Aula P10
Linhas transgénicas de peixe-zebra

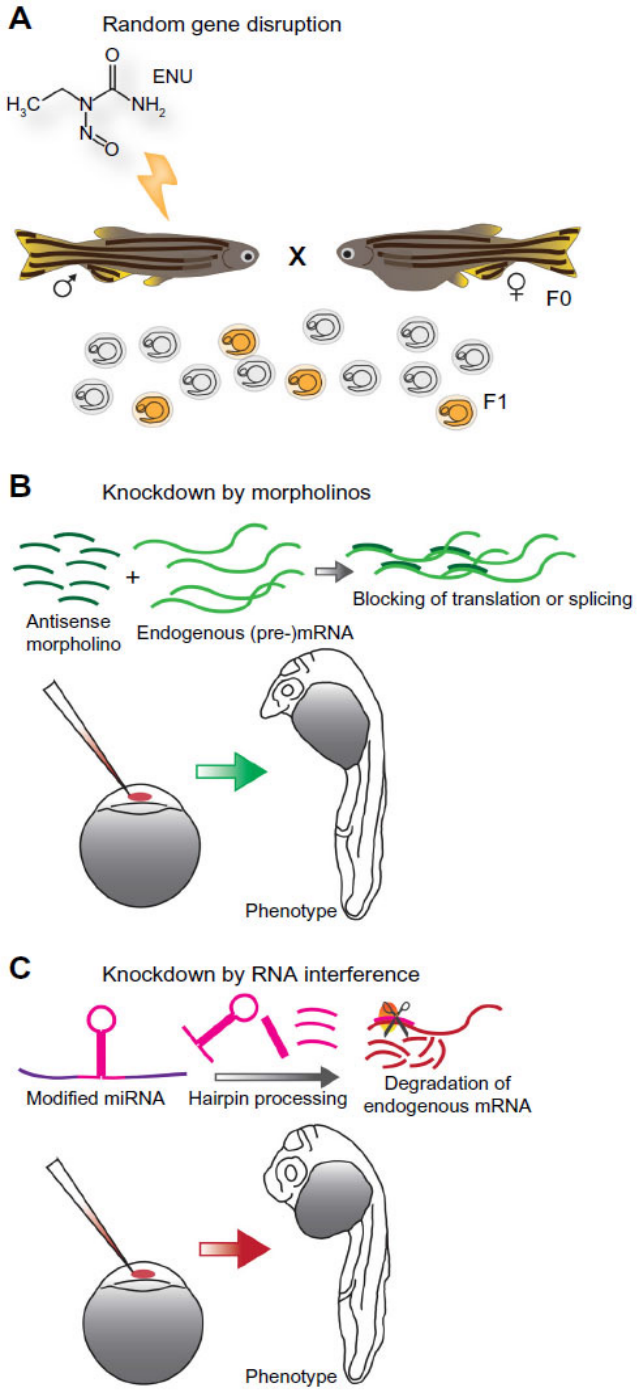
Nesta aula vamos aprender como se fazem e para que servem as linhas transgénicas de peixe-zebra

Peixes-zebra transgénicos

O peixe-zebra transgénico teve essencialmente genes exógenos adicionados ao seu genoma e, como tal, pode ser usado para muitas aplicações experimentais diferentes, como sobreexpressão estável, expressão de uma mutação dominante negativa e análise geral da regulação genética.

Toolbox for zebrafish: Mutants and Knockdowns

Ver: A molecular toolbox for genetic manipulation of zebrafish
http://dx.doi.org/10.2147/AGG.S57585
by Sassen & Köster 2015



Knockout and knockdown of zebrafish gene function.
Notes: (**A**) *N*-Ethylnitrosourea induces point mutations in the genome, which can be inherited to the next generation (mutation carriers indicated as orange- colored embryos). (**B**) A transient knockdown can be achieved by the injection of an antisense morpholino in the early embryo, which then blocks the translation or splicing of the mRNA encoding the protein of interest.
(**C**) A quite new knockdown technique in zebrafish is the use of modified miRNAs matching the mRNA encoding the protein interest, which is then degraded by the endogenous RNA interference machinery.
Abbreviations: mRNA, messenger RNA; miRNA, microRNA.

Toolbox for zebrafish: Knockouts: CRISPR-Cas9

Ver: A molecular toolbox for genetic manipulation of zebrafish

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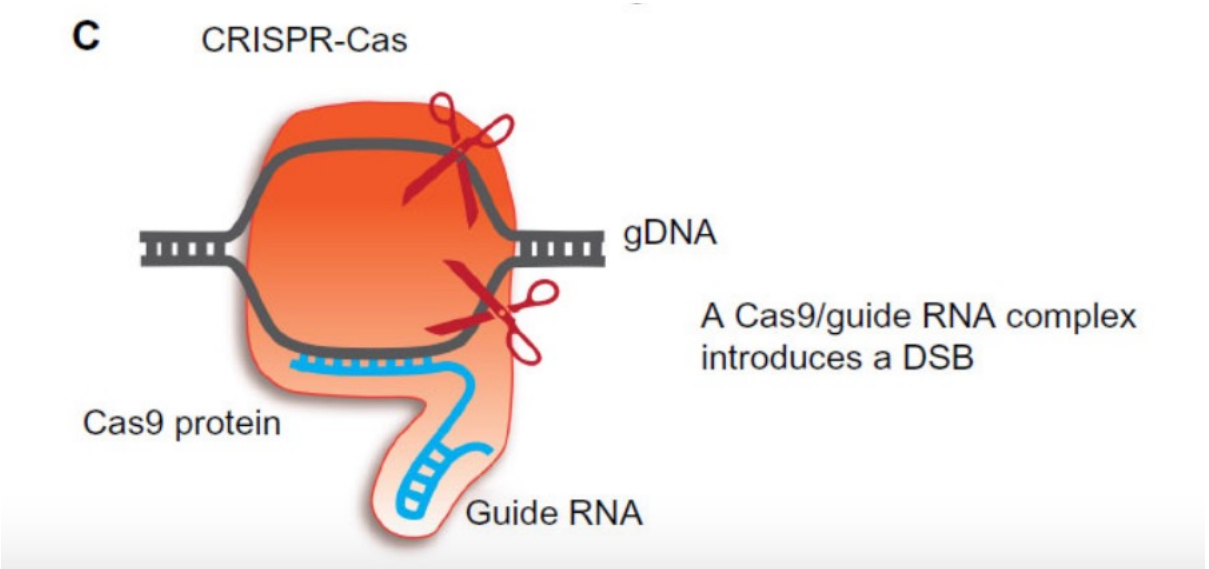
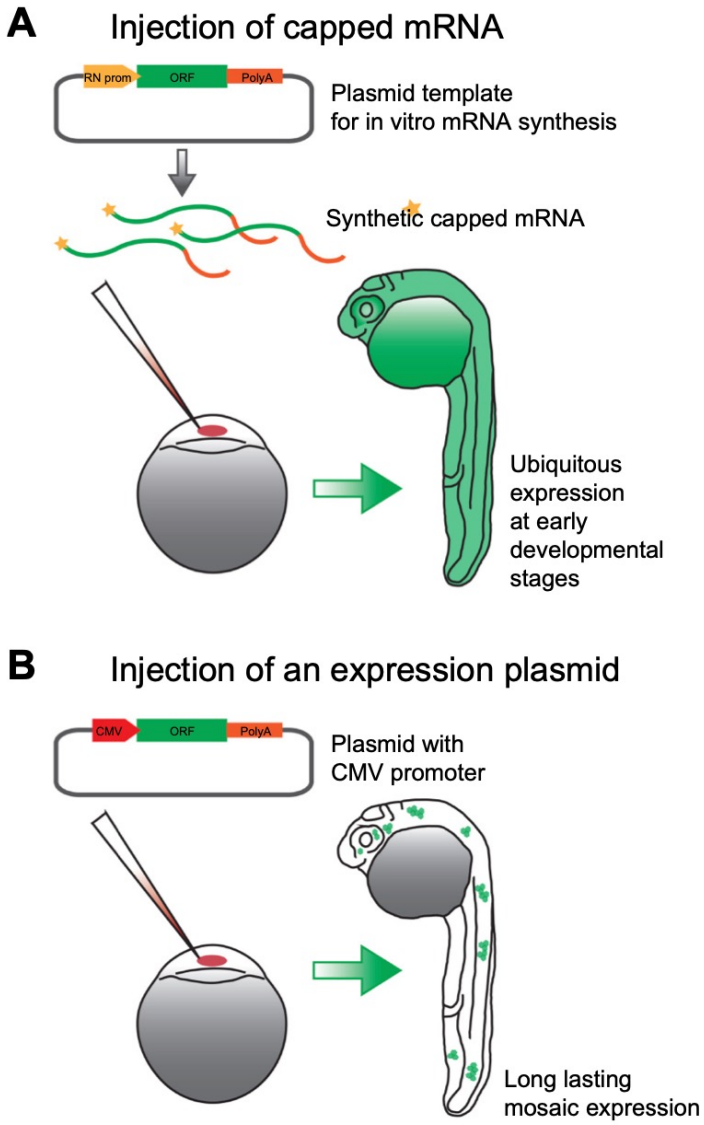
by Sassen & Köster 2015

The clustered regularly interspaced short palindromic repeat (CRISPR)-Cas system simply requires the co-delivery of the Cas9 endonuclease and a guide RNA matching the genomic sequence of interest. By recognizing the target site by the guide RNA, Cas9 can introduce a Double Strand Break (DSB) which will be resolved by INDELs (inserts or deletions).



Toolbox for zebrafish – expressão de genes – há várias opções



~ 95%
success

~ 5%
success

Review

Tol2: a versatile gene transfer vector in vertebrates

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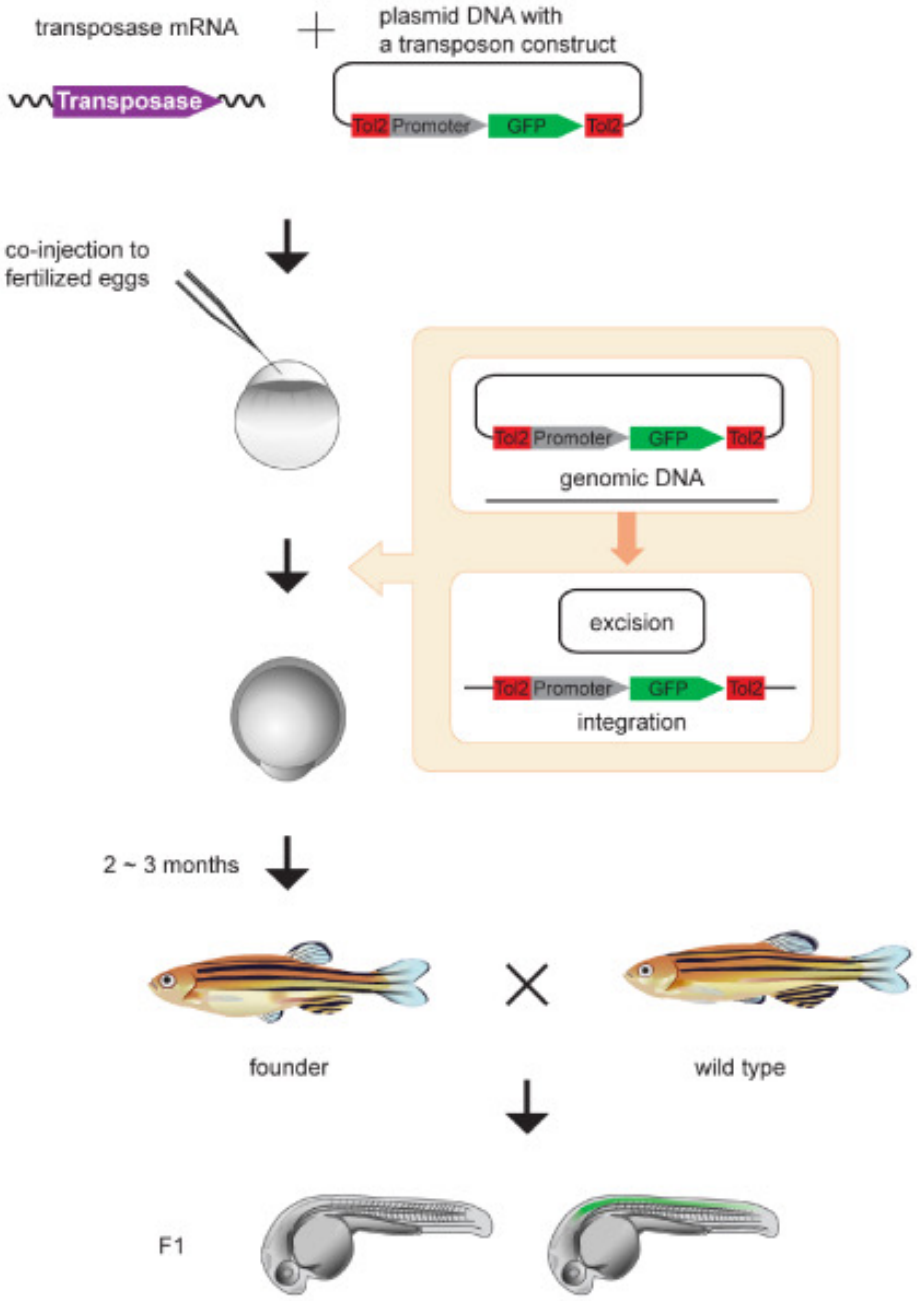
Abstract

The medaka fish Tol2 element is an autonomous transposon that encodes a fully functional transposase. The transposase protein can catalyze transposition of a transposon construct that has 200 and 150 base pairs of DNA from the left and right ends of the Tol2 sequence, respectively. These sequences contain essential terminal inverted repeats and subterminal sequences. DNA inserts of fairly large sizes (as large as 11 kilobases) can be cloned between these sequences without reducing transpositional activity. The Tol2 transposon system has been shown to be active in all vertebrate cells tested thus far, including zebrafish, Xenopus, chicken, mouse, and human. In this review I describe and discuss how the Tol2 transposon is being applied to transgenic studies in these vertebrates, and possible future applications.

Transgenesis in zebrafish.
The synthetic transposase mRNA and a transposon donor plasmid containing a *Tol2* construct with a promoter and the gene encoding green fluorescent protein (GFP) are co-injected into zebrafish fertilized eggs. The *Tol2* construct is excised from the donor plasmid [2] and integrated into the genome. *Tol2* insertions created in germ cells are transmitted to the F_1 generation. Germ cells of the injected fish are mosaic, and, by crossing the injected fish (founder) with wild-type fish, nontransgenic fish and transgenic fish heterozygous for the *Tol2* insertion are obtained [4]. In this figure, the promoter is tentatively defined as a spinal cord specific enhancer/promoter and the spinal cord of the embryo is depicted in green.



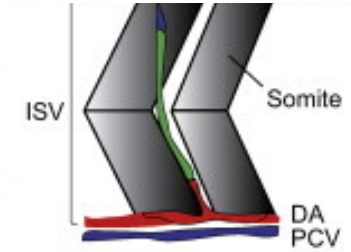
Mosaic F0

Stable F1

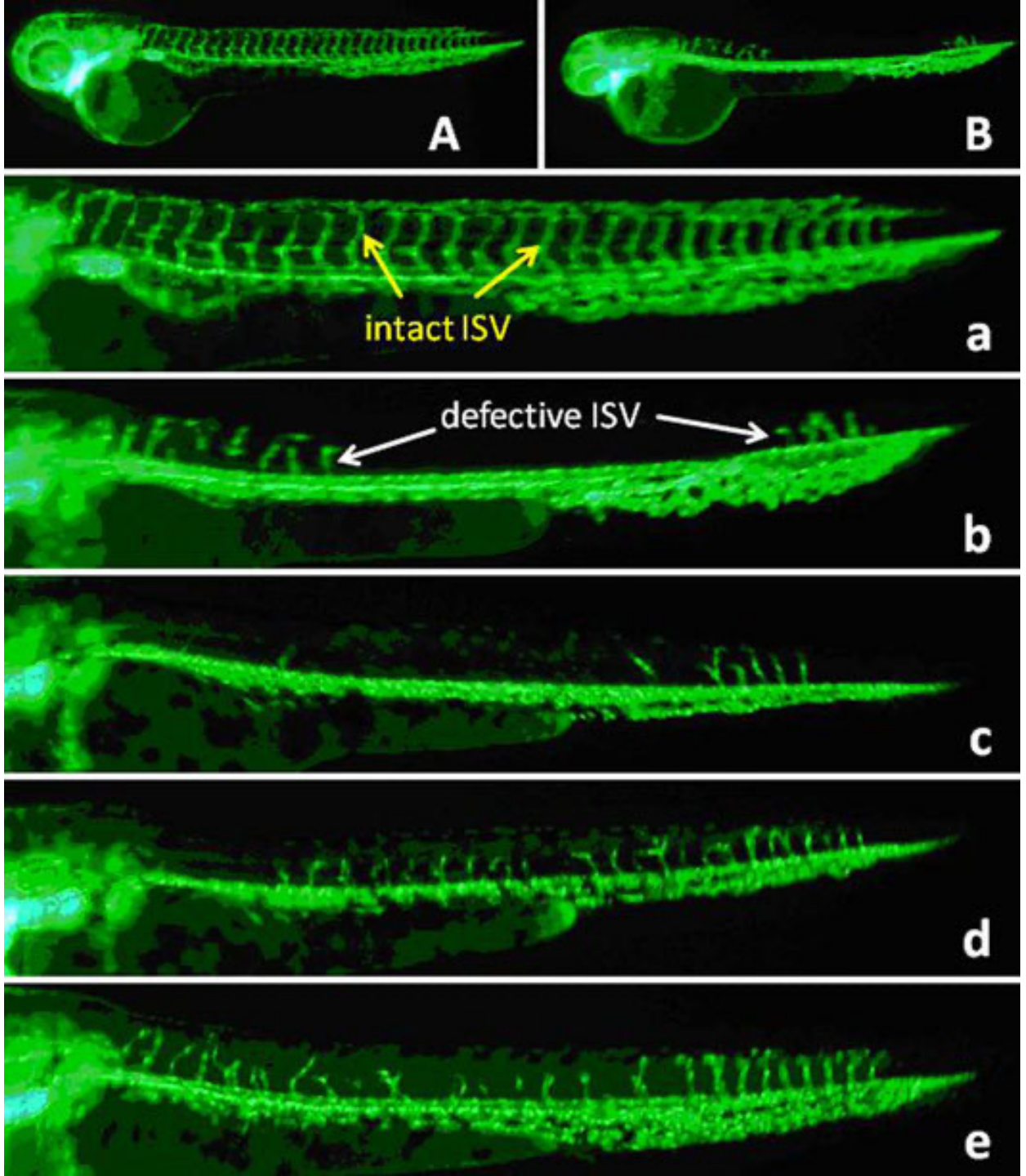
~50% success

Transgenic reporter tg(*fli1:eGFP)*

For labelling the endothelial cells lining blood vessels

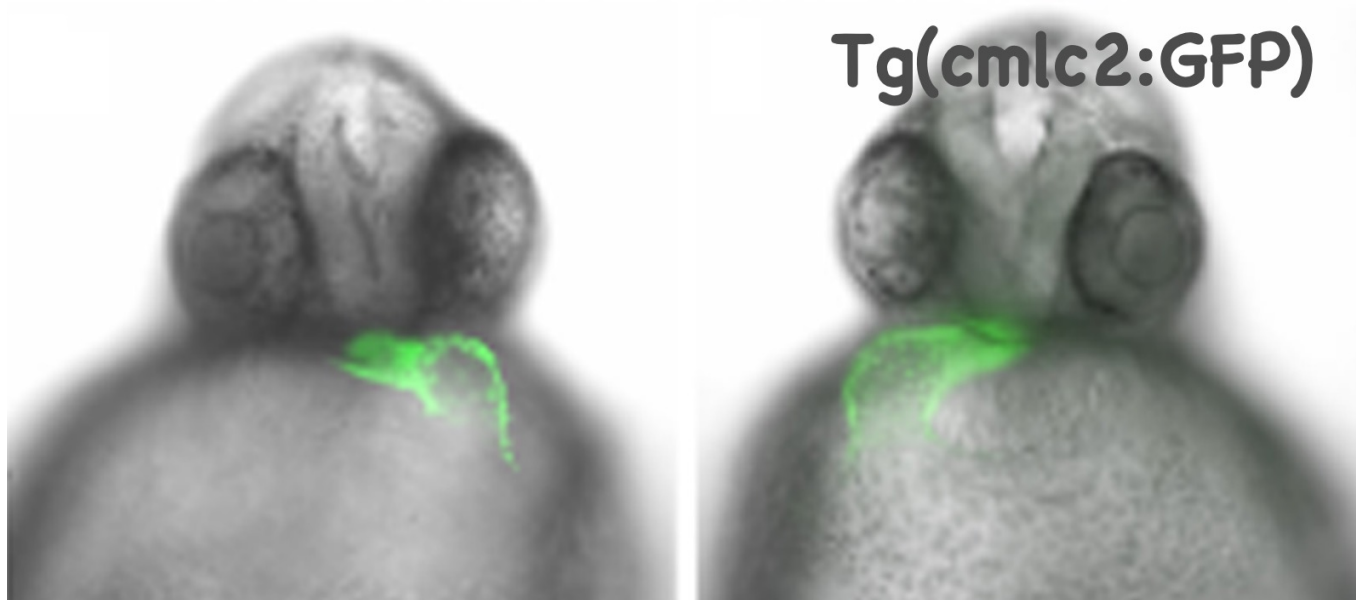


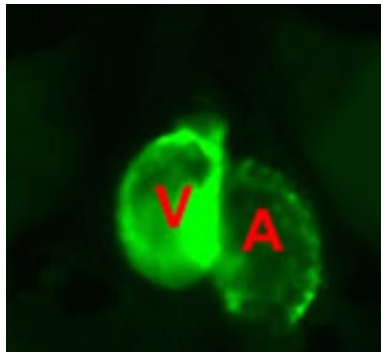
Friend leukemia integration 1 transcription factor encodes a transcription factor containing an ETS DNA-binding domain (winged helix-turn-helix DNA binding domain). It is involved in vascular development.

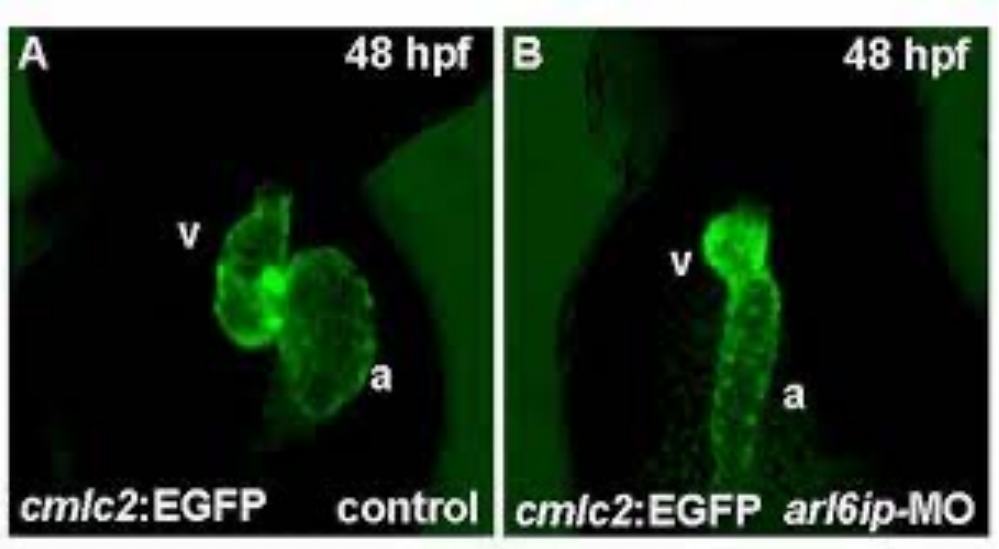


Transgenic reporter tg(*cmlc2:eGFP)*

For labelling cardiac myosin light chain2 Also known as myl7



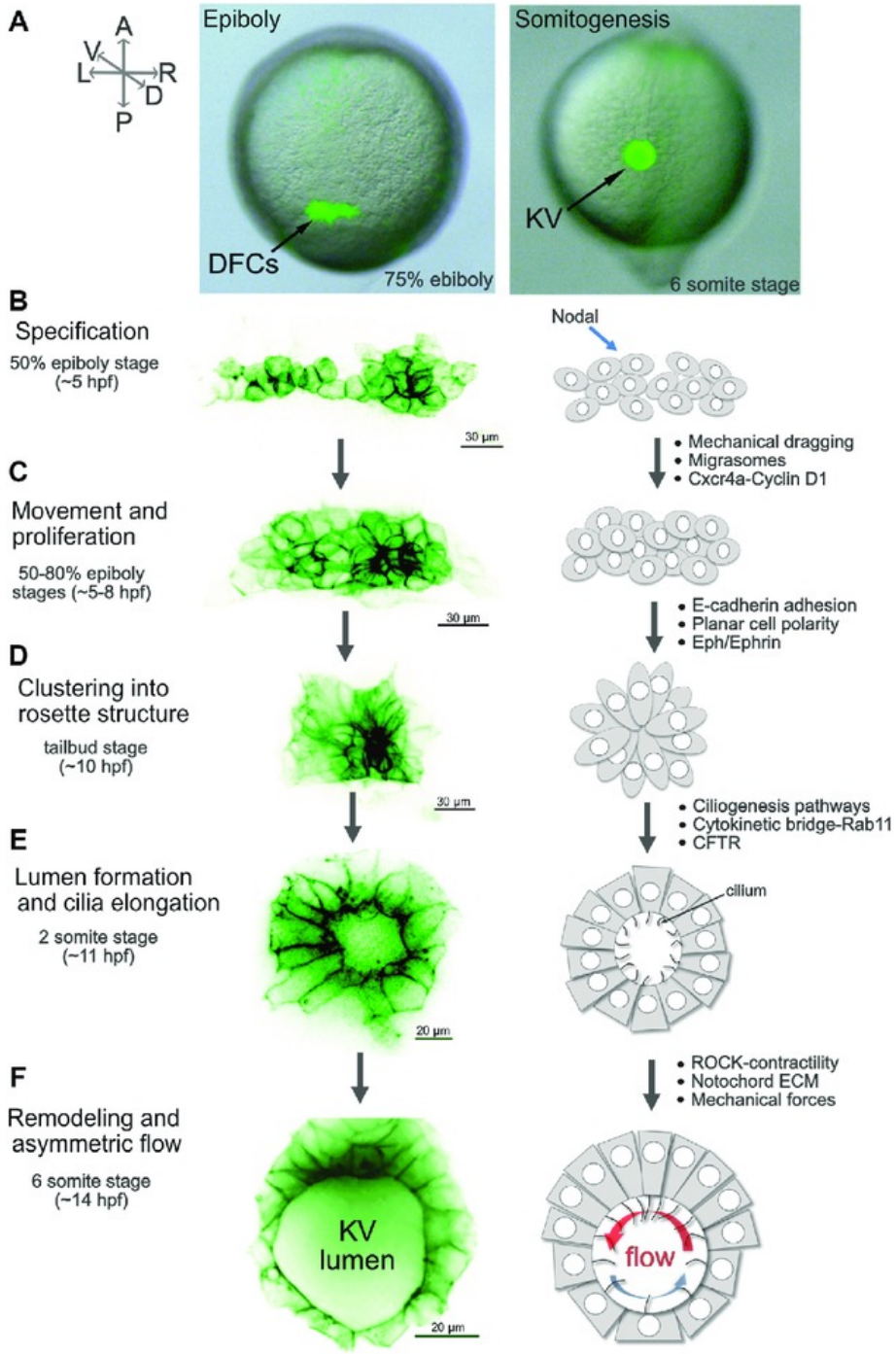




MYL7 (Myosin Light Chain 7) Diseases associated with MYL7 include Myasthenic Syndrome, Among its related pathways are Activation of cAMP-Dependent PKA and Cytoskeleton remodeling Regulation of actin cytoskeleton by Rho GTPases. *calcium ion binding*.

Transgenic reporter tg(*sox17:eGFP)*

For labelling the DFCs and the left-right organizer



This gene encodes a member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and in the determination of the cell fate.

The encoded protein may act as a transcriptional regulator after forming a protein complex with other proteins.

Transgenic reporter tg(*sox17:eGFP)*

Later in development for labelling liver, pancreas and the intestinal bulb

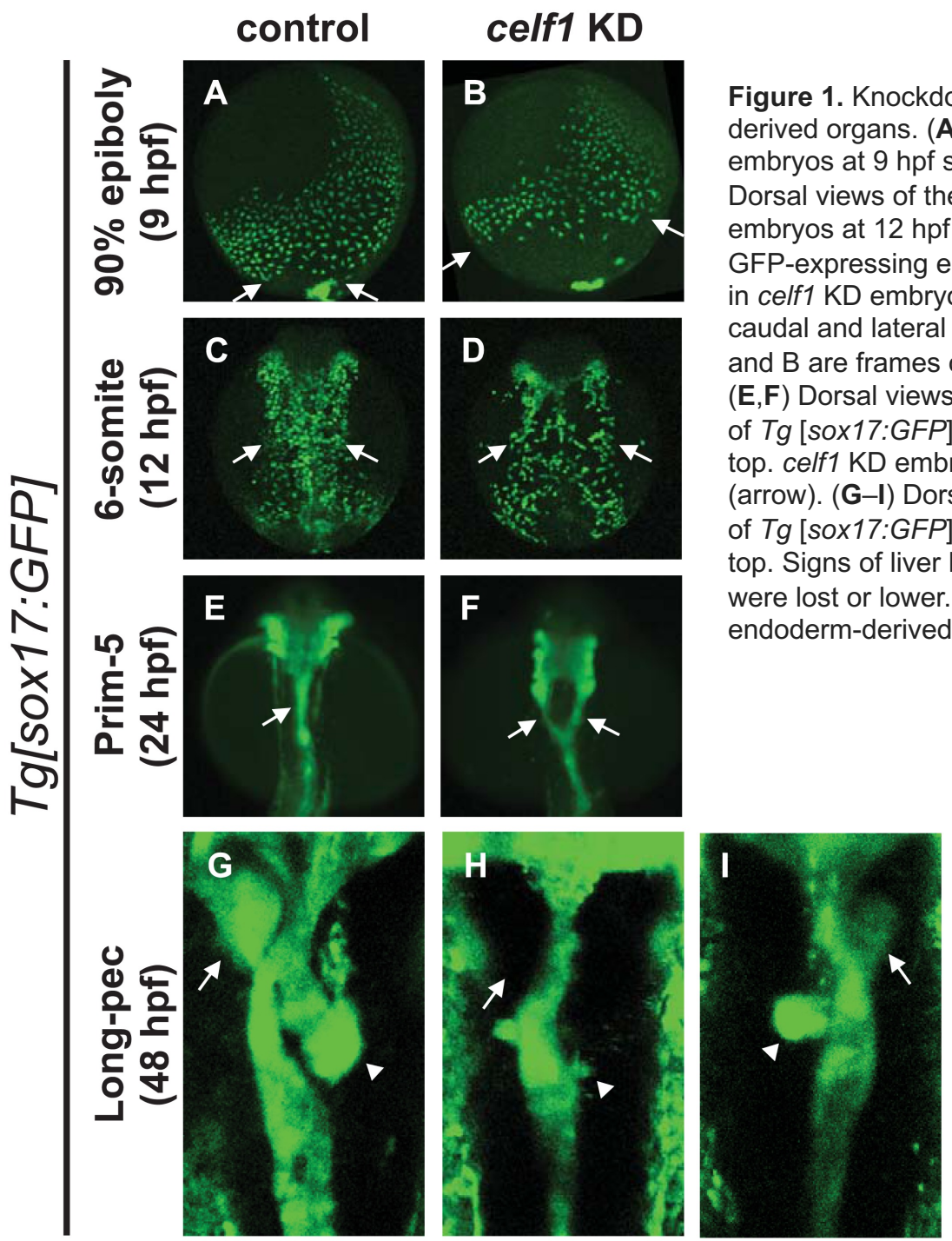
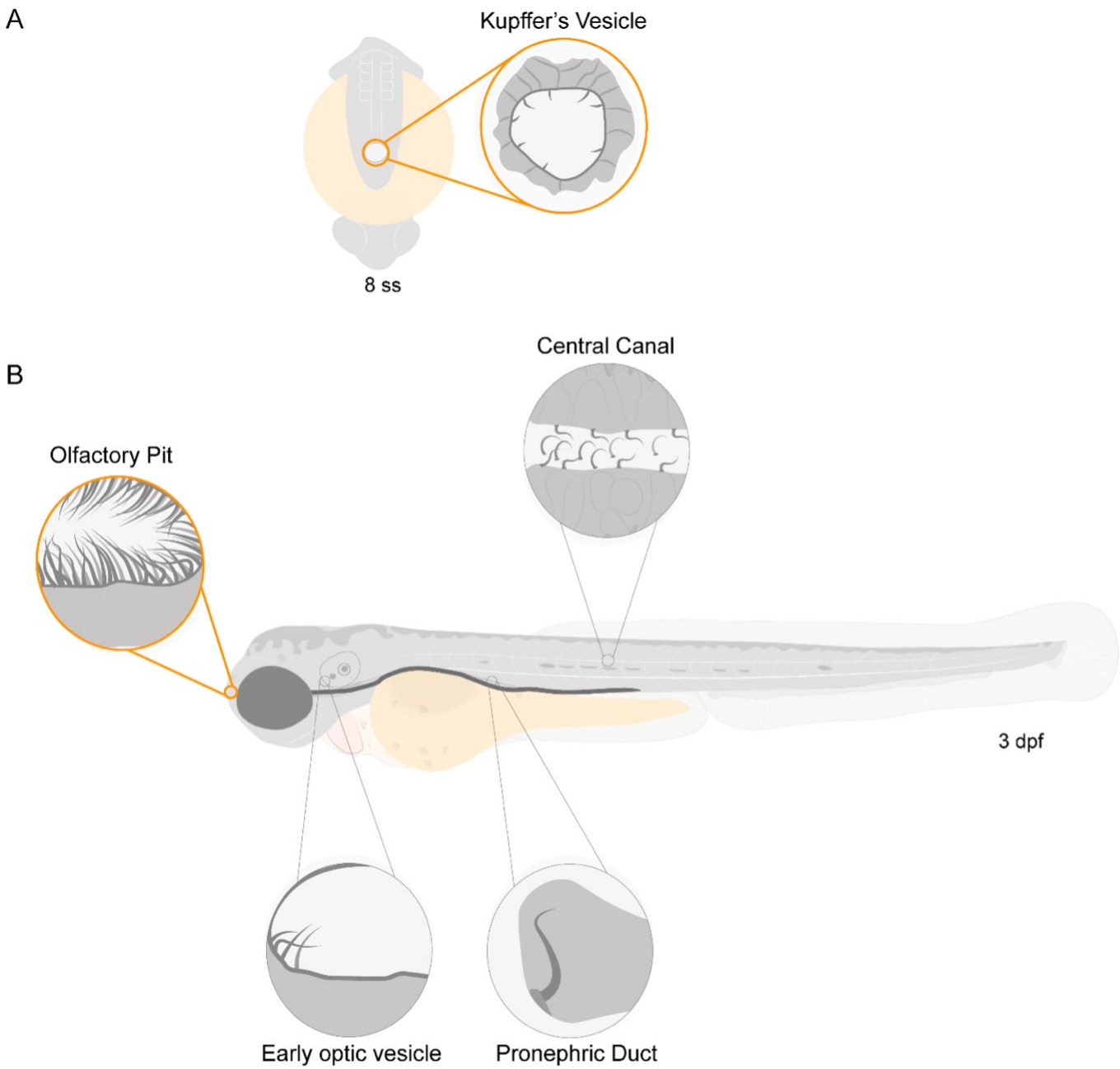


Figure 1. Knockdown of *celf1* leads to defects in endoderm-derived organs. (**A**–**D**) Lateral views of *Tg*[*sox17:GFP*] transgenic embryos at 9 hpf showing endodermal cells and DFCs (**A**,**B**). Dorsal views of the mid-trunk region of *Tg* [*sox17:GFP*] transgenic embryos at 12 hpf (**C**,**D**). Anterior to the top. The migration of GFP-expressing endoderm cells to the dorsal midline was delayed in *celf1* KD embryos. Arrows in panels A, B and C, D point at the caudal and lateral edges of endoderm cells, respectively. Panels A and B are frames of supplementary movies 1 and 2, respectively. (**E**,**F**) Dorsal views of the pharyngeal and foregut regions of *Tg* [*sox17:GFP*] transgenic embryos at 24 hpf. Anterior to the top. *celf1* KD embryos showed a splitting of the anterior gut (arrow). (**G**–**I**) Dorsal views of the mid-trunk region of *Tg* [*sox17:GFP*] transgenic embryos at 48 hpf. Anterior to the top. Signs of liver buds (arrows) and pancreas buds (arrowheads) were lost or lower. *celf1* KD embryos resulted in defects of endoderm-derived organs (**H**,**I**) and left-right patterning (**I**).

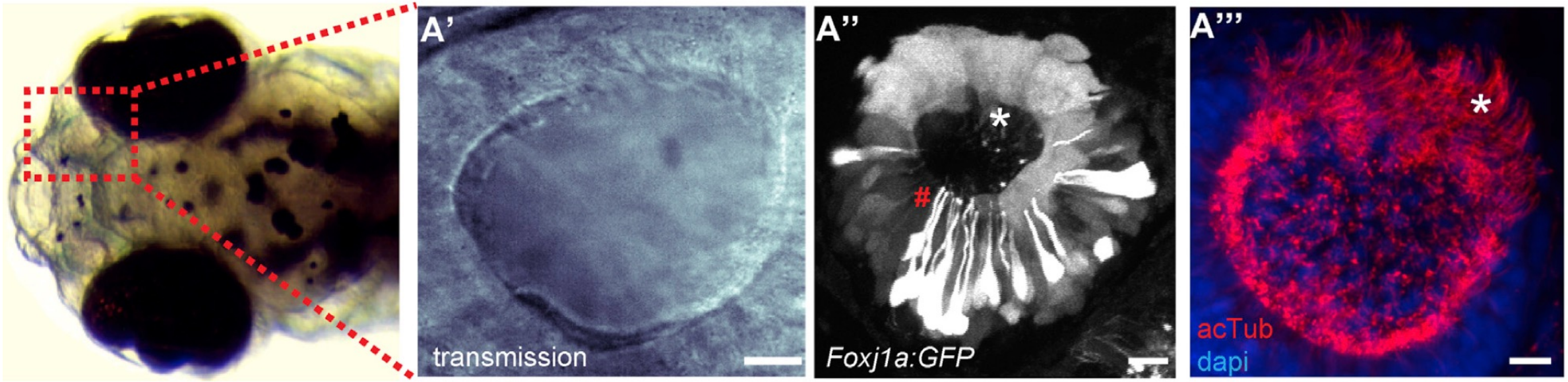
Transgenic reporter tg(*foxj1a:eGFP)*

For labelling motile ciliated cells In the embryo and larvae



Transgenic reporter
tg(*foxj1a:eGFP)*

For labelling motile ciliated cells
In the olfactory pit of the zebrafish (nasal cavity)



Encodes a member of the Forkhead/winged helix (FOX) family of transcription factors