

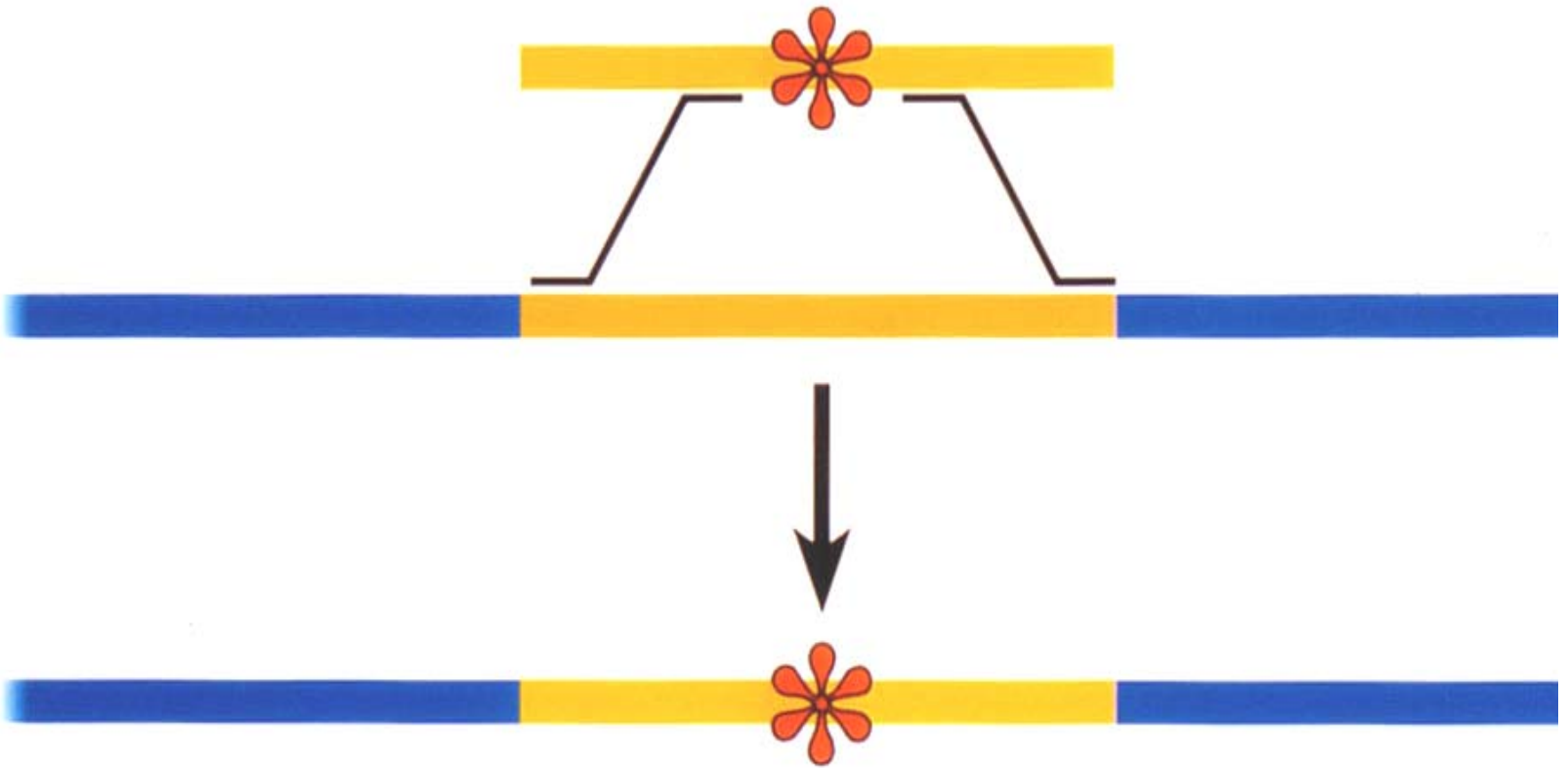
# Nobel Lecture

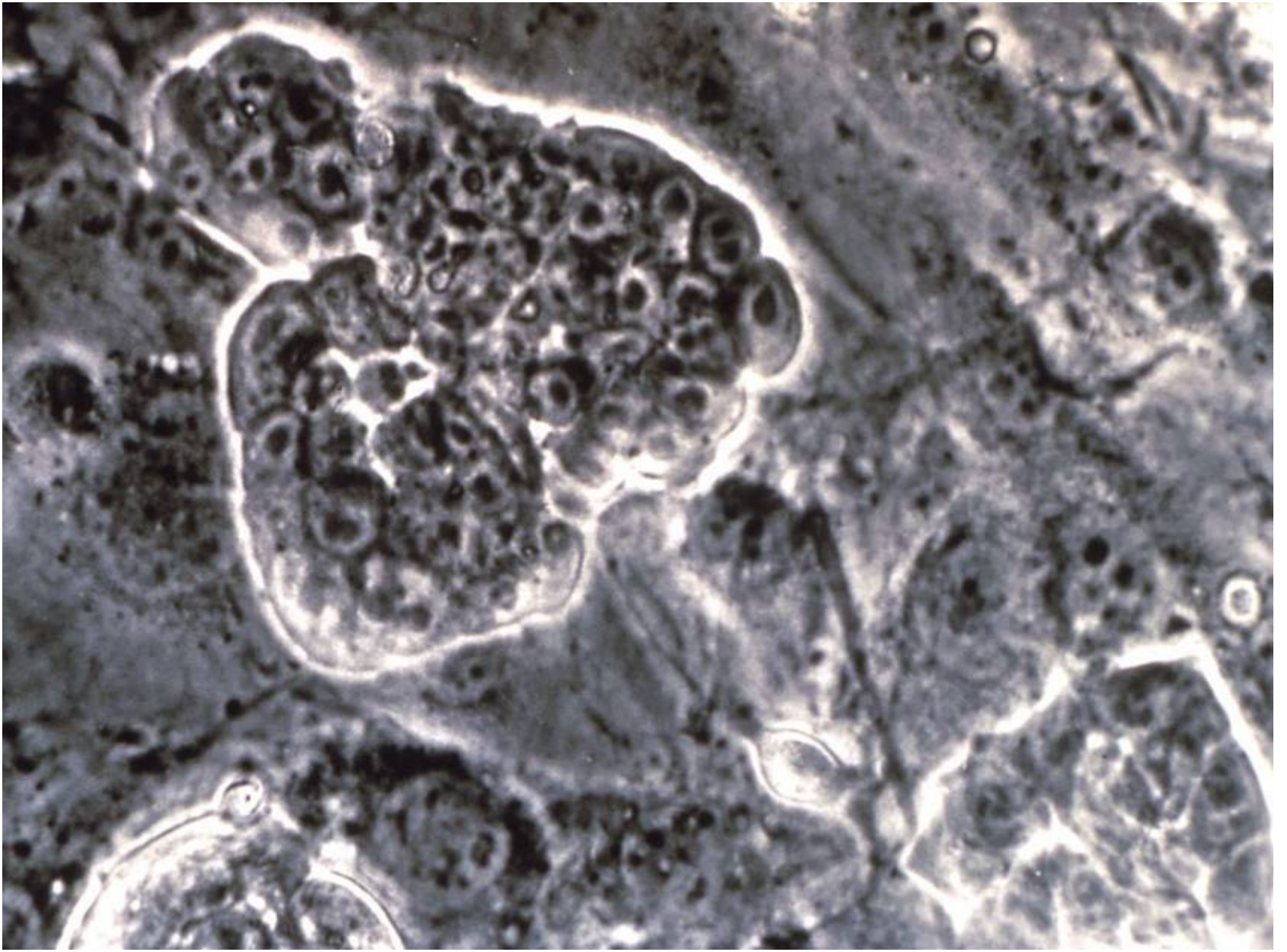
## Gene Targeting into the 21<sup>st</sup> Century

Mario R. Capecchi

Howard Hughes Medical Institute  
Department of Human Genetics  
University of Utah School of Medicine

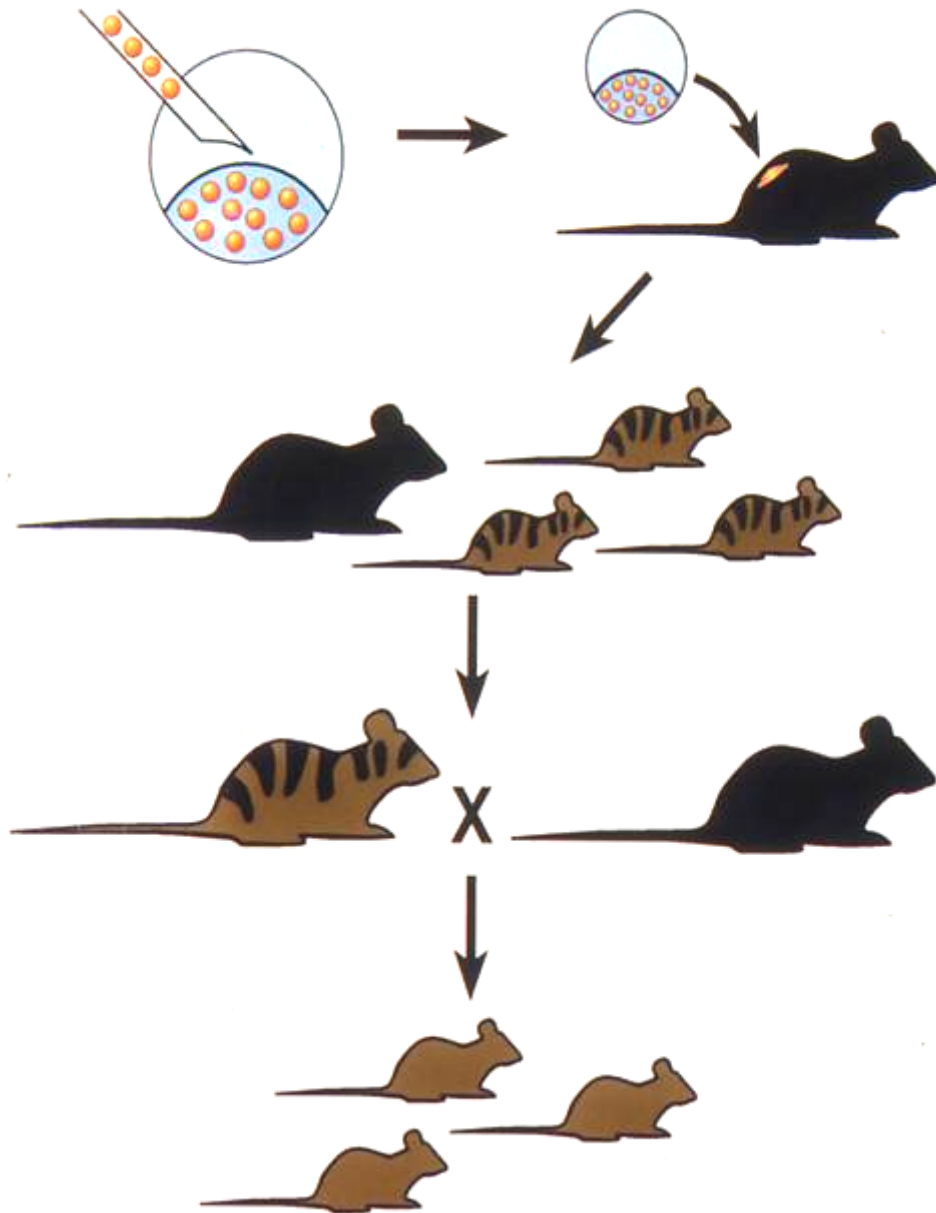
December 7<sup>th</sup>, 2007

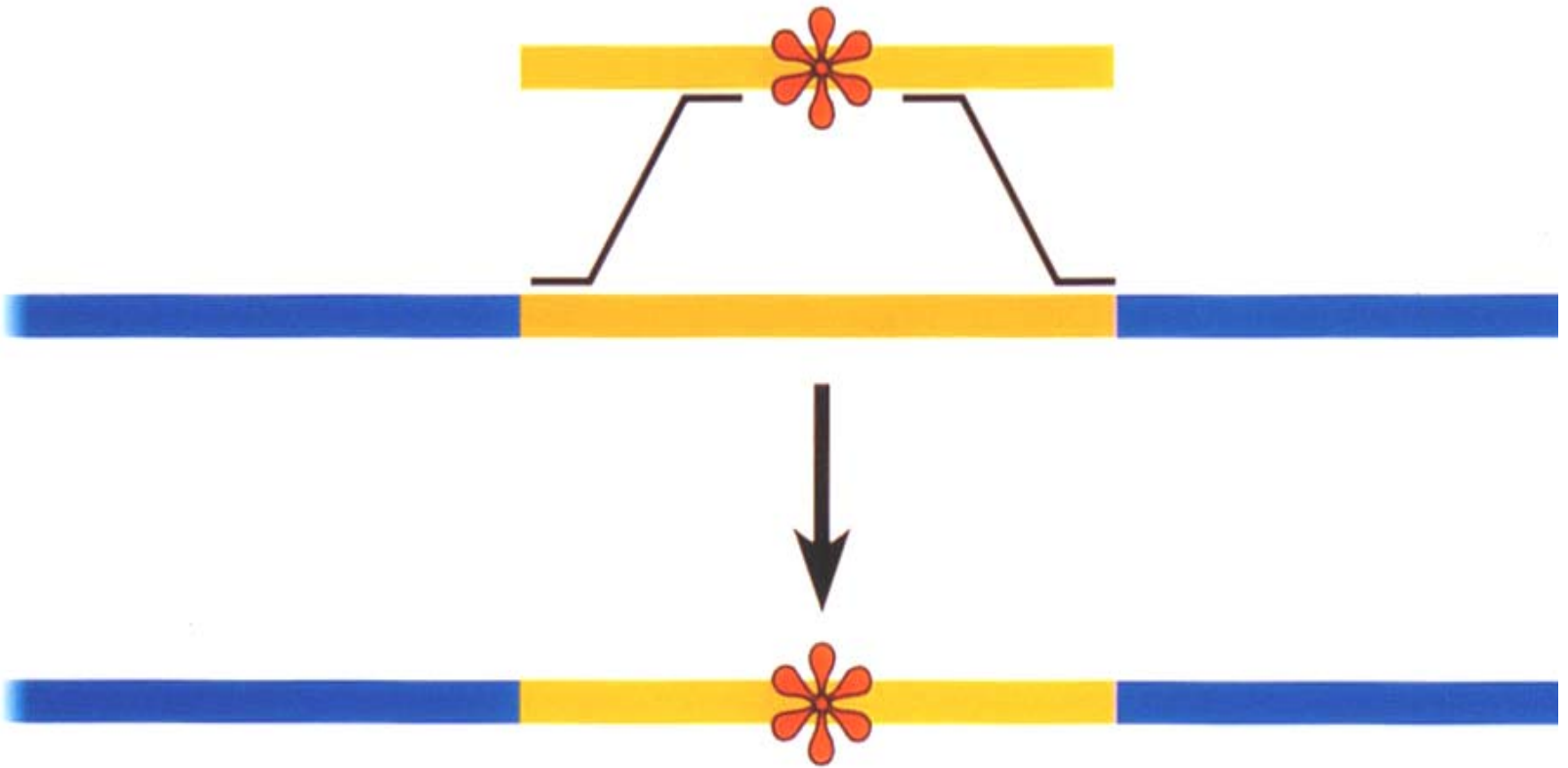




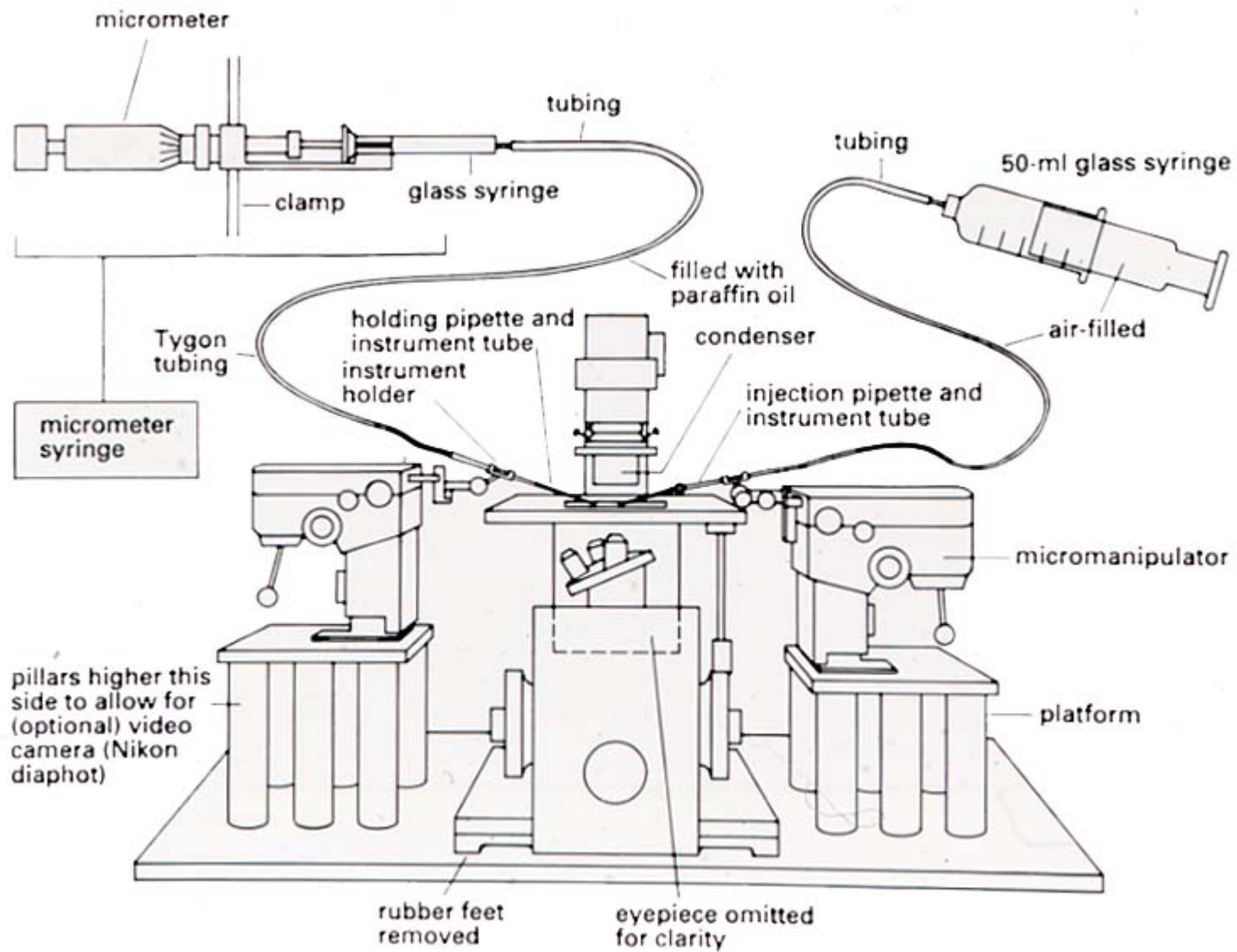


## Generation of Germ Line Chimeras



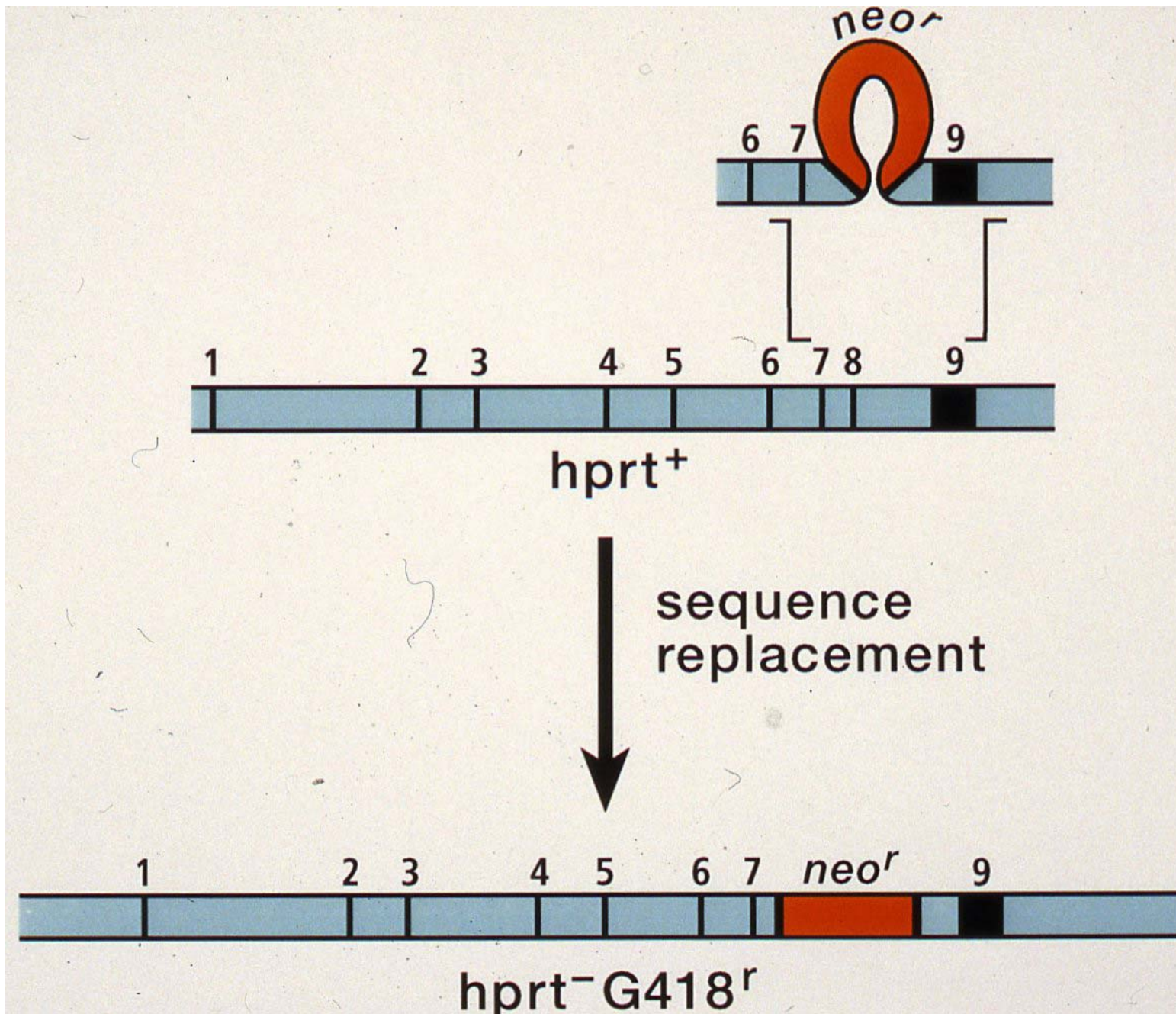


**How did the idea to  
do Gene Targeting  
arise?**

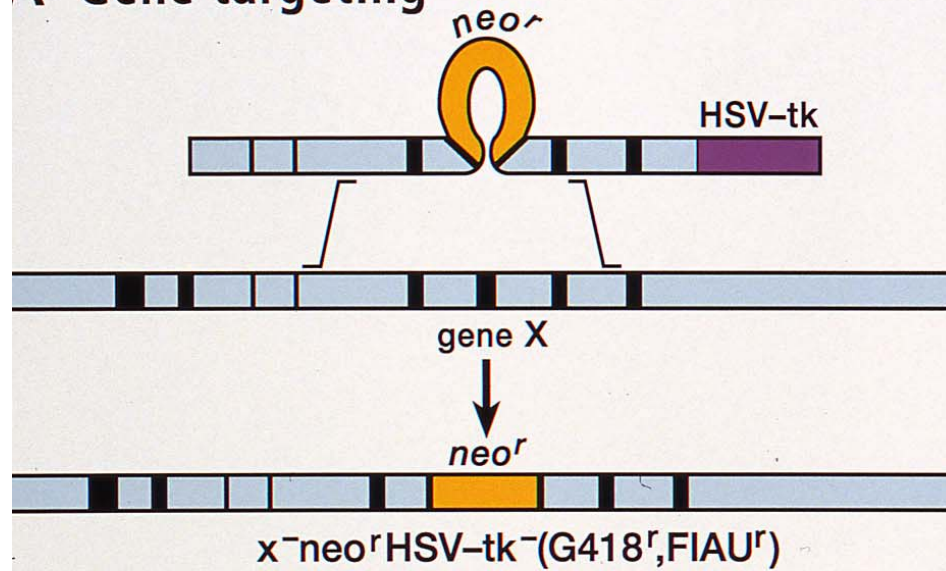




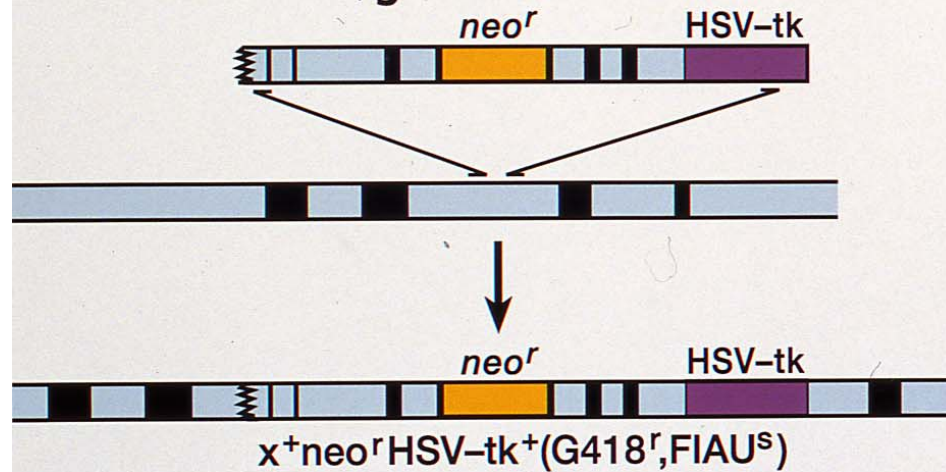




## A Gene targeting



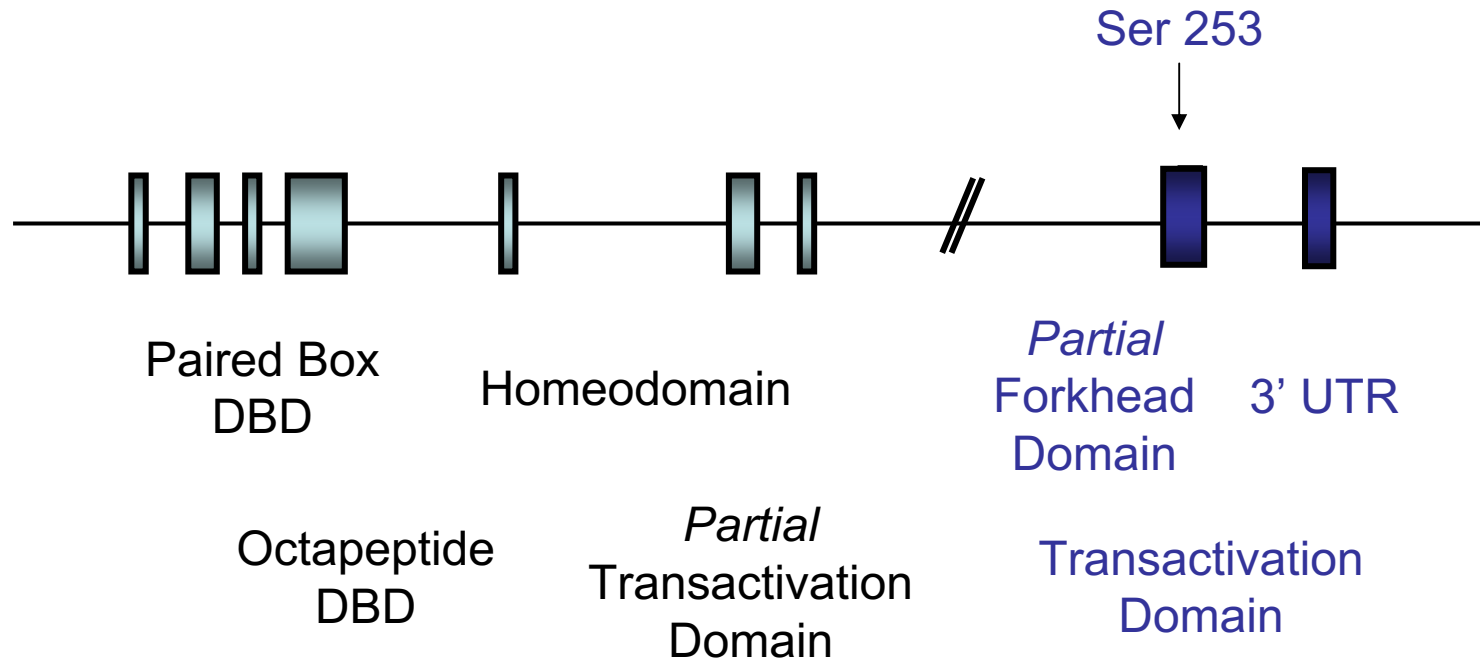
## B Random integration



# Considerations for Models

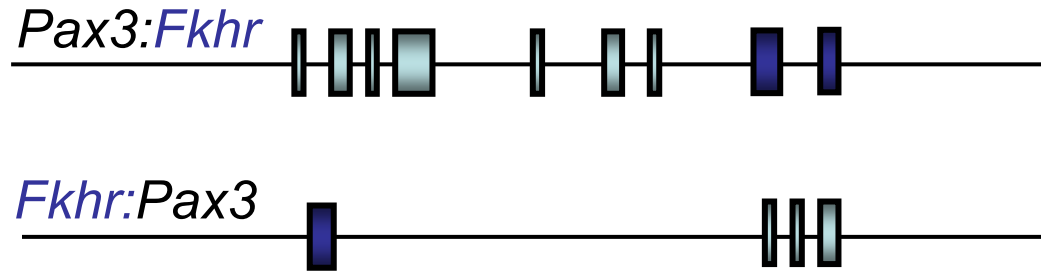
1. Inducing Event
2. Time of Induction
3. Stoichiometry
4. Micro-molecular  
and -cellular environment

# PAX3-FKHR Structure



# **Modeling Alveolar Rhabdomyosarcoma**

# *What's Important?*



*Pax3:Fkhr* gene is created.

*Fkhr:Pax3* is created.

*Pax3* allele is destroyed.

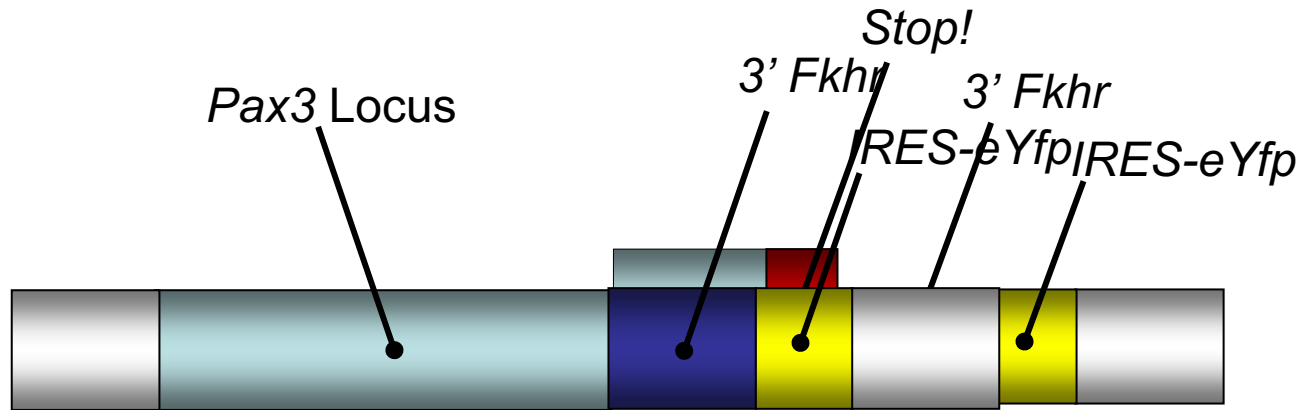
*Fkhr* allele is destroyed.

# **Chromosomal Translocations:**

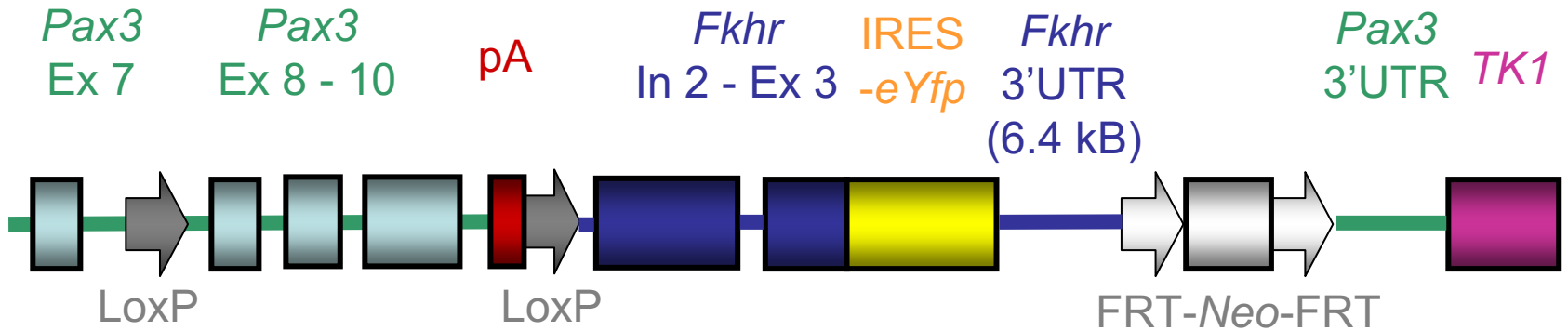
**Why not generate real  
translocations?**



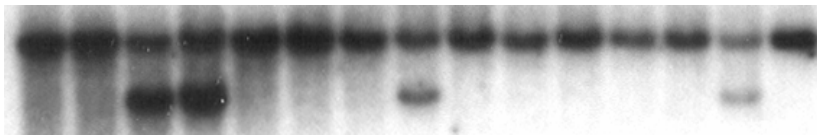
# *Pax3* becomes *Pax3:Fkhr* with Time- & Tissue Specificity



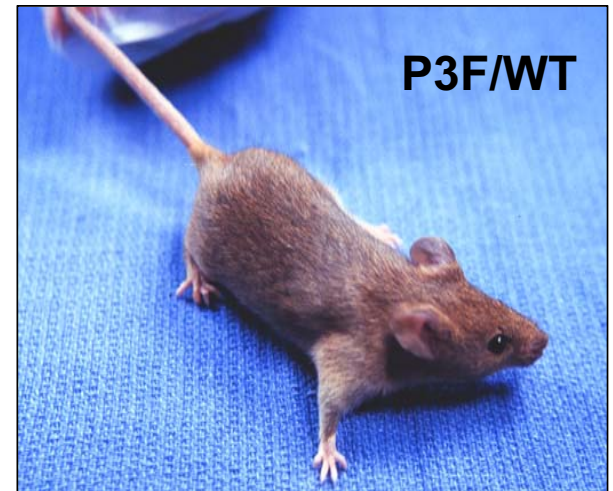
# Conditional Knock-In Construct

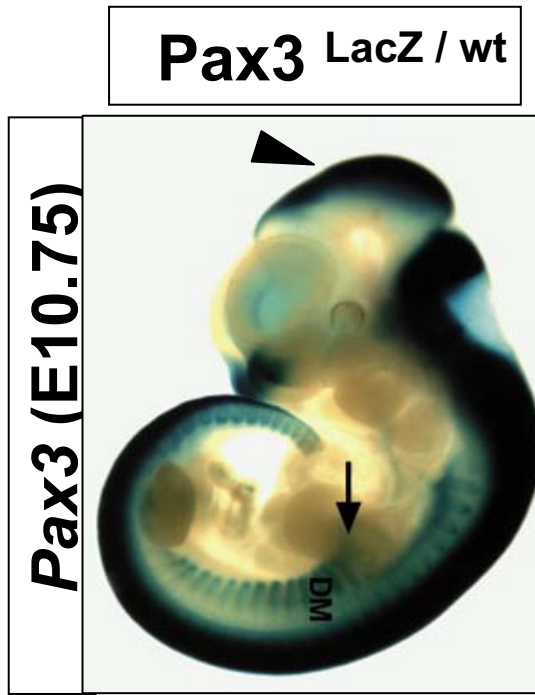


Germline Offspring

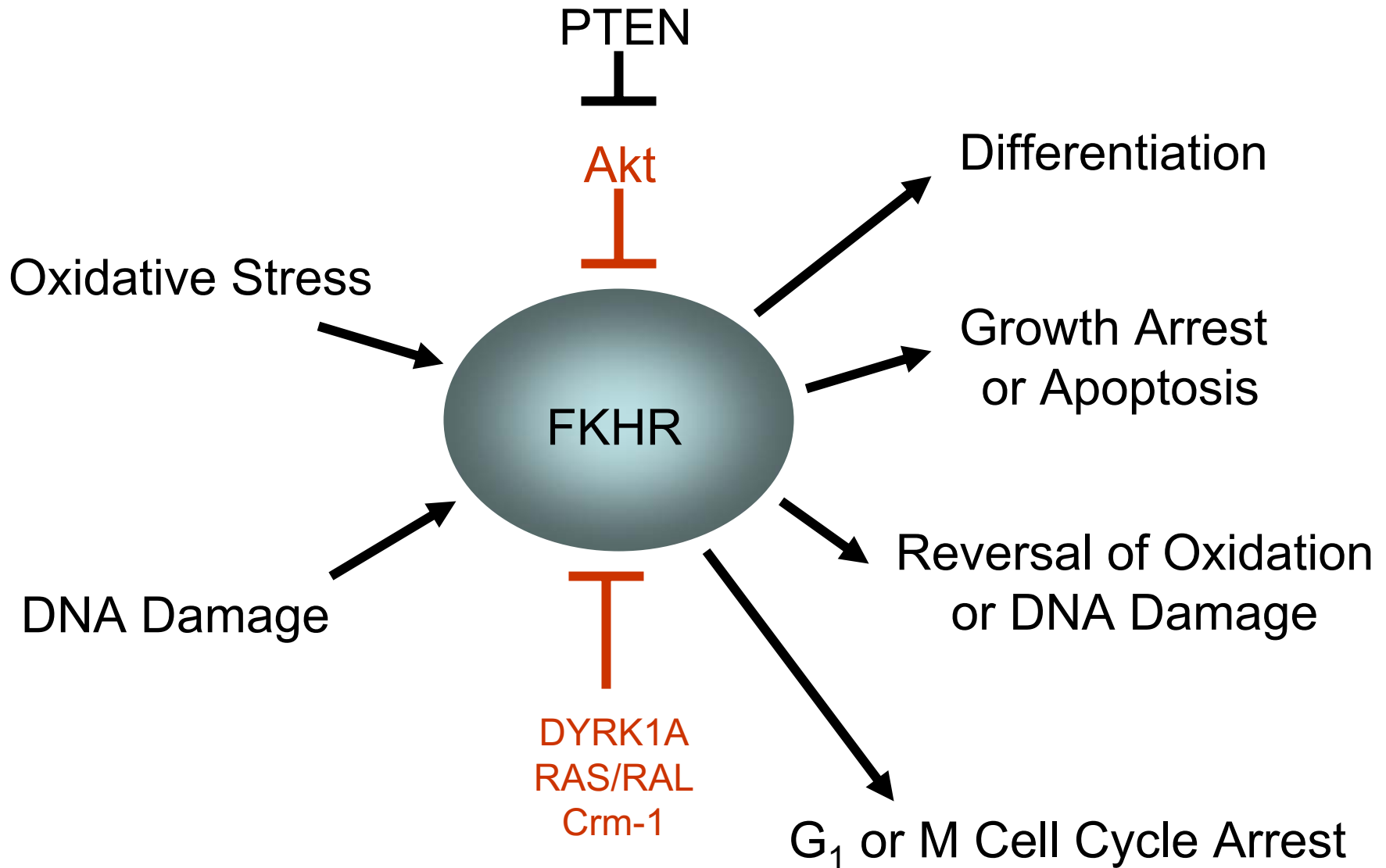


← WT  
← P3F

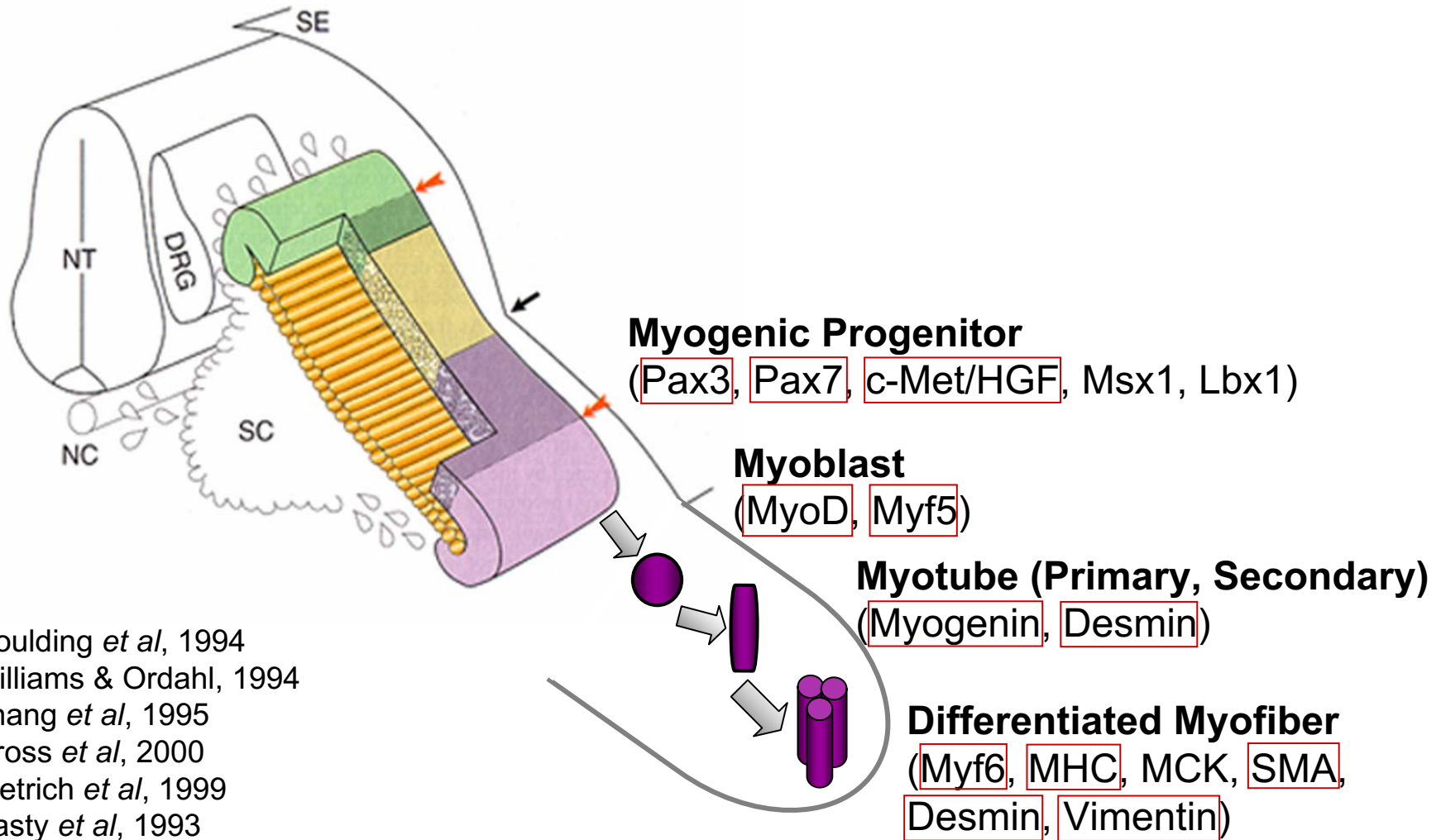




# FKHR Integrates Repair Signals

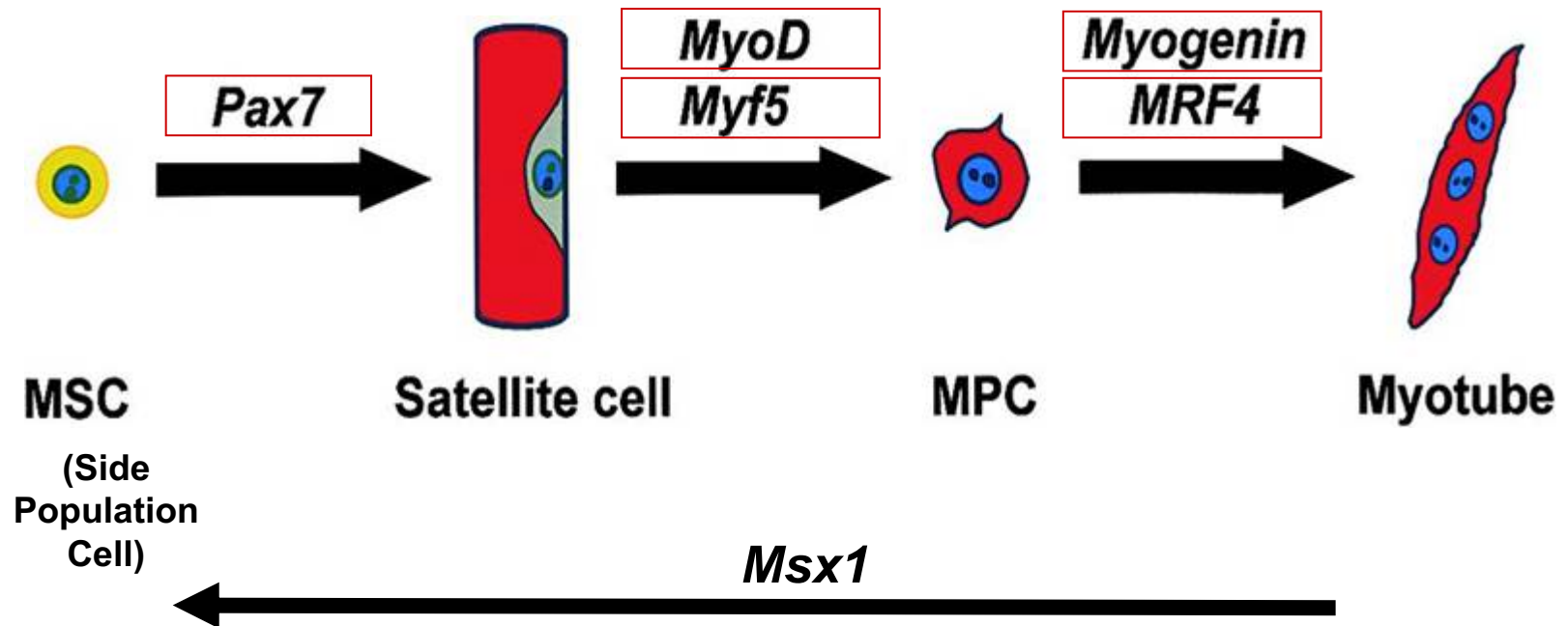


# RMS Cells are not from a Distinct Embryonic Subset

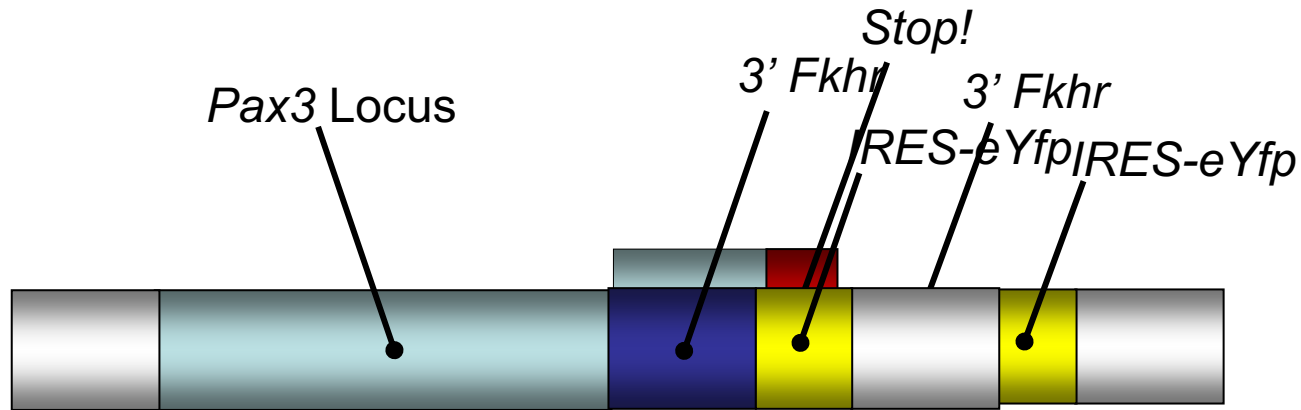


Goulding *et al*, 1994  
Williams & Ordahl, 1994  
Zhang *et al*, 1995  
Gross *et al*, 2000  
Dietrich *et al*, 1999  
Hasty *et al*, 1993  
Kablar *et al*, 1997  
Schafer *et al*, 1994

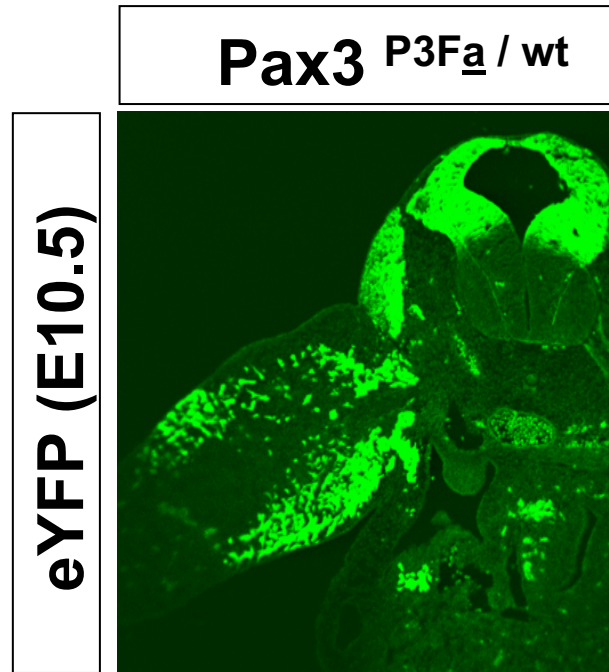
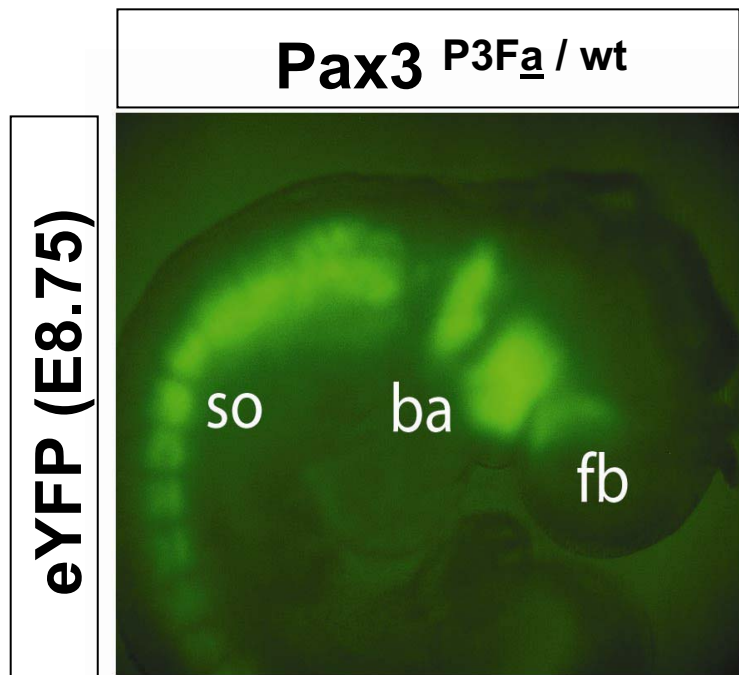
# RMS Cells are not from a Distinct Postembryonic Subset



# *Pax3* becomes *Pax3:Fkhr* with Time- & Tissue Specificity



# Pax3:Fkhr is expressed in the *Pax3* domain





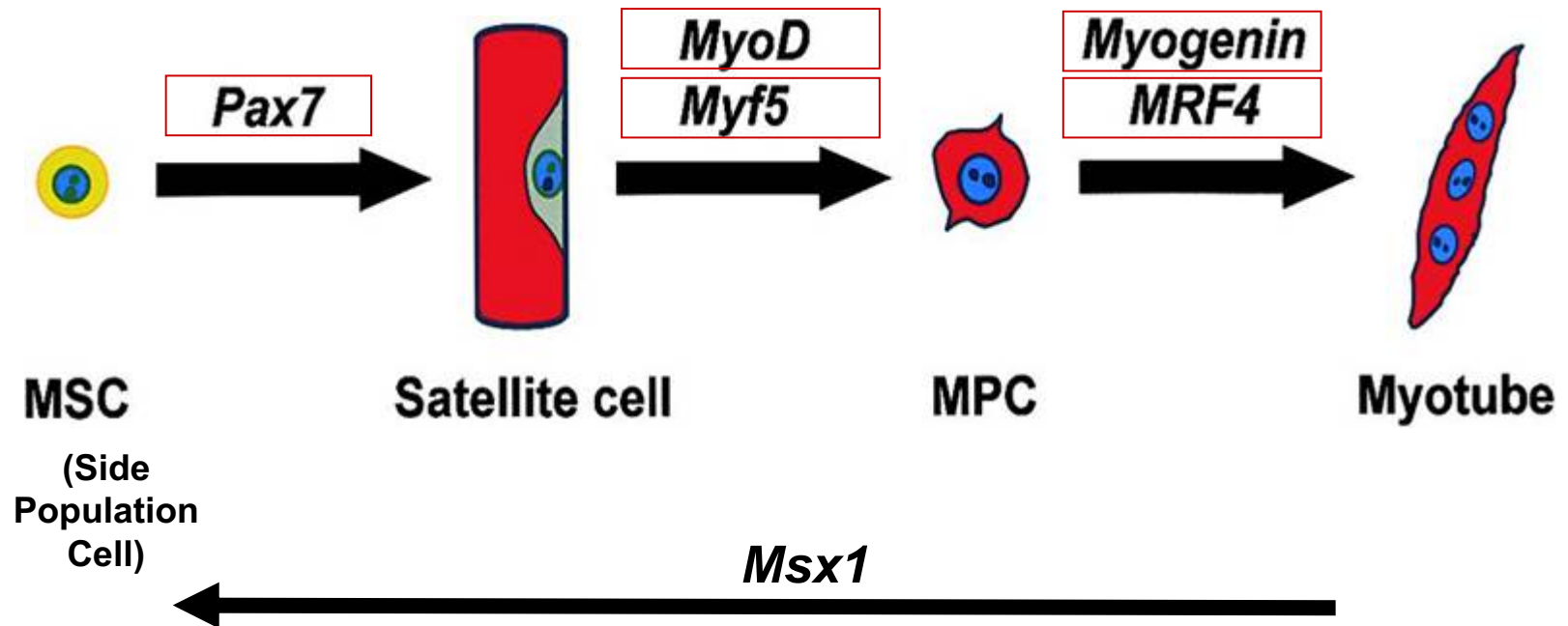


**Pax3** P3Fm / wt

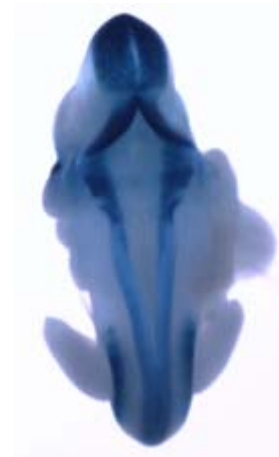
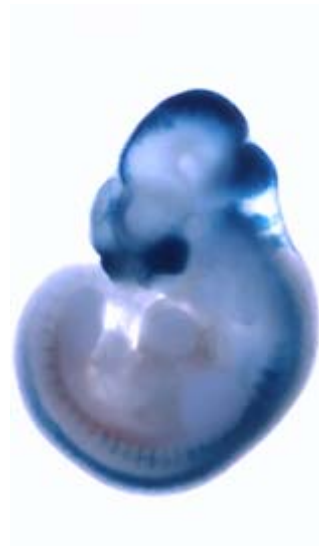
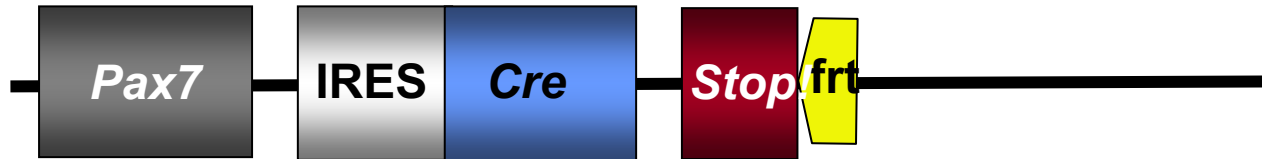


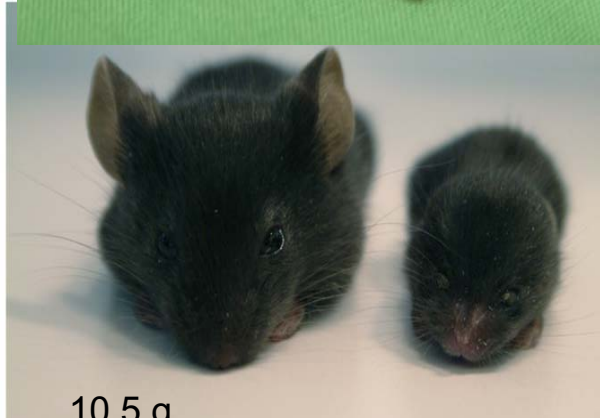
**Pax3** P3F $\underline{a}$  / wt

# RMS Cells are not from a Distinct Postembryonic Subset



# Hardwire *Pax7-Cre*

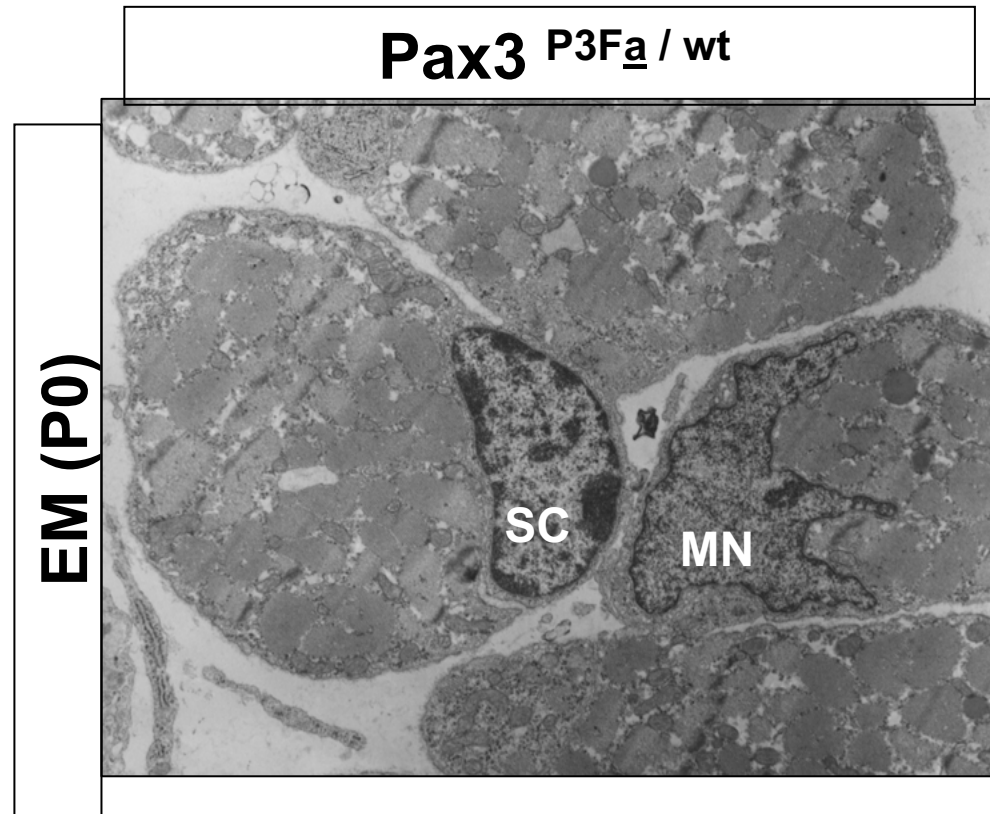




**Pax3** P3Fm / wt

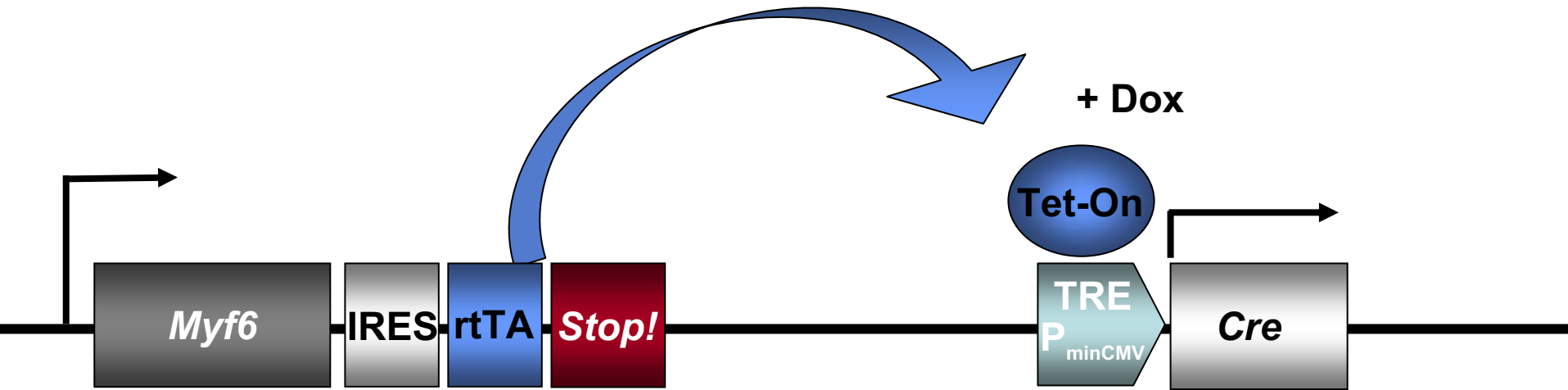
**Pax3** P3Fa / wt  
*activated by*  
**Pax7** ICNm / wt

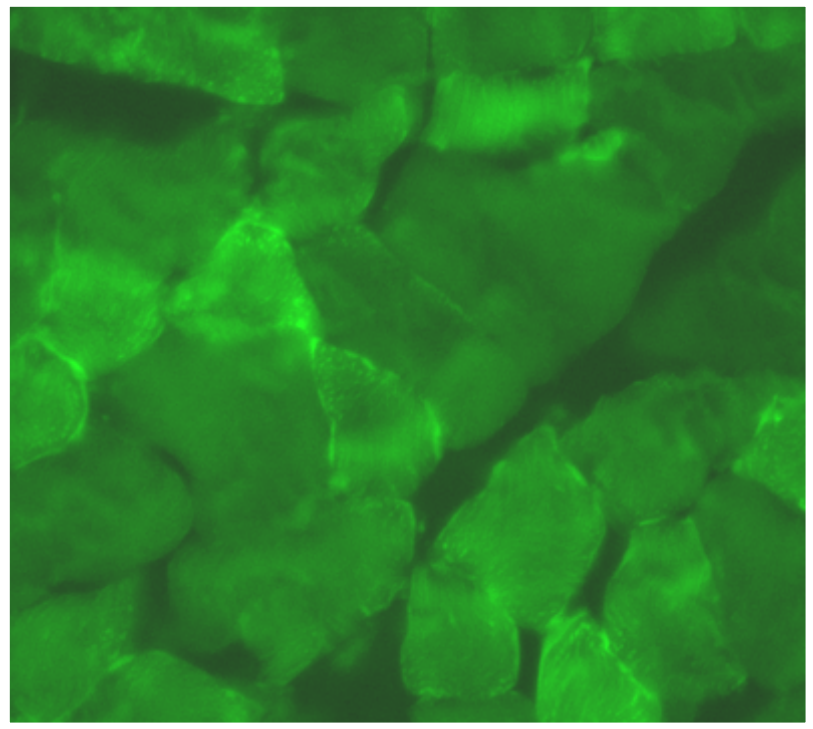
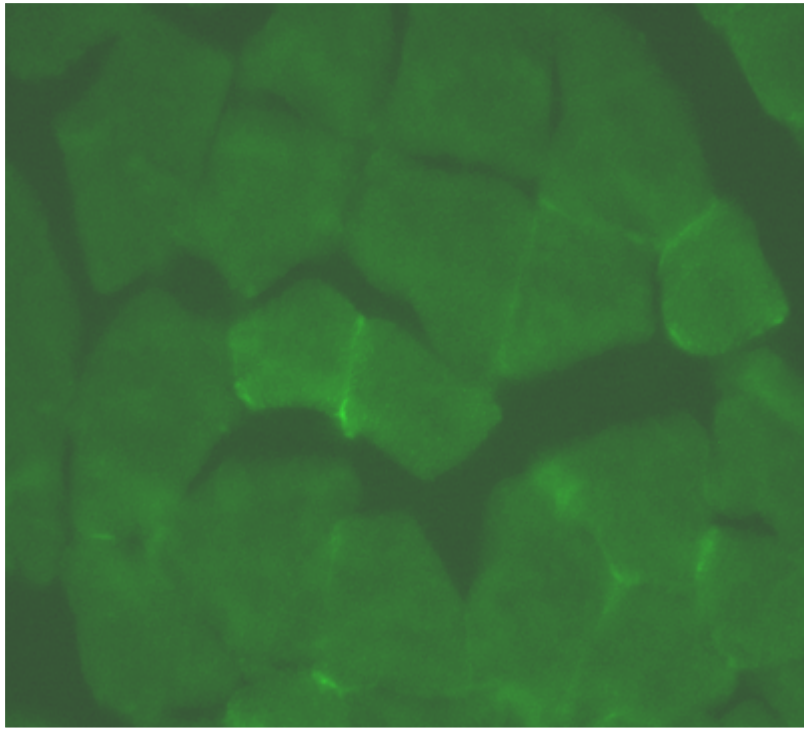
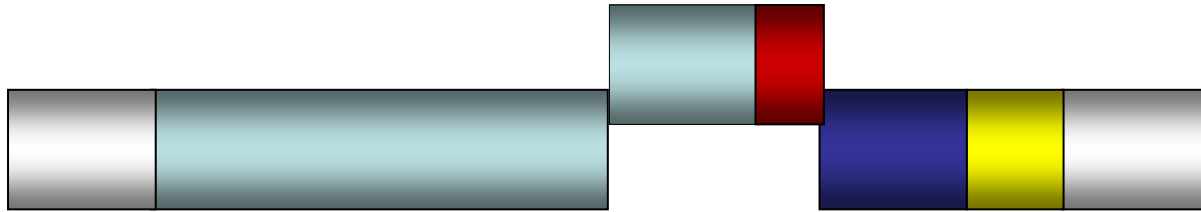
# Satellite Cells are Present, but Reduced?



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

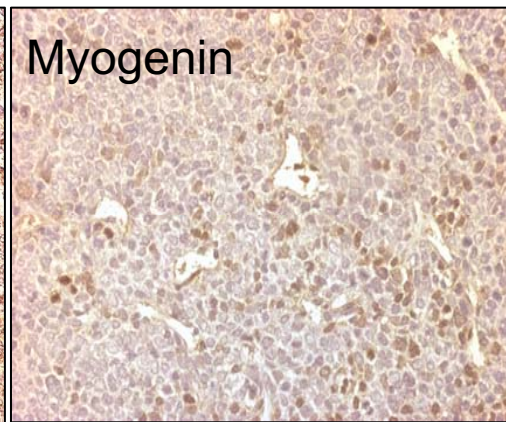
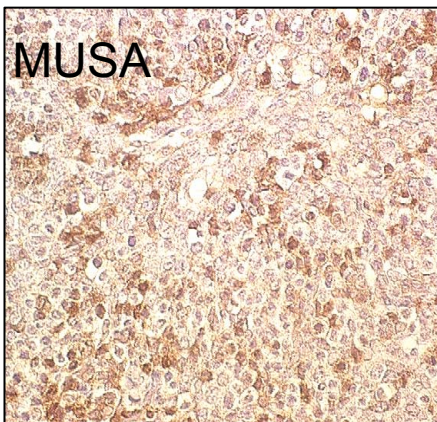
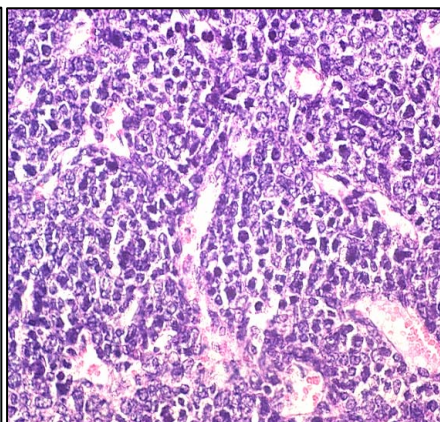
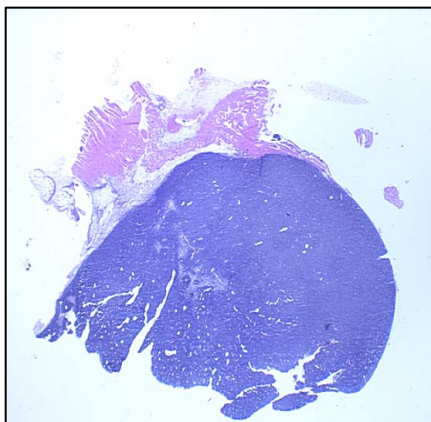
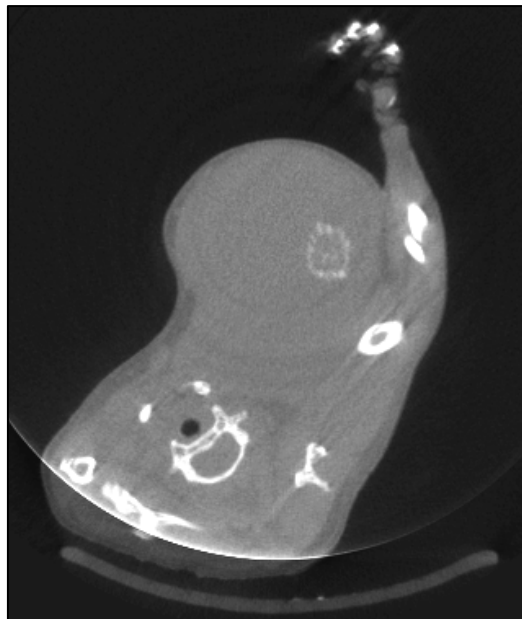
# Temporal, Muscle-Specific Conditional *Cre*



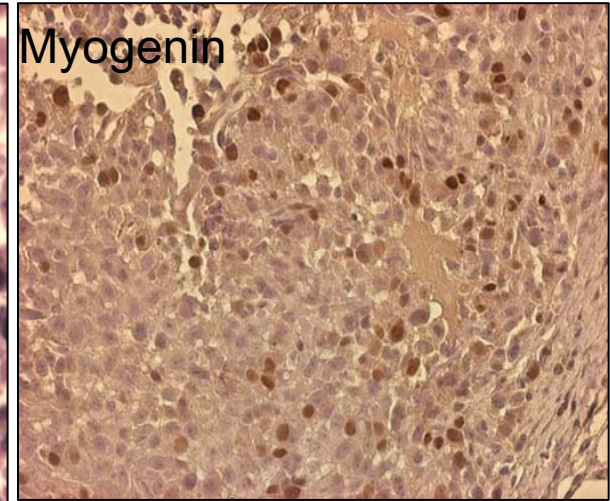
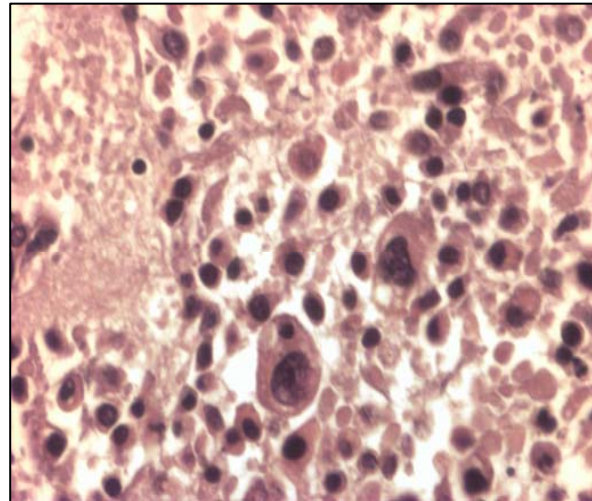
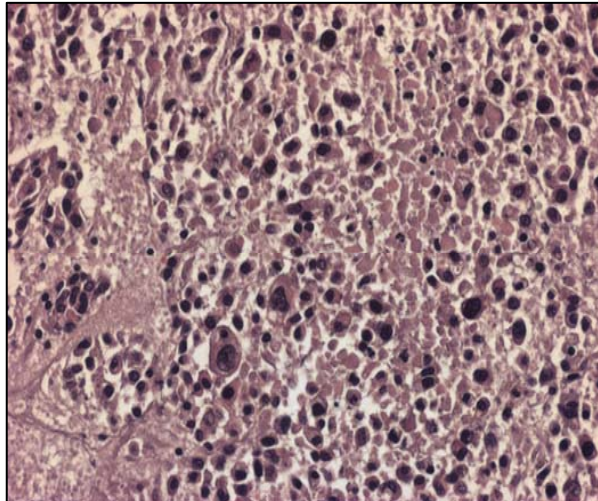
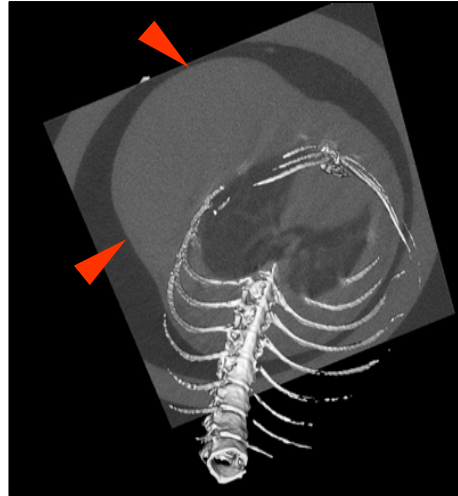




# ARMS in Pax3<sup>P3Fm/WT</sup> Myf6<sup>tCreM/WT</sup> mice



# ARMS in Pax3<sup>P3Fm/P3Fm</sup> Myf6<sup>ICNm/wt</sup> Trp53<sup>F2-10/wt</sup> mice



# **Developing a Mouse Model of Synovial Sarcoma**