

Supplementary Materials: Transcriptomic Insights into the Response of Placenta and Decidua Basalis to the CpG Oligodeoxynucleotide Stimulation in Non-Obese Diabetic Mice and Wild-Type Controls

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Table S1. Statistics of raw and mapped reads from RNA-Seq analysis in Non-Obese Diabetic Mice (NOD) and Wild-Type Controls (WT) with CpG oligodeoxynucleotide (CpG ODN) or Control ODN.

Variable	NOD		WT	
	CpG ODN	Control ODN	CpG ODN	Control ODN
Raw reads	50,915,916	48,993,770	53,112,240	42,140,620
Raw nucleotide bases	5,142,507,516	4,948,370,770	5,364,336,240	4,256,202,620
≥Q20 (%) ^a	94.87	94.97	94.81	96.57
Hi-Q ^b reads	48,231,724	46,449,852	50,378,402	40,840,510
Hi-Q nucleotide bases	47,264,282,860	4,555,716,219	4,932,417,478	4,036,119,559
≥Q20 (%)	99.12	98.86	98.79	99.14
Mapped reads	45,955,512	44,248,507	48,377,489	39,287,064
(Rate)	(95.3%)	(95.3%)	(96.0%)	(96.2%)

^a Percent of sequence with error estimates better than 10^{-2} (Q20); ^b Hi-Q is short for high quality.

Table S2. Primer sequences used for RT-qPCR.

Gene Symbol	Primer Sequences	
<i>β-actin</i>	Forward	TGGCTCCTAGCACCATGAAG
	Reverse	AACGCAGCTCAGTAACAGTCC
<i>Adipoq</i>	Forward	CCGCTTATGTGTATCGCTCAG
	Reverse	CCGTGATGTGGTAAGAGAAGTAGTAG
<i>Apoa4</i>	Forward	GACCTACGTGACCGCATGAT
	Reverse	TCTGCATGCGCTGGATGTAT
<i>Apom</i>	Forward	GTGGACATAACCGATTGACTGAAG
	Reverse	GTGGTGACCGATTGTAGAGGA
<i>Arg1</i>	Forward	CTCCAAGCCAAAGTCCTTAGAG
	Reverse	AGGAGCTGTCATTAGGGACATC
<i>Arg2</i>	Forward	TCCTCCACGGGCAAATCC
	Reverse	GCTGGACCATATTCCACTCCTA
<i>B2m</i>	Forward	GAGATGTCAGATATGTCCTTCAGCA
	Reverse	TCGATCCCAGTAGACGGTCTT
<i>C1qa</i>	Forward	AGGACTGAAGGGCGTGAAAG
	Reverse	CGTGTGGTTCTGGTATGGACTC
<i>C1qb</i>	Forward	CACACCTGTTACTGCTGCTTCTA
	Reverse	CCTTTCTCTCCAAACTCACCAAG
<i>C1qc</i>	Forward	GCGATGAGGTGTGGCTATCA
	Reverse	GGAAGAGGTCTGAGTGAGGATG
<i>C3</i>	Forward	CCAGCTCCCCATTAGCTCTG
	Reverse	GCACTTGCCTCTTTAGGAAGTC
<i>Cd74</i>	Forward	CTCCGAAATCTGCCAAACC
	Reverse	ATCTTCCAGTTCACGCCATC

Table S2. Cont.

Gene Symbol		Primer Sequences
<i>Ceacam11</i>	Forward	ACTCAGAATGACACAGGGCTTTA
	Reverse	GGAAGTAATGGAAGAATCGCAC
<i>Cfb</i>	Forward	GTCTGATGAGAGGAGTAGCGATG
	Reverse	CATGCTATACACAGCCTGGAGA
<i>Cfd</i>	Forward	CATGCTCGGCCCTACATGG
	Reverse	CACAGAGTCGTCATCCGTCAC
<i>Ctsk</i>	Forward	TTGTGACCGTGATAATGTGAACC
	Reverse	TCCGAGCCAAGAGAGCATATC
<i>Cxcl15</i>	Forward	TCCTGCTGGCTGTCCTTAAC
	Reverse	ACTGCTATCACTTCCTTTCTGTTG
<i>Cyp26a1</i>	Forward	CTCTCCAACCTGCACGATTC
	Reverse	TGCTCCAGACAACCTGCTGAC
<i>Ctss</i>	Forward	CAAGTGGGCATGAACGATATG
	Reverse	GTGTCAGGCAATGTCCGATTA
<i>Cybb</i>	Forward	ATTGTGATAATGCCACCAGTCTG
	Reverse	CCAGCCAGTAAGGTAGATATTGTAGC
<i>Degs2</i>	Forward	CTCTTGGCGATGGCTGCTAT
	Reverse	GAAGGATGTAGCGTAAGGTAGGC
<i>F10</i>	Forward	GAGGGACACCTACGACTATGAT
	Reverse	GCCCAGTCTTTCTGAGGCA
<i>F2</i>	Forward	CCGAAAGGGCAACCTAGAGC
	Reverse	GGCCAGAACACGTCTGTG
<i>Fcgr4</i>	Forward	CGAGGACAATTCTATCAAGTGGTT
	Reverse	ACTTAGTGGTCTGAAGCAATAGCC
<i>Fgb</i>	Forward	CAGGATGGGACCCACAGAAC
	Reverse	GTTCTCCCCCACCAGTTGAG
<i>H2-D1</i>	Forward	CTCCGTCCACTGACTCTTAC
	Reverse	GAGAACTGAGGGCTCTGGATG
<i>Il10</i>	Forward	GAAGACAATAACTGCACCCACTT
	Reverse	GCAACCCAAGTAACCCTTAAAGT
<i>Itgam</i>	Forward	GCTTCAGTGCTTCCATTACCTC
	Reverse	GAATCCACTCTGGTTGTGTTGAT
<i>Igj</i>	Forward	ACGACGAAGCGACCATTCT
	Reverse	GGGAGGTGGGATCAGAGATATT
<i>Il1r2</i>	Forward	GCCAGGAATACAACATCACTAGG
	Reverse	GGGTAAGCAGCCGAGATAAAC
<i>Klk7</i>	Forward	GTGCTGGCATTCTGACTCTAA
	Reverse	AGACTTGAGTGTAGACGCCTGG
<i>Ltf</i>	Forward	GGAGCCTTGAGGTGTCTGAGA
	Reverse	AGGTGGCACTCCTTGTATTCTG
<i>Masp1</i>	Forward	CTTCTGTGGGGTAGCCTTTT
	Reverse	TGAGCTGTGTAGGGTTGGTTC
<i>Mbl2</i>	Forward	TGACAGTGGTTTATGCAGAGAC
	Reverse	CGTCACGTCCATCTTTGCC
<i>Mgp</i>	Forward	CTGTGCTACGAATCTCACGAAAG
	Reverse	CTTGTTCGTTCTCTGGACTC

Table S2. Cont.

Gene Symbol	Primer Sequences
<i>Mpeg1</i>	Forward GACAACCAGAATAGCCAGAACAC
	Reverse TCTAAGGTGATGCCTGGATAGAAG
<i>NOS2</i>	Forward GTTCTCAGCCCAACAATACAAGA
	Reverse GTGGACGGGTCGATGTCAC
<i>Plin1</i>	Forward TCTCGACACACCATGCAAAC
	Reverse TCTGGTCGTCATGGCTCTC
<i>Plg</i>	Forward ATAGGCACAACAGGACACCAGA
	Reverse GGA CT CGCAGGATGGAATCT
<i>Rbp4</i>	Forward CGAGTCCGTCTTCTGAGCAA
	Reverse GCAGAGCGAAGGTGTCGTAG
<i>Retnla</i>	Forward CGTGGAGAATAAGGTCAAGGAAC
	Reverse ACGAGTAAGCACAGGCAGTTG
<i>Retn</i>	Forward TTCAACTCCCTGTTTCAAATG
	Reverse GGCTGCTGTCCAGTCTATCCT
<i>Ren1</i>	Forward GACTCCTGGCAGATCACGAT
	Reverse ACCTGGCTACAGTTCACAACATATT
<i>Sla</i>	Forward GGCTGATGGTCTATGCTGTGT
	Reverse TCTGCTCCCTCTGAACCTTCT
<i>Smpd1</i>	Forward ATCCCTCCAGGACATTGTCTTAA
	Reverse GCTAGTGGTCCGGCTCAGAGTT
<i>Sprr2i</i>	Forward GAAGAAGAGGAACTCCATCTCACAT
	Reverse CAGCAGGATTATCATCTCAGGTTAA
<i>Tacstd2</i>	Forward GCTGATGCCGCCTACTACTT
	Reverse CTACCGCTACCGAGACGACA
<i>Tlr1</i>	Forward TTGCTGGTGTTAGGAGATGCTTAT
	Reverse CTGACGGACACATCCAGAAGA
<i>Ttr</i>	Forward CGTACTGGAAGACA CT TGGCAT
	Reverse GCCGTGGTGTGTAGGAGTAT
<i>Ubb</i>	Forward AGTGACGAGAGGCTTTGTCC
	Reverse CGAAGATCTGCATTTTGACCTGT

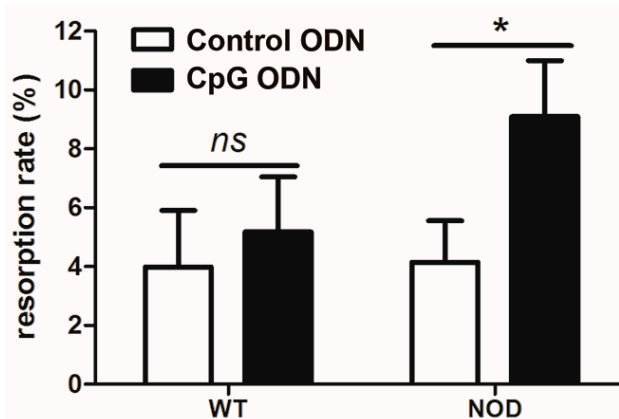


Figure S1. CpG ODN-induced embryo-resorption in NOD mice. CpG ODN in 200 μ L PBS at 25 μ g/dam was injected intraperitoneally on Embryonic Day 6.5 (E6.5). Control ODN treatment was performed at the same time and dose. Data represent mean of the biological replicates \pm SEM ($n = 7$ in control ODN-treated WT mice, $n = 7$ in CpG ODN-treated WT mice, $n = 16$ in control ODN-treated NOD mice, and $n = 14$ in CpG ODN-treated NOD mice), Nonparametric test (Mann-Whitney test), *ns*, not significant; * $p < 0.05$.

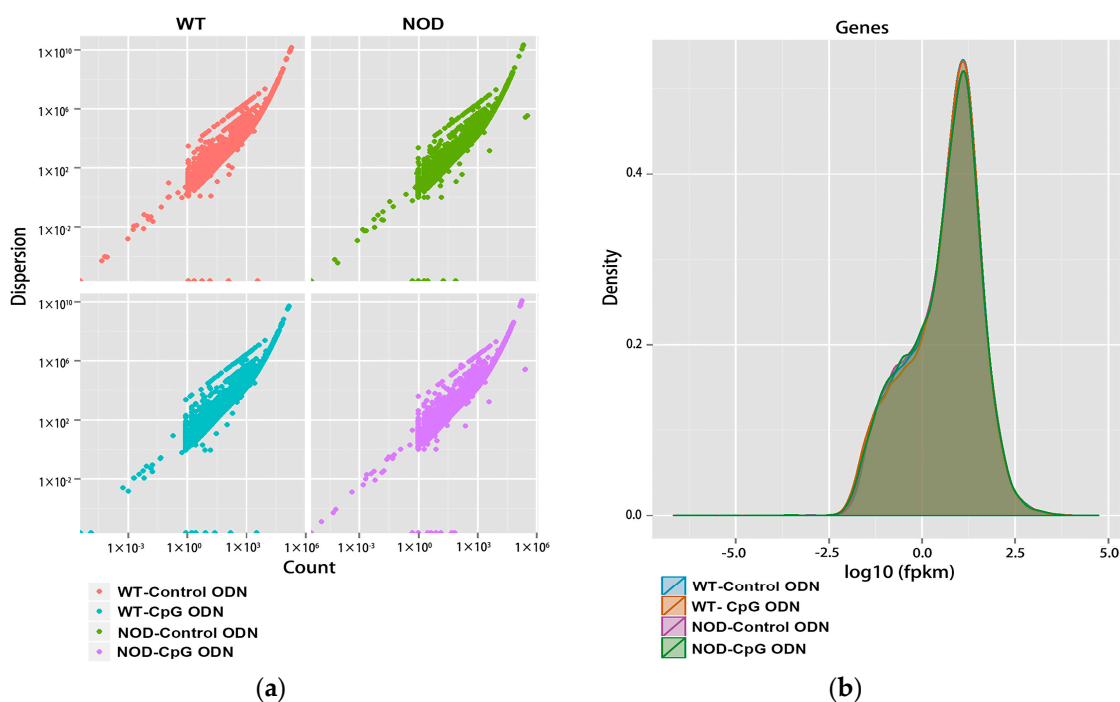


Figure S2. Expression pattern of genes from all samples. (a) Count vs. dispersion plot by condition for all genes; (b) Density plot of individual conditions.

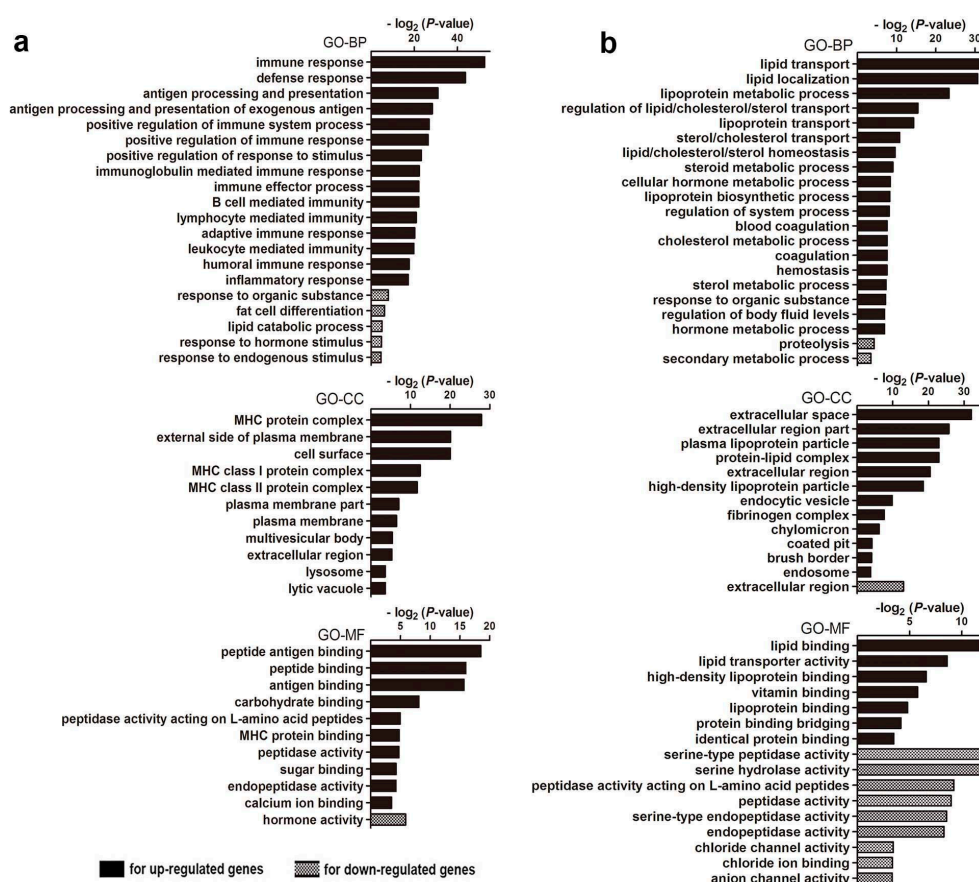


Figure S3. Gene ontology (GO) analysis of DEGs in WT and NOD mice treated with control ODN or CpG ODN. Enriched gene ontology (GO) terms with significant *p*-values. (a) are based on the DEGs between CpG ODN and control ODN-treated WT mice; (b) are based on the DEGs between CpG ODN and control ODN-treated NOD mice. GO-BP: biological processes; GO-CC: cellular components; GO-MF: molecular functions.

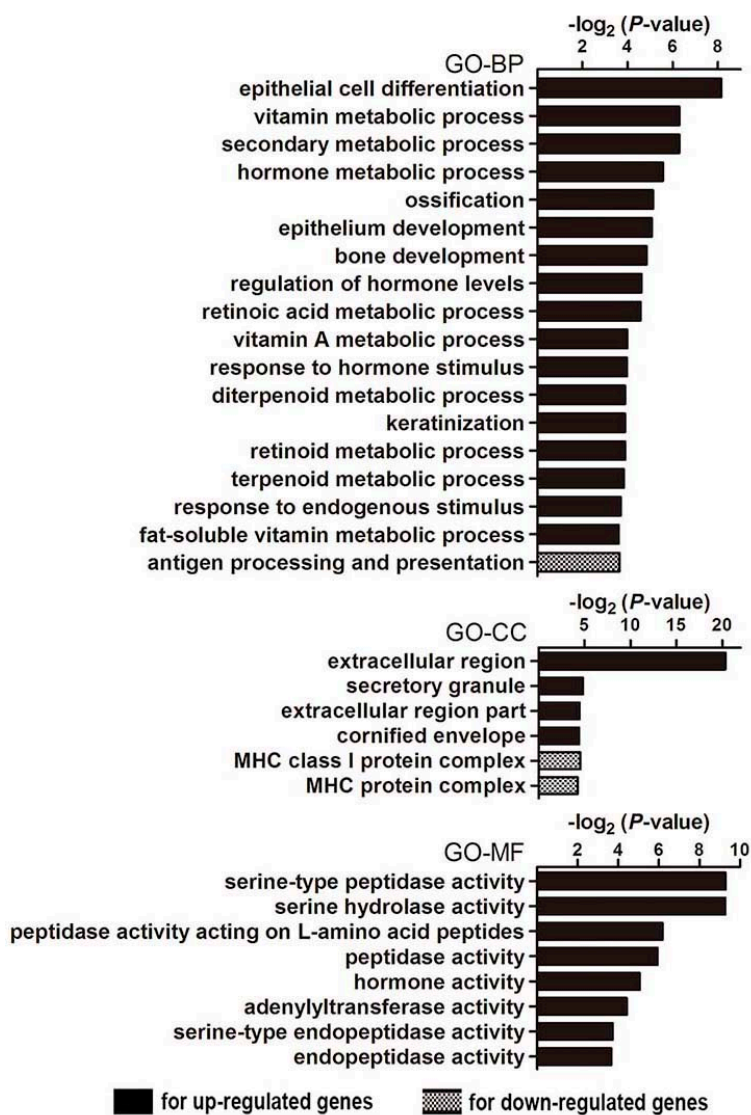


Figure S4. GO analysis of DEGs between WT and NOD mice treated with CpG ODN. Enriched GO terms with significant *p*-values. GO-BP: biological processes; GO-CC: cellular components; GO-MF: molecular functions.

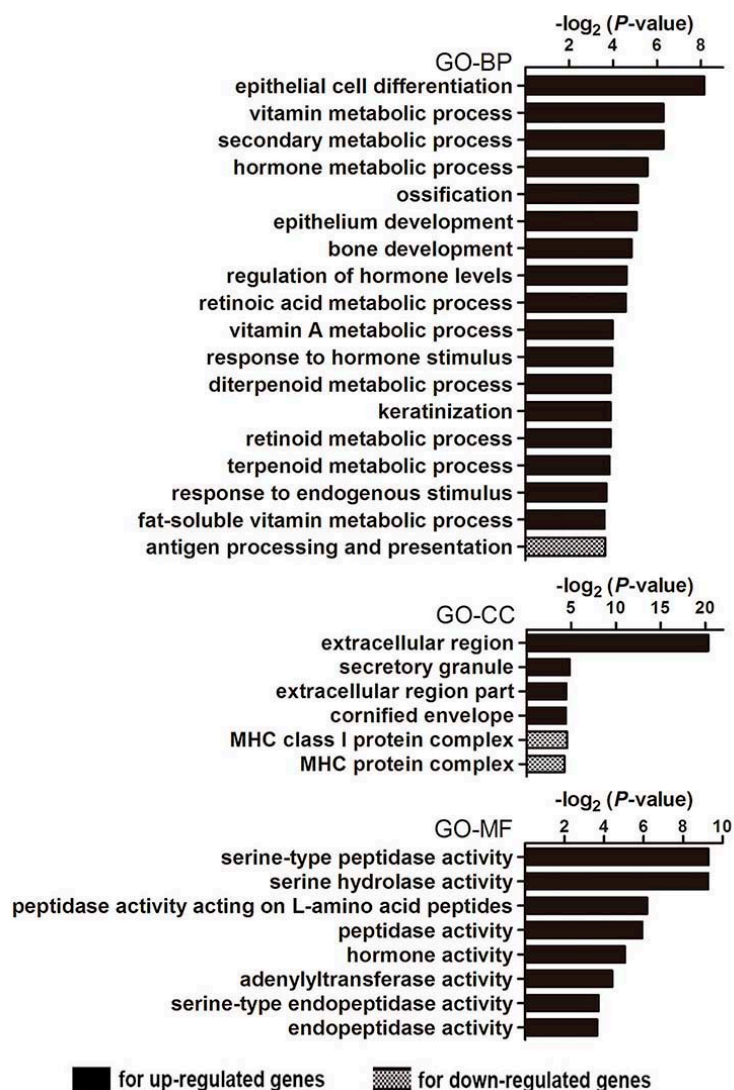


Figure S5. GO analysis of DEGs between WT and NOD mice treated with control ODN. Enriched GO terms with significant p -values. GO-BP: biological processes; GO-CC: cellular components; GO-MF: molecular functions.

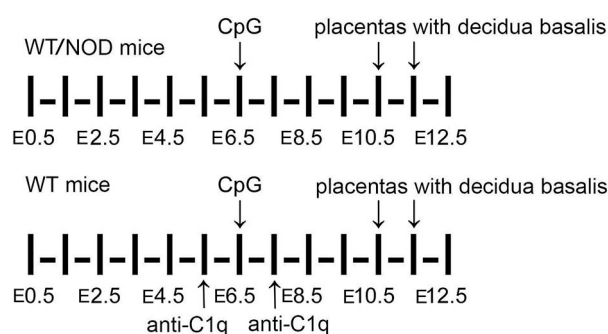


Figure S6. Methods for CpG-stimulation and C1q-blocking. To simulated intrauterine infection, CpG ODN was injected intraperitoneally into pregnant mice at a dose of 25 μg on Embryonic Day 6.5 (E6.5). Control mice were injected with control ODN at the same dose and time. For blocking the function of C1q, neutralizing anti-C1q antibody was injected intraperitoneally into pregnant WT mice at a dose of 50 μg on E5.5 and E7.5 with CpG ODN at a dose of 25 μg on E6.5. On E10.5 or E11.5, placentas with decidua basalis were collected separately and immediately frozen in liquid nitrogen.