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Supplementary Information for

Extracellular Vesicles Mediate Improved Functional Outcomes in Engineered Cartilage Produced from MSC/Chondrocyte Co-Cultures

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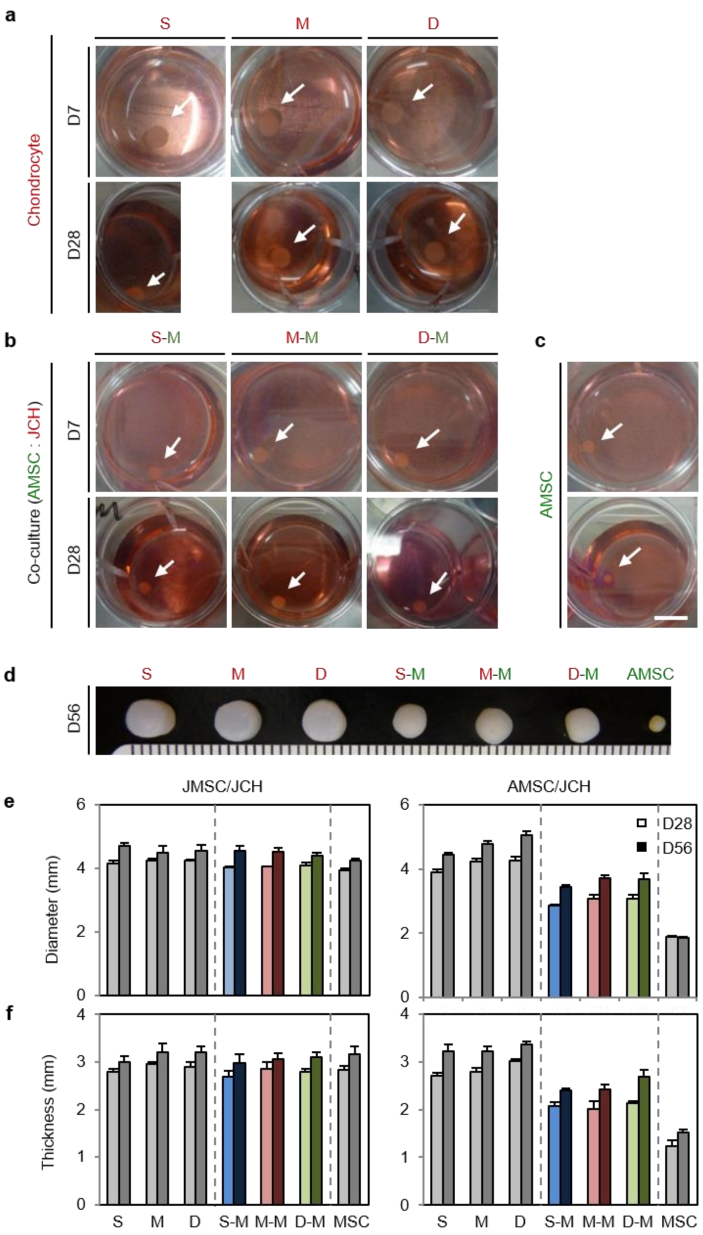


Fig. S1. Growth of zonal JCH-, AMSC- and co-cultured constructs. (a) Zonal JCHs, **(b)** AMSC/JCH co-cultures and **(c)** AMSCs alone in MeHA hydrogels at day 7 and 28. **(d)** Gross images of cell-laden constructs at day 56 (AMSC/JCH co-cultures). **(e and f)** Construct dimensions (diameter and thickness) on day 28 and day 56 (left column = JMSC/JCH and right column = AMSC/JCH; lighter bars = day 28 and darker bars = day 56).

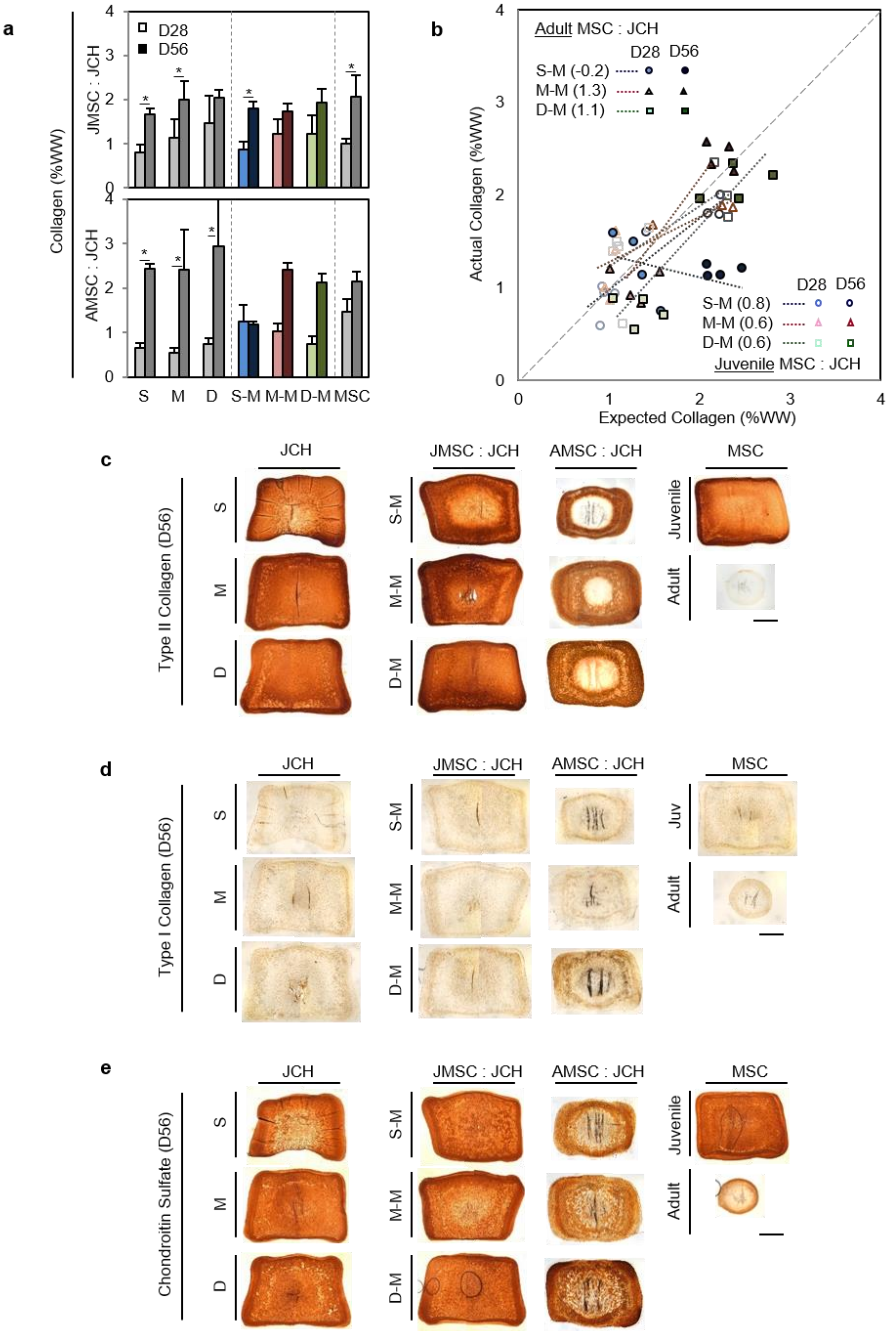


Fig. S2. Collagen content and immunohistochemistry for collagens and proteoglycans. (a) Collagen content (%WW). **(b)** Plot of co-culture efficacy for collagen content. **(c-e)** Immunohistochemistry for **(c)** type II collagen, **(d)** type I collagen, and **(e)** chondroitin sulfate at day 56 (JCH = left, Co-cultures = middle, AMSC = right; scale bar = 1mm; *p*<0.05).

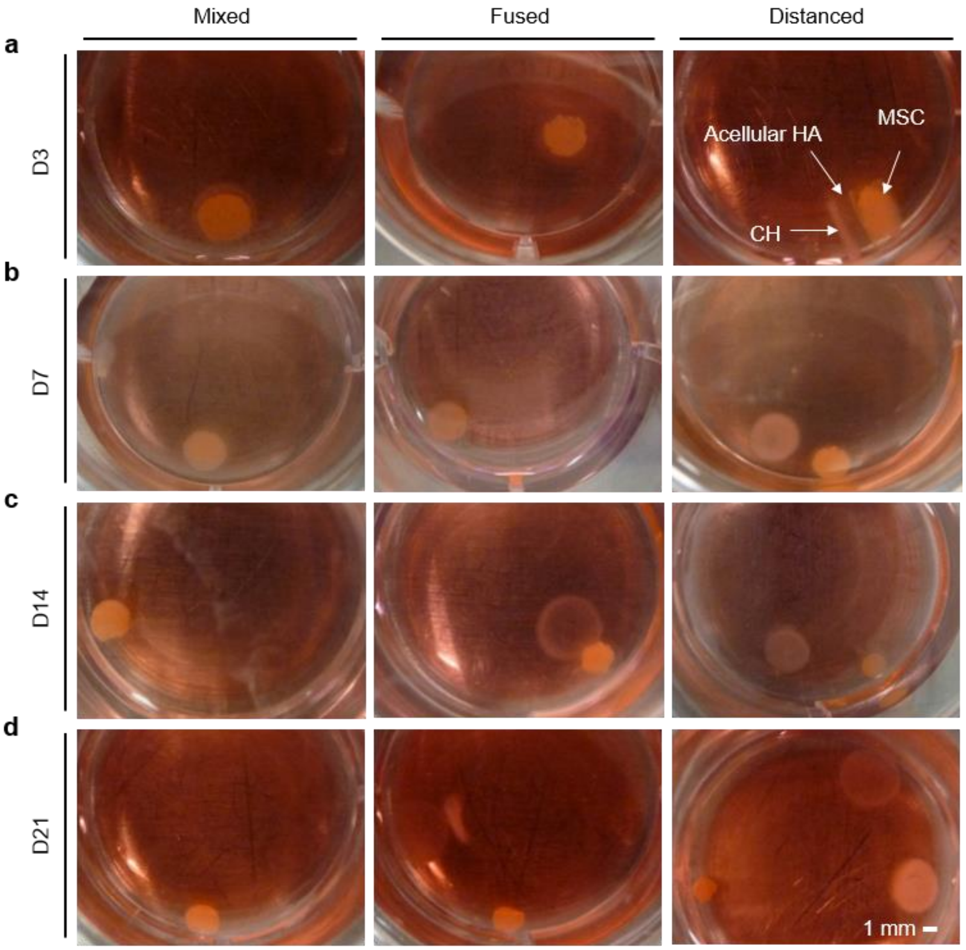


Fig. S3. Gross appearance of 'mixed', 'fused' and 'distanced' co-cultures of JCHs and AMSCs in MeHA hydrogel constructs. Images of mixed (left), fused (middle), and distanced (right) constructs on day 3, 7, 14 and 21 (scale bar = 1mm).

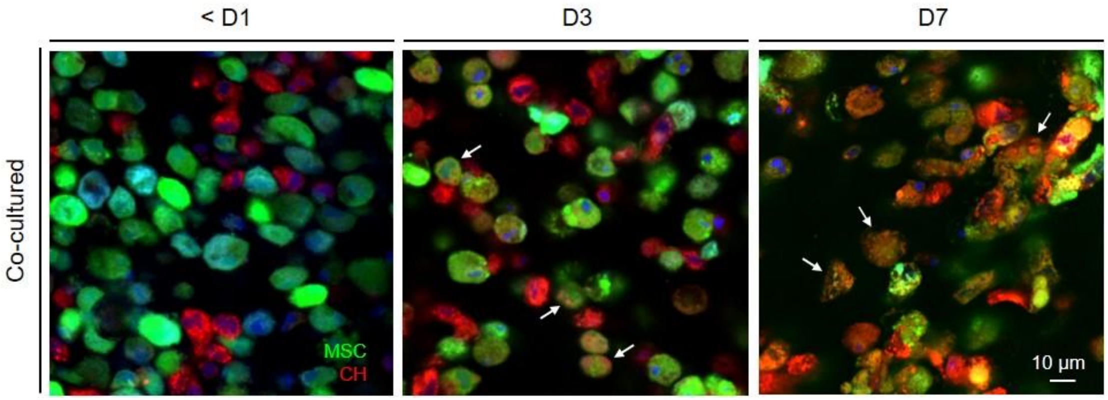


Fig. S4. Transfer of intracellular contents in AMSC/JCH co-cultures. Confocal images of JCH (red) and AMSCs (green) in MeHA hydrogels on days 3 and 7, showing increasing number of double positive cells (DP, arrow heads) (scale bar = 10µm).

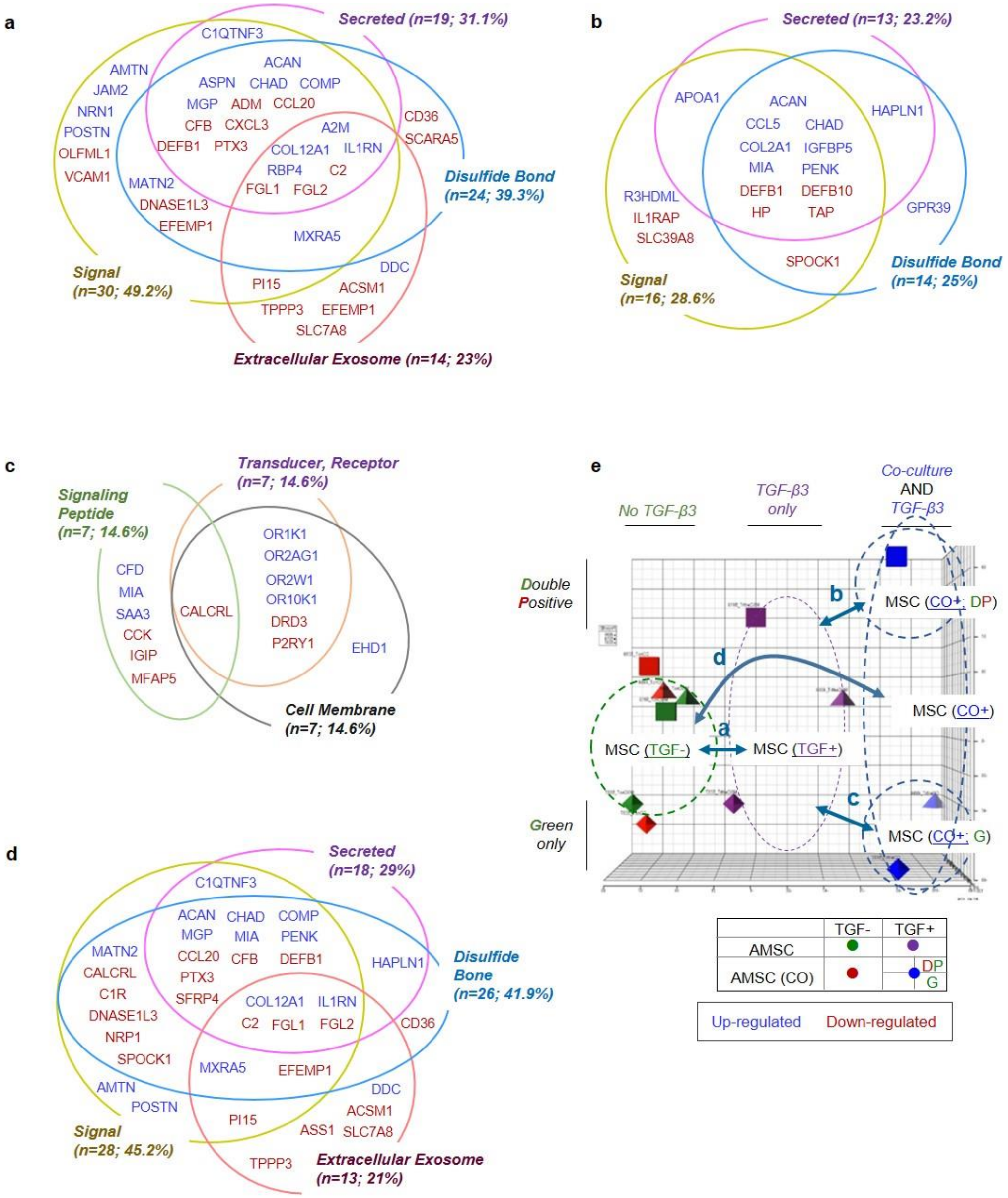


Fig. S5. Cluster analysis showing pathways induced by TGF-β3 and/or factors secreted from juvenile chondrocytes to adult MSCs in co-culture, displayed via Venn diagrams. Blue font indicates upregulation, and red font indicates down regulation. **(a)** Adult MSCs alone without TGF-β3 (TGF-_G) vs. with TGF-β3 (TGF+_G), **(b)** Adult MSCs alone with TGF-β3 (TGF+_G) vs. adult MSCs that were co-cultured with TGF-β3 and became double positive (CO+_DP), **(c)** Adult MSCs with TGF-β3 (TGF+_G) vs. adult MSCs that were co-cultured with TGF-β3 and remained green only (CO+_G), **(d)** Adult MSCs without TGF-β3 (TGF-_G) vs. adult MSCs that were co-cultured with TGF-β3 (CO+), **(e)** PCA analysis from Fig. 4e.

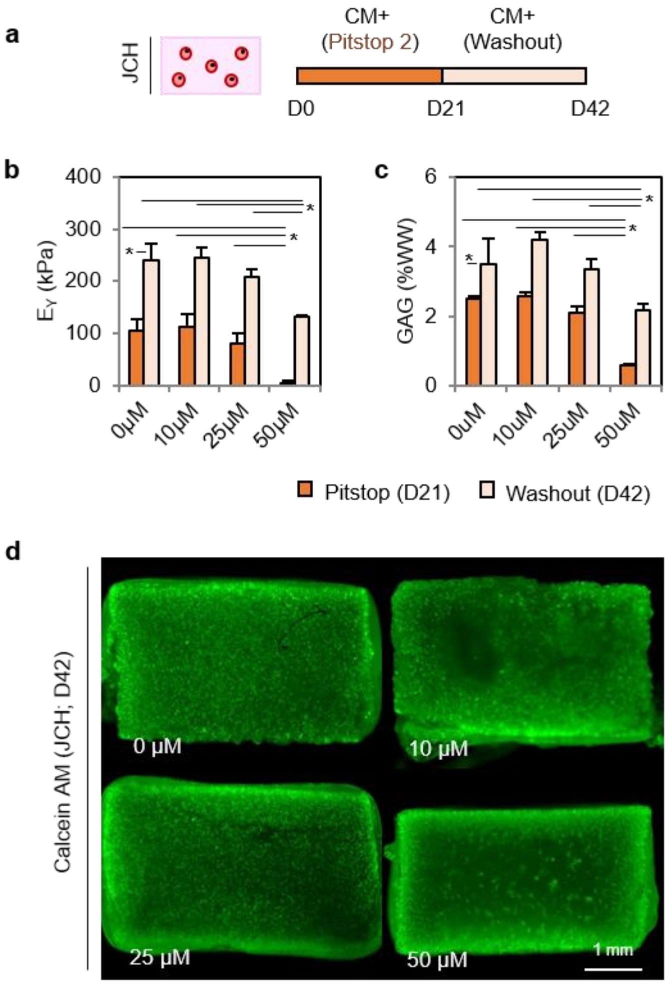


Fig. S6. Calibration of inhibitors of clathrin-mediated vesicle formation in JCH-laden constructs. (a) To determine the dose-dependent impact and reversibility of Pitstop2 in exocytic release of extracellular proteins, JCH-laden constructs were cultured in CM+ in the presence of Pitstop2 at a varying concentration (0, 10, 25 and 50μM) for the first 21 days (D0-21) followed by culture for an additional 21 days (D22-42) in the absence of Pitstop2 (washout). Although some attenuation in cellularity and construct properties was noted with the highest dose (50μM) employed ($p<0.001$), functional properties and GAG content increased after removal of Pitstop2. **(b)** Analysis of mechanical properties (E_Y: kPa), **(c)** GAG content (%WW). **(d)** Cells from all groups were viable and produced comparable ECM. Viability staining (Calcein-AM labeling on day 42) indicated that a dose of 25μM would not inhibit normal chondrocyte matrix accumulation or viability (scale bar = 1mm, n=3-4/group; $p<0.05$).

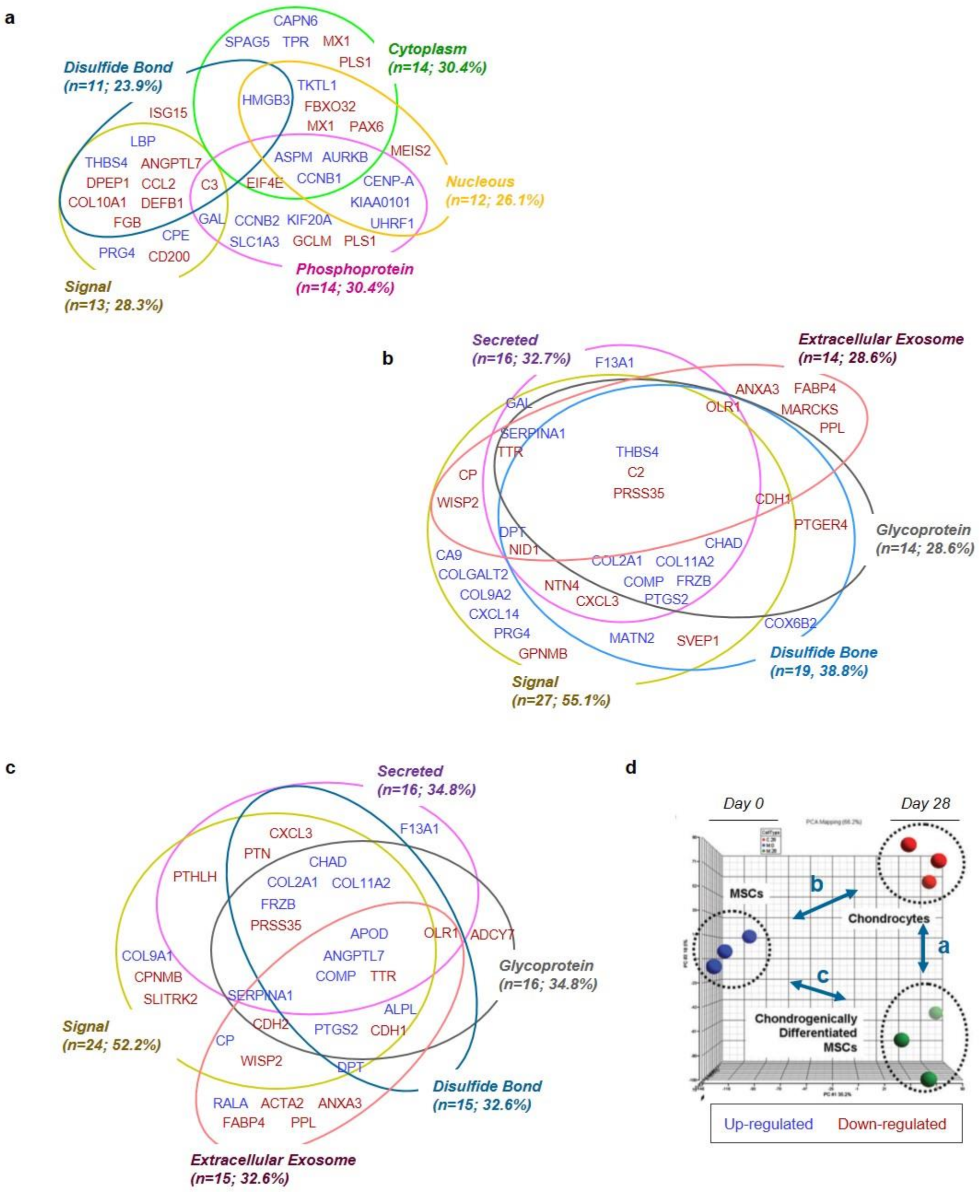


Fig. S7. Cluster analysis showing the effect of TGF-β3 on juvenile MSCs and chondrocytes in CM at day 28. Blue font indicates upregulation, and red font indicates down regulation. **(a)** Chondrogenically induced juvenile MSCs with TGF-β3 (CM+) vs. juvenile chondrocytes in CM+, **(b)** Undifferentiated juvenile MSCs without TGF-β3 (CM-) vs. juvenile chondrocytes in CM+, **(c)** Chondrogenically induced juvenile MSCs in CM+ vs. undifferentiated juvenile MSCs in CM- **(d)** PCA analysis adapted with permission from *Huang et al. (1).*

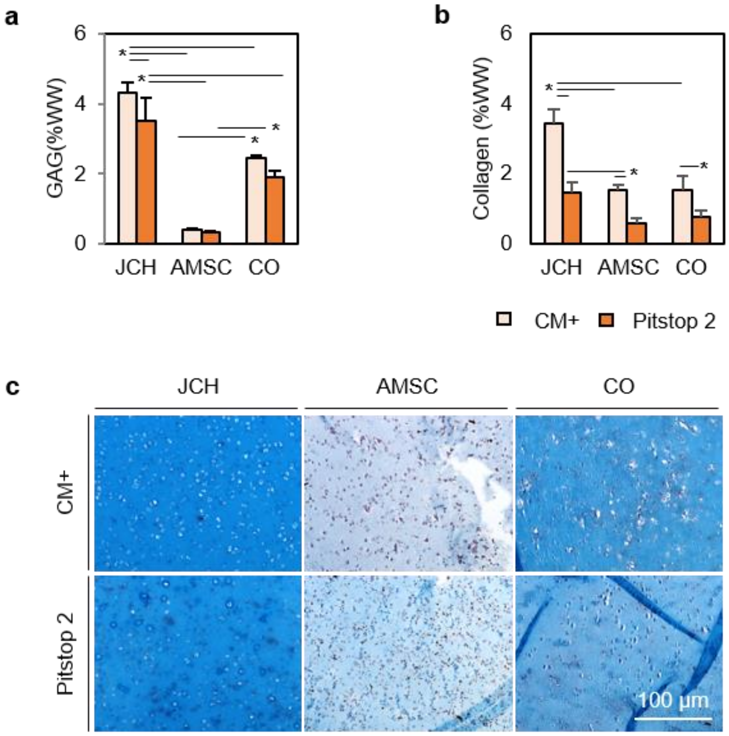


Fig. S8. Biochemical content of cell-laden constructs cultured with Pitstop 2. (a) GAG (%WW), **(b)** Collagen (%WW), **(c)** Alcian blue staining on day 42 (scale bar = 100µm, n=3-4/group; $p<0.05$).

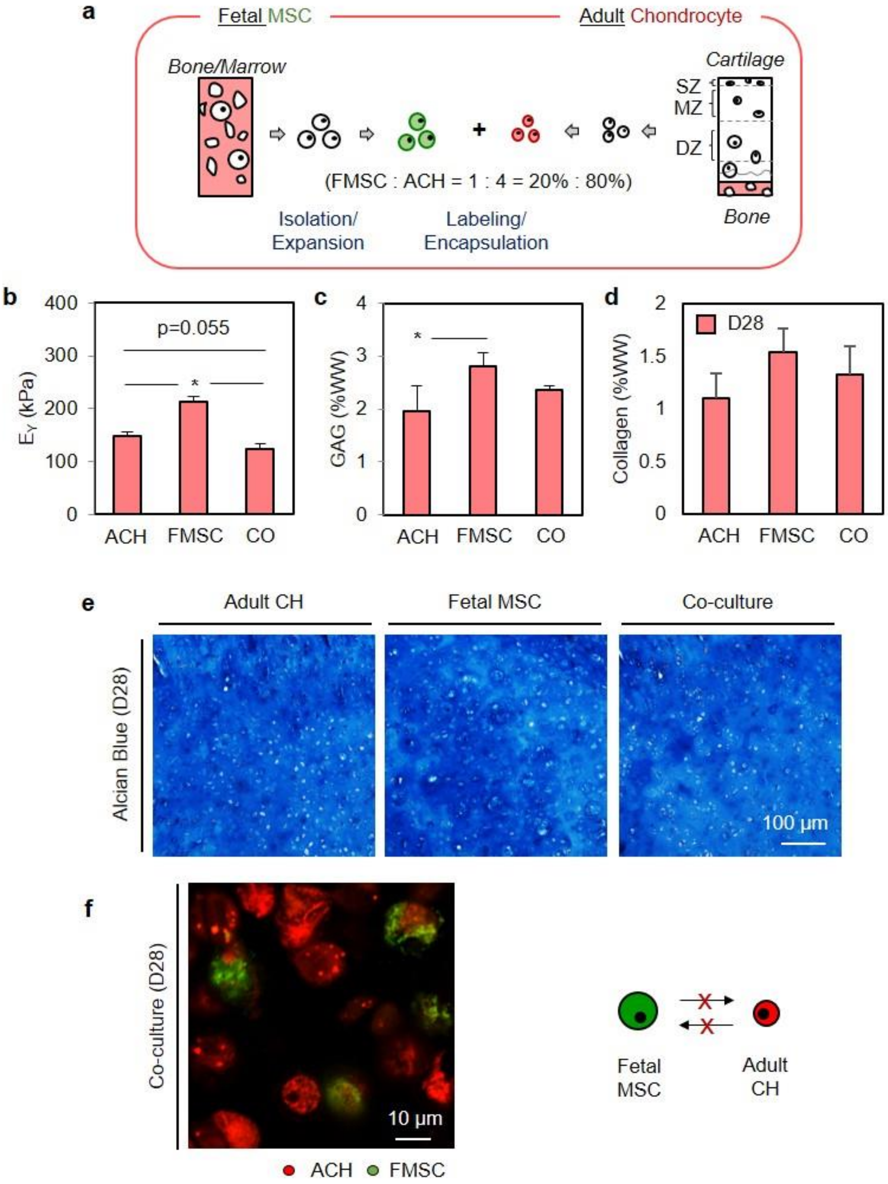
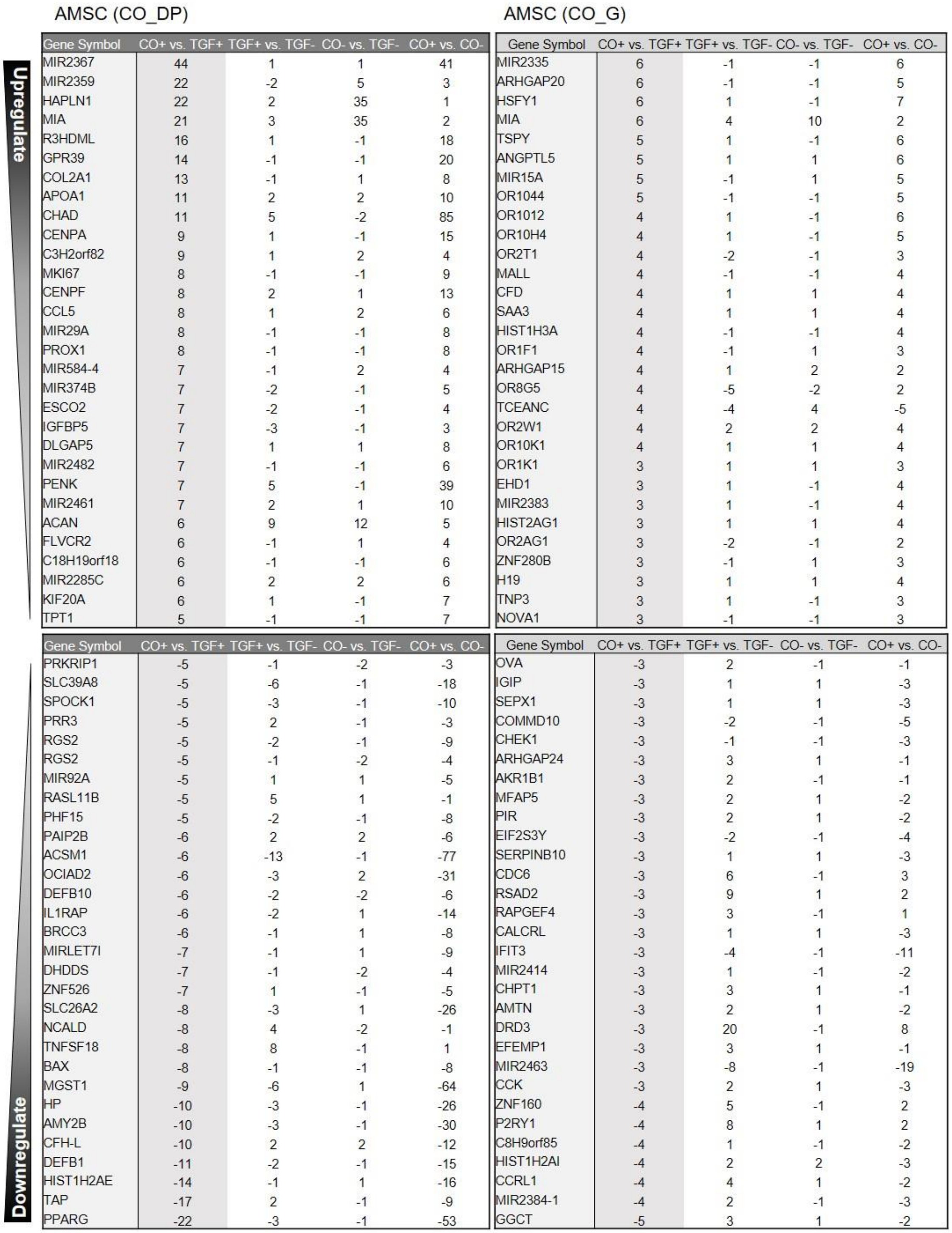


Fig. S9. Co-culture of fetal MSCs (FMSC) with adult chondrocytes (ACH). (a) Fetal MSCs and adult chondrocytes were expanded and labeled with CellTracker (MSC= green, CH= red). Fetal MSCs were mixed with adult chondrocytes (MSC:CH = 1:4). **(b)** Equilibrium modulus (E_Y; kPa), **(c)** GAG (%WW), **(d)** Collagen (%WW), **(e)** Alcian blue staining on day 28, **(f)** Confocal microscopy showed no vesicular transport in either direction (Fetal MSC = Green; Adult chondrocyte = red) (scale bar = 100µm, n=3-4/group; $p<0.05$).

Table S1. Differences in expression and fold changes in AMSCs that had become double positive (CO±_DP) compared to those that remained green (CO±_G) over 10 days of co-culture, in the absence or presence of TGF-β3, compared to AMSCs cultured on their own (TGF±_G).



AMSC (CO_DP)

Upregulate

Gene Symbol	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs. TGF-	CO+ vs. CO-
MIR2367	44	1	1	41
MIR2359	22	-2	5	3
HAPLN1	22	2	35	1
MIA	21	3	35	2
R3HDML	16	1	-1	18
GPR39	14	-1	-1	20
COL2A1	13	-1	1	8
APOA1	11	2	2	10
CHAD	11	5	-2	85
CENPA	9	1	-1	15
C3H2orf82	9	1	2	4
MKI67	8	-1	-1	9
CENPF	8	2	1	13
CCL5	8	1	2	6
MIR29A	8	-1	-1	8
PROX1	8	-1	-1	8
MIR584-4	7	-1	2	4
MIR374B	7	-2	-1	5
ESCO2	7	-2	-1	4
IGFBP5	7	-3	-1	3
DLGAP5	7	1	1	8
MIR2482	7	-1	-1	6
PENK	7	5	-1	39
MIR2461	7	2	1	10
ACAN	6	9	12	5
FLVCR2	6	-1	1	4
C18H19orf18	6	-1	-1	6
MIR2285C	6	2	2	6
KIF20A	6	1	-1	7
TPT1	5	-1	-1	7

Downregulate

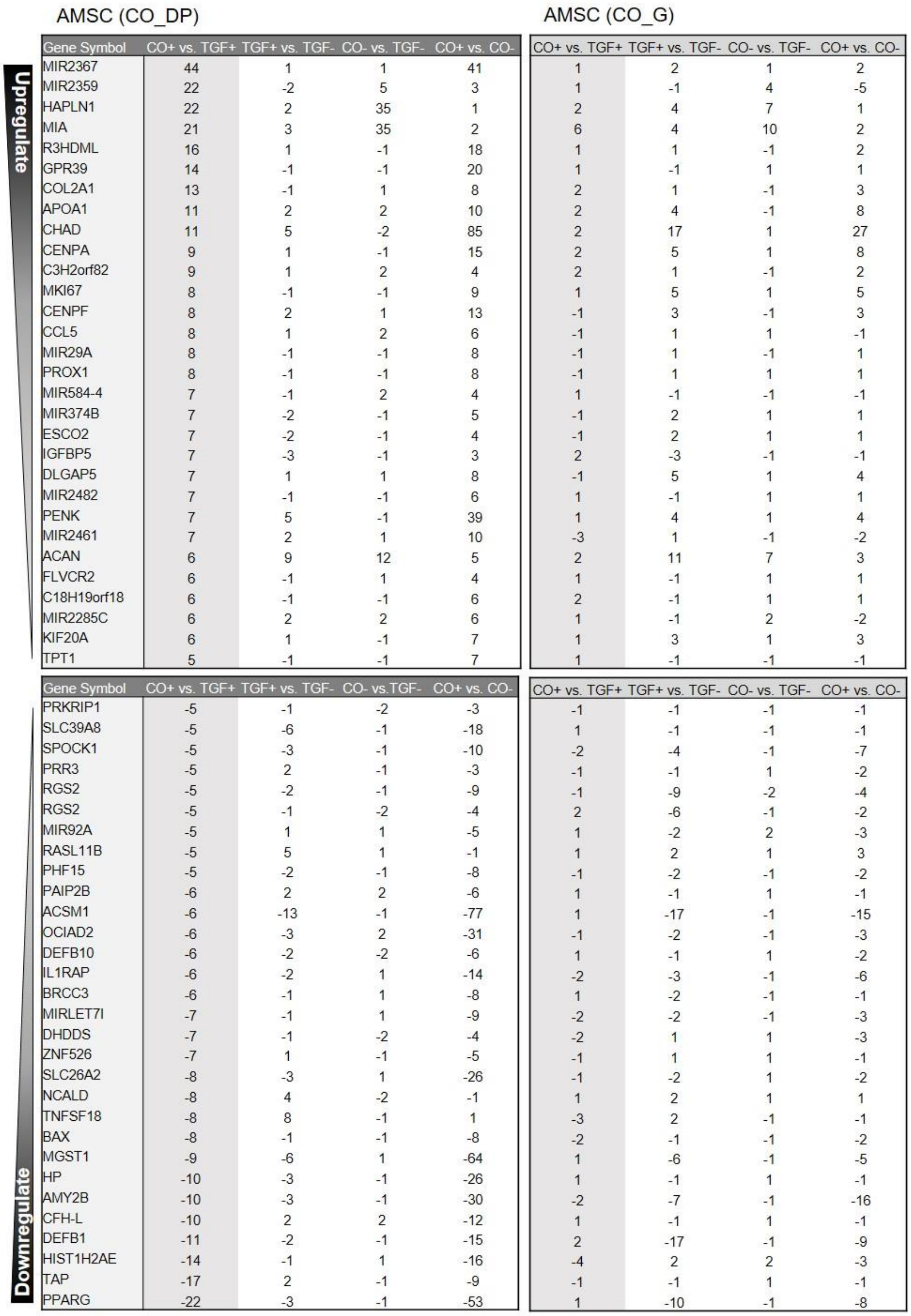
Gene Symbol	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs. TGF-	CO+ vs. CO-
PRKRIP1	-5	-1	-2	-3
SLC39A8	-5	-6	-1	-18
SPOCK1	-5	-3	-1	-10
PRR3	-5	2	-1	-3
RGS2	-5	-2	-1	-9
RGS2	-5	-1	-2	-4
MIR92A	-5	1	1	-5
RASL11B	-5	5	1	-1
PHF15	-5	-2	-1	-8
PAIP2B	-6	2	2	-6
ACSM1	-6	-13	-1	-77
OCIAD2	-6	-3	2	-31
DEFB10	-6	-2	-2	-6
IL1RAP	-6	-2	1	-14
BRCC3	-6	-1	1	-8
MIRLET7I	-7	-1	1	-9
DHDDS	-7	-1	-2	-4
ZNF526	-7	1	-1	-5
SLC26A2	-8	-3	1	-26
NCALD	-8	4	-2	-1
TNFSF18	-8	8	-1	1
BAX	-8	-1	-1	-8
MGST1	-9	-6	1	-64
HP	-10	-3	-1	-26
AMY2B	-10	-3	-1	-30
CFH-L	-10	2	2	-12
DEFB1	-11	-2	-1	-15
HIST1H2AE	-14	-1	1	-16
TAP	-17	2	-1	-9
PPARG	-22	-3	-1	-53

AMSC (CO_G)

Gene Symbol	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs. TGF-	CO+ vs. CO-
MIR2335	6	-1	-1	6
ARHGAP20	6	-1	-1	5
HSFY1	6	1	-1	7
MIA	6	4	10	2
TSPY	5	1	-1	6
ANGPTL5	5	1	1	6
MIR15A	5	-1	1	5
OR1044	5	-1	-1	5
OR1012	4	1	-1	6
OR10H4	4	1	-1	5
OR2T1	4	-2	-1	3
MALL	4	-1	-1	4
CFD	4	1	1	4
SAA3	4	1	1	4
HIST1H3A	4	-1	-1	4
OR1F1	4	-1	1	3
ARHGAP15	4	1	2	2
OR8G5	4	-5	-2	2
TCEANC	4	-4	4	-5
OR2W1	4	2	2	4
OR10K1	4	1	1	4
OR1K1	3	1	1	3
EHD1	3	1	-1	4
MIR2383	3	1	-1	4
HIST2AG1	3	1	1	4
OR2AG1	3	-2	-1	2
ZNF280B	3	-1	1	3
H19	3	1	1	4
TNP3	3	1	-1	3
NOVA1	3	-1	-1	3

Gene Symbol	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs. TGF-	CO+ vs. CO-
OVA	-3	2	-1	-1
IGIP	-3	1	1	-3
SEPX1	-3	1	1	-3
COMMD10	-3	-2	-1	-5
CHEK1	-3	-1	-1	-3
ARHGAP24	-3	3	1	-1
AKR1B1	-3	2	-1	-1
MFAP5	-3	2	1	-2
PIR	-3	2	1	-2
EIF2S3Y	-3	-2	-1	-4
SERPINB10	-3	1	1	-3
CDC6	-3	6	-1	3
RSAD2	-3	9	1	2
RAPGEF4	-3	3	-1	1
CALCRL	-3	1	1	-3
IFIT3	-3	-4	-1	-11
MIR2414	-3	1	-1	-2
CHPT1	-3	3	1	-1
AMTN	-3	2	1	-2
DRD3	-3	20	-1	8
EFEMP1	-3	3	1	-1
MIR2463	-3	-8	-1	-19
CCK	-3	2	1	-3
ZNF160	-4	5	-1	2
P2RY1	-4	8	1	2
C8H9orf85	-4	1	-1	-2
HIST1H2AI	-4	2	2	-3
CCRL1	-4	4	1	-2
MIR2384-1	-4	2	-1	-3
GGCT	-5	3	1	-2

Table S2. Impact of molecular factors from JCH on the expression of AMSCs that had become double positive (CO±_DP) compared to those that remained green (CO±_G) within the same constructs, and their gene-matched fold changes.



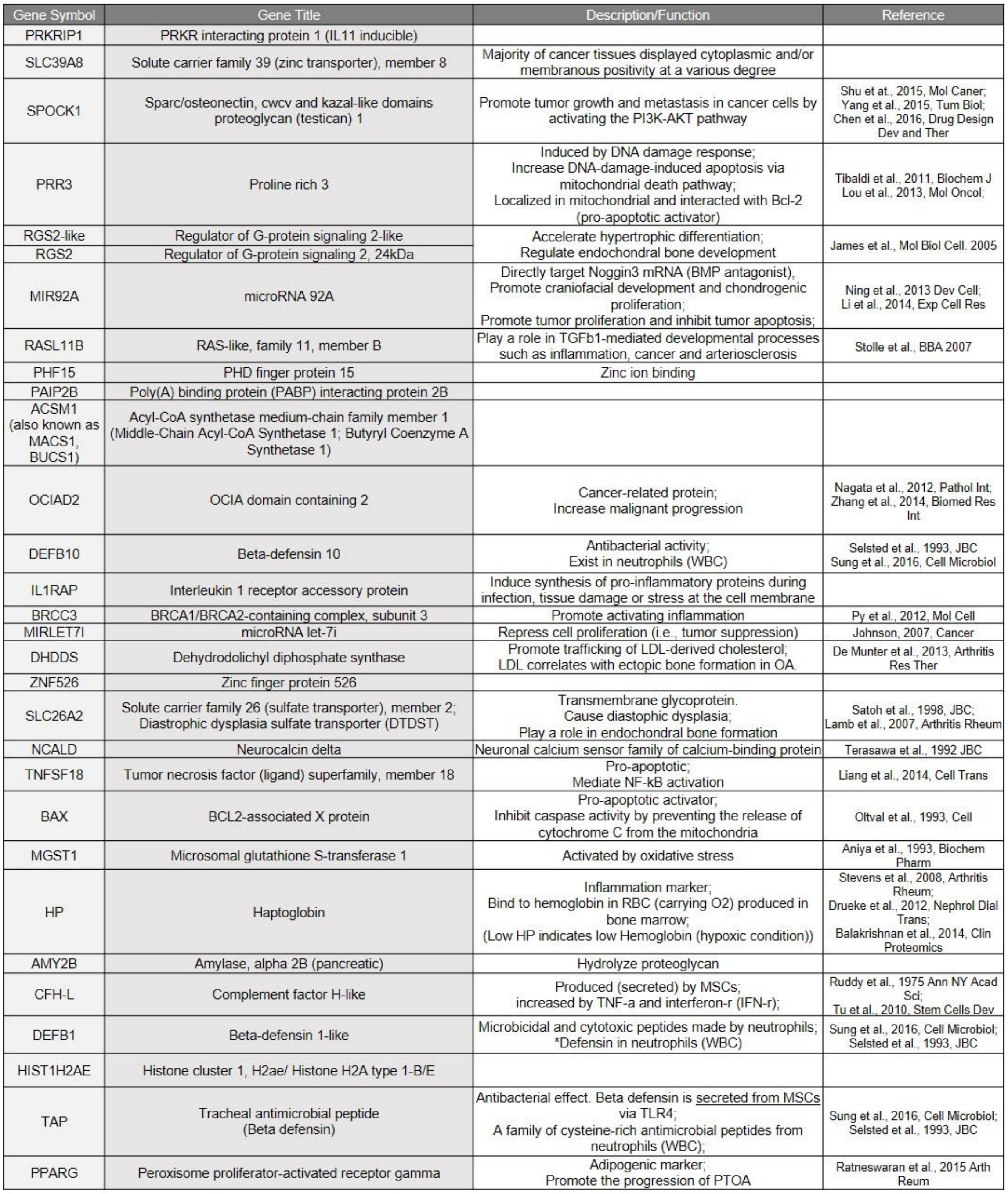
		AMSC (CO_DP)				AMSC (CO_G)			
	Gene Symbol	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs. TGF-	CO+ vs. CO-	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs. TGF-	CO+ vs. CO-
Upregulate	MIR2367	44	1	1	41	1	2	1	2
	MIR2359	22	-2	5	3	1	-1	4	-5
	HAPLN1	22	2	35	1	2	4	7	1
	MIA	21	3	35	2	6	4	10	2
	R3HDML	16	1	-1	18	1	1	-1	2
	GPR39	14	-1	-1	20	1	-1	1	1
	COL2A1	13	-1	1	8	2	1	-1	3
	APOA1	11	2	2	10	2	4	-1	8
	CHAD	11	5	-2	85	2	17	1	27
	CENPA	9	1	-1	15	2	5	1	8
	C3H2orf82	9	1	2	4	2	1	-1	2
	MKI67	8	-1	-1	9	1	5	1	5
	CENPF	8	2	1	13	-1	3	-1	3
	CCL5	8	1	2	6	-1	1	1	-1
	MIR29A	8	-1	-1	8	-1	1	-1	1
	PROX1	8	-1	-1	8	-1	1	1	1
	MIR584-4	7	-1	2	4	1	-1	-1	-1
	MIR374B	7	-2	-1	5	-1	2	1	1
	ESCO2	7	-2	-1	4	-1	2	1	1
	IGFBP5	7	-3	-1	3	2	-3	-1	-1
	DLGAP5	7	1	1	8	-1	5	1	4
	MIR2482	7	-1	-1	6	1	-1	1	1
	PENK	7	5	-1	39	1	4	1	4
	MIR2461	7	2	1	10	-3	1	-1	-2
	ACAN	6	9	12	5	2	11	7	3
	FLVCR2	6	-1	1	4	1	-1	1	1
	C18H19orf18	6	-1	-1	6	2	-1	1	1
	MIR2285C	6	2	2	6	1	-1	2	-2
	KIF20A	6	1	-1	7	1	3	1	3
	TPT1	5	-1	-1	7	1	-1	-1	-1

	Gene Symbol	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs.TGF-	CO+ vs. CO-	CO+ vs. TGF+	TGF+ vs. TGF-	CO- vs. TGF-	CO+ vs. CO-
Downregulate	PRKRIP1	-5	-1	-2	-3	-1	-1	-1	-1
	SLC39A8	-5	-6	-1	-18	1	-1	-1	-1
	SPOCK1	-5	-3	-1	-10	-2	-4	-1	-7
	PRR3	-5	2	-1	-3	-1	-1	1	-2
	RGS2	-5	-2	-1	-9	-1	-9	-2	-4
	RGS2	-5	-1	-2	-4	2	-6	-1	-2
	MIR92A	-5	1	1	-5	1	-2	2	-3
	RASL11B	-5	5	1	-1	1	2	1	3
	PHF15	-5	-2	-1	-8	-1	-2	-1	-2
	PAIP2B	-6	2	2	-6	1	-1	1	-1
	ACSM1	-6	-13	-1	-77	1	-17	-1	-15
	OCIAD2	-6	-3	2	-31	-1	-2	-1	-3
	DEFB10	-6	-2	-2	-6	1	-1	1	-2
	IL1RAP	-6	-2	1	-14	-2	-3	-1	-6
	BRCC3	-6	-1	1	-8	1	-2	-1	-1
	MIRLET7I	-7	-1	1	-9	-2	-2	-1	-3
	DHDDS	-7	-1	-2	-4	-2	1	1	-3
	ZNF526	-7	1	-1	-5	-1	1	1	-1
	SLC26A2	-8	-3	1	-26	-1	-2	1	-2
	NCALD	-8	4	-2	-1	1	2	1	1
	TNFSF18	-8	8	-1	1	-3	2	-1	-1
	BAX	-8	-1	-1	-8	-2	-1	-1	-2
	MGST1	-9	-6	1	-64	1	-6	-1	-5
	HP	-10	-3	-1	-26	1	-1	1	-1
	AMY2B	-10	-3	-1	-30	-2	-7	-1	-16
	CFH-L	-10	2	2	-12	1	-1	1	-1
	DEFB1	-11	-2	-1	-15	2	-17	-1	-9
	HIST1H2AE	-14	-1	1	-16	-4	2	2	-3
	TAP	-17	2	-1	-9	-1	-1	1	-1
	PPARG	-22	-3	-1	-53	1	-10	-1	-8

Table S3. Details on genes that showed positive fold changes in AMSCs with co-culture**.**

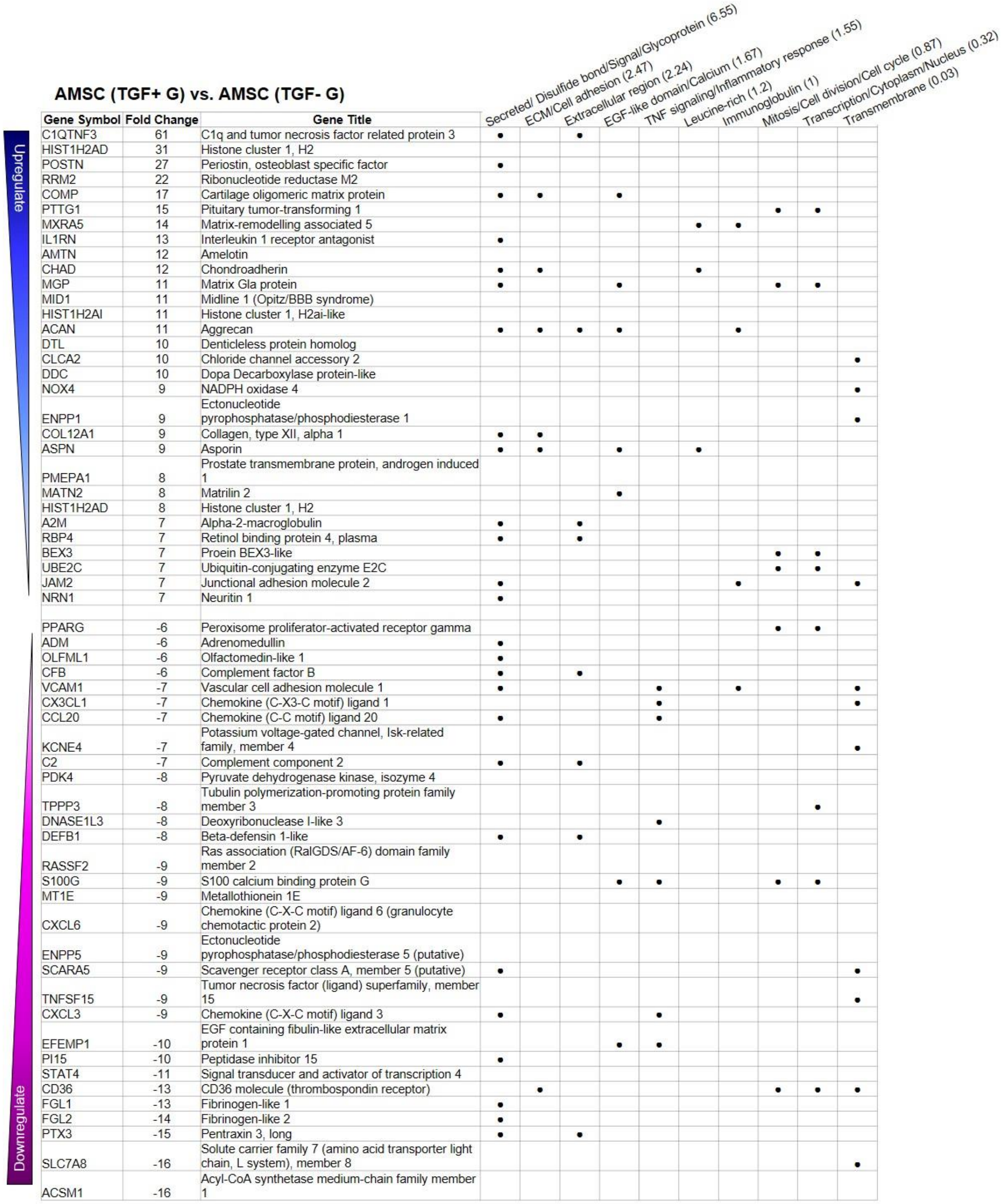
Gene Symbol	Gene Title	Description/ Function	Reference
MIR2367	microRNA 2367	Bos taurus	NCBI Gene
MIR2359	microRNA 2359	Bos taurus	NCBI Gene
HAPLN1	Hyaluronan/PG link protein 1 (CRTL1: cartilage link protein 1)	Contribute to ECM stability and flexibility; Regulated by SOX9	Binette et al., 1994 JBC Spicer et al., 2003 JBC Ikuyo et al., 2004 JBC Fang et al., 2014 Arteriosc Thromb Vasc Biol
MIA	Melanoma inhibitory activity (CD-RAP: cartilage-derived retinoic acid sensitive protein)	Found in articular cartilage; Regulated by SOX9	Sakano et al., 1999 JBMR Bosserhoff et al., 2003 Biomaterials
R3HDML	R3H domain containing like	Peptidase inhibitor (a putative serine protease inhibitor); Cysteine-rich secretory protein; May act as an anti-fungal agent	Gibbs et al., 2008 Endocr Rev
GPR39	G protein-coupled receptor 39	Block Hedgehog signaling that causes heterotopic ossification	Regard et al., 2013 Nat Med Bassilana et al., 2014 Nat Chem Biol
COL2A1	Collagen, type II, alpha 1	Major component of articular cartilage	
APOA1	Apolipoprotein A-1	Main functional protein of HDL (high-density lipoprotein); Transports endogenous microRNAs to recipient cells; Decrease accumulation of atherosclerosis by removing LDL	Vickers et al., 2011 Nat Cell Biol Chen et al., 2012 Trends Cell Biol Wu et al., 2014 Arterioscler Thromb Vasc Biol Michell et al., 2016 BBA
CHAD	Chondroadherin	A leucine rich repeat ECM protein in cell-matrix interaction; Binds cells via integrin and cell surface PGs; Acts as an anchor to ECM by binding to collagens	Hessle et al., 2013 PLOSOne Batista et al, 2014 Mat Biol Tillgren et al, 2015 JBC
CENPA	Centromere protein A (Histone H3 variant)	Recruited to chromatin in DNA damage; Essential for growth and development;	Van Hooser et al., 2001 JCS Zeitlin et al., 2009 PNAS
C3H2orf82	Chromosome 3 open reading frame, human C2orf82	Chromosome 3 C2orf82 homolog	NCBI Gene
MKI67	Marker of proliferation Ki-67 (Ki-67)	Reflect cellular proliferation rate; Exclusively expressed in proliferating cells (maintaining proliferation); Interact with KIF15	Gerdes et al., 1984 J Immunol Bullwinkel et al, 2006 JCP
CENPF	Centromere protein F (Mitosin)	Involved in proliferation, differentiation and stress response associated with ATF4	Yang et al, 2003 Cell Res Zhou et al., 2005 JBC Yan et al., 2012 Proteomics
CCL5	Chemokine (C-C motif) ligand 5; (RANTES; regulated on activation, normal T cell expressed and secreted)	CCR3 was expressed at the mRNA level and increased during MSC-derived chondrogenesis; Chemokine receptors were expredded in chondrocytes; Expressed in normal and OA cartilage; Exert anti-apoptotic effect mediated by Erk/Akt pathway;	Borzi et al., 2000 Arthritis Rheum Alaaeddine et al., 2001 Arthritis Rheum Mazzetti et al., 2004 Arthritis Rheum Djouad et al., 2007 Arthritis Res Ther Hofer et al., 2016 Stem Cell Res Ther
MIR29A	microRNA 29A	Up-regulated in normal cartilage; Inhibit osteonectin expression	Goldring et al., 2012 Trends Mol Med Shang et al., 2013 J Cell Mol Med Li et al., 2016 Mol. Cells
PROX1	Prospero homeobox 1	Regulate FGFRs expressed in proliferating or hypertrophic chondrocytes	Liu et al., 2002 Genes Dev Audette et al., 2015 Development
MIR584-4	microRNA 584-4	Bos taurus	NCBI Gene
MIR374B	microRNA 374B	Inhibit angiogenesis by targeting VEGF regulated by CCL3 through CCR1 and CCR5	Sohn et al., 2015 J Cancer Liao et al., 2015 Oncotarget
ESCO2	Establishment of sister chromatid cohesion N-acetyltransferase 2	An acetyltransferase that converts chromatin-bound cohesins to a tether-competent state; Induce mitosis and proliferation	Kim et al., 2008 BBRC Banerji et al., 2016 Dev Dyn
IGFBP5	Insulin-like growth factor binding protein 5	Regulate the availability of IGF-1, an anabolic factor involved in matrix synthesis and chondrocyte survival	Buechli et al., 2013 Stem Cells Dev
DLGAP5	Disc, large (Drosophila) homolog-associated protein 5	Promote cell proliferation, migration and invasion	Liao et al., 2013 PLOSOne
MIR2482	microRNA 2482	Bos taurus	NCBI Gene
PENK	Proenkephalin	Attenuate inflammation by stimulating secretion of IL-10	Milwid et al, 2014 Mol Ther
MIR2461	microRNA 2461	Bos taurus	NCBI Gene
ACAN	Aggrecan	Major component of articular cartilage	
FLVCR2	Feline leukemia virus subgroup C cellular receptor family, member 2-like (FLVCR2)	Import heme and transport calcium-chelator complex; Regulate growth and calcium metabolism	Braiser et al., 2004 Exp Cell Res Duffy et al., 2010 Mol Cell Biol
C18H19orf18	Chromosome 18 open reading frame, human C19orf18	Chromosome 18 C19orf18 homolog	NCBI Gene
MIR2285C	microRNA 2285C	Bos taurus	NCBI Gene
KIF20A	Kinesin family member 20A	Expressed in healthy DNA; Mitotic cell cycle	Kim et al., 2014 PLOSOne
TPT1	Tumor protein, translationally-controlled 1-like (TCTP)	Anti-apoptosis	Liu et al., 2005 Mol Cell Biol Susini et al., 2008 Cell Death Diff Thebault et al., 2016 Sci Rep

Table S4. Details on genes that showed negative fold changes in AMSCs with co-culture.



Gene Symbol	Gene Title	Description/Function	Reference
PRKRIP1	PRKR interacting protein 1 (IL11 inducible)		
SLC39A8	Solute carrier family 39 (zinc transporter), member 8	Majority of cancer tissues displayed cytoplasmic and/or membranous positivity at a various degree	
SPOCK1	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	Promote tumor growth and metastasis in cancer cells by activating the PI3K-AKT pathway	Shu et at., 2015, Mol Caner; Yang et al., 2015, Tum Biol; Chen et al., 2016, Drug Design Dev and Ther
PRR3	Proline rich 3	Induced by DNA damage response; Increase DNA-damage-induced apoptosis via mitochondrial death pathway; Localized in mitochondrial and interacted with Bcl-2 (pro-apoptotic activator)	Tibaldi et al., 2011, Biochem J Lou et al., 2013, Mol Oncol;
RGS2-like	Regulator of G-protein signaling 2-like	Accelerate hypertrophic differentiation; Regulate endochondral bone development	James et al., Mol Biol Cell. 2005
RGS2	Regulator of G-protein signaling 2, 24kDa		
MIR92A	microRNA 92A	Directly target Noggin3 mRNA (BMP antagonist), Promote craniofacial development and chondrogenic proliferation; Promote tumor proliferation and inhibit tumor apoptosis;	Ning et al., 2013 Dev Cell; Li et al., 2014, Exp Cell Res
RASL11B	RAS-like, family 11, member B	Play a role in TGFb1-mediated developmental processes such as inflammation, cancer and arteriosclerosis	Stolle et al., BBA 2007
PHF15	PHD finger protein 15	Zinc ion binding	
PAIP2B	Poly(A) binding protein (PABP) interacting protein 2B		
ACSM1 (also known as MACS1, BUCS1)	Acyl-CoA synthetase medium-chain family member 1 (Middle-Chain Acyl-CoA Synthetase 1; Butyryl Coenzyme A Synthetase 1)		
OCIAD2	OCIA domain containing 2	Cancer-related protein; Increase malignant progression	Nagata et al., 2012, Pathol Int; Zhang et al., 2014, Biomed Res Int
DEFB10	Beta-defensin 10	Antibacterial activity; Exist in neutrophils (WBC)	Selsted et al., 1993, JBC Sung et al., 2016, Cell Microbiol
IL1RAP	Interleukin 1 receptor accessory protein	Induce synthesis of pro-inflammatory proteins during infection, tissue damage or stress at the cell membrane	
BRCC3	BRCA1/BRCA2-containing complex, subunit 3	Promote activating inflammation	Py et al., 2012, Mol Cell
MIRLET7I	microRNA let-7i	Repress cell proliferation (i.e., tumor suppression)	Johnson, 2007, Cancer
DHDDS	Dehydrodolichyl diphosphate synthase	Promote trafficking of LDL-derived cholesterol; LDL correlates with ectopic bone formation in OA.	De Munter et al., 2013, Arthritis Res Ther
ZNF526	Zinc finger protein 526		
SLC26A2	Solute carrier family 26 (sulfate transporter), member 2; Diastrophic dysplasia sulfate transporter (DTDST)	Transmembrane glycoprotein. Cause diastophic dysplasia; Play a role in endochondral bone formation	Satoh et al., 1998, JBC; Lamb et al., 2007, Arthritis Rheum
NCALD	Neurocalcin delta	Neuronal calcium sensor family of calcium-binding protein	Terasawa et al., 1992 JBC
TNFSF18	Tumor necrosis factor (ligand) superfamily, member 18	Pro-apoptotic; Mediate NF-kB activation	Liang et al., 2014, Cell Trans
BAX	BCL2-associated X protein	Pro-apoptotic activator; Inhibit caspase activity by preventing the release of cytochrome C from the mitochondria	Oltval et al., 1993, Cell
MGST1	Microsomal glutathione S-transferase 1	Activated by oxidative stress	Aniya et al., 1993, Biochem Pharm
HP	Haptoglobin	Inflammation marker; Bind to hemoglobin in RBC (carrying O2) produced in bone marrow; (Low HP indicates low Hemoglobin (hypoxic condition))	Stevens et al., 2008, Arthritis Rheum; Drueke et al., 2012, Nephrol Dial Trans; Balakrishnan et al., 2014, Clin Proteomics
AMY2B	Amylase, alpha 2B (pancreatic)	Hydrolyze proteoglycan	
CFH-L	Complement factor H-like	Produced (secreted) by MSCs; increased by TNF-a and interferon-r (IFN-r);	Ruddy et al., 1975 Ann NY Acad Sci; Tu et al., 2010, Stem Cells Dev
DEFB1	Beta-defensin 1-like	Microbicidal and cytotoxic peptides made by neutrophils; *Defensin in neutrophils (WBC)	Sung et al., 2016, Cell Microbiol; Selsted et al., 1993, JBC
HIST1H2AE	Histone cluster 1, H2ae/ Histone H2A type 1-B/E		
TAP	Tracheal antimicrobial peptide (Beta defensin)	Antibacterial effect. Beta defensin is secreted from MSCs via TLR4; A family of cysteine-rich antimicrobial peptides from neutrophils (WBC);	Sung et al., 2016, Cell Microbiol; Selsted et al., 1993, JBC
PPARG	Peroxisome proliferator-activated receptor gamma	Adipogenic marker; Promote the progression of PTOA	Ratneswaran et al., 2015 Arth Reum

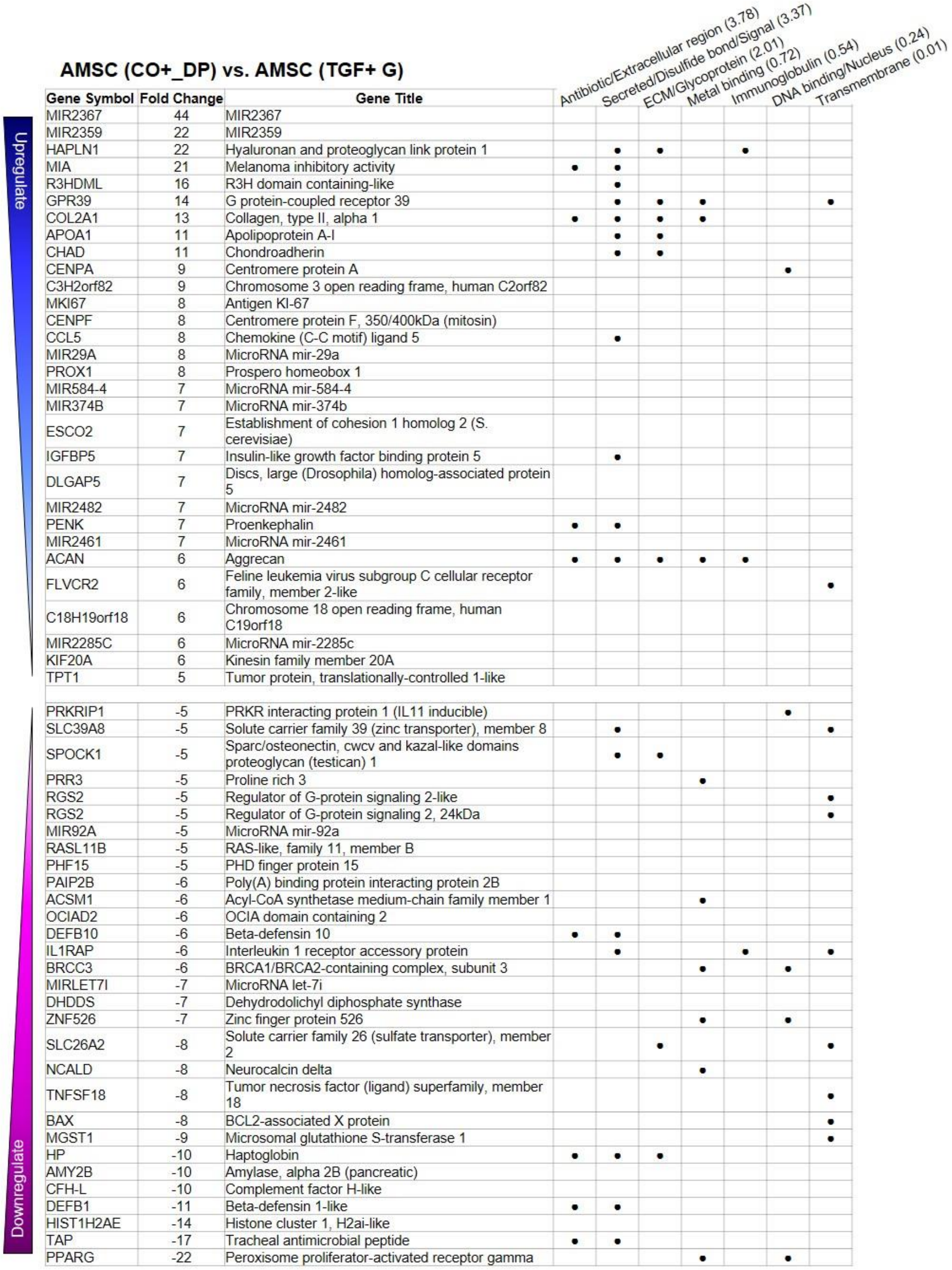
Table S5. Expression of genes and fold changes based on biological themes, enriched functional-related gene clusters, and expression levels based on the effect of TGF-β3 in adult MSCs (AMSC).



AMSC (TGF+ G) vs. AMSC (TGF- G)

Gene Symbol	Fold Change	Gene Title	Secreted/ Disulfide bond/Signal/Glycoprotein (6.55)	ECM/Cell adhesion (2.47)	Extracellular region (2.24)	EGF-like domain/Calcium (1.67)	TNF signaling/Inflammatory response (1.55)	Leucine-rich (1.2)	Immunoglobulin (1)	Mitosis/Cell division/Cell cycle (0.87)	Transcription/Cytoplasm/Nucleus (0.32)	Transmembrane (0.03)
C1QTNF3	61	C1q and tumor necrosis factor related protein 3	●		●							
HIST1H2AD	31	Histone cluster 1, H2										
POSTN	27	Periostin, osteoblast specific factor	●									
RRM2	22	Ribonucleotide reductase M2										
COMP	17	Cartilage oligomeric matrix protein	●	●		●						
PTTG1	15	Pituitary tumor-transforming 1								●	●	
MXRA5	14	Matrix-remodelling associated 5						●	●			
IL1RN	13	Interleukin 1 receptor antagonist	●									
AMTN	12	Amelotin										
CHAD	12	Chondroadherin	●	●				●				
MGP	11	Matrix Gla protein	●			●				●	●	
MID1	11	Midline 1 (Opitz/BBB syndrome)										
HIST1H2AI	11	Histone cluster 1, H2ai-like										
ACAN	11	Aggrecan	●	●	●	●			●			
DTL	10	Denticleless protein homolog										
CLCA2	10	Chloride channel accessory 2										●
DDC	10	Dopa Decarboxylase protein-like										
NOX4	9	NADPH oxidase 4										●
ENPP1	9	Ectonucleotide pyrophosphatase/phosphodiesterase 1										●
COL12A1	9	Collagen, type XII, alpha 1	●	●								
ASPN	9	Asporin	●	●		●		●				
PMEPA1	8	Prostate transmembrane protein, androgen induced 1										
MATN2	8	Matrilin 2				●						
HIST1H2AD	8	Histone cluster 1, H2										
A2M	7	Alpha-2-macroglobulin	●		●							
RBP4	7	Retinol binding protein 4, plasma	●		●							
BEX3	7	Proein BEX3-like								●	●	
UBE2C	7	Ubiquitin-conjugating enzyme E2C								●	●	
JAM2	7	Junctional adhesion molecule 2	●						●			●
NRN1	7	Neuritin 1	●									
PPARG	-6	Peroxisome proliferator-activated receptor gamma								●	●	
ADM	-6	Adrenomedullin	●									
OLFML1	-6	Olfactomedin-like 1	●									
CFB	-6	Complement factor B	●		●							
VCAM1	-7	Vascular cell adhesion molecule 1	●				●		●			●
CX3CL1	-7	Chemokine (C-X3-C motif) ligand 1					●					●
CCL20	-7	Chemokine (C-C motif) ligand 20	●				●					
KCNE4	-7	Potassium voltage-gated channel, Isk-related family, member 4										●
C2	-7	Complement component 2	●		●							
PDK4	-8	Pyruvate dehydrogenase kinase, isozyme 4										
TPPP3	-8	Tubulin polymerization-promoting protein family member 3									●	
DNASE1L3	-8	Deoxyribonuclease I-like 3					●					
DEFB1	-8	Beta-defensin 1-like	●		●							
RASSF2	-9	Ras association (RalGDS/AF-6) domain family member 2										
S100G	-9	S100 calcium binding protein G				●	●			●	●	
MT1E	-9	Metallothionein 1E										
CXCL6	-9	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)										
ENPP5	-9	Ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative)										
SCARA5	-9	Scavenger receptor class A, member 5 (putative)	●									●
TNFSF15	-9	Tumor necrosis factor (ligand) superfamily, member 15										●
CXCL3	-9	Chemokine (C-X-C motif) ligand 3	●				●					
EFEMP1	-10	EGF containing fibulin-like extracellular matrix protein 1				●	●					
PI15	-10	Peptidase inhibitor 15	●									
STAT4	-11	Signal transducer and activator of transcription 4										
CD36	-13	CD36 molecule (thrombospondin receptor)		●						●	●	●
FGL1	-13	Fibrinogen-like 1	●									
FGL2	-14	Fibrinogen-like 2	●									
PTX3	-15	Pentraxin 3, long	●		●							
SLC7A8	-16	Solute carrier family 7 (amino acid transporter light chain, L system), member 8										●
ACSM1	-16	Acyl-CoA synthetase medium-chain family member 1										

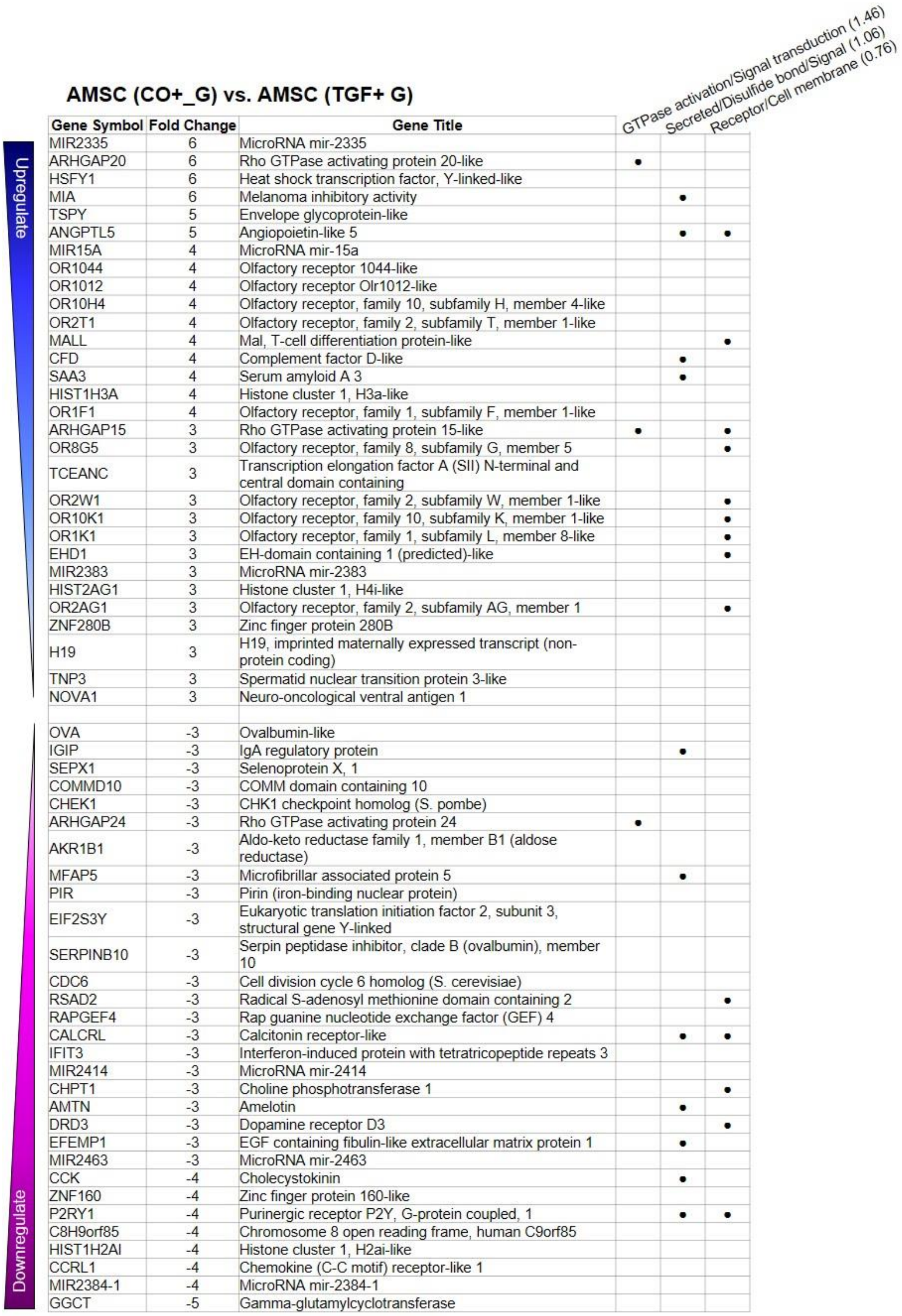
Table S6. Expression of genes and fold changes based on biological themes, enriched functional-related gene clusters, and their expression levels based on the effect of TGF-β3 and/or molecular factors from co-cultured JCH (CO+_DP) in AMSCs.



AMSC (CO+_DP) vs. AMSC (TGF+ G)

	Gene Symbol	Fold Change	Gene Title	Antibiotic/Extracellular region (3.78)	Secreted/Disulfide bond/Signal (3.37)	ECM/Glycoprotein (2.01)	Metal binding (0.72)	Immunoglobulin (0.54)	DNA binding/Nucleus (0.24)	Transmembrane (0.01)
Upregulate	MIR2367	44	MIR2367							
	MIR2359	22	MIR2359							
	HAPLN1	22	Hyaluronan and proteoglycan link protein 1		•	•		•		
	MIA	21	Melanoma inhibitory activity	•	•					
	R3HDML	16	R3H domain containing-like		•					
	GPR39	14	G protein-coupled receptor 39		•	•	•			•
	COL2A1	13	Collagen, type II, alpha 1	•	•	•	•			
	APOA1	11	Apolipoprotein A-I		•	•				
	CHAD	11	Chondroadherin		•	•				
	CENPA	9	Centromere protein A						•	
	C3H2orf82	9	Chromosome 3 open reading frame, human C2orf82							
	MKI67	8	Antigen KI-67							
	CENPF	8	Centromere protein F, 350/400kDa (mitosin)							
	CCL5	8	Chemokine (C-C motif) ligand 5		•					
	MIR29A	8	MicroRNA mir-29a							
	PROX1	8	Prospero homeobox 1							
	MIR584-4	7	MicroRNA mir-584-4							
	MIR374B	7	MicroRNA mir-374b							
	ESCO2	7	Establishment of cohesion 1 homolog 2 (S. cerevisiae)							
	IGFBP5	7	Insulin-like growth factor binding protein 5		•					
	DLGAP5	7	Discs, large (Drosophila) homolog-associated protein 5							
	MIR2482	7	MicroRNA mir-2482							
	PENK	7	Proenkephalin	•	•					
	MIR2461	7	MicroRNA mir-2461							
	ACAN	6	Aggrecan	•	•	•	•	•		
	FLVCR2	6	Feline leukemia virus subgroup C cellular receptor family, member 2-like							•
	C18H19orf18	6	Chromosome 18 open reading frame, human C19orf18							
	MIR2285C	6	MicroRNA mir-2285c							
	KIF20A	6	Kinesin family member 20A							
	TPT1	5	Tumor protein, translationally-controlled 1-like							
Downregulate	PRKRIP1	-5	PRKR interacting protein 1 (IL11 inducible)						•	
	SLC39A8	-5	Solute carrier family 39 (zinc transporter), member 8		•					•
	SPOCK1	-5	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1		•	•				
	PRR3	-5	Proline rich 3				•			
	RGS2	-5	Regulator of G-protein signaling 2-like							•
	RGS2	-5	Regulator of G-protein signaling 2, 24kDa							•
	MIR92A	-5	MicroRNA mir-92a							
	RASL11B	-5	RAS-like, family 11, member B							
	PHF15	-5	PHD finger protein 15							
	PAIP2B	-6	Poly(A) binding protein interacting protein 2B							
	ACSM1	-6	Acyl-CoA synthetase medium-chain family member 1				•			
	OCIAD2	-6	OCIA domain containing 2							
	DEFB10	-6	Beta-defensin 10	•	•					
	IL1RAP	-6	Interleukin 1 receptor accessory protein		•			•		•
	BRCC3	-6	BRCA1/BRCA2-containing complex, subunit 3				•		•	
	MIRLET7I	-7	MicroRNA let-7i							
	DHDDS	-7	Dehydrodolichyl diphosphate synthase							
	ZNF526	-7	Zinc finger protein 526				•		•	
	SLC26A2	-8	Solute carrier family 26 (sulfate transporter), member 2			•				•
	NCALD	-8	Neurocalcin delta				•			
	TNFSF18	-8	Tumor necrosis factor (ligand) superfamily, member 18							•
	BAX	-8	BCL2-associated X protein							•
	MGST1	-9	Microsomal glutathione S-transferase 1							•
	HP	-10	Haptoglobin	•	•	•				
	AMY2B	-10	Amylase, alpha 2B (pancreatic)							
	CFH-L	-10	Complement factor H-like							
	DEFB1	-11	Beta-defensin 1-like	•	•					
	HIST1H2AE	-14	Histone cluster 1, H2ai-like							
	TAP	-17	Tracheal antimicrobial peptide	•	•					
	PPARG	-22	Peroxisome proliferator-activated receptor gamma				•		•	

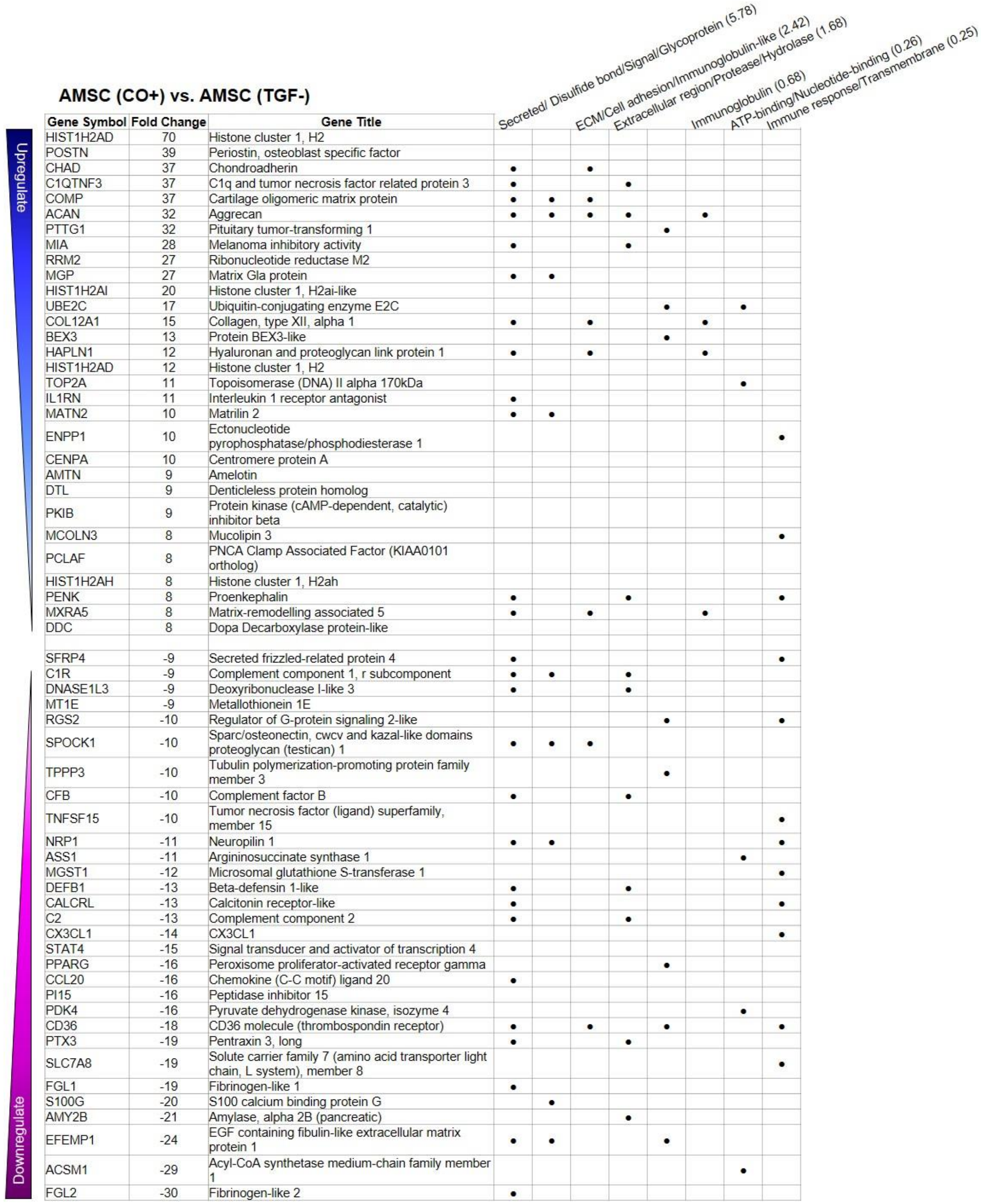
Table S7. Expression of genes and fold changes based on biological themes, enriched functional-related gene clusters, and their expression levels based on the effect of TGF-β3 and co-culture for cells where vesicular transfer did not occur (CO+_G) in AMSCs.



AMSC (CO+_G) vs. AMSC (TGF+ G)

Gene Symbol	Fold Change	Gene Title	GTPase activation/Signal transduction (1.46)	Secreted/Disulfide bond/Signal (1.06)	Receptor/Cell membrane (0.76)
MIR2335	6	MicroRNA mir-2335			
ARHGAP20	6	Rho GTPase activating protein 20-like	●		
HSFY1	6	Heat shock transcription factor, Y-linked-like			
MIA	6	Melanoma inhibitory activity		●	
TSPY	5	Envelope glycoprotein-like			
ANGPTL5	5	Angiopoietin-like 5		●	●
MIR15A	4	MicroRNA mir-15a			
OR1044	4	Olfactory receptor 1044-like			
OR1012	4	Olfactory receptor Olr1012-like			
OR10H4	4	Olfactory receptor, family 10, subfamily H, member 4-like			
OR2T1	4	Olfactory receptor, family 2, subfamily T, member 1-like			
MALL	4	Mal, T-cell differentiation protein-like			●
CFD	4	Complement factor D-like		●	
SAA3	4	Serum amyloid A 3		●	
HIST1H3A	4	Histone cluster 1, H3a-like			
OR1F1	4	Olfactory receptor, family 1, subfamily F, member 1-like			
ARHGAP15	3	Rho GTPase activating protein 15-like	●		●
OR8G5	3	Olfactory receptor, family 8, subfamily G, member 5			●
TCEANC	3	Transcription elongation factor A (SII) N-terminal and central domain containing			
OR2W1	3	Olfactory receptor, family 2, subfamily W, member 1-like			●
OR10K1	3	Olfactory receptor, family 10, subfamily K, member 1-like			●
OR1K1	3	Olfactory receptor, family 1, subfamily L, member 8-like			●
EHD1	3	EH-domain containing 1 (predicted)-like			●
MIR2383	3	MicroRNA mir-2383			
HIST2AG1	3	Histone cluster 1, H4i-like			
OR2AG1	3	Olfactory receptor, family 2, subfamily AG, member 1			●
ZNF280B	3	Zinc finger protein 280B			
H19	3	H19, imprinted maternally expressed transcript (non-protein coding)			
TNP3	3	Spermatid nuclear transition protein 3-like			
NOVA1	3	Neuro-oncological ventral antigen 1			
OVA	-3	Ovalbumin-like			
IGIP	-3	IgA regulatory protein		●	
SEPX1	-3	Selenoprotein X, 1			
COMMD10	-3	COMM domain containing 10			
CHEK1	-3	CHK1 checkpoint homolog (S. pombe)			
ARHGAP24	-3	Rho GTPase activating protein 24	●		
AKR1B1	-3	Aldo-keto reductase family 1, member B1 (aldose reductase)			
MFAP5	-3	Microfibrillar associated protein 5		●	
PIR	-3	Pirin (iron-binding nuclear protein)			
EIF2S3Y	-3	Eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked			
SERPINB10	-3	Serpin peptidase inhibitor, clade B (ovalbumin), member 10			
CDC6	-3	Cell division cycle 6 homolog (S. cerevisiae)			
RSAD2	-3	Radical S-adenosyl methionine domain containing 2			●
RAPGEF4	-3	Rap guanine nucleotide exchange factor (GEF) 4			
CALCRL	-3	Calcitonin receptor-like		●	●
IFIT3	-3	Interferon-induced protein with tetratricopeptide repeats 3			
MIR2414	-3	MicroRNA mir-2414			
CHPT1	-3	Choline phosphotransferase 1			●
AMTN	-3	Amelotin		●	
DRD3	-3	Dopamine receptor D3			●
EFEMP1	-3	EGF containing fibulin-like extracellular matrix protein 1		●	
MIR2463	-3	MicroRNA mir-2463			
CCK	-4	Cholecystokinin		●	
ZNF160	-4	Zinc finger protein 160-like			
P2RY1	-4	Purinergic receptor P2Y, G-protein coupled, 1		●	●
C8H9orf85	-4	Chromosome 8 open reading frame, human C9orf85			
HIST1H2AI	-4	Histone cluster 1, H2ai-like			
CCRL1	-4	Chemokine (C-C motif) receptor-like 1			
MIR2384-1	-4	MicroRNA mir-2384-1			
GGCT	-5	Gamma-glutamylcyclotransferase			

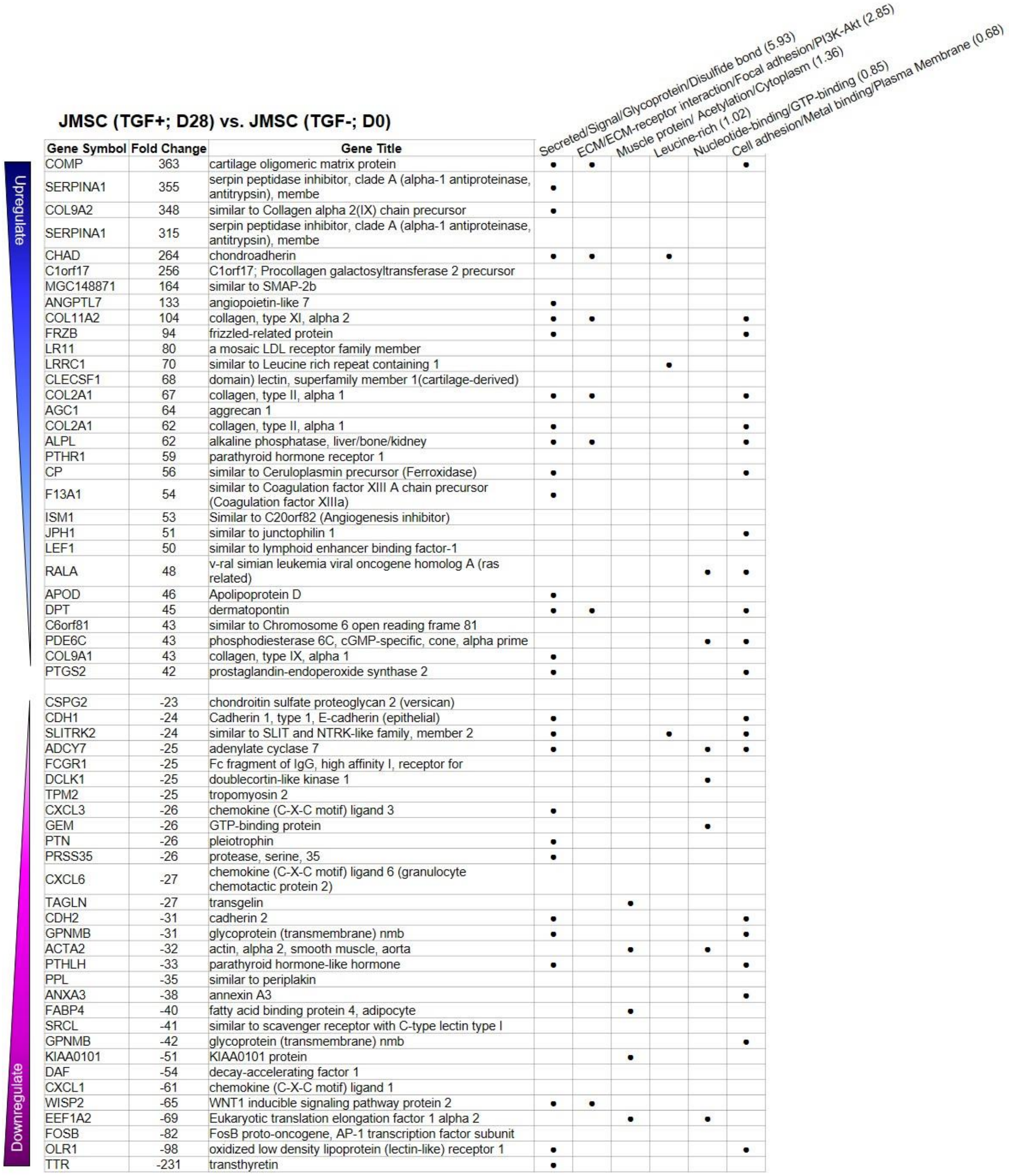
Table S8. Expression of genes and fold changes based on biological themes, enriched functional-related gene clusters, and their expression levels based on the effect of TGF-β3 and molecular factors from co-cultured JMSCs in adult MSCs.



AMSC (CO+) vs. AMSC (TGF-)

Gene Symbol	Fold Change	Gene Title	Secreted/ Disulfide bond/Signal/Glycoprotein (5.78)		ECM/Cell adhesion/Immunoglobulin-like (2.42)	Extracellular region/Protease/Hydrolase (1.68)		Immunoglobulin (0.68)	ATP-binding/Nucleotide-binding (0.26)	Immune response/Transmembrane (0.25)
HIST1H2AD	70	Histone cluster 1, H2								
POSTN	39	Periostin, osteoblast specific factor								
CHAD	37	Chondroadherin	•		•					
C1QTNF3	37	C1q and tumor necrosis factor related protein 3	•			•				
COMP	37	Cartilage oligomeric matrix protein	•	•	•					
ACAN	32	Aggrecan	•	•	•	•		•		
PTTG1	32	Pituitary tumor-transforming 1					•			
MIA	28	Melanoma inhibitory activity	•			•				
RRM2	27	Ribonucleotide reductase M2								
MGP	27	Matrix Gla protein	•	•						
HIST1H2AI	20	Histone cluster 1, H2ai-like								
UBE2C	17	Ubiquitin-conjugating enzyme E2C					•		•	
COL12A1	15	Collagen, type XII, alpha 1	•		•			•		
BEX3	13	Protein BEX3-like					•			
HAPLN1	12	Hyaluronan and proteoglycan link protein 1	•		•			•		
HIST1H2AD	12	Histone cluster 1, H2								
TOP2A	11	Topoisomerase (DNA) II alpha 170kDa							•	
IL1RN	11	Interleukin 1 receptor antagonist	•							
MATN2	10	Matrilin 2	•	•						
ENPP1	10	Ectonucleotide pyrophosphatase/phosphodiesterase 1								•
CENPA	10	Centromere protein A								
AMTN	9	Amelotin								
DTL	9	Denticleless protein homolog								
PKIB	9	Protein kinase (cAMP-dependent, catalytic) inhibitor beta								
MCOLN3	8	Mucolipin 3								•
PCLAF	8	PNCA Clamp Associated Factor (KIAA0101 ortholog)								
HIST1H2AH	8	Histone cluster 1, H2ah								
PENK	8	Proenkephalin	•			•				•
MXRA5	8	Matrix-remodelling associated 5	•		•			•		
DDC	8	Dopa Decarboxylase protein-like								
SFRP4	-9	Secreted frizzled-related protein 4	•							•
C1R	-9	Complement component 1, r subcomponent	•	•		•				
DNASE1L3	-9	Deoxyribonuclease I-like 3	•			•				
MT1E	-9	Metallothionein 1E								
RGS2	-10	Regulator of G-protein signaling 2-like					•			•
SPOCK1	-10	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	•	•	•					
TPPP3	-10	Tubulin polymerization-promoting protein family member 3					•			
CFB	-10	Complement factor B	•			•				
TNFSF15	-10	Tumor necrosis factor (ligand) superfamily, member 15								•
NRP1	-11	Neuropilin 1	•	•						•
ASS1	-11	Argininosuccinate synthase 1							•	
MGST1	-12	Microsomal glutathione S-transferase 1								•
DEFB1	-13	Beta-defensin 1-like	•			•				
CALCRL	-13	Calcitonin receptor-like	•							•
C2	-13	Complement component 2	•			•				
CX3CL1	-14	CX3CL1								•
STAT4	-15	Signal transducer and activator of transcription 4								
PPARG	-16	Peroxisome proliferator-activated receptor gamma					•			
CCL20	-16	Chemokine (C-C motif) ligand 20	•							
PI15	-16	Peptidase inhibitor 15								
PDK4	-16	Pyruvate dehydrogenase kinase, isozyme 4							•	
CD36	-18	CD36 molecule (thrombospondin receptor)	•		•		•			•
PTX3	-19	Pentraxin 3, long	•			•				
SLC7A8	-19	Solute carrier family 7 (amino acid transporter light chain, L system), member 8								•
FGL1	-19	Fibrinogen-like 1	•							
S100G	-20	S100 calcium binding protein G		•						
AMY2B	-21	Amylase, alpha 2B (pancreatic)				•				
EFEMP1	-24	EGF containing fibulin-like extracellular matrix protein 1	•	•			•			
ACSM1	-29	Acyl-CoA synthetase medium-chain family member 1							•	
FGL2	-30	Fibrinogen-like 2	•							

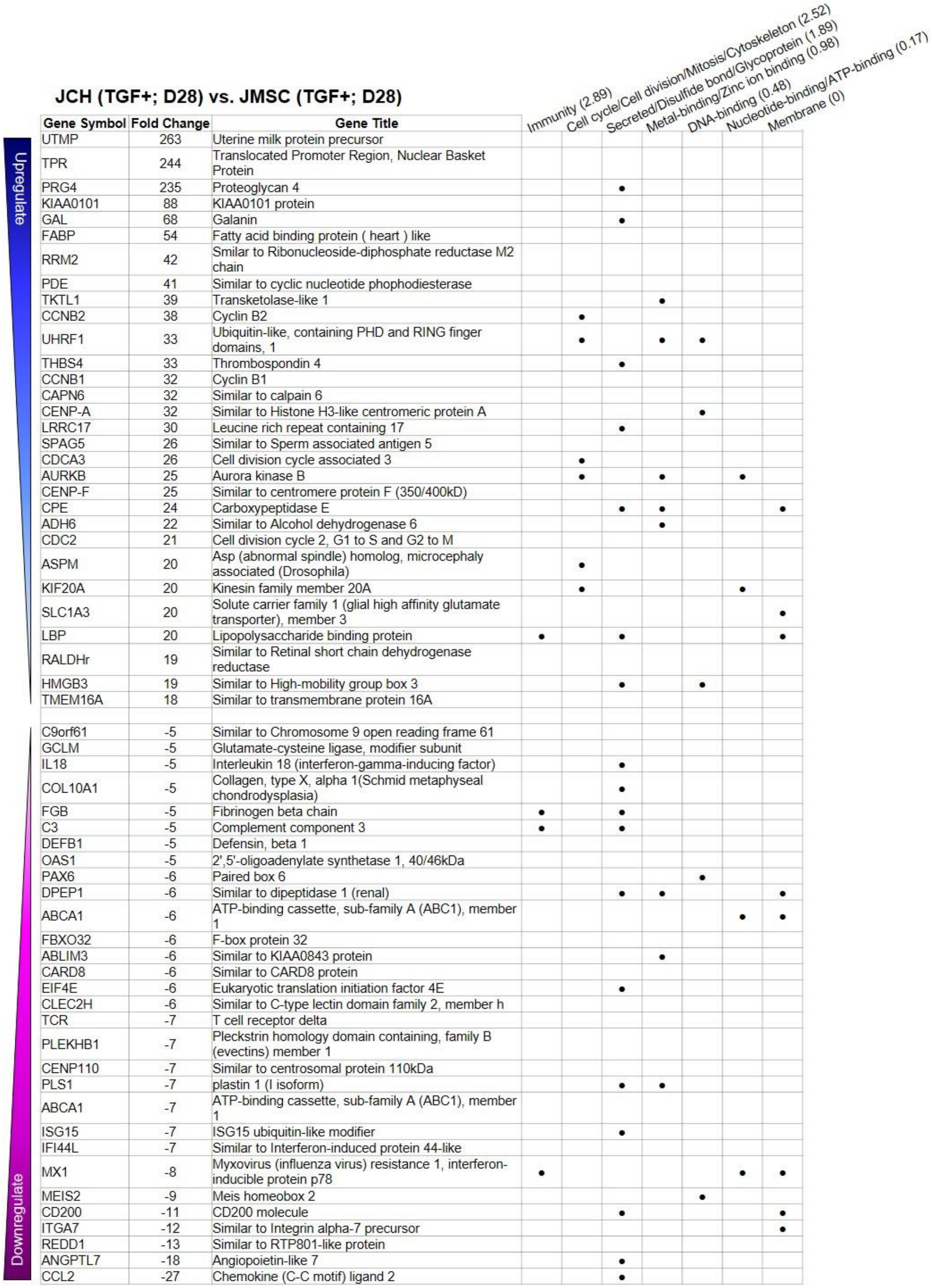
Table S9. Expression of genes with fold changes based on biological themes, enriched functional-related gene clusters, and their expression based on the effect of TGF-β3 on juvenile MSCs.



JMSC (TGF+; D28) vs. JMSC (TGF-; D0)

	Gene Symbol	Fold Change	Gene Title	Secreted/Signal/Glycoprotein/Disulfide bond (5.93)	ECM/ECM-receptor interaction/Focal adhesion/PI3K-Akt (2.85)	Muscle protein/ Acetylation/Cytoplasm (1.36)	Leucine-rich (1.02)	Nucleotide-binding/GTP-binding (0.85)	Cell adhesion/Metal binding/Plasma Membrane (0.68)
Upregulate	COMP	363	cartilage oligomeric matrix protein	•	•				•
	SERPINA1	355	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), membe	•					
	COL9A2	348	similar to Collagen alpha 2(IX) chain precursor	•					
	SERPINA1	315	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), membe						
	CHAD	264	chondroadherin	•	•		•		
	C1orf17	256	C1orf17; Procollagen galactosyltransferase 2 precursor						
	MGC148871	164	similar to SMAP-2b						
	ANGPTL7	133	angiopoietin-like 7	•					
	COL11A2	104	collagen, type XI, alpha 2	•	•				•
	FRZB	94	frizzled-related protein	•					•
	LR11	80	a mosaic LDL receptor family member						
	LRRC1	70	similar to Leucine rich repeat containing 1				•		
	CLECSF1	68	domain) lectin, superfamily member 1(cartilage-derived)						
	COL2A1	67	collagen, type II, alpha 1	•	•				•
	AGC1	64	aggrecan 1						
	COL2A1	62	collagen, type II, alpha 1	•					•
	ALPL	62	alkaline phosphatase, liver/bone/kidney	•	•				•
	PTHR1	59	parathyroid hormone receptor 1						
	CP	56	similar to Ceruloplasmin precursor (Ferroxidase)	•					•
	F13A1	54	similar to Coagulation factor XIII A chain precursor (Coagulation factor XIIIa)	•					
	ISM1	53	Similar to C20orf82 (Angiogenesis inhibitor)						
	JPH1	51	similar to junctophilin 1						•
	LEF1	50	similar to lymphoid enhancer binding factor-1						
	RALA	48	v-ral simian leukemia viral oncogene homolog A (ras related)					•	•
	APOD	46	Apolipoprotein D	•					
	DPT	45	dermatopontin	•	•				•
	C6orf81	43	similar to Chromosome 6 open reading frame 81						
	PDE6C	43	phosphodiesterase 6C, cGMP-specific, cone, alpha prime					•	•
	COL9A1	43	collagen, type IX, alpha 1	•					
	PTGS2	42	prostaglandin-endoperoxide synthase 2	•					•
Downregulate	CSPG2	-23	chondroitin sulfate proteoglycan 2 (versican)						
	CDH1	-24	Cadherin 1, type 1, E-cadherin (epithelial)	•					•
	SLITRK2	-24	similar to SLIT and NTRK-like family, member 2	•			•		•
	ADCY7	-25	adenylate cyclase 7	•				•	•
	FCGR1	-25	Fc fragment of IgG, high affinity I, receptor for						
	DCLK1	-25	doublecortin-like kinase 1					•	
	TPM2	-25	tropomyosin 2						
	CXCL3	-26	chemokine (C-X-C motif) ligand 3	•					
	GEM	-26	GTP-binding protein					•	
	PTN	-26	pleiotrophin	•					
	PRSS35	-26	protease, serine, 35	•					
	CXCL6	-27	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)						
	TAGLN	-27	transgelin			•			
	CDH2	-31	cadherin 2	•					•
	GPNMB	-31	glycoprotein (transmembrane) nmb	•					•
	ACTA2	-32	actin, alpha 2, smooth muscle, aorta			•		•	
	PTHLH	-33	parathyroid hormone-like hormone	•					•
	PPL	-35	similar to periplakin						
	ANXA3	-38	annexin A3						•
	FABP4	-40	fatty acid binding protein 4, adipocyte			•			
	SRCL	-41	similar to scavenger receptor with C-type lectin type I						
	GPNMB	-42	glycoprotein (transmembrane) nmb						•
	KIAA0101	-51	KIAA0101 protein			•			
	DAF	-54	decay-accelerating factor 1						
	CXCL1	-61	chemokine (C-X-C motif) ligand 1						
	WISP2	-65	WNT1 inducible signaling pathway protein 2	•	•				
	EEF1A2	-69	Eukaryotic translation elongation factor 1 alpha 2			•		•	
	FOSB	-82	FosB proto-oncogene, AP-1 transcription factor subunit						
	OLR1	-98	oxidized low density lipoprotein (lectin-like) receptor 1	•					•
	TTR	-231	transthyretin	•					

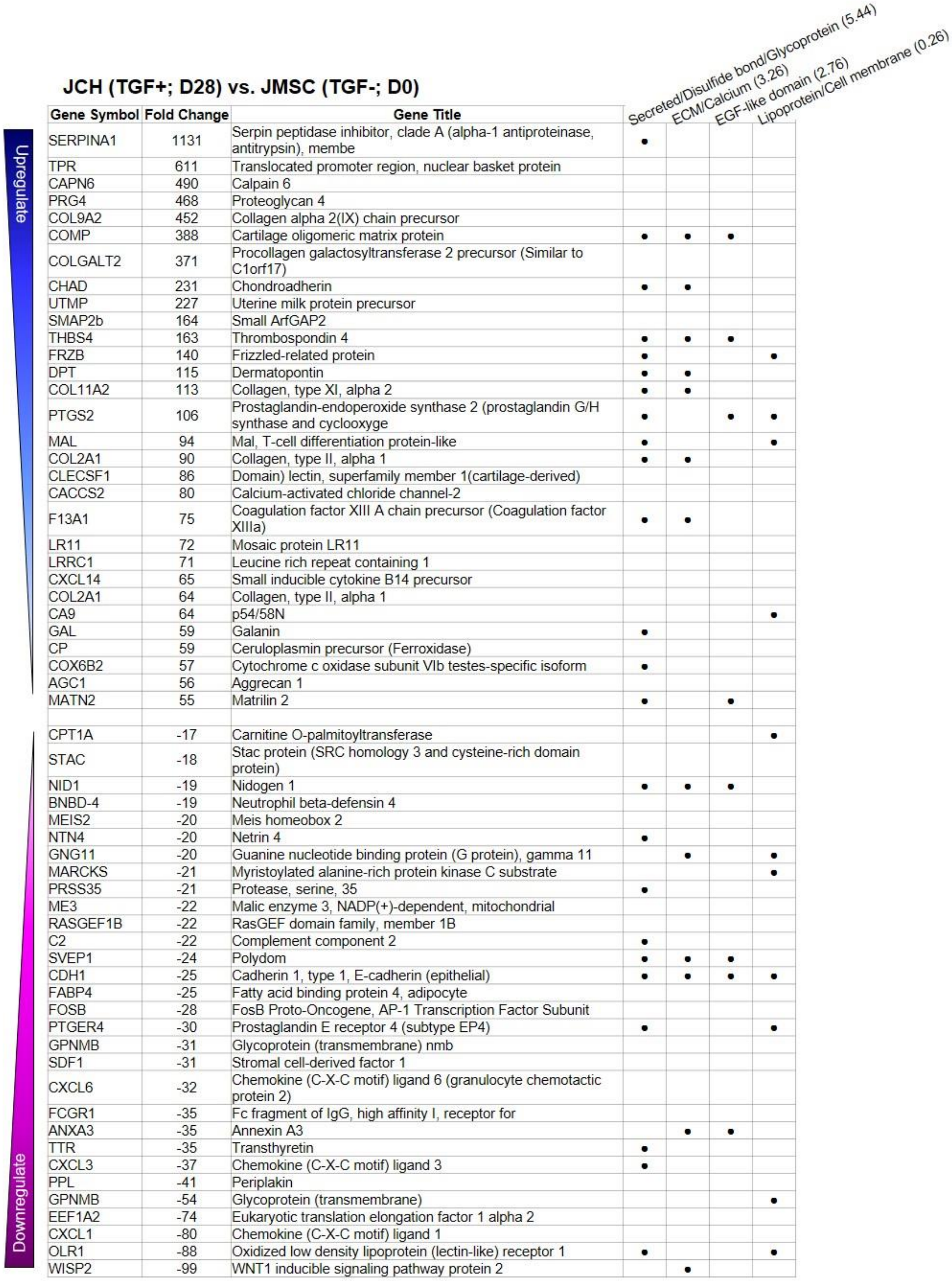
Table S10. Expression of genes with fold changes based on biological themes, enriched functional-related gene clusters, and their expression levels for juvenile MSCs compared to chondrocytes at day 28.



JCH (TGF+; D28) vs. JMSC (TGF+; D28)

Gene Symbol	Fold Change	Gene Title	Immunity (2.89)	Cell cycle/Cell division/Mitosis/Cytoskeleton (2.52)	Secreted/Disulfide bond/Glycoprotein (1.89)	Metal-binding/Zinc ion binding (0.98)	DNA-binding (0.48)	Nucleotide-binding/ATP-binding (0.17)	Membrane (0)
UTMP	263	Uterine milk protein precursor							
TPR	244	Translocated Promoter Region, Nuclear Basket Protein							
PRG4	235	Proteoglycan 4			●				
KIAA0101	88	KIAA0101 protein							
GAL	68	Galanin			●				
FABP	54	Fatty acid binding protein (heart) like							
RRM2	42	Smilar to Ribonucleoside-diphosphate reductase M2 chain							
PDE	41	Similar to cyclic nucleotide phophodiesterase							
TKTL1	39	Transketolase-like 1				●			
CCNB2	38	Cyclin B2		●					
UHRF1	33	Ubiquitin-like, containing PHD and RING finger domains, 1		●		●	●		
THBS4	33	Thrombospondin 4			●				
CCNB1	32	Cyclin B1							
CAPN6	32	Similar to calpain 6							
CENP-A	32	Similar to Histone H3-like centromeric protein A					●		
LRRC17	30	Leucine rich repeat containing 17			●				
SPAG5	26	Similar to Sperm associated antigen 5							
CDCA3	26	Cell division cycle associated 3		●					
AURKB	25	Aurora kinase B		●		●		●	
CENP-F	25	Similar to centromere protein F (350/400kD)							
CPE	24	Carboxypeptidase E			●	●			●
ADH6	22	Similar to Alcohol dehydrogenase 6				●			
CDC2	21	Cell division cycle 2, G1 to S and G2 to M							
ASPM	20	Asp (abnormal spindle) homolog, microcephaly associated (Drosophila)		●					
KIF20A	20	Kinesin family member 20A		●				●	
SLC1A3	20	Solute carrier family 1 (glial high affinity glutamate transporter), member 3							●
LBP	20	Lipopolysaccharide binding protein	●		●				●
RALDHr	19	Similar to Retinal short chain dehydrogenase reductase							
HMGB3	19	Similar to High-mobility group box 3			●		●		
TMEM16A	18	Similar to transmembrane protein 16A							
C9orf61	-5	Similar to Chromosome 9 open reading frame 61							
GCLM	-5	Glutamate-cysteine ligase, modifier subunit							
IL18	-5	Interleukin 18 (interferon-gamma-inducing factor)			●				
COL10A1	-5	Collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)			●				
FGB	-5	Fibrinogen beta chain	●		●				
C3	-5	Complement component 3	●		●				
DEFB1	-5	Defensin, beta 1							
OAS1	-5	2',5'-oligoadenylate synthetase 1, 40/46kDa							
PAX6	-6	Paired box 6					●		
DPEP1	-6	Similar to dipeptidase 1 (renal)			●	●			●
ABCA1	-6	ATP-binding cassette, sub-family A (ABC1), member 1						●	●
FBXO32	-6	F-box protein 32							
ABLIM3	-6	Similar to KIAA0843 protein				●			
CARD8	-6	Similar to CARD8 protein							
EIF4E	-6	Eukaryotic translation initiation factor 4E			●				
CLEC2H	-6	Similar to C-type lectin domain family 2, member h							
TCR	-7	T cell receptor delta							
PLEKHB1	-7	Pleckstrin homology domain containing, family B (evectins) member 1							
CENP110	-7	Similar to centrosomal protein 110kDa							
PLS1	-7	plastin 1 (I isoform)			●	●			
ABCA1	-7	ATP-binding cassette, sub-family A (ABC1), member 1							
ISG15	-7	ISG15 ubiquitin-like modifier			●				
IFI44L	-7	Similar to Interferon-induced protein 44-like							
MX1	-8	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78	●					●	●
MEIS2	-9	Meis homeobox 2					●		
CD200	-11	CD200 molecule			●				●
ITGA7	-12	Similar to Integrin alpha-7 precursor							●
REDD1	-13	Similar to RTP801-like protein							
ANGPTL7	-18	Angiopoietin-like 7			●				
CCL2	-27	Chemokine (C-C motif) ligand 2			●				

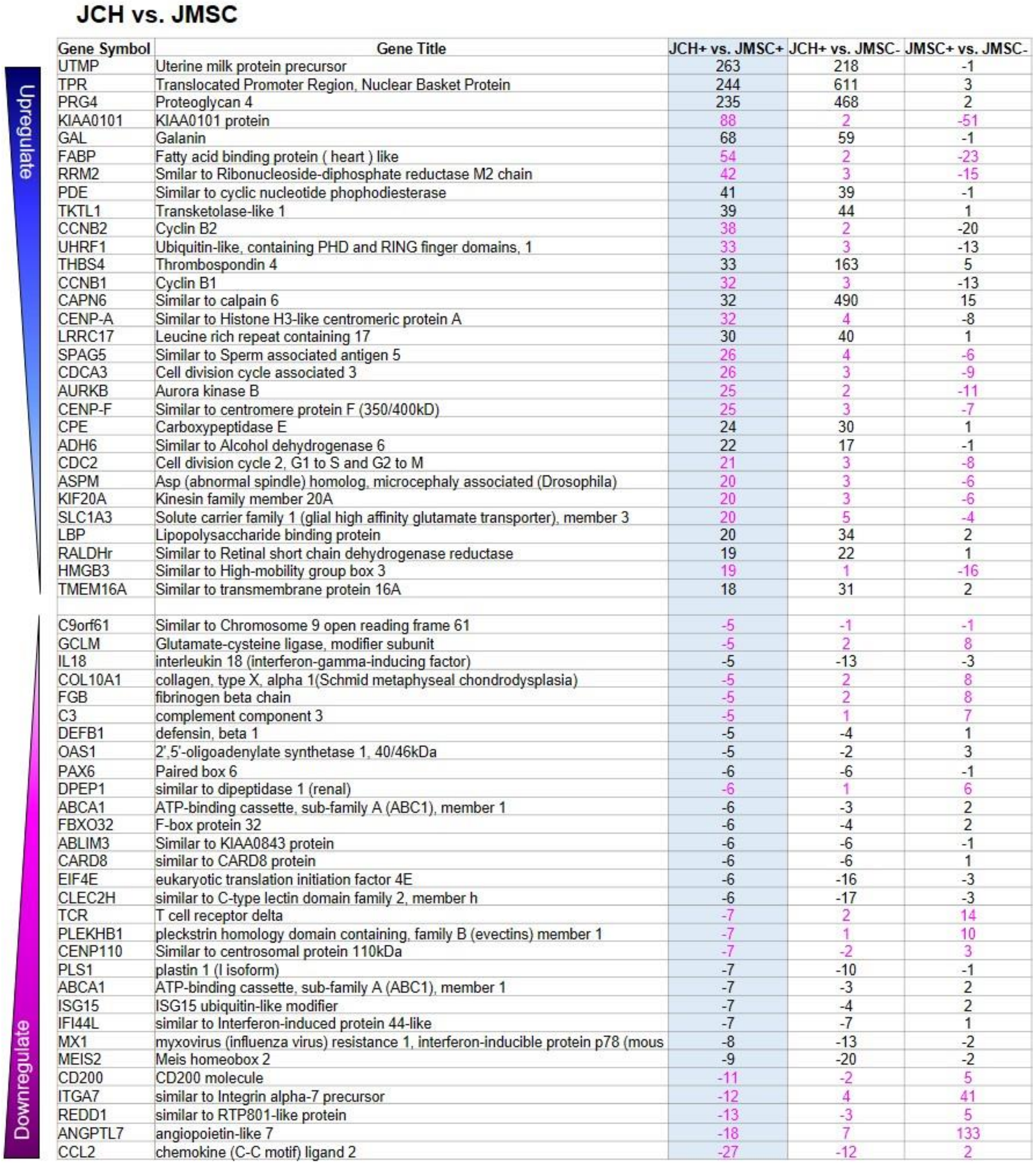
Table S11. Expression of genes with fold changes based on biological themes, enriched functional-related gene clusters, and their expression levels between juvenile chondrocytes with TGF-β3 and undifferentiated juvenile MSCs.



JCH (TGF+; D28) vs. JMSC (TGF-; D0)

Gene Symbol	Fold Change	Gene Title	Secreted/Disulfide bond/Glycoprotein (5.44)	ECM/Calcium (3.26)	EGF-like domain (2.76)	Lipoprotein/Cell membrane (0.26)
SERPINA1	1131	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), membe	●			
TPR	611	Translocated promoter region, nuclear basket protein				
CAPN6	490	Calpain 6				
PRG4	468	Proteoglycan 4				
COL9A2	452	Collagen alpha 2(IX) chain precursor				
COMP	388	Cartilage oligomeric matrix protein	●	●	●	
COLGALT2	371	Procollagen galactosyltransferase 2 precursor (Similar to C1orf17)				
CHAD	231	Chondroadherin	●	●		
UTMP	227	Uterine milk protein precursor				
SMAP2b	164	Small ArfGAP2				
THBS4	163	Thrombospondin 4	●	●	●	
FRZB	140	Frizzled-related protein	●			●
DPT	115	Dermatopontin	●	●		
COL11A2	113	Collagen, type XI, alpha 2	●	●		
PTGS2	106	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxyge	●		●	●
MAL	94	Mal, T-cell differentiation protein-like	●			●
COL2A1	90	Collagen, type II, alpha 1	●	●		
CLECSF1	86	Domain) lectin, superfamily member 1(cartilage-derived)				
CACCS2	80	Calcium-activated chloride channel-2				
F13A1	75	Coagulation factor XIII A chain precursor (Coagulation factor XIIIa)	●	●		
LR11	72	Mosaic protein LR11				
LRRC1	71	Leucine rich repeat containing 1				
CXCL14	65	Small inducible cytokine B14 precursor				
COL2A1	64	Collagen, type II, alpha 1				
CA9	64	p54/58N				●
GAL	59	Galanin	●			
CP	59	Ceruloplasmin precursor (Ferroxidase)				
COX6B2	57	Cytochrome c oxidase subunit VIb testes-specific isoform	●			
AGC1	56	Aggrecan 1				
MATN2	55	Matrilin 2	●		●	
CPT1A	-17	Carnitine O-palmitoyltransferase				●
STAC	-18	Stac protein (SRC homology 3 and cysteine-rich domain protein)				
NID1	-19	Nidogen 1	●	●	●	
BNBD-4	-19	Neutrophil beta-defensin 4				
MEIS2	-20	Meis homeobox 2				
NTN4	-20	Netrin 4	●			
GNG11	-20	Guanine nucleotide binding protein (G protein), gamma 11		●		●
MARCKS	-21	Myristoylated alanine-rich protein kinase C substrate				●
PRSS35	-21	Protease, serine, 35	●			
ME3	-22	Malic enzyme 3, NADP(+)-dependent, mitochondrial				
RASGEF1B	-22	RasGEF domain family, member 1B				
C2	-22	Complement component 2	●			
SVEP1	-24	Polydom	●	●	●	
CDH1	-25	Cadherin 1, type 1, E-cadherin (epithelial)	●	●	●	●
FABP4	-25	Fatty acid binding protein 4, adipocyte				
FOSB	-28	FosB Proto-Oncogene, AP-1 Transcription Factor Subunit				
PTGER4	-30	Prostaglandin E receptor 4 (subtype EP4)	●			●
GPNMB	-31	Glycoprotein (transmembrane) nmb				
SDF1	-31	Stromal cell-derived factor 1				
CXCL6	-32	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)				
FCGR1	-35	Fc fragment of IgG, high affinity I, receptor for				
ANXA3	-35	Annexin A3		●	●	
TTR	-35	Transthyretin	●			
CXCL3	-37	Chemokine (C-X-C motif) ligand 3	●			
PPL	-41	Periplakin				
GPNMB	-54	Glycoprotein (transmembrane)				●
EEF1A2	-74	Eukaryotic translation elongation factor 1 alpha 2				
CXCL1	-80	Chemokine (C-X-C motif) ligand 1				
OLR1	-88	Oxidized low density lipoprotein (lectin-like) receptor 1	●			●
WISP2	-99	WNT1 inducible signaling pathway protein 2		●		

Table S12. Differences in expression profiles and their fold changes in juvenile chondrocytes and MSCs over 28 days of culture, in the absence or presence of TGF-β3.



JCH vs. JMSC

Gene Symbol	Gene Title	JCH+ vs. JMSC+	JCH+ vs. JMSC-	JMSC+ vs. JMSC-
UTMP	Uterine milk protein precursor	263	218	-1
TPR	Translocated Promoter Region, Nuclear Basket Protein	244	611	3
PRG4	Proteoglycan 4	235	468	2
KIAA0101	KIAA0101 protein	88	2	-51
GAL	Galanin	68	59	-1
FABP	Fatty acid binding protein (heart) like	54	2	-23
RRM2	Smilar to Ribonucleoside-diphosphate reductase M2 chain	42	3	-15
PDE	Similar to cyclic nucleotide phophodiesterase	41	39	-1
TKTL1	Transketolase-like 1	39	44	1
CCNB2	Cyclin B2	38	2	-20
UHRF1	Ubiquitin-like, containing PHD and RING finger domains, 1	33	3	-13
THBS4	Thrombospondin 4	33	163	5
CCNB1	Cyclin B1	32	3	-13
CAPN6	Similar to calpain 6	32	490	15
CENP-A	Similar to Histone H3-like centromeric protein A	32	4	-8
LRRC17	Leucine rich repeat containing 17	30	40	1
SPAG5	Similar to Sperm associated antigen 5	26	4	-6
CDCA3	Cell division cycle associated 3	26	3	-9
AURKB	Aurora kinase B	25	2	-11
CENP-F	Similar to centromere protein F (350/400kD)	25	3	-7
CPE	Carboxypeptidase E	24	30	1
ADH6	Similar to Alcohol dehydrogenase 6	22	17	-1
CDC2	Cell division cycle 2, G1 to S and G2 to M	21	3	-8
ASPM	Asp (abnormal spindle) homolog, microcephaly associated (Drosophila)	20	3	-6
KIF20A	Kinesin family member 20A	20	3	-6
SLC1A3	Solute carrier family 1 (glial high affinity glutamate transporter), member 3	20	5	-4
LBP	Lipopolysaccharide binding protein	20	34	2
RALDHr	Similar to Retinal short chain dehydrogenase reductase	19	22	1
HMGB3	Similar to High-mobility group box 3	19	1	-16
TMEM16A	Similar to transmembrane protein 16A	18	31	2
C9orf61	Similar to Chromosome 9 open reading frame 61	-5	-1	-1
GCLM	Glutamate-cysteine ligase, modifier subunit	-5	2	8
IL18	interleukin 18 (interferon-gamma-inducing factor)	-5	-13	-3
COL10A1	collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)	-5	2	8
FGB	fibrinogen beta chain	-5	2	8
C3	complement component 3	-5	1	7
DEFB1	defensin, beta 1	-5	-4	1
OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa	-5	-2	3
PAX6	Paired box 6	-6	-6	-1
DPEP1	similar to dipeptidase 1 (renal)	-6	1	6
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	-6	-3	2
FBXO32	F-box protein 32	-6	-4	2
ABLIM3	Similar to KIAA0843 protein	-6	-6	-1
CARD8	similar to CARD8 protein	-6	-6	1
EIF4E	eukaryotic translation initiation factor 4E	-6	-16	-3
CLEC2H	similar to C-type lectin domain family 2, member h	-6	-17	-3
TCR	T cell receptor delta	-7	2	14
PLEKHB1	pleckstrin homology domain containing, family B (evectins) member 1	-7	1	10
CENP110	Similar to centrosomal protein 110kDa	-7	-2	3
PLS1	plastin 1 (I isoform)	-7	-10	-1
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	-7	-3	2
ISG15	ISG15 ubiquitin-like modifier	-7	-4	2
IFI44L	similar to Interferon-induced protein 44-like	-7	-7	1
MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mous	-8	-13	-2
MEIS2	Meis homeobox 2	-9	-20	-2
CD200	CD200 molecule	-11	-2	5
ITGA7	similar to Integrin alpha-7 precursor	-12	4	41
REDD1	similar to RTP801-like protein	-13	-3	5
ANGPTL7	angiopoietin-like 7	-18	7	133
CCL2	chemokine (C-C motif) ligand 2	-27	-12	2

Reference

1. Huang AH, Stein A, & Mauck RL (2010) Evaluation of the complex transcriptional topography of mesenchymal stem cell chondrogenesis for cartilage tissue engineering. *Tissue Eng Part A* 16(9):2699-2708.