Crossing the oxidative DNA damage threshold - consequences resulting from the loss of the NEIL1 DNA glycosylase

R. Stephen Lloyd
Center for Research on Occupational and Environmental Toxicology and
Department of Molecular & Medical Genetics
Oregon Health & Science University

DNA Repair & Related Transactions

October 2001

Erling Seeberg

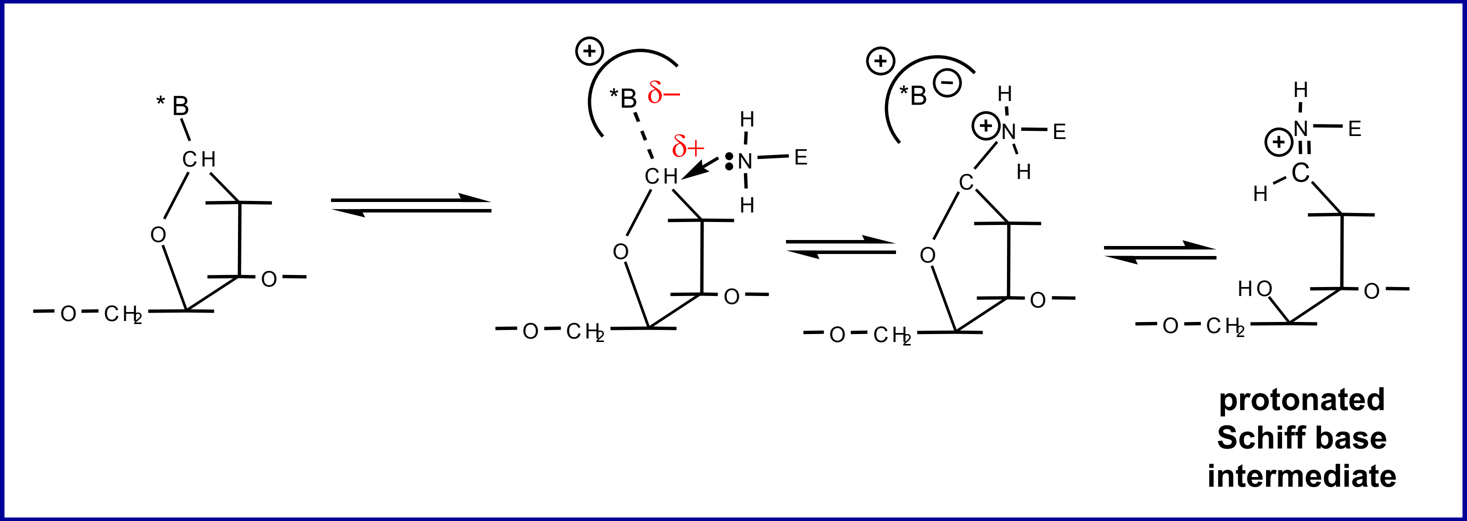
Arthur Grollman

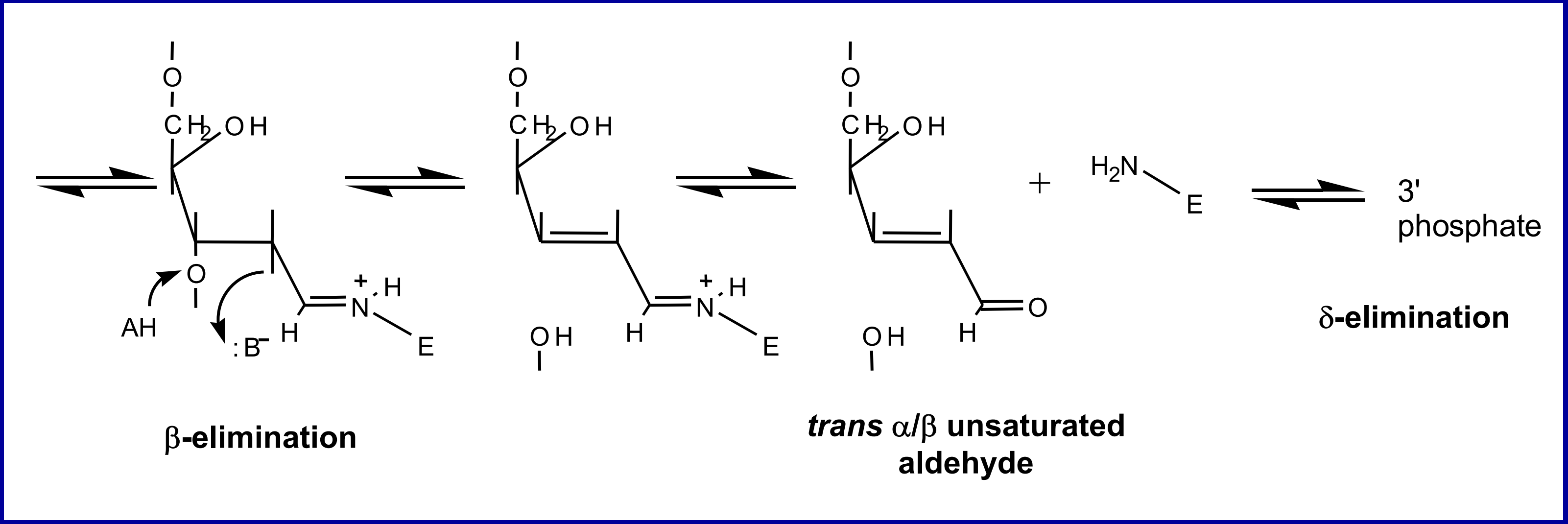
Stephen Lloyd

But…..we were not alone

Sankar Mitra	3/2002	(NEH1)
Susan Wallace	4/2002	(NEIL1)
Mitra, Hazra, Izumi	7/2002	(NEIL2)
Erling Seeberg	9/2002	(HFPG1)
Akira Yasui	9/2002	(NEIL1)
Arthur Grollman	1/2003	(NEIL1)

Reaction Mechanism of DNA Glycosylase/AP Lyases





McCullough et al, 2001

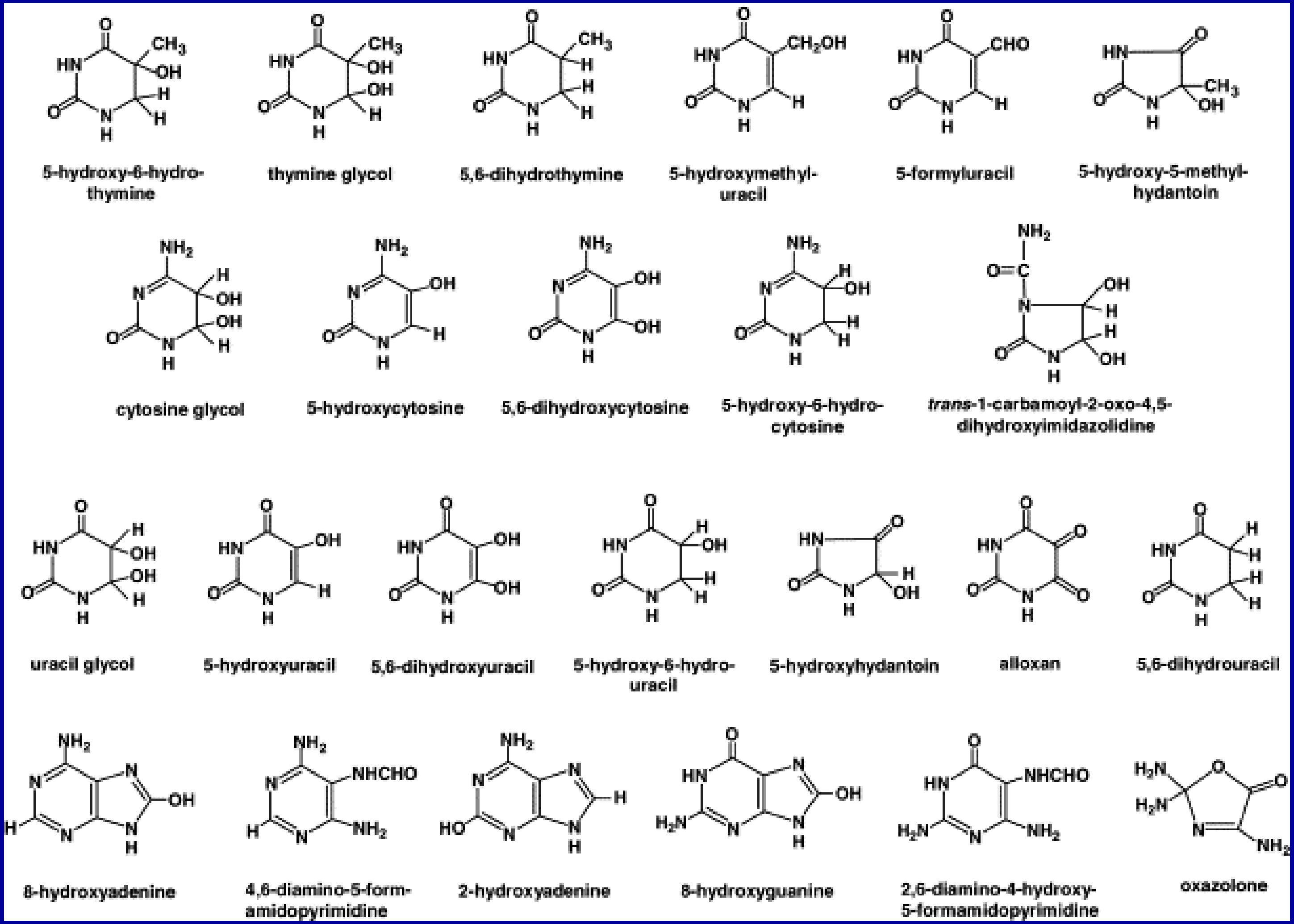
Catalytic Mechanism

Glycosylase/β, δ elimination catalyst

Higher catalytic efficiency on single-stranded vs double-stranded DNA and S-phase expression ⇒ replication-associated and/or transcription-coupled repair (Dou et al, 2003)

Stimulated by the hus1,Rad1, Rad9 (9-1-1 complex)

Major Products of Oxidative Damage to DNA Bases



Evans et al, 2004

Substrate Specificities of NEIL1

IR-damaged genomic DNA (Miral Dizdaroglu)

Fapy A* and Fapy G

Defined Oligodeoxynucleotides

Thymine glycol (both 5S,6R*, and 5R,6S)

5-OH U (↑ proximity to ssb), 5-OH C

me-FapyG

DHT, DHU

↓ 8-oxoG $\xrightarrow{\text{oxidation}}$ spiroiminodihydantoin * ↑↑↑

guanidinohydantoin * ↑↑↑

* = Lack of recognition redundancy for:
FapyA, 5S,6R TG, SP, GH

May suggest a critical role for NEIL1 in maintenance of genomic integrity

Radiation Sensitivity of *neil1* si RNA Depleted Cells

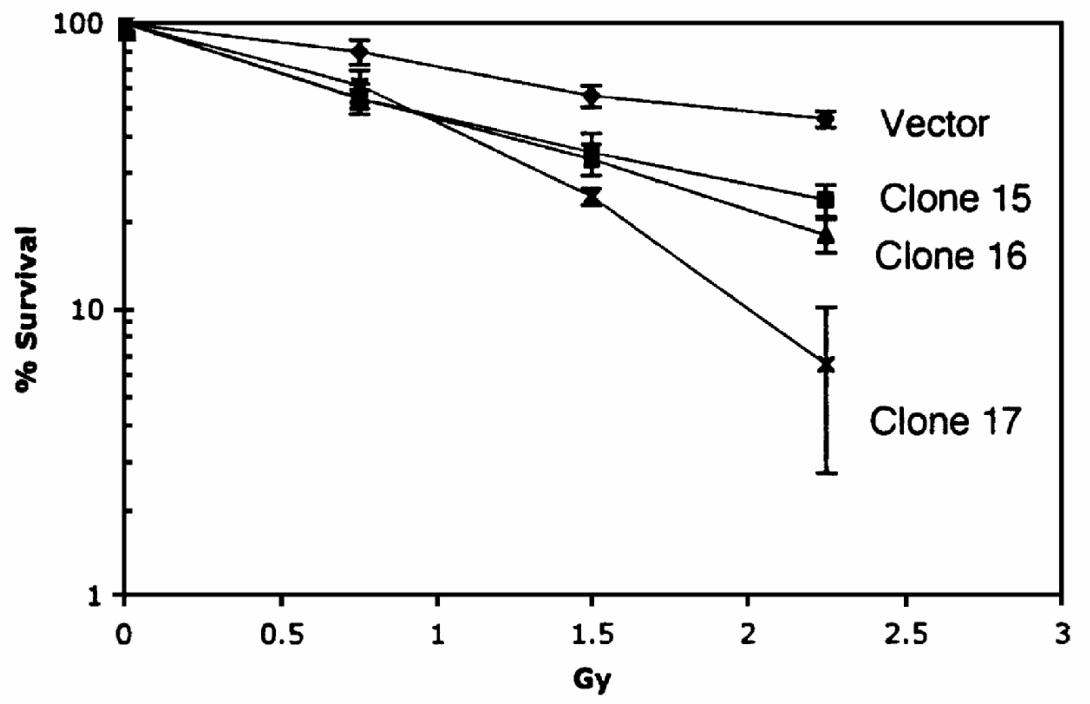


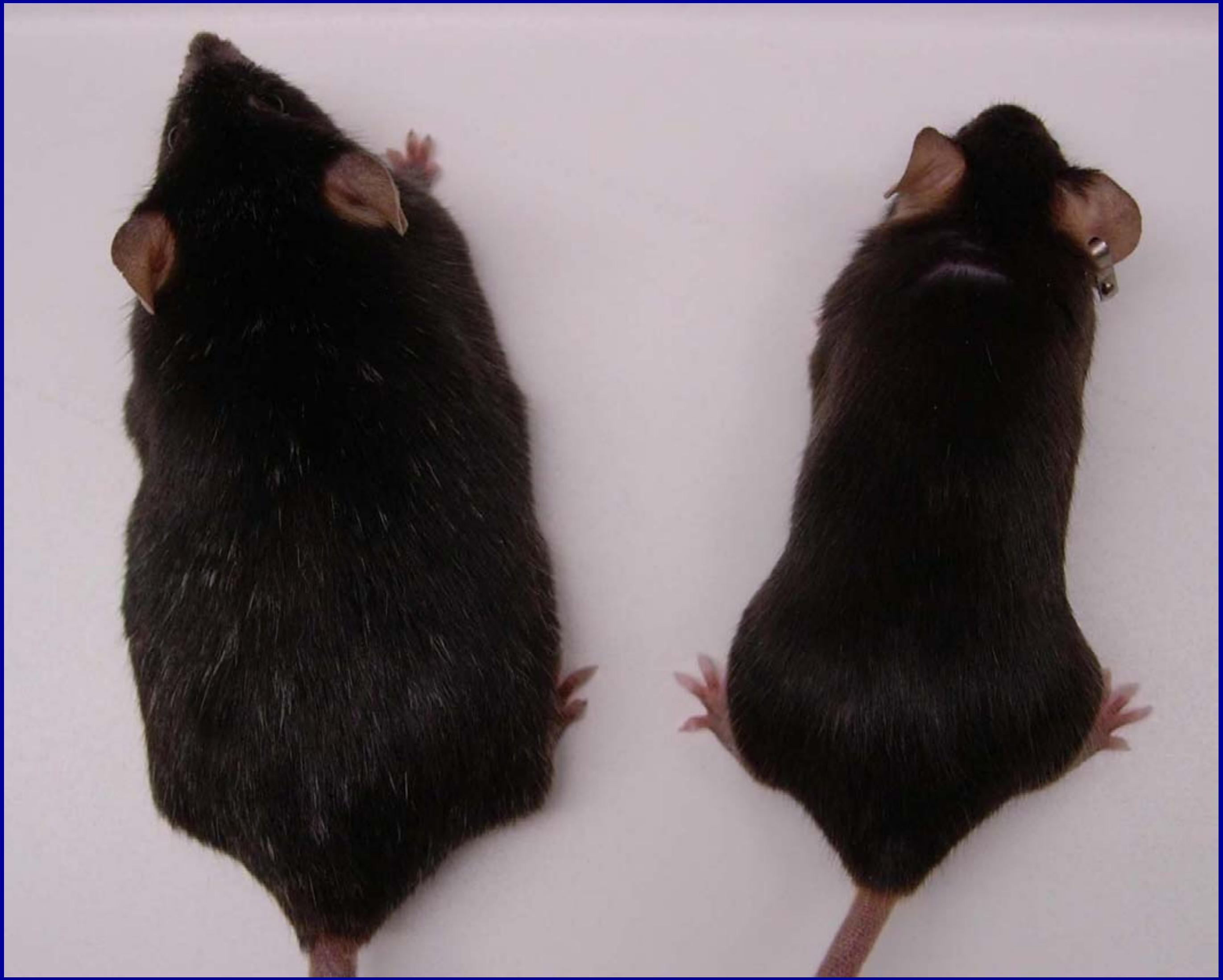
Fig. 5. Radiation survival curves for control embryonic stem cells containing the empty vector pH1P-neo or three clones containing the *Neil1*-hairpin expression plasmid, pNEIL1HP.

Rosenquist et al. 2003

Glycosylase Knockout Mice

KOs made in ung, mpg, ogg1, nth, mutY etc with essentially no phenotype until combined with other BER or p53 mutants

neil1$^{-/-}$ Male with Control WT Age-matched Male



General Phenotype

- Manifests predominantly in males; mild to no phenotype in females
- Mice fed low fat (10% caloric intake fat)
- No exogenous oxidative stressors
- Chow consumption indistinguishable between *neil1* KO and WT
- Majority of male KO show late onset obesity

Paternal Inheritance

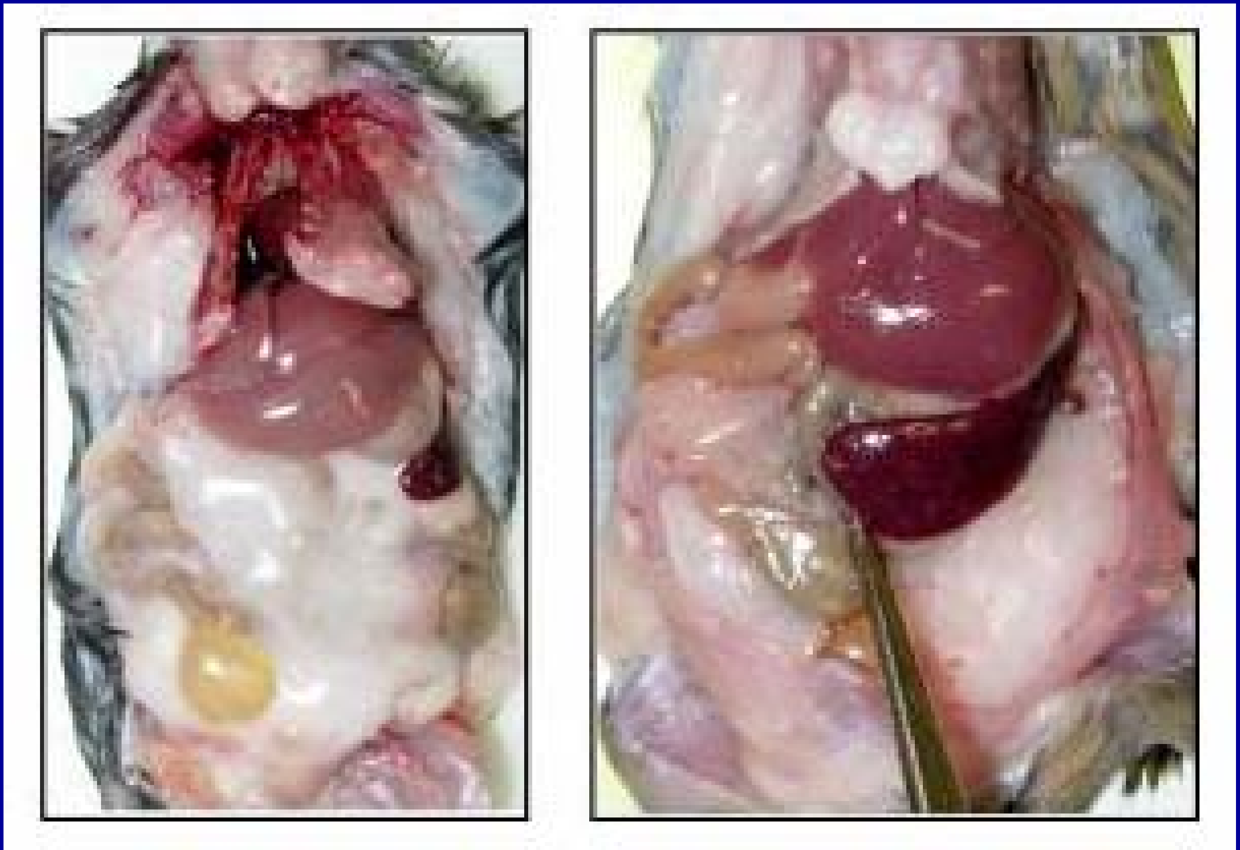
(S. Ballinger, UAB)

- Extensive colony of *neil1* KO that reveal a strong paternal inheritance of final steady-state weight
- However, DEXA analyses reveal same % body fat content

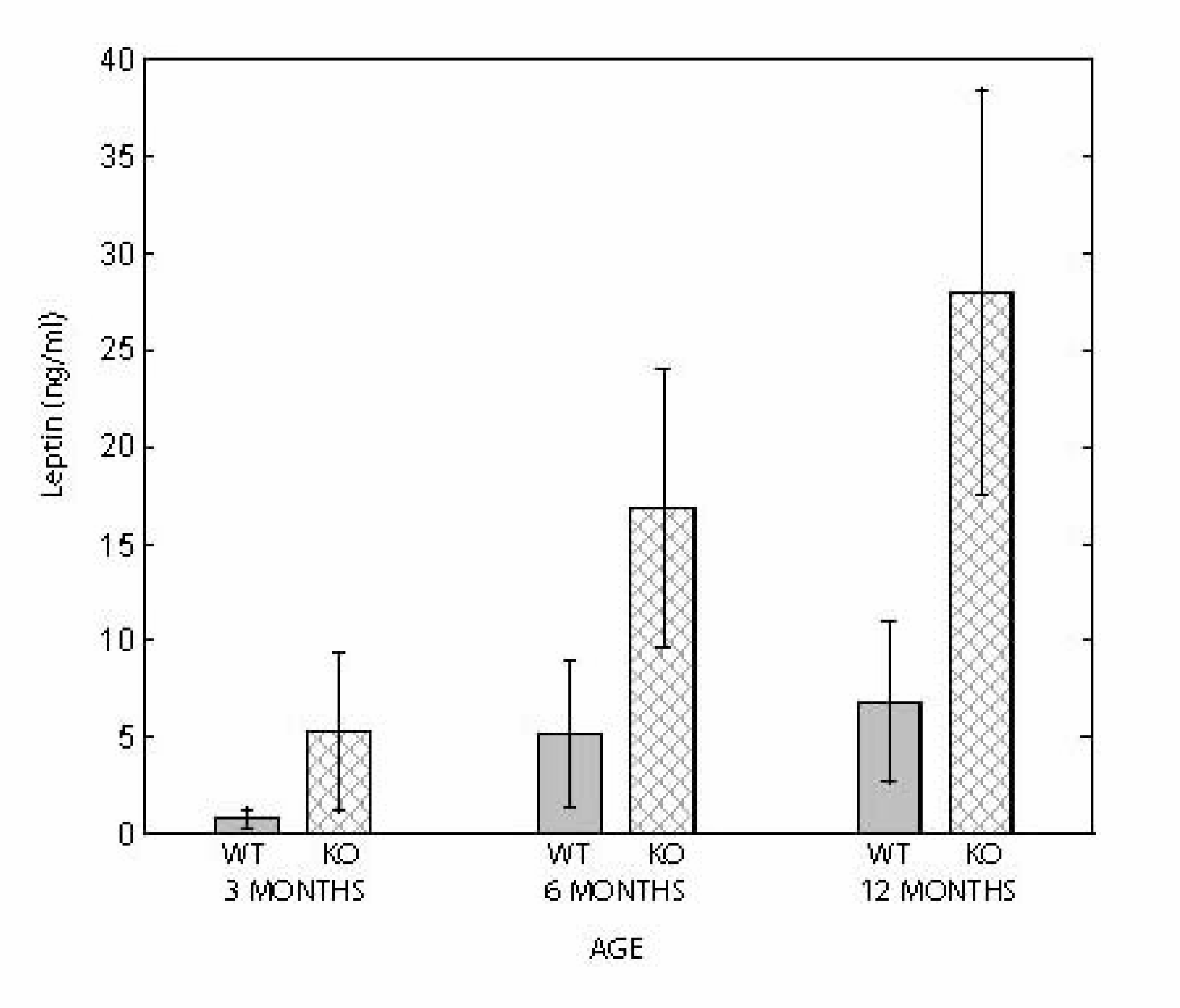
WT = 15-18%; KO (~40g) = 36% fat; KO (~ 50g) = 39% fat

Fat Deposition in Abdomen of *neil1*$^{-/-}$

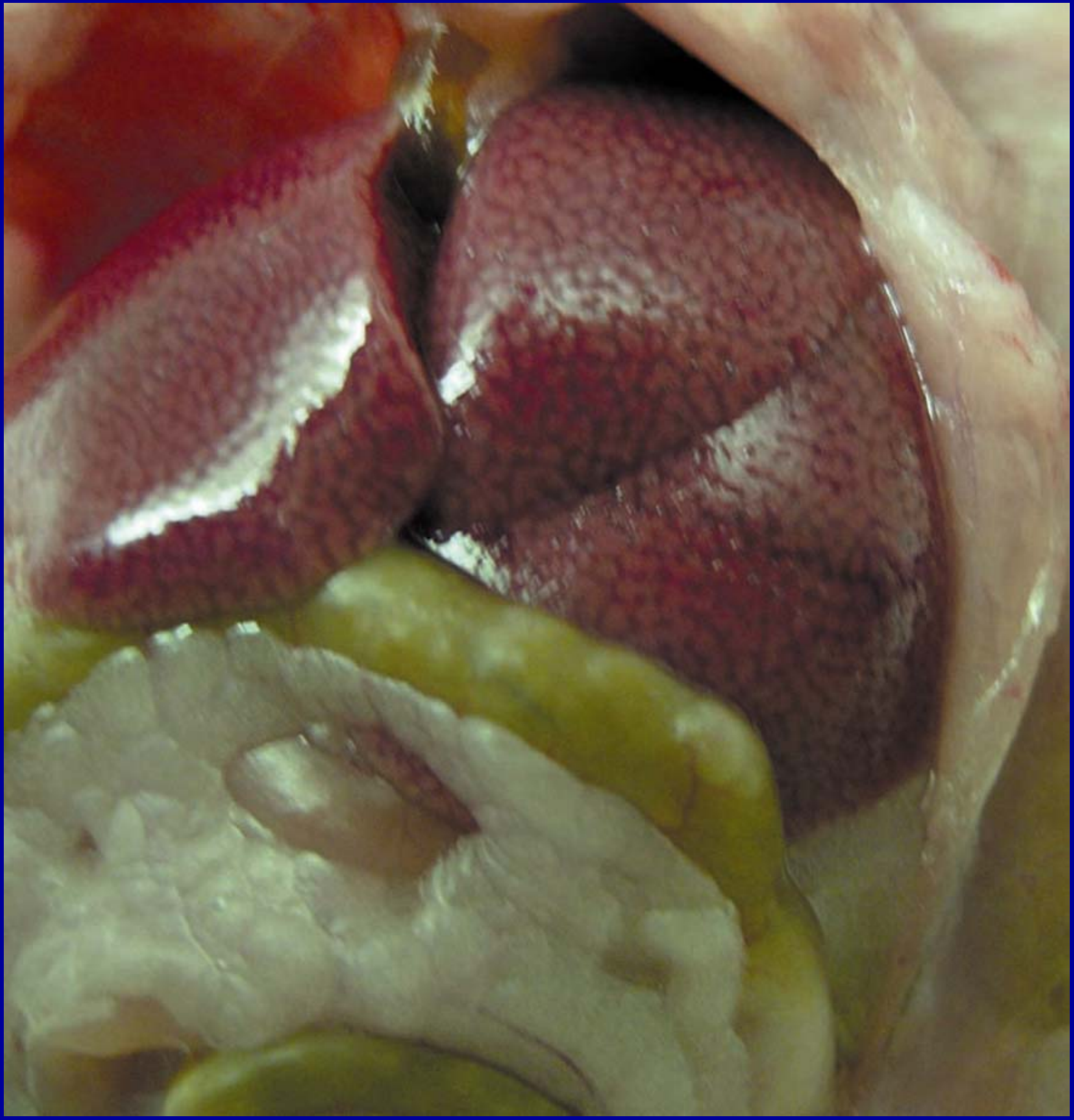
Fat Deposition in Abdomen of *neil1-/-*



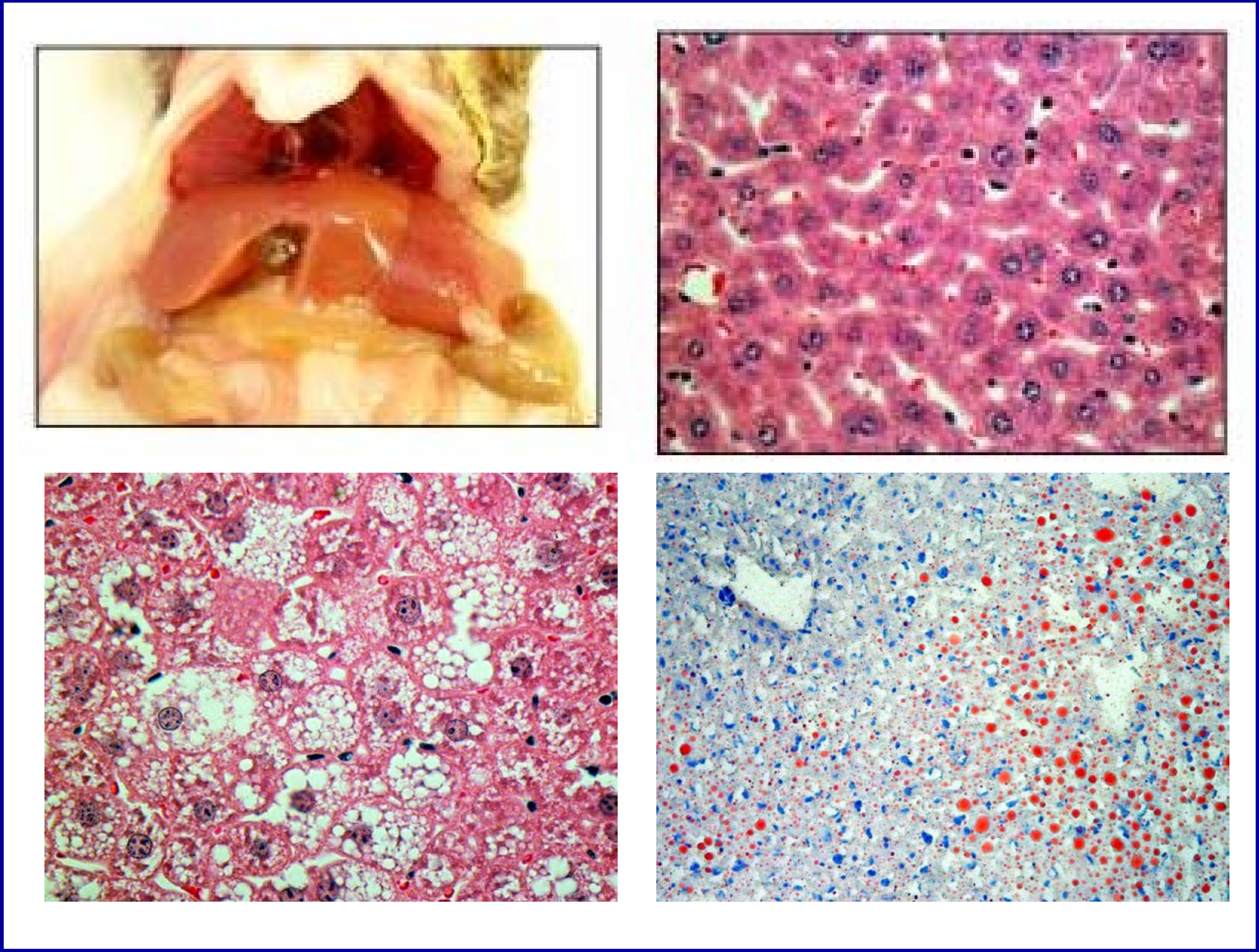
Age-dependent Increase in Leptin Values in *neil1*$^{-/-}$



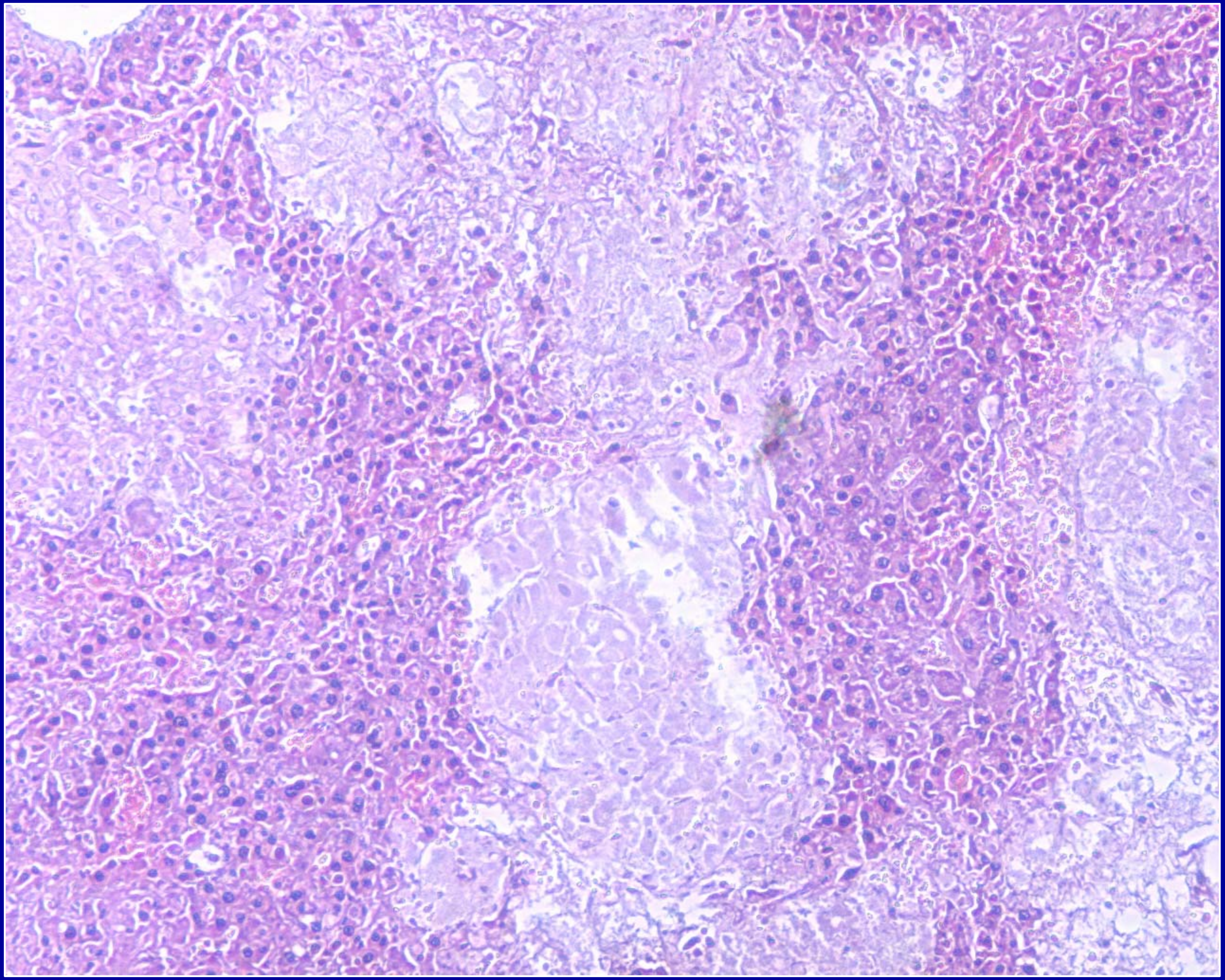
Fatty Liver Disease in *neil1$^{-/-}$* Mice



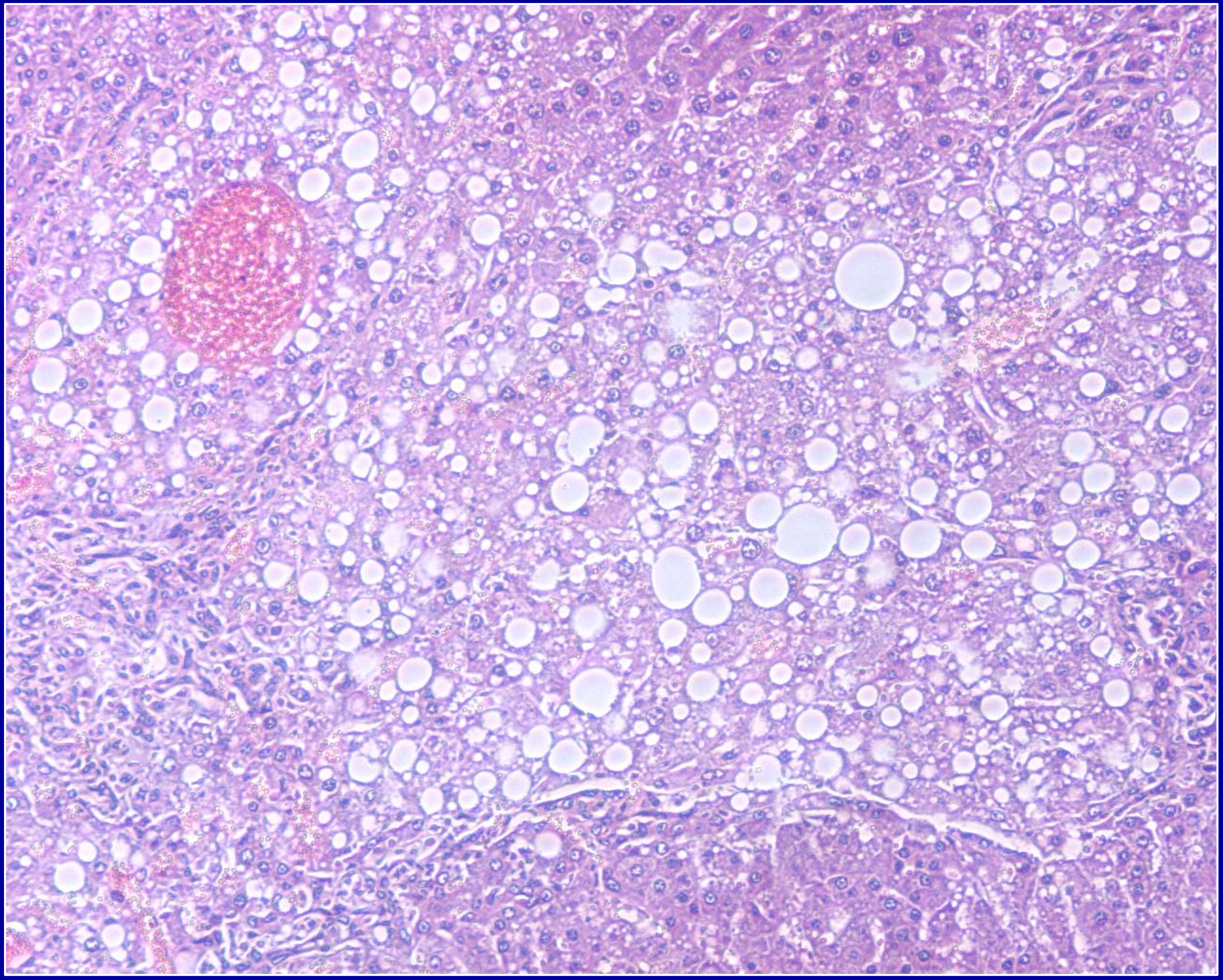
Fatty Liver Disease in *neil1*$^{-/-}$



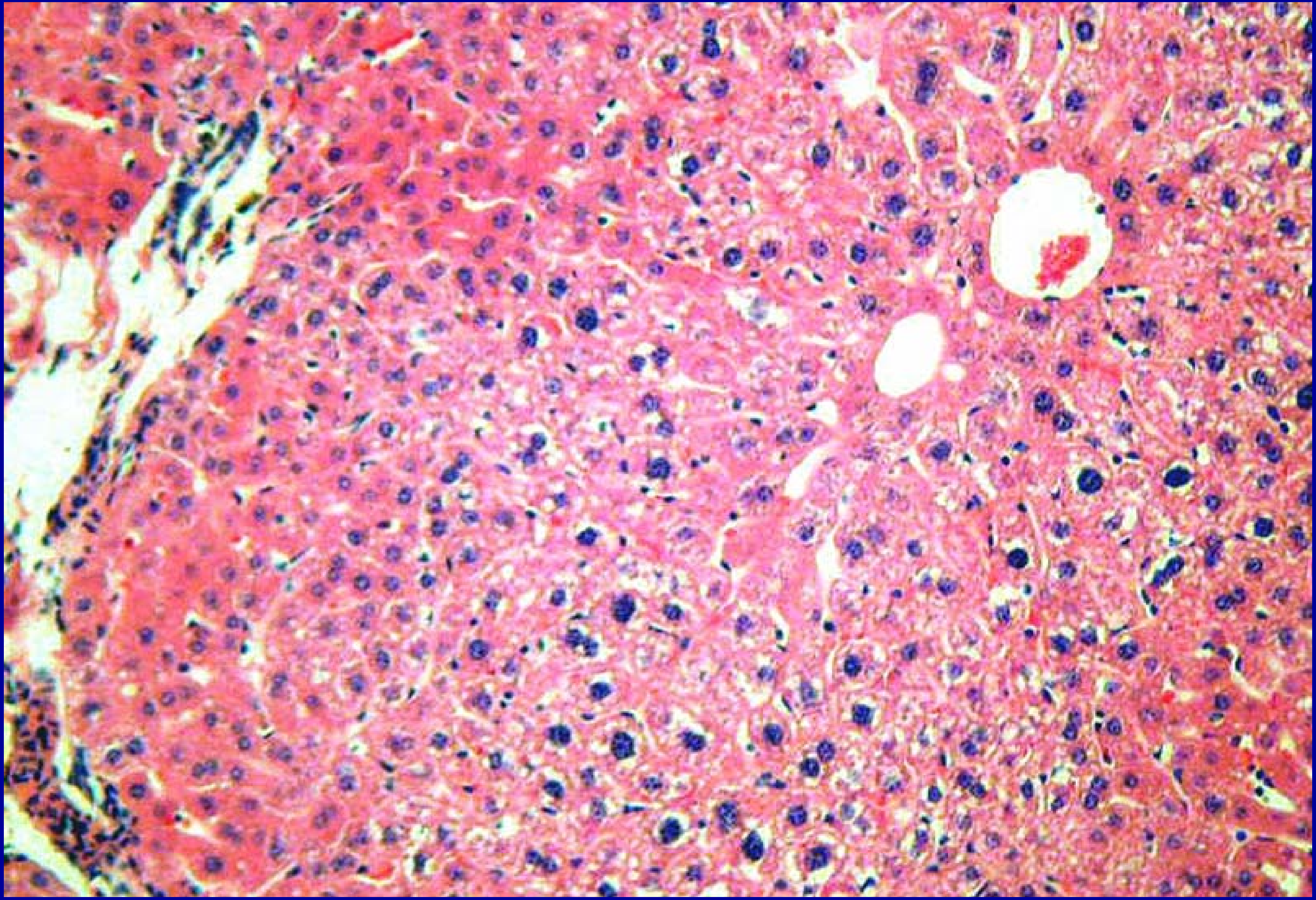
KO male (12 month) hepatic necrosis



KO male (16 month)
hepatic fibrotic sclerosis



KO female liver



Plasma Lipids

	WT	KO
Triglycerides (TG)	230 ng/μl	440 ng/μl
Free fatty Acids (FFA)	21 ng/μl	51 ng/μl
Cholesterol Esters (CE)	332 ng/μl	445 ng/μl

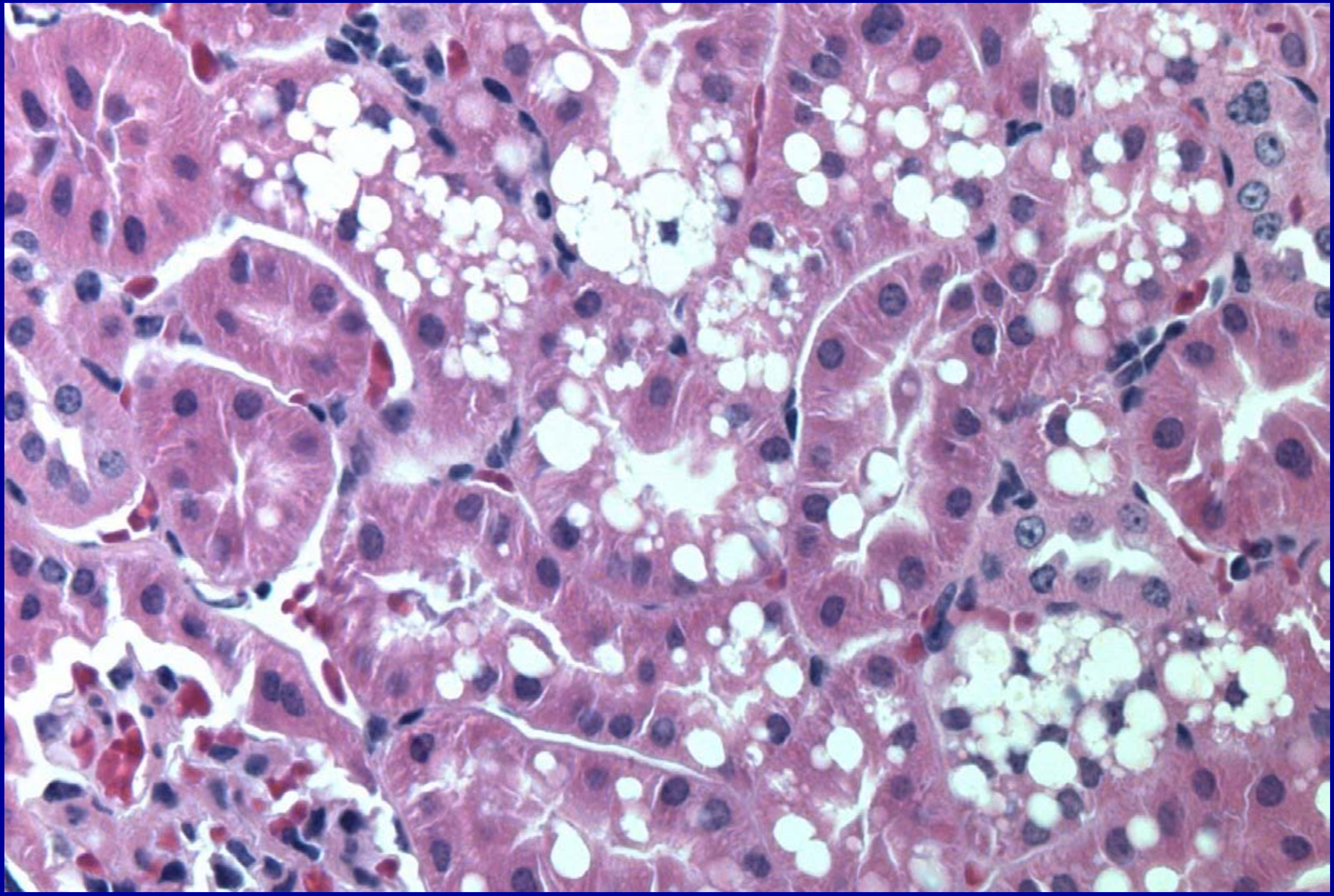
Anti-inflammatory Fatty Acids

DHA docosahexaenoic acid (22:6)

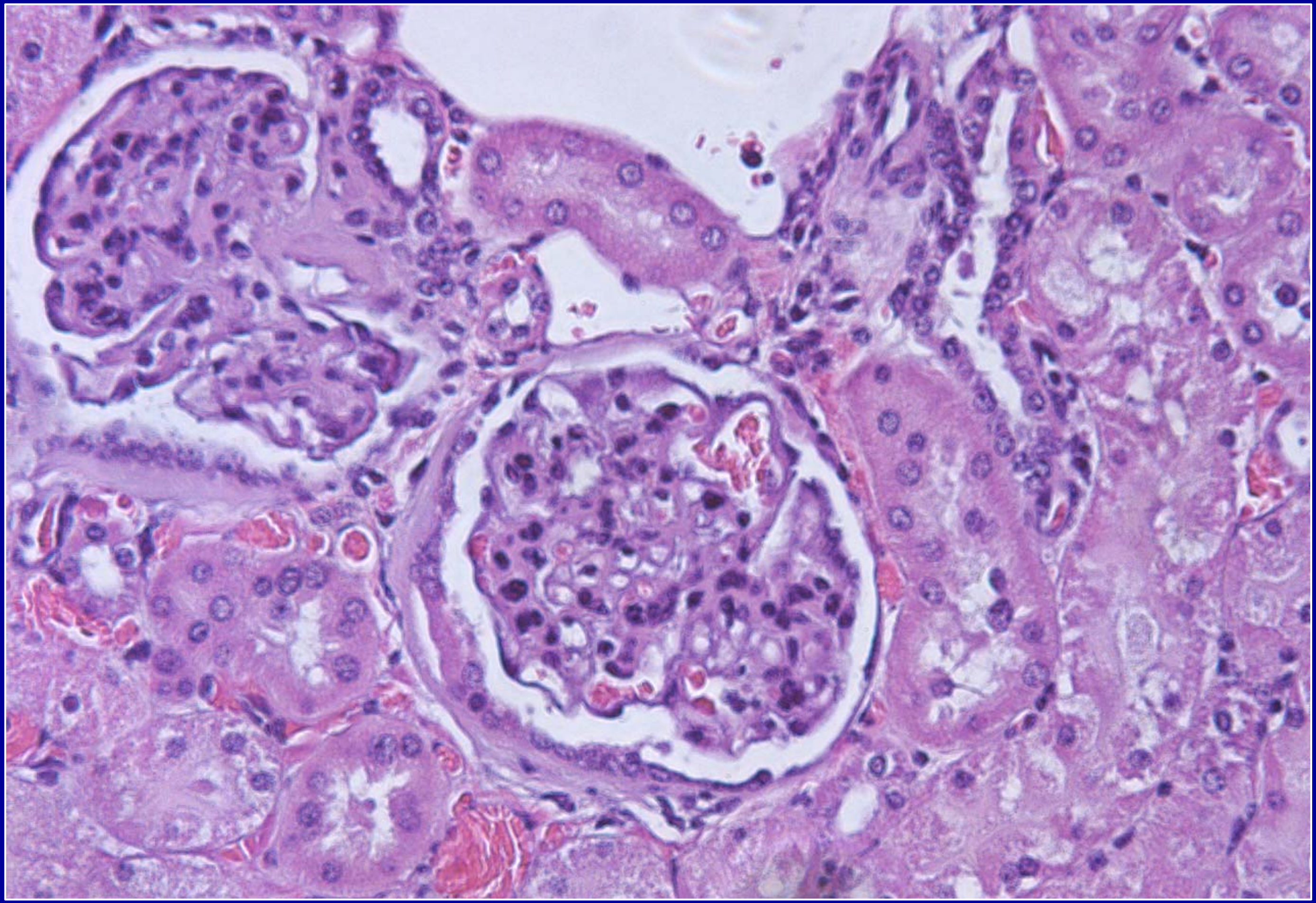
EPA eicosapentaenoic acid (20:5)

	WT	KO
DHA in TG	11 ng/μl	25 ng/μl
EPA in TG	4.9	9.0
DHA in FFA	0.6	2.3
EPA in FFA	0.3	0.7
DHA in CE	14	30
EPA in CE	10	20
Total DHA	**25.6**	**57.3**
Total EPA	**15.2**	**29.7**

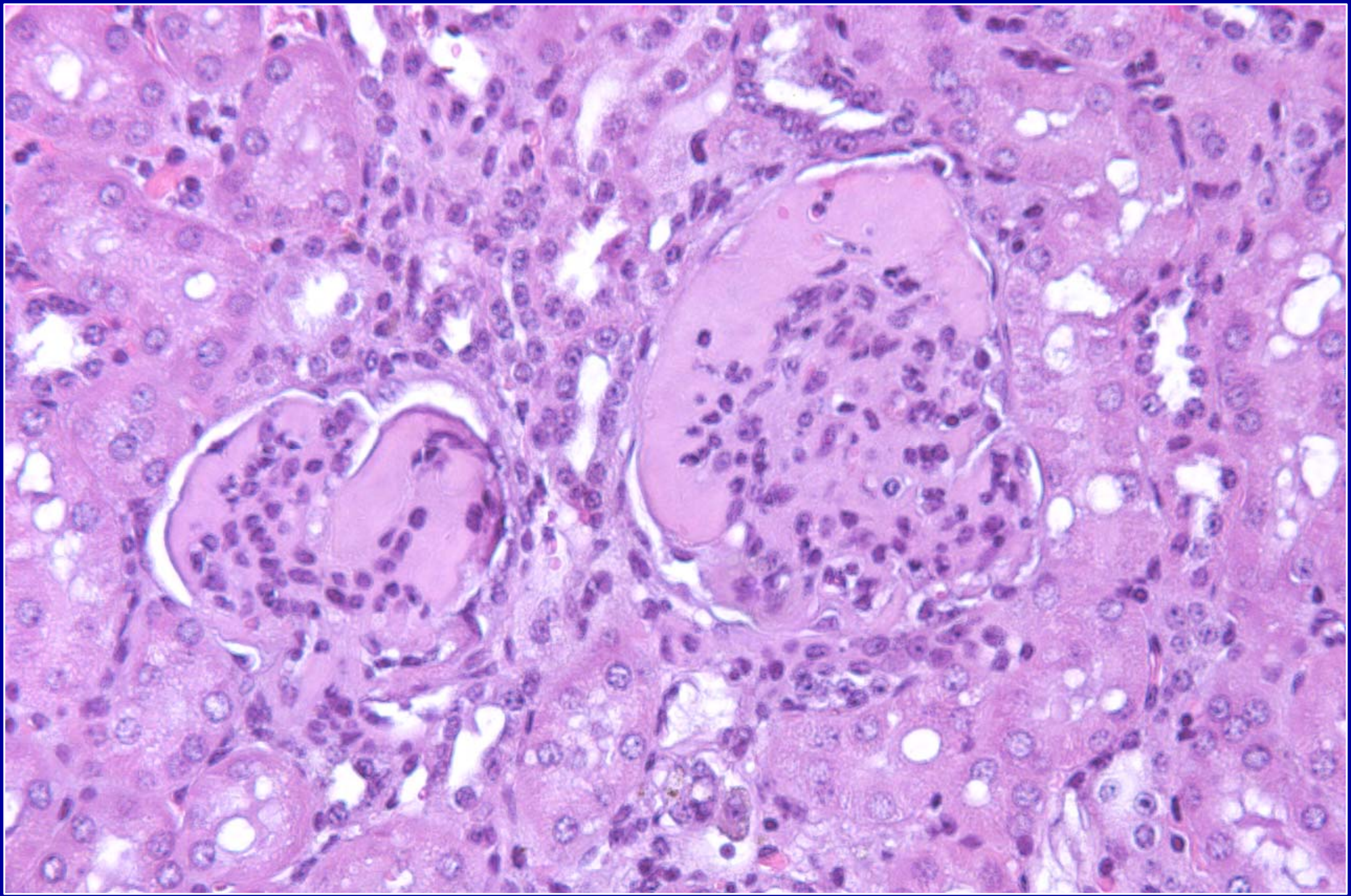
KO male kidney: aqueous vacuolization consistent with type II diabetes



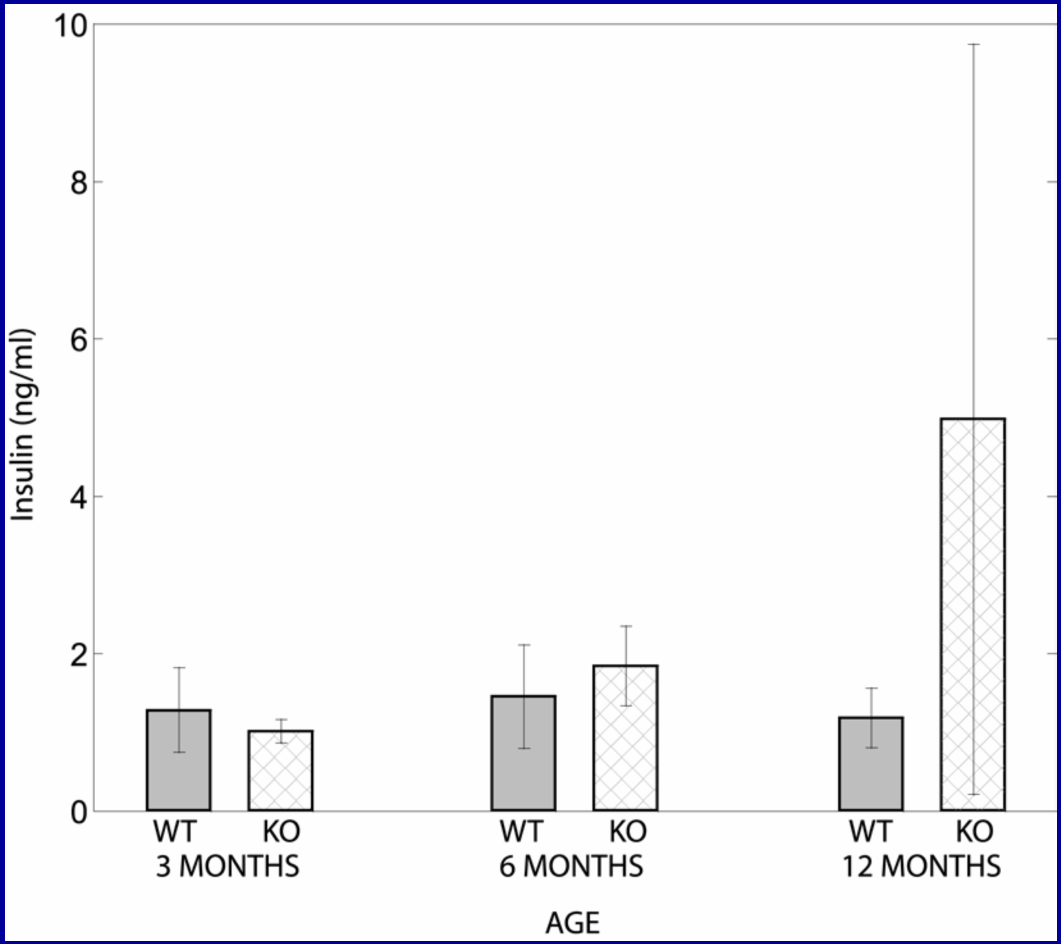
KO male (12 months) hypertrophy of Bowman’s capsule



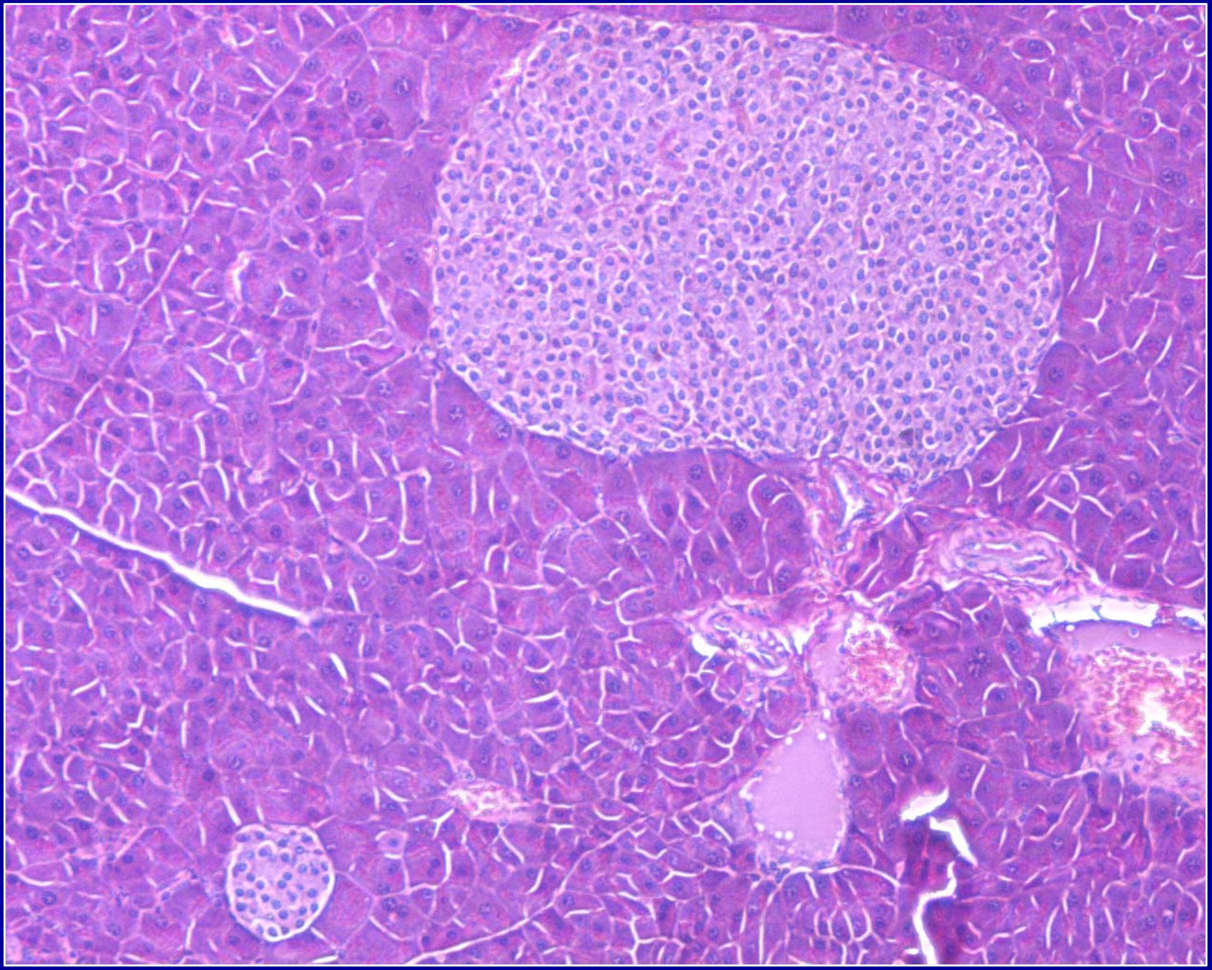
KO male (12 months) membranous glomerulopathy



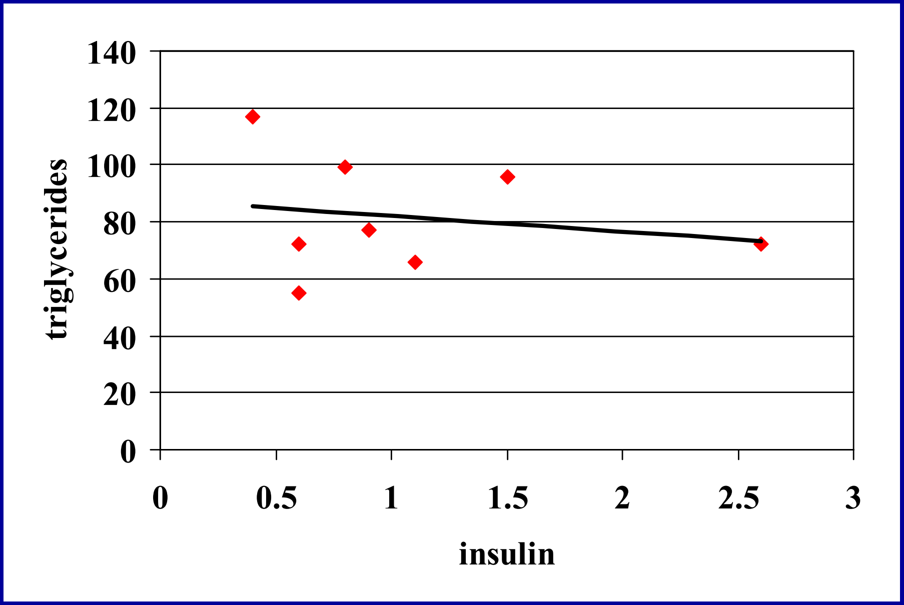
Age-dependent Increase in Insulin Values in *neil1*$^{-/-}$

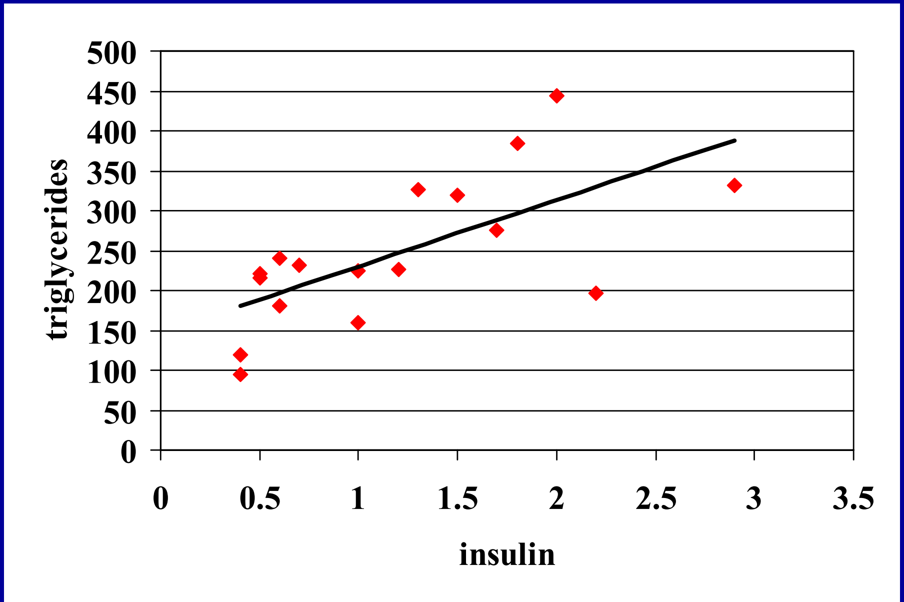


KO male (12 months) pancreas



Relationship of triglycerides and insulin levels in the wild-type and *neil1*$^{-/-}$ mice





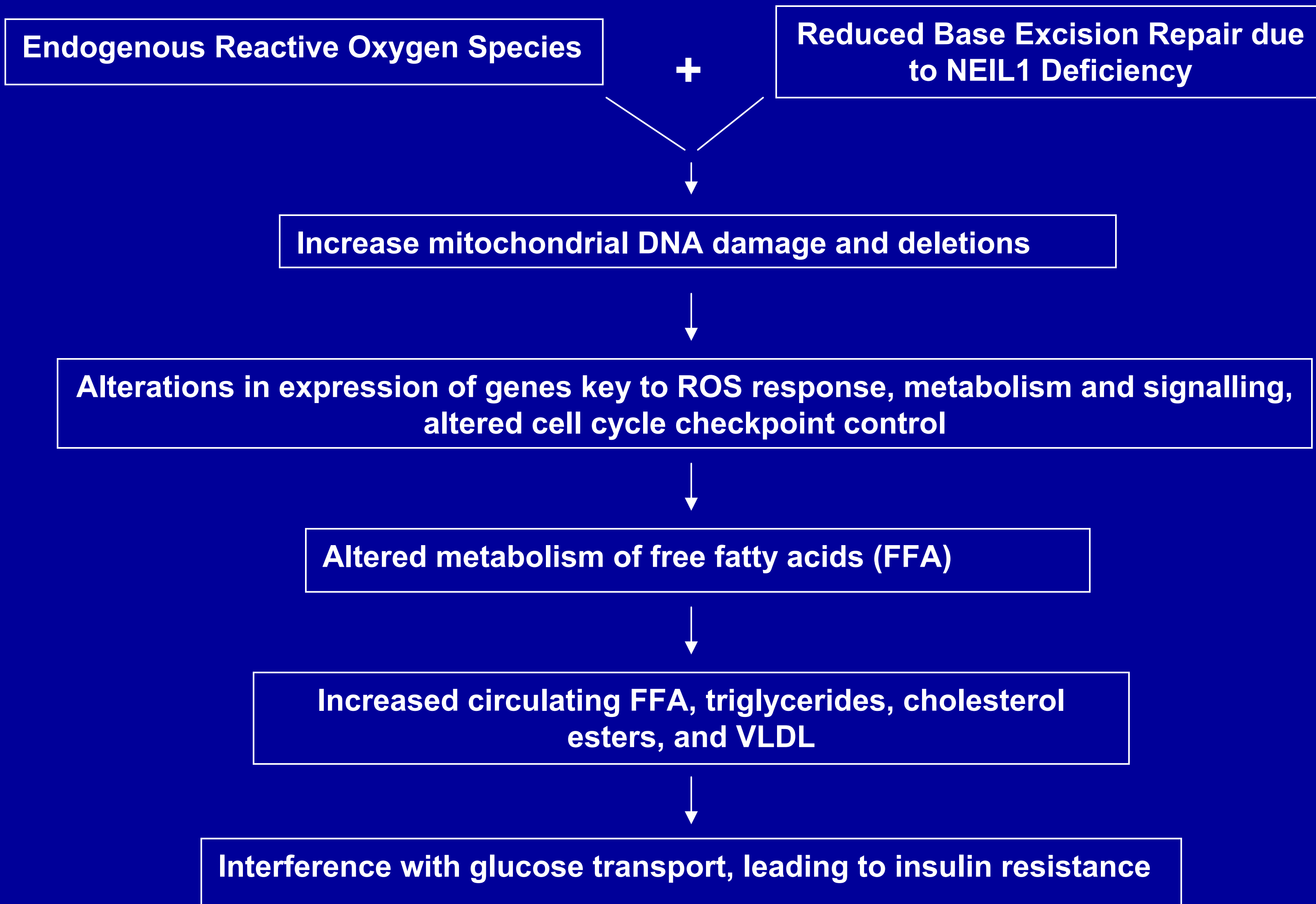
Metabolic Syndrome Phenotype

- √ Severe obesity
- √ Fatty liver disease
- √ Insulin resistance
- √ Dyslipidemia
- **x** Hypertension

Constellation of Diseases:

Also observed in heterozygotic mice, but later in life

Cancer proneness: late onset, affecting liver and epidermal tissues


Endogenous Reactive Oxygen Species
+
Reduced Base Excision Repair due to NEIL1 Deficiency
Increase mitochondrial DNA damage and deletions
Alterations in expression of genes key to ROS response, metabolism and signalling, altered cell cycle checkpoint control
Altered metabolism of free fatty acids (FFA)
Increased circulating FFA, triglycerides, cholesterol esters, and VLDL
Interference with glucose transport, leading to insulin resistance

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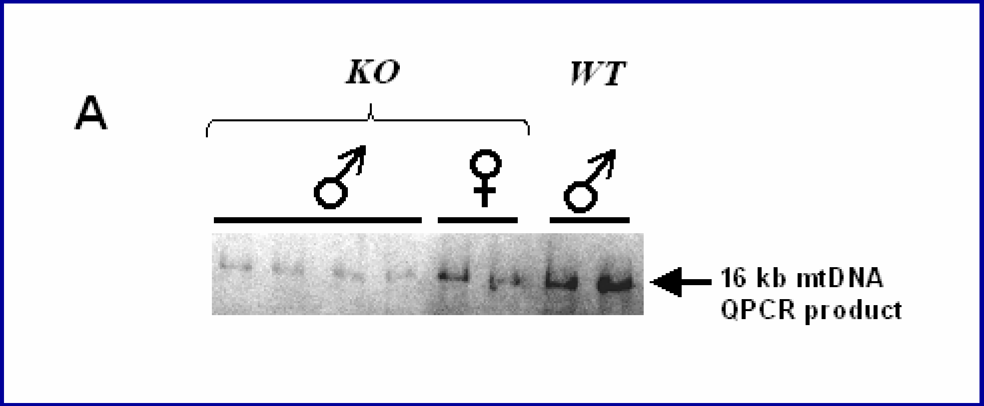
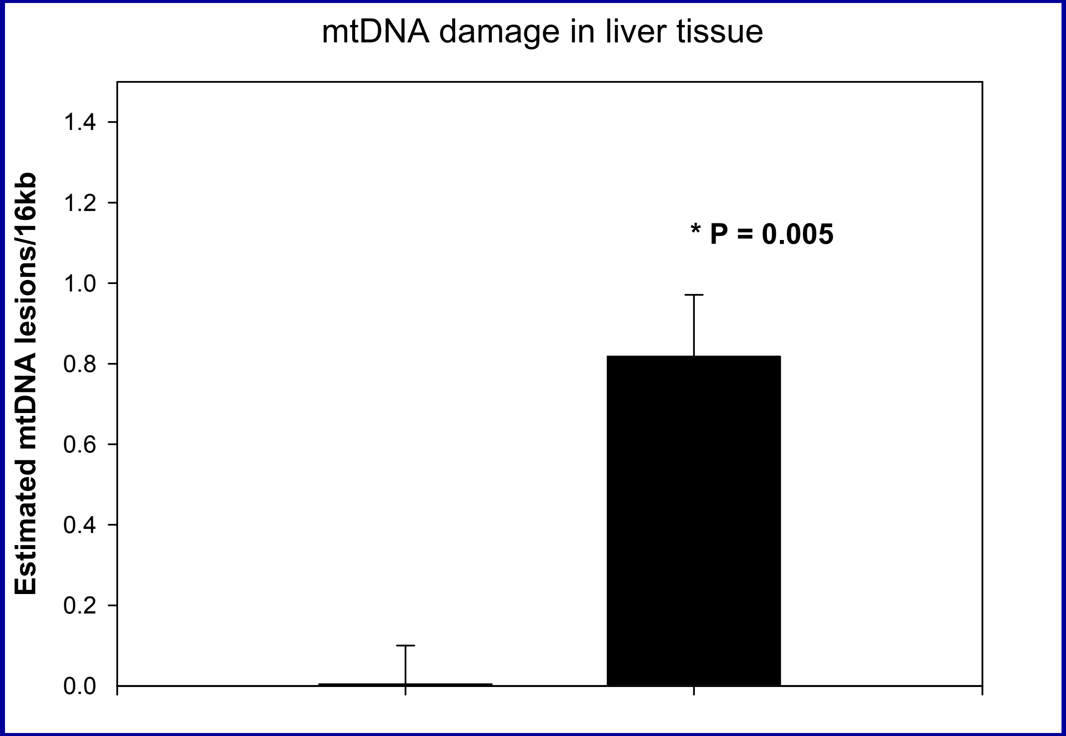
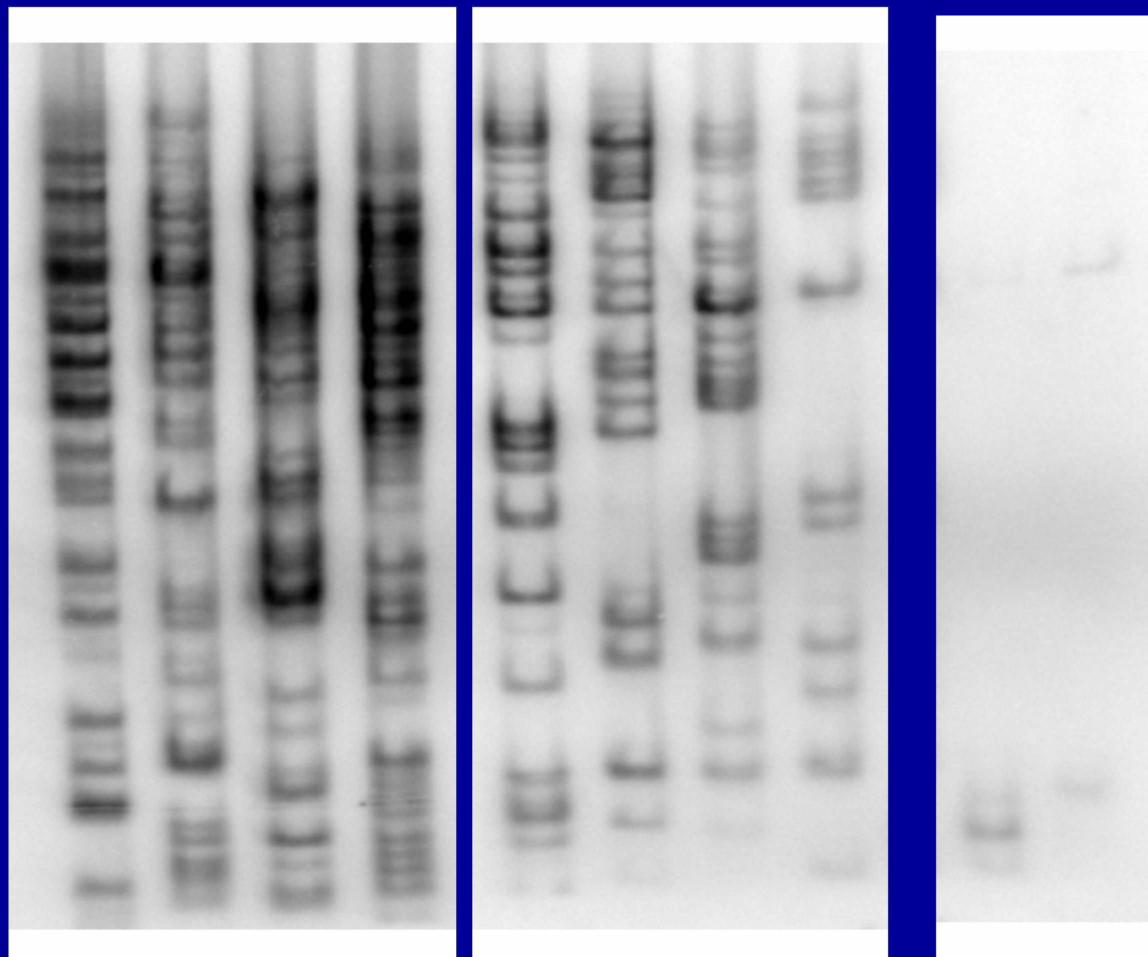
mtDNA Damage in *neil1*$^{-/-}$





Mitochondrial Deletions (males)



Overweight

Severely Overweight

Controls

How could the loss of a DNA repair glycosylase lead to this constellation of diseases?

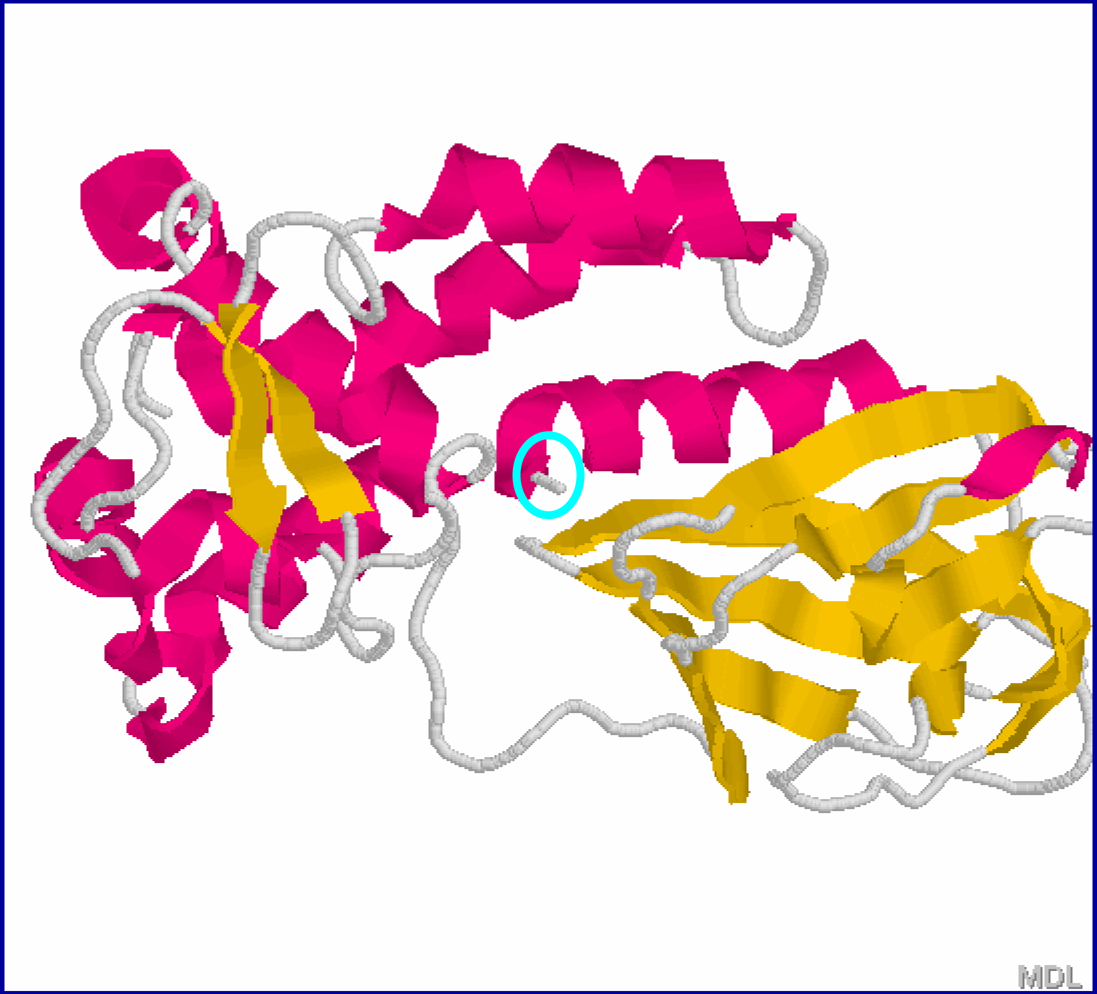
Current Hypothesis

NEIL1 deficiency lowers the threshold at which cumulative oxidative stress-induced DNA damage destabilizes metabolic homeostasis

Heterozygotes develop same phenotype but at a slower rate

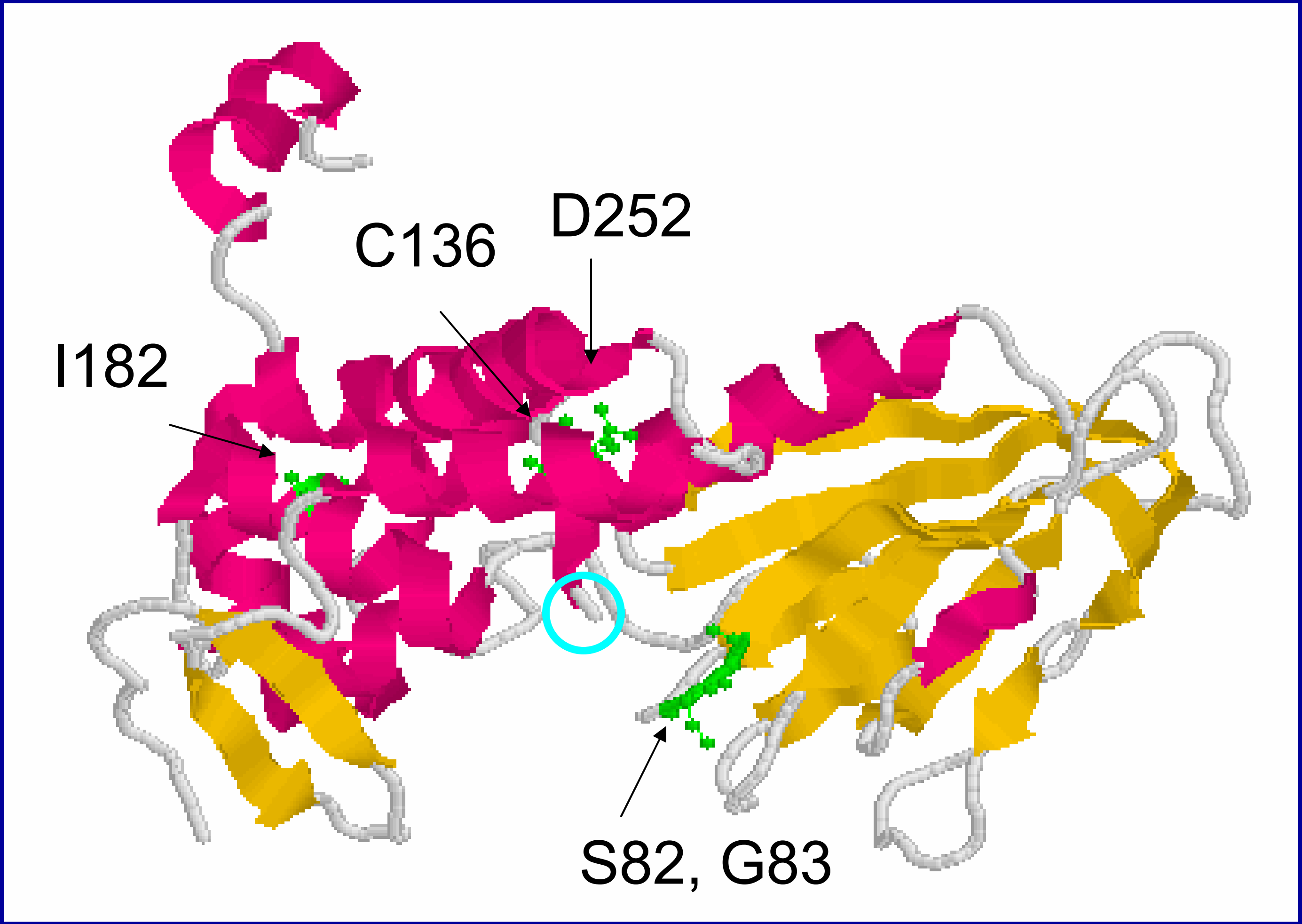
Potential implications for human health

NEIL1 Crystal Structure

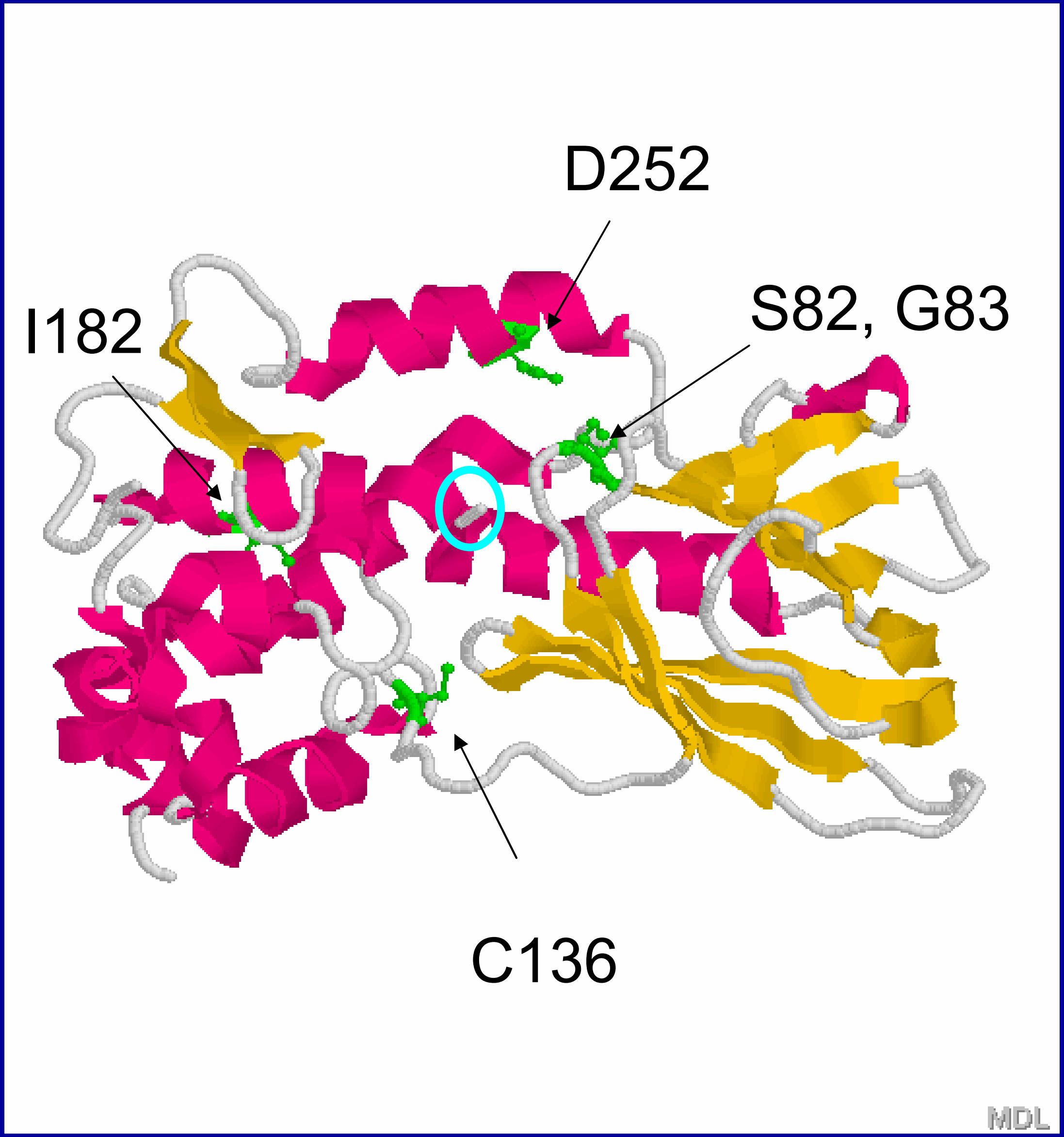


PDB: 1TDH
Doublie et al, 2004

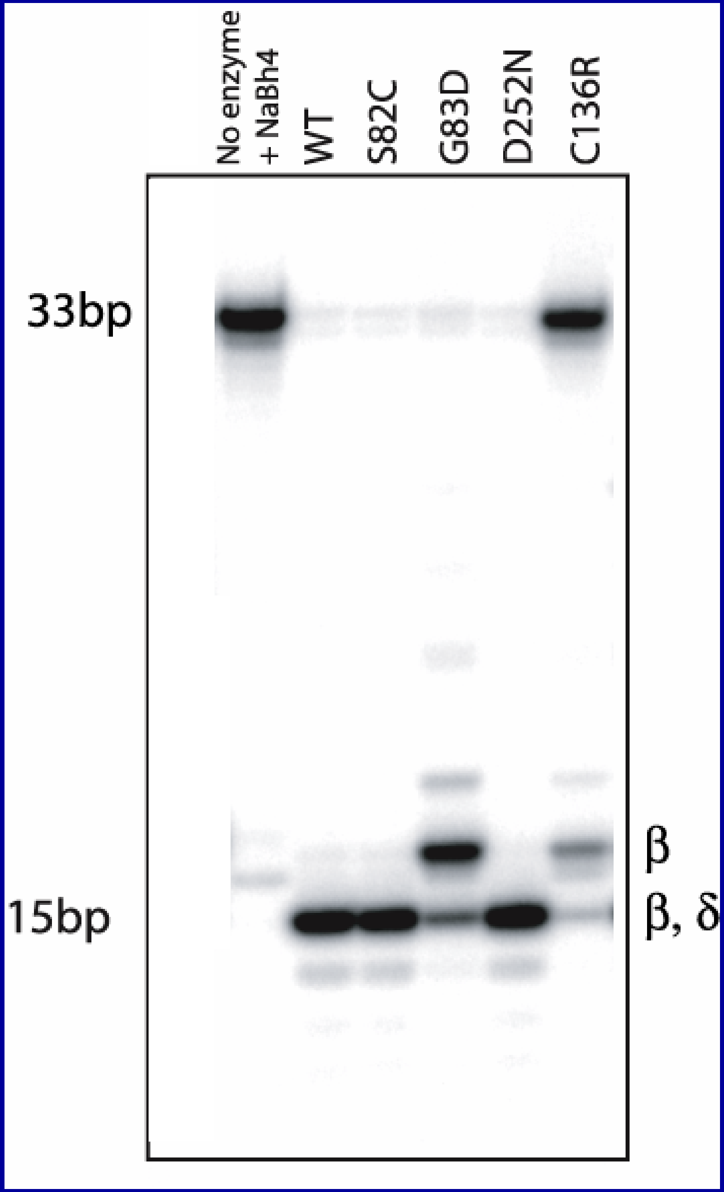
Location of Polymorphic Variants of NEIL1



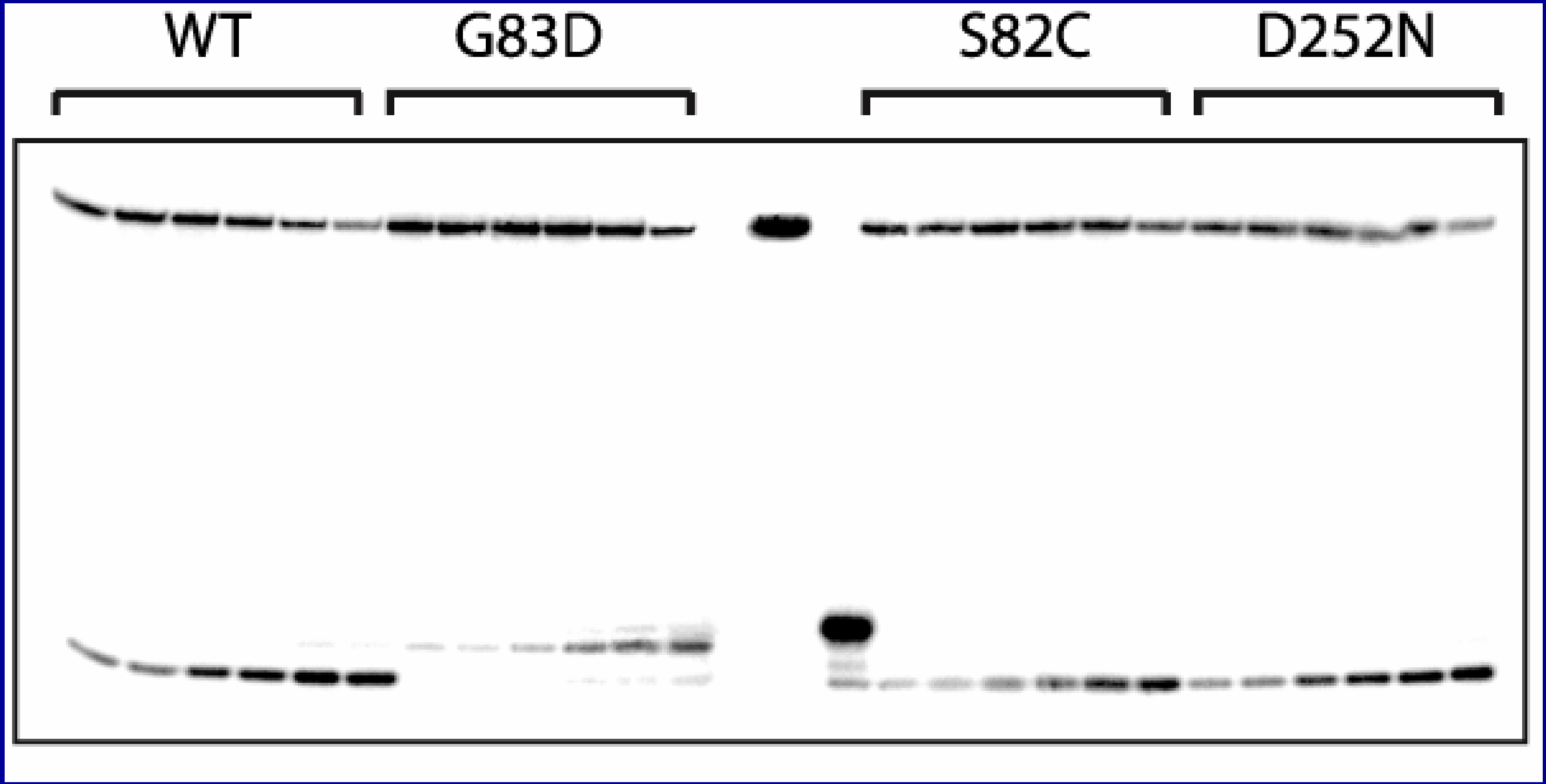
PDB: 1TDH
Doublie et al, 2004

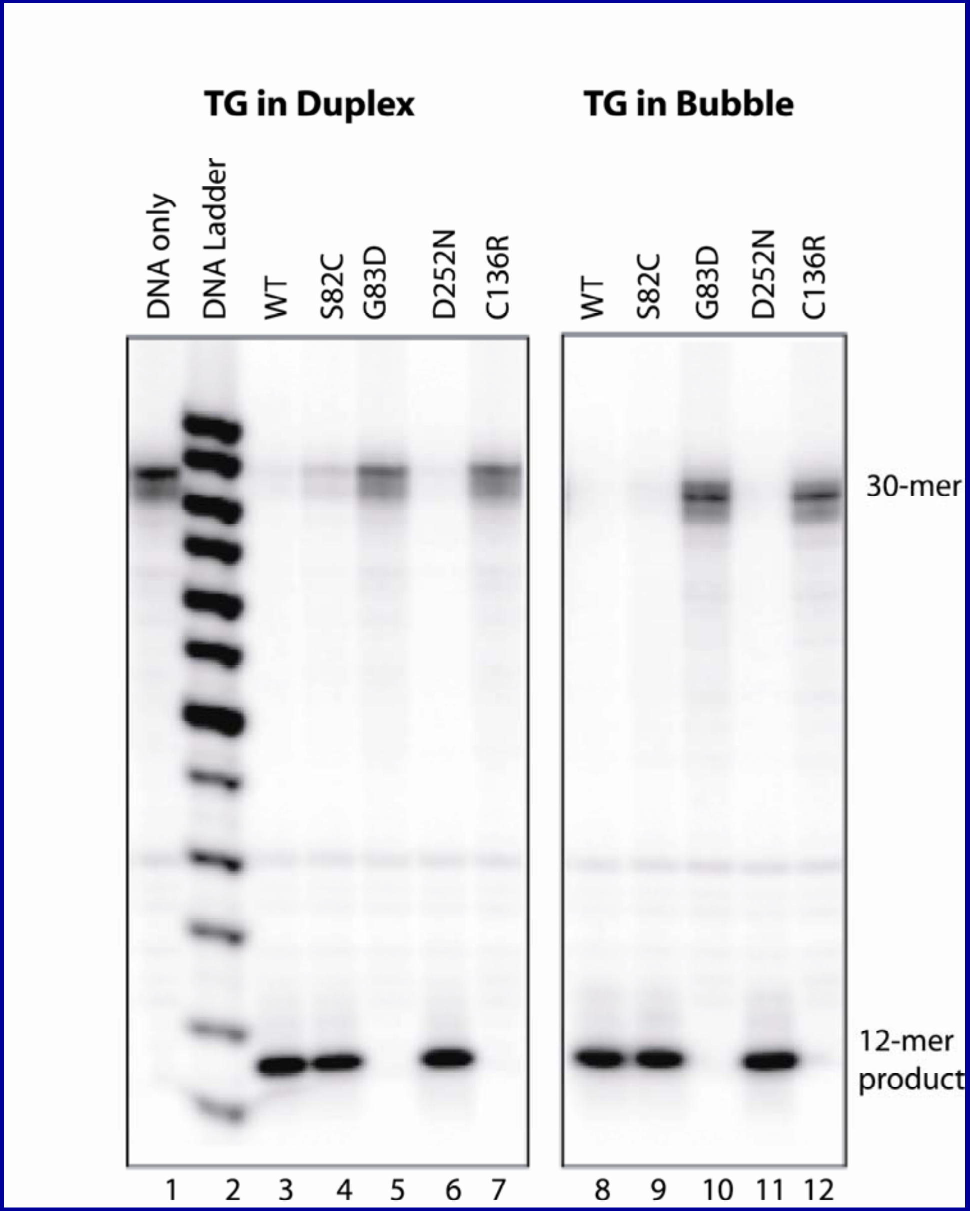

D252
I182
S82, G83
C136
MDL

Survey of Abasic Site Lyase Activities of NEIL1 Variants



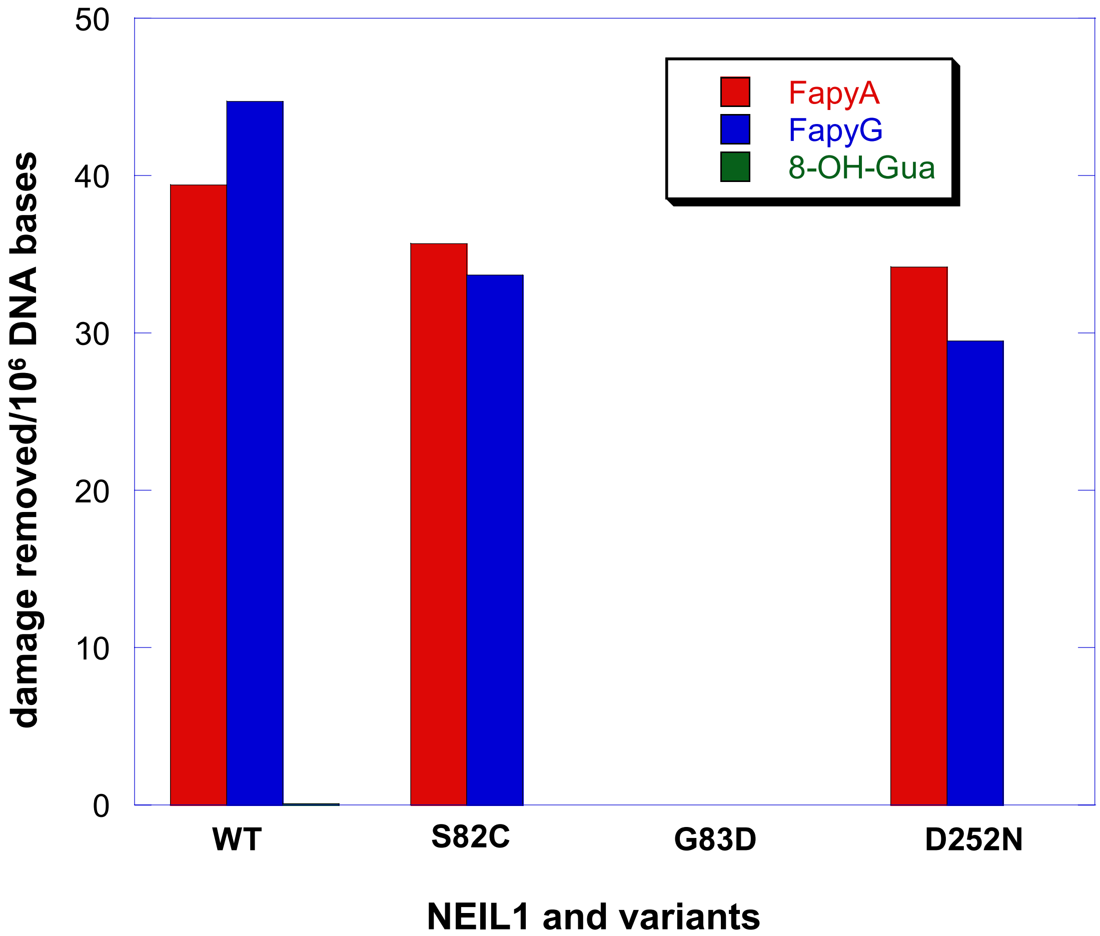
Kinetics of Abasic Site Lyase Activities





TG in Duplex
TG in Bubble
DNA only
DNA Ladder
WT
S82C
G83D
D252N
C136R
WT
S82C
G83D
D252N
C136R
30-mer
12-mer
product
1
2
3
4
5
6
7
8
9
10
11
12

NEIL1 Enzymatic Activity



Kinetic constants for excision of FapyAde and FapyGua by human wild-type NEIL1, D252N-NEIL1 and S82C-NEIL1 from DNA γ-irradiated in aqueous buffered solution saturated with N_2O

Substrate	V_{max} (nM min^{-1})	k_{cat} (min^{-1})	K_M (nM)	$k_{cat}/K_M \times 10^5$ (min^{-1} nM^{-1})
Wild type NEIL1				
FapyAde	5.17 ± 0.58 [a]	0.0114 ± 0.0020	2420 ± 284	0.470 ± 0.104
FapyGua	3.79 ± 0.45	0.0083 ± 0.0018	3572 ± 469	0.233 ± 0.052
D252N-NEIL1				
FapyAde	3.34 ± 0.51	0.0074 ± 0.0021	1677 ± 277	0.438 ± 0.130
FapyGua	3.17 ± 0.38	0.0070 ± 0.0016	3493 ± 466	0.200 ± 0.045
S82C-NEIL1				
FapyAde	3.04 ± 0.65	0.0067 ± 0.0030	1378 ± 308	0.485 ± 0.020
FapyGua	3.73 ± 1.01	0.0082 ± 0.0417	6841 ± 1817	0.127 ± 0.064

[a]Values represent the mean ± standard deviation (*n* = 6). $k_{cat} = V_{max}$/[enzyme]. The enzyme concentration was 454 nM. Concentration ranges of FapyAde and FapyGua were 0.32 μM–4.29 μM and 0.92 μM–8.69 μM, respectively.

Human Polymorphic Variants of *neil1*

Residue #	Frequency*	Activity
S82C	1.1 %	Wild type
G83D	1.1 %	No glycosylase
R136C	1.1 %	No glycosylase
I182M	0.5%	?
D252N	2.4%	Wild type

** NCBI SNP database*

Hypothesis:

Humans carrying inactive polymorphic variants in NEIL1 will be at greater risk of developing the Metabolic Syndrome.

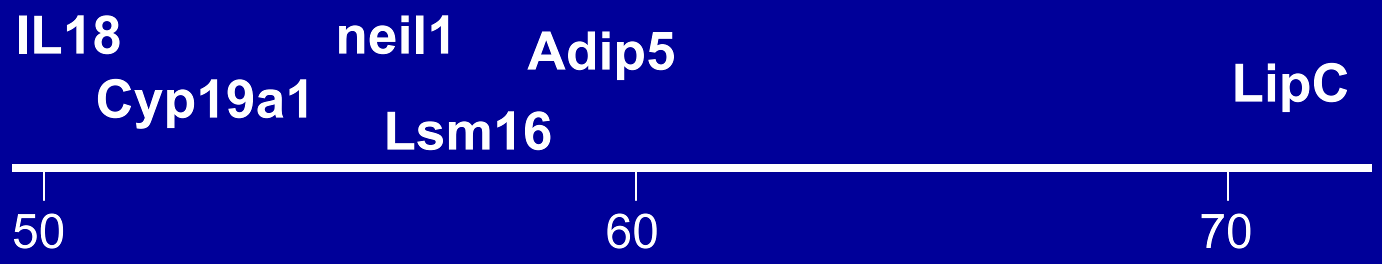
Goal:

Screen normal human controls and Metabolic Syndrome patients for variants in NEIL1.

Ongoing Collaborative Investigations:

Large scale sequencing of Hertfordshire Metabolic Syndrome Cohort (>10,000)

Could KO of *neil1* affect gene expression in local vicinity and produce phenotype?



- IL18 KO mice: hyperphagic, obese, insulin^R
- Cyp19a1 KO mice: increased gonadal fat
- Lsm16 KO mice: reduced body fat
- Adip5: increases gonadal fat
- LipC alleles: increased obesity

- None match *neil1* KO phenotype
- Gene chip analyses – no significant changes of genes in the region

Metabolic Syndrome & Ataxia Telangiectasia
(Schneider et al, 2006)

AT children

- Cerebellar ataxia, skin and eye telangiectasia, malignancies, immune deficiencies
- Insulin resistance *

Metabolic Syndrome & AT

ApoE $^{-/-}$ background ATM $^{-/-}$, ATM $^{+/-}$, ATM $^{-/-}$ fed Western diet 40% fat calories

absolute weight unchanged

% body fat 23% $^{+/+}$; 33% $^{+/-}$; 36% $^{-/-}$

systolic & diastolic BP increases in $^{+/-}$, $^{-/-}$

cholesterol, TG, FFA unchanged

atherosclerosis very elevated in $^{+/-}$, $^{-/-}$

	Apo E $^{-/-}$ ATM $^{-/-}$	NEIL1 $^{-/-}$
weight	Unaffected	Increased
% fat	Increased	Increased
BP	Increased	ND
Cholesterol/TG/ FFA	Unaffected	Increased
Atherosclerosis	Severe	none

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Jaruga Pawel

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USGS June 29, 2005

Location: human 15q25
mouse 9a5,3

Tissue distribution in human
liver
pancreas
thymus

S-phase specific expression in tissue culture

Up-regulated following oxidative stress

Hazra et al , 2003
Das et al, 2003
Morland et al, 2002

Intracellular Distribution

nucleolus

nucleus

mitochondria

Association with Rad9-Rad1-hus1 9-1-1 checkpoint sensor (Guan et al, 2007)