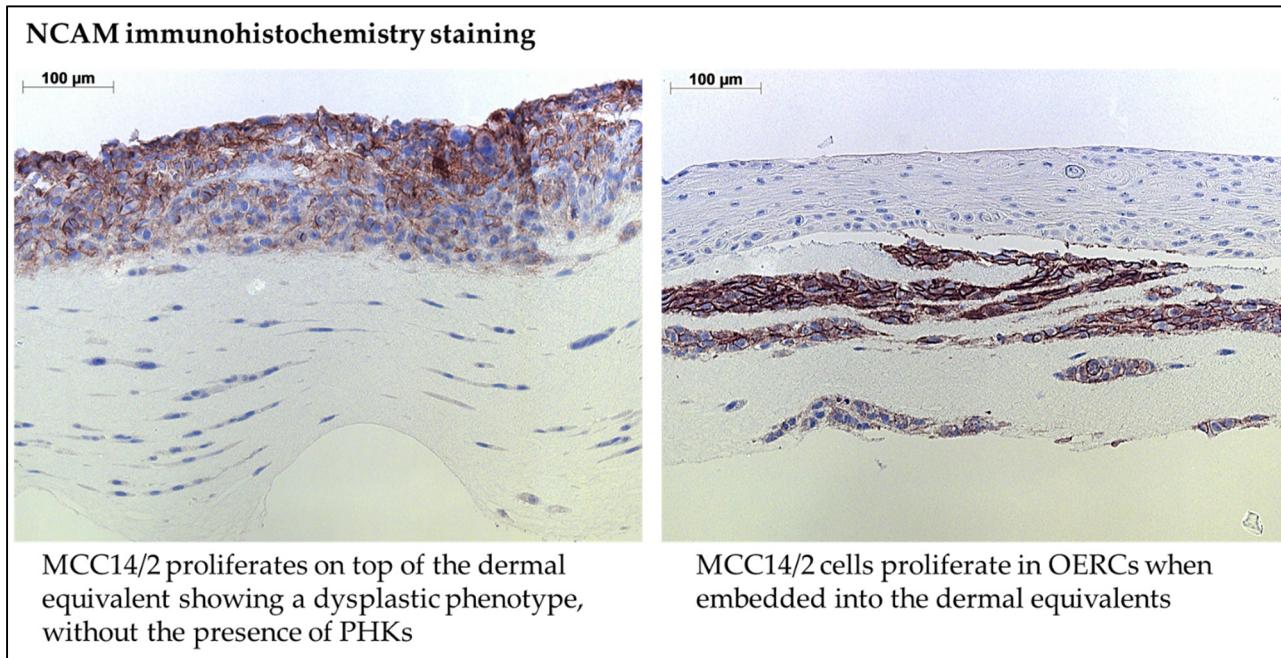


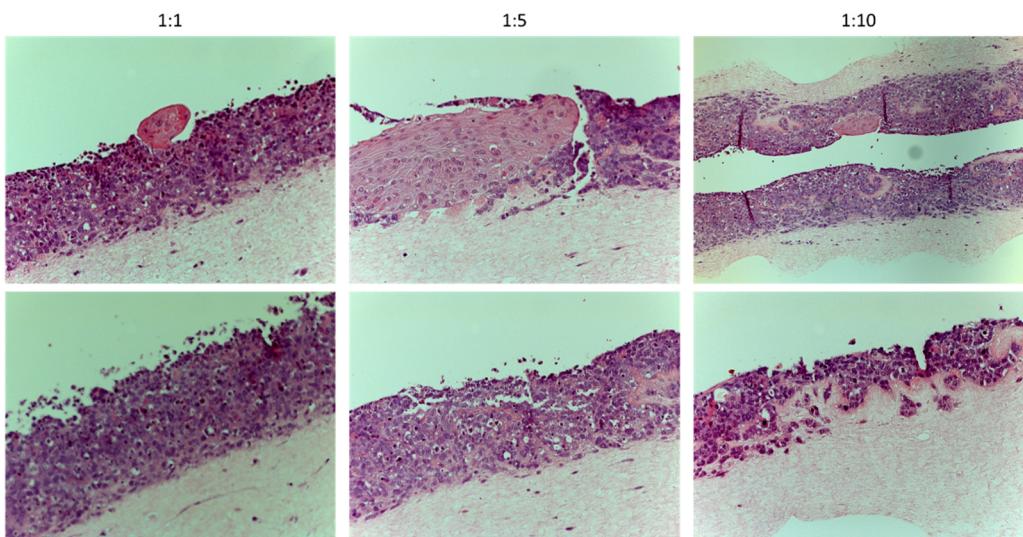
# Supplementary Materials: Organotypic Epithelial Raft Cultures as a New Three-Dimensional In Vitro Model of Merkel Cell Carcinoma

Arturo Temblador, Dimitrios Topalis, Joost van den Oord, Graciela Andrei and Robert Snoeck

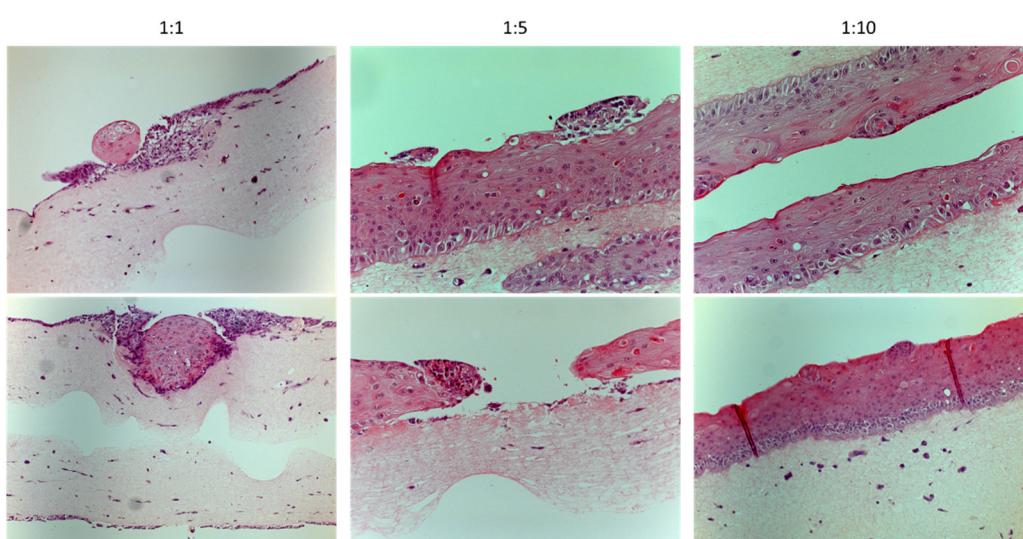


**Figure S1.** Specificity of NCAM staining for MCC cells. The MCPyV- MCC cell line MCC14/2 was grown either on top of the dermal equivalent (left image) or embedded into the dermal equivalent (right image). Murine fibroblasts in the dermal equivalent are clearly negative for NCAM. Images of the H&E staining were taken at an overall 200 $\times$  magnification.

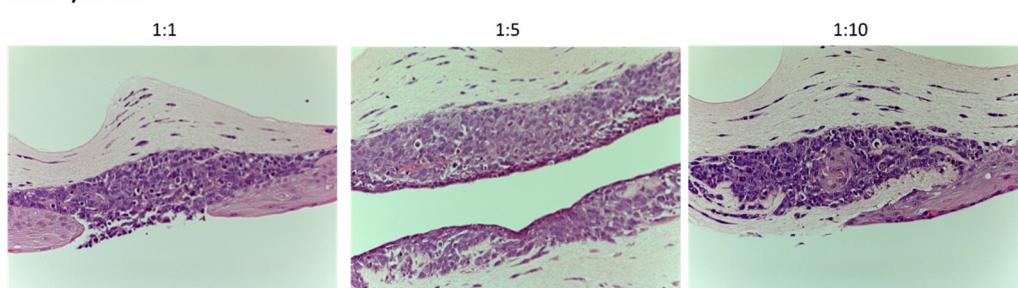
**MKL-1:PHKs**



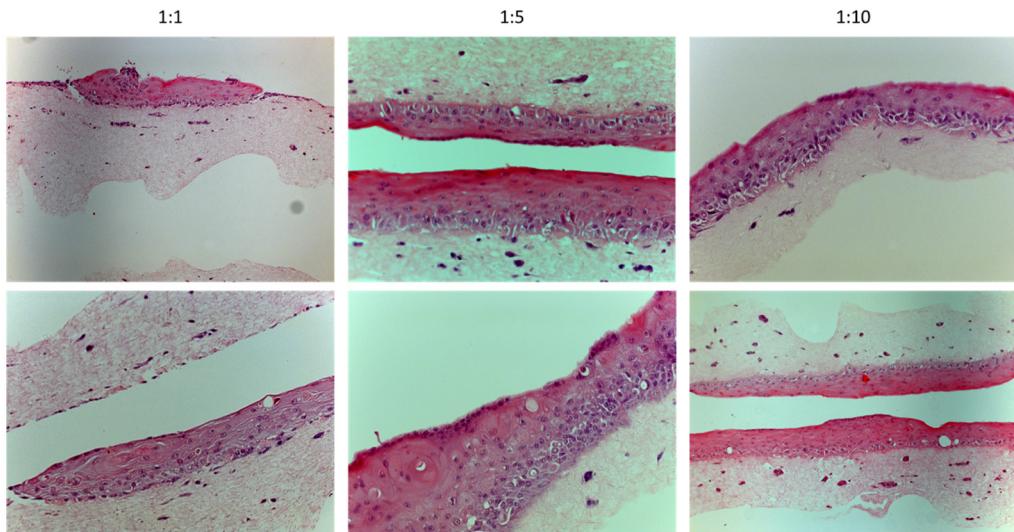
**MCC26:PHKs**



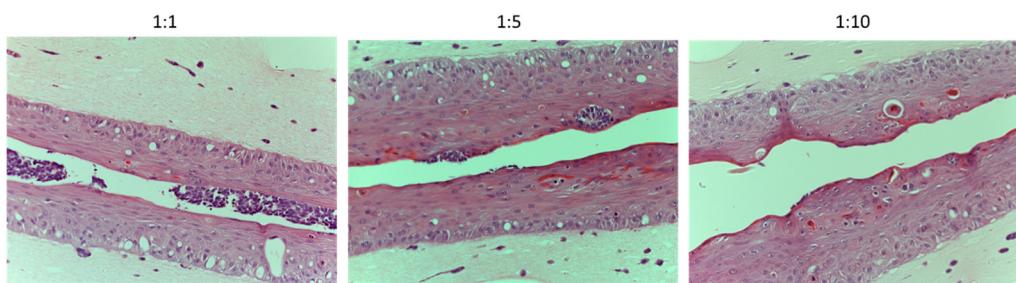
**MCC14/2:PHKs**



**MCC13:PHKs**



**MS-1:PHKs**



**Figure S2.** Organotypic epithelial raft cultures (OERCs) of Merkel cell carcinoma (MCC) cell lines co-cultures with primary human keratinocytes (PHKs) at a ratio of 1:1, 1:5 and 1:10. The MCPyV- MCC cell lines (MCC14/2, MCC26 and MCC13) and the MCPyV<sup>+</sup> MCC cell lines (MS-1, MKL-1 and WAGA) were used. Different strategies can be assayed for the development of organotypic epithelial raft cultures (OERCs). Images of the H&E staining were taken an overall 200× magnification.

**Table S1.** Panel of genes present in the Human Extracellular Matrix & Adhesion Molecules array plate. The description of each gene symbol is provided under the table.

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>A</b>	18S	GAPDH	HPRT1	GUSB	ACTB	B2M	RPLPO	HMBS	TBP	PGK1	UBC	PPIA
<b>B</b>	ADAMTS1	ADAMTS13	ADAMTS8	CD44	CDH1	CNTN1	COL11A1	COL12A1	COL14A1	COL15A1	COL16A1	COL1A1
<b>C</b>	COL4A2	COL5A1	COL6A1	COL6A2	COL7A1	COL8A1	VCAN	CTGF	CTNNA1	CTNNB1	CTNND1	CTNND2
<b>D</b>	ECM1	FN1	HAS1	ICAM1	ITGA1	ITGA2	ITGA3	ITGA4	ITGA5	ITGA6	ITGA7	ITGA8
<b>E</b>	ITGAL	ITGAM	ITGAV	ITGB1	ITGB2	ITGB3	ITGB4	ITGB5	KAL1	LAMA1	LAMA2	LAMA3
<b>F</b>	LAMB1	LAMB3	LAMC1	MMP1	MMP10	MMP11	MMP12	MMP13	MMP14	MMP15	MMP16	MMP2
<b>G</b>	MMP3	MMP7	MMP8	MMP9	NCAM1	PECAM1	SELE	SELL	SELP	SGCE	SPARC	SPG7
<b>H</b>	SPP1	TGFBI	THBS1	THBS2	THBS3	TIMP1	TIMP2	TIMP3	CLEC3B	TNC	VCAM1	VTN

**Gene Symbol**

18S	Eukaryotic 18S rRNA
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HPRT1	hypoxanthine phosphoribosyltransferase 1
GUSB	glucuronidase, beta
ACTB	actin, beta
B2M	beta-2-microglobulin
RPLPO	ribosomal protein, large, P0
HMBS	hydroxymethylbilane synthase
TBP	TATA box binding protein

<i>PGK1</i>	phosphoglycerate kinase 1
<i>UBC</i>	ubiquitin C
<i>PPIA</i>	peptidylprolyl isomerase A (cyclophilin A)
<i>ADAMTS1</i>	ADAM metallopeptidase with thrombospondin type 1 motif, 1
<i>ADAMTS13</i>	ADAM metallopeptidase with thrombospondin type 1 motif, 13
<i>ADAMTS8</i>	ADAM metallopeptidase with thrombospondin type 1 motif, 8
<i>CD44</i>	CD44 molecule (Indian blood group)
<i>CDH1</i>	cadherin 1, type 1, E-cadherin (epithelial)
<i>CNTN1</i>	contactin 1
<i>COL11A1</i>	collagen, type XI, alpha 1
<i>COL12A1</i>	collagen, type XII, alpha 1
<i>COL14A1</i>	collagen, type XIV, alpha 1
<i>COL15A1</i>	collagen, type XV, alpha 1
<i>COL16A1</i>	collagen, type XVI, alpha 1
<i>COL1A1</i>	collagen, type I, alpha 1
<i>COL4A2</i>	collagen, type IV, alpha 2
<i>COL5A1</i>	collagen, type V, alpha 1
<i>COL6A1</i>	collagen, type VI, alpha 1
<i>COL6A2</i>	collagen, type VI, alpha 2
<i>COL7A1</i>	collagen, type VII, alpha 1
<i>COL8A1</i>	collagen, type VIII, alpha 1
<i>VCAN</i>	versican
<i>CTGF</i>	connective tissue growth factor
<i>CTNNA1</i>	catenin (cadherin-associated protein), alpha 1, 102kDa
<i>CTNNB1</i>	catenin (cadherin-associated protein), beta 1, 88kDa
<i>CTNND1</i>	catenin (cadherin-associated protein), delta 1
<i>CTNND2</i>	catenin (cadherin-associated protein), delta 2 (neural plakophilin-related arm-repeat protein)
<i>ECM1</i>	extracellular matrix protein 1
<i>FN1</i>	fibronectin 1
<i>HAS1</i>	hyaluronan synthase 1
<i>ICAM1</i>	intercellular adhesion molecule 1
<i>ITGA1</i>	integrin, alpha 1
<i>ITGA2</i>	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
<i>ITGA3</i>	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
<i>ITGA4</i>	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
<i>ITGA5</i>	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
<i>ITGA6</i>	integrin, alpha 6
<i>ITGA7</i>	integrin, alpha 7
<i>ITGA8</i>	integrin, alpha 8
<i>ITGAL</i>	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
<i>ITGAM</i>	integrin, alpha M (complement component 3 receptor 3 subunit)
<i>ITGAV</i>	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
<i>ITGB1</i>	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
<i>ITGB2</i>	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
<i>ITGB3</i>	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
<i>ITGB4</i>	integrin, beta 4
<i>ITGB5</i>	integrin, beta 5
<i>KAL1</i>	Kallmann syndrome 1 sequence
<i>LAMA1</i>	laminin, alpha 1

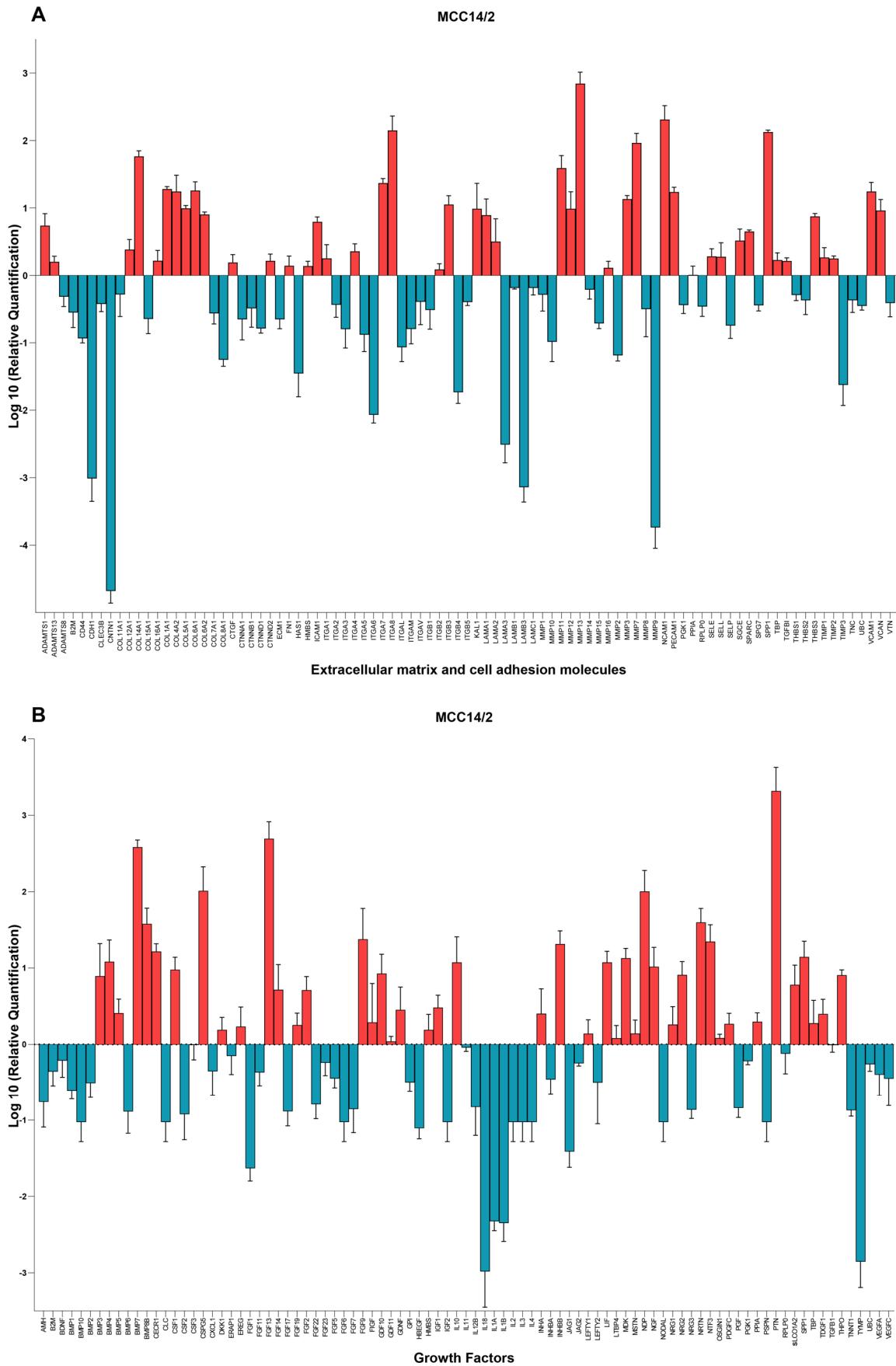
<i>LAMA2</i>	laminin, alpha 2
<i>LAMA3</i>	laminin, alpha 3
<i>LAMB1</i>	laminin, beta 1
<i>LAMB3</i>	laminin, beta 3
<i>LAMC1</i>	laminin, gamma 1 (formerly LAMB2)
<i>MMP1</i>	matrix metallopeptidase 1 (interstitial collagenase)
<i>MMP10</i>	matrix metallopeptidase 10 (stromelysin 2)
<i>MMP11</i>	matrix metallopeptidase 11 (stromelysin 3)
<i>MMP12</i>	matrix metallopeptidase 12 (macrophage elastase)
<i>MMP13</i>	matrix metallopeptidase 13 (collagenase 3)
<i>MMP14</i>	matrix metallopeptidase 14 (membrane-inserted)
<i>MMP15</i>	matrix metallopeptidase 15 (membrane-inserted)
<i>MMP16</i>	matrix metallopeptidase 16 (membrane-inserted)
<i>MMP2</i>	matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
<i>MMP3</i>	matrix metallopeptidase 3 (stromelysin 1, progelatinase)
<i>MMP7</i>	matrix metallopeptidase 7 (matrilysin, uterine)
<i>MMP8</i>	matrix metallopeptidase 8 (neutrophil collagenase)
<i>MMP9</i>	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
<i>NCAM1</i>	neural cell adhesion molecule 1
<i>PECAM1</i>	platelet/endothelial cell adhesion molecule
<i>SELE</i>	selectin E
<i>SELL</i>	selectin L
<i>SELP</i>	selectin P (granule membrane protein 140kDa, antigen CD62)
<i>SGCE</i>	sarcoglycan, epsilon
<i>SPARC</i>	secreted protein, acidic, cysteine-rich (osteonectin)
<i>SPG7</i>	spastic paraplegia 7 (pure and complicated autosomal recessive)
<i>SPP1</i>	secreted phosphoprotein 1
<i>TGFBI</i>	transforming growth factor, beta-induced, 68kDa
<i>THBS1</i>	thrombospondin 1
<i>THBS2</i>	thrombospondin 2
<i>THBS3</i>	thrombospondin 3
<i>TIMP1</i>	TIMP metallopeptidase inhibitor 1
<i>TIMP2</i>	TIMP metallopeptidase inhibitor 2
<i>TIMP3</i>	TIMP metallopeptidase inhibitor 3
<i>CLEC3B</i>	C-type lectin domain family 3, member B
<i>TNC</i>	tenascin C
<i>VCAM1</i>	vascular cell adhesion molecule 1
<i>VTN</i>	vitronectin

**Table S2.** Panel of genes present in the Human Growth Factors array plate. The description of each gene symbol is provided under the table.

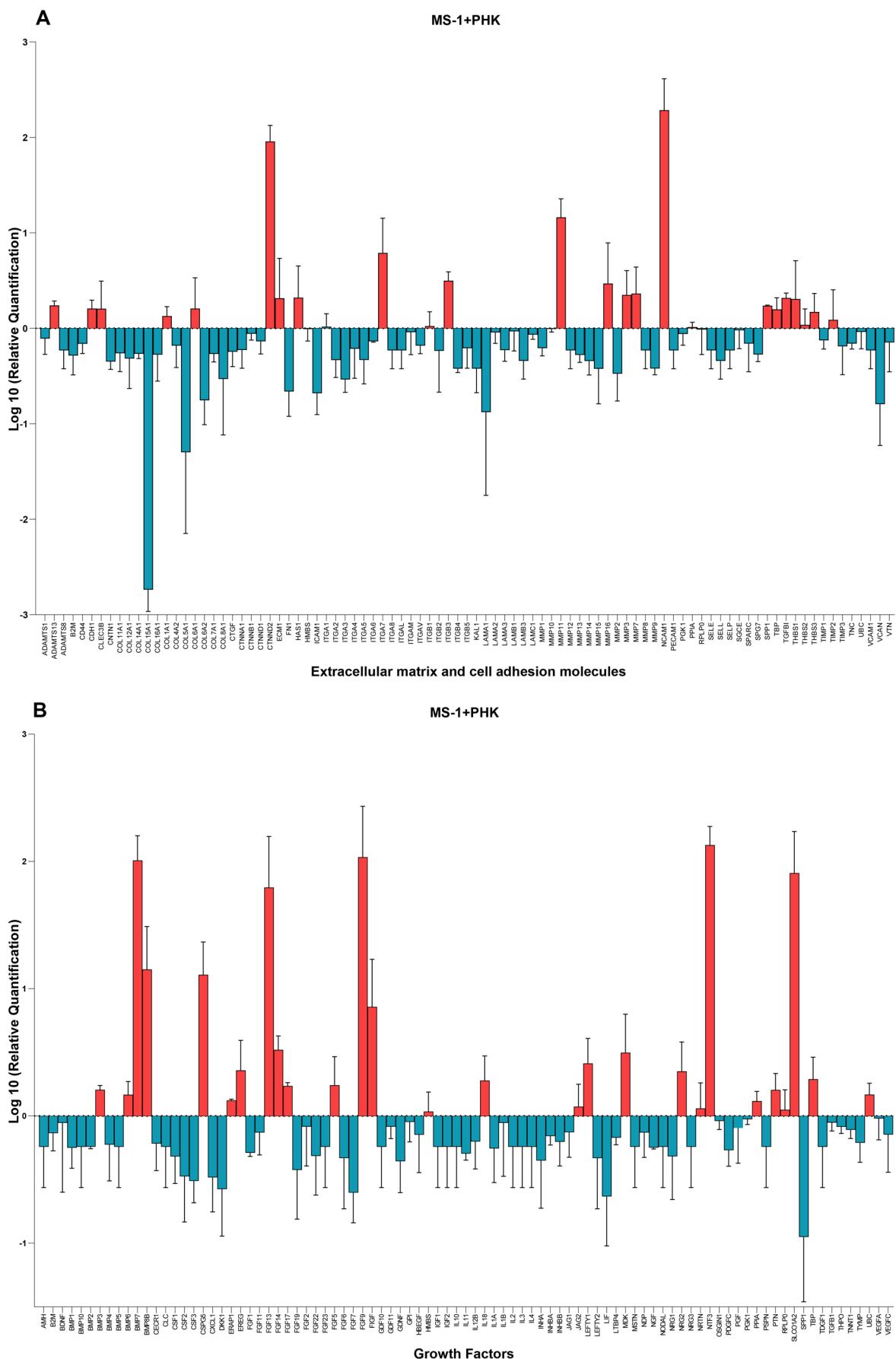
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>A</b>	18S	GAPDH	HPRT1	GUSB	ACTB	B2M	RPLPO	HMBS	TBP	PGK1	UBC	PPIA
<b>B</b>	AMH	BDNF	BMP1	BMP10	BMP2	BMP3	BMP4	BMP5	BMP6	BMP7	BMP8B	CECR1
<b>C</b>	CLC	CSF1	CSF2	CSF3	CSPG5	CXCL1	DKK1	ERAP1	TYMP	EREG	FGF1	FGF11
<b>D</b>	FGF13	FGF14	FGF17	FGF19	FGF2	FGF22	FGF23	FGF5	FGF6	FGF7	FGF9	FIGF
<b>E</b>	GDF10	GDF11	MSTN	GDNF	GPI	HBEGF	IGF1	IGF2	IL10	IL11	IL12B	IL18
<b>F</b>	IL1A	IL1B	IL2	IL3	IL4	INHA	INHBA	INHBB	JAG1	JAG2	LEFTY1	LEFTY2
<b>G</b>	LIF	LTBP4	MDK	NDP	NGF	NODAL	NRG1	NRG2	NRG3	NRTN	NTF3	OSGIN1

<b>H</b>	<i>PDGFC</i>	<i>PGF</i>	<i>PSPN</i>	<i>PTN</i>	<i>SLCO1A2</i>	<i>SPP1</i>	<i>TDGF1</i>	<i>TGFB1</i>	<i>THPO</i>	<i>TNNT1</i>	<i>VEGFA</i>	<i>VEGFC</i>
<b>Gene Symbol</b>	<b>Description</b>											
<i>18S</i>												
<i>GAPDH</i>												
<i>HPRT1</i>												
<i>GUSB</i>												
<i>ACTB</i>												
<i>B2M</i>												
<i>RPLP0</i>												
<i>HMBS</i>												
<i>TBP</i>												
<i>PGK1</i>												
<i>UBC</i>												
<i>PPIA</i>												
<i>AMH</i>												
<i>BDNF</i>												
<i>BMP1</i>												
<i>BMP10</i>												
<i>BMP2</i>												
<i>BMP3</i>												
<i>BMP4</i>												
<i>BMP5</i>												
<i>BMP6</i>												
<i>BMP7</i>												
<i>BMP8B</i>												
<i>CECR1</i>												
<i>CLC</i>												
<i>CSF1</i>												
<i>CSF2</i>												
<i>CSF3</i>												
<i>CSPG5</i>												
<i>CXCL1</i>												
<i>DKK1</i>												
<i>ERAP1</i>												
<i>TYMP</i>												
<i>EREG</i>												
<i>FGF1</i>												
<i>FGF11</i>												
<i>FGF13</i>												
<i>FGF14</i>												
<i>FGF17</i>												
<i>FGF19</i>												
<i>FGF2</i>												
<i>FGF22</i>												
<i>FGF23</i>												
<i>FGF5</i>												
<i>FGF6</i>												
<i>FGF7</i>												
<i>FGF9</i>												

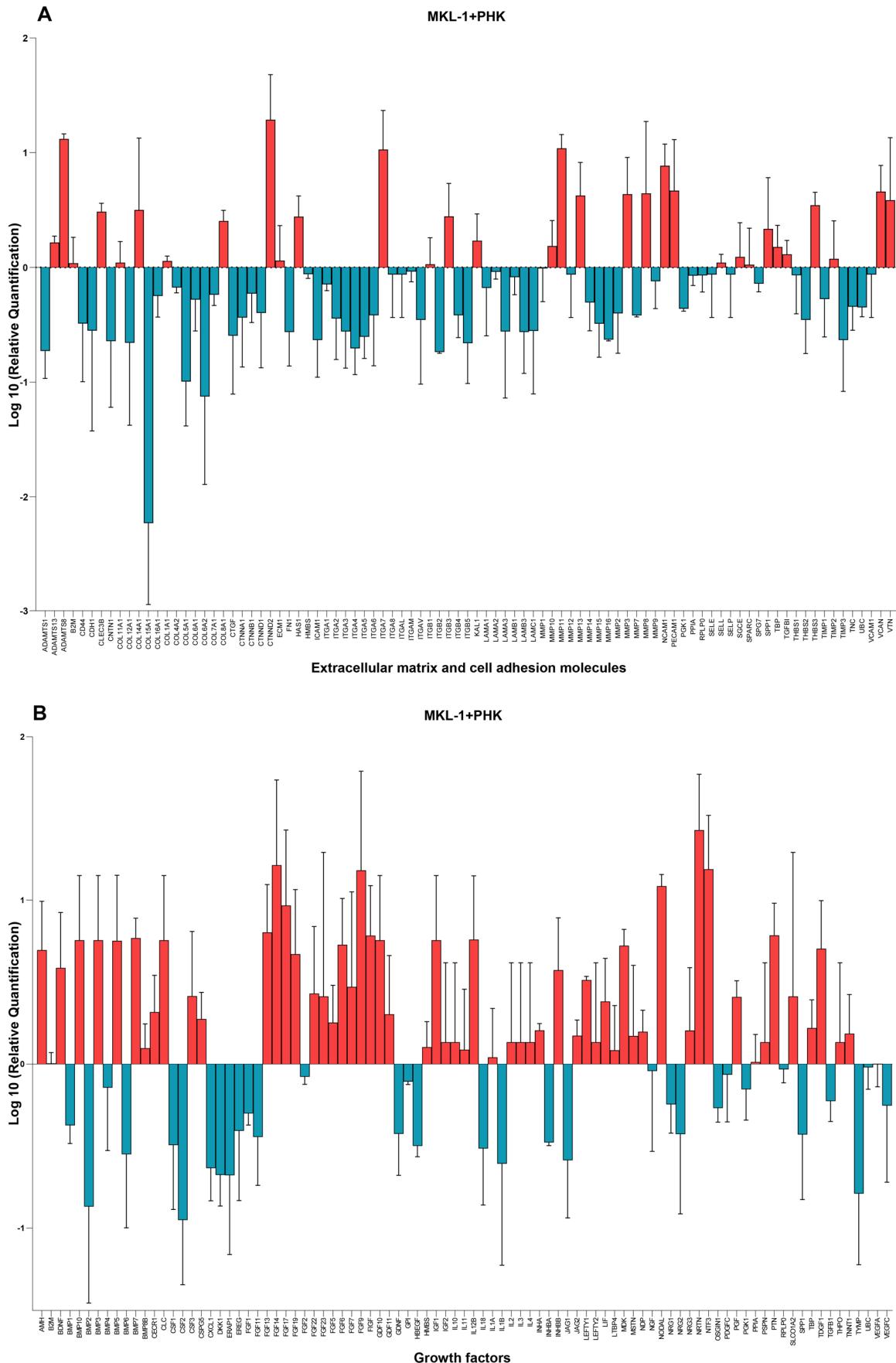
<i>FIGF</i>	c-fos induced growth factor (vascular endothelial growth factor D)
<i>GDF10</i>	growth differentiation factor 10
<i>GDF11</i>	growth differentiation factor 11
<i>MSTN</i>	myostatin
<i>GDNF</i>	glial cell derived neurotrophic factor
<i>GPI</i>	glucose phosphate isomerase
<i>HBEGF</i>	heparin-binding EGF-like growth factor
<i>IGF1</i>	insulin-like growth factor 1 (somatomedin C)
<i>IGF2</i>	insulin-like growth factor 2 (somatomedin A)
<i>IL10</i>	interleukin 10
<i>IL11</i>	interleukin 11
<i>IL12B</i>	interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)
<i>IL18</i>	interleukin 18 (interferon-gamma-inducing factor)
<i>IL1A</i>	interleukin 1, alpha
<i>IL1B</i>	interleukin 1, beta
<i>IL2</i>	interleukin 2
<i>IL3</i>	interleukin 3 (colony-stimulating factor, multiple)
<i>IL4</i>	interleukin 4
<i>INHA</i>	inhibin, alpha
<i>INHBA</i>	inhibin, beta A
<i>INHBB</i>	inhibin, beta B
<i>JAG1</i>	jagged 1 (Alagille syndrome)
<i>JAG2</i>	jagged 2
<i>LEFTY1</i>	left-right determination factor 1
<i>LEFTY2</i>	left-right determination factor 2
<i>LIF</i>	leukemia inhibitory factor (cholinergic differentiation factor)
<i>LTBP4</i>	latent transforming growth factor beta binding protein 4
<i>MDK</i>	midkine (neurite growth-promoting factor 2)
<i>NDP</i>	Norrie disease (pseudoglioma)
<i>NGF</i>	nerve growth factor (beta polypeptide)
<i>NODAL</i>	nodal homolog (mouse)
<i>NRG1</i>	neuregulin 1
<i>NRG2</i>	neuregulin 2
<i>NRG3</i>	neuregulin 3
<i>NRTN</i>	neurturin
<i>NTF3</i>	neurotrophin 3
<i>OSGIN1</i>	oxidative stress induced growth inhibitor 1
<i>PDGFC</i>	platelet derived growth factor C
<i>PGF</i>	placental growth factor
<i>PSPN</i>	persephin
<i>PTN</i>	pleiotrophin
<i>SLCO1A2</i>	solute carrier organic anion transporter family, member 1A2
<i>SPP1</i>	secreted phosphoprotein 1
<i>TDGF1</i>	teratocarcinoma-derived growth factor 1
<i>TGFB1</i>	transforming growth factor, beta 1
<i>THPO</i>	thrombopoietin
<i>TNNT1</i>	troponin T type 1 (skeletal, slow)
<i>VEGFA</i>	vascular endothelial growth factor A
<i>VEGFC</i>	vascular endothelial growth factor C



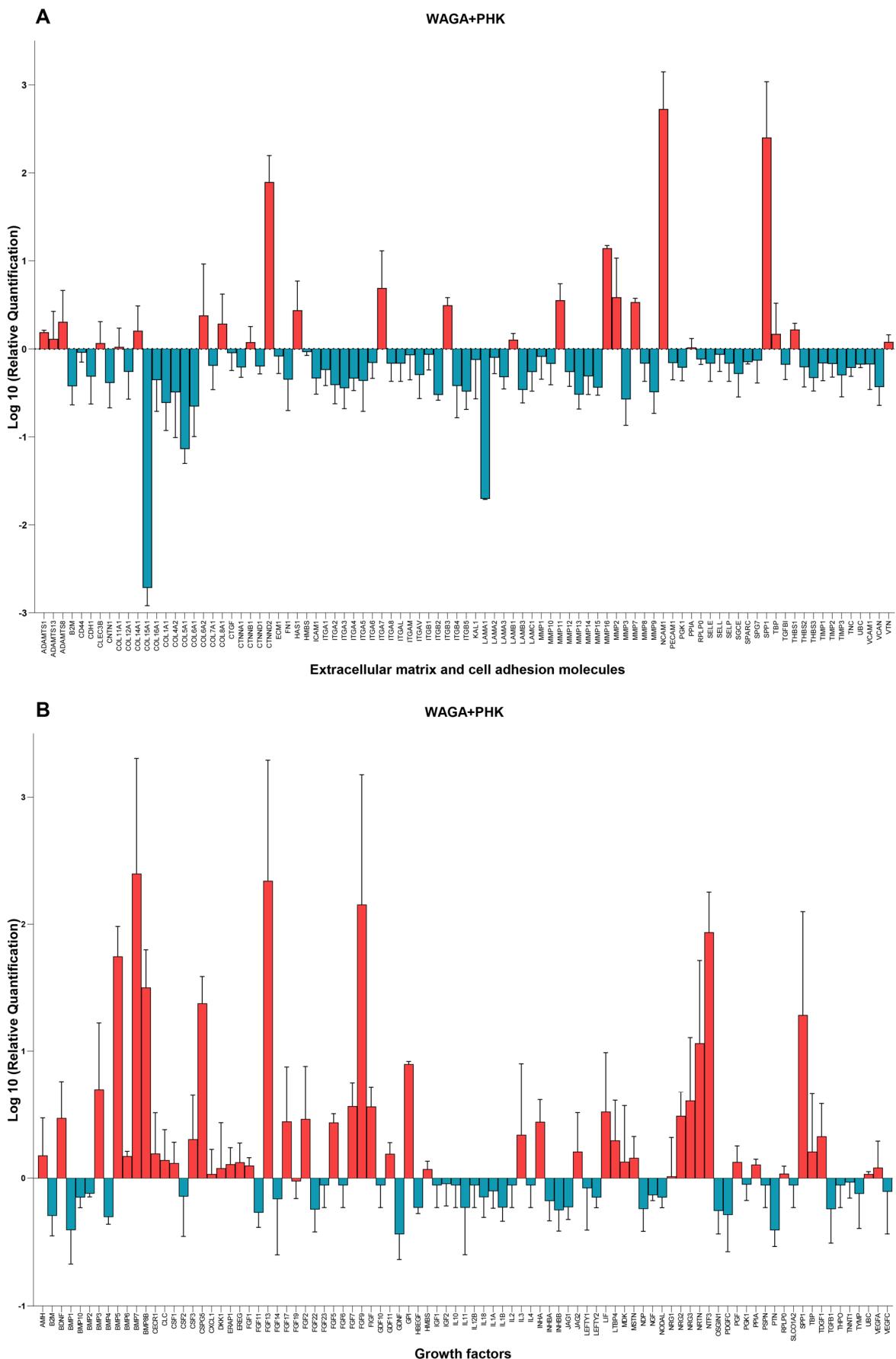
**Figure S3.** Gene expression profiles of organotypic epithelial raft cultures (OERCs) of MCC14/2 cells grown alone on top of the dermal equivalents. The plots depict the fold-change expression of (A) extracellular matrix and cell adhesion molecules and (B) growth factors relative to their expression in OERCs of PHKs. Data represent mean values  $\pm$  SD of three independent experiments.



**Figure S4.** Gene expression profiles of co-cultures of MS-1 cells with primary human keratinocytes (PHKs). The plots depict the fold-change expression of (A) extracellular matrix and cell adhesion molecules and (B) growth factors relative to their expression in OERCs of PHKs. Data represent mean values  $\pm$  SD of three independent experiments.



**Figure S5.** Gene expression profiles of co-cultures of MKL-1 cells with primary human keratinocytes (PHKs). The plots depict the fold-change expression of (A) extracellular matrix and cell adhesion molecules and (B) growth factors relative to their expression in OERCs of PHKs. Data represent mean values  $\pm$  SD of three independent experiments.



**Figure S6.** Gene expression profiles of co-cultures of WAGA cells with primary human keratinocytes (PHKs). The plots depict the fold-change expression of (A) extracellular matrix and cell adhesion molecules and (B) growth factors relative to their expression in OERCs of PHKs. Data represent mean values  $\pm$  SD of three independent experiments.