

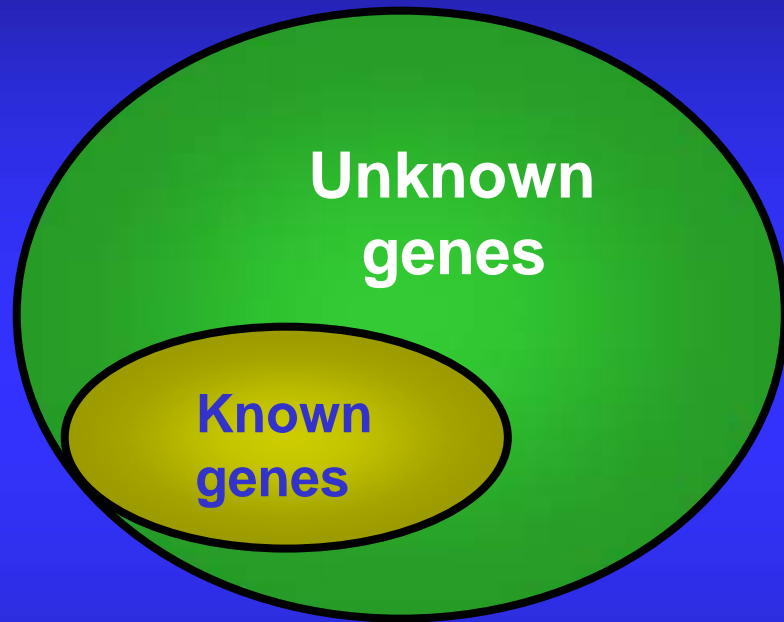
Functional analysis of vascular genes in zebrafish

Cheol-Hee Kim

Chungnam Natl. Univ., Korea

2005-4-16 , ()

Post Genome Era



25,000 Human genes

Genome Function



Fishing new functional genes

Disease & Drug targets

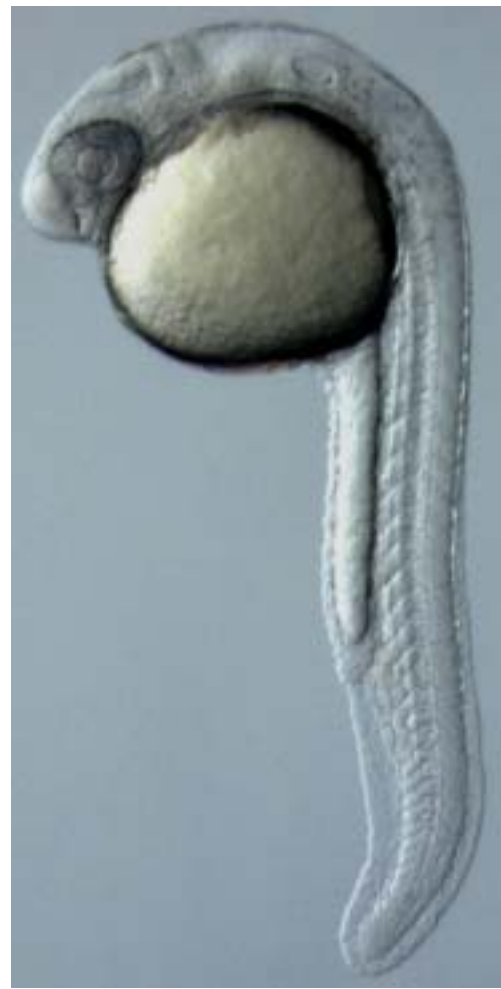
Why zebrafish ?



- A small vertebrate
 - Genetic analysis
 - Embryological manipulation
 - Genome duplication (4 copies)
1. 100 ~ 200 eggs/fish
 2. Rapid development
 3. Transparent
 4. Simple & inexpensive (1/100 ~ 1/1000)
 5. 'yeast-style' haploid genetics (UV-inactivated sperm)
 6. Sperm freezing

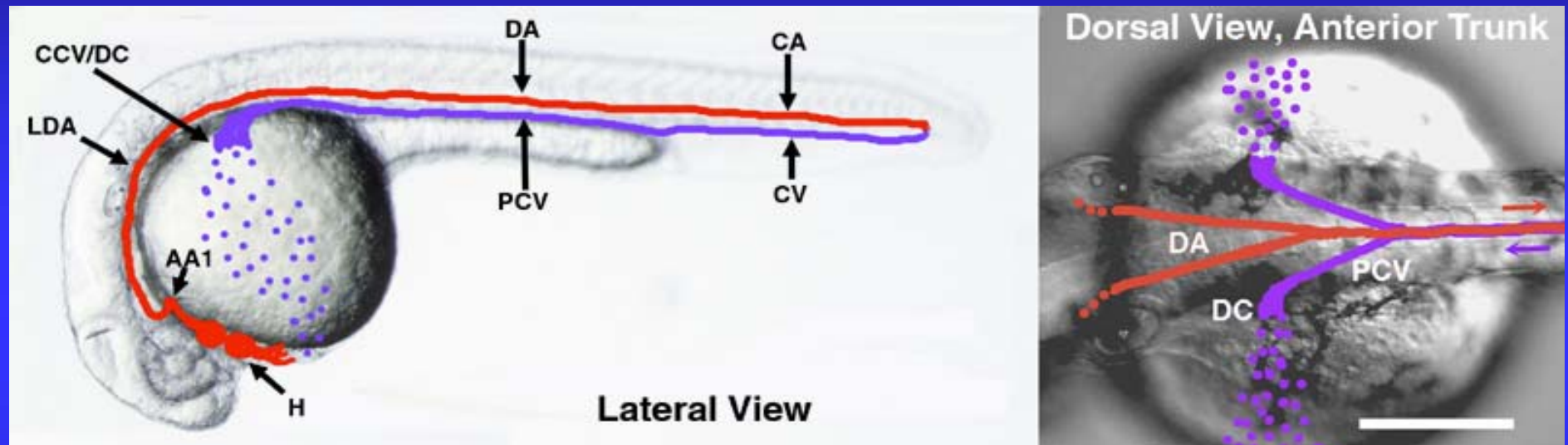


day 28 human



day 1 zebrafish

Zebrafish Vascular System at 24h



H : Heart

AA1 : Mandibular arch

LDA : Lateral dorsal aorta

CCV/DC : Common cardinal vein

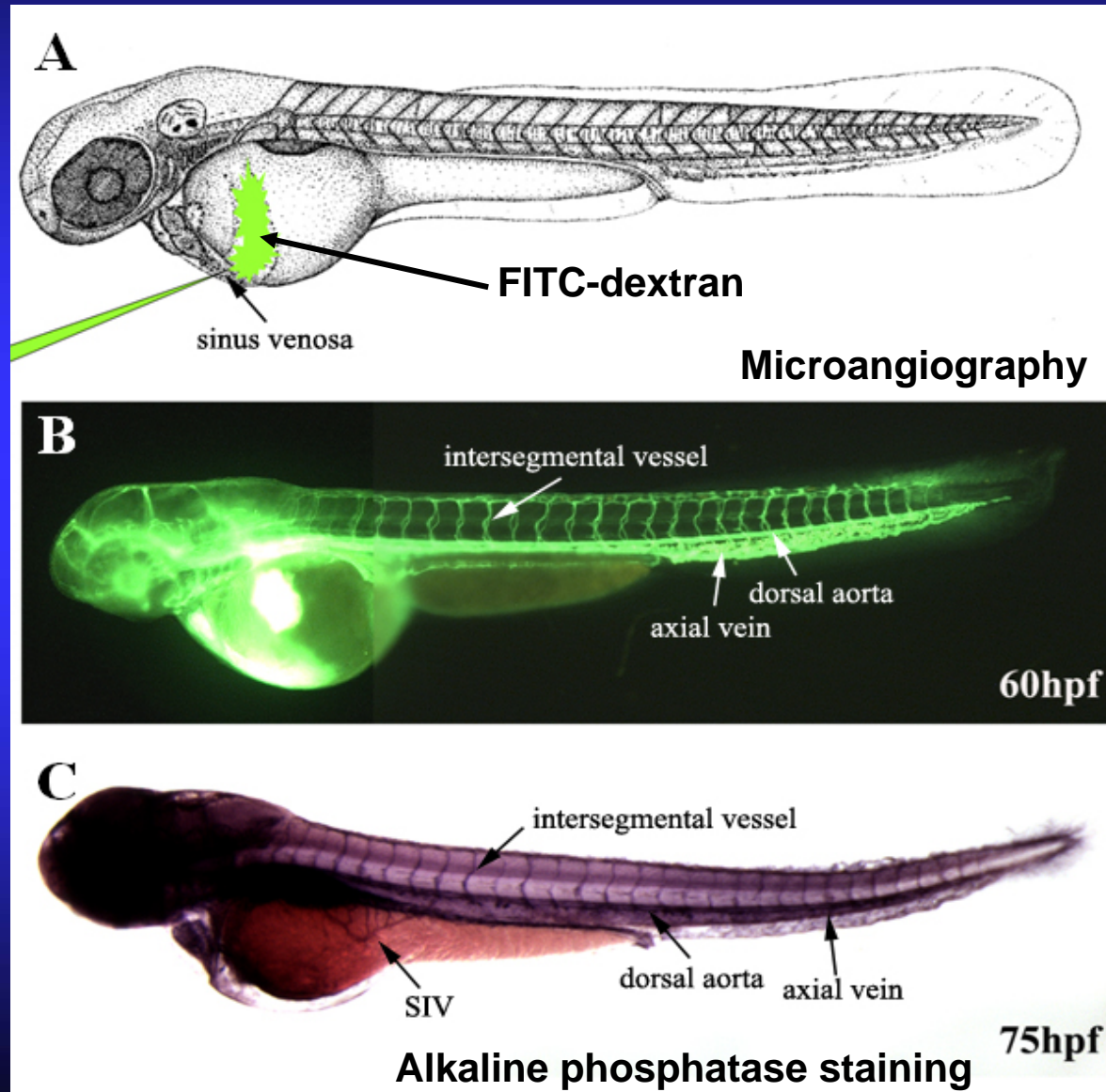
DA : Dorsal aorta

PCV : Posterior cardinal vein

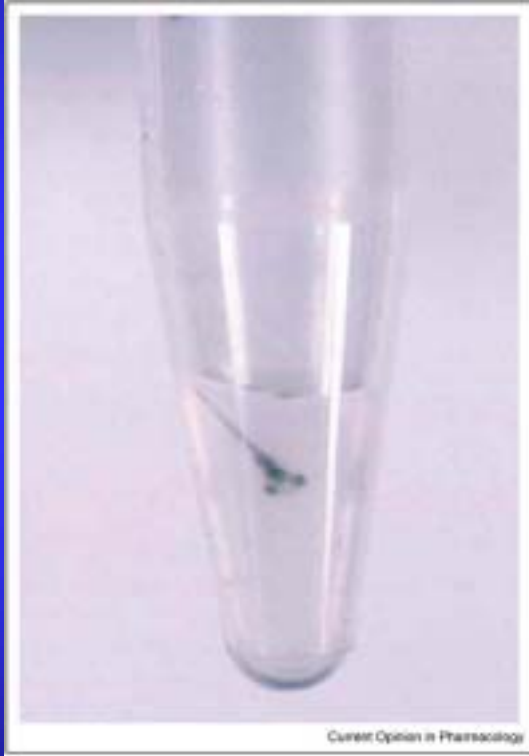
CA : Caudal artery

CV : Caudal vein

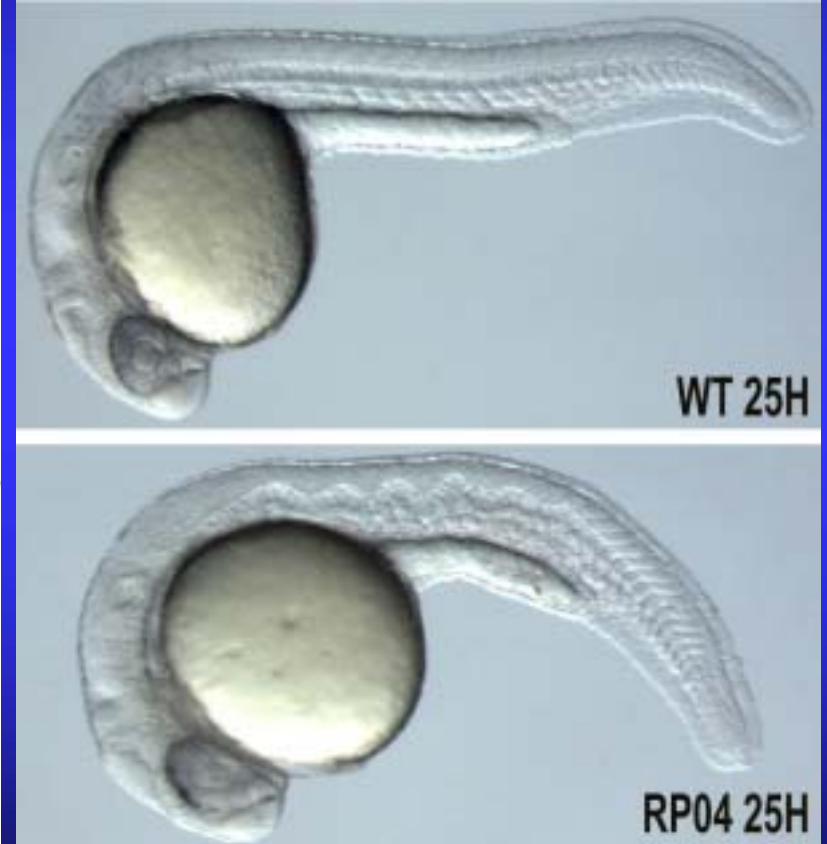
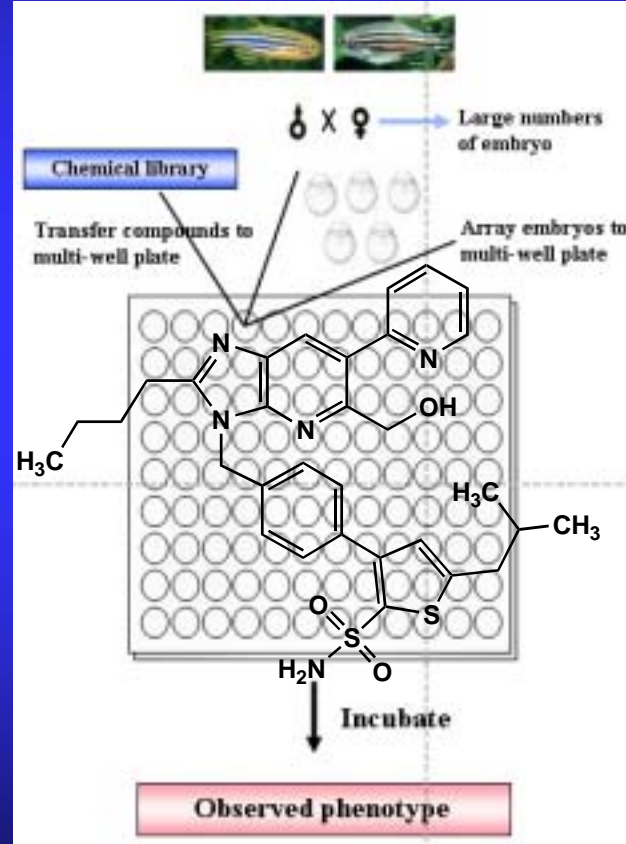
Detection of vascular development



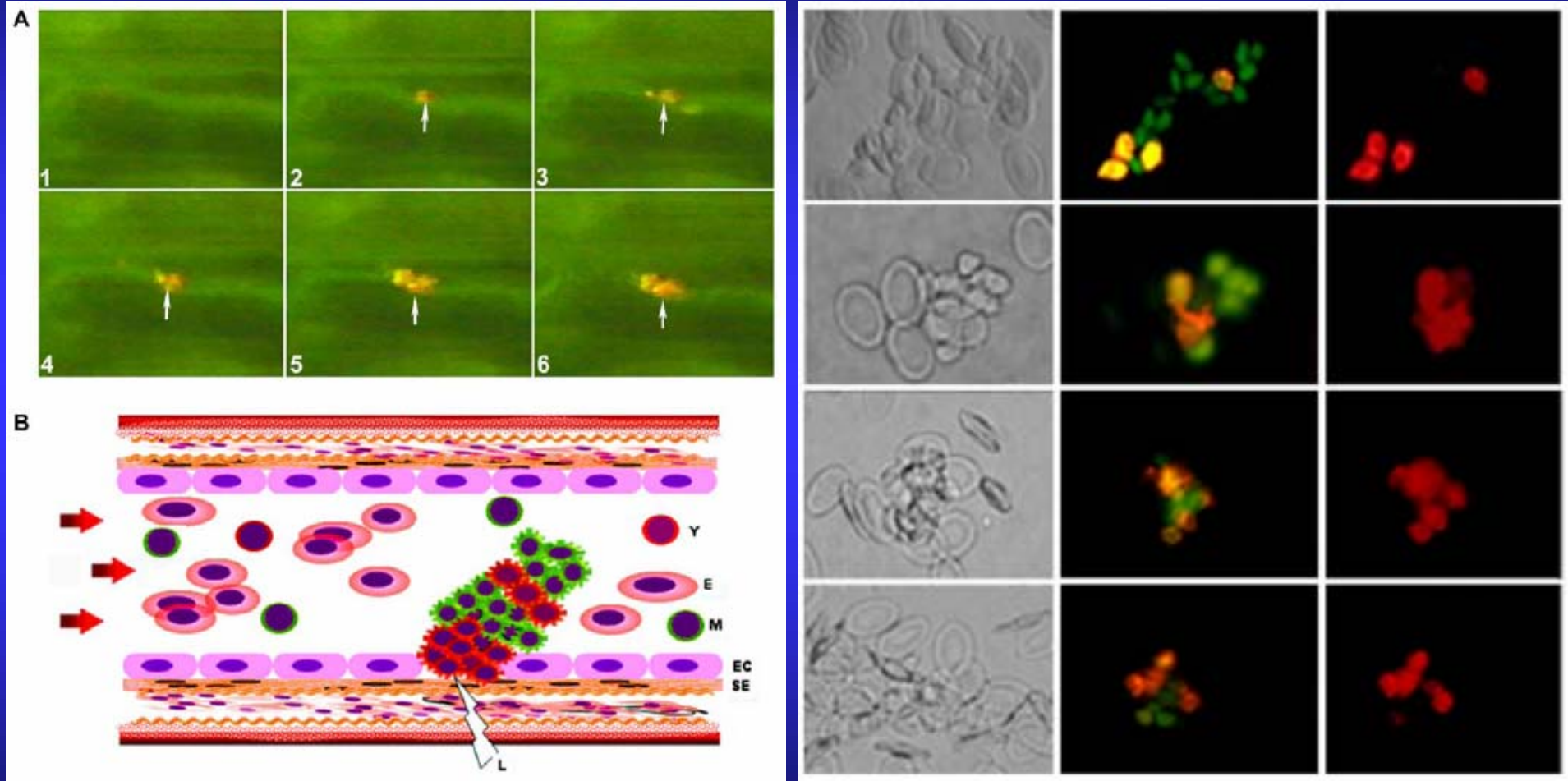
Chemical genomics in zebrafish (phenotype-based HTS)



A 7d-zebrafish in 50 μ l



Thrombosis after laser-induced microinjury



Thattliyath, 2005

Expression cloning of human genes in zebrafish

In silico screening

Korean UniGene Information (KUGI)

Human UniGene Collection

10,000 full-length open reading frame (FL-ORF) clones



In vitro synthesis of mRNAs



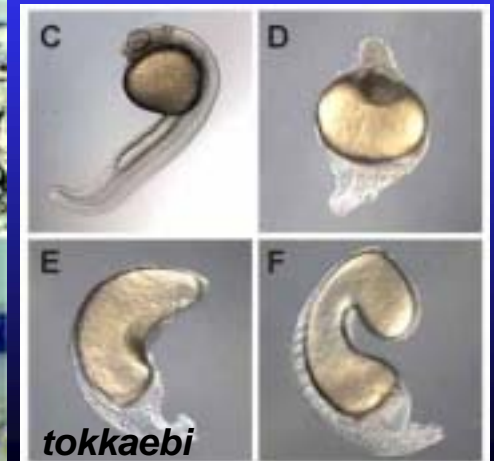
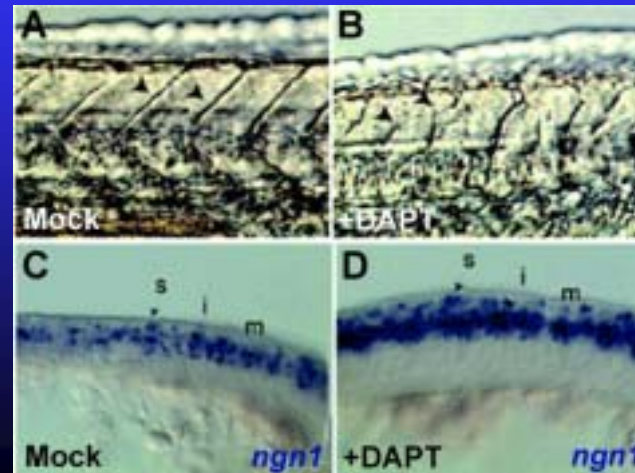
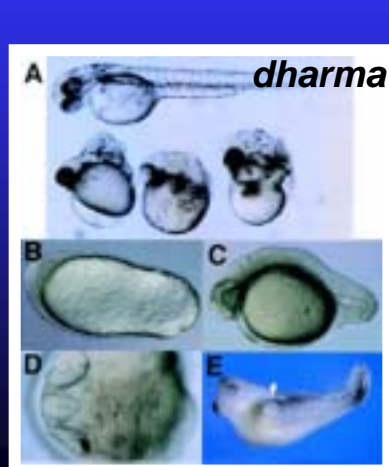
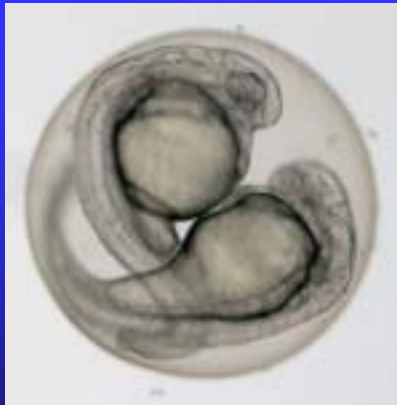
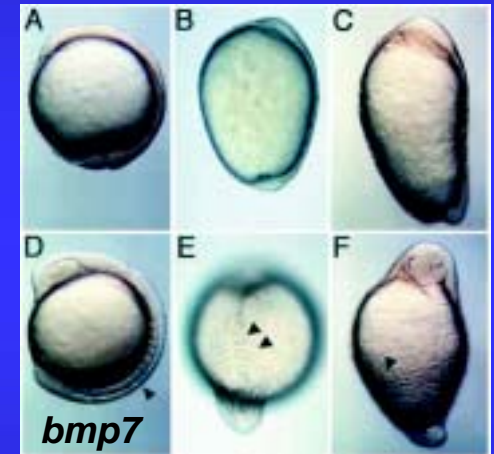
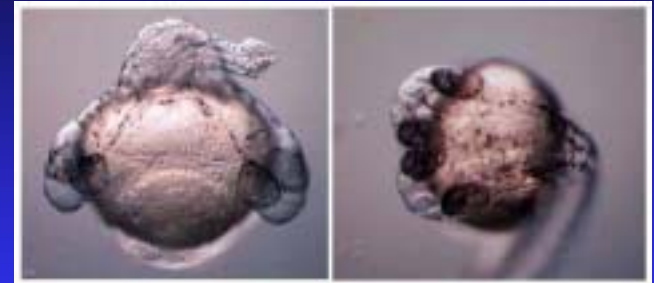
Overexpression by microinjection



In vivo screening of functional genes

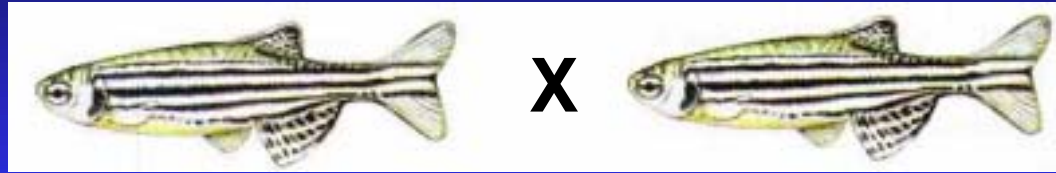


New Disease & Drug targets



Saturation mutagenesis in zebrafish

P

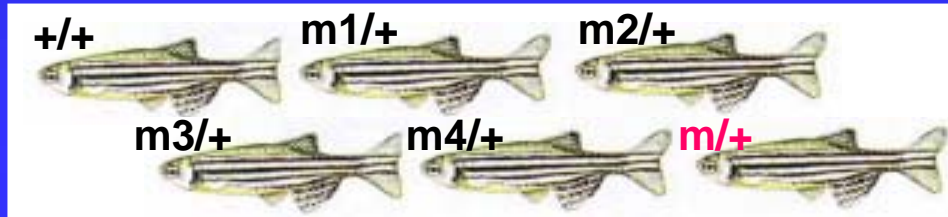


ENU-treated male

Wild-type female

~ 500 mutants / male

F1



X



Wild-type

F2

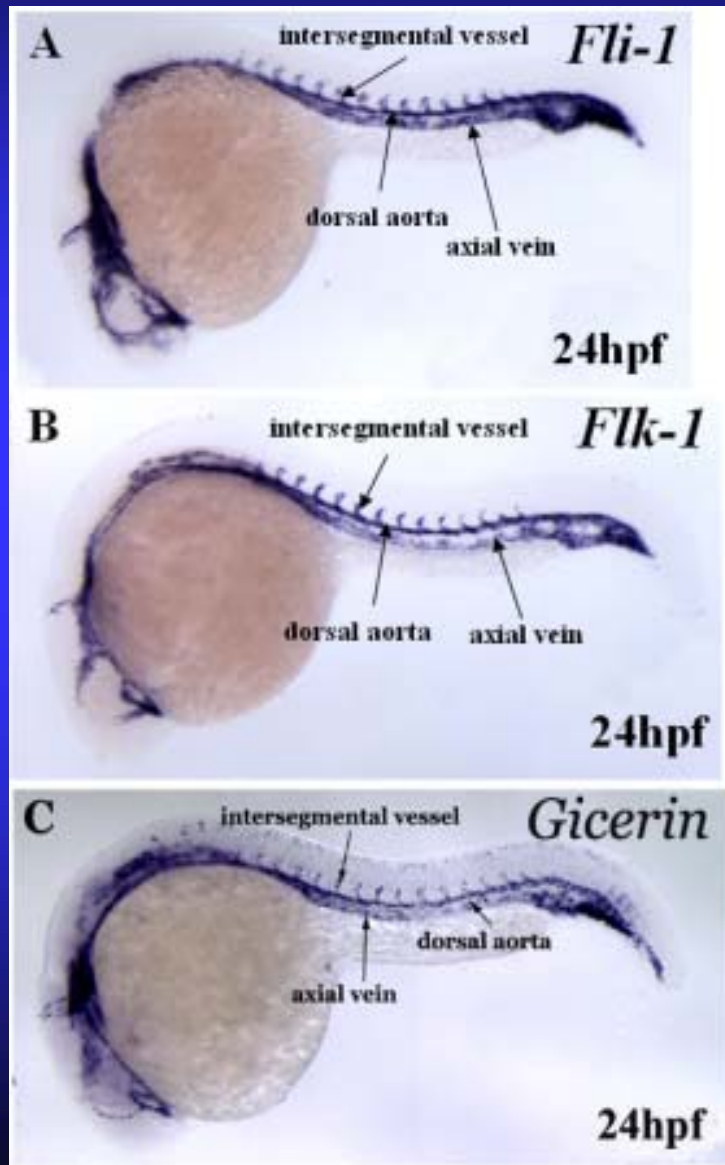


Backcross / Incross

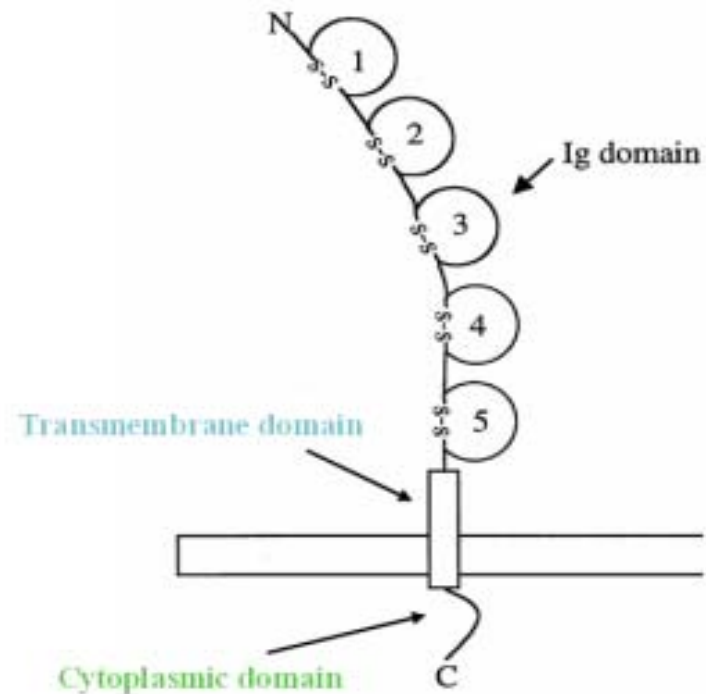
F3



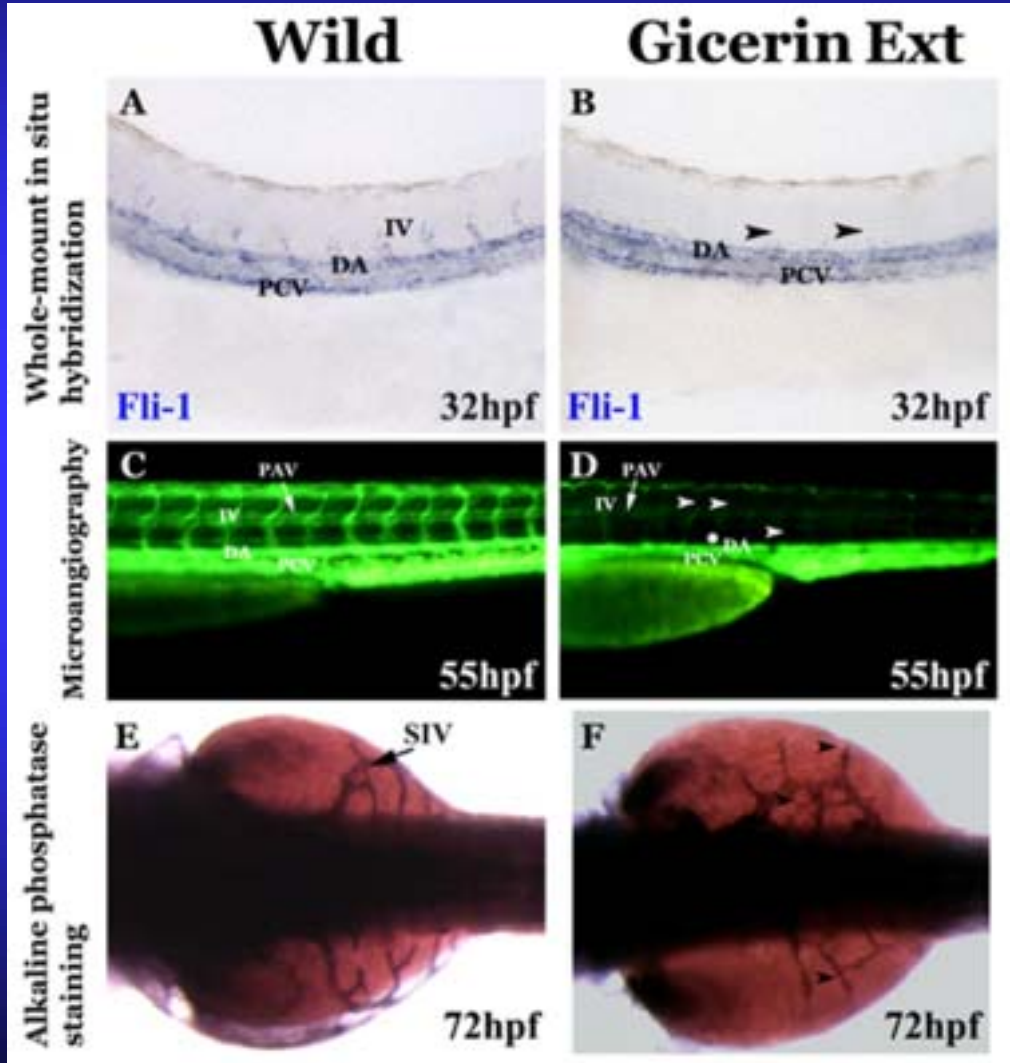
Vascular specific expression of *gicerin*



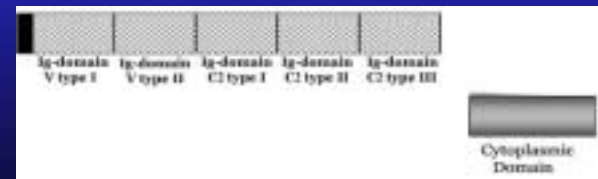
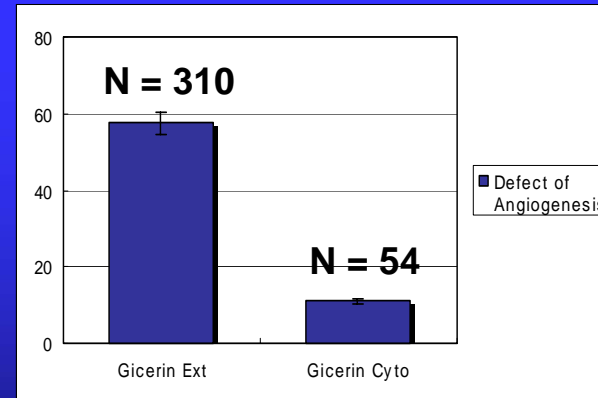
Fli-1 (Friend leukemia integration-1)
Flk-1 (Fetal liver kinase-1; VEGFR2)



DN form of Gicerin inhibits angiogenesis



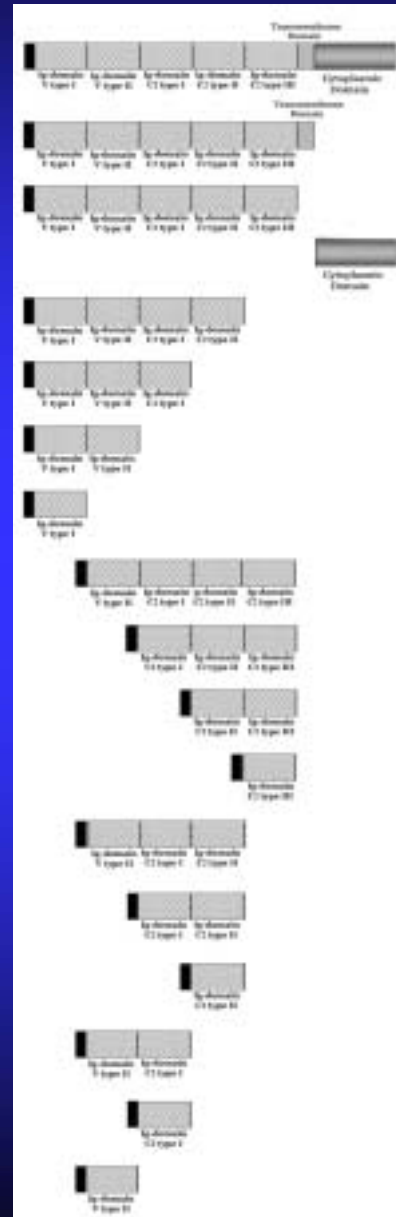
intersegmental vessel formation
subintestinal vein-sprouting



Gicerin Ext (DN)

Gicerin Cyto

Gicerin deletion constructs



Gicerin

Gicerin C

Gicerin Ext (DN)

Gicerin Cyto

Gicerin Ig I- IV

Gicerin Ig I- III

Gicerin Ig I - II

Gicerin Ig I

Gicerin Ig II- V

Gicerin Ig III- V

Gicerin Ig IV- V

Gicerin Ig V

Gicerin Ig II- IV

Gicerin Ig III-IV

Gicerin Ig IV

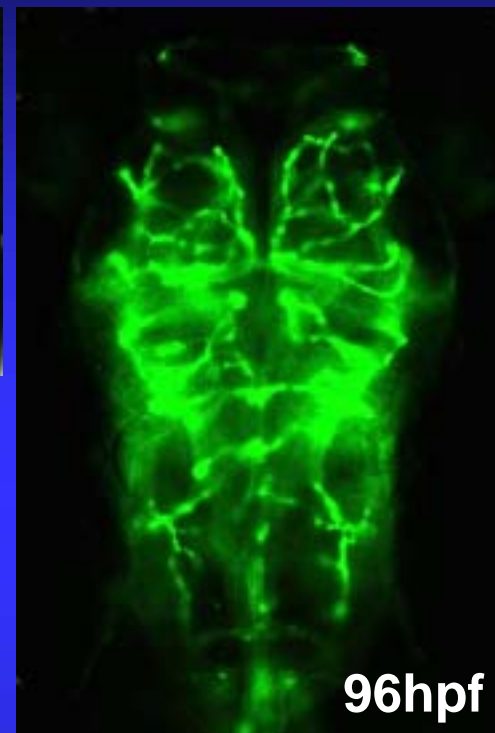
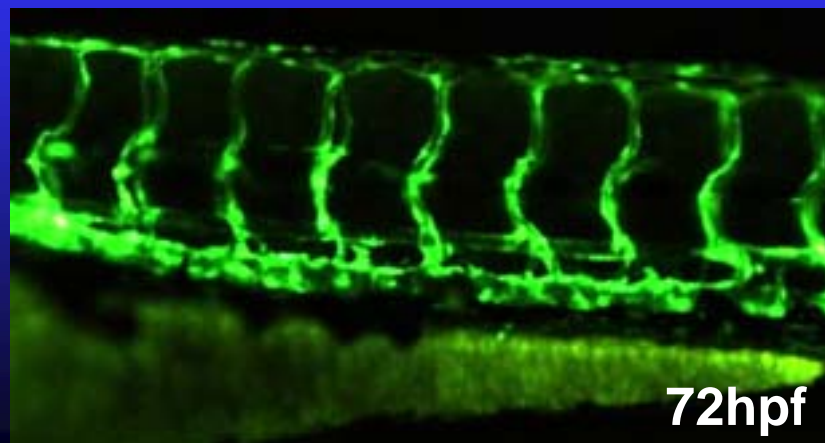
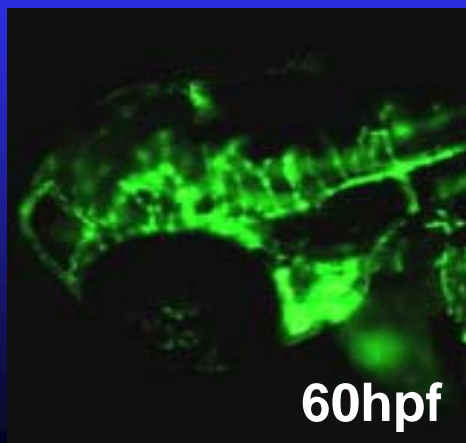
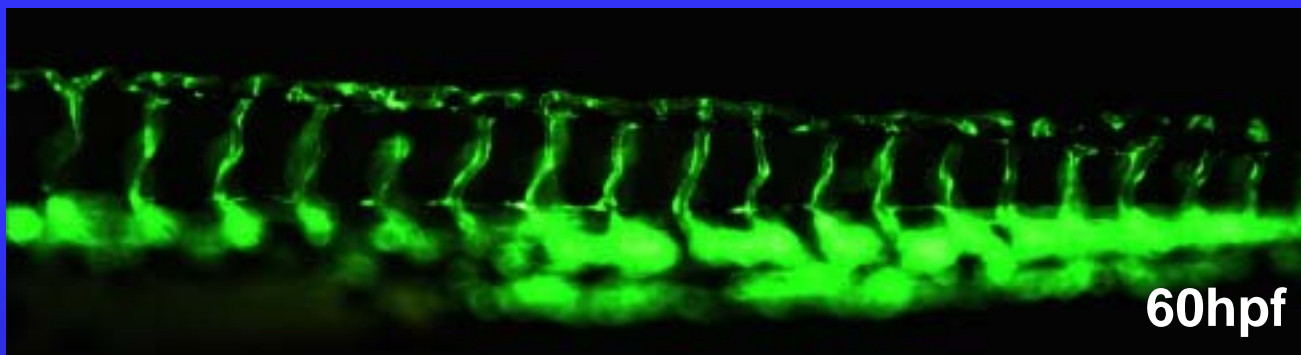
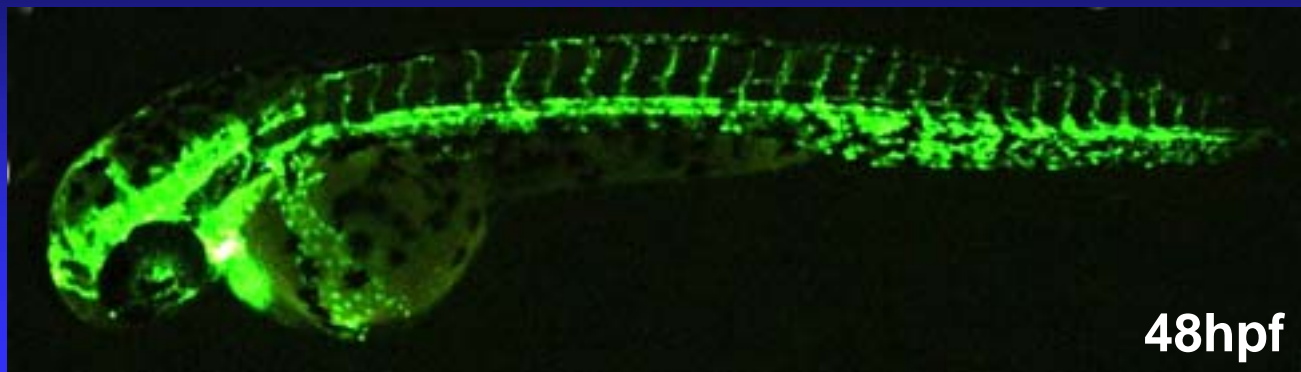
Gicerin Ig II- III

Gicerin Ig III

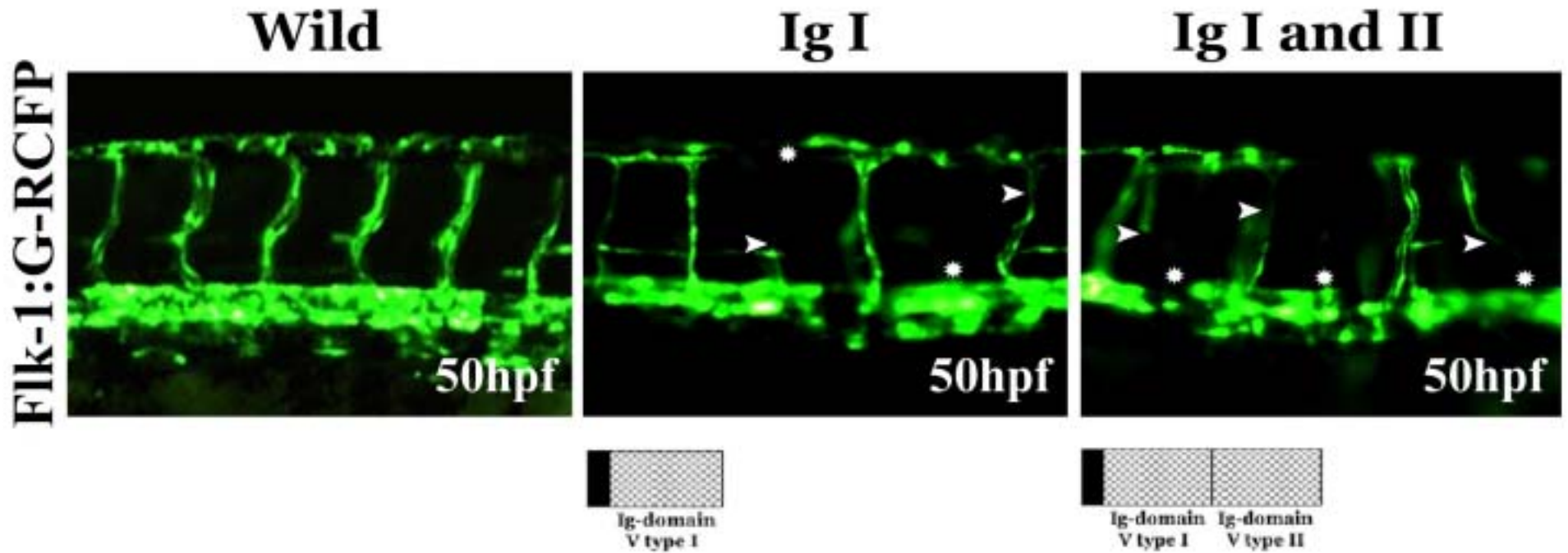
Gicerin Ig II

Anti-angiogenic peptide?

flk1-GFP transgenic zebrafish

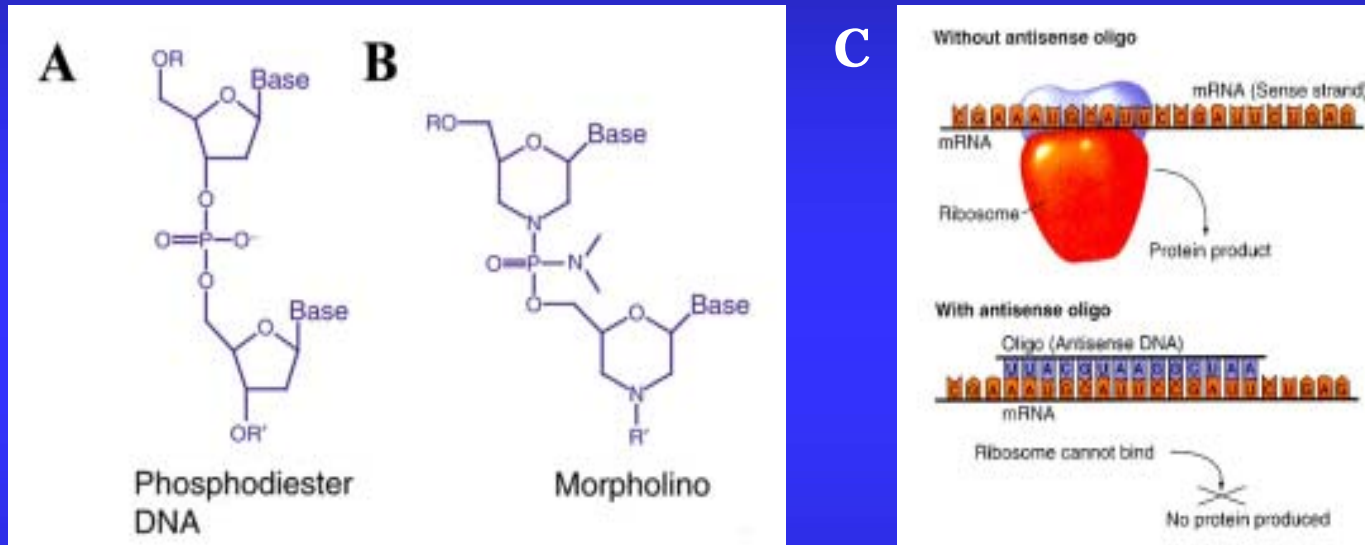


Gicerin DN-forms & vascular defects



Knock-down analysis of Gicerin

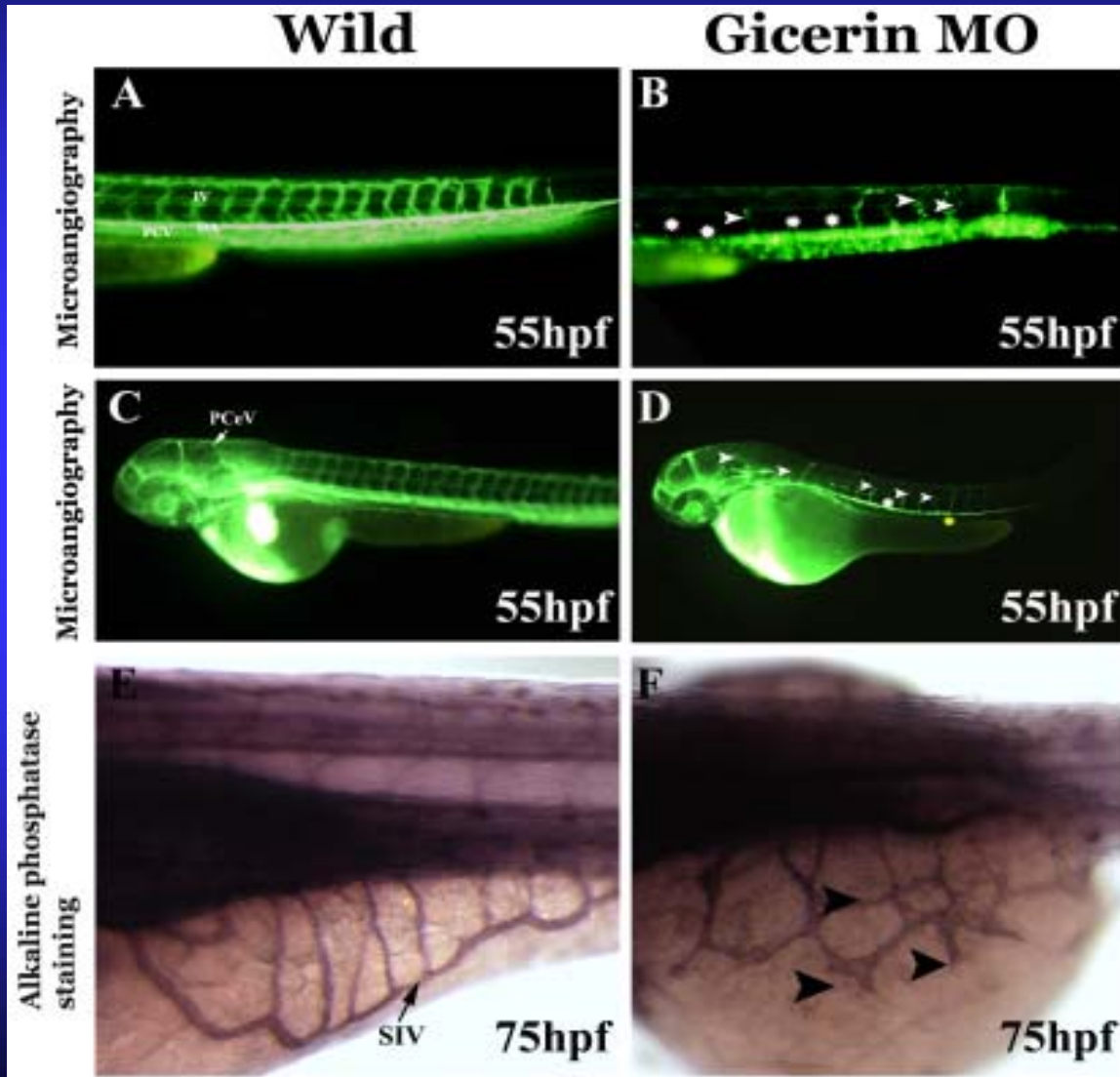
Morpholino (anti-sense oligonucleotide)



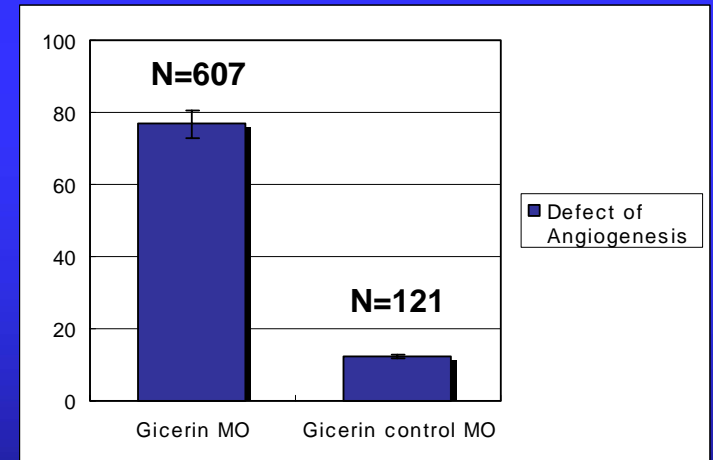
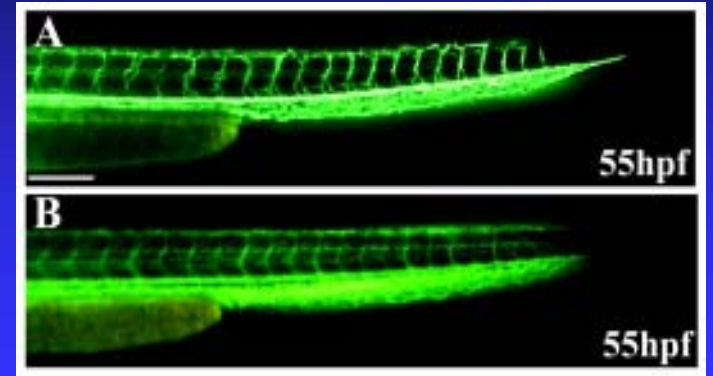
Gicerin Morpholino ;
5' AGCAGTGCGGTGTAGGTCATTTCTC 3'

Gicerin 5 base-mismatch control Morpholino;
5' AG**G**CGTGCGG**A**GTAG**C**TCATTT**G**TC 3'

Angiogenesis in MO-injected embryos



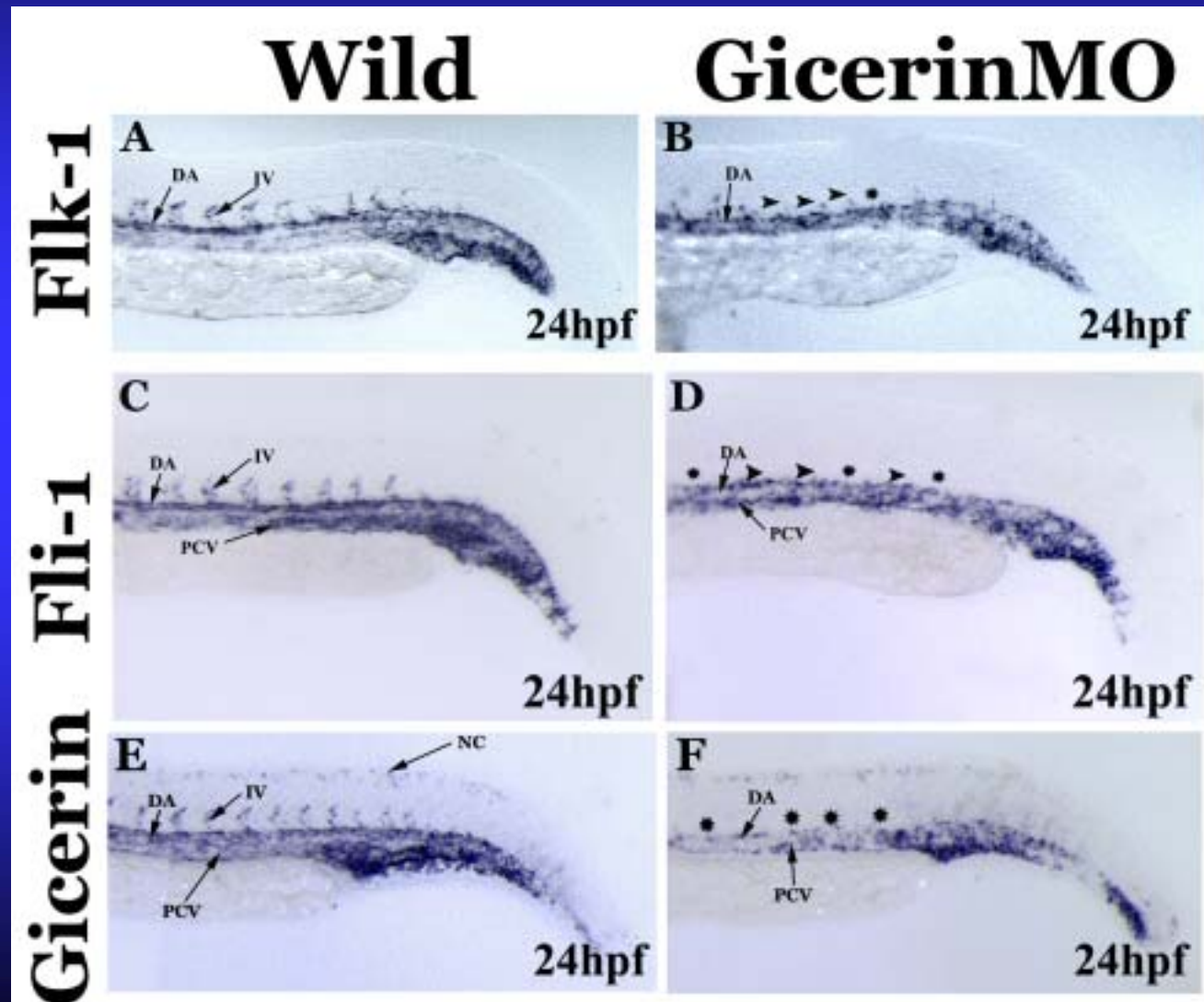
Wild
Con.MO



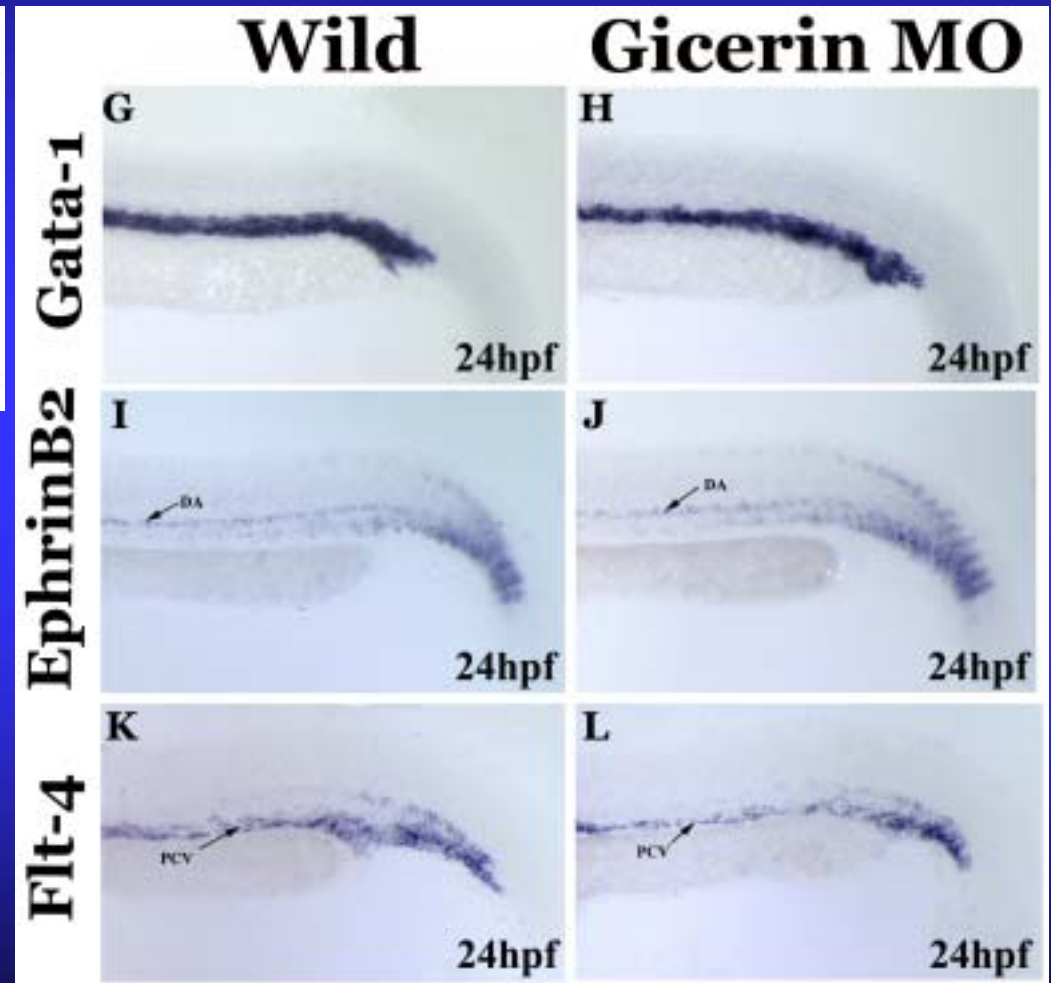
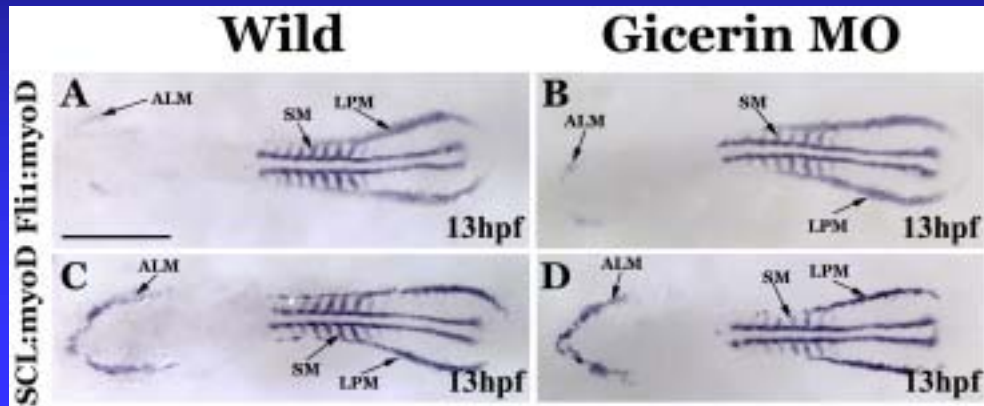
Gicerin MO : 1 ng/embryo

Gicerin control MO : 1.5 ng/embryo

Gicerin MO inhibits angiogenesis

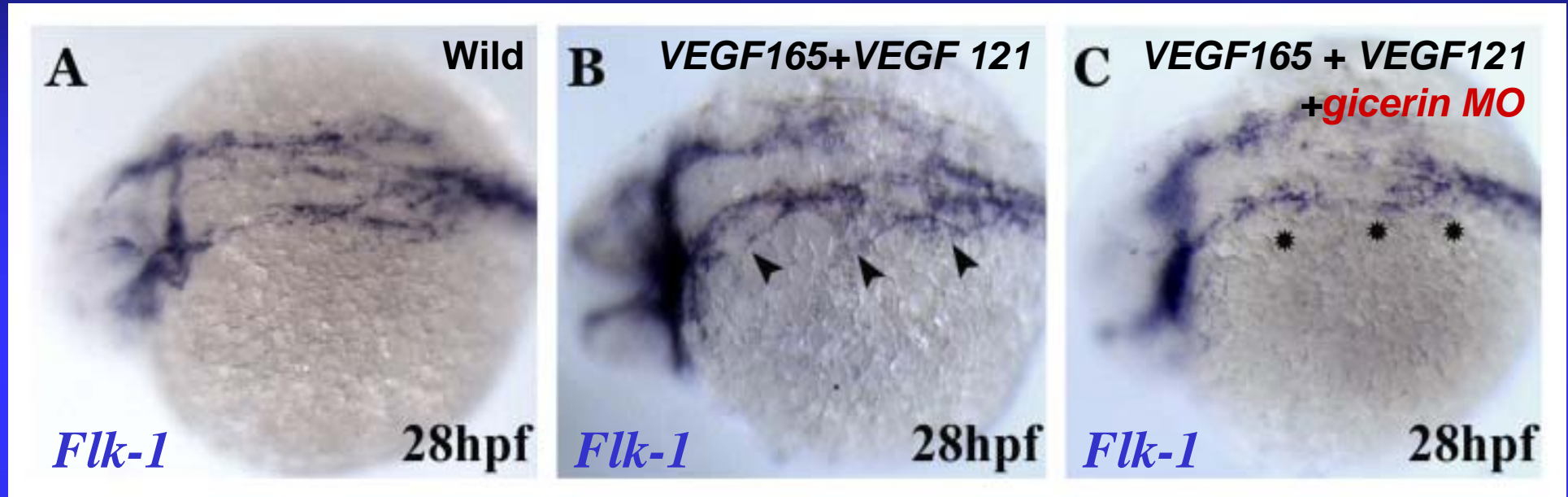


Vasculogenesis is not affected by gicerin MO



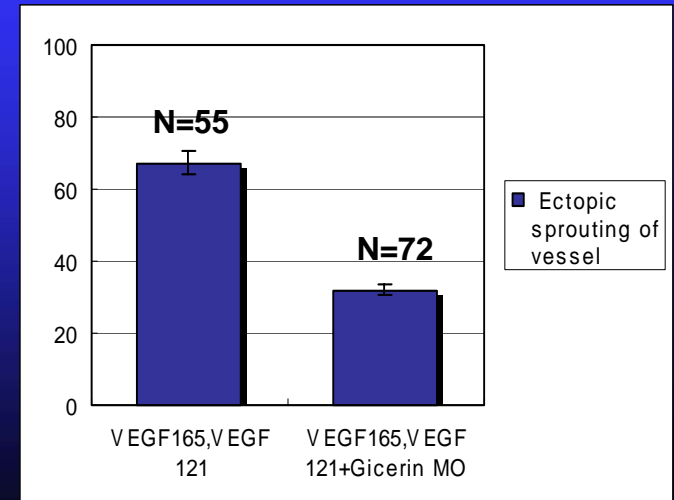
Gata-1 : erythroid precursor
SCL : haemangioblast
EphrinB2 : artery
Fli-4 : vein
Fli1-1 : vascular marker

Gicerin MO inhibits VEGF-induced angiogenesis



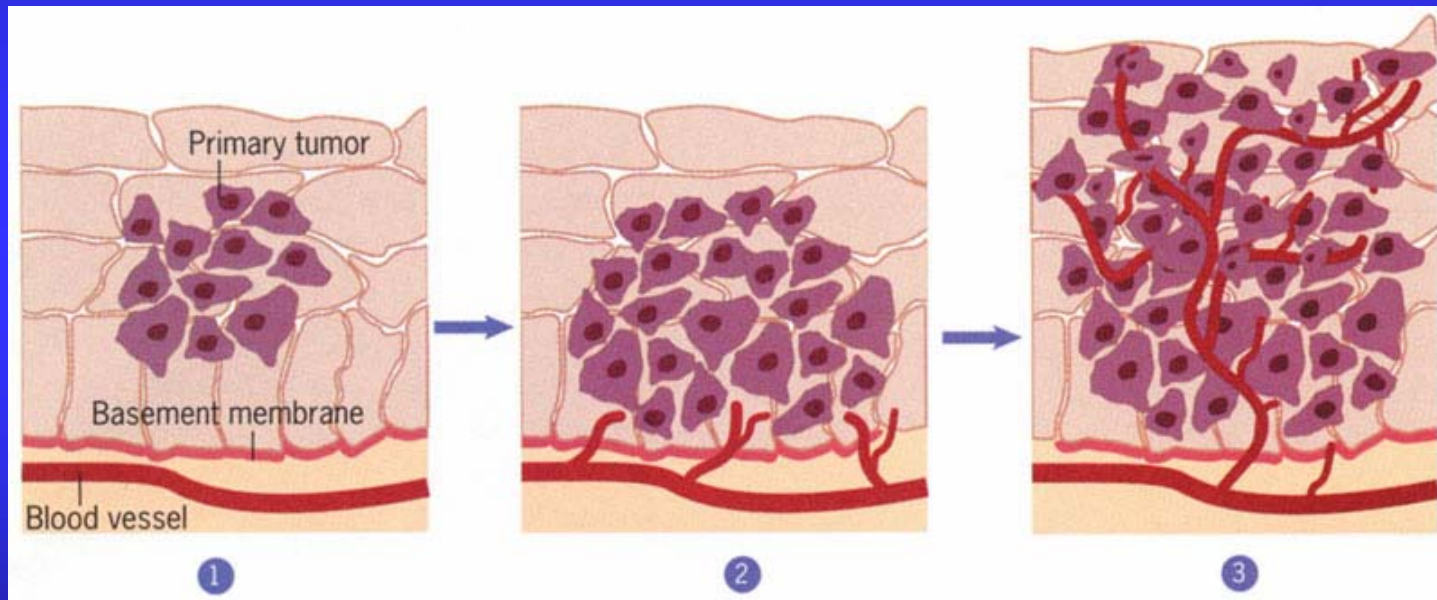
VEGF165+VEGF121: 30 pg/embryo

Gicerin MO : 1.5 ng/embryo



Angiogenesis inhibitors for anticancer therapy

CD146, MCAM, Mel-CAM, MUC18, P1H12, A32 antigen, S-Endo-1
Tumor endothelial marker (*Croix, Science 2000*)
Immunoglobulin superfamily (NCAM, PECAM, ICAM, VCAM)



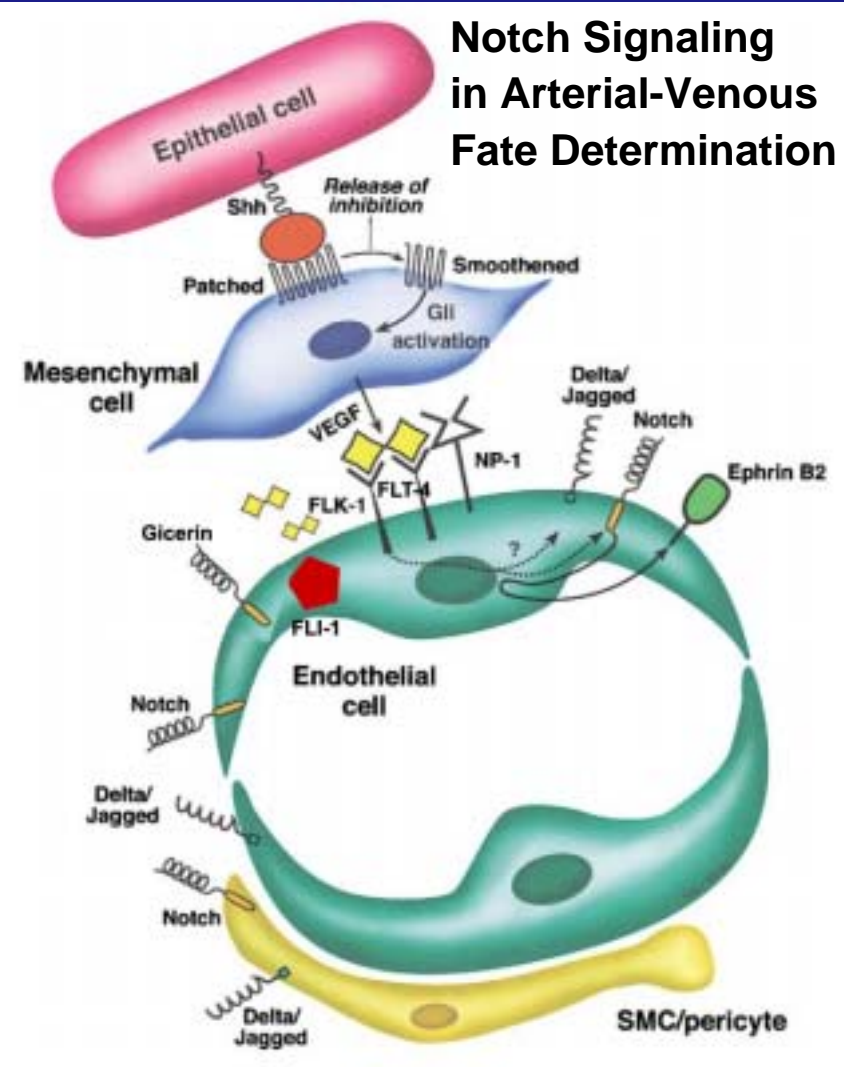
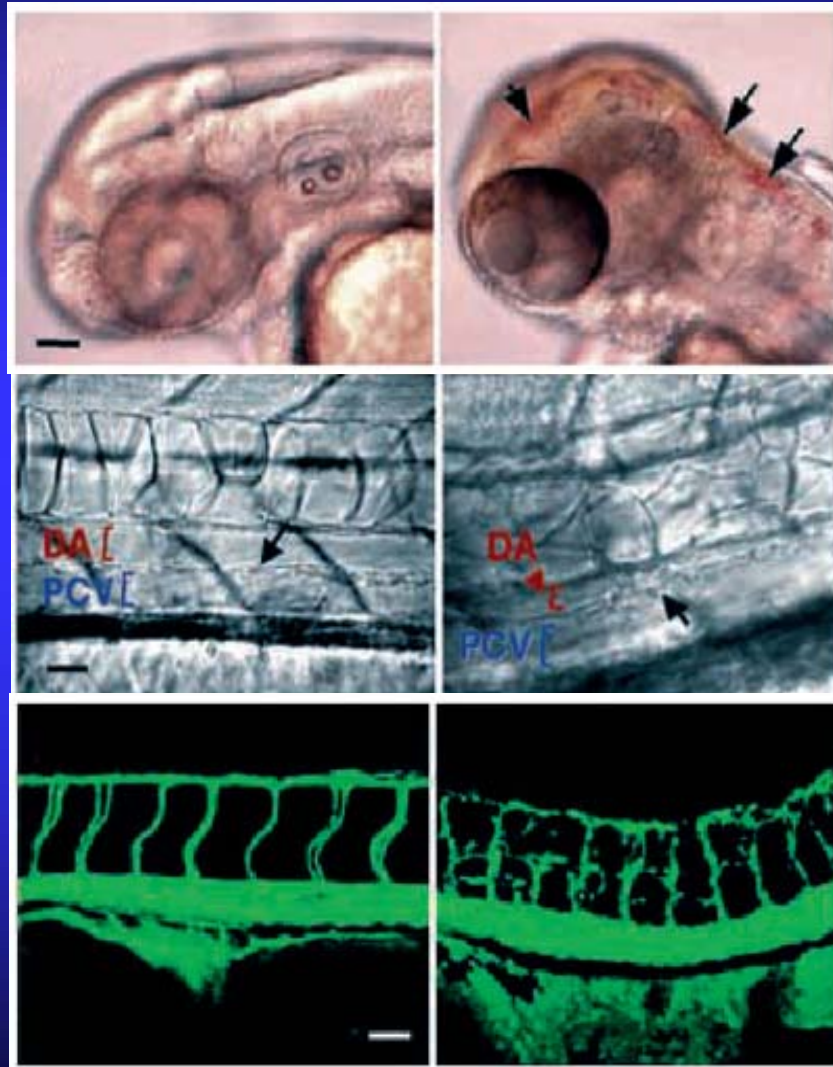
Endothelial precursor cells (EPC)

Human umbilical vein endothelial cells (HUVEC)

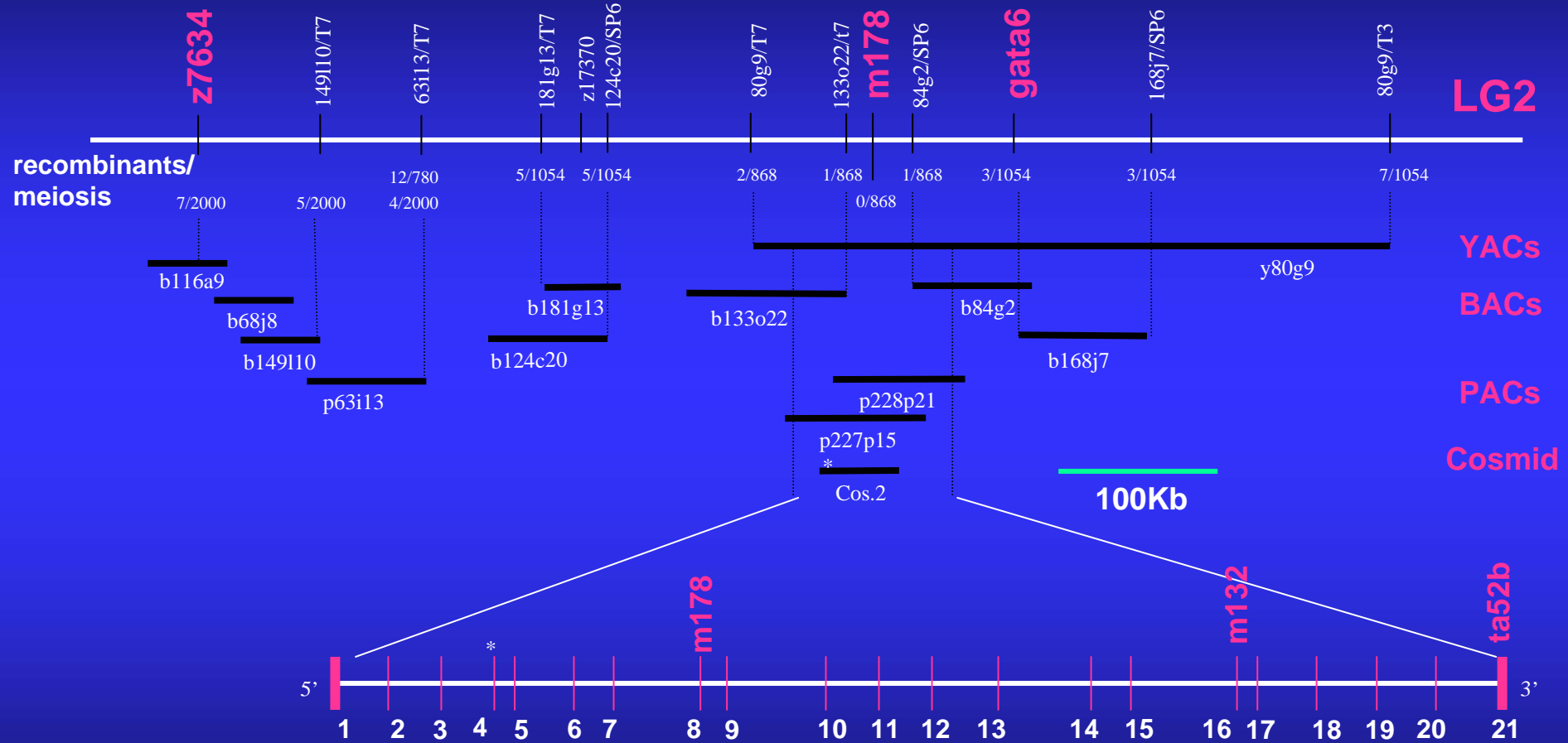
Human microvascular endothelial cells (HMVEC)

Anti-angiogenic peptide?

Vascular Defects in *mind bomb* mutants

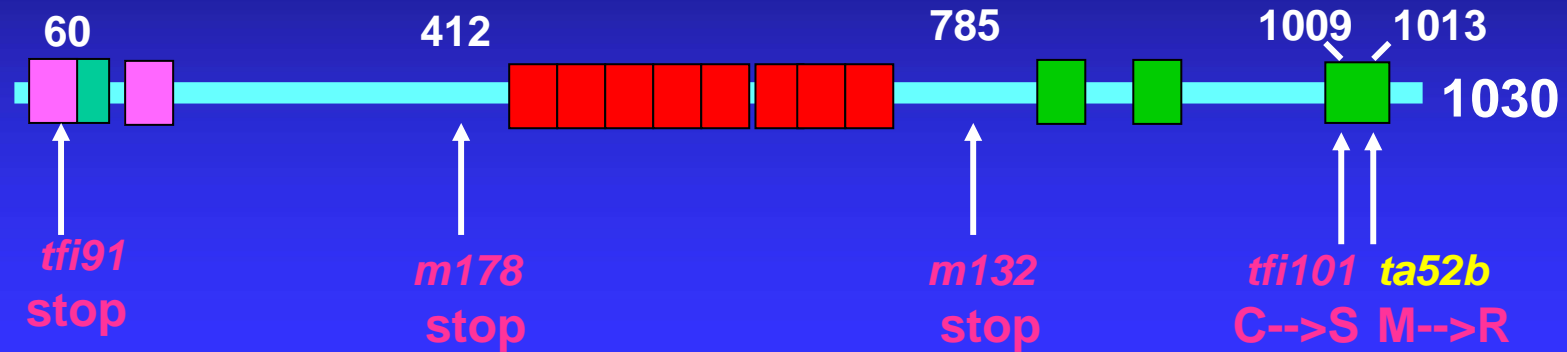






Physical map of *mind bomb* locus



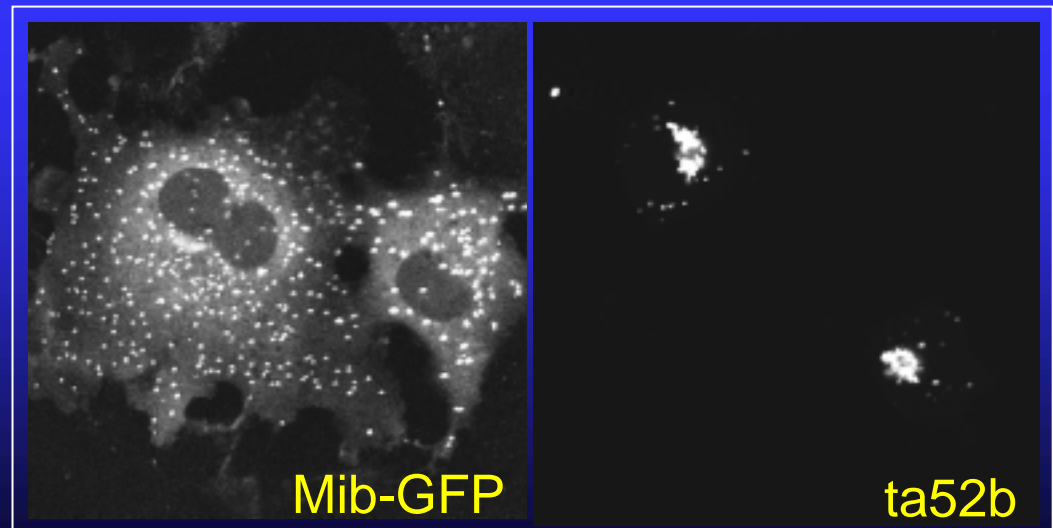
mindbomb (mib) is a novel gene

Mib protein structure and mutations

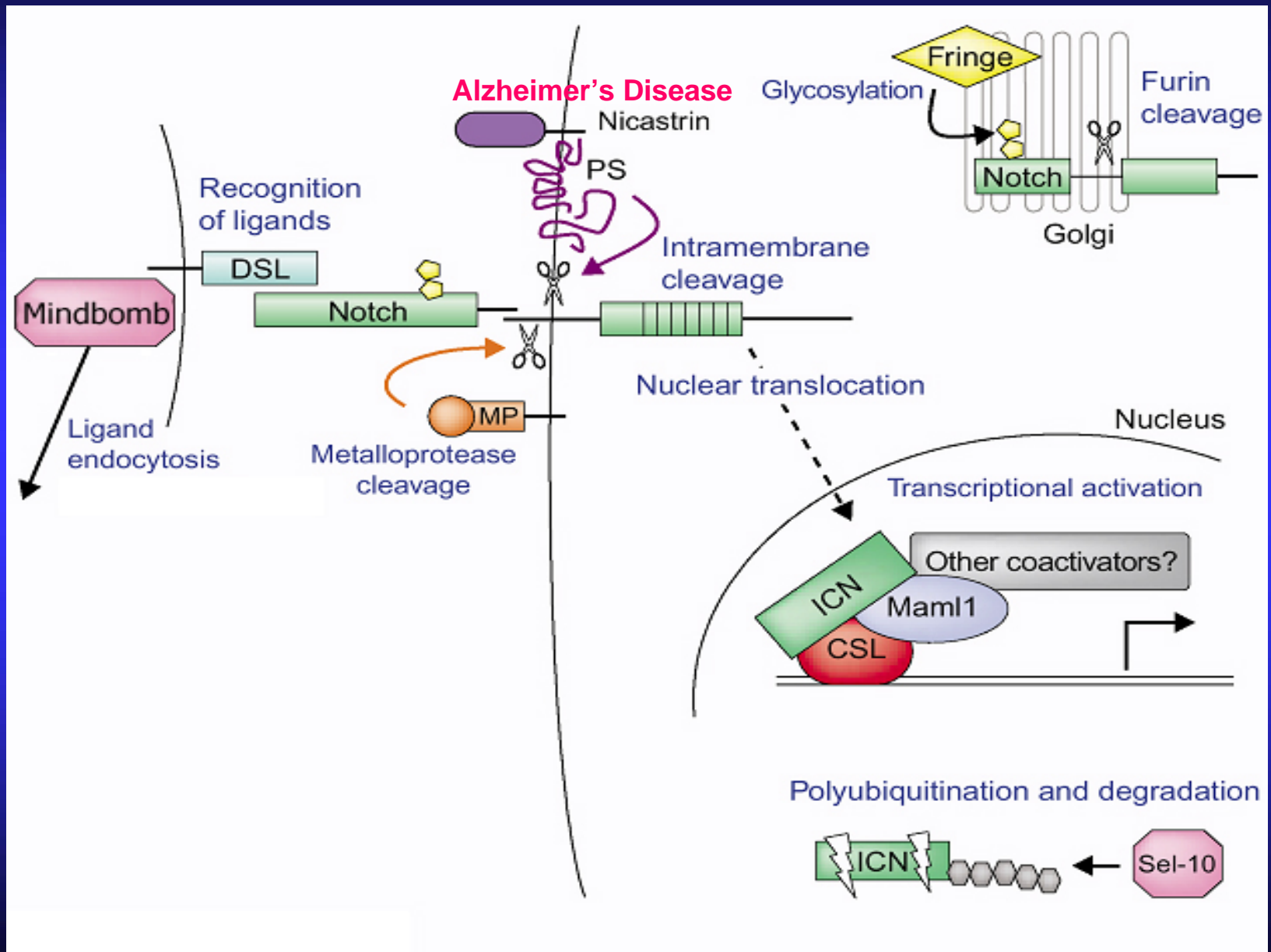


-  mib/herc2 domain
-  zz zinc finger domain
-  ankyrin repeat domain
-  RING finger domain

Subcellular localization of proteins



Notch signaling pathway

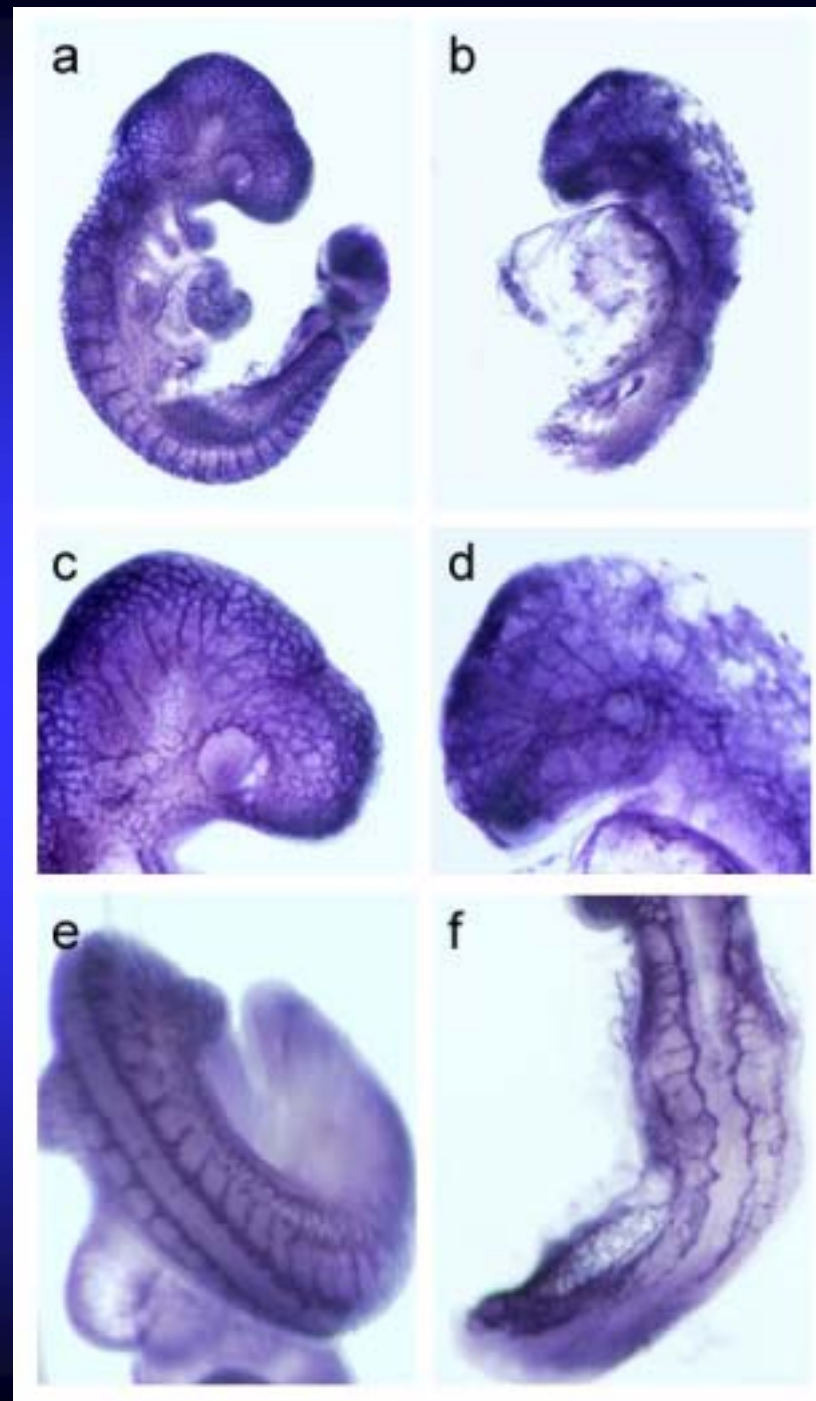


Comparison of Mib proteins between human and zebrafish

human mib.txt	1:	MVEGVGARVVRGPDWKWGKQDGGEGHVGTVRSFESPEEVVVVVDNGTAANYRCSGAYDLR	60
mouse mib.txt	1:	MVEGVGARVVRGPDWKWGKQDGGEGHVGTVRSFESPEEVVVVVDNGTAANYRCSGAYDLR	60
zebra mib.txt	1:	ME-GVGARVIRGPDWKWGKQDGGEGHVGTVRSFESPEEVVVVVDNGTAANYRCSGAYDVR	59
human mib.txt	61:	ILDSAPTGIKHDGTMCDTCRQQPIIGIRWKCAECTNYDLCTVCYHGDKHHLRHRFYRITT	120
mouse mib.txt	61:	ILDSAPTGIKHDGTMCDTCRQQPIIGIRWKCAECTNYDLCTVCYHGDKHHLRHRFYRITT	120
zebra mib.txt	60:	ILDSAPTGIKHDGTMCDTCRQQPIIGIRWKCAECTNYDLCTTCYHGDKHHLRHRFYRITT	119
human mib.txt	121:	PGSERVLLESRRKSKKITARGIFAGARVVRGVWQWEDQDGGNGRRGKVTEIQDWSASSP	180
mouse mib.txt	121:	PGSERVLLESRRKSKKITARGIFAGARVVRGVWQWEDQDGGNGRRGKVTEIQDWSASSP	180
zebra mib.txt	120:	PGSERVLLESRRKSKKITARGIFAGGRVVRGVWQWEDQDGGNGRRGKVTEIQDWSAASP	179
human mib.txt	181:	HSAAAYVLWDNGAKNLYRVGFEGMSDLKCVQDAKGGSFYRDHCPVLGEQNGNRNPPGGLQIG	240
mouse mib.txt	181:	HSAAAYVLWDNGAKNLYRVGFEGMSDLKCVQDAKGGSFYRDHCPVLGEQNGNRNPPGGLQIG	240
zebra mib.txt	180:	HSAAAYVLWDNGAKNLYRVGFEGMSDLKCVQDAKGGTFYRDHCPVLGEQNGNRNPPGGLQIG	239
human mib.txt	241:	DLVNIDLdleIVQSLQHGHHGGWTDGMFETLTTTGTVCGIDEDHDIVVQYPSGnrWTFNPA	300
mouse mib.txt	241:	DLVNIDLdleIVQSLQHGHHGGWTDGMFETLTTTGTVCGIDEDHDIVVQYPSGnrWTFNPA	300
zebra mib.txt	240:	DLVNIDLdleIVQSLQHGHHGGWTDGMFETLTTTGTVCGIDEDHDIVVQYPSGnrWTFNPA	299
human mib.txt	301:	VLTKANIVRSGDAACGAKGGTSOFQVGDLVQVCYDLERIKLLQRGHGENAEAMLPTLGKV	360
mouse mib.txt	301:	VLTKANIVRSGDAACGAEGGTSOFQVGDLVQVCYDLERIKLLQRGHGENAEAMLPTLGKV	360
zebra mib.txt	300:	VLTKANVVRSGEVAAGAEGGSSOFMVGDLVQVICYDIDRIKLLQRGHGENAEAMLPTLGKV	359

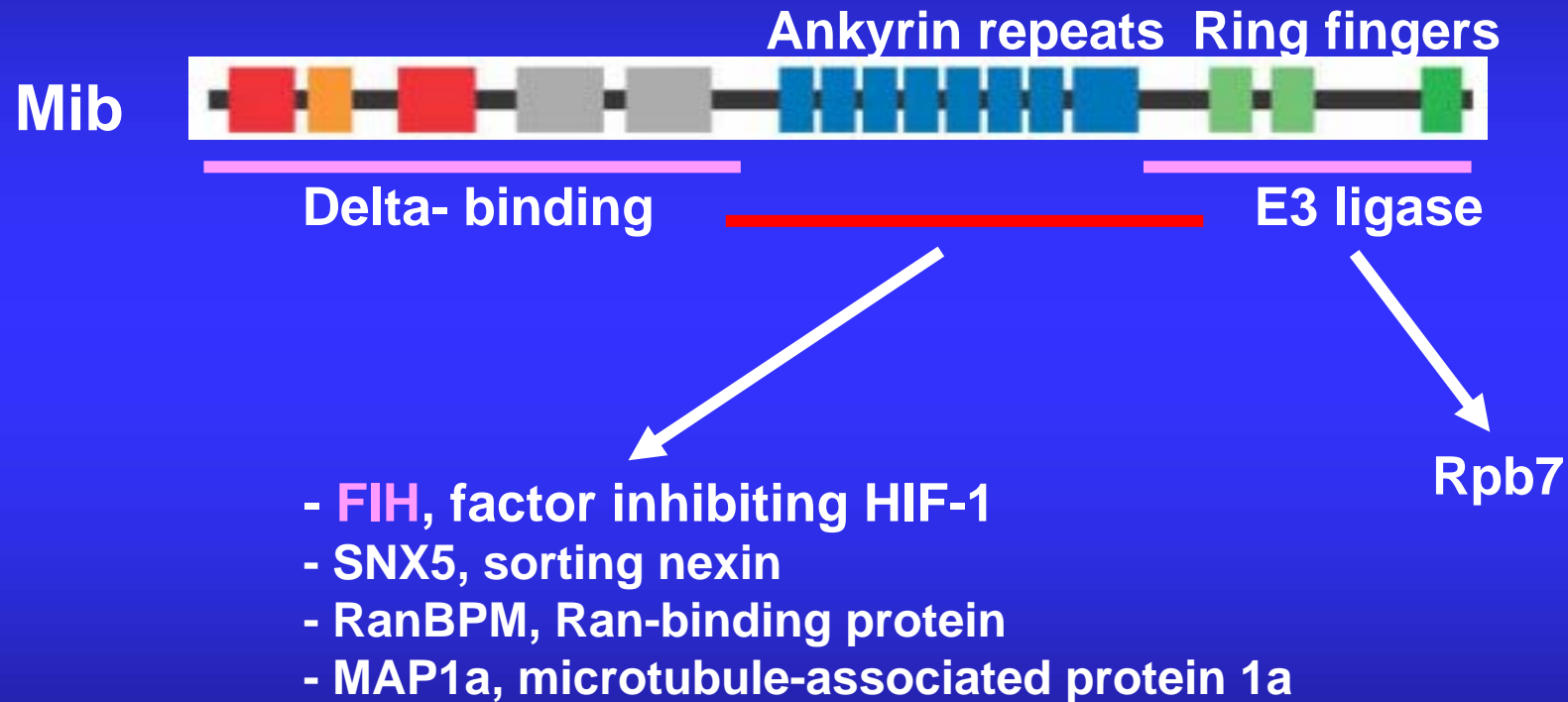
Vascular defects in *mib* KO mouse

Dr. Young-Yun Kong
(POSTECH)

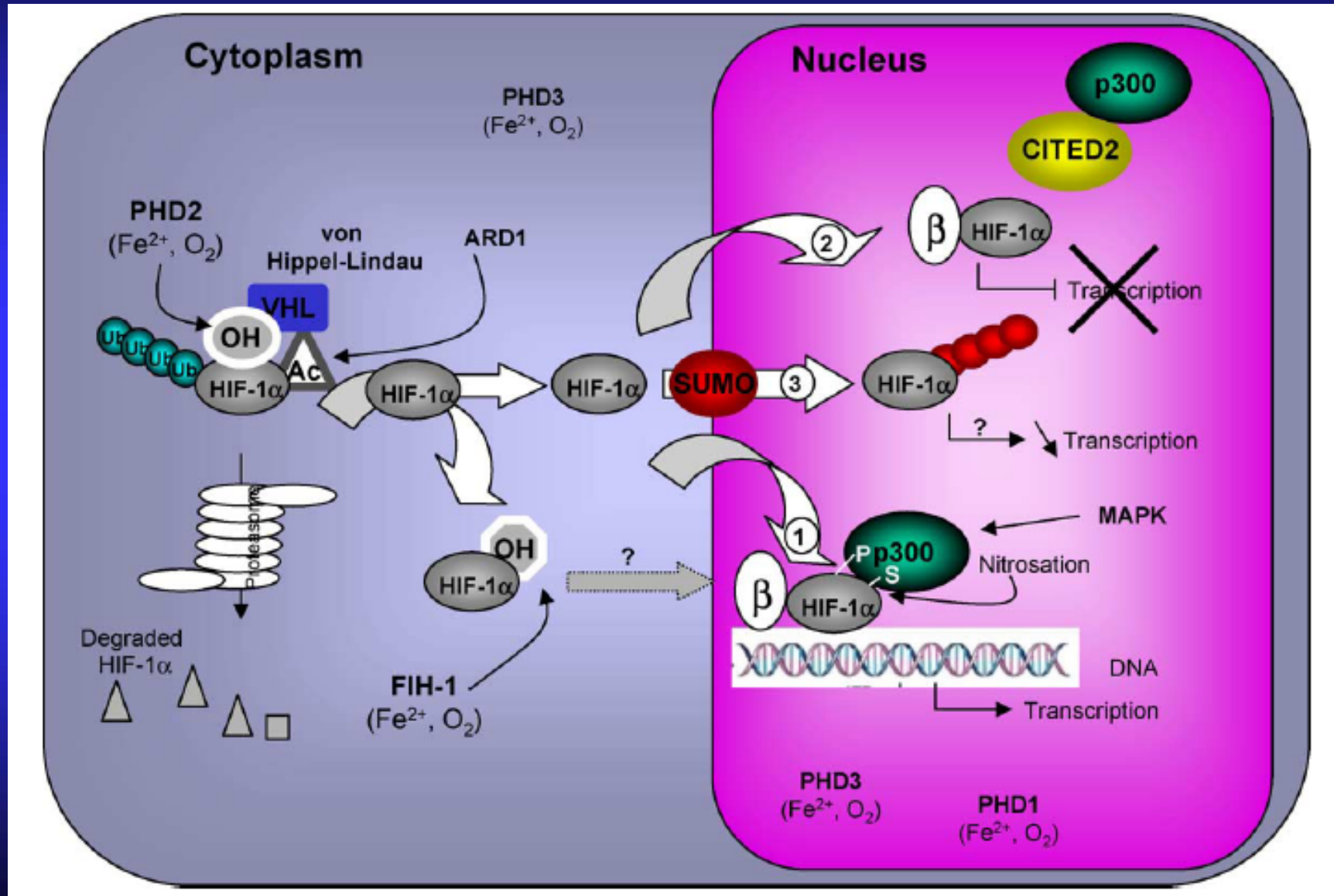


Flk1 immunostaining at E9.5

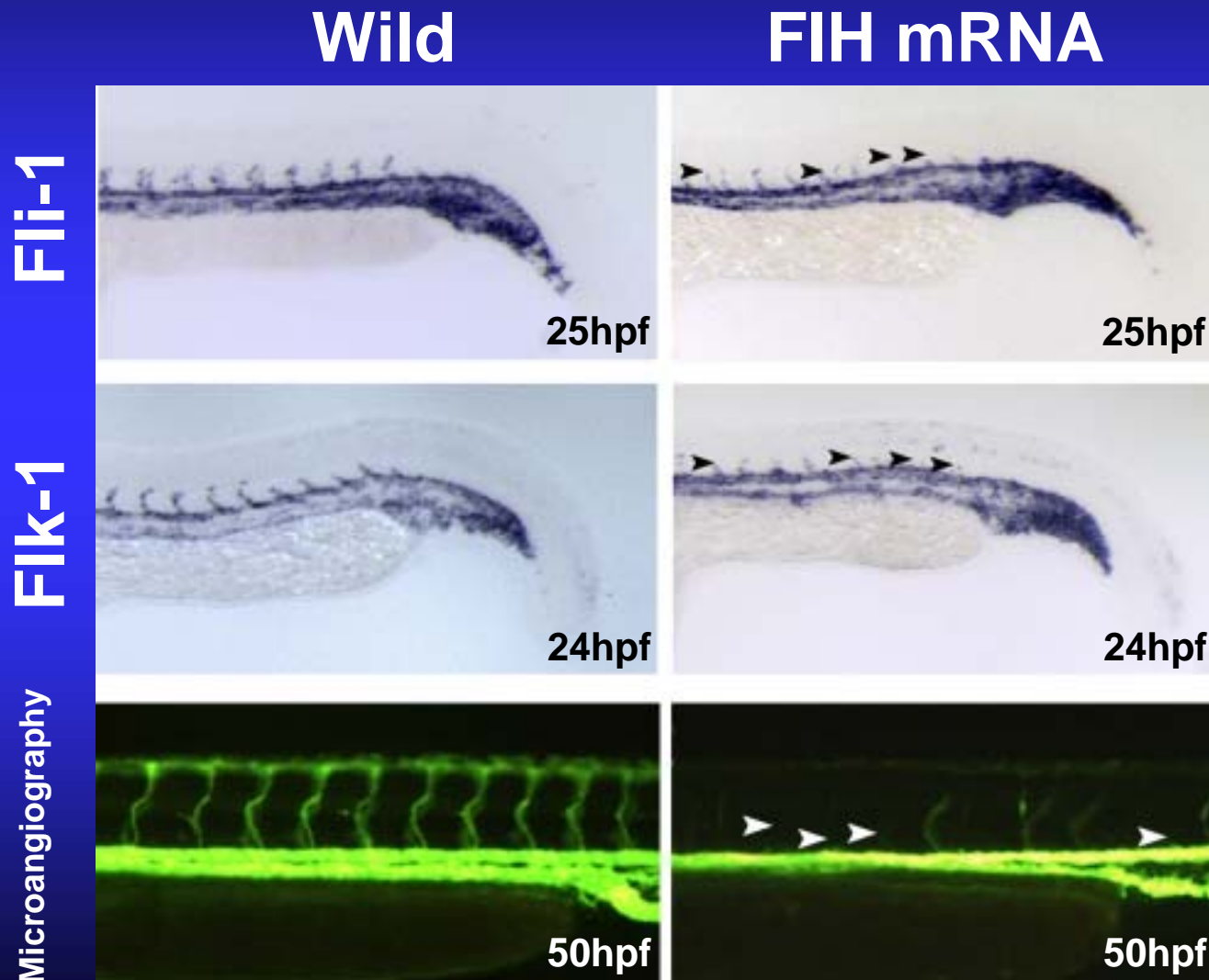
Screening of Mib-binding proteins



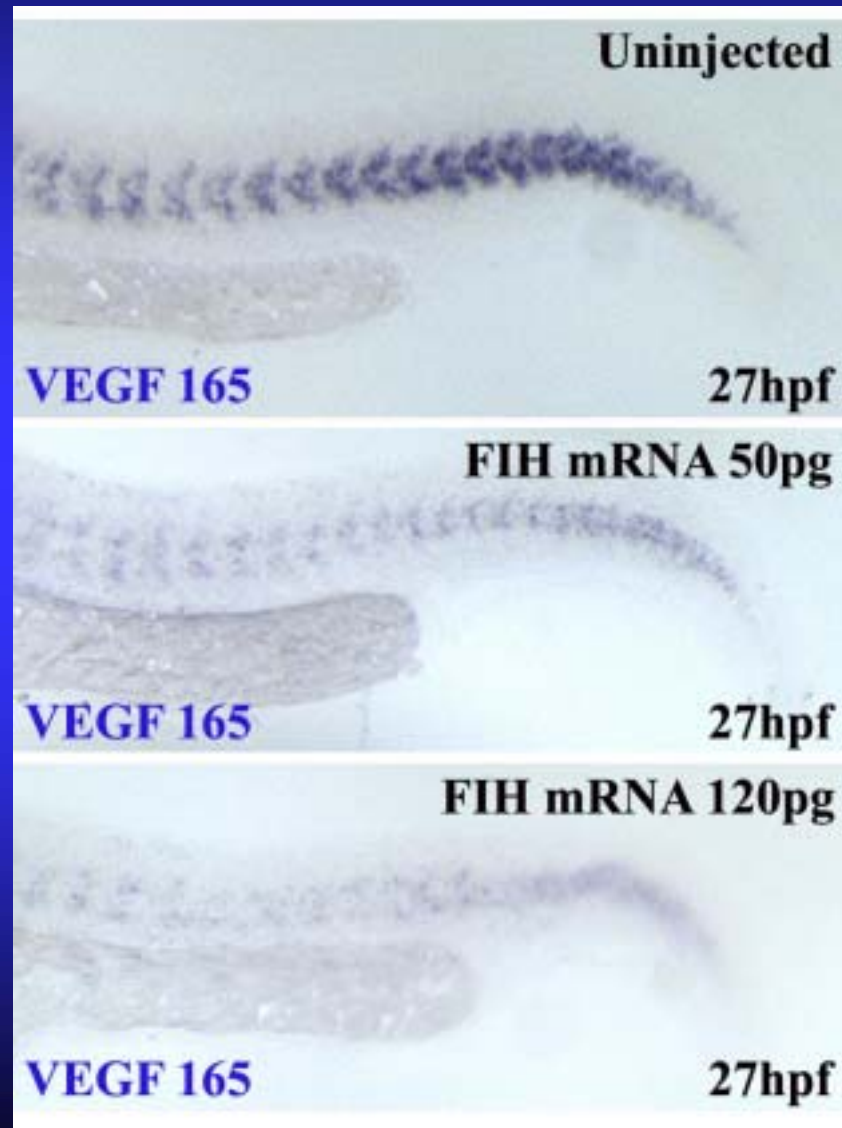
FIH, an Asparaginyl Hydroxylase of HIF-1



FIH inhibits angiogenesis

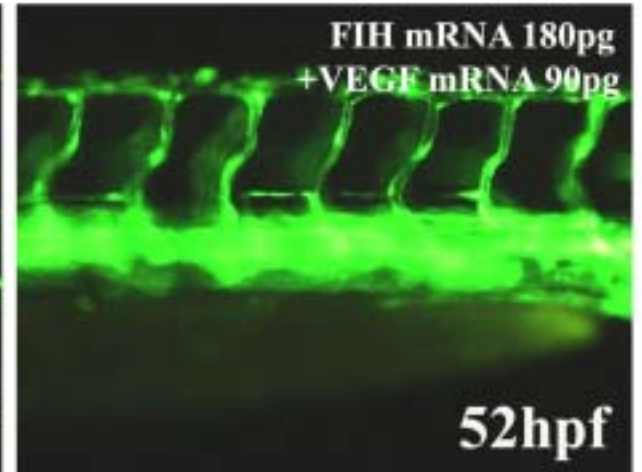
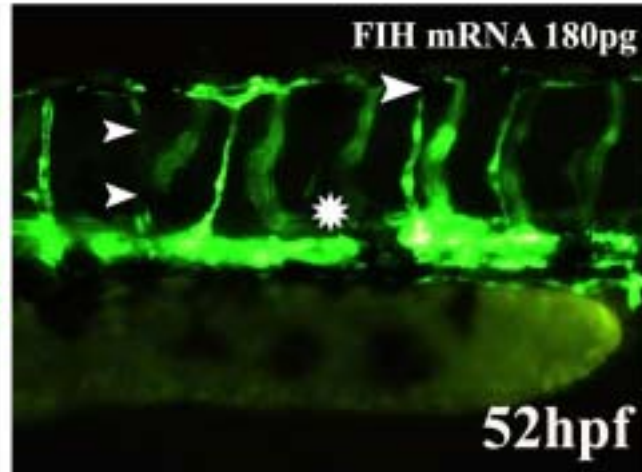
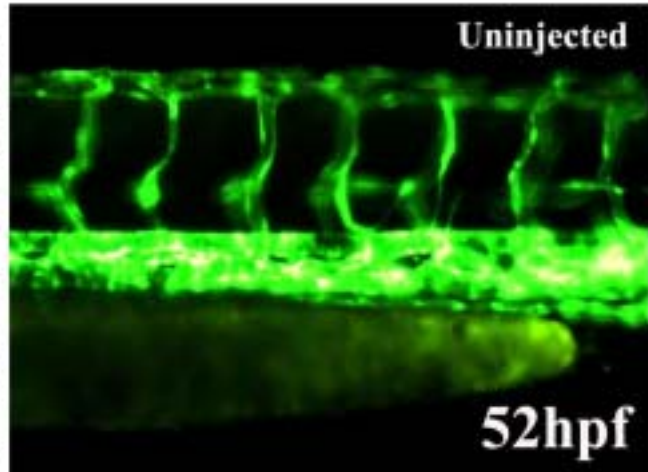


FIH inhibits VEGF expression

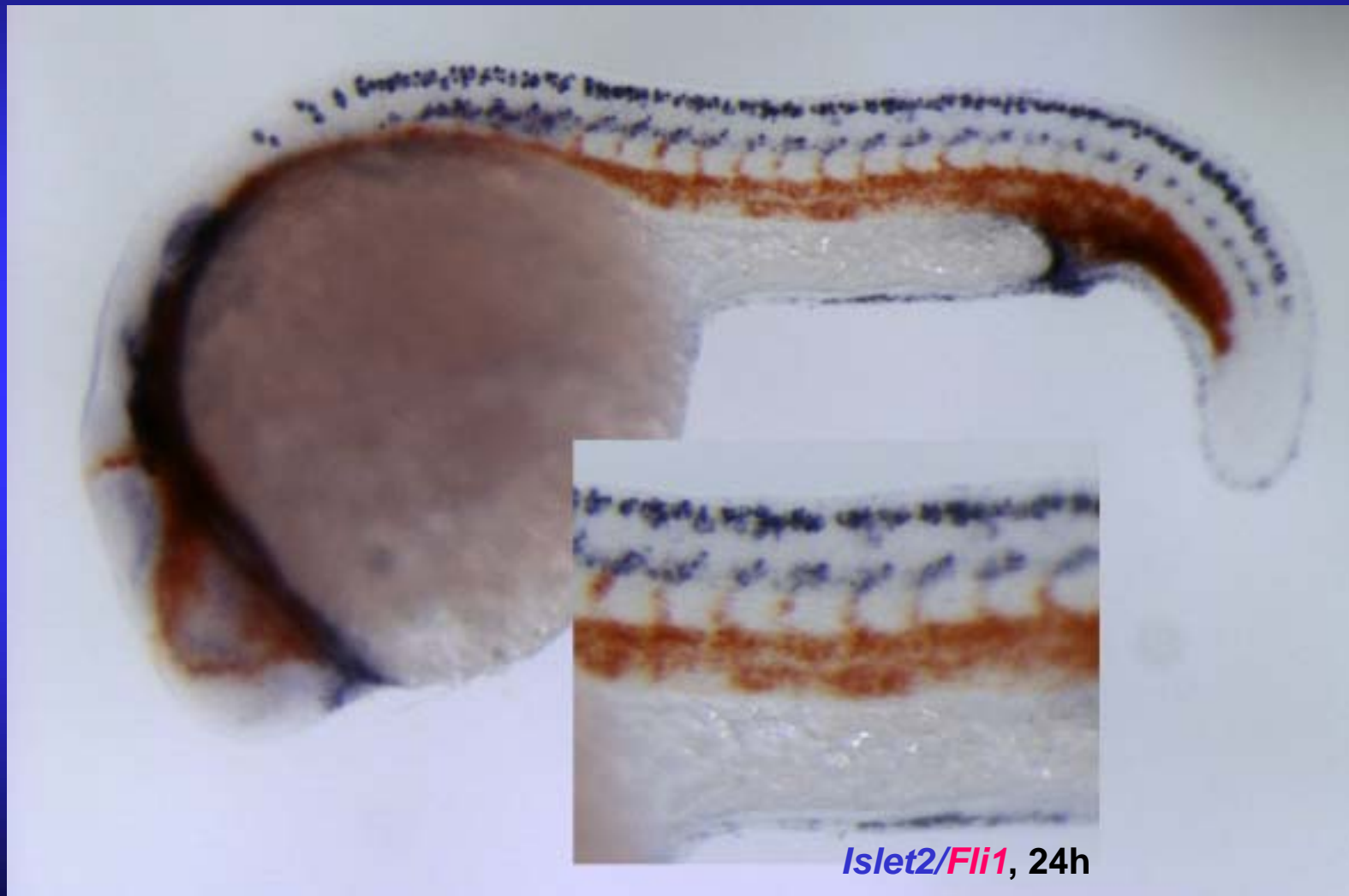


VEGF rescue angiogenesis in FIH-injected embryos

Flk-1 : GFP



**Future study:
Interactions between neural and vessel cells**



MCH as a new transgene reporter in zebrafish (Melanin-concentrating Hormone)

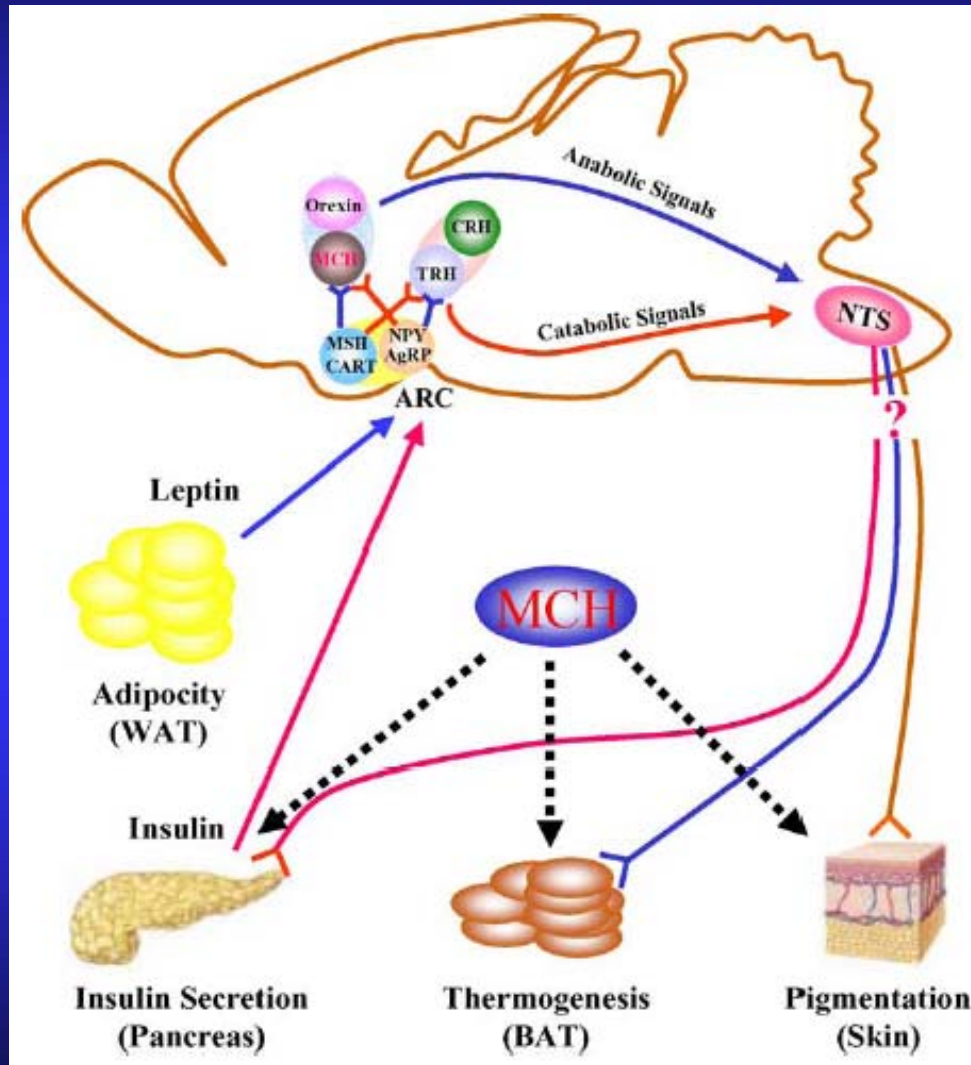
Skin color

Obesity

Energy homeostasis

Behavior & Emotion

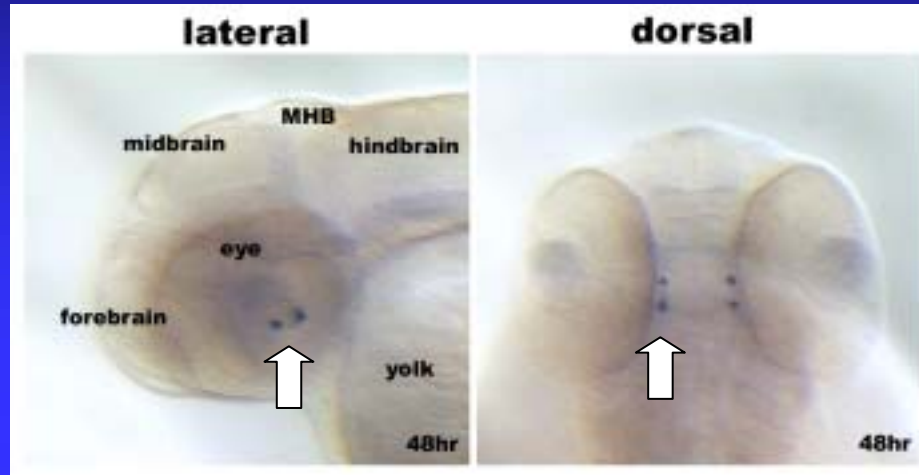
MCH circuitry in the brain and its regulation of peripheral physiology



Peptides, 2004

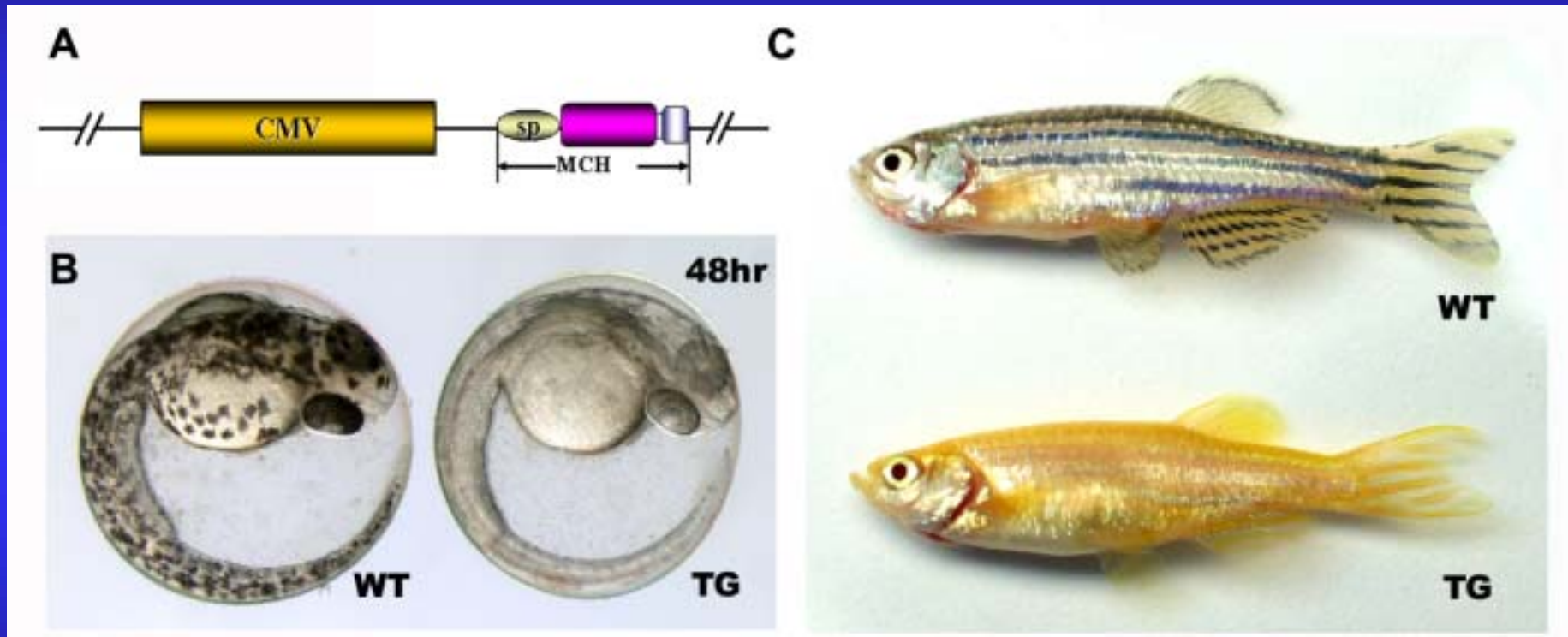
ARC; arcuate nucleus, a-MSH; a-melanocyte-stimulating hormone, CRF; corticotropin-releasing factor, CART; cocaine and amphetamine-related transcript, NPY; neuropeptide Y, NTS; nucleus of the solitary tract, TRH; thyroid-releasing hormone, AgRP; agouti-related protein

MCH-positive neurons in ventral hypothalamus



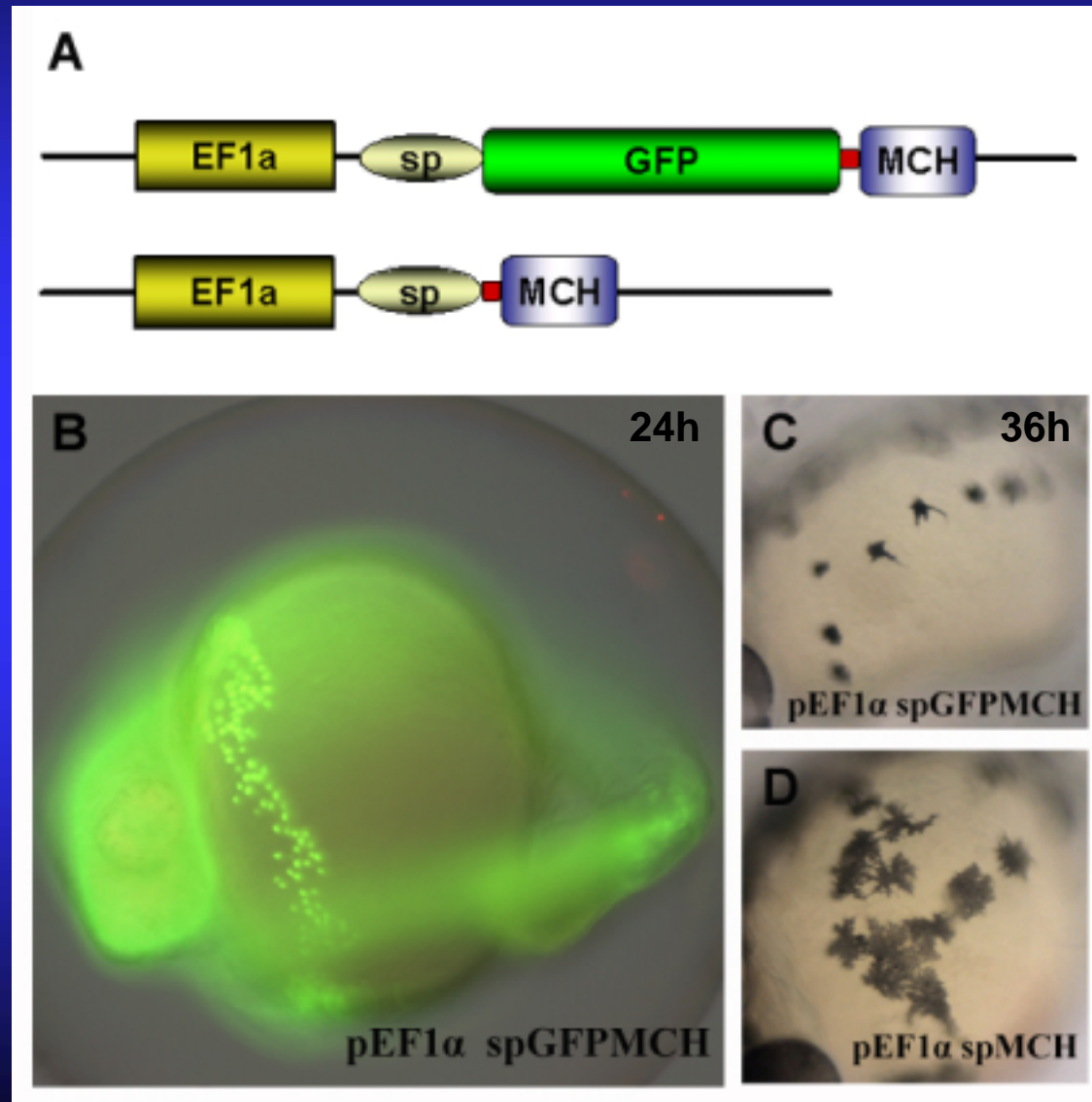
TH, tyrosine hydroxylase

MCH transgenic zebrafish

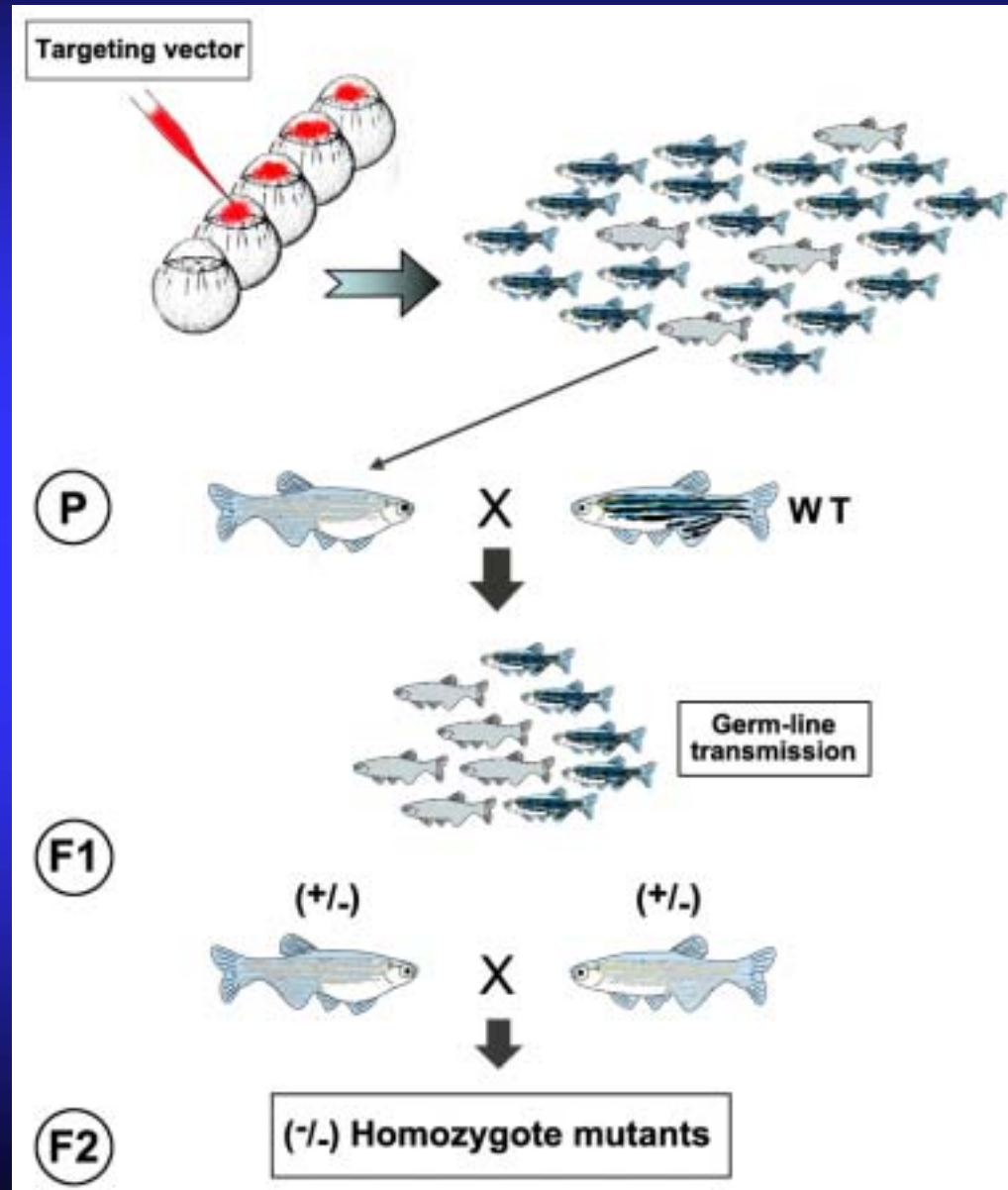


1. Transgene reporter in insertional mutagenesis
2. Biosensor for environmental pollutions
3. Screening of MCH antagonists (obesity)

Expression and activity of spGFP-MCH



Transposon-based mutagenesis



Thank you

