

Figure S1

Characterization of human osteoblast-like cell (HOB) preparations used for transduction

Osteoblasts outgrowing from bone fragments in culture dishes were harvested and characterised as described in Materials and Methods. Cells were propagated for 2 passages (2-6 PD) before transduction. Cultures were transduced with LV vectors only if more than 80% of cell showed an osteoblast phenotype. Osteoblast phenotype was determined as cell ability to express alkaline phosphatase activity (ALP), measured on cell cytopins by a cytochemical method and to produce mineralised extra-cellular matrix. Panels show the characterization of a HOB preparation (no. 1704), representative of all the preparations used in experiments. (A) ALP expression in HOBs (purple colour), compared to that of the human osteosarcoma cell line SAOS-2 (B) and to the negative staining of the human fibroblast cell line MRC5 (C). Parallel cultures of the same HOB preparation were maintained for three weeks in differentiating medium, to appreciate the production of mineralized calcium phosphate deposits, estimated as Alizarin red staining (D). HOBs did not express the MET receptor (E) detectable as immuno-histochemical staining (brown) with a monoclonal antibody in the gastric carcinoma cell line GTL-16 mixed in the cytopsin (F), where HOBs might be identified by ALP production (purple colour in G).

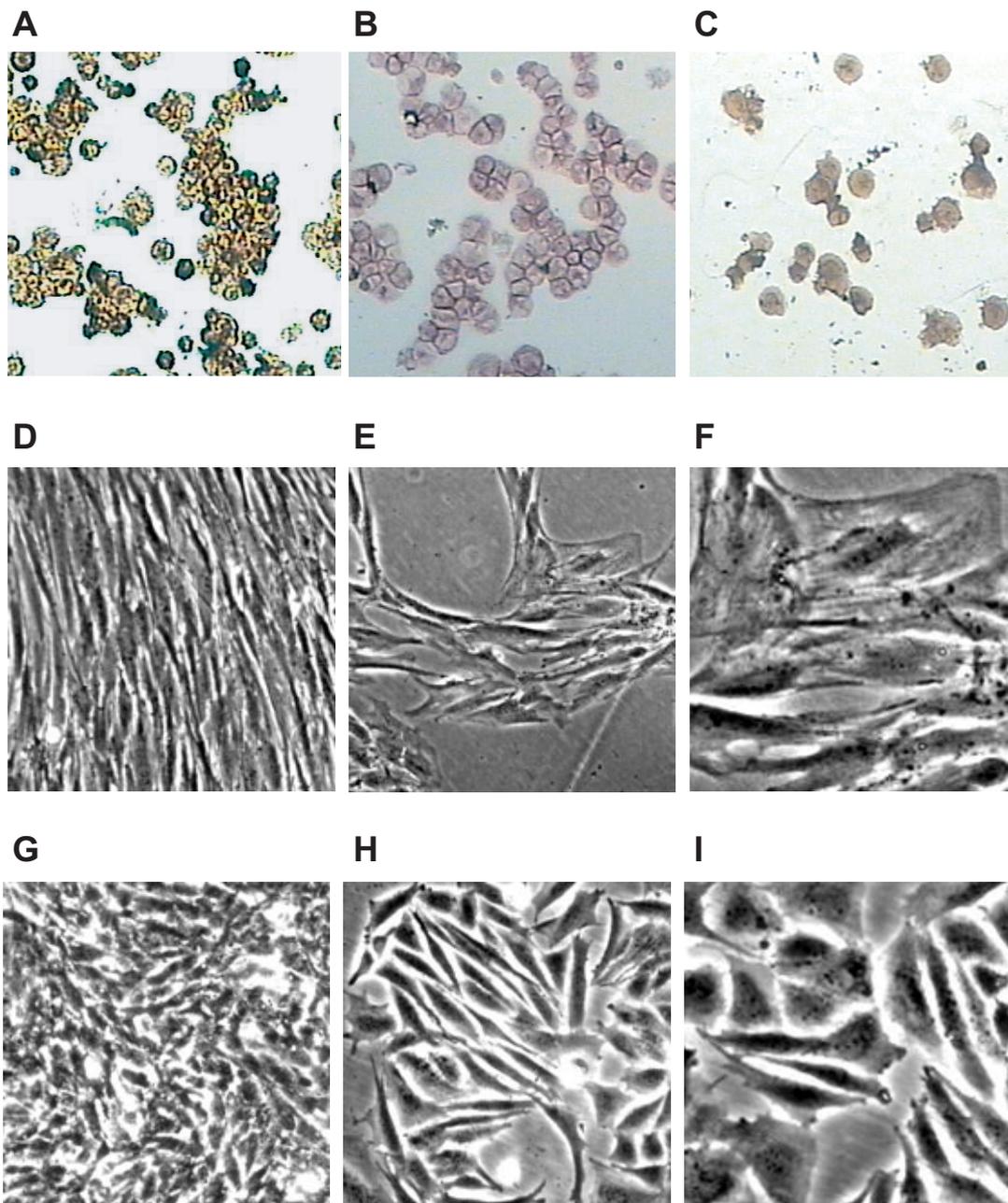


Figure S2

Characterization of MET overexpressing HOB clones

(A) Cytopins of HOB clone cultures were stained. The MET receptor was detected as immuno-histochemical staining (brown) with a monoclonal antibody. (B) The osteoblastic phenotype was determined as cell ability to express alkaline phosphatase activity (ALP) in non-differentiating medium (purple colour). (C) ALP expression was detectable in the same cells that show MET receptor expression using double staining. The following panels show the morphology of monolayer cultures of parental HOBs (D-F) and MET overexpressing HOBs (G-I) at different magnification.