



HAL
open science

Vitamin D limits inflammation-linked microRNA expression in adipocytes in vitro and in vivo: A new mechanism for the regulation of inflammation by vitamin D

Esma Karkeni, Lauriane Bonnet, Julie Marcotrchino, Franck Tourniaire, Julien Astier, Jianping Ye, Jean-Francois Landrier

► To cite this version:

Esma Karkeni, Lauriane Bonnet, Julie Marcotrchino, Franck Tourniaire, Julien Astier, et al.. Vitamin D limits inflammation-linked microRNA expression in adipocytes in vitro and in vivo: A new mechanism for the regulation of inflammation by vitamin D. *Epigenetics*, 2018, 13 (2), pp.156-162. hal-01998541

HAL Id: hal-01998541

<https://amu.hal.science/hal-01998541v1>

Submitted on 29 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1 **Vitamin D limits inflammation linked microRNA expression in adipocytes in vitro and in**
2 **vivo : A new mechanism of the regulation of inflammation by vitamin D**

3

4 Esma Karkeni¹, Lauriane Bonnet¹, Julie Marcotorchino¹, Franck Tourniaire¹, Julien Astier¹,
5 Jianping Ye², Jean-François Landrier¹

6

7 ¹ NORT, Aix-Marseille Université, INRA, INSERM, 13000, Marseille, France

8 ² Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge,
9 Louisiana 70808, USA.

10

11 Abbreviated title : Vitamin D blunts inflammatory miRs

12 Keyterms : vitamin D, adipose tissue, adipocyte, anti-inflammatory strategy, miR, inflammation.

13

14 *Corresponding author and person to whom reprint requests should be addressed:*

15 Jean-François Landrier, UMR 1260 INRA/1062 INSERM/Université d'Aix-Marseille, 27 Bd Jean
16 Moulin, 13385 Marseille cedex 05, France. Phone: +33 4 91 29 41 17; Fax: +33 4 91 78 21 01; E-
17 mail: jean-francois.landrier@univ-amu.fr

18

19 Disclosure statement : The authors have nothing to disclose.

20

21 **Abstract**

22

23 Inflammation of adipose tissue is believed to be a contributing factor to many chronic diseases
24 associated with obesity. Vitamin D (VD) is now known to limit this metabolic inflammation by
25 decreasing inflammatory marker expression and leukocyte infiltration in adipose tissue. In this
26 study, we investigated the impact of VD on microRNA (miR) expression in inflammatory
27 conditions in human and mouse adipocytes, using high throughput methodology (miRNA PCR
28 arrays). Firstly, we identified three miRs (miR-146a, miR-150 and miR-155) positively regulated
29 by TNF α in human adipocytes. Interestingly, the expression of these miRs was strongly prevented
30 by 1,25(OH) $_2$ D preincubation. These results were partly confirmed in 3T3-L1 adipocytes (for miR-
31 146a and miR-150). The ability of VD to control the expression of these miRs was confirmed in
32 diet-induced obese mice: the levels of the three miRs were increased following High Fat (HF) diet
33 in epididymal white adipose tissue and reduced in HF diet fed mice supplemented with VD. The
34 involvement of NF- κ B signaling in the induction of these miRs was confirmed in vitro and in vivo
35 using aP2-p65 transgenic mice. Finally, the ability of VD to deactivate NF- κ B signaling, via p65
36 and I κ B phosphorylation inhibition in murine adipocyte was observed, and could constitute a
37 driving molecular mechanism.

38 This study demonstrated for the first time that VD modulates the expression of miRs in adipocytes
39 in vitro and in adipose tissue in vivo through its impact on NF- κ B signaling pathway, which could
40 represent a new mechanism of regulation of inflammation by VD.

41

42 **Keywords:** Inflammation; vitamin D; adipocyte; miRs; aP2-p65 transgenic mice, NF- κ B

43

44 **Introduction**

45

46 Adipose tissue was originally considered as a passive reservoir for energy storage, mechanical and
47 heat insulation, and participating to the regulation of thermogenesis ¹. Now, this tissue is also
48 known to synthesize and secrete a large variety of pro-inflammatory and anti-inflammatory
49 adipokines, cytokines and chemokines ², that act at both autocrine/paracrine and endocrine level ³.
50 Other markers such as microRNAs (miRs) have been associated with inflammatory response in
51 adipose tissue during obesity ⁴⁻⁶. miRs are small non-coding RNAs that can modulate
52 transcriptional networks, influencing most of biological processes. miRs regulate gene expression
53 by degrading complementary mRNA targets or by inhibition of traduction, depending of the
54 complementarity of sequence with the target mRNA ⁷. Several miRs are involved in adipocyte
55 differentiation ⁸, fat cell behavior ⁹ and oxidative stress ⁴. The relevance of miRs in adipose tissue
56 biology is increasingly recognized, being intrinsically linked to different pathways, including
57 obesity-related inflammation. This is notably the case for miR-155 which was reported to be
58 increased in inflamed adipocytes ¹⁰, but also in subcutaneous adipose tissue biopsies of obese
59 subjects ¹¹, and which displays a strong impact on the regulation of inflammatory tone and
60 adipocyte differentiation ¹¹

61 Similarly to the increased pro-inflammatory markers, a low vitamin D (VD) status is common and
62 well documented in obesity ¹². Whether low VD status is the consequence of obesity, or whether
63 this factor accelerates the development of obesity in humans is presently unknown. However, the
64 VD insufficiency could influence the development of obesity-related diseases, including
65 inflammation and insulin resistance ¹²⁻¹⁶. Indeed, it was shown that 1,25(OH)₂D significantly
66 decreased the release of cytokines and chemokines by human preadipocytes ¹⁷ and by human
67 adipocytes ¹⁸. We also demonstrated the anti-inflammatory properties of 1,25(OH)₂D in murine
68 and human adipocytes, in both basal and TNF α -stimulated conditions ¹⁹. Similarly, 1,25(OH)₂D

69 reduced the expression of cytokines in human adipose tissue biopsies submitted to IL-1 β
70 stimulation in vitro ²⁰. The molecular mechanisms have been investigated, and VDR and NF- κ B
71 signaling pathways and p38 MAP kinases were shown to be involved in 3T3-L1 adipocytes ¹⁹ and
72 in human adipocytes ²¹. Interestingly, Zoico et al. recently demonstrated that VD, similarly to
73 1,25(OH)₂D, was able to blunt the LPS-mediated pro-inflammatory effect in human adipocytes ²².
74 Using a microarray approach, we recently demonstrated that 1,25(OH)₂D was able to downregulate
75 a large set of chemokines in human and murine adipocytes, and consequently reduced macrophage
76 migration mediated by adipocyte-conditioned medium ²³. Finally, we demonstrated for the first
77 time this effect in vivo, in acute and chronic models of adipose inflammation ²³.

78

79 In the present study, we aimed to explore the impact of VD on adipose inflammation associated
80 miRs. To this end, we evaluated the impact of 1,25(OH)₂D on miR expression in inflammatory
81 conditions in human and mouse adipocytes, using a highthroughput methodology (miRNA PCR
82 arrays). We also confirmed in vivo the anti-inflammatory effects of VD in a chronic mice model of
83 adipose tissue inflammation and identified molecular mechanisms involved in miR regulation by
84 VD.

85

86 **Results**

87

88 **1,25(OH)₂D modulates microRNA expression in human and murine adipocytes**

89 To examine, in detail, the impact of 1,25(OH)₂D on the modulation of miRs in human adipocytes
90 submitted to inflammatory stress, human adipocytes primocultures were preincubated with or
91 without 1,25(OH)₂D for 24 h followed by incubation with TNF α . Using a miRNA PCR array
92 approach, we quantified 84 miRs and 6 housekeeping genes. Results analysis showed that 76 miRs
93 were expressed in adipocytes in basal condition. Among these miRs, only 3 were positively
94 regulated by TNF α in our conditions. As shown in Figure 1A, the inflammatory response mediated
95 by TNF α significantly increased the expression levels of miR-146a, miR-150 and miR-155 (4.3,
96 1.9 and 2.1-fold, respectively, Figure 1). Interestingly, their expression decreased in adipocytes
97 preincubated with 1,25(OH)₂D in inflammatory conditions by 2.4, 2.1 and 1.6-fold, respectively
98 compared with TNF α condition (Figure 1).

99 Our results were partly confirmed in 3T3-L1 adipocytes, where the levels of miR-146a and miR-
100 155 increased in TNF α -treated cells (by 6.7 and 8-fold, respectively) and decreased in the
101 adipocytes pretreated with 1,25(OH)₂D and incubated with TNF α (by 3.1 and 1.3-fold,
102 respectively, Figure 2). No effect on miR-150 was observed in this cell model.

103

104 **Vitamin D₃ supplementation limits microRNA expression in epididymal white adipose tissue**

105 To further examine the effect of VD on miR expression in vivo, a model of chronic inflammation
106 induced by a HF diet was used. In this preclinical model, the induction of the miR-146a, miR-150
107 and miR-155 levels was observed after 10 weeks of HF diet (by 2.4, 1.4 and 1.5-fold, respectively)
108 compared with control mice (Figure 3). Interestingly, VD supplementation for 10 weeks strongly
109 limited the expression of these three miRs in epididymal adipose tissue (2.1, 1.3 and 1.3-fold,
110 respectively, Figure 3).

111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130

1,25(OH)₂D limits microRNA expression through NF-κB signaling in 3T3-L1 adipocytes.

To identify signaling pathways involved in miR-146a and miR-155 regulation by TNFα, 3T3-L1 cells were incubated with specific inhibitors of MAP kinases (JNK, p38) and NF-κB signaling, and then stimulated or not with TNFα. NF-κB signaling inhibition resulted in a significant decrease in miR-146a and miR-155 expression (by 2.7- and 1.6-fold compared with TNFα condition) (Figure 4A) whereas no effect of JNK and p38 inhibitors were observed,. To confirm the role of NF-κB in miR-146a and miR-155 regulation in vivo, we used a transgenic model overexpressing NF-κB p65 subunit in adipose tissue (aP2-p65 mice ²⁴). Results showed that miR-146a and miR-155 were significantly increased in epididymal adipose tissue of aP2-p65 mice compared with controls (by 2.2- and 10-fold, respectively; Figure 4B). Altogether these data strongly support the specific role of NF-κB on the regulation of these two miRs.

Thus, we examined the impact of 1,25(OH)₂D preincubation (10 nM and 100 nM) for 24 hours followed by a 24-hour incubation with TNFα on NF-κB signaling. To this end, the phosphorylation levels of p65 and IκB were quantified by ELISA. Expectedly, the phosphorylation levels of p65 and IκB were significantly increased into 3T3-L1 adipocytes incubated with TNFα (by 4.1- and 3.6-fold, respectively), whereas incubation with 1,25(OH)₂D strongly limited the phosphorylation of p65 and IκB, suggesting that 1,25(OH)₂D reduced NF-κB activation in 3T3-L1 adipocytes (Figure 4C).

131 **Discussion**

132

133 Our results indicate that obesity-related inflammation increased the production of miR-146a, miR-
134 150 and miR-155 in adipocytes. We also reported a beneficial role of VD or its active form
135 (1,25(OH)₂D) on these miR expression in human and murine adipocytes, but also in inflamed
136 adipose tissue of mice. Molecular mechanisms for these effects were investigated and the NF-κB
137 signaling pathway is suggested to be involved in miR regulation and to be targeted by 1,25(OH)₂D.
138 If the anti-inflammatory effect of VD has largely been reported based on cytokines and chemokines
139 expression in adipocytes as described in the introduction, its impact regarding its ability to
140 modulate inflammatory-linked miRs has never been reported.

141

142 In a preliminary study, we identified miR-146a, miR-150 and miR-155 as adipocyte inflammatory
143 stimulus modulated miR. These miRs have been largely suspected to be involved in inflammatory
144 tone regulation. Indeed, miR-155, which is described as a typical multifunctional microRNA ²⁵,
145 has been associated with the regulation of different immune-related processes, such as
146 haematopoiesis ²⁶, innate immunity ²⁷, cancer ²⁸ and B-cell and T-cell differentiation ²⁹. In the
147 context of obesity onset and/or obesity comorbidities, we have recently shown that miR-155 was
148 induced in adipocytes submitted to inflammatory stress and in subcutaneous adipose tissue
149 biopsies of obese subjects ¹¹. We also showed that this miR induced inflammatory response,
150 chemokine expression, and macrophage migration in 3T3-L1 adipocytes ¹¹. A recent study also
151 demonstrated that the deletion of miR-155 in female mice prevented diet-induced obesity,
152 improved insulin sensitivity and energy uncoupling machinery and abrogated HF diet induced
153 adipocyte hypertrophy and white adipose tissue inflammation ³⁰. Together these data confirm the
154 importance of miR-155 in adipose tissue function, notably in the control of inflammation.

155 To the best of our knowledge, no specific effect of miR-146a in adipocyte or adipose tissue biology
156 has been reported. However, in inflammatory context, it has been reported that miR-146a is
157 involved in the control of innate immunity. Indeed, miR-146a was found to be inducible upon
158 stimulation with LPS in a NF- κ B-dependent manner in human monocytes³¹. Such induction of
159 miR-146a could contribute to the establishment of endotoxin tolerance in monocytes and to the
160 regulation of TNF α production³². In addition, exposure of human lung alveolar epithelial cells
161 resulted in a pronounced increase of miR-146a levels, that in turn induced a decrease of IL-8 and
162 RANTES chemokines³³. Finally, miR-146a deficiency during diabetes led to increased expression
163 of M1 activation markers and pro-inflammatory cytokines and suppression of M2 markers in
164 macrophages³⁴. Together these data strongly suggest that miR-146a displays an anti-inflammatory
165 effect. However, its role in adipose tissue will require further investigations.

166 Regarding miR-150, inflammatory or anti-inflammatory properties are less clear. A recent study
167 demonstrated that miR-150 could physiologically modulate metabolic activities and inflammatory
168 response both in cells and animals by regulating lipid metabolism and inflammatory response.
169 Indeed, overexpression of miR-150 in macrophages resulted in an increase in lipid accumulation,
170 associated with an elevated expression of several pro-inflammatory cytokines³⁵. Conversely, when
171 miR-150 knockout mice were challenged with a HF diet, these mice presented reduced whole body
172 weight with less fat accumulation, improved systemic glucose tolerance and insulin sensitivity³⁵.
173 In contrast, another study reported that miR-150 knockout mice showed exacerbated obesity-
174 associated tissue inflammation and systemic insulin resistance³⁶. Such versatility has already been
175 suggested in human cancer, where the role of miR-150 is context-dependent, i.e. this miR can have
176 either oncogenic or tumor suppressor activity in cells that originate from different tissues³⁷. Thus
177 its specific role in adipose tissue and notably in the control of inflammatory tone will require
178 further investigations.

179 To identify molecular mechanisms involved in miR regulation several approaches were combined.
180 First, we demonstrated that miR-146a and miR-155 regulations were primarily NF- κ B dependent
181 in 3T3-L1 adipocytes, through the use of specific NF- κ B inhibitor that blunted TNF α -mediated
182 induction of these two miRs. The involvement of this signaling pathway was also confirmed in
183 vivo, through the use of aP2-p65 transgenic mice which display a specific adipose tissue
184 overexpression of p65²⁴ and that presented a strong induction of these two miRs. Based on this
185 putative role of NF- κ B in the induction of miR-146a and miR-155, the effect of the VD on the
186 phosphorylation of two intermediates of the NF- κ B signaling pathway (p65 and I κ B) has been
187 investigated. As previously demonstrated^{19,23}, we reported that 1,25(OH)₂D has a strong limiting
188 effect on NF- κ B signaling in 3T3-L1 adipocytes through the reduction of the phosphorylation
189 levels of p65 and I κ B. This effect could be related to the ability of 1,25(OH)₂D to bind VDR and
190 to interact with IKK³⁸ or could result from the induction of phosphatases involved in the
191 dephosphorylation of p65 and I κ B. Altogether these data strongly suggested that the inhibition of
192 miRs by VD is largely mediated by its ability to reduce NF- κ B signaling.

193 Interestingly, we observed both in vitro in human and murine adipocytes and in vivo in mice, a
194 global upregulation of the miRs in pro-inflammatory conditions, suggesting that these three miRs,
195 independently of their inflammatory effect (pro- or anti-), actively participated to inflammatory
196 process in adipocytes and in adipose tissue. We also observed that VD, through the down-
197 regulation of these three miRs, displayed an overall blocking effect of the inflammatory pathway
198 in adipocytes. If the modulation of miRs by VD is reported here for the first time in the context of
199 obesity associated comorbidities, similar ability has been widely described in cancer context.
200 Indeed, in prostate cancer cells³⁹, in bladder cancer cells⁴⁰ or in lung cancer⁴¹, it has been shown
201 that 1,25(OH)₂D differentially regulated miR expression profiles.

202 These original observations not only reinforce the role of VD as an anti-inflammatory agent in
203 adipose tissue and adipocytes, but it also demonstrates the relevance of the miR regulation as new

204 anti-inflammatory mechanism in adipose tissue / adipocytes, that could represent an interesting
205 new target to limit metabolic inflammation.

206

207 **Material and Methods**

208

209 **Reagents** - Dulbecco's modified Eagle's medium (DMEM) was purchased from Life
210 Technologies (Cergy Pontoise, France), and fetal bovine serum (FBS) was obtained from PAA
211 Laboratories (Les Mureaux, France). Isobutylmethylxanthine, dexamethasone and insulin were
212 purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). TRIzol reagent was obtained
213 from Life Technologies (Saint Aubin, France). QuantiTect SYBR Green PCR Master Mix,
214 miScript Universal Primer and miScript Primer assays were purchased from Qiagen (Courtabœuf,
215 France).

216

217 **Cell culture** - Adipocyte cells were grown at 37 C in a 5% CO₂ humidified atmosphere. The human
218 preadipocytes (3 independent cultures) were obtained from Promocell and cultured according to
219 the manufacturer's instructions. The mature adipocytes (day 12) were incubated with 1,25(OH)₂D
220 (100 nM, 24 h) followed by a 24-hour incubation with TNF α (15 ng/mL). Experiments were
221 performed in triplicate, on 3 independent cultures.

222 The 3T3-L1 preadipocytes (ATCC, Manassas, VA) were seeded in 3.5 cm-diameter dishes at a
223 density of 15×10^4 cells/well, and grown in DMEM supplemented with 10% FBS, at 37 C, as
224 previously reported⁴². To induce differentiation, two-day postconfluent 3T3-L1 preadipocytes
225 (day 0) were stimulated for 72 h with 0.5 mM isobutylmethylxanthine, 0.25 μ mol/L
226 dexamethasone and 1 μ g/mL insulin in DMEM supplemented with 10% FBS. The cultures were
227 then treated with DMEM supplemented with 10% FBS and 1 μ g/mL insulin. The mature
228 adipocytes (d 8) were incubated with 1,25(OH)₂D (100 nM, 24 h) followed by a 24-hour incubation
229 with TNF α (15 ng/mL). Experiments were performed in triplicate, on 3 independent cultures.

230

231 **3T3-L1 adipocytes incubations** - To identify signaling pathways involved in microRNA
232 regulation, 3T3-L1 cells were treated with specific inhibitors of MAP (Mitogen Activated Protein)
233 kinases (JNK, p38) and NF- κ B signaling (JNK inhibitor II (10 μ M), SB 202190 (20 μ M) and BAY
234 117082 (10 μ M), respectively) for 1 h (all obtained from Calbiochem, Merck Millipore, Darmstadt,
235 Germany) and then stimulated or not with TNF α (15 ng/mL) for 24 h. All the treatments were
236 performed on day 8.

237

238 **NF- κ B activation** - The levels of p65 (Ser536) and I κ B α (Ser32/36) phosphorylation were
239 quantified using the ELISA Instant One kit according to the manufacturer's instructions
240 (eBiosciences SAS, Paris, France).

241

242 **miRNA PCR arrays** - The miRNA PCR arrays (Qiagen, Courtabœuf, France) were used to
243 quantify miRs extracted from human culture adipocytes, according to the manufacturer's
244 instructions. Reactions were performed in a 12.5 μ L volume containing 6.25 μ L of 2X QuantiTect
245 SYBR Green PCR Master Mix (Qiagen, Courtabœuf, France), 1.25 μ L of 10X miScript Universal
246 Primer (Qiagen, Courtabœuf, France). After an initial incubation step of 15 min at 95°C,
247 amplification reaction was performed in 40 cycles comprising 3 steps (94°C, 15 s; 55°C, 30 s and
248 70°C, 30 s). For each condition, the expression was quantified in duplicate and the SNORD68 was
249 used as the endogenous control in the comparative cycle threshold (C_T) method.

250

251 **RNA isolation and qPCR** - Total cellular RNA was extracted using TRIzol reagent according to
252 the manufacturer's instructions. To quantify miR-146a, miR-150 and miR-155, cDNAs were first
253 synthesized from 1 μ g of total RNA in 20 μ L using 5X miScript Hiflex Buffer, 10X nucleic mix
254 and miScript reverse transcriptase according to the manufacturer's instructions (Qiagen). Real-
255 time quantitative RT-PCR analyses were performed using the Mx3005P Real-Time PCR System

256 (Stratagene, La Jolla, CA) as previously described ⁴³. Reactions were performed in a 12.5 μ L
257 volume containing 6.25 μ L of 2X QuantiTect SYBR Green PCR Master Mix (Qiagen), 1.25 μ L of
258 10X miScript Universal Primer (Qiagen), 1.25 μ L of 10X miScript Primer Assay (Mm_miR146_1
259 miScript Primer Assay, Mm_miR-150_1 miScript Primer Assay, Mm_miR-155_1 miScript Primer
260 Assay and Hs_SNORD68 miScript Primer Assay (Qiagen)) and 2.5 μ L of RNase-free water. For
261 each condition, the expression was quantified in duplicate, and the SNORD68 was used as the
262 endogenous control in the comparative cycle threshold (C_T) method.

263

264 **Animal experiments** – The protocol was approved through the local ethics committee. Six-week-
265 old male C57BL/6J mice were obtained from Janvier (Le Genest Saint Isle, France). The mice
266 were fed ad libitum (chow diet A04, Safe, Augy, France), with full access to drinking water. The
267 animals were maintained at 22°C under a 12 h light: 12 h dark cycle with a 50% humidity level.
268 To assess the impact of vitamin D (cholecalciferol) on miRs expression, three experimental groups
269 of mice were fed ad libitum with a low (10% of total energy as fat; TD06416; Harlan, Indianapolis,
270 Indiana, USA) or HF diet, providing 45% of total energy as fat (TD06415, Harlan, Indianapolis,
271 Indiana, USA), supplemented (3000 IU/kg of body weight, i.e. around 90 UI/j/mouse, Sigma-
272 Aldrich, Saint Quentin Fallavier, France) or not (300 IU/kg of body weight, i.e. around 9
273 UI/j/mouse) with cholecalciferol. The mice (10 per group) were assigned into one of the three
274 experimental groups depending on the diet, i.e., control, HF and HF plus vitamin D (HFVD), for
275 10 weeks as previously described ⁴⁴. Epididymal adipose tissue was collected and stored at -80°C.
276 aP2-p65 mice were generated on the C57BL/6J background as described elsewhere ^{24, 45}. All of
277 the mice were housed in the animal facility at the Pennington Biomedical Research Center with a
278 12:12-h light-dark cycle and constant temperature (22–24°C). The male mice were fed with a chow
279 diet (MF 5001, 11% calorie in fat) and the epididymal fat tissue was collected at 20 weeks. The
280 mice were housed at four per cage with free access to water and diet.

281

282 **Statistical analysis** - The data are expressed as the means \pm SEM. Significant differences between
283 the control and treated group were determined using ANOVA, followed by the PLSD Fischer post
284 hoc test using Statview software, and $p < 0.05$ was considered statistically significant. Significant
285 differences between two groups were determined using *t* test.

286

287 **Acknowledgments**

288

289 This work was partially funded through grants from the Fondation Coeur et Artères (FCA).

290

291

292 **References**

- 293 1. Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an
294 endocrine and secretory organ. *Proc Nutr Soc* 2001; 60:329-39.
- 295 2. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol*
296 2011; 29:415-45.
- 297 3. Proenca AR, Sertie RA, Oliveira AC, Campaaa AB, Caminhotto RO, Chimin P, Lima FB.
298 New concepts in white adipose tissue physiology. *Braz J Med Biol Res* 2014; 0:0.
- 299 4. Arner P, Kulyte A. MicroRNA regulatory networks in human adipose tissue and obesity.
300 *Nat Rev Endocrinol* 2015; 11:276-88.
- 301 5. Ge Q, Brichard S, Yi X, Li Q. microRNAs as a new mechanism regulating adipose tissue
302 inflammation in obesity and as a novel therapeutic strategy in the metabolic syndrome. *J Immunol*
303 *Res* 2014; 2014:987285.
- 304 6. Hulsmans M, De Keyzer D, Holvoet P. MicroRNAs regulating oxidative stress and
305 inflammation in relation to obesity and atherosclerosis. *Faseb J* 2011; 25:2515-27.
- 306 7. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis,
307 function and decay. *Nat Rev Genet* 2010; 11:597-610.
- 308 8. Ortega FJ, Moreno-Navarrete JM, Pardo G, Sabater M, Hummel M, Ferrer A, Rodriguez-
309 Hermosa JI, Ruiz B, Ricart W, Peral B, et al. MiRNA expression profile of human subcutaneous
310 adipose and during adipocyte differentiation. *PLoS One* 2010; 5:e9022.
- 311 9. Oger F, Gheeraert C, Mogilenko D, Benomar Y, Molendi-Coste O, Bouchaert E, Caron S,
312 Dombrowicz D, Pattou F, Duez H, et al. Cell-specific dysregulation of microRNA expression in
313 obese white adipose tissue. *J Clin Endocrinol Metab* 2014; 99:2821-33.
- 314 10. Ortega FJ, Moreno M, Mercader JM, Moreno-Navarrete JM, Fuentes-Batllevell N, Sabater
315 M, Ricart W, Fernandez-Real JM. Inflammation triggers specific microRNA profiles in human
316 adipocytes and macrophages and in their supernatants. *Clin Epigenetics* 2015; 7:49.
- 317 11. Karkeni E, Astier J, Tourniaire F, El Abed M, Romier B, Gouranton E, Lin W, Borel P,
318 Salles J, Walrand S, et al. Obesity-associated inflammation induces microRNA-155 expression in
319 adipocytes and adipose tissue: outcome on adipocyte function. *J Clin Endocrinol Metab*
320 2016; jc20153410.
- 321 12. Landrier JF, Karkeni E, Marcotorchino J, Bonnet L, Tourniaire F. Vitamin D modulates
322 adipose tissue biology: possible consequences for obesity? *Proc Nutr Soc* 2015:1-9.
- 323 13. Garcia OP, Long KZ, Rosado JL. Impact of micronutrient deficiencies on obesity. *Nutr*
324 *Rev* 2009; 67:559-72.
- 325 14. Kaidar-Person O, Person B, Szomstein S, Rosenthal RJ. Nutritional deficiencies in
326 morbidly obese patients: a new form of malnutrition? Part A: vitamins. *Obes Surg* 2008; 18:870-
327 6.
- 328 15. Marcotorchino J, Tourniaire F, Landrier JF. Vitamin D, adipose tissue, and obesity. *Horm*
329 *Mol Biol Clin Investig* 2013; 15:123-8.

- 330 16. Mezza T, Muscogiuri G, Sorice GP, Prioletta A, Salomone E, Pontecorvi A, Giaccari A.
331 Vitamin D deficiency: a new risk factor for type 2 diabetes? *Ann Nutr Metab* 2012; 61:337-48.
- 332 17. Gao D, Trayhurn P, Bing C. 1,25-Dihydroxyvitamin D₃ inhibits the cytokine-induced
333 secretion of MCP-1 and reduces monocyte recruitment by human preadipocytes. *Int J Obes (Lond)*
334 2012; 37:357-65.
- 335 18. Lorente-Cebrian S, Eriksson A, Dunlop T, Mejhert N, Dahlman I, Astrom G, Sjolín E,
336 Wahlen K, Carlberg C, Laurencikiene J, et al. Differential effects of 1 α ,25-
337 dihydroxycholecalciferol on MCP-1 and adiponectin production in human white adipocytes. *Eur*
338 *J Nutr* 2012; 51:335-42.
- 339 19. Marcotorchino J, Gouranton E, Romier B, Tourniaire F, Astier J, Malezet C, Amiot MJ,
340 Landrier JF. Vitamin D reduces the inflammatory response and restores glucose uptake in
341 adipocytes. *Mol Nutr Food Res* 2012; 56:1771-82.
- 342 20. Wamberg L, Cullberg KB, Rejnmark L, Richelsen B, Pedersen SB. Investigations of the
343 anti-inflammatory effects of vitamin D in adipose tissue: results from an in vitro study and a
344 randomized controlled trial. *Horm Metab Res* 2013; 45:456-62.
- 345 21. Mutt SJ, Karhu T, Lehtonen S, Lehenkari P, Carlberg C, Saarnio J, Sebert S, Hypponen E,
346 Jarvelin MR, Herzig KH. Inhibition of cytokine secretion from adipocytes by 1,25-
347 dihydroxyvitamin D(3) via the NF-kappaB pathway. *Faseb J* 2012; 26:4400-7.
- 348 22. Zoico E, Franceschetti G, Chirumbolo S, Rossi AP, Mazzali G, Rizzatti V, Budui S,
349 Zamboni M. Phenotypic shift of adipocytes by cholecalciferol and 1 α ,25
350 dihydroxycholecalciferol in relation to inflammatory status and calcium content. *Endocrinology*
351 2014; 155:4178-88.
- 352 23. Karckeni E, Marcotorchino J, Tourniaire F, Astier J, Peiretti F, Darmon P, Landrier JF.
353 Vitamin D limits chemokine expression in adipocytes and macrophage migration in vitro and in
354 male mice. *Endocrinology* 2015; 156:1782-93.
- 355 24. Tang T, Zhang J, Yin J, Staszkiwicz J, Gawronska-Kozak B, Jung DY, Ko HJ, Ong H,
356 Kim JK, Mynatt R, et al. Uncoupling of inflammation and insulin resistance by NF-kappaB in
357 transgenic mice through elevated energy expenditure. *J Biol Chem* 2010; 285:4637-44.
- 358 25. Faraoni I, Antonetti FR, Cardone J, Bonmassar E. miR-155 gene: a typical multifunctional
359 microRNA. *Biochim Biophys Acta* 2009; 1792:497-505.
- 360 26. Masaki S, Ohtsuka R, Abe Y, Muta K, Umemura T. Expression patterns of microRNAs
361 155 and 451 during normal human erythropoiesis. *Biochem Biophys Res Commun* 2007; 364:509-
362 14.
- 363 27. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is
364 induced during the macrophage inflammatory response. *Proc Natl Acad Sci U S A* 2007;
365 104:1604-9.
- 366 28. Gironella M, Seux M, Xie MJ, Cano C, Tomasini R, Gommeaux J, Garcia S, Nowak J,
367 Yeung ML, Jeang KT, et al. Tumor protein 53-induced nuclear protein 1 expression is repressed
368 by miR-155, and its restoration inhibits pancreatic tumor development. *Proc Natl Acad Sci U S A*
369 2007; 104:16170-5.
- 370 29. Kluiver J, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, Kroesen BJ, van den
371 Berg A. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large
372 B cell lymphomas. *J Pathol* 2005; 207:243-9.
- 373 30. Gaudet AD, Fonken LK, Gushchina LV, Aubrecht TG, Maurya SK, Periasamy M, Nelson
374 RJ, Popovich PG. miR-155 Deletion in Female Mice Prevents Diet-Induced Obesity. *Sci Rep*
375 2016; 6:22862.
- 376 31. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of
377 microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc*
378 *Natl Acad Sci U S A* 2006; 103:12481-6.

- 379 32. Nahid MA, Pauley KM, Satoh M, Chan EK. miR-146a is critical for endotoxin-induced
380 tolerance: IMPLICATION IN INNATE IMMUNITY. *J Biol Chem* 2009; 284:34590-9.
- 381 33. Perry MM, Williams AE, Tsitsiou E, Lerner-Svensson HM, Lindsay MA. Divergent
382 intracellular pathways regulate interleukin-1beta-induced miR-146a and miR-146b expression and
383 chemokine release in human alveolar epithelial cells. *FEBS Lett* 2009; 583:3349-55.
- 384 34. Bhatt K, Lanting LL, Jia Y, Yadav S, Reddy MA, Magilnick N, Boldin M, Natarajan R.
385 Anti-Inflammatory Role of MicroRNA-146a in the Pathogenesis of Diabetic Nephropathy. *J Am*
386 *Soc Nephrol* 2015.
- 387 35. Luo N, Garvey WT, Wang D-Z, Fu Y. MicroRNA-150 Regulates Lipid Metabolism and
388 Inflammatory Response. *J Metabolic Syndr* 2013; 2.
- 389 36. Ying W, Tseng A, Chang RC, Wang H, Lin YL, Kanameni S, Brehm T, Morin A, Jones
390 B, Splawn T, et al. miR-150 regulates obesity-associated insulin resistance by controlling B cell
391 functions. *Sci Rep* 2016; 6:20176.
- 392 37. Moles R, Bellon M, Nicot C. STAT1: A Novel Target of miR-150 and miR-223 Is Involved
393 in the Proliferation of HTLV-I-Transformed and ATL Cells. *Neoplasia* 2015; 17:449-62.
- 394 38. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC. Vitamin D receptor inhibits nuclear factor
395 kappaB activation by interacting with IkappaB kinase beta protein. *J Biol Chem* 2013; 288:19450-
396 8.
- 397 39. Kutmon M, Coort SL, de Nooijer K, Lemmens C, Evelo CT. Integrative network-based
398 analysis of mRNA and microRNA expression in 1,25-dihydroxyvitamin D3-treated cancer cells.
399 *Genes Nutr* 2015; 10:484.
- 400 40. Ma Y, Hu Q, Luo W, Pratt RN, Glenn ST, Liu S, Trump DL, Johnson CS.
401 1alpha,25(OH)2D3 differentially regulates miRNA expression in human bladder cancer cells. *J*
402 *Steroid Biochem Mol Biol* 2015; 148:166-71.
- 403 41. Guan H, Liu C, Chen Z, Wang L, Li C, Zhao J, Yu Y, Zhang P, Chen W, Jiang A. 1,25-
404 Dihydroxyvitamin D3 up-regulates expression of hsa-let-7a-2 through the interaction of
405 VDR/VDRE in human lung cancer A549 cells. *Gene* 2013; 522:142-6.
- 406 42. Landrier JF, Gouranton E, El Yazidi C, Malezet C, Balaguer P, Borel P, Amiot MJ.
407 Adiponectin expression is induced by vitamin E via a peroxisome proliferator-activated receptor
408 gamma-dependent mechanism. *Endocrinology* 2009; 150:5318-25.
- 409 43. Landrier JF, Malezet-Desmoulins C, Reboul E, Marie Lorec A, Josephe Amiot M, Borel
410 P. Comparison of different vehicles to study the effect of tocopherols on gene expression in
411 intestinal cells. *Free Radic Res* 2008; 42:523-30.
- 412 44. Marcotorchino J, Tourniaire F, Astier J, Karkeni E, Canault M, Amiot MJ, Bendahan D,
413 Bernard M, Martin JC, Giannesini B, et al. Vitamin D protects against diet-induced obesity by
414 enhancing fatty acid oxidation. *J Nutr Biochem* 2014; 25:1077-83.
- 415 45. Tourniaire F, Romier-Crouzet B, Lee JH, Marcotorchino J, Gouranton E, Salles J, Malezet
416 C, Astier J, Darmon P, Blouin E, et al. Chemokine Expression in Inflamed Adipose Tissue Is
417 Mainly Mediated by NF-kappaB. *PLoS One* 2013; 8:e66515.

418

419

420 **Legends for figures**

421

422 **Figure 1. 1,25(OH)₂D limits microRNA expression levels in human adipocytes.** Cells were
423 preincubated with 1,25(OH)₂D (100 nM) for 24 h and incubated with TNF α (15 ng/mL) for 24 h.
424 The miR expression levels were quantified through miRNA PCR arrays approach. SNORD68 was
425 used as the endogenous control. The values are presented as the means \pm SEM. Bars not sharing
426 the same letters are significantly different, $p < 0.05$. Experiments were reproduced in triplicate, at
427 least 3 independent times.

428

429 **Figure 2. 1,25(OH)₂D limits microRNA expression levels in murine adipocytes.** 3T3-L1
430 adipocytes were preincubated with 1,25(OH)₂D (100 nM) for 24 h and incubated with TNF α (15
431 ng/mL) for 24 h. The expression levels of miR-146a and miR-155 were quantified through qPCR
432 in 3T3-L1 adipocytes. SNORD68 was used as the endogenous control. The values are presented
433 as the means \pm SEM. Bars not sharing the same letters are significantly different, $p < 0.05$.
434 Experiments were reproduced in triplicate, at least 3 independent times.

435

436 **Figure 3. Vitamin D limits miR-146a, miR-150 and miR-155 expression in mice epididymal**
437 **adipose tissue.** The expression levels of miR-146a, miR-150 and miR-155 were quantified through
438 qPCR in epididymal adipose tissue of mice fed standard, HF or VD-supplemented HF diets for 10
439 weeks (n=10) and expressed relative to SNORD68 in the white adipose tissue. The data are
440 expressed as relative expression ratios. The values are presented as the means \pm SEM. Bars not
441 sharing the same letters are significantly different, $p < 0.05$.

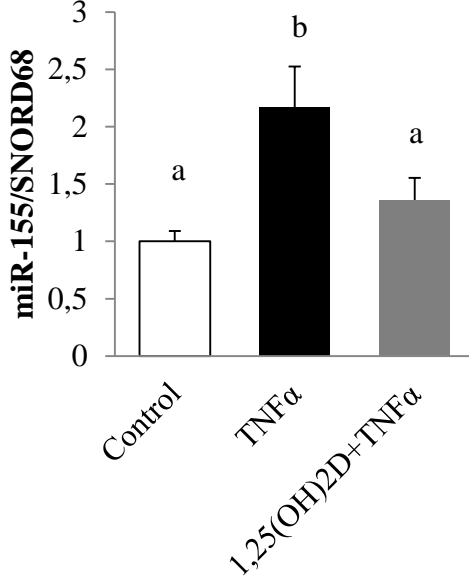
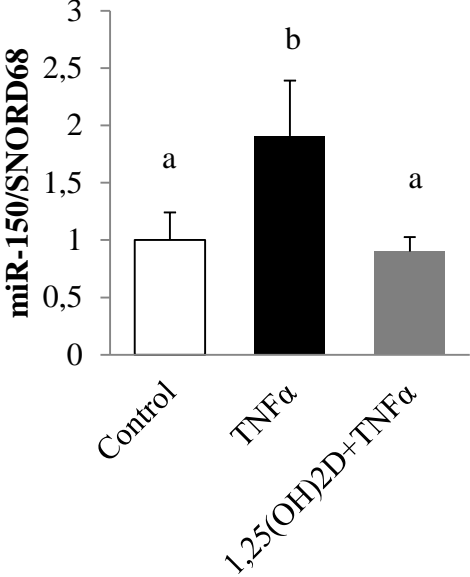
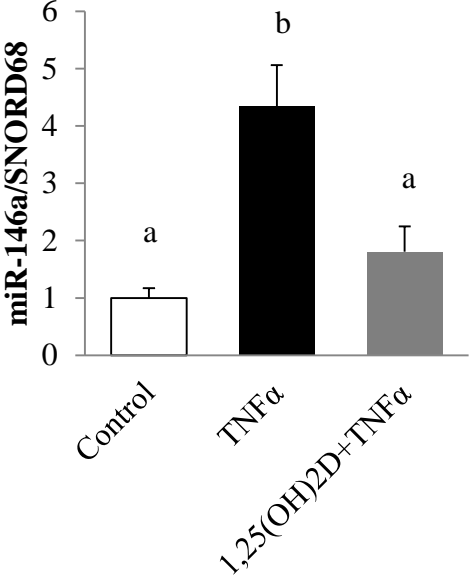
442

443 **Figure 4. 1,25(OH)₂D limits NF- κ B activation. A.** 3T3-L1 cells were treated with specific
444 inhibitors of MAP (Mitogen Activated Protein) kinases (JNK, p38) or NF- κ B signaling (JNK

445 inhibitor II (10 μ M), SB 202190 (20 μ M) and BAY 117082 (10 μ M), respectively) for 1 h and
446 then stimulated with TNF α (15 ng/mL) for 24 h, and miR-146a and miR-155 expression were
447 measured by qPCR. SNORD68 was used as the endogenous control. The data are expressed as
448 relative expression ratios. The values are presented as means \pm SEM. Bars not sharing the same
449 letters are significantly different, $p < 0.05$. Experiments were reproduced in triplicate, at least 3
450 independent times. **B.** The expression of miR-146a and miR-155 were quantified by qPCR in
451 epididymal adipose tissue of aP2-p65 transgenic mice. SNORD68 was used as the endogenous
452 control. The data are expressed as relative expression ratios. The values are presented as means \pm
453 SEM. Bars not sharing the same letters are significantly different, $p < 0.05$. **C.** The cells were
454 preincubated with 1,25(OH) $_2$ D (10 and 100 nM) for 24 h in dose-dependent manner and incubated
455 with TNF α (15 ng/mL) for 5 min. The phosphorylation levels of the NF- κ B subunits (p65 and I κ B)
456 were evaluated using ELISA. The data are expressed as relative expression ratios. The values are
457 presented as the means \pm SEM. Values not sharing the same letters are significantly different, $p <$
458 0.05 . Experiments were reproduced in triplicate, at least 3 independent times.

459

Figure 1



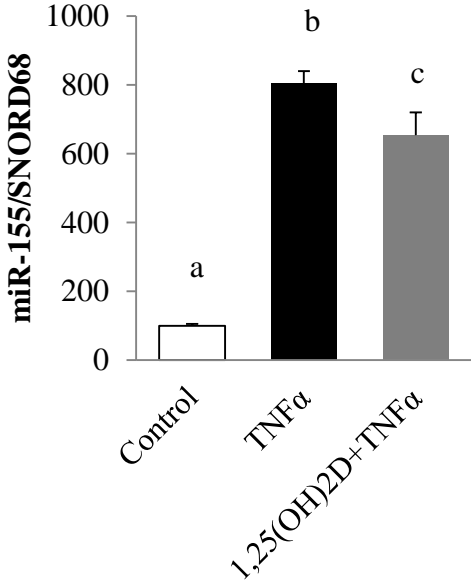
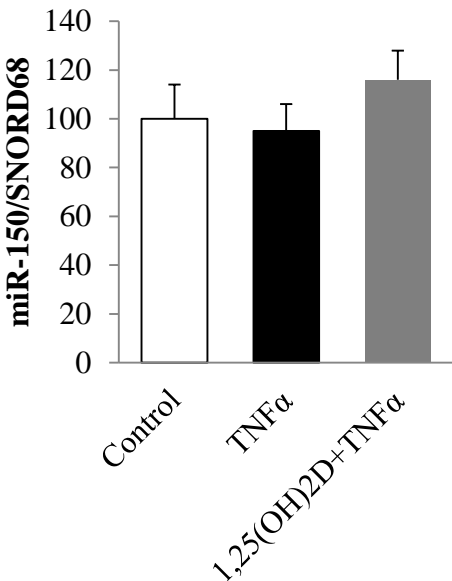
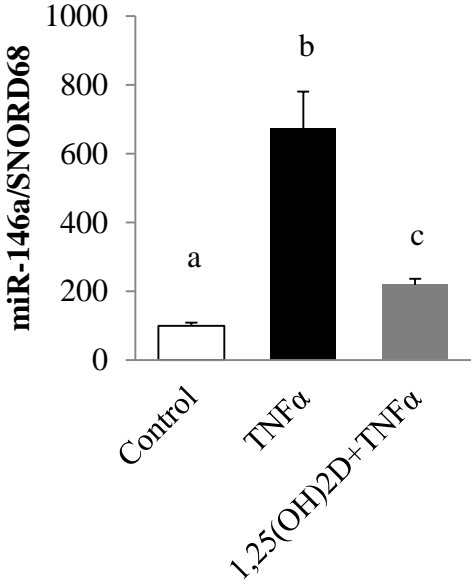
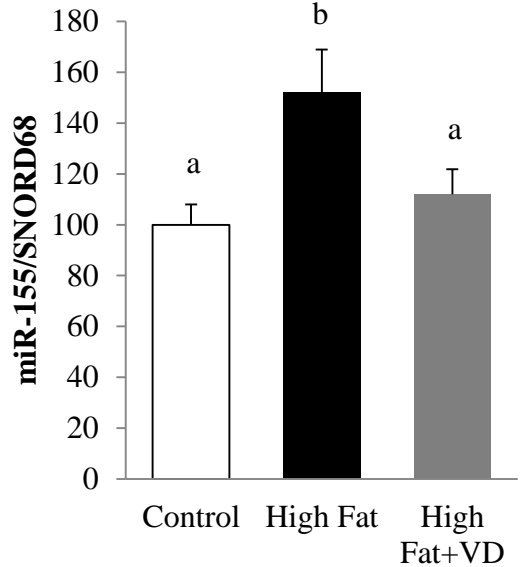
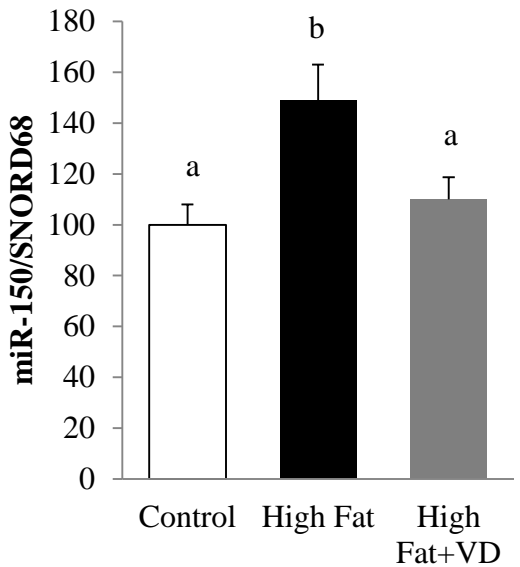
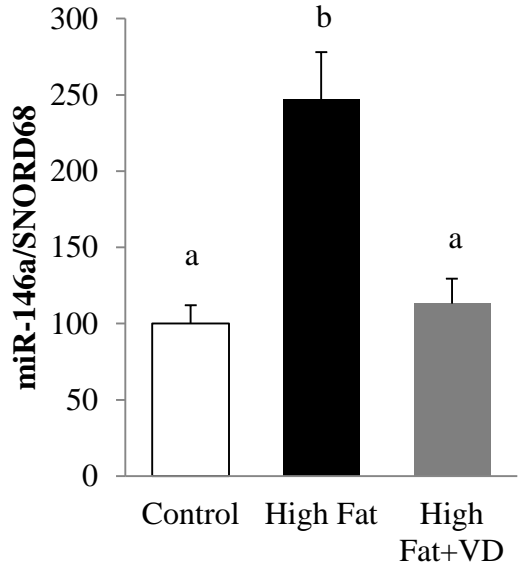
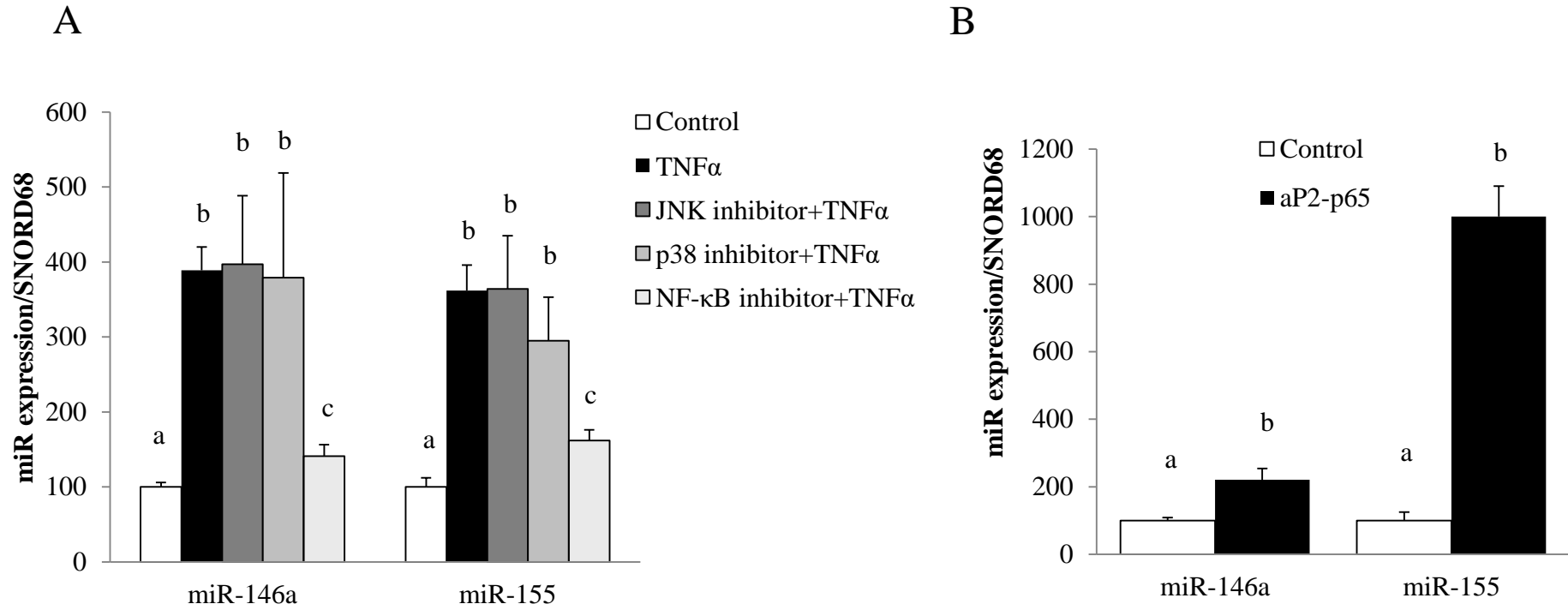


Figure 3





C

	Control	1,25(OH) ₂ D (10 nM)	1,25(OH) ₂ D (100 nM)	TNF α	1,25(OH) ₂ D (10 nM) +TNF α	1,25(OH) ₂ D (100 nM) +TNF α
p65 phosphorylation	0.61±0.01 ^a	0.87±0.014 ^a	0.73±0.01 ^a	2.53±0.013 ^b	1.14±0.008 ^a	1.27±0.025 ^a
I κ B phosphorylation	0.61±0.007 ^a	0.44±0.005 ^a	0.62 ±0.013 ^a	2.2±0.021 ^b	0.99±0.01 ^a	0.85±0.01 ^a