

Figure S1. HDAC mRNA expression in PBMCs. PBMCs from patients with GO (n=32) and controls (n=11) were used. The mRNA expression levels of HDACs (class I: HDAC1, 2 and 3; class IIa: HDAC4, 5 and 7; class IIb: HDAC6 and 10) were determined by reverse transcription-quantitative PCR. The results are represented as the median and interquartile range. GO, Graves' orbitopathy; HDAC, histone deacetylase; PBMCs, peripheral blood mononuclear cells.

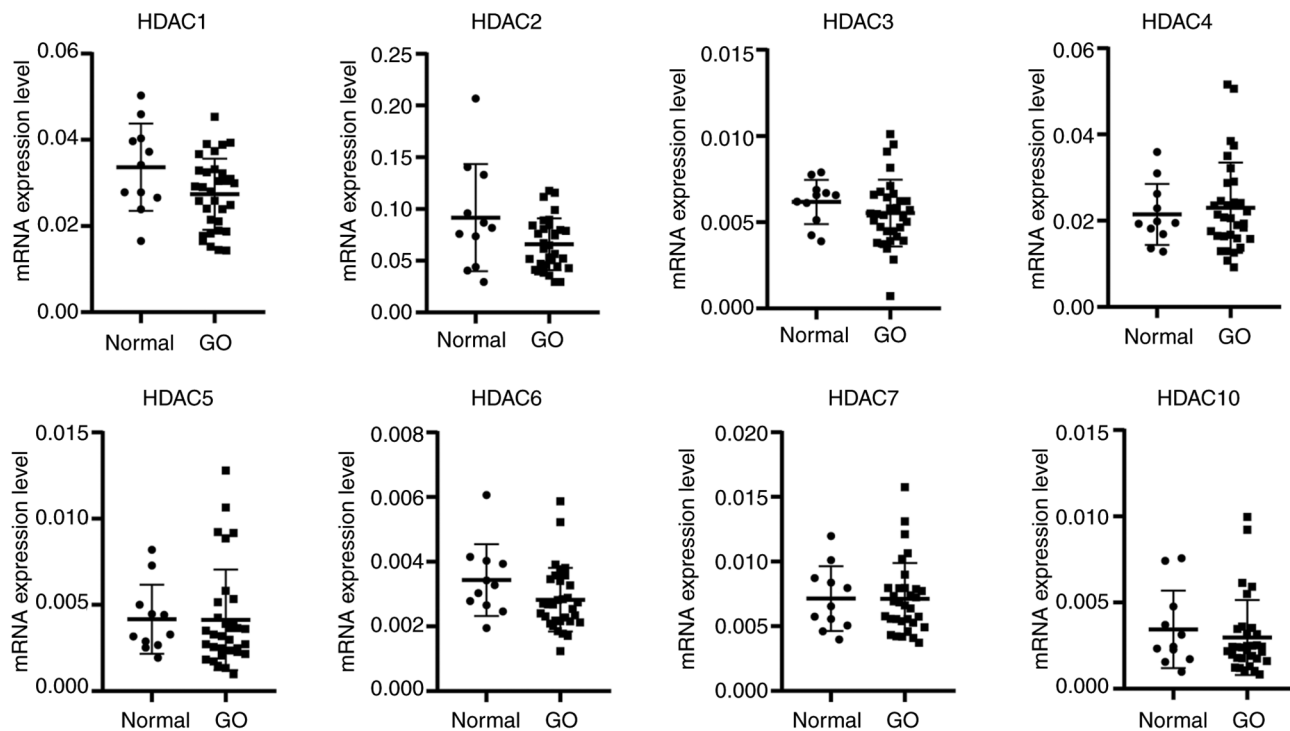


Figure S2. HDAC7 protein expression in GO and normal orbital tissues. HDAC7 protein expression levels in GO orbital (n=7) and control (n=7) adipose tissues were analyzed using western blot analysis. Western blot gel images are presented with β -actin as the loading control. The relative density normalized to β -actin is shown as the median and interquartile range. Statistical significance was determined using Mann-Whitney U-test. *P<0.05. GO, Graves' orbitopathy; HDAC7, histone deacetylase 7.

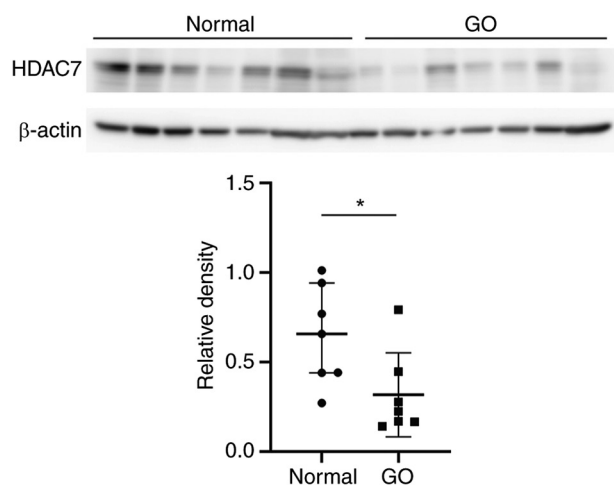


Figure S3. Effect of silencing HDAC6 on inflammation and fibrosis. Orbital fibroblasts from patients with GO (n=3) and controls (n=3) were transfected with si-HDAC6 (10 nM) or si-con for 24 h and further maintained for 48 h. (A) Silencing efficiency of si-HDAC6 was demonstrated in both GO and normal orbital fibroblasts (n=3) compared with si-con. (B) Following transfection, orbital fibroblasts were stimulated with IL-1 β (10 ng/ml) for 24 h. The proinflammatory cytokines IL-6 and IL-8 were analyzed using western blotting. (C) si-HDAC6- or si-con-transfected cells were treated with TGF- β (5 ng/ml) for 24 h. Profibrotic proteins, including fibronectin, Col I α , Col 3 and α -SMA, were examined by western blot analysis. Values were normalized to β -actin and presented relative to those of the control. All experiments were conducted twice on samples from three individuals. Data are presented as the mean \pm SD. Statistical significance was determined using Mann-Whitney U-test to compare the effect of silencing HDAC6 under IL-1 β or TGF- β stimulation. *P<0.05. α -SMA, α -smooth muscle actin; Col, collagen; con, control; GO, Graves' orbitopathy; HDAC6, histone deacetylase 6; si, small interfering RNA.

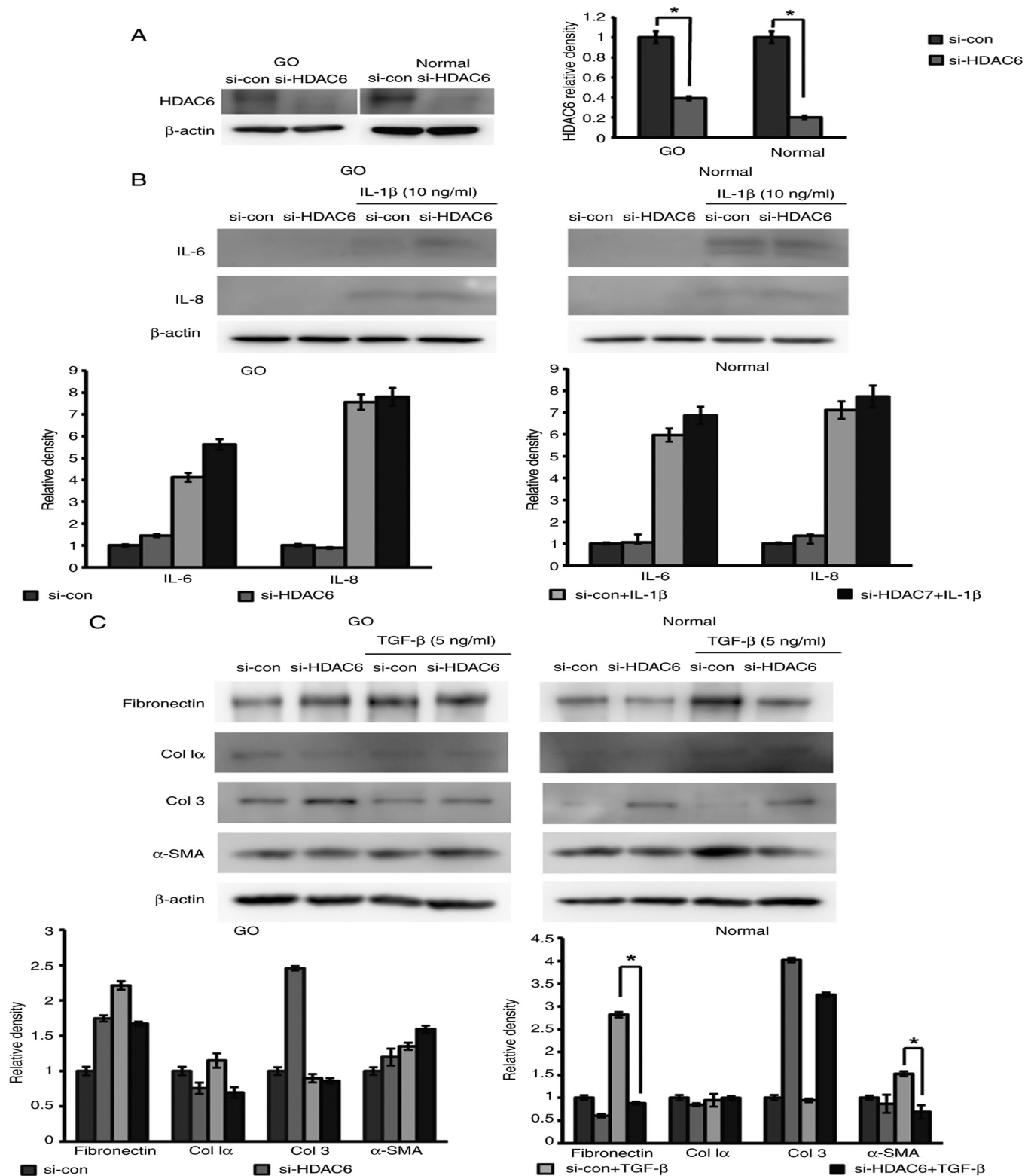


Figure S4. HDAC3 and HDAC7 expression following panobinostat treatment and si-HDAC7 transfection. (A) Orbital fibroblasts were cultured with panobinostat (100 nM) for 24 h. HDAC3 and HDAC7 protein expression levels were determined by western blotting. (B) After 24 h of siRNA transfection (si-HDAC7 and si-con) followed by 48 h of maintenance, the protein expression levels of HDAC3 and HDAC7 were measured by western blotting. All experiments were conducted twice in orbital fibroblasts obtained from 3 different individuals. Representative gel images are shown. con, control; GO, Graves' orbitopathy; HDAC, histone deacetylase; si/siRNA, small interfering RNA.

