Supplementary Information for

PRMT5 regulates ATF4 transcript splicing and oxidative stress response

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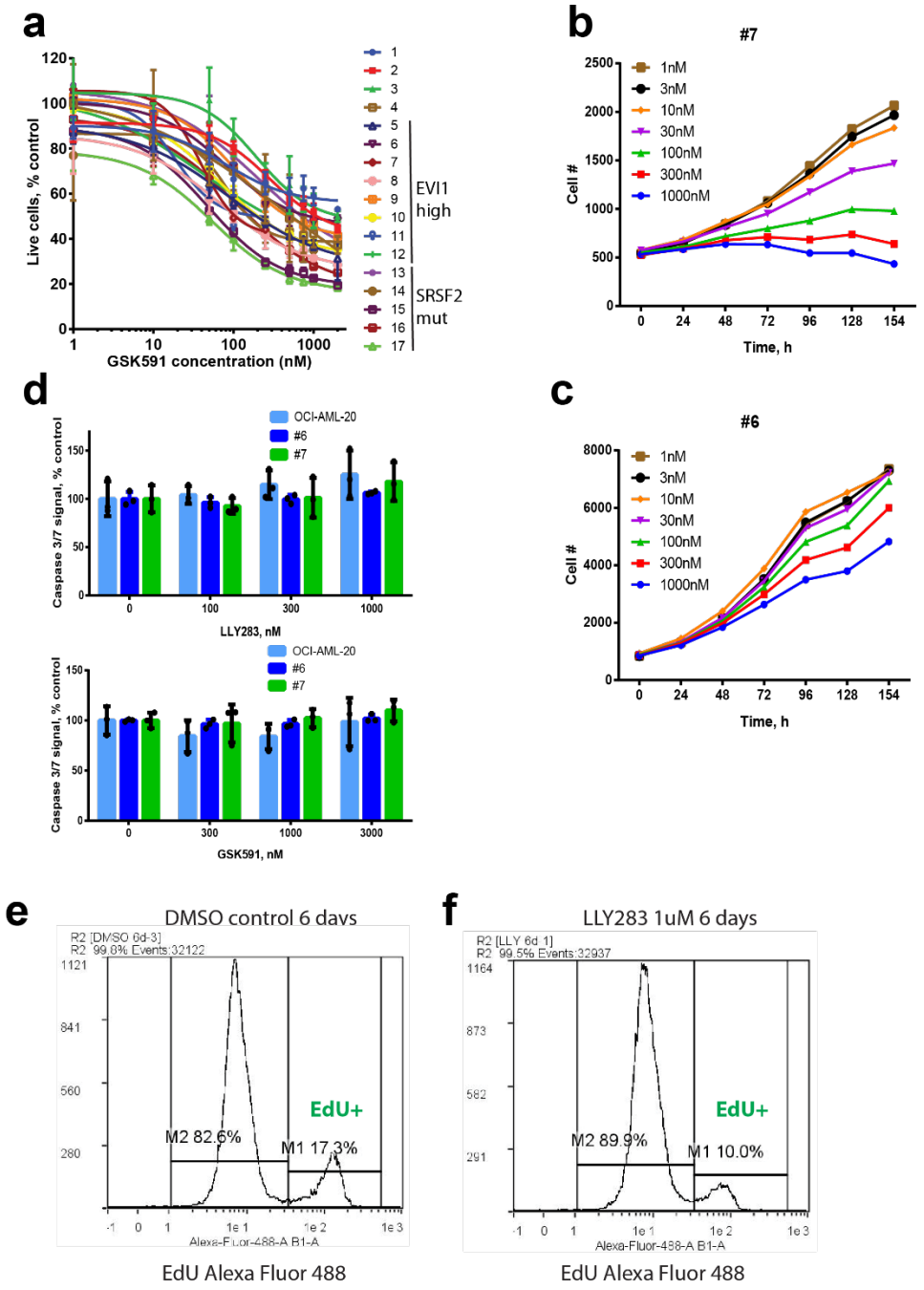
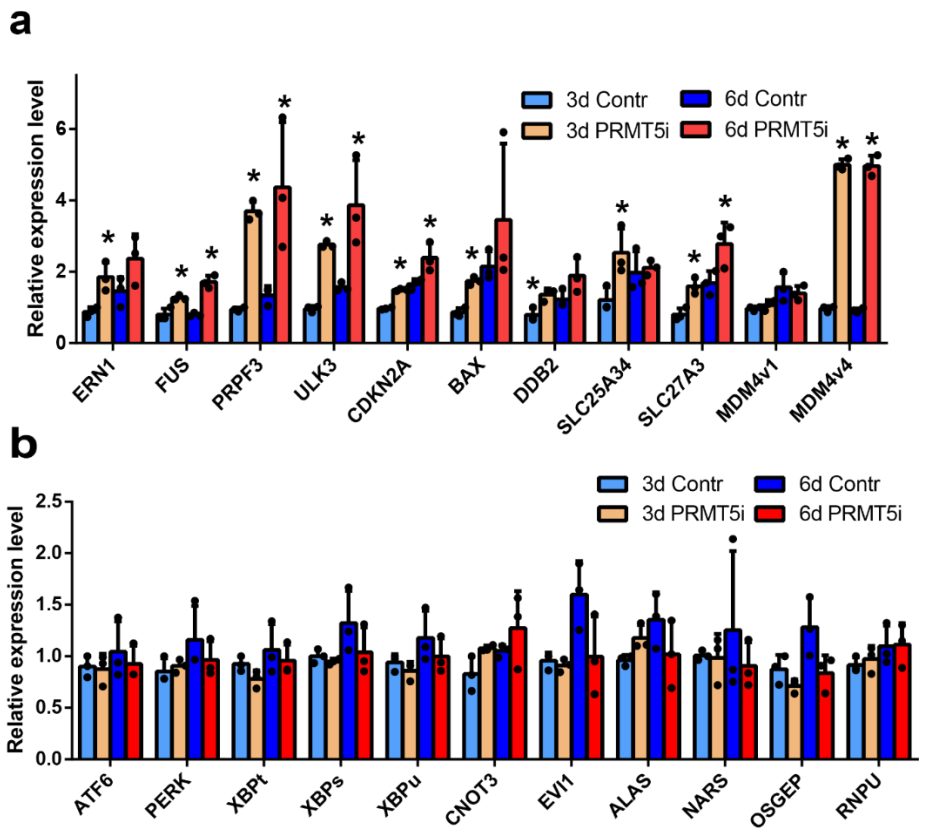
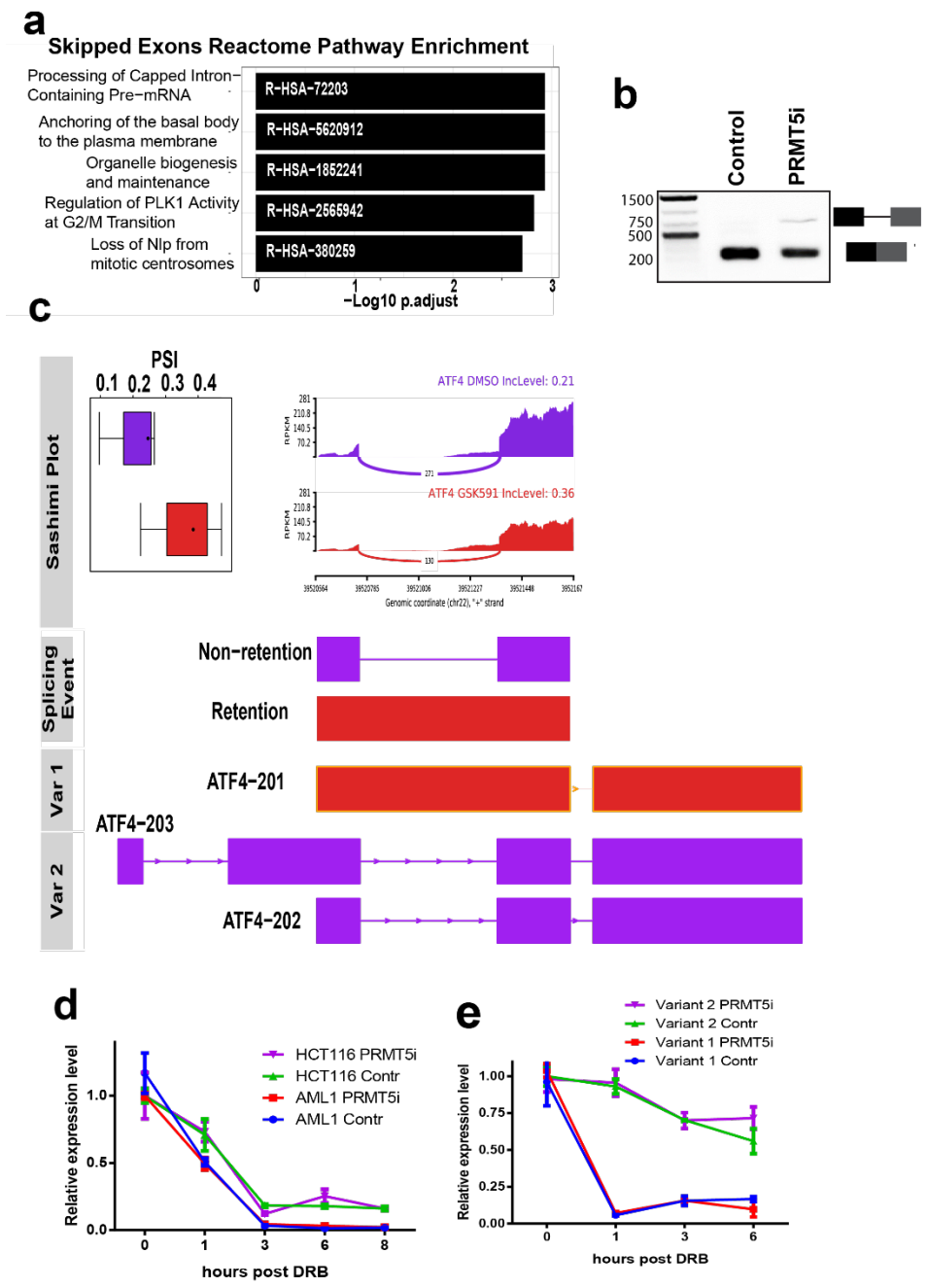


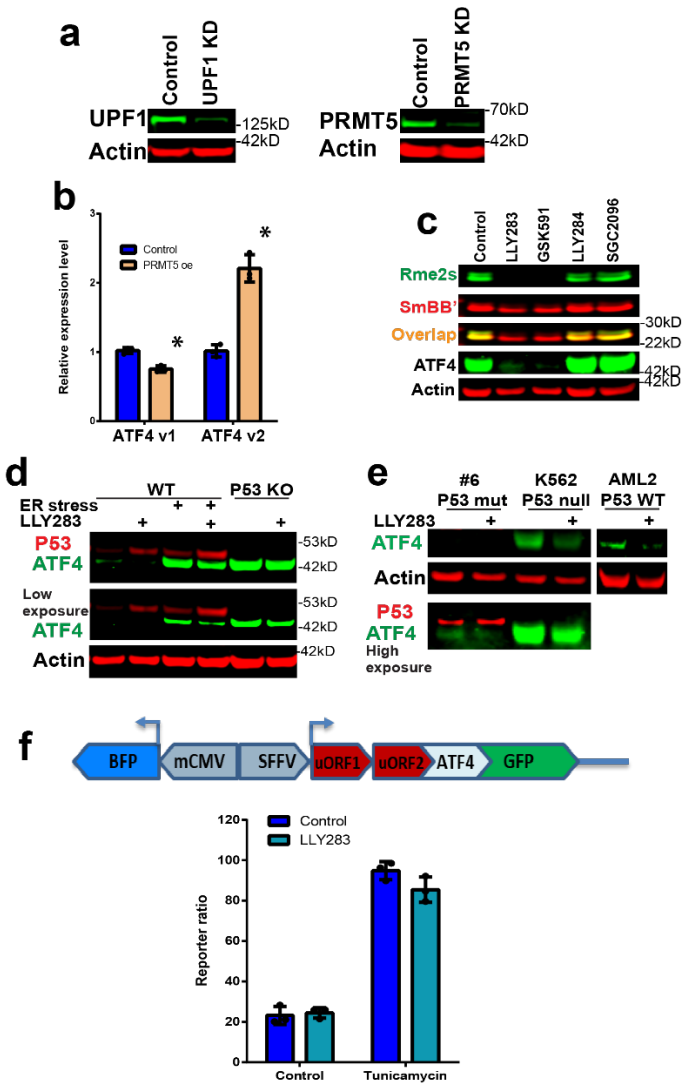
Suppl. Fig. 1. PRMT5 inhibition reduces cell proliferation and S-phase but does not affect apoptosis. **a)** Dose-response of PRMT5 inhibition suppressing cell proliferation in leukemia patient samples for IC_{50} values shown in Fig. 1a. (mean+/- SEM, n=3) **b-c)** Kinetics of cell growth response in #7 and #6 (P53 mutant) EVI1-high patient samples. **d)** Apoptosis levels are not affected by PRMT5 inhibition as measured by cleaved caspase 3/7 fluorescent signal after 6-day compound treatment, N=3, mean±SD. **e-f)** Flow cytometry histograms to illustrate data in Fig. 1c.



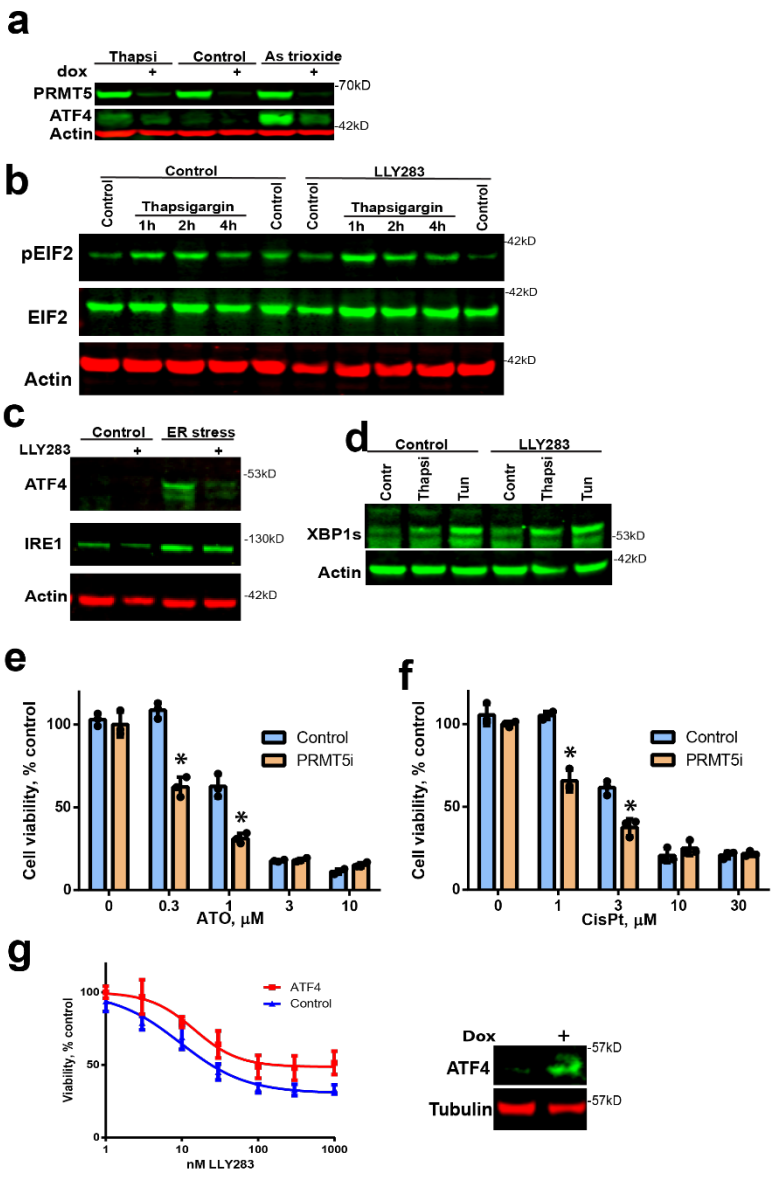
Suppl. Fig. 2. PRMT5 regulates gene expression. **a)** Validation of genes upregulated by PRMT5 inhibition. **b)** Genes associated with the UPR pathway and others that were found not to be regulated by PRMT5 inhibition. N=3, means±SD, * $p \leq 0.05$, relative to time-matched control.



Suppl. Fig. 3. PRMT5 regulates splicing programs. **a)** Significantly enriched Reactome pathways in PRMT5 inhibition elicited exon-skipping gene set. **b)** ATF4 splice form PCR agarose gel representation. Experiment performed as in Fig.3c, UCSD-AML-1 cells. **c)** ATF4 splice form regulation analysis. Top panel: sashimi plots illustrate the difference in inclusion level of introns between DMSO and GSK591 (1 μM) treatment, while boxplots summarize the distribution of percentage spliced-in (PSI) of introns. Bottom panels illustrate the non-retention versus retention splicing events analyzed for sashimi plots and which splicing event is involved in each ATF4 transcript variant. **d)** Transcript stability for c-MYC. **e)** ATF4 variant stability in HCT116 cells under the same experimental conditions as in Fig. 3d.

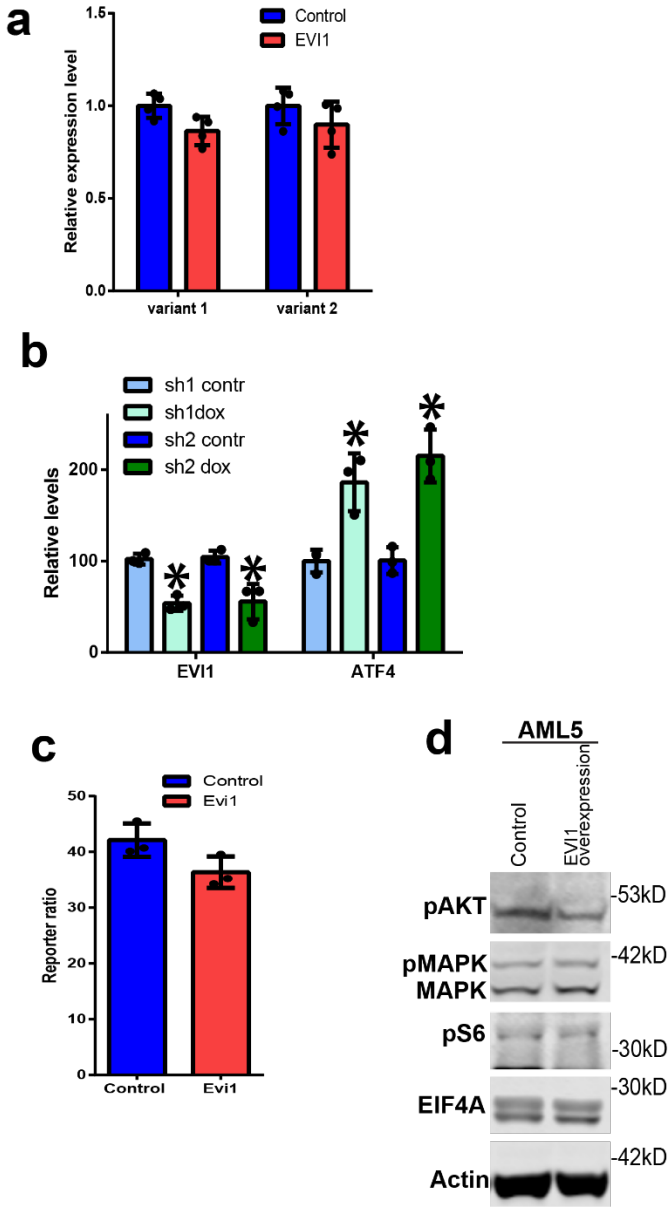


Suppl. Fig. 4. PRMT5 loss of function and knockdown leads to reduced ATF4 levels a) UPF1 and PRMT5 knockdown validation for data in Fig. 3e. **b)** Overexpression of PRMT5 upregulates V2 and downregulates V1 transcripts of ATF4 in OCI-AML5 cells, N=3, means±SD, * $p<0.05$. **c)** PRMT5 inhibitors LLY283 (100 nM) and GSK591 (1 µM) but not their negative control compounds LLY284 (100 nM) and SGC2096 (1 µM) reduce SmBB' arginine methylation and ATF4 protein levels at six days. **d)** PRMT5 inhibition (LLY283, 100nM) reduces the levels of ATF4 protein irrespective of P53 status. *TP53* knockout (KO) HCT116 cells have high baseline levels of ATF4 protein. The comparison between low ATF4 levels at baseline (*TP53* WT), induced (ER stress thapsigargin, eight hours, 300 nM), and *TP53* KO HCT116 cells. **e)** The status of p53 does not influence the downregulation of ATF4 upon PRMT5 inhibition and this downregulation occurs in other genetic background leukemias. *TP53* mutant and null cells were treated with 100-300 nM LLY283 for six days. **f)** Reporter system to evaluate the translation of ATF4. Heterologous bidirectional promoters drive transcription of ATF4-GFP and BFP (the latter is used for normalization). Cells expressing this reporter are GFP positive if ATF4-GFP is translated. PRMT5i does not affect ATF4 translation reporter under baseline control conditions or when ATF4 translation is induced by 4 h tunicamycin (Tun) exposure (N=3, mean±SD).

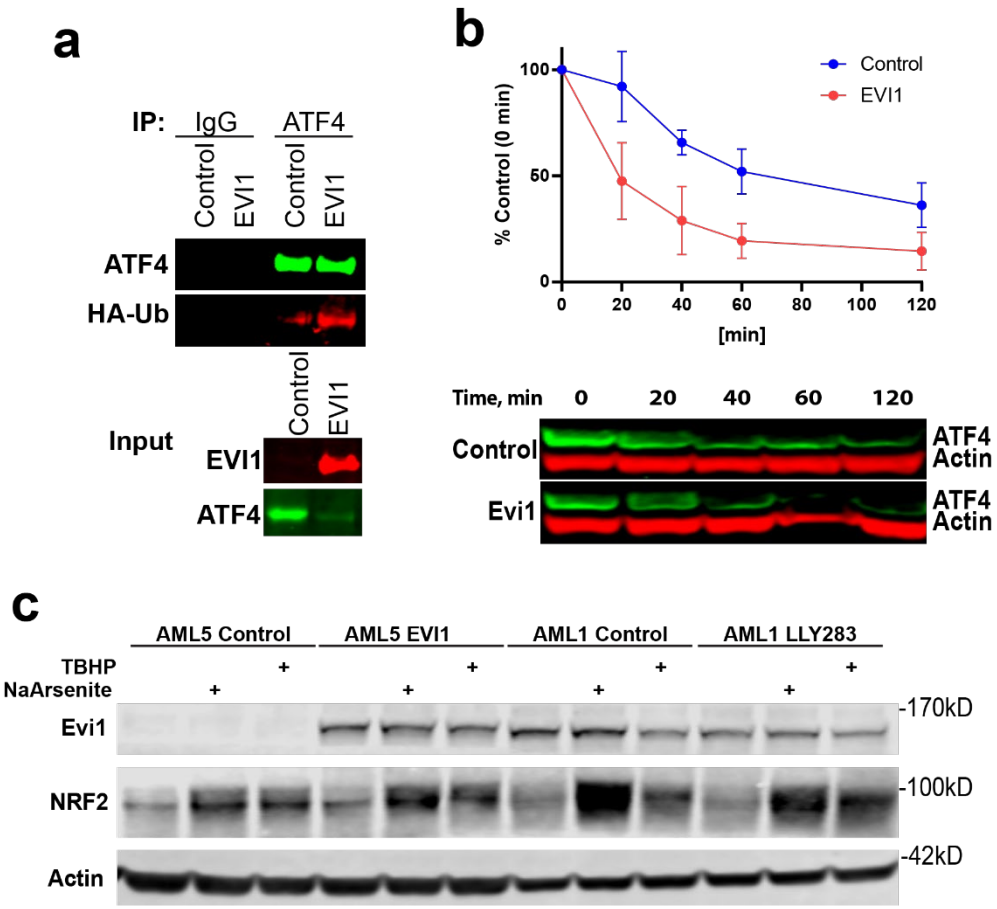


Suppl. Fig. 5. PRMT5 loss of function affects ATF4 levels, sensitivity to oxidative stress but does not influence other branches of UPR. **a)** PRMT5 knockdown downregulates levels of ATF4, the experiment performed as in Fig. 5a with doxycycline treatment to induce PRMT5 shRNA for 6 days. **b)** PRMT5 inhibition does not affect EIF2 phosphorylation (pEIF2). Tg denotes thapsigargin treatment (300 nM) that increases pEIF2. **c-d)** IRE1 or XBP1 levels were not affected by PRMT5 inhibition in control or ER stress (tunicamycin, Tun or thapsigargin, (Thapsi) conditions. **e-f)** PRMT5 inhibition (LLY238, 30 nM, 3 days) sensitizes UCSD-AML-1 cells to arsenic trioxide (ATO) and cisplatin (CisPt) exposure for 72 h. Cell viability was determined using resazurin assay, N=3, mean±SD *p< 0.05. **g)** Overexpression of ATF4 partially rescues cell proliferation defect of PRMT5 inhibition in OCI-AML-1 cells. Cell viability was determined using resazurin assay N=3, mean±SEM. Inducible overexpression of

ATF4 in UCSD-AML-1 cells is shown on the right. Cells were exposed to doxycycline (dox) for two days, and ATF4 levels were evaluated by blotting.



Suppl. Fig. 6. EVI1 overexpression downregulates ATF4 protein levels. **a)** EVI1 overexpression does not affect transcript levels of ATF4 variants 1 and 2. EVI1 was overexpressed as in Fig. 6b, and variant abundance was analyzed as in Fig. 3. **b)** Quantitation of Fig. 6d blots, (N=3, * $p<0.05$, mean±SD).**c)** EVI1 overexpression does not affect the translation of ATF4. ATF4 reporter was analyzed as in Suppl. Fig. 4e. **d)** EVI1 overexpression does not affect Akt, MAPK, or S6 kinase phosphorylation or EIF4A levels.



Suppl. Fig. 7. EVI1 regulates ATF4 translation. **a)** EVI1 overexpression leads to higher ATF4 ubiquitylation. HEK293 cells were transfected with EVI1, ATF4, and HA-ubiquitin. Immunoprecipitated ATF4 was blotted for ATF4 and HA-ubiquitin levels. **b)** EVI1 overexpression decreases the stability of endogenous ATF4 protein in OCI-AML5 cells as determined by assessing ATF4 protein levels upon cycloheximide (100 µg/ml) exposure. Quantitation of N=3, mean±SEM, and representative blotting are shown. **c)** NRF2 levels are not affected by Evi1 overexpression or PRMT5 inhibition. AML5 control or EVI1 overexpressing cells and UCSD-AML-1 cells that were treated with PRMT5i LLY283 (30 nM) for 4 days were exposed to sodium arsenite (10 µM) or TBHP (0.2 mM) for 6 h to induce NRF2.

Table 1. Primers used in the gene expression analysis.

Primer target	Forward	Reverse
18s ribosomal control	GTA ACC CGT TGA ACC CCA TT	CCA TCC AAT CGG TAG TAG CG
RPLP0 (36B4) (ribosomal protein control	TGGAGACGGATTACACCTTCCC	TCTTCCTTGGCTTCAACCTTAGC
TBP control	GGGCATTATTTGTGCACTGAGA	TAGCAGCACGGTATGAGCAACT
ASNS	AAAGTTGCATCCGTGGAAATGG	AGTCCAAGCCCCCTGATAAAAG
PSAT1	GGCTTGGTTCTGGAGTGGATTA	GCTCCACTGGACAAACGTAGAA
TRIB3	GCTTTGTCTTCGCTGACCGTGA	CTGAGTATCTCAGGTCCCACGT
CHOP	GGGGTACCTATGTTTCACCTCC	GCTGTGCCACTTTCCTTTCATT
PHGDH	ACGACAGGCTTGCTGAATGAC	AGATTTCCCCTTCACCATGTCC
SHMT2	CACTGGTATCAGGTGGTACTGA	CTTGCTCTTCACCTCTAAGCCA
PPP1R15A	CCGAGTGGCCATCTATGTACC	AGCGCACCTTTCTGGCCTTTA
SLC7A5	CTACATGCTGGAGGTCTACGG	CTTCACGCTGTAGCAGTTCAC
SLC3A2 (CD98)	GTGAAGACAGGCTCTTGATTGC	TATGCTCCCCAGTAGAACCAGA
SLC2A3	GACCCAGAGATGCTGTAATGGT	GCCAAATTGGAAAGAGCCGATT
SLC7A1	TCATTTAAGGTTCCCTTCCTGC	CACAGGCCATAGCCAAAGTAGA
SLC7A11	CTCATAATACGCCCTGCAGCTA	CAGCTGTAATGAGCTTGATCGC
CHAC1	GTGGTGACGCTCCTTGAAGATC	GAAGGTGACCTCCTTGGTATCG
ERN1	CGGCCTCGGGATTTTTGGAA	AGGCTGCCATCATTAGGATCTG
FUS	CAAGATGGATTCCAGGGGTGA	GTGAAAAGGGGGAAGAGGAACA
PRPF3	ACGAGAGCTAAAGGAGGTGTTT	TTCCTCTCCTCGATTTGTCGTG
ULK3	CTACGCCAAGAAGGACACTCGT	ATCTCCGTGAGGAGGTTCTCCA
CDKN2A	CGCTGCCCATCATCATGA	CCAACGCACCGAATAGTTACG
BAX	AAACTGGTGCTCAAGGCCC	CCACAAAGATGGTCACGGTCTG
DDB2	AATGGAGGGAACAACTAGGCTG	GAGATTCCAAAGCTCTTTGCCG
SLC25A34	AAGCAACAGTGGCTCCCTGAG	TCCACCGGCTGATTGTATAGC
SLC27A3	CTGGAGACACCTTCAGGTGGAA	CCATCCGAACTTTCTGCTGTTT
MDM4 var1	GCTGCCGTAAGTTTTACCAACA	ACAGTGAACATTTCACCTTGCG
MDM4 var 4	ATCAGGTACGACCAAAACTGCC	ACTTTCCTCTGCACTTTGCTGT
ATF6	ATGAAGTTGTGTCAGAGAACC	CTCTTTAGCAGAAAATCCTAG
PERK	CAGTGGCAATGAGAAGTGGAAT	AGGCAGATGCAATTGGAGTACA
XBPt total	CGCTGAGGAGGAAACTGAAAAA	CGCTGTCTTAACTCCTGGTTCT
XBPs spliced	CTGAGTCCGCAGCAGGTG	GTCCAGAATGCCCAACAGGATA
CNOT3	CAAGAAGAAGGGCGACAAGGAT	AGGTCCAGGTCATCGTAGAGAA
EVI1	TTTGCAAAGCCTGGAGAAACAC	GTTGCTAGGGTCCGTGAAAAC
ALAS	GTCTTCTGCAAAGCCAGTCTTG	CCCTCCATCGGTTTTCACACTA
NARS	CCCAGAGCCAAAATGTGTGAAG	TACCATCTCGCAACACCAGAAA
OSGEP	CGTGTTGTATGTGAGTGGAGGA	CAGGATCCCTGAGAATGAGACG
HNRNPU	CTGGGAATCGTGGCGGATATAA	TCGTCCTCTGAAGTTCTGGTTG
CMYC	AGGGTCAAGTTGGACAGTGTCA	TGGTGCATTTTCGGTTGTTG
ACA19	GTGCACATTTCATTGACCTGCT	GAAGGAGACTGGCAGCATTACT
ATF4 variant 2	CTTAAGCCATGGCGCTTCTCA	AGGAAGCTCATTTCGGTCATGT
ATF4 variant 2 (additional confirmation)	TTAAGCCATGGCGCTTCTCAC	CCCATGAGGTTTGAAGTGCTTG
ATF4 variant 1	TAAGCCATGGCGTGAGTACC	TCGTTAAATCGCTTCCCCCTTG
ATF4 variant 1 (additional confirmation)	CGCCTCATAAGTGGAAGGATGA	TGAGAATCAGAAGCCAACTCCC
ATF4 variant 1 (additional confirmation)	CGCCTCATAAGTGGAAGGATGA	AGGAAGCTCATTTCGGTCATGT

Legends for supplementary datasets 1 and 2

Dataset 1. Gene expression analysis for transcripts regulated by PRMT5 inhibition. Full data is available through GEO (GSE163305).

Dataset 2. Splicing analysis for splicing events regulated by PRMT5 inhibition. Intron retention and exon skipping were identified using rMATS-turbo with settings "t paired", "readLength 75", and "variable-read-length" enabled.