Adaptive laboratory evolution (ALE) has emerged as a new approach with which to pursue fundamental biological inquiries and, in particular, new insights into the systemic function of a gene product. Two E. coli knockout strains were constructed: one that blocked the Pentose Phosphate Pathway (gnd KO) and one that decoupled the TCA cycle from electron transport (sdhCDAB KO). Despite major perturbations in central metabolism, minimal growth rate changes were found in the two knockout strains. More surprisingly, many similarities were found in their initial transcriptomic states that could be traced to similarly perturbed metabolites despite the differences in the network location of the gene perturbations and concomitant re-routing of pathway fluxes around these perturbations. However, following ALE, distinct metabolomic and transcriptomic states were realized. These included divergent flux and gene expression profiles in the gnd and sdhCDAB KOs to overcome imbalances in NADPH production and nitrogen/sulfur assimilation, respectively, that were not obvious limitations of growth in the unevolved knockouts. Therefore, this work demonstrates that ALE provides a productive approach to reveal novel insights of gene function at a systems level that cannot be found by observing the fresh knockout alone.