

Supplementary Material

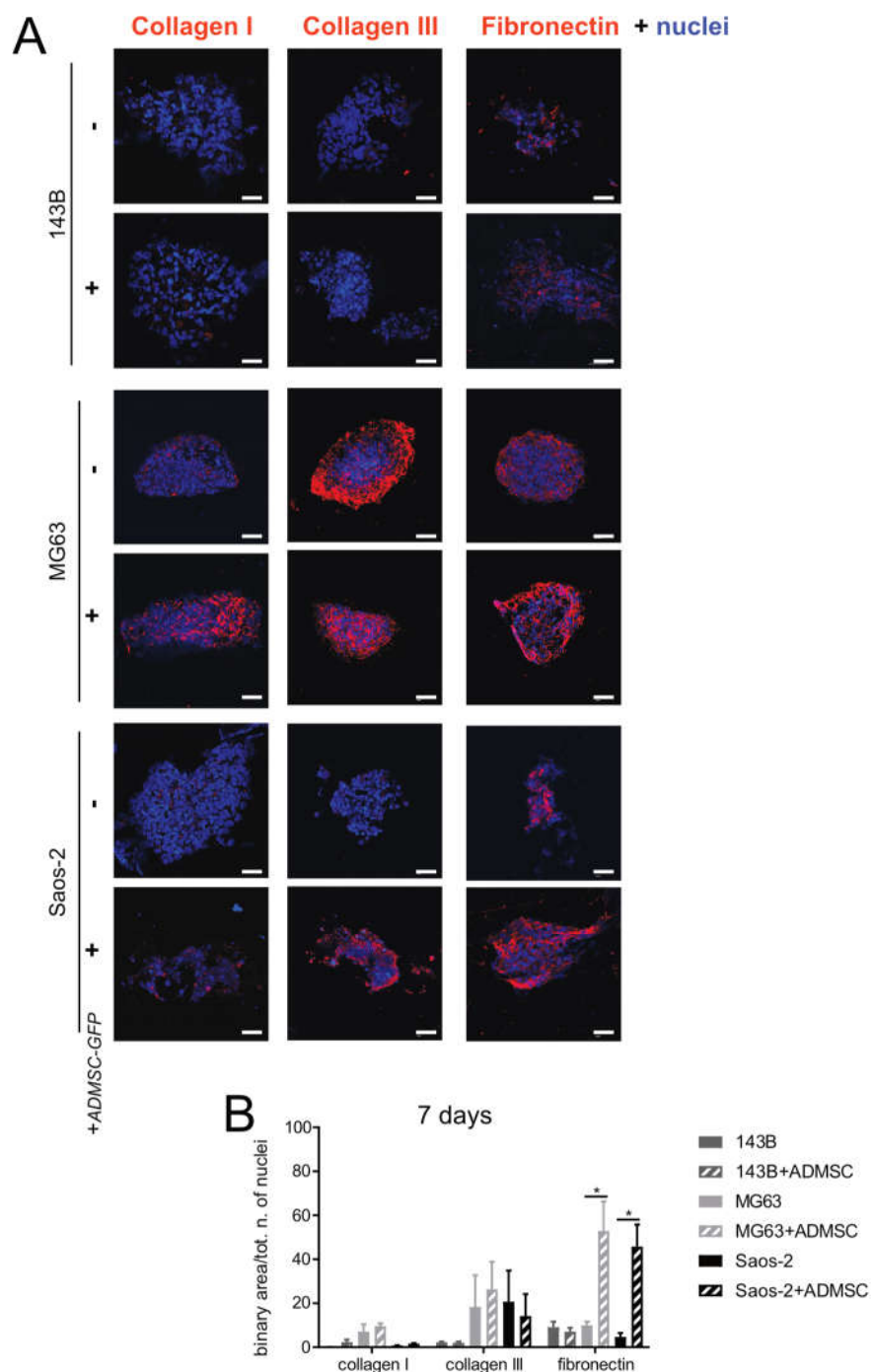


Figure S1. Day 7 expression of ECM proteins. A) Representative confocal images of immunofluorescence of OS spheroids from 3 different cell lines cultured as homotypic (OS cells alone), or as heterotypic mixed with mesenchymal stromal cells (OS/ADMSC-GFP spheroids), grown for 7 days. The spheroids were included in OCT, sliced and stained with specific antibodies for collagen Type I, collagen Type III and fibronectin (red) and counterstained with Hoechst (blue). (scale bar 50 μ m); B) Quantification of IF shown in A, expressed as the total area occupied by ECM protein, and divided for the total number of nuclei. Data presented as mean \pm SEM of n=10 different fields from 3 independent experiments (unpaired two-tailed Mann-Whitney test; ***p<0.001; **p<0.01; *p<0.05).

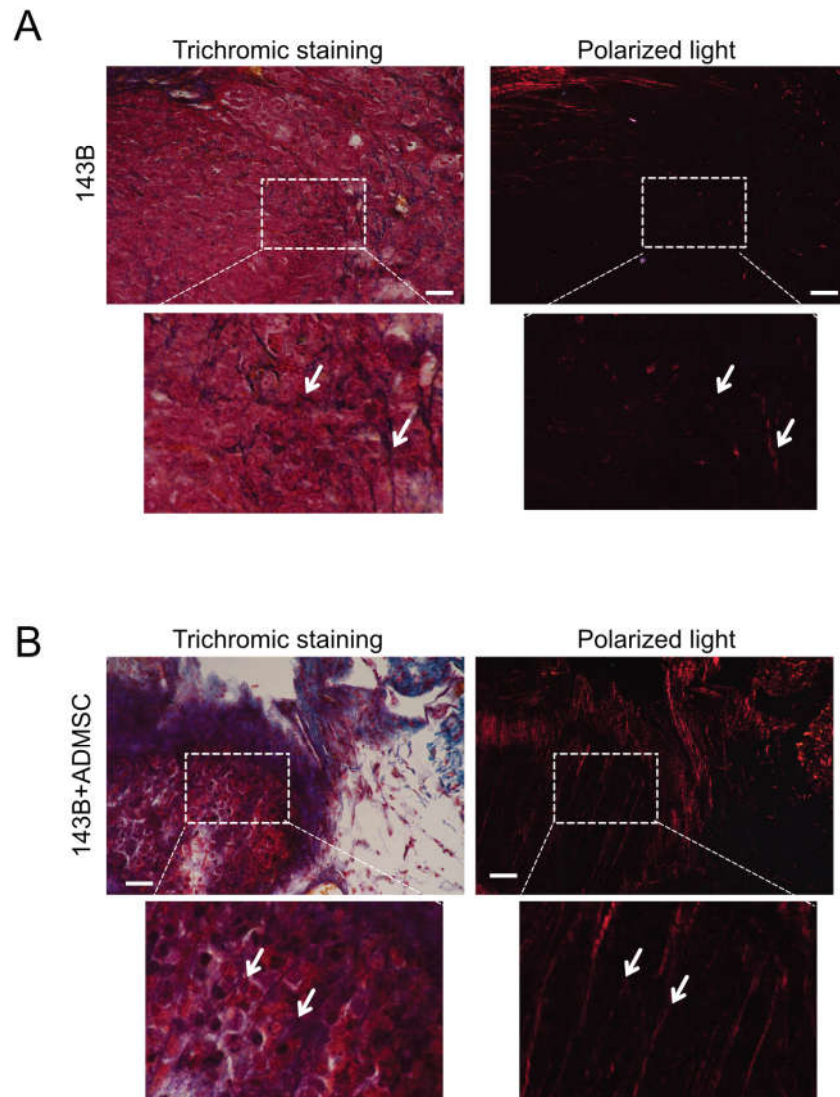


Figure S2. Collagen expression in intratibial OS tumors. A and B) Trichromatic staining (left) and polarized light (right) of orthotopic intratibial OS 143B tumors without ADMSC (A) or with ADMSC in a 3:1 ratio (B) were included in paraffin and sliced at 7 μ m. Collagen appears in blue in the trichromatic staining and in red in polarized light images. Scale bar 100 μ m. Arrows indicate positive staining.

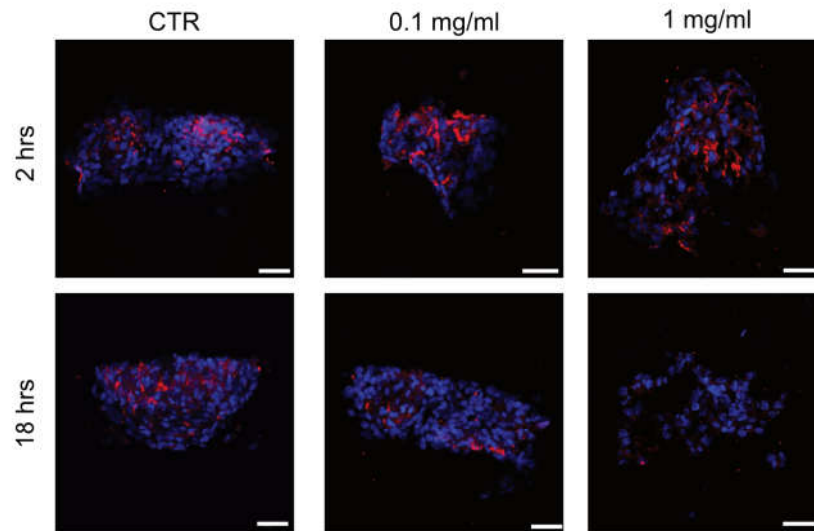


Figure S3. Collagenase treatment does not affect collagen Type III staining. Spheroids from MG63/ADMSC-GFP grown for 14 days, treated with collagenase IA as indicated, included in OCT, sliced at 5 μm and stained for collagen Type III, counterstained with Hoechst (blue). Scale bar 50 μm .