Ubiquitination role in TCR signaling and costimulation via GITR Muller, J.[1], Zhang, G.[2], Silva, H.M.[2], Neubert, T.[2], Dustin, M.[3] [1]New York University Langone Medical Center, Sackler Institute / Immunology, New York, United States, [2]New York University Langone Medical Center, Skirball Institute of Biomolecular Medicine, New York, United States, [3]Oxford University, Kennedy Institute of Rheumatology, Oxford, United Kingdom

Essential steps in TCR sorting, signaling, and degradation, are mediated by ubiquitination of components in TCR microclusters. Signaling through the costimulatory family of TNF receptors (GITR, OX40, 4-1BB, etc.) utilize a series of ubiquitination events that regulate the signaling process. A number of substrates of ubiquitin ligase like Cbl-B, ITCH, and CIAP1/2 have been identified. However, a detailed analysis of the global pattern of ubiquitination from TCR signaling is lacking. To address this we have utilized SILAC labeling and proteomics to profile the dynamic changes in ubiquitination that result from TCR signaling alone or in combination with GITR signaling. We have identified 4,500 ubiquitination sites with 44 regulated by TCR or TCR and GITR signaling. Using bioinformatics, we have identified key ubiquitin ligases that are responsible for the dynamic ubiquitination observed in our screen and confirmed their function by knock down, IP and ubiquitination of targets by western blot. Moreover, we have used IP with linkage specific antibodies to identify the specificity of the chains on specific ubiquitinated signaling molecules. Our work demonstrates the application of ubiquitin proteomics to primary murine antigen specific T cells, cross talk between TCR and GITR signaling, and the ubiquitination events downstream of the TCR and GITR that regulate their signaling.