

Supplementary Material

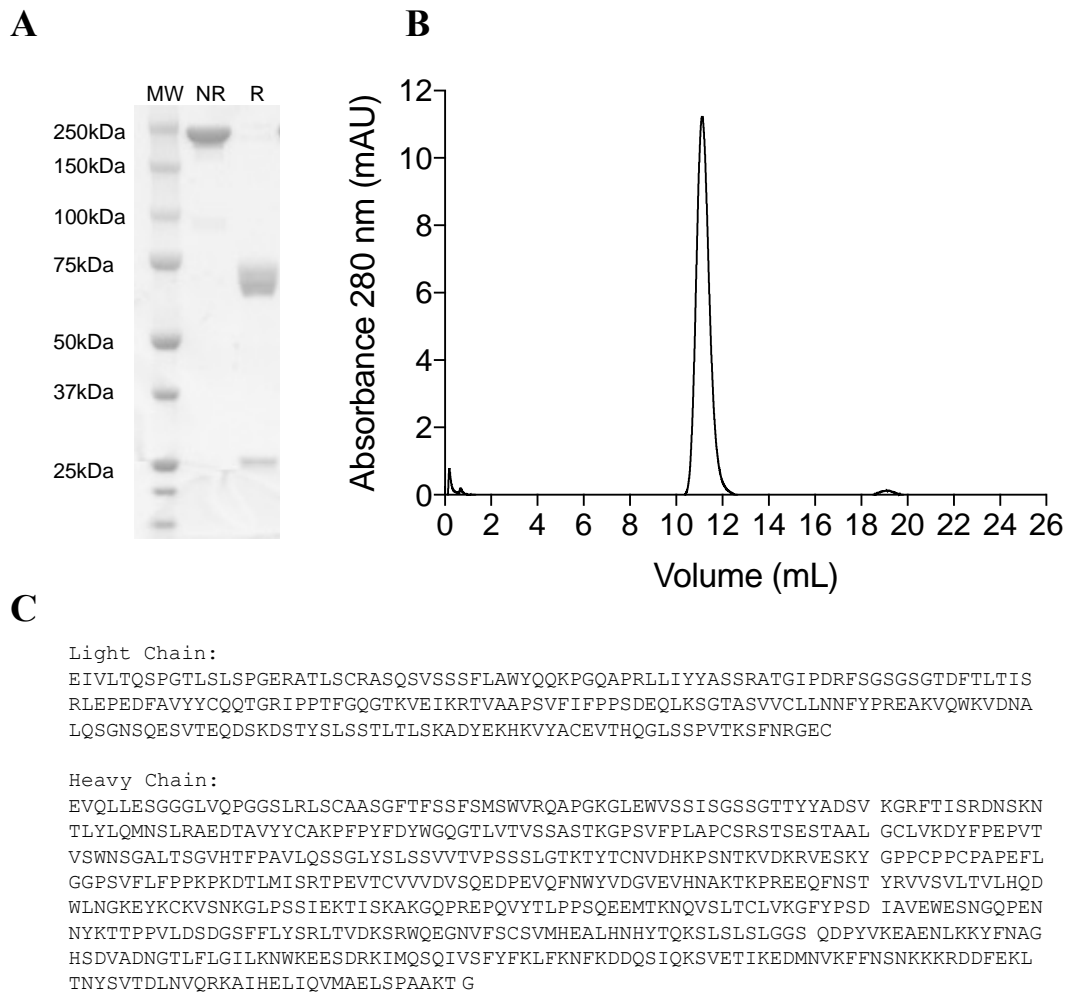


Figure S1 L19-IFN γ TG biochemical characterization and amino acid sequence

(A) SDS-page, 10% gel in reducing (R) and non-reducing (NR) conditions. (B) Size exclusion chromatography profile. (C) Amino acid sequence.

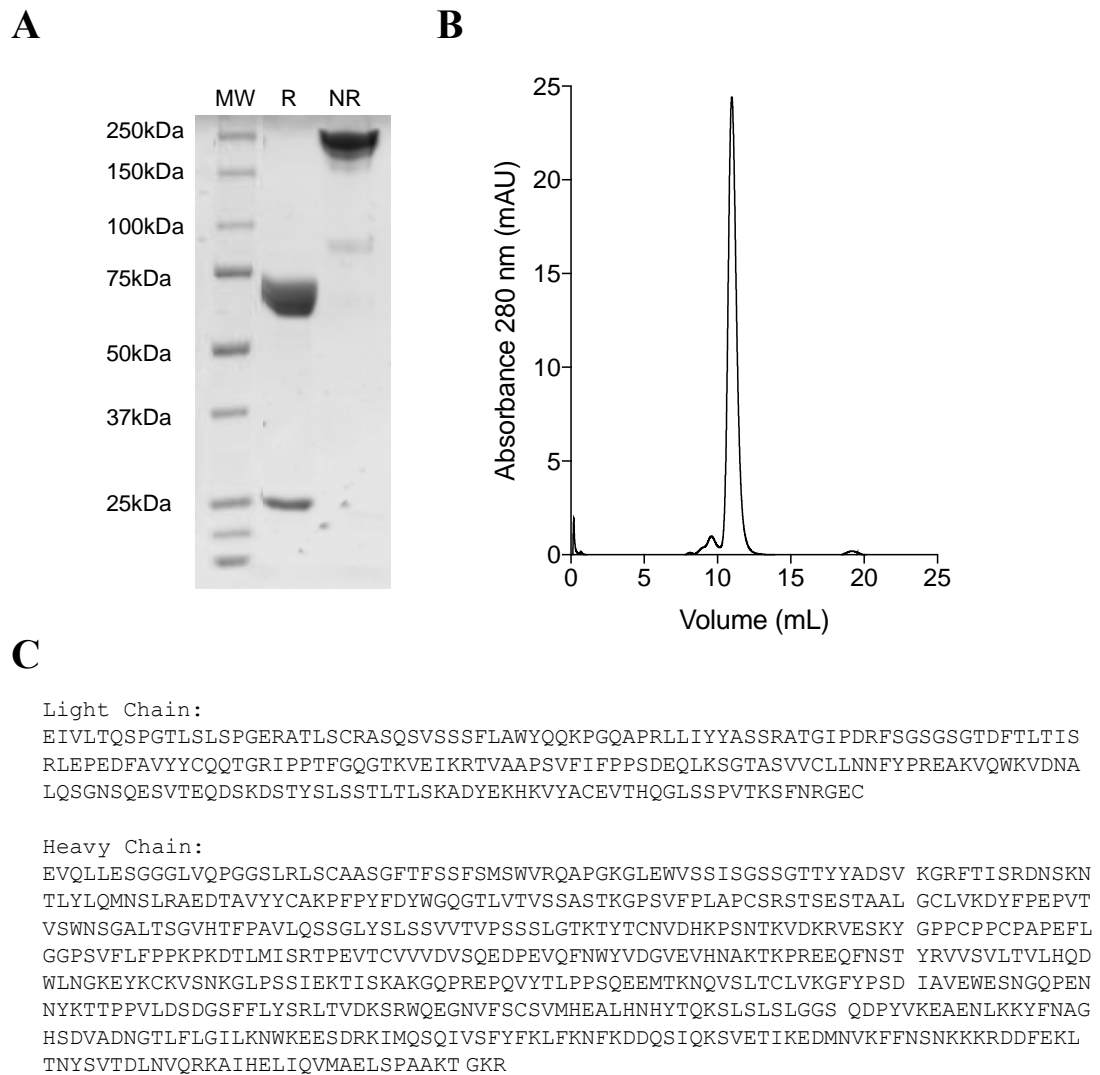


Figure S2 L19-IFN γ KR biochemical characterization and amino acid sequence
(A) SDS-page, 10% gel in reducing (R) and non-reducing (NR) conditions. **(B)** Size exclusion chromatography profile. **(C)** Amino acid sequence.

Light Chain:

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTIS
RLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy Chain:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSV KGRFTISRDN SKN
TLYLQMNSLRAEDTAVYYCAKPFPPYFDYWGGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVT
VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKY GPFCPPCPAPEFL
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQD
WLNKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGGS QDPYVKEAENLK KYFNAG
HSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKLFKNFDDQSIQKSVETIKEDMNVKFFNSNKKKRDDFEKL
TNYSVTDLNVQRKAIHELIQVMAELSPA AKTGKRG

Figure S3 L19-IFN γ KRG amino acid sequence

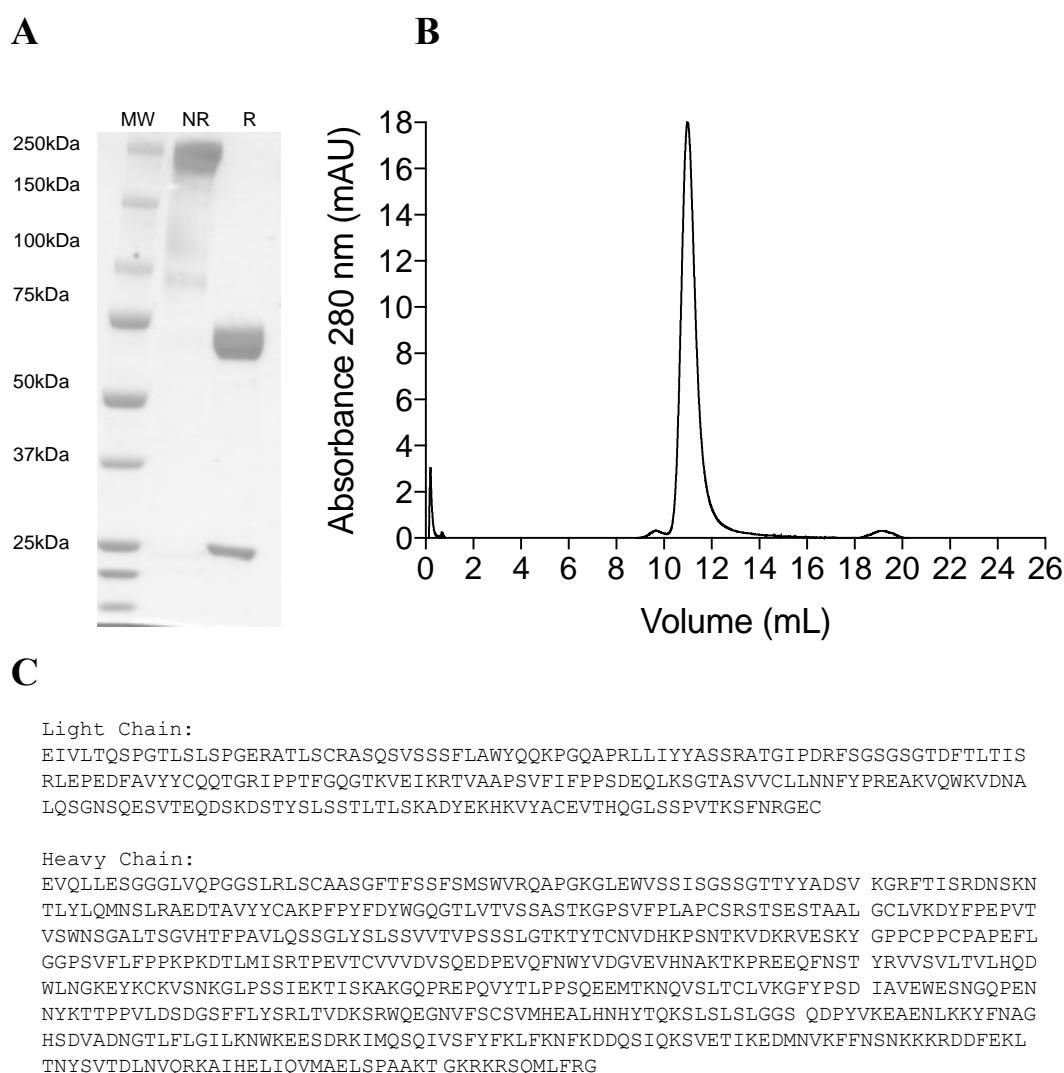


Figure S4 L19-IFN γ WT biochemical characterization and amino acid sequence
(A) SDS-page, 10% gel in reducing (R) and non-reducing (NR) conditions. **(B)** Size exclusion chromatography profile. **(C)** Amino acid sequence.

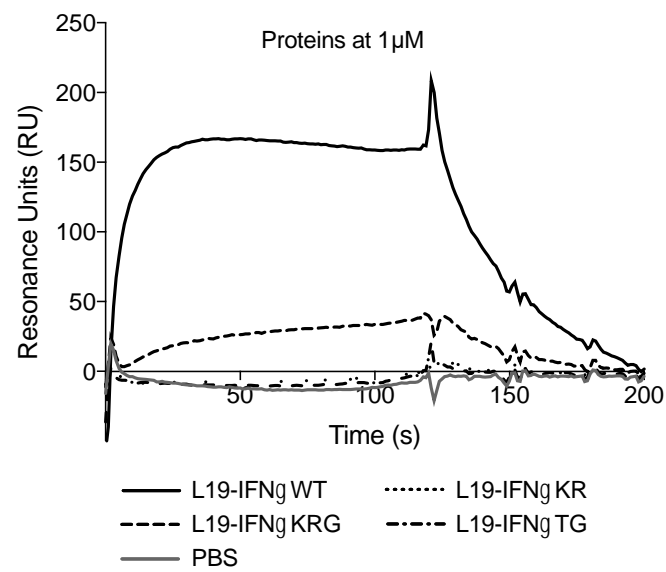
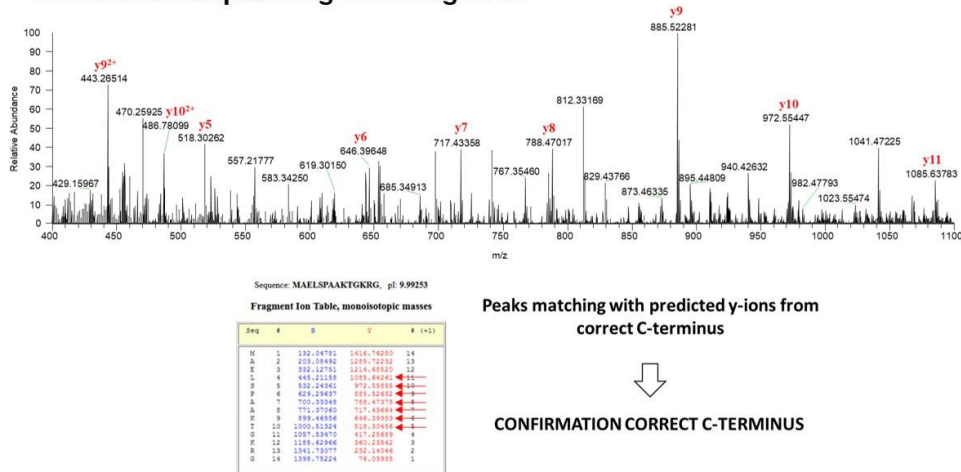


Figure S5 SPR analysis on IFN γ R1 CM5 coated chip

Proteins at 1 μ M were analyzed on an IFN γ R1 CM5 coated chip.

A C-terminus sequencing L19-IFNg-KRG



B C-terminus sequencing L19-IFNg-KR

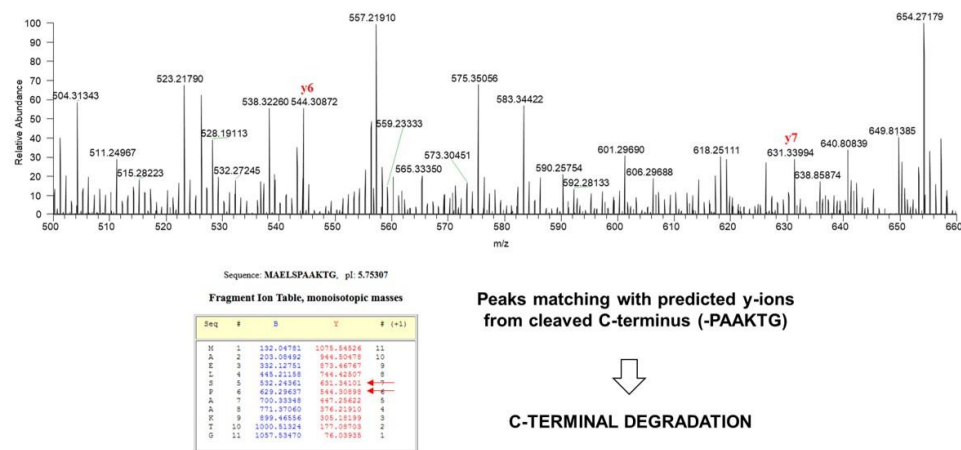


Figure S6 C-terminus sequence confirmation by Intact Mass Analysis

For L19-IFNg-KRG variant (A), several y-ions from the predicted fragmentation list were detected in the fragmentation spectrum, confirming its identity. For L19-IFNg-KR variant (B), no y-ions from the predicted fragmentation list were detected in the fragmentation spectrum. However, y-ions from the predicted fragmentation list of L19-IFNg-TG variant were identified indicating a C-terminal degradation.

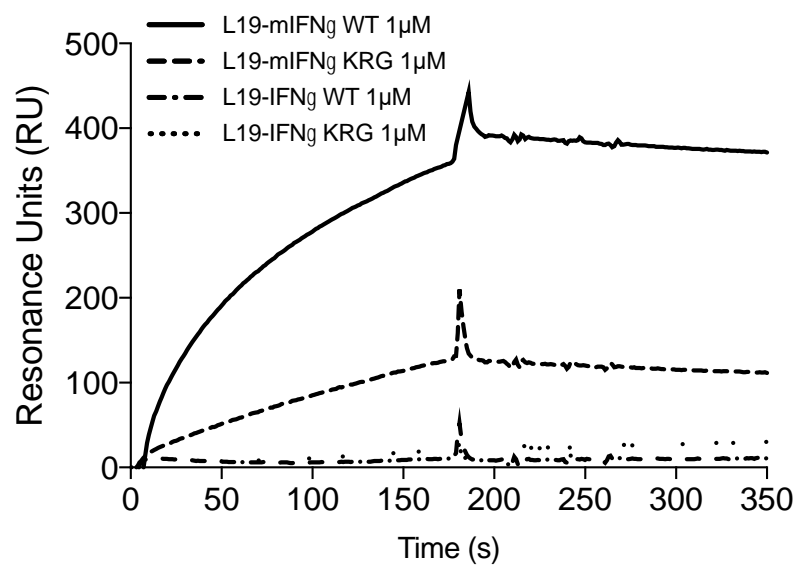


Figure S7 SPR analysis on mIFN γ R1 CM5 coated chip

Proteins at 1 μ M were analyzed on a mIFN γ R1 CM5 coated chip.

Light Chain:

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTIS
RLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy Chain:

EVQLLESQGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSV KGRFTISRDNSKN
TLYLQMNSLRAEDTAVYYCAKPPYFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVT
VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGKTYTCNVDPKPSNTKVDKRVESKY GPCCPPCPAPEFL
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQD
WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG GSGGGGSGGGSGGGSGGGG
HGTVIESLESNNYFNSSGIDVEEKSFLDIWRNWQKDGDMKILQSQIISFYLRLEVLKDNQAISNNISVIESHLI
TTFSSNSKAKKDAFMSIAKFEVNNPQVQRQAFNELIRVVHQLLPESSLRKRKRG

Figure S8 L19-mIFN γ KRG amino acid sequence

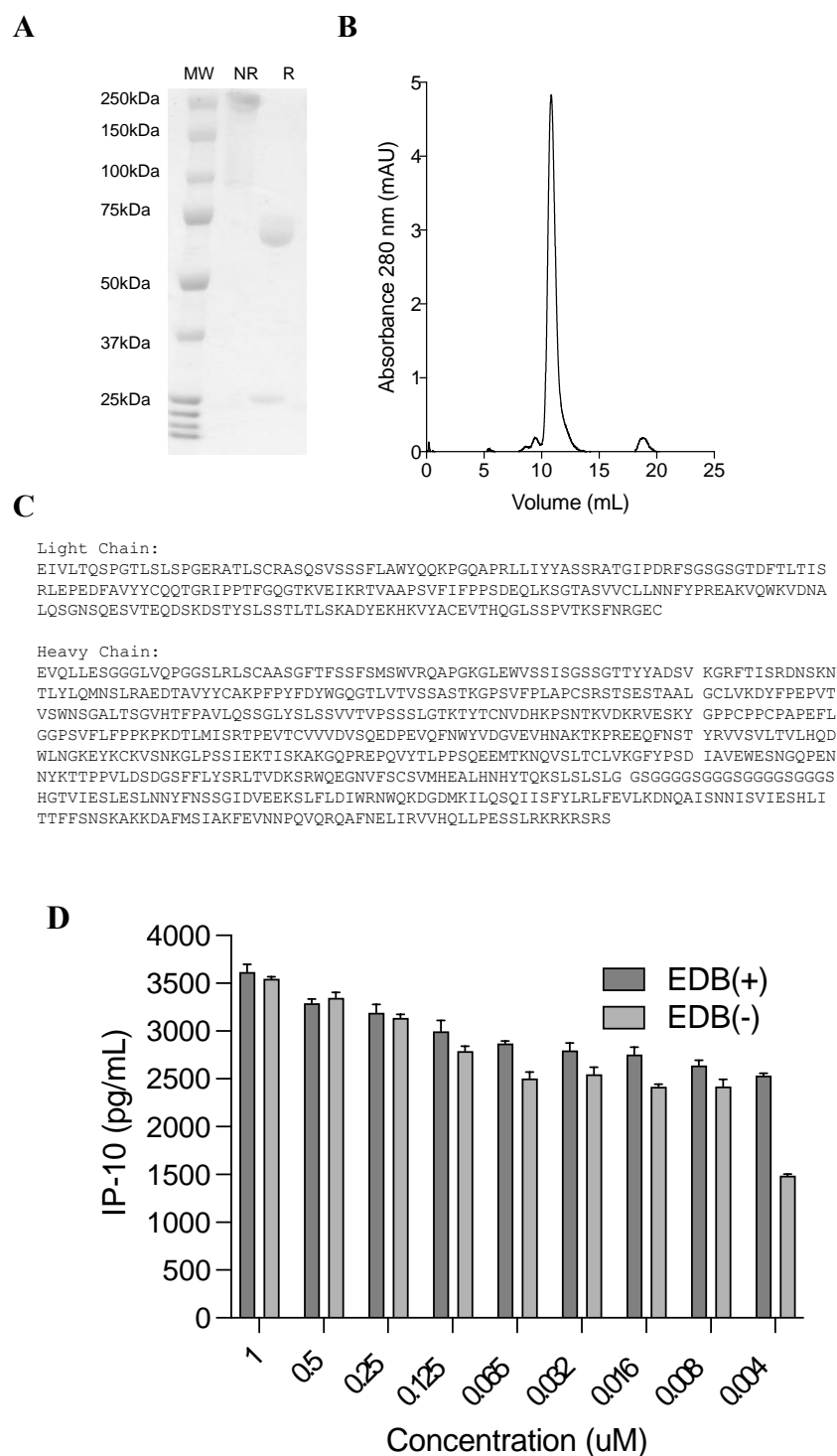


Figure S9 L19-mIFN γ WT biochemical characterization and amino acid sequence

(A) SDS-page, 10% gel in reducing (R) and non-reducing (NR) conditions. (B) Size exclusion chromatography profile. (C) Amino acid sequence. (D) IP-10

release on TIB-49 cells exposed to titration of L19-mIFN γ KRG in coated EDB(+) and non-coated EDB(-) wells.

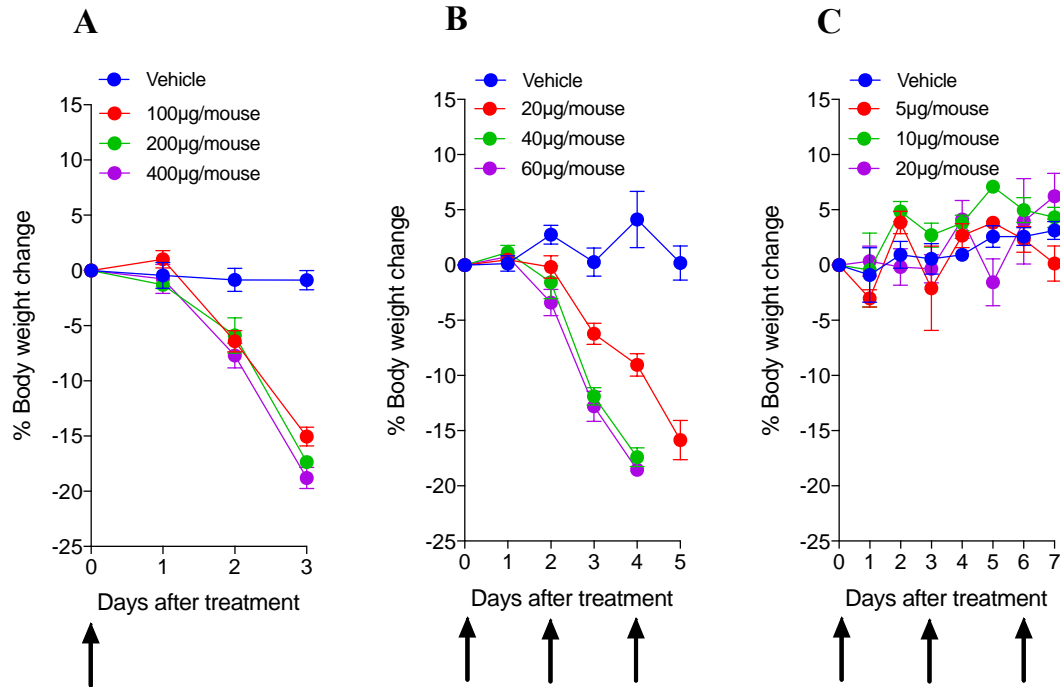


Figure S10 Dose escalation study

WEHI-164 tumor bearing mice intravenously injected at the following dose and schedules: (A) 400 μ g, 200 μ g, and 100 μ g with a single injection. (B) 60 μ g, 40 μ g and 20 μ g every 48h. (C) 20 μ g, 10 μ g and 5 μ g every 72h interval. Body weight loss equal or superior to 15% respect to the average body weight was considered as an endpoint. Data represent mean % weight (\pm SEM), n = 4 mice per group.

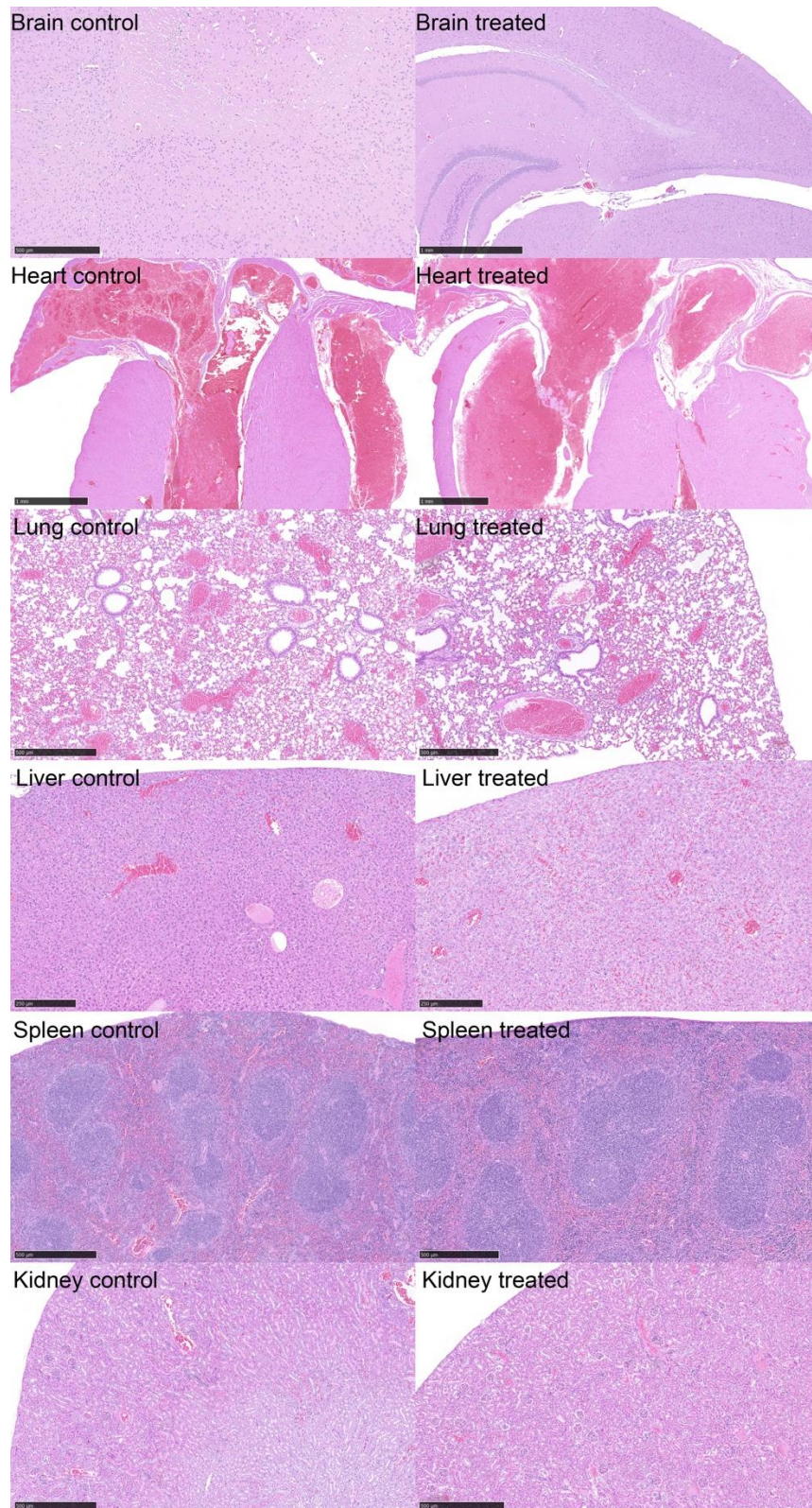


Figure S11 Histopathological analysis on tumor bearing mice

H&E staining of organs after L19-mIFN γ KRG (right panels) treatment compared to Saline (left panels).

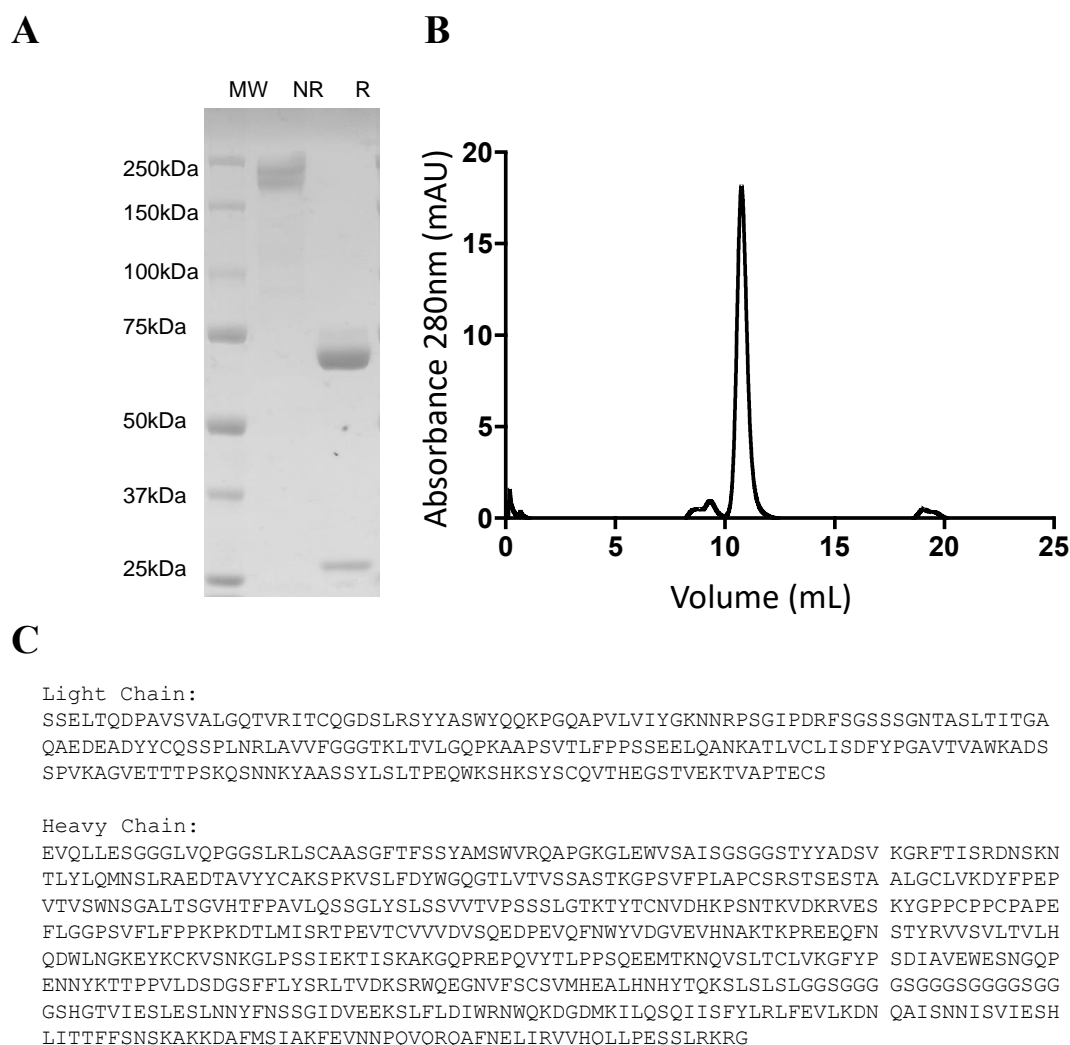
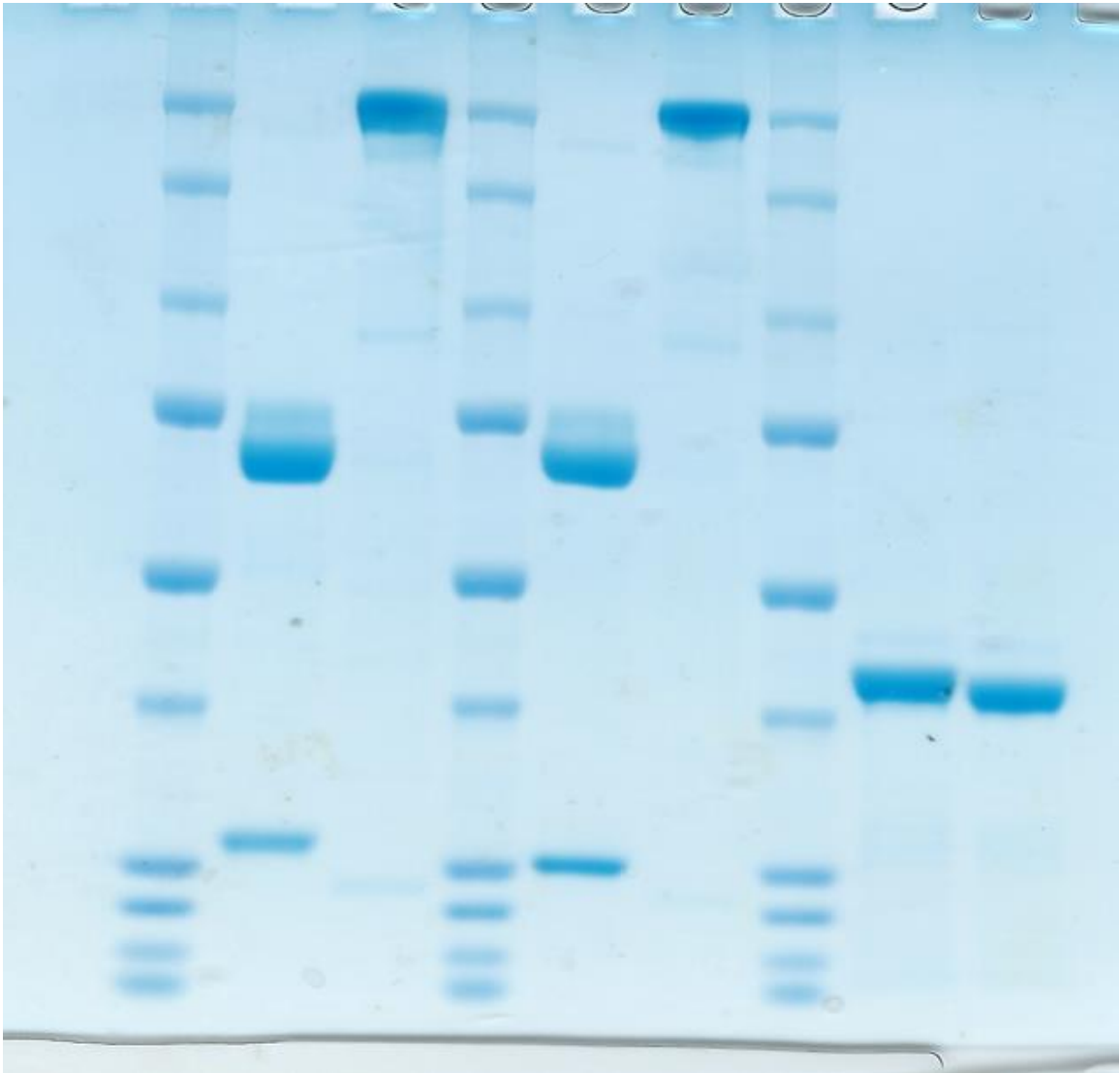


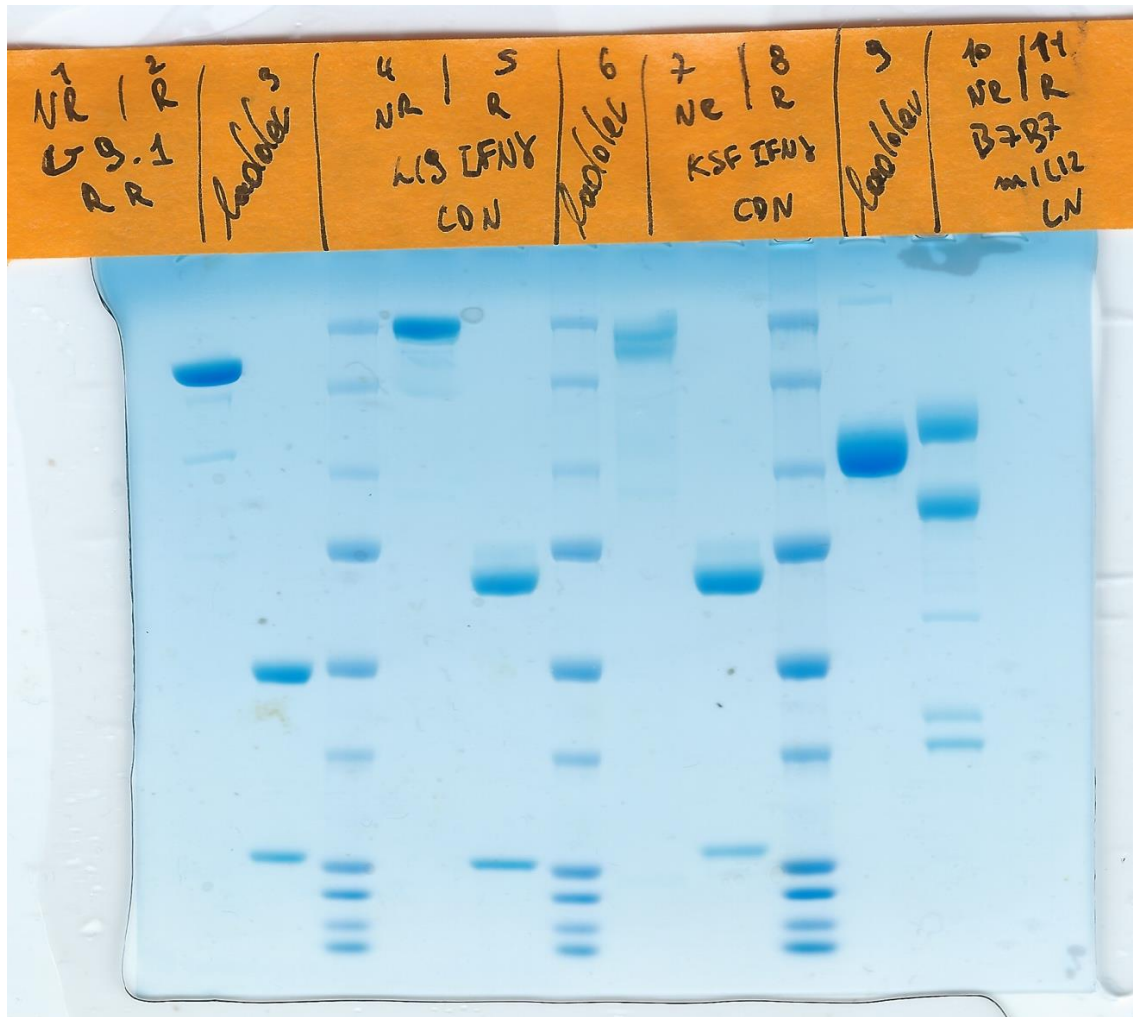
Figure S12 KSF-mIFNg KRG biochemical characterization and amino acid sequence

(A) SDS-page, 10% gel in reducing (R) and non-reducing (NR) conditions. (B) Size exclusion chromatography profile. (C) Amino acid sequence.

Original SDS-Page images



Original SDS-Page from Figure 1. Lanes description from left to right, in **bold** the figure present in the manuscript: molecular weight ladder / Protein reducing condition / Protein non-reducing condition / molecular weight ladder / **L19-IFNg KRG Figure 1 reducing condition** / **L19-IFNg KRG Figure 1 non-reducing condition** / molecular weight ladder / Protein reducing condition / Protein non-reducing condition



Original SDS-Page from Figure 3. lanes description from left to right, in **bold** the figure present in the manuscript: Protein non-reducing condition / Protein reducing condition / molecular weight ladder / **L19-mIFN γ KRG Figure 3 non-reducing condition** / **L19-mIFN γ KRG Figure 3 reducing condition** / molecular weight ladder / Protein non-reducing condition / Protein reducing condition / molecular weight ladder / Protein non-reducing condition / Protein reducing condition