Supplementary Material

Supplementary method: Detection of cell viability by MTS assay

The 90% confluent 3T3-L1 cells were cultured and induced in the H-DMEM (with 10% FBS) containing the differentiation cocktail with rhOPN (10 µM, 50 µM, 100 µM and 200 µM) for the whole 6 days, respectively. The cell viability was measured by MTS assay. Briefly, the 3T3-L1 cells were first exposed to rhOPN throughout the differentiation period. And then the medium was replaced with the fresh serum-free DMEM. Subsequently, 20 μL of MTS solution were added, and the cells were further cultured for 0.5-2 h. All of the operations above needed to be protected from light. Finally, optical density values (OD) at wavelength of 490 nm were detected.

Supplementary results: Levels of sOPN in adipocytes

As shown in (Supplementary Fig. 1Sa), with the elongation of the induction time, the amount of lipid droplets gradually increased, peaked at day 6 and persisted till day 8. Thus, the time of 6 days was chosen as the optimal induction time for the subsequent experiments. In addition, the results from adenoviral infection showed that the fluorescence intensity of GFP reached a peak upon infected with $1 \times 10^8$PFU of Ad-GFP-aP2-OPN for 48 h (Supplementary Fig. 1Sb), demonstrating that $1 \times 10^8$PFU was the optimal infective titer for the further experiments. Moreover, the data of ELISA displayed that with infection of $1 \times 10^8$PFU Ad-GFP-aP2-OPN for 48 h, the levels of sOPN significantly increased (Supplementary Fig. 1Sc), suggesting that the infection of Ad-GFP-aP2-OPN can increase the sOPN expression and secretion. However, treatment with various concentration of rhOPN for 6 days did not manifest any cytotoxicity (Supplementary Fig. 1Sd).

Fig. 1S Levels of sOPN in adipocytes. 90% confluent 3T3-L1 preadipocytes were cultured and induced in the DMI medium for 0, 2, 4, 6 and 8 days, respectively. Lipid droplet accumulation was visualized and quantification by oil red O staining (a). Another batch of 3T3-L1 cells with 70% confluence were infected with Ad-GFP-ap2-OPN ($1 \times 10^9$PFU, $1 \times 10^8$PFU and $1 \times 10^7$PFU) or empty adenovirus vector (as a Vehicle) and induced with DMI for 6 days. Fluorescence was visualized under a fluorescent inverted microscope (b). The levels of sOPN from the medium were measured by ELISA (c). In addition, 90% confluent 3T3-L1 cells were induced in DMI medium containing with rhOPN (10, 50, 100, 200 µM) for 6 days. The cell viability was measured by MTS assay (d). All data were expressed as the mean ± S.E.M of 3 independent experiments. * * p < 0.01, vs. Ctrl or Vehicle group. Ctrl: Control, Vehicle: empty vector or PBS, ns: no significance, scale bar: 100 µm.