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**Fig. S1 Dual-degrading PEG properties and ADSC viability.** (a) Schematic depicting follicleand ADSC-driven degradation of PEG hydrogels crosslinked with plasmin- and matrix metalloprotease-sensitive "YKNS/VPMS" crosslinkers. (b-d) ADSC viability (green = live, red = dead) in PEG hydrogels crosslinked with "YKNS" and "YKNS/VPMS." (e) Storage moduli of PEG hydrogels crosslinked with "YKNS" and "YKNS/VPMS" (n=6).



**Fig. S2 Schematic depicting experimental approach and culture conditions.** Follicles encapsulated in PEG hydrogels were cultured in conditioned media obtained from ADSCs (a) cultured in flasks (2D CM), (b) encapsulated in PEG (3D CM), or (c) co-cultured with follicles (co-culture).



**Fig. S3 Sex hormone secretion from follicles and ADSCs.** Culture medium was collected from follicles co-cultured with ADSCs (green), follicles cultured in 3D CM (black), and ADSCs encapsulated in 3D PEG hydrogels (red) for analysis of (a) androstenedione, (b) estradiol, and (c) progesterone. Hormones were not analyzed for follicles cultured in 2D CM or ADSCs cultured in 2D because the low survival rate of follicles in this condition indicates that it is not suitable for translation to human follicles in future studies.



**Fig. S4 Differentiation markers.** Gene expression in human adipose derived stem cells (ADSCs) cultured in standard 2D conditions (blue), 3D encapsulated in PEG without follicles (red), and 3D encapsulated in PEG with primary murine follicles (green) on days 4 and 10 of culture relative to gene expression of ADSCs in 2D culture on Day 1. (A) Markers of adipogenesis: PPAR-g, perilpin 4 (PLIN4), and fatty acid binding protein 4 (FABP4) show non-significant changes in expression. (B) Markers of osteogenesis: osterix (SP7) and RUNX2 were up-regulated in both 3D encapsulation without follicle and 3D encapsulation with primary murine follicle conditions while alkaline phosphatase (ALPL) was up-regulated in ADSCs in 3D culture with follicle on Day 4 only. (C) Markers of chondrogenesis: aggrecan (ACAN) up-regulated in all culture groups with

significantly less up-regulation in ADSCs cultured in 3D with follicle on day 4 of culture while SOX9 was up-regulated in both 3D encapsulation without follicle and 3D encapsulation with primary murine follicle conditions. (mean + SEM, n=48, p<0.05, vs ADSCs in 2D culture control group)



**Fig S5. Normalized concentrations of key cytokines.** Normalized concentrations of (a) IL-6, (b) KC, and (c) VEGF-A in the medium of follicles co-cultured with ADSCs (green), and cultured in 3D (black) and 2D (blue) ADSC conditioned medium (n=3).

Pluripotency Markers <sup>1,2,3</sup>	
CXCL 12	C-X-C motif chemokine 12
POSTN	Periostin
OCT4	Octamer-binding transcription factor 4
SOX2	SRY-Box 2
NODAL	Nodal protein
SNAI2	Snail family transcriptional repressor 2
UTF-1	Undifferentiated embryonic cell transcription factor 1
NANOG	Homeobox transcription factor Nanog
Differentiation Markers	
Adipocyte Markers <sup>4,5</sup>	
ΡΡΑRγ	Peroxisome proliferator-activated receptor gamma
ADIPOQ	Adiponectin
FABP4	Fatty acid binding protein 4
PLIN4	Perilipin 4
Osteoblast Markers <sup>6,7,8,9</sup>	
RUNX2	Runt-related transcription factor 2
SP7	Osterix
IBSP	Integrin-binding sialoprotein
ALPL	Alkaline phosphatase
Chondrocyte Markers <sup>10,11,12</sup>	
SOX9	SRY-Box 9
ACAN	Aggrecan
COL2A1	Collagen 2 alpha 1 chain
Myoblast Markers <sup>13,14</sup>	
PAX3	Paired box 3
MYOG	Myogenin
MYOD1	Myogenic differentiation 1
Paracrine Factors <sup>15,16,17</sup>	
VEGFA	Vascular endothelial growth factor A
FGF-2	Fibroblast growth factor
TGFB1	Transforming growth factor beta 1
TGFB2	Transforming growth factor beta 2
PGF	Placental growth factor
HGF	Hepatocyte growth factor

Supplementary Table 1. Genes analyzed from human adipose-derived stem cells

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**Supplementary Table 2.** A complete list of cytokines tested in this study and their lower detection limits for each condition

		Lower Detection Limit (pg/mL)		
Category	Cytokine	Co-	3D CM	2D CM
		Culture		
Angiogenesis- related factors	Angiopoietin 2 (ANG2)	0.85	0.34	2.06
	Follistatin	2.52	40.28	4.09
	Keratinocyte chemoattractant (KC)	0.17	0.86	0.15
	Vascular endothelial growth factor A (VEGF-A)	0.08	0.56	0.18
	Vascular endothelial growth factor C (VEGF-C)	6.40	2.33	32.00
	Vascular endothelial growth factor D (VEGF-D)	3.38	1.69	42.85
	Fibroblast growth factor 2 (FGF-2)	6.24	80.00	5.14
	Granulocyte colony-stimulating factor (G-CSF)	0.16	1.15	0.28
	Placental growth factor 2 (PLGF-2)	1.24	0.08	3.20
Inflammation- related factors	Macrophage inflammatory protein-1a (MIP-1a)	15.38	0.39	4.73
	Interleukin-1β (IL-1β)	16.00	16.17	0.44
	Interleukin-6 (IL-6)	0.32	0.17	0.18
	Interleukin-17A (IL-17A)	0.12	1.60	1.07
	Monocyte chemotactic protein-1 (MCP-1)	14.62	13.68	0.62
	Tumor necrosis factor a (TNFa)	0.12	0.73	6.48
	Stromal cell-derived factor 1 (SDF-1)	3.24	19.29	2.29
	Soluble Fas ligand (sFasL)	0.54	1.71	31.79
	Platelet endothelial cell adhesion molecule (PECAM)	9.56	5.49	0.27
EGF-like factors	Epidermal growth factor (EGF)	0.58	0.64	25.44
	Amphiregulin	0.21	8.08	0.16
	Betacellulin	3.63	0.32	0.63
Paracrine- related factors	Endothelin-1	0.92	0.73	1.17
	Prolactin	2.21	1.72	0.96
	Hepatocyte growth factor (HGF)	10.01	3.87	32.00
	Leptin	0.32	0.49	0.22
TGFβ	Endoglin	0.97	0.09	0.35
superfamily	Activin receptor-like kinase (sALK-1)	3.20	16.17	1.78

(file uploaded separately)

**Supplementary File E1.** Cytokine measurements in media from follicles co-cultured with ADSCs and follicles cultured in conditioned medium from 2D and 3D ADSCs.