

**Supplementary information, Figure S3** Base preference of 400 RVDs from the screening of the TALE-(XX')<sub>3</sub> library. The binding efficiencies and specificities of the

variable RVDs (XX') in each customized TALE were assayed based on the fold induction of EGFP reporters, by comparing with the basal level of EGFP in HEK293T cells transfected with the reporter plasmid and the customized TALE plasmid containing unmatched TALE repeats. The EGFP fluorescence intensity assayed from FACS analysis was normalized to the corresponding mCherry fluorescence intensity. The fold induction of the EGFP reporter of all 400 types of TALE-(XX')<sub>3</sub> (corresponding to four reporters) was categorized by the 13<sup>th</sup> residue (X'), and the data are listed in alphabetical order according to the 12<sup>th</sup> residue (X) of the variable RVD-carrying TALE. The x-axis labels indicate the variable RVDs (XX') tested in TALE-(XX')<sub>3</sub>. The color-coded bars represent the fold induction of the different reporters (A, green; T, red; C, blue; and G, yellow) in this and the subsequent figures.