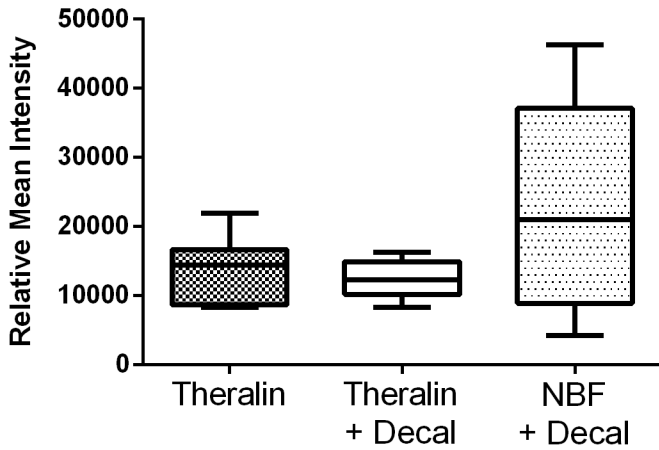
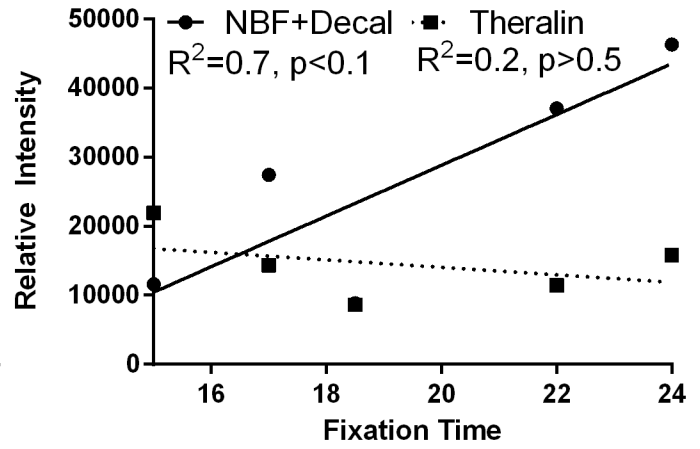


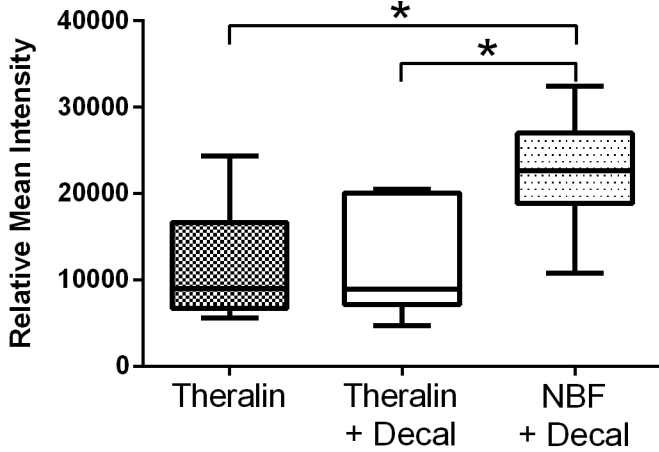
Beta Actin (clone #2)



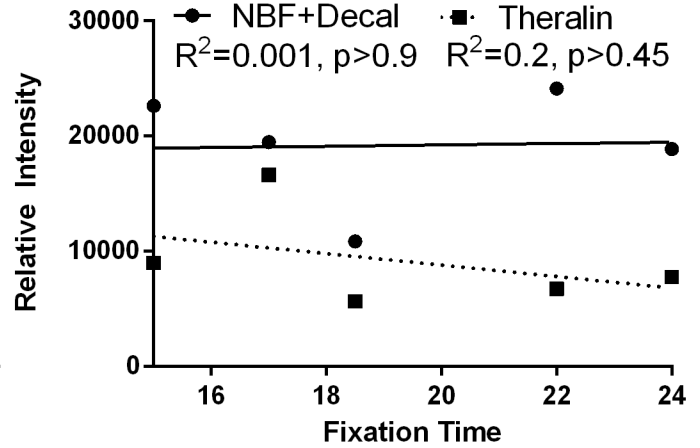
Beta Actin (clone #2)



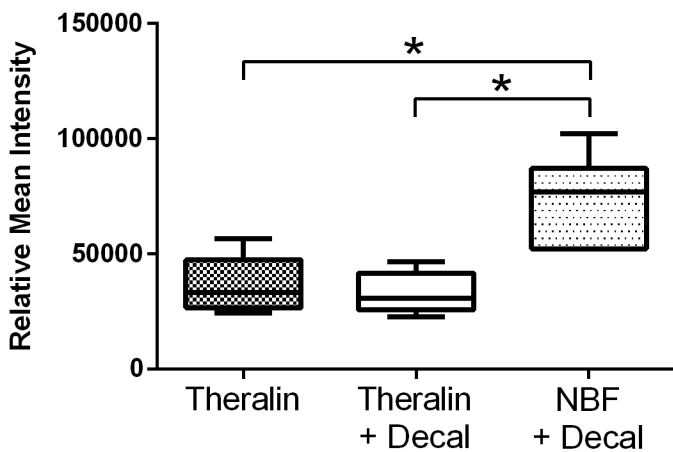
Doublecortin (clone #1)



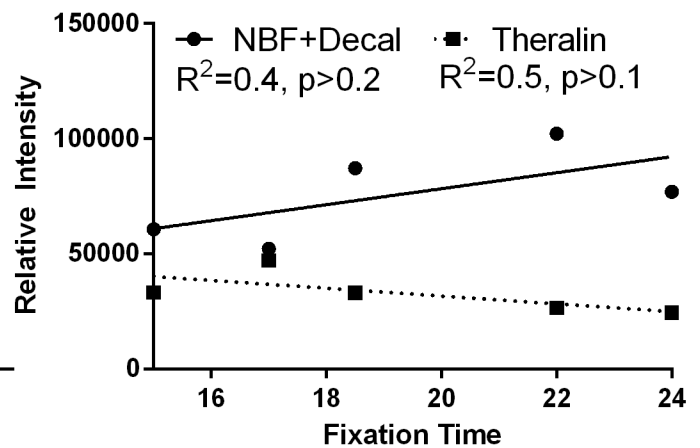
Doublecortin (clone #1)



Doublecortin (clone #2)



Doublecortin (clone #2)



Supplemental Figure 1. Formalin fixation introduces a fixation artifact, whereby measurable β -Actin levels correlate with fixation time, while Doublecortin levels are increased following formalin fixation, independent of fixation time. (Top row) β -Actin is increased following formalin fixation and decalcification of bone tumor samples compared to Theralin fixed or Theralin fixed and decalcified patient matched samples (n=7, laser-capture microdissected tumor cells, boxes = 25th to 75th percentile with whiskers going to the smallest and largest value). Data shown is based on a different antibody clone than data in Fig. 7. A subset of patient samples where exact fixation times were available (n=5) show a direct correlation between fixation time and the level of measurable β -Actin when fixed in formalin but not after Theralin fixation. (Center and Bottom row) Doublecortin is increased following formalin fixation with acid decalcification compared to Theralin fixed or Theralin fixed and acid decalcified patient matched samples (n=7, laser-capture microdissected tumor cells, boxes = 25th to 75th percentile with whiskers going to the smallest and largest value, * p<0.05). This is independent on the specific antibody clone used. However, in contrast to β -Actin, Doublecortin levels do not correlate with fixation time.