**Lamin A/C mutation associated with lipodystrophy
influences adipogenic differentiation of stem cells through
interaction with Notch signaling**

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1 **Lamin A/C mutation associated with lipodystrophy influences adipogenic differentiation of**
2 **stem cells through interaction with Notch signaling**

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20 ABSTRACT

21 Lamin A/C is involved in many cellular functions due to its ability to bind chromatin and
 22 transcription factors and affect their properties. Mutations of *LMNA* gene encoding lamin A/C
 23 affect differentiation capacity of stem cells. However, signaling pathways involved in interaction
 24 with lamins in cellular differentiation remain unclear. Lipodystrophy associated with *LMNA*
 25 mutation R482L causes loss of fat tissue. In this study we investigated the role of *LMNA*
 26 mutation R482L in modulating Notch signaling activity in adipogenic differentiation of
 27 mesenchymal stem cells. Notch was activated using lentiviral Notch intracellular domain.
 28 Activation of Notch was estimated by expression of Notch-responsive genes by qPCR and by
 29 activation of luciferase CSL-reporter construct. Effect of *LMNA* mutation on Notch activation
 30 and adipogenic differentiation was analyzed in cells bearing lentiviral *LMNA WT* or *LMNA*
 31 *R482L*. We show that *LMNA R482L* contributes to down regulation of Notch activation in
 32 undifferentiated and differentiated cells and decreases adipogenic differentiation, when Notch is
 33 activated. Thus, lamin A/C interacts with Notch signaling thereby influencing cellular
 34 differentiation and point mutation in *LMNA* could halt this interaction.

35 key words: lamin A/C, Notch signaling, Dunnigan-type familial partial lipodystrophy,
 36 adipogenic differentiation

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39 INTRODUCTION

40 The nuclear lamina consists of intermediate filament proteins called lamins and primarily
41 localized at the inner side of the nuclear envelope (Aebi et al. 1986). Most differentiated somatic
42 cells express two A-type lamins, lamin A and lamin C, which are alternative splice variants of
43 one gene, *LMNA* (Lin and Worman 1993). The lamina has long been thought to ensure structural
44 support for the nuclear envelope. The discoveries that different mutations in *LMNA* are
45 associated with tissue-specific disorders has led to the suggestion that lamins have regulatory
46 functions and are essential for maintaining the differentiated state of post-embryonic somatic
47 tissues (Hutchison and Worman 2004).

48 How lamins regulate gene expression and cell differentiation remains largely unclear.
49 Direct binding of lamins to DNA, chromatin, nucleosomes and histones has been shown in many
50 studies, but the physiological relevance of these interactions is still unclear (Naetar et al. 2017).
51 Long heterochromatic domains associated with lamins have been identified and named lamina-
52 associated domains. A number of studies have shown that anchoring genes to the lamina
53 correlates with tissue-specific gene repression leading to the concept that tethering of genomic
54 regions to the lamina is required for stable repression of genes during differentiation (Amendola
55 and van Steensel 2014; Braun and Barrales 2016). In particular, it has been shown that lamin
56 A/C could bind to specific promoter subregions that regulate gene expression during adipogenic
57 differentiation (Lund et al. 2013). The underlying mechanism for how this interaction contributes
58 to gene regulation is unknown.

59 One of the diseases caused by autosomal-dominant inherited mutations in A-type lamins
60 is Dunnigan-type familial partial lipodystrophy (FPLD; OMIM #151660) (Speckman et al.
61 2000). FPLD primarily affects adipocytes and is characterized by progressive loss of
62 subcutaneous fat from upper and lower extremities (Hegele 2001). In FPLD, *LMNA* missense

63 mutations substitute a highly conserved arginine at position 482, or lysine at position 486 in the
64 C-terminal globular domain of lamin A/C (Shackleton et al. 2000). Several studies have shown
65 that A-type lamins can influence adipogenic differentiation of stem cells (Boguslavsky et al.
66 2006; Lund et al. 2013; Malashicheva et al. 2015), but cellular pathways affected by lamin A
67 interactions remain under investigated.

68 Lamin A/C interacts with the nuclear envelope protein emerin, which is absent or
69 mutated in X-linked Emery-Dreifuss muscular dystrophy (EDMD1) (Clements et al. 2000;
70 Sakaki et al. 2001). Anchorage of emerin at the nuclear rim is dependent upon lamin A/C
71 interaction (Sullivan et al. 1999). Failure to interact with lamin A in R482L mutated fibroblasts
72 from a FPLD patient has been shown for emerin (Capanni et al. 2003). Emerin, in turn, is able to
73 suppress Notch signaling (Lee et al. 2017). Involvement of lamins in regulating Notch has also
74 been shown for progerin, a mutant form of lamin A, resulted from a deletion in *LMNA* gene,
75 which causes premature-ageing disease Hutchinson-Gilford Progeria Syndrome (HGPS)
76 (Scaffidi and Misteli 2008).

77 The focus of this study was to analyze lamin A/C interaction with Notch pathway during
78 adipogenic differentiation. For this we studied the effect of the *LMNA* point mutation on Notch
79 activity and adipogenic differentiation of mesenchymal stem cells. We used lentiviruses bearing
80 either *LMNA WT* encoding wild type lamin A/C or lentivirus bearing *LMNA R482L* encoding
81 mutant lamin A/C associated with familial partial lipodystrophy. We show that *LMNA R482L*
82 contributes to down regulation of Notch activation in undifferentiated and differentiated stem
83 cells and decreases adipogenic differentiation, when Notch is activated.

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87 MATERIALS AND METHODS

88 **Human samples**

89 The study was performed according to Helsinki declaration and approval was obtained
90 from the local Ethics Committee of Almazov Federal Medical Research Centre. Written
91 informed consent was obtained from all subjects prior tissue biopsy.

92 **Cell culture**

93 Human and rat cardiac mesenchymal stem cells (cMSC) from myocardial tissue were
94 isolated, propagated and differentiated as described previously (Smits et al. 2009). Briefly,
95 myocardial tissue was dissected onto small clamps and digested by collagenase II (Worthington)
96 solution (1 mg/ml) for 2 hours at 37°C. Then cell suspension was passed through the 40 mkm
97 strainer, centrifuged at 300 g for 5 min and cells were resuspended in growth medium (22.5 %
98 EGM-2 (Stem Cell, USA), 67.5 % M199, 10 % fetal bovine serum (Hyclone), 1x nonessential
99 amino, 50 units/mL penicillin and 50 µg/mL streptomycin (Thermo Fisher Scientific), seeded on
100 0.1% gelatine-coated 96-well plate and clonally expanded. After expansion the cells were
101 maintained in Petri dishes (Corning, USA) in growth medium, at 37°C and 5% CO₂. cMSC
102 immunophenotype was verified with flow cytometer GuavaEasyCyte6 (Millipore, USA) using
103 CD33, CD45, CD117, CD90, CD105, CD73, CD146, CD56 monoclonal antibodies (BD, USA)
104 as previously described (Dmitrieva et al. 2015).

105 **Lentiviral constructs and transduction**

106 *LMNA WT* and *LMNA R482L* bearing lentiviral constructs were described previously
107 (Malashicheva et al. 2015) as well as Notch-intracellular domain (NICD) bearing lentiviral
108 construct (Kostina et al. 2016). Lentiviral packaging plasmids were a generous gift of D.Trono
109 (École Polytechnique Fédérale de Lausanne, Switzerland); pLVTHM was modified by the
110 addition of the T7 tag and chloramphenicol resistance gene (cm), resulting in the pLVTHM-T7-

111 cm vector. 12xCSL-luc construct was kind gift of prof. U. Lendahl (Karolinska Institute,
112 Stockholm, Sweden). The reporter sequence was subcloned into pLVTHM-T7-cm vector.

113 **Lentiviral production and transduction**

114 Lentiviral production was performed as described previously (Kostina et al. 2016).
115 Briefly, 100-mm dishes of nonconfluent 293T cells were cotransfected with 15 µg pLVTHM-T7-
116 NICD, 5.27 µg pMD2.G, and 9.73 µg packaging pCMV-dR8.74psPAX2 by calcium-phosphate
117 method. The following day the medium was changed to fresh, and the cells were incubated for
118 24 hours to obtain high-titer virus production. Produced lentivirus was concentrated from
119 supernatant by ultracentrifugation method at 20000 g for 2h, resuspended in 1% BSA/PBS and
120 frozen in aliquots at -80°C. Concentrated viral particles were added one hour after cMSC
121 trypsinisation; 6 h later the culture medium was changed.

122 Transgene expression from lentiviruses was verified as follows. For NICD-bearing virus
123 transduction of cMSC was verified by westernblotting for NICD. For *LMNA*-bearing viruses rat
124 cMSC were transduced with viruses bearing human *LMNA* and then immunocytochemical
125 staining with antibodies against human lamin A/C was done. The saturating efficiency of cMSC
126 transduction was 40–60% .

127 **Immunocytochemical staining**

128 After growing on cover slides, cells were fixed for 20 minutes in 1% paraformaldehyde
129 and permeablized in 1% BSA/0.1% Triton X-100/PBS for three minutes, followed by blocking
130 in 1% BSA/PBS for one hour. Then cells were incubated for one hour with primary antibodies:
131 against lamin A/C (Leica). Secondary antibodies conjugated with r Alexa488 (Invitrogen) were
132 used. DAPI was used to visualize nuclei. Microphotographs were taken using AxioObserver
133 Microscope (Zeiss) at x20 and x40 magnification with AxioVision software.

134

135 **Differentiation efficiency and analysis of microphotographs**

136 Intact cells and cells transduced with virus carrying wild type *LMNA* *WT* or mutant
137 *LMNA* cLMNA R482L were used in experiments. Adipogenic differentiation was induced in
138 control and transduced cells by addition 0.5 mM 3_isobutyl_1_methylxanthine, 1 μ M
139 dexamethasone, and 1 μ M insulin to the standard culture medium. All experiments were
140 performed with three biological replications, i.e. three independent experimental procedures. 14
141 days after the induction of differentiation the cells were stained with Oil Red O (Sigma, USA) to
142 reveal fully differentiated cells. The images of cell differentiation were analyzed for the ratio of
143 differentiated and undifferentiated cell areas with MosaiX software (Carl Zeiss Microsystems,
144 Germany). Expression of adipogenic markers were estimated after 3 days of differentiation using
145 qPCR.

146 **qPCR**

147 RNA from cultured cells was isolated using ExtractRNA (Eurogene, Russia). Total RNA
148 (1 μ g) was reverse transcribed with MMLV RT kit (Eurogen, Russia). Real-time PCR was
149 performed with 1 μ L cDNA and SYBRGreen PCRMastermix (Eurogen, Russia) in the Light
150 Cyclor system using specific forward and reverse primers for target genes. The thermocycling
151 conditions were as follows: 95 $^{\circ}$ C for five minutes, followed by 45 cycles at 95 $^{\circ}$ C for 15
152 seconds and 60 $^{\circ}$ C for one minute. A final heating step of 65 $^{\circ}$ C to 95 $^{\circ}$ C was performed to
153 obtain melting curves of the final PCR products. Corresponding gene expression level was
154 normalized to HPRT or GAPDH from the same samples. Changes in target genes expression
155 levels were calculated as fold differences using the comparative $\Delta\Delta$ CT method. Primers for
156 human *NOTCH1*, *HEY1*, *CCND3*, *CMYC*, *SLUG*, *PPARG*, *AP2* could be represented at request.

157 **Promoter activity assay**

Cells were transduced with lentivirus containing 12xCSL-luc reporter described above. In the construct the expression of the firefly luciferase gene is regulated by twelve CSL binding sites upstream of a minimal TK promoter and the level of CSL promoter activity indicates the transcriptional activation of Notch pathway. Cells were lysed using Luciferase Assay System (Promega) according to the manufacturer recommendations 48 h after transduction. Luciferase activity was measured with Synergy2 (BioTek, CIII A). Samples were normalized by protein content using Pierce BCA Protein Assay Kit (Thermo Scientific).

Statistics

Values are expressed as mean $\pm$ SD of triplicate experiments. Groups were compared using Student's t-test. A value of $P \leq 0.05$ was considered significant. Statistical analysis was performed by using R software (version 2.12.0; R Foundation for Statistical Computing, Vienna, Austria).

171 RESULTS

172 ***LMNA R482L* causes disruption of lamin organization in cMSC**

173 We analyzed distribution of lamin A/C in cMSC transduced with lentiviruses bearing
 174 wild type and mutant *LMNA*. For this we transduced rat cMSC with lentiviruses containing
 175 human sequences of *LMNA WT* and *LMNA R482L*, and then stained the cells with antibody
 176 specifically recognizing human lamin A/C (Fig.1). In control non-transduced cells and in the
 177 cells transduced with *LMNA WT* nuclei show lamin A/C at the nuclear rim with no alterations in
 178 nuclear morphology. The cells transduced with *LMNA R482L* show nuclear blebbing and
 179 alterations in nuclear morphology.

180 ***LMNA R482L* causes down regulation of Notch target genes in cMSC**

181 All further experiments were done using human cMSC. To estimate interaction of lamin
 182 A/C and Notch pathway we analyzed effect of mutant *LMNA* on expression of Notch target
 183 genes in human cMSC. We transduced cMSC with lentiviruses bearing *LMNA WT* or *LMNA*
 184 *R482L* and activated Notch signaling by transducing the cells with Notch-intracellular domain
 185 (NICD) and analyzed expression of *HEY1*, *SLUG*, *CMYC* (c-Myc), *CCND3* (cyclin D3), genes
 186 known to be among main Notch transcriptional targets (Andersson et al. 2011). NICD
 187 substantially activated expression of *HEY1* indicating activation of Notch signaling (Fig. 2).
 188 *LMNA R482L* caused statistically significant decrease in expression of *HEY1*, *SLUG*, *CMYC* (c-
 189 Myc), *CCND3* compared to *LMNA WT*. This data indicates that mutant lamin A/C counteracts
 190 Notch signaling activation.

191 ***LMNA R482L* impairs adipogenic differentiation of cMSC in the presence of Notch**
 192 **activation**

193 To prove adipogenic capacity of cMSC we induced adipogenic differentiation in the cells
194 by addition of specific factors to growth medium. After 14 days the cells demonstrated strong
195 adipogenic phenotype with accumulation of fat droplets revealed by Oil Red O staining (Fig. 3).
196 Adipogenic induction was also confirmed by early expression of specific adipogenic markers
197 *AP2* and *PPARG* 3 days after induction of adipogenic differentiation (Fig. 3).

198 To estimate interaction of lamin A/C and Notch pathway in adipogenic differentiation we
199 analyzed effect of mutant *LMNA* on expression of Notch target genes in differentiating cMSC.
200 We transduced cMSC with lentiviruses bearing *LMNA WT* or *LMNA R482L* and activated Notch
201 signaling by transducing the cells with Notch-intracellular domain (NICD) and induced
202 adipogenic differentiation in control and transduced cells. Fig. 4 shows that Notch activation
203 itself inhibited adipogenic differentiation, which was observed by counting the differentiated cell
204 area and by expression of *AP2* and *PPARG* (Fig 4a, Fig. 4b). In the absence of Notch activation
205 *LMNA R482L* did not have prominent effect on adipogenic differentiation of cMSC comparing to
206 *LMNA WT*. In contrast, in the presence of Notch activation *LMNA R482L* caused statistically
207 significant decrease in amount of differentiated cells as well as decrease in the expression of
208 adipogenic markers *AP2* and *PPARG*. Thus, we observed inhibiting action of *LMNA R482L* on
209 adipogenic differentiation of cMSC only in the presence of activated Notch signaling.

210 ***LMNA R482L* causes down regulation of Notch target genes in differentiated cMSC**

211 Next we sought to confirm contribution of Notch signaling to the observed influence of
212 *LMNA R482L* on downregulation of adipogenic differentiation. We transduced cMSC with
213 lentiviruses bearing *LMNA WT* or *LMNA R482L* and activated Notch signaling by transducing
214 the cells with Notch-intracellular domain (NICD) and induced adipogenic differentiation of
215 cMSC (Fig. 5). Expression of *HEY1* and *NOTCH1* decreased in the cells bearing *LMNA R482L*
216 compared to *LMNA WT*, when Notch was activated. Correspondingly, luciferase activity of CSL
217 reporter decreased in cMSC with *LMNA R482L* compared to *LMNA WT*.

218 DISCUSSION

219 Our data show that *LMNA R482L* contributes to down regulation of Notch activation in
220 undifferentiated and differentiated mesenchymal stem cells and decreases adipogenic
221 differentiation, when Notch is activated, thus supporting the hypothesis that lamin A/C interacts
222 with Notch pathway.

223 Mutations of *LMNA* that cause laminopathies with various phenotypes affect different
224 types of tissues such as muscular, fat or bone and reflect the ability of lamins A/C to influence
225 different types of cellular differentiation (Malashicheva et al. 2015). Direct binding of lamins to
226 different regions of chromatin in the course of cellular differentiation led to the concept that
227 differentiation might be dependent on repression/activation of genes involved in this binding
228 (Naetar et al. 2017), however specific pathways involved in these interactions are hardly known.

229 Structural anomalies of nuclear lamina caused by mutations in *LMNA* could contribute to
230 altered interaction of lamins with chromatin. Lamin A/C fails to functionally assemble in
231 fibroblasts bearing R482L derived from a FPLD patient (Capanni et al. 2003). In line with this
232 in our experiments lentiviral *LMNA R482L* caused nuclear structural abnormalities seen as
233 nuclear blebbing in cMSC.

234 Overexpression of lamin A has been reported to suppress adipogenic differentiation
235 (Bermeo et al. 2015; Boguslavsky et al. 2006; Tilgner et al. 2009; Vadrot et al. 2014). Moreover
236 it has recently been shown, that R482W mutation in *LMNA* affects the regulatory activity of
237 sterol response element binding protein 1 (SREBP1), a transcription factor that regulates
238 hundreds of genes involved in lipid metabolism and adipocyte differentiation (Vadrot et al.
239 2014), thus proposing the mechanism for FPLD-associated *LMNA* mutation inhibition of
240 adipogenesis. However, in our cellular model using mesenchymal stem cells of cardiac origin we
241 did not observe the inhibition of adipogenic differentiation by *LMNA R482L* alone comparing to

242 *LMNA* WT, but only, when Notch was activated. This difference could be related to variations in
243 cellular context and origin.

244 Notch is one of the key pathways ensuring development, differentiation and maintenance
245 of adult tissues (Andersson et al. 2011). Notch is known to be involved in regulation of
246 adipogenic differentiation (Shan et al. 2016) and mainly inhibits adipogenic differentiation.
247 Implication of Notch in the interactions with lamins A/C has been shown for progerin, a protein
248 resulted from a deletion of *LMNA* gene and causing premature aging (Scaffidi and Misteli 2008).
249 Our data show that *LMNA* R482L causes down regulation of activated Notch and acts
250 synergistically with Notch in inhibiting adipogenic differentiation.

251 In a recent report emerin, lamin binding partner, has been shown to be able to directly
252 interact with Notch intracellular domain thereby suppressing Notch activity (Lee et al. 2017).
253 According to another report emerin binding to R482L mutated lamin is impaired in the cells of
254 FLPD patient (Capanni et al. 2003). Our findings on Notch downregulation by *LMNA* R482L in
255 undifferentiated and differentiated cells are well in line with these reports. Interactions of lamin
256 with Notch might be, thus, mediated by emerin.

257 In conclusion, our data suggest that lamin A/C interacts with Notch signaling. Details and
258 outcomes of this interaction deserve further research for better understanding the role of A-type
259 lamins in cellular differentiation as well as the in pathology.

260 **Author contributions**

261 K.P. performed experiments, analyzed data, wrote manuscript, R.D., E.I., A.B., A.K. performed
262 experiments, analyzed data, A.M. designed research, analyzed data, wrote the manuscript.

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352 FIGURE CAPTIONS

353 Figure 1

354 Verification of transgene *LMNA* expression in cMSC. Rat cMSC were infected with saturating
355 concentrations of lentiviruses bearing human *LMNA WT* or *LMNA R482L* genes. The cells were
356 stained with anti lamin A antibody recognizing only human lamin A/C. Upper row: 20x
357 magnification; lower row 40x magnification. Human cMSC were used as a control (control) and
358 give uniform nuclear staining of lamin A/C at the nuclear rim. Transduction of rat cMSC with
359 lentivirus bearing human *LMNA WT* shows nuclei with normal appearance and without any
360 blebbing (*LMNA WT*). Transduction of rat cMSC with lentivirus bearing human *LMNA R482L*
361 shows nuclear blebbing (*LMNA WT*) in some of the transduced cells as an effect of the mutation

362

363 Figure 2

364 *LMNA R482L* affects Notch target gene expression in cMSC. Notch was activated in cMSC by
365 lentiviral transduction of Notch intracellular domain (NICD) and expression of Notch target
366 genes was verified with the presence of either *LMNA WT* (WT) or *LMNA R482L* (R482L). The
367 results are represented as mean±SD *p<0.05

368

369 Figure 3

370 cMSC are capable to adipogenic differentiation. Upper panel represents images of
371 undifferentiated (left) and differentiated cells 14 days after induction of adipogenic
372 differentiation with visible adipose droplets revealed by Oil red O staining. Lower panel
373 represents analysis of adipogenic marker expression by qPCR in undifferentiated cells (Undiff)

374 and after 14 days of adipogenic differentiation (Diff). The results are represented as mean±SD;
 375 **p <0.05

376

377 Figure 4

378 *LMNA R482L* affects differentiation ability of cMSC, when Notch is activated. Adipogenic
 379 differentiation of cMSC was induced with/without Notch induction via transduction with
 380 lentivirus bearing Notch-intracellular domain (NICD) and in the presence of either *LMNA WT*
 381 (WT) or *LMNA R482L* (R482L). (A) Left panel: images of differentiated cells after Oil Red O
 382 staining. Right panel: results of differentiated cell area counts. The results are represented as
 383 mean±SD, *p<0.05

384

385 Figure 5

386 *LMNA R482L* affects Notch target gene expression in differentiated cMSC. Adipogenic
 387 differentiation of cMSC was induced with/without Notch induction via transduction with
 388 lentivirus bearing Notch-intracellular domain (NICD) and in the presence of either *LMNA WT*
 389 (WT) or *LMNA R482L* (R482L. mRNA level of *NOTCH1* and *HEY1* was measured by qPCR.
 390 Notch-dependent transcription was measured as luciferase activity of CSL-luc reporter. The
 391 results are represented as mean±SD, *p<0.05

392

393

394

Figure 1

Verification of transgene LMNA expression in cMSC. Rat cMSC were infected with saturating concentrations of lentiviruses bearing human LMNA WT or LMNA R482L genes. The cells were stained with anti lamin A antibody recognizing only human lamin A/C. Upper row: 20x magnification; lower row 40x magnification. Human cMSC were used as a control (control) and give uniform nuclear staining of lamin A/C at the nuclear rim. Transduction of rat cMSC with lentivirus bearing human LMNA WT shows nuclei with normal appearance and without any blebbing (LMNA WT). Transduction of rat cMSC with lentivirus bearing human LMNA R482L shows nuclear blebbing (LMNA WT) in some of the transduced cells as an effect of the mutation

83x42mm (300 x 300 DPI)

Figure 2
LMNA R482L affects Notch target gene expression in cMSC. Notch was activated in cMSC by lentiviral transduction of Notch intracellular domain (NICD) and expression of Notch target genes was verified with the presence of either LMNA WT (WT) or LMNA R482L (R482L). The results are represented as mean±SD
*p<0.05

202x149mm (300 x 300 DPI)

Figure 3
cMSC are capable to adipogenic differentiation. Upper panel represents images of undifferentiated (left) and differentiated cells 14 days after induction of adipogenic differentiation with visible adipose droplets revealed by Oil red O staining. Lower panel represents analysis of adipogenic marker expression by qPCR in undifferentiated cells (Undiff) and after 14 days of adipogenic differentiation (Diff). The results are represented as mean $\pm$ SD; **p < 0.05

137x131mm (300 x 300 DPI)

Figure 4
LMNA R482L affects differentiation ability of cMSC, when Notch is activated. Adipogenic differentiation of cMSC was induced with/without Notch induction via transduction with lentivirus bearing Notch-intracellular domain (NICD) and in the presence of either LMNA WT (WT) or LMNA R482L (R482L). (A) Left panel: images of differentiated cells after Oil Red O staining. Right panel: results of differentiated cell area counts. The results are represented as mean±SD, *p<0.05

209x199mm (300 x 300 DPI)

LMNA R482L affects Notch target gene expression in differentiated cMSC. Adipogenic differentiation of cMSC was induced with/without Notch induction via transduction with lentivirus bearing Notch-intracellular domain (NICD) and in the presence of either LMNA WT (WT) or LMNA R482L (R482L). mRNA level of NOTCH1 and HEY1 was measured by qPCR. Notch-dependent transcription was measured as luciferase activity of CSL-luc reporter. The results are represented as mean $\pm$ SD, *p<0.05

104x237mm (300 x 300 DPI)