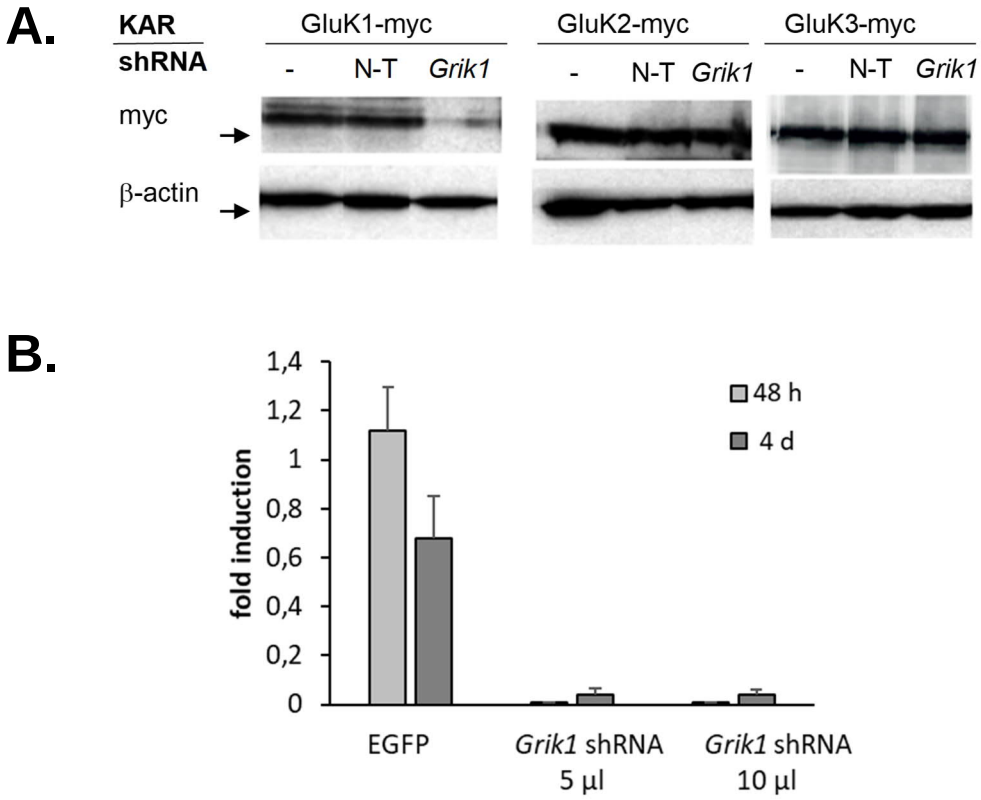
Figure 8 - Supplement 1



Validation of the Grik1 shRNA

A. A western blot illustrating that the Grik1 shRNA strongly inhibited expression of GluK1-myc but not GluK2-myc or GluK3-myc in HEK293T cells.
B. RT-qPCR results showing that the lentiviral Grik1 shRNA construct strongly inhibited expression of endogenous GluK1 in dorsal root gangion (DRG) neurons within 48 h from infection.

Methods:

Seven different shRNA sequences against rat Grik1 in pLKO.1 vector (five obtained from Sigma Aldrich and two from TRC shRNA library, Biomedicum Helsinki Functional Genomics Unit) were tested for their efficiency to suppress expression of GluK1-myc and GluK2-myc and GluK3 –myc in HEK293T cells as described (Sakha et al., 2016). The TRC Clone ID: TRCN0000100308 was selected based on its efficiency and specificity to knock down expression of the target protein in the heterologous system. The shRNA target sequence (CCTGGACATTATCAGTCTCAA) was subcloned into a modified pLKO.1 vector where the puromycin resistance cassette was replaced with GFP under the synapsin -1 promoter (pLKO.1-syn1-EGFP). Lentiviral particles were produced in HEK293T cells as described (Vesikansa et al., 2012). The lentiviral Grik1 shRNA was then further tested in primary dorsal root ganglion neurons (DRG) where GluK1 KAR subunits are endogenously expressed. The neurons were prepared and cultured as described (Kysenius et al. 2012) and infected with the shRNA encoding lentiviral vectors at 4 and 7 DIV. For quantification of the Grik1 mRNA levels, total RNA

was isolated from two independent cultures at 9 DIV and 12 DIV and RT-qPCR with Grik1 selective primers was carried out essentially as described (Sakha et al., 2016).

References:

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Vesikansa A, Sakha P, Kuja-Panula J, Molchanova S, Rivera C, Huttunen HJ, Rauvala H, Taira T, Lauri SE. (2012) Expression of GluK1c underlies the developmental switch in presynaptic kainate receptor function. Sci Rep.2:310.

Contributions :

Tiina-Kaisa Kukko-Lukjanov, Western blots in HEK cells, lentiviral vectors
Ester Orav, RT-qPCR
Kärt Mätlik , DRG cultures