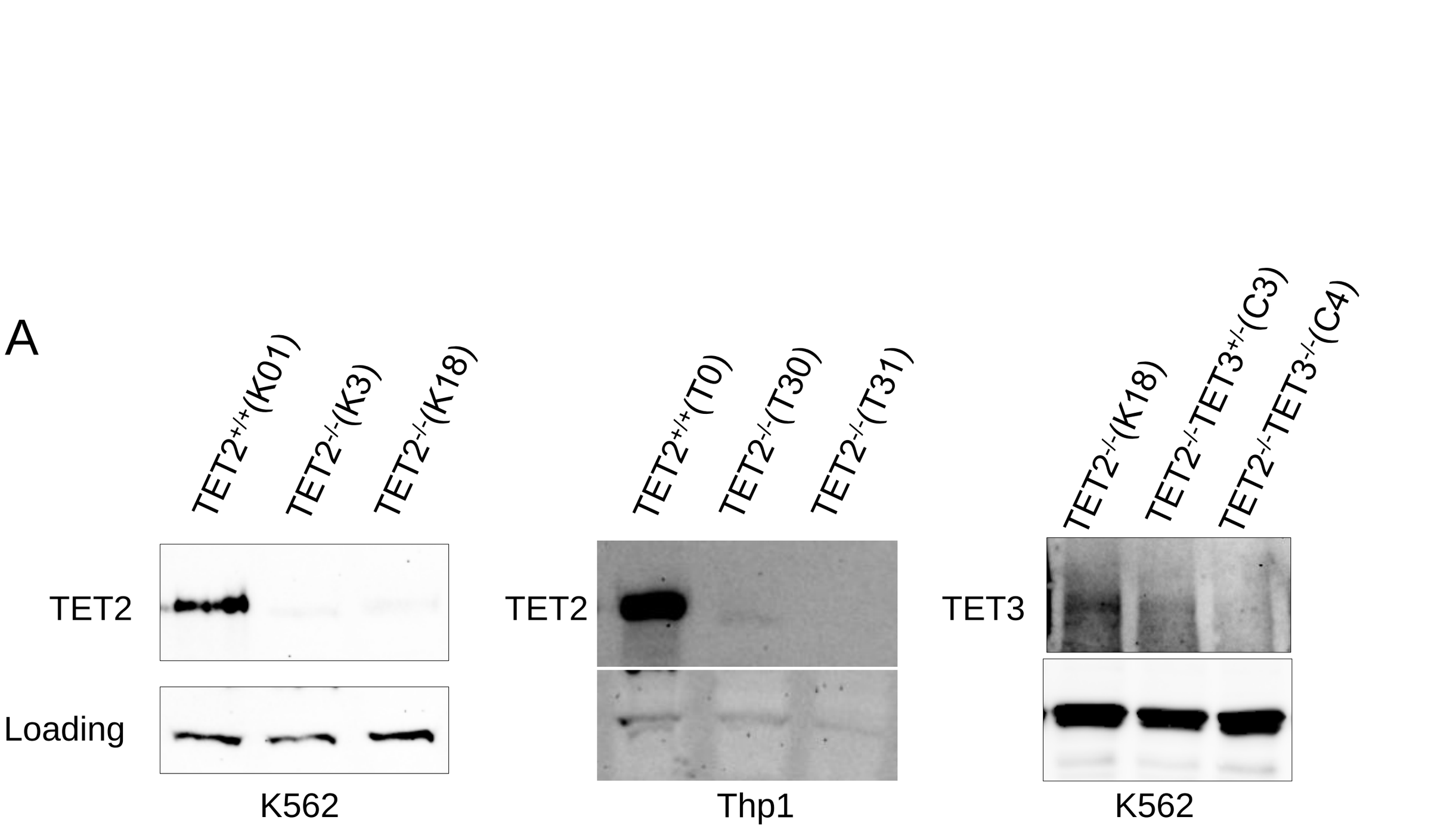
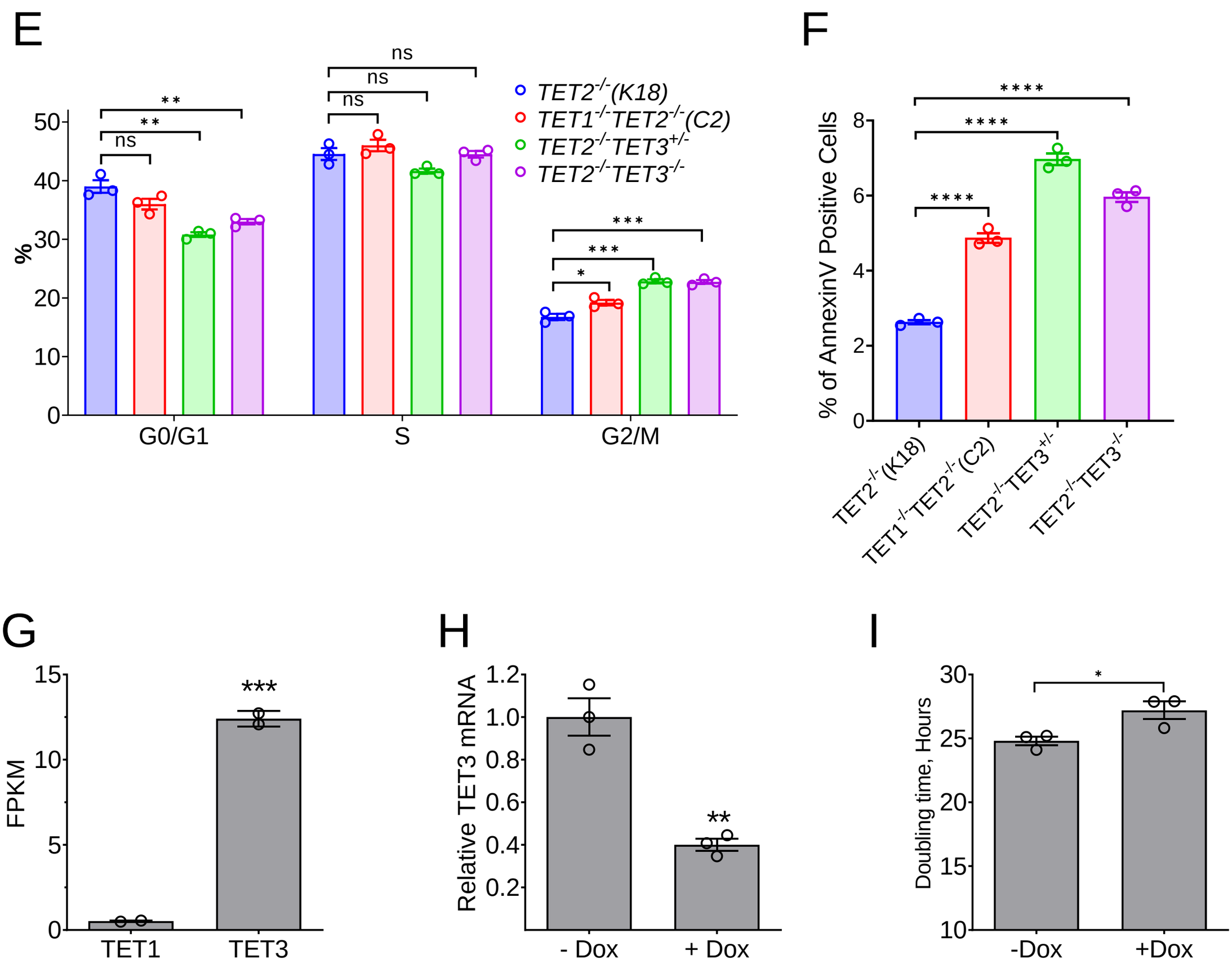


Supplementary Figure 1

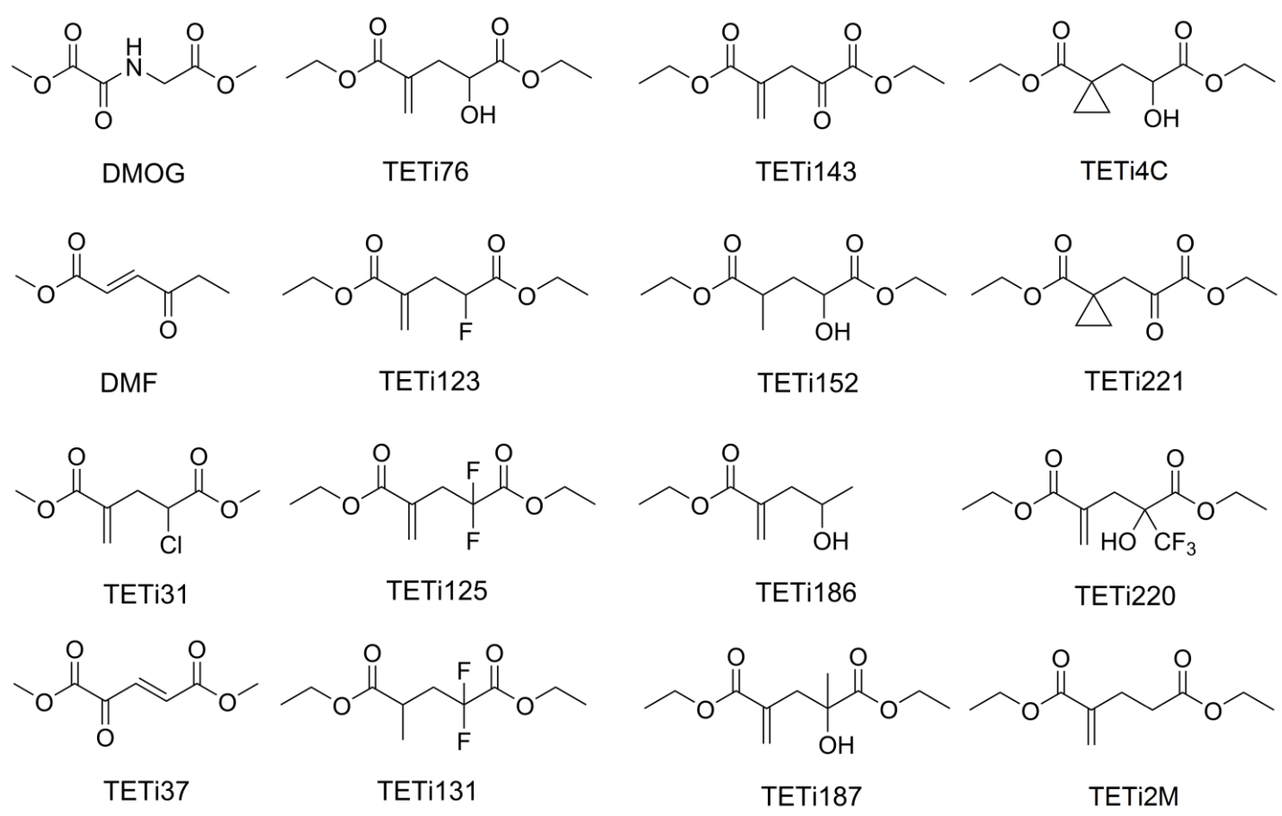


Supplementary Figure 1. A, Western blot analysis of isogenic TET2 and TET3 knockout cells. **B,** Dot blot analysis of 5hmC/5mC in different isogenic TET dioxygenase knockout cells. BioRad imager was used for quantification of 5hmC/5mC. **C,** Inducible expression of 3XFlag-IDH1^{R132C} in K562 TET2$^{+/+}$ and TET2$^{-/-}$ cells. Cells were treated with 1 μg/ml doxycycline (Dox) for 3 days. Anti-Flag antibody was used in western blot for the detection of induced IDH1^{R132C}. **D,** Cells grow at similar rate without (w/o) Dox treatment. Three independent clones from each of TET2$^{+/+}$-IDH1^{R132C} and TET2$^{-/-}$-IDH1^{R132C} were monitored for the growth in duplicate at least three times. The total cell output was plotted as a function of time. **E-F,** Further deletion of *TET1* or *TET3* in *TET2-/-* background prolonged the G2/M cell cycle (propidium iodide staining) and also the increased basal levels of apoptotic cell death (Annexin V staining). **G,** *TET1* and *TET3* mRNA level. **H,** *TET3* mRNA levels upon induction of shRNA targeting *TET3*. **I,** Cell growth of SIGM5-tet-on-shTET3 upon Dox induction. Data are shown as mean±SEM; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns: not significant.

A

Chemicals	R^1	R^2	R^3
aKG	=O	-	H
2HG	-OH	-H	-H
NOG/DMOG	structure in Fig. S2B		
DMF	structure in Fig. S2B		
TETi31	-Cl	-H	$=CH_2$
TETi37	$-^3CH=^4CH-$	-	=O
TETi76	-OH	-H	$=CH_2$
TETi123	-F	-H	$=CH_2$
TETi125	-F	-F	$=CH_2$
TETi131	-F	-F	$-CH_3$
TETi143	=O	-	$=CH_2$
TETi152	-OH	-H	$-CH_3$
TETi186	structure in Fig. S2B		
TETi187	-OH	$-CH_3$	$=CH_2$
TETi220	-OH	$-CF_3$	$=CH_2$
TETi221	=O	-	cyclopropyl
TETi2M	H	H	=CH2
TETi4C	-OH	H	cyclopropyl
TETi46	=O	-	$-CH_3$
TETi48	=O	-	$-C_2H_5$

B



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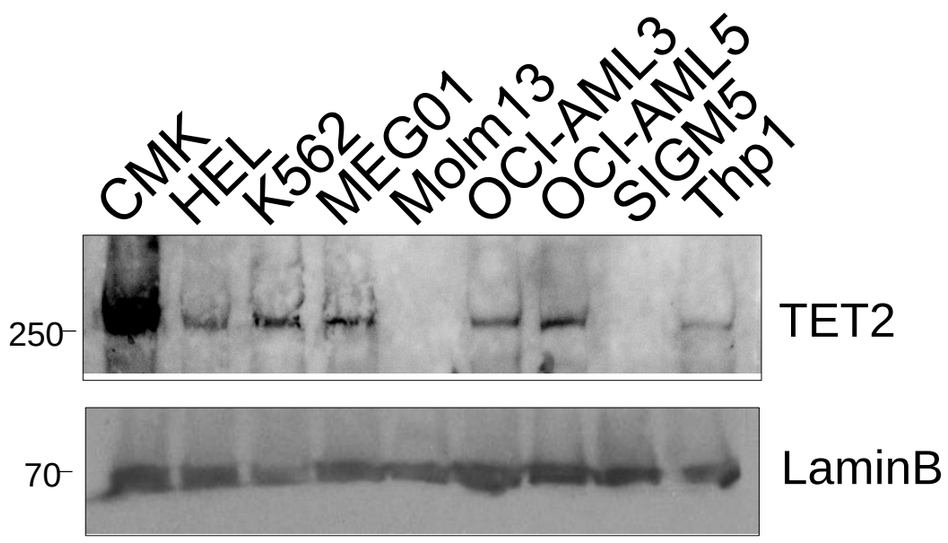

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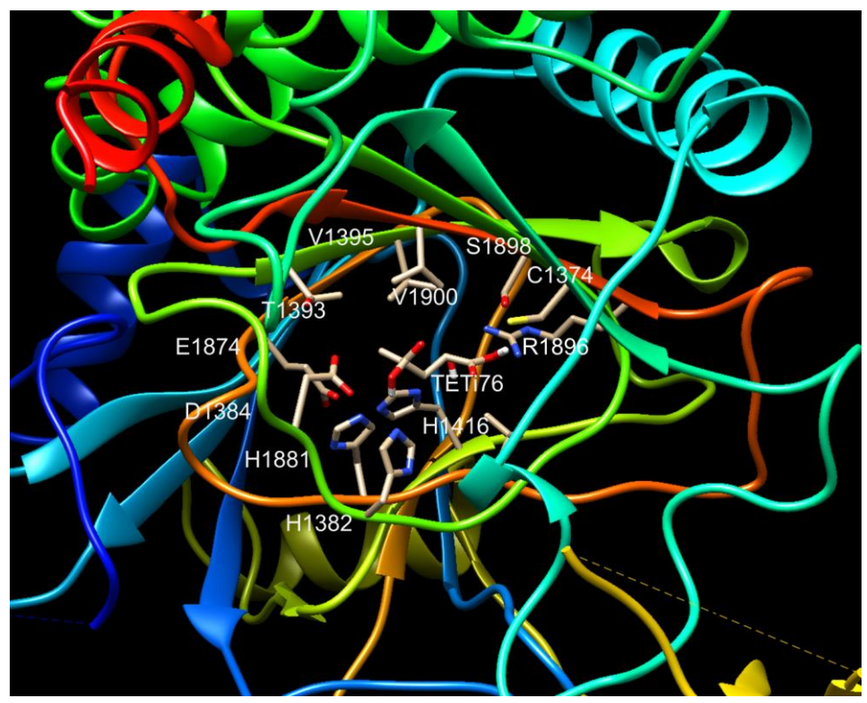


E

```
Q6N021 TET2_HUMAN  1323   ESHLQNLSTLMAPTYKKLAPDAYNNQIEYEHRAPECRLGLKEGRPFSGVTACLDFCAHAH
Q8NFU7 TET1_HUMAN  1613   EDNLQSLATRLAPIYKQYAPVAYQNQVEYENVARECRLGSKEGRPFSGVTACLDFCAHPH
O43151 TET3_HUMAN   883   RKSFQDLATEVAPLYKRLAPQAYQNQVTNEEIAIDCRLGLKEGRPFAGVTACMDFCAHAH
                          .. :*.*:* :** **: ** **:**:  *. * :**** ******:*****:***** *

Q6N021 TET2_HUMAN  1383   RDLHNMQNGSTLVCTLTREDNREFGGKPEDEQLHVLPLYKVSDVDEFGSVEAQEEKKRSG
Q8NFU7 TET1_HUMAN  1673   RDIHNMNNGSTVVCTLTREDNRSLGVIPQDEQLHVLPLYKLSDTDEFGSKEGMEAKIKSG
O43151 TET3_HUMAN   943   KDQHNLYNGCTVVCTLTKEDNRCVGKIPEDEQLHVLPLYKMANTDEFGSEENQNAKVGSG
                          :* **: **.*:*****:**** .*  *:**********:::.***** *  : *  **

Q6N021 TET2_HUMAN  1755   EHHSPSHIIHNYSAAPGMFNSSLHALHLQNKENDMLSHTANGLSKMLPALNH---DR---
Q8NFU7 TET1_HUMAN  1939   PNHQPSFLTSP--------------------QDLASSPMEEDE---------------
O43151 TET3_HUMAN  1389   ALAGPSLTEKPWALGAGDFNSALKGSPGF---QDKLWNPMKGEEGRIPAAGASQLDRAWQ
                             **                            :*      :  .

Q6N021 TET2_HUMAN  1809   --------TACVQGGLH---KLSD----ANGQ-----EKQPLALVQ--GVASGAEDNDEV
Q8NFU7 TET1_HUMAN  1962   ----------------------------QHSEADEPPSDEPLSDDPLSPAEEKLPHIDEY
O43151 TET3_HUMAN  1446   SFGLPLGSSEKLFGALKSEEKLWDPFSLEEGPAEEPPSKGAVKEEK--GGGGAEEEEEEL
                                                       ..      ..  :          . :*

Q6N021 TET2_HUMAN  1847   WSDSEQSFLDPDIGGVAVAPTHGSILIECAKRELHATTPLKNPNRNHPTRISLVFYQHKS
Q8NFU7 TET1_HUMAN  1994   WSDSEHIFLDANIGGVAIAPAHGSVLIECARRELHATTPVEHPNRNHPTRLSLVFYQHKN
O43151 TET3_HUMAN  1504   WSDSEHNFLDENIGGVAVAPAHGSILIECARRELHATTPLKKPNRCHPTRISLVFYQHKN
                          *****: *** :*****:**:***:*****:********:::*** ****:********.

Q6N021 TET2_HUMAN  1907   MNEPKHGLALWEAKMAEKA---REKEEECEKYGPDYVPQKSHGKKVKREP---AEPHETS
Q8NFU7 TET1_HUMAN  2054   LNKPQHGFELNKIKFEAKEAKNKKMKAS---------EQK--------DQAANEGPEQSS
O43151 TET3_HUMAN  1564   LNQPNHGLALWEAKMKQLAERARARQEEAARLGLGQQEAKLYGKKRKWGGTVVAEPQQKE
                          :*:*:**: * : *:       :  : .          *                *.:..

Q6N021 TET2_HUMAN  1961   EPTYLRFIKSLAERTMSVTTDSTVTTSPYAFTRVTGPYNRYI
Q8NFU7 TET1_HUMAN  2097   E--VNELNQIPSHKALTLTHDNVVTVSPYALTHVAGPYNHWV
O43151 TET3_HUMAN  1624   KKG-----VVPTRQALAVPTDSAVTVSSYAYTKVTGPYSRWI
                          :          :.::::  *..**.* ** *:*:***.:::
```

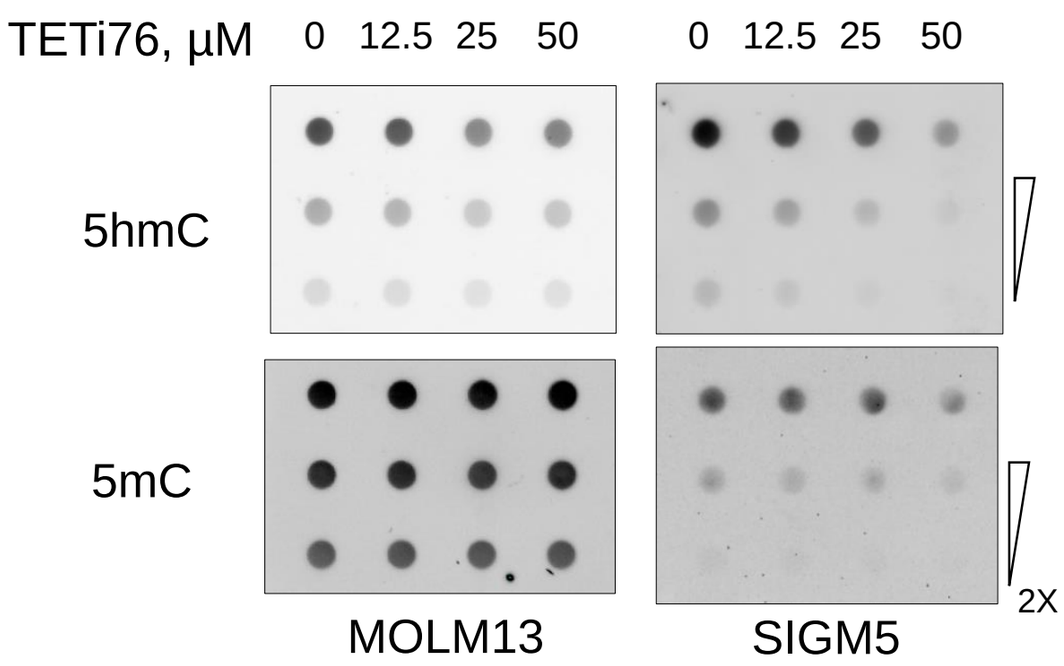
F

G

Supplementary Figure 2

Supplementary Figure 2. A-B, Two dimensional structures for TETi. **C,** Western blot analysis of TET2 protein levels in different leukemia cell lines indicated on the top of each lane. **D,** Binding mode of TETi76 in NOG binding site. **E,** Alignment of the catalytic domain of TET1, TET2 and TET3 proteins. **F,** Coomassie blue staining of SDS PAGE gel of recombinant $TET2^{CD}$ used in cell free assays. **G,** R- and S- enantiomers of TETi76 showed similar activity in TET2 activity inhibition *in vitro*. Catalytic domains of recombinant TET2 was incubated with different concentrations of TETi76 or enantiomers (R or S) and the 5hmC was monitored by ELISA using anti 5hmC antibodies.

A



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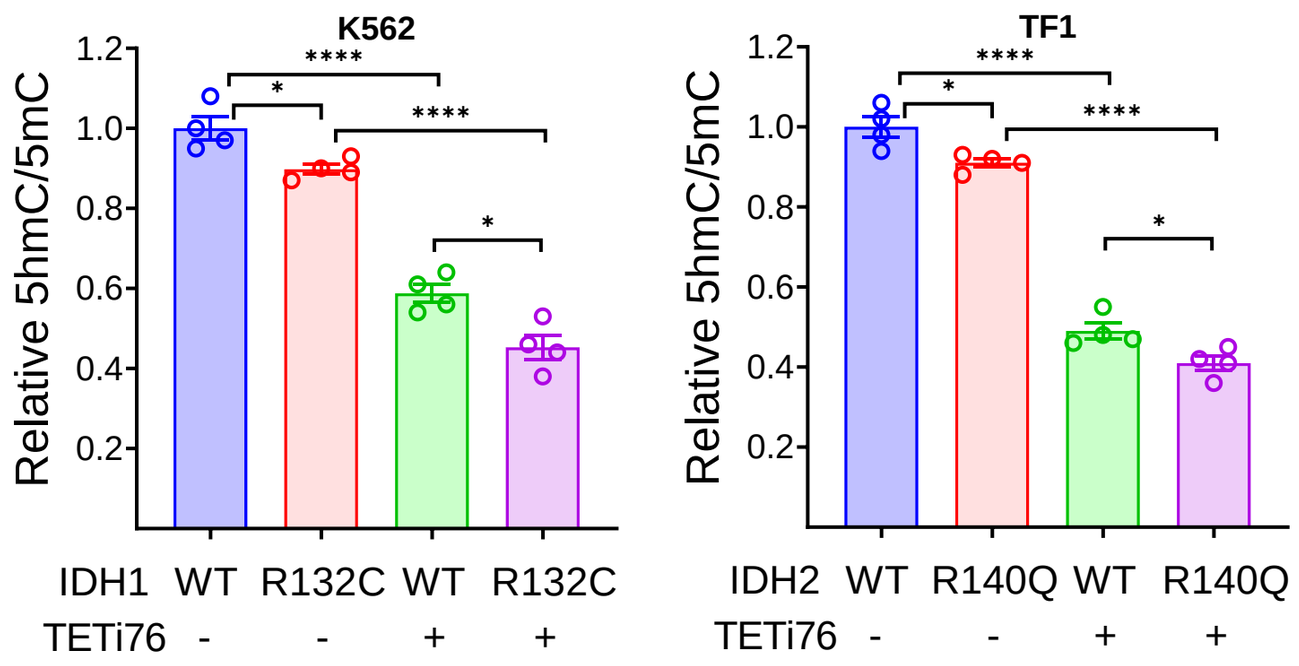

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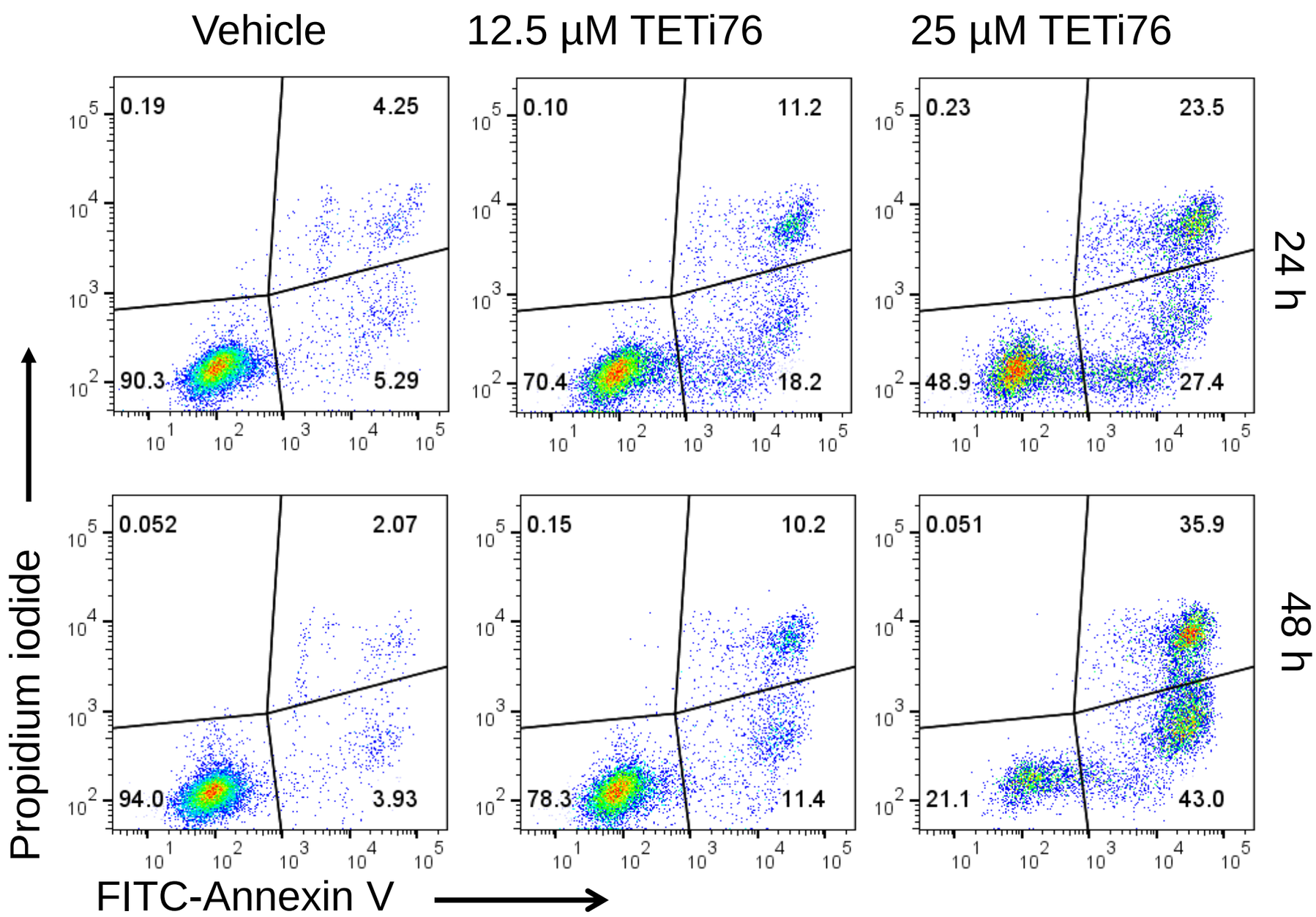

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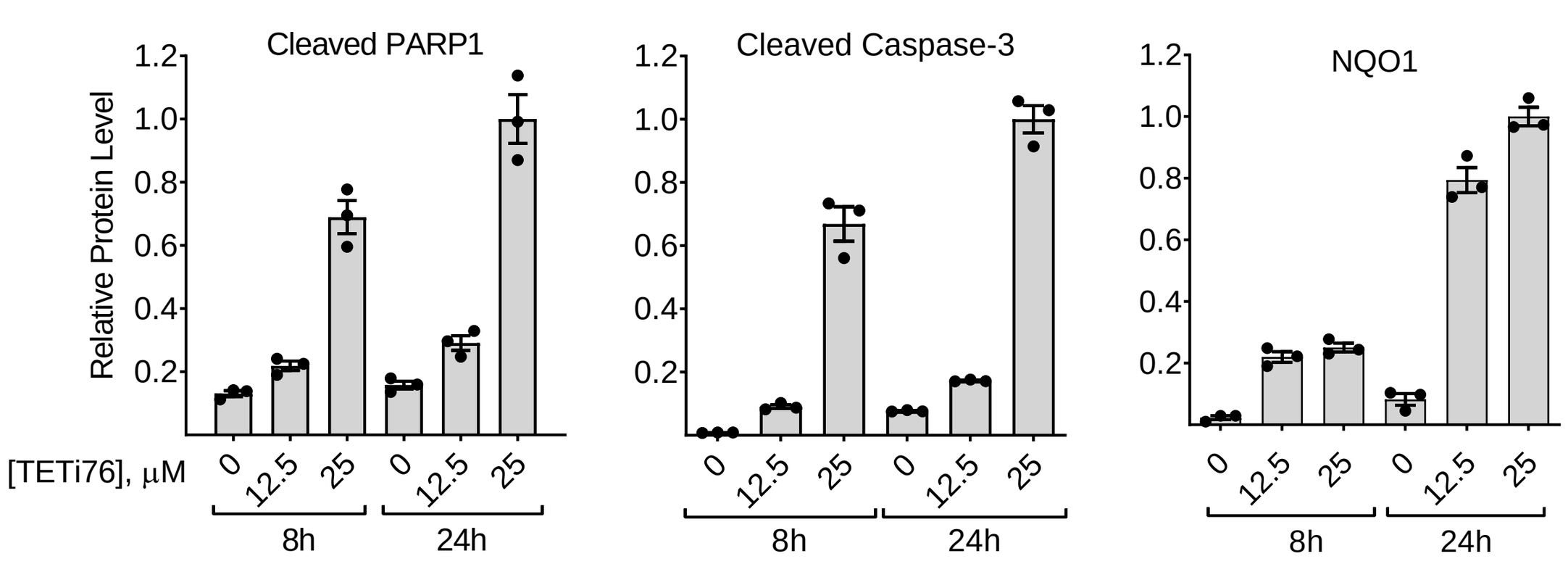
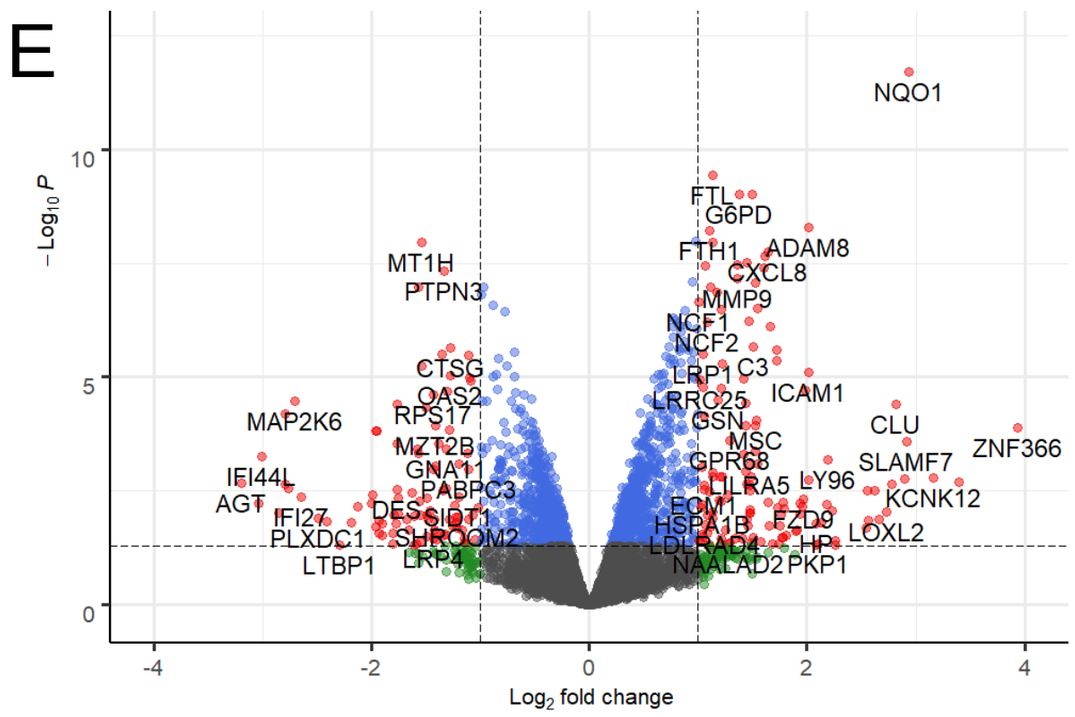
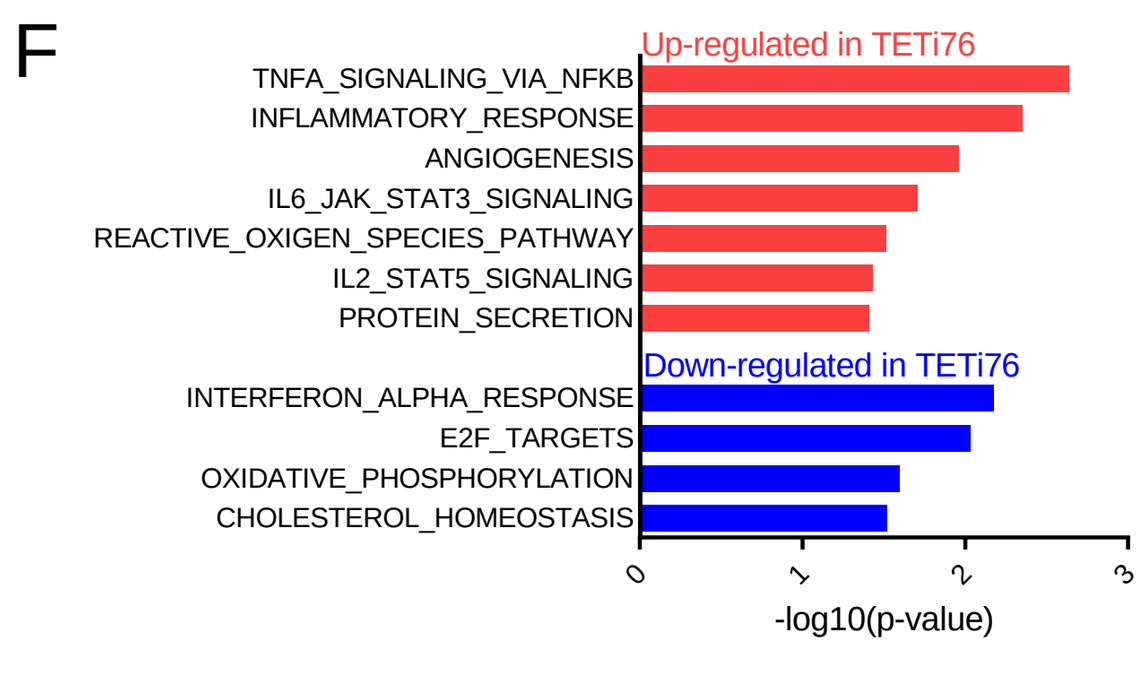
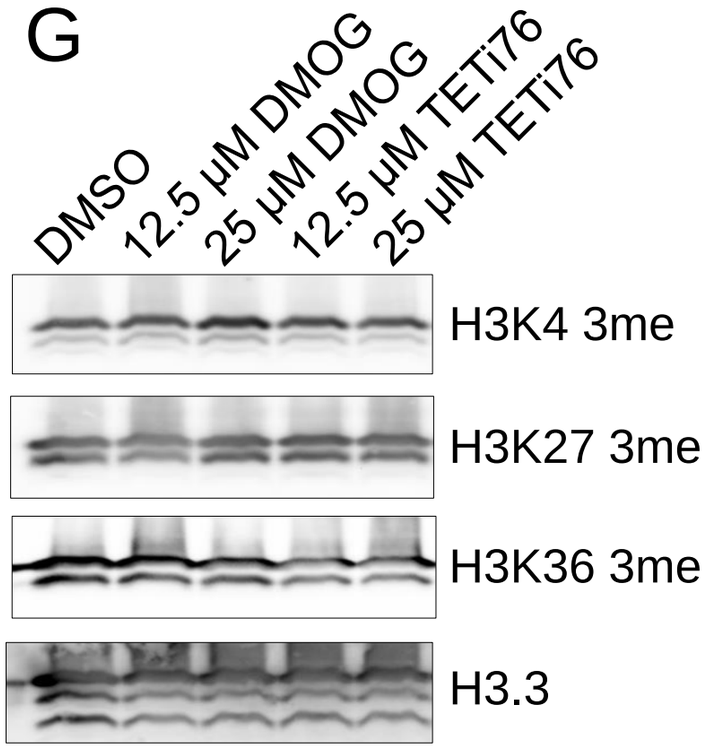
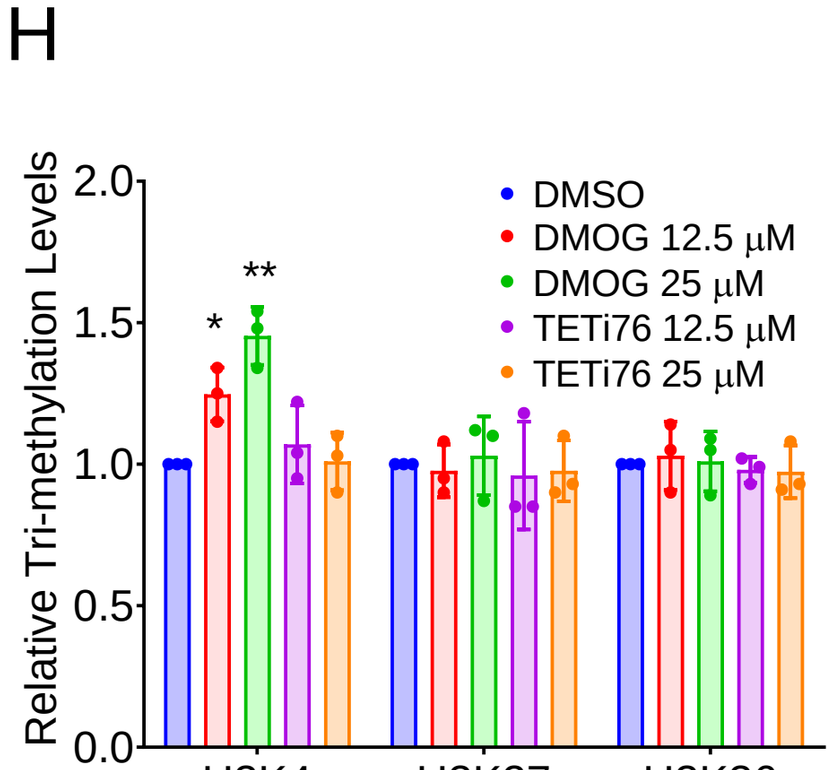


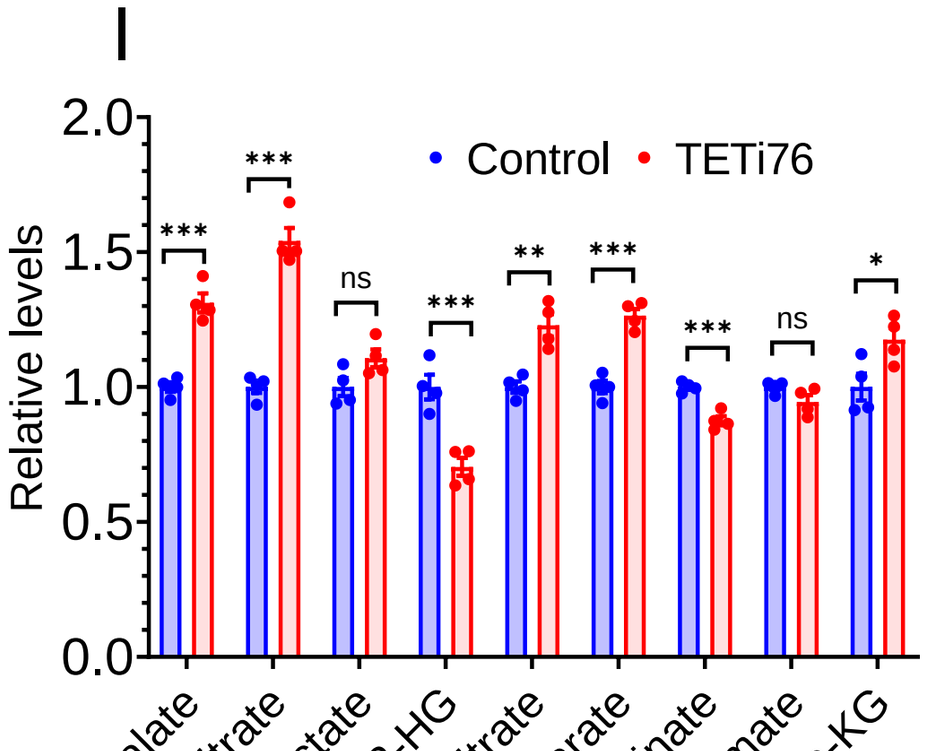
Supplementary Figure 3



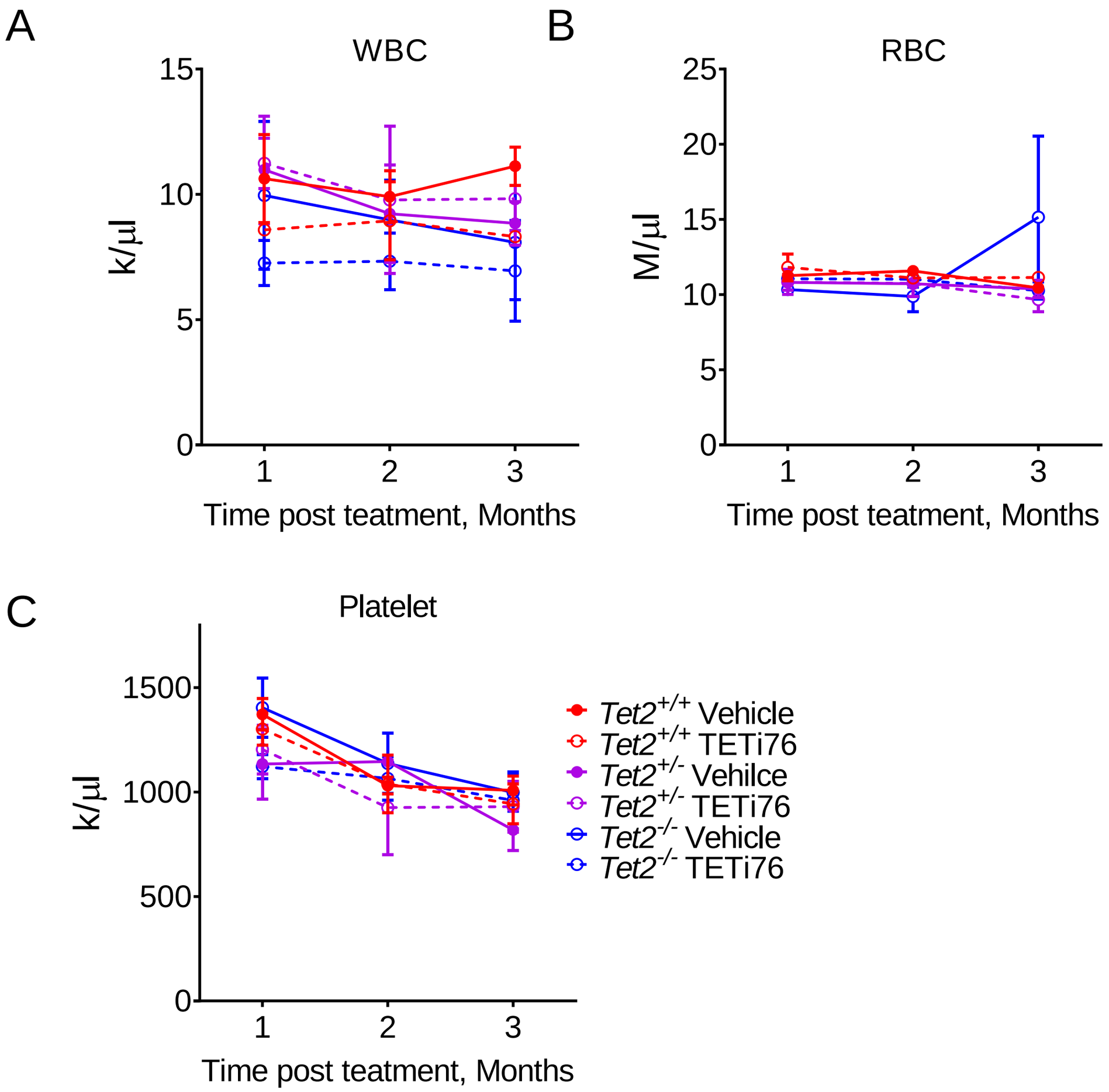








Supplementary Figure 3. A, TETi treatment mimics loss of TET activity in leukemia cells. Cells were treated with indicated concentrations of TETi76 in the presence of 100 μM sodium ascorbate for 12 hours. DNA was extracted for 5hmC and 5mC analysis by dot blot. **B,** Neomorphic IDH1/2 mutations lead to partial loss of TET-dioxygenase which can be further inhibited by TETi76. Cells were treated 25 μM TETi76 in the presence of 100 μM sodium ascorbate for 12 hours. DNA was extracted for dot blot analysis of 5hmC/5mC. Both K562 and K562-IDH1^{R132C} cells were pretreated with 1 μg/ml doxycycline for 2 days. **C,** Quantification of apoptosis analysis of SIGM5 cells treated with TETi76 by annexin V and propidium iodide staining using flow cytometer. **D,** Quantification of cleaved PARP1, cleaved Caspase-3 and NQO1 levels using Image Lab 6.0 (Biorad). **E,** Volcano plot analysis of RNAseq data of SIGM5 cells treated with TETi76 (12.5 μM) for 24 hours. **F,** Pathway analysis performed by hallmark gene set enrichment analysis of RNAseq data of SIGM5 cells treated with TETi76. **G-H,** TETi76 does not affect histone methylation. SIGM5 cells were treated with indicated compounds for 12 hours and western blot analysis and quantification were performed for histone methylation upon TETi76 or DMOG treatment. **I,** Mass spectral analysis of indicated metabolite upon TETi76 treatment.



Supplementary Figure 4. A-C, TETi76 had no adverse effect on normal HSPCs growth *in vivo*. *Tet2*$^{+/+}$, *Tet2*$^{+/-}$ and *Tet2*$^{-/-}$ mice (n=3/group) were treated orally with TETi76 (50mg/Kg), 5 days/week for 3 months. Peripheral blood samples were counted once a month. **A**. WBC: white blood cell. **B**. RBC: red blood cell; **C**. Platelet, remained in normal range for each genotype.