

Age, Diet and Epidermal Signaling Modulate Dermal Fibroblasts' Adipogenic Potential

Table S1. Dermal fibroblasts migration—comparisons by day.

GROUP	DAY	LSMEAN	LOWER.CL	UPPER.CL	SE	DF	2D	3D	4D	6D	7D
YOUNG LFD	1d	0.00	-9.16	9.16	4.62	101.00	1.000	0.987		<0.001	<0.001
YOUNG LFD	2d	-0.00	-9.16	9.16	4.62	101.00		0.987		<0.001	<0.001
YOUNG LFD	3d	4.25	-4.92	13.41	4.62	101.00				0.004	<0.001
YOUNG LFD	4d	NA	NA	NA	NA	NA					
YOUNG LFD	6d	28.52	19.36	37.68	4.62	101.00					0.006
YOUNG LFD	7d	54.65	43.70	65.60	5.52	101.00					
YOUNG HFD	1d	0.00	-14.49	14.49	7.30	101.00	1.000	0.728	<0.001		
YOUNG HFD	2d	1.58	-12.91	16.07	7.30	101.00		0.813	<0.001		
YOUNG HFD	3d	14.45	-0.04	28.93	7.30	101.00			0.004		
YOUNG HFD	4d	53.26	38.77	67.75	7.30	101.00					
YOUNG HFD	6d	NA	NA	NA	NA	NA					
YOUNG HFD	7d	NA	NA	NA	NA	NA					
OLD LFD	1d	0.00	-12.96	12.96	6.53	101.00	1.000	1.000		1.000	0.999
OLD LFD	2d	0.00	-12.96	12.96	6.53	101.00		1.000		1.000	0.999
OLD LFD	3d	1.25	-11.70	14.21	6.53	101.00				1.000	1.000
OLD LFD	4d	NA	NA	NA	NA	NA					
OLD LFD	6d	1.90	-11.06	14.86	6.53	101.00					1.000
OLD LFD	7d	3.10	-9.86	16.06	6.53	101.00					
OLD HFD	1d	16.18	7.02	25.34	4.62	101.00	0.001	<0.001			
OLD HFD	2d	42.91	33.75	52.08	4.62	101.00		0.025			
OLD HFD	3d	63.54	54.38	72.70	4.62	101.00					
OLD HFD	4d	NA	NA	NA	NA	NA					
OLD HFD	6d	NA	NA	NA	NA	NA					
OLD HFD	7D	NA	NA	NA	NA	NA					

Table S2. *Cepba* mRNA expression in DFs with keratinocytes coculture—comparisons by day.

	GROUP	TIME	LSMEAN	LOWER.CL	UPPER.CL	SE	DF	D2	D4	D7
2M LFD	Ad-Foxn1	d0	2.85	-1.06	6.77	1.97	104.00	0.357	0.033	0.007
2M LFD	Ad-Foxn1	d2	7.45	3.53	11.36	1.97	104.00		0.727	0.403
2M LFD	Ad-Foxn1	d4	10.20	6.70	13.70	1.77	104.00			0.944
2M LFD	Ad-Foxn1	d7	11.59	8.09	15.09	1.77	104.00			
2M LFD	Ad-GFP	d0	2.85	-1.06	6.77	1.97	104.00	0.704	0.370	0.129
2M LFD	Ad-GFP	d2	5.86	1.95	9.77	1.97	104.00		0.947	0.675
2M LFD	Ad-GFP	d4	7.39	3.47	11.30	1.97	104.00			0.938
2M LFD	Ad-GFP	d7	9.00	5.09	12.92	1.97	104.00			
2M HFD	Ad-Foxn1	d0	3.85	0.35	7.35	1.77	104.00	1.000	0.936	0.892
2M HFD	Ad-Foxn1	d2	3.81	-0.71	8.33	2.28	104.00		0.953	0.916
2M HFD	Ad-Foxn1	d4	5.40	1.48	9.31	1.97	104.00			0.998
2M HFD	Ad-Foxn1	d7	5.90	1.38	10.42	2.28	104.00			
2M HFD	Ad-GFP	d0	3.85	0.35	7.35	1.77	104.00	0.455	0.893	0.142
2M HFD	Ad-GFP	d2	7.76	3.85	11.68	1.97	104.00		0.885	0.917
2M HFD	Ad-GFP	d4	5.73	1.81	9.64	1.97	104.00			0.519
2M HFD	Ad-GFP	d7	9.56	5.65	13.48	1.97	104.00			
18M LFD	Ad-Foxn1	d0	5.83	1.92	9.75	1.97	104.00	0.093	0.998	0.409
18M LFD	Ad-Foxn1	d2	12.40	8.49	16.32	1.97	104.00		0.139	0.855
18M LFD	Ad-Foxn1	d4	6.35	2.44	10.27	1.97	104.00			0.521
18M LFD	Ad-Foxn1	d7	10.17	6.26	14.09	1.97	104.00			
18M LFD	Ad-GFP	d0	5.83	1.92	9.75	1.97	104.00	<0.001	0.136	0.114
18M LFD	Ad-GFP	d2	18.20	14.70	21.70	1.77	104.00		0.089	0.058
18M LFD	Ad-GFP	d4	11.92	8.00	15.83	1.97	104.00			1.000
18M LFD	Ad-GFP	d7	11.82	8.32	15.32	1.77	104.00			
18M HFD	Ad-Foxn1	d0	1.75	-1.21	4.71	1.49	104.00	0.052	<0.001	0.076
18M HFD	Ad-Foxn1	d2	8.83	4.31	13.35	2.28	104.00		0.164	0.986
18M HFD	Ad-Foxn1	d4	15.13	11.21	19.04	1.97	104.00			0.048
18M HFD	Ad-Foxn1	d7	7.79	3.88	11.71	1.97	104.00			
18M HFD	Ad-GFP	d0	1.75	-1.21	4.71	1.49	104.00	0.042	0.087	0.365
18M HFD	Ad-GFP	d2	9.06	4.53	13.58	2.28	104.00		0.966	0.701
18M HFD	Ad-GFP	d4	7.65	3.73	11.56	1.97	104.00			0.910
18M HFD	AD-GFP	D7	5.79	1.88	9.71	1.97	104.00			

Table S3. *Ppar γ* mRNA expression in DFs with keratinocytes coculture—comparisons by day.

	GROUP	TIME	LSMEAN	LOWER.CL	UPPER.CL	SE	DF	D2	D4	D7
2M LFD	Ad-Foxn1	d0	2.54	-1.30	6.38	1.94	104.00	0.658	0.008	0.006
2M LFD	Ad-Foxn1	d2	5.69	1.85	9.53	1.94	104.00		0.180	0.139
2M LFD	Ad-Foxn1	d4	10.99	7.55	14.42	1.73	104.00			0.999
2M LFD	Ad-Foxn1	d7	11.32	7.89	14.76	1.73	104.00			
2M LFD	Ad-GFP	d0	2.54	-1.30	6.38	1.94	104.00	0.699	0.067	0.045
2M LFD	Ad-GFP	d2	5.51	1.67	9.35	1.94	104.00		0.499	0.400
2M LFD	Ad-GFP	d4	9.36	5.52	13.20	1.94	104.00			0.998
2M LFD	Ad-GFP	d7	9.81	5.97	13.65	1.94	104.00			
2M HFD	Ad-Foxn1	d0	1.24	-2.19	4.68	1.73	104.00	0.637	0.010	0.102
2M HFD	Ad-Foxn1	d2	4.59	0.16	9.03	2.24	104.00		0.338	0.746
2M HFD	Ad-Foxn1	d4	9.57	5.73	13.41	1.94	104.00			0.930
2M HFD	Ad-Foxn1	d7	7.78	3.34	12.21	2.24	104.00			
2M HFD	Ad-GFP	d0	1.24	-2.19	4.68	1.73	104.00	0.311	0.063	0.031
2M HFD	Ad-GFP	d2	5.75	1.91	9.59	1.94	104.00		0.879	0.749
2M HFD	Ad-GFP	d4	7.79	3.95	11.63	1.94	104.00			0.994
2M HFD	Ad-GFP	d7	8.49	4.66	12.33	1.94	104.00			
18M LFD	Ad-Foxn1	d0	3.74	-0.10	7.58	1.94	104.00	<0.001	0.005	0.006
18M LFD	Ad-Foxn1	d2	19.19	15.35	23.03	1.94	104.00		0.116	0.104
18M LFD	Ad-Foxn1	d4	13.02	9.18	16.86	1.94	104.00			1.000
18M LFD	Ad-Foxn1	d7	12.88	9.04	16.72	1.94	104.00			
18M LFD	Ad-GFP	d0	3.74	-0.10	7.58	1.94	104.00	<0.001	0.042	0.009
18M LFD	Ad-GFP	d2	16.21	12.78	19.65	1.73	104.00		0.205	0.355
18M LFD	Ad-GFP	d4	11.08	7.24	14.92	1.94	104.00			0.975
18M LFD	Ad-GFP	d7	12.17	8.73	15.60	1.73	104.00			
18M HFD	Ad-Foxn1	d0	2.47	-0.44	5.37	1.46	104.00	0.033	<0.001	<0.001
18M HFD	Ad-Foxn1	d2	9.88	5.44	14.31	2.24	104.00		0.801	0.615
18M HFD	Ad-Foxn1	d4	12.56	8.72	16.40	1.94	104.00			0.987
18M HFD	Ad-Foxn1	d7	13.49	9.65	17.33	1.94	104.00			
18M HFD	Ad-GFP	d0	2.47	-0.44	5.37	1.46	104.00	0.118	0.001	<0.001
18M HFD	Ad-GFP	d2	8.47	4.03	12.90	2.24	104.00		0.695	0.561
18M HFD	Ad-GFP	d4	11.70	7.86	15.54	1.94	104.00			0.996
18M HFD	AD-GFP	D7	12.33	8.49	16.17	1.94	104.00			

Table S4. *Fabp4*mRNA expression in DFs with keratinocytes coculture—comparisons by day.

	GROUP	TIME	LSMEAN	LOWER.CL	UPPER.CL	SE	DF	D2	D4	D7
2M LFD	Ad-Foxn1	d0	0.06	-1.61	1.73	0.84	104.00	0.985	<0.001	<0.001
2M LFD	Ad-Foxn1	d2	0.48	-1.18	2.15	0.84	104.00		<0.001	<0.001
2M LFD	Ad-Foxn1	d4	11.52	10.03	13.01	0.75	104.00			0.010
2M LFD	Ad-Foxn1	d7	14.93	13.44	16.42	0.75	104.00			
2M LFD	Ad-GFP	d0	0.06	-1.61	1.73	0.84	104.00	0.980	<0.001	<0.001
2M LFD	Ad-GFP	d2	0.52	-1.14	2.19	0.84	104.00		<0.001	<0.001
2M LFD	Ad-GFP	d4	10.17	8.51	11.84	0.84	104.00			0.134
2M LFD	Ad-GFP	d7	12.77	11.11	14.44	0.84	104.00			
2M HFD	Ad-Foxn1	d0	0.05	-1.44	1.54	0.75	104.00	0.978	<0.001	<0.001
2M HFD	Ad-Foxn1	d2	0.54	-1.38	2.47	0.97	104.00		<0.001	<0.001
2M HFD	Ad-Foxn1	d4	10.40	8.73	12.06	0.84	104.00			0.963
2M HFD	Ad-Foxn1	d7	9.78	7.85	11.70	0.97	104.00			
2M HFD	Ad-GFP	d0	0.05	-1.44	1.54	0.75	104.00	0.880	<0.001	<0.001
2M HFD	Ad-GFP	d2	0.88	-0.78	2.55	0.84	104.00		<0.001	<0.001
2M HFD	Ad-GFP	d4	8.16	6.49	9.82	0.84	104.00			0.038
2M HFD	Ad-GFP	d7	11.38	9.72	13.05	0.84	104.00			
18M LFD	Ad-Foxn1	d0	0.18	-1.49	1.84	0.84	104.00	0.749	<0.001	<0.001
18M LFD	Ad-Foxn1	d2	1.37	-0.30	3.03	0.84	104.00		<0.001	<0.001
18M LFD	Ad-Foxn1	d4	11.17	9.50	12.83	0.84	104.00			0.233
18M LFD	Ad-Foxn1	d7	13.43	11.76	15.09	0.84	104.00			
18M LFD	Ad-GFP	d0	0.18	-1.49	1.84	0.84	104.00	0.560	<0.001	<0.001
18M LFD	Ad-GFP	d2	1.65	0.16	3.14	0.75	104.00		<0.001	<0.001
18M LFD	Ad-GFP	d4	9.64	7.98	11.31	0.84	104.00			0.032
18M LFD	Ad-GFP	d7	12.78	11.29	14.27	0.75	104.00			
18M HFD	Ad-Foxn1	d0	0.04	-1.22	1.30	0.63	104.00	0.990	<0.001	<0.001
18M HFD	Ad-Foxn1	d2	0.39	-1.53	2.32	0.97	104.00		<0.001	<0.001
18M HFD	Ad-Foxn1	d4	12.82	11.15	14.48	0.84	104.00			0.220
18M HFD	Ad-Foxn1	d7	15.12	13.45	16.78	0.84	104.00			
18M HFD	Ad-GFP	d0	0.04	-1.22	1.30	0.63	104.00	0.992	<0.001	<0.001
18M HFD	Ad-GFP	d2	0.37	-1.55	2.29	0.97	104.00		<0.001	<0.001
18M HFD	Ad-GFP	d4	10.85	9.19	12.52	0.84	104.00			0.005
18M HFD	AD-GFP	D7	14.91	13.24	16.57	0.84	104.00			

Table S5. *Zfp423* mRNA expression in DFs with keratinocytes coculture—comparisons by day.

METHOD	GROUP	TIME	LSMEAN	LOWER.CL	UPPER.CL	SE	DF	D2	D4	D7
AD-FOXN1	2m LFD	d0	9.84	6.88	12.80	1.49	97.00	0.917	0.754	0.139
AD-FOXN1	2m LFD	d2	11.20	8.24	14.16	1.49	97.00		0.989	0.447
AD-FOXN1	2m LFD	d4	11.83	9.18	14.47	1.33	97.00			0.598
AD-FOXN1	2m LFD	d7	14.18	11.53	16.83	1.33	97.00			
AD-FOXN1	2m HFD	d0	6.89	4.25	9.54	1.33	97.00	0.877	1.000	0.979
AD-FOXN1	2m HFD	d2	5.40	2.44	8.35	1.49	97.00		0.890	0.729
AD-FOXN1	2m HFD	d4	6.91	3.95	9.87	1.49	97.00			0.982
AD-FOXN1	2m HFD	d7	7.75	4.34	11.17	1.72	97.00			
AD-FOXN1	18m LFD	d0	11.22	8.26	14.18	1.49	97.00	0.063	0.911	0.708
AD-FOXN1	18m LFD	d2	16.53	13.58	19.49	1.49	97.00		0.010	0.003
AD-FOXN1	18m LFD	d4	9.82	6.86	12.78	1.49	97.00			0.977
AD-FOXN1	18m LFD	d7	8.96	6.00	11.92	1.49	97.00			
AD-FOXN1	18m HFD	d0	5.33	2.38	8.29	1.49	97.00	<0.001	0.096	0.137
AD-FOXN1	18m HFD	d2	18.23	14.81	21.64	1.72	97.00		0.004	0.002
AD-FOXN1	18m HFD	d4	10.27	7.31	13.22	1.49	97.00			0.998
AD-FOXN1	18m HFD	d7	9.92	6.97	12.88	1.49	97.00			
AD-GFP	2m LFD	d0	9.84	6.88	12.80	1.49	97.00	0.924	0.977	0.850
AD-GFP	2m LFD	d2	11.16	8.20	14.12	1.49	97.00		0.730	0.998
AD-GFP	2m LFD	d4	8.98	6.02	11.94	1.49	97.00			0.617
AD-GFP	2m LFD	d7	11.55	8.59	14.50	1.49	97.00			
AD-GFP	2m HFD	d0	6.89	4.25	9.54	1.33	97.00	0.984	0.969	1.000
AD-GFP	2m HFD	d2	6.18	3.22	9.14	1.49	97.00		1.000	0.987
AD-GFP	2m HFD	d4	5.99	3.04	8.95	1.49	97.00			0.973
AD-GFP	2m HFD	d7	6.90	3.94	9.85	1.49	97.00			
AD-GFP	18m LFD	d0	11.22	8.26	14.18	1.49	97.00	<0.001	0.590	0.783
AD-GFP	18m LFD	d2	20.67	17.25	24.08	1.72	97.00		<0.001	<0.001
AD-GFP	18m LFD	d4	8.56	5.61	11.52	1.49	97.00			0.980
AD-GFP	18m LFD	d7	9.34	6.69	11.98	1.33	97.00			
AD-GFP	18m HFD	d0	5.33	2.38	8.29	1.49	97.00	<0.001	0.686	0.820
AD-GFP	18m HFD	d2	16.86	13.44	20.27	1.72	97.00		<0.001	<0.001
AD-GFP	18m HFD	d4	7.67	4.71	10.62	1.49	97.00			0.995
AD-GFP	18m HFD	d7	7.17	4.21	10.12	1.49	97.00			

Table S6. *Zfp521* mRNA expression in DFs with keratinocytes coculture—comparisons by day.

METHOD	GROUP	TIME	LSMEAN	LOWER.CL	UPPER.CL	SE	DF	D2	D4	D7
AD-FOXN1	2m LFD	d0	25.29	20.98	29.59	2.17	97.00	0.997	<0.001	<0.001
AD-FOXN1	2m LFD	d2	25.94	21.64	30.24	2.17	97.00		<0.001	<0.001
AD-FOXN1	2m LFD	d4	6.97	3.12	10.82	1.94	97.00			0.712
AD-FOXN1	2m LFD	d7	4.05	0.20	7.90	1.94	97.00			
AD-FOXN1	2m HFD	d0	21.24	17.39	25.08	1.94	97.00	0.005	0.026	0.002
AD-FOXN1	2m HFD	d2	11.27	6.97	15.57	2.17	97.00		0.950	0.958
AD-FOXN1	2m HFD	d4	12.91	8.61	17.21	2.17	97.00			0.750
AD-FOXN1	2m HFD	d7	9.60	4.63	14.57	2.50	97.00			
AD-FOXN1	18m LFD	d0	17.62	13.32	21.92	2.17	97.00	0.897	0.001	<0.001
AD-FOXN1	18m LFD	d2	15.48	11.18	19.78	2.17	97.00		0.011	<0.001
AD-FOXN1	18m LFD	d4	5.76	1.46	10.07	2.17	97.00			0.856
AD-FOXN1	18m LFD	d7	3.32	-0.98	7.62	2.17	97.00			
AD-FOXN1	18m HFD	d0	12.11	7.81	16.41	2.17	97.00	0.811	0.065	0.018
AD-FOXN1	18m HFD	d2	15.05	10.09	20.02	2.50	97.00		0.010	0.002
AD-FOXN1	18m HFD	d4	4.42	0.12	8.72	2.17	97.00			0.964
AD-FOXN1	18m HFD	d7	2.95	-1.35	7.26	2.17	97.00			
AD-GFP	2m LFD	d0	25.29	20.98	29.59	2.17	97.00	0.715	<0.001	<0.001
AD-GFP	2m LFD	d2	28.53	24.23	32.84	2.17	97.00		<0.001	<0.001
AD-GFP	2m LFD	d4	8.44	4.13	12.74	2.17	97.00			0.954
AD-GFP	2m LFD	d7	6.85	2.54	11.15	2.17	97.00			
AD-GFP	2m HFD	d0	21.24	17.39	25.08	1.94	97.00	0.013	0.093	<0.001
AD-GFP	2m HFD	d2	12.20	7.90	16.51	2.17	97.00		0.893	0.748
AD-GFP	2m HFD	d4	14.38	10.08	18.68	2.17	97.00			0.322
AD-GFP	2m HFD	d7	9.13	4.82	13.43	2.17	97.00			
AD-GFP	18m LFD	d0	17.62	13.32	21.92	2.17	97.00	0.557	0.002	<0.001
AD-GFP	18m LFD	d2	21.97	17.00	26.93	2.50	97.00		<0.001	<0.001
AD-GFP	18m LFD	d4	6.03	1.73	10.34	2.17	97.00			0.920
AD-GFP	18m LFD	d7	4.18	0.34	8.03	1.94	97.00			
AD-GFP	18m HFD	d0	12.11	7.81	16.41	2.17	97.00	0.373	0.130	0.041
AD-GFP	18m HFD	d2	17.47	12.50	22.44	2.50	97.00		0.002	<0.001
AD-GFP	18m HFD	d4	5.36	1.06	9.66	2.17	97.00			0.961
AD-GFP	18m HFD	d7	3.85	-0.45	8.16	2.17	97.00			

Table S7. List of antibodies used for flow cytometry assay.

Flow Cytometry Assay				
Antibody	Probe Fluorochrome	Vendor	Cat. No	Clone
CD3	PE	BD Biosciences	557749	SP34-2
CD31	PE	BD Biosciences	561073	MEC 13.3
CD45	PE	BD Biosciences	560695	104
CD24	APC	BD Biosciences	562349	M1/69
Sca1 (Ly-6A/E)	PE-Cy7	BD Biosciences	558162	D7
CD26	FITC	BD Biosciences	559652	H194-112
CD26	PE-Cy7	Biolegend	137809	H194-112
PDGF α	FITC	BD Biosciences	558774	APA5

Table S8. List of TagMan gene expression assays.

Gene	TaqMan® Pimer and Probe Sets ID
<i>Pparγ</i>	Mm00440940
<i>Cebpα</i>	Mm01265914_s1
<i>Fabp4</i>	Mm00445878_m1
<i>leptin</i>	Mm00434759_m1
<i>Bmp2</i>	Mm01340178_m1
<i>Igf2</i>	Mm00439564_m1
<i>Zfp 521</i>	Mm00521009_m1
<i>Zfp 423</i>	Mm00473699_m1
<i>Foxn1</i>	Mm01298129
<i>Hprt1</i>	Mm01545399_m1

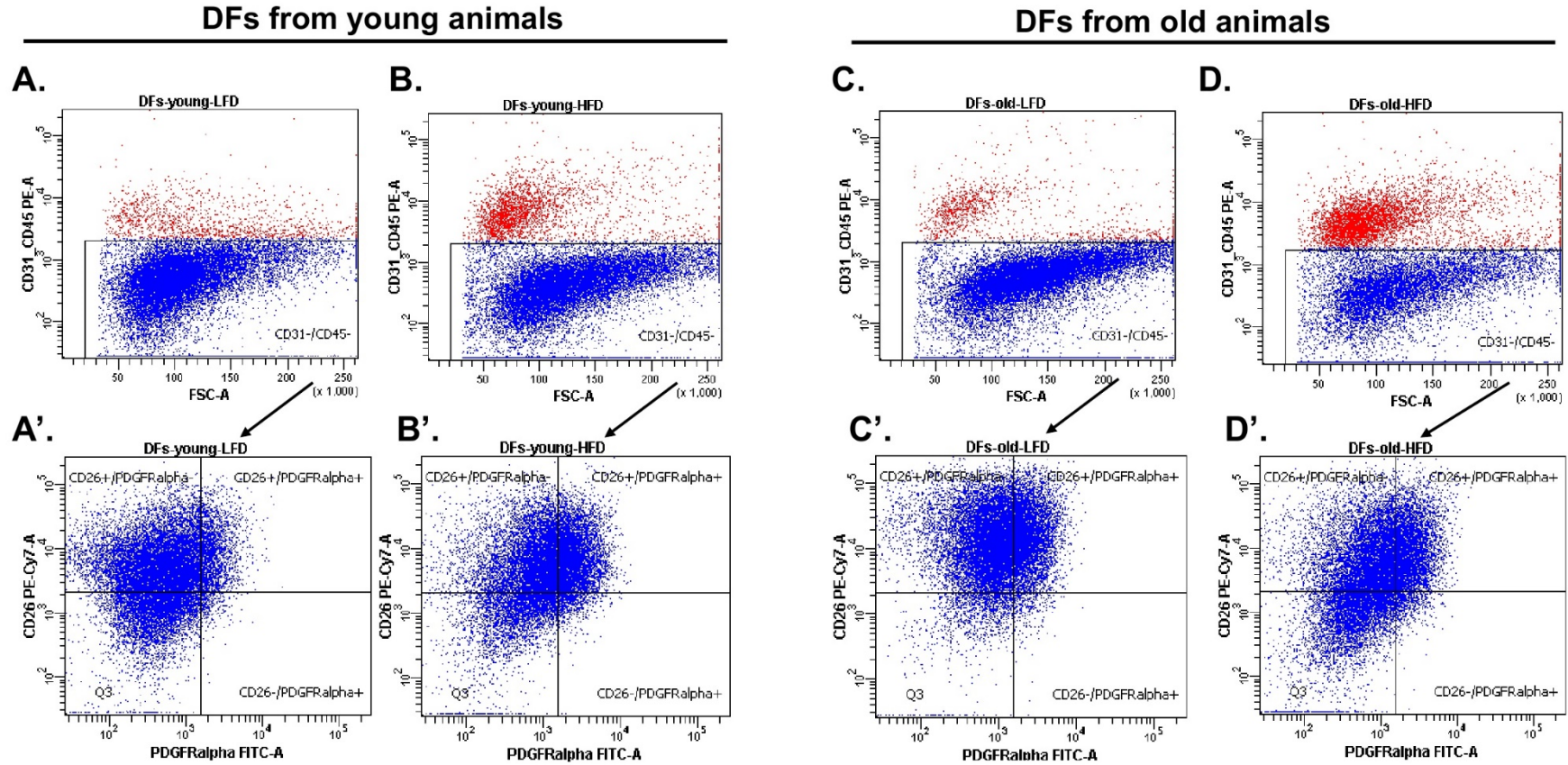


Figure S1

Flow cytometry analysis of DFs isolated from the skin of young, old, LFD or HFD C56BL/6(B6) mice. Representative dot-plots for Lin- (CD31-/CD45-) population in a group of DFs isolated from (A) young LFD, (B) young HFD, (C) old LFD, (D) old HFD mice. Further separation of CD26⁺ (CD31-/CD45-) and/ or PDGFRα⁺ (CD31-/CD45-) population in DFs isolated from (A') young LFD, (B') young HFD, (C') old LFD, (D') old HFD mice. The data are representative of n = 3–5 animals per age and diet. HFD, high-fat diet; LFD, low-fat diet.

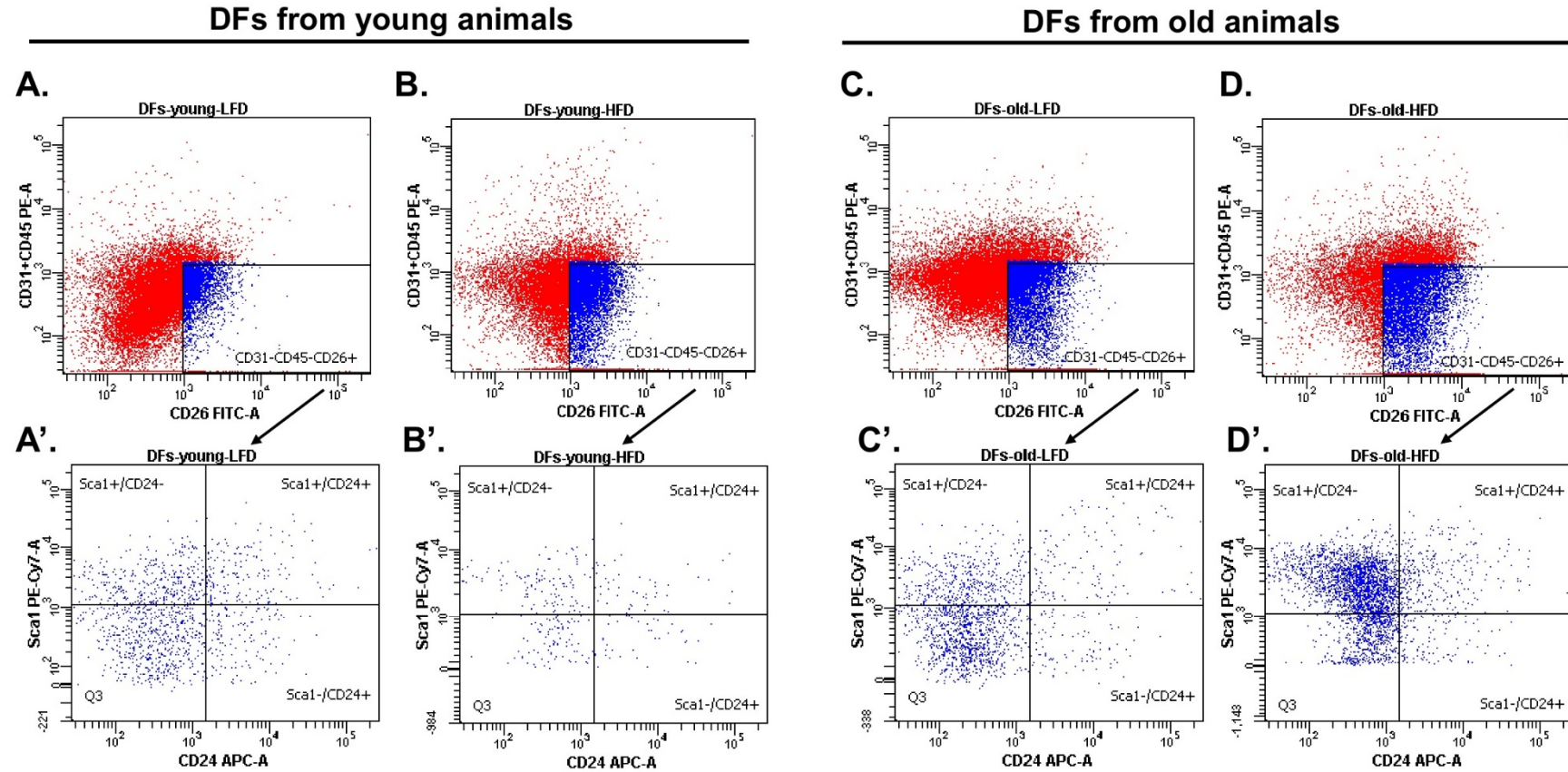


Figure S2

Representative dot-plots of CD26⁺ cells within Lin⁻ population in DFs isolated from (A) young LFD, (B) young HFD, (C) old LFD, (D) old HFD mice. Sca1⁺, CD24⁺ and Sca1⁺/CD24⁺ cells within CD26⁺ population of DFs isolated from (A') young LFD, (B') young HFD, (C') old LFD, (D') old HFD mice. The data are representative of n = 3–5 animals per age and diet. HFD, high-fat diet; LFD, low-fat diet.

