



**Supplemental Figure S10: Improved off-target performance of dC9Sun-D3A in sorted cells compared to puromycin treated cells**

(A) Mean mCG values over 33 CGs in the *UNC5C* promoter for HeLa cells sorted by flow cytometry (mCherry control - green, dC9Sun-D3A + *SHB* sgRNA - orange) compared to puromycin selected HeLa cells (puromycin control - purple, dC9Sun-D3A + *SHB* sgRNA - pink). mCG measured by bsPCR-seq with amplicon spanning 33 CGs in the *UNC5C* promoter (n=3, replicates for cell sorting and puromycin treatment, respectively). (B) Mean mCG value at 1 CG in CTCF core binding site located in an *SHB* intron for HeLa cells sorted by flow cytometry (mCherry control - green, dC9Sun-D3A + *SHB* sgRNA - orange) compared to puromycin selected HeLa cells (puromycin control - purple, dC9Sun-D3A + *SHB* sgRNA - pink). mCG measured by bsPCR-seq with amplicon spanning the *SHB* CTCF core binding site (n=3, replicates for cell sorting, n=2 for puromycin treatment).