

Defining the role(s) of non-classical tumour suppressor Wwox in
cellular function using *Drosophila melanogaster* genetic modelling

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by

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Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Amanda Choo Yen Ying

Date

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Abbreviations

°C – degrees Celsius

% – percentage

µg – micrograms

µL – microlitre

A – adenosine (in context of DNA)

A – alanine (in context of amino acid)

Akt – v-akt murine thymoma viral oncogene homolog/protein kinase B

ATP – adenosine triphosphate

bp – base pairs

C – cytosine

CFS – common fragile site

CDD – conserved domain database

cDNA – complementary DNA

CIN – chromosomal instability

CoVa – cytochrome c oxidase subunit Va

CoVb – cytochrome c oxidase subunit Vb

da – daughterless

DEPC – diethyl pyrocarbonate

DNA – deoxyribonucleic acid

dNTP – deoxyribonucleoside triphosphate

DMSO – dimethyl sulfoxide

EDTA – ethylene diamine tetra-acetic acid

en - engrailed

ETC – electron transport chain

EV – empty vector

ey – eyeless

F – phenylalanine

FLP – flippase

Foxo – forkhead box, sub-group O

FRT – flippase recognition target

G – guanosine

GFP – green fluorescent protein

GSH – glutathione

GWAS – genome wide association studies

HDL-C – high density lipoprotein-cholesterol
hh – hedgehog
HIF1 α - hypoxia inducible factor 1 α
IDH – isocitrate dehydrogenase
IMS – intermembrane space
kb – kilobase
kDa – kilodalton
L – lysine
LB – Luria Broth
LiCl – lithium chloride
LOH – loss of heterozygosity
M – Molar
MARCM – mosaic analysis with a repressible cell marker
mg – milligram
ml – milliliter
mM – millimolar
mRNA – messenger RNA
N – asparagine
NAD⁺ – nicotinamide adenine dinucleotide (oxidised)
NADH – nicotinamide adenine dinucleotide (reduced)
NAD(P)⁺ - nicotinamide adenine dinucleotide phosphate (oxidised)
NAD(P)H - nicotinamide adenine dinucleotide phosphate (reduced)
ND23 – NADH:ubiquinone reductase 23kD subunit precursor
ND42 – NADH:ubiquinone reductase 42kD subunit precursor
ND75 – NADH:ubiquinone reductase 75kD subunit precursor
NLS – nuclear localisation sequence
ng – nanograms
ORF – open reading frame
P – proline
PBS – phosphate buffered saline
PBST – PBS + Tween
PCR – polymerase chain reaction
pmol – picomole
QTL – quantitative trait loci
R – arginine
XII

RNA – ribonucleic acid
RNAi – RNA interference
ROS – reactive oxygen species
Rp49 – Ribosomal protein 49
rcf – relative centrifugal force
Scrib – scribbled planar cell polarity protein
SDR – short-chain dehydrogenase reductase
SDS – sodium dodecyl sulfate
Sima - similar
SOC – super-optimal broth with catabolite repression
SOD – superoxide dismutase
T – thymine (in context of DNA)
T – threonine (in context of amino acid)
Tgo - tango
TCA – tricarboxylic acid
TMRE – tetramethylrhodamine, ethyl ester
TNF α - tumor necrosis factor α
Tub – tubulin
U – uracil
UAS – upstream activator sequence
UTR – untranslated region
V – volts
VDRC - Vienna Drosophila Resource Centre
W – tryptophan
WW1 – 1st WW domain of WWOX
WW2 – 2nd WW domain of WWOX
WNP – WWOX mutant line carrying a triple mutation in the tryptophan 58 (W58F),
asparagine 81 (N81A) and proline 84 (P84A) residues
WWOX – WW domain-containing oxidoreductase
Y – tyrosine

***Drosophila* nomenclature**

The *Drosophila* nomenclature used is according to conventional notation as stated on the *Drosophila* database, Flybase (www.flybase.org). Genes are represented by italicised text (e.g. *Wwox*) and proteins are represented by non-italicised text (e.g. Wwox).

Abstract

The *WWOX* gene has been identified as the gene that spans the *FRA16D* common chromosomal fragile site (CFS), which is a frequent site of DNA instability in cancer. Perturbation of the *WWOX* gene has been reported in various cancers, with low *WWOX* levels correlating with poorer prognosis. Individuals who inherit a non-functional copy of *WWOX* have also been found to be at greater risk of developing cancer. *WWOX* has been implicated in various cellular pathways, however the role of *WWOX* in tumourigenesis is not yet fully defined. There is therefore a need to determine the normal function(s) of *WWOX* and how perturbation of these roles is likely to contribute to cancer. A model was previously established to examine the cellular function of the *Drosophila* orthologue, *Wwox* and to identify novel functional interactors. Loss of *Wwox* in *Drosophila* was not found to result in any obvious cellular dysfunction that manifested as a phenotype. The aim of this study was to identify the types of cellular dysfunction brought about by other genes that could be modulated by *Wwox*. As *Wwox* has previously been implicated in metabolic processes, particularly aerobic metabolism and redox homeostasis, an RNA interference (RNAi) screen was performed to identify the types of metabolic stress that can be modulated by altered *Wwox* levels. *Wwox* was found to regulate cellular homeostasis in cells with mitochondrial dysfunction, with a requirement for the active site of its short-chain dehydrogenase/reductase (SDR) enzyme. Other genetic effectors of the mitochondrial dysfunction were also identified as candidates for further investigation into the pathway(s) in which *Wwox* participates. The contributions of *Wwox* to two other models of cellular dysfunction were also examined. *Wwox* was found to have a role in a *Drosophila* model of intrinsic tumour suppression. In addition, *Wwox* was also shown to affect cells with chromosomal instability (CIN), with loss of *Wwox* resulting in oxidative stress, DNA damage and subsequently apoptosis of CIN cells. This study has identified roles for *Wwox* in three different novel models of cellular dysfunction. These findings provide further insight into the tumourigenic potential of *WWOX* and could contribute to the ultimate aim of designing therapeutics for treatment of cancers with low *WWOX* levels.