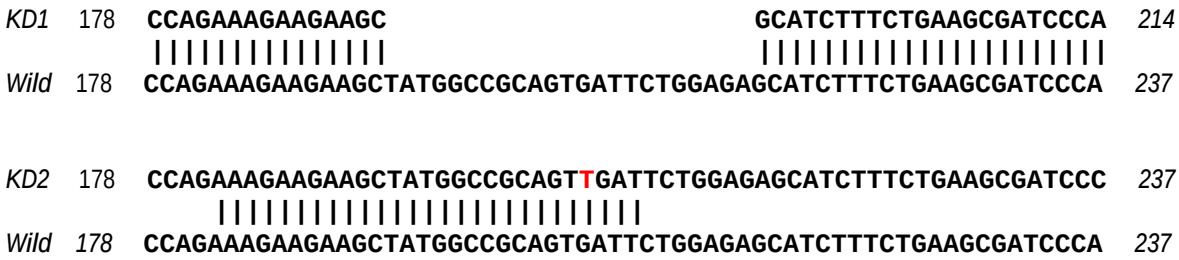

Fig. S1. Generation of BTK-KD mutants. Parental Jeko cells were generated following the procedure described by Mali et. al . Candidate clones were screened by immunoblotting with anti-BTK antibody (#8547, CST). BTK protein-null clones were subjected to genotyping by sequencing 10 independent PCR products flanking the site of gRNA targeting regions. In BTK-KD1 and BTK-KD2 cells, a 23-base deletion and a single base deletion were found, respectively. In both clones, wild type allele were also present. Given that BTK is X-linked and the Jeko cells were derived from a female patient, the inactivated X-chromosome is most likely refractory to gRNA targeting and remains wild type, which does not produce any detectable amount of BTK protein due to X-inactivation. Disruption of the active X-chromosome leads to loss of BTK expression and results mutants BTK KD1 and BTK KD2.