AMDCC

THE ANIMAL MODELS OF DIABETIC
COMPLICATIONS CONSORITUM

Michigan/Chicago unit

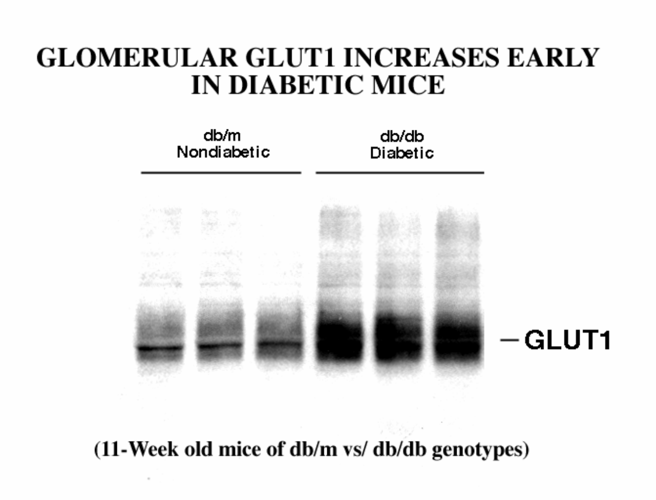
Modifications in Mouse Models to Enhance Nephropathy/Neuropathy- GLUT1 overexpression

- Increased oxidative stress

- Increased glucose metabolic flux or alteration in GLUT expression profiles that will lead to changes in glucose metabolism

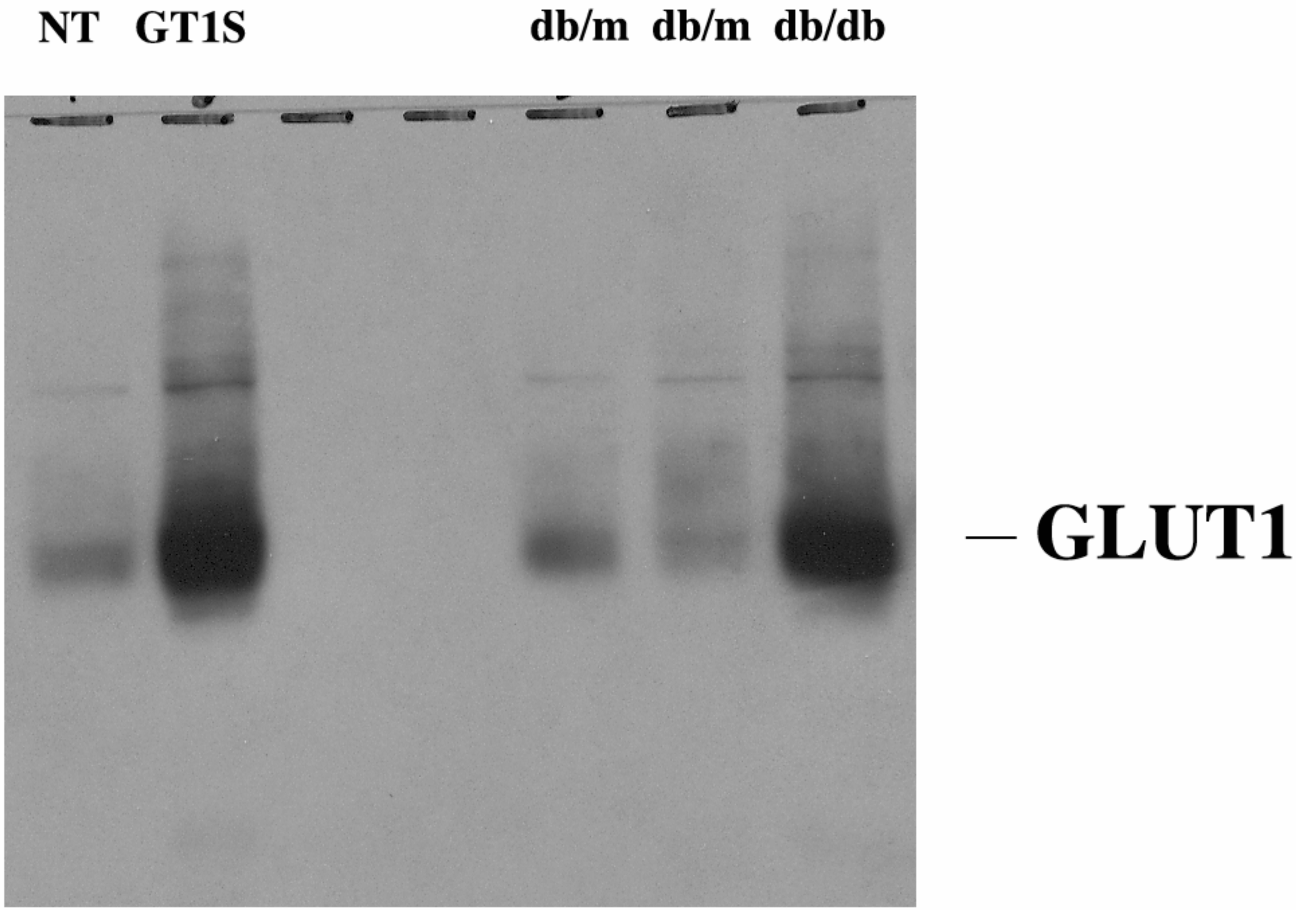
- Altered signaling that may enhance glucose toxicity

GLUT1tg C57Bl/6J model (GT1S)

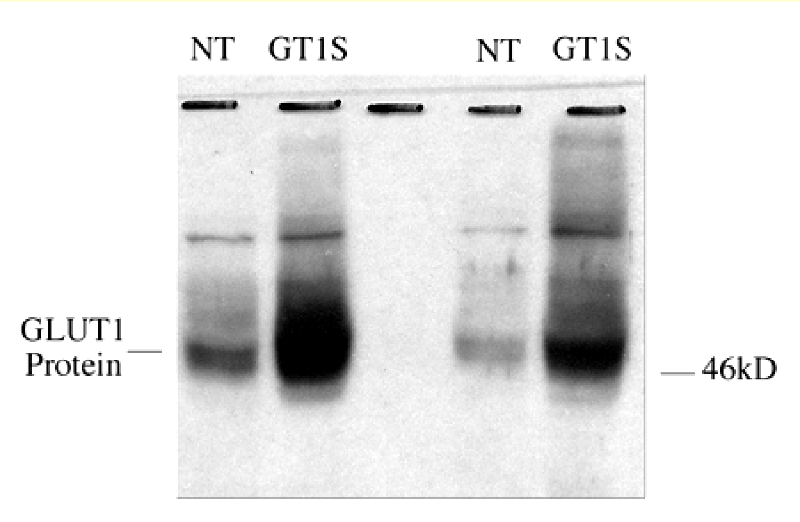


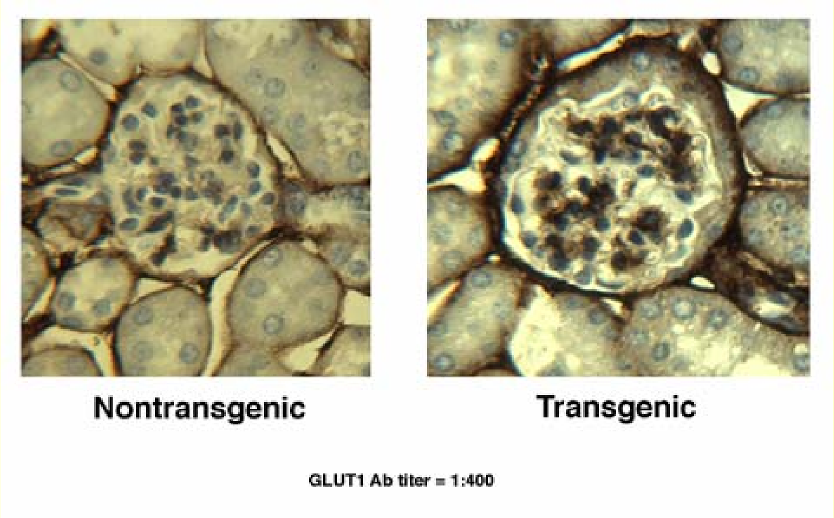
- GLUT1 is increased in glomerular cells in diabetes
- GLUT1 leads to enhanced PKCα and AR activity, fibronectin synthesis
- Modified β-actin promoter drives GLUT1 in many tissues: high in mesangial cells
- Bred onto db/db C57BL/6J
- Effect of “high fat” feeding (breeder chow; ca 7% fat)

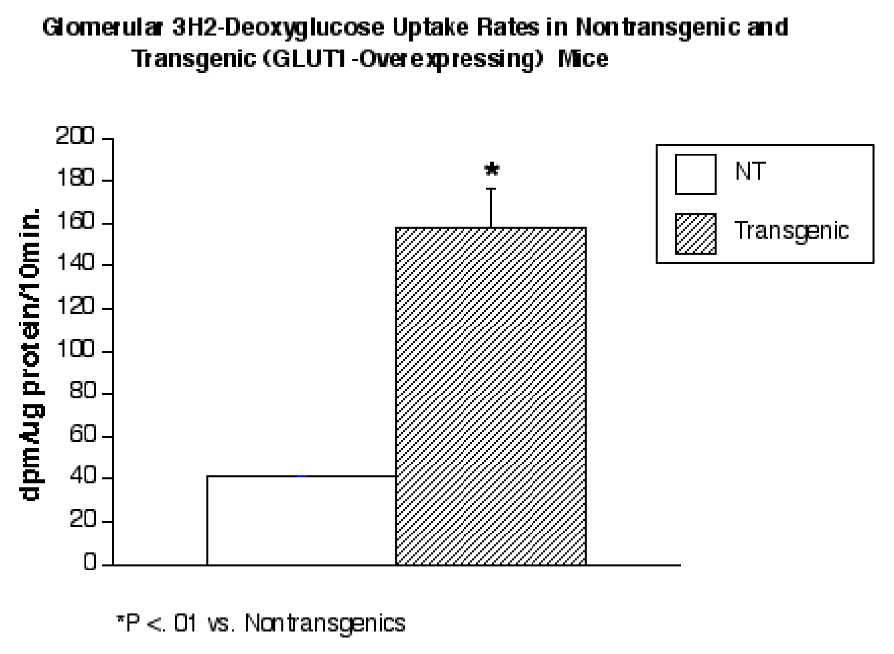
Glomerular GLUT1 Protein is Increased in Both GT1S Transgenic Mice and Type 2 Diabetic Mice



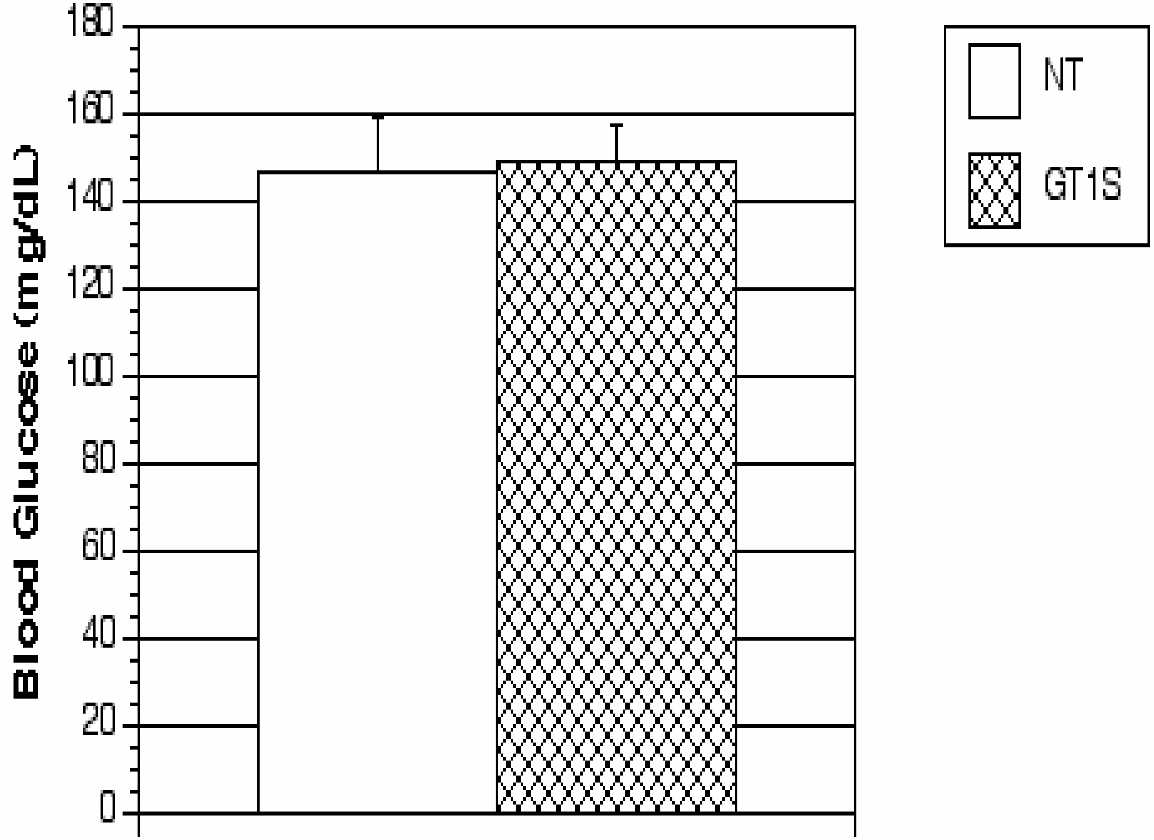
GLUT1tg C57BL/6J (GT1S) model





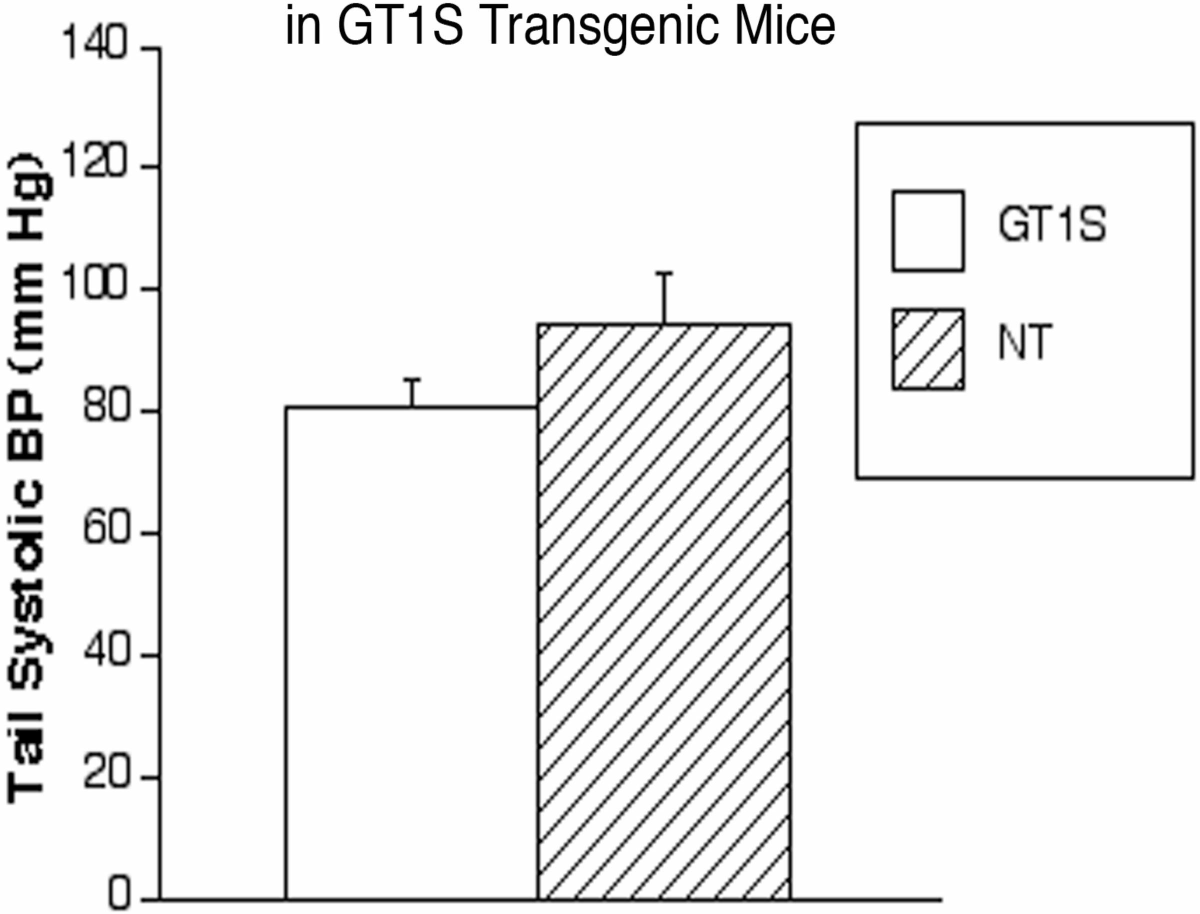


Blood Glucose Concentrations in GT1S Line Mice



N = 4-9 in each group
P > 0.1

Tail Cuff Blood Pressures were Unchanged in GT1S Transgenic Mice

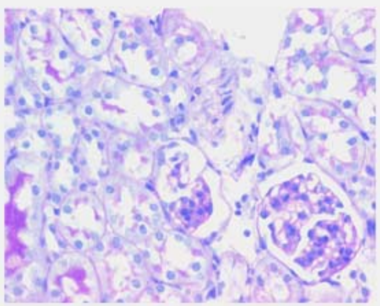


N = 5-10 mice in each group.

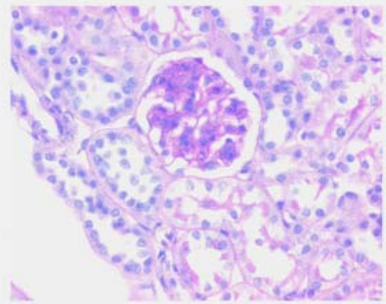
20 BP measurements per mouse.

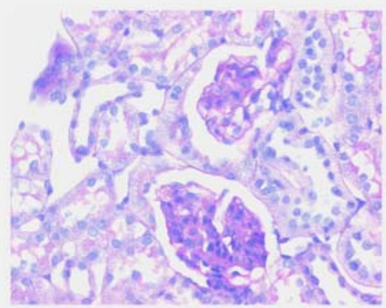
PAS Staining of Glomeruli from Nontransgenic and GT1S Transgenic Mice at 26 Weeks of Age

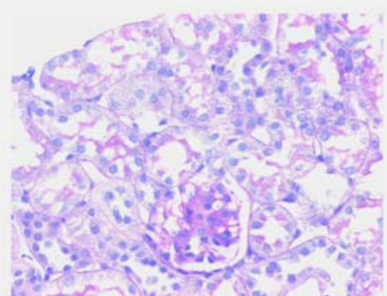
Nontransgenic



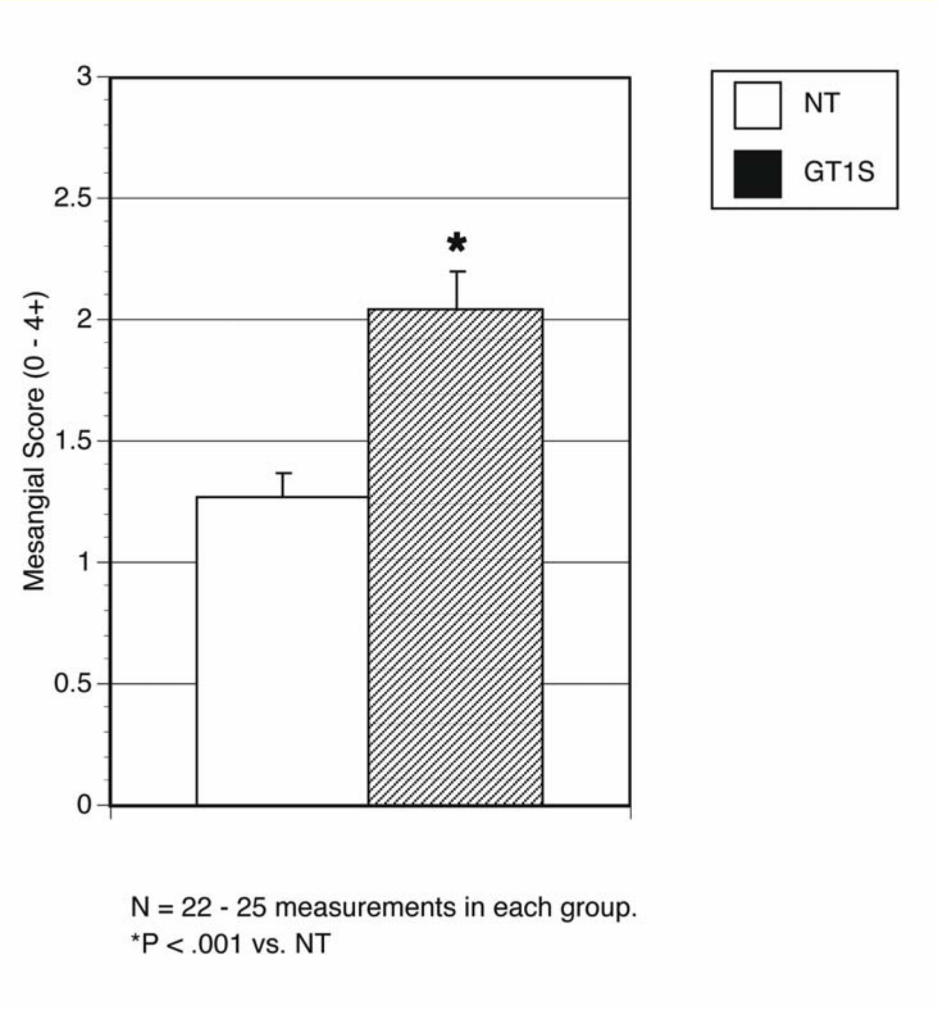
GT1S Transgenic



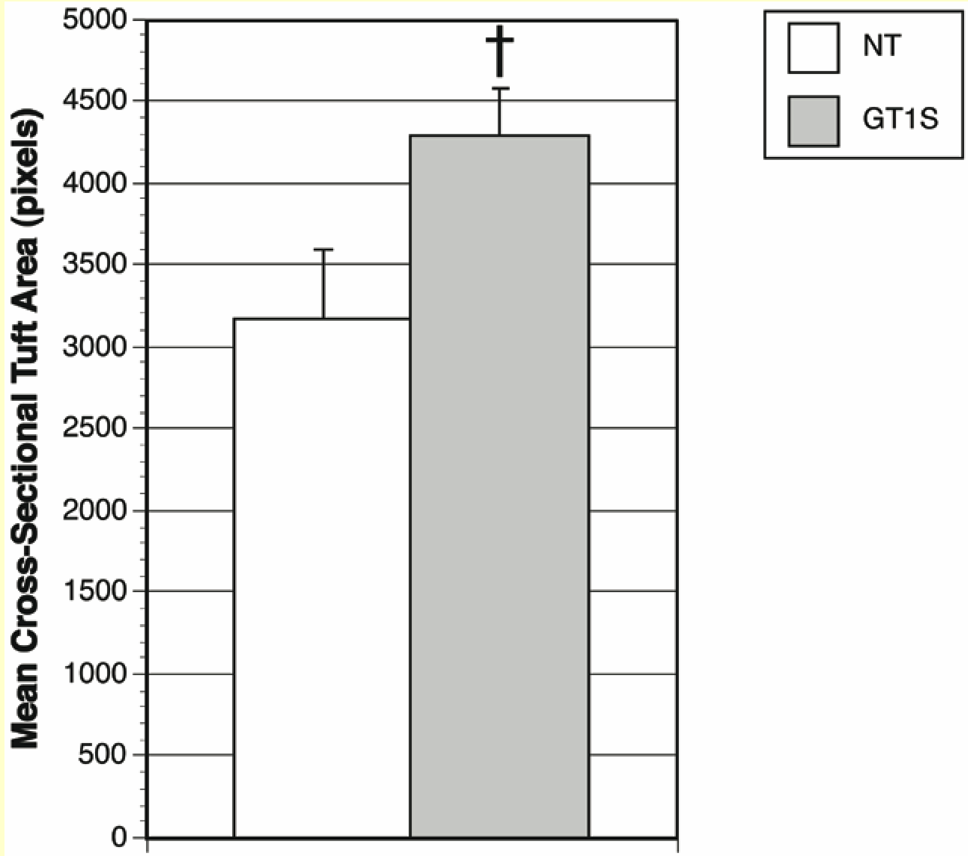




Mesangial Matrix Scores in NT and GT1S Transgenic Glomeruli at Age 26 Weeks



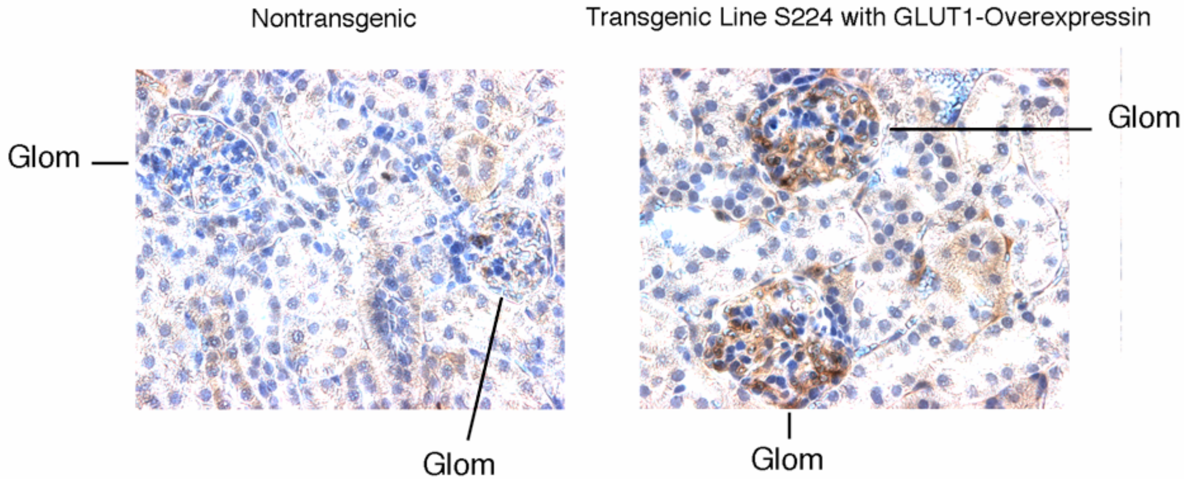
Mean Cross-Sectional Glomerular Tuft Areas in NT and GT1S Transgenic Mice at Age 26 Weeks



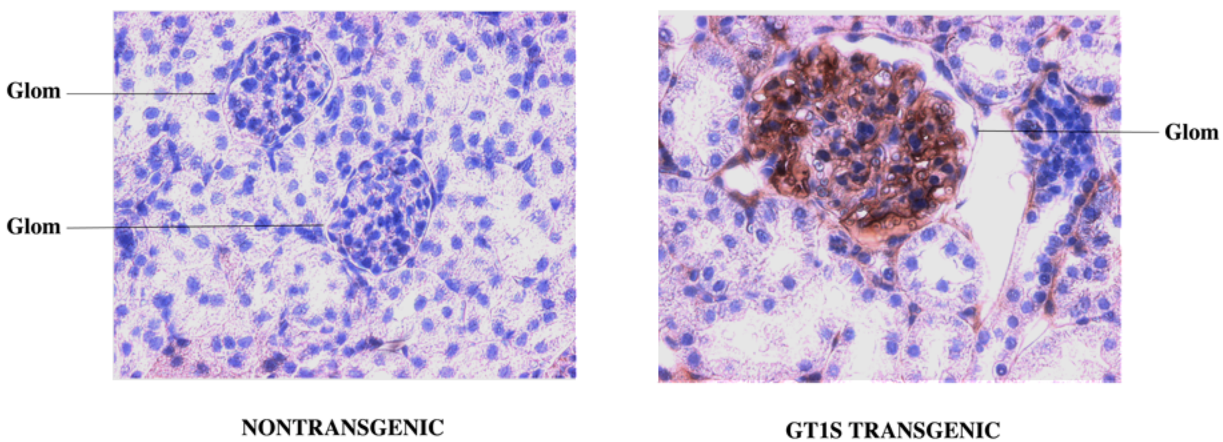
N = 18 -22 measurements per group.

†P < .05 vs. NT

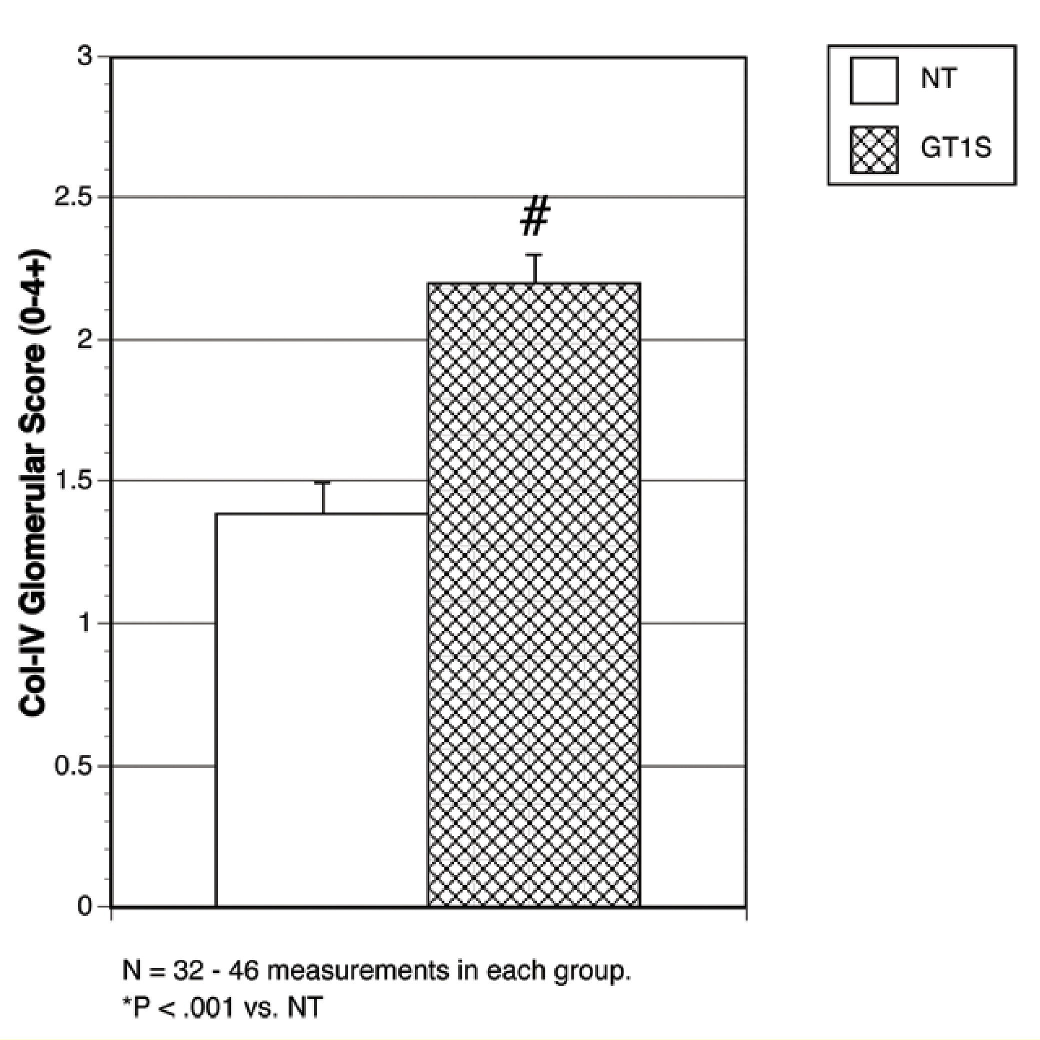
Immunolabelling of Type IV Collagen in Nontransgenic and Transgenic Line S224 GLUT1-Overexpressing Mice Age 26 Weeks



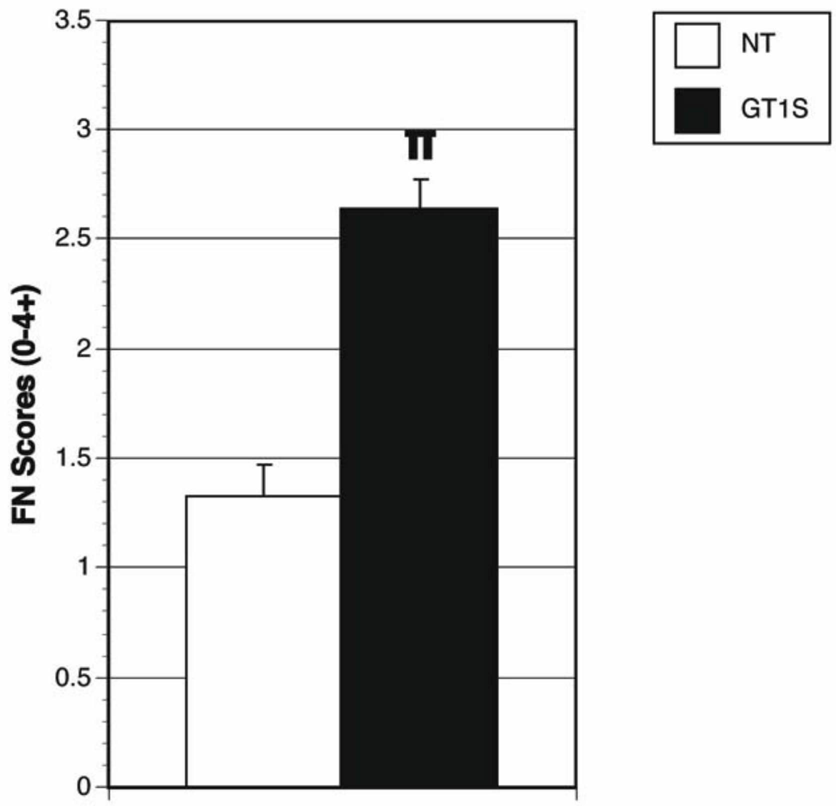
Glomerular Fibronectin in Nontransgenic and Line 224 GT1S Transgenic Mice at Age 26 Weeks



Type IV Collagen Accumulation in Glomeruli of GT1S Transgenic Mice at Age 26 weeks



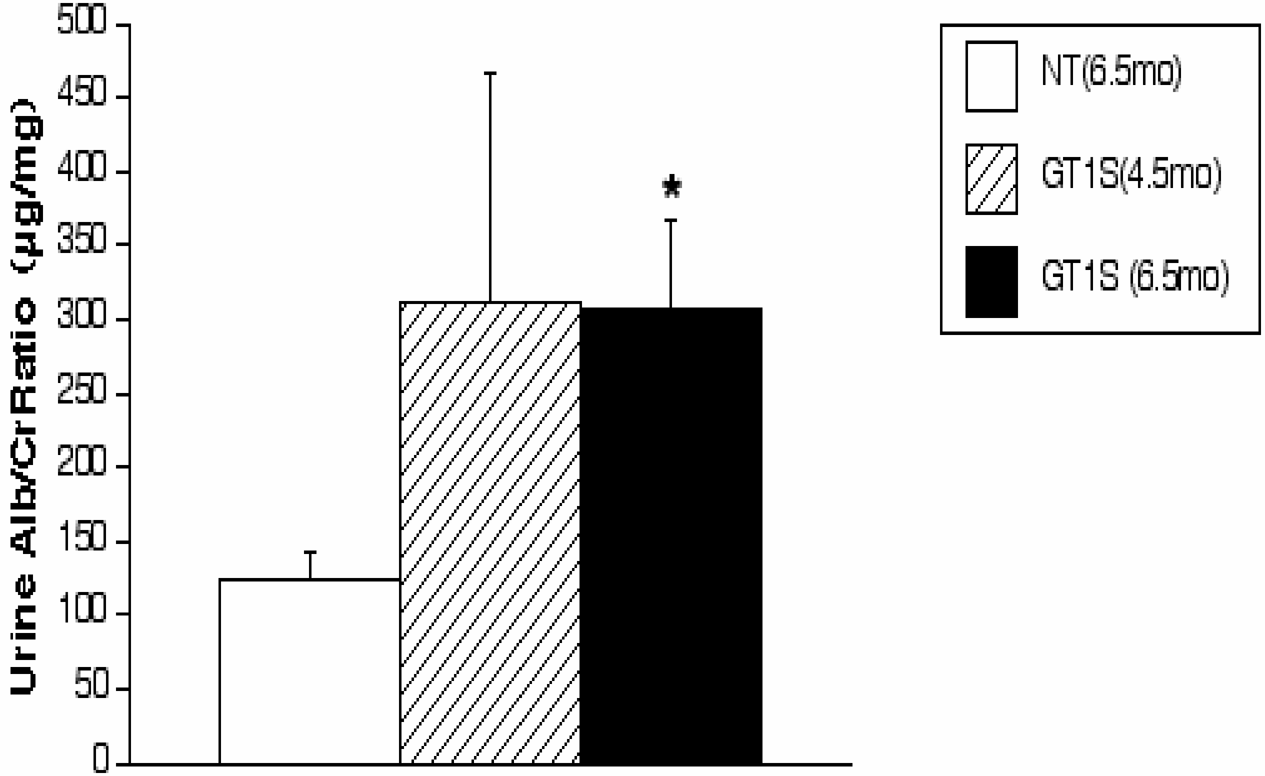
FN Staining Scores in Nontransgenic and GT1S Transgenic Glomeruli at Age 26 Weeks



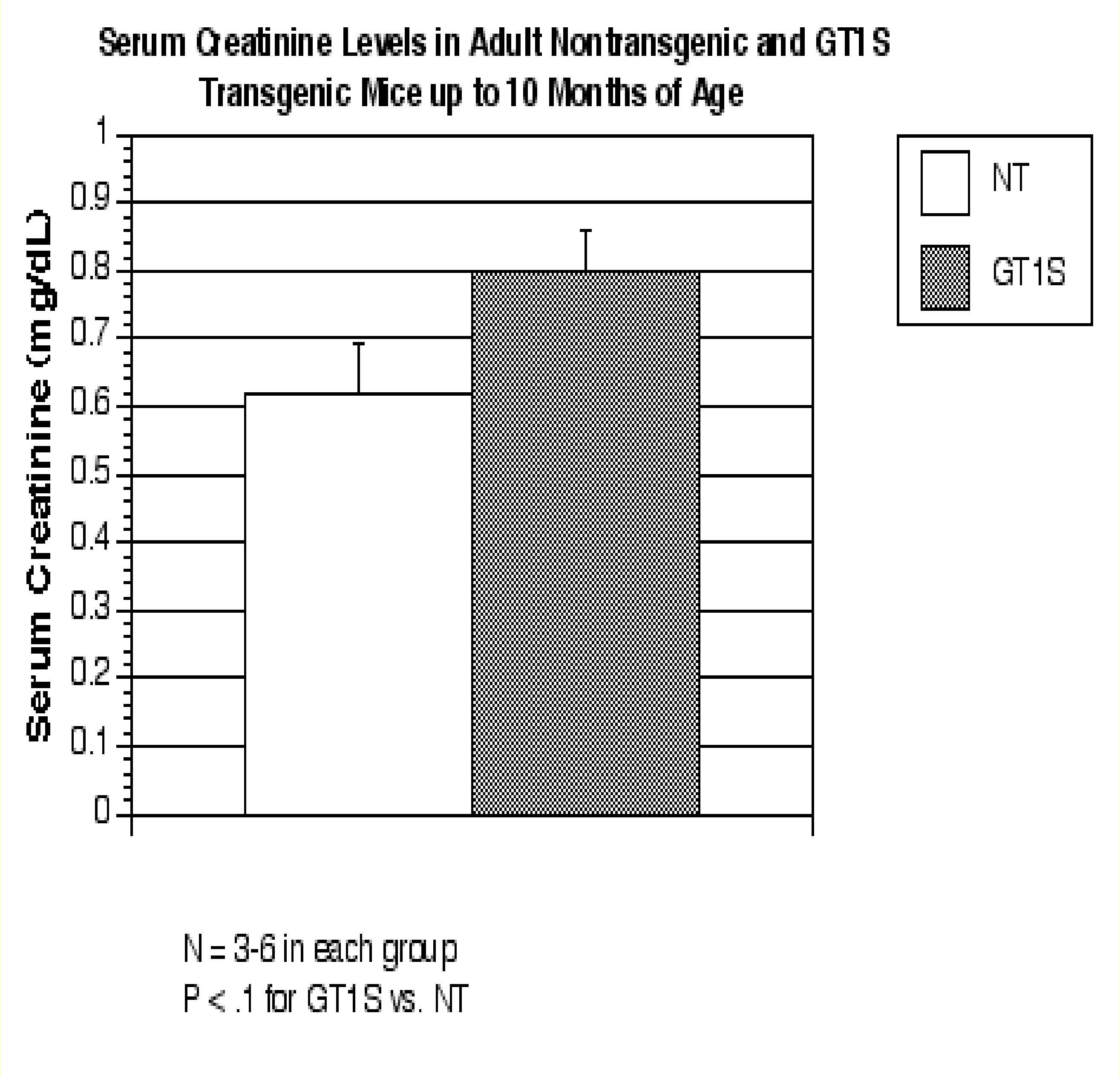
N = 14 - 21 measurements in eqch group.

$^{\pi}$P < .001 vs. NT

Urinary Albumin/Cr Ratios for GT1S Line Mice

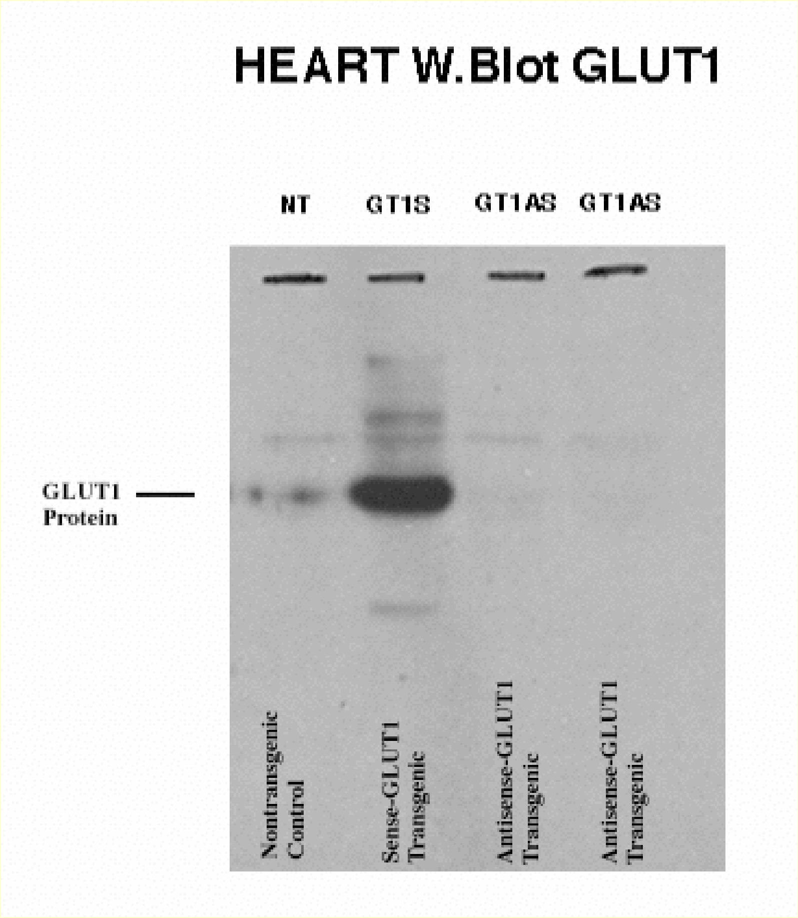


N = 3 - 5 mice per group.

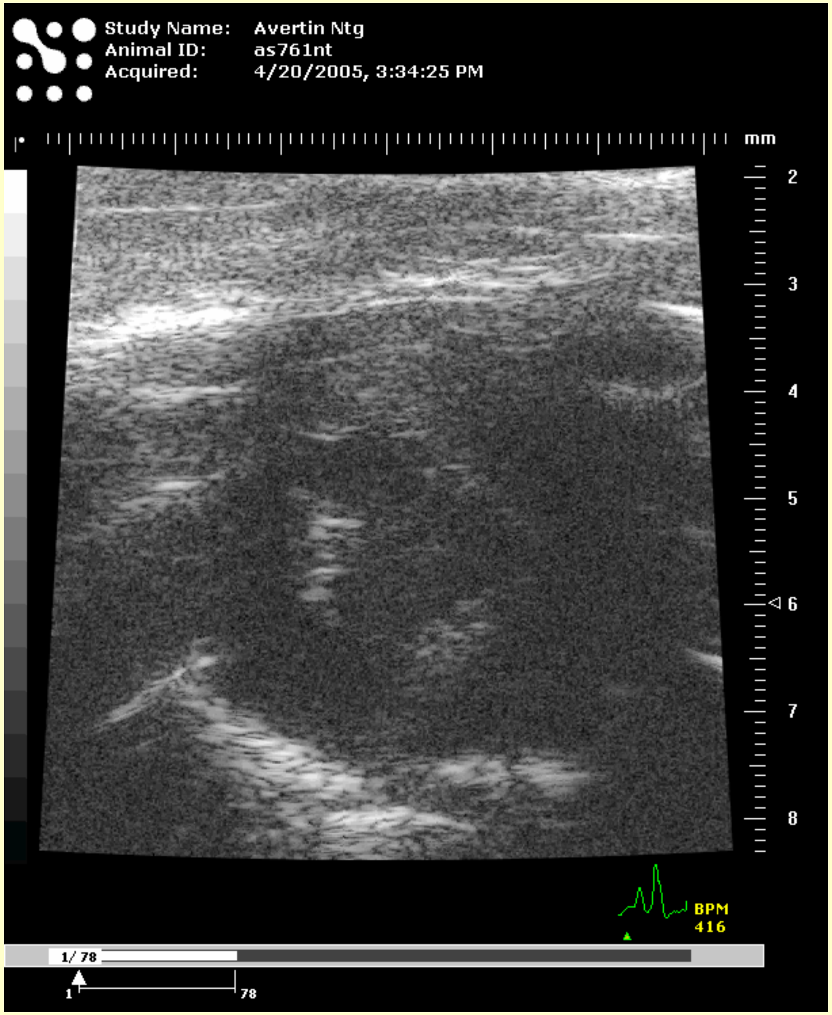


Serum Creatinine Levels in Adult Nontransgenic and GT1S Transgenic Mice up to 10 Months of Age
Serum Creatinine (mg/dL)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NT
GT1S
N = 3-6 in each group
P < .1 for GT1S vs. NT

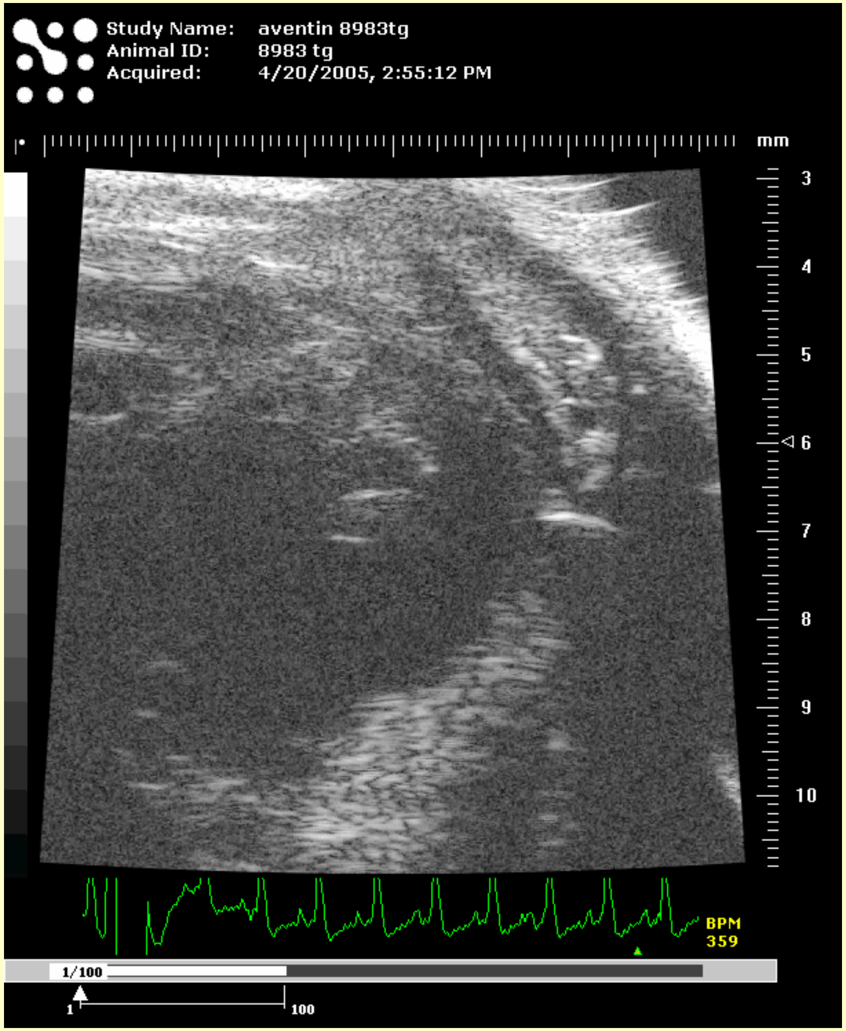
GLUT1 Protein is Increased Approximately 7-Fold in the Hearts of GT1S Transgenic Mice



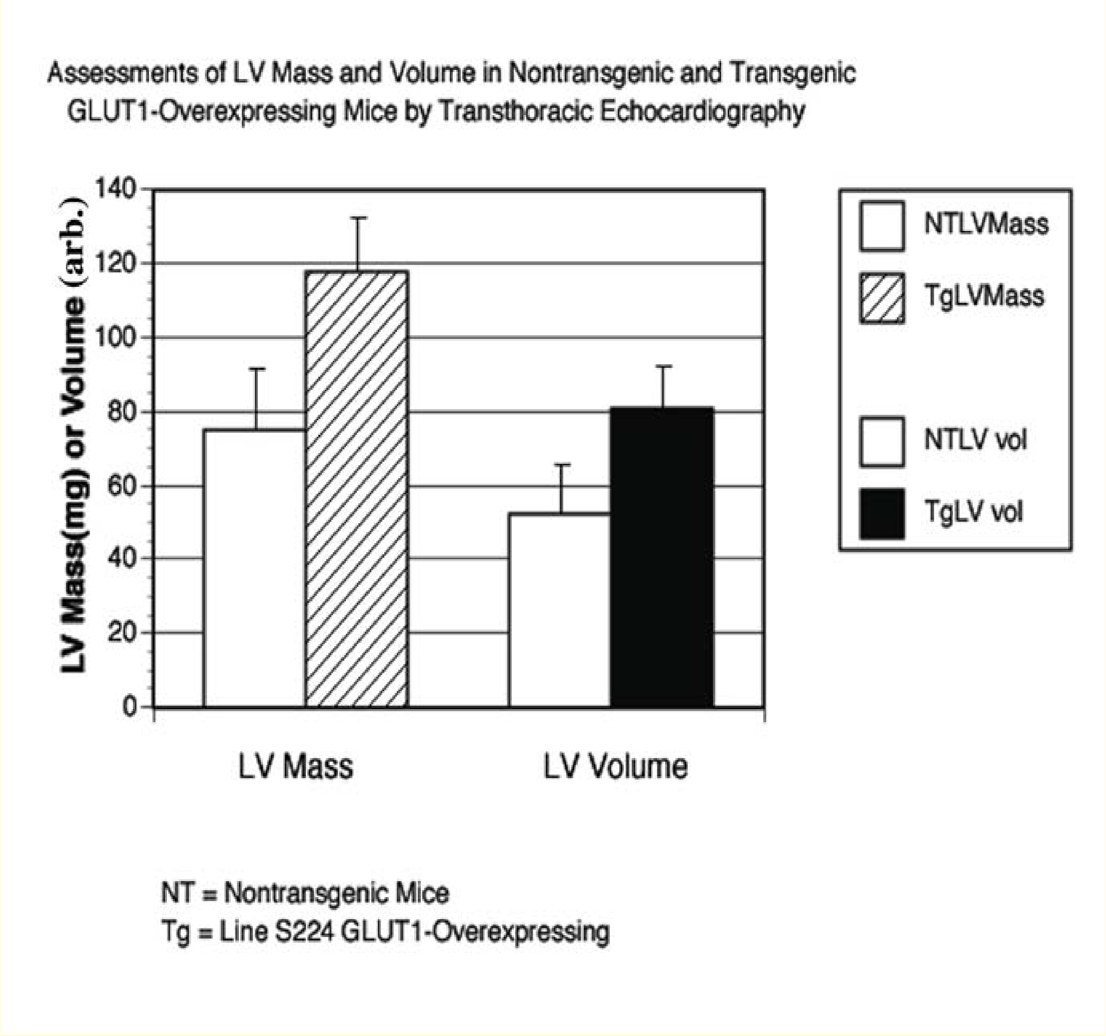
Nontransgenic Heart at Age 16 weeks



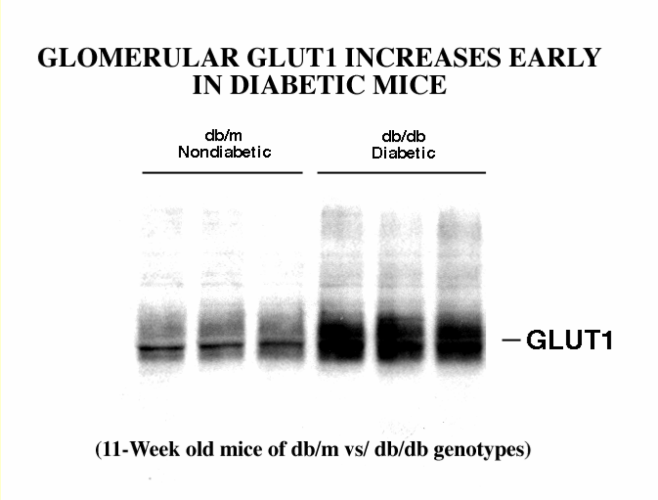
GT1S Transgenic Heart at Age 16 Weeks



Increased LV Mass and Volume in GT1S Transgenic Hearts



GLUT1tg C57Bl/6J db/db model (GT1S)



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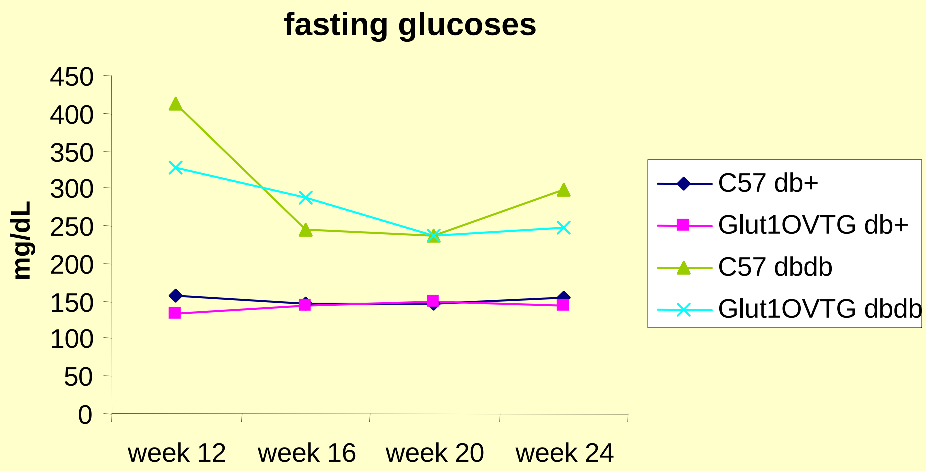
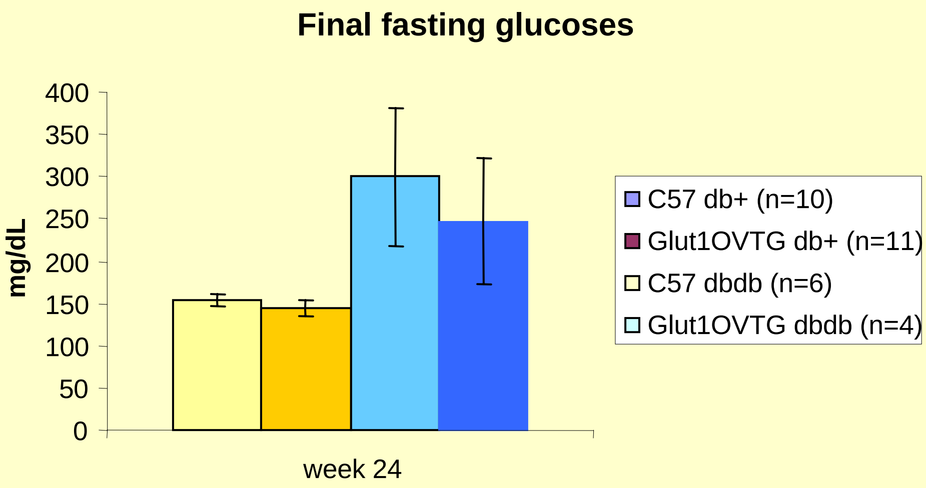
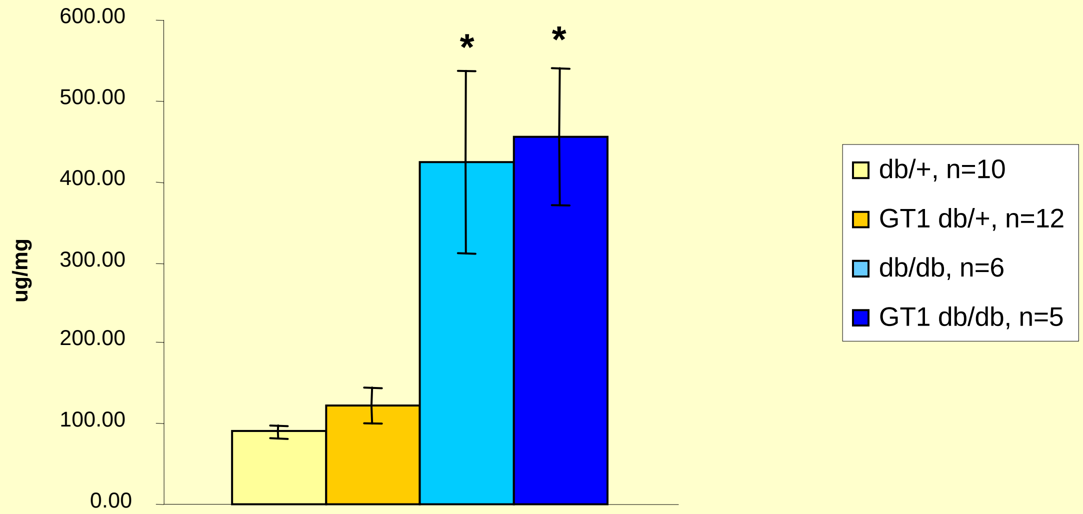
Fasting blood sugar
GLUT1 tg C57BL/6J db/db model



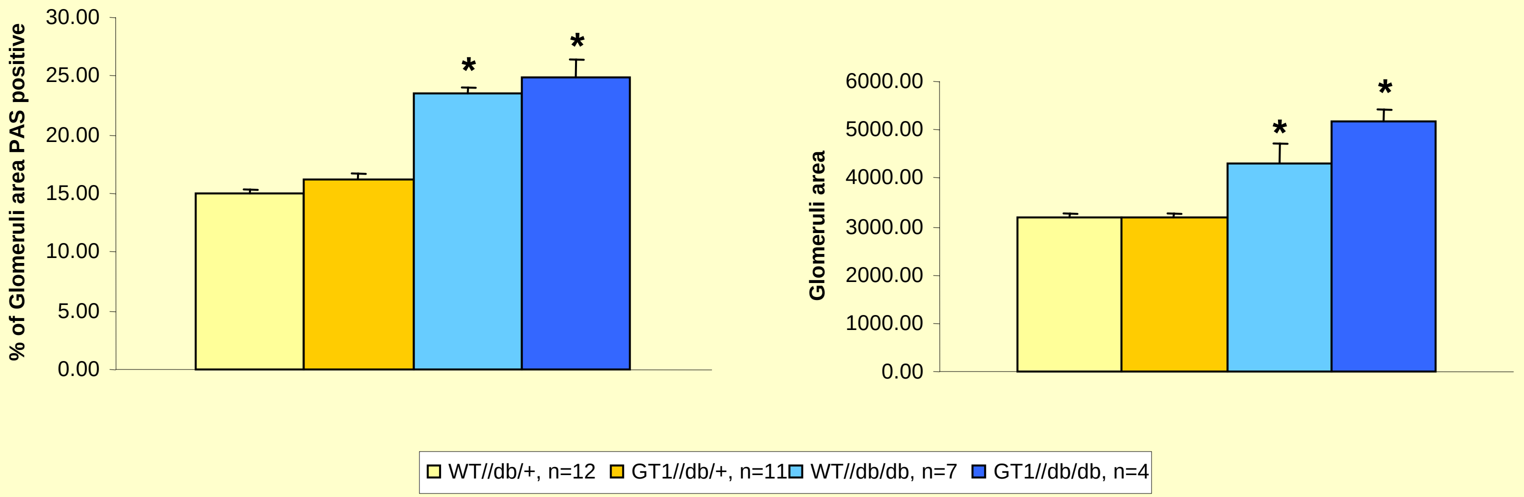


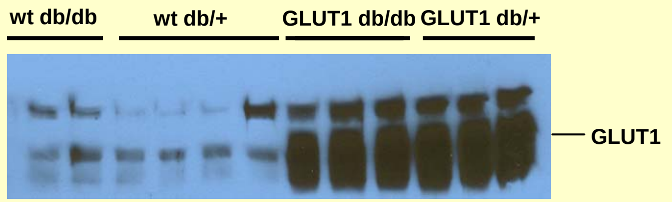
Albuminuria
GLUT1 tg C57BL/6J db/db model-24 wk



***p<0.01 vs. db/+**

PAS expansion
GLUT1 tg C57BL/6J db/db model-24 wk



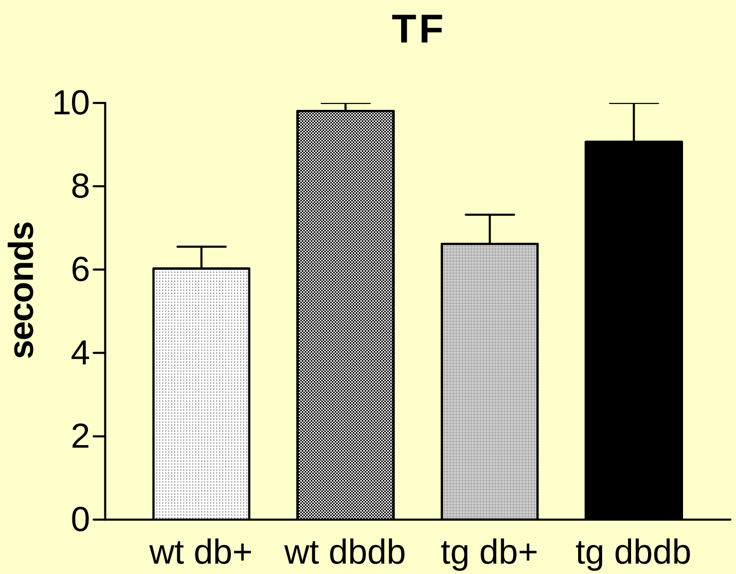


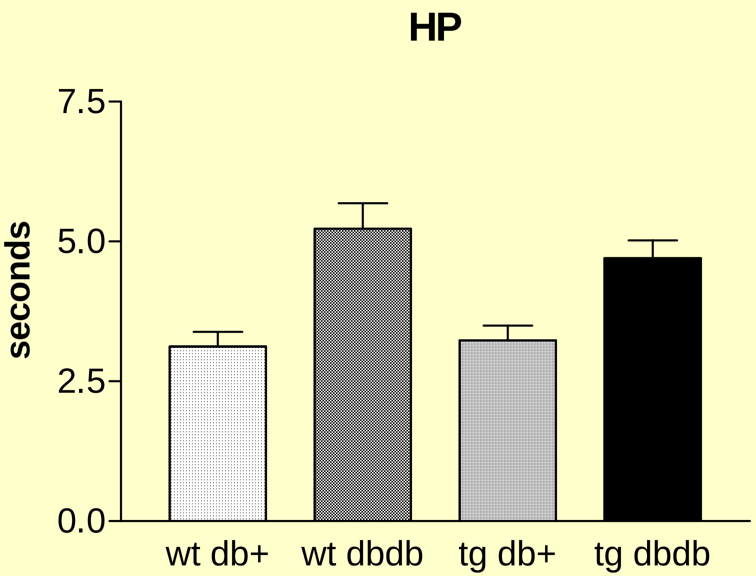
*p<0.001 vs. db/+

AMDCC Definition of Neuropathy

- Evidence of clinical loss of sensory function
  - Tail flick and hind paw withdrawal latencies (TF and HP)
- Electrophysiological evidence of nerve impairment
  - Sensory and motor distal latencies and nerve conduction velocity (NCV)
- Anatomical evidence of nerve fiber loss
  - Intraepidermal fiber density (IEFD

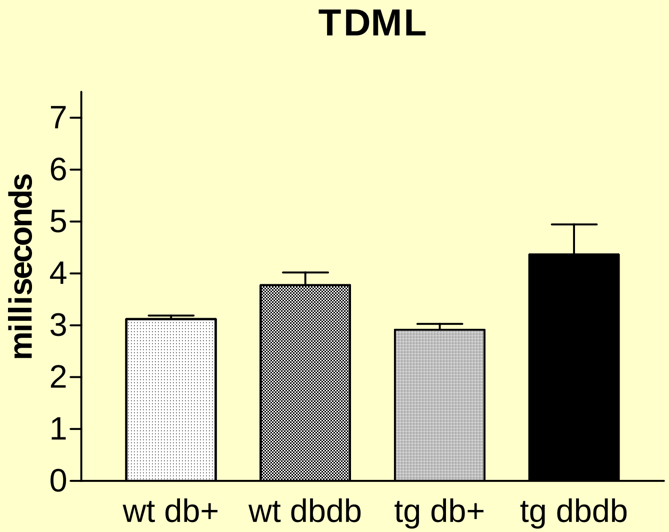
Sensory Tests
GLUT1tg C57Bl/6J dbdb Mice

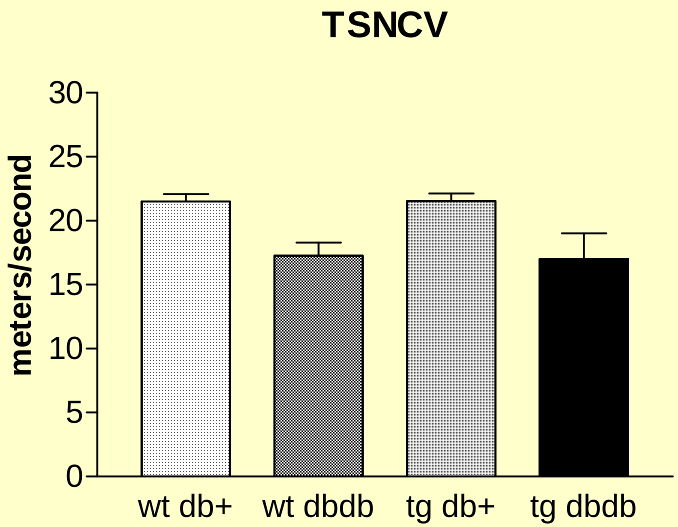


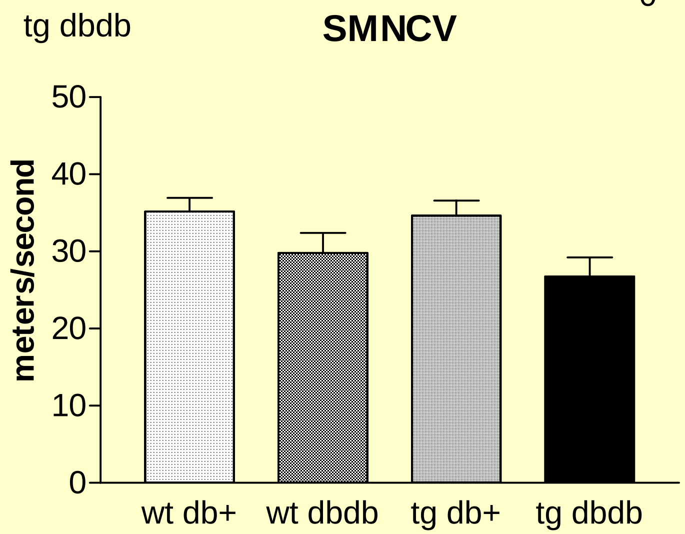


No Significant Differences

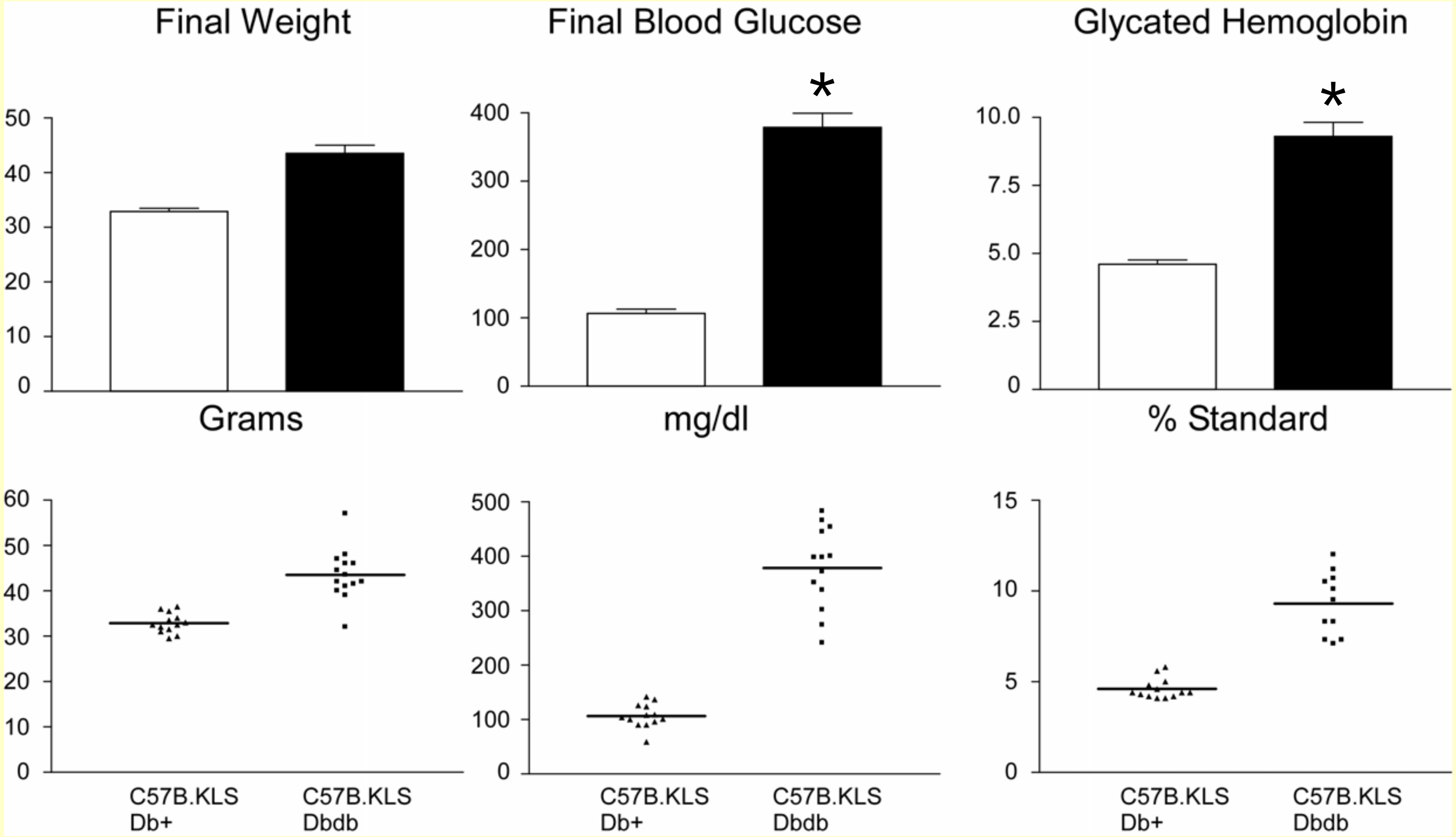
Nerve Conduction Velocity GLUT1tg C57Bl/6J dbdb Mice





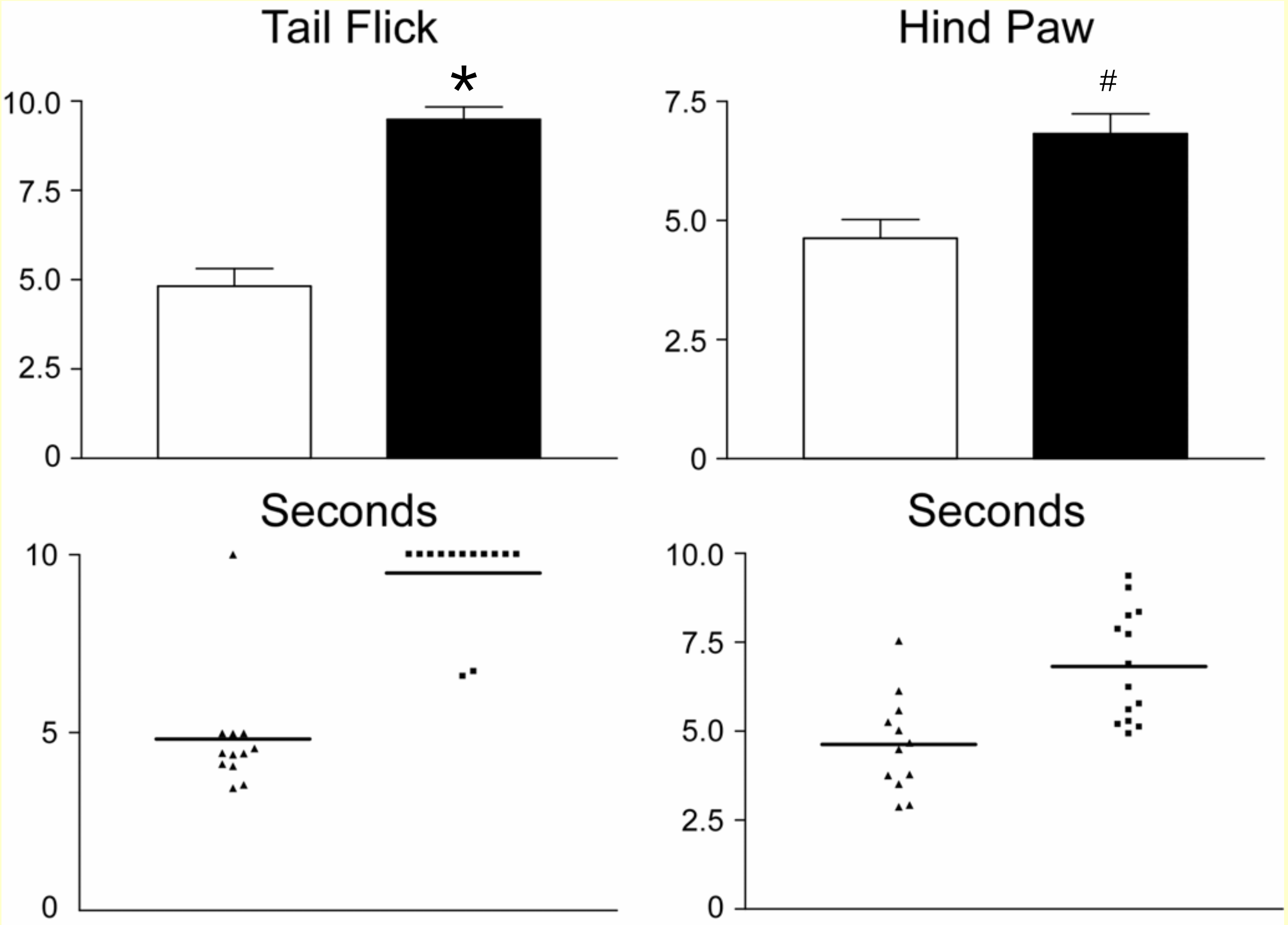


C57BlKS dbdb



* P< 0.001

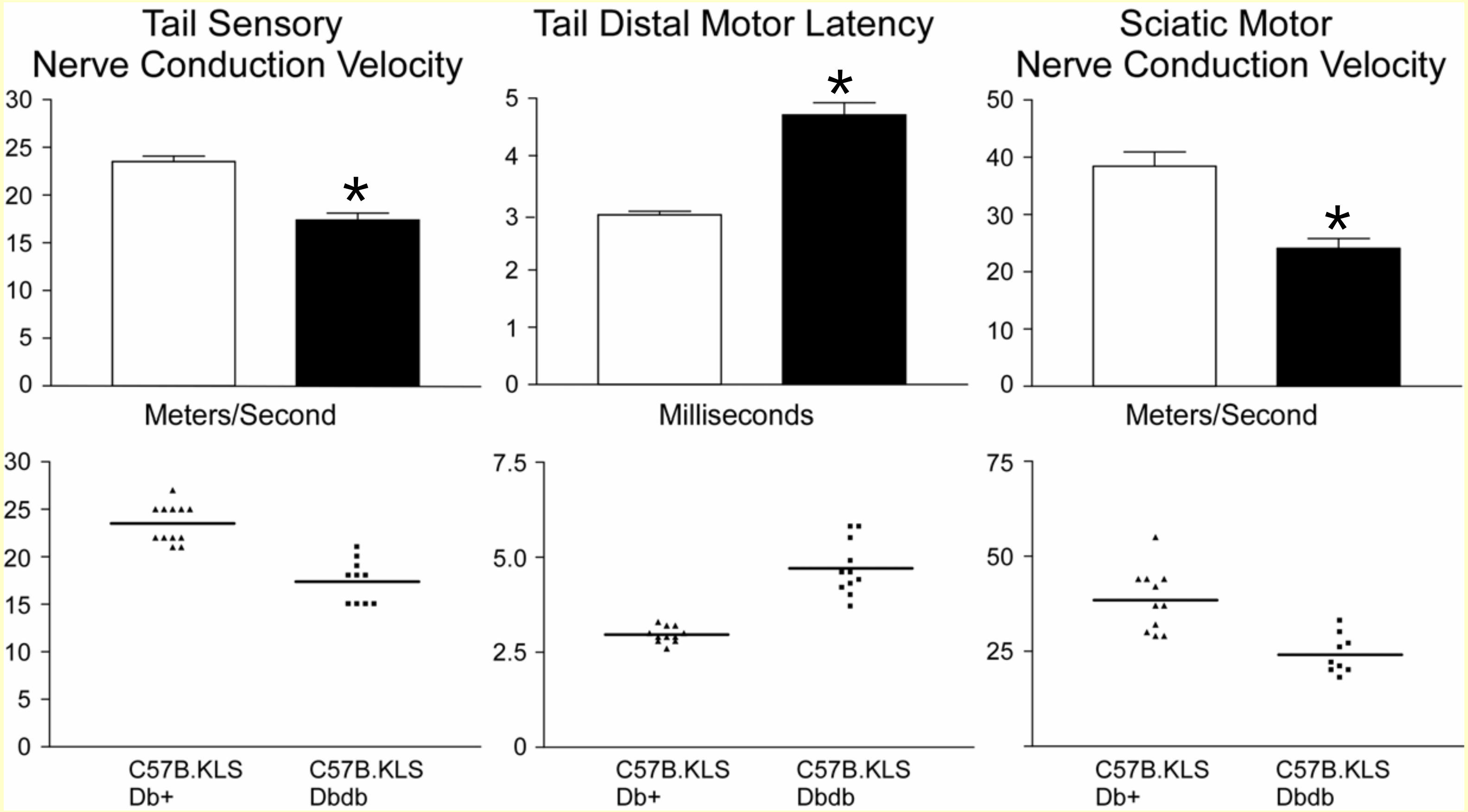
C57BlKS dbdb: Sensory Tests



* P< 0.001

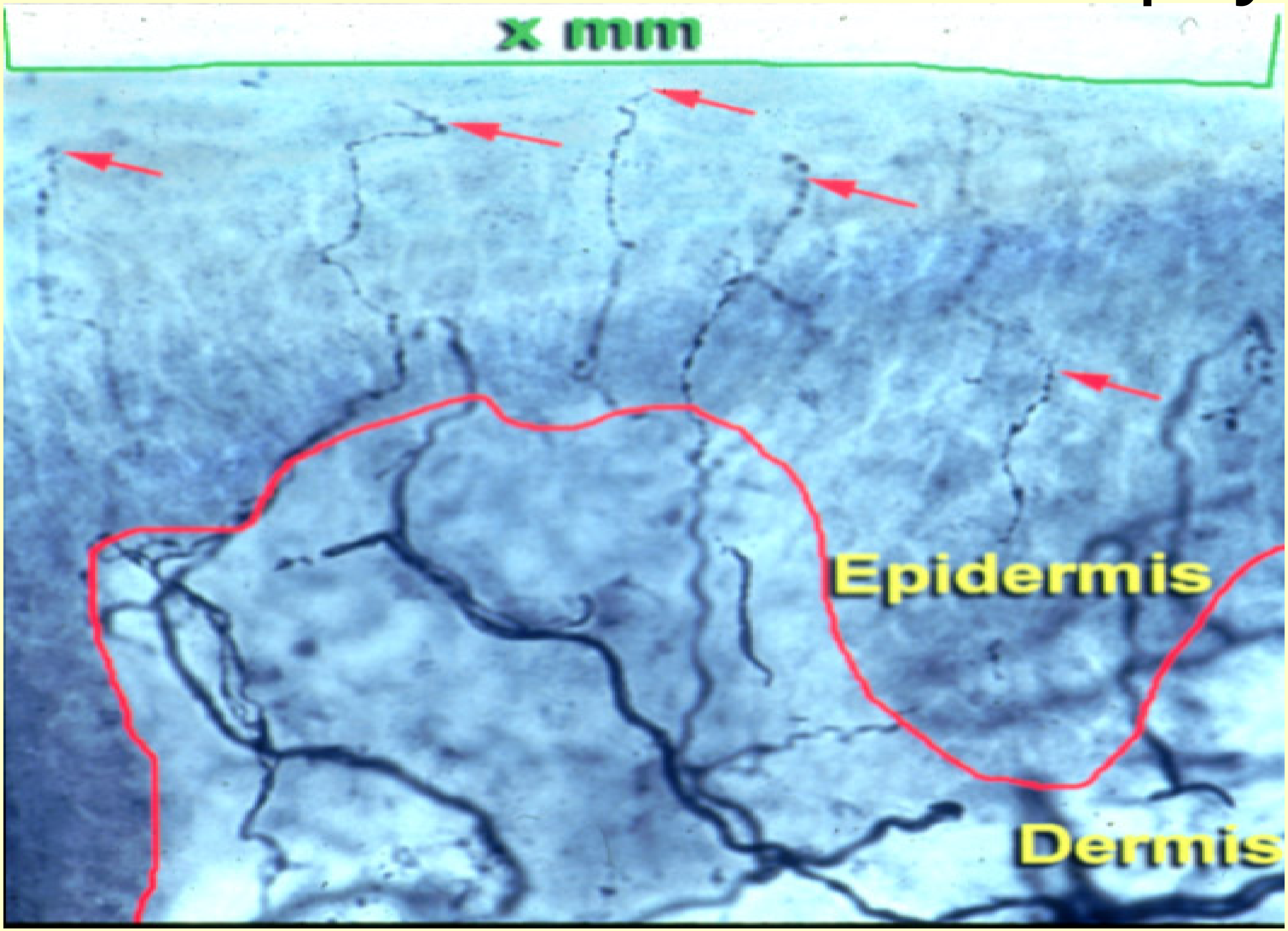
# P < 0.05

C57BlKS dbdb: Nerve Conductions

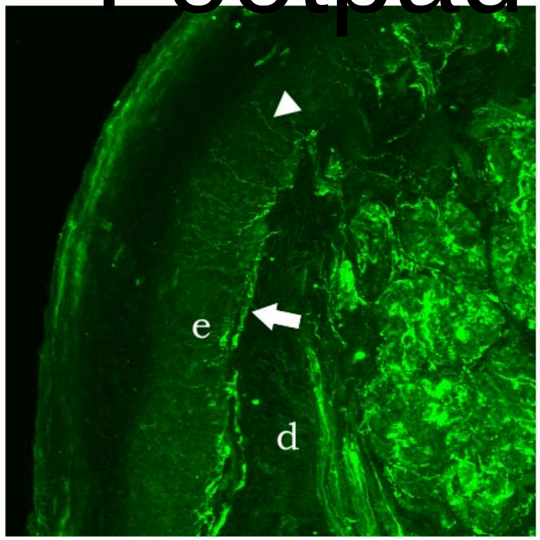


* P< 0.001

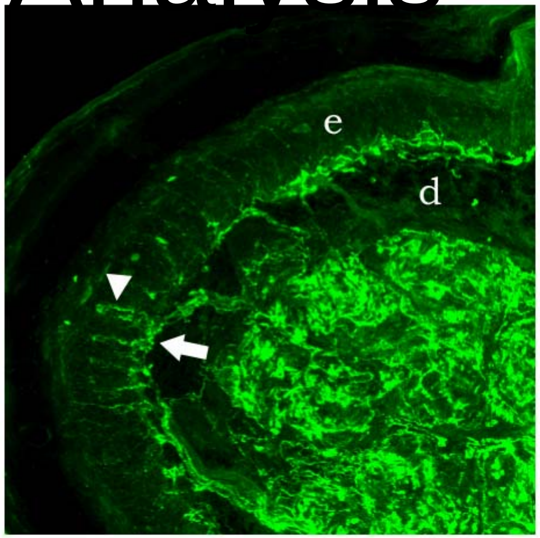
Normal Cutaneous Innervation Assessed with Skin Biopsy



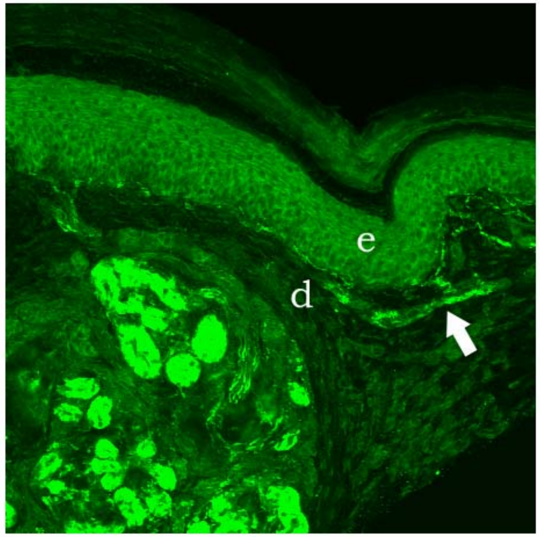
Footpad Analysis



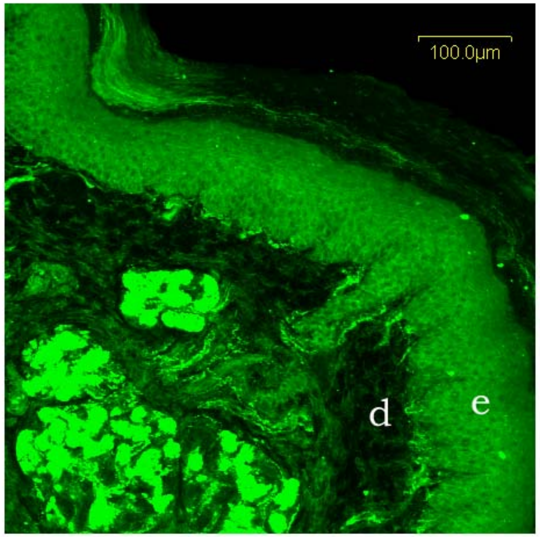
- Fresh
- 2% Zamboni's 24 hrs



- Fresh
- 2% Zamboni's 24 hrs



- Fresh
- 4% PFA 24 hrs

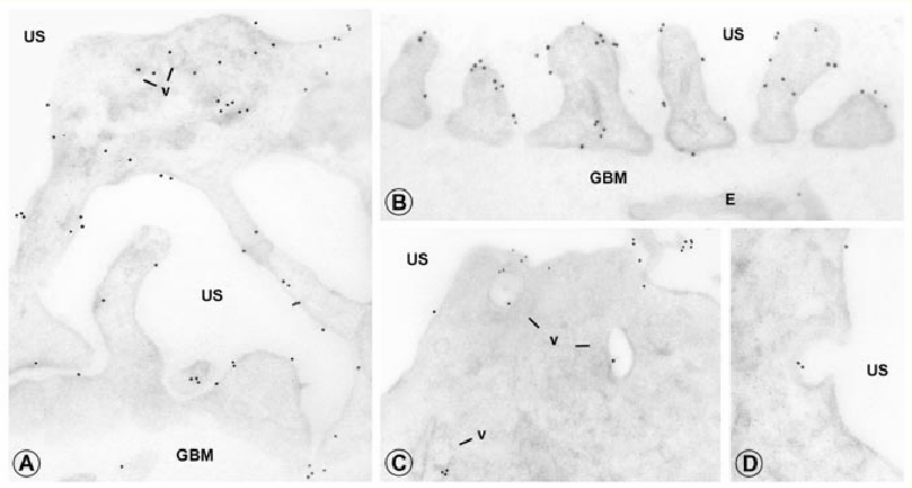


- Fresh
- 4% PFA 24 hrs
- 2% Zamboni's 24 hrs

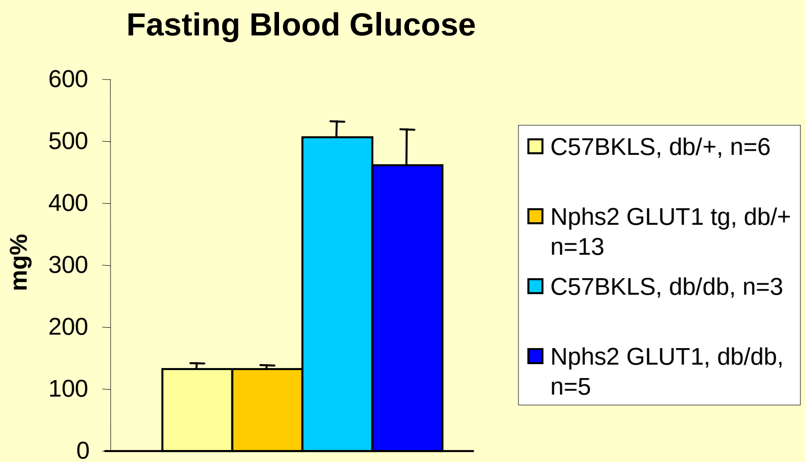
Nphs2 GLUT1tg db/db C57BLKS model

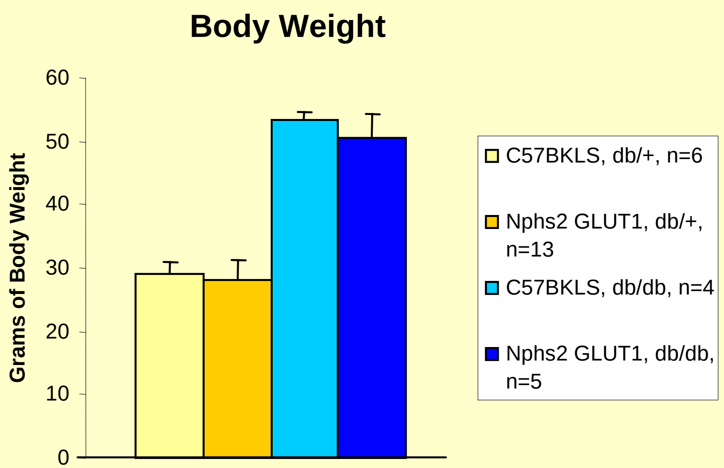
- GLUT1 is highly expressed in podocytes
- High glucose and diabetes in humans and rodents lead to podocyte loss
- Nphs2 promoter drives GLUT1 in podocytes
- db/m C57BKS eggs injected to allow direct examination on diabetic background

GLUT1 in human kidney podocytes

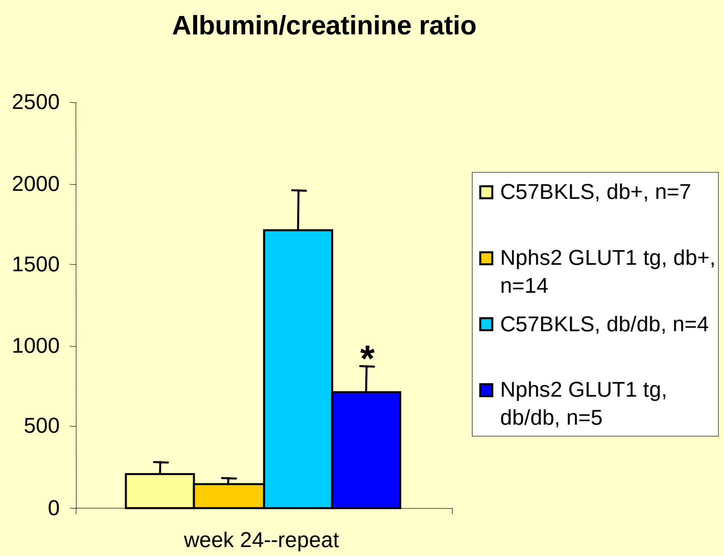


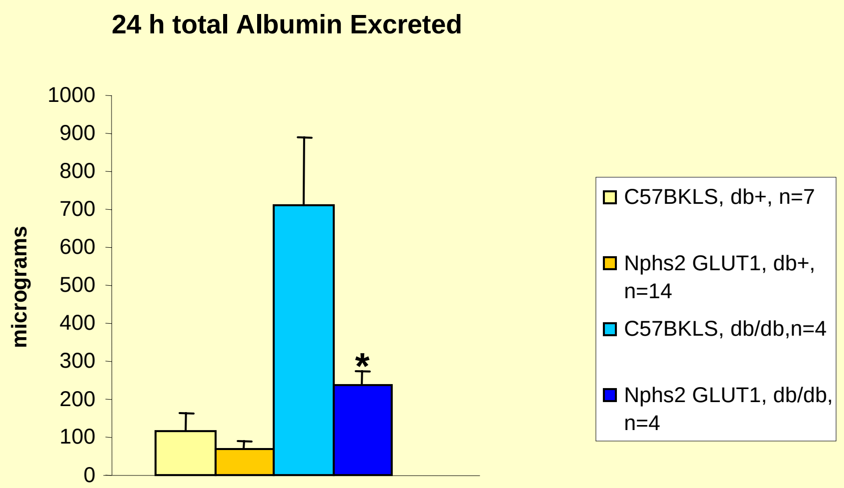
Fasting blood sugar and weights-24 wk Nphs2 GLUT1tg db/db C57BLKS model





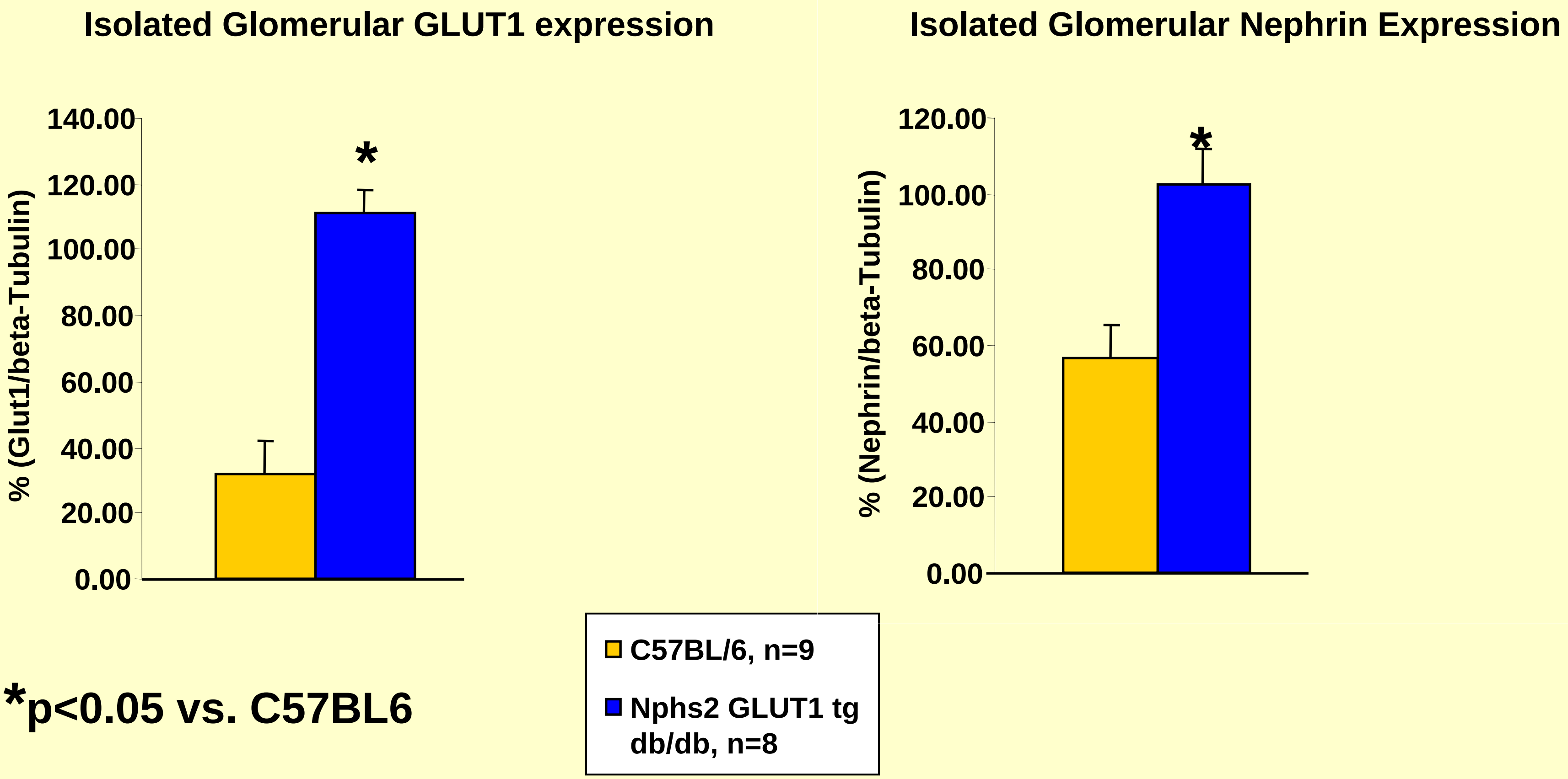
Albuminuria-24 wk
Nphs2 GLUT1tg db/db C57BLKS model





***p<0.001 vs. C57BLKS db/db**

GLUT1 and nephrin expression- 6-8 wk Nphs2 GLUT1tg db/db C57BLKS model



*p<0.05 vs. C57BL6

Summary

- GLUT1 tg in nondiabetic C57BL6 mice results in enlarged glomeruli, increased proteinuria and mesangial matrix expansion.
- GLUT1 tg in nondiabetic C57BL6 mice results in cardiac hypertrophy in the absence of hypertension.
- GLUT1 tg in C57BL6 db/db diabetic mice fed with higher fat chow fails to increase proteinuria and mesangial matrix expansion significantly by 6 months of age.
- “High fat” feeding in C57BL/6 db/db mice leads to sustained hyperglycemia and more robust nephropathy than in mice fed a normal fat chow.
- Similarly, “high fat” feeding in C57BL/6 db/db mice leads to more robust neuropathy than in mice fed a normal fat chow, but not as robust as in C57BLKS mice.
- Podocyte specific GLUT1 overexpression unexpectedly leads to reduced proteinuria in C57BLKS db/db mice