Science Advances

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Supplementary Materials for

Elovanoids are a novel class of homeostatic lipid mediators that protect neural cell integrity upon injury

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Published 27 September 2017, *Sci. Adv.* **3**, e1700735 (2017)
DOI: 10.1126/sciadv.1700735

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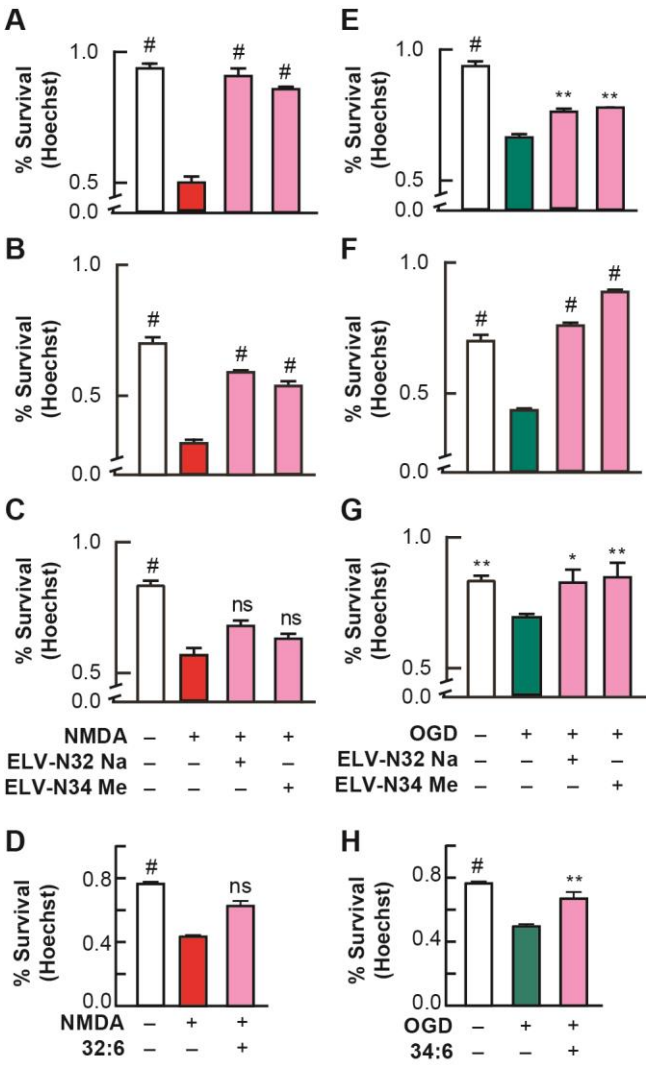

fig. S1. ELV-N32 and ELV-N34 elicit protection of cerebral-cortical mixed neuronal cell cultures exposed to OGD or NMDA. Cell survival assessed by Hoechst positive nuclei counting and unbiased image analysis after cerebral-cortical mixed neurons in culture (DIV 12) were exposed to NMDA (50 μM) **(A** to **C)** or OGD **(E** to **G)**, respectively, in the presence of either ELV-N32 Na or ELV-N34 Me (500 nM). These are results from three separate experiments. (#p<0.0001, **p<0.001 and *p<0.05, n=9, one-way ANOVA, followed by Holm-Sidak's multiple comparisons test). 32:6 (250 nM) could attenuate NMDA excitotoxicity **(D)** and 34:6 (250 nM) elicits neuroprotection to cortical neurons in culture (DIV 28) exposed to OGD (#p<0.0001, and **p<0.001) **(H)**.

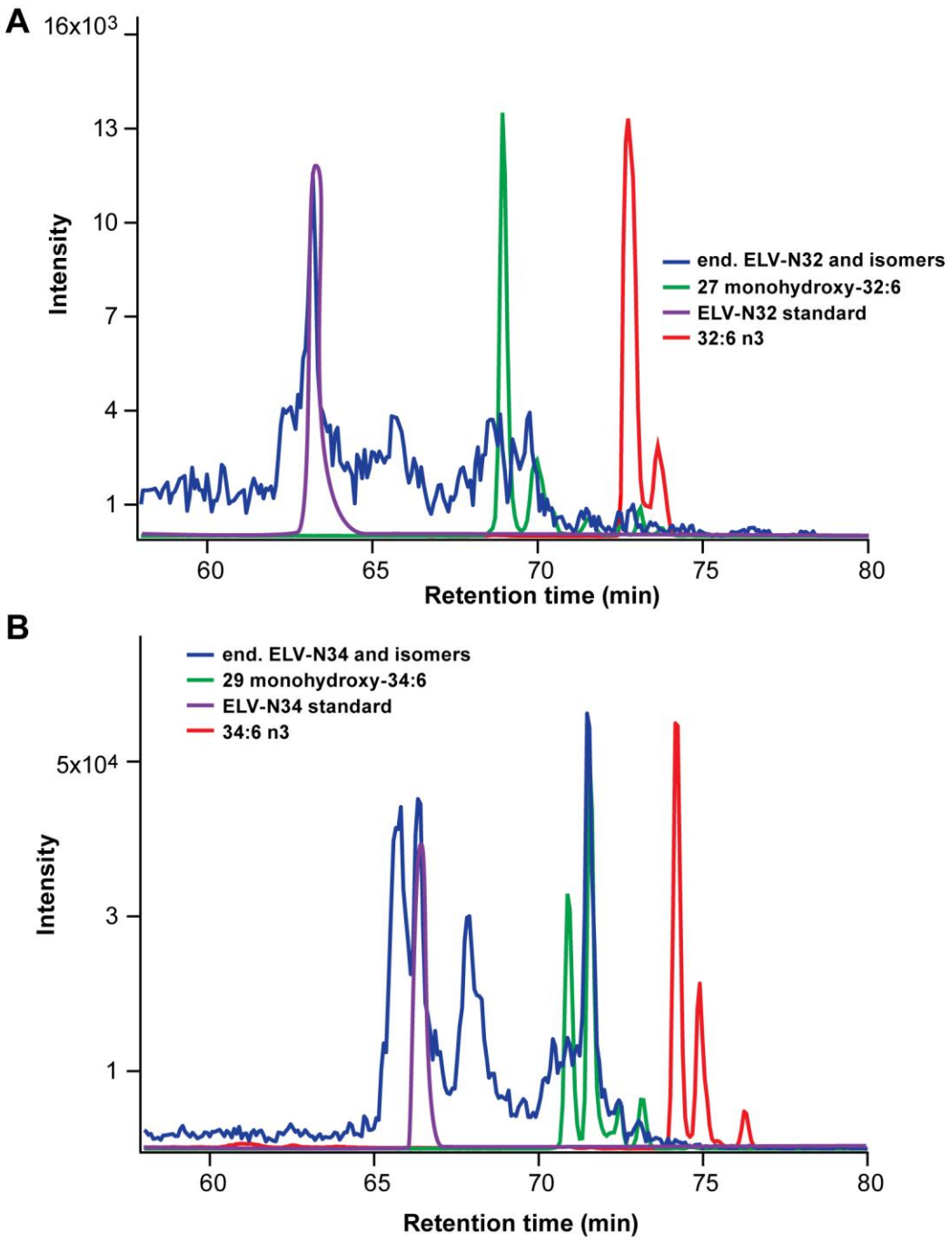

fig. S2. ELV-N32 and ELV-N34 in neuronal cell cultures. Cells were incubated with 32:6n3 and 34:6n3 5 μM each, under OGD conditions. **(A)** 32:6n3 (red line), endogenous mono-hydroxy-32:6 (green line) and ELV-N32 (blue line) are shown with ELV-N32 standard (purple line). MRM of ELV-N32 matches well with the MRM of the ELV-N32 standard. In **(B)**, the same features were shown in 34:6n3 and ELV-N34. There are more peaks in ELV-N34 MRMs, which implies possible isomers.

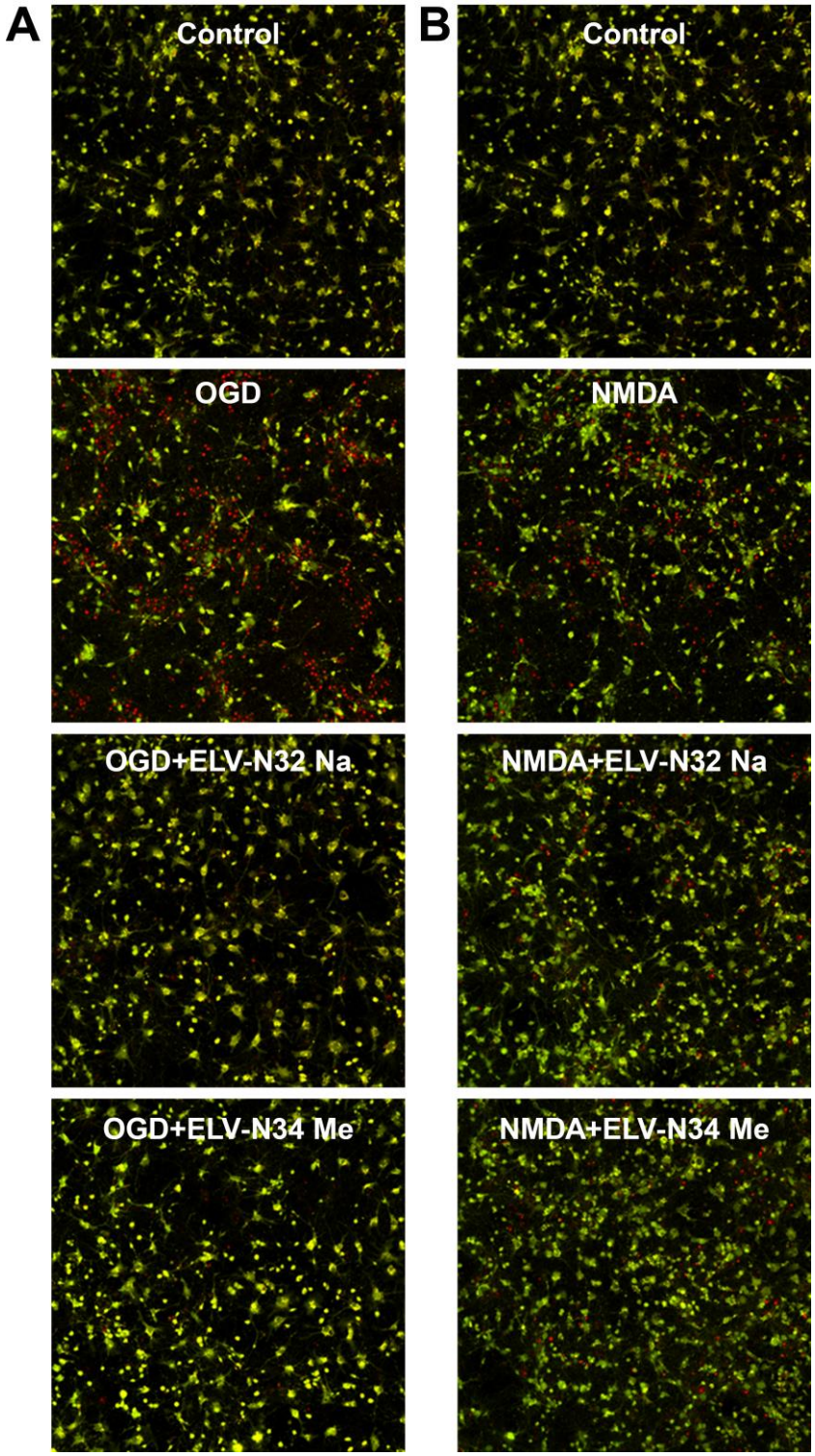

fig. S3. Representative images of calcein-stained cerebral-cortical mixed neuronal cells exposed to OGD or NMDA. Neuroprotection elicited by ELV-N32 Na or ELV-N34 Me (500 nM) as assessed by calcein-positive cell counting after exposure to OGD stress **(A)** or NMDA excitotoxicity **(B)**.

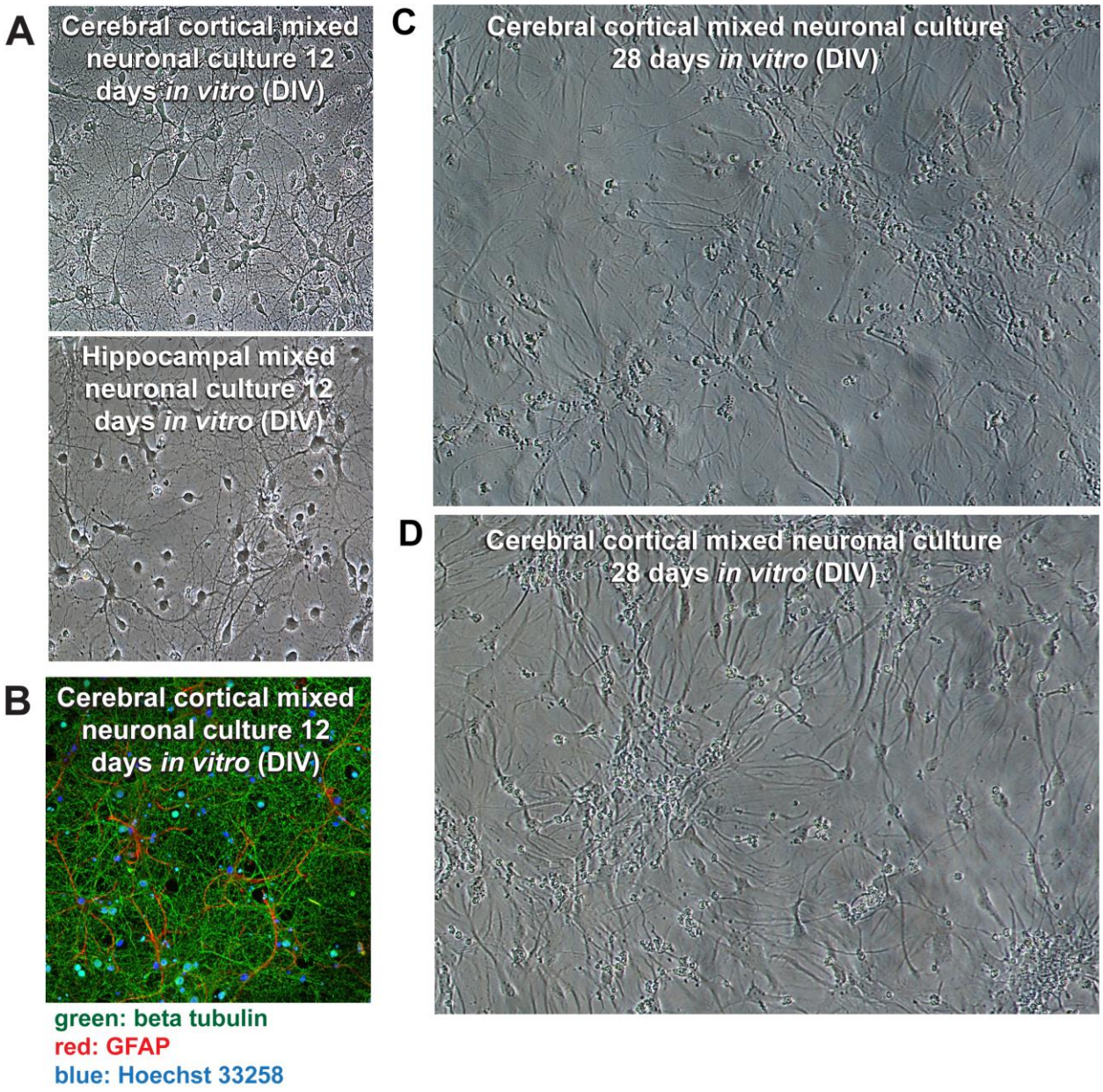

fig. S4. Representative bright-field and fluorescent images of cerebral-cortical mixed neuronal and hippocampal mixed neuronal cultures. (A) Bright field images (10X) of cerebral cortical mixed neuronal and hippocampal mixed neurons in culture 12 days *in vitro* (DIV12) showing morphology. **(B)** Representative immunofluorescent image of cerebral cortical mixed neurons in culture 12 days *in vitro* (DIV12) stained for βIII tubulin, GFAP and Hoechst. As determined by immunostaining, the mixed neuronal cultures had about 85%-90% neurons, while the rest were glial cells. **(C** and **D)** Bright field images (10X) of cerebral cortical mixed neurons in culture 28 days *in vitro* (DIV28) showing morphology.

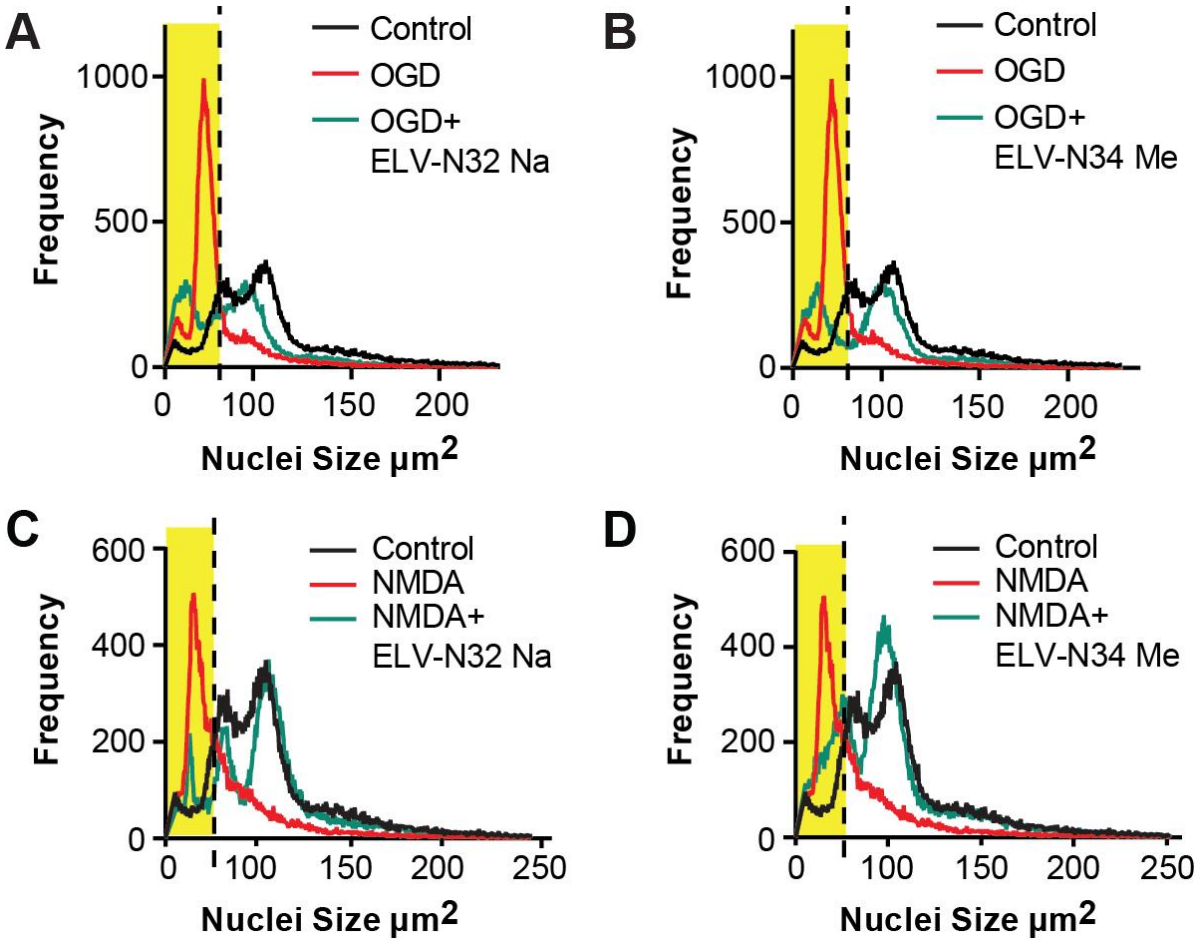

fig. S5. Absolute frequency histograms of cerebral-cortical mixed neuronal or hippocampal mixed neuronal cultures exposed to OGD or NMDA. (A to **D)** Representative absolute frequency histograms from an unbiased image analysis method that was applied to count Hoechst-positive nuclei. Frequency distribution of pyknotic vs. nonpyknotic nuclei is shown in the presence of OGD + ELV-N32 Na **(A)** or OGD + ELV-N34 Me **(B)** or NMDA + ELV-N32 Na **(C)** or NMDA + ELV-N34 Me **(D)** respectively. When the cells were subjected to OGD stress or NMDA excitotoxicity, they underwent pyknosis, as shown by the leftward shift of the nuclear peak, highlighted in a yellow box on the left side of the black dashed line, which defines the nuclear size cutoff for defining pyknotic vs nonpyknotic nuclei. For different experiments, the cutoff value varied within a range from 48 – 62µm^2 with the majority of pyknotic nuclei measuring about 30 – 50 µm^2, while most of the non-pyknotic nuclei ranging between 80 – 140 µm^2. Again, upon treatment with either ELV-N32 Na or ELV-N34 Me, there was a positive rightward shift towards the control nuclear population peak, indicating that cellular survival was elicited by these novel lipid mediators, elovanoids.

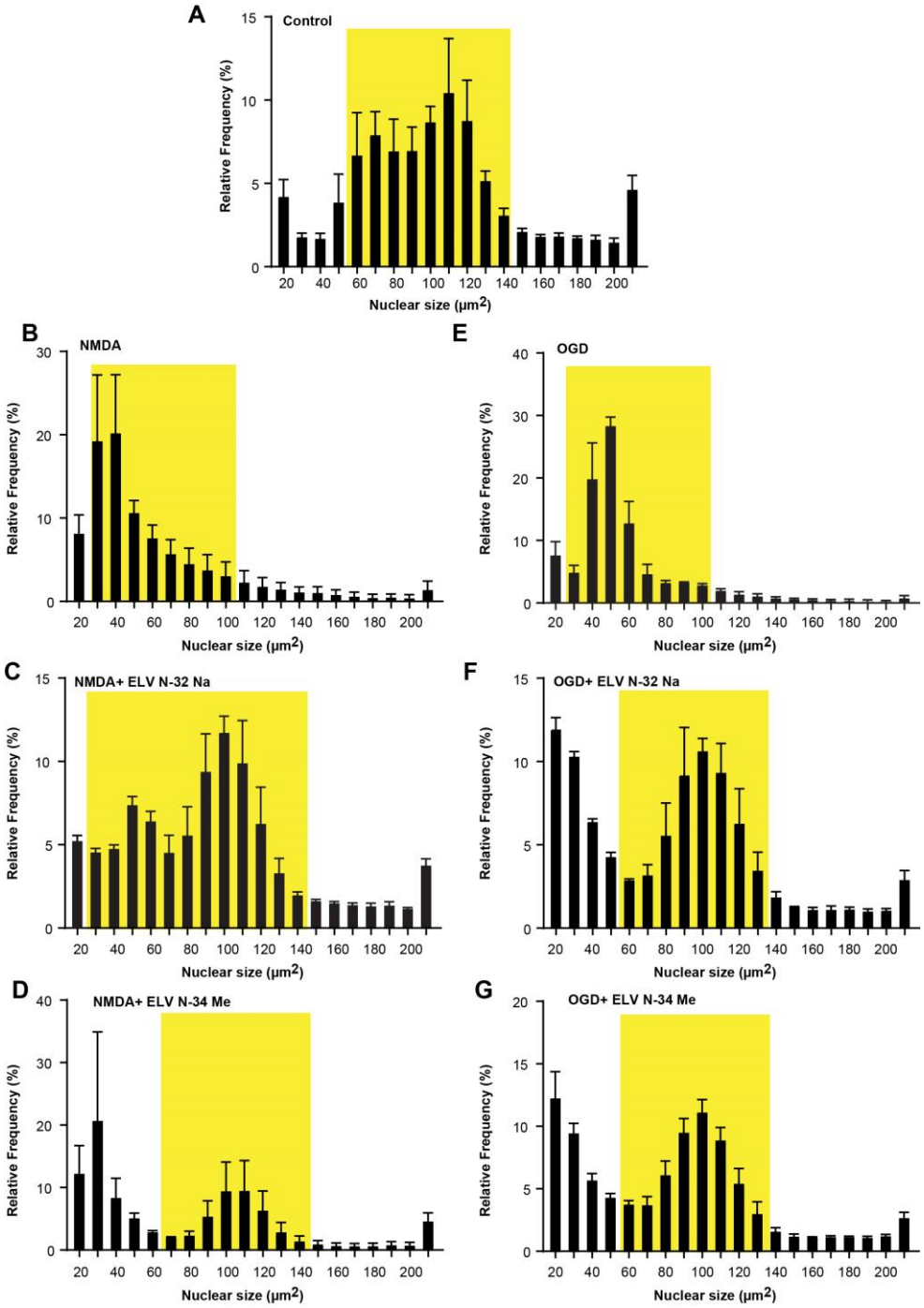

fig. S6. Percent relative frequency histograms. (A to **G)** Representative histograms showing % relative frequencies of nuclear sizes (μm^2) of Hoechst positive neuronal nuclei subjected to different conditions – Control **(A)**, NMDA **(B)**, NMDA + ELV-N32 Na **(C)**, NMDA + ELV-N34 Me **(D)**, OGD **(E)**, OGD + ELV-N32 Na **(F)**, OGD + ELV-N34 Me **(G)** respectively. The frequency histogram of the control population shows the majority of nonpyknotic nuclei ranging between 80 – 140 μm^2, while the nuclei subjected to NMDA excitotoxicity or OGD stress shows the majority of pyknotic nuclei ranging between 30 – 60 μm^2. Again, on treatment with ELV-N32 Na or ELV-N34 Me, the nuclear population shifts rightward towards 80 – 140 μm^2. The data shown above are from three separate experiments and represented as mean ± SEM.

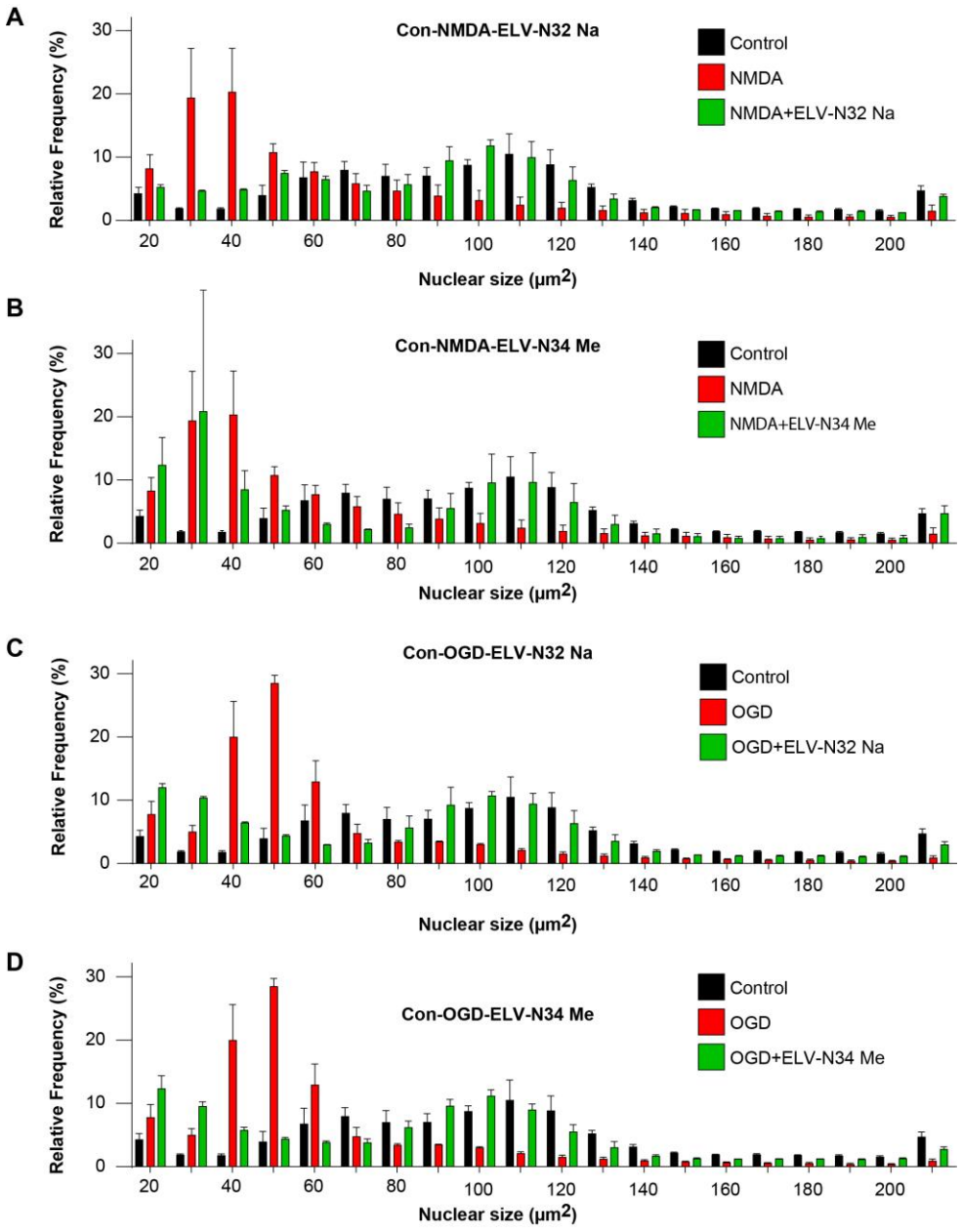

fig. S7. Clustered histograms of percent relative frequencies. (A to **D)** Representative clustered histograms showing % relative frequencies of nuclear sizes (μm^2) of Hoechst positive neuronal nuclei subjected to different conditions – Control – NMDA – NMDA + ELV-N32 Na **(A)**, Control – NMDA – NMDA + ELV-N34 Me **(B)**, Control – OGD – OGD + ELV-N32 Na **(C)**, Control – OGD – OGD + ELV-N34 Me **(D)** respectively. In the frequency histograms, control is depicted in black, NMDA/OGD stress in red, and the treatment conditions in green. The frequency histogram of the control population shows the majority of nonpyknotic nuclei ranging between 80 – 140 μm^2, while the nuclei subjected to NMDA excitotoxicity or OGD stress shows the majority of pyknotic nuclei ranging between 30 – 60 μm^2. Again, on treatment with ELV-N32 Na or ELV-N34 Me, the nuclear population shifts rightward towards 80 – 140 μm^2. The data shown above are from three separate experiments and represented as mean ± SEM.