SUPPLEMENTARY MATERIALS AND METHODS

In vitro differentiation

In vitro differentiation of hMSC-bm (unseparated population, ALDEFLUOR-positive population, ALDEFLUOR-negative population) was evaluated in triplicates.

Adipogenesis differentiation was assayed by using the mesenchymal stem cell adipogenesis kit (Chmicon). Briefly, the sorted cells were plated in 1 ML of Mesenchymal stem cell expansion medium at a density of 60000 cells per well in a 24-well culture dish and incubated at 37°C in a 5% CO_2 humidified incubator overnight. The medium was then replaced with the adipogenesis induction medium which was change every two days for 3 weeks (Instead of using induction medium, Mesenchymal stem cell maintenance medium was used Day 7 and Day 15). After 21 days of differentiation, adipocytes were fixed in 4% paraformaldehyde and the lipid droplets were stained with Oil Red O solution as described by the manufacturer.

Osteogenic differentiation was assayed by using the mesenchymal stem cell osteogenesis kit (Chmicon). Briefly, the sorted cells were plated in 1 ML of Mesenchymal stem cell expansion medium at a density of 60000 cells per well in a vitronectin/collagen pre-coated 24-well culture dish and incubated at 37°C in a 5% CO_2 humidified incubator overnight. The medium was then replaced with osteogenesis induction medium which was change every two days. After 15 days of differentiation, osteocytes were fixed in 70% ethonal and stained with Alizarin Red solution as described by the manufacturer.

Conditioned medium, Antibody array and Luminex bead assay

To prepare conditioned media, breast cancer cell lines (BCC: SUM159 or SUM149) alone, MSC alone or the co-culture of BCC and MSC (1:1 mixture) were plated in 100-mm tissue culture dishes in the mixture of IHM and MSCM (1:1 mixture). For the transwell-culture, BCC and MSC were plated in either top-chamber or bottom-chamber. Following overnight incubation, the medium was replaced with MEBM serum-free medium and cultured for four days (We have optimized the culture period and chosen the four-day culture as the optimum). The supernatants were collected and filtered through 0.22um syringe filter, aliquoted, and stored at -80°C until testing. For the cells treated with different cytokines or cytokine antibodies, cells were plated in 100-mm tissue culture dishes in regular culture medium. Following overnight incubation, the medium was replaced with serum-free MEBM in the presence of the cytokines or cytokine antibodies and cultured for four days (We optimized the concentration of each treatment and chose the optimal concentration). Except for IL6 and anti-IL6 which were purchased from Sigma, all of other cytokine were purchased from R&D Biosytems. Assay for cytokine antibody arrays was carried out as per manufacturer's instructions (RayBio Human Cytokine Array V). In the end, we used the X-ray film (Blue Basic Cat. 9023, ISC Bioexpress) for detection. The films were scanned and the dots were quantified with ImageJ software (http://rsb.info.nih.gov/ij/). We confirmed the antibody array results using the Luminex bead assay (Figure S5). All analyte-specific reagents were purchased

from R&D Systems (Minneapolis, MN). Manufacturer's suggested protocols were followed without deviation throughout.

Intra-tibia injection

A few hours before transplantation, mice were irradiated with 300 cGy from an x-ray irradiator (Mark I, Model 25, J.L. Shepherd). All procedures were approved by the Animal Care Committee of University of Michigan. The intramedullary injection into the tibia shaft was carried out according to previously published methods (31-32). Briefly, a commercial depilatory agent (Nair) was used to exfoliate the region surrounding the knee joint. The knee was then flexed 90° and the proximal side of the tibia drawn to the anterior. Next a 26-gauge needle was inserted into the joint surface of the tibia through the patellar tendon and then down into the bone marrow cavity with steady pressure. Subsequently a change to a 28-gauge needle was made in order to transfer the MSCs. Approximately $1x10^6$ DsRed- and luciferase-labeled MSCs were suspended in 10 ul PBS and transplanted directly through the bored bone holes into the bone marrow cavity of NOD/SCID mice using a Hamilton syringe (Reno, NV) equipped with a 28-gauge needle. 10 ul PBS were injected to the right tibia of the control group. Six mice were assayed for each group.