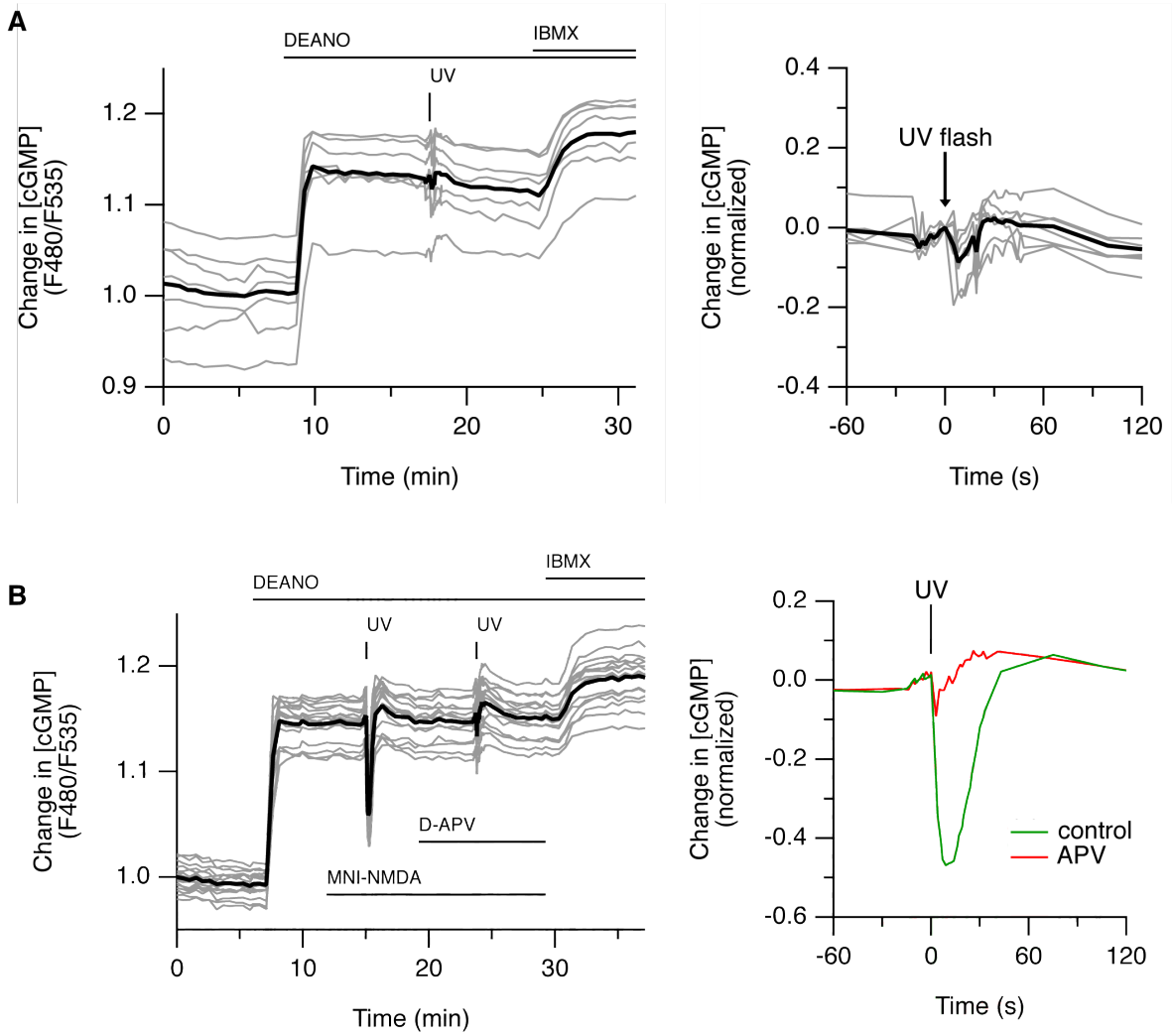
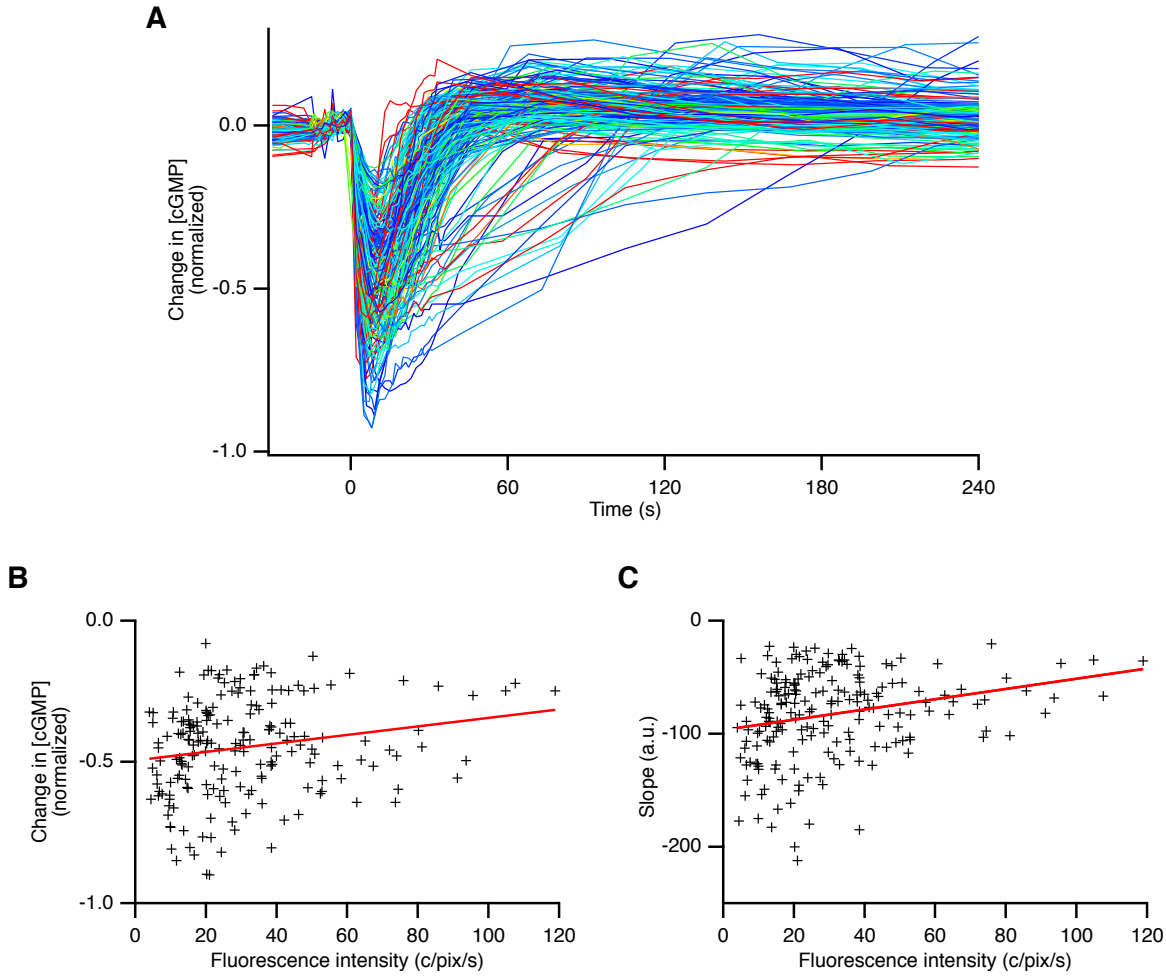
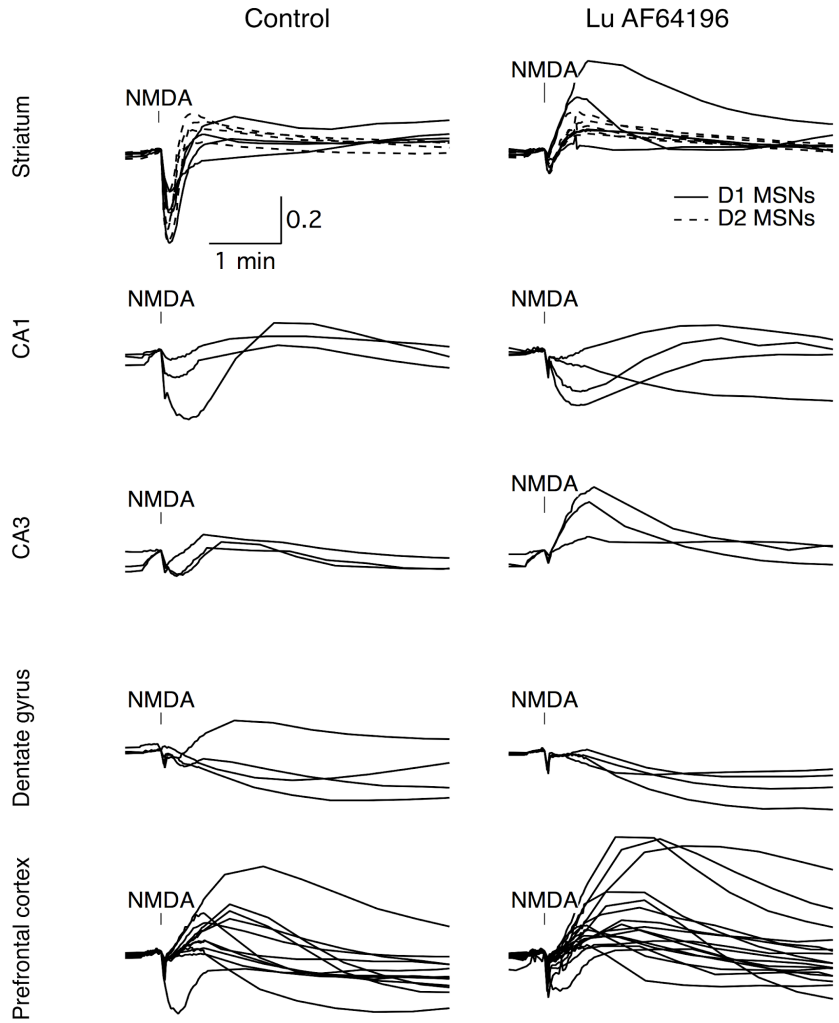


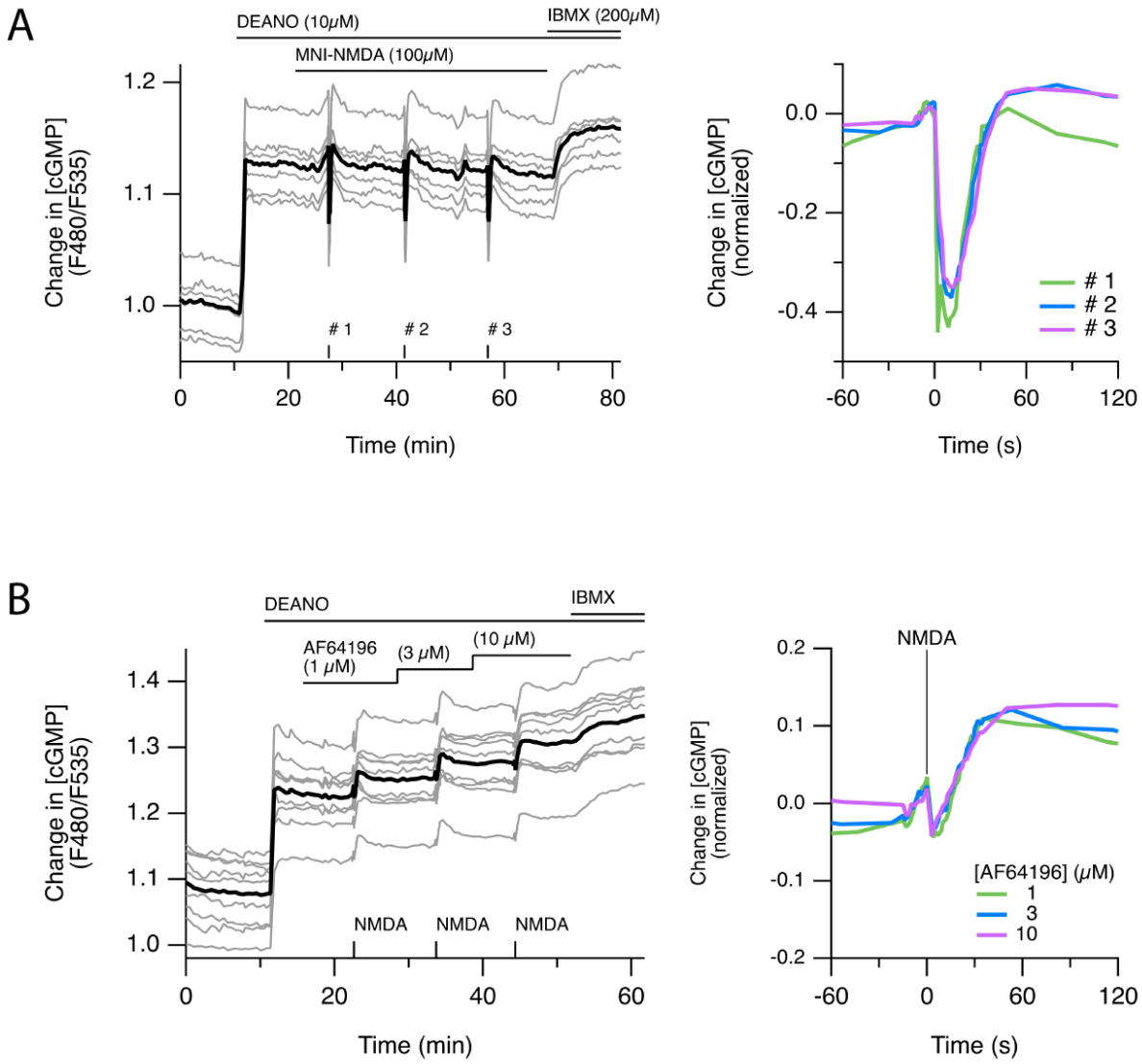
Supplementary Figure 1: **Uncaging protocol controls.** Mouse brain slices expressing the cGMP biosensor cyGNAL. A: The flash of UV light has negligible effect on the ratio measurement. The protocol was the same as in Figure 2A, except that there was no MNI-NMDA in the bath. B: The ratio change in response to NMDA uncaging depends on NMDA receptors. A first NMDA uncaging was performed to elicit a control cGMP response. A second uncaging was performed in the presence of the selective NMDA receptor antagonist D-APV (40 µM).



Supplementary Figure 2: **PDE1 effect on cGMP has little correlation with biosensor expression level.** Experiments with a control NMDA uncaging (such as Figure 2 and the control uncaging of Figure 5), were pooled together (106 neurones). Only neurones that were well defined in the focal plane were analysed. The intensity was quantified as the average intensity of the 10 % brightest pixels within the region of interest. This average intensity is linearly related to biosensor concentration. A: all responses to NMDA uncaging are overlaid, each trace corresponding to one neurone, colour-coded to represent the intensity, from blue (dimmest) to red (brightest). B: maximal decrease in [cGMP] after NMDA uncaging plotted as a function of fluorescence intensity. C: slope of the decrease in [cGMP] as a function of fluorescence intensity. The red lines in B and C represent the linear fit of these data.



Supplementary Figure 3: **PDE1 activity on cAMP in the striatum, hippocampus and cortex.** NMDA was uncaged while monitoring cAMP with the biosensor Epac-S^{H150} as shown in Figure 3. Each trace represents the average ratio of one experiment comprising 1 to 13 neurones. Traces are normalised between baseline and maximal response to forskolin (12.5 µM) and IBMX (200 µM). The vertical bar indicates the uncaging of NMDA (100 µM). The experiments were performed in striatum (same data as shown on Figure 3), hippocampus (CA1 and CA3), dentate gyrus and prefrontal cortex, in control conditions (left panels) or with 10 µM Lu AF64196 (right panels).



Supplementary Figure 4: **Dose dependency of Lu AF64196 effect on cGMP.** (A) Three successive NMDA applications on brain slices through uncaging have reproducible effects on cGMP signalling. (B) Maximal effect is achieved at 1μM Lu AF64196, since higher concentrations (3 and 10 μM) did not produce further reduction in the cGMP response.