Supplementary Material

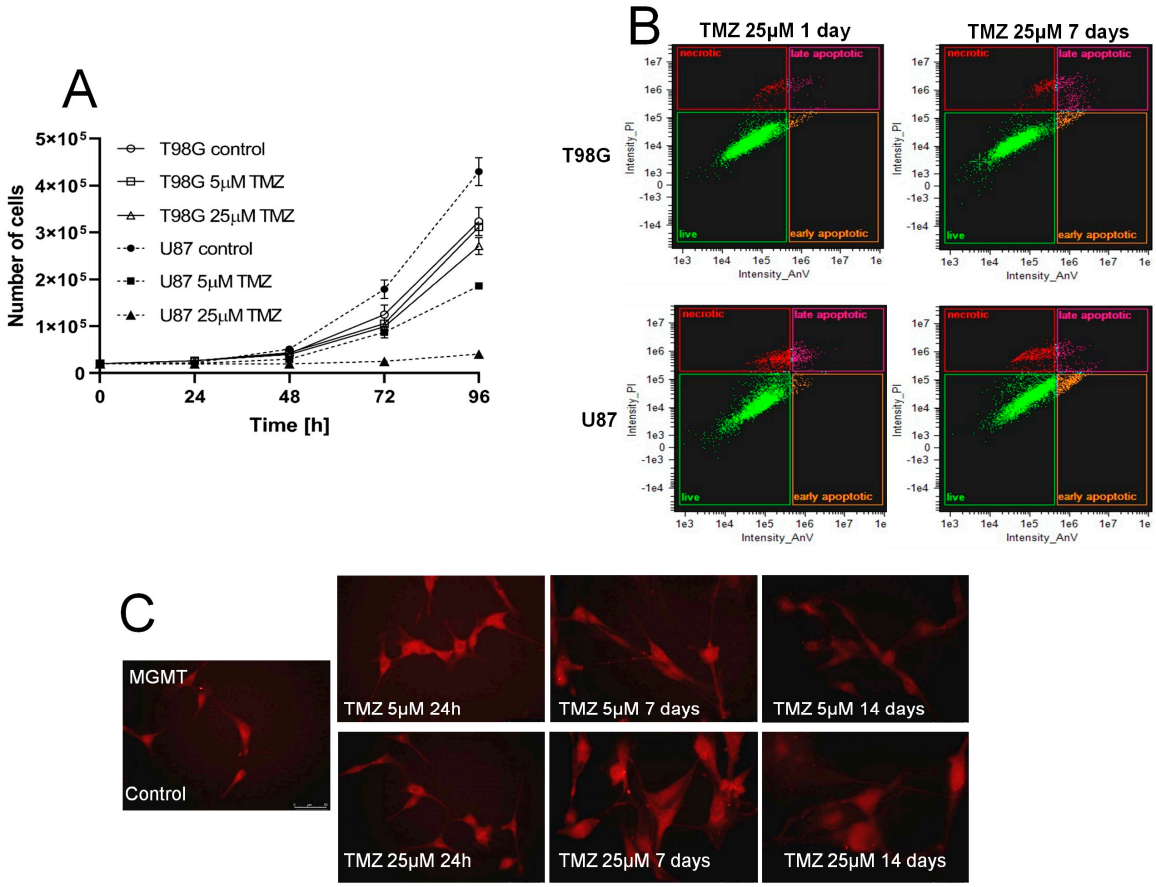

Figure S1. Effect of TMZ on the proliferation. (**A**), apoptosis (**B**) and MGMT levels (**C**) in GBM populations. (A) T98G and U87 cells were incubated in the presence of 5 or 25 μM TMZ. Their proliferation was estimated after the next 24–96 hours with Coulter Counter. (B) T98G and U87 cells were treated with 25 μM TMZ and their apoptotic response was estimated after 24 hours and 7 days with AnnexinV/PI assay. At least 5000 single cells were analyzed, gated according to their area/aspect ratio. (C) TMZ effect on MGMT levels in U87 cells undergone the short- and long-term 5/25 μM TMZ treatment. Note relatively high U87 sensitivity to TMZ and the lack of TMZ effects on MGMT levels in U87 cells.

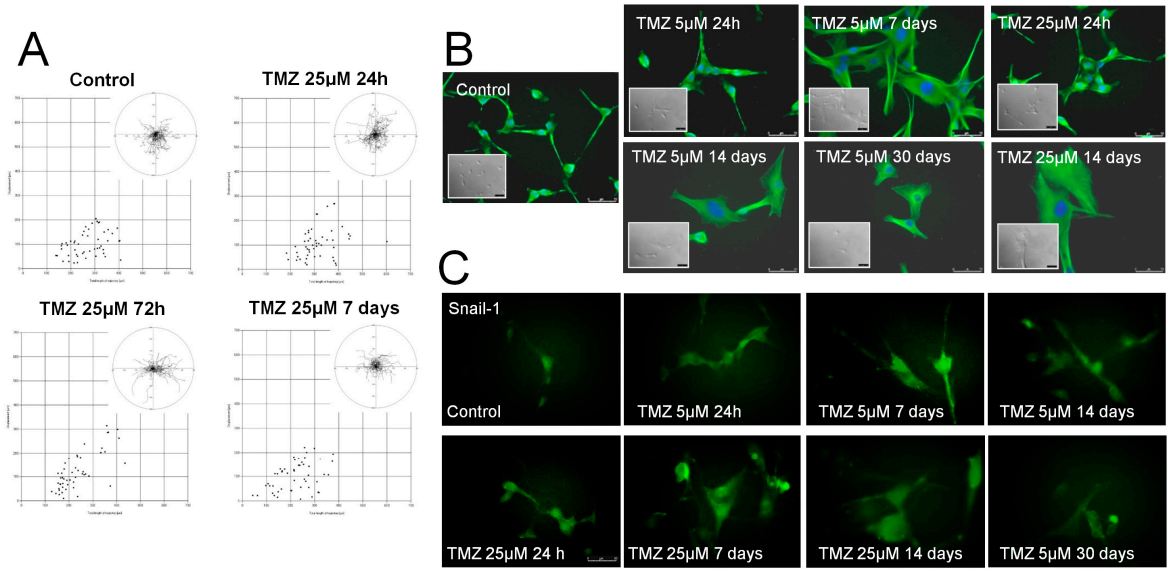

Figure S2. Effect of TMZ on the motility, morphology/architecture of microtubular cytoskeleton and Snail-1 levels in U87 cells. (**A**) U87 cells were seeded at the density of 2 000/well of 12-well plate and incubated for 24 hours before the administration of 25 μM TMZ. Their motility was estimated with time-lapse video microscopy at the indicated time-points. Circular diagrams and dot-plots show trajectories, movement parameters (distance and displacement) at the single cell level. (**B**) Cells were incubated in the presence of 5/25 μM TMZ, fixed, permeabilized and stained against α-tubulin (green)/DNA (blue) to visualize cell morphology and microtubular architecture at the indicated time-points). (**C**) Effect of TMZ on the expression levels of Snail-1 in U87 populations. Scale bars 50 μm. Note the increased spreading degree and the lack of Snail-1 up-regulation in TMZ-treated U87 cells.

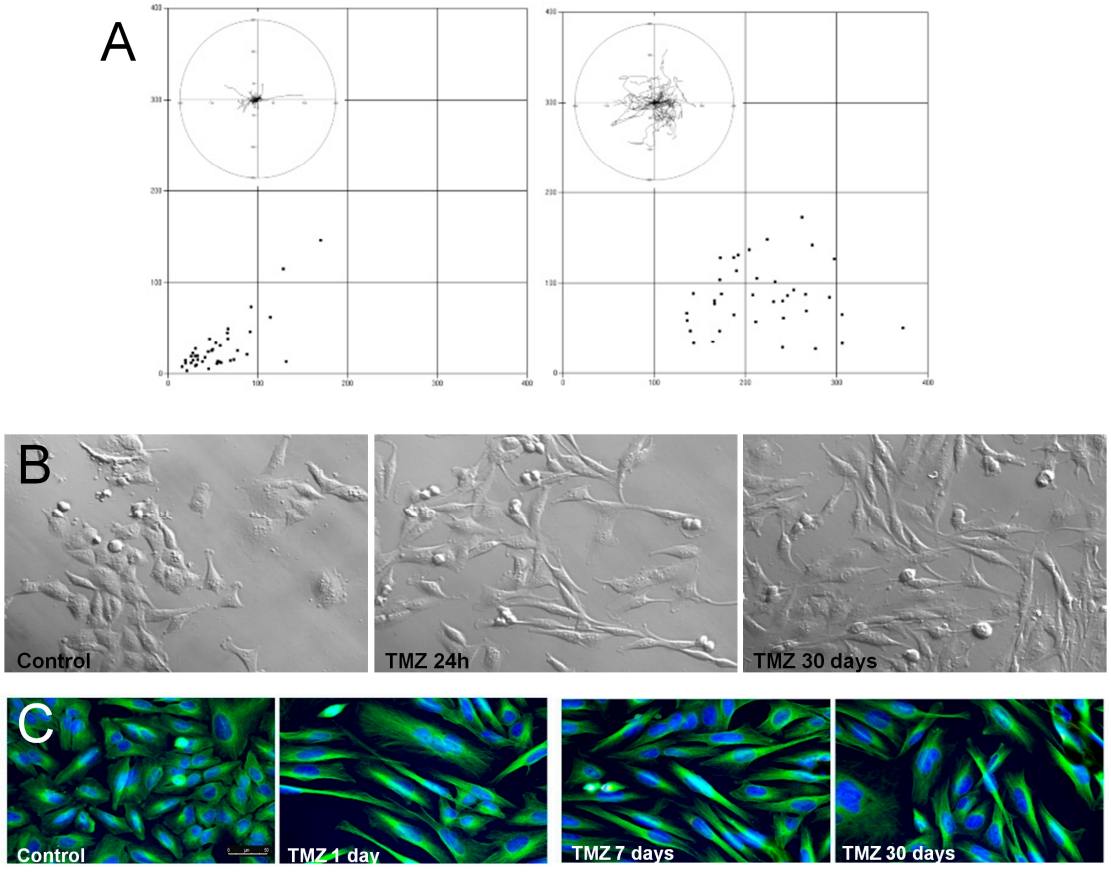

Figure S3. (**A**) Motility of T98G (left) and U87 cells (right) estimated with time-lapse videomicroscopy and presented in circular diagrams/dot-plots, which show single cell trajectories/movement parameters. (**B**) T98G cells were cultivated in the presence of 25 μM TMZ for 1 and 30 days. Their morphology was

estimated with Nomarski Interference Contrast (NIC) microscopy. (**C**) Architecture of microtubular cytoskeleton (tubulin-green/DNA-blue) was estimated with immunofluorescence. Cells were incubated in the presence of 25 μM TMZ, fixed, permeabilized and stained against α-tubulin (green)/DNA (blue) to visualize cell morphology/microtubular architecture at the indicated time-points. Scale bar 50 μm. Note spindle-like shapes of long-term TMZ-treated T98G cells.

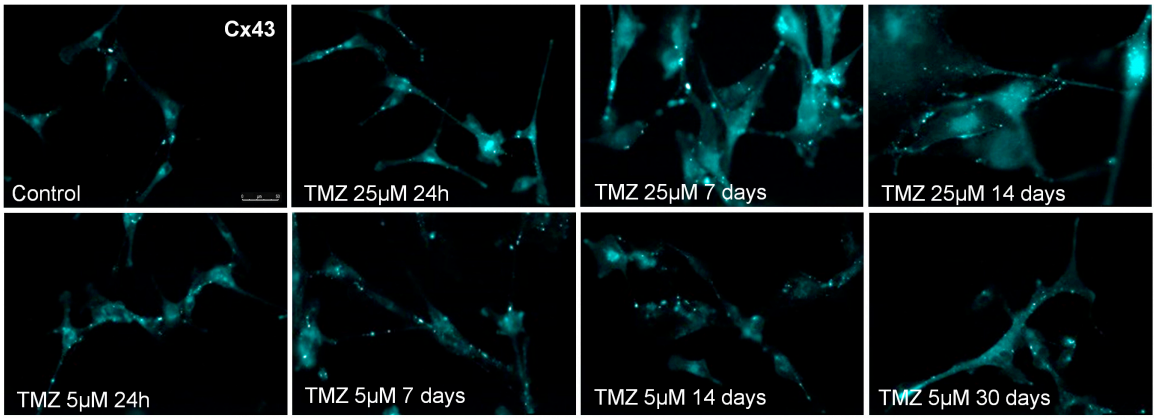

Figure S4. Effect of TMZ on the expression levels of Cx43 in U87 populations. Cells were treated with 5/25 μM TMZ for up to 30 days and Cx43 levels were estimated with immunofluorescence. Scale bar 50 μm.