Supplementary Information

Enhanced Biocompatibility of 3D Printed Resin Parts via Wet Autoclave Postprocessing: implications for stem cell organ-on-a-chip cultures

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Supporting Figures

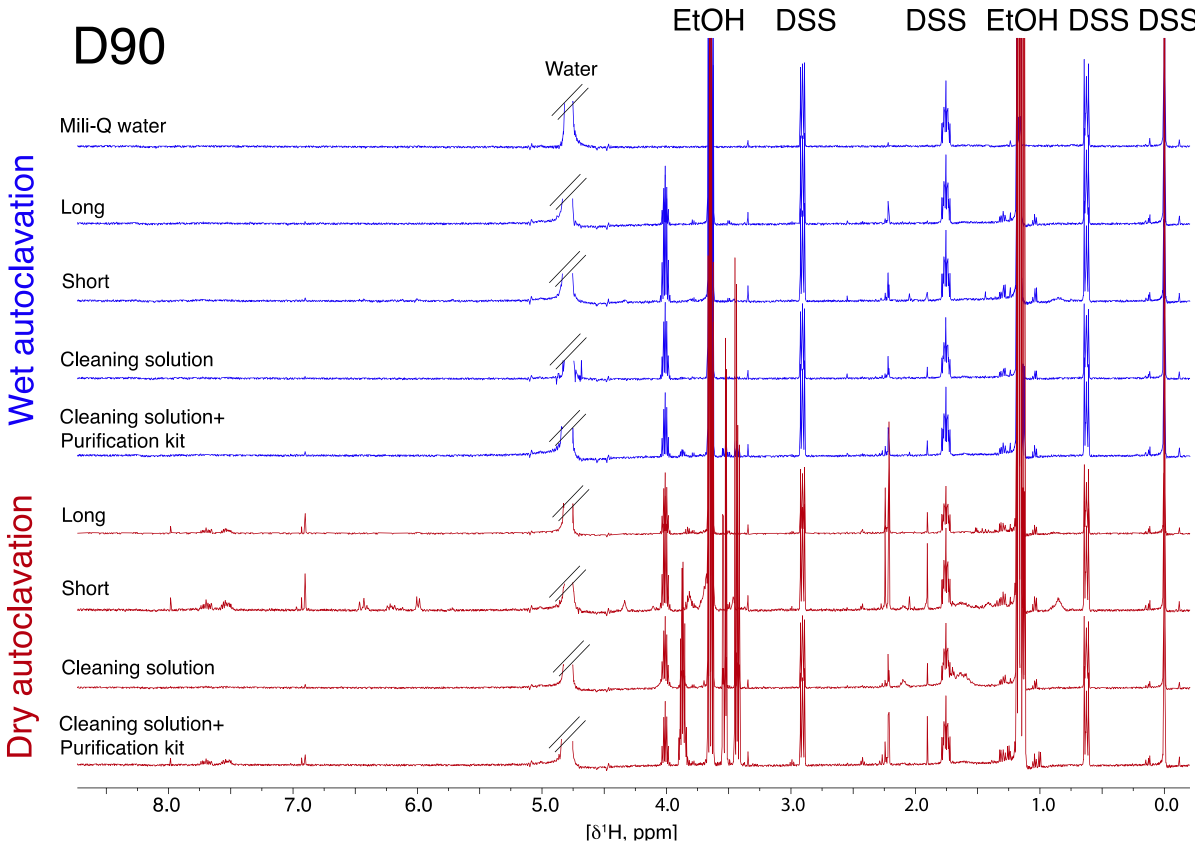

Figure S1. NMR traces showing the peaks after different processing.

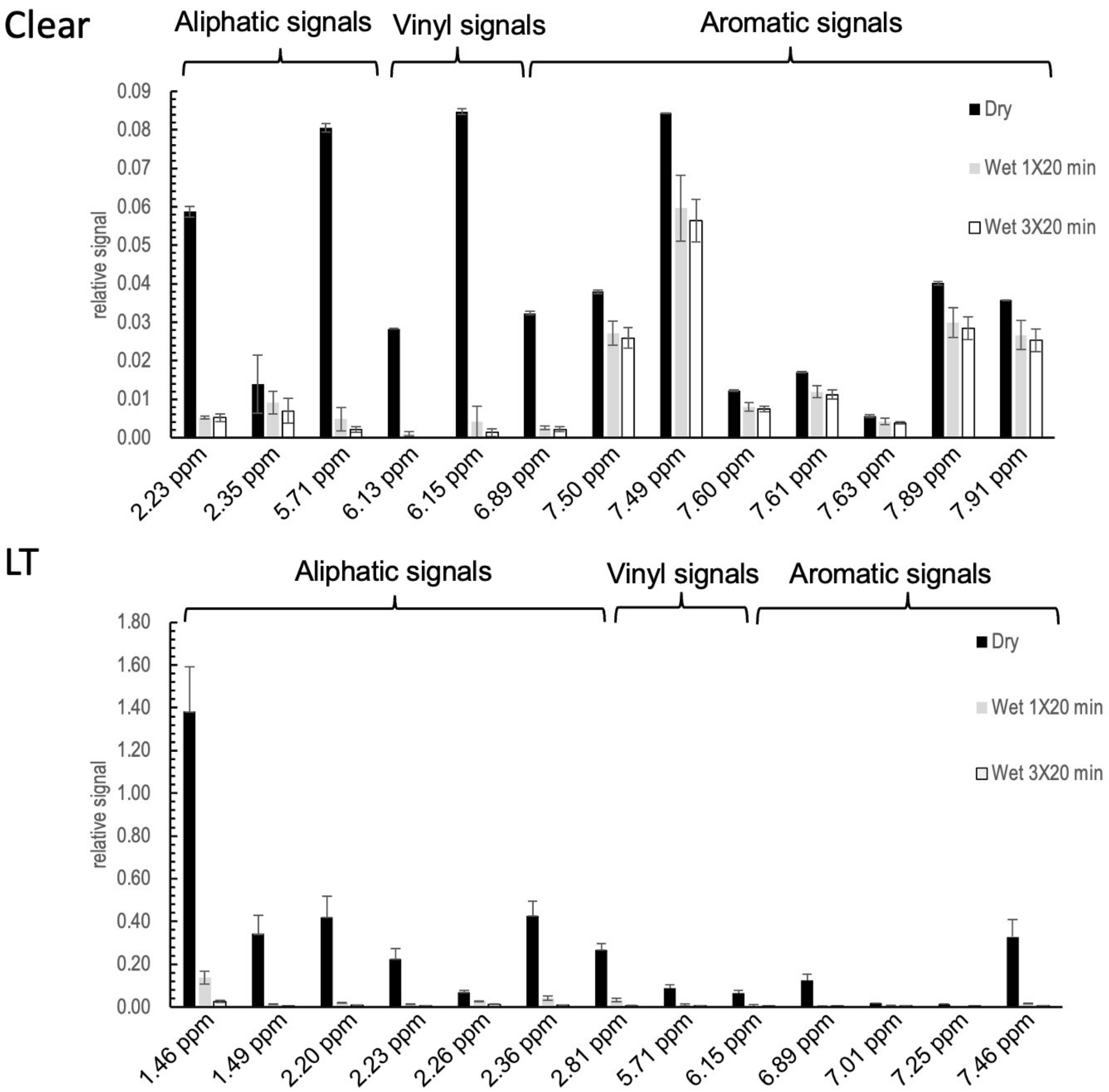

Figure S2. Quantified peaks from three independent experiments using Formlab Clear and Dental LT resins.

Integrity and resolution of resins after autoclavation

Next, we investigated how well prints structurally survive wet autoclavation. A test structure was printed and subjected to different autoclavation procedures (**Figure 3**). The results show that D90 gave high resolution prints using a Phrozen 8k printer. There was no physical change in the D90 structure after autoclavation. BM Clear resulted in poorer prints and one explanation is probably due to the larger pixel size used in the Formlab printer compared to the Phrozen printer. The Biomed Clear also survived autoclavation without changes to the structure. As a control, Formlabs Clear resin resulted in well-defined prints comparable to Biomed Clear. The prints, however, did not survive autoclavation and started to delaminate (yellow rectangle, **Figure 3**) which agrees with the manufacturer's specifications. Dental LT resulted in very poor prints, comparably, but no further aberrations were observed after autoclavation.

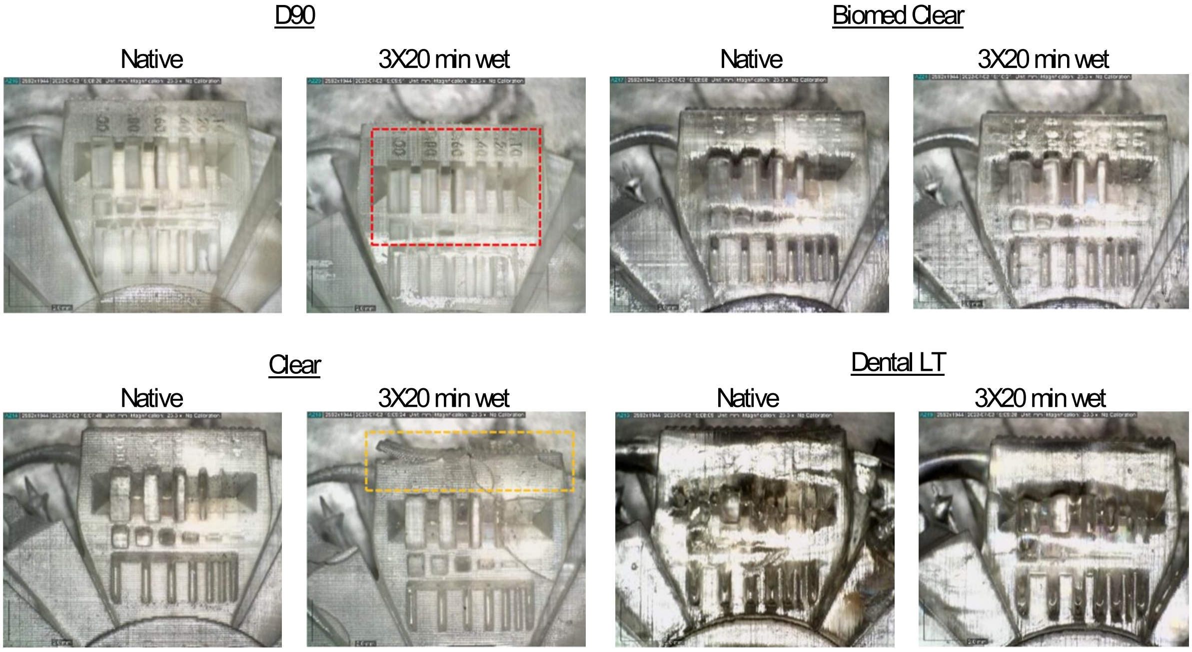

Figure S3. Print resolution and effect of autoclavation. The test prints were processed using the short protocol (Native) and subjected to 3X20 minute wet autoclavation. Red box shows a well-defined print with no aberrations after autoclavation. Yellow rectangle shows examples of delaminated prints.

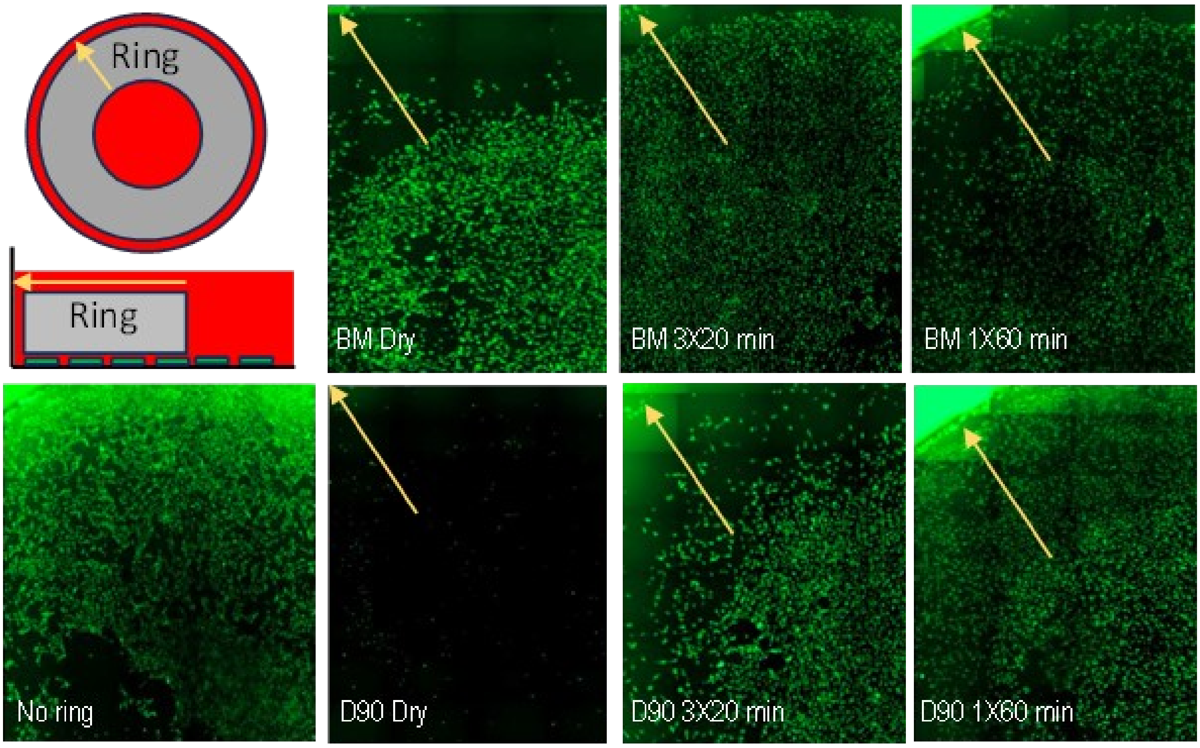

Figure S4. Tile scan of microscope pictures of live/dead cells cultured with rings of BM and D90. The rings sit on top of the plated cells in the periphery of a well, leaving the center without 3D printed material. The top left panel shows a principal sketch of the setup from a top and side view where the ring is in grey, medium in red, and cells are in green. ADSCs were cultured for 6 days without splitting, but the medium was changed after three days. The rings were removed before imaging due to significant autofluorescence. Arrowheads indicate the wall of the titer plate with large autofluorescence. The length of the arrow indicates approximately the width (3 mm) of the ring.

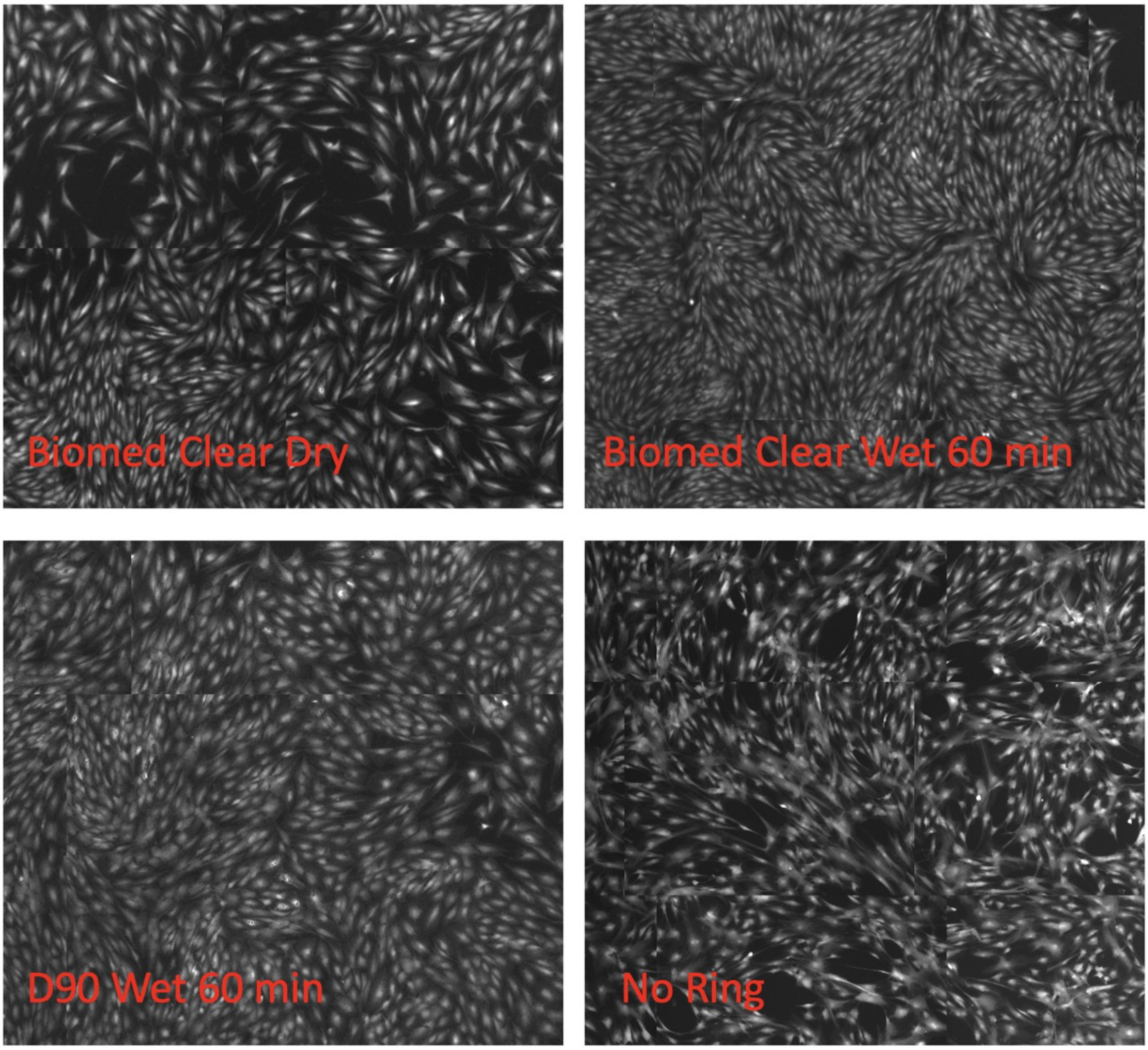

Figure S5 Morphology and of undifferentiated ADSC after a live dead stain using different post processing. The morphology of cells is similar to cells grown without rings.

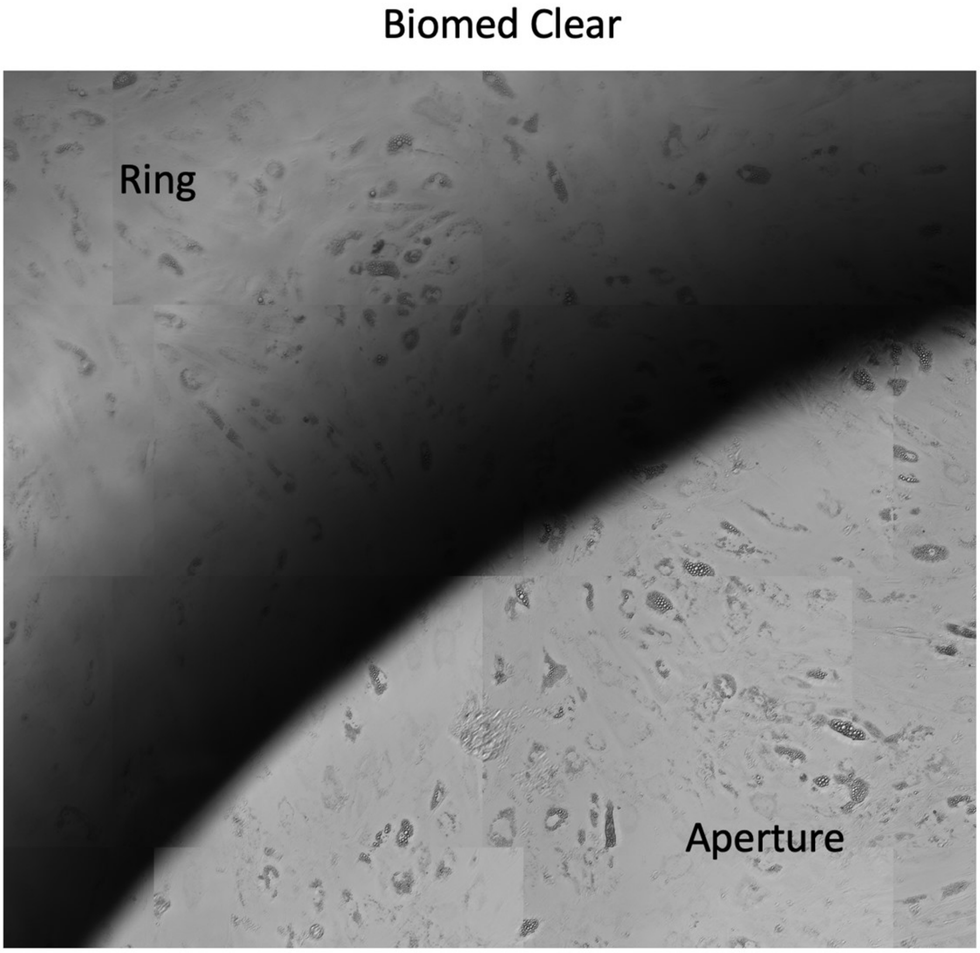

Figure S6. Adipocytes growth in the presence of a 3D-printed ring made from Biomed Clear.

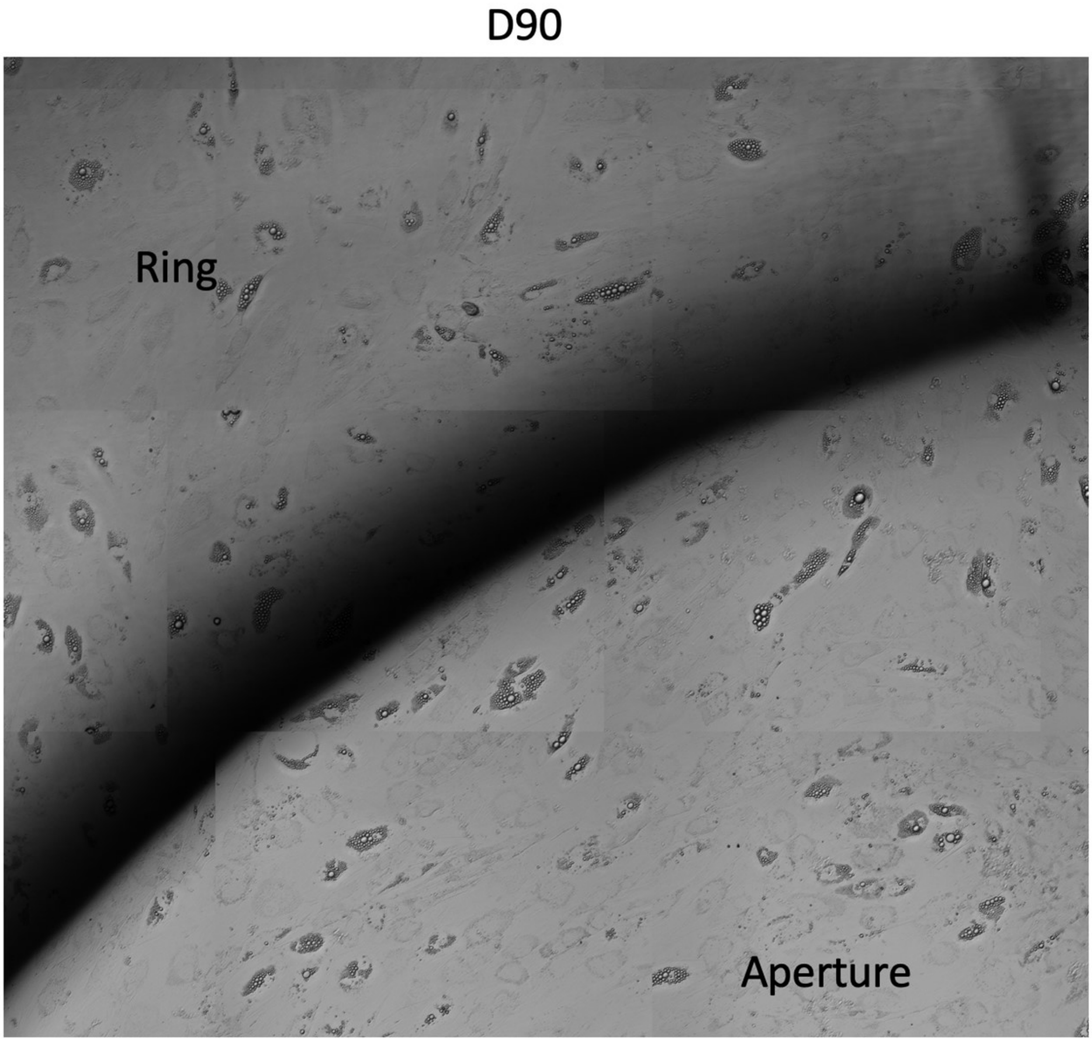

Figure S7. Adipocytes growth in the presence of a 3D-printed ring made from Biotough D90 MF.

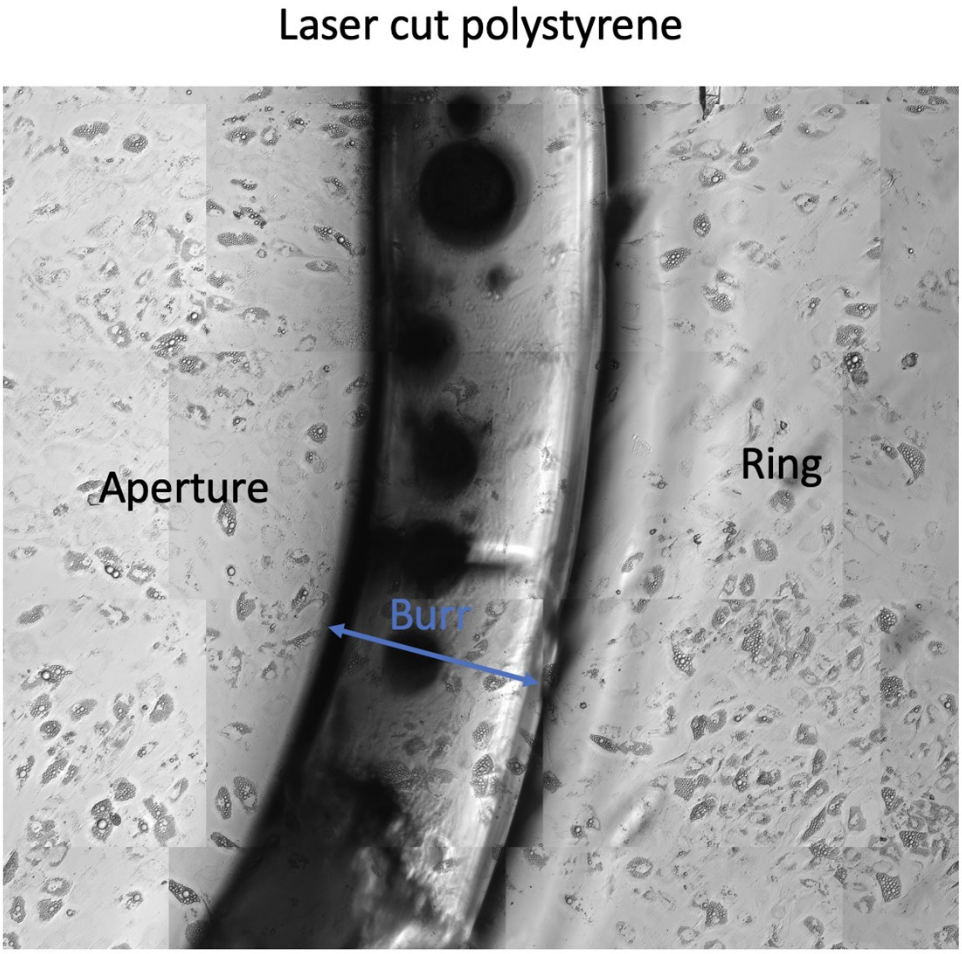

Figure S8. Adipocytes growth in the presence of a laser-cut ring made from polystyrene.

Supporting Tables

Table S1. Full lists of regulated genes

See separate file

Table S2. List of regulated genes for ADSCs grown in the presence of rings 3D-printed from Biomed Clear. Adjusted P<0.05.

BM								
GeneID	**Full name**	**Function**	**Base mean**	**log2(FC)**	**StdErr**	**Wald-Stats**	**P-value**	**P-adj**
ITGA4	integrin subunit alpha 4	Cell adhesion	10.91	7.62	1.80	4.24	2.28E-05	1.01E-02
PLD1	phospholipase D1	produce phosphatidic acid, possibly needed for endocytosis	10.66	5.86	1.44	4.06	4.85E-05	1.61E-02
PTPRG	protein tyrosine phosphatase receptor type G	Cell growth, differentiation	14.13	3.74	1.00	3.73	1.91E-04	4.42E-02
FABP3	fatty acid binding protein 3	Fatty acid metabolism	28.41	3.71	0.72	5.19	2.15E-07	2.62E-04
NRDC	nardilysin convertase	Migration, proliferation	212.04	1.31	0.31	4.27	1.99E-05	9.61E-03
USP4	ubiquitin specific peptidase 4	Substrates involved in TGF-β and Wnt/β-catenin pathways	88.02	1.30	0.35	3.71	2.07E-04	4.59E-02
SCD	stearoyl-CoA desaturase	Fatty acid synthesis	193.22	1.29	0.30	4.30	1.69E-05	9.01E-03
TXNRD1	thioredoxin reductase 1	Oxireductase	150.95	1.27	0.33	3.80	1.47E-04	3.91E-02
FDFT1	farnesyl-diphosphate farnesyltransferase 1	Sterol synthesis (cholesterol)	415.86	1.14	0.20	5.81	6.07E-09	1.08E-05
ACO1	aconitase 1	Carbon metabolism	150.65	1.06	0.28	3.85	1.20E-04	3.36E-02
SPARC	Secreted protein acidic and cystein rich	ECM synthesis, calcification of collagen	3060.06	-0.75	0.11	-6.69	2.25E-11	1.20E-07
SERPINF1	Serpin family F member 1	VEGF inhibiting, downregulated by hypoxia, suppresses migration and proliferation in endothelial cells	1200.65	-0.84	0.14	-5.96	2.55E-09	6.79E-06
MFAP4	microfibril associated protein 4	ECM	239.01	-0.91	0.24	-3.78	1.55E-04	3.92E-02
POSTN	periostin	ECM, related to integrins	2860.24	-0.95	0.19	-4.97	6.77E-07	6.01E-04
COL1A1	collagen type I alpha 1 chain	ECM	643.83	-1.08	0.21	-5.16	2.46E-07	2.62E-04
BGN	biglycan	ECM, related to decorin	145.38	-1.22	0.31	-3.94	8.22E-05	2.58E-02
TGFBI	transforming growth factor beta induced	ECM, cell-collagen interaction, bone formation	328.61	-1.28	0.29	-4.43	9.60E-06	6.39E-03
IGFBP3	insulin like growth factor binding protein 3	Secreted, limits proliferation	169.89	-1.36	0.33	-4.12	3.76E-05	1.43E-02
DPT	dermatopontin	ECM, related to decorin	176.22	-2.03	0.47	-4.30	1.68E-05	9.01E-03
TIMP3	TIMP metallopeptidase inhibitor 3	ECM remodelling	69.03	-2.12	0.52	-4.10	4.20E-05	1.49E-02
TOP2B	DNA topoisomerase II beta	Alters DNA topology	15.05	-6.57	1.75	-3.76	1.67E-04	4.03E-02
OSBPL6	oxysterol binding protein like 6	lipid receptor, cholesterol trafficing regulator	16.11	-6.68	1.72	-3.88	1.02E-04	3.03E-02
GDE1	glycerophosphodiester phosphodiesterase 1	glycerophospholipid metabolism	17.95	-6.85	1.65	-4.15	3.26E-05	1.34E-02
SKIV2L2	Ski2 like RNA helicase 2	located exosome	34.22	-7.77	1.62	-4.81	1.52E-06	1.16E-03

Table S3. List of regulated genes for ADSCs grown in the presence of rings 3D-printed from Biotough D90 MF. Adjusted P<0.05.

D90

GeneID	Full name	Function	Base mean	log2(FC)	StdErr	Wald-Stats	P-value	P-adj
SIPA1L1	signal induced proliferation associated 1 like 1	reorganization of actin cytoskeleton	9.48	5.71	1.34	4.25	2.13E-05	4.94E-03
OGDH	oxoglutarate dehydrogenase	carbon metabolism	10.89	5.30	1.52	3.49	4.77E-04	4.88E-02
FABP3P2	fatty acid binding protein 3 pseudogene 2	Fatty acid metabolism	5.74	4.94	1.38	3.57	3.50E-04	4.59E-02
HOOK3	hook microtubule tethering protein 3	microtubule-organell binding	12.43	4.94	1.31	3.77	1.63E-04	2.75E-02
ANKRD28	ankyrin repeat domain 28	Cell migration, adhesion	9.35	4.93	1.25	3.94	8.30E-05	1.50E-02
STK4	serine/threonine kinase 4	apoptosis	8.04	4.80	1.28	3.76	1.73E-04	2.87E-02
STARD5	StAR related lipid transfer domain containing 5	cholesterol transporter	6.84	4.55	1.29	3.53	4.11E-04	4.82E-02
FABP3	fatty acid binding protein 3	Fatty acid metabolism	54.36	4.03	0.45	8.91	5.21E-19	2.12E-15
PDS5A	PDS5 cohesin associated factor A	Gene expression	11.81	3.94	1.00	3.92	8.84E-05	1.56E-02
CAPN7	calpain 7	unknown	10.73	3.66	1.05	3.48	5.09E-04	4.98E-02
ACSL4	acyl-CoA synthetase long chain family member 4	lipid synthesis, fatty acid degradation	31.52	2.40	0.59	4.07	4.79E-05	9.73E-03
PCSK9	proprotein convertase subtilisin/kexin type 9	cholesterol and fatty acid metabolism	38.69	2.37	0.46	5.17	2.30E-07	1.04E-04
ZNF581	coiled-coil domain containing 106	transcription	23.33	2.07	0.51	4.03	5.59E-05	1.11E-02
ABI3BP	ABI family member 3 binding protein	ECM, cell adhesion	98.62	2.01	0.40	5.07	3.93E-07	1.68E-04
LSS	lanosterol synthase	Cholesterol synthesis	41.01	1.95	0.53	3.69	2.27E-04	3.42E-02
AKR1C1	aldo-keto reductase family 1 member C1	Inactivates ketosteroid hormones, specific adipose	266.33	1.89	0.33	5.76	8.41E-09	5.69E-06
PLCD1	phospholipase C delta 1	intracellular signal transduction	32.42	1.87	0.41	4.62	3.86E-06	1.01E-03
NQO1	NAD(P)H quinone dehydrogenase 1	ROS protection	311.70	1.66	0.23	7.20	6.14E-13	9.98E-10
LASP1	LIM and SH3 protein 1	reorganization of cytoskeleton during cell motility	30.54	1.61	0.45	3.59	3.35E-04	4.46E-02
AKR1C4	aldo-keto reductase family 1 member C4	Inactivates ketosteroid hormones, specific adipose	56.69	1.55	0.31	5.00	5.88E-07	2.08E-04
SCD	stearoyl-CoA desaturase	Fatty acid synthesis	297.91	1.53	0.31	4.92	8.81E-07	2.98E-04
SOD2	superoxide dismutase 2	ROS protection	42.87	1.49	0.43	3.50	4.68E-04	4.88E-02
USO1	USO1 vesicle transport factor	vesicle transport factor	74.97	1.49	0.43	3.48	4.97E-04	4.92E-02
PAM	peptidylglycine alpha-amidating monooxygenase	biosynthesis of many signaling peptides and some fatty acid amides	122.51	1.47	0.42	3.49	4.86E-04	4.88E-02
AKR1C2	aldo-keto reductase family 1 member C2	Inactivates ketosteroid hormones, specific adipose	281.97	1.44	0.22	6.44	1.23E-10	1.25E-07
PGLS	6-phosphogluconolactonase	carbon metabolism	52.74	1.41	0.35	4.02	5.83E-05	1.13E-02
TXNRD1	thioredoxin reductase 1	Oxireductase	216.03	1.38	0.20	6.85	7.62E-12	1.03E-08
FDPS	farnesyl diphosphate synthase	Sterol synthesis (cholesterol)	129.47	1.35	0.33	4.09	4.35E-05	9.05E-03
USP14	ubiquitin specific peptidase 14	deubiquitinase, antiviral immunity	52.99	1.31	0.37	3.52	4.34E-04	4.82E-02
FDPSP1	farnesyl diphosphate synthase pseudogene 1	Sterol synthesis (cholesterol)	104.46	1.31	0.30	4.41	1.04E-05	2.48E-03
PGD	phosphogluconate dehydrogenase	carbon metabolism	79.94	1.30	0.31	4.20	2.66E-05	5.83E-03
HEBP1	heme binding protein 1	promotes calcium mobilization	59.56	1.30	0.35	3.73	1.90E-04	3.09E-02

FADS2	fatty acid desaturase 2	Fatty acid metabolism, Fatty acid desaturation	153.16	1.29	0.26	5.04	4.76E-07	1.84E-04
PGDP1	phosphogluconate dehydrogenase pseudogene 1	carbon metabolism	69.36	1.26	0.30	4.17	3.04E-05	6.51E-03
TRIM22	tripartite motif containing 22	antiviral immunity	68.01	1.24	0.26	4.87	1.14E-06	3.43E-04
ACAT2	sterol O-acyltransferase 2	Lipid metabolism	246.11	1.21	0.26	4.76	1.98E-06	5.56E-04
ATF4	activating transcription factor 4	Transcription	136.43	1.21	0.34	3.51	4.53E-04	4.87E-02
TKT	transketolase	carbon metabolism	663.86	1.20	0.25	4.88	1.07E-06	3.34E-04
DHCR7	7-dehydrocholesterol reductase	Cholesterol synthesis	83.93	1.17	0.33	3.56	3.73E-04	4.74E-02
VIM	vimentin	cytoskeleton component	1432.80	1.07	0.28	3.79	1.53E-04	2.64E-02
FDFT1	farnesyl-diphosphate farnesyltransferase 1	Sterol synthesis (cholesterol)	520.97	1.02	0.14	7.33	2.35E-13	4.76E-10
PERP	p53 apoptosis effector related to PMP22	desmosome cell-cell junctions	68.50	0.92	0.25	3.67	2.40E-04	3.52E-02
CD44	CD44 molecule (IN blood group)	Cell-cell interaction, cell adhesion, migration	417.11	0.92	0.26	3.56	3.77E-04	4.74E-02
FTLP3	ferritin light chain pseudogene 3	regulate iron storage	1374.28	0.91	0.19	4.66	3.15E-06	8.53E-04
IDI1	isopentenyl-diphosphate delta isomerase 1	Sterol synthesis (cholesterol)	118.44	0.89	0.26	3.49	4.86E-04	4.88E-02
AKR1C3	aldo-keto reductase family 1 member C3	Inactivates ketosteroid hormones, specific adipose	289.34	0.88	0.17	5.35	8.99E-08	4.29E-05
FTL	ferritin light chain	regulate iron storage	3874.08	0.86	0.18	4.91	9.29E-07	3.02E-04
AHNAK	AHNAK nucleoprotein	Cell structure, migration	93.79	0.81	0.23	3.53	4.12E-04	4.82E-02
GPI	glucose-6-phosphate isomerase	carbon metabolism	236.27	0.78	0.20	4.01	6.07E-05	1.15E-02
PHGDH	phosphoglycerate dehydrogenase	carbon metabolism	471.36	0.59	0.12	5.02	5.29E-07	1.95E-04
SARS	seryl-tRNA synthetase 1	protein translation	551.99	-0.51	0.14	-3.72	1.99E-04	3.11E-02
AP2M1	adaptor related protein complex 2 subunit mu 1	protonpumping in endosomes and lysosomes	266.85	-0.51	0.14	-3.73	1.95E-04	3.10E-02
ANXA2	annexin A2	cell growth, osteoclast formation, cell motility, cell-cell/cell-ECM interaction	981.01	-0.51	0.14	-3.55	3.80E-04	4.74E-02
ANXA2P2	annexin A2 pseudogene 2	endocytosis, cholesterol transport	1049.72	-0.51	0.14	-3.63	2.80E-04	3.92E-02
CLU	clusterin	extracellular molecular chaperone, degradation of missfolded proteins	3773.21	-0.62	0.10	-6.20	5.56E-10	5.02E-07
LUM	lumican	ECM assembly, decorin like	376.83	-0.64	0.18	-3.52	4.39E-04	4.82E-02
ERP44	endoplasmic reticulum protein 44	ER protein	209.21	-0.67	0.19	-3.50	4.61E-04	4.87E-02
MFAP2	microfibril associated protein 2	(ECM?),antigen of elastin-associated microfibrils	171.65	-0.89	0.24	-3.66	2.57E-04	3.66E-02
COL6A3	collagen type VI alpha 3 chain	ECM	607.69	-0.89	0.25	-3.53	4.17E-04	4.82E-02
POSTN	periostin	ECM, related to integrins	3014.77	-0.93	0.26	-3.62	2.97E-04	4.09E-02
SERPINF1	serpin family F member 1	VEGF inhibiting, downregulated by hypoxia, suppresses migration and proliferation in endothelial cells	1228.84	-0.96	0.14	-6.64	3.12E-11	3.62E-08
ISLR	immunoglobulin superfamily containing leucine rich repeat	Cell adhesion	138.08	-0.96	0.20	-4.83	1.36E-06	3.94E-04
SPARC	secreted protein acidic and cysteine rich	ECM synthesis, calcification of collagen	3043.34	-1.00	0.17	-5.85	4.85E-09	3.58E-06
GOLGA7	golgin A7	Golgi protein	97.06	-1.03	0.29	-3.59	3.25E-04	4.40E-02
GNL2	G protein nucleolar 2	RNA binding	68.03	-1.12	0.28	-3.94	8.14E-05	1.50E-02
SAP30BP	SAP30 binding protein	transcriptional repressor	70.68	-1.16	0.33	-3.54	3.99E-04	4.82E-02
MFAP4	microfibril associated protein 4	ECM, cell adhesion or intercellular interactions	232.18	-1.20	0.26	-4.61	4.01E-06	1.02E-03

FBLN5	fibulin 5	ECM, cell adhesion	288.75	-1.24	0.22	-5.53	3.21E-08	1.74E-05
DCN	decorin	ECM assembly	2119.30	-1.39	0.17	-8.09	6.13E-16	1.66E-12
APOD	apolipoprotein D	upregulated by steroids, varied function, lipid metabolism, excreated	81.14	-1.61	0.38	-4.23	2.31E-05	5.22E-03
COL3A1	collagen type III alpha 1 chain	ECM	2245.28	-1.63	0.30	-5.46	4.71E-08	2.39E-05
KLF10	KLF transcription factor 10	transcriptional repressor	27.37	-1.71	0.47	-3.67	2.43E-04	3.52E-02
ERCC8	ERCC excision repair 8, CSA ubiquitin ligase complex subunit	DNA repair	34.95	-2.23	0.49	-4.53	5.78E-06	1.42E-03
DPT	dermatopontin	ECM, related to decorin	161.04	-2.31	0.25	-9.28	1.76E-20	1.43E-16
SFRP2	secreted frizzled related protein 2	modulates Wnt signaling	55.69	-2.69	0.44	-6.08	1.19E-09	9.67E-07
TNC	tenascin C	ECM, adhesion, proliferation, migration	71.81	-3.29	0.58	-5.63	1.82E-08	1.05E-05
MMP3	matrix metallopeptidase 3	ECM remodelling	24.82	-3.74	0.65	-5.73	9.82E-09	6.14E-06
SGPL1	sphingosine-1-phosphate lyase 1	cholesterol and fatty acid metabolism, apoptosis	6.95	-4.15	1.18	-3.52	4.24E-04	4.82E-02
YTHDC1	YTH N6-methyladenosine RNA binding protein C1	mRNA splicing	4.53	-5.05	1.43	-3.53	4.09E-04	4.82E-02
TEAD2	TEA domain transcription factor 2	transcription factor, apoptosis, YAP/TAZ	4.60	-5.06	1.44	-3.50	4.57E-04	4.87E-02
PI16	peptidase inhibitor 16	extracellular, not clearly defined	4.29	-5.74	1.63	-3.52	4.32E-04	4.82E-02
WDR78	dynein axonemal intermediate chain 4	not clear	4.46	-5.76	1.56	-3.70	2.16E-04	3.31E-02
KDM3A	lysine demethylase 3A	splicing of cell-cycle genes following DNA damage	9.28	-6.82	1.35	-5.05	4.48E-07	1.82E-04

Table S4. Genes related to the pentose phosphate pathway. Red indicates genes not significantly differentially expressed. Green indicates genes differentially expressed at adjusted P<0.05. Metabolites in bold connect to the glycolysis.

Step	Gene	Enzyme	Log2(FC) BM	adjusted P BM	Log2(FC) D90	adjusted P D90	Reaction	Co-reaction
Glucose-6-phosphate								
1	G6PD	Glucose-6-phosphate dehydrogenase	0.612	1	1.16	0.151	(3) Glucose-6-phosphate -> (3) 6-phosphoglucono-B-lactone	(3) NADP+ -> (3) NADPH + H+
6-phosphoglucono-B-lactone								
2	PGLS	6-phosphogluconolactonase	0.608	1	1.41	0.0113	(3) 6-phosphoglucono-B-lactone -> (3) 6-phosphogluconate	(3) H20 -> (3) H+
6-phosphogluconate								
3	PGD	6-phosphogluconate dehydrogenase	0.521	1	1.3	0.00583	(3) 6-phosphogluconate -> (3) Ribulose 5-phosphate	(3) NADP+ -> (3) NADPH + H+
Ribulose 5-phosphate								
4-1	RPE	Phosphopentose epimerase	-	-	0.82	0.998	Ribulose 5-phosphate <-> Xylose 5-phosphate	
Xylose 5-phosphate								
4-2	RPIA	Phosphopentose isomerase	0.194	1	-0.336	0.998	Ribulose 5-phosphate <-> Ribose 5-phosphate	
Ribose 5-phosphate								
5-1	TKT	Transketolase	0.297	1	1.2	0.000334	Various together with Transaldolase + Fructose 6-phosphate <-> Glyceraldehyde 3-phosphate	
Glyceraldehyde 3-phosphate								
5-2	TALDO1	Transaldolase	-0.22	1	0.673	0.357	Various together with Transketolase + Fructose 6-phosphate <-> Glyceraldehyde 3-phosphate	
Glyceraldehyde 3-phosphate								