Time: 2024.10.05-2024.10.13

1. **Experiment:** Sequence design
2. **Time:** 2024.10.05-2024.10.13
3. **Member:** Fan Yang, Qiwen Jiang, Xinxin Zhang, Kaiqing Zhang, Meng Sun
4. **Method:**
   (1) In this study, a homologous recombination strategy was employed for the experimental design. The selected peptide sequences, namelyB0, B34, B51 and G9, G16, G35, G42, G57, G71, along with their corresponding reverse sequences, were conjugated via a flexible linker. Reverse sequences were incorporated due to the flexibility of peptide linker and the inherent directionality of protein-receptor binding. Furthermore, glycosylphosphatidylinositol a (GPI) membrane-anchoring sequence was fused to the construct to retain the IL-2 mimetics on the cell surface rather than their secretion into the extracellular space, facilitating activation of NK cells. Additionally, Flag tag was coupled with the recombinant sequences for subsequent detection and isolation purposes.
   (2) The specific sequences of the genetic elements are listed in the table below.

Table.1 β binder

β binder	Sequence
B0	MEEKLEELKKKLAELDGKYIYEKCYGTEEEAKKALEELKAALEELAKAEKEAAAA AA
B34	AAEAARRAARAAFDARLTAAERKYLAAQDDPEAAAAWLAEIAAIEAERTAAERAW AA
B51	EEERRRQIEALKRAAAAAEYEYALAKELAAKDPAYAPLAEALKAELERLKAELAAL EAA

Table.2 γ binder

γ binders	Sequence
G9	SAAAKEAAKALATALRLAGTRLFTAGAVAAKIDPAAGAALFAAGAAAFAAAAALEK ALA
G16	MSLAEAIRDAGVAAALASGDPAHLDAAKAAIAAAVSPEEAARWAAVLDEDYARAR AAAA
G35	AAEEEAERLRRAAAELAERLARAALLAALRAALAARLAANALKIAAAAAALAAAA A
G42	YLEEAVAALKKLRDDLAAQLAKAKAAADTPEMKALAAETQALLELATKQLEKAEA KLK
G57	EELARLAEELAAARREALRAELEALRREQEERLREEEEERRRREEEEK
G71	DVATLKALAAQYRAARAAVREEAARLAAAEPERAAEILAEGAALAAAFDAKAAAA AAAAAAAA

Table.3 Recombinant Elements

Name	Sequence
β-Homology Arm	AGCTGTGACCGGCGCCTACT
β-Restriction Enzyme Cutting Site	CTAGA
Signal Peptide（SP）	ATGGCCCTGTGGATGAGACTATTGCCACTGCTTGCCCTGC TCGCACTGTGGGGTCCCGATCCTGCTGCCGCA

FLAG	GACTACAAGGACGACGACGACAAG
Glycosylphosphatidylinositol（GPI）	GGAGGCACCTCTCTGTCCGAGAAGACAGTGCT GCTGCTGGTGACACCATTTCTGGCCGCCGCAT GGTCTCTGCATCCATGA
γ-Restriction Enzyme Cutting Site	GGATC
γ-Homology Arm	CGCGGCCGCTGAGGGCAGAG
BamHI	GGATCCGCTAGCCCCGGGGCGGCCG
Xbal	TTGACCTCCATAGAAGATTCTAGA

5. Result:

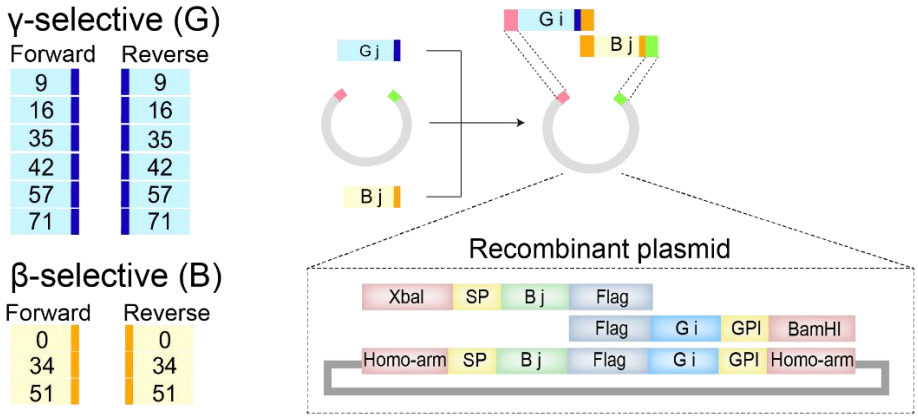

Figure.1 Construction and screening of IL-2 mimics library. Twelve IL-2Rβ binding mimics six IL-2Rγ mimics form 72 combinations.