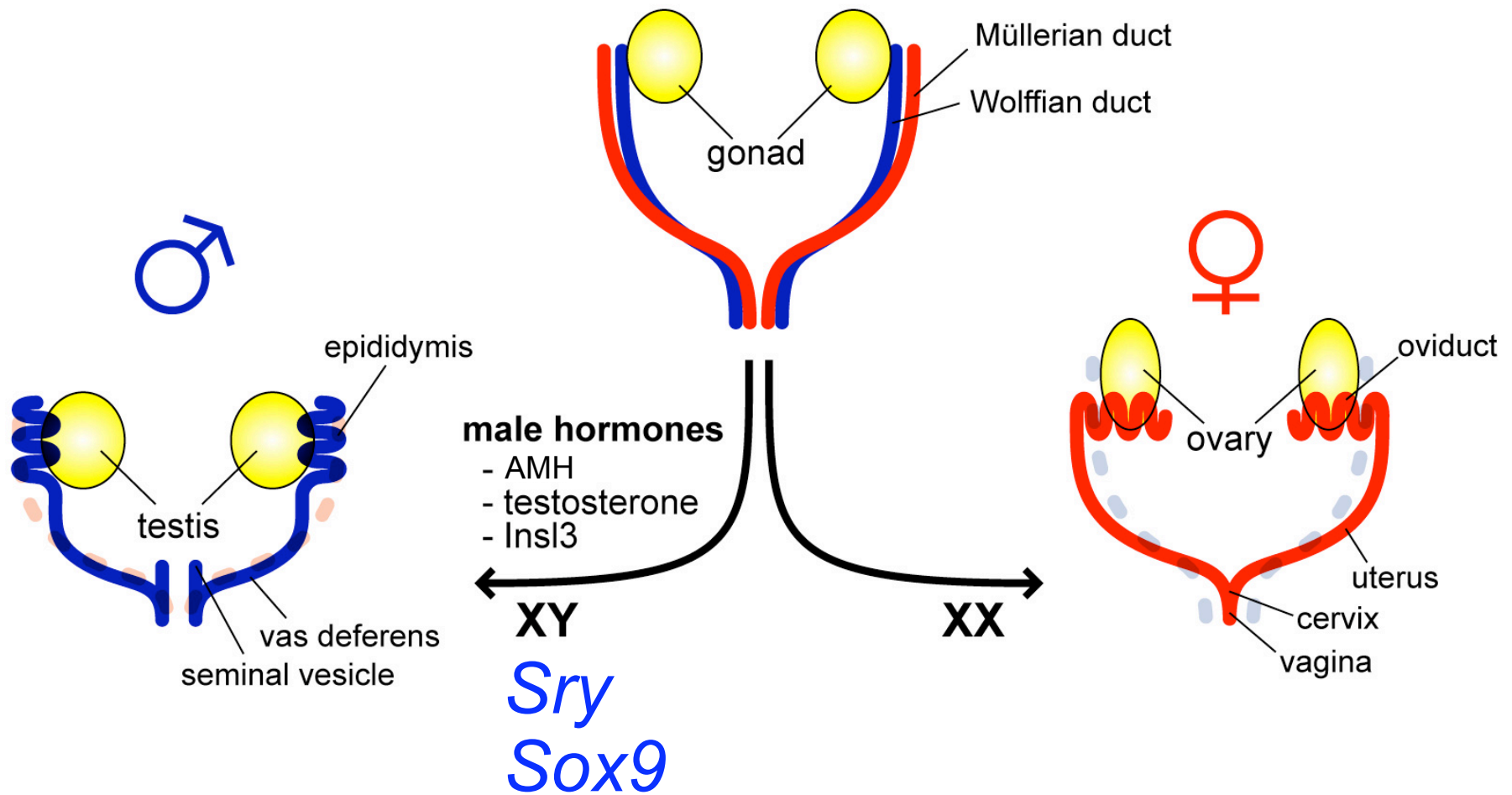


Developmental mechanisms of organ formation in the mouse

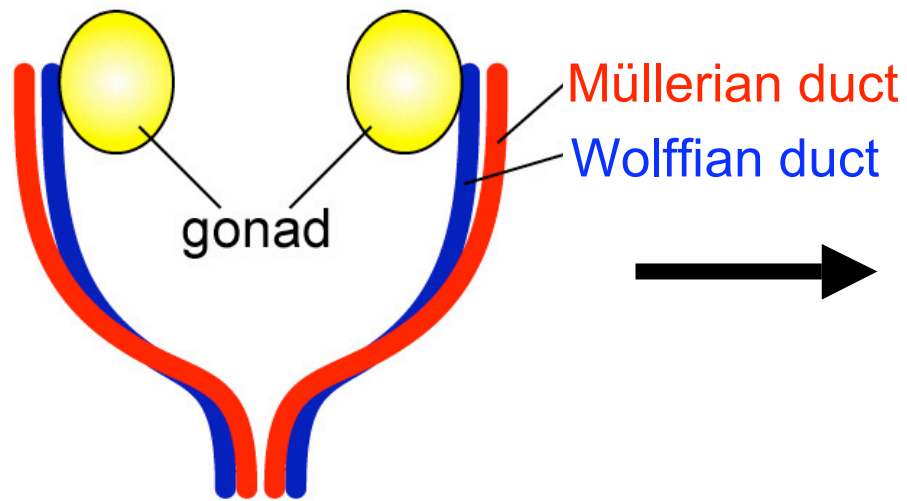


Richard Behringer
M.D. Anderson Cancer Center

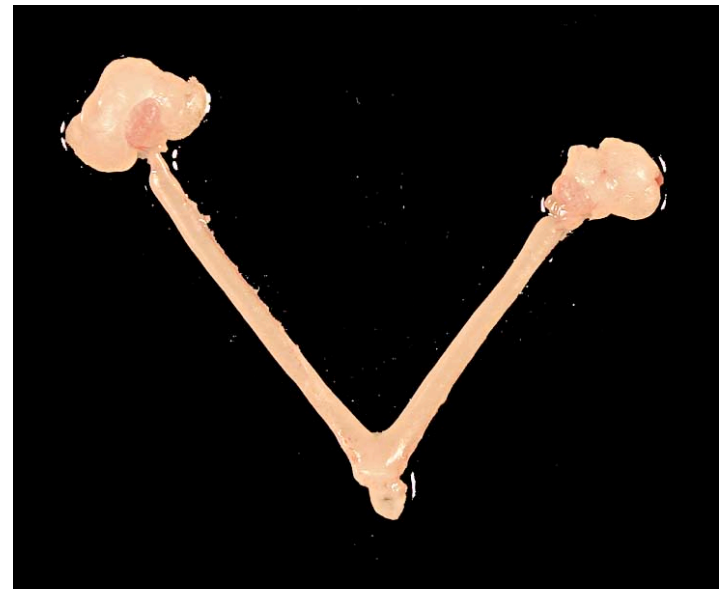
Mammalian sex differentiation



Müllerian duct gives rise to female reproductive tract



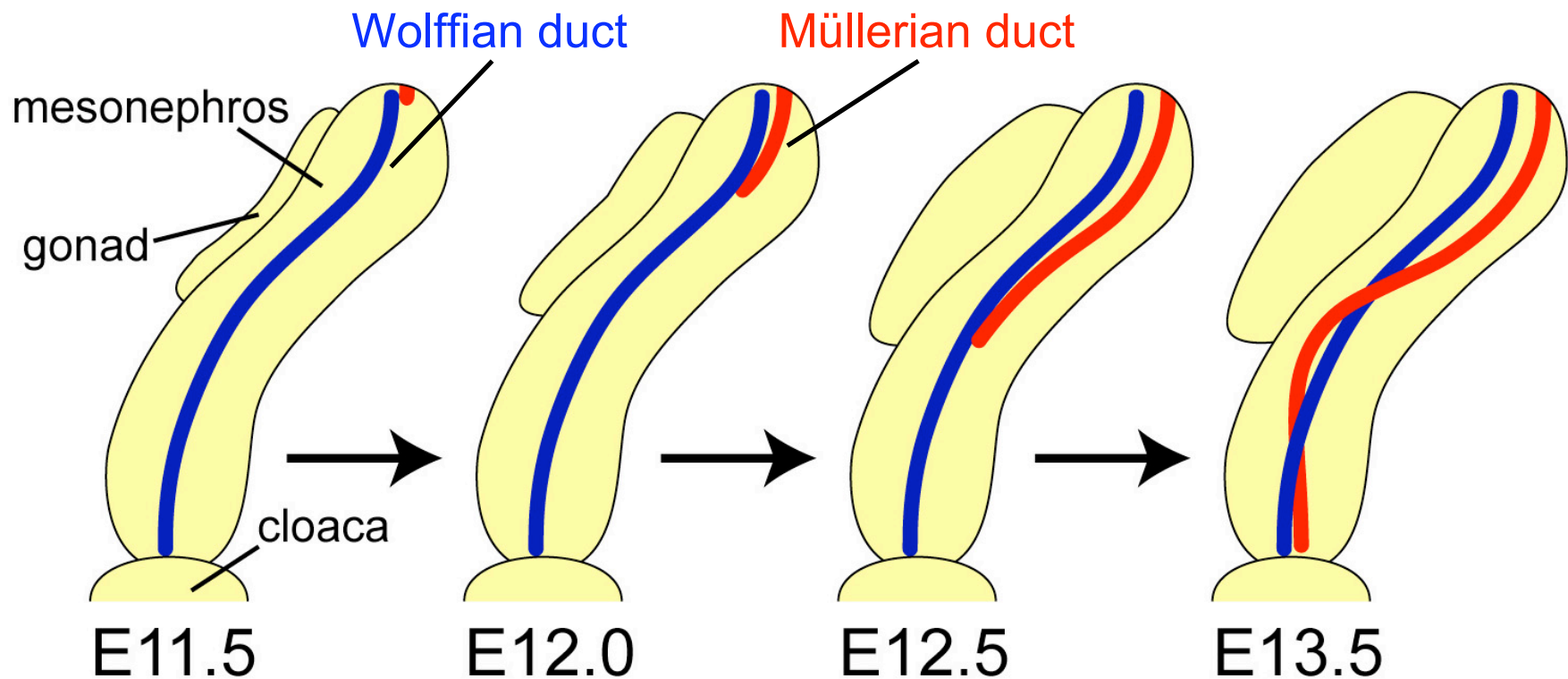
Embryonic day
(E) 13.5



adult

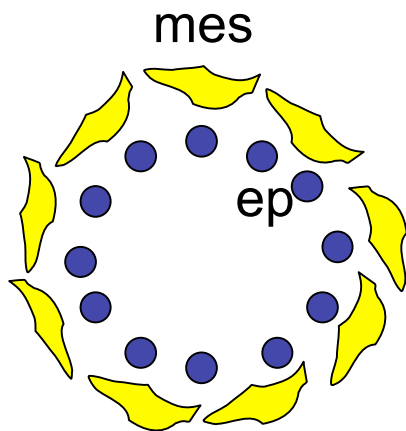
Mullerian duct formation

(rostral to caudal)



Müllerian duct differentiates into epithelial and mesenchymal tissues

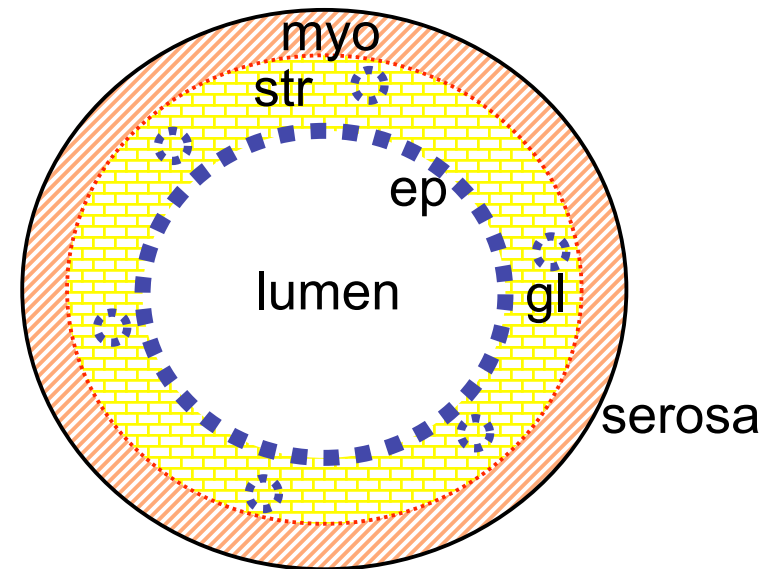
Müllerian duct



ep: epithelium
mes: mesenchyme

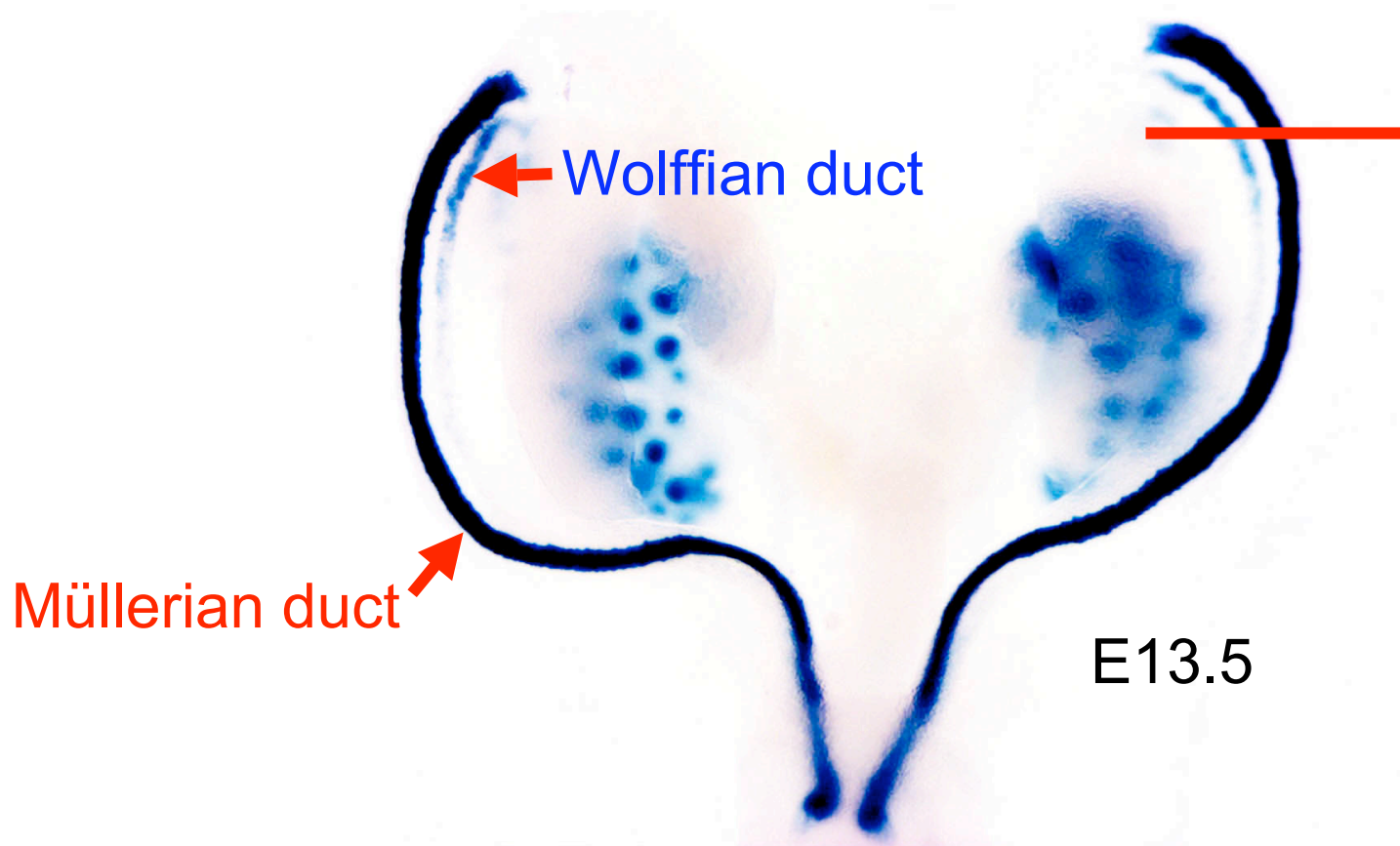


uterus

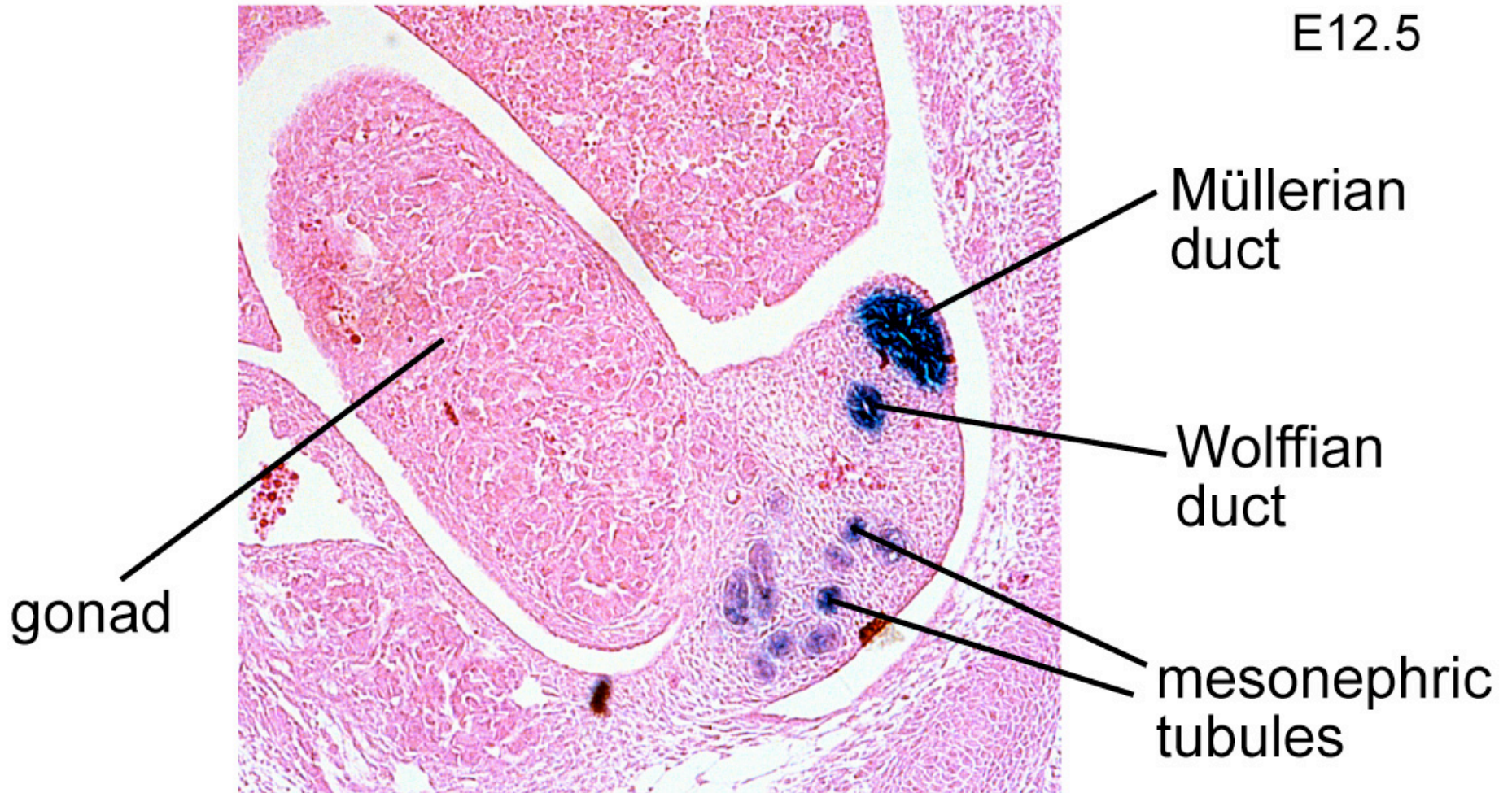


gl: gland
myo: myometrium
str: stroma

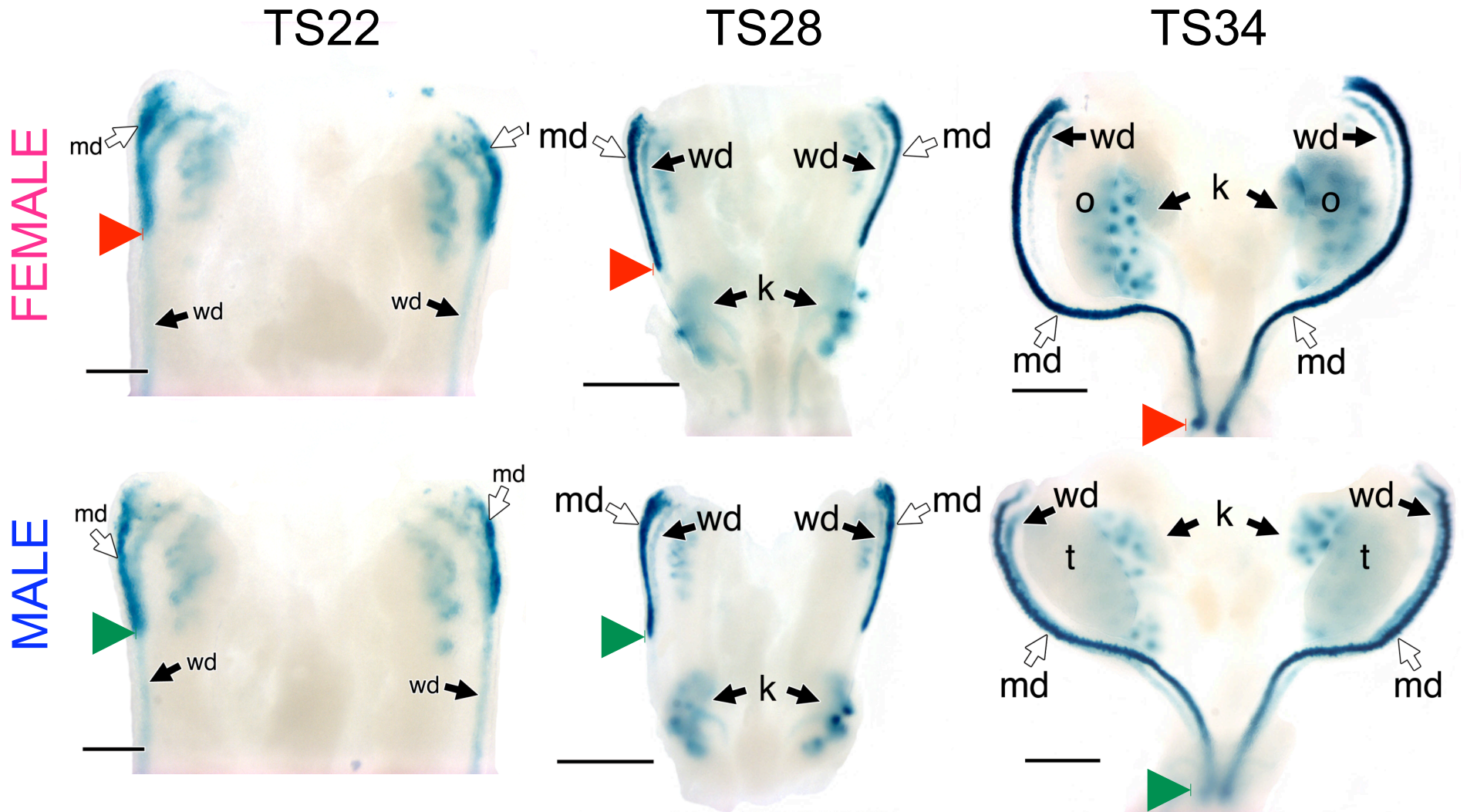
Lim1-lacZ genital duct marker



Müllerian duct

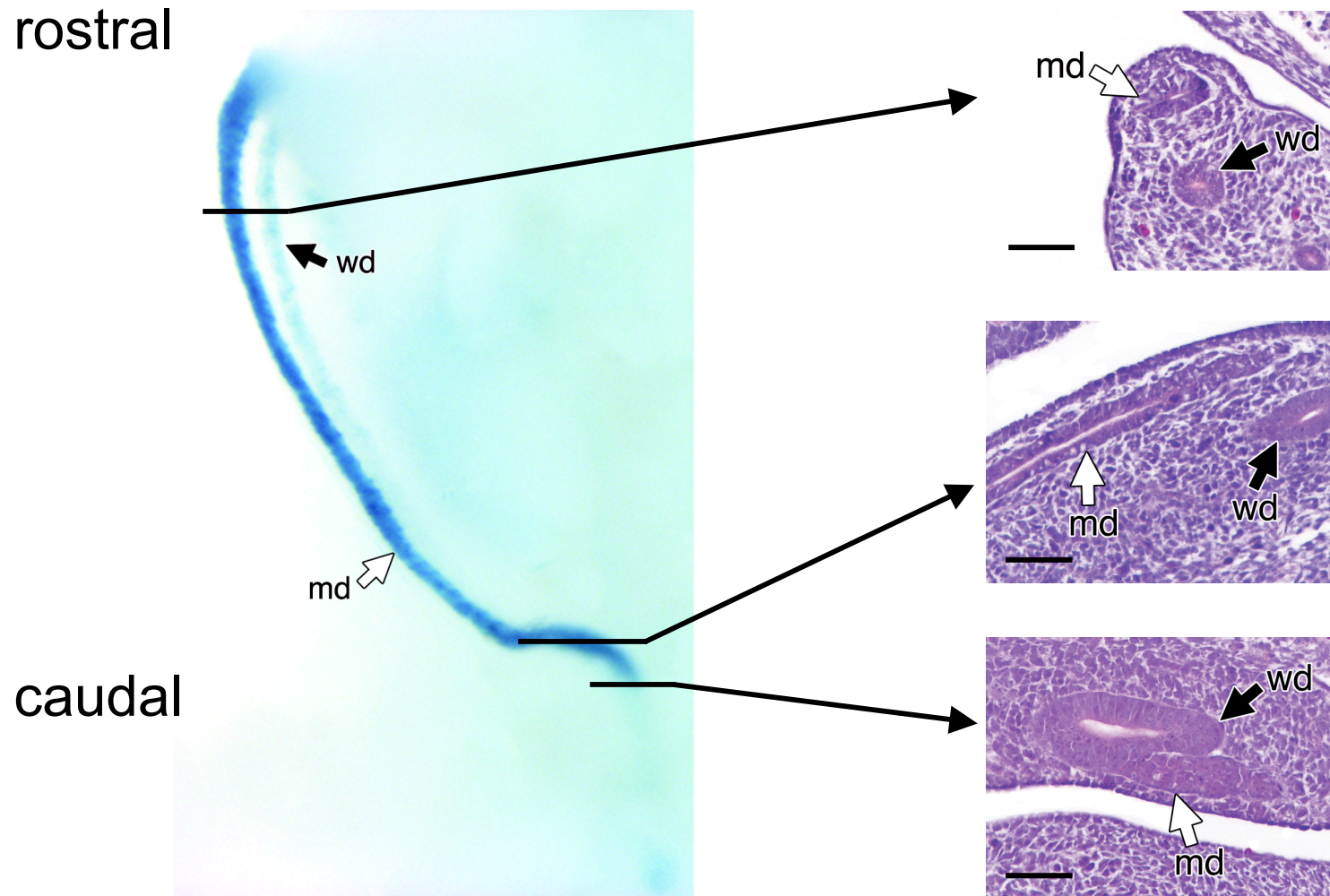


Müllerian duct formation



Grant Orvis

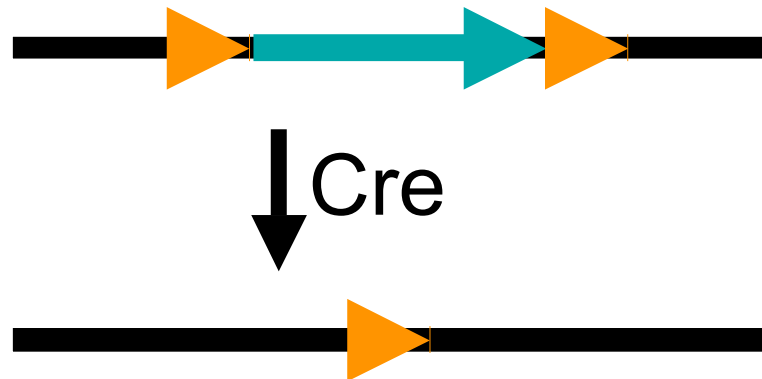
Müllerian duct relationship with Wolffian duct



What are the mechanisms for
Müllerian duct formation/elongation?

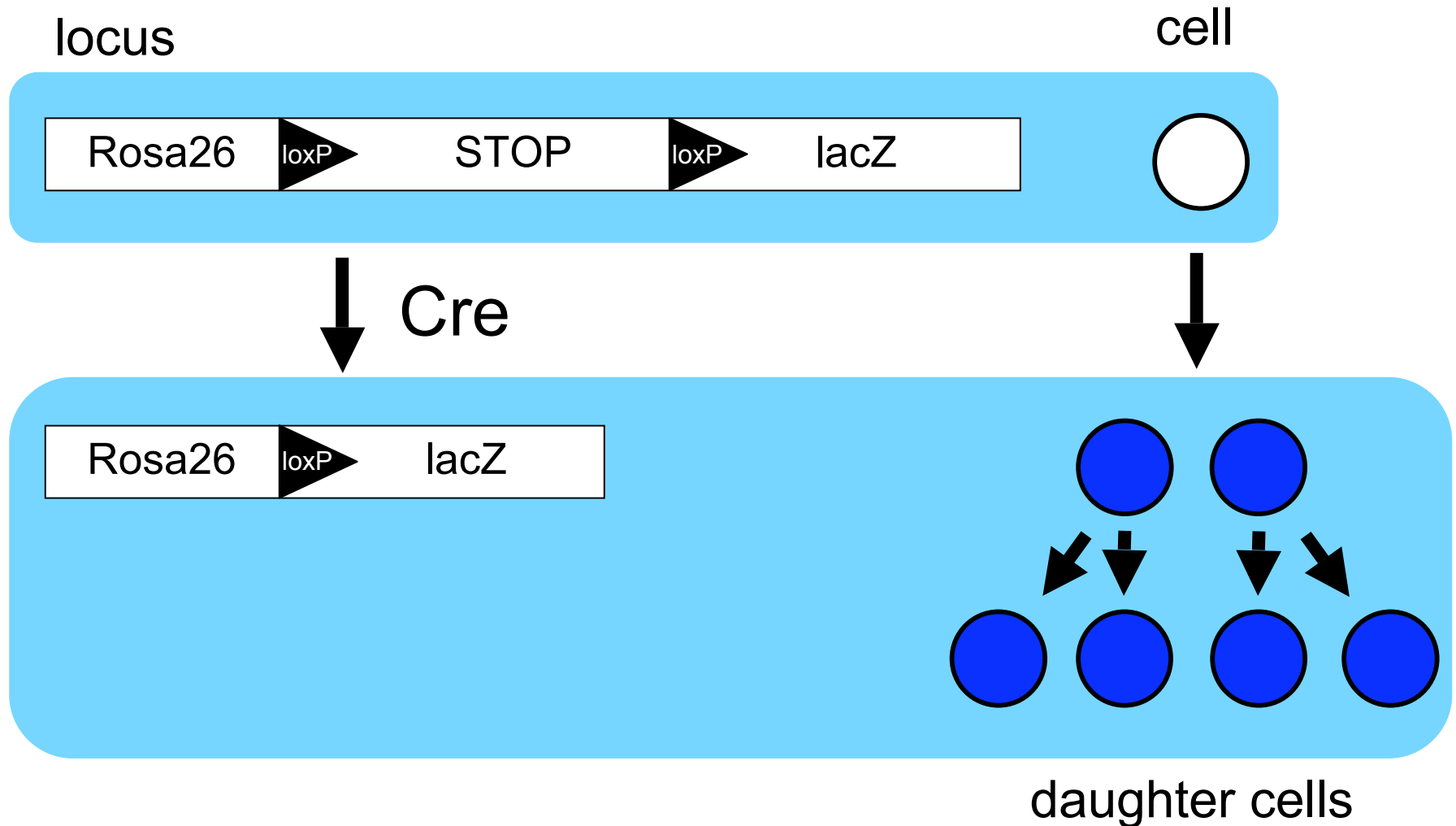
Cre/*loxP*

- Cre is a DNA recombinase
- Cre specifically recognizes 34 bp *loxP* sites
- flox - flanked by *loxP* sites



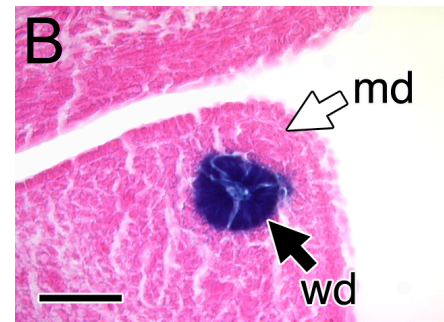
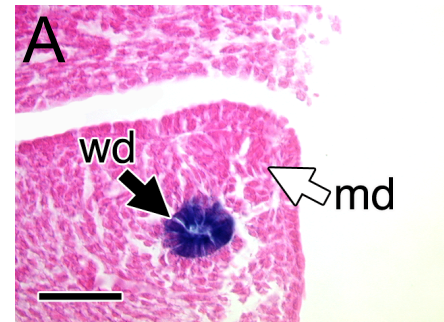
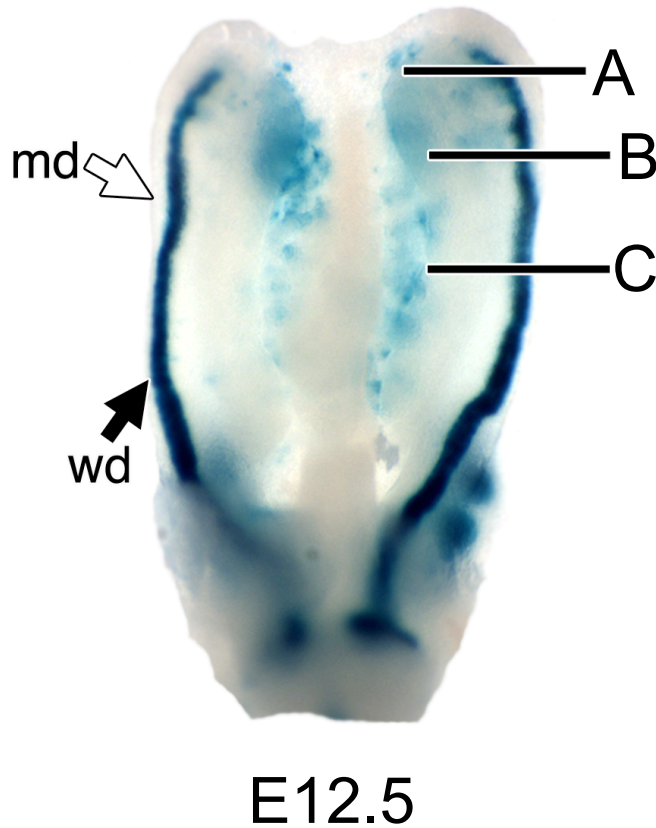
Fate mapping

Rosa26 Cre reporter mouse (*R26R*)

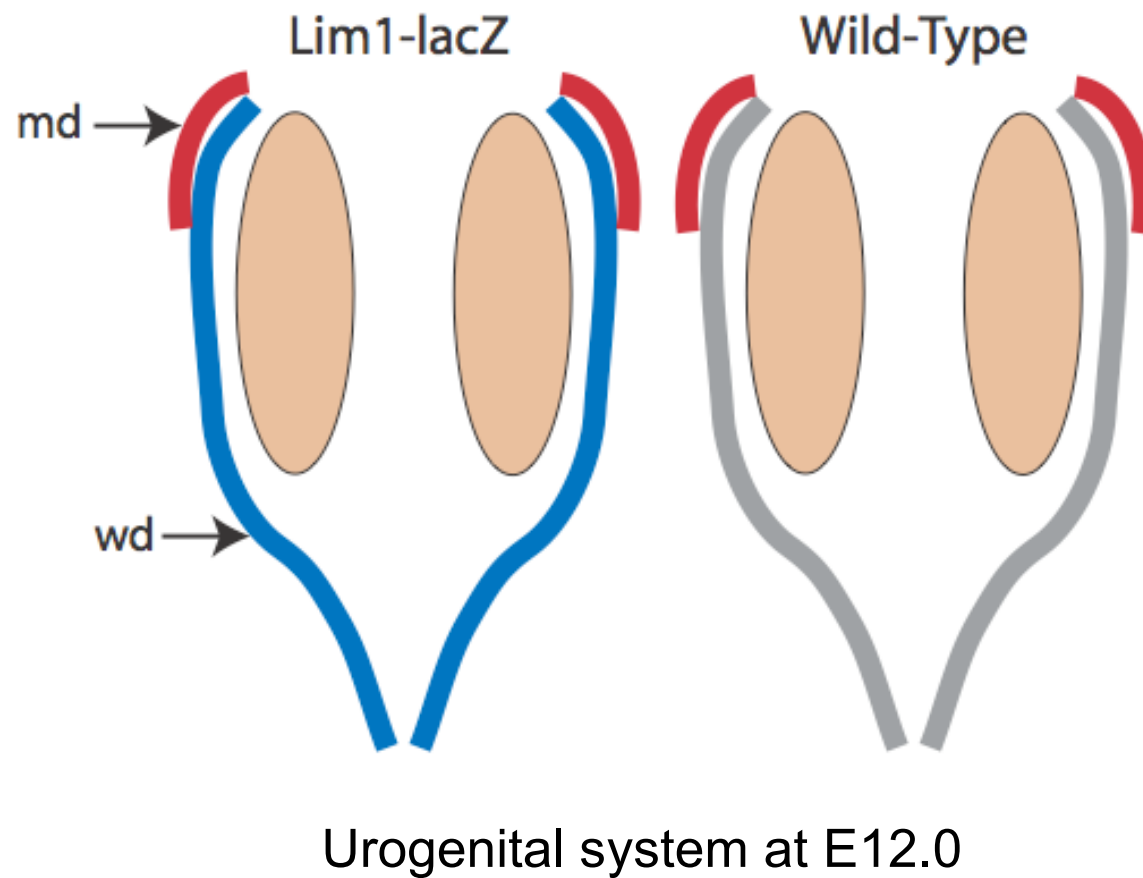


Wolffian duct does not produce the Müllerian duct

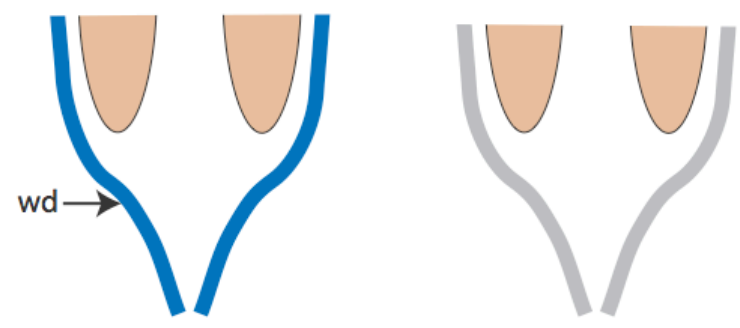
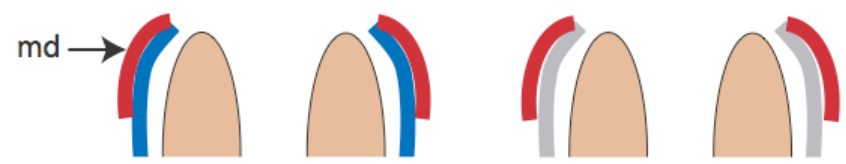
Hoxb7-Cre (Wolffian duct) x *R26R*



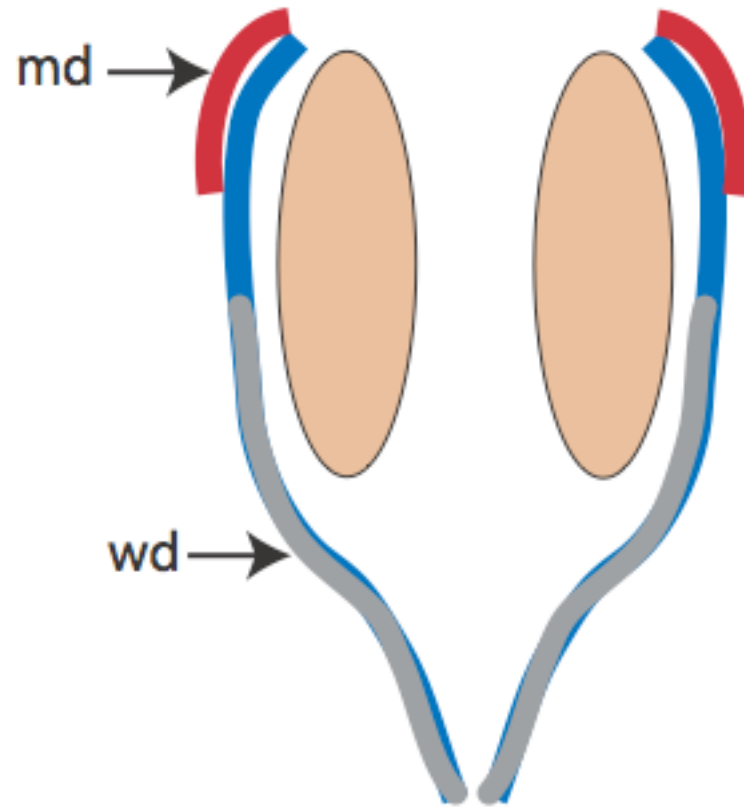
Lim1-lacZ recombinant explant assay



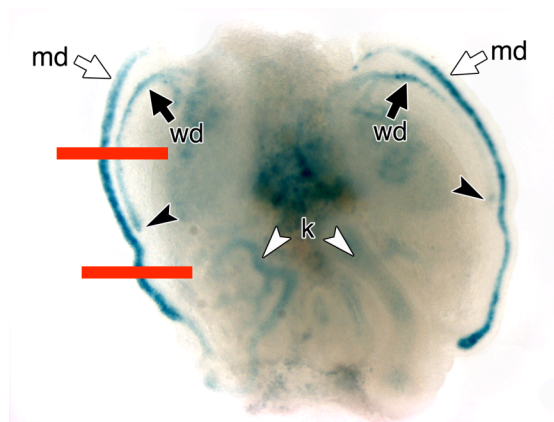
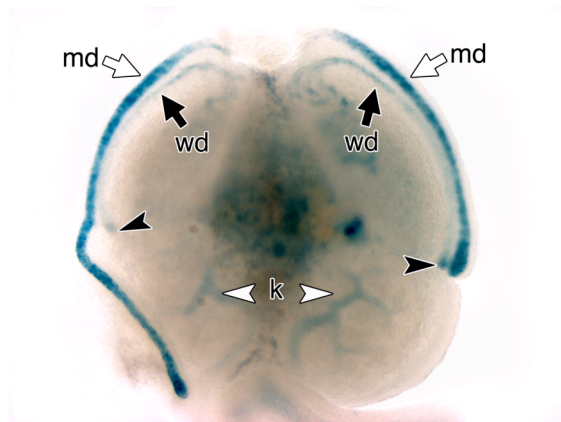
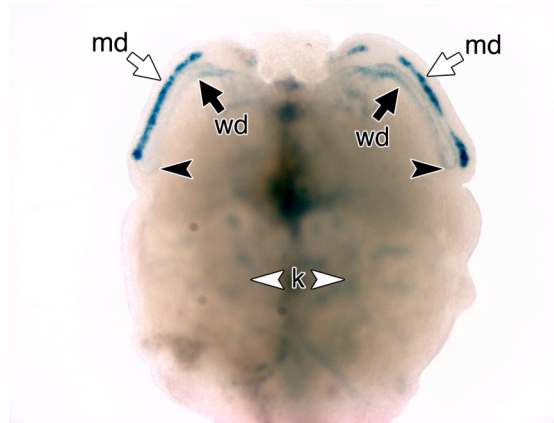
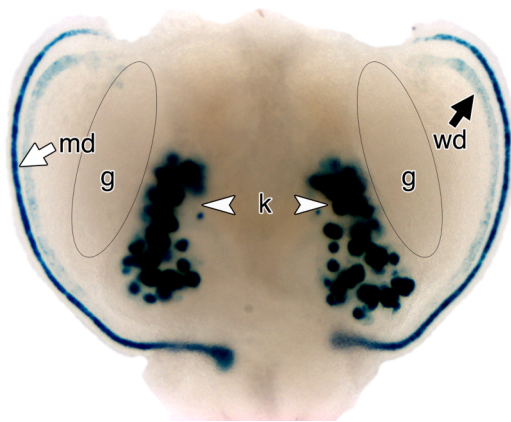
Cut



Paste



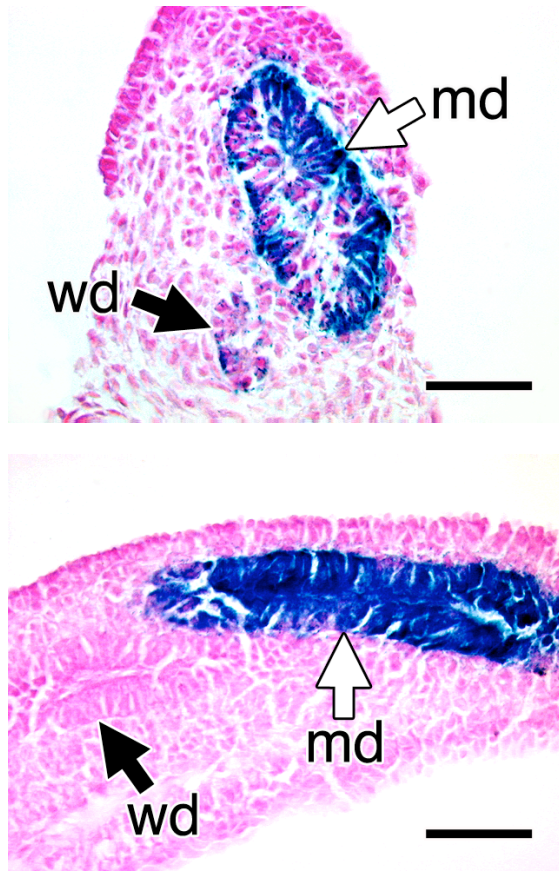
Explant culture results



Müllerian duct of the *Lim1-lacZ* rostral ridge grew into the wild-type caudal ridge

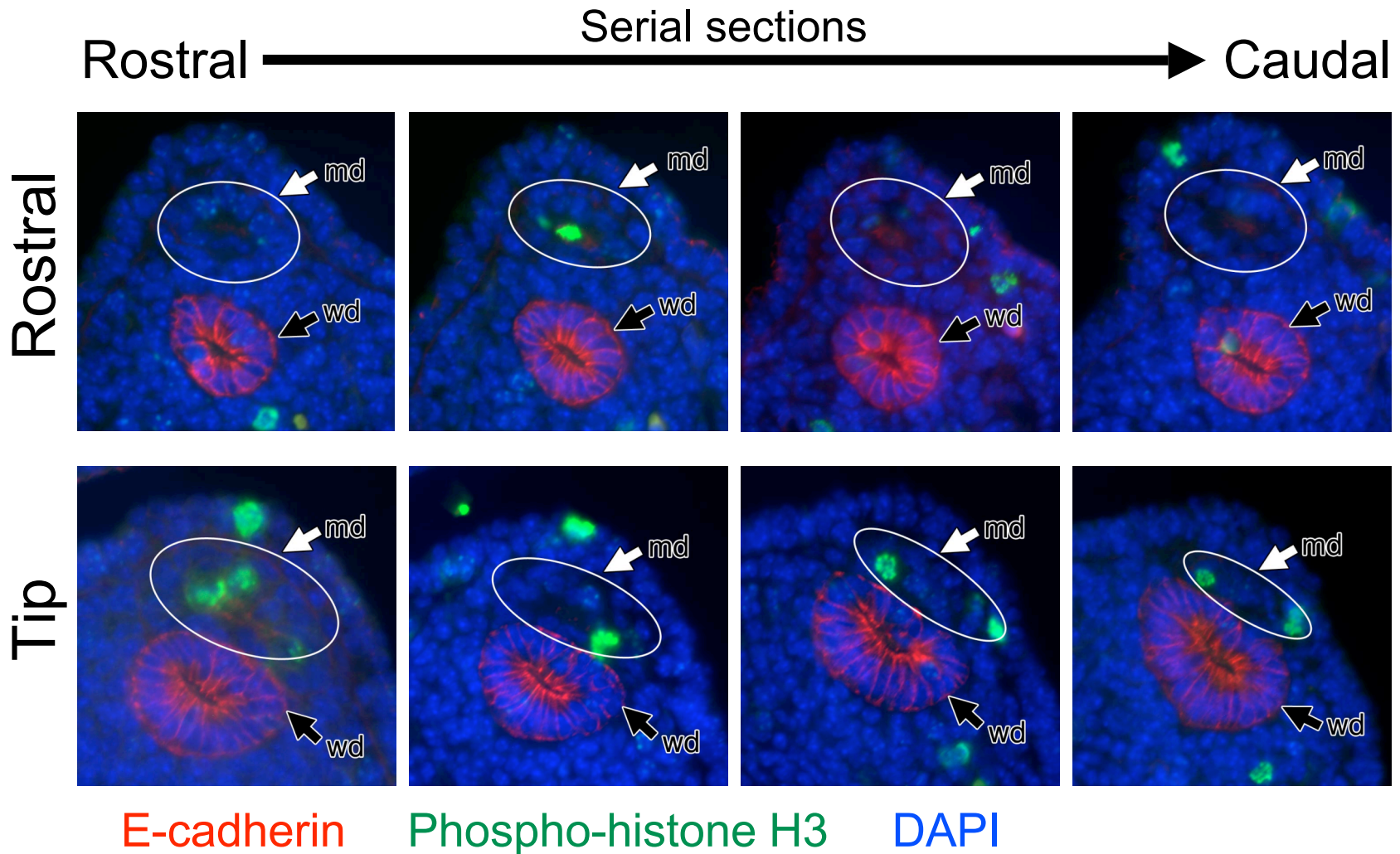
n = 4

Explant culture results

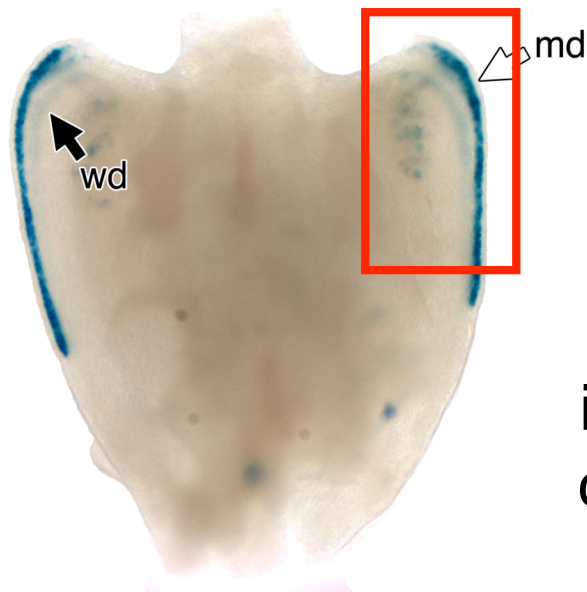


- In the rostral (*Lim1-lacZ* +) ridge, both the Müllerian and Wolffian ducts express *lacZ*
- The *lacZ* + Müllerian duct grew into the caudal (wild-type) ridge

Cells at tip of duct proliferate

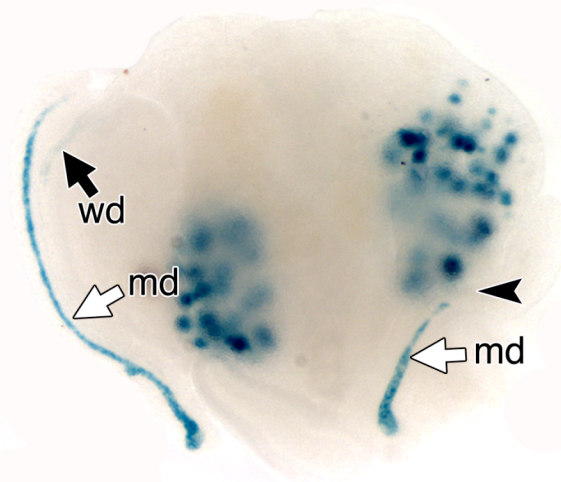


Tip cells generate the Müllerian duct



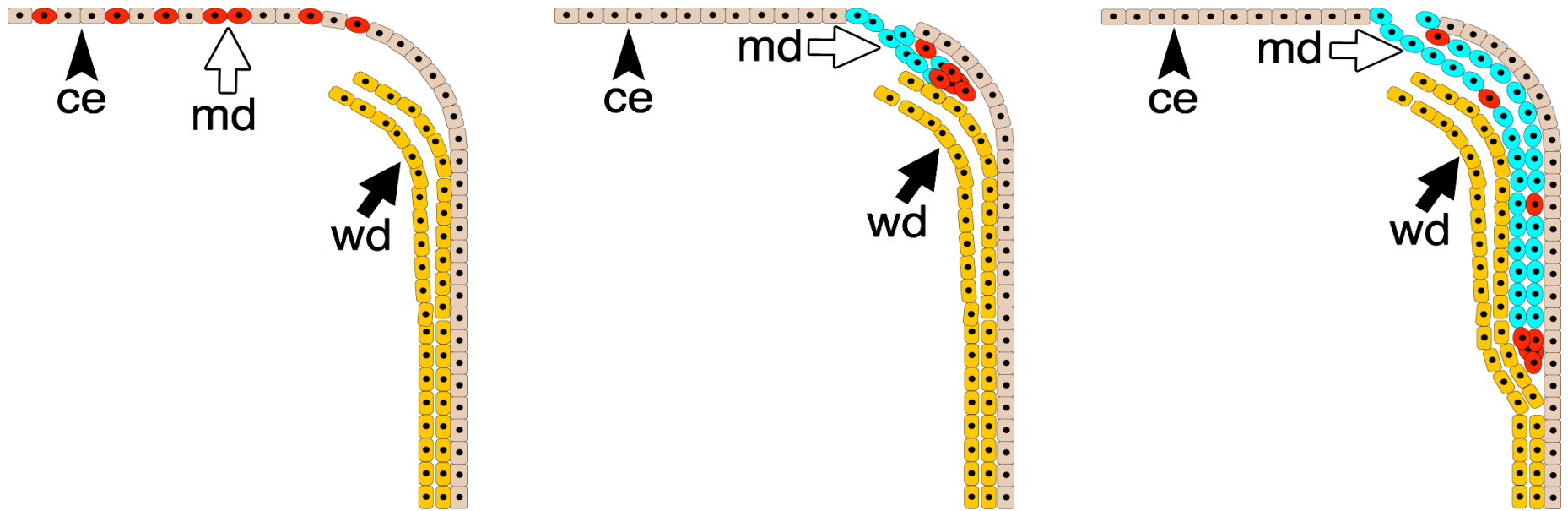
Starting control

→
in vitro
culture



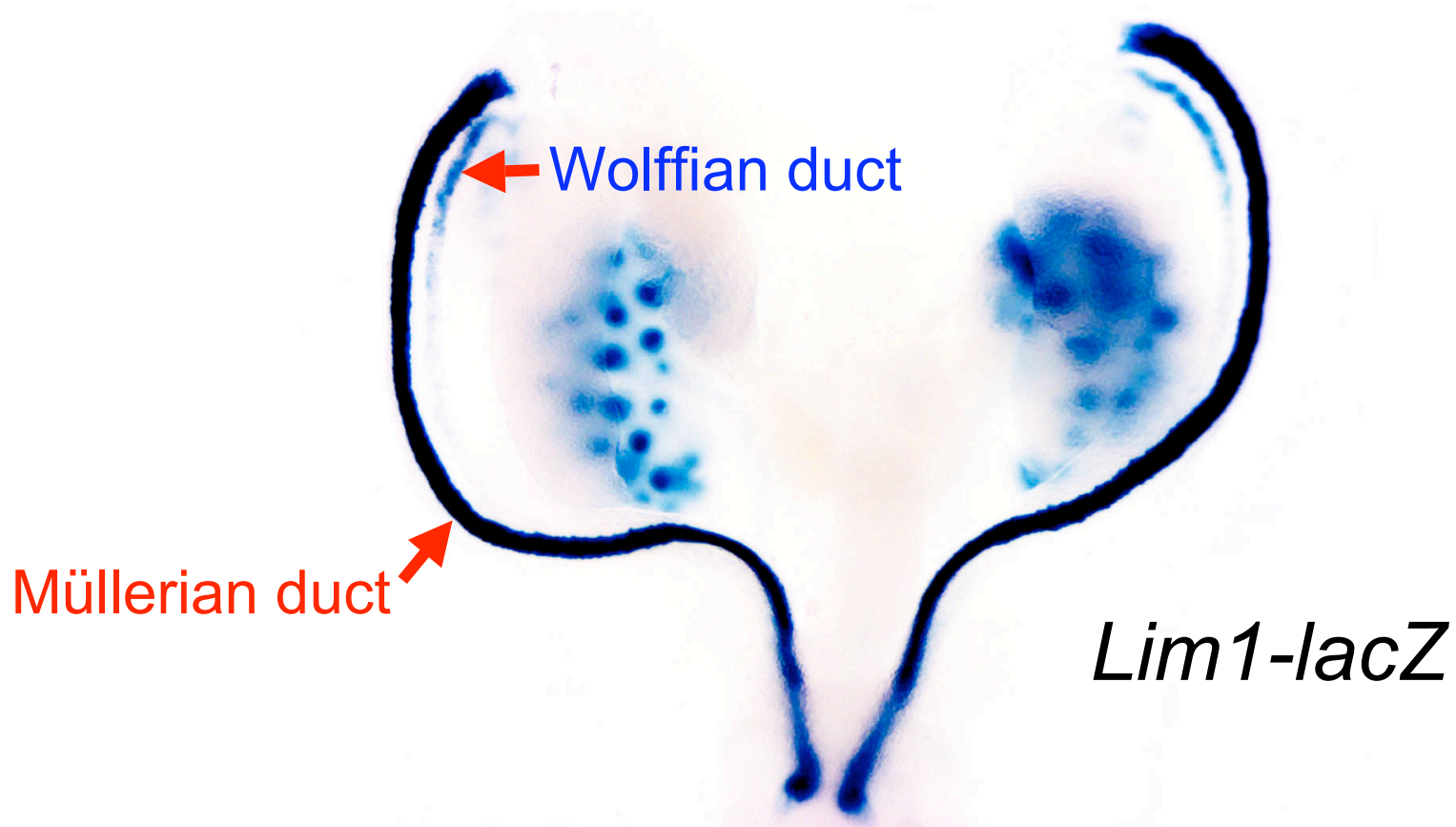
Rostral duct
removed

Rooting Tubulogenesis

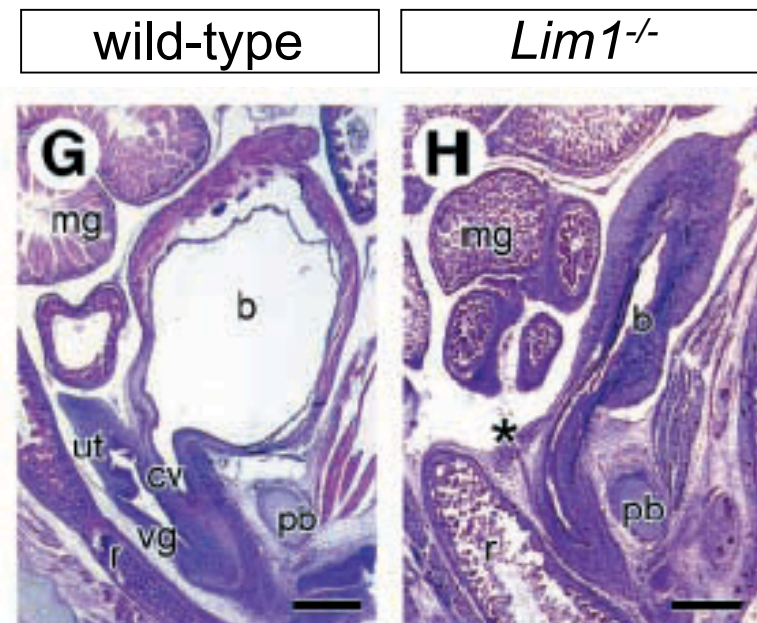
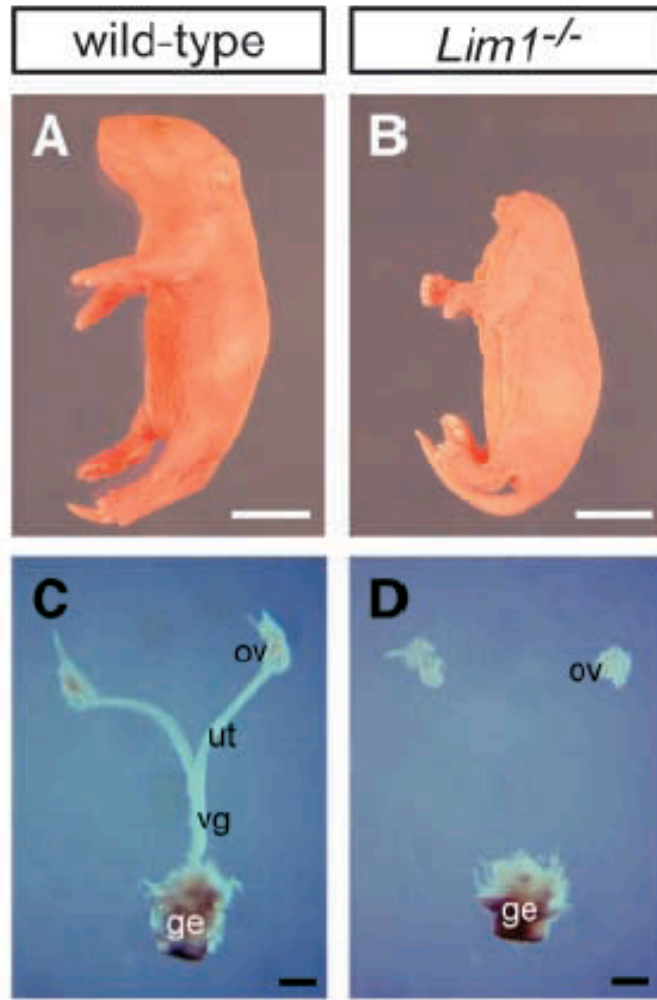


Tubule is created primarily by
cell proliferation at the caudal most tip

Lim1 expressed in WD and MD

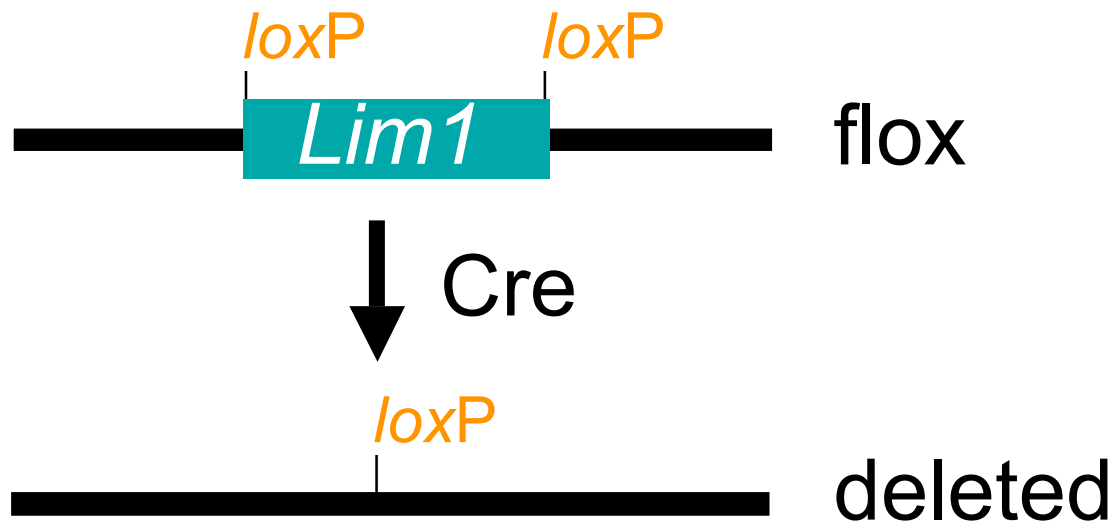


Lim1 is required for female reproductive tract formation

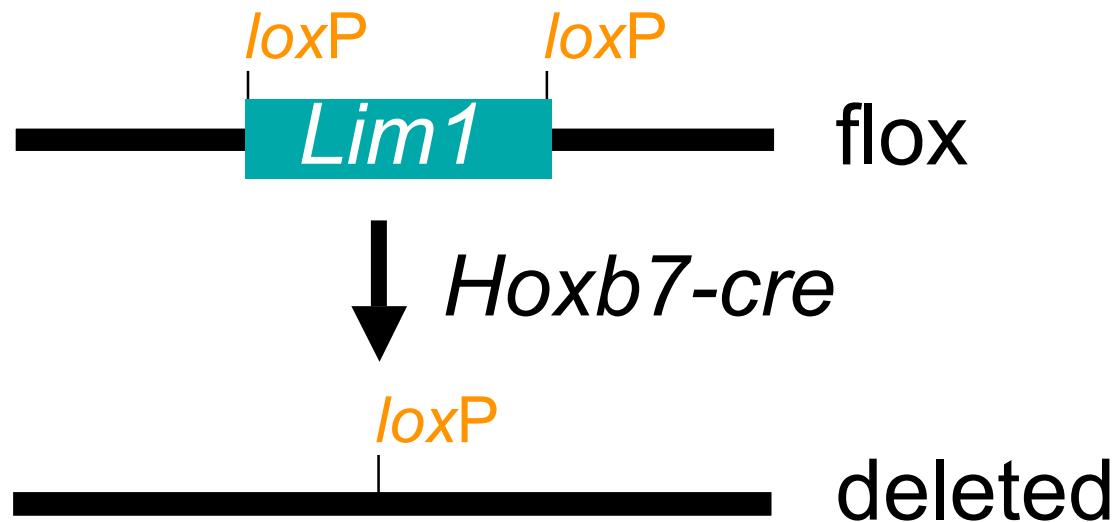


Akio Kobayashi

Lim1 conditional null allele

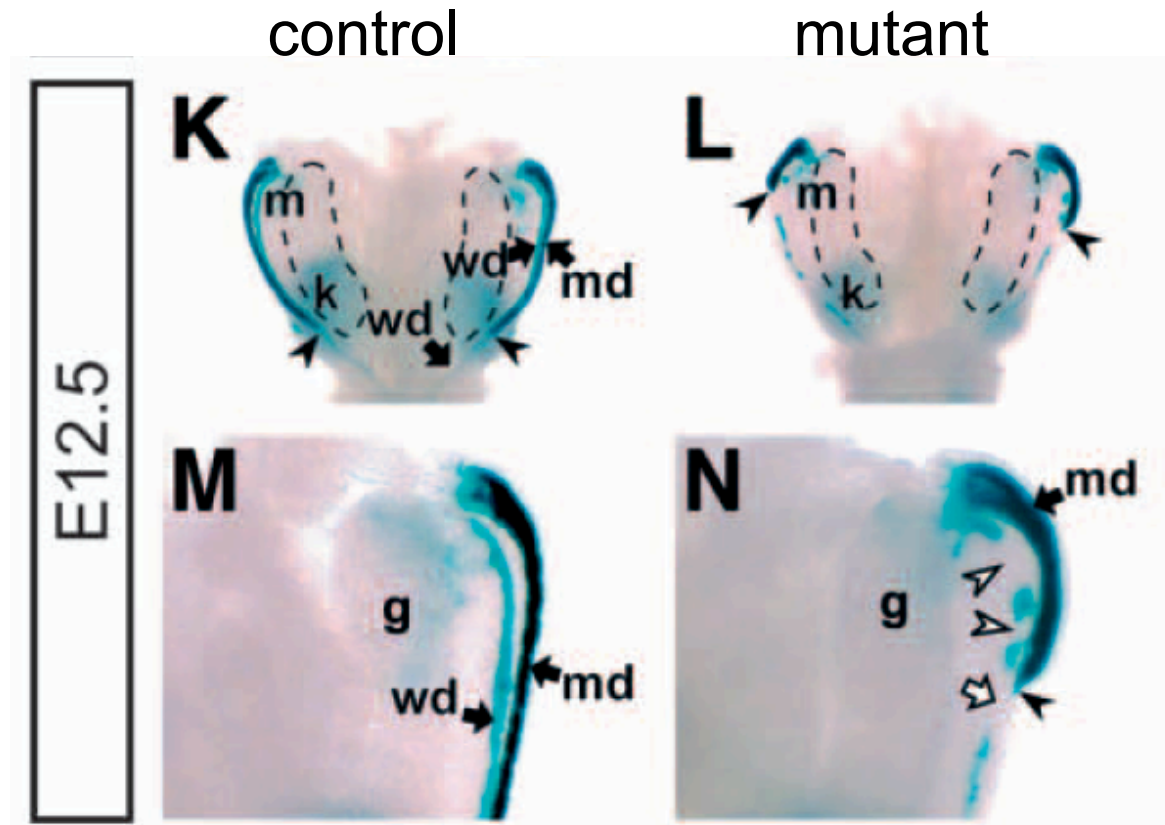


Lim1 conditional knockout in Wolffian duct epithelium



Müllerian duct formation depends on Wolffian duct

Hoxb7-Cre (Wolffian duct); *Lim1 lacZ/flox*



Akio Kobayashi

Loss of female reproductive tract
is secondary to WD defects



What is the role of *Lim1* in the
Müllerian duct epithelium?

Wnt7a



- *Wnt7a* expressed in Müllerian duct epithelium from E11.75 to adult

Summary

- *Wnt7a-cre* transgene is active in MD epithelium & epithelium of adult tract
- *Lim1;Wnt7a-cre* females lack uterus and cervix
- MD elongation initiates but becomes arrested
- *Lim1* is essential in MD epithelium for uterus formation

Acknowledgements



- Grant Orvis
- Cheng-Chiu Huang
- Chuan-Wei Jang
- David Stewart
- Liesl Nel-Themaat

