Mouse Models of Diabetic Nephropathy

Mount Sinai / Jefferson / Einstein / Minnesota
Erwin Böttinger, PI
Kumar Sharma, Co-PI

Group Members

- *Mount Sinai: Phenotyping, Molecular Pathology & Validation*
  - Erwin Bottinger (PI)
  - Sandhya Xavier (Fellow)
  - Liping Yu, Technician
- *Albert Einstein: Strain Generation & Characterization*
  - Maureen Charron (Co-Investigator)
  - Scott Henderson, Technician
  - Katalin Susztak, Co-Investigator
- *Thomas Jefferson: Experimental Diabetes, HPLC Core, Phenotyping*
  - Kumar Sharma (Co-PI)
  - Steve Dunn, Technician and Core Manager
  - Kevin Williams, (Co-Investigator)
  - Peter McCue, Pathology
- *U Minnesota: Phenotyping (Morphometry)*
  - Michael Steffes, Consultant

Progress Report

- *Strain Generation*

  Katalin Susztak (AECOM)/Erwin Bottinger (MSSM)
  - **gGT-CD36 tg > NO EXPRESSION in 11 lines screened**
  - **Alternate strategy: sglt2l-CD36 tg > 9 Founders**

- *Experimental Models*

  Bottinger (MSSM)

  completed
  - db/db C57BLKS longitudinal, standardized phenotyping
  - Ins2-Akita longitudinal, standardized phenotyping
  - flk-1 RAGEtg with low dose STZ to 28 wks
  - Cd2ap+/- with STZ to 28 wks
  - Db/db and Ins2-Akita (glomerular RNA profiles - molecular phenotying: up to 28 wk-of-age)

  ongoing
  - **flk-1 RAGEtg x Ins2-Akita CD1xB6 > 24 wk-of-age phenotyping**
  - **Cd2ap+/- x Ins2-Akita 129xB6 > 24 wk-of-age phenotyping**

  Kumar Sharma (TJU)

  Manuscript in preparation
  - **Decorin knockout with low dose STZ**

- *Phenotyping Cores*

  HPLC creatinine… Kumar Sharma/Steve Dunn (TJU)

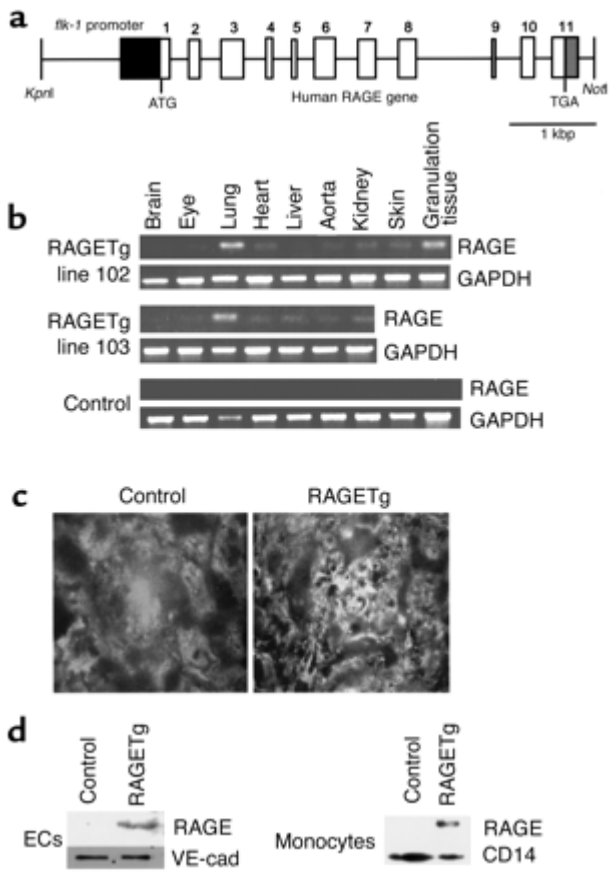
  Genomics…. Erwin Bottinger (MSSM)

Ins2Akita x flk-RAGE tg

Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice
Yasuhiko Yamamoto and Hiroshi Yamamoto;
J Clin Invest, 2001

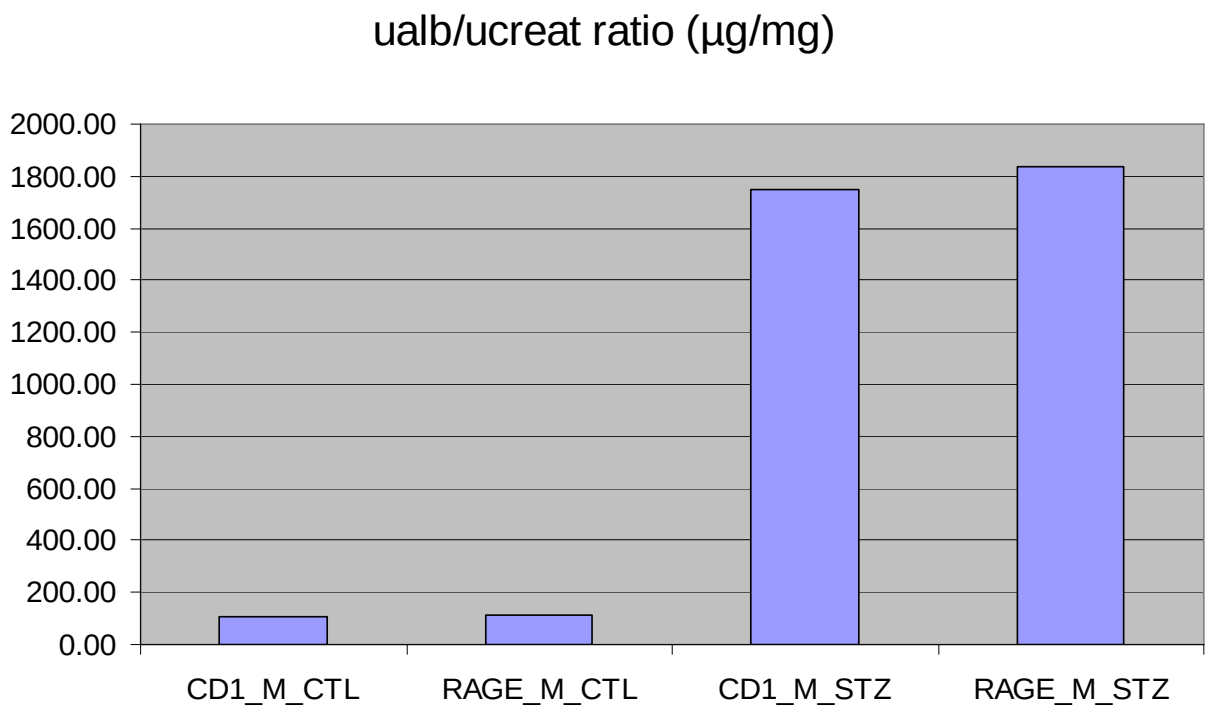
DIABETES INDUCTION

inducible nitric oxide synthase (iNOS) under the control of the insulin promoter (iNOSTg)



Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

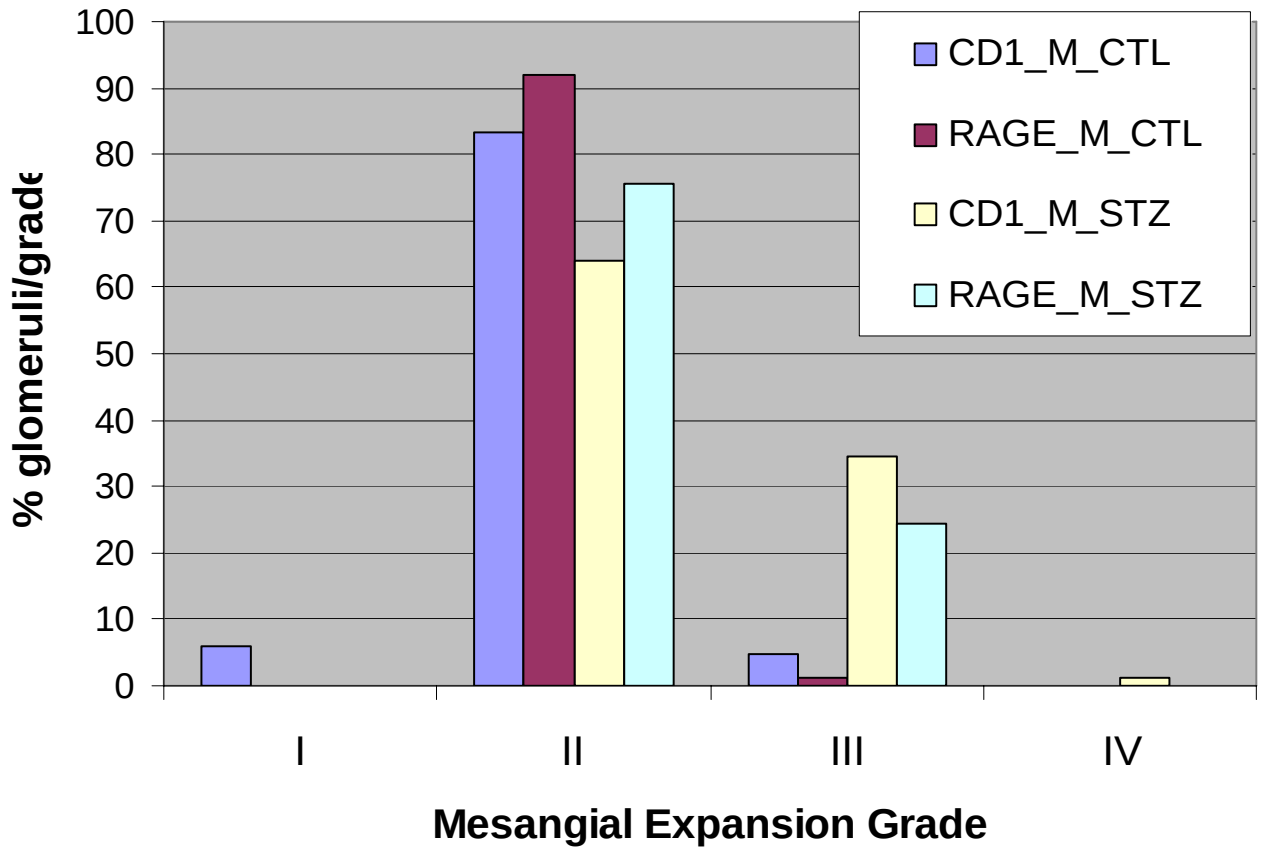
Experimental Design
- Female and Male mice
- CD1 inbred
- STZ 8 wk
- Sac 20 wk



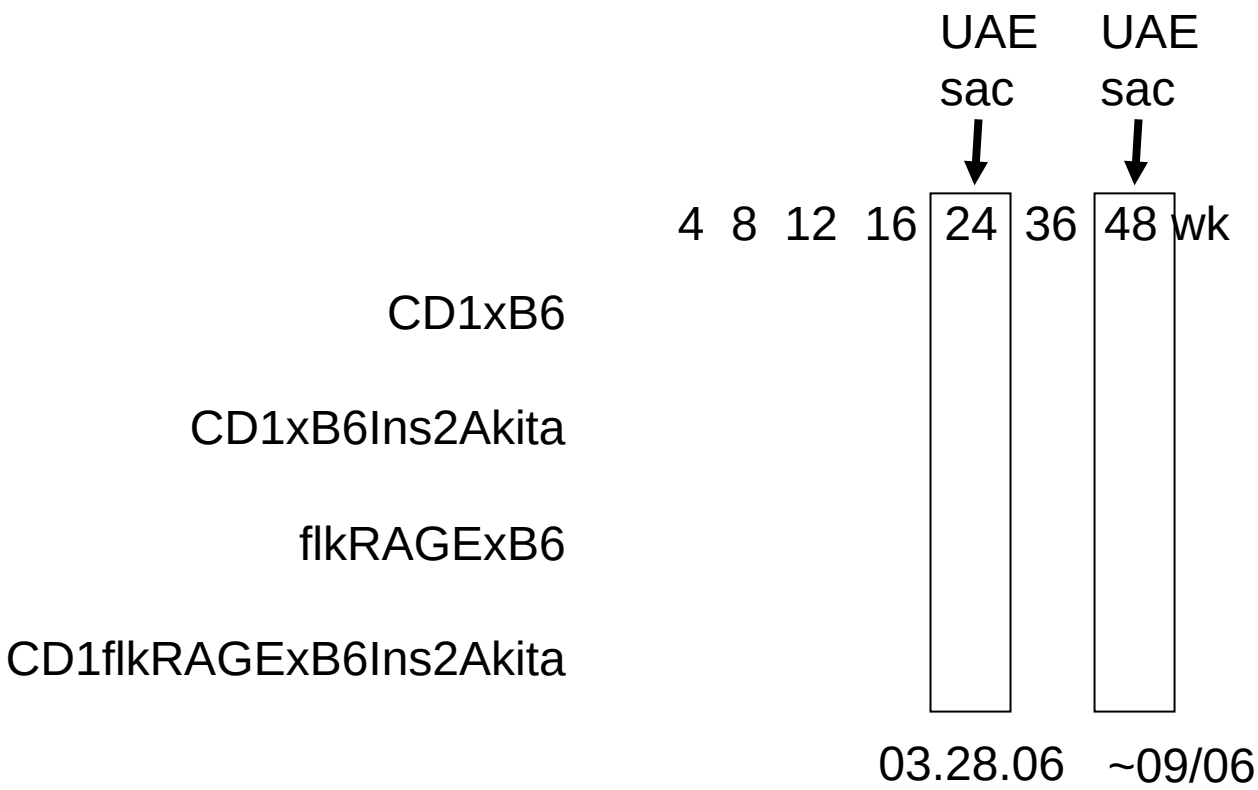
Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

Experimental Design
- Male mice
- CD1 inbred
- STZ 8 wk
- Sac 20 wk

Pathology Report
- No nodular lesion
- No tub.int. lesions
- No vascular leseions

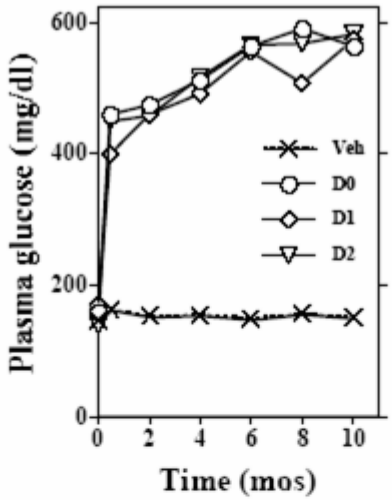


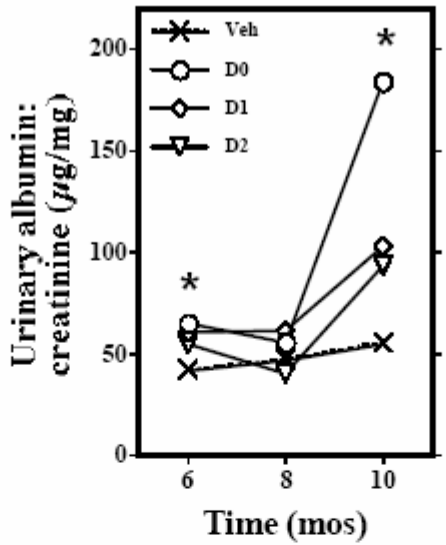
Ins2Akita x flk-RAGE tg

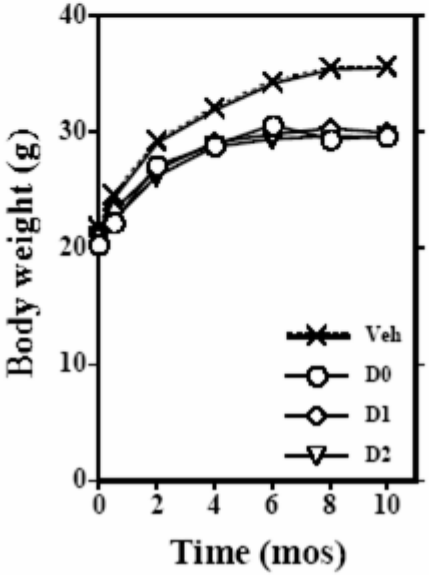


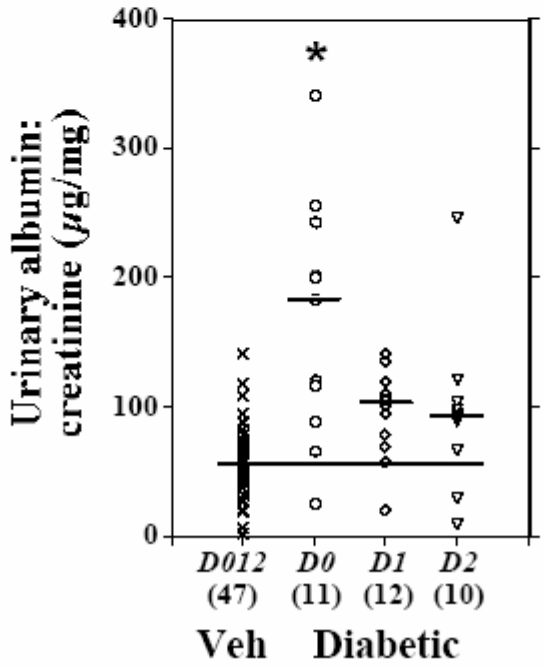
Decorin and diabetic nephropathy

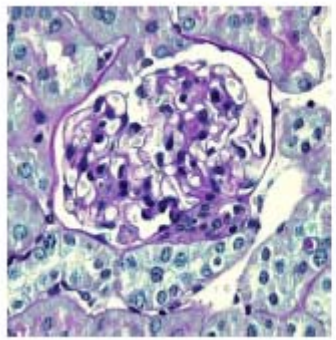
- **Background: Decorin is a small proteoglycan that inhibits TGF-β via direct binding to active TGF-β. Decorin is stimulated in human mesangial cells by high glucose and stimulated in several mouse models of diabetic kidney disease.**
- **Hypothesis: Decorin protects against diabetic kidney disease.**
- **Back-cross *Dcn* KO allele onto the C57BL/6J background; induce STZ diabetes in male littermates with different *Dcn* gene doses**
  - **Albuminuria**
  - **Creatinine clearance by HPLC**
  - **Histologic parameters of diabetic nephropathy**

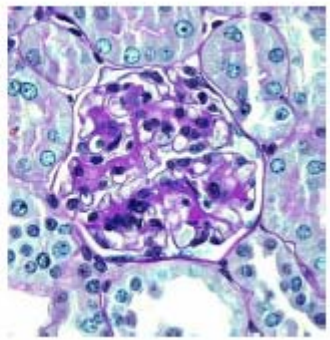

Plasma glucose (mg/dl)
600
400
200
0
0
2
4
6
8
10
Time (mos)
Veh
D0
D1
D2

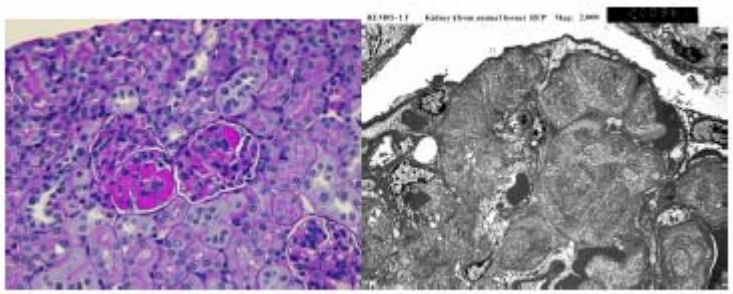

Urinary albumin: creatinine (μg/mg)
200
150
100
50
0
6
8
10
Time (mos)
Veh
D0
D1
D2
*
*

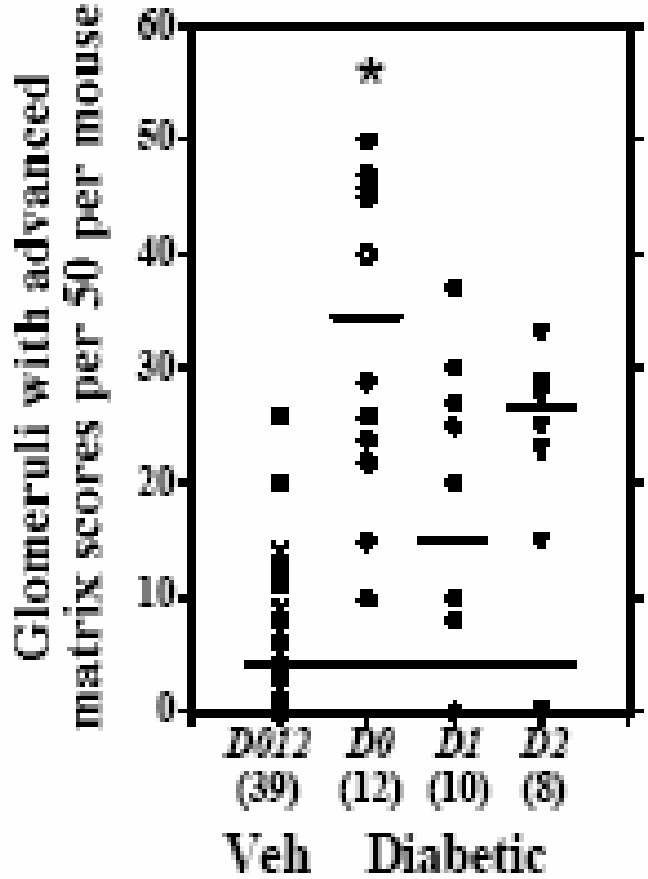

Body weight (g)
40
30
20
10
0
0
2
4
6
8
10
Time (mos)
Veh
D0
D1
D2


Urinary albumin: creatinine (μg/mg)
400
300
200
100
0
*
D012 (47)
D0 (11)
D1 (12)
D2 (10)
Veh
Diabetic





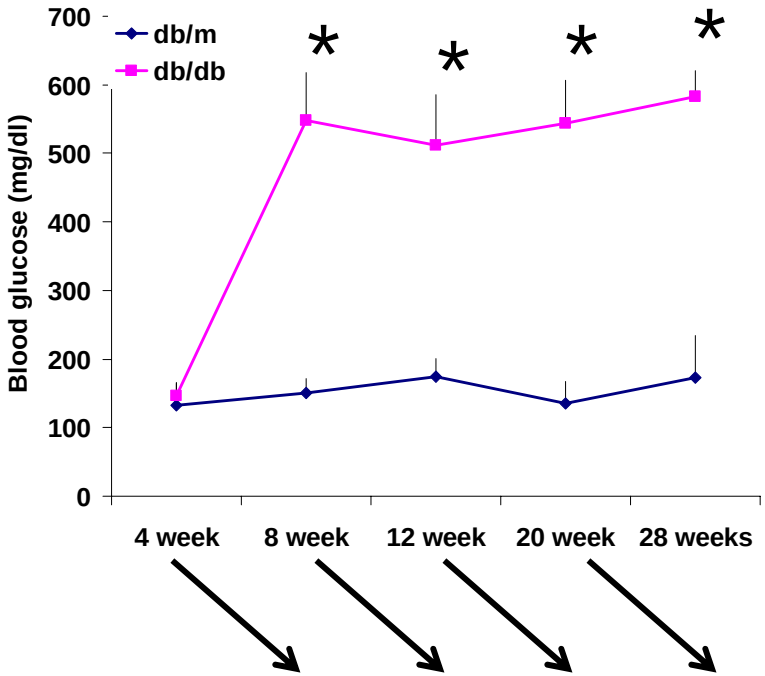



Glomeruli with advanced matrix scores per 50 per mouse
60
50
40
30
20
10
0
*
D012
(39)
D0
(12)
D1
(10)
D2
(8)
Veh
Diabetic

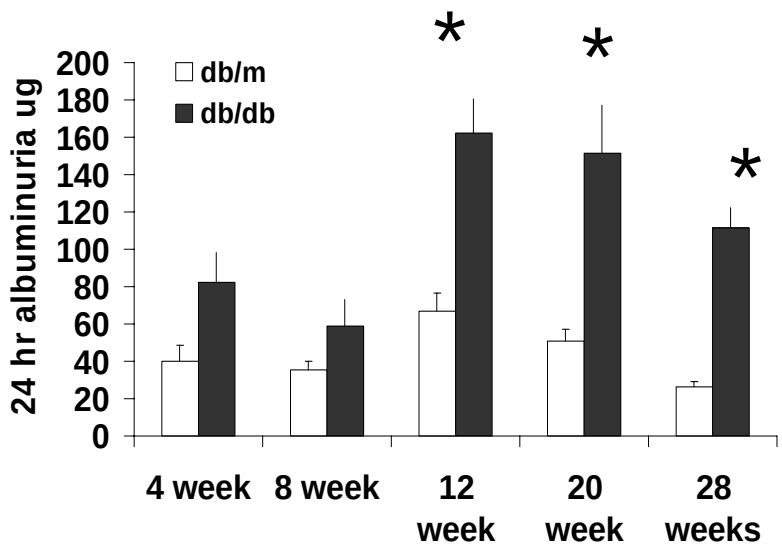
db/db C57BLKS/J Molecular Phenotyping

MOUNT SINAI SCHOOL OF MEDICINE

Blood Glucose (BG)



Urinary Albumin Excretion (UAE)



Glomerular and tubular RNA preparations

↓

MOE430 whole genome profiling

Experiments:

	db/db	db/m		Akita	B6
4 weeks	4	5	4-5 weeks	1	3
8 weeks	10	7	10 weeks	3	0
12 weeks	3	3	18-20 weeks	5	2
20 weeks	4	4	28 weeks	2	2

Unique genes: 14097

Filtering: Genes whose median expression value cross experiments < 2 are excluded from analysis.

SAM and GoMiner are used to identify differentially expressed genes and pathways.

Cutoff for selecting differentially expressed genes: FDR <= 0.05
Cutoff for selecting enriched GO category: FDR <= 0.01

Experiment	Differentially expressed genes
db/db 8, 12&20 weeks and Akita all vs db/db 4 weeks and db/m all and B6 all (All diabetic vs all non-diabetic)	2691
db/db 20 weeks vs db/db 4, 8&12 weeks and db/m all	2641
db/db 12&20 weeks vs db/db 4&8 weeks and db/m all	2132
db/db 8, 12&20 weeks vs db/db 4 weeks and db/m all (Diabetic vs non-diabetic)	1550
db/db 12&20 weeks vs db/m 12&20 weeks	1195
db/db 8&12 weeks vs db/m 8&12 weeks	697
db/db 8 weeks vs db/m 8 weeks	402
db/db 8&12 weeks vs db/db 4&20 weeks and db/m all	350
db/db 20 weeks vs db/m 20 weeks	231
Akita all vs B6 all (Diabetic vs non-diabetic)	220
Akita 18-20&28 weeks vs B6 18-20&28 weeks	23
db/db 12 weeks vs db/m 12 weeks	18
Akita 4-5&10 weeks vs B6 4-5 weeks	6

Gene Set Enrichment Analysis
(Diabetic vs Non-diabetic for db/db & db/m)

Top leading pathways (up-regulated in diabetic): Total 453 pathways (genesets) analyzed

GeneSet/Pathway	SIZE	SOURCE
MAP00350_Tyrosine_metabolism	23	GenMAPP
calcineurinPathway	19	Increased intracellular calcium activates the phosphatase calcineurin in differentiating keratinocytes.
erkPathway	26	Cell growth is promoted by Ras activation of the anti-apoptotic p44/42 MAP kinase pathway.
inflamPathway	25	BioCarta
dcPathway	21	Dendritic cells internalize and present antigen, after which they migrate to lymphocyte-rich tissues and induce T and B cell differentiation.
intrinsicPathway	22	BioCarta
MAP00280_Valine_leucine_and_isoleucine_degradation	25	GenMAPP
MAP00260_Glycine_serine_and_threonine_metabolism	15	GenMAPP
fmlppathway	36	The fMLP receptor is a G-protein coupled receptor in neutrophiles that recognizes formylated bacterial peptides and activates NADPH oxidase.
MAP00310_Lysine_degradation	15	GenMAPP
ST_G_alpha_i_Pathway_	31	Signalling Transduction KE
CR_TRANSPORT_OF_VESICLES	20	PNAS_2015
MAP00030_Pentose_phosphate_pathway	15	GenMAPP
P53_UP	31	Kannan_et_al_2001
mef2dPathway	16	BioCarta
MAP00330_Arginine_and_proline_metabolism	32	GenMAPP
cell_adhesion	144	The attachment of a cell, either to another cell or to the extracellular matrix, via cell adhesion molecules.
MAP00071_Fatty_acid_metabolism	26	GenMAPP
MAP00590_Prostaglandin_and_leukotriene_metabolism	15	GenMAPP

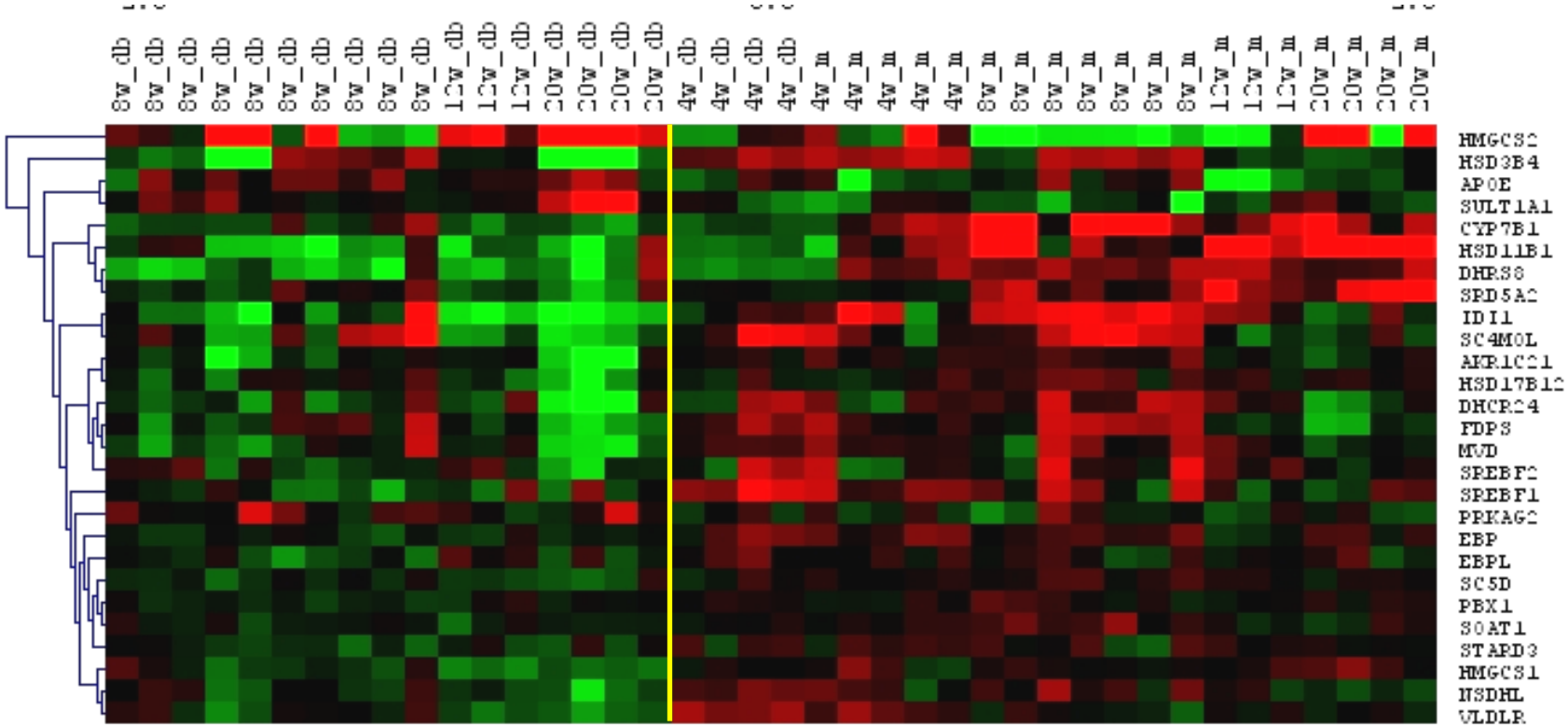
Experiments:

db/db 8, 12&20 weeks (diabetic) vs
db/db 4 weeks and db/m 4, 8, 12&20 weeks (non-diabetic)

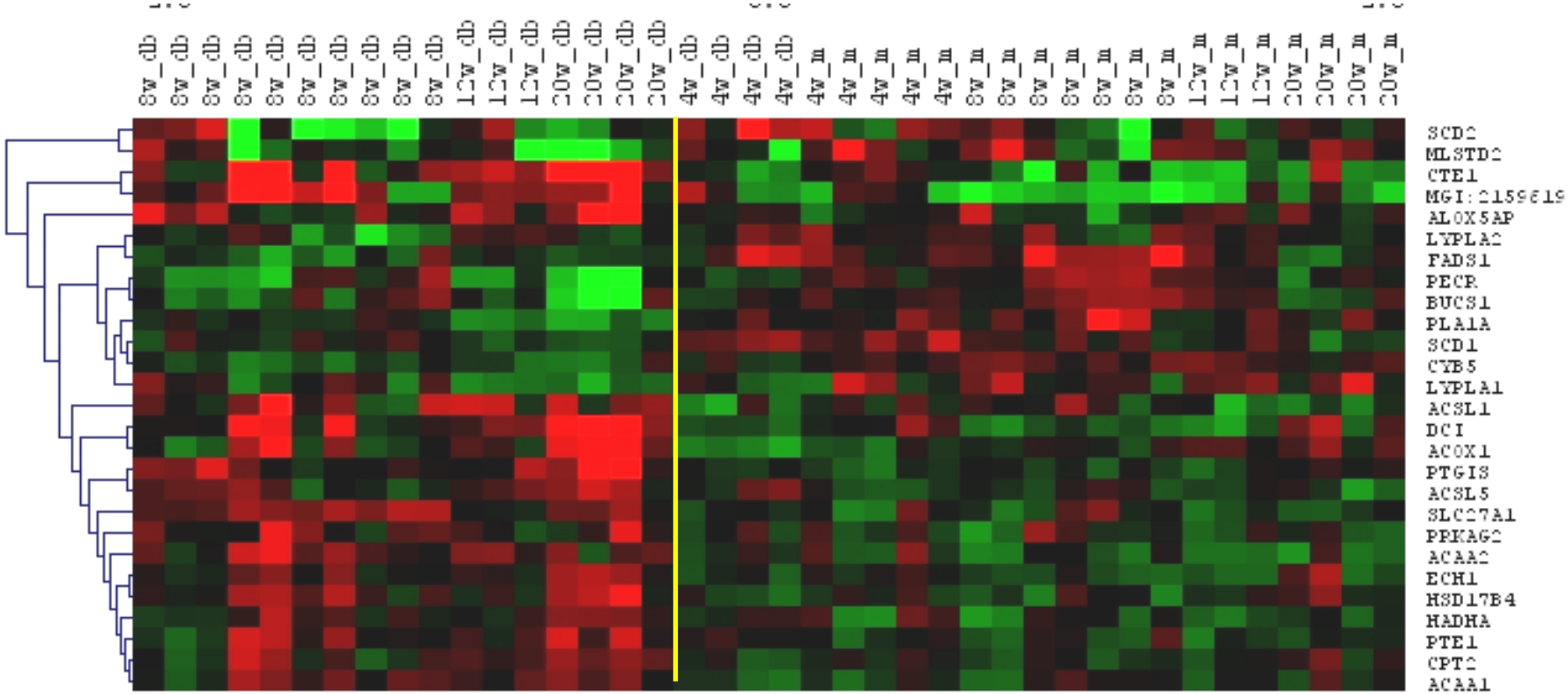
Selected GO categories:

GO:0044255_cellular_lipid_metabolism
GO:0006629_lipid_metabolism
GO:0008610_lipid_biosynthesis
GO:0006694_steroid_biosynthesis
GO:0006986_response_to_unfolded_protein
GO:0016125_sterol_metabolism
GO:0016126_sterol_biosynthesis
GO:0008202_steroid_metabolism
GO:0006631_fatty_acid_metabolism
GO:0006790_sulfur_metabolism

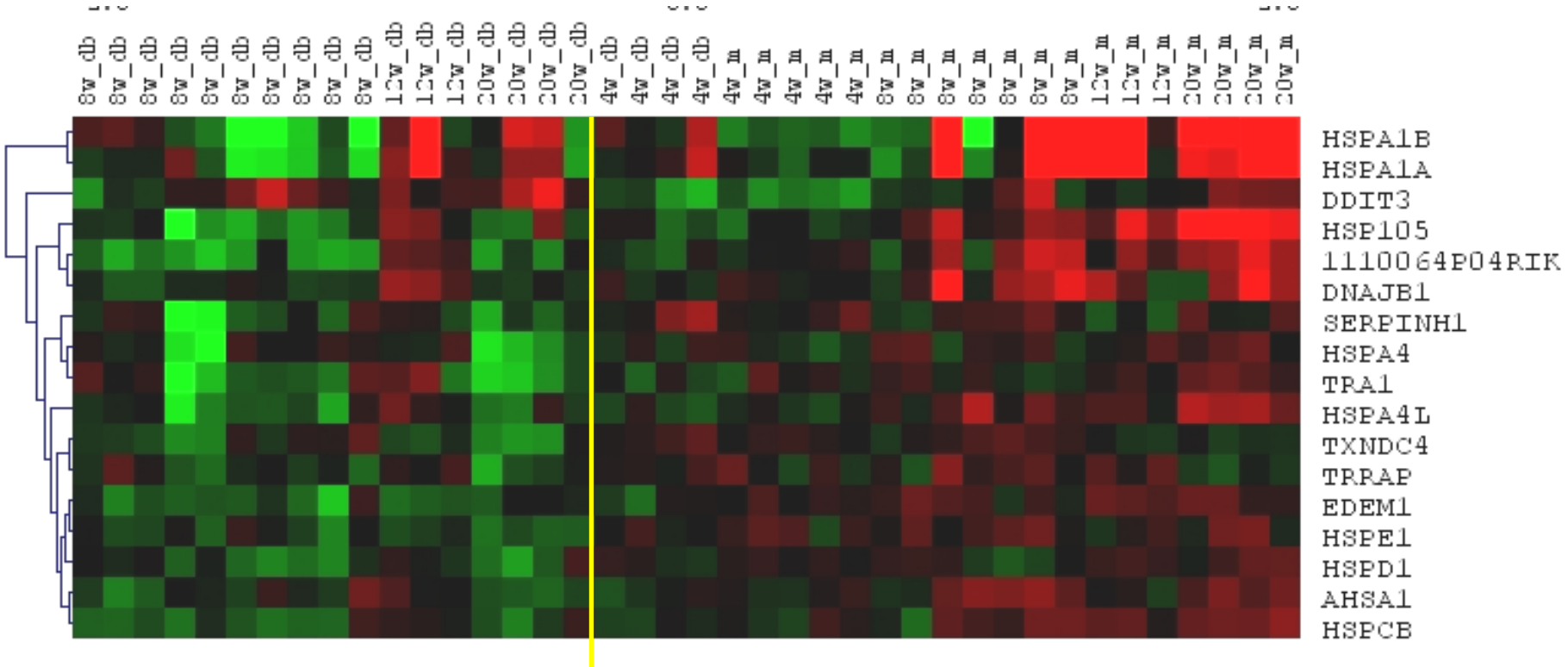
Steroid metabolism & biosynthesis
Sterol metabolism & biosynthesis



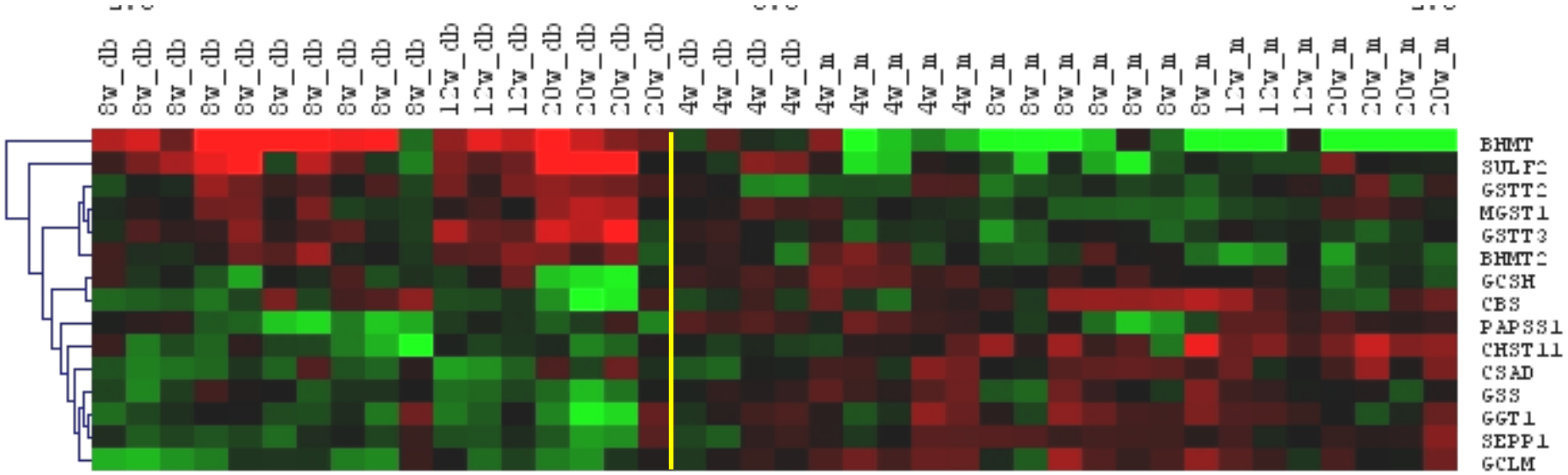
Fatty acid metabolism



Response to unfold protein



Sulfur metabolism



Reduced Podocyte Number in Glomerular Diseases

MOUNT SINAI SCHOOL OF MEDICINE

- Type 2 Diabetes Mellitus [Pagtalunan et al., JCI, 1997]
- Type 1 Diabetes Mellitus [Steffes et al., Kidney Int, 2001]
- IgA Nephropathy [Lemley et al., Kidney Int, 2002]
- Focal Segmental Glomerulosclerosis

- Experimental Models
  - Puromycin aminonucleoside model [Kim et al., JASN 2001]
  - TGF-β1 transgenic mice [Schiffer et al., JCI 2001]
  - Cd2ap knockout mice [Schiffer et al., JBC 2004]

  - **T1DM ($Ins2^{Akita}$) and T2DM ($Lepr^{db/db}$) mice [Susztak et al., Diabetes (In Press)]**

MOUNT SINAI SCHOOL OF MEDICINE

Original Article

Glucose-Induced Reactive Oxygen Species Cause Apoptosis of Podocytes and Podocyte Depletion at the Onset of Diabetic Nephropathy

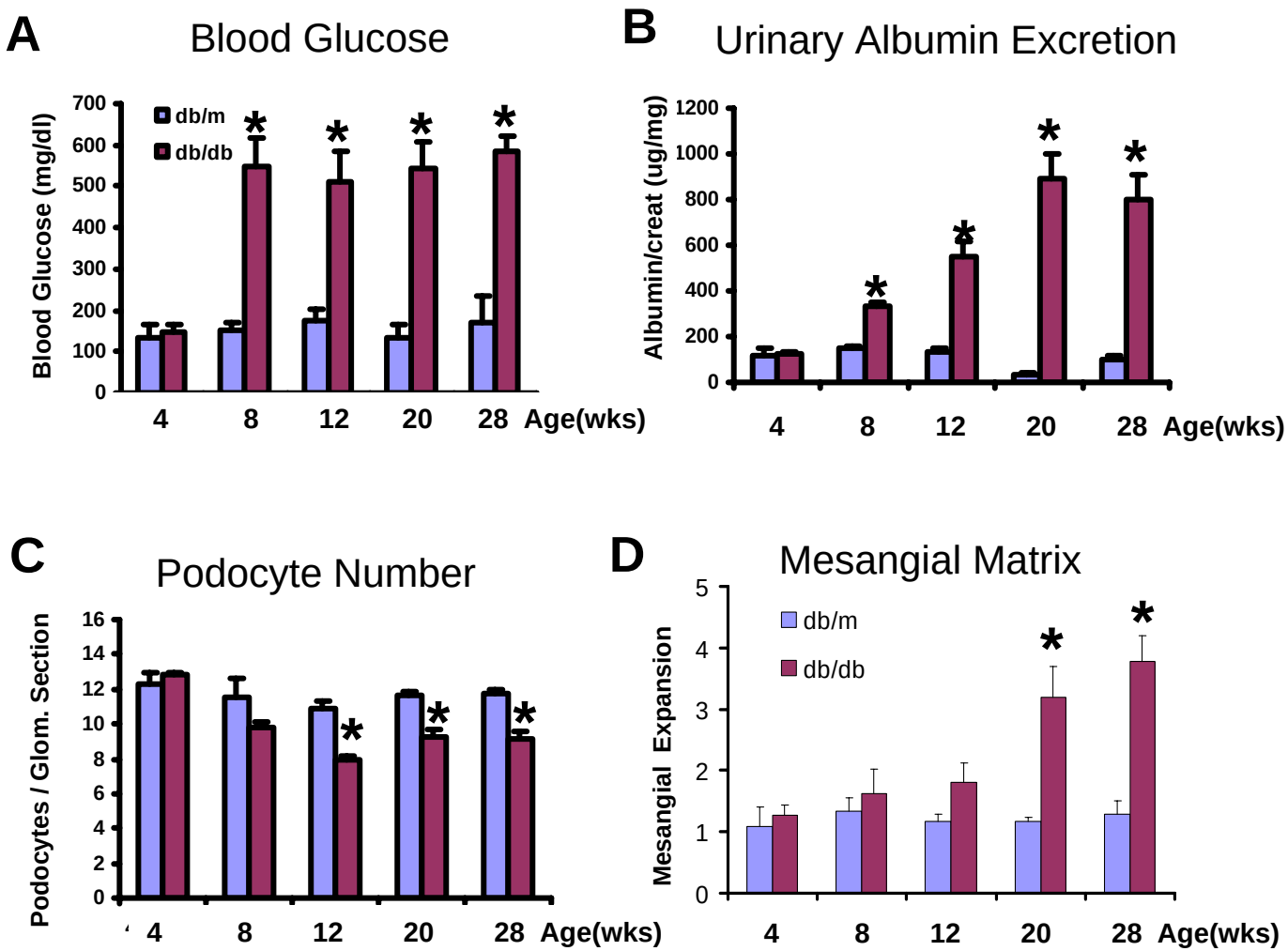
Katalin Susztak,[1] Amanda C. Raff,[1] Mario Schiffer,[1] and Erwin P. Böttinger[2]

DIABETES,
VOL. 55, January 2006

Katalin Susztak

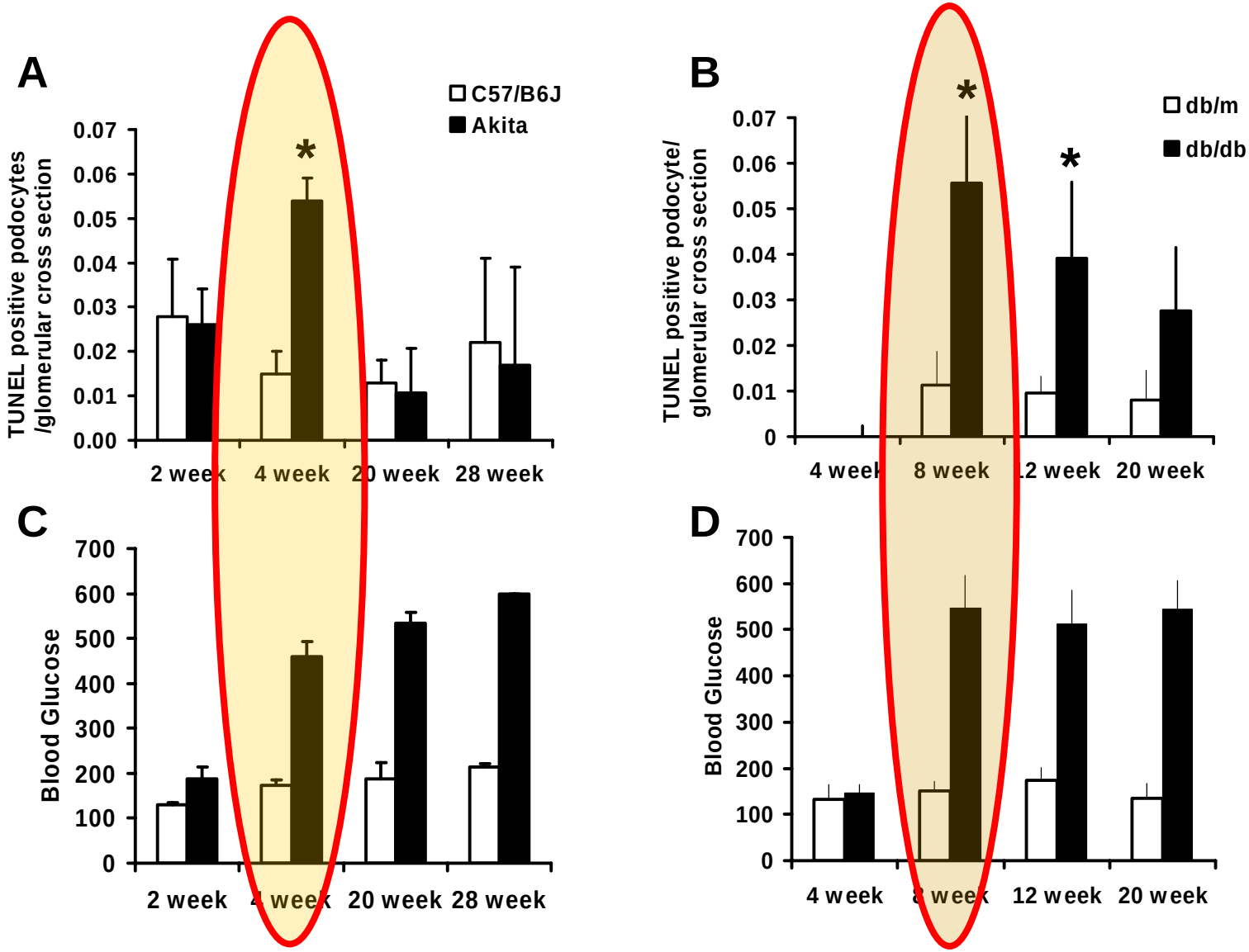
Natural History of Kidney Disease in T2 Diabetic Lepr$^{db/db}$ (db/db) Mice

MOUNT SINAI SCHOOL OF MEDICINE



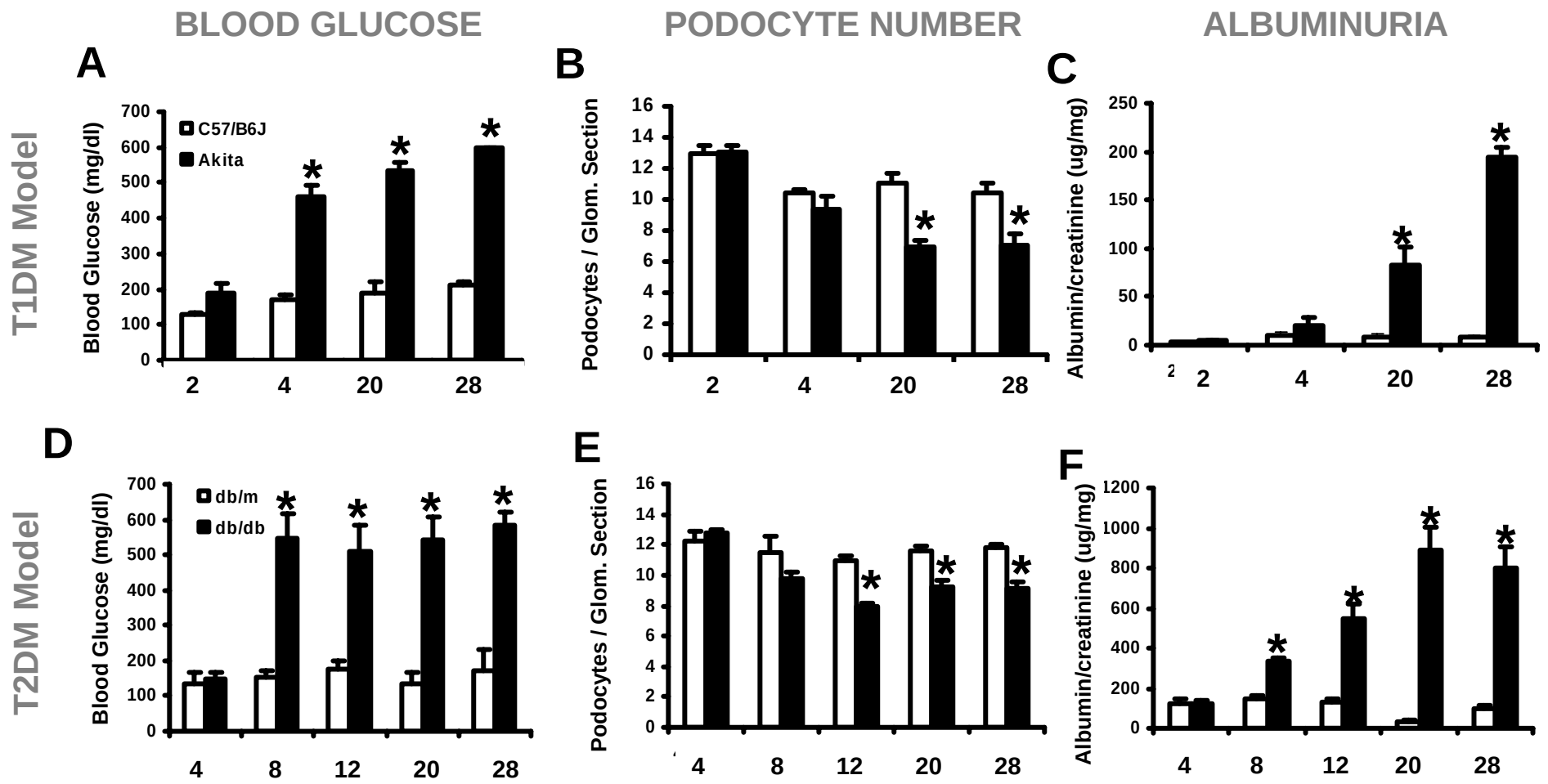
Podocyte Apoptosis Coincides With Onset o Hyperglycemia in Murine T1DM and T2DM

MOUNT SINAI SCHOOL OF MEDICINE

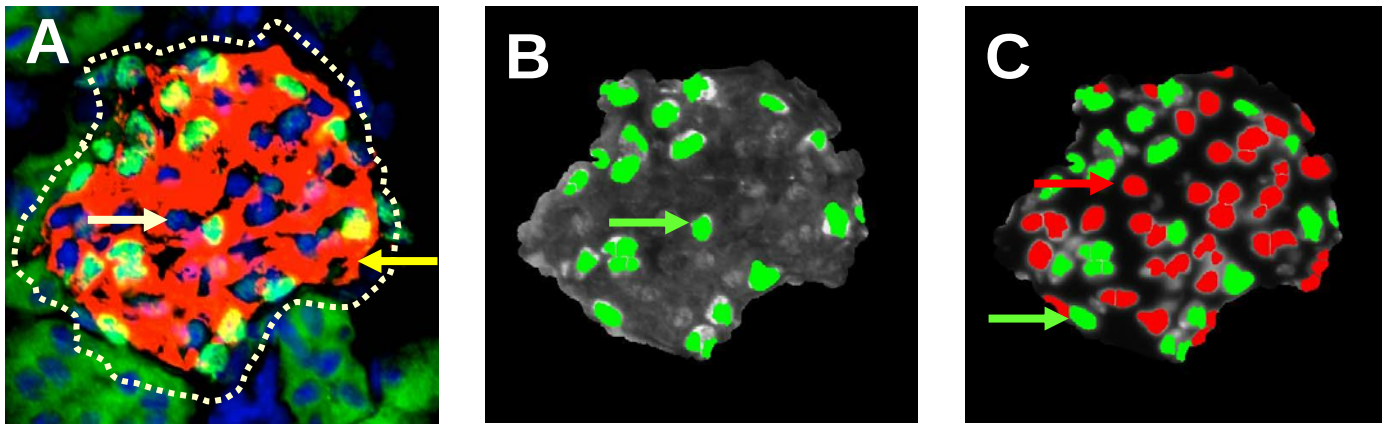


Diabetes, Podocyte Number, and Albuminuria in Murine T1DM and T2DM

MOUNT SINAI SCHOOL OF MEDICINE



Glomerular Cell Count Method using Digital Image Analysis



MetaMorph Digital Image Analysis Software
Nuclei Count Application Module

Thanks to

Advisors
NIH Programs
JDRF
Consortium