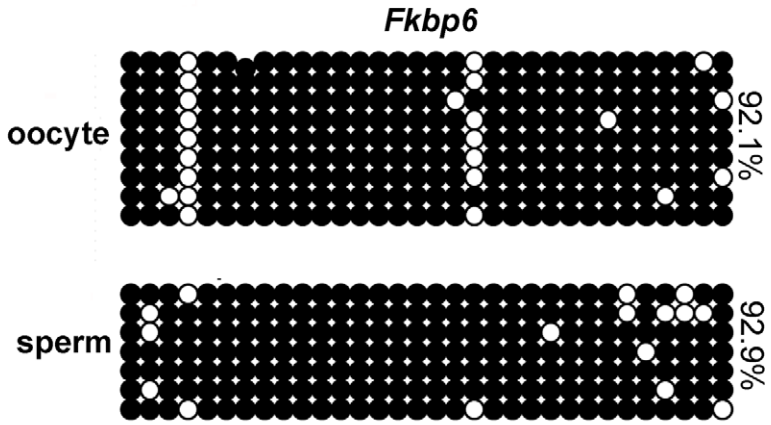

Fig. S1. Clonal analysis of methylation at *Fkbp6* in WT sperm and oocytes. Bisulphite sequencing shows that *Fkbp6* is highly methylated in oocyte as has been previously reported, but also confirms COBRA results suggesting that the gene is methylated in sperm collected from cauda epididymis.

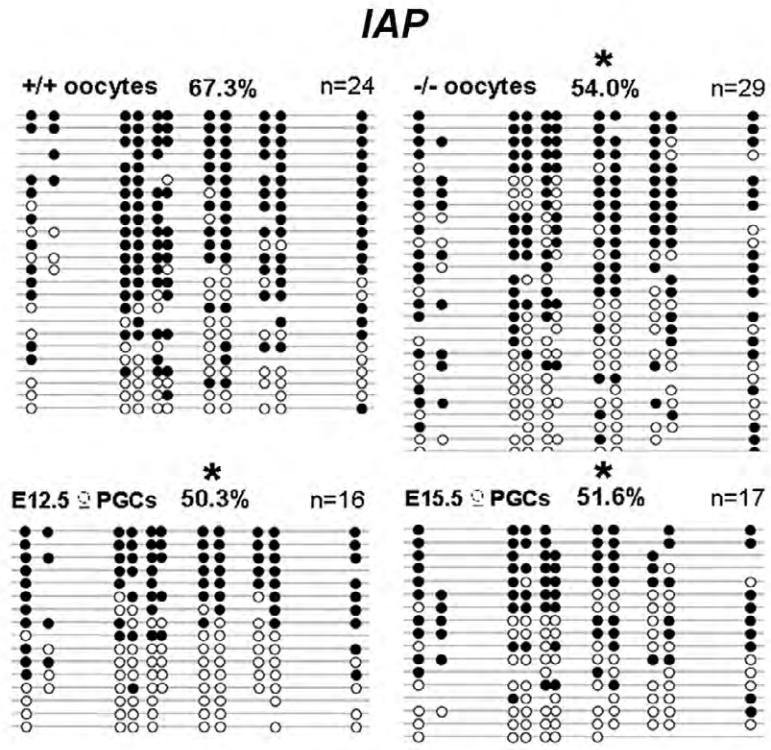

Fig. S2. Clonal analysis of methylation at IAP in WT primordial germ cells, WT oocytes and *Dnmt3l*$^{-/-}$ oocytes. Bisulphite sequencing shows that loss of DNMT3L from murine oocytes causes the methylation of IAP sequences to be reduced to a level comparable to that seen during methylation reprogramming in female PGCs at embryonic day (E) 12.5 and E15.5. WT and *Dnmt3L* KO oocytes indicated by +/+ and −/−, respectively. Asterisk denotes a significant difference in methylation (P<0.05) compared to +/+ sample, as determined by Chi-squared test. The number of sequences analysed for each sample is indicated.

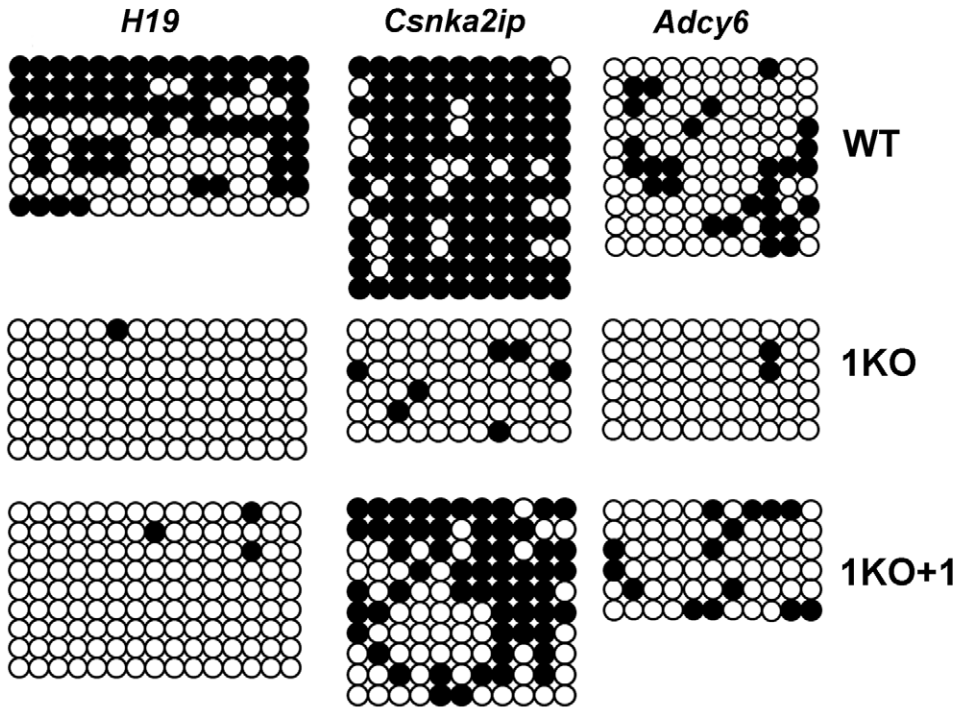

Fig. S3. Additional bisulphite sequencing data indicating methylation rescue on non-imprinted genes. Genes from each group (*H19*- imprinted; *Csnka2ip* –testis; *Adcy6*- brain) were analysed in WT ES cells, cells lacking DNMT1 (1KO) and 1KO cells rescued with an expression construct carrying Dnmt1 (1KO+1).

Table S1. Primers used for analysis of transcription and methylation levels

Application	Oligo sequence
RT-PCR	
ACTB-F	5'-gga ctt cga gca aga gat gg-3'
ACTB-R	5'-agc act gtg ttg gcg tac ag-3'
Adcy6-F	5'-gag atg gct tcg atc atg tc-3'
*Adcy*6-R	5'-cac tgt ctg agg atc aag atc-3'
Csnka2ip-F	5'-tca gct acg atc aat tca tcc-3'
Csnka2ip-R	5'-agg acg agg gtt tgg ctt tcc-3'
DAZL-F	5'-cgt gga tgt gca gaa gat ag-3'
DAZL-R	5'-aac tgt ggt gga gga gga tg-3'
Dpep3-F	5'-tgc agt ctg ttt gct aac gt-3'
Dpep3-R	5'-ggt atg tag ata cat cct cc-3'
DPEP3-F	5'-ttg acc tca ttc acc gca tg-3'
DPEP3-R	5'-tgt act gca ggt gaa ggt aag-3'
Fkbp6-F	5'-gtt cct gtt caa gcc agc ct-3'
Fkbp6-R	5'-ctg aga gtg cac aga act tg-3'
FKBP6-F	5'-gat cag cac ccc ctg aag ag-3'
FKBP6-R	5'-tct cct gct gta gaa cca tc-3'
Grin3b-F	5'-tcc tgg cag ctg cca cag ag-3'
Grin3b-R	5'-aga gca cta gag caa tgt cc-3'
Hprt-F	5'-gga gat gat ctc tca act tt-3'
Hprt-R	5'-cca aca aca aac ttg tct gg-3'
IAP-F	5'-caa agg ctt aca aga ctg gc-3'
IAP-R	5'-tgt cct ctg atc att tct gc-3'
Piwil1-F	5'-aga ctt cag aac cct ctt cc-3'
Piwil1-R	5'-gat gac att gta gtg tgt tgg-3'
PIWIL1-F	5'-cct ggc ttc act act tcc atc-3'
PIWIL1-R	5'-cca gtc aat atc atc cac tct g-3'
Rhox13-F	5'-ccg gaa tct gaa tag cag cg-3'
Rhox13-R	5-'acg aga gtg atg acg act cg-3'
RHOXF1-F	5'-gaa gtt cac gct gtt gca gg-3'
RHOXF1-R	5'-ctg atg tcg cct aca tct gg-3'
SYCP1-F	5'-cgt acc acc gag atc aag cag c-3'
SYCP1-R	5'-cct tcc tga tag tga cag tc-3'
Sycp3-F	5'-aat ctg gga agc cac ctt tg-3'
Sycp3-R	5'-ctt caa tta tcc cag cag atc-3'
SYCP3-F	5'-tct ggg aag ccg tct gtg ga-3'
SYCP3-R	5'-cca gca tat tct gca ctt cac ccc c-3'
TEX12-F	5'-tct cca gtg cca gat agt cc-3'
TEX12-R	5'-gca tct act gct gct ctc tc-3'
Tssk2-F	5'-gga tgg aag aca ggc tgg ctg-3'
Tssk2-R	5'-ctt gtt cct tgc acc tgt gcc-3'

RT-qPCR	
Adcy6-F	5’-gag atg gct tcg atc atg tc-3’
Adcy6-R	5’-cac tgt ctg agg atc aag atc-3’
Crocc-F	5’-gcg gag cgc agg ccg cta g-3’
Crocc-R	5’-agc ctg gag ctg agg ctg gag-3’
DAZL	Sequences as for RT-PCR
Dnmt1-F	5’-ggctttccagatagctaccg-3’
Dnmt1-R	5’-gcaggcagagcttaatctcc-3’
Dnmt3l-F	5’-ctc atc cct acc tac ggg ttc-3’
Dnmt3l-R	5’-cag tct tcc agt acc aca tcc-3’
Dpep3	Sequences as for RT-PCR
DPEP3	Sequences as for RT-PCR
Efna2-F	5’-cag cga ggc ttc aag cgc tg-3’
Efna2-R	5’-gag gtt ggg agg tgt ggc ag-3’
Fkbp6	Sequences as for RT-PCR
FKBP6	Sequences as for RT-PCR
Ggn1-F	5’-aag tgc tac tgc cgc cat caa c-3’
Ggn1-R	5’-gca agt cgt agt gct cca gg-3’
Grin3b-F	5’-tcc tgg cag ctg cca cag ag-3’
Grin3b-R	5’-aga gca cta gag caa tgt cc-3’
HPRT-F	5’-agc cct ggc gtc gtg att agt-3’
HPRT-R	5’-ccc gtt gag cac aca gag gcc ta-3’
IAP	Sequences as for RT-PCR
Piwil1	Sequences as for RT-PCR
PIWIL1	Sequences as for RT-PCR
Rhox9-F	5’-cga gga tga caa cat cca gg-3’
Rhox9-R	5’-tgg gga agc gat tct ctt gg-3’
Rhox13	Sequences as for RT-PCR
RHOXF1-F	5’-cta ctg cct gag tgt ata cc-3’
RHOXF1-R	5’-tca tgc cgt tct cgt ggt tc-3’
Snrpn-F	5’-tgc tac gtg ggg aga act tg-3’
Snrpn-R	5’-cct ggg gaa tag gta cac ctg-3’
Spag12-F	5’-tta caa tcc tgg atg cag tc-3’
Spag12-R	5’-att cgc aga ggc agc cgc cga a-3’
Spata16-F	5’-gca gca gga cag ttc agg ac-3’
Spata16-R	5’-cgt agg tag cat gtg acg agc-3’
SYCP1	Sequences as for RT-PCR
Sycp3	Sequences as for RT-PCR
SYCP3	Sequences as for RT-PCR
Syt2-F	5’-gga gcc aaa tgt ggc tcc ggc-3’
Syt2-R	5’-cac agc cat ggc gat cag ag-3’
Tbpl1-F	5’-aac cgg aag tga gtg gct ag-3’
Tbpl1-R	5’-gcg tcg gac gcggag aag aa-3'
TEX12	Sequences as for RT-PCR
Trim52-F	5’-taa gat ggc cac ctc tac ac-3’

Trim52-R	5’-ccg gtc ctg ctc atc ttc ct-3’
COBRA and bisulfite sequencing	
Csnka2ip-outer-F	5’-tga tat ttt ttg att tga ggg-3’
Csnka2ip-outer-R	5’-ccc taa ata tca tct aaa cta cac ct-3’
Csnka2ip-inner-F	5’-gag gtt ttt gtt tgt tat tga gtg aag-3’
Csnka2ip-inner-R	5’-atc taa atc aca aaa aaa ttc cac a-3’
Dazl-outer-F	5’-taa ttt ttt agt gtt ggt gta gg-3’
Dazl-outer-R	5’-taa atc ccc taa ccc cc-3’
Dazl-inner-F	5’-gtt tta ttt tgg ggg ttg-3’
Dazl-inner-R	5’-aaa ctc tct ttc cac cac-3’
Dpep3-outer-F	5’-gtg tta tta gga atg ttt gga g-3’
Dpep3-outer-R	5’-cac taa cac aca taa atc taa cca-3’
Dpep3-inner-F	5’-gga gta gtt agg gtg tag gtt att-3’
Dpep3-inner-R	5’-att tct aac cac taa caa acc c-3’
Fkbp6-outer-F	5’-tat agg ttt tag agg tgg aag t-3’
Fkbp6-outer-R	5’-cca aac ata aca tta aaa cca-3’
Fkbp6-inner-F	5’-ggg tgt ttg tta ttt atg gt-3’
Fkbp6-inner-R	5-cca aaa ctc cat cat cct act tta c-3’
Grin3b-outer-F	5’-gtg gaa ggt att ggg gag tat tag-3’
Grin3b-outer-R	5’-cct cct aac tct tca tcc aaa taa a-3’
Grin3b-inner-F	5’- ttt aga ggg att tta ggg tta gg-3’
Grin3b-inner-R	5’- aca act cta act ccc cta cca c-3’
H19-outer-F	5’-gag tat tta gga ggt ata aga att-3’
H19-outer-R	5’-atc aaa aac taa cat aaa ccc ct-3’
H19-inner-F	5’-gta agg aga tta tgt tta ttt ttg g-3’
H19-inner-R	5’-cct cat taa tcc cat aac tat-3’
IAP-F	5’-ttg ata gtt gtg ttt taa gtg gta aat aaa-3’
IAP-R	5’-caa aaa aaa cac cac aaa cca aaa t-3’
Piwil1-outer-F	5’-ggg tgg att tgt ata gtt gtt t-3’
Piwil1-outer-R	5’-tcc aat cct atc cta acc cc-3’
Piwil1-inner-F	5’-tgt agt ggg ttt tgg tat agg g-3’
Piwil1-inner-R	5’-acc ccc tac aac aaa ctt ctc-3’
Rhox13-outer-F	5’-agt ggg tag aaa gtt att ggt agt t-3’
Rhox13-outer-R	5’-cct cct caa aat cta aaa tat cct-3’
Rhox13-inner-F	5’-att att ttg agg gga gtg tg-3’
Rhox13-inner-R	5’-cac aac cta taa ctc ctc cac t-3’
Snrpn-outer-F	5’-tat gta ata tga tat agt tta gaa att ag-3’
Snrpn-outer-R	5’-aat aaa ccc aaa tct aaa ata ttt taa tc-3’
Snrpn-inner-F	5’-aat ttg tgt gat gtt tgt aat tat ttg g-3’
Snrpn-inner-R	5’-ata aaa tac act ttc act act aaa atc c-3’
Sycp3-outer-F	5’-agt aaa gat ggt tag gtt agg tgg-3’
Sycp3-outer-R	5’-aac cta acc cca att cct tc-3’
Sycp3-inner-F	5’-gtg agg tgt tta tta tgg aag tg-3’
Sycp3-inner-R	5’-cca act tcc tac cta aat acc c-3’

Pyrosequencing	
Adcy6-F	5'-tgt ttt ggt tta ttt gga aga agt-3'
Adcy6-R*(biotinylated)*	5'-act tat aca aat att cca atc taa aca att-3'
Adcy6-seq	5'-ggt tta ttt gga aga agt at-3'
Crocc-F	5'-ttt gtg tag ggt tga gtg g-3'
Crocc-R*(biotinylated)*	5'-ccc aaa act acc cta tct aac ac-3'
Crocc-seq	5'-gtt ggg gat agg ttt-3'
EFNA-F	5'-tgg agg tga gta tta atg att att t-3'
EFNA-R*(biotinylated)*	5'-acc cca ctc aca aat ata at-3'
EFNA-seq	5'-gtg agt att aat gat tat ttg gat-3'
Fkbp6-F	5'-tgg ttt tag tta tta att ttg tag taa tgt-3'
Fkbp6-R*(biotinylated)*	5'-aca act atc tcc aaa aac ctt ac-3'
Fkbp6-seq	5'-gtg ttt tgt ttt tgt gat aga-3'
Piwil1-F	5'-att tgg tag ttg ggg ttg tt-3'
Piwil1-R(biotinylated)	5'-ccc cct aca aca aac ttc tc-3'
Piwil1-seq	5'-ggt agt tgg ggt tgt ta-3'
GRIN3B-F	5'-agg ttt ttt agg ggt ttg tta t-3'
GRIN3B-R*(biotinylated)*	5'-ata cta caa cct act ccc ctt ac-3'
GRIN3B-seq	5'-aga ttt tgt aga tga gta ttt att-3'
In situ hybridisation	
Ddx4	Toyooka et al. (2000)
Fkbp6-F	5'-atg gac aag cct ttc gat tct-3'
Fkbp6-R	5'- ctg aag atc tgc ttc cac agg-3'

Toyooka, Y., Tsunekawa, N., Takahashi, Y., Matsui, Y., Satoh, M. and Noce, T. (2000). Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. Mech. Dev. 93, 139-149.

Table S2. Commercially available methylation pyrosequencing assays used

Gene	***Assay number***
ADCY6	PM00160440
Dazl	PM00291270
DAZL	PM00013636
DPEP3	PM00175077
FKBP6	PM00028973
Grin3b	PM00217091
Peg1	PM00384230
PIWIL1	PM00051884
Rhox13	PM00434042
SNRPN	PM00168252
Sycp3	PM00219744
SYCP3	PM00052514
Sycp1	PM 00337001