

Huttala et al.:

Presence of Vasculature Results in Faster Insulin Response in Adipocytes in Novel *In Vitro* Vascularized Adipose Tissue Model

Supplementary Data

Tab. S1: Primer sequences used in the one step RT-qPCR and respective annealing temperatures (T)

Gene	Primer sequence	Annealing T used
<i>36B4</i>	Forward- ATGCTCAACATCTCCCCCTTCTCC Reverse- GGGAAAGGTGTAATCCGTCTCCACAG	60.7
<i>Adiponectin</i>	Forward- GGCCGTGATGGCAGAGAT Reverse- CCTTCAGCCCGGTTACT	56
<i>FABP4</i>	Forward- GCTTTTGTAGGTACCTGGAAACTT Reverse- ACACTGATGATCATGTTAGGTTTGG	57
<i>Glut1</i>	Forward- GCT GTG CTT ATG GGC TTC TC Reverse- CAC ATA CAT GGG CAC AAA GC	57.5
<i>Glut4</i>	Forward- CGT CGG GCT TCC AAC AGA TA Reverse- CAC CGC AGA GAA CAC AGC AA	59.2
<i>Leptin</i>	Forward- AGG GAG ACC GAG CGC TTT C Reverse- TGC ATC TCC ACA CAC CAA ACC	60.2
<i>PPARα</i>	Forward- GGC GAA CGA TTC GAC TCA AG Reverse- TCC AAA ACG AAT CGC GTT GT	56.3
<i>PPARγ</i>	Forward- GATCCAGTGGTTGCAGATTACAA Reverse- GAGGGAGTTGGAAGGCTCTTC	61
<i>PPARγ2</i>	Forward- CAGTGTGAATTACAGCAAACC Reverse- ACAGTGTATCAGTGAAGGAAT	52

doi:10.14573/altex.1811271s

ALTEX 36(3), SUPPLEMENTARY DATA

This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International license (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is appropriately cited.

Tab. S2: Primers used in the Fluidigm run and their target genes

Gene	Full name	Entrez ID	Ensemble	Left and right PRIMER
VEGFA	Vascular endothelial growth factor A	7422	ENSG00000112715	tcttcaagccatcgtgtg ggccttggtaggttgat
VEGFR-2 / FLK-1	Kinase Insert Domain Receptor (A Type III Receptor Tyrosine Kinase)	3791	ENSG00000128052	gtgaccaacatggatcgtg tgcttcacagaagaccatgc
VEGFR-1 / FLT-1	fms-related tyrosine kinase 1	2321	ENSG00000102755	aatcattccgaagcaagggtg ttctcccacagtcccaac
PDGF	PDGF-BB, platelet-derived growth factor	5155	ENSG00000100311	aattcaagcacacgcatgac gacggacgagggaaacaata
PDGFRb	Platelet-derived growth factor receptor, beta polypeptide	5159	ENSG00000113721	gtgaaccgactgcagactgt agggtgaggccccgactct
FGF2	Fibroblast Growth Factor 2 (Basic)	2247	ENSG00000138685	agagcgaccctcacatcaag actgccagctcttcagt
FGFR2	Fibroblast Growth Factor Receptor 2	2263	ENSG00000066468	cagagaccaacgttcaagca gagggaagcatggttcgtaa
ANGPT1	Angiopoietin 1	284	ENSG00000154188	aggctggaaggaatataaaatgg cctccagtcatttaactca
ANGPT2	Angiopoietin 2	285	ENSG00000091879	gcatgtgtcctccaact ctcagggtgactggatgtt
AGGF1	angiogenic factor with G patch and FHA domains 1	55109	ENSG00000164252	tggaccatttgcctcaaat ttctgtgcagctctcaaac
TIE2	TEK Tyrosine Kinase, Endothelial	7010	ENSG00000120156	tacacctgcctcatgctcag ttcacaagcctctcacacg
Dkk1	Dickkopf WNT Signaling Pathway Inhibitor 1	22943	ENSG00000107984	tccgaggagaaattgaggaa cctgaggcacagctgatga
DLL4	Delta-Like 1 (Drosophila), DII4	54567	ENSG00000128917	gcgagaagaaagtggacagg acagtagggtcccgtgaatc
Notch 1	Notch homolog 1	4851	ENSG00000148400	actgtgaggacctggtggac ttgagggttgggagggtc
Notch 4	Notch homolog 4	4855	ENSG00000204301	cacgtgaaccatgtgagtc agcagttctgtccatcgtagc
CD 34	CD34 Molecule	947	ENSG00000174059	gagcaggctgatgctgatg cataagttggagtttctggaa
CDH5	VE-cadherin 5	1003	ENSG00000179776	ctctcatcctcaccatcac gaccagctgctcgtggat
GJA1	gap junction protein alpha 1	2697	ENSG00000152661	ggcgtgaggaaagtaccaaa cctccagcagttgagtaggc
OCLN	occludin	100506658	ENSG00000197822	tccaatggcaaagtgaatga gcagggtcttttgaagg
SMO	Smoothed, Frizzled Family Receptor	6608	ENSG00000128602	gggaggctactctcatcc ggcagctgaaggttaatgagc
MMP9	matrix metalloproteinase-9	4318	ENSG00000100985	atccggcacctctatggtc ctgagggtggacagtggtg
FN1	Fibronectin	2335	ENSG00000115414	accaacctacggatgactcg gctcatcatctggccatttt
ITGA5	Integrin, Alpha 5 , Fibronectin Receptor, Alpha Polypeptide	3678	ENSG00000161638	agcctcagaaggaggaggac ggttaatggggtgattggtg
CSPG4 (NG2)	Chondroitin Sulfate Proteoglycan 4	1464	ENSG00000173546	gtctttgaggctgcctgic ctgtgtgacctggaagagca
S1PR1 (S1P1)	sphingosine-1-phosphate	1901	ENSG00000170989	aaattccaccgacccatgta agttattgctcccggtgtgg
NOS3	endothelial nitric oxide synthase, eNOS	4846	ENSG00000164867	ccttctcctggacatcacc ccacttccactcctctgtagc
EGFL7	EGF-like-domain, multiple 7	51162	ENSG00000172889	ttctggtgttggcagtggtg ggtacacacgctgcacga
EGFR	Epidermal growth factor receptor	1956	ENSG00000146648	cagcgtactcctgtcattca tgactcagagagctcagga
TGFB	transforming growth factor beta 1	7040	ENSG00000105329	cacgtggagctgtaccagaa gaaccctgtgatgccactt
TGFB1	transforming growth factor beta receptor 1	7046	ENSG00000106799	tgttgtaaccaaggaaagc cactctgtggttggagcaa
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	2597	ENSG00000111640	cgaccacttgtcaagctca aggggtcatatggcaactg
HPRT	hypoxanthine phosphoribosyltransferase 1	3251	ENSG00000165704	gaccagtcaacagggagcat gtgtcaattatatctccacaatcaag

Tab. S3: Heatmap of vasculature-related genes analyzed in vascularized adipose tissue model
 Concentrations calculated from the standard curve were normalized for housekeeping gene GAPDH, blue indicating down-regulation and red up-regulation. n=3, each analyzed in three technical replicates.

Classification	Gene	Vascularized adipose tissue model	
		without insulin	with 500nM insulin
Angiogenesis master switch	VEGFA	0.36±0.09	0.49±0.11
	VEGFR-2 / FLK-1	1.84±1.35	2.41±0.56
	VEGFR-1/ FLT-1	1.08±0.84	1.23±0.21
Proangiogenic	FGF2	0.94±0.30	0.70±0.14
	FGFR2	3.34± 2.65	7.43± 3.99
	EGFL7	0.74±0.55	1.17±0.22
	EGFR	3.34±0.34	2.34±0.59
Lateral signaling (tip cell selection)	DLL4	1.49±1.15	2.37±0.49
	Notch 1	1.45±0.74	1.82±0.62
	Notch 4	1.59±1.31	1.70±0.31
	AGGF1	1.83± 0.39	2.17±0.44
Maturation	PDGF	0.67±0.52	1.36±0.20
	PDGFRb	2.17±0.85	2.73±0.26
	ANGPT1	3.83±2.10	7.03±0.81
	ANGPT2	2.23±1.66	3.11±0.72
	TIE2	0.43±0.39	0.60±0.12
Arborization/pruning	Dkk1	2.23±0.42	1.56±0.36
	SMO	1.87±0.48	2.36±0.44
	TGFB	0.59±0.14	0.69±0.09
	TGFBR1	1.20±0.13	1.54±0.10
Cell adhesion	CD34	5.27±3.80	7.76±1.50
	VE-cadherin 5	0.69±0.58	1.39±0.25
	GJA1	1.53±0.52	1.66±0.21
	Occludin	1.91±0.76	1.75±0.97
Matrix remodeling	Fibronectin	1.57±0.44	0.89±0.16
	ITGA5	0.43±0.06	0.54±0.06
	NG2	1.09±0.63	1.82±0.88
Metabolism	S1PR1	2.06±1.54	2.04±0.52
	eNOS	1.95±2.44	2.16±3.13
	HPRT	0.82±0.09	0.78±0.13