Kent Academic Repository

Full text document (pdf)

Citation for published version

Vohra, Muhammad Sufyan and Ahmad, Bilal and Serpell, Christopher J. and Parhar, Ishwar S. and Wong, Eng Hwa (2020) Murine in vitro cellular models to better understand adipogenesis and its potential applications. Differentiation, 115 . pp. 62-84. ISSN 0301-4681.

DOI

https://doi.org/10.1016/j.diff.2020.08.003

Link to record in KAR

https://kar.kent.ac.uk/82817/

Document Version

Author's Accepted Manuscript

Copyright & reuse
Content in the Kent Academic Repository is made available for research purposes. Unless otherwise stated all content is protected by copyright and in the absence of an open licence (eg Creative Commons), permissions for further reuse of content should be sought from the publisher, author or other copyright holder.

Versions of research
The version in the Kent Academic Repository may differ from the final published version.
Users are advised to check **http://kar.kent.ac.uk** for the status of the paper. **Users should always cite the published version of record.**

Enquiries
For any further enquiries regarding the licence status of this document, please contact:
researchsupport@kent.ac.uk

If you believe this document infringes copyright then please contact the KAR admin team with the take-down information provided at **http://kar.kent.ac.uk/contact.html**

KAR Kent Academic Repository

University of Kent

Murine *in vitro* cellular models to better understand adipogenesis and its potential applications

Muhammad Sufyan Vohra[1], Bilal Ahmad[2], Christopher J. Serpell[3], Ishwar S. Parhar[4], Eng Hwa Wong*

[1] *School of Medicine, Faculty of Health and Medical Sciences, Taylor's University Lakeside Campus, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia*

[2] *School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University Lakeside Campus, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia*

[3] *School of Physical Sciences, Ingram Building, University of Kent, Canterbury, Kent, CT2 7NH, United Kingdom*

[4] *School of Medicine and Health Sciences, Monash University, Sunway Campus, PJ 46150 Selangor, Malaysia*

***Corresponding author:**

Eng Hwa Wong, School of Medicine, Faculty of Health and Medical Sciences Taylor's University Lakeside Campus No1 Jalan Taylor's, 47500 Subang Jaya, Malaysia. Email Address: EngHwa.Wong@taylors.edu.my Contact No. +60 12-269 8587

Abstract

Adipogenesis has been extensively studied using *in vitro* models of cellular differentiation, enabling long-term regulation of fat cell metabolism in human adipose tissue (AT) material. Many studies promote the idea that manipulation of this process could potentially reduce the prevalence of obesity and its related diseases. It is essential to understand the molecular basis of fat cell development if we are to tackle this pandemic disease, by identifying therapeutic targets and new biomarkers. This review explores murine cell models and their applications for study of the adipogenic differentiation process *in vitro*. We focus on the benefits and limitations of the different cell line models, with the aim of aiding data interpretation and selection of appropriate model cell lines model for future advances in adipose biology.

Keywords: Adipose tissue, Adipogenesis, Cellular models, Differentiation, Anti-obesity

1 Introduction

Obesity is one of the most widespread problematic health conditions, having tripled worldwide since 1980 (Chooi, Ding and Magkos, 2019). The condition is caused by accumulation of excess body fat in human body, to the extent that it is associated with several life-threatening diseases, predominantly hypertension, diabetes, heart disease, osteoarthritis and cancer (Włodarczyk and Nowicka, 2019,Lohmann, Goodwin, Chlebowski et al., 2016).

33 The alarming prevalence and severity of obesity has drastically increased both in children and
34 adults, and corresponding morbidity and mortality has increased markedly over the past two
35 decades (Abarca-Gómez, Abdeen, Hamid et al., 2017,Wolfenden, Ezzati, Larijani et al., 2019).
36 According to NCD Risk Factor Collaboration, a network of health scientists around the world,
37 suggested global occurrence of obesity could possibly reach 21% in women and 18% in men
38 by 2025, if the trend continues (Collaboration, 2016). Therefore, understanding obesity and its
39 intervention must become public health priorities worldwide.

40 This complex multifactorial disease is generally caused by a decrease in energy
41 expenditure and/or increased energy intake for a prolonged period of time, resulting in an
42 energy imbalance (Ross, Flynn and Pate, 2016). Environmental factors, like high caloric food
43 consumption and sedentary lifestyle are strongly associated with the dysfunctionality of
44 adipose tissue (AT) formation, which undergoes molecular and cellular alterations affecting
45 metabolism, insulin sensitivity and promoting local and systemic inflammation (de Ferranti
46 and Mozaffarian, 2008,van Meijel, Blaak and Goossens, 2019). AT increases through
47 differentiation of pre-adipocytes to greater numbers of adipocytes, referred to as hyperplasia
48 and/or by increase of size of existing adipocytes to accommodate further lipids, known as
49 hypertrophy (Jo, Gavrilova, Pack et al., 2009).

50 AT is a complex organ, able to regulate whole-body energy supply through the
51 accumulation of triglycerides. Besides adipocytes, these tissues also contain several other cell
52 types, including fibroblasts, blood cells, endothelial cells, macrophages, and other immune
53 cells. All these cells continuously interact to tune metabolic response and tissue expansion.
54 Alongside its passive function, AT also plays an intricate role in whole-body homeostasis as
55 an endocrine organ. AT produces adipokines and numerous other bioactive factors that
56 communicate with other organs and moderate a variety of metabolic pathways (Booth,
57 Magnuson, Fouts et al., 2016,PNandhini, Desai and Sahoo, 2019).

58 White, brown, and beige are names given to the three types of fat cells in AT. These
59 cells have distinct locations and functions in the human body, differing in abundance of
60 mitochondria and in thermogenic genes expression (Giralt and Villarroya, 2013). The majority
61 of fats in adult humans are stored in white adipocytes of white adipose tissue (WAT) featuring
62 a single large lipid droplet which serves as a storage depot for excess energy (Han, Zaretsky,
63 Andrade-Oliveira et al., 2017). In contrast, brown adipose tissue (BAT) cells contain multiple
64 lipid droplets and function by generating heat through mitochondrial uncoupling of lipid

oxidation that burns energy through thermogenesis (Villarroya, Cereijo, Villarroya et al., 2017). As for the third type of fat, beige adipocytes were recently discovered and showed similar functions to both white and brown adipocytes. Like BAT, beige adipocytes have enhanced thermogenic capacity, high uncoupling protein-1 (UCP1) expression, and energy expenditure when activated (Mottillo, Desjardins, Crane et al., 2016). Nevertheless, all these types of fat cells work together to maintain whole-body energy homeostasis.

All fat tissues are refined by a cell differentiation process, wherein preadipocytes differentiate into mature adipocytes and become fully functional. This is a complex process known as adipogenesis, that comprises of numerous stages extensively regulated by the specific expression of proteins and transcription factors leading to adipocyte development. Among them, Peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer-binding protein-α (C/EBPα) are considered the main regulators of adipogenesis (Rosen, Walkey, Puigserver et al., 2000). They induce expression of each other mutually and have their cooperation in activating a few other adipocyte genes has been previously reported (Munawar, Prakash and Vangalapati, 2018). However, newer studies on adipogenesis have revealed that several other transcription factors including C/EBPβ, C/EBPδ, as well as some of the Krupel-like factors (KLF), induce expression of PPARγ (Hammarstedt, Gogg, Hedjazifar et al., 2018). Nevertheless, transcriptional repressors including GATA2, KLF2, and CHOP have shown to reduce PPARγ expression in adipogenesis. To store or utilize energy, mature adipocytes respond to different metabolic stimuli. The various cell types are able to communicate with each other via adipokines, cytokines or lipid/glucose fluxes. Over time, adipocytes eventually lose their differentiation or thermogenic capability due to senescence (González-Casanova, Pertuz-Cruz, Caicedo-Ortega et al., 2020).

Our understanding of preadipocyte differentiation using *in vitro* culture models has advanced significantly in recent years (Ruiz-Ojeda, Rupérez, Gomez-Llorente et al., 2016). These cellular systems have become invaluable tools to determine the mechanisms involved in adipocyte proliferation, differentiation, adipokine secretion and gene/protein expression. An *in vitro* model which accurately recapitulates the properties of native human AT would greatly benefit therapy development and pathology studies. Genes, proteins, and signaling pathways involved in regulation of adipocytes are now rapidly identified using modern technologies like protein arrays, microarrays, and genetic manipulation. Yet, these techniques are only valuable if the most effective cell model is used in the research efforts. Moreover, the cell lines serve as useful systems to explore biochemical characteristic and functions of key adipogenic factors

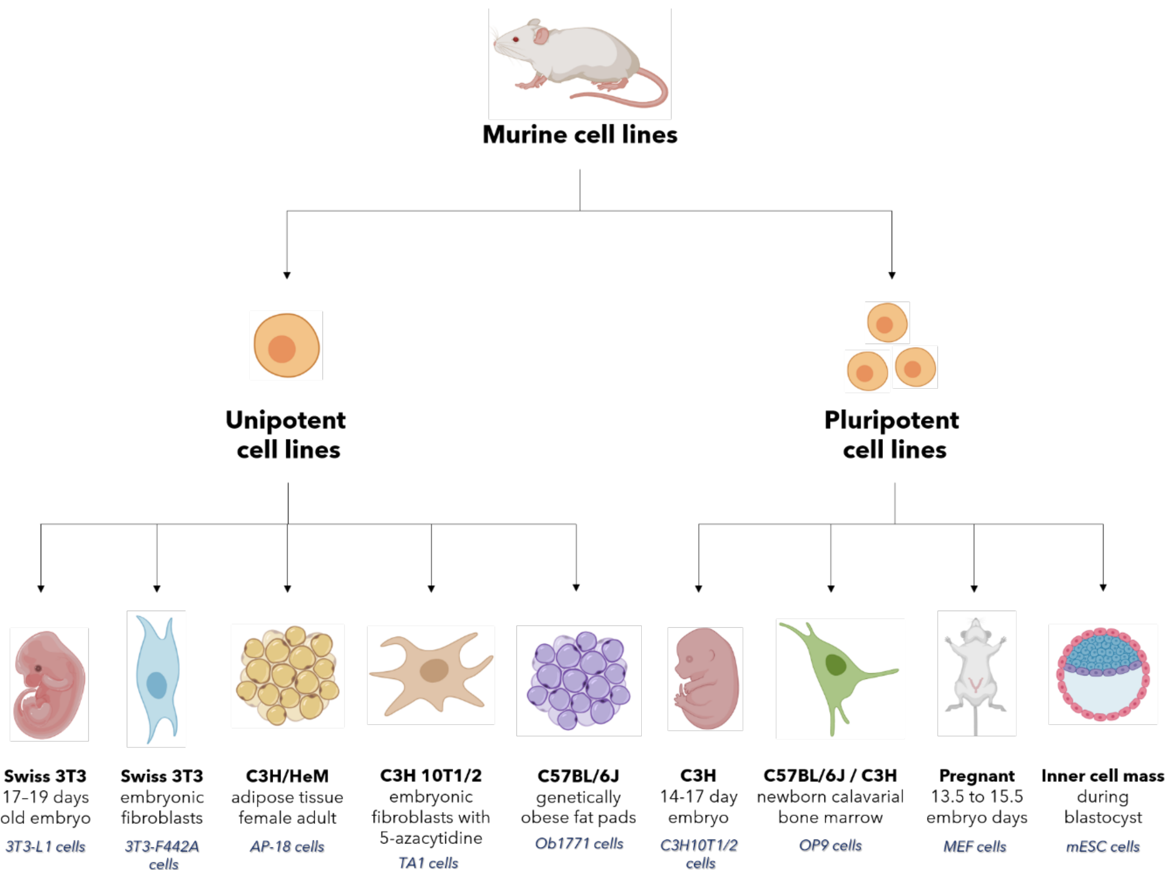
98 and pathways. Additionally, murine derived cell culture systems have also assisted with several
99 adipogenesis studies as they are easy to cultivate and translate to *in vivo*, which has further
100 enhanced our knowledge on adipose biology (Wang, Scherer and Gupta, 2014).

101 The aim of this review is to provide a better knowledge and understanding of murine *in*
102 *vitro* cellular models for the study of adipogenesis, focusing mainly on the cell differentiation
103 and their applications for anti-adipogenic effects. This article discusses information relevant to
104 the culture systems, highlighting benefits and limitations of the cell lines as well as their
105 applications in adipocyte biology, and provides guidance for those seeking to select an
106 appropriate model for their work. Our goal is to support a better understanding of the science
107 of adipocytes and AT, as well as their mechanisms will assist with the development of novel
108 therapeutic approaches and agents that can effectively treat these conditions.

109 2 Cellular models for study of adipogenesis

110 The availability of a vast range of cell models has enabled extensive study on
111 differentiation of adipocyte using cell culture systems. These models represent the stages of
112 adipocyte development, detailing the molecular and cellular events in transition from
113 fibroblast-like preadipocytes into adipocyte cells (Ruiz-Ojeda et al., 2016). Hence, biologists
114 and biochemists have been able to explore new and existing mechanisms using different
115 sources of adipose cell models which have immensely facilitated research into the
116 differentiation process and identification of regulatory elements that assist with coordinated
117 expression during differentiation of adipocyte genes.

118 Unipotent preadipocyte and pluripotent cell lines are the two primary classes of *in vitro*
119 cell models present for the study of adipogenesis (Figure 1) (Moreno-Navarrete and Fernández-
120 Real, 2017). Preadipocyte models are unipotent cells which are useful in understanding the
121 molecular events responsible for preadipocyte conversion, whereas, multipotent fibroblasts
122 cells are pluripotent models committed to different lineages and used to study the cellular
123 determination of the separate cell fates, including adipocytes.



124 **Figure 1: Murine cell line - Schematic diagram showing the source of each unipotent and**
125 **pluripotent cell line from the *in vitro* model to study cellular differentiation for**
126 **adipogenesis**

127 Preadipocyte clonal cell lines (3T3-L1; 3T3-F442A; TA1; AP-18; Ob1771) (refer to
128 Figure 1) present a homogenous cellular population with the same differentiation stages. The
129 ability of these preadipocyte cells to passage indefinitely present them the lines as effective
130 resources for study. Alternatively, pluripotent cell models can differentiate into various cell
131 types other than adipocytes. These cell lines include C3H10T1/2, OP9 cells, mouse embryonic
132 stem cells (mESCs) and mouse embryonic fibroblasts (MEFs) (see Figure 1). In particular,
133 C3H10T1/2 have been beneficial for acknowledging the events responsible for lineage

134 determination. These cell lines have various practical features that make them suitable for
135 adipocyte studies, especially OP9 cells which hold the capacity to rapidly differentiate.
136 Likewise, mESCs provide an effectively infinite supply of cells when combined with retinoic
137 acid (RA) and pro-adipogenic agents. More interestingly, MEFs are established and maintained
138 easily, they proliferate rapidly, and can yield various cell types within several days, from just
139 a single embryo (Yusuf, Gopurappilly, Dadheech et al., 2013). It should be noted that the
140 development stage of each cell line has minute variations in their requirements for
141 differentiation (Kassotis, Masse, Kim et al., 2017). Nonetheless, they also have similar
142 functionalities to mesenchymal stem cells (MSCs) and maintain stable morphology for a long
143 period in culture. Table 1 provides a summary of murine *in vitro* cell models and their
144 applications for understanding adipogenesis.

145 Several researchers have thoroughly examined the adipogenic or anti-adipogenic
146 potential of many pharmacological compounds including hormones and growth factors due to
147 the availability of murine *in vitro* cell models. Identifying specific development markers allows
148 us to align the development programs of each cell line. Comprehensive knowledge of the
149 differentiation process could assist with manipulating adipocyte cell numbers to control
150 specific diseases - study of adipocyte differentiation, expansion and endocrine function at a
151 complex level will support the development of therapies against obesity and its metabolic
152 complications.

153 **Table 1: Adipogenic applications of murine *in vitro* cell line model**

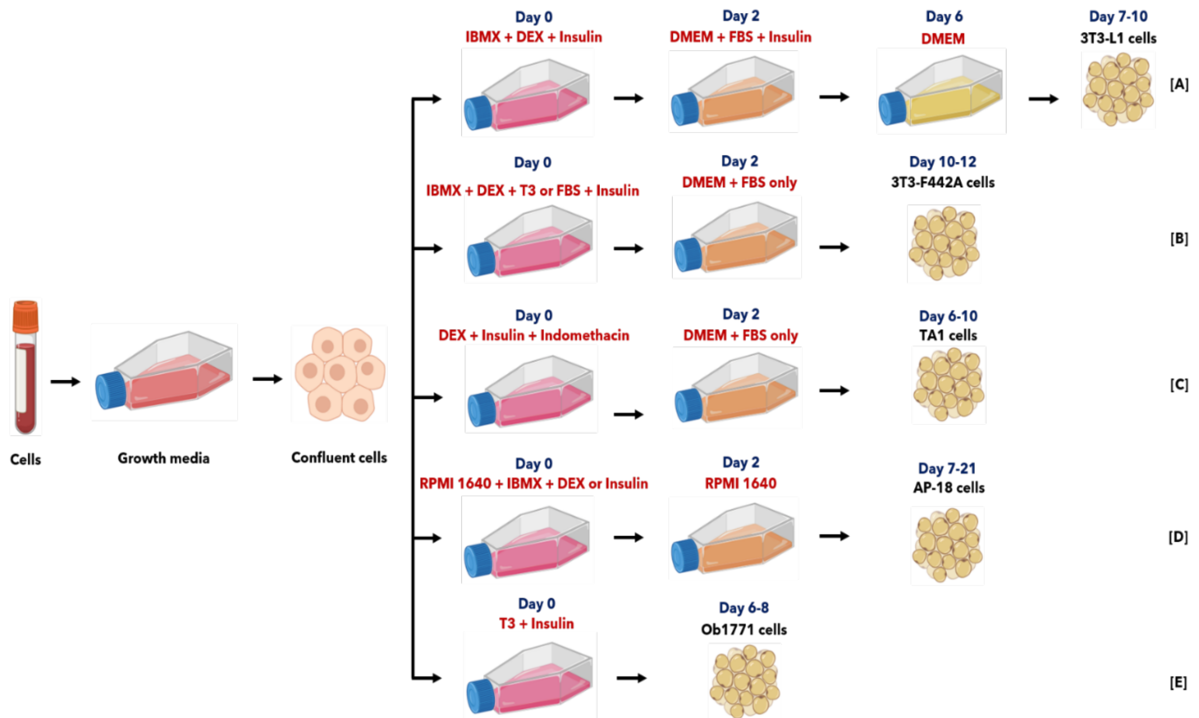
Cell line	Adipogenic agents	Differentiation time	Reported applications	References
3T3-L1	Dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX) and Insulin	7-10 days	• Understanding role of adipocyte-related proteins and genes • Used in co-culture and three-dimensional culture system for adipose tissue • Screening anti-adipogenic compounds, anti-adipogenic peptides, adipogenic agents in food products and anti-adipogenic crude extracts	(Kim, Lee, Kim et al., 2020,Zhao, Hu, Wang et al., 2019)
3T3-F442A	Insulin, Fetal bovine serum (FBS), Tri-iodothyronine (T3), IBMX and DEX	10-12 days	• Examination of adipogenic agents in differentiation processes • Screening anti-adipogenic compounds, effective adipogenic peptides, adipogenesis transcriptional factors and anti-adipogenic crude extracts	(Hemmeryckx, Vranckx, Bauters et al., 2019,Khalilpourfarshbafi, Murugan, Sattar et al., 2019)

TA1	DEX, Insulin and Indomethacin	6-10 days	• Understanding role and function of adipocyte-related proteins • Screening effective agents in adipose differentiation process • Estimating pre-existing and new genes involved in adipogenesis • Used in identification of early adipogenic markers	(Shinohara, Murata and Shimizu, 1992,Ninomiya-Tsuji, Torti and Ringold, 1993)
AP-18	Combination of DEX and IBMX or Insulin	7-21 days	• Potential in identification of mechanism for subcutaneous adipocytes biology	(Chen, Takahashi, Yoshida et al., 2010)
Ob1771	Insulin and T3	6-8 days	• Examination of different molecules in differentiation process of adipogenesis • Screening effective fatty acids in adipogenesis biology and agents involved in obesity and its related conditions	(Abderrahim-Ferkoune, Bezy, Astri-Roques et al., 2004)
C3H10T1/2	IBMX, DEX, Insulin, and Troglitazone/Rosiglitazone	12 days	• Screening natural compounds and crude extract for anti-adipogenic effects • Examination of the role and function of adipocyte-related proteins in adipogenesis • Estimation of the regulatory effects of non-coding RNA in adipogenic differentiation	(Schwind, Schetting and Montenarh, 2017,Hussain, Rehman, Luckett et al., 2020)
OP9	Serum replacement method (SRM), Insulin oleate method (IOM) and Adipogenic cocktail method (ACM)	2-3 days	• Identification of key regulators in adipocyte related disease conditions • Screening compounds on early and late differentiation of adipogenesis • Examination of natural crude extracts for anti-adipogenic effects • Used in high-throughput RNA screening and techniques	(Wolins, Quaynor, Skinner et al., 2006)
Mouse Embryonic Stem cells (mESCs)	Retinoic acid (RA), Insulin, T3 and Rosiglitazone	21 days	• Characterisation of pre-existing genes and new adipogenic regulatory genes • Used in advanced and high-throughput techniques • Identification of genetic and epigenetic mechanisms involved in adipogenesis • Potential in exploring developmental fate of	(Rosen and MacDougald, 2006,Ota, Tong, Goto et al., 2017)

			adipocytes origin and screening compounds on differentiation of adipogenesis	
Mouse Embryonic Fibroblasts (MEFs)	Insulin, DEX, IBMX, Troglitazone and FBS	14 days	• MEFs from genetically modified or knockout mice used for study the effects of genes in adipogenesis • Evaluation of the effects of proteins or genes in adipogenesis • Screening anti-adipogenic compounds and anti-adipogenic crude extracts	(Yusuf et al., 2013,Hou, Chen, Wang et al., 2020)

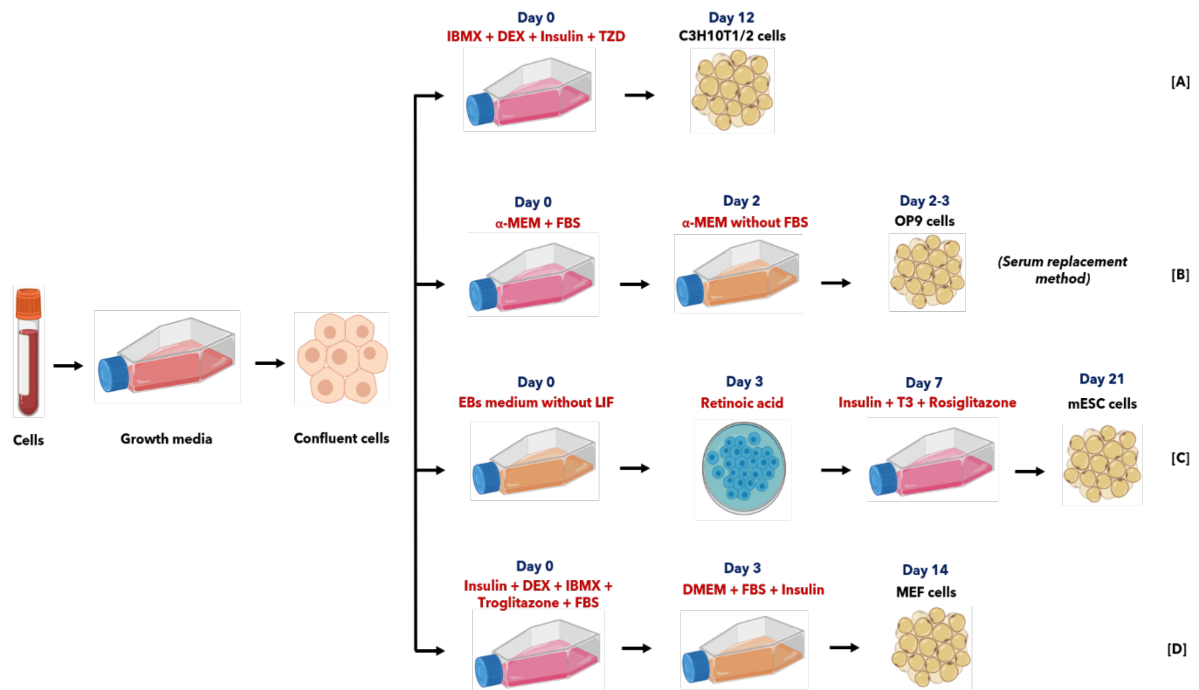
154

155



156 **Figure 2 A-E: Differentiation process - Schematic diagram presents adipocytes**
157 **differentiation process of unipotent murine cell line**

158



159 **Figure 3 A-D: Differentiation process - Schematic diagram presents adipocytes**
160 **differentiation process of pluripotent murine cell line**

161 **2.1 3T3-L1 Cell Line**

162 In 1974, Green and Kehinde reported the discovery of the 3T3-L1 cell line (Green and
163 Kehinde, 1974,Antony, Debroy, Manisha et al., 2019). 3T3-L1 is an established embryonic
164 murine preadipocyte cell line with distinctive characteristics, extensively used for the study of
165 adipocyte biology. The cell line was originally manufactured by selecting cells from the resting
166 state of a disaggregated 17–19 days old Swiss 3T3 mouse embryo. Its significance was
167 recognized when it was injected into mice, which formed fat pads that could not be
168 differentiated from their normal AT (Green and Kehinde, 1979,Kuri-Harcuch, Velez-delValle,
169 Vazquez-Sandoval et al., 2019). It is known that mature 3T3-L1 cells possess the majority of
170 the ultrastructural properties of adipocytes in culture, i.e. same as that of an animal tissue
171 (Novikoff, Novikoff, Rosen et al., 1980,Xiu, Xinong, Tianjia et al., 2017). Furthermore, 3T3-
172 L1 culture displays spontaneous lipid accumulation when converted into its adipocyte-like
173 phenotype. Adipogenic cocktails, also known as adipogenic agents, are defined
174 prodifferentiative agents required for conversion of undifferentiated cells into differentiated
175 adipocyte cells. Insulin, dexamethasone (DEX), and 3-isobutyl-1-methylxanthine (IBMX) are
176 the most commonly used adipogenic cocktails in 3T3-L1 cell differentiation.

177 3T3-L1 cells are first cultured in a basal medium containing high glucose concentration,
178 generally consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal
179 bovine serum (FBS) and antibiotics. A humidified atmosphere of 37 °C containing 5% CO_2 is
180 a standard protocol essential for cell culture. The medium is changed every 2–3 days, until the
181 cells reach confluency. Once confluence is reached, 3T3-L1 differentiation is initiated by
182 exchanging the growth media with the adipogenic cocktail medium, also known as induction
183 medium. After 48 hours of exposure, the induction media is substituted with the growth
184 medium containing insulin. Thereafter, the media is removed on day 6 and un-supplemented
185 growth media is added. Subsequently, by day 7-10, the cells start to accumulate triglycerides
186 in the form of lipid droplets identified as fully differentiated adipocyte-like cells. These mature
187 fat cells grow in number and size over cultivation time and also express multiple metabolic
188 characteristics (Figure 2-A) (Kim et al., 2020,Zhao et al., 2019).

189 Several researchers have tried to introduce numerous adipogenic agents to create more
190 efficient methods of obtaining improved differentiation efficiency of 3T3-L1 cells (Subra,
191 Fontana, Visentin et al., 2003,Katafuchi, Garbers and Albanesi, 2010). Thiazolidinediones
192 (TZDs) are agonists of PPARγ that are frequently used as an additional component for 3T3-L1

193 differentiation. A further report by Zebish et al. suggested that the use of rosiglitazone (one of
194 the TZDs) as an additional adipogenic agent resulted in differentiation within 2 weeks which
195 persists for up to 10 cell passages (Zebisch, Voigt, Wabitsch et al., 2012). The experimental
196 results indicated an increase of lipid accumulation and high glucose uptake with Troglitazone
197 and DEX (Vishwanath, Srinivasan, Patil et al., 2013). Additionally, this combination was
198 proven to generate better quality adipocytes over a shorter period of time in comparison to the
199 combination of IBMX and DEX. Another study by Hua et al. on 3T3-L1 cells suggested that
200 prolonged treatment of IBMX improved differentiation efficiency (Hua, Ke, Wang et al.,
201 2016). A recent analysis of six different commonly used adipogenic cocktails and their
202 protocols suggested that the concentration of 0.5 mM IBMX, 1 μM DEX, and 10 μg/mL insulin
203 is most effective for 3T3-L1 cell differentiation (Zhao et al., 2019).

204 3T3-L1 cells are widely applicable for the study of adipocyte biology. Over the last
205 decade, the 3T3-L1 *in vitro* model has been widely used to study molecular
206 and cellular processes of adipogenesis. Studies using this cell line have focused on evaluating
207 the role, function and possible mechanisms of adipocyte-related proteins to get a better insight
208 of adipogenesis and its regulations. For example, Matrix Gla protein (MGP) has been identified
209 in 3T3-L1 cells for involvement in fat metabolism and as a novel serum marker in central
210 obesity (Li, Li, He et al., 2020). Likewise, the function of LIGHT/TNFSF14 was discovered in
211 the diversion of energy in favour of immune activation that limited the adipogenic and
212 thermogenic programs (Kou, Liu, Liu et al., 2019). Recently, liver kinase B1 (LKBI) was
213 identified by up-regulation of brown adipocyte expression markers including UCP1, PGC-1α
214 and PRDM16 in 3T3-L1 cells (Xi, Xue, Wu et al., 2019). Consequently, this work has helped
215 us determine the use of these proteins as different biomarkers of obesity and their usefulness
216 to acknowledge the development of obesity in adipocyte tissue.

217 This cell line has also enabled characterization of the proteins involved in obesity and its
218 complications to identify its remedies. For example, W. Tang and Fan characterized Sirtuin 6
219 (SIRT6) as resulting in reduced insulin resistance and increased glucose metabolism in 3T3-
220 L1 cells (Tang and Fan, 2019). Similarly, an investigation by Kobayashi et al. on WW domain
221 containing E3 ubiquitin protein ligase 1 (WWP1) in 3T3-L1 cells and on an *in vivo* model
222 revealed that it is an obesity-inducible E3 ubiquitin ligase that assists with protection against
223 obesity-associated oxidative stress (Kobayashi, Hoshino, Abe et al., 2019). Several other
224 proteins have also been explored in 3T3-L1 cells for their effects on the adipocyte
225 differentiation process. For instance, Tyrphostin-AG17 was found effective in preventing

226 adipogenesis and lipid synthesis by activating caspase-3 mechanism which induced adipocyte
227 apoptosis (Camacho, Segoviano-Ramírez, Sánchez-Garcia et al., 2018). In addition, the 3T3-
228 L1 cell line was also used to understand the mechanisms underlying obesity and other
229 metabolic disorders to investigate the regulators associated with adipose development. In this
230 case, The role of pigment epithelium-derived factor (PEDF) has been established in lipid
231 metabolism, which has been recognized for negatively regulating adipogenesis through various
232 signaling intermediates (Huang, Hsu, Chen et al., 2018). Table 2 provides detailed information
233 on the proteins examined for adipogenic properties in 3T3-L1 preadipocytes cells.

234 The large number of studies which use the 3T3-L1 cell line reflect its ability to rapidly
235 screen and assess the inhibition of adipogenesis by measurement of intracellular triglyceride
236 contents and lipid accumulation, making the cell line a valuable model for screening natural
237 therapeutic agents. Some negatively regulated adipogenic compounds including bisphenol-A
238 (BPA) and polychlorinated biphenyls 138 (PCBs) have been shown to contribute to the
239 induction of obesity using 3T3-L1 cells (De Filippis, Li and Rosen, 2018,Kim, Kim, Oh et al.,
240 2018). Further data on compounds investigated on 3T3-L1 cells and their possible adipogenic
241 actions have been given in Table 3.

242 Co-culture is the growth of two different cell types together in the same environment.
243 The study of co-culture systems assists with observing the interactions in functional structures,
244 being somewhat closer to interactions *in vivo* (Marino, Bishop, de Ridder et al., 2019,Paschos,
245 Brown, Eswaramoorthy et al., 2015,Hendriks, Riesle and van Blitterswijk, 2007). Dodson, and
246 his coworkers (1997) discovered that co-culture systems are applicable for the study of obesity
247 in humans. They developed a defined system from myogenic satellite cells and muscle-derived
248 preadipocytes to examine soluble factors involved in their communication (Dodson, Vierck,
249 Hossner et al., 1997). A recent investigation by Hao and her team, established a 3T3-L1 cell
250 co-culture system with human prostate cancer cells to determine the inhibition ability of
251 arctigenin as an effective agent that can co-target obesity in obese-related prostate cancer (Hao,
252 Diaz, del Rio Verduzco et al., 2020). According to another study, a co-cultured system of
253 differentiated 3T3-L1 and RAW264.7 cells was used to study the mechanism of macrophage-
254 adipocytes interaction in innate and adaptive immunity (Lu, Ma, Zhao et al., 2020).

255 This 3T3-L1 co-culture system was also used to screen plant-derived components for
256 biological activity. For example, Brassinin (BR) was recognized for inhibition of obesity-
257 induced inflammation via Nrf2-HO-1 signaling pathway (Kang, Kim, Hwang et al., 2019). The

258 3T3-L1 cell line was also used to further evaluate the effects of saponin fraction from red
259 ginseng on treatment of obesity through a co-culture system (Kim, Kang, Suh et al., 2018).
260 Hence, evidence suggest that the use of 3T3-L1 cell line in a co-culture system has been proven
261 effective for its effects on anti-obesity and obesity related inflammations.

262 Two-dimensional (2D) cell culture techniques usually require low-cost maintenance and
263 are easily manipulated according to their conditions for cell growth. Yet, the tissues of the *in*
264 *vivo* micro-environment are not fully duplicated through this approach. Therefore, three-
265 dimensional (3D) cell culture systems were introduced to overcome the limitation of the 2D
266 culture method (Marino et al., 2019). A scaffold-free method has now been used to generate
267 3D adipose spheroids from primary, immortal human and 3T3-L1 preadipocyte cells,
268 developed by Turner, Tang, Weiss and Janorkar (Turner, Tang, Weiss et al., 2015)**.** This
269 demonstrates that 3D culture of 3T3-L1 cells can effectively identify new biomarkers and
270 effective therapeutics.

271 Many researchers have determined that co-culture systems better mimic the *in vivo* tissue
272 microenvironment of cell morphology and structural complexity in a 3D platform, as well as
273 biological processes and functions, such as proliferation, differentiation and gene or protein
274 expression. A recent high-throughput proteomic analysis of 3D co-cultured system of 3T3-L1
275 cells was utilized to explore the differential protein expression between 2D and 3D co-cultured
276 system using the iTRAQ-bases technique. This encouraged development of an insulin resistant
277 model that produced an *in vitro* obesity model identical to the conditions of *in vivo*,
278 considering the mechanisms underpinning metabolic syndromes (Lee, Park, Kim et al., 2019),
279 consequently introducing new ways by utilizing 3T3-L1 cells to tackle obesity and its related-
280 metabolic disorders.

281 In recent years, an integrated AT on-chip nano-plasmonic biosensing platform to
282 investigate obesity-associated inflammation was developed using 3T3-L1 cells. The system
283 was created for drug-efficacy screening and as a prognostic tool to create personalized
284 treatment plans for risk prevention against obesity (Zhu, He, Verano et al., 2018). These cells
285 were also served as a model system to develop a visual difference mapping (VDM) platform,
286 a new method used to determine the program of adipogenesis. The system analyses the
287 conversion process of fibroblast-like cells into a rounded shape with formation of lipid droplets
288 (Lustig, Feng, Payan et al., 2019). These advanced techniques and high-throughput screening

289 could assist in finding effective and potent therapies to combat obesity and its related metabolic
290 disorders.

291 In summary, the 3T3-L1 cell line has been used extensively in the last 50 years in
292 lipogenesis and lipolysis research due to its abundant supply of homogeneous cells through
293 culture, making it a good model to screen compounds for their potential antilipolytic effects
294 (Pereira-Fernandes, Vanparys, Vergauwen et al., 2014). These preadipocyte cells are also
295 suitable models to study molecular mechanisms and transcription factors in the adipogenesis
296 process due to their adherent properties (Poulos, Dodson and Hausman, 2010). 3T3-L1 cells
297 are ideal for the study of long-term regulation of adipocyte functions as they provide a
298 monolayer culture of newly differentiated fat cells (Adler-Wailes, Guiney, Wolins et al., 2010).
299 Though some research suggests that 3T3-L1 cells have low differentiation efficiency if they
300 are repeatedly thawed from liquid nitrogen (Zhao et al., 2019). In addition, the cells are unable
301 to differentiate robustly into adipocytes if they become confluent and are passaged extensively
302 (Wolins et al., 2006,Hock, 2016,Hernández-Mosqueira, Velez-delValle and Kuri-Harcuch,
303 2015). Hence, culturing 3T3-L1 cells can become demanding and limit utility in a generation
304 of stable cell lines. Nonetheless, extensive research demonstrates that 3T3-L1 cells are most
305 effective, with significantly lower-costs than other mature adipocytes cell line models.

306 **Table 2: List of proteins investigated for their adipogenic effects using 3T3-L1 *in vitro* cell**
307 **model (↑ Increased; ↓ Decreased)**

No.	Protein	Description	Mechanism	Comments	Reference
1.	Matrix Gla protein (MGP)	Vitamin K-dependent protein	↓ CEBPα ↓ FABP4	Involved in fat metabolism and novel serum marker	(Li et al., 2020)
2.	Sirtuin 6 (SIRT6)	Stress responsive protein deacetylase	↑ Glucose uptakes ↓ Insulin resistance	Controls insulin resistance and glucose update	(Tang and Fan, 2019)
3.	LIGHT/TNFSF14	Tumor necrosis factor superfamily protein 14	↓ UCP1 ↓ PPARγ ↓ PRDM16	Involves in beige fat biogenesis	(Kou et al., 2019)
4.	Liver kinase B1 (LKB1)	Serine/threonine protein kinase	↑ UCP1 ↑ PGC-1α ↑ PRDM16 ↑ PLIN	Browning of white adipocytes and increases lipid metabolism	(Xi et al., 2019)
5.	WW domain containing E3 ubiquitin protein ligase 1 (WWP1)	HECT E3 ubiquitin ligases	↓ Oxidative stress	Protective role in oxidative stress in WAT	(Kobayashi et al., 2019)
6.	Protein kinase D1 (PKD1)	G protein-coupled receptor	↓ C/EBPα ↓ C/EBPδ	Deletion of PKD1 improve insulin sensitivity and reduced liver steatosis	(Löffler, Mayer, Viera et al., 2018)
7.	Polymerase I and transcription release factor (PTRF)	Intracellular protein	↑ Hypertrophy ↑ Senescence	Behave as an adipokine and detrimental effects	(Perez-Diaz, Garcia-Sobreviela,

				in visceral fat accumulation	Gonzalez-Irazabal et al., 2018)
8.	Reticulon 3 (RTN_3)	Endoplasmic reticulum protein	↑ SREBP-1c ↑ AMPK activity	Induced obesity and increased hypertriglyceridemia	(Xiang, Fan, Huang et al., 2018)
9.	Erb-B2 Receptor Tyrosine Kinase 4 (ErbB4)	Epidermal growth factor (EGF) receptor family	↑ Inflammation ↑ Subcutaneous and visceral fat	ErbB4 deletion involved in metabolic syndrome	(Zeng, Wang, Kloepfer et al., 2018)
10.	Neuregulin-4 (Nrg4)	EGF family of proteins	↑ Angiogenesis	Disruption of Nrg4 decreased in obesity	(Nugroho, Ikeda, Barinda et al., 2018)
11.	S100A4	S100 calcium-binding protein family	↓ Inflammation ↑ Akt signaling	Inhibit adipogenesis and reduced inflammation factors	(Hou, Jiao, Yuan et al., 2018)
12.	Pentraxin 3 (PTX_3)	Long pentraxin protein family	↑ NPY/NPYR ↑ Oxidative stress	Involved in development of obesity	(Chang, Shin, Choi et al., 2018)
13.	Tyrphostin-AG17	Reversible Inhibitor of epidermal growth factor	↑ Adipocytes apoptosis by activating caspase-3	Anti-obesity effect	(Camacho et al., 2018)
14.	CD38	Type II transmembrane glycoprotein	↓ PPARγ ↓ aP2 ↓ C/EBPα ↓ SREBP1-c ↓ FAS	CD38 deficiency impairs adipogenesis and lipogenesis	(Wang, Miao, Wang et al., 2018)
15.	Pigment epithelium-derived factor (PEDF)	Serine protease inhibitor glycoprotein	↑ CD36	Negatively regulates the adipogenesis	(Huang et al., 2018)
16.	9-PAHSA	Endogenous mammalian lipid	↑ UCP1 ↑ PGC-1α ↑ PRDM16 ↑ C/EBPβ	Browning effects, anti-inflammatory and anti-obesity effects	(Wang, Liu and Fang, 2018)

308

309 **Table 3: List of compounds, structure ID and possible mechanisms investigated in 3T3-**
310 **L1 preadipocyte cell line (↑ Increased; ↓ Decreased)**

No.	Agents	Structure ID	Description	Mechanism	Comments	Reference
1.	2,6-Dimethoxy-1,4-benzoquinone (DMBQ)	PubChem CID:68262	Present in fermented wheat germ	↓ CEBPα ↓ aP2 ↓ FAS ↓ PPARγ ↑ AMPK signaling ↓ SREBP-1c	Suppressed adipogenesis	(Son, Jang, Jung et al., 2019)
2.	Acrylamide (ACR)	PubChem CID:6579	Present in starch-rich food	↓ AMPK signaling ↑ PPARγ ↑ C/EBPα ↑ aP2 ↑ SREBP-1c ↑ FAS	Upregulated of adipogenesis	(Lee, Kim, Choi et al., 2019)
3.	Salt	PubChem CID:5234		↑ PPARγ ↑ C/EBPα	High salt increased adipogenesis and	(Lee, Sorn, Lee

				↑ SREBP-1c ↑ ACC ↑ FAS ↑ aP2	contribute in obesity	et al., 2019)
4.	Adenanthin	PubChem CID:15011073	A natural *ent*-kaurane diterpenoid extracted from the herb *Isodon adenantha*	↓ PPARγ ↓ FABP4 ↓ C/EBPβ	Anti-adipogenic effect	(Hu, Li, Tian et al., 2019)
5.	Vitexin	PubChem CID:5280441	Flavone obtained from *Crataegus pinnatifida* (hawthorn leaf)	↑ AMPK signaling ↓ C/EBPα ↓ FAS	Anti-adipogenic effect	(Peng, Sun, Xu et al., 2019)
6.	α, β-Amyrin	PubChem CID: 73170 and 73145	Triterpenoids isolated from *Protium heptaphyllum*	↓ PPARγ ↓ C/EBPα ↓ GLUT4	Anti-adipogenic effect	(de Melo, de Oliveira, Silva et al., 2019)
7.	Platycodin D (PD)	PubChem CID:162859	Active compound of *Platycodi radix*	↓ PPARγ ↓ C/EBPα ↑ UCP1 ↑ PGC-1α ↑ AMPK signaling	Anti-adipogenic effects and thermogenic actions	(Kim, Park, Jung et al., 2019)
8.	Oxyresveratrol	PubChem CID:5321884	Present in mulberry twigs and fruits (*Morus alba L.*)	↑ UCP1 ↑ Foxo3a	Increased energy expenditure through thermogenesis	(Choi, Song, Lee et al., 2019)
9.	7-Hydroxymatairesinol (7-HMR)	PubChem CID:45273284	7-HMR is Plant lignan	↓ PPARγ ↓ C/EBPα ↓ aP2	Inhibit adipogenesis and lipid uptake	(Biasiotto, Zanella, Predolini et al., 2018)
10.	2-bromo-4′-methoxychalcone (compound 5) and 2-iodo-4′-methoxychalcone (compound 6)	PubChem CID:11173046 (compound 6)	Synthetic halogen containing chalcone derivatives	↑ AMPK signaling ↑ ACC	Anti-obesity effect	(Hsieh, Chang, Tsai et al., 2018)
11.	Bisphenol-A (BPA)	PubChem CID:6623	Lipophilic compound, used in the manufacture of plastic items	↓ Adipocytes marker ↑ IL-6 ↑ TNFα	Increased inflammation and contribute in obesity	(De Filippis et al., 2018)
12.	Trans-1-methyoxy-4-propenyl-benzene (Trans-anethole)	PubChem CID:637563	Flavoring substance present in the essential oils of various plants	↑ PGC-1α ↑ PRDM16 ↑ UCP1 ↓ C/EBPα ↓ PPARα ↓ PPARγ ↓ FAS ↓ ACC ↑ HSL ↑ ATGL ↑ AMPK signaling	Induced white fat browning and anti-adipogenic effect	(Kang, Mukherjee, Min et al., 2018)
13.	Plumbagin	PubChem CID:10205	Naphthoquinone found in roots of *Plumbago zeylanica*	↓ Triglycerides content	Anti-adipogenic effect	(Pai, Martis, Joshi et al., 2018)
14.	Polychlorinated biphenyls 138 (PCBs)	PubChem CID:35823	Persistent organic pollutants (POPs) present in environment	↓ TNFα ↑ Survivin ↑ PLIN	Increased lipid droplets and induction of obesity	(Kim et al., 2018)

15.	Ginsenosides Rg1	PubChem CID:441923	Saponins present in leaves of *Panax quinquefoliu*	↓ PPARγ ↓ C/EBPα ↓ SERBP-1c ↓ FAS ↓ FABP4 ↑ AMPK/ACC signaling	Inhibiting lipogenesis and anti-adipogenic effect	(Liu, Wang, Liu et al., 2018)
16.	Epigallocatechin-3-gallate (EGCG)	PubChem CID:65064	Polyphenol catechin present in Green tea	↑ AMPK signaling ↑ UCP1 ↓ ACC ↓ PPARγ ↓ C/EBPα ↓ SERBP-1c ↓ FAS	Suppressed adipogenesis in white adipocytes	(Mi, Liu, Tian et al., 2018)

311

312 ## 2.2 3T3-F442A Cell Line

313 In 1976, Green developed 3T3-F442A cells from Swiss 3T3 embryonic fibroblasts. The
314 cells were isolated from the 3T3 clone-18 line that converted into fat cell clusters at a high
315 frequency, and increased size as compared to the 3T3-L1 cell line (Green and Kehinde,
316 1976,Sadie-Van Gijsen, 2019). This demonstrates the cells' ability to develop morphological
317 characteristics of mature adipocytes both *in vitro* and *in vivo* considering their spherical shape,
318 increased lipogenic activity, accumulation of triglycerides, and adipocyte-specific marker
319 expression. The reliability of this preadipocyte model was first tested on the nude mouse by
320 subcutaneously injecting cells that produced ectopic fat which became histologically (Green
321 and Kehinde, 1979) and biochemically (Mandrup, Loftus, MacDougald et al., 1997)
322 indistinguishable from the host normal AT. Thereby, suggesting that the studies of the adipose
323 conversion and its regulation on this model is supported hugely by the cell behavior in animals.

324 3T3-F442A cells generally require treatment of differentiating agents such as insulin and
325 FBS to undergo adipose differentiation, which typically suggest high adipogenic activity (Kuri-
326 Harcuch and Green, 1978). Though some researchers have also used triiodothyronine (T3),
327 IBMX and DEX agents (Hemmeryckx et al., 2019). For differentiation, the preadipocyte cells
328 are cultivated and maintained in a high basal medium containing DMEM supplemented with
329 FBS and antibiotics. Evidence suggests that differentiation can be prevented by maintaining
330 the cells at their pre-confluency stage. Once the cells reach confluency, the culture medium is
331 exchanged for 48 hours with an induction medium. Thereafter, it is substituted with DMEM
332 medium containing only FBS, for up to a week, replacing the media every 48 hours. Over an
333 approximate period of 2 weeks, the cells will differentiate into adipocytes, which can be

334 confirmed by quantification of lipid containing cells and cell viability assay (Figure 2-B)
335 (Hemmeryckx et al., 2019,Khalilpourfarshbafi, Murugan, Sattar et al., 2019).

336 Interestingly, defined small molecules can be used to induce commitment into
337 adipocytes, thus this cell line has become a valuable tool to understand some of the mechanisms
338 involved in the early stages of differentiation. For example, adipose differentiation of 3T3-
339 F442A cells occurred rapidly when in the presence of low amounts of staurosporine, a selective
340 serine–threonine kinase inhibitor, and absence of other adipogenic factors (Ayala-Sumuano,
341 Velez-Del Valle, Beltrán-Langarica et al., 2008). Two stages were identified in early
342 adipogenesis. In the first stage, staurosporine administration lasts for up to 4 hours in which
343 GSK3β is activated. The second stabilization stage continues through from 4 to 48 hours after
344 removal of staurosporine from the culture medium. The cells then enter into the clonal
345 expansion stage and express the adipose specific phenotype (Ayala-Sumuano, Velez-delValle,
346 Beltrán-Langarica et al., 2011,Diaz-Velasquez, Castro-Muñozledo and Kuri-Harcuch, 2008).
347 Identifying these stages of early adipogenesis has assisted with study of the early molecular
348 events that regulate the induction and stabilization stages of adipogenesis process in further
349 detail, including the participating genes and the effect of different compounds on these
350 processes.

351 Q. Q. Tang and Lane introduced an analog of staurosporine, stauprimide, also likely to
352 activate the GSK3β to mediate adipose differentiation action in 3T3- F442A (Tang and Lane,
353 2012). A debate on the action of DEX found that it enhanced 3T3-F442A cell differentiation
354 when it was induced by staurosporine, but caused impairment in lipid metabolism (Ayala-
355 Sumuano, Velez-delValle, Beltrán-Langarica et al., 2013). As a result, it was concluded that
356 DEX may have a complex dual role in the impairment of AT homeostasis because it stimulates
357 the differentiation process of preadipocytes. However, it also alters the lipid metabolism and
358 insulin sensitivity of differentiated fat cells (Ayala-Sumuano et al., 2013). Thus, it has been
359 acknowledged through the 3T3-F442A model that the actions of DEX may impair lipid
360 homeostasis, induce insulin resistance in the organism and cause obesity. These dual actions
361 explain the effects of DEX *in vivo*, along with several other studies that suggest high
362 glucocorticoid levels could cause metabolic syndrome (Masuzaki, Paterson, Shinyama et al.,
363 2001).

364 Farnesol is another inducer that can cause differentiation in 3T3-F442A cells. It is a
365 mevalonate-derived inducer of adipocyte differentiation that also plays the role of an insulin

366 sensitizer (Torabi and Mo, 2016). This cell line also has the capacity to differentiate into
367 osteoblasts due to action of bone morphogenetic protein (BMP) and retinoic acid (RA) that
368 stimulates cell proliferation, represses adipogenesis, and promotes osteoblast formation
369 (Skillington, Choy and Derynck, 2002). In support of this notion, exposure to a specific inducer
370 can cause these cells to oblige to either adipocytes or osteoblasts.

371 3T3-F442A cells are less commonly utilized for adipogenic study, compared to 3T3-L1
372 cells, considering they have minimal differences between their differentiation protocols. 3T3-
373 F442A cells are used to test compounds to evaluate their potential effects on adipogenesis and
374 their underlying mechanisms. Tocotrienols, specific components in vitamin E family, showed
375 inhibition in differentiation of 3T3-F442A preadipocytes. The compounds reduced triglyceride
376 contents by decreasing glucose uptake and lowering the amount of GLUT4 and HMG-CoA
377 reductase proteins. Consequently, tocotrienols could inhibit adipocyte differentiation and
378 enhance energy expenditure which can render this class of vitamins useful in creation of novel
379 dietary approaches for prevention and treatment of obesity and diabetes (Torabi, Yeganehjoo,
380 Shen et al., 2016). Alongside, the 3T3-F442A cell line and *in vivo* mice models were used to
381 examine drug supplements such as OBEX or pterostilbene to evaluate their potential anti-
382 adipogenic effects. Both drugs proved effective in reduction of adiposity in mice and in
383 downregulation of key adipogenesis transcriptional factors (Carreira, Andrade, Gonzalez-
384 Izquierdo et al., 2018,Gomez-Zorita, Belles, Briot et al., 2017).

385 Many studies have been conducted on 3T3-F442A preadipocytes clonal cells to examine
386 the potential effects of peptides and their possible molecular mechanisms in adipogenesis. For
387 example, Egg white hydrolysate (EWH) was explored for differentiation, insulin signaling and
388 inflammatory effects, which indicated that EWH encouraged adipocyte differentiation by
389 combining insulin mimetic and insulin sensitizing actions on 3T3-F442A cells. Treatment with
390 EWH also resulted in increased expression of adiponectin and suppressed cytokine mediated
391 inflammatory response in these cells (Jahandideh, Chakrabarti, Davidge et al., 2017).

392 Importantly, 3T3-F442A cells have facilitated the study of transcriptional factors
393 involved in the differentiation process of adipocytes. For example, cytoglobin (Cygb) is a
394 hexacoordinated haemoglobin protein, which when overexpressed in preadipocytes cells
395 contributes to adipogenic differentiation as validated by higher lipid droplets and increased
396 PPARγ, CEBPα and FABP4 expressions (Doğan, Demirci, Kıratlı et al., 2017). A few other
397 proteins like Wnt-1 inducible signaling pathway protein-1 (WISP1) and intestinal

398 chemosensory signaling proteins when investigated found reduced adipocytes differentiation
399 in 3T3-F442A cells and therapeutic ability in obesity related diseases (Ferrand, Béreziat,
400 Moldes et al., 2017,Avau, Bauters, Steensels et al., 2015).

401 3T3-F442A cells can also be used to screen the effects of crude extracts from natural
402 sources in aim of isolating and identifying active substances such as grape powder extract
403 (GPE), which have been clarified for their risk/benefit in obesity and insulin resistance. Results
404 revealed that polyphenolic extract induces browning of adipogenesis through increased glucose
405 uptake and upregulation of AMPK signaling that upregulated energy expenditure and lipolysis
406 in 3T3-F442A cells (Torabi and DiMarco, 2016). Hence, identifying the molecular
407 mechanisms in adipogenic differentiation pathway could assist in developing new strategies to
408 diagnose and prevent obesity and related diseases. Table 4 provides information on the
409 compounds and peptides that have been explored for their adipogenic effects in 3T3-F442A
410 cells.

411 As with 3T3-L1, the 3T3-F442A cell line has also been used in co-culture systems to
412 examine the *in vitro* effects that influence the process of adipogenesis (Christiaens, Sujatha,
413 Hellemans et al., 2010). 3D culture of mature adipocytes has been developed within a hydrogel
414 scaffold using 3T3-L1 and 3T3-F442A cell lines with primary human white preadipocytes cells
415 to create a robust adipose 3D model, that caused increase in adipocytes phenotypic and
416 genotypic markers (Louis, Pannetier, Souguir et al., 2017). Although there is limited research
417 related to co-culture and 3D culture of 3T3-F442A cells, this cell line has the capacity for
418 exploration of adipogenesis through these advanced systems. It has further been acknowledged
419 that these cells are also capable of accumulating higher fat than 3T3-L1 cells and develop
420 morphological characteristics of mature white adipocytes both in *in vivo* and *in vitro*,
421 representing 3T3-F442A cell line as a good model for the study of biology of adipogenesis.

422 **Table 4: List of compounds and proteins investigated for adipogenic effects using 3T3-**
423 **F442A cells (↑ Increased; ↓ Decreased)**

No.	Compounds	Description	Mechanism	Comments	Reference
1.	Withaferin A (WFA)	A steroidal lactone derived from *Withania somnifera*	↓ PPARγ ↓ C/EBPα ↓ TNFα ↓ IL-6	Decreased adipogenesis and reduced inflammation	(Khalilpourfarshbafi et al., 2019)
2.	OBEX	Oral nutritional supplement contained many natural antioxidants	↓ PPARγ ↑ UCP1 ↑ PGC-1α ↓ GLUT4 ↓ Adiponectin	Decreased adiposity and increased browning	(Carreira et al., 2018)

3.	Pterostilbene	A naturally oral drug derived stilbenoid	↓ Triglycerides ↓ Glucose incorporation into lipids	Decreased adipocytes differentiation and increased glucose uptake	(Gomez-Zorita et al., 2017)
4.	d-δ-tocotrienol	Present in the vitamin E family	↓ GLUT4 ↓ HMG CoA reductase ↓ Akt protein	Decreased differentiation and enhanced energy expenditure	(Torabi et al., 2016)
5.	Farnesol	A mevalonate-derived sesquiterpene	↑ GLUT4 ↑ PPARγ ↑ FABP4 ↑ Adiponectin	Induced adipocytes differentiation and insulin sensitizer	(Torabi and Mo, 2016)
6.	Egg white hydrolysate (EWH)	Dietary protein	↑ Adiponectin ↑ PPARγ ↑ C/EBPα ↑ Akt phosphorylation ↓ COX-2	Promoted differentiation and reduced cytokine induced inflammation	(Jahandideh et al., 2017)
7.	Wnt1 inducible signaling pathway protein-1 (WISP1)	Member of CCN protein family	↓ PPARγ ↓ Adiponectin ↓ LPL ↓ FABP4	Novel regulator of adipogenesis	(Ferrand et al., 2017)
8.	Cytoglobin (Cygb)	New globin family member of hexacoordinated protein	↑ PPARγ ↑ C/EBPα ↑ FABP4	Cygb involved in adipogenesis and indicator for obesity	(Doğan et al., 2017)
9.	Gustatory G-protein, gustducin, and bitter taste receptors (TAS2R)	Involves in intestinal chemosensory signaling pathways	↓ Adiposity ↑ UCP1	Inhibited adipogenesis and increased browning	(Avau et al., 2015)
10.	Gelatinase A (MMP-2)	Type IV collagenase known as matrix metalloproteinase-2	↑ Differentiation ↑ Pro-adipogenic marker	Impaired adipogenesis	(Bauters, Scroyen, Van Hul et al., 2015)
11.	Grape powder extracted polyphenols (GPEP)	Grape products are rich in phenolic compounds	↑ FAS ↑ LPL ↑Adiponectin ↑ GLUT4 ↑ AMPK signaling	Increased energy expenditure and lipolysis effects	(Torabi and DiMarco, 2016)

2.3 TA1 Cell Line

A new preadipocyte cell line was introduced in 1984 by Chapman and his colleagues. These TA1 cells were isolated and characterized as stable adipogenic cells extracted by treating a C3H 1OT1/2 mouse embryonic fibroblast with 5-azacytidine, a DNA methylation inhibitor (Chapman, Knight, Dieckmann et al., 1984). TA1 cells express the functional and morphological characteristics of mature adipocytes through the appearance of lipid droplets and adipocyte specific RNAs.

The differentiation of TA1 cells from preadipocytes to adipocytes is dramatically accelarated by the adipogenic agents DEX and insulin (Chapman, Knight and Ringold, 1985). However, in 1987, an anti-inflammatory drug known as indomethacin was recognized as a potent adipogenic inducer that stimulates differentiation in a shorter period of 3 days, with 90% adipocyte capacity in comparison to DEX-treated cells (Knight, Chapman, Navre et al., 1987). TA1 cells are maintained and cultured in 37°C with humidified air of 5% CO_2 with Eagle's basal DMEM medium, FBS and antibiotics, changing the medium every 2 days until the cells reached confluency. To induce differentiation, the cells must be exposed to the adipogenic cocktail for approximately 2 days. For the remaining days, the cells must be first washed with DMEM media and then cultured in DMEM with only fetal serum. Thereafter, the cells should begin to accumulate lipid droplets and reach differentiation approximately 6-10 days after becoming confluent (Figure 2-C) (Shinohara et al., 1992,Ninomiya-Tsuji et al., 1993).

There are very few studies as to the application of this cell line for adipogenesis, yet it holds great potential. Chapman and his colleagues first discovered fat-specific protein-27 (fsp-27) using TA1 cells in 1984. They cloned the protein using a screening approach to identify the cDNAs related to adipocytes differentiation (Chapman et al., 1984). However, it was later characterized by Danesch and his teams that fsp-27 plays a critical role in regulating the key adipogenic transcription of C/EBPs (Danesch, Hoeck and Ringold, 1992,Williams, Chang, Danesch et al., 1992). This cell line was also used to evaluate the mechanisms of lipogenic activity using cachectin factors which showed that cachectin reversely and specifically inhibits the expression of adipose specific genes, thereby immediately inhibiting the lipogenic activity in TA1 preadipocyte cells (Torti, Dieckmann, Beutler et al., 1985).

TA1 preadipocyte cells were treated with the tumor necrosis factors (TNF) to establish their molecular mechanisms of adipogenesis. TNF resulted in reduced expression of several adipose-inducible genes which inhibit and reverse the expression of adipose genes to fully

457 differentiated cells (Torti, Torti, Larrick et al., 1989). In later studies, TA1 cells were examined
458 for the inhibitory role of the cell signaling protein, yielding genetic and pharmacologic
459 evidence that TNF mediates its effects by two distinct or overlapping pathways (Reid, Torti
460 and Ringold, 1989). Further research helped facilitate proto-oncogenes such as *c-fos* and *c-jun*
461 that are transiently induced by TNF in TA1 cells (Haliday, Ramesha and Ringold, 1991).
462 Ninomiya and her team provoked the idea that TNF induced *c-myc* expression in TA1
463 adipocyte cells. They concluded that TNF plays a central role in inhibition and reversal of
464 adipocyte differentiation (Ninomiya-Tsuji et al., 1993).

465 Further studies have been conducted using TA1 cells to examine the cellular effects of
466 chemical agents in the adipose differentiation process. For example, isoproterenol and
467 ractopamine were investigated for lipid metabolism in TA1 preadipocytes. It was found that
468 glycerol release increased and fatty acid synthase activity decreased by these agents in a dose-
469 dependent manner (Weber, Merkel and Bergen, 1992). Finally, the functions of collagens were
470 evaluated using the TA1 preadipocyte cells which demonstrated that the active synthesis of
471 collagens are required in adipose conversion of preadipocytes into adipocytes (Ibrahimi,
472 Bonino, Bardon et al., 1992).

473 TA1 cells have been reported to express more preexisting genes particularly involved
474 in fatty acid and triglyceride synthesis as compared to other preadipocyte cell lines. TA1 cells
475 also display dramatic changes in gene expression and create a large number of new gene
476 products during adipocyte differentiation (Chapman et al., 1984). Subsequently, the qualities
477 of the TA1 cell line make it a suitable model to evaluate the effects of preexisting and new
478 genes that are specifically included in adipogenesis program. Evidence suggests that TA1 cells
479 show adipocyte characteristics, like expressing early adipocyte specific genes within
480 approximately 3 days of reaching its confluency (Torti et al., 1985). Due to this, the cell line
481 may also be used for the identification of early adipogenic markers and their underlying
482 mechanisms in adipogenesis. Additionally, these cells respond to physiological events in *in*
483 *vivo* (RINGOLD, CHAPMAN, KNIGHT et al., 1988). TA1 cells may therefore be a plausible
484 *in vitro* model for the study of lipid metabolism regulation. Furthermore, fully differentiated
485 cells can be used to elucidate the effects of lipolytic and lipogenic agents in adipogenesis
486 (Weber et al., 1992).

487 Interestingly, a recent study comparing 3T3-L1, 3T3-F442A and TA1 cells found that TA1
488 cells expressed higher levels of leptin than that of other cells in adipocyte differentiation

489 (Slieker, Sloop and Surface, 1998). Regardless of the limited studies in the last 20 years,
490 numerous scientists have made insightful discoveries for the understanding of adipose biology
491 using TA1 cells. In summary, this cell line requires further characterization to aid in the
492 understanding of the adipogenesis process, and introduce new discoveries to tackle obesity and
493 its related morbidities.

494 **2.4 AP-18 Cell Line**

495 AP-18 is a another preadipocyte cell line, discovered in 2005. It was developed from the
496 normal AT of an adult female C3H/HeM mouse, specifically derived from the subcutaneous
497 fat of the skin behind its ears (Doi, Masaki, Takahashi et al., 2005). These cells have the ability
498 to accumulate lipids in the form of triglycerides, and they also express characteristics of
499 preadipocyte and mature adipocyte genes.

500 This cell line requires differentiation agents DEX, IBMX and insulin, as with 3T3-L1
501 cells (Chen et al., 2010). The AP-18 cells are suspended in RPMI-1640 media supplemented
502 with FBS, sodium pyruvate, L-glutamine and antibiotics at 37 ˚C and 10% CO_2. After growth,
503 cells are treated with trypsin (0.5%) and diluted to 1:4 in a culture plate. Once the cells reach
504 confluency, the medium is changed to high glucose RPMI-1640 medium with a combination
505 of DEX and IBMX, otherwise insulin alone. Subsequently, the cells must be refreshed every 3
506 days with high glucose RPMI-1640 for the following 7–21 days, wherein they will begin
507 revealing adipocyte characteristics (Figure 2-D) (Chen et al., 2010). Through this protocol, it
508 was discovered that AP-18-cells developed lipid accumulation at a rapid rate when cells were
509 cultured alone, or with a relatively low concentration of insulin. The result is 70 to 90%
510 adipocyte development within 2 to 3 weeks.

511 Notably, when characterized for mRNA profile of adipogenesis, AP-18 cells induced
512 expression of key transcriptional factors for adipocyte differentiation, including C/EBPβ,
513 C/EBPα, PPARγ, aP2 and Adipisin (Doi et al., 2005). A study conducted by C. Chen and her
514 team also confirmed that AP-18 cells differentiate into mature adipocytes by a decrease in
515 expression of preadipocyte factor 1 (Pref-1) and increase in expression of lipoprotein lipase
516 (LPL), retinoid X receptor-α (RXRα), PPARγ, GLUT4, adiponectin, resistin and leptin which
517 have shown similar patterns to 3T3-L1 cells (Chen et al., 2010).

518 Through analysis of this cell line, two apparent advantages were identified from the
519 somewhat minimal research available on AP-18 cells. They hold the ability to grow for many
520 generations or passage in culture at a slow growth rate whilst differentiating into adipocytes
521 without changing their morphology (Doi et al., 2005). Secondly, AP-18 cells have been
522 recognized as a useful model for investigating the mechanism of subcutaneous adipocyte
523 biology because they are one of the preadipocyte cell lines that are derived from a normal AT
524 (Doi et al., 2005). Yet, genes are expressed at a slower and lower rate in comparison to 3T3-
525 L1 cell line. It is also important to note that AP-18 cells have a doubling time of 50-60 hours,

526 in comparison to embryo-derived 3T3-L1 cells, that double in 22 hours (Doi et al., 2005).
527 Nevertheless, Chen Chen and her team concluded that the AP-18 cells represent specific white
528 adipocyte phenotypes under more physiological conditions than 3T3-L1 cells (Chen et al.,
529 2010). Considering that this cell line has not yet been used for any research related to adipocyte
530 differentiation, there is a need for further characterization to assist with developing new targets
531 for obesity treatment through creating an understanding of its prevalence.

2.5 Ob1771 Cell Line

Ob1771 is another established preadipocyte cell line, obtained from subcloned Ob17 cells from the fat pads of a genetically obese C57BL6J mouse. This cell line exhibits a fibroblastic shaped appearance and has shown an exponential growth rate. It has also undergone 35 passages with no detectable changes and has doubling times of 12.5 and 19 hours in 10% and 1% FBS, respectively (Sadie-Van Gijsen, 2019,Negrel, Grimaldi and Ailhaud, 1978).

A standard growth medium supplemented with insulin and T3 is required for adipocyte differentiation of the Ob1771 strain (Abderrahim-Ferkoune et al., 2004). Interestingly, T3 (a thyroid growth hormone necessary for regulating the expression of differentiation-dependent genes) further stimulates the transcription of insulin-like growth factor-I (IGF-I) proteins in this cell line. A combination of IGF-I protein and T3 in medium is required for terminal differentiation of Ob17 preadipocyte cells (Kamai, Mikawa, Endo et al., 1996,Grimaldi, Djian, Negrel et al., 1982). Similar to other preadipocyte cells, Ob1771 cells are also grown and maintained in DMEM supplemented with FBS and antibiotics. After cells become confluent, they are shifted to the differentiation induction medium with insulin and T3, changing the media every other day (Abderrahim-Ferkoune et al., 2004). Within a short period of time, key adipocyte characteristics will appear, including the formation of triacylglycerol and lipid accumulation (Figure 2-E).

These characteristics are closely associated with the appearance of lipolytic and lipogenic enzymes. LPL is an early marker of adipocyte conversion and its expression is dependent upon the growth arrest stage (Amri, Dani, Doglio et al., 1986). Likewise, there is also a late differential marker whose expression is initiated by the accumulation of triacylglycerol (Ibrahimi, Abumrad, Maghareie et al., 1999,Ailhaud, Amri, Bertrand et al., 1990). Hence, expression of both early and late markers in this cell line can help track the process of adipogenesis.

The use of chemically defined molecules has been investigated with these cells, reflecting the line's reliability as a faithful *in vitro* model to investigate factors involved in the chronological events of adipogenesis. For example, a study on the role of spermidine concluded that it effects terminal differentiation of adipose cells and has a permissive effect on growth hormones (Amri, Barbaras, Doglio et al., 1986). Similarly, arachidonic acid is an adipogenic factor that plays a major role in controlling mitosis by increasing intracellular cyclic AMP

564 concentrations and promoting breakdown of inositol phospholipids, subsequently, causing
565 terminal differentiation in Ob1771 cells (Gaillard, Negrel, Lagarde et al., 1989).

566 The Ob1771 cell line assisted in elucidating the effects of small molecules on the
567 adipogenesis phenome. For example, fatty acids were investigated using Ob1771 cells, which
568 revealed their role as signal transducing molecules, suggesting that they are involved in adipose
569 cell differentiation (Amri, Ailhaud and Grimaldi, 1994,Ailhaud, Amri and Grimaldi, 1995).
570 Later in 1997, another intrinsic adipogenic inducer for the Ob1771 cells was discovered,
571 showing that calcitriol (1α, 25-$(OH)_2$ vitamin D3 or VD) was able to trigger the terminal
572 differentiation of the cells when cultured in the presence of thyroid hormone-deprived medium
573 (Dace, Martin-El Yazidi, Bonne et al., 1997).

574 Furthermore, the application of this cell line was used to discover the relationship
575 between obesity and its related diseases. A potential link was recognized between insulin
576 resistance and high blood pressure when investigated using Ob1771 cells, causing increased
577 angiotensinogen secretion in AT, especially of obese subjects (Aubert, Safonova, Negrel et al.,
578 1998). Additionally, extracellular and intracellular signaling pathways are involved in
579 adipocyte differentiation; it was found that leukemia inhibitory factor (LIF) and its receptors
580 are also responsible for early adipogenesis events (Aubert, Dessolin, Belmonte et al., 1999). In
581 a recent investigation, the efficacy of pro-nucleotides (prodrugs) were tested in Ob1771
582 preadipocyte cells. This prodrug was considered useful for blocking obesity and related
583 conditions due to its availability and characteristics, thus providing a potential new therapeutic
584 approach (Laux, Pande, Shoshani et al., 2004).

585 The greatest advantage of Ob1771 is its ability to rapidly multiply its cells as compared to
586 other established preadipocyte cell lines. In context, the cells have a doubling time of 12.5 and
587 19 hours in 10% and 1% FBS, respectively. On the other hand, 3T3-L1 and 3T3-F442A cells
588 from the Swiss mouse have a doubling time of 24 hours in 10% FBS and 100 hours in 1% FBS.
589 Furthermore, some studies suggested that low serum concentrations have a slight effect on
590 Ob1771 cells (Doglio, Dani, Grimaldi et al., 1986); in the absence of added insulin, adipose
591 conversion can occur significantly. Unlike other established preadipocyte cell lines, including
592 3T3-L1 and 3T3-F442A, Ob1771 cells strictly depend on the addition of insulin. Hence, it
593 appears that this cell line is a useful system to study differentiation of adipocyte cells and their
594 growth factor requirements involved in cell multiplication, in contrast to preadipocytes cell line
595 from the non-genetically obese mouse.

596 **2.6 C3H10T1/2 Cell Line**

597 In 1973, Reznikoff et al. discovered C3H10T1/2 cells, a mesenchymal cell line derived
598 from a C3H mouse embryo aged between 14 to 17 days (Reznikoff, Brankow and Heidelberger,
599 1973). In culture, these cells show a fibroblastic-like morphology and present similar
600 functionalities to MSCs (Reznikoff, Bertram, Brankow et al., 1973). This multipotent fibroblast
601 cell line can present several new types of cells such as adipose, muscles, bone and cartilage
602 tissues when treated with an inhibitor of DNA methylation (Taylor and Jones, 1979).

603 The transformation of C3H10T1/2 cells into mature adipocytes requires adipogenic
604 agents such as IBMX, DEX and insulin which have proven sufficient to induce differentiation
605 within 12 days (Schwind et al., 2017). Troglitazone or rosiglitazone can also be used to cause
606 differentiation (Hussain et al., 2020). In a culture containing DMEM media and heat-
607 inactivated FBS, L-glutamate and antibiotics, C3H10T1/2 cells are cultivated at 37°C in a
608 humidified atmosphere of 5% CO_2. Media must be replaced every 3 days until confluency is
609 reached. The cells are then induced for adipocyte differentiation in DMEM supplemented with
610 an adipogenic cocktail for 2-3 more days. Eventually they start revealing their adipocyte
611 characteristics, but until then, they must be maintained in the same culture (Figure 3-A) (Haider
612 and Larose, 2020,Moseti, Regassa, Chen et al., 2020). Additionally, BMP4, a member of the
613 transforming growth factor type β superfamily is able to induce commitment of C3H10T1/2
614 cells to preadipocytes which develop into cells of the adipocyte phenotype when subjected to
615 an adipocyte differentiation protocol (Tang, Otto and Lane, 2004).

616 Through investigation on *in vitro* cell models, several pharmacological studies were
617 conducted which assisted with the development of anti-obesity drugs from natural resources
618 that aim to induce weight loss and reduce fat accumulation. This suggests that use of
619 C3H10T1/2 cells has mainly focused on evaluation of the anti-adipogenic effects of phytogenic
620 compounds, and determination of their role and functions in the adipogenesis processes. For
621 example, oxyresveratrol, a natural compound, and pyrvinium, an anthelminthic drug have
622 showed anti-adipogenic properties in C3H10T1/2 cells (Choi et al., 2019,Wang, Dai, Luo et
623 al., 2019). Similarly, the anti-adipogenic effects of hybrid molecules i.e. triazole and indole
624 derivates were investigated in both *in vitro* C3H10T1/2 and *in vivo* Syrian golden hamster
625 model (Rajan, Puri, Kumar et al., 2018). The cell line also allows measurement of negative
626 effects, ultimately contributing to the development of obese conditions including bisphenol-A
627 (BPA) and benzyl butyl phthalate (BBP) (De Filippis et al., 2018,Zhang and Choudhury, 2017).

628 C3H10T1/2 cells were also used to determine the effects of proteins and their related
629 genes in adipocyte development to create an effective strategy to combat abnormal
630 adipogenesis and related metabolic conditions. For instance, this cell line was used to
631 understand the mechanism underlying the protective role of taurine, a non-proteinogenic amino
632 acid proven useful in improving obesity by mediating the browning of WAT and activating the
633 AMPK pathway (Guo, Li, Peng et al., 2019). An earlier study on adiponectin receptor agonist,
634 AdipoRon in these cells was found to downregulate the expression of adipogenic transcription
635 factors and adipocyte-specific genes by promoting the phosphorylation of AMPK (Wang, Lu
636 and Liu, 2017).

637 It is vital to understand the importance of regular adipocyte proteins in the process of
638 adipogenesis in order to find remedies for obesity related disorders. Hence, neprilysin (NEP),
639 ahnak and CD38 were investigated in C3H10T1/2 cells to explore their role and functions in
640 adipogenesis (Wang et al., 2018,Kim, Han, Byun et al., 2017,Shin, Seong and Bae, 2016). This
641 cell line was also used to investigate the regulatory effects of long chain non-coding RNA
642 (lncRNA) in obesity and adipogenic differentiation. According to another investigation, novel
643 treatments for obesity found lncRNA *Plnc1* controls adipocyte differentiation by regulating
644 PPARγ (Zhu, Zhang, Li et al., 2018). Screening crude extracts from medicinal plants is a good
645 strategy to discover anti-obesity drugs as it can help derive potential anti-obesity compounds.
646 Hence, C3H10T1/2 cells were used to uncover the effects of natural plant extracts, as detailed
647 in the table provided below (Table 5). In conclusion, we believe that such discoveries will
648 support the potential benefits of novel anti-adipogenic and anti-lipogenic agents in future
649 clinical studies. Table 5 presents the detailed information of compounds, proteins and extracts
650 investigated in C3H10T1/2 cells.

651 The C3H10T1/2 cell line is beneficial as it maintains a stable morphology even after long
652 periods in culture. Another advantage of this mouse embryo cell line is the ability to examine
653 the molecular genetic regulation of both the developmental determination of vertebrate stem
654 cell lineages and their subsequent differentiation. Nonetheless, it acts as a good model to
655 understand the events responsible for 10T1/2 lineage determination, a simple genetic control
656 that mediates the formation of myogenic, chondrogenic and adipogenic lineages.

657 **Table 5: List of compounds, proteins and extracts which is investigated in C3H10T1/2**
658 **cells (↑ Increased; ↓ Decreased)**

No.	Compounds	Description	Mechanism	Comments	Reference

1.	25-Hydroxycholesterol	Specific oxysterol	↓ PPARγ ↓ C/EBPα ↓ FABP4	Inhibitory effect on adipogenesis	(Moseti et al., 2020)
2.	Pyrvinium	Classical anthelminthic drug	↓ C/EBPα ↓ PPARγ	Suppressed adipogenic differentiation	(Wang et al., 2019)
3.	Oxyresveratrol	Stilbenoid present in mulberry twigs and fruits (*Morus alba L.*)	↑ UCP1 ↑ Foxo3a	Increased energy expenditure through browning	(Choi et al., 2019)
4.	Bisphenol-A (BPA)	Lipophilic compound, used in the manufacture of plastic items	↓ PPARγ ↓ C/EBPα ↓ FABP4 ↓ FASN ↑ IL-6 ↑ TNFα	Increased inflammation and contributed in obesity	(De Filippis et al., 2018)
5.	Medicarpin (Med)	Natural pterocarpan involves in various beneficial biological roles	↑ PRDM16 ↑ PGC-1α ↑ UCP1 ↑ AMPK pathway	Promoted lipolysis activity	(Imran, Yoon, Lee et al., 2018)
6.	Licarin A (LA)	Obtained from Mexican medicinal plant *Aristolochia taliscana*	↑ PGC-1α ↑ PRDM16 ↑ UCP1	Induced browning and lipolytic effects	(Yoon, Imran and Kim, 2018)
7.	Cryptotanshinone (CT)	Natural compound from *Salvia miltiorrhiza* plant	↑ UCP1 ↑ PRDM16 ↑ PFC-1α ↑ AMPK	Activated browning of white adipocytes	(Imran, Rahman, Yoon et al., 2017)
8.	Triazole and Indole derivates (Hybrid molecules)		↓ PPARγ ↓ C/EBPα ↑ Wnt3a/β pathway	Ameliorated dyslipidemia and high anti-adipogenic effect	(Rajan et al., 2018)
9.	Protocatechuic acid (PCA)	A catechol-type *O*-diphenol phenolic acid (3,4-dihydroxybenzoic acid) present in plants	↑ RUNX2 ↓ PPARγ ↓ C/EBPα ↓ aP2 ↑ Wnt3a/β-catenin	Alleviated osteogenic differentiation and reduced adipocytes differentiation	(Rivera-Piza, An, Kim et al., 2017)
10.	Benzyl butyl phthalate (BBP)	An endocrine disrupting chemical (EDC)	↑ aP2 ↑ PPARγ ↓ Sirt1 ↓ PGC-1α	Contributed in obesity development	(Zhang and Choudhury, 2017)
11.	Xanthoangelol (XA) and 4-hydroxyderrcin (4-HD)	Chalcones obtained from *Angelica keiskei*	↓ Activator protein 1 (AP1) ↓ c-Jun N-terminal Kinase (JNK) ↓ NF-κB	Reduced inflammation induced by obesity	(Li, Goto, Ikutani et al., 2016)
12.	Artepillin C (ArtC)	Brazilian propolis (a resinous plant-based material)	↑ PPARγ ↑ UCP1 ↑ PRDM16	Induced browning in white adipocytes	(Nishikawa, Aoyama, Kamiya et al., 2016)
13.	Epigallocatechin gallate (EGCG)	Green tea polyphenol component	↓ Cells proliferation and migration ↓ Adipisin	Inhibited differentiation and anti-adipogenic effects	(Chani, Puri, Sobti et al., 2016)
14.	Kinsenoside	Obtained from *Anoectochilus formosanus* plant	↑ AMPK ↑ CPT1 ↑ PGC-1α	Increased browning and enhanced catabolic effects	(Cheng, Wang, Chou et al., 2015)

15.	Taurine	Non-protein amino acid	↑ PGC-1α ↑ UCP ↑ AMPK	Increased browning of white adipocytes	(Guo et al., 2019)
16.	Long noncoding RNA *Plnc1*	Type of non-codding RNA and regulates cell function	↑ PPARγ ↑C/EBPα ↑ aP2	Regulator in adipocyte differentiation	(Zhu et al., 2018)
17.	E1A-stimulated genes 1 (CREG1)	Secreted glycoprotein and involves control of cell growth and differentiation	↑ UCP1	Increased browning of adipogenesis	(Kusudo, Hashimoto, Kataoka et al., 2018)
18.	CD38	Type II transmembrane glycoprotein	↓ PPARγ ↓ AP2 ↓ C/EBPα ↓ SREBP1-c ↓ FASN ↑ Sirt1 signaling	CD38 deficiency impaired adipogenesis and lipogenesis	(Wang et al., 2018)
19.	AdipoRon	Adiponectin receptor agonist	↓ PPARγ ↓ C/EBPβ ↓ C/EBPα ↓ FABP4 ↓ FAS ↑ AMPK pathway ↑ ACC	Inhibitory effect on adipogenesis	(Wang et al., 2017)
20.	Enone fatty acids	Synthesized dietary polyunsaturated fatty acids (PUFAs)	↑ UCP1 ↓ Inflammatory cytokine	Decreased dysfunctions of adipocytes induced inflammation	(Yang, Li, Nishimura et al., 2017)
21.	Neprilysin (NEP)	Integral plasma membrane or zinc metallopeptidase protein	↑ PPARγ ↑ C/EBPα ↑ aP2 ↑ PI3K/Akt signaling	Accelerated adipogenesis	(Kim et al., 2017)
22.	Ahnak	Neuroblastomas or nucleoprotein protein	Smad1-dependent PPARγ expression	Regulated adipocyte differentiation	(Shin et al., 2016)
23.	Cytochrome P450 1B1 (CYP1B1)	Member of the cytochrome P450 superfamily of enzymes	CYP1B1 deficiency (-) ↓ PPARγ ↓ CD36 ↓ FAS ↓ SCD-1 ↑ UCP-2 ↑ CPT-1a ↑ AMPK pathway	CYP1B1 deficiency ameliorated obesity and glucose intolerance	(Liu, Huang, Li et al., 2015)
24.	Erucic acid	Natural extract from Rosemary	↓ PPARγ	Reduced adipogenesis and enhanced osteogenesis	(Takahashi, Dohi, Egashira et al., 2020)
25.	Phytanic acid (PA)	Branched-chain of fatty acid present in dietary food	↑ PGC-1α ↑ PRDM16 ↑ UCP1	Promoted beige adipogenic differentiation	(Wang, Mao and Du, 2019)
26.	Spirulina maxima 70% ethanol extract (SM70EE)	Microalga that is rich in essential nutrients and contains pigment proteins such as chlorophyll a and C-phycocyanin	↓ PPARγ ↓ SREBP1-c ↓ C/EBPα ↓ C/EBPβ ↓ aP2 ↓ FAS	Reduced adipogenesis and activated thermogenesis	(Seo, Kim, Choi et al., 2018)

			↓ ACC ↑ PRDM16 ↑ PGC-1α ↑ UCP1		
27.	Mulberry extract (ME) and Mulberry wine extract (MWE)	Extract of edible fruit of *Morus alba L.*	↑ UCP1 ↑ PGC-1α ↑ PRDM16 ↑ CPT-1	Increased mitochondrial biogenesis by browning	(You, Yuan, Lee et al., 2015)
28.	Peanut sprout extracts (PS)	Peanut extract from *Arachis hypogaea L.*	↑ AMPK ↓ aP2 ↑ PGC-1α ↑ CPT1	Increased fatty acid oxidation and enhanced beige adipogenesis	(Seo, Jo, Kim et al., 2019)

659

660 **2.7 OP9 Cell Line**

661 OP9 is a stromal cell line taken from the calvarial bone marrow of a newborn C57BL/6J
662 and C3H mouse, that is genetically deficient in functional macrophage colony-stimulating
663 factor (M-CSF) (Nakano, Kodama and Honjo, 1994). This cell line is a tractable alternative
664 model system for the study of adipogenesis that shows rapid accumulation of triglyceride
665 droplets within 72 hours of differentiation (Gao, Yan, Li et al., 2010,Lane, Doyle, Fortin et al.,
666 2014). The functionalities of these cells are similar to MSCs as they acquire the ability to
667 support and facilitate the study of molecular mechanisms involved in the development and
668 differentiation of hematopoietic cells. During co-culture with mouse embryonic stem cells (ES
669 cells), OP9 cells are able to induce differentiation of the cells into blood cells of erythroid,
670 myeloid, and B cell lineages.(Gao et al., 2010,Ueno, Sakita-Ishikawa, Morikawa et al., 2003).

671 Wolins and his colleagues described three different methodologies used for
672 differentiation of OP9 cells; serum replacement, insulin oleate, and adipogenic cocktail
673 methods (Wolins et al., 2006). The first step of the serum replacement method (SRM) is to
674 grow the OP9 cells to confluence and then culture them for 2 additional days in propagation
675 medium with α-minimum essential medium eagle (α-MEM), FBS, L-glutamine, and
676 antibiotics. Then, the cells are cultured for a further 4 days in a serum replacement medium
677 containing α-MEM media without FBS and antibiotics. Besides the purpose of studying insulin
678 effects, it is important to change the medium to OP9 propagation medium after day 2 of
679 differentiation, because the media contains high concentrations of insulin which may affect cell
680 morphology (Figure 3-B). The second method is the insulin oleate method (IOM**),** whereby the
681 propagation medium of OP9 cells are replaced with the insulin oleate medium when the cells
682 attach to the plate. They contain α-MEM, FBS, insulin, oleate to albumin (5.5:1 molar
683 ratio), DEX, IBMX, L-glutamine, and antibiotics. As for the adipogenic cocktail method
684 (ACM), the OP9 cells are grown to confluence and then cultured for a further 2 days in
685 adipocyte differentiation medium including DMEM, FBS, L-glutamine and antibiotics. The
686 cells are cultured for 2 days in differentiation medium 1: DMEM, FBS, insulin, DEX, IBMX,
687 L-glutamine and antibiotics. Then again, the cells are cultured for an additional 2 days in
688 differentiation medium 2: DMEM, FBS, insulin, L-glutamine and antibiotics. All three
689 treatment methods require OP9 cells to be maintained in propagation medium until they display
690 the adipocyte morphology and the accumulation of triglycerides and abundant intracellular
691 lipid droplets. This cell line expresses high levels of adipocyte specific markers like PPARγ,

C/EBPα, C/EBPβ and SREBP-1c. Moreover, at its pre-confluent stage, OP9 cells express detectable levels of PPARγ and C/EBPα (Wolins et al., 2006).

OP9 cells are used to identify key regulators and mechanistic in adipocyte related disease conditions for the development of a potential and effective treatment. An example is thymic adiposity, a condition which is characterized by deposition of adipocyte in age-related thymic involution causing detrimental effects on the thymic microenvironments, associated with obesity (Lamas, Lopez, Carrio et al., 2016). Some of the key regulators in thymic adipogenesis were investigated in OP9 cells using a label-free quantitative approach. This proteomic analysis revealed that transforming growth factor β (TGF-β) may have a role in thymic adiposity, indicated by inhibition in OP9-DL1 and primary thymic stromal cells. As a result, it has been acknowledged that activation of TGF-β serves as a useful tool for the prevention of thymic adiposity (Tan, Wang, Wang et al., 2019). A previous study on metabolomic alterations of thymic adipogenesis was tested in OP9 adipogenic differentiation using a liquid chromatography-mass spectrometry technique. The study suggested to address the underlying mechanism in thymic adipogenesis (Tan, Wang, Wang et al., 2017).

Other research on a Ca^{2+} permeable channel known as transient receptor potential mucolipin 1 (TRPML1) showed involvement of intracellular membrane trafficking including lysosomal degradation and lysosomal exocytosis, although its impairment was associated with several pathophysiological conditions including obesity (Dhakal and Lee, 2019). However, when the exact role of TRPML1 was investigated in OP9 cells it was recognized that it has diverse roles. Firstly, TRPML1 is crucial for the differentiation of adipocytes and secondly, it mediates the lipid metabolism by membrane trafficking, exosome formation, and exosomal release. Subsequently, results indicated that TRPML1 is a key factor for treatment of obesity-related diseases (Kim, Muallem, Kim et al., 2019).

This cell line has also been used to identify the underlying mechanism for alcohol-elicited dysfunction of WAT that causes visceral adipose tissue expansion resulting in hypoxia and low inflammation within the tissue, introducing a new mechanism to target in treatment of ethanol-related diabetes (He, Li, Zheng et al., 2015). It is essential to improve methods that detect the events in adipocyte differentiation in order to accelerate our understanding for treatment of metabolic and other disorders involving lipid accumulation. These treatments could potentially become powerful tools for future drug screening and mechanistic studies on adipocytic differentiation. Similarly, a new method was recently developed to study lipid

724 accumulation through refractive index. The robustness of a digital holographic
725 microscopy (DHM) system was tested using OP9 cells. This label-free, non-perturbing method
726 allows detection of lipid droplets in differentiating adipocytes, without the need for washing,
727 staining, or other liquid manipulations (Campos, Rappaz, Kuttler et al., 2018).

728 OP9 mouse stromal cells have also been used to investigate the effects of different
729 compounds and their underlying mechanisms on early and late differentiation of adipogenesis.
730 With regards to drug screening, this cell line has proven a suitable model to evaluate the
731 responses of novel drug therapies (Jiang, Di Wu, Weng et al., 2017). For example, quercetin
732 showed anti-adipogenic and anti-lipolysis effects in OP9 stromal cells, indicating that the
733 compound has potential anti-obesity therapeutic effect by upregulating ATGL and HSL
734 expression and downregulating FAS, LPL and aP2 expression (Seo, Kang, Kim et al., 2015).
735 OP9 cell lines were also used to confirm repression of lipid droplets formation. To which it
736 was revealed that together with low cytotoxicity the extract suppresses adipogenesis associated
737 lipid accumulation during differentiation of OP9 preadipocytes (Kato, Kato, Shibata et al.,
738 2015).

739 OP9 cells in co-culture systems were found useful in evaluation of adipocyte biology.
740 For example, a co-culture system using mouse bone marrow and OP9 cells indicated a novel
741 function in control of hematopoiesis by identifying IL-1 as a therapeutic target for aged and
742 obese individuals (Kennedy and Knight, 2015). Use of the OP9 cell line aims to generate a
743 systemic model that identifies the genes essential for adipogenesis that can also become
744 applicable to high-throughput RNA screening. Such models can help identify novel therapeutic
745 targets and map disease pathways involved in obesity. Subsequently, Lane and her co-workers
746 generated a new clonal population of OP9 cells, known as OP9-K. These cells were able to
747 differentiate rapidly and revealed adipocyte morphology like rounded cell shape, lipid
748 accumulation, and coalescence of lipids into a large droplet. This study contributes to the
749 development of rapid screens that can deepen our understanding of adipose biology and test
750 obesity therapeutics (Lane et al., 2014). Similarly, OP9-DL1 is another cell derived from the
751 OP9 cell line characterized in order to study the commitment, differentiation, and proliferation
752 of T-lineage *in vitro* that ectopically expresses Notch ligand Delta-like 1 (Dll1) by mimicking
753 the thymic microenvironment to support T cell development *in vitro* (Schmitt and Zúñiga-
754 Pflücker, 2002) (Holmes and Zúñiga-Pflücker, 2009).

755 These cells are derived non-clonally, which means that the heterogeneity of cells can
756 be assessed for undergoing adipocytic differentiation (Campos et al., 2018). In addition, OP9
757 cells can be preserved at high density levels without loss of potential to differentiate into
758 adipocytes, even at high passage numbers. Consequently, these features allow the cells to be
759 maintained in culture and within a few days the OP9 adipocytes become available for
760 experiment (Kassotis et al., 2017). OP9 cells are given any one of three adipogenic stimuli,
761 while rapidly accumulating triglycerides, to form numerous large lipid droplets to express
762 adipocyte marker proteins, and assume adipocyte morphology whilst expressing late adipocyte
763 marker proteins. However, not every protocol of OP9 cells may be optimized for adipocyte
764 differentiation and manipulation, and when maintained at low cell density, OP9 cells adopt a
765 spindly morphology and differentiate into adipocytes poorly (Ruiz-Ojeda et al., 2016).

766 Accordingly, OP9 cells can differentiate into adipocytes within a period of 2 days and
767 easily express detectable levels of transcription factors after confluency has been reached
768 (Wolins et al., 2006). To conclude, the OP9 cell line represents a new model to understand the
769 mechanisms of differentiation and investigate the effects of drugs on the biology of adipocytes.
770 It is also useful for fast high-throughput studies such as non-perturbing quantification of lipid
771 droplets and digital holographic microscopy. In summary, the capacity to rapidly differentiate
772 and present several practical features, OP9 cell line is a suitable model for adipocyte studies.

773 **2.8 Mouse embryonic stem cells (mESCs)**

774 Martin, Evans and Kaufman described an alternative model for the study of adipogenesis
775 biology known as mouse embryonic stem cells (mESCs). These proliferating, pluripotent stem
776 cells deliver an unlimited supply of cells which can directly differentiate into adipocytes using
777 a combination of RA and pro-adipogenic agents (Rosen and MacDougald, 2006). The mESCs
778 are extracted from the inner cell mass during the developing blastocyst stage of murine embryo
779 (Evans and Kaufman, 1981,Martin, 1981). They display numerous properties, including a
780 stable and normal diploid karyotype, with the capacity to self-renew indefinitely and have the
781 potential to reconstitute all embryonic lineages. Furthermore, mESCs also have the ability to
782 integrate into an embryo and contribute to all cell lineage, whilst fully participating in fetal
783 development when transplanted back into the mouse blastocyst (Nichols and Smith,
784 2009,Bradley, Evans, Kaufman et al., 1984). They promote proliferation and can be maintained
785 in the presence of LIF to remain in an undifferentiated state (Stavridis and Smith, 2003). Yet,
786 appropriate culture conditions without LIF tend to aggregate into embryoid bodies (EBs),
787 causing differentiation *in vitro* into several derivatives of all ectodermal, mesodermal, and
788 endodermal cells. Consequently, producing all the cell types in the body (Keller, 1995).
789 Nevertheless, mESCs potentially offer a unique *in vitro* cell culture system to study the initial
790 stages of mammalian development.

791 These highly efficient and reliable adipocytes can be differentiated with RA and pro-
792 adipogenic agents like insulin, T_3 and rosiglitazone (PPARγ agonist) (Rosen and MacDougald,
793 2006). Two main phases were recognized in the adipogenesis process of mESCs. The
794 permissive period is the first phase of adipogenesis which requires RA and begins after the
795 formation of EBs, causing commitment of mESC cells. In the second phase, cells are treated
796 with adipogenic agents that cause terminal differentiation of pre-adipocytes into adipocytes
797 and lead to outgrowth with lipid droplet-containing adipose cells (Phillips, Vernochet and Dani,
798 2003). The mESCs are cultured and maintained at 37 °C with 5% to 10% CO_2 in DMEM media
799 on gelatin-coated plates with FBS, LIF, L-glutamate, sodium pyruvate, antibiotics and β-
800 Mercaptoethanol. To induce differentiation, mESCs cells will differentiate in up to 21 days
801 forming a large cluster colony of mature adipocytes, confirmed by staining EBs with Oil Red
802 O stain (refer to Figure 3-C) (Ota et al., 2017,Dani, 1999).

803 This embryonic stem cell line is an invaluable model for the characterization of genes
804 expressed and identification of new adipogenic regulatory genes. In recent years, mESCs have

805 been utilized in understanding of the adipogenesis mechanisms associated with obesity. Chen
806 and his colleagues examined RNA pol III transcripts in mESCs for their functional role,
807 through which they discovered that Maf1 assists in promoting the mesoderm induction and
808 adipocyte differentiation. Conversely, if Maf1 expression decreases in mESCs, then
809 preadipocytes will display impaired adipogenesis; increased expression will enhance
810 differentiation (Chen, Lanz, Walkey et al., 2018). The capacity for adipogenesis is determined
811 using epigenetic regulators: the role of histone H3K27 demethylase encoded by Utx gene**,** has
812 been investigated during the differentiation process of mESCs. Furthermore, results indicate
813 that Utx mediates adipogenesis by regulating *c-myc* in a differentiation stage-specific manner.
814 Targeting Utx signaling pathways is potentially valuable for the treatment of obesity, diabetes,
815 and congenital Utx-deficiency disorders (Ota, Tong, Goto et al., 2017).

816 In 2018, advanced tools for automated cell sorting in lineage analysis were established.
817 Label-free quantitative imaging was used to classify the cell population on intermediate states
818 during the differentiation of mESCs into adipocytes. The methodology was developed to
819 distinguish undifferentiated cells from cells in other stages, and to estimate the optimal number
820 of clusters in differentiated cells (Masia, Glen, Stephens et al., 2018). Other high-throughput
821 techniques were developed by Guerrero-Robles and his team who introduced a new biosensor
822 technique known as electrical bioimpedance spectroscopy (EBIS) using mESCs, MEFs and
823 3T3-L1 cells. This method helped to identify and measure the cell lineage population, cell
824 differentiation and undifferentiation process of adipogenesis (Guerrero-Robles, Vazquez-
825 Zapien, Mata-Miranda et al., 2017).

826 Earlier in 2011, ultrasound standing wave traps (USWT) were used to analyze the gene
827 expression of mESCs. The technique promised safe cell manipulation techniques for a variety
828 of applications, including tissue engineering and regenerative medicine (Bazou, Kearney,
829 Mansergh et al., 2011). mESCs are also used in the bioengineered 3D culture system created
830 by Unser and Mooney et al. to open up a new arena for studying the morphology of brown
831 adipogenesis and its implications in obesity and metabolic disorders (Unser, Mooney, Corr et
832 al., 2016). Previously, 3D culture of mESCs has been developed using electro-spun polymer
833 scaffolds, this *in vitro* 3D model mimicking the *in vivo* environment required to effectively
834 study adipogenesis (Kang, Xie, Powell et al., 2007). These studies promoted the idea that
835 mESCs could be a potential method to understand the process of adipogenesis, especially when
836 integrated with high through-put techniques.

Previous studies with immortalized mouse stromal cell line or other mesenchymal precursor cells isolated from adult tissues have been used to determine mesenchymal cell fate decisions. However, these systems were not found informative with respect to the developmental origin of mesenchymal stem cells and adipocytes (Billon, Kolde, Reimand et al., 2010). Thus, a more suitable source of embryonic cells should be used to address this issue and elucidate the exact pathways and intermediates between the embryonic stem cell and the mature adipocyte. Accordingly, mESCs have the capability to enable exploration of the developmental fate of adipocytes from their gene expression (Billon et al., 2010).

A major limitation of mESCs is the heterogeneity of the culture combined with the low efficiency of the adipocyte differentiation (Schaedlich, Knelangen, Navarrete Santos et al., 2010). It was later discovered that the addition of ascorbic acid (AA) in adipogenic cocktail causes robust and efficient differentiation of mESCs to mature adipocytes (Cuaranta-Monroy, Simandi and Nagy, 2015). In conclusion, this pluripotent stem cell provides a remarkable *in vitro* model to study the genetics and epigenetic mechanisms involved in adipogenesis and could also be promising in areas including anti-obesity drug screening and tissue engineering in order to understand the obesity-related complex diseases.

853 **2.9 Mouse Embryonic Fibroblasts (MEFs)**

854 Mouse Embryonic Fibroblasts (MEFs) are an actively used model in the study of adipose
855 cells. This primary cell line is derived from a pregnant female mouse during 13.5 to 15.5
856 embryonic days by removing the head, limbs, tail and internal organs from the embryos. The
857 remaining minced carcasses are then rinsed with phosphate buffer saline (PBS), and are cut
858 into smaller pieces. These pieces are trypsinized and seeded into a culture medium for single-
859 cell suspension forming them into a largely homogeneous population of cells after a few
860 passages (Singhal, Sassi, Lan et al., 2016,Nagy, Gertsenstein, Vintersten et al., 2006). Primary
861 MEFs have proven useful, but their lifespan is limited. Moreover, the isolation of these
862 fibroblast cells is time-consuming and labour intensive, especially as they take a long time to
863 be prepared for the experiment. After repeated transmissions, the fibroblasts will reach
864 senescence and finally die off (Amand, Hanover and Shiloach, 2016).

865 Researchers have developed immortal MEFs with permanent growth features using two
866 approaches. The first approach is using serial passages of MEF cells and the second approach
867 is the transformation of the primary MEFs by overexpressing oncogenes using viral infections
868 (Xu, 2005). These methods develop an immortalized MEF with the desired genetic
869 manipulations, making MEF cell line maintenance time efficient, with indefinite growth. MEFs
870 are well-known for their use as feeder layers during culture of mESCs as they provide factors
871 that enhance proliferation and maintenance of undifferentiated states (Hogan, Costantini and
872 Lacy, 1986). They also assist with the study of biological properties including cell cycle
873 regulation, immortalization, transformation, senescence, apoptosis and differentiation (Yusuf
874 et al., 2013). In addition, these fibroblast cells can differentiate into adipogenic, chondrogenic,
875 and osteogenic lineages expressing typical differentiation markers (Dastagir, Reimers,
876 Lazaridis et al., 2014).

877 As with other cell lines, MEFs are also cultured and maintained in a growth medium
878 (DMEM media and FBS) at 37 °C and 5% CO_2. To induce differentiation in these cells, cells
879 must reach confluence, after which media is changed to adipocyte induction medium. Once
880 triglyceride concentration is quantified and lipid content is visualized, differentiation can be
881 confirmed by Oil red O staining (refer to Figure 3-D) (Yusuf et al., 2013) (Hou et al., 2020).

882 The advancement of genomic manipulation has assisted with the creation of genetically
883 engineered mice and knockout mice as efficient tools for human disease research, including
884 the discovery, refinement, and utility of many currently available therapeutic

885 regimes. Likewise, the cells isolated from these mutant mice could become powerful tools to
886 study the molecular and cellular mechanisms of mutated genes under well-defined culture
887 conditions (Dobrowolski, Fischer and Naumann, 2018). Marian E Durkin, and her team
888 established a protocol to obtain MEF cells from genetically manipulated mouse embryos. MEF
889 cells obtained through this procedure are suitable for use in biochemical assays and further
890 experiments of genetic manipulation (Durkin, Qian, Popescu et al., 2013). Thus, it was
891 acknowledged that genetically modified MEF cells can be used to better understand the
892 adipogenesis process. Recently, MEF cells were also investigated for the metabolic footprint
893 of early adipocyte commitment. Data indicated that ceramide induced apoptosis is essential in
894 initiating adipogenesis by providing lipophilic components that activate adipogenic
895 transcription factor expression and facilitate lipid droplet formation. Therefore, Sirt1 may
896 target treatment of obesity and other ceramide-associated metabolic syndromes (Hou et al.,
897 2020). In another study, MEF cells derived from *Irx3*-knockout mice were developed to
898 identify the role of Irx3 in beige preadipocyte functions and differentiation. Results suggested
899 that complete loss of Irx3 in MEF cells could lead to reduced cell cycle progression, impaired
900 mitochondrial respiration as well as loss of cell identity and an inability to undergo adipogenic
901 differentiation (Bjune, Dyer, Røsland et al., 2020). Consequently, MEF cells developed from
902 genetically modified or knockout mice are approved to study the effects of different genes in
903 adipogenesis program.

904 Similarly, MEF cells can also assist in evaluating the effects of protein or its gene in
905 adipogenesis in order to create new therapeutic targets to treat obesity and its associated
906 diseases. The 14-3-3ζ scaffold protein was investigated in MEF cells which found the novel
907 adipogenic factors that blocked the obesity-associated expansion of AT (Mugabo, Sadeghi,
908 Fang et al., 2018). Another example is the loss of CD38 expression in MEF cells, which impairs
909 adipogenesis and lipogenesis (Wang et al., 2018). Follistatin (Fst) is a glycoprotein which
910 resulted in increased browning of WAT by increasing UCP1, PRDM16 and PGC-1α expression
911 in MEF cells (Singh, Braga, Reddy et al., 2017), whilst, Pin1 is a peptidylprolyl cis/trans
912 isomerase that was found in enhanced adipocytes differentiation in MEF cells by increased
913 expression of PPARγ and ERK pathway (Han, Lee, Bahn et al., 2016). Meanwhile, Interferon-
914 alpha (IFNα) was found to inhibit adipocyte differentiation at early stages of adipogenesis by
915 decreasing the expression of PPARγ and C/EBPα (Lee, Um, Rhee et al., 2016). Additionally,
916 ADAMTS5, a metalloproteinase superfamily protein has been identified as a promotor of
917 angiogenesis effects in MEF cells as well as *in vivo* (Bauters, Scroyen, Deprez-Poulain et al.,

918 2016). Table 6 presents a list of proteins and their identified mechanism in MEFs cell in term
919 of adipogenesis.

920 These cell lines were also integrated with high throughput techniques to identify and
921 characterize diverse cells types and the cell differentiation process in adipogenesis. For
922 example, EBIS was developed to identify populations of undifferentiated mESCs, MEFs and
923 the differentiation process from preadipocytes (3T3-L1) to mature adipocytes (Guerrero-
924 Robles et al., 2017). As with primary cultures, MEFs also have certain limitations due to their
925 origin. Hence, the cellular heterogeneity of embryonic tissue and the culture of these cells often
926 presents difficulties. However, a few steps could ensure a greater degree of homogeneity
927 (Garfield, 2010).

928 Several studies revealed that fibroblast cells were identical to MSCs, and can
929 differentiate into bone, fat and cartilage cells. In particular, MEFs are easily established and
930 maintained, they proliferate rapidly and a can provide a large number of cells from a single
931 embryo (Yusuf et al., 2013). Moreover, immortalized MEFs have the potential to expand
932 through several passages. Accordingly, these properties make MEF an attractive cell culture
933 model to further explore and create a better understanding of adipogenesis.

934 **Table 6: List of compounds, protein and their mechanism investigated in MEF cells (↑**
935 **Increased; ↓ Decreased)**

No.	Compound	Description	Mechanism	Comments	Reference
1.	RepSox	Inhibitor of transforming growth factor-beta receptor I (TGF-β-RI)	↑ UCP1 ↑ PRDM16 ↑ PGC-1α	Induced browning of adipogenesis	(Tu, Fu and Xie, 2019)
2.	2,6-Dimethoxy-1,4-benzoquinone (DMBQ)	Present in fermented wheat germ	↓ PPARγ ↓ C/EBPα ↓ FAS ↓ aP2 ↑ AMPK signaling	Decreased adipogenesis	(Son et al., 2019)
3.	Adenanthin	Natural *ent*-kaurane diterpenoid from *Isodon adenantha*	↓ PPARγ ↓ FABP4 ↓ C/EBPβ	Anti-obesity effects	(Hu et al., 2019)
4.	Di-2-ethylhexyl phthalate (DEHP)	A ubiquitous plasticizer and Endocrine disruptor chemical (EDC)	↑ PPARγ	Contributed in development of obesity	(Hunt, Wang, Chen et al., 2017)
5.	N-acetylcysteine (NAC)	A nutritional supplement from cysteine	↑ MAPK pathway ↓ aP2 ↓ PPARγ ↓ C/EBPβ	Inhibited lipid accumulation	(Pieralisi, Martini, Soto et al., 2016)
6.	Chrysin	An active flavonoid present in many herb	↓ PPARγ2 ↓ LPL ↓ aP2	Decreased adipogenic differentiation	(Gao, Ding, Shui et al., 2016)

7.	4-(1-(4-iso-propylbenzyl)-1H-1,2,3-triazol-4-yl) benzene-1,2-diol (2e)		↑ binding of 2e with Estrogen receptor (ERRγ)	Induced browning	(Xu, Mao, Ding et al., 2015)
8.	14-3-3ζ scaffolds protein	Regulator of cellular signaling cascades	PPARγ *Lpin1*	Novel adipogenic protein	(Mugabo et al., 2018)
9.	CD38	Transmembrane glycoprotein	CD38 deficiency (-) ↓ PPARγ ↓ AP2 ↓ C/EBPα ↓ SREBP1-c ↓ FASN	CD38 deficiency impaired adipogenesis and lipogenesis in AT	(Wang et al., 2018)
10.	$p16^{INK4a}$	Cell cycle regulator and tumor suppressor	$p16^{INK4a}$ deficiency (-) ↓ Adipogenesis	Involved in AT formation	(Wouters, Deleye, Hannou et al., 2017)
11.	Follistatin (Fst)	An autocrine glycoprotein and express in most tissues	↑ UCP1 ↑ PRDM16 ↑ PGC-1α ↑ GLUT4	Increased browning of WAT	(Singh et al., 2017)
12.	FK506-binding protein 51 (FKBP51)	Intracellular protein act as cochaperone in heat shock protein 90 (Hsp90) machinery		Involved in regulation of adipogenesis	(Zhang, Qiu, Wang et al., 2017)
13.	EP3 receptor	Gi protein-coupled prostaglandin receptor	↓ PPARγ ↑ HSL	Included in bodily lipid and glucose metabolism	(Xu, Fu, Miao et al., 2016)
14.	Pin1	Peptidylprolyl cis/trans isomerase and isomerizes Ser/Thr-Pro motifs	↑ PPARγ ↑ ERK pathway	Regulator of adipocyte differentiation	(Han et al., 2016)
15.	Interferon-alpha (IFN-α)	Key immunoregulatory cytokine	↓ PPARγ ↓ C/EBPα	Decreased adipocyte differentiation and high antiadipogenic effects	(Lee et al., 2016)
16.	A Disintegrin And Metalloproteinase with Thrombospondin type 1 motifs; member 5 (ADAMTS5)	Metalloproteinase superfamily protein		Increased adipogenesis and shows angiogenesis effects	(Bauters et al., 2016)
17.	BCL11B	Zinc finger-type transcription factor	↑ C/EBPβ ↓ Wnt/β-catenin signaling	Function as regulator of adipogenesis	(Inoue, Ihara, Tsukamoto et al., 2016)
18.	BACH1	BTB and CNC homology 1 (BACH1) repressor	↓ PPARγ	Decreased adipocyte differentiation	(Matsumoto, Kondo, Shiraki et al., 2016)
19.	Perilipin2 (Plin2)	Also known as adipose differentiation-related protein (ADRP)	Stabilized upon lipolytic stimuli ↑ lipolysis	Positive regulator of lipolysis	(Takahashi, Shinoda, Kamada et al., 2016)
20.	Transglutaminase 2 (TG2)	Multifunctional crosslinking enzyme	↓ PPARγ ↓ C/EBPα ↑ β-catenin	Negative regulator of adipogenesis	(Myneni, Melino and Kaartinen, 2015)
21.	Serine/threonine kinase 40 (Stk40)	A putative serine/threonine kinase protein	↓ C/EBP proteins	Decreased adipogenesis	(Yu, He, Wang et al., 2015)
22.	Gelatinase A (MMP-2)	Type IV collagenase known as matrix metalloproteinase-2	↑ PPARγ ↑ aP2 ↑ Adiponectin	Impaired adipogenesis	(Bauters et al., 2015)

23.	FTO gene	The fat mass and obesity-associated protein	↑ RUNX1T1-S ↑ FABP4 ↑ PPARγ ↑ C/EBPα ↑ PLIN1	Increased adipogenesis	(Merkestein, Laber, McMurray et al., 2015)
24.	Ewing sarcoma gene (EWS)	Putative RNA-binding protein	↑ C/EBPβ ↑ C/EBPδ	Essential during early differentiation	(Park and Lee, 2015)
25.	MEFs injection		Formed a single fat pad	Used as cell-based therapies for the treatment of leptin-deficient states	(Ferguson, Blenden, Hutson et al., 2018)
26.	Glyphosate-based herbicides (GF)	Active ingredient of herbicide	↓ PPARγ ↑ Oxidative stress	Reduced Prefiltration and differentiation	(Martini, Gabrielli, Brandani et al., 2016)

936

1045

1046 **3 Conclusion**

1047 Cellular differentiation is commonly used for adipogenesis studies. The process is used
1048 to transform preadipocytes into mature adipocytes via adipogenic cocktails. These cocktails
1049 are also known as adipogenic agents and are defined prodifferentiative agents required for
1050 conversion of undifferentiated cells into differentiated adipocyte cells (Moreno-Navarrete and
1051 Fernández-Real, 2017). Cultivating the cells in the growth media is a fundamental step to
1052 prepare the preadipocyte cells for induction. Of note, a humidified atmosphere of 37°C with 5-
1053 10% CO_2 is essential. Once the cells reach confluency, the cells are exposed to the adipogenic
1054 agents, which generally vary for each cell line. The three major inducers most commonly used
1055 for differentiation include insulin, DEX and IBMX (Zhao et al., 2019). The confluent cells are
1056 cultured in the differentiation agents, refreshing the medium periodically. Over time, the cells
1057 reveal adipocyte-like characteristics such as formation of lipid droplets which approve their
1058 differentiation (Moreno-Navarrete and Fernández-Real, 2017).

1059 There are minor differences in the differentiation protocol for each cell line that has been
1060 discussed in Table 1. For example, additional adipogenic agents are usually necessary in FBS
1061 medium for 3T3-L1 cell conversion, however, 3T3-L1 cells can be differentiated with
1062 adipogenic serum without the addition of IBMX/DEX. The absence of bovine serum or growth
1063 hormone in the culture medium can prevent the 3T3-F442A cells from undergoing adipose
1064 differentiation, and hereby the cells can be maintained at their pre-confluency stage
1065 (Hemmeryckx et al., 2019). It was also identified in 1987, that an anti-inflammatory drug
1066 known as indomethacin was a potent adipogenic inducer that stimulates differentiation in a
1067 shorter period of 3 days, with 90% adipocyte capacity in comparison to DEX-treated cells
1068 (Knight et al., 1987). The greatest advantage of Ob1771 is its doubling time that allow its cells
1069 to rapidly multiply. Furthermore, some studies suggested that low serum concentrations have
1070 a slight effect on Ob1771 cells. Wherein, during the absence of added insulin, adipose
1071 conversion can occur significantly. We have also identified that OP9 cells can be differentiated
1072 by three different methods (Wolins et al., 2006). All three treatment methods require OP9 cells
1073 to be maintained in propagation medium until they differentiate into adipocytes morphology
1074 that accumulate triglycerides and abundant intracellular lipid droplets.

1075 Applications of these cell lines are similar, as they all assist in understanding the role
1076 of adipocyte-related proteins and genes. However, 3T3-L1 cell line is majorly used in co-
1077 culture and three-dimensional culture systems for AT. The majority of these cell lines can be

1078 used to screen anti-adipogenic compounds, anti-adipogenic peptides, adipogenic agents in food
1079 products and anti-adipogenesis crude extracts. TA1 cells also have the potential to identify
1080 early adipogenic markers. OP9 and mESCs are the more frequently used cell lines in advanced
1081 and high-throughput techniques, since the differentiation time for the cell lines are short,
1082 averaging around 10 days. Whilst some require the medium to be refreshed at short-time
1083 intervals, others require a change of medium. Studies indicate that Ob1771 cells are easy to
1084 differentiate within a short period, unlike AP-18 cells, which require RPMI 1640 medium and
1085 a rigorous differentiation process that can take up to 21 days for differentiation.

1086 We note that, in addition to the heterogeneous mesenchymal cell populations discussed
1087 above, another important cellular model in the study of adipose biology is adipose-derived
1088 tissue from mouse stromal vascular fractions (SVF), comprising of adipose stromal/stem cells
1089 (ASC) (Cawthorn, Scheller and MacDougald, 2012,Bourin, Bunnell, Casteilla et al., 2013).
1090 ASCs provide cell renewal and repair functions, as well as maintenance of homeostasis in AT
1091 (Zhang, Liu, Yong et al., 2015). While these cells are significantly more heterogeneous than
1092 the examples we have focused upon, in common with MSCs, these cells can transform into
1093 adipogenic and other lineage *in vitro* (Kelly, Tanaka, Baron et al., 1998,Zheng, Cao, Li et al.,
1094 2006,Kilroy, Dietrich, Wu et al., 2018), and harvesting MSCs from AT can yield better
1095 accessibility and greater abundance of MSCs. Furthermore, ASC fractions are thought to be
1096 primarily composed of immune cells, which enables us to make links between the immune
1097 system and obesity-related health problems, taking into account several studies that have
1098 established that chronic inflammation of AT is characterized by the influx of immune cells into
1099 AT caused by obesity (Grant and Dixit, 2015). We refer readers to recent review papers (Sadie-
1100 Van Gijsen, 2019,Jankowski, Dompe, Sibiak et al., 2020,Chu, Nguyen Thi Phuong, Tien et al.,
1101 2019) for further detail upon the differentiation, characterization, and applications of ASCs.

1102 An extensive number of studies have described that the development of obesity and
1103 related metabolic diseases are mainly instigated by dysregulation of AT. Thus, developing new
1104 strategies in this regard requires critical knowledge of molecular pathways regulating adipocyte
1105 development and metabolism. Importantly, employing cellular models has provided essential
1106 evidence of the contribution of AT to energy homeostasis. These cell lines have become
1107 suitable models for study of adipogenesis and its obesity-related metabolic alterations.
1108 Nevertheless, they have also been useful for studying adipocyte renewal, expansion and donor
1109 and depot-specific differences.

1110 There are several benefits and limitations of different cell line models which must be
1111 acknowledged; hence, this review assists with interpreting data and selecting a good cell line
1112 model by creating a better understanding on the science of adipocytes and AT, as well as their
1113 mechanism. The review also provides a detailed insight of available *in vitro* cell models which
1114 enables the determination of the crucial factors and pathways that will assist in targeting new
1115 pharmacological interventions against obesity and diabetes. Whilst 3D cultures and co-cultures
1116 of adipocytes with other cell types have been used as crucial tools to elucidate the multiple
1117 metabolic connections between fat and other tissues.

1118 **Funding**

1119 This study was financially supported by Taylor's University Flagship Research Grant -
1120 Scheme Project No: TUFR/2017/003/05. The funders had no role in the study design,
1121 collection, and analysis of data, manuscript draft preparation, or decision to publish.

1122 **Conflicts of Interest**

1123 The authors report no conflict of interest.

1124 **Acknowledgment**

1125 This article is written with the support of the following universities

1126 1. Taylor’s University Lakeside Campus, 47500 Subang Jaya, Malaysia.
1127 2. University of Kent, Canterbury, Kent, United Kingdom.
1128 3. Monash University, Sunway Campus, PJ 46150 Selangor, Malaysia.

1129 **Ethical approval**

1130 Not required.

1131 **List of Abbreviation**

PPARγ	Peroxisome Proliferator-Activated Receptor-γ
AT	Adipose Tissue
WAT	White Adipocytes Tissue
BAT	Brown Adipocytes Tissue
UCP1	Uncoupling protein-1
PPARα	Peroxisome Proliferator-Activated Receptor-α
mESCs	Mouse Embryonic Stem Cells
MEFs	Mouse Embryonic Fibroblasts
RA	Retinoic acid
T3	Triiodothyronine
MSCs	Mesenchymal Stem Cells
DEX	Dexamethasone
IBMX	3-isobutyl-1-methylxanthine
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
PGC-1α	Peroxisome Proliferator-Activated Receptor-gamma Coactivator-1α
PRDM16	PR domain containing 16
C/EBPα	CCAAT/enhancer-binding protein-α
C/EBPβ	CCAAT/enhancer-binding protein-β
C/EBPδ	CCAAT/enhancer-binding protein-δ
aP2	Adipocyte Protein 2
Pref-1	Preadipocyte factor 1
LPL	Lipoprotein lipase
SREBP-1c	Sterol regulatory element-binding protein-1c
FABP4	Fatty Acid-Binding Protein 4
FAS	Fatty acid synthase
HSL	Hormone-sensitive lipase
LPL	Lipoprotein lipase
CPT1	Carnitine palmitoyltransferase I
SVF	Stromal vascular fractions
ACS	Adipose stromal/stem cells
IL-6	Interleukin 6
IL-1β	Interleukin 1β
RUNX2	Runt-related transcription factor 2
DGATs	Diacylglycerol acyltransferases
SCD1	Stearoyl-CoA desaturase 1
ATGL	Adipose triglyceride lipase
AMPK	AMP-activated protein kinase
ACC	Acetyl-CoA carboxylase
Akt	Protein Kinase B
GLUT4	Glucose transporter type 4
FOXO3a	Forkhead box O3
PLIN	Perilipin 1
TNFα	Tumor necrosis factor-α
NPY	Neuropeptide Y
NPYR	Neuropeptide Y receptor
EBs	Embryoid bodies

LIF	Leukemia inhibitory factor
MAPK	Mitogen-activated protein kinase

1132

1133 **References**

1134 [1] Chooi, Y.C., Ding, C. and Magkos, F., 2019. The epidemiology of obesity,
1135 Metabolism. 92, 6-10.
1136 [2] Włodarczyk, M. and Nowicka, G., 2019. Obesity, DNA damage, and development of
1137 obesity-related diseases, International journal of molecular sciences. 20, 1146.
1138 [3] Lohmann, A.E., Goodwin, P.J., Chlebowski, R.T., Pan, K., Stambolic, V. and
1139 Dowling, R.J., 2016. Association of obesity-related metabolic disruptions with cancer
1140 risk and outcome, Journal of Clinical Oncology. 34, 4249-4255.
1141 [4] Abarca-Gómez, L., Abdeen, Z.A., Hamid, Z.A., Abu-Rmeileh, N.M., Acosta-Cazares,
1142 B., Acuin, C., Adams, R.J., Aekplakorn, W., Afsana, K. and Aguilar-Salinas, C.A.,
1143 2017. Worldwide trends in body-mass index, underweight, overweight, and obesity
1144 from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies
1145 in 128· 9 million children, adolescents, and adults, The Lancet. 390, 2627-2642.
1146 [5] Wolfenden, L., Ezzati, M., Larijani, B. and Dietz, W., 2019. The challenge for global
1147 health systems in preventing and managing obesity, Obesity Reviews. 20, 185-193.
1148 [6] Collaboration, N.R.F., 2016. Trends in adult body-mass index in 200 countries from
1149 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with
1150 19· 2 million participants, The Lancet. 387, 1377-1396.
1151 [7] Ross, S.E., Flynn, J.I. and Pate, R.R., 2016. What is really causing the obesity
1152 epidemic? A review of reviews in children and adults, Journal of sports sciences. 34,
1153 1148-1153.
1154 [8] de Ferranti, S. and Mozaffarian, D., 2008. The perfect storm: obesity, adipocyte
1155 dysfunction, and metabolic consequences, Clinical chemistry. 54, 945-955.
1156 [9] van Meijel, R.L., Blaak, E.E. and Goossens, G.H., 2019. Adipose tissue metabolism
1157 and inflammation in obesity, Mechanisms and Manifestations of Obesity in Lung
1158 Disease. Elsevier, pp. 1-22.
1159 [10] Jo, J., Gavrilova, O., Pack, S., Jou, W., Mullen, S., Sumner, A.E., Cushman, S.W. and
1160 Periwal, V., 2009. Hypertrophy and/or hyperplasia: dynamics of adipose tissue
1161 growth, PLoS computational biology. 5.
1162 [11] Booth, A., Magnuson, A., Fouts, J. and Foster, M.T., 2016. Adipose tissue: an
1163 endocrine organ playing a role in metabolic regulation, Hormone molecular biology
1164 and clinical investigation. 26, 25-42.
1165 [12] PNandhini, L., Desai, A. and Sahoo, J., 2019. Adipose Tissue as an Endocrine Organ,
1166 Lipocrinology: The Relationship between Lipids and Endocrine Function.
1167 [13] Giralt, M. and Villarroya, F., 2013. White, brown, beige/brite: different adipose cells
1168 for different functions?, Endocrinology. 154, 2992-3000.
1169 [14] Han, S.-J., Zaretsky, A.G., Andrade-Oliveira, V., Collins, N., Dzutsev, A., Shaik, J.,
1170 da Fonseca, D.M., Harrison, O.J., Tamoutounour, S. and Byrd, A.L., 2017. White
1171 adipose tissue is a reservoir for memory T cells and promotes protective memory
1172 responses to infection, Immunity. 47, 1154-1168. e6.
1173 [15] Villarroya, F., Cereijo, R., Villarroya, J. and Giralt, M., 2017. Brown adipose tissue as
1174 a secretory organ, Nature Reviews Endocrinology. 13, 26.
1175 [16] Mottillo, E.P., Desjardins, E.M., Crane, J.D., Smith, B.K., Green, A.E., Ducommun,
1176 S., Henriksen, T.I., Rebalka, I.A., Razi, A. and Sakamoto, K., 2016. Lack of
1177 adipocyte AMPK exacerbates insulin resistance and hepatic steatosis through brown
1178 and beige adipose tissue function, Cell metabolism. 24, 118-129.
1179 [17] Rosen, E.D., Walkey, C.J., Puigserver, P. and Spiegelman, B.M., 2000.
1180 Transcriptional regulation of adipogenesis, Genes & development. 14, 1293-1307.

1181 [18] Munawar, T.M., Prakash, D.S. and Vangalapati, M., 2018. Development of response
1182 surface methodology for optimization of parameters and quantitative analysis of
1183 chebulinic acid from composition of medicinal herbs by HPLC, Saudi Journal of
1184 Biological Sciences.
1185 [19] Hammarstedt, A., Gogg, S., Hedjazifar, S., Nerstedt, A. and Smith, U., 2018.
1186 Impaired adipogenesis and dysfunctional adipose tissue in human hypertrophic
1187 obesity, Physiological reviews. 98, 1911-1941.
1188 [20] González-Casanova, J.E., Pertuz-Cruz, S.L., Caicedo-Ortega, N.H. and Rojas-Gomez,
1189 D.M., 2020. Adipogenesis Regulation and Endocrine Disruptors: Emerging Insights
1190 in Obesity, BioMed Research International. 2020.
1191 [21] Ruiz-Ojeda, F.J., Rupérez, A.I., Gomez-Llorente, C., Gil, A. and Aguilera, C.M.,
1192 2016. Cell models and their application for studying adipogenic differentiation in
1193 relation to obesity: a review, International journal of molecular sciences. 17, 1040.
1194 [22] Wang, Q.A., Scherer, P.E. and Gupta, R.K., 2014. Improved methodologies for the
1195 study of adipose biology: insights gained and opportunities ahead, Journal of lipid
1196 research. 55, 605-624.
1197 [23] Moreno-Navarrete, J.M. and Fernández-Real, J.M., 2017. Adipocyte differentiation,
1198 Adipose tissue biology. Springer, pp. 69-90.
1199 [24] Yusuf, B., Gopurappilly, R., Dadheech, N., Gupta, S., Bhonde, R. and Pal, R., 2013.
1200 Embryonic fibroblasts represent a connecting link between mesenchymal and
1201 embryonic stem cells, Development, growth & differentiation. 55, 330-340.
1202 [25] Kassotis, C.D., Masse, L., Kim, S., Schlezinger, J.J., Webster, T.F. and Stapleton,
1203 H.M., 2017. Characterization of adipogenic chemicals in three different cell culture
1204 systems: implications for reproducibility based on cell source and handling, Scientific
1205 reports. 7, 42104.
1206 [26] Kim, J.H., Lee, S., Kim, H.Y. and Cho, E.J., 2020. Acer okamotoanum inhibits
1207 adipocyte differentiation by the regulation of adipogenesis and lipolysis in 3T3-L1
1208 cells, International Journal of Molecular Medicine. 45, 589-596.
1209 [27] Zhao, X., Hu, H., Wang, C., Bai, L., Wang, Y., Wang, W. and Wang, J., 2019. A
1210 comparison of methods for effective differentiation of the frozen-thawed 3T3-L1
1211 cells, Analytical biochemistry. 568, 57-64.
1212 [28] Hemmeryckx, B., Vranckx, C., Bauters, D., Lijnen, H.R. and Scroyen, I., 2019. Does
1213 butein affect adipogenesis?, Adipocyte. 8, 209-222.
1214 [29] Khalilpourfarshbafi, M., Murugan, D.D., Sattar, M.Z.A., Sucedaram, Y. and
1215 Abdullah, N.A., 2019. Withaferin A inhibits adipogenesis in 3T3-F442A cell line,
1216 improves insulin sensitivity and promotes weight loss in high fat diet-induced obese
1217 mice, PloS one. 14.
1218 [30] Shinohara, O., Murata, Y.-I. and Shimizu, M., 1992. Enhancement of differentiation
1219 of cultured adipogenic cells (TA1) by pertussis toxin, Biochemistry and Cell Biology.
1220 70, 650-655.
1221 [31] Ninomiya-Tsuji, J., Torti, F.M. and Ringold, G.M., 1993. Tumor necrosis factor-
1222 induced c-myc expression in the absence of mitogenesis is associated with inhibition
1223 of adipocyte differentiation, Proceedings of the National Academy of Sciences. 90,
1224 9611-9615.
1225 [32] Chen, C., Takahashi, K., Yoshida, A., Takizawa, Y., Lee, Y., Nakui, M., Doi, H.,
1226 Takebayashi, Y., Fukumoto, M. and Yamada, T., 2010. Characterization of a novel
1227 murine preadipocyte line, AP-18, isolated from subcutaneous tissue: Analysis of
1228 adipocyte-related gene expressions, Cell biology international. 34, 293-299.
1229 [33] Abderrahim-Ferkoune, A., Bezy, O., Astri-Roques, S., Elabd, C., Ailhaud, G. and
1230 Amri, E.-Z., 2004. Transdifferentiation of preadipose cells into smooth muscle-like

1231 cells: role of aortic carboxypeptidase-like protein, Experimental cell research. 293,
1232 219-228.
1233 [34] Schwind, L., Schetting, S. and Montenarh, M., 2017. Inhibition of protein kinase CK2
1234 prevents adipogenic differentiation of mesenchymal stem cells like C3H/10T1/2 cells,
1235 Pharmaceuticals. 10, 22.
1236 [35] Hussain, S., Rehman, A.U., Luckett, D.J., Blanchard, C.L., Obied, H.K. and Strappe,
1237 P., 2020. Phenolic Compounds with Antioxidant Properties from Canola Meal
1238 Extracts Inhibit Adipogenesis, International Journal of Molecular Sciences. 21, 1.
1239 [36] Wolins, N.E., Quaynor, B.K., Skinner, J.R., Tzekov, A., Park, C., Choi, K. and
1240 Bickel, P.E., 2006. OP9 mouse stromal cells rapidly differentiate into adipocytes:
1241 characterization of a useful new model of adipogenesis, Journal of lipid research. 47,
1242 450-460.
1243 [37] Rosen, E.D. and MacDougald, O.A., 2006. Adipocyte differentiation from the inside
1244 out, Nature reviews Molecular cell biology. 7, 885.
1245 [38] Ota, K., Tong, K.I., Goto, K., Tomida, S., Komuro, A., Wang, Z., Nishio, K. and
1246 Okada, H., 2017. The H3K27 demethylase, Utx, regulates adipogenesis in a
1247 differentiation stage-dependent manner, PloS one. 12.
1248 [39] Hou, W., Chen, Q., Wang, H., Qiu, P., Lyu, X., Chen, W., Chua, M.L., Chinn, Y.E.,
1249 Deng, C.-X. and Wang, R., 2020. The metabolic footprint during adipocyte
1250 commitment highlights ceramide modulation as an adequate approach for obesity
1251 treatment, EBioMedicine. 51, 102605.
1252 [40] Green, H. and Kehinde, O., 1974. Sublines of mouse 3T3 cells that accumulate lipid,
1253 Cell. 1, 113-116.
1254 [41] Antony, J., Debroy, S., Manisha, C., Thomas, P., Jeyarani, V. and Choephel, T., 2019.
1255 In-vitro cell line Models and Assay methods to study the Anti-diabetic Activity,
1256 Research Journal of Pharmacy and Technology. 12, 2200-2206.
1257 [42] Green, H. and Kehinde, O., 1979. Formation of normally differentiated subcutaneous
1258 fat pads by an established preadipose cell line, Journal of cellular physiology. 101,
1259 169-171.
1260 [43] Kuri-Harcuch, W., Velez-delValle, C., Vazquez-Sandoval, A., Hernández-Mosqueira,
1261 C. and Fernandez-Sanchez, V., 2019. A cellular perspective of adipogenesis
1262 transcriptional regulation, Journal of cellular physiology. 234, 1111-1129.
1263 [44] Novikoff, A.B., Novikoff, P.M., Rosen, O.M. and Rubin, C.S., 1980. Organelle
1264 relationships in cultured 3T3-L1 preadipocytes, The Journal of Cell Biology. 87, 180-
1265 196.
1266 [45] Xiu, L., Xinong, L., Tianjia, L. and Bao, L., 2017. Research Progress of the
1267 Differentiation of 3T3-L1 Preadipocytes into Mature Adipocytes, Chinese Academy
1268 of Medical Sciences. 39, 727-731.
1269 [46] Subra, C., Fontana, E., Visentin, V., Testar, X. and Carpéné, C., 2003. Tyramine and
1270 benzylamine partially but selectively mimic insulin action on adipose differentiation
1271 in 3T3-L1 cells, Journal of physiology and biochemistry. 59, 209-216.
1272 [47] Katafuchi, T., Garbers, D.L. and Albanesi, J.P., 2010. CNP/GC-B system: A new
1273 regulator of adipogenesis, Peptides. 31, 1906-1911.
1274 [48] Zebisch, K., Voigt, V., Wabitsch, M. and Brandsch, M., 2012. Protocol for effective
1275 differentiation of 3T3-L1 cells to adipocytes, Analytical biochemistry. 425, 88-90.
1276 [49] Vishwanath, D., Srinivasan, H., Patil, M.S., Seetarama, S., Agrawal, S.K., Dixit, M.
1277 and Dhar, K., 2013. Novel method to differentiate 3T3 L1 cells in vitro to produce
1278 highly sensitive adipocytes for a GLUT4 mediated glucose uptake using fluorescent
1279 glucose analog, Journal of cell communication and signaling. 7, 129-140.

1280 [50] Hua, Y., Ke, S., Wang, Y., Irwin, D.M., Zhang, S. and Wang, Z., 2016. Prolonged
1281 treatment with 3-isobutyl-1-methylxanthine improves the efficiency of differentiating
1282 3T3-L1 cells into adipocytes, Analytical biochemistry. 507, 18-20.
1283 [51] Li, C., Li, J., He, F., Li, K., Li, X. and Zhang, Y., 2020. Matrix Gla protein regulates
1284 adipogenesis and is serum marker of visceral adiposity, Adipocyte. 9, 68-76.
1285 [52] Kou, Y., Liu, Q., Liu, W., Sun, H., Liang, M., Kong, F., Zhang, B., Wei, Y., Liu, Z.
1286 and Wang, Y., 2019. LIGHT/TNFSF14 signaling attenuates beige fat biogenesis, The
1287 FASEB Journal. 33, 1595-1604.
1288 [53] Xi, P., Xue, J., Wu, Z., Wang, H., Han, J., Liang, H. and Tian, D., 2019. Liver kinase
1289 B1 induces browning phenotype in 3 T3-L1 adipocytes, Gene. 682, 33-41.
1290 [54] Tang, W. and Fan, Y., 2019. SIRT6 as a potential target for treating insulin resistance,
1291 Life sciences. 116558.
1292 [55] Kobayashi, M., Hoshino, S., Abe, T., Okita, N., Tagawa, R., Nagai, W., Konno, R.,
1293 Suzuki, Y., Furuya, K. and Ishikawa, N., 2019. Identification of WWP1 as an obesity-
1294 associated E3 ubiquitin ligase with a protective role against oxidative stress in
1295 adipocytes, Biochemical and biophysical research communications. 508, 117-122.
1296 [56] Camacho, A., Segoviano-Ramírez, J.C., Sánchez-Garcia, A., García-Juarez, J.,
1297 Hernandez-Puente, C.A., Calvo-Anguiano, G., Maltos-Uro, S.R., Olguin, A., Gojon-
1298 Romanillos, G. and Gojon-Zorrilla, G., 2018. Tyrphostin AG17 inhibits adipocyte
1299 differentiation in vivo and in vitro, Lipids in health and disease. 17, 128.
1300 [57] Huang, K.-T., Hsu, L.-W., Chen, K.-D., Kung, C.-P., Goto, S. and Chen, C.-L., 2018.
1301 Decreased PEDF Expression Promotes Adipogenic Differentiation through the Up-
1302 Regulation of CD36, International journal of molecular sciences. 19, 3992.
1303 [58] De Filippis, E., Li, T. and Rosen, E.D., 2018. Exposure of adipocytes to bisphenol-A
1304 in vitro interferes with insulin action without enhancing adipogenesis, PloS one. 13,
1305 e0201122.
1306 [59] Kim, Y.A., Kim, H.Y., Oh, Y.J., Kwon, W.Y., Lee, M.H., Bae, J.Y., Woo, M.S., Kim,
1307 J.-M. and Yoo, Y.H., 2018. Polychlorinated biphenyl 138 exposure-mediated lipid
1308 droplet enlargement endows adipocytes with resistance to TNF-α-induced cell death,
1309 Toxicology letters. 292, 55-62.
1310 [60] Marino, S., Bishop, R.T., de Ridder, D., Delgado-Calle, J. and Reagan, M.R., 2019.
1311 2D and 3D in vitro co-culture for cancer and bone cell interaction studies, Bone
1312 Research Protocols. Springer, pp. 71-98.
1313 [61] Paschos, N.K., Brown, W.E., Eswaramoorthy, R., Hu, J.C. and Athanasiou, K.A.,
1314 2015. Advances in tissue engineering through stem cell-based co-culture, Journal of
1315 tissue engineering and regenerative medicine. 9, 488-503.
1316 [62] Hendriks, J., Riesle, J. and van Blitterswijk, C.A., 2007. Co-culture in cartilage tissue
1317 engineering, Journal of tissue engineering and regenerative medicine. 1, 170-178.
1318 [63] Dodson, M., Vierck, J., Hossner, K., Byrne, K. and McNamara, J., 1997. The
1319 development and utility of a defined muscle and fat co-culture system, Tissue and
1320 Cell. 29, 517-524.
1321 [64] Hao, Q., Diaz, T., del Rio Verduzco, A., Magyar, C.E., Zhong, J., Elshimali, Y.,
1322 Rettig, M.B., Henning, S.M., Vadgama, J.V. and Wang, P., 2020. Arctigenin inhibits
1323 prostate tumor growth in high-fat diet fed mice through dual actions on adipose tissue
1324 and tumor, Scientific Reports. 10, 1-11.
1325 [65] Lu, Y., Ma, J., Zhao, J., Song, Z., Zhou, C., Liu, X., Teng, W., Wang, W., Zhang, Q.
1326 and Yan, W., 2020. The Role of MKP-5 in Adipocyte-Macrophage Interactions
1327 during Obesity, Obesity Facts. 1-16.
1328 [66] Kang, B., Kim, C.Y., Hwang, J., Suh, H.J. and Choi, H.S., 2019. Brassinin, a
1329 phytoalexin in cruciferous vegetables, suppresses obesity-induced inflammatory

1330 responses through the Nrf2-HO-1 signaling pathway in an adipocyte-macrophage co-
1331 culture system, Phytotherapy Research. 33, 1426-1437.
1332 [67] Kim, C.Y., Kang, B., Suh, H.J. and Choi, H.-S., 2018. Red ginseng-derived saponin
1333 fraction suppresses the obesity-induced inflammatory responses via Nrf2-HO-1
1334 pathway in adipocyte-macrophage co-culture system, Biomedicine &
1335 Pharmacotherapy. 108, 1507-1516.
1336 [68] Turner, P.A., Tang, Y., Weiss, S.J. and Janorkar, A.V., 2015. Three-dimensional
1337 spheroid cell model of in vitro adipocyte inflammation, Tissue Engineering Part A.
1338 21, 1837-1847.
1339 [69] Lee, S.Y., Park, S.B., Kim, Y.E., Yoo, H.M., Hong, J., Choi, K.-J., Kim, K.Y. and
1340 Kang, D., 2019. iTRAQ-Based Quantitative Proteomic Comparison of 2D and 3D
1341 Adipocyte Cell Models Co-cultured with Macrophages Using Online 2D-nanoLC-
1342 ESI-MS/MS, Scientific reports. 9, 1-13.
1343 [70] Zhu, J., He, J., Verano, M., Brimmo, A.T., Glia, A., Qasaimeh, M.A., Chen, P.,
1344 Aleman, J.O. and Chen, W., 2018. An integrated adipose-tissue-on-chip
1345 nanoplasmonic biosensing platform for investigating obesity-associated inflammation,
1346 Lab on a Chip. 18, 3550-3560.
1347 [71] Lustig, M., Feng, Q., Payan, Y., Gefen, A. and Benayahu, D., 2019. Noninvasive
1348 continuous monitoring of adipocyte differentiation: From macro to micro scales,
1349 Microscopy and Microanalysis. 25, 119-128.
1350 [72] Pereira-Fernandes, A., Vanparys, C., Vergauwen, L., Knapen, D., Jorens, P.G. and
1351 Blust, R., 2014. Toxicogenomics in the 3T3-L1 cell line, a new approach for
1352 screening of obesogenic compounds, Toxicological Sciences. 140, 352-363.
1353 [73] Poulos, S.P., Dodson, M.V. and Hausman, G.J., 2010. Cell line models for
1354 differentiation: preadipocytes and adipocytes, Experimental biology and medicine.
1355 235, 1185-1193.
1356 [74] Adler-Wailes, D.C., Guiney, E.L., Wolins, N.E. and Yanovski, J.A., 2010. Long-term
1357 ritonavir exposure increases fatty acid and glycerol recycling in 3T3-L1 adipocytes as
1358 compensatory mechanisms for increased triacylglycerol hydrolysis, Endocrinology.
1359 151, 2097-2105.
1360 [75] Hock, F.J., 2016. Drug discovery and evaluation: Pharmacological assays, Springer.
1361 [76] Hernández-Mosqueira, C., Velez-delValle, C. and Kuri-Harcuch, W., 2015. Tissue
1362 alkaline phosphatase is involved in lipid metabolism and gene expression and
1363 secretion of adipokines in adipocytes, Biochimica et Biophysica Acta (BBA)-General
1364 Subjects. 1850, 2485-2496.
1365 [77] Löffler, M.C., Mayer, A.E., Viera, J.T., Valdes, A.L., El-Merahbi, R., Ade, C.P.,
1366 Karwen, T., Schmitz, W., Slotta, A. and Erk, M., 2018. Protein kinase D1 deletion in
1367 adipocytes enhances energy dissipation and protects against adiposity, The EMBO
1368 journal. 37.
1369 [78] Perez-Diaz, S., Garcia-Sobreviela, M.P., Gonzalez-Irazabal, Y., Garcia-Rodriguez, B.,
1370 Espina, S., Arenaz, I. and Arbones-Mainar, J.M., 2018. PTRF acts as an adipokine
1371 contributing to adipocyte dysfunctionality and ectopic lipid deposition, Journal of
1372 physiology and biochemistry. 74, 613-622.
1373 [79] Xiang, R., Fan, L.-l., Huang, H., Chen, Y.-q., He, W., Guo, S., Li, J.-J., Jin, J.-y., Du,
1374 R. and Yan, R., 2018. Increased Reticulon 3 (RTN3) Leads to Obesity and
1375 Hypertriglyceridemia by Interacting With Heat Shock Protein Family A (Hsp70)
1376 Member 5 (HSPA5), Circulation. 138, 1828-1838.
1377 [80] Zeng, F., Wang, Y., Kloepfer, L.A., Wang, S. and Harris, R.C., 2018. ErbB4 deletion
1378 predisposes to development of metabolic syndrome in mice, American Journal of
1379 Physiology-Endocrinology and Metabolism. 315, E583-E593.

1380 [81] Nugroho, D.B., Ikeda, K., Barinda, A.J., Wardhana, D.A., Yagi, K., Miyata, K., Oike,
1381 Y., Hirata, K.-i. and Emoto, N., 2018. Neuregulin-4 is an angiogenic factor that is
1382 critically involved in the maintenance of adipose tissue vasculature, Biochemical and
1383 biophysical research communications. 503, 378-384.
1384 [82] Hou, S., Jiao, Y., Yuan, Q., Zhai, J., Tian, T., Sun, K., Chen, Z., Wu, Z. and Zhang, J.,
1385 2018. S100A4 protects mice from high-fat diet-induced obesity and inflammation,
1386 Laboratory Investigation. 98, 1025.
1387 [83] Chang, E.-J., Shin, M.-K., Choi, B., Kim, E.-Y., Park, J.-E., Hwang, E.S., Lee, H.J.,
1388 Kim, M.K., Kim, S.W. and Kim, J.-E., 2018. Elevated pentraxin 3 in obese adipose
1389 tissue promotes adipogenic differentiation by activating neuropeptide Y signaling,
1390 Frontiers in immunology. 9, 1790.
1391 [84] Wang, L.F., Miao, L.J., Wang, X.N., Huang, C.C., Qian, Y.S., Huang, X., Wang,
1392 X.L., Jin, W.Z., Ji, G.J. and Fu, M., 2018. CD 38 deficiency suppresses adipogenesis
1393 and lipogenesis in adipose tissues through activating Sirt1/PPAR γ signaling pathway,
1394 Journal of cellular and molecular medicine. 22, 101-110.
1395 [85] Wang, Y.-M., Liu, H.-X. and Fang, N.-Y., 2018. 9-PAHSA promotes browning of
1396 white fat via activating G-protein-coupled receptor 120 and inhibiting
1397 lipopolysaccharide/NF-kappa B pathway, Biochemical and biophysical research
1398 communications. 506, 153-160.
1399 [86] Son, H.J., Jang, Y.J., Jung, C.H., Ahn, J. and Ha, T.Y., 2019. 2, 6-Dimethoxy-1, 4-
1400 benzoquinone Inhibits 3T3-L1 Adipocyte Differentiation via Regulation of AMPK
1401 and mTORC1, Planta medica. 85, 210-216.
1402 [87] Lee, M.R., Kim, J.E., Choi, J.Y., Park, J.J., Kim, H.R., Song, B.R., Choi, Y.W., Kim,
1403 K.M., Song, H. and Hwang, D.Y., 2019. Anti-obesity effect in high-fat-diet-induced
1404 obese C57BL/6 mice: Study of a novel extract from mulberry (Morus alba) leaves
1405 fermented with Cordyceps militaris, Experimental and therapeutic medicine. 17,
1406 2185-2193.
1407 [88] Lee, M., Sorn, S., Lee, Y. and Kang, I., 2019. Salt induces adipogenesis/lipogenesis
1408 and inflammatory adipocytokines secretion in adipocytes, International journal of
1409 molecular sciences. 20, 160.
1410 [89] Hu, J., Li, X., Tian, W., Lu, Y., Xu, Y., Wang, F., Qin, W., Ma, X., Puno, P.-T. and
1411 Xiong, W., 2019. Adenanthin, a Natural ent-Kaurane Diterpenoid Isolated from the
1412 Herb Isodon adenantha Inhibits Adipogenesis and the Development of Obesity by
1413 Regulation of ROS, Molecules. 24, 158.
1414 [90] Peng, Y., Sun, Q., Xu, W., He, Y., Jin, W., Yuan, L. and Gao, R., 2019. Vitexin
1415 ameliorates high fat diet-induced obesity in male C57BL/6J mice via the AMPKα-
1416 mediated pathway, Food & function. 10, 1940-1947.
1417 [91] de Melo, K.M., de Oliveira, F.T.B., Silva, R.A.C., Quinderé, A.L.G., Marinho Filho,
1418 J.D.B., Araújo, A.J., Pereira, E.D.B., Carvalho, A.A., Chaves, M.H. and Rao, V.S.,
1419 2019. α, β-Amyrin, a pentacyclic triterpenoid from Protium heptaphyllum suppresses
1420 adipocyte differentiation accompanied by down regulation of PPARγ and C/EBPα in
1421 3T3-L1 cells, Biomedicine & Pharmacotherapy. 109, 1860-1866.
1422 [92] Kim, H.-L., Park, J., Jung, Y., Ahn, K.S. and Um, J.-Y., 2019. Platycodin d, a novel
1423 activator of amp-activated protein kinase, attenuates obesity in db/db mice via
1424 regulation of adipogenesis and thermogenesis, Phytomedicine. 52, 254-263.
1425 [93] Choi, J., Song, N.-J., Lee, A., Lee, D., Seo, M.-J., Kim, S., Chang, S.-H., Yang, D.,
1426 Hwang, Y.-J. and Hwang, K.-A., 2019. Oxyresveratrol Increases Energy Expenditure
1427 through Foxo3a-Mediated Ucp1 Induction in High-Fat-Diet-Induced Obese Mice,
1428 International journal of molecular sciences. 20, 26.

1429 [94] Biasiotto, G., Zanella, I., Predolini, F., Archetti, I., Cadei, M., Monti, E., Luzzani, M.,
1430 Pacchetti, B., Mozzoni, P. and Andreoli, R., 2018. 7-Hydroxymatairesinol improves
1431 body weight, fat and sugar metabolism in C57BJ/6 mice on a high-fat diet, British
1432 Journal of Nutrition. 120, 751-762.
1433 [95] Hsieh, C.-T., Chang, F.-R., Tsai, Y.-H., Wu, Y.-C. and Hsieh, T.-J., 2018. 2-Bromo-
1434 4′-methoxychalcone and 2-Iodo-4′-methoxychalcone Prevent Progression of
1435 Hyperglycemia and Obesity via 5′-Adenosine-Monophosphate-Activated Protein
1436 Kinase in Diet-Induced Obese Mice, International journal of molecular sciences. 19,
1437 2763.
1438 [96] Kang, N.H., Mukherjee, S., Min, T., Kang, S.C. and Yun, J.W., 2018. Trans-anethole
1439 ameliorates obesity via induction of browning in white adipocytes and activation of
1440 brown adipocytes, Biochimie. 151, 1-13.
1441 [97] Pai, S., Martis, E., Joshi, S., Munshi, R. and Juvekar, A., 2018. Plumbagin exerts
1442 antiobesity effects through inhibition of pancreatic lipase and adipocyte
1443 differentiation, Phytotherapy research. 32, 1631-1635.
1444 [98] Liu, H., Wang, J., Liu, M., Zhao, H., Yaqoob, S., Zheng, M., Cai, D. and Liu, J.,
1445 2018. Antiobesity effects of ginsenoside Rg1 on 3T3-L1 preadipocytes and high fat
1446 diet-induced obese mice mediated by AMPK, Nutrients. 10, 830.
1447 [99] Mi, Y., Liu, X., Tian, H., Liu, H., Li, J., Qi, G. and Liu, X., 2018. EGCG stimulates
1448 the recruitment of brite adipocytes, suppresses adipogenesis and counteracts TNF-α-
1449 triggered insulin resistance in adipocytes, Food & function. 9, 3374-3386.
1450 [100] Green, H. and Kehinde, O., 1976. Spontaneous heritable changes leading to increased
1451 adipose conversion in 3T3 cells, Cell. 7, 105-113.
1452 [101] Sadie-Van Gijsen, H., 2019. Adipocyte biology: It is time to upgrade to a new model,
1453 Journal of cellular physiology. 234, 2399-2425.
1454 [102] Mandrup, S., Loftus, T.M., MacDougald, O.A., Kuhajda, F.P. and Lane, M.D., 1997.
1455 Obese gene expression at in vivo levels by fat pads derived from sc implanted 3T3-
1456 F442A preadipocytes, Proceedings of the National Academy of Sciences. 94, 4300-
1457 4305.
1458 [103] Kuri-Harcuch, W. and Green, H., 1978. Adipose conversion of 3T3 cells depends on a
1459 serum factor, Proceedings of the National Academy of Sciences. 75, 6107-6109.
1460 [104] Khalilpourfarshbafi, M., Murugan, D.D., Sattar, M.Z.A., Sucedaram, Y. and
1461 Abdullah, N.A., 2019. Withaferin A inhibits adipogenesis in 3T3-F442A cell line,
1462 improves insulin sensitivity and promotes weight loss in high fat diet-induced obese
1463 mice, PloS one. 14, e0218792.
1464 [105] Ayala-Sumuano, J.-T., Velez-Del Valle, C., Beltrán-Langarica, A., Hernández, J.M.
1465 and Kuri-Harcuch, W., 2008. Adipogenic genes on induction and stabilization of
1466 commitment to adipose conversion, Biochemical and biophysical research
1467 communications. 374, 720-724.
1468 [106] Ayala-Sumuano, J.-T., Velez-delValle, C., Beltrán-Langarica, A., Marsch-Moreno,
1469 M., Cerbón-Solorzano, J. and Kuri-Harcuch, W., 2011. Srebf1a is a key regulator of
1470 transcriptional control for adipogenesis, Scientific reports. 1, 178.
1471 [107] Diaz-Velasquez, C.E., Castro-Muñozledo, F. and Kuri-Harcuch, W., 2008.
1472 Staurosporine rapidly commits 3T3-F442A cells to the formation of adipocytes by
1473 activation of GSK-3β and mobilization of calcium, Journal of cellular biochemistry.
1474 105, 147-157.
1475 [108] Tang, Q.Q. and Lane, M.D., 2012. Adipogenesis: from stem cell to adipocyte, Annual
1476 review of biochemistry. 81, 715-736.
1477 [109] Ayala-Sumuano, J.-T., Velez-delValle, C., Beltrán-Langarica, A., Marsch-Moreno,
1478 M., Hernandez-Mosqueira, C. and Kuri-Harcuch, W., 2013. Glucocorticoid

1479 paradoxically recruits adipose progenitors and impairs lipid homeostasis and glucose
1480 transport in mature adipocytes, Scientific reports. 3, 2573.
1481 [110] Masuzaki, H., Paterson, J., Shinyama, H., Morton, N.M., Mullins, J.J., Seckl, J.R. and
1482 Flier, J.S., 2001. A transgenic model of visceral obesity and the metabolic syndrome,
1483 Science. 294, 2166-2170.
1484 [111] Torabi, S. and Mo, H., 2016. Trans, trans-farnesol as a mevalonate-derived inducer of
1485 murine 3T3-F442A pre-adipocyte differentiation, Experimental Biology and
1486 Medicine. 241, 493-500.
1487 [112] Skillington, J., Choy, L. and Derynck, R., 2002. Bone morphogenetic protein and
1488 retinoic acid signaling cooperate to induce osteoblast differentiation of preadipocytes,
1489 The Journal of cell biology. 159, 135-146.
1490 [113] Torabi, S., Yeganehjoo, H., Shen, C.-L. and Mo, H., 2016. Peroxisome proliferator–
1491 activated receptor γ down-regulation mediates the inhibitory effect of d-δ-tocotrienol
1492 on the differentiation of murine 3T3-F442A preadipocytes, Nutrition Research. 36,
1493 1345-1352.
1494 [114] Carreira, M.C., Andrade, S., Gonzalez-Izquierdo, A., Amil, M., Folgueira, C.,
1495 Monteiro, M.P., Sanz, E., Crujeiras, A.B. and Casanueva, F.F., 2018. Anti-obesity
1496 activity of OBEX is regulated by activation of thermogenesis and decreasing
1497 adiposity gain, Scientific reports. 8, 17155.
1498 [115] Gomez-Zorita, S., Belles, C., Briot, A., Fernández-Quintela, A., Portillo, M.P. and
1499 Carpéné, C., 2017. Pterostilbene inhibits lipogenic activity similar to resveratrol or
1500 caffeine but differently modulates lipolysis in adipocytes, Phytotherapy research. 31,
1501 1273-1282.
1502 [116] Jahandideh, F., Chakrabarti, S., Davidge, S.T. and Wu, J., 2017. Egg white
1503 hydrolysate shows insulin mimetic and sensitizing effects in 3T3-F442A pre-
1504 adipocytes, PloS one. 12, e0185653.
1505 [117] Doğan, A., Demirci, S., Kıratlı, B. and Şahin, F., 2017. Cytoglobin: a potential marker
1506 for adipogenic differentiation in preadipocytes in vitro, Cytotechnology. 69, 157-165.
1507 [118] Ferrand, N., Béreziat, V., Moldes, M., Zaoui, M., Larsen, A.K. and Sabbah, M., 2017.
1508 WISP1/CCN4 inhibits adipocyte differentiation through repression of PPARγ activity,
1509 Scientific reports. 7, 1749.
1510 [119] Avau, B., Bauters, D., Steensels, S., Vancleef, L., Laermans, J., Lesuisse, J., Buyse,
1511 J., Lijnen, H.R., Tack, J. and Depoortere, I., 2015. The gustatory signaling pathway
1512 and bitter taste receptors affect the development of obesity and adipocyte metabolism
1513 in mice, PLoS One. 10, e0145538.
1514 [120] Torabi, S. and DiMarco, N.M., 2016. Polyphenols extracted from grape powder
1515 induce lipogenesis and glucose uptake during differentiation of murine preadipocytes,
1516 Experimental Biology and Medicine. 241, 1776-1785.
1517 [121] Christiaens, V., Sujatha, R., Hellemans, K.H., Pipeleers, D. and Lijnen, H.R., 2010.
1518 Functional interactions between pancreatic beta cells and (pre) adipocytes, Endocrine.
1519 38, 118-126.
1520 [122] Louis, F., Pannetier, P., Souguir, Z., Le Cerf, D., Valet, P., Vannier, J.P., Vidal, G.
1521 and Demange, E., 2017. A biomimetic hydrogel functionalized with adipose ECM
1522 components as a microenvironment for the 3D culture of human and murine
1523 adipocytes, Biotechnology and bioengineering. 114, 1813-1824.
1524 [123] Bauters, D., Scroyen, I., Van Hul, M. and Lijnen, H.R., 2015. Gelatinase A (MMP-2)
1525 promotes murine adipogenesis, Biochimica et Biophysica Acta (BBA)-General
1526 Subjects. 1850, 1449-1456.

1527 [124] Chapman, A.B., Knight, D., Dieckmann, B. and Ringold, G., 1984. Analysis of gene
1528 expression during differentiation of adipogenic cells in culture and hormonal control
1529 of the developmental program, Journal of Biological Chemistry. 259, 15548-15555.
1530 [125] Chapman, A.B., Knight, D.M. and Ringold, G.M., 1985. Glucocorticoid regulation of
1531 adipocyte differentiation: hormonal triggering of the developmental program and
1532 induction of a differentiation-dependent gene, The Journal of cell biology. 101, 1227-
1533 1235.
1534 [126] Knight, D.M., Chapman, A.B., Navre, M., Drinkwater, L., Bruno, J.J. and Ringold,
1535 G.M., 1987. Requirements for triggering of adipocyte differentiation by
1536 glucocorticoids and indomethacin, Molecular Endocrinology. 1, 36-43.
1537 [127] Danesch, U., Hoeck, W. and Ringold, G., 1992. Cloning and transcriptional regulation
1538 of a novel adipocyte-specific gene, FSP27. CAAT-enhancer-binding protein (C/EBP)
1539 and C/EBP-like proteins interact with sequences required for differentiation-
1540 dependent expression, Journal of Biological Chemistry. 267, 7185-7193.
1541 [128] Williams, P.M., Chang, D.J., Danesch, U., Ringold, G.M. and Heller, R.A., 1992.
1542 CCAAT/enhancer binding protein expression is rapidly extinguished in TA1
1543 adipocyte cells treated with tumor necrosis factor, Molecular Endocrinology. 6, 1135-
1544 1141.
1545 [129] Torti, F.M., Dieckmann, B., Beutler, B., Cerami, A. and Ringold, G.M., 1985. A
1546 macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia,
1547 Science. 229, 867-869.
1548 [130] Torti, F.M., Torti, S.V., Larrick, J.W. and Ringold, G.M., 1989. Modulation of
1549 adipocyte differentiation by tumor necrosis factor and transforming growth factor
1550 beta, The Journal of cell biology. 108, 1105-1113.
1551 [131] Reid, T.R., Torti, F. and Ringold, G., 1989. Evidence for two mechanisms by which
1552 tumor necrosis factor kills cells, Journal of Biological Chemistry. 264, 4583-4589.
1553 [132] Haliday, E.M., Ramesha, C.S. and Ringold, G., 1991. TNF induces c-fos via a novel
1554 pathway requiring conversion of arachidonic acid to a lipoxygenase metabolite, The
1555 EMBO journal. 10, 109-115.
1556 [133] Weber, P.D., Merkel, R. and Bergen, W., 1992. Adipogenic cell line TA1: A suitable
1557 model to study the effect of β-adrenergic agonists on lipid metabolism, Proceedings of
1558 the Society for Experimental Biology and Medicine. 201, 47-53.
1559 [134] Ibrahimi, A., Bonino, F., Bardon, S., Ailhaud, G. and Dani, C., 1992. Essential role of
1560 collagens for terminal differentiation of preadipocytes, Biochemical and biophysical
1561 research communications. 187, 1314-1322.
1562 [135] RINGOLD, G.M., CHAPMAN, A.B., KNIGHT, D.M., NAVRE, M. and TORTI,
1563 F.M., 1988. Hormonal control of adipocyte differentiation and adipocyte gene
1564 expression, Proceedings of the 1987 Laurentian Hormone Conference. Elsevier, pp.
1565 115-140.
1566 [136] Slieker, L.J., Sloop, K.W. and Surface, P.L., 1998. Differentiation method-dependent
1567 expression of leptin in adipocyte cell lines, Biochemical and biophysical research
1568 communications. 251, 225-229.
1569 [137] Doi, H., Masaki, N., Takahashi, H., Komatsu, H., Fujimori, K. and Satomi, S., 2005.
1570 A new preadipocyte cell line, AP-18, established from adult mouse adipose tissue,
1571 The Tohoku Journal of Experimental Medicine. 207, 209-216.
1572 [138] Negrel, R., Grimaldi, P. and Ailhaud, G., 1978. Establishment of preadipocyte clonal
1573 line from epididymal fat pad of ob/ob mouse that responds to insulin and to lipolytic
1574 hormones, Proceedings of the National Academy of Sciences. 75, 6054-6058.

1575 [139] Kamai, Y., Mikawa, S., Endo, K., Sakai, H. and Komano, T., 1996. Regulation of
1576 insulin-like growth factor-I expression in mouse preadipocyte Ob1771 cells, Journal
1577 of Biological Chemistry. 271, 9883-9886.
1578 [140] Grimaldi, P., Djian, P., Negrel, R. and Ailhaud, G., 1982. Differentiation of Ob17
1579 preadipocytes to adipocytes: requirement of adipose conversion factor (s) for fat cell
1580 cluster formation, The EMBO journal. 1, 687-692.
1581 [141] Amri, E.-Z., Dani, C., Doglio, A., Grimaldi, P. and Ailhaud, G., 1986. Coupling of
1582 growth arrest and expression of early markers during adipose conversion of
1583 preadipocyte cell lines, Biochemical and biophysical research communications. 137,
1584 903-910.
1585 [142] Ibrahimi, A., Abumrad, N., Maghareie, H., Golia, M., Shoshani, I., Désaubry, L. and
1586 Johnson, R.A., 1999. Adenylyl cyclase P-site ligands accelerate differentiation in
1587 Ob1771 preadipocytes, American Journal of Physiology-Cell Physiology. 276, C487-
1588 C496.
1589 [143] Ailhaud, G., Amri, E., Bertrand, B., Barcellini-Couget, S., Bardon, S., Catalioto, R.,
1590 Dani, C., Deslex, S., Djian, P. and Doglio, A., 1990. Obesity: Towards a Molecular
1591 Approach, AR Liss, New York. 219-236.
1592 [144] Amri, E.-Z., Barbaras, R., Doglio, A., Dani, C., Grimaldi, P. and Ailhaud, G., 1986.
1593 Role of spermidine in the expression of late markers of adipose conversion Effects of
1594 growth hormone, Biochemical Journal. 239, 363-370.
1595 [145] Gaillard, D., Negrel, R., Lagarde, M. and Ailhaud, G., 1989. Requirement and role of
1596 arachidonic acid in the differentiation of pre-adipose cells, Biochemical Journal. 257,
1597 389-397.
1598 [146] Amri, E., Ailhaud, G. and Grimaldi, P., 1994. Fatty acids as signal transducing
1599 molecules: involvement in the differentiation of preadipose to adipose cells, Journal
1600 of Lipid Research. 35, 930-937.
1601 [147] Ailhaud, G., Amri, E. and Grimaldi, P., 1995. Fatty acids and adipose cell
1602 differentiation, Prostaglandins, leukotrienes and essential fatty acids. 52, 113-115.
1603 [148] Dace, A., Martin-El Yazidi, C., Bonne, J., Planells, R. and Torresani, J., 1997.
1604 Calcitriol is a positive effector of adipose differentiation in the OB 17 cell line:
1605 relationship with the adipogenic action of triiodothyronine, Biochemical and
1606 Biophysical Research Communications. 232, 771-776.
1607 [149] Aubert, J., Safonova, I., Negrel, R. and Ailhaud, G., 1998. Insulin down-regulates
1608 angiotensinogen gene expression and angiotensinogen secretion in cultured adipose
1609 cells, Biochemical and biophysical research communications. 250, 77-82.
1610 [150] Aubert, J., Dessolin, S., Belmonte, N., Li, M., McKenzie, F.R., Staccini, L.,
1611 Villageois, P., Barhanin, B., Vernallis, A. and Smith, A.G., 1999. Leukemia inhibitory
1612 factor and its receptor promote adipocyte differentiation via the mitogen-activated
1613 protein kinase cascade, Journal of Biological Chemistry. 274, 24965-24972.
1614 [151] Laux, W.H., Pande, P., Shoshani, I., Gao, J., Boudou-Vivet, V., Gosselin, G. and
1615 Johnson, R.A., 2004. Pro-nucleotide inhibitors of adenylyl cyclases in intact cells,
1616 Journal of Biological Chemistry. 279, 13317-13332.
1617 [152] Doglio, A., Dani, C., Grimaldi, P. and Ailhaud, G., 1986. Growth hormone regulation
1618 of the expression of differentiation-dependent genes in preadipocyte Ob1771 cells,
1619 Biochemical Journal. 238, 123-129.
1620 [153] Reznikoff, C.A., Brankow, D.W. and Heidelberger, C., 1973. Establishment and
1621 characterization of a cloned line of C3H mouse embryo cells sensitive to
1622 postconfluence inhibition of division, Cancer research. 33, 3231-3238.
1623 [154] Reznikoff, C.A., Bertram, J.S., Brankow, D.W. and Heidelberger, C., 1973.
1624 Quantitative and qualitative studies of chemical transformation of cloned C3H mouse

1625 embryo cells sensitive to postconfluence inhibition of cell division, Cancer research.
1626 33, 3239-3249.
1627 [155] Taylor, S.M. and Jones, P.A., 1979. Multiple new phenotypes induced in 10T12 and
1628 3T3 cells treated with 5-azacytidine, Cell. 17, 771-779.
1629 [156] Haider, N. and Larose, L., 2020. Activation of the PDGFRα-Nrf2 pathway mediates
1630 impaired adipocyte differentiation in bone marrow mesenchymal stem cells lacking
1631 Nck1, Cell Communication and Signaling. 18, 1-12.
1632 [157] Moseti, D., Regassa, A., Chen, C. and Kim, W.K., 2020. 25-Hydroxycholesterol
1633 Inhibits Adipogenic Differentiation of C3H10T1/2 Pluripotent Stromal Cells,
1634 International Journal of Molecular Sciences. 21, 412.
1635 [158] Tang, Q.-Q., Otto, T.C. and Lane, M.D., 2004. Commitment of C3H10T1/2
1636 pluripotent stem cells to the adipocyte lineage, Proceedings of the National Academy
1637 of Sciences. 101, 9607-9611.
1638 [159] Wang, Z., Dai, Z., Luo, Z. and Zuo, C., 2019. Identification of Pyrvinium, an
1639 Anthelmintic Drug, as a Novel Anti-Adipogenic Compound Based on the Gene
1640 Expression Microarray and Connectivity Map, Molecules. 24, 2391.
1641 [160] Rajan, S., Puri, S., Kumar, D., Babu, M.H., Shankar, K., Varshney, S., Srivastava, A.,
1642 Gupta, A., Reddy, M.S. and Gaikwad, A.N., 2018. Novel indole and triazole based
1643 hybrid molecules exhibit potent anti-adipogenic and antidyslipidemic activity by
1644 activating Wnt3a/β-catenin pathway, European journal of medicinal chemistry. 143,
1645 1345-1360.
1646 [161] Zhang, J. and Choudhury, M., 2017. The plasticizer BBP selectively inhibits
1647 epigenetic regulator sirtuin during differentiation of C3H10T1/2 stem cell line,
1648 Toxicology in Vitro. 39, 75-83.
1649 [162] Guo, Y.-Y., Li, B.-Y., Peng, W.-Q., Guo, L. and Tang, Q.-Q., 2019. Taurine-mediated
1650 browning of white adipose tissue is involved in its anti-obesity effect in mice, Journal
1651 of Biological Chemistry. 294, 15014-15024.
1652 [163] Wang, S.J., Lu, W.Y. and Liu, K.Y., 2017. Adiponectin receptor agonist AdipoRon
1653 suppresses adipogenesis in C3H10T1/2 cells through the adenosine
1654 monophosphate-activated protein kinase signaling pathway, Molecular medicine
1655 reports. 16, 7163-7169.
1656 [164] Kim, J., Han, D., Byun, S.-H., Kwon, M., Cho, S.-J., Koh, Y.H. and Yoon, K., 2017.
1657 Neprilysin facilitates adipogenesis through potentiation of the phosphatidylinositol 3-
1658 kinase (PI3K) signaling pathway, Molecular and cellular biochemistry. 430, 1-9.
1659 [165] Shin, S., Seong, J.K. and Bae, Y.S., 2016. A hnak stimulates BMP 2-mediated
1660 adipocyte differentiation through S mad1 activation, Obesity. 24, 398-407.
1661 [166] Zhu, E., Zhang, J., Li, Y., Yuan, H., Zhou, J. and Wang, B., 2018. Long noncoding
1662 RNA Plnc1 controls adipocyte differentiation by regulating peroxisome proliferator–
1663 activated receptor γ, The FASEB Journal. 33, 2396-2408.
1664 [167] Imran, K.M., Yoon, D., Lee, T.-J. and Kim, Y.-S., 2018. Medicarpin induces lipolysis
1665 via activation of protein kinase A in brown adipocytes, BMB reports. 51, 249.
1666 [168] Yoon, D., Imran, K.M. and Kim, Y.-S., 2018. Distinctive effects of licarin A on
1667 lipolysis mediated by PKA and on formation of brown adipocytes from C3H10T1/2
1668 mesenchymal stem cells, Toxicology and applied pharmacology. 340, 9-20.
1669 [169] Imran, K.M., Rahman, N., Yoon, D., Jeon, M., Lee, B.-T. and Kim, Y.-S., 2017.
1670 Cryptotanshinone promotes commitment to the brown adipocyte lineage and
1671 mitochondrial biogenesis in C3H10T1/2 mesenchymal stem cells via AMPK and p38-
1672 MAPK signaling, Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology
1673 of Lipids. 1862, 1110-1120.

1674 [170] Rivera-Piza, A., An, Y.J., Kim, D.K., Lee, S.-H., Kim, J.-B., Choi, J.-S. and Lee, S.-
1675 J., 2017. Protocatechuic acid enhances osteogenesis, but inhibits adipogenesis in
1676 C3H10T1/2 and 3T3-L1 cells, Journal of medicinal food. 20, 309-319.
1677 [171] Li, Y., Goto, T., Ikutani, R., Lin, S., Takahashi, N., Takahashi, H., Jheng, H.F., Yu,
1678 R., Taniguchi, M. and Baba, K., 2016. Xanthoangelol and 4-hydroxyderrcin suppress
1679 obesity-induced inflammatory responses, Obesity. 24, 2351-2360.
1680 [172] Nishikawa, S., Aoyama, H., Kamiya, M., Higuchi, J., Kato, A., Soga, M., Kawai, T.,
1681 Yoshimura, K., Kumazawa, S. and Tsuda, T., 2016. Artepillin C, a typical Brazilian
1682 propolis-derived component, induces brown-like adipocyte formation in C3H10T1/2
1683 cells, primary inguinal white adipose tissue-derived adipocytes, and mice, PloS one.
1684 11, e0162512.
1685 [173] Chani, B., Puri, V., Sobti, R.C. and Puri, S., 2016. Epigallocatechin gallate inhibits
1686 mouse mesenchymal stem cell differentiation to adipogenic lineage, Journal of stem
1687 cells & regenerative medicine. 12, 16.
1688 [174] Cheng, K.-T., Wang, Y.-S., Chou, H.-C., Chang, C.-C., Lee, C.-K. and Juan, S.-H.,
1689 2015. Kinsenoside-mediated lipolysis through an AMPK-dependent pathway in
1690 C3H10T1/2 adipocytes: Roles of AMPK and PPARα in the lipolytic effect of
1691 kinsenoside, Phytomedicine. 22, 641-647.
1692 [175] Kusudo, T., Hashimoto, M., Kataoka, N., Li, Y., Nozaki, A. and Yamashita, H., 2018.
1693 CREG1 promotes uncoupling protein 1 expression and brown adipogenesis in vitro,
1694 The Journal of Biochemistry. 165, 47-55.
1695 [176] Yang, H.E., Li, Y., Nishimura, A., Jheng, H.F., Yuliana, A., Kitano-Ohue, R.,
1696 Nomura, W., Takahashi, N., Kim, C.S. and Yu, R., 2017. Synthesized enone fatty
1697 acids resembling metabolites from gut microbiota suppress macrophage-mediated
1698 inflammation in adipocytes, Molecular nutrition & food research. 61, 1700064.
1699 [177] Liu, X., Huang, T., Li, L., Tang, Y., Tian, Y., Wang, S. and Fan, C., 2015. CYP1B1
1700 deficiency ameliorates obesity and glucose intolerance induced by high fat diet in
1701 adult C57BL/6J mice, American journal of translational research. 7, 761.
1702 [178] Takahashi, A., Dohi, H., Egashira, Y. and Hirai, S., 2020. Erucic acid derived from
1703 rosemary regulates differentiation of mesenchymal stem cells into
1704 osteoblasts/adipocytes via suppression of peroxisome proliferator-activated receptor γ
1705 transcriptional activity, Phytotherapy Research.
1706 [179] Wang, H., Mao, X. and Du, M., 2019. Phytanic acid activates PPARα to promote
1707 beige adipogenic differentiation of preadipocytes, The Journal of Nutritional
1708 Biochemistry. 67, 201-211.
1709 [180] Seo, Y.-J., Kim, K.-J., Choi, J., Koh, E.-J. and Lee, B.-Y., 2018. Spirulina maxima
1710 extract reduces obesity through suppression of adipogenesis and activation of
1711 browning in 3T3-L1 cells and high-fat diet-induced obese mice, Nutrients. 10, 712.
1712 [181] You, Y., Yuan, X., Lee, H.J., Huang, W., Jin, W. and Zhan, J., 2015. Mulberry and
1713 mulberry wine extract increase the number of mitochondria during brown
1714 adipogenesis, Food & function. 6, 401-408.
1715 [182] Seo, S.H., Jo, S.-M., Kim, J., Lee, M., Lee, Y. and Kang, I., 2019. Peanut Sprout
1716 Extracts Attenuate Triglyceride Accumulation by Promoting Mitochondrial Fatty
1717 Acid Oxidation in Adipocytes, International journal of molecular sciences. 20, 1216.
1718 [183] Nakano, T., Kodama, H. and Honjo, T., 1994. Generation of lymphohematopoietic
1719 cells from embryonic stem cells in culture, Science. 265, 1098-1101.
1720 [184] Gao, J., Yan, X.-L., Li, R., Liu, Y., He, W., Sun, S., Zhang, Y., Liu, B., Xiong, J. and
1721 Mao, N., 2010. Characterization of OP9 as authentic mesenchymal stem cell line,
1722 Journal of Genetics and Genomics. 37, 475-482.

1723 [185] Lane, J.M., Doyle, J.R., Fortin, J.-P., Kopin, A.S. and Ordovás, J.M., 2014.
1724 Development of an OP9 derived cell line as a robust model to rapidly study adipocyte
1725 differentiation, PloS one. 9, e112123.
1726 [186] Ueno, H., Sakita-Ishikawa, M., Morikawa, Y., Nakano, T., Kitamura, T. and Saito,
1727 M., 2003. A stromal cell–derived membrane protein that supports hematopoietic stem
1728 cells, Nature immunology. 4, 457.
1729 [187] Lamas, A., Lopez, E., Carrio, R. and Lopez, D.M., 2016. Adipocyte and leptin
1730 accumulation in tumor-induced thymic involution, International journal of molecular
1731 medicine. 37, 133-138.
1732 [188] Tan, J., Wang, Y., Wang, S., Wu, S., Yuan, Z. and Zhu, X., 2019. Label-free
1733 quantitative proteomics identifies transforming growth factor β1 (TGF-β1) as an
1734 inhibitor of adipogenic transformation in OP9-DL1 cells and primary thymic stromal
1735 cells, Cell & bioscience. 9, 48.
1736 [189] Tan, J., Wang, Y., Wang, S., Zhang, N., Wu, S., Yuan, Z. and Zhu, X., 2017.
1737 Untargeted metabolomics analysis of adipogenic transformation in OP9-DL1 cells
1738 using liquid chromatography-mass spectrometry: Implications for thymic
1739 adipogenesis, Cell biology international. 41, 447-456.
1740 [190] Dhakal, S. and Lee, Y., 2019. Transient Receptor Potential Channels and Metabolism,
1741 Molecules and cells. 42, 569.
1742 [191] Kim, M.S., Muallem, S., Kim, S.H., Kwon, K.B. and Kim, M.S., 2019. Exosomal
1743 release through TRPML1-mediated lysosomal exocytosis is required for adipogenesis,
1744 Biochemical and biophysical research communications. 510, 409-415.
1745 [192] He, Z., Li, M., Zheng, D., Chen, Q., Liu, W. and Feng, L., 2015. Adipose tissue
1746 hypoxia and low-grade inflammation: a possible mechanism for ethanol-related
1747 glucose intolerance?, British Journal of Nutrition. 113, 1355-1364.
1748 [193] Campos, V., Rappaz, B., Kuttler, F., Turcatti, G. and Naveiras, O., 2018. High-
1749 throughput, nonperturbing quantification of lipid droplets with digital holographic
1750 microscopy, Journal of lipid research. 59, 1301-1310.
1751 [194] Jiang, Z., Di Wu, W.Y., Weng, J., Lai, P., Shi, P., Guo, X., Huang, G., Deng, Q.,
1752 Tang, Y. and Zhao, H., 2017. Defined, serum/feeder-free conditions for expansion
1753 and drug screening of primary B-acute lymphoblastic leukemia, Oncotarget. 8,
1754 106382.
1755 [195] Seo, Y.-S., Kang, O.-H., Kim, S.-B., Mun, S.-H., Kang, D.-H., Yang, D.-W., Choi, J.-
1756 G., Lee, Y.-M., Kang, D.-K. and Lee, H.-S., 2015. Quercetin prevents adipogenesis
1757 by regulation of transcriptional factors and lipases in OP9 cells, International journal
1758 of molecular medicine. 35, 1779-1785.
1759 [196] Kato, S., Kato, Y., Shibata, H., Saitoh, Y. and Miwa, N., 2015. Repressive effects of
1760 oat extracts on intracellular lipid-droplet formation in adipocytes and a three-
1761 dimensional subcutaneous adipose tissue model, Materials Science and Engineering:
1762 C. 49, 269-273.
1763 [197] Kennedy, D.E. and Knight, K.L., 2015. Inhibition of b lymphopoiesis by adipocytes
1764 and il-1–producing myeloid-derived suppressor cells, The Journal of Immunology.
1765 195, 2666-2674.
1766 [198] Schmitt, T.M. and Zúñiga-Pflücker, J.C., 2002. Induction of T cell development from
1767 hematopoietic progenitor cells by delta-like-1 in vitro, Immunity. 17, 749-756.
1768 [199] Holmes, R. and Zúñiga-Pflücker, J.C., 2009. The OP9-DL1 system: generation of T-
1769 lymphocytes from embryonic or hematopoietic stem cells in vitro, Cold Spring
1770 Harbor Protocols. 2009, pdb. prot5156.
1771 [200] Evans, M.J. and Kaufman, M.H., 1981. Establishment in culture of pluripotential cells
1772 from mouse embryos, Nature. 292, 154-156.

1773 [201] Martin, G.R., 1981. Isolation of a pluripotent cell line from early mouse embryos
1774 cultured in medium conditioned by teratocarcinoma stem cells, Proceedings of the
1775 National Academy of Sciences. 78, 7634-7638.
1776 [202] Nichols, J. and Smith, A., 2009. Naive and primed pluripotent states, Cell stem cell. 4,
1777 487-492.
1778 [203] Bradley, A., Evans, M., Kaufman, M.H. and Robertson, E., 1984. Formation of germ-
1779 line chimaeras from embryo-derived teratocarcinoma cell lines, Nature. 309, 255-256.
1780 [204] Stavridis, M. and Smith, A., 2003. Neural differentiation of mouse embryonic stem
1781 cells, Biochemical Society Transactions. 31, 45-49.
1782 [205] Keller, G.M., 1995. In vitro differentiation of embryonic stem cells, Current opinion
1783 in cell biology. 7, 862-869.
1784 [206] Phillips, B.W., Vernochet, C. and Dani, C., 2003. Differentiation of embryonic stem
1785 cells for pharmacological studies on adipose cells, Pharmacological research. 47, 263-
1786 268.
1787 [207] Dani, C., 1999. Embryonic stem cell-derived adipogenesis, Cells Tissues Organs. 165,
1788 173-180.
1789 [208] Chen, C.-Y., Lanz, R.B., Walkey, C.J., Chang, W.-H., Lu, W. and Johnson, D.L.,
1790 2018. Maf1 and Repression of RNA Polymerase III-Mediated Transcription Drive
1791 Adipocyte Differentiation, Cell reports. 24, 1852-1864.
1792 [209] Ota, K., Tong, K.I., Goto, K., Tomida, S., Komuro, A., Wang, Z., Nishio, K. and
1793 Okada, H., 2017. The H3K27 demethylase, Utx, regulates adipogenesis in a
1794 differentiation stage-dependent manner, PloS one. 12, e0173713.
1795 [210] Masia, F., Glen, A., Stephens, P., Langbein, W. and Borri, P., 2018. Label-free
1796 quantitative chemical imaging and classification analysis of adipogenesis using mouse
1797 embryonic stem cells, Journal of biophotonics. 11, e201700219.
1798 [211] Guerrero-Robles, C., Vazquez-Zapien, G., Mata-Miranda, M., Noriega-Gonzalez, J.
1799 and Gonzalez-Diaz, C., 2017. Electrical bioimpedance spectroscopy as biosensor
1800 technique to identify cells lineages and cell differentiation process, 2017 39th Annual
1801 International Conference of the IEEE Engineering in Medicine and Biology Society
1802 (EMBC). IEEE, pp. 3568-3571.
1803 [212] Bazou, D., Kearney, R., Mansergh, F., Bourdon, C., Farrar, J. and Wride, M., 2011.
1804 Gene expression analysis of mouse embryonic stem cells following levitation in an
1805 ultrasound standing wave trap, Ultrasound in medicine & biology. 37, 321-330.
1806 [213] Unser, A.M., Mooney, B., Corr, D.T., Tseng, Y.-H. and Xie, Y., 2016. 3D brown
1807 adipogenesis to create “Brown-Fat-in-Microstrands”, Biomaterials. 75, 123-134.
1808 [214] Kang, X., Xie, Y., Powell, H.M., Lee, L.J., Belury, M.A., Lannutti, J.J. and Kniss,
1809 D.A., 2007. Adipogenesis of murine embryonic stem cells in a three-dimensional
1810 culture system using electrospun polymer scaffolds, Biomaterials. 28, 450-458.
1811 [215] Billon, N., Kolde, R., Reimand, J., Monteiro, M.C., Kull, M., Peterson, H., Tretyakov,
1812 K., Adler, P., Wdziekonski, B. and Vilo, J., 2010. Comprehensive transcriptome
1813 analysis of mouse embryonic stem cell adipogenesis unravels new processes of
1814 adipocyte development, Genome biology. 11, R80.
1815 [216] Schaedlich, K., Knelangen, J.M., Navarrete Santos, A., Fischer, B. and Navarrete
1816 Santos, A., 2010. A simple method to sort ESC-derived adipocytes, Cytometry Part A.
1817 77, 990-995.
1818 [217] Cuaranta-Monroy, I., Simandi, Z. and Nagy, L., 2015. Differentiation of adipocytes in
1819 monolayer from mouse embryonic stem cells, Embryonic Stem Cell Protocols.
1820 Springer, pp. 407-415.
1821 [218] Singhal, P.K., Sassi, S., Lan, L., Au, P., Halvorsen, S.C., Fukumura, D., Jain, R.K.
1822 and Seed, B., 2016. Mouse embryonic fibroblasts exhibit extensive developmental

1823 and phenotypic diversity, Proceedings of the National Academy of Sciences. 113,
1824 122-127.
1825 [219] Nagy, A., Gertsenstein, M., Vintersten, K. and Behringer, R., 2006. Preparing mouse
1826 embryo fibroblasts, Cold Spring Harbor Protocols. 2006, pdb. prot4398.
1827 [220] Amand, M.M.S., Hanover, J.A. and Shiloach, J., 2016. A comparison of strategies for
1828 immortalizing mouse embryonic fibroblasts, Journal of Biological Methods. 3.
1829 [221] Xu, J., 2005. Preparation, culture, and immortalization of mouse embryonic
1830 fibroblasts, Current protocols in molecular biology. 70, 28.1. 1-28.1. 8.
1831 [222] Hogan, B., Costantini, F. and Lacy, E., 1986. Manipulating the mouse embryo: a
1832 laboratory manual.
1833 [223] Dastagir, K., Reimers, K., Lazaridis, A., Jahn, S., Maurer, V., Strauß, S., Dastagir, N.,
1834 Radtke, C., Kampmann, A. and Bucan, V., 2014. Murine embryonic fibroblast cell
1835 lines differentiate into three mesenchymal lineages to different extents: new models to
1836 investigate differentiation processes, Cellular Reprogramming (Formerly" Cloning
1837 and Stem Cells"). 16, 241-252.
1838 [224] Dobrowolski, P., Fischer, M. and Naumann, R., 2018. Novel insights into the genetic
1839 background of genetically modified mice, Transgenic research. 27, 265-275.
1840 [225] Durkin, M.E., Qian, X., Popescu, N.C. and Lowy, D.R., 2013. Isolation of mouse
1841 embryo fibroblasts, Bio Protoc. 3, e908.
1842 [226] Bjune, J.-I., Dyer, L., Røsland, G.V., Tronstad, K.J., Njølstad, P.R., Sagen, J.V.,
1843 Dankel, S.N. and Mellgren, G., 2020. The homeobox factor Irx3 maintains adipogenic
1844 identity, Metabolism. 103, 154014.
1845 [227] Mugabo, Y., Sadeghi, M., Fang, N.N., Mayor, T. and Lim, G.E., 2018. Elucidation of
1846 the 14-3-3ζ interactome reveals critical roles of RNA-splicing factors during
1847 adipogenesis, Journal of Biological Chemistry. 293, 6736-6750.
1848 [228] Singh, R., Braga, M., Reddy, S.T., Lee, S.-J., Parveen, M., Grijalva, V., Vergnes, L.
1849 and Pervin, S., 2017. Follistatin targets distinct pathways to promote brown adipocyte
1850 characteristics in brown and white adipose tissues, Endocrinology. 158, 1217-1230.
1851 [229] Han, Y., Lee, S.H., Bahn, M., Yeo, C.-Y. and Lee, K.Y., 2016. Pin1 enhances
1852 adipocyte differentiation by positively regulating the transcriptional activity of
1853 PPARγ, Molecular and cellular endocrinology. 436, 150-158.
1854 [230] Lee, K., Um, S.H., Rhee, D.K. and Pyo, S., 2016. Interferon-alpha inhibits
1855 adipogenesis via regulation of JAK/STAT1 signaling, Biochimica et Biophysica Acta
1856 (BBA)-General Subjects. 1860, 2416-2427.
1857 [231] Bauters, D., Scroyen, I., Deprez-Poulain, R. and Lijnen, H.R., 2016. ADAMTS5
1858 promotes murine adipogenesis and visceral adipose tissue expansion, Thrombosis and
1859 haemostasis. 116, 694-704.
1860 [232] Garfield, A.S., 2010. Derivation of primary mouse embryonic fibroblast (PMEF)
1861 cultures, Mouse Cell Culture. Springer, pp. 19-27.
1862 [233] Tu, W.-z., Fu, Y.-b. and Xie, X., 2019. RepSox, a small molecule inhibitor of the
1863 TGFβ receptor, induces brown adipogenesis and browning of white adipocytes, Acta
1864 Pharmacologica Sinica. 1.
1865 [234] Hunt, B.G., Wang, Y.-L., Chen, M.-S., Wang, S.-C. and Waltz, S.E., 2017. Maternal
1866 diethylhexyl phthalate exposure affects adiposity and insulin tolerance in offspring in
1867 a PCNA-dependent manner, Environmental research. 159, 588-594.
1868 [235] Pieralisi, A., Martini, C., Soto, D., Vila, M., Calvo, J.C. and Guerra, L.N., 2016. N-
1869 acetylcysteine inhibits lipid accumulation in mouse embryonic adipocytes, Redox
1870 biology. 9, 39-44.

1871 [236] Gao, M., Ding, Z., Shui, Y., Zhou, F. and Gao, J., 2016. Chrysin inhibits adipogenic
1872 differentiation of mouse embryonic fibroblasts, Zhongguo Zhong yao za zhi=
1873 Zhongguo zhongyao zazhi= China journal of Chinese materia medica. 41, 106-111.
1874 [237] Xu, S., Mao, L., Ding, P., Zhuang, X., Zhou, Y., Yu, L., Liu, Y., Nie, T., Xu, T. and
1875 Xu, Y., 2015. 1-Benzyl-4-phenyl-1H-1, 2, 3-triazoles improve the transcriptional
1876 functions of estrogen-related receptor γ and promote the browning of white adipose,
1877 Bioorganic & medicinal chemistry. 23, 3751-3760.
1878 [238] Wouters, K., Deleye, Y., Hannou, S.A., Vanhoutte, J., Maréchal, X., Coisne, A.,
1879 Tagzirt, M., Derudas, B., Bouchaert, E. and Duhem, C., 2017. The tumour suppressor
1880 CDKN2A/p16INK4a regulates adipogenesis and bone marrow-dependent
1881 development of perivascular adipose tissue, Diabetes and Vascular Disease Research.
1882 14, 516-524.
1883 [239] Zhang, L., Qiu, B., Wang, T., Wang, J., Liu, M., Xu, Y., Wang, C., Deng, R.,
1884 Williams, K. and Yang, Z., 2017. Loss of FKBP5 impedes adipocyte differentiation
1885 under both normoxia and hypoxic stress, Biochemical and biophysical research
1886 communications. 485, 761-767.
1887 [240] Xu, H., Fu, J.-L., Miao, Y.-F., Wang, C.-J., Han, Q.-F., Li, S., Huang, S.-Z., Du, S.-
1888 N., Qiu, Y.-X. and Yang, J.-C., 2016. Prostaglandin E2 receptor EP3 regulates both
1889 adipogenesis and lipolysis in mouse white adipose tissue, Journal of molecular cell
1890 biology. 8, 518-529.
1891 [241] Inoue, J., Ihara, Y., Tsukamoto, D., Yasumoto, K., Hashidume, T., Kamimura, K.,
1892 Nakai, Y., Hirano, S., Shimizu, M. and Kominami, R., 2016. Identification of
1893 BCL11B as a regulator of adipogenesis, Scientific reports. 6, 32750.
1894 [242] Matsumoto, M., Kondo, K., Shiraki, T., Brydun, A., Funayama, R., Nakayama, K.,
1895 Yaegashi, N., Katagiri, H. and Igarashi, K., 2016. Genomewide approaches for BACH
1896 1 target genes in mouse embryonic fibroblasts showed BACH 1-Pparg pathway in
1897 adipogenesis, Genes to Cells. 21, 553-567.
1898 [243] Takahashi, Y., Shinoda, A., Kamada, H., Shimizu, M., Inoue, J. and Sato, R., 2016.
1899 Perilipin2 plays a positive role in adipocytes during lipolysis by escaping proteasomal
1900 degradation, Scientific reports. 6, 1-14.
1901 [244] Myneni, V., Melino, G. and Kaartinen, M., 2015. Transglutaminase 2—a novel
1902 inhibitor of adipogenesis, Cell death & disease. 6, e1868.
1903 [245] Yu, H., He, K., Wang, L., Hu, J., Gu, J., Zhou, C., Lu, R. and Jin, Y., 2015. Stk40
1904 represses adipogenesis through translational control of CCAAT/enhancer-binding
1905 proteins, J Cell Sci. 128, 2881-2890.
1906 [246] Merkestein, M., Laber, S., McMurray, F., Andrew, D., Sachse, G., Sanderson, J., Li,
1907 M., Usher, S., Sellayah, D. and Ashcroft, F.M., 2015. FTO influences adipogenesis by
1908 regulating mitotic clonal expansion, Nature communications. 6, 6792.
1909 [247] Park, J.H. and Lee, S.B., 2015. An essential role for E wing sarcoma gene (EWS) in
1910 early white adipogenesis, Obesity. 23, 138-144.
1911 [248] Ferguson, D., Blenden, M., Hutson, I., Du, Y. and Harris, C.A., 2018. Mouse
1912 Embryonic Fibroblasts Protect ob/ob Mice From Obesity and Metabolic
1913 Complications, Endocrinology. 159, 3275-3286.
1914 [249] Martini, C.N., Gabrielli, M., Brandani, J.N. and Vila, M.d.C., 2016. Glyphosate
1915 inhibits PPAR gamma induction and differentiation of preadipocytes and is able to
1916 induce oxidative stress, Journal of biochemical and molecular toxicology. 30, 404-
1917 413.
1918 [250] Cawthorn, W.P., Scheller, E.L. and MacDougald, O.A., 2012. Adipose tissue stem
1919 cells meet preadipocyte commitment: going back to the future, Journal of lipid
1920 research. 53, 227-246.

1921 [251] Bourin, P., Bunnell, B.A., Casteilla, L., Dominici, M., Katz, A.J., March, K.L., Redl,
1922 H., Rubin, J.P., Yoshimura, K. and Gimble, J.M., 2013. Stromal cells from the
1923 adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-
1924 derived stromal/stem cells: a joint statement of the International Federation for
1925 Adipose Therapeutics and Science (IFATS) and the International Society for Cellular
1926 Therapy (ISCT), Cytotherapy. 15, 641-648.
1927 [252] Zhang, Q., Liu, L.-N., Yong, Q., Deng, J.-C. and Cao, W.-G., 2015. Intralesional
1928 injection of adipose-derived stem cells reduces hypertrophic scarring in a rabbit ear
1929 model, Stem cell research & therapy. 6, 145.
1930 [253] Kelly, K.A., Tanaka, S., Baron, R. and Gimble, J.M., 1998. Murine bone marrow
1931 stromally derived BMS2 adipocytes support differentiation and function of osteoclast-
1932 like cells in vitro, Endocrinology. 139, 2092-2101.
1933 [254] Zheng, B., Cao, B., Li, G. and Huard, J., 2006. Mouse adipose-derived stem cells
1934 undergo multilineage differentiation in vitro but primarily osteogenic and
1935 chondrogenic differentiation in vivo, Tissue engineering. 12, 1891-1901.
1936 [255] Kilroy, G., Dietrich, M., Wu, X., Gimble, J.M. and Floyd, Z.E., 2018. Isolation of
1937 murine adipose-derived stromal/stem cells for adipogenic differentiation or flow
1938 cytometry-based analysis, Adipose-Derived Stem Cells. Springer, pp. 137-146.
1939 [256] Grant, R.W. and Dixit, V.D., 2015. Adipose tissue as an immunological organ,
1940 Obesity. 23, 512-518.
1941 [257] Jankowski, M., Dompe, C., Sibiak, R., Wąsiatycz, G., Mozdziak, P., Jaśkowski, J.M.,
1942 Antosik, P., Kempisty, B. and Dyszkiewicz-Konwińska, M., 2020. In Vitro Cultures
1943 of Adipose-Derived Stem Cells: An Overview of Methods, Molecular Analyses, and
1944 Clinical Applications, Cells. 9, 1783.
1945 [258] Chu, D.-T., Nguyen Thi Phuong, T., Tien, N.L.B., Tran, D.K., Minh, L.B., Thanh,
1946 V.V., Gia Anh, P., Pham, V.H. and Thi Nga, V., 2019. Adipose tissue stem cells for
1947 therapy: An update on the progress of isolation, culture, storage, and clinical
1948 application, Journal of clinical medicine. 8, 917.

1949