Journal of Alzheimer's Disease 27 (2011) 1–2
IOS Press

Supplementary Data

Wild Type and Tangier Disease ABCA1 Mutants Modulate Cellular Amyloid-β Production Independent of Cholesterol Efflux Activity

Woojin S. Kim[a,b], Andrew F. Hill[c,d], Michael L. Fitzgerald[e], Mason W. Freeman[e,f], Genevieve Evin[d,g] and Brett Garner[h,i,*]
[a]*Neuroscience Research Australia, Sydney, NSW, Australia*
[b]*School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia*
[c]*Department of Biochemistry and Molecular Biology, Bio21 Institute, University of Melbourne, VIC, Australia*
[d]*Mental Health Research Institute of Victoria, VIC, Australia*
[e]*Lipid Metabolism Unit, Center for Computational and Integrative Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
[f]*Department of Medicine, Massachusetts General Hospital, Boston, MA, USA*
[g]*Department of Pathology, University of Melbourne, VIC, Australia*
[h]*Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW, Australia*
[i]*School of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia*

Handling Associate Editor: Ralph Martins

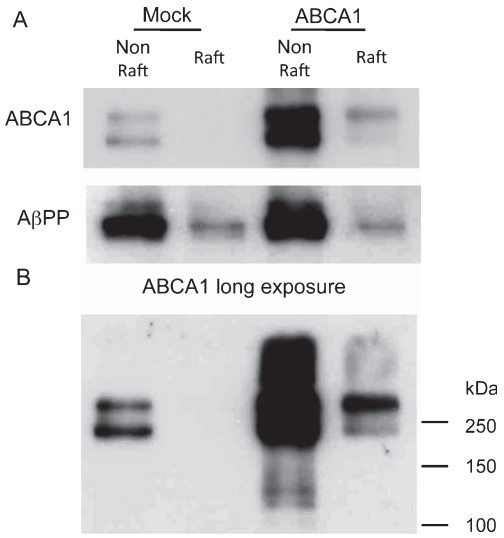
Accepted 30 June 2011

*Correspondence to: Prof. Brett Garner, Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522, Australia. Tel.: +61 2 4298 1576; Fax: +61 2 4221 8130; E-mail: brettg@uow.edu.au.

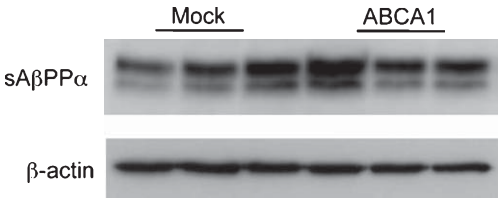
ISSN 1387-2877/11/$27.50

Supplementary Table 1
PCR primers

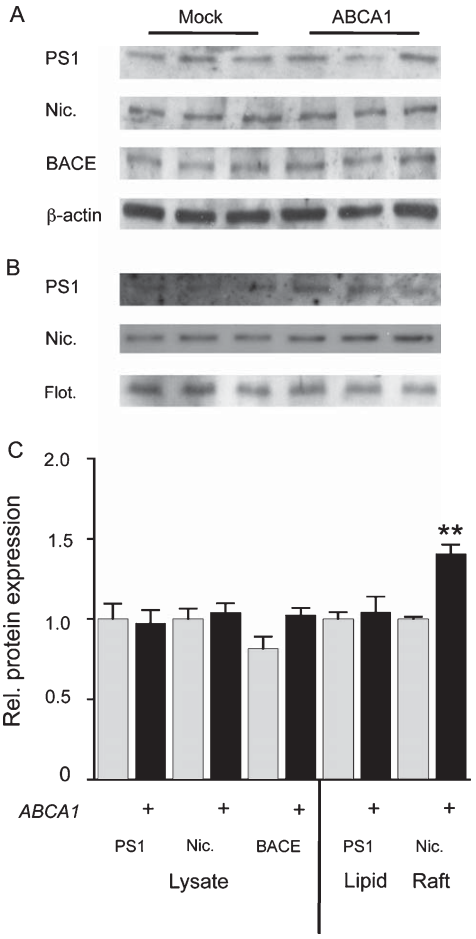
Gene	GenBank accession	Primer 5′–3′ Forward & Reverse	Product size (bp)
ABCA1	NM_005502	F AACTCTACATCTCCCTTCCCG	123
		R CTCCTGTCGCATGTCACTCC	
AβPP	NM_000484	F ACATGCACATGAATGTCCAG	169
		R CACCAGTTCTGGATGGTCAC	
β-actin	NM_001101	F GAATTCTGGCCACGGCTGCTTCCAGCT	162
		R AAGCTTTTTCGTGGATGCCACAGGACT	



Supplementary Figure 1. Analysis of ABCA1 and AβPP proteins in lipid raft and non-raft membrane fractions. A) CHO-AβPP cells were transiently transfected with mock empty vector or ABCA1 and lipid raft and non-raft membrane fractions were isolated. Equal amounts of protein was loaded onto each well and analyzed by western blotting with ABCA1 and AβPP antibodies. B) A longer exposure (3 h) of film of the ABCA1 western blot was used to enhance the signal.



Supplementary Figure 2. Impact of ABCA1 on endogenous sAβPPα production by human SK-N-SH neurons. SK-N-SH neuroblastoma cells grown in 175 cm^2 flasks were transiently transfected with mock empty vector or ABCA1 and the culture media sAβPPα measured by western blotting. Western blotting of cell lysates for β-actin was used as a control for cellular protein levels.



Supplementary Figure 3. Impact of ABCA1 on γ-secretase and BACE1 proteins in cell lysates and lipid raft membrane fractions. A) CHO-AβPP cells were transiently transfected with mock empty vector or ABCA1 and cell lysates (A) and lipid raft membrane fractions (B) were analysed for presenilin 1 (PS1), nicastrin (Nic.), BACE1 (BACE) by Western blotting as indicated. β-Actin and flotilin-2 (Flot.) were used to confirm equal protein loading of the lysates and raft fractions, respectively. Quantification of the signal intensity relative to the relevant control proteins is shown in the histogram (C). Note we did not detect a reliable signal for BACE1 in the lipid raft membrane fractions in these experiments (not shown). Data are representative of two experiments performed in triplicate. $^{**}p < 0.01$.