

Supplementary Material

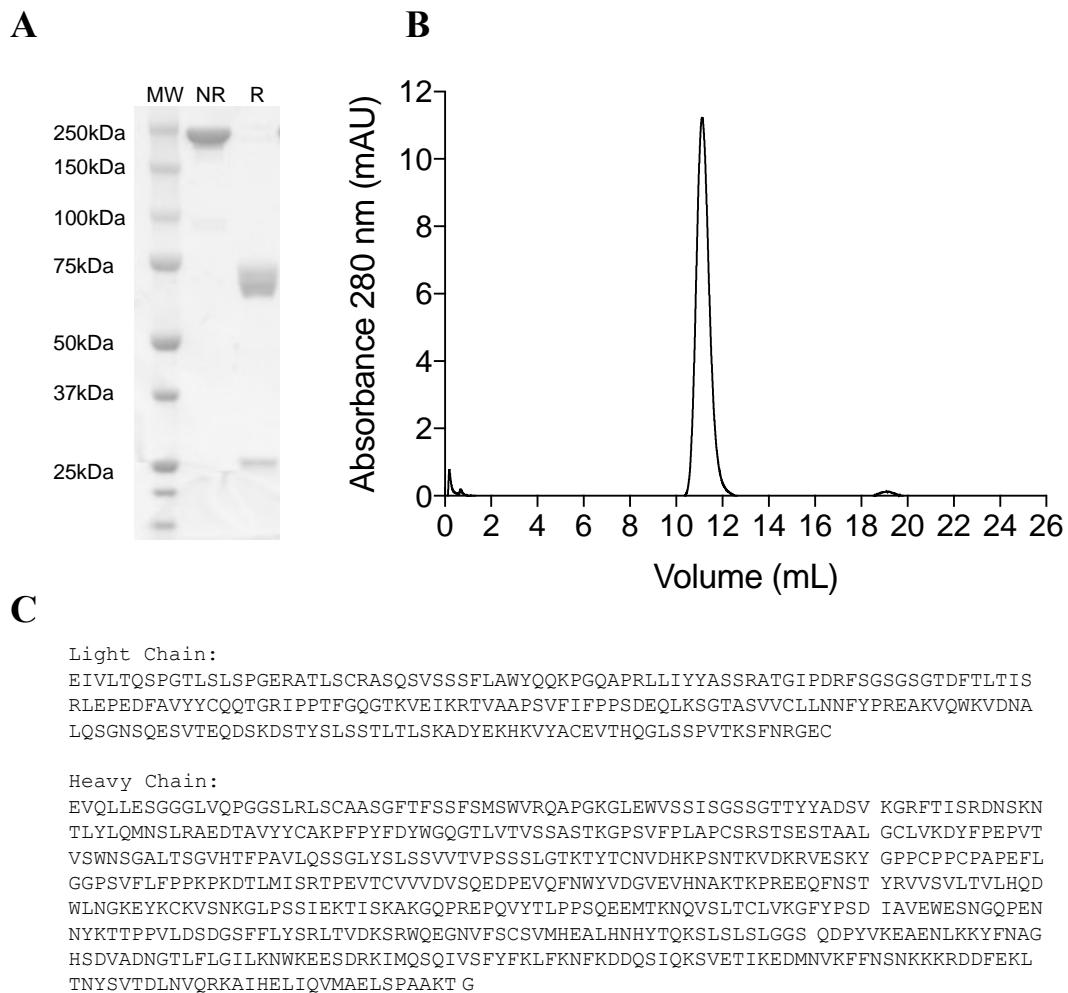


Figure S1 L19-IFNg TG biochemical characterization and amino acid sequence
(A) SDS-page, 10% gel in reducing (R) and non-reducing (NR) conditions. **(B)** Size exclusion chromatography profile. **(C)** Amino acid sequence.

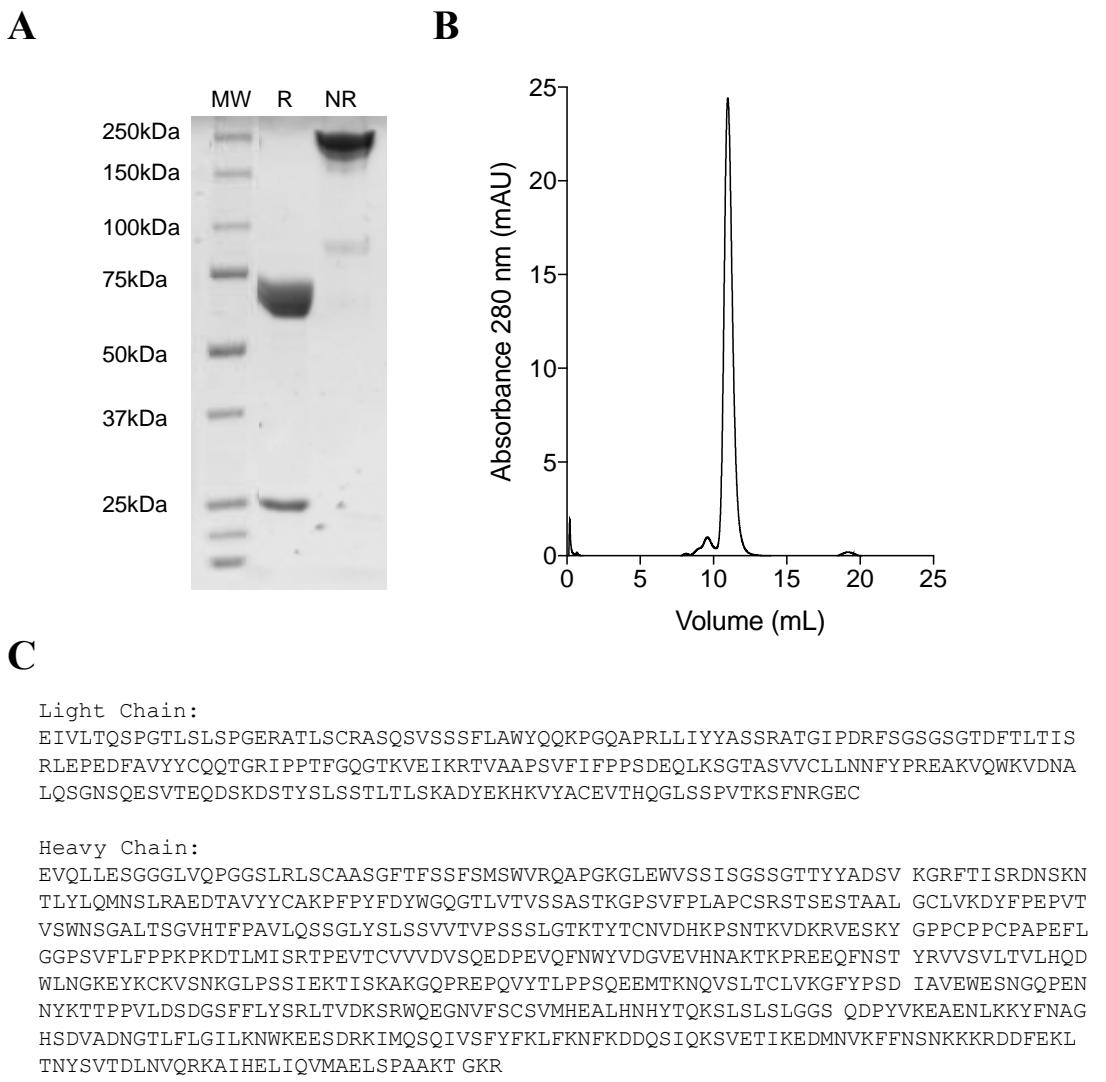
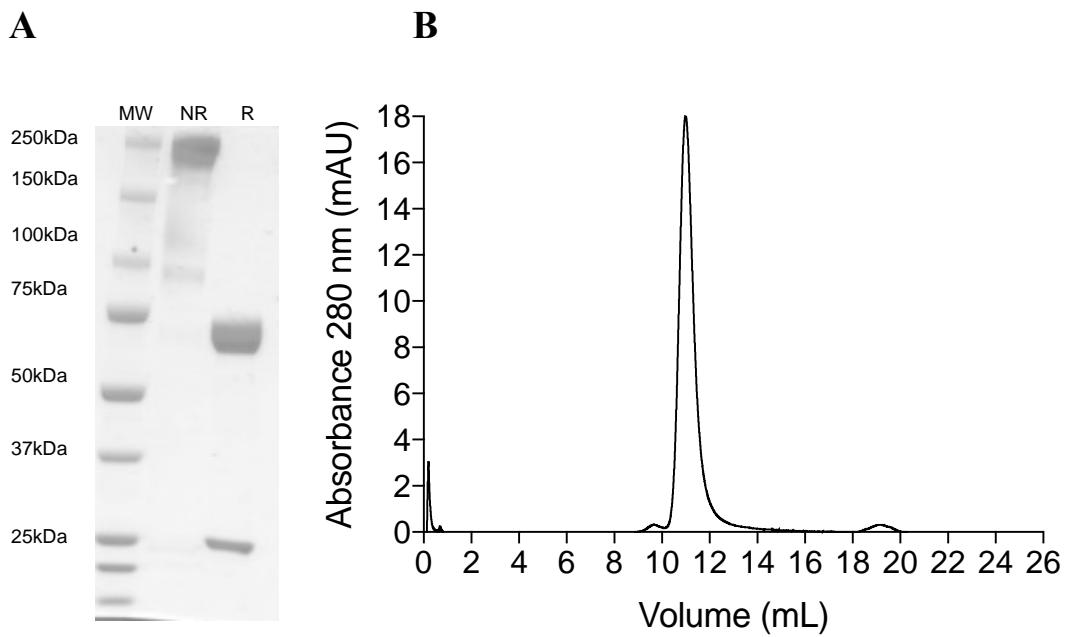


Figure S2 L19-IFNg KR biochemical characterization and amino acid sequence
(A) SDS-page, 10% gel in reducing (R) and non-reducing (NR) conditions. **(B)** Size exclusion chromatography profile. **(C)** Amino acid sequence.

Light Chain:
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTIS
RLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRC

Heavy Chain:
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWSSISGSSGTTYYADSV KGRFTISRDNSKN
TLYLQMNSLRaedtAVYYCAKPFPYFDWGGTlTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPV
VSWNSGALTSGVHTFPavLQSSGLYSLSSVTVPSSSLGTKTTCNVDHKPSNTKVDKRVESKY GPPCPCCPAPEFL
GGPSVFLFPPPKDLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQD
WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD IAVEWESNGQOPEN
NYKTTPPVLDSDGSFFLYSRlTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLISLGGS QDPYVKEAENLKKYFNAG
HSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKLFKNFKDDQSIQKSVETIKEDMNVKFFNSNKKRDDFEKL
TNYSVTDLNVQRKAIHELIQVMAELSPAAGTGKRG

Figure S3 L19-IFNg KRG amino acid sequence



C

Light Chain:
 EIVLTQSPGTLSLSPGERATLSCRASQS VSSSSFLAWYQQKPGQAPRLLI YYASSRATGIPDRFSGSGSGTDFTLTIS
 RLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKRTVAAPS VFI FPPSDEQLKSGTASV C LNNFY PREAKVQWKVDNA
 LQSGNSQESVTEQDSKD STYLSSTLTL SKADYEKHKVY ACEVTHQGLSSPVT KSFNR GEC

Heavy Chain:
 EVQLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYYADSV KGRFTISRDNSKN
 TLYLQMNSLRAEDTAVYYCAKPF PYFDYWGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVT
 VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKT YTCNVDHKPSNTKVDKRVESKY GPPCPCPAPEFL
 GGPSVFLFPPPKPDKTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQD
 WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD IAVEWESNGOPEN
 NYKTPPVLDSDGSFFLYSRLTVDKSRWQE GNVFCSVMHEALHNHTQKSLSL LGGS QDPYVKEAENLKKYFNAG
 HSDVADNGTLFLGILKNWKEESDRKIMQS QIVSFYFKLFKNFKDDQSIQKS VETIKEDMNVKFFNSNKKRDDFEKL
 TNYSVTDLNVQRKAIHELIQVMAELSPA AKT GKRKRSQMLFRG

Figure S4 L19-IFNg 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.

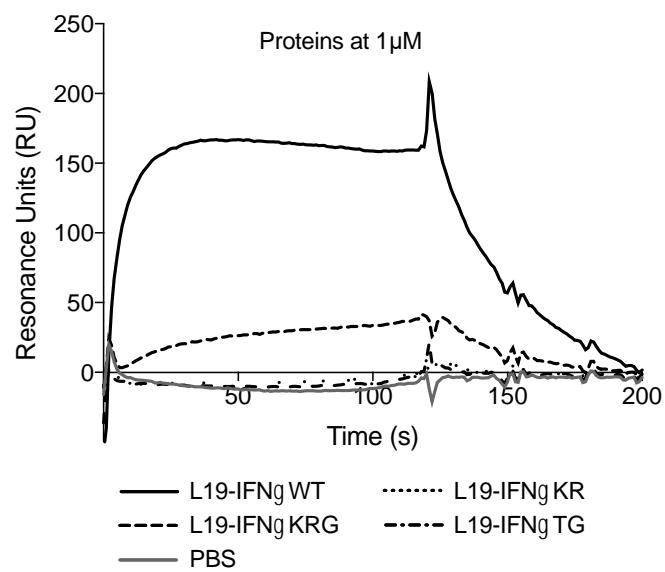
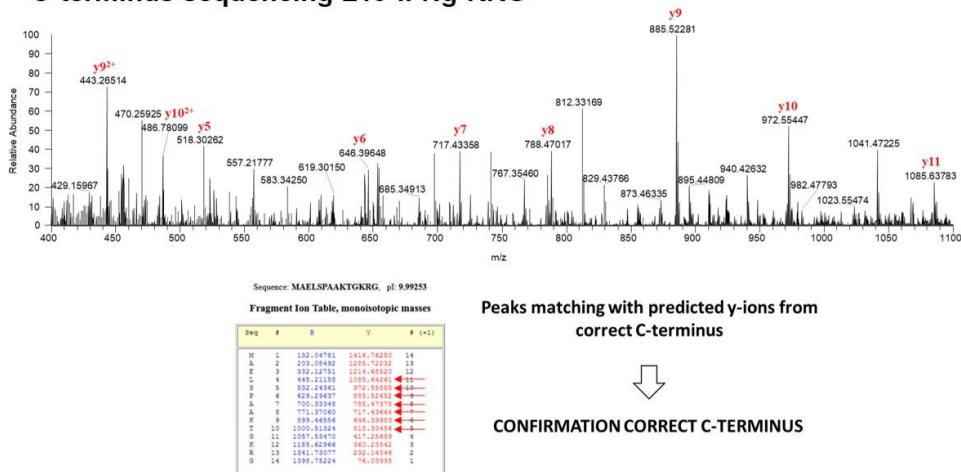


Figure S5 SPR analysis on IFNgR1 CM5 coated chip

Proteins at 1 μ M were analyzed on an IFNgR1 CM5 coated chip.

A C-terminus sequencing L19-IFNg-KRG



B C-terminus sequencing L19-IFNg-KR

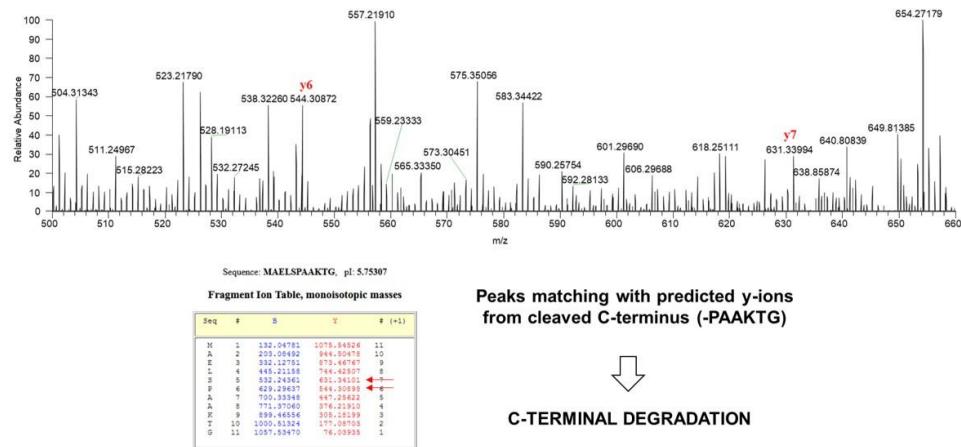


Figure S6 C-terminus sequence confirmation by Intact Mass Analysis

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.

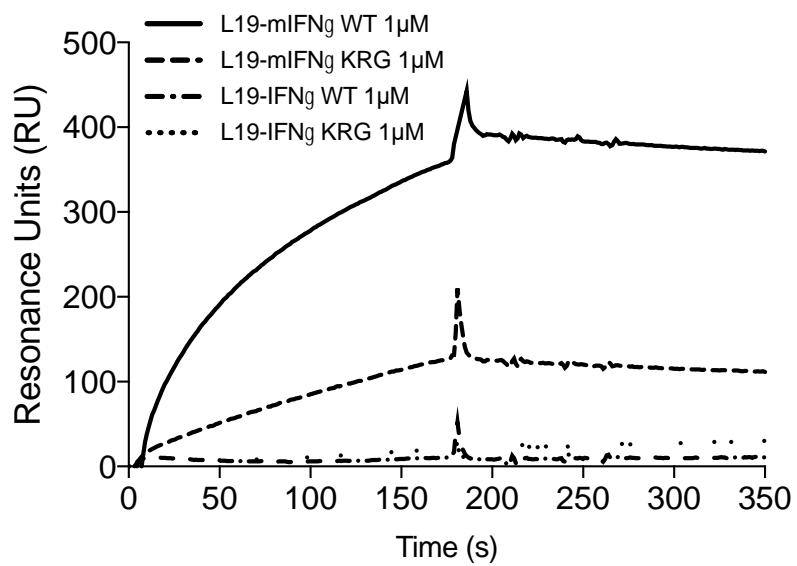


Figure S7 SPR analysis on mIFNgR1 CM5 coated chip

Proteins at 1 μ M were analyzed on a mIFNgR1 CM5 coated chip.

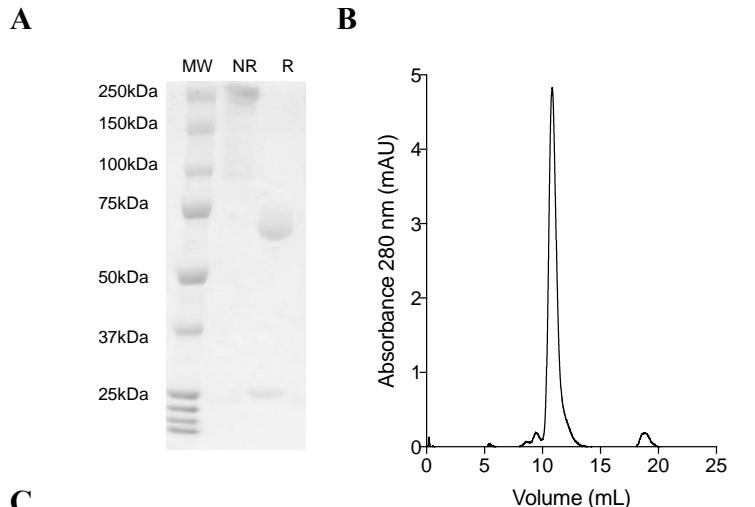
Light Chain:

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTIS
RLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

Heavy Chain:

EVQLLESGGGLVQPGGLRLSCAASGFTFSSFSMSVRQAPGKLEWVSSISGSSGTTYYADSV KGRFTISRDN SKN
TLYLQMNSLRAEDTAVYYCAKPF PYFDYWGQGT LTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPV
VSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKY GPPCP PCPAPEFL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN A KTPREEQFNST YRVVSVLTVLHQD
WLNGKEYKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD IAVEWE SNGQ PEN
NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSL LG GSGGGGSGGGGGGGGGGG
HGT VIESLESLN NYFNSSGIDVEEKSLFLDIWRNWQKDGDMKILQS QIISFYLRLFEVLKD NQAISNNISVIESHLI
TFFSNSKA KKDAFMSIAKFEVNNPQVQRQAFNELIRV VHQLLPESSLRKRG

Figure S8 L19-mIFNg KRG amino acid sequence



C

Light Chain:
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYASSRATGIPDRFSGSGSGTDFLTIS
RLEPEDFAVYYCQQQTGRIPPTFGQQGTKEVIRKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy Chain:
EVOLLESGGGLVQPGGSRLSCAASGFTFSFFSMSWVQAPGKGLEWVSSISGSSGTYYADSV KGRFTISRDN SKN
TLYLQMNSLRAEDTA VVYCAKPFYFWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAAL GCLVKDYFPEPVT
VSNNSGALTSGVHTP AVLQSSGLYSLSSVTVFSSSLG TKTYYTCNVDHKP SNTKVDKR VESKY GPPCPPCPA PEF L
GGPSVFLFPPKPKDTLMISRTPEVTCVV DVQS QEDP EVQFNWYV DGV EHVNAKTKPREEQFN ST YRVV SVL VLHQD
WLNGKEYKCKVS NKG LPSI EKTIS KAKG P REPOV YLPPS QEEEMT KQV S LTCL VKGF YPSD IAEWE SNG QPEN
NYKTPVFLSDGSF FLYS RLTV DKSR WQEG NVFC S VMHE ALHN HYTQ KSL SLS LG GS GGG GSGGG GGG GGG GGG
HGT VIES LESL NN YF NSS GID VE EKSL FLDI WRN WQ KGD MKI LQ S QII SFY RL FEV LKD NQ A IS NN S VIE SHLI
TTFFSNSKA KKDA FMSIA KFEVNNPQ VQR QAFN EL IRV VHQLL PESS LRKR KRS RS

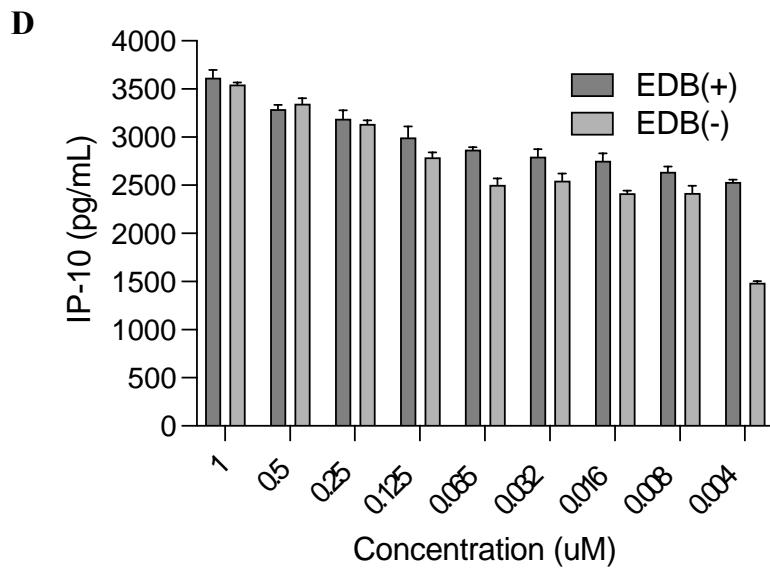


Figure S9 L19-mIFNg WT biochemical characterization and amino acid sequence

(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-mIFNg KRG in coated EDB(+) and non-coated EDB(-) wells.

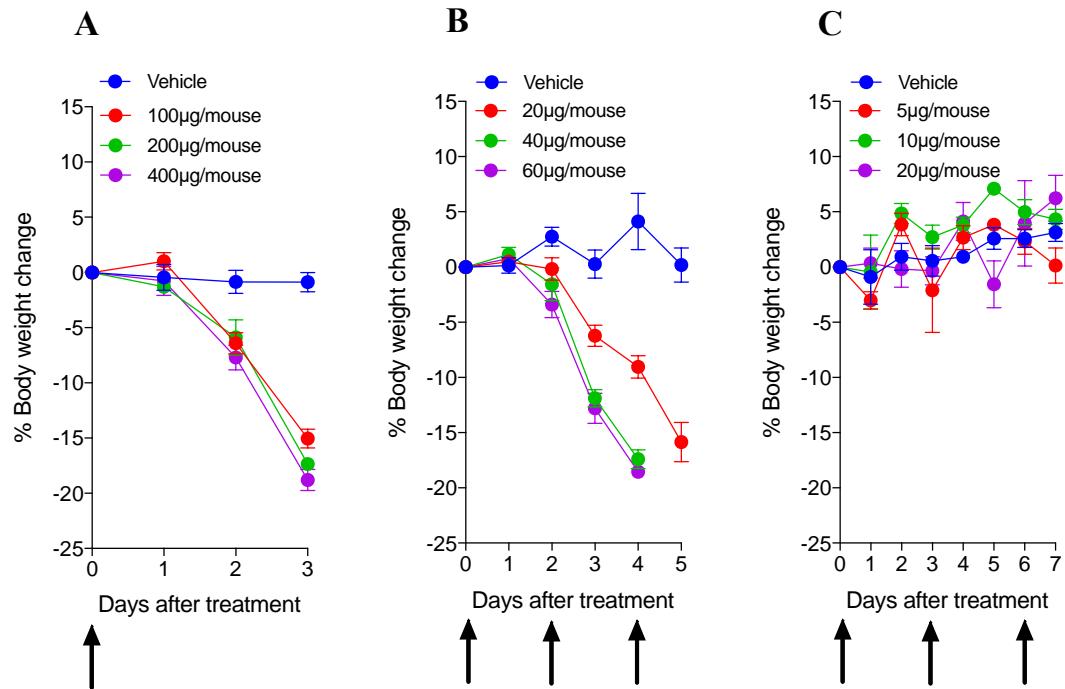


Figure S10 Dose escalation study

WEHI-164 tumor bearing mice intravenously injected at the following dose and schedules: (A) 400µg, 200µg, and 100µg with a single injection. (B) 60µg, 40µg and 20µg every 48h. (C) 20µg, 10µg and 5µg 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.

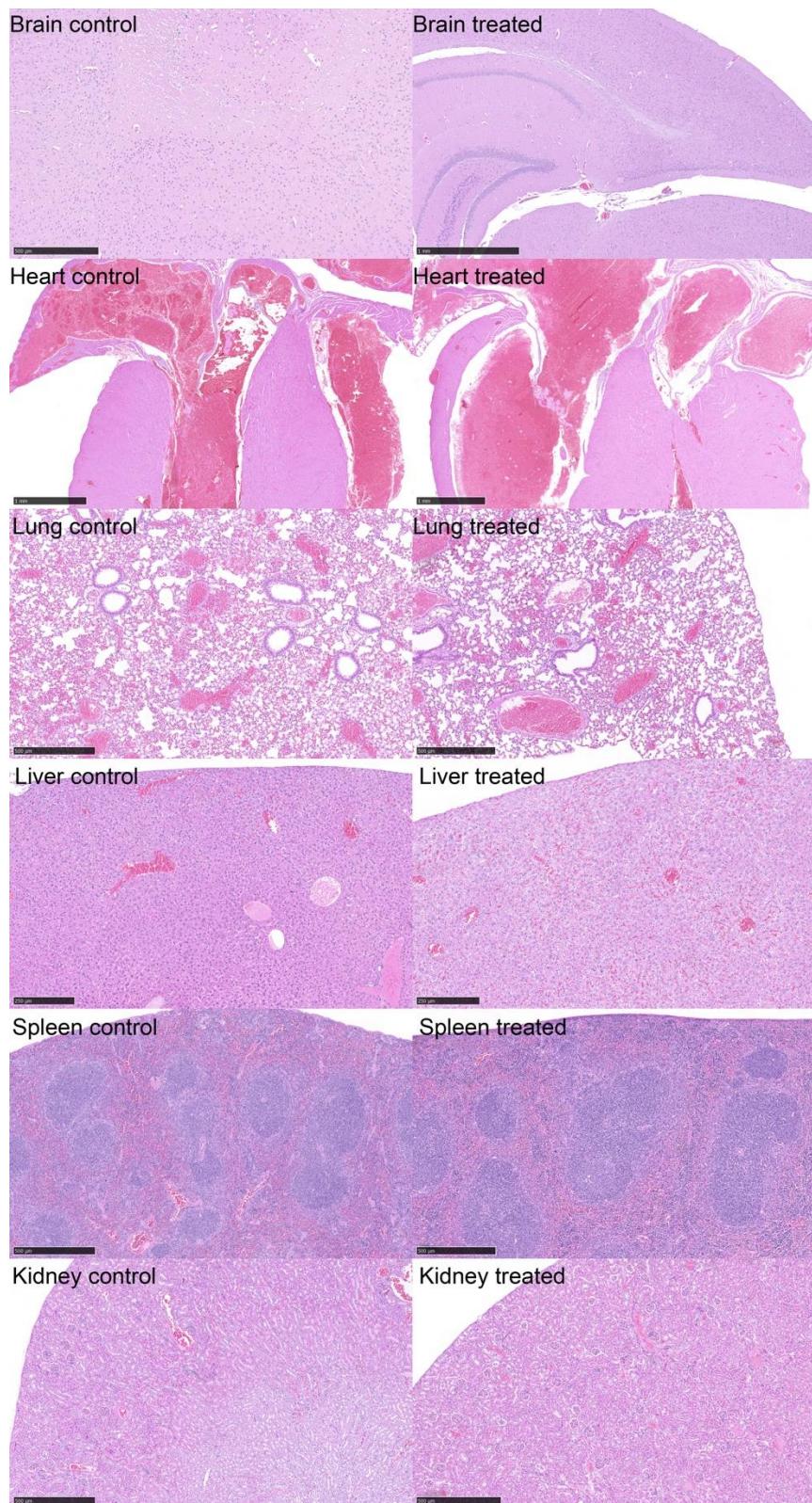
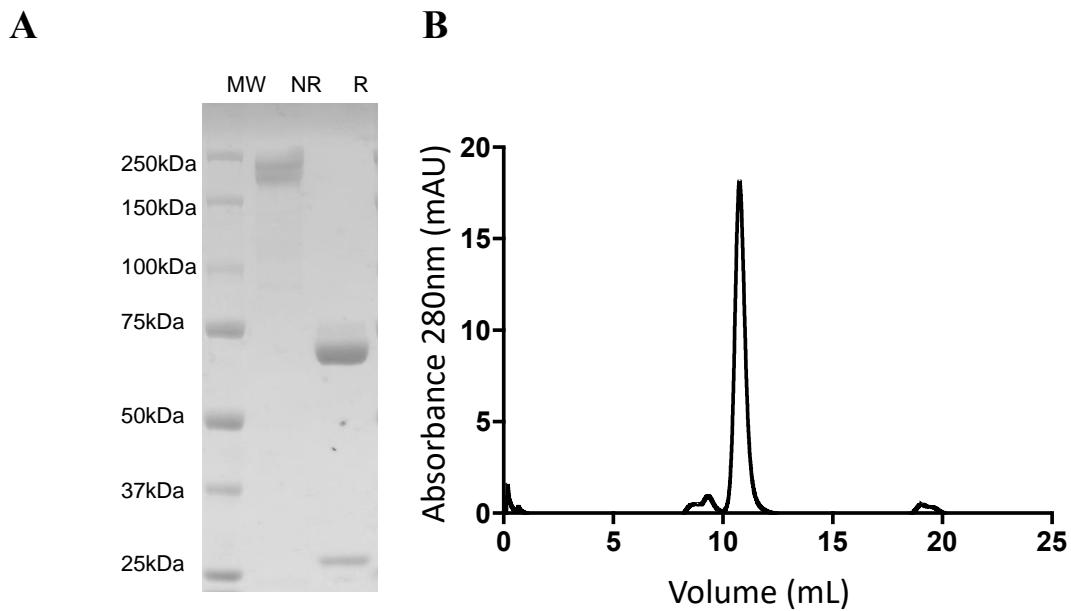


Figure S11 Histopathological analysis on tumor bearing mice

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



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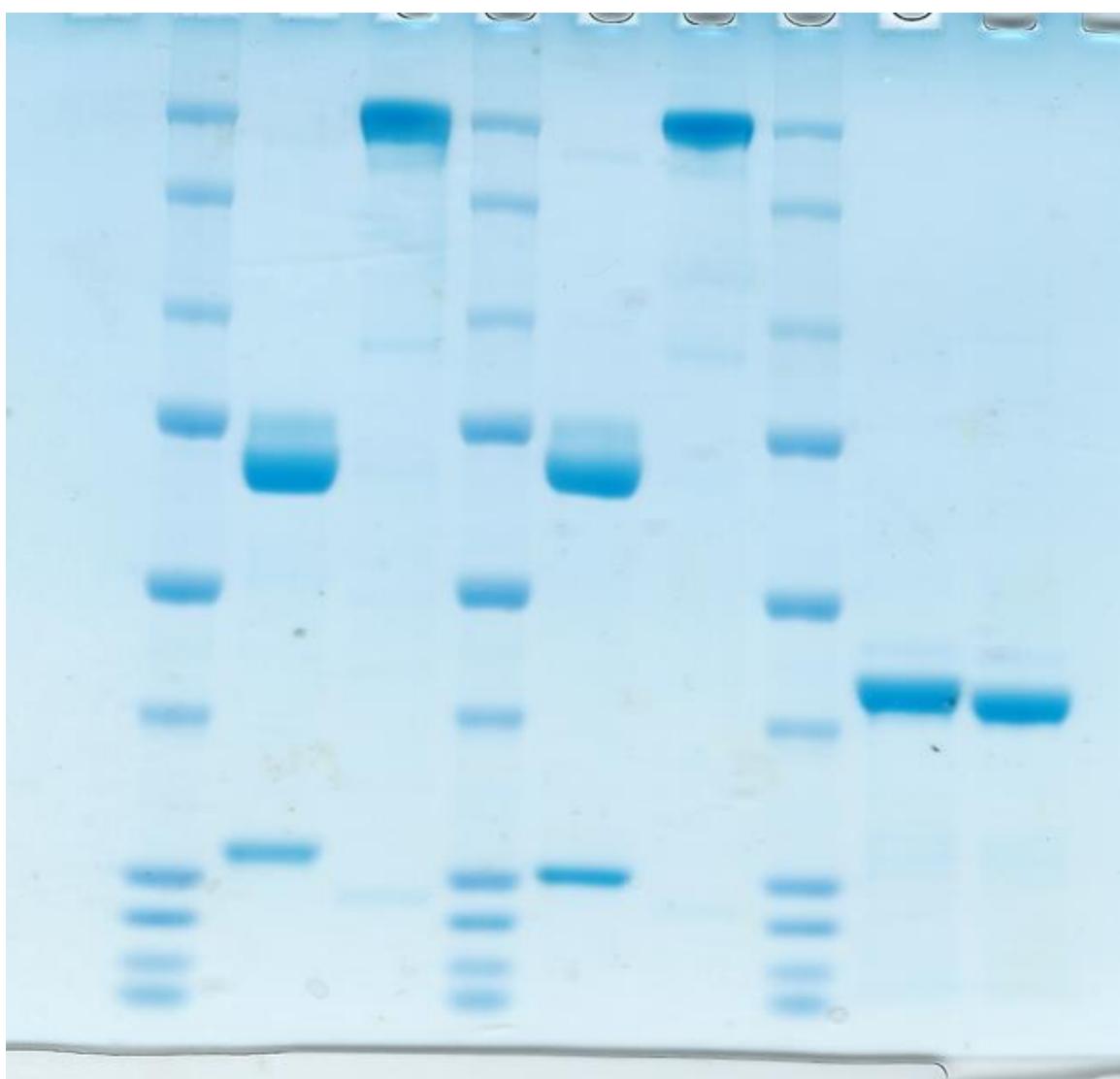
Light Chain:
 SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVVLVIYGKNNRPSGIPDRFSGSSGNTASLTITGA
 QAEDeadYYCQSSPLNRLAVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVLCLISDFYPGAVTVAWKADS
 SPVKAGVETTPSKQSNNKYAASSYSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS

Heavy Chain:
 EVQLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKLEWVAISGSGGSTYYADSV KGRFTISRDN SKN
 TLYLQMSLRAEDTA VYYCAKSPKVSLFDYWGQGT LTVSSASTKGPSVFPLAPCSRSTSESTA ALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSLGTKT YTCNVDHKPSNTKVDKRVES KYGPPCP PCPAPE
 FLGGPSVFLFPPPKD TLMISRTPEVTCVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFM STYRVVSVLTVLH
 QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP SDIAVEWE SNGQP
 ENNYKTTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSC SVMHEALHNHTQKSLSL LGGS GGG GSGHGT
 VIESLESLN NYFNSSGIDVEEKSLFLDIWRNWQKDGMKILQS QIISFYLR LF E VLKD N QAI SNN VI
 SH LITTFFSNSKA KKDA FM SI AKFEVNNPQVQRQAFNELIRV VHQLL PESS LRKRG

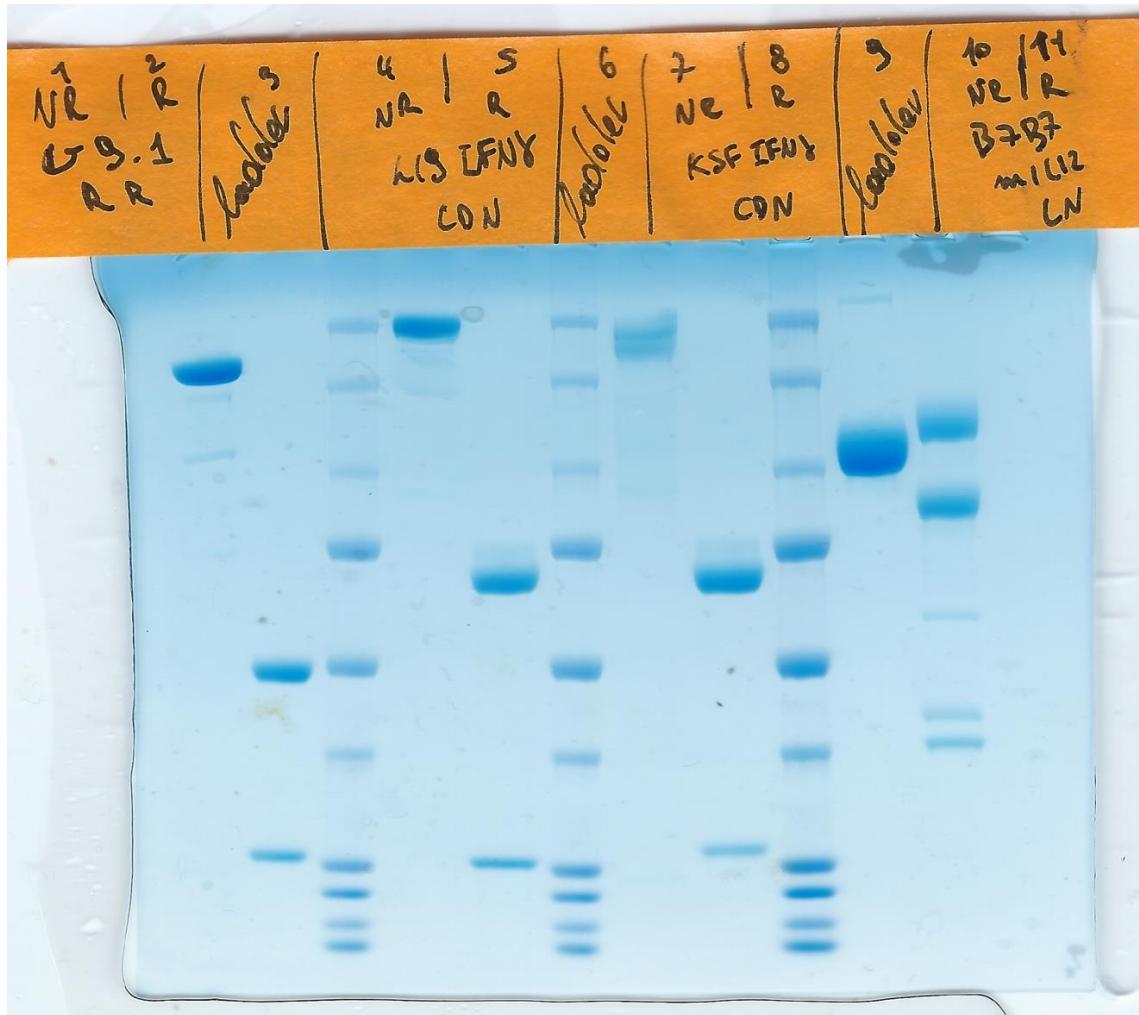
Figure S12 KSF-mIFNg KRG biochemical characterization and amino acid sequence

(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-mIFNg KRG Figure 3 non-reducing condition / L19-mIFNg 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