Figure S1. Model of Methotrexate Responsiveness and STAT3 Mediated Signaling in LGL leukemia.

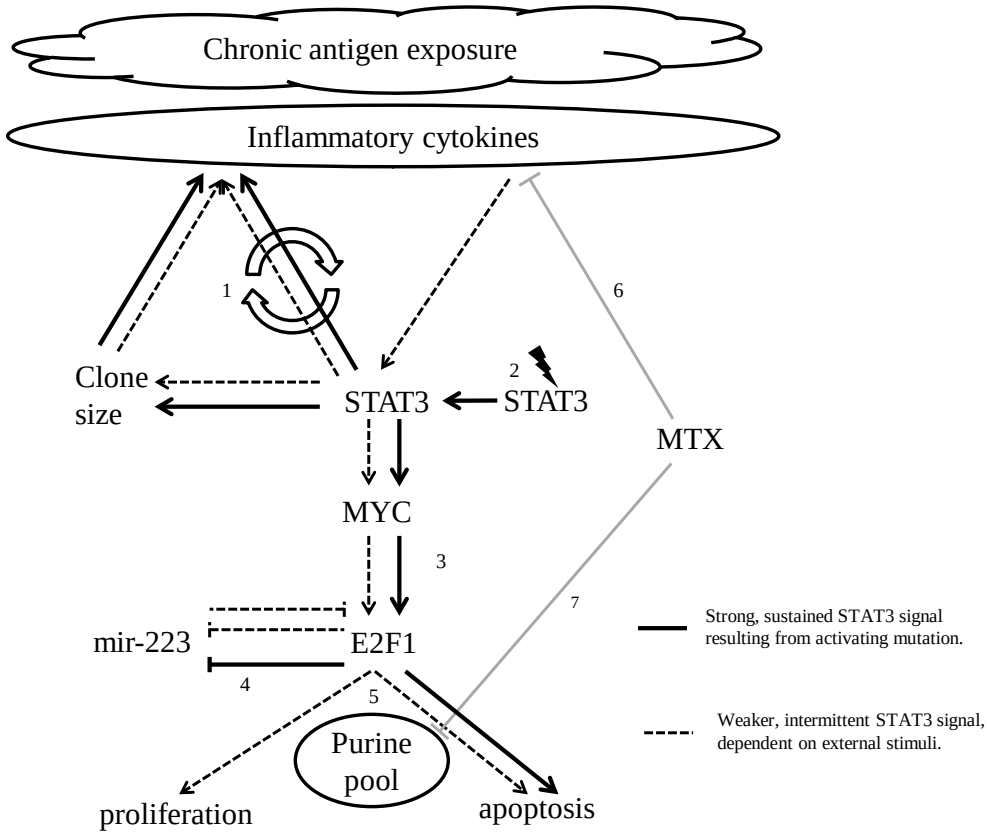

Figure S1. Proposed Model of Methotrexate Responsiveness and STAT3 Mediated Signaling in LGL leukemia.

LGL leukemia is thought to arise in the context of chronic antigen exposure that maintains LGL survival associated with production of multiple inflammatory cytokines. Downstream effectors including STAT3 amplify this autoimmune loop(1) by increasing the release of inflammatory cytokines through direct transcriptional regulation and indirectly through the support of larger clonal populations. STAT3 mutations(2), Y640F mutations in particular, provide stronger and more sustained STAT3 signaling. This could lead to larger clone size and greater cytokine production resulting in more symptomatic disease. MTX and other immunosuppressives reduce the levels of such inflammatory cytokines(6).

Key downstream targets of STAT3, such as MYC and E2F1(3) were upregulated in patients that responded to MTX. Levels of mir-223 were low in Y640F mutant samples, consistent with high E2F1 expression(4) as they are reciprocally regulated. The E2F1 transcriptional program pushes cell cycle transition while monitoring for proper conditions including available purines(5). We postulate that high E2F1 expression maintained by strong and sustained STAT3 signaling in Y640F mutation positive responders sensitizes leukemic LGL to apoptosis in the setting of purine depletion caused by low dose MTX(7).