Supp Figure 1: Validation of mitogenic stimulation by growth factor treatment.

Methods. To assess whether growth factor treatment during explant culture results in greater subsequent S phase entry, a distinct set of cells from three donors were treated with 1 µM EdU (5-ethynyl-2'-deoxyuridine, Click-iT™ EdU, Thermo Fisher Scientific C10338) at each feed for the duration of monolayer culture. Visualization of integrated EdU was obtained by imaging the companion Alexa Fluor™ 555 with an EVOS M5000 imaging system (Thermo Fisher Scientific) and the percentage of positive cells was quantified across 5 randomly selected images per condition per donor using ImageJ imaging software (Fiji).

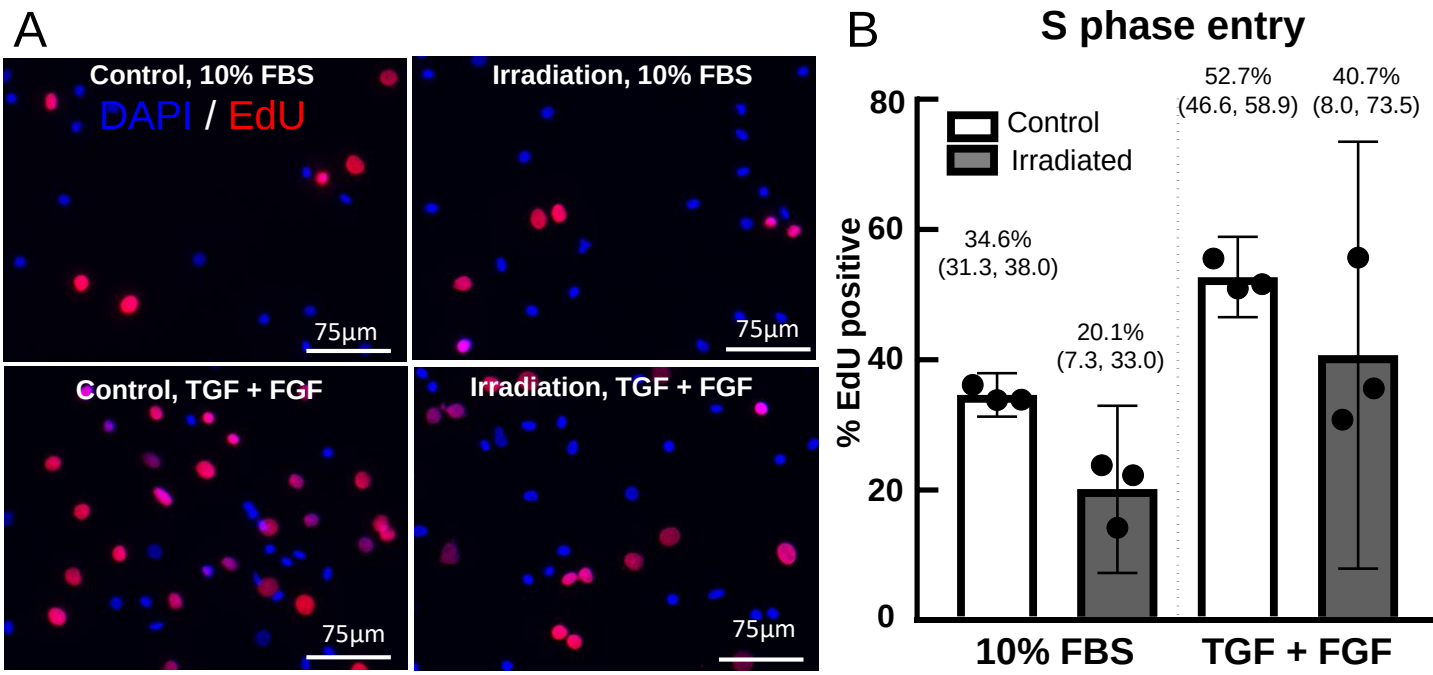
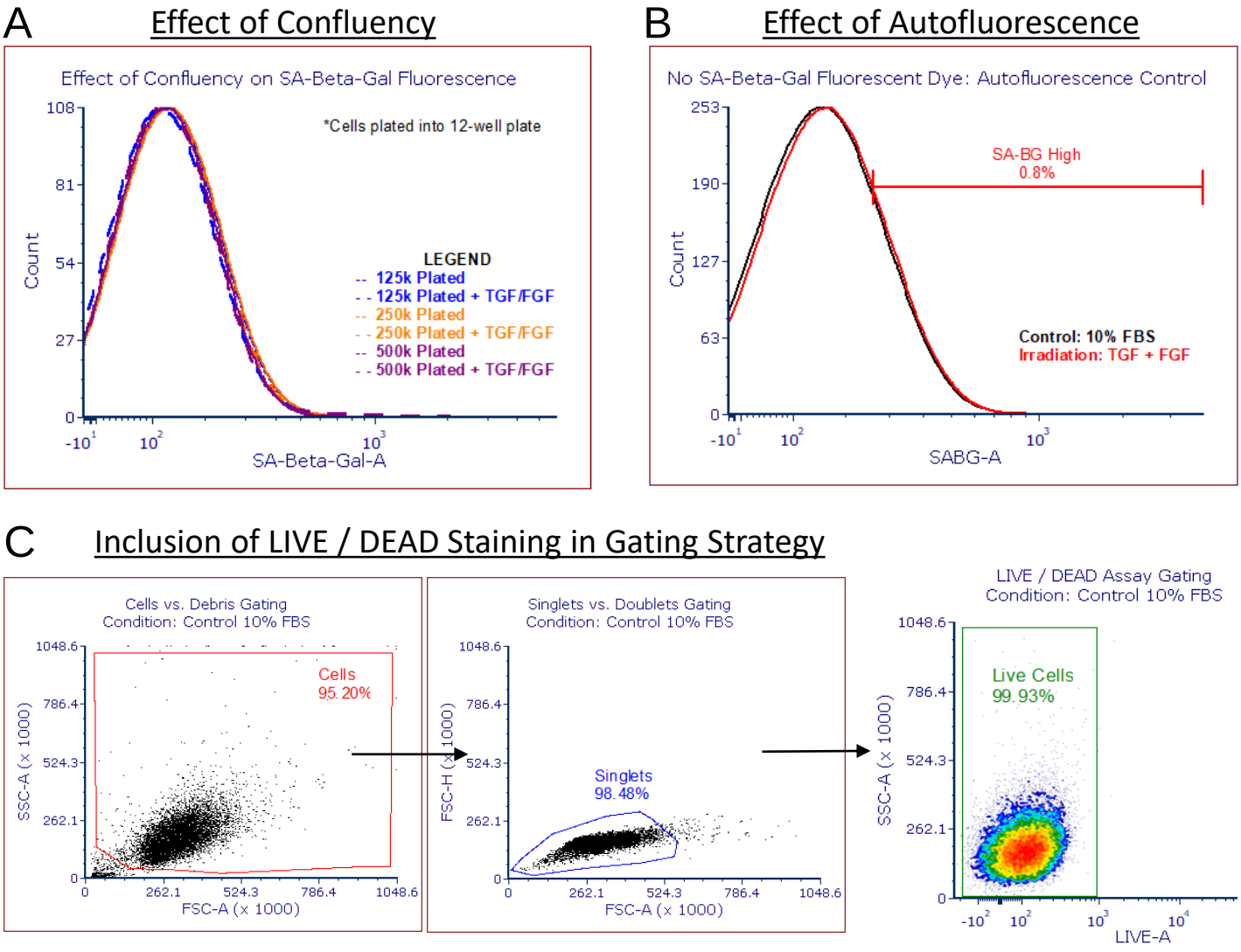


Fig. S1. EdU incorporation during subsequent monolayer culture in response to the growth factors TGF-β1 and bFGF (during explant culture only). (A) EdU by AF555 at the end of monolayer culture. (B) Percentage of cells with positive signal for EdU. The main effects of irradiation ($p<0.01$) and growth factors ($p<0.05$) provided a significant source of variation by two-way ANOVA (n=3).

Supp Figure 2: Validation of SA-β-Gal flow cytometry readout



Gating strategy used for analysis of most donors

Additional LIVE/DEAD Analysis

Fig. S2. (A) Differing confluence and cell morphology was achieved through 3 plating densities with and without growth factors (B) Autofluorescence was tested by leaving out the fluorescent SA-β-gal substrate. Senescent cells (Irradiation plus growth factors) were compared the control 10% group. In this experiment, 0.8% of cells from the IR with TGF/FGF would have been deemed as SA-β-gal high based on autofluorescence alone. (C) The default gating strategy did not use an explicit live/dead cell discriminator, but applying a fixable live/dead analysis (Thermo Fisher Scientific, L34962) showed that in this case 99.93% of cells reaching analysis are live.

Supp Figure 3: Acute DNA damage after irradiation

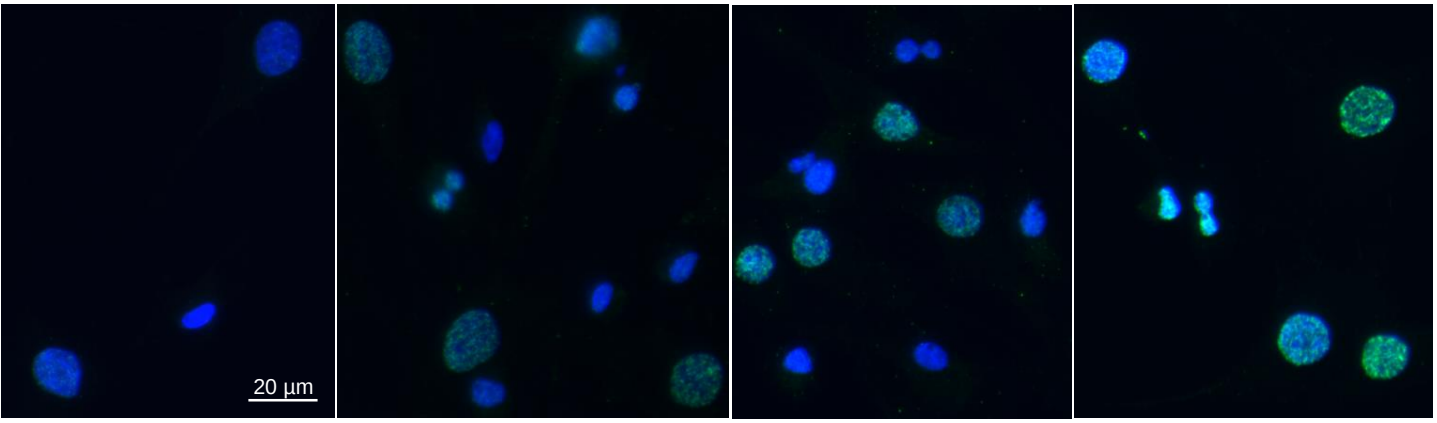


Fig. S3. Primary human chondrocytes in monolayer culture were treated with 10 Gy irradiation to initiate DNA damage. At 30, 60, and 120 minutes after irradiation (or a no irradiation control), individual wells were fixed and analyzed by immunofluorescence for γH2AX. The appearance of foci throughout the nucleus indicates a robust DNA damage response.