Appendix for "Tet enzymes are essential for embryogenesis and completion of embryonic genome activation"

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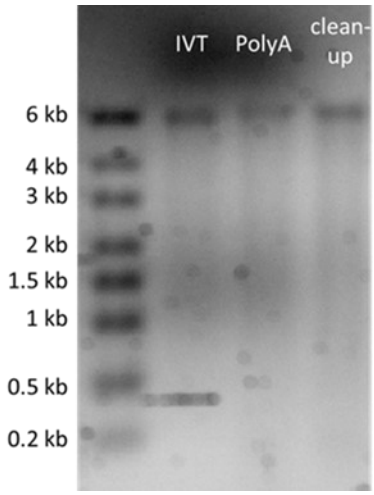
A



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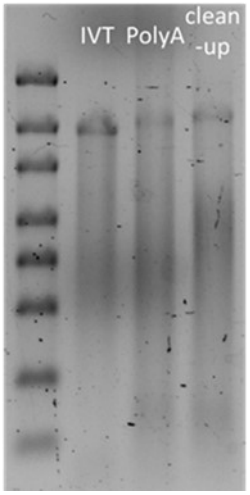
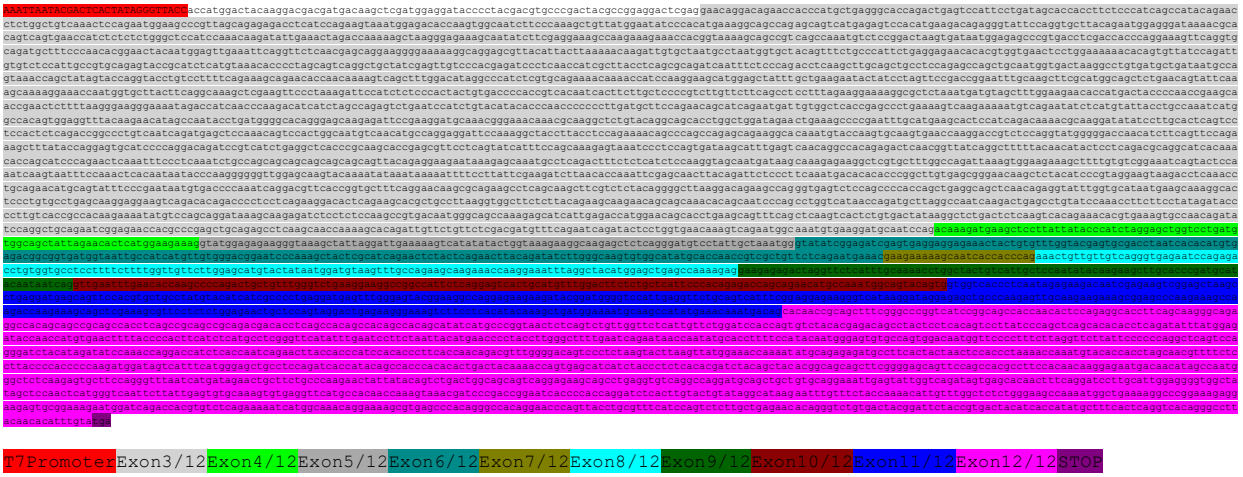


C

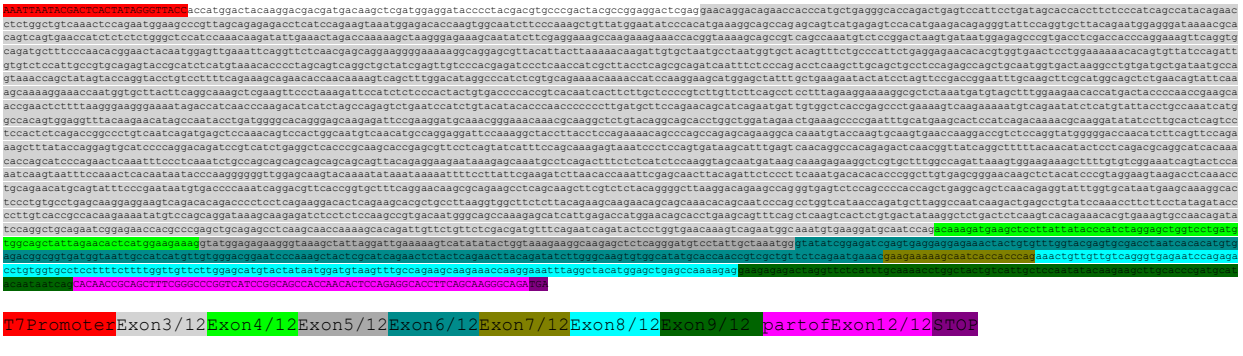
Template for IVT (5867 bp): Tet2 full-length (amplified from FH-Tet2-pEF-5)



T7PromoterExon3/12Exon4/12Exon5/12Exon6/12Exon7/12Exon8/12Exon9/12Exon10/12Exon11/12Exon12/12STOP

D

Template for IVT (3986 bp): Tet2 truncated (amplified from FH-Tet2-pEF-5 linearized with SpeI, mimicking Tet2 KO from Dai *et al*. Nature, 2016)

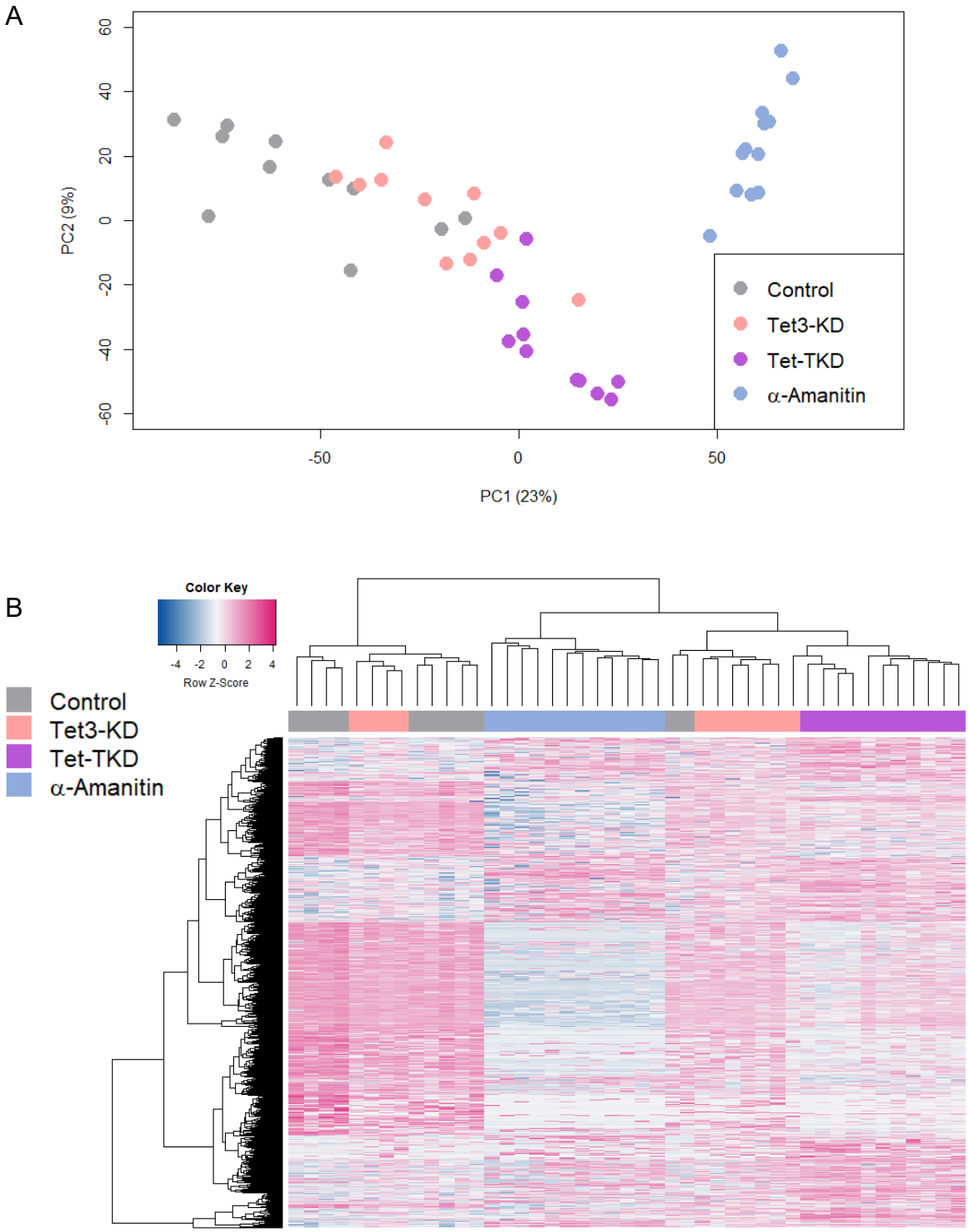


T7PromoterExon3/12Exon4/12Exon5/12Exon6/12Exon7/12Exon8/12Exon9/12 partofExon12/12STOP

Appendix Figure S1: Generation of mRNA for Tet2 rescue experiments

(A+B) 1% denaturing agarose gel showing *in vitro* transcribed **(A)** full-length Tet2-mRNA and **(B)** truncated Tet2-mRNA used for rescue experiments after *in vitro* transcription (IVT), poly-adenylation reaction (Poly-A) and the clean-up of the reaction (clean-up).

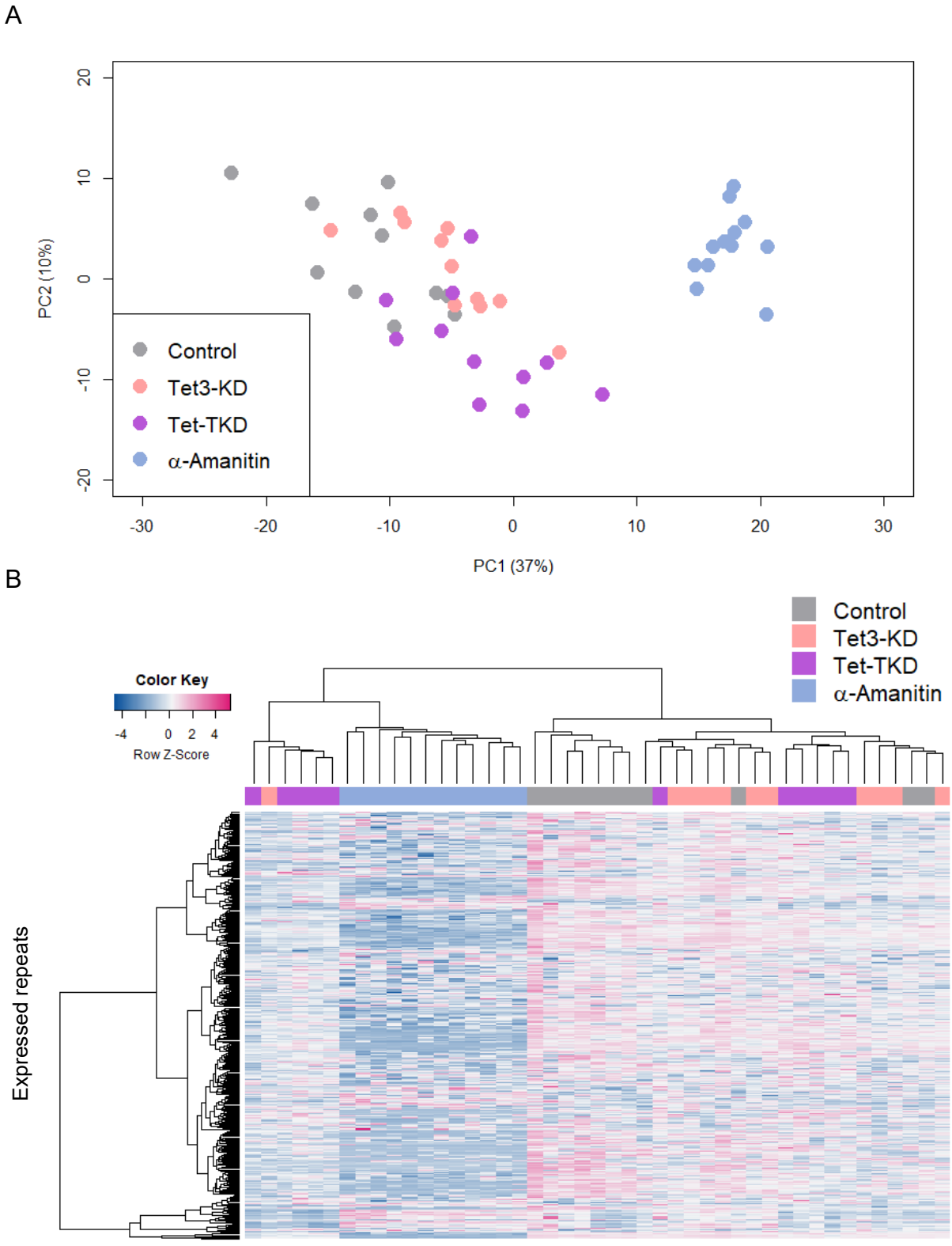
(C+D) Sequences of generated PCR products used as template for IVT. Different exons are highlighted. **(C)** Full-length Tet2. **(D)** Truncated Tet2 without catalytical domain, mimicking the Tet2-KO from Dai *et al.,* 2016.



Appendix Figure S2: RNA-seq analysis of single copy genes in control, Tet3 and Tet-TKD 2-cell and α-amanitin inhibited embryos

(A) Principle component analysis of single embryo RNA-seq samples (each dot represents one 2-cell embryo).

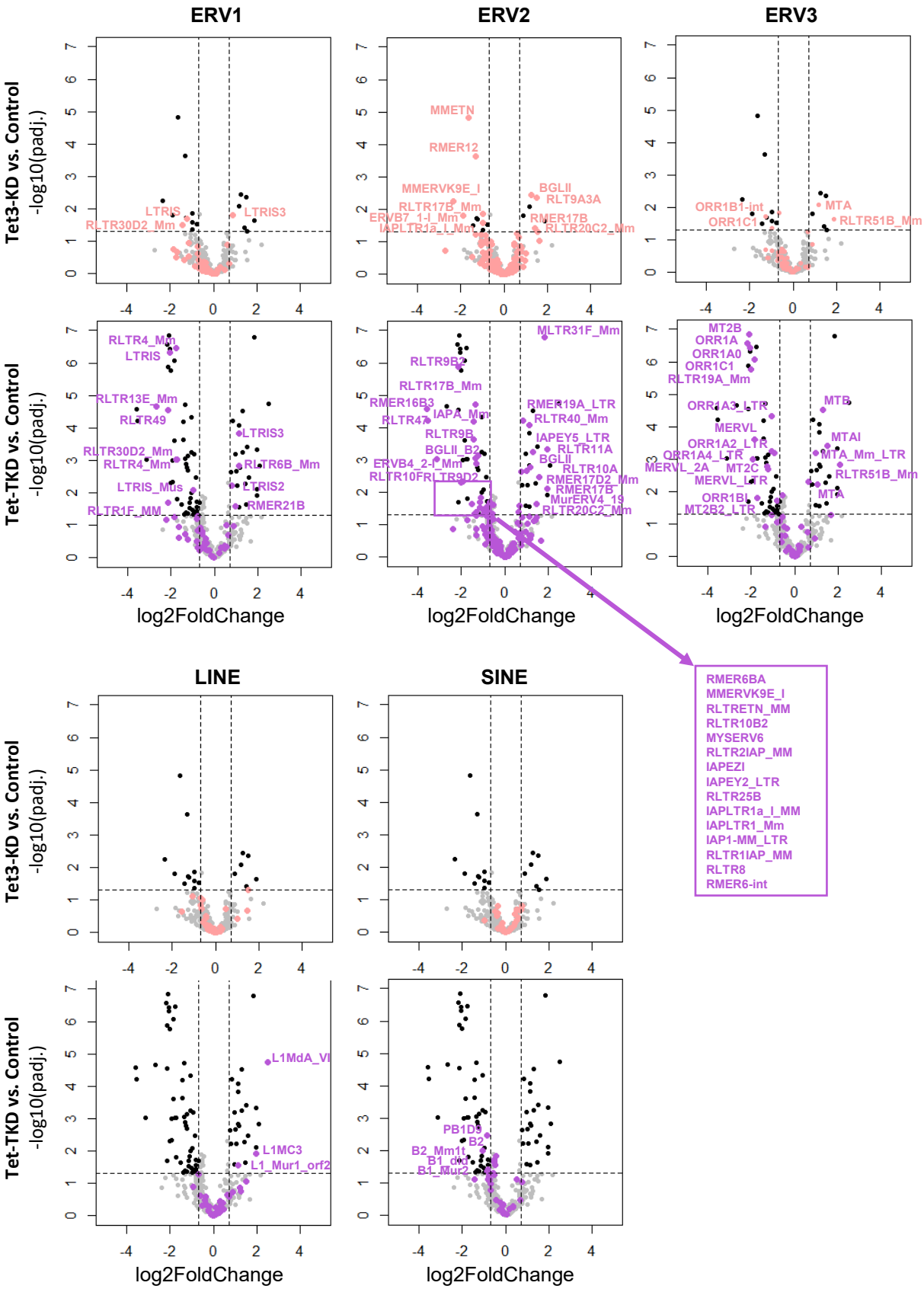
(B) Heatmap of differentially expressed genes in derived 2-cell embryos. The map shows row-normalized expression levels for control, Tet3-KD, Tet-TKD and α-amanitin treated 2-cell embryos using unsupervised hierarchical clustering.



Appendix Figure S3: Expression analysis of transposable elements (TEs) in control, Tet3-KD, Tet-TKD and α-amanitin treated embryos

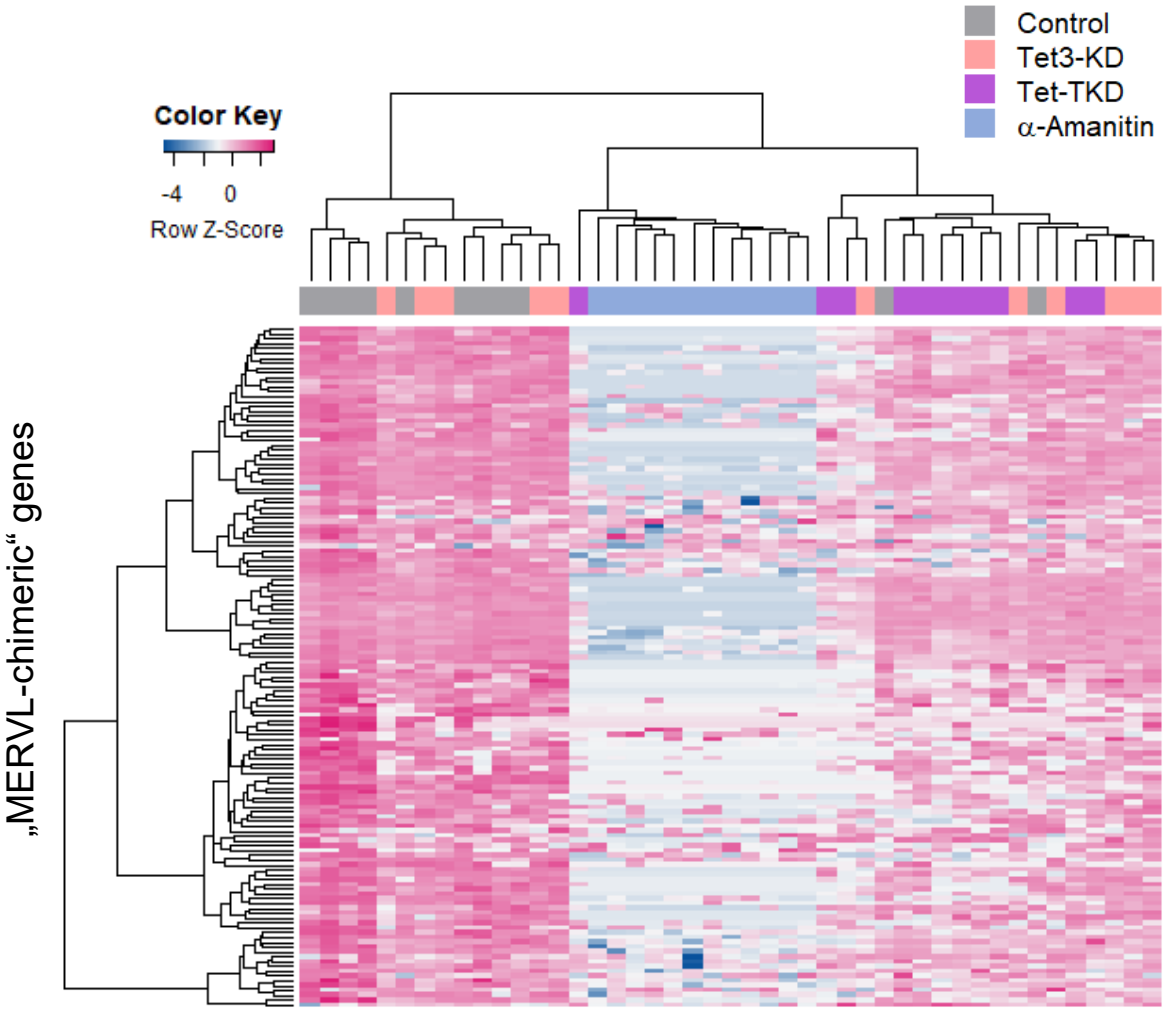
(A) Principle component analysis of expression levels of TEs. Each dot represents a single 2-cell embryo.

(B) Heatmap of row-normalized expression levels of TEs normalized with single genes.

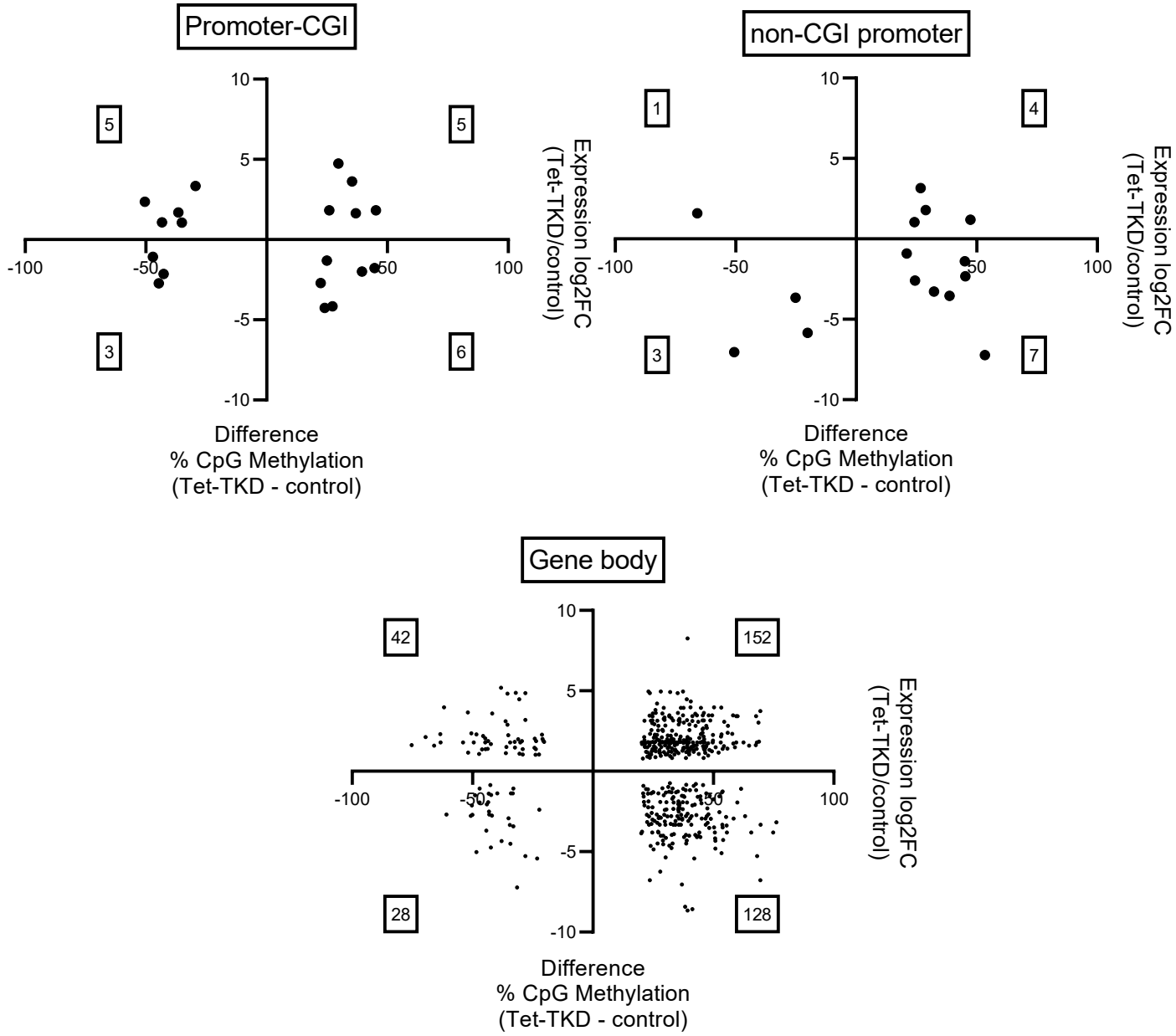


Appendix Figure S4: Differential expression of repeats in Tet3-KD and Tet-TKD 2-cell embryos compared to control 2-cell embryos.

Specific, differentially expressed repeats of the different classes are highlighted in the specific volcano plots.

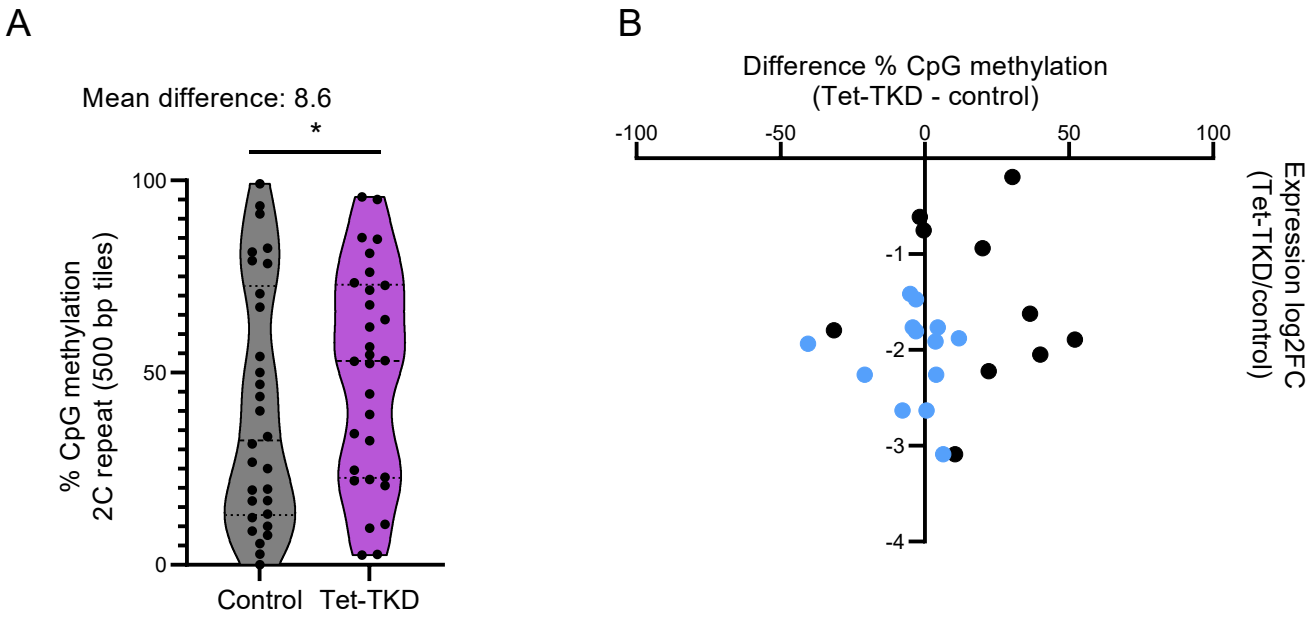


Appendix Figure S5: Hierarchical clustering of expression profiles of 2C-specific chimeric genes. Heatmap of row-normalized expression levels of genes defined by MacFarlan *et al*., 2012 as MERVL-chimeric transcripts for control, Tet3-KD, Tet-TKD and α-amanitin treated embryos.



Appendix Figure S6: Correlation analysis of DNA methylation and gene expression for promoter CGIs, non-CGI promoter and gene bodies.

Shown are genes which are differentially expressed and show significant differentially methylated tiles. Number in boxes represent number of genes per quadrant.



Appendix Figure S7: Correlation of DNA methylation and gene expression of MERVL-driven chimeric genes

(A) DNA Methylation of 500 bp tiles overlapping repeats of MERVL-driven chimeric genes. Data is represented as medium smoothed violin plot with indicated median and quartiles as dotted line; each dot represents one 2C repeat (two-tailed paired t-test; * = $p<0.05$). **(B)** Correlation plot of DNA methylation differences of tiles from **(A)** and corresponding gene expression. Significant differentially expressed genes are colored in blue.

Appendix Table S1: Morpholino (MO) mediated knockdown (KD) of Tet1-3 in early embryogenesis

Location of genomic binding sides for MOs targeting the 5'-UTRs of Tet1-3 mRNAs. MO-set 1 is marked in green, MO-set 2 is marked in blue. Start-codon is indicated in yellow. Tet3oo was described in Jin *et al.,* 2016.

Morpholino	Targeted 5'-UTR
Tet1-MOs	TCCAGATTCTCCTCAAAAGGAAGATTTTGCAAAGGACCAATGACACTGTGCCCC GCATCCTTGCATCTTTACTTACTCTGCAGCCATGTCTCGGT
Tet2-MOs	GGTGTAACTGTAATGCTGTGTCTTTACTTTTTTCTGTGTCTTTAGGATTCATTC AAAGGGCAGCCTTGTGGATGGCCCCGAAGCAAGCCTGATGGAACAGGACAGAAC CACCCAT
Tet3oo- and Tet3-MOs	AGCTGCTGCTTTGGGGTCGCACATGTTCCTCCCAGAAACCCCTCAACAATATGC TGTGGAAATAAATGCTCGTGAAGGAACGGGGCCCTGGGCACAAGGGGCGACTGT CAAGACAGGCTCAGAGCTCAGCCCAGTTGATGGACCTGTTCCAGGTCAGATG
Control-MO	Mutated human beta-globin intron

Appendix Table S2: Enrichment analyses of differentially expressed genes

ChIP Enrichment Analysis of downregulated genes in Tet3-KD and Tet-TKD 2-cell embryos. List was obtained from Enrichr (Kuleshov *et al.,* 2016; Lachmann *et al.,* 2010), with an adjusted p-value of <0.1 for Tet-TKD and <0.25 for Tet3-KD applied to all samples from mouse embryonic stem cells from ChEA 2016 database, duplicated gene names were removed keeping the term with the highest adjusted p-value (padj.). Non-overlapping binding factors between both groups are marked bold (Stat3, Zfp42 and Tet1), factors that have an adjusted p-value in Tet3-KD of <0.25 & >0.1 are marked italic. PMID = Pubmed ID of study used to obtain the list of targets.

Enrichment analysis of downregulated genes to ChEA database (2016, mESCs)							
Tet3-KD				Tet-TKD			
Term	PMID	padj.	Overlap	Term	PMID	padj.	Overlap
KDM5B	21448134	1,22E-22	145/3724	KDM5B	21448134	3,18E-99	719/3724
MYC	19030024	6,65E-20	142/3868	MYC	19030024	5,59E-61	651/3868
NELFA	20434984	4,24E-14	85/2000	NELFA	20434984	5,50E-51	396/2000
ZFX	18555785	4,72E-12	110/3249	JARID1A	20064375	3,51E-40	392/2171
E2F1	18555785	2,12E-11	128/4172	E2F1	18555785	1,48E-38	621/4172
ASH2L	23239880	2,85E-09	104/3336	MYCN	18555785	1,20E-32	382/2261
JARID1A	20064375	1,98E-05	67/2171	ZFX	18555785	2,56E-32	497/3249
MYCN	18555785	1,98E-05	69/2261	KLF4	18555785	1,11E-20	365/2444
MYBL2	22936984	1,10E-04	66/2250	CHD1	19587682	5,26E-15	153/843
KLF4	18555785	2,26E-04	69/2444	ASH2L	23239880	9,22E-13	429/3336
CHD1	19587682	3,59E-03	29/843	*SIN3A*	21632747	6,96E-09	174/1186
TRIM28	19339689	4,26E-03	76/3072	*SIN3B*	21632747	5,33E-08	500/4302
CNOT3	19339689	1,80E-02	42/1547	MYBL2	22936984	6,65E-08	287/2250
TCFCP2L1	18555785	1,86E-02	51/1987	TRIM28	19339689	1,31E-06	365/3072
YY1	21170310	5,42E-02	16/464	*HCFC1*	20581084	1,35E-06	58/306
THAP11	20581084	5,82E-02	25/864	YY1	21170310	4,07E-06	77/464
NANOG	18555785	9,40E-02	17/542	THAP11	20581084	1,01E-05	123/864
SIN3B	21632747	1,37E-01	89/4302	*POU5F1*	18555785	1,52E-05	86/555
SOX2	19030024	1,55E-01	23/863	**STAT3**	18555785	4,62E-05	86/572
POU5F1	18555785	1,91E-01	16/555	*SOX2*	18555785	1,50E-04	75/497
SIN3A	21632747	1,96E-01	29/1186	**ZFP42**	18358816	1,58E-04	184/1480
HCFC1	20581084	2,32E-01	10/306	CNOT3	19339689	1,63E-04	191/1547
				NANOG	18555785	0,0012619	76/542
				TCFCP2L1	18555785	0,00167237	229/1987
				TET1	21451524	0,09525211	196/1839

Appendix References

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