

Supplementary Data

Table S1 and Table S2 are addition to

2.2. DSB DNA-damage response following 2 Gy irradiation in SOX2⁻ - cells (Results)

Table S1. List of exact values and p-values for all SOX2⁻ - nuclei analyzed for the presence of γ H2AX⁺ - foci.

Antibody (γ H2AX)	Control	30min	18h	72h
Total Foci Area (μm^2)	1.01 \pm 0.11	2.46 \pm 0.18	0.94 \pm 0.04	1.01 \pm 0.09
Control		p < 0.0001	p = 0.9763	p = 0.9994
30min			p < 0.0001	p = 0.0007
18h				p = 0.9972
Total Foci Intensity (AU)	4100 \pm 400	9600 \pm 700	7500 \pm 200	5400 \pm 500
Control		p = 0.005	p = 0.0516	p = 0.9000
30min			p = 0.0020	p = 0.0404
18h				p = 0.5172

Table S2. List of exact values and p-values for all SOX2⁻ - nuclei analyzed for the presence of 53BP1⁺ - foci.

Antibody (53BP1)	Control	30min	18h	72h
Total Foci Area (μm^2)	0.62 \pm 0.10	1.15 \pm 0.07	1.15 \pm 0.08	0.98 \pm 0.10
Control		p < 0.0001	p = 0.0857	p = 0.3016
30min			p = 0.0037	p < 0.0001
18h				p = 0.6835
Total Foci Intensity (AU)	3700 \pm 400	12000 \pm 2000	6800 \pm 400	6100 \pm 500
Control		p < 0.0001	p = 0.0587	p = 0.1609
30min			p < 0.0001	p < 0.0001
18h				p = 0.8239

Table S3 and Table S4 are addition to

2.3. DSB DNA-damage induced by 2Gy irradiation is most persistent in neuronal progenitor cells (Results)

Table S3. List of exact values and p-values for all SOX2⁺ - nuclei analyzed for the presence of γ H2AX⁺ - foci.

Antibody (γ H2AX)	Control	30min	18h	72h
Total Foci Area (μm^2)	1.1 \pm 0.05	3.70 \pm 0.06	3.21 \pm 0.06	2.60 \pm 0.09
Control		p < 0.0001	p < 0.0001	p < 0.0001
30min			p < 0.0001	p < 0.0001
18h				p < 0.0001
Total Foci Intensity (AU)	4800 \pm 200	16100 \pm 300	12400 \pm 200	9000 \pm 300
Control		p < 0.0001	p < 0.0001	p < 0.0001
30min			p < 0.0001	p < 0.0001
18h				p < 0.0001

Table S4. List of exact values and p-values for all SOX2⁺ - nuclei analyzed for the presence of 53BP1⁺ - foci.

Antibody (53BP1)	Control	30min	18h	72h
Total Foci Area (μm^2)	1.03 \pm 0.06	3.23 \pm 0.18	3.08 \pm 0.09	2.81 \pm 0.06
Control		p < 0.0001	p < 0.0001	p < 0.0001
30min			p = 0.7785	p = 0.0396
18h				p = 0.0346
Total Foci Intensity (AU)	6600 \pm 200	23000 \pm 1000	21500 \pm 400	20300 \pm 500
Control		p < 0.0001	p < 0.0001	p < 0.0001
30min			p = 0.4333	p = 0.0261
18h				p = 0.1224

Figure S1 part of 4.5 *Microscopy and Image Analysis* (Material and Methods)

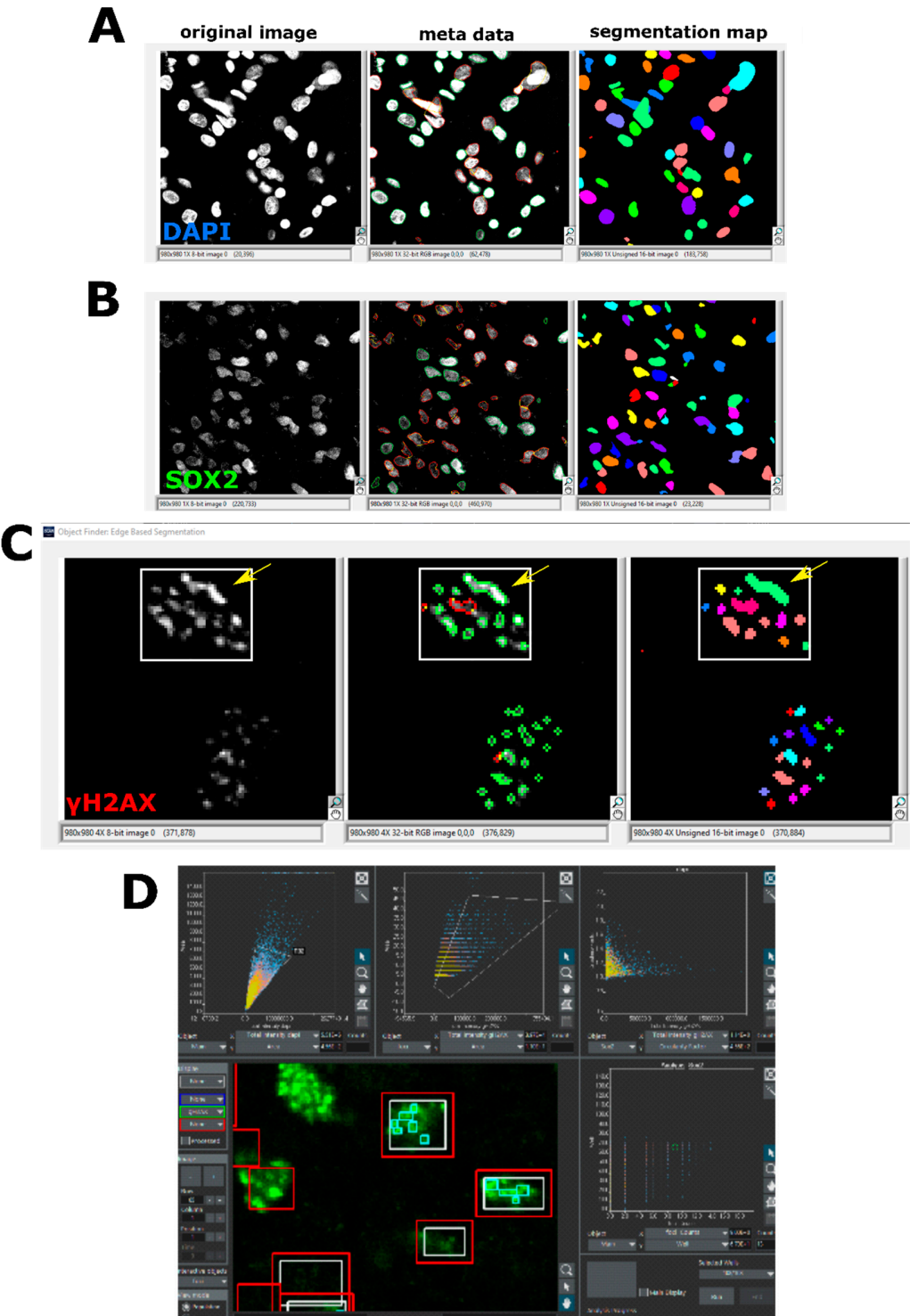


Figure S1. Image segmentation and object classification. (A) On the original image (left panel), at first as a main object DAPI⁺ - nuclei were chosen based on edge segmentations module (ScanR, Olympus). This created nuclei image-mask (right panel) that was used as platform for further analysis. (B) SOX2⁺ - nuclei segmentation was based on edge criteria. SOX2⁺-cells were identified only if the signal appeared within the cell nucleus (C) γ H2AX⁺ - foci segmentation was performed based on edge segmentation. In white rectangle are foci within one nucleus that are good representation of software limitations and at the same time, non-objective individual foci counting. Yellow arrow is pointing at a big focus that was segmented by software, even though 3 or 4 foci could be visually identified (no universal agreement on this issue). Therefore, to omit a potential bias in single focus quantification, total foci area appeared as better parameter, since total area is independent of that if foci are grouped together and area calculated, or if foci are separated individually and total area summed at the end. (D) Example of “gating” principle in ScanR where all the unwanted nuclei (double- or small dying nuclei) were excluded. Red rectangles represent DAPI⁺ / SOX2⁻ - nuclei, white rectangles represent DAPI⁺ / SOX2⁺ - nuclei and green rectangles are γ H2AX⁺ - foci. All gating parameters were kept constant throughout the analysis, for all experimental groups.