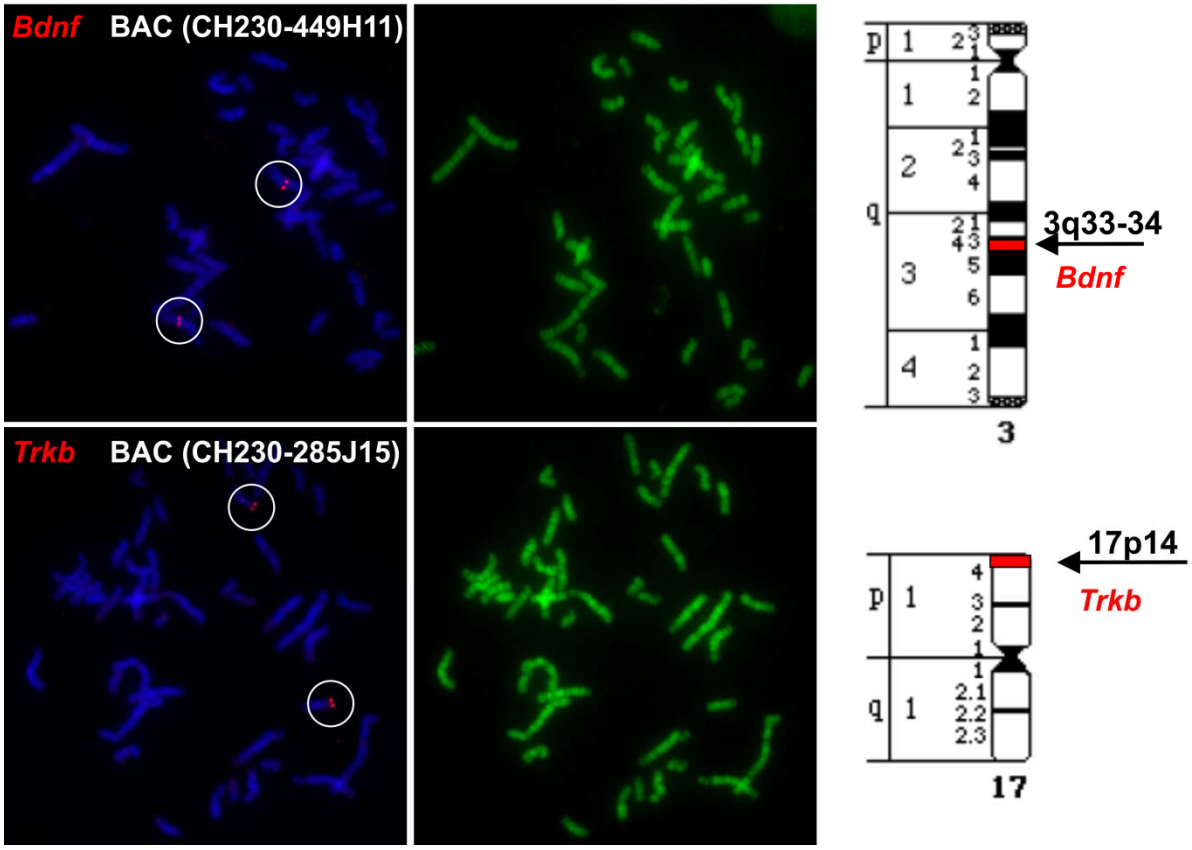
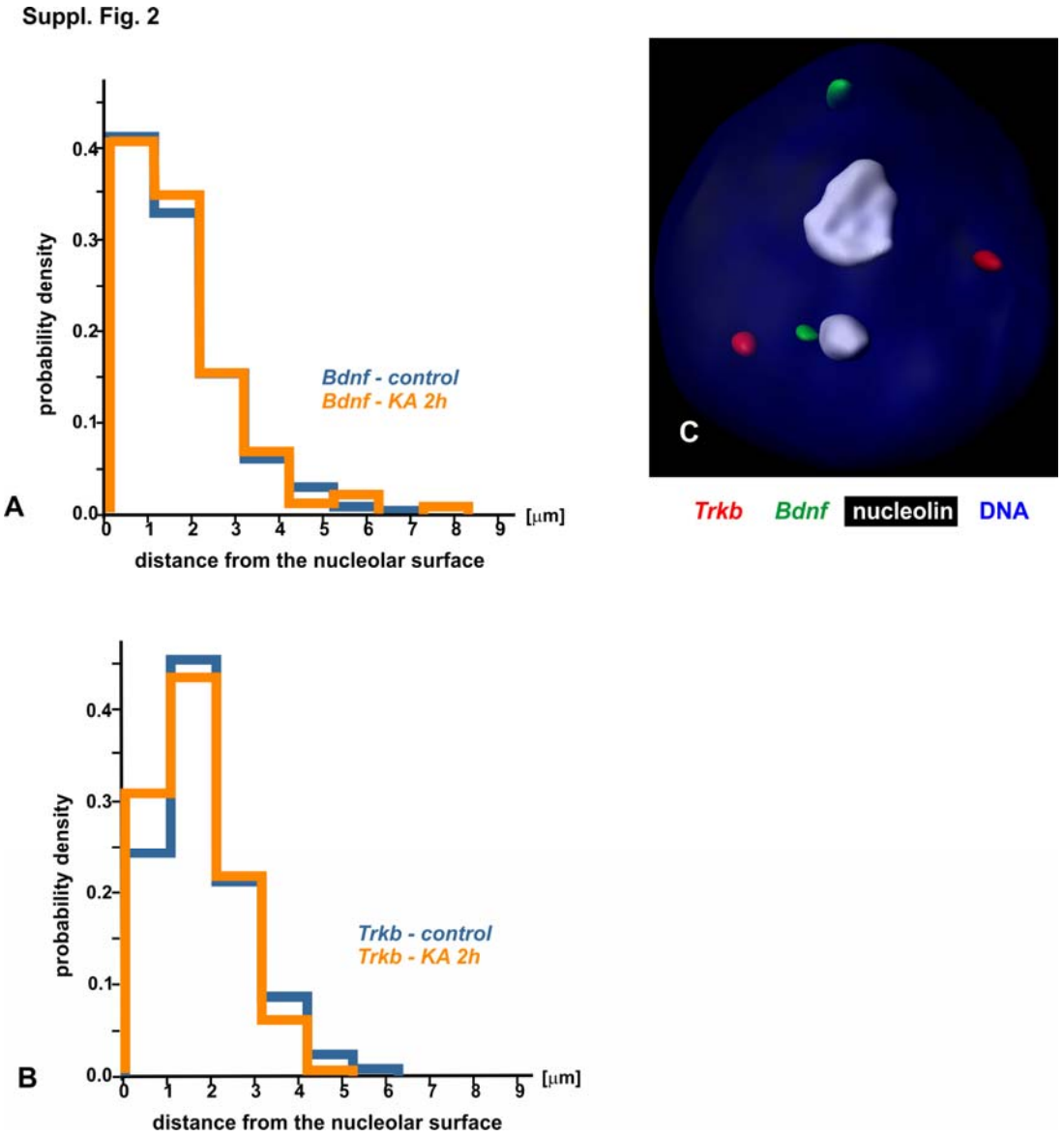
Suppl. Fig. 1



Suppl. Fig. 1. Validation of FISH probes

(A, B) Rat primary fibroblast metaphase spreads hybridized with TRITC labeled probes for Bdnf (A) and Trkb (B); BACs used for probes generation, CH230-449H21 and CH230-285J18 for Bdnf and Trkb respectively, were ordered from CHORI. The chromosome ideograms show exact localization of Bdnf and Trkb.

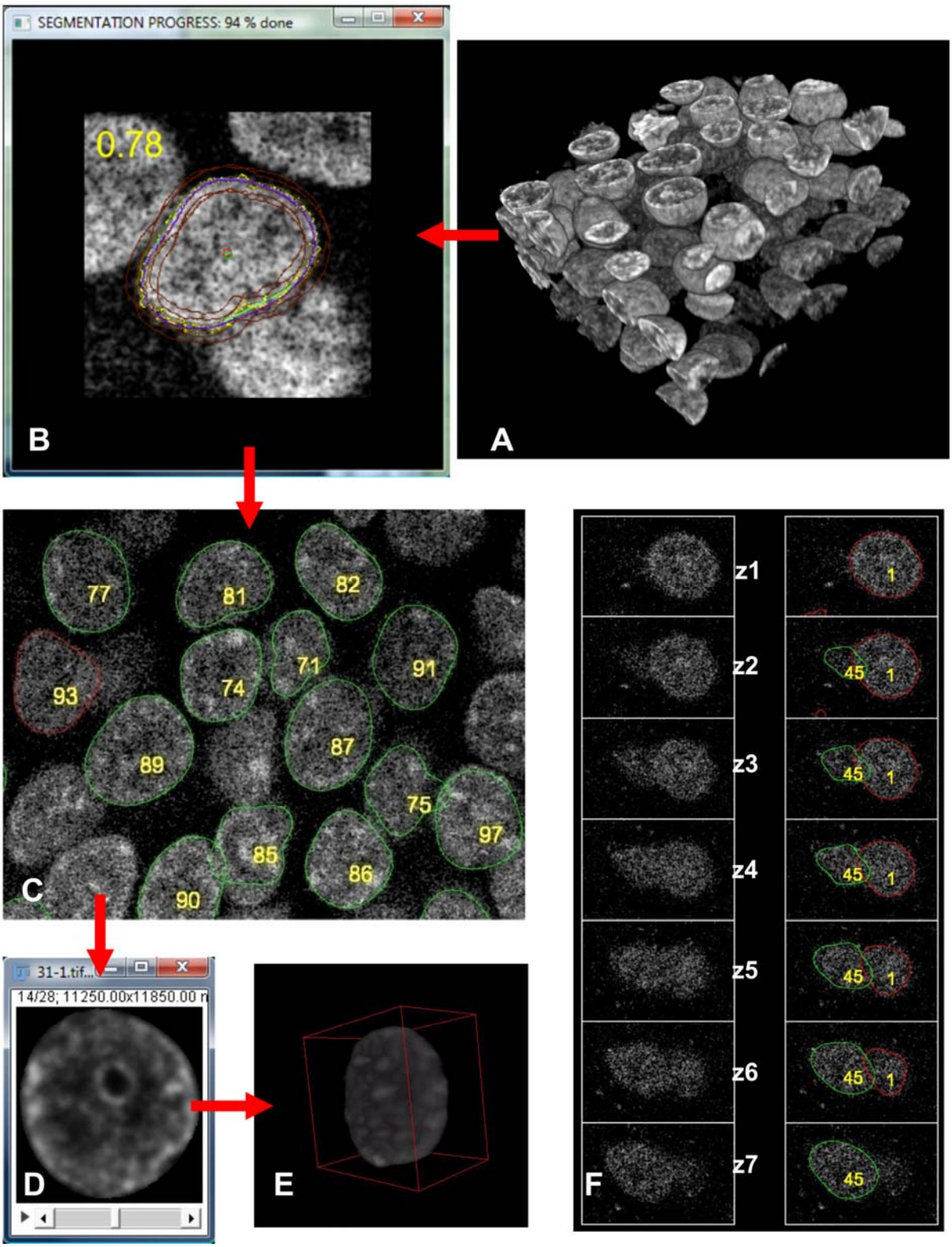


Suppl. Fig. 2. Spatial relationship of the Bdnf and Trkb genes to the nucleolus

A, B) Quantitative analysis of the intranuclear positions of Bdnf (A) and Trkb (B) alleles in relation to the nucleolar surface, in the nuclei of the dentate gyrus granule neurons from control animals (blue lines) and kainate-treated animals sacrificed at the 2 hour time point after the onset of the status epilepticus (orange lines). Shown are the histograms of the probability density (vertical axis) of finding the nucleus with a given minimal distance between the respective alleles and the nucleolus surface (horizontal axis);

C) The example of a 3D reconstructed segmented neuronal nucleus from the control tissue after immuno-FISH for Bdnf (green) and Trkb (red) alleles, and the nucleolus (white) identified by nucleolin immunoreactivity.

Suppl. Fig. 3


SEGMENTATION PROGRESS: 94 % done
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Suppl. Fig. 3. Schematic presentation of 3D segmentation procedure

A) An example of confocal stack taken from the 30 μm-thick section of the rat hippocampal dentate gyrus. Note the very dense packing of the nuclei of granule neurons visualized using TOPRO-3 staining. The red arrows indicate the direction of the work-flow of the software.

B) Individual nucleus recognized by "Segmentation magick". This screen snapshot of a running software was taken while the continuous boundary tracing was being done.

C) The result of the segmentation of the stack shown in (A). The segmented nuclei are numbered, and their outlines are highlighted in colors. Green outlines indicate nuclei that were completely segmented by the software, whereas red outlines indicate nuclei that are recognized by the software as not properly segmented.

D) The representation of every segmented nucleus is saved in the form of a single small image-stack.

E) The 3D projection of the nucleus shown in (D)

F) The picture demonstrates the accuracy of the software. Note that even the nuclei that appear to overlap due to the limitations in confocal mresolution can be separated by the software.