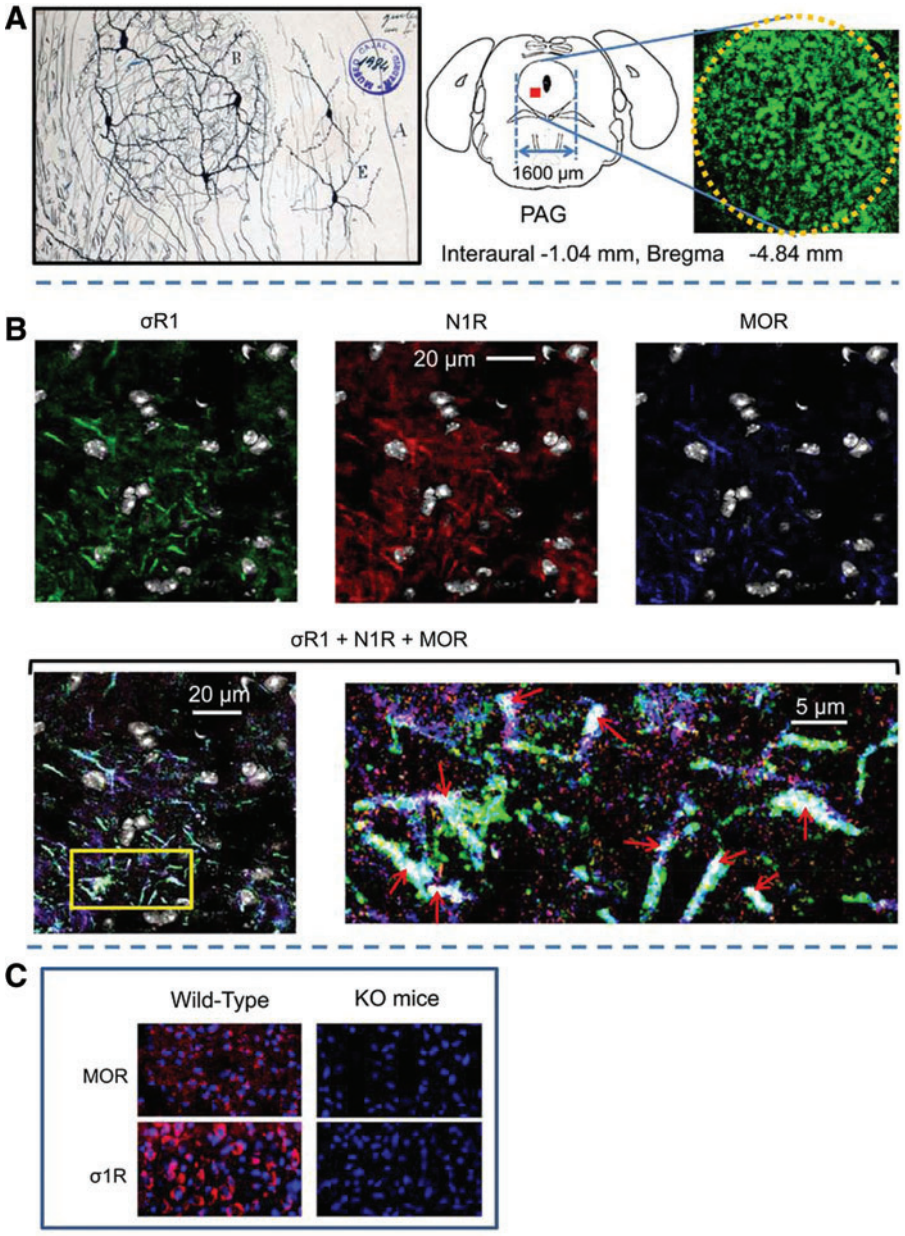

SUPPLEMENTARY FIG. S3. Co-localization of the NMDAR NR1 subunit with σ1R and the MOR, and of σ1R with the MOR in mouse PAG neurons. (A) *Left panel*, Original Cajal drawing showing a view of the ventro-lateral region of PAG, Golgi method. A: cerebral aqueduct; B and C axons in Darkschewitsch nucleus; E, fiber-rich region of the central gray matter. *Right panel*, Mouse PAG (*yellow dotted circle*) and Coronal drawing of mouse brain showing the PAG region analyzed in this triple co-localization of σ1R, NMDAR NR1 subunit, and MOR. **(B)** Confocal laser-scanning microphotographs taken from coronal sections of the mouse midbrain PAG. The individual labeling and triple co-localization of NMDAR NR1 subunit (*red*), σ1R (*green*) and MOR (*blue*) was studied in the dorsolateral region (*red square* in the PAG diagram). Nuclei are in *gray* (DAPI) and the MOR, NR1, and σ1R were observed with Alexa Fluor 647, 555 and 488, respectively. In this ventro-lateral PAG region, triple co-localization of the MOR, σ1R, and NR1 subunits is mostly observed in fibers, probably corresponding to synapses. Note that high-power magnification *panel* (nuclei were removed) shows triple co-localization as *white* regions (*red arrows*). Coincidence between NR1 and σ1R is shown in *yellow* color. The MOR co-localizes with σ1R (*purple* color) and NR1 (*light green* color). For details, see Materials and Methods section and Figure 4. A positive control sample labeled with conventional secondary detection methods was included in the pilot studies to confirm the specificity of the staining observed with Zenon labeling. A negative control was processed with the same protocol but with the omission of the primary antibody to assess nonspecific labeling. **(C)** Immunodetection of the MOR or of σ1R in the PAG of wild-type and knock-out mice (both antibodies in *red* and the nuclei in *blue*). Note the negative staining for either MOR or σ1R in the respective KO mice. MOR, mu-opioid receptor; PAG, periaqueductal grey matter.