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Nkp46:epa1 Interaction Occurs through Multiple O- and N- Glycosylation Sites

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NK cells are part of the innate immune system and induce apoptosis in target cells. The interaction between NK cells and their target cells is mediated by activating and inhibitory receptors on the NK cell surface. NKp46 is an activating receptor that interacts with several ligands, one of which is the epithelial adhesin Epa1 in the yeast Candida glabrata. Candidiasis is a common complication in immunocompromised patients. This study focused on the interaction between NKp46 and Epa1 using recombinant glycosylated NKp46 proteins. Previous studies indicated that this interaction is O-glycan dependent at the T225 site of NKp46. To study this, we generated a recombinant NKp46-T225A variant. The recombinant NKp46 was expressed in HEK293S GnTI$^-$ and HEK293T cells, while the Epa1 was produced in an E. coli bacterial expression system. MST and ITC were used to study the interaction between NKp46 and Epa1. Additional NKp46 variants were generated through O- and N-deglycosylation, and their binding affinities with Epa1 were analyzed using these biophysical methods. For the first time, we provided a glycosylation profile of NKp46 by performing mass spectrometry. Our findings indicate that NKp46 contains multiple O-glycans and one N-glycan in its stalk domain. And, Epa1 interacts with NKp46 through all these glycan sites, meaning its binding is not solely dependent on the O-glycan at T225.