

Figure S1. SET is associated to the ER in AML cells.

(A) Immunofluorescence of SET (green) and different organelle markers (red) in HL60 cells. Endoplasmic reticulum (ER) marker Calnexin; Golgi apparatus marker Syntaxin 6; mitochondrial marker mitotracker and lysosomal marker lysotracker. Confocal microscopy showed co-localization (yellow) of the ER marker calnexin and SET. Nucleus are stained with DAPI (blue). (B) Immunofluorescence of SET (green) and the ER marker Calnexin (red) in FLT3-WT AML cells OCI-AML3 and FLT3-ITD cells MV4-11. Representative picture of each staining is shown. Immunofluorescences were visualized by confocal microscopy. Scale bar represent 5 μ m.

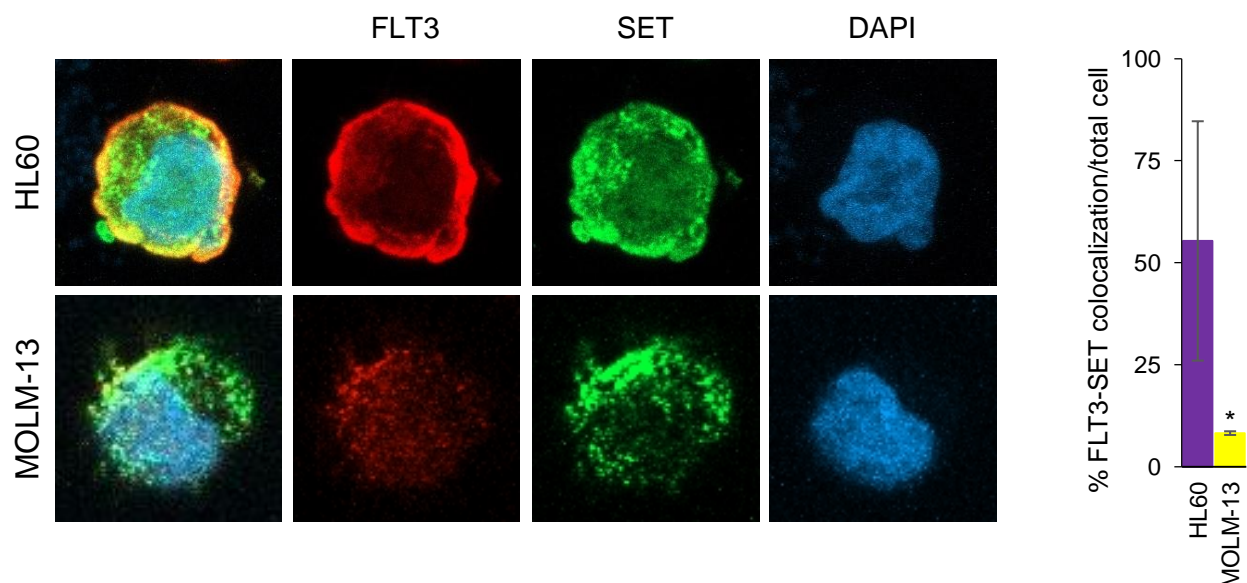


Figure S2. SET and FLT3 co-localize in FLT3-WT cells but hardly co-localize in FLT3-ITD AML cells. Immunofluorescence of SET (green) and FLT3 (red) in AML cell lines HL60 (FLT3-WT) and MOLM-13 (FLT3-ITD). Nuclei were stained with DAPI (blue). Confocal microscopy showed co-localization (yellow) of both protein in FLT3-WT cells and hardly in FLT3-ITD cells. Representative pictures are shown. Immunofluorescences were visualized by confocal microscopy. Scale bars representing quantification of mean values \pm SD of SET and FLT3 co-localization referred to total cell volume, demonstrating significantly less co-localization of SET/FLT3 in FLT3-ITD cells compare to FLT3-WT cells. The results are expressed as mean values \pm SEM. Experiments were performed in triplicate four times. * $p<0.05$ FLT3-ITD cells vs. FLT3-WT cells.

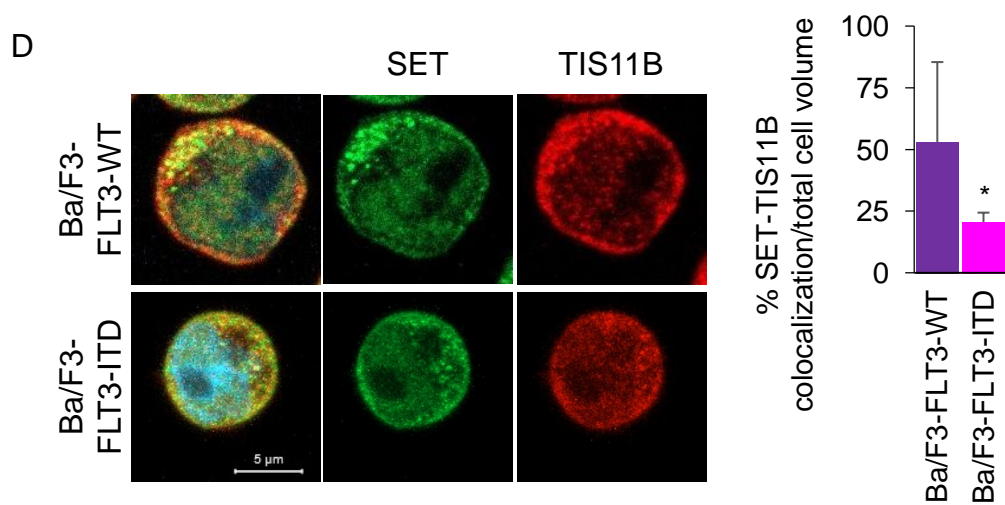
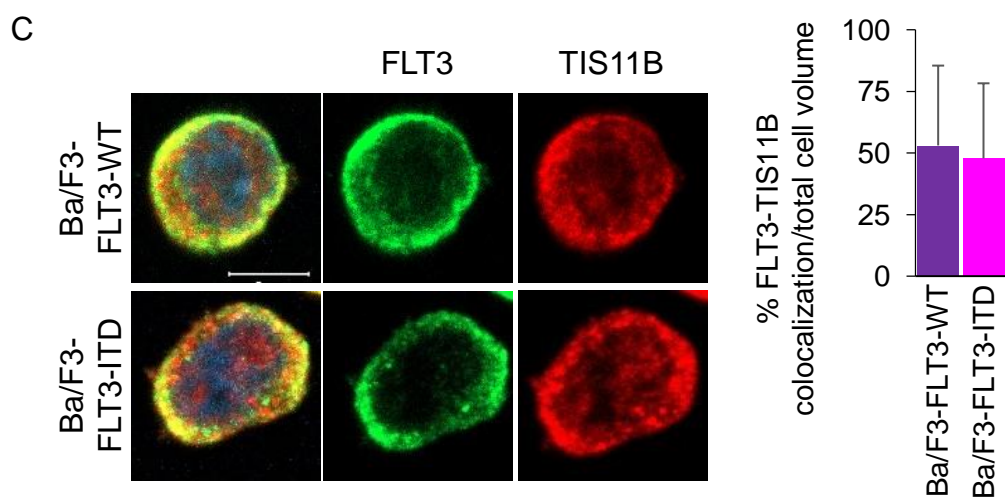
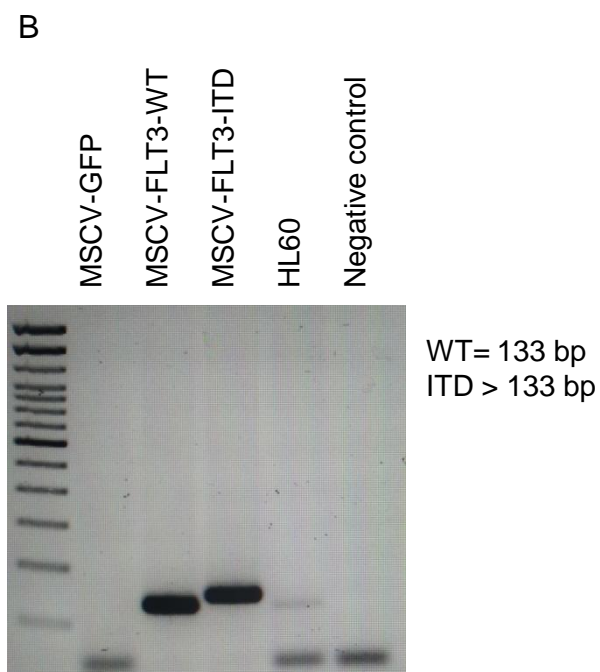
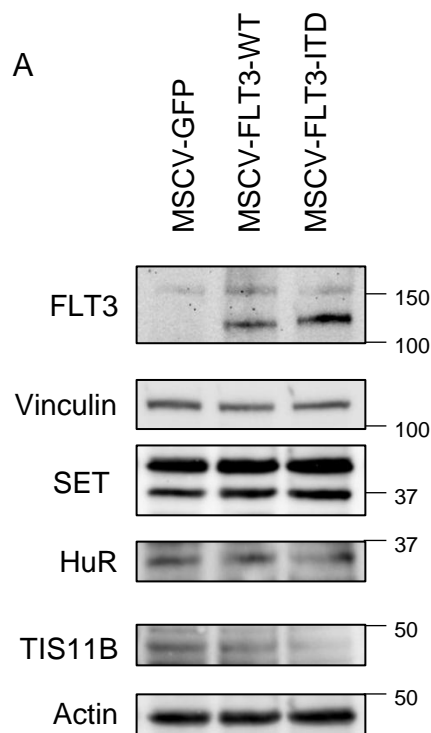


Figure S3. SET and FLT3 interact in FLT3-WT AML cells but not in FLT3-ITD AML cells. (A) Western blot analysis of Ba/F3 over expressing FLT3-WT or FLT3-ITD for FLT3, SET, HuR, TIS11B, RAC1 and normalized with Actin; indicating expression of those proteins in all cell lines. Representative WB are shown. (B) Agarose gel from PCR analysis of DNA from Ba/F3 over expressing the empty vector, FLT3-WT or FLT3-ITD; using the primers Primers: 11F:5'caatttaggtatgaaagcc 3' and 11R: 5'caaactctaaattttctct 3'. The FLT3-WT product is 133 bp and the FLT3-ITD product is bigger than 133 bp. (C) Immunofluorescence of FLT3 (green) and TIS11B (red) in Ba/F3 over expressing FLT3-WT or FLT3-ITD showed co-localization of both proteins (yellow). (D) Immunofluorescence of SET (green) and TIS11B (red) in Ba/F3 over expressing FLT3-WT or FLT3-ITD showed co-localization of both proteins in FLT3-WT and barely in FLT3-ITD over expressing cells. Nucleus are stained with DAPI (blue). Scale bar represent 5 μ m. Graphs represent mean values \pm SD of SET and FLT3 co-localization referred to total cell volume.

NM_004119.3 FLT3 [organism=Homo sapiens] [GenelD=2322] [transcript=1]
GCGCCGCTCCAGGCGGCATCGCAGGGCTGGGCCGGCGCGGCCTGGGGACCCCGGGCTCCGGAGGCCATGCCGGCGTTGGCGCGGAC
GGCGGCCAGCTGCCGCTGCTCGTTGTTTTCTGCAATGATATTTGGGACTATTACAAATCAAGATCTGCCTGTGATCAAGTGTGTT
TTAATCAATCATAAGAACAATGATTCATCAGTGGGGAAGTCATCATATCCCATGGTATCAGAATCCCCGGAAGACCTCGGGTGT
GCGTTGAGACCCCAGAGCTCAGGGACAGTGTACGAAGCTGCCGCTGTGGAAGTGGATGTATCTGCTTCCATCACACTGCAAGTGTG
GTCGACGCCCCAGGGAACATTTCTGTCTCTGGGTCTTTAAGCACAGCTCCCTGAATTGCCAGCCACATTTTGATTTACAAAACAGA
GGAGTTGTTTCCATGGTCATTTTGAAAATGACAGAAACCCAAGCTGGAGAATACCTACTTTTTATTAGAGTGAAGCTACCAATTAC
ACAATATTGTTTACAGTGAGTATAAGAAATACCCTGCTTTACACATTAAGAAGACCTTACTTTAGAAAAATGGAAAACAGGACGCC
CTGGTCTGCATATCTGAGAGCGTTCCAGAGCCGATCGTGAATGGGTGCTTTGCGATTACAGGGGGAAAGCTGTAAAGAAGAAAGT
CCAGCTGTTGTTAAAAAGGAGGAAAAAGTGCTTCATGAATTATTTGGGACGGACATAAGGTGCTGTGCCAGAAATGAACTGGGCAGG
GAATGCACCAGGCTGTTTACAATAGATCTAAATCAAACCTCTCAGACCACATTGCCACAATTATTTCTTAAAGTAGGGGAACCTT
TGGATAAGGTGCAAAGCTGTTTATGTGAACCATGGATTGGGCTCACCTGGGAATTAGAAAACAAAGCACTCGAGGAGGGCAACTAC
TTTGAGATGAGTACCTATTCAACAAACAGAACTATGATACGGATTCTGTTTGCTTTTGATCATCAGTGGCAAGAAACGACACCGGA
TACTACACTTGTTCTCTTCAAAGCATCCCAGTCAATCAGCTTTGGTTACCATCGTAGAAAAGGGATTTATAAATGCTACCAATTCA
AGTGAAGATTATGAAATTGACCAATATGAAGAGTTTTGTTTTCTGTGAGGTTTAAAGCCTACCCACAAATCAGATGTACGTGGACC
TTCTCTCGAAAATCATTTCTTGTGAGCAAAAGGGTCTTGATAACGGATACAGCATATCCAAGTTTTGCAATCATAAGCACCAGCCA
GGAGAATATATATTCCATGCAGAAAATGATGATGCCCAATTTACCAAAATGTTACGCTGAATATAAGAAGGAAACCTCAAGTGCTC
GCAGAAGCATCGGCAAGTCAGGCGTCTGTTTCTCGGATGGATACCCATTACCATCTTGACCTGGAAGAAGTGTTTCAGACAAGTCT
CCCAACTGCACAGAAGAGATCACAGAAGGAGTCTGGAATAGAAAAGGCTAACAGAAAAGTGTTTGGACAGTGGGTGTCGAGCAGTACT
CTAAACATGAGTGAAGCCATAAAAGGGTCTGTTCAAGTGCTGTGCATACAATTCCTTGGCACATCTTGTGAGACGATCCTTTTA
AACTCTCCAGGCCCCCTTCCCTTTTATCCAAGACAACATCTCATTCTATGCAACAATTTGGTGTGTTGCTCCTCTTCATTGTGTTTTA
ACCCTGCTAATTTGTACAAGTACAAAAAGCAATTTAGGTATGAAAGCCAGCTACAGATGGTACAGGTGACCGGCTCCTCAGATAAT
GAGTACTTCTACGTTGATTTTCAAGAGAATATGAATATGATCTCAAATGGGAGTTTCCAAGAGAAAATTTAGAGTTTGGGAAGGTACTA
GGATCAGGTGCTTTTGGAAAAGTGATGAACGCAACAGCTTATGGAATTAGCAAAAACAGGAGTCTCAATCCAGGTTGCCGTCAAAATG
CTGAAAGAAAAAGCAGACAGCTCTGAAAGAGAGGCACTCATGTGAGAATCAAGATGATGACCCAGCTGGGAAGCCACGAGAATATT
GTGAACCTGCTGGGGGCGTGCACACTGTCAGGACCAATTTACTTGATTTTTGAATACTGTTGCTATGGTGATCTTCTCAACTATCTA
AGAAGTAAAAGAGAAAAATTTACAGGACTTGGACAGAGATTTTCAAGGAACACAATTTAGTTTTTACCCCACTTTCCAATCACAT
CCAAATTCAGCATGCCTGGTTCAAGAGAAGTTCAGATACACCCGGACTCGGATCAAATCTCAGGGCTTCATGGGAATTCATTTAC
TCTGAAGATGAAATTGAATATGAAAACCAAAAAAGGCTGGAAGAAGAGGAGGACTTGAATGTGCTTACATTTGAAGATCTTCTTTGC
TTTGCATATCAAGTTGCCAAAGGAATGGAATTTCTGGAATTTAAGTCGTGTGTTTACAGAGACCTGGCCGCCAGGAACGTGCTTGTC
ACCCACGGGAAAGTGGTGAAGATATGTGACTTTGGATTGGCTCGAGATATCATGAGTGATTCCAATCTATGTTGTCAGGGGCAATGCC
CGTCTGCCTGTAAATGGATGGCCCCGAAAGCCTGTTTGAAGGCATCTACACCATTAAGAGTGATGTCTGGTCATATGGAATATTA
CTGTGGGAAATCTTCTCACTTGGTGTGAATCCTTACCCTGGCATTCCGGTTGATGCTAATCTTACAACTGATTCAAAATGGATTT
AAAATGGATCAGCCATTTTATGCTACAGAAGAAATATACATTATAATGCAATCCTGCTGGGCTTTTACTCAAGGAAACGGCCATCC
TTCCCTAATTTGACTTCGTTTTTAGGATGTCAGCTGGCAGATGCAGAAGAAGCGATGTATCAGAATGTGGATGGCCGTGTTTCGGAA
TGTCTCTCACACCTACCAAAACAGGCGACCTTTACGAGAGAGATGGATTTGGGGCTACTCTCTCCGACGGCTCAGGTGGAAGATTG
TAGAGGAACAATTTAGTTTTAAGGACTTCATCCCTCCACCTATCCCTAACAGGCTGTAGATTACCAAAACAAGATTAATTTATCAC
TAAAGAAAATCTATTATCAACTGCTGCTTACCAGACTTTTCTCTAGAAGCTGTCTGCGTTTACTCTTGTTTTCAAAGGGACTTTT
GTAAAATCAAATCATCTGTGACAAGGCAGGAGGAGCTGATAATGAACTTTATTGGAGCATTGATCTGCATCCAAGGCCTTCTCAGG
CTGGCTTGAGTGAATTGTGTACCTGAAGTACAGTATATTCTTGTAATATACATAAAACAAAAGCATTGCTAAGGAGAAGCTAATAT
GATTTTTTAAGTCTATGTTTTAAATAATATGTAATTTTTTCACTATTTAGTGATATATTTTATGGGTGGGAATAAAATTTTCTACT
ACAGAATTGCCATTATTGAATTATTTATCATGGTATAATTAGGGCAAGTCTTAAGTGGAGTTCACGAACCCCTGAAATTGTGCACC
CATAGCCACCTACACATTCCTTCCAGAGCACGTGTGCTTTTACCCCAAGATACAAGGAATGTGTAGGCAGCTATGGTTGTACAGCC
TAAGATTTCTGCAACAACAGGGGTTGTATTGGGGGAAGTTTATAATGAATAGGTGTTCTACCATAAAGAGTAATACATCACCTAGAC
ACTTTGGCGGCCTTCCAGACTCAGGGCCAGTCAGAAGTAACATGGAGGATTAGTATTTTCAATAAAGTTACTCTTGCCCCACA

Figure S4. mRNA sequence of FLT3. Start and stop codons are colored in blue, 5'UTR is colored in orange and 3'UTR in green. Highlighted in yellow is the canonical sequence for TIS11B binding.

FLT3 amino acid sequence

10	20	30	40	50
MPALARDGGQ	LPLLVVFSAM	IFGTITNQDL	PVIKCVLINH	KNNDSSVGKS
60	70	80	90	100
SSYPMVSESP	EDLGCALRPQ	SSGTVYEAAA	VEVDVSASIT	LQVLVDAPGN
110	120	130	140	150
ISCLWVFKHS	SLNCQPHFDL	QNRGVVSMVI	LKMTETQAGE	YLLFIQSEAT
160	170	180	190	200
NYTILFTVSI	RNTLLYTLRR	PYFRKMENQD	ALVCISESVP	EPIVEWVLCD
210	220	230	240	250
SQGESCKEES	PAVVKKEEKV	LHELFGTDIR	CCARNELGRE	CTRLFTIDLN
260	270	280	290	300
QTPQTTLPQL	FLKVGEPLWI	RCKAVHVNHG	FGLTWELENK	ALEEGNYFEM
310	320	330	340	350
STYSTNRTMI	RILFAFVSSV	ARNDTGYITC	SSSKHPSQSA	LVTIVEKGF
360	370	380	390	400
NATNSSDYE	IDQYEEFCFS	VRFKAYPQIR	CTWTFSRKSF	PCEQKGLDNG
410	420	430	440	450
YSISKFCNHK	HQPGEYIFHA	ENDDAQFTKM	FTLNIRRKPQ	VLAEASASQA
460	470	480	490	500
SCFSDGYPLP	SWTWKKCSDK	SPNCTEEITE	GVWNRKANRK	VFGQWVSSST
510	520	530	540	550
LNMSIAIKGF	LVKCCAYNSL	GTSCETILLN	SPGPFPIQD	NISFYATIGV
560	570	580	590	600
CLLFIVVLTL	LICHKKYKQF	RYESQLQMVQ	VTGSSDNEYF	YVDFREYEYD
610	620	630	640	650
LKWEFPR	ENLEFGKVLGSGA	FGKVMNATAY	GISKTGVSIQ	VAVKMLKEKA
660	670	680	690	700
DSSEREALMS	ELKMMTQLGS	HENIVNLLGA	CTLSGPIYLI	FEYCCYGDLL
710	720	730	740	750
NYLRSKREKF	HRTWTEIFKE	HNFSFYPTFQ	SHPNSSMPGS	REVQIHPDSD
760	770	780	790	800
QISGLHGNSF	HSEDEIEYEN	QKRLEEEEDL	NVLTFFEDLLC	FAYQVAKGME
810	820	830	840	850
FLEFKSCVHR	DLAARNVLVT	HGKVVKICDF	GLARKDIMSDS	NYVVRGNARL
860	870	880	890	900
PVKWMAPESL	FEGIYTIKSD	VWSYGILLWE	IFSLGVNPYP	GIPVDANFYK
910	920	930	940	950
LIQNGFKMDQ	PFYATEEIIYI	IMQSCWAFDS	RKRPSFPNLT	SFLGCQLADA
960	970	980	990	
EEAMYQNVDG	RVSECPHTYQ	NRRPFSREMD	LGLLSPQAQV	EDS

Figure S5. FLT3 amino acid sequence. The extracellular domain is highlighted in pink. Transmembrane domain is highlighted in green. Positively charge amino acid from the cytosolic domain, indicating potential SET-binding sites are highlighted in yellow and colored. The amino acid implicated in the ITD mutation are highlighted in red

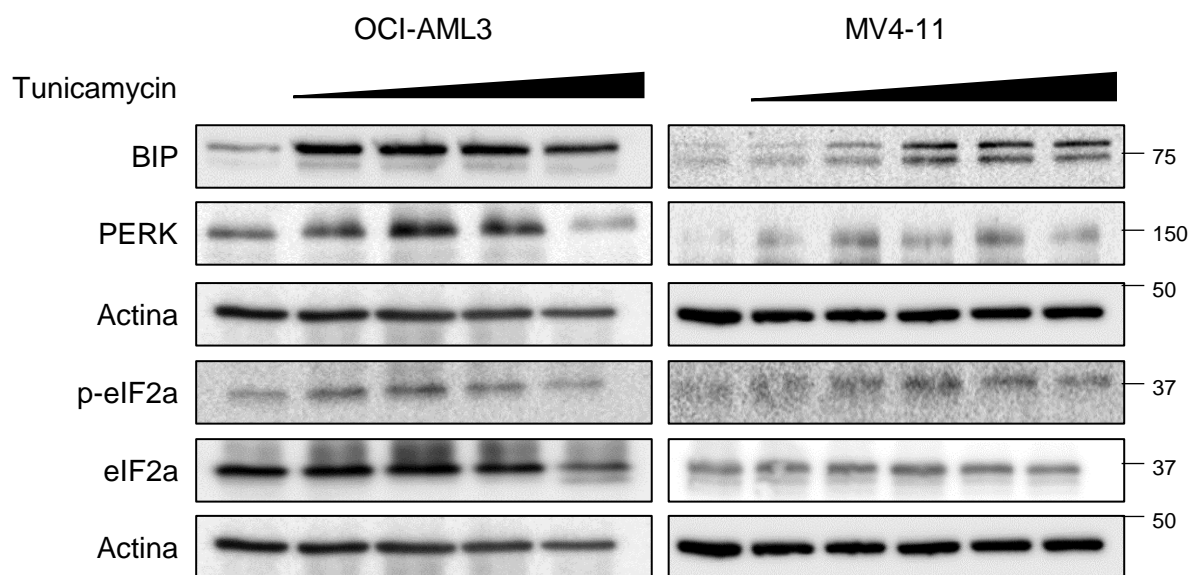
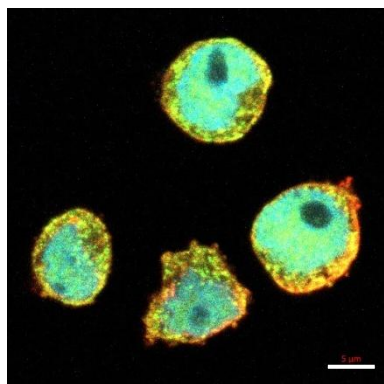


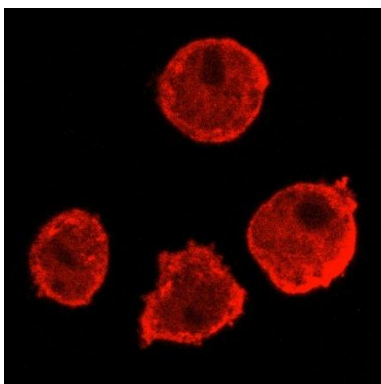
Figure S6. Tunicamycin induces UPR response. AML cells were treated with increasing concentrations of tunicamycin (0, 25, 50, 100, 200, 400 ng/ml) for 24 h and western blot analysis of the UPR markers were performed. Representative WB are shown.

A

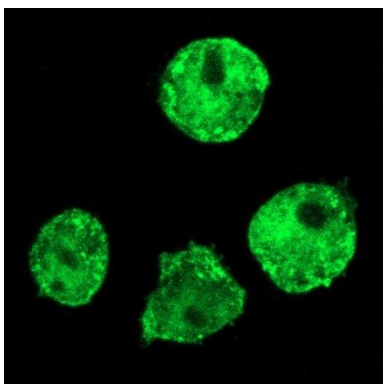
OCI-AML3



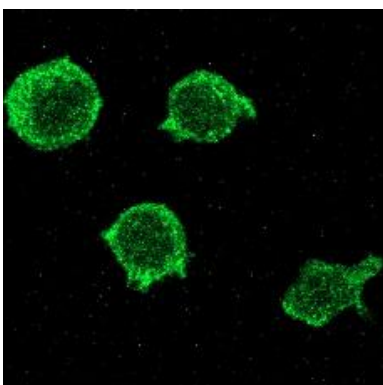
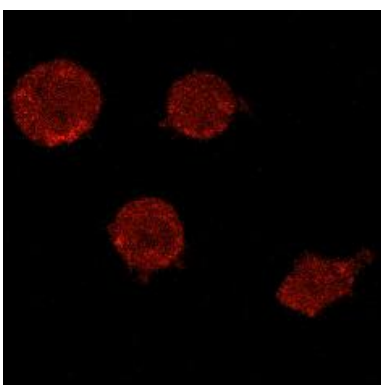
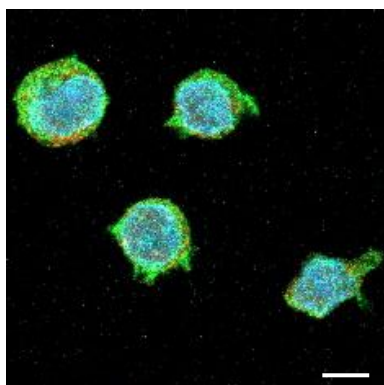
FLT3



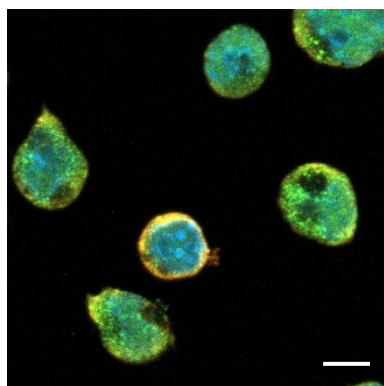
SET



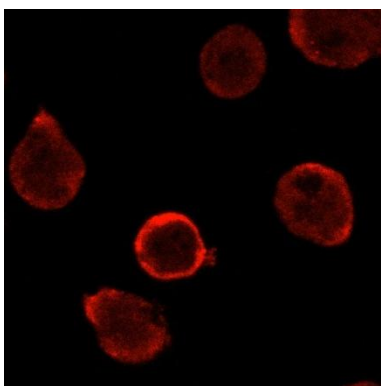
MV4-11



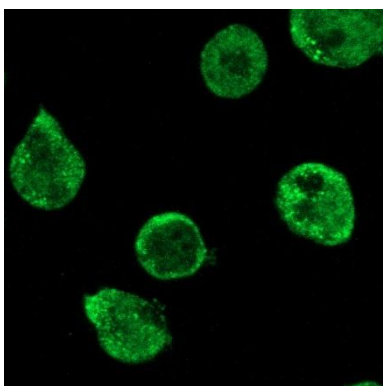
Ba/F3-
FLT3-WT



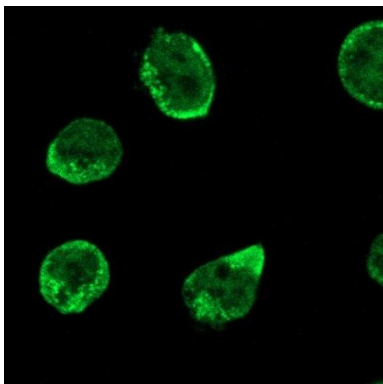
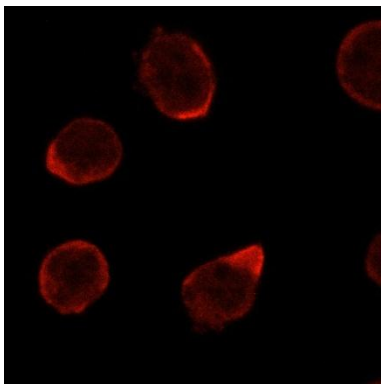
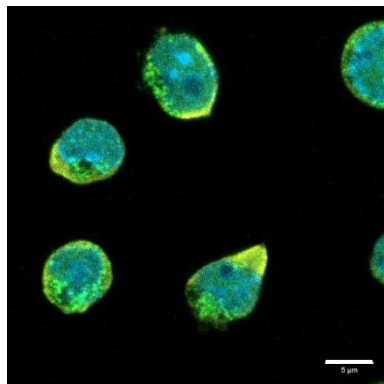
FLT3



SET



Ba/F3-
FLT3-ITD

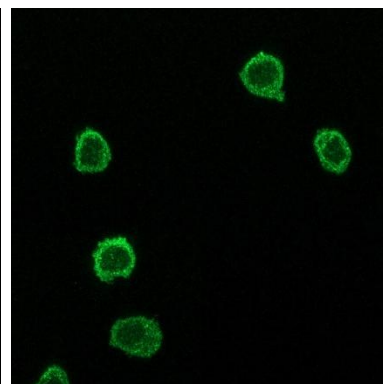
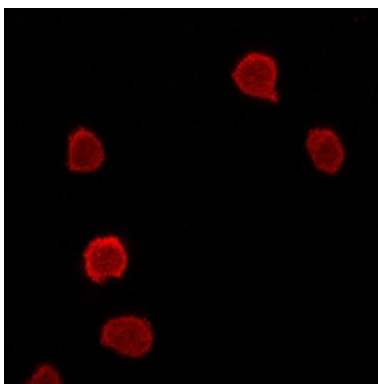
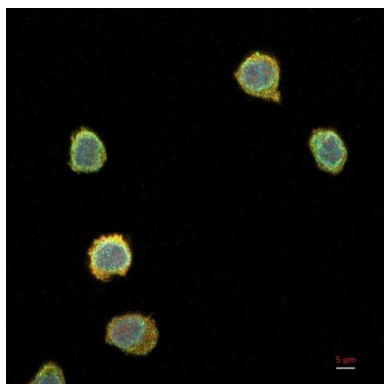


B

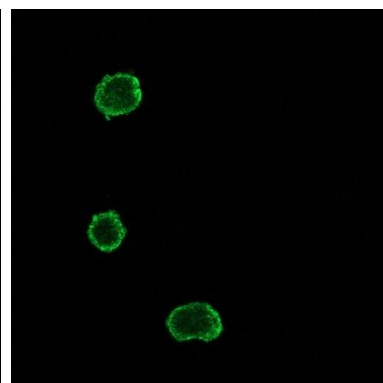
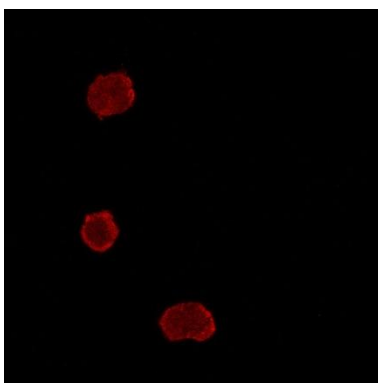
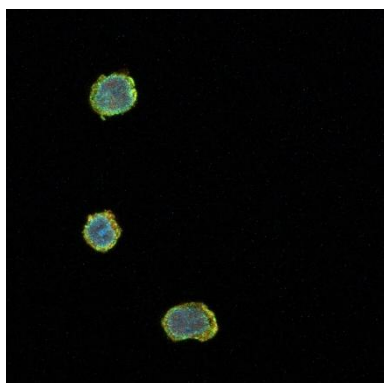
TIS11B

FLT3

OCI-AML3



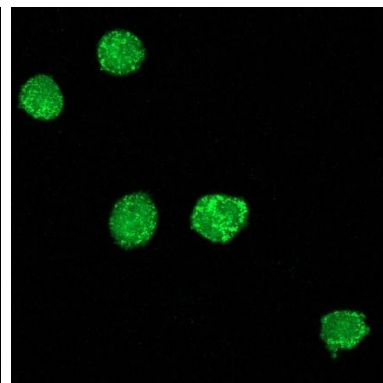
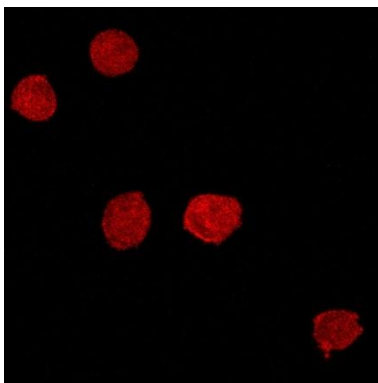
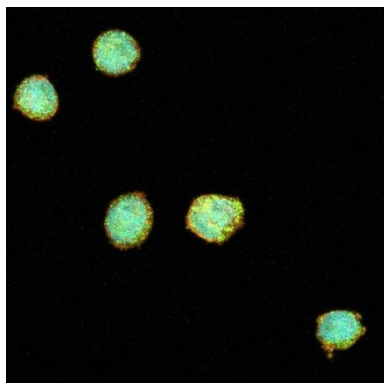
MV4-11



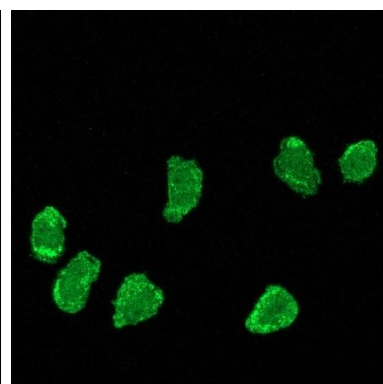
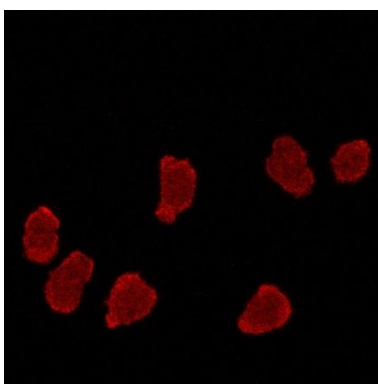
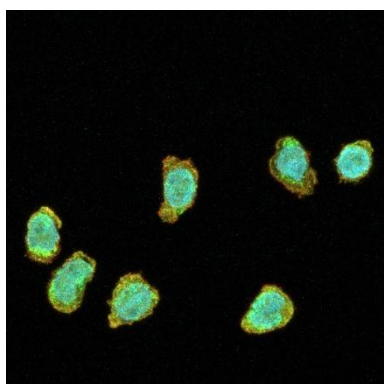
TIS11B

SET

OCI-AML3

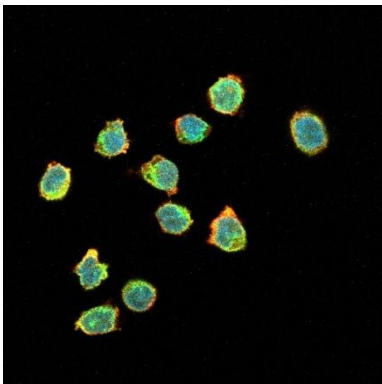


MV4-11

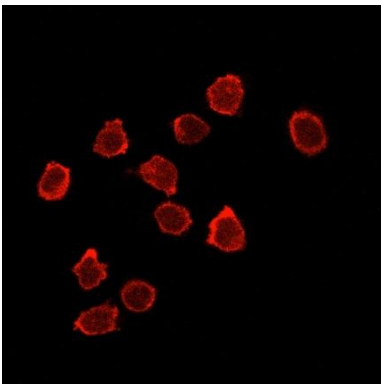


OCI-AML3

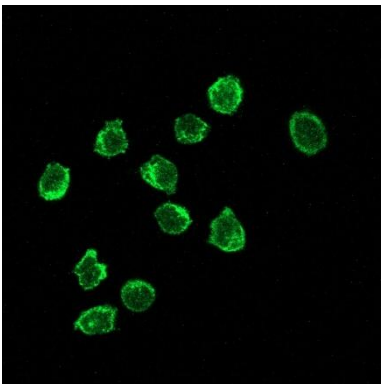
DMSO



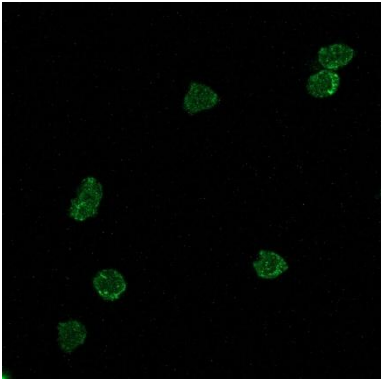
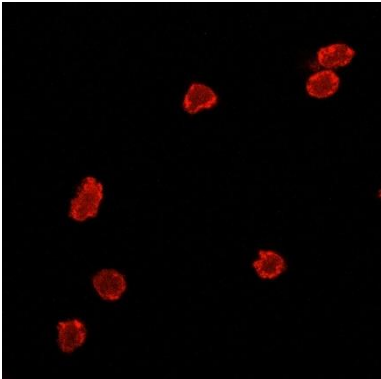
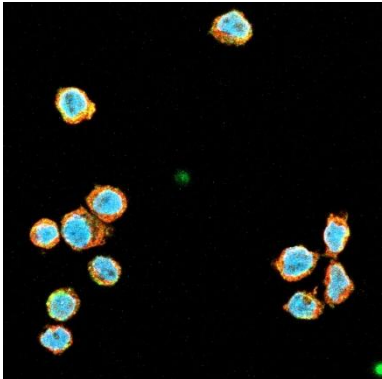
FLT3



SET

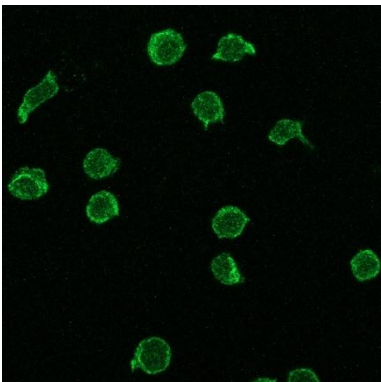
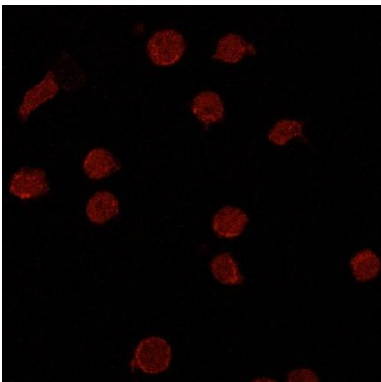
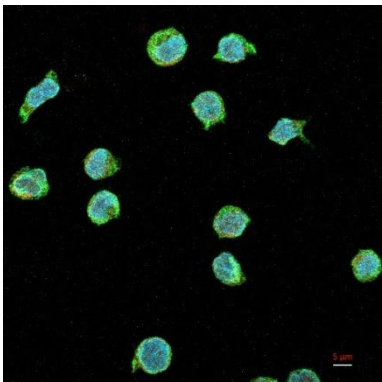


Tunicamycin

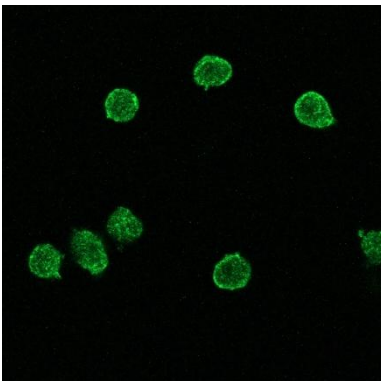
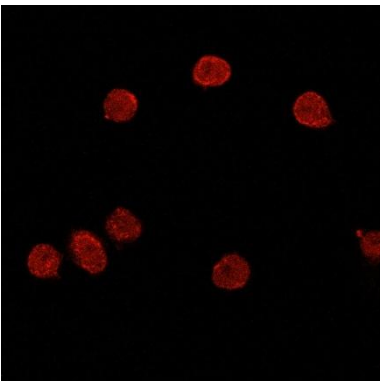
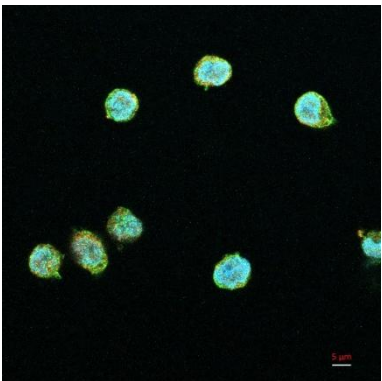


MV4-11

DMSO



Tunicamycin



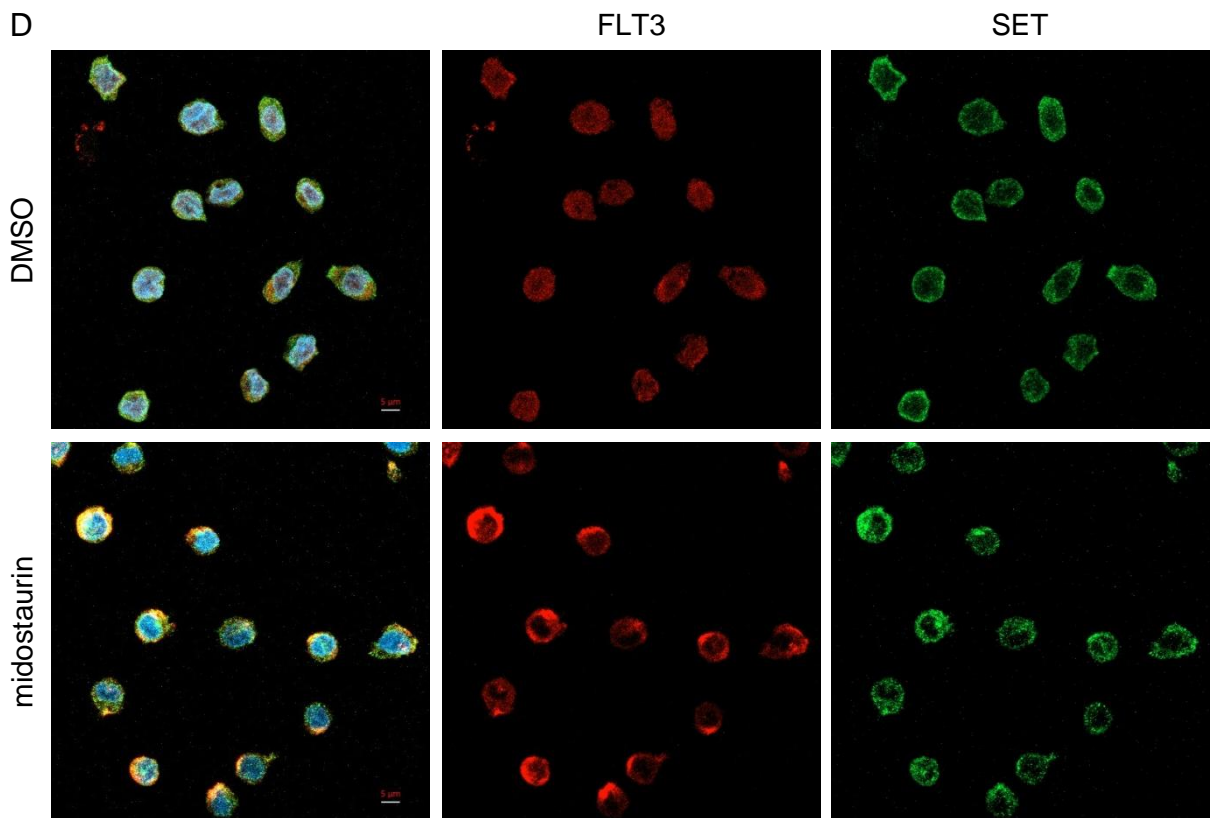


Figure S7. Panoramic view of immunofluorescence. (A) Immunofluorescence of SET (green) and FLT3 (red) in FLT3-WT cell lines (OCI-AML3 and Ba/F3-FLT3-WT) and FLT3-ITD cell lines (MV4-11 and Ba/F3-FLT3-ITD). Confocal microscopy showed co-localization (yellow) of both protein. (B) Immunofluorescence of FLT3 (green) and TIS (red) and SET (green) and TIS (red) in FLT3-WT cell line OCI-AML3 and FLT3-ITD cell line MV4-11. Confocal microscopy showed co-localization (yellow) of both protein. (C) Immunofluorescence of SET (green) and FLT3 (red) in FLT3-WT cell line OCI-AML3 and FLT3-ITD cell line MV4-11 treated with and without tunicamycin (200 $\mu\text{g/ml}$) for 24 h. Confocal microscopy showed co-localization (yellow) of both protein. (D) Immunofluorescence of SET (green) and FLT3 (red) in FLT3-WT cell line OCI-AML3 and FLT3-ITD cell line MV4-11 treated with and without midostaurin (20 ng/ml) for 24 h. Confocal microscopy showed co-localization (yellow) of both protein. Nuclei were stained with DAPI (blue). Representative pictures are shown. Immunofluorescences were visualized by confocal microscopy. Experiments were performed in triplicate four times.

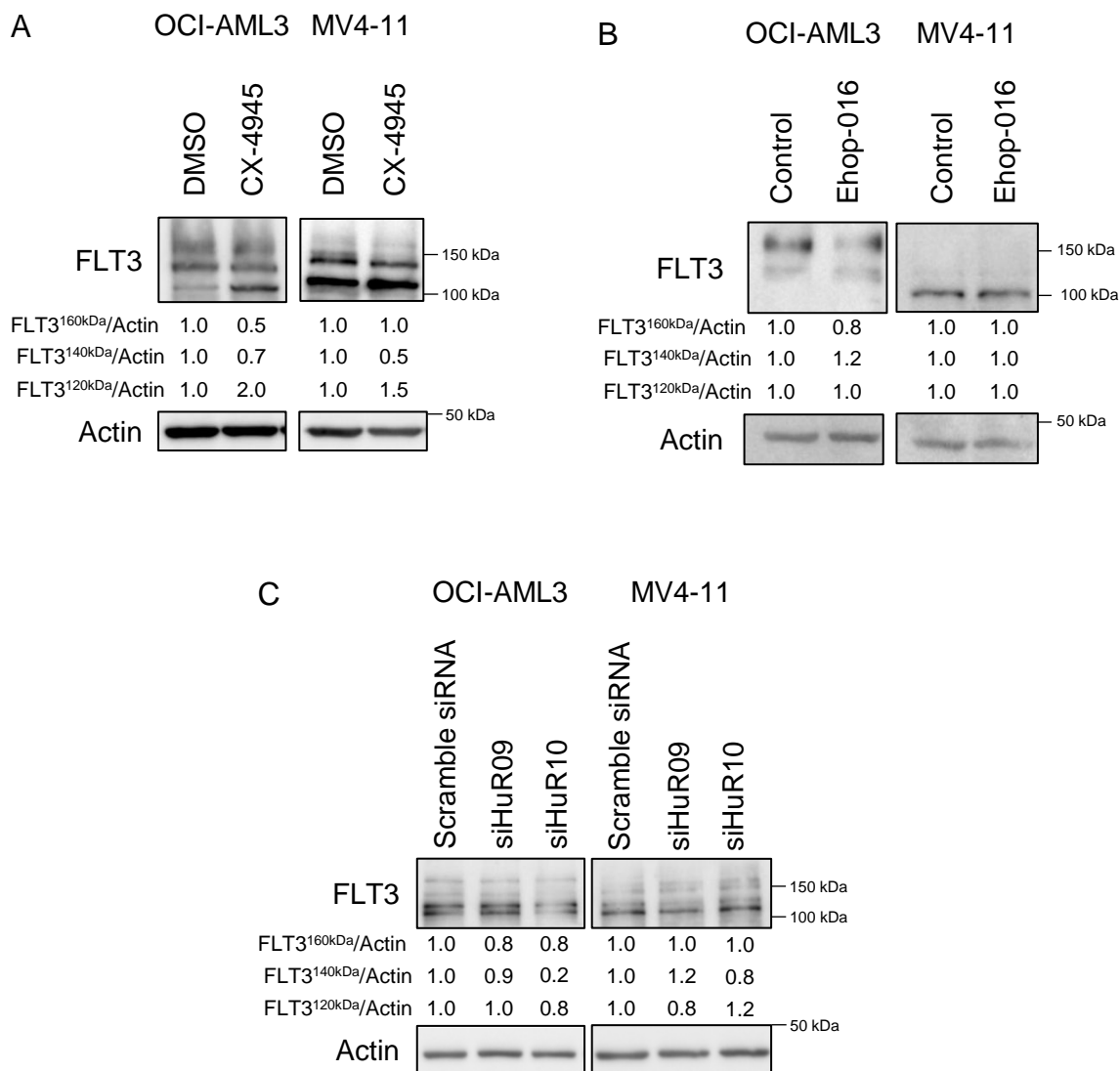


Figure S8. Relative bands of FLT3. Western blot analysis of FLT3 after CX-4945 (**A**) and Ehop-016 treatment (**B**) and HuR silencing (**C**) in OCI-AML and MV4-11 cells. Quantification of the relative FLT3 bands demonstrated a decreased in the 150kDa mature band along with an increase in <150 kDa immature bands in FLT3-WT cells but not in FLT3-ITD cells. A representative WB is shown.

Table S1. List of drugs used

Reagent	Ref#	Source	Concentration
CX-4945	S2248	Selleckchem Chemicals	5µM
Ehop-016	S7319	Selleckchem Chemicals	
FTY720	S5002	Selleckchem Chemicals	5µM
Midostaurin	M1323	SIGMA	20 nM
DT-061	S8774	Selleckchem Chemicals	2.5 and 4 µM

Table S2. List of antibodies used

Target	Ref#	Source	Application
Alexa fluor 488 donkey anti-goat	A-11055	Invitrogen	IF
Alexa fluor 488 donkey anti-mouse	A21202	Invitrogen	IF
Alexa fluor 555 donkey anti-rabbit	A31572	Invitrogen	IF
Alexa fluor 568 donkey anti-mouse	A10037	Invitrogen	IF
Amersham ECL Mouse IgG, HRP-linked whole Ab (from sheep)	NA931	Amersham	WB
Anti-rabbit IgG, HRP-linked Antibody	7074	Cell signaling	WB
Donkey anti-goat IgG HRP affinity purified PAB	04HAF109	R&D systems	WB
PE Mouse anti-Human CD135	558996	BD Pharmingen	FACS
ELAV1/ HuR	12582	cell signalling	WB
ELAV1/ HuR	7468	MERCK	RIP
FLT3	3462	cell signalling	WB, IP
RAC1	66122-1-Ig	Proteintech	WB
SET	A302-261A	Bethyl	IP, WB
SET	sc-5655	Santa Cruz Biotechnology	IF
ZFP36L1/2 (TIS11B)	12306-1-AP	Proteintech	IF, WB
β-Actin	A5441	Sigma	WB