

## Article

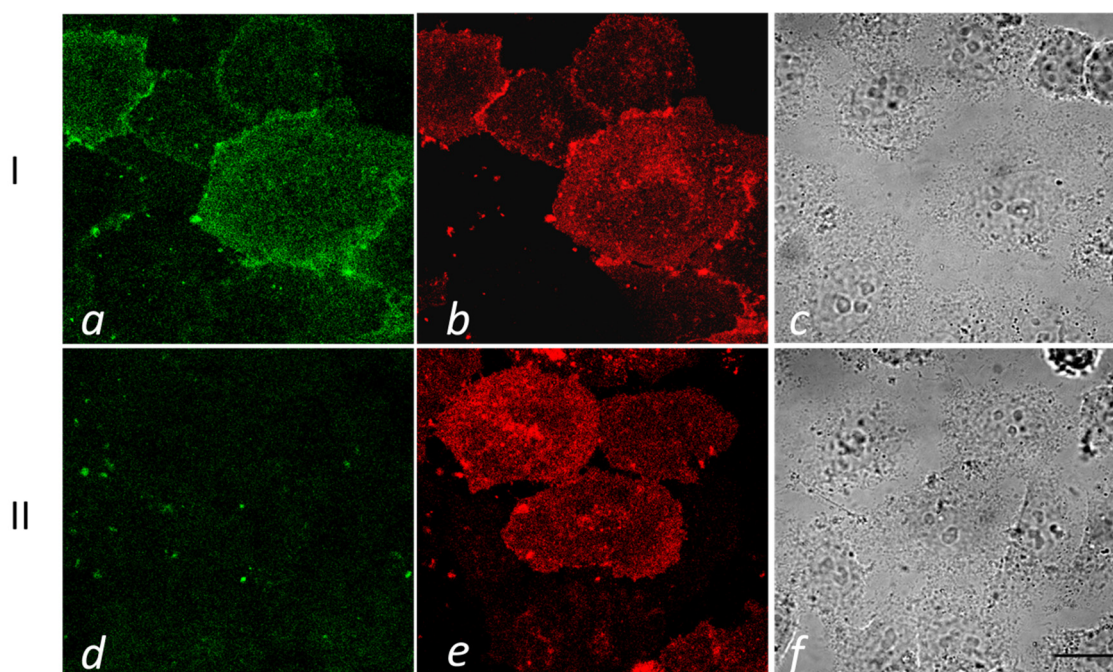
# AgTx2-GFP, Fluorescent Blocker Targeting Pharmacologically Important K<sub>v</sub>1.x (x = 1, 3, 6) Channels

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## *Interaction of AgTx2-GFP with Kv1.3 channels on the membrane of HEK293 cells.*

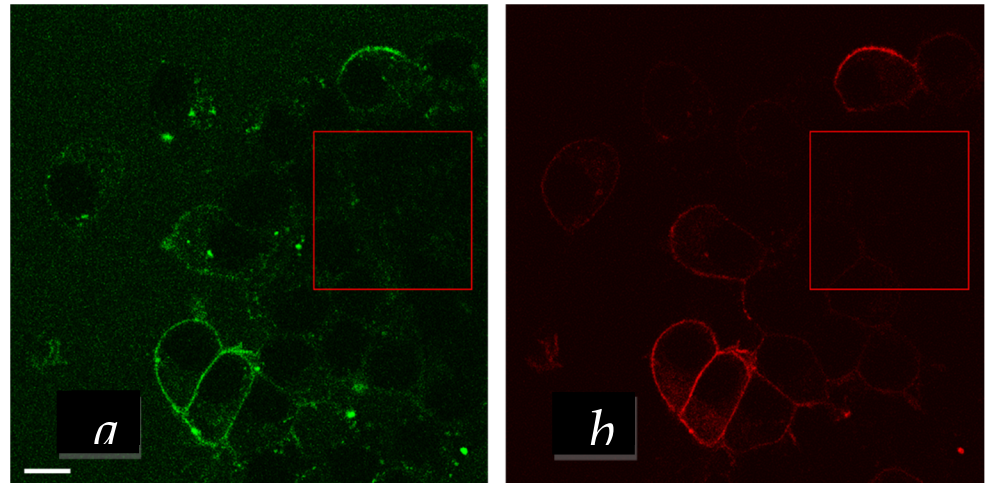
HEK293 cells (from the Russian collection of cell cultures, the Institute of Cytology RAS, Saint Petersburg, Russia) were grown in Dulbecco's modified Eagle's medium DMEM/F12 (Paneco, Russia) containing 5% fetal bovine serum (FBS, HyClone, Utah, USA) and 2 mM L-glutamine (complete medium). Transient transfection of cells with pmKate2-KCNA3-del was performed as described in [36] using GenJector-U reagent according to the manufacturer's protocol.

Confocal microscopy of HEK293 cells was performed as described in *Material and methods* section.

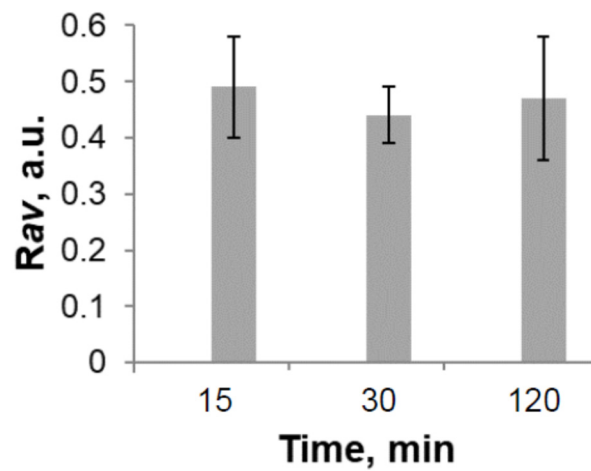


**Figure S1.** Confocal imaging of AgTx2-GFP interaction (green; *a, d*) with mKate2-Kv1.3 channels (red; *b, e*) transiently expressed in HEK293 cells. Confocal images were measured from the basal layer (plasma membrane) of living HEK293 cells. (rows I, II) Binding of AgTx2-GFP (40 nM, 20 min incubation) to mKate2-Kv1.3 (row I) and its displacement from the complexes (row II) by AgTx2 (40 nM AgTx2-GFP, 400 nM AgTx2, 20 min incubation). The scale bar is 20  $\mu$ m. Panels (*c, f*) – transmitted light images of cells. .

*AgTx2-GFP demonstrates no nonspecific binding on the membrane of cells in the absence of Kv1.3 channels.*



**Figure S2.** Confocal imaging of an interaction of AgTx2-GFP (8 nM, green) with Neuro 2A cells expressing mKate2-Kv1.3 (red). Red square marks a region with cells, which do not express mKate2-Kv1.3 (b) and demonstrate absence of nonspecific binding of AgTx2-GFP (a). Bar is 10  $\mu\text{m}$ .



**Figure S3.** Time dependence of AgTx2-GFP binding to Kv1.3 channels on the membrane of Neuro 2A cells that was measured as the dependence of  $R_{av}$  (mean  $\pm$  SD) on the time of cells incubation with 8 nM of AgTx2-GFP.