Paquet et al. 2009 – Supplementary Information

Supplementary Figure 1

Integration of Gateway cloning technology can further improve the applicability of the transgenesis vectors.

(**A**) Since our constructs are suitable to express any gene in a tissue of interest to study various diseases in transgenic zebrafish, we have further improved the system to allow introducing various other genes and promoter. We achieved this by combining our vectors with the commercially available Gateway cloning system (1), which uses size independent recombination instead of standard restriction and ligation for cloning. Genes of interest (G.o.i.) or promoters of interest (P.o.i.) are PCR-amplified and cloned into a Gateway-compatible TOPO-cloning vector, an Entry vector, which already contains attL sites needed for recombination. Once the gene or promoter of interest is inserted into the Entry vector, it can be recombined into a Destination vector. We have modified our Driver and Responder constructs to Destination vectors by replacing Tau and the HuC promoter by an attR flanked Gateway cassette. This cassette contains the ccdB gene for negative selection, as this gene will block growth of bacteria, until the gene or promoter of interest replaces it by recombination. To obtain ready-to-use vectors for generation of transgenic zebrafish, the Entry and Destination vectors are mixed together with the enzyme for LR-recombination and after a few hours bacteria can be transformed, which will only grow if they contain the Destination vector with the gene or promoter of interest. With the *Tol2* transposon system founder animals are generated at high efficiency, and their transgenic offspring can be easily identified due to concomitant DsRed expression. The combination of all these advantages greatly streamlines the generation of transgenic zebrafish. (**B,C**) We have tested the functionality of our

vectors by recombining two independent promoters, zebrafish HuC (2) and goldfish alpha-tubulin (αTub) (3), into the Driver vectors and human Tau P301L into the responder vector, and coinjecting Driver and responder vectors into zebrafish eggs. After 48 hpf the embryos displayed coexpression of Tau and DsRed in neurons in the spinal cord (**B** for HuC**, C** for αTub) and brain (not shown).

Supplementary Figure 2

Genetic inheritance of Driver and Responder constructs over three generations of hTau-P301L transgenic fish

(A) 15 out of 76 raised founder fish (19,7%) were identified with DsRed positive offspring. Three generations originating from one founder fish were analyzed for genetic inheritance by counting DsRed negative and positive embryos. About one quarter of the offspring in all generations carried Driver and Responder, as they were DsRed positive. **(B)** The inheritance was further analyzed by PCR for Driver and Responder genotype of an F2 outcross. Of 225 F3 embryos about one quarter contained both Driver and Responder, either one of them or none. **(C)** Immunostaining of embryos from three subsequent generations of transgenic fish. Protein levels and expression domains are highly similar over three generations demonstrating stable inheritance and activity of the Gal4-UAS transgene.

Supplementary Figure 3

BBB is normal in Tau transgenic zebrafish

(A-D) Restricted permeability of cerebral microvessels to sulfo-NHS-biotin. 8 week-old-fish were injected with sulfo-NHS-biotin as described (4). No difference in the BBB permeability of Tau-fish (**A,B**) versus controls (**C,D**) was observed, indicating a normal development and function of the BBB also in transgenic fish.

Supplementary Figure 4

Phosphorylation and conformation specific Tau antibodies show no crossreactivity in neurons of control embryos.

(A-S) Double whole mount immunostainings for phosphorylated Tau, Tau with pathologic conformation and DsRed of 48 hpf old transgenic zebrafish embryos expressing only DsRed but not Tau. No crossreactivity is observed in neurons of DsRed expressing embryos for all antibodies used: 12E8 (**A**, note unspecific cross-reactivity with blood cells (arrows), but not with neurons), AT180 (**D**), AT270 (**G**), PHF1 (**K**), AT8 (N), 422 (**Q**) and MC1 (**S**). Lateral views of trunk with anterior to the left. **(W,X)** Immunohistochemical stainings of spinal cord paraffin sections of comparable areas of 5-week-old non-transgenic zebrafish with Antibody DA9 (**W**) and AT8 (**X**). No crossreactivity is observed in all stainings.

Supplementary References

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3. Koster, R.W., and Fraser, S.E. 2001. Tracing transgene expression in living zebrafish embryos. *Dev Biol* 233:329-346.
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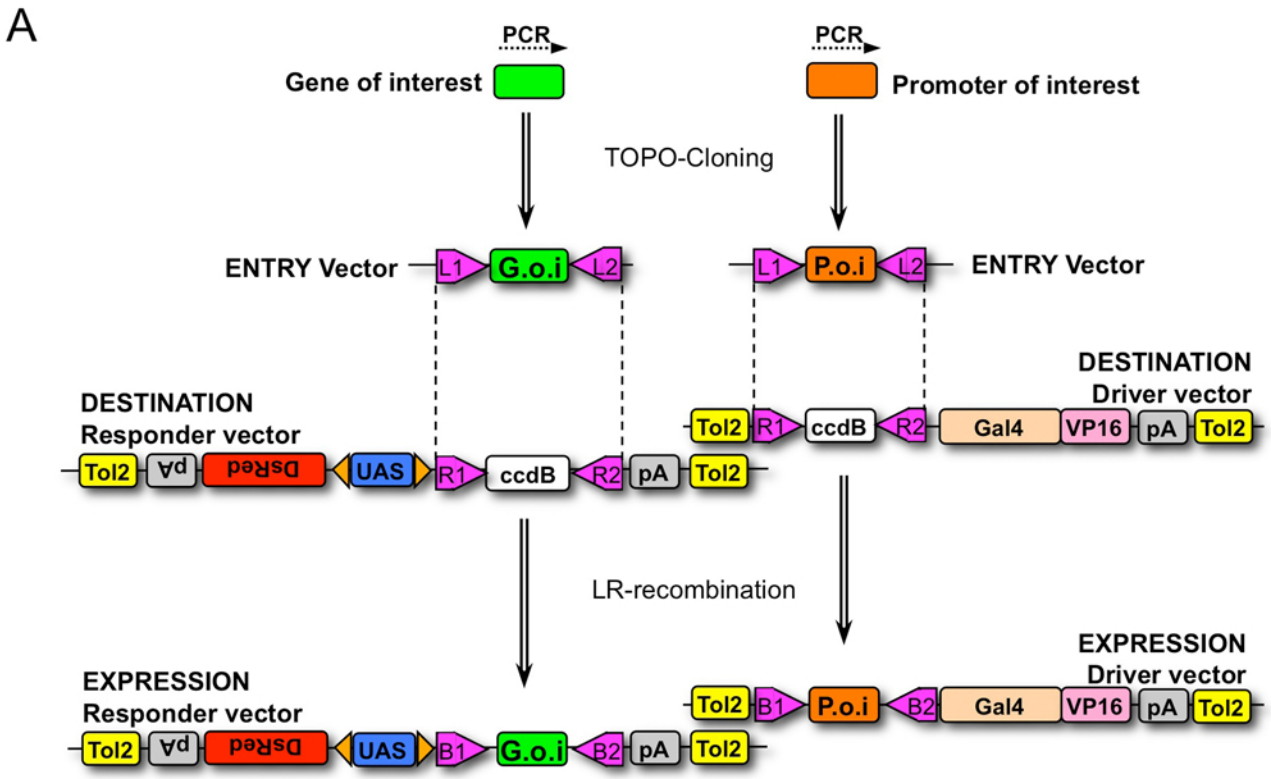
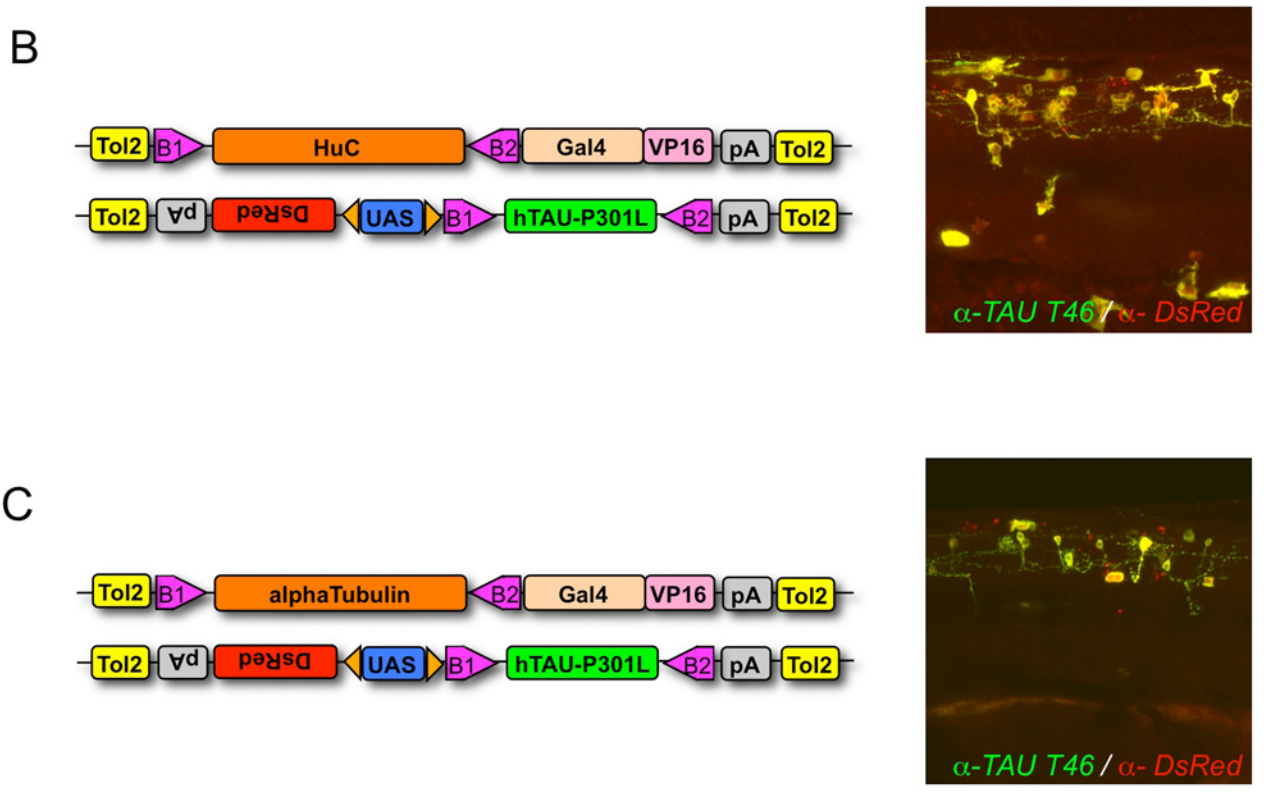
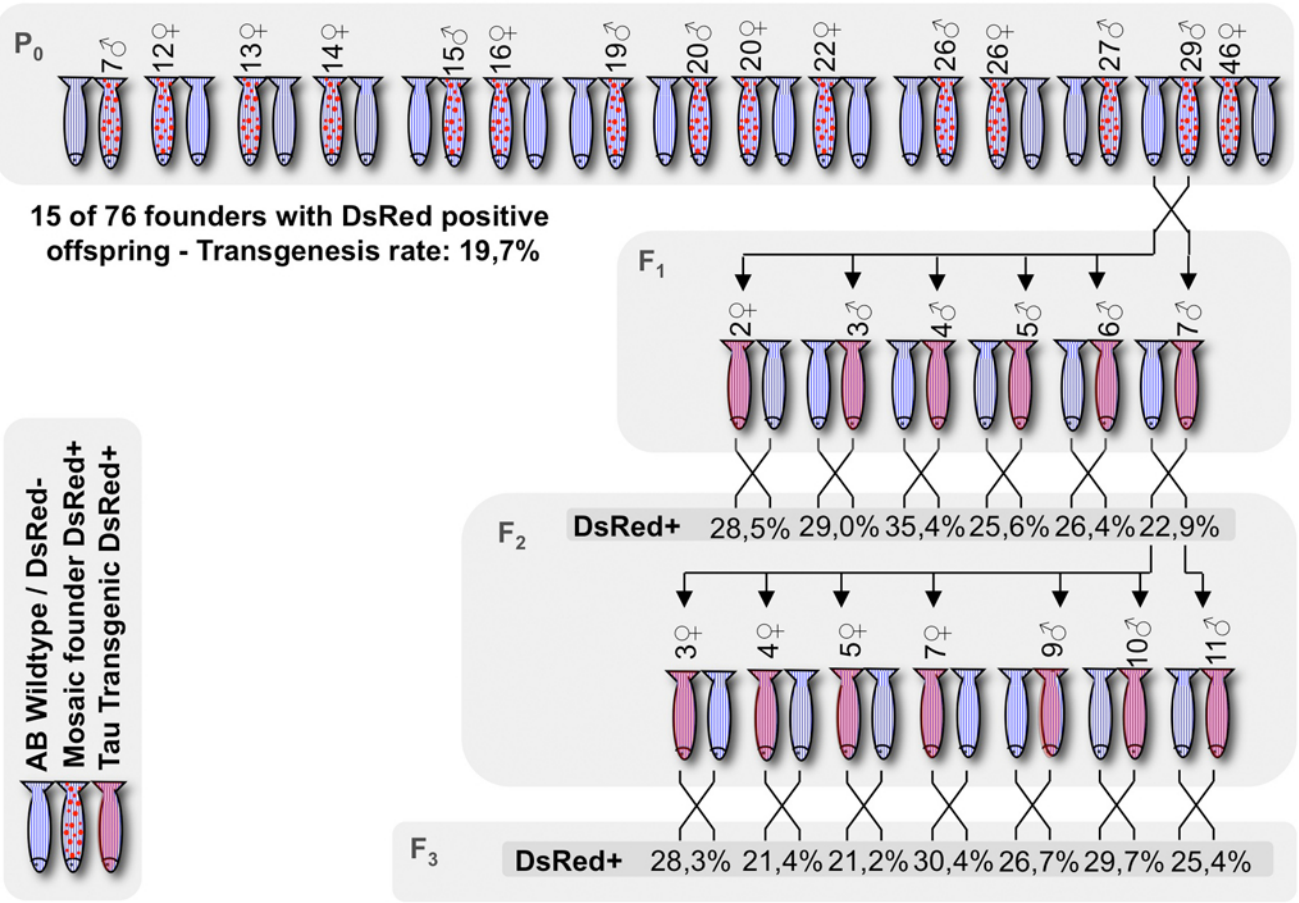
Supplementary Figure 1





Supplementary Figure 2

A



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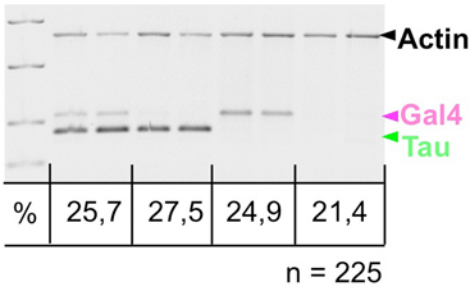

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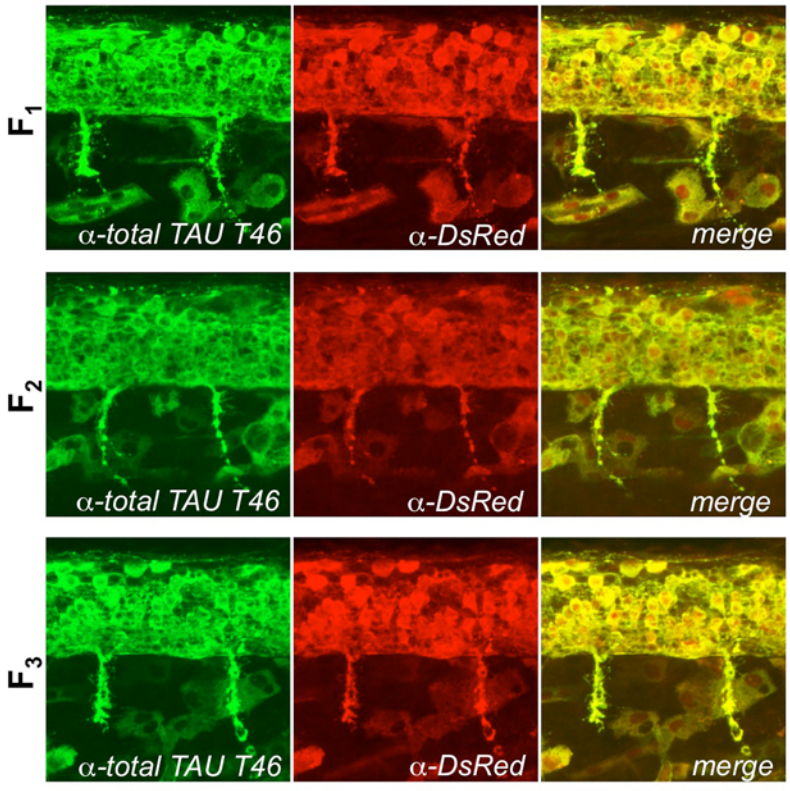
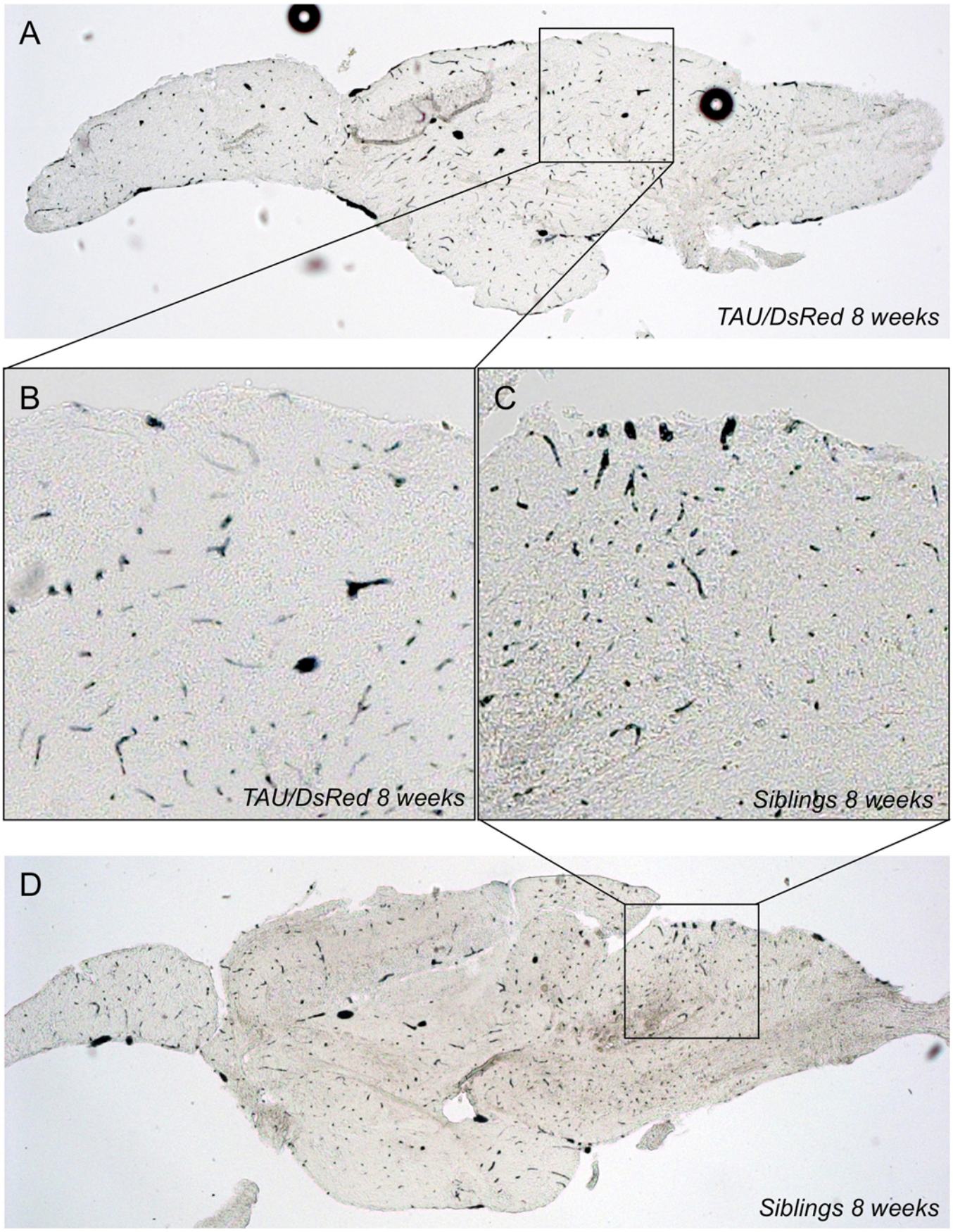


Supplementary Figure 3



Supplementary Figure 4