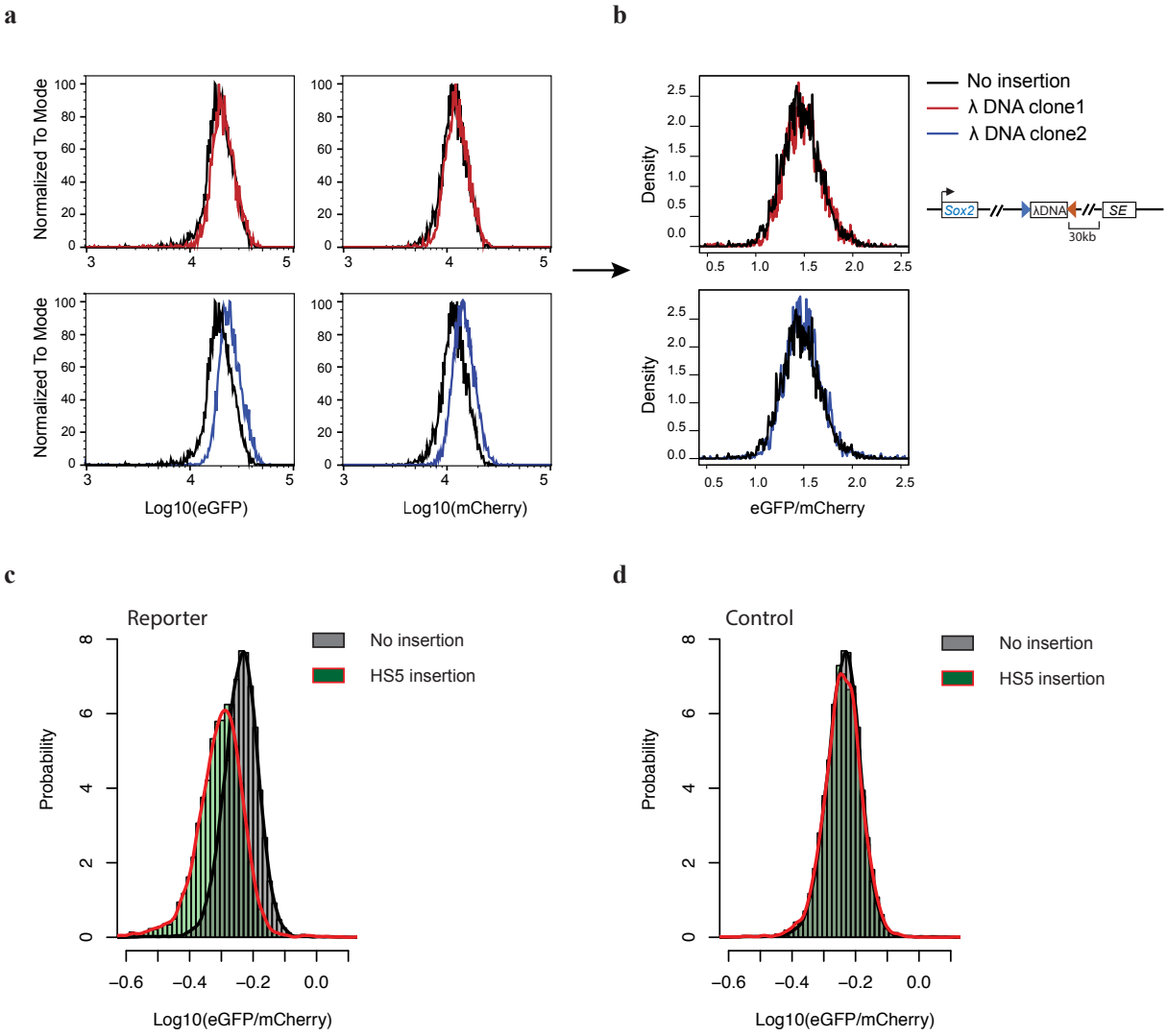
Supplementary Fig1.

Supplementary Figure. 1 | Efficiency of insertion by recombinase-mediated cassette exchange.

a, Diagram of recombinase-mediated cassette exchange (RMCE) in the insulator reporter cell line. Flippase expression plasmid and the donor plasmid carrying the insertion sequence were co-electroporated into cells. The replacement only happens on the CAST allele. **b,** Genotyping insertion clones of λDNA fragments generated by RMCE. PCR primers were designed from genomic locations that spanned the insertion position. Top band, insertion fragments; Bottom band, PCR products from the no insertion allele.

Supplementary Fig2.



Supplementary Figure. 2 | Normalization of *Sox2* expression.

a-b, FACS profiles of two clones with the insertion of the same λDNA fragment. **a,** Histograms showing eGFP and mCherry signals of the two clones; **b,** Density plots of normalized signal (eGFP/mCherry) of cells from the two clones. For every cell, the ratio of eGFP signal over mCherry signal was calculated. **c,** A histogram shows the normalized Sox2-eGFP expression of cells with the human β-globin HS5 insulator inserted between the *Sox2* gene and its super-enhancer. The CTCF motif of the HS5 insulator was in forward orientation and Sox2-eGFP expression was reduced by 14.1%. **d,** A histogram shows the normalized Sox2-eGFP of cells with the human β-globin HS5 insulator inserted downstream of the *Sox2* super-enhancer. The CTCF motif of the HS5

insulator was in forward orientation.

Unprocessed gel of Supplementary Fig. 1b