

SUPPLEMENTAL MATERIAL

LEGENDS FOR SUPPLEMENTAL TABLES

Supplemental Table 1. Genes that show significantly different responses to E2 between wild-type and DPM mice. All genes that were significantly differentially regulated in the comparison of E2 response in DPM mice and WT mice ($p < .02$ and fold change > 1.25) are listed. First two pages: genes that show a differential increase in E2 response (higher expression in response to E2) in DPM vs WT aortas. These represent genes that are likely to be repressed by rapid signaling in WT. Last page: genes that show a differential decrease in E2 response DPM vs WT (that are likely to be activated by rapid signaling in WT). “logFC” (in column 3) indicates log, base 2, differential E2 effect, equal to $(\text{DPM_E2} - \text{DPM_veh}) - (\text{WT_E2} - \text{WT_veh})$. “logFC wt” and “logFC DPM” indicate log2 pairwise differences in expression levels for DPM or for WT +/-E2. “Regl type” indicates the type of regulation for WT / DPM, where “up” means at least 1.1x increased with E2, “down” means at least 1.1x decreased with E2, and “NR” means non-regulated. Cases with more than 1.25x increases or decreases in expression are shaded in pink and green, respectively. One gene, *Rsad2*, was identified by three different Affymetrix probe set IDs. Note that, while eNOS did not make the p value threshold for this list, it did show regulation in the same direction as seen in control versus striatin knock-down EAhy926 endothelial cells treated with E2 for 24 hrs (Fig. 3 A&B): showing 1.29-fold higher expression in WT aortas versus DPM aortas treated with E2 for 4hrs. Entries shaded in grey are genes of particular interest in regard to estrogen’s effects in the

vascular system. All of the IPA pathways or functions connected to that gene are indicated (after "IPA:"), followed by the number(s) of any IPA networks that gene was found in (referring to networks 1 through 7 in Fig. 4C and supplemental figures 1 through 3), followed by additional notes and/or citations from the literature.

Supplemental Table 2. Ingenuity pathway analysis suggests the relevance of rapid signaling response genes for cardiovascular disease & estrogen metabolism. The top pathways or disease models having significant association with genes that are differentially E2 regulated in DPM versus WT mouse aortas are shown. Functions or pathways associated with cardiovascular disease, angiogenesis or muscle function are boxed in red. Those associated with endocrine function are boxed in blue. Where a given group (e.g. Cardiovascular Disease) has more than one subgroup for which differentially regulated genes are significantly enriched, a range of the p-values for the subgroups is given. Where there are no subgroups, a ratio of the number of differentially regulated genes versus all genes in the group is given.

Supplemental Table 3. Transcription factor binding sites (TFBSes) enriched or anti-enriched in top DPM vs. WT differentially E2-responsive genes (with $P < .01$ and fold-change > 1.25). "FG sites" (column 2): the number of binding sites found in the differentially regulated foreground datasets. "fg/bkg" (column 3): enrichment ratio equal to the foreground frequency over the background frequency. "p. for FDR.05" (column 5): the value that the calculated binomial p. value (binom. p) must be less than for a false discovery rate of less than 0.05, by the Benjamini Hochberg method. The single letter codes in Consensus Sequences are; M:A/C, R:A/G, W:A/T, S:C/G, Y:C/T, K:G/T, V:A/C/G, H:A/C/T, D:A/G/T, B:C/G/T. Note that the fact that a higher number of

significantly over- and under-represented TFBSes were found in the upregulated gene set relative to the downregulated gene set does not mean that fewer TFBS sites are associated with differential downregulation, but may instead be due to the lower statistical power afforded by analysis of the 24 downregulated genes versus the 48 upregulated genes.

Supplemental Table 4. Characteristics of mice used in the carotid injury study. Results are shown for the 22 WT and 23 DPM mice. Genotypes were established before the study and also confirmed at the end of the study using a small piece of tail from each mouse. There was no significant difference in body weights for all pair-wise comparisons. Wet uterine weights were low in all vehicle treated mice and high in all mice that received estrogen treatment ($p < .001$, with no significant differences for the Veh WT vs. DPM or E2 WT vs. DPM comparisons). Circulating estrogen level in the serum of each mouse was measured by radioimmunoassay (17β -estradiol (E2) Double Antibody - ^{125}I RIA Kit, MP Biomedical). E2 levels were low in both WT and DPM vehicle treated mice and increased equally in both with estrogen treatment ($p < .001$, with no significant differences for the Veh WT vs. DPM or E2 WT vs. DPM comparisons). The decrease in wet uterine weight and E2 levels in the absence of E2 treatment serves to confirm complete ovariectomy (-E2), and the increase in both these measures confirms successful restoration of estrogenic signaling with E2 treatment.

LEGENDS FOR SUPPLEMENTAL FIGURES

Supplemental Figure 1. Top IPA networks 2 & 3. See Fig. 4C for details.

Supplemental Figure 2. Top IPA networks 4 & 5. See Fig. 4C for details.

Supplemental Figure 3. Top IPA networks 6 & 7. See Fig. 4C for details.

Supplemental Figure 4. ER β and ER α each interact with striatin in cell extracts and, directly, in vitro. **(A)** GST striatin pull-down of ER α and ER β from MCF7 cells. MCF cell lysates were incubated with GST beads alone or GST striatin 1-203 followed by SDS-PAGE and immunoblotting with anti-ER β or anti-ER α respectively. **(B)** Coimmunoprecipitation of striatin and ER β . Lysates from Cos1 cells overexpressing either ER β or ER α (transfected with either an ER α or ER β expression plasmid), were immunoprecipitated with nonimmune IgG, anti-ER α or anti-ER β antibody, then immunoblotted for striatin, ER α and ER β respectively. **(C)** ER β associates with striatin in vitro. Recombinant HIS-tagged striatin (purified from BL21 E.Coli) was mixed with recombinant ER β and/or ER α (CalBiochem) in the presence or absence of E2 at 10nM for 20 min, before collection on Ni-NTA agarose beads.

GENES SHOWING DIFFERENTIALLY-INCREASED E2 RESPONSE IN DPM MOUSE AORTAS VS WT (e.g. normally repressed by rapid signaling)

Gene Symbol	Affymetrix ID	log ₂ FC	P.Value	logFC WT	logFC DPM	Regl type	Gene Title & Notes
Rala	1450870_at	0.441264	9.57E-07	-0.013894	0.427369	NR/up	v-ral simian leukemia viral oncogene homolog A (ras related). IPA: Endocrine system devel. & func. IPA network: 7.
Mrpl15	1435232_x_at	1.261658	9.99E-05	-0.529136	0.732523	down/up	mitochondrial ribosomal protein L15
Fam96a	1416634_at	0.763892	0.000173	-0.003932	0.75996	NR/up	family with sequence similarity 96, member A
Myo6	1433942_at	0.503341	0.000256	0.00799	0.511331	NR/up	myosin VI
Aldh1a2	1422789_at	0.627127	0.000283	0.030716	0.657843	NR/up	aldehyde dehydrogenase family 1, subfamily A2. IPA: Cardiovasc. system dev. & func., Embryonic dev.; IPA Network: 4, Butanoate metab. & Retinol metab. Catalyzes formation of RA from retinaldehyde. Required for coronary artery development, PNAS 2010 107:9234-9.
Cnot7	1430519_a_at	0.657077	0.000286	-0.067995	0.589083	NR/up	CCR4-NOT transcription complex, subunit 7. IPA network: 2. Alters cell proliferation, upregulated in SMCs with contact. Biochem J 1998 336:471-81.
Mrpl22	1435995_at	0.37114	0.000331	-0.138093	0.233047	down/up	mitochondrial ribosomal protein L22
Dus1l	1423943_at	1.04636	0.000361	-0.457864	0.588496	down/up	dihydrouridine synthase 1-like (S. cerevisiae)
Dctn5	1415748_a_at	0.600467	0.000835	-0.126151	0.474316	NR/up	dynactin 5
Btbd1	1456222_at	0.402436	0.000974	-0.019011	0.383425	NR/up	BTB (POZ) domain containing 1
Gsr	1421817_at	0.431938	0.001097	0.010613	0.442552	NR/up	glutathione reductase. IPA: Cardiovasc. disease & Glutathione depletion - hepatocellular hypertrophy. IPA network: 3.
Gfm1	1416570_s_at	0.59937	0.001186	-0.300658	0.298712	down/up	G elongation factor, mitochondrial 1
Dhx32	1420427_a_at	0.671714	0.0016	-0.379708	0.292006	down/up	DEAH (Asp-Glu-Ala-His) box polypeptide 32
Ccdc56	1423840_at	0.38079	0.001635	-0.020242	0.360548	NR/up	coiled-coil domain containing 56
Rps11	1424000_a_at	0.325646	0.001938	0.006828	0.332474	NR/up	ribosomal protein S11
D16Ert472e	1429897_a_at	0.70015	0.001993	-0.667659	0.03249	down/NR	DNA segment, Chr 16, ERATO Doi 472, expressed
Mtch2	1430474_a_at	0.322809	0.002013	-0.032989	0.28982	NR/up	mitochondrial carrier homolog 2 (C. elegans)
Lyst	1434674_at	0.595577	0.002039	-0.324499	0.271078	down/up	lysosomal trafficking regulator. IPA: Immunological disease. IPA network 2.
Trappc5	1448999_at	0.852565	0.002277	-0.404606	0.44796	down/up	trafficking protein particle complex 5
Cdc5l	1428092_at	0.53789	0.002479	-0.27235	0.265539	down/up	cell division cycle 5-like (S. pombe)
Pigk	1435352_at	0.974096	0.002714	-0.349667	0.624429	down/up	phosphatidylinositol glycan anchor biosynthesis, class K
Pex11a	1419365_at	1.187609	0.002723	-0.520822	0.666787	down/up	peroxisomal biogenesis factor 11 alpha. IPA: Connective tissue dev. & func. IPA network 6.
Sar1b	1428163_at	0.351213	0.002767	-0.149126	0.202088	down/up	SAR1 gene homolog B (S. cerevisiae). IPA: Metabolic disease.
Anapc16	1449339_at	0.803261	0.002889	-0.629397	0.173865	down/up	anaphase promoting complex subunit 16, also called D10Ert641e: DNA segment, Chr 10, ERATO Doi 641, expressed
Mela	1456182_x_at	2.461061	0.003215	0.022721	2.483782	NR/up	melanoma antigen
Nol11	1423479_at	0.479308	0.004011	-0.117945	0.361362	NR/up	nucleolar protein 11
Slimo2	1448543_at	0.49541	0.004132	-0.076983	0.418427	NR/up	slowmo homolog 2 (Drosophila)
Ltbp2	1418061_at	0.846913	0.004726	-0.248598	0.598316	down/up	latent transforming growth factor beta binding protein 2. IPA: Connective tissue dev. & func. IPA network 6. Involved in regulating elasticity of arteries. EMBO J. 2007 26:3283-95.
Tle3	1419655_at	0.369146	0.004883	-0.270232	0.098914	down/NR	transducin-like enhancer of split 3, homolog of Drosophila E(spl)
1110008F13Rik	1416699_at	0.341016	0.005019	-0.089483	0.251534	NR/up	RIKEN cDNA 1110008F13 gene
Hsd17b8	1454987_a_at	0.432042	0.005592	-0.014883	0.417159	NR/up	hydroxysteroid (17-beta) dehydrogenase 8, also called H2-Ke6: H2-K region expressed gene 6. IPA: Endocrine system dev. & func., & Androgen & estrogen metab. IPA network 4. Can inactivate estradiol & testosterone by oxidation but can also reduce estrone to form estradiol.
Ube2a	1417609_at	0.634673	0.005917	-0.09523	0.539443	NR/up	ubiquitin-conjugating enzyme E2A, RAD6 homolog (S. cerevisiae). IPA: Hypoxia-inducible factor signaling. IPA network 4.
Rcbtb2	1416389_a_at	1.323983	0.00592	-0.426328	0.897656	down/up	RCC1 and BTB (POZ) domain containing protein 2. IPA: Cardiac arteriopathy.
Chmp7	1451300_a_at	0.414837	0.006364	-0.072277	0.34256	NR/up	CHMP family, member 7
Mrpl3	1422463_a_at	0.909036	0.006859	-0.309015	0.600021	down/up	mitochondrial ribosomal protein L3
Trak1	1428327_at	0.331576	0.007172	-0.123281	0.208296	NR/up	trafficking protein, kinesin binding 1
Eif3l	1423728_at	0.370731	0.007183	-0.121381	0.24935	NR/up	eukaryotic translation initiation factor 3, subunit L
Snrk	1448864_at	0.444404	0.007212	0.037173	0.481578	NR/up	SNF related kinase
Capn2	1416257_at	0.417232	0.007332	-0.340907	0.076325	down/NR	calpain 2. IPAL: Connective tissue dev. & func.
Idua	1439251_at	0.453573	0.007691	0.008065	0.461637	NR/up	iduronidase, alpha-L-, also called Gm3932: predicted gene 3932
Heatr6	1424686_at	0.462983	0.007805	-0.315935	0.147049	down/up	HEAT repeat containing 6
Nip7	1453324_at	0.662525	0.007838	-0.46035	0.202175	down/up	nuclear import 7 homolog (S. cerevisiae)
Gpatch1	1419013_at	0.330581	0.00786	-0.007356	0.323225	NR/up	G patch domain containing 1
Mvp	1448618_at	0.69518	0.008187	-0.411736	0.283444	down/up	major vault protein
Ugt1a1	1424783_a_at	0.796954	0.008277	-0.14576	0.651193	down/up	UDP glucuronosyltransferase 1 fam., polypepts. A1-2,5,6A-B,7C,9&10. IPA: Metab. disease, Endocrine system dev. & func., Androgen & estrogen metab., Retinol metab. IPA network 3.
Gart	1416283_at	0.591267	0.008794	-0.352505	0.238763	down/up	phosphoribosylglycinamide formyltransferase
D10Wsu52e	1420129_s_at	0.348212	0.008918	-0.140655	0.207557	down/up	DNA segment, Chr 10, Wayne State University 52, expressed
Fiz1	1423733_a_at	0.431515	0.008975	-0.065073	0.366441	NR/up	Flt3 interacting zinc finger protein 1. IPA network 6. Interacts with FLT3 receptor essential for hematopoietic and lymphoid cell development.
Gbe1	1420654_a_at	1.057358	0.008997	-0.458577	0.598781	down/up	glucan (1,4-alpha-), branching enzyme 1. IPA: Metabolic disease, & Cardiac arteriopathy. IPA network 5. Coronary artery disease & metabolic syndrome associated with variants, Hum Genet 2003 114:87-98.
Hsd17b10	1438391_x_at	0.592136	0.009107	-0.128387	0.463748	NR/up	hydroxysteroid (17-beta) dehydrogenase 10. IPA: Metabolic disease, Fatty acid elong in mitochondria, Androgen & estrogen metab., Butanoate metab. IPA network 4. Metabolizes fatty acids & steroids, including estrogen.
Myo1e	1420159_at	1.372814	0.009172	-0.899922	0.472892	down/up	myosin IE. IPA network 4

Supplemental Table 1 (part 1)

GENES SHOWING DIFFERENTIALLY-INCREASED E2 RESPONSE IN DPM MOUSE AORTAS (continued)

Gene Symbol	Affymetrix ID	log ₂ FC	P.Value	logFC WT	logFC DPM	Regl type	Gene Title & Notes
Scamp3	1416294_at	0.435727	0.00934	-0.546639	-0.110911	down/NR	secretory carrier membrane protein 3
Pja1	1426448_at	0.539518	0.009404	-0.02171	0.517807	NR/up	praja1, RING-H2 motif containing
Zfyve27	1424550_at	0.933858	0.009586	-0.69778	0.236078	down/up	zinc finger, FYVE domain containing 27
Txndc9	1424512_a_at	0.359624	0.009855	-0.130864	0.228759	NR/up	thioredoxin domain containing 9
Abca1	1421839_at	0.517126	0.010036	-0.418586	0.09854	down/NR	ATP-binding cassette, sub-family A (ABC1), member 1. IPA: Immunological disease, Hormone rec. reg'd cholesterol metab., Cardiac ateriopathy. Plasma membrane cholesterol efflux transporter.
Rab28	1423990_at	0.331518	0.010101	0.045105	0.376624	NR/up	RAB28, member RAS oncogene family
Pdcd2l	1426845_at	0.362858	0.010227	-0.060128	0.30273	NR/up	programmed cell death 2-like
Krt18	1448169_at	0.883369	0.010287	-0.437052	0.446317	down/up	keratin 18. IPA: Endocrine system dev. & func., Embryonic dev. IPA networks 1 & 2.
Smcp1	1431744_a_at	0.705675	0.010745	-0.391858	0.313817	down/up	similar to SMAP1 /// stromal membrane-associated protein 1
Tcf19	1423809_at	0.495042	0.011539	-0.079781	0.415261	NR/up	transcription factor 19
Asns	1433966_x_at	0.835336	0.011653	-0.39358	0.441757	down/up	asparagine synthetase
Ext1	1417730_at	0.44978	0.011853	-0.108428	0.341352	NR/up	exostosins (multiple) 1. IPA: Embryonic dev., & Cardiac ateriopathy. IPA network 1.
Tmem119	1451344_at	0.418689	0.011889	0.055807	0.474496	NR/up	transmembrane protein 119
Ctcf	1418330_at	0.420168	0.01191	0.004279	0.424448	NR/up	CCCTC-binding factor. IPA: Embryonic dev. Key mammalian insulator protein regulating enhancer/silencer-promoter interactions.
Hmgcs1	1433446_at	0.532722	0.012044	-0.300907	0.231816	down/up	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1. IPA: Butanoate metab., & Cardiac ateriopathy. IPA network 7. Cytoplasmic HMG-CoA synthase required for cholesterol synthesis.
Zfp62	1425495_at	0.721616	0.012235	-0.427656	0.293961	down/up	zinc finger protein 62
Scarb2	1460235_at	0.530261	0.013225	-0.026617	0.503643	NR/up	scavenger receptor class B, member 2
A630072M18Rik	1427081_at	0.437341	0.01348	-0.28079	0.156551	down/up	RIKEN cDNA A630072M18 gene
Stard7	1449628_s_at	0.409192	0.013492	-0.261784	0.147408	down/up	START domain containing 7
Kcap5	1437349_at	0.42722	0.013499	-0.168905	0.258316	down/up	cytoskeleton associated protein 5
Ostc	1449139_at	0.716881	0.013854	-0.157622	0.559259	down/up	oligosaccharyltransferase complex subunit
Nacc2	1429582_at	1.042051	0.014134	-0.61489	0.427161	down/up	nucleus accumbens associated 2, BEN & BTB (POZ) domains
Ccdc127	1449096_at	0.389945	0.014497	0.04997	0.439914	NR/up	coiled-coil domain containing 127
Nufip1	1448802_at	0.374458	0.014711	-0.164684	0.209773	down/up	nuclear fragile X mental retardation protein interacting protein 1
Unc50	1421982_a_at	0.684779	0.014716	-0.275809	0.40897	down/up	unc-50 homolog (C. elegans)
Mrps24	1438563_s_at	0.511888	0.015014	-0.039831	0.472056	NR/up	mitochondrial ribosomal protein S24
Vopp1	1423812_s_at	0.715475	0.015194	-0.01318	0.702295	NR/up	vesicular, overexpressed in cancer, prosurvival protein 1
Pasma4	1460339_at	0.444316	0.015511	-0.317778	0.126538	down/NR	proteasome (prosome, macropain) subunit, alpha type 4. IPA: Cardiac ateriopathy.
Glrx3	1417340_at	0.59679	0.015604	0.022922	0.619713	NR/up	glutaredoxin 3
C3orf37	1423786_at	0.67609	0.015673	-0.209438	0.466651	down/up	chromosome 3 open reading frame 37 also caled 8430410A17Rik: RIKEN cDNA 8430410A17 gene. IPA: Cardiac ateriopathy.
Cd83	1416111_at	1.006113	0.016025	-0.093971	0.912142	NR/up	CD83 antigen. IPA: Endocrine system disorders, & Immunological disease. IPA network 1. Cell surface marker in T-cell responses.
Mecr	1417098_s_at	1.3068	0.01643	-0.264387	1.042413	down/up	mitochondrial trans-2-enoyl-CoA reductase. IPA: Fatty acid elongation in mitochondria. IPA network 5.
Topbp1	1452241_at	0.771422	0.016439	-0.268768	0.502653	down/up	topoisomerase (DNA) II binding protein 1
4931408A02Rik	1454048_a_at	0.786894	0.016479	-0.75717	0.029725	down/NR	RIKEN cDNA/// similar to Protein C21orf63 homolog precursor
Toe1	1426527_at	0.35676	0.016699	-0.206543	0.150218	down/up	target of EGR1, member 1 (nuclear)
Eif4e3	1417978_at	0.542701	0.016732	-0.19701	0.34569	down/up	eukaryotic translation initiation factor 4E member 3
Abcd3	1416679_at	0.595709	0.017117	0.105716	0.701425	NR/up	ATP-binding cassette, sub-family D (ALD), member 3
Srfbp1	1420509_at	0.501018	0.017798	-0.295459	0.205559	down/up	serum response factor binding protein 1. Transcriptional cofactor for SRF, with important functions in cardiac muscle.
1110012L19Rik	1451302_at	1.312574	0.017987	-0.382243	0.930331	down/up	RIKEN cDNA 1110012L19 gene
Mrpl49	1423218_a_at	0.347116	0.018473	0.017574	0.36469	NR/up	mitochondrial ribosomal protein L49
Gosr1	1419371_s_at	0.398681	0.018483	-0.037232	0.361448	NR/up	golgi SNAP receptor complex member 2. IPA: Cardiac ateriopathy. IPA network 3.
Ptpmt1	1426581_at	0.490081	0.018873	-0.102706	0.387376	NR/up	protein tyrosine phosphatase, mitochondrial 1
---	1436570_at	0.700152	0.019098	-0.282007	0.418146	down/up	---
Sh2b2	1450718_at	0.431976	0.019151	-0.025157	0.406818	NR/up	SH2B adaptor protein 2. IPA: Connective tissue dev. & func. IPA network 7.
Tcta	1420124_s_at	0.981671	0.019205	-0.588165	0.393507	down/up	T-cell leukemia translocation altered gene
TIK2	1454018_at	0.670817	0.019223	-0.059918	0.610899	NR/up	tousled-like kinase 2 (Arabidopsis)
Pasma6	1437144_x_at	0.448054	0.019413	-0.066611	0.381444	NR/up	proteasome (prosome, macropain) subunit, alpha type 6
Rpn1	1438943_x_at	0.338279	0.019498	-0.163809	0.174469	down/up	ribophorin I
Prg4	1449824_at	1.158903	0.019825	-1.172303	-0.0134	down/NR	proteoglycan 4 (megakaryocyte stimulating factor). Also called hemangiopoietin. IPA: Connective tissue dev. & func. IPA network 2. Increases EC proliferation via PI3K/Akt pathways.
Pdzd11	1451269_at	0.4464	0.019901	-0.235187	0.211213	down/up	PDZ domain containing 11

GENES SHOWING DIFFERENTIALLY-REDUCED E2 RESPONSE IN DPM MOUSE AORTAS VS WT (e.g. normally activated by rapid signaling)

Gene Symbol	Affymetrix ID	log ₂ FC	P.Value	logFC WT	logFC DPM	Regl type	Gene Title & Notes
Phxr4	1422272_at	-0.50478	0.000318	0.289874	-0.214904	up/down	per-hexamer repeat gene 4
Reep2	1424386_at	-0.76553	0.000691	0.455596	-0.30993	up/down	receptor accessory protein 2
Ccdc82	1421383_at	-0.47891	0.000779	0.534244	0.055336	up/NR	coiled-coil domain containing 82
Lhpp	1452889_at	-0.64619	0.001411	0.272322	-0.373864	up/down	phospholys. Phosphohis. Inorg. pyrophosphate phosphatase
Eif5b	1435592_at	-0.65124	0.001533	0.574537	-0.076703	up/NR	eukaryotic translation initiation factor 5B
Wwc2	1448611_at	-0.53982	0.001612	0.458205	-0.08162	up/NR	WW, C2 and coiled-coil domain containing 2
Flt1	1451756_at	-1.23246	0.002577	0.360735	-0.871724	up/down	FMS-like tyrosine kinase 1. IPA: Cardiovasc. disease, Cardio. dev. & func., Connective tissue dev. & func., Embryonic dev., Increases glomerular injury, Hypoxia-inducible factor signaling, & Cardiac transformation. IPA network 7. Major VEGF receptor which binds VEGF and Placental GF. Increases SMC proliferation with VEGF. Cardiovasc Res 2002 55:201-12.

Supplemental Table 1 (part 2)

GENES SHOWING DIFFERENTIALLY-REDUCED E2 RESPONSE IN DPM MOUSE AORTAS VS WT (continued)

Gene Symbol	Affymetrix ID	log ₂ FC	P.Value	logFC WT	logFC DPM	Regl type	Gene Title & Notes
Rsad2	1421008_at	-1.35449	0.003083	0.971703	-0.382786	up/down	radical S-adenosyl methionine domain containing 2. IPA: Connective tissue dev. & func. Interferon induced. IPA network 1. Protein levels increase in atherosclerotic lesions, Olofsson et al Atheroscler Thromb Vasc Biol 2005 25:e113-6.
Zfp1	1417119_at	-0.40894	0.003128	0.154025	-0.254911	up/down	zinc finger like protein 1
Mast2	1417324_at	-0.51783	0.003228	0.844786	0.326956	up/up	microtubule associated serine/threonine kinase 2
Sfrp1	1460187_at	-1.38103	0.003307	1.157861	-0.223165	up/down	secreted frizzled-related protein 1. IPA: Connective tissue dev. & func., & Embryonic dev. IPA networks 6 & 7. Soluble mediator of WNT signaling. Promotes neo-vascularization after ischemia. Am J Pathol 2007 172:37-49.
Tmem141	1435259_s_at	-0.33656	0.00384	0.099056	-0.237507	NR/down	transmembrane protein 141
Asb4	1423422_at	-0.88284	0.004045	0.646927	-0.235914	up/down	ankyrin repeat and SOCS box-containing 4
Rbm39	1442745_x_at	-0.88713	0.004071	0.379284	-0.507849	up/down	RNA binding motif protein 39. IPA network 7. Transcriptional coactivator through AP1 & ER. Dutta et al. 2008 J Virol 2008 82:10792-802.
Rsad2	1436058_at	-1.67063	0.004112	0.787739	-0.882895	up/down	radical S-adenosyl methionine domain containing 2 (see 1st Rsad2 entry).
Rsad2	1421009_at	-2.24679	0.0043	1.079372	-1.167421	up/down	radical S-adenosyl methionine domain containing 2 (see 1st Rsad2 entry)
5730528L13Rik	1430701_a_at	-0.40567	0.004329	0.38504	-0.020635	up/NR	RIKEN cDNA 5730528L13 gene
9530028C05	1455581_x_at	-0.5218	0.005754	0.386236	-0.135565	up/NR	hypothetical protein 9530028C05
Elmo1	1424523_at	-0.51117	0.006375	0.401389	-0.109779	up/NR	engulfment and cell motility 1, ced-12 homolog (C. elegans). IPA: Cardiac arteriopathy. IPA network 5. Important for phagocytosis & cell migration.
---	1420285_at	-0.76349	0.007254	0.737821	-0.025668	up/NR	---
Ifit3	1449025_at	-1.81175	0.007946	0.884413	-0.92734	up/down	interferon-induced protein with tetratricopeptide repeats 3. IPA network 1. One of four interferon-induced genes that are downregulated by E2 in DPM vs WT mice (Rsad2, Ifit1, 2 & 3).
Ifit2	1418293_at	-1.49923	0.008146	1.130714	-0.368518	up/down	interferon-induced protein with tetratricopeptide repeats 2. IPA network 1. One of four interferon-induced genes that are downregulated by E2 in DPM vs WT mice (Rsad2, Ifit1, 2 & 3).
Rad23a	1422964_at	-0.81638	0.008264	0.486714	-0.329671	up/down	RAD23a homolog (S. cerevisiae)
Zfp319	1439008_at	-0.51419	0.008459	0.052877	-0.461312	NR/down	zinc finger protein 319
Adck4	1448490_at	-1.20178	0.008663	0.316076	-0.885705	up/down	aaRF domain containing kinase 4
Gas6	1417399_at	-0.49931	0.008906	0.634029	0.134723	up/NR	growth arrest specific 6. IPA: Cardiovascular disease, Embryonic dev., & Increases glomerular injury. IPA network 1. Stimulates cell proliferation & involved in thrombosis. Knock out reduces thrombosis, Nat Med 2001 7:215-21.
Sept4	1455422_x_at	-0.61656	0.00896	0.35422	-0.262342	up/down	septin 4
Gpatch2	1420528_at	-0.40024	0.009172	0.039037	-0.361207	NR/down	G patch domain containing 2
Stat2	1450403_at	-1.32774	0.010267	0.170134	-1.157602	up/down	signal transducer and activator of transcription 2. IPA network 1. Important component of a transcription factor complex required for myogenic differentiation and for proliferation of myoblasts. J. Biol. Chem. 2008 283:34029-36.
Aph1a	1424979_at	-0.38072	0.010415	0.114716	-0.266005	NR/down	anterior pharynx defective 1a homolog (C. elegans). IPA: Embryonic dev. IPA network 1.
Plekhj1	1424179_at	-0.41595	0.010605	0.159318	-0.256635	up/down	pleckstrin homology domain containing, family J member 1
Lifr	1425107_a_at	-0.37708	0.01179	0.401774	0.024695	up/NR	leukemia inhibitory factor receptor. IPA: Cardiac arteriopathy. IPA network 1.
Zcchc14	1418169_at	-0.3338	0.013442	0.213286	-0.120513	up/NR	zinc finger, CCHC domain containing 14
Pbld	1449462_at	-0.40951	0.013635	0.302111	-0.107402	up/NR	phenazine biosynthesis-like protein domain containing, also called 3110049J23Rik: RIKEN cDNA 3110049J23 gene
Znfx1	1427091_at	-0.653	0.014208	0.221525	-0.431476	up/down	zinc finger, NFX1-type containing 1
Ifit1	1450783_at	-2.64905	0.014406	1.62336	-1.025687	up/down	interferon-induced protein with tetratricopeptide repeats 1. IPA network 1. One of four interferon-induced genes that are downregulated by E2 in DPM vs WT mice (Rsad2, Ifit1, 2 & 3).
---	1425918_at	-1.44187	0.014654	1.194069	-0.247803	up/down	---
Baz2a	1438192_s_at	-0.65223	0.014733	0.355732	-0.2965	up/down	bromodomain adjacent to zinc finger domain, 2A
Traf7	1424320_a_at	-0.46464	0.01519	0.164705	-0.299933	up/down	TNF receptor-associated factor 7
Ap1s1	1416087_at	-0.584	0.015427	0.43943	-0.144571	up/down	adaptor protein complex AP-1, sigma 1
Armcx3	1424373_at	-0.54818	0.016064	0.463014	-0.085169	up/NR	armadillo repeat containing, X-linked 3
Midn	1449188_at	-0.65609	0.016197	0.576582	-0.079513	up/NR	midnolin
Tg	1450790_at	-0.51381	0.016209	0.470563	-0.043249	up/NR	thyroglobulin. IPA: Endocrine system disorders, Immunological disease, & Cardiovasc. system dev. & func. IPA network 7.
Pomt2	1451396_at	-0.34483	0.016769	0.068947	-0.275882	NR/down	protein-O-mannosyltransferase 2
Itgb5	1417534_at	-0.49394	0.017518	0.356429	-0.137511	up/down	integrin beta 5. Loss enhanced pathological angiogenesis in the context of Itgb3 absence: Reynolds et al. 2002 Nat Med 8:27-34.
Icam2	1448862_at	-0.69145	0.017643	0.675906	-0.015539	up/NR	intercellular adhesion molecule 2. IPA network 7. Regulates lymphocyte adhesion, perhaps by blocking LFA-1 dependent cell adhesion.
Pdxdc1	1436372_a_at	-0.62195	0.017889	0.319978	-0.301969	up/down	pyridoxal-dependent decarboxylase domain containing 1
Wdr48	1426723_at	-0.45608	0.018238	0.172274	-0.283806	up/down	WD repeat domain 48
Unc45a	1455912_x_at	-0.70739	0.018754	0.485939	-0.221449	up/down	Unc-45 homolog A (C. elegans). IPA network 3. An HSP90 co-chaperone required for myosin folding, and for progesterone receptor activation by release from HSPs, e.g. Mol Cell Biol 2006 26:1722-30.
Sic20a1	1448568_a_at	-0.38191	0.019757	0.227738	-0.154526	up/down	solute carrier family 20, member 1

Supplemental Table 1 (part 3)

Top IPA disorders, systems & pathways for DPM vs. WT differential E2 responsive genes

Top Diseases & Disorders

Name	p-value	# Molecules
Endocrine System Disorders	5.00E-03 - 4.84E-02	2
Immunological Disease	5.00E-03 - 4.84E-02	4
Metabolic Disease	5.00E-03 - 4.84E-02	7
Cardiovascular Disease	9.86E-03 - 3.94E-02	4

Top Physiological Systems: Development & Function

Name	p-value	# Molecules
Endocrine System Development and Function	7.00E-03 - 3.89E-02	4
Cardiovascular System Development and Function	9.86E-03 - 4.84E-02	3
Connective Tissue Development and Function	9.86E-03 - 4.88E-02	8
Embryonic Development	9.86E-03 - 4.84E-02	8

Top Canonical Pathways

Name	p-value	Ratio
Fatty Acid Elongation in Mitochondria	1.19E-02	2/45 (0.044)
Androgen and Estrogen Metabolism	1.53E-02	3/142 (0.021)
Butanoate Metabolism	1.77E-02	3/132 (0.023)
Retinol Metabolism	2.31E-02	2/62 (0.032)

Top Tox Lists

Name	p-value	Ratio
Increases Glomerular Injury	3.31E-03	2/9 (0.222)
Glutathione Depletion - Hepatocellular Hypertrophy	6.71E-02	1/8 (0.125)
Hormone Receptor Regulated Cholesterol Metabolism	7.63E-02	1/8 (0.125)
Hypoxia-Inducible Factor Signaling	1.28E-01	2/70 (0.029)

Cardiotoxicity list

Name	p-value	# Molecules
Cardiac Arteriopathy	2.93E-02 - 2.52E-01	11
Cardiac Transformation	3.89E-02 - 3.89E-02	1

 = Associated with endocrine function.

 = Associated with cardiovascular function.

TFBSes over/under-represented in differentially regulated gene promoters

Enriched in DOWN-regulated promoters only (DPM_E2/Veh < WT_E2/Veh)

TFBS site	FG sites	fg/bkg	binom. p	p. for FDR.05	Consensus Sequence	Name
V\$BLIMP1_Q6	36	2.80804	8.39E-08	0.00025641	AGRAAGKGAAAGKR	B-lymphocyte-induced maturation protein, encoded by PRDM1
V\$CEBP_Q1	190	1.274896	0.001161	0.001282051	NNTKTGGWNAANN	CCAAT/enhancer binding protein
V\$CEBP_Q2_Q1	148	1.329416	0.000867	0.001111111	NTTRCNNAANN	CCAAT/enhancer binding protein
V\$HMG1Y_Q6	281	1.220426	0.001172	0.001367521	GGAAAWT	HMG IY
V\$VJUN_Q1	8	5.225764	0.000194	0.000769231	NYGATGACGTCATNCY	v-Jun

Anti-Enriched in DOWN-regulated promoters only

TFBS site	FG sites	fg/bkg	binom. p	p. for FDR.05	Consensus Sequence	Name
V\$AHRHIF_Q6	50	0.567998	1.44E-05	0.00042735	NRCGTGNNG	AHRHIF
V\$HMG1Y_Q3	142	0.764701	0.000916	0.001196581	NNKKNAWTTTNYTNN	HMG1Y
V\$P53_Q2	159	0.74768	0.000132	0.000683761	NGRCWTGYCY	tumor suppressor p53
V\$XVENT1_Q1	14	0.41788	0.000255	0.001025641	SBNCTATTGYSY	Xenopus ventral 1

Enriched in UP-regulated promoters only (DPM_E2/Veh > WT_E2/Veh)

TFBS site	FG sites	fg/bkg	binom. p	p. for FDR.05	Consensus Sequence	Name
V\$BRCA_Q1	757	1.171617	1.91E-05	0.001880342	KTNNGTTG	BRCA1 containing protein complex (BRCA1 or BRCA1:USF2)
V\$CDXA_Q2	3128	1.180784	1.76E-20	8.54701E-05	AWTWMTR	Cdx-1
V\$E2F1_Q3	1117	1.109034	0.000594	0.003675214	TTGGCGCGRAANNNGM	E2F-1
V\$ELF1_Q6	88	1.583791	5.2E-05	0.002393162	RNWMBAGGAART	Elf-1
V\$EV1_Q4	175	1.48099	9.69E-07	0.001111111	DGATADGAHWAGATA	ectopic viral integration site 1 encoded factor
V\$FAC1_Q1	378	1.409576	2.16E-10	0.00034188	NNNCAMAACACRNA	fetal Alz-50 clone 1
V\$FOXQ3_Q1	479	1.257542	1.16E-06	0.001196581	NAWTGTTTTRTTT	fork head box D3
V\$FOXP1_Q1	58	1.801057	4.19E-05	0.002307692	TTATTTGTRTTKKYTWKTWT	FOXP1a (repressor)
V\$FREACT_Q1	88	1.498892	0.000315	0.003247863	WNNANATAAAAYANNNN	Fork head RElated ACTivator-7
V\$GATA1_Q6	63	1.504862	0.001935	0.004444444	MNAGATAANR	GATA-binding factor 1
V\$HNF3_Q1	179	1.393833	2.15E-05	0.001965812	KNNTRTTTTRTTTA	HNF-3 (HNF3/fork head homolog 3)
V\$HNF3_Q6	456	1.23342	1.3E-05	0.001794872	NWRARYAAAYANN	HNF-3 alpha, beta or gamma
V\$HNF3ALPHA_Q6	377	1.204777	0.000394	0.003418803	TRTTTGYTYWN	HNF-3alpha
V\$HOXA7_Q1	139	1.283088	0.004487	0.004786325	YCAATCT	HOXA7
V\$IRF_Q6_Q1	358	1.320341	4.2E-07	0.000940171	RAAANTGAAAN	IRF-1,2,3,4,5,7,8,9,10 or ISGF3
V\$MYC_Q2	276	1.205962	0.0023	0.004529915	CACGTGS	c-Myc
V\$NKX62_Q2	214	1.246079	0.001712	0.004358974	NWADTAAWTANN	NK related homeobox factor 6-2
V\$OCT1_Q3	286	1.236475	0.000474	0.003504274	NNNRTAATNANN	octamer factor 1, POU2F1
V\$OCT1_Q4	55	1.809332	5.49E-05	0.002478632	NNNNNNNNWATGCAATNNNNNA	octamer factor 1, POU2F1
V\$PAX2_Q2	1523	1.197057	4.93E-12	0.00017094	NNNAAASNN	paired box factor 2, pax-2
V\$PBX1_Q1	359	1.184804	0.001681	0.004273504	ANCAATCAW	homeo domain factor Pbx-1
V\$PBX1_Q2	12	3.214869	0.000507	0.003589744	NNCATCAATCAANNW	homeo domain factor Pbx-1
V\$PU1_Q6	402	1.242483	2.51E-05	0.002051282	WAGGAGAG	Pu.1, mouse, human
V\$SRY_Q1	985	1.130312	0.000141	0.002905983	AAACWAM	sex-determining region Y gene product
V\$STAT4_Q1	1961	1.107056	6.72E-06	0.001623932	SNWTTCCN	signal transducer and activator of transcription 4
V\$STAT5A_Q3	788	1.119674	0.00164	0.004188034	NAWTTTCYN	signal transducer and activator of transcription 5a
V\$STAT5A_Q4	1678	1.112953	1.23E-05	0.001709402	NNNTTCYN	signal transducer and activator of transcription 5a

Anti-Enriched in UP-regulated promoters only

TFBS site	FG sites	fg/bkg	binom. p	p. for FDR.05	Consensus Sequence	Name
V\$AP2REP_Q1	462	0.839414	0.000115	0.002820513	CAGTGGG	AP-2 repressor
V\$CACBINDINGPRO	383	0.867133	0.004474	0.004700855	GRGGSTGGG	CAC-binding protein
V\$CADC_Q1	446	0.859288	0.001097	0.003931624	CCACRCCC	CACD
V\$CHCH_Q1	2598	0.916982	4.71E-06	0.001538462	CGGGNN	Churchill
V\$DBP_Q6	521	0.843644	6.7E-05	0.002649573	AGCAHAC	DBP
V\$E2F_Q2	996	0.883987	6.67E-05	0.002564103	GGCGSG	E2F (1, 3a & 4)
V\$EGR_Q6	316	0.796208	2.86E-05	0.002136752	GTGGGSGCRRS	EGR-1, 2 & 3
V\$GATA2_Q1	633	0.878274	0.000847	0.003846154	NNNGATRNNN	GATA-binding factor 2
V\$GC_Q1	499	0.818365	3.77E-06	0.001452991	NRGGGCGGGGCKNK	GC box (Pol II basal promoter element)
V\$HAND1E47_Q1	20	0.538098	0.003027	0.004615385	NNNNGNRTCTGGMWTT	Hand1:E47 heterodimer
V\$HEB_Q6	143	0.770592	0.001365	0.004017094	RCCWGCTG	HEB
V\$KROX_Q6	60	0.488601	4.99E-10	0.00042735	CCCCCCCCCRCCCC	EGR-1,2,3 & 4
V\$MAZ_Q6	313	0.772318	1.9E-06	0.001282051	GGGGAGGG	MAZ
V\$MAZR_Q1	144	0.751815	0.000385	0.003333333	NSGGGGGGGGMCMN	MAZ related factor
V\$MOVQ1_Q1	1202	0.858353	4.24E-08	0.000683761	GNGGGGG	MOVQ-B
V\$PAX4_Q3	388	0.844809	0.000679	0.003760684	NNNNNYCACCCB	Pax-4
V\$SP1_Q2_Q1	443	0.770877	1.02E-08	0.000512821	CCCCGCCCCN	Sp1
V\$SP1_Q4_Q1	312	0.753987	1.88E-07	0.000854701	NNGGGCGGGGNN	Sp1
V\$SP1_Q6	294	0.803851	0.000114	0.002735043	GGGGCGGGGC	Sp1
V\$SP1_Q6_Q1	388	0.81471	3.09E-05	0.002222222	GGGGCGGGGC	Sp1
V\$WT1_Q6	1365	0.861411	1.14E-08	0.000598291	SMCNCCNSC	WT1
V\$ZF5_Q1	463	0.845692	0.00022	0.003162393	GSAGCGGR	ZF5

Enriched in UP- AND DOWN-regulated promoters

TFBS site	FG sites	fg/bkg	binom. p	p. for FDR.05	Consensus Sequence	Name
V\$CDXA_Q1	526	1.185866	0.000116	0.000598291	MTTATATR	Cdx-1
V\$STAT6_Q2	842	1.136603	0.000227	0.000854701	NNYTTCY	signal transducer and activator of transcription 6

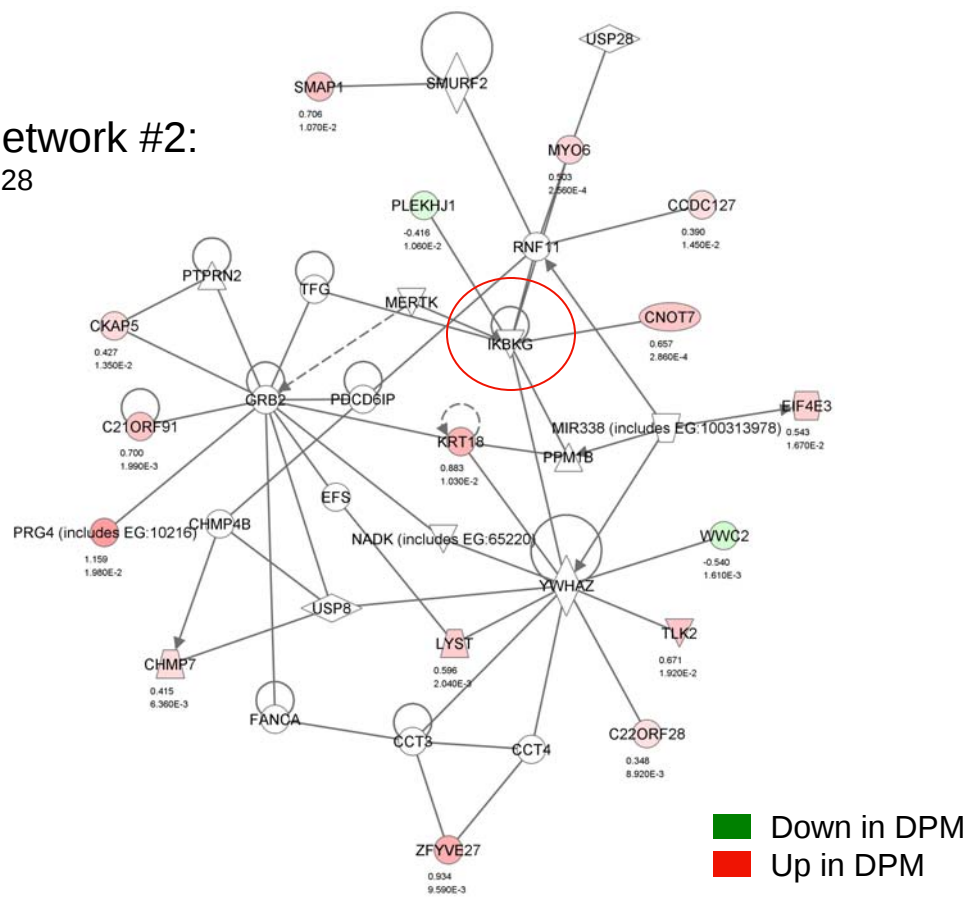
Anti-Enriched in UP- AND DOWN-regulated promoters

TFBS site	FG sites	fg/bkg	binom. p	p. for FDR.05	Consensus Sequence	Name
V\$AP2ALPHA_Q1	649	0.778794	2.04E-11	8.54701E-05	GCCNNNRGS	AP-2 alpha
V\$AP2GAMMA_Q1	412	0.768418	2.05E-08	0.00017094	GCCYNNNGGS	AP-2 gamma
V\$ETF_Q6	393	0.800234	4.23E-06	0.00034188	GVGGMGG	ETF
V\$HNF4_Q6_Q3	911	0.889216	0.000253	0.000940171	NGGNCA	HNF4, or HNF4alpha, half site 2
V\$LRF_Q2	286	0.800944	0.0001	0.000512821	VNNRMCCCC	mouse LRF, human FBI-1, rat OCZF

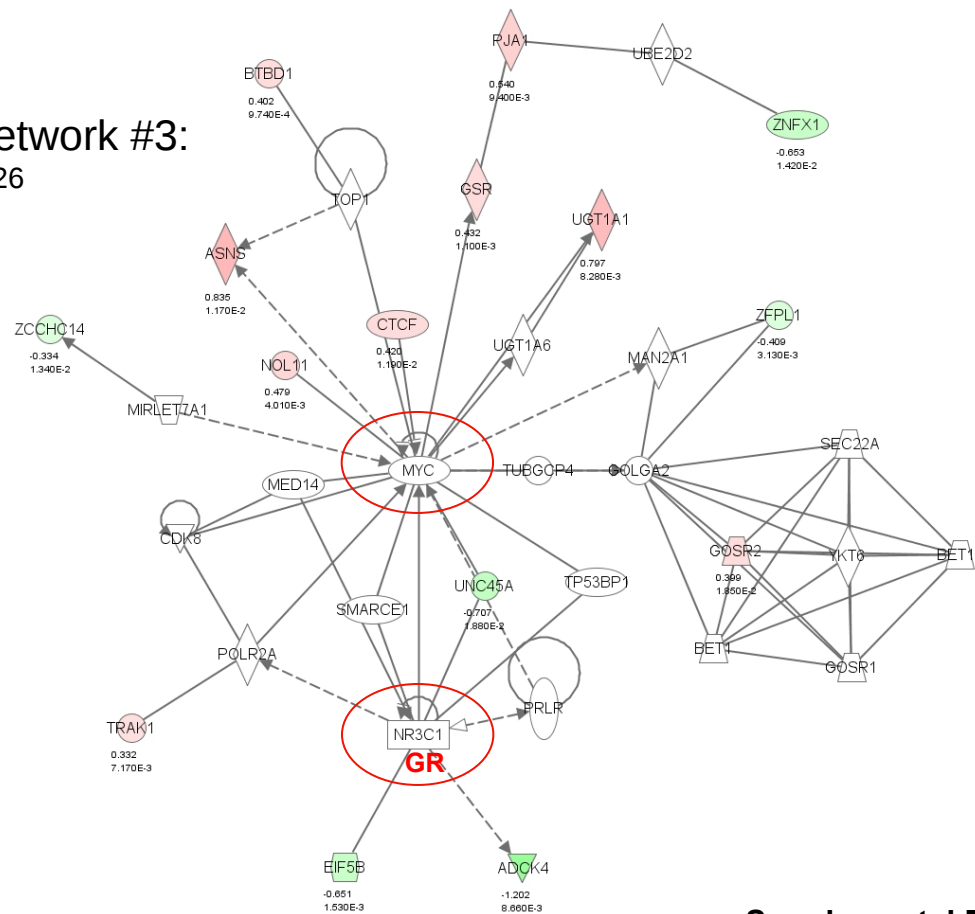
	Body Weight (grams)	Uterine Weight (μg)	Circulating E₂ (pg/ml)
WT, Veh. (n=11)	21.2±0.63	13.0±0.85	66±20
WT, E2 (n=11)	22.2±0.50	143.4±5.82	1227±114
DPM Veh. (n=12)	22.8±0.69	11.7±0.55	35±6
DPM E2 (n=11)	22.3±0.58	117.5±5.89	868±150

A

IPA Network #2:
 $P < 10^{-28}$

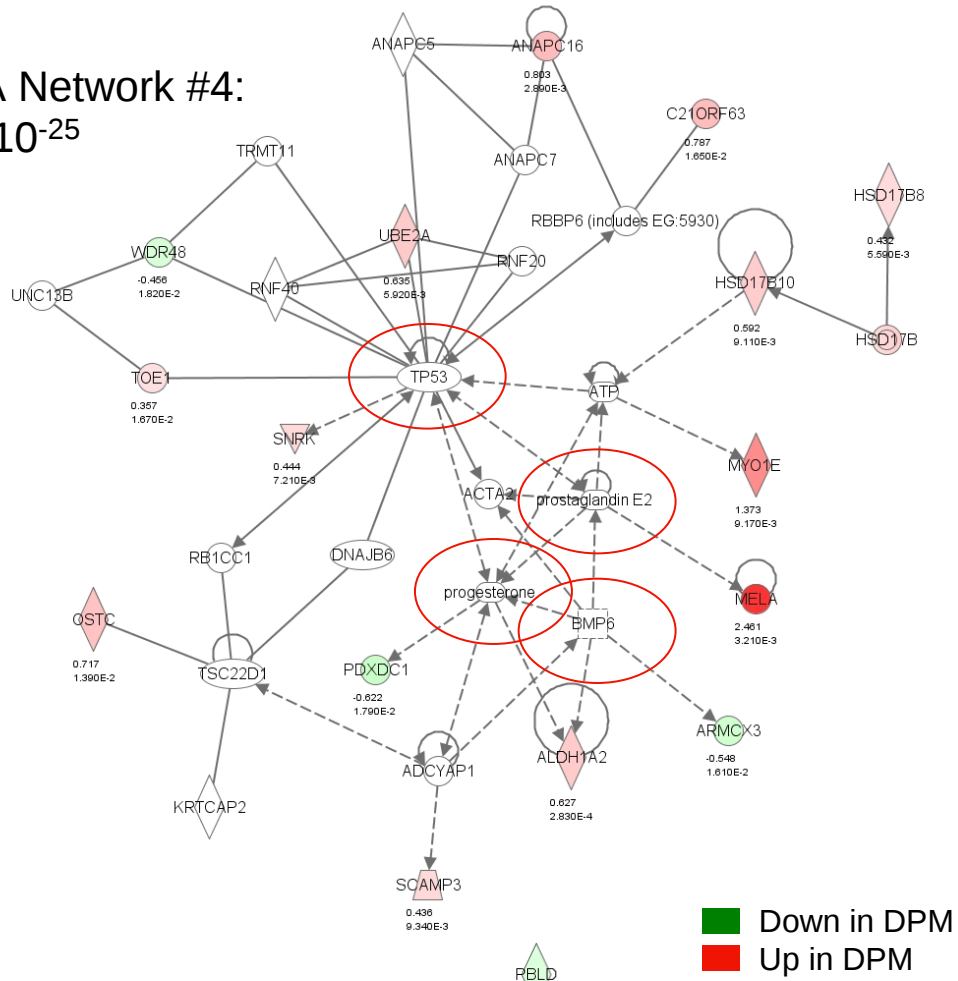
**B**

IPA Network #3:
 $P < 10^{-26}$

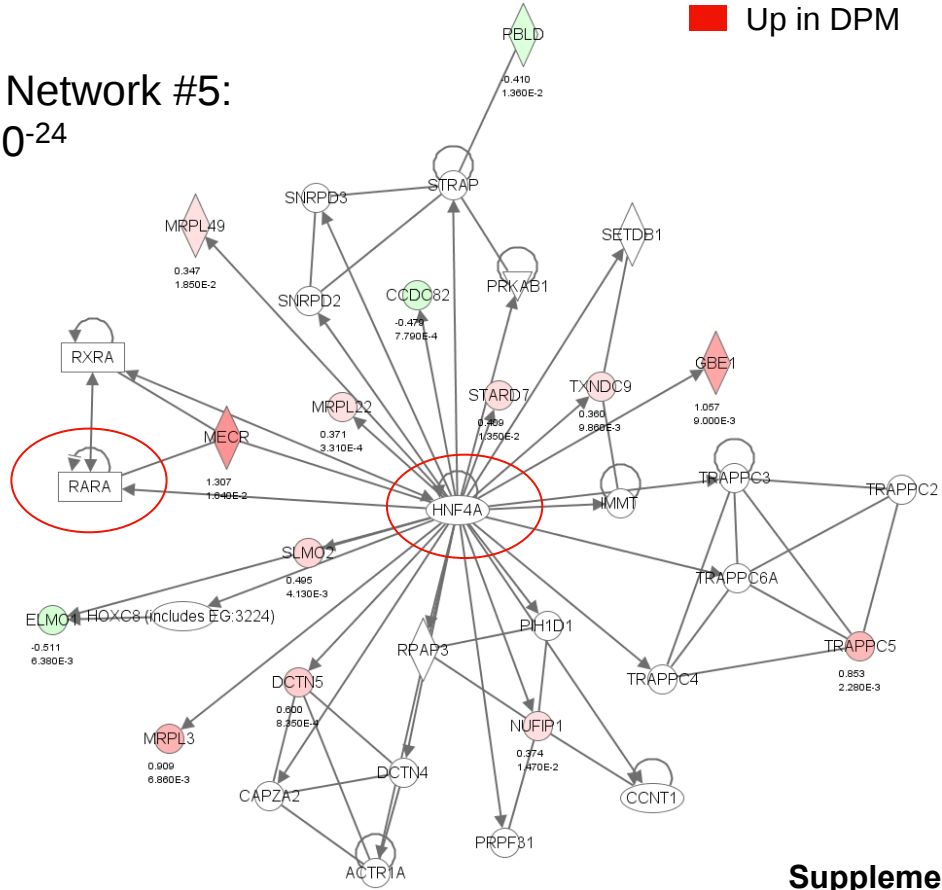


Supplemental Figure 1

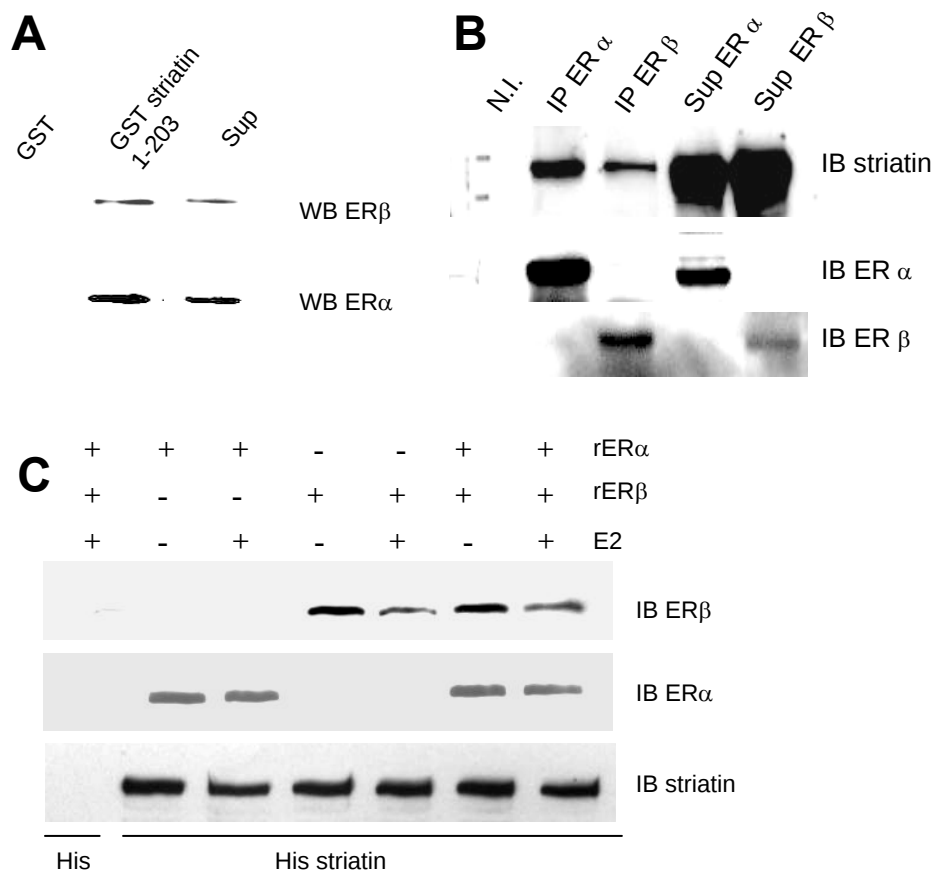
A IPA Network #4:
P<10⁻²⁵



B IPA Network #5:
P<10⁻²⁴



Supplemental Figure 2



Supplemental Figure 4