

Supplementary Material

High Throughput Patch Clamp Screening in Human Alpha6-Containing Nicotinic Acetylcholine Receptors

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Supplementary Figure Legends

Supplementary Figure 1. RT-PCR and Western Blot Analysis of the nAChR mRNAs

Encoding the $\alpha 6/3$, $\beta 2$ and $\beta 3$ Subunits

A: RT-PCR was performed with primer sets specific for each subunit as indicated in the legend.

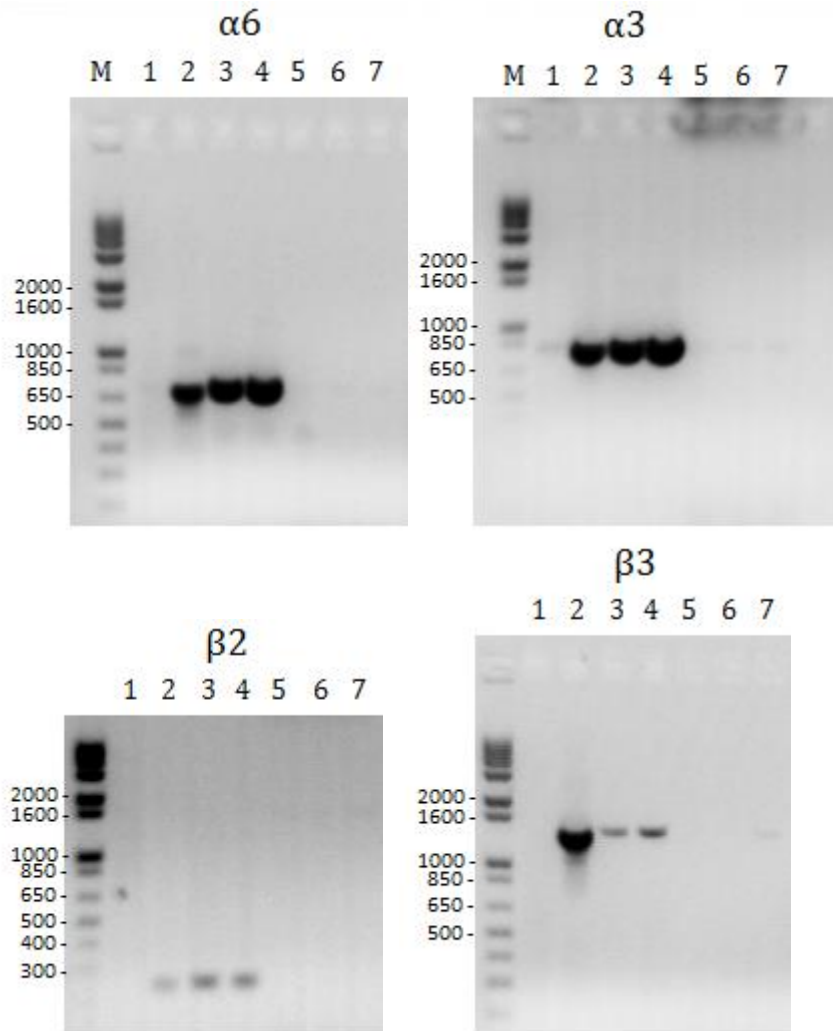
Products were run on a 1% agarose gel stained with ethidium bromide (negative images are presented). Results show that clones B4 and G5 (lanes 3 and 4), but not untransfected HEK (lane 1), express mRNAs encoding the chimera of $\alpha 6$ (top left) and $\alpha 3$ (top right), $\beta 2$ (bottom left) and $\beta 3$ (bottom right) subunits. The plasmids containing the corresponding cDNAs (lane 2) served as positive controls. Specificity of the reaction for mRNA over residual genomic DNA is indicated by the lack of bands in lanes 5-7 where RNA untreated with reverse transcriptase was used. Both clones express similar amounts of the mRNAs encoding $\alpha 6/3$ and $\beta 2$. Clone G5 appears to express a slightly higher amount of the mRNA encoding $\beta 3$ than clone B4. **B:** Western Blot Analysis with antibodies against $\alpha 3$ and $\beta 2$. Lysates of $\alpha 6/3\beta 2\beta 3$ clones B4, B14 and G5, and wild-type HEK, were analyzed by Western blotting with antibodies against the $\alpha 3$ and $\beta 2$ nicotinic acetylcholine receptor subunits. The $\alpha 3$ and $\beta 2$ antibodies each detected bands of ~57 kD in all of the candidate $\alpha 6/3\beta 2\beta 3$ clones. These molecular weights correspond well to the predicted molecular weights of 57 kD. The bands were not present in the wild-type HEK cells. The immunoblotting results confirm expression of the $\alpha 6/3$ and $\beta 2$ subunits in the candidate $\alpha 6/3\beta 2\beta 3$ nAChR clones.

Supplementary Figure 2. DMSO Tolerance

The effect of DMSO (0 to 2.5%) on the (-)-nicotine concentration response relationship was tested in two independent experiments. **A:** Representative current traces in plate view. Shaded wells were invalidated. **B:** Nicotine concentration response curves at 0 and 2.5% DMSO. Data points represent Mean \pm SD (3 – 4 replicate wells/concentration). Average EC₅₀ values from two independent experiments were 0.73, 0.88, 1.44 and 3.69 μ M, respectively at DMSO concentrations 0, 0.5, 1.5 and 2.5%.

Supplementary Figure 1

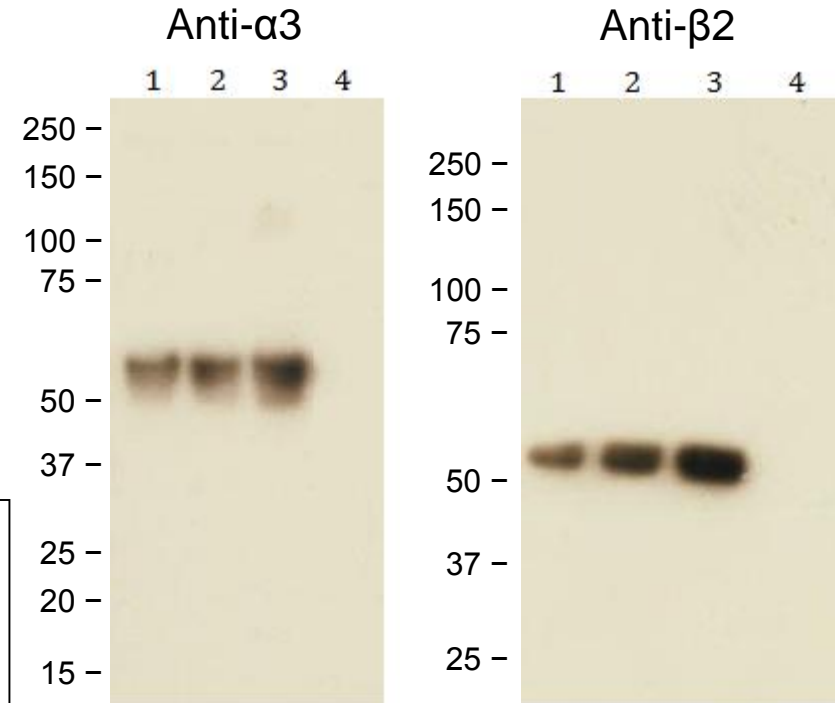
A. RT-PCR



Legend

- M. Size Markers
- 1. Negative control HEK293 cDNA
- 2. Plasmid DNA
- 3. $\alpha 6/3\beta 2\beta 3$ clone B4 cDNA
- 4. $\alpha 6/3\beta 2\beta 3$ clone G5 cDNA
- 5. Negative control HEK293 mRNA
- 6. $\alpha 6/3\beta 2\beta 3$ clone B4 mRNA
- 7. $\alpha 6/3\beta 2\beta 3$ clone G5 mRNA

B. Western Blot



Legend

- 1. $\alpha 6/3\beta 2\beta 3$ clone B4
- 2. $\alpha 6/3\beta 2\beta 3$ clone B14
- 3. $\alpha 6/3\beta 2\beta 3$ clone G5
- 4. Untransfected HEK

Supplementary Figure 2

