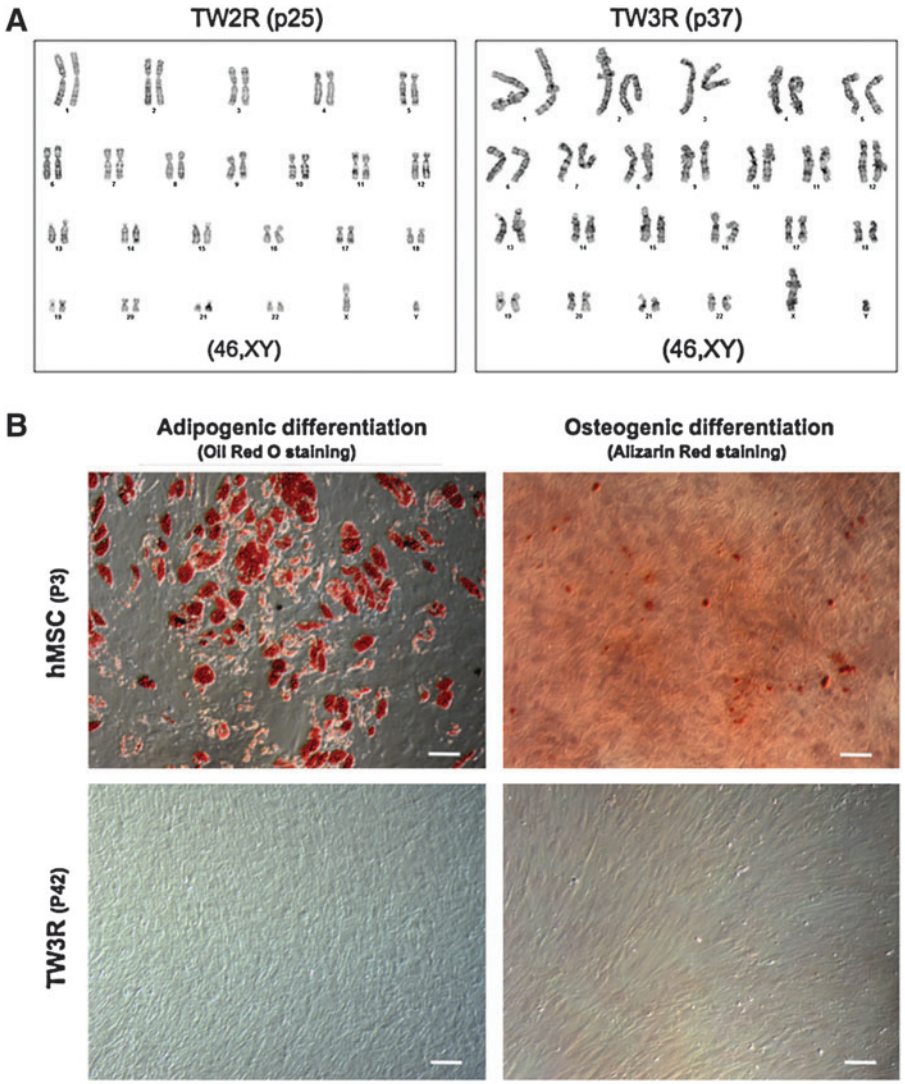
Supplementary Data

Supplementary Method

Adipogenic and osteogenic differentiation of mesenchymal stem cells

Human mesenchymal stem cells (hMSCs) (P3) and TW3R cells (P42) were differentiated into adipocytes and osteocytes by using differentiation medium (Lonza Walk-ersville Inc., Walkersville, MD) following the manufacturer's instructions (www.lonza.com). For adipogenic differentiation, hMSCs or TW3R cells were plated at 1×10^5 cells in 1 mL MSC medium per 1 well of a 12-well plate and allowed to reach 100% confluence, followed by incubation for 3 cycles in induction/maintenance medium. Three complete cycles of induction/maintenance later, the hMSCs or TW3R cells continued to be cultured for 7 more days in supplemented Adipogenic Maintenance Medium. To determine the adipogenic differentiation, cultures were rinsed with phosphate-buffered saline, fixed with 10% buffered formalin, and stained with Oil Red O. For osteogenic differentiation, hMSCs or TW3R cells were plated at 1.5×10^4 cells in 1 mL MSC medium per 1 well of a 12-well plate and incubated for 24 h. The medium in the culture was then replaced with an osteogenic induction medium and incubated for 21 days. Cultures were then fixed and stained with fresh alizarin red solution to detect calcium deposition.



SUPPLEMENTARY FIG. S1. TW2R and TW3R feeders maintained normal karyotypes but lost differentiation potential. G-banding karyotyping has shown that both TW2R (passage 25) and TW3R (passage 37) maintained a normal male karyotype (46, XY) **(A)**. However, the immortalized cell lines, after extensive culture, have lost the adipogenic and osteogenic differentiation potential that lower-passage MSCs possess. **(B)** Scale Bar: 100μm. hMSCs, human mesenchymal stem cells.