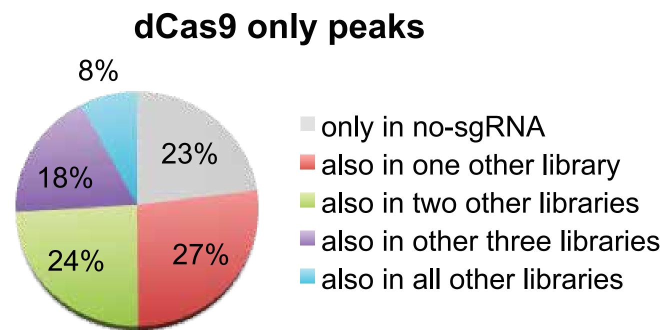
a

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

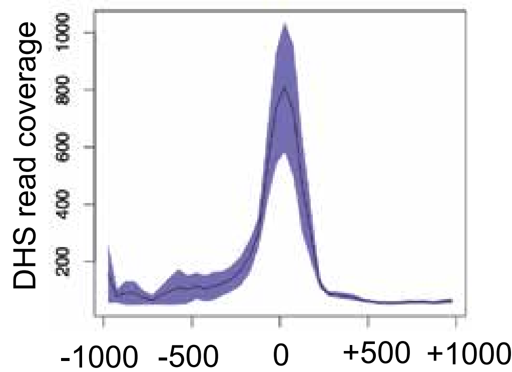
b



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.

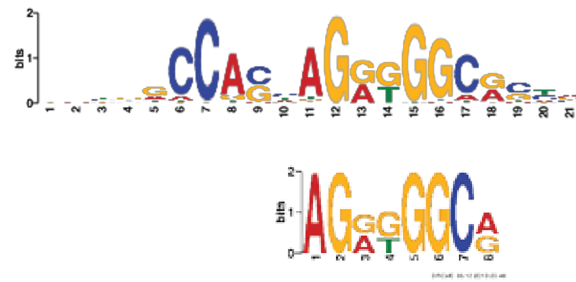
a

peaks enrich for open chromatin



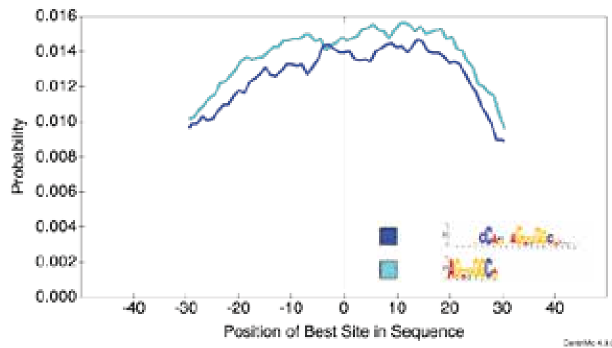
b

861 (41%) peaks contain GC−rich motif



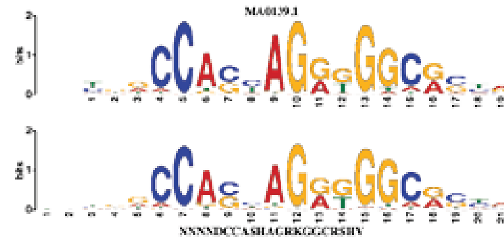
c

The GC-rich motif is enriched at the center



d

The GC-rich motif matches CTCF motif



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

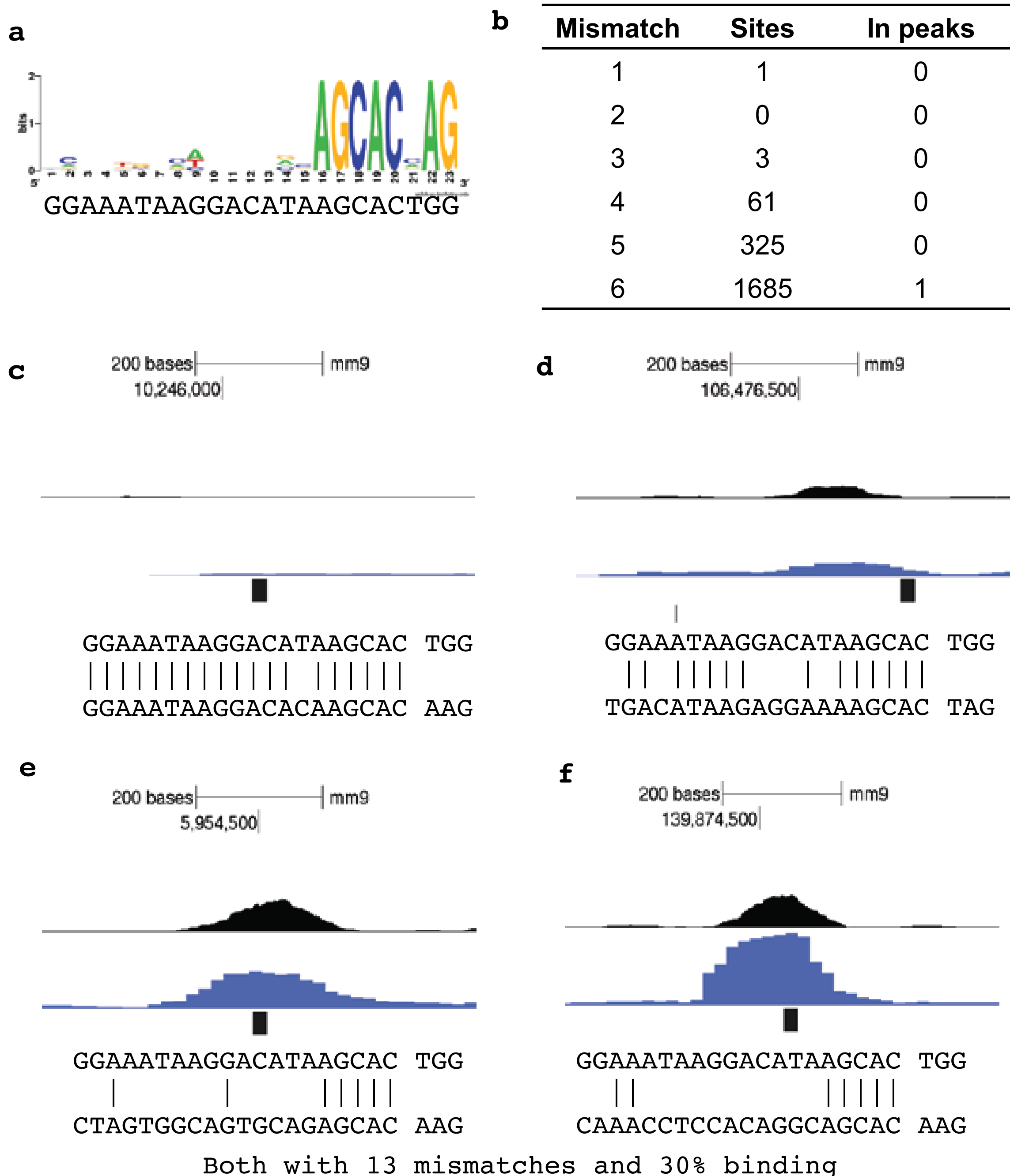
Phc1-sg2

GATGACGAAACCAATGGTACTGG

Nanog-sg3

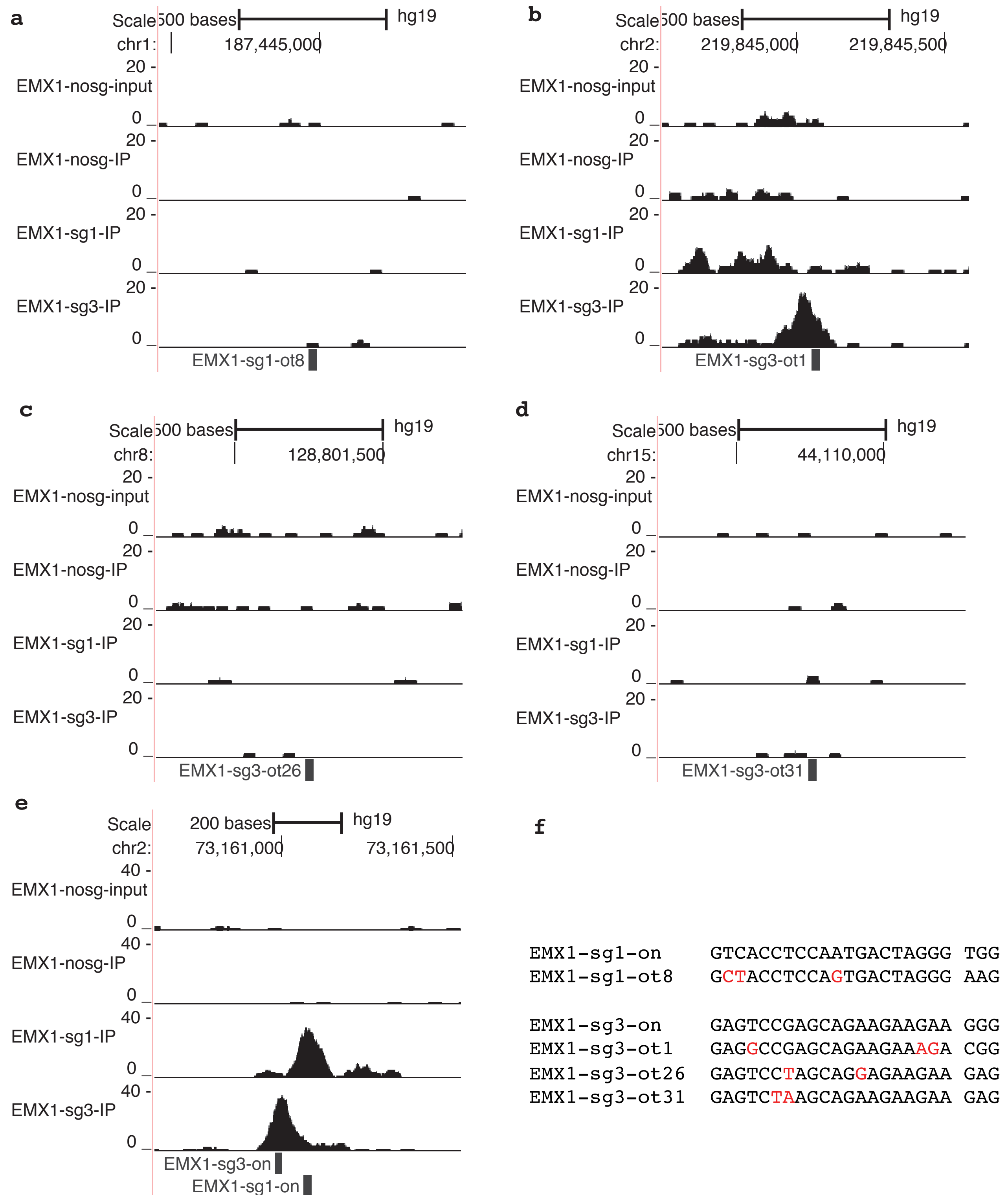
GTCTGTAGAAAGAATGGAAGAGG

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.

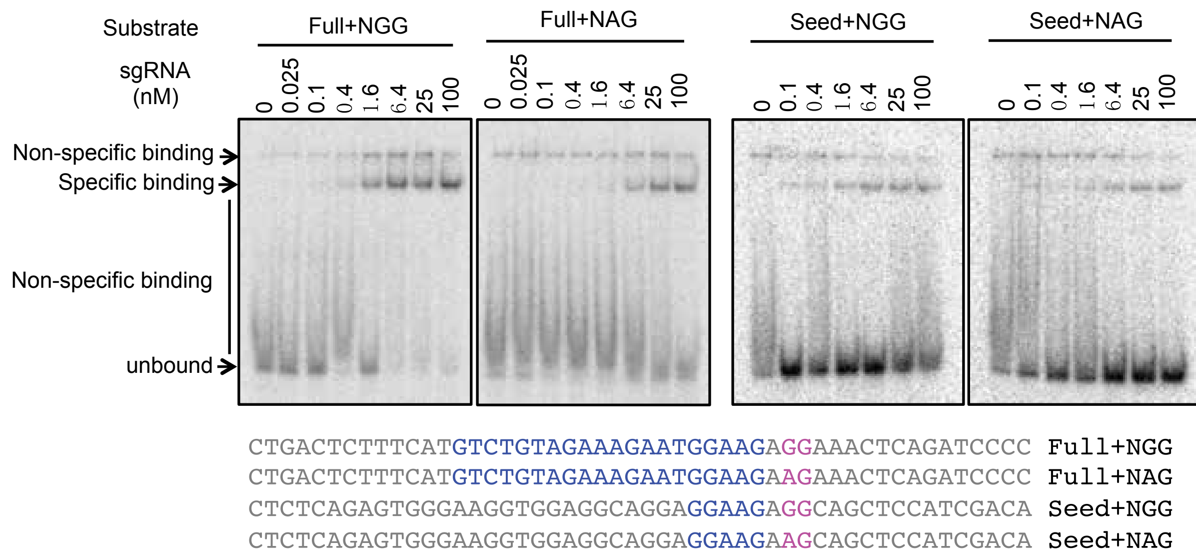


Mismatch	Sites	In peaks
1	1	0
2	0	0
3	3	0
4	61	0
5	325	0
6	1685	1

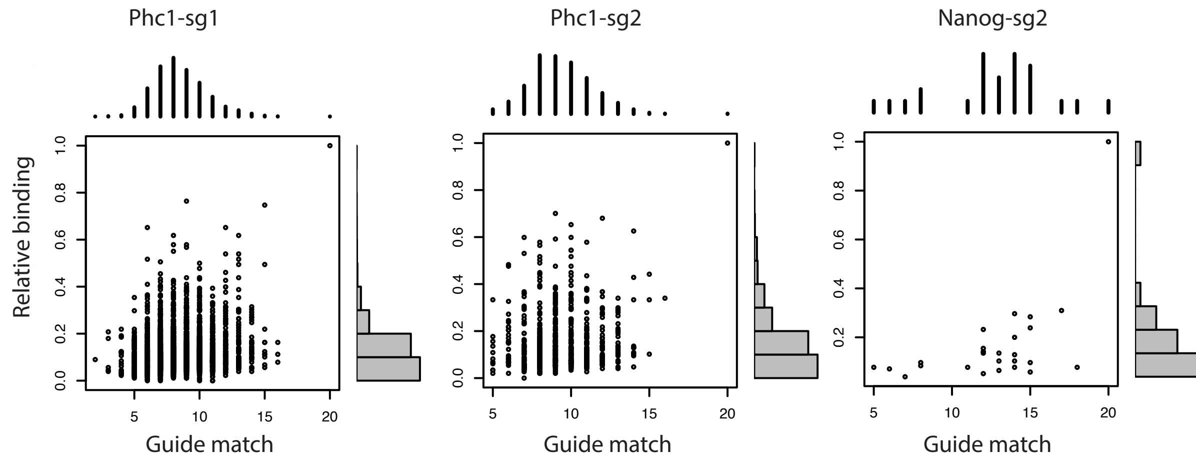
Supplementary Figure 4 | Lack of ChIP enrichment at seed+NAG sites. (**a**) of the 996 (33%) Phc1-sg1 ChIP peaks without seed+NGG sites, only 18 (1.8%) contain seed+NAG (AGCACNGG) within 50bp of peak summits, which is not higher than random (2.7%). Motif logo of the 18 NAG peaks showing the lack of base pairing outside the seed region. (**b**) The number of seed+NAG sites in the genome (column 2) and within ChIP peaks (column 3) that contain specific number of mismatches in the guide region (column 1). None of the seed+NAG sites with less than 6 mismatches showed ChIP signals strong enough to be defined as peaks. (**c**) The best match contains only 1 mismatch and showed no ChIP signals. (**d**) Only one site with 6 mismatches is within a peak, yet the seed+NAG site is not in the center of the peak. (**e-f**) Similar to (**c-d**) but showing the two strongest peaks that are associated with seed+NAG sites. For (**c-f**), the top track is ChIP signal, and the bottom track is open chromatin. The scale is the same as Fig. 2b.



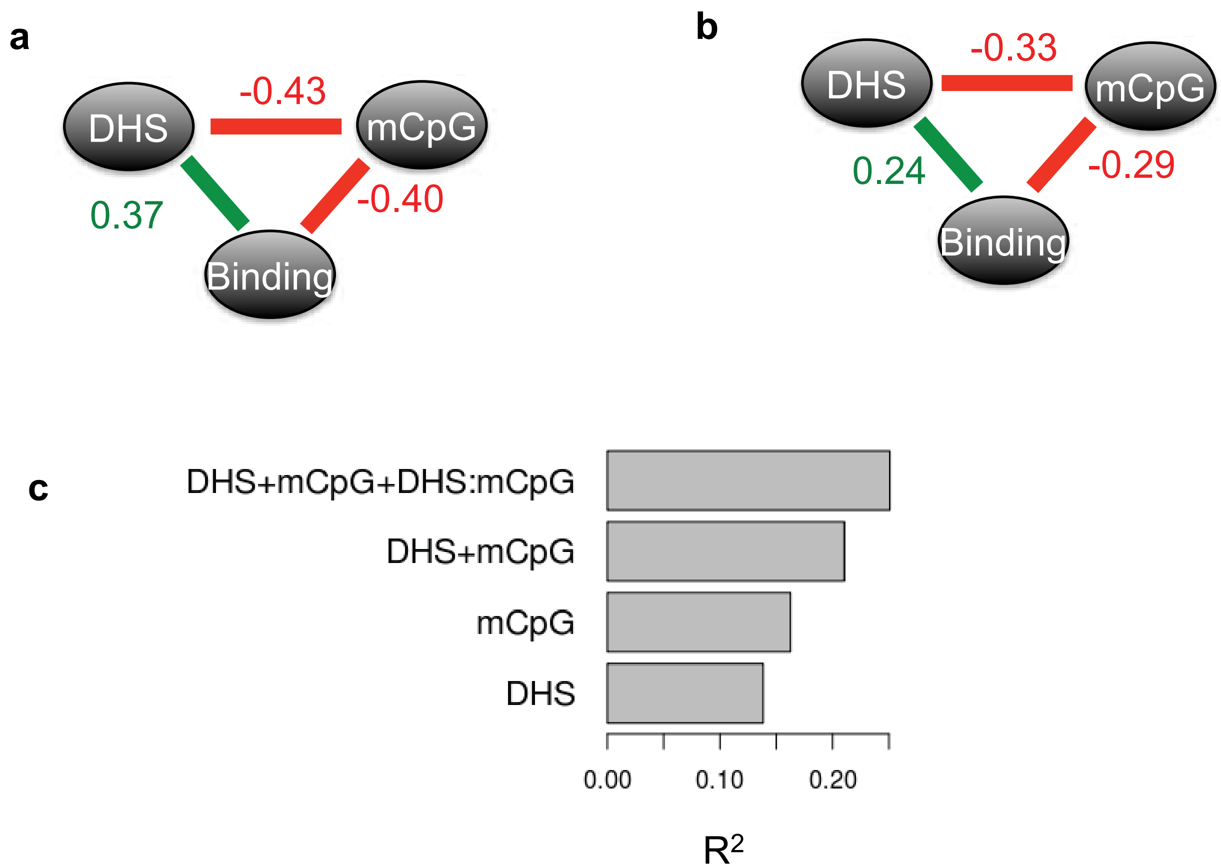
Supplementary Figure 5 | ChIP signals in HEK293FT cells. ChIP read density at four off-targets (**a-d**) and on-targets (**e**). Sequences of the guide match and PAM are shown in (**f**), with mismatches highlighted in red. The four tracks are: input DNA, dCas9 transfected with no sgRNA, dCas9 transfected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

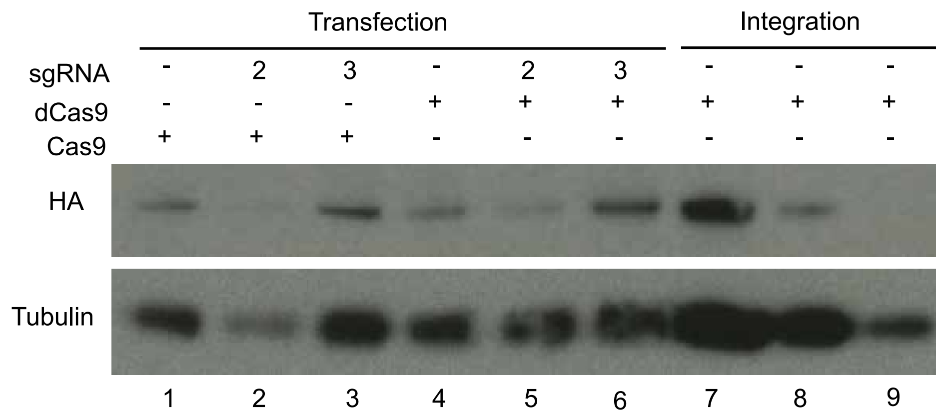


Supplementary Figure 7 | Scatter and histogram plots of guide match and relative binding for sgRNA Phc1-sg1, Phc1-sg2, and Nanog-sg2. The legends were the same as Fig 3a.



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.