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CORRESPONDENCE

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FNDC4 and FNDC5 reduce SARS-CoV-2 entry points and spike glycoprotein S1-induced pyroptosis, apoptosis, and necroptosis in human adipocytes

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A sedentary lifestyle and obesity are important risk factors for severe COVID-19 complications and death [1, 2]. Excessive adipose tissue (AT) might contribute to more extensive viral spread with increased shedding, immune activation, and cytokine amplification [3]. Several factors secreted by contracting muscle, termed myokines, mediate the beneficial effects of exercise in a wide range of diseases, including obesity [4]. Thus, we conducted a study to explore the potential role of myokines of the fibronectin type III domain-containing family, FNDC4 and FNDC5, in mechanisms underlying the increased susceptibility to COVID-19 complications in obesity.

Patients with obesity (see Supplementary Table 1 for the clinical characteristics of the cohort) exhibited overexpression of all the components for SARS-CoV-2 host cell entry (*ACE2, CD147, DPP4*, and *NRP1*) and the spike protein processing enzyme *FURIN* in visceral AT (VAT) as well as *NRP1* upregulation in subcutaneous fat (SAT) (Supplementary Fig. 1), which might increase their susceptibility to SARS-CoV-2 infection. To date, the potential beneficial effects of FNDC4 and FNDC5 on COVID-19 outcomes include anti-obesity [5–7] and anti-inflammatory [4, 5] activities, such as inhibiting macrophage M1 polarization and proinflammatory cytokine production in AT. Interestingly, RNAseq-based data revealed that FNDC5 affects multiple genes related to SARS-CoV-2 infection in human subcutaneous adipocytes [8]. In agreement with this potential antiviral action, we observed that treatment of human visceral adipocytes with different concentrations of FNDC4 and FNDC5 decreased the mRNA levels of the SARS-CoV-2 host cell receptors *ACE2, CD147*, *NRP1*, and, to a lesser extent, *DPP4* (Fig. 1A, B). Interestingly, *FNDC4* and *FNDC5* gene silencing resulted in increased *ACE2*, *DPP4, FURIN*, and *NRP1* mRNA levels in adipocytes (Fig. 1C), highlighting the relevance of these myokines in modulating critical points of SARS-CoV-2 entry and priming in AT.

Another mechanism linking cytokine storm and organ damage during SARS-CoV-2 infection is cell death [9, 10]. Several SARS-CoV-2 viral proteins can induce inflammatory cell death called PANoptosis, which engages three programmed cell death pathways: pyroptosis, apoptosis, and necroptosis. We found that treatment with SARS-CoV-2 spike protein subunit 1 (S1) (10 ng/mL) for 24 h induced inflammatory cell death by pyroptosis through the activation of inflammasome components (NLRP3, ASC, and active caspase-1), cleavage of gasdermin D (GSDMD) (Fig. 1D) and secretion of the downstream proinflammatory mediator IL-1β into adipocyte culture media (Fig. 1E). Interestingly, the coincubation of S1 with FNDC4 (10 ng/mL) or FNDC5 (10 ng/mL) blunted inflammasome activation and GSDMD processing (Fig. 1D) as well as IL-1β release ($P < 0.05$) (Fig. 1E). In addition to pyroptosis, apoptosis was also induced by S1, as evidenced by the cleavage of the apoptotic caspase-8 and the downstream effector caspase-3 as well as by the percentage of TUNEL-positive cells (Fig. 1F, G). Cotreatment with FNDC4 or FNDC5 reduced the S1-induced activation of caspases 8 and 3 and adipocyte apoptosis ($P < 0.05$). Next, we examined whether S1 induces necroptosis by analyzing RIP1/RIP3 necrosome activation and phosphorylation of its downstream substrate MLKL. Differentiated adipocytes stimulated with S1 showed a tendency towards increased RIP1 phosphorylation ($P = 0.076$), robust RIP3 and MLKL phosphorylation (both $P < 0.05$) (Fig. 1H), and the upregulation ($P < 0.05$) of alarmin HMGB1 expression (Fig. 1H) and release (Fig. 1I). Cotreatment with FNDC4 and FNDC5 significantly reduced RIP3 and MLKL phosphorylation and HMGB1 expression and release (Fig. 1H, I). Collectively, these data suggest that S1 sensitizes adipocytes to undergo PANoptosis and that FNDC4 and FNDC5 blunt this inflammatory cell death.

Finally, we evaluated the circulating levels of the SARS-CoV-2 receptor ACE2 and the myokines FNDC4 and FNDC5 as well as their potential association with risk factors for severe COVID-19, including sex (male) and type 2 diabetes in patients with obesity. Circulating ACE2 levels were increased in patients with obesity and insulin resistance ($P < 0.05$) and were higher in males than in females ($P < 0.05$) (Fig. 1J). Plasma FNDC4 and FNDC5 levels were decreased in patients with obesity regardless of insulin resistance, and no sexual dimorphism was found (Fig. 1K, L). Multiple regression analyses revealed that total BF ($P < 0.05$) and visceral fat

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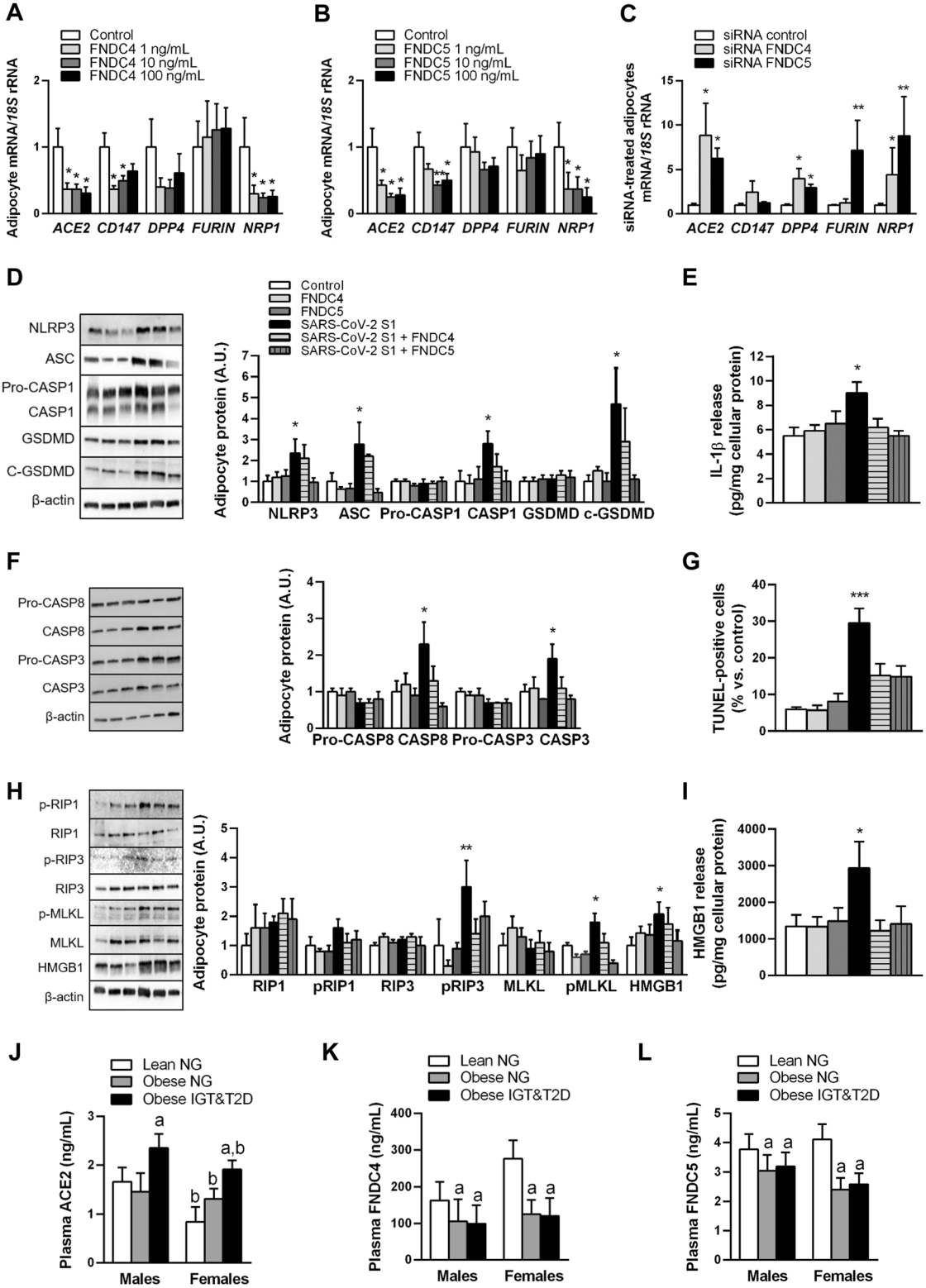

Fig. 1 FNDC4 and FNDC5 reduced SARS-CoV-2 cell entry points and SARS-CoV-2 spike subunit 1-mediated PANoptosis in human visceral adipocytes. Bar graphs show the gene expression of several SARS-CoV-2 host cell receptors and S1/S2 protein priming in adipocytes stimulated with different concentrations of FNDC4 (**A**) or FNDC5 (**B**) or after gene silencing of *FNDC4* or *FNDC5* (**C**). Gene expression in unstimulated or siRNA control cells was set to 1. Effect of coincubating adipocytes with SARS-CoV-2 spike protein subunit S1 (10 ng/mL) and FNDC4 (10 ng/mL) or FNDC5 (10 ng/mL) on the protein expression and secretion of several factors involved in pyroptosis (**D**, **E**), apoptosis (**F**, **G**) and necroptosis (**H**, **I**). Representative blots are shown on the left side of the histograms. The protein expression in unstimulated adipocytes was set to 1. Sex-dependent differences in fasting plasma ACE2 (**J**), FNDC4 (**K**) and FNDC5 (**L**) levels in normal-weight controls and patients with obesity and normoglycemia (NG), impaired glucose tolerance (IGT) or type 2 diabetes (T2D). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. unstimulated cells or siRNA control cells. [a]$P < 0.05$ effect of obesity and/or insulin resistance; [b]$P < 0.05$ effect of sex (male)

($P < 0.05$) accounted for 22.1% and 13.6% of plasma FNDC4 and FNDC5 variance, respectively.

In conclusion, AT serves as a SARS-CoV-2 viral reservoir and target organ that is overactivated in obesity and can thus induce PANoptosis. The myokines FNDC4 and FNDC5 inhibit SARS-CoV-2 entry points and S1-induced inflammatory cell death in human visceral adipocytes. The low FNDC4 and FNDC5 levels in patients with obesity might increase COVID-19 susceptibility due to increased expression of SARS-CoV-2 receptors in VAT and the amplification of SARS-CoV-2 S1-induced inflammatory cell death in visceral adipocytes.

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AUTHOR CONTRIBUTIONS

AR designed the study. AR, VC, VV, RM, JG-A, SB, CS, JE, and GF collected and analyzed the data. VV, CS, JE, and GF enrolled the patients. AR wrote and edited the manuscript. VC, JG-A, SB, PP, and GF revised the manuscript critically for important intellectual content. All the authors provided final approval of the version to be published. AR and GF, the guarantors of this work, contributed equally, had full access to all the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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