

Supplementary material - Cannabis significantly alters DNA methylation of the human ovarian follicle in a concentration-dependent manner

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Supplementary Table SIV – All differentially methylated regions (DMR) between cases and controls

Chromosome	Location	DMR State	Size (bp)	Number of CpGs	Significant CpGs	#CpGs	#CpGs	#CpGs	q value	Mean methylation difference	Max methylation difference	Genomic Feature	annotated Genomic Annotation
chr10	32792786	Hypo	115	4	4 (Hypo)	0	0	4	3.85E-30	-52.45	-56.30	YARS2	exon and promoter
chr12	1097887	Hypo	803	3	3 (Hypo)	0	0	3	6.21E-38	-50.42	-53.77	IDI1	intron
chr10	12884599	Hypo	865	3	3 (Hypo)	3	0	0	2.86E-10	-44.36	-56.83	LOC100128276	intergenic
chrX	12647251	Hypo	22	4	4 (Hypo)	0	0	4	2.48E-16	-41.81	-44.40	DCAF12L1	enhancer
chr19	3981605	Hypo	71	4	4 (Hypo)	0	0	4	5.95E-12	-40.27	-49.37	LINC01975	exon
chr9	6621466	Hypo	56	4	4 (Hypo)	0	0	4	4.47E-11	-39.66	-42.92	MIR8055	1to5kb
chr10	52596457	Hypo	48	3	3 (Hypo)	0	0	3	1.26E-23	-39.64	-43.79	ZNF137P	exon
chr9	13483853	Hypo	571	3	3 (Hypo)	2	1	0	2.16E-22	-38.96	-43.99	MIR3689C	intron
chr11	63074755	Hypo	922	4	4 (Hypo)	0	0	4	1.87E-12	-37.56	-42.54	HAR1A	intron
chr9	80450825	Hypo	60	3	3 (Hypo)	0	0	3	4.37E-20	-36.08	-37.80	TSPAN14	1to5kb
chr18	7148150	Hypo	50	4	4 (Hypo)	0	0	4	2.28E-11	-35.39	-41.17	ASGR1	intergenic
chr20	4118125	Hypo	183	5	5 (Hypo)	5	0	0	1.02E-06	-34.88	-42.00	OTOP1	exon
chr20	79890249	Hypo	88	4	4 (Hypo)	0	0	4	1.99E-06	-34.34	-43.17	LINC01978	intergenic
chr9	56987407	Hypo	613	3	3 (Hypo)	0	0	3	8.94E-17	-33.66	-35.22	BMP7-AS1	intergenic
chr4	15812619	Hypo	175	3	3 (Hypo)	3	0	0	4.25E-07	-33.59	-43.18	PTPRN2-AS1	intron
chrX	11306167	Hypo	71	8	8 (Hypo)	0	8	0	1.26E-18	-33.09	-41.29	ZFP37	1to5kb
chr4	30289803	Hypo	995	25	25 (Hypo)	0	0	25	3.31E-07	-32.11	-45.11	LINC01597	promoter
chr2	30752011	Hypo	75	4	4 (Hypo)	0	0	4	3.03E-08	-31.38	-36.19	LINC02644	intergenic
chr5	30289744	Hypo	45	6	6 (Hypo)	0	0	6	3.11E-05	-31.26	-38.08	LINC01597	promoter
chr2	16785004	Hypo	3	3	3 (Hypo)	0	0	3	4.56E-10	-31.13	-37.30	MYO10	intron
chr8	30284141	Hypo	115	5	5 (Hypo)	0	0	5	4.38E-06	-30.69	-33.78	LINC01597	enhancer
chr4	30289175	Hypo	336	17	17 (Hypo)	0	0	17	1.06E-09	-30.45	-35.45	LINC01597	promoter

chr6	30585552	Hypo	933	9	9 (Hypo)	9	0	0	3.37E-04	-30.34	-39.52	<i>MLLT10P1</i>	intergenic
chr9	46185010	Hypo	304	9	9 (Hypo)	0	0	9	7.53E-09	-30.21	-33.88	<i>SPATC1L</i>	promoter
chr20	422534	Hypo	59	3	3 (Hypo)	3	0	0	4.77E-07	-30.02	-32.14	<i>C2CD4C</i>	1to5kb
chr4	13003720	Hypo	936	6	6 (Hypo)	5	1	0	4.38E-15	-29.68	-36.44	<i>FAR2P1</i>	1to5kb
chr17	2399815	Hypo	478	5	5 (Hypo)	4	1	0	1.81E-16	-29.51	-34.68	<i>ZFYVE28</i>	intron
chr12	30723760	Hypo	656	11	11 (Hypo)	11	0	0	4.77E-07	-29.23	-32.70	<i>MLLT10P1</i>	exon and promoter
chr9	30585061	Hypo	391	9	9 (Hypo)	0	9	0	3.34E-04	-28.85	-34.35	<i>MLLT10P1</i>	intergenic
chr17	11306204	Hypo	412	12	12 (Hypo)	5	7	0	1.48E-11	-28.54	-34.96	<i>ZFP37</i>	1to5kb
chr8	30488847	Hypo	852	9	9 (Hypo)	9	0	0	1.31E-04	-28.49	-31.63	<i>MLLT10P1</i>	1to5kb
chr4	94862775	Hypo	21	3	3 (Hypo)	0	0	3	1.66E-07	-28.45	-29.53	<i>LINC00879</i>	intergenic
chr8	11308935	Hypo	373	12	12 (Hypo)	7	5	0	1.68E-16	-28.25	-33.86	<i>FAM225B</i>	1to5kb
chr12	11305999	Hypo	199	9	9 (Hypo)	9	0	9	1.84E-19	-28.01	-33.12	<i>ZFP37</i>	1to5kb
chr5	11308450	Hypo	155	6	6 (Hypo)	3	3	0	1.13E-06	-27.71	-28.66	<i>FAM225B</i>	1to5kb
chr20	32300477	Hypo	116	3	3 (Hypo)	0	0	3	5.87E-07	-27.51	-30.46	<i>MRAP</i>	intron
chr10	1417291	Hypo	109	3	3 (Hypo)	2	1	0	2.25E-10	-27.07	-28.85	<i>LOC286083</i>	intron
chr21	30316528	Hypo	568	6	6 (Hypo)	6	0	0	1.60E-06	-26.78	-29.26	<i>LINC01597</i>	intergenic
chr3	94871748	Hypo	140	10	10 (Hypo)	10	0	0	8.90E-04	-26.71	-28.84	<i>TEKT4</i>	exon
chr6	112336	Hypo	64	4	4 (Hypo)	4	0	0	1.08E-17	-26.71	-28.20	<i>MIR8078</i>	promoter
chr12	63732464	Hypo	53	6	3 (Hypo); 1 (Hyper)	0	0	6	1.05E-05	-12.65	-27.50	<i>LIME1</i>	promoter
chr10	4108547	Hypo	465	6	3 (Hyper); 3 (Hypo)	5	1	0	3.68E-06	-0.14	-43.17	<i>LOC105373394</i>	intergenic
chr4	19459873	Hypo	60	5	3 (Hyper); 2 (Hypo)	0	0	5	2.76E-08	10.56	40.93	<i>TMEM44-AS1</i>	enhancer
chr11	34688342	Hypo	130	7	7 (Hyper)	0	0	7	7.17E-06	26.49	27.52	<i>CLIC6</i>	intron
chr3	1050997	Hypo	35	3	3 (Hyper)	3	0	0	3.14E-13	26.54	27.11	<i>FGFRL1</i>	intergenic
chr13	35657904	Hypo	12	3	3 (Hyper)	3	0	0	1.26E-12	28.11	29.91	<i>RMRP</i>	promoter
chr20	43994666	Hypo	22	3	3 (Hyper)	0	3	0	1.37E-04	28.15	31.54	<i>PARVB</i>	promoter

chr2	50085800	Hyperr	68	8	8 (Hyper)	0	0	8	3.59E-05	28.25	32.85	<i>MLC1</i>	promoter
chr7	28990823	Hyperr	65	3	3 (Hyper)	0	0	3	4.18E-07	28.25	32.07	<i>TOGARAM2</i>	intron
chr19	38729914	Hyperr	602	3	3 (Hyper)	1	2	0	5.97E-09	28.34	29.41	<i>ACTR3BP5</i>	intergenic
chr20	23214288	Hyperr	74	3	3 (Hyper)	0	0	3	4.93E-06	28.47	29.89	<i>DISC1-IT1</i>	intergenic
chr21	11588861	Hyperr	21	5	5 (Hyper)	5	0	0	3.20E-26	28.49	36.23	<i>DANT1</i>	intron
chr4	95023726	Hyperr	9	3	3 (Hyper)	0	0	3	2.86E-07	28.78	29.46	<i>LINC00557</i>	intron
chr9	400162	Hyperr	201	8	8 (Hyper)	8	0	0	5.64E-05	29.22	33.26	<i>PKP3</i>	1to5kb
chr20	12888135	Hyperr	39	3	3 (Hyper)	0	0	3	4.44E-06	29.55	33.18	<i>GPRC5A</i>	1to5kb
chr17	52316930	Hyperr	834	3	3 (Hyper)	0	0	3	2.95E-08	30.36	36.38	<i>KRT83</i>	cds
chr2	13446600	Hyperr	771	3	3 (Hyper)	3	0	0	2.92E-13	31.49	34.94	<i>ZFAT-AS1</i>	interg4enic
chr20	13328604	Hyperr	446	3	3 (Hyper)	0	3	0	8.07E-29	31.55	35.37	<i>ADAM8</i>	1to5kb
chr12	46161087	Hyperr	655	45	45 (Hyper)	45	0	0	1.18E-04	31.81	42.64	<i>FTCD</i>	exon
chr1	521699	Hyperr	357	3	3 (Hyper)	0	0	3	5.34E-16	31.90	33.03	<i>PIGG</i>	promoter
chr21	536425	Hyperr	926	3	3 (Hyper)	0	3	0	2.47E-12	33.85	44.09	<i>PIGG</i>	exon
chr20	52995723	Hyperr	102	12	12 (Hyper)	0	12	0	6.59E-13	34.20	39.77	<i>RN7SK</i>	promoter
chr20	18101252	Hyperr	128	3	3 (Hyper)	0	0	3	7.84E-08	34.42	38.19	<i>SAAL1</i>	intron
chr22	71587803	Hyperr	718	3	3 (Hyper)	0	0	3	3.19E-19	34.87	37.96	<i>KRTAP5-11</i>	1to5kb
chr11	534692	Hyperr	17	4	4 (Hyper)	0	4	0	5.68E-11	35.29	39.71	<i>PIGG</i>	1to5kb
chr21	13143495	Hyperr	49	4	4 (Hyper)	0	4	0	1.38E-11	35.30	36.11	<i>LINC02370</i>	1to5kb
chr20	61389938	Hyperr	583	3	3 (Hyper)	1	2	0	3.83E-17	35.53	37.18	<i>CDH4</i>	intron
chr22	10580450	Hyperr	40	3	3 (Hyper)	0	0	3	2.78E-08	36.48	43.44	<i>PRDM1</i>	intron
chr20	2303797	Hyperr	211	3	3 (Hyper)	3	0	0	7.53E-08	37.91	47.45	<i>MXD4</i>	intron
chr20	24166623	Hyperr	316	3	3 (Hyper)	0	3	0	3.57E-14	38.93	44.52	<i>DTYMK</i>	promoter
chr2	16018636	Hyperr	133	3	3 (Hyper)	0	0	3	5.50E-15	40.74	46.77	<i>FABP6</i>	promoter

Supplementary Table SV – AmpliSeq RNASep targets and differential expression between cases and controls

Gene symbol	Fold change (Case vs. Control)	FDR step up (Case vs. Control)	DNA methylation status in this study (hypo/hyper)	DNA methylation correlated with THC in this study (yes/no)	Previously reported to be DE (yes/no)	Top 30 DMR in this study (yes/no)	Major Pathway/Family
<i>ABR</i>	-1.31	2.32E-01	hyper	yes	yes	no	GPCR Signaling
<i>DCAF12L1</i>	1.33	4.97E-01		yes	no	yes	Cell structure
<i>FTCD</i>	-10.2	1.66E-04		yes	no	no	Folate pool regulation
<i>KCNMA1</i>	1.08	8.73E-01		yes	yes	no	Potassium channel
<i>KRT83</i>	not detected	-	hypo	yes	no	no	Cell structure
<i>MLC1</i>	-3.01	6.12E-01		yes	no	no	Cell structure
<i>MRAP</i>	not detected	-		yes	no	no	Steroid synthesis
<i>PIGG</i>	-1.25	1.21E-01		yes	no	yes	Transcription regulation
<i>SMYD3</i>	1.31	2.15E-01		yes	yes	no	Chromatin regulation
<i>SPATC1L</i>	-2.25	1.04E-01		yes	no	no	Cell structure
<i>TEKT4</i>	-1.46	8.92E-01		yes	no	no	Cell structure
<i>ZFP37</i>	-1.81	3.59E-02		yes	no	yes	Zinc finger protein
<i>TP73</i>	-2.17	7.81E-01		no	yes	no	Apoptosis
<i>ETV2</i>	1.57	4.64E-01		no	yes	no	Transcription regulation
<i>KLHL30</i>	not detected	-		no	yes	no	Post-translational modification
<i>PRKCZ</i>	-1.12	6.67E-01		no	yes	no	Apoptosis
<i>MGAT3</i>	-14.9	7.55E-04	hyper	no	yes	no	Post-translational modification
<i>LSP1</i>	2.36	3.10E-02	hypo	no	yes	no	Actin binding
<i>LZTS2</i>	-1.47	2.50E-02		no	yes	no	Apoptosis
<i>RFC1</i>	1.36	6.16E-02		no	yes	no	Transcription regulation
<i>ABHD8</i>	1.15	4.00E-01		no	yes	no	ECM remodeling
<i>GRXCR2</i>	not detected	-		no	yes	no	Post-translational modification

<i>TOX2</i>	1.23	2.61E-01		no	yes	no	Transcription regulation
<i>MFAP2</i>	-4.63	5.10E-02		no	yes	no	ECM remodeling
<i>EPHA2</i>	1.06	8.93E-01		no	yes	no	Apoptosis
<i>MPPED1</i>	not detected	-		no	yes	no	ECM remodeling
<i>AFF3</i>	1.06	8.98E-01		no	yes	no	Transcription regulation
<i>IDI1</i>	1.56	1.17E-01		no	no	yes	Steroid synthesis
<i>YARS2</i>	-1.17	3.60E-01		no	no	yes	tRNA synthesis
<i>ADAM8</i>	-1.16	6.41E-01		no	no	yes	ECM remodeling
<i>ZNF137P</i>	not detected	-		no	no	yes	Zinc finger protein
<i>TSPAN14</i>	1.2	3.63E-01		no	no	yes	Trafficking
<i>KRTAP5-11</i>	-80.2	3.88E-04	hyper	no	no	yes	Keratin formation
<i>CDH4</i>	not detected	-		no	no	yes	Cell adhesion
<i>ZFYVE28</i>	-1.8	9.70E-02		no	no	yes	Zinc finger protein
<i>FABP6</i>	not detected	-		no	no	yes	Fatty acid synthesis
<i>DTYMK</i>	1.17	4.51E-01		no	no	yes	Purine metabolism
<i>FGFRL1</i>	-1.28	2.30E-01		no	no	yes	Cell proliferation
<i>RMRP</i>	-3.31	1.58E-02	hyper	no	no	yes	Mitochondrial RNA processing
<i>HAR1A</i>	not detected	-		no	no	yes	non-coding RNA
<i>ASGR1</i>	-1.24	7.88E-01		no	no	yes	Endocytosis
<i>CNR1</i>	1.04	7.39E-01		no	no	no	Cannabis receptor/metabolism
<i>CNR2</i>	1.36	7.53E-01		no	no	no	Cannabis receptor/metabolism
<i>GPR55</i>	1.61	8.31E-01		no	no	no	Cannabis receptor/metabolism
<i>FAAH</i>	-1.39	3.85E-01		no	no	no	Cannabis receptor/metabolism
<i>DNMT1</i>	-1.46	8.99E-02		no	no	no	DNA methyltransferase
<i>DNMT3A</i>	-1.11	5.46E-01		no	no	no	DNA methyltransferase
<i>DNMT3B</i>	-1.24	8.66E-01		no	no	no	DNA methyltransferase
<i>ZFP36</i>	1.54	4.85E-02		no	yes	no	Zinc finger protein
<i>ZFP57</i>	14.1	3.14E-02	hypo	no	no	no	Zinc finger protein

ZFP64	-1.04	8.44E-01		no	yes	no	Zinc finger protein
ZNF235	-3.09	1.88E-03	not DM	no	yes	no	Zinc finger protein
ZNF295	not detected	-		no	yes	no	Zinc finger protein
ZNF423	-2.85	1.00E-01		no	yes	no	Zinc finger protein
ZNF14	-1.46	6.35E-02		no	yes	no	Zinc finger protein
ZNF592	-1.06	6.97E-01		no	yes	no	Zinc finger protein
ZNF644	1.45	8.91E-02		no	yes	no	Zinc finger protein
ZSCAN29	not detected	-		no	yes	no	Zinc finger protein
GNAI1	1.14	4.48E-01		no	no	no	GPCR Signaling
GNAI2	1.34	4.95E-02		no	no	no	GPCR Signaling
GNAI3	1.12	3.96E-01		no	no	no	GPCR Signaling
GNAO1	1.17	8.70E-01		no	no	no	GPCR Signaling
GNA12	1.01	9.35E-01		no	no	no	GPCR Signaling
GNA13	1.06	6.27E-01		no	no	no	GPCR Signaling
ADCY1	1.36	8.74E-01		no	no	no	GPCR Signaling
ADCY2	1.04	9.54E-01		no	no	no	GPCR Signaling
ADCY3	-1.3	4.85E-01		no	no	no	GPCR Signaling
ADCY4	-2.26	4.30E-01		no	no	no	GPCR Signaling
ADCY5	2.27	4.78E-01		no	no	no	GPCR Signaling
ADCY6	-1.09	7.21E-01		no	no	no	GPCR Signaling
ADCY7	-1	1.00E+00		no	no	no	GPCR Signaling
ADCY8	not detected	-		no	no	no	GPCR Signaling
ADCY9	-1.01	9.52E-01		no	no	no	GPCR Signaling
MAPK1	1.3	1.77E-02		no	no	no	ERK1/2 Signaling
MAPK3	1.27	7.79E-02		no	no	no	ERK1/2 Signaling
MAPK4	1.16	8.02E-01		no	no	no	ERK1/2 Signaling
MAPK8	1.52	2.32E-01		no	no	no	ERK1/2 Signaling
MAPK11	-2.42	7.01E-02		no	no	no	ERK1/2 Signaling
AKT1	1	9.84E-01		no	no	no	AKT Signaling
AKT2	1.15	2.36E-01		no	no	no	AKT Signaling
AKT3	1.02	8.86E-01		no	no	no	AKT Signaling
PRKACA	1.32	6.01E-02		no	no	no	PKA Signaling
PRKAR1A	1.36	2.61E-02		no	no	no	PKA Signaling
PRKACB	1.16	3.75E-01		no	no	no	PKA Signaling

<i>PRKAR2A</i>	1.16	2.17E-01		no	no	no	PKA Signaling
<i>PRKAR2B</i>	1.14	2.50E-01		no	no	no	PKA Signaling
<i>PRKAG2</i>	1.08	8.62E-01		no	no	no	PKA Signaling
<i>PRKACG</i>	-16.5	1.15E-02	not DM	no	no	no	PKA Signaling
<i>SLC30A1</i>	1.02	9.23E-01		no	no	no	Zinc homeostasis
<i>SLC39A4</i>	1.51	5.90E-02		no	no	no	Zinc homeostasis
<i>SLC39A10</i>	1.08	8.96E-01		no	no	no	Zinc homeostasis
<i>MT2A</i>	not detected	-		no	no	no	Zinc homeostasis
<i>LHCGR</i>	-1.03	9.21E-01		no	no	no	Cell markers
<i>FSHR</i>	-1.79	3.81E-01		no	no	no	Cell markers
<i>CYP19A1</i>	-2.11	3.44E-02	not DM	no	no	no	Cell markers
<i>AMH</i>	-3.1	1.75E-03	not DM	no	no	no	Cell markers
<i>PTPRC</i>	not detected	-		no	no	no	Cell markers