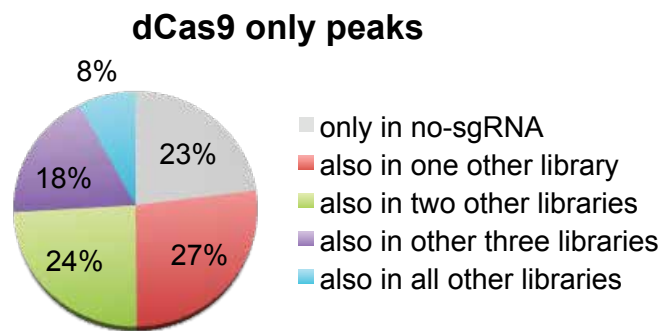


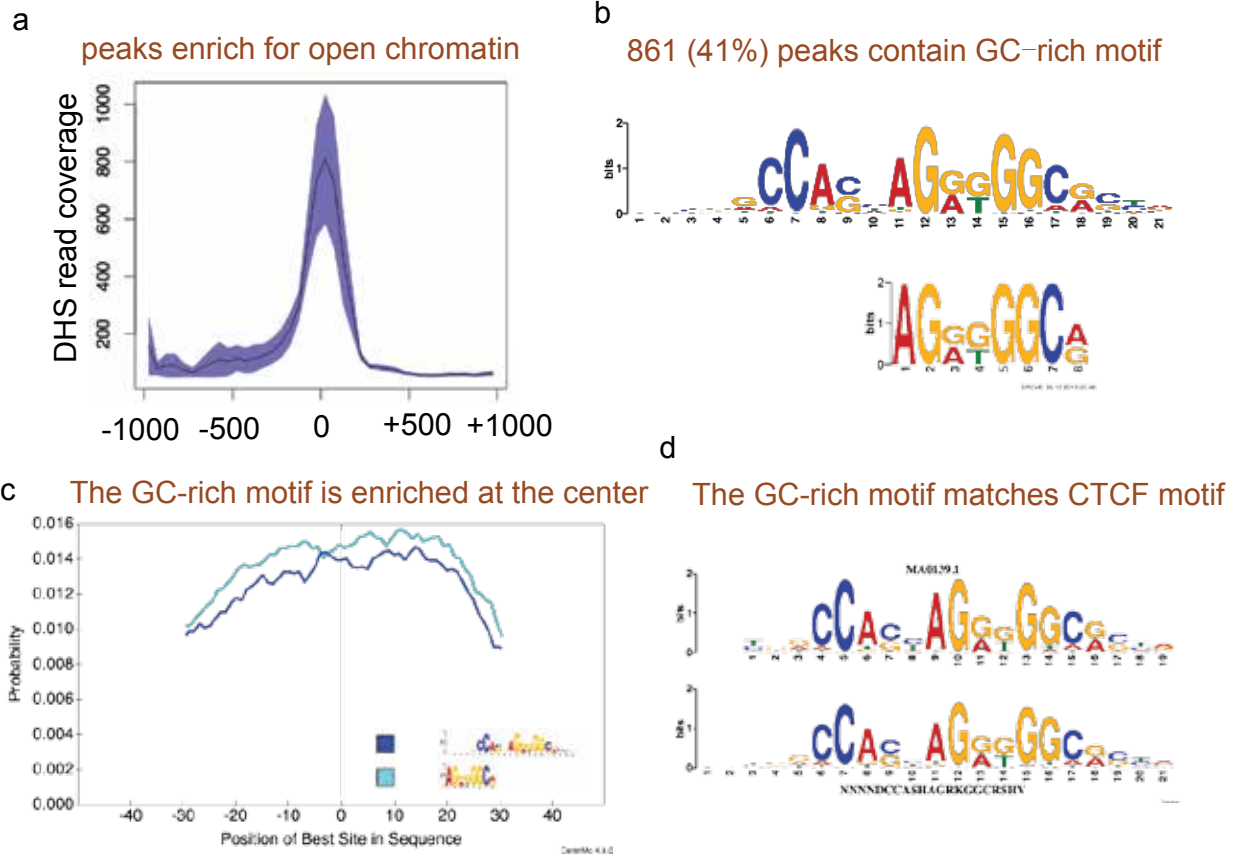
a

IP	Peaks called (MACS) over input
No-sgRNA / dCas9 only	2,115
Phc1-sg1	21,328
Phc1-sg2	4,568
Nanog-sg2	4,424
Nanog-sg3	19,857

b



Supplementary Figure 1 | Conventional peak calling comparing IP to input. (a) The number of peaks called by MACS using default settings. (b) The fraction of dCas9-only peaks that are also detected in one, two, three, or all other four IP samples.



Supplementary Figure 2 | Characteristics of dCas9-only peaks. (a) Peaks are enriched for open chromatin regions. Shown is the average density of Dnase Hypersensitivity reads per 50bp bin in a 2kb window centered on peak summits. Blue area indicates standard error. (b) De novo motif finding within 50bp of peak summits by MEME-ChIP uncovered two related GG/CC-rich motifs. (c) Relative position of the motif within the peak, 0 indicates peak summits. (d) The longer motif (bottom) closely resembles CTCF binding motif (top, $p < 1e-23$)

Phc1-sg1



GGAAATAAGGACATAAGCACTGG

Nanog-sg2



GATCTCTAGTGGGAAGTTTCAGG

Phc1-sg2



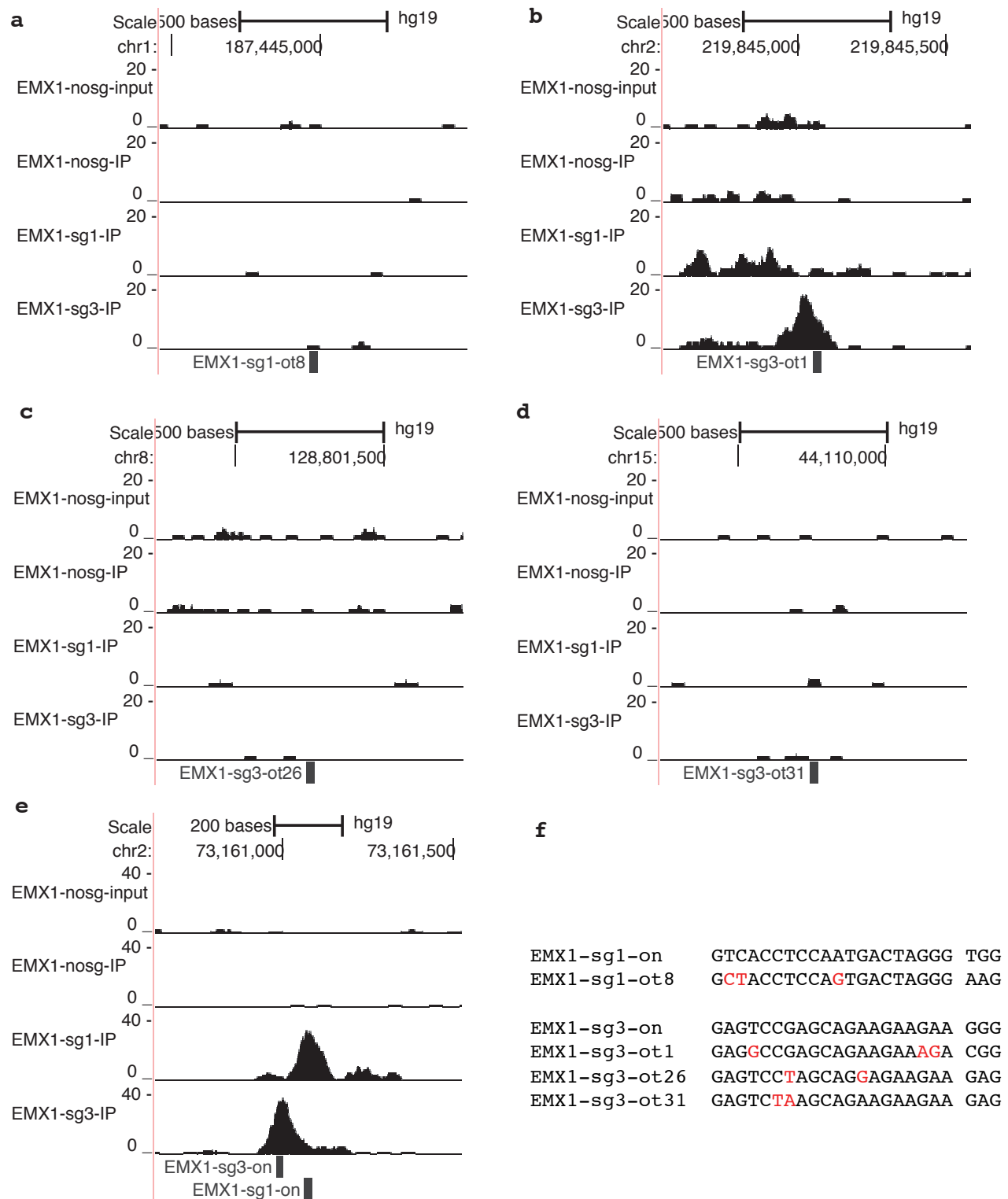
GATGACGAAACCAATGGTACTGG

Nanog-sg3

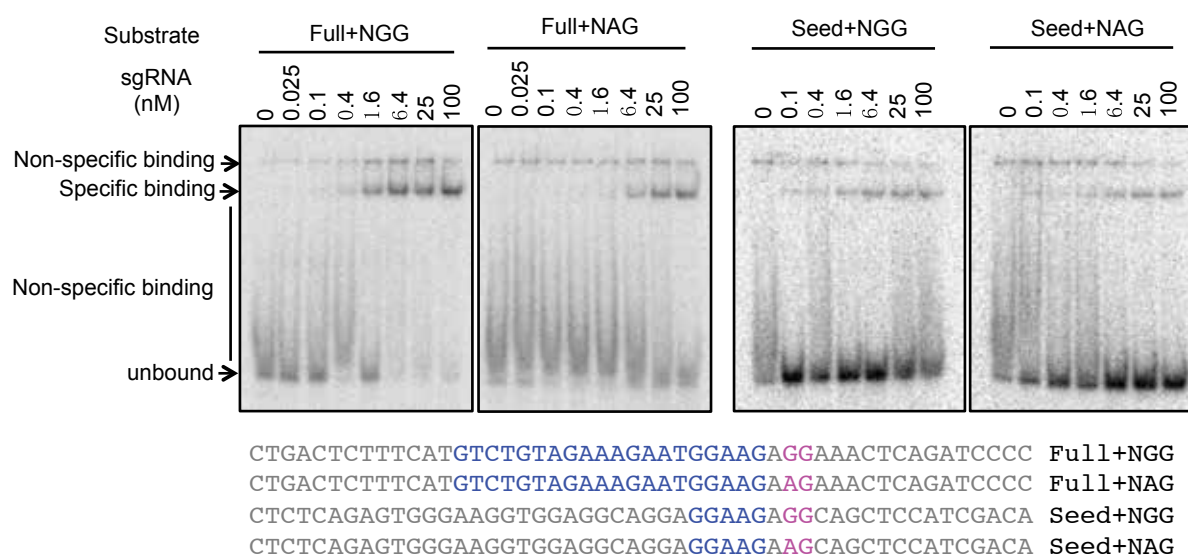


GTCTGTAGAAAGAATGGAAGAGG

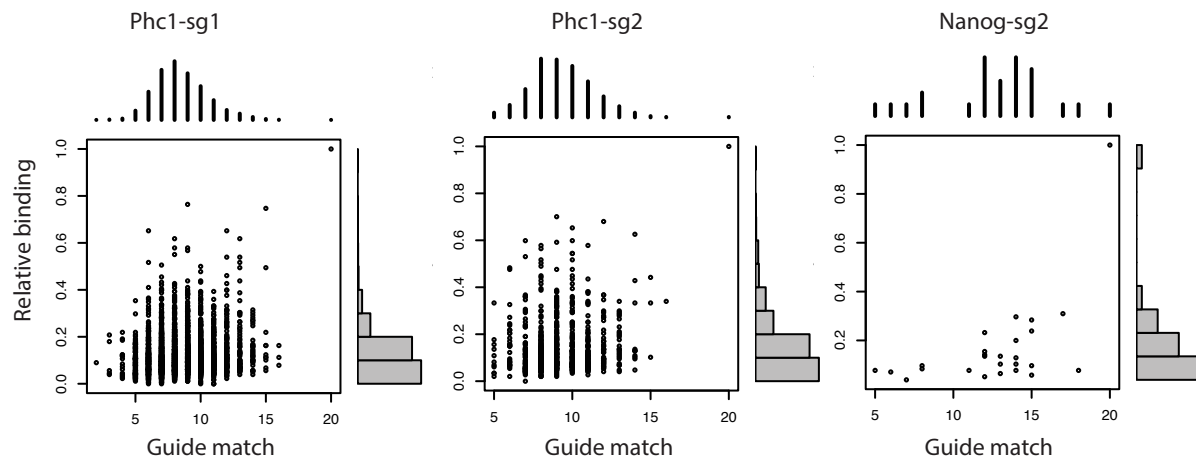
Supplementary Figure 3 | De novo motif discovery in ChIP peaks. Motifs detected by MEME-ChIP using default settings and sequences within 50 bps of peak summits. The guide RNA sequences were shown below the motif.



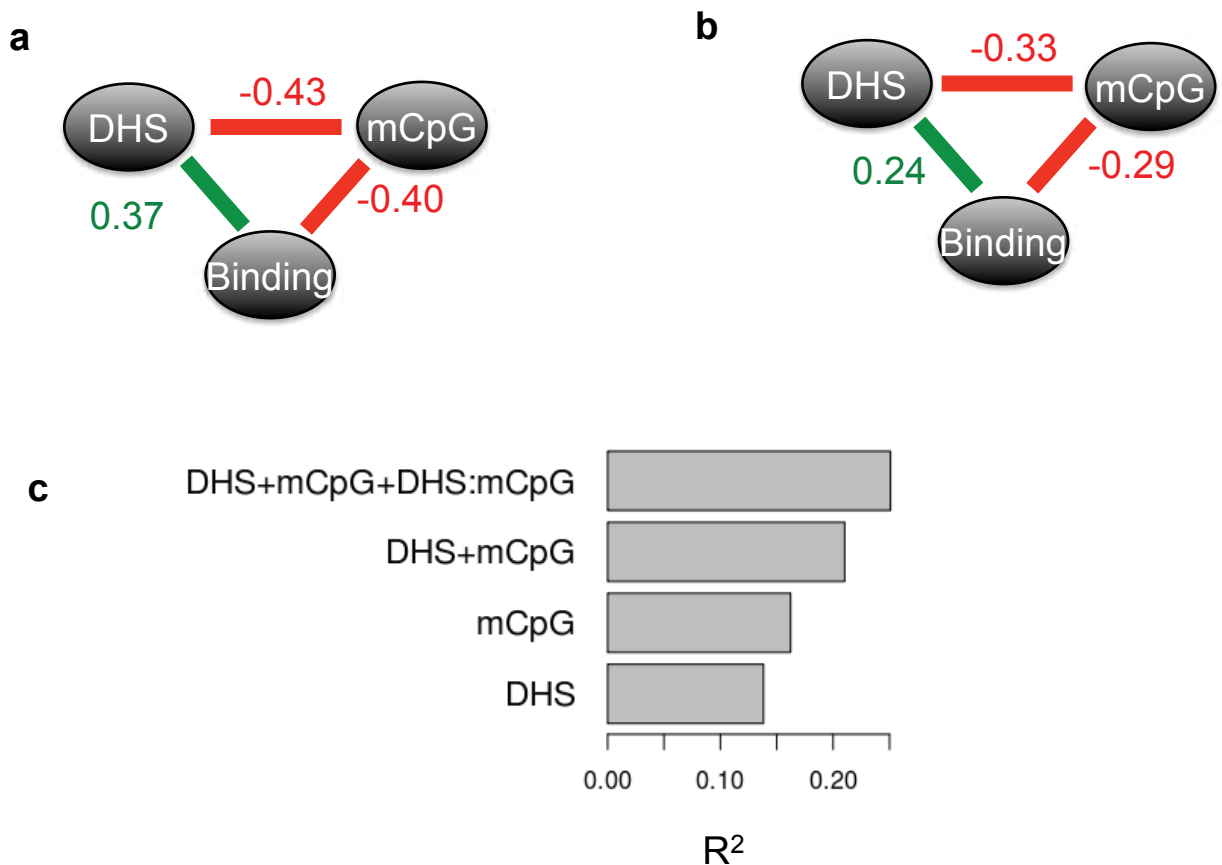
Supplementary Figure 5 | ChIP signals in HEK293FT cells. ChIP read density at four off-targets (a-d) and on-targets (e). Sequences of the guide match and PAM are shown in (f), with mismatches highlighted in red. The four tracks are: input DNA, dCas9 transfected with no sgRNA, dCas9 transfected with EMX1-sg1, and dCas9 transfected with EMX1-sg3.



Supplementary Figure 6 | Gel shift assay for NAG substrates. The assay were done under the same condition as Fig. 2c. Sequences are shown at the bottom, with AG in pink and guide-matched bases in blue. Gels for NGG substrates were taken from Fig. 2c for comparison.

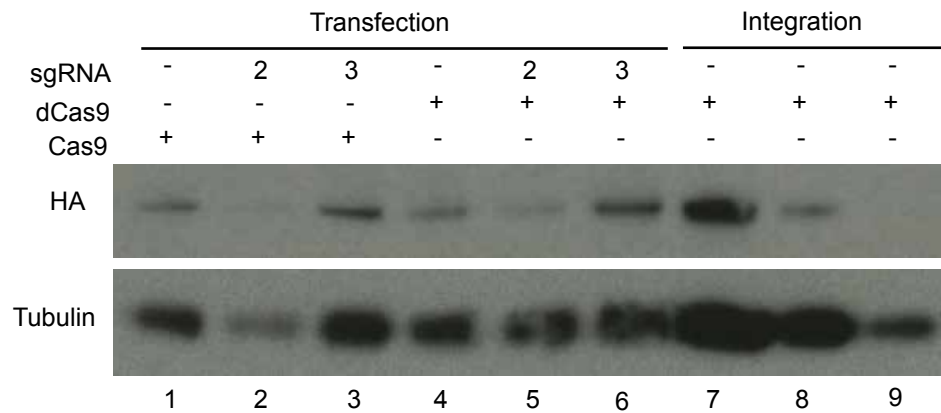


Supplementary Figure 7 | Scatter and histogram plots of guide match and relative binding for sgRNA Phc1-sg1, Phc1-sg2, and Nanog-sg2. The legends were the same as Fig 3a.



Supplementary Figure 8 | CpG methylation is negatively correlated with DHS and ChIP signals. (a) Pearson correlation coefficients between DHS, CpG methylation, and binding. (b) Partial Pearson correlation coefficients between DHS, CpG methylation, and binding. (c) The fraction of variation in binding explained by DHS, CpG methylation, DHS and CpG methylation without interaction, or DHS and CpG methylation with interaction.

a



b

Study	Plates	Cas9 plasmid used per well (ug)	10 cm equivalent (ug)
Schwank et al, 2013, Cell Stem Cell	48-well	0.7	38.5
Cong et al, 2013, Science	24-well	0.8	22
This study	10-cm	20	20
Hsu et al, 2013, Nature Biotechnology	24-well	0.5	13.7
Pattanayak et al, 2013, Nature Biotechnology	6-well	1	6.1

Supplementary Figure 9 | Cas9/dCas9 expression. (a) Western blot using lysates from cells with either transiently transfected Cas9 (lanes 1-3), or dCas9 (lanes 4-6), or cells stably integrated with dCas9 (lanes 7-9). All Cas9/dCas9 proteins contain an HA tag. Tubulin was used as loading control. Cells for lanes 2 and 5 were also transfected with Nanog-sg2, and lanes 3 and 6 were from cells transfected with Nanog-sg3. Lanes 7-9 were the same lysate with 1:1, 1:2, and 1:4 dilution. After normalizing to the loading control, the HA band in lane 1 is about 2.6 times of the HA band in lane 8, suggesting that the expression of dCas9 in our stable cells is much lower than the cells with transiently transfected dCas9. (b) Comparison of Cas9 plasmid used in various studies, including the references, type of plates used, and the amount of Cas9/dCas9 plasmids transfected per well, and the equivalent amount on a 10 cm plate based on the area on each plate.