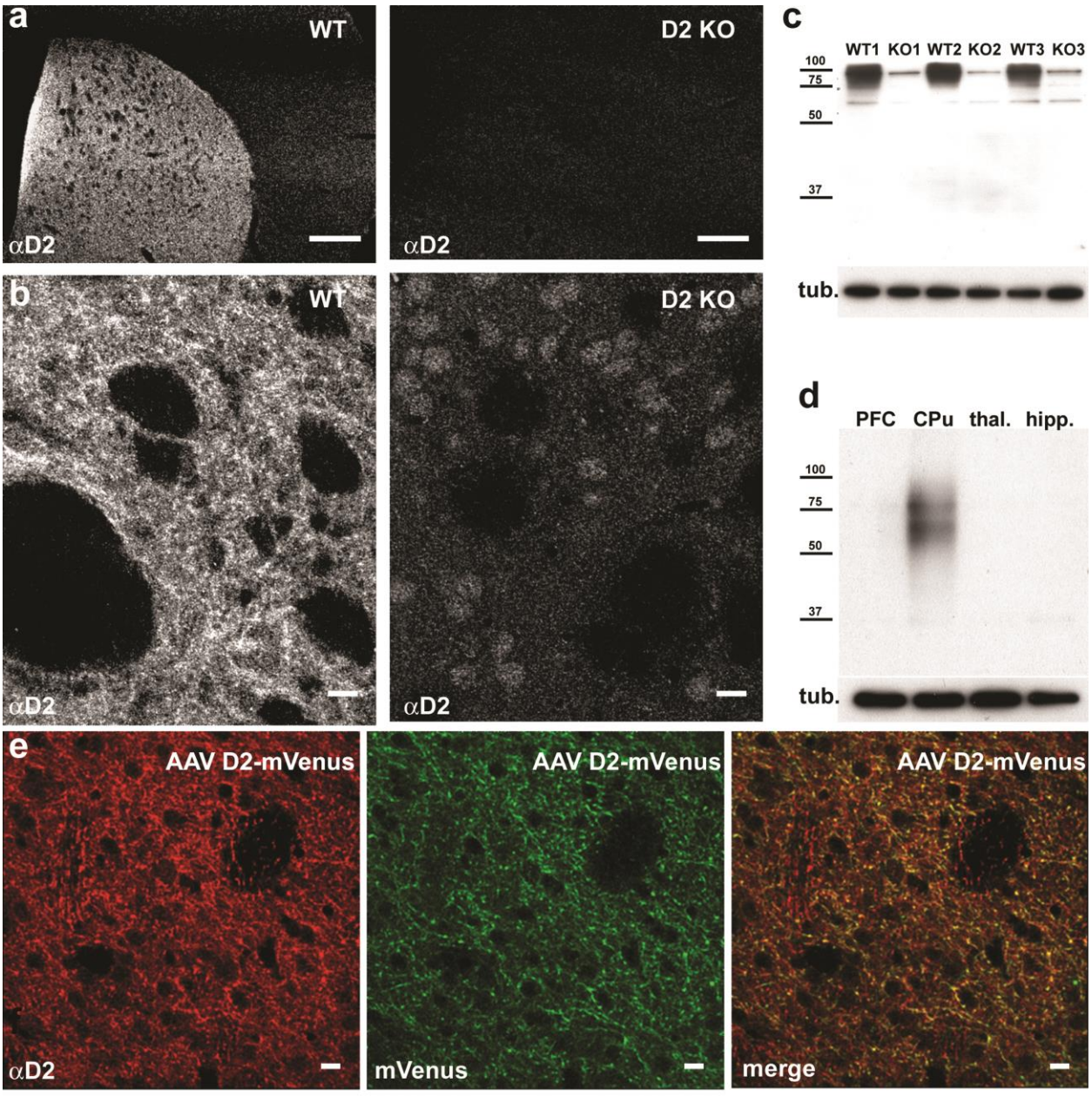
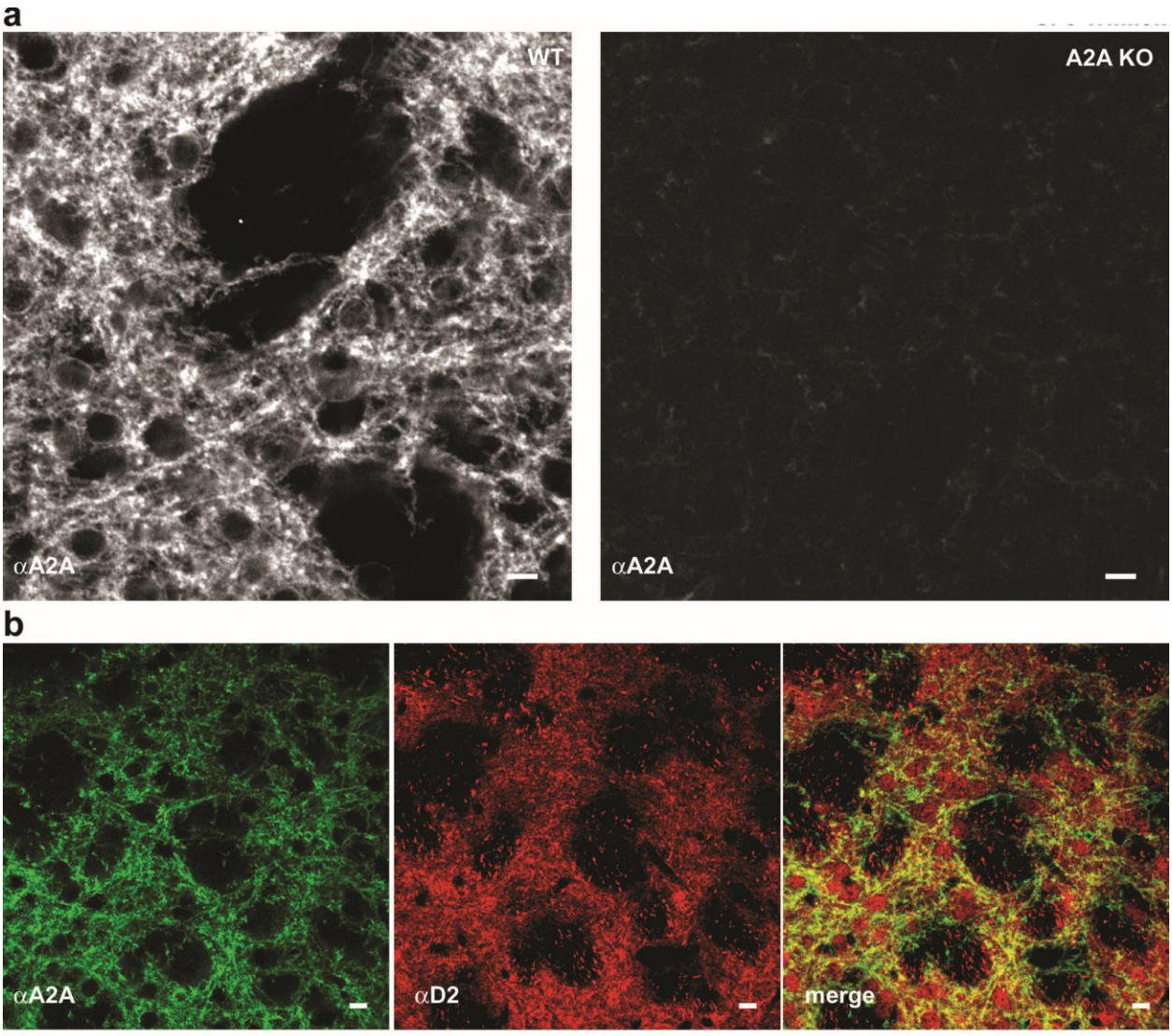
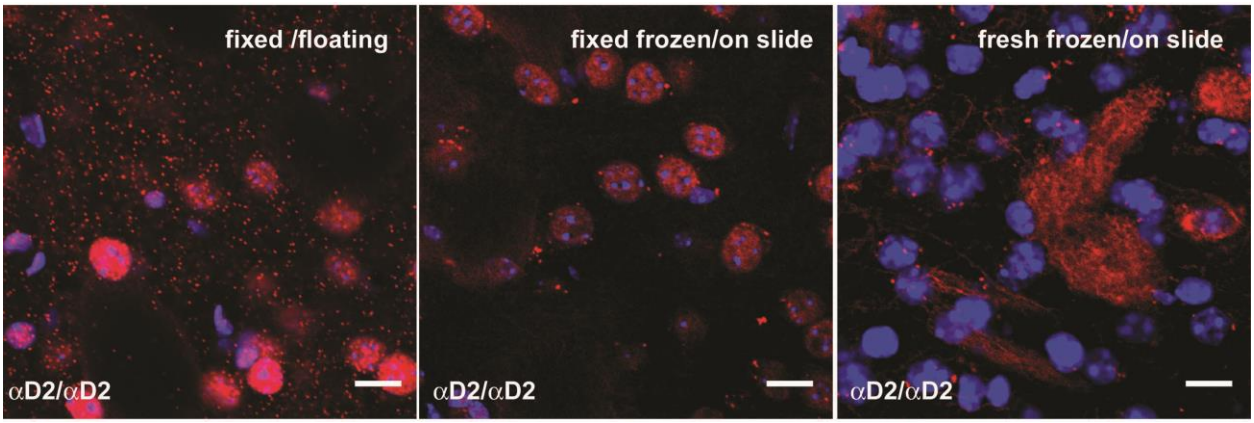
Supplementary Figure 1:



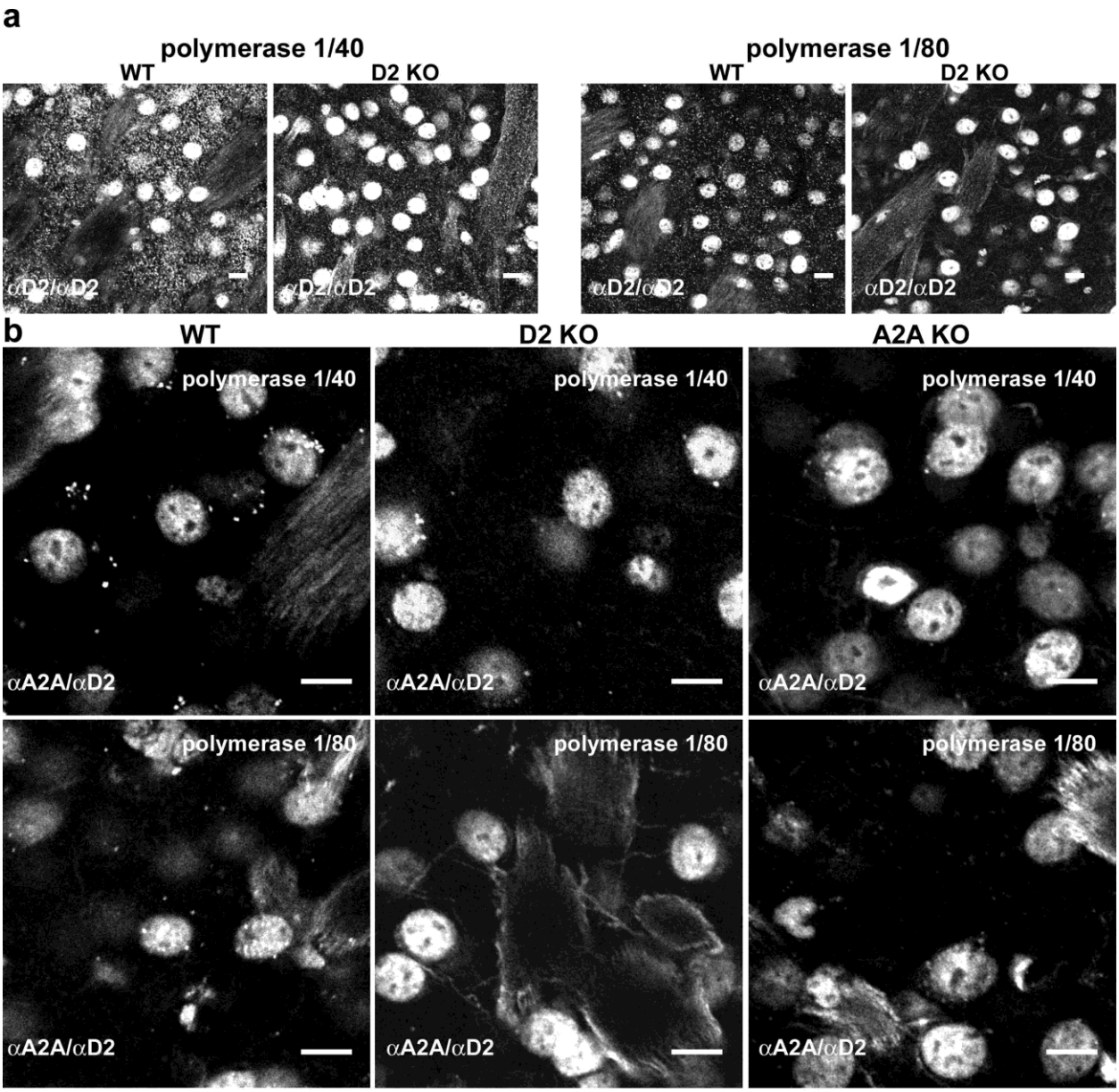
Supplementary Figure 2:



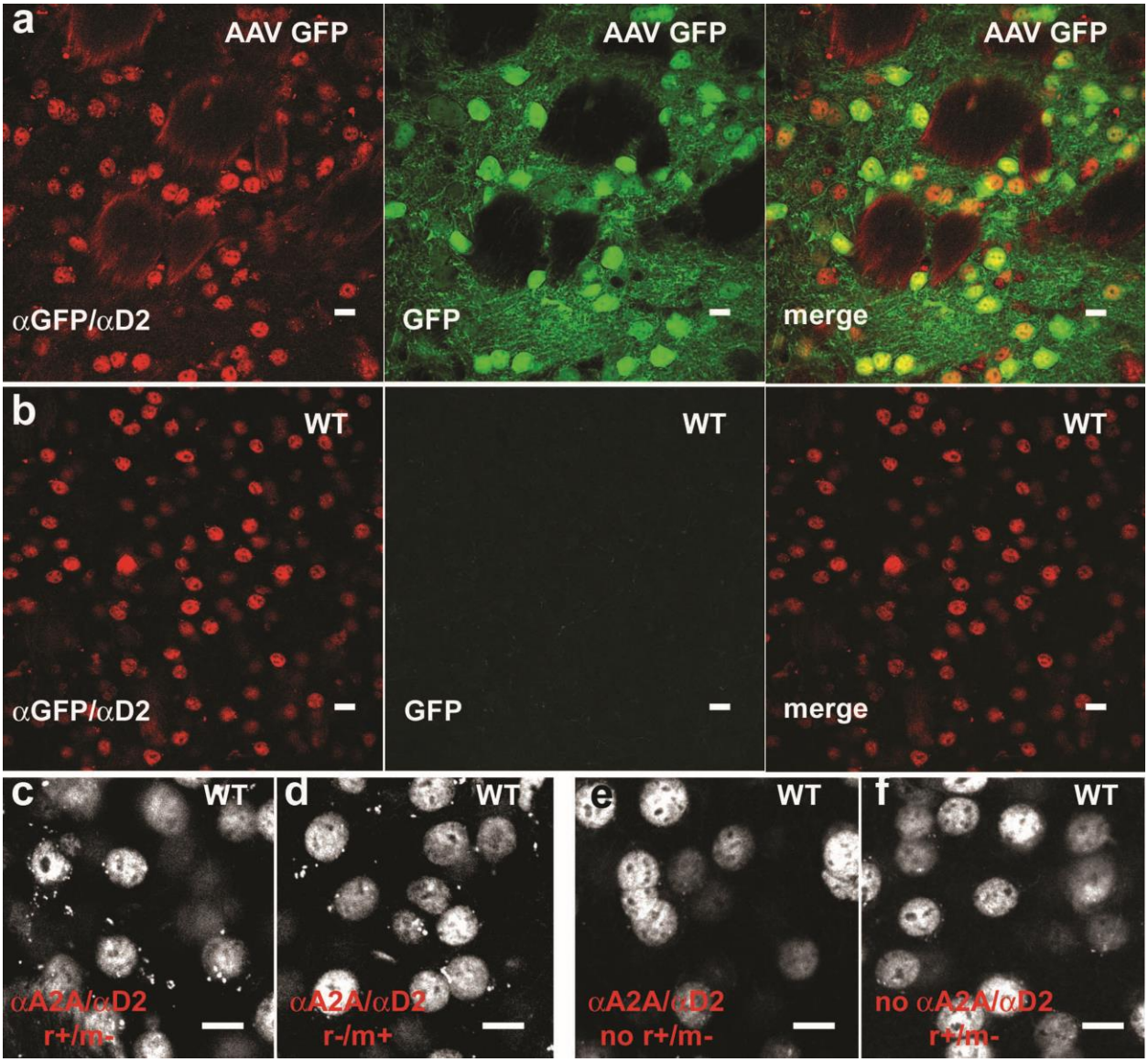
Supplementary Figure 3:



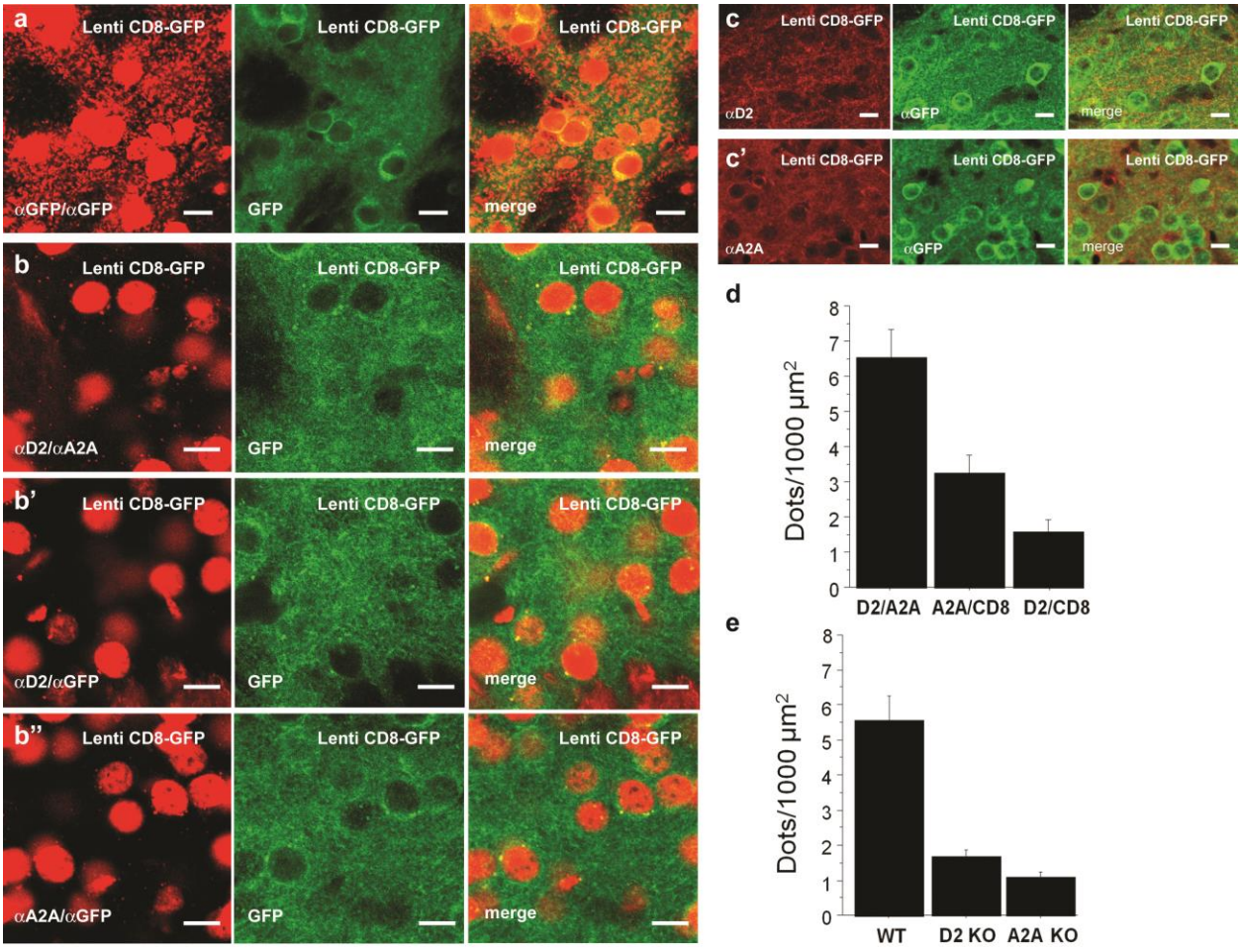
Supplementary Figure 4:



Supplementary Figure 5:



Supplementary Figure 6:



p<0.01) and A2AR (l; unpaired t-test: t=-4.2; p<0.01) demonstrates the difference of PLA signal density between WT and KO mice. Scale bars=10 μm.

Figure 3: Detection of proximity ex vivo in striatal sections using PLA.

Proximity detection was performed on striatal slices overexpressing the $D2_L$-R-mVenus (AAV D2-mVenus) using antibodies directed against D2-R and GFP (a-c). This can lead to intraprotomer as well as potential interprotomer proximity. (a) PLA signal. (b) Direct fluorescence of mVenus. (c) merge.

Proximity between D2R and A2AR was detected by PLA in the striatum of WT mice (d and g). The signal was virtually absent in both D2R (e and h) and A2AR (f and i) KO mice. (d-f are pseudocolors) (j) Quantification of PLA signals for D2R and A2AR proximity confirmed the significant difference of PLA signal density between WT and KO mice (ANOVA: p<0.01; post-hoc comparison: WT/A2A KO, p<0.01; WT/D2 KO, p=0.01; A2A KO/D2 KO, p=0.46). Scale bars=10 μm.

SF 1: Specificity of the anti-D2R antibody.

(a) D2R immunoreactivity was high in the striatum of WT mice compared to adjacent cortical areas using standard fluorescent immunohistochemistry (left) and was virtually absent in D2R KO mice (right). Scale bars=750 μm. (b) Higher magnification images revealed that most of the signal was indeed strongly decreased in D2R KO mice (right) compared to controls (left) but a residual signal was observed, mainly in cell somas. (c) Immunoblot analysis (12% acrylamide) revealed some residual labeling by the antibody in D2R KO mice. In addition to the specific D2R signal there was a non-specific band migrating at a similar molecular weight (≈ 75kDa) as

well as another migrating at a lower molecular weight (≈ 60 kDa). (d) Consistent with the immunohistochemical pattern of D2R expression, D2R were highly enriched in the CPu compared to other brain areas as revealed by immunoblot analyses (8% acrylamide). PFC=prefrontal cortex; CPu= caudate putamen; thal.=thalamus; hipp.=hippocampus. (e) The $D2_L$-R-mVenus transgene introduced by viral transduction was strongly stained by the anti-D2R antibody. Scale bars (except in (a))=10 μm.

SF 2: Specificity of the anti-A2AR antibody and colocalization with D2R.

(a) A2AR immunoreactivity in the striatum of WT mice (left) was absent in A2AR KO mice (right). (b) Co-staining of striatal slices for A2AR (left) and D2R (middle) showed a high level of colocalization of both receptors (right) in the striatum of WT mice. Scale bars=10 μm.

SF 3: Fixation protocols and nuclear background.

Single recognition of D2R with PLA was much more efficient in fixed tissue and floating sections (left) compared to fixed frozen tissue mounted on slides (middle) or fresh frozen tissue mounted on slides (right). The fixation step, however, led to unspecific nuclear signal as shown by colocalization of PLA signal (red) with DAPI (blue); this staining was absent in fresh frozen sections. Scale bars=10 μm.

SF 4: Impact of the polymerase concentration on PLA signal.

(a) Using a polymerase concentration of 1/40 for the single recognition of the D2R increased the nonspecific PLA signal in D2R KO mice (left panels) and substantially increased the signal in WT mice. The 1/80 concentration of polymerase seemed to be optimal as there was virtually no

signal in D2R KOs but measurable PLA signal in WT mice. (b) Using the polymerase at a concentration of 1/40 for the dual recognition of D2R and A2A seemed to be optimal since it gave a stronger signal/noise ratio (compare the intensity of PLA with that of the nuclear background). The PLA signal was absent in D2R (middle column) and A2A KO (right column) in both conditions. Scale bars=10 μm.

SF 5: Negative controls for dual recognition.

PLA signal (left) for dual recognition of D2R and GFP was virtually absent when soluble GFP was overexpressed (middle: direct fluorescence from GFP) instead of $D2_L$-R-mVenus (a) (compare with Figure 2a-c) or in WT mice (b). For the dual recognition of D2R and A2AR, switching the polarity of PLA probes gave a similar signal in WT mice (c and d). The PLA signal was absent when either one secondary (e) or one primary (f) antibody was omitted from the reaction. Scale bars=10 μm.

SF 6: Effect of overexpression of a membrane protein on the PLA signal.

These experiments were performed with an affinity-purified rabbit polyclonal anti-D2R antibody we generated (see methods and (e) for validation). (a) Overexpression of the non-neuronal membrane protein CD8-GFP gave a strong PLA signal (left) when single recognition of GFP was performed (middle: direct fluorescence from GFP). CD8-GFP overexpression did not alter the detection of endogenous D2R-A2AR heteromers (b). In contrast, a lower PLA signal was detected for GFP-D2R (b') or GFP-A2AR (b") dual recognition, despite evidence for colocalization of C8-GFP with D2R (single confocal slice) (c) and A2AR (c'). These data

suggest that random collisions in the plasma membrane from overexpression are much less effective in generating a PLA signal that true proximity. (d) Quantification of PLA signals (ANOVA: $p<0.001$; post-hoc comparison: D2R-A2AR/D2R-CD8, $p<0.001$; D2R-A2AR/A2AR-CD8, $p<0.01$; D2R-CD8/A2AR-CD8, $p=0.1$). (e) The quantification of PLA signal for dual recognition of D2R and A2AR with the new anti-D2R antibody confirmed the amount of PLA signal for the detection of those heteromers but also improved the signal/noise respective to the PLA signal in D2R KO mice (ANOVA: $p<0.001$; post-hoc comparison: WT/A2AR KO, $p<0.001$; WT/D2R KO, $p=0.001$; A2AR KO/D2R KO, $p=0.32$). Scale bars=10 μm.

Authors contributions:

P.T., M-L.R., E.U. and J.A.J conceived the study and designed the experiments; P.T., J.C. performed the experiments; R.A.P generated the CD8-GFP construct and lentivirus and tested its efficiency for intracerebral injection. H.D.V. supervised and helped with imaging; C.S. raised and provided the D2R KO mice; M.S. and M.G. provided reagents; P.T. analyzed the data; P.T. and J.A.J wrote the manuscript; all the authors participated to the interpretation of the data and edited the manuscript.