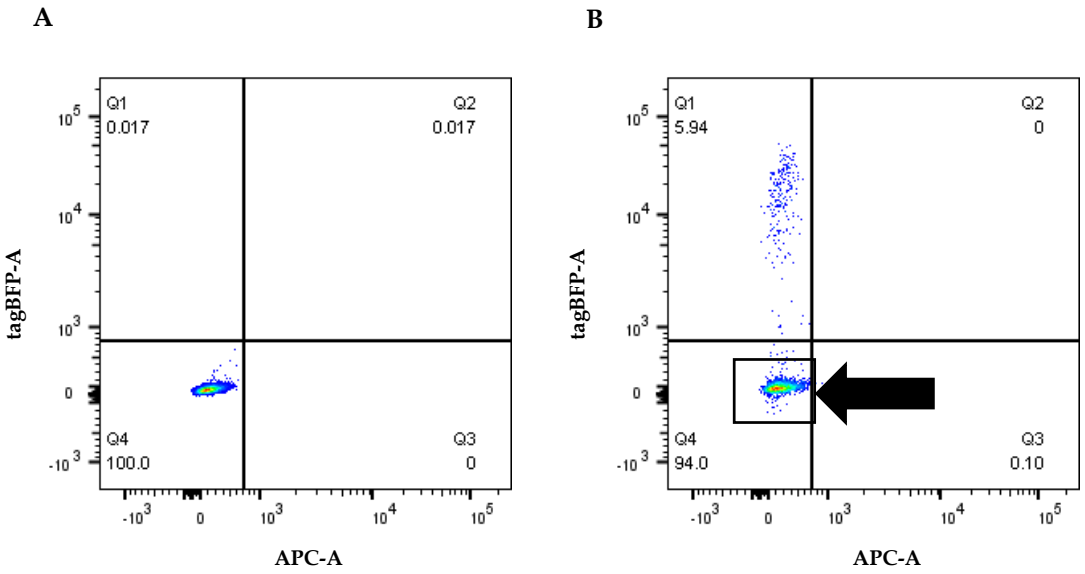
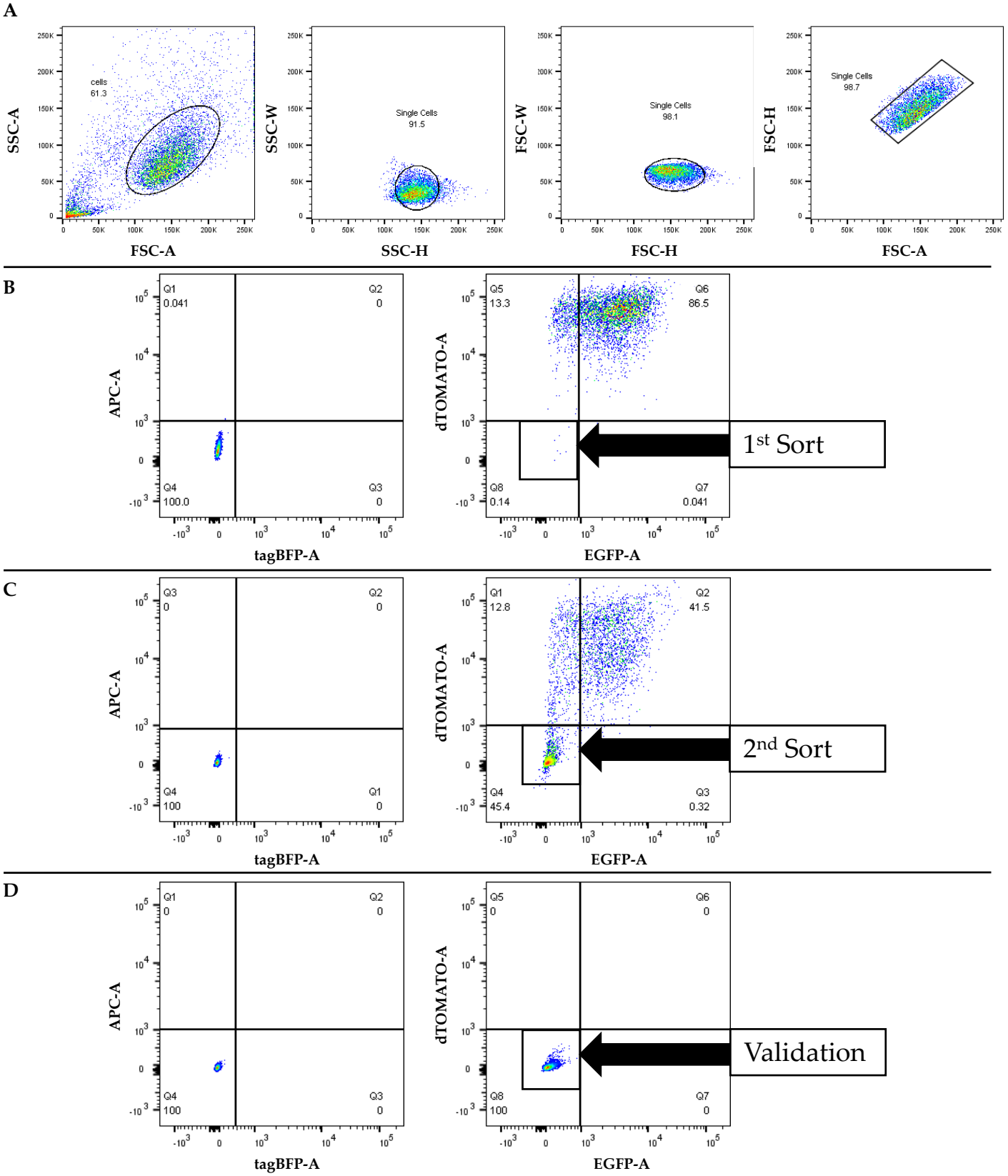
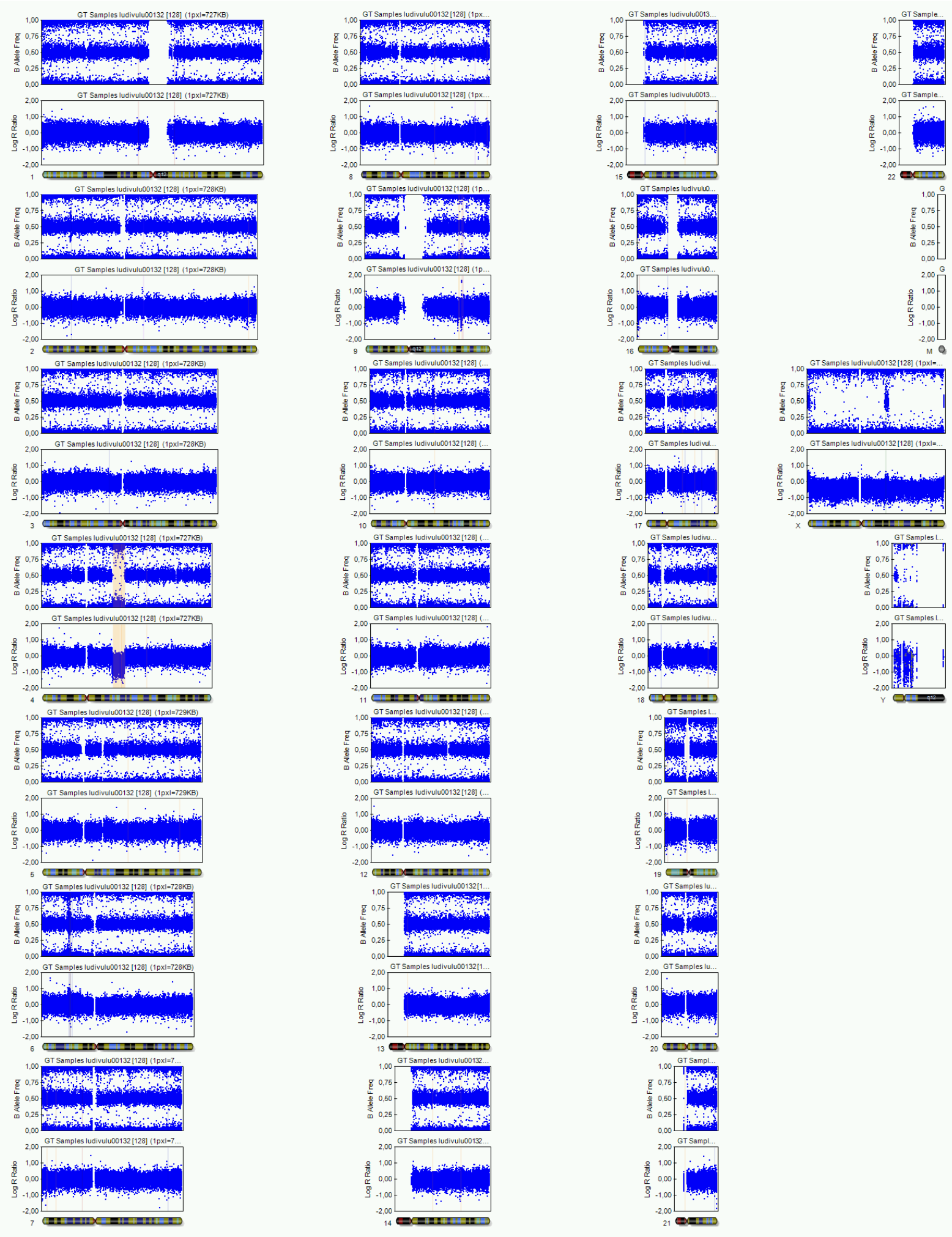


Supplementary Figure 1: Removal of tagBFP^{+} cells using FACS prior to sorting the dTOMATO^{+}/EGFP^{+} transfected cells. **(A)** Untransfected control used to set the gates. **(B)** tagBFP^{+} and tagBFP^{-} cells, black arrow and gate indicates the tagBFP negative fraction.

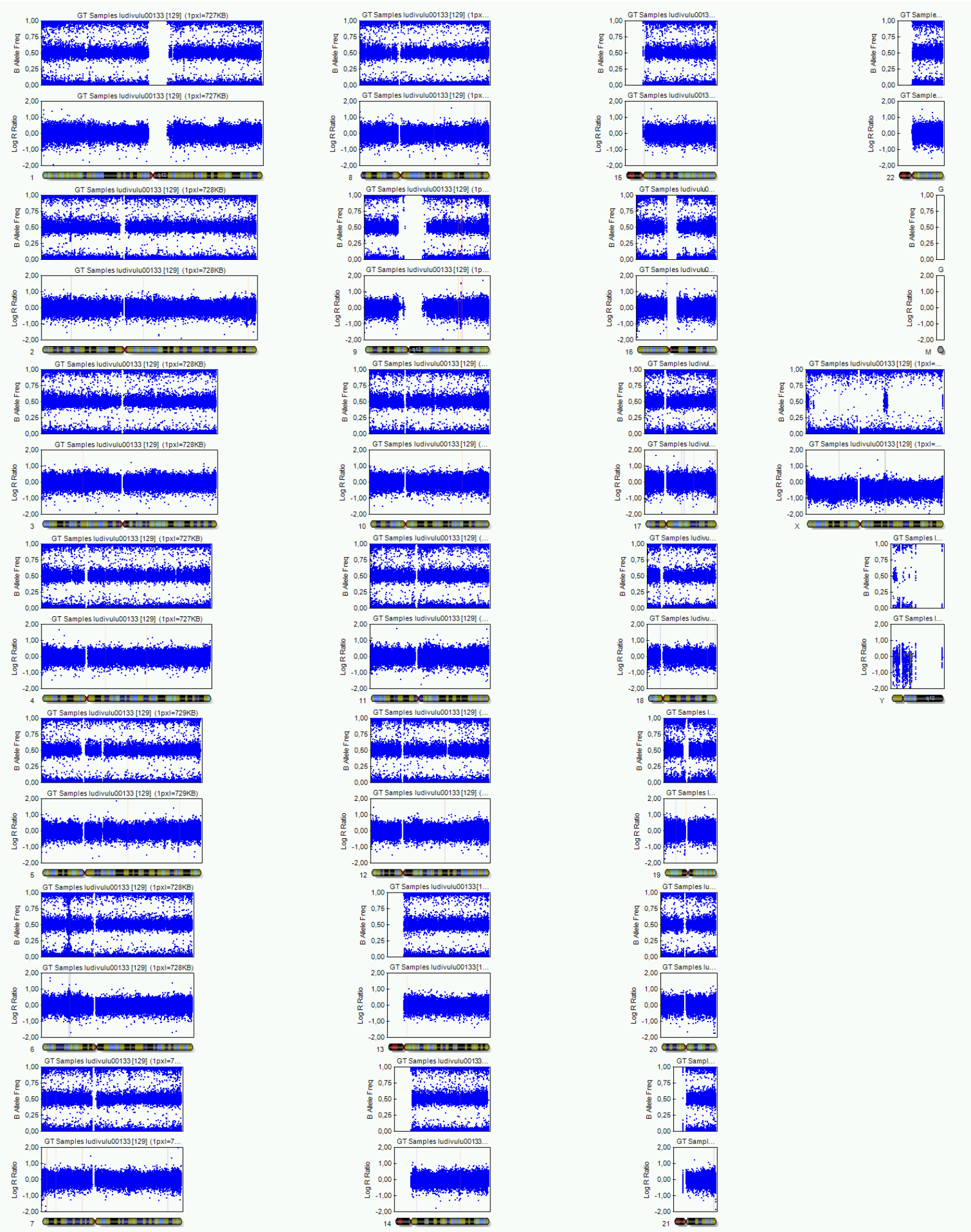
Supplementary Figure 2. 102 bp sequence insertion in Cl. 14. Seq_1 refers to the gene-corrected clone SNCA Cl.33. Seq_2 refers to Cl.14. The highlighted “grey” sequence refers to the 102 bp insertion. The highlighted “blue” base pair at 126 bp refers to the site of the A30P mutation.



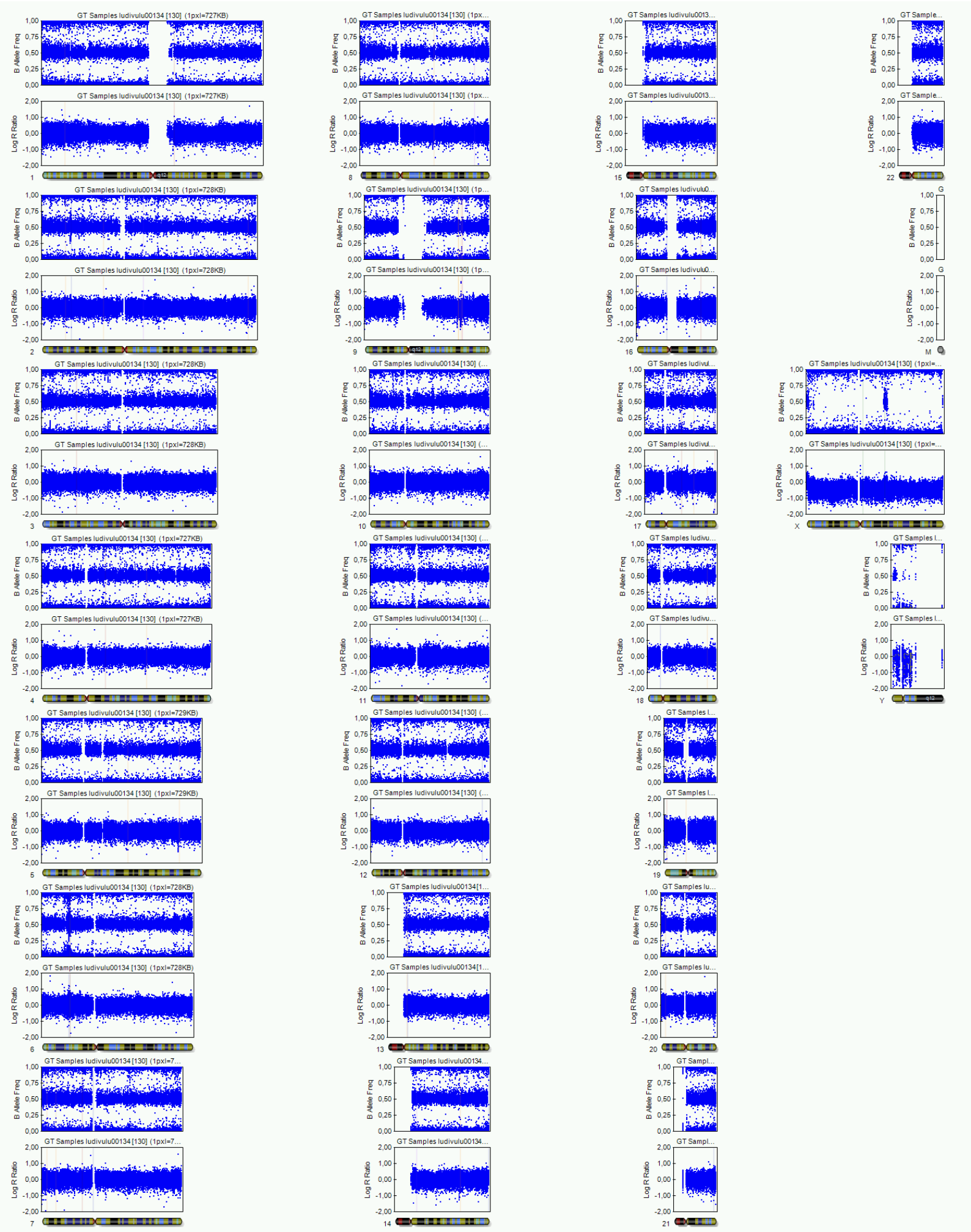
Supplementary Figure 3. Transposase removal of biallelic constructs using FACS and validation of footprint-free isogenic cell lines. **(A)** Set up of gating parameters for sorting a live cell population with doublet-discrimination, **(B)** Left panel shows tagBFP- (Pacific Blue) cells, right panel shows iPSCs expressing both dTOMATO (PE) and EGFP (FITC) fluorescent constructs. Gate and arrow indicate sorted cell population (0.14%) after the transposase removal of the construct. The cells sorted were expanded and resorted in **(C)**, left panel shows no BFP cells, the right panel contains a mixed population with and without the fluorescent constructs, 45.4% of the cells sorted in (B) had the constructs been successfully excised, this population (black arrow) is then resorted and purified. (D) Right panel, 100% of the cells sorted in step C (black arrow) have had their constructs excised.



Supplementary Figure 4: Molecular karyotype of the single-cell gene-corrected cell line Clone 5. Single nucleotide polymorphism assay shows a 14Mbp deletion on the long arm of Chromosome 4.



Supplementary Figure 5: Molecular karyotype of the single-cell gene-corrected cell line Clone 13. Single nucleotide polymorphism assay shows a normal karyotype for a male individual



Supplementary Figure 6: Molecular karyotype of the single-cell gene-corrected cell line Clone 33. Single nucleotide polymorphism assay shows a normal karyotype for a male individual