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The Alzheimer's disease risk factor APOE4 drives pro-inflammation in human astrocytes via HDAC-dependent repression of TAGLN3

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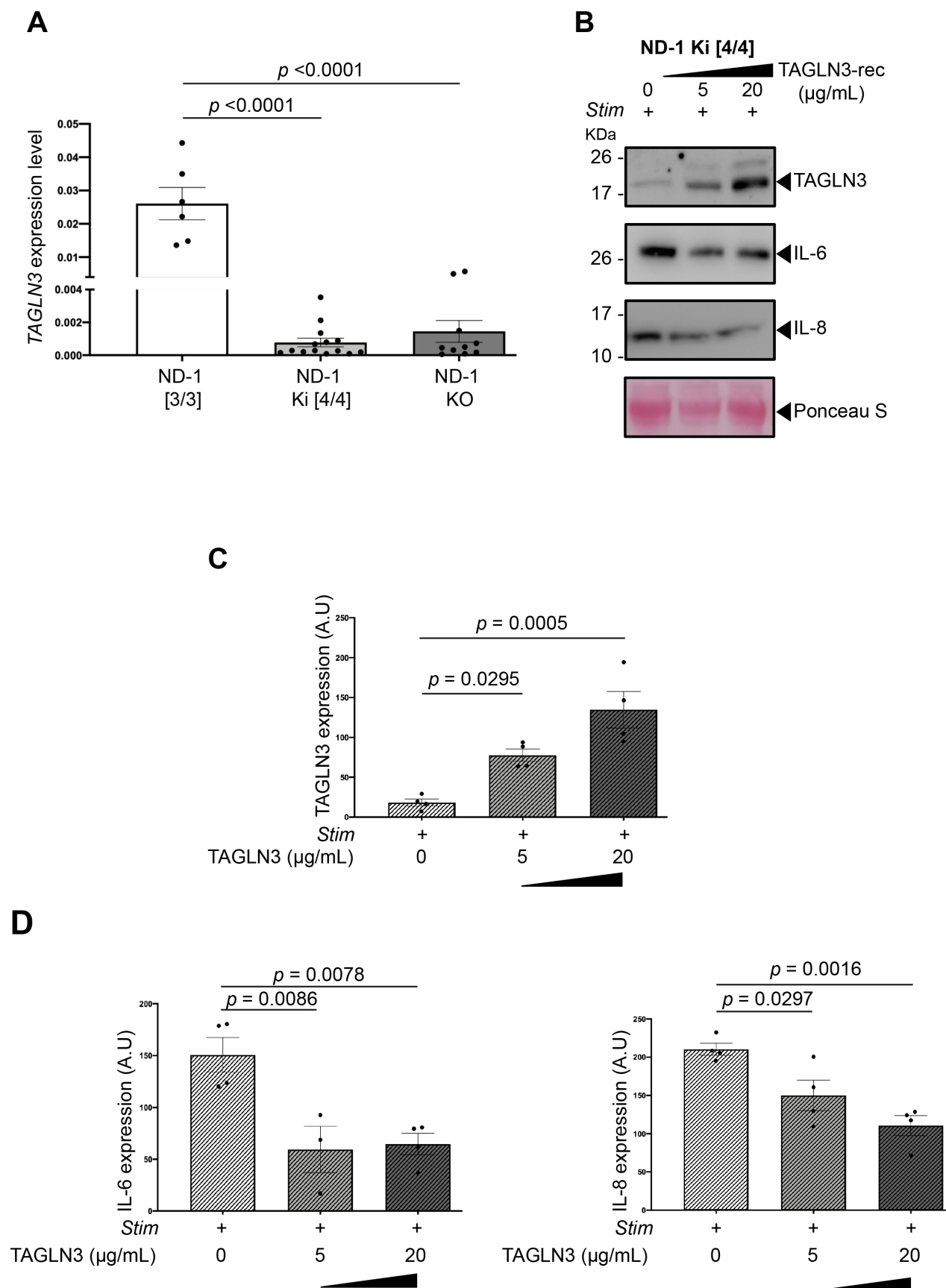
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Extended Data Figure 4.

NF- κ B activity underlies the APOE4-specific proinflammatory phenotype in astrocytes. Cell lysates from [*APOE3/E3*] (ND-1 [3/3]) and their respective [*APOE4/E4*]-Ki isogenic astrocytes (ND-1 Ki [4/4/]), either in unstimulated or stimulated (Stim) conditions, were prepared for cell fractioning. In **a**) representative Western blots to validate the fractioning (Cytosolic and nuclear fractions). Note that the nuclear Histone 3 was almost exclusively detected in the nuclear fraction, as expected. **b**) From the two fractions, different members of the NF- κ B cascade, namely p50 (top), Phosphorylated p65 (P-p65, middle) and p52 (bottom) were analysed by Western blots. Representative immunoblots (left panels) and quantitative analyses (bar charts, right) are shown. Astrocytes from the original ND-1 [*APOE3/E3*] line and two independent isogenic clones [*APOE4/E4*]-Ki were analysed, with 2 independent batches of differentiation for each line. Data are presented as mean \pm SEM using unpaired two-tailed Student's t-Test. In **b**, A.U. stands for arbitrary units (see methods).

Extended Data Fig.5

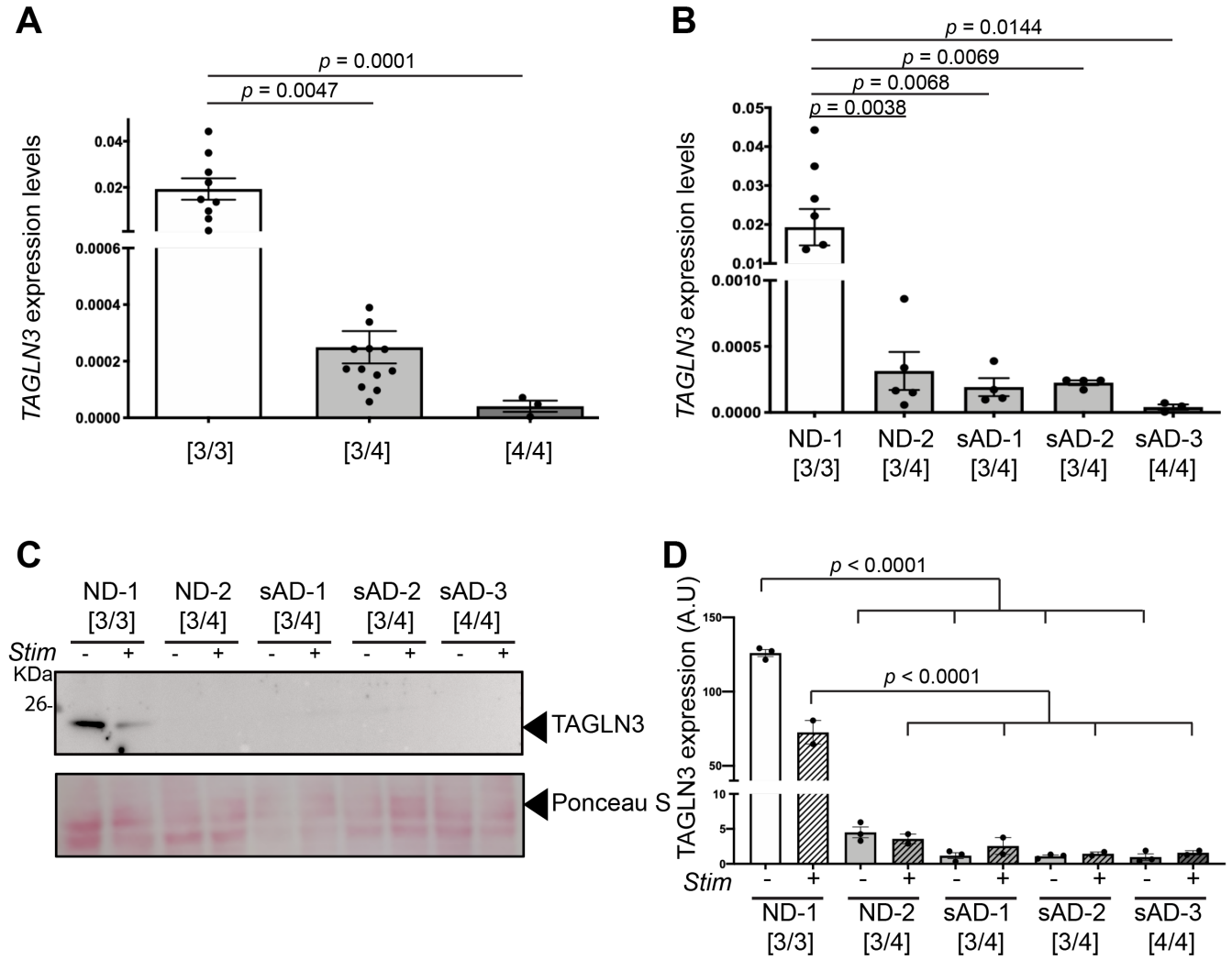


Extended Data Figure 5.

TAGLN3 stands as a new neuroinflammatory modulator in human astrocytes.

a) Quantification of *TAGLN3* mRNA levels, using qPCR analyses, from [*APOE3/E3*], [*APOE4/E4*]-Ki and [*APOE*-KO] astrocytes. **b-d)** Stimulated [*APOE4/E4*]-Ki astrocytes were treated with 5 or 20 $\mu\text{g/mL}$ of TAGLN3, as recombinant protein (TAGLN3-rec), or with its control vehicle, 24 hours prior to being analysed by Western blot analyses for TAGLN3, IL-6 and IL-8 (**b**). Quantification of Western blot analyses are shown in **c** and **d**. In all different assays, astrocytes from the original ND-1 [*APOE3/E3*] line and three independent isogenic clones [*APOE4/E4*]-Ki and APOE-KO (when applicable) were analysed, with 3 to 7 independent batches of differentiation for each line. Data are presented as mean \pm SEM using one-way ANOVA followed by post hoc Dunnett's test (a, c and d). In **c** and **d**, A.U. stands for arbitrary units (see methods).

Extended Data Fig.6



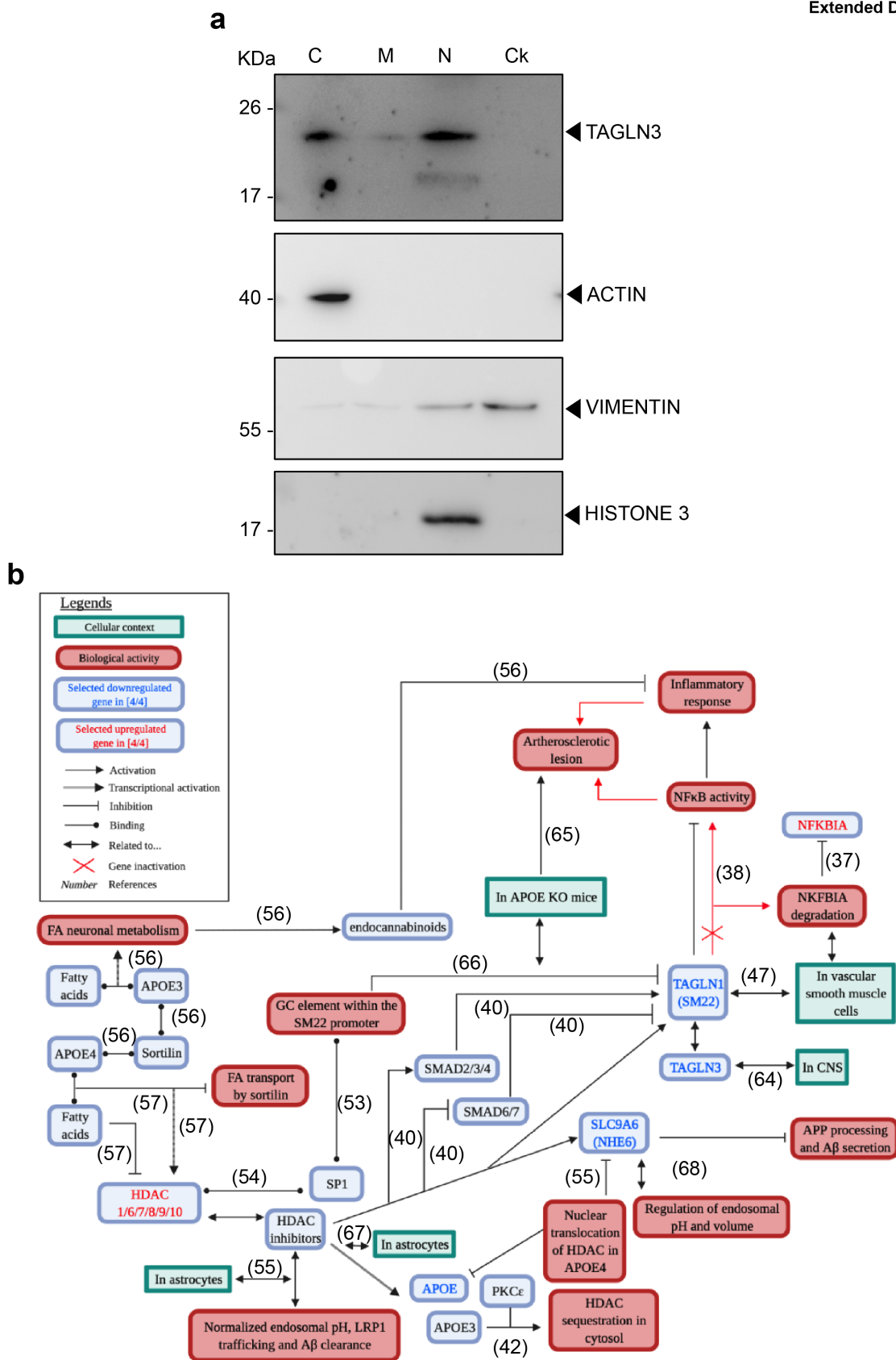
Extended Data Figure 6.

TAGLN3 downregulation in patient-specific astrocytes carrying the *APOE4* allele

a,b) *TAGLN3* mRNA expression levels from astrocytes derived from hiPSCs of individuals carrying different APOE polymorphisms [*APOE3/E3*], [*APOE3/E4*] or [*APOE4/E4*]. For each genotype, the *TAGLN3* expression of different hiPSC lines and different batches of differentiation were pooled (**a**) or represented in a patient/donor-specific manner (**b**). **c,d)** Western blotting analyses for TAGLN3 from hiPSC-astrocytes from individuals carrying different APOE polymorphisms [*APOE3/E3*], [*APOE3/E4*] or [*APOE4/E4*]. Non-stimulated (Stim-) and stimulated (Stim+) astrocytes were analysed.

Representative Western Blot (**c**) and quantitative analyses (**d**) are shown. For each hiPSC line, 2 to 3 independent batches of differentiation were analysed. Data are presented as mean \pm SEM using one-way ANOVA followed by post hoc Dunnett's test. In **d**, A.U. stands for arbitrary units (see methods).

Extended Data Fig.7



Extended Data Figure 7. TAGLN3 intracellular distribution and a comprehensive gene

interactions map underlying the novel APOE4-TAGLN3 axis. a) Representative western Blot

revealing the presence of TAGLN3 in in the cytosolic (C) and nuclear (N) fractions of [APOE3/E3]

astrocytes. TAGLN3 was barely detected in the membrane (M) fraction and totally absent from the

cytoskeleton (Ck) fraction. ACTIN, VIMEMTIN and HISTONE 3 were used as positive control of the

C, Ck and N fractions respectively. **b)** Gene interactions map revealing TAGLN3 downregulation as a

novel candidate controlling inflammation in an APOE-dependent manner. [APOE3/E3] astrocytes and

their respective isogenic [APOE4/E4]-Ki astrocytes, either nonstimulated (No Stim) or stimulated

(Stim) with a pro-inflammatory cocktail, were prepared for DNA microarray analysis. Raw data were

analysed to identify upregulated and downregulated genes in each experimental condition. Fold

changes (FC) cut-off used for analyses were ≥ 1.5 and ≤ 0.6 for upregulated and downregulated genes,

respectively. Leveraging on those analyses and using PredictSearch® as a powerful text mining

software, we established a gene network linking different genes found dysregulated in an APOE-

isoform dependent manner to identify possible mechanisms at play in the pro-inflammatory phenotype

observed in astrocytes carrying the APOE4 allele. The numbers indicate references (see main and

supplementary references).

Supplementary references (related to Extended data Figure 7)

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