Mouse Clr-g, a ligand for NK cell activation receptor NKR-P1F: crystal structure and biophysical properties

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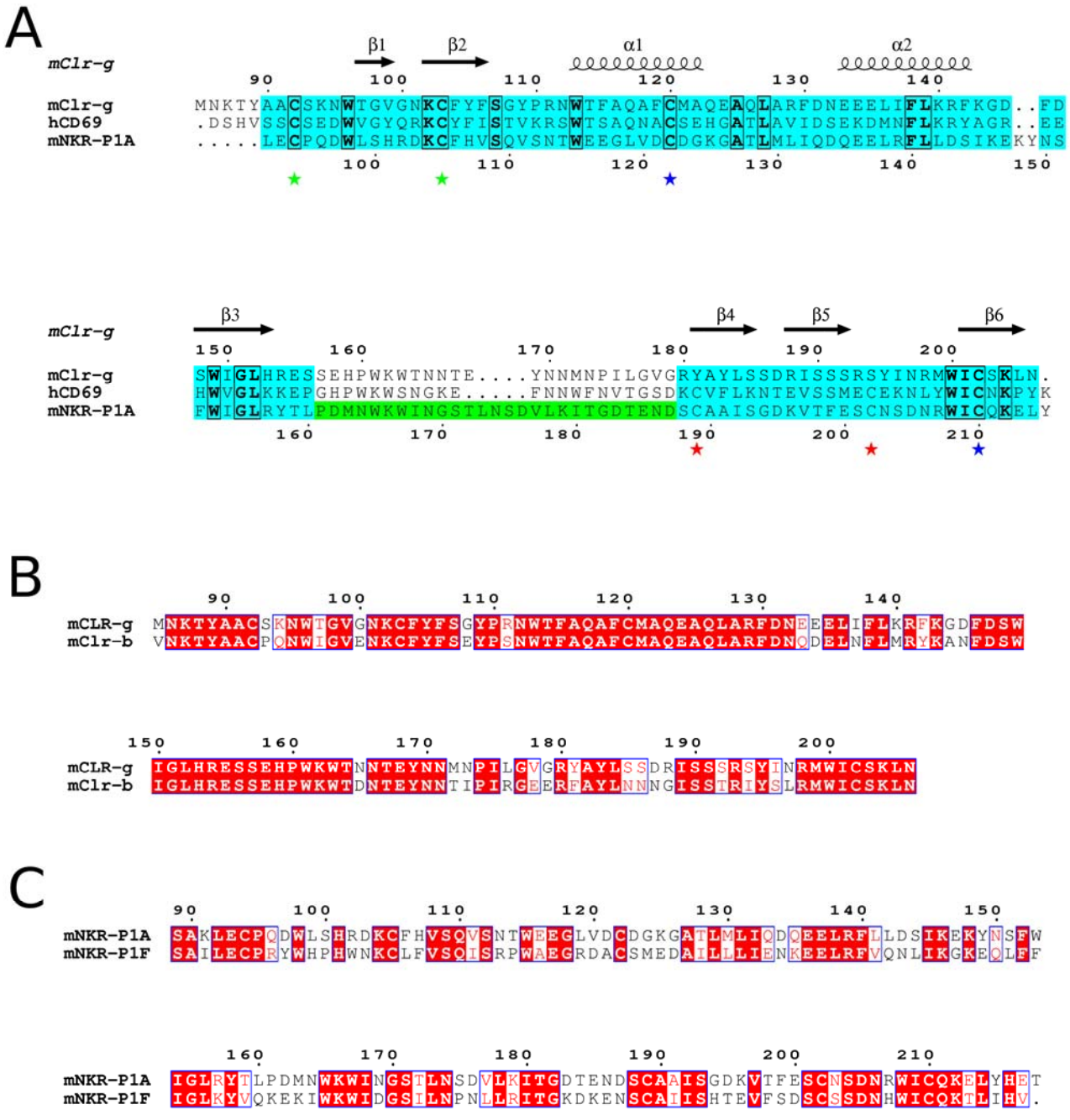
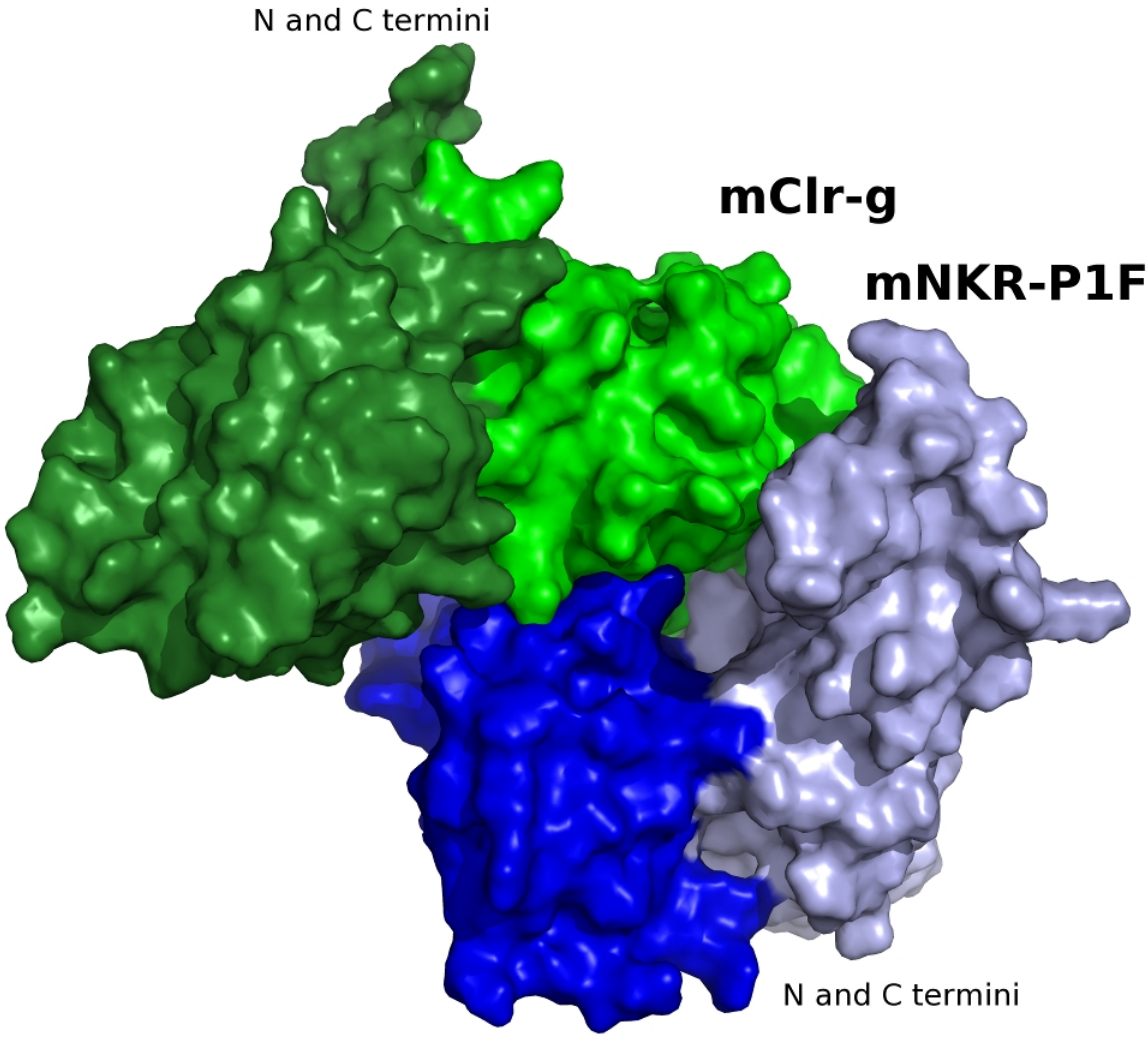
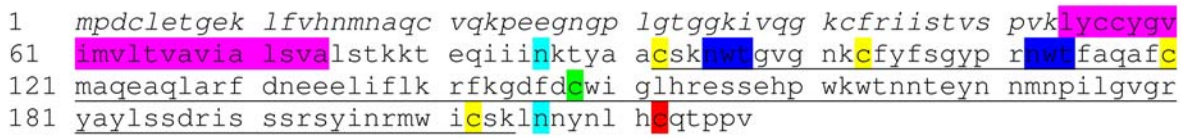


SUPPLEMENTAL FIGURE 1. (A) Structure-based sequence alignment of the extracellular part of mClr-g, hCD69 and mNKR-P1A. Secondary structure of mClr-g is denoted. The upper numbering corresponds to mClr-g and the lower one to mNKR-P1A. The secondary-structure matching parts are in cyan, the extended loop of mNKR-P1A is in green. Disulfide bonds are denoted by stars with color marking bonded pairs (green and blue in all three proteins, red in hCD69 and mNKR-P1A only). Secondary structure was assigned with DSSP (Kabsch and Sander, 1983, *Biopolymers* 22(12): 2577-2637). Secondary structure matching was performed by EBI-SSM. (B) Sequence alignment of the extracellular part of mClr-g with mClr-b: a basis for homology modelling. Prepared using CLUSTALW (35). (c) Sequence alignment of the extracellular part of mNKR-P1A with mNKR-P1F: a basis for homology modelling. Prepared using CLUSTALW. The figure was prepared using ESPRIPT (Gouet, P., Courcelle, E., Stuart, D. I., and F. Metoz. 1999. ESPript: multiple sequence alignments in PostScript. *Bioinformatics* 15: 305-308.).

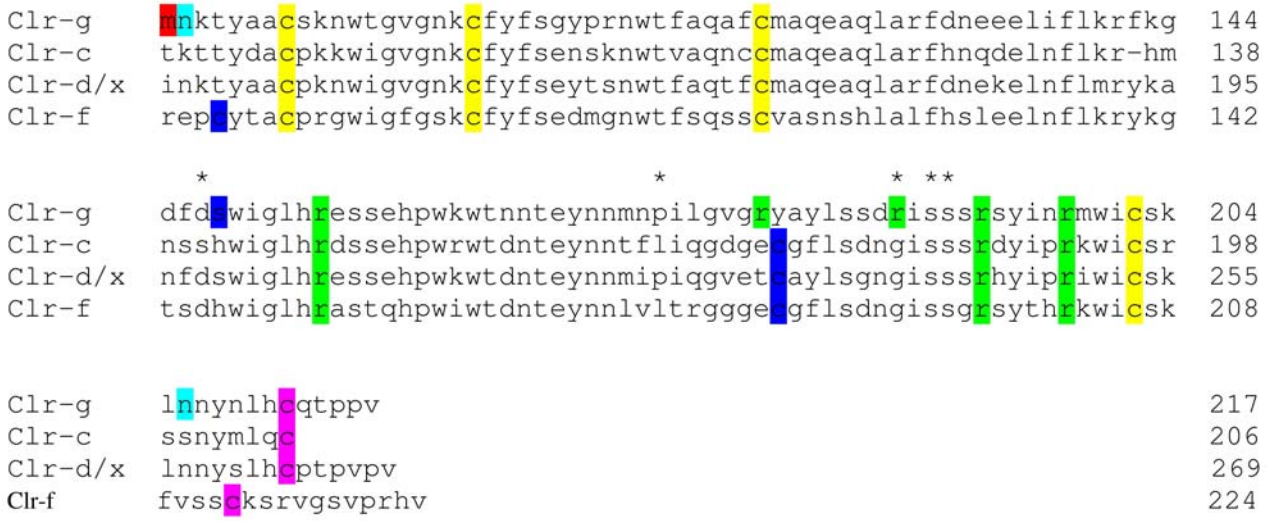


SUPPLEMENTAL FIGURE 2. A model of the mouse NKR-P1F:Clr-g complex, respecting the electrostatic complementarity of both molecules, in molecular surface representation. The model was created by the RosettaDock protein-protein docking server using input model no. 2 as explained in the manuscript (position of mClr-g is expected between the extended loops of mNKR-P1F so that the loops embrace a monomer of the mClr-g dimer). Dimers of the extracellular domains of the receptor and ligand are used in the model. The individual protein chains within the dimers are differentiated by color hue.

A



B



SUPPLEMENTAL FIGURE 3. (A) Bioinformatic overview of mClr-g using UniProtKB/Swiss-Prot file Q9WVF9.1. This particular splice variant of mClr-g is 217 amino acids long, and is composed of intracellular N-terminal fragment (Met[1] – Lys[53], *in italics*), the transmembrane domain (Leu[54] – Ala[74], *purple*), and the large C-terminal extracellular portion (Leu[75] – Val[217]) that includes the CTL domain (Cys[92] – Lys[204], *underlined*) stabilized by two predicted canonical disulfide bonds formed by Cys[92] and Cys[103], and Cys[120] and Cys[202] (*in yellow*). The unpaired Cys[148] within the CTL domain is shown in *green*, and the C-terminal Cys[212] predicted to be responsible for covalent dimerization of the receptor in *red.* The expression construct used in this study is marked in *light blue.* Putative *N*-glycosylation sites at Asn[95] and Asn[112] are marked in *dark blue*

(B) Multiple sequence alignment of mNKR-P1F reactive mClr isoforms mClr-g (accession Q9WVF9.1), mClr-c (accession AEP22558.1), mClr-d/x (accession NP_081838.1), and mClr-f (accession Q8C1T8.1). Cysteine residues forming the canonical disulfide bonds are shown in *yellow*, those supposed to be involved in covalent receptor dimerization in *purple*, and additional cysteines are in *dark blue* (including Cys[148] mutated to Ser in the expressed protein, the extent of which is indicated in *light blue* and includes the initiation Met[85] replacing Ile shown in *red*). Arginines Arg 154, 180, 188, 193, and 198 predicted to be involved in mNKR-P1F interaction are shown in *green*, and additional predicted interaction residues are marked with asterisks.