

Supplementary Figure S5 | HDR-, HITI- and oaHDR-mediated gene knock-in efficiency in dividing cells, a Schematic representation of gene targeting by HDR and oaHDR in the GFPcorrection HEK293 and hESC lines. Each cell line is stably expressing the chromosomal reporter construct. Once the truncated GFP (tGFP) donor is correctly integrated into the target sequence, GFP can be expressed and detected. If donor sequence is inserted by HITI, no GFP expression is detected. **b** Surveyor nuclease assay performed transfected with Cas9, gRNA and tGFP donor DNA. Different gRNAs (gRNA1, gRNA2 and gRNA3) are transfected respectively in GFP-correction HEK293 line. gRNA cutting efficiency is calculated from the band intensity, indicated at bottom (%). c, d GFP knock-in efficiency in HEK293 (c) and hES (d) cells. gRNA for HDR: gRNA 1. Genome cut-only gRNA: gRNA 2. Donor cut-only gRNA: gRNA 3. Both genome and donor cut gRNA: gRNA2+3. Data from three independent experiments resulted in Unpaired Student's t-test of \*P < 0.05 and \*\*P < 0.01 (c, d). Data are represented as mean  $\pm$  s.e.m.