



Supplementary Figure 1 Sample Flow diagram of Human mesenchymal stem cell (hMSC) isolation from trabecular bone and subsequent characterization. A: Flow of patient samples through the study from recruitment to inclusion in functional assays. Femoral heads were collected from patients undergoing total hip arthroplasty (26 idiopathic ONFH, 34 OA; total = 60). MSCs were successfully isolated from 20 ONFH and 30 OA samples. Of these, 10 ONFH and 10 OA lines could be expanded to passage 2 (P2) and were therefore eligible for downstream analyses. These lines were subsequently included in proliferation assays (MTT), osteogenesis assays (Alizarin Red and osteogenic gene expression), adipogenesis assays (Oil Red

O and adipogenic gene expression), and qPCR analyses for NF- κ B and BMP pathway-related genes. The diagram illustrates patient attrition and the distribution of MSC lines across assays, providing a transparent overview of sample handling and analysis; B: Characterization of hMSCs by flow cytometry. Representative samples were labelled with antibodies against CD45, CD34, CD73, CD90, and CD105, and analysed by Flow Cytometry. The hMSC phenotype was confirmed by the expected marker profile: CD45⁻ , CD34⁻ , CD90⁺ , CD73⁺ , CD105.