

FIGURE S1. The levels of $\alpha 3$ are independent of the transfection ratio. Confocal microscopy analysis of the $\alpha 3$ subunit in HEK293 cells using the transfection ratio 1:1:1 between α , β and γ . *A*, the cell surface level of $\alpha 3(M)$ in combination with $\beta 2$ and $\gamma 2L$ averaged to 0.43 (95% c.i.=0.28-0.66, when $\alpha 3(I)$ was normalized to 1 ($n=3$)). *B*, the total level of $\alpha 3(M)$ from transfections as in *A* averaged to 0.56 (95% c.i.=0.36-0.87, $n=3$)). *C*, the cell surface level of $\alpha 3(M)$ normalized to $\alpha 3(I)$ in combination with $\beta 3$ and $\gamma 2L$ was 0.5 (95% c.i.=0.38-0.67,) *D*, total level of $\alpha 3(M)$ from transfections as in *C* was 0.75 (95% c.i.=0.56-1.0). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

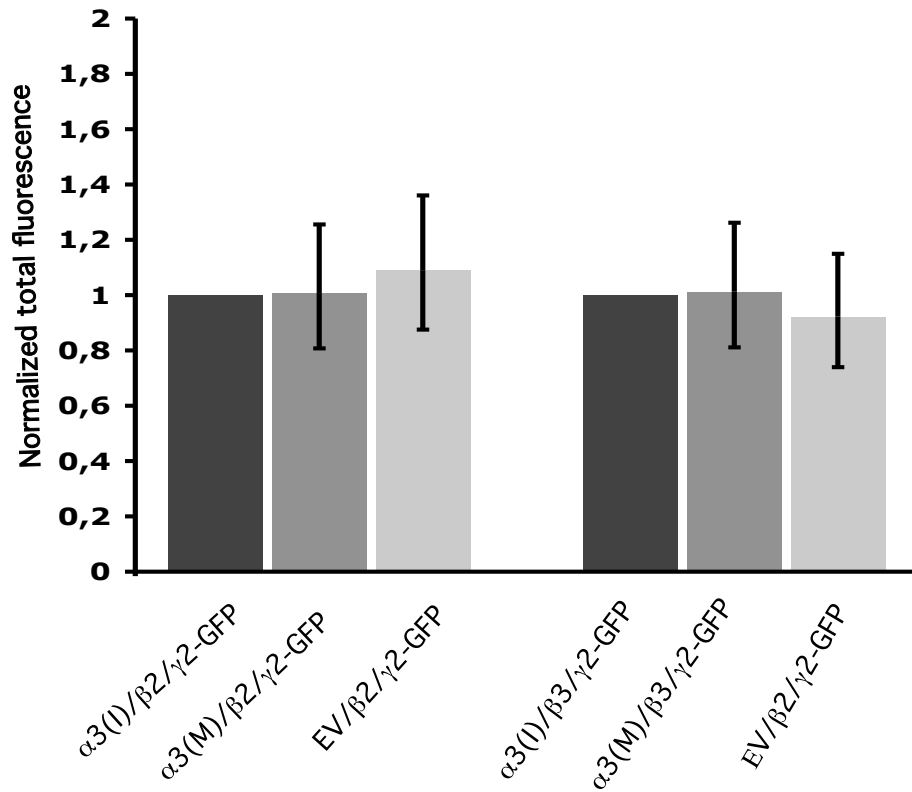


FIGURE S2. The expression of $\gamma 2L$ is independent of the subunit combination. Confocal microscopy analysis of $\gamma 2L$ -GFP using different subunit combinations with the transfection ratio 1:1:1. Left, as indicated the rate of $\gamma 2L$ -GFP expression in combination with $\alpha 3(I)$, $\alpha 3(M)$ or empty vector (EV) together with $\beta 2$. The measurements are normalized to $\gamma 2L$ -GFP in combination with $\alpha 3(I)$ and $\beta 2$ ($n=3$, $p=0.9$) indicating no statistical differences between the transfections. Right, the rate of $\gamma 2L$ -GFP expression in combination with $\alpha 3(I)$ or $\alpha 3(M)$ or EV together with $\beta 3$ ($n=3$, $p=0.4$). As with $\beta 2$, there are no statistical differences in the expression of $\gamma 2L$ -GFP between the transfections.

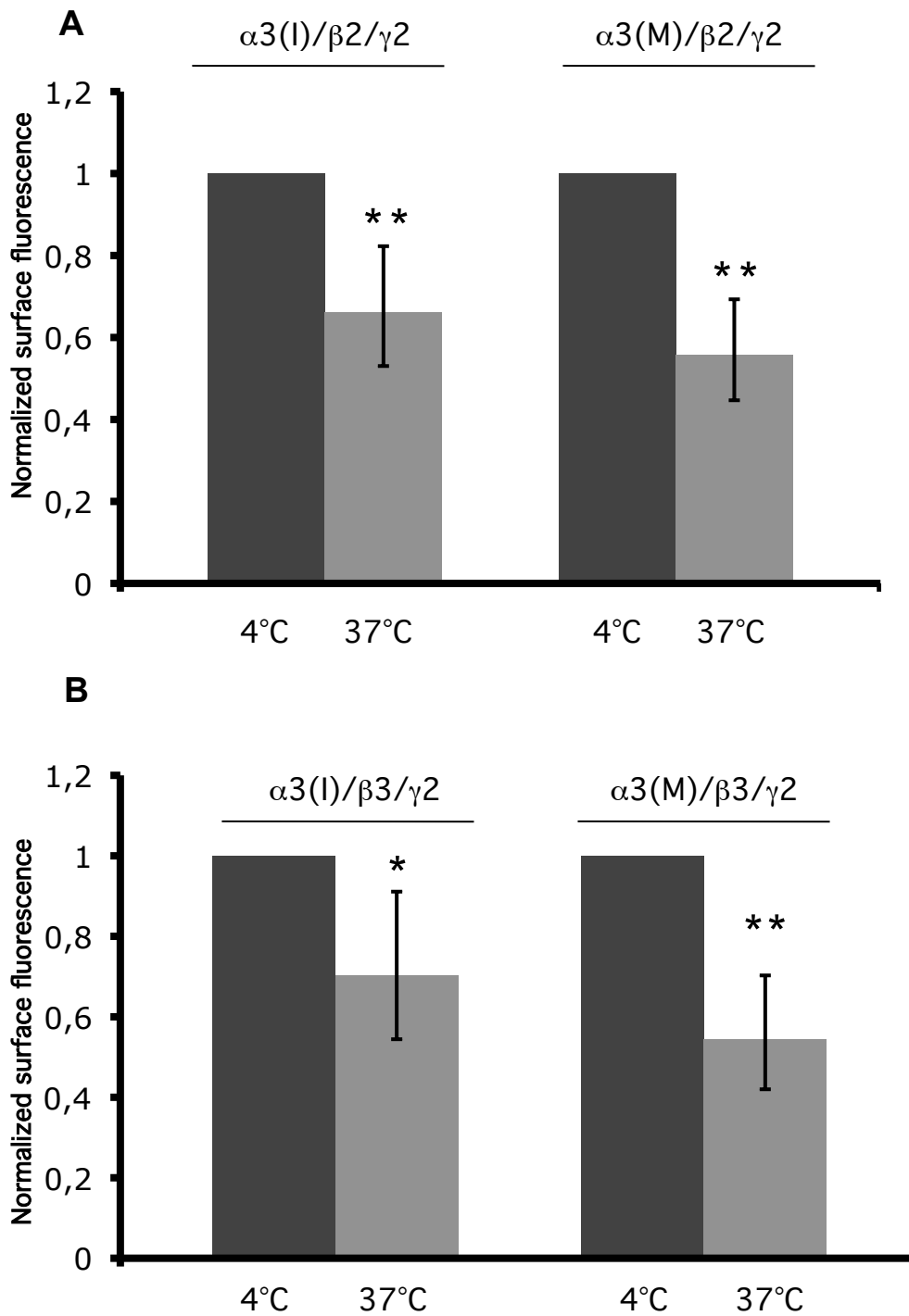


FIGURE S3. Quantification of the effect of internalization in HEK293 cells expressing GABA_A receptor subunits. The $\alpha3$ subunits were prebound with the $\alpha3$ -antibody at 4°C for 30 min. Cells were rinsed and incubated at 37°C for 60 min to allow internalization, while control cells were left at 4°C to prevent internalization. *A* Internalization of the $\alpha3(I)$ or $\alpha3(M)$ when assembled with $\beta2$ and $\gamma2$. Cell surface intensity of $\alpha3(I)$ or $\alpha3(M)$ in control and internalization experiments as indicated. The rate of internalization was normalized to control cells that were left at 4°C (n=3). Values are represented as mean with 95% c.i.. * p <0.05, ** p <0.01. *B* Internalization of the $\alpha3(I)$ or $\alpha3(M)$ when assembled with $\beta3$ and $\gamma2$. Cell surface intensity in control and internalization experiments as indicated (n=3) ** p =0.001.

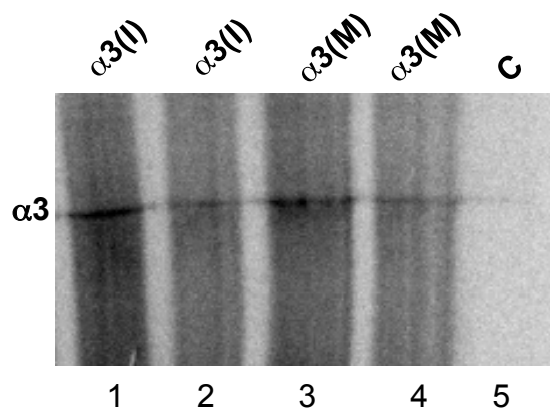


FIGURE S4. In vitro translation of $\alpha 3(I)$ and $\alpha 3(M)$. In vitro transcripts coding for $\alpha 3(I)$ and $\alpha 3(M)$ were made and in vitro translated using rabbit retic lysate. Lane 1 and 3, 2 μg of SP6 transcript is used and in lane 2 and 4 translation is from 20 μg of SP6 in vitro transcript. The excess of RNA added to the reaction in lane 2 and 4 has an inhibitory effect on translation. In lane 5 (C) no RNA was added to the lysate.