Supplementary Figure 3 Primary osteoblasts from wild type and Fhl2−/− calves. (A) Proliferation rate is determined by incorporation of EdU. (B-E) Analysis of primary osteoblasts after culture in mineralization medium for 0, 5, 10, and 15 days. (B) Relative activity of alkaline phosphatase of cell extracts, (C) mineralization shown by alizarin red staining, (D) quantification of mineralization after von Kossa staining. (E) The rate of expression of the representative bone markers Ocn, Phex, and Akp2 was analyzed by qRT-PCR in primary osteoblasts of wild type versus Fhl2−/− cells in relation to Hprt expression. (F) Primary osteoblasts infected with a Runx2-coding virus (Runx2) or a control virus (ctr), were incubated in mineralization medium for 10 days. The mineralized area was quantified after von Kossa staining. The increase in mineralization by ectopic Runx2 expression is not dependent on Fhl2. Experiments were repeated at least 3 times. Bars represent mean ±SD (n=3). * Statistically significant difference (p<0.05).