

RNA-mediated paternal heredity of diet-induced obesity and metabolic disorders

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Supplementary Information

- Supplementary Figure Legends
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- 4 supplementary Tables (S1 through S4)
- Supplementary references

Supplementary figure legends

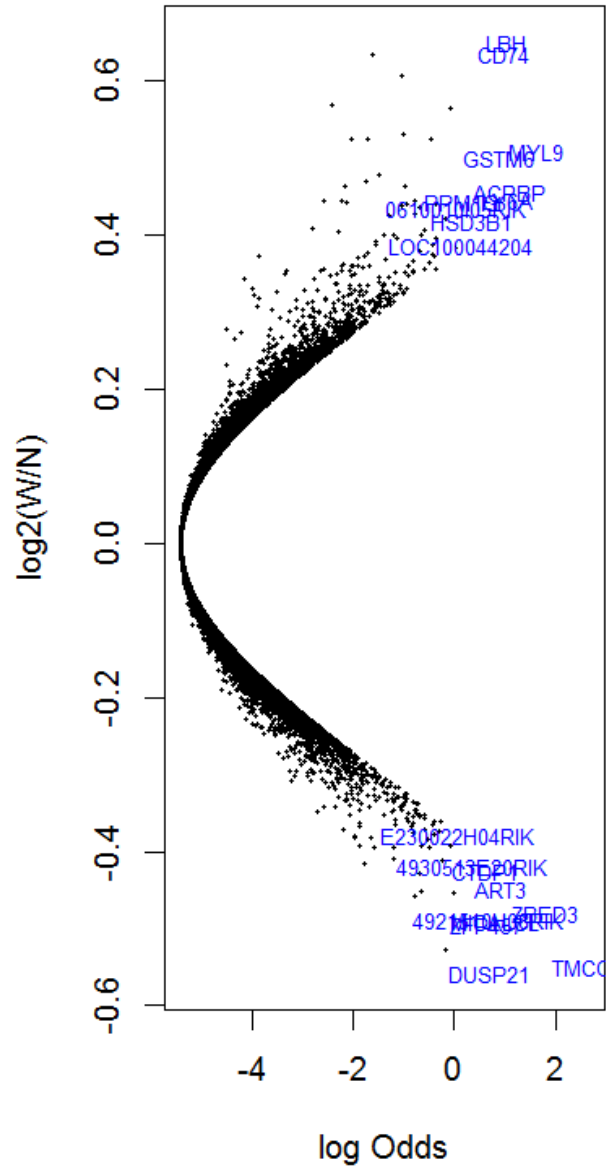
Supplementary Figure S1. **Volcano Plot.** The Log Odds (or B value) on the x-axis is the odds (or probability) that the gene is differentially expressed between Standard and Western-like Diet testis. A log Odds value of 0 corresponds to 50-50 chance that the gene is differentially expressed. The y-axis indicates the log₂ value of fold changes between the two conditions. B. MA Plot. The x-axis is the average expression level among the two conditions, indicating the basal expression level. And the y-axis is the fold change (log₂ scale), which indicates the difference level between the two. Each gene is represented on the plot as a single dot. Genes, up- or down-regulated in the Western-like Diet compared to the Standard Diet, correspond to blue spots and are listed in Table S2.

Supplementary Figure S2. **Spectrometric RNA quality checks.** RNAs prepared for microinjection were verified using Agilent Bioanalyser 2100 apparatus. The profiles indicate that both standard and western sperm RNA samples contain no apparent ribosomal RNA which are consistent with pure RNA sperm preparation.

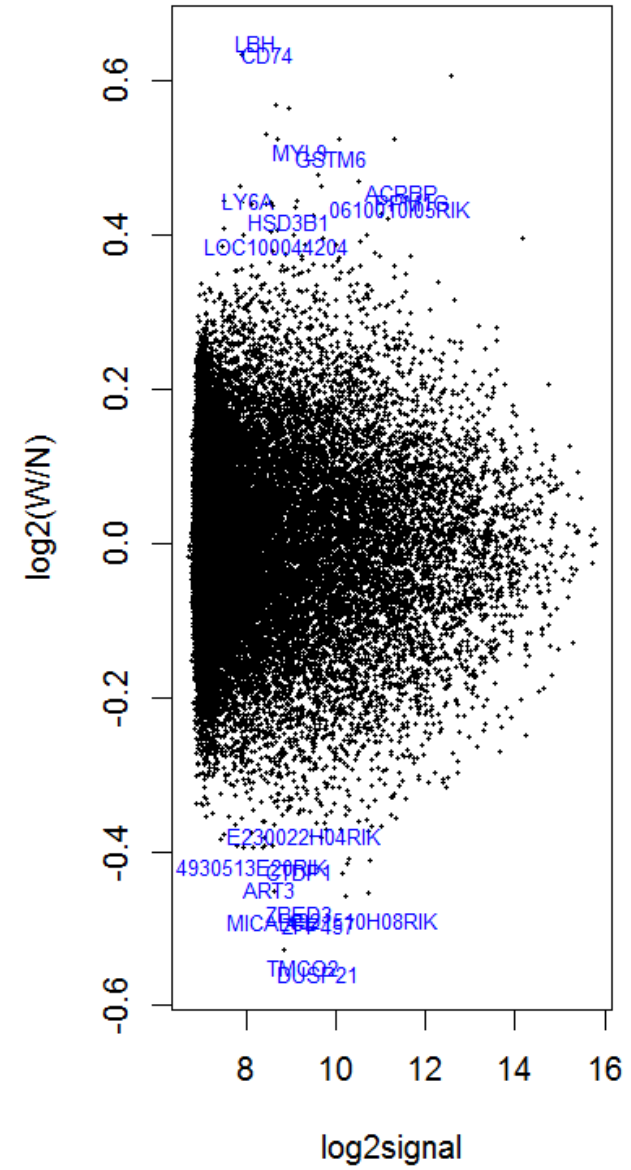
Supplementary references

- 1 Roovers, E. F. *et al.* Piwi proteins and piRNAs in mammalian oocytes and early embryos. *Cell Rep* **10**, 2069-2082 (2015).
- 2 Rosenkranz, D. & Zischler, H. proTRAC--a software for probabilistic piRNA cluster detection, visualization and analysis. *BMC Bioinformatics* **13**, 5 (2012).

Volcano Plot



MA Plot



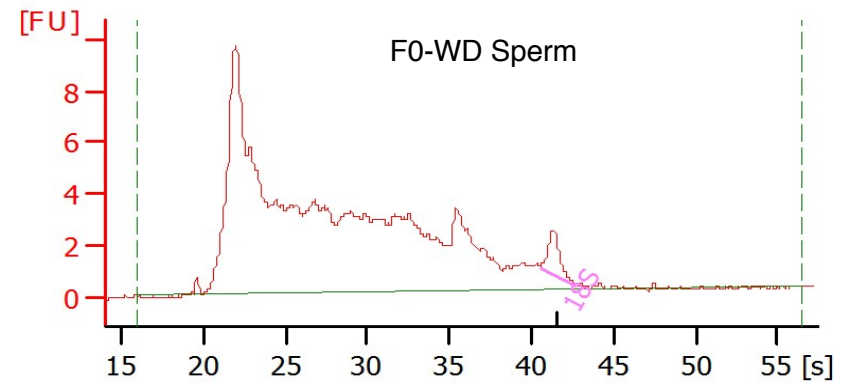
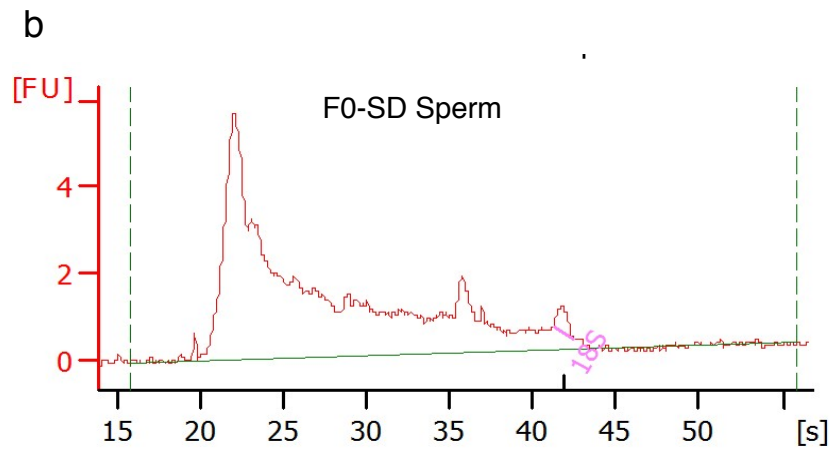
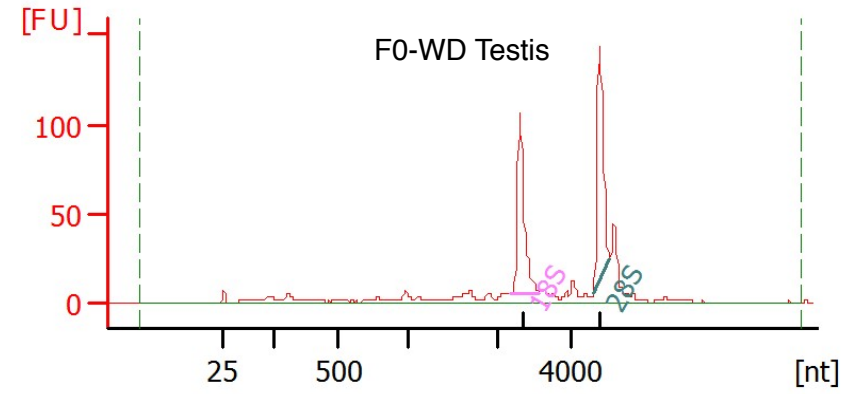
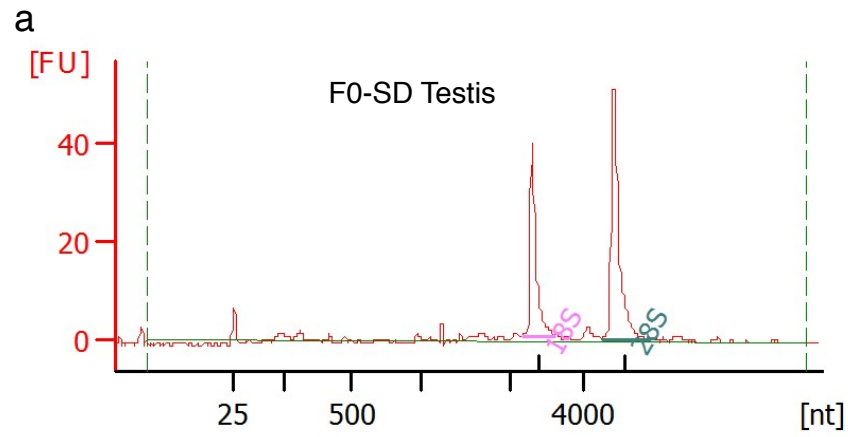


Table S1 Experimental diet nutritional composition

	Standard Diet ^a	Western Diet ^a
Carbohydrates (kcal%)	59,9 (of which sugar 3,2%)	46,1 (of which sugar 34%)
Proteins (kcal%)	16,1	15,3
Lipids (kcal%)	3,1	17,9
Caloric intake (kcal/kg)	2791	4035

^aStandard Diet and Western Diet formulation according to SAFE

Supplementary Table S2. Genes up- or down-regulated in the Western Diet compared to the Standard Diet.

TargetID	IlluminaProbe	log2signal	log2(W/N)	p-value	N	N3	N4	W	W2	W4
DUSP21	6480196	9,58580985	-0,5573977	8,37E-05	712	1085,7	1242,9	658	540,8	621,6
TMCO2	1170377	9,25456911	-0,550455	3,91E-06	655,4	840,3	861,2	484,6	488,6	471,9
OTTMUSG00C	5130528	8,81610352	-0,5273555	0,00029025	524	688,3	510,6	420,7	285,6	384,8
ZFP457	6420563	9,62767069	-0,4944659	8,59E-05	877,5	1199,4	931,9	682,4	573,3	653,8
MICALCL	6520224	8,5827955	-0,4909786	6,86E-05	361,7	635,5	473,7	310,4	344,5	277,2
4921510H08R	4900639	10,5933185	-0,4878514	8,20E-05	1527,8	2663,4	1809,3	1242,1	1265,8	1178,4
ZBED3	1740161	9,20381296	-0,4792123	1,50E-05	683,5	724,2	793,4	474,7	488,1	467,7
PSMD6	6180088	10,2093418	-0,4584601	0,00072592	1081,1	1836,1	1619,9	979,6	1210,3	727,8
1700015E13R	3940408	10,722115	-0,4538694	0,0002383	1392,4	2593,9	2594,5	1247,9	1347	1485,1
LOC213411	4670632	8,60459717	-0,4523619	0,00059628	410,8	671,2	391,3	365,9	319,2	276,3
ART3	7000500	8,50666247	-0,4484799	5,82E-05	336,5	553,4	477,2	257	380,1	271,9
HIPK4	2350546	10,1310882	-0,427455	0,00062764	862,2	1829	1668,2	818	1018,2	910,5
CTDP1	520746	9,18156581	-0,4253574	9,21E-05	632,9	765,1	732,6	499,5	459,6	478,8
4930513E20R	460187	8,15378688	-0,4183476	0,00013285	281,1	394,2	364,3	222,5	234,1	254,7
SUGT1	580152	10,7660794	-0,4114791	0,00033175	1563	2793	2226,8	1361,5	1503,1	1402,8
NMT2	6450373	7,93415203	-0,3940891	0,00047263	226	384,4	285,8	182,3	217,7	219,4
MED7	6420156	7,76468368	-0,3931397	0,00059852	228,2	305,8	246,9	172	173,3	206,5
TMEM209	6420367	8,58318223	-0,3916577	0,00025476	350,9	551,1	506,8	290,8	355,8	319,8
A430037M23I	6060544	7,43603018	-0,3833058	0,00049556	167,2	216,7	239,6	135,2	164,9	142,5
E230022H04R	4880390	9,31165764	-0,379095	0,00020248	762	798,1	736,7	562,3	528,1	504,5
LOC10004783	6130202	7,50182954	-0,3769548	0,00041841	195	226,8	219,9	141,4	183,4	141,2
UCHL5IP	4570079	8,11939124	-0,3759743	0,00074363	257,9	411,7	338,2	230,8	252,2	221,8
RHBDL2	2970307	10,7146008	-0,3726464	0,00036209	1728,8	2221,6	2189,7	1423,9	1420,5	1349,5
ATP5SL	5270131	9,77181887	-0,3720594	0,00054409	710,8	1331,1	1234	566,3	758,5	891,9
SOX5	5050082	11,0081401	-0,3682525	0,00077092	2282,3	2958,8	2302,2	1946,2	1631,3	1547,4
ZFH3	150605	7,76360962	-0,3649002	0,00043974	209,6	313,6	255,7	173,4	207,9	176,3
WDR35	5080072	9,55754216	-0,3620437	0,00074968	750,9	1039,5	946	607,2	598,8	695,1
KHSRP	4920593	8,39745294	-0,3616897	0,00084951	301,5	463,1	460,3	248,8	273,9	342,8
LOC10004067	2940673	10,5172777	-0,3612726	0,00041008	1714,1	1770,5	1824,1	1339,9	1079,4	1260,8
FANCL	6620240	8,37418001	-0,3608961	0,00034809	343,6	436	404,1	261	296,4	287,6
TIMP3	1400309	8,91589322	0,35602822	0,00054995	369,6	520,3	461,9	422	655,7	506,8
LAMB2	6840209	7,89516815	0,35652882	0,00038822	163,7	272,6	235,2	195,9	328,4	269,9
1700029F12RI	1770504	11,2915265	0,35995174	0,00086015	2524,9	2248,3	2319,5	2832,7	2221,7	3040,8
AMHR2	3310739	8,52406641	0,36366088	0,00047346	307,7	411,2	305,2	339,2	470,2	397,5
CD151	1400630	10,0151647	0,36677298	0,00075086	798,1	1122,2	1005,9	817	1356,5	1219,8
SPTLC1	450048	9,21445583	0,37225431	0,00041237	447,6	612,9	605,5	588,8	733,2	607,9
ALDH1A2	6330008	8,86333918	0,37434794	0,00042977	384,4	425,1	478,2	413,6	549,1	564
MRPL4	4810100	8,56874317	0,37854329	0,00063127	317,7	350,1	378,3	323,6	428,2	511,4
GLOXD1	6480609	7,46384962	0,38515349	0,00020998	123,3	193,3	171,6	156,9	241,1	195
LOC10004420	3830048	8,67174533	0,38627035	0,00018334	279	489,5	380,3	323,4	581,1	466,2
LOC10004650	4610397	9,28995922	0,38718891	0,00042973	480,5	640,1	624,2	549,8	666,3	858,1
DEFB19	2030088	14,1621756	0,39594323	0,00039391	15501,3	20021,7	15849,5	16406,8	25160	18816,3
TGM2	7000187	9,06624757	0,40106689	0,00059245	340,1	607,5	576,7	396,6	875,1	565,4
ATP6V0D1	6290673	8,70114094	0,40676388	0,00053496	346,2	364	425,1	343,9	522	531,1
HSD3B1	360463	8,94033029	0,41807307	0,00013645	382	489,9	470,3	493,5	648,1	493,5
SLC25A5	5870092	11,1597012	0,42048999	0,0002995	1814,5	2316,3	2223,7	1865,2	3058,4	2653,4
EG668668	7570762	11,0204684	0,42660908	0,00069992	1603,9	1901	2268,5	1710,1	2876,5	2314
0610010I05RI	3990427	11,4512637	0,43483371	0,00020984	2889,8	2495,8	2352,3	2987,6	3011,7	3171,2
INHA	240739	8,5961062	0,43813215	0,00103142	348,1	306,1	390	348,8	529	430,5
PPM1G	3370546	11,6981224	0,44411152	0,00018829	3492,3	2588,7	3114,6	3632,6	3346,5	3964,7
LY6A	1690187	8,02745599	0,44528579	4,91E-05	185,2	297,7	225,1	218,1	405,5	280,8
ACRBP	2320017	11,4902449	0,45586514	4,42E-05	2786,5	2404,2	2708,5	3214	2993,7	3291,4

The experimental design is shown in Figure 1. Total RNAs from animal testes at four months old were prepared and subjected to analysis (Microarray Service CS3021 Illumina Gene Exp Process Ref: 338908 Qiagen). The resulting microarray data compared three males fed the WD diet (W, W2 and W4) to standards (N, N3 and N4). The RNA levels of WD-fed mice were compared to controls. Colon D ratios (W/S): colour ranged from green for lower expression to red for higher expression in WD.

Table S4: Mapping of the deregulated piRNAs to known piRNA clusters

Chromosome localization	Start	End	Cluster number	Number of deregulated piRNA/cluster
chr1	57348009	57362762	9	1
chr1	91551282	91560929	22	1
chr1	92988215	93000921	23	2
chr10	85848012	85855029	88	1
chr10	94670068	94680641	93	1
chr10	127690863	127704362	96	1
chr10	62651034	62694021	69	2
chr10	86560003	86596028	89	2
chr10	85740002	85774027	86	3
chr10	66680002	66709815	70	4
chr11	107990084	108001964	167	1
chr11	5526240	5536022	103	2
chr11	103420011	103453008	162	2
chr12	98379001	98455979	204	13
chr13	53393064	53407849	226	1
chr14	24078000	24124942	248	1
chr14	24421041	24433017	250	1
chr14	44759007	44791890	255	1
chr14	44932114	44946021	256	1
chr15	73094010	73110057	276	1
chr15	79924089	79934896	287	1
chr15	83349097	83369988	299	1
chr15	74625035	74667908	279	3
chr15	78642001	78675022	283	3
chr15	59245061	59304022	275	6
chr17	22511012	22522028	340	1
chr17	66148680	66159006	372	1
chr17	66164005	66233002	373	3
chr17	27283000	27370953	350	6
chr18	67028034	67088022	389	8
chr19	37252052	37264978	403	1
chr2	127687043	127706875	443	1
chr2	151139086	151162029	456	1
chr2	151245426	151267020	457	2
chr2	151315256	151336029	458	3
chr2	151398016	151420600	459	3
chr2	92536004	92613016	430	11
chr3	20460000	20469958	494	1
chr3	124398138	124419933	520	1
chr4	57353369	57369005	535	1
chr4	62216001	62243018	538	1
chr4	123888001	123899426	556	1
chr4	123831081	123846028	555	2

chr4	94277004	94338846	540	6
chr5	130149032	130159006	633	1
chr5	113316068	113373001	614	3
chr5	149786048	149834611	657	7
chr6	85983003	85994796	685	1
chr6	128179025	128204765	709	1
chr6	128440015	128453028	710	1
chr6	127778004	127842026	708	5
chr7	52903534	52918016	737	1
chr7	125323006	125337723	758	3
chr7	73098005	73154023	740	7
chr7	73774005	73842029	741	8
chr7	69879006	69940023	739	10
chr8	27291000	27301030	773	1
chr8	37089005	37096684	775	1
chr8	110118003	110134528	803	1
chr8	92185128	92202847	794	3
chr9	44139170	44153876	832	1
chr9	54206007	54266025	840	9
chr9	67689001	67763015	849	10

97% deregulated piRNAs were mapped to known clusters using the piRNA cluster data base provided on the web site <http://www.smallrnagroup.uni-mainz.de>^{1,2}. Only piRNA clusters with deregulated piRNA are listed.