

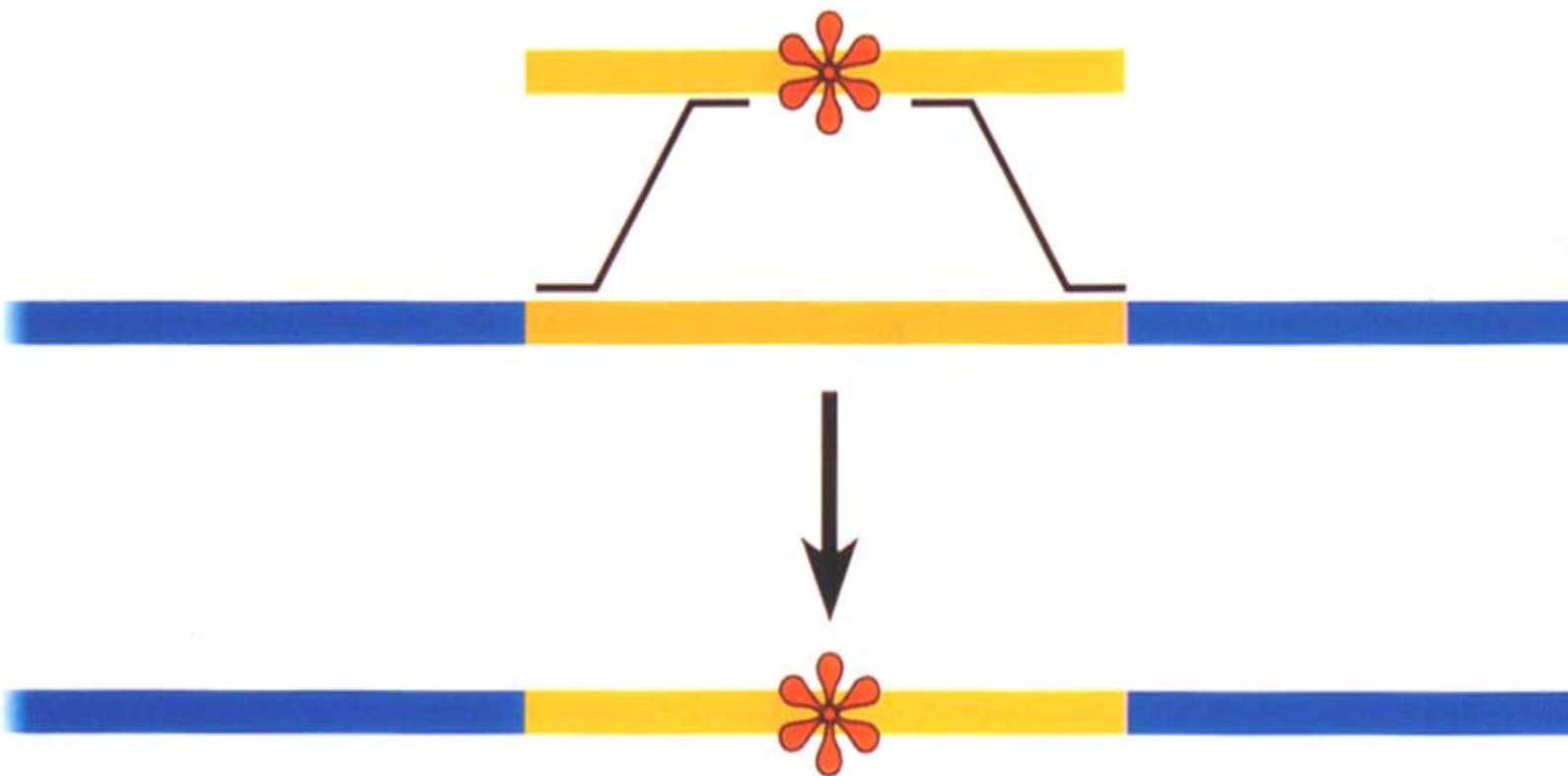
Nobel Lecture

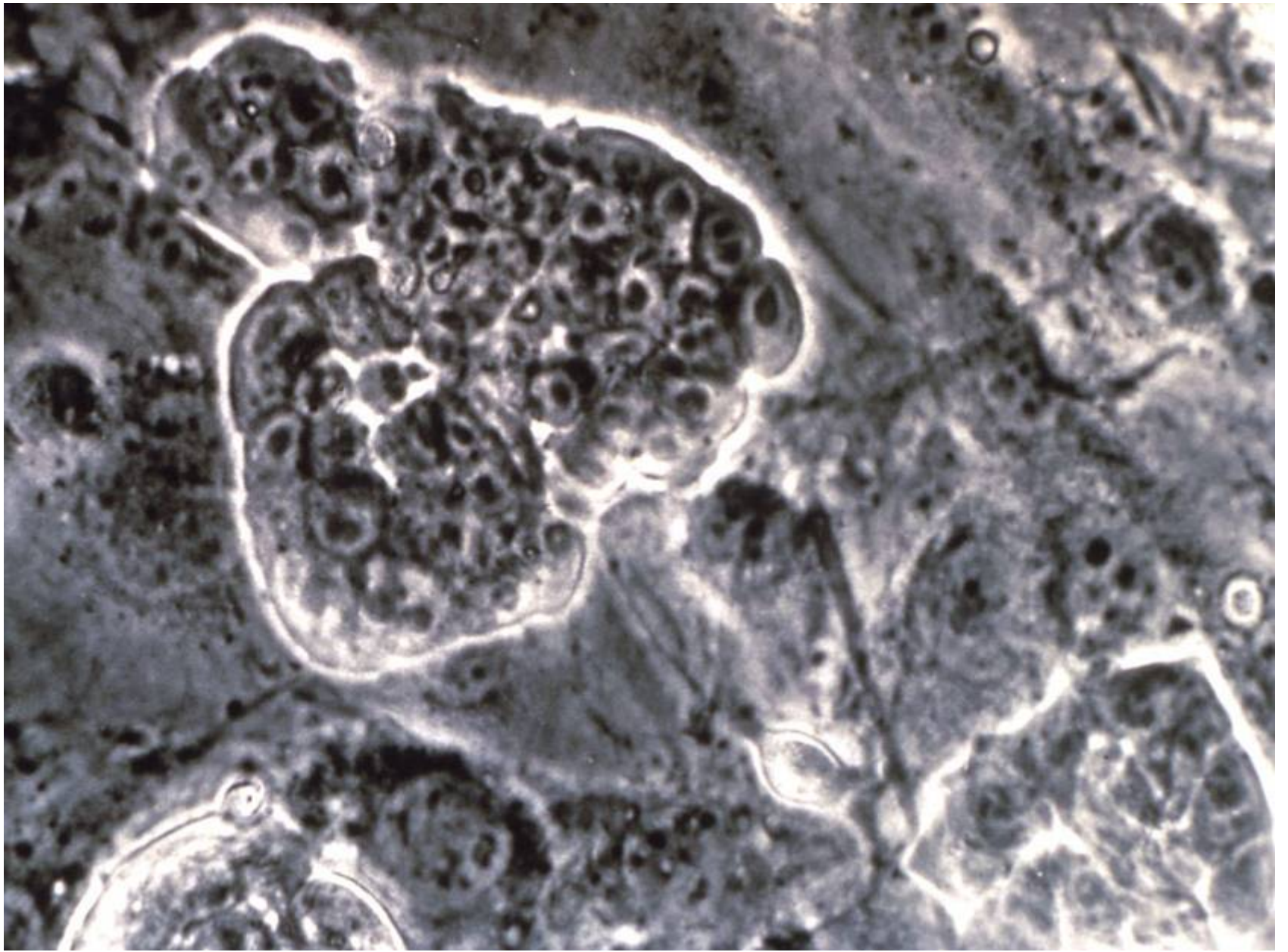
Gene Targeting into the 21st Century

Mario R. Capecchi

Howard Hughes Medical Institute
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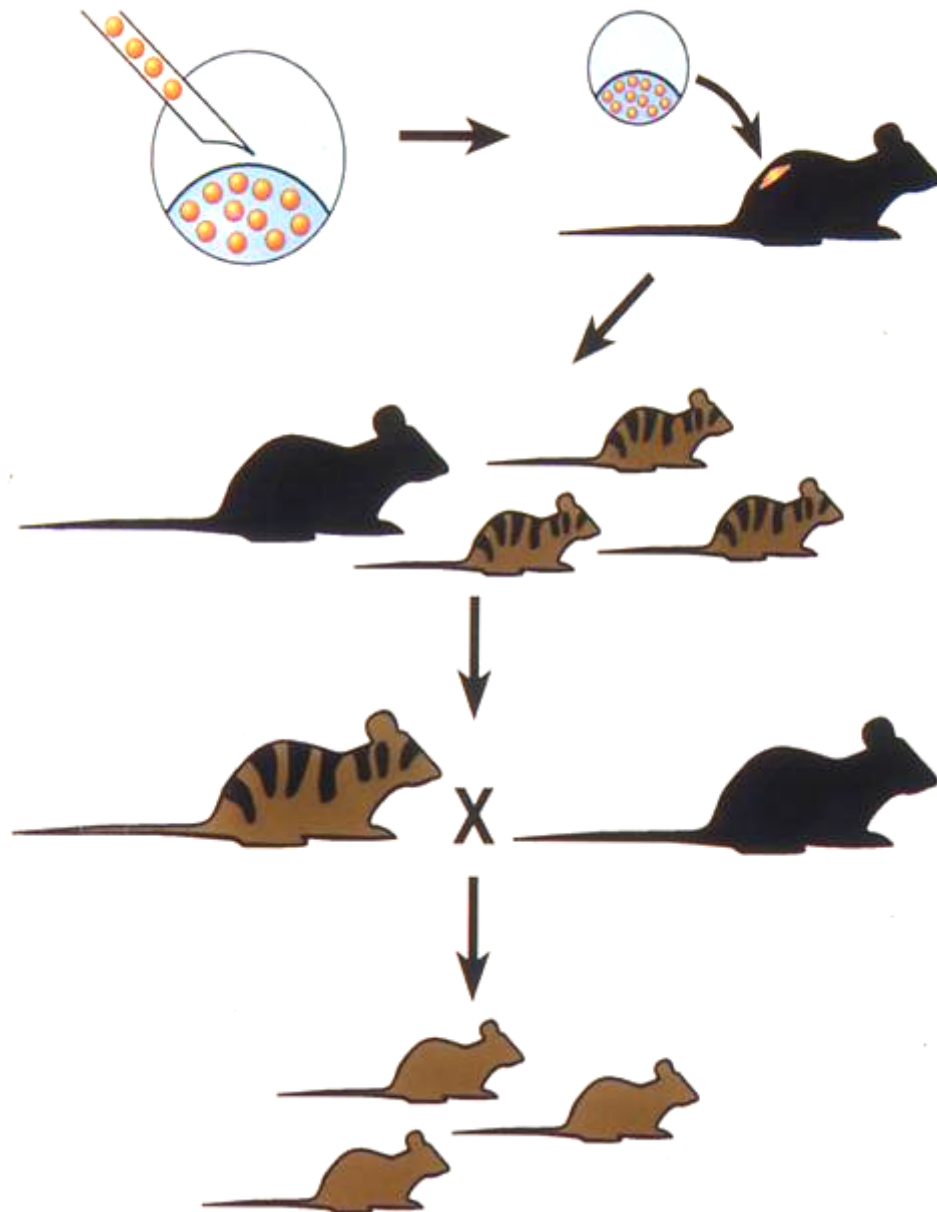
December 7th, 2007

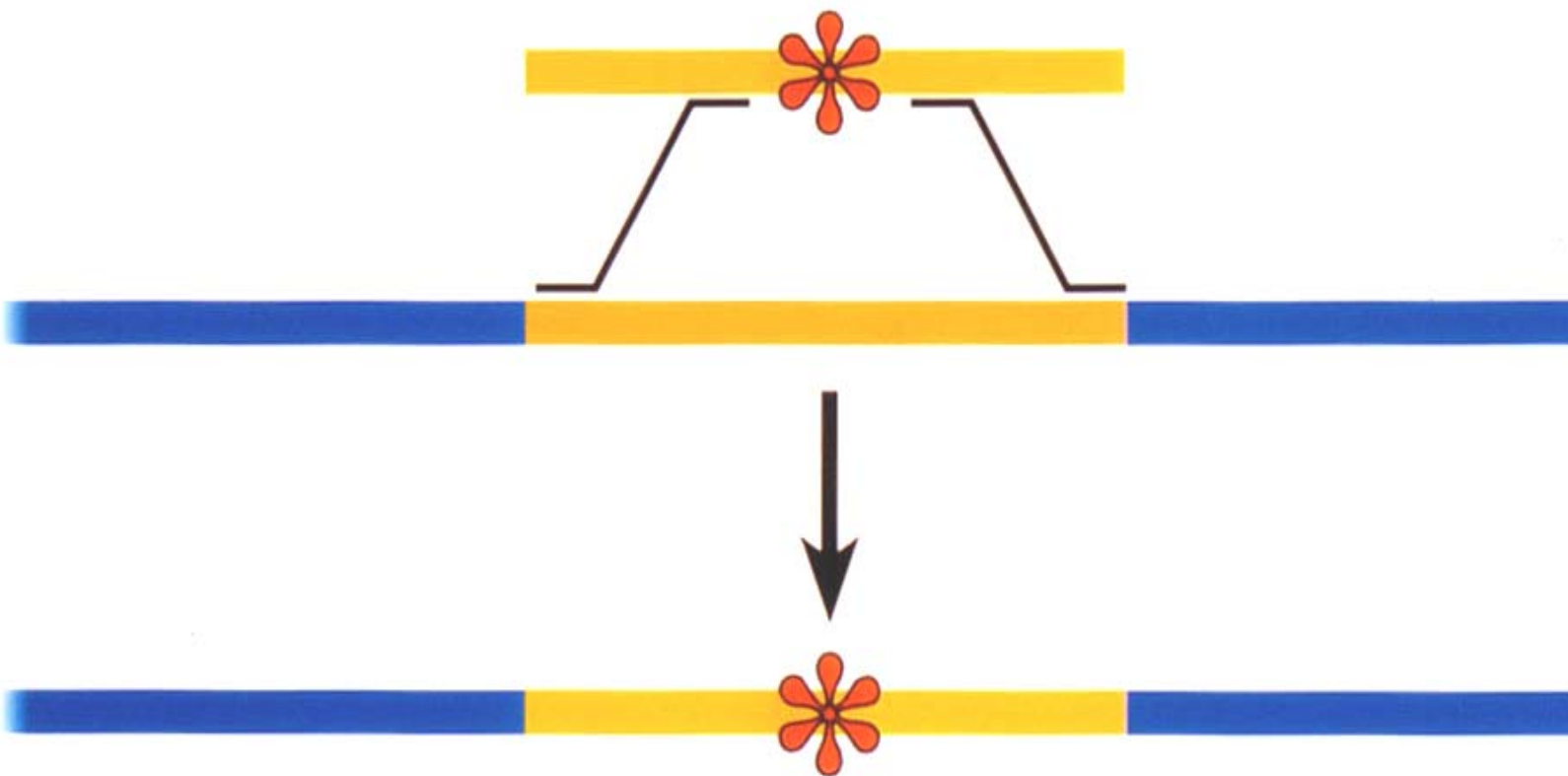




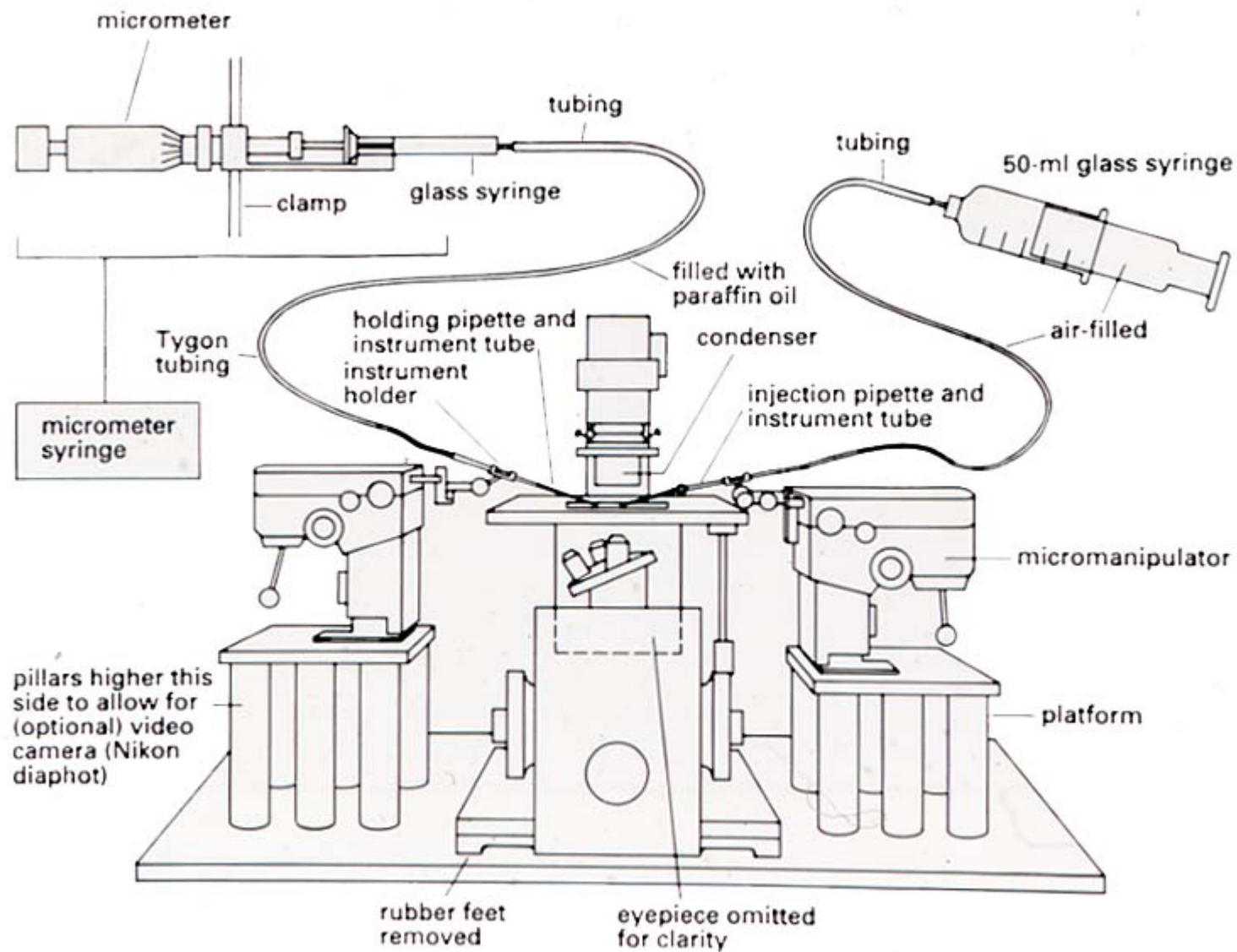


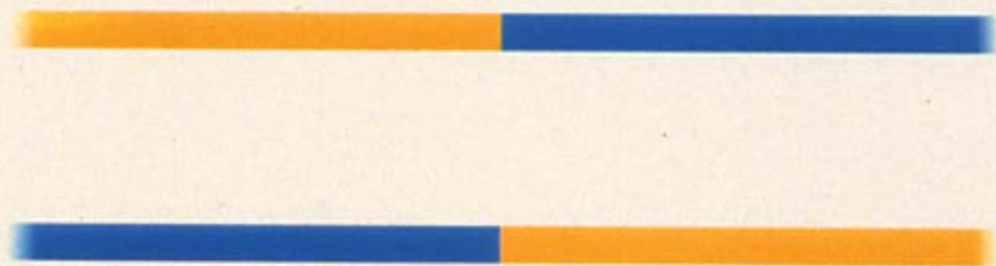
Generation of Germ Line Chimeras

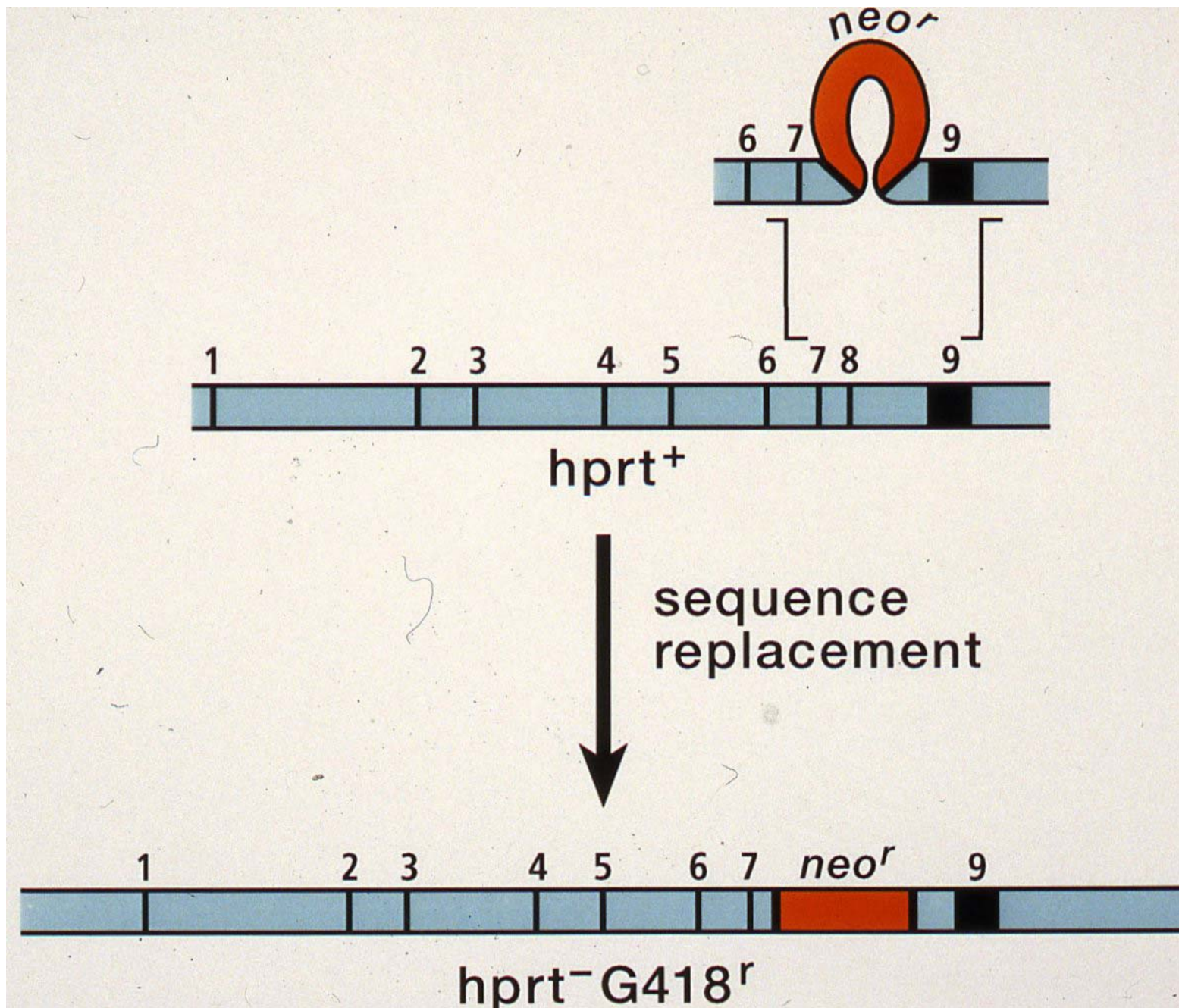




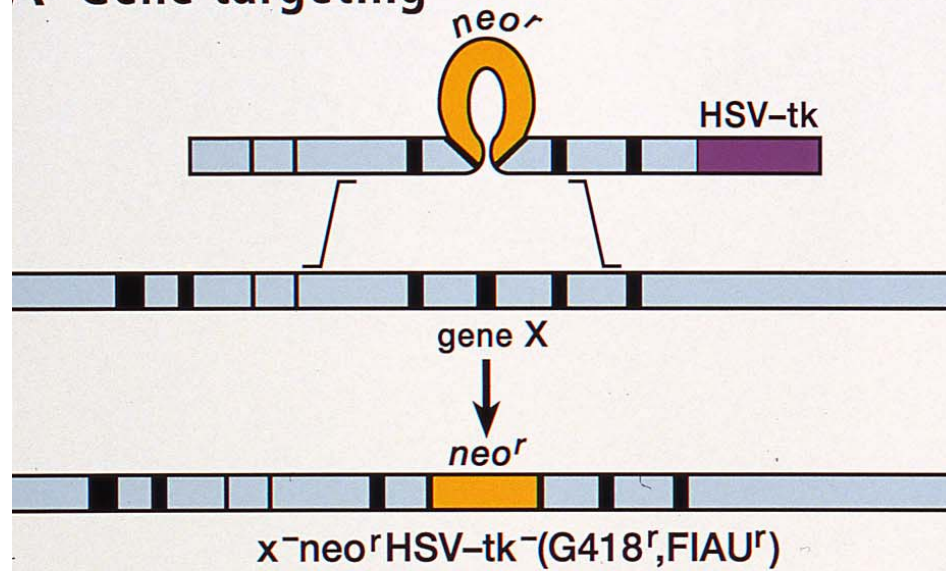
**How did the idea to
do Gene Targeting
arise?**



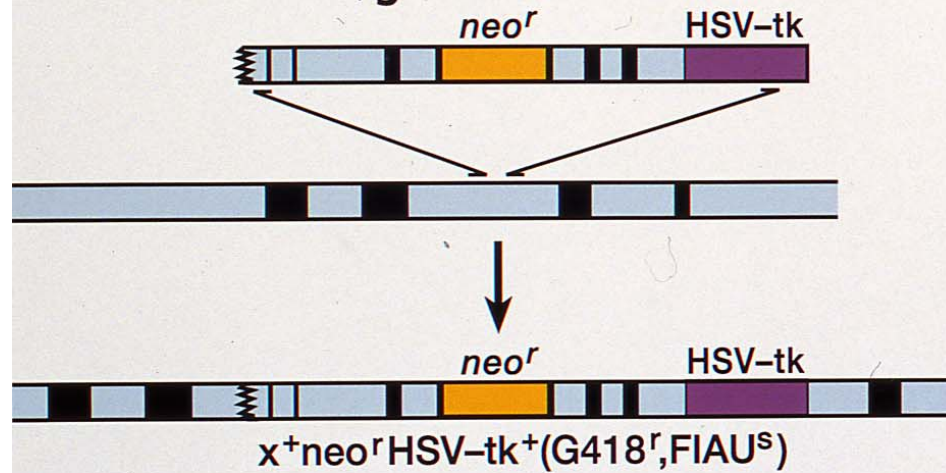




A Gene targeting



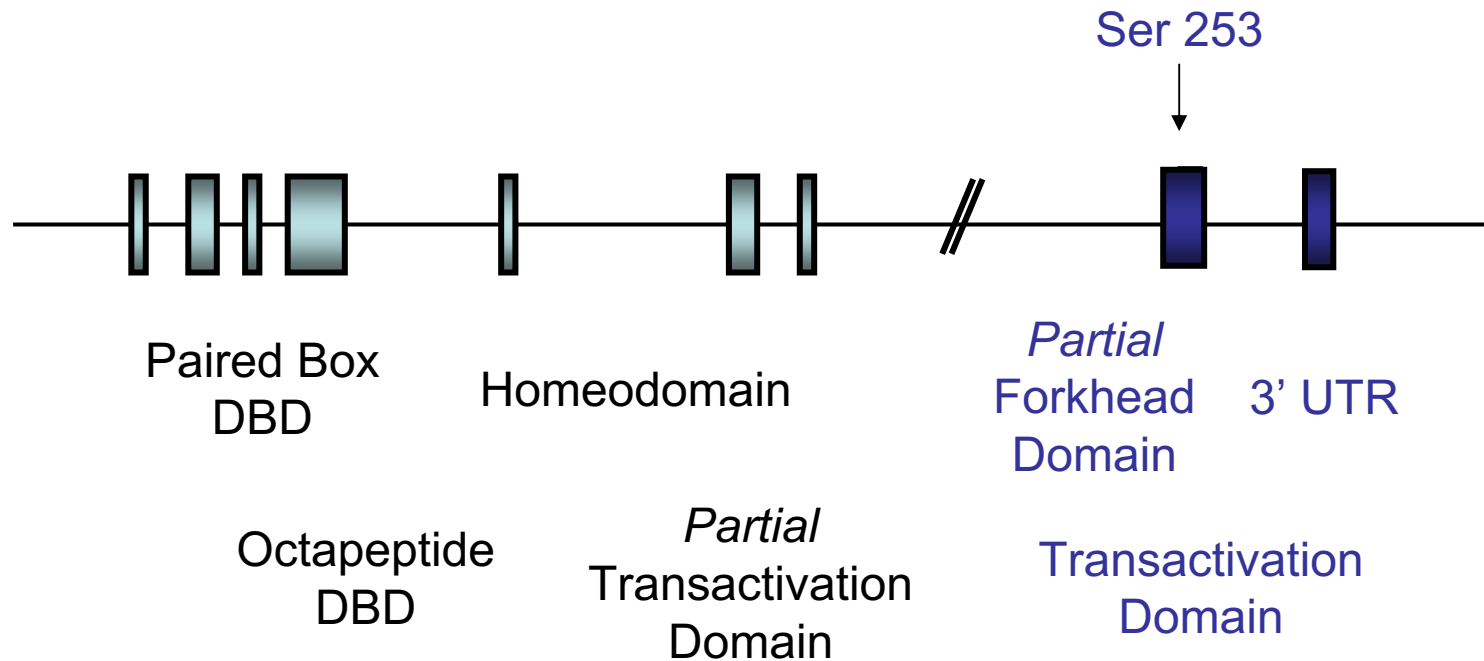
B Random integration



Considerations for Models

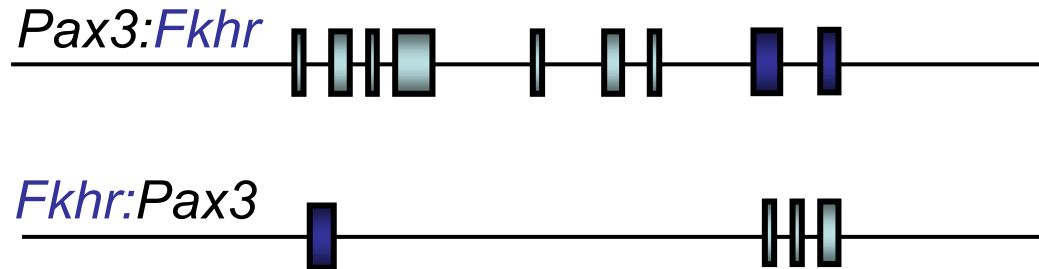
1. Inducing Event
2. Time of Induction
3. Stoichiometry
4. Micro-molecular
and -cellular environment

PAX3-FKHR Structure



Modeling Alveolar Rhabdomyosarcoma

What's Important?



Pax3:Fkhr gene is created.

Fkhr:Pax3 is created.

Pax3 allele is destroyed.

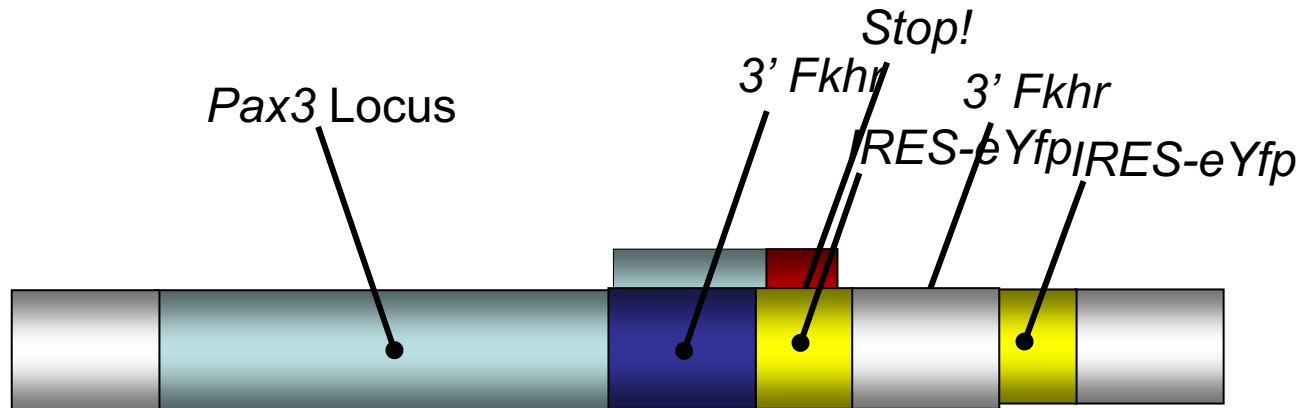
Fkhr allele is destroyed.

Chromosomal Translocations:

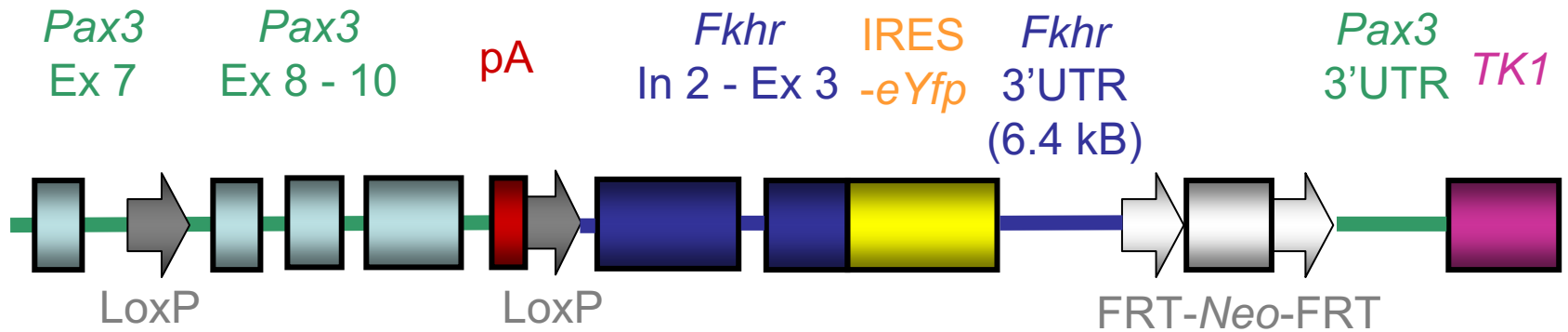
**Why not generate real
translocations?**

Pax3* becomes *Pax3:Fkhr

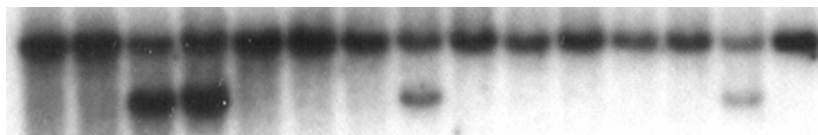
with Time- & Tissue Specificity



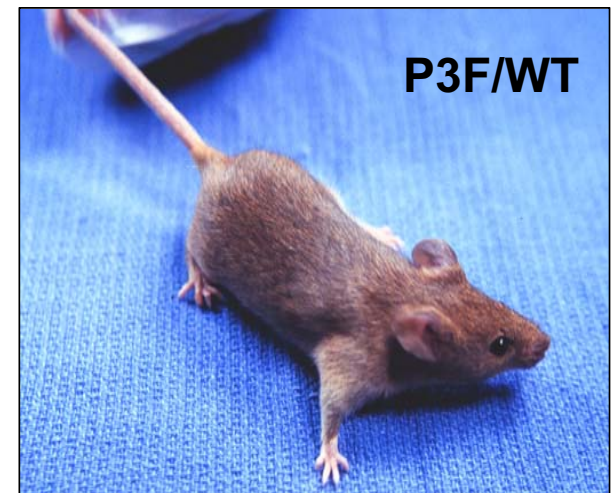
Conditional Knock-In Construct

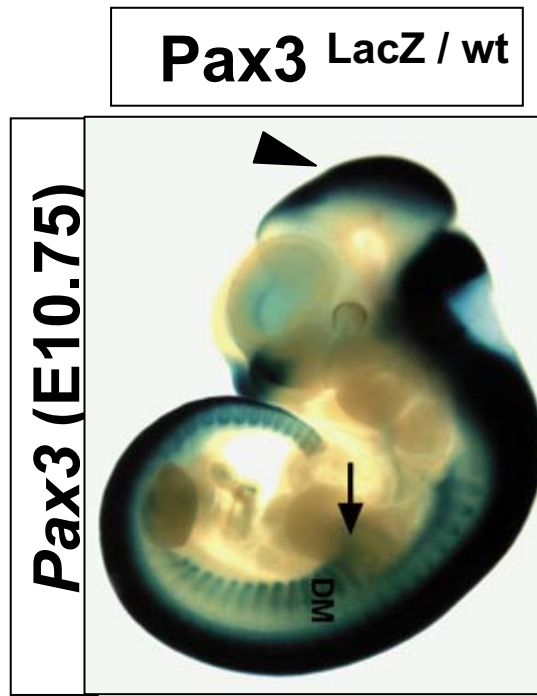


Germline Offspring

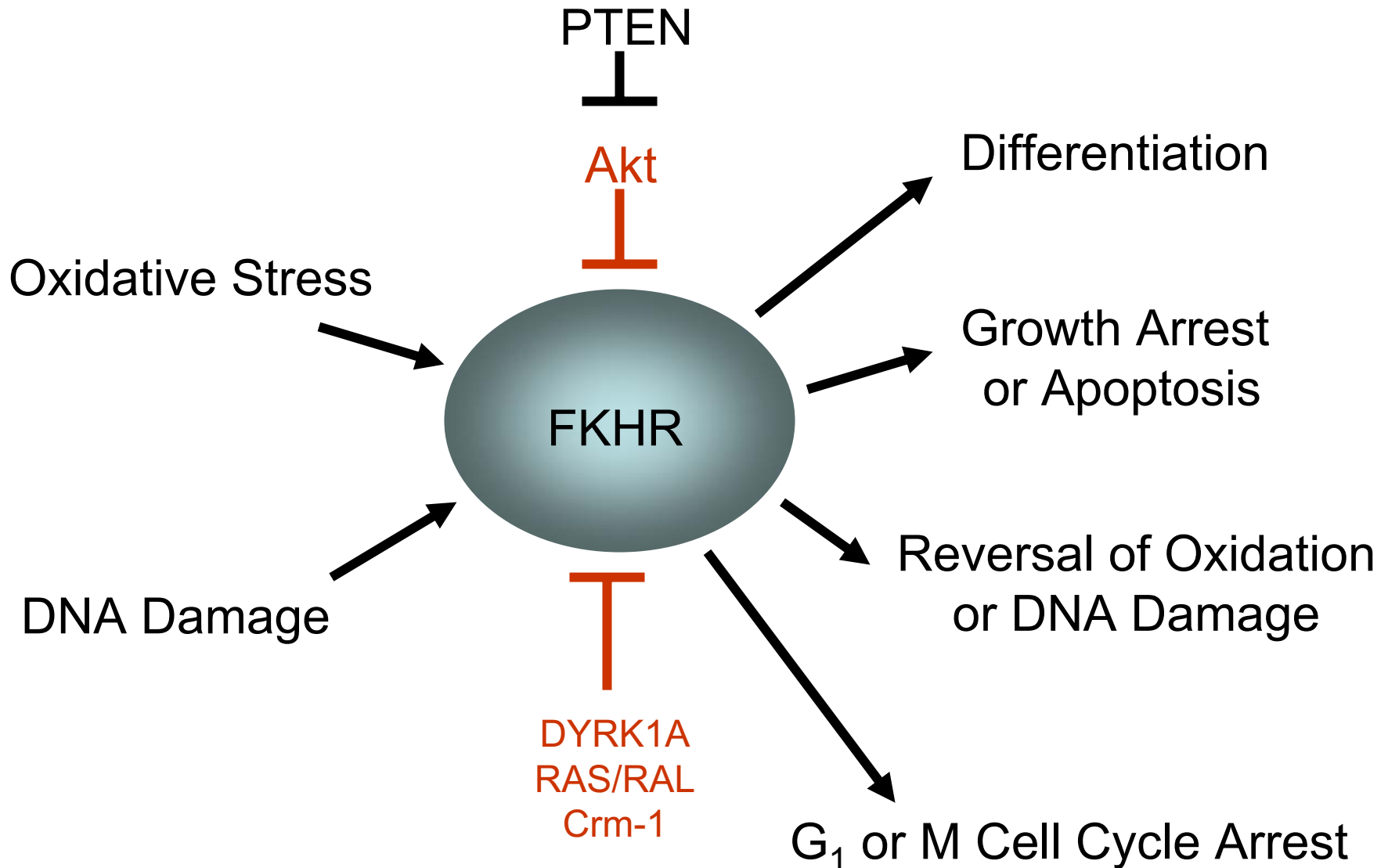


← WT
← P3F

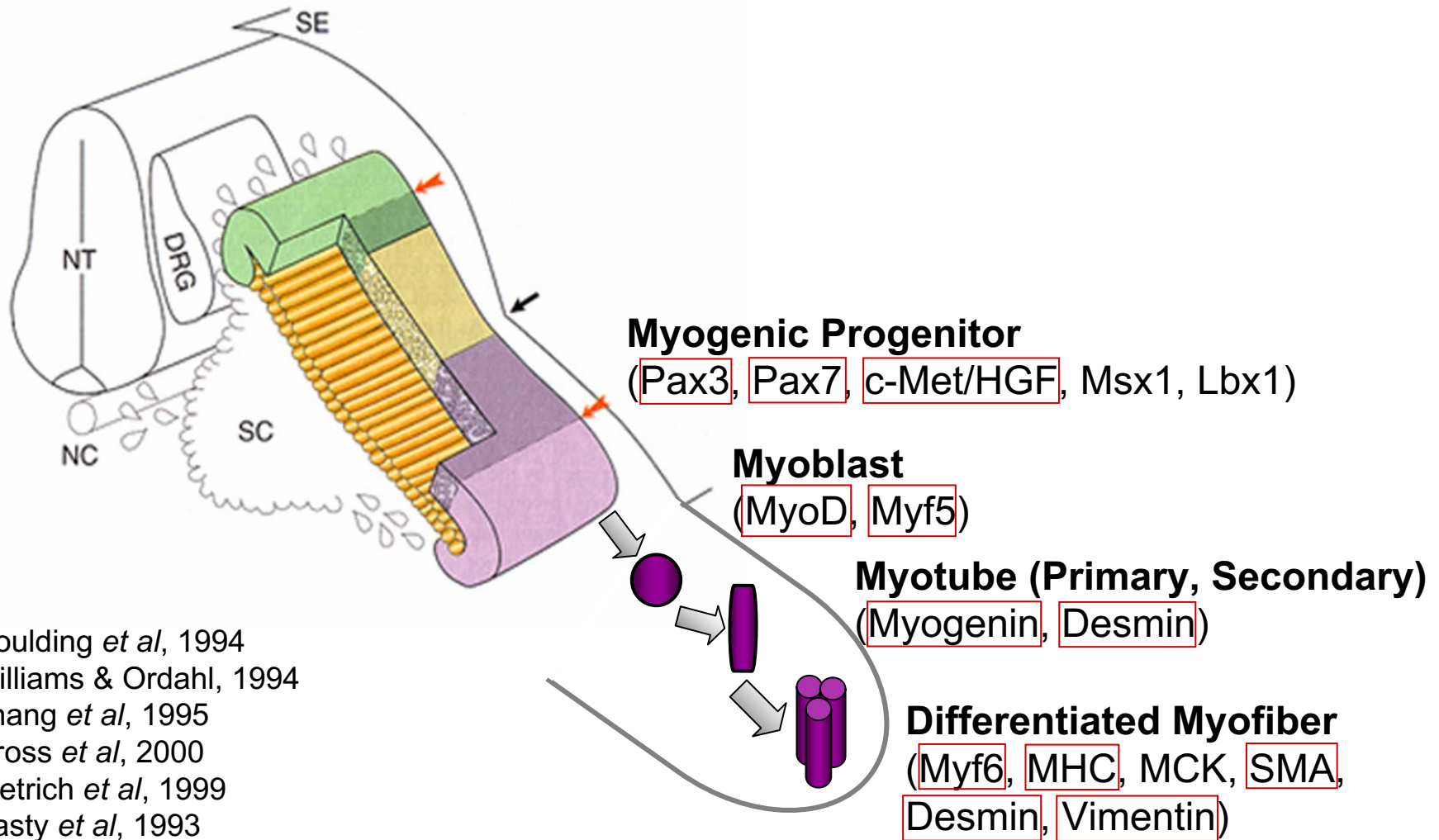




FKHR Integrates Repair Signals

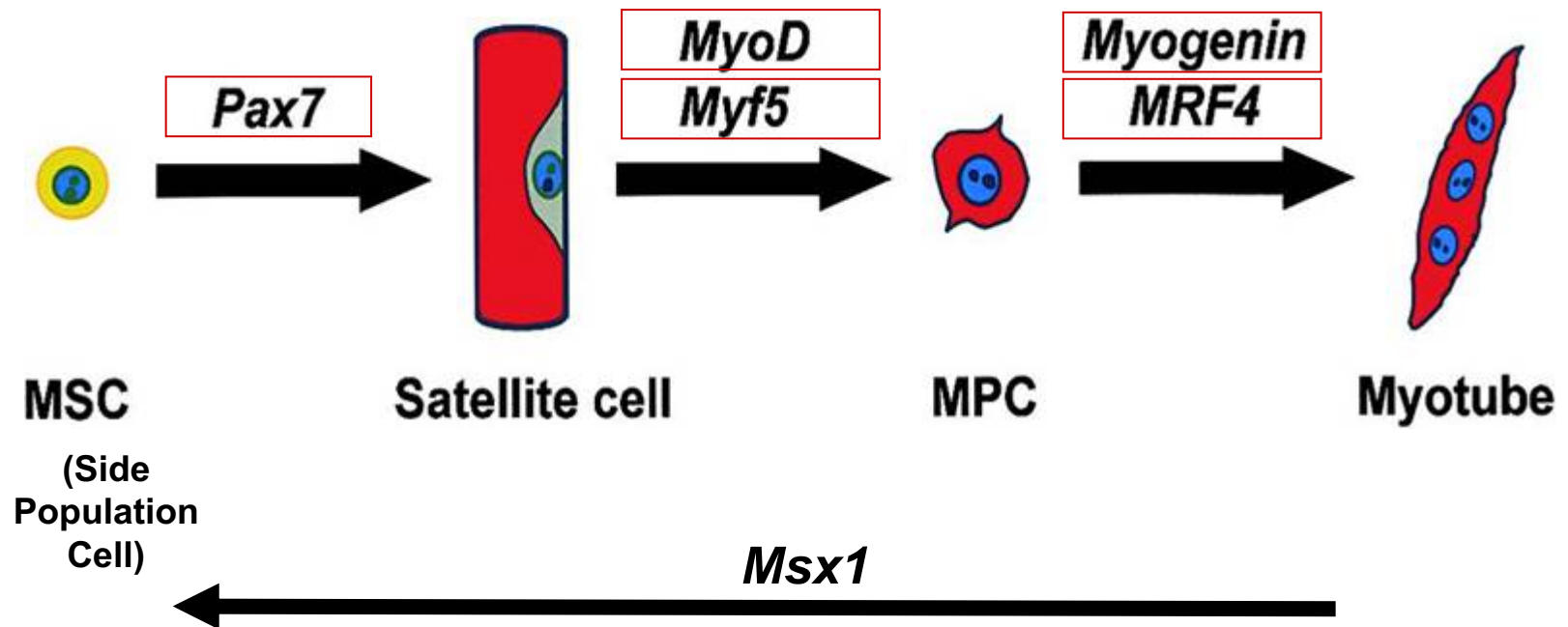


RMS Cells are not from a Distinct Embryonic Subset



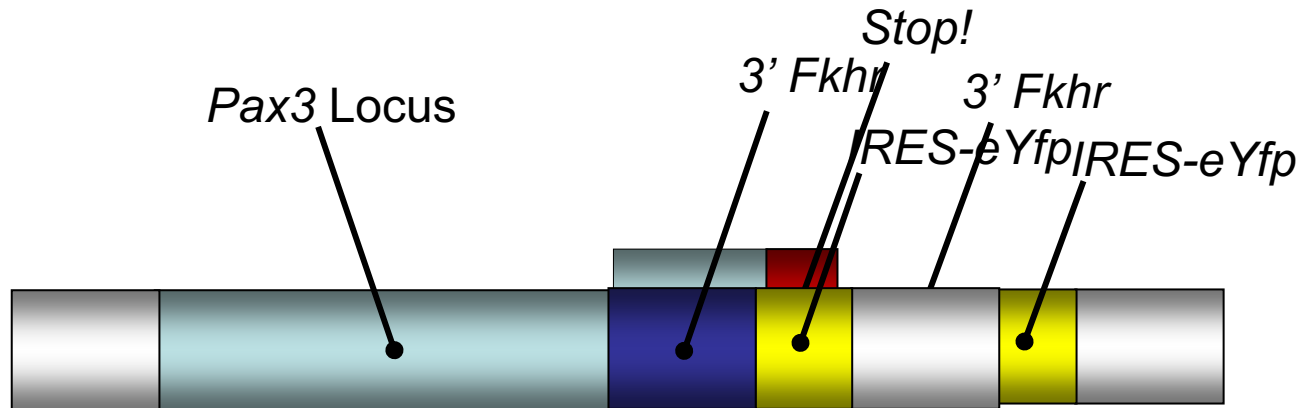
Goulding *et al*, 1994
Williams & Ordahl, 1994
Zhang *et al*, 1995
Gross *et al*, 2000
Dietrich *et al*, 1999
Hasty *et al*, 1993
Kablar *et al*, 1997
Schafer *et al*, 1994

RMS Cells are not from a Distinct Postembryonic Subset

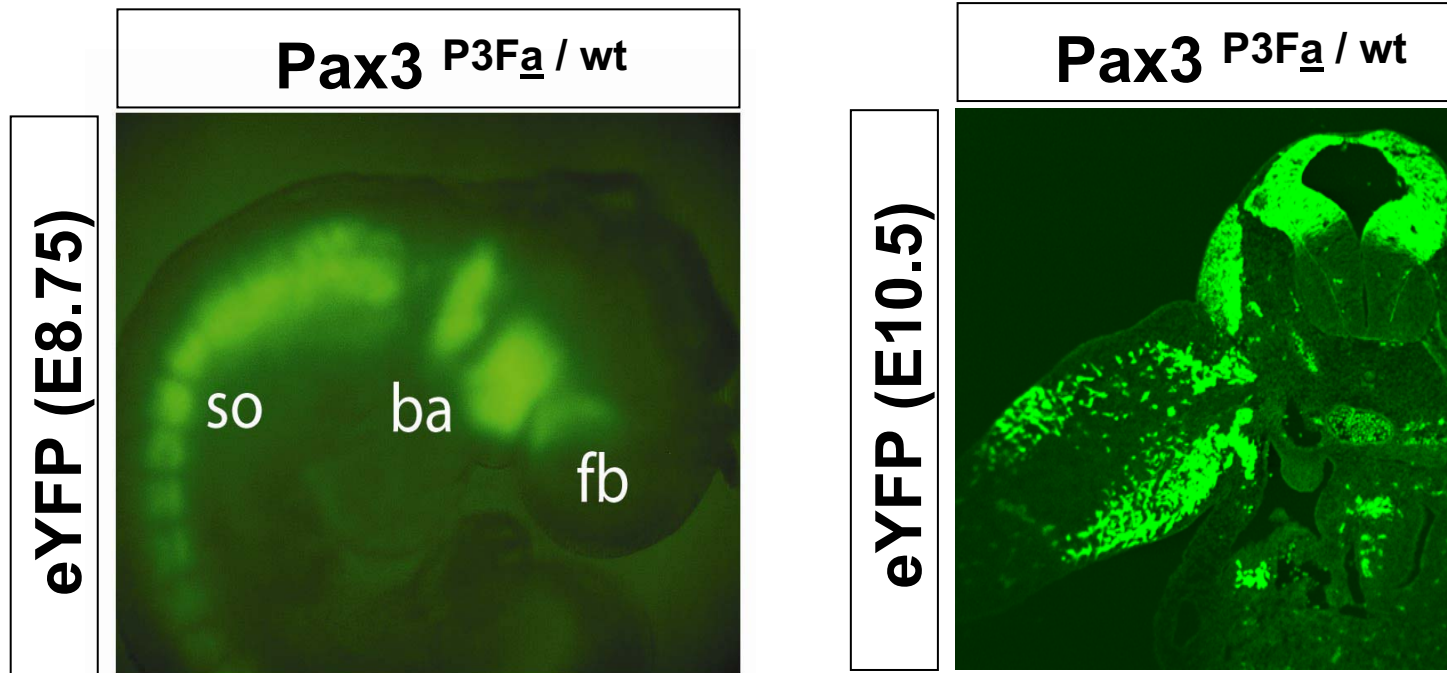


Pax3* becomes *Pax3:Fkhr

with Time- & Tissue Specificity



Pax3:Fkhr is expressed in the *Pax3* domain



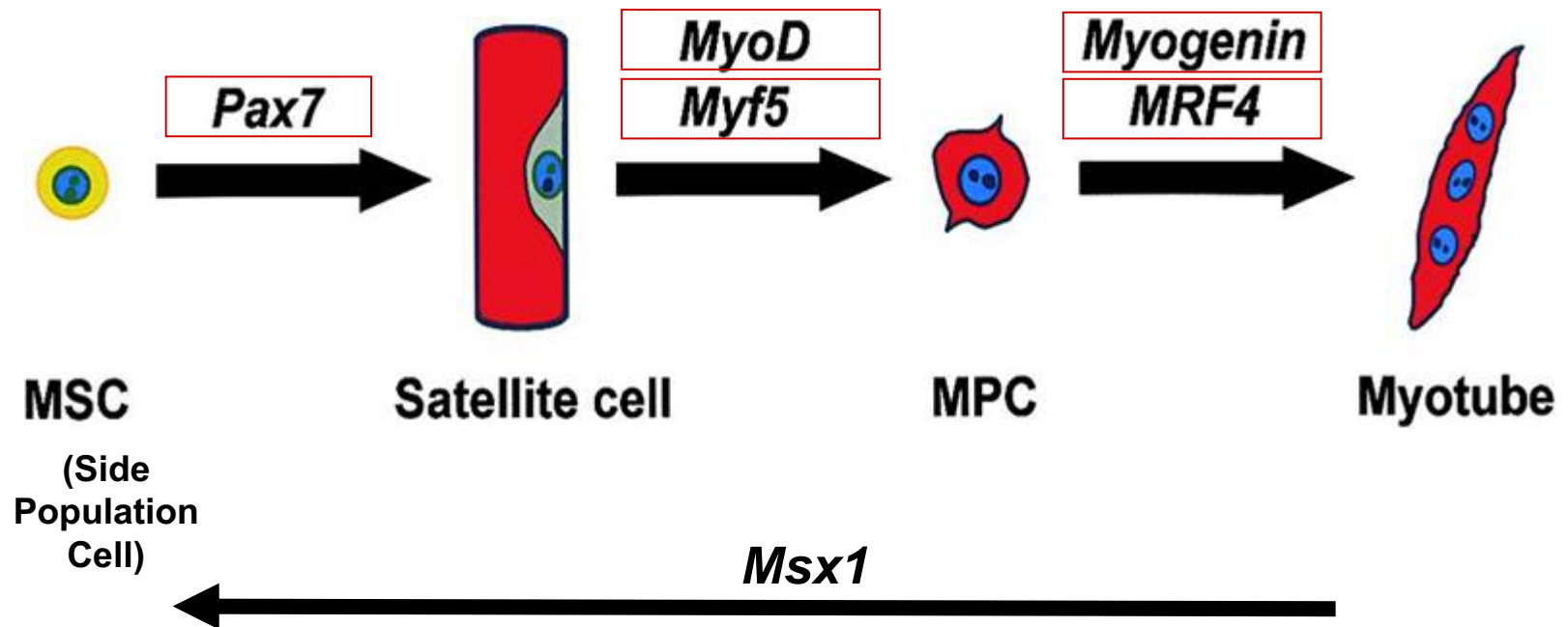


Pax3 P3Fm / wt

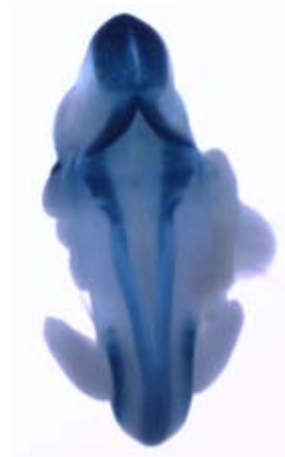
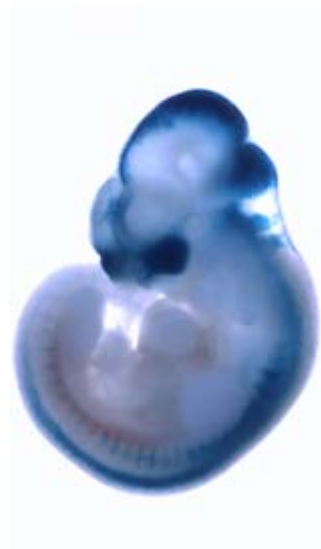
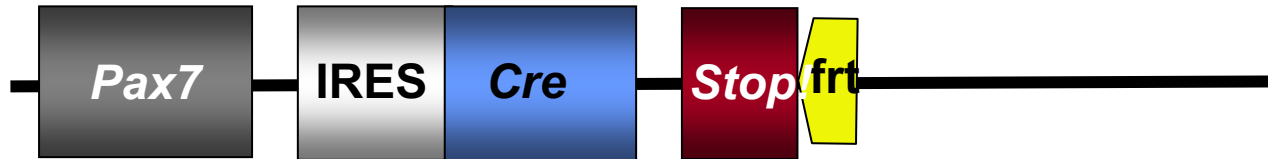


Pax3 P3Fa / wt

RMS Cells are not from a Distinct Postembryonic Subset

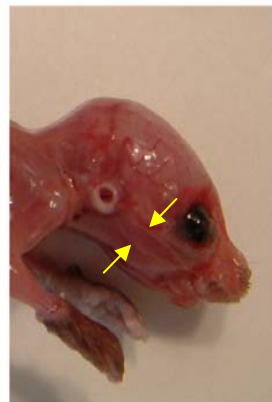


Hardwire *Pax7*-Cre





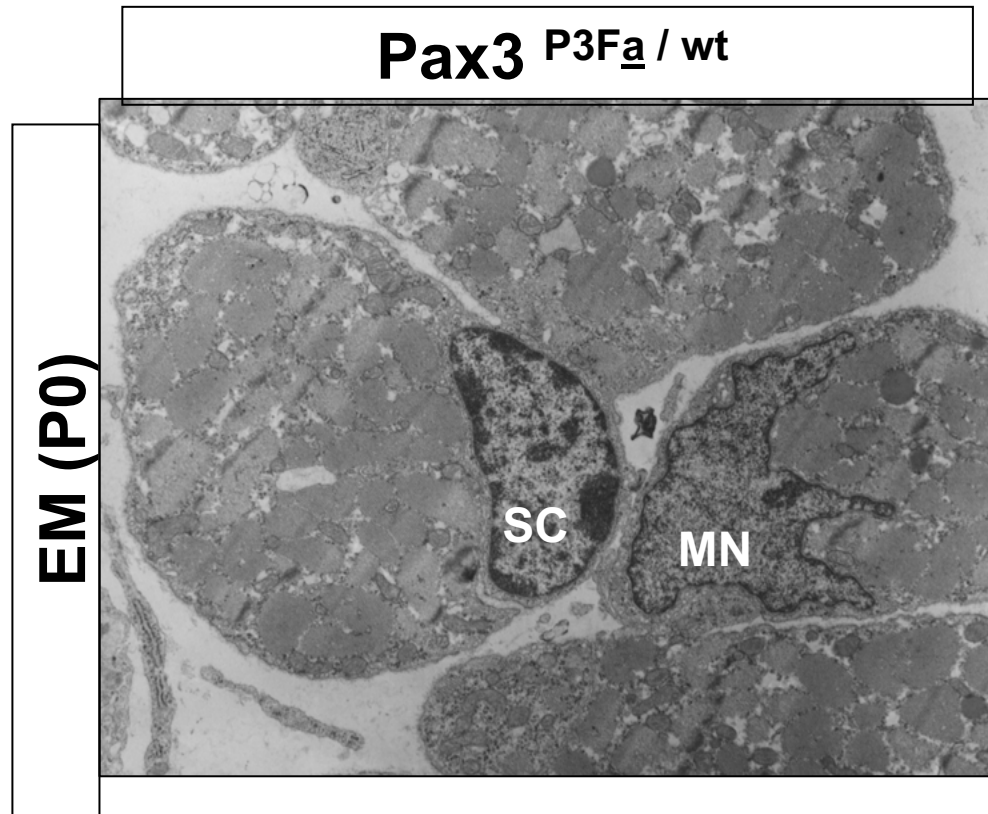
10.5 g
3 ~



Pax3 $P3F_{\underline{m}}$ / wt

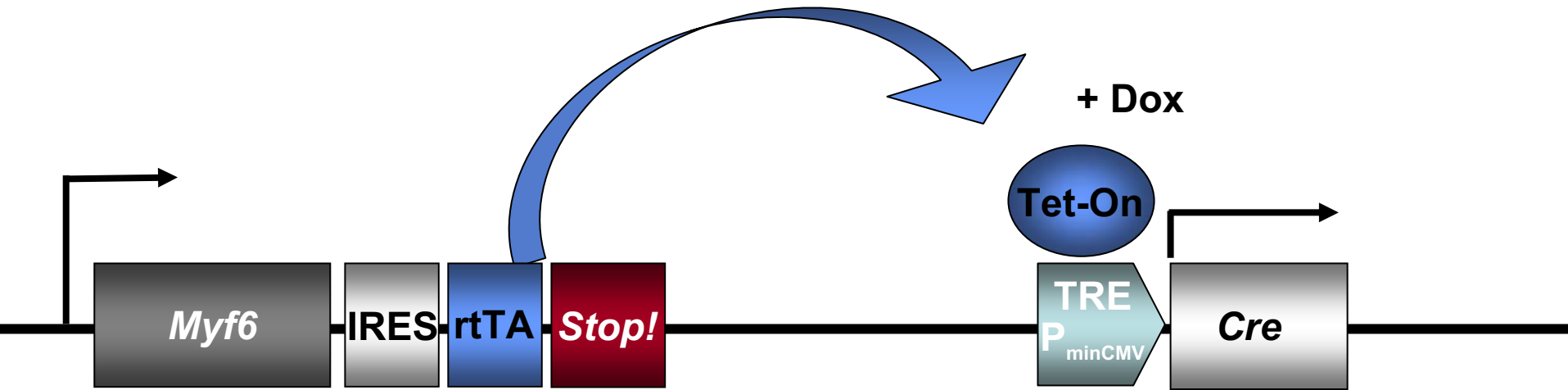
Pax3 $P3F_{\underline{a}}$ / wt
activated by
Pax7 $ICNm$ / wt

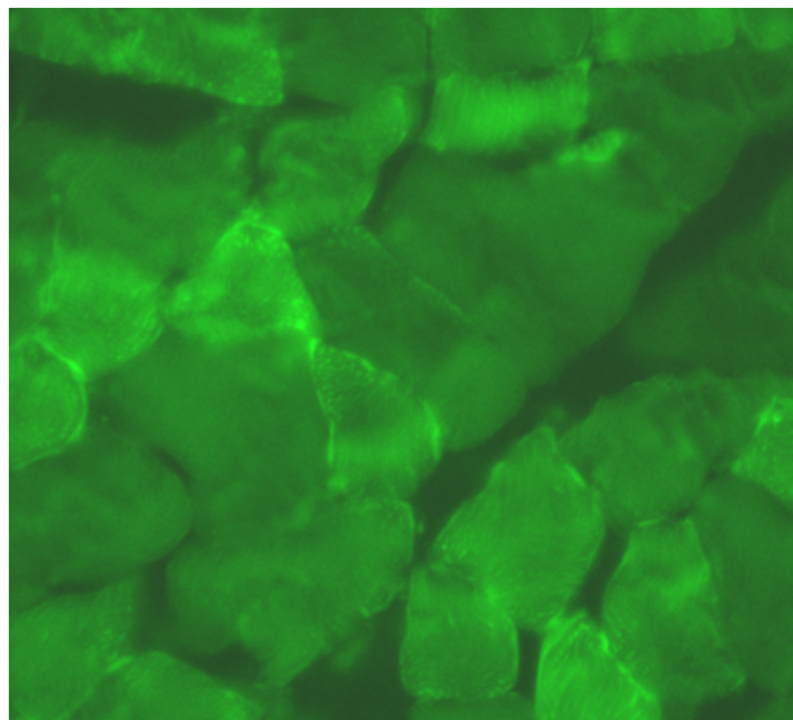
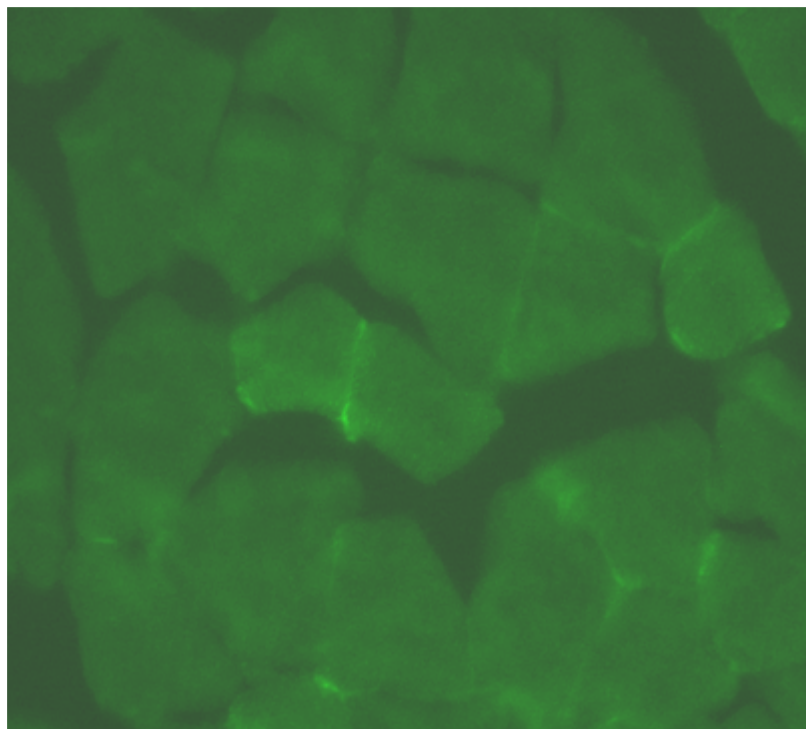
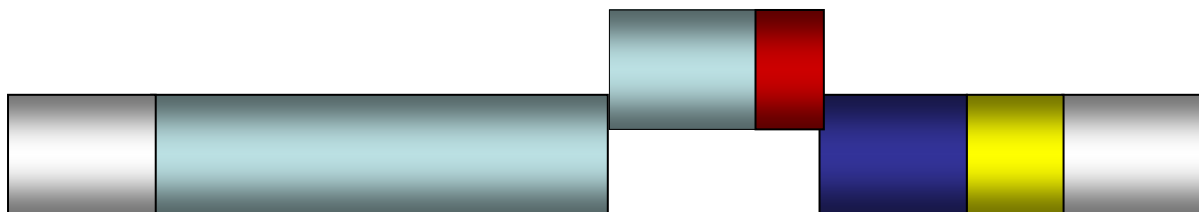
Satellite Cells are Present, but Reduced?



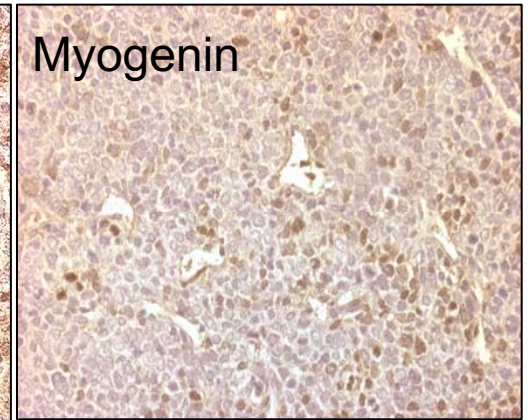
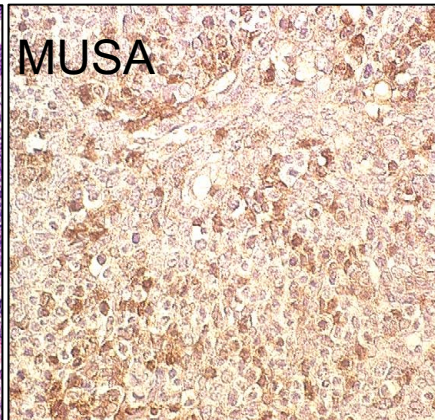
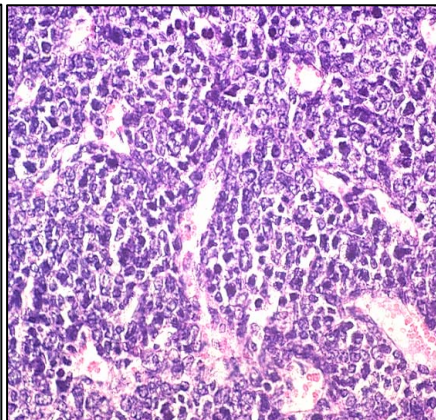
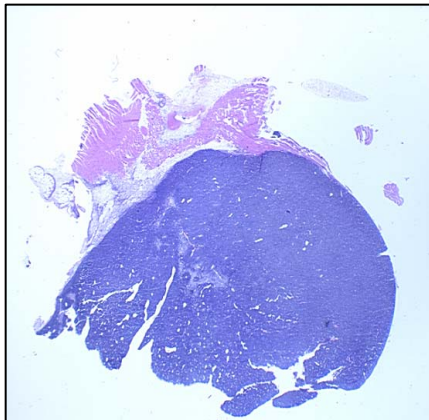
Despite the fusion gene Pax3:Fkhr
being expressed in every satellite cell
during and post fetal development
these mice did not develop
Alveolar Rhabdomyosarcomas.

Temporal, Muscle-Specific Conditional *Cre*

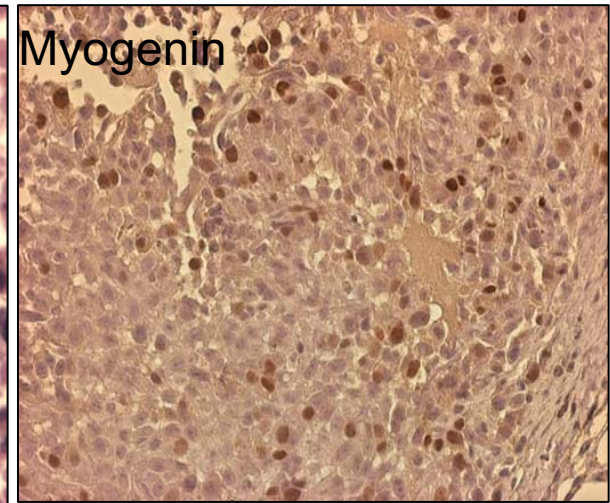
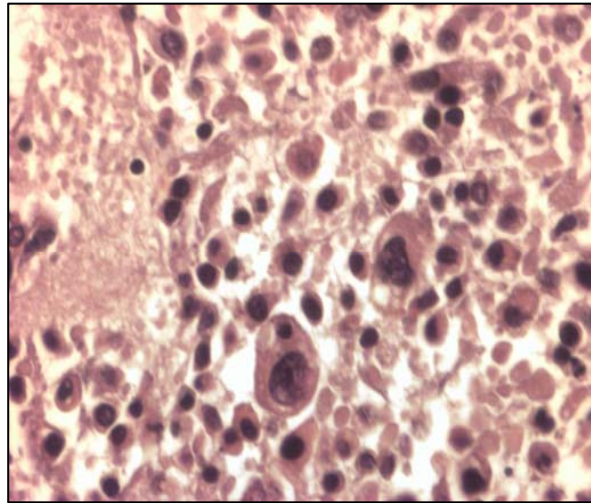
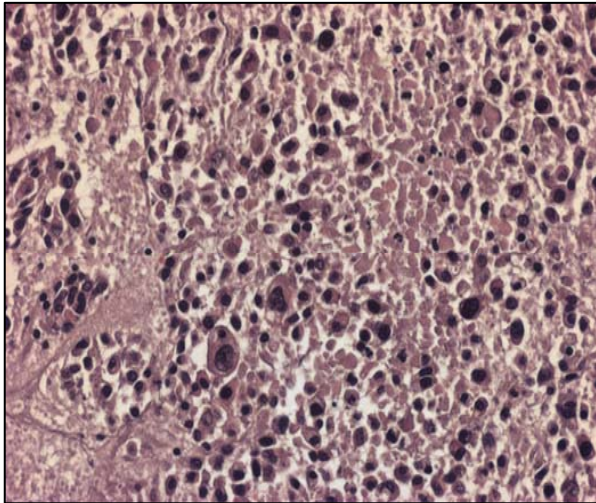
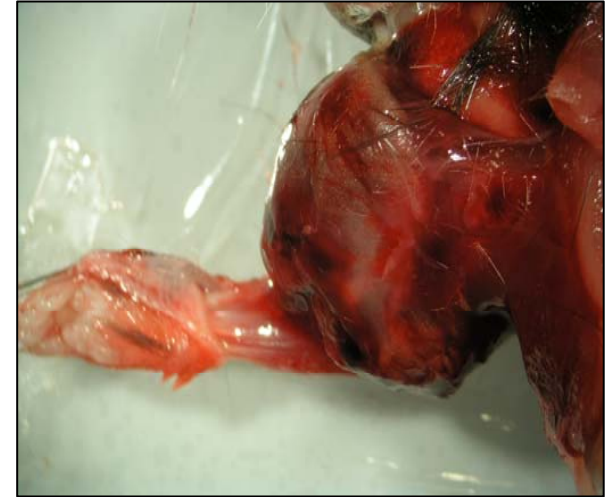
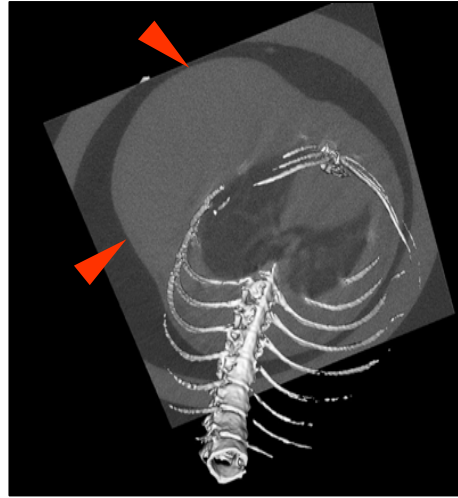
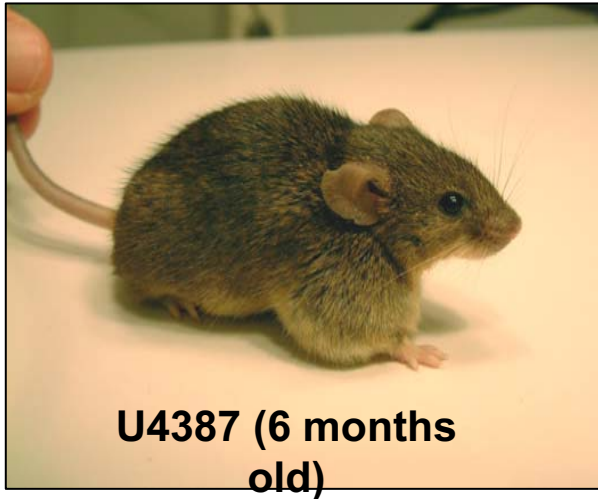




ARMS in Pax3^{P3Fm/WT} Myf6^{tCreM/WT} mice



ARMS in Pax3^{P3Fm/P3Fm} Myf6^{ICNm/wt} Trp53^{F2-10/wt} mice



Developing a Mouse Model of Synovial Sarcoma