

## Supplementary Materials

# Induction of the CD24 Surface Antigen in Primary Undifferentiated Human Adipose Progenitor Cells by the Hedgehog Signaling Pathway

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### Supplementary Materials for the Results Section

**Table S1.** Generation *in vitro* of CD24 positive cells from primary hASCs: Statistical significance for flow cytometer analysis, ordinary one-way ANOVA (multiple comparisons).

Comparisons test	Significant	Summary	p-value
<i>UrSuppe</i> vs. <i>US-24</i>	No	ns	0.9576
<i>UrSuppe</i> vs. <i>US-24 + Ins</i>	No	ns	0.4420
<i>UrSuppe</i> vs. <i>US-24 + SAG</i>	Yes	*	0.0383
<i>UrSuppe</i> vs. <i>US-24 + SAG + Ins</i>	Yes	***	0.0004
<i>US-24</i> vs. <i>US-24 + Dex</i>	No	ns	0.9344
<i>US-24</i> vs. <i>US-24 + SAG</i>	No	ns	0.4698
<i>US-24</i> vs. <i>US-24 + SAG + Dex</i>	No	ns	0.9433
<i>US-24</i> vs. <i>US-24 + Ins</i>	No	ns	0.9953
<i>US-24</i> vs. <i>US-24 + Ins + Dex</i>	No	ns	0.9378
<i>US-24</i> vs. <i>US-24 + SAG + Ins</i>	Yes	**	0.0097
<i>US-24</i> vs. <i>US-24 + SAG + Ins + Dex</i>	No	ns	0.9989
<i>US-24</i> vs. <i>US-24 + TNF<math>\alpha</math></i>	No	ns	0.9815
<i>US-24</i> vs. <i>US-24 + TNF<math>\alpha</math> + SAG</i>	No	ns	0.9827
<i>US-24</i> vs. <i>US-24 + TNF<math>\alpha</math> + SAG + Ins</i>	No	ns	0.9993
<i>US-24 + SAG</i> vs. <i>US-24+SAG+Dex</i>	Yes	*	0.0333
<i>US-24 + SAG</i> vs. <i>US-24 + SAG + Ins</i>	No	ns	0.7026
<i>US-24 + SAG</i> vs. <i>US-24 + SAG + TNF<math>\alpha</math></i>	No	ns	0.0546
<i>US-24 + SAG+Ins</i> vs. <i>US-24 + Ins</i>	No	ns	0.0934
<i>US-24 + SAG+Ins</i> vs. <i>US-24-SAG + Ins + Dex</i>	Yes	**	0.0012
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + TNF<math>\alpha</math></i>	Yes	**	0.0014

ns: non significative p value; \*: p value <0.05; \*\*: p value <0.01; \*\*\*: p value <0.001; \*\*\*\*: p value <0.0001

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**Table S2.** Generation *in vitro* of CD24 positive cells from primary hASCs: Statistical significance for RT-qPCR analysis, ordinary one-way ANOVA (multiple comparisons).

Comparisons test	Significant	Summary	p-value
<i>UrSuppe</i> vs. <i>US-24</i>	No	ns	>0.9999
<i>UrSuppe</i> vs. <i>US-24 + Ins</i>	No	ns	>0.9999
<i>UrSuppe</i> vs. <i>US-24 + SAG</i>	Yes	*	0.0298
<i>UrSuppe</i> vs. <i>US-24 + SAG + Ins</i>	Yes	***	0.0004
<i>US-24</i> vs. <i>US-24 + Dex</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + SAG</i>	No	ns	0.4580
<i>US-24</i> vs. <i>US-24 + SAG + Dex</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + Ins</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + Ins + Dex</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + SAG + Ins</i>	Yes	**	0.0064
<i>US-24</i> vs. <i>US-24 + SAG + Ins + Dex</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + TNF<math>\alpha</math></i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + TNF<math>\alpha</math> + SAG</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + TNF<math>\alpha</math> + SAG + Ins</i>	No	ns	>0.9999
<i>US-24 + SAG</i> vs. <i>US-24+SAG+Dex</i>	Yes	*	0.0291
<i>US-24 + SAG</i> vs. <i>US-24+SAG+Ins</i>	No	ns	>0.9999
<i>US-24 + SAG</i> vs. <i>US-24+SAG+TNF<math>\alpha</math></i>	No	ns	0.0621
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + Ins</i>	Yes	**	0.0062
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + Dex</i>	Yes	***	0.0004
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + TNF<math>\alpha</math></i>	Yes	**	0.0083

ns: non significative p value; \*: p value <0.05; \*\*: p value <0.01; \*\*\*: p value <0.001; \*\*\*\*: p value <0.0001

**Table S3.** CD24 upregulation in hASCs mediated by recombinant natural Hh ligands and synthetic SAG agonist: Statistical significance for RT-qPCR analysis, ordinary one-way ANOVA (multiple comparisons).

Comparisons test	Significant	Summary	p-value
<i>US-24</i> vs. <i>US-24 + SAG + Ins</i>	Yes	****	<0.0001
<i>US-24</i> vs. <i>US-24 + SHH + Ins</i>	Yes	**	0.0018
<i>US-24</i> vs. <i>US-24 + DHH + Ins</i>	Yes	**	0.0073
<i>US-24</i> vs. <i>US-24 + IHH + Ins</i>	No	ns	0.1393
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SHH + Ins</i>	Yes	****	<0.0001
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + DHH + Ins</i>	Yes	****	<0.0001
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + IHH + Ins</i>	Yes	****	<0.0001

ns: non significative p value; \*: p value <0.05; \*\*: p value <0.01; \*\*\*: p value <0.001; \*\*\*\*: p value <0.0001

**List of abbreviations in Table S1, S2, & S3:** Ins: Insulin; SAG: Smoothened Agonist, synthetic Hedgehog signaling pathway agonist; Dex: Dexamethasone; TNF $\alpha$ : Tumor Necrosis Factor alpha; DHH: Desert Hedgehog; IHH: Indian Hedgehog; SHH: Sonic Hedgehog; US-24: UrSuppe-24, special defined serum-free medium permissive to the Hedgehog signaling pathway.

**Table S4.** Overview of measured genes.

	<b>Name</b>	<b>Description</b>	<b>Reference</b>
<i>PREF1</i> ( <i>DLK1</i> )	Preadipocyte Factor 1 (Delta-Like 1 homolog) is a transmembrane protein that inhibits adipogenesis. It can be considered as the "seal" of the undifferentiated status of the hASCs. It belongs to the non-canonical Notch ligands family (together with Dlk2). Pref-1 also exists as a biologically active soluble form. However, its receptor is still unknown.		Hudak <i>et al.</i> [1] Hei [2] Da Silva <i>et al.</i> [3]
<i>SOX9</i>	It is a member of the HMG-box class DNA-binding proteins and is a Pref-1 target. Sox9 directly binds to the promoters of two important pro-adipogenic transcription factors, c/EBP $\alpha$ , and c/EBP $\beta$ , to suppress their activity, thus repressing adipocyte differentiation		Wang and Sul [4] Gulyaeva <i>et al.</i> [5] Kamachi and Kondoh [6]
<i>WISP2</i>	Wnt1-inducible signaling pathway protein 2 is an endogenous and secreted auto- and paracrine non-conventional WNT ligand, promoting precursors cells' proliferation and inhibiting their adipogenic commitment and differentiation.		Hammarstedt <i>et al.</i> [7] Grünberg <i>et al.</i> [8]
<i>PPARG</i>	Peroxisome Proliferator-Activated Receptor gamma is a ligand-dependent transcription factor that is a member of the nuclear hormone receptor superfamily. It plays a crucial role in adipose tissue development and differentiation, and therefore, it is considered the master regulator of adipogenesis.		Tontonoz <i>et al.</i> [9] Barak <i>et al.</i> [10] Rosen <i>et al.</i> [11] Ahmadina <i>et al.</i> [12]
<i>UCP1</i>	Uncoupling Protein 1, also known as thermogenin. As opposed to white adipocytes, both brown and beige adipocytes possess thermogenic properties and can produce high heat levels via uncoupled respiration. This is achieved by expressing high levels of UCP1 to dissipate energy in the form of heat by uncoupling the mitochondrial respiration.		Chouchani <i>et al.</i> [13]
<i>RUNX2</i>	It is a transcription factor essential for osteoblast differentiation and chondrocyte maturation.		Komori [14] Toshihisa [15]

**Table S5.** CD24 positive hASCs and transforming growth factor  $\beta$  family: Statistical significance for flow cytometer analysis, ordinary one-way ANOVA (multiple comparisons).

Comparisons test.	Significant	Summary	p-value
<i>UrSuppe</i> vs. <i>US-24</i>	No	ns	0.9442
<i>UrSuppe</i> vs. <i>US-24 + SAG + Ins</i>	Yes	****	<0.0001
<i>US-24</i> vs. <i>US-24 + SAG + Ins</i>	Yes	****	<0.0001
<i>US-24</i> vs. <i>US-24 + SAG + Ins + BMP4</i>	No	ns	0.2351
<i>US-24</i> vs. <i>US-24 + BMP4</i>	No	ns	0.997
<i>US-24</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>1</i>	No	ns	0.9863
<i>US-24</i> vs. <i>US-24 + TGF-<math>\beta</math>1</i>	No	ns	0.9926
<i>US-24</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>3</i>	No	ns	0.9625
<i>US-24</i> vs. <i>US-24 + TGF-<math>\beta</math>3</i>	No	ns	0.9621
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + BMP4</i>	Yes	**	0.0025
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>1</i>	Yes	****	<0.0001
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>3</i>	Yes	***	0.0001
<i>US-24 + BMP4</i> vs. <i>US-24 + SAG + Ins + BMP4</i>	No	ns	0.0910
<i>US-24 + TGF-<math>\beta</math>1</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>1</i>	No	ns	0.6767
<i>US-24 + TGF-<math>\beta</math>3</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>3</i>	No	ns	0.4173

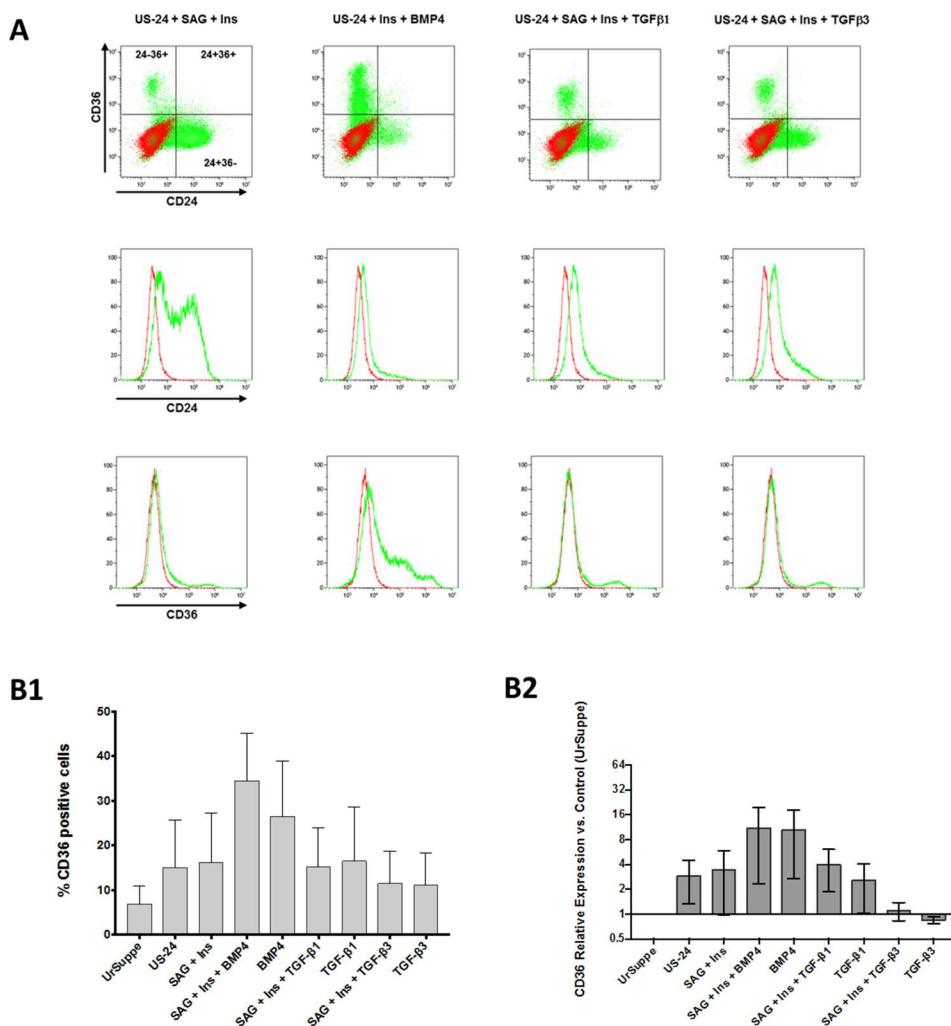
ns: non significative p value; \*: p value <0.05; \*\*: p value <0.01; \*\*\*: p value <0.001; \*\*\*\*: p value <0.0001

**Table S6.** CD24 positive hASCs and transforming growth factor  $\beta$  family: Statistical significance for RT-qPCR analysis, ordinary one-way ANOVA (multiple comparisons).

Comparisons test	Significant	Summary	p-value
<i>UrSuppe</i> vs. <i>US-24</i>	No	ns	>0.9999
<i>UrSuppe</i> vs. <i>US-24 + SAG + Ins</i>	Yes	**	0.0010
<i>US-24</i> vs. <i>US-24 + SAG + Ins</i>	Yes	**	0.0029
<i>US-24</i> vs. <i>US-24 + SAG + Ins + BMP4</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + BMP4</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>1</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>TGF-<math>\beta</math>1</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>3</i>	No	ns	>0.9999
<i>US-24</i> vs. <i>TGF-<math>\beta</math>3</i>	No	ns	>0.9999
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + BMP4</i>	Yes	**	0.0045
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>1</i>	Yes	*	0.0187
<i>US-24 + SAG + Ins</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>3</i>	Yes	**	0.0016
<i>US-24 + BMP4</i> vs. <i>US-24 + SAG + Ins + BMP4</i>	No	ns	>0.9999
<i>US-24 + TGF-<math>\beta</math>1</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>1</i>	No	ns	>0.9999
<i>US-24 + TGF-<math>\beta</math>3</i> vs. <i>US-24 + SAG + Ins + TGF-<math>\beta</math>3</i>	No	ns	>0.9999

ns: non significative p value; \*: p value <0.05; \*\*: p value <0.01; \*\*\*: p value <0.001; \*\*\*\*: p value <0.0001

List of abbreviations in Table S5 & S6: Ins: Insulin; SAG: Smoothened Agonist, synthetic Hedgehog signaling pathway agonist; BMP4: Bone morphogenic protein 4; TGF- $\beta$ 1: Transforming growth factor beta 1; TGF- $\beta$ 3: Transforming growth factor beta 3; US-24: Ur-Suppe-24, special defined serum-free medium permissive to the Hedgehog signaling pathway.



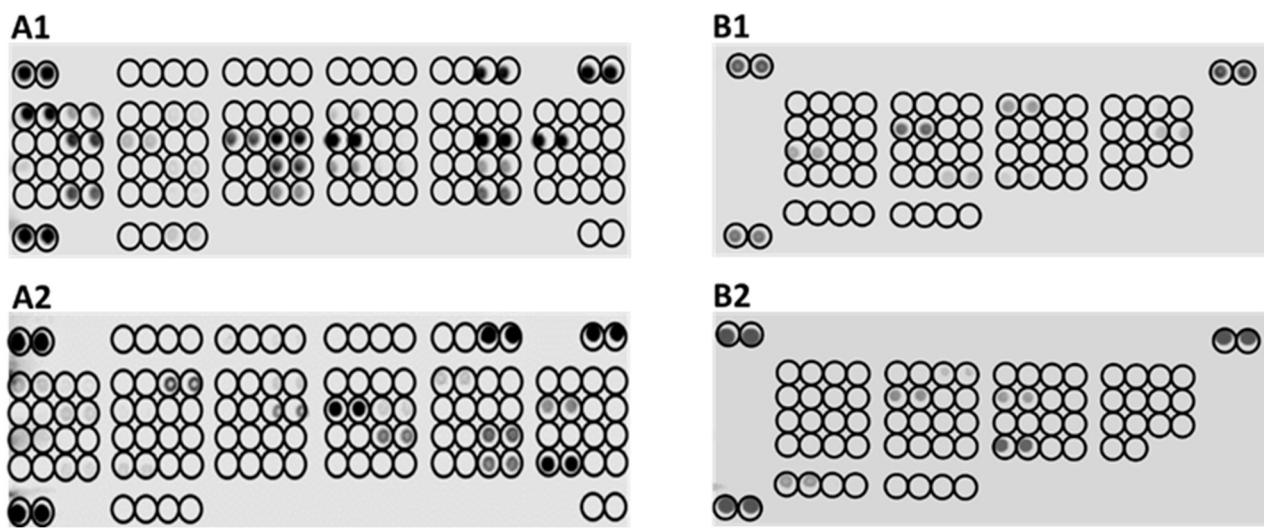
**Figure S1.** Analysis of CD24 and CD36 expression in hASCs. The cells were expanded in *UrSuppe* up to passage P2. Once the confluence was reached, the usual cell culture medium was replaced by *US-24*. Additionally, these factors were added, as indicated: SAG with Ins (Insulin), BMP4 with Ins, TGF- $\beta$ 1 with Ins, and TGF- $\beta$ 3 with Ins. **A)** Cells were analyzed by flow cytometry for the expression of CD24-CD36: representative histograms and plots depicting the CD24-CD36 expression in response to different treatments. Red line: the isotype control; the green line: the specific antibody. **B1)** Quantification of flow cytometry analysis of CD36 populations, percentage of CD36 positive cells ( $n = 3$ , error bars represents S.E.M.). **B2)** hASC were analyzed by RT-qPCR for the expression and quantification of CD36 mRNA ( $n=3$ , error bars represent S.E.M.).

**List S1.** Descriptions and references regarding the 14 genes discussed in the article's paragraph 2.5 "Monitoring Angiogenic Markers after Triggering the Hh Signaling Pathway in hASCs"

1. **Vascular endothelial growth factor A (VEGFA):** Classically known to be involved in vascular development during embryogenesis (vasculogenesis), as well as blood vessel formation (angiogenesis) and tissue remodeling in the adult organism. Recent findings highlight adipose tissue VEGF-A's roles in the control of adipose tissue function and systemic energy metabolism by modulating the adipose vasculature. VEGF-A overexpression in WAT facilitates angiogenesis and causes a "beiging effect" in subcutaneous WAT [16–18].
2. **CD202b:** Also known as TIE2, it is the receptor, with similar affinities, for Angiopoietin-1 (Ang1) and Angiopoietin-2 (Ang2). Ang1 promotes vessel maturation, whereas Ang2 is a competitive antagonist, favoring immature vessel remodeling [19,20]

3. **CD309:** Also known as Vascular Endothelial Growth Factor Receptor 2 [17].
4. **CD143:** Angiotensin-converting enzyme, or ACE, is a central component of the renin-angiotensin system (RAS), which controls blood pressure by regulating the volume of fluids in the body [21].
5. **Angiopoietin 1 (ANGPT1):** Ang1 binds to the CD202b receptor and triggering its autophosphorylation. It promotes vessel maturation via increased mural cell, matrix contacts, and reduced permeability [19,20].
6. **Angiopoietin 2 (ANGPT2):** Naturally occurring antagonist for both ANGPT1 and TIE2. Expressed only at the sites of vascular remodeling [19,20].
7. **Angiotensinogen (AGT):** It is the protein precursor that is sequentially cleaved by the enzymes renin and angiotensin-converting enzyme (ACE) into angiotensin I (Ang I) and II (Ang II), respectively [21,22].
8. **Angiotensin II receptor type 1 (AGTR1),** also known as the **AT<sub>1</sub> receptor:** It is the best-characterized angiotensin receptor. In the adipose tissue, its activation induces proliferation and has anti-adipogenic effects [21,22].
9. **Angiotensin II receptor type 2 (AGTR2),** also known as the **AT<sub>2</sub> receptor:** In the adipose tissue, upon activation with the active hormone, Angiotensin II, this receptor induces the production of crucial lipogenic enzymes and leads to lipogenesis, thus increasing lipid storage and promoting adipogenic differentiation [21,22].
10. **Interferon-gamma (IFNG):** It is a cytokine primarily known for its roles in immunological responses and one of the major T-cell inflammatory cytokines. The biological effects of IFNG are elicited through the activation of intracellular molecular signaling networks, mainly via the JAK/STAT pathway. This signaling often results in the activation of the transcription factor called "signal transducer and activator of transcription" (STAT) 1 [23]. Depending on the cellular, microenvironmental, and/or molecular context, IFN- $\gamma$  can function as an angiogenesis inhibitor [24]. In adipocytes, IFNG treatment results in anti-adipogenic effects [25–27].
11. **CD130 or glycoprotein 130 (gp130):** It is a transmembrane protein that can transduce signals from many different ligands with diverse biological functions. These include the cytokines IL-6, IL-11, IL-27, IL-30, IL-31, oncostatin M (OSM), leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), ciliary neurotrophic factor (CNTF), cardiotrophin-like cytokine (CLC), and ciliary neurotrophic factor (CNTF). The members of this ligands family often employ the Jak-STAT signaling pathway to regulate different cellular processes. STAT3 and STAT5 promote adipogenesis, whereas STAT1 inhibits adipocyte development [23,28,29].
12. **Stromal cell-derived factor 1 (SDF1),** also known as **C-X-C motif chemokine 12 (CXCL12):** It is a chemokine protein and the physiologic ligand for the chemokine receptors CXCR4 and CXCR7. The SDF-1/CXCR4 or CXCR7 complex activates several pathways that mediate chemotaxis, migration, and angiopoietic factors' secretion. SDF-1 is also chemotactic for mesenchymal stem cells, and in adulthood, it plays an essential role in angiogenesis by recruiting endothelial progenitor cells (EPCs). Using human ASCs, it has been reported that the SDF-1/CXCR7 signaling axis was required for proliferation, whereas the SDF-1/CXCR4 axis was involved in cell migration. SDF-1 is secreted by both progenitor cells and mature adipocytes. On the other hand, its receptors are expressed differently: CXCR4 is predominant on mature adipocytes and very scarce on progenitors, while CXCR7 is the opposite, i.e., abundant on progenitors and deficient on more mature cells [30–32].
13. **C-X-C motif chemokine ligand 10 (CXCL10),** also known as **Interferon gamma-induced protein 10 (IP-10)** or **small-inducible cytokine B10:** It is secreted by several cell types in response to IFN- $\gamma$ . CXCL10 has been attributed to several roles, such as chemoattraction for monocytes/macrophages, T cells, NK cells, and dendritic cells, promoting T cell adhesion to endothelial cells, antitumor activity, and inhibition of bone marrow colony formation. It is also known as a potent inhibitor of angiogenesis *in vivo*. This chemokine elicits its effects by binding to the cell surface chemokine receptor CXCR3 [33,34].

**14. CXCR7:** It is a seven transmembrane-spanning receptor and belongs to the atypical chemokine receptors (ACKRs) family. Therefore, it was initially named ACKR3. In 2005, considering its structure's similarity to CXC receptors, it was renamed CXCR7 according to chemokine receptor nomenclature. Some studies have indicated that CXCR7 is a scavenger or decoy receptor which does not associate with Gi-proteins. Other evidence suggests that CXCR7 physically interacted with CXCR4, leading to a change of CXCR4 signaling and cellular functions. CXCR4 was thought for a long time to be the exclusive receptor for CXCL12/SDF-1. Later on, CXCR7 was found to be a second receptor for CXCL12 at a 10-fold higher binding affinity compared to CXCR4. It is mainly expressed on progenitor cells during adipogenesis rather than on maturing adipocytes [32,35–37].



**Figure S2.** Comparing the adipokines (**A**) and chemokines (**B**) secretion profile of hASCs. **1)** Profile of hASCs cultured in the *UrSuppe*, control cells. **2)** Profile of hASCs induced with *US-24* medium with SAG and insulin. Array procedure according to the manufacturer of the kit (bio-techno, #ARY024, #ARY017).

**Table S7.** Human adipokine array.

Coordinate.	Analyte/Control	Coordinate	Analyte/Control
A1, A2	Reference Spots	C19, C20	IL-6
A5, A6	Adiponectin/Acrp30	C21, C22	CXCL8/IL-8
A7, A8	Angiopoietin-1	C23, C24	IL-10
A9, A10	Angiopoietin-2	D1, D2	IL-11
A11, A12	Angiopoietin-like 2	D3, D4	LAP (TGF- $\beta$ 1)
A13, A14	Angiopoietin-like 3	D5, D6	Leptin
A15, A16	BAFF/BLyS/TNFSF13B	D7, D8	LIF
A17, A18	BMP-4	D9, D10	Lipocalin-2/NGAL
A19, A20	Cathepsin D	D11, D12	CCL2/MCP-1
A23, A24	Reference Spots	D13, D14	M-CSF
B1, B2	Cathepsin L	D15, D16	MIF
B3, B4	Cathepsin S	D17, D18	Myeloperoxidase
B5, B6	Chemerin	D19, D20	Nidogen-1/Entactin
B7, B8	Complement Factor D	D21, D22	Oncostatin M (OSM)
B9, B10	C-Reactive Protein/CRP	D23, D24	Pappalysin-1/PAPP-A
B11, B12	DPPIV/CD26	E1, E2	PBEF/Visfatin
B13, B14	Endocan	E3, E4	Pentraxin-3/SG-14
B15, B16	EN-RAGE	E5, E6	Pref-1/DLK-1/FA1
B17, B18	Fetuin B	E7, E8	Proprotein Convertase 9/PCSK9
B19, B20	FGF basic	E9, E10	RAGE
B21, B22	FGF-19	E11, E12	CCL5/RANTES
B23, B24	Fibrinogen	E13, E14	Resistin
C1, C2	Growth Hormone	E15, E16	Serpin A8/AGT
C3, C4	HGF	E17, E18	Serpin A12
C5, C6	ICAM-I/CD54	E19, E20	Serpin E1/PAI-1
C7, C8	IGFBP-2	E21, E22	TIMP-1
C9, C10	IGFBP-3	E23, E24	TIMP-3
C11, C12	IGFBP-4	F1, F2	Reference Spots
C13, C14	IGFBP-6	F5, F6	TNF- $\alpha$
C15, C16	IGFBP-rp1/IGFBP-7	F7, F8	VEGF
C17, C18	IL-1 $\beta$ /IL-1F2	F23, F24	Negative Controls

**Table/List on the left:** Coordinates and explanations of the 58 different spots present on the human adipokine array membrane.

**Table S8.** Human chemokine array.

Coordinate	Analyte/Control
A1, A2	Reference Spots
A19, A20	Reference Spots
B3, B4	CCL21/ Exodus-2/ 6Ckine
B5, B6	CCL28/ MEC
B7, B8	CXCL16/ SRPSOX
B9, B10	Chemerin/ TIG-2/ RARRES2
B11, B12	CXCL5/ ENA-78
B13, B14	CCL26/ Eotaxin-3
B15, B16	CX3CL1/ Neurotactin/ Fractalkine
B17, B18	CXCL1/ GRO $\alpha$
C3, C4	HCC-1/ CCL14, HCC-3
C5, C6	I-309/ CCL1, TCA3
C7, C8	IL-8/ CXCL8
C9, C10	IL-16/ LCF
C11, C12	IP-10/ CXCL10
C13, C14	I-TAC/ CXCL11
C15, C16	Lymphotactin/ XCL1/ Lptn, ATAC/ SCM-1 $\alpha$
C17, C18	MCP-1/ CCL2, MCAF
D3, D4	MCP-3/ CCL7
D5, D6	MDC/ CCL22/ STCP-1/ ABCD-1
D7, D8	Midkine
D9, D10	MIG/ CXCL9
D11, D12	MIP-1 $\alpha$ / $\beta$ / CCL3/ CCL4
D13, D14	MIP-1 $\delta$ / CCL15/ Leukotactin 1/ MIP-5/ HCC-2
D15, D16	MIP-3 $\alpha$ / CCL20/ LARC/ Exodus-1
D17, D18	MIP-3 $\beta$ / CCL19/ ELC/ Exodus-3
E3, E4	NAP-2/ CXCL7/ CTAP III
E5, E6	PARC/ CCL18/ MIP-4/ AMAC-1
E7, E8	PF4/ CXCL4
E9, E10	RANTES/ CCL5/ SISd
E11, E12	SDF-1/ CXCL12/ PBSF
E13, E14	TARC/ CCL17
E15, E16	VCC-1/ CXCL17/ DMC
F3, F4	Fibrinogen (Sample Control)
F5, F6	gp130 (Sample Control)/ IL6ST/ CD130
F7, F8	Transferrin R (Sample Control)/ TfR, CD71
F9, F10	Negative Control
G1, G2	Reference Spots

**Table/List on the left:** Coordinates and explanations of the 31 different spots present on the human chemokine array membrane.

## Supplementary Materials for Material and Methods Section

**Table S9.** Detailed information of the antibodies used for the flow cytometric measurements.

Name	# Catalog	Company	Concentration of use [ng]
7-AAD	559925	Becton Dickinson	2.5 µL
CD24-APC	130-095-954	Miltenyi	50
CD36-APC	130-095-475	Miltenyi	50
CD36-FITC	130-120-064	Miltenyi	50
CD130-APC	362005	BioLegend	50
CD143-PE	FAB929P	R&D System	50

Patrick C. Baer *et al.* reported as data not shown that they were able to detect the CD34 marker on the surface of hASCs only when the anti-CD34 antibody (manufactured by BD Biosciences) was labeled with PE and not with FITC [38]. Anti-hCD34-FITC and anti-hCD34-PE were from the same antibody clone; therefore, the different fluorochromes and the coupling reactions were responsible for the observed discrepancy. We observed the same phenomenon also with anti-CD24 antibodies. Daniel T. Gillian *et al.* provide a detailed list of commonly used mouse and human anti-CD24 antibodies with their associated epitope targets, wherever identified [39]. We used the antibody clone 3D12, which is listed in Table 2 in Daniel T. Gillian *et al.* We found that only antibodies labeled with "large" fluorochromes, such as APC or PE, worked perfectly, while the FITC derivative of the same clone not. So anti-hCD24-PE (# 130-098-861) and anti-hCD24-APC (# 130-095-954) work perfectly, while anti-hCD24-FITC (# 130-099-118) did not. These three antibodies derive from clone 3D12 and are manufactured by Miltenyi Biotech. This problem probably also exists with antibodies produced by other companies, such as the anti-hCD24-FITC clone ML5, manufactured by BioLegend (# 311103). So we invite the colleagues to pay attention to this possible fluorochrome-based complication with anti-hCD24 (and anti-hCD34) antibodies when working with hASCs or other non-blood related cells.

**Table S10.** Reverse transcription detailed procedure.

Reagent	Amount
<i>Mix 1:</i>	
RNA	Up to 5 µg
Oligo dT	0.5 µg
Random Primers	0.5 µg
H <sub>2</sub> O	Final volume 5 µL
<i>Mix 2:</i>	
Buffer 5x	2 µL
MgCl <sub>2</sub>	1 µL [2.5 mM]
dNTPs	0.5 µL [0.5 mM]
Inhibitor RNasi	0.25 µL [20 Units]
RT Enzyme	0.5 µL
H <sub>2</sub> O	Final volume 5 µL

**Procedure:** Add Mix 1, incubate 5 min at 70 °C, cool to 10 °C and incubate 5 min in ice. Add Mix 2 and incubate 5 min at 25 °C, 42 °C for 1h and 70 °C for 15 min.

**Table S11.** RT-qPCR cycle conditions.

Phase	T (°C)	Time (min)	Repetition
Denaturation	95 °C	2:00	
Denaturation	95 °C	0:05	
Annealing + Extension	60 °C	0:20	{ ×40
Denaturation	95 °C	0:05	
Melting Curve	65–95 °C	18:00	

**Table S12.** Primer sequences.

Gene Name	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>AGT</i>	ATT GAG CAA TGA CCG CAT CAG G	GGC TTG TTA AGT TGT TGG GTA G
<i>AGTR1</i>	CAT CCC AGA AAG TCG GCA CCA G	GCC TTC CAG CTT TGG GAC AAT C
<i>AGTR2</i>	ATT ACG TCC CAG CGT CTG AGA G	TCA CAA GCC CGA AGT GAA GAC C
<i>ANGPT1*</i>	TCG TGA GAG TAC GAC AGA CCA	TCT CCG ACT TCA TGT TTT CCA C
<i>ANGPT2*</i>	ACC CCA CTG TTG CTA AAG AAG A	CCA TCC TCA CGT CGC TGA ATA
<i>CD24</i> [40]	CTC CTA CCC ACG CAG ATT TAT TC	AGA GTG AGA CCA CGA AGA GAC
<i>CD130*</i>	GCA ACA CAC AAG TTT GCT GAT T	CCT TCC CAA GGG CAT TCT CTG
<i>CD143*</i>	GGA GGA ATA TGA CCG GAC ATC C	TGG TTG GCT ATT TGC ATG TCC TT
<i>CD202b*</i>	TTA GCC AGC TTA GTT CTC TGT GG	AGC ATC AGA TAC AAG AGG TAG GG
<i>CD309*</i>	GTG ATC GGA AAT GAC ACT GGA G	CAT GTT GGT CAC TAA CAG AAG CA
<i>CXCL10*</i>	GTG GCA TTC AAG GAG TAC CTC	TGA TGG CCT TCG ATT CGT GAT T
<i>CXCL12/SDF1*</i>	ATT CTC AAC ACT CCA AAC TGT GC	ACT TTA GCT TCG GGT CAA TGC
<i>CXCR7</i>	GCA AAG TGC TCA GCA CTA AG	TTC CCT GGC TCT GAG TAG TC
<i>DHH*</i>	CGA GCG TTG TAA GGA GCG G	CCC TCA GTC ACT CGT AGG C
<i>DLL1</i>	ACT CCG CGT TCA GCA ACC CCA T	TGG GTT TTC TGT TGC GAG GTC ATC AGG
<i>DLL4</i>	ACT GCG AGA AGA AAG TGG ACA GGT	ACA TGA GCC CAT TCT CCA GGT CAT
<i>GLI1</i> [41]	TGC AGT AAA GCC TTC AGC AAT G	TTT TCG CAG CGA GCT AGG AT
<i>GLI2*</i>	CCC CTA CCG ATT GAC ATG CG	GAA AGC CGG ATC AAG GAG ATG
<i>GLI3*</i>	GAA GTG CTC CAC TCG AAC AGA	GTC GCT GCA TAG TGA TTG CG
<i>HHAT*</i>	CGG GAT GTG GAG GTA TTT TGA TG	CAC CAG AGG TAG TCG TAG CC-
<i>HES1</i> [42]	GTC AAC ACG ACA CCG GAT AAA CCA	TTT CCA GAA TGT CCG CCT TCT CCA
<i>IHH*</i>	TCC GTC AAG TCC GAG CAC T	GTC CTG AGT CTC GAT GAC CTG
<i>IFNG*</i>	TCG GTA ACT GAC TTG AAT GTC CA	TCG CTT CCC TGT TTT AGC TGC
<i>JAG1</i> [42]	ACT GCT CAC ACC TGA AAG ACC ACT	AGG ACC ACA GAC GTT GGA GGA AAT
<i>NOTCH1*</i>	TGG ACC AGA TTG GGG AGT TC	GCA CAC TCG TCT GTG TTG AC
<i>PPARG</i> [43]	TGA CAG CGA CTT GGC AAT ATT TAT T	TTG TAG CAG GTT GTC TTG AAT GTC T
<i>PREF1</i> [44]	TGA CCA GTG CGT GAC CTC T	GGC AGT CCT TTC CCG AGT A
<i>PTCH1*</i>	CCA GAA AGT ATA TGC ACT GGC A	GTC CTC GTC CAT TTG CTT GGG
<i>RLP13A</i>	CCT GGA GGA GAA GAG GAA AGA GA	TTG AGG ACC TCT GTG TAT TTG TCA A
<i>RUNX2*</i>	TCA ACG ATC TGA GAT TTG TGG G	GGG GAG GAT TTG TGA AGA CGG
<i>SHH</i>	AGA GTA GCC CTA ACC GCT CCA G	CCA GGA GCC AGG TGC CAT TTT G
<i>SMO*</i>	GAA GTG CCC TTG GTT CGG A	GCA GGG TAG CGA TTC GAG TT
<i>SOX9*</i>	AGC GAA CGC ACA TCA AGA C	CTG TAG GCG ATC TGT TGG GG
<i>UCP1*</i>	AGG ATC GGC CTC TAC GAC AC	GCC CAA TGA ATA CTG CCA CTC
<i>VEGFA</i>	CAT GCC AAG TGG TCC CAG GCT	CAG CCC CCG CAT CGC ATC AG
<i>WISP2*</i>	GCG ACC AAC TCC ACG TCT G	TCC CCT TCC CGA TAC AGG C

\* Primer from PrimerBank (<https://pga.mgh.harvard.edu/primerbank/>).

**Table S13.** Materials.

Name	# Catalog	Company
SAG	AG-CR1-3585	Adipogen
Omnifix 100 mL Syringe	4614003F	BBraun
CytoFLEX Daily QC Fluorospheres	B53230	Beckman & Coulter
VersaComp Antibody Capture Bead Kit	B22804	Beckman & Coulter
VersaLyse Lysing Solution	B59266AA	Beckman & Coulter
Cell Strainers 40 $\mu$ m	352340	Becton Dickinson
Cell Strainers 100 $\mu$ m	352360	Becton Dickinson
Dulbecco's PBS ( $\text{Ca}^{2+}$ $\text{Mg}^{2+}$ )	3-05F00-I	BioConcept
Sso Advanced Universal SYBR Green Supermix	1725271	Biorad
Dulbecco's PBS (without $\text{Ca}^{2+}$ $\text{Mg}^{2+}$ )	L0615-500	Biowest
Dexamethasone	11015	Cayman Chemicals
Albumin CSL 20%	22918180119611	CLS Behring
Privigen Immunoglobulin	44206-437-05	CLS Behring
Gosselin™ Containers with Snap Cap	PC1000-05	Corning
PureCoat™ Fibronectin Peptide 75 cm <sup>2</sup> Flask	356242	Corning
BioCoat™ Fibronectin-coated Plates	354402	Corning
Syringe Filters	FPE-204-030	Jet Biofil
IRDye® 800CW Streptavidin	926-32230	Li-Cor
Accugene™ Molecular Biology Water	51200	Lonza
Nucleospin RNA kit	740955.250	Macherey-Nagel
GoScript Reverse Transcription System	A5001	Promega
BMP4 human	CYT-081	Prospec
DHH	CYT-362	Prospec
IHH	CYT-195	Prospec
SHH	CYT-676	Prospec
TGF- $\beta$ 1 human	CYT-716	Prospec
TGF- $\beta$ 3 human	CYT-685	Prospec
TNF- $\alpha$ human	CYT-223	Prospec
Proteome Profiler Human Adipokine Array Kit	ARY024	R&D Systems
Proteome Profiler Human Chemokine Array Kit	ARY017	R&D Systems
Insulin	I9278-5ML	Sigma-Aldrich
Trypan Blue	15250-061	Thermo Fisher
TrypLE Select	12563-029	Thermo Fisher
Conical Tube 50 mL	91050	TPP
Collagenase Type B AOF	CLSAFA	Worthington Biochemical Corp.

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