

Biased G-protein-coupled receptor (GPCR) signaling of CCL22 chemokine mutations in chronic lymphoproliferative disorder of NK cells (CLPD-NK)

Shunsuke Kimura^{*1}, Constance Baer^{*2}, Mitra S. Rana³, Andrew Kleist^{4,5}, David J. Feith^{6,7}, Wencke Walter², Manja Meggendorfer², Thomas L. Olson^{6,7}, HeeJin Cheon^{6,7,8}, Kristine C. Olson^{6,7}, Aakrosh Ratan^{6,9,10}, Martha-Lena Mueller², James M. Foran¹¹, Laura Janke¹, Chunxu Qu¹, Ravi Kalathur³, Claudia Haferlach², Wolfgang Kern², Elisabeth Paietta¹², M. Madan Babu^{3,13}, Thomas P. Loughran Jr.^{6,7}, Ilaria Iacobucci¹, Torsten Haferlach² and Charles G Mullighan^{1,14}

¹Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA; ²MLL Munich Leukemia Laboratory, Munich, Germany; ³Protein Technologies Center, Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN, USA; ⁴Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI, USA; ⁵Medical Scientist Training Program, Medical College of Wisconsin, Milwaukee, WI, USA; ⁶University of Virginia Cancer Center, Charlottesville, VA, USA; ⁷Department of Medicine, Division of Hematology/Oncology, University of Virginia School of Medicine, Charlottesville, VA, USA; ⁸Medical Scientist Training Program, University of Virginia School of Medicine, Charlottesville, VA, USA; ⁹Department of Public Health Sciences, University of Virginia School of Medicine, Charlottesville, VA, USA; ¹⁰Center for Public Health Genomics, University of Virginia, Charlottesville, VA, USA; ¹¹Division of Hematology and Medical Oncology, Mayo Clinic, Jacksonville, FL, USA; ¹²Oncology, Montefiore Medical Center, Bronx, NY, USA; ¹³Center for Data Driven Discovery, St Jude Children's Research Hospital, Memphis, TN, USA; ¹⁴Hematological Malignancies Program, St. Jude Children's Research Hospital, Memphis, TN, USA; ^{*}Contributed equally



Introduction

- Chronic lymphoproliferative disorder of natural killer (NK)-cells (CLPD-NK) is a subset of large granular lymphocyte (LGL) leukemia with clonal NK cell expansion [1].
- CLPD-NK has been included as a provisional entity in the 2016 WHO classification of lymphoid neoplasm and characterized by clonal expansion of NK cells with no standardized therapy [2].
- Several mutations have been identified in CLPD-NK such as *STAT3* and *TET2* (each 15–30%), however, the genetic basis of the remaining cases is unknown [3,4].
- IL-15 is key cytokine for NK cell differentiation and activation [5].

Aim

- To perform a comprehensive genomic analysis to elucidate
- the genomic landscape
 - investigate mechanism of pathogenesis
 - improve clinical understandings

Samples and methods

- ### 1. Genomic part
- CLPD-NK (NK-LGL: chronic type) (Fig. 1–6)
- Total 121 cases
 - Defined by immunophenotyping
- Discovery cohort**
- N = 59
 - Whole genome seq
 - RNA-seq
- Validation cohort**
- N = 62
 - Targeted seq (CCL22, STAT3)
- ### 2. Functional part
- In vitro assay**
- G protein signaling (Fig. 8)
 - β-arrestin recruitment (Fig. 9)
 - CCR4 Internalization (Fig 10)
 - Chemotaxis (Fig. 11)
- Transplantation** (Fig. 12,13)
- NSG-Tg(Hu-IL15) mice
 - NSG mice

References

1. Barila, G. et al. Best Pract Res Clin Haematol 32, 207-216 (2019).
2. Swerdlow, S.H. et al. Blood 127, 2375-90 (2016).
3. Koskela, H.L. et al. N Engl J Med 366, 1905-13 (2012).
4. Olson, T.L. et al. Blood (2021). [Online ahead of print. blood.2020005831.]
5. Lucas, M. et al. Immunity 26, 503-17 (2007).

Results; Genomic analysis and structural modeling



Fig. 1. Clinical features and mutational status of 59 cases of CLPD-NK in discovery cohort.

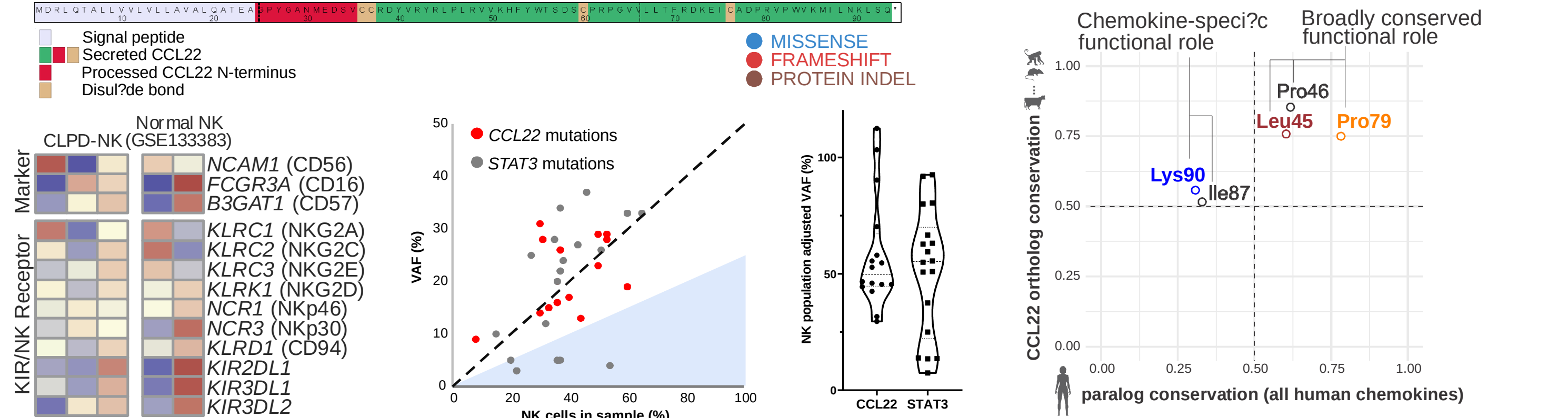


Fig. 3. CCL22 mutations are all clonal and in major clone.

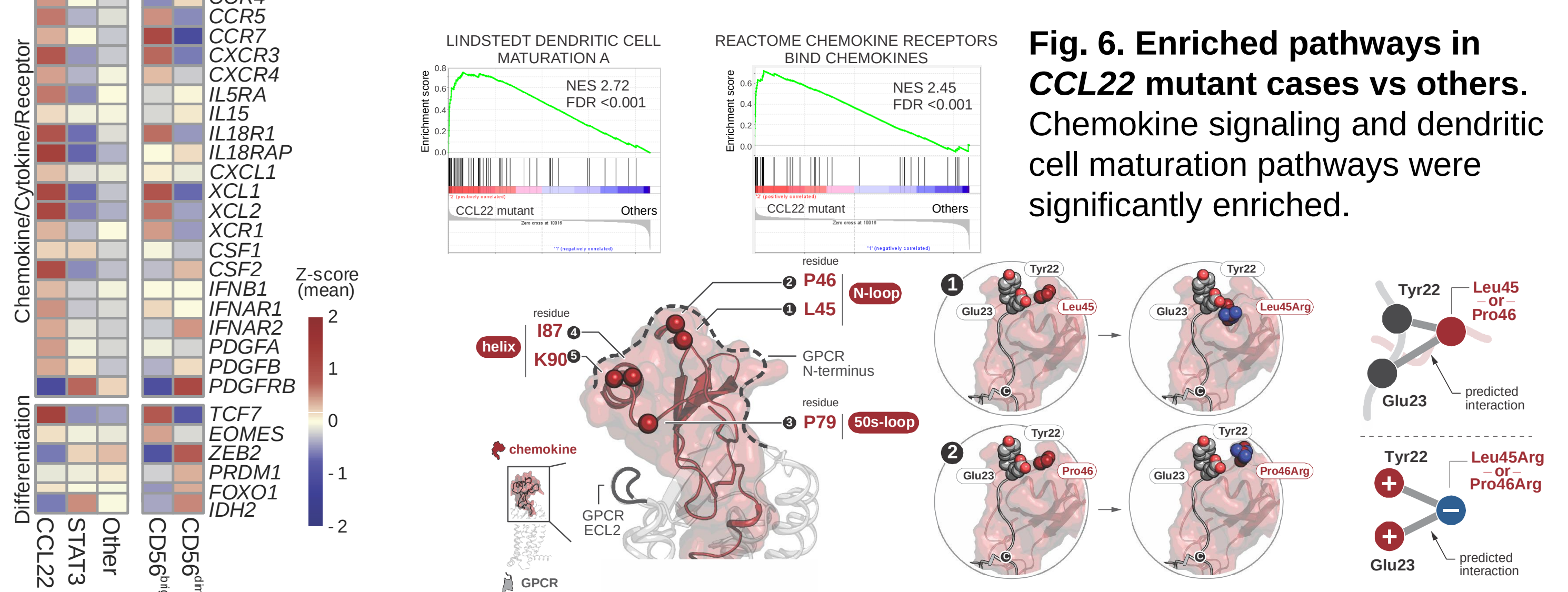


Fig. 5. CCL22 mutant showed similar expression profiles to normal CD56^{bright} NK cells.

Results; In vitro and vivo functional analysis of CCL22 mutations

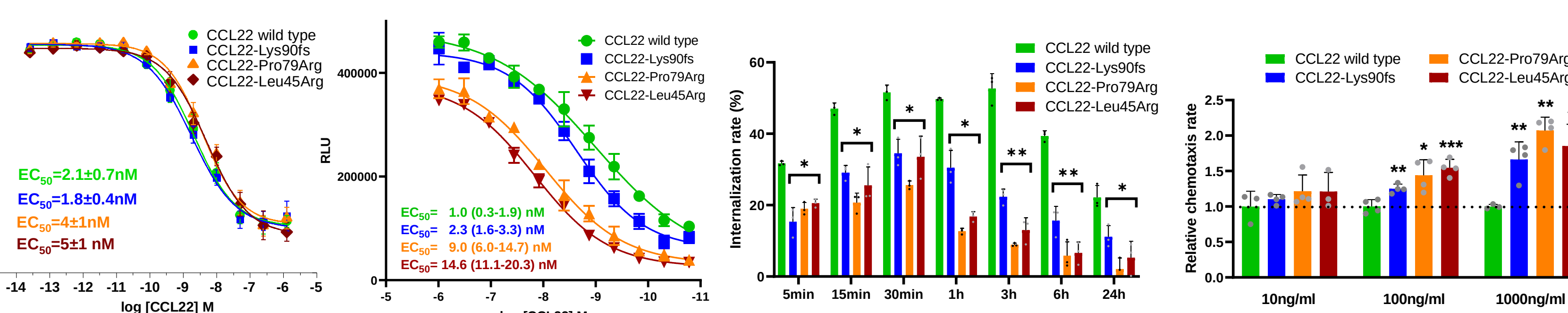
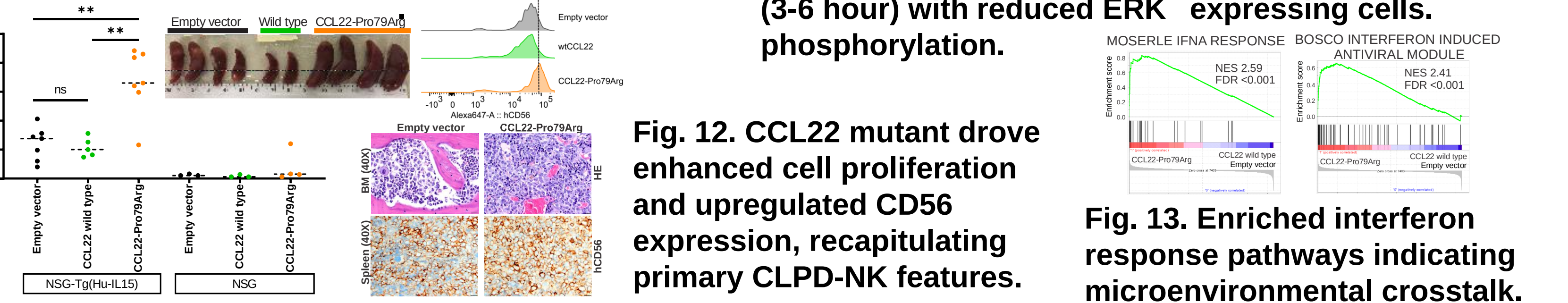
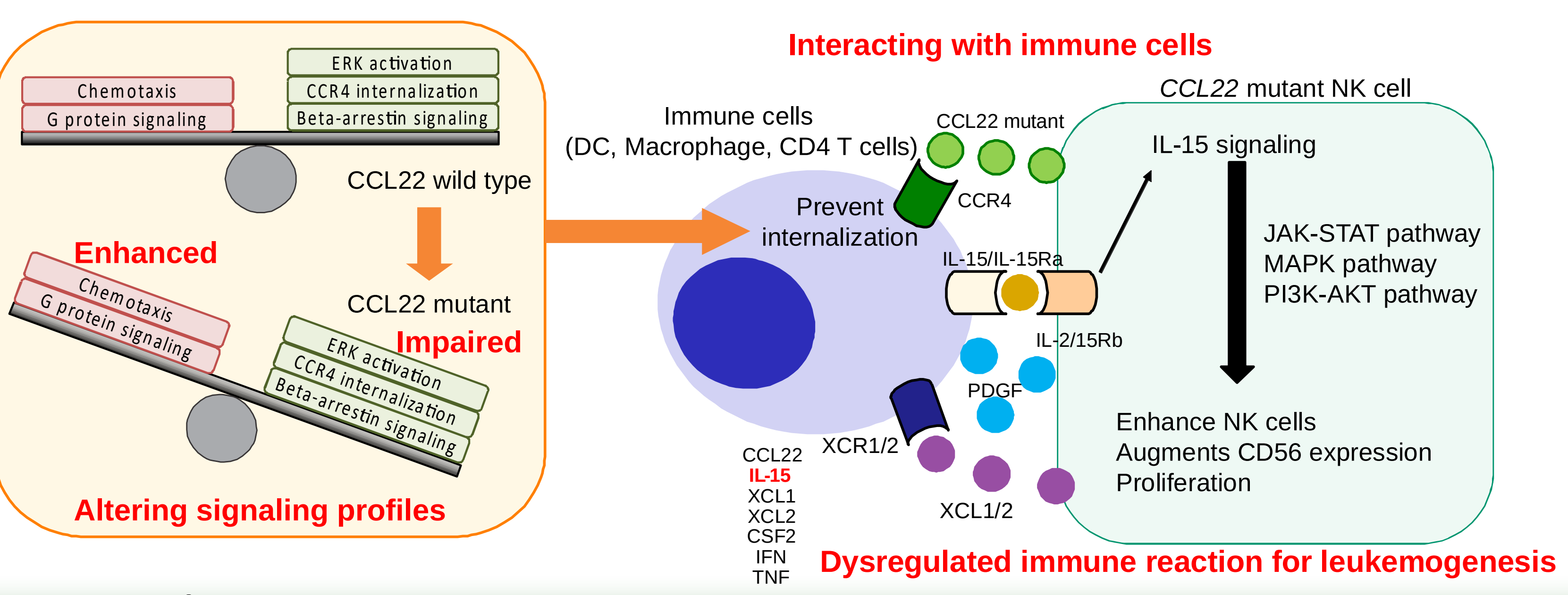


Fig. 7. CCL22 mutations are on well-conserved positions.



Summary of CCL22 mutated CLPD-NK

- Distinct subgroup resembling CD56^{bright} normal NK cell with upregulated cytokine signaling and dendritic cell maturation pathways.
- Induces functional selectivity to enhance chemotaxis with impaired internalization
- Importance of microenvironmental crosstalk to have selective proliferation.



Conflict of interest: Nothing to declare. Shunsuke Kimura; Shunsuke.kimura@stjude.org