AMDCC
Neuropathy Phenotyping Core

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Outline

- Respond to EAC comments
- Define neuropathy and illustrate phenotyping
- Summarize current phenotyping efforts
- Highlight key models
- Collaborations

EAC Recommendations and Comments

- *Validation of Intraepidermal Fiber Density (IEFD)*
  - IEFD strongly correlates with neuropathy in human patients (Brain 127:1606-1615, 2004)
  - IEFD may be expressed as number of axons per linear mm of epidermis or as axonal area per epidermal area
  - Comparisons between these two methods are underway and results are expected by the November 2004 conference call

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EAC Recommendations and Comments

- *Neuropathy Progression*
  - In human patients, diabetic neuropathy is defined by clinical sensory loss and abnormal psychophysical tests
  - These changes often correlate with decreased fiber density, increased incidence of degenerating fibers and deinnervation of target tissues
  - Serial biopsies are not feasible in mice, therefore only one time point is available for study
  - By exaggerating diabetic complications we hope to create a more human-like neuropathology
  - We look forward to testing other AMDCC models for potential neuropathic changes

EAC Recommendations and Comments

- *Differences Between Nerve Conduction Data and Pathology*

  – Differences in nerve conduction velocities are evident; however, axon pathologies have not been observed

  – Profound changes are noted in target tissues, specifically, IEFD

  – Alterations in gene expression targeted to sensory neurons (Synapsin Cre Floxed SOD2, Nestin Cre Floxed SOD2) and Schwann cells (Po Cre) should create a more severe phenotype

EAC Recommendations and Comments

- *Measuring Changes in the Sympathetic Nervous System*
  - This type of testing would only be performed if the model indicated a profound change in sympathetic function
  - Sweat imprint would be used to assess sympathetic function in the extremities
  - This technique could then be compared directly to innervation of the sweat glands in foot pad biopsies

EAC Recommendations and Comments

- *Discrimination Between Normal and Degenerating Nerve Fibers in Foot Pad Biopsies*
  - We are unaware of any markers of degeneration detectable with light microscopy
  - This distinction between normal and degenerating fibers requires transmission electron microscopy (TEM)
  - Not appropriate as a neuropathy screening procedure but would be useful as a follow-up assay

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EAC Recommendations and Comments

- *Measures of Noradrenergic Function*
  - The feasibility and cost of these measures is under investigation
  - It is currently possible to use immunohistochemistry to examine tyrosine hydroxylase containing fibers in the foot pad biopsies

EAC Recommendations and Comments

- *Providing Neuropathy Phenotyping for AMDCC Investigators*
  - Sending foot pad samples to Michigan for IEFD screening may be the first, best step in examining potential neuropathy models
  - Subsequent measures of neuropathy including sensory testing and NCV could be performed at Michigan (terminal phenotyping, requires 5 animals per group)

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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

Evidence of Clinical Loss of Sensory Function

- Tail flick and hind paw thermal testing
  - The time from the application of the stimulus to time of withdrawal equals the latency
  - Measured every 12 weeks and at tissue harvest
  - Reliable and reproducible
  - Hind paw latencies correlate well with IEFD
  - Non-invasive

Tail Flick

Hind Paw Withdrawal

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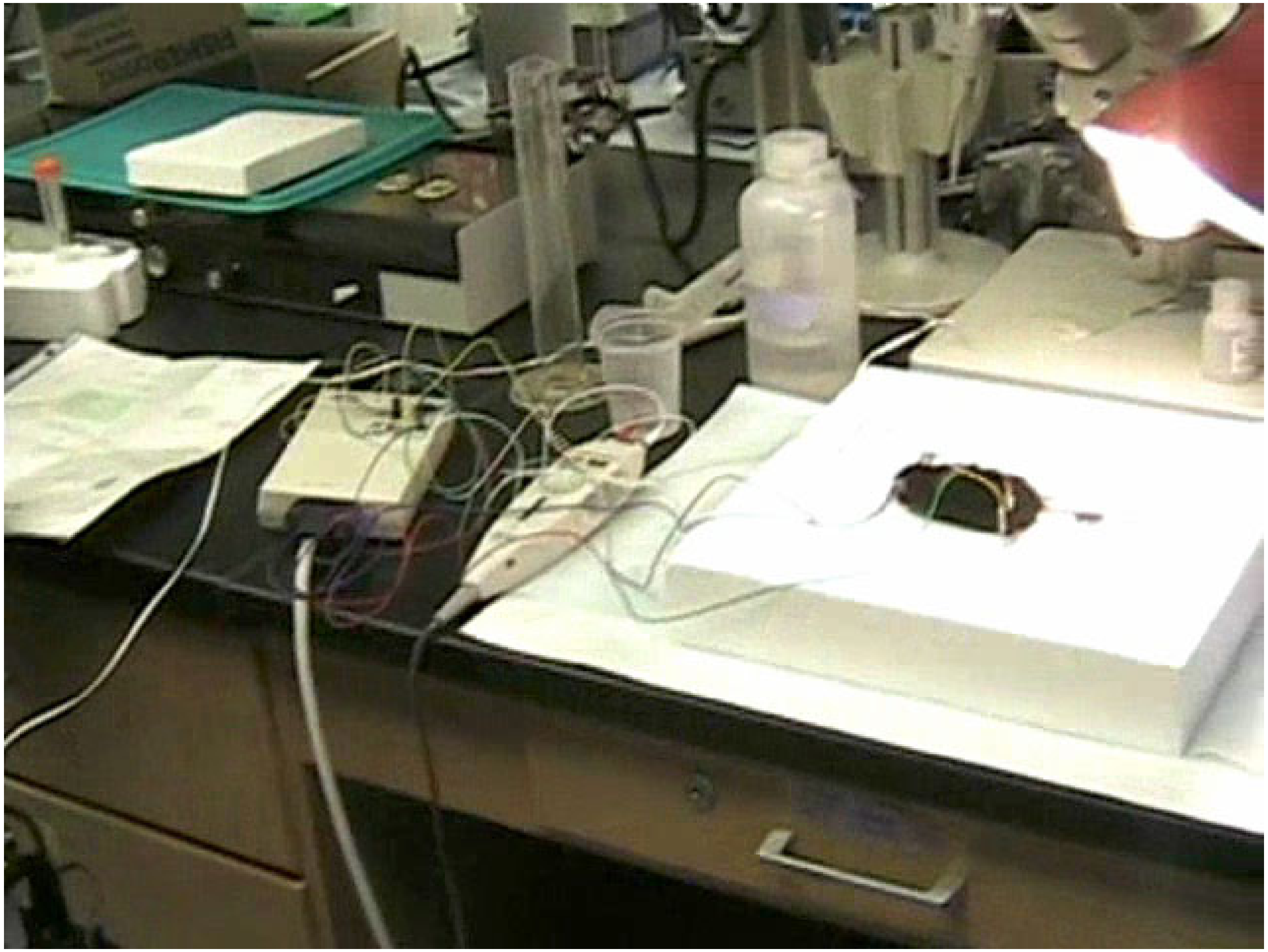
Electrophysiological Evidence of Nerve Impairment

- Nerve Conductions
- Tail motor distal latency, tail sensory and sciatic motor conduction velocity
  - Sensitive measures of peripheral nerve function
  - Requires anesthesia
  - Performed at 12 weeks post-diabetes and at tissue Harvest

Tail Distal Motor Latency

Sciatic Nerve Motor Conduction Velocity



Anatomical Evidence of Nerve Fiber Loss with Skin Biopsies

- Intraepidermal Fiber Density
  - PGP9.5 (ubiquitin C-terminal hydrolase)
  - Pan-axonal marker
  - Confocal image capture and analysis
    - Data may be expressed as number of fibers per mm of epidermis or as area of fibers per area epidermis
  - Samples may also be examined for specific neurotransmitters

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Skin Biopsy Technique

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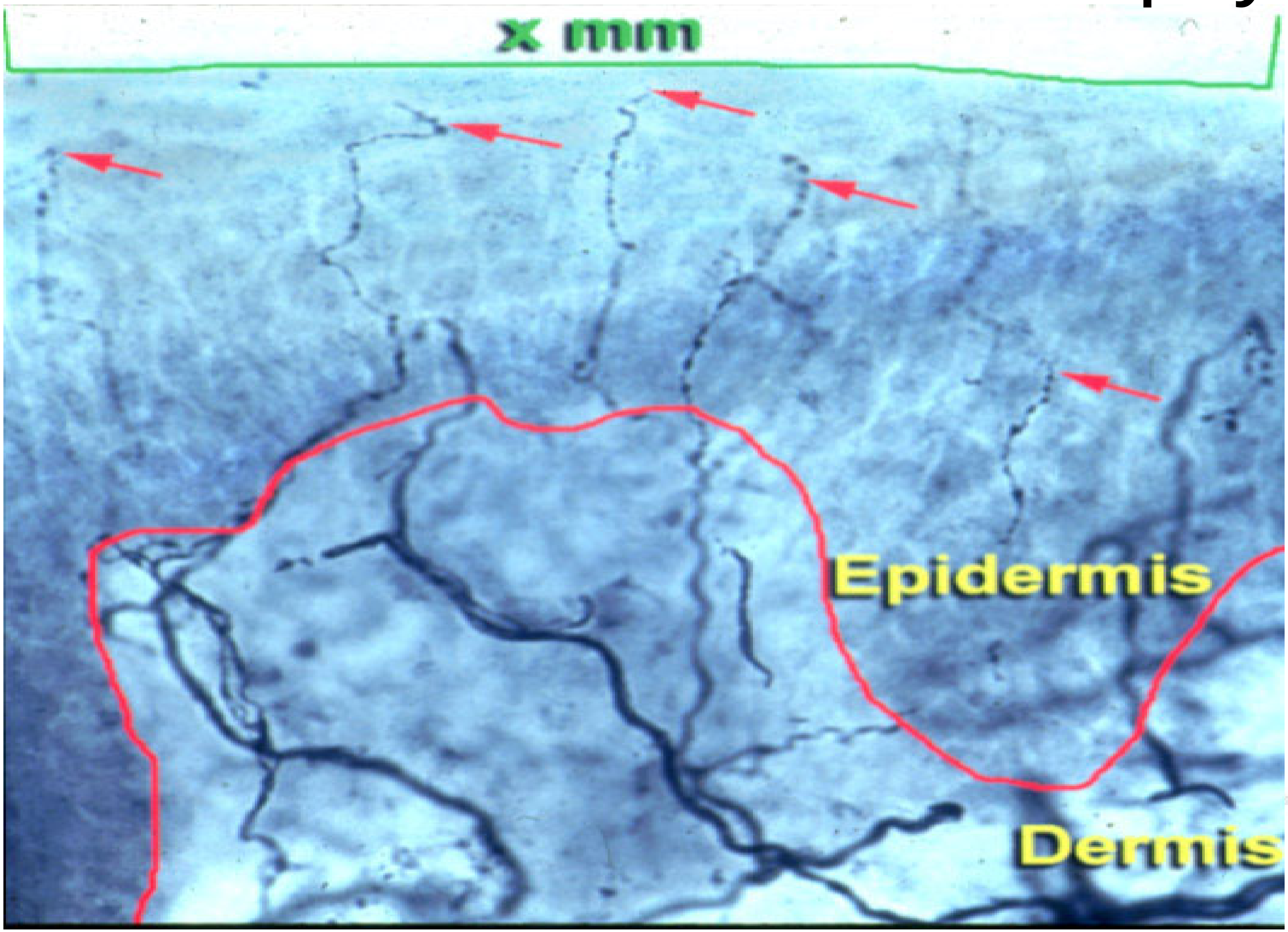
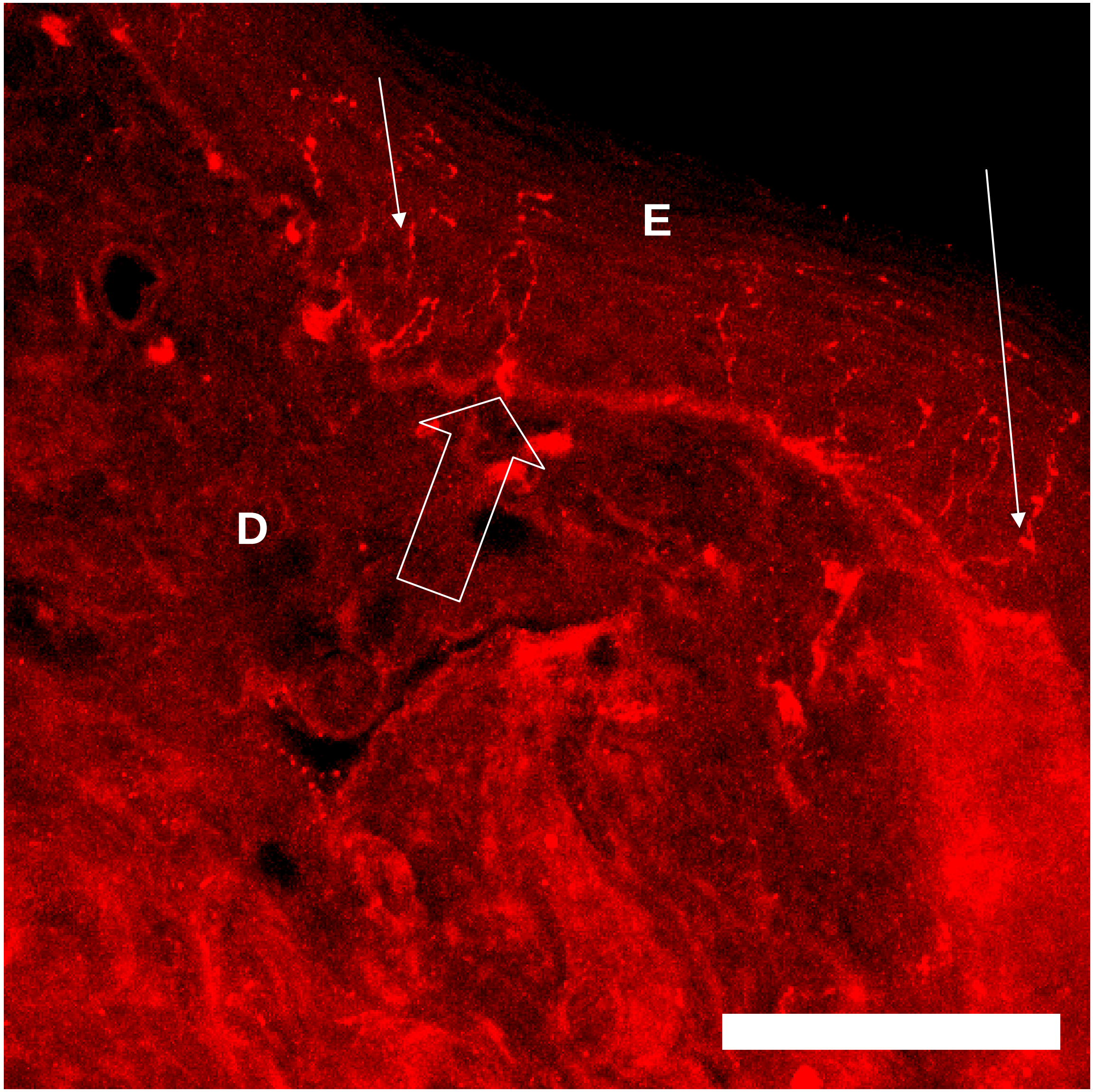
Normal Cutaneous Innervation Assessed with Skin Biopsy

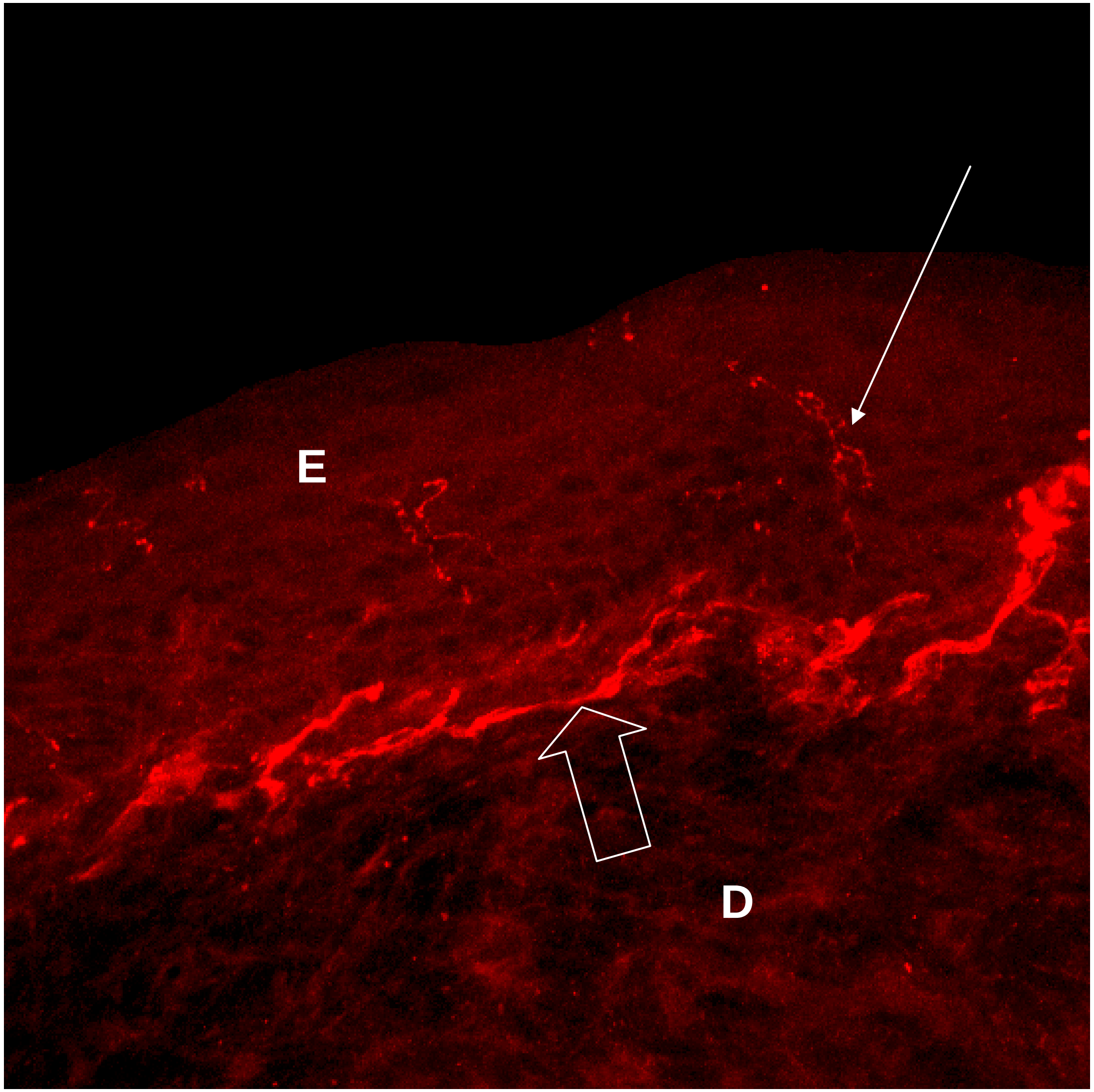


SOD2+/+ Db+

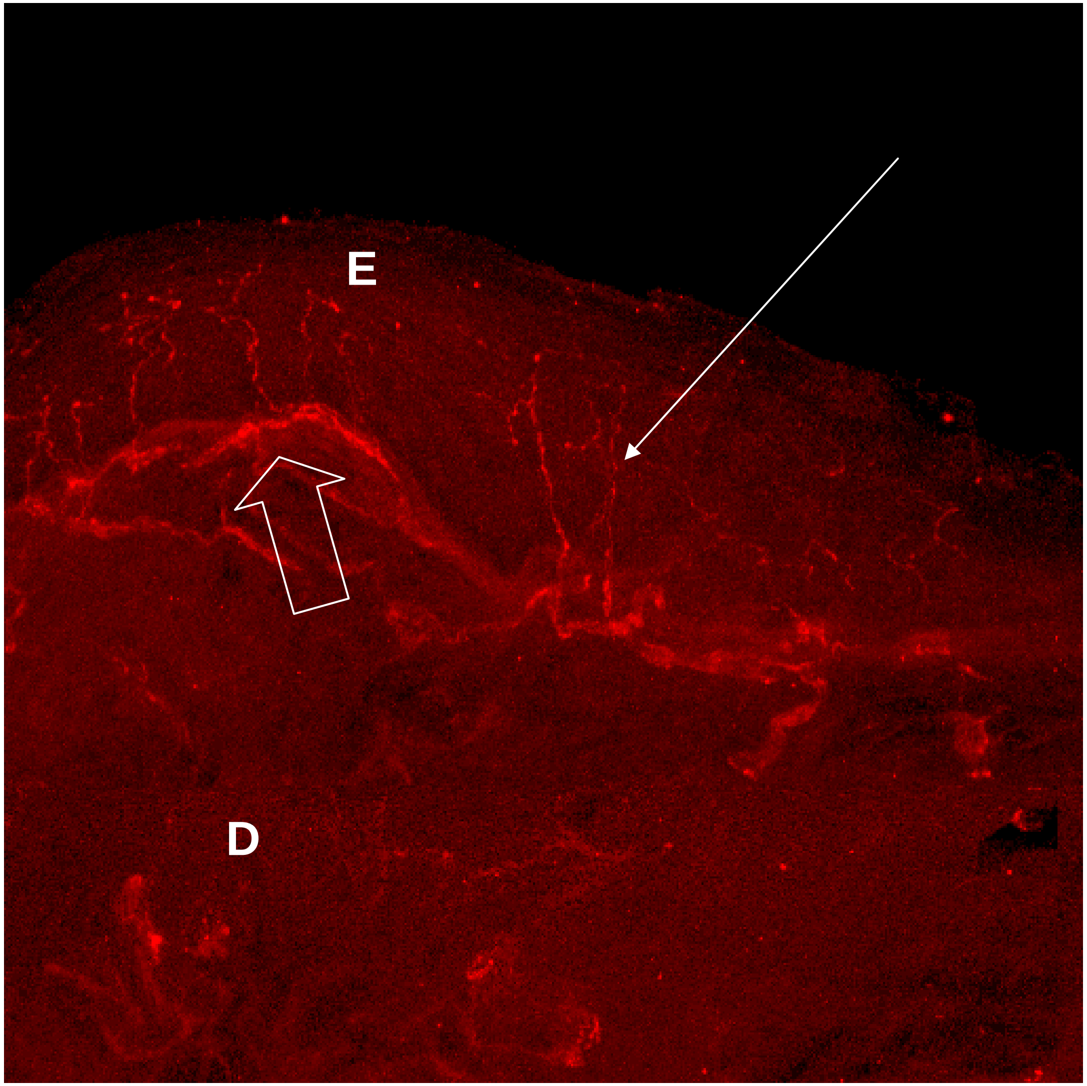


Bar = 50 υm

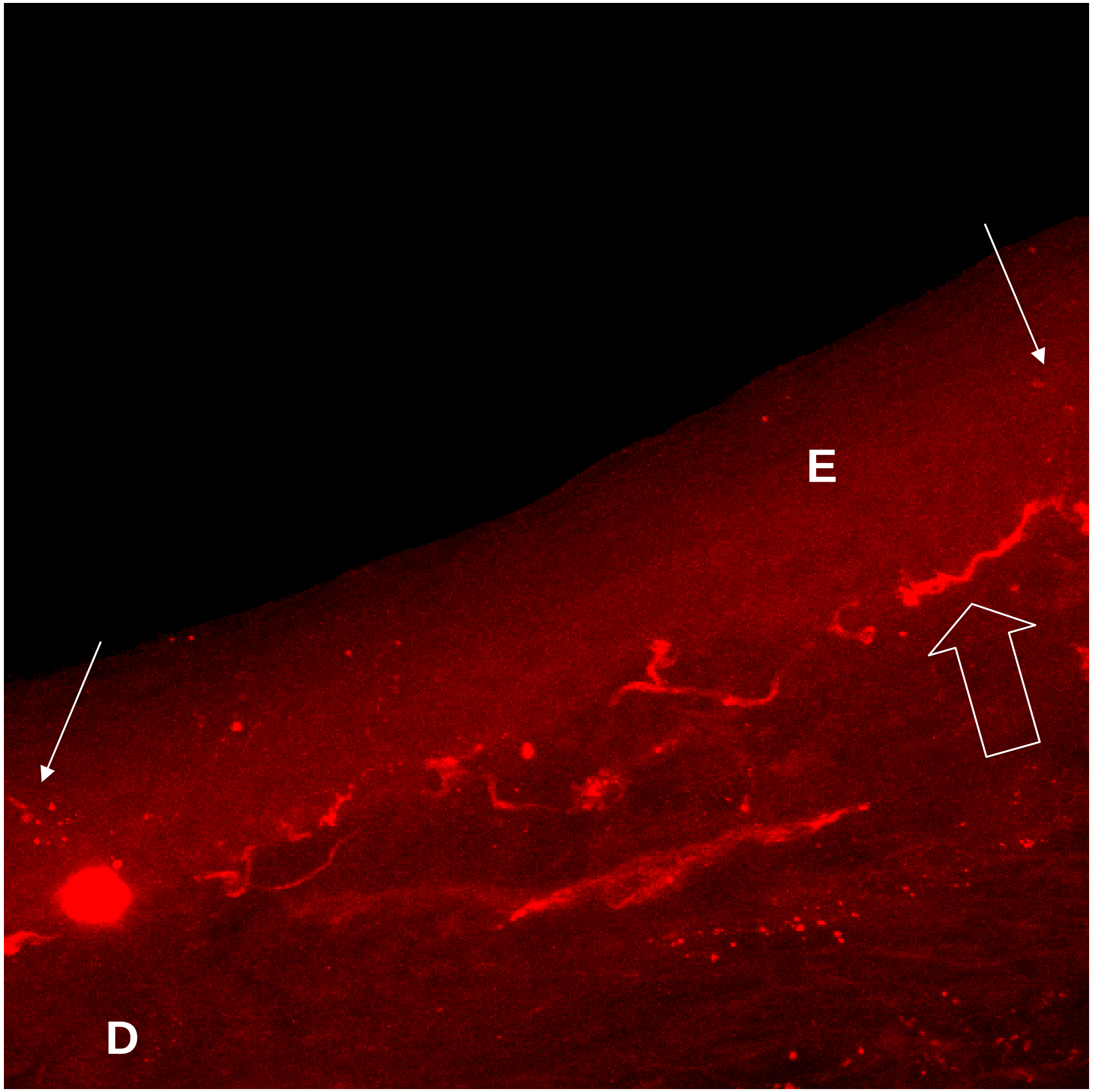
SOD2+/+ Dbdb



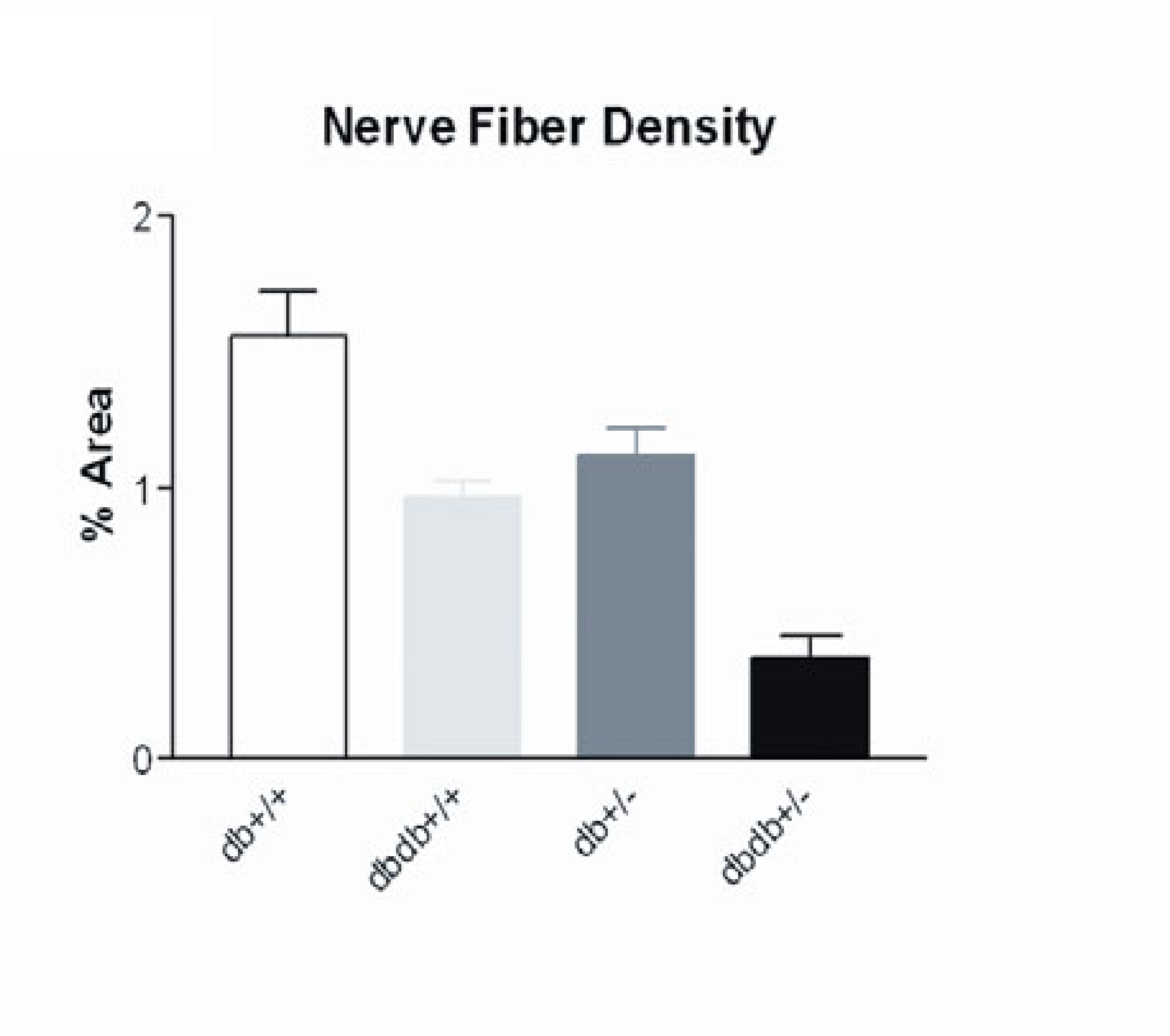
SOD2+/- Db+



SOD2+/- Dbdb



IntraEpidermal Fiber Density



Current Models (page 1)

ROS

signaling

Animal model	Background strain	current status	Phenotyping (begin-end)	Consortium collaborators
SOD2 +/- STZ	C57BL/6J	phenotyping complete; no increase in neuropathy or nephropathy	3-02 to 8-03	
SOD2 +/- db/db	C57BL/6J	Phenotyping complete; no increase in neuropathy or nephropathy	3-03 to 9 -03	
Nphs2 Cre//SOD2 loxP/loxP	C57BL/6J	phenotyping ongoing; no obvious increase nephropathy	12-03 to 12-04	Vanderbilt
Nphs2 Cre//SOD2 loxP/loxP	C57BL/6J 129SvJ	phenotyping nearing completion; no significant increase nephropathy	1-04 to 12-04	Vanderbilt
nestin Cre//SOD2 loxP/loxP	C57BL/6J	phenotyping ongoing	3-04 to 2-05	Vanderbilt
synapsin Cre//SOD2 loxP/loxP	C57BL/6J	Breeding complicated; phenotyping ongoing	4-04 to 4-05	Vanderbilt
Po Cre// SOD2 loxP/loxP	C57BL/6J	receiving mice—On hold	12-04 to 12-05	Vanderbilt
GCLC +/- STZ	C57BL/6J	On hold	6-03 to 5-04	
GCLC +/- db/db	C57BL/6J	phenotyping virtually complete; excellent neuropathy; no increase nephropathy	7-03 to 6-04	
AR tg//SOD2+/- STZ	C57BL/6	phenotyping ongoing; increased neuropathy in diabetics with double mutation	1-04 to 12-04	
GCLC +/-//SOD2 +/- STZ	C57BL/6	To begin breeding—On hold	12-04 to 12-05	
fyn -/-	C57BL/6J 129SvJ	phenotyping complete severe albuminuria in nondiabetics	6-03 to 8-03	
fyn -/- STZ	C57BL/6J 129SvJ	phenotyping completed: increased albuminuria in diabetics with mutation	6-03 to 8-03	
fyn -/-	129SvJ	rederivation completed; phenotyping ongoing. No albuminuria in nondiabetics	9-03 to 8-04	
fyn -/- and +/- STZ	129SvJ	phenotyping ongoing	9-03 to 9-04	
IRS-1 -/-	C57BL/6J	Breeding colony—On hold	?	

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Current Models (page 2)

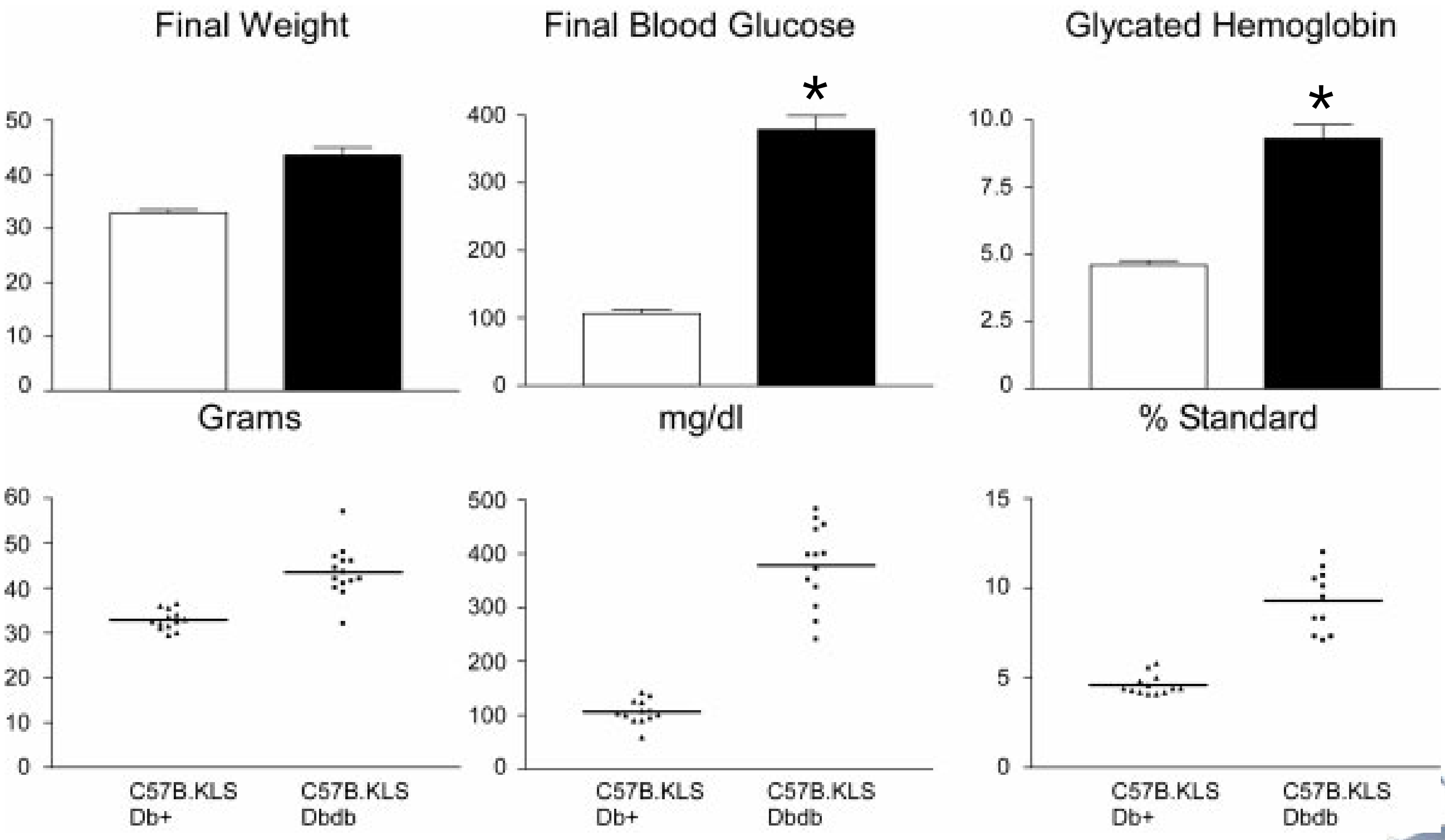
GLUTs

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	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)

Phenotyping: Current

	Tail Flick Hind Paw Every 8 weeks	NCV Measured 12 and 24 weeks post-diabetes	IEFD Measured at tissue harvest
C57BLKS Animals being added	X	X	X
GCLC +/- and +/+ **Dbdb and Db+**	X	X	Ongoing
C57Bl6 Dbdb "Breeder" **Chow**	4 animals		
nestin Cre// **SOD2 LoxP/LoxP**	X (9 animals)	X (9 animals)	
synapsin Cre// **SOD2 LoxP/LoxP**	Animals Breeding		
fyn (-/-)(+/-)(+/+) STZ treated and control	X	X	
GLUT4 (-/-) (+/+) STZ treated and control	X	X	

C57BlKS



* P< 0.001

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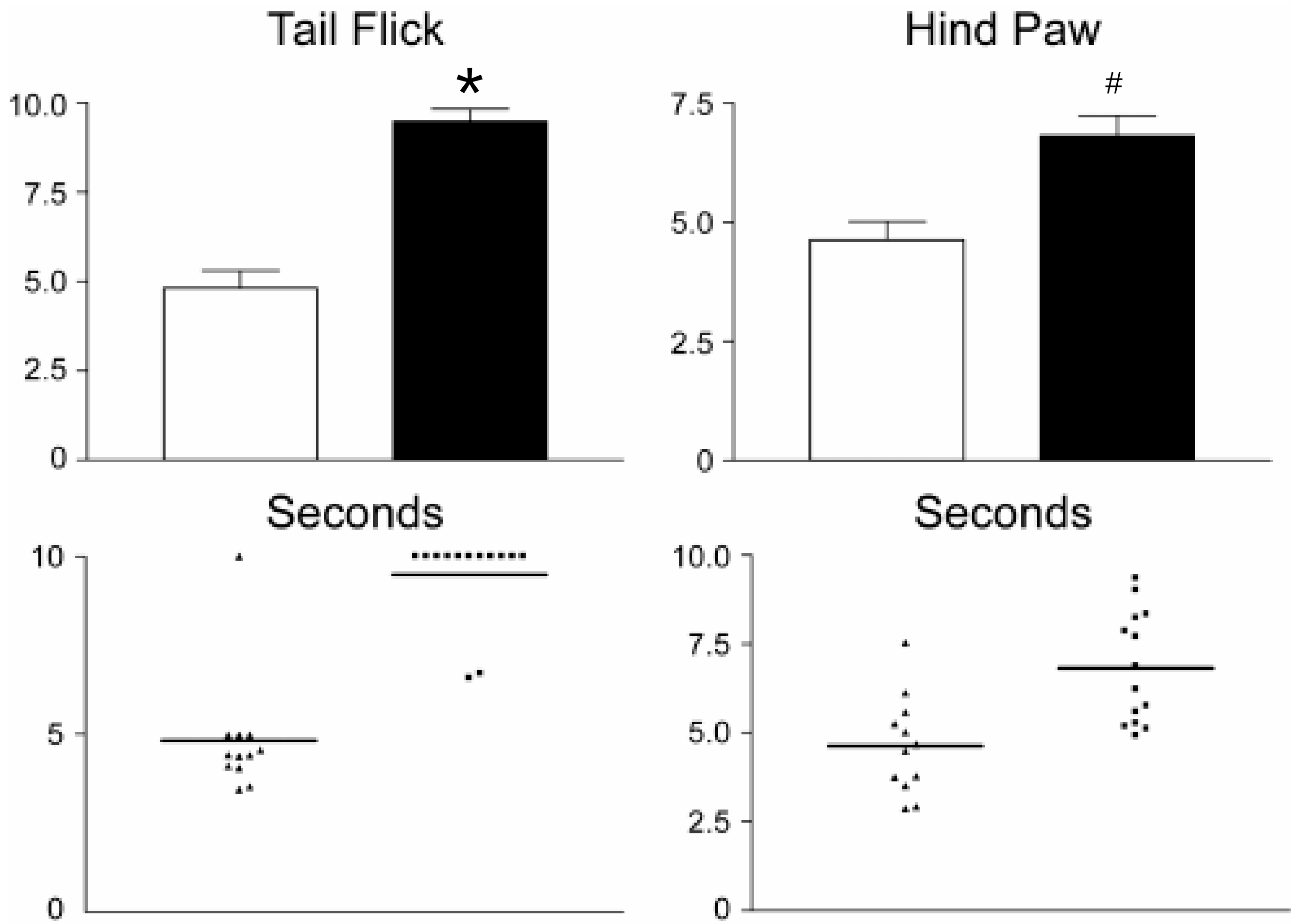
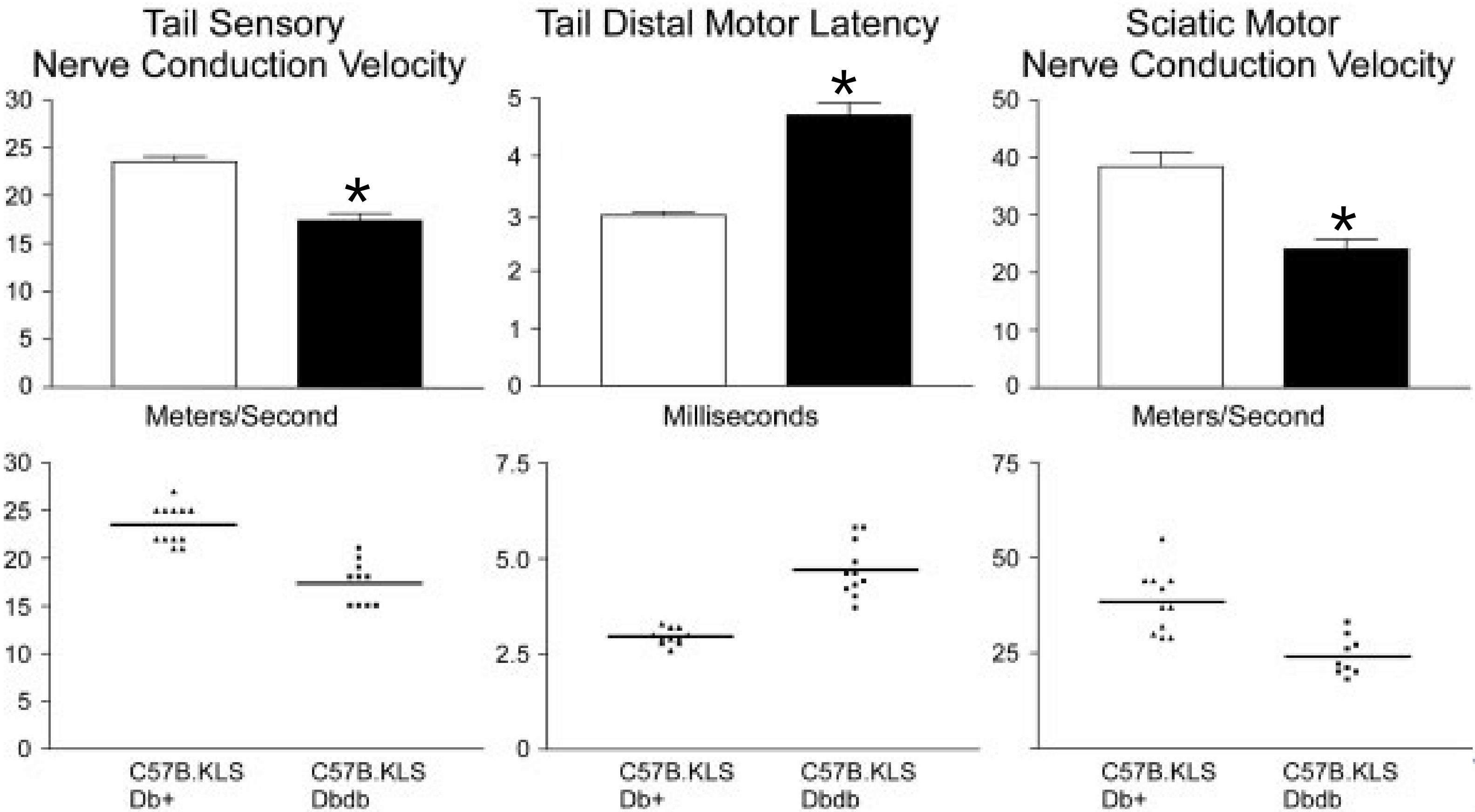
C57BlKS : Sensory Tests



* P< 0.001

# P < 0.05

C57BlKS : Nerve Conductions



* P< 0.001

Collaborations

- Firouz Daneshgari, Cleveland Clinic Foundation
  - Measures of bladder elasticity and emptying
  - Plans to examine innervation density similar to foot pad biopsy method
- Jan Breslow, Rockefeller University
  - Will send foot pad biopsies to Michigan for IEFD
- Timothy Kern, Case Western Reserve University
  - Developing plans to send whole eyes from Michigan mice
- David Clemmons and Timothy Nichols, University of North Carolina
  - Have sent ankle and hip skin, dorsal root ganglia and sciatic nerves from hypercholesterolemic pigs

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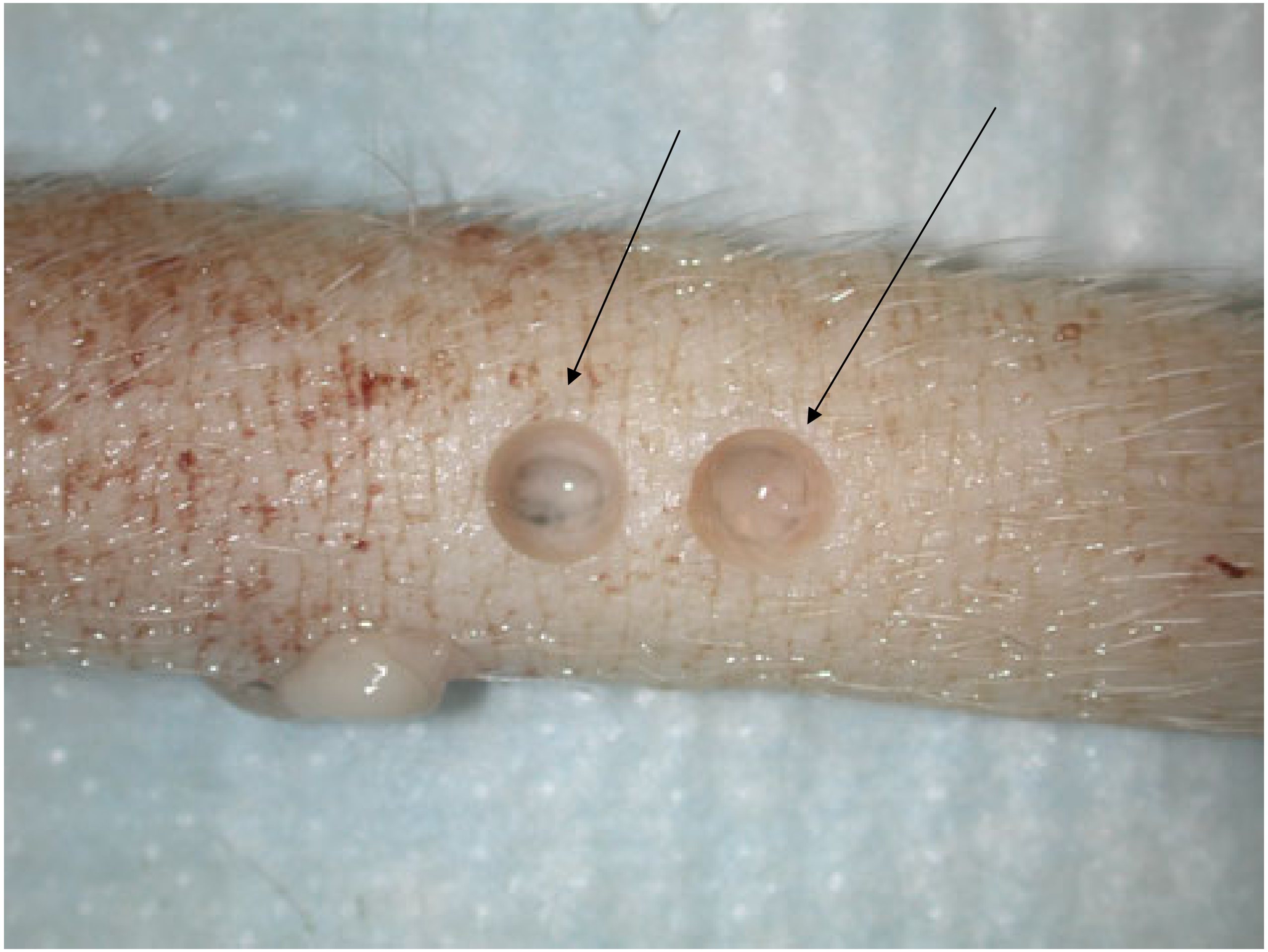
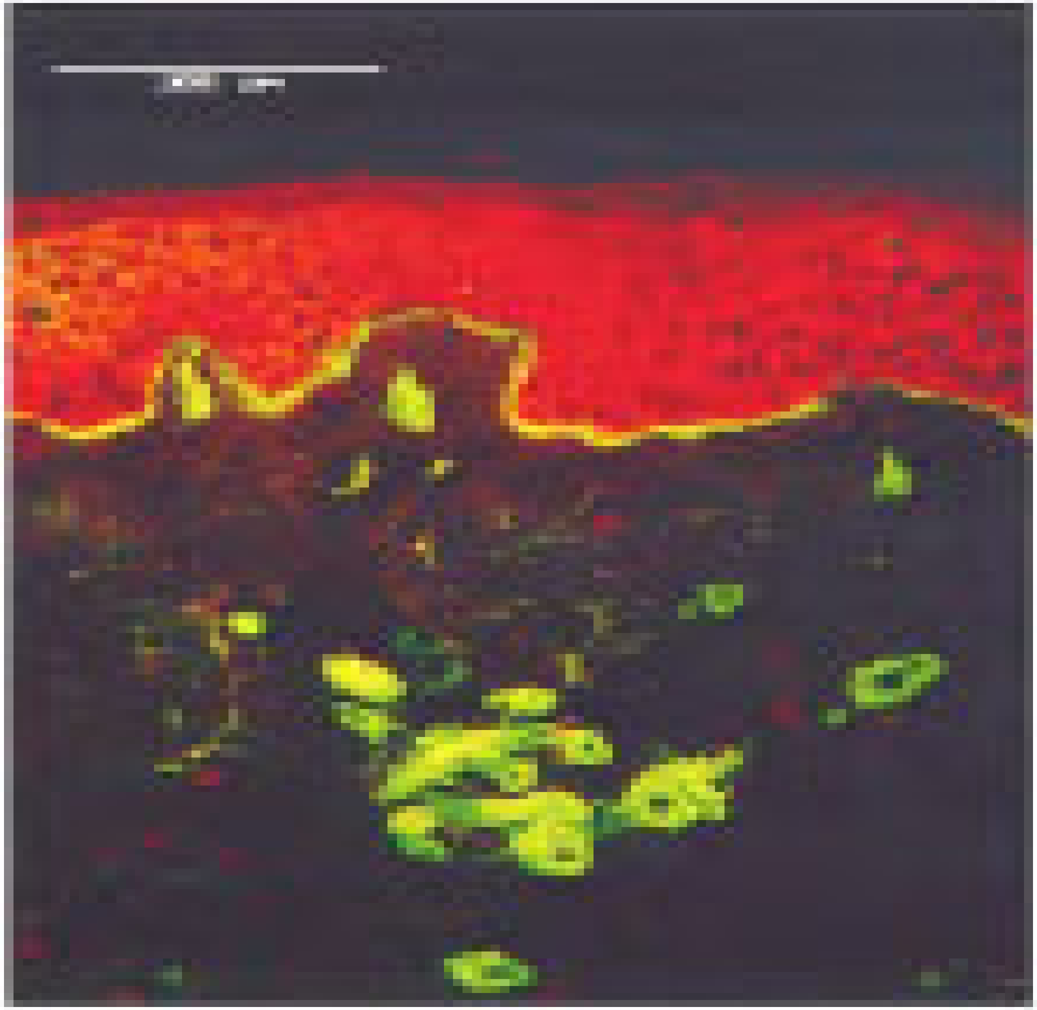
Porcine Skin

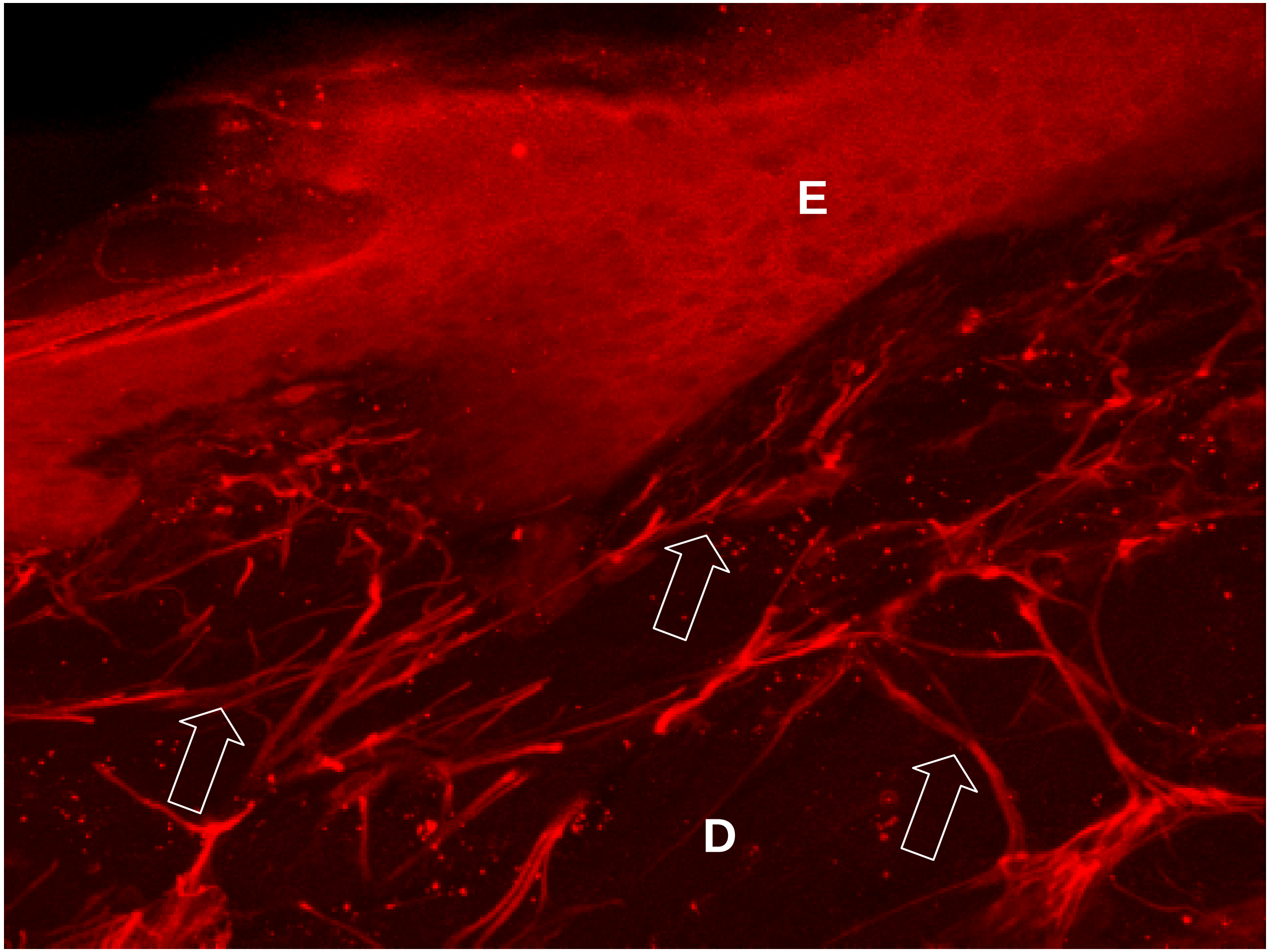


Porcine Skin



J. Anat. 190:601-611, 1997

IEFD Porcine Skin



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AMDCC Neuropathy Core is fully functional and can perform screening via footpads for IEFD or terminal phenotyping

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