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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

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Specific recommendations from EAC

- Focus on 3-4 models
- Document variability of STZ diabetes
- Recommended against urinary podocyte measurements
- Develop podocyte counting protocol. Ratio podocytes to glomerular area.

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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	
ROS	AR tg//SOD2+/- STZ	C57BL/6	phenotyping ongoing; increased neuropathy in diabetics with double mutation	1-04 to 12-04	
ROS	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	?	

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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)

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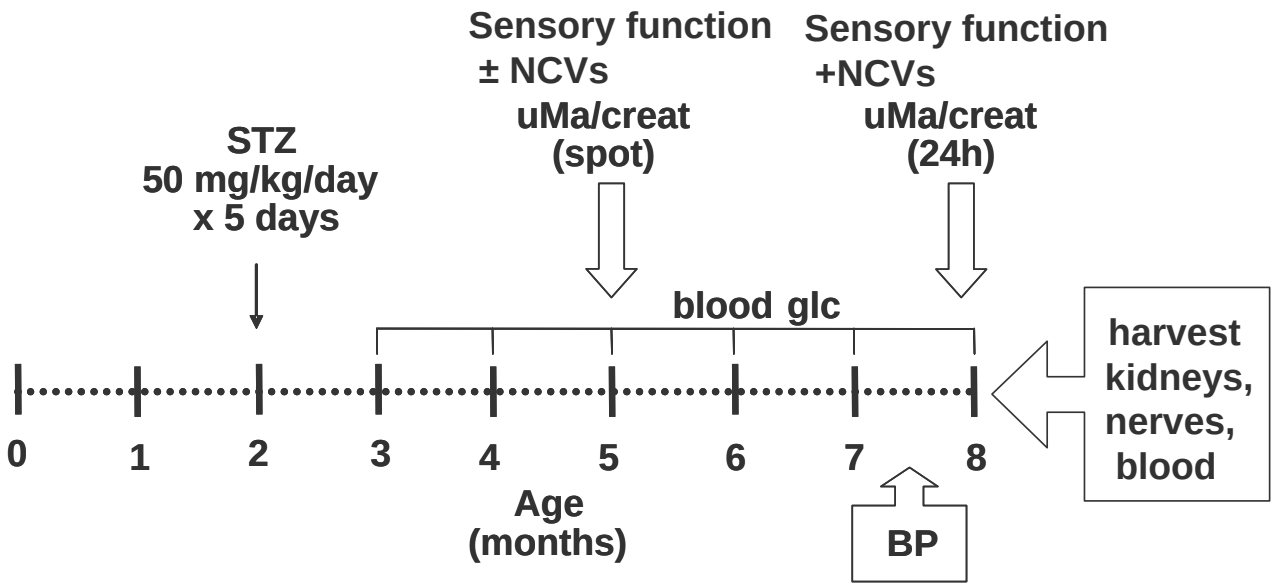
General schema for evaluation



Ongoing evaluation

	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
C57BKLS Animals being added		X	X			X
GCLC +/- db/db Animals being added	X		X	X		Ongoing
GCLC +/- db/db high fat diet	Starting trial →					
C57BKLS db/db high fat diet	Starting trial →					
Nphs2 Cre// SOD2 loxP/loxP	X					
Nestin Cre// SOD2 loxP/loxP	X	X (9 animals)	X (9 animals)			
Synapsin Cre// SOD2 loxP/loxP	Starting trial →					

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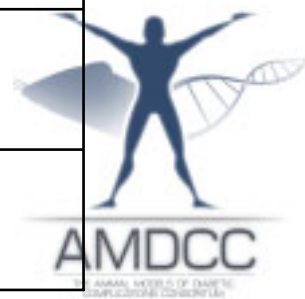
Ongoing evaluation

	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
fyn -/- and +/- 129 and mixed bkgrd STZ treated and control	X	X	X			
GLUT4 -/- STZ treated and control Adding animals	X	X	X	X	X	
Nphs2 cre// GLUT4 loxP/loxP	X			X		
Nphs2 GLUT1 db/db C57BLKS	Starting trial →					
β actin GLUT1 db/db	Starting trial →					



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.

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signaling	fyn -/- and +/- STZ	129SvJ	phenotyping ongoing	9-03 to 9-04	
signaling	IRS-1 -/-	C57BL/6J	Breeding colony—On hold	?	

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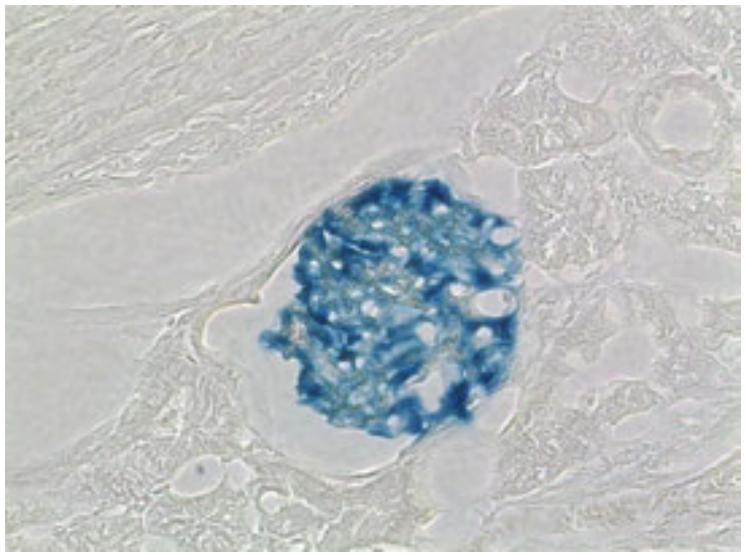
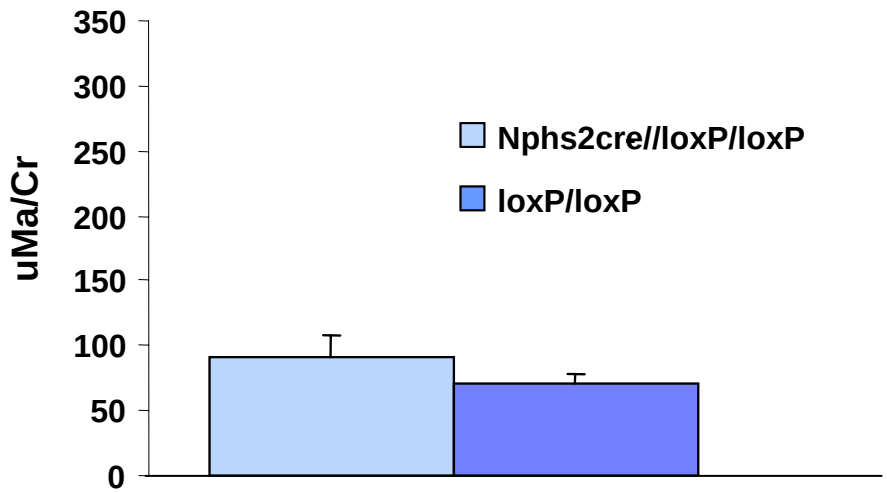
Nphs2 cre SOD2 loxP/loxP C57BL/6J/129SvJ -STZ diabetes

nphs2 cre x Rosa26



Microalbuminuria (24 wk)



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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

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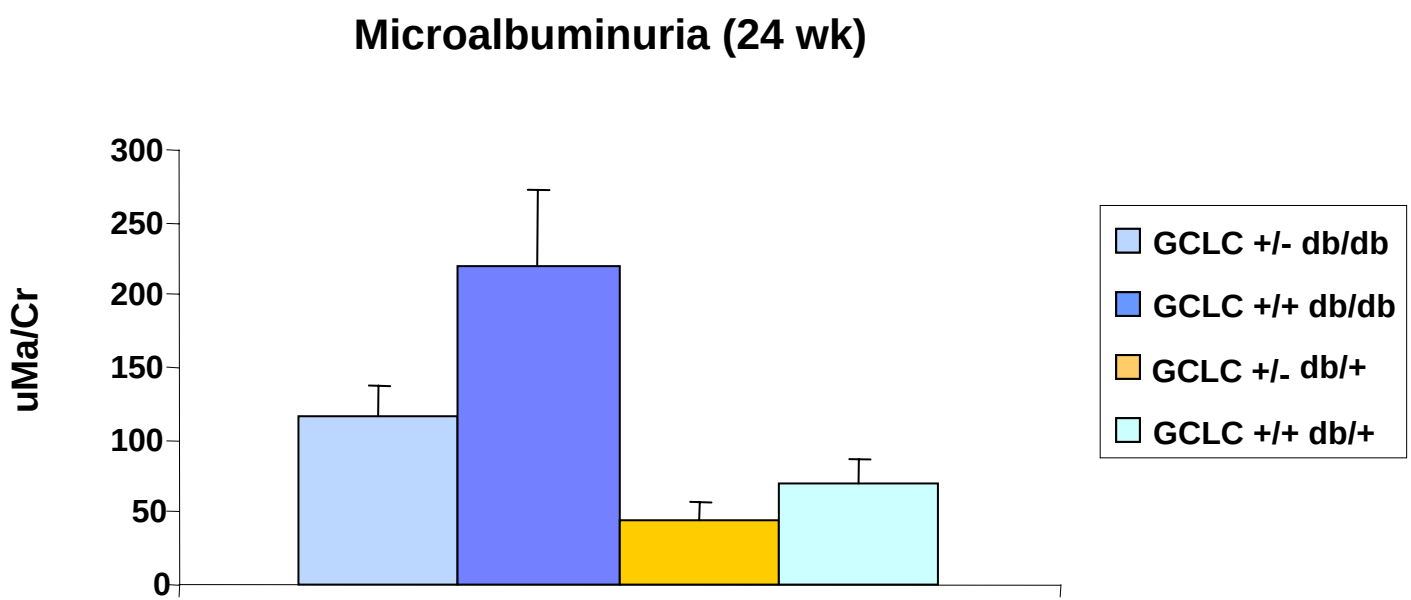
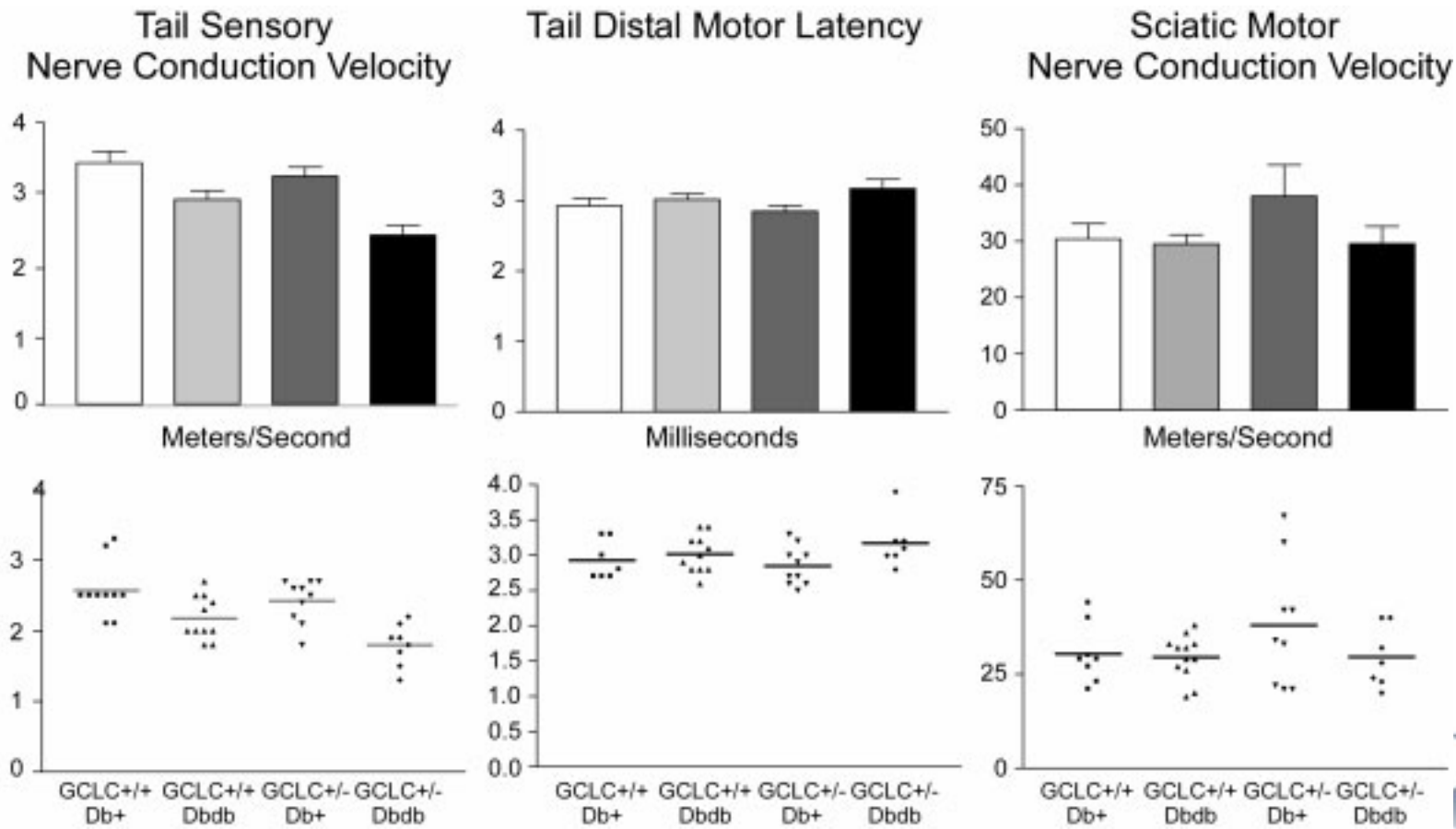
Nephropathy-GCLC+/- db/db C57BL/6J model



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



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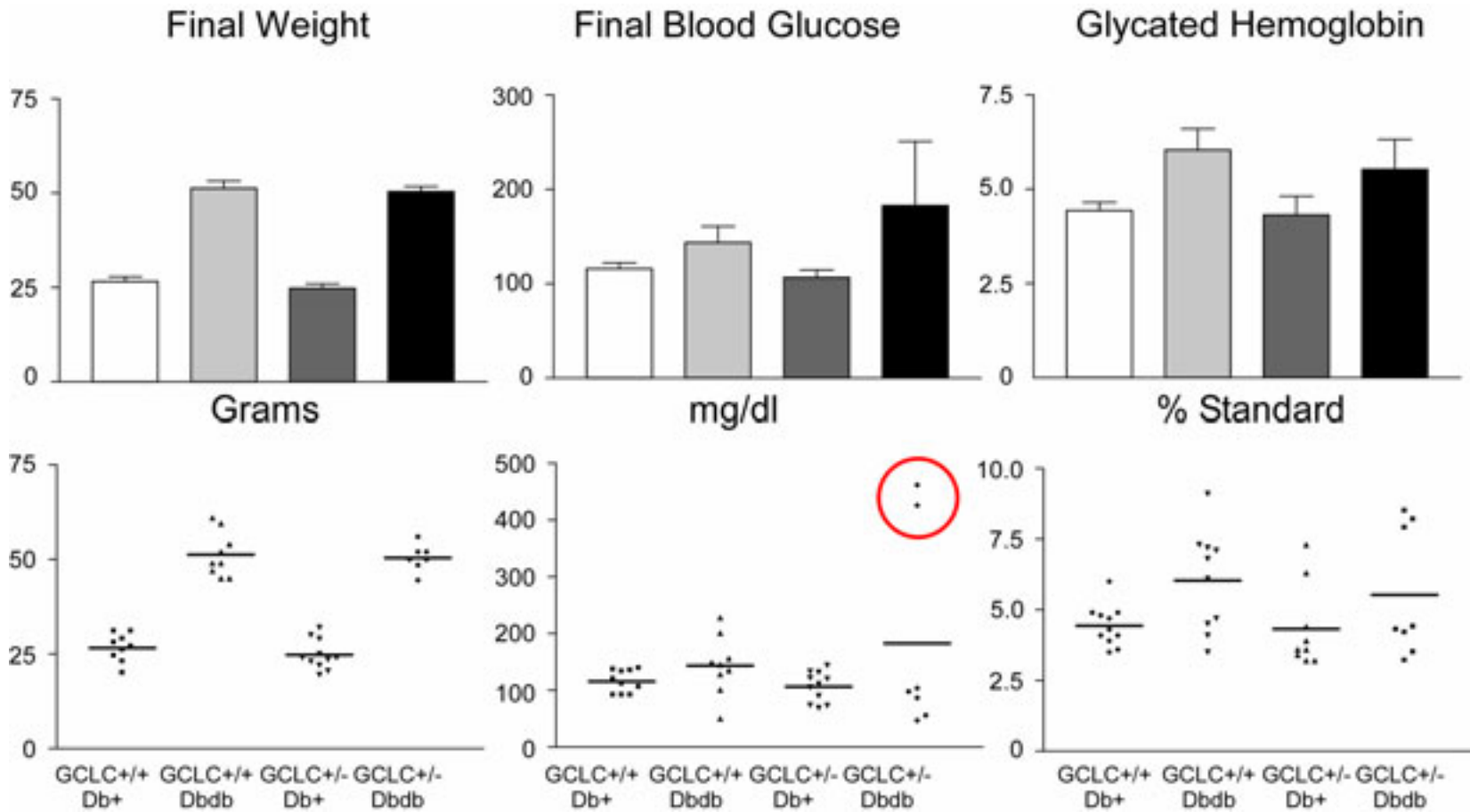
GCLC+/- db/db C57BL/6J model



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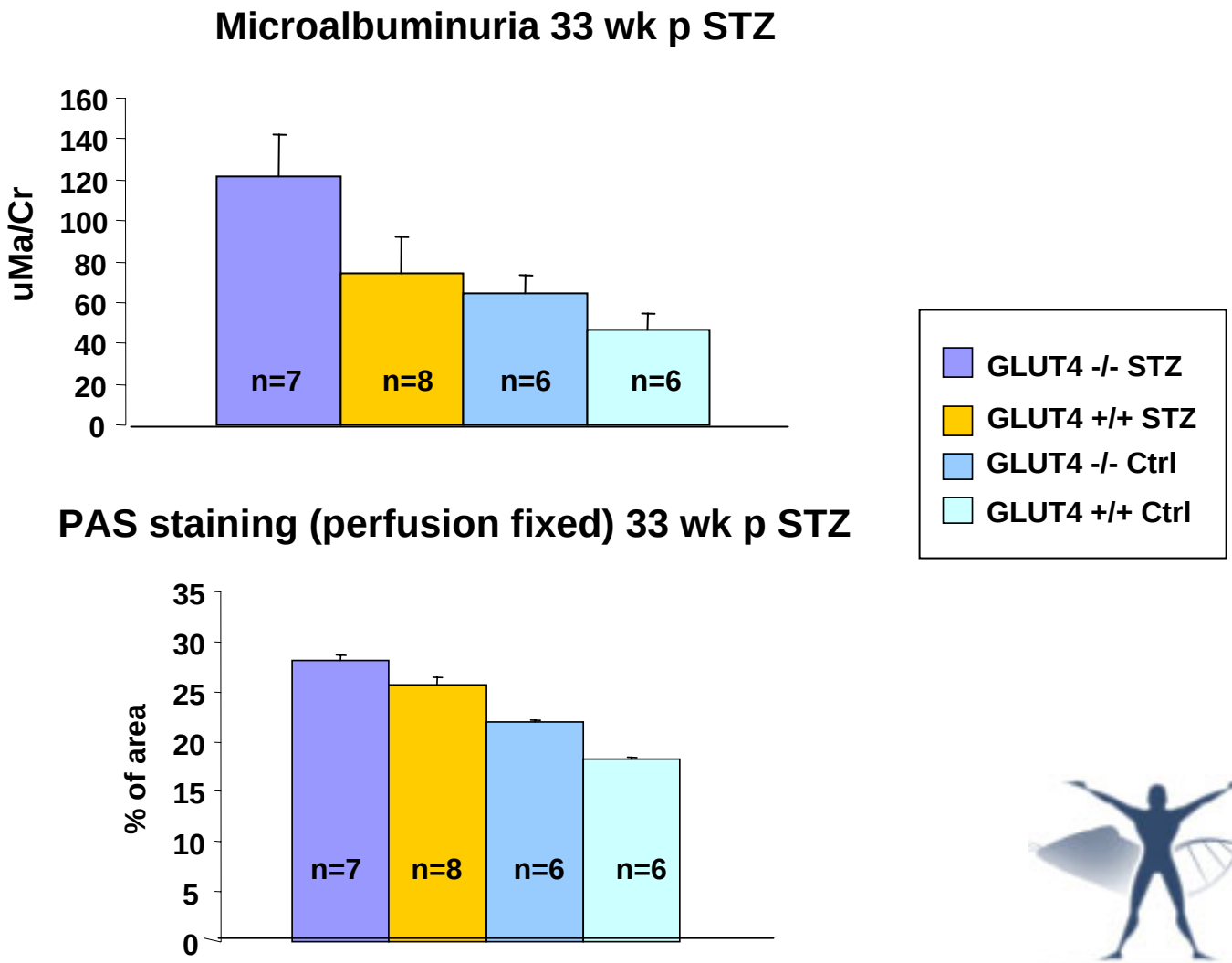
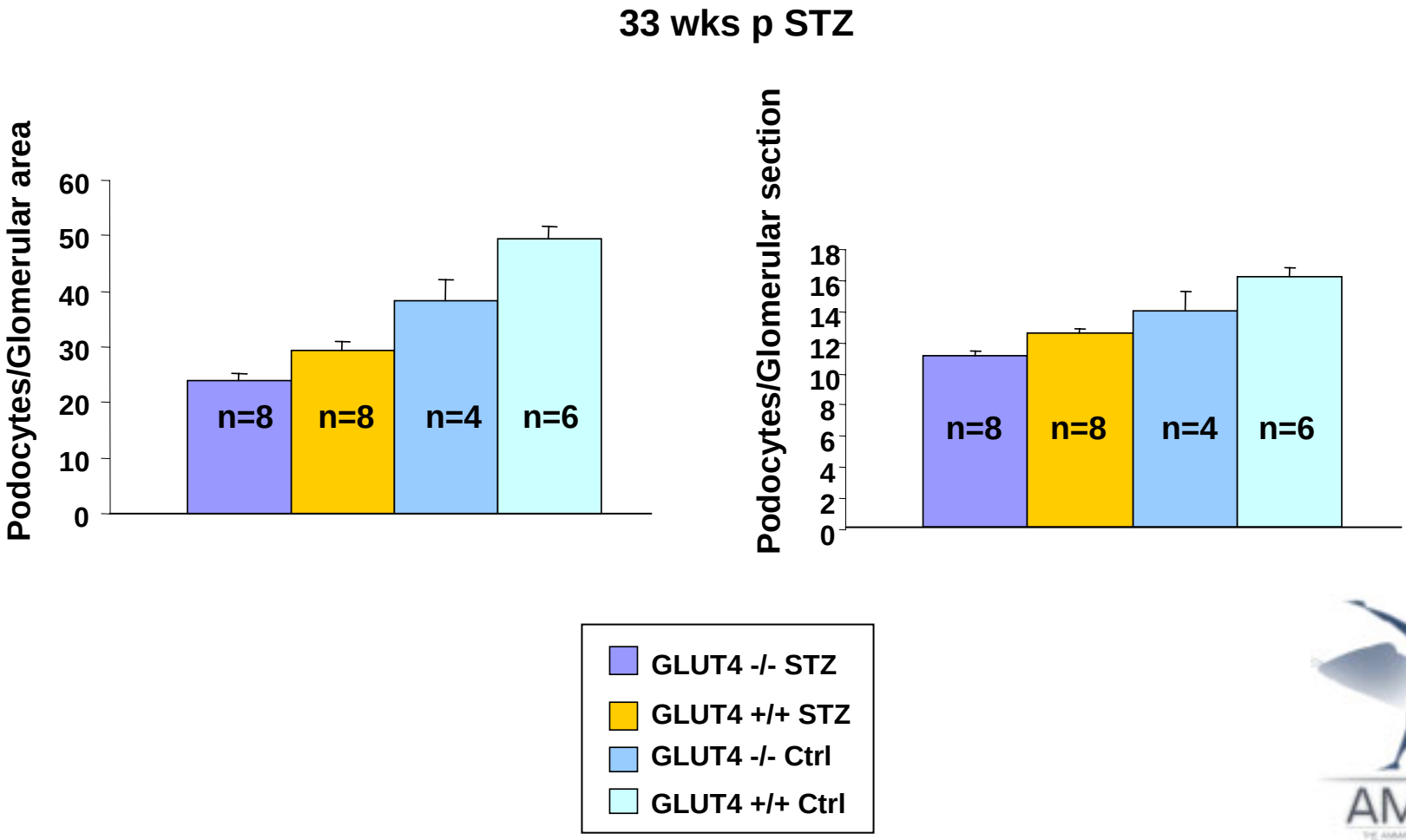
GLUT4 -/- C57BL/6J model

- 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.

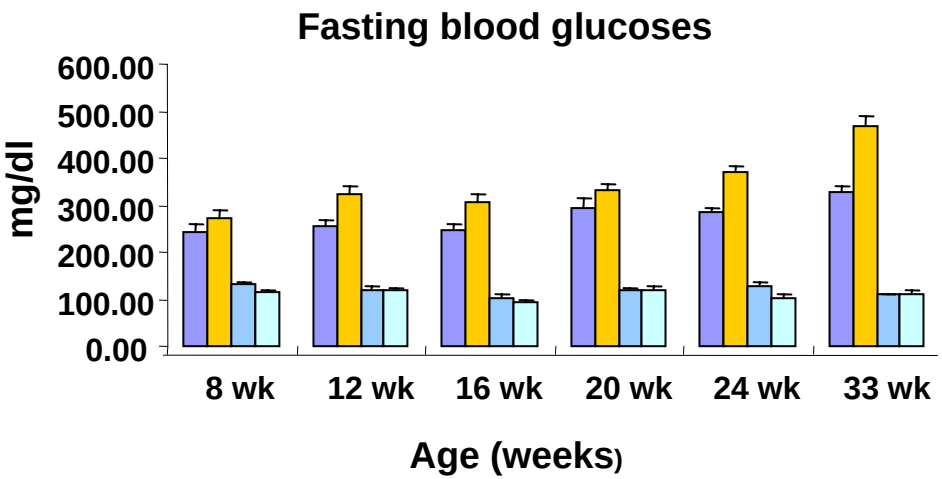
Nephropathy-GLUT4 -/- STZ C57BL/6J model

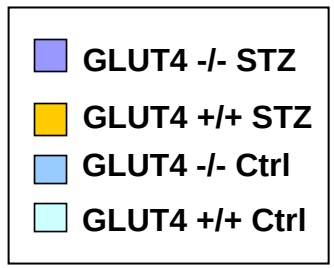


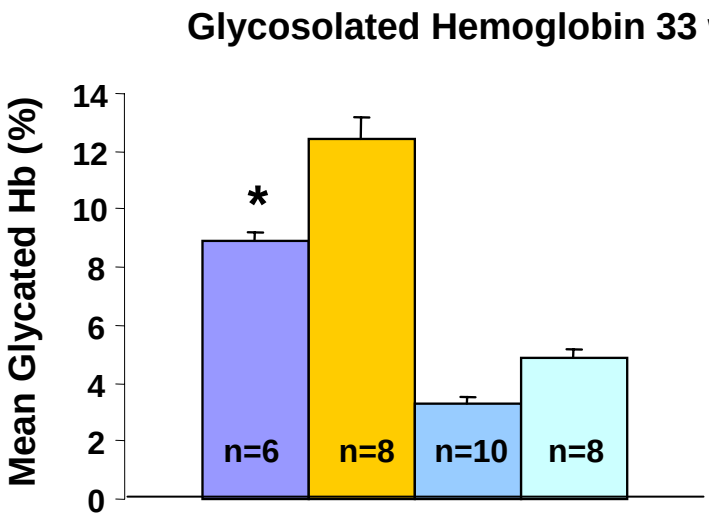
Podocytes in GLUT4 -/- C57BL/6J STZ model



GLUT4 -/- C57BL/6J model







p=0.0006

* vs. GLUT4 +/+ STZ

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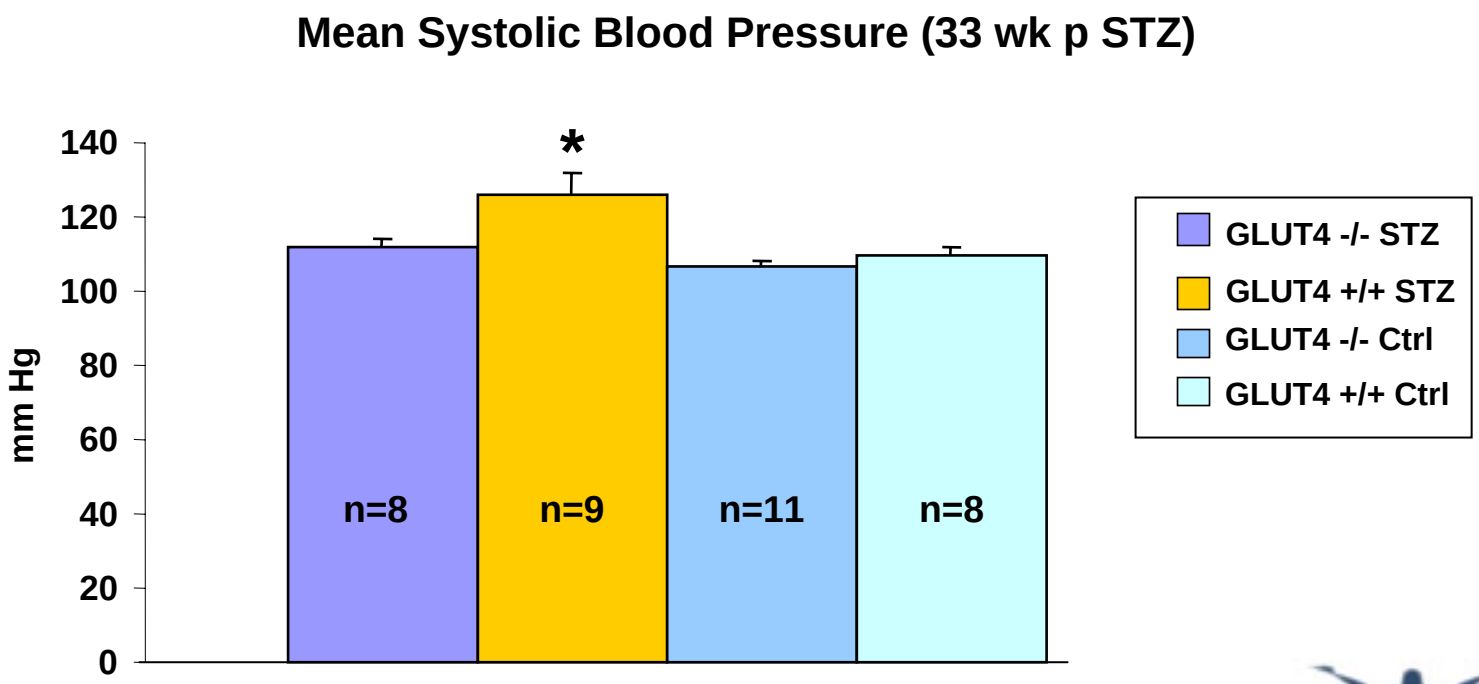
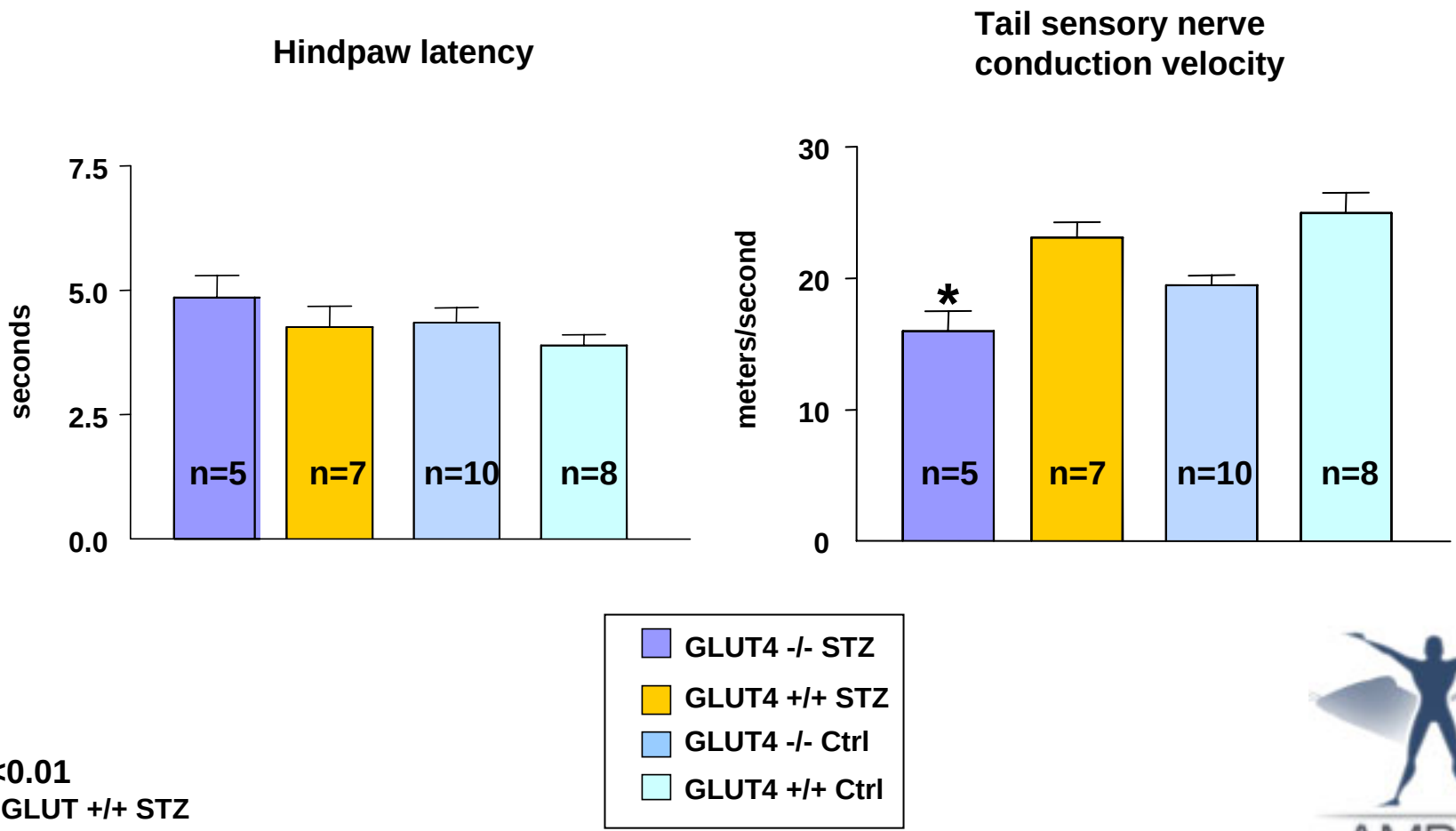
GLUT4 -/- STZ C57BL/6J model



- p=0.0138
- vs. other groups

Neuropathy-GLUT4 -/- STZ C57BL/6J model



•P<0.01
vs. GLUT +/+ STZ

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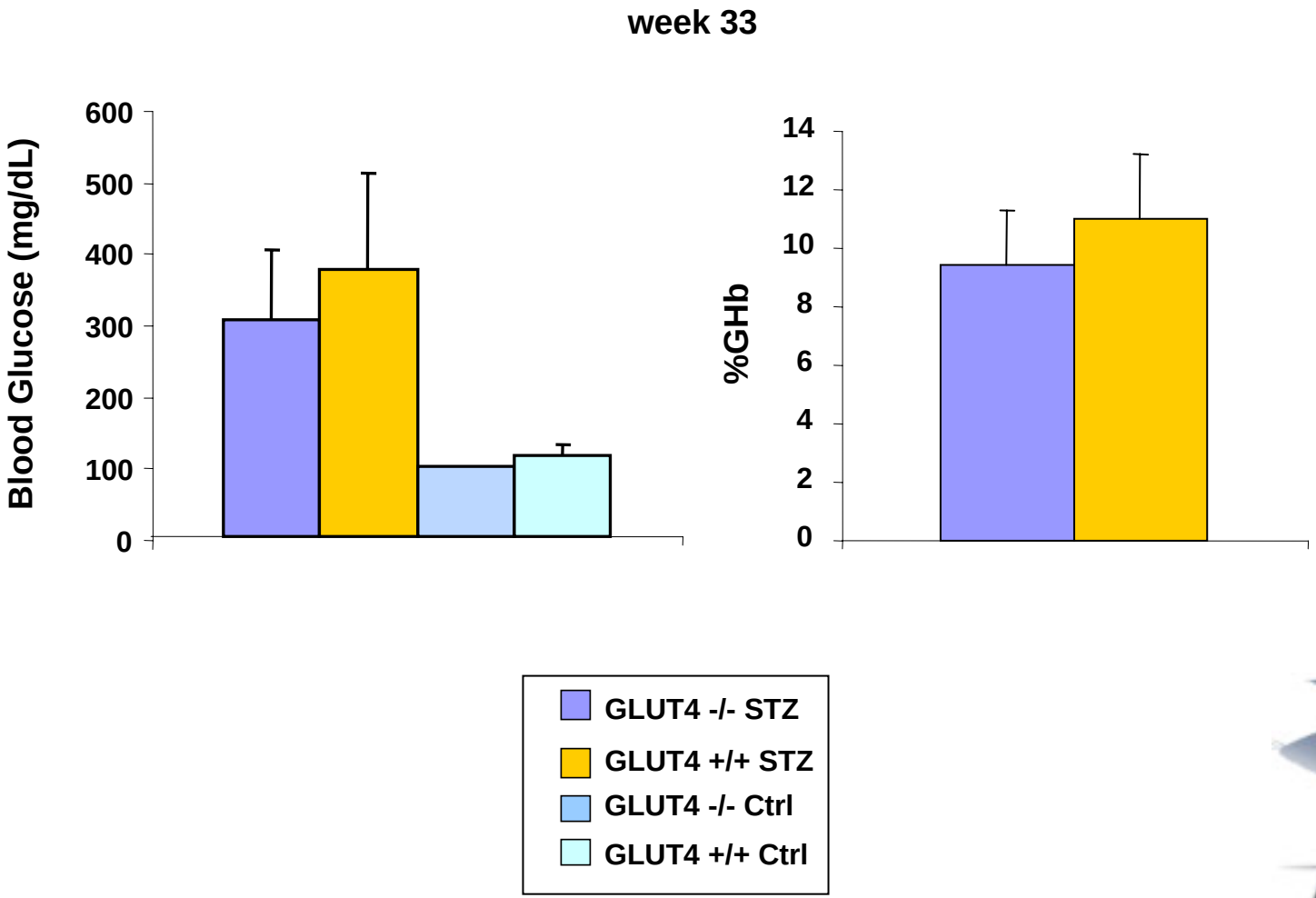
Nphs2 Cre //Glut4 loxP/loxP STZ model (mixed FVB/C57BL/6J background)



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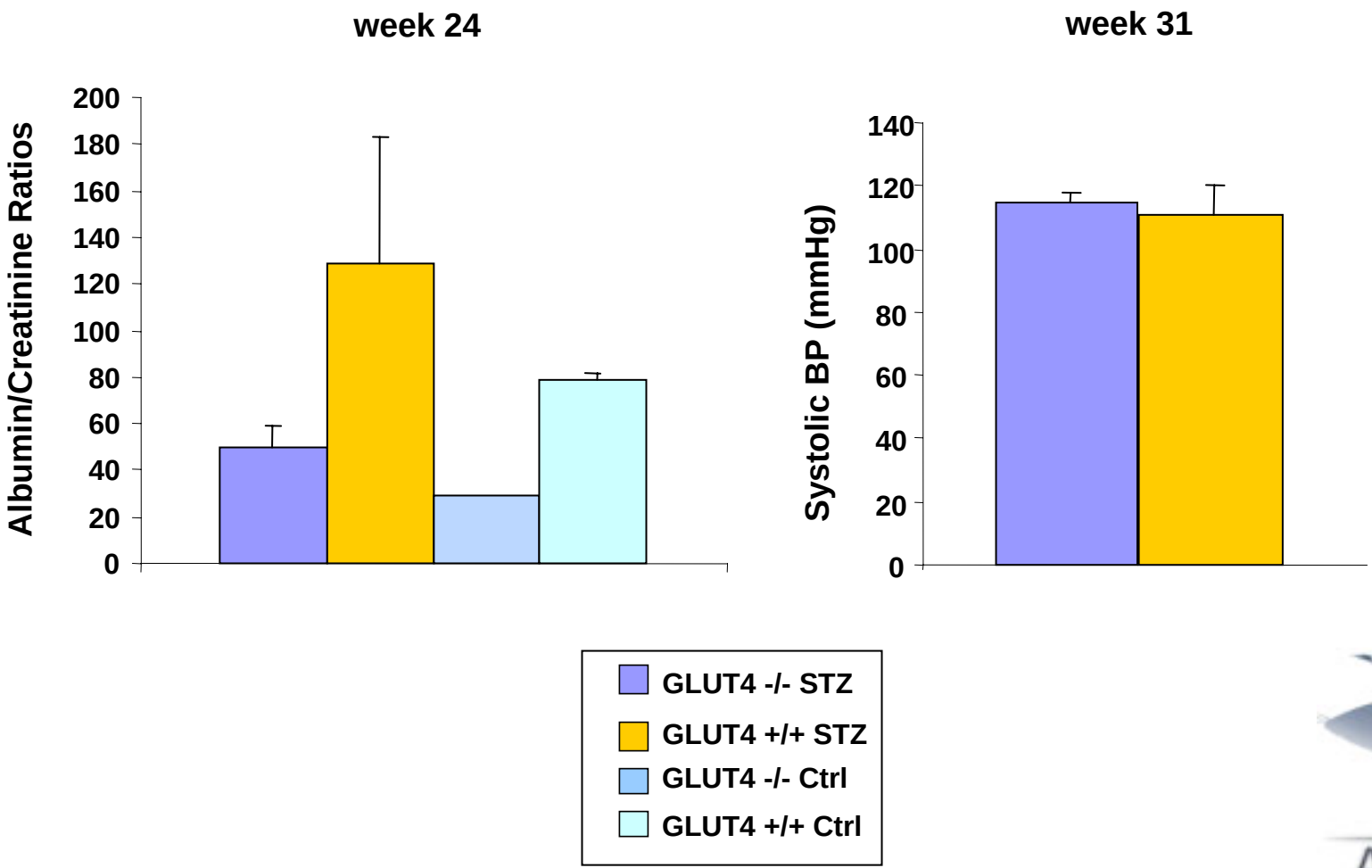
Nphs2 Cre //Glut4 loxP/loxP STZ model (mixed FVB/C57BL/6J background)



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Current Models (page 2)

GLUTs

Animal model	Background strain	current status	Phenotyping (begin-end)	Consortium collaborators
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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)

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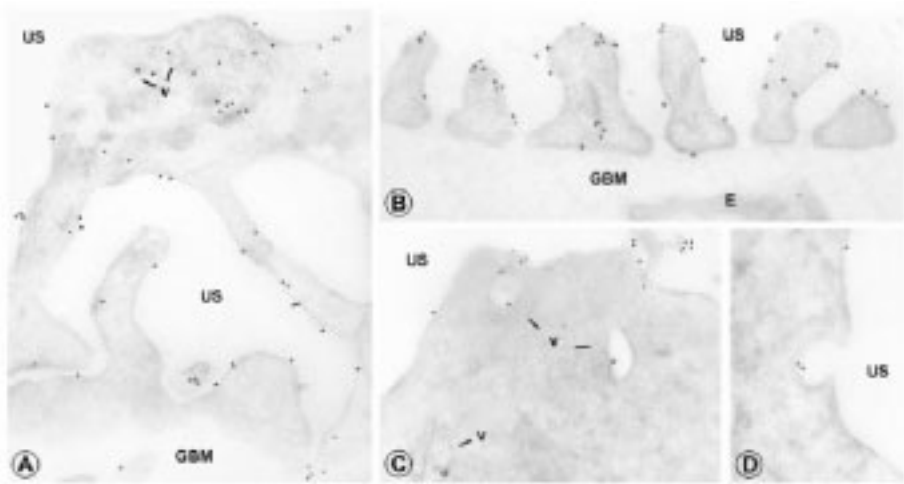
Nphs2 GLUT1tg db/db C57BLKS model

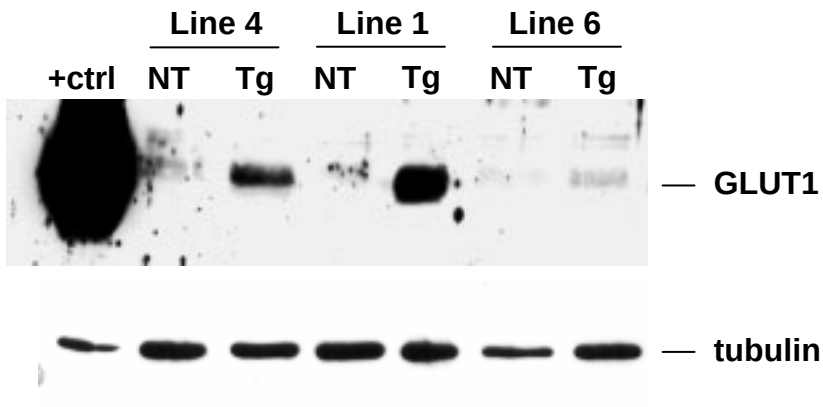
- GLUT1 is highly expressed in podocytes
- High glucose and diabetes in humans and rodents lead to podocyte loss
- Nphs2 promoter drives GLUT1 in podocytes
- db/m C57BKS eggs injected to allow direct examination on diabetic background

GLUT1 in human kidney podocytes

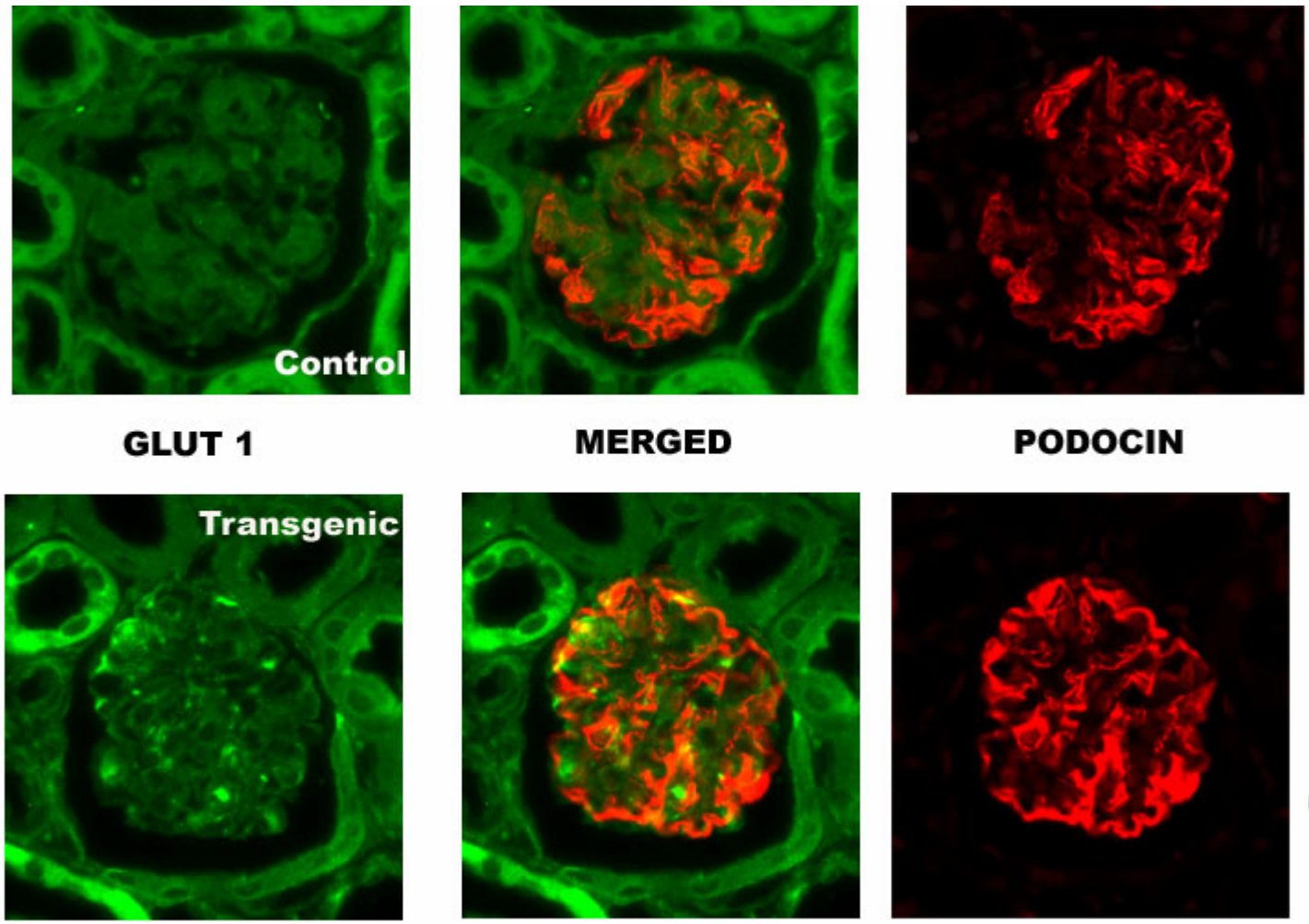


Nphs2 GLUT1tg C57BLKS db/+ model

Glomerular GLUT1 immunoblot



Nphs2 GLUT1tg C57BLKS db/+ model (line 4)



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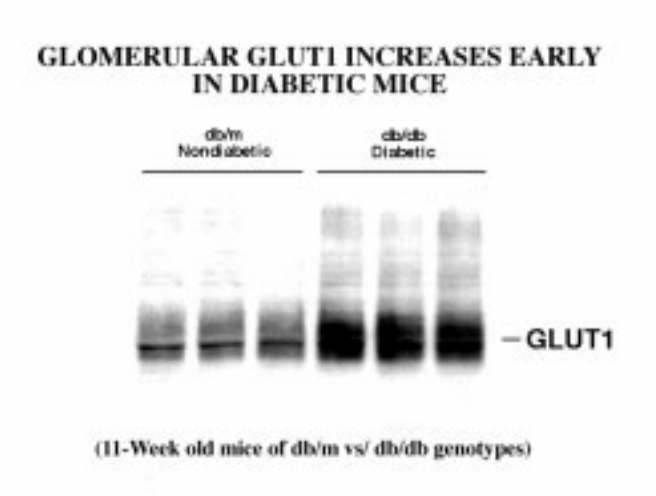
GLUT1tg C57Bl/6J model (GT1S)



- GLUT1 is increased in glomerular cells in diabetes and in mesangial cells and podocytes cultured in high glucose
- GLUT1 leads to enhanced PKCα and AR activity, fibronectin synthesis
- Modified β-actin promoter drives GLUT1 in many tissues: high in mesangial cells

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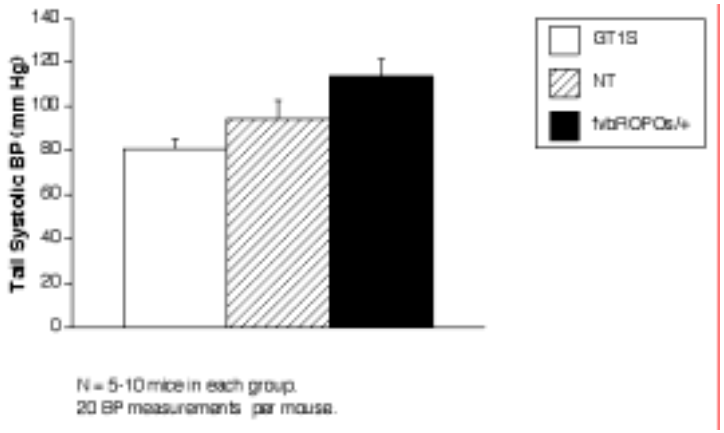
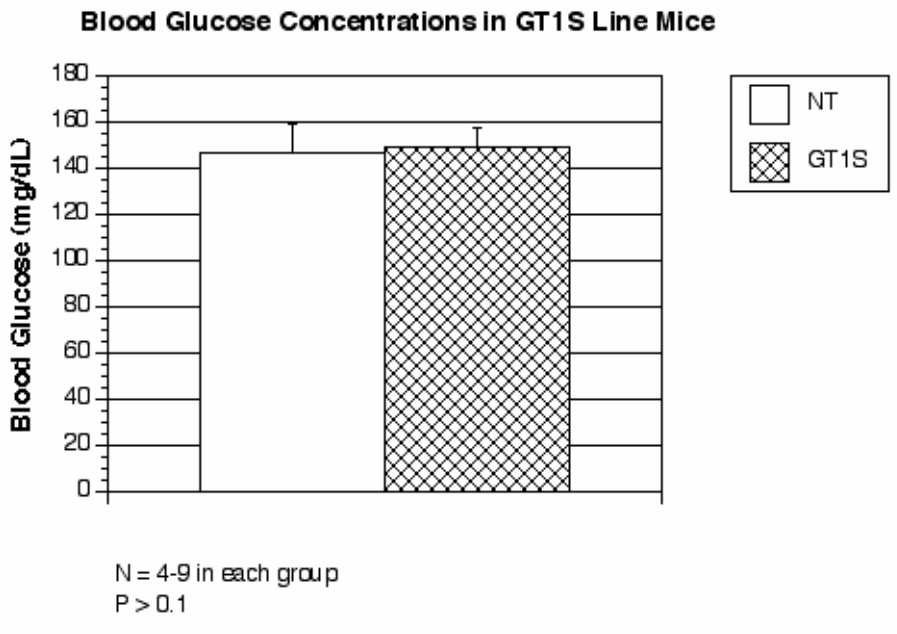
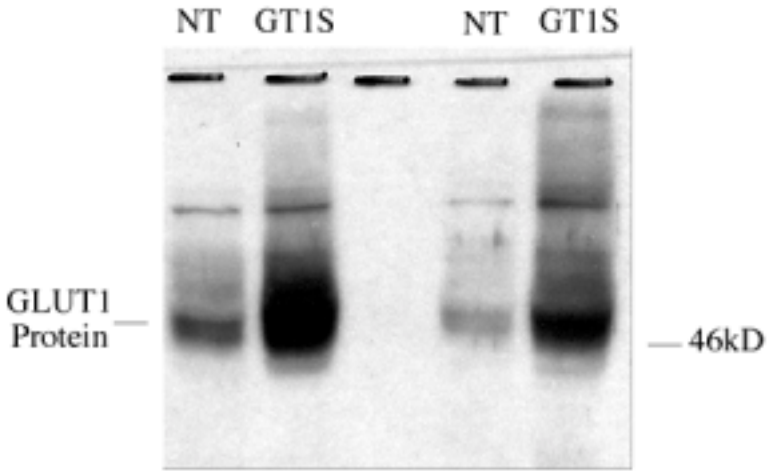
GLUT1tg C57BL/6J model

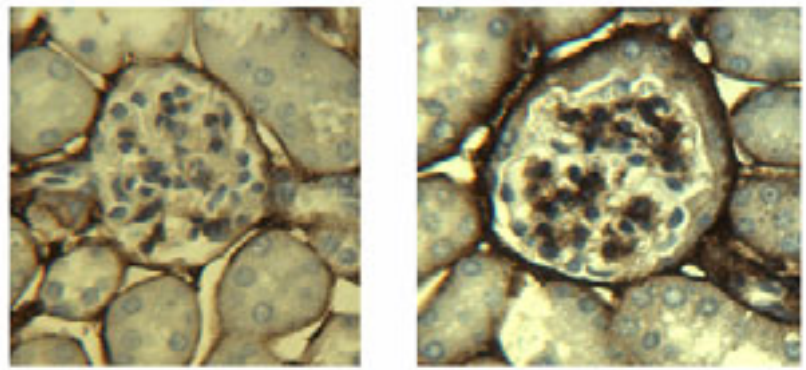
Systolic tail cuff BP (adults)





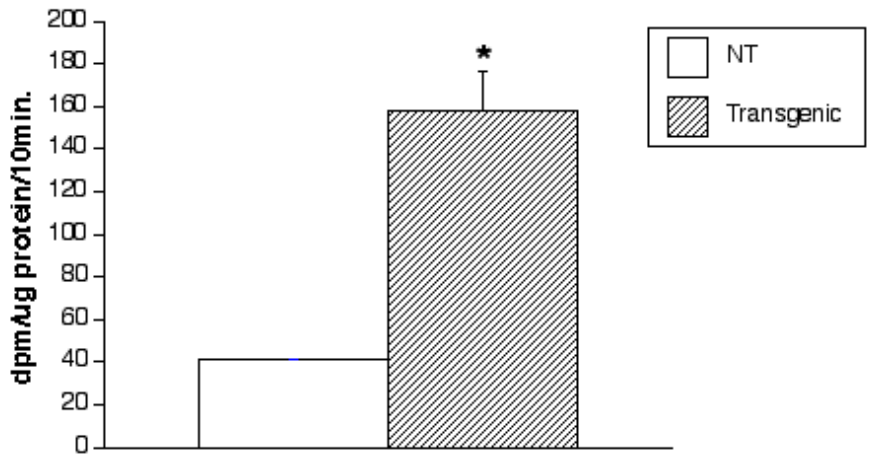
GLUT1tg C57BL/6J (GT1S) model





GLUT1 Ab titer = 1:400

Glomerular 3H2-Deoxyglucose Uptake Rates in Nontransgenic and Transgenic (GLUT1-Overexpressing) Mice



*P <. 01 vs. Nontransgenics

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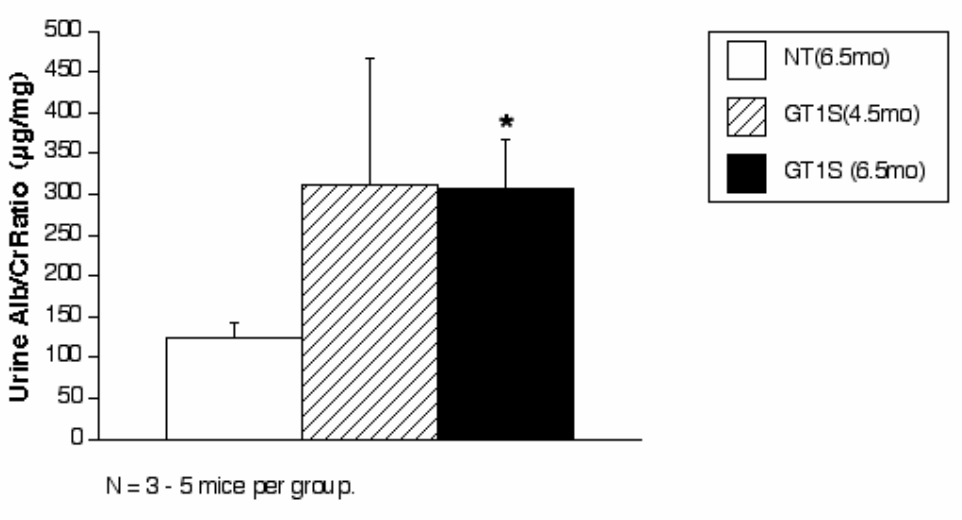
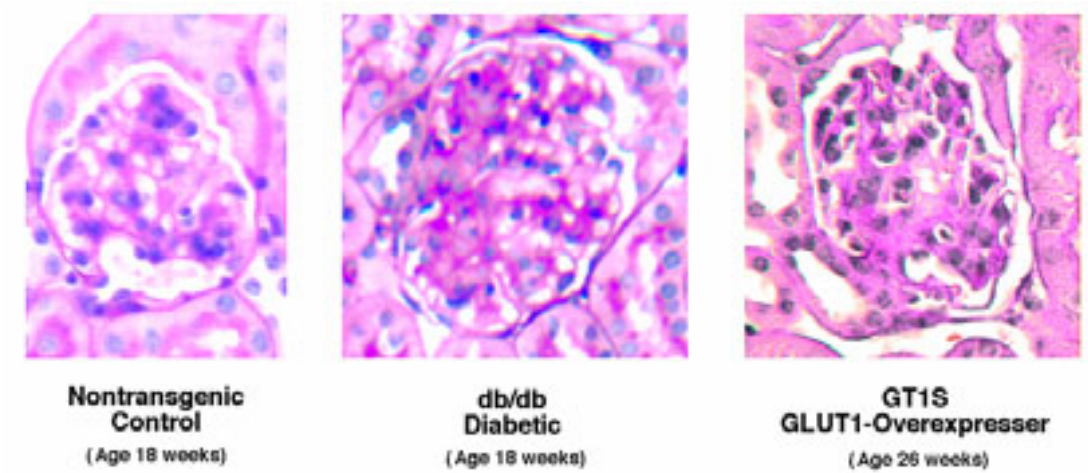
Nephropathy-GLUT1tg C57BL/6J (GT1S) model

Albumin/Creatinine Ratios



PAS staining



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Nephropathy-GLUT1tg C57BL/6J (GT1S) model

Glomerular TGFβ1 protein levels (wks)

Current Models (page 2)

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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)

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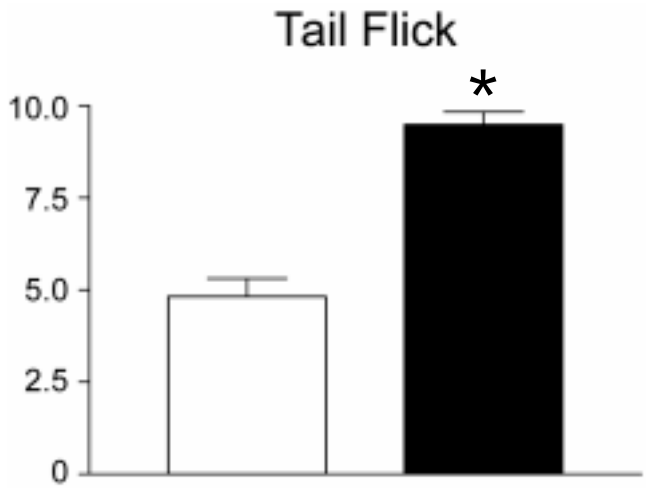
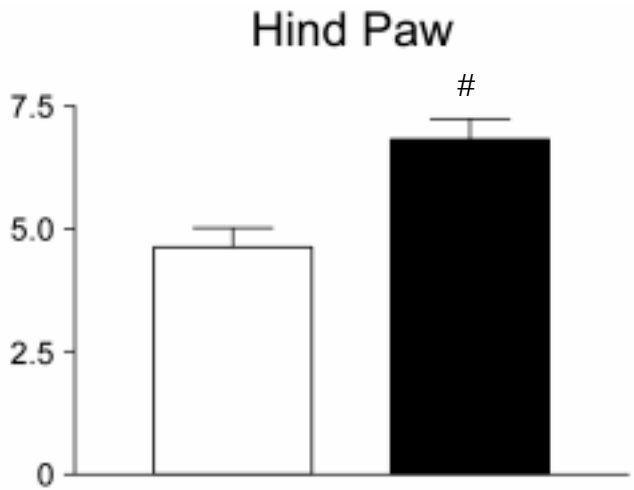
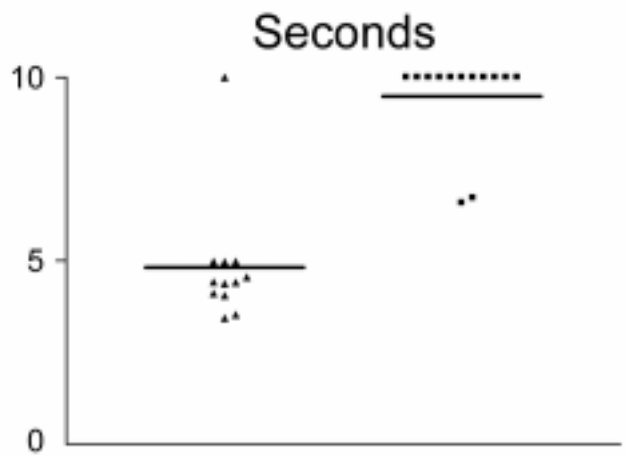
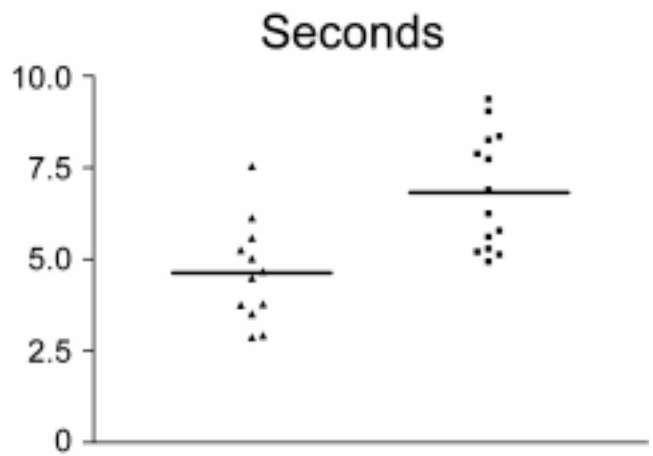
Neuropathy-C57BLKS db/db model









* P< 0.001

# P < 0.05

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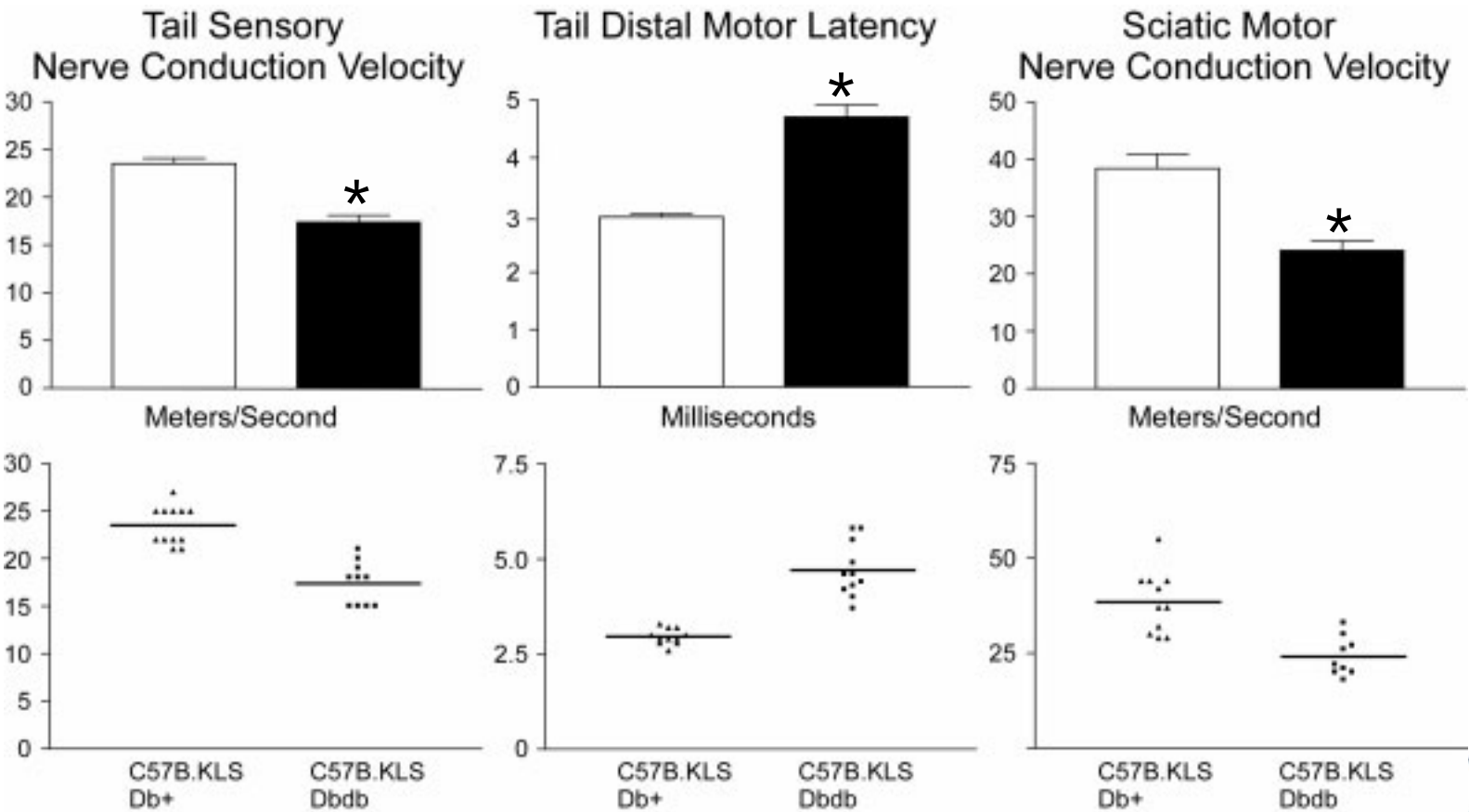
Neuropathy-C57BLKS db/db model



* P< 0.001

Summary

- No increased albuminuria in STZ-diabetic podocyte specific SOD2 knockout mice on mixed background or in GCLC +/- db/db C57BL/6J mice.

- Increased nephropathy and probably neuropathy in STZ-GLUT4 -/- animals despite less severe diabetes; No increase in albuminuria in podocyte specific GLUT4 -/- animals, suggesting that the podocyte reduction in GLUT4 is not critical.

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Summary (cont.)

- Podocyte specific GLUT1 transgenic mice have been established on db/+ C57BLKS background. Trials are underway.
- GLUT1 transgenic mice exhibit nephropathy in absence of diabetes.
- Robust neuropathic changes were found in C57BLKS mice.

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Plans for year 4

- GLUT4 -/- model. Breed onto db/db and /or Akita on C57BL/6J backgrounds.
- GLUT1 transgenic mice. Complete studies on db/db background. GFRs in these studies.
- Podocyte specific GLUT1 transgenic C57BKS mice. Complete db/db trial.
- db/db C57BL/6J model and GCLC +/- model. High fat feeding.
- All models: enhance hyperlipidemia.

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