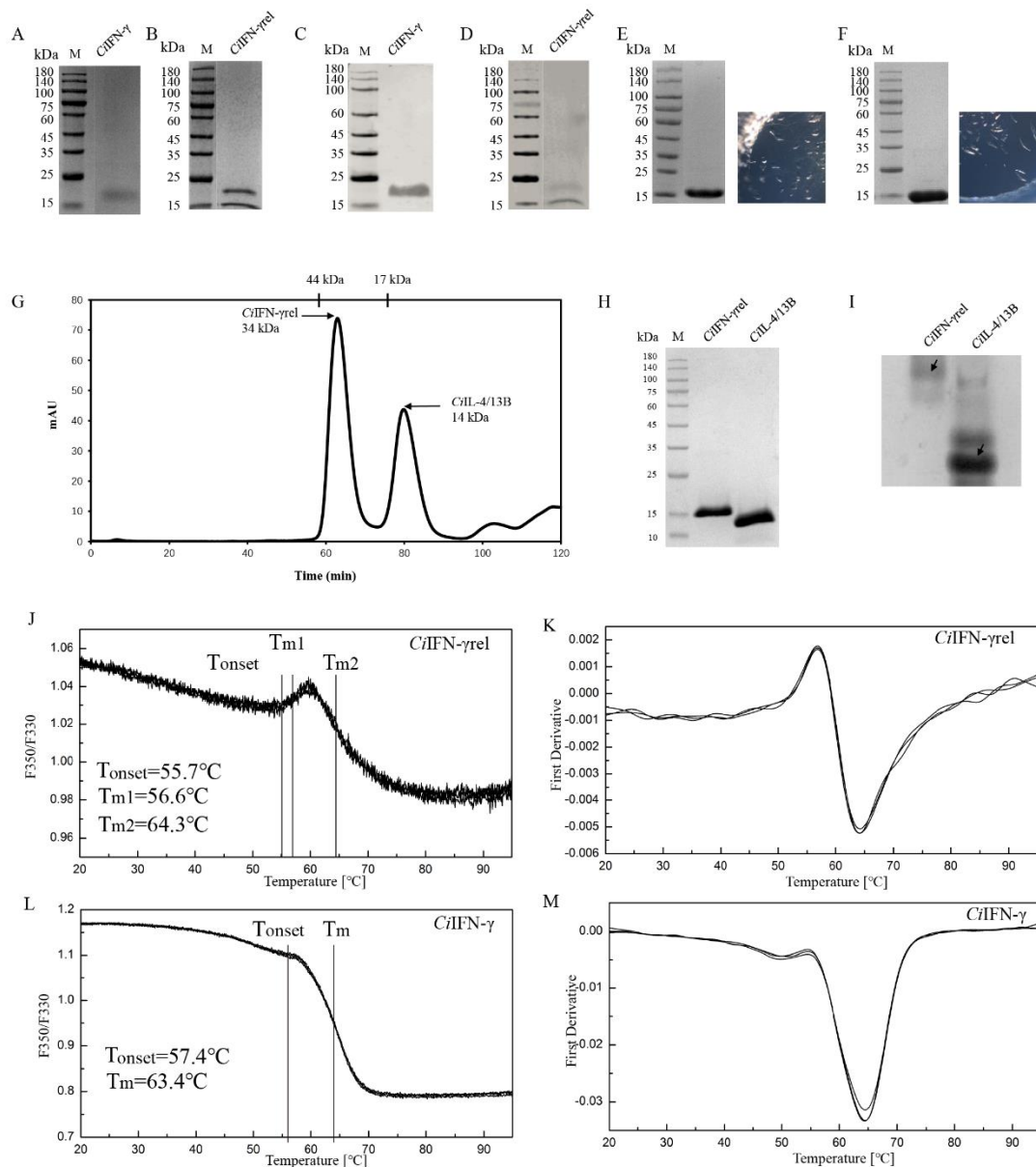
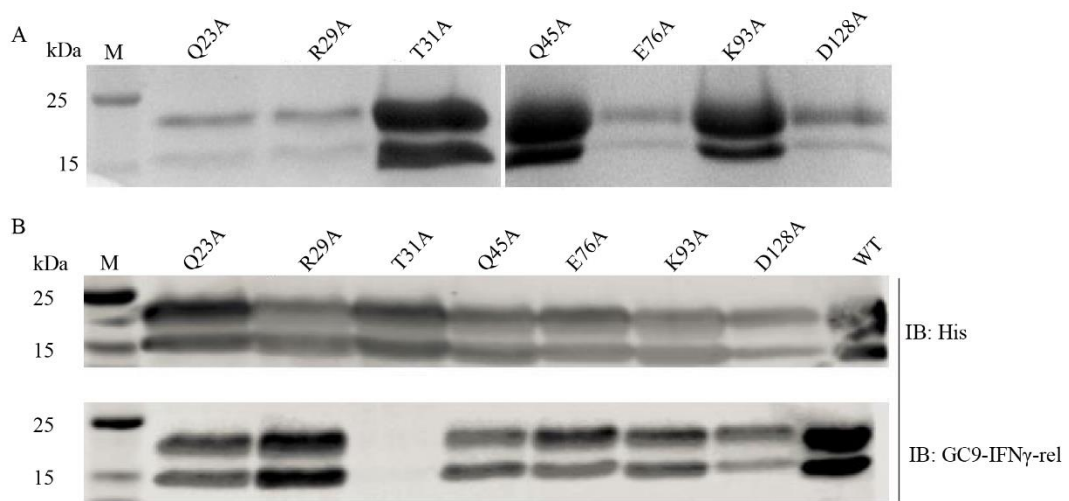


Supplemental figure 1



**Supplemental Fig. 1** Purification of recombinant *CiIFN-γ* protein expressed in CHO-S cells (A) and *CiIFN-γrel* protein expressed in HEK293F cells (B). Western blotting analysis of recombinant IFN- $\gamma$  (C) and IFN- $\gamma$ rel (D). Purification and crystallization of native (E) and selenomethionine (F) recombinant *CiIFN-γrel* proteins. Size exclusion chromatography of purified recombinant *CiIFN-γrel* and *CiIL-4/13B* proteins using a Superdex 75 column (G) and PAGE analyses under reducing (H) and non-reducing conditions (I). NanoDSF thermogram of *CiIFN-γrel* and *CiIFN-γ* proteins. (J-M). F350/330 ratio of *CiIFN-γrel* (J) and *CiIFN-γ* (L). The first derivative of F350/F330 data of *CiIFN-γrel* (K) and *CiIFN-γ* (M).

Supplemental figure 2



**Supplemental Fig. 2** SDS-PAGE (A) and Western blotting (B) analysis of mutant IFN- $\gamma$ rel proteins. Mutant IFN- $\gamma$ rel proteins were expressed in HEK293F cells. Monoclonal antibodies against IFN- $\gamma$ rel (GC9-IFN $\gamma$ rel) and a 6 x His tag were used for Western blotting.

Supplemental Table I. Primers used in the study

| Primer name                       | Sequence from 5' to 3'             |
|-----------------------------------|------------------------------------|
| PET- $\gamma$ rel F               | CATGCCATGGCTTTTCGCTCCGCCGCAGCAAAGC |
| PET- $\gamma$ rel R               | CGGGATCCTTACTGAACCTTTTGTGTTTCTCGG  |
| EF-1 $\alpha$ -F                  | CAGCACAAACATGGGCTGGTTC             |
| EF-1 $\alpha$ -R                  | ACGGGTACAGTTCCAATACCTCCA           |
| <i>Il-1<math>\beta</math></i> -qF | TCTCCTCGTCTGCTGGGTGT               |
| <i>Il-1<math>\beta</math></i> -qR | CAAGACCAGGTGAGGGGAAG               |
| <i>Il-6</i> -qF                   | CAGCAGAATGGGGGAGTTATC              |
| <i>Il-6</i> -qR                   | CTCGCAGAGTCTTGACATCCTT             |
| <i>Cxcl8-11</i> -qF               | TCTACCCTCCTAGCCCTCACTG             |
| <i>Cxcl8-11</i> -qR               | TCATGGTGCTTTGTTGGCAAGGA            |
| <i>Cxcl11.1b</i> -qF              | CGGACACCAAAGGTGTTTATG              |
| <i>Cxcl11.1b</i> -qR              | CAGCGTGACAACAACCTCCAG              |
| <i>MhcII</i> -qF                  | CCCTTCTCTACAGACCCTCAGTC            |
| <i>MhcII</i> -qR                  | CTCCATACGGAATGCAGTCATTC            |
| <i>Tbet</i> -qF                   | TGCGTACGAGAGCGAATACC               |
| <i>Tbet</i> -qR                   | CTGGGGATGAGATGGTTCGG               |
| <i>Mxl</i> -qF                    | CGCTGAAGACCATCGCCGATGT             |
| <i>Mxl</i> -qR                    | AGCTCCATCTTGAATTGGGTCCTC           |
| <i>STAT1</i> -qF                  | TCAAAGAATGGTGAACCTAAGATGC          |
| <i>STAT1</i> -qR                  | GAATCATCAGGTGCGCTGGTATAGT          |
| <i>Tap1</i> -qF                   | ACAGGAGCCAGTTCTGTTCG               |
| <i>Tap1</i> -qR                   | TGCTTCTGACCTCCGAAAC                |
| <i>Ciita</i> -qF                  | TCTGGGCACATACAGCAGAC               |
| <i>Ciita</i> -qR                  | GAGGGCTGGCACACCTTTAT               |
| <i>CstII</i> -qF                  | AGGGCAATGAGGGCTGTAAC               |
| <i>CstII</i> -qR                  | CCAGCGTCAATAGCCACAGA               |
| <i>Cstd</i> -qF                   | AGAAACCCTGACACCCAACC               |
| <i>Cstd</i> -qR                   | CCCTGGATCAGAGGGATTGC               |

Note: The *Nco* I and *Bam*H I sites are underlined. Double underlined bases (CT) are introduced to avoid open reading frame shift. Mutated bases are in bold.

*Supplemental Table II Mass spectrometric analysis of IFN- $\gamma$ rel protein expressed in HEK293F cells*

| Name                | Calculated pI value | Matched peptides shown in bold   | Sequence Coverage | Possible variable modifications |
|---------------------|---------------------|--|-------------------|---------------------------------|
| IFN- $\gamma$ rel-1 | 9.2                 | <b>MAFRFRRSKSEMTHLETNIHSLQEHY</b><br><b>KTRGTEWVSKSVFPHLNQLNSKAS</b><br><b>CTCQALLLERMLNIYEELFQDMKSE</b><br><b>HKEGRKDL D HLMDEVK KLRGNYKEE</b><br>HKVWKELQEMNSVKVKNGTIRGGAL<br>NDFLMVFDRASTEKHKKVQH HHHHHH<br><b>MAFRFRRSKSEMTHLETNIHSLQEHY</b><br><b>KTRGTEWVSKSVFPHLNQLNSKAS</b><br><b>CTCQALLLERMLNIYEELFQDMKSE</b><br><b>HKEGRKDL D HLMDEVK KLRGNYKEE</b><br>HKVWKELQEMNSVKVKNGTIRGGAL<br>NDFLMVFDRASTEKHKKVQH HHHHHH | 74%               | O-GlcNAcylation                 |
| IFN- $\gamma$ rel-2 | 9.2                 | <b>MAFRFRRSKSEMTHLETNIHSLQEHY</b><br><b>KTRGTEWVSKSVFPHLNQLNSKAS</b><br><b>CTCQALLLERMLNIYEELFQDMKSE</b><br><b>HKEGRKDL D HLMDEVK KLRGNYKEE</b><br>HKVWKELQEMNSVKVKNGTIRGGAL<br>NDFLMVFDRASTEKHKKVQH HHHHHH  | 74%               | -                               |