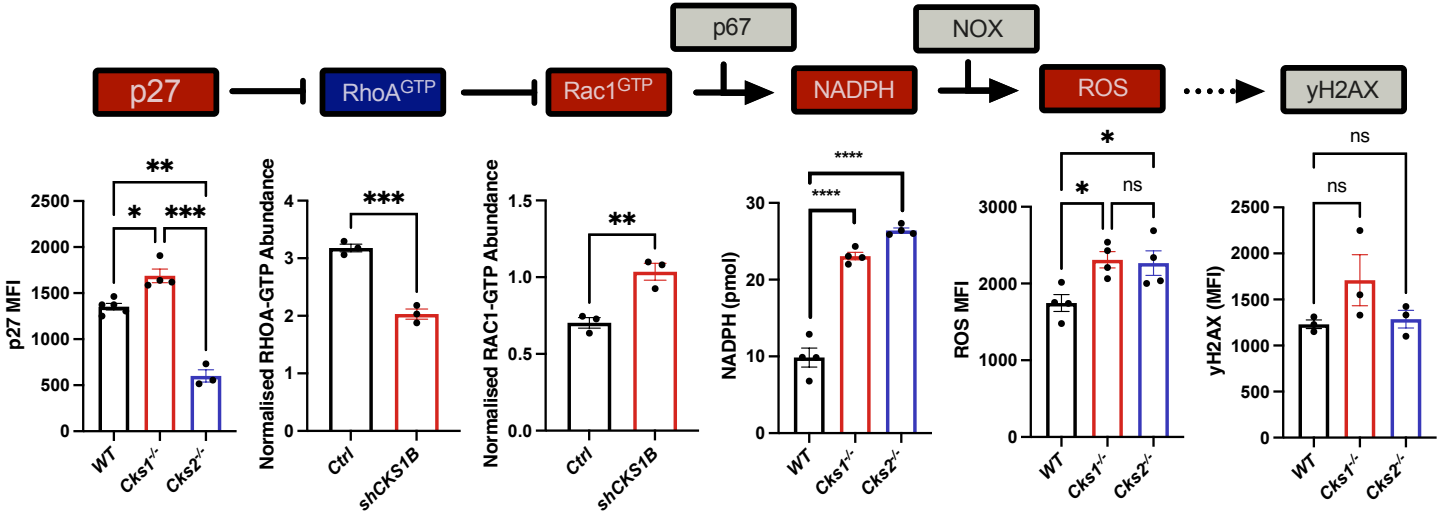

Rebuttal Figure 1. The top row is a graphical representation of our previously published mechanism from Grey et al. 2022 and 2023. Red indicates increased protein levels, blue decreased protein levels and grey indicates no changes detected. From left to right, key mechanistic insights demonstrating production of intracellular ROS. Intracellular flow cytometry in HSPCs from WT and *Cks1*$^{-/-}$ and *Cks2*$^{-/-}$ mice demonstrating altered p27 protein levels. High levels of p27 block GTP loading of RhoA, keeing RhoA in its GDP bound form. RhoA and Rac1 have reciprocal inhibitory activities when in their GTP bound forms, and with reduced RhoA-GTP, we demonstrated this led to increased amounts of Rac1-GTP. High levels of Rac1-GTP, in concert with p67-Phox can lead to increased NADP/NADPH metabolism, and we detect higher levels of the NADPH metabolite in knockout HSPCs. NADPH can act as a reservoir for NOX proteins to drive intracellular ROS production, and we detect significantly higher levels of intracellular ROS, as measured by CellRox deep red with the addition of verapamil. This reagents specifically fluoresces when intracellular ROS (produced in mitochondria) is present. It has previously been proposed that these specific intracellular ROS lead to DNA damage and long-term HSPC senescence. We do not detect increased DNA damage in our HSCs and indeed we do not see a long-term bone marrow injury from high levels of ROS in the mice. Hence, this detailed mechanistic pathway supports our hypothesis that increased intracellular ROS can drive altered RTK signalling and our pilot data in Figure 2 of this grant demonstrates that this is the case for the RTK Tie2.