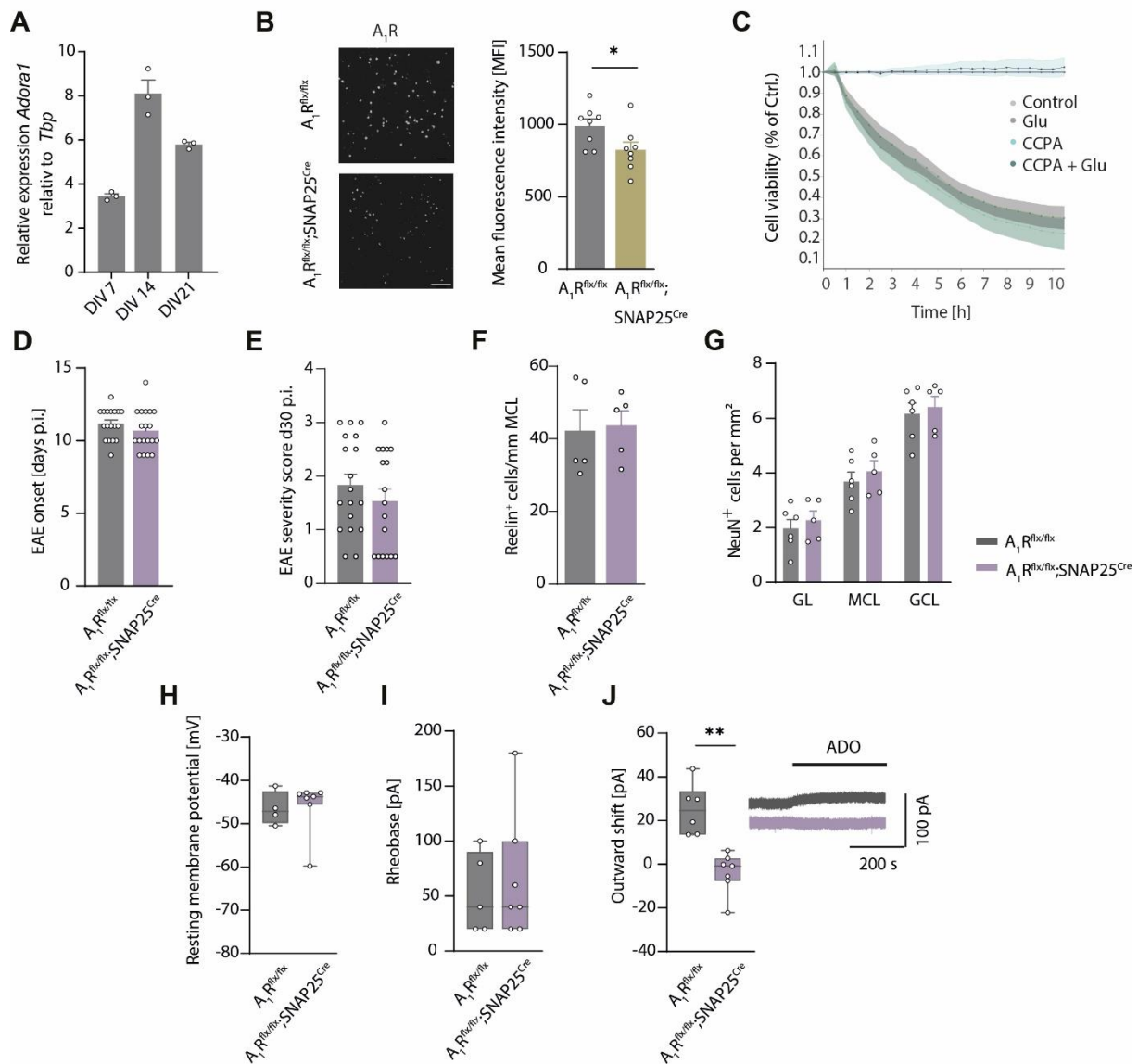


Supplementary Figure 4



Supplementary Figure 4 | Neuronal A₁R is not involved in glutamate excitotoxicity *in vitro* and neuronal injury in experimental autoimmune encephalomyelitis. (A) Primary neuronal cultures of the mouse olfactory bulb. Relative RNA expression of *Adora1* measured by qRT-PCR at day of days *in vitro* (DIV) 7, 14 and 21. (B) Representative image and quantification of mean fluorescence intensity in immunocytochemical staining of A₁R in A1R^{flx/flx} and A1R^{flx/flx};SNAP25^{Cre} neuronal cultures ($n = 4$ biological replicates per group with two technical replicates each). (C) Cell viability of wildtype olfactory bulb primary neuronal cultures DIV16-18 over a time course of ten hours after treatment with glutamic acid (5 μM) preincubated with 100 nM 2-chloro-N6-cyclopentyladenosine (CCPA) or medium control compared to the respective controls ($n = 4$ biological replicates with at least 5 technical replicates each). (D) Day of onset in experimental autoimmune encephalomyelitis (EAE) (BF₁₀ = 0.307). (E) EAE severity score at the final day 30 p.i. in A1R^{flx/flx} and A1R^{flx/flx};SNAP25^{Cre} ($n = 18$ per group, BF₁₀ = 0.669). (F) Reelin-positive cells in the mitral cell layer based on immunohistochemical staining at peak of EAE day 15 p.i. in A1R^{flx/flx} and A1R^{flx/flx} SNAP25^{Cre} ($n = 5$ per group, BF₁₀ = 0.531). (G) Quantification of NeuN-positive cells in the glomerular layer (GL, BF₁₀ = 0.651), mitral cell layer (MCL, BF₁₀ = 0.958) and granular cell layer (GCL, BF₁₀ = 0.666) of the olfactory bulb 30 days p.i. (A1R^{flx/flx}, $n = 6$; A1R^{flx/flx};SNAP25^{Cre}, $n = 5$).

(H) Resting membrane potential in mitral cells of $A_1R^{flx/flx}$ ($n = 4$) and $A_1R^{flx/flx};SNAP25^{Cre}$ ($n = 7$) ($BF_{10} = 0,494$). **(I)** Rheobase (first suprathreshold step of current injection) in mitral cells of $A_1R^{flx/flx}$ ($n = 5$) and $A_1R^{flx/flx};SNAP25^{Cre}$ ($n = 7$) ($BF_{10} = 0,505$). **(J)** Adenosine-induced (100 μM) outward current in mitral cells of $A_1R^{flx/flx}$ ($n = 6$) and $A_1R^{flx/flx};SNAP25^{Cre}$ ($n = 7$) ($P = 0,003$). * $P < 0.05$, ** $P < 0,01$.