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Supplemental Information

Obestatin Increases the Regenerative Capacity of Human Myoblasts Transplanted Intramuscularly in an Immunodeficient Mouse Model

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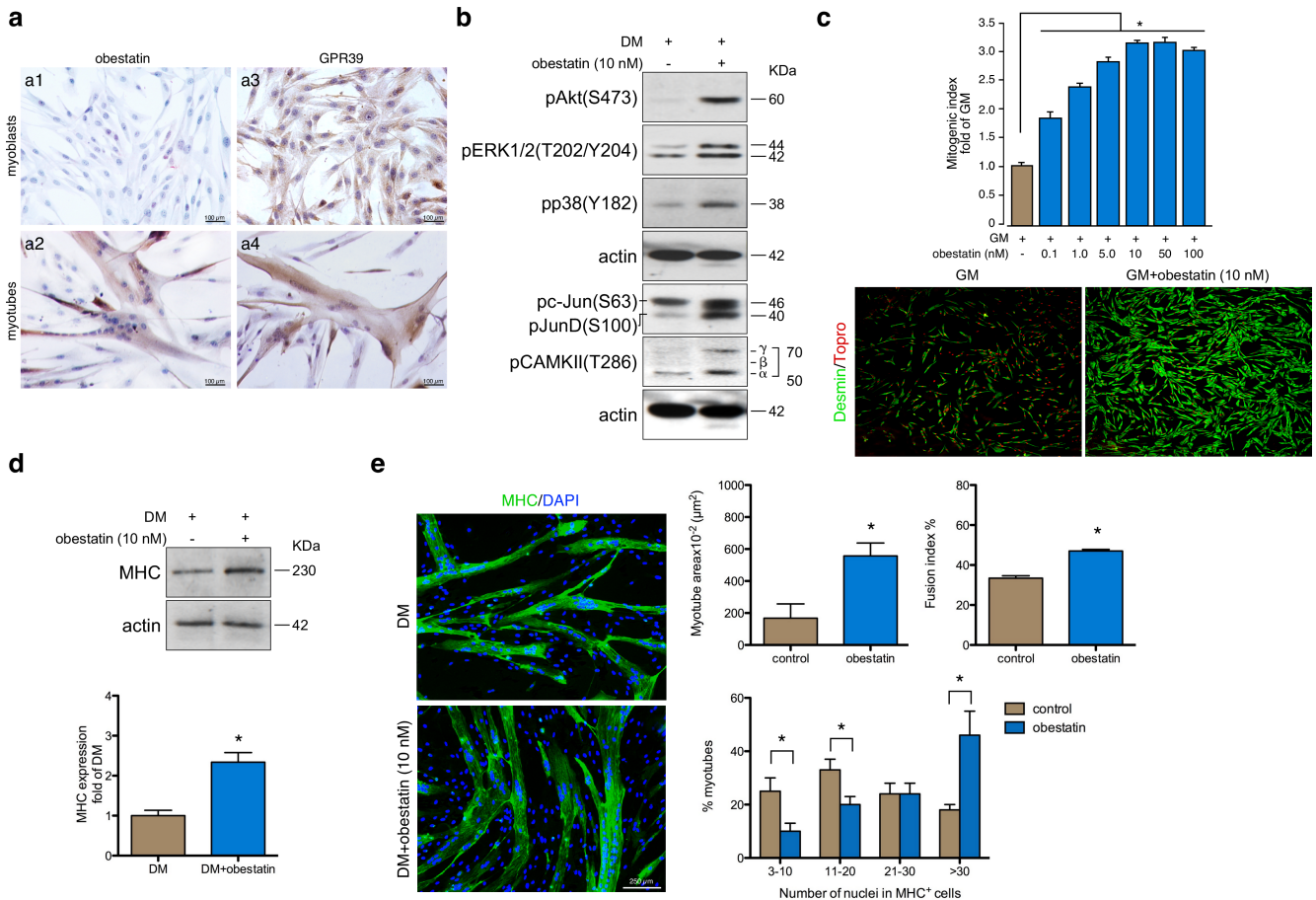


Figure S1 Validation of obestatin/GPR39 functionality in human primary myoblasts. (a) Immunocytochemical detection of obestatin and GPR39 in human primary myoblasts (a1 and a3) and myotubes (a2 and a4) (C25 cells). (b) Immunoblot analysis of the effect of obestatin (10 nM, 5 min) on the activation of Akt [pAkt(S473)], ERK1/2 [pERK1/2(T202/Y204)], p38 [pp38(Y182)], c-Jun [pc-Jun(S63) and pJunD(S100)] and CAMKII [pCAMKII(T286): α , β and γ isoforms] in human primary myoblasts (C25 cells). (c) *Upper panel*, dose-response effect of obestatin (0.1–100 nM) in human primary myoblast (C25 cells) proliferation (48 h, n=5). Quantification of cell number was carried out by cell counting using desmin, marker for myoblasts, and Topro to counterstain nuclei (*lower panel*). (d) Effect of obestatin (10 nM) on differentiating human primary myoblasts (C25 cells). MHC expression was determined by immunoblot 6 days after stimulation (n=3). (e) *Left panel*, immunofluorescence detection of MHC in human primary myotubes in DM (control) or DM + obestatin (10 nM) 6 days after stimulation (n=6). DAPI was used to counterstain nuclei. *Right panel*, the extent of differentiation was evaluated by the MHC⁺ myotube area (*left upper panel*), fusion index (*right upper panel*) and the percentages of MHC⁺ myotubes containing the indicated numbers of nuclei (*bottom panel*). Data in c, d and e are expressed as mean \pm SEM. **P* < 0.05 versus control values.

Table S1

Antibody	Application	Dilution	Species specificity	Species origin and immunoglobulin isotype	Supplier	Reference
Actin	WB	1:5000	Human	Rabbit polyclonal IgG	Abcam	ab1801
Desmin	ICC/IF	1:300	Human	Rabbit polyclonal IgG	Abcam	15200
DVL2	WB	1:500	Human	Rabbit monoclonal	Cell Signaling	3224
GPR39	ICC	1:100	Human	Rabbit polyclonal IgG	Abcam	ab39227
ki67 Clone MIB1	IHCf/r/Fl	1:100	Human/mouse	Mouse mAb IgG1	DAKO	M7240
Lamin A/C	IHCf/r/Fl	1:100	Human	Mouse mAb IgG2b	Novocastra, Leica	NCL-LAM-A/C
Lamin A/C	IHCf/r/Fl	1:200	Human	Rabbit polyclonal IgG	Abcam	ab108595
Laminin	IHCf/r/Fl	1:200	Human/mouse	Rabbit polyclonal IgG	DAKO	Z0097
Myogenin	IHCf/r/Fl	1:200	Human/mouse	Mouse mAb IgG1	Hybridoma Bank	F5D
Myosin Heavy Chain	ICC/IF	1:200	Human/mouse	Mouse mAb IgG2b	Hybridoma Bank	MF20
Myosin Heavy Chain	IHCf/r/Fl	1:20	Human/mouse	Mouse mAb IgG2b	Hybridoma Bank	MF20
Obestatin	ICC	1:100	Human	Rabbit polyclonal IgG	Abcam	ab41704
Pax7	IHCf/r/Fl	1:20	Human	Mouse mAb IgG1	Hybridoma Bank	Pax-7
pH3(S10)	IHCf/r/Fl	1:200	Human/mouse	Rabbit polyclonal IgG	Molipore	06-570
Rac1	WB	1:1000	Human	Mouse mAb IgG2b	Thermo	1862341
Rho	WB	1:1000	Human/mouse	Rabbit polyclonal IgG	Thermo	1862332
Cleaved caspase-3 (Asp175)	IHCf/r/Fl	1:500	Human/mouse	Rabbit polyclonal IgG	Cell Signaling	9661
Spectrin	IF	1:1000	Human	Mouse mAb IgG2b	Novocastra, Leica	NCL-SPEC1
Secondary antibody	Use	Dilution			Supplier	Ref
Anti-rabbit HRP	WB	1:10000	Rabbit	Goat polyclonal Cy3	Jackson	11-035-003
Goat anti-mouse Alexa Fluor 488 IgG2b	IHCf/r/Fl	1:1000	Mouse	Goat polyclonal Alexa 488	Life Technologies	A21141
Goat anti-mouse Alexa Fluor 594 IgG1	IHCf/r/Fl	1:1000	Mouse	Goat polyclonal Alexa 594	Life Technologies	A21125
Goat anti-rabbit Alexa Fluor 594	IHCf/r/Fl	1:1000	Rabbit	Goat polyclonal Cy3	Abcam	150089

Applications key: ICC: immunocytochemistry; ICC/IF: cell-immunofluorescence; IHCf: immunohistochemistry-frozen tissue; IHCf/r/Fl: frozen tissue-immunofluorescence; WB: western blot; Ig: immunoglobulin; mAb: monoclonal antibody