**Supplementary information for**

# Sperm epigenetic alterations contribute to inter- and transgenerational effects of paternal exposure to long-term psychological stress via evading offspring embryonic reprogramming

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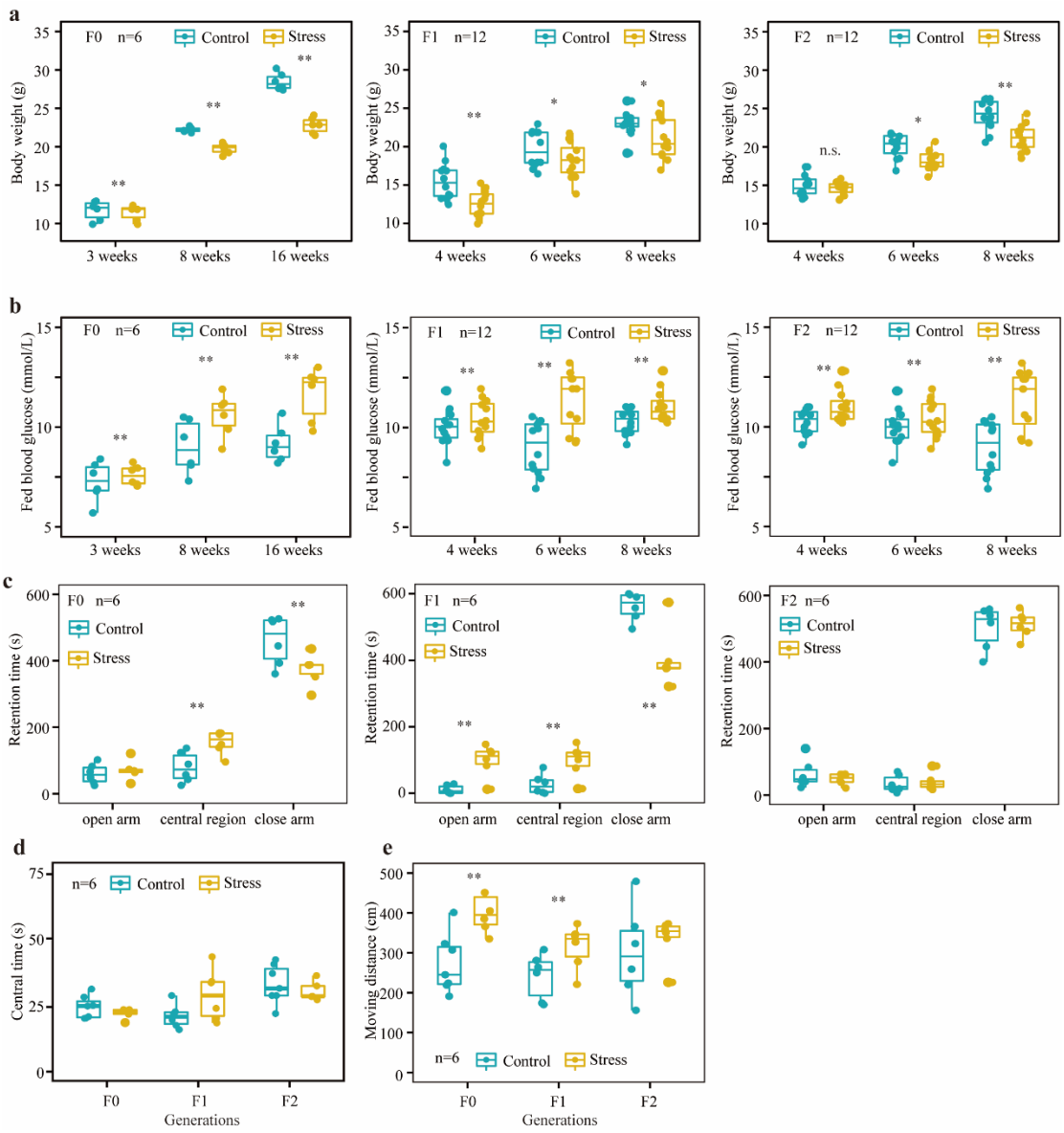
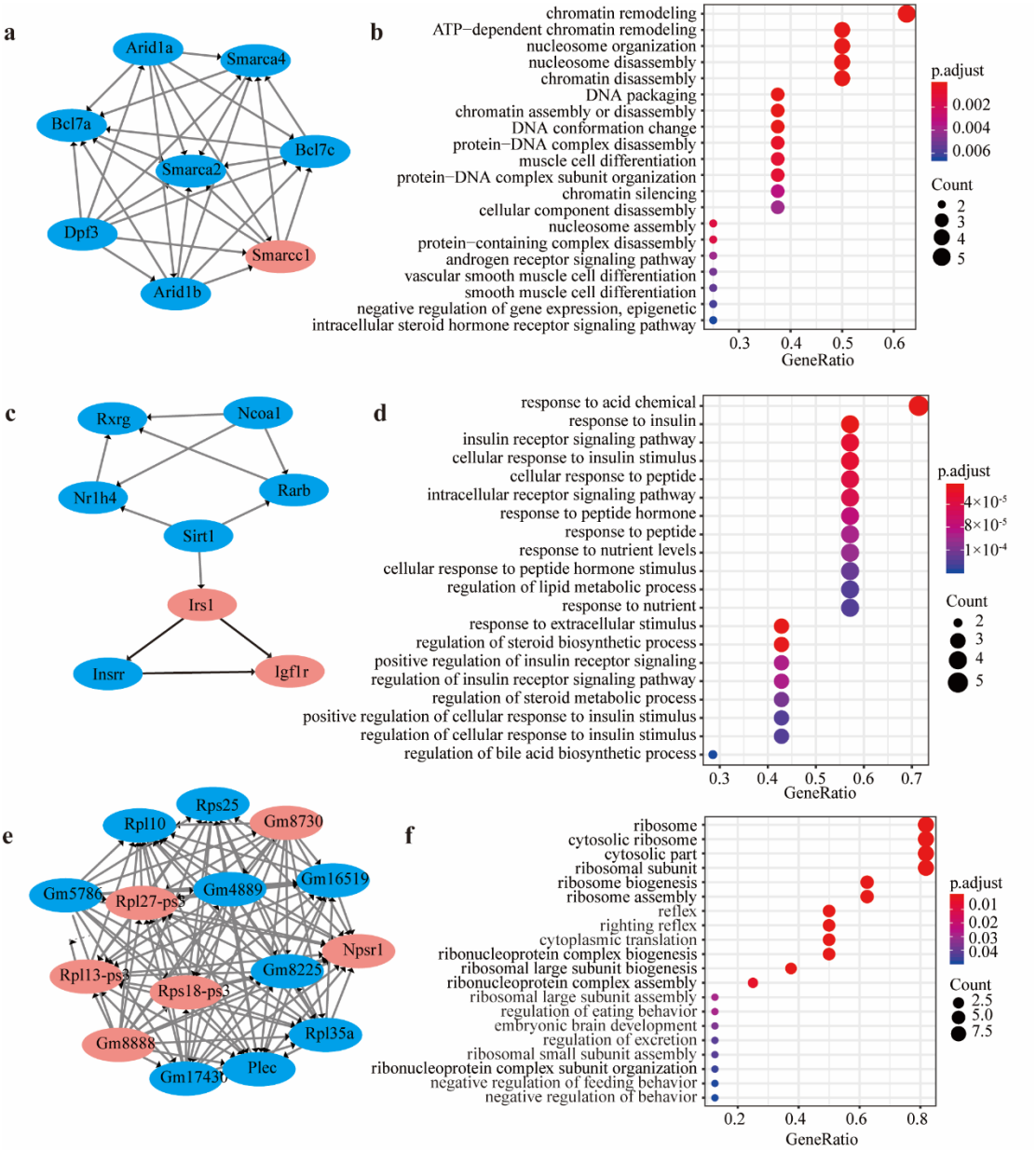
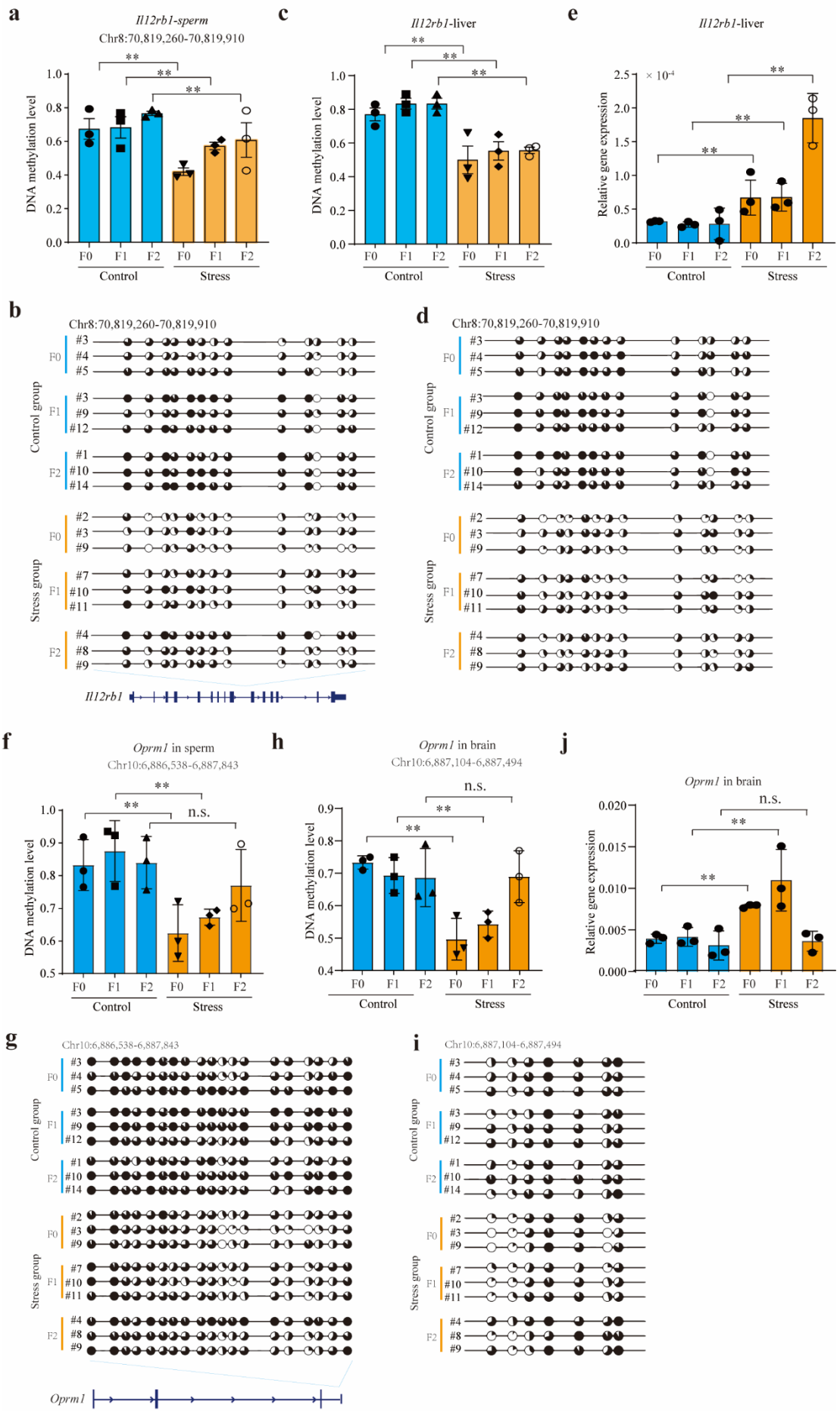


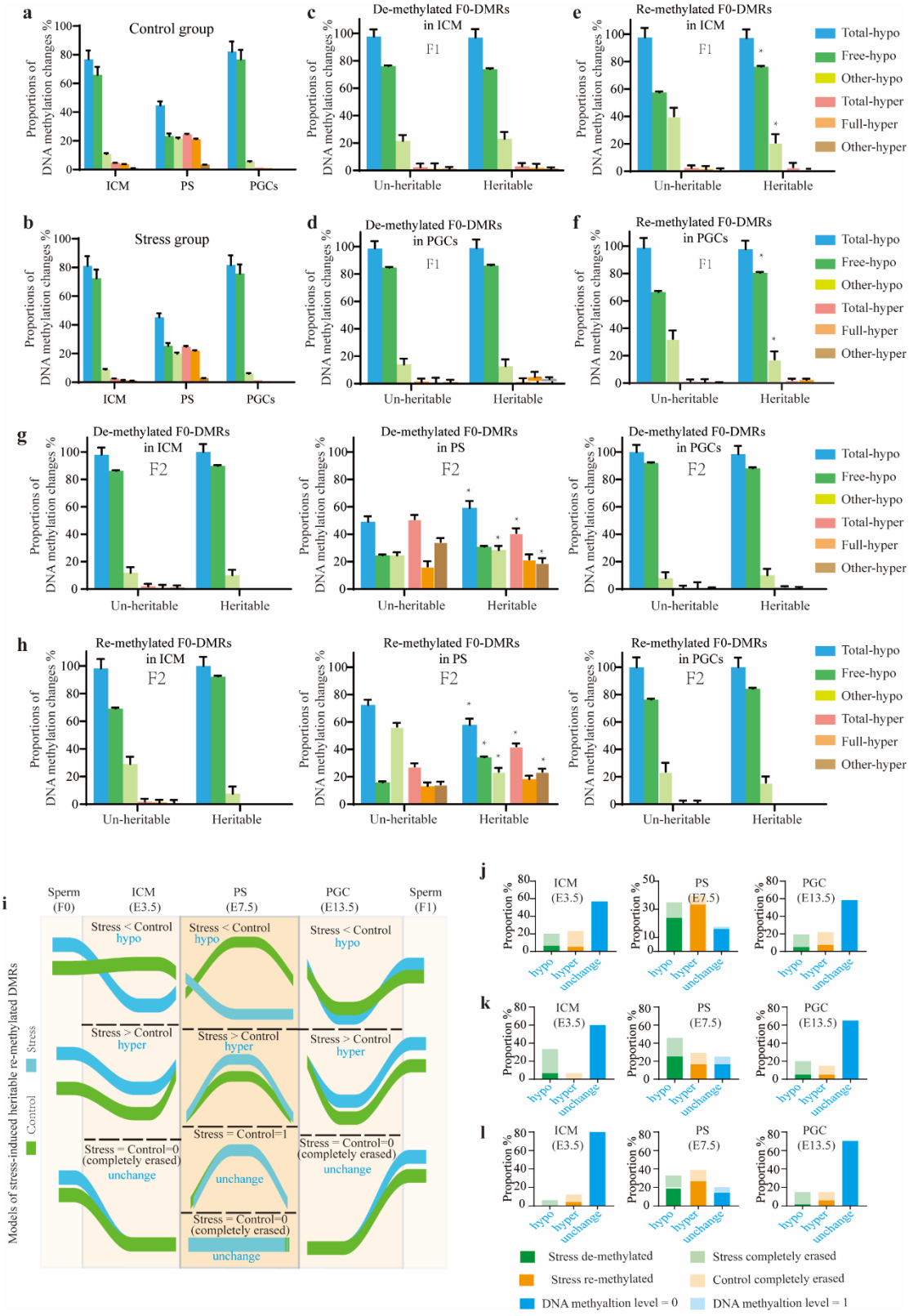

**Supplementary Fig. S1. Chronic psychological stress induced paternal inheritance of developmental, metabolic, behavioral, and reproductive disorders in mice.** **a**, Stress-induced transgenerational inheritance of developmental retardation. **b**, Stress-induced transgenerational inheritance of disorder in blood glucose metabolism. **c**, Stress-induced intergenerationally inherited behavioral disorder. Elevated plus maze (EPM) test to assess anxiety-like behavior of the mice. **d**, Open field test to assess anxiety-like behavior of the mouse. **e**, Difference in moving distances between stress and control groups during the open field test. **: *t*-test *p*-value <0.01; n.s.: no significant, *t*-test *p*-value >0.05.




**Supplementary Fig. S2. Stress-induced DMRs associate with paternally inherited phenotypes.** **a**, Interaction of the genes that enriched in MCODE subgroup 1. Red nodes represented genes that related to re-methylated DMRs, while blue nodes represented genes that related to de-methylated DMRs. **b**, Gene Ontology (GO) analysis of the genes in subgroup 1. **c**, Interaction of the genes that enriched in subgroup 2. **d**, GO analysis of the genes in the subgroup 2. **e**, Interaction of the genes that enriched in subgroup 3. **f**, GO analysis of the genes in the subgroup 3.

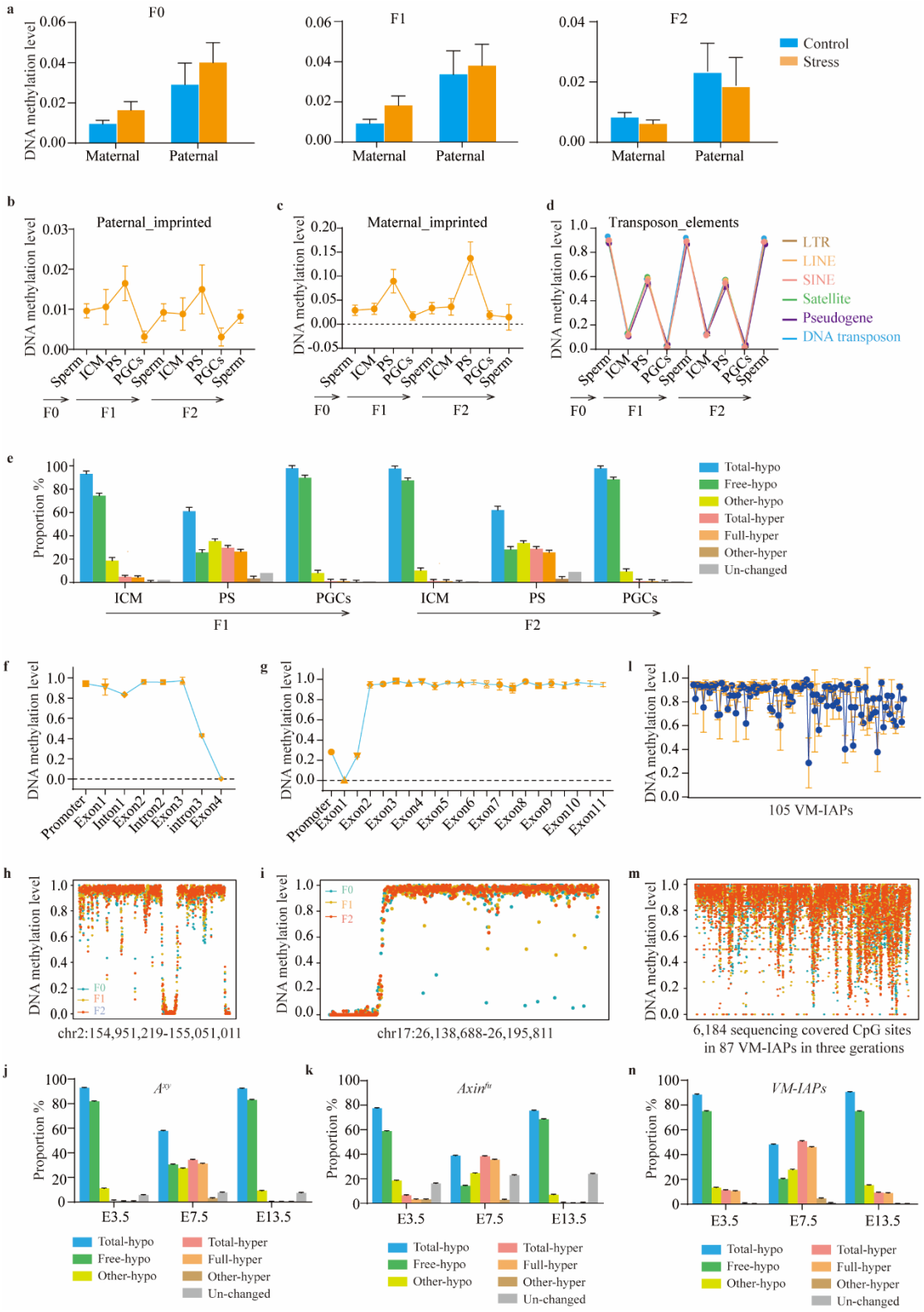

a
Il12rb1-sperm
Chr8:70,819,260-70,819,910
DNA methylation level
F0 F1 F2 F0 F1 F2
Control
Stress
c
Il12rb1-liver
DNA methylation level
e
Il12rb1-liver
Relative gene expression
× 10⁻⁴
b
Chr8:70,819,260-70,819,910
Control group
Stress group
Il12rb1
d
Chr8:70,819,260-70,819,910
f
Oprm1 in sperm
Chr10:6,886,538-6,887,843
n.s.
h
Oprm1 in brain
Chr10:6,887,104-6,887,494
j
Oprm1 in brain
g
Chr10:6,886,538-6,887,843
Oprm1
i
Chr10:6,887,104-6,887,494

**Supplementary Fig. S3. Epigenetically inherited DMRs not only were transmitted to sperm, but also were inherited by tissues, and subsequently mediated expression alterations of their related genes.** DNA methylation levels **(a)** and patterns **(b)** of a transgenerationally-inherited de-methylated DMR, Chr8:70,818,260-70,819,910, in sperm samples of all generations. DNA methylation levels **(c)** and patterns **(d)** of this DMR in liver tissues. **e**, Expression patterns of the *Il12rb1* in liver tissues. DNA methylation levels **(f)** and patterns **(g)** of an intergenerationally-inherited de-methylated DMR, Chr10:6,886,538-6,887,843, in sperm samples of all generations. DNA methylation levels **(h)** and patterns **(i)** of this DMR in brain tissues. **j**, Expression patterns of the *Oprm1* in brain tissues. **: *t*-test p-value <0.01; n.s.: no significant, *t*-test *p*-value >0.05.




**Supplementary Fig. S4. DNA methylation reestablishment proportions and levels of the heritable DMRs have been altered in the PS stage.** DNA methylation alterations in embryo samples in in comparative analysis with the paternal sperm in control group **(a)** and stress group **(b)**. Dynamic DNA methylation patterns of both heritable and un-heritable de-methylated F0-DMRs in F1-ICM **(c)** and F1-PGC **(d)**. Dynamic DNA methylation patterns of both heritable and un-heritable re-methylated F0-DMRs

in F1-ICM **(e)**, F1-PGC **(f)**. Dynamic DNA methylation patterns of both heritable and un-heritable de-methylated F0-DMRs **(g)** and re-methylated F0-DMRs **(h)** in all three embryonic stages of the F2 generation. **i**, Dynamic DNA methylation models of the stress-induced heritable re-methylated DMRs during reprogramming process. In each stage, all DMRs were classified into three types: hypo (stress group was de-methylated), hyper (stress group was re-methylated), and unchanged (there was no difference between two groups). **j**, Proportion of each DMR type in each embryo stage of the heritable re-methylated DMRs in the F1 generation. **k**, Proportion of each DMR type in each embryo stage of the heritable de-methylated DMRs in the F2 generation. **l**, Proportion of each DMR type in each embryo stage of the heritable re-methylated DMRs in the F2 generation.




**Supplementary Fig. S5. Dynamic DNA methylation patterns of the imprinted genes and transposable elements during reprogramming. a,** DNA methylation levels of both paternal and maternal imprinted genes in all three generations. DNA methylation patterns of the paternal imprinted genes **(b)**, maternal imprinted genes **(c)** and transposable elements **(d)** in sperm and embryo samples of both $F_1$ and $F_2$ generations. **e,** Dynamic DNA methylation patterns of the transposable elements during reprogramming. DNA methylation patterns of the *Agouti* locus **(f)** and the *Fused* locus

**(g)**. Distribution of the DNA methylation levels of all CpG sites in the *Agouti* locus **(h)** and the *Fused* locus **(i)** in all three generations. Dynamic DNA methylation patterns of the *Agouti* locus **(j)** and the *Fused* locus **(k)** during reprogramming. **l,** Distribution of the DNA methylation levels of the 105 variably methylated IAPs (VM-IAPs). **m,** Distribution of the DNA methylation levels of all CpG sites in these VM-IAPs. **n,** Dynamic DNA methylation patterns of these VM-IAPs during reprogramming.