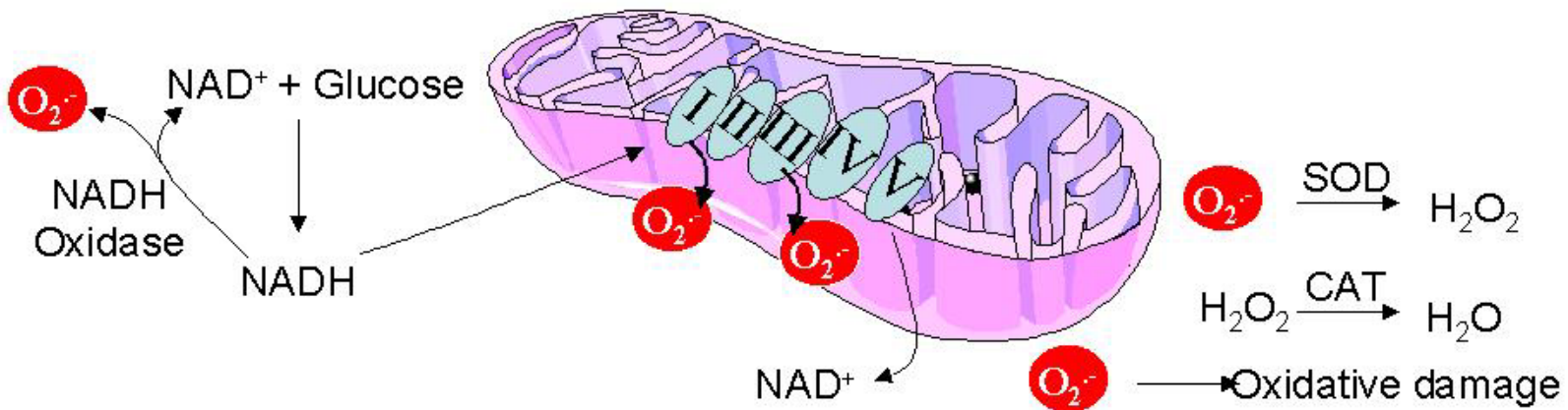
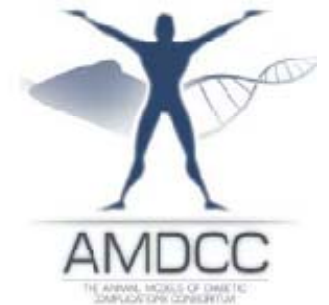
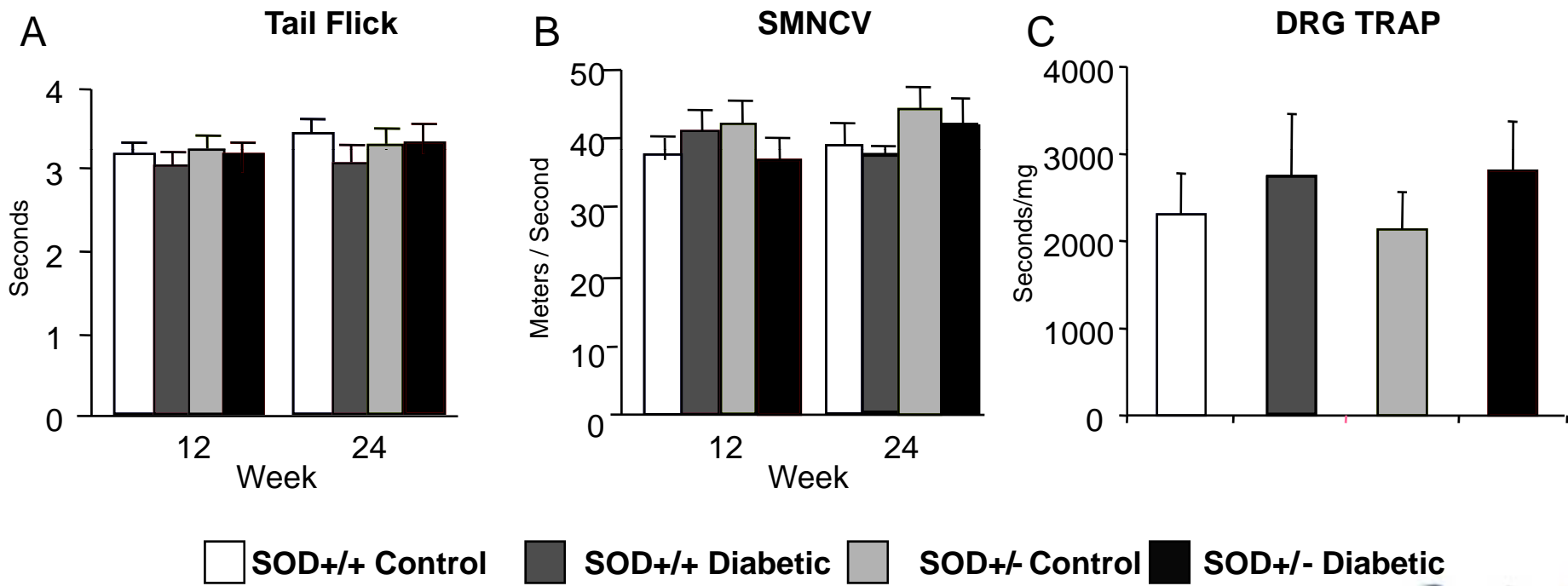


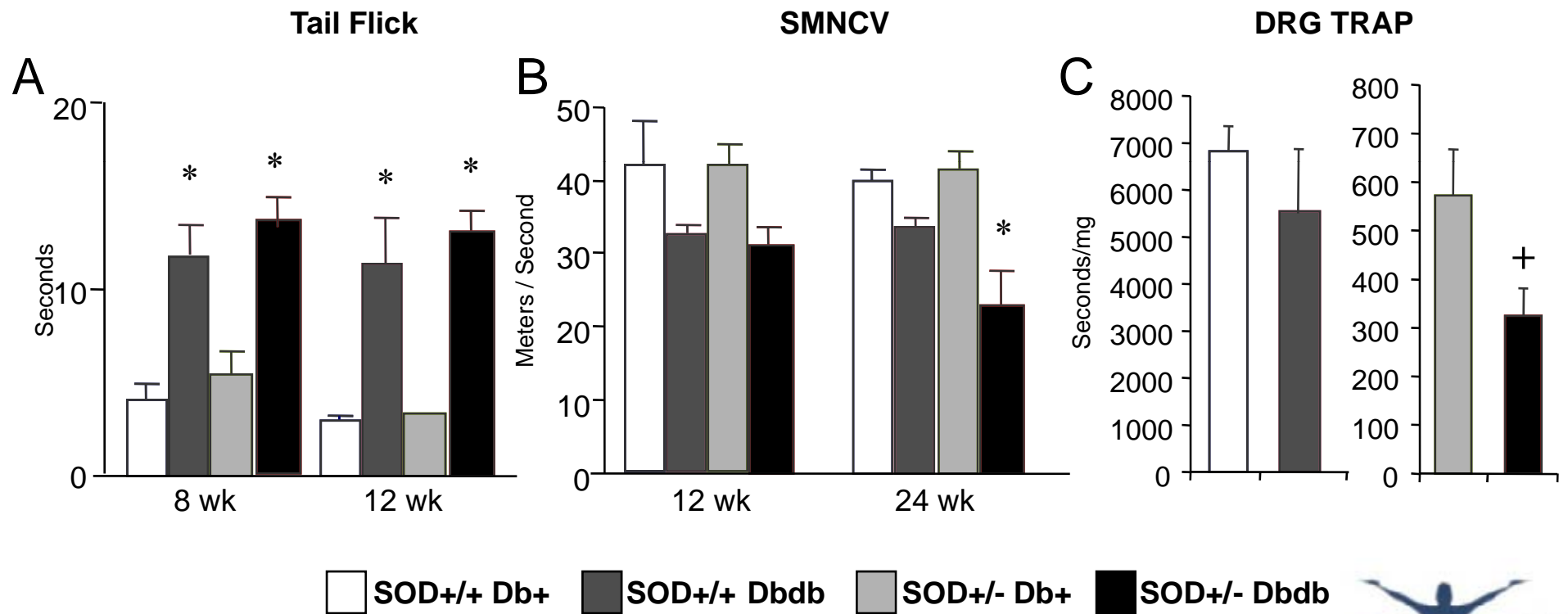
Hyperglycemia Leads to the Formation of Superoxides and Oxidative Damage



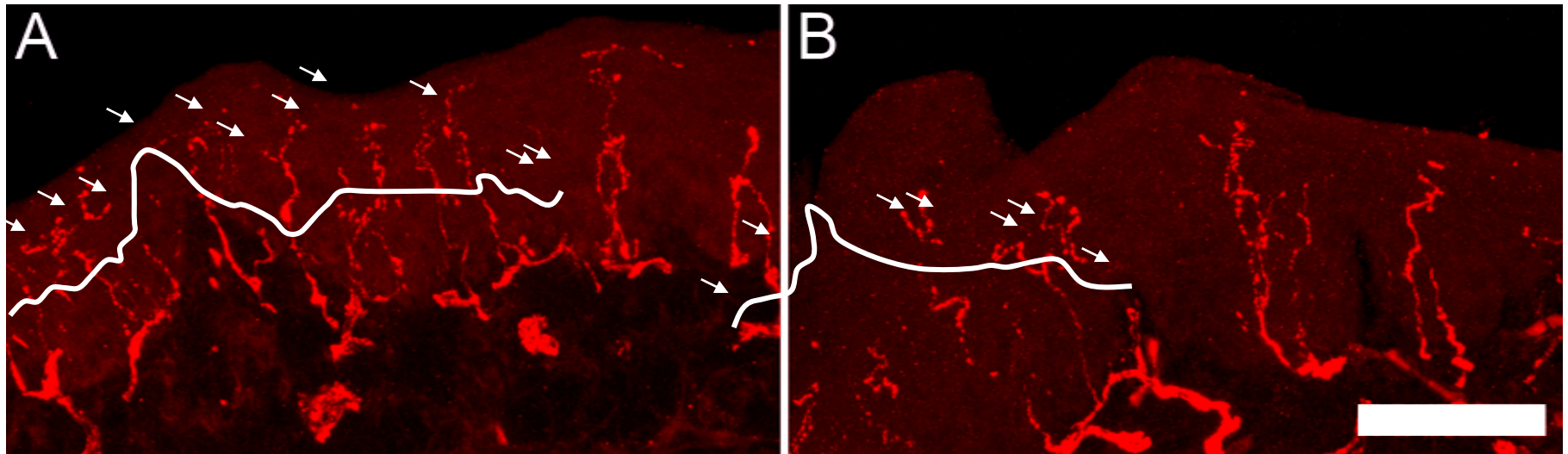
SOD2 Underexpression Does Not Produce Neuropathy in C57Bl/6 Mice



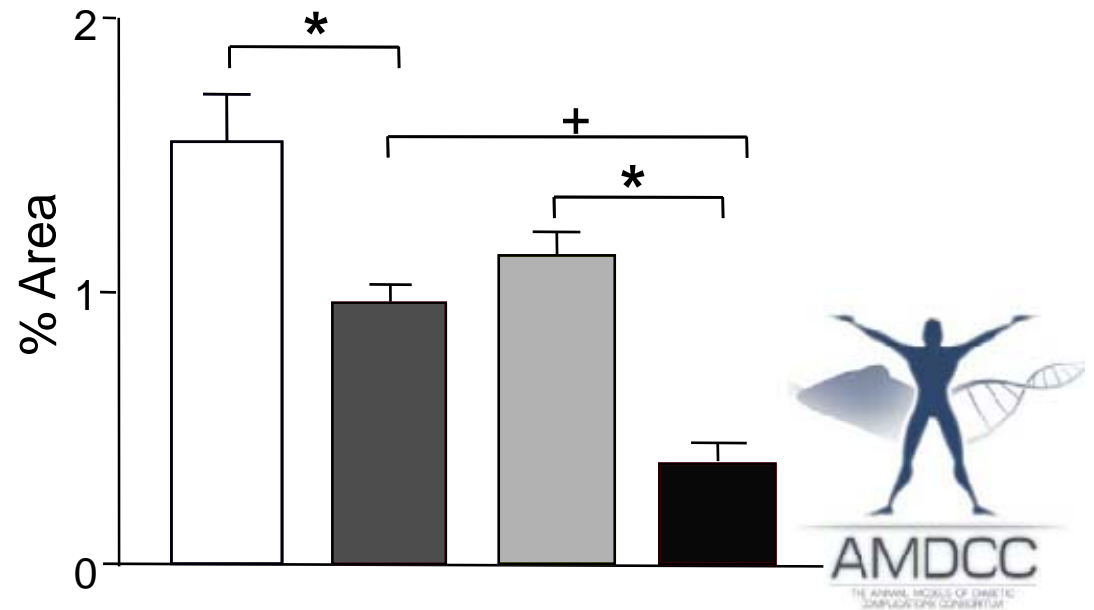
SOD2 Underexpression Increases Neuropathy in C57B1/6 dbdb Mice



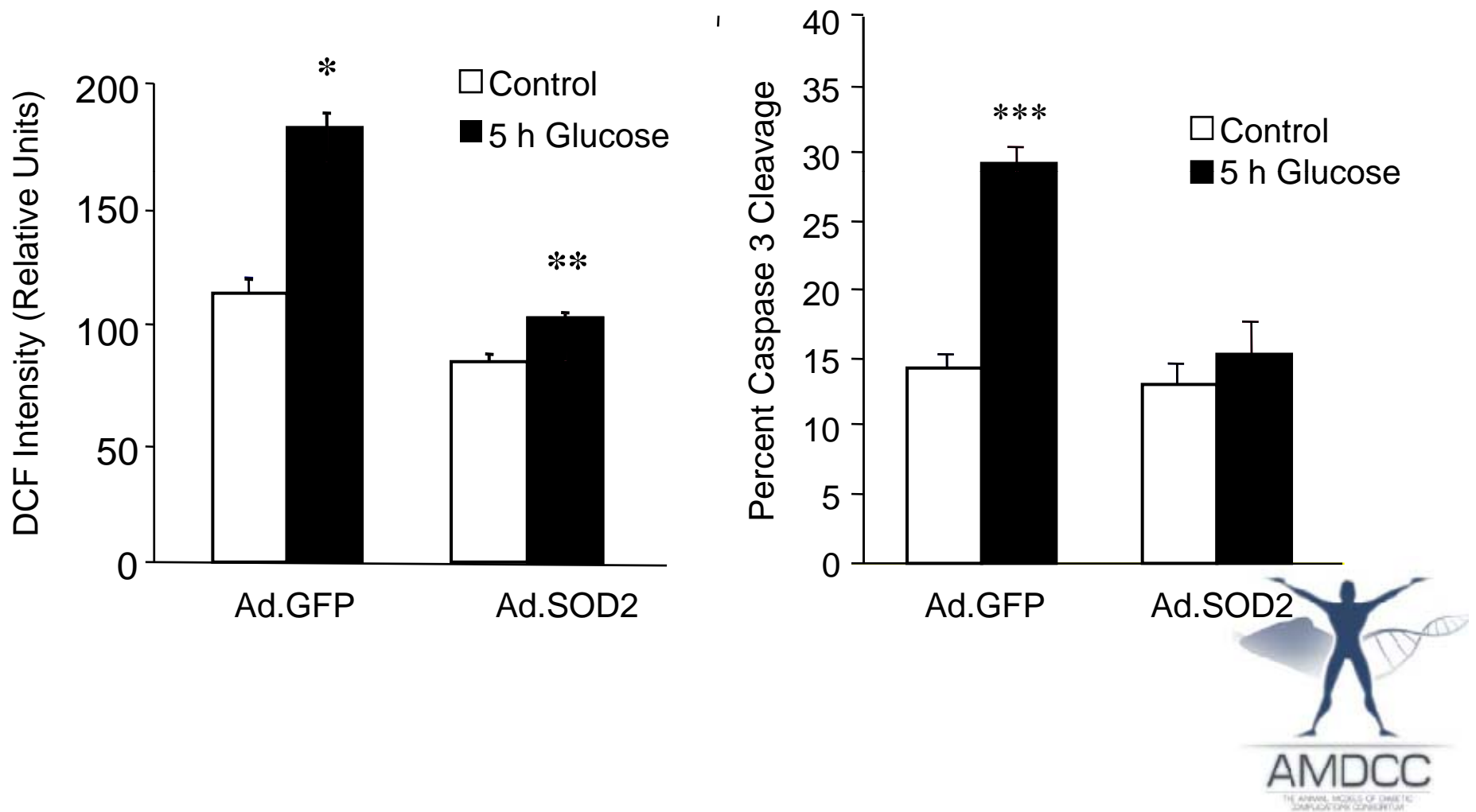
SOD2 Underexpression Increases Neuropathy in Db/db Mice- IENF



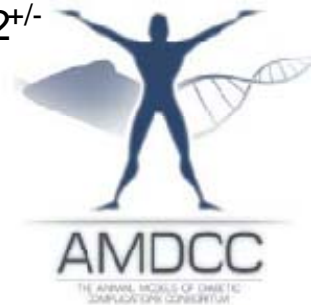
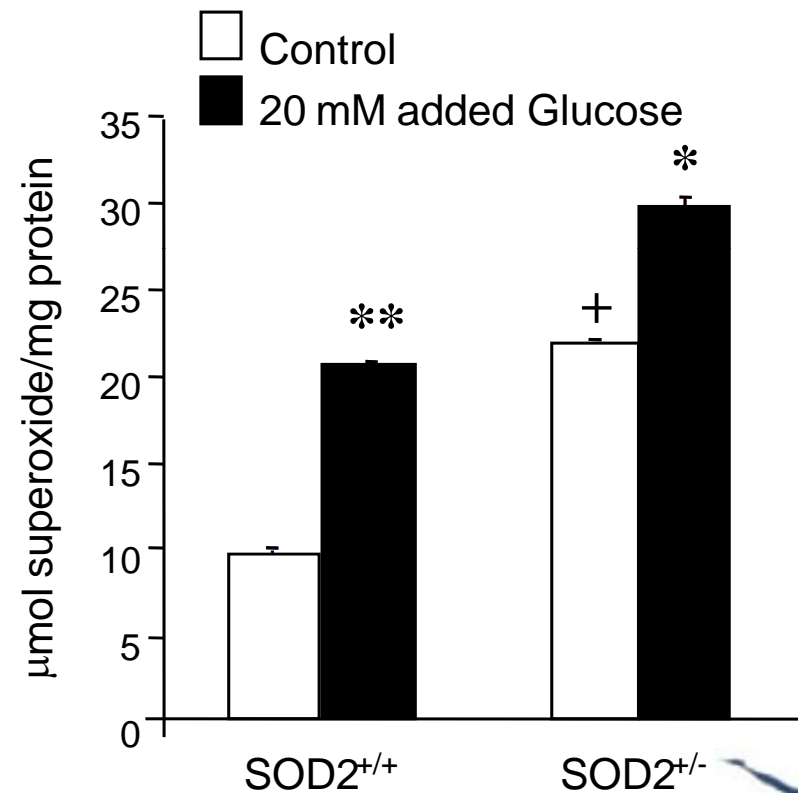
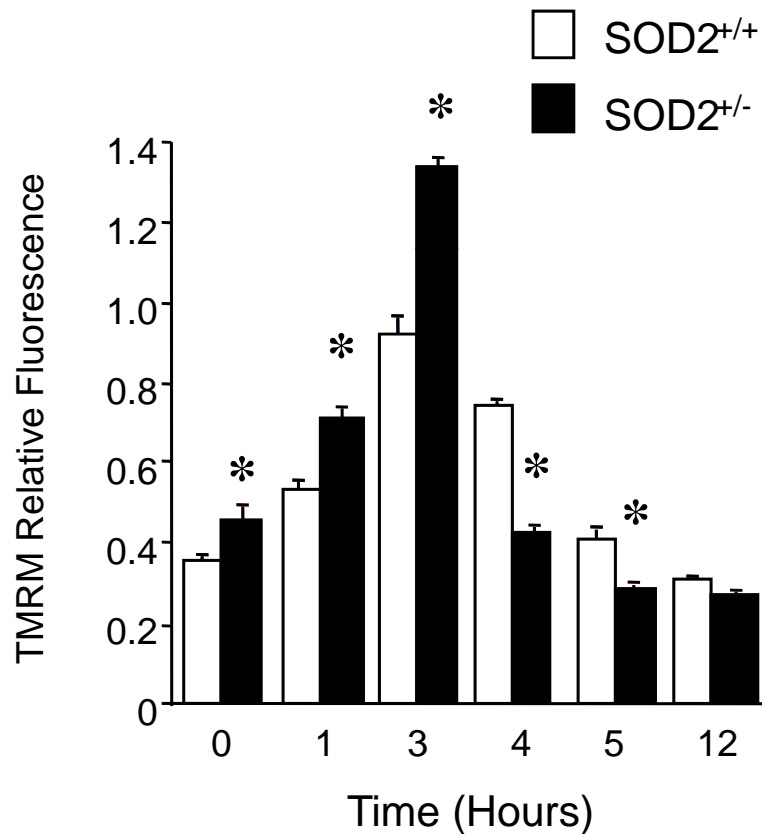
C



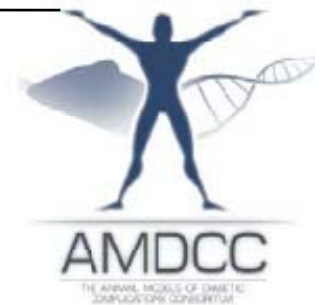
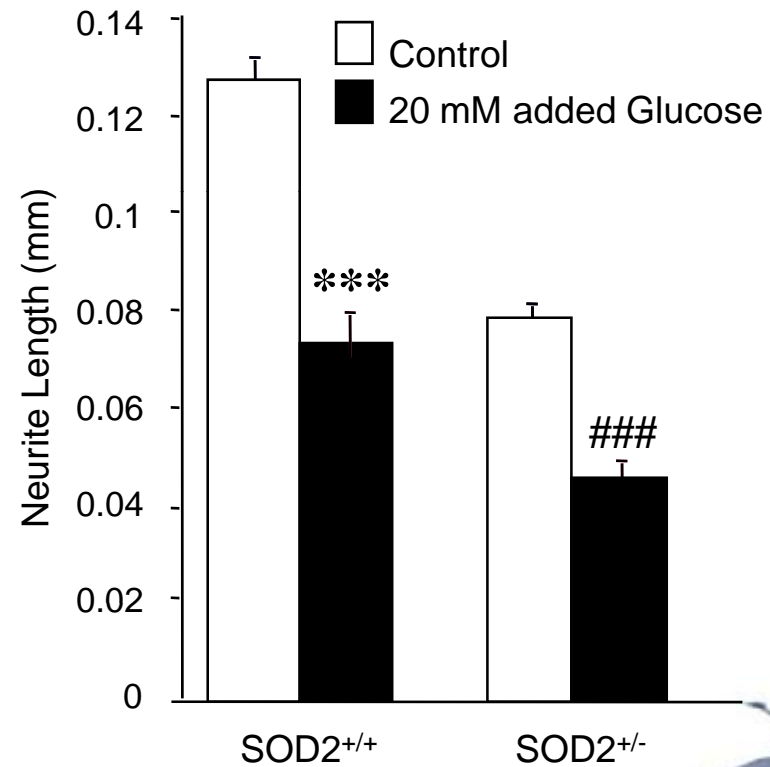
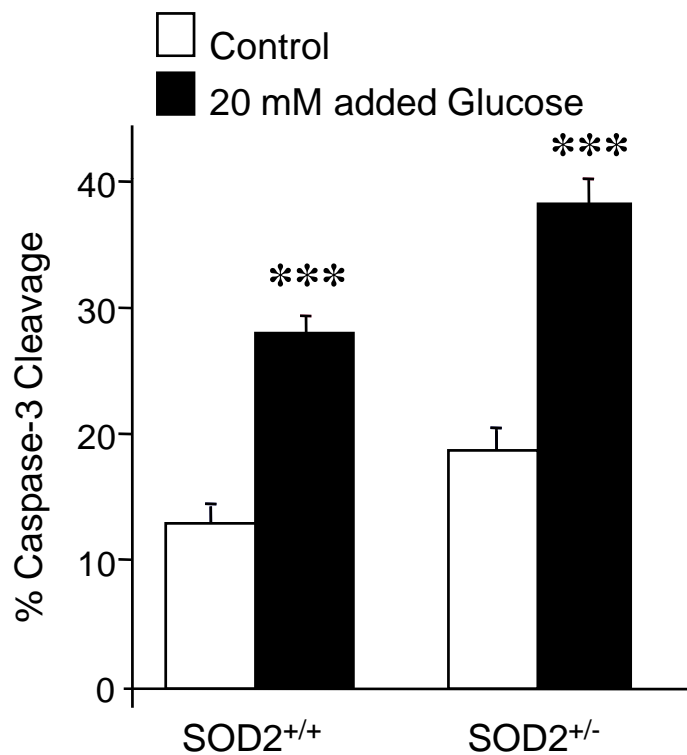
SOD2 Overexpression Prevents Oxidative Stress and DRG Neuron Injury



SOD2 Underexpression Increases $\Delta\Psi_M$ Hyper- and De-polarization and $O_2^{\cdot-}$



SOD2 Underexpression Increases DRG Neuron Injury

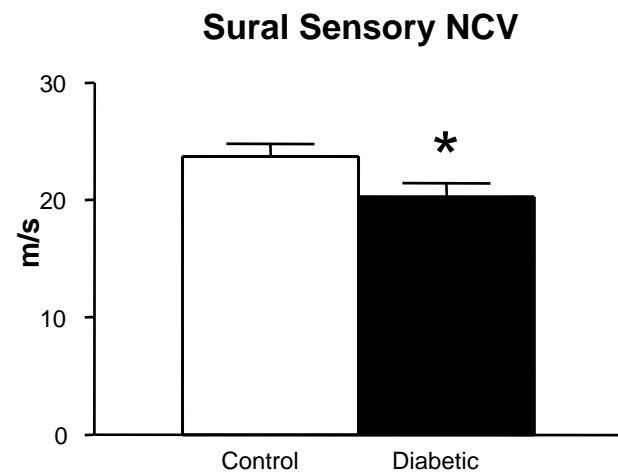
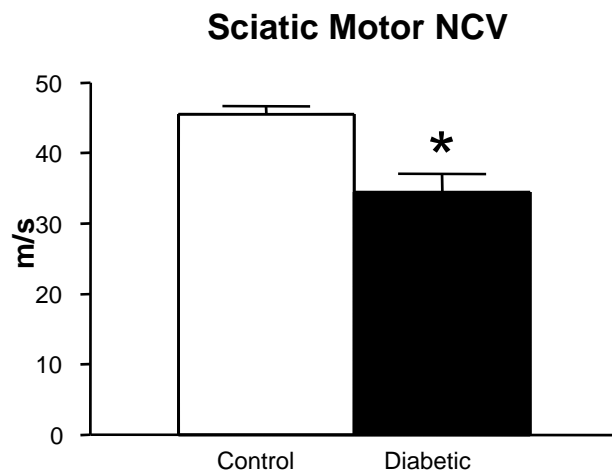
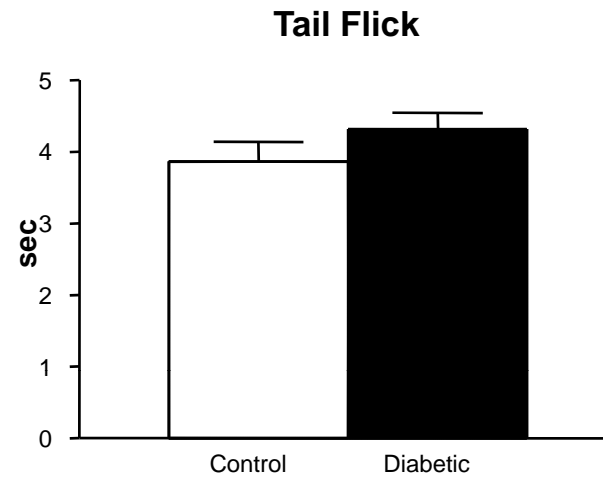
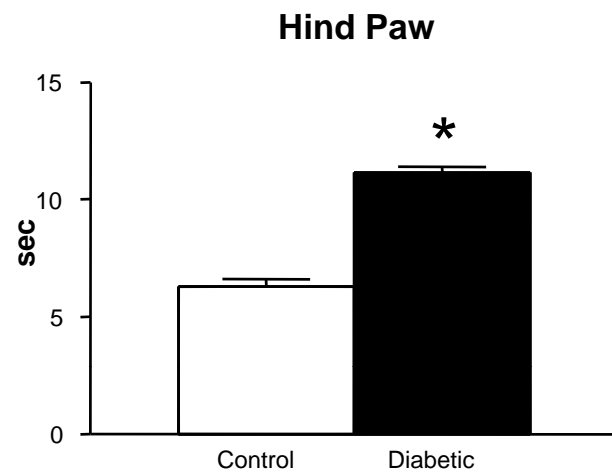


Phenotyping of New Models in Collaboration with other AMDCC Investigators

- DBA2/J STZ \pm TZD (Brosius)
 - Metabolome and gene expression (Pennathur & Kretzler)
 - Informatics analyses (NCIBI)
- CD1 STZ mice (Abel)



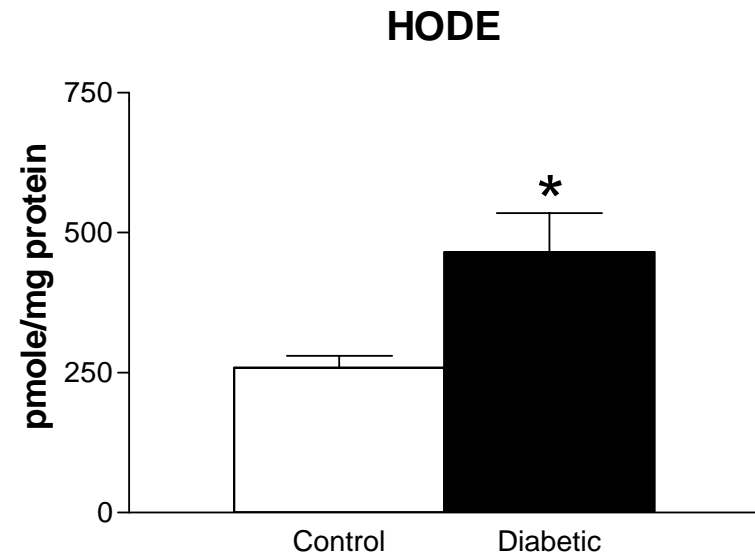
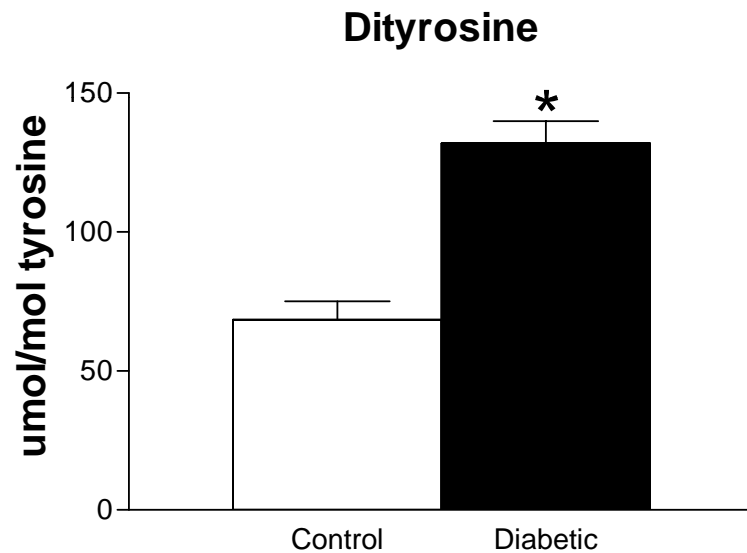
Neuropathy Phenotyping



Measured at 26 weeks diabetic, $p < 0.05$



Oxidative Stress in Sciatic Nerve



Measured at 26 weeks diabetic, $p < 0.05$



Affymetrix Analysis Pipeline

GenePattern: Data analysis software

Genes are regulated if both the p-value and CyberT false discovery rate < 0.05

9 Samples, 4 control & 5 diabetic

- 12,150 gene measured
- 141 genes down regulated
- 385 genes up regulated

NCIBI - <http://ncibi.org>



Functional clustering analysis

Using DAVID 2007

- Database for Annotation, Visualization and Integrated Discovery
- Contains annotation from GO, KEGG, BioCarta, SWISS-PROT, and others.

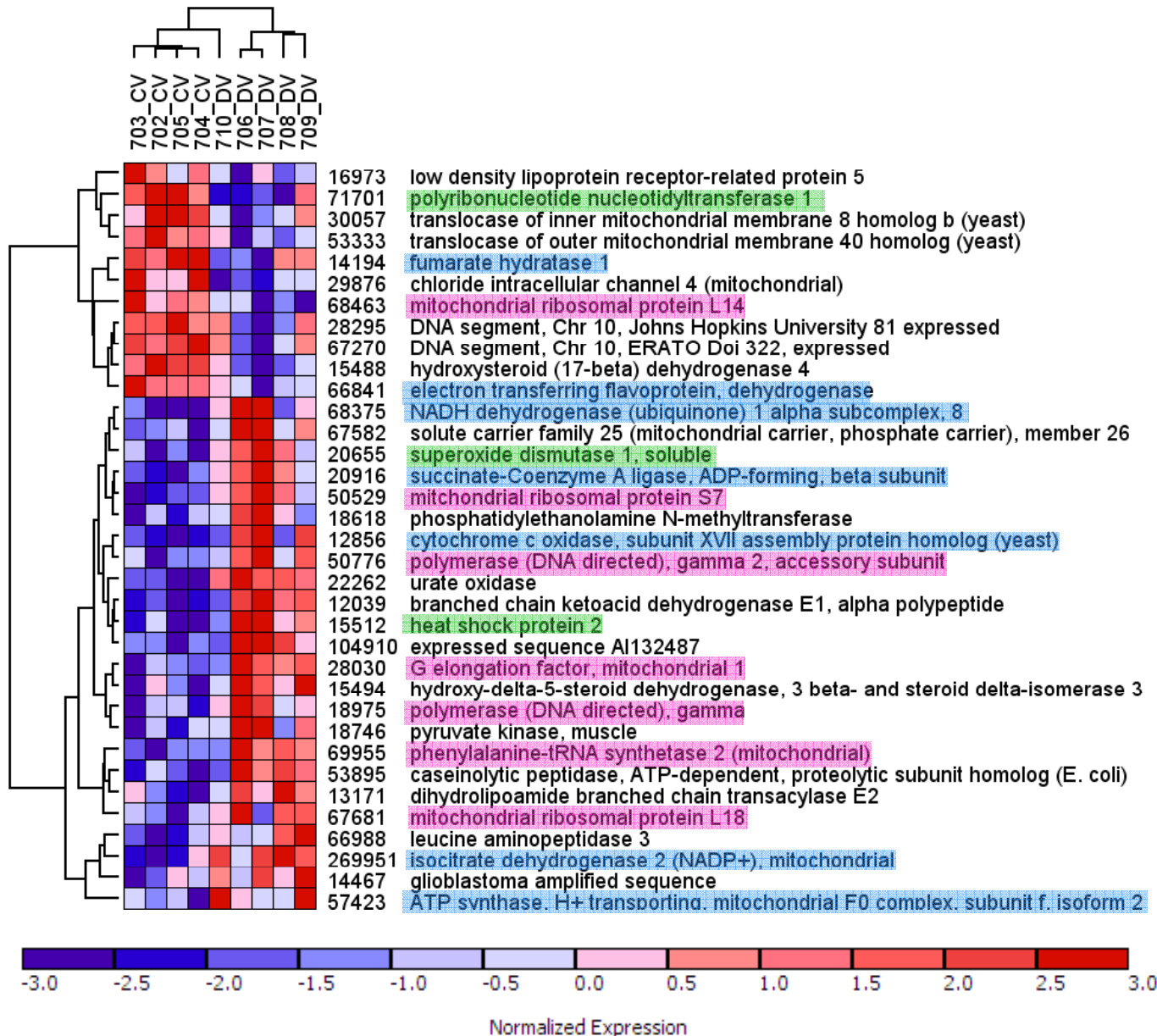
Top 5 Cluster types

- Metabolism
- Metal Binding
- RNA processing
- Transport
- Mitochondria

Genes in these categories are especially relevant to oxidative stress



Mitochondria & Metabolism Genes



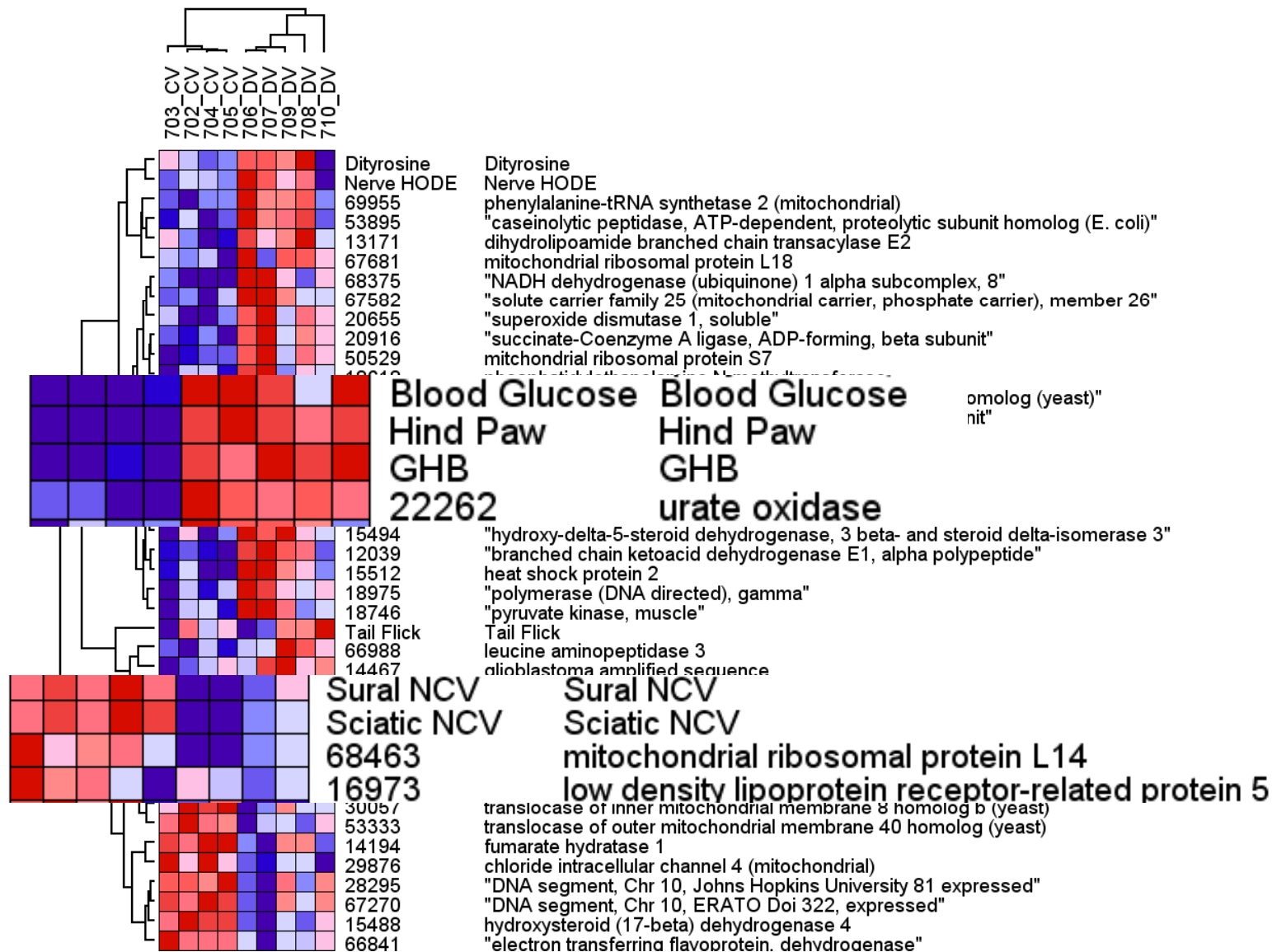
TCA and ETC cycles

Oxidative stress Response

Mitochondrial DNA and protein synthesis



Neuropathy Correlation



Conserved Promoter Modules

Upregulated Genes

Promoter module	Count	Frequency	Genomic frequency	Fold Change in Frequency
NF1F_NR2F_01	10	0.19	0.05	3.9

NRF2 is a key regulator of the anti-oxidant response element

Indicative of a compensatory response

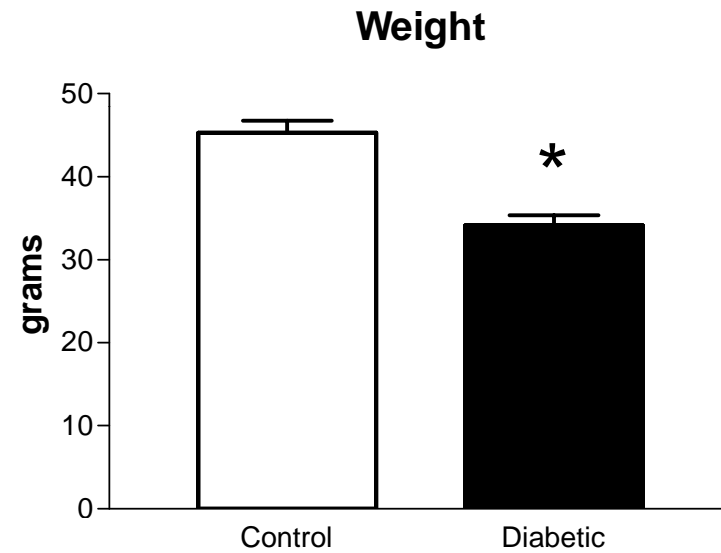
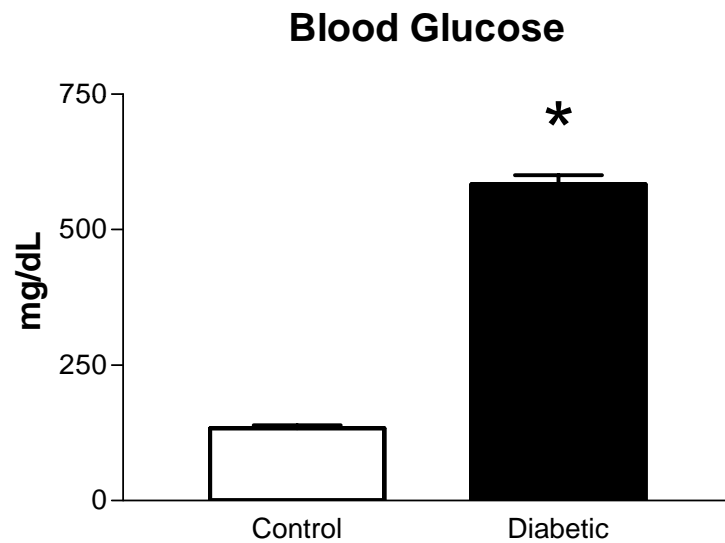


Animal Models of Diabetic Complications Consortium

- Completing oxidative stress measures and microarrays for C57Bl6 \pm STZ \pm high fat diet; KLS db+ and dbdb
- Sciatic nerve (current)
- Dorsal root ganglia (planned)
- Gene, promoter and pathway informatics with NCIBI



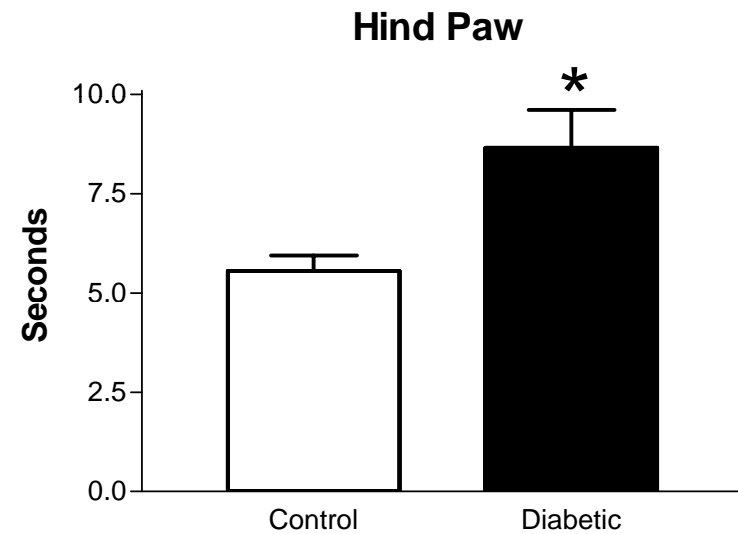
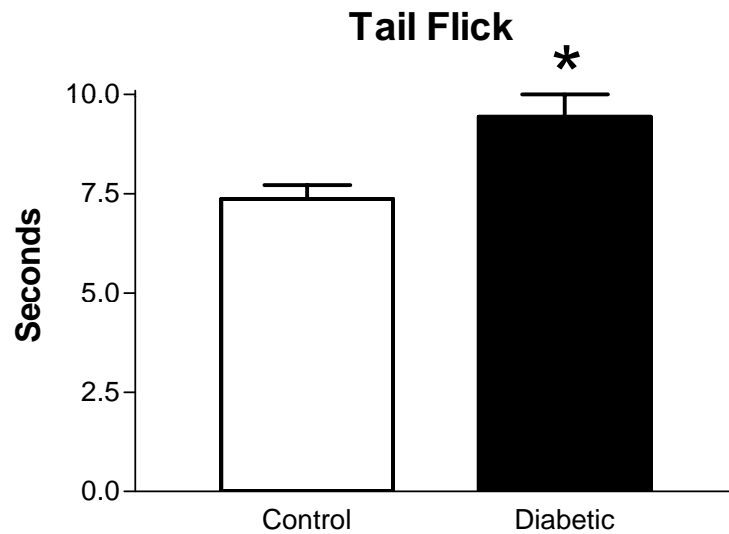
Metabolic Measures in CD1 Mice



*, $p < 0.01$



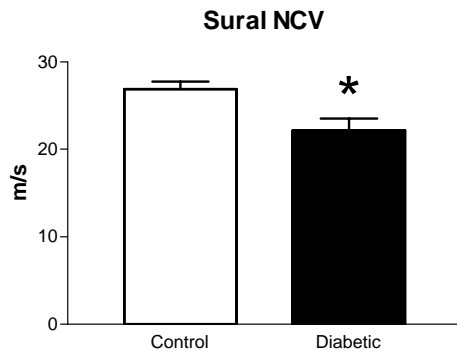
Behavioral Measures of CD1 Mice



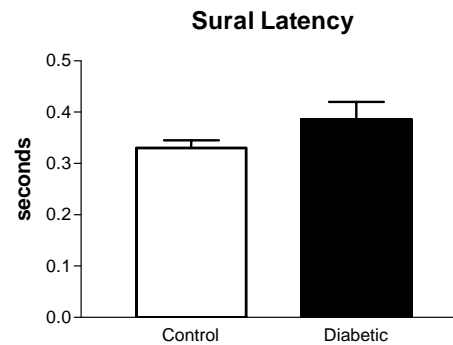
*, $p < 0.01$



Nerve Conduction Measures of CD1 Mice

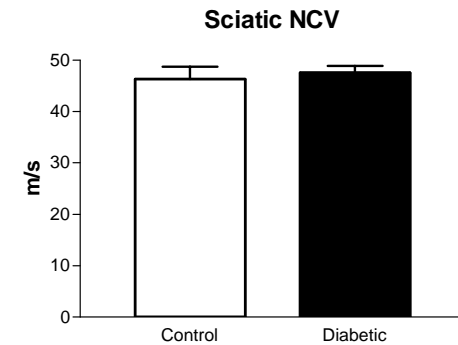


*, $p < 0.01$



$p = 0.08$, Power = 0.46 at current n .

Likely significant with more animals



Animal Models of Diabetic Complications Consortium: NeuroDiab

- History of AMDCC (2001-present)
- Goal: Develop mouse models that replicate as closely as possible the human disease
- Grants focused on nephropathy and macrovascular disease, 1 neuropathy, 1 uropathy
- “Unexpected” lessons were learned: strain, diet, gender, duration of diabetes



Animal Models of Diabetic Complications Consortium: NeuroDiab

- Guidelines are needed to define the rodent complications
- Standard induction of STZ diabetes
- Nephropathy: *Am Soc of Nephrology* 16:27-45 and *Diabetes* 54:2628-2637
- Neuropathy: external advisors: Calcutt, Low, Zochodne



Animal Models of Diabetic Complications Consortium: NeuroDiab

- 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 nerve conduction velocities (NCV)
- Anatomical evidence of nerve fiber loss
 - Intraepidermal fiber density (IEFD)



Animal Models of Diabetic Complications Consortium: NeuroDiab

- Collective wisdom of a larger number of investigators (e.g. AMDCC Nephropathy consortium)
- NIDDK symposium: 4/4-4/5, 2007
- Wise group: Neuropathy Study Group of EASD
- Support from NIDDK and JDRF for this initiative



Animal Models of Diabetic Complications Consortium: NeuroDiab

- Goal: to develop guidelines for what constitutes diabetic neuropathy in rodent models
- Simple, standardized tests to phenotype rodent models
- Not testing specific hypotheses, but simply confirming the presence of DN in rodent models

