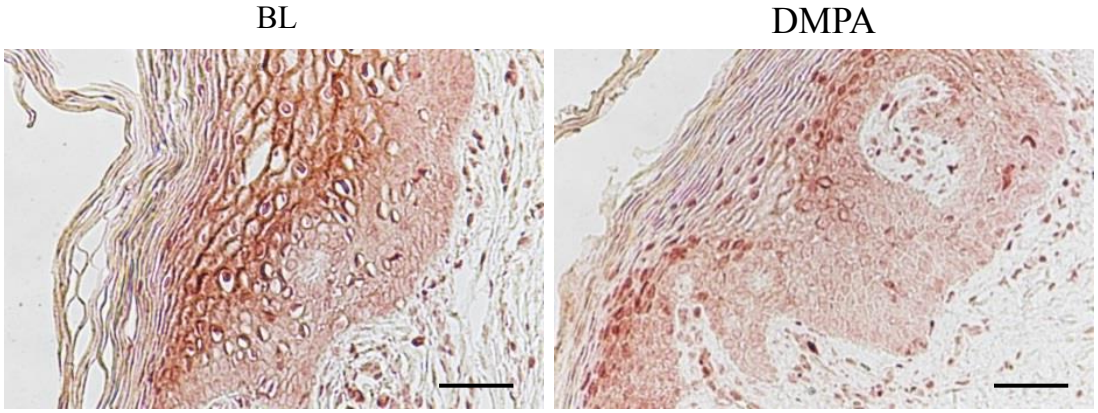
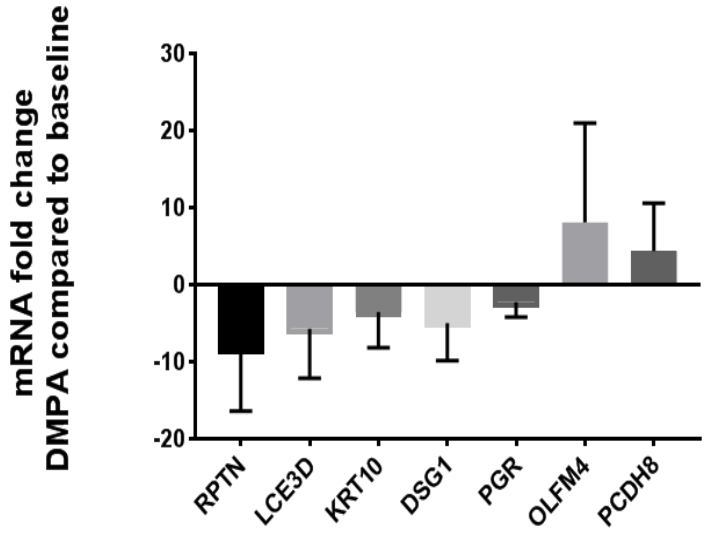
Supplemental information

Supplemental figures:



Supplemental Figure S1. Representative image of immunohistochemical detection of DMKN in the vaginal epithelium at the baseline and after use of DMPA. Bar - 100 μm

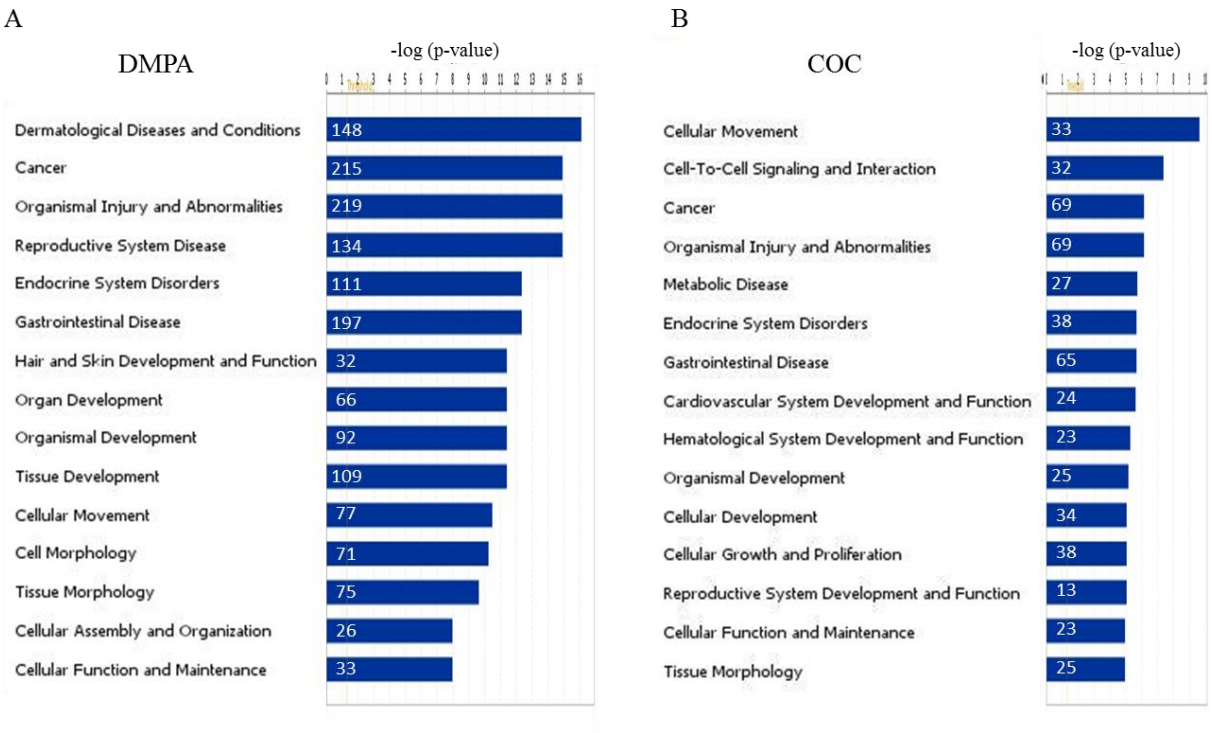


Supplemental Figure S2. Real-time qPCR validation of changes in expression of selected genes in the ectocervical epithelium of the DMPA users. Bars represent mean ±SD of fold changes in the DMPA users relative to corresponding baseline values. All genes were normalized to GAPDH. Information on primers used in PCR is given in the Supplemental Table S6.

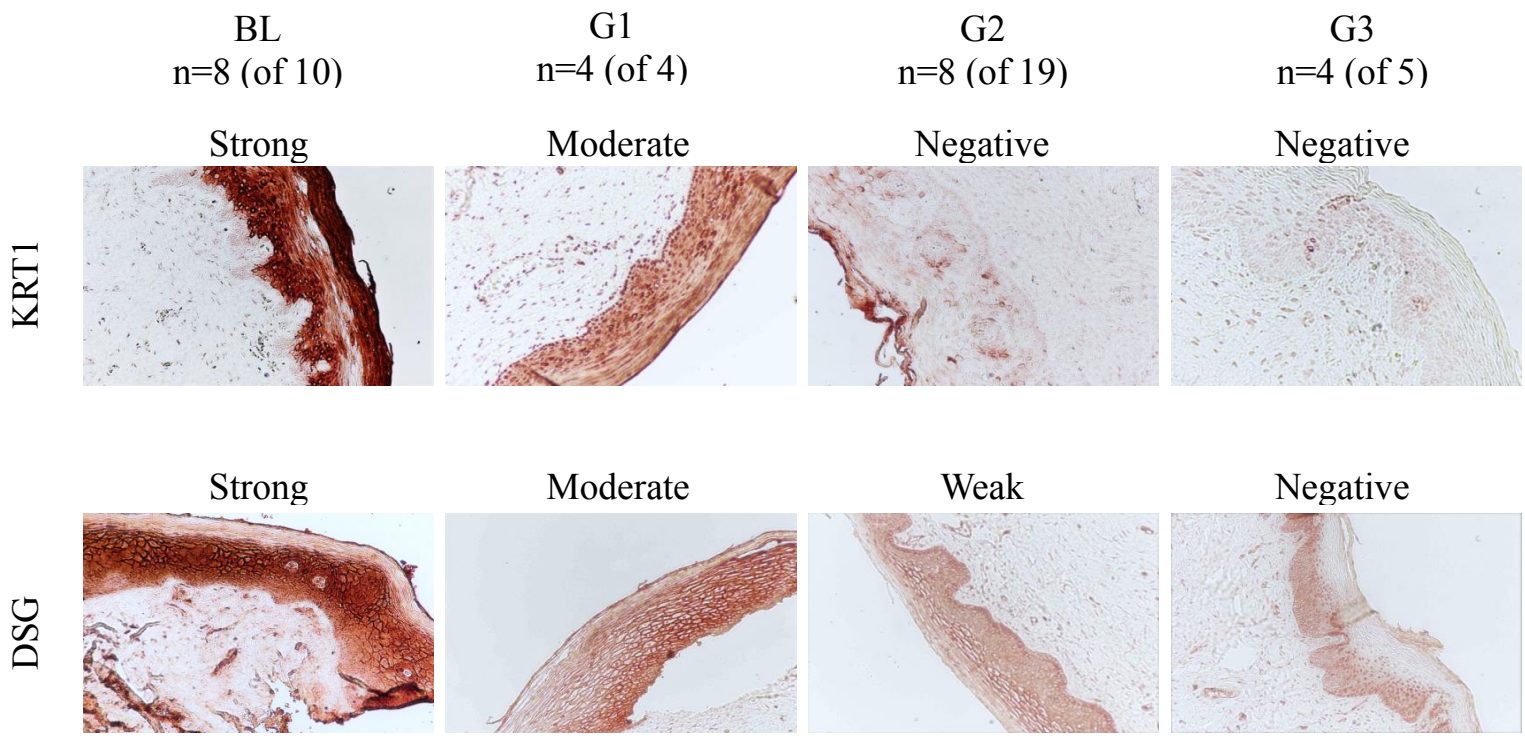
Symbol	RPTN	DMKN	ALOX 12B	DSG1	LCE3D	KRT10	TGM3	CYP26 B1	GYS2	PGR	PRSS23	WISP2	VCAN	PCDH8	RDH10	OLFM4	DUSP4	CAPN 14	GABRP	STS
RPTN		4	4	4	4	4	4	4	3	1	0	0	0	2	3	3	4	4	4	4
DMKN	4		4	4	4	4	4	4	4	1	3	1	0	2	3	3	3	4	4	4
ALOX12B	4	4		4	4	4	4	4	4	0	0	0	1	2	2	4	2	3	4	4
DSG1	4	4	4		4	4	3	4	4	0	1	0	0	1	4	3	4	4	4	4
LCE3D	4	4	4	4		3	4	3	4	0	0	0	0	2	1	2	0	4	4	4
KRT10	4	4	4	4	3		3	4	0	1	2	1	1	2	4	2	4	3	4	4
TGM3	4	4	4	3	4	3		3	4	0	0	0	0	4	1	4	2	2	3	4
CYP26B1	4	4	4	4	3	4	3		0	4	4	4	4	3	3	3	4	3	4	3
GYS2	3	4	4	4	4	0	4	0		0	0	0	0	1	0	3	0	3	0	4
PGR	1	1	0	0	0	1	0	4	0		4	4	4	4	2	2	4	1	4	0
PRSS23	0	2	0	1	0	2	0	4	0	4		4	4	3	2	1	3	1	4	0
WISP2	0	1	0	0	0	1	0	4	0	4	4		4	4	1	2	4	0	4	0
VCAN	0	0	0	0	0	1	0	4	0	4	4	4		4	0	0	2	0	4	0
PCDH8	2	2	2	1	2	2	4	3	1	4	3	4	4		1	4	4	2	4	3
RDH10	3	3	2	4	1	4	1	3	0	2	2	1	0	1		3	4	4	4	4
OLFM4	3	3	4	3	2	2	4	3	3	2	1	2	0	4	3		4	3	4	4
DUSP4	4	3	2	4	1	4	2	4	0	4	3	4	2	4	4	4		4	4	4
CAPN14	4	4	3	4	4	3	2	3	3	1	1	0	0	2	4	3	4		4	4
GABRP	4	4	4	4	2	4	3	4	0	4	4	4	4	4	4	4	4	4		4
STS	4	4	4	4	4	4	4	3	4	0	0	0	0	3	4	4	4	4	4	

4	****	p<0.0001
3	***	p<0.001
2	**	p<0.01
1	*	p<0.05
0	ns	p≥0.05

Supplemental Figure S3. Significance of Spearman r correlation between selected dysregulated genes from the ectocervix of DMPA users (related to Figure 4). Values 4, 3, 2, 1, 0 describe significance and correspond to p<0.0001, p<0.001, p<0.01, p< 0.05, and ns (p≥0.05), respectively (determined using GraphPad Prizm 7)

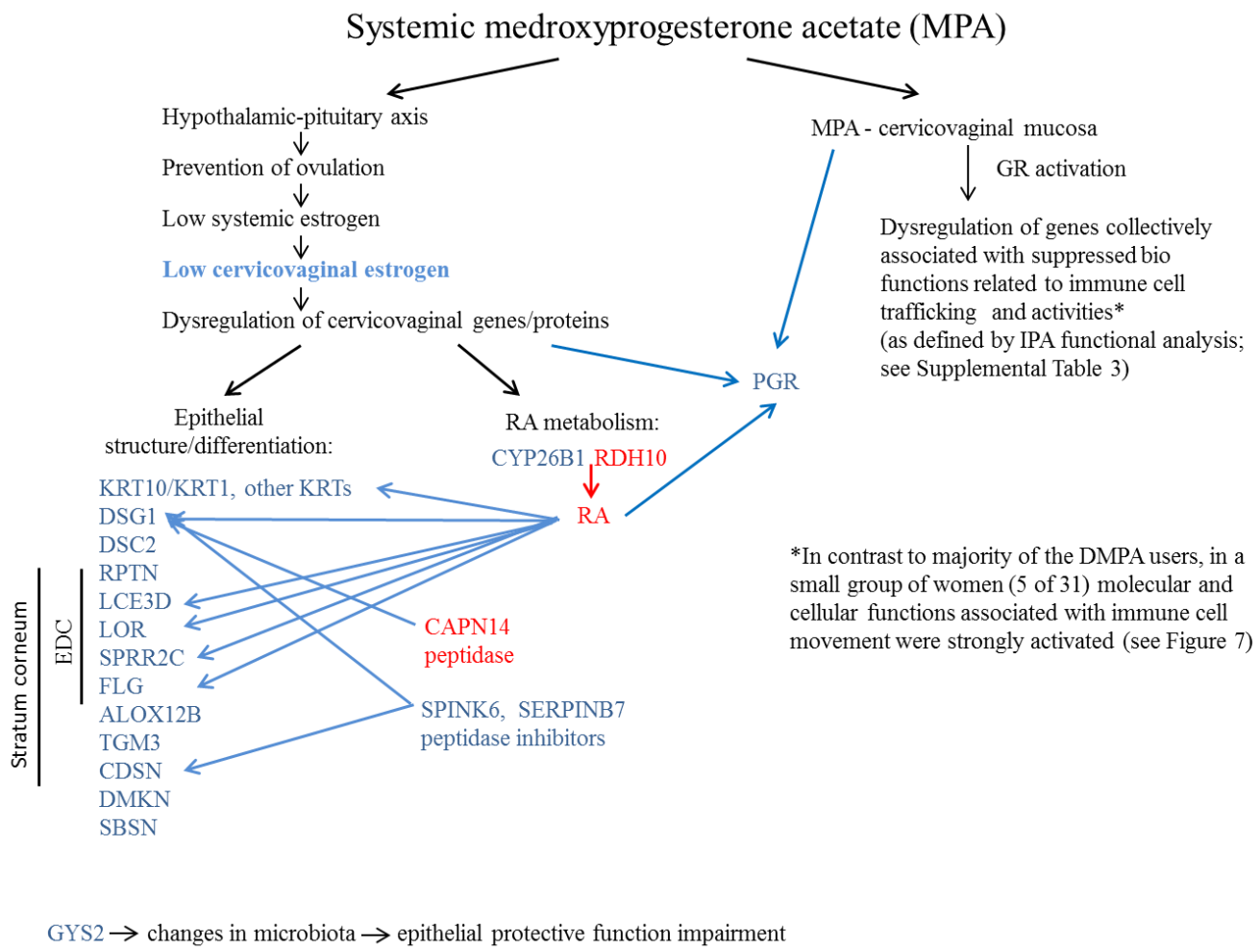


Supplemental Figure S4. IPA-determined biological functions most significantly associated with genes dysregulated in the ectocervical epithelium of the HC users. (A) DMPA, (B) COC. Numbers of dysregulated genes involved into enriched functions are shown on bars.



Supplemental Figure S5. Representative images of KRT10 and DSG1 in vaginal samples obtained at baseline (BL) and after use of DMPA in participant groups defined according to ectocervical gene expression analysis.

Immunostaining was evaluated using the following scale: strong staining (~100% of cells staining in an appropriate tissue and sub-cellular location); moderate staining (~50% staining in an appropriate tissue and sub-cellular location); weak staining (< 50% staining in an appropriate tissue and sub-cellular location); negative (< 5% staining in an appropriate tissue and sub-cellular location). Appropriate epithelial location for both proteins: suprabasal; appropriate sub-cellular location: cytoplasmic for KRT10 and membrane staining for DSG1. The staining was found to be mostly consistent within each group, except for DSG1 in G3: one sample (out of 4) displayed moderate staining.
Magnification 20x



Supplemental Figure S6. Schematic summary of cervicovaginal mucosal barrier gene/protein differentially expressed in DMPA users. Blue symbols are suppressed molecules, red symbols - upregulated molecules. Blue arrows denote inhibiting effect, red arrow – enhancing effect.

Supplemental tables:

Supplemental Table S1. Genes differentially expressed between DMPA and COC groups at baseline (BL).

Supplemental Table S2. Genes significantly (FDR adjusted P<0.05) dysregulated in the ectocervical epithelium of DMPA users (xlsx).

Supplemental Table S3. Genes significantly (FDR adjusted P<0.05) dysregulated in the ectocervical epithelium of COC users (xlsx).

Supplemental Table S4. Genes commonly deregulated by DMPA and COC.

Supplemental Table S5. Proteins encoded by the most up-regulated genes.

Supplemental Table S6. Primers used for qRT-PCR.

Supplemental Table S7. Predicted activation state of functions after use of DMPA (xlsx).

Supplemental Table S8. Predicted activation state of functions after use of COC (xlsx).

Supplemental Table S9. Dysregulated genes in top gene sets enriched in the DMPA users as defined by GO categories (xlsx).

Supplemental Table S10. Genes significantly dysregulated in the DMPA group 1 (xlsx).

Supplemental Table S11. Genes significantly dysregulated in the DMPA group 2 (xlsx).

Supplemental Table S12. Genes significantly dysregulated in the DMPA group 3 (xlsx).

Supplemental Table S13. Expression alterations of selected cervicovaginal genes from three groups of DMPA users

Supplemental Table S14. Demographic and clinical characteristics of participants in groups.

Supplementary Table S15. RNA quality.

Supplemental Table S16. Antibodies.

Supplemental tables S2, S3, S7, S8, S9, S10, S11 and S12 are presented in Excel format

Supplemental Table S1. Genes differentially expressed between DMPA and COC groups at baseline (BL)

Symbol	DMPA_BL/ COC_BL FC	Name	Parametric p-value	Entrez ID
KANSL1	-1.54	KAT8 regulatory NSL complex subunit 1	8.00E-07	284058
CLK4	-1.45	CDC-like kinase 4	4.92E-05	57396
KLF9	-1.41	Kruppel-like factor 9	7.82E-05	687
GOPC	-1.41	golgi-associated PDZ and coiled-coil motif containing	8.18E-05	57120
S100A8	1.42	S100 calcium binding protein A8	9.61E-05	6279

Supplemental Table S4. Genes commonly deregulated by DMPA and COC

Symbol	Fold change		Name	Parametric p-value		Cell movement	Cell differentiation
	DMPA	COC		DMPA	COC		
PADI1	1.69	1.82	peptidyl arginine deiminase, type I	**0.00088**	**0.00043**		
PRR5L	-1.37	-1.25	proline rich 5 like	**0.00011**	**0.00066**		
CRIM1	-1.22	-1.25	cysteine rich transmembrane BMP regulator 1 (chordin-like)	0.00912	0.00192		
PGBD5	-1.74	-1.32	piggyBac transposable element derived 5	**< 1e-07**	0.00141		
TMTC1	-1.36	-1.34	transmembrane and tetratricopeptide repeat containing 1	0.00843	**0.00045**		
PGF	-1.27	-1.34	placental growth factor	0.00765	0.00141	x	x
COL21A1	-1.30	-1.34	collagen, type XXI, alpha 1	0.00133	0.00259		
CSGALNACT2	-1.26	-1.36	chondroitin sulfate N-acetylgalactosaminyltransferase 2	0.00864	**0.00031**		
TGFBR3	-1.35	-1.36	transforming growth factor, beta receptor III	**0.00016**	0.00100	x	x
CXCL14	-1.27	-1.38	chemokine (C-X-C motif) ligand 14	**0.00021**	**0.00004**	x	x
TPBG	-1.25	-1.39	trophoblast glycoprotein	0.00672	**0.00003**	x	x
F2R	-1.45	-1.41	coagulation factor II (thrombin) receptor	0.00290	0.00186	x	x
ABCA6	-1.28	-1.46	ATP-binding cassette, sub-family A (ABC1), member 6	0.00748	**0.00019**		
DIO2	-1.90	-1.49	deiodinase, iodothyronine, type II	**0.00002**	**0.00024**	x	
LOX	-1.75	-1.55	lysyl oxidase	**0.00000**	**0.00042**	x	
ANK2	-1.63	-1.55	ankyrin 2, neuronal	0.00307	0.00113		
VCAN	-1.86	-1.57	Versican	**0.00003**	**0.00018**	x	x
CYP26B1	-1.95	-1.60	cytochrome P450, family 26, subfamily B, polypeptide 1	**< 1e-07**	**0.00006**	x	x
SFRP4	-1.86	-1.61	secreted frizzled-related protein 4	**0.00029**	0.00211	x	x
PGR	-2.23	-1.62	progesterone receptor	**0.00000**	**0.00021**	x	x

Supplemental Table S5. Proteins encoded by the most up-regulated genes

Protein	Major functional characteristics	Roles/activities	Regulation	Ref
OLFM4	Interacts with a number of proteins and regulates a variety of cell signaling pathways and essential biofunctions, including Wnt pathway, apoptosis, cell adhesion, inflammation.	Tissue- and cell-specific; may exert opposite effects in different cell types.	Regulated by multiple factors, including NF-kB, Wnt, retinoic acid and estrogen.	(1)
STS	Hydrolyzes several 3-beta-hydroxysteroid sulfates, which serve as metabolic precursors for estrogens, androgens, and cholesterol. A pivotal player in estrogen synthesis and metabolism.	Role in estrogen-dependent reproductive organ diseases such as endometriosis, adenomyosis, uterine fibroids, and gynecological and breast cancers. Role in skin barrier defects.	Role in local estrogen biosynthesis from estrone sulfate and DHEA-sulfate.	(2) (3)
GABRP	This subunit of GABA A receptor can assemble with other GABA A receptor subunits and alter the receptor sensitivity to modulatory agents such as GABA and pregnanolone.	Elevated in breast cancers with ER^- and immature cell type phenotypes. In basal-like breast cancer maintains migratory phenotype via GABRP-ERK1/2-cytokeratin axis. Role in generation of receptive endometrium. Correlation between GABRP expression and preeclampsia. Promotes apoptosis in trophoblast cell line.	In reproductive tissues and cancer cells can be regulated by beta estradiol.	(4) (5) (6) (7)
PCDH8	Belongs to protocadherin gene family (of the cadherin superfamily)	Role in epithelial-mesenchymal transition. Pathogenesis and progression of multiple cancers; context-depended: from tumor suppression to tumor promotion; poorly resolved mechanism.	Regulated by ER in breast cancer cells.	(8) (9)
DUSP4	Dual-specificity MAP kinase phosphatase (MKP), nuclear, mitogen- and stress-inducible; acts as negative feedback regulators of MAPK activity of ERK and p38 kinases.	Up-regulated in liver, breast and pancreatic cancer cell lines. Expressed in ovarian serous borderline tumors, but down-regulated in ovarian serous carcinoma, lung and breast carcinoma, and in glioblastoma.	Expression of DUSP1/2/5/10 are linked to suppression of ER in breast cancer cell lines, although DUSP4 was not reported for these cells.	(10) (11)

References

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Supplemental Table S6. Primers used for qRT-PCR*

Gene Symbol	Primer catalog number
RPTN	PPH18382A
LCE3D	PPH14903B
KRT10	PPH05868E
DSG1	PPH00655E
PGR	PPH01007F
OLFM4	PPH15541A
PCDH8	PPH08225A

*Primers were purchased from Qiagen, Valencia, CA

Information on the primers can be found at the following links

RPTN: http://www.sabiosciences.com/primerinfo.php?pcatn=PPH18382A

LCE3D: http://www.sabiosciences.com/primerinfo.php?pcatn=PPH14903B

KRT10: http://www.sabiosciences.com/primerinfo.php?pcatn=PPH05868E

DSG1: http://www.sabiosciences.com/primerinfo.php?pcatn=PPH00655E

PGR: http://www.sabiosciences.com/primerinfo.php?pcatn=PPH01007F

OLFM4: http://www.sabiosciences.com/primerinfo.php?pcatn=PPH15541A

PCDH8: http://www.sabiosciences.com/primerinfo.php?pcatn=PPH08225A

Table S13. Expression alterations of selected cervicovaginal genes from three groups of DMPA users

Gene symbol	Gene name	DMPA/BL fold change		
		G1	G2	G3
Down-regulated genes				
DSG1	desmoglein 1		-2.22	-30.15
RPTN	Repetin		-8.20	-25.11
LCE3D	late cornified envelope 3D		-2.12	-24.76
GYS2	glycogen synthase 2 (liver)		-1.35	-12.96
DMKN	dermokine	-1.55	-1.95	-10.73
ALOX12B	arachidonate 12-lipoxygenase, 12R type		-2.16	-10.24
FLG	Filaggrin			-9.85
KRT1	keratin 1			-9.21
SBSN	suprabasin		-1.39	-8.07
TGM3	transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)		-3.57	-7.44
CD36	CD36 molecule (thrombospondin receptor)		-2.14	-7.42
SPRR2C	small proline-rich protein 2C (pseudogene)		-1.71	-6.18
KRT6B	keratin 6B		-1.35	-5.48
SPINK6	serine peptidase inhibitor, Kazal type 6		-2.26	-5.22
KRT16	keratin 16			-4.85
KRT78	keratin 78			-4.73
DSC2	desmocollin 2			-4.71
SERPINB7	serpin peptidase inhibitor, clade B (ovalbumin), member 7		-1.90	-4.50
KRT14	keratin 14		-1.67	-4.37
AADACL2	arylacetamide deacetylase-like 2		-2.28	-4.23
KRT10	keratin 10		-2.57	-4.20
LOR	Loricrin			-3.92
KRT2	keratin 2			-3.77
HAL	histidine ammonia-lyase		-1.86	-3.07
CDSN	corneodesmosin		-1.52	-1.86
CYP26B1	cytochrome P450, family 26, subfamily B, polypeptide 1	-2.13	-2.08	-1.82
WISP2	WNT1 inducible signaling pathway protein 2	-4.96	-2.95	
PGR	progesterone receptor	-4.51	-2.38	
PRSS23	protease, serine, 23	-3.72	-2.24	
VCAN	Versican	-3.60	-1.98	
Up-regulated genes				
OLFM4	olfactomedin 4		6.25	6.67
GABRP	gamma-aminobutyric acid (GABA) A receptor, pi		5.26	
PCDH8	protocadherin 8		5.56	3.33
CAPN14	calpain 14		3.13	7.69

DUSP4	dual specificity phosphatase 4	3.03	1.64
SLC15A2	solute carrier family 15 (H+/peptide transporter), member 2	2.17	
STS	steroid sulfatase (microsomal), isozyme S	2.27	3.70
CYP4B1	cytochrome P450, family 4, subfamily B, polypeptide 1	2.27	
RDH10	retinol dehydrogenase 10 (all-trans)	2.22	2.8
KRT19	keratin 19	1.52	
KRT18	keratin 18	1.56	
CXCL6	chemokine (C-X-C motif) ligand 6		11.90
CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)		6.25
IL8	interleukin 8		4.55
C3	complement component 3		4.00
CCL19	chemokine (C-C motif) ligand 19		2.94
CFI	complement factor I		2.56
CXCL13	chemokine (C-X-C motif) ligand 13		2.50
CCL21	chemokine (C-C motif) ligand 21	-1.36	1.82

Supplemental Table S14: Demographic and clinical characteristics of participants in groups

Characteristics	G1 (n=4)	G2 (n = 19)	G3 (n = 5)	P value[a]
Age, years, mean (SD)	24.3 (5.4)	34.8 (7.5)	34.2 (6.3)	0.053
Race, n (%)				0.84
Black	2 (50.0)	7 (36.8)	2 (40.0)	
White	2 (50.0)	5 (26.3)	1 (20.0)	
Mixed	0 (0)	6 (31.6)	2 (40.0)	
Other	0 (0)	1 (5.3)	0 (0)	
Hispanic, n(%)	0 (0)	7 (36.8)	2 (50.0)	
Non-Hispanic, n(%)	4 (100)	12 (63.2)	2 (50.0)	0.46
BMI[b], mean (SD)	30.2 (7.4)	34.2 (8.3)	26.7 (5.9)	0.096
Sexual partner status, n (%)				0.91
Lives with partner	1 (25.0)	8 (42.1)	2 (40.0)	
Does not live with partner	3 (75.0)	9 (47.4)	2 (40)	
No partner	0 (0)	2 (10.5)	1 (20.0)	
Sexual relationship (years)	3.7 (6.4)	7.6 (5.9)	9.0 (8.7)	0.29

	G1 (n=4)	G2+G3 n=24	P value
Age, years, mean (SD)	24.3 (5.4)	34.7 (7.1)	0.0162
Sexual relationship (years)	3.7 (6.4)	7.9 (6.3)	0.126

	G1+G2	G3	
BMI[b], mean (SD)	33.5 (8.2)	26.7(5.9)	0.063

[a] Continuous values were compared using t-test for normally distributed data or Kruskal-Wallis test for non-normally distributed data; for categorical data Fisher's exact tests or chi square statistics were used.

[b] The BMI is the weight in kilograms divided by the square of height in meters

Supplementary Table S15. RNA quality.

V2- baseline, V5 - HC use; D - DMPA group, O - COC group

hyb_ID	Sample Name	RNA 260280	RIN	Visit
A0167028	101,V2	1.91	7.4	V2D
A0167029	103,V2	1.9	7.1	V2D
A0167030	105,V2	1.9	7.0	V2D
A0167031	118,V2	1.91	7.5	V2D
A0167032	122,V2	1.94	7.1	V2D
A0167033	125,V2	1.94	8.0	V2D
A0167034	126,V2	1.95	6.9	V2D
A0167035	127,V2	1.98	7.4	V2D
A0167036	128,V2	1.82	6.2	V2D
A0167037	129,V2	1.94	6.6	V2D
A0167038	130,V2	1.96	7.4	V2D
A0167039	131,V2	1.85	7.0	V2D
A0167040	132,V2	1.99	7.5	V2D
A0167041	134,V2	1.94	6.7	V2D
A0167042	135,V2	1.97	6.5	V2D
A0167043	204, V2	1.95	6.7	V2D
A0167044	206, V2	1.91	6.4	V2D
A0167045	208, V2	1.85	7.2	V2D
A0167046	209, V2	1.77	7.2	V2D
A0167047	210, V2	1.78	7.1	V2D
A0167048	211, V2	1.91	7.4	V2D
A0167049	212, V2	1.94	7.7	V2D
A0167050	220, V2	1.89	7.1	V2D
A0167051	304, V2	1.83	7.7	V2D
A0167052	305, V2	1.83	6.6	V2D
A0167053	308, V2	1.82	7.6	V2D
A0167054	311, V2	1.82	7.7	V2D
A0167055	317, V2	1.82	7.1	V2D
A0167056	318, V2	1.5	6.7	V2D
A0167057	319, V2	1.76	6.7	V2D
A0167058	320, V2	1.8	7.1	V2D
A0167059	101, V5	1.96	3.5	V5D
A0167060	103, V5	1.95	6.4	V5D
A0167061	105, V5	1.96	8.1	V5D
A0167062	118, V5	1.85	6.4	V5D
A0167063	122, V5	1.92	8.1	V5D

A0167064	125, V5	1.88	7.3	V5D
A0167065	126, V5	1.55	8.0	V5D
A0167066	127, V5	1.94	7.1	V5D
A0167067	128, V5	1.59	4.1	V5D
A0167068	129, V5	1.66	2.6	V5D
A0167069	130, V5	1.83	6.6	V5D
A0167070	131, V5	1.84	6.8	V5D
A0167071	132, V5	1.97	6.9	V5D
A0167072	134, V5	1.93	6.6	V5D
A0167073	135, V5	1.96	5.7	V5D
A0167074	204, V5	1.94	6.9	V5D
A0167075	206, V5	1.74	7.0	V5D
A0167076	208, V5	1.78	7.2	V5D
A0167077	209, V5	1.98	7.3	V5D
A0167078	210, V5	1.86	6.5	V5D
A0167079	211, V5	1.89	7.6	V5D
A0167080	212, V5	1.88	6.3	V5D
A0167081	220, V5	1.96	7.1	V5D
A0167082	304, V5	1.89	7.9	V5D
A0167083	305, V5	1.84	7.6	V5D
A0167091	303 V2	1.87	3.2	V2O
A0167092	307 V2	1.95	6.9	V2O
A0167093	309 V2	2.06	7.5	V2O
A0167094	312 V2	2.02	7.2	V2O
A0167095	313 V2	1.89	8.2	V2O
A0167096	314 V2	2	7.6	V2O
A0167097	315 V2	1.98	6.6	V2O
A0167098	104 V2	1.96	8.1	V2O
A0167099	106 V2	1.86	6.6	V2O
A0167100	107 V2	1.93	7.2	V2O
A0167101	108 V2	1.9	8.4	V2O
A0167102	109 V2	1.91	7.9	V2O
A0167103	110 V2	1.94	6.6	V2O
A0167104	111 V2	1.93	7.9	V2O
A0167105	113 V2	1.93	7.2	V2O
A0167106	114 V2	1.91	6.3	V2O
A0167107	115 V2	1.91	6.7	V2O
A0167108	116 V2	1.89	7.9	V2O
A0167109	117 V2	1.89	7.3	V2O
A0167110	119 V2	1.92	6.3	V2O
A0167111	120 V2	1.89	6.7	V2O

A0167112	123 V2	1.94	7.9	V2O
A0167113	124 V2	1.92	7.7	V2O
A0167114	207 V2	1.96	7.1	V2O
A0167115	213 V2	1.91	5.1	V2O
A0167116	214 V2	1.89	7.5	V2O
A0167117	215 V2	1.95	7.3	V2O
A0167118	216 V2	1.87	6.4	V2O
A0167119	217 V2	1.92	7.0	V2O
A0167120	218 V2	1.81	7.8	V2O
A0167121	219 V2	1.94	7.9	V2O
A0167122	302 V5	1.83	8.4	V5O
A0167123	303 V5	1.94	6.8	V5O
A0167124	307 V5	1.95	7.6	V5O
A0167125	309 V5	1.97	7.5	V5O
A0167126	312 V5	1.89	7.9	V5O
A0167127	313 V5	1.98	9.0	V5O
A0167128	314 V5	1.9	7.8	V5O
A0167129	315 V5	1.83	7.4	V5O
A0167130	104 V5	1.86	8.3	V5O
A0167131	106 V5	1.87	7.8	V5O
A0167132	107 V5	1.9	7.4	V5O
A0167133	108 V5	1.88	8.3	V5O
A0167134	109 V5	1.67	8.4	V5O
A0167135	110 V5	1.91	8.0	V5O
A0167136	111 V5	1.86	8.2	V5O
A0167137	113 V5	1.94	8.3	V5O
A0167138	114 V5	1.89	6.2	V5O
A0167139	115 V5	1.94	8.5	V5O
A0167140	116 V5	1.88	7.9	V5O
A0167141	117 V5	1.96	8.5	V5O
A0167142	119 V5	1.91	6.6	V5O
A0167143	120 V5	1.92	7.2	V5O
A0167144	123 V5	1.9	9.4	V5O
A0167145	124 V5	1.82	9.4	V5O
A0167146	207 V5	1.85	8.1	V5O
A0167147	213 V5	1.91	7.0	V5O
A0167148	214 V5	1.91	7.8	V5O
A0167149	215 V5	1.74	6.2	V5O
A0167150	216 V5	1.9	6.8	V5O
A0167151	217 V5	1.87	9.2	V5O
A0167152	218 V5	1.91	7.3	V5O

A0167153	219 V5	1.85	7.1	V5O
A0167966	308, V5	1.7	8.0	V5D
A0167967	311, V5	1.79	6.5	V5D
A0167968	317, V5	1.87	8.0	V5D
A0167969	318, V5	1.82	7.8	V5D
A0167970	319, V5	1.91	7.4	V5D
A0167971	320, V5	1.9	8.2	V5D
A0167972	302 V2	1.91	6.4	V2O

Supplemental Table S16. Antibodies

Antibody	Source	Cat. #	Host	Dilution
Primary				
KRT-10	Santa Cruz	sc23877	Mouse (monoclonal)	`1:50
DSG-1	Novus Biologicals	NBP1-84567	Rabbit (polyclonal)	`1:25
DMKN	Novus Biologicals	NBP1-86840	Rabbit (polyclonal)	`1:100
Secondary				
Horse anti-mouse	Vector	BA-2000		`1:200
Goat anti-rabbit	Vector	BA-100		`1:200

Supplemental method

Quantitative real time PCR

Total RNA (1 μg) was reverse transcribed using Reverse Transcription System kit from Promega Corp. (Madison, WI). Reverse transcription was primed with Oligo(dT)15 according to the manufacturer's instructions. Quantitative real-time PCR was performed in 20 μl reaction volume containing 1 μg cDNA using LightCycler FastStart DNA Master SYBR I kit (Roche, IN) according to the manufacturer's instructions. The reaction parameters were as follows: 95°C for 10 min followed by 45 cycles at 95°C for 15 s, 55°C for 5 s, 72°C for 15 s. Each sample was run in triplicates; the threshold cycle (Ct) of GAPDH was used to normalize target gene expression (ΔCt). The relative change in gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Experiments were performed at least three times, the mean and SD were calculated using Graph Pad software (version 7.01). Information on primers is given in the Table S6.

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	✓	2
		• Structured abstract recommended	✓	2
		• Information on target population or study sample	✓	2
Introduction				
Background	2	• Scientific background and explanation of rationale	✓	3-4
		• Theories used in designing behavioral interventions		
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	✓	20
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented		
		• Recruitment setting	✓	20
		• Settings and locations where the data were collected	✓	20
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:		
		○ Content: what was given?	✓	20
		○ Delivery method: how was the content given?	✓	20
		○ Unit of delivery: how were the subjects grouped during delivery?	✓	20
		○ Deliverer: who delivered the intervention?	✓	20
		○ Setting: where was the intervention delivered?	✓	20
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	✓	20
		○ Time span: how long was it intended to take to deliver the intervention to each unit?		
		○ Activities to increase compliance or adherence (e.g., incentives)		
Objectives	5	• Specific objectives and hypotheses	✓	4
Outcomes	6	• Clearly defined primary and secondary outcome measures	✓	5-11
		• Methods used to collect data and any methods used to enhance the quality of measurements	✓	20-23
		• Information on validated instruments such as psychometric and biometric properties		
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules		
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	✓	20
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	✓	20
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	✓	5

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	✓	5, 20
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	✓	9
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)		
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	✓	22-23
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	✓	22-23
		• Methods for imputing missing data, if used		
		• Statistical software or programs used	✓	21-23
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)		
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	✓	20
		○ Assignment: the numbers of participants assigned to a study condition	✓	20
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention		
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition		
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition		
		• Description of protocol deviations from study as planned, along with reasons		
Recruitment	13	• Dates defining the periods of recruitment and follow-up		
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	✓	5,20
		• Baseline characteristics for each study condition relevant to specific disease prevention research		
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition		
		• Comparison between study population at baseline and target population of interest		
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences	✓	5,22

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	✓	5,20
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses		
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	✓	5-11
		• Inclusion of null and negative findings		
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any		
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	✓	9-10
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)		
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	✓	12-19
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	✓	12-19
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	✓	18-19
		• Discussion of research, programmatic, or policy implications	✓	18-19
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	✓	18-19
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	✓	12-19

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/