Supplementary Information

Locus-specific ChIP combined with NGS analysis reveals genomic regulatory regions that physically interact with the *Pax5* promoter in a chicken B cell line

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Supplementary Table and Figure Legends

Supplementary Table S1. gRNA and primers used in this study

Supplementary Table S2. Information on iChIP-Seq and in vitro enChIP-Seq analyses

Supplementary Table S3. List of genomic regions detected by iChIP-Seq

Supplementary Figure S1. Genomic regions that interact with the *Pax5* promoter region. (**A**) Thirty four genomic regions were commonly detected in Data set #1 (Fold enrichment, >7) and #2 (Fold enrichment, >7). (**B**) Ninety (75.6%) genomic regions in Data set #1 (Fold enrichment, >7) were detected in Data set #2 (Fold enrichment, >2). (**B**) Sixty two (59.0%) genomic regions in Data set #2 (Fold enrichment, >7) were detected in Data set #1 (Fold enrichment, >2).

Supplementary Figure S2. Isolation of the *Pax5* promoter region by *in vitro* enChIP. (**A**) Positions of gRNA and the primer set. (**B**) Target sequence of Pax5 gRNA. The protospacer adjacent motif (PAM) is underlined. (**C**) DNA yields of *in vitro* enChIP. DNA isolated by *in vitro* enChIP was used for quantitative real-time PCR.

Supplementary Figure S3. Representative peak positions detected by iChIP-Seq (#1). Three peak positions in chromosome 11 (**A–C**) and one peak position in chromosome 21 (**D**) are shown. The peak positions corresponding to iChIP-Seq (#2) are shown in Figure 4.

Supplementary Figure S4. Representative peak positions not confirmed by *in vitro* enChIP-Seq. (**A–C**) Two peak positions in chromosome 21 that were detected by iChIP-Seq but not by *in vitro* enChIP-Seq.

Supplementary Figure S5. Prediction of potential off-target binding sites. Potential off-target binding sites for Pax5 gRNA were predicted using a CRISPR design tool (http://crispr.mit.edu/) and the chicken genome reference galGal4.

Supplementary Figure S6. Intra-chromosomal interaction with the *Pax5* promoter region. Intra-chromosomal interactions in Data set #1 (Fold enrichment, >2) and #2 (Fold enrichment, >2) are shown.

Supplementary Figure S7. Distribution of identified genomic regions in chromosome 21.

Supplementary Figure S8. The LexA BE insertion site and Pax5 gRNA-targeted site in the *Pax5* promoter. The genomic region upstream from nucleotide -1 is shown.

Supplementary Figure S9. Representative peak positions constitutively detected by iChIP-Seq in KI(B) and KI(MΦ). Two representative peak positions on chromosome 21 are shown.

Supplementary Figure S10. *in vitro* enChIP-Seq with DT40(MΦ). (**A**) Trans-differentiation of DT40 into a macrophage-like cell by stable expression of chicken C/EBPβ. (**B**) Silencing of *Pax5* and *AID* transcription in the macrophage-like cell DT40(MΦ). Expression levels of *Pax5* and *AID* mRNA were quantified by real-time RT-PCR and

normalized against *GAPDH* mRNA (mean ± s.e.m., n = 3); the mRNA levels in DT40 were defined as 1. (**C**) *M-CSFR* transcription in DT40(MΦ). *M-CSFR* transcription was confirmed by RT-PCR analysis. (**D**) Unsuccessful isolation of the *Pax5* promoter region from DT40(MΦ) by *in vitro* enChIP. The images of *in vitro* enChIP-Seq with DT40 in Figure 3C are also shown here for comparison with that of *in vitro* enChIP-Seq with DT40(MΦ).

Supplementary Figure S11. CRISPR-mediated locus deletion. (**A**) Schematic depiction of the loci in chromosome 11, which were identified as those interacting with the *Pax5* promoter region in a B cell–specific manner. One allele is shown. The interacting regions are shown with gray triangles. Numbers indicate target sites of sgRNAs. (**B**) Scheme of CRISPR-mediated locus deletion. (**C**) Target sequences of sgRNAs. (**D** and **E**) Scheme of CRISPR-mediated locus deletion in DT40. WT indicates intact DT40.

Supplementary Figure S12. Confirmation of CRISPR-mediated locus deletion by genotyping PCR. (**A**) Depiction of the genomic regions in each cell clone. Primer positions used in genotyping PCR are shown. (**B**) Results of genotyping PCR.

Supplementary Materials and Methods

Trans-differentiation of DT40 cells into macrophage-like cells.

For trans-differentiation into macrophage-like cells, DT40 cells (1×10^7) were co-transfected with 110 μg of linearized cC/EBPβ/pEF (Fujita et al. 2015) and 3 μg of puromycin-resistance gene by electroporation. The cells were selected in the presence of puromycin (0.35 μg/ml). The surviving cell colonies were individually picked and expanded. An established cell line was used as DT40(MΦ) in Supplementary Figure S8.