AMDCC

THE ANIMAL MODELS OF DIABETIC COMPLICATIONS CONSORITUM

Michigan/Chicago unit

Modifications in Mouse Models to Enhance Nephropathy/Neuropathy

- Increased oxidative stress
- Increased glucose metabolic flux or alteration in GLUT expression profiles that will lead to changes in glucose metabolism
- Altered signaling that may enhance glucose toxicity

Current Models (page 1)

	Animal model	Background strain	current status	Phenotyping (begin-end)	Consortium collaborators
ROS	SOD2 +/- STZ	C57BL/6J	phenotyping complete; no increase in neuropathy or nephropathy	3-02 to 8-03	
ROS	SOD2 +/- db/db	C57BL/6J	Phenotyping complete; no increase in neuropathy or nephropathy	3-03 to 9 -03	
ROS	Nphs2 Cre//SOD2 loxP/loxP	C57BL/6J	phenotyping ongoing; no obvious increase nephropathy	12-03 to 12-04	Vanderbilt
ROS	Nphs2 Cre//SOD2 loxP/loxP	C57BL/6J 129SvJ	phenotyping nearing completion; no significant increase nephropathy	1-04 to 12-04	Vanderbilt
ROS	nestin Cre//SOD2 loxP/loxP	C57BL/6J	phenotyping ongoing	3-04 to 2-05	Vanderbilt
ROS	synapsin Cre//SOD2 loxP/loxP	C57BL/6J	Breeding complicated; phenotyping ongoing	4-04 to 4-05	Vanderbilt
ROS	Po Cre// SOD2 loxP/loxP	C57BL/6J	receiving mice—On hold	12-04 to 12-05	Vanderbilt
ROS	GCLC +/- STZ	C57BL/6J	On hold	6-03 to 5-04	
ROS	GCLC +/- db/db	C57BL/6J	phenotyping virtually complete; excellent neuropathy; no increase nephropathy	7-03 to 6-04	
signaling	AR tg//SOD2+/- STZ	C57BL/6	phenotyping ongoing; increased neuropathy in diabetics with double mutation	1-04 to 12-04	
signaling	GCLC +/-//SOD2 +/- STZ	C57BL/6	To begin breeding—On hold	12-04 to 12-05	
signaling	fyn -/-	C57BL/6J 129SvJ	phenotyping complete severe albuminuria in nondiabetics	6-03 to 8-03	
signaling	fyn -/- STZ	C57BL/6J 129SvJ	phenotyping completed: increased albuminuria in diabetics with mutation	6-03 to 8-03	
signaling	fyn -/-	129SvJ	rederivation completed; phenotyping ongoing. No albuminuria in nondiabetics	9-03 to 8-04	
signaling	fyn -/- and +/- STZ	129SvJ	phenotyping ongoing	9-03 to 9-04	
signaling	IRS-1 -/-	C57BL/6J	Breeding colony—On hold	?	

Current Models (page 2)

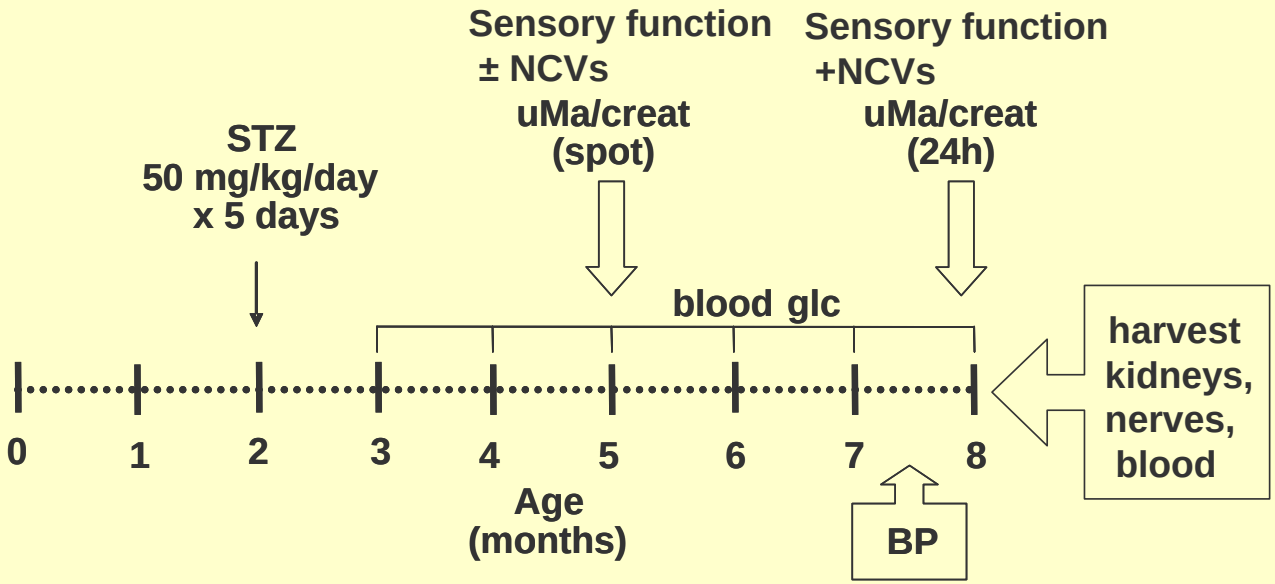
GLUTs

Animal model	Background strain	current status	Phenotyping (begin-end)	Consortium collaborators
GLUT4 -/- STZ	C57BL/6J	phenotyping complete; increased nephropathy; modest increase neuropathy	11-01 to 5-04	Einstein
GLUT4 -/- db/db	C57BL/6J	On hold	11-03 to 10-04	Einstein
Nphs2 Cre //Glut4 loxP/loxP	FVB/ C57BL/6J	phenotyping ongoing	4-04 to 12-05	Utah
GLUT1 tg	C57BL/6J	phenotyping ongoing; increased glomerulosclerosis in nondiabetics	7-03 to 1-05	Hopkins
GLUT1 tg STZ	C57BL/6J	expanding lines	9-03 to 11-04	Hopkins
GLUT1 tg db/db	C57BL/6J	phenotyping ongoing	10-03 to 1-05	Hopkins
Nphs2 GLUT1 tg db/db	C57BLKS	Transgenic lines with overexpression established; beginning phenotyping	12-04 to 10-05	Hopkins
Nestin GLUT1 tg db/db	C57BLKS	making construct—On hold	3-04 to 10-05	Hopkins
Nphs2 cre //PPARγ loxP/loxP	C57BL/6J	uncertain	12-04 to 8-05	UCLA
conditional Nphs2 cre	C57BL/6J	Crossed with other lines	breeding stock	
synapsin cre	C57BL/6J	crossed with other lines	breeding stock	
nestin cre	C57BL/6J	crossed with other lines	breeding stock	
db/db	C57BL/6J	breeding; phenotyping complete	9-03; breeding stock	
db/db	C57BLKS	breeding; increased neuropathy	2-04 to 10-04; breeding stock	
Selenocysteine floxed (trsp) x synapsin cre STZ	C57BL/6J	breeding	8-04	Koenig (Michigan)
Selenocysteine floxed (trsp) x podocin cre STZ	C57BL/6J	breeding	8-04	Koenig (Michigan)

Current models

	uMa/Cr Every 12 weeks 24 hr urine at 24 wks or end of trial	Tail Flick Hind Paw Every 8 weeks	NCV Measured 12 and 24 weeks post-diabetes	Glom. PAS area Measured at tissue harvest	Glom. podocyte Measured at tissue harvest	IEFD Measured at tissue harvest	Biochemistry Measured at tissue harvest
C57BLKS db/db	NA	X	X	NA	NA	X	X
GCLC +/- db/db high fat diet	Ongoing	X	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
GLUT1 tg db/db high fat diet	Ongoing	X	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
Nphs2 GLUT1 tg C57BLKSdb/db	Ongoing	X	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
Nphs2 Cre// SOD2 loxP/loxP	X	NA	NA	X	Ongoing	NA	Ongoing
Nestin Cre// SOD2 loxP/loxP	X	X	X	Ongoing	Ongoing	Ongoing	Ongoing
GLUT4 -/- and Nphs2 Cre// GLUT4 loxP/loxP	X	X	X	X	X	X	Ongoing
GLUT4 -/- Akita	Breeding	Breeding	Breeding	Breeding	Breeding	Breeding	Breeding
PDX+/-	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing

General schema for evaluation



AMDCC Collaborations

- Podocyte specific GLUT4 -/- mice: Utah group (Abel) bred GLUT4 loxP/loxP with nphs2 cre.
- Podocyte specific SOD2 -/- mice: Vanderbilt group (Breyer, Harris) gave SOD2 loxP/loxP mice.
- GLUT4 -/- mice: Einstein (Charron), Sinai (Bottinger) groups provided mice and will help with metabolic assessment. Cleveland Clinic (Daneshgari) group evaluated cystopathy.
- Podocyte specific PPARg -/-. Sent cre mice to UCLA (Hsueh) group.
- AR tg mice. Sent to Rockefeller (Breslow) group.
- PDX +/-. Received from Rockefeller (Breslow) group.

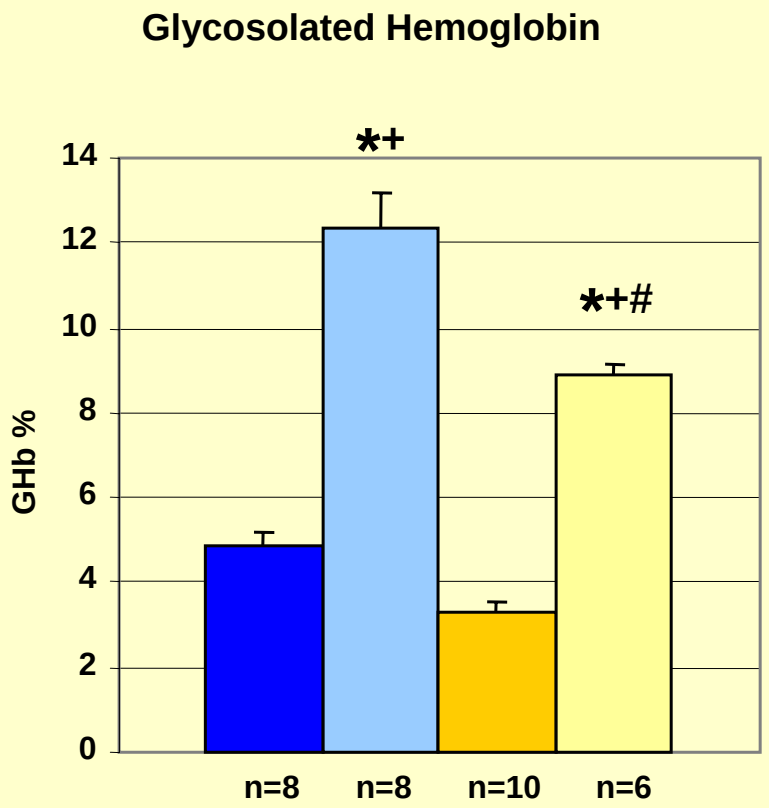
GLUT4 -/- STZ C57bl/6J model

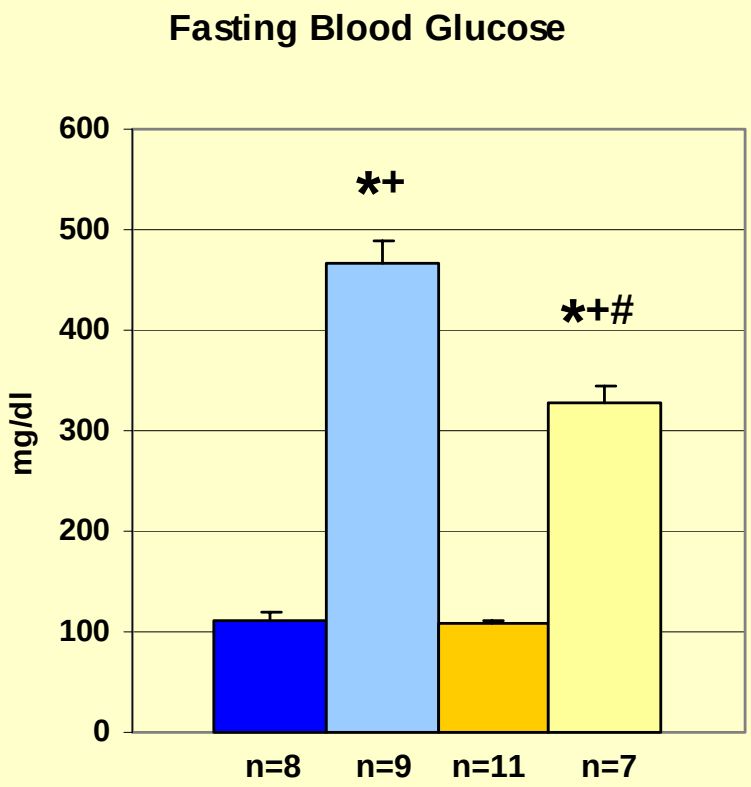
- Four groups: GLUT4 +/+ Control
  GLUT4 +/+ Diabetic
  GLUT4 -/- Control
  GLUT4 -/- Diabetic
- Male mice injected with STZ (50 mg/kg BW) for 5 days @ 9-10 wks of age
- Experiment duration - 33 wk post STZ
- One-way ANOVA followed by Scheffe

GLUT4 -/- C57BL/6J model

- GLUT4 -/- mice: insulin resistance but no overt diabetes, diminished fat stores and FFA levels.
- GLUT4 expressed in renal glomerular mesangial cells and podocytes.
- GLUT4 -/- led to proteinuria on mixed background. Pathogenesis unclear.
- Examined both total body GLUT4 -/- (collab. Bottinger/Charron) and podocyte-specific GLUT4 -/- (collab. Abel) STZ models
- Background c57BL/6J (total body) or mixed c57BL/6J and FvB (podo. specific)

GLUT4 -/-
33 weeks post STZ





* vs. GLUT4 +/+ Control

+ vs. GLUT4 -/- Control

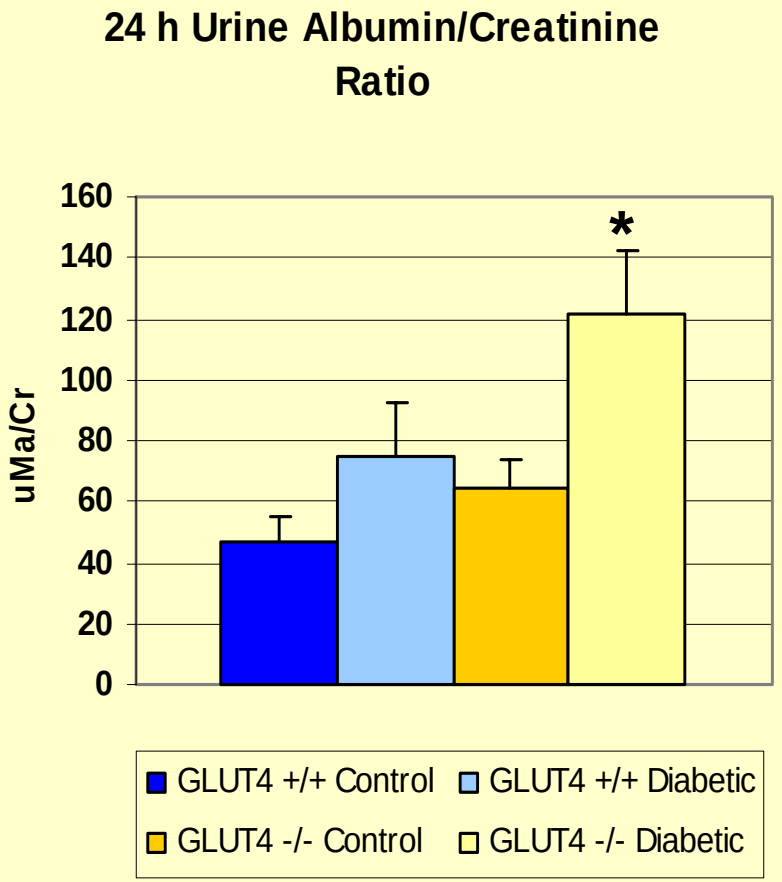
vs. GLUT4 +/+ Diabetic

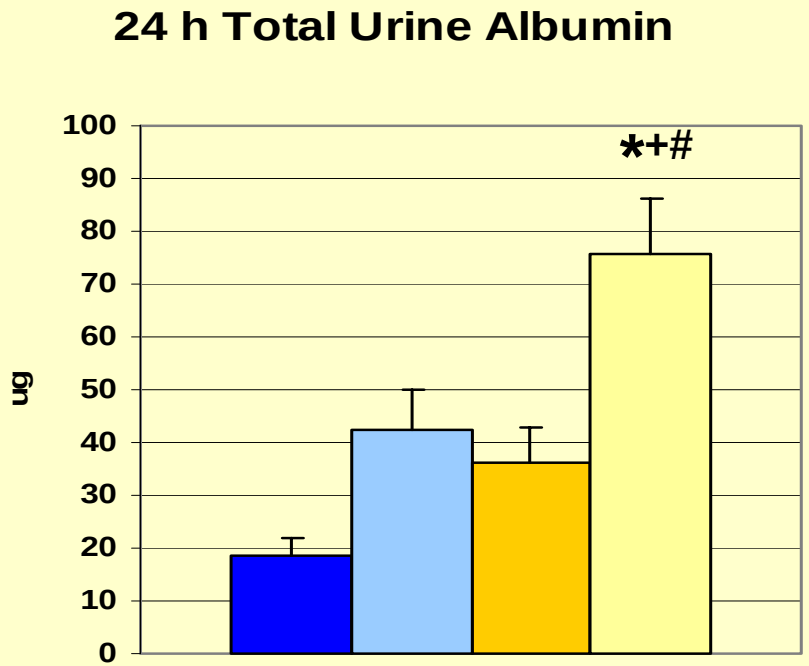
p<0.0006

GLUT4 +/+ Control
GLUT4 +/+ Diabetic
GLUT4 -/- Control
GLUT4 -/- Diabetic

p<0.0001

GLUT4 -/-
33 weeks post STZ





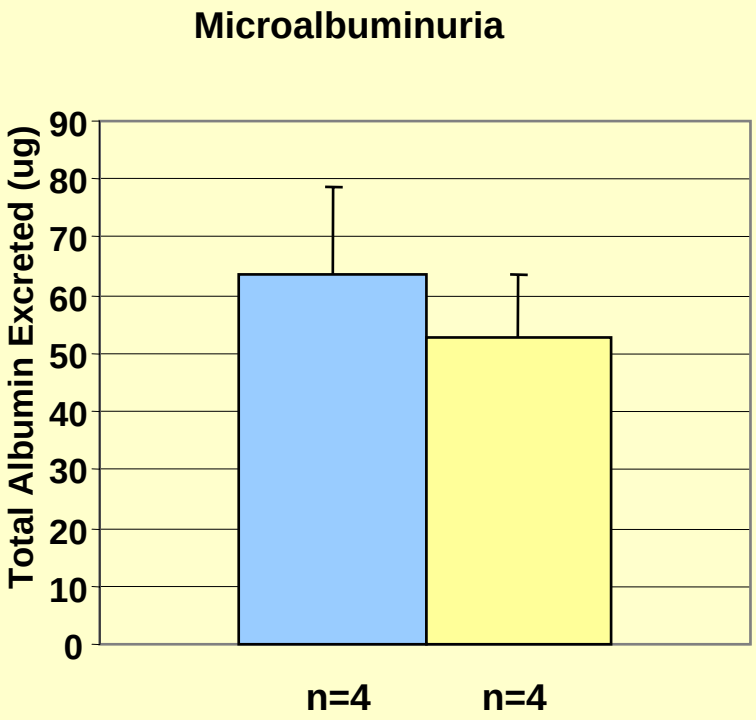
* vs. GLUT4 +/+ Control

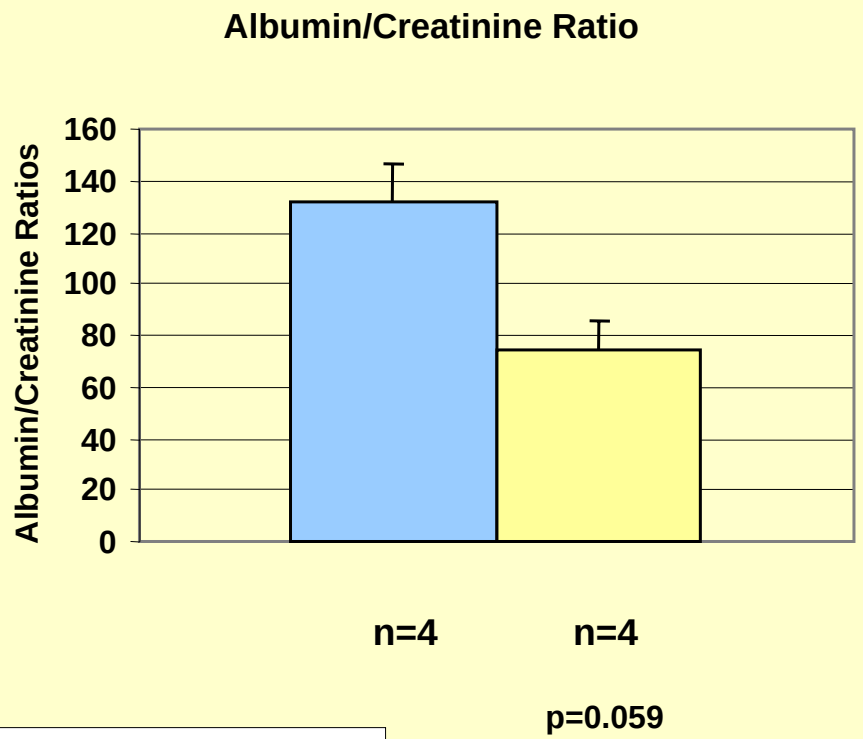
\+ vs. GLUT4 -/- Control

\# vs. GLUT4 +/+ Diabetic

p<0.0325

Kidney Podocyte-specific GLUT4 KO C57BL/6J/129/SvJ

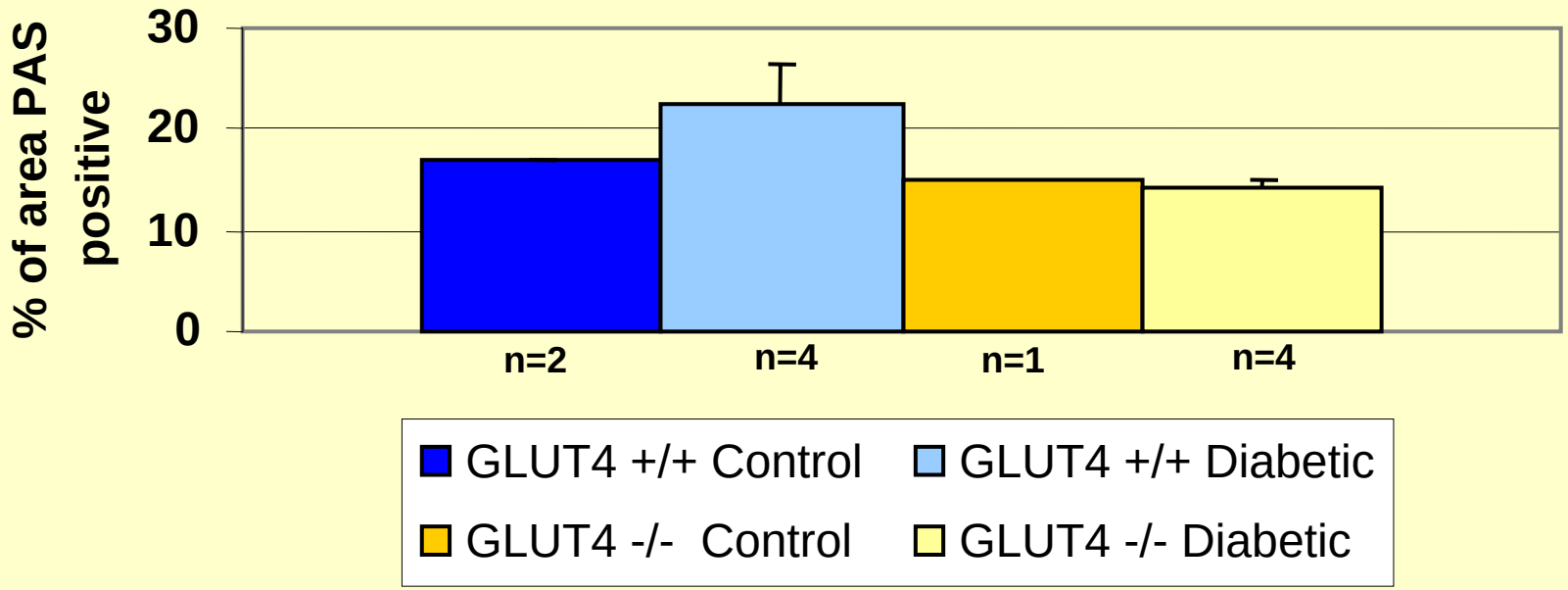




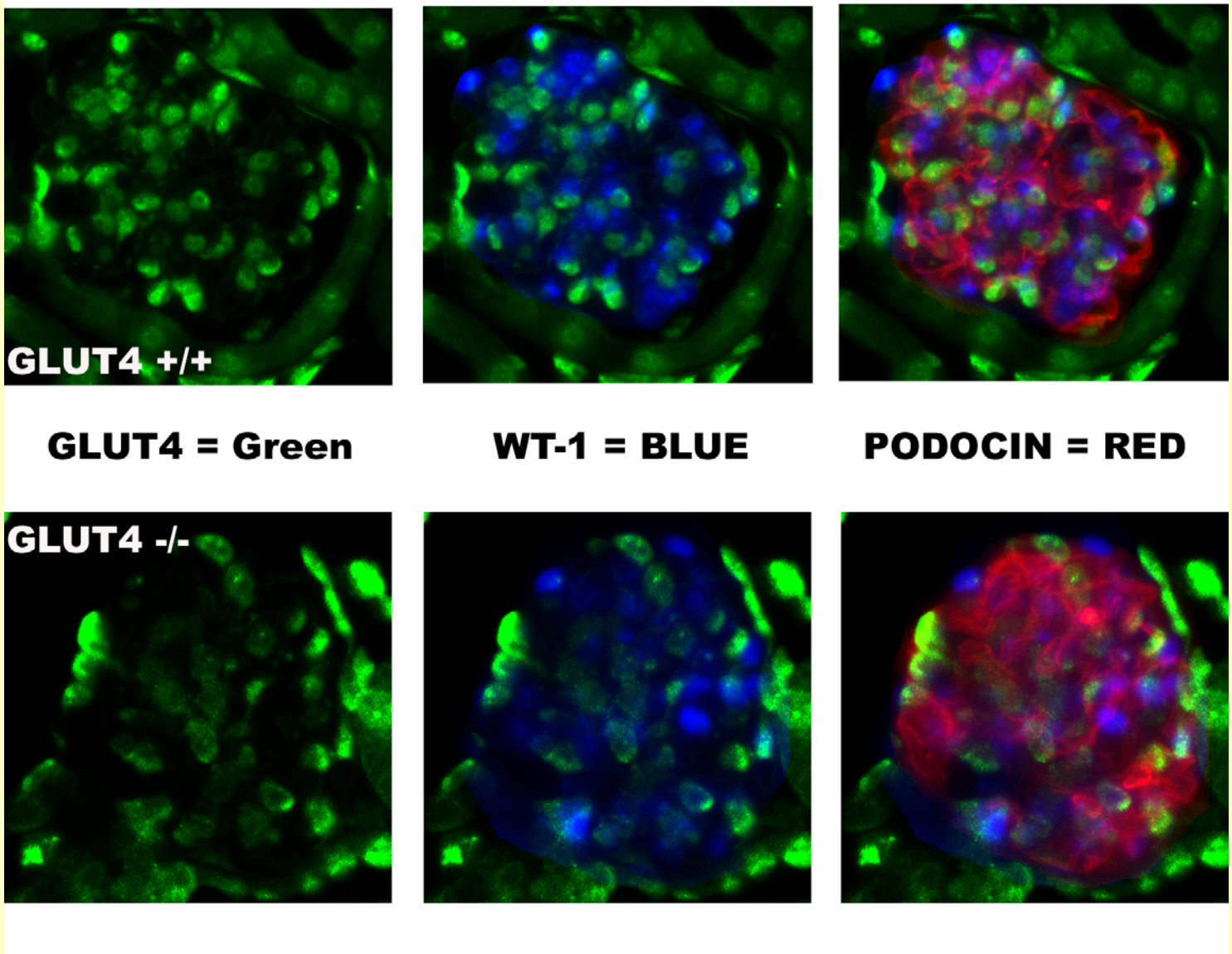
GLUT4 +/+ Diabetic GLUT4 -/- Diabetic

Kidney Podocyte-specific GLUT4 KO C57BL/6J/129/SvJ

Glomerulus PAS staining @ 33 weeks post STZ



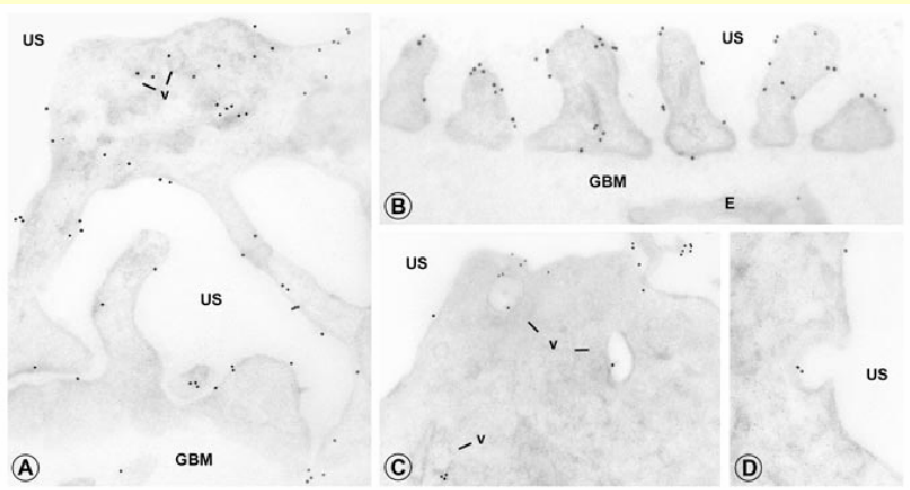
Nphs2 Cre // GLUT4 loxP/loxP



Nphs2 GLUT1tg db/db C57BLKS model

- GLUT1 is highly expressed in podocytes
- High glucose and diabetes in humans and rodents lead to podocyte loss
- Nphs2 promoter drives GLUT1 in podocytes
- db/m C57BKS eggs injected to allow direct examination on diabetic background

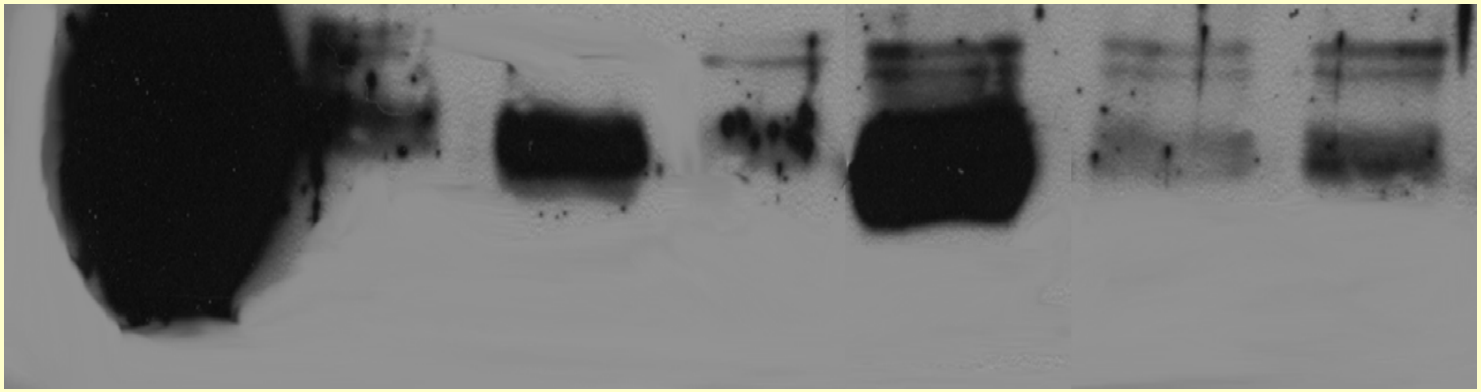
GLUT1 in human kidney podocytes



Nphs2 GLUT1 tg C57BLKS model

GLUT1

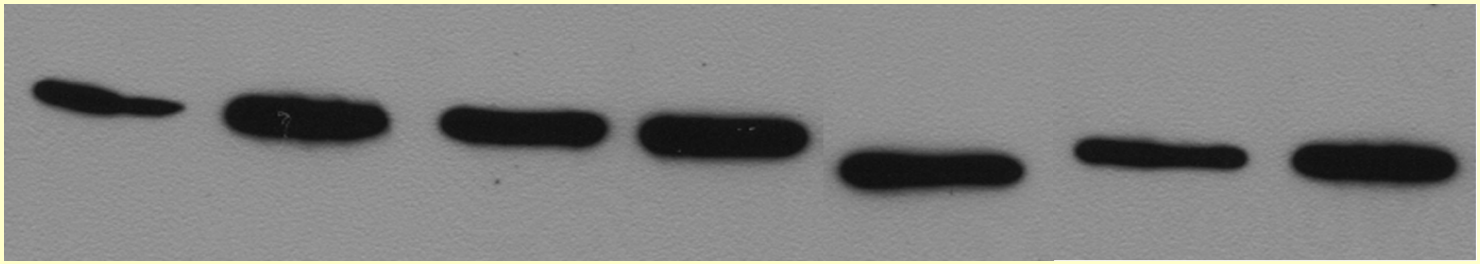
A



-GLUT1

CONTROL TG CONTROL TG CONTROL TG

B



-TUBULIN

Positive control

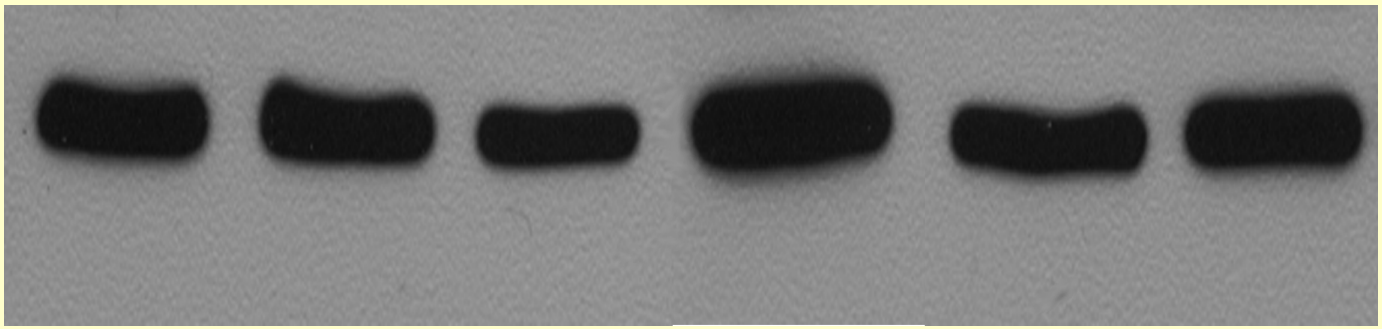
LINE 4

LINE 1

LINE 6

Nphs2 GLUT1 tg C57BLKS model

PODOCIN

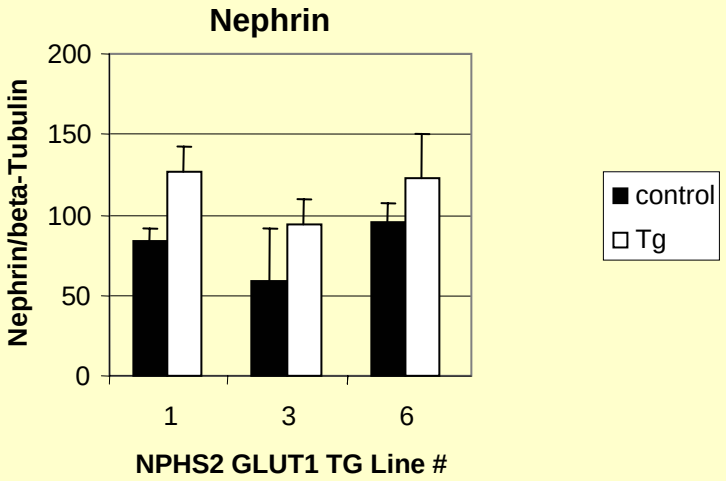


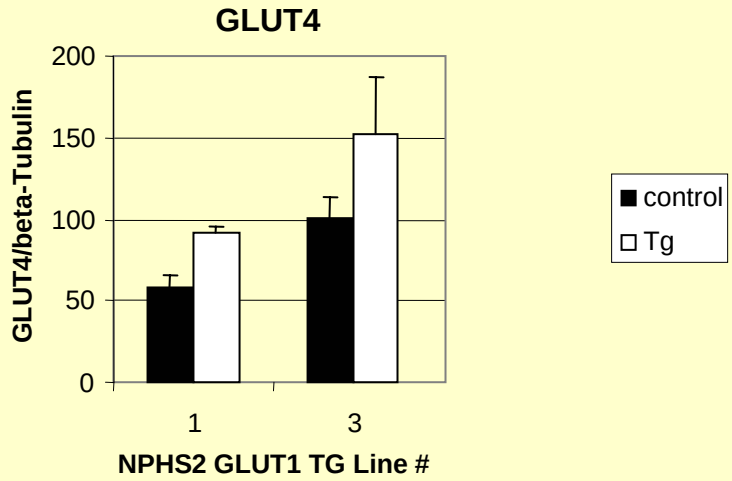
-Podocin

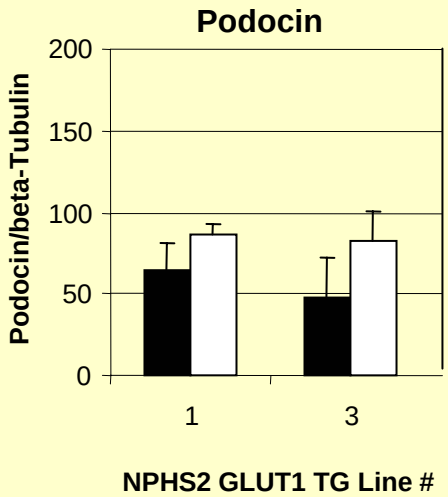
CONTROL TG CONTROL TG CONTROL TG

LINE 4 LINE 1 LINE 6

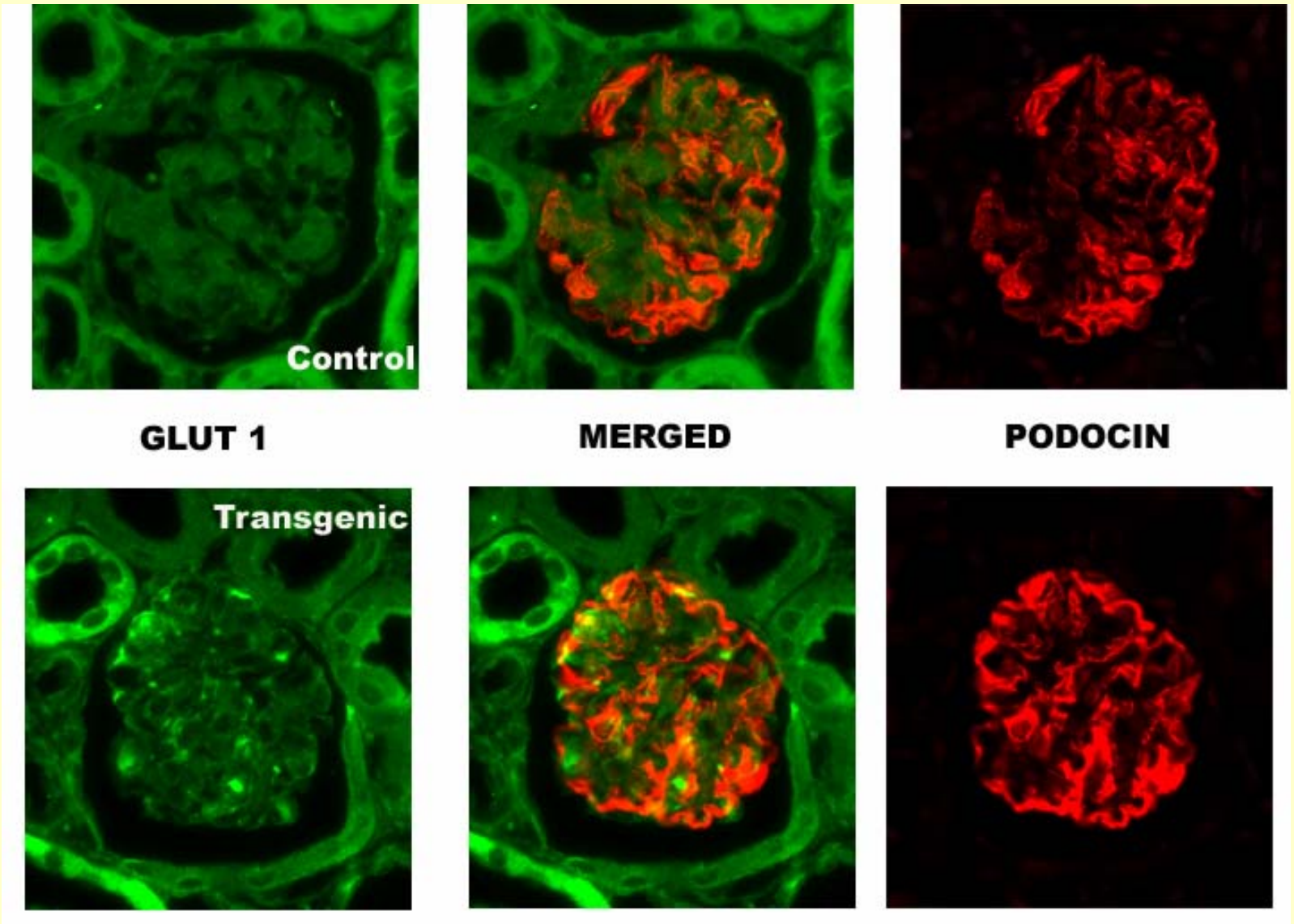
Nphs2 GLUT1 tg C57BLKS model



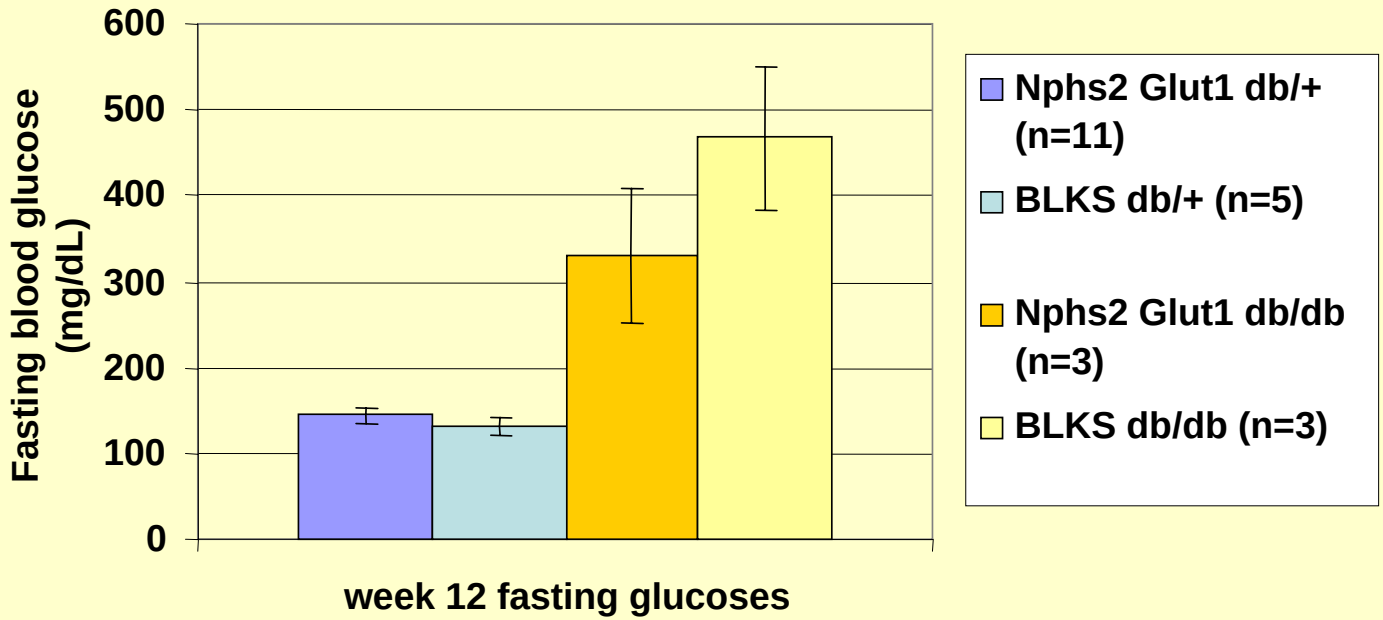




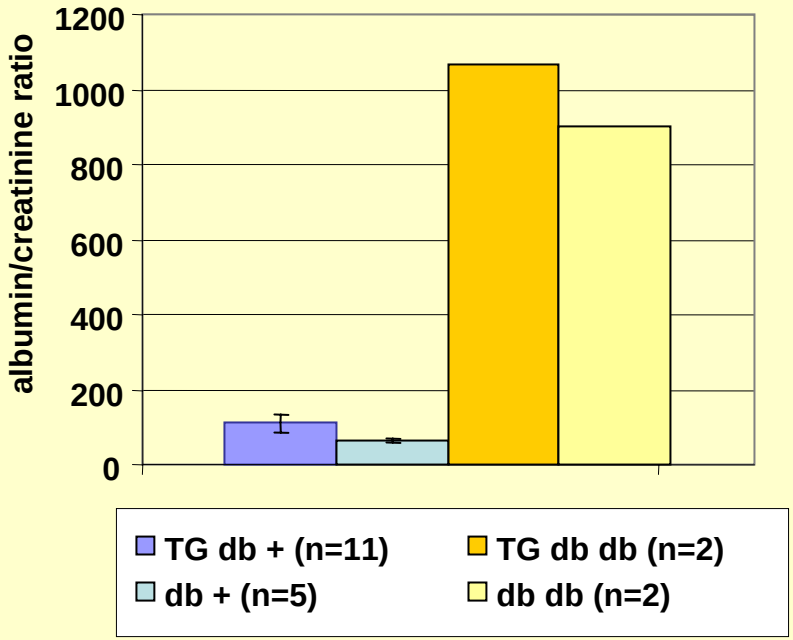
Nphs2 GLUT1 tg C57BLKS db+ model



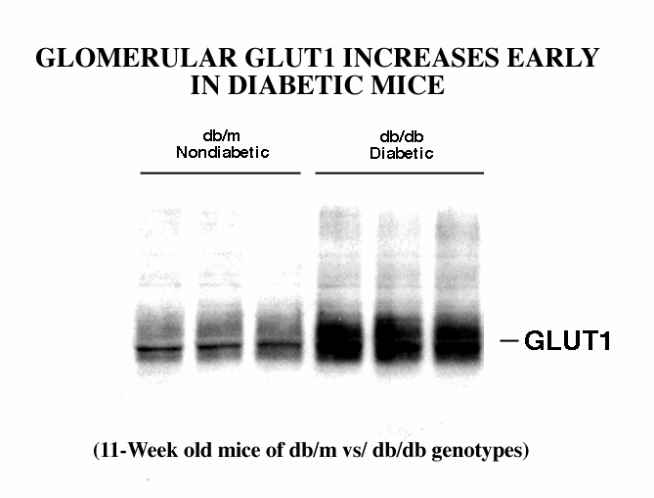
Nphs2 GLUT1 tg C57BLKS model--FBS 12 wk



Nphs2 GLUT1 tg C57BLKS model –Albuminuria 12 wk

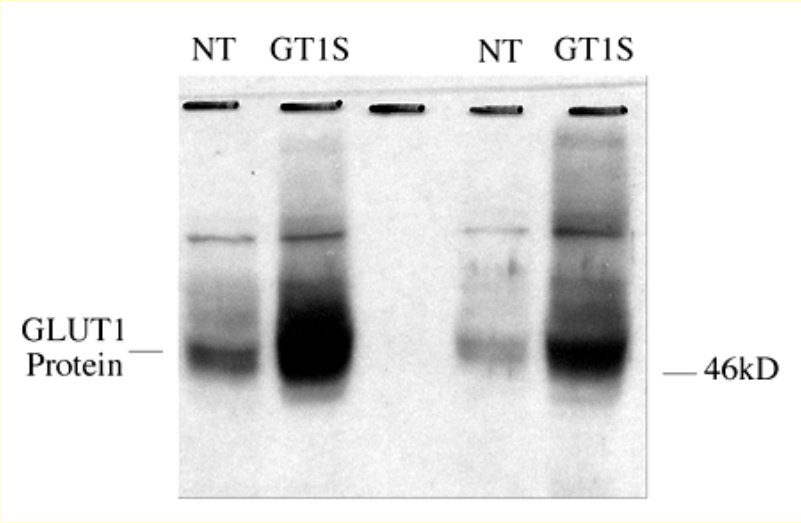


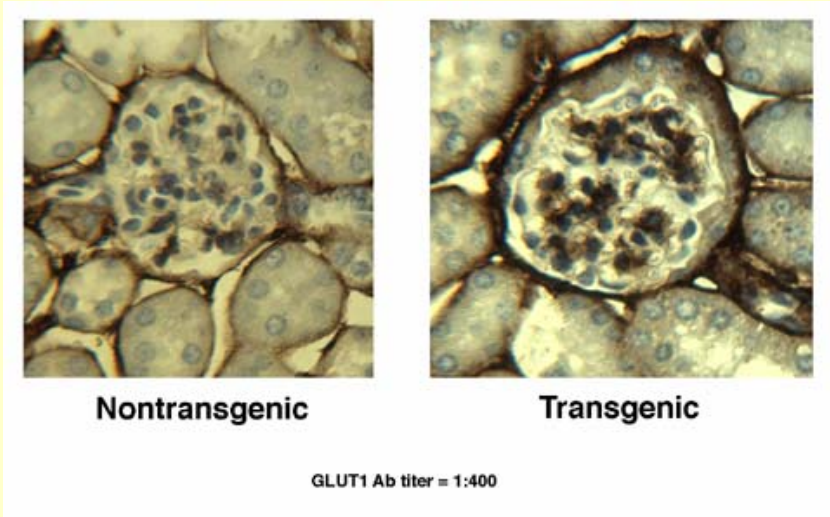
GLUT1tg C57Bl/6J model (GT1S)

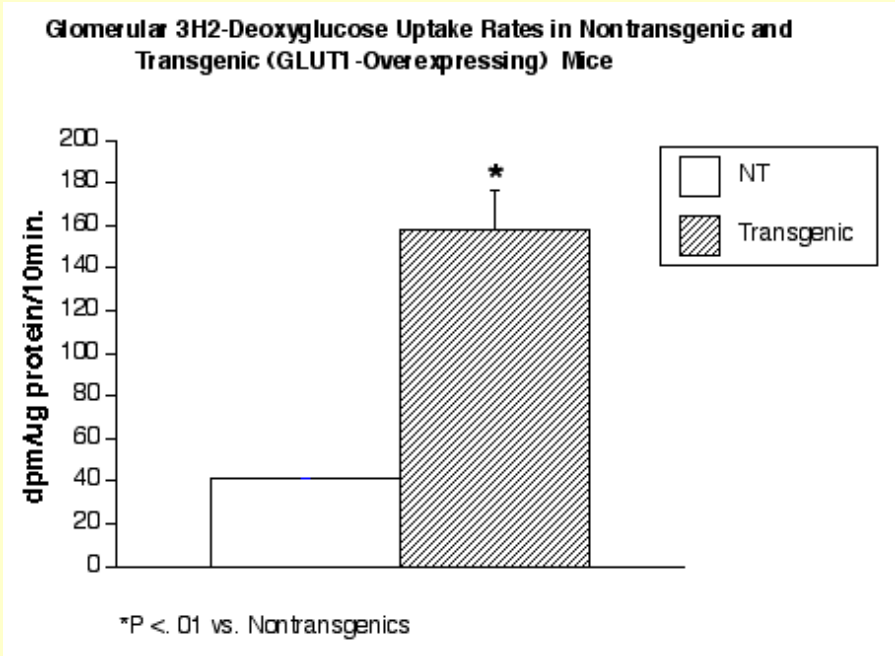


- GLUT1 is increased in glomerular cells in diabetes
- GLUT1 leads to enhanced PKCα and AR activity, fibronectin synthesis
- Modified β-actin promoter drives GLUT1 in many tissues: high in mesangial cells
- Bred onto db/db C57BL/6J
- Effect of high fat feeding

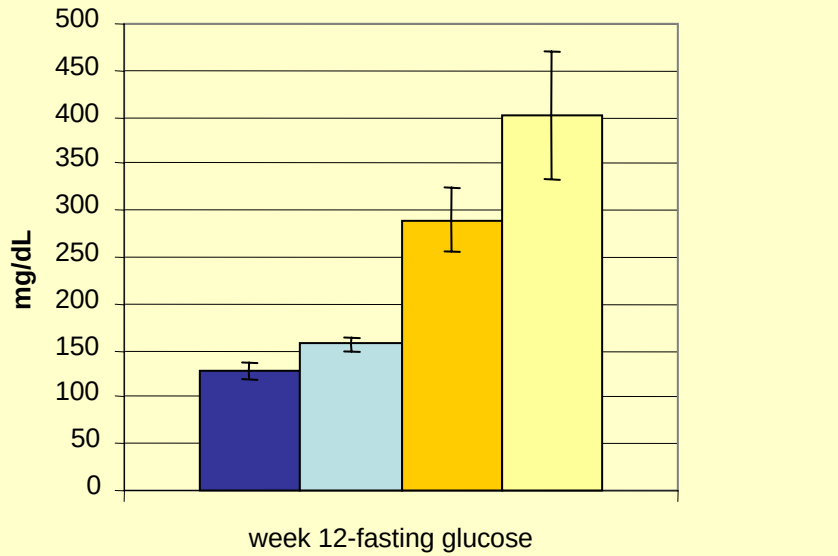
GLUT1tg C57BL/6J (GT1S) model



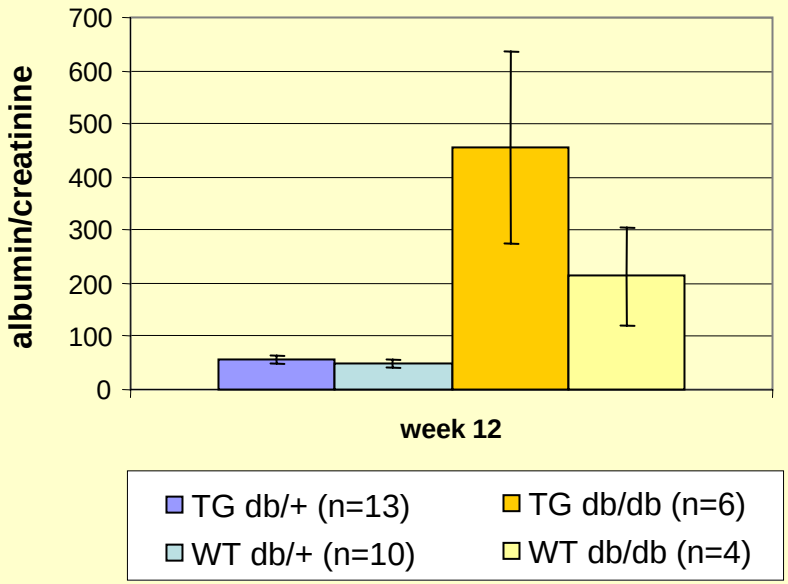




Effects of high fat feeding on fasting blood sugar GLUT1 tg C57BL/6J db/db model



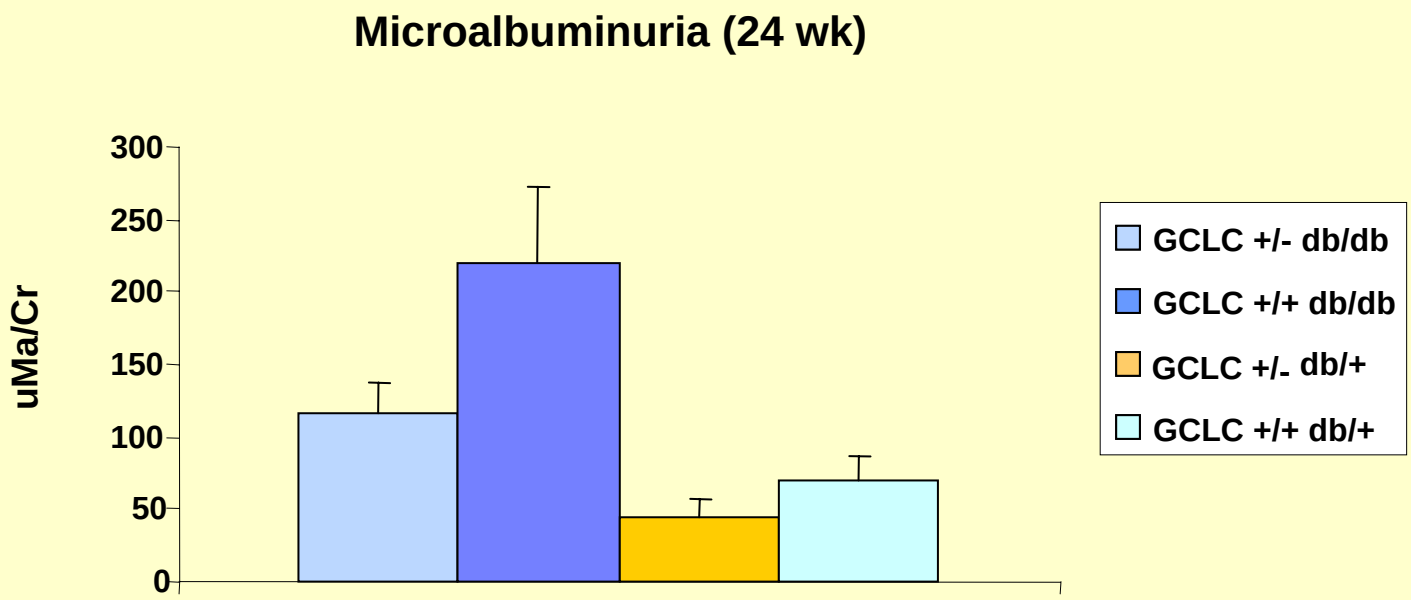
Effects of high fat feeding on albuminuria GLUT1 tg C57BL/6J db/db model



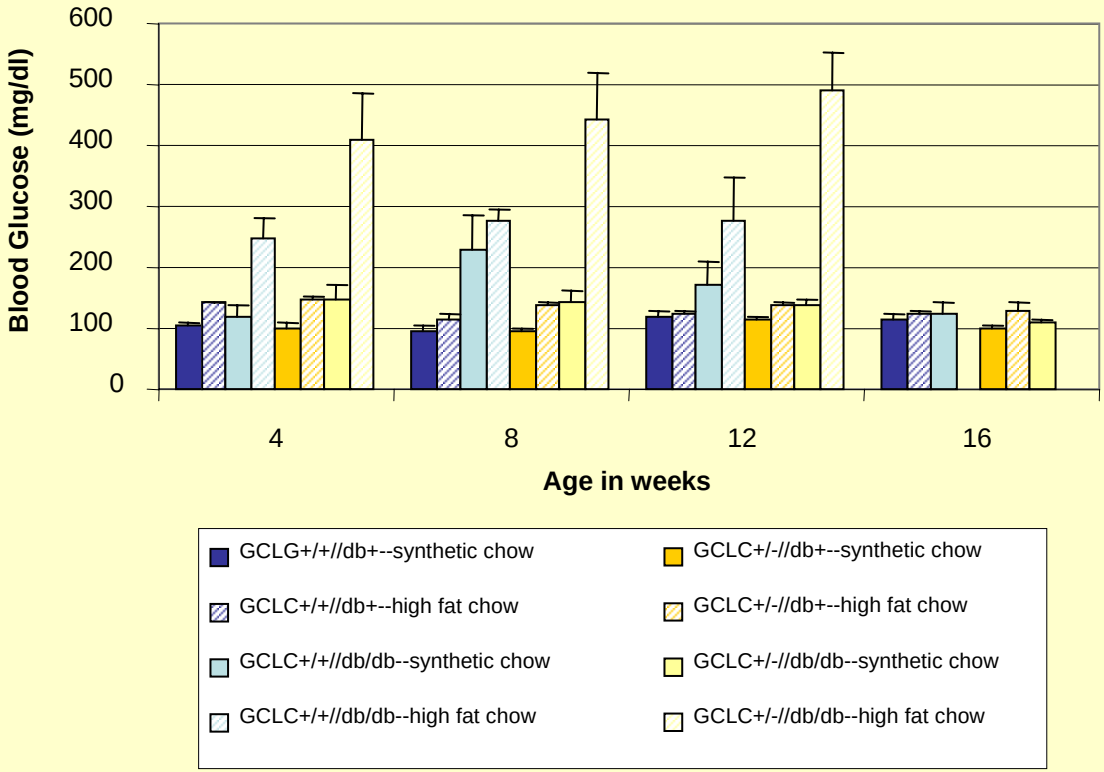
GCLC +/- db/db C57BL/6J model

- γ-glutamate cysteine ligase heavy chain
- GCLC -/- embryonically lethal
- GCLC +/- mild oxidative stress
- GCLC +/- mice placed in db/db c57BL/6J background
- Effect of semisynthetic diet on db/db diabetes determined
- Now effect of high fat feeding

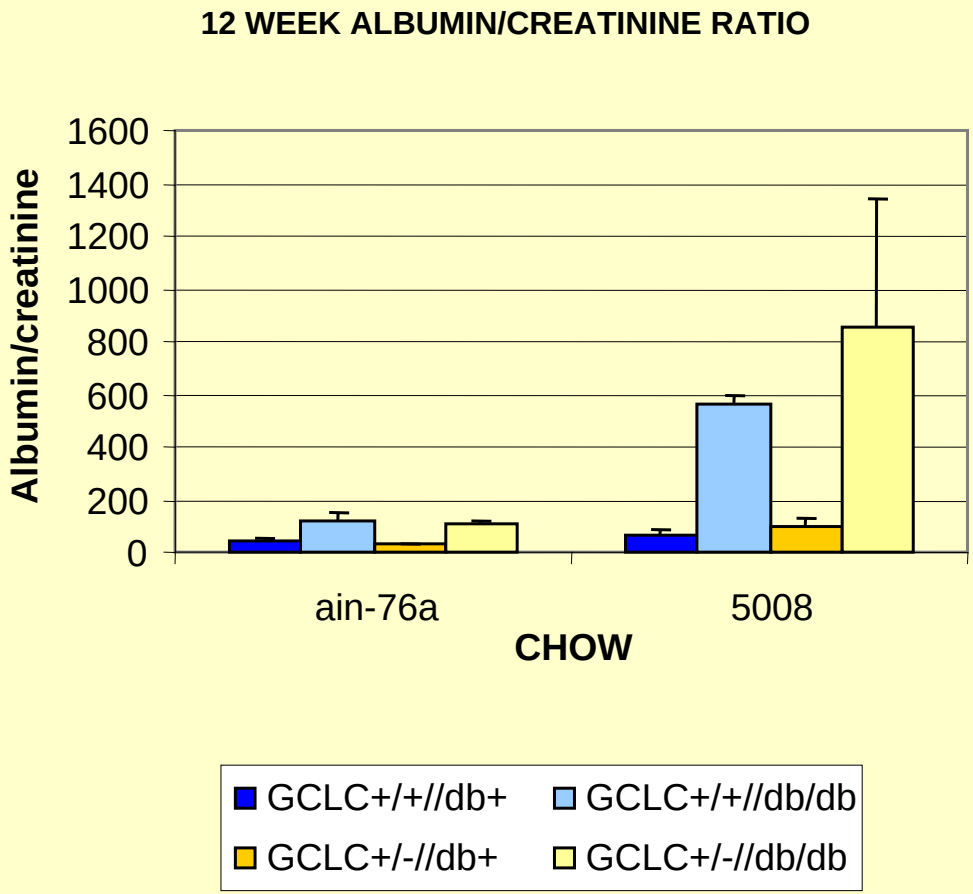
Nephropathy-GCLC+/- db/db C57BL/6J model



Effects of high fat feeding on fasting blood sugar GCLC +/- C57BL/6J db/db model



Effects of high fat feeding on albuminuria GCLC +/- C57BL/6J db/db model



Summary

- Nphs2 GLUT1 tg C57BLKS db/db mice develop severe hyperglycemia and albuminuria by 12 wks, but albuminuria is not yet clearly greater than in wt db/db mice.
- High fat feeding results in substantially enhanced hyperglycemia
- GLUT1 tg C57BL/6J db/db mice on high fat feeding develop severe albuminuria by 12 wks that appears to be greater than wt db/db mice.
- GCLC +/- C57BL/6J db/db mice on high fat feeding develop more profound hyperglycemia and albuminuria than wt db/db mice.