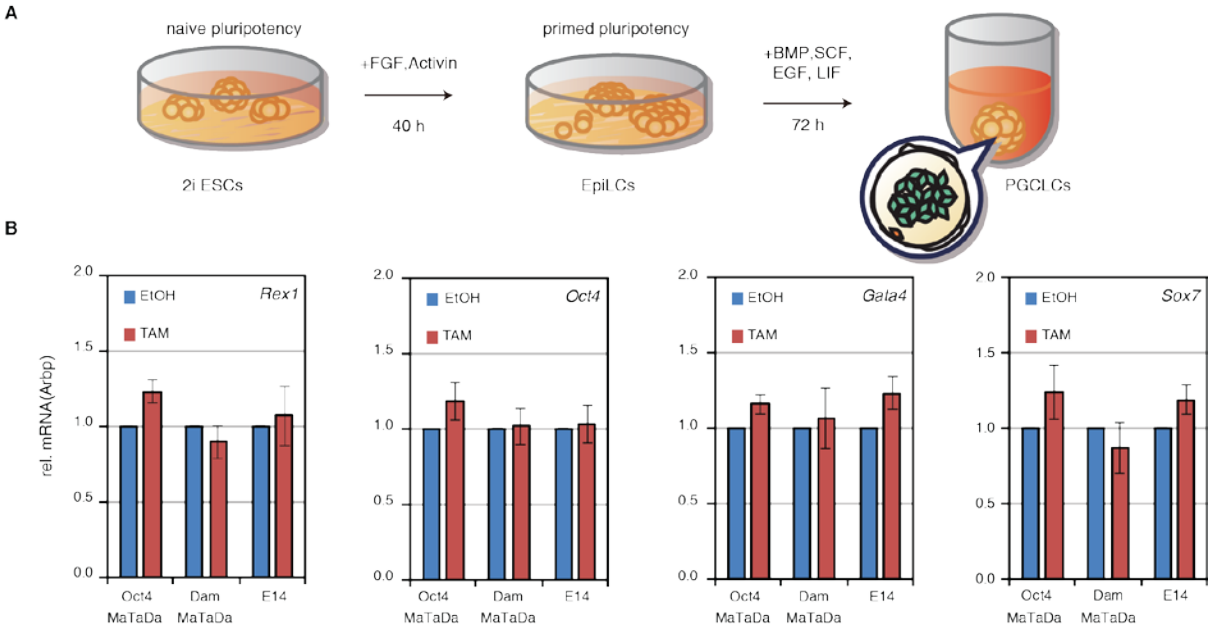

Figure S1. MaTaDa does not affect cell fate

(**A**) ESCs cultures in 2i media remain in a naïve pluripotency. The addition of FGF and Activin drives differentiation into EpiLCs, a primed pluripotent state. Supplementation with BMP, SCF, EGF and LIF promotes the transition into primordial germ cells. **(B)** qPCR of key marker genes of pluripotency and endodermal fate reveals that OCT4 MaTaDa has no effect upon cell fate.

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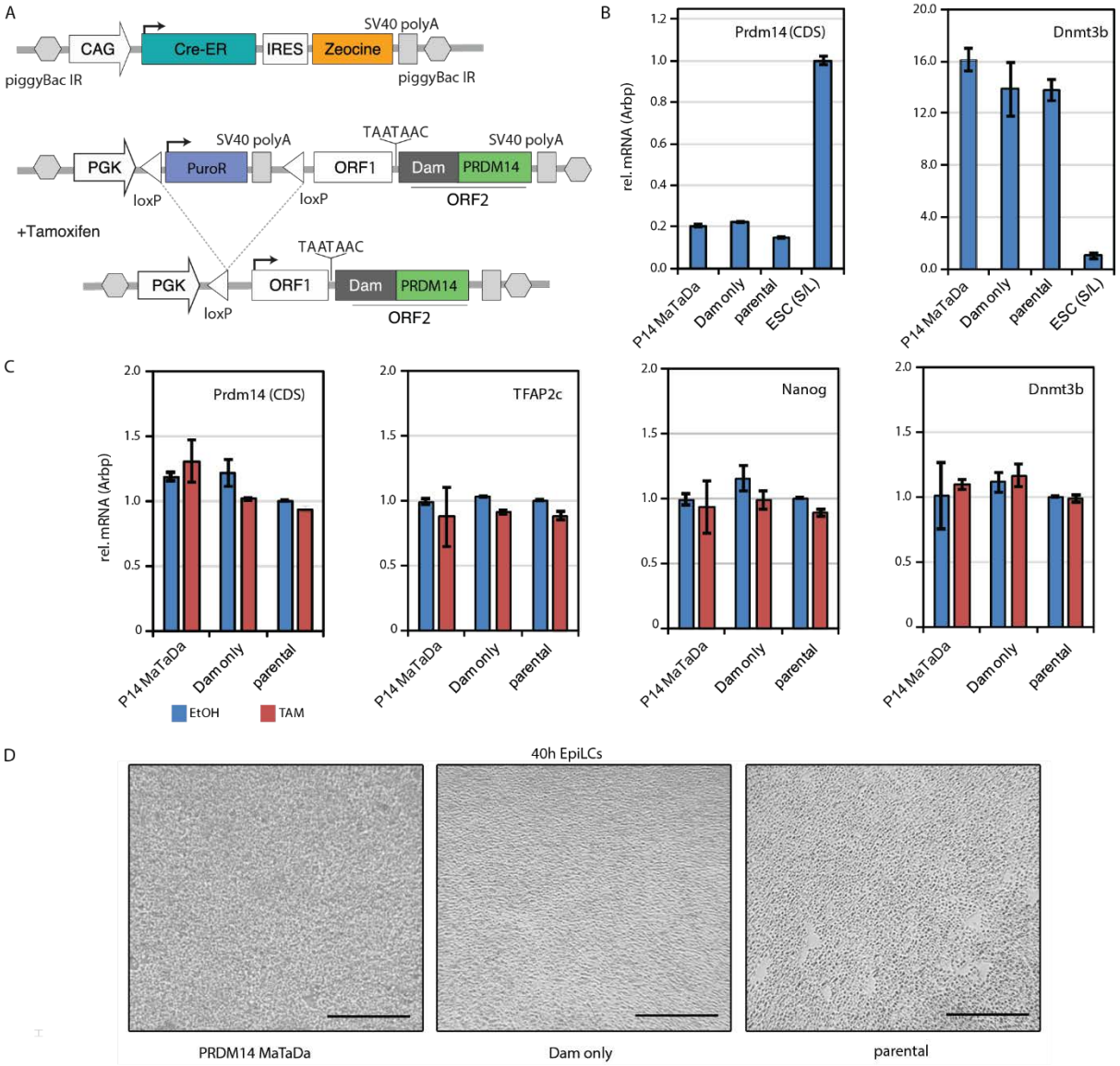

Figure S2. Conditional expression of Dam-PRDM14 does not disrupt cellular behaviour (**A**) Cre-ER is constitutively expressed and translocates to the nucleus upon tamoxifen treatment. The PGK promoter drives expression of a floxed puromycin resistance cassette (dark blue). Upon Cre induction the floxed cassette is excised allowing expression of ORF1 (246 amino acids). Rare translational re-initiation results in low-level expression of the Dam-PRDM14 fusion protein (ORF2; grey/green). **(B)** PRDM14 is not overexpressed and Dnmt3b remains high in EpiLCs following PRDM14 MaTaDa induction. **(C)** The expression of key marker genes and PRDM14 targets in mESCs are unaffected by PRDM14 MaTaDa. (**D**) Brightfield microscopy demonstrated that PRDM14 MaTaDa does not impact EpiLC differentiation.

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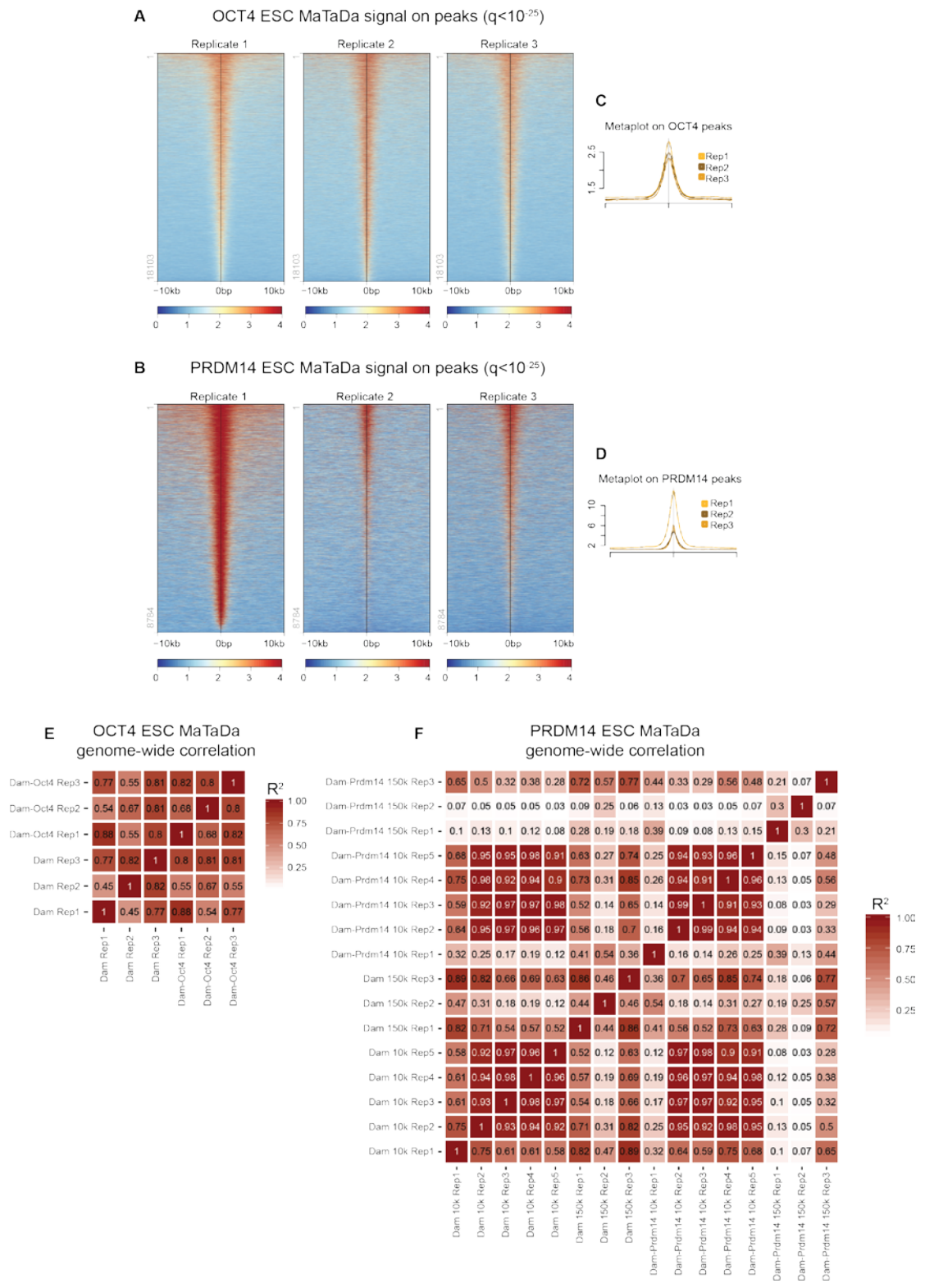

Figure S3. MaTaDa Peaks are reproducible

(**A,B**) OCT4 (**A**) and PRDM14 (**B**) ESC MaTaDa peaks are consistently detected between biological replicates. Signal is plotted over a 10 kb window either side of the peak midpoint ($q<10^{-25}$) for each of the three replicates. (**C,D**) Metaplots of the three replicates of OCT4 (**C**) and PRDM14 (**D**) ESC MaTaDa signal over a 10kb window on either side of the peak midpoint. (**E,F**) Correlation matrix showing R^2 values for genome-wide correlation between OCT4 (**E**) and PRDM14 (**F**) ESC MaTaDa replicates.

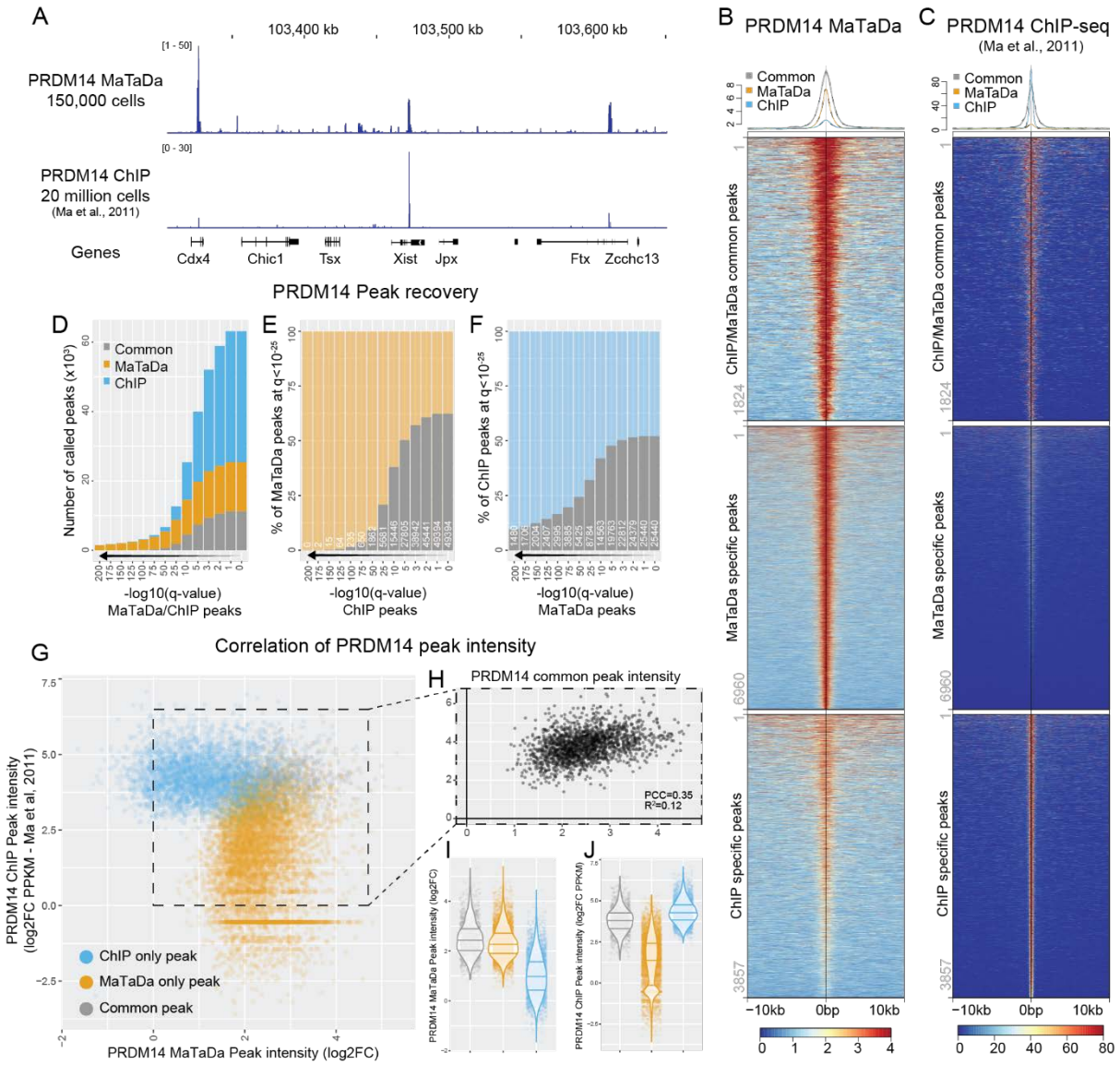

Figure S4. Correlation between Prdm14 MaTaDa and ChIP-seq in ESC. (**A,B**) PRDM14 MaTaDa (**A**) and ChIP-seq (**B**) ESC signal is plotted over a 10 kb window either side of the peak midpoint, for peaks common to MaTaDa and ChIP-seq (top), specific to MaTaDa only (middle) and specific to ChIP-seq only (bottom) at $q<10^{-25}$. Above are metaplots of the MaTaDa (**A**) and ChIP-seq (**B**) signal over a 10kb window on either side of the peak midpoint. (**C**) Genome browser view of PRDM14 binding at the Xist locus (MaTaDa; average of three replicates) compared to ChIP. MaTaDa data are represented as fold enrichment of Dam-fusion over Dam only; ChIP-seq data are represented as aligned reads. (**D-F**) Number (**D**) or percentage (**E,F**) of peaks called upon changing the q-value for peak-detection, either for MaTaDa and ChIP-seq in parallel (**D**), or compared to a fixed q-value $<10^{-25}$ for MaTaDa (**E**) or ChIP-seq (**F**). Common peaks are grey, MaTaDa-specific peaks are orange, ChIP-seq specific peaks are blue. (**G**) Scatterplot of peak intensity for common, MaTaDa-specific and ChIP-seq-specific peaks at $q<10^{-25}$. (**H**) Scatterplot of peak intensity for peaks common to MaTaDa and ChIP-seq at $q<10^{-25}$. (**I,J**) Distribution of peak intensity in PRDM14 MaTaDa (**I**) and ChIP-seq (**J**) for common, MaTaDa-specific and ChIP-seq-specific peaks at $q<10^{-25}$.

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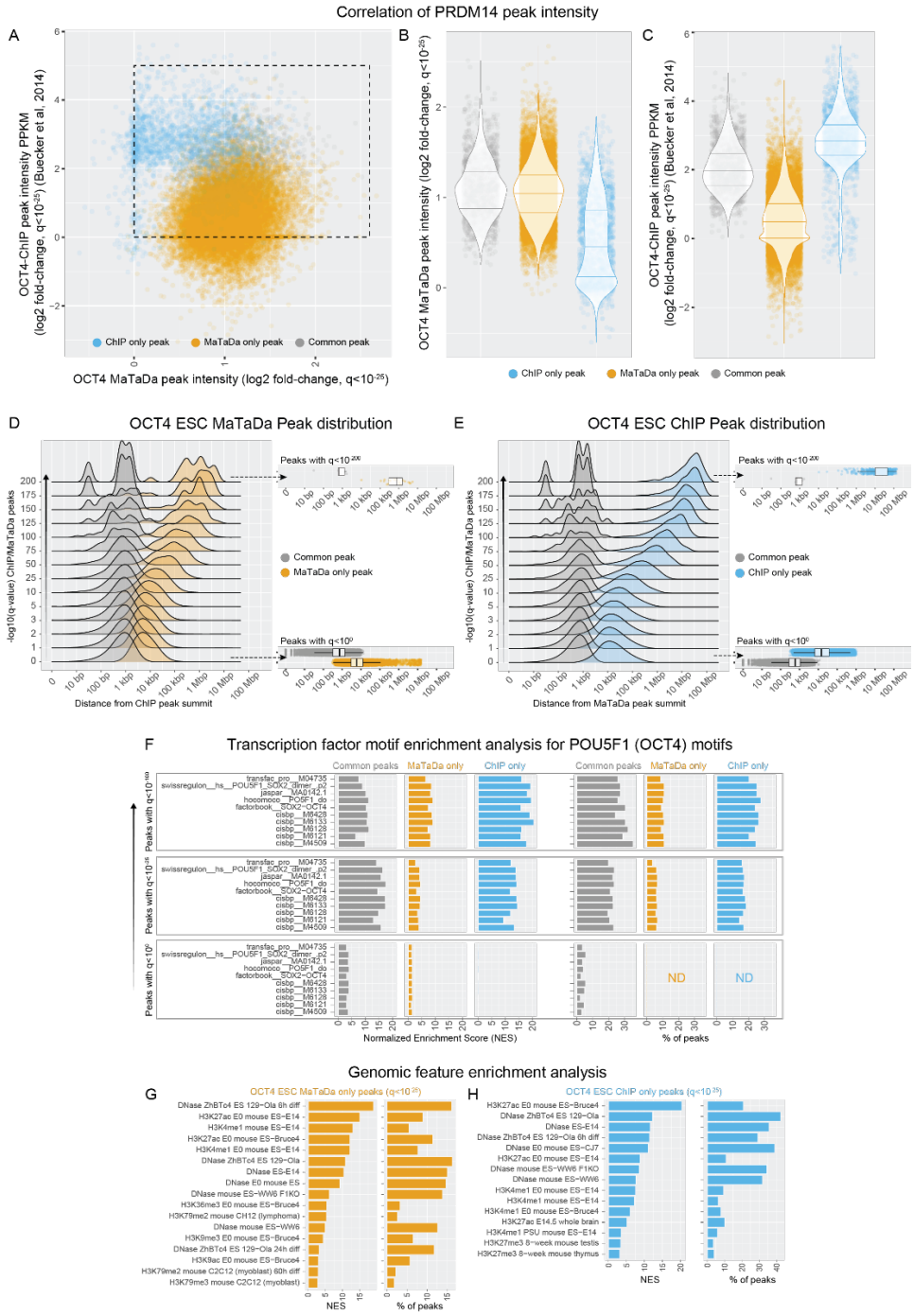

Figure S5. Overlap of OCT4 MaTaDa and ChIP peaks in ESC.
(**A**) Scatterplot of peak intensity for common, MaTaDa-specific and ChIP-seq-specific peaks at $q<10^{-25}$. ChIP-seq peaks are from (Buecker et al., 2014). Dashed outline for common peaks only is presented in **Fig 2H**. (**B,C**) Distribution of peak intensity in OCT4 MaTaDa (**B**) and ChIP-seq (**C**) for common, MaTaDa-specific and ChIP-seq-specific peaks at $q<10^{-25}$. **(D,E)** Distribution of peak density of the nearest common and MaTaDa-specific (**D**) or ChIP-specific (**E**) peaks relative to OCT4 ESC ChIP (**D**) or MaTaDa (**E**) peak summits at variable q-values. Insets show distribution of the individual peaks respectively at $q<10^{-200}$ and $q<10^{0}$. (**F**) Transcription factor motif enrichment analysis of all available OCT4-motifs in the i-cisTarget database for OCT4 ESC peaks common to MaTaDa and ChIP, or specific to either technique at three different q-values ($q<10^{-100}$, top; q<10-25, middle; $q<10^{0}$, bottom). Normalized enrichment score (NES) and percentage of peaks containing the motif are indicated. ND, not determined. (**G,H**) Genomic feature enrichment analysis of OCT4 MaTaDa-specific (**G**) or ChIP-specific (**H**) peaks ($q<10^{-25}$). Normalized enrichment score (NES) and percentage of peaks containing the feature are indicated.

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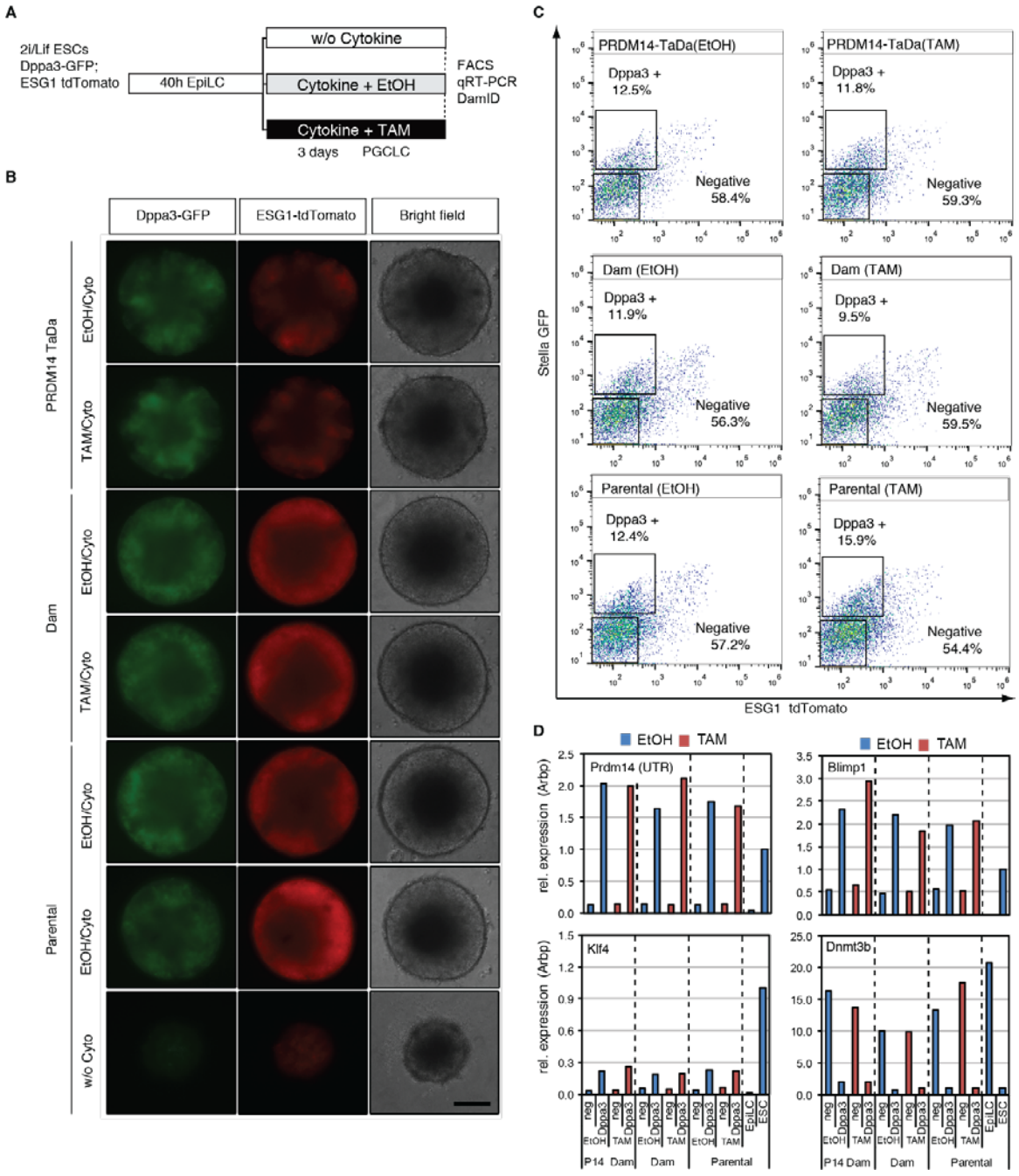

Figure S6. PGCLCs specify normally in the presence of PRDM14-Dam
(**A**) Experimental outline; 2i/LIF cultured, Dppa3/GFP, Esg1/tdTomato transgenic ESCs and harbouring 1.) no additional transgene (parental), 2.) Dam-PRDM14 or, 3.) Dam inducible transgenes were differentiated into EpiLCs for 40h. During PGCLC specification cell were treated with either ethanol (EtOH) or tamoxifen (TAM) for 72h. (**B**) GFP and tdTomato fluorescence as well as brightfield microscopy images of transgenic PGCLCs cultured in the presence of ethanol or tamoxifen (scale bar 100µm) (**C**) FACS plots of the different transgenic PGCLCs treated with ethanol or tamoxifen during 72h of PGCLC specification. Sorted PGCLCs (Dppa3 positive) and control cells (negative) are indicated in the panels. (**D**) qPCR analysis of PGC (Prdm14, Blimp1), ESC (Klf4) and EpiLC (Dmnt3b) marker genes on parental, Dam-PRDM14 or Dam transgenic cell FACS purified PGCLCs (Dppa3) and control cells (neg), treated with tamoxifen or ethanol.

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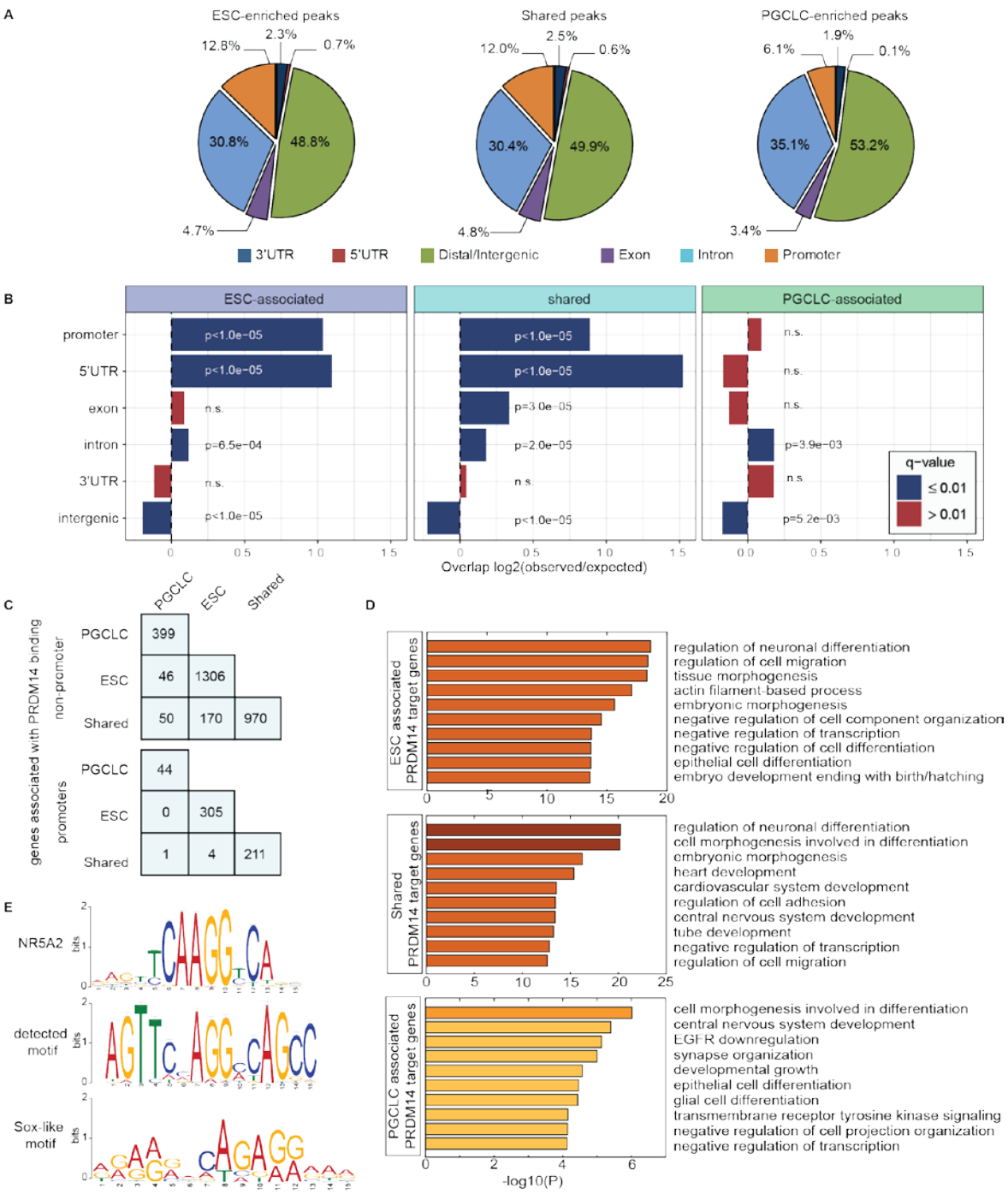

Figure S7. Genome wide analysis of PRDM14 binding
(**A**) Genomic distribution of PRDM14 peaks. (**B**) Enrichment of PRDM14 relative to genomic features (Genomic association test). (**C**) PGCLC- and ESC-enriched peaks are associated with distinct sets of genes. (**D**) Gene ontology terms associated with ESC-enriched, shared and PGCLC-enriched PRDM14 peaks. (**E**) Two motifs one of which resembles the NR5A2 motif and the other which is Sox-like were detected de novo by MEME (Bailey et al., 2015).

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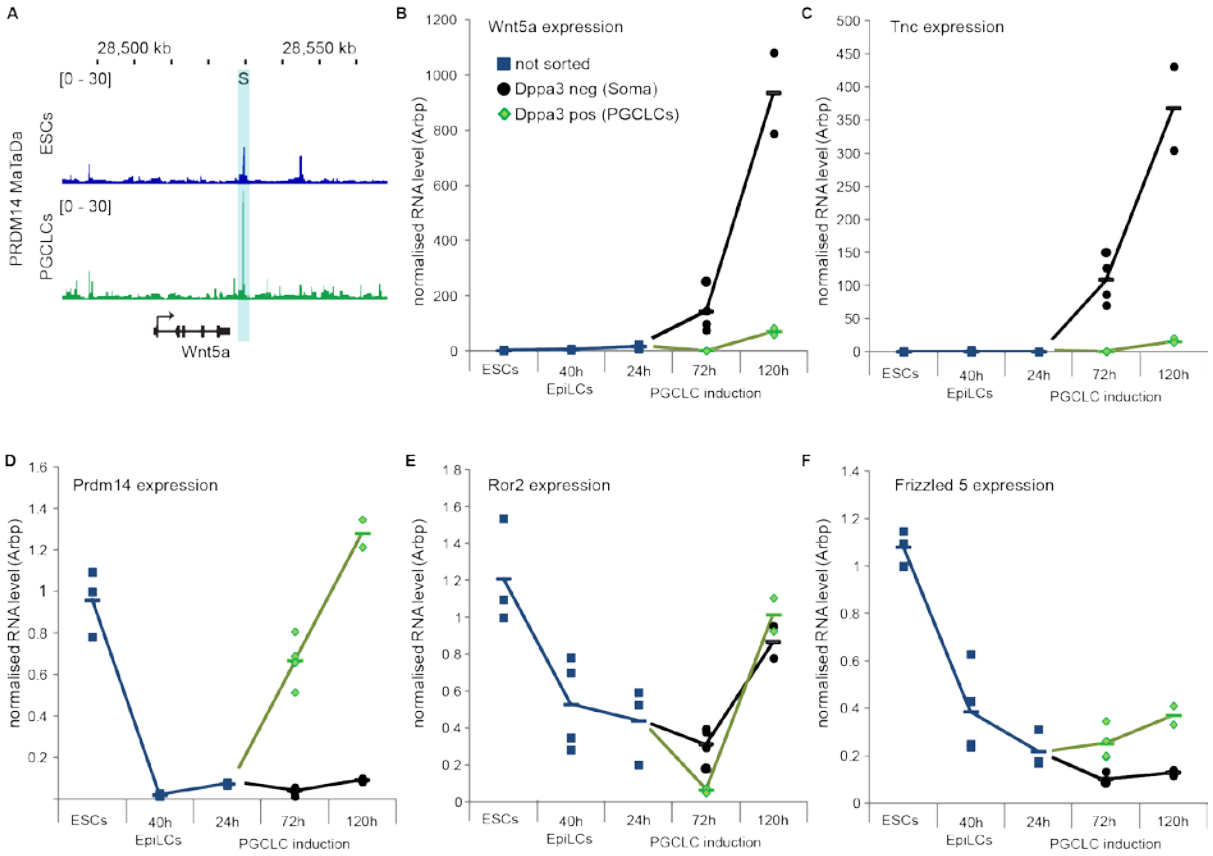

Figure S8: Gene expression kinetics

(**A**) Genome browser view of PRDM14 occupancy in ESCs and PGCLCs at the *Wnt5a* locus (both average of three replicates). Shared (S) binding site between ESC and PGCLC is shown. **(B-F)** Relative gene expression of indicated genes detected by qRT-PCR in 2i/LIF ESCs, EpiLCs (40h after induction) and at 3 time points of PGCLC development (24h, 72h and 120h after induction). Gene expression was normalized to *Arbp* expression. 72h and 120h PGCLCs were FACS purified using the Dppa3-GFP reporter gene. Dppa3-positive PGCLCs are depicted in green and Dppa3-negative somatic cells are depicted in black. Biological replicates (data points) and average gene expression (bar) are shown. (number of biological replicates: ESCs 3; EpilCs 4; 24h PGCLCs 3; 27h PGCLCs 4; 120h PGCLCs 2)

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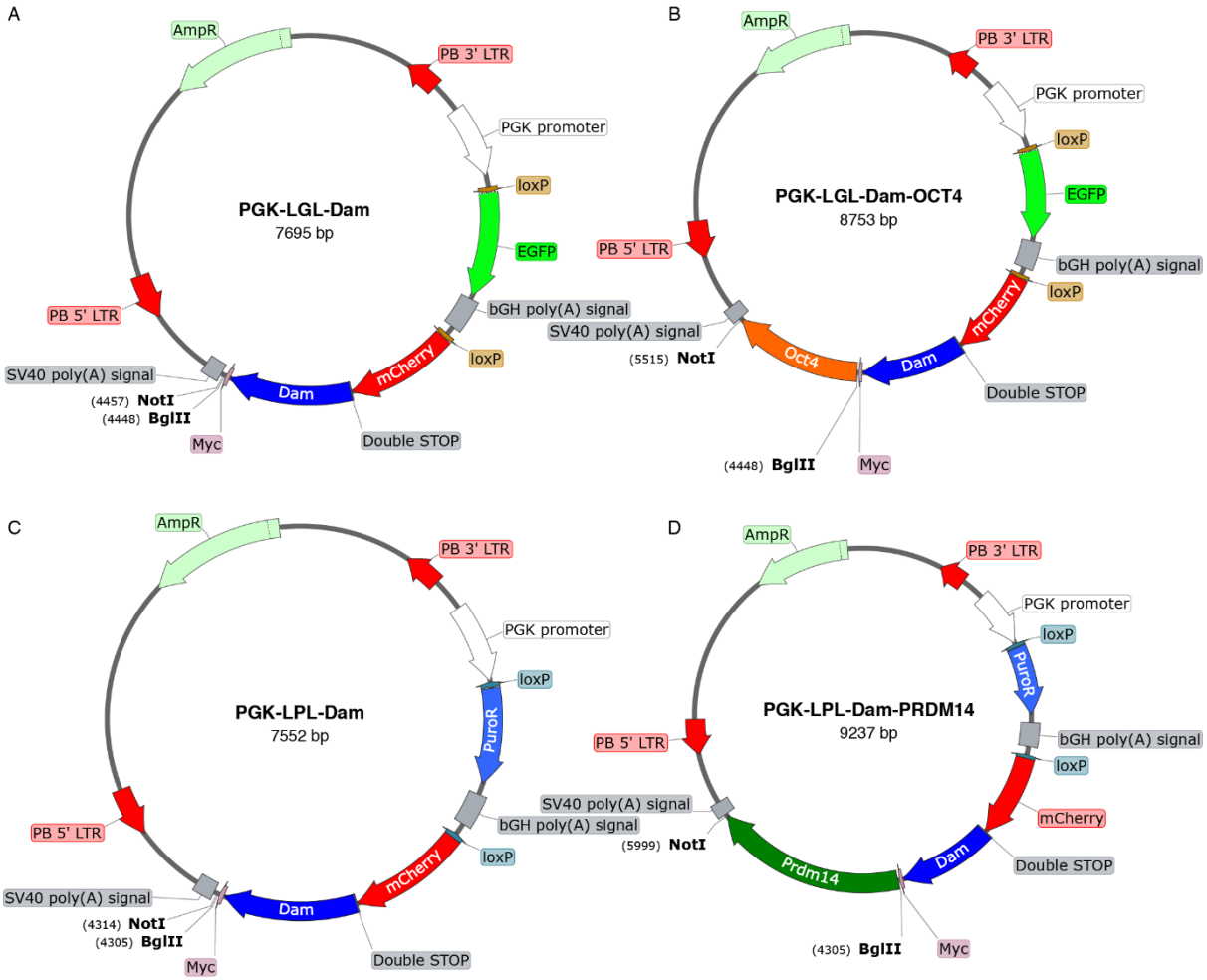

Figure S9: MaTaDa Constructs

(A) PGK-LGL-Dam is a conditional vector for establishing stable cell lines that can inducibly express low levels of Dam methylase. The vector contains PB 3'LTRs that allow integration into mammalian genomes with the piggyBac transposase. PGK drives the expression of a floxed GFP cassette. Upon Cre treatment this cassette will be excised allowing transcription of mCherry (ORF1, LT3) and Dam. ORF1 will be highly translated but the presence of two stop codons and a single nucleotide frameshift before Dam results in extremely low levels of Dam expression. (**B**) PGK-LGL-Dam with OCT4 CDS inserted in the multiple cloning site. (**C**) PGK-LPL-Dam, a modification of PGK-LGL-Dam with the GFP cassette replaced with a puromycin resistance cassette for antibiotic selection. (**D**) PGK-LPL-Dam with the PRDM14 CDS inserted into the multiple cloning site.

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