

SUPPLEMENTAL DATA

for

Global deficits in development, function, and gene expression in the endocrine pancreas in a deletion mouse model of Prader-Willi syndrome

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The Supplemental Data for this manuscript contains one Figure and three Tables (one in a separate Excel file).

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FIGURE LEGEND, SUPPLEMENTAL DATA (see page 7 for Figure S1)

Figure S1. Analysis of apoptosis and replication rate for β -cells in TgPWS compared with WT controls. **A:** evaluation of β -cell apoptosis in pancreas sections of TgPWS and WT mice at E17.5 and P1 by immunostaining of activated caspase-3 (red) and insulin (green). Arrows indicate doubly-stained β -cells. Cell nuclei were stained with DAPI (blue). See Fig. 5D for quantitative analysis of β -cell apoptosis data. **B:** evaluation of replication of β -cells by assessment of BrdU incorporation in TgPWS and control pancreas at E17.5. Arrows indicate proliferative β -cells. **C:** quantification of BrdU labeled β -cells in TgPWS and control islets. Values represent means \pm SE of percentage of BrdU positive cells normalized to insulin positive cells from three animals of each genotype; $P = 0.90$.

Table S1. PCR primers to examine gene expression in pancreas and NIT-1 insulinoma cells.

PCR primer name ^a	Gene ^b	PCR primer sequence
RN2318 F	<i>Polr2a</i> ^c	5' -ACTCCTTCACTCACTGTCTTCCTGTT-3'
RN2319 R	<i>Polr2a</i> ^c	5' -TCCTGATCTTCTGCCACCACTGT-3'
RN2530 F	<i>Ins2</i> ^d	5' -AAAACCATCAGCAAGCAGGAA-3'
RN2531 R	<i>Ins2</i> ^d	5' -CATCCACAGGGCCATGTTG-3'
RN1331 F	<i>Frat3</i>	5' -CTGGCGATTGTGATGGAGGG-3'
RN1332 R	<i>Frat3</i>	5' -GAAGTCCTGCT GACAGCCTG-3'
RN2353 F	<i>Mkrn3</i>	5' -CTCCTCTGGCTTTGTCATTCTTA-3'
RN2354 R	<i>Mkrn3</i>	5' -GCACGCTTCTGGCTCATCC-3'
RN2351 F	<i>Magel2</i>	5' -ATCCGAGAATACAAAGACGACAGC-3'
RN2352 R	<i>Magel2</i>	5' -TGACGATGATGTAAGTGTGGGTTT-3'
RN2320 F	<i>Ndn</i>	5' -GCTGTGTAAGGGTGGGGTTGA-3'
RN2321 R	<i>Ndn</i>	5' -GCACTGTGGATTTGGGATACTCTAA-3'
RN2355 F	<i>Mirh1</i>	5' -AAATGACTCTGGAATAATAATCTGA-3'
RN2356 R	<i>Mirh1</i>	5' -TAAGAGCCTTAGAAATACACAGTCT-3'
RN2324 F	<i>U1A</i>	5' -TAGGAGTTGTCAGCAAGGCAAT-3'
RN2325 R	<i>U1A-Snurfl-Snrpn</i>	5' -CAGTAGTTCTTCTCAAGTGAAGCGAT-3'
RN3062 F	<i>Snurfl-Snrpn</i>	5' -TTGGTTCTGAGGAGTGATTTGC-3'
RN2325 R	<i>Snurfl-Snrpn</i>	5' -CAGTAGTTCTTCTCAAGTGAAGCGAT-3'
RN3278 F	<i>Snord107</i>	5' -CGGAGACCTTGACTGGATTT-3'
RN3279 R	<i>Snord107</i>	5' -GTGAGGCACATTGACTAGAT-3'
RN3280 F	<i>Snord64</i>	5' -GGCTGGACTTATGATGAGCTA-3'
RN3281 R	<i>Snord64</i>	5' -CTTCAGAGTAATCATTGAGC-3'
RN3282 F	<i>Snord116</i>	5' -GGATCTATGATGATTCCCAG-3'
RN3283 R	<i>Snord116</i>	5' -GGACCTCAGTCCGATGAGA-3'
RN3284 F	<i>Snord115</i>	5' -GGGTCAATGATGACAACCCA-3'
RN3285 R	<i>Snord115</i>	5' -GGCCTCAGCGTAATCCTATT-3'

^a F, forward primer; R, reverse primer. ^b Blue, paternally expressed imprinted gene within mouse chromosome 7C.

^c Control gene for RNA amount. ^d Control gene for pancreatic β -cells.

Table S2. Genes deregulated in TgPWS vs. WT pancreas at P1 by microarray^a.

Gene Name	Fold change	P value	PaGE confidence	QRT-PCR confirmation	NCBI Reference Sequence	Mouse chromosome location [position in Mb and strand]
<i>Ndn</i>	-3.62	0.006	0.81	Yes ^b	NM_010882	7 [69,493,163-69,494,814 F]
<i>Hspa9a</i>	-1.71	0.004	0.94	No	NM_010481	18 [35,097,293-35,113,998 R]
<i>Sfrs10</i>	-1.55	0.018	0.94	-	NM_009186	16 [22,246,442-22,265,994 R]
<i>Iapp</i> (2)	3.32	0.038	0.83	Yes	NM_010491	6 [142,246,948-142,252,337 F]
<i>Sgne1</i> (3)	2.89	0.030	0.85	Yes	NM_009162	2 [113,616,705-113,669,196 R]
<i>Sst</i> (2)	2.87	0.036	0.83	Yes	NM_009215	16 [23,889,670-23,890,930 R]
<i>Ppy</i> (2)	2.82	0.019	0.90	Yes	NM_008918	11 [101,961,245-101,962,633 R]
<i>Tmem27</i>	2.72	0.011	0.89	Yes	NM_020626	X [160,526,762-160,556,792 F]
<i>Mt1</i>	2.68	0.015	0.85	-	NM_013602	8 [96,703,141-96,704,225 F]
<i>Tor2a</i>	2.62	0.041	0.83	-	NM_152800	2 [32,612,811-32,617,756 F]
<i>Arl7</i>	2.54	0.028	0.84	-	NM_177305	1 [90,569,702-90,598,451 R]
<i>Fabp4</i> (2)	2.49	0.023	0.86	-	NM_024406	3 [10,204,347-10,208,576 R]
<i>Ins1</i> (3)	2.49	0.031	0.83	-	NM_008386	19 [52,338,939-52,339,497 F]
<i>Neat1</i>	2.47	0.008	0.86	-	NR_003513	19 [5,842,302-5,845,478 F]
<i>Ins2</i> (4)	2.46	0.029	0.82	Yes	NM_008387	7 [149,864,561-149,885,415 R]
<i>Gtl2/Meg3</i> (7)	2.44	0.003	0.86	-	NM_144513	12 [110,784,505-110,809,929 F]
<i>Zscan18</i>	2.39	0.034	0.83	-	NM_001017955	7 [13,353,442-13,357,393 R]
<i>Elac2</i>	2.35	0.015	0.84	-	NM_023479	11 [64,792,537-64,815,578 F]
<i>Camk2n1</i>	2.30	0.023	0.83	-	NM_025451	4 [138,011,101-138,014,058 F]
<i>Fzd9</i>	2.28	0.029	0.86	-	XM_284144	5 [135,724,808-135,727,100 R]
<i>Rtn4</i>	2.22	0.031	0.83	-	NM_024226	11 [29,592,938-29,642,916 F]
<i>Tbcd</i>	2.21	0.019	0.90	-	NM_029878	11 [121,313,261-121,478,477 F]
<i>Rras2</i>	2.13	0.006	0.87	-	NM_025846	7 [121,190,302-121,261,295 R]
<i>Penk1</i> (2)	2.11	0.026	0.85	-	NM_001002927	4 [4,060,684-4,065,935 R]
<i>Coll1a1</i> (2)	2.08	0.006	0.90	-	NM_007742	11 [94,797,567-94,813,201 F]
<i>Pyy</i>	1.90	0.030	0.82	-	NM_145435	11 [101,967,994-101,969,146 R]
<i>Pim3</i>	1.90	0.017	0.84	-	NM_145478	15 [88,692,905-88,696,148 F]

<i>Bicc1</i>	1.84	0.019	0.84	-	NM_031397	10 [70,387,850-70,622,438 R]
<i>Gabarapl1</i>	1.83	0.047	0.82	-	NM_020590	6 [129,483,218-129,492,349 F]
<i>Igfbp6</i>	1.82	0.036	0.90	-	NM_008344	15 [101,974,793-101,979,942 F]
<i>Isl1</i>	1.80	0.024	0.86	Yes	NM_021459	13 [117,088,489-117,099,896 R]
<i>Ngfr</i>	1.76	0.041	0.82	-	NM_033217	11 [95,430,142-95,449,012 R]
<i>Nfib</i>	1.72	0.044	0.82	-	NM_001113209	4 [81,941,952-82,351,493 R]
<i>Ctrl</i>	1.72	0.027	0.83	-	NM_023182	8 [108,455,900-108,457,747 R]
<i>Tmem45a</i>	1.71	0.018	0.82	-	NM_019631	16 [56,806,382-56,886,279 R]
<i>Wfdc15b</i>	1.66	0.011	0.86	-	NM_138685	2 [164,040,190-164,047,399 R]
<i>Mapre3</i>	1.64	0.002	0.87	-	NM_133350	5 [31,117,127-31,168,432 F]
<i>Ahnak</i>	1.63	0.023	0.90	-	NM_009643	19 [9,063,774-9,151,409 F]
<i>Ihpk2</i>	1.63	0.018	0.84	-	NM_029634	9 [108,698,325-108,708,663 F]
<i>Srrm2</i>	1.62	0.005	0.86	-	NM_175229	17 [23,940,154-23,961,706 F]
<i>Armcx2</i>	1.60	0.014	0.86	-	NM_026139	X [131,338,684-131,343,535 R]
<i>Igf2</i>	1.60	0.041	0.83	-	NM_010514	7 [149,836,673-149,852,721 R]
<i>Il17rd</i>	1.60	0.033	0.86	-	NM_134437	14 [27,852,187-27,920,472 F]
<i>Pmp22</i>	1.60	0.036	0.83	-	NM_008885	11 [62,942,484-62,973,048 F]
<i>Rev3l</i>	1.59	0.007	0.90	-	NM_011264	10 [39,451,966-39,595,012 F]
<i>Igf1r (2)</i>	1.59	0.009	0.86	-	NM_010513	7 [75,097,143-75,378,553 F]
<i>Malat1/Neat2 (2)</i>	1.59	0.005	0.90	-	NR_002847	19 [5,796,214-5,796,614 F]
<i>Fbn1</i>	1.58	0.010	0.83	-	NM_007993	2 [125,126,330-125,333,729 R]
<i>Sdccag33</i>	1.58	0.031	0.82	-	XM_129060	18 [83,653,127-84,255,954 R]
<i>Ctsf</i>	1.58	0.029	0.83	-	NM_019861	19 [4,855,129-4,860,912 F]
<i>mt-Cytb (2)</i>	1.57	0.001	0.90	-	AB125774	MT [14,145-15,288 F]
<i>Amoil1</i>	1.56	0.025	0.86	-	NM_001081395	9 [14,346,410-14,419,444 R]
<i>Ndst1</i>	1.55	0.008	0.86	-	NM_008306	18 [60,845,632-60,908,304 R]
<i>Col4a1 (2)</i>	1.54	0.009	0.88	-	NM_009931	8 [11,198,433-11,312,748 R]
<i>Rpl38</i>	1.54	0.023	0.82	-	NM_023372	11 [114,529,862-114,533,645 F]
<i>mt-Atp6</i>	1.52	0.019	0.83	-	AF093677	MT [7,927-8,607 F]

<i>Lamc1</i>	1.52	0.010	0.86	-	NM_010683	1 [155,066,053-155,179,916 R]
<i>S100a11</i>	1.52	0.033	0.84	-	NM_016740	3 [93,324,417-93,330,209 F]
<i>Rhoj</i>	1.52	0.032	0.85	-	NM_023275	12 [76,409,309-76,502,006 F]
<i>Rhbdf1</i>	1.52	0.015	0.86	-	NM_010117	11 [32,109,587-32,122,280 R]
<i>D4Wsu53e</i>	1.52	0.045	0.81	-	NM_023665	4 [134,479,540-134,483,285 F]
<i>Rpl12</i>	1.51	0.013	0.86	-	NM_009076	2 [32,817,239-32,819,563 F]
<i>Atad4</i>	1.50	0.006	0.83	-	NM_146026	11 [96,790,616-96,796,962 F]
<i>Timp2</i>	1.50	0.037	0.86	-	NM_011594	11 [118,164,905-118,216,639 R]
<i>Ppp2r2b</i>	1.50	0.007	0.86	-	NM_028392	18 [42,797,293-43,218,894 R]

^a Genes found deregulated by both t-test and Patterns from Gene Expression (PaGE) analyses are shown. Only genes with a *P* value ≤ 0.05 (t-test) and a PaGE confidence interval ≥ 0.08 (False Discovery Rate of 20%) were called differentially expressed. All microarray values are presented as the ratio of the median expression fold changes for TgPWS and WT. The data is sorted by fold change (≥ 1.5) in decreasing format first for down-regulated genes and then for up-regulated genes. A total of 62 genes were called up-regulated and 3 genes were down-regulated in TgPWS compared with WT pancreas. The numbers in parentheses indicate the number of times a particular transcript spotted several times on the array was found significantly deregulated with a fold change ≥ 1.5 by both t-test and PaGE; for clarity, only the replicate with the largest fold-change is shown in this Table. The quantitative (Q)RT-PCR expression data is also summarized (-, not tested), as are the chromosome locations and orientation (F, forward; R, reverse) of the deregulated genes.

^b *Ndn* is within the TgPWS deletion interval and is not expressed in TgPWS tissues by standard RT-PCR (1; data not shown).

Reference

1. **Stefan M, Portis T, Longnecker R, Nicholls RD.** A nonimprinted Prader-Willi Syndrome (PWS)-region gene regulates a different chromosomal domain in *trans* but the imprinted PWS loci do not alter genome-wide mRNA levels. *Genomics* 85: 630-640, 2005.

Figure S1

