Supplemental Figure S14. Calibration experiments to determine mCherry gating for single integration sorting. (A) pZDonor plasmid containing a *ACTB* promoter upstream of the eGFP was integrated into K562 using ZFN-mediated site-specific integration. The populations of single integrations (P2) and double integrations (P3) were evaluated according to the GFP and mCherry density plot. (**B**) Cells were sorted by their mCherry levels, according to differential gating on mCherry (P1(7% of the mCherry positive cells), P5(12.1%), P6(19.3%), P7(26%), P8(32.7%), P9 (40.5%), P10(59.3%), P11 (78.2%). As a control, the range containing both single integrations and double integrations was sorted as well (P4(7.4%)). (**C**) Cells were reanalyzed 7 days after the sorting according to mCherry levels. While all the gates up to P9 (P1,P5,P9 are shown) exhibited only 1% or less of the cells in the P3 gate, i.e. cells with double integration, in P10 1.8% of the cells were present in this gate, and 9.5% in P11. P9 (highlighted in a red frame) was thus chosen as the gate allowing collection of most cells, with minimal rate of cells that may possess double integrations. In the positive control P4, 36% of the cells were in P3 a week after the sorting.