Sequence Motif	E (i) and K (i+n) position in sequence
EK (i, i+1)	E – K– E – K i i+1 i i+1
EKX (i, i+1)	E – K – X – E – K – X i i+1 i i+1
EEKK (i, i+2)	E – E – K – K – E – E – K – K i i+2 i i+2
EXKX (i, i+2)	E – X – K – X – E – X – K – X i i+2 i i+2

Table S1. Sequence motif nomenclature. Sequence motifs EK(i,i+1), EKX(i,i+1), EEKK(i,i+2), and EXKX(i,i+2) with depictions of i and i+n to define amino acid positions and distances between charged residues.

Peptide	Full Peptide Sequence
EK	EKEKEKEKEKEK
EEKK	EEKKEEKKEEKK
EKP	EKPEKPEKPEKP
EPKP	EPKPEPKPEPKP
EKS	EKSEKSEKSEKS
ESKS	ESKSESKSESKS
EKG	EKGEKGEKGEKG
EGKG	EGKGEGKGEGKG

Table S2. Short i,i+1 and i,i+2 EK peptides containing P, S, and G. Twelve amino acid zwitterionic peptide sequences conjugated synthesized by solid phase peptide synthesis conjugated to KLH and BSA. KLH conjugates were used for injection into mice, and BSA conjugates were used for detection of anti-peptide antibodies.

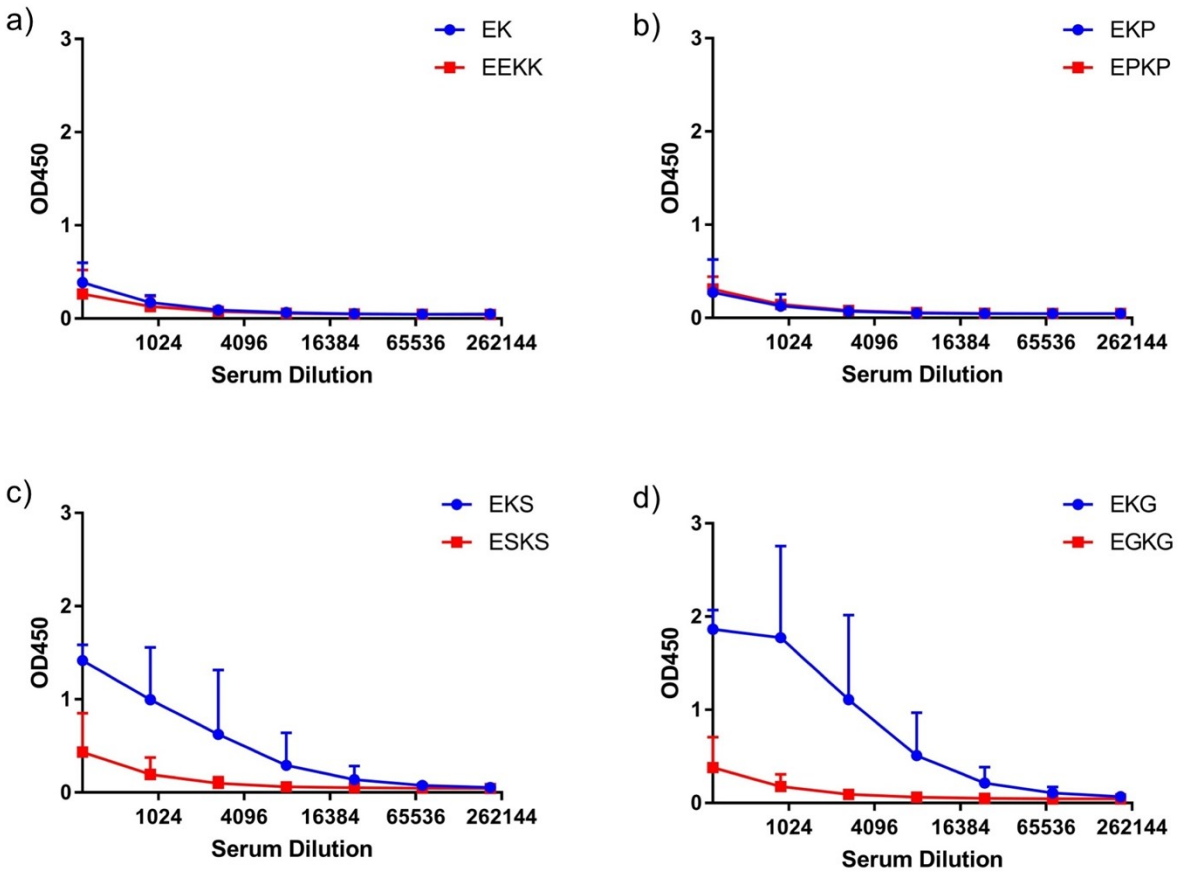

Fig. S1. OD450 signals detecting anti-peptide IgG from KLH-peptide injected mice. Signals were detected for the indicated serum dilutions. **(A)** EK (i,i+1) and EEKK (i,i+2), **(B)** EKP (i,i+1) and EPKP (i,i+2), **(C)** EKS (i,i+1) and ESKS (i,i+2), and **(D)** EKG (i,i+1) and EGKG (i,i+2).

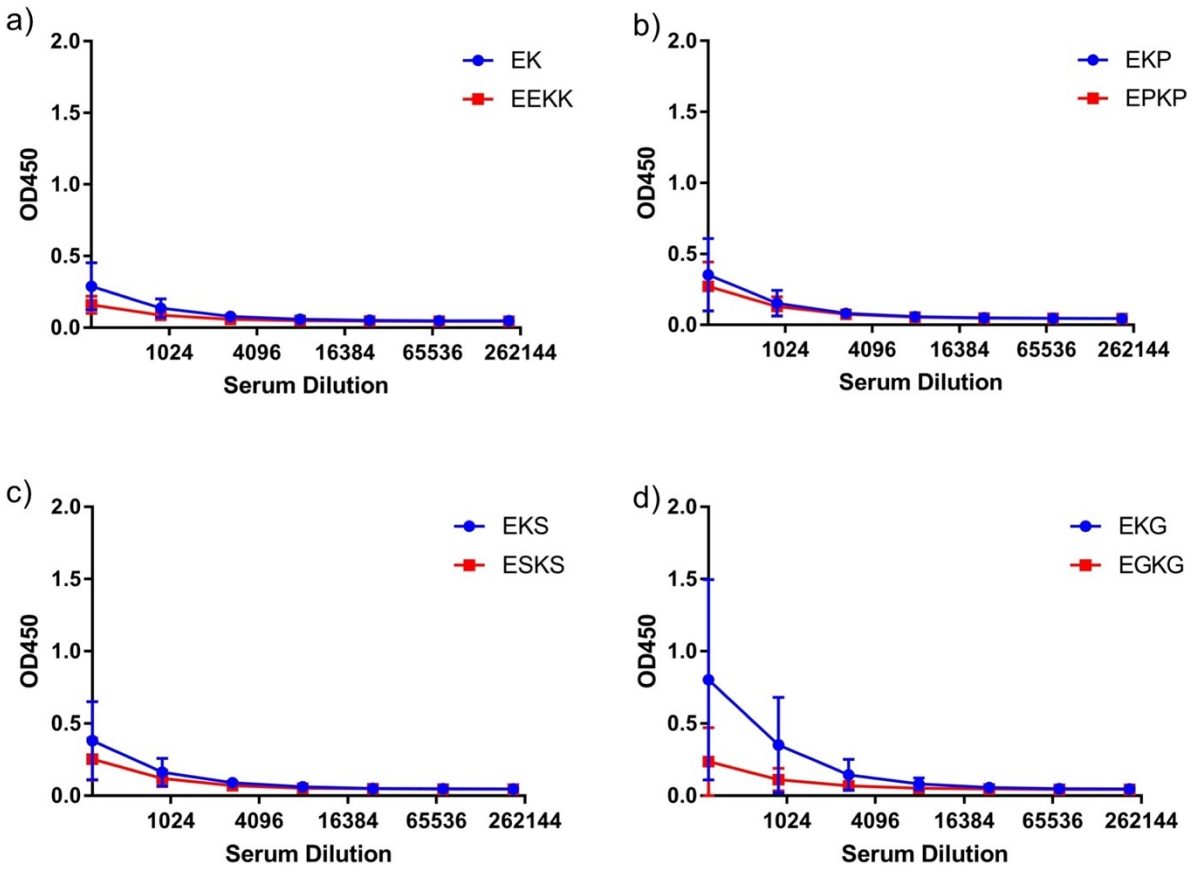

Fig. S2. OD450 signals detecting anti-peptide IgM from KLH-peptide injected mice. Signals were detected for the indicated serum dilutions. **(A)** EK (i,i+1) and EEKK (i,i+2), **(B)** EKP (i,i+1) and EPKP (i,i+2), **(C)** EKS (i,i+1) and ESKS (i,i+2), and **(D)** EKG (i,i+1) and EGKG (i,i+2).

	Percent Stable Structure	H_2O H-Bond per Residue
EK	0.72	4.68
EEKK	0.62	4.67
EKP	0.08	4.26
EPKP	0	3.83
EKS	0.68	3.90
ESKS	0.58	3.44
EKG	0.29	3.89
EGKG	0.28	3.38

Table S3. Structural and hydrogen bonding patterns for i,i+1 and i,i+2 EK peptide motifs containing P, S, and G. Percent stable structure (non-random coil structure) as well as average hydrogen bonds from water per residue calculated from peptide simulations.

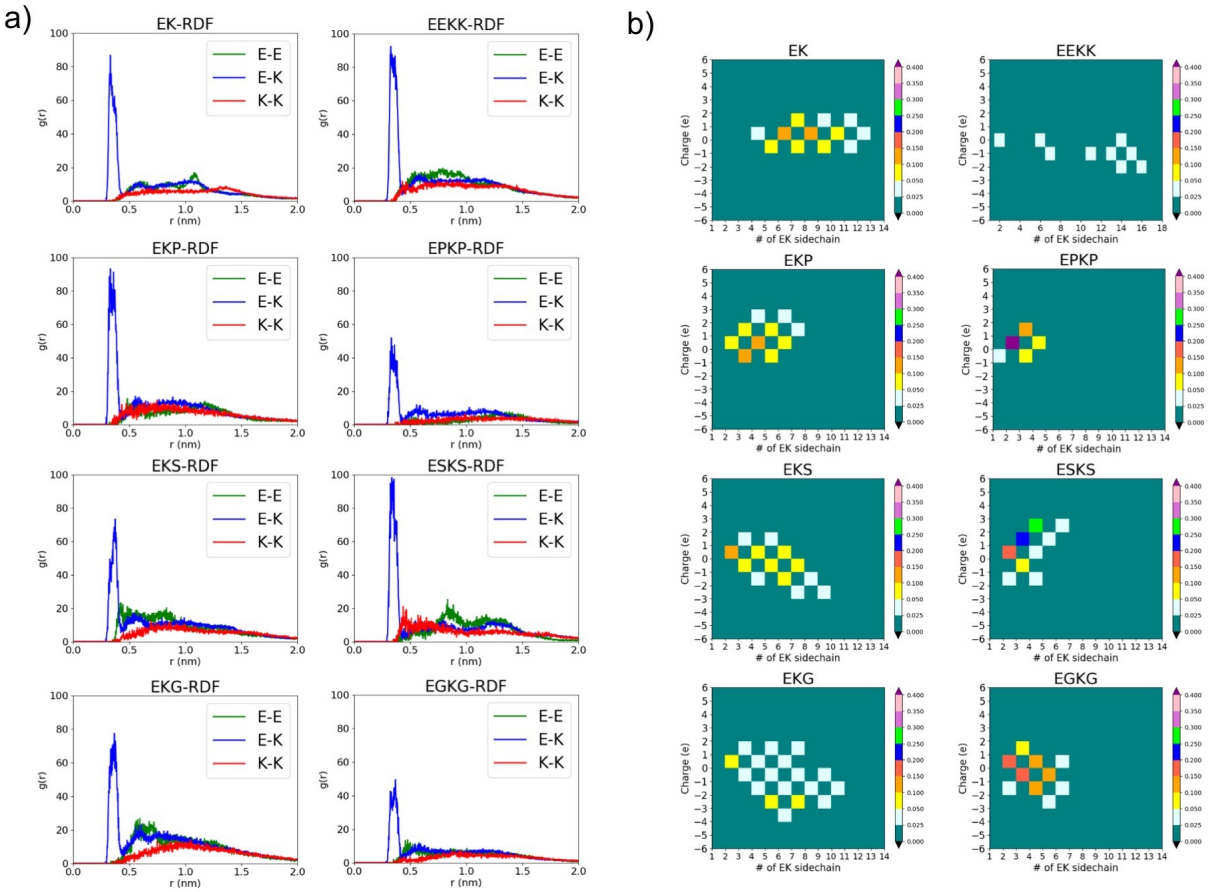

Fig. S3. Charge distribution and charge clustering behavior of i,i+1 and i,i+2 EK peptide motifs containing P, S, and G. (A) Radial Distribution Functions (RDF) of modeled peptides examining the radial distances between charged atoms on the side chains of glutamic acid and lysine residues (E-K), between glutamic acid and glutamic acid residues (E-E), and lysine and lysine residues (K-K). For E residues, the position of C in the COO^- group is used. For K residues, the position of N in the NH_3^+ group is used. **(B)** Charge clustering plots of indicated zwitterionic peptides. The cluster is defined by the distance between C of COO^- on E side chains and N of NH_3^+ on K side chains with a threshold of 0.75 nm. The number of side chains (E or K) in the cluster and the charge of each cluster are plotted on the x and y axis respectively. The color represents the frequency of the cluster to occur.

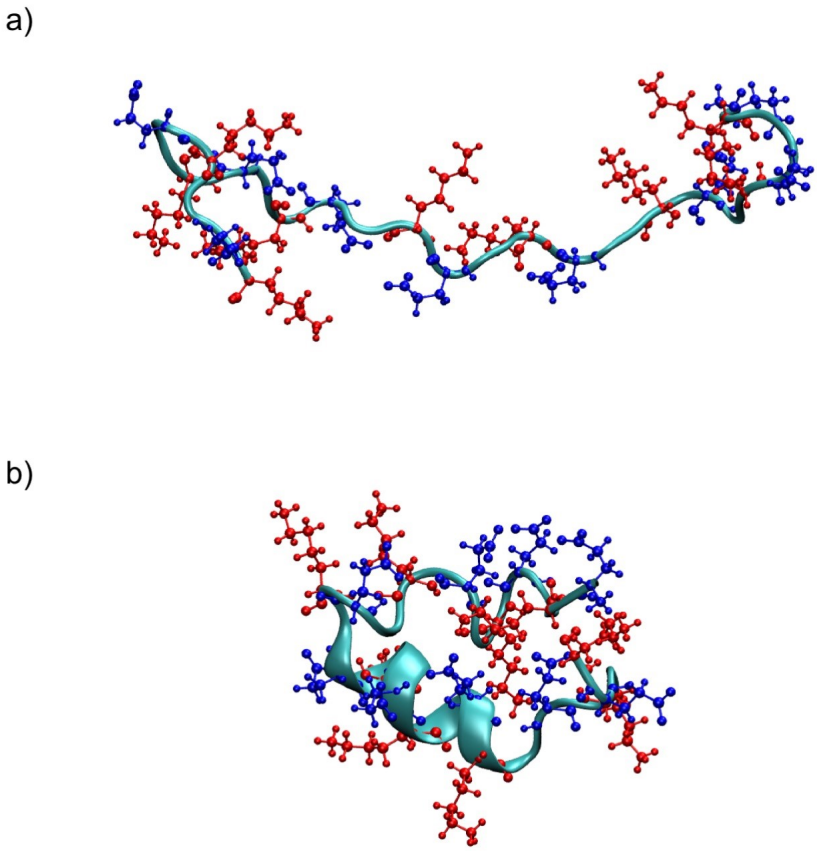

Fig. S4. Representative configurations of charge clustering events. Configurations of **(A)** EKP and **(B)** EKS showing charge clustering effects for EKS. Lysine (K) side-chains are labeled red, and glutamic acid (E) side chains are labeled blue.

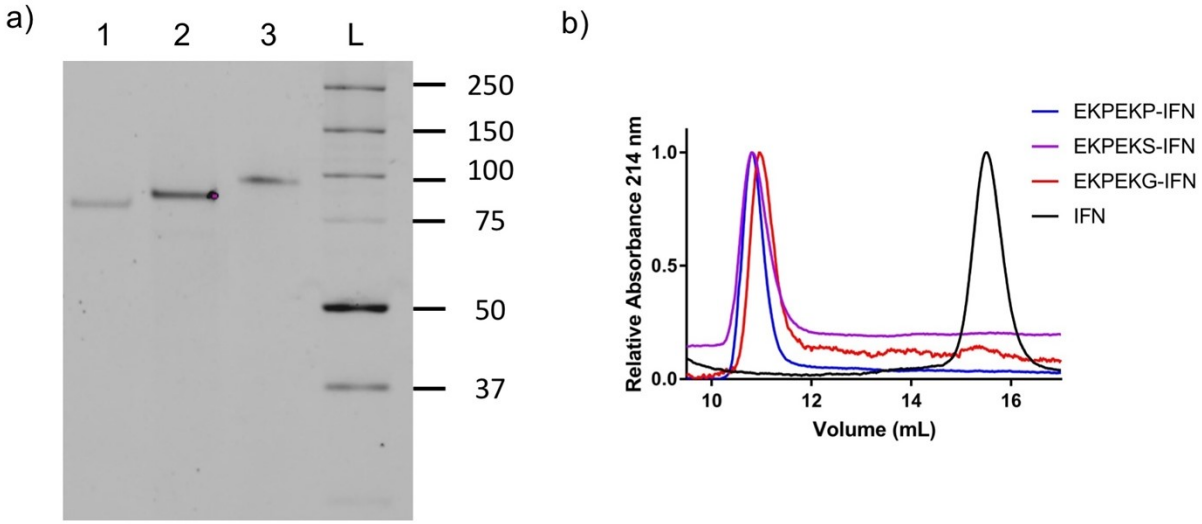

Fig. S5. Purification and biophysical properties of high molecular weight i,i+1 EK peptide motifs containing of P, S, and G fused to IFN. (A) SDS-PAGE gel of purified (1) EKPEKG-IFN, (2) EKPEKS-IFN, and (3) EKPEKP-IFN which were expressed in HEK293F cells and purified using anti-HA agarose resin. Bio-rad Precision Plus Standard was used as the molecular weight marker. **(B)** Size Exclusion Chromatography elution profile of EKPEKP-IFN, EKPEKS-IFN, EKPEKG-IFN, and IFN.

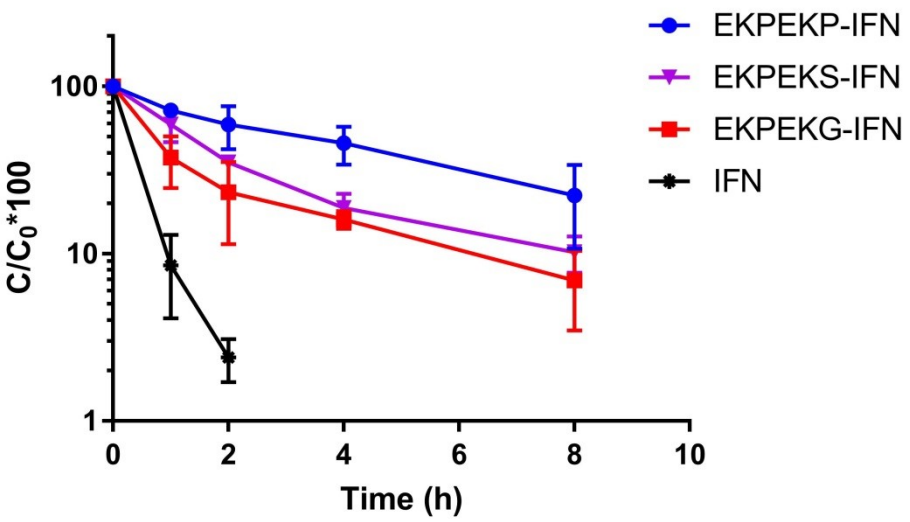

Fig. S6. Pharmacokinetics of high molecular weight i,i+1 EK peptide motifs containing of P, S, and G fused to IFN. Normalized concentration of EKPEKP-IFN, EKPEKS-IFN, EKPEKG-IFN, and IFN in the blood serum following one intravenous injection.

	$\tau_{1/2}$ (h)	AUC (C/C_0*h)	MRT (h)
IFN	0.29	41.2	0.41
EKPEKP-IFN			
Injection 1	1.72	236	2.48
Injection 3	1.73	249	2.50
EKPEKS-IFN			
Injection 1	1.47	209	2.13
Injection 3	0.81	116	1.16
EKPEKG-IFN			
Injection 1	0.89	125	1.28
Injection 3	0.61	87.4	0.88

Table S3. Pharmacokinetic parameters of high molecular weight i,i+1 EK peptide motifs containing of P, S, and G fused to IFN. Pharmacokinetic parameters following single I.V. injection of IFN and the first and third injection of EKPEKP-IFN, EKPEKS-IFN, and EKPEKG-IFN.