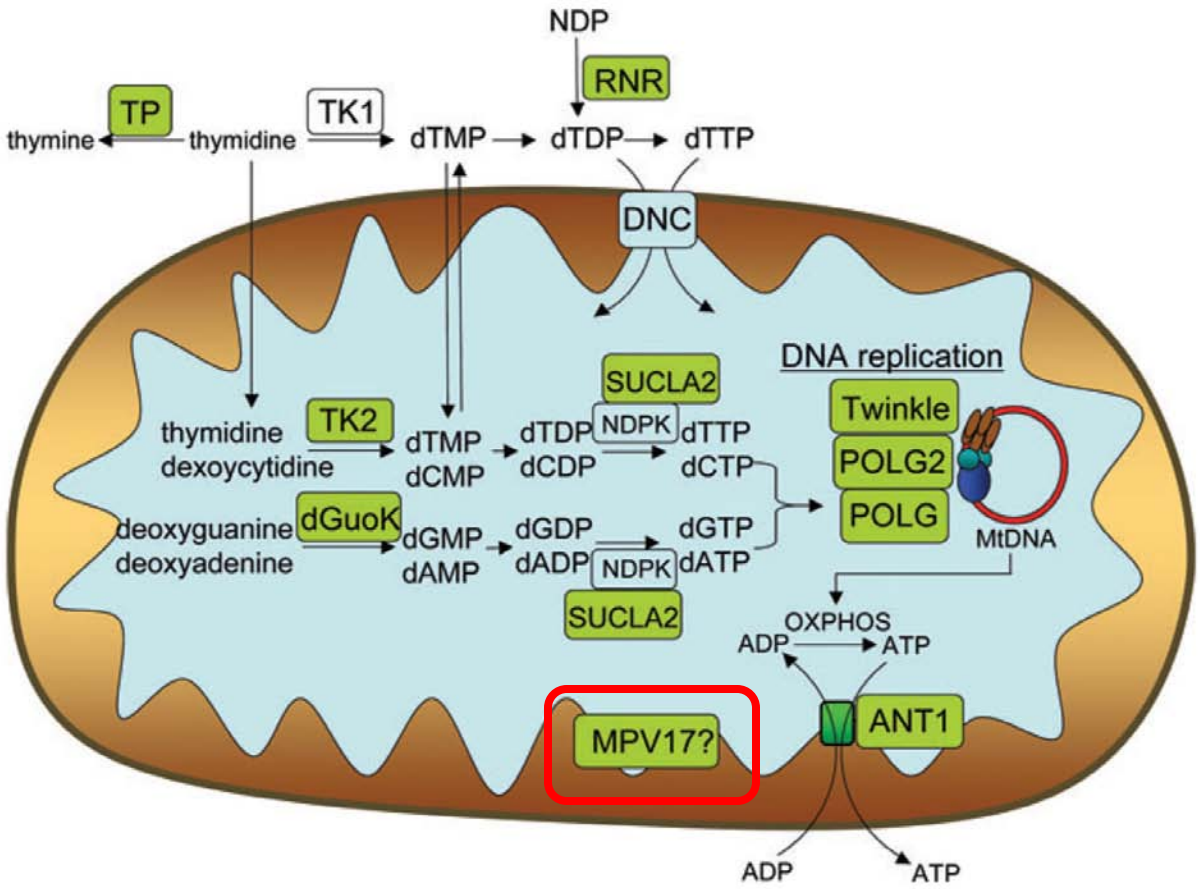
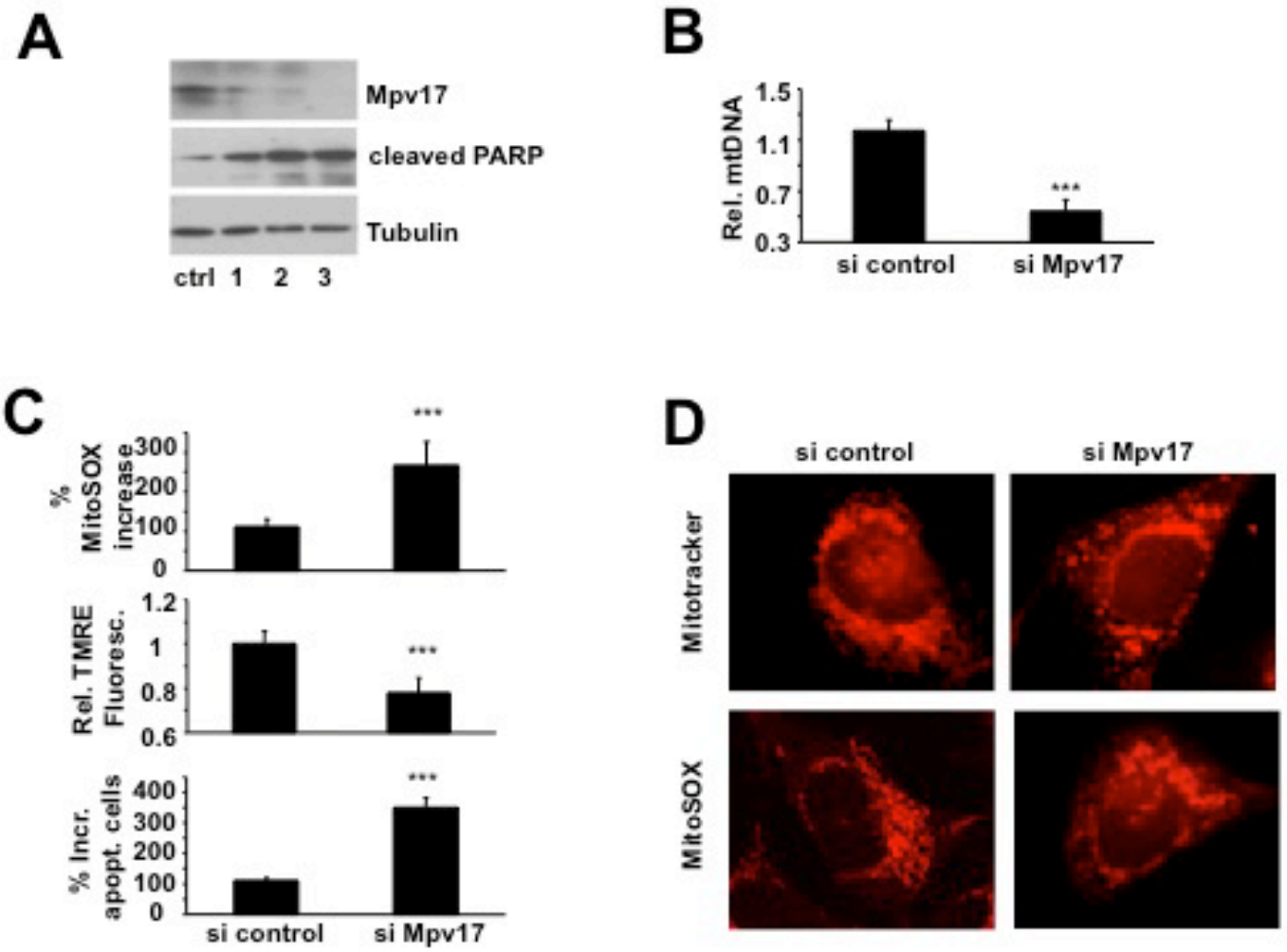
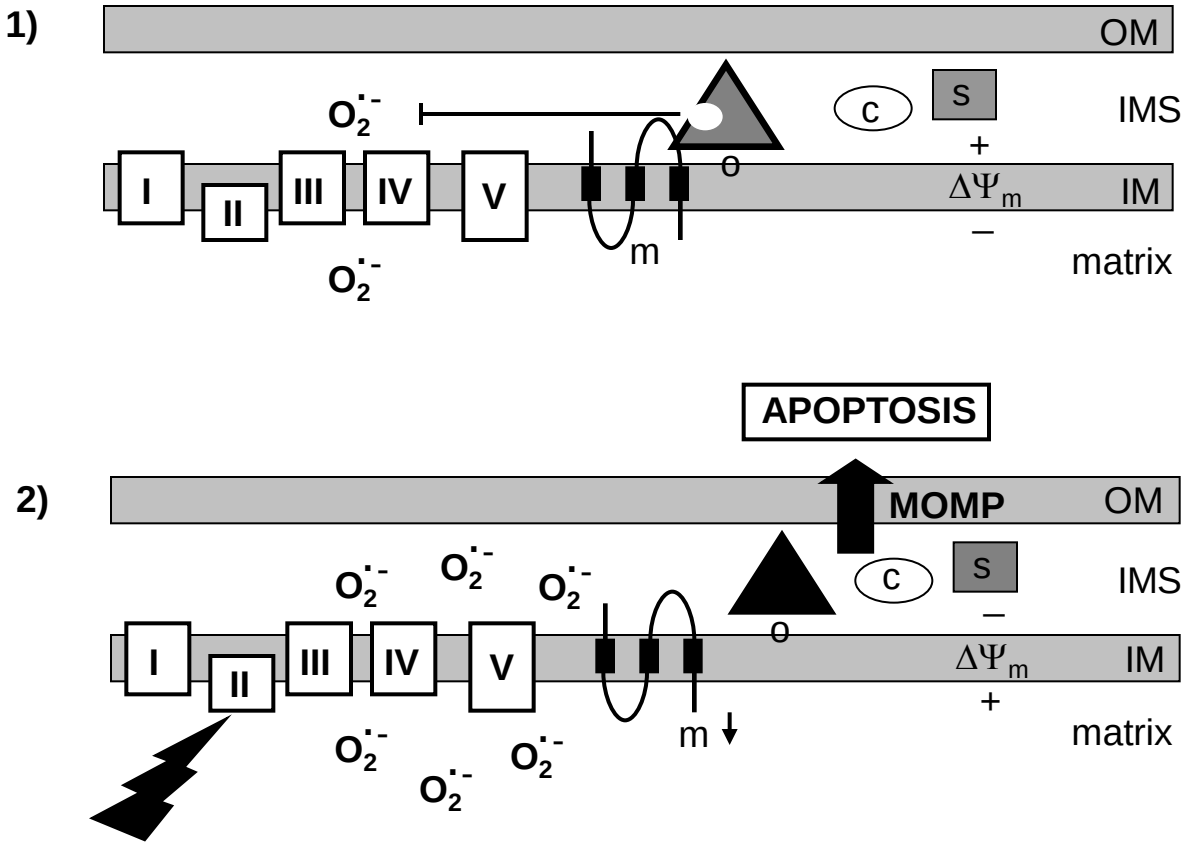
Mitochondrial DNA depletion pathways

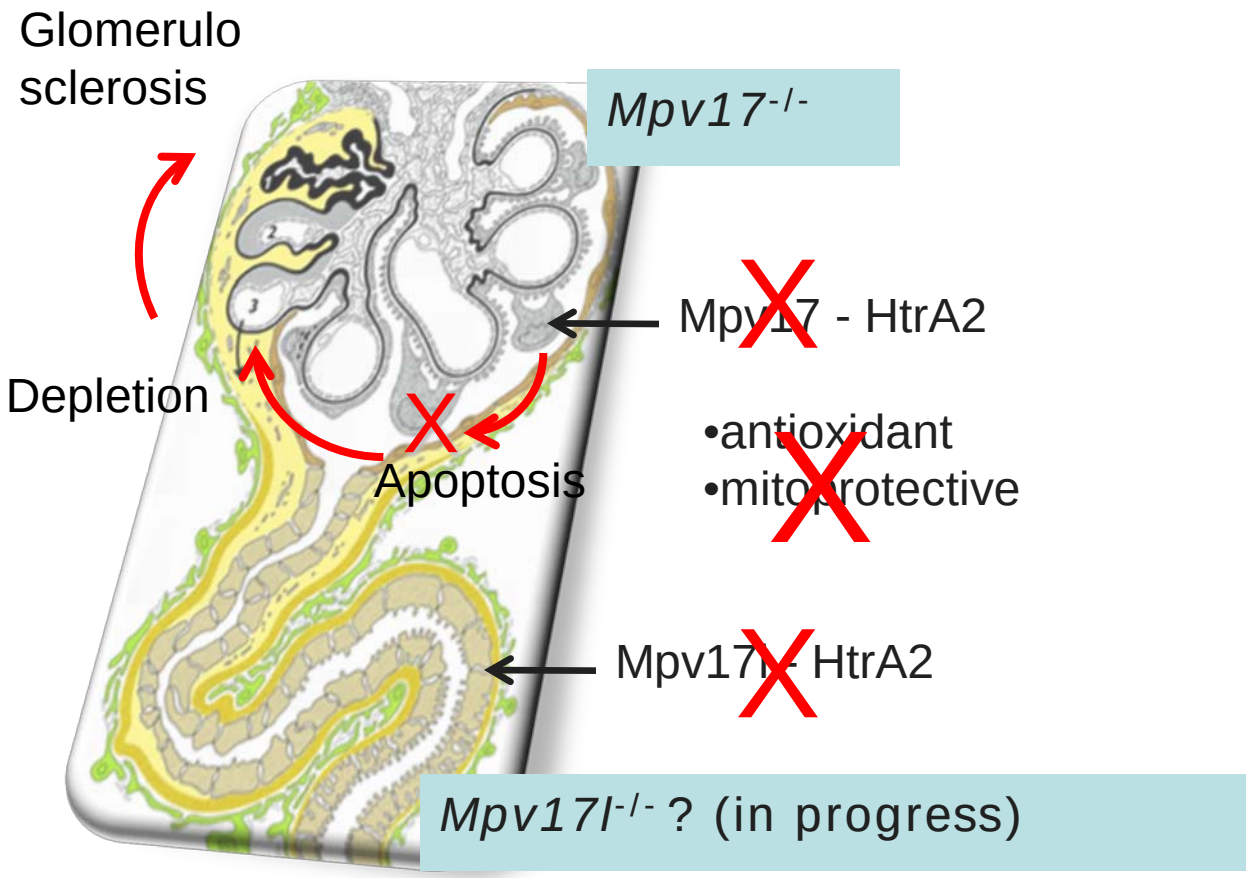



A
Mpv17
cleaved PARP
Tubulin
ctrl 1 2 3
B
Rel. mtDNA
1.5
1.1
0.7
0.3
***
si control
si Mpv17
C
% MitoSOX increase
300
200
100
0
***
Rel. TMRE Fluoresc.
1.2
1
0.8
0.6
***
% Incr. apopt. cells
400
300
200
100
0
***
si control
si Mpv17
D
si control
si Mpv17
Mitotracker
MitoSOX



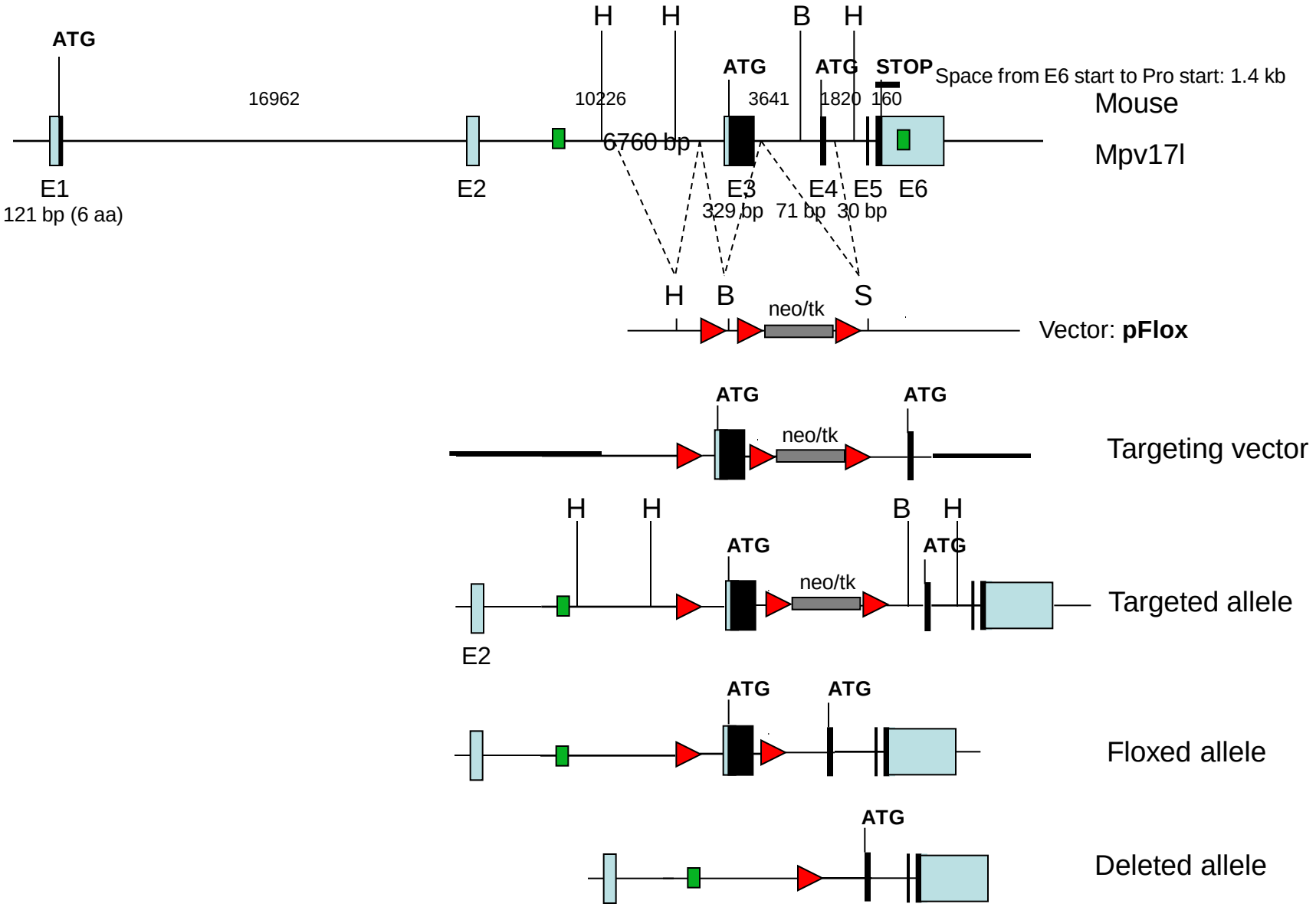
Krick et al., PNAS 2008

Role of mitochondrial Mpv17-family protein / HtrA2 complexes in kidney homeostasis and epithelial injury



HtrA2-/- ? (in progress)

Strategy for Targeting Mpv17-like Gene



Sensitizing Tubular Epithelia To Chronic Injury: Tubular epithelial deletion of Mpv17l-floxed allele

- 1st Attempt (2006 – 2008)
  - Germline
  - 3' arm recombination
- 2nd Attempt (2008 -)
  - 4 chimeras (carry targeted allele)
  - Agouti pups
  - Only wildtype, but no targeted allele
  - Haploid germ cell toxicity ?
- Diabetes models
  - Low dose STZ
  - $Ins2^{Akita}$ FVB/N
- Phenotyping

Protecting Podocytes and Tubular Epithelial From Injury: Mpv17-like and Mpv17 Inducible Transgenic Mice

- System
  - Tet-O-Mpv17l: 3 founder lines established
  - Pax8-rtTA mice imported [Nature Medicine …..]
  - Inducible tubular epithelial expression: Tet-O-Mpv17l x Pax8-rtTA
  - Tet-O-Mpv17: transgenic vector completed
  - Nphs2-rtTA

- Diabetes models
  - Low dose STZ
  - $Ins2^{Akita}$ FVB/N

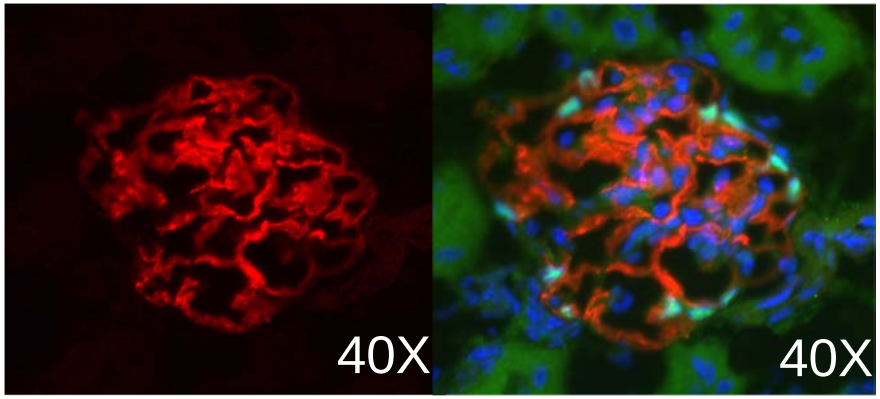
- Phenotyping

Identification of Susceptibility Genes for Progression of Diabetic Glomerulopathy

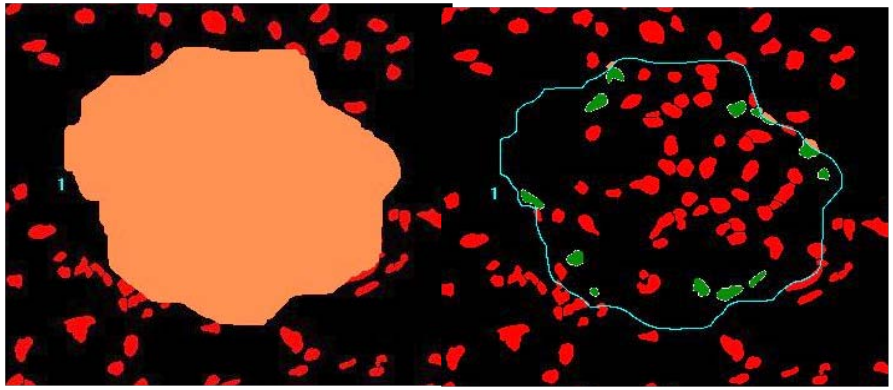
- Phenotype traits analysis in C57BL/6J, DBA/2J, and BXD RI strains
  (coll. Matt Breyer, Eli Lilly & Co)
  - Glomerular cell count (DAPI-positive nuclei/glomerular section)
  - Endocapillary cell count (WT1-negative cells/glomerular section)
  - Podocyte cell count (WT1-positive cells/glomerular section)
  - Podocyte cell density (WT1-positive cells/1000 μm^2 glomerular section area)
  - Kidney weight (gram)
  - Glomerular section area (μm^2)
  - Glomerular matrix fraction (Silver methenamine-positive area/glom. section area)
  - Gene expression (microarray)
- Statistical analysis using GeneNetworks.org
  (coll. Rob Williams, Memphis)
- Goals
  - Apply QTL and gene mapping approach to identify loci/gene(s) that mediate susceptibility to podocyte depletion and glomerular matrix expansion

Methods: morphometry

Microscope images: Syn, WT-1,DAPI

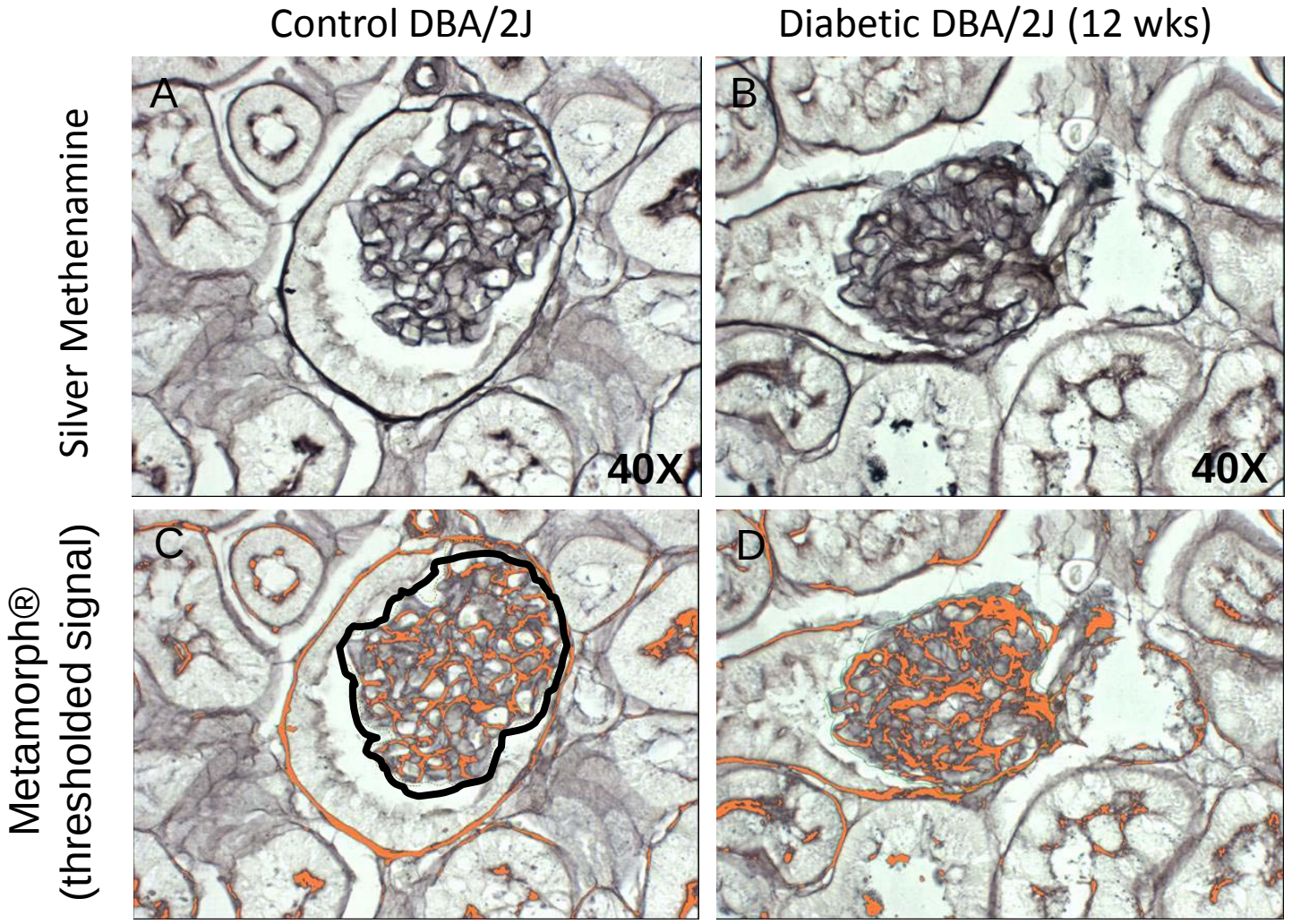


MetaMorph images



- **Podocytes density = podocytes number/glomerular surface area(1,000 μm^2)**
- **50 glomeruli/mouse.**

Quantitative Glomerular Matrix Analysis



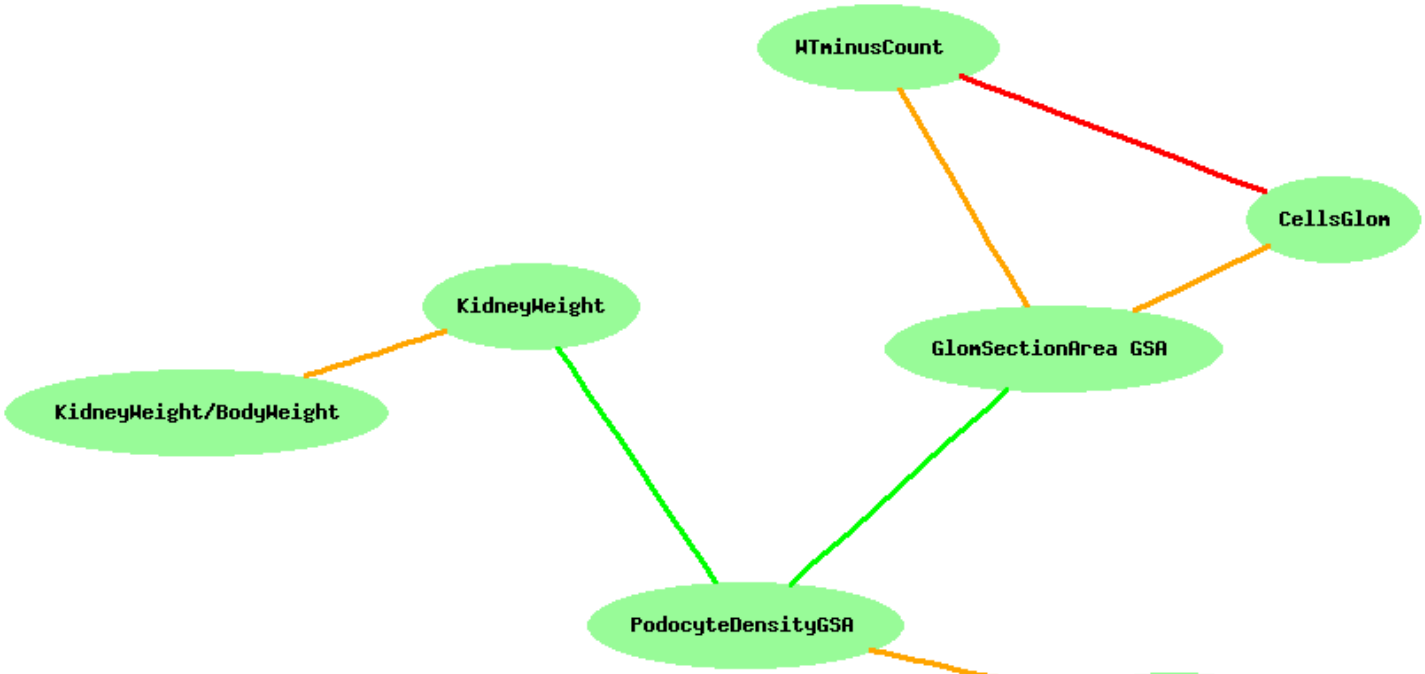
Glomerular Traits Analysis

	(CellsGlom)	(KidneyWeight/BodyWeight)	(PodocyteCount)	(KidneyWeight)	(GlomSectionArea GSA)	(PodocyteDensityGSA)	(WTminusCount)
(CellsGlom)	1.000	-0.203 31	0.331 31	-0.191 31	0.545 31	-0.102 31	0.952 31
(KidneyWeight/BodyWeight)	-0.153 31	1.000	-0.299 31	0.670 31	0.295 31	-0.431 31	-0.109 31
(PodocyteCount)	0.440 31	-0.285 31	1.000	-0.399 31	0.178 31	0.677 31	0.125 31
(KidneyWeight)	-0.230 31	0.553 31	-0.344 31	1.000	0.329 31	-0.544 31	-0.125 31
(GlomSectionArea GSA)	0.559 31	0.291 31	0.131 31	0.230 31	1.000	-0.569 31	0.550 31
(PodocyteDensityGSA)	-0.079 31	-0.407 31	0.663 31	-0.424 31	-0.628 31	1.000	-0.285 31
(WTminusCount)	0.969 31	-0.094 31	0.220 31	-0.170 31	0.597 31	-0.288 31	1.000

Spearman's R

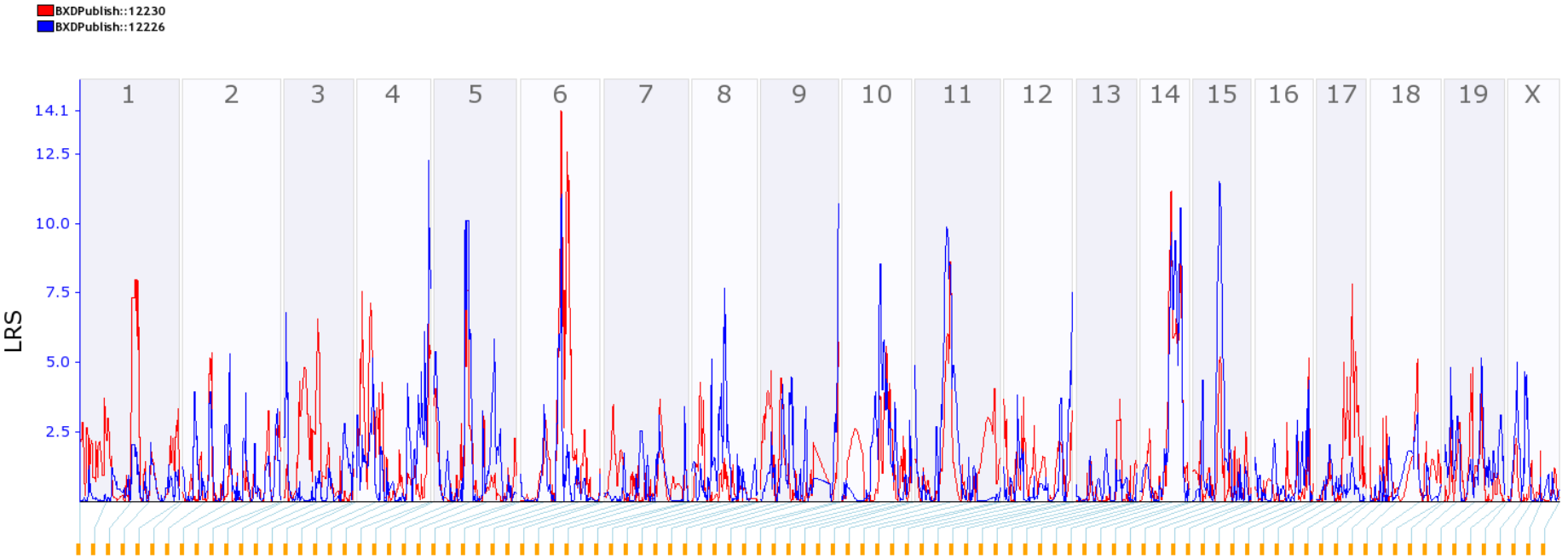
Pearson's R

Glomerular Traits Correlations

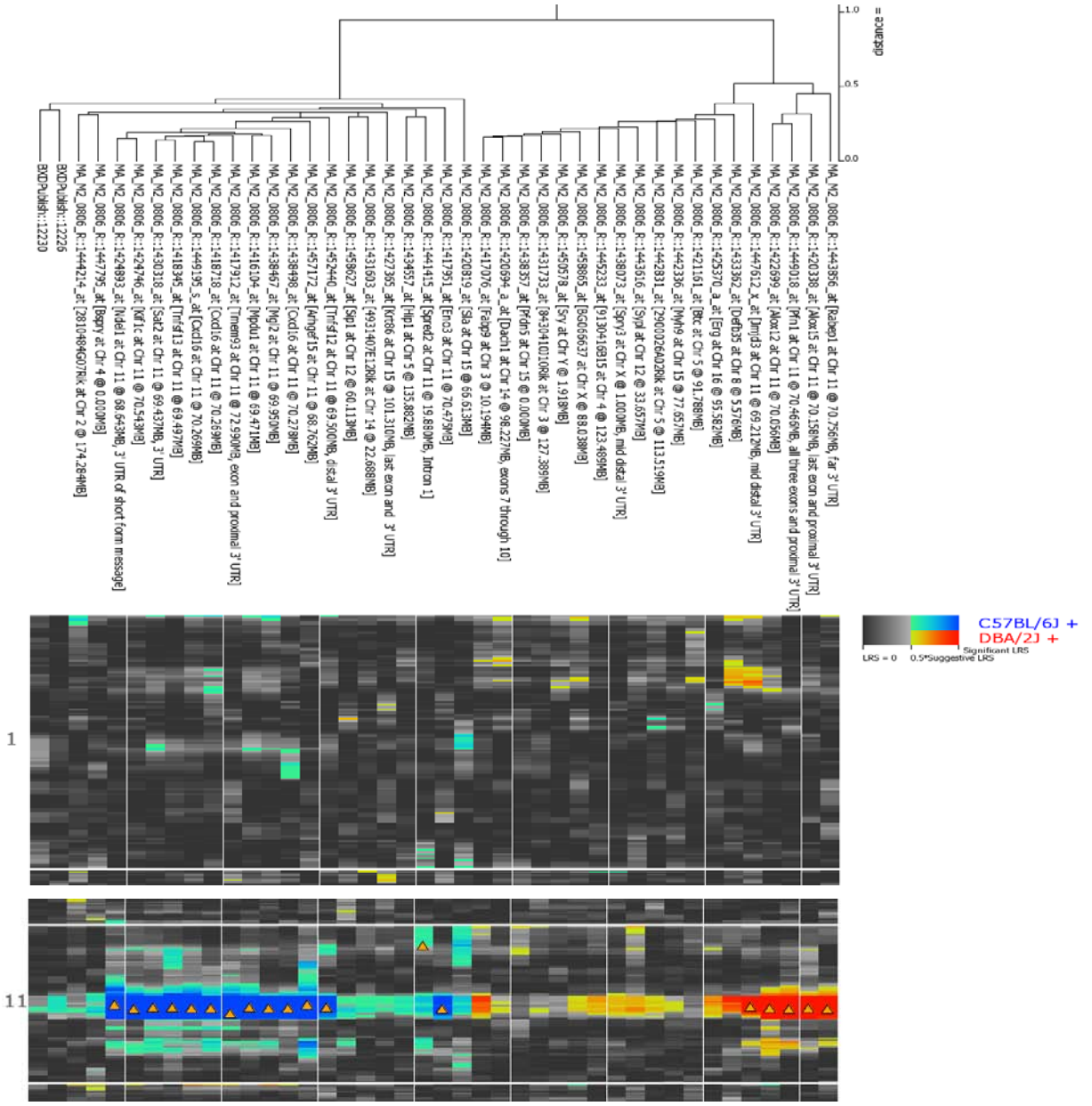


Line Type 1:	-1	to	-0.7	
Line Type 2:	-0.7	to	-0.5	
Line Type 3:	-0.5	to	0	
Line Type 4:	0	to	0.5	
Line Type 5:	0.5	to	0.7	
Line Type 6:	0.7	to	1	

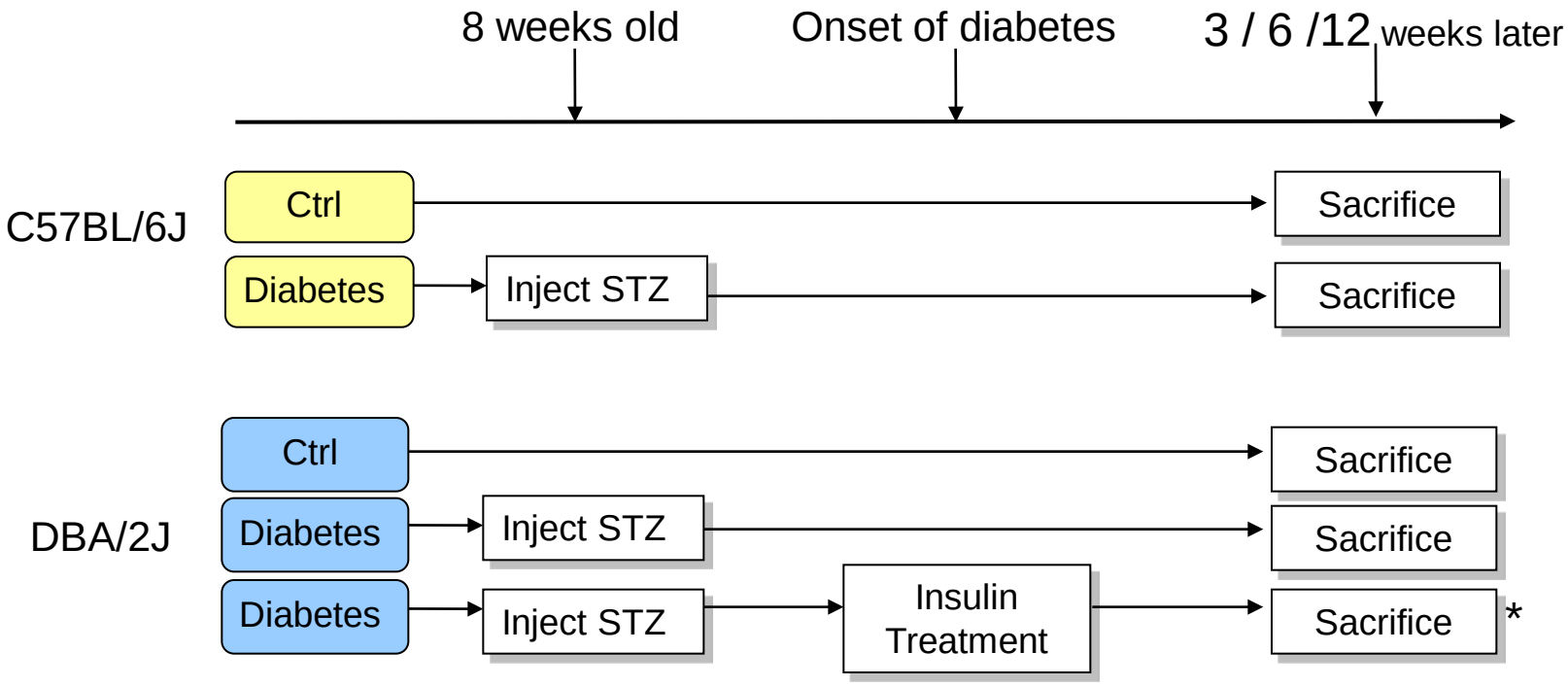
Co-mapping of Podocyte Count and Density Traits to Chr 6, 11, 14



Heatmap of Expression Traits Correlated With Podocyte Traits



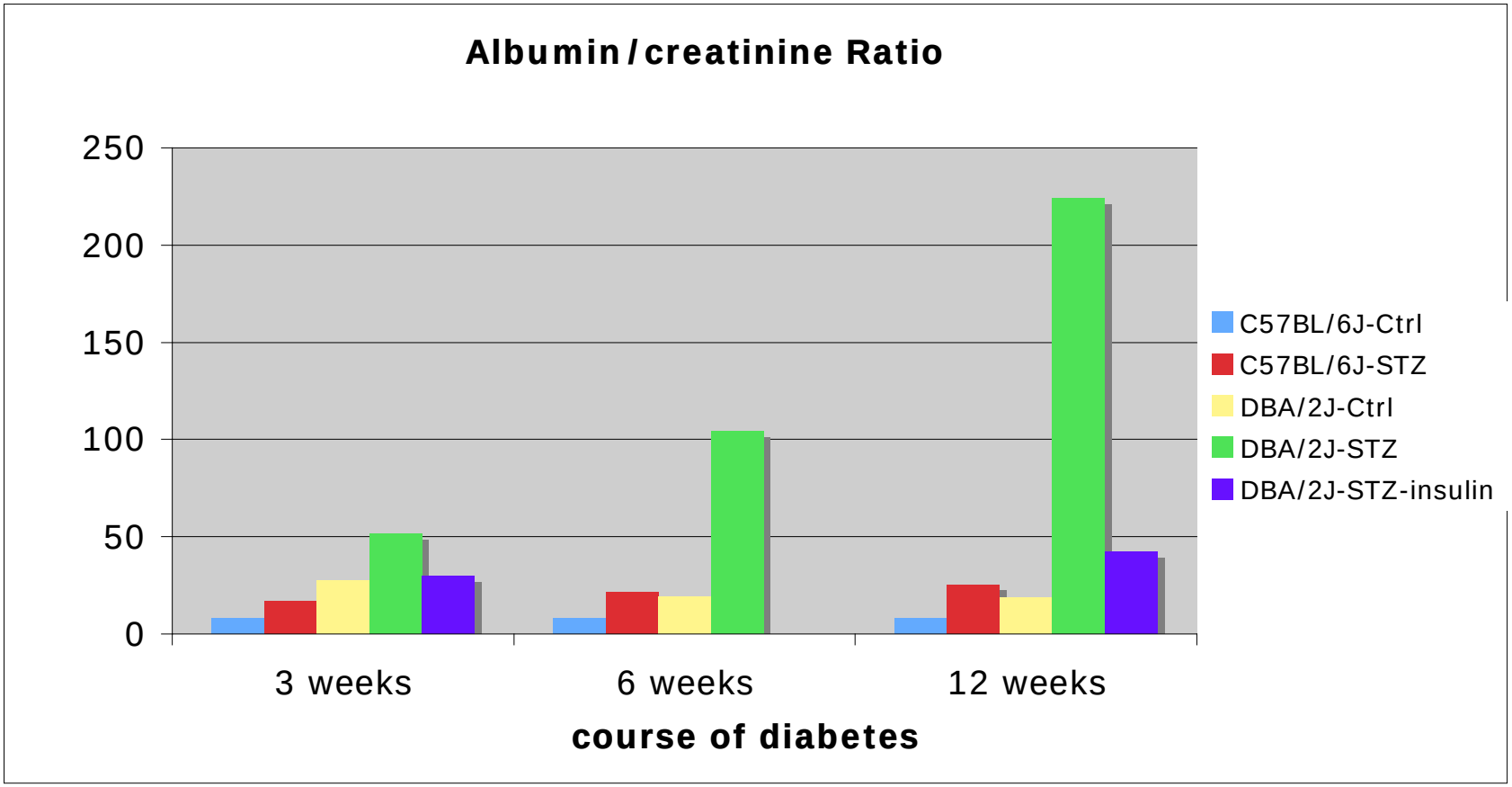
Experimental Design

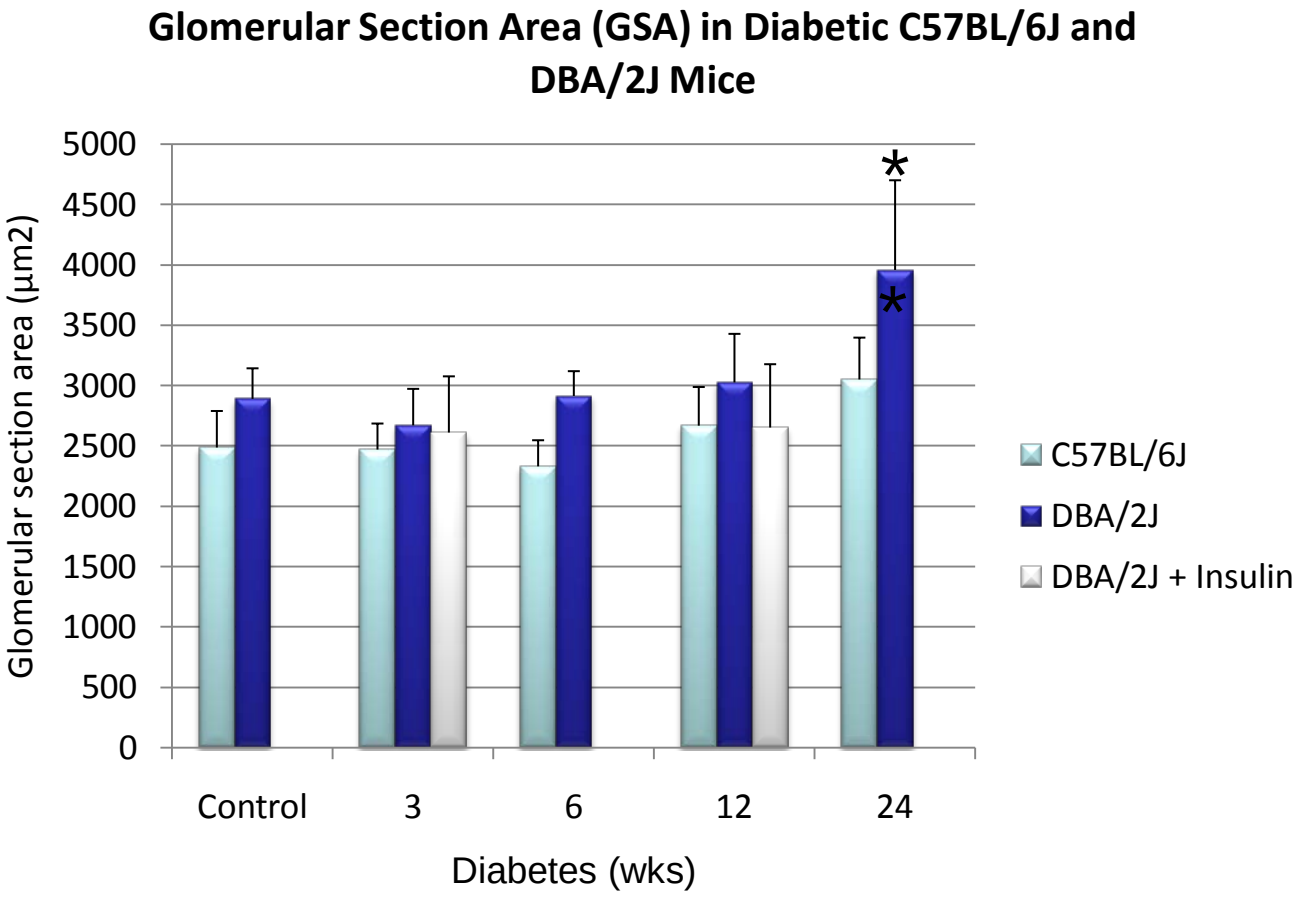


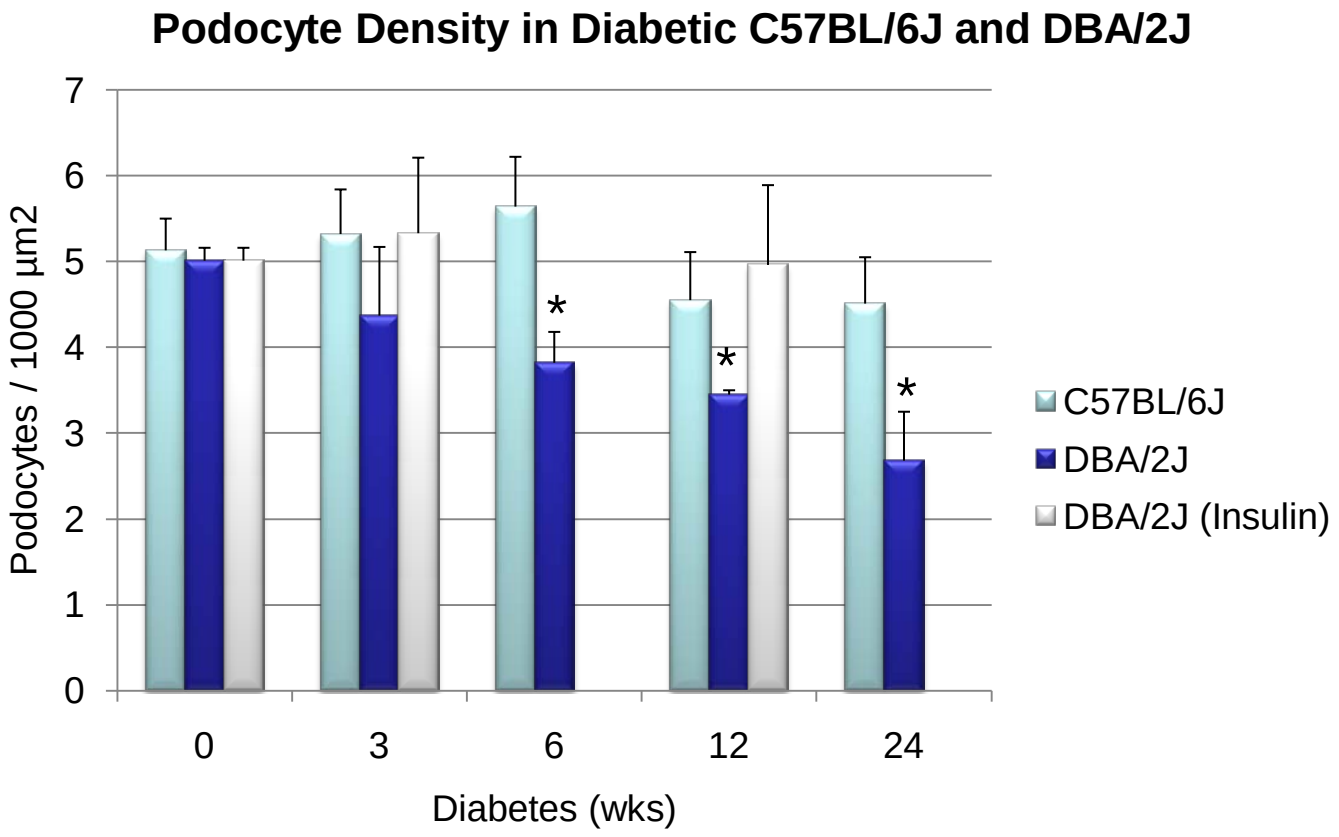
- For each time point, n=3-5 mice/group.

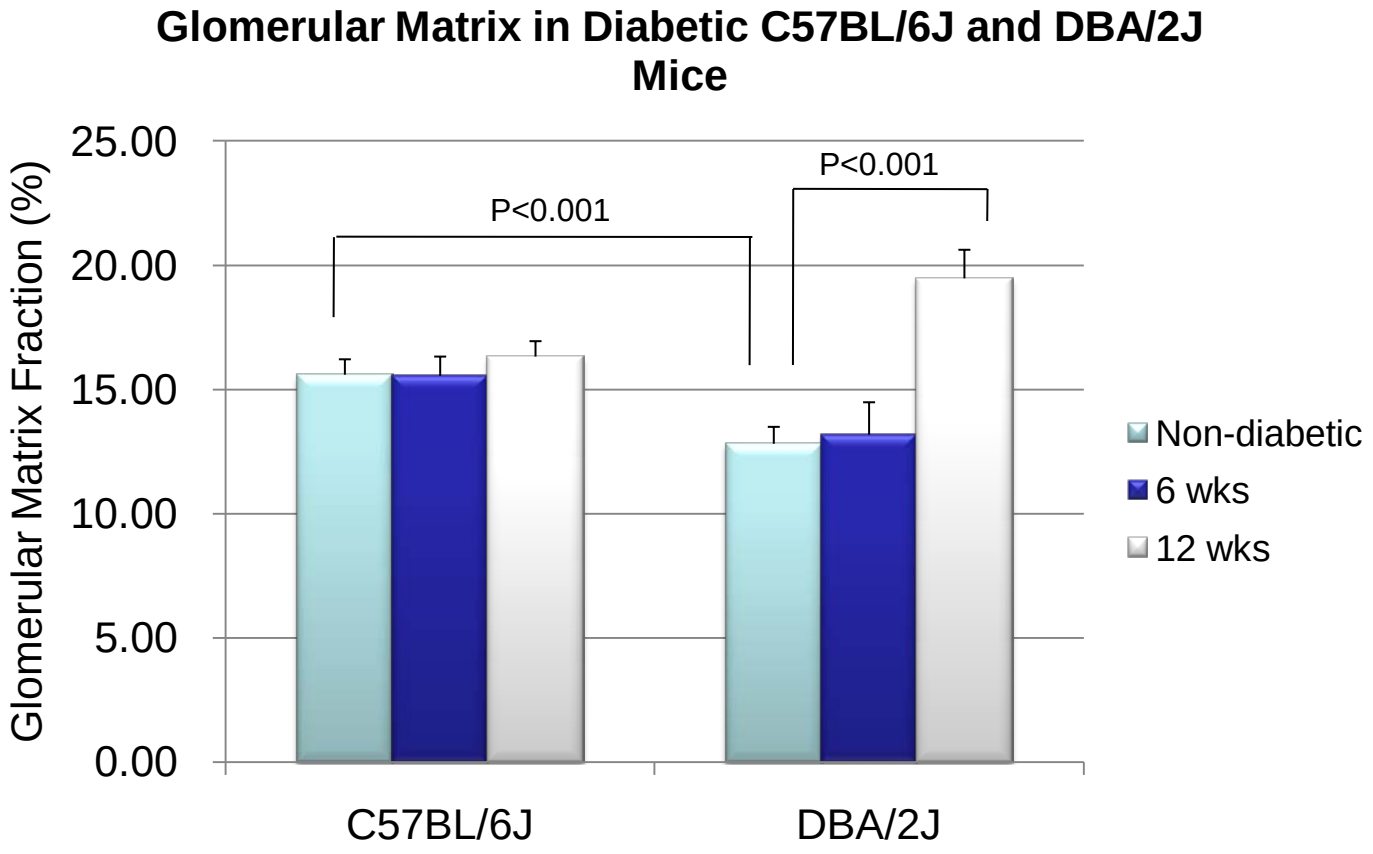
* sacrifice insulin-treated mice only at 3/12 weeks.

Results: 24 hours Proteinuria

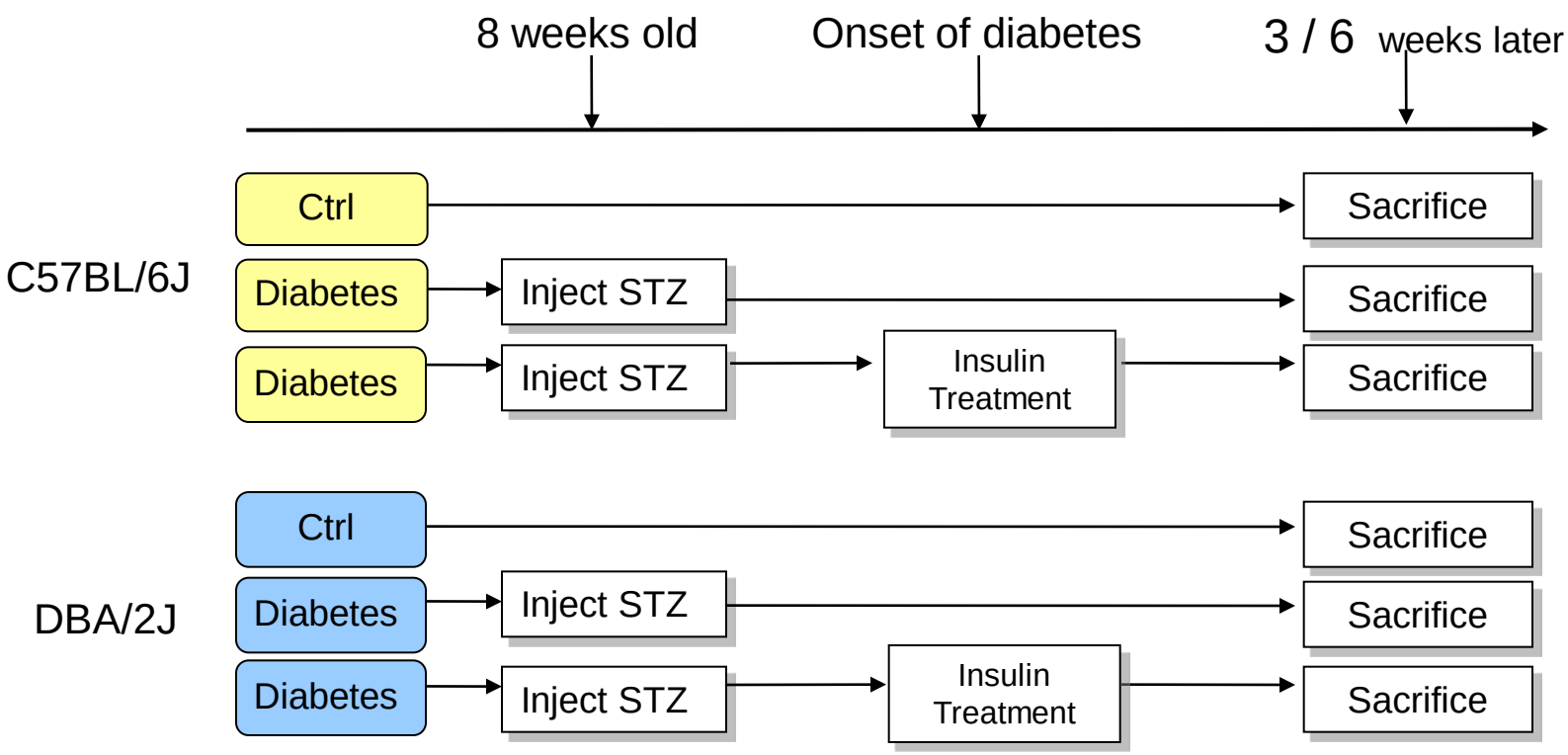



Glomerular Section Area (GSA) in Diabetic C57BL/6J and DBA/2J Mice
Glomerular section area (µm2)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
*
*
Control
3
6
12
24
Diabetes (wks)
C57BL/6J
DBA/2J
DBA/2J + Insulin

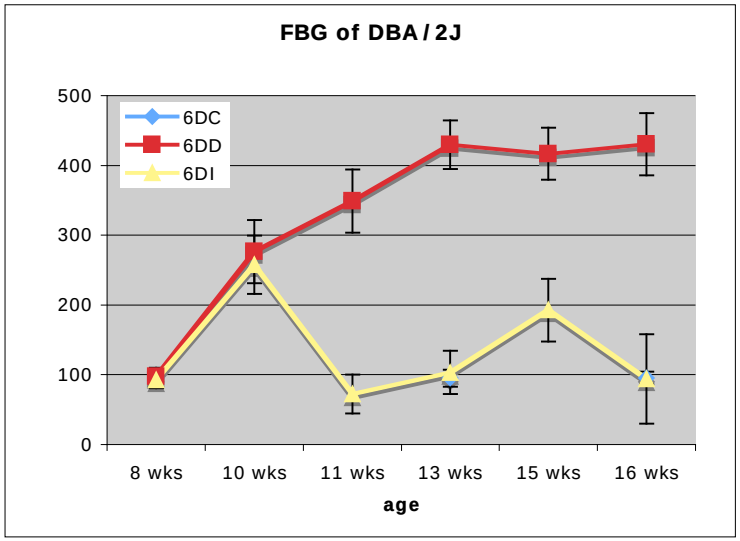

Podocyte Density in Diabetic C57BL/6J and DBA/2J
Podocytes / 1000 µm2
0
1
2
3
4
5
6
7
0
3
6
12
24
Diabetes (wks)
*
*
*
C57BL/6J
DBA/2J
DBA/2J (Insulin)

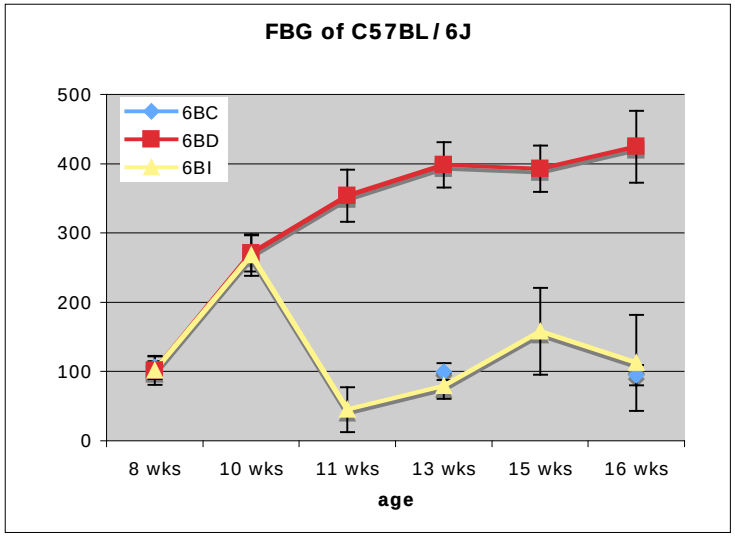

Glomerular Matrix in Diabetic C57BL/6J and DBA/2J Mice
Glomerular Matrix Fraction (%)
25.00
20.00
15.00
10.00
5.00
0.00
P<0.001
P<0.001
C57BL/6J
DBA/2J
Non-diabetic
6 wks
12 wks

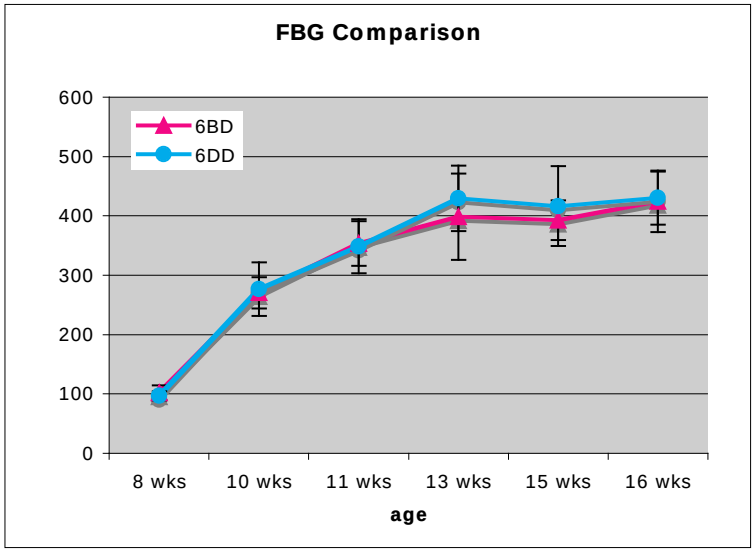
Experimental Design



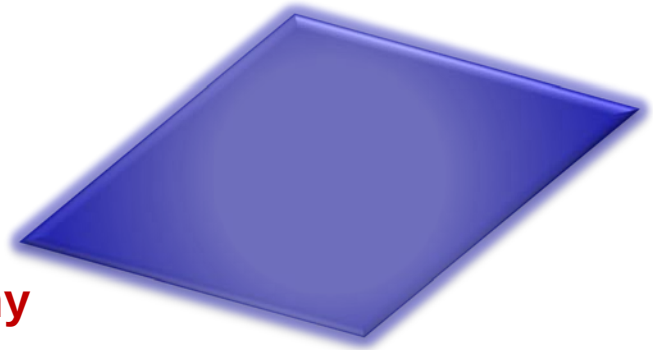
- At each time point, n=8-9 mice/group to do microarray.
- At 6 week time point, sacrifice diabetic D2 and B6 (n=5) for podocytes density calculation.


FBG of DBA / 2J
500
400
300
200
100
0
6DC
6DD
6DI
8 wks
10 wks
11 wks
13 wks
15 wks
16 wks
age


FBG of C57BL / 6J
500
400
300
200
100
0
6BC
6BD
6BI
8 wks
10 wks
11 wks
13 wks
15 wks
16 wks
age


FBG Comparison
600
500
400
300
200
100
0
6BD
6DD
8 wks
10 wks
11 wks
13 wks
15 wks
16 wks
age

Glomerular Transcriptomes in C57BL/6J and DBA/2J Mice



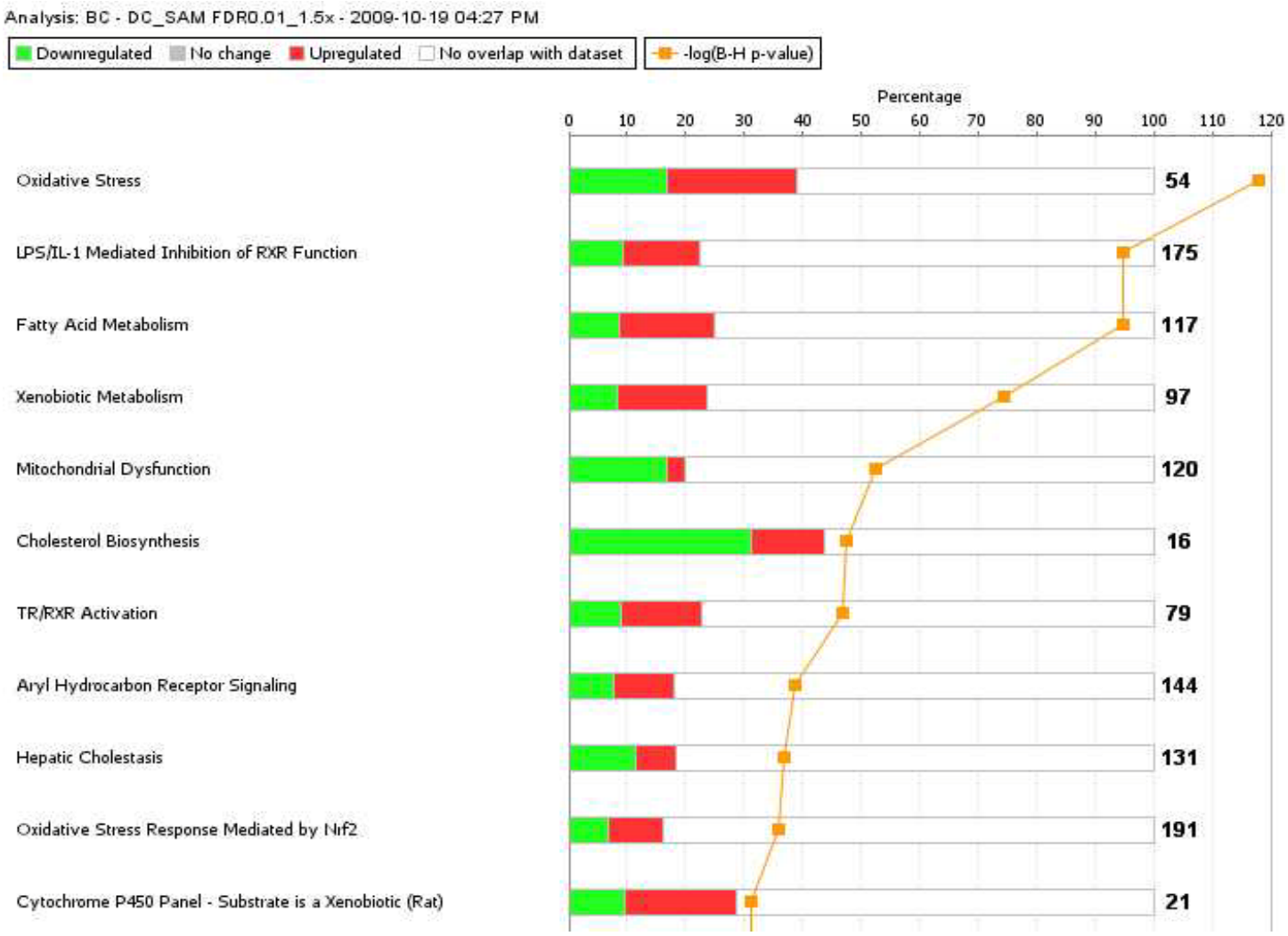
(BC : DC)
Δ = 1731 genes
- genetic strain

(DD : DC)
Δ = 1612 genes
- **↑BG**
- **Glomerulopathy**
- **STZ**

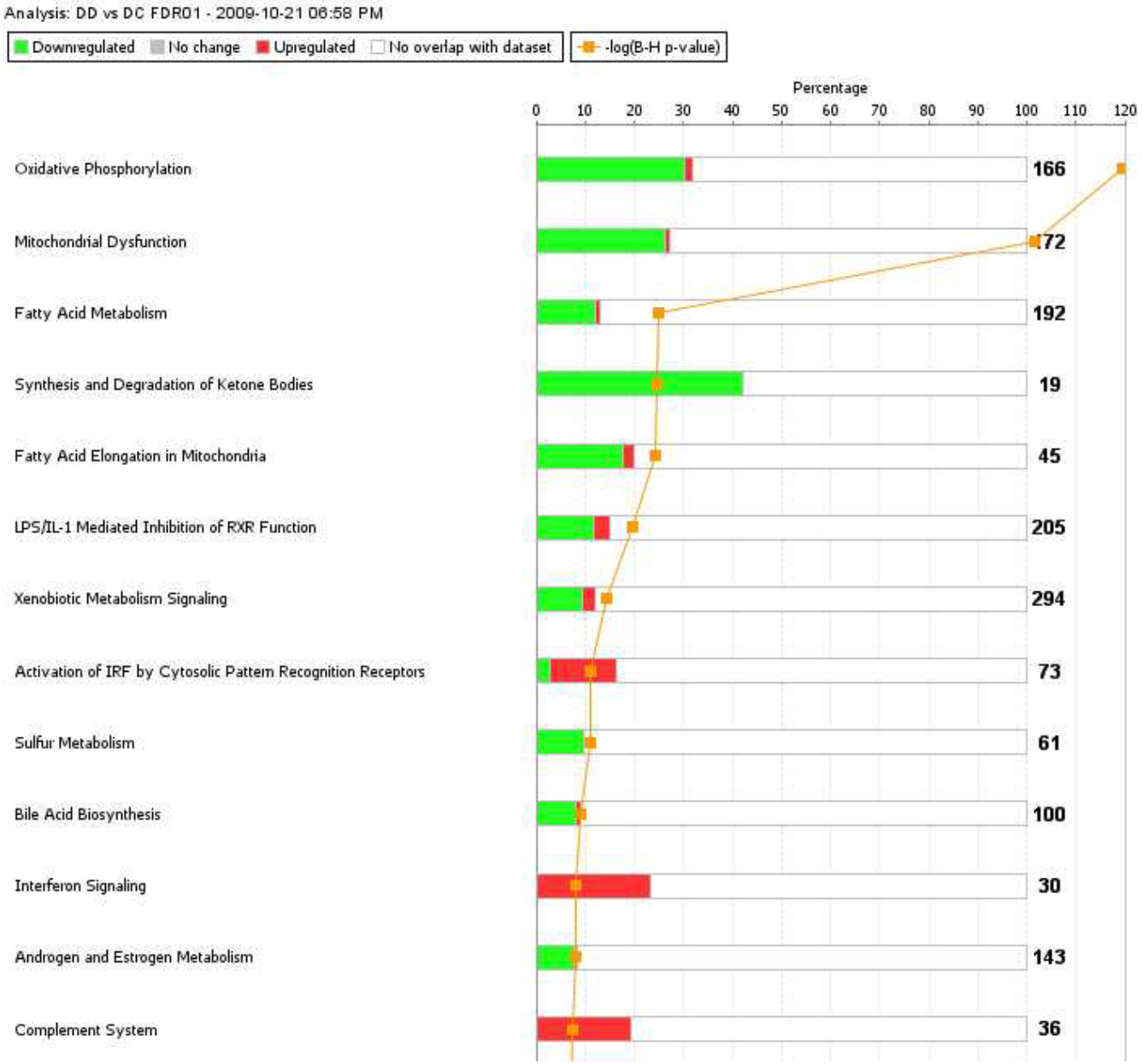
(DDI : DC)
Δ = 43 genes
- **↑Insulin**
- **STZ**

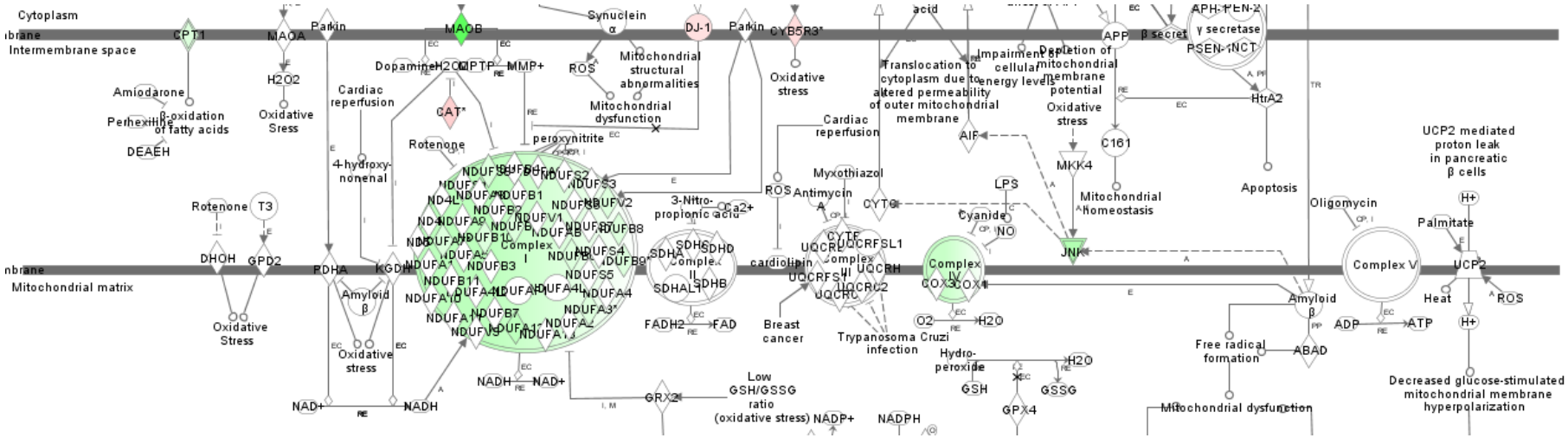
(DDI : DD)
Δ = 304 genes
- **↑BG**
- **Glomerulopathy**

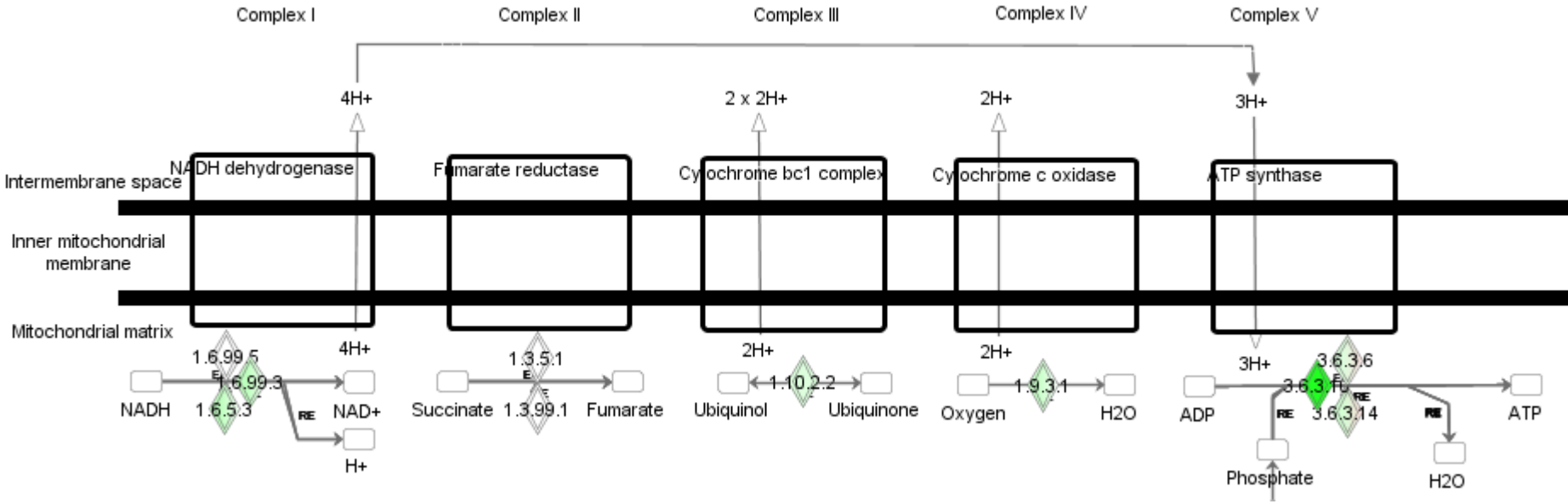
Pathways Affected By Genetic Background In Non-diabetic C57BL/6J And DBA/2J Mice



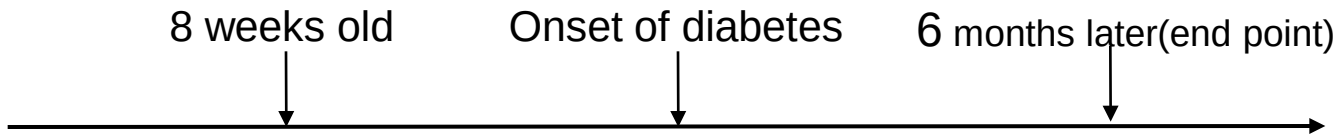
Pathways Affected By Diabetes In C57BL/6J And DBA/2J Mice



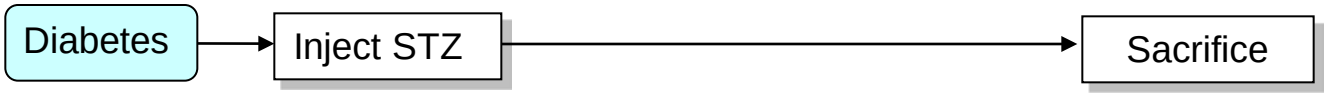

Cytoplasm
Intermembrane space
Mitochondrial matrix
CPT1
MAOA
MAOB
Parkin
Synuclein α
DJ-1
CYB5R3*
APP
β secret
γ secretase
PSEN-1
NCT
APH-1
PEN-2
HtrA2
AIF
C16:1
MKK4
JNK
Apoptosis
Mitochondrial Homeostasis
Amiodarone
Perhexiline
β-oxidation of fatty acids
DEAEH
H2O2
Oxidative Sress
Cardiac reperfusion
4-hydroxy-nonenal
Dopamine
H2O2
DTPT
MPP+
ROS
CAT*
Mitochondrial structural abnormalities
Mitochondrial dysfunction
peroxynitrite
Rotenone
Oxidative stress
Translocation to cytoplasm due to altered permeability of outer mitochondrial membrane
Impairment of cellular energy levels
Depletion of mitochondrial membrane potential
Oxidative stress
Cardiac reperfusion
Myxothiazol
Antimycin A
CYTC
Cyanide
NO
LPS
3-Nitro-propionic acid
Ca2+
cardiolipin
Complex I
Complex II
Complex III
Complex IV
Complex V
UCP2 mediated proton leak in pancreatic β cells
Oligomycin
Palmitate
UCP2
Heat
ROS
ADP
ATP
H+
Rotenone
T3
DHOH
GPD2
PDHA
KGDH
Amyloid β
Oxidative Stress
Oxidative stress
NAD+
NADH
GRX2
Low GSH/GSSG ratio (oxidative stress)
NADP+
NADPH
FADH2
FAD
Breast cancer
Trypanosoma Cruzi infection
O2
H2O
Hydro-peroxide
GSH
GSSG
GPX4
Free radical formation
ABAD
Amyloid β
Mitochondrial dysfunction
Decreased glucose-stimulated mitochondrial membrane hyperpolarization


Complex I
Complex II
Complex III
Complex IV
Complex V
4H+
2 x 2H+
2H+
3H+
NADH dehydrogenase
Fumarate reductase
Cytochrome bc1 complex
Cytochrome c oxidase
ATP synthase
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
1.6.99.5
1.6.99.3
1.6.5.3
NADH
NAD+
H+
1.3.5.1
1.3.99.1
Succinate
Fumarate
1.10.2.2
Ubiquinol
Ubiquinone
1.9.3.1
Oxygen
H2O
3.6.3.6
3.6.3.10
3.6.3.14
ADP
ATP
Phosphate
H2O

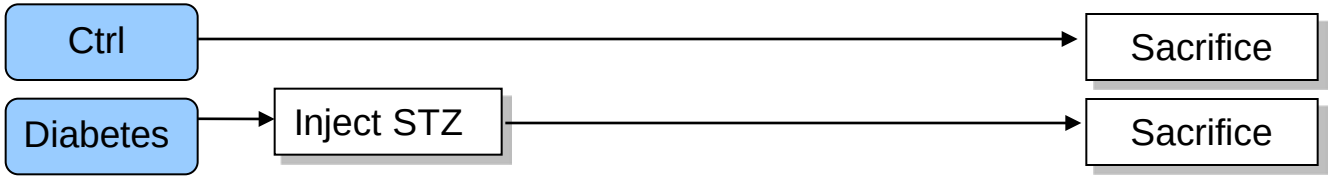
Experimental Design



Two parental strains: D2/B6



each BXD strain



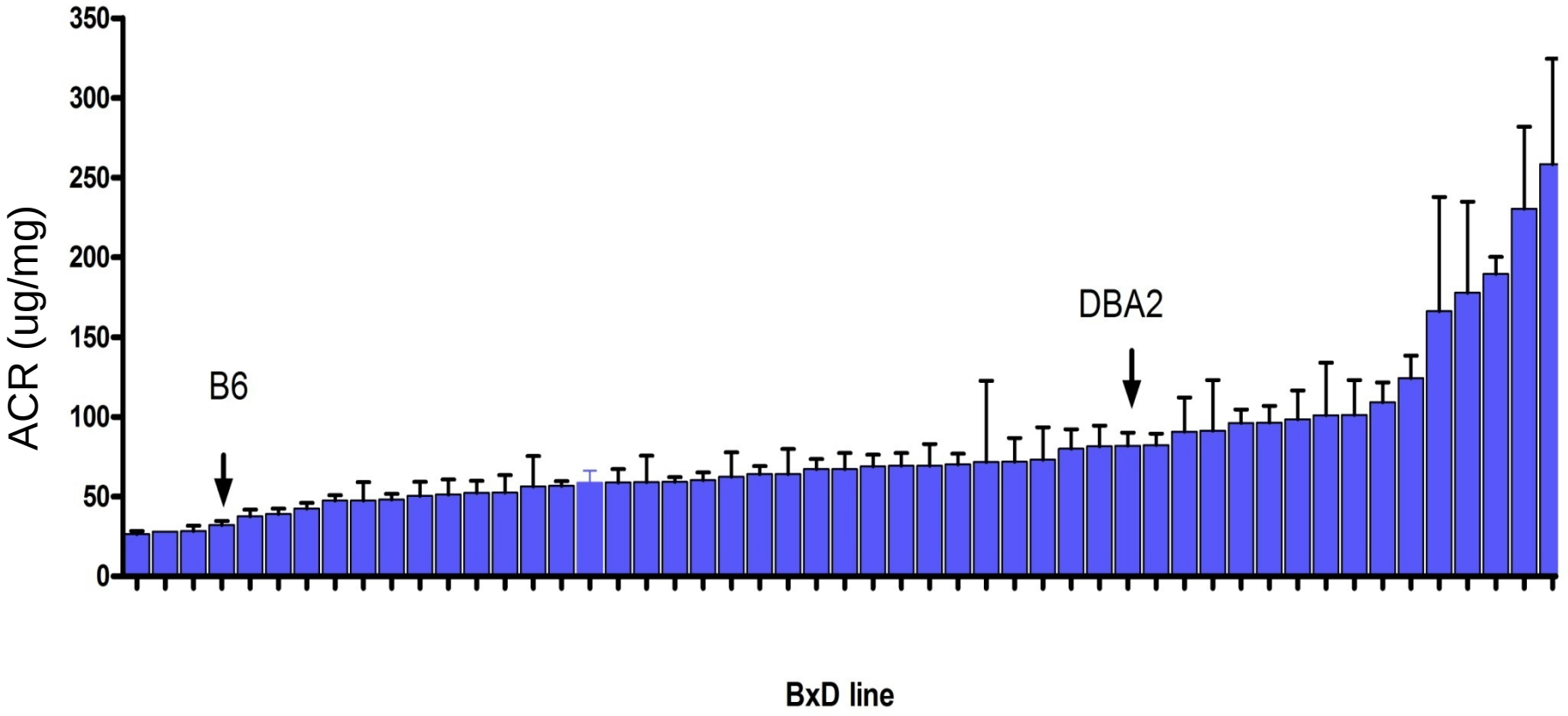
- For each strain, n=4-7 mice/group.
- Phenotypic trait: Podocytes density.

  collaborate with Dr. Mathew Breyer's group.
- Genetic mapping.

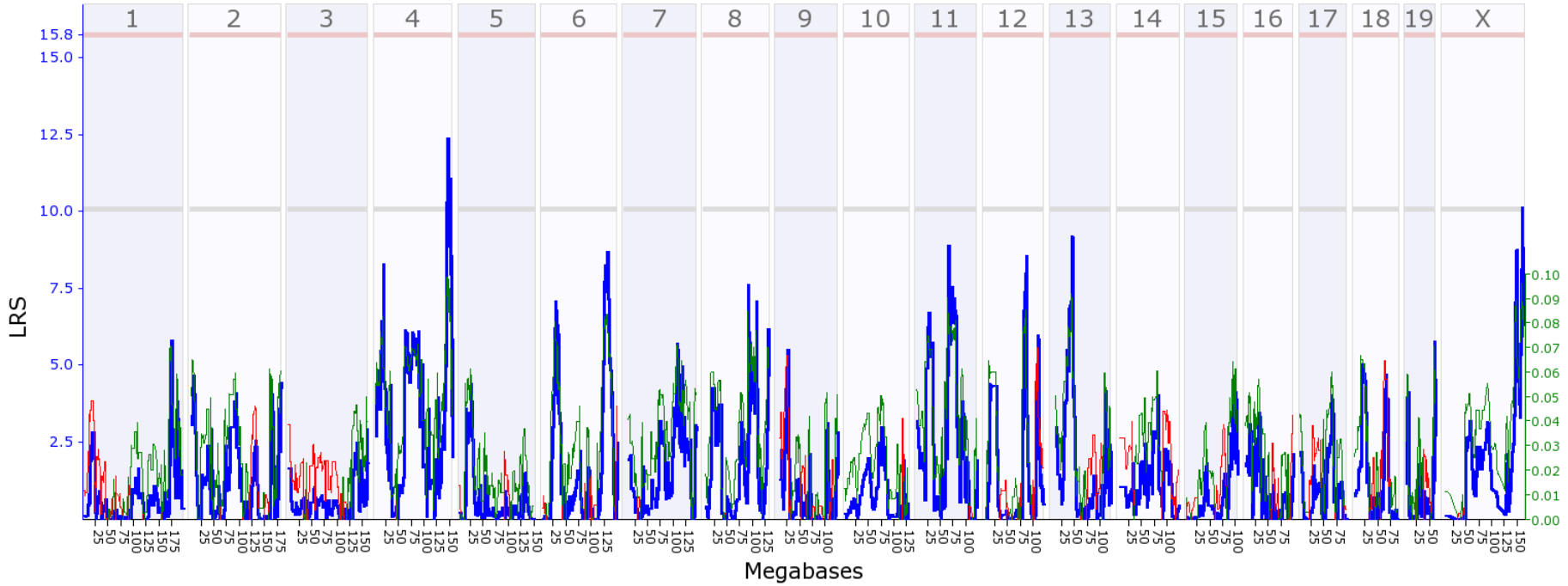
  collaborate with Robet Williams's group.

Baseline (non-diabetic) ACR in recombinant BxD lines

Determined on spot urines from 457 individual mice (12-14 wks of age)



QTL Log Baseline ACR BxD RI



Identification of Susceptibility Genes for Progression of Diabetic Glomerulopathy

- Quantitative traits analysis in C57BL/6J, DBA/2J, and BXD RI strains (coll. Matt Breyer, Eli Lilly & Co)
  - Glomerular cell count (DAPI-positive nuclei/glomerular section)
  - Endocapillary cell count (WT1-negative cells/glomerular section)
  - Podocyte cell count (WT1-positive cells/glomerular section)
  - Podocyte cell density (WT1-positive cells/1000 μm^2 glomerular section area)
  - Kidney weight (gram)
  - Glomerular section area (μm^2)
  - Glomerular matrix fraction (Silver methenamine-positive area/glom. section area)
  - Gene expression (microarray)
- Statistical analysis using GeneNetworks.org (coll. Rob Williams, Memphis)
- Goals
  - Apply QTL and gene mapping approach to identify loci/gene(s) that mediate susceptibility to podocyte depletion and glomerular matrix expansion

RATIONALE:

- Tubulointerstitial disease of patients with DNP correlates well with renal function and progression

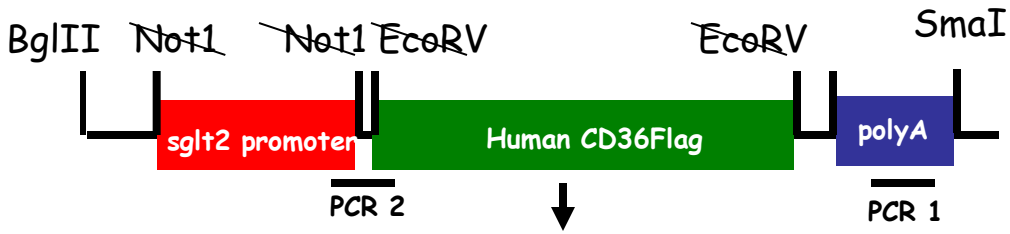
- Most Mouse Models of Diabetic Nephropathy do not develop tubulointerstitial disease

- Tubular apoptosis is present in human DNP samples, but absent in mouse DNP models

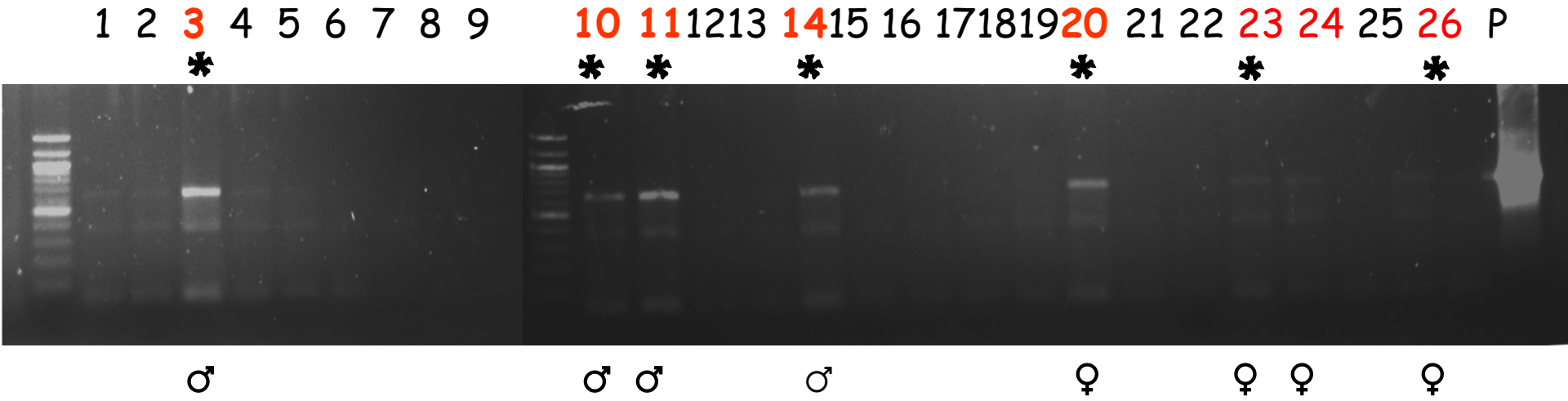
- CD36 expression correlated with tubular apoptosis

- Decreased CD36 expression in diabetic mice vs. Increased Cd36 expression in patients with diabetes

Proximal Tubular Specific CD36 Overexpression in Mice To Induce Tubular Degenration



Injection (FvB Oocyte)

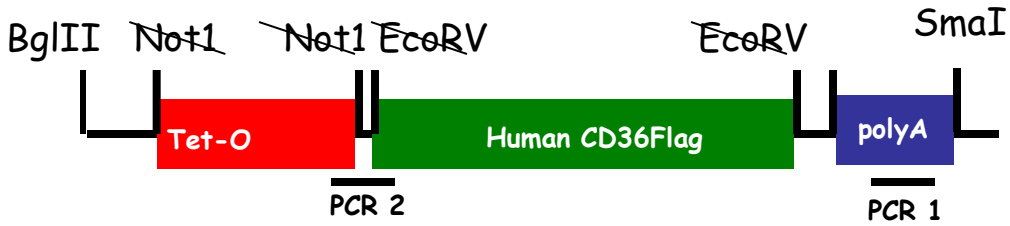


Animals were made diabetic by STZ
Phenotyping performed---indicating increased tubular apoptosis
MPV detected in this colony
Animals are being rederived

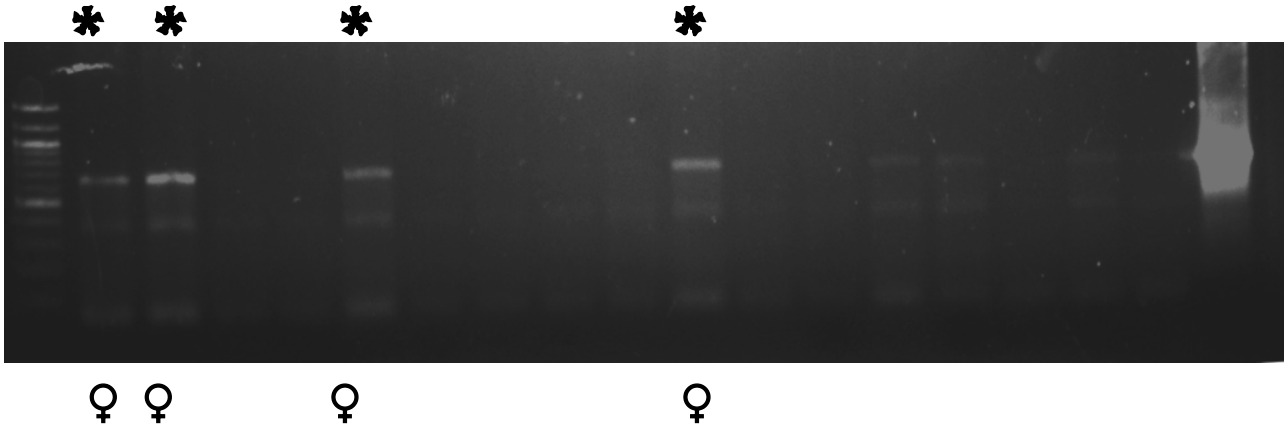
EAC comments; Analyze Tubular specific promoters

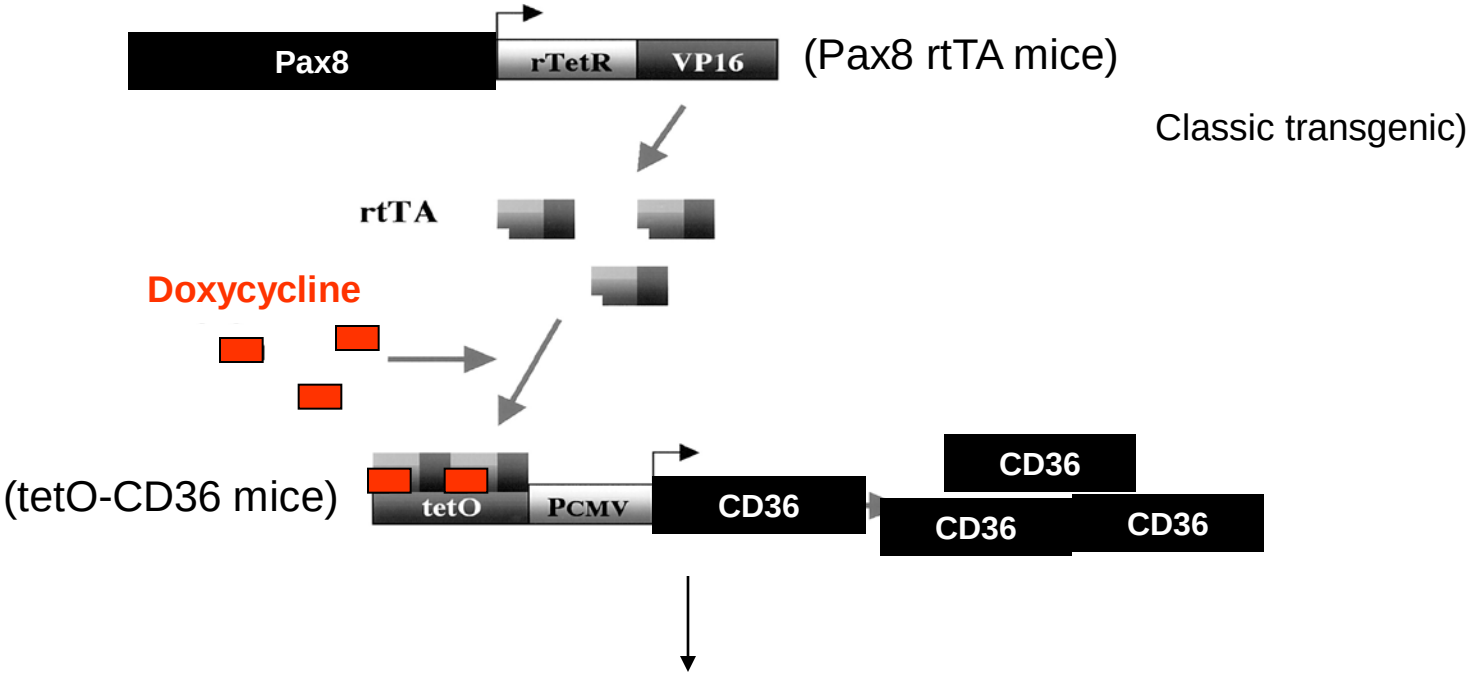
TG mice	Cre mRNA expression/CTL in whole kidney lysates	Deletion mRNA reduction KO/WT
Sglt2cre	x10	<10%
Kspcre	In progress	
GGTcre		
PEPCKcre	x60	~50%
NapIcre..	In progress	
Pax8rtTA/tet-O-cre	**x1000**	**90%**
NPHS2cre	x1000	80%

Proximal Tubular Specific CD36 Overexpression in Mice To Induce Tubular Degenration



Injection (FvB Oocyte)

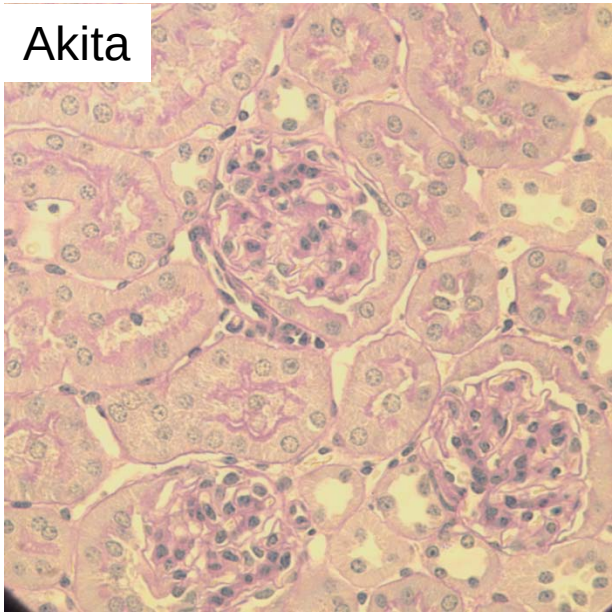


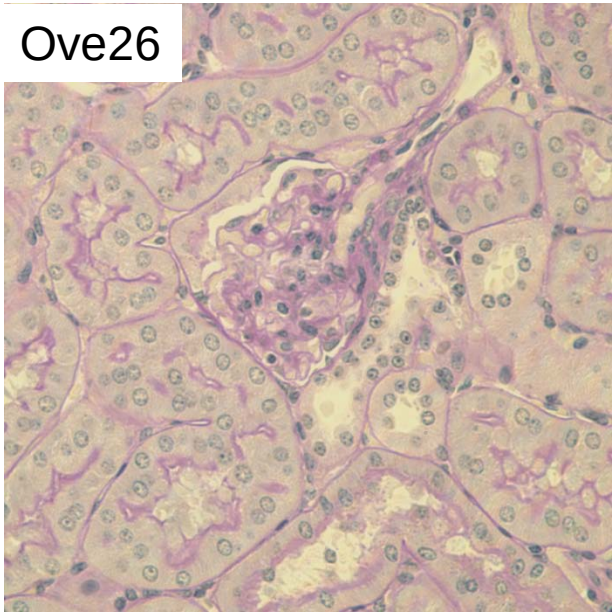


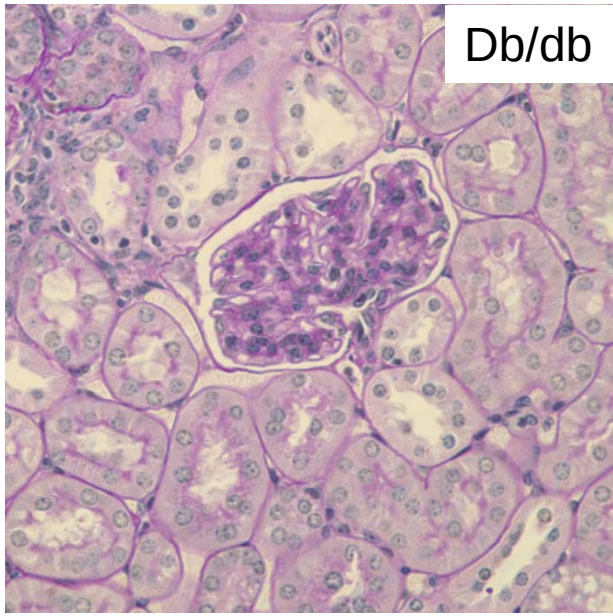
Tissue and cell specific regulation of a Transgene

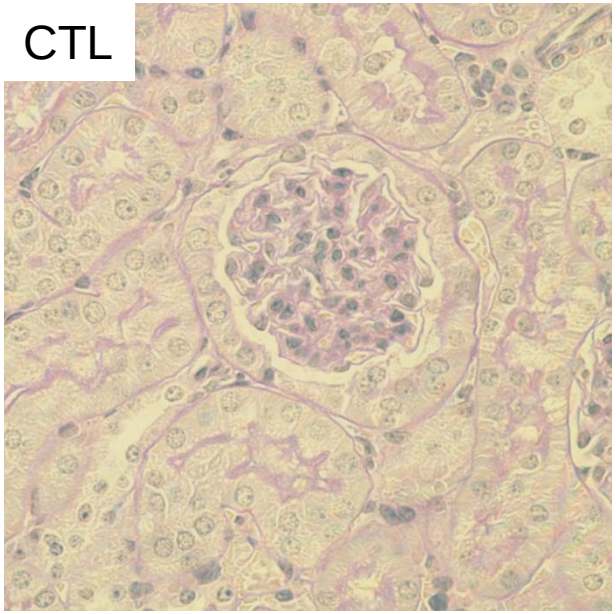
FvB as a background for diabetic nephropathy

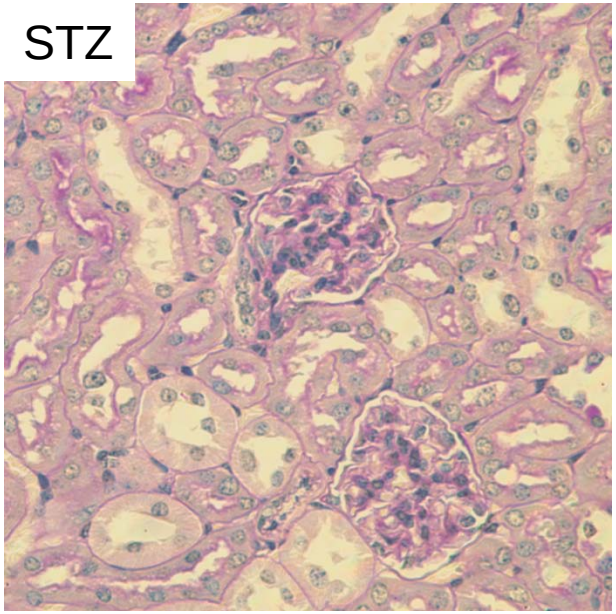
Renal Histology 20 week old diabetic mice on Fvb background



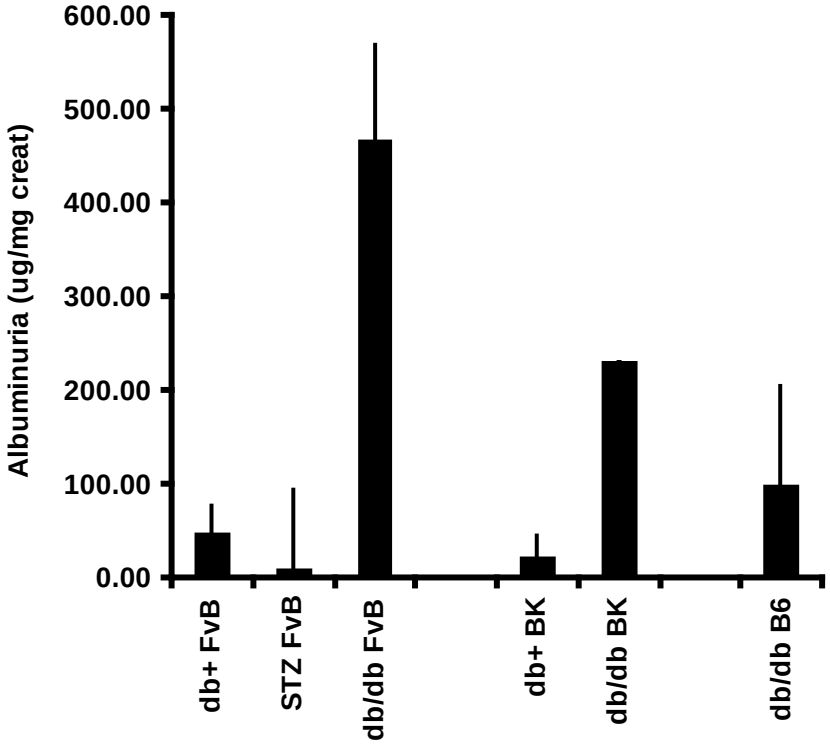


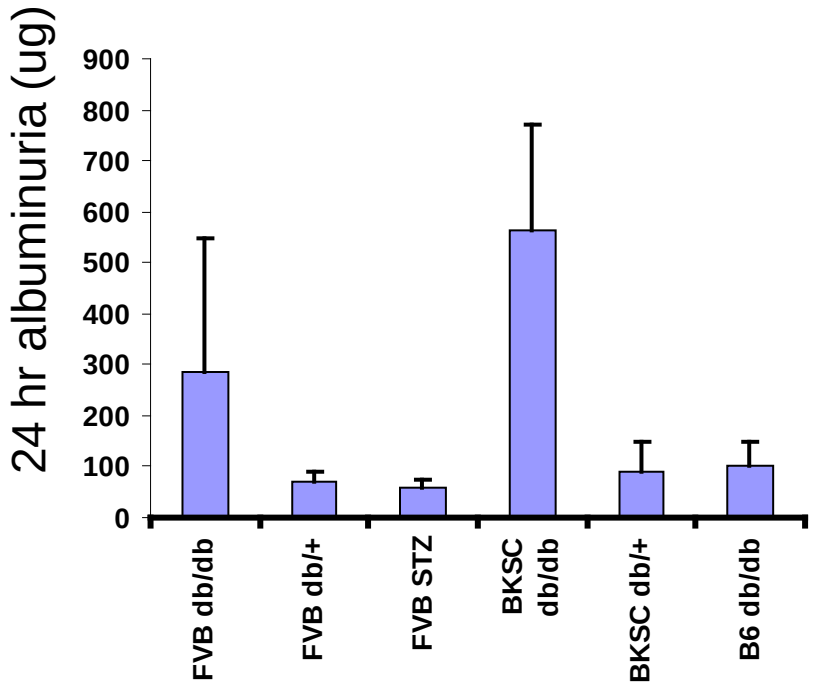




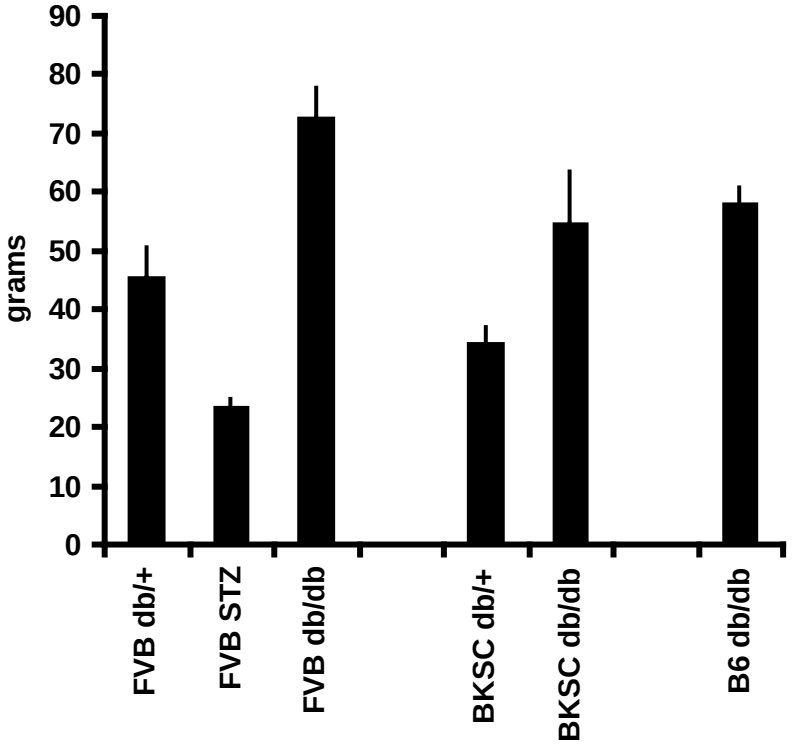


Albuminuria in different Diabetic Animals





Body Weight



Blood Glucose