A

nanobody-MFG-E8-hIFN-β amino acid sequence

MFG-E8 signal sequence | FLAG peptide | anti-spike nanobody

MPRPRLLAALCGALLCAPSLLVADYKDDDDKDVQLQESGGGLVQPGGSLRLSCAASGLTLDYYAIGWFRQAPGKEREGVS

CISSSDGSTYYADSVKGRFTTSRDNAKNTVYLQMNSLKPEDTAVYYCAAVPSTYYSGTYYYTCHPGGMDYWGKGTQVTVS

linker | human MFG-E8(D48E)

SGGGGSLDICSKNPCHNGGLCEEISQEVRGEVFPSYTCTCLKGYAGNHCETKCVEPLGLENGNIANSQIAASSVRVTFLG

LQHWVPELARLNRAGMVNAWTPSSNDDNPWIQVNLLRRMWVTGVVTQGASRLASHEYLKAFKVAYSLNGHEFDFIHDVNK

KHKEFVGNWNKNAVHVNLFETPVEAQYVRLYPTSCHTACTLRFELLGCELNGCANPLGLKNNSIPDKQITASSSYKTWGL

HLFSWNPSYARLDKQGNFNAWVAGSYGNDQWLQVDLGSSKEVTGIITQGARNFGSVQFVASYKVAYSNDSANWTEYQDPR

linker | human IFN-β

TGSSKIFPGNWDNHSHKKNLFETPILARYVRILPVAWHNRIALRLELLGCGGGGSMSYNLLGFLQRSSNFQCQKLLWQLN

GRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEK

LEKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN

B

nanobody-MFG-E8-mIFN-β amino acid sequence

MFG-E8 signal sequence | FLAG peptide | anti-spike nanobody

MPRPRLLAALCGALLCAPSLLVADYKDDDDKDVQLQESGGGLVQPGGSLRLSCAASGLTLDYYAIGWFRQAPGKEREGVS

CISSSDGSTYYADSVKGRFTTSRDNAKNTVYLQMNSLKPEDTAVYYCAAVPSTYYSGTYYYTCHPGGMDYWGKGTQVTVS

linker | human MFG-E8(D48E)

SGGGGSLDICSKNPCHNGGLCEEISQEVRGEVFPSYTCTCLKGYAGNHCETKCVEPLGLENGNIANSQIAASSVRVTFLG

LQHWVPELARLNRAGMVNAWTPSSNDDNPWIQVNLLRRMWVTGVVTQGASRLASHEYLKAFKVAYSLNGHEFDFIHDVNK

KHKEFVGNWNKNAVHVNLFETPVEAQYVRLYPTSCHTACTLRFELLGCELNGCANPLGLKNNSIPDKQITASSSYKTWGL

HLFSWNPSYARLDKQGNFNAWVAGSYGNDQWLQVDLGSSKEVTGIITQGARNFGSVQFVASYKVAYSNDSANWTEYQDPR

linker | mouse IFN-β

TGSSKIFPGNWDNHSHKKNLFETPILARYVRILPVAWHNRIALRLELLGCGGGGSNYKQLQLQERTNIRKCQELLEQLNGKINL

TYRADFKIPMEMTEKMQKSYTAFAIQEMLQNVFLVFRNNFSSTGWNETIVVRLLDELHQQTVFLKTVLEEKQEERLTWEMSST

ALHLKSYYWRVQRYLKLMKYNSYAWMVVRAEIFRNFLIIRRLTRNFQN

C

nanobody-hIFN-β amino acid sequence

MFG-E8 signal sequence | FLAG peptide | anti-spike nanobody

MPRPRLLAALCGALLCAPSLLVADYKDDDDKDVQLQESGGGLVQPGGSLRLSCAASGLTLDYYAIGWFRQAPGKEREGVS

CISSSDGSTYYADSVKGRFTTSRDNAKNTVYLQMNSLKPEDTAVYYCAAVPSTYYSGTYYYTCHPGGMDYWGKGTQVTVS

linker | human IFN-β

SGGGGSMSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQDSSS

TGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLT

GYLRN

D

nanobody-mIFN-β amino acid sequence

MFG-E8 signal sequence | FLAG peptide | anti-spike nanobody

MPRPRLLAALCGALLCAPSLLVADYKDDDDKDVQLQESGGGLVQPGGSLRLSCAASGLTLDYYAIGWFRQAPGKEREGVS

CISSSDGSTYYADSVKGRFTTSRDNAKNTVYLQMNSLKPEDTAVYYCAAVPSTYYSGTYYYTCHPGGMDYWGKGTQVTVS

linker | mouse IFN-β

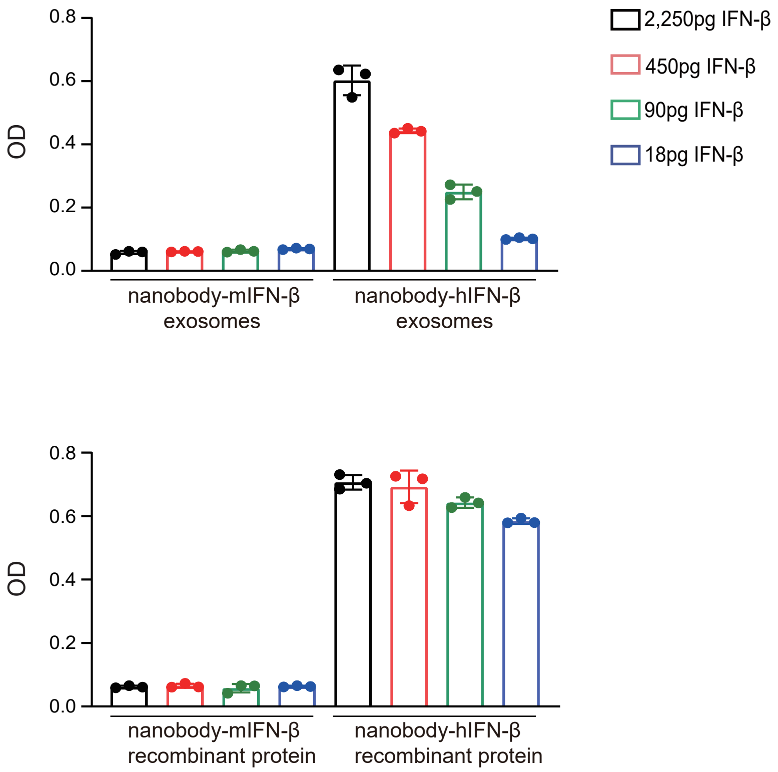
SGGGGSNYKQLQLQERTNIRKCQELLEQLNGKINLTYRADFKIPMEMTEKMQKSYTAFAIQEMLQNVFLVFRNNFSSTGWN

ETIVVRLLDELHQQTVFLKTVLEEKQEERLTWEMSSTALHLKSYYWRVQRYLKLMKYNSYAWMVVRAEIFRNFLIIRRLTRNF

QN

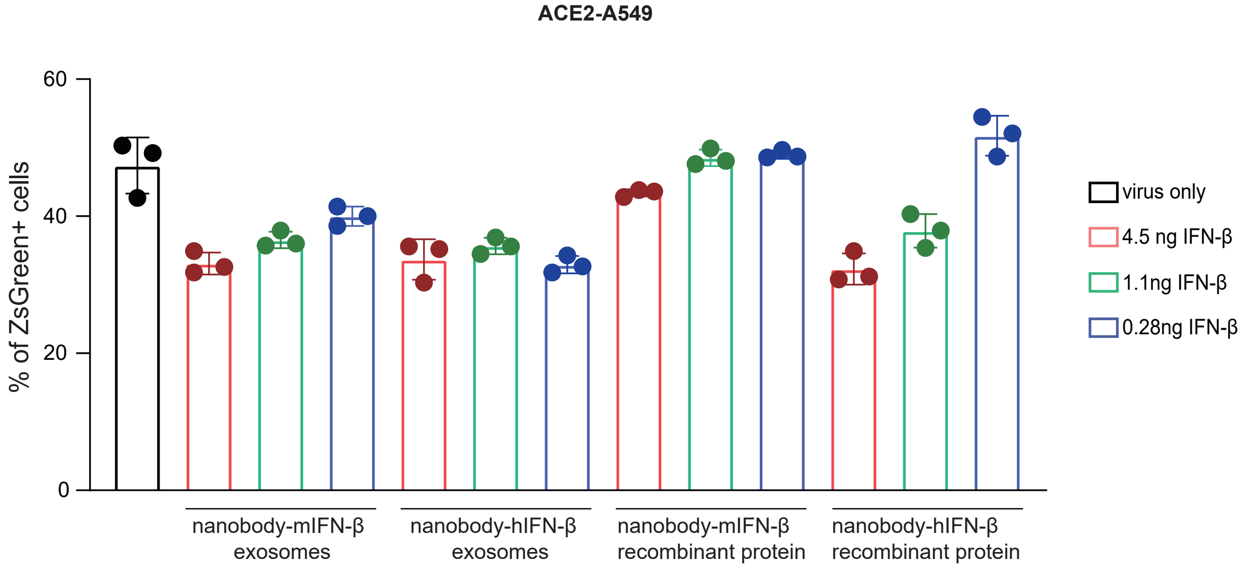
Supplemental Figure1. Amino acid sequences of the nanobody-MFG-E8-IFN-β and the soluble nanobody-IFN-β recombinant proteins

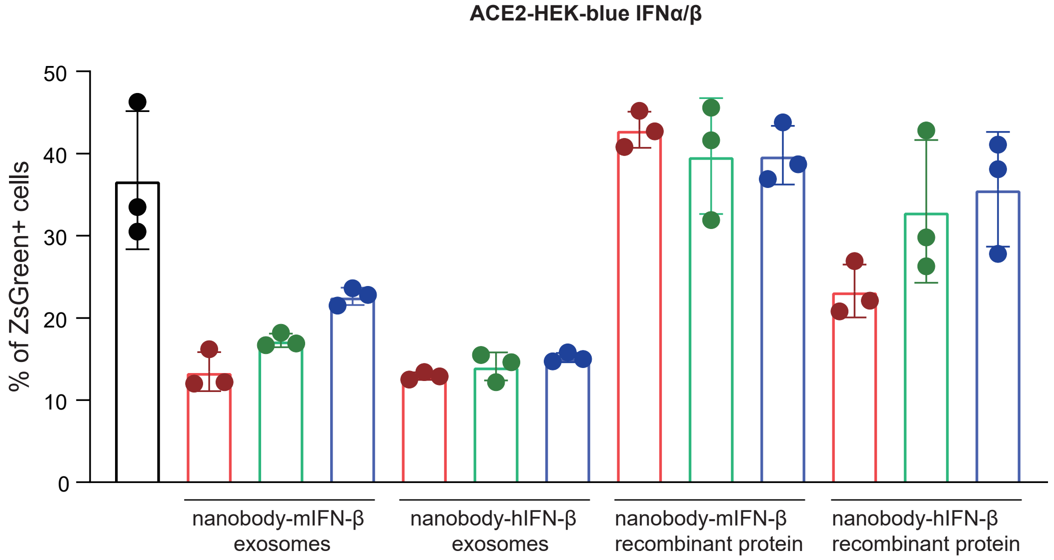
(A) The amino acid sequence of anti-spike nanobody-MFG-E8-hIFN-β. (B) The amino acid sequence of anti-spike nanobody-MFG-E8-mIFN-β. (C) The amino acid sequence of soluble anti-spike nanobody-hIFN-β recombinant protein. (D) The amino acid sequence of soluble anti-spike nanobody-mIFN-β recombinant protein.



Supplemental Figure2. Bioactivity of the engineered exosomes and the soluble nanobody-IFN-β recombinant proteins

The serial dilutions of the engineered exosomes or soluble nanobody-IFN-β recombinant proteins were applied to the HEK-blue IFNα/β cells. After 24 h, the IFN-induced secreted embryonic alkaline phosphatase (SEAP) level was determined using a spectrophotometer at 650 nm.





Supplemental Figure3. Anti-viral efficiency of the engineered exosomes

The anti-viral effect of high dose (containing 4.5ng IFN-β), middle dose (containing 1.1ng IFN-β), and low dose (containing 0.28ng IFN-β) of nanobody-IFN-β conjugated exosomes or soluble nanobody-IFN-β recombinant proteins were applied in the infection assay. The percentage of ZsGreen positive cells was analyzed by flow cytometry.