Emma Treco HW 1 He 11 Feb 2023

Impact of Traf3 Copy Number on IL-6 Signaling

Treco, E; Hornick, E; Bishop, G. The Bishop Lab. 2022-23

Introduction High specificity and diversity are key features of adaptive immunity and are critical for protection against reinfection. Plasma cells (PCs) are major contributors to these featues of adaptive immunity, as each PC is specialized to constitutively produce a singular product: high-affinity antibodies (note: PCs are different from memory B cells which are dormant). After immature B cells with high-affinity B cell receptors differentiate into PCs, they are sustained by IL-6R signaling. IL-6 is also a growth factor forr malignant PCs and therefore must be tightly regulated. Previous work from our laboratory has shown that the adaptor protein Tumor Necrosis Factor Receptor (TNFR)-Associated Factorr 3 (TRAF3) is an important negative regulator of IL-6R signaling in B cells. Upon IL-6 stimulation of the receptor, TRAF3 recruits phosphatase PTPN22 to the IL-6R signaling complex. PTPN22 reverses IL-6 dependent activation of JAK1 and STAT3 by removing the activating phosphorylation, which suppresses downstream effects such as PC development. B cells deficient in TRAF3 (knockouts) have increased IL-6 dependent phosphorylation of JAK1 and STAT3 as a reesult. Most of our work to date has been performed on B cells with a homozygous loss of *Traf3*, but further investigation of how *Traf3* copy number affects TRAF3-dependent phenotypes is justified based on: - an increasing number of patients with heterozygous loss-of-function *Traf3* mutations - preliminay work indicating B cells TRAF3 levels may decline with age Here we focused on how IL-6 signaling is affected by loss of one *Traf3* allele.

Figure 2 shows that *Traf3* heterozygous primary mouse B cells have statistically enhanced early STAT3 phosphorylation compared to B cells from B-*Traf3* WT mice.

Materials and Methods Western blotting was used to determine the differences in the amount of protein produced by WT, hets, and KO. Figure 2 shows the pooled data from western blots asseessing the differences in the amount of STAT3 phosphorylation in response to IL-6. The data I have is the quantification of the sizes of the bands in the western blots that I have ran and imaged over the last year. There is no pre-processing required for these data. I plan to use R to graph these data.

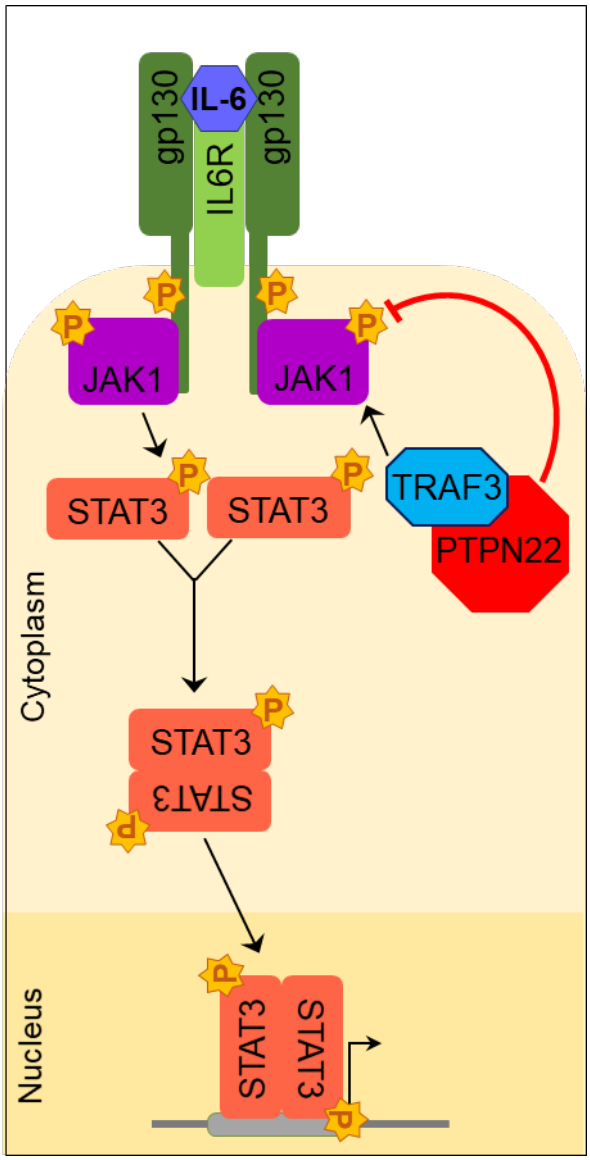

Figure 1: Figure 1. IL-6R signaling pathway.

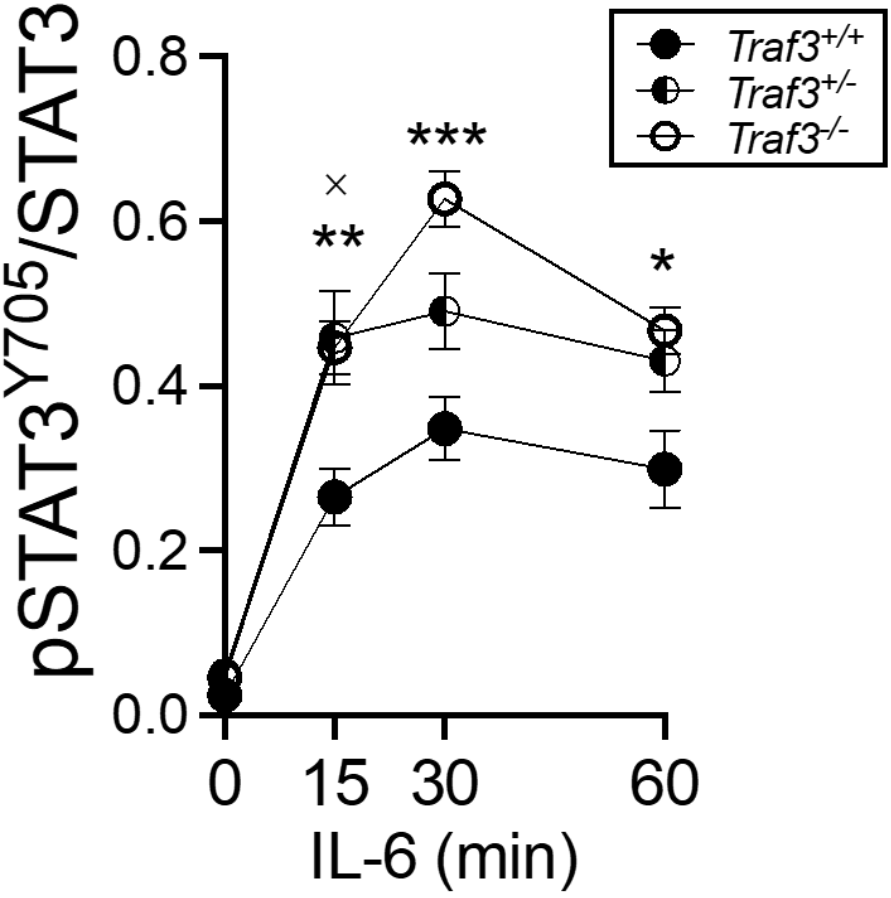

Figure 2: Figure 2. Wildtype, *Traf3* heterozygous, and *Traf3* homozygous primary mouse cells stimulated with IL-6 for given time, then blotted for pSTAT3, STAT3, and actin. * $p<0.05$, ** $p<0.1$, and * * * $p< 0.001$ between WT and KO. X $p<0.05$ between WT and het.