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Supplemental Material to:

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Human and mouse ZFP57 proteins are functionally interchangeable in maintaining genomic imprinting at multiple imprinted regions in mouse ES cells

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Supplemental Figure Legends

Figure 1S. Bisulphite sequencing result showing the unique clones for the IG-DMR of the *Dlk1-Dio3* imprinted region

Genomic DNA samples from the ES clones plated on the gelatin-coated plates were subjected to bisulphite mutagenesis, PCR amplification and bacterial colony sequencing. Filled circle, a methylated CpG. Unfilled circle, an unmethylated CpG. Cross (X), a CpG site that its methylation status cannot be determined. Each row stands for a unique clone with a distinctive CpG methylation pattern or unique unconverted non-CpG sites after bisulphite mutagenesis. Unique clones were derived from different starting template DNA molecules. The number to the right of a unique clone indicates the number of the sequenced bacterial colonies for each bisulphite DNA sample that displayed an identical CpG methylation pattern and did not have unique unconverted non-CpG sites. These are called non-unique clones. Percentage of methylated CpG sites based on just the unique clones is listed directly below the diagram illustrating methylated and unmethylated CpG sites. Each unique clone was only counted once for calculation of percentage of DNA methylation even if it contained multiple non-unique clones. Numbers 1 to 19 indicate the same ES clones that were subjected to COBRA analysis in Figure 4.

A, four independent *Zfp57*-null mouse ES clones expressing the wild-type human ZFP57-3XFLAG protein (hZFPWT).

B, four independent *Zfp57*-null mouse ES clones expressing the R248H mutant human ZFP57-3XFLAG protein.

C, four independent *Zfp57*-null mouse ES clones expressing the H277N mutant human ZFP57-3XFLAG protein.

D, four independent *Zfp57*-null mouse ES clones expressing the H458D mutant human ZFP57-3XFLAG protein.

E, control (Ctrl) ES clones. 17 and 18, two independent *Zfp57*-null mouse ES clones expressing mouse ZFP57 tagged with a Myc epitope and six histidines at the C-terminal end. 19, parental (P) mouse ES cells with two floxed alleles of *Zfp57*.

Figure 2S. Bisulphite sequencing result showing the unique clones for the *Snrpn* DMR region

Genomic DNA samples from the ES clones plated on the gelatin-coated plates were subjected to bisulphite mutagenesis, PCR amplification and bacterial colony sequencing. Filled circle, a methylated CpG. Unfilled circle, an unmethylated CpG. Cross (X), a CpG site with unknown methylation status. Each row stands for a unique clone. The number to the right of a unique clone indicates the number of the non-unique clones within this group. Percentage of methylated CpG sites based on just the unique clones is listed directly below the diagram illustrating methylated and unmethylated CpG sites. Numbers 1 to 19 indicate the same ES clones that were subjected to COBRA analysis in Figure 4.

A, four independent *Zfp57*-null mouse ES clones expressing the wild-type human ZFP57-3XFLAG protein (hZFPWT).

B, four independent *Zfp57*-null mouse ES clones expressing the R248H mutant human ZFP57-3XFLAG protein.

C, four independent *Zfp57*-null mouse ES clones expressing the H277N mutant human ZFP57-3XFLAG protein.

D, four independent *Zfp57*-null mouse ES clones expressing the H458D mutant human ZFP57-3XFLAG protein.

E, control (Ctrl) ES clones. 17 and 18, two independent *Zfp57*-null mouse ES clones expressing mouse ZFP57 tagged with a Myc epitope and six histidines at the C-terminal end. 19, parental (P) mouse ES cells with two floxed alleles of *Zfp57*.

Figure 3S. Bisulphite sequencing result showing the unique clones for the *Zac1* DMR region

Genomic DNA samples from the ES clones plated on the gelatin-coated plates were subjected to bisulphite mutagenesis, PCR amplification and bacterial colony sequencing. Filled circle, a methylated CpG. Unfilled circle, an unmethylated CpG. Cross (X), a CpG site with unknown methylation status. Each row stands for a unique clone. The number to the right of a unique clone indicates the number of the non-unique clones within this group. Percentage of methylated CpG sites based on just the unique clones is listed directly below each panel. Numbers 1 to 19 indicate the same ES clones that were subjected to COBRA analysis in Figure 4.

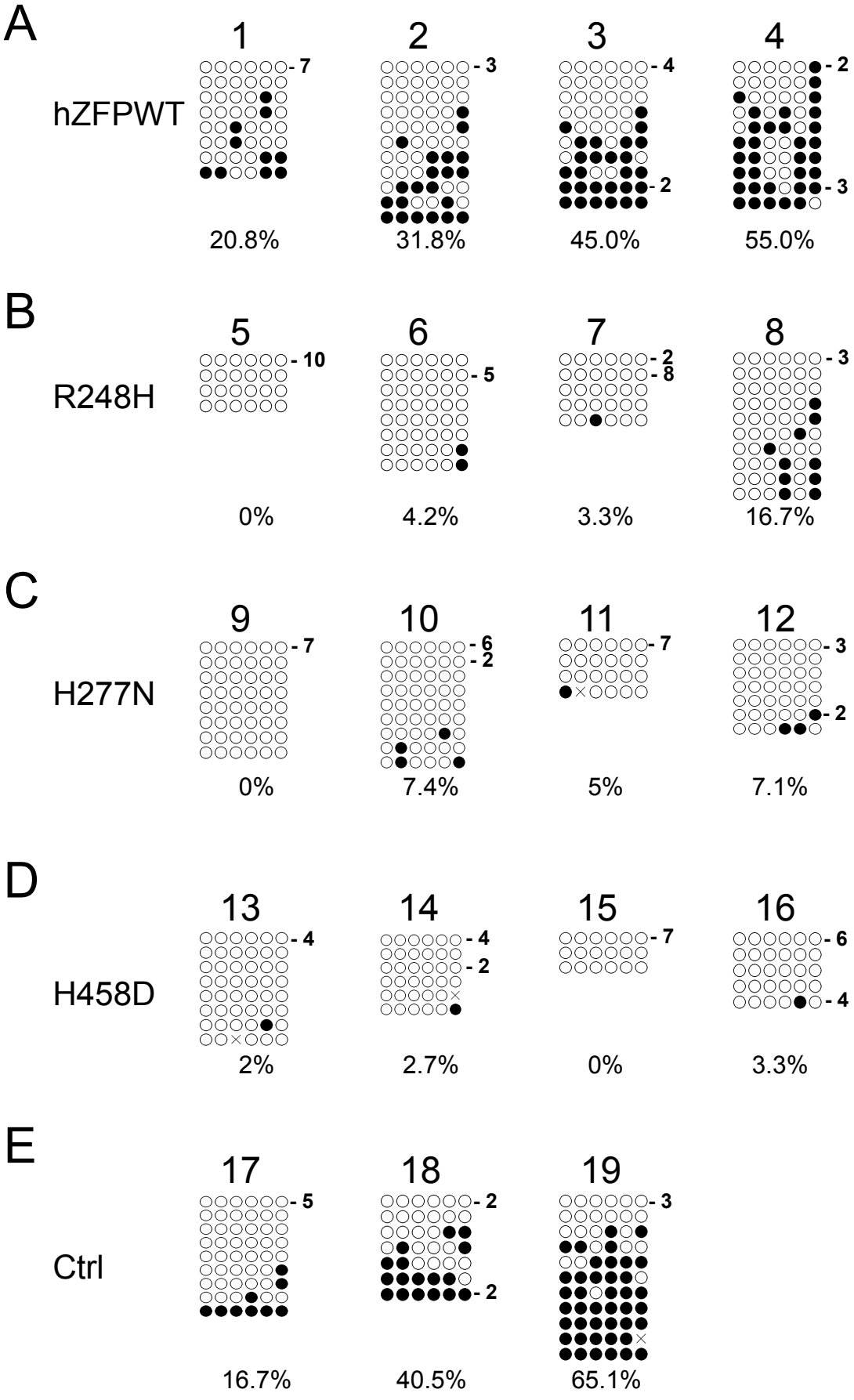
A, four independent *Zfp57*-null mouse ES clones expressing the wild-type human ZFP57-3XFLAG protein (hZFPWT).

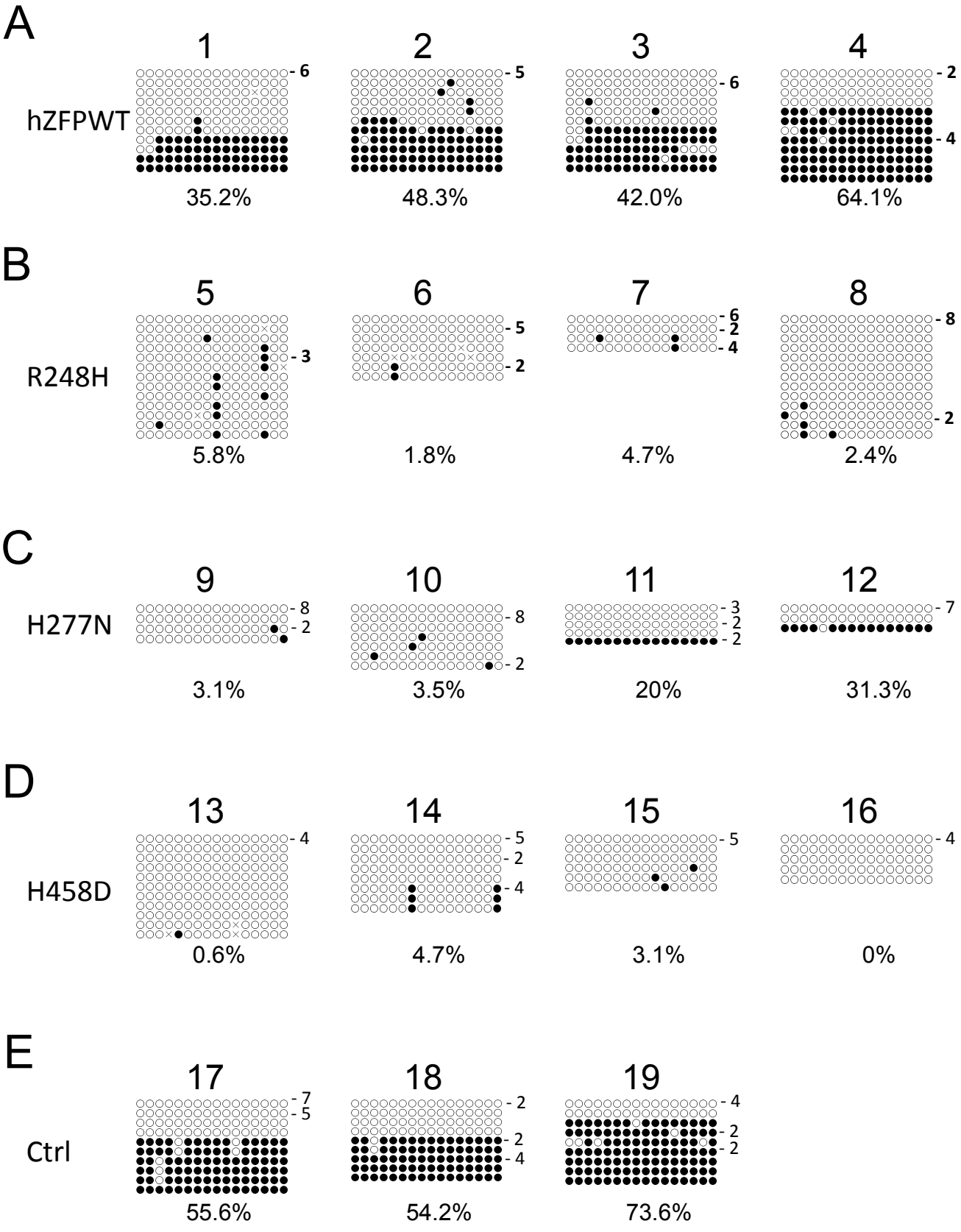
B, four independent *Zfp57*-null mouse ES clones expressing the R248H mutant human ZFP57-3XFLAG protein.

C, four independent *Zfp57*-null mouse ES clones expressing the H277N mutant human ZFP57-3XFLAG protein.

D, four independent *Zfp57*-null mouse ES clones expressing the H458D mutant human ZFP57-3XFLAG protein.

E, control (Ctrl) ES clones. 17 and 18, two independent *Zfp57*-null mouse ES clones expressing mouse ZFP57 tagged with a Myc epitope and six histidines at the C-terminal end. 19, parental (P) mouse ES cells with two floxed alleles of *Zfp57*.


A
hZFPWT
1
- 7
20.8%
2
- 3
31.8%
3
- 4
- 2
45.0%
4
- 2
- 3
55.0%
B
R248H
5
- 10
0%
6
- 5
4.2%
7
- 2
- 8
3.3%
8
- 3
16.7%
C
H277N
9
- 7
0%
10
- 6
- 2
7.4%
11
- 7
5%
12
- 3
- 2
7.1%
D
H458D
13
- 4
2%
14
- 4
- 2
2.7%
15
- 7
0%
16
- 6
- 4
3.3%
E
Ctrl
17
- 5
16.7%
18
- 2
- 2
40.5%
19
- 3
65.1%


A
1
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3
4
hZFPWT
- 6
- 5
- 6
- 2
- 4
35.2%
48.3%
42.0%
64.1%
B
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7
8
R248H
- 3
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- 2
- 6
- 2
- 4
- 8
- 2
5.8%
1.8%
4.7%
2.4%
C
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H277N
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- 8
- 2
- 3
- 2
- 2
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H458D
- 4
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- 5
- 4
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- 7
- 5
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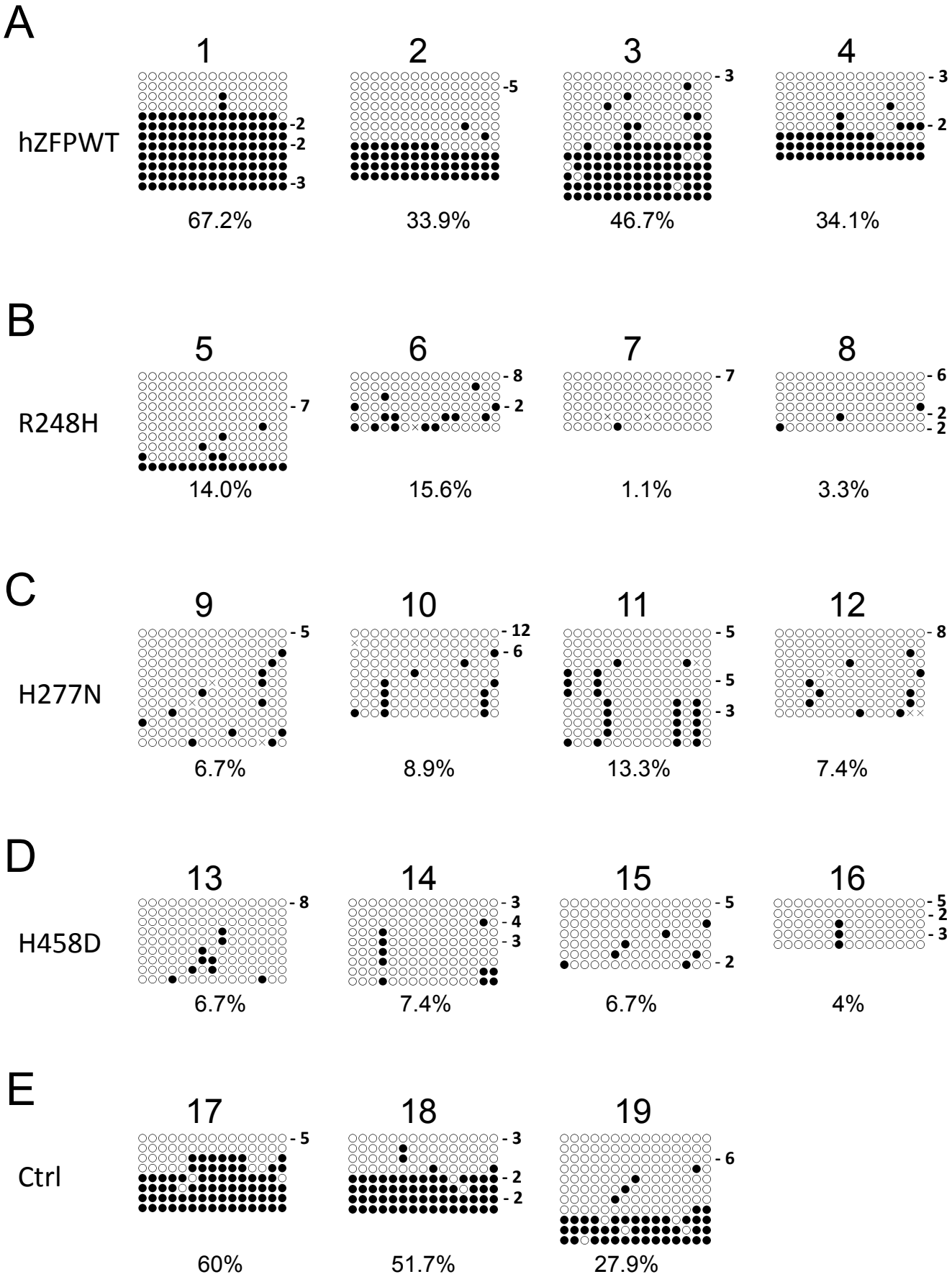
- 2
- 4
- 4
- 2
- 2
55.6%
54.2%
73.6%


A
1
2
3
4
hZFPWT
-2
-2
-3
-5
-3
-3
-2
67.2%
33.9%
46.7%
34.1%
B
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6
7
8
R248H
-7
-8
-2
-7
-6
-2
-2
14.0%
15.6%
1.1%
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H277N
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7.4%
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H458D
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-5
-2
-3
6.7%
7.4%
6.7%
4%
E
17
18
19
Ctrl
-5
-3
-2
-2
-6
60%
51.7%
27.9%