

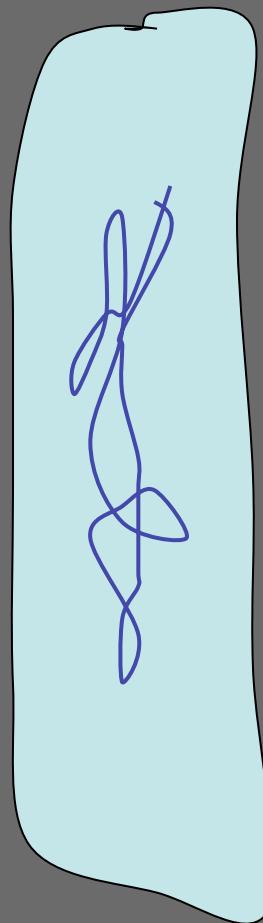
# Extensive Tissue Variation In the Nuclear Envelope Proteome

BSPR/EBI Conference 2011  
From the Visible to the Hidden Proteome  
July 12, 2011

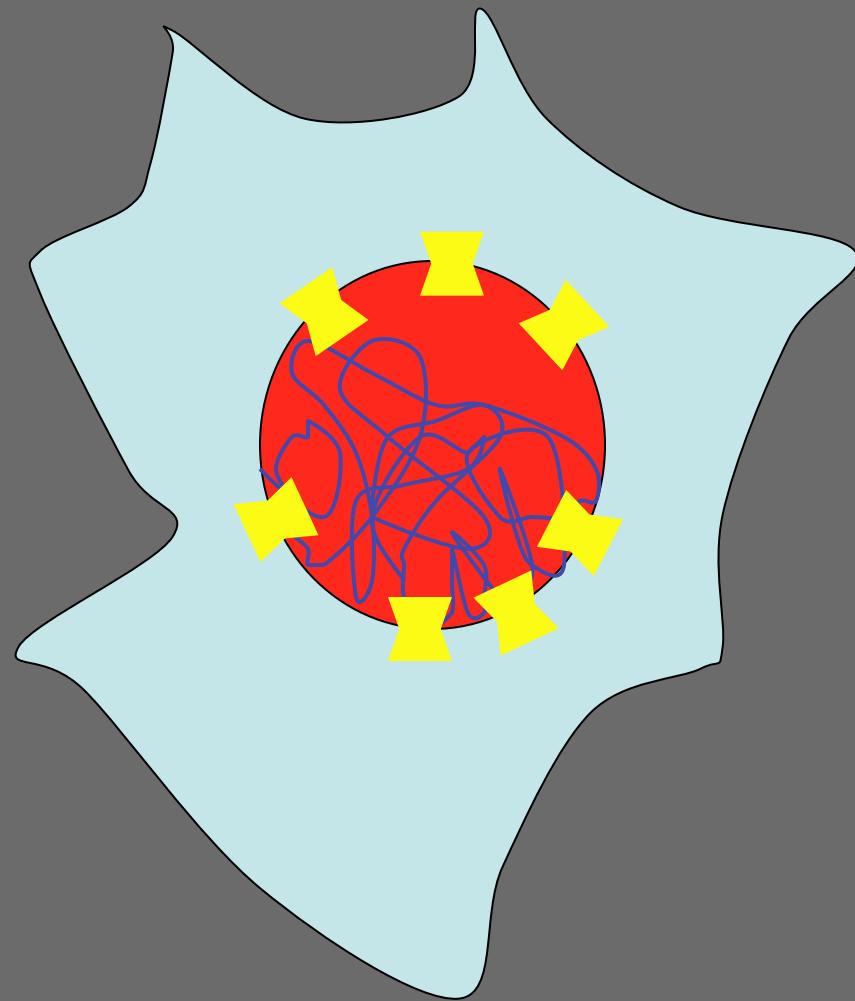
Eric C Schirmer  
Wellcome Trust Centre *for* Cell Biology  
University of Edinburgh

# The Nuclear Envelope In Evolution

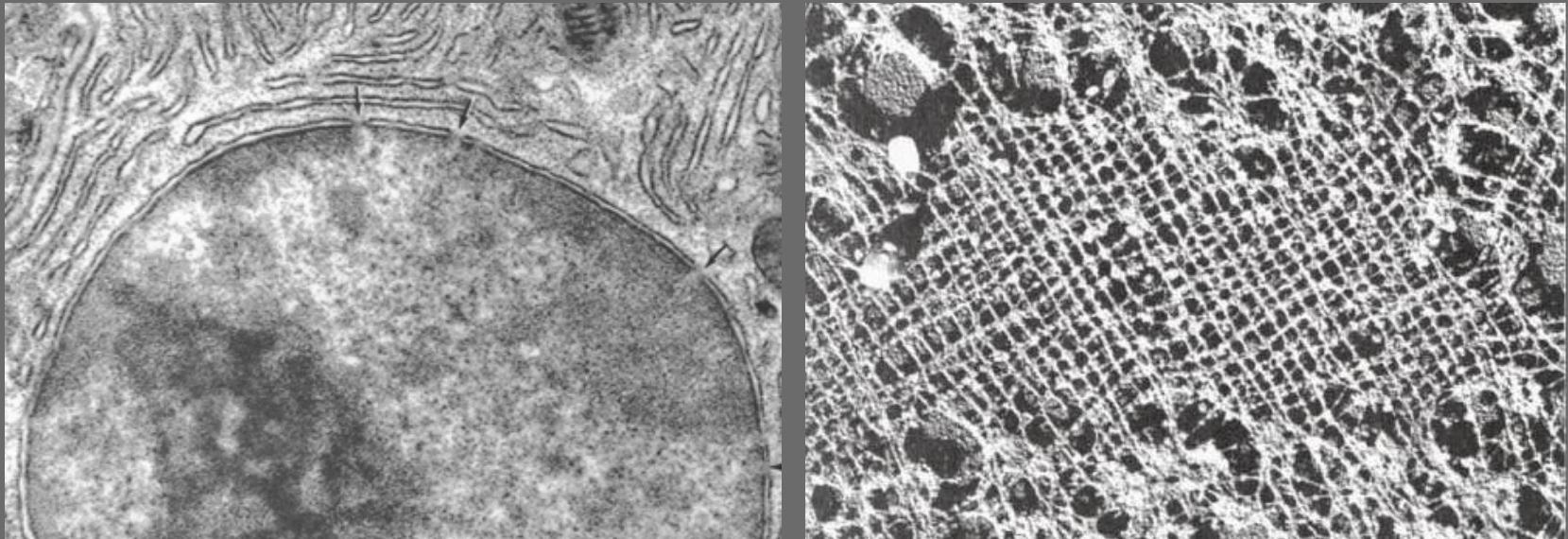
Prokaryote



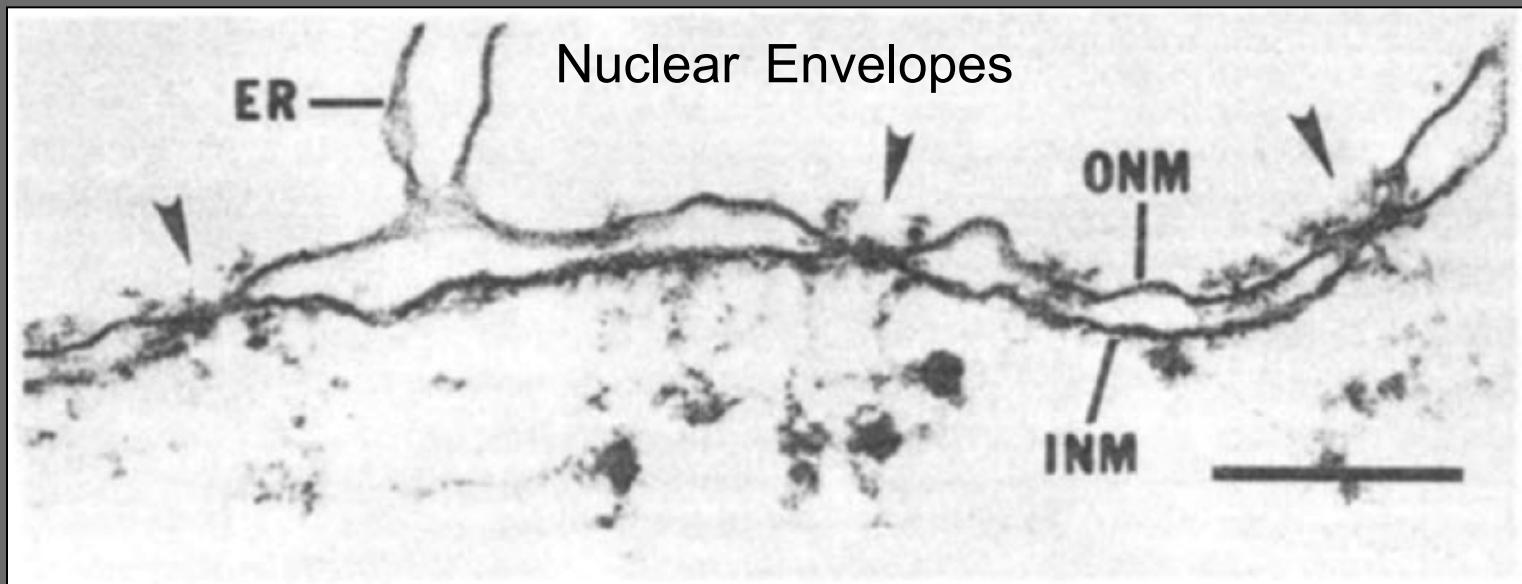
Eukaryote



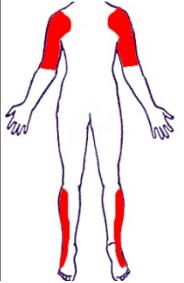
# Organisation of The Nuclear Envelope



Aebi et al., *Nature* 1986



Gerace and Burke *Annu Rev Cell Biol* 1989



## Muscle

Muscular dystrophy

*LMNA/EMERIN*  
*SUN2/Nesprin*



## Nerves

Neuropathy

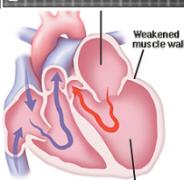
*LMNA/Torsin A*



## Skin

Dermopathy

*LMNA/FACE1*



## Heart

Cardiomyopathy

*LMNA/LAP2 $\alpha$*



## Bone

Melorheostosis

*MAN1/LBR*



## Fat

Lipodystrophy

*LMNA*



## Aging

Progeria

*LMNA*

# How Can Nuclear Envelope Protein Mutations Produce Tissue-Specific Disease Pathology?

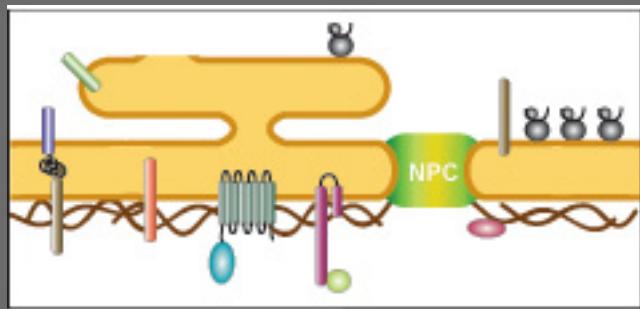
**Conundrum:** mutations in widely expressed NE proteins cause different tissue-specific diseases

**Hypothesis:** as yet unidentified tissue specific NE proteins direct the specific tissue pathologies

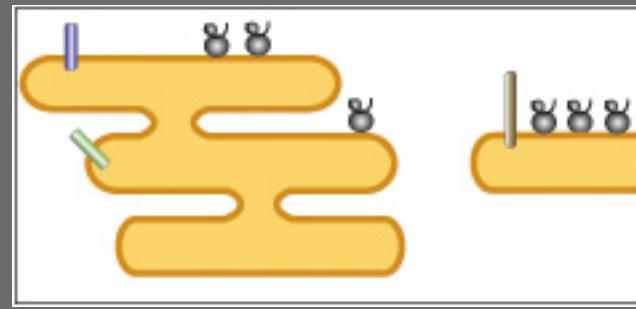
**Experiment:** test if NE proteome differs among tissues

# Subtractive Proteomics

*isolated  
nuclear envelopes*

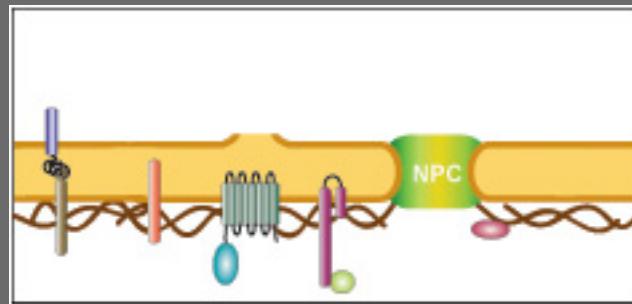


*isolated  
microsomal membranes*



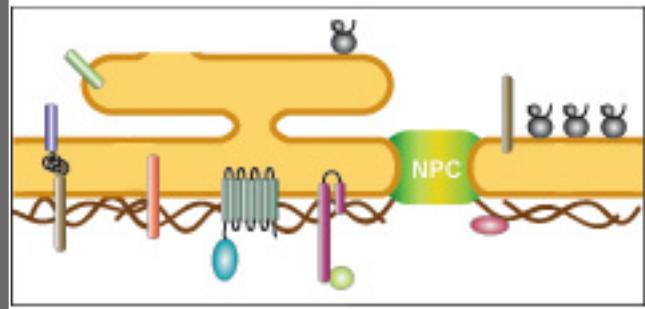
*in silico  
nuclear envelopes*

=

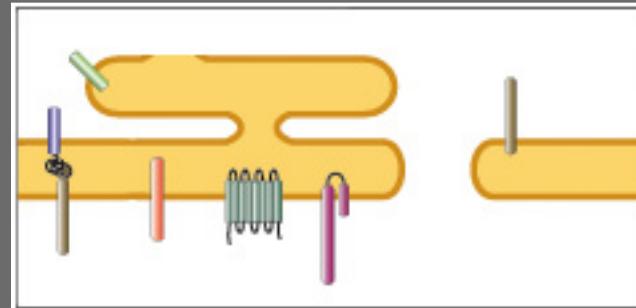


# Multiple Enriched Fractions

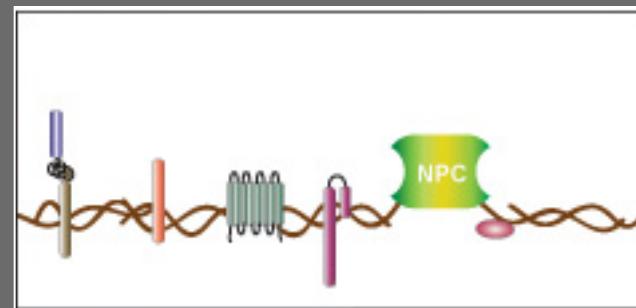
*isolated nuclear envelopes*



*alkali-extracted*



*salt/detergent-extracted*

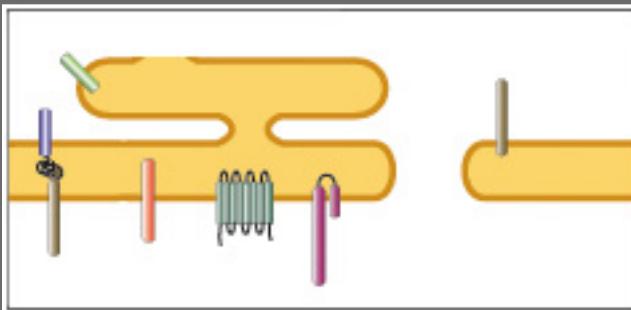


Schirmer et al., Science 2003

Schirmer and Gerace, TiBS 2005

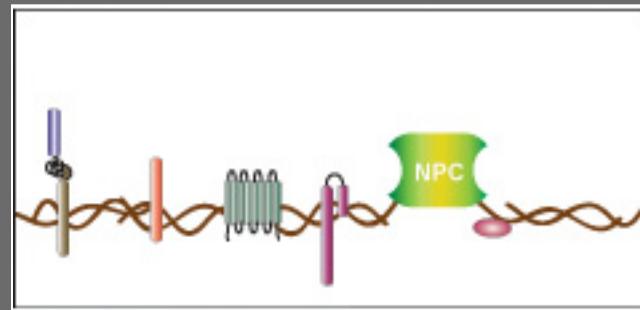
# Multiple Digestions

*Membrane Proteins*



*extremely hydrophobic*

*Intermediate Filaments*



*extremely insoluble*

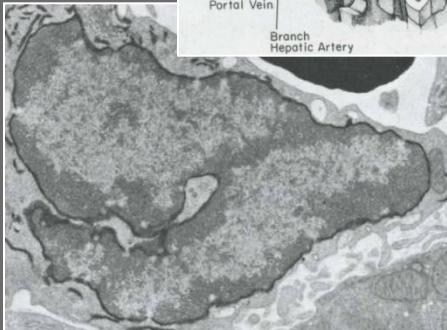
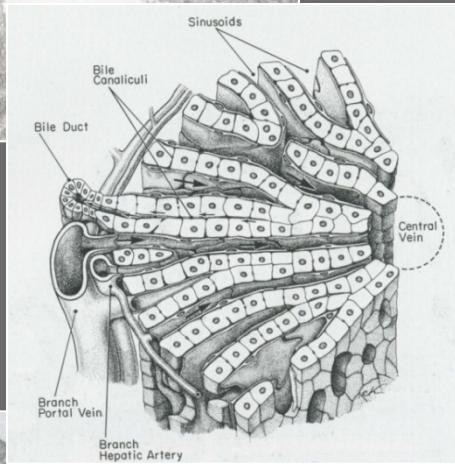
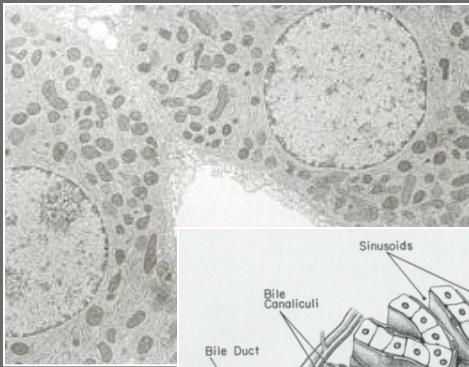
Trypsin digestion → Soluble → MudPIT



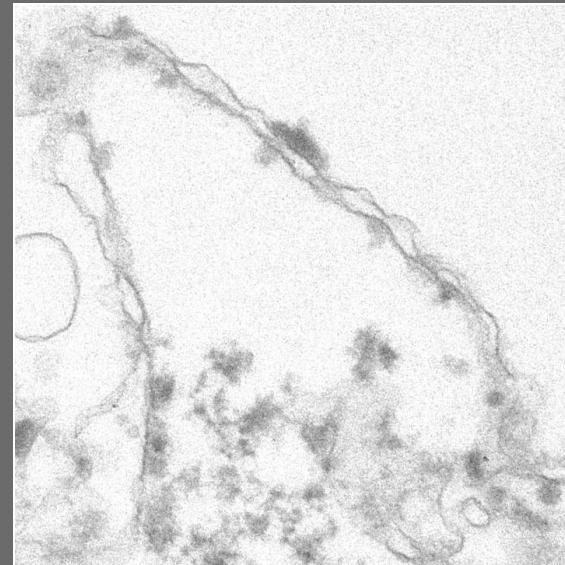
Pellet → PK digestion → MudPIT

# Nuclear Envelope Proteomics of Liver

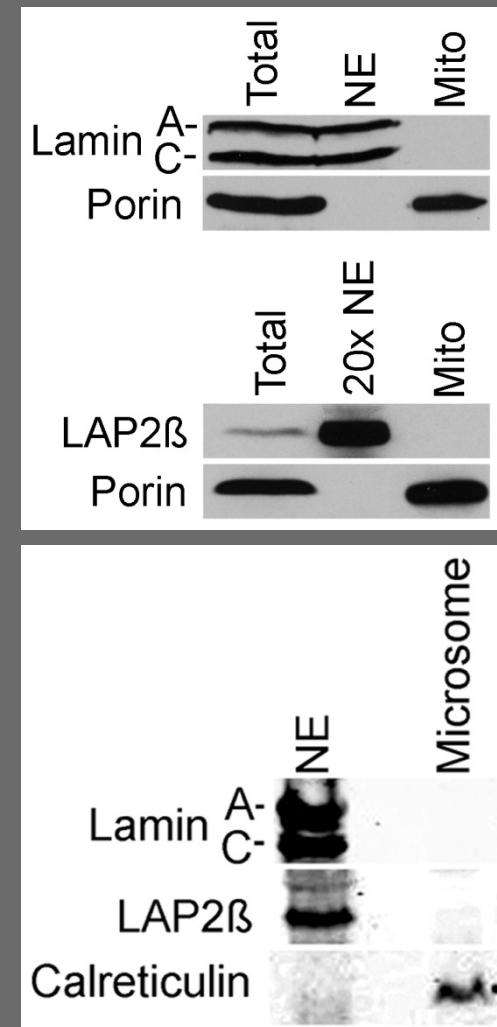
## Liver Sections



## Nuclear Envelopes



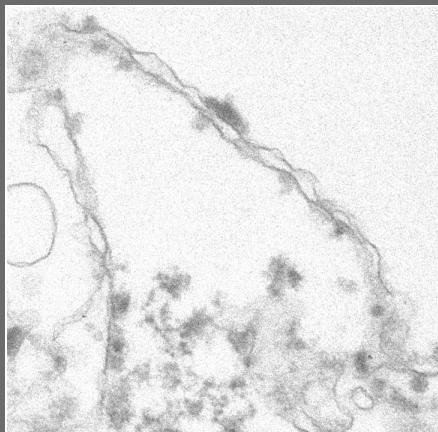
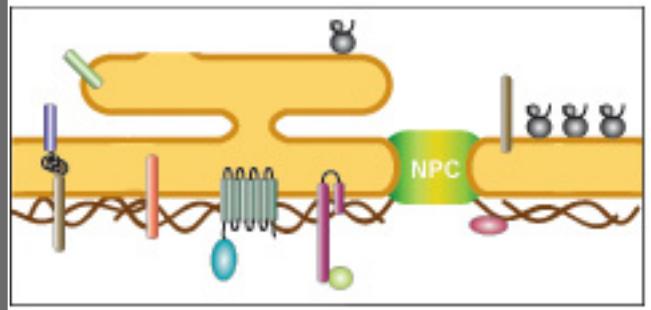
## Fraction Purity



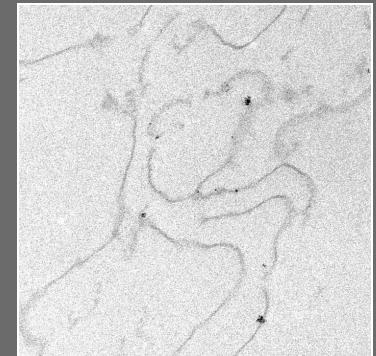
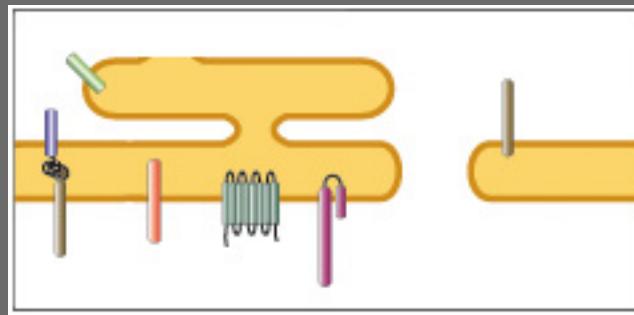
Schirmer et al., Science 2003  
Korfali et al., in revision

# Fractions Further Cleaned By Extractions

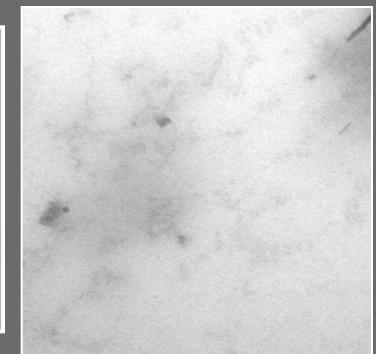
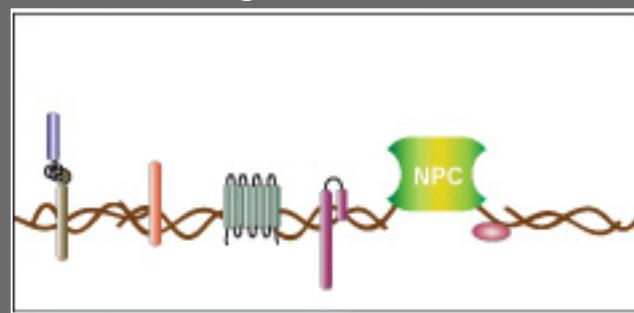
*isolated nuclear envelopes*



*alkali-extracted*



*salt/detergent-extracted*

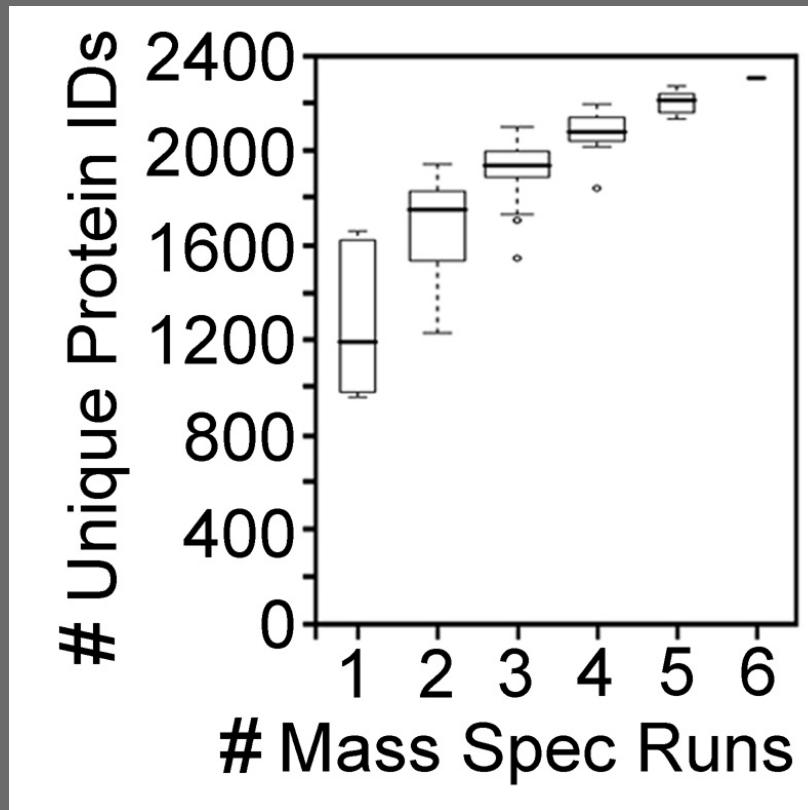


0.1 M NaOH  
Extracted

1%  $\beta$ -octylglucoside, 400 mM KCl  
Extracted

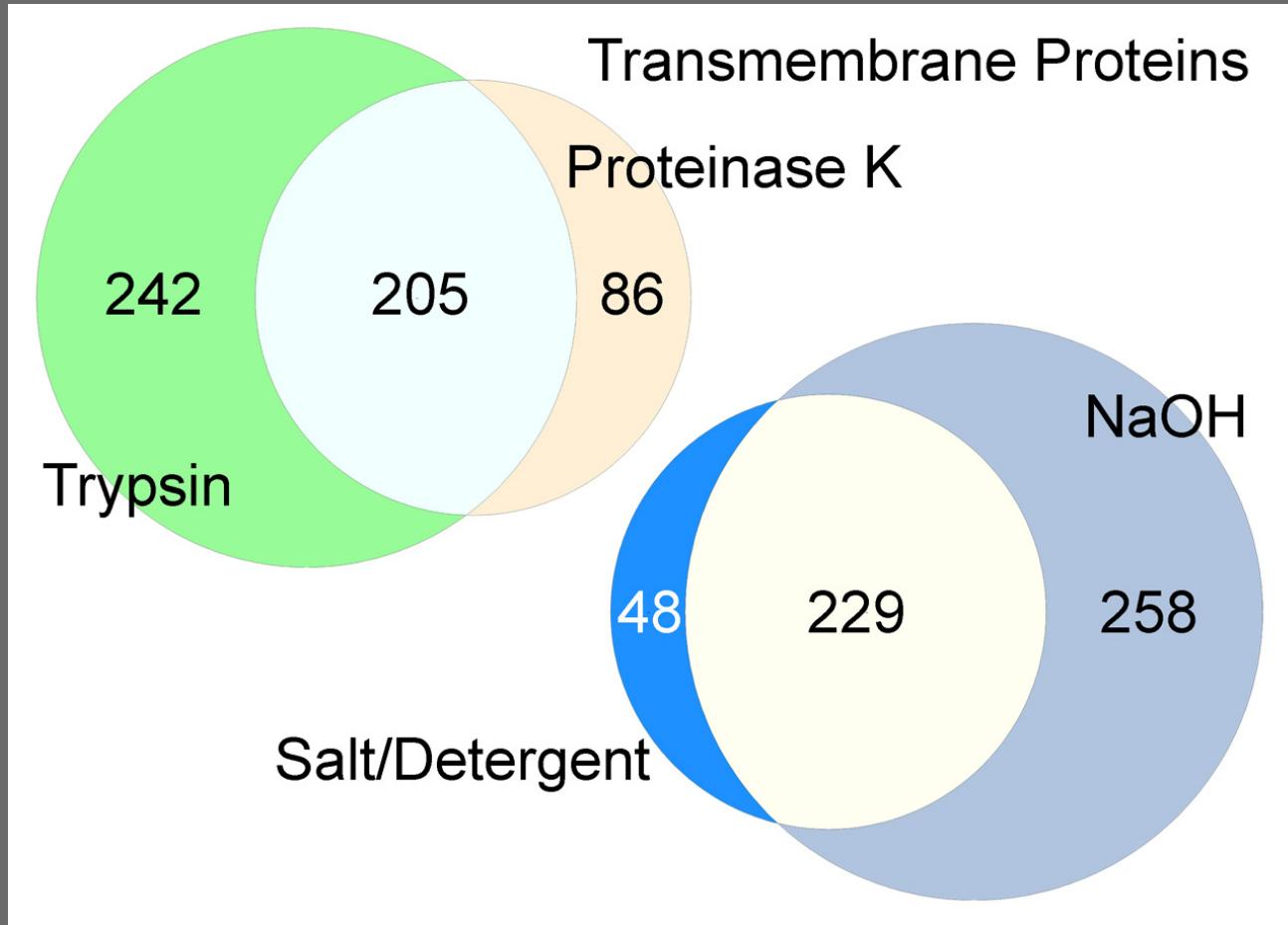
Schirmer et al., Science 2003  
Schirmer and Gerace, TiBS 2005

# Multiple Runs Needed With Complex Fractions

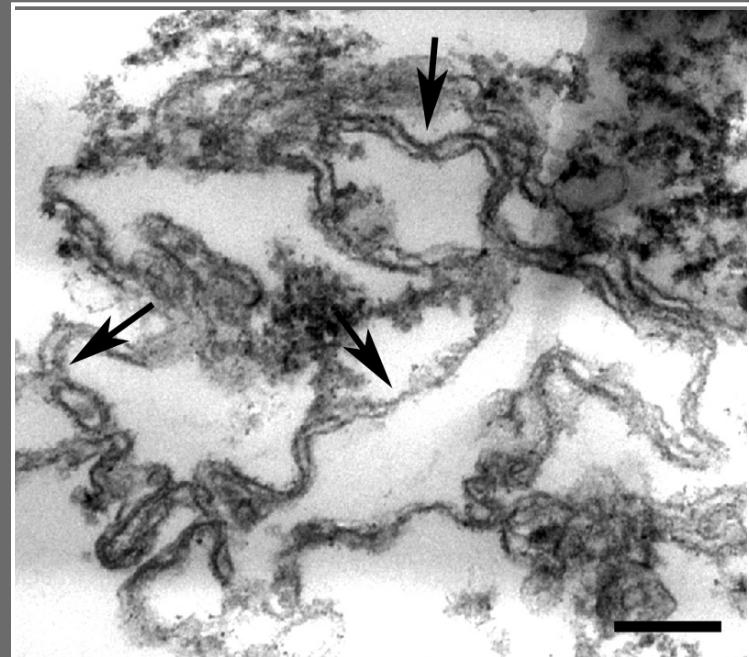
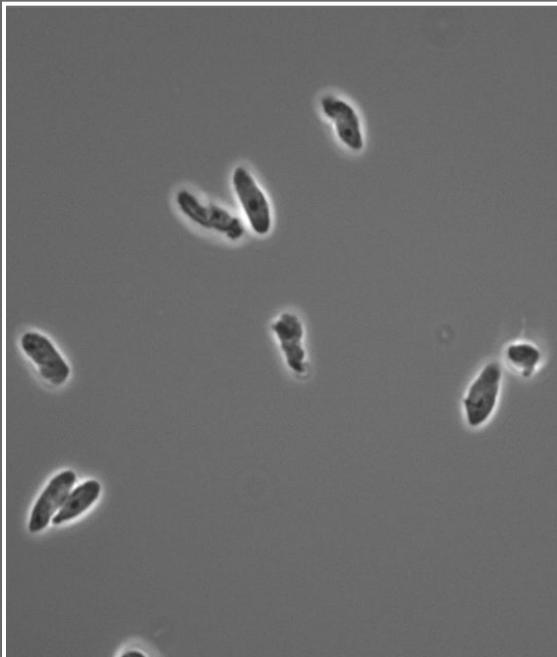
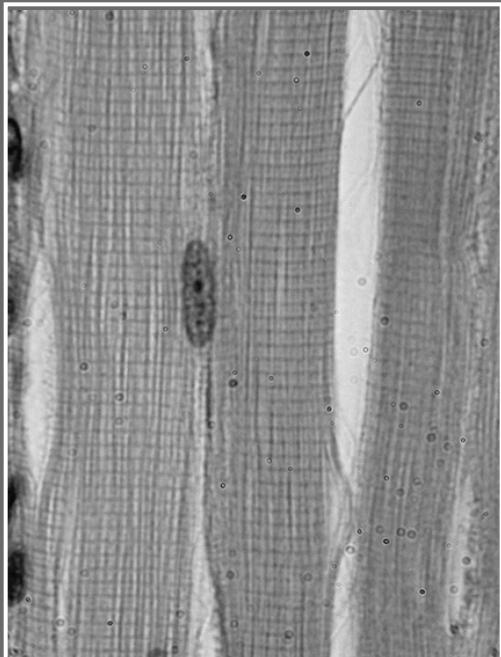


*Same trypsin sample divided equally for four runs  
plus same PK sample divided equally for two more runs*

# Both Multiple Extractions AND Multiple Digests Increased Transmembrane Proteins Identified

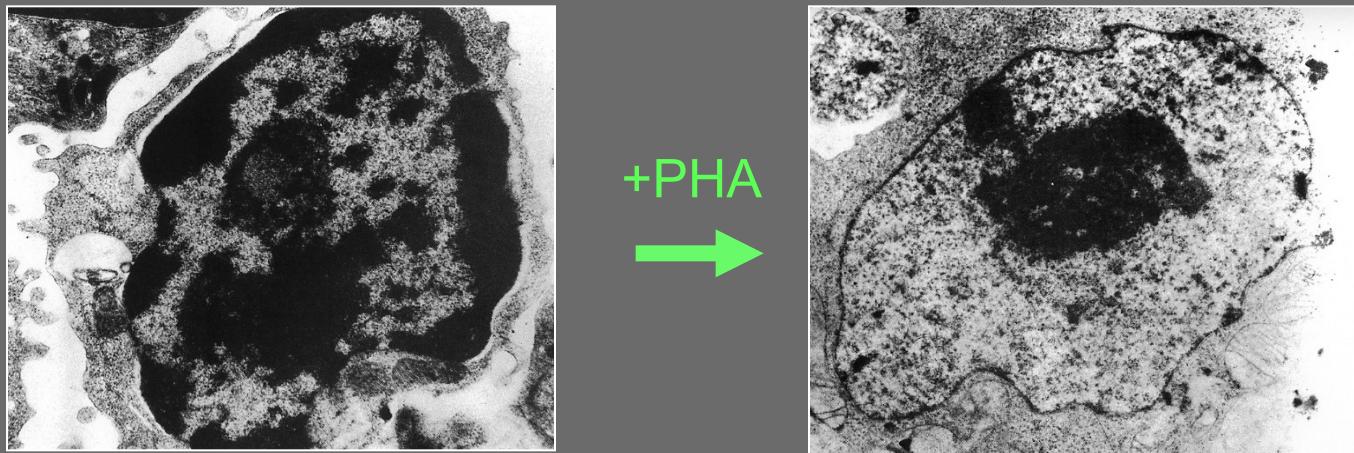
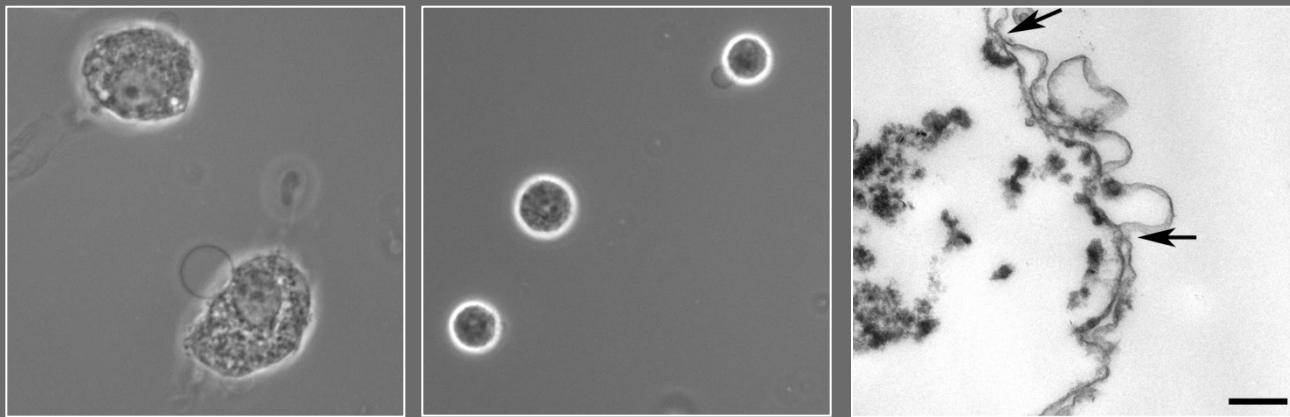


# Nuclear Envelope Proteomics of Skeletal Muscle

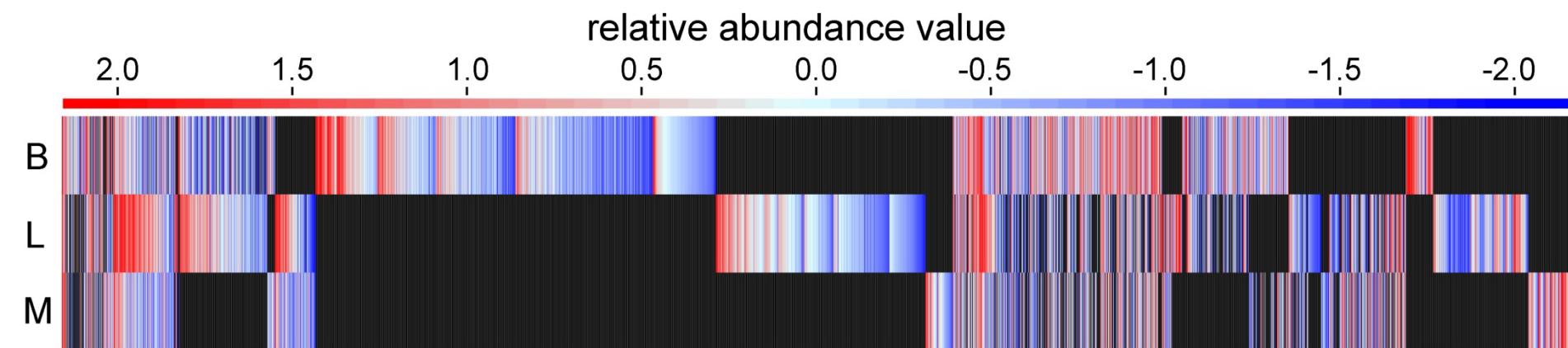


Wilkie et al., *Methods Mol Biol* 2008  
Wilkie et al., *Mol Cell Proteomics* 2011

# Nuclear Envelope Proteomics of Blood Leukocytes



# Tissue Variation In The Nuclear Envelope Proteome



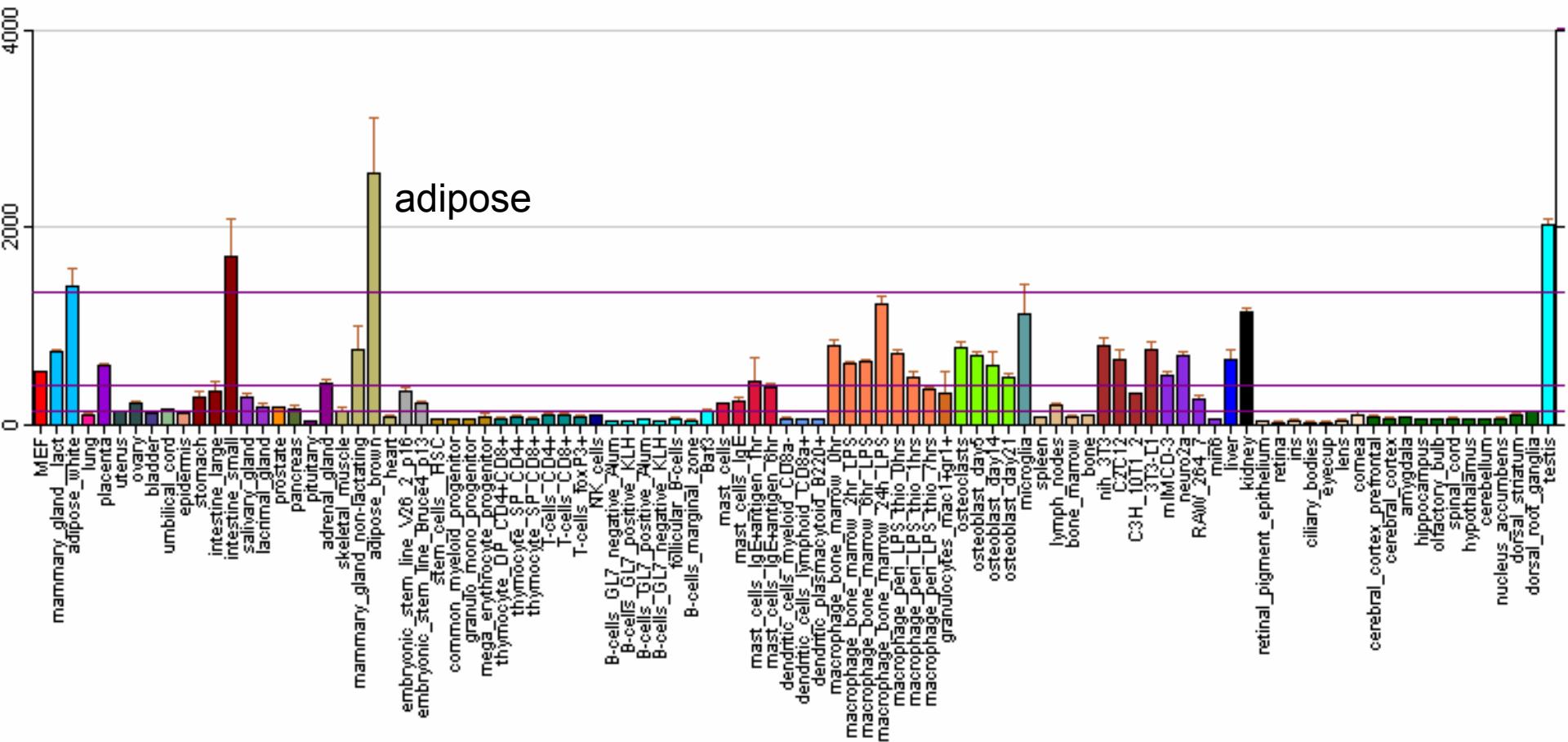
B = Blood, L = Liver, M = Muscle

Black no peptides identified

dNSAF scores used to estimate relative abundance also yield variation

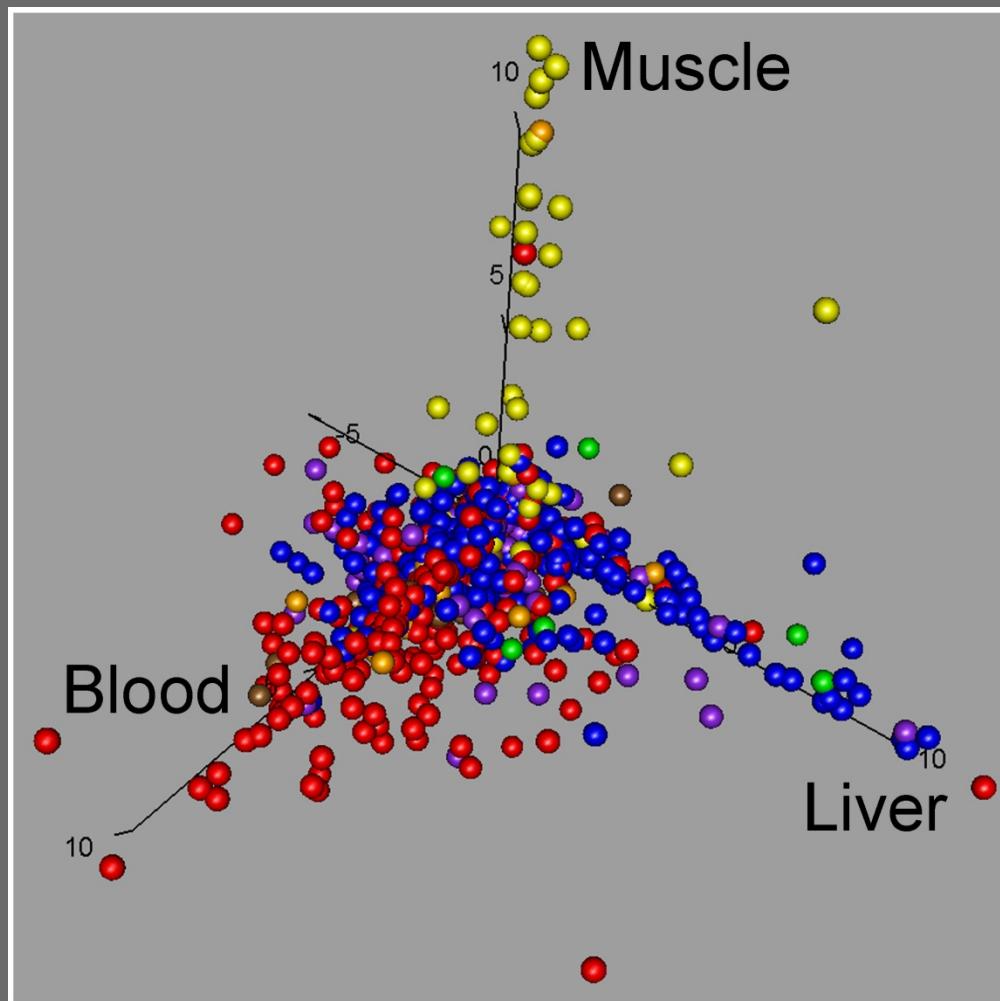
# NETs In The BioGPS Transcriptome Database

NET29

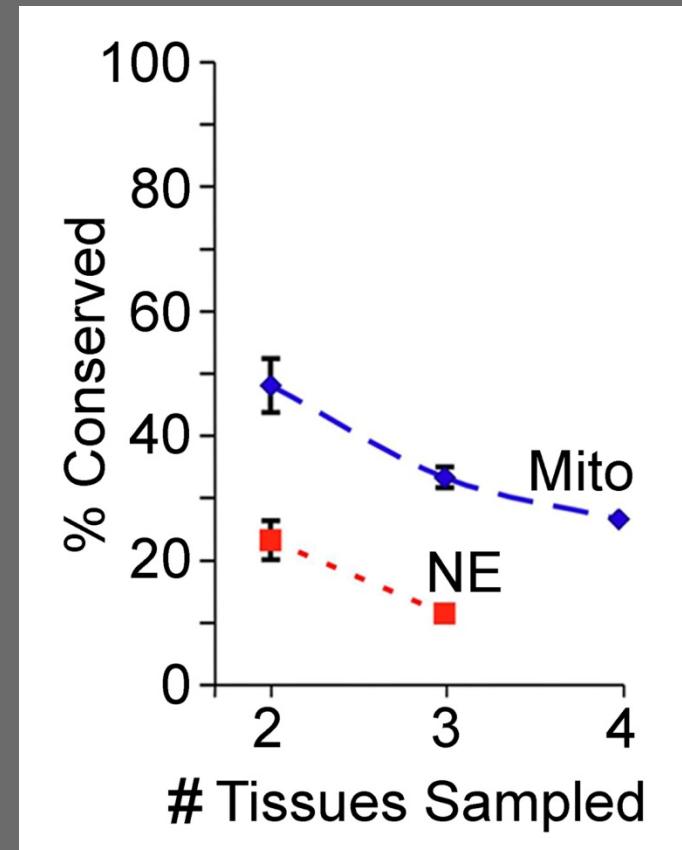
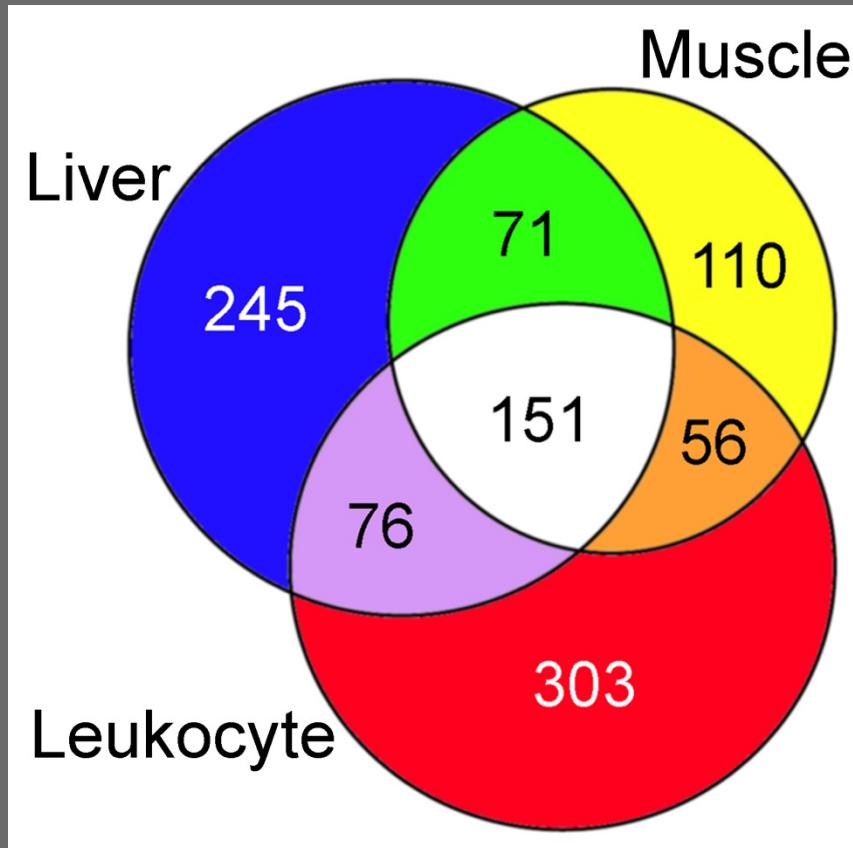


# Proteome Tissue Variation Correlates With Transcriptome Tissue Variation

From BioGPS

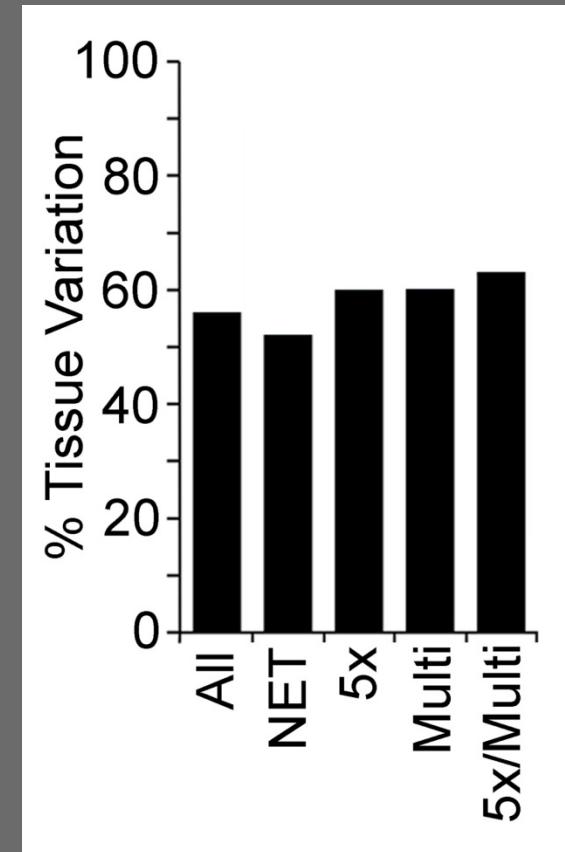
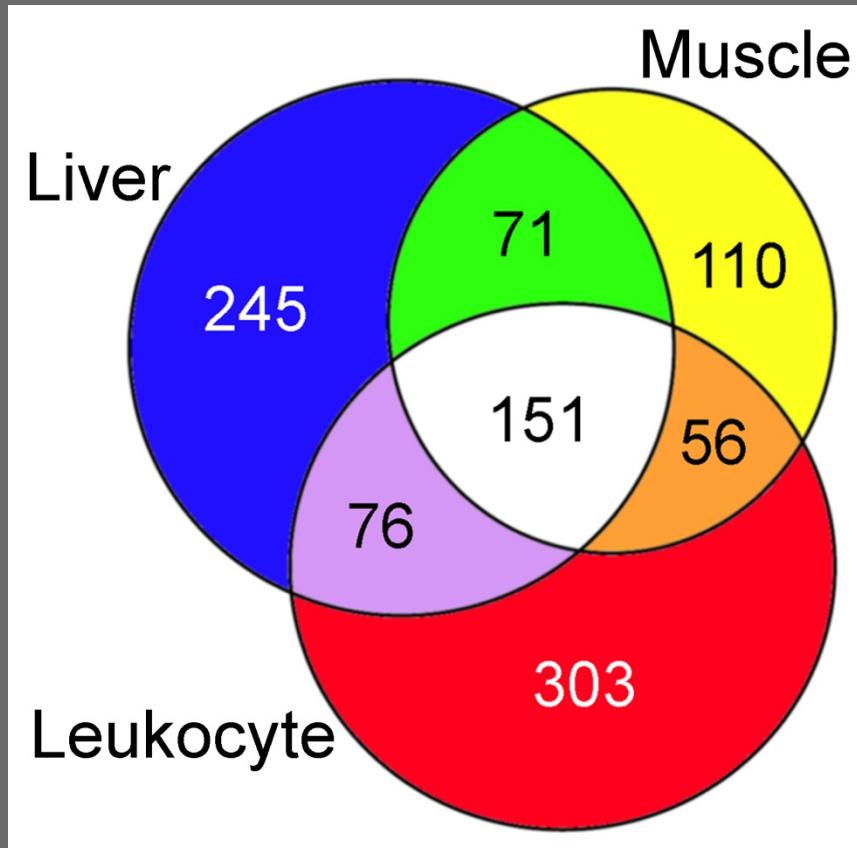


# General Organelle Proteome Tissue Variation



*Mitochondria data from  
Mootha et al., Cell 2003*

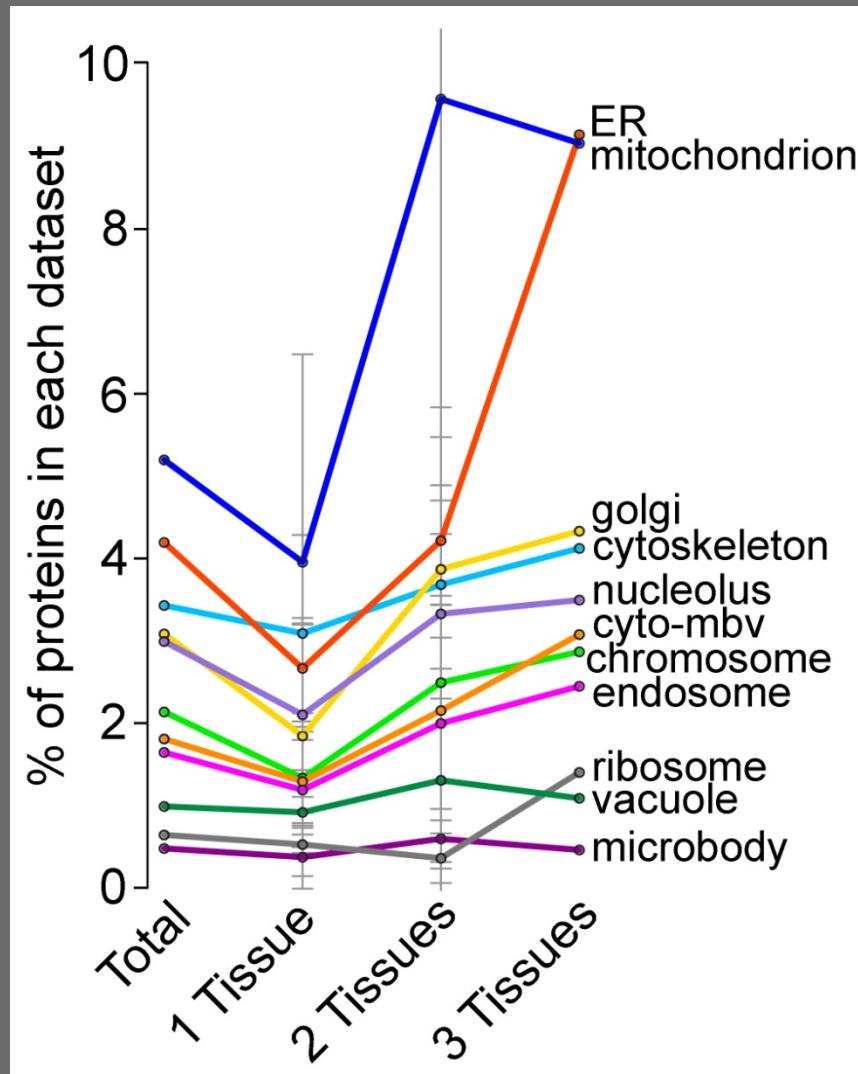
# General Organelle Proteome Tissue Variation



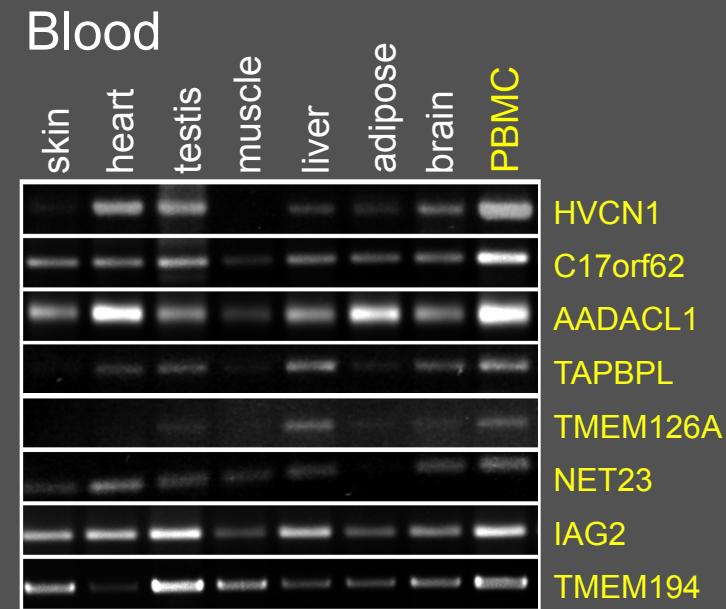
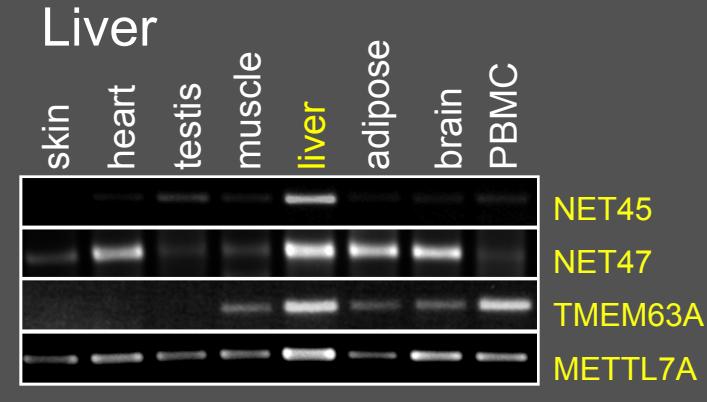
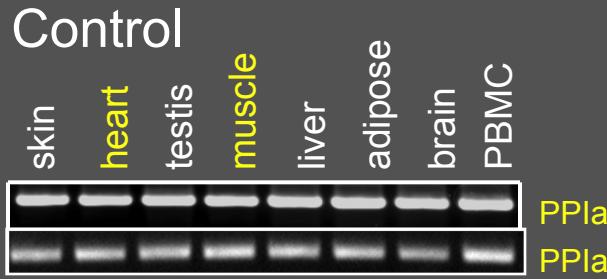
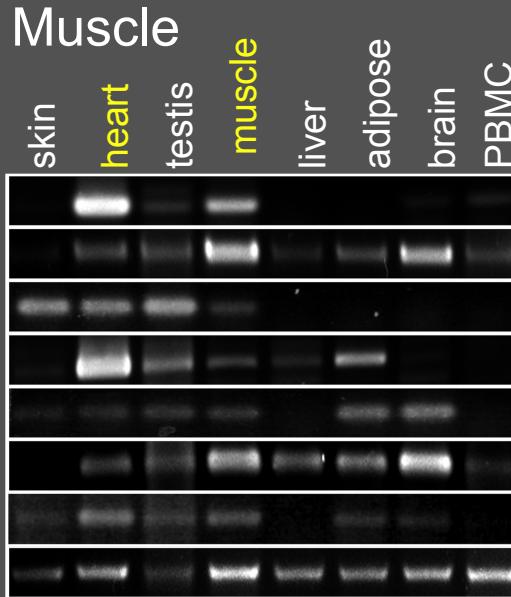
similar variation observed using increased stringency

# Putative ‘Contaminants’ ID’ d By GO-Terms NOT Reproducibly ID’ d, Except ER and Mitochondria

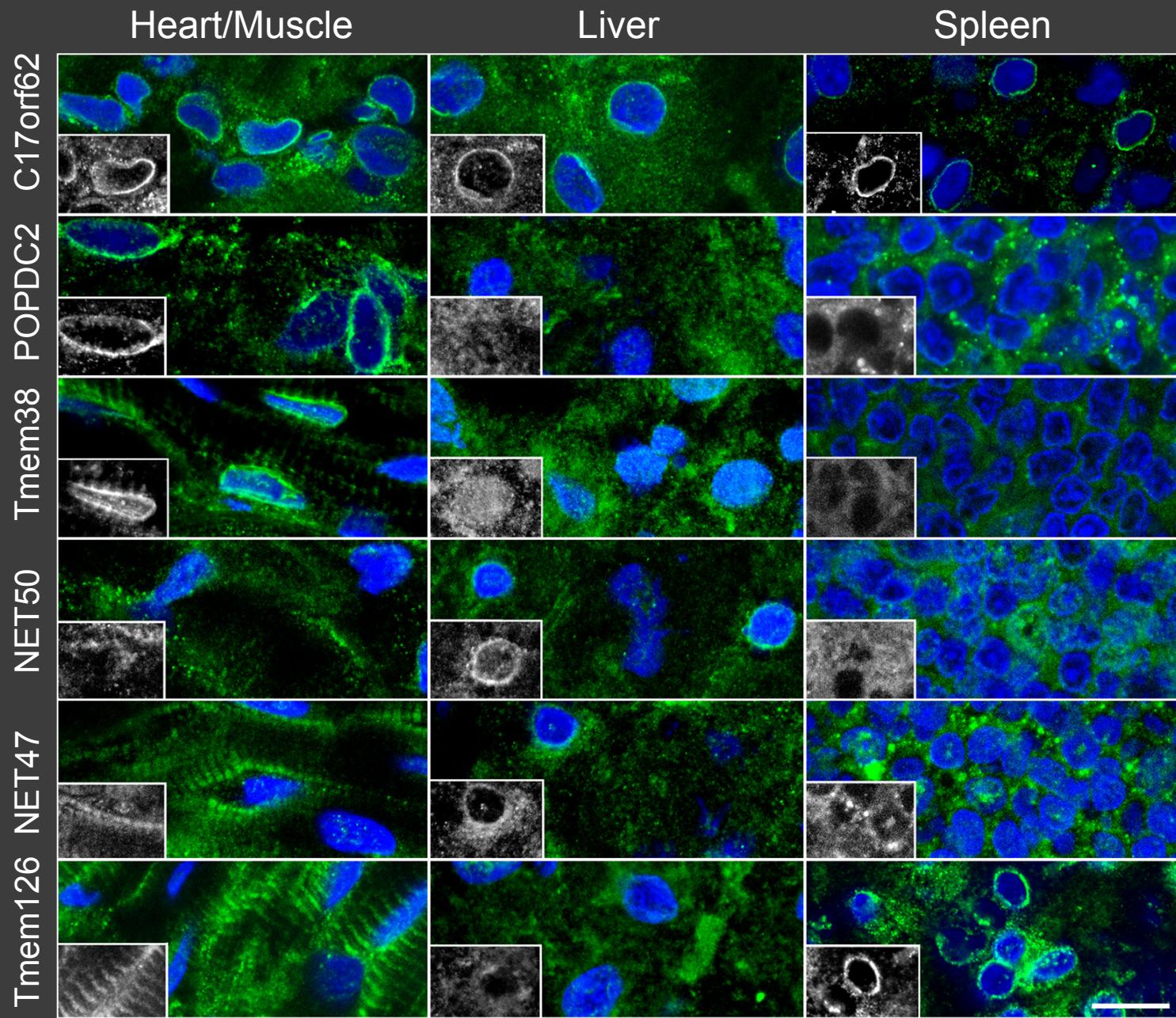
GO-Localisation Terms



# Some Novel Blood and Muscle NETs Exhibit Tissue Preferential/ Restricted Expression

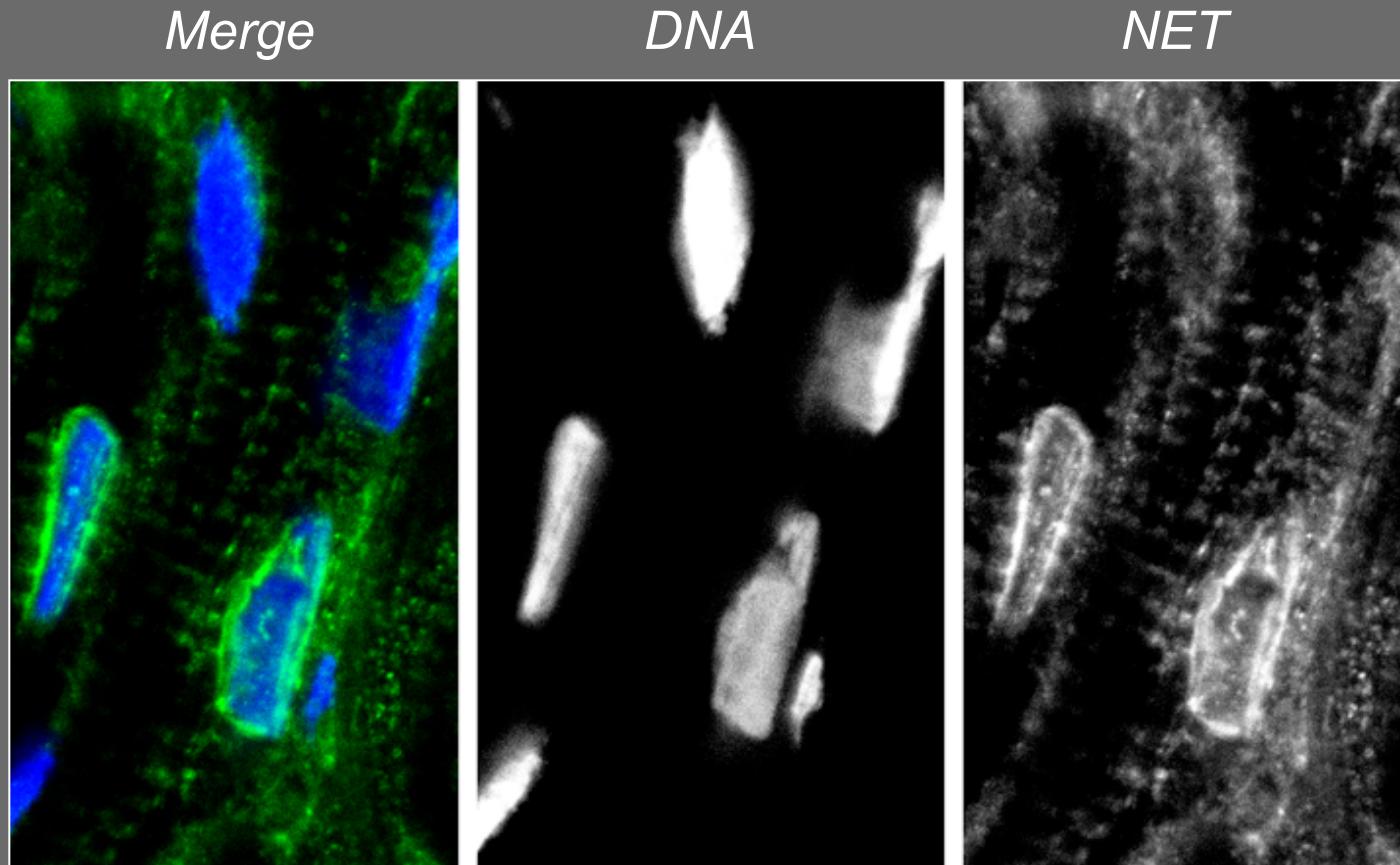


# NETs Only Appear In Tissues Where Recovered



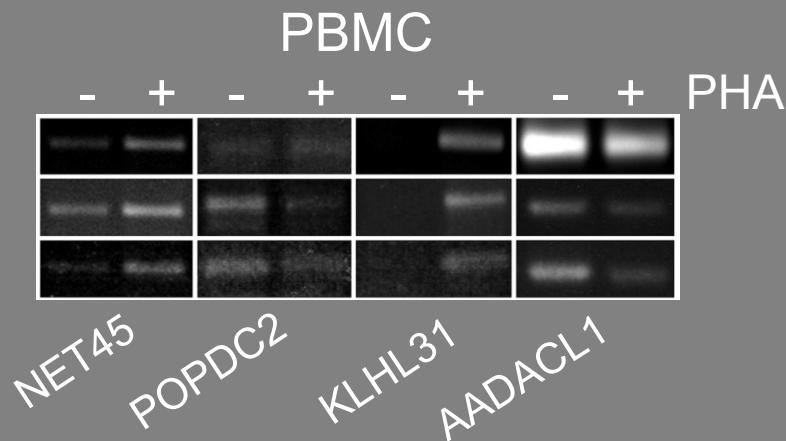
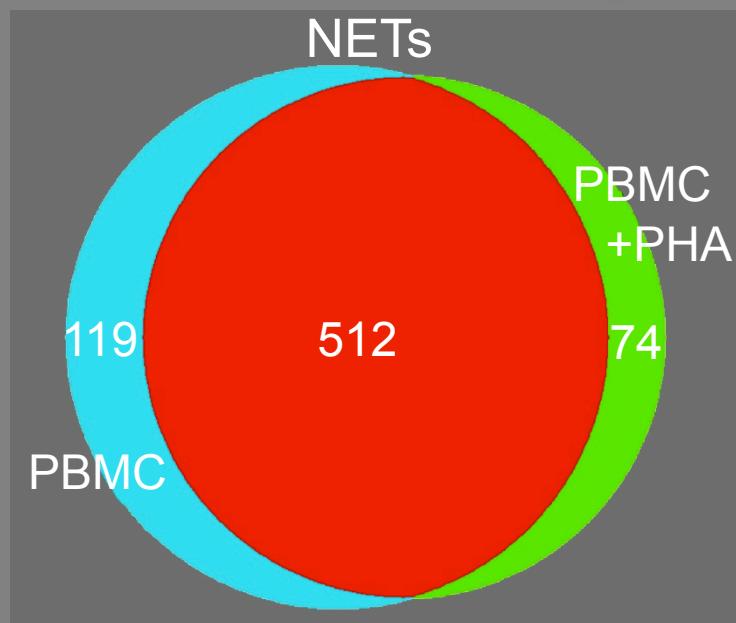
Vlastimil  
Sršen

# Even Within A Tissue, Cell Type Specificity Is Frequently Observed

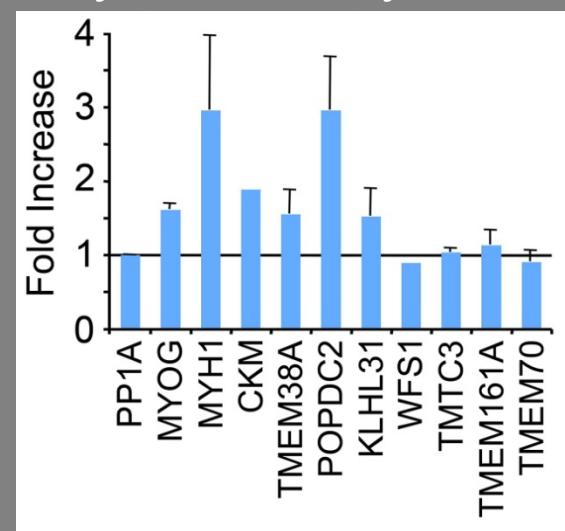


*Heart cryosections*

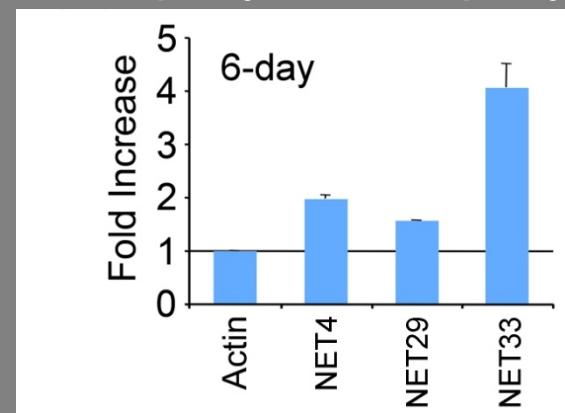
# Levels of Some NETs Increase During Differentiation Of Specific Tissues

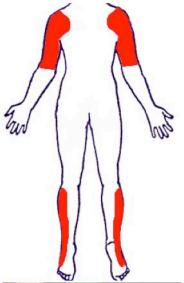


Myoblast to Myotube



Pre-adipocyte to Adipocyte





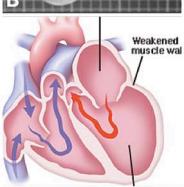
Muscle  
Muscular  
dystrophy  
*LMNA/EMERIN*  
*SUN2/Nesprin*



Nerves  
Neuropathy  
*LMNA/Torsin A*



Skin  
Dermopathy  
*LMNA/FACE1*



Heart  
Cardiomyopathy  
*LMNA/LAP2 $\alpha$*



Bone  
Melorheostosis  
*MAN1/LBR*

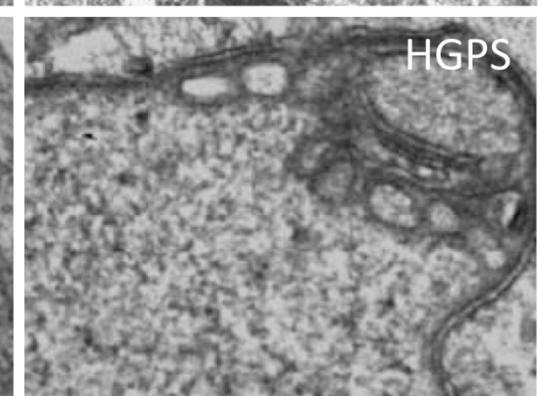
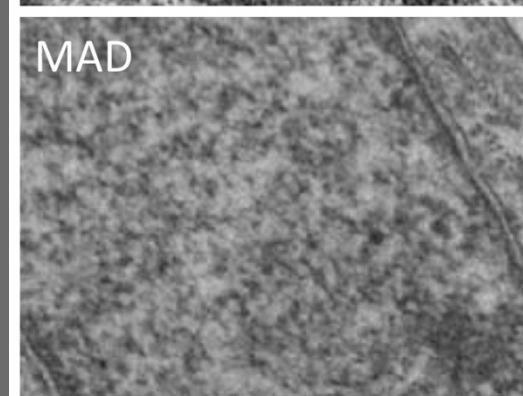
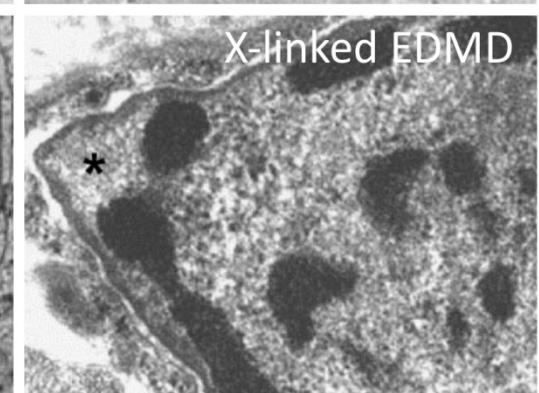
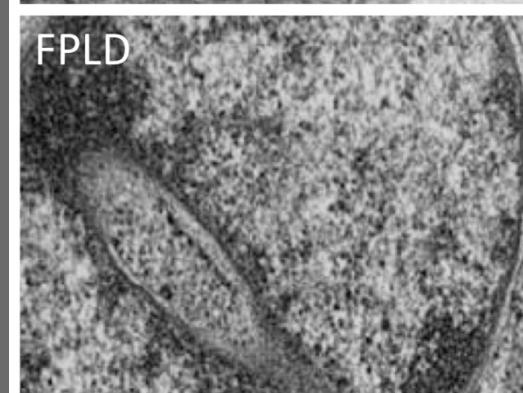
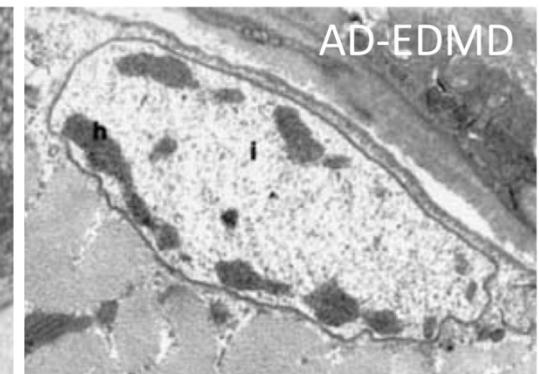
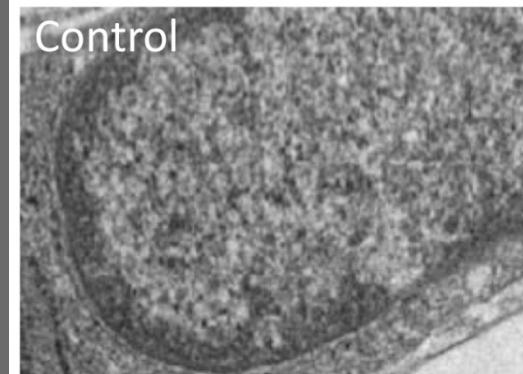


Fat  
Lipodystrophy  
Mandibuloacral Dysplasia  
*LMNA*



Aging  
Progeria  
*LMNA*

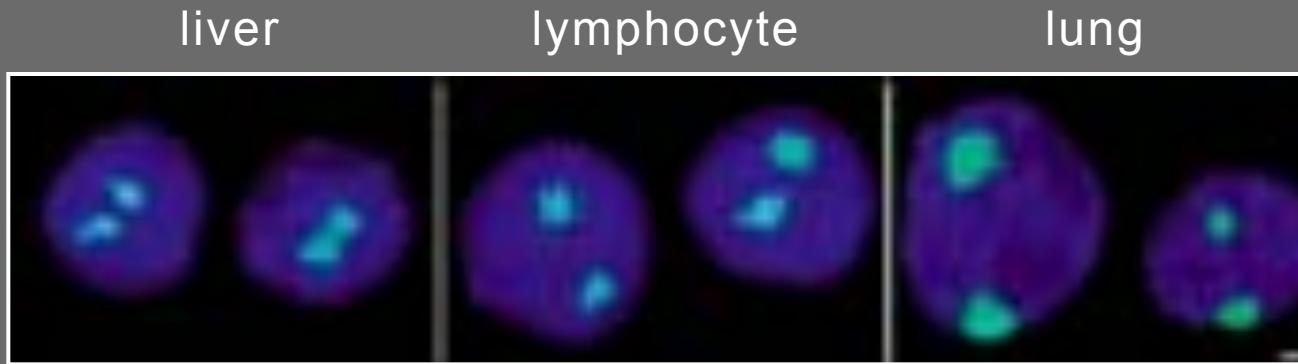
# Chromatin In Nuclear Envelope Diseases



Images taken from *Maraldi et al Adv Enzyme Reg 2006*  
and *Ognibene et al Muscle Nerve 1999*

# Chromosome Positioning Is Tissue-Specific

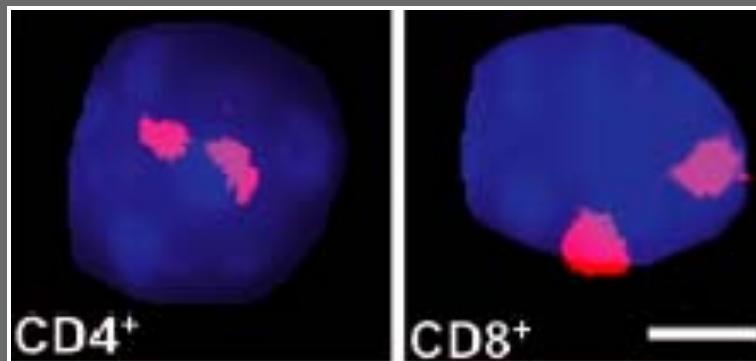
Chromosome 5 is internal or peripheral depending on cell type



*Parada et al., 2004 Genome Biol. 5, R44*

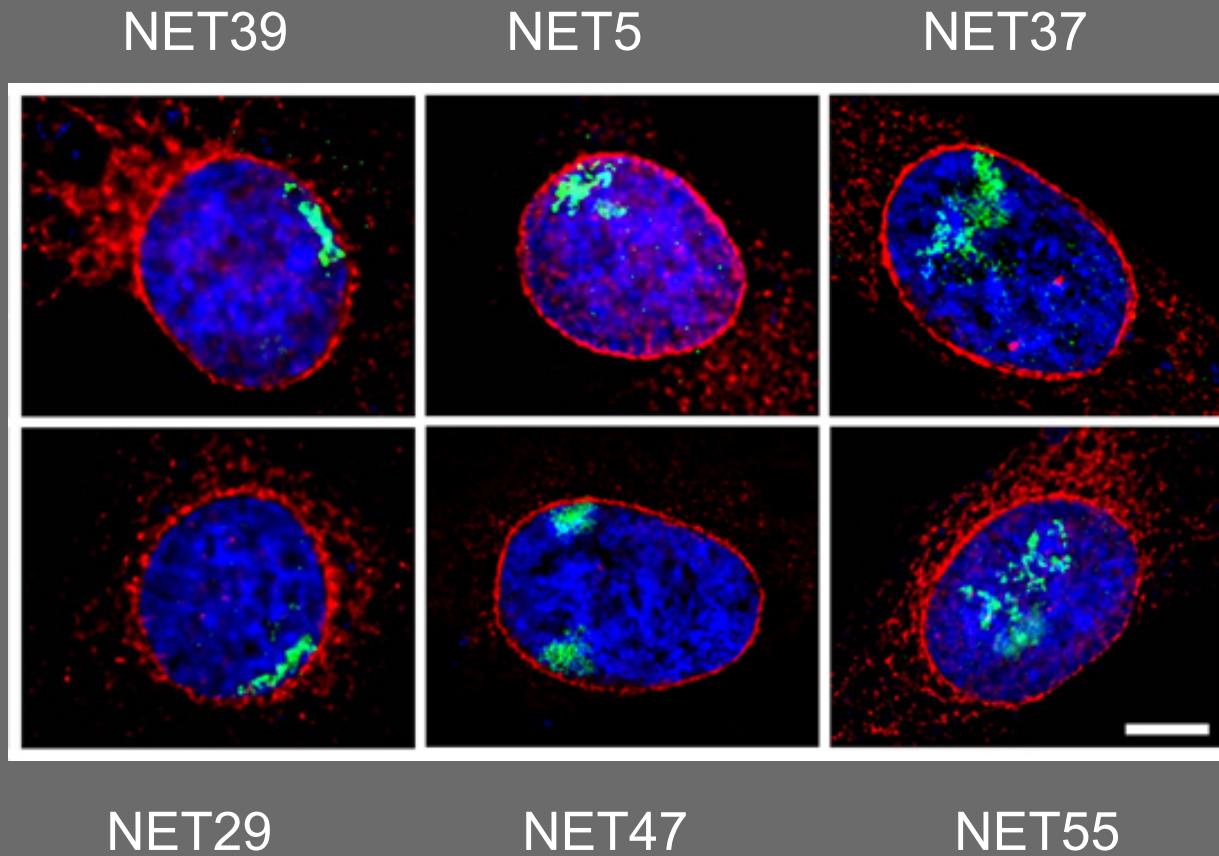
Blood cell differentiation

chromosome 6 position changes

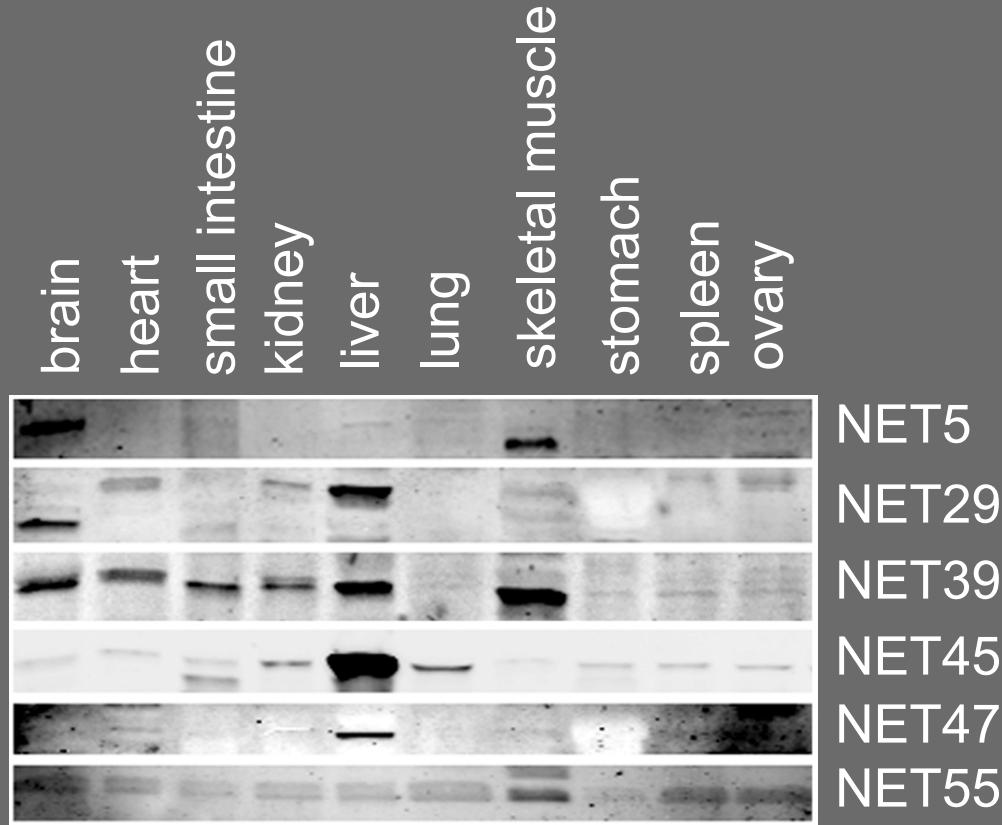


*Kim et al., 2004 Cytogenet Genome Res. 105, 292*

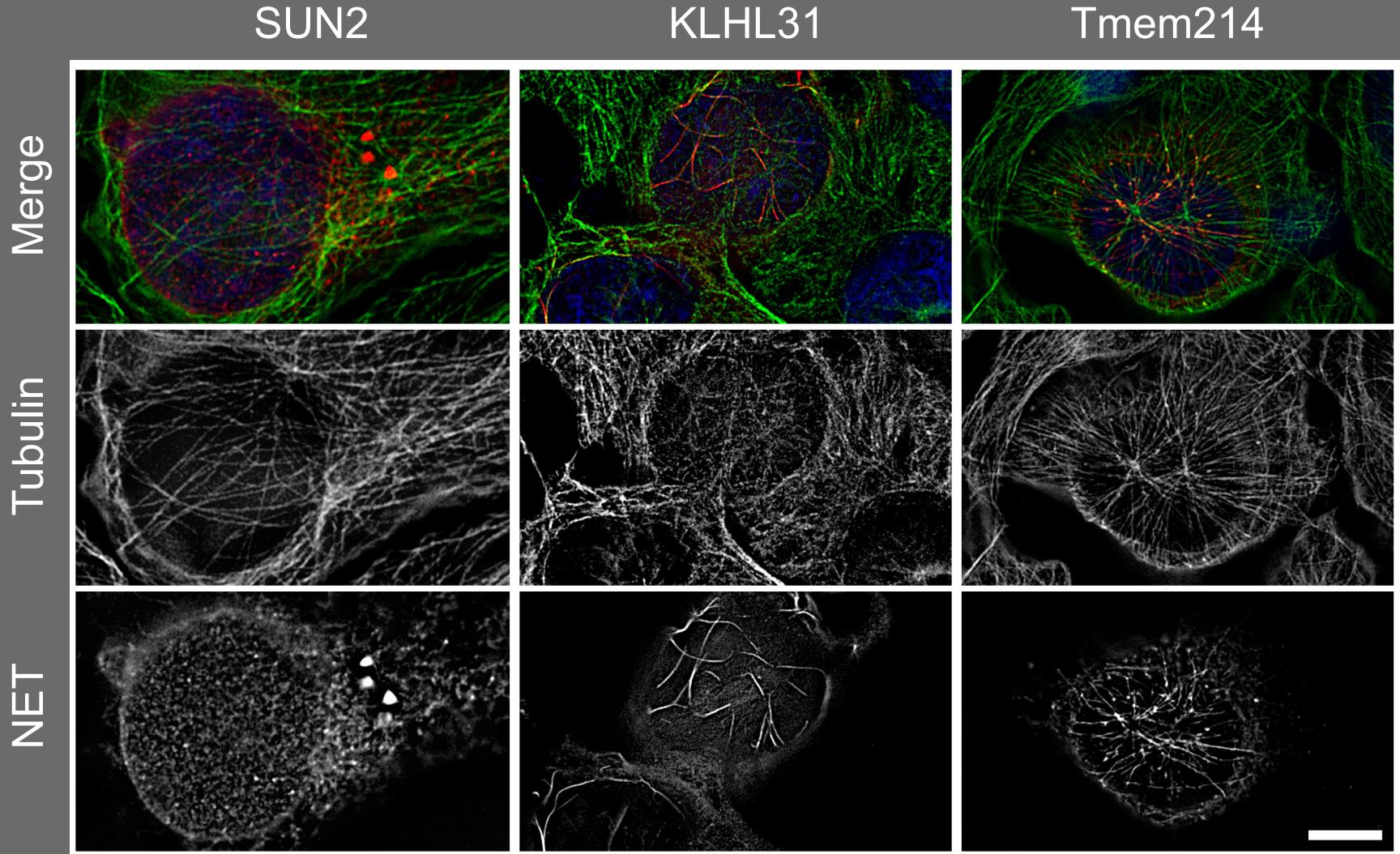
# Some NETs Promote Chromosome Repositioning



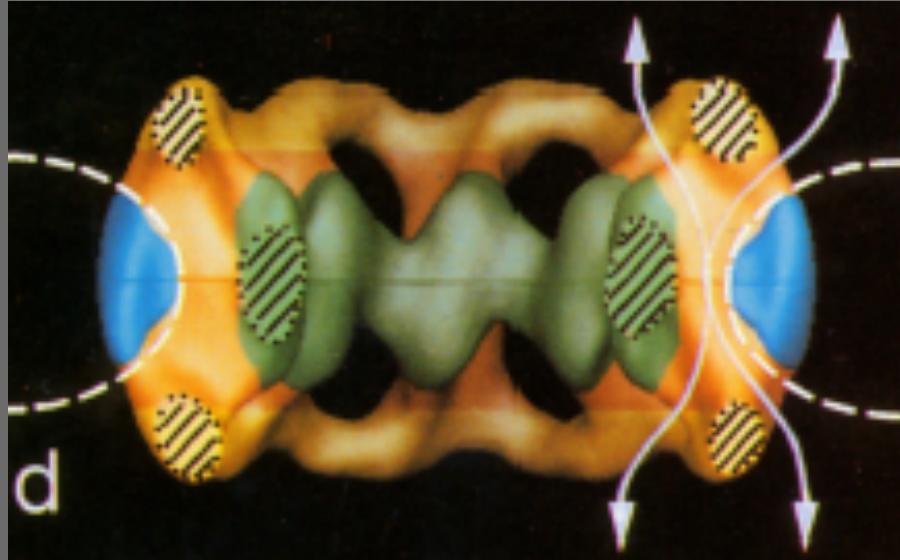
# NETs That Reposition Chromosomes Have Restricted Tissue Expression Profiles



# Some Muscle NETs Track With Microtubule Networks At The Nuclear Surface



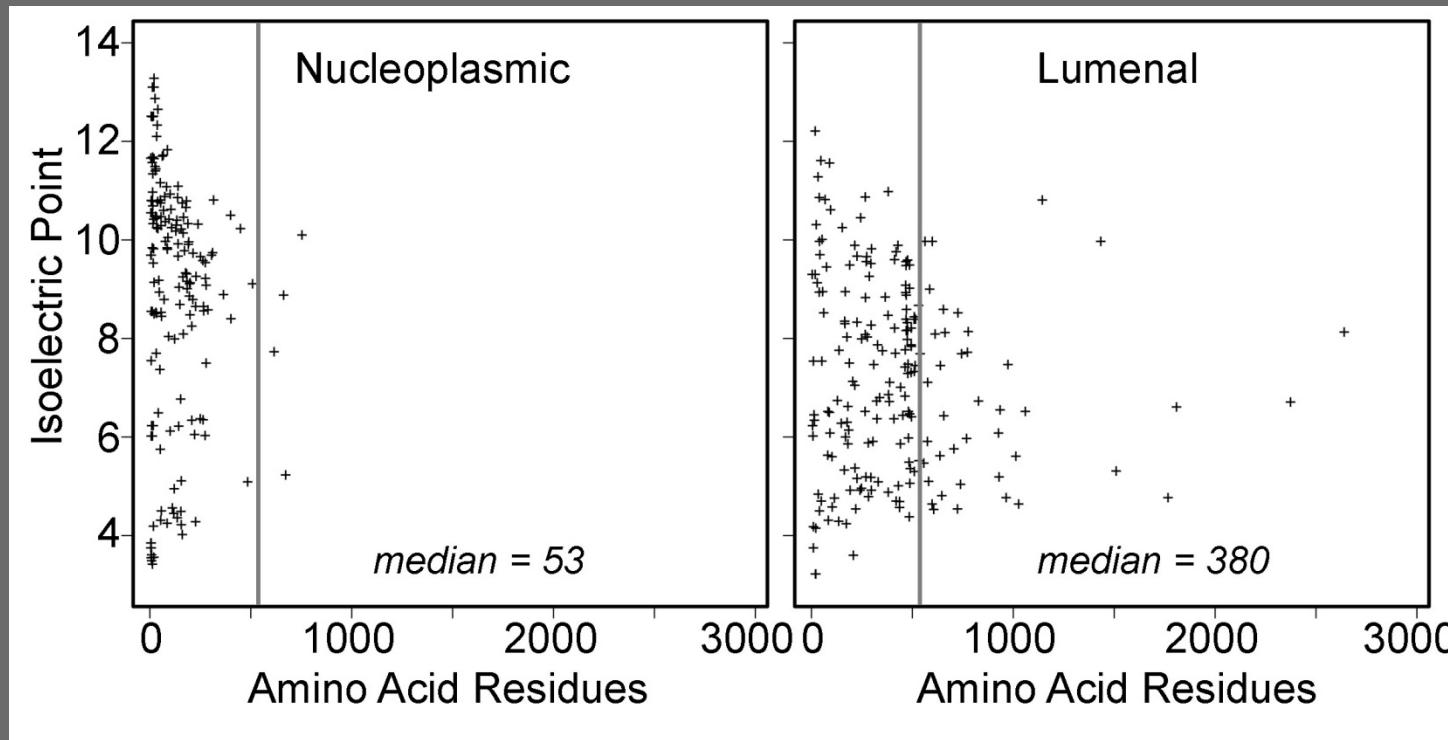
## Datasets Also Inform About NET Translocation



Central channel can handle  
cargoes 39 nm diameter  
(assembled ribosome 25 nm)

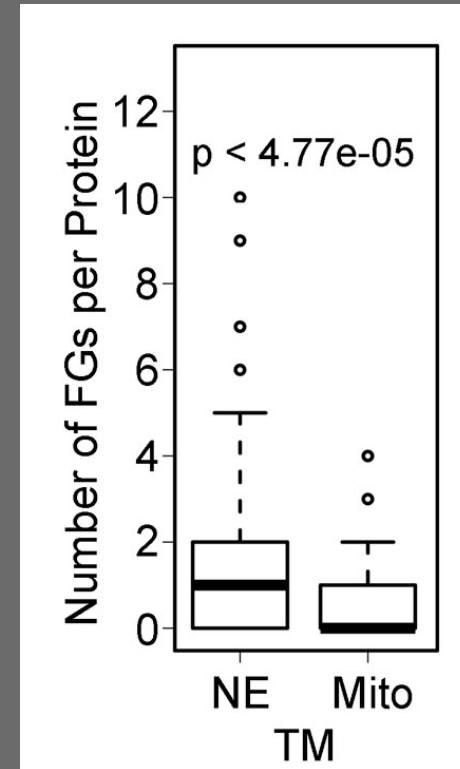
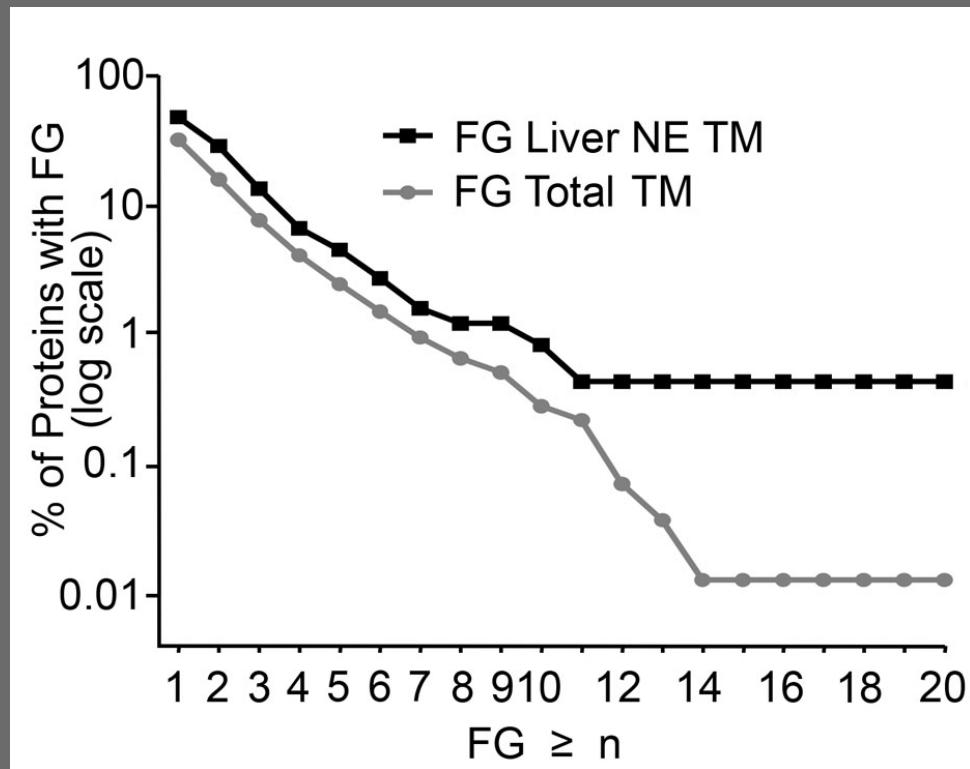
Peripheral channel can handle  
cargoes of 10 nm diameter  
roughly 60 kDa protein

# A Size Limit Is Observed for Nucleoplasmic Mass

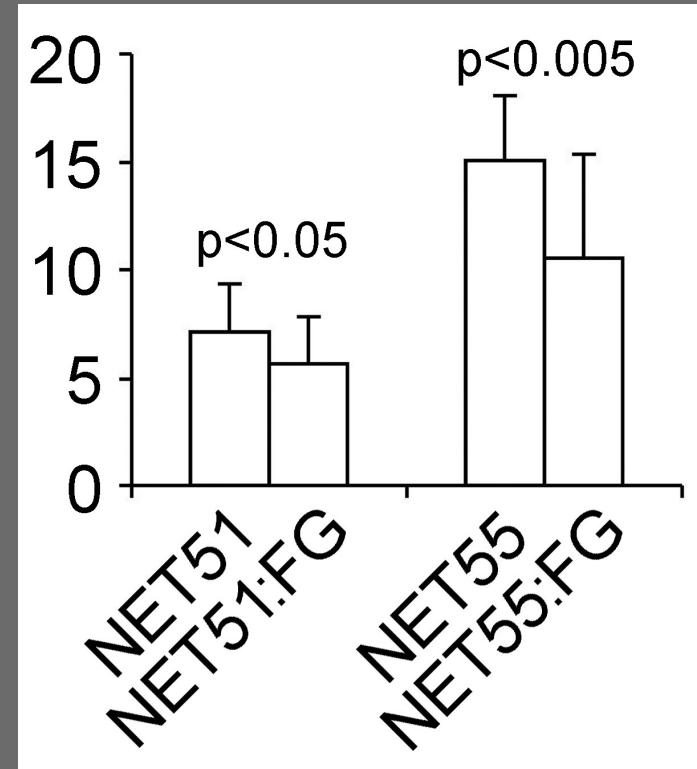
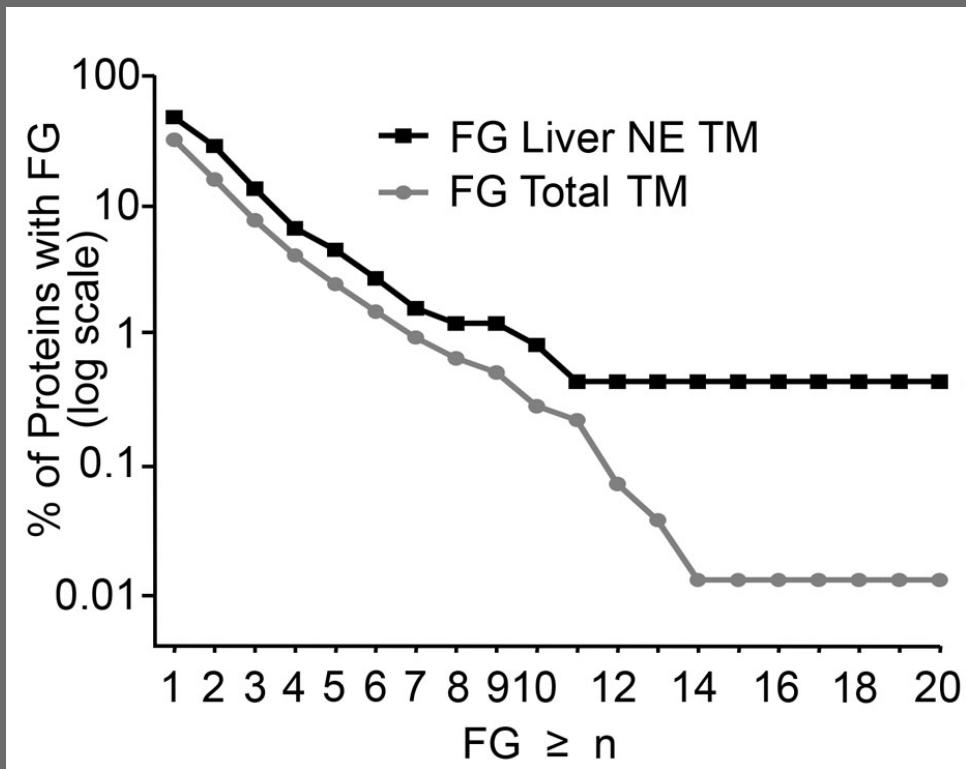


Nearly ALL transmembrane proteins in NE datasets smaller than exclusion limit for transport through peripheral channels

# NE Datasets Also Enriched for Proteins with FGs



# FGs Facilitate Trafficking from the ER to the Inner Nuclear Membrane



# Conclusions

The NE varies considerably between tissues

Multiple runs, multiple extractions, and multiple digests  
all facilitate comprehensive identifications in complex fractions

Tissue-specific NETs have a range of functions from  
chromosome positioning to cytoskeletal organisation to  
nucleocytoplasmic transport and cell cycle regulation

Analysis of other organelle proteomes is likely to also yield  
interesting tissue-specific components and functions

# The Wellcome Trust Centre for Cell Biology



*Nikolaj Zuleger*

*Jose de las Heras*

*Nadia Korfali*

*Poonam Malik*

*Vlastimil Srsen*

*Dzmitry Batrakou*

*Gerlinde Otti*

*Vasiliki Lazou*

*Gavin Wilkie*

*Centre for Cell Biology*

*David Kelly*

*Alastair Kerr*



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**Collaborators**

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**Laurence Florens**

**Selene Swanson**

**MRC HGU**

**Wendy Bickmore**

**Lee Finlan**

**Paul Perry**

**Shelagh Boyle**

**U Edinburgh Dept Pathology**

**David J Harrison**

**Wolfson Centre, Oswestry**

**Glenn E. Morris**

**Durham University**

**Chris Hutchison**

