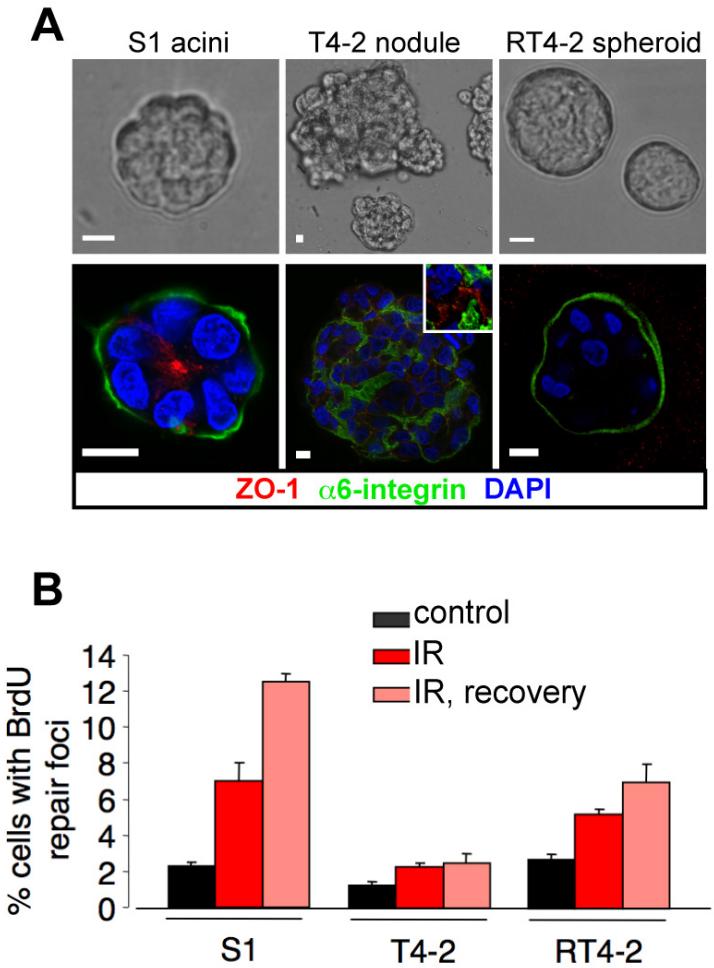

Fig. S1. Basoapical polarity and DSB repair activity in 3D culture of cells. S1 cells, T4-2 cells and T4-2 cells undergoing phenotypic reversion (RT4-2) were cultured in 3D for 10 days in the presence of EHS gel. (*A*) Bright field images of 3D cultures (top) and immunostaining (bottom) for the basal polarity marker α6-integrin (green) and the apical tight junction marker ZO1 (red). The inset represents an enlargement of part of the image. Size bar, 10 μm. (*B*) Percentages of nuclei with BrdU-labeled repair foci, directly after irradiation (IR) and after 24 hours recovery (IR, recovery). Controls were mock-irradiated. Results are averages of two experiments.

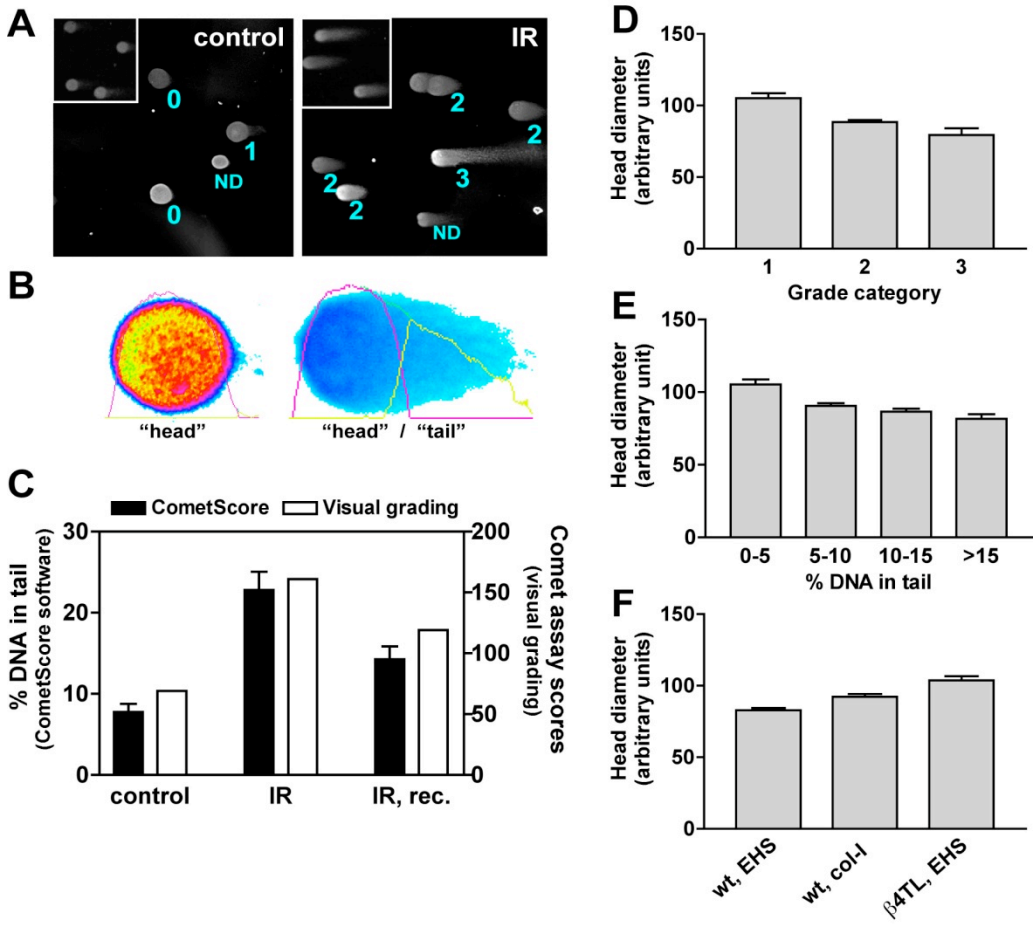

Fig. S2. Implementation of the comet assay for 3D culture of cells. Multicellular structures obtained in 3D culture were released from the ECM gels, embedded in low-melting point agarose, permeabilized, and subjected to electrophoresis following the manufacturer's recommendations (Trevigen). (*A*) A four-grade system was used to quantify the severity of the damage (i.e., the amount of DNA fragments migrating in the "tails"). Comet images from single-cell suspensions are shown for comparison in the insets. In 3D samples, structures with head diameters similar to diameters from single cells were not analyzed (ND). (*B*) For validation, 3D S1 acini processed with the comet assay were analyzed with the CometScore software. Shown are false-color images and "head" and "tail" intensity profiles of representative multicellular structures that were sham-irradiated (left; one multicellular structure shown) or exposed to 3 Gy radiations (right; one multicellular structure shown). (*C*) Comparison between visual and software analysis of sham-irradiated cells (control), cells exposed to 3 Gy gamma radiations (IR), and irradiated cells after two hours recovery (IR, rec.). (*D-E*) Average head diameter of S1 acini exposed to 3 Gy radiations and classified according to the severity of the DNA damage assessed visually (*D*) or using the CometScore software (*E*). (*F*) Average head diameter of wild-type (wt) and ß4TL S1 cells cultivated in EHS gels and in collagen-I gels.

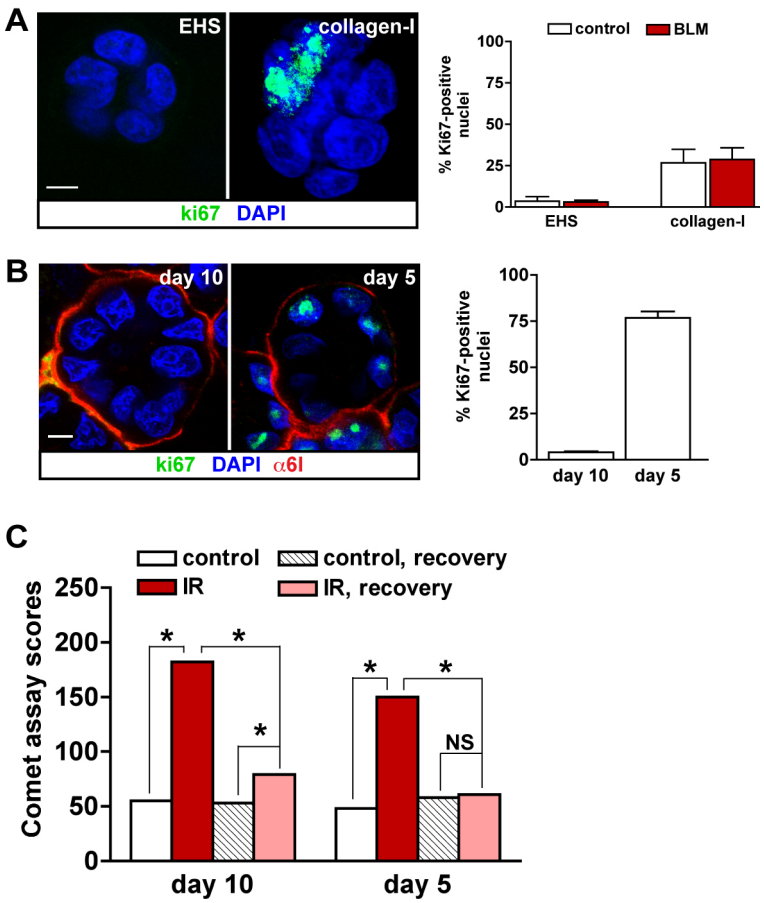

Fig. S3. Relationship between polarity, proliferation and DSB repair in 3D cultures of S1 cells. (*A*) Analysis of cell proliferation in S1 multicellular structures embedded in EHS or in collagen-I gels using the Ki67 cell cycle marker. The percentage of ki67-positive nuclei in vehicle (control) and BLM-treated cells was determined by immunostaining. (*B*) Immunostaining for Ki67 (green) and α6-integrin (α6I; red) in S1 acini cultured in EHS gel for five or 10 days. The bar graph represents the percentages of Ki67-positive nuclei. (*C*) Comet assay scores after exposure to 3 Gy of gamma radiations (IR), directly after IR and after a 2-hour recovery period. * $p<0.0001$, Chi-square, $n\geq331$, three independent biological replicates. Size bars, 5 µm

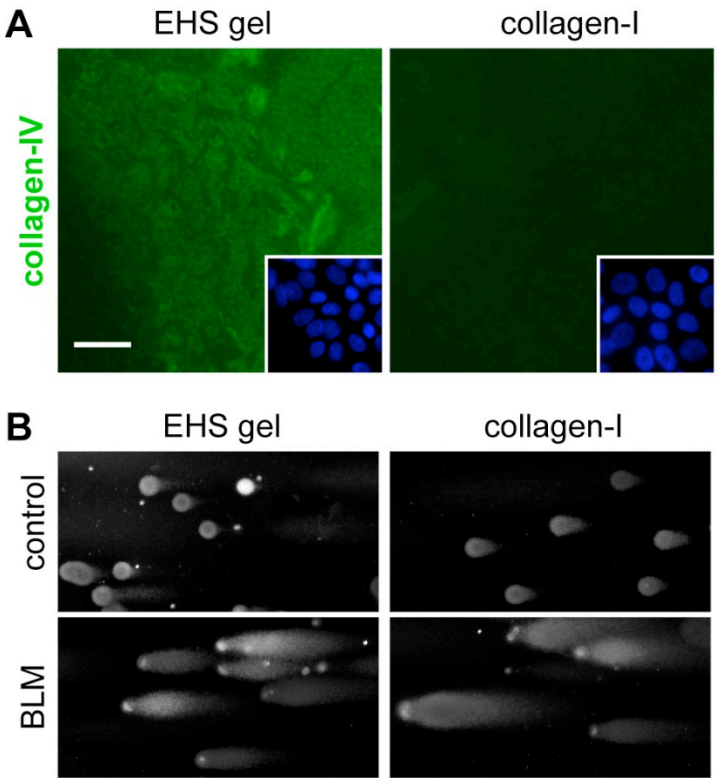

Fig. S4. Basal polarity and BLM sensitivity in flat monolayers of S1 cells (2D culture). Cells were cultured on dried EHS and collagen-I gels. (*A*) Immunostaining for BM component collagen-IV (green). The corresponding DAPI images of nuclei for this field are shown in small size in the insets. (*B*) Images of comet tails (comet assay) from single cells after treatment with vehicle (control) or with BLM. Size bar, 10 μm

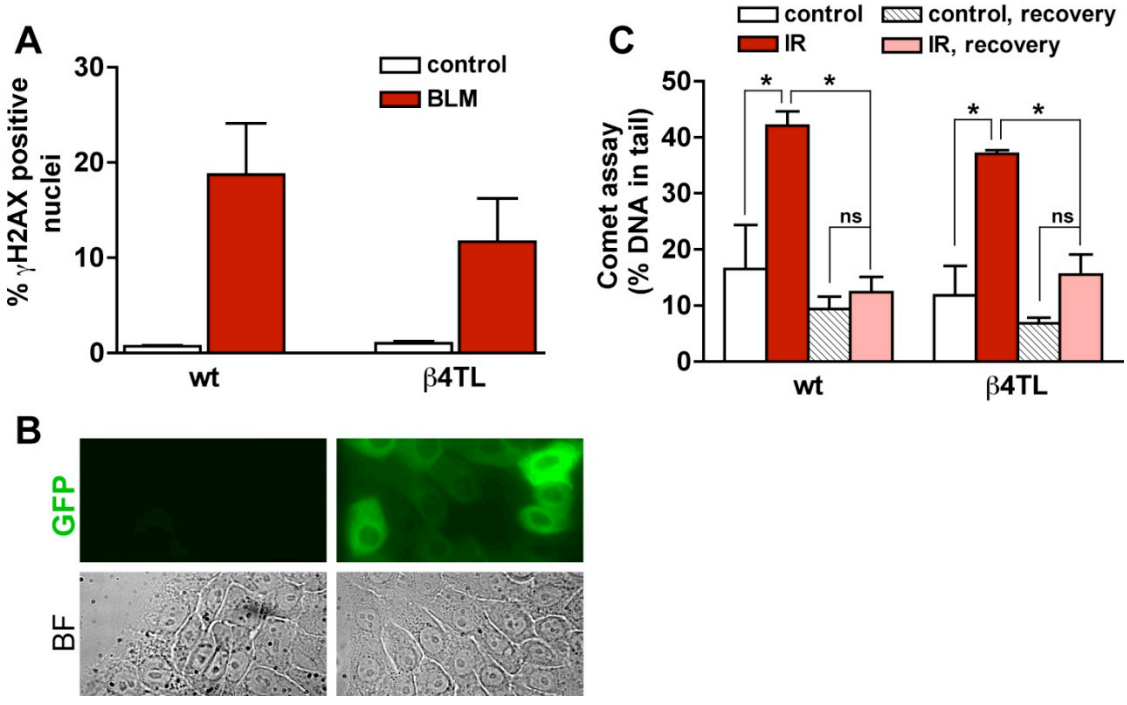

Fig. S5. H2AX phosphorylation and DSB repair activity in flat monolayers of wild-type and ß4TL S1 cells (2D culture). *(A)* Percentages of wild-type (wt) and ß4TL cells with γH2AX staining after treatment with bleomycin (BLM) or with vehicle (control). (*B*) Expression of truncated ß4-integrin monitored by GFP expression in live cells. BF, bright field images corresponding to the GFP fluorescence images shown in the upper panel. (*C*) Measure of DSBs by comet assay on individual cells directly after gamma irradiation (IR) and after two hours of recovery (IR, recovery). Controls were mock-irradiated. * $p<0.001$, Bonferroni, n=4

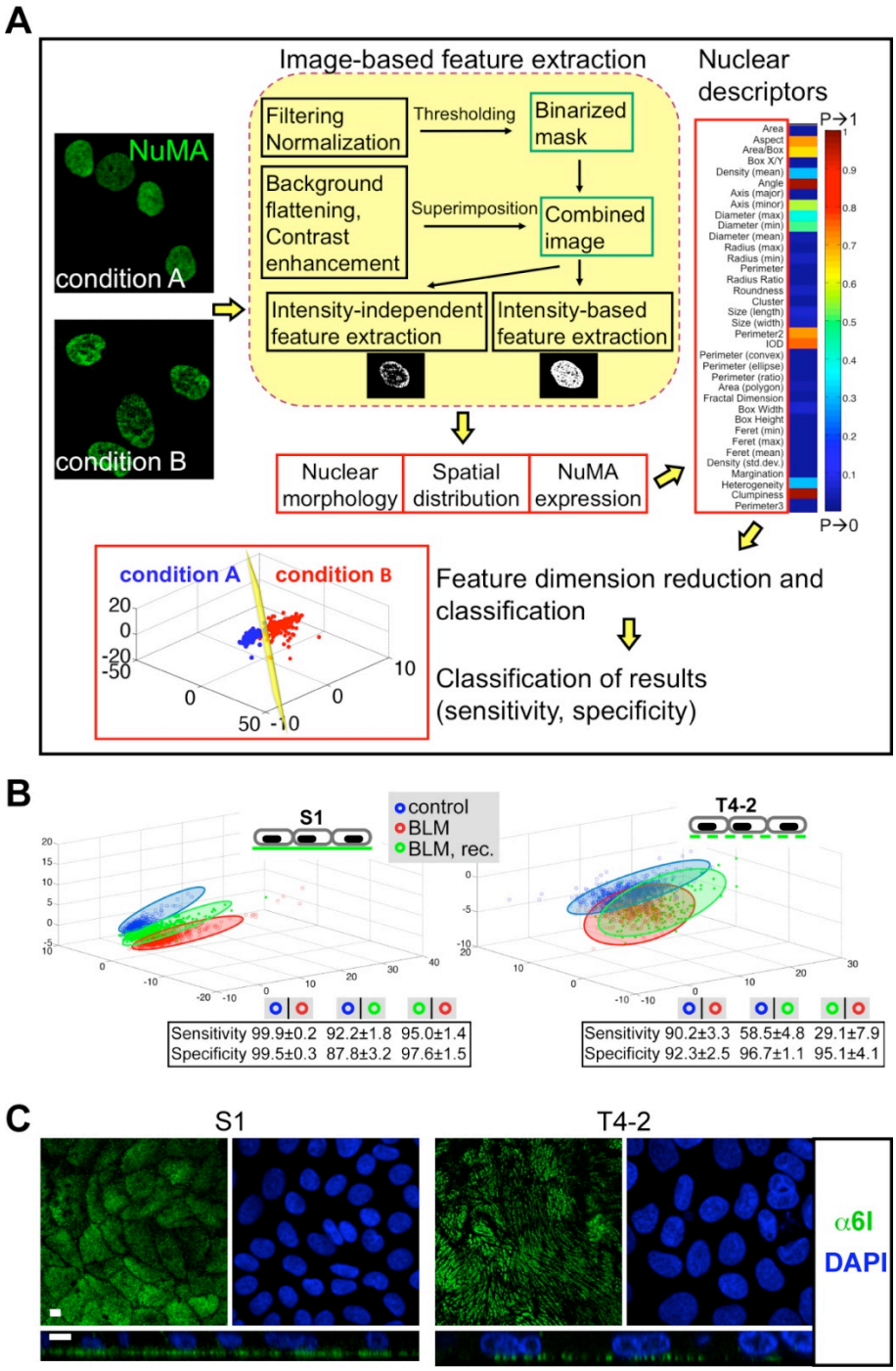

Fig. S6. Analysis of NuMA morphometric descriptors in S1 and T4 cells. (*A*) Schematic of the high-content image analysis and data mining pipeline. (*B*) Visualization of nuclear descriptors in S1 (left) and T4-2 (right) flat monolayers of cells (2D culture) after treatment with vehicle (control), BLM, and BLM followed by 20 hours recovery in BLM-free medium (BLM, rec.). (*C*) Immunostaining for α6-integrin (α6I) in S1 and T4-2 flat monolayers of cells. Maximal intensity projections (top panels) and orthogonal views of confocal z-stacks (bottom panels) are shown. Nuclei are counterstained with DAPI. Size bar, 5 µm