

Autophagy promotes MSC-mediated vascularization in cutaneous wound healing via regulation of VEGF secretion

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Supplementary Figure S1 Isolation and characterization of human mesenchymal stem cells (MSCs). (a) Representative images of single-cell cloning formation from MSCs at day 10. (b) Alizarin red staining of MSCs after 4 weeks of induction in the osteogenic medium (100×). The expression of osteogenesis proteins OCN, SP7 and Runx-2 were detected by western blot. (c) Oil red staining of MSCs after 3 weeks of induction in the adipogenic medium (100×). The expression of adipogenesis proteins LPL and PPAR were detected by western blot. (d) The ultrastructural morphology of autophagosome in MSCs and the protein of Atg7, Beclin-1 and LC3I/II were detected by western blot.

Supplementary Figure S2 Dio-labeled MSCs (red) and LC3-positive cells (green) at 48h after subcutaneous injection. The co-stained cells represented the autophagy activation of MSCs. Scale bar = 50µm.

Supplementary Figure S3 There was no distinction between control group and si-control group. (a) The expression of proteins VEGF, Atg7, Beclin-1 and LC3I/II were detected by western blot. (b) Immunohistochemistry staining on CD31 of wound area tissues 2-week post-operative (^{NS}P > 0.05, n=3). Scale bar = 50µm. (c, d) Images of wound size on the back of mouse and quantification of wound healing rate (%). Scale bar = 5mm.

Supplementary Figure S4 Dio-labeled MSCs (red) were detected by immunofluorescence assay 48h after injection. Scale bar = 50 μ m.

Supplementary Figure S5 Rapamycin had no effect on the wound healing through local application. (a) Images of wound size on the back of mouse and quantification of wound healing rate (%). Scale bar = 5mm. (b) Hematoxylin and eosin (H&E) staining of wound area tissues at 2-week post-operative. The red boxes indicate the skin wound healing. Scale bar = 2mm. (c) Immunohistochemistry staining on CD31 of wound area tissues and quantification of positive area of CD31 at 2-week post-operative (^{NS}P > 0.05, n=3). Scale bar = 50 μ m.

Supplementary Figure S6 VEGF was transfected by siRNA. (a) The protein of VEGF was decreased at 48 hours after transfection (n=3). (b) The gene of VEGF was decreased at 48h after transfection (^{NS}P > 0.05, n=6).

Supplement Table S1 Specific primer sequences used for reverse transcriptase-polymerase chain reaction analysis.