Deciphering the genetic barcode of cancer susceptibility using mouse models of astrocytoma, MPNST, and NF1

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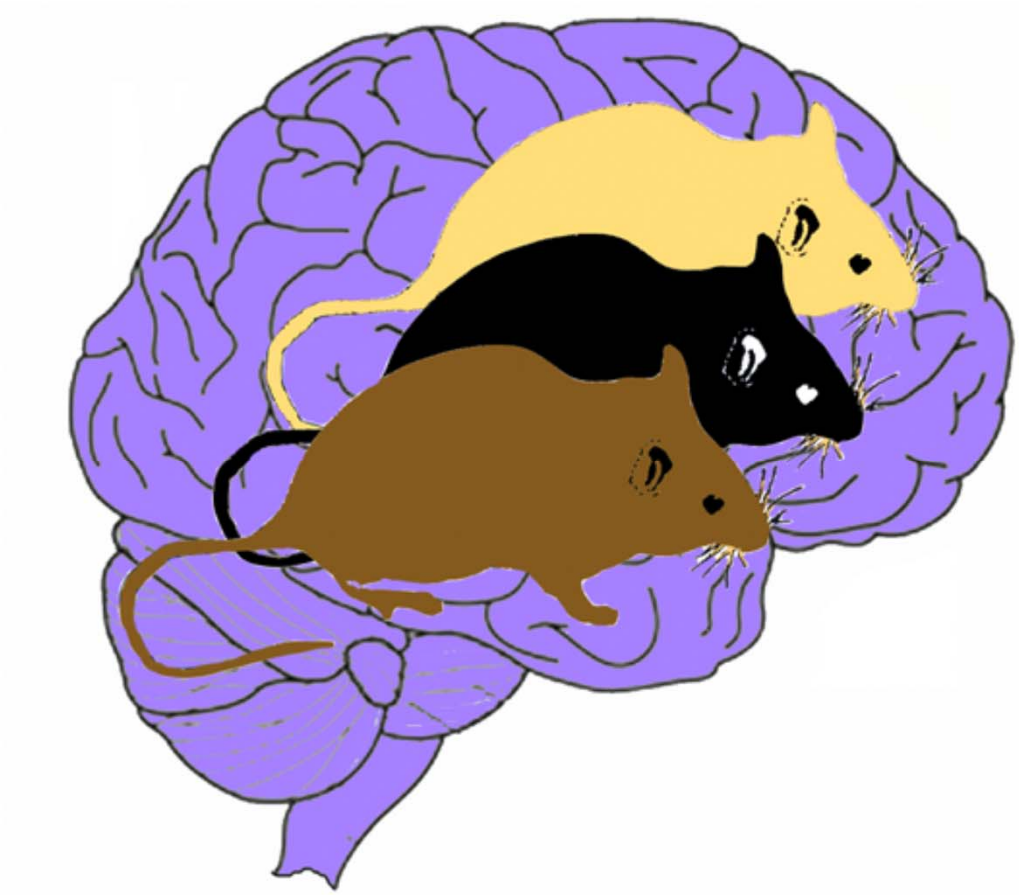
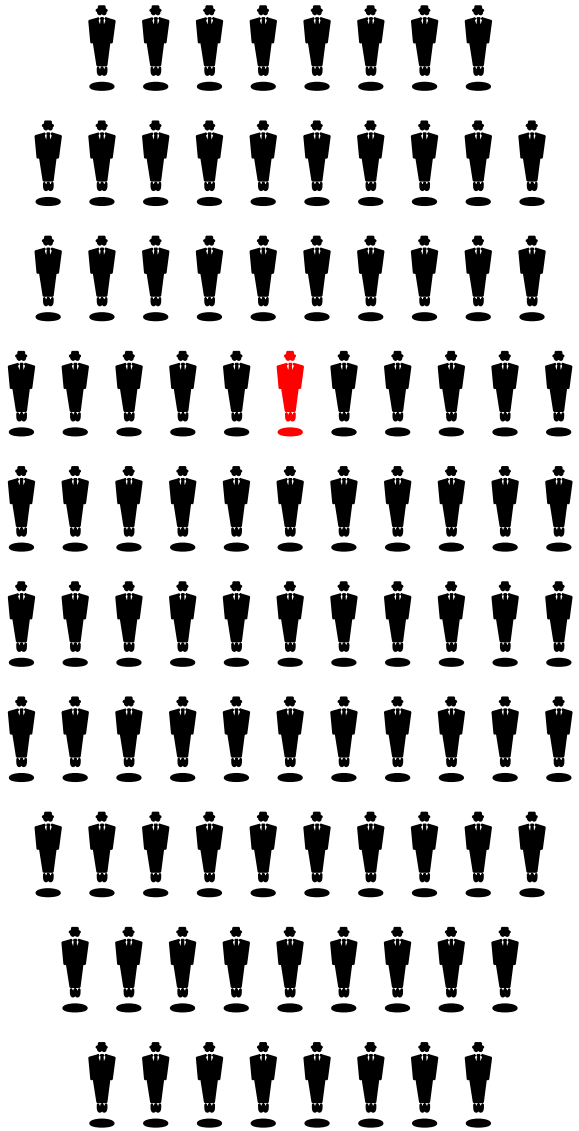


In any population, why do certain individuals develop cancer?



- **Genetic Predisposition**
- **Diet/Environmental Factors**
- **Sporadic Events (bad luck!)**

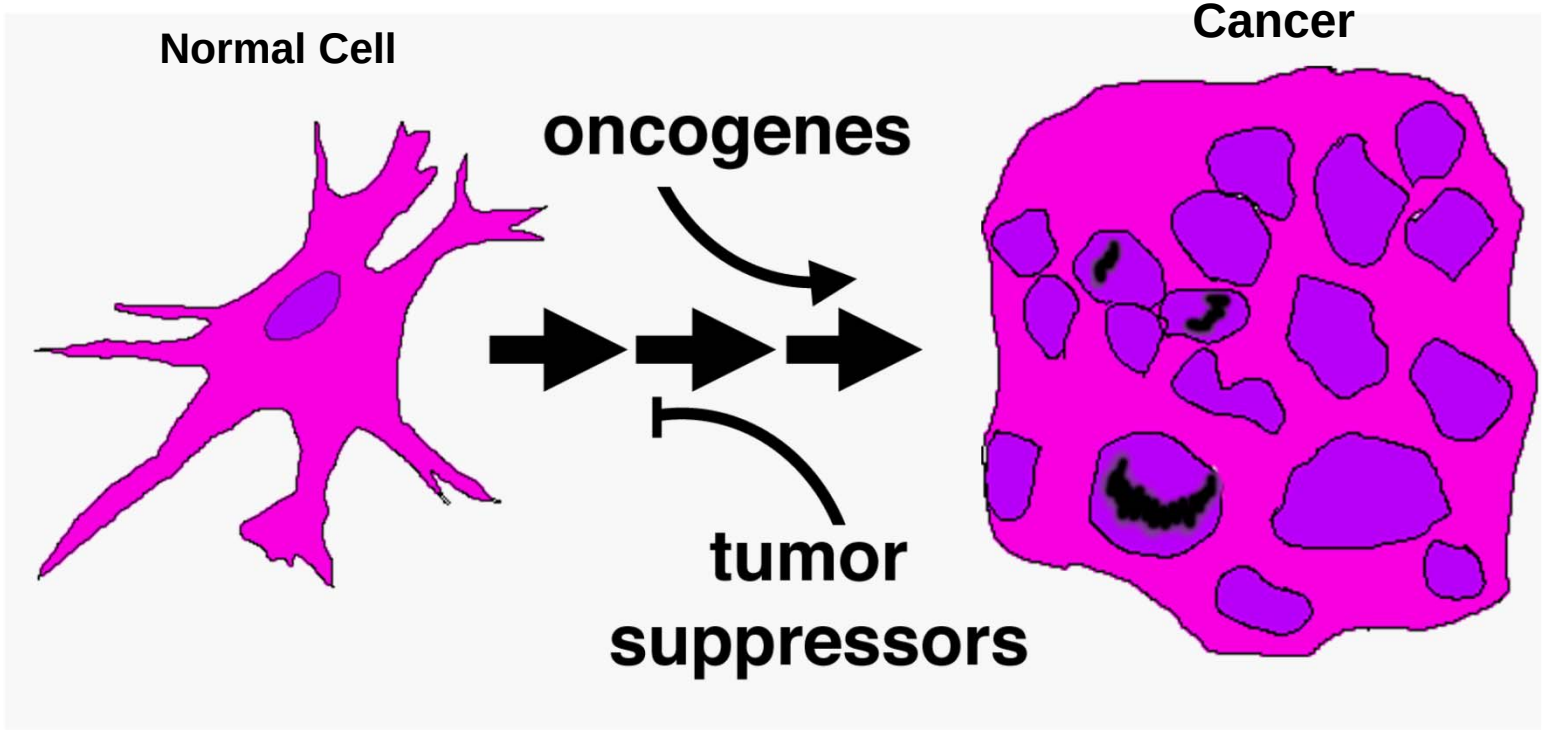
Combinatorial effects within a population can give rise to rare cancer

- **Genetic Predisposition**
- **Diet/Environmental Factors**
- **Sporadic Events (bad luck!)**

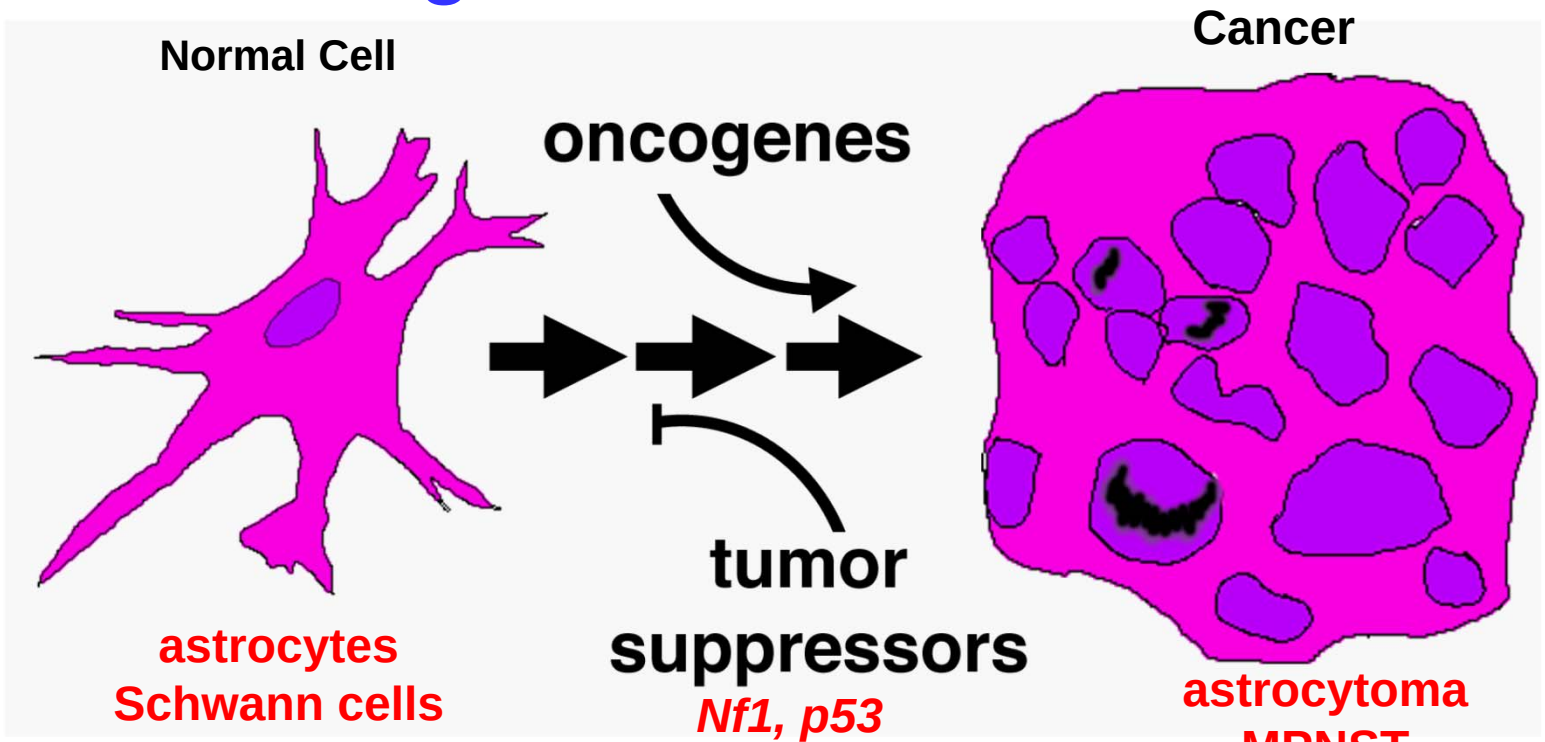
→ **Understand basic biology of cancer**

→ **Apply what we learn to developing therapy**

Cancer is a process of accumulating genetic mutations

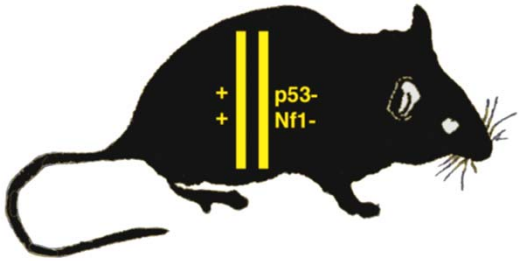


Cancer is a process of accumulating genetic mutations



Cancer is a disease of gene alteration

High-penetrance cancer genes
(rare mutations with powerful effects)
e.g. p53, Rb1, APC, Nf1, H-Ras, myc



***Nf1;p53cis* mouse model of**
Neurofibromatosis type 1

Low-penetrance modifier genes
(common variants with partial effects)

e.g. *Pla2g2a, STK15, Ptprj, Sipa1*

Imprinted genes/Epigenetic effects
(genome modifications inherited from one parent or the other)
Evidence in rhabdomyosarcoma, oligodendroglioma, and paraganglioma

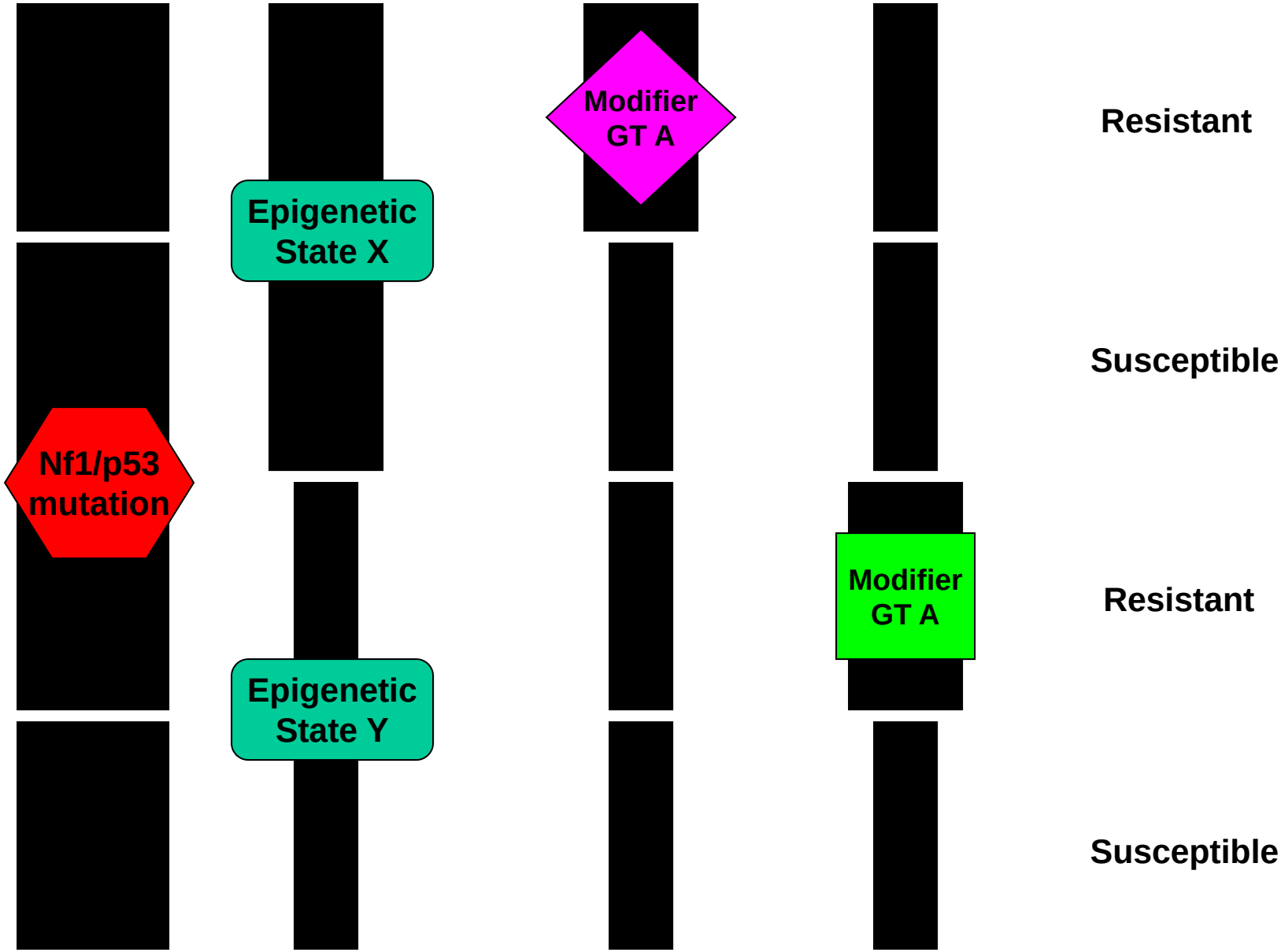
The Genetics of Cancer Susceptibility

Cancer Risk

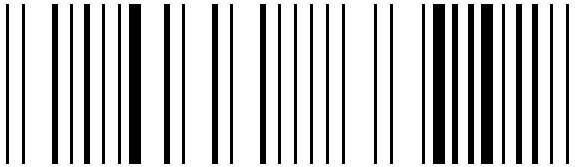
High-penetrance tumor suppressors

- **Resistant?**
- **Susceptible?**
- **Resistant?**
- **Susceptible?**

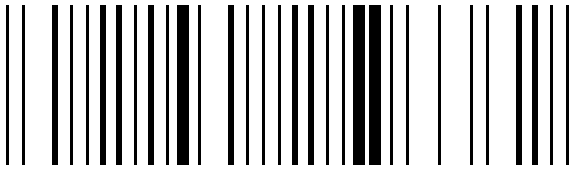
Reading the Barcode of Cancer Susceptibility



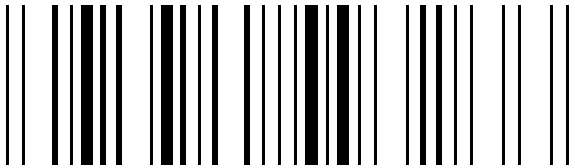
Apparent “sporadic” cancers may be the result of more complicated genetic “barcodes”



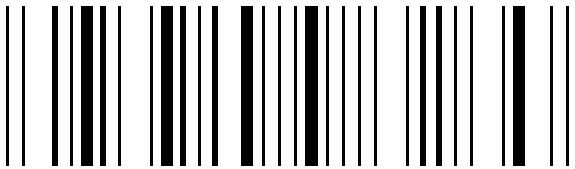
Resistant



Susceptible



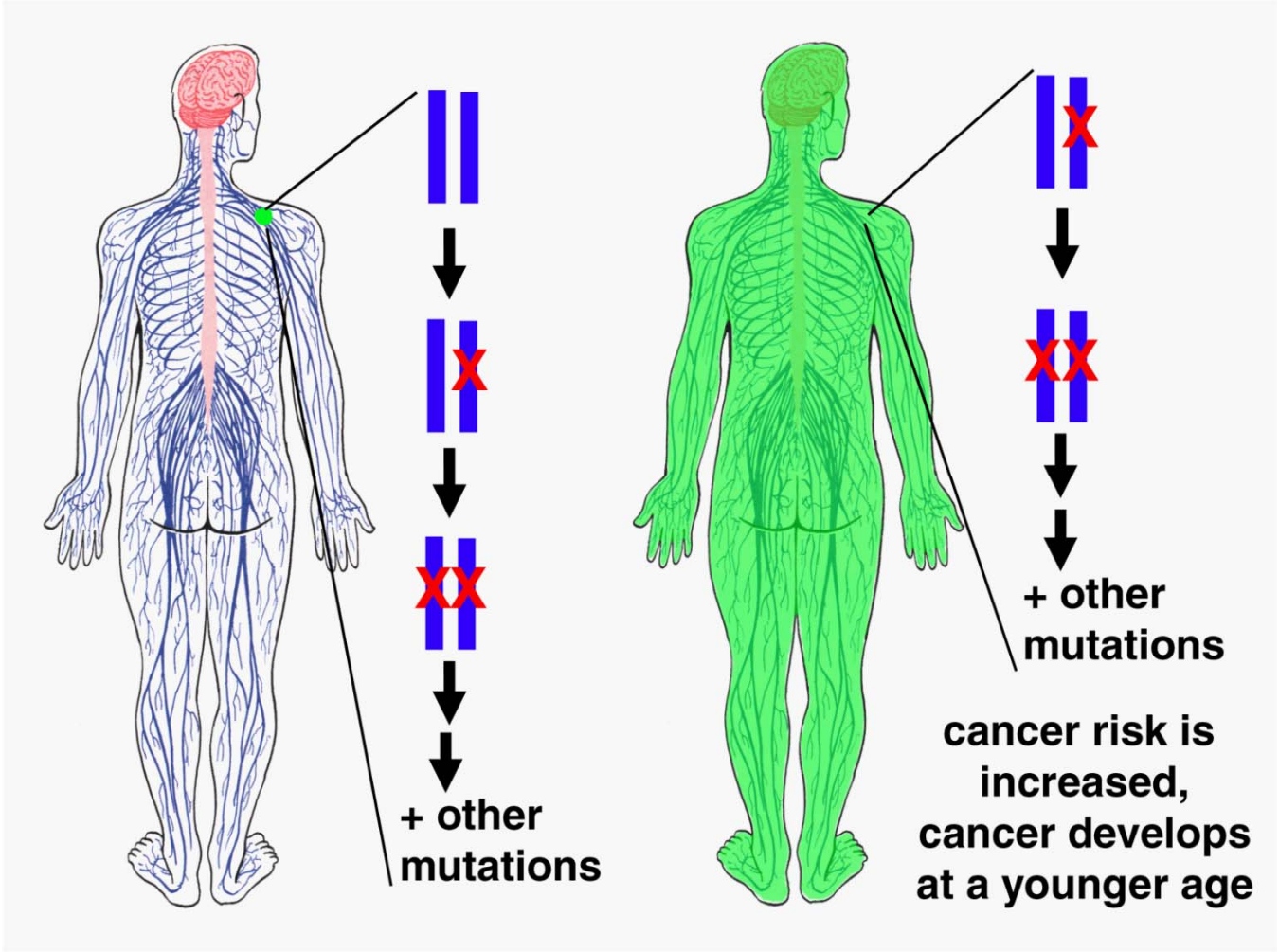
Resistant



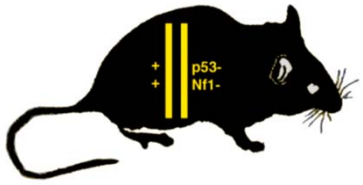
Susceptible

- **These “barcodes” are present before cancer develops, independent of environmental exposures, providing an opportunity for personalized prevention of cancer**

Deciphering Susceptibility Codes:
Sporadic vs. Familial Cancer vs. Mouse Cancer



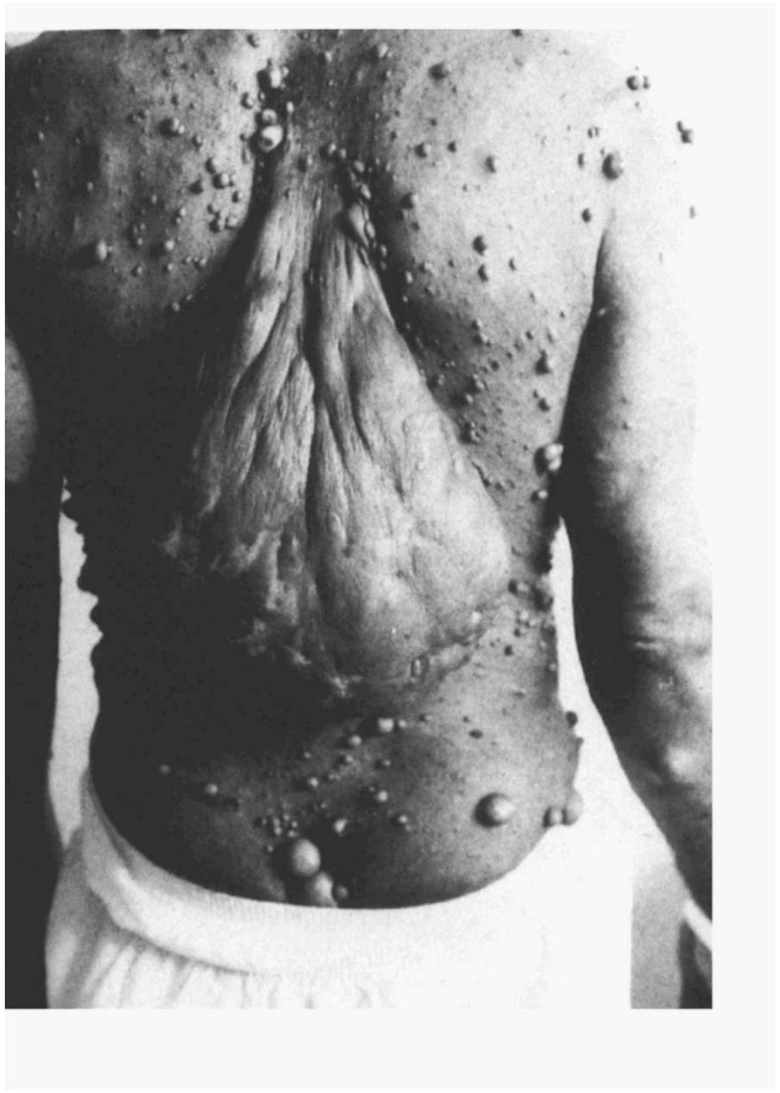
shorter lifespan, controlled breeding, controlled diet/ environment



(not to scale)

Neurofibromatosis type 1

- **Autosomal dominant, affecting 1 in 3500**
- **100% penetrant, but variable expressivity**
- **Evidence for modifier genes from twin studies**
- **Characterized by benign lesions in many different organ systems, including:**
  - **-neurofibromas**
  - **-optic nerve gliomas (WHO I)**
  - **-learning disabilities**
  - **-bone fragility**
  - **-changes in brain anatomy (MRI)**
- **Increased risk for malignancies**
  - **-malignant peripheral nerve sheath tumors**
  - **-astrocytomas/glioblastomas (WHO II-IV)**
  - **-pheochromocytomas**
  - **-rhabdomyosarcomas**
  - **-myeloid leukemia**
- **p53 is mutated in the transformation of neurofibromas into MPNSTs**



Glia cell tumors of the central and peripheral nervous systems are increased in Neurofibromatosis type 1 patients

Astrocytes (normal cell)

- Maintain homeostasis in the central nervous system through interactions with neurons, blood vessels, and basement membranes

Astrocytoma/Glioblastoma Multiforme

- The most common malignant tumor of the CNS
- Incidence of 15 in 100,000 in the general population
- Affects 2% of NF1 patients
- The median survival rate for high-grade astrocytoma (glioblastoma) is 0.4 years
- Only 6% of diagnosed high-grade astrocytoma patients live to 3 years post-diagnosis
- Diffusely infiltrating behavior makes surgical resection difficult to impossible
- Mutation of p53 occurs during astrocytoma initiation

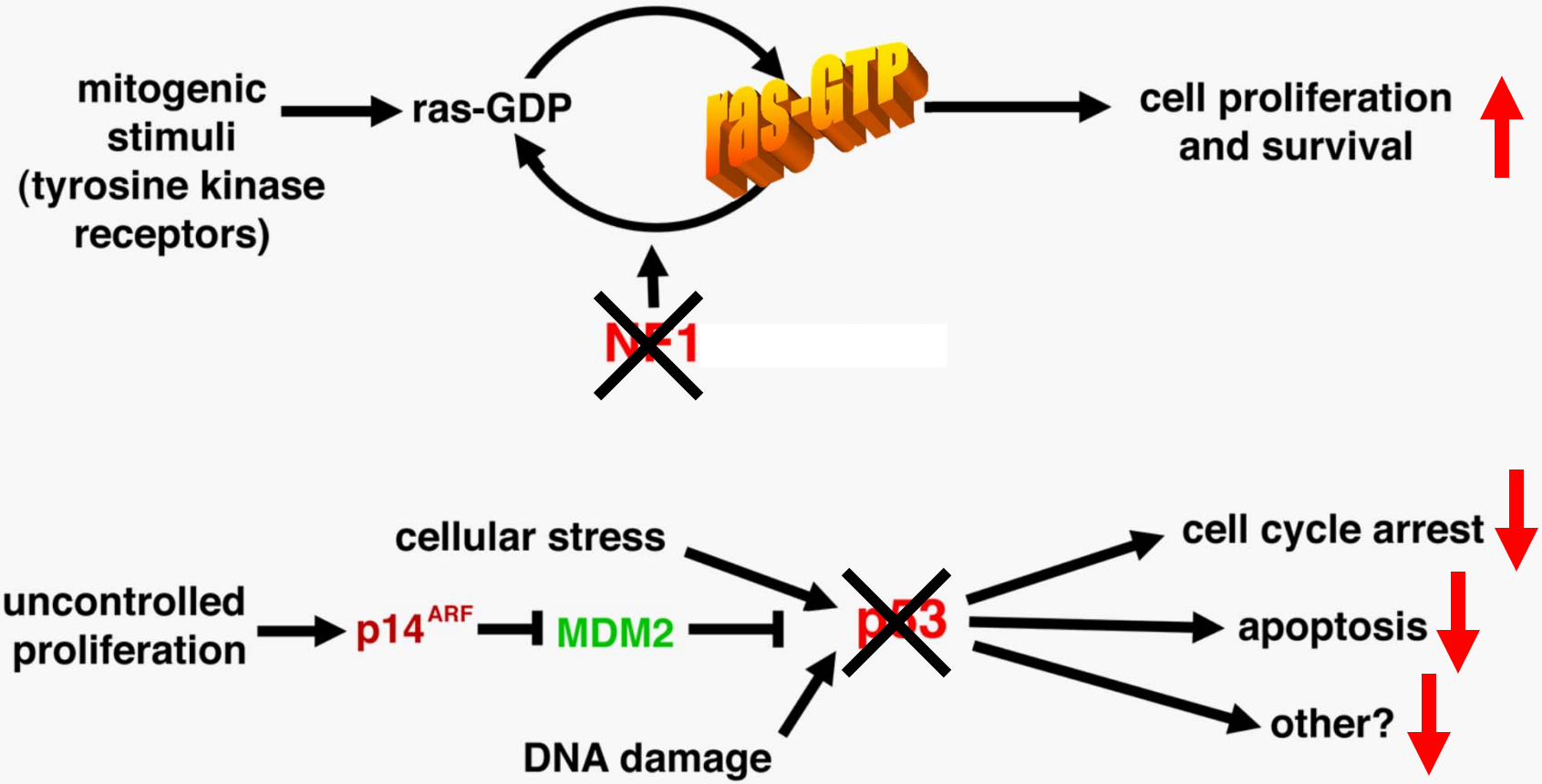
Schwann cells (normal cell)

- Insulate electrical currents of axons in the peripheral nervous system

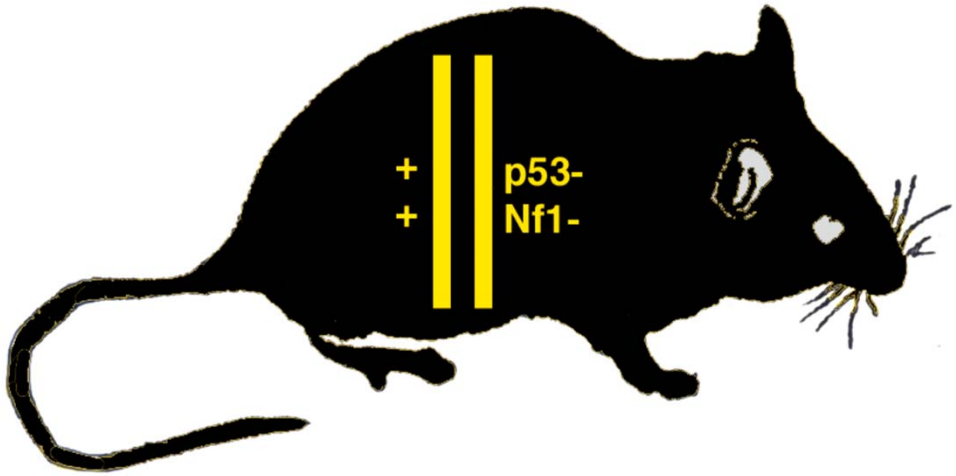
Malignant Peripheral Nerve Sheath Tumor

- Incidence of 1 in 100,000 in the general population
- Affects up to 10% of NF1 patients
- Tumors can infiltrate along nerve tracks and metastasize
- 5-year survival rate has been estimated between 16-52%, depending on degree of resection of tumors, size, and location
- Mutation of p53 occurs during progression of benign tumors to malignancy

Nf1 and p53 are tumor suppressor genes acting at different points in the control of cell growth and survival

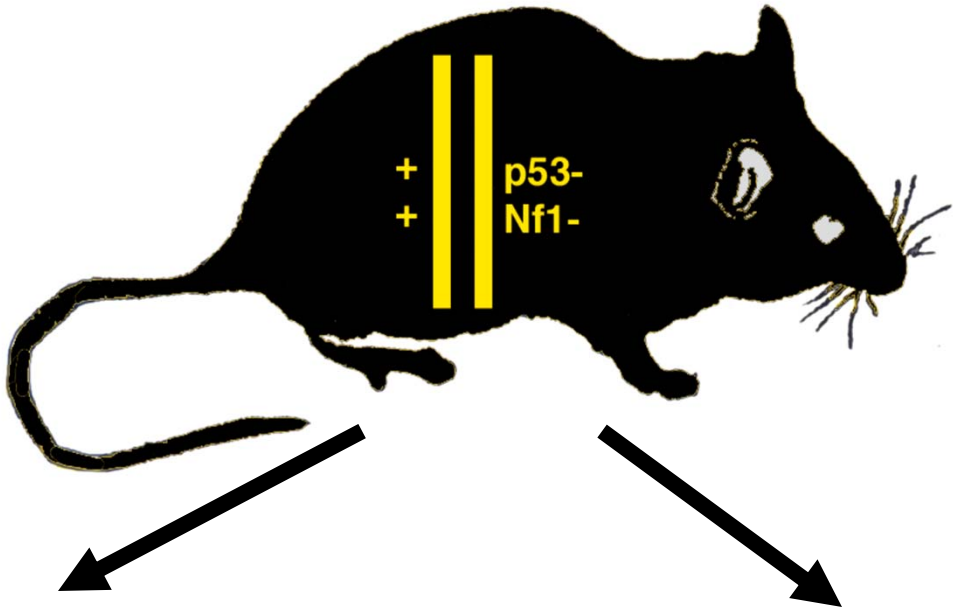


The *Nf1;p53cis*, C57BL/6J mouse model of astrocytoma and peripheral nerve sheath tumor



- The average tumor latency is 7 months
- Mutations are maintained by simple Mendelian inheritance
- The p53 pathway is lost and the ras pathway is upregulated at physiologically relevant levels by a single chromosomal loss event
- The genetic background is well defined

The *Nf1;p53cis*, C57BL/6J mouse model of NF1



malignant astrocytomas/glioblastomas

- 73% mutant mice on C57BL/6J
- modified by 129S4/SvJae and CBA/J
- progeny of mutant moms have increased susceptibility
- F1 analysis shows modifiers on chr 11 near *Nf1* and *p53*

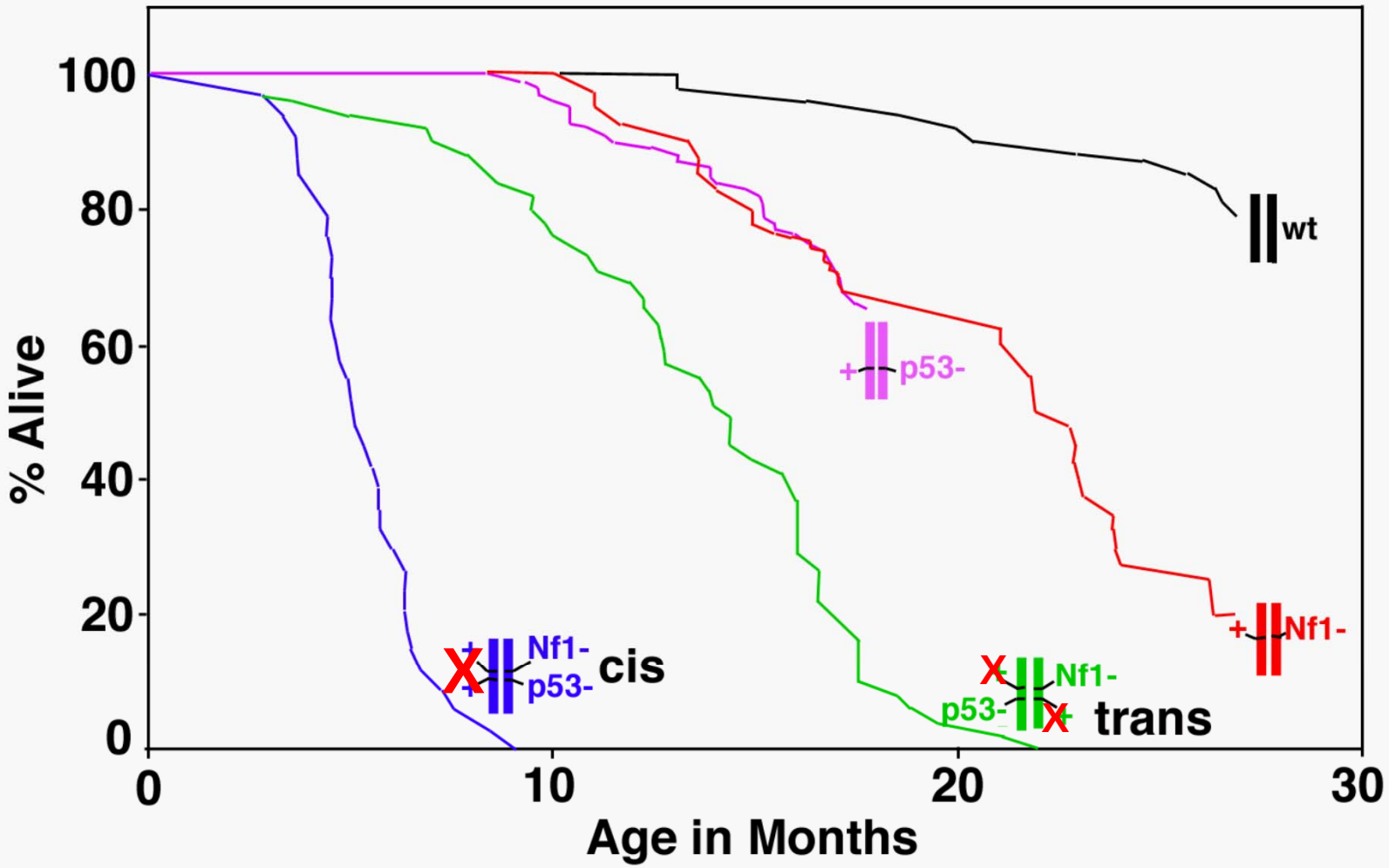
Reilly et al (2004) PNAS 101:13008-13

malignant peripheral nerve sheath tumors

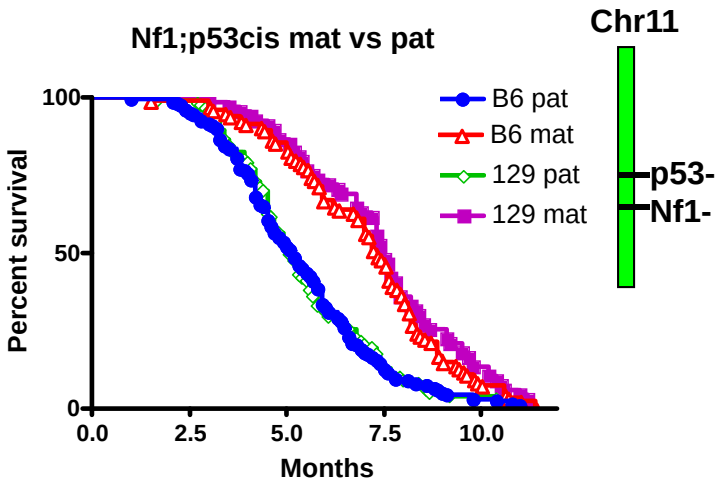
- 65% mutant mice on C57BL/6J
- modified by A/J and DBA/2J
- progeny of mutant dads have increased susceptibility
- backcross mapping shows linkage to chr 15 and 19

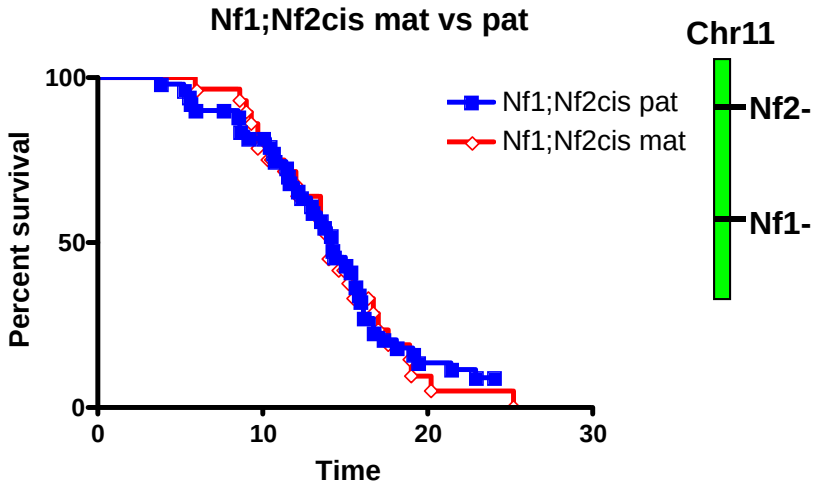
Reilly et al (2006) Cancer Res, 66:62-8

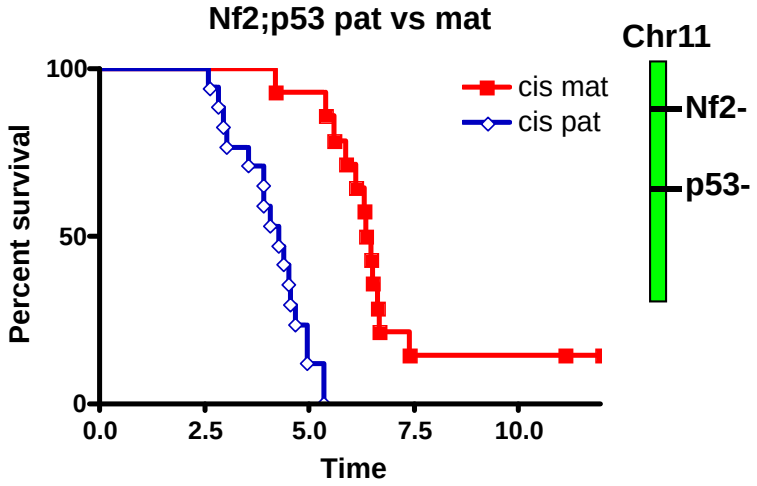
Nf1 and *p53* cooperate in tumor suppression

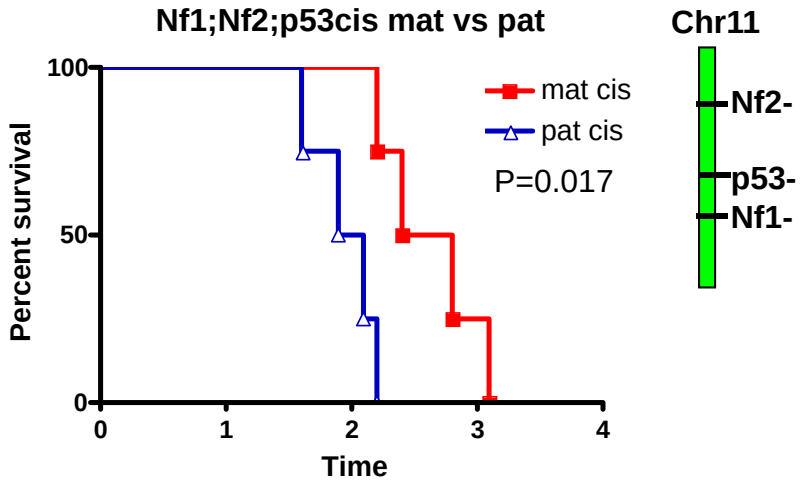


Imprinting on mouse chromosome 11 cooperates with p53 mutation in tumorigenesis









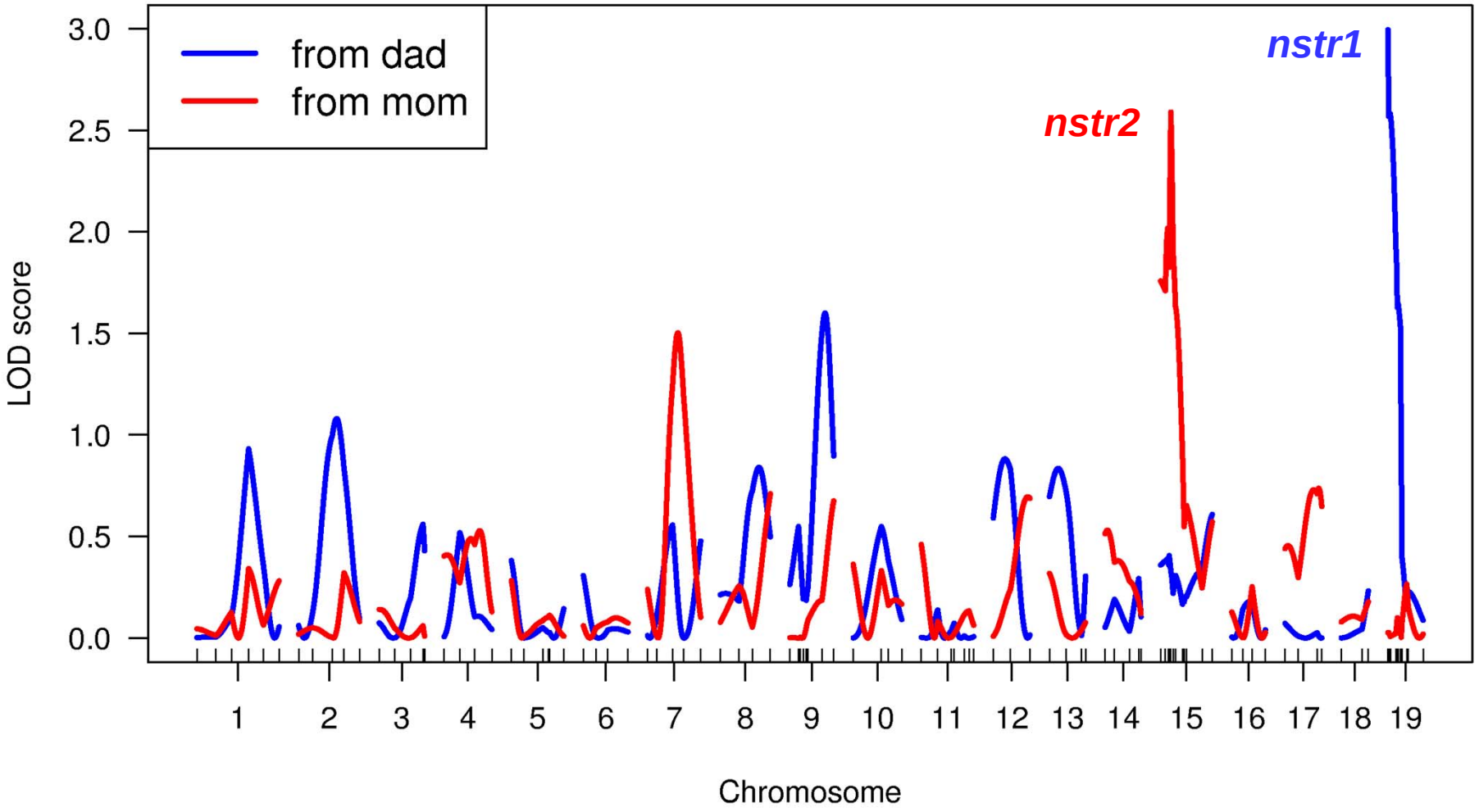
Mapping modifiers of MPNSTs by backcrossing:
Polymorphisms in the A/J strain modify tumorigenesis

Progeny	Total	With sarcoma	Percentage
NPcis, B6, mother cis	47	18	38%
NPcis, B6, father cis	91	59	65%
NPcis, B6XA, mother cis	80	20	25%
NPcis, B6XA, father cis	20	9	45%
NPcis, B6XAXB6, mother cis	97	38	39%
NPcis, B6XAXB6, father cis	144	89	62%

- Inheritance of mutant chromosome 11 affects incidence of sarcomas regardless of strain background.
- The A strain confers dominant resistance to sarcomas regardless of inheritance of chromosome 11.

Parents of backcross progeny determine which loci affect susceptibility

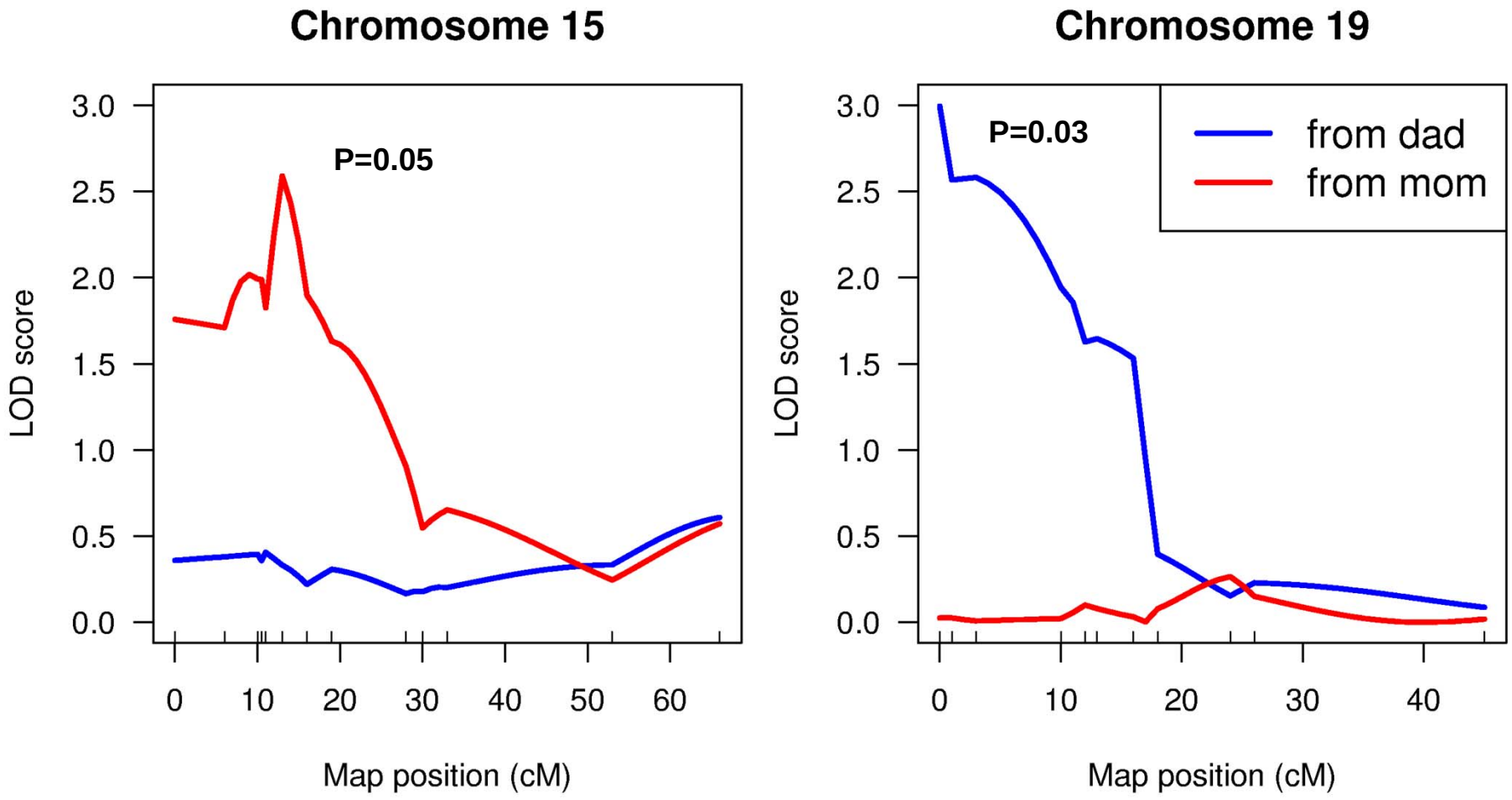
Progeny of Nf1;p53cis (B6XA) female X wt B6 male (N=97)
vs. Progeny of wt B6 female X Nf1;p53cis (B6XA) male (N=144)



nerve sheath tumor resistance loci

Karl Broman, John Hopkins University

nstr1 on mouse chromosome 19 and *nstr2* on mouse chromosome 15 control susceptibility to GEM PNSTs



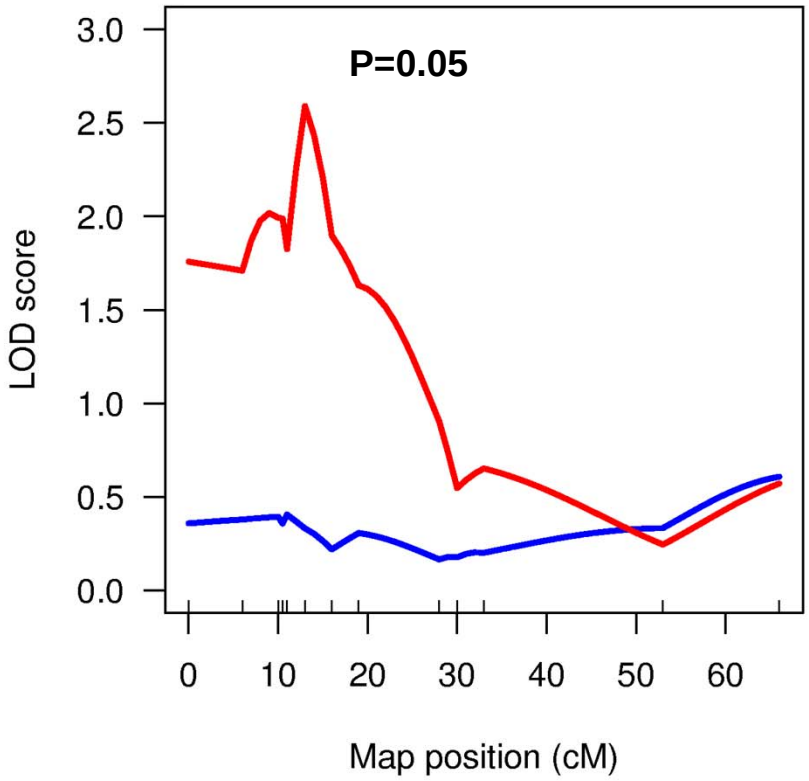
nstr1 and *nstr2* are syntenic with chromosomal regions altered in human MPNSTs

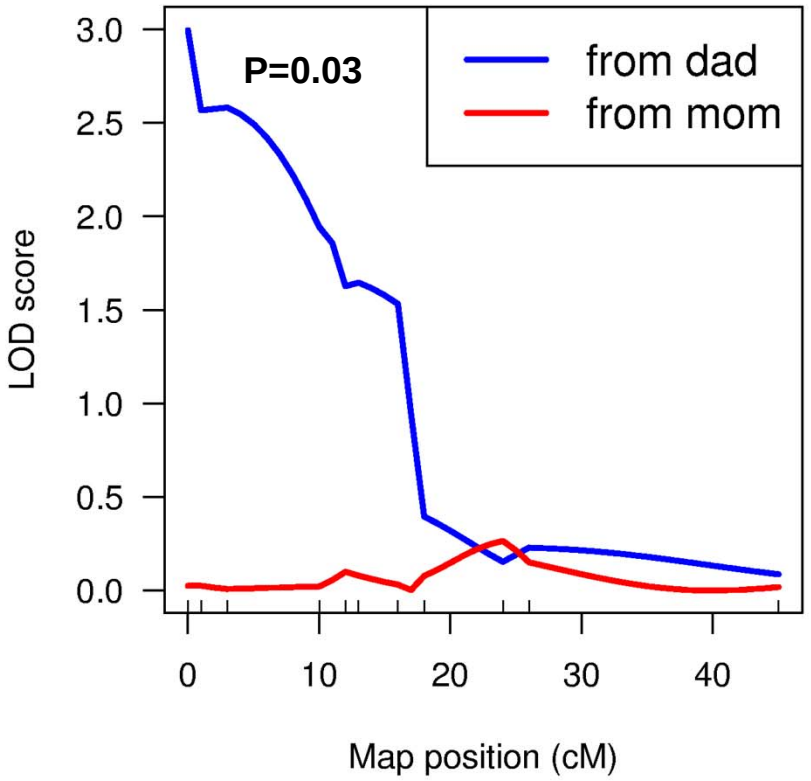
Alterations in human MPNSTs (CGAP, N=90)

+8 - 12 cases
-8 - 10 cases
addition 8q22 - 1 case
addition 8q24 - 3 cases
translocation 8q22 - 1 case
translocation 8q23 - 1 case

+11 - 2 cases
-11 - 17 cases
11q13 deletion - 2 cases
11q13 addition - 1 case
11q13 translocation - 3 cases

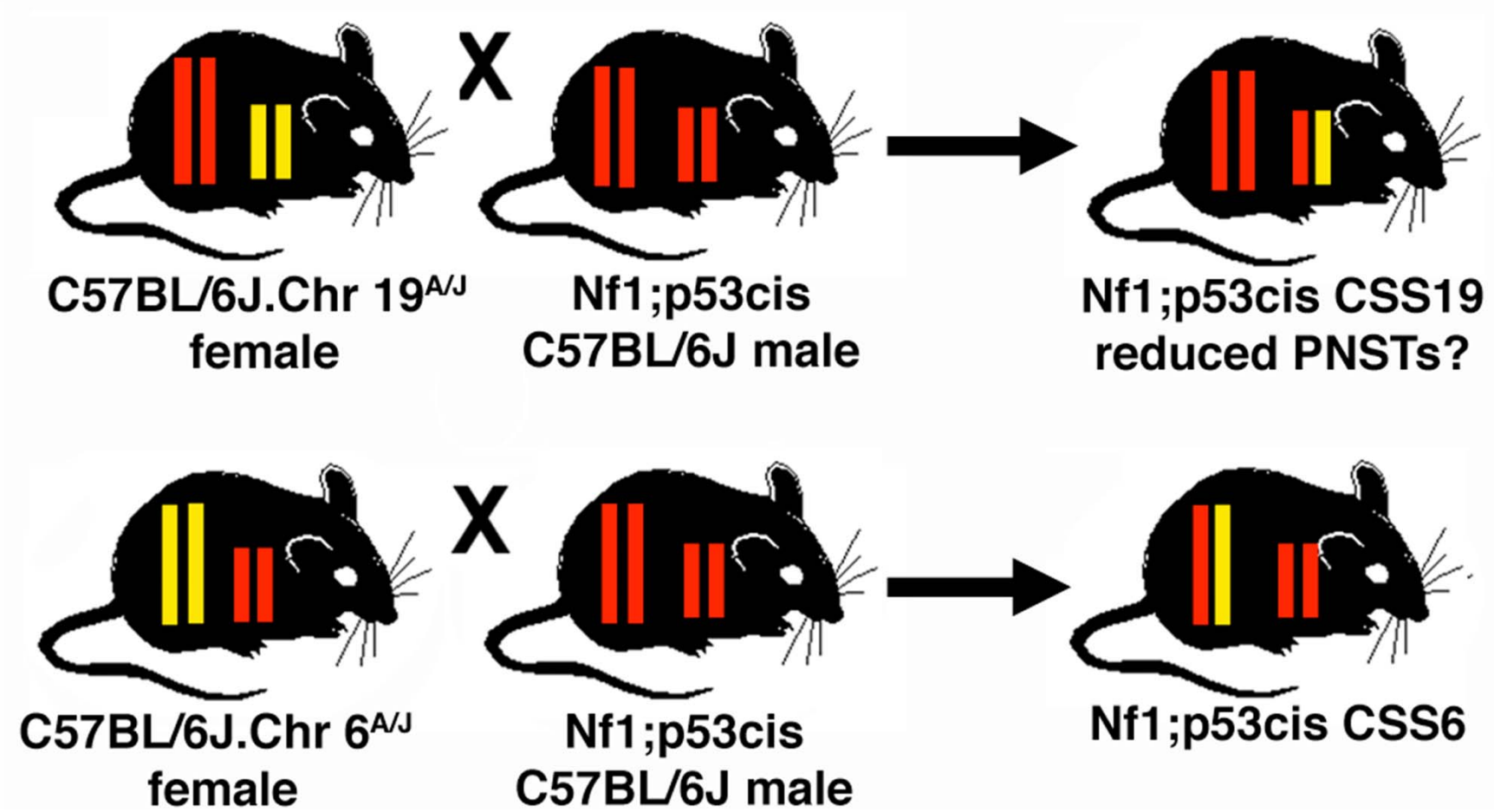
Corresponding human chromosomes





www.ncbi.nlm.nih.gov/Homology/
www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=cancerchromosomes

Using Chromosome Substitution Strains to test mechanisms of resistance

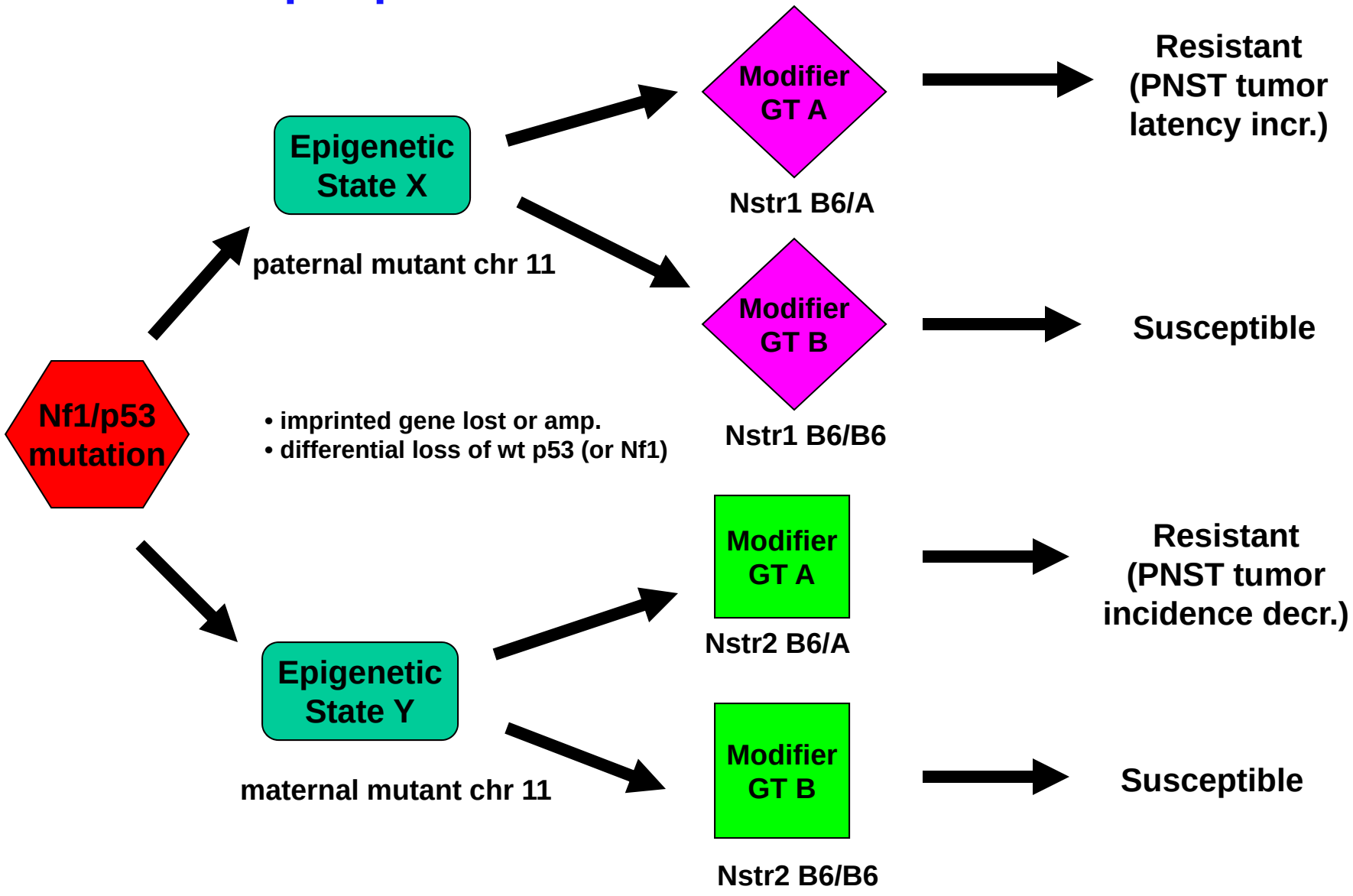


Chr 19 (*nstr1*) and Chr 15 (*nstr2*) modifiy PNST susceptibility differently

	N	% GEM PNST	χ^2 test P value	Median Age w/ GEM PNST (mo)	Mean Age w/ GEM PNST (mo)	T-test P value
NPcis^{pat};CSS-19	30	77%	0.5	5.7	5.9	0.06
NPcis^{pat};CSS-6	19	84%		4.9	4.9	
NPcis^{mat};CSS-15	27	37%	0.03	7.3	7.5	0.24
NPcis^{mat};CSS-8	20	70%		6.8	6.9	

- **Nstr2 (chr 15) reduces number of PNSTs, but does not affect latency**
- **Nstr1 (chr 19) may increase latency of PNSTs, but does not affect penetrance**
- **Although *NPcis*^{mat};CSS-15 mice develop significantly fewer GEM PNSTs, they develop significantly more astrocytoma and hematopoietic tumors**

A genetic/epigenetic network for susceptibility to peripheral nerve sheath tumors

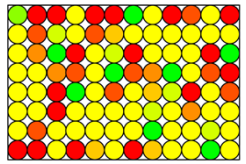


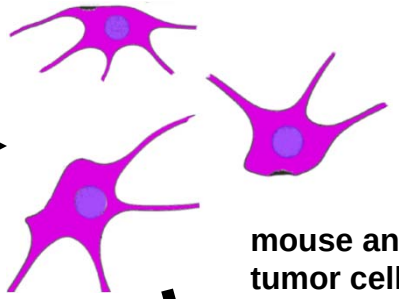
Using mouse models of cancer to develop new therapies

Candidate targets identified by modifier screens and basic biology studies

Available libraries of therapeutics

"High-throughput" screening of compounds in astrocytoma cells

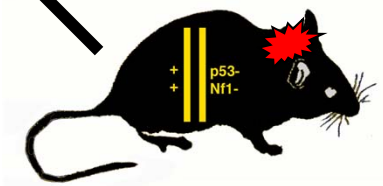




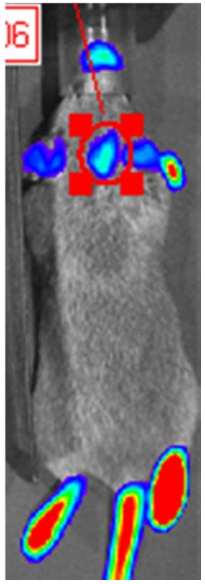
mouse and human tumor cells of different grades and primary astrocytes

toxicology studies In mice

treatment studies in subcutaneous or intracranial models (immune-competent and immune-deficient)

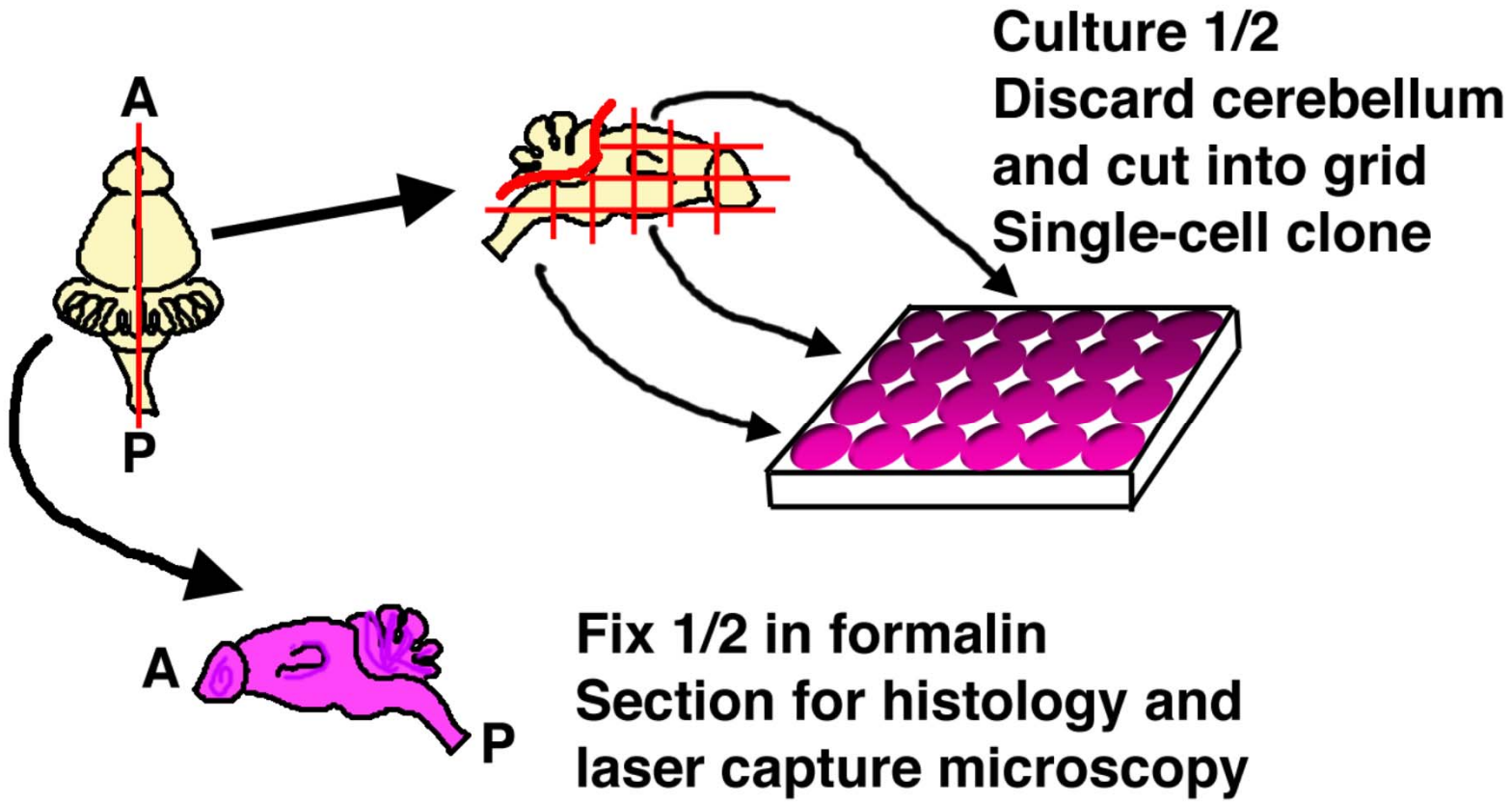


Nf1-/+;p53-/+cis;ELUX Tg spontaneous astrocytoma model



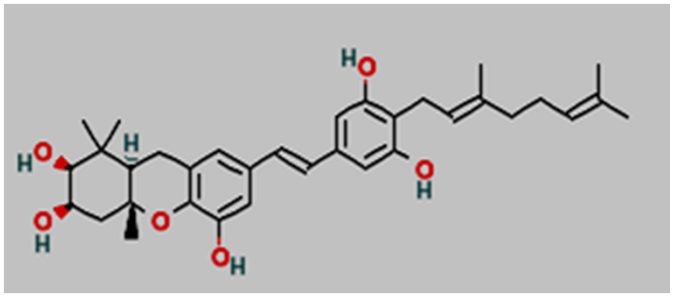
Follow tumor growth and quantify tumor burden using bioluminescence

Generation of cell lines from low-grade astrocytomas



- 14 cells lines of differing tumor grades, sex, and genetic background have been generated thus far by this method

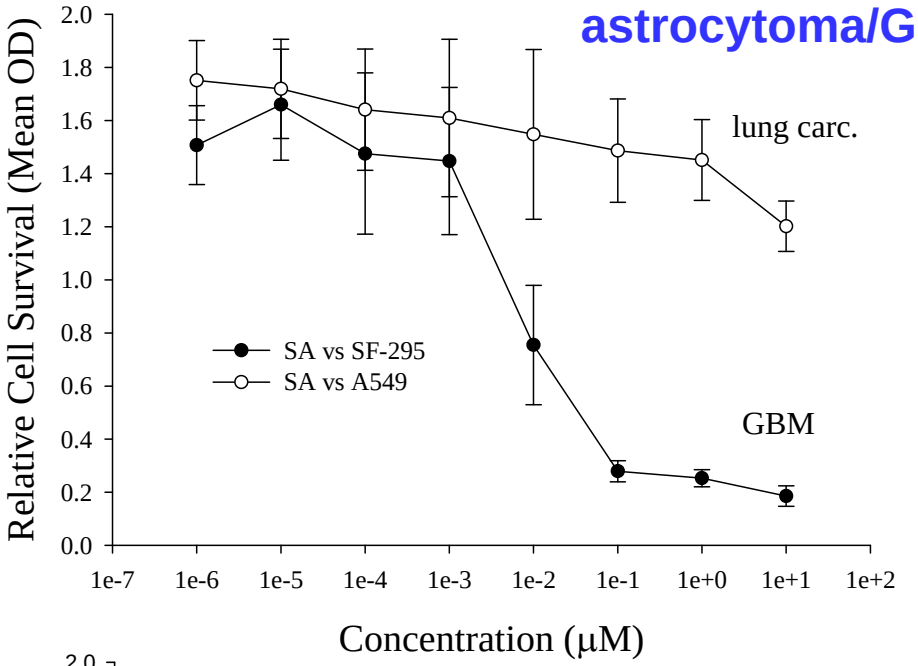
Using the Nf1-/+;p53-/+cis mouse model to investigate a potential anti-astrocytoma therapeutic derived from natural compounds



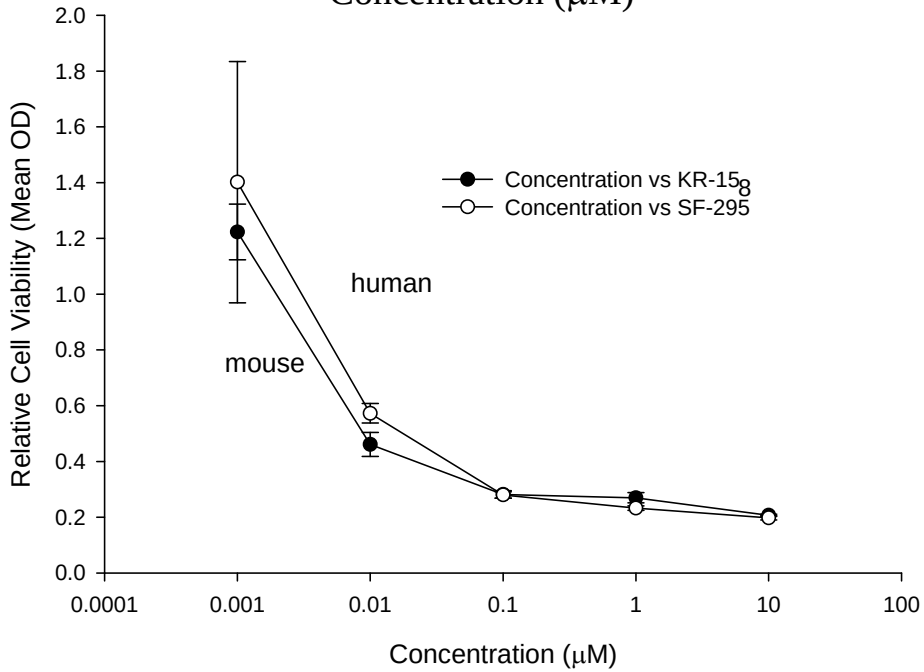
Schweinfurthin A

In collaboration with the Molecular Targets Development Program

Schweinfurthin A specifically inhibits mouse and human astrocytoma/GBM cell growth



Schweinfurthin A shows differential activity in a screen of the NCI60 cell lines: specificity towards brain tumor cell lines



Mouse and human glioma cells are equally sensitive to Schweinfurthin A

Summary

- Cancer susceptibility is determined by the interaction of high-penetrance cancer genes, low-penetrance cancer genes, and epigenetic effects
- Epistatic and combinatorial effects can mask the genetic component of cancer susceptibility
- Mouse models of cancer and human familial cancer syndromes are useful to dissect the components of cancer susceptibility
- Mouse tumor cell lines are useful surrogates for human cell lines in preclinical drug testing and allow testing in immune-competent animals
- Brain tumor specific therapeutics may provide a new, more effective approach for the treatment of astrocytoma and glioblastoma.

Acknowledgements

Modifiers of Cancer
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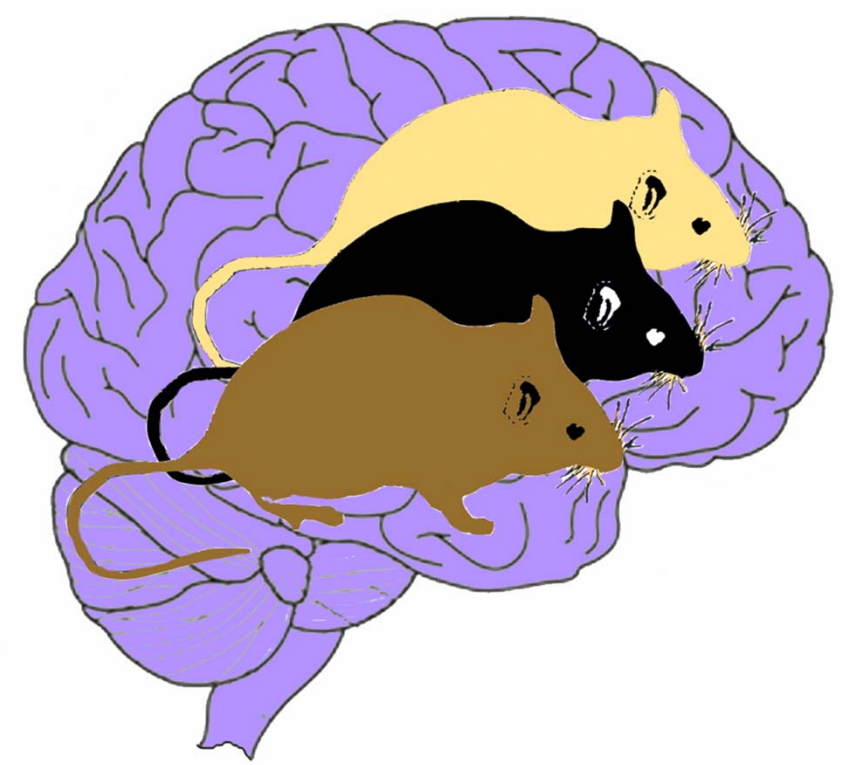

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