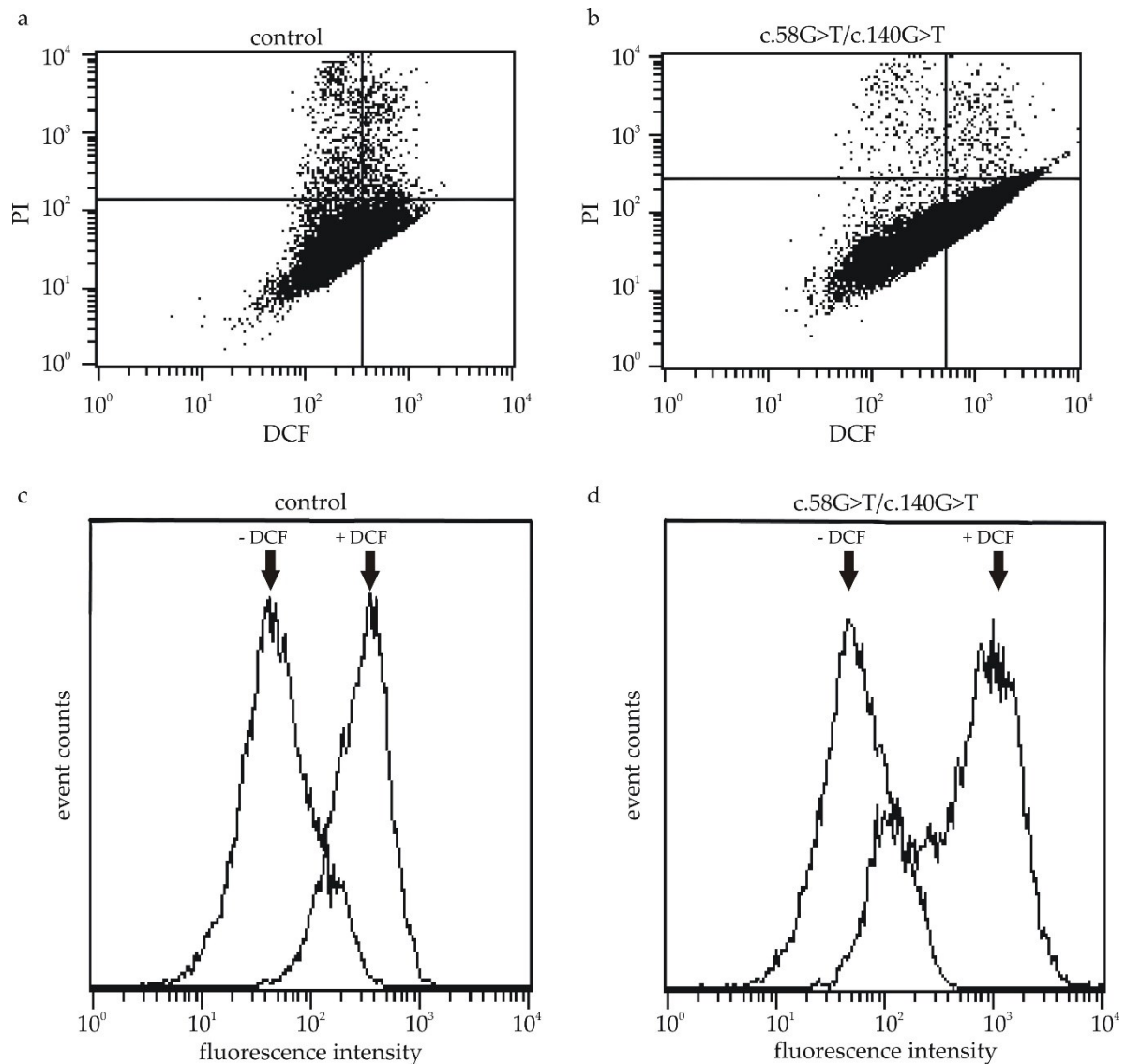


Supplementary Figure S4



Supplementary Figure S4: Example of FACS analysis of ROS level by DCF fluorescence. (a) Dot plot of control cells and (b) of mutated cells simultaneously stained with propidium iodide (PI) and DCF. **Lower left quadrant:** double negative cells. **Lower right quadrant:** DCF positive cells and PI negative cells, reflecting cells suffering from oxidative stress (OS). **Upper left quadrant:** PI positive cells (necrotic/apoptotic cells). **Upper right quadrant:** PI and DCF positive cells. (c) Histogram of fluorescence distribution of control cells and (d) NPC2-deficient cells. **-DCF** = cells not treated with DCF, used as negative control to set the cut off for the fluorescence analysis. **+DCF** = cells treated with DCF. For both examples one can observe the right shift of the DCF fluorescence, indicating cells suffering from OS. Noticeably, the shift to a higher fluorescence intensity is more pronounced in the NPC2-deficient cell line.