

## Top Panel: Proximity Ligation Assay (PLA) experimental images.

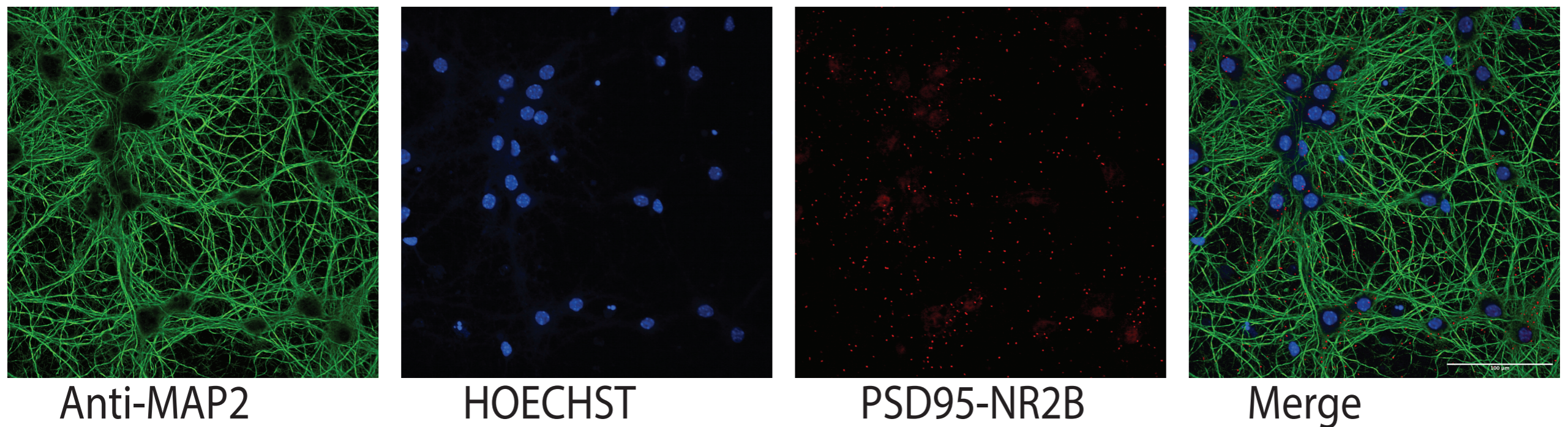
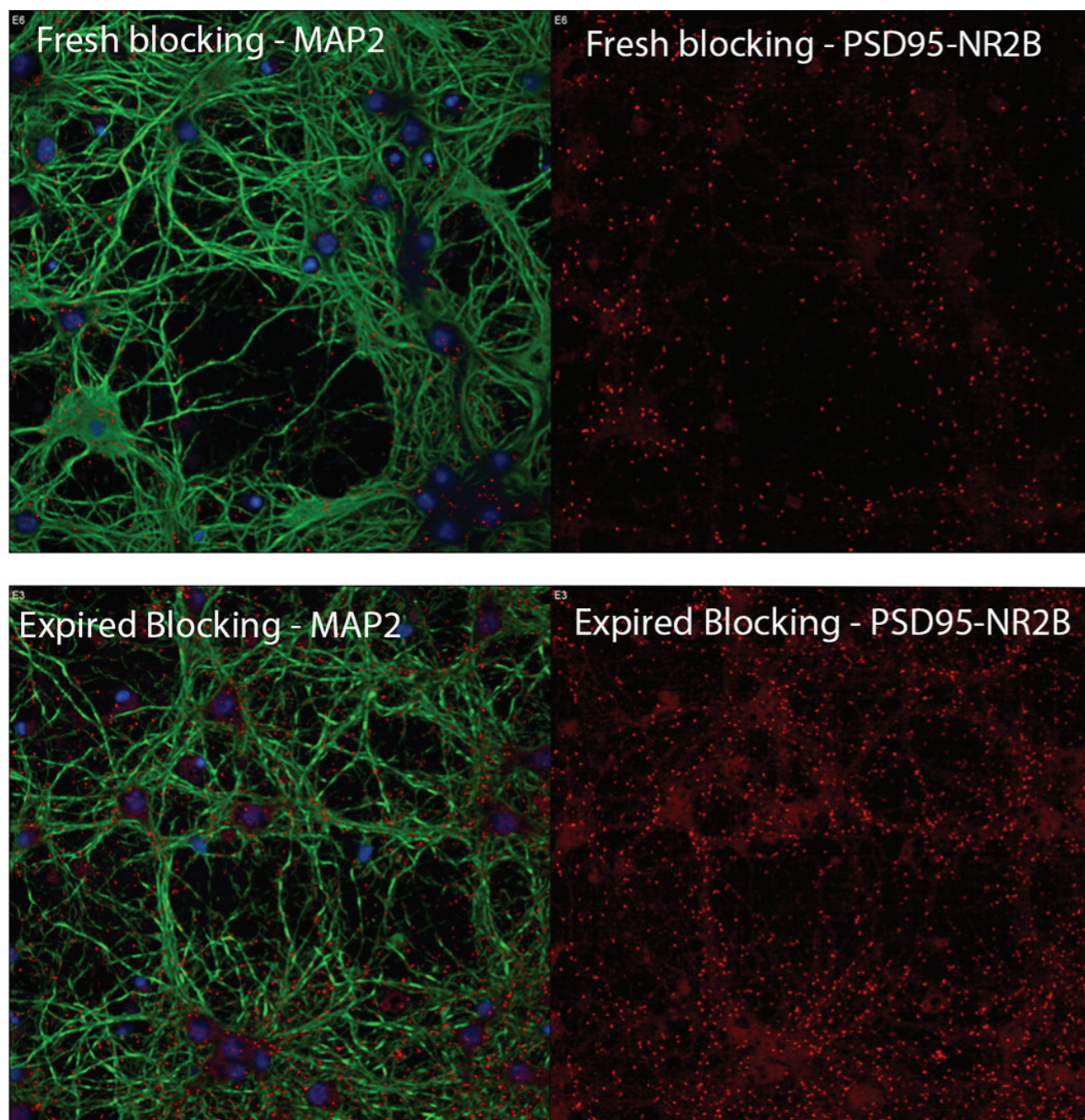


Fig 1. Representative images of PLA showing PSD95-NR2B interactions and MAP2 staining in primary neurons. (Image acquired using 40x water objective with (NA 1.1). Scale bar :100 $\mu$ m)

## Bottom Panel: Blocking buffer efficiency influences the PSD95-NR2B detection.



Test the blocking buffer before running the experiment. Even when blocking stored at recommended temperature 4°C, its efficiency is deteriorated (before expiration). Here, I tested my blocking using Anti-MAP2/HOECHST staining.

The old (expired) blocking buffer produced weak and patchy MAP2 signal compared to the fresh one. Additionally, many faint and diffused puncta can be visualised in the expired buffer. In contrast, fresh blocking resulted in distinct, round and bright puncta.