Expanded View Figures

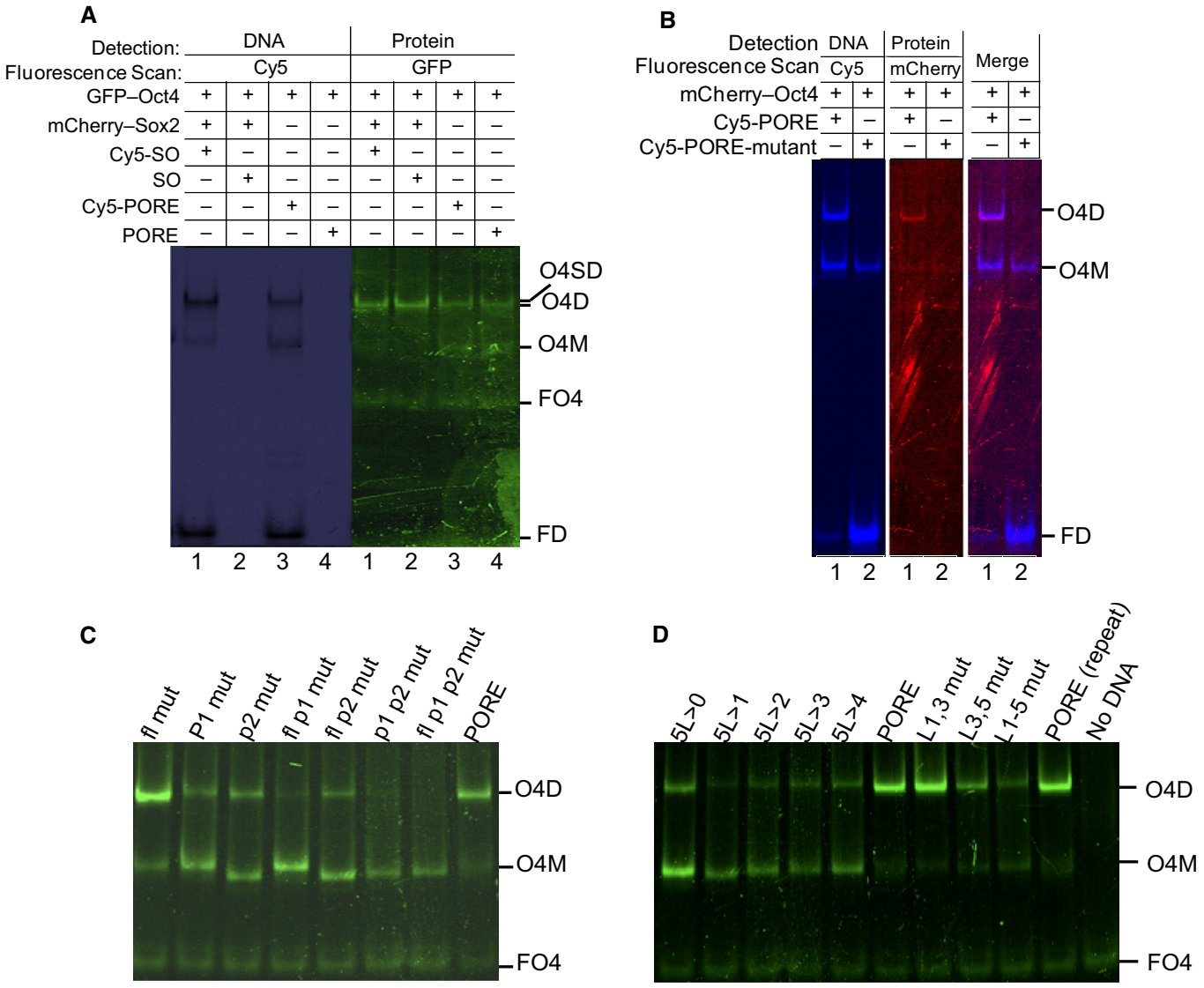


Figure EV1. Validating FP-EMSA.

A Homodimer formation was tested using DNA labelled with Cy5 or unlabelled as indicated. The presence of a Cy5 tag does not affect protein binding as dimerisation in lanes 2 and 4 is comparable with lanes 1 and 3.

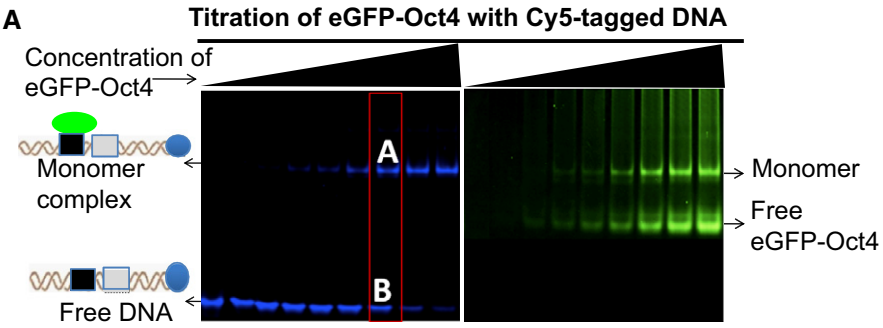
B Homodimerisation is independent of the fluorescent protein tag. mCherry–Oct4 was tested instead of GFP-Oct4 with PORE-wt (lane 1) and PORE-mt (lane 2).

C Mutations inside the PORE motif affect homodimer formation, whereas mutations outside the PORE motif do not. The wild-type PORE is indicated; fl, P1 and P2 indicate oligonucleotides with mutations in the flanking sequence, palindromic repeat 1 and repeat 2, respectively.

D Effects of mutations of base pairs in the non-palindromic linker of the PORE motif (AAATG). 5L > 4-0 indicates sequential reduction in the size of the linker; other point mutation oligonucleotides are indicated.

Data information: O4D: GFP-Oct4 homodimer complex, O4SD: GFP-Oct4 and mCherry-Sox2 heterodimer complex, O4M: GFP-Oct4 monomer complex, FO4: free GFP-Oct4, and FD: free DNA. All oligonucleotide sequences are in Appendix Table S1. $n = 3$.
Source data are available online for this figure.

A



$$\text{Bound fraction} = \frac{\text{total complexes}}{\text{free DNA} + \text{total complexes}} = \frac{A}{A+B}$$

The apparent dissociation constant, aK_d, can be experimentally determined by ploting the bound fraction with respect to the concentration of the fusion protein.

B Sigmoidal fitting equation

$$Y(x) = Y_0 + \frac{Y_{max}}{1 + e^{\left(\frac{X_{half} - X}{R}\right)}}$$

Where Y(x) represents X (concentration of total protein) dependent bound fraction; Y0 is the base value; Ymax represents maximum bound fraction; Xhalf represents the concentration where the titration will reach to 50% of bound fraction; and R represents the rise rate. Smaller rate causes a faster rise.

Figure EV2. Quantification of aK_d by FP-EMSA.

A Blue panel: DNA; green panel: protein detection. For quantification purpose, only the blue panel needs to be taken into consideration. The bound fraction was calculated according to the equation provided.

B Sigmoid fitting function used to fit the bound fraction versus total protein concentration plot.

Source data are available online for this figure.

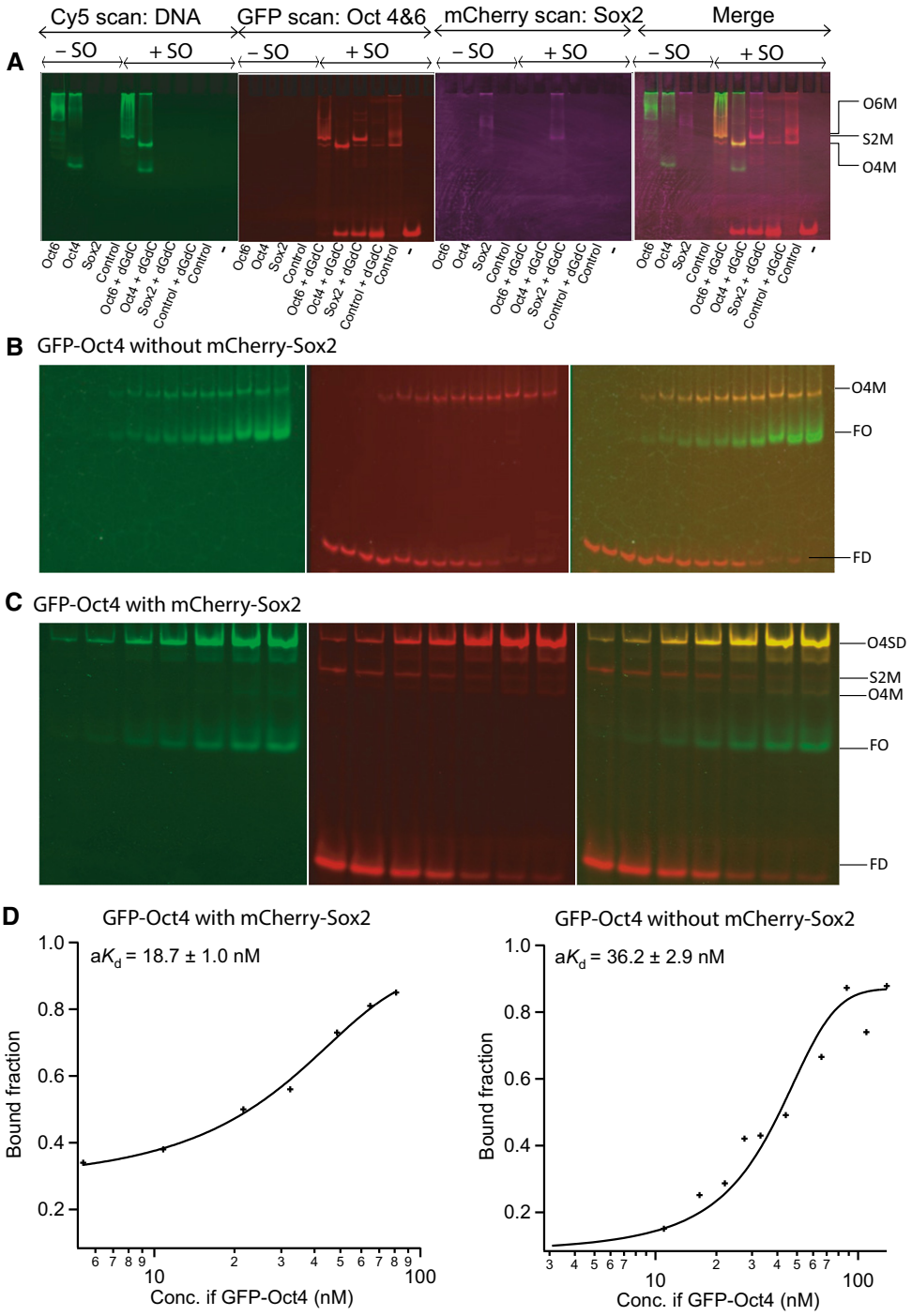


Figure EV3. Control experiments for the titration of Oct6 in the presence and absence of Sox2.

A Confirmative monomeric band was observed by studying proteins in the absence (−SO) or the presence (+SO) of the *Nanog* Sox/Oct sequence. Control is lysate from untransfected cells. dGdC is included as a non-specific competitor.

B, C FP-EMSA analysis of titrations of GFP-Oct4, binding to the *Nanog* Sox/Oct oligonucleotide (10 nM) in the absence (B) or the presence (C) of 132 nM mCherry-Sox2.

D Bound fractions observed from titrations shown in (B, C) were plotted against the respective concentration of Oct4 for quantifying the aK_d of those titrations.

Data information: O4SD: GFP-Oct4-mCherry-Sox2 dimer complex, O6M: GFP-Oct6 monomer complex, S2M: mCherry-Sox2 monomer complex, O4M: GFP-Oct4 monomer complex, FO: free GFP-Oct4, FD: free DNA. $n = 2$.

Source data are available online for this figure.

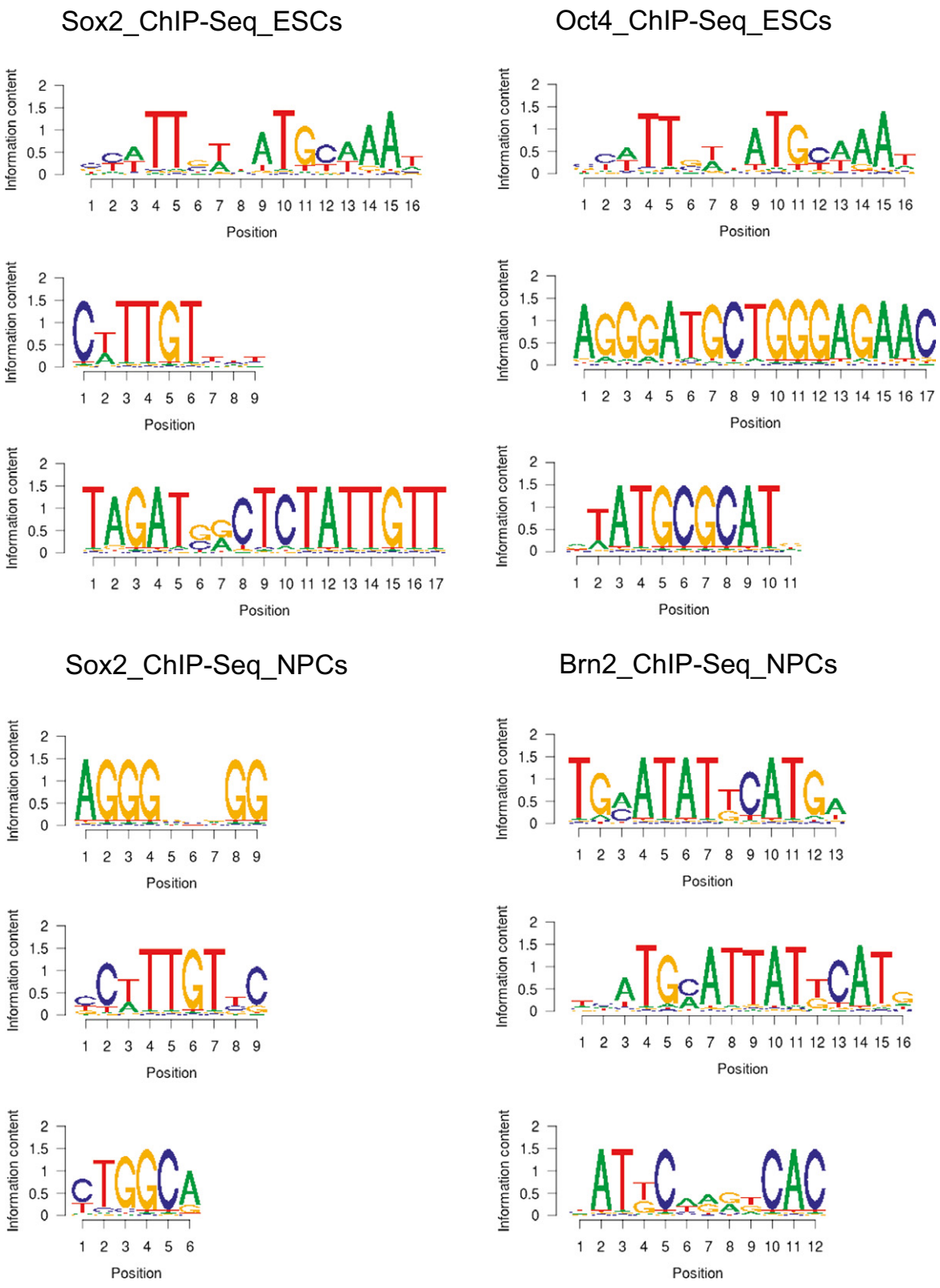


Figure EV4. *De novo* motif discovery from the published ChIP-Seq data (Lodato *et al*, 2013).
The top *de novo* sequence motifs (based on enrichment) detected by *XXmotif* (see Materials and Methods) in ChIP-Seq data for Oct4 in ESCs, Sox2 in ESCs (upper panel), Sox2 in NPCs and Brn2 in NPCs (lower panel). The top 3 enriched motifs for each are shown by the position frequency matrices visualised by WebLogo.