Deletion of CD44 promotes adipogenesis by regulating PPARγ and cell cycle-related pathways

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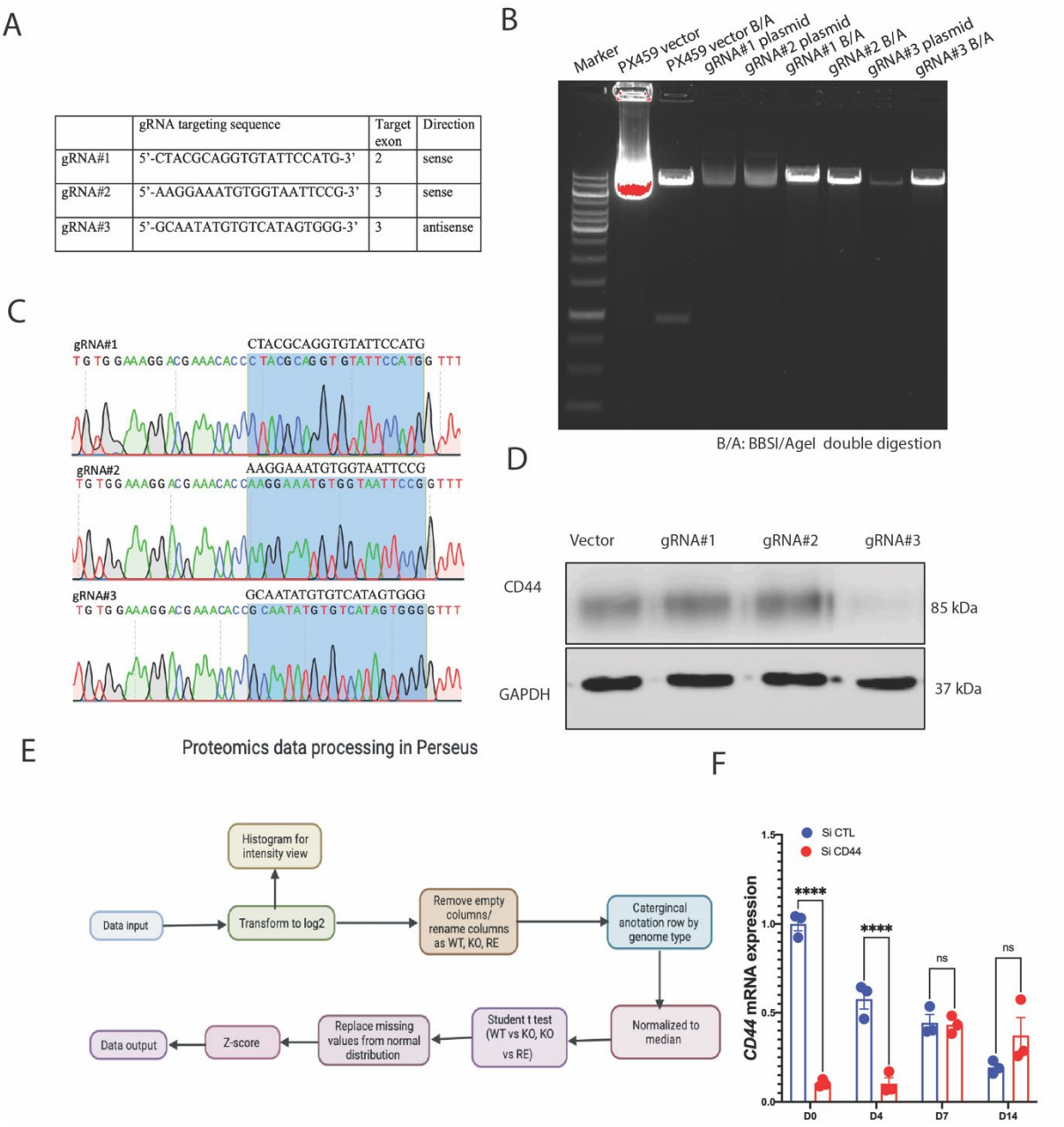
LC-MS analysis

LC buffers were the following: buffer A (0.1% formic acid in Milli-Q water (v/v)) and buffer B (80% acetonitrile and 0.08% formic acid in Milli-Q water (v/v). Aliquots of 2µL of each sample were loaded at 10µL/min onto a trap column (100µm × 2cm, PepMap nanoViper C18 column, 5µm, 100Å) equilibrated in 5% buffer B. The trap column was washed for 5min at the same flow rate and then the trap column was switched in-line with a Thermo Scientific, resolving C18 column (75µm × 50cm, PepMap RSLC C18 column, 2µm, 100Å). The peptides were eluted from the column at a constant flow rate of 300nL/min with a linear gradient from 5% buffer B (for Fractions 1-10, 7% for Fractions 11-20) to 35% buffer B in 130min, and then to 98% buffer B by 132 min. The column was then washed with 98% buffer B for 20min and re-equilibrated in 5% or 7% buffer B for 17min. The MS spectra was acquired using data dependent mode (DDA). A scan cycle comprised MS1 scan (m/z range from 335-1800, with a maximum ion injection time of 50ms, a resolution of 120,000 and automatic gain control (AGC) value of $3x10^6$) followed by 15 sequential dependant MS2 scans (with an isolation window set to 0.7m/z, resolution at 60,000, maximum ion injection time at 200ms and AGC $1x10^5$). To ensure mass accuracy, the mass spectrometer was calibrated on the first day that the runs were performed.



	gRNA targeting sequence	Target exon	Direction
gRNA#1	5'-CTACGCAGGTGTATTCCATG-3'	2	sense
gRNA#2	5'-AAGGAAATGTGGTAATTCCG-3'	3	sense
gRNA#3	5'-GCAATATGTGTCATAGTGGG-3'	3	antisense

Supplemental Figure 1: Generation of CD44KO 3T3-L1 cells using Crispr Cas9 gene editing. (A) gRNA design using the Broad institute web portal (http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design). (B) BbsI and AgeI double enzyme digestion of gRNA containing PX459 plasmid. (C) Sequencing confirmation of the insertion of gRNA in PX459 plasmid. (D) CD44 protein expression after gRNA transfection. (E) Workflow of proteomics data process. (F) CD44 gene expression during transient knockdown of CD44 by siRNA in 3T3-L1 cells. 3T3-L1 cells were transfected with CD44 siRNA (Si CD44) or scramble siRNA (Si CTL) for 48hrs before being induced to differentiate. CD44 mRNA was measured by real-time PCR up to 14 days post the induction of cell differentiation. Two-way ANOVA with Sodak's multiple comparisons were performed for statistical analysis. ****p<0.001; ns: no significance. N=3.