

**Genetic variation in TGF β 1 dependency
for vascular development**

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BROAD GOALS:

- **What are the nature of genetic variants that modify vascular development, vascular structure and integrity?**
- **Do these variants influence the severity of vascular diseases?**
- **Could these variants be novel targets for therapy?**

POLYMORPHISMS IN *TGFB1* ALTER DISEASE RISKS

Polymorphisms in *TGFB1* modify risk for cardiovascular and fibrotic diseases

Disease	Variant allele→ ↑ risk/severity	Circulating TGFβ1 Level	Population size (total-or affected/unaffected)	Reference
Myocardial infarction (MI)	Pro ²⁵	Low	561/629	Cambien <i>et al.</i> , (1996)
	Leu ^{10*}	Low	315/591	Yokota <i>et al.</i> (2000)
Hypertension/Blood pressure	Arg ²⁵	High	1190	Cambien <i>et al.</i> , (1996)
	Pro ^{10@}	High	2241	Yamada <i>et al.</i> (2002)
End-stage dilated cardiomyopathy	Pro ¹⁰ (not Arg ²⁵)	High	253/94	Holweg et al 2001
Coronary vasculopathy after heart transplant	Pro ¹⁰	High	252	Densem et al (2000)
Proliferative diabetic retinopathy	Arg ²⁵	High	73/172 NIDDMs	Beranek <i>et al.</i> (2002)
Arthritis	Leu ¹⁰	Low	155/110	Sugiura <i>et al.</i> , (2002)
Systemic sclerosis	Pro ¹⁰	High	149/147	Crilly <i>et al.</i> , (2002)
Hepatic fibrosis	Pro ¹⁰	High	48/97	Gewaltig <i>et al.</i> , (2002)
Cystic Fibrosis severity	Pro ¹⁰	High	171	Arkwright <i>et al.</i> (2000)

What other genetic variants might interact with *TGFB1* to modify disease risk?

Why use the mouse?

Inbred mice genetically simple

Show considerable inter-strain genetic variation

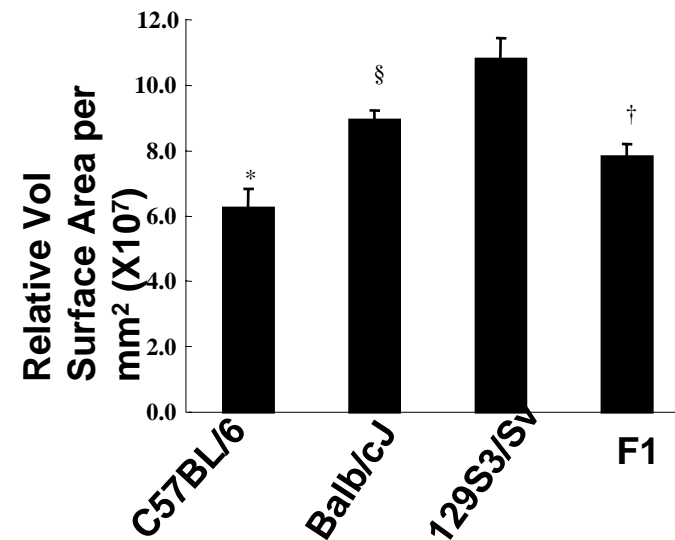
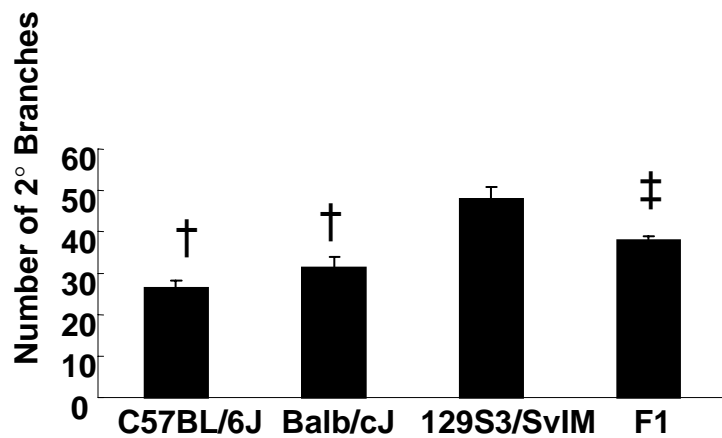
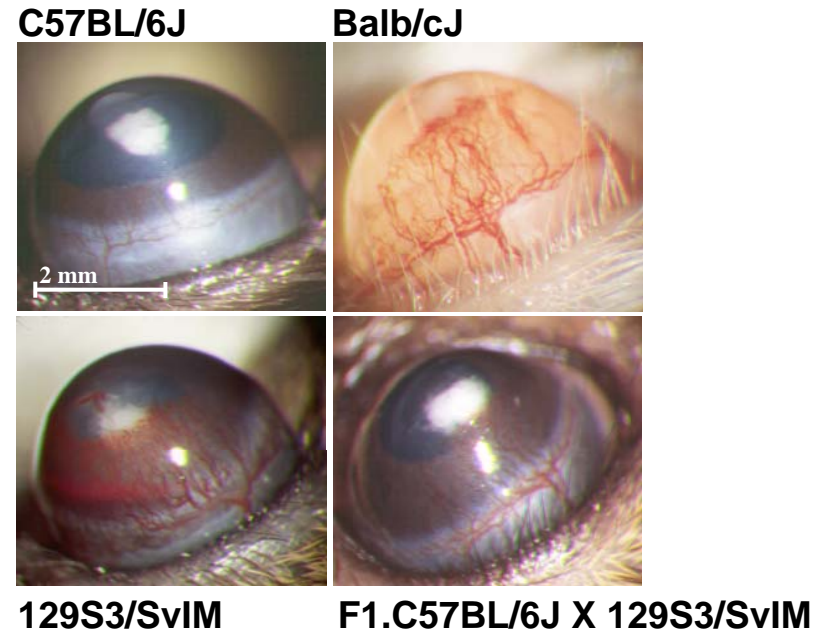
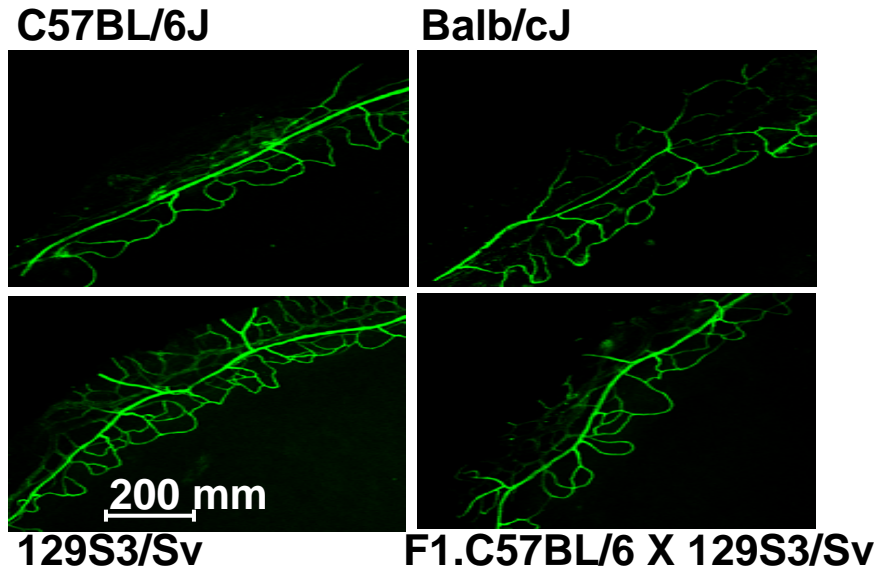
Easy to breed

Can study genetic interactions - difficult in humans

Modifiers found in mice generally conserved in human

**Innate variation in vascular architecture
between inbred mouse strains:
Limbal vasculature of the eye**

**Strain variation in FGF-induced
adult corneal angiogenesis**



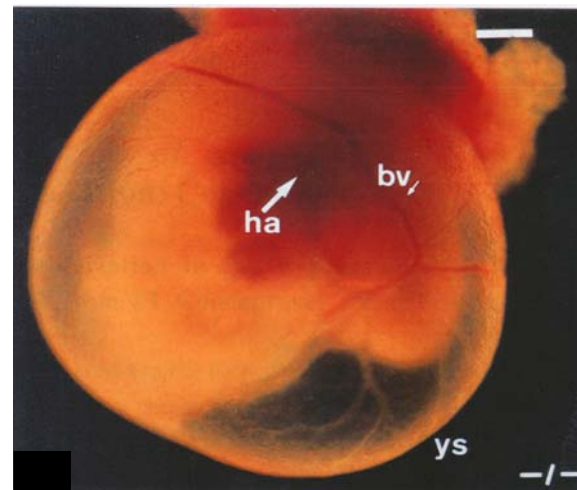
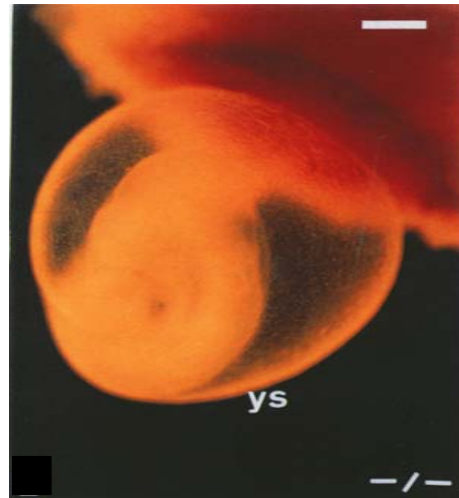
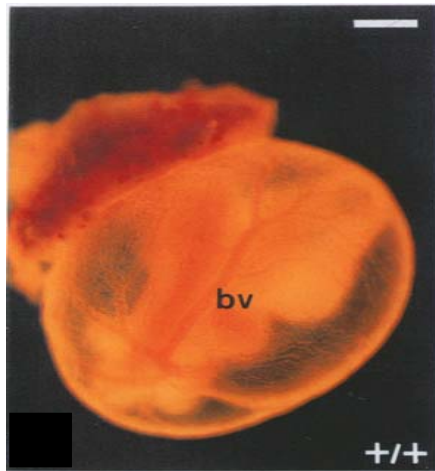
Chan et al. (2004) IOVS

Most *Tgfb1*^{-/-} mice die from defects in vascular development

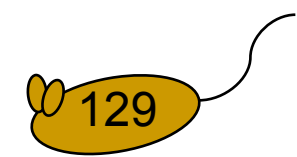
Dickson et al (1995) Development

Genetic background determines *Tgfb1* developmental redundancy

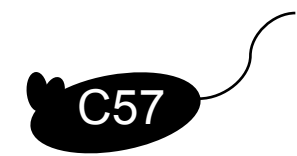
Bonyadi et al (1997)
Nat. Genet.



15% death (BC4)



70% death (BC4)



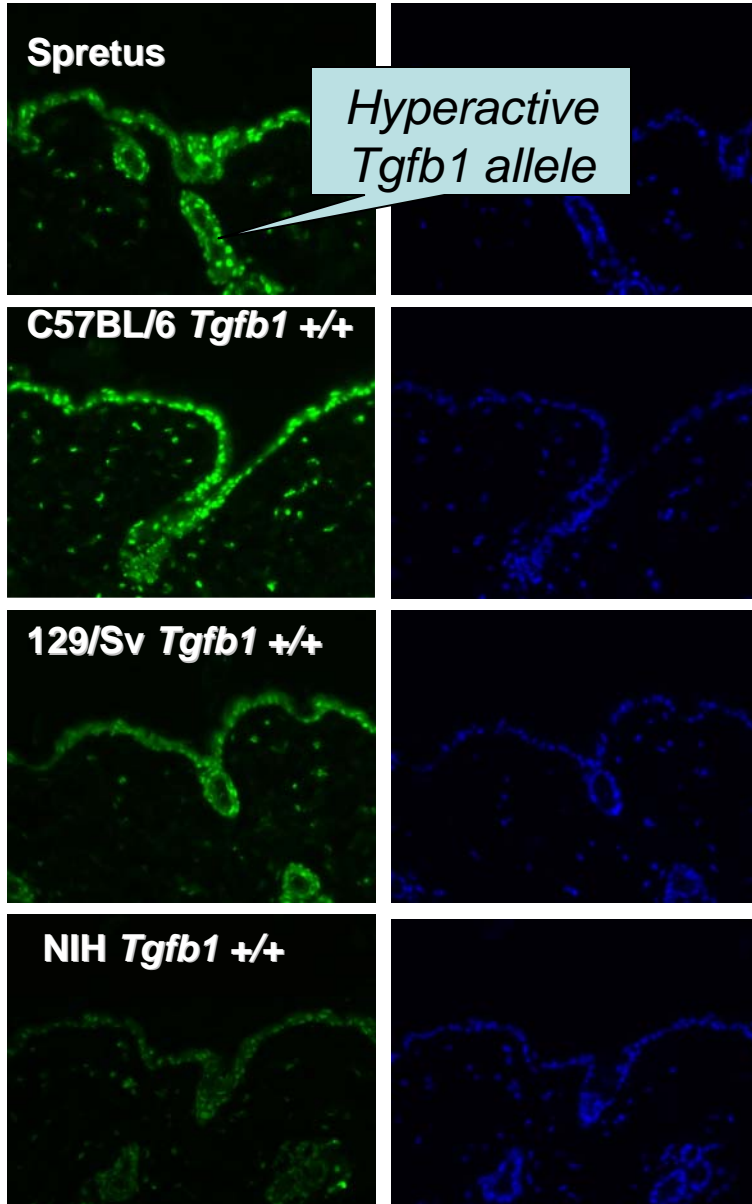
100% death

Mouse strains innate variation in levels of basal TGFβ signaling activity

P-Smad2 levels in skin :

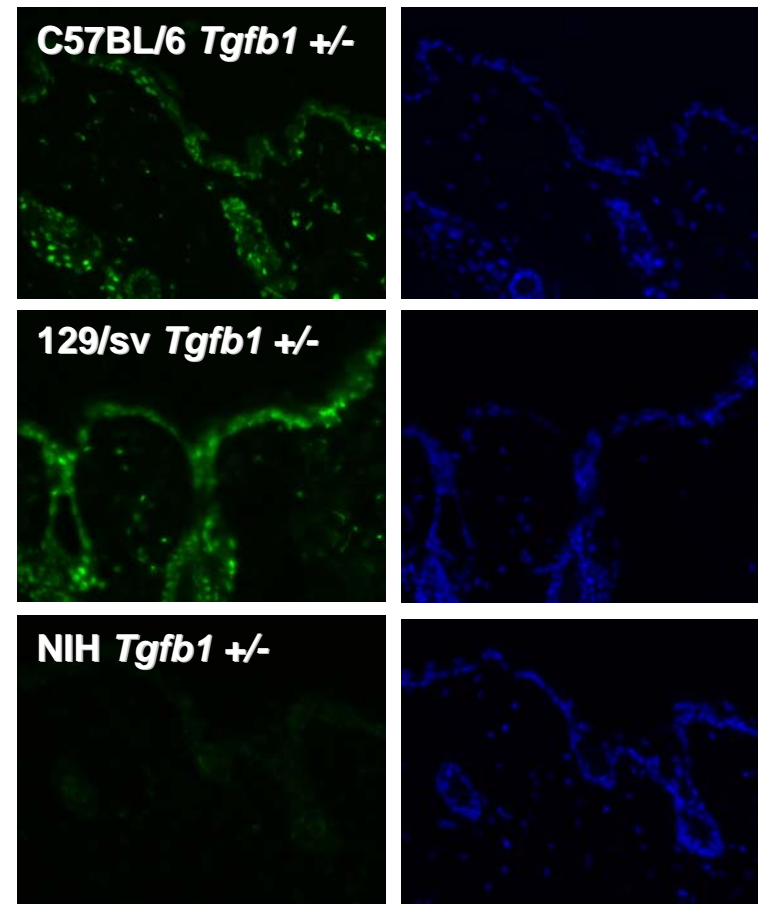
P-Smad2

DAPI



P-Smad2

DAPI



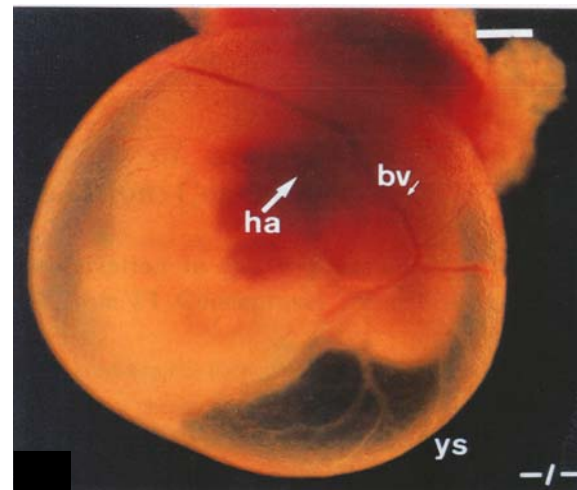
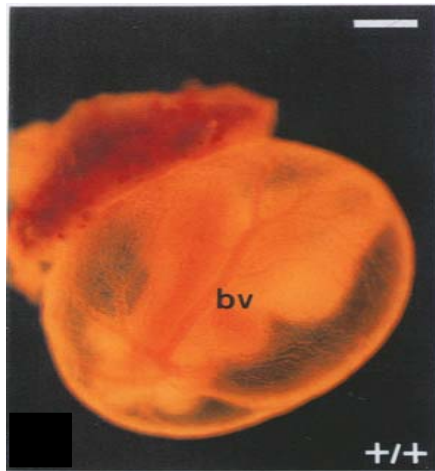
Mao et al. 2006 PNAS

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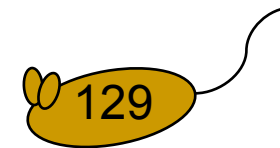
Dickson et al (1995) *Development*

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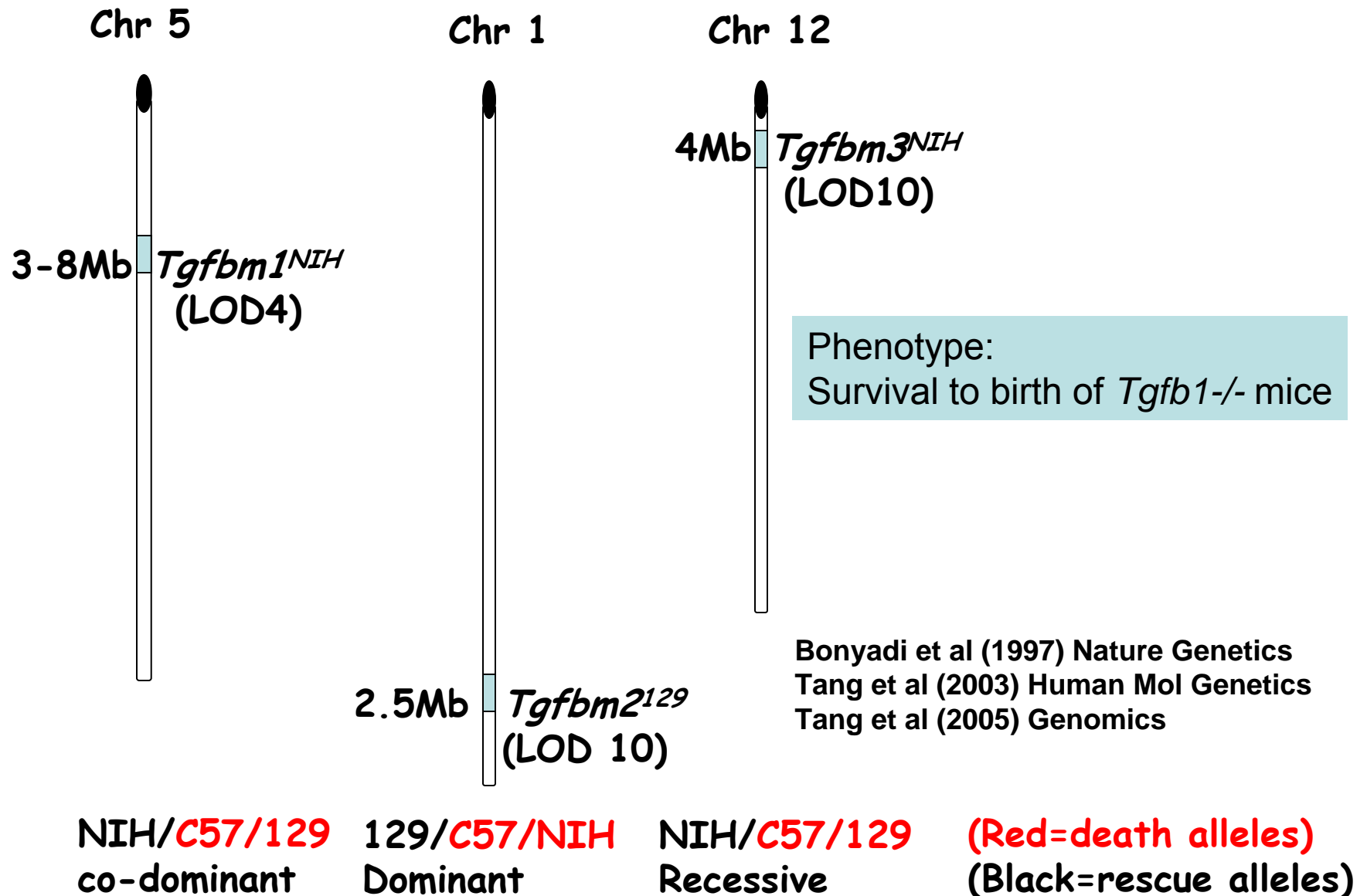


70% death (BC4)



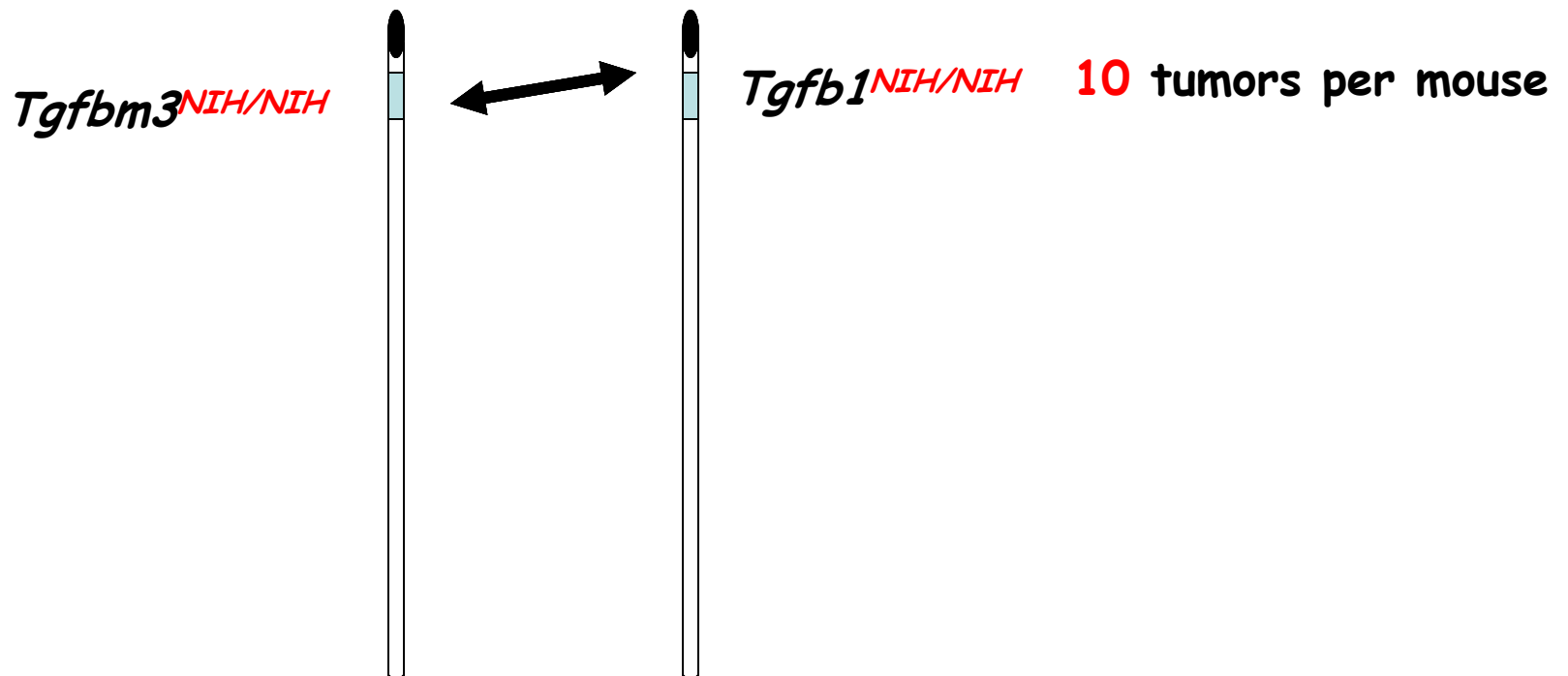
100% death

Classical genetic mapping techniques identified three *Tgfb1* modifier loci that determine the dependence of vascular development on *Tgfb1*



Tgfbm3 independently identified as a *Tgfb1*-interacting locus (*Skts15*)
in an unbiased genome-wide scan for skin tumor susceptibility loci

Mao et al. 2006 PNAS



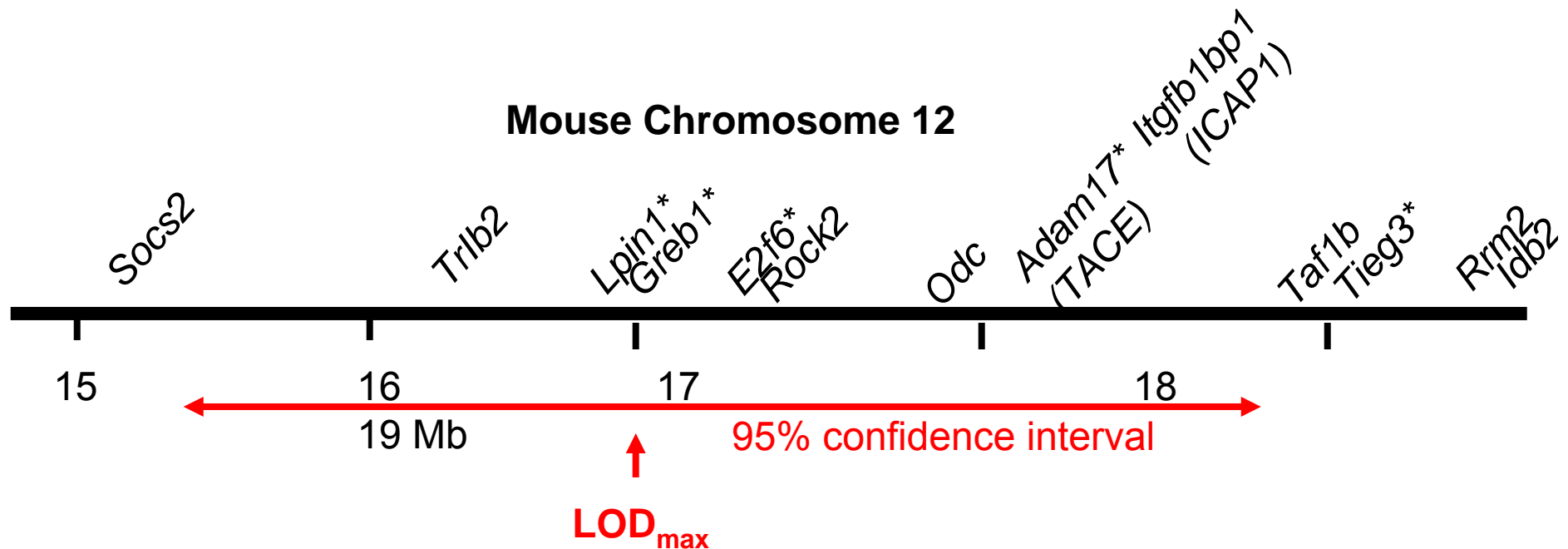
High tumor susceptibility only when both
Tgfb1 and *Skts15/Tgfbm3* are homozygous NIH

Such genetic interaction may MASK effect of single
gene association studies

Progressing from Locus to Gene

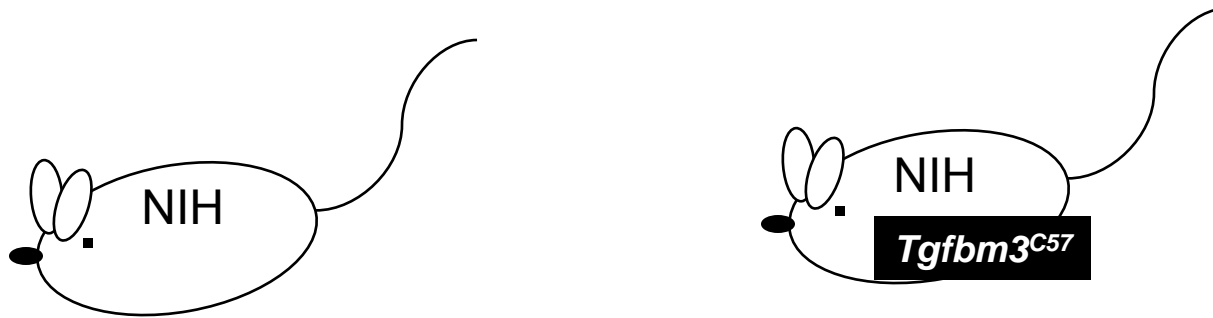
The TGF β 1-interacting locus, *Tgfbm3*

- The 4Mb genomic region of *Tgfbm3* contains functionally-related genes involved in cell proliferation, migration and apoptosis - some known to be influenced by TGF β (Tang et al *Genomics* 2005).
- All candidate genes sequenced: Some alleles several variant proteins.

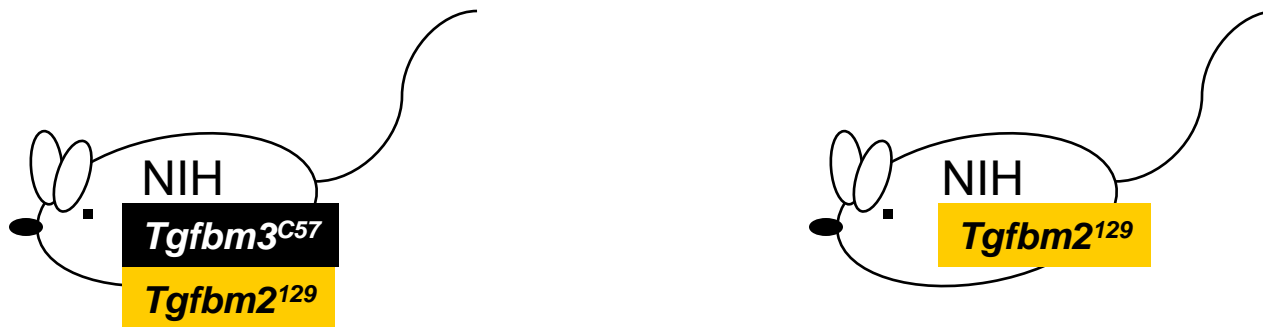


Progressing from Locus to Gene

Generation of **panel** of NIH mice congenic for *Tgfbm3*^{C57} by repeated backcrossing (N>5)



Discovery of contaminant 129 genomic DNA at *Tgfbm2* chromosome 1
Contaminant DNA derived from original *Tgfb1*^{+/-} ES cells (1.1Mb)
Implies biological selection through *Tgfb1*^{+/-} heterozygous advantage

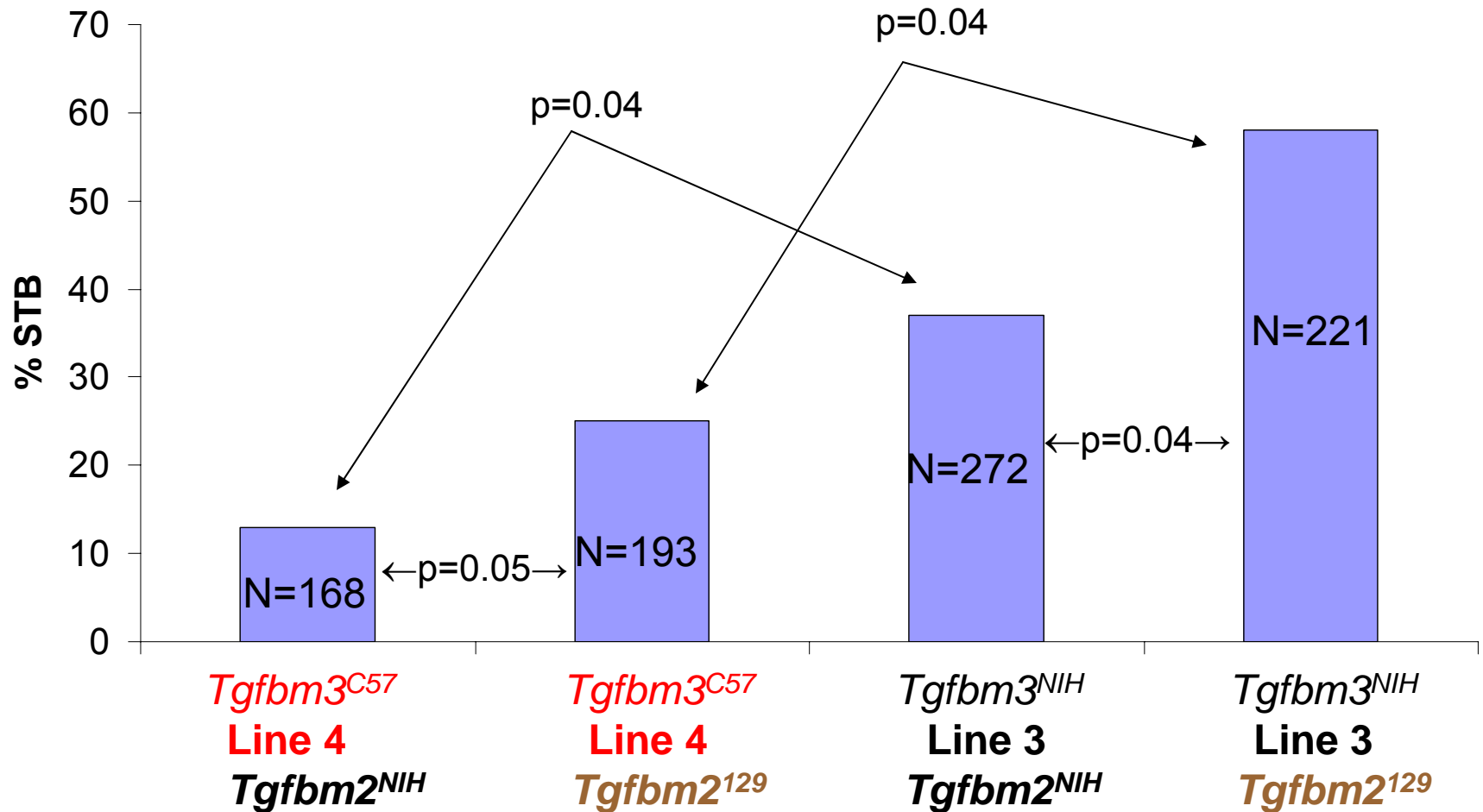


Acquisition of four types of NIH congenic:

expect ↑ survival due to *Tgfbm2*¹²⁹
expect ↓ survival due to *Tgfbm3*^{C57}

Line 3 and Line 4 NIH. *Tgfbm3*^{C57} congenic mice map major component of *Tgfbm3* effect to very small interval

NIH congenic *Tgfb1*^{-/-} survival to birth rates



Conclusions:

Genetic variation in levels of TGF β 1 signaling between mouse strains (p-SMAD2)

Differential *Tgfb1* gene expression \rightarrow some but not all of this effect

Three loci that determine *Tgfb1* redundancy for vascular development
mapped by genetic linkage

Tgfbm3 independently identified as a *Tgfb1*-interacting skin tumor susceptibility locus
In unbiased screen

Tgfbm1, *Tgfbm2* and *Tgfbm3* validated using congenic mice

Tgfbm2 and *Tgfbm3* mapped to $< 1.1\text{Mb}$ using congenic mice

RELEVANCE TO HUMAN DISEASE?
Do the *TGFBM*'s interact with *TGFB1*
to influence vascular disease?
Targets for therapy?

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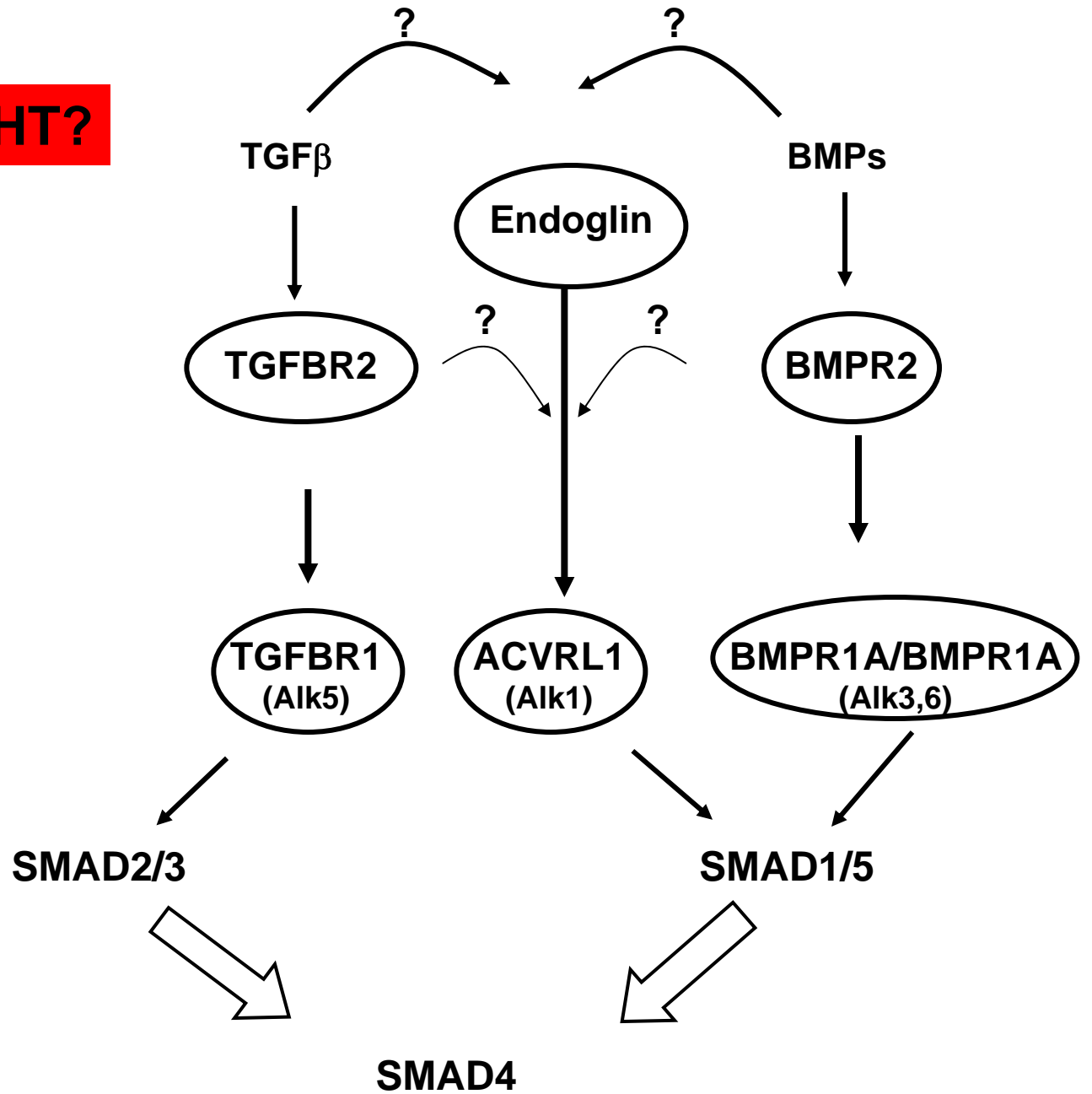
J. Mao PhD

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RELEVANCE TO HHT?



RELEVANCE TO HHT?

Clinical manifestation of *ENG* and *ACVRL1* mutations (vascular) distinct from *TGFB1*, *TGFBR1* and *TGFBR2* mutations (ECM, connective tissue).

Some clinical overlap between *ENG* and *ACVRL1* mutations and those in BMP signaling pathway

Is there interaction between $TGF\beta 1$ and endoglin/ *Acvrl1* pathways?

