Genetic variation in TGFβ1 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

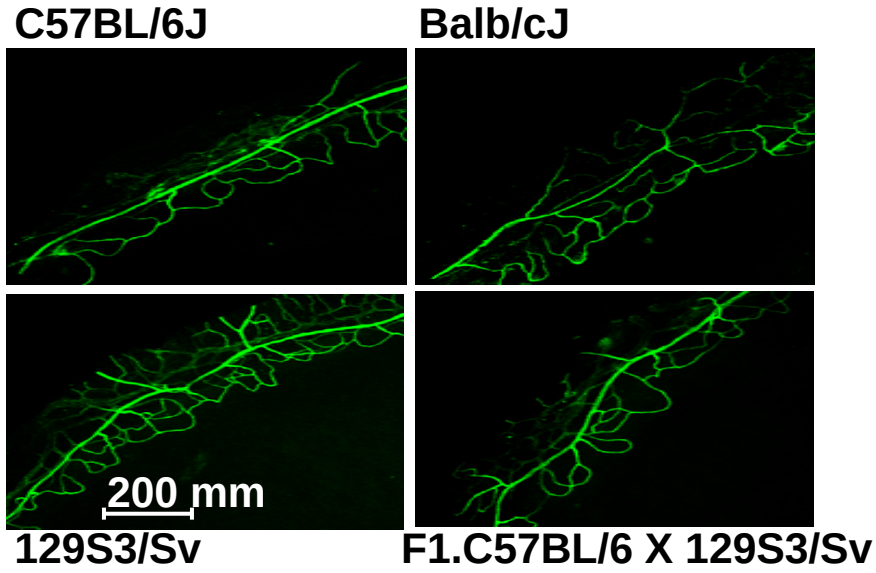
Disease	Variant allele→ ↑ risk/severity	Circulating TGFβ1 Level	Population size (total-or affected/unaffected)	Reference
Myocardial infarction (MI)	Pro^{25}	Low	561/629	Cambien *et al.*, (1996)
	Leu^{10*}	Low	315/591	Yokota *et al.* (2000)
Hypertension/Blood pressure	Arg^{25}	High	1190	Cambien *et al.*, (1996)
	$Pro^{10@}$	High	2241	Yamada *et al.* (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 *et al.* (2002)
Arthritis	Leu^{10}	Low	155/110	Sugiura *et al.*, (2002)
Systemic sclerosis	Pro^{10}	High	149/147	Crilly *et al.*, (2002)
Hepatic fibrosis	Pro^{10}	High	48/97	Gewaltig *et al.*, (2002)
Cystic Fibrosis severity	Pro^{10}	High	171	Arkwright *et al.* (2000)

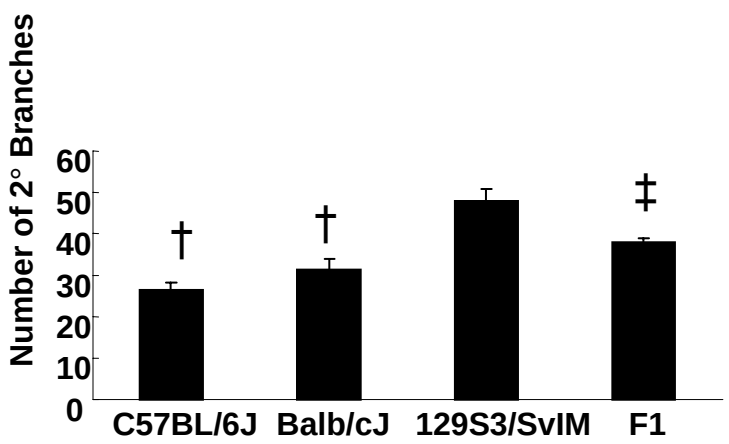
What other genetic variants might interact with *TGFB1* to modify disease risk?

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

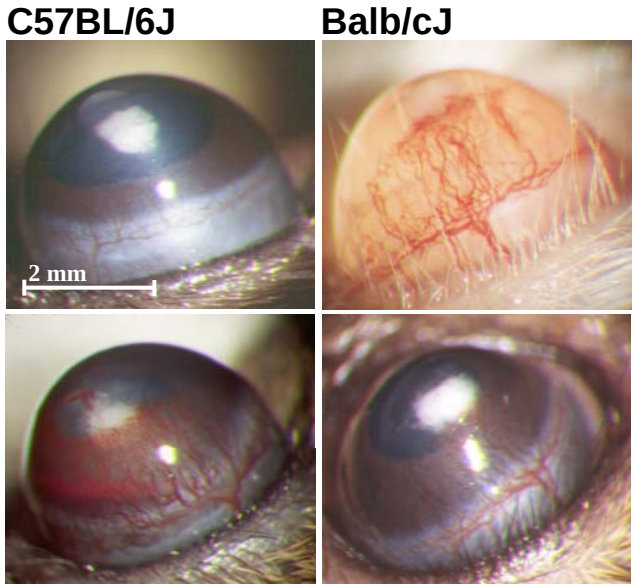
Innate variation in vascular architecture between inbred mouse strains: Limbal vasculature of the eye

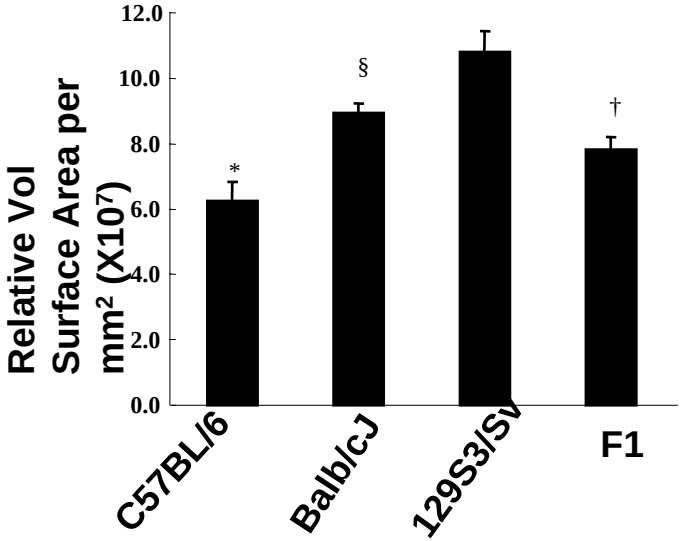




Chan et al. (2004) IOVS

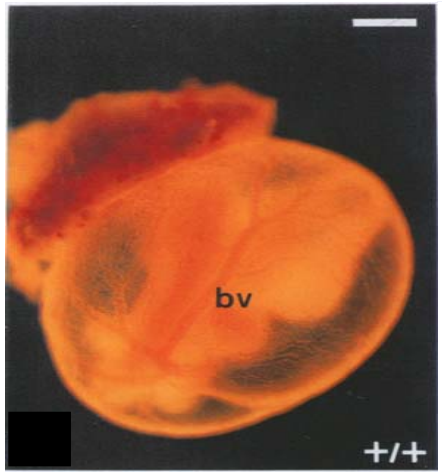
Strain variation in FGF-induced adult corneal angiogenesis

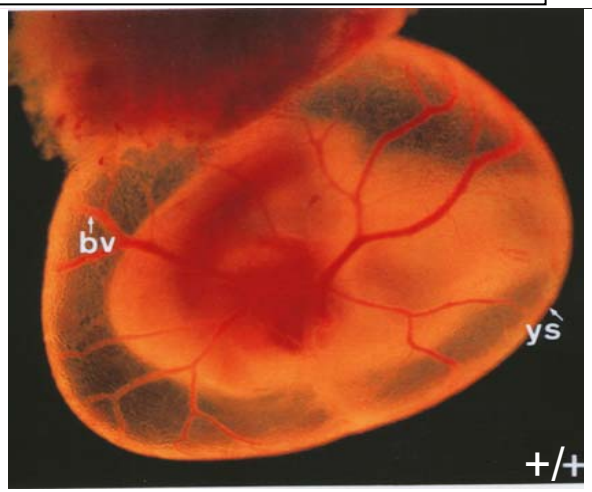


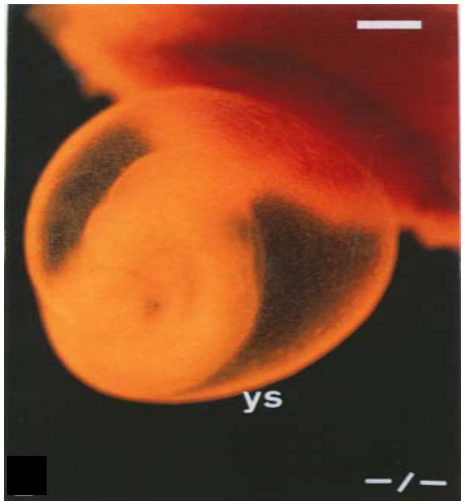


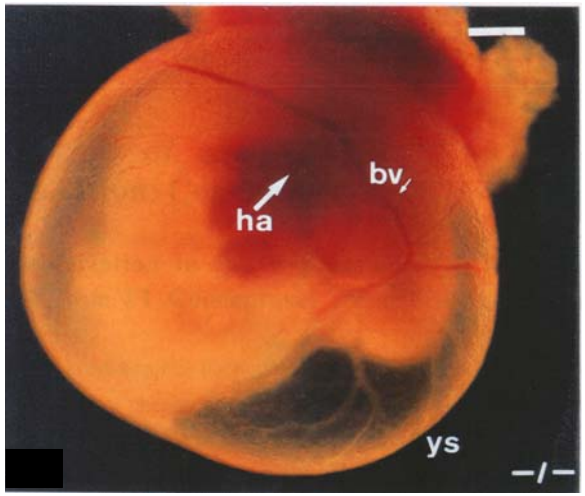
Most *Tgfb1-/-* mice die from defects in vascular development

Dickson et al (1995) Development



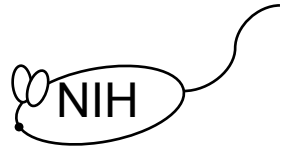




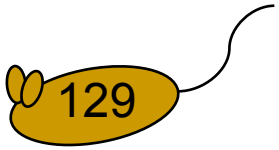


Genetic background determines *Tgfb1* developmental redundancy

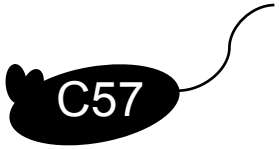
Bonyadi et al (1997) Nat. Genet.



15% death (BC4)



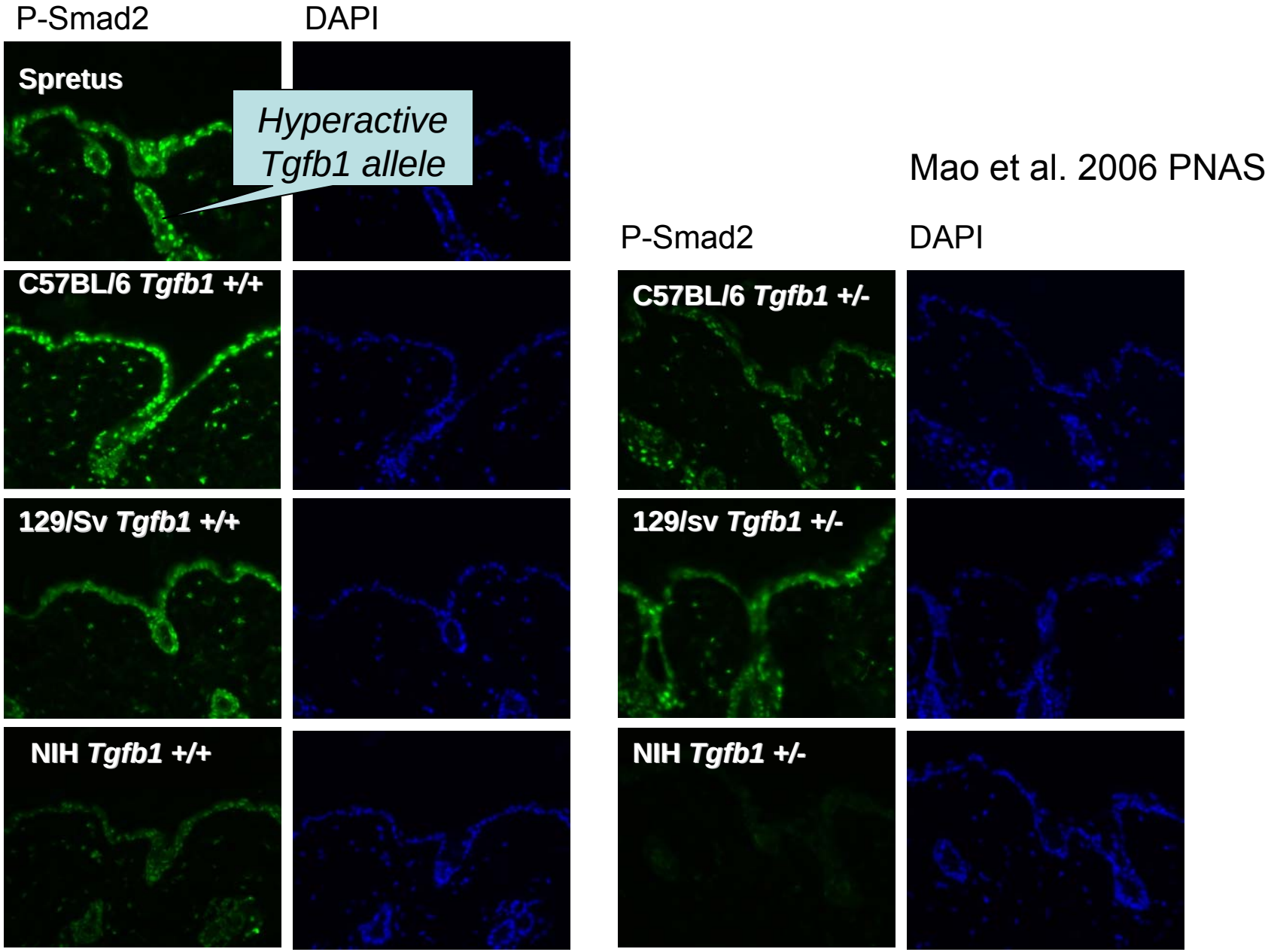
70% death (BC4)



100% death

Mouse strains innate variation in levels of basal TGFβ signaling activity

P-Smad2 levels in skin :



Mao et al. 2006 PNAS

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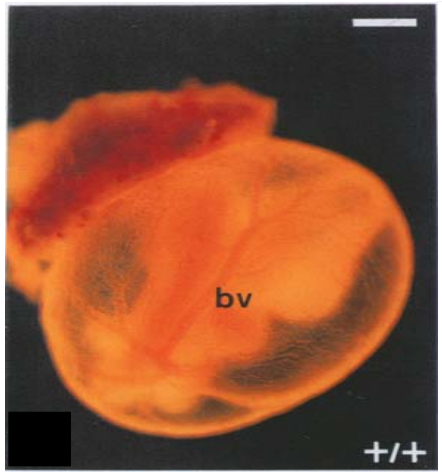

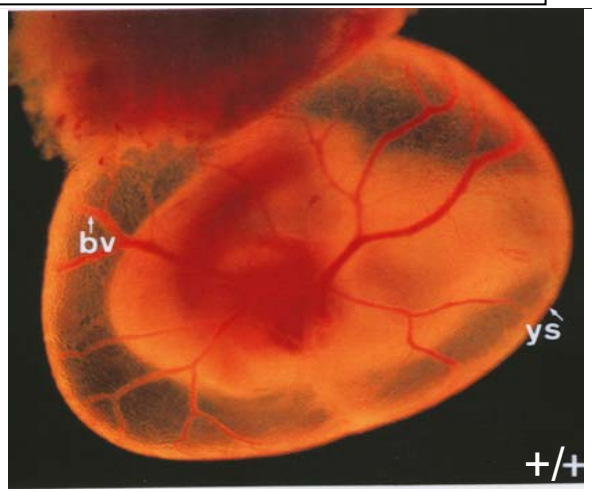

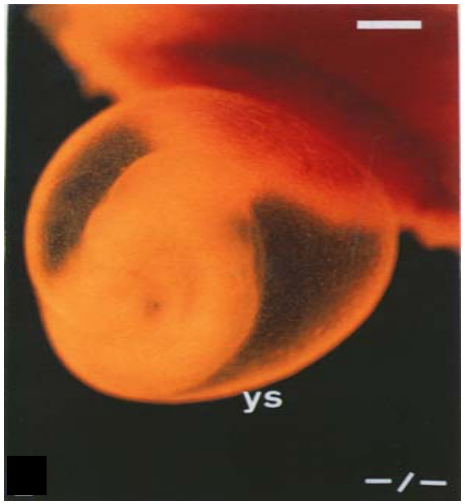

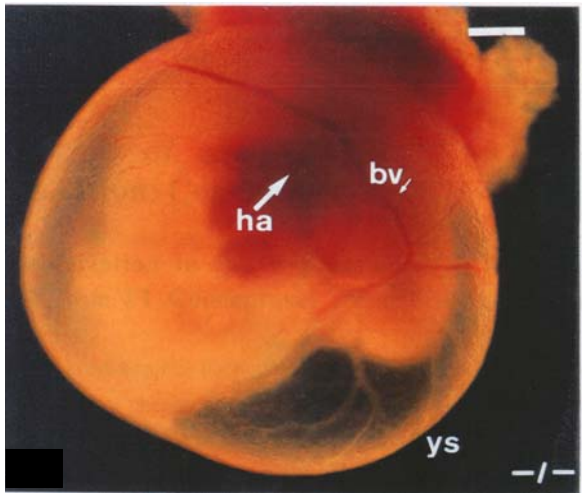

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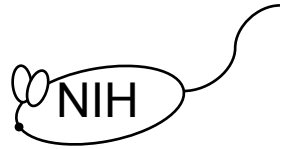

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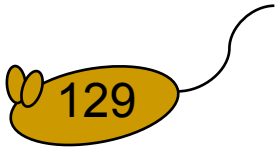

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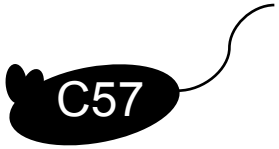
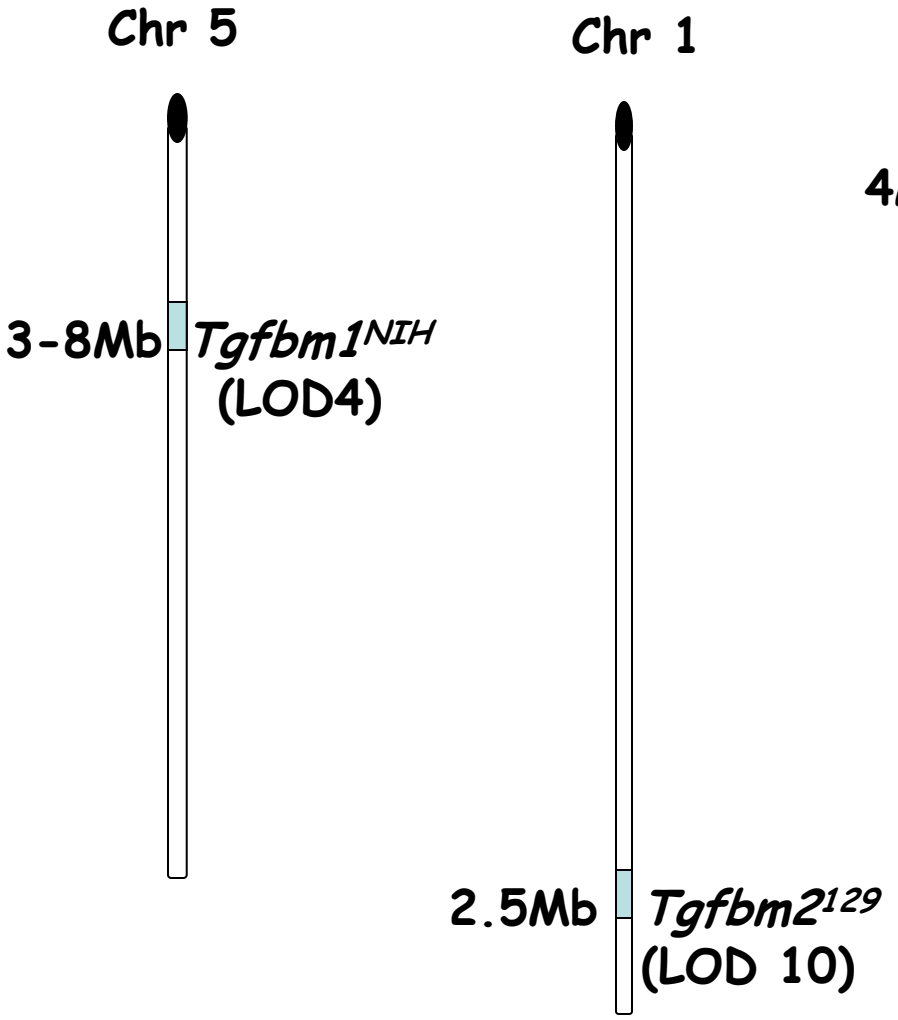


100% death

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



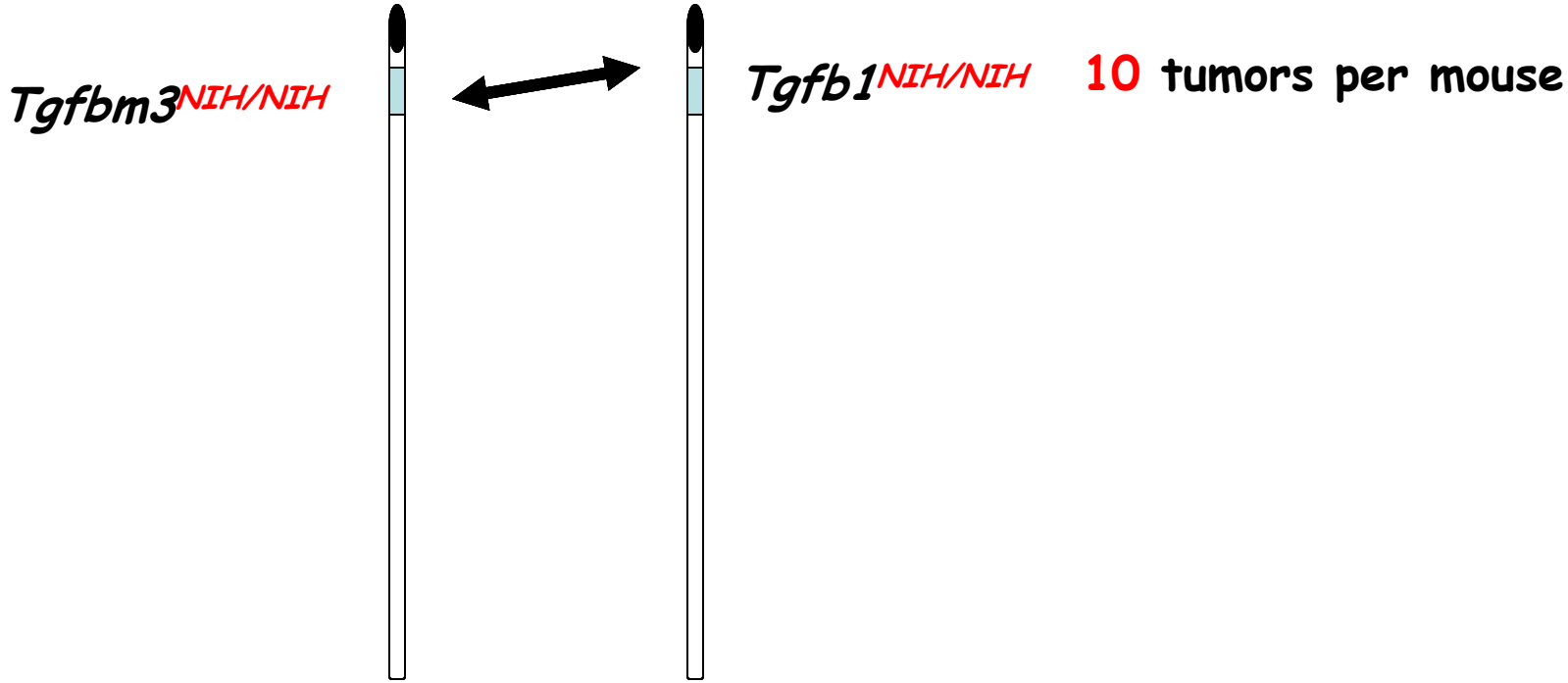
Phenotype:
Survival to birth of *Tgfb1-/-* mice

Bonyadi et al (1997) Nature Genetics
Tang et al (2003) Human Mol Genetics
Tang et al (2005) Genomics

NIH/C57/129	129/C57/NIH	NIH/C57/129	(Red=death alleles)
co-dominant	Dominant	Recessive	(Black=rescue alleles)

***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



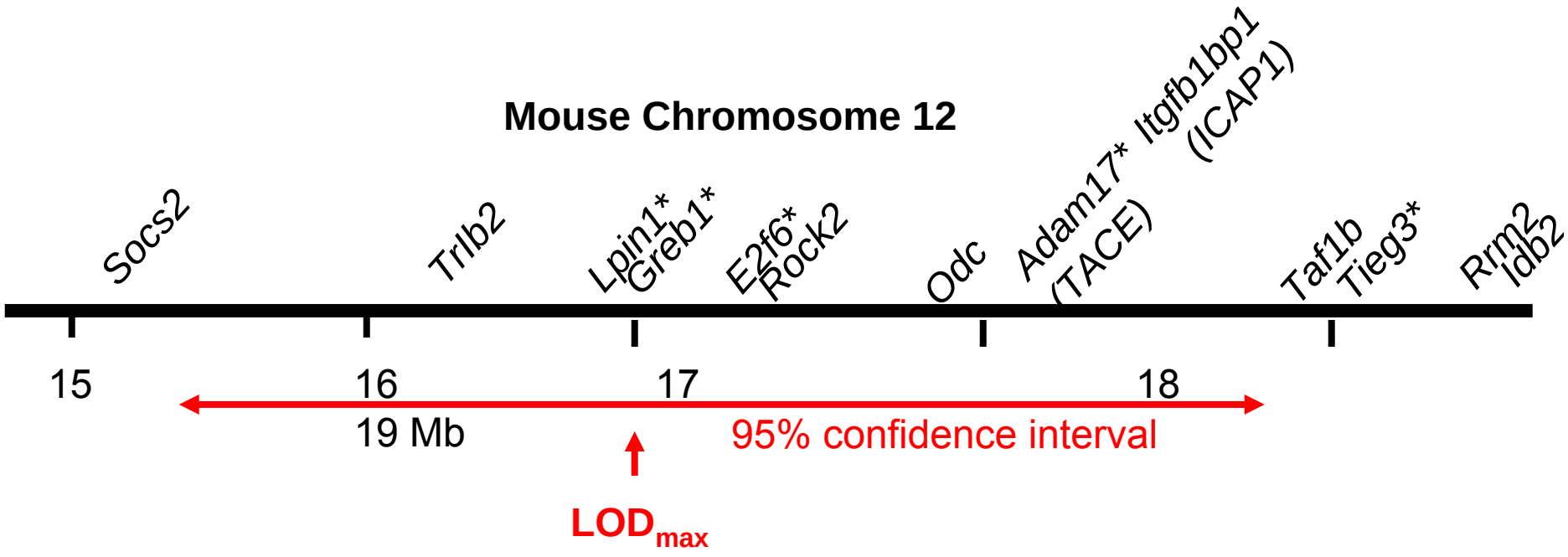
High tumor susceptibility only when <u>both</u>
***Tgfb1* and *Skts15/Tgfbm3* are homozygous NIH**

Such genetic interaction may MASK effect of single gene association studies

Progressing from Locus to Gene

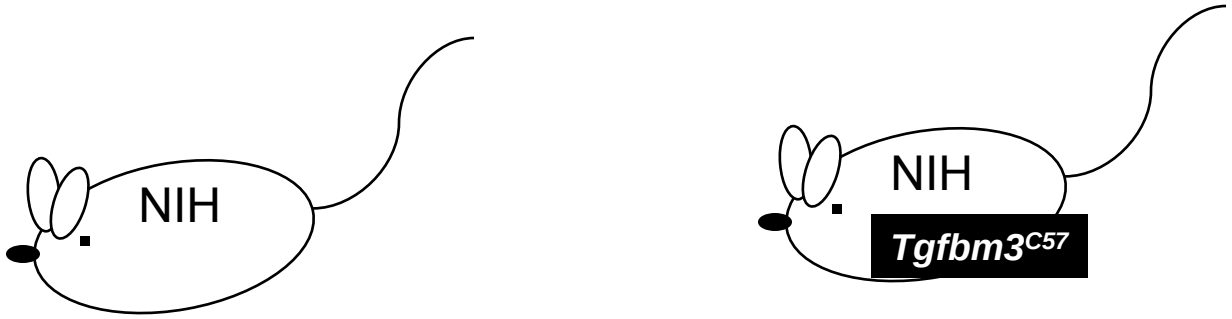
The TGFβ1-interacting locus, *Tgfbm3*

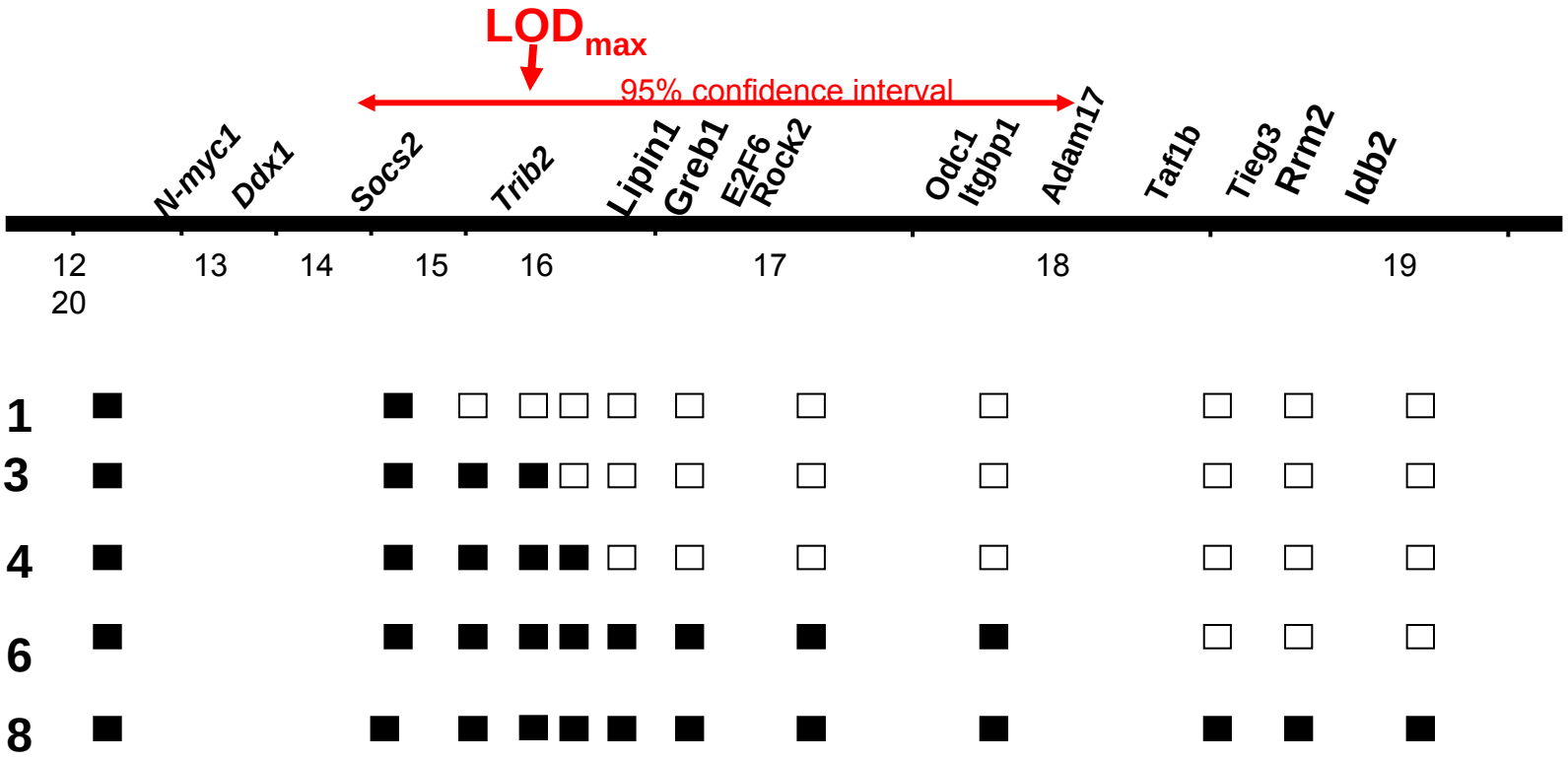
- The 4Mb genomic region of *Tgfbm3* contains functionally-related genes involved in cell proliferation, migration and apoptosis
  - some known to be influenced by TGFβ
  (Tang et al *Genomics* 2005).
- All candidate genes sequenced: Some alleles several variant proteins.



Progressing from Locus to Gene

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





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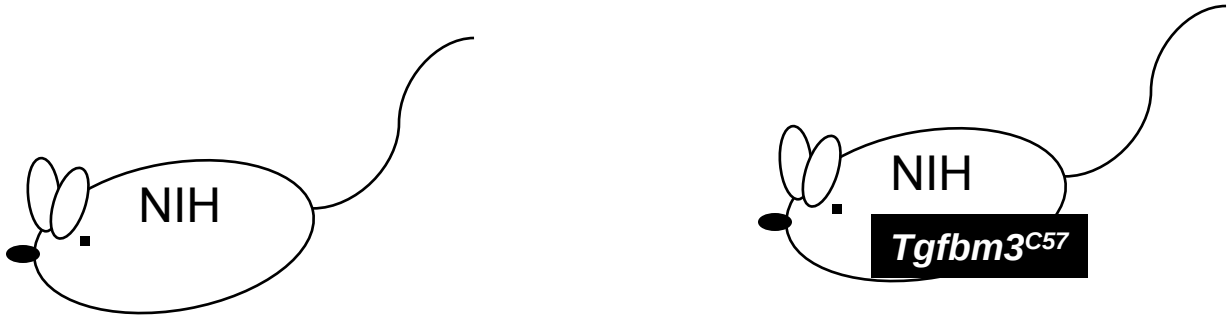
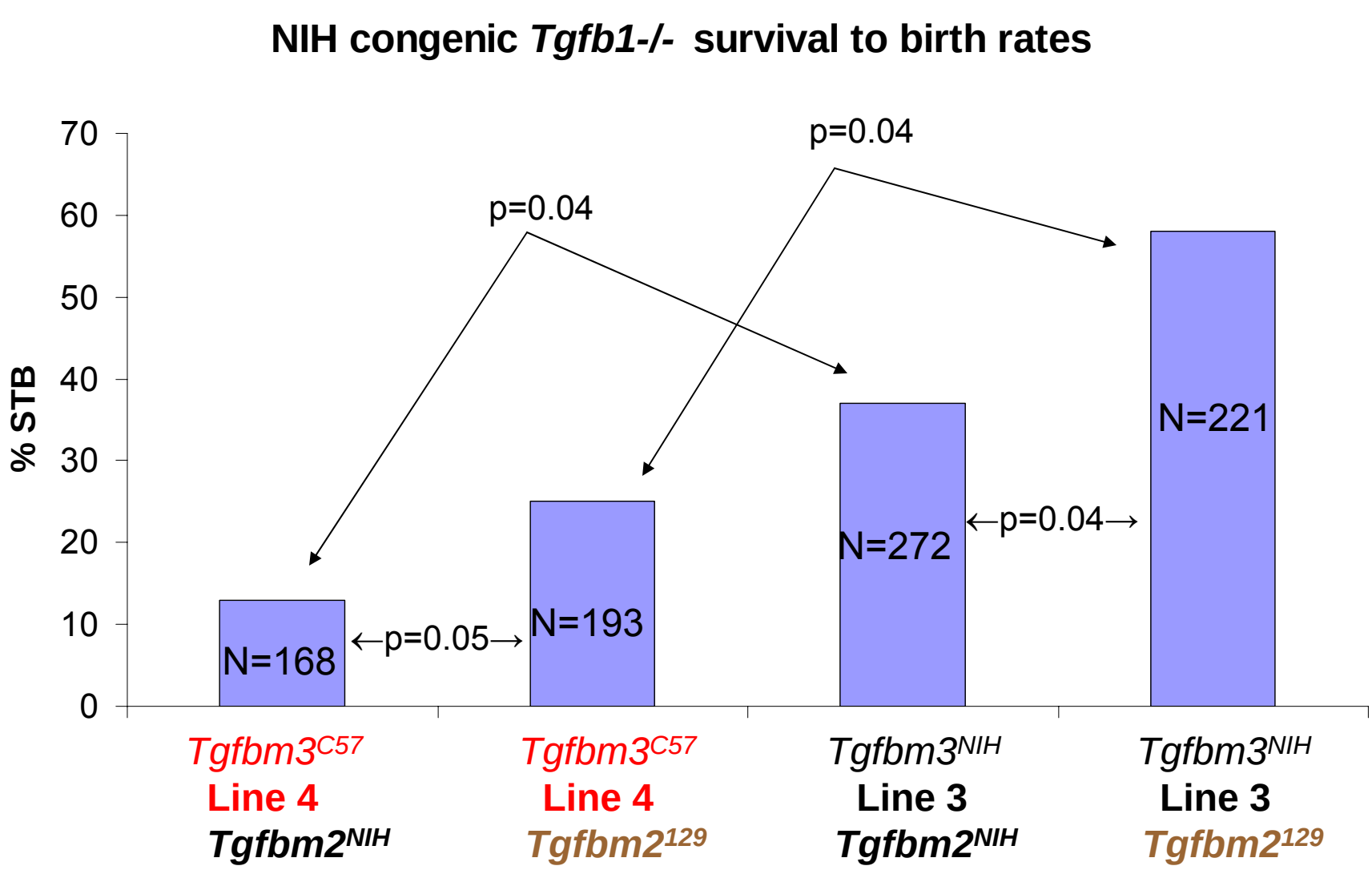


Discovery of contaminant 129 genomic DNA at *Tgfbm2* chromosome 1
Contaminant DNA derived from original *Tgfb1*+/- ES cells (1.1Mb)
Implies biological selection through *Tgfb1*+/- heterozygous advantage

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



Conclusions:

Genetic variation in levels of TGFβ1 signaling between mouse strains (p-SMAD2)

Differential *Tgfb1* gene expression → 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* < *1.1Mb* 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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Kyeong Sook Lee PhD
Marie Lee PhD
Frederic Clermont PhD
Elise Saunier PhD
Minh Thu Luu
Mamie Nakajima Higgins

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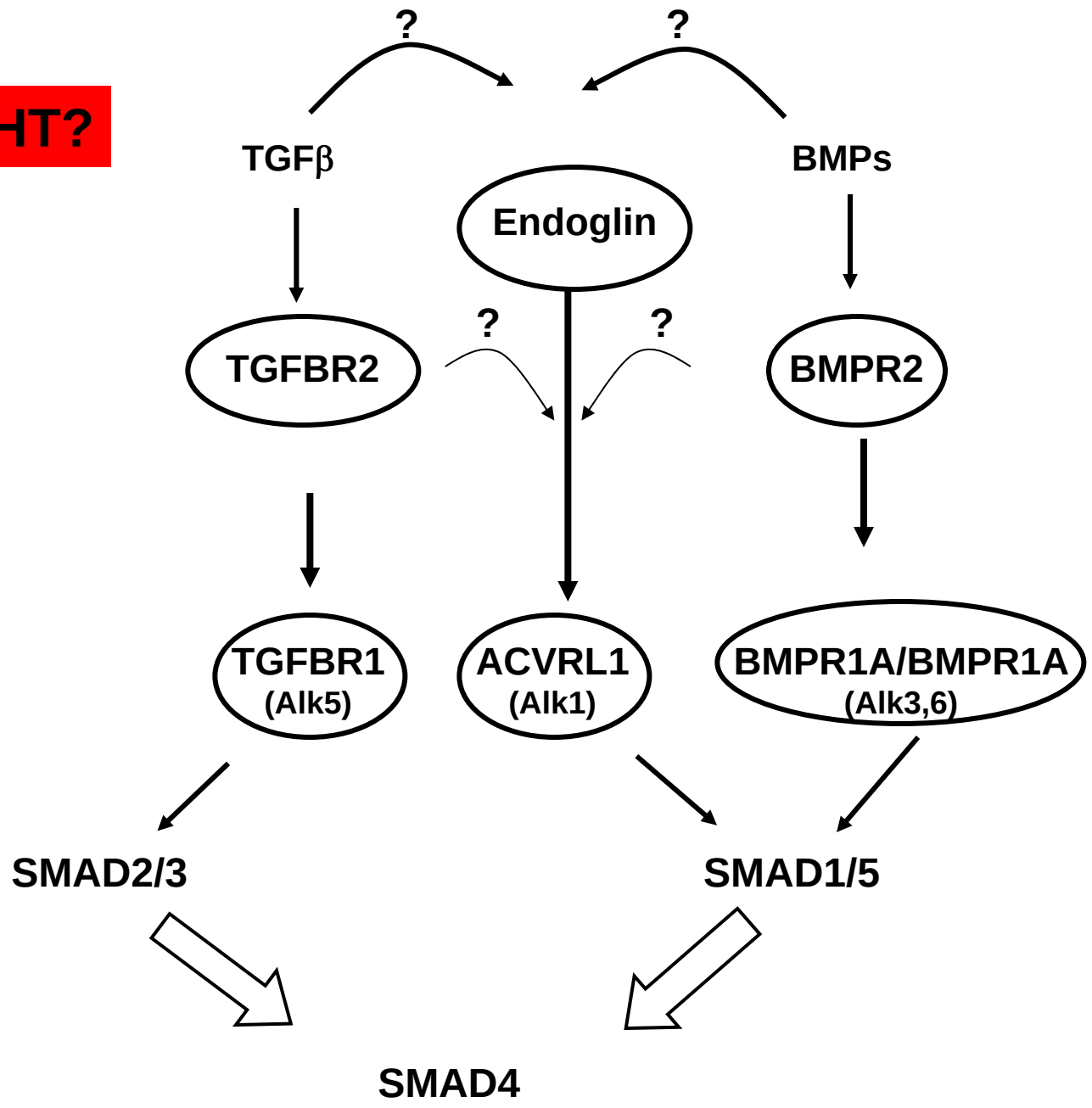
Cancer Center

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 TGFβ1 and endoglin/ Acvrl1 pathways?