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Supplemental information

DNA methyltransferase 3 alpha and TET methylcytosine dioxygenase 2 restrain mitochondrial DNA-mediated interferon signaling in macrophages

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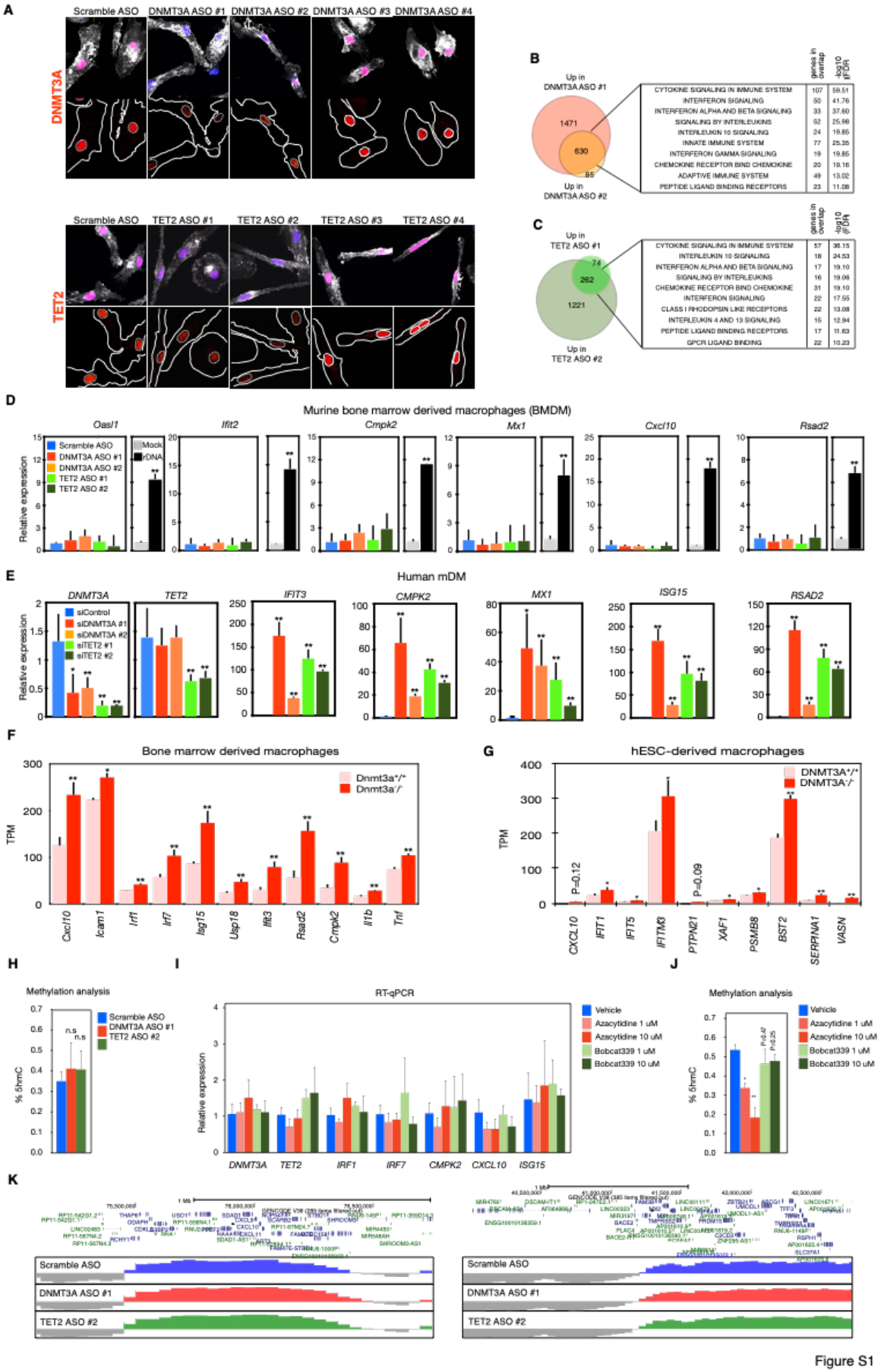

A
Scramble ASO
DNMT3A ASO #1
DNMT3A ASO #2
DNMT3A ASO #3
DNMT3A ASO #4
DNMT3A
TET2 ASO #1
TET2 ASO #2
TET2 ASO #3
TET2 ASO #4
TET2
B
Up in DNMT3A ASO #1
1471
630
85
Up in DNMT3A ASO #2
genes in overlap
-log10 #FDR
CYTOKINE SIGNALING IN IMMUNE SYSTEM 107 58.51
INTERFERON SIGNALING 50 41.76
INTERFERON ALPHA AND BETA SIGNALING 33 37.60
SIGNALING BY INTERLEUKINS 52 25.98
INTERLEUKIN 10 SIGNALING 24 19.85
INNATE IMMUNE SYSTEM 77 25.35
INTERFERON GAMMA SIGNALING 19 19.85
CHEMOKINE RECEPTOR BIND CHEMOKINE 20 19.16
ADAPTIVE IMMUNE SYSTEM 49 13.02
PEPTIDE LIGAND BINDING RECEPTORS 23 11.08
C
Up in TET2 ASO #1
74
262
1221
Up in TET2 ASO #2
genes in overlap
-log10 #FDR
CYTOKINE SIGNALING IN IMMUNE SYSTEM 57 36.15
INTERLEUKIN 10 SIGNALING 18 24.53
INTERFERON ALPHA AND BETA SIGNALING 17 19.10
SIGNALING BY INTERLEUKINS 16 19.06
CHEMOKINE RECEPTOR BIND CHEMOKINE 31 19.10
INTERFERON SIGNALING 22 17.55
CLASS I RHODOPSIN LIKE RECEPTORS 22 13.08
INTERLEUKIN 4 AND 13 SIGNALING 15 12.94
PEPTIDE LIGAND BINDING RECEPTORS 17 11.63
GPCR LIGAND BINDING 22 10.23
D
Murine bone marrow derived macrophages (BMDM)
Oasl1
Ifit2
Cmpk2
Mx1
Cxcl10
Rsad2
Relative expression
Scramble ASO
DNMT3A ASO #1
DNMT3A ASO #2
TET2 ASO #1
TET2 ASO #2
Mock
rDNA
E
Human mDM
DNMT3A
TET2
IFIT3
CMPK2
MX1
ISG15
RSAD2
Relative expression
siControl
siDNMT3A #1
siDNMT3A #2
siTET2 #1
siTET2 #2
F
Bone marrow derived macrophages
TPM
Dnmt3a+/+
Dnmt3a-/-
Cxcl10
Icam1
Irf1
Irf7
Isg15
Usp18
Ifit3
Rsad2
Cmpk2
Il1b
Tnf
G
hESC-derived macrophages
TPM
DNMT3A+/+
DNMT3A-/-
P=0.12
P=0.09
CXCL10
IFIT1
IFIT5
IFITM3
PTPN21
XAF1
PSMB8
BST2
SERPINA1
VASN
H
Methylation analysis
% 5hmC
Scramble ASO
DNMT3A ASO #1
TET2 ASO #2
n.s
n.s
I
RT-qPCR
Relative expression
Vehicle
Azacytidine 1 uM
Azacytidine 10 uM
Bobcat339 1 uM
Bobcat339 10 uM
DNMT3A
TET2
IRF1
IRF7
CMPK2
CXCL10
ISG15
J
Methylation analysis
% 5hmC
Vehicle
Azacytidine 1 uM
Azacytidine 10 uM
Bobcat339 1 uM
Bobcat339 10 uM
P=0.42
P=0.25
K
Scramble ASO
DNMT3A ASO #1
TET2 ASO #2
Scramble ASO
DNMT3A ASO #1
TET2 ASO #2

Figure S1

Figure S1-related to Figure 1 and Figure 2. DNMT3A or TET2 loss of expression leads to up-regulation of interferon signalling.

A) Protein quantification by IF. Scale bars= 5 μm.
B) Venn plot of the overlap between DEG in both DNMT3A ASOs
C) Venn plot of the overlap between DEG in both TET2 ASOs.
D) RT-qPCR of ISGs in BMDM treated with recombinant DNA and with human specific ASOs (n=3).
E) RT-qPCR of *DNTM3A*, *TET2* and ISG in mDM incubated with siRNAs (n=3 donors).
F) Bar plot of ISG in BMDM from *Dnmt3a*$^{-/-}$ vs. *Dnmt3a*$^{+/+}$ mice.
G) Bar plot showing of ISG in *DNMT3A*$^{-/-}$ vs. *DNMT3A*$^{+/+}$ iPSC-derived macrophages.
H) Bar graphs of global 5-hydroxymethyl-cytosine analysis in Scramble ASO, DNMT3A ASO #1 or TET2 ASO #2 (n=3 donors).
I) Bar graphs of expression analysis by RT-qPCR of *CXCL10*, *IRF7*, *MX1*, *IFIT1*, *IFIT2* and *CMPK2* in mDM treated with 1uM or 10uM of 5´-azacytidine or Bobcat339 for 24h in mDM (n=3 donors).
J) Bar graphs of global methylation analysis in mDM treated with 5´-azacytidne but not Bobcat339 (n=3 donors).
K) Composites of HiC values of PC1 in genomic regions around the CXCL10 (left) or MX1 (right) locus in mDM treated with DNMT3A ASO #1 or TET2 ASO #2 vs. Scramble ASO. (n=1 pool of three donors).

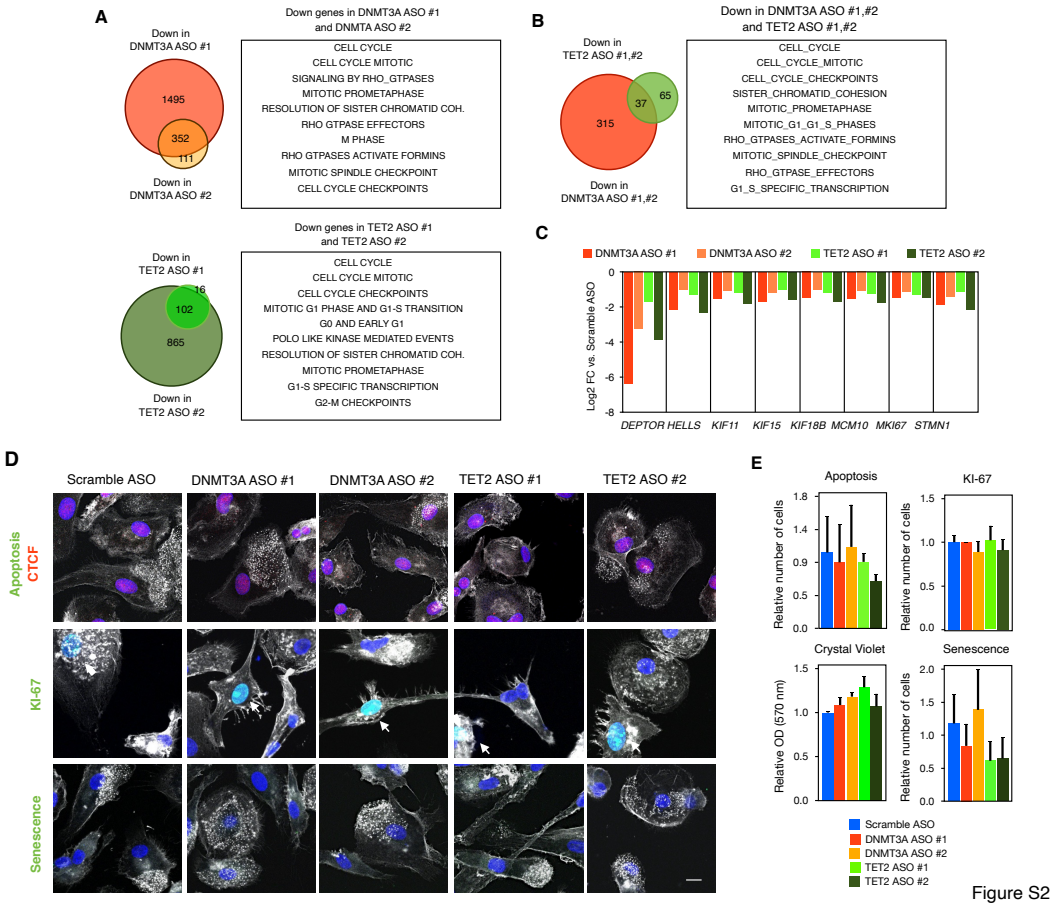


Figure S2

Figure S2-related to Figure 1 and Figure 2. DNMT3A or TET2 loss of expression does not alter cell proliferation, apoptosis, or senescence in mDM but activates signaling by IFNA or TLR agonist.

A) Venn diagram of the overlap of down-regulated genes from DNMT3A ASOs #1 and #2 and enriched gene ontology terms.

B) Venn diagram of the overlap of down-regulated genes from TET2 ASOs #1 and #2 and enriched gene ontology terms.

C) RNA expression of regulators of cell cycle in mDM treated with both DNMT3A ASO and TET2 ASO.

D) IF analysis of ApoTracker, CTCF, KI-67 and CellEvent (Senescence) in Scramble ASO, DNMT3A ASO or TET2 ASO in mDMs. Scale bars= 2 μm.

E) Quantification by flow cytometry (ApoTracker, KI-67 and CellEvent) or by luminescence (crystal violet) of mDM treated with DNMT3A ASO or TET2 ASO ($n \geq 4$ donors)

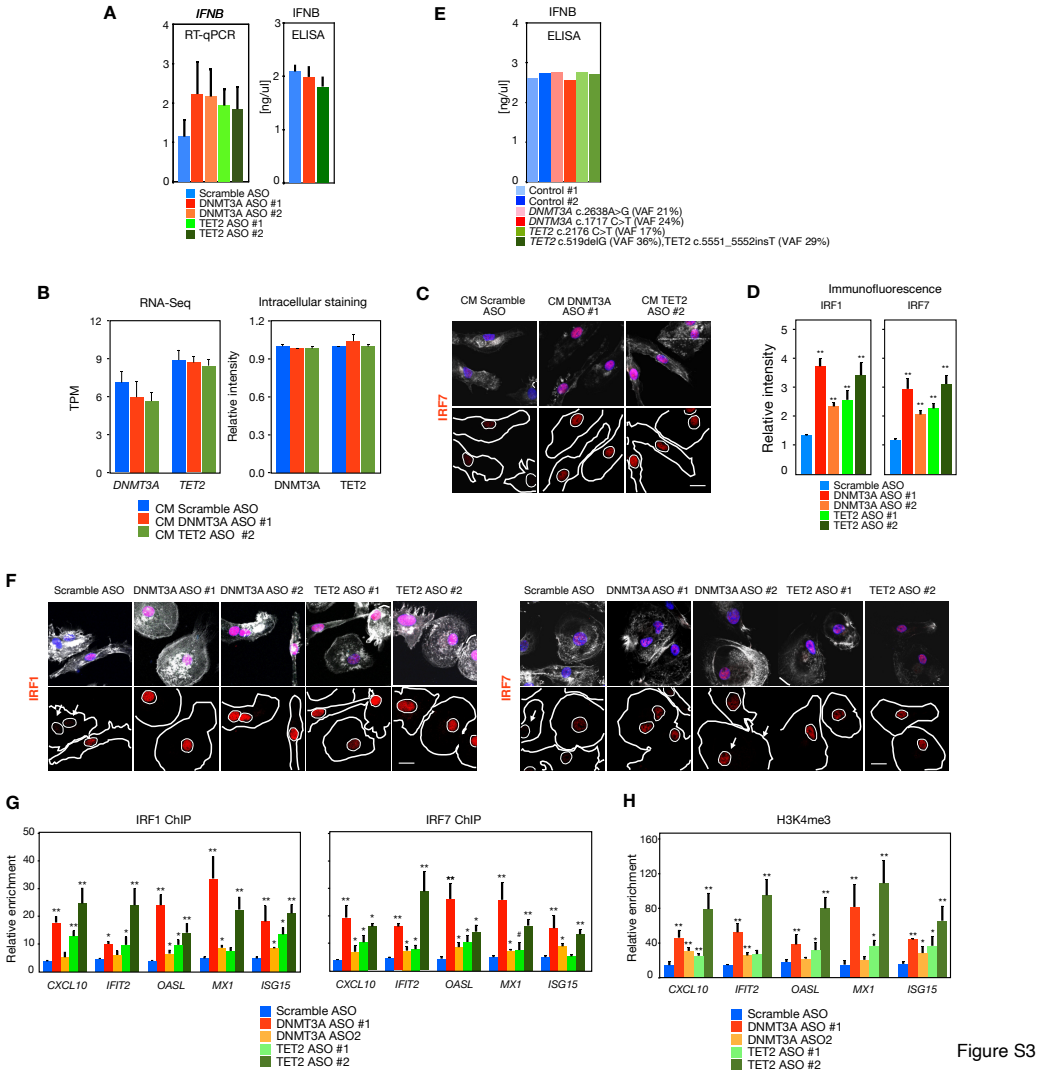


Figure S3-related to Figure 2. Reduced DNMT3A or TET2 expression leads to increased IRF1 and IRF7 and changes in the histone landscape of ISGs.

(A) RT-qPCR (left) or protein secretion by ELISA (right) of IFNB in mDM incubated with DNMT3A ASOs or TET2 ASOs (n≥3/donor)

(B) Analysis of DNMT3A or TET2 mRNA (left) or protein (right) expression in macrophages incubated conditioned media from macrophages treated with DNMT3A ASO #1 or TET2 ASO #2 (n≥4 donors)

(C) Protein expression analysis of IRF7 by IF in macrophages incubated conditioned media from macrophages treated with DNMT3A ASO #1 or TET2 ASO #2 (n=4 donors). Scale bars= 5 μm.

(D) Protein expression by IF of IRF1 or IRF7 in macrophages with reduced DNMT3A or TET2 expression (n=3 donors).

(E) Secretion analysis of IFNB by ELISA in mDM control and those harbouring *DNMT3A* mutations of *TET2* mutations.

(F) Immunofluorescence analysis of IRF1 and IRF7 in mDM incubated with DNMT3A ASO #1 or TET2 ASO #2. Scale bars= 5 μm.

(G) ChIP-qPCR of IRF1 and IRF7 over the promoter of *CXCL10*, *IFIT2*, *OASL*, *MX1* and *ISG15* in mm treated with DNMT3A ASO or TET2 ASO as compared to Scramble ASO (n=4 donors).

(H) H3K4me3 ChIP-qPCR on the promoter of *CXCL10*, *IFIT2*, *OASL*, *MX1*, *ISG15* in macrophages with reduced DNMT3A ASOs or TET2 ASOs ~~expression~~ (n=4 donors).

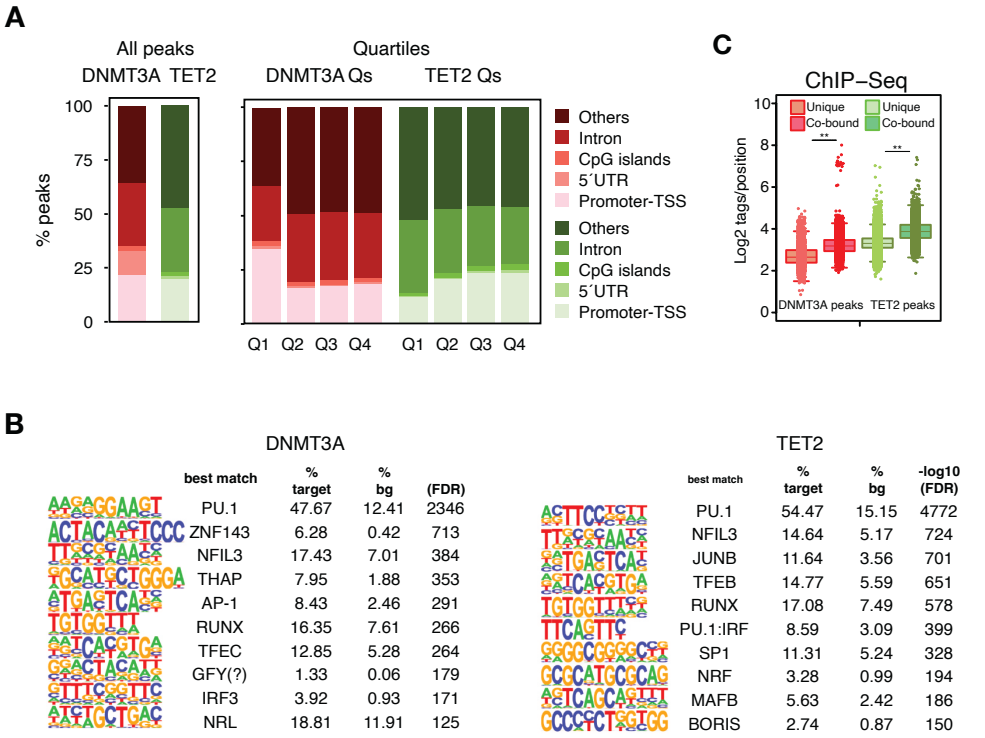


Figure S4

Figure S4-related to Figure 4. DNMT3A and TET2 bind to genomic regions with enriched in PU.1, ZNF143, AP-1 and RUNX motifs.

(A) Bar graphs showing the peak distribution of the quartiles of intensity of DNMT3A or TET2 ChIP-Seq. Data show higher percentage of promoter-TSS peaks in of the top Q1 quartile of DNMT3A ChIP-Seq and in Q4 of TET2 ChIP-Seq peaks.

(B) *De novo* motif analysis of peaks DNTM3A unique peaks, DNMT3A-TET2 cobound peaks or TET2 peaks (n=4 donors).

(C) Boxplot showing the number of tags per position in DNMT3A or TET2 ChIP-Seq when analysed DNMT3A or TET2 unique peaks, or DNMT3A-TET2 cobound peaks (n=4 donors).

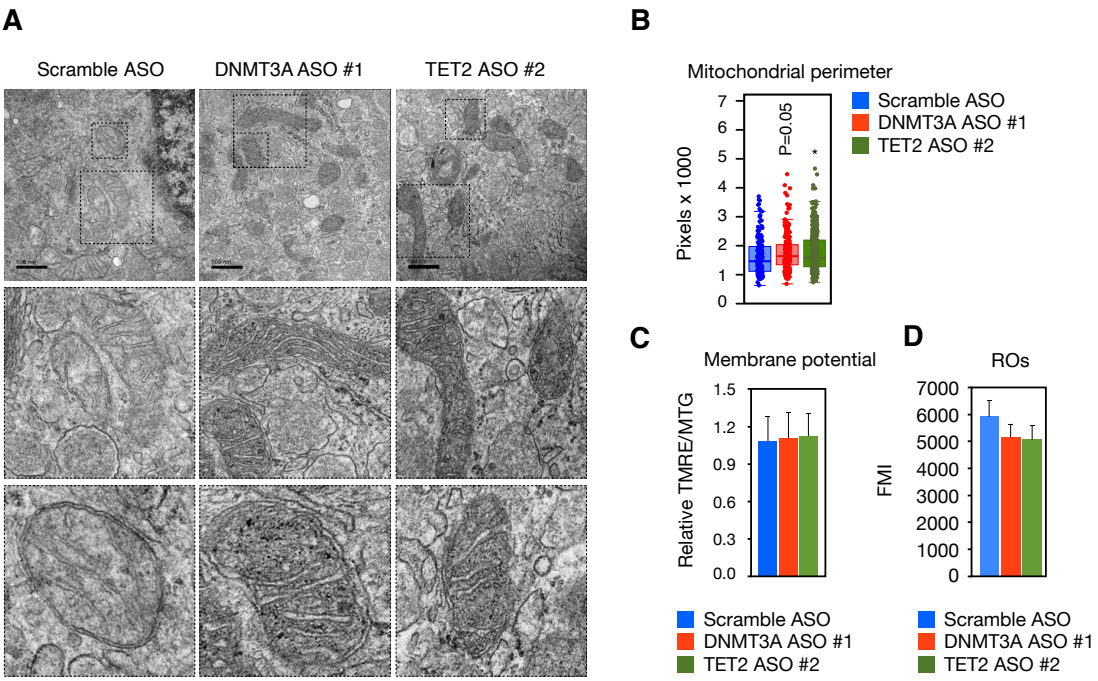


Figure S5

Figure S5-related to Figure 5. Larger mitochondria are observed in macrophages with reduced DNMT3A or TET2 expression, but this does not affect mitochondrial function nor alter levels of reactive oxygen species.

(A) Transmission electron microscopy images n mDM treated with DNMT3A ASO and TET2 ASO (n=1 donor). Squares with broken lines denote magnification areas in images below. Scale bars denote 500 nm.
(B) Quantification of mitochondria perimeter (n>100 mitochondria per condition)
(C) Mitochondria membrane potential in DNMT3A or TET2 treated mDM (left) assessed by flow cytometry ratio of TMRE/MTG. Larger mitochondria is observed in DNMT3A ASO or TET2 ASO treated mDM
(D) Levels of reactive oxygen species in DNMT3A ASO or TET2 ASO treated mDM (n≥4 donors)

Mann Whitney U-test was used to calculate statistical significance in all panels except in E where Student´s T test was used. * P<0.05; ** P<0.01.

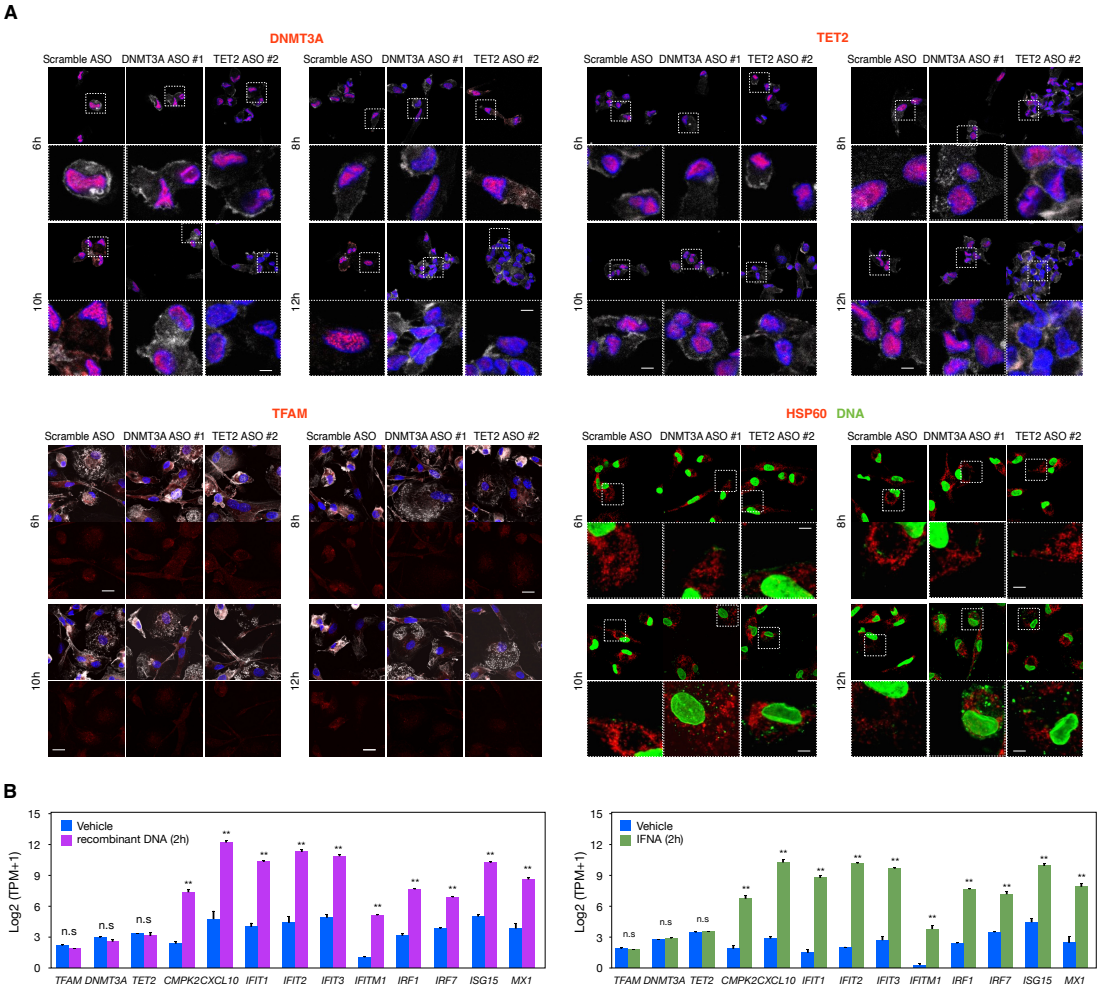


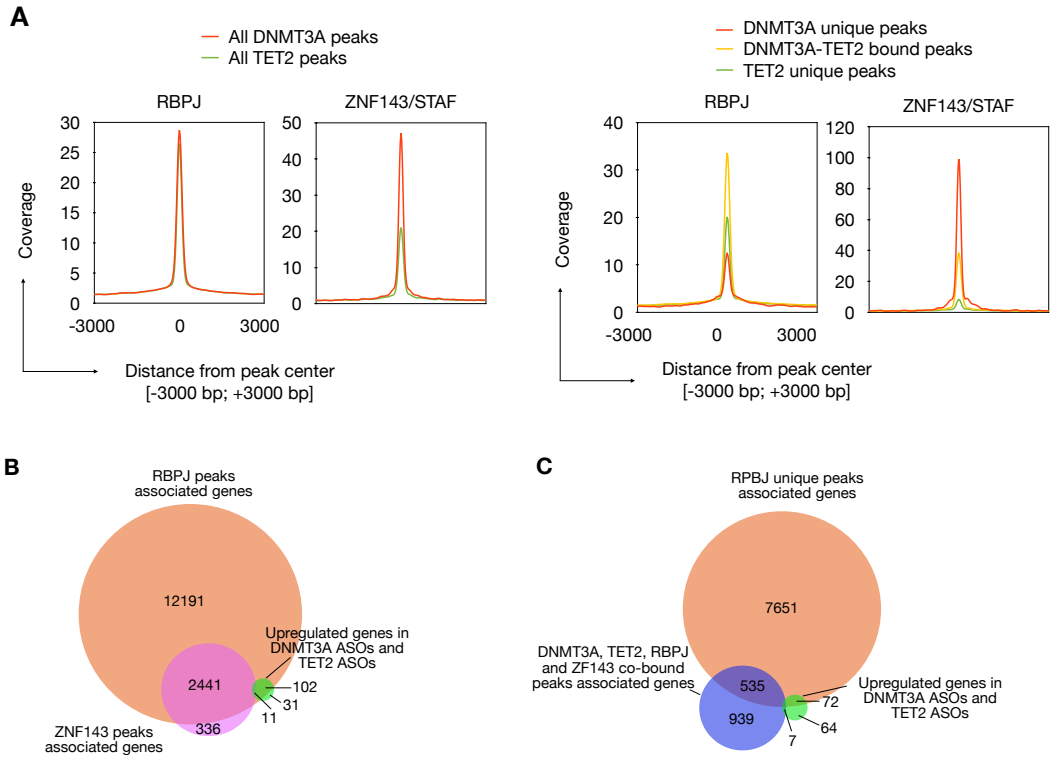
Figure SF6

Figure S6-related to Figure 6. TFAM downregulation precedes the formation of cytosolic DNA.

A) Protein analysis by IF of DNMT3A and TET2 at 8h whereas TFAM downregulation occurs at 10h and accumulation of cytosolic DNA occurs at 12h. Scale bars= 1 μm.

B) Activation of ISG by recombinant DNA (left) or IFNA (right) assessed by RNA-Seq does not lead to lower expression of *DNMT3A*, *TET2* or *TFAM* (n=3 donors).

Mann Whitney U-test was used to calculate statistical significance. * P<0.05; ** P<0.01.



Supplementary Figure 7

Figure S7-related to Figure 7. DNMT3A, TET2, RBPJ and ZNF143 are part of a collaborative network of transcription factors in mDM.

A) Histogram showing the enrichment in ZNF143 binding in DNMT3A peaks (left), in DNMT3A unique peaks (right) and the enrichment of RBPJ in peaks co-bound by DNTM3A and TET2 (right).
B) Venn diagram showing the overlap between of RBPJ peaks and ZNF143 peaks associated genes and the genes upregulated by DNMT3A ASO and TET2 ASOs.
C) Venn diagram of upregulated genes by DNMT3A ASO and TET2 ASOs and RBPJ unique peaks.

	Diagnosis	Age	Gender	*DNMT3A*	*TET2*	CVD
Sample 1	Normal	79	F	No mutation	No mutation	No
Sample 2	Normal	70	F	No mutation	No mutation	No
Sample 3	CCUS	72	F	c.2638A>G, p.M880V, VAF 21%	No mutation	No
Sample 4	MDS	76	F	c.1717C>T, p.Q573*, VAF 24%	No mutation	No
Sample 5	CCUS	88	M	No mutation	c.2176C>T, p.Q726*, VAF 17%	Yes
Sample 6	CCUS	69	M	No mutation	c.519delG, p.P174Lfs*9, VAF 36%; c.5551_5552insT, p.E1851Vfs*8, VAF 29%	Yes

Table S1-related to Figure 2, 5. Demographics of patient samples used. CVD denotes cardiovascular disease.