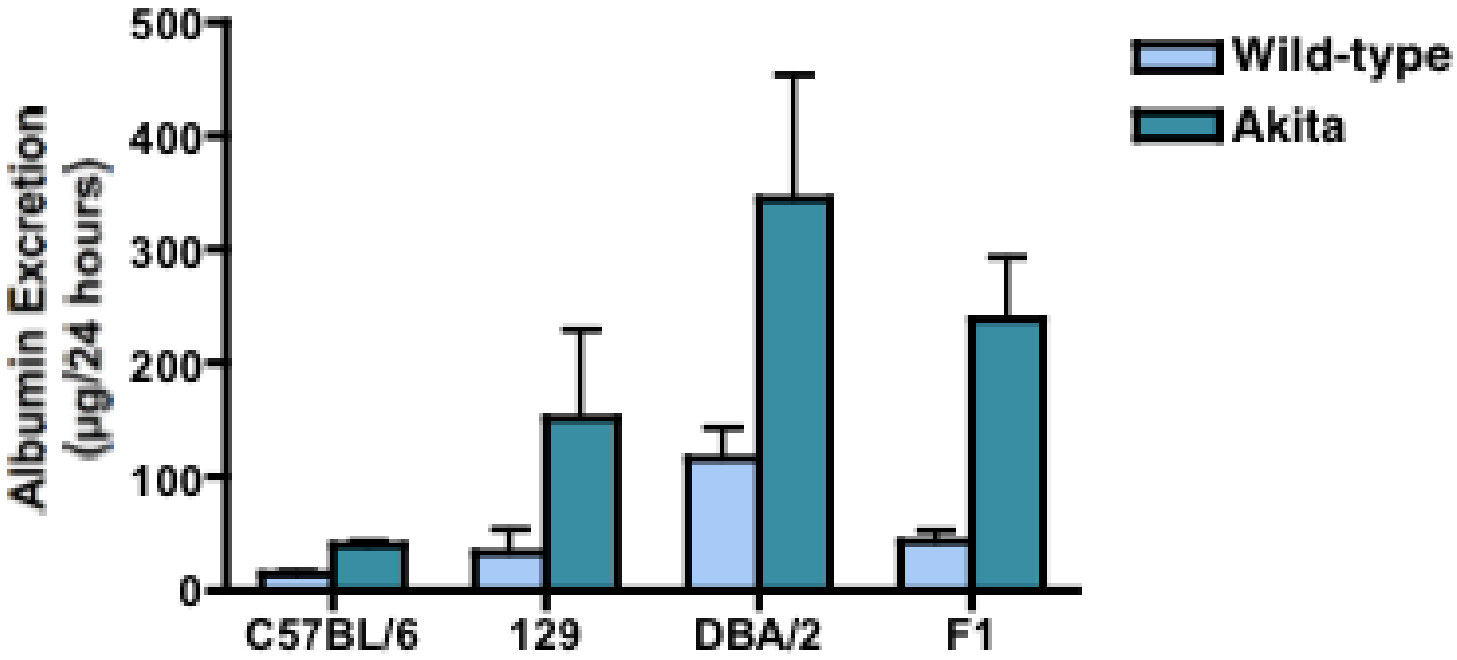
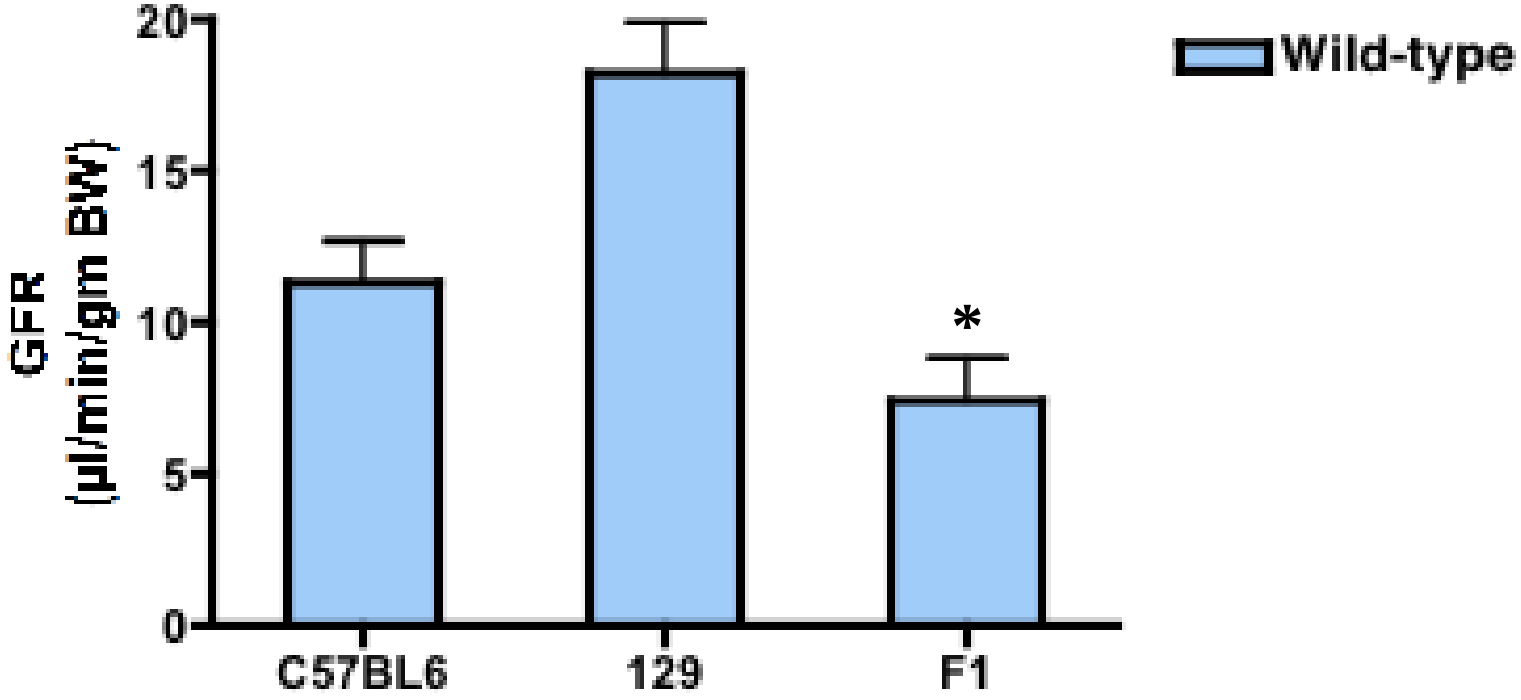
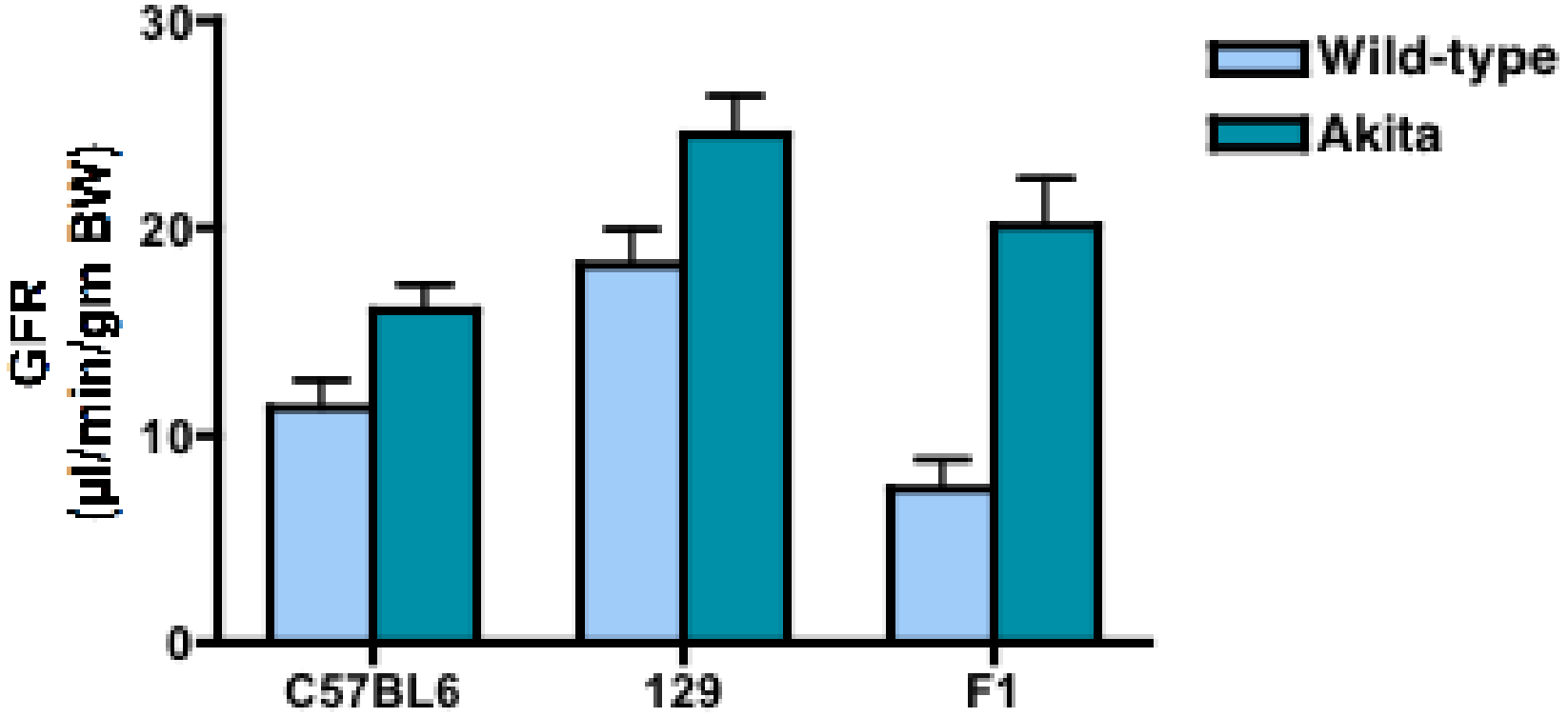
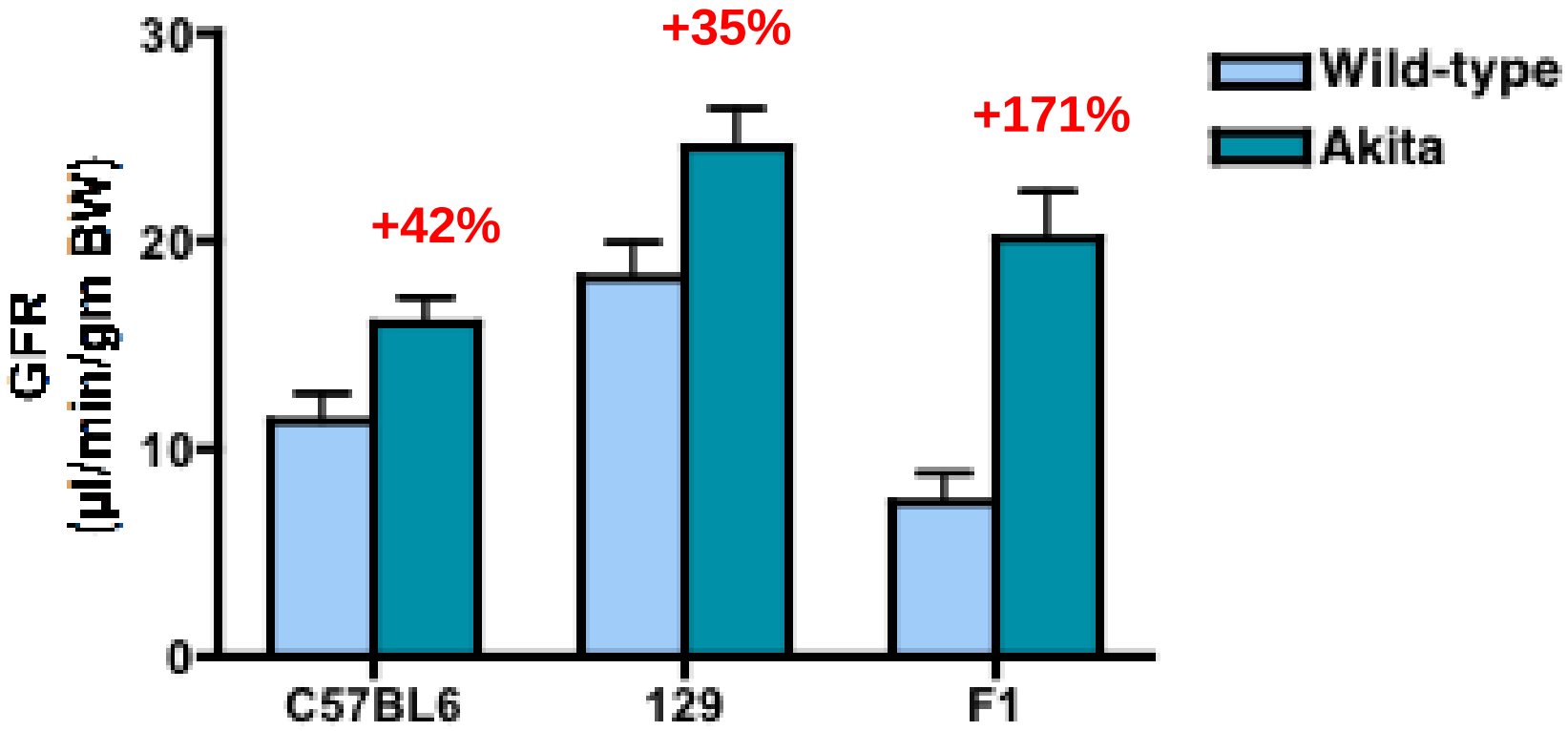
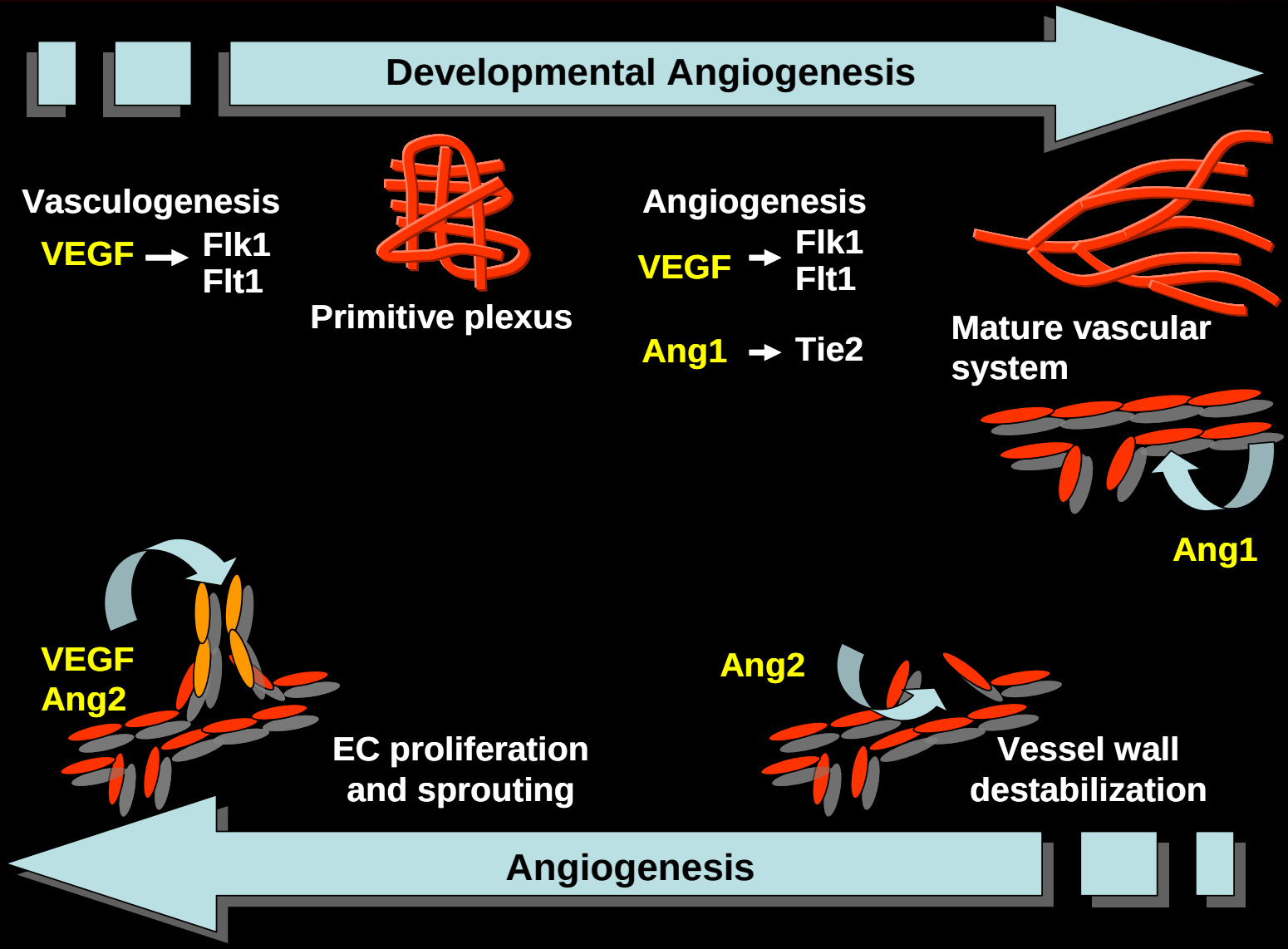

Albumin Excretion
(µg/24 hours)
500
400
300
200
100
0
C57BL/6
129
DBA/2
F1
Wild-type
Akita


GFR
(µl/min/gm BW)
20
15
10
5
0
*
C57BL6
129
F1
Wild-type

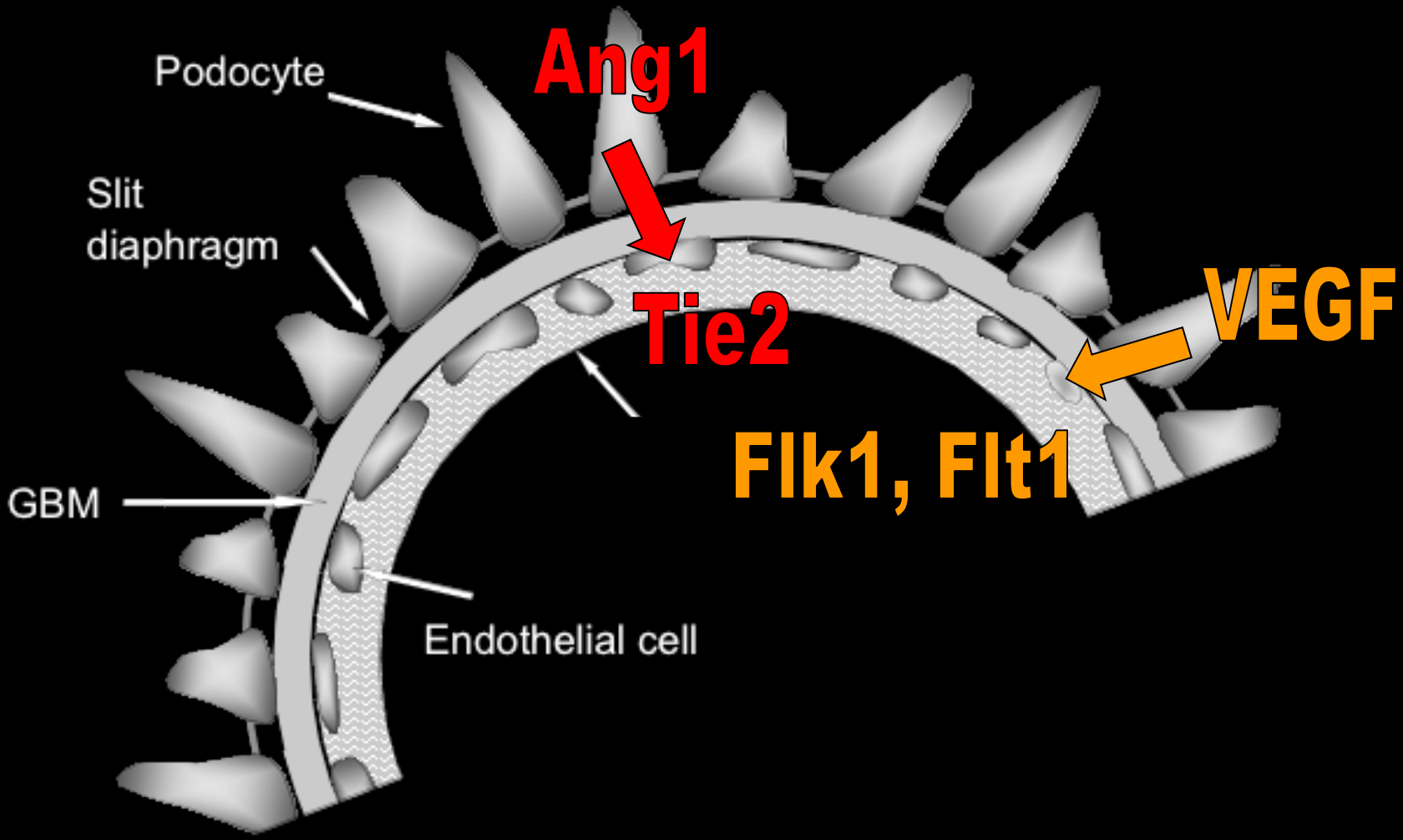

GFR
(µl/min/gm BW)
30
20
10
0
C57BL6
129
F1
Wild-type
Akita


30
20
10
0
GFR
(µl/min/gm BW)
+42%
+35%
+171%
C57BL6
129
F1
Wild-type
Akita

Background - Angiogenesis

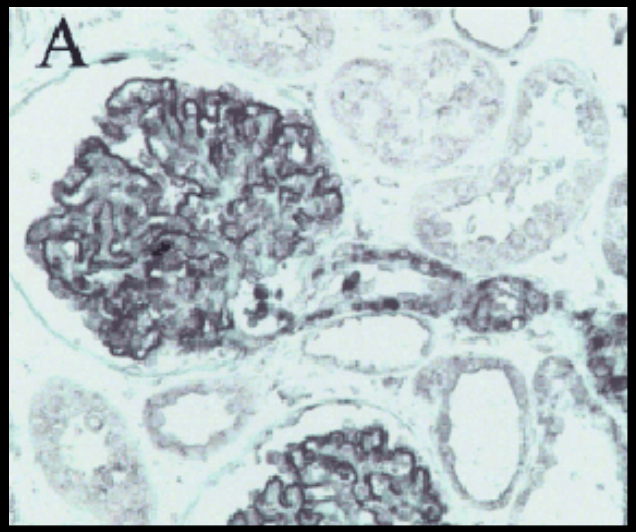


Ang1 and VEGF in the glomerulus

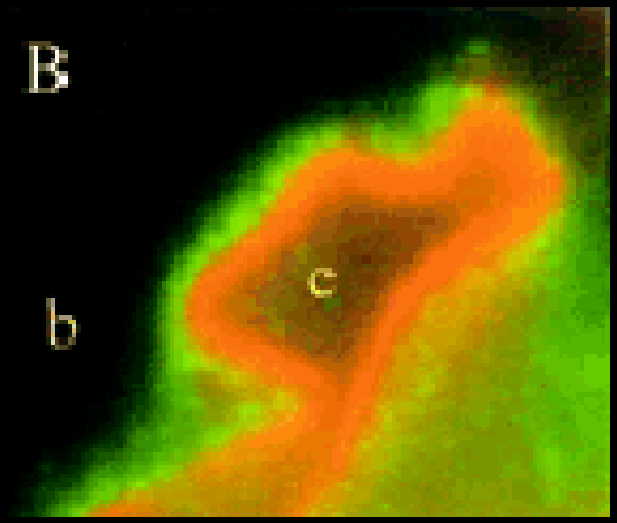


Ang1 production in the glomerulus

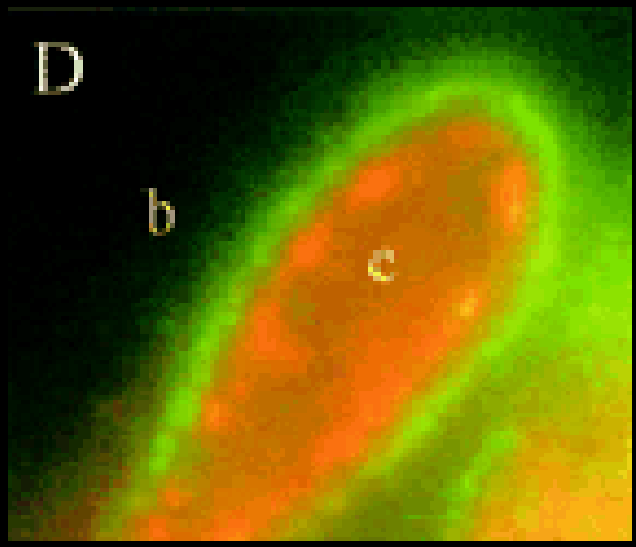
Ang1



Ang1



VEGF



Satchell et al, *JASN*, 2002

Comp-Ang1 - renoprotective effect in db/db mouse: S. Lee Et al. *NDT* 2007

Ang-1 suppresses diabetic retinopathy: *Am J Pathol*; A. Joussen et al. 2002

Angiopoietin-1, Tie2 standard KO

Ang1 KO: Suri et al. Cell, 1996

Tie2 KO: Dumont et al. Genes Dev, 1994

Puri et al. EMBO J, 1995

Sato et al. Nature, 1995

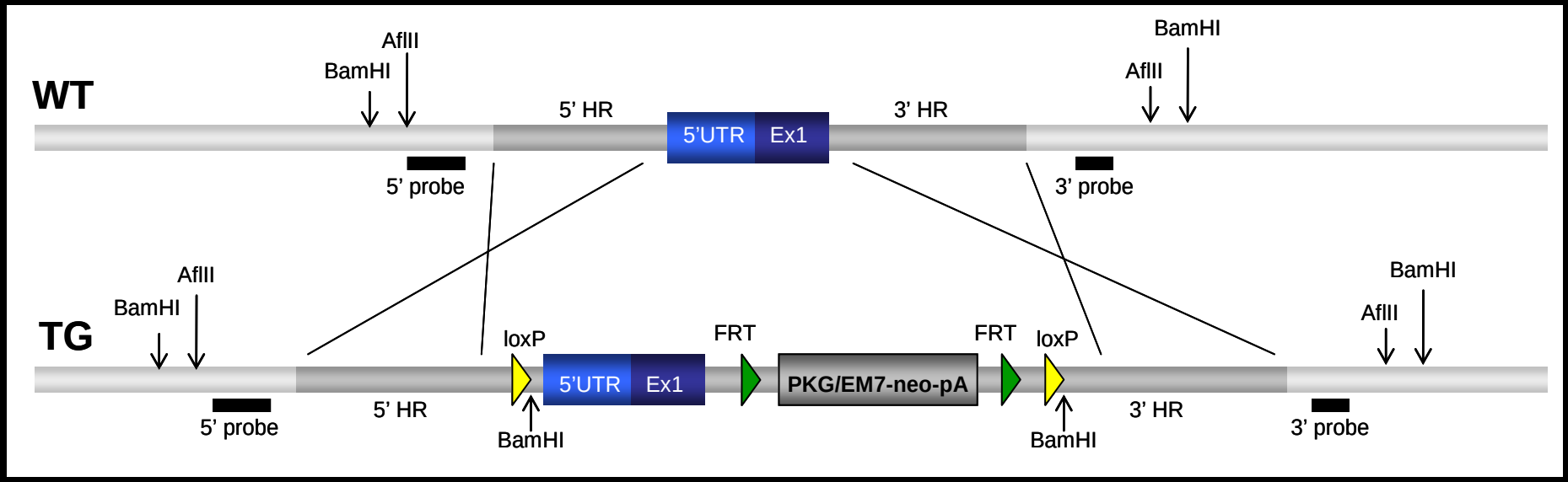
<u>Phenotype</u>

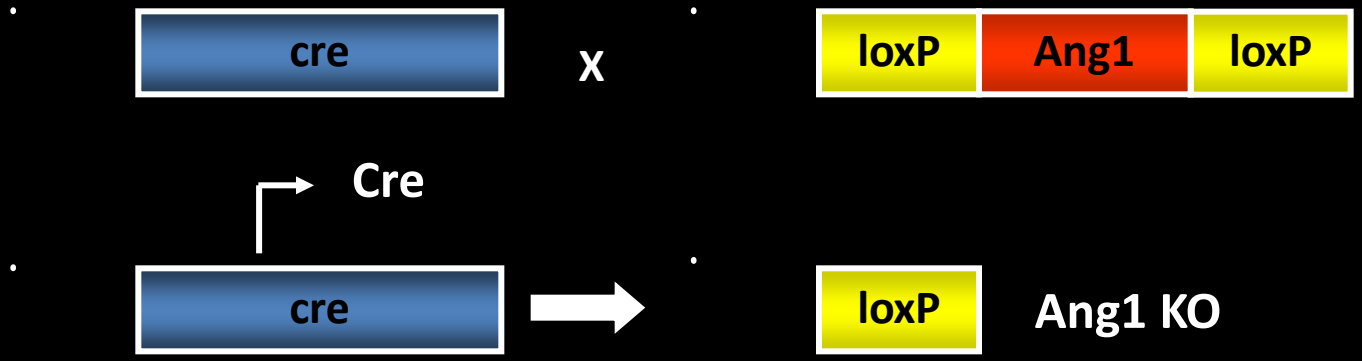
Embryonic lethal E9.5 – E12.5

Immature structures of the heart

Less complex vasculature in the forebrain, head region, eye, and yolk sac

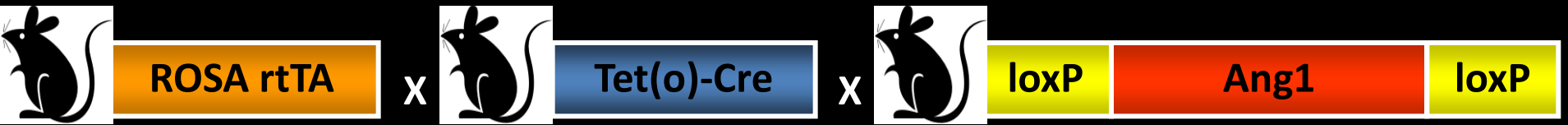
Floxed Ang1

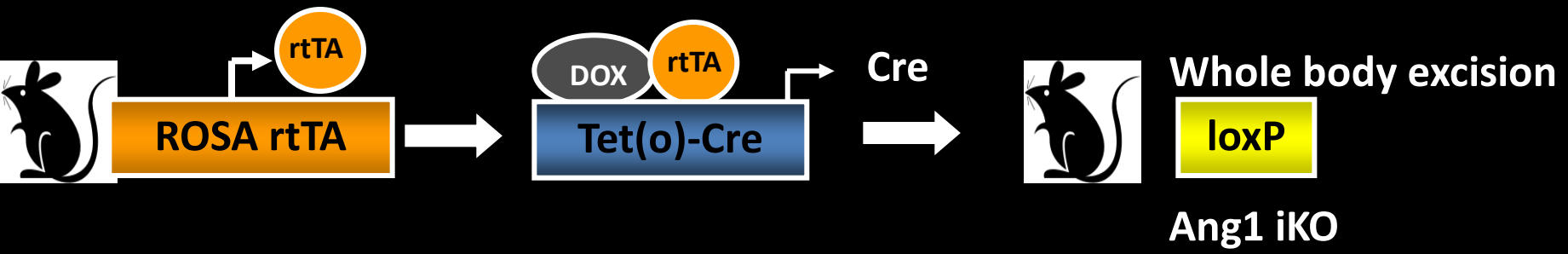




Jeansson, Quagin, et al. (unpublished)

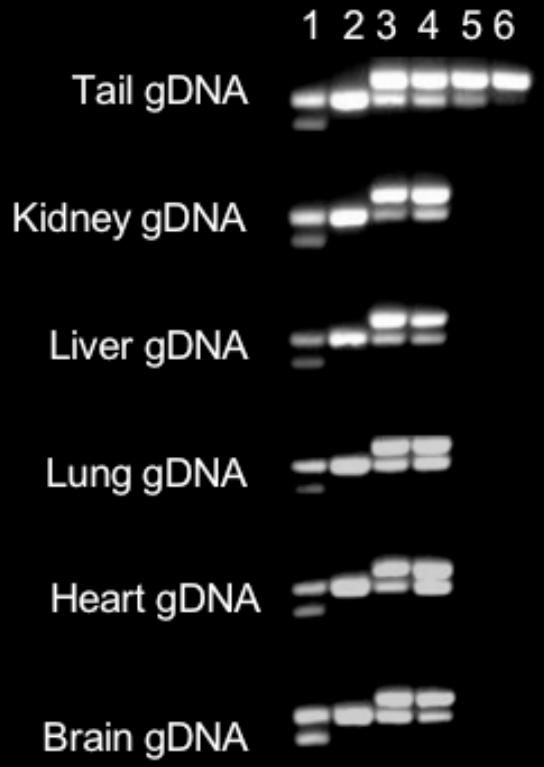
Inducible whole body deletion; Ang iKO





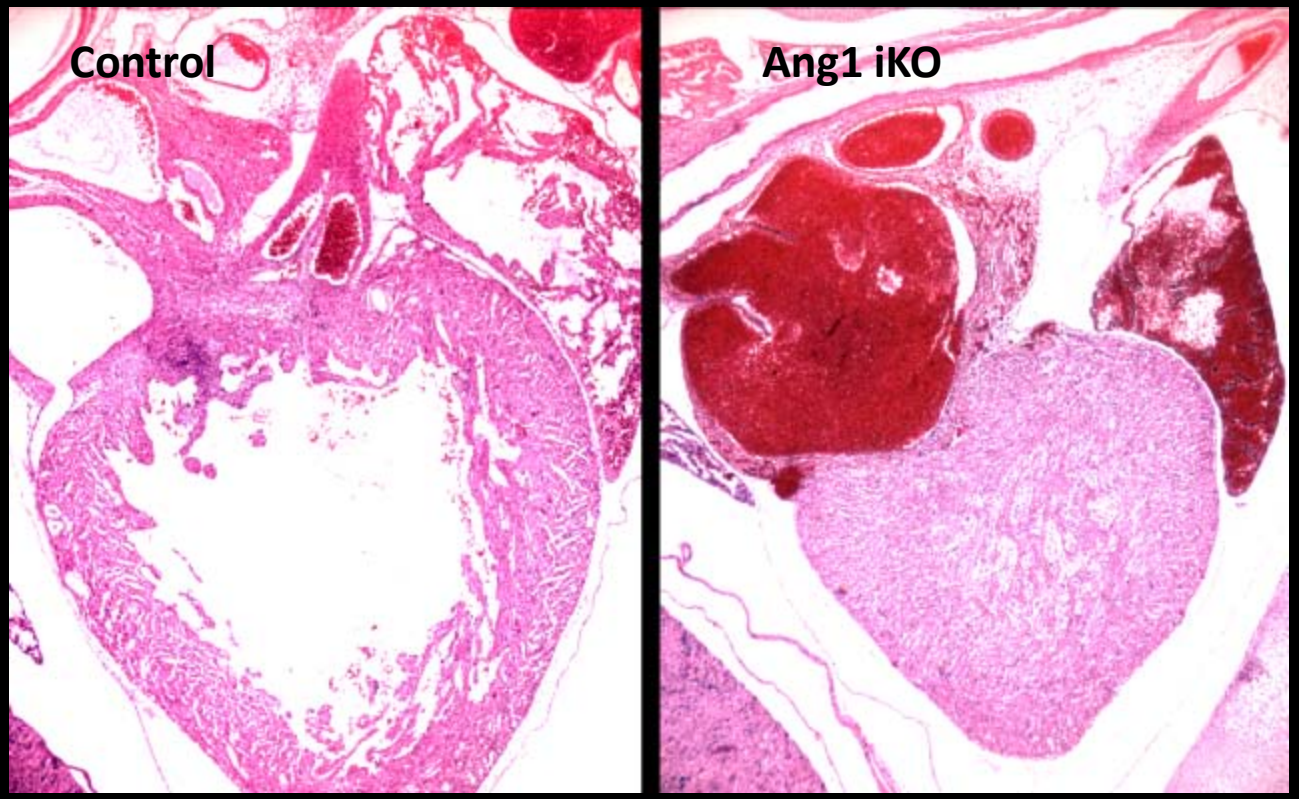
Ang1 iKO: Breeding & DOX

	DOX	Phenotype
Ang1 iKO	E10.5	Die at P1, heart, vasculature
Ang1 iKO	E16.5	Non found
Ang1 iKO	4 wks	Non found



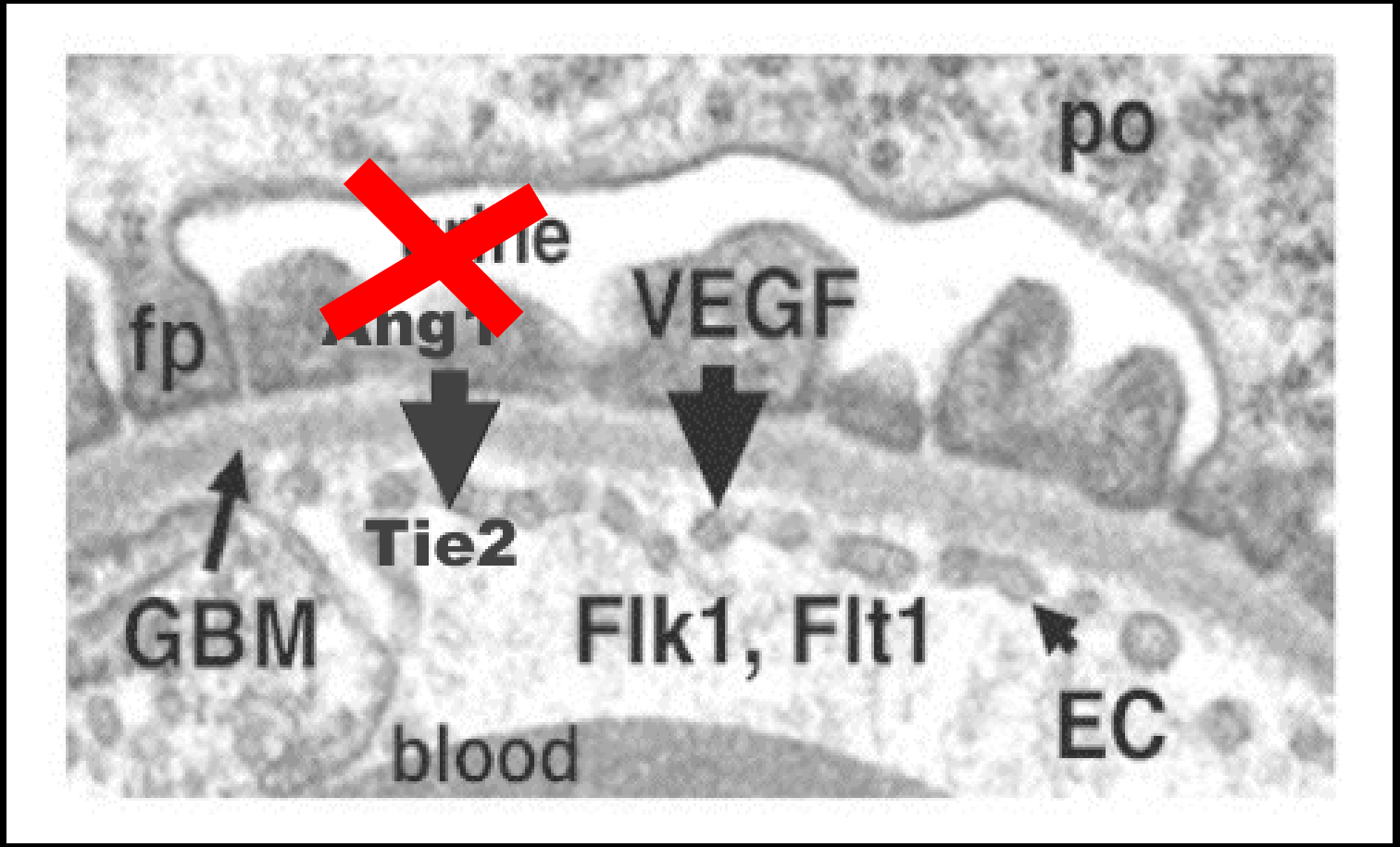
Inducing whole body iKO of Ang1 in adults and at E16.5 does not give a phenotype…

Inducing at E10.5 is lethal (P1) and pups show cardiovascular abnormalities



Podocyte specific KO of Ang1

Glomerular filtration barrier



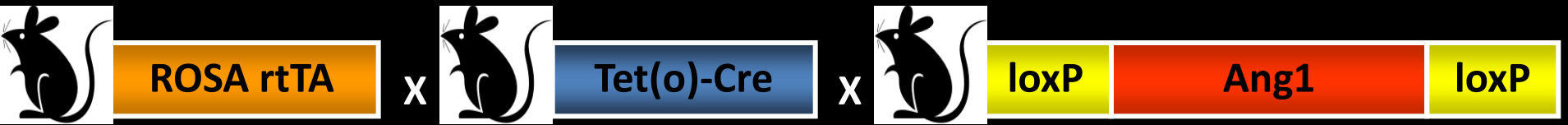
Ang1 podKO

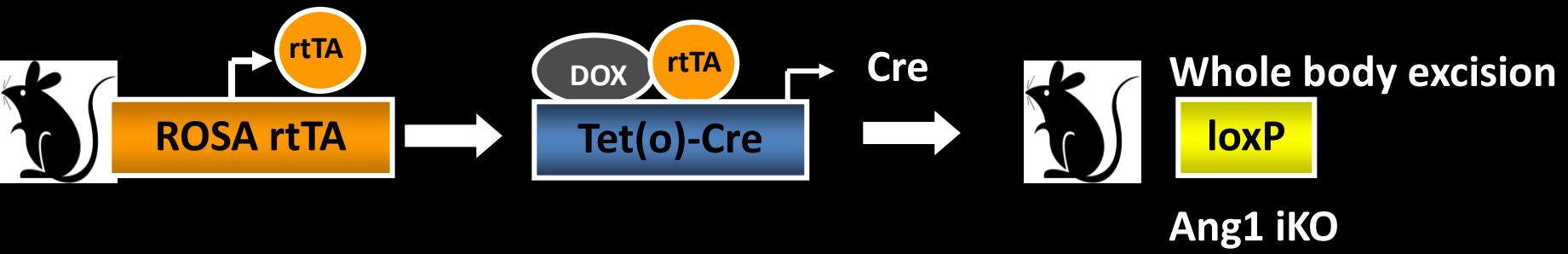
PODOCYTE

Podocyte specific KO of Ang1 does not have a phenotype…!

	Litters / pups / mut	DOX	Dissected
Ang1 pod-KO	31 / 256 / 63		P0 – 12 mo
Ang1 pod-iKO	15 / 103 / 16	4 wks	2-12 mo
		E0	P0, 2 – 12 mo

Inducible whole body deletion; Ang iKO



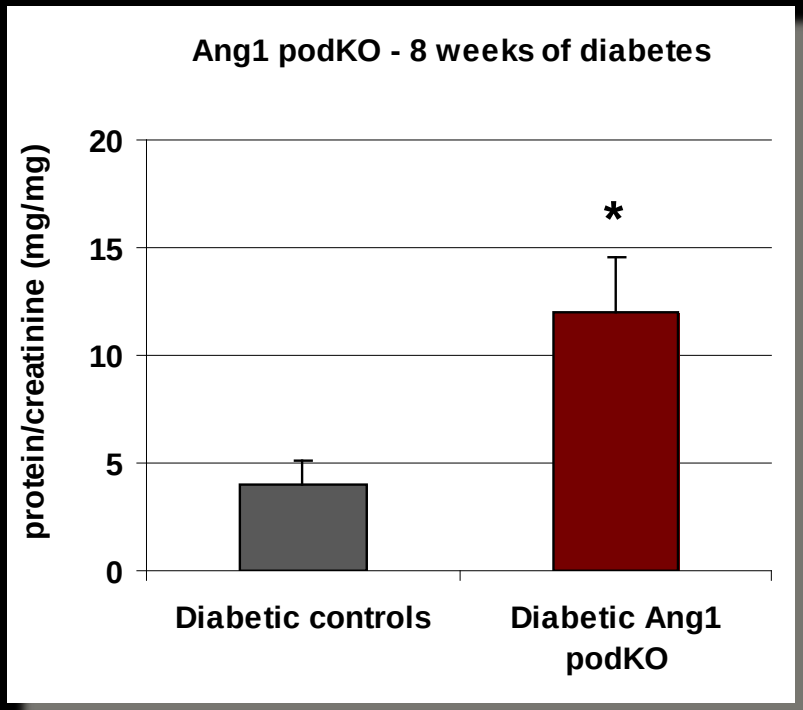


Ang1 podKO & Ang1 iKO - Diabetes

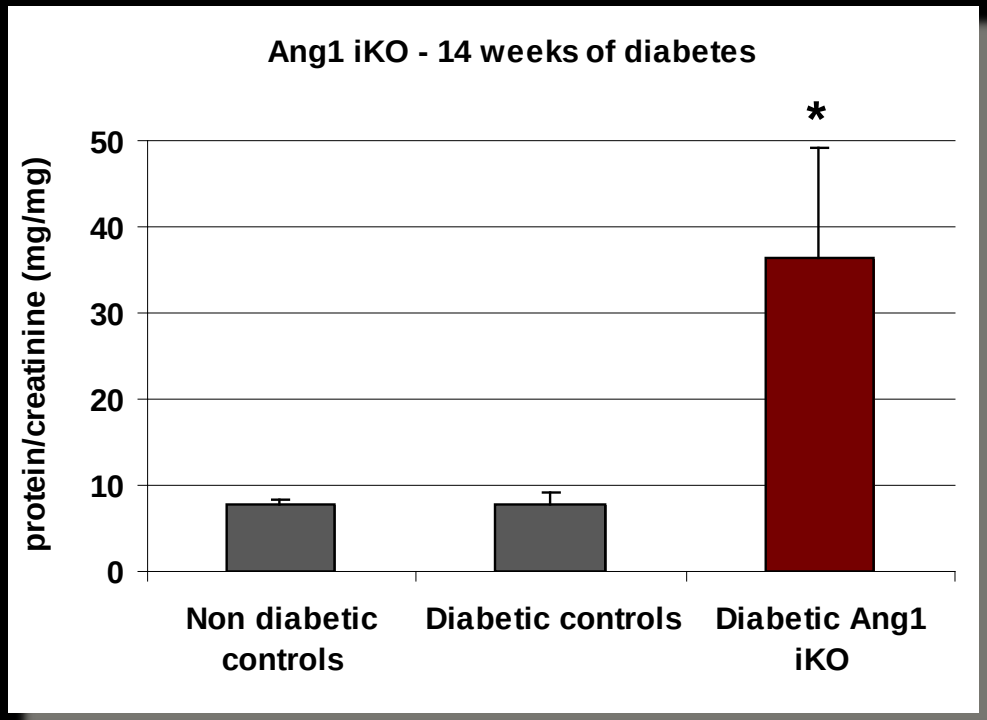
DIABETES

Urine protein/creatinine

Ang1 podKO



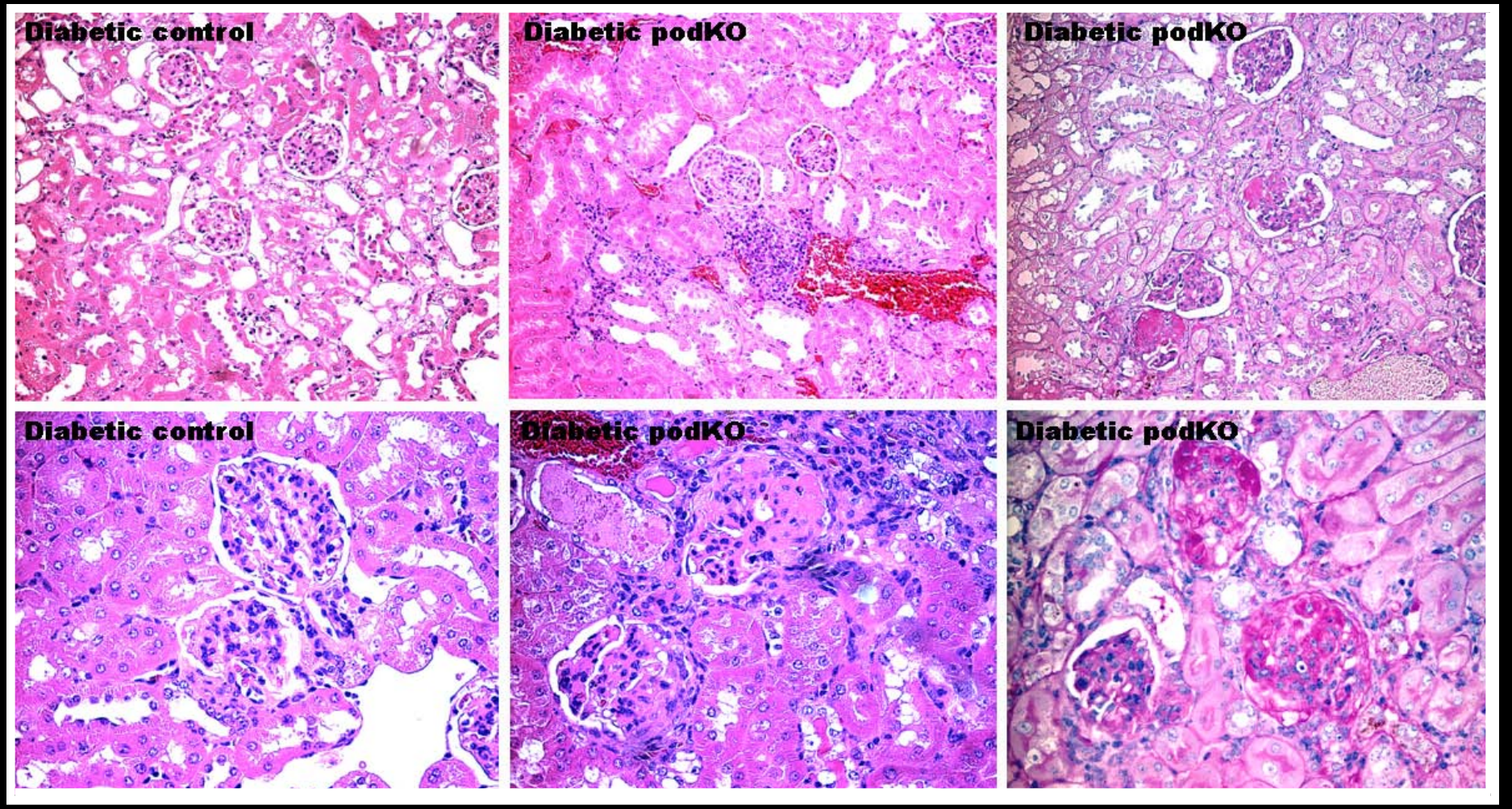
Ang1 iKO



Jeansson, Quagin, et al. (unpublished)

Diabetes in Ang1 podKO

DIABETES



Jeansson, Quagin, et al. (unpublished)

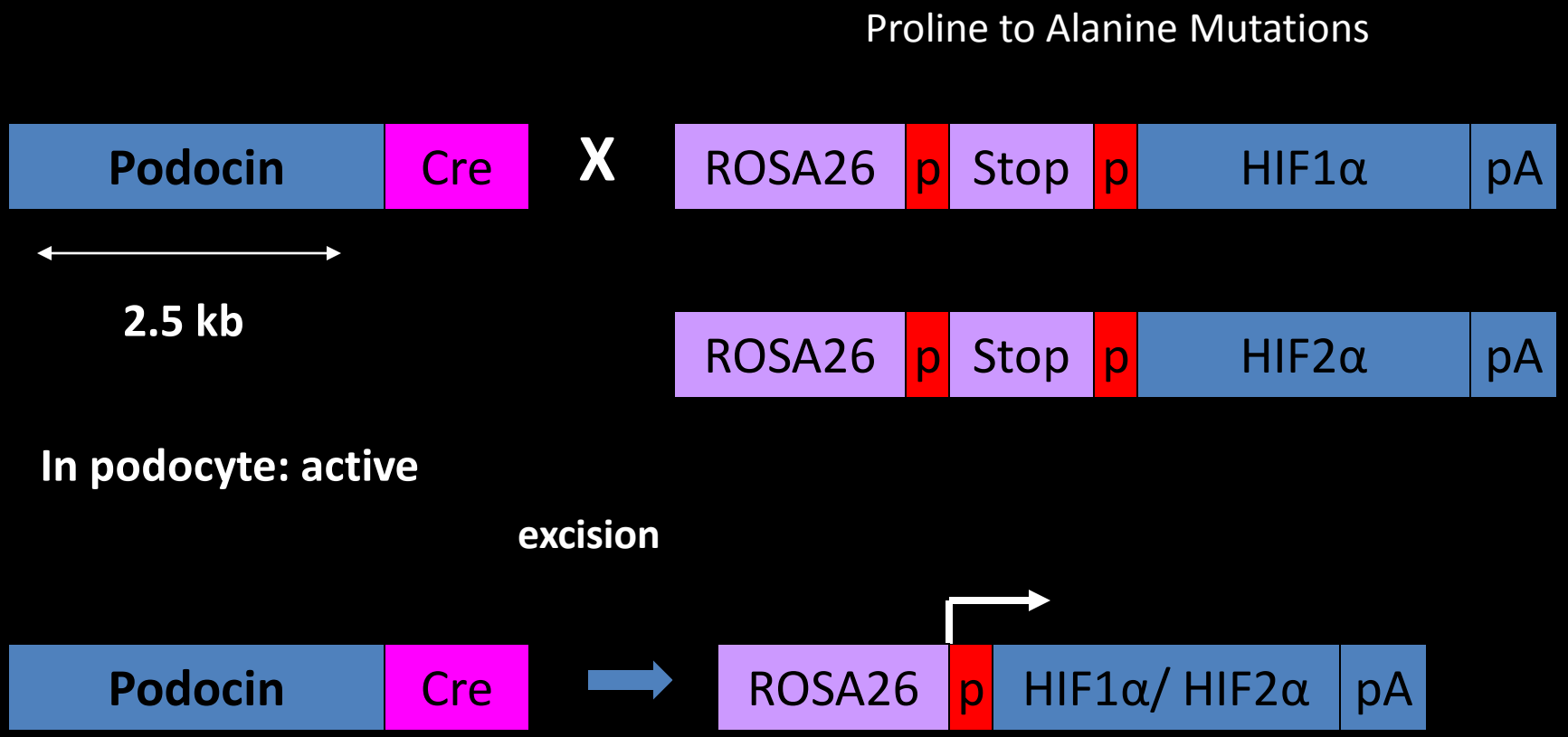
Conclusions I

- Both increase and decrease in VEGF is bad for the adult glomerulus
- Increase VEGF - features of early diabetic nephropathy
- Decrease VEGF in a diabetic mouse = features of late diabetic nephropathy and/or accelerated injury
- DEATH with ascites and nephrotic syndrome and sclerosis (dangerous Rx)

Conclusions II

- Loss of Ang1 also accelerates glomerular injury in diabetic model
- ‘endothelial dysfunction’ is central

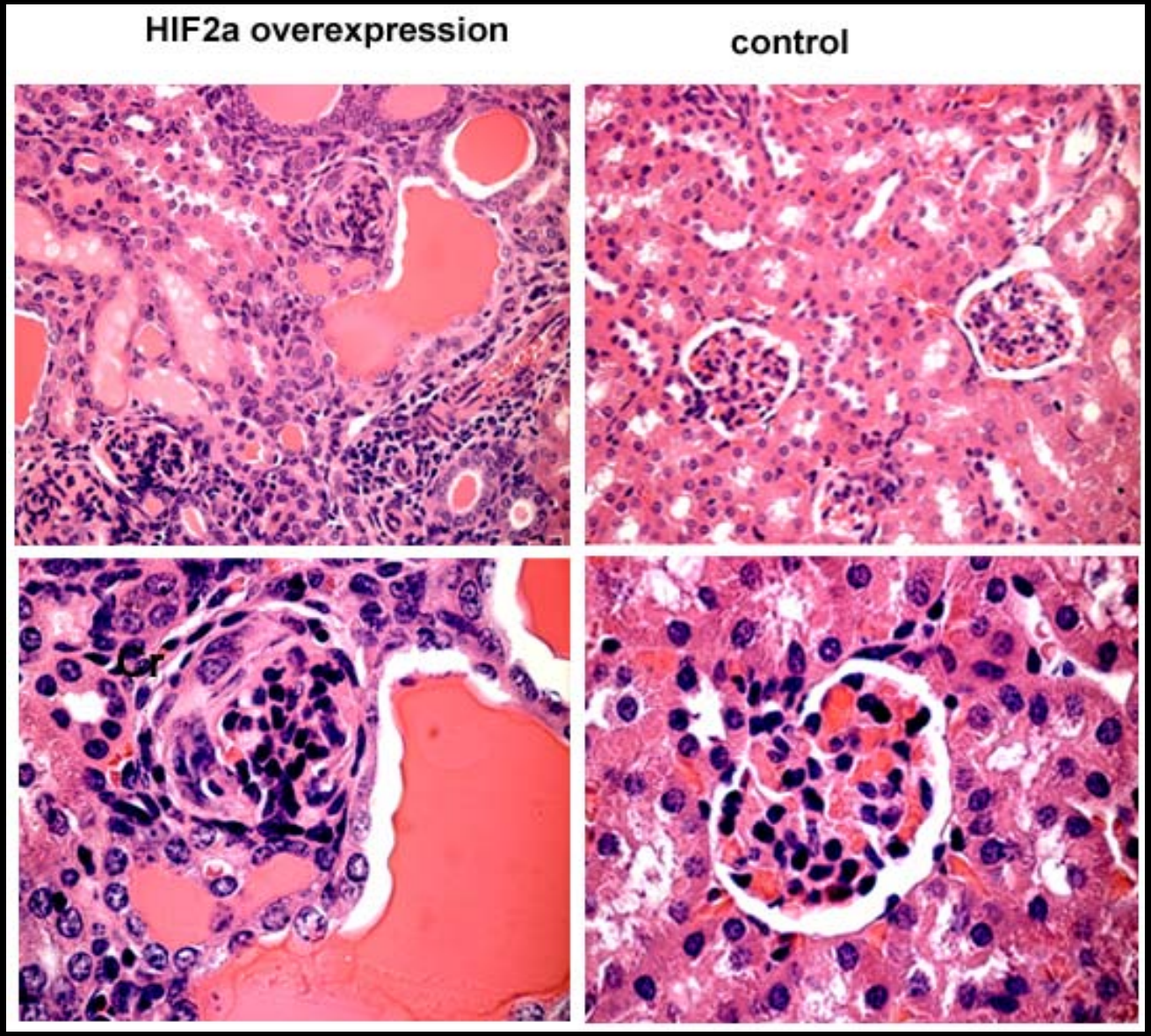
Does HIF1□or HIF2□overexpression enhance complications of diabetes?



W. Kim, UNC, EMBO J , 25 (19), 2006

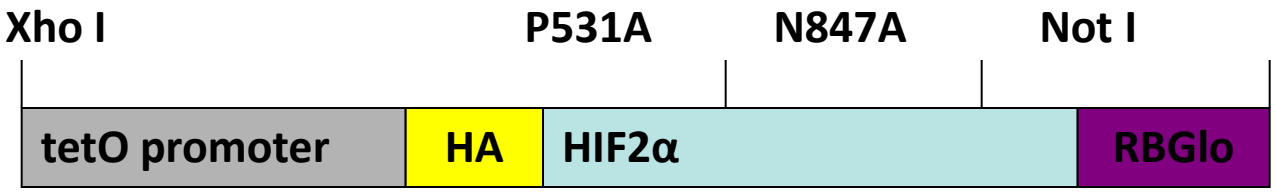
	VHL knockout	HIF2□ overexpression		HIF1□ overexpression	
		Homozygote (n=25)	Heterozygote (n = 40)	Homo (n=12)	Het (n=24)
Onset of the phenotype	3 - 4 weeks proteinuria crescents	3 - 4 weeks proteinuria crescents	5 months abnormal glomeruli	no phenotype	

HIF2α overexpression in podocytes led to abnormal glomeruli similar to VHL knockout mutant mice

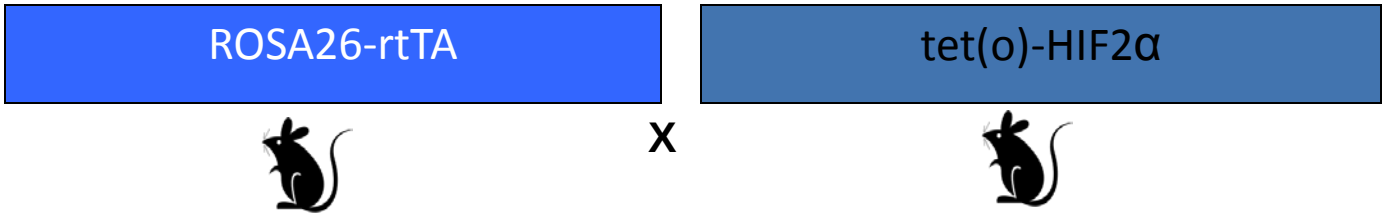


1 month of age

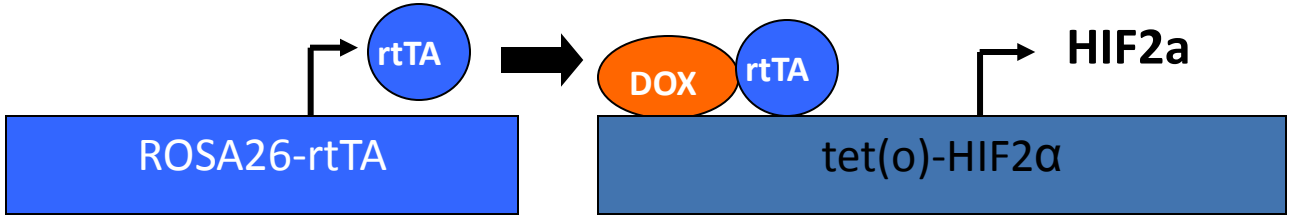
Timed Upregulation; Whole body induction



Pro531Ala - no proteasomal degradation
Asn847Ala – constitutive transcription



With DOX:

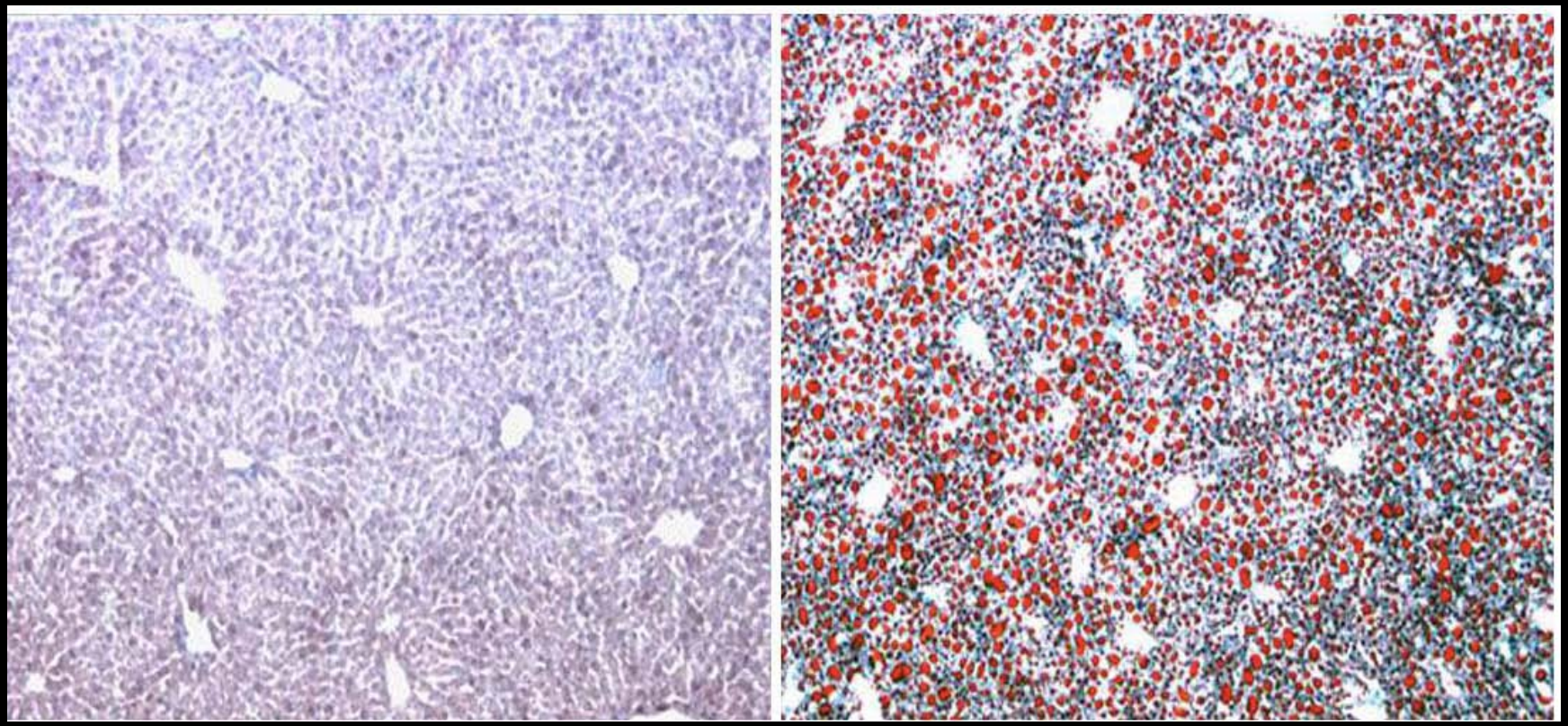


Embryonic induction - lethal

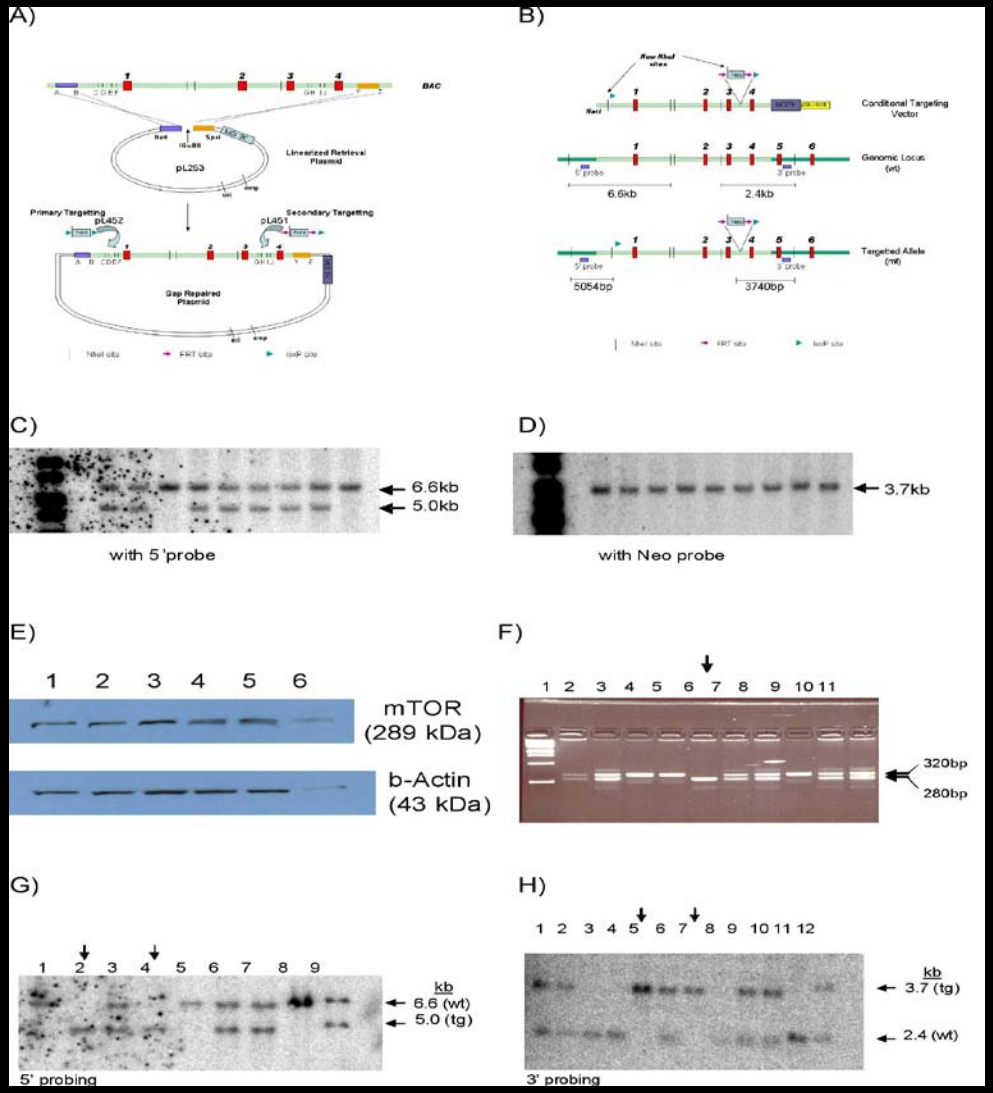
Microvesicular Steatosis/Liver

Control on Dox

HIF2 alpha on Dox



Does mTOR inhibition protect against diabetic complications?



Floxed mTOR mice; cell-sp adult KO (hypo-Morphic, floxed null alleles)

Predictions: knockdown of mTOR in various cell types will protect from complications

SUMMARY

- VEGF or Angiopoietin 1 KO in podocytes or adut whole body - enhances diabetic complications (*endo role)
- HIF2 alpha overexpressors - 5 lines - validated - reversible, robust - diabetes will be induced to look at role of HIFs
- mTOR inhibition - believed to be protective - generated allelic series for mTOR to investigate role
- Not shown: cell-specific driver lines - podos, mesangial, glomerular endos (underway), retinal pericytes