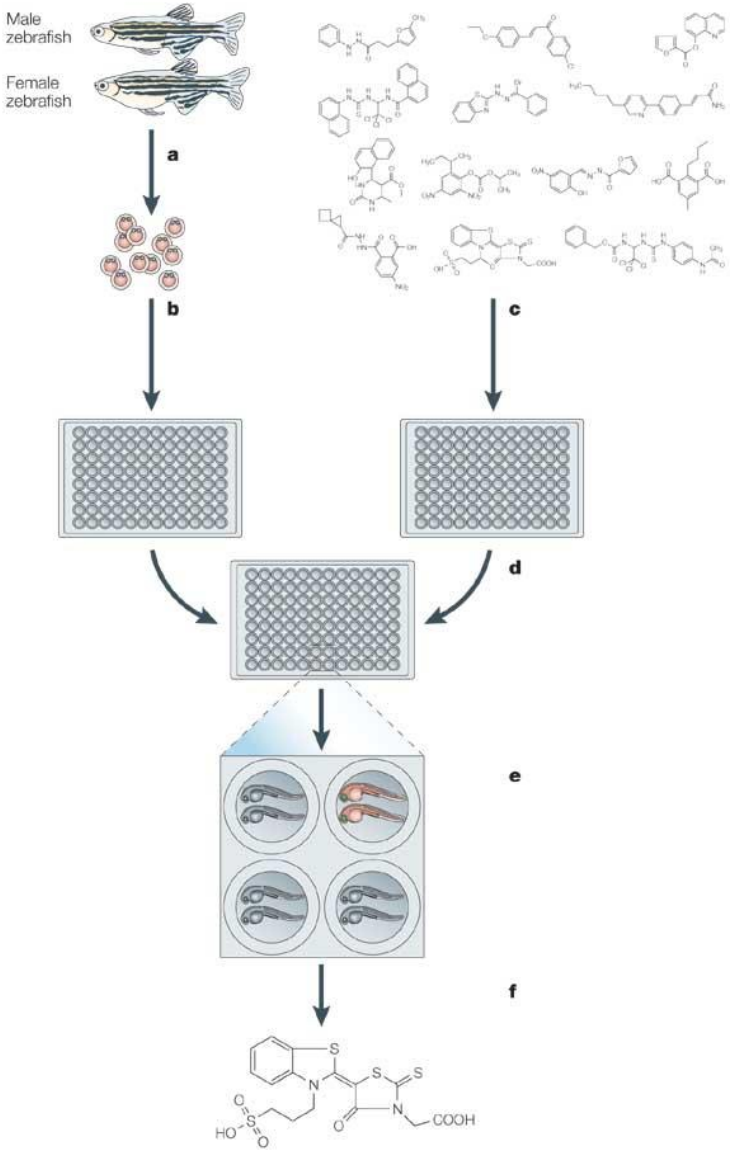
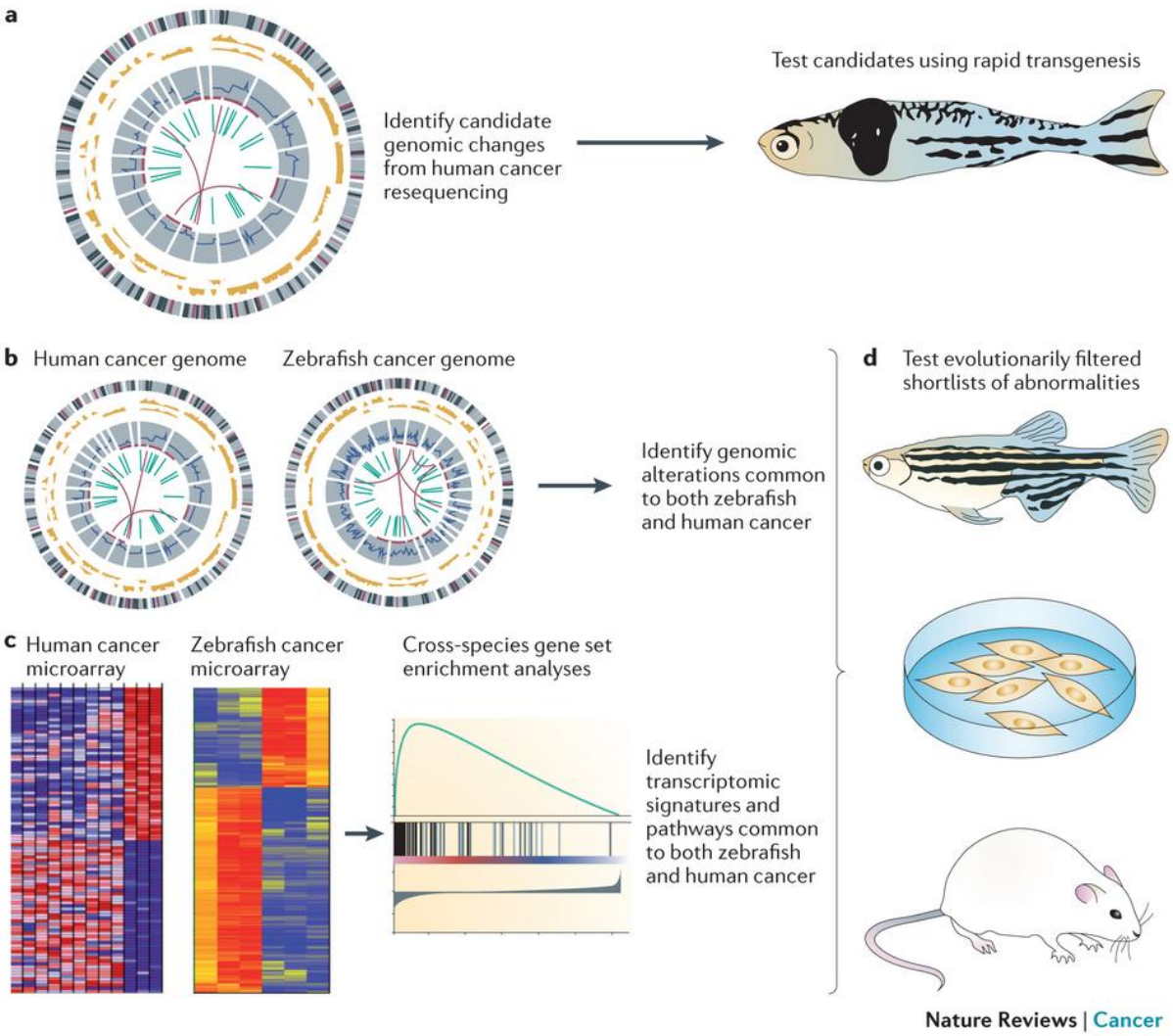


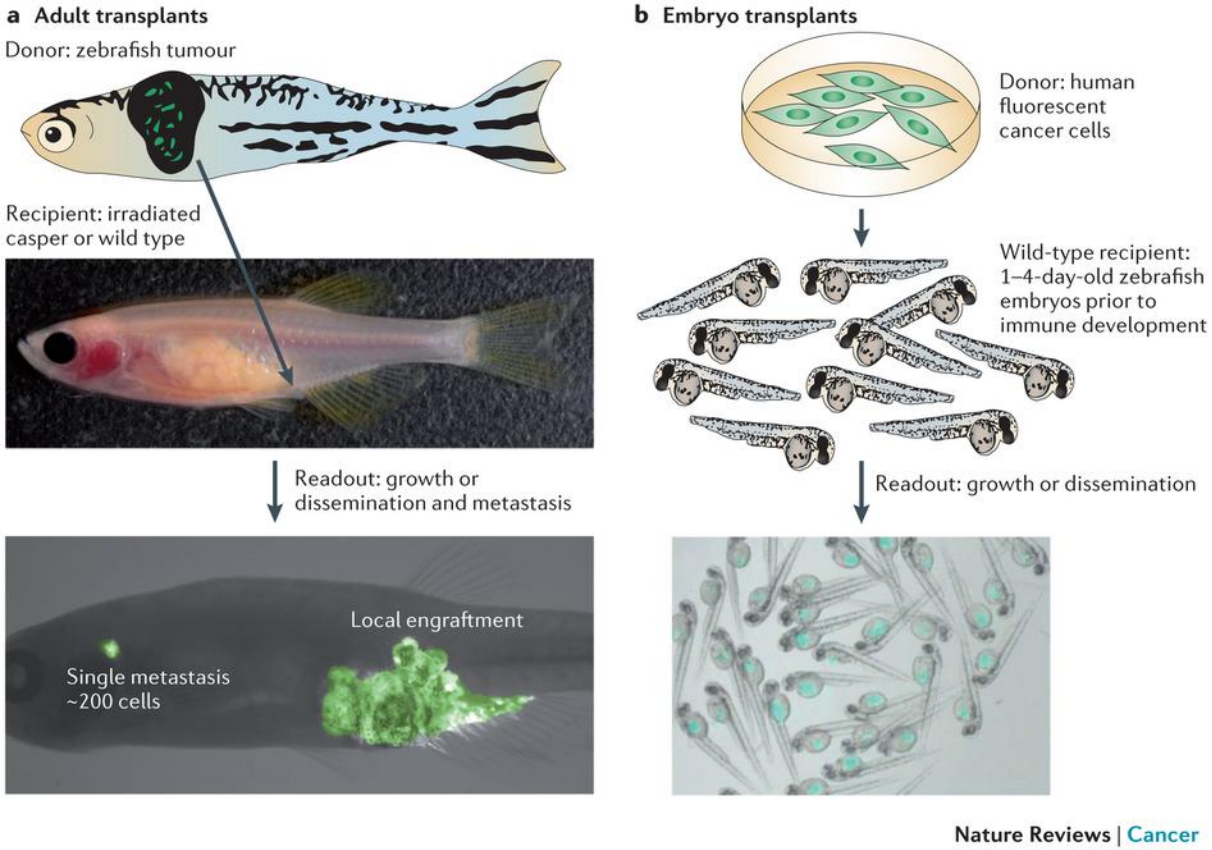
Nature Reviews | Drug Discovery

Zebrafish as an animal model in cancer research



Zebrafish cancer: the state of the art and the path forward, Nature Reviews Cancer 2013

Transplantation tools in zebrafish



Nature Reviews | Cancer

Zebrafish as an animal model in cancer research

Cancer	Oncogene	Tumour suppressor	Use in cancer biology
Melanoma	*mitfa–BRAF*^{V600E}	*tp53*$^{-/-}$	Genetic and chemical modifier screens
	mitfa:EGFP:*NRAS*^{Q61K}	*tp53*$^{-/-}$	
	kita–Gal4 × *uas-HRAS*		
Pancreatic	*ptf1a–KRAS*^{G12V}–GFP		Genetic modifier screens
	ptf1a:Gal4–VP16 × *uas–KRAS*^{G12V}–GFP		
T cell lymphoma or leukaemia	*rag2–myc*		Cancer modelling and *in vivo* imaging
	rag2–lox–dsRED2–lox–EGFP–mMyc × *hsp70*–cre		Inducible cancer model
	rag2–NOTCH1		NOTCH1 interaction with Bcl-2
	rag2–myc × *rag2–bcl2*		Mechanisms of leukaemia dissemination
B cell leukaemia	*Xenopus* Spp. EF1α or zebrafish B actin–TEL–AML1 (ETV6–RUNX1)		Initiating events in B cell leukaemia
Numerous	b-actin–lox–GFP–lox–*KRAS*^{G12D} × *hsp70*–cre		Inducible cancer model
	krt4:Gal4VP16;14 × *uas:smoa1*–EGFP × *uas:myrAKT1*		Cooperation of hedgehog and AKT pathways
Rhabdomyosarcoma	*rag2–KRAS*^{G12D}		Identification of tumour-initiating cell populations
Neuroblastoma	dβh:EGFP–*MYCN*		Cooperation of MYCN and ALK
	dβh:EGFP and dβh:*ALK*^{F1174L}		Cooperation of MYCN and ALK
AML	*pu1–MYST3/NCOA2*–EGFP		First model of AML in zebrafish
MPNST		*tp53*$^{-/-}$	Conservation of tumour-suppressor pathways in zebrafish
			Major tumour type found in p53-deficient zebrafish
Lipoma	*krt4*–myr*AKT1*		Platform for the study of drugs to treat lipoma and/or obesity
Ewing's sarcoma	*hsp70* or β-actin–*EWSR1–FLI1*	*tp53*$^{-/-}$	Conserved function of EWS–FLI1 fusion protein from human to fish
Liver	*fabp10*:LexPR; LexA:EGFP × cryB:mCherry; LexA:EGFP–*kras*^{G12V}		Inducible KRAS-G12V hepatocellular cancer model
	fabp10:TA; TRE:*xmrk*; *krt4*:GFP		Inducible EGFR-homologue hepatocellular cancer model
Pancreatic neuroendocrine	*zmyod–MYCN*		Pancreatic neuroendocrine model as a platform for downstream MYCN targets
Myeloproliferative neoplasms	*sp1–NUP98–HOXA9*		NUP98–HOXA9-induced oncogenesis from defects in haematopoiesis and aberrant DNA damage response
Corticotroph adenoma and neoplasm	*POMC–PTTG*		Identification of CDK inhibitors as possible treatment of corticotroph tumours
Testicular germ cell tumour	*fugu flck*–SV40 large T		Platform for modifier screens of testicular tumours

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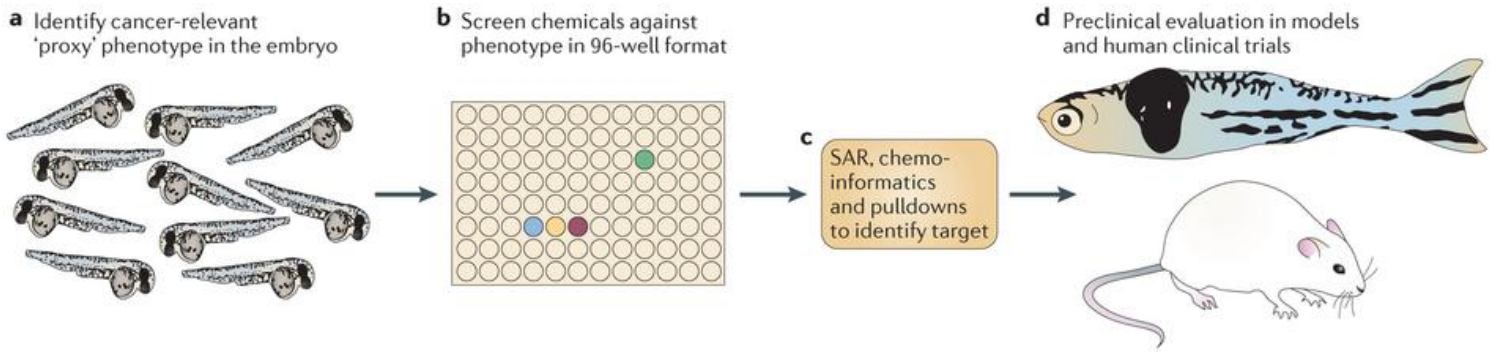

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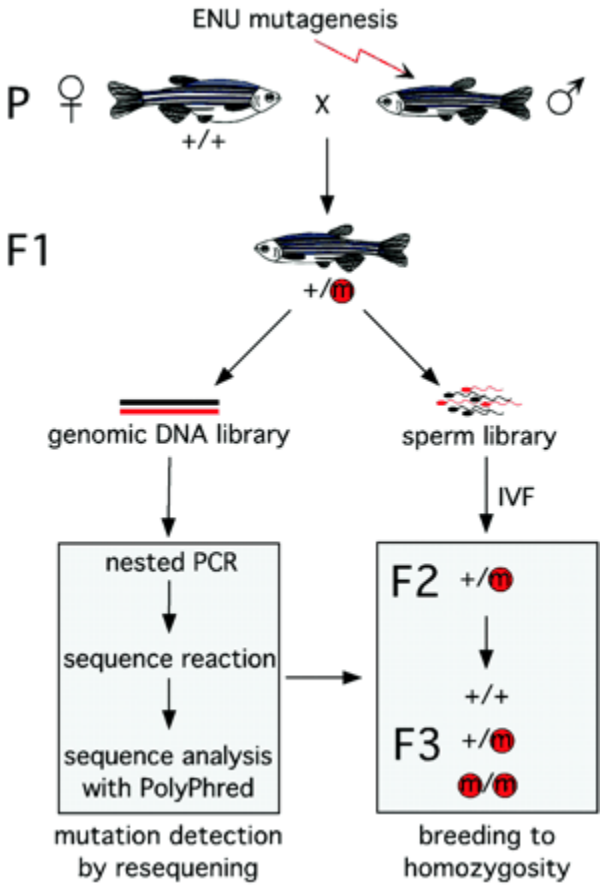
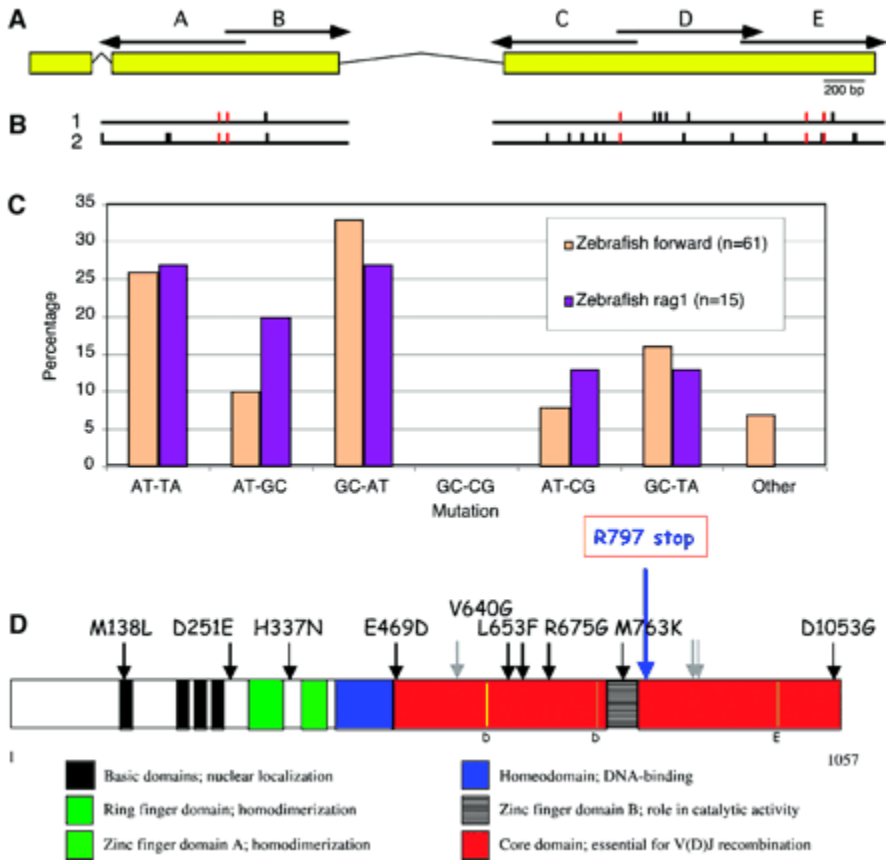
Overview of target-selected mutagenesis in zebrafish



Target-Selected Inactivation of the Zebrafish rag1 Gene, Science 2002



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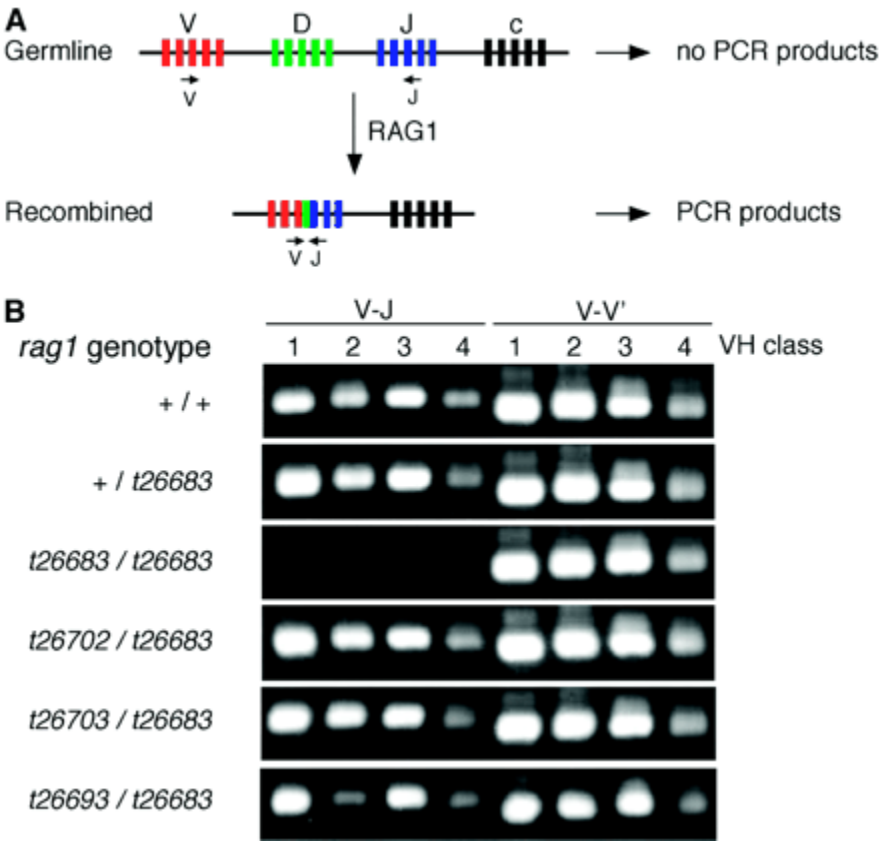
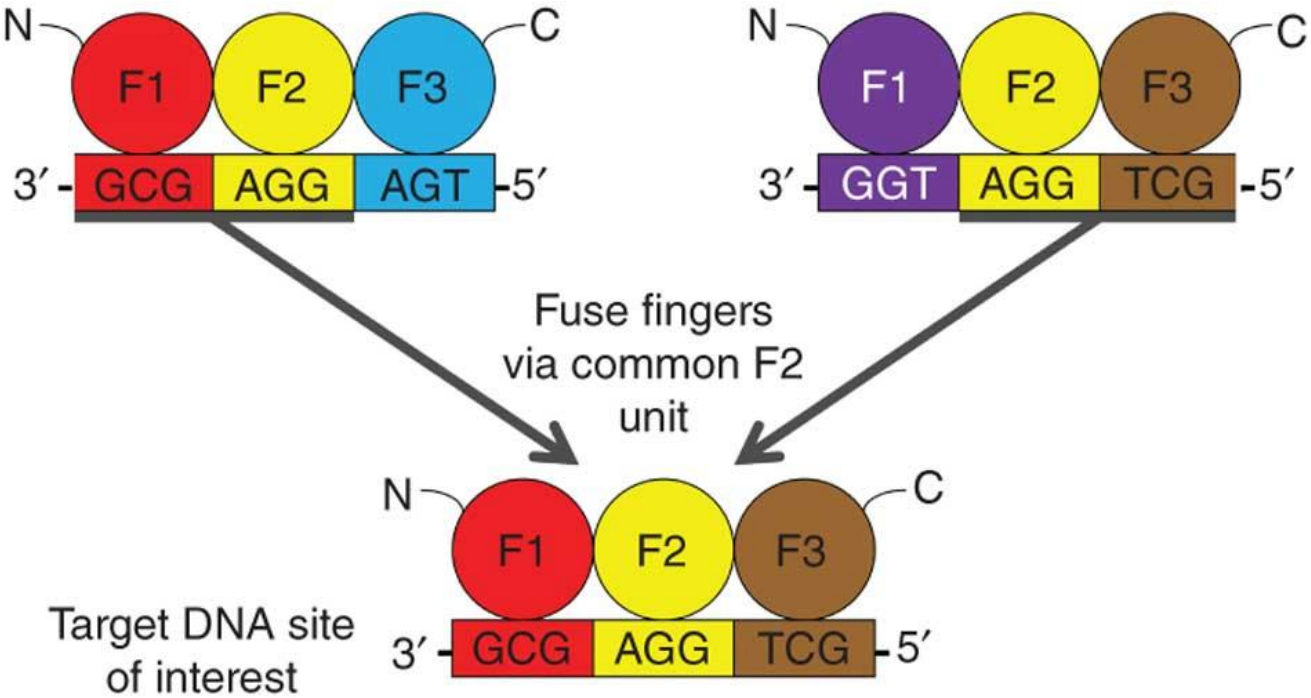


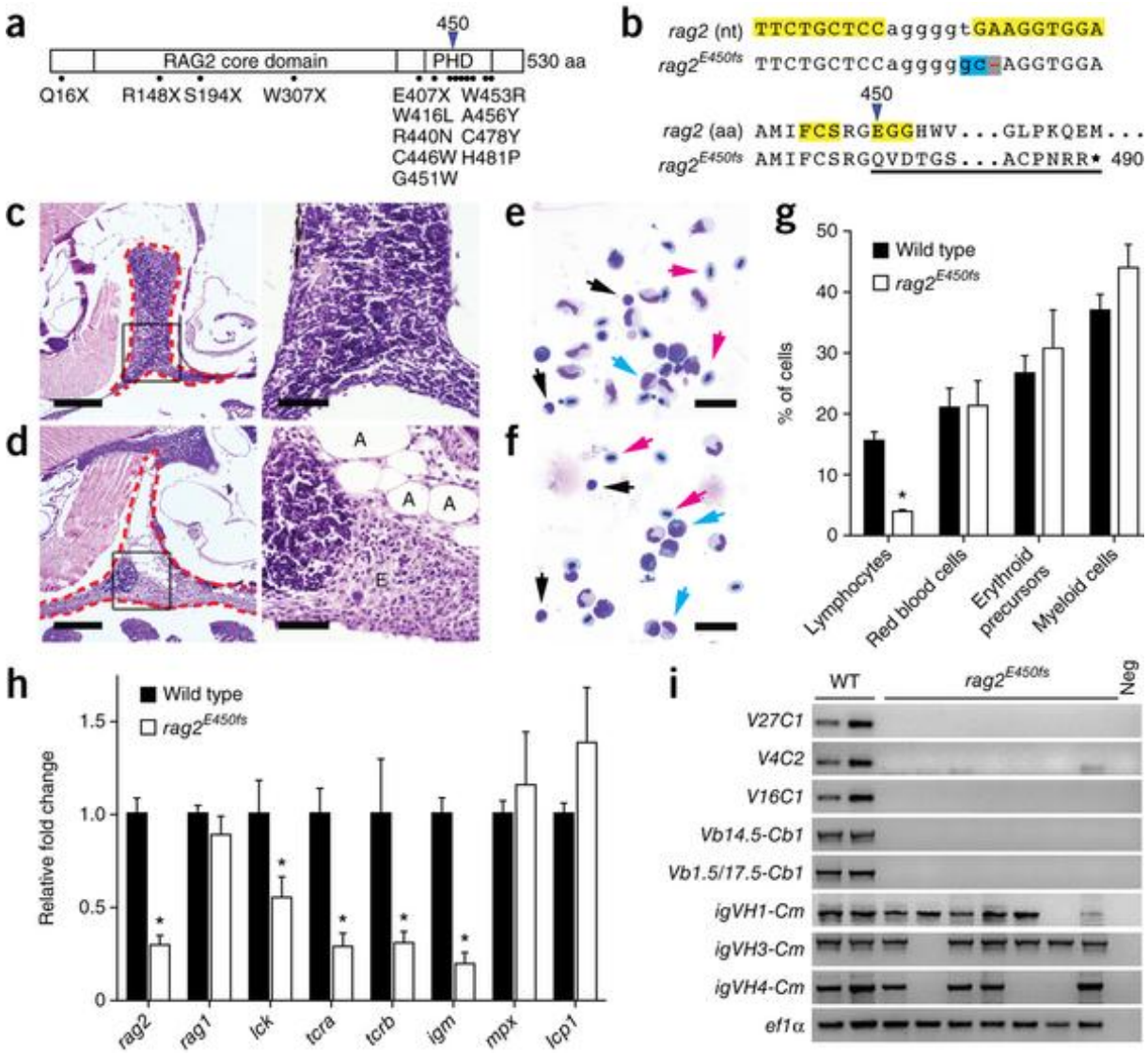
Target-Selected Inactivation of the Zebrafish rag1 Gene, Science 2002

Schematic overview of context-dependent assembly

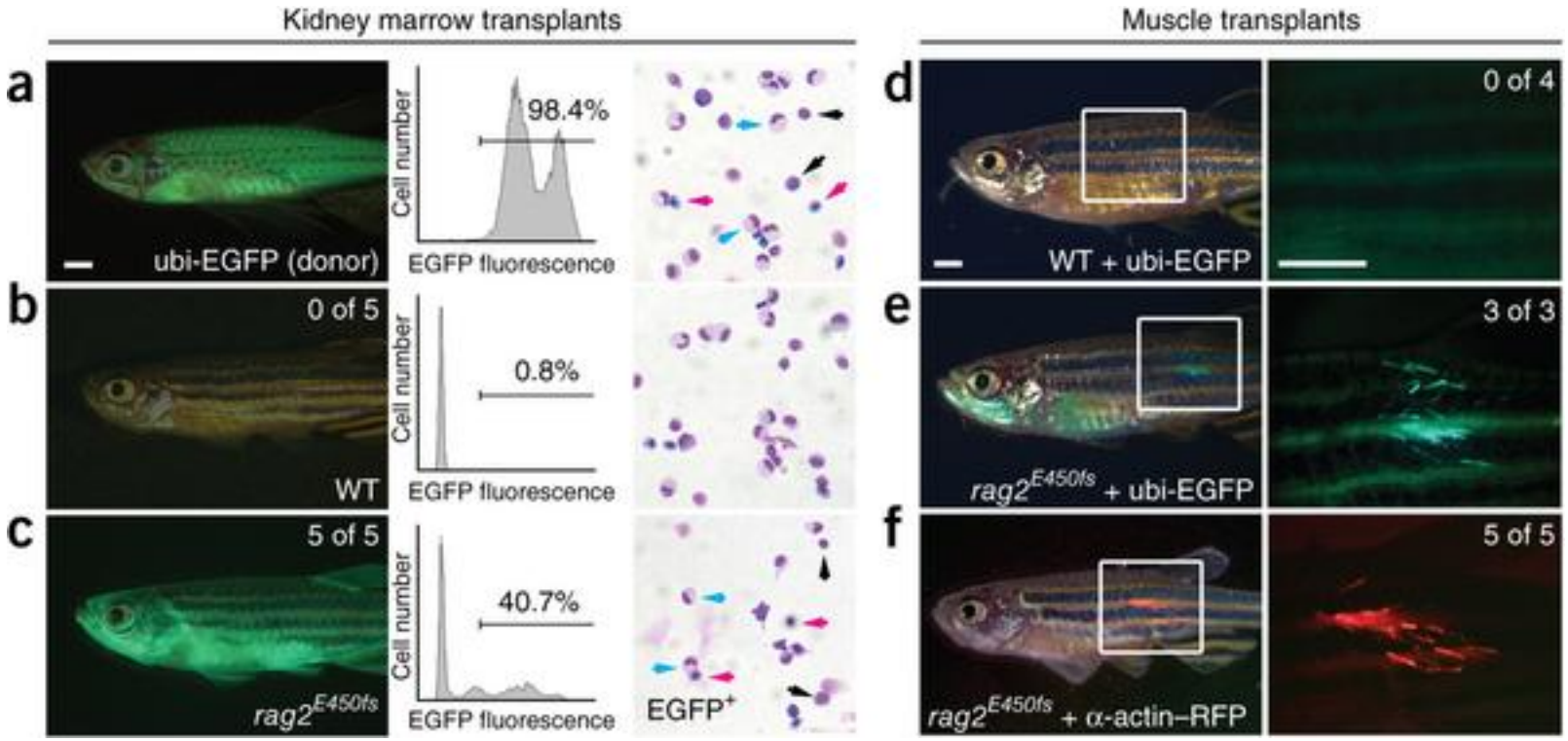


Selection-free zinc-finger-nuclease engineering by context-dependent assembly (CoDA), Nature Methods 2011

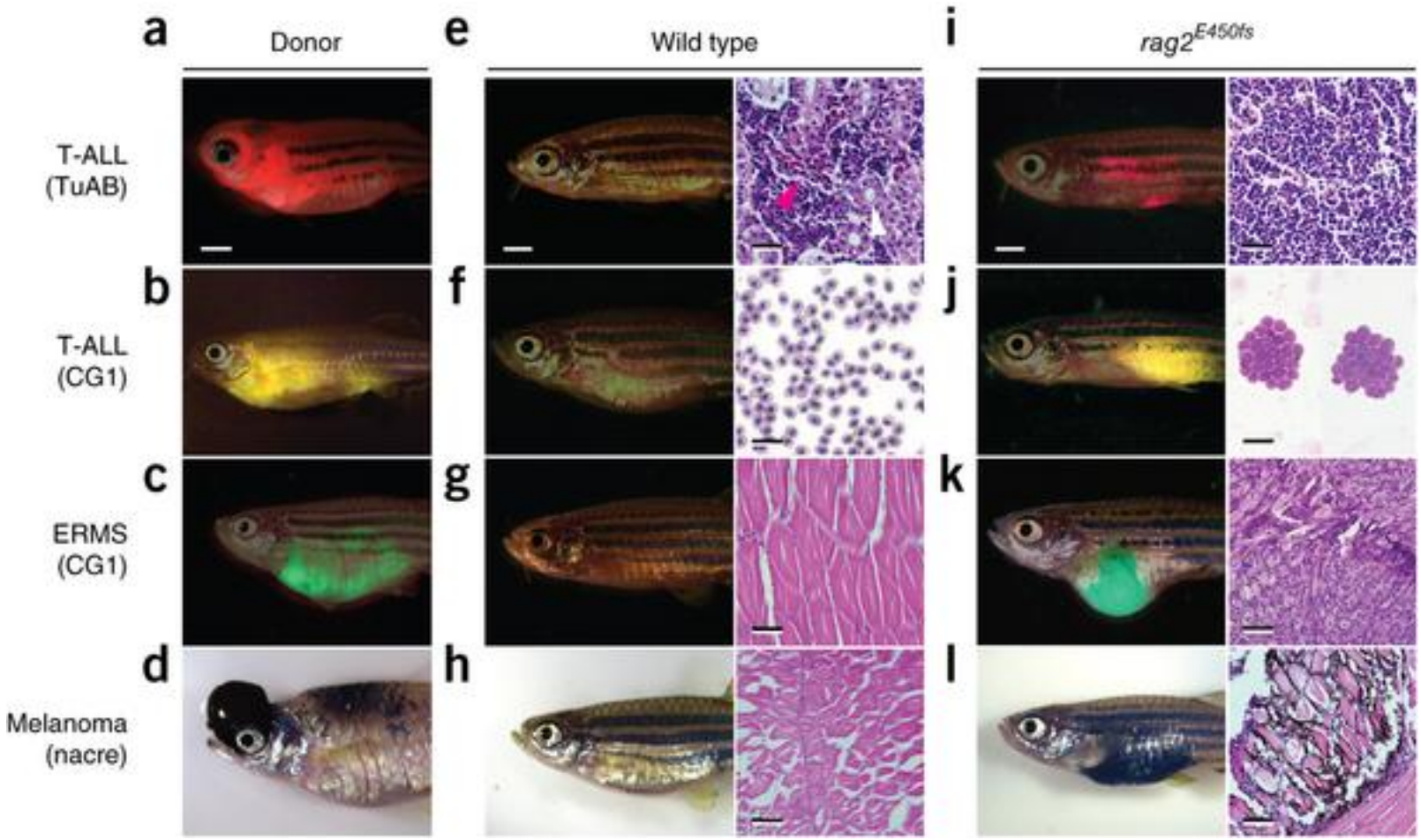
rag2E450fs mutant zebrafish lack mature T cells and have a reduced B-cell repertoire



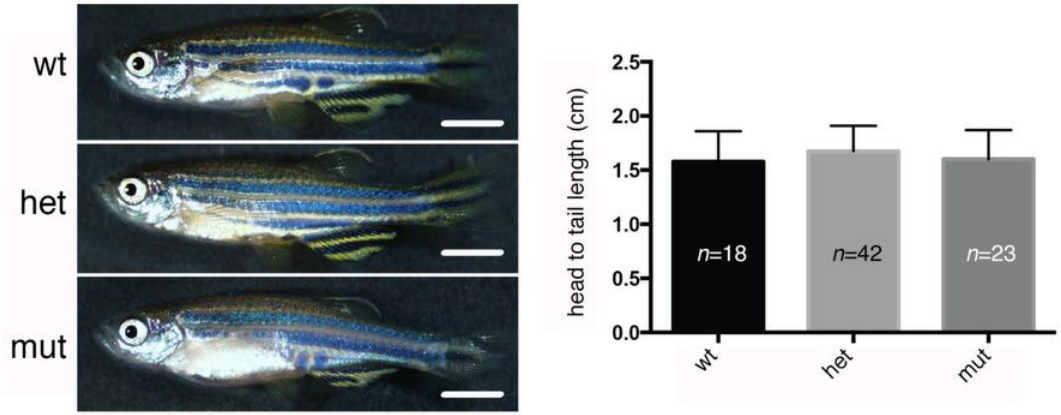
rag2E450fs mutant fish engraft hematopoietic and muscle stem cells



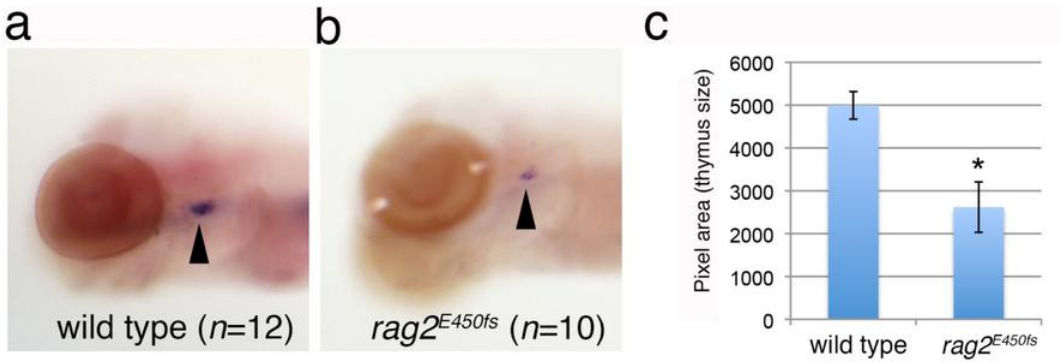
Engraftment of zebrafish tumors into rag2E450fs mutant fish



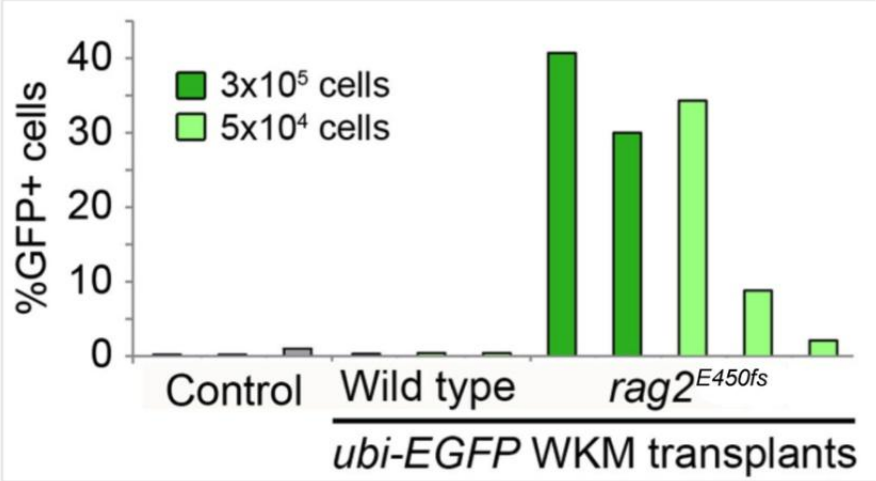
Homozygous rag2E450fs mutants are healthy and viable similar to wild-type and heterozygous siblings



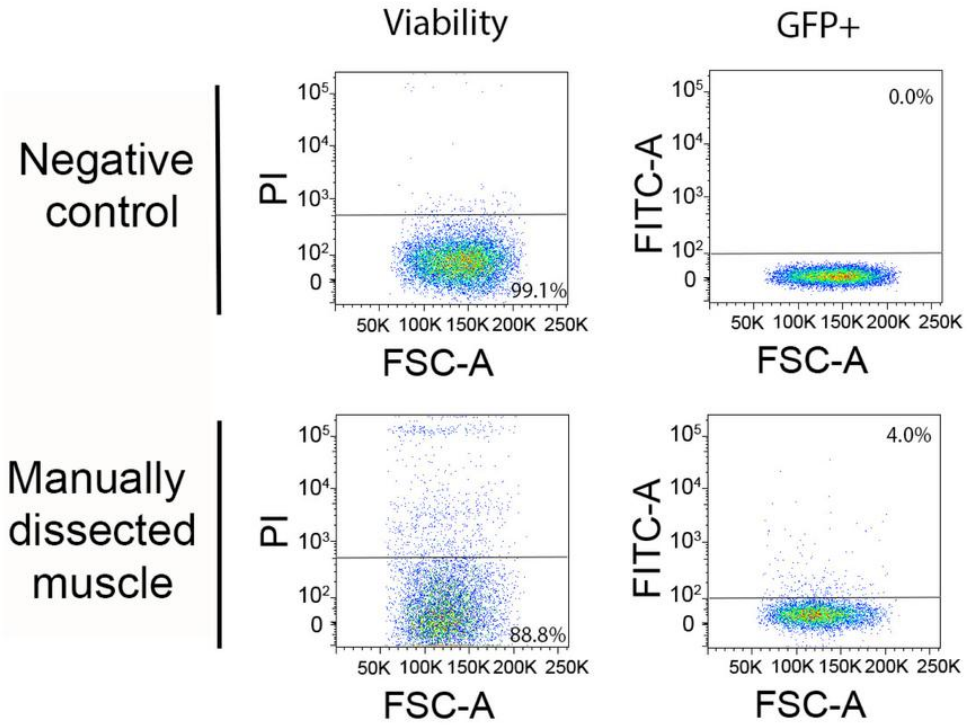
Homozygous rag2E450fs mutants have reduced T-cell numbers and thymus size at 5 days of life



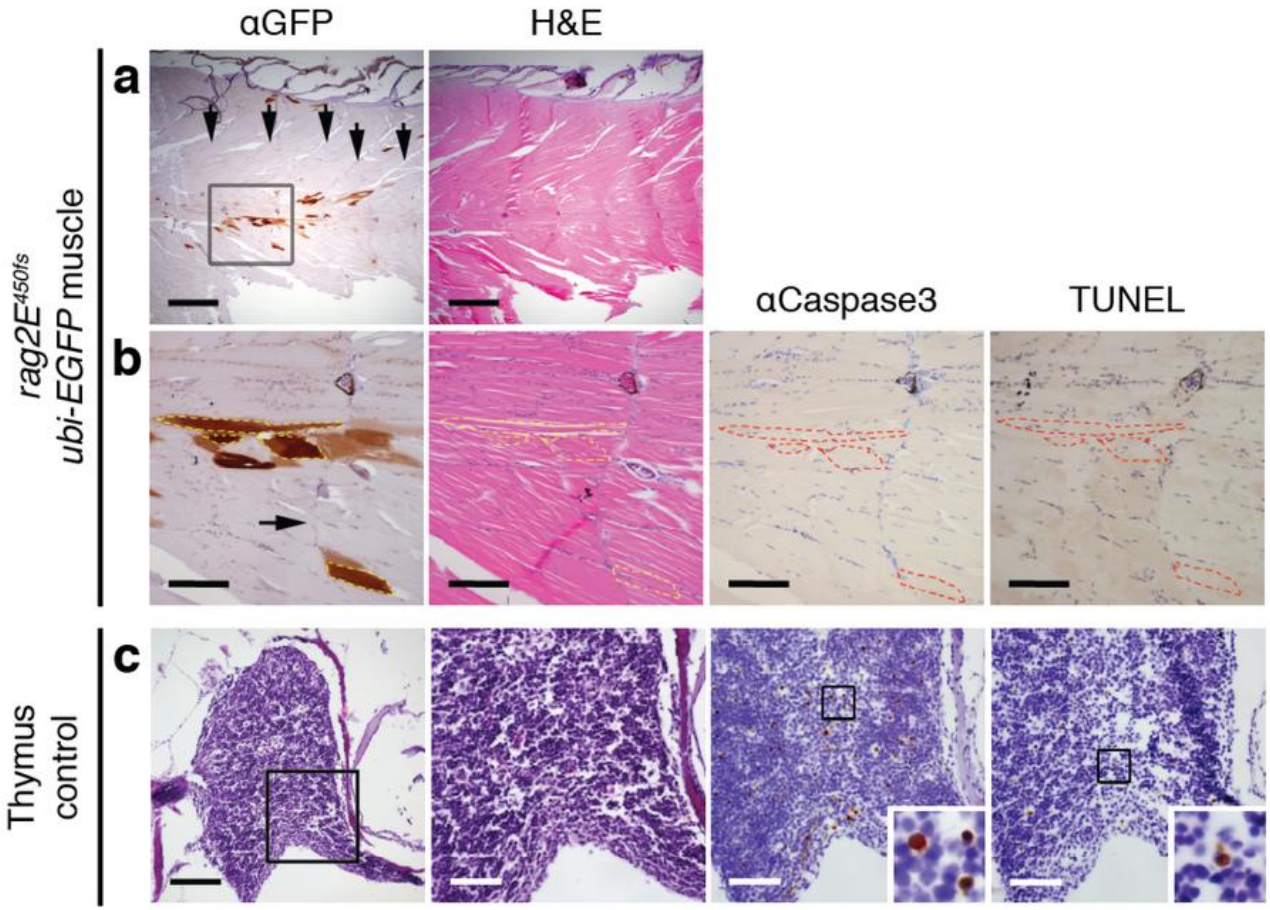
Percentage of ubi-EGFP+ blood cell engraftment in recipient fish at 45 days post transplantation



Manual dissection and cell harvesting protocols produce viable muscle cells



Homozygous rag2E450fs mutant zebrafish engraft EGFP+ muscle from ubi-EGFP transgenic donor animals



Genotyping strategy to identify rag2E450fs mutants