Expression of human mitochondrial transcriptional factor A (hTFAM) improves cognitive function in Alzheimer's disease model mice

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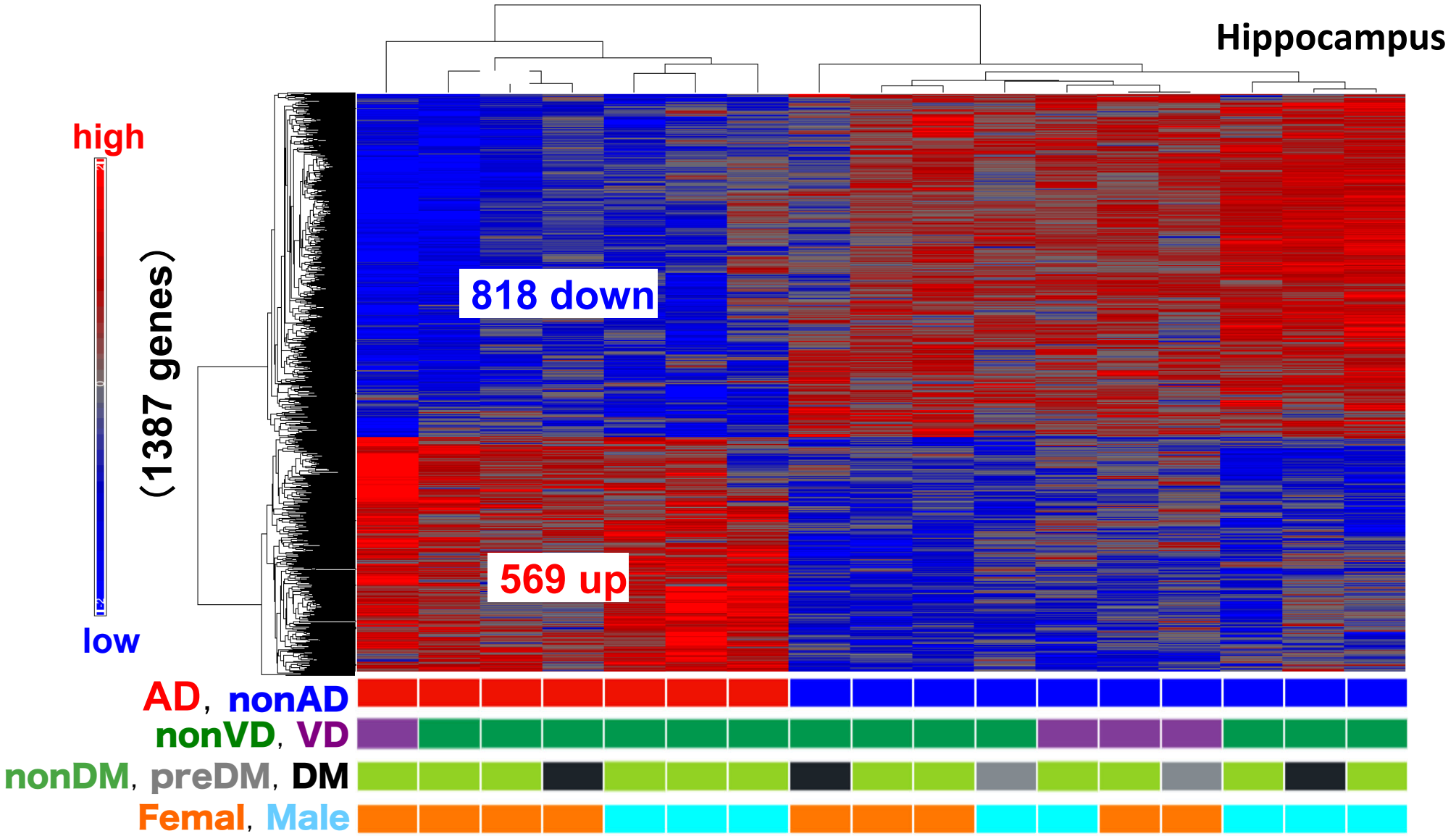
Introduction

More than 20 million people worldwide suffer from dementia, and this number is expected to exceed 80 million by 2040 because of the rapid increase in the numbers of elderly (Ferri CP et al. 2005). The prevalences of all-cause dementia and Alzheimer's disease (AD) in the general population of Japanese elderly have increased significantly over the past 20 years, especially among subjects aged ≧75 years (Sekita A et al. 2010). Thus, it is important to establish effective prevention strategies for dementia, and particularly for AD. To reach this goal, it is essential to understand the risk factors for developing dementia, including AD, in the elderly population.

Several recent studies have indicated effects of insulin and glucose metabolism on the risk of developing dementia, especially AD (Kuusisto J et al. 1997; de la Monte SM and JR Wands 2008; Schrijvers EM et al. 2010). The results of the Hisayama study suggested that hyperinsulinemia and hyperglycemia caused by insulin resistance accelerate the formation of neuritic plaques (NPs) in combination with the effect of the *APOE e*4 allele, a major risk factor for AD (Matsuzaki T et al. 2010).

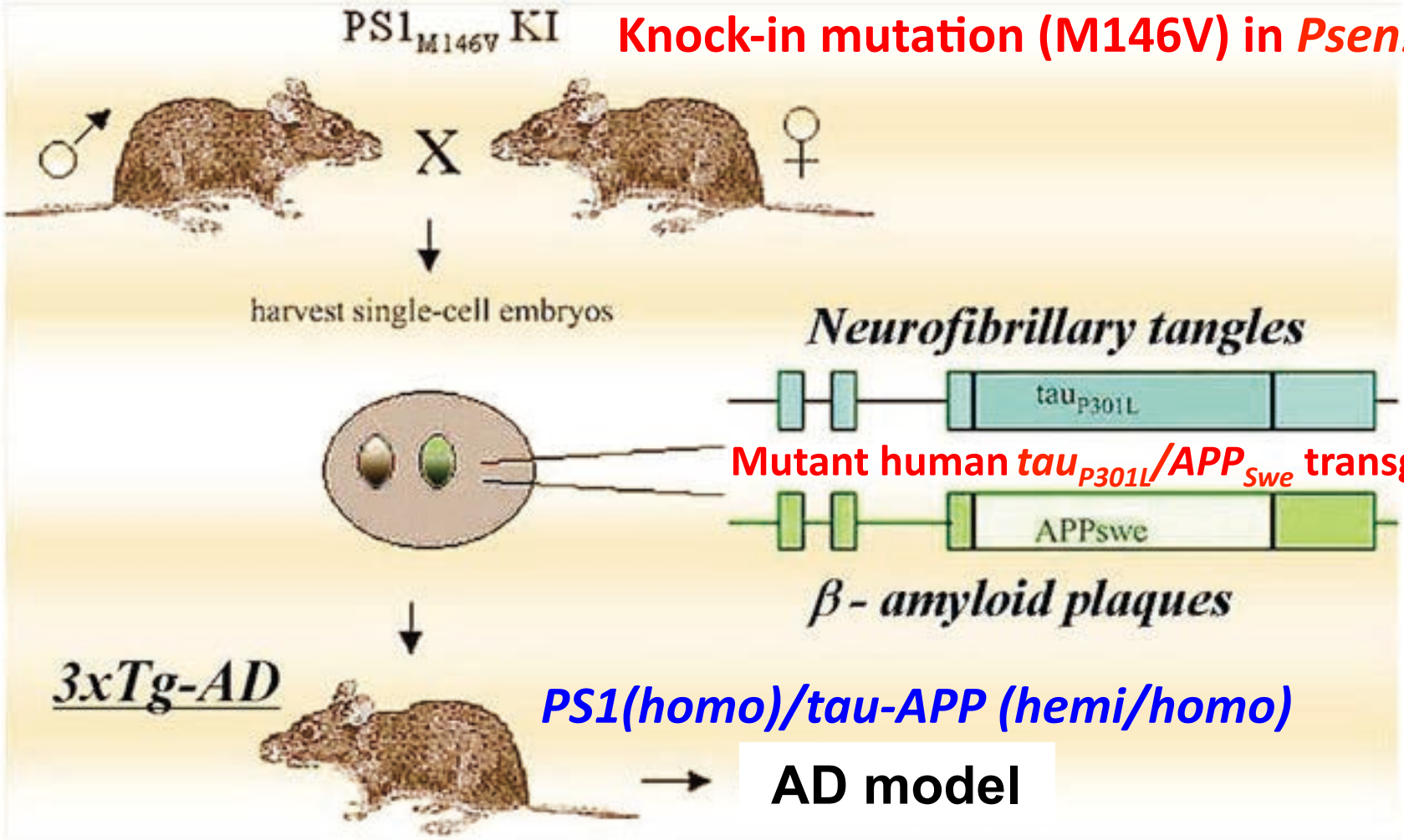
To identify molecular pathological alterations in AD brains, we performed interspecies comparative microarray analyses using RNA prepared from postmortem human brain tissues donated for the Hisayama study (Katsuki S 1966; Matsuzaki T *et al.* 2010; Sekita A *et al.* 2010), and hippocampal RNAs from the triple-transgenic mouse model of AD (3xTg-AD) (Oddo S et al. 2003). We found altered expression profiles of diabetes mellitus (DM)-related genes in AD brains, which were independent of peripheral DM-related abnormalities Finally the altered gene expression profile indicates severe mitochondrial dysfunction in AD brain.

Altered gene expression profile in AD brain



Hokama M. *et al*, Altered expression of diabetes-related genes in Alzheimer's disease brains: the hisayama study. *Cereb Cortex* 24:2476-2488, 2014.

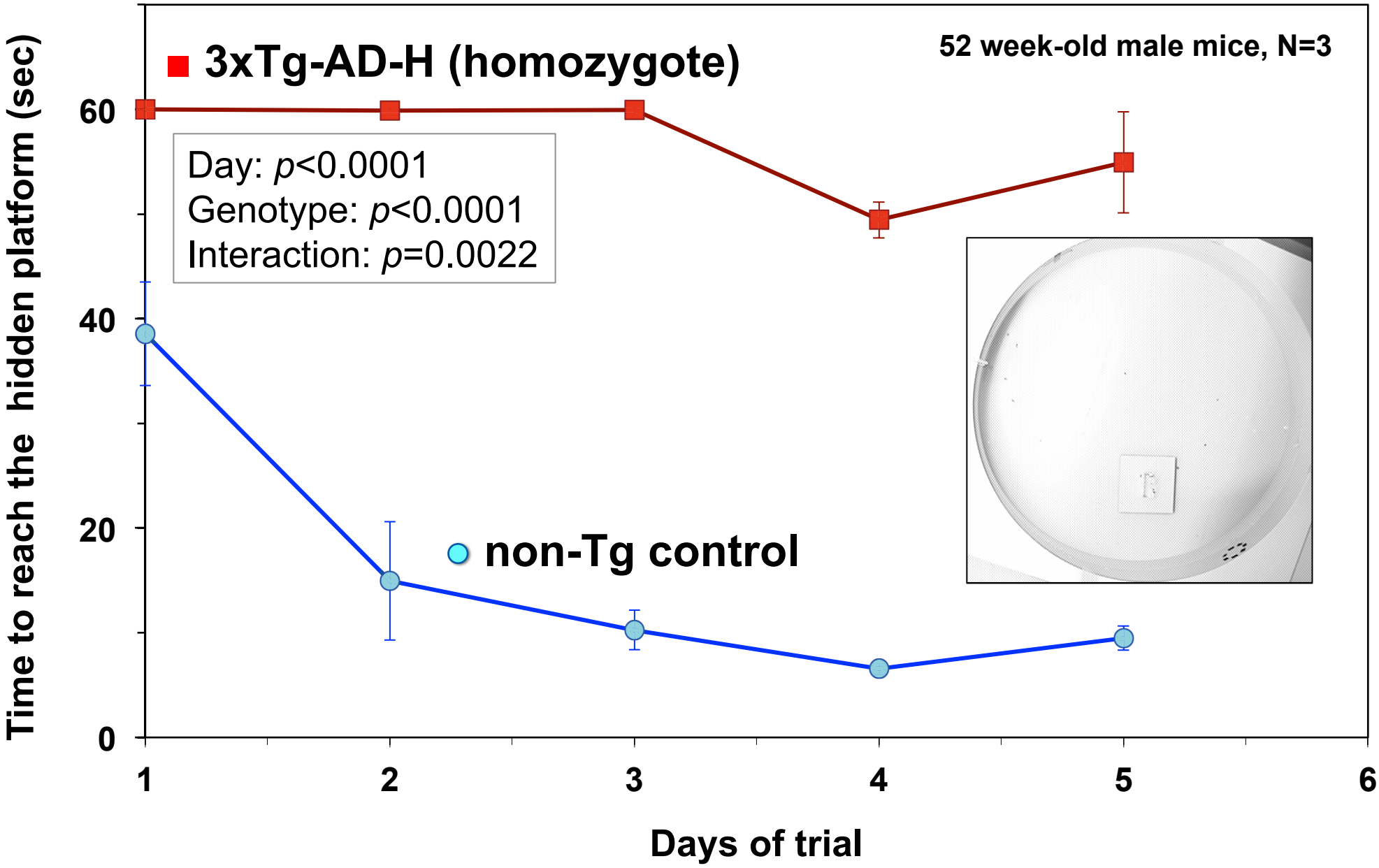
Triple transgenic AD mouse model



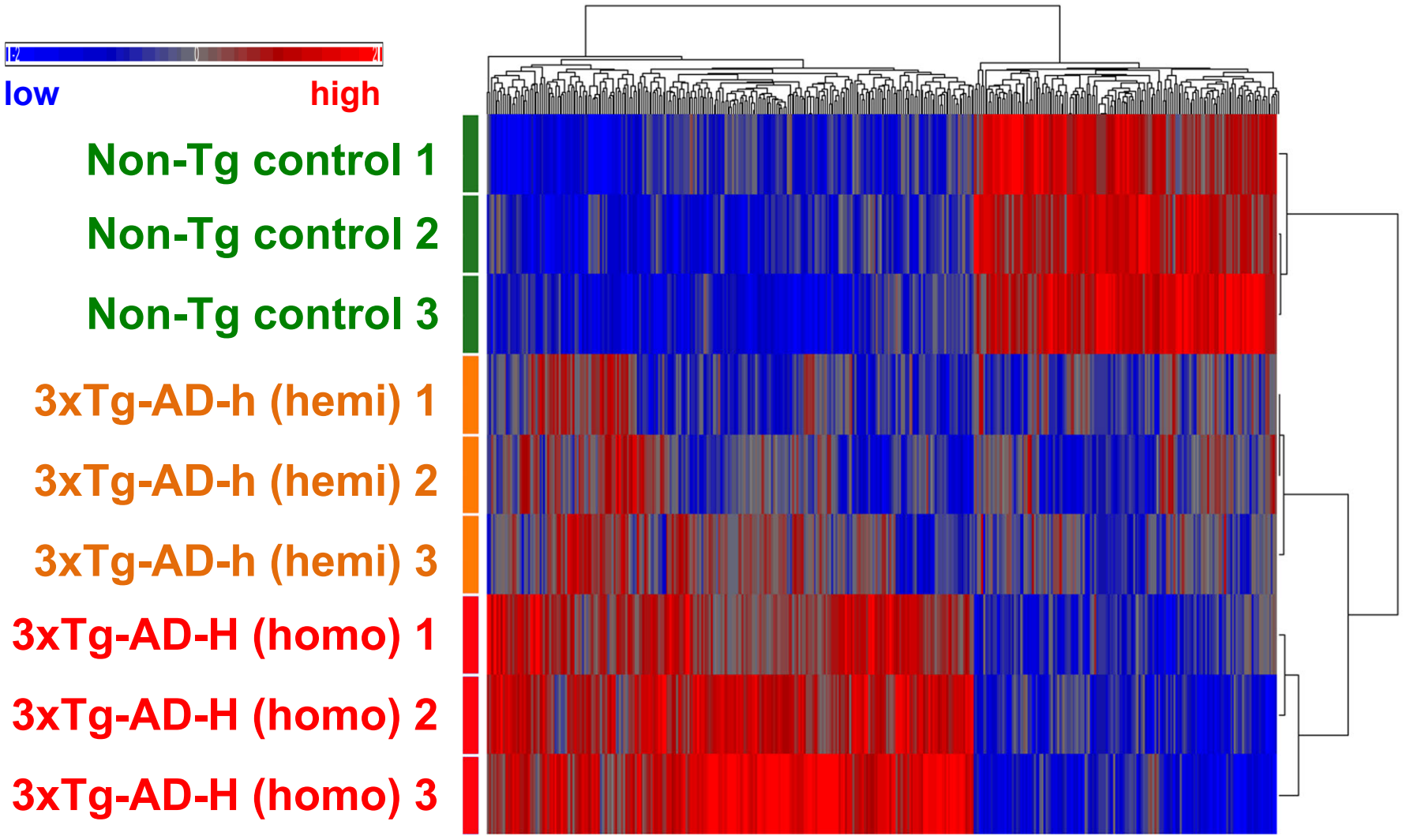
Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y., and LaFerla, F.M. . Neuron. 39:409-421, 2003

3xTg-AD model mice exhibit plaques and tangles, intracellular Aβ and synaptic dysfunction

Impaired spatial learning in 3xTg-AD mice



Altered gene expression profile in 3xTg-AD mice



Transcript clusters (406) exhibiting significantly altered expression between 3xTg-AD-H and non-Tg control hippocampal RNA with a fold change >1.3 and FDR (q<0.05)

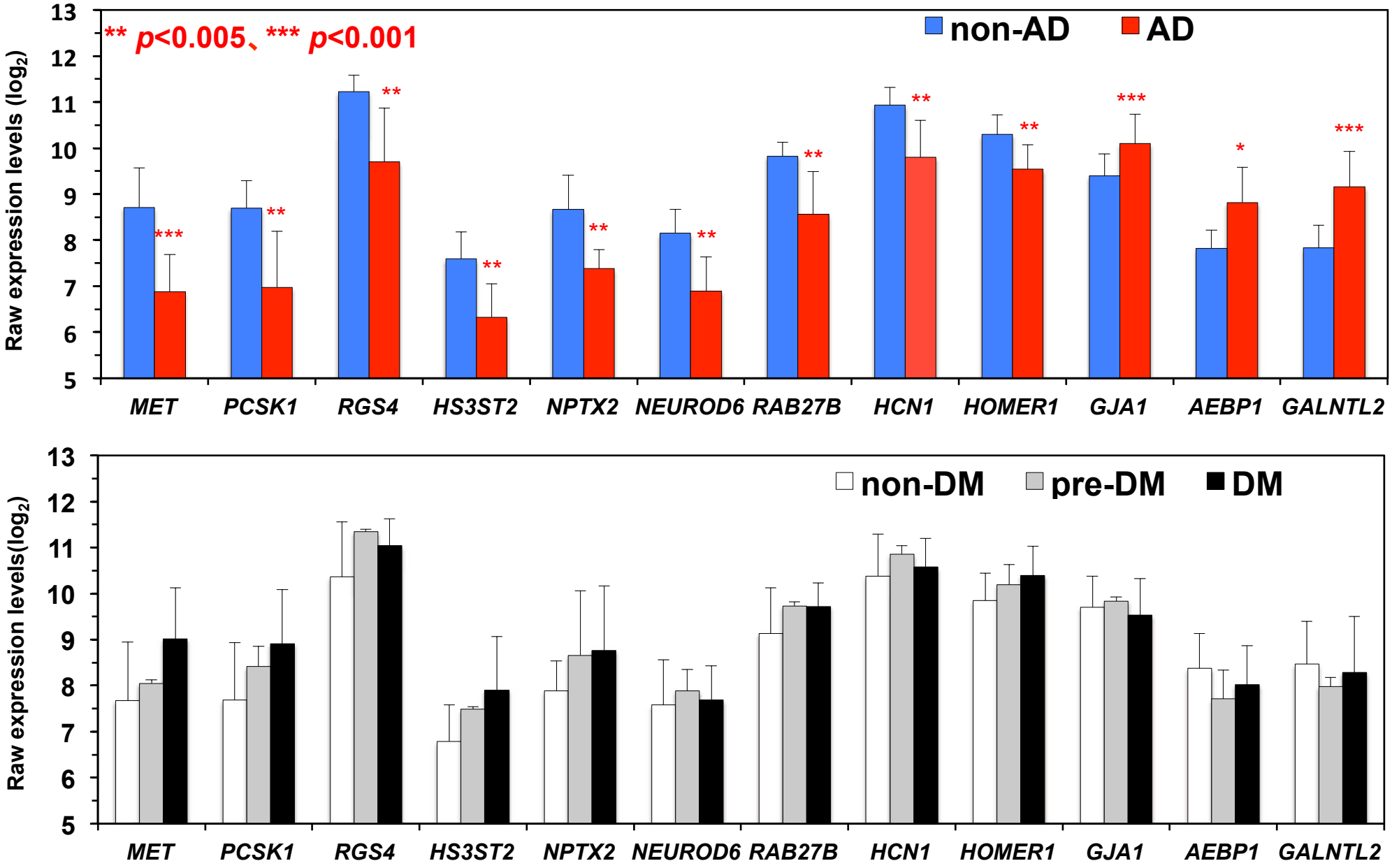
Genes whose expression is significantly altered in human and mouse with AD pathology are enriched in selective disease & disorders

Diseases and Disorders	AD hippocampus		3xTg-AD (homo)	
	Top p-value	# Genes	p-value	# Genes
Genetic Disorder	6.77E-14	105	3.72E-05	62
Neurological Disease	8.66E-15	85	4.64E-05	43
Gastrointestinal Disease	4.52E-08	74	5.25E-03	35
Cancer	4.52E-08	59	3.12E-04	14
Skeletal and Muscular Disorders	1.33E-07	59	6.13E-03	32
Inflammatory Disease	6.65E-05	53	1.22E-02	35
Psychological Disorders	2.92E-14	50	2.83E-05	27
Immunological Disease	1.85E-05	48	3.72E-05	31
Metabolic Disease	2.90E-06	47	6.13E-03	30
Cardiovascular Disease	8.39E-06	46	4.02E-03	22
Endocrine System Disorders	2.90E-06	45	8.04E-04	29
Connective Tissue Disorders	6.65E-05	36	1.22E-02	23
Hematological Disease	5.60E-05	35	4.53E-03	27
Organismal Injury and Abnormalities	4.16E-06	18	6.13E-03	7
Nutritional Disease	1.54E-04	15	1.49E-03	11
Ophthalmic Disease	2.90E-06	12	3.98E-03	4
Dermatological Disease and Conditions	5.39E-04	8	3.54E-03	19
Total genes eligible		147		109

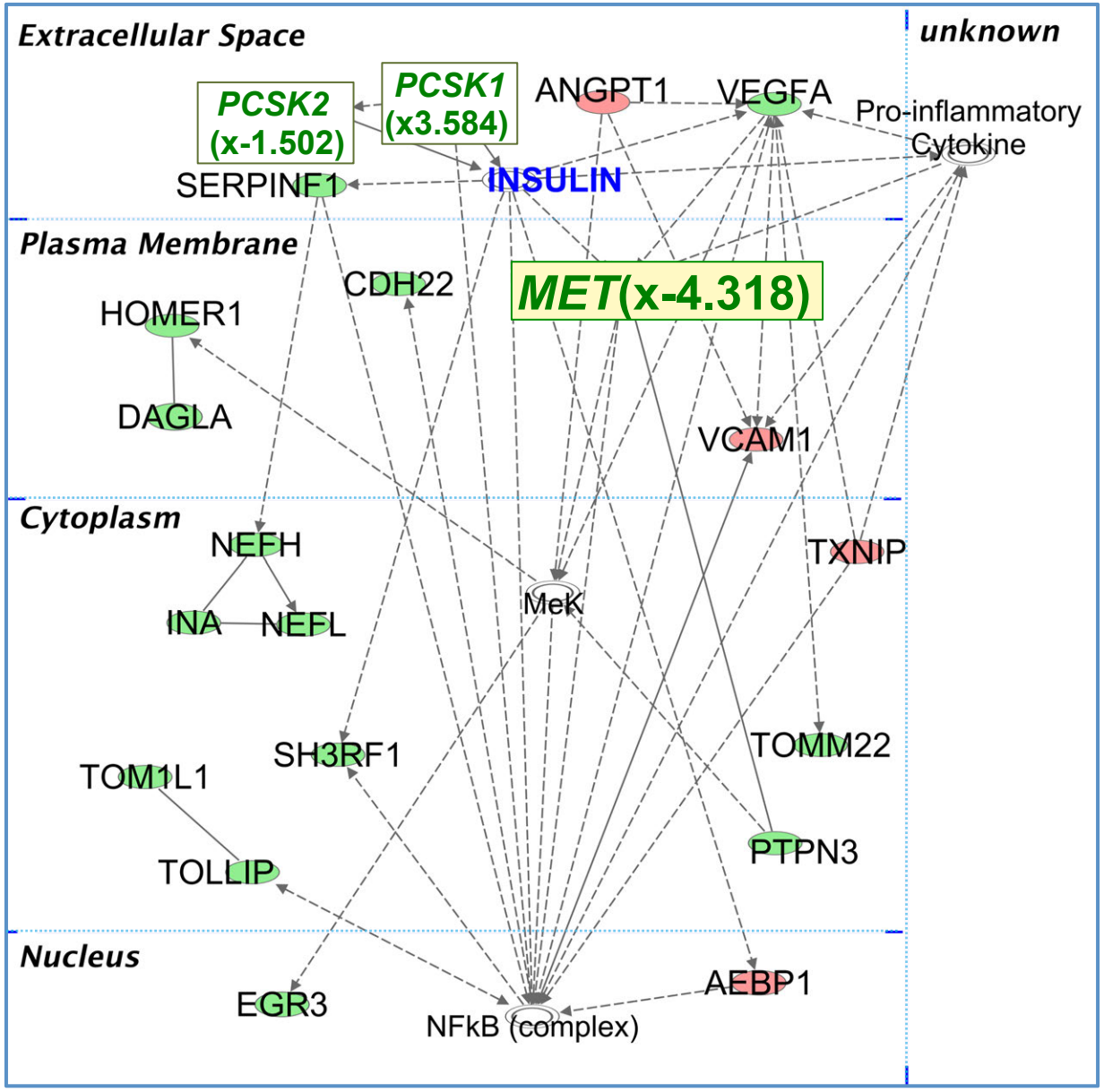
Genes whose expression is significantly altered in human and mouse with AD pathology are enriched in selective genetic disorders

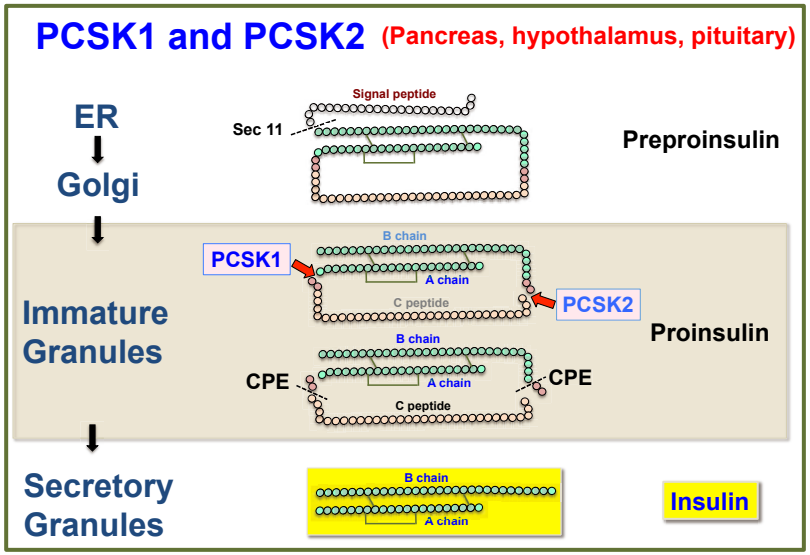
Diseases and disorders	AD hippocampus		3xTg-AD (homo)	
	p-value	# Genes	*p-value*	# Genes
schizophrenia	6.77E-14	29	–	–
bipolar disorder	6.08E-06	26	4.64E-05	20
coronary artery disease	1.04E-04	25	8.39E-03	16
Crohn's disease	2.46E-04	23	–	–
non-insulin-dependent diabetes mellitus	4.15E-03	23	1.41E-02	17
amyotrophic lateral sclerosis	2.30E-08	22	–	–
Huntington's disease	2.79E-07	22	–	–
Alzheimer's disease	1.77E-04	21	1.41E-02	13
Parkinson's disease	2.21E-03	14	3.13E-02	9
obesity	1.54E-04	12	1.36E-02	7
genetic disorder	3.05E-09	105	3.83E-02	62

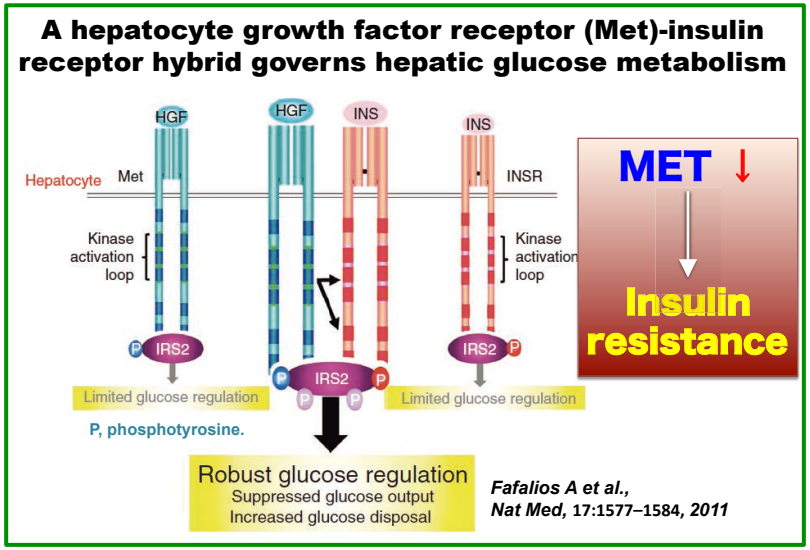
DM has little effect on gene expression profiles in brain



AD network 1: Cell-To-Cell Signaling and Interaction, Hematological System Development and Function, Immune Cell Trafficking

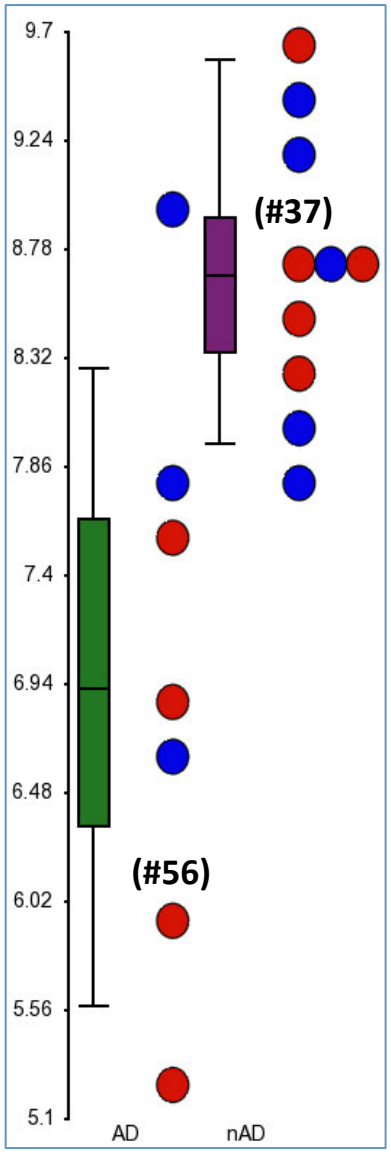




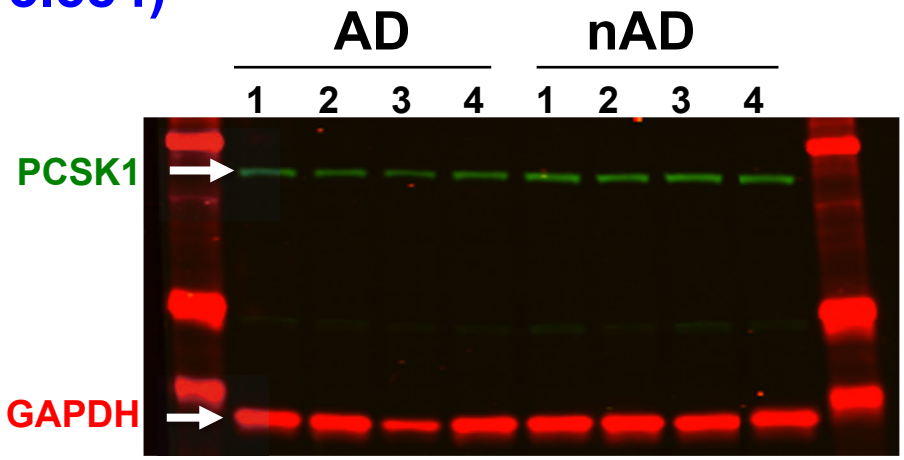


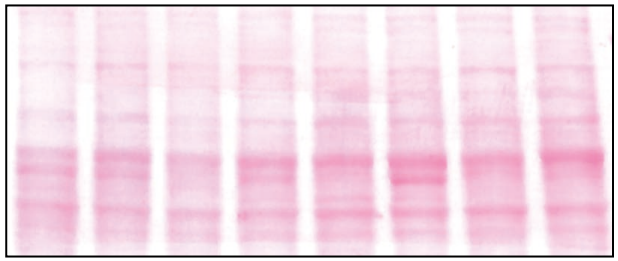
Expression of PCSK1 protein in AD hippocampus

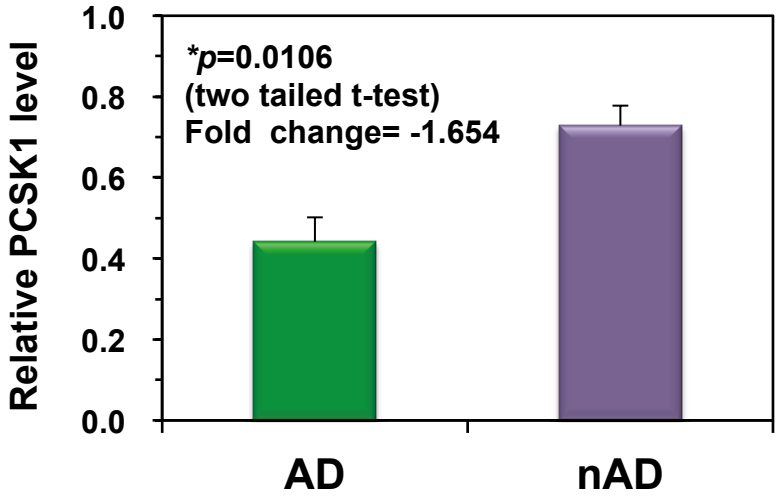
PCSK1 mRNA (x-3.584)



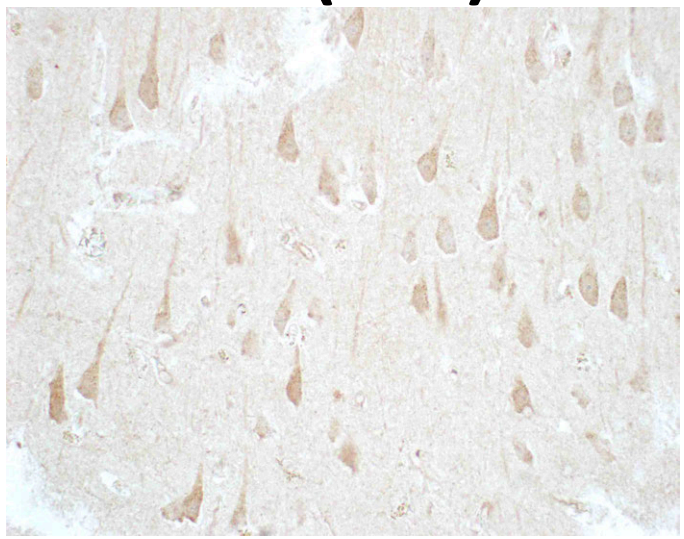
■:AD ■:nAD ●:F ●:M



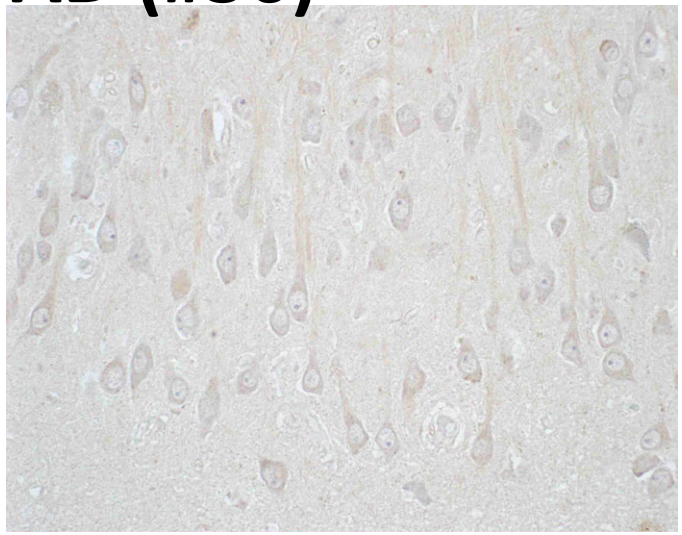




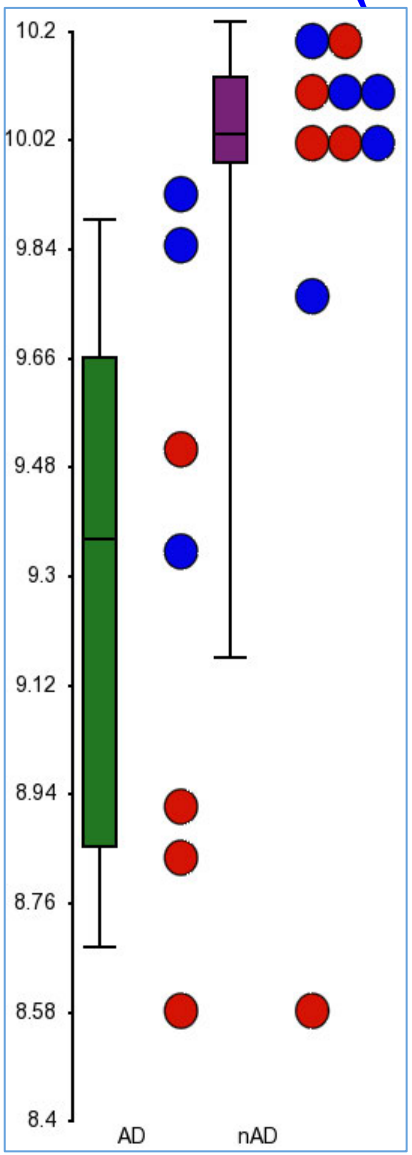
Non-AD (#37)

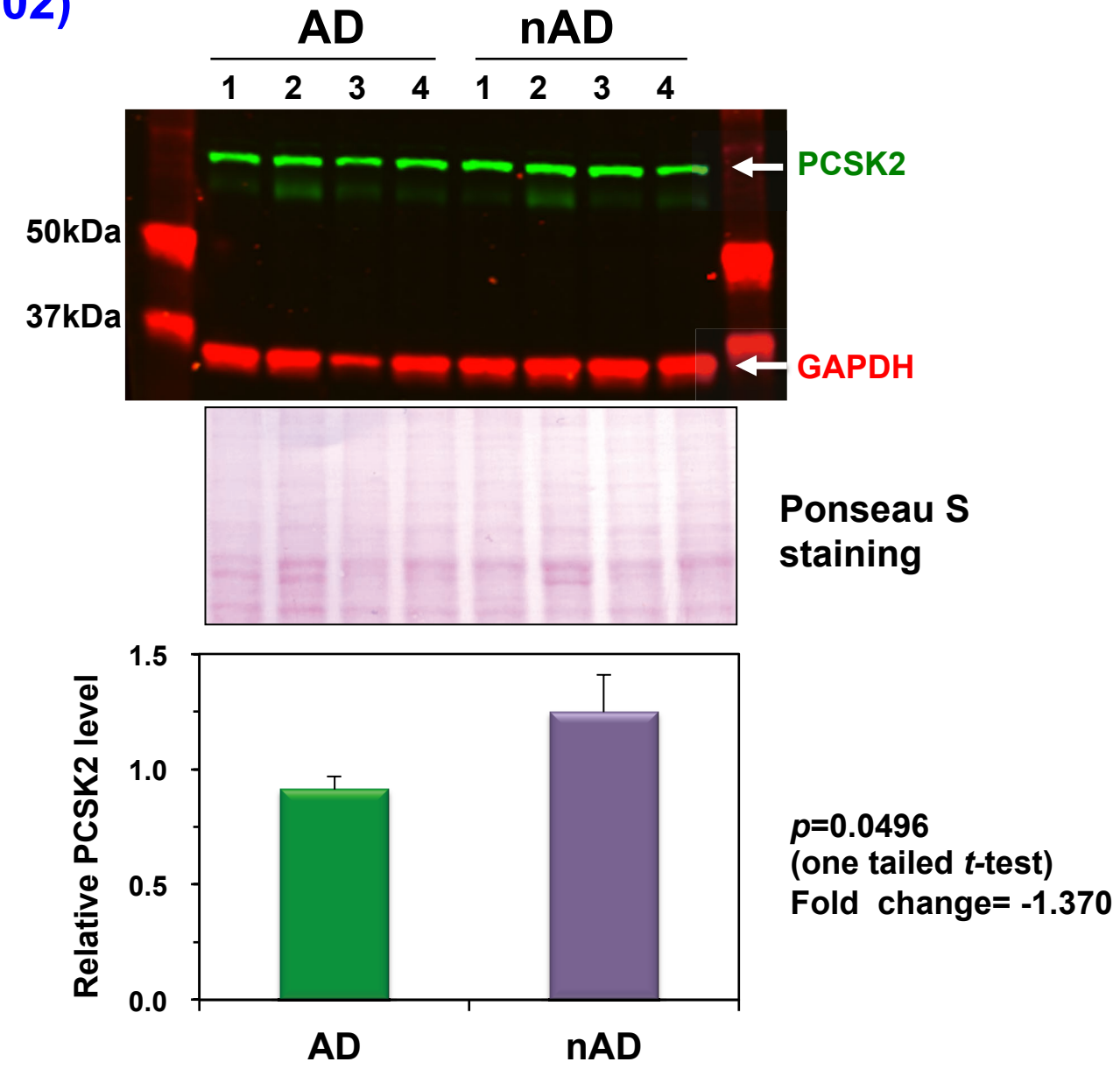


AD (#56)



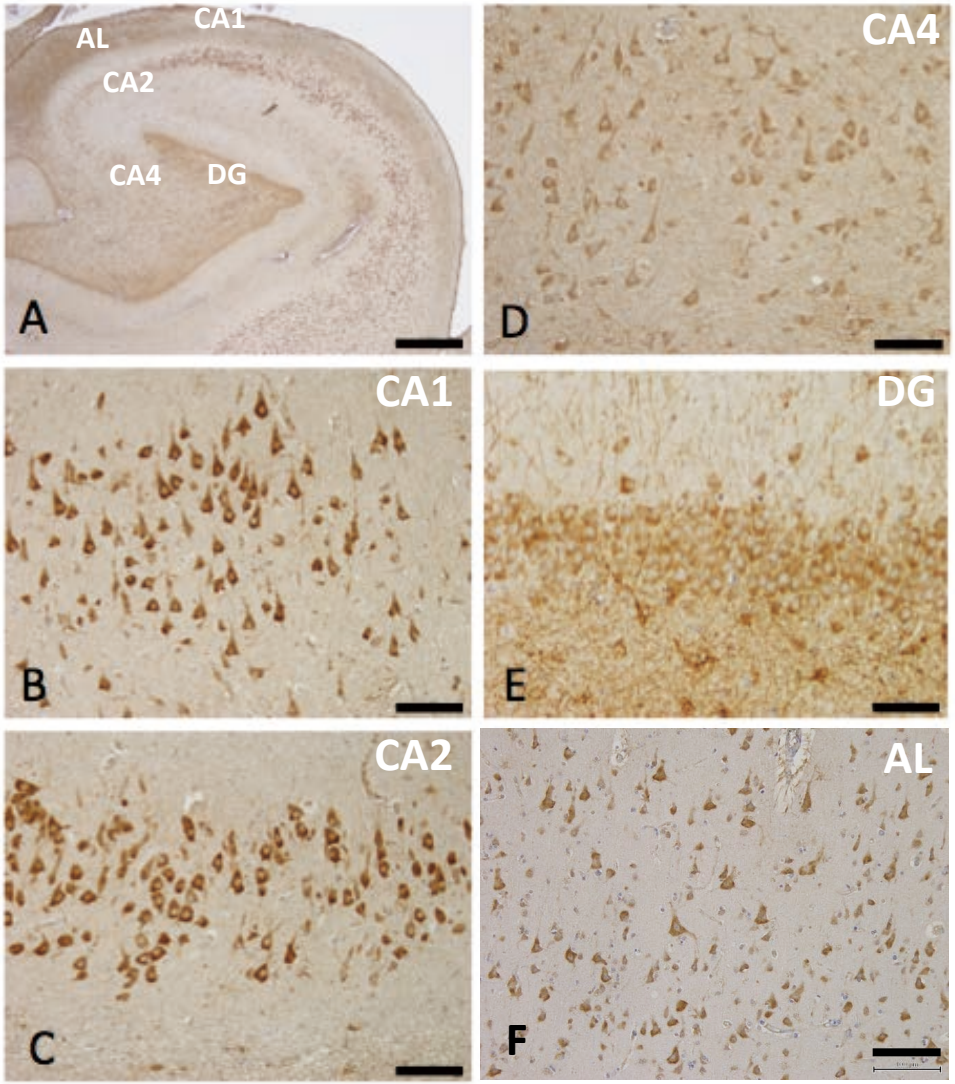
Expression of PCSK2 protein in AD hippocampus



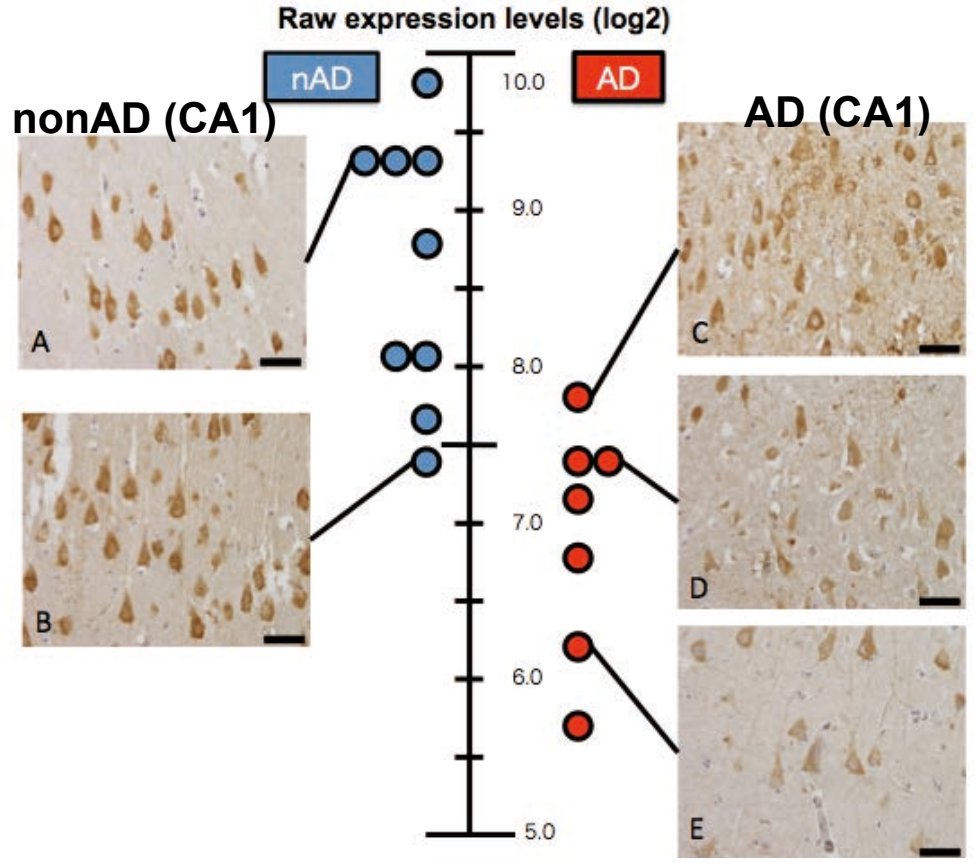


Down regulation of MET expression in AD brain

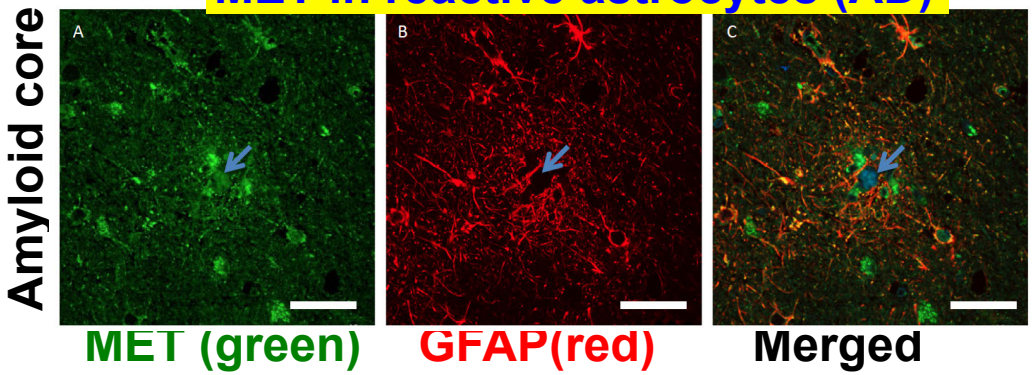
MET expression control brain



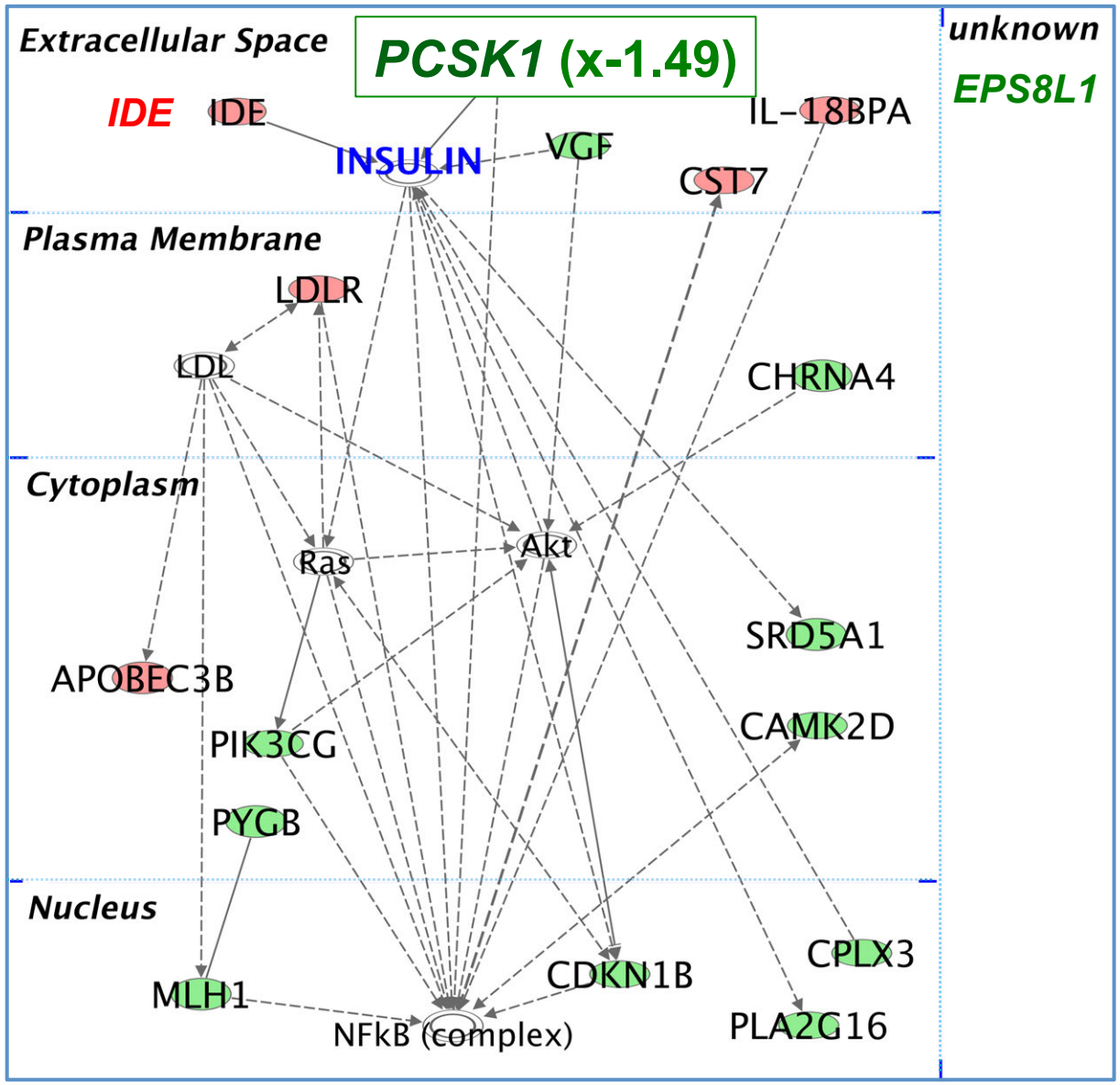
Hamasaki H, Honda H, Suzuki SO, Hokama M, Kiyohara Y, Nakabeppu Y & Iwaki T. **Down-regulation of MET in hippocampal neurons of Alzheimer's disease brains**. *Neuropathol* **34**, 284-290, 2014.



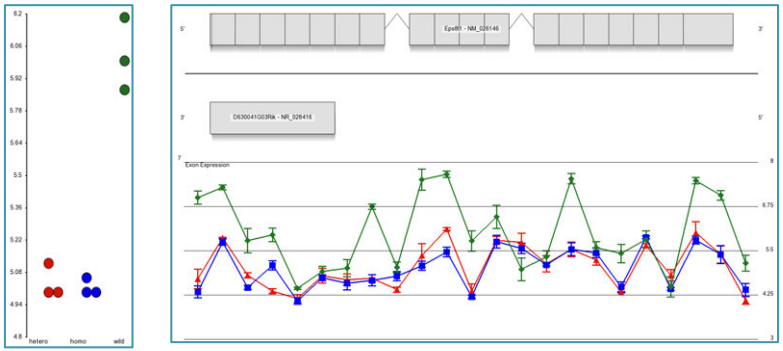
MET in reactive astrocytes (AD)



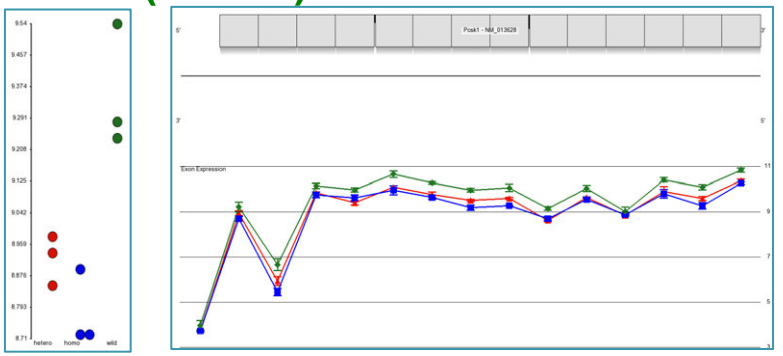
Mouse network 1: Lipid Metabolism, Small Molecule Biochemistry, Cell Cycle



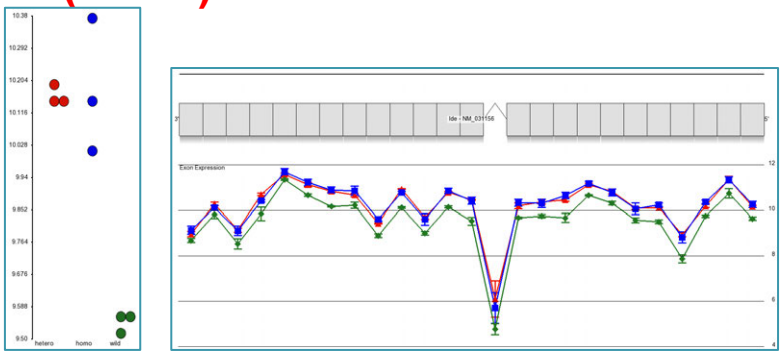
EPS8L1 (x-2.02) EGFR kinase substrate 8-like protein 1



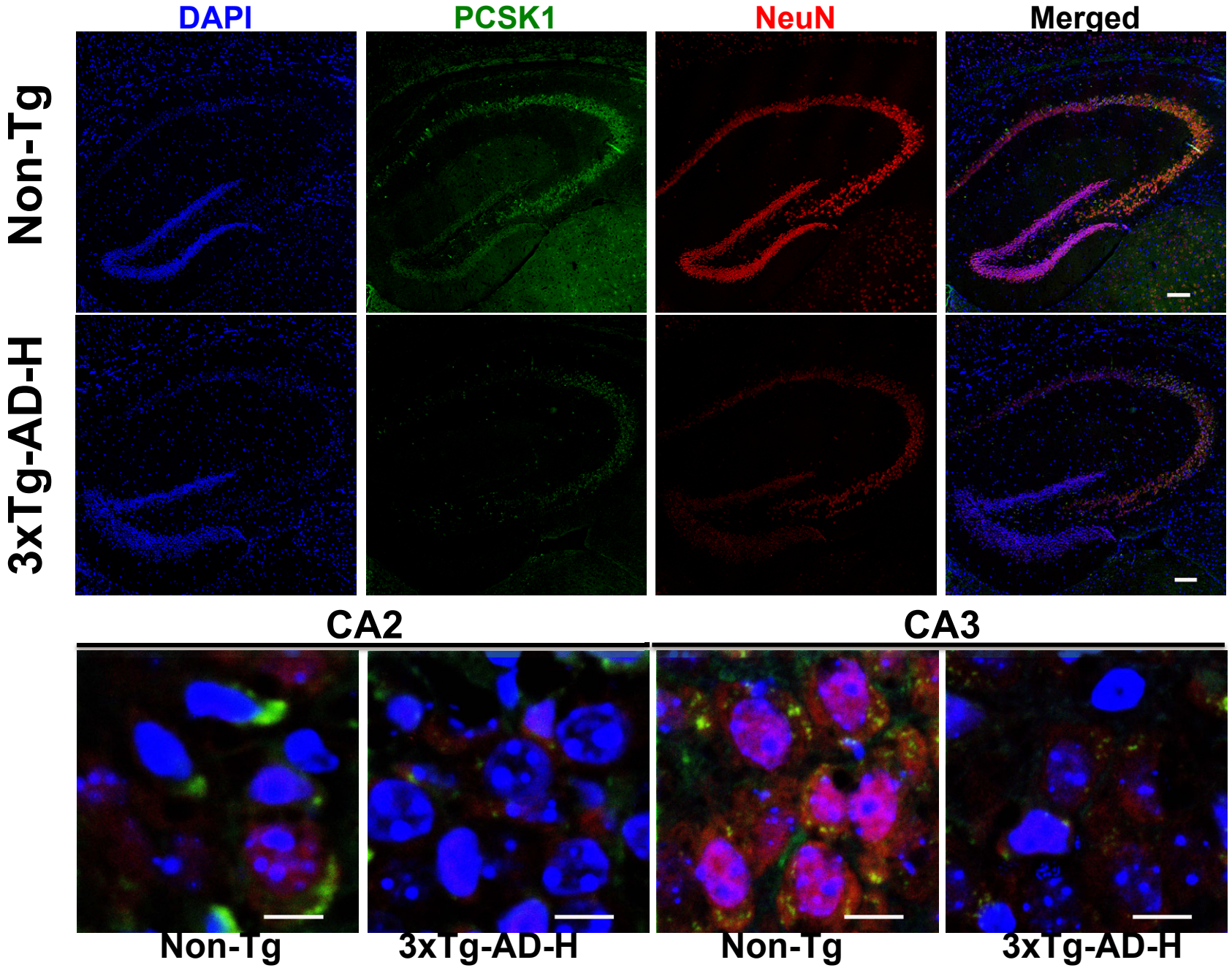
PCSK1 (x-1.49) proprotein convertase 1



IDE (x1.55) Insulin-degrading enzyme



Decreased expression of PCSK1 in 3xTg-AD-H hippocampus



Impaired glucose metabolism in AD patients

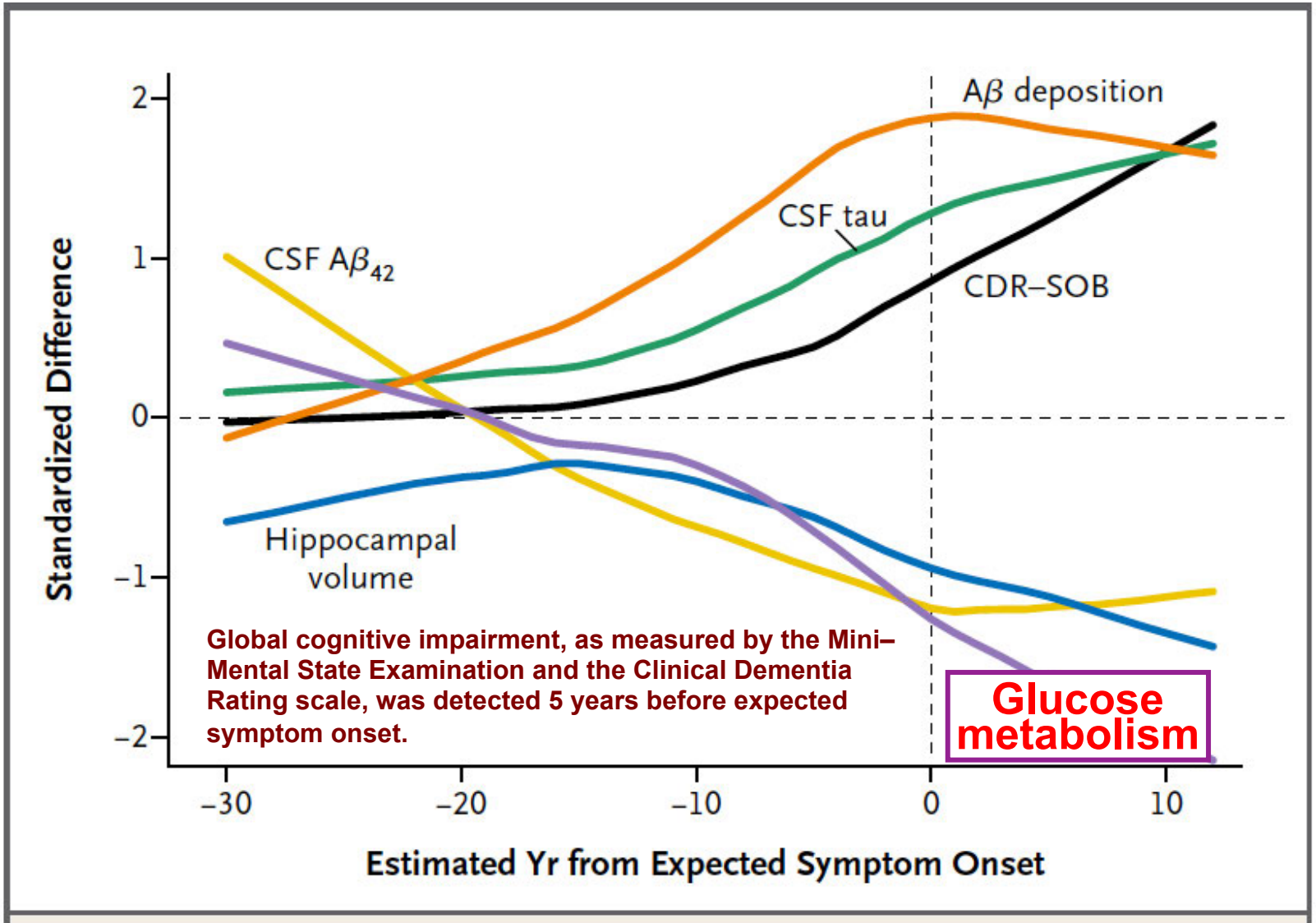
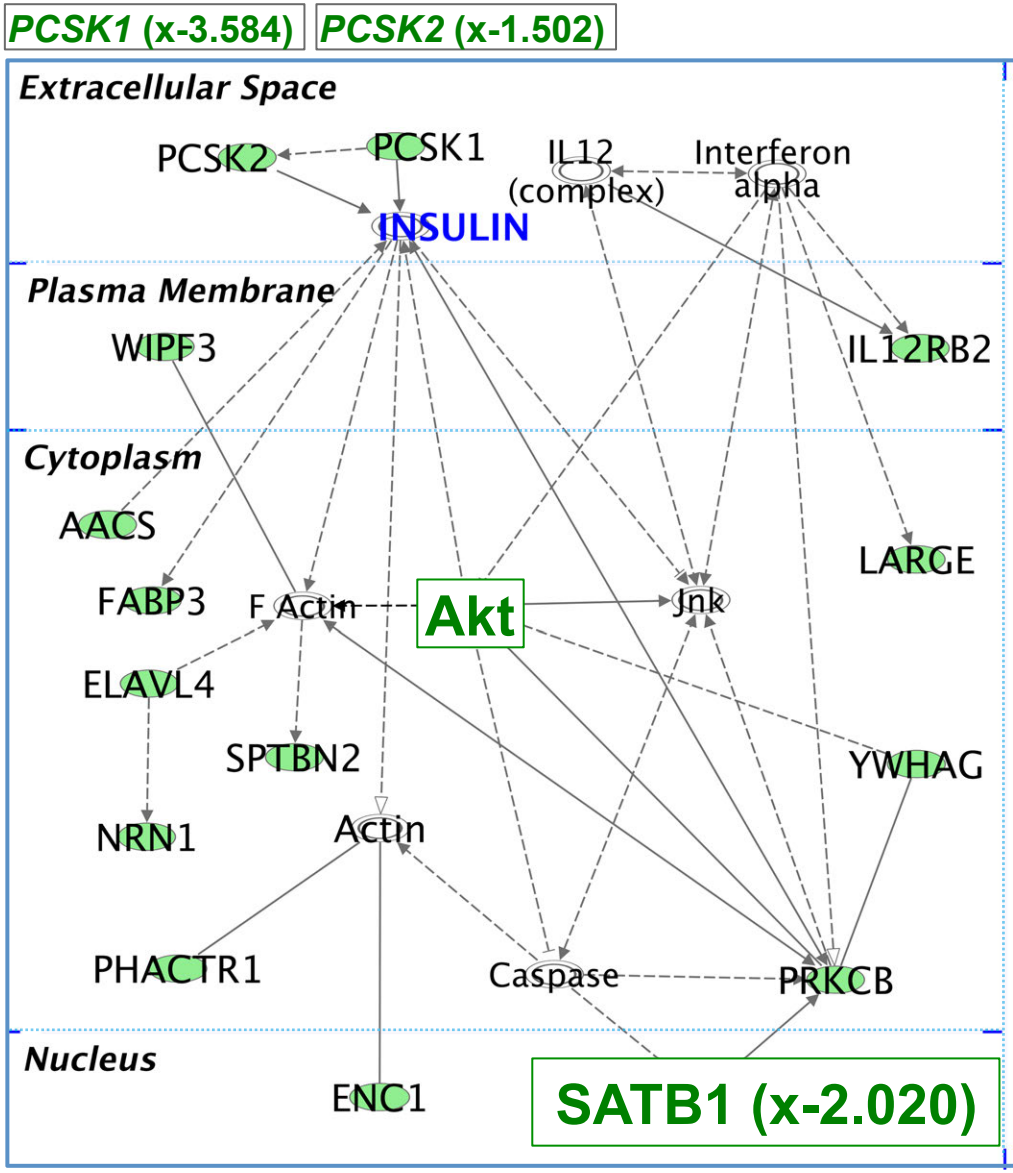


Figure 2. Comparison of Clinical, Cognitive, Structural, Metabolic, and Biochemical Changes as a Function of Estimated Years from Expected Symptom Onset.

Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease, NEJM367, 795, 2012 (DIAN cohort)

AD network 3: Cell Death, Lipid Metabolism, Small Molecule Biochemistry



NEUROD6 (x-2.439)

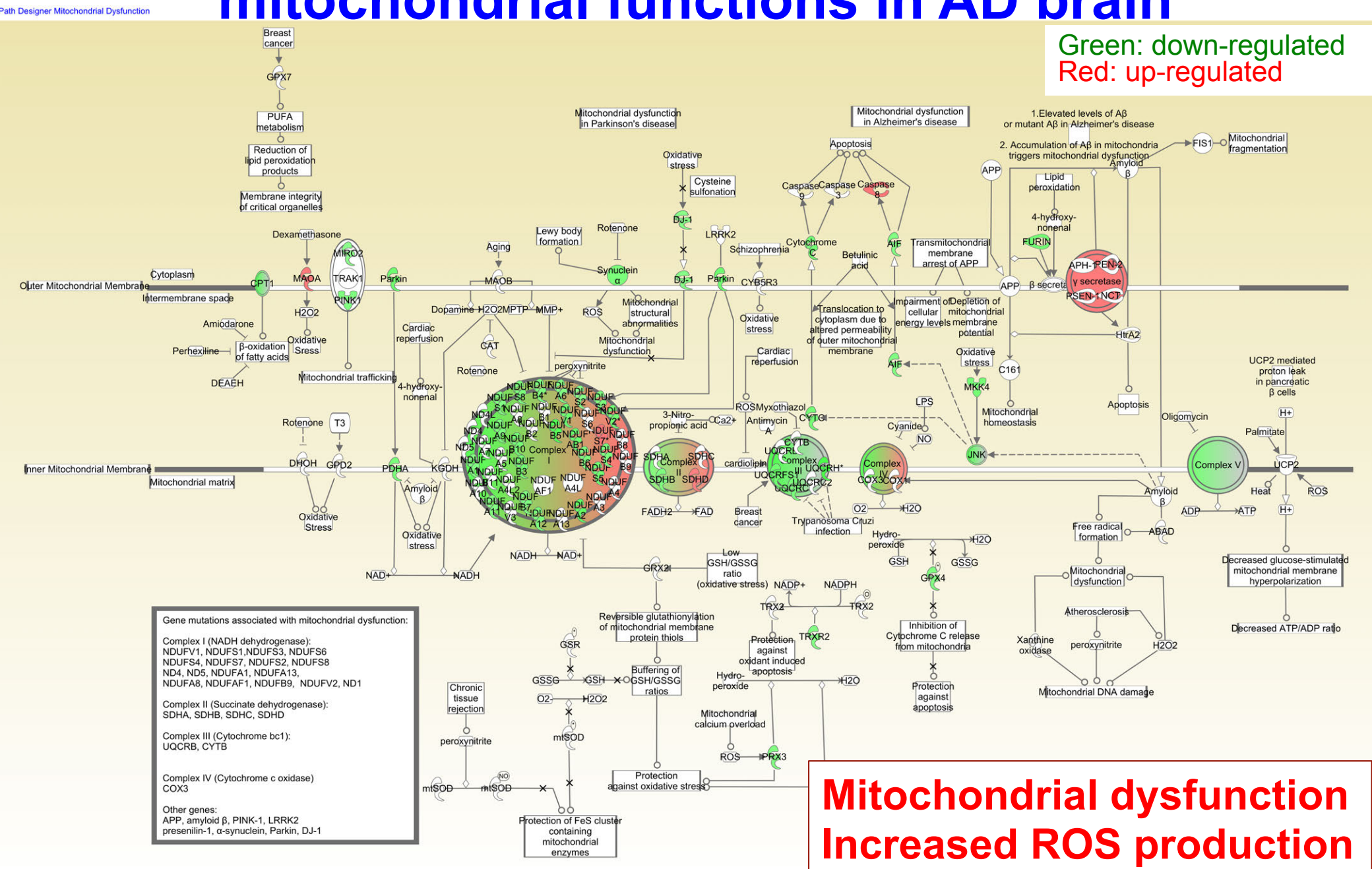
Kay JN, et al., *Nature Neurosci* 14: 965, 2011
Neurod6 expression defines new retinal amacrine cell subtypes and regulates their fate.

(Expression of *NeuroD6* is induced by Satb2)

Balamotis, MA et al., Mol Cell Biol, 2011
Satb1 ablation alters temporal expression of immediate early genes and reduces dendritic spine density during postnatal brain development

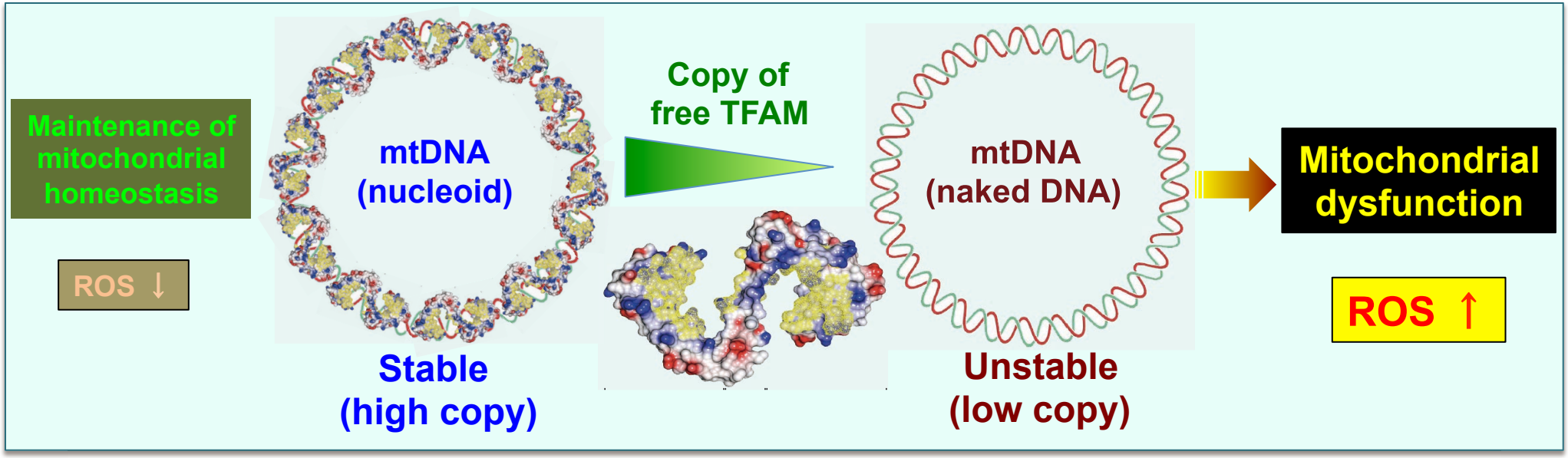
Uittenbogaard M et al., ASN Neuro. 2010,2:e00034.c
The neurogenic basic helix-loop-helix transcription factor NeuroD6 confers tolerance to oxidative stress by triggering an antioxidant response and sustaining the mitochondrial biomass.

Altered expression of genes involved in mitochondrial functions in AD brain



TFAM plays an essential role in the mitochondrial homeostasis by maintaining mtDNA copy number

① **Mitochondrial transcription factor A (TFAM): Roles in maintenance of mtDNA and cellular functions.** D Kang et al. *Mitochondrion* 7 (2007) 39–44



TFAM stabilizes mitochondrial DNA through formation of nucleoid and thus regulates the copy number of mtDNA.

② **Reverse of Age-Dependent Memory Impairment and Mitochondrial DNA Damage in Microglia by an Overexpression of Human Mitochondrial Transcription Factor A in Mice.** Hayashi et al. *J Neurosci,* 2008, 28(34):8624–8634

Abstract

Mitochondrial dysfunction is considered to have a pivotal role for developing Alzheimer's disease (AD). Accumulation of Amyloid β (Aβ) in neurons induces mitochondrial dysfunction and reactive oxygen species (ROS) production. It has been reported that 8-oxoguanine (8-oxoG), one of the major oxidation products in DNA/RNA and nucleotides, accumulates in AD brain. Oxidative DNA damage especially in mitochondria can lead to synaptic dysfunction and neuronal loss, and thereby causing neurodegenation. In the present study, we examined effects of human mitochondrial transcriptional factor A (hTFAM) transgene on the pathology of a mouse model of AD (3xTg-AD) harboring PS1M146V, APP(Swe), and MAPT(P301L) transgenes. TFAM is now known to contribute not only in transcription of mitochondrial DNA but also its maintenance, and thus protecting mitochondria from oxidative stress. In the Morris Water Maze test, 13-month-old 3xTg-AD hemizygous mice carrying hemizygous hTFAM transgene exhibited significant improvement of learning and memory deficit compared to 3xTg-AD hemizygous mice. Accumulation of Aβ was markedly decreased in cerebral cortices and hippocampi of the 3xTg-AD/hTFAM mice. Moreover, 3xTg-AD/hTFAM mice exhibited much less 8-oxo-dG immunoreactivity in cerebral cortices and hippocampi in comparison to 3xTg-AD mice which accumulated higher level of 8-oxo-dG. To clarify the mechanism of improvement of AD phenotype by hTFAM, we are currently performing gene expression profiling using hippocampal RNA prepared from these animals. These results will provide the new insight to understand the molecular mechanisms of AD pathology and possible new strategies for the therapy of AD.