

Supplementary Material

A synthetic peptide rescues rat cortical neurons from anesthetic-induced cell death, perturbation of growth and synaptic assembly

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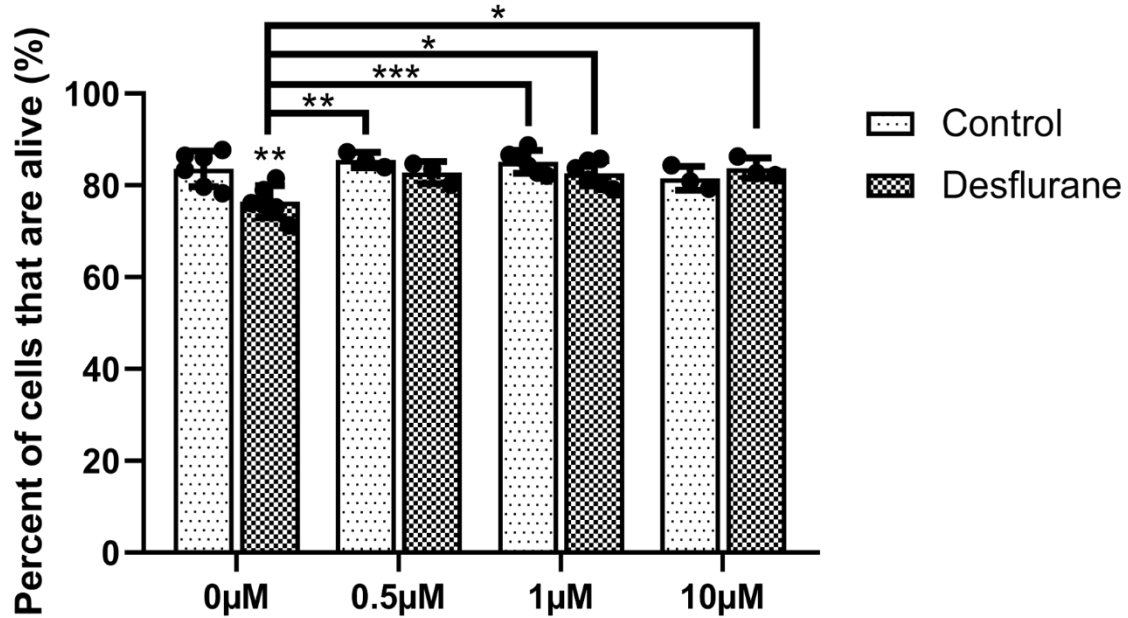


Figure S1: P110 prevents desflurane-induced cell death in a concentration-dependent manner. P110 did not affect cell viability by itself (0.5μM: $M = 85.52\%$, $SD = 1.66\%$ alive, $p > 0.9999$ compared to control, $n = 3$; 1μM: $M = 85.12\%$, $SD = 2.5\%$ alive, $p > 0.9999$ compared to control, $n = 6$; 10μM: $M = 81.52\%$, $SD = 2.59\%$ alive, $p > 0.9999$ compared to control, $n = 3$) and instead reversed desflurane-induced cell death (0.5μM: $M = 82.76\%$, $SD = 2.39\%$ alive, $p > 0.9999$ compared to control, $p = 0.1424$ compared to desflurane alone, $n = 3$; 1μM: $M = 82.61\%$, $SD = 2.74\%$ alive, $p > 0.9999$ compared to control, $p = 0.0347$ compared to desflurane alone, $n = 6$; 10μM: $M = 83.72\%$, $SD = 2.21\%$ alive, $p > 0.9999$ compared to control, $p = 0.0482$ compared to desflurane alone, $n = 3$). Two-Way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Experiments and results were validated at least twice. Bars indicate $\pm SD$ across biological replicates. Each data-point indicates the mean viability reported in each biological replicate.

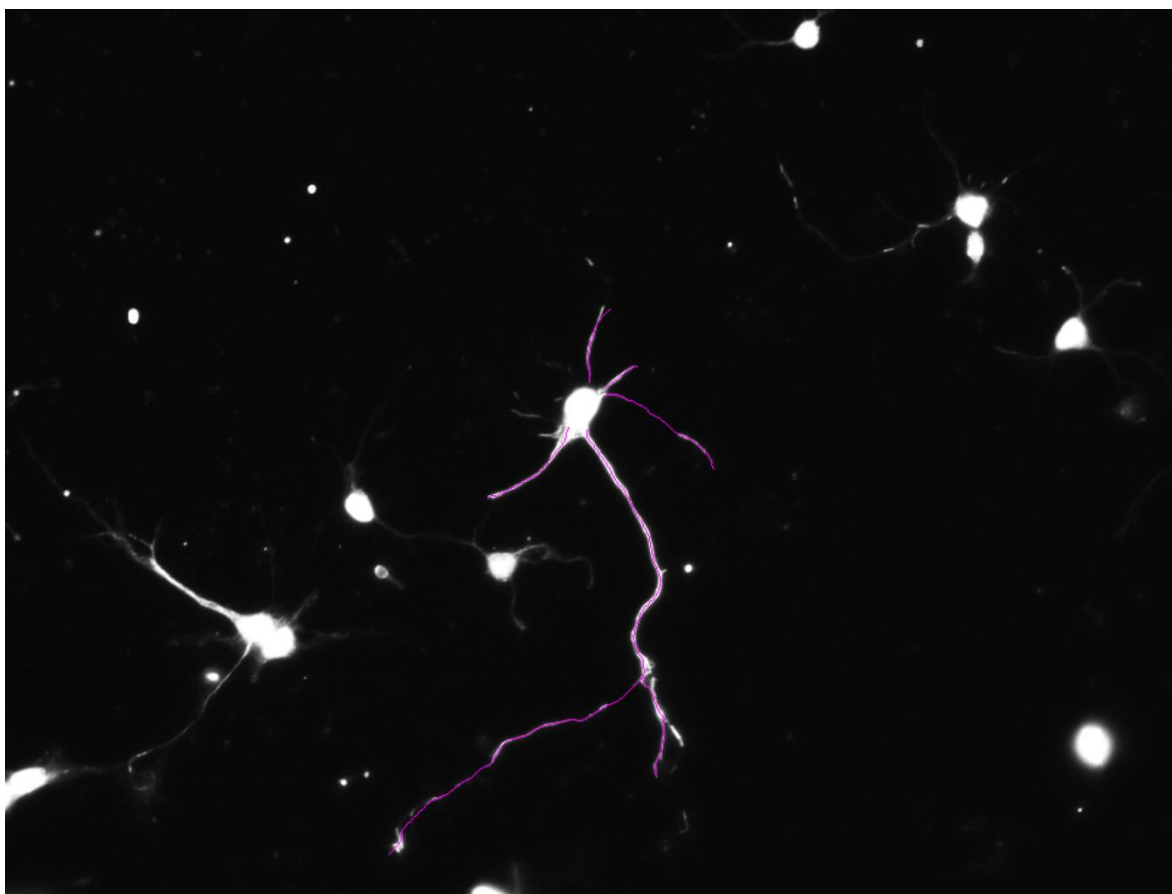


Figure S2: Example of neuronJ tracing of neurites of a cell at day *in vitro* 3. The central cell in each field of view following a standardized imaging protocol was traced and the average neurite lengths per aggregate for each biological replicate.

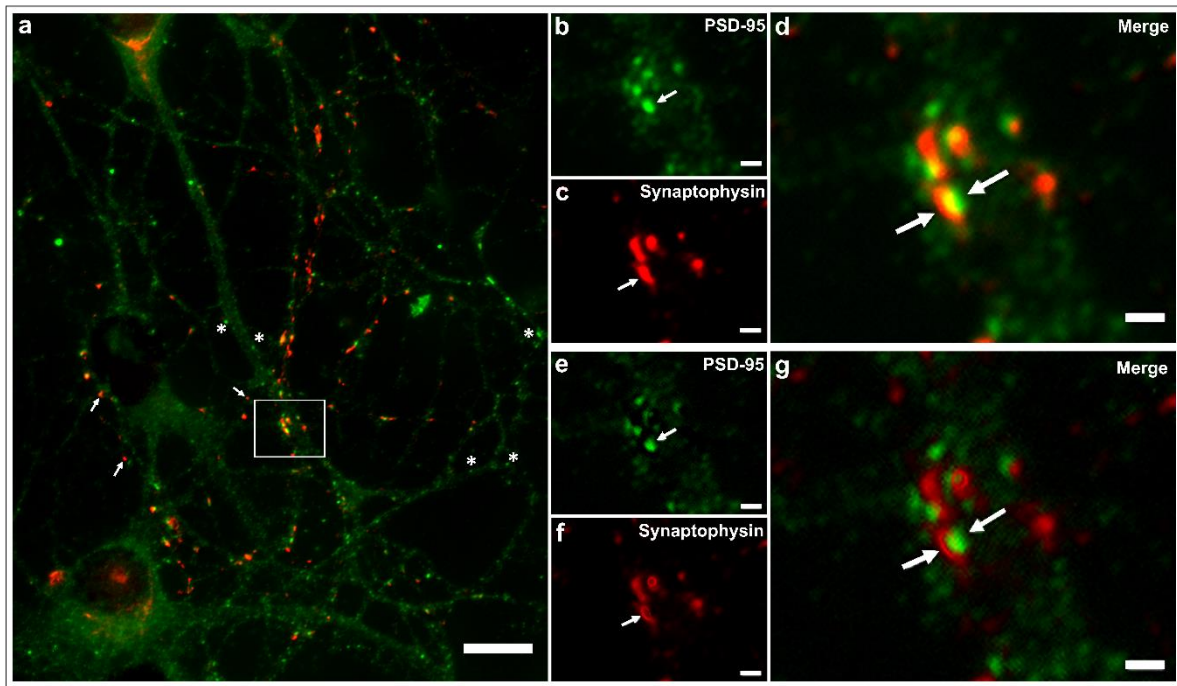


Figure S3: Example of synaptic network staining for quantification of synaptic density. Representative fluorescent image that is labelled for the post-synaptic marker PSD-95 (green) and the presynaptic marker synaptophysin (red) **(a)**. Close-up of a synaptic punctum where the juxtaposition of PSD-95 **(b)** and synaptophysin **(c)** produces a characteristic yellow, which was quantified as a punctum **(d)**. A sharpened image is shown in the final row to verify and display the localization of these signals at the synaptic site **(e-g)**. Average puncta density was manually quantified as number of puncta per unit length wherever these characters for a synapse were met. Scale bars indicate 10µm **(a)** and 1µm **(b-g)**.