

Supplemental Information

Table 2

Relative body weight of organs of *Wwox*-null mice compared to wild-type mice.

	<u>Wild Type</u> <u>gm (BW%)</u>	<u>Knockout</u> <u>gm (BW%)</u>
Spleen	0.058 (0.71%)	0.009 (0.28%)
Thymus	0.068 (0.83%)	0.020 (0.63%)
Liver	0.32 (3.9%)	0.11 (3.3%)
Kidney	0.13 (1.6%)	0.044 (1.4%)
Heart	0.060 (0.73%)	0.018 (0.57%)
Brain	0.39 (4.8%)	0.35 (11.2%)
Testis	0.025 (0.28%)	0.016 (0.28%)
Adrenal	0.003 (0.036%)	0.002 (0.063%)
Pan/Mes. Nodes	0.041 (0.50%)	0.016 (0.50%)
Br Adipose Tissue	0.18 (2.2%)	0.018 (0.57%)
Pituitary	0.001 (0.012%)	0.001 (0.032%)

Both knockout mice and their wild-type littermates were killed at 3 weeks age. Autopsies were performed after CO₂ asphyxiation, mice and organs were weighed relative body weight were calculated; organ weight/whole body weight.

Supplementary Information

Table 3:
Serum Chemistry levels in *Wwox*^{-/-} mice

Parameter	<u>KO</u>	<u>HET</u>	<u>WT</u>
BUN mmol/L	7.7	3.9	4.5
Creatinine mmol/L	18	18	18
Uric acid mmol/L	140	72	83
Phosphorus mmol/L	5.84	3.79	3.93
Calcium mmol/L	1.19	2.15	2.02
Na mmol/L	36.1	141.2	142.2
K mmol/L	27.29	10.67	11.08
Cl mmol/L	40	106.2	106.3
Mg mmol/L	0.66	1.05	1.1
GGT U/L	12.1	0	0
ALT (SGPT) U/L	16	19	20
AST (SGOT) U/L	179	410	431
AlkP U/L	131	882	929
Lipase U/L	24.2	22.1	19.9
Cholesterol mmol/L	1.5	4.2	4.6
Triglyceride mmol/L	0.97	1.53	1.33
Bilirubin–total mmol/L	6	4.4	3.2
Total protein g/L	22	46.3	45.5
Albumin g/L	6.44	28.88	28.53
Glucose mmol/L	2.87	8.16	8.29

Wild-type, heterozygous and homozygous littermate mice were sacrificed at 2-3 weeks of age. Autopsies were performed after CO₂ asphyxiation, blood was collected from renal vein from each genotype, pooled and serum analysis was carried out by Frimorfo Inc. (Fribourg, Switzerland). A representative analysis at 3 weeks of age is shown demonstrating a marked metabolic defect in KO mice compared to control littermates.

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Table 4. mRNA profiling in *Wwox*-deficient mice.

Downregulated genes in *Wwox*-null mice

Functional Group 1 – Chromatin – Nucleosome assembly

1	1452540_a_at	histone 1, H2bn
2	1421932_at 1421933_at 1421934_at 1450416_at	chromobox homolog 5 (Drosophila HP1a)
3	1422155_at 1452540_a_at 1460314_s_at	histone 2, H2be
4	1460314_s_at	histone 1, H3a
5	1422947_at 1422948_s_at 1428014_at	histone 1, H4b
6	1452540_a_at	histone 1, H2bg
7	1436596_at	H2A histone family, member V
8	1460314_s_at	histone 1, H3a
9	1427762_x_at 1452540_a_at	

Functional group 2 - Cell Cycle

1	1452954_at	ubiquitin-conjugating enzyme E2C
2	1456077_x_at	cell division cycle 25 homolog C (S. cerevisiae)
3	1429658_a_at 1429659_at 1429660_s_at	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)
4	1450920_at	cyclin B2
5	1417910_at 1417911_at	cyclin A2

Unclustered genes

1	1423074_at 1423075_at	lectin, mannose-binding 2
2	1424278_a_at	baculoviral IAP repeat-containing 5
3	1422533_at	A kinase (PRKA) anchor protein (yotiao) 9
4	1418194_at	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase

	1418195_at	
5	1424128_x_at 1451246_s_at	aurora kinase B
6	1437187_at	E2F transcription factor 7
7	1432065_at	RIKEN cDNA 310003L13 gene
8	1449528_at	c-fos induced growth factor
9	1427410_at 1427411_s_at	deleted in lymphocytic leukemia, 2
10	1433547_s_at	RIKEN cDNA 4921532K09 gene
11	1430979_a_at	peroxiredoxin 2
12	1442051_at	gb:BE691662 /DB_XREF=gi:10079286 /DB_XREF=uv65a11.x1 /CLONE= ...
13	1452459_at	calmodulin binding protein 1
14	1424629_at 1424630_a_at 1451417_at	breast cancer 1
15	1421890_at 1421891_at 1421892_at	sialyltransferase 5
16	1451611_at	HRAS like suppressor 3
17	1451480_at	E2F transcription factor 4
18	1431095_a_at 1438037_at 1453757_at	hect domain and RLD 5
19	1422279_at	Friend virus susceptibility 1

Upregulated genes in *Wwox*-null mice

Functional Group 1 – DNA packaging

1	1441140_at	arginine glutamic acid dipeptide (RE) repeats
2	1443952_at	nuclear receptor subfamily 1, group D, member 1
3	1420039_s_at 1433557_at 1449713_at	chromobox homolog 7

Unclassified Genes

1	1421916_at 1421917_at	platelet derived growth factor receptor, alpha polypeptide
2	1453294_at	RIKEN cDNA 1700012B15 gene

3	1429235_at 1429236_at	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 2
4	1418352_at	hydroxysteroid (17-beta) dehydrogenase 2
5	1437833_at 1456189_x_at	latent transforming growth factor beta binding protein 3
6	1444018_at	RIKEN cDNA B930098A02 gene
7	1427029_at	serine protease HETRA3
8	1457337_at	RIKEN cDNA 2810434M15 gene
9	1423062_at 1458268_s_at	insulin-like growth factor binding protein 3
10	1443153_at	RIKEN cDNA 6030460N08 gene
11	1420106_at 1449733_s_at	seven in absentia 1A
12	1419039_at 1419040_at	cytochrome P450, family 2, subfamily d, polypeptide 22
13	1449545_at	fibroblast growth factor 18
14	1441050_at	RIKEN cDNA 5730526G10 gene
15	1423460_at	PERQ amino acid rich, with GYF domain 1

Using an Affymetrix microarray chip, the differential expression of mRNAs in the *Wwox*-deficient mice are compared to their wild type littermates. mRNAs extracted from spleen, kidney, brain, femur and calvarial bone were analyzed using mouse gene-chip 430 2.0 arrays. Genes were clustered according to their function by using Davide Ease 2.1 (Douglas A Hosack, Glynn Dennis Jr, Brad T Sherman, HClifford Lane, Richard A Lempicki. Identifying Biological Themes within Lists of Genes with EASE. [Genome Biology 2003 4\(6\):P4.](#)).