Supplemental Figure 1. Representative sampling of the phenotypic characterization of cell populations derived form osteosarcoma patients. Semiquantitative RT-PCR of bone-matrix genes in N (normal osteoblasts) and B (tumor-derived cells).

Supplemental Figure 2. (A) Hierarchical clustering based on the quantitative protein expression data clearly separates the different experimental groups (controls and osteosarcoma samples). Red indicates up-regulated proteins and green represents down-regulated proteins. Hierarchical clustering of protein quantification values obtained by DIGE experiments and samples were carried out using Genesis software (14), with Euclidean distance and average linkage parameters. Clustering of probe-sets corresponding to microarray experiments and samples were performed using the same software, but Pearson correlation distance and complete linkage parameters were used.

(B) Gene expression profiles of Affymetrix HG-U133A2 probe-sets corresponding to the osteosarcoma protein signature. Probe-set values were normalized before hierarchical clustering analysis. Red in this figure indicates high expression, whereas green indicates low expression. Both background correction and normalization were done using the RMA (Robust Multichip Average) algorithm (15). After calculation of the expression for each probe-set in all the microarrays, the biomaRt package of Bioconductor (16) was used to convert Swissprot/Uniprot access numbers of the proteomic osteosarcoma signature into Affymetrix U133A2 identifiers.

Supplemental Figure 3. Functional network between enriched GO categories and differentially abundant proteins. To assess overrepresentation of GO categories the Hypergeometric test was used with a p-value of 0.05. Yellow nodes are GO categories and red/green nodes are proteins. Red represents induction in osteosarcoma and green represents repression. Enrichment of Gene Ontology (GO) categories was determined using the standard hypergeometric test (17) with p-value<0.05. The network of GO and selected proteins was graphically represented using Cytoscape software (18).

Supplemental Table 1. Detailed information of the differential proteins between primary osteosarcoma cell lines and their normal bone counterparts detected by 2D-DIGE.