

Supplementary Figure S1:

Housekeeping gene mRNA expression validation. The expression of a housekeeping gene such as GAPDH is assumed to be constant and used to correct for variations in RNA quality/stability as well as potential variability in the RT-PCR enzymatic reaction. Limited variations in GAPDH expression between samples can be expected and are compensated for using standard calculations. To assess the stability of GAPDH as a housekeeping gene for qRT-PCR between different samples, raw data of GAPDH c(t) values of all investigated samples were plotted for each PFD and intact bone (IB). Error bars indicate standard deviation of all individual animals for each PFD and IB. Since GAPDH expression was found to be reasonably stable between PFDs, the qRT-PCR analysis was based on normalization to GAPDH as a housekeeping gene.

Table 1. Experimental Design

| Day of Tissue Harvest – Post Fracture Day (PFD) | Number of Animals | Methods |
|---|-------------------|--|
| 1 | n=8 | Histology and IHC |
| | n=5 | Protein extraction |
| | n=5 | RNA extraction |
| 3 | n=8 | Histology and IHC |
| | n=5 | Protein extraction |
| | n=5 | RNA extraction |
| 5 | n=8 | Histology and IHC |
| 7 | n=8 | Histology and IHC |
| | n=5 | Protein extraction |
| | n=5 | RNA extraction |
| 14 | n=8 | Histology and IHC |
| | n=5 | Protein extraction |
| | n=4 | RNA extraction |
| 21 | n=8 | Histology and IHC |
| Intact Bone (IB) | n=4 | Histology and IHC |
| | n=5 | Protein extraction |
| | n=5 | RNA extraction |
| | n=1 | Protein extraction for musculoskeletal tissue analysis |