Supplementary Information

MA-5 attenuates disease-associated transcriptomic aging and pathological microglial states in Gaucher disease

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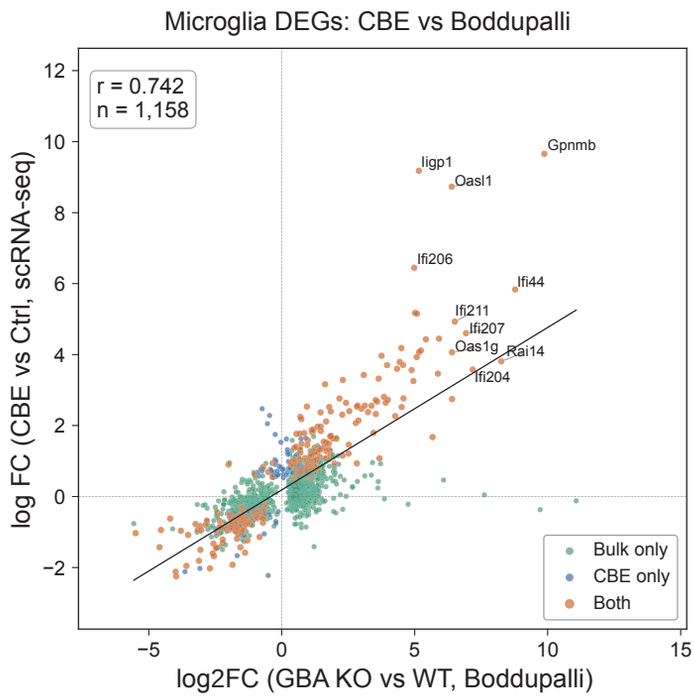
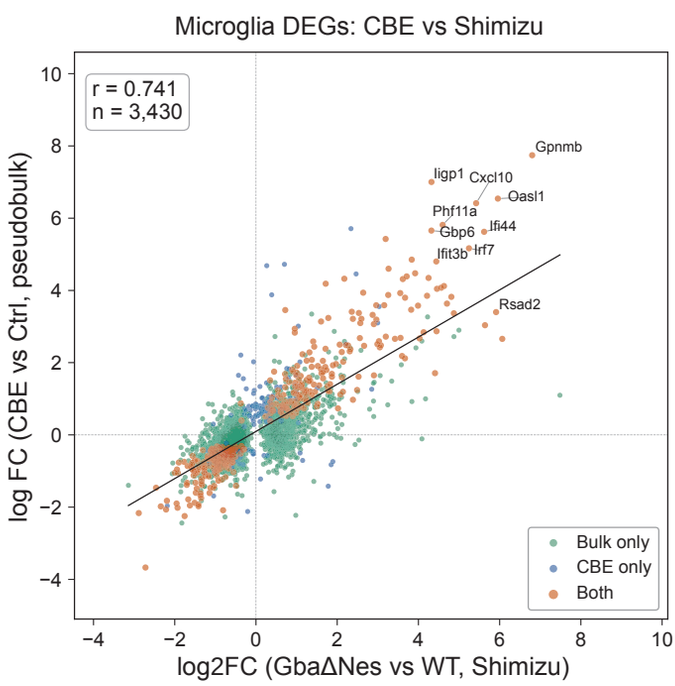
The PDF file includes:

Supplementary Figure S1 to S5
Supplementary Table 1-7

a



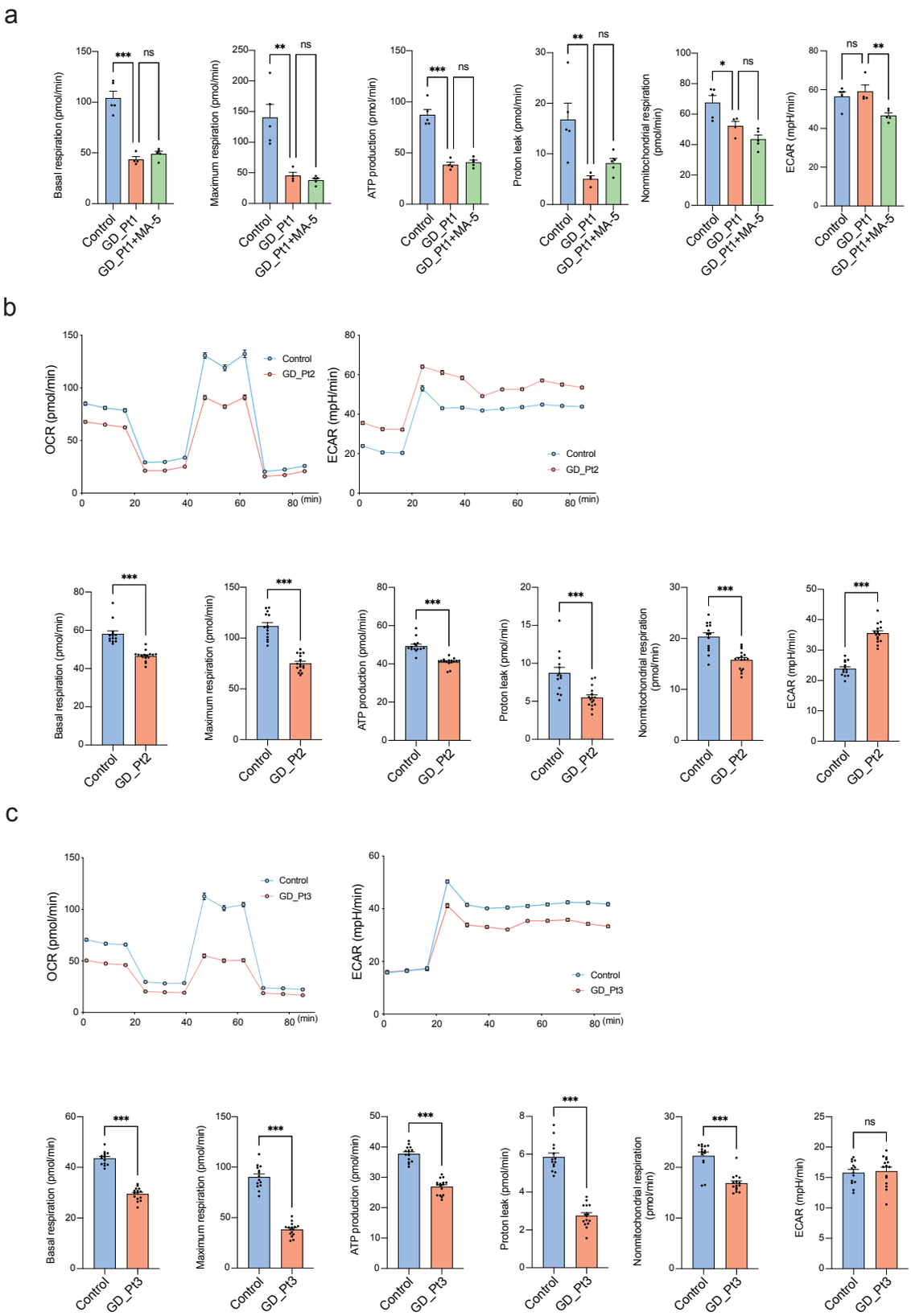
b



Supplementary Figure 1

The CBE model selectively recapitulates the conserved microglial inflammatory program of genetic Gaucher disease.

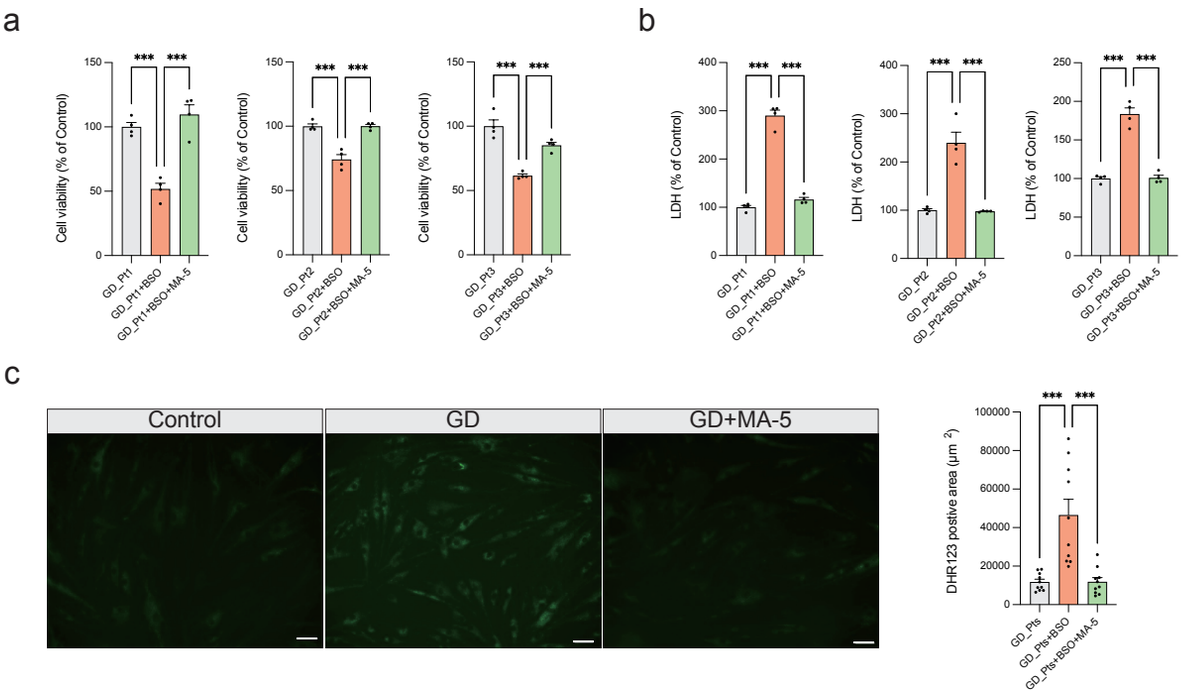
(a) Scatter plot comparing differential gene expression changes in CBE model microglia and GBA knockout microglia from the scRNA-seq dataset of Boddupalli et al.37; Genes with adjusted $P < 0.05$ in either dataset was included (n = 1,158). A strong positive correlation was observed between the two datasets (Pearson r = 0.742), indicating substantial concordance of microglial transcriptional changes across models. Representative shared upregulated genes included interferon- and disease-associated microglial genes such as Gpnmb, Iigp1, Oasl1, and Irf7. (b) Scatter plot comparing differential gene expression changes in CBE pseudobulk microglia and sorted microglia from the GbaΔNes model reported by Shimizu et al.38 Genes with adjusted $P < 0.05$ in either dataset was included (n = 3,430). The two datasets showed a similarly strong positive correlation (Pearson r = 0.741), with concordant upregulation of inflammatory genes including Cxcl10, Irf7, and Ifi44.



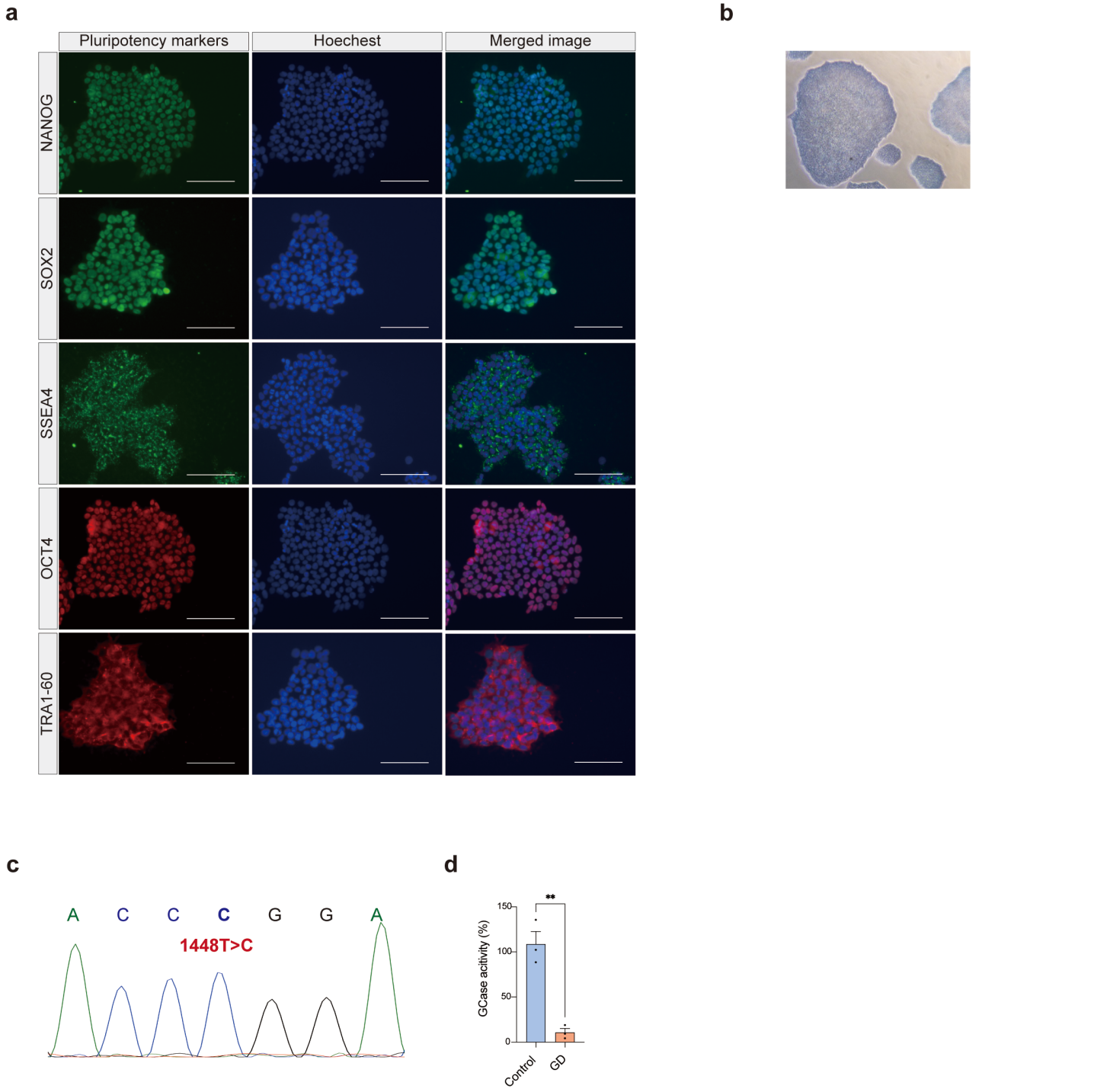
Supplementary Figure 2

(a) Mitochondrial function analysis in Patient 1 fibroblasts measured by Seahorse flux analyzer. Parameters include basal respiration, maximum respiration, ATP production, proton leak, non-mitochondrial respiration, and ECAR (extracellular acidification rate) comparing control (blue), GD (red), and GD+MA-5 (green). Statistical significance: * p <0.05, ** p <0.01, *** p <0.001, ns=not significant. (b) Mitochondrial function in Patient 2 fibroblasts. Top: Time-course measurements of OCR (oxygen consumption rate) and ECAR comparing control (black) and Patient 2 (blue). Bottom: Quantified metabolic parameters showing significant impairment in GD cells (***p<0.001). (c) Mitochondrial function in Patient 3 fibroblasts. Top: Time-course measurements of OCR and ECAR. Bottom: Quantified metabolic parameters showing significant impairment in most parameters (***p <0.001).

Statistical analysis: Two groups compared by Student's t-test; three groups compared by one-way ANOVA with Tukey's test.



Supplementary Figure 3
Oxidative stress resistance in GD patient-derived fibroblasts. (a) Cell viability in fibroblasts from three GD patients with and without BSO (L-buthionine-(S,R)-sulfoximine) treatment and MA-5. (b) LDH (lactate dehydrogenase) release in fibroblasts from three GD patients with and without BSO treatment and MA-5. (c) Representative fluorescence images and quantification of DHR123-positive area in GD patient fibroblasts with BSO and MA-5 treatment. Statistical significance was determined by *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA Tukey test.

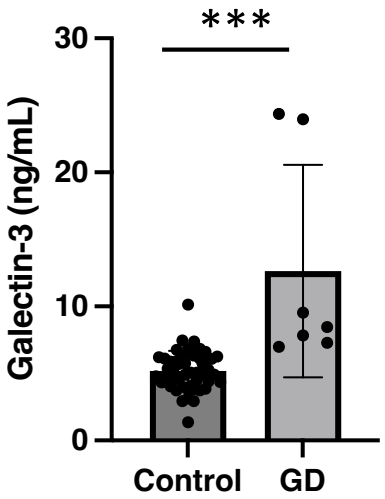


Supplementary Figure 4
Characterization of patient-derived iPSCs. (a) Immunofluorescence staining for pluripotency markers (NANOG, SOX2, SSEA4, OCT4, and TRA1-60) with Hoechst nuclear counterstaining in GD patient-derived iPSCs. (b) Alkaline phosphatase staining of iPSC colonies. (c) Sanger sequencing chromatogram confirming the GBA 1448T>C mutation (left) and GCase enzyme activity in control and GD iPSCs (right). Statistical significance was determined by *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA Tukey test.

a

		Control (n=48)	GD (n=7)
Galectin-3	(ng/mL)	5.20 ± 0.21	12.64 ± 2.99
Age	(yr)	31.8 ± 1.1	26.9 ± 4.8
Gender	(M/F)	48/0	3/4

b



Supplementary Figure 5
Plasma galectin-3 levels in patients with Gaucher disease.
(a) Age distribution of patients with GD (n = 7) and controls (n = 48) in whom plasma galectin-3 levels were measured. There was no significant difference in age between the two groups, as assessed by an unpaired t-test.
(b) Plasma galectin-3 levels in patients with GD and controls. Plasma galectin-3 levels were significantly elevated in patients with GD ($p < 0.05$).