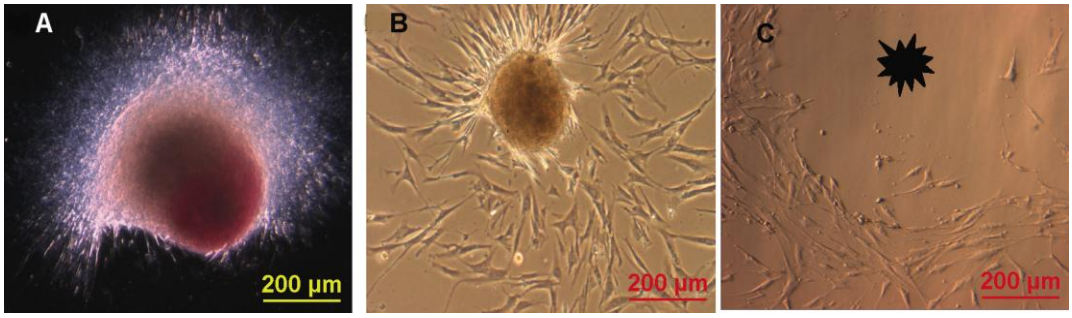
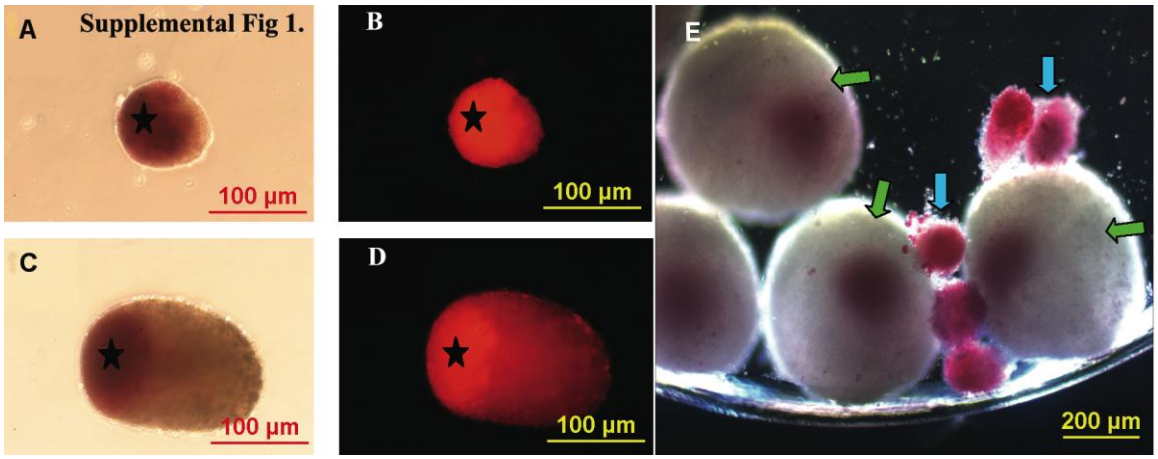


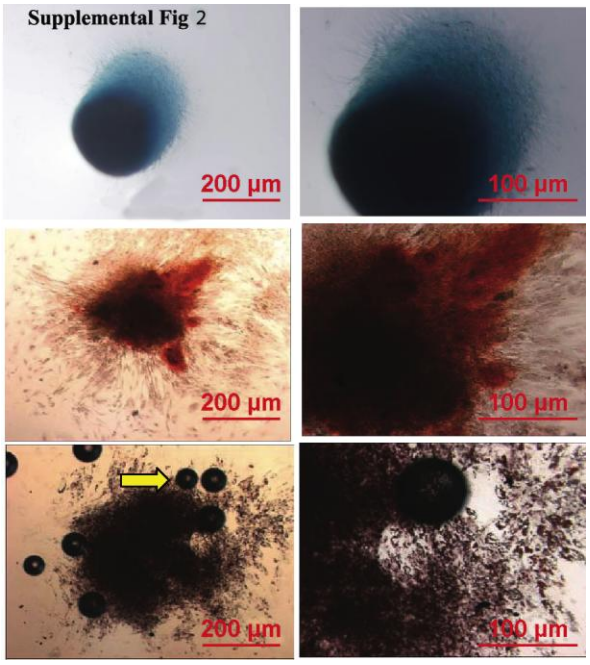
Supplemental Figure 1. Differential growth and polarization of ASC spheroids grown in suspension culture.

ASC spheroids were prepared using cells pre-labeled with fluorescent dye (DiI). Initially, the spheroids were small and spherical (A (bright light); and B (fluorescent)), but when maintained in growth factor enriched serum-free medium (HGFM-SF), the spheroid grew and polarized (C and D). Note the intense fluorescent pole (star) that remains even after extensive growth of the spheroid. ASC spheroids demonstrate differential growth in suspension culture depending on the specific culture conditions. Image E illustrates smaller, circular spheroids (blue arrows) with uniform red fluorescence that have been grown in traditional DMEM medium with 10% FBS (D-10). These are in stark contrast to the large spheroids (green arrows) grown in HGFM-SF medium. The 'starting' DiI cells are still visible as an intense red 'core', surrounded by new-growth cells and matrix with a diluted (absent) fluorescent signal.



Supplemental Figure 2. Differentiation of human ASC spheroids after placement in adherent culture.

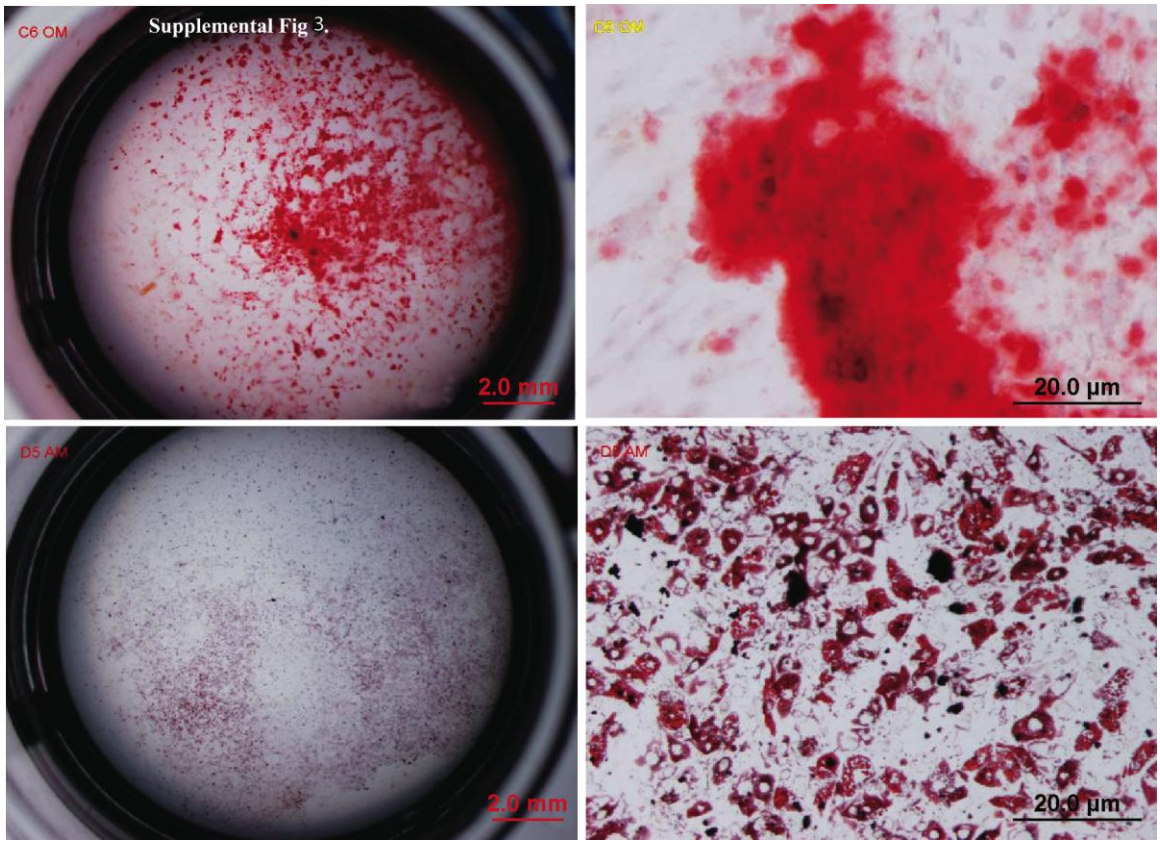
ASC spheroids were cultured for 4 weeks in serum-free suspension culture, and then placed into adherent culture (tissue culture plastic). After 4 days in adherent culture the cells were exposed to adipogenic, chondrogenic or osteogenic induction media for 2 weeks. Spheroids were then fixed and stained with Alcian Blue for chondrogenesis (top images), Alizarin Red for osteogenesis (middle images), or Oil Red O for adipogenesis (bottom images). Note the presence of free lipid droplets in the medium (arrow) in lower images, indicative of adipogenic differentiation.



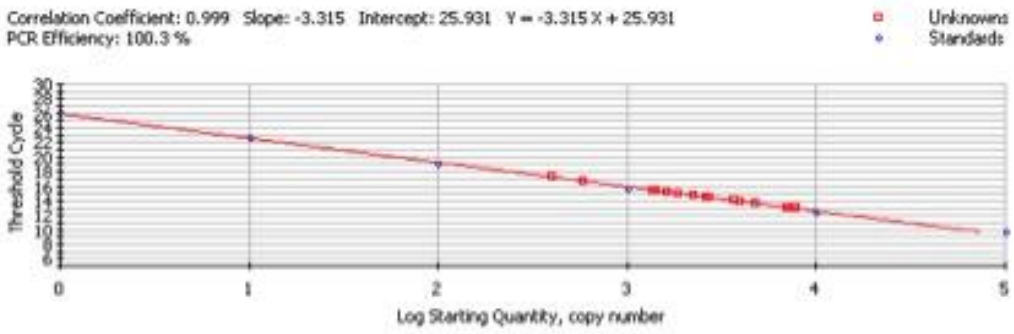
Supplemental Figure 3. Differentiation of 2-D monolayer ASCs derived from adherent, serially passaged spheroids.

Human ASCs were maintained for 12 days in serum-free suspension culture (HGFM-SF), and then placed into adherent culture (tissue culture plastic) in D-10 medium. The adherent spheroids were passaged every 3-4 days to new culture wells 10 separate times. Passage 10 spheroid-derived cells were then expanded to near-confluence in D-10 medium over 6 weeks. The

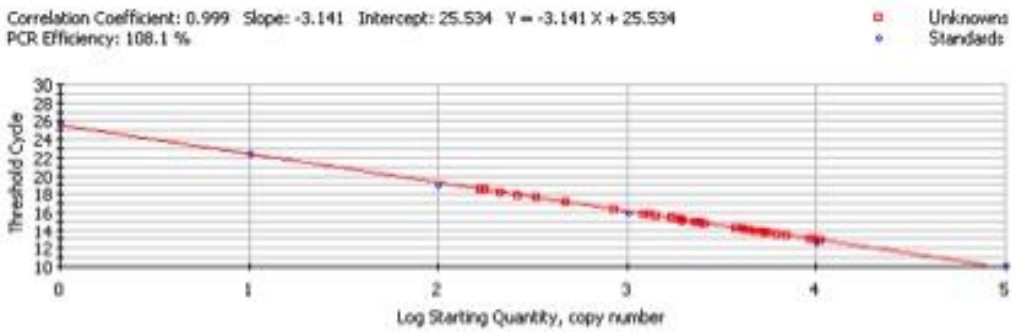
expanded P10 cells were then cultured in either adipogenic or osteogenic induction media for 2 weeks. Cells were then fixed and stained with Alizarin Red for osteogenesis (top images), or Oil Red O for adipogenesis (bottom images). Images on left show the entire culture well, while images on right show representative microscopic view.



Supplemental Figure 4: Standard Curve Graph for SYBR-490: Adipogenic Markers



Supplemental Figure 5: Standard Curve Graph for SYBR-490: Osteogenic Markers



Supplemental Figure 6: Standard Curve Graph for SYBR-490: Chondrogenic Markers

Supplemental Tables

Tables showing standard deviation on the PCR data graphed in Figure 8.

Supplemental Table 1

LPL Control vs AM at 3 wks	Variable 1	Variable 2
Mean	0.020762	1.252387
Variance	0.000383	0.177107
Observations	3	3
Pearson Correlation	-0.97559	
Hypothesized Mean Difference	0	
df	2	
t Stat	-4.84867	
P(T<=t) one-tail	0.020001	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.040001	
t Critical two-tail	4.302653	

Supplemental Table 2

PPAR2 Control vs AM at 3 wks	Variable 1	Variable 2
Mean	0.009153	1.707977
Variance	1.87E-05	4.368772
Observations	3	3
Pearson Correlation	-0.73438	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.40562	
P(T<=t) one-tail	0.147525	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.295051	
t Critical two-tail	4.302653	

Supplemental Table 3

FABP4 Control vs AM at 3 wks	Variable 1	Variable 2
Mean	0.062508	1.005848
Variance	0.003381	0.812801
Observations	3	3
Pearson Correlation	-0.96972	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.70547	
P(T<=t) one-tail	0.115112	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.230225	
t Critical two-tail	4.302653	

Supplemental Table 4

LPL Control vs AM at 6 wks	Variable 1	Variable 2
Mean	0.041073	0.49027
Variance	0.000941	0.103642
Observations	3	3
Pearson Correlation	0.386994	
Hypothesized Mean Difference	0	
df	2	
t Stat	-2.49891	
P(T<=t) one-tail	0.064852	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.129704	
t Critical two-tail	4.302653	

Supplemental Table 5

PPAR2 Control vs AM at 6 wks	Variable 1	Variable 2

Mean	0.085042	0.439802
Variance	0.015255	0.131218
Observations	3	3
Pearson Correlation	0.352993	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.81284	
P(T<=t) one-tail	0.10577	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.211539	
t Critical two-tail	4.302653	

Supplemental Table 6

FABP4 Control vs AM at 6 wks	Variable 1	Variable 2
Mean	0.063073	0.434706
Variance	0.002398	0.107693
Observations	3	3
Pearson Correlation	0.870406	
Hypothesized Mean Difference	0	
df	2	
t Stat	-2.24629	
P(T<=t) one-tail	0.076873	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.153747	
t Critical two-tail	4.302653	

Supplemental Table 7

RUNX2 Control vs OM at 3 wks	Variable 1	Variable 2
Mean	0.557695	0.268591
Variance	0.231749	0.003094
Observations	3	3
Pearson Correlation	-0.83916	
Hypothesized Mean Difference	0	
df	2	
t Stat	0.946686	
P(T<=t) one-tail	0.221862	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.443724	
t Critical two-tail	4.302653	

Supplemental Table 8

AP Control vs OM at 3 wks	Variable 1	Variable 2
Mean	0.349271	0.213835
Variance	0.105966	0.003427

Observations	3	3
Pearson Correlation	-0.21115	
Hypothesized Mean Difference	0	
df	2	
t Stat	0.684517	
P(T<=t) one-tail	0.282163	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.564326	
t Critical two-tail	4.302653	

Supplemental Table 9

RUNX2 Control vs OM at 6 wks	Variable 1	Variable 2
Mean	0.463116	0.790479
Variance	0.072851	0.527358
Observations	3	3
Pearson Correlation	0.996095	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.23812	
P(T<=t) one-tail	0.170644	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.341289	
t Critical two-tail	4.302653	

Supplemental Table 10

AP Control vs OM at 3 wks	Variable 1	Variable 2
Mean	0.500895	0.510108
Variance	0.091036	0.01932
Observations	3	3
Pearson Correlation	0.875071	
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.083	
P(T<=t) one-tail	0.470706	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.941411	
t Critical two-tail	4.302653	

Supplemental Table 11

Collagen I Control vs CM at 3 wks	Variable 1	Variable 2
Mean	0.14170997	0.136470011
Variance	0.037593371	0.012706905
Observations	3	3
Pearson Correlation	0.959839588	
Hypothesized Mean Difference	0	
df	2	
t Stat	0.09936183	
P(T<=t) one-tail	0.464956675	
t Critical one-tail	2.91998558	

P(T<=t) two-tail	0.929913351
t Critical two-tail	4.30265273

Supplemental Table 12

Collagen I Control vs CM at 3 wks	Variable 1	Variable 2
Mean	0.594373	1.508529
Variance	0.228741	1.918067
Observations	3	3
Pearson Correlation	0.644261	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.39228	
P(T<=t) one-tail	0.14922	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.29844	
t Critical two-tail	4.302653	

Supplemental Table 13

Aggrecan Control vs CM at 3 wks	Variable 1	Variable 2
Mean	0.356179	0.619536
Variance	0.034008	0.282747
Observations	3	3
Pearson Correlation	0.554147	
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.99999	
P(T<=t) one-tail	0.211327	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.422655	
t Critical two-tail	4.302653	

Supplemental Table 14

Collagen I Control vs CM at 6 wks	Variable 1	Variable 2
Mean	0.020490384	0.059263853
Variance	0.000236223	0.003261218
Observations	3	3
Pearson Correlation	0.151695379	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.1814533	
P(T<=t) one-tail	0.179436943	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.358873885	
t Critical two-tail	4.30265273	

Supplemental Table 15

Collagen II Control vs CM at 6 wks	Variable 1	Variable 2
Mean	2.332844	21.35508

Variance	3.469658	1324.319
Observations	3	3
Pearson Correlation	0.823733	
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.94479	
P(T<=t) one-tail	0.222247	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.444494	
t Critical two-tail	4.302653	

Supplemental Table 16

Aggrecan Control vs CM at 6 wks	Variable 1	Variable 2
Mean	0.984337	8.19944
Variance	1.056371	180.5887
Observations	3	3
Pearson Correlation	0.939871	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.00158	
P(T<=t) one-tail	0.211022	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.422044	
t Critical two-tail	4.302653	

Supplemental Table 17
Standard Curve PCR Data for Adipogenic Differentiation Markers

Data Analysis Parameters

Calculated threshold using the **maximum correlation coefficient approach** is **685.4**.

Per-well baseline cycles have been determined automatically.

Data analysis window is set at **95.00%** of a cycle, centered at **end** of the cycle.

Weighted Mean digital filtering has been applied. Global filtering is **off**.

Type	Identifier	Rep	Ct	Log	SQ	SQ	SQ	Ct	Ct	Set
A02	Standard		1	9.79	5.000	1.00E+05	1.00E+05	N/A	9.79	N/A
A03	Standard		2	12.40	4.000	1.00E+04	1.00E+04	N/A	12.40	N/A
A04	Standard		3	15.66	3.000	1.00E+03	1.00E+03	N/A	15.66	N/A
A05	Standard		4	19.14	2.000	1.00E+02	1.00E+02	N/A	19.14	N/A
A06	Standard		5	22.80		1.00E+01	1.00E+01	N/A	22.80	N/A

A07	Standard		6	26.06	1.000	1.00E+00	1.00E+00	N/A	26.06	N/A
A08	Unknown		1	16.77	0.000	5.80E+02	5.80E+02	N/A	16.77	N/A
A09	Unknown		2	17.32	2.763	3.97E+02	3.97E+02	N/A	17.32	N/A
A10	Unknown		3	15.49	2.599	1.41E+03	1.41E+03	N/A	15.49	N/A
A11	Unknown		4	15.55	3.149	1.35E+03	1.35E+03	N/A	15.55	N/A
B01	Unknown		5	15.29	3.131	1.63E+03	1.63E+03	N/A	15.29	N/A
B02	Unknown		6	15.12	3.211	1.83E+03	1.83E+03	N/A	15.12	N/A
B03	Unknown		7	14.84	3.262	2.22E+03	2.22E+03	N/A	14.84	N/A
B04	Unknown		8	14.56	3.346	2.69E+03	2.69E+03	N/A	14.56	N/A
B05	Unknown		9	14.12	3.431	3.66E+03	3.66E+03	N/A	14.12	N/A
B06	Unknown		10	14.61	3.563	2.61E+03	2.61E+03	N/A	14.61	N/A
B07	Unknown		11	13.03	3.416	7.80E+03	7.80E+03	N/A	13.03	N/A
B08	Unknown		12	13.07	3.892	7.58E+03	7.58E+03	N/A	13.07	N/A
B09	Unknown		13	13.21	3.880	6.90E+03	6.90E+03	N/A	13.21	N/A
B10	Unknown		14	13.17	3.839	7.05E+03	7.05E+03	N/A	13.17	N/A
B11	Unknown		15	13.74	3.848	4.76E+03	4.76E+03	N/A	13.74	N/A
					3.678					

Supplemental Table 18
Standard Curve PCR Data for Osteogenic Differentiation Markers
Data Analysis Parameters

Calculated threshold using the **maximum correlation coefficient approach** is **539.5**.

Per-well baseline cycles have been determined automatically.

Data analysis window is set at **95.00%** of a cycle, centered at **end** of the cycle.

Weighted Mean digital filtering has been applied. Global filtering is **off**.

Type	Identifier	Rep	Ct	Log	SQ	SQ	SQ	Ct	Ct	Set
A02	Standard		1	10.15	5.000	1.00E+05	1.00E+05	N/A	10.15	N/A
A03	Standard		2	12.75	4.000	1.00E+04	1.00E+04	N/A	12.75	N/A
A04	Standard		3	16.04	3.000	1.00E+03	1.00E+03	N/A	16.04	N/A
A05	Standard		4	18.99	2.000	1.00E+02	1.00E+02	N/A	18.99	N/A
A06	Standard		5	22.40		1.00E+01	1.00E+01	N/A	22.40	N/A

A07	Standard	6	25.75	1.000	1.00E+00	1.00E+00	N/A	25.75	N/A
A08	Unknown	1	17.13	0.000	4.75E+02	4.75E+02	N/A	17.13	N/A
A09	Unknown	2	17.62	2.676	3.31E+02	3.31E+02	N/A	17.62	N/A
A10	Unknown	3	15.73	2.519	1.32E+03	1.32E+03	N/A	15.73	N/A
A11	Unknown	4	16.33	3.121	8.51E+02	8.51E+02	N/A	16.33	N/A
B01	Unknown	5	14.98	2.930	2.29E+03	2.29E+03	N/A	14.98	N/A
B02	Unknown	6	13.64	3.360	6.13E+03	6.13E+03	N/A	13.64	N/A
B03	Unknown	7	15.27	3.788	1.86E+03	1.86E+03	N/A	15.27	N/A
B04	Unknown	8	15.24	3.269	1.90E+03	1.90E+03	N/A	15.24	N/A
B05	Unknown	9	18.47	3.278	1.77E+02	1.77E+02	N/A	18.47	N/A
B06	Unknown	10	17.94	2.247	2.62E+02	2.62E+02	N/A	17.94	N/A
B07	Unknown	11	13.82	2.418	5.36E+03	5.36E+03	N/A	13.82	N/A
B08	Unknown	12	14.00	3.729	4.69E+03	4.69E+03	N/A	14.00	N/A
B09	Unknown	13	15.20	3.671	1.94E+03	1.94E+03	N/A	15.20	N/A
B10	Unknown	14	15.22	3.288	1.92E+03	1.92E+03	N/A	15.22	N/A
B11	Unknown	15	15.39	3.284	1.70E+03	1.70E+03	N/A	15.39	N/A
B12	Unknown	16	15.63	3.230	1.42E+03	1.42E+03	N/A	15.63	N/A
C01	Unknown	17	14.99	3.154	2.28E+03	2.28E+03	N/A	14.99	N/A
C02	Unknown	18	15.29	3.358	1.83E+03	1.83E+03	N/A	15.29	N/A
C03	Unknown	19	13.09	3.261	9.14E+03	9.14E+03	N/A	13.09	N/A
C04	Unknown	20	13.48	3.961	6.89E+03	6.89E+03	N/A	13.48	N/A
C05	Unknown	21	13.92	3.838	4.98E+03	4.98E+03	N/A	13.92	N/A
C06	Unknown	22	13.85	3.697	5.23E+03	5.23E+03	N/A	13.85	N/A
C07	Unknown	23	15.74	3.718	1.31E+03	1.31E+03	N/A	15.74	N/A
C08	Unknown	24	15.84	3.117	1.22E+03	1.22E+03	N/A	15.84	N/A
C09	Unknown	25	18.22	3.087	2.13E+02	2.13E+02	N/A	18.22	N/A
C10	Unknown	26	18.57	2.327	1.65E+02	1.65E+02	N/A	18.57	N/A
				2.216					

C11	Unknown		27	13.95	3.687	4.87E+03	4.87E+03	N/A	13.95	N/A
C12	Unknown		28	14.09	3.643	4.40E+03	4.40E+03	N/A	14.09	N/A
D01	Unknown		29	14.85	3.402	2.53E+03	2.53E+03	N/A	14.85	N/A
D02	Unknown		30	14.91	3.382	2.41E+03	2.41E+03	N/A	14.91	N/A
D03	Unknown		31	13.79	3.739	5.48E+03	5.48E+03	N/A	13.79	N/A
D04	Unknown		32	14.18	3.616	4.13E+03	4.13E+03	N/A	14.18	N/A
D05	Unknown		33	13.97	3.682	4.81E+03	4.81E+03	N/A	13.97	N/A
D06	Unknown		34	14.33	3.565	3.67E+03	3.67E+03	N/A	14.33	N/A
D07	Unknown		35	13.04	3.978	9.50E+03	9.50E+03	N/A	13.04	N/A
D08	Unknown		36	12.89	4.024	1.06E+04	1.06E+04	N/A	12.89	N/A
D09	Unknown		37	14.31	3.573	3.74E+03	3.74E+03	N/A	14.31	N/A
D10	Unknown		38	14.21	3.604	4.02E+03	4.02E+03	N/A	14.21	N/A
A02	Standard		1	10.15	5.000	1.00E+05	1.00E+05	N/A	10.15	N/A
A03	Standard		2	12.75	4.000	1.00E+04	1.00E+04	N/A	12.75	N/A
A04	Standard		3	16.04	3.000	1.00E+03	1.00E+03	N/A	16.04	N/A

Supplemental Table 19
Standard Curve PCR Data for Chondrogenic Differentiation Markers

Data Analysis Parameters

Calculated threshold using the **maximum correlation coefficient approach** is **1,081.1**.

Per-well baseline cycles have been determined automatically.

Data analysis window is set at **95.00%** of a cycle, centered at **end** of the cycle.

Weighted Mean digital filtering has been applied. Global filtering is **off**.

Type	Identifier	Rep	Ct	Log	SQ	SQ	SQ	Ct	Ct	Set
A03	Standard		2	13.32	4.000	1.00E+04	1.00E+04	N/A	13.32	N/A
A04	Standard		3	16.56	3.000	1.00E+03	1.00E+03	N/A	16.56	N/A
A05	Standard		4	19.55	2.000	1.00E+02	1.00E+02	N/A	19.55	N/A
A06	Standard		5	22.79	1.000	1.00E+01	1.00E+01	N/A	22.79	N/A

A07	Standard	6	26.07	0.000	1.00E+00	1.00E+00	N/A	26.07	N/A
A08	Unknown	1	18.88	2.245	1.76E+02	1.76E+02	N/A	18.88	N/A
A09	Unknown	2	17.96	2.537	3.44E+02	3.44E+02	N/A	17.96	N/A
A10	Unknown	3	15.35	3.359	2.28E+03	2.28E+03	N/A	15.35	N/A
A11	Unknown	4	15.54	3.298	1.99E+03	1.99E+03	N/A	15.54	N/A
B01	Unknown	5	14.00	3.783	6.06E+03	6.06E+03	N/A	14.00	N/A
B02	Unknown	6	16.38	3.032	1.08E+03	1.08E+03	N/A	16.38	N/A
B03	Unknown	7	15.13	3.429	2.68E+03	2.68E+03	N/A	15.13	N/A
B04	Unknown	8	15.54	3.299	1.99E+03	1.99E+03	N/A	15.54	N/A
B05	Unknown	9	18.08	2.498	3.15E+02	3.15E+02	N/A	18.08	N/A
B06	Unknown	10	17.96	2.535	3.43E+02	3.43E+02	N/A	17.96	N/A
B07	Unknown	11	16.15	3.106	1.28E+03	1.28E+03	N/A	16.15	N/A
B08	Unknown	12	16.08	3.129	1.35E+03	1.35E+03	N/A	16.08	N/A
B09	Unknown	13	15.43	3.332	2.15E+03	2.15E+03	N/A	15.43	N/A
B10	Unknown	14	16.10	3.121	1.32E+03	1.32E+03	N/A	16.10	N/A
B11	Unknown	15	20.77	1.650	4.47E+01	4.47E+01	N/A	20.77	N/A
B12	Unknown	16	21.19	1.517	3.29E+01	3.29E+01	N/A	21.19	N/A
C01	Unknown	17	16.09	3.124	1.33E+03	1.33E+03	N/A	16.09	N/A
C02	Unknown	18	15.84	3.205	1.60E+03	1.60E+03	N/A	15.84	N/A
C03	Unknown	19	14.18	3.727	5.33E+03	5.33E+03	N/A	14.18	N/A
C04	Unknown	20	14.37	3.666	4.63E+03	4.63E+03	N/A	14.37	N/A
C05	Unknown	21	14.01	3.781	6.03E+03	6.03E+03	N/A	14.01	N/A
C06	Unknown	22	14.26	3.701	5.02E+03	5.02E+03	N/A	14.26	N/A
C07	Unknown	23	15.18	3.413	2.59E+03	2.59E+03	N/A	15.18	N/A
C08	Unknown	24	15.18	3.413	2.59E+03	2.59E+03	N/A	15.18	N/A
C09	Unknown	25	16.57	2.974	9.42E+02	9.42E+02	N/A	16.57	N/A
C10	Unknown	26	16.43	3.018	1.04E+03	1.04E+03	N/A	16.43	N/A
C11	Unknown	27	13.93	3.804	6.37E+03	6.37E+03	N/A	13.93	N/A

C12	Unknown	28	14.41	3.656	4.53E+03	4.53E+03	N/A	14.41	N/A
D01	Unknown	29	14.39	3.660	4.57E+03	4.57E+03	N/A	14.39	N/A
D02	Unknown	30	13.90	3.814	6.52E+03	6.52E+03	N/A	13.90	N/A
D03	Unknown	31	14.95	3.484	3.05E+03	3.05E+03	N/A	14.95	N/A
D04	Unknown	32	15.27	3.384	2.42E+03	2.42E+03	N/A	15.27	N/A
D05	Unknown	33	14.41	3.655	4.52E+03	4.52E+03	N/A	14.41	N/A
D06	Unknown	34	14.98	3.475	2.99E+03	2.99E+03	N/A	14.98	N/A
D07	Unknown	35	14.08	3.757	5.72E+03	5.72E+03	N/A	14.08	N/A
D08	Unknown	36	13.73	3.867	7.37E+03	7.37E+03	N/A	13.73	N/A
D09	Unknown	37	14.73	3.553	3.58E+03	3.58E+03	N/A	14.73	N/A
D10	Unknown	38	14.62	3.590	3.89E+03	3.89E+03	N/A	14.62	N/A