

Supplementary Information

Multilayered Regulation of GluK3 Kainate Receptors is Mediated by Neto Subunits and Zinc

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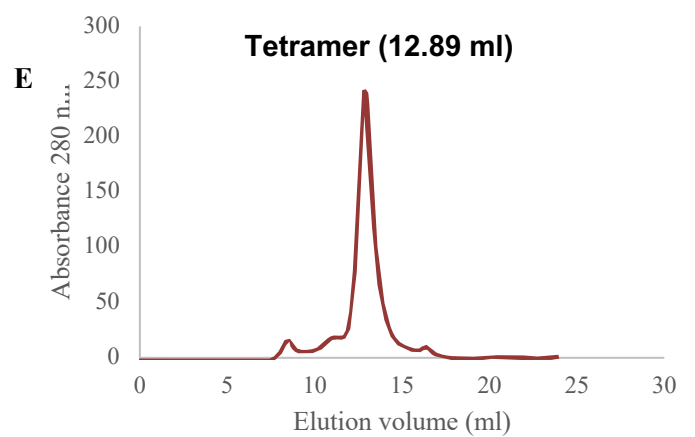
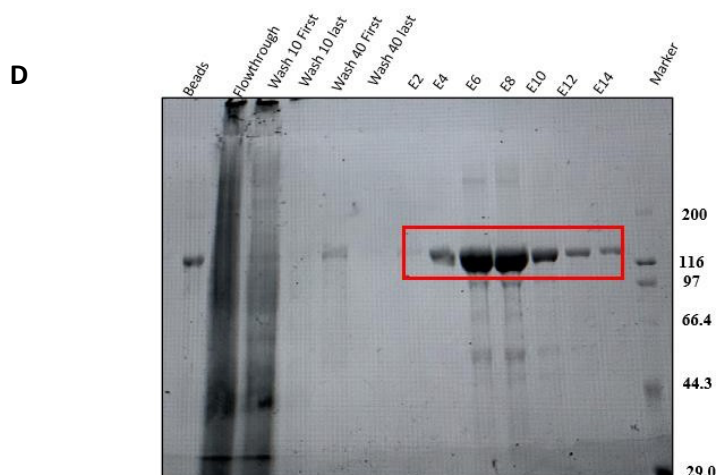
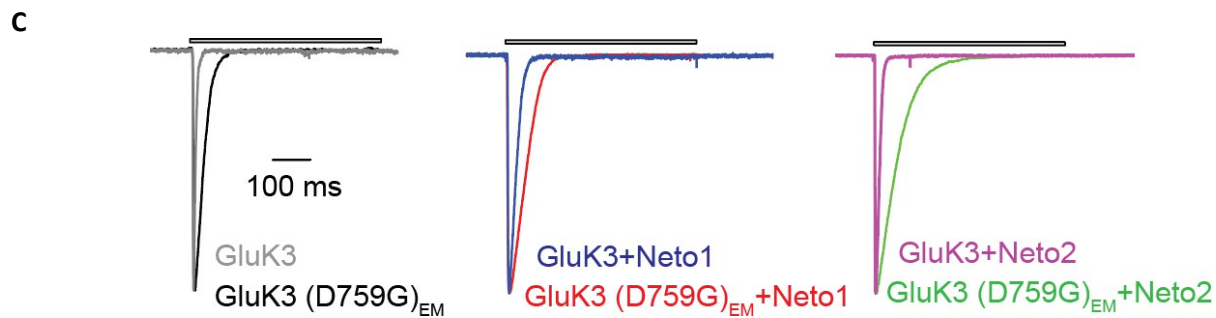
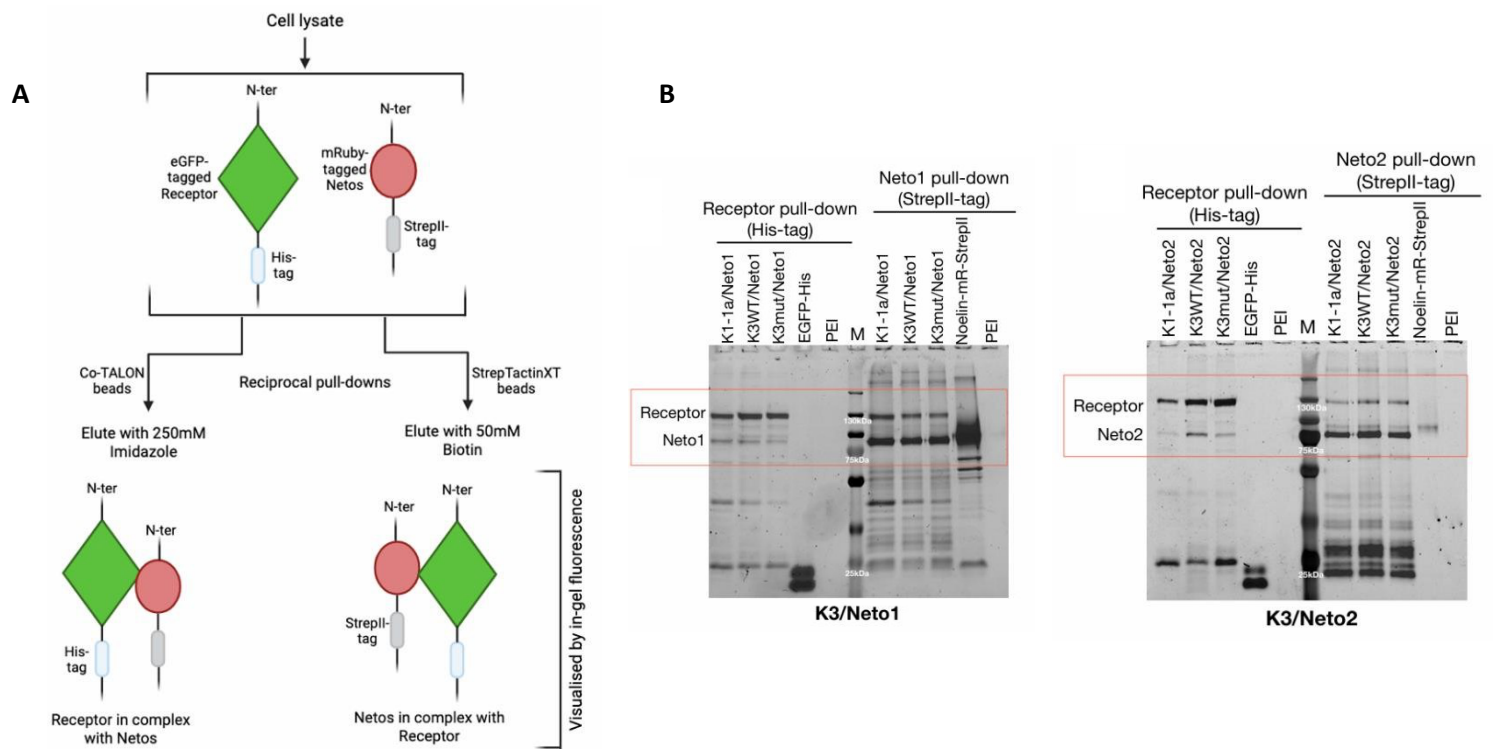
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Supplementary Figure 1

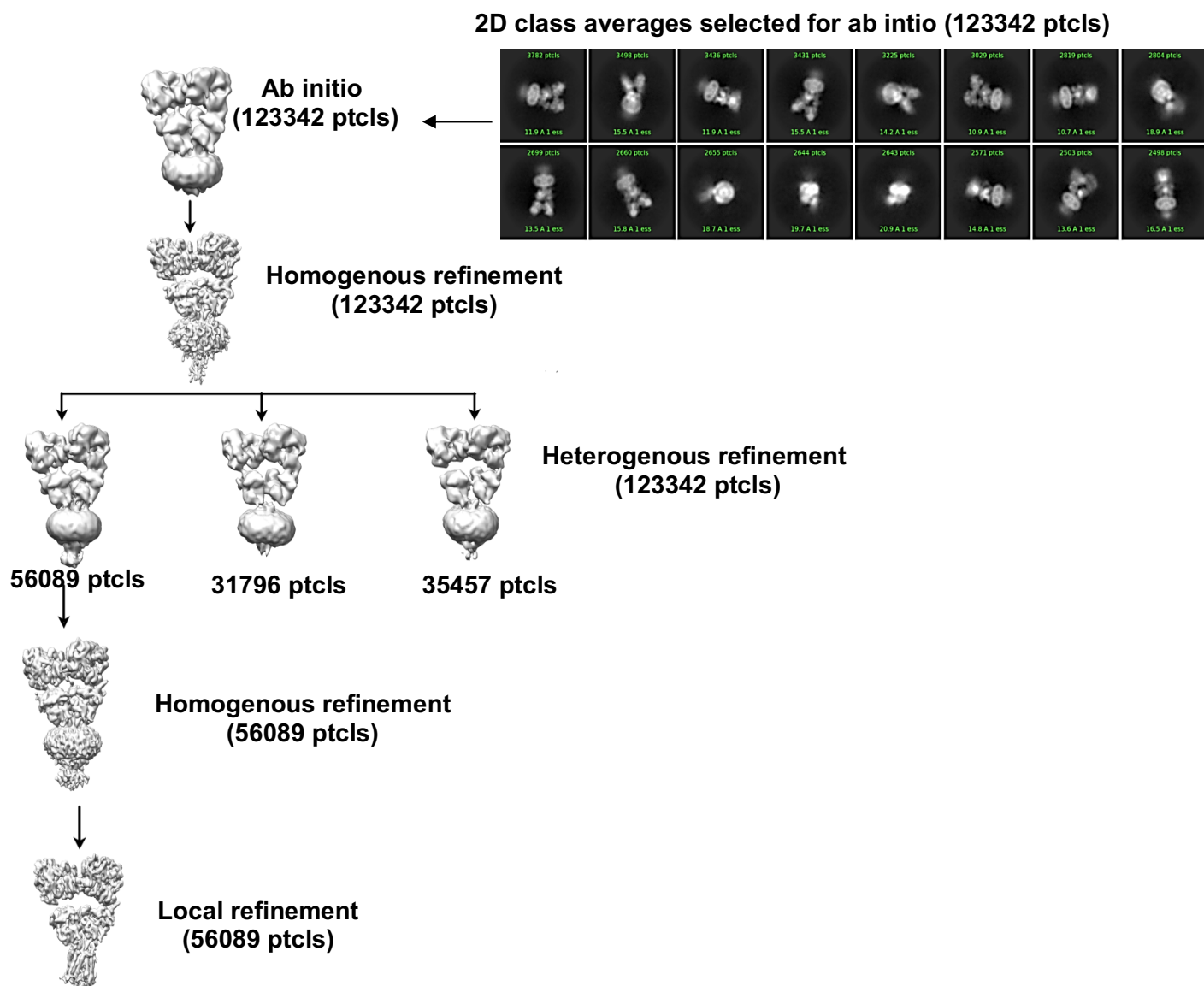
A. Schematic representation to depict the strategy used for co-immunoprecipitation of GluK3 with Netos.

B. Uncropped gel images for Co-immunoprecipitation analysis of GluK3 with Netos. Panel B represents the GluK3-Neto1 complex and GluK3-Neto2 complex reciprocal pull-downs.. The demarcated box in red shows the cropped image dimensions used in the image in Figure 1A.

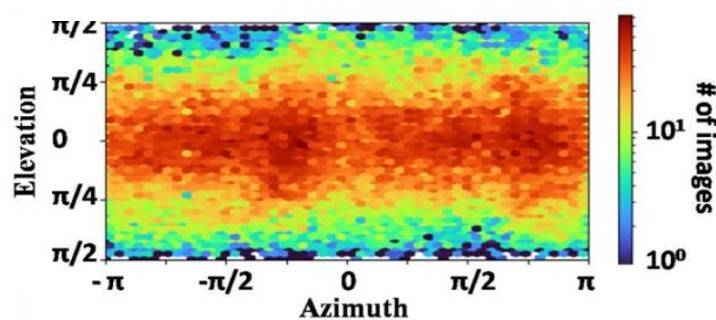
C. Representative current traces evoked by 30 mM glutamate (100 ms application) from HEK293 cells expressing GluK3 and GluK3 (D756G) or absence of Neto1 or Neto2.

D. SDS-PAGE analysis of the GluK3D759G receptors post immobilized metal affinity chromatography. Red box points at the band corresponding to protein of interest. Chromatogram showing the gel filtration profile for GluK3D759G is shown in **(E)**.

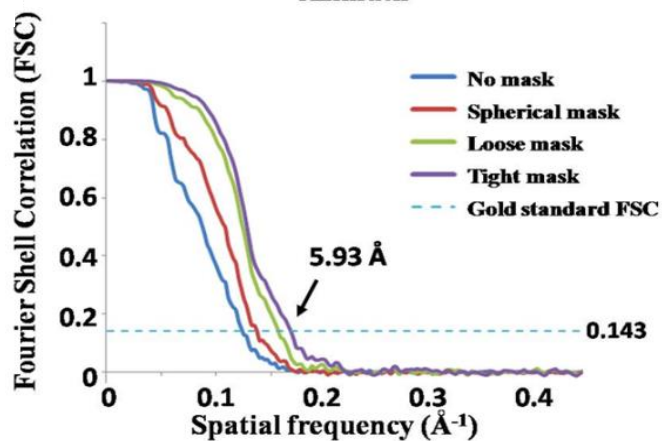
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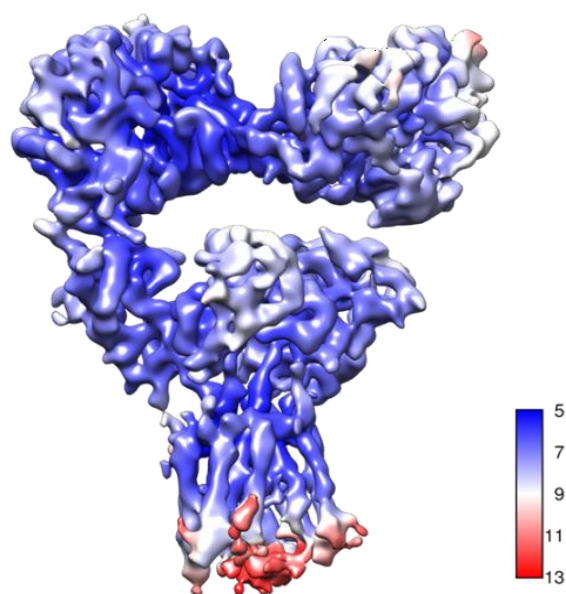
B



C



D



Supplementary Figure 2

A. Flow chart depicting the steps involved in CryoEM data processing of GluK3D759G_{UBP310+spermine}. 2D class averages with good features were selected and used to perform ab initio reconstruction with 123342 particles. In successive steps, heterogenous refinement was performed to sort the conformationally distinct classes. The best 3D class so obtained was selected for further rounds of refinement by Homogenous and local refinement steps implemented in cryoSPARCv3.1.

B-D. Representation of Local resolution, particle distribution and FSC curve for GluK3D759G. Viewing direction distribution of particles incorporated in final 3D map across azimuth and elevation angles(B). (C) FSC curve for GluK3-Spermine-UBP310 indicating a resolution of 5.9 Å at the gold standard cut off (0.143). Local resolution map for GluK3D759G estimated in cryoSPARCv3.1 (D).