Figure S1

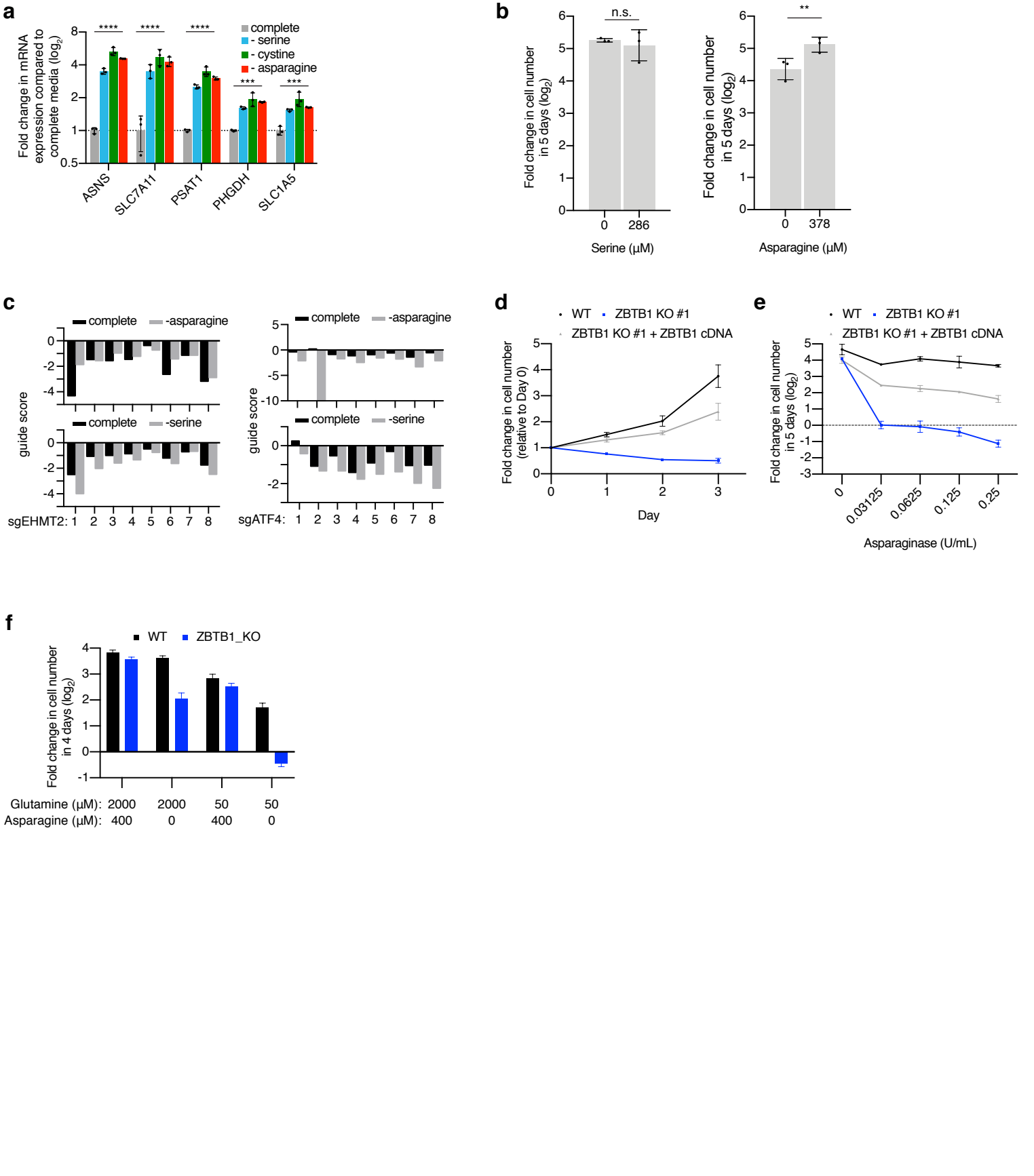
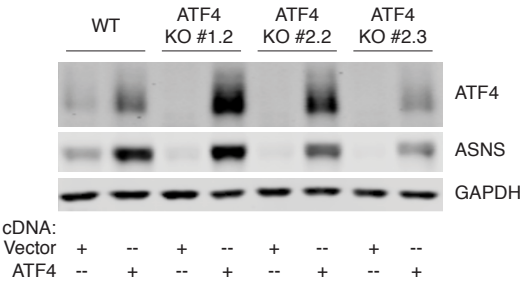

a
Fold change in mRNA expression compared to complete media (log2)
complete
- serine
- cystine
- asparagine
****
***
ASNS
SLC7A11
PSAT1
PHGDH
SLC1A5
b
Fold change in cell number in 5 days (log2)
n.s.
**
0
286
Serine (μM)
0
378
Asparagine (μM)
c
guide score
complete
-asparagine
-serine
sgEHMT2: 1 2 3 4 5 6 7 8
sgATF4: 1 2 3 4 5 6 7 8
d
WT
ZBTB1 KO #1
ZBTB1 KO #1 + ZBTB1 cDNA
Fold change in cell number (relative to Day 0)
Day
e
Fold change in cell number in 5 days (log2)
0
0.03125
0.0625
0.125
0.25
Asparaginase (U/mL)
f
WT
ZBTB1_KO
Fold change in cell number in 4 days (log2)
Glutamine (μM): 2000 2000 50 50
Asparagine (μM): 400 0 400 0

Figure S2

a


WT
ATF4 KO #1.2
ATF4 KO #2.2
ATF4 KO #2.3
ATF4
ASNS
GAPDH
cDNA:
Vector + -- + -- + -- + --
ATF4 -- + -- + -- + -- +

b

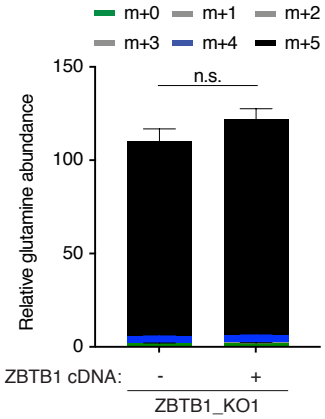

m+0
m+1
m+2
m+3
m+4
m+5
n.s.
Relative glutamine abundance
150
100
50
0
ZBTB1 cDNA: - +
ZBTB1_KO1

Figure S3

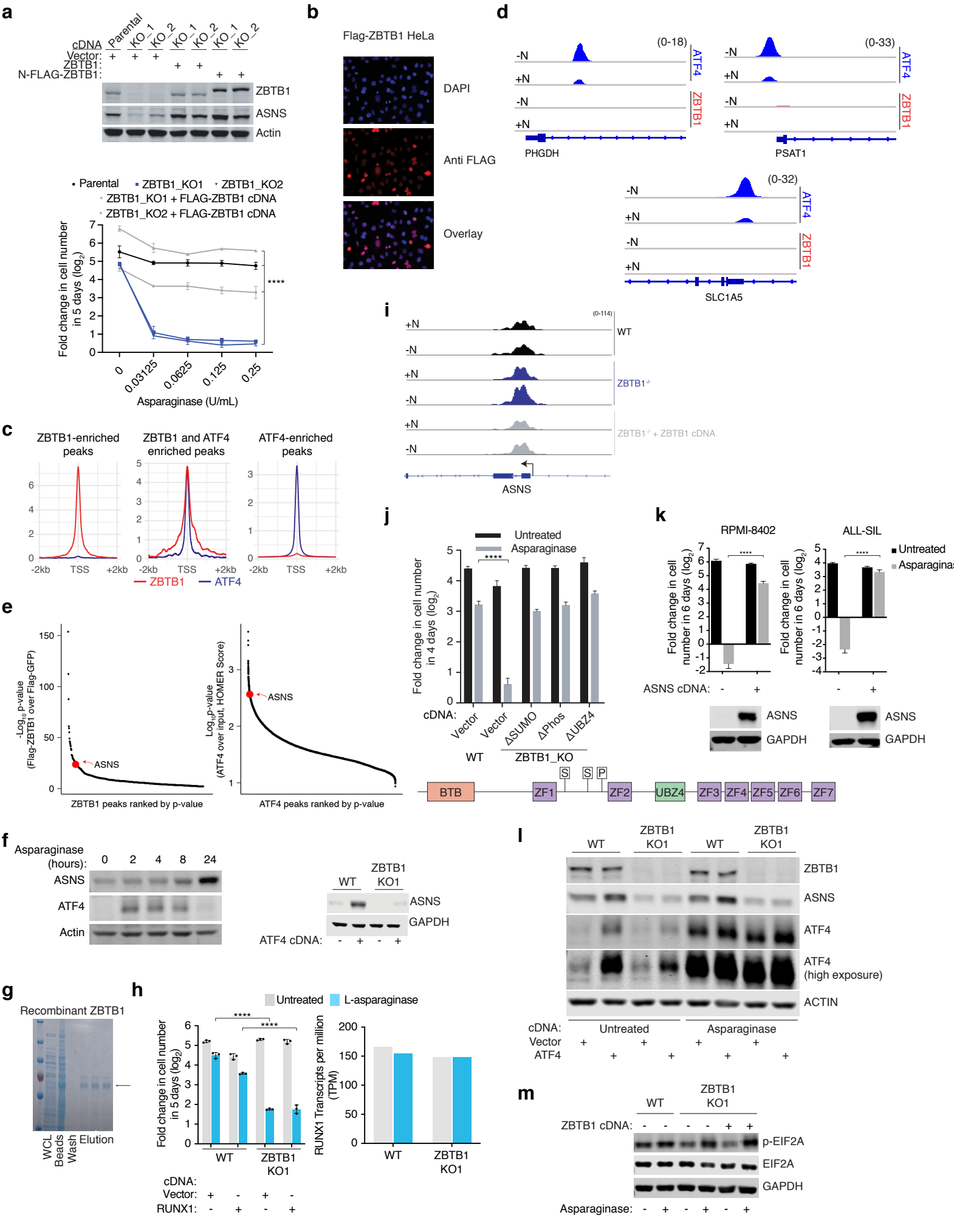
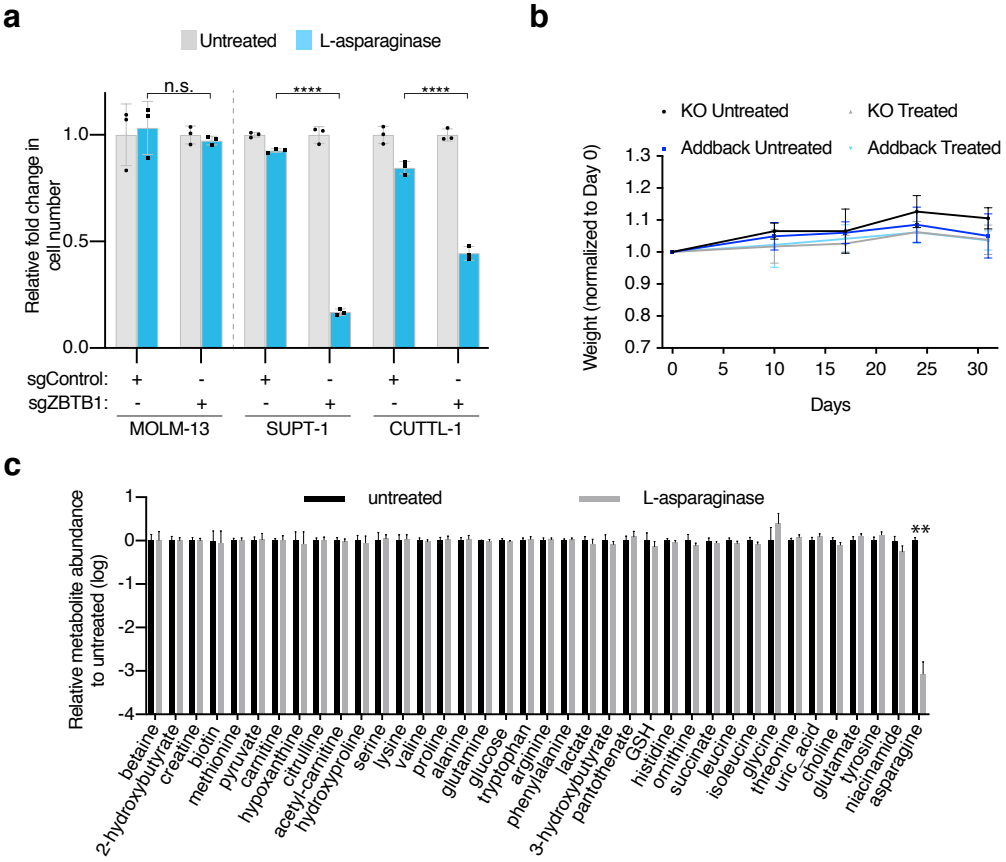

a
cDNA
Parental
KO_1
KO_2
KO_1
KO_2
KO_1
KO_2
Vector:
ZBTB1:
N-FLAG-ZBTB1:
ZBTB1
ASNS
Actin
• Parental
▪ ZBTB1_KO1
▾ ZBTB1_KO2
▵ ZBTB1_KO1 + FLAG-ZBTB1 cDNA
◦ ZBTB1_KO2 + FLAG-ZBTB1 cDNA
Fold change in cell number in 5 days (log2)
****
0
0.03125
0.0625
0.125
0.25
Asparaginase (U/mL)
b
Flag-ZBTB1 HeLa
DAPI
Anti FLAG
Overlay
d
(0-18)
(0-33)
(0-32)
ATF4
ZBTB1
-N
+N
PHGDH
PSAT1
SLC1A5
c
ZBTB1-enriched peaks
ZBTB1 and ATF4 enriched peaks
ATF4-enriched peaks
-2kb
TSS
+2kb
ZBTB1
ATF4
i
(0-114)
WT
ZBTB1-/-
ZBTB1-/- + ZBTB1 cDNA
ASNS
e
-Log10 p-value (Flag-ZBTB1 over Flag-GFP)
ASNS
ZBTB1 peaks ranked by p-value
Log10 p-value (ATF4 over input, HOMER Score)
ATF4 peaks ranked by p-value
j
Untreated
Asparaginase
****
Fold change in cell number in 4 days (log2)
cDNA:
Vector
Vector
ΔSUMO
ΔPhos
ΔUBZ4
WT
ZBTB1_KO
BTB
ZF1
S
S
P
ZF2
UBZ4
ZF3
ZF4
ZF5
ZF6
ZF7
k
RPMI-8402
ALL-SIL
Fold change in cell number in 6 days (log2)
Untreated
Asparaginase
ASNS cDNA:
ASNS
GAPDH
f
Asparaginase (hours):
0
2
4
8
24
ASNS
ATF4
Actin
ZBTB1
WT
KO1
ASNS
GAPDH
ATF4 cDNA:
l
WT
ZBTB1 KO1
ZBTB1
ASNS
ATF4
ATF4 (high exposure)
ACTIN
cDNA:
Untreated
Asparaginase
Vector
ATF4
g
Recombinant ZBTB1
WCL
Beads
Wash
Elution
h
Untreated
L-asparaginase
****
Fold change in cell number in 5 days (log2)
WT
ZBTB1 KO1
cDNA:
Vector:
RUNX1:
RUNX1 Transcripts per million (TPM)
m
WT
ZBTB1 KO1
ZBTB1 cDNA:
p-EIF2A
EIF2A
GAPDH
Asparaginase:

Figure S4


a
Untreated
L-asparaginase
n.s.
****
****
Relative fold change in cell number
1.0
0.5
0.0
sgControl: + - + - + -
sgZBTB1: - + - + - +
MOLM-13
SUPT-1
CUTTL-1
b
KO Untreated
KO Treated
Addback Untreated
Addback Treated
Weight (normalized to Day 0)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0
5
10
15
20
25
30
Days
c
untreated
L-asparaginase
Relative metabolite abundance to untreated (log)
1
0
-1
-2
-3
-4
**
betaine
2-hydroxybutyrate
creatine
biotin
methionine
pyruvate
carnitine
hypoxanthine
citrulline
acetyl-carnitine
hydroxyproline
serine
lysine
valine
proline
alanine
glutamine
glucose
tryptophan
arginine
phenylalanine
lactate
3-hydroxybutyrate
pantothenate
GSH
histidine
ornithine
succinate
leucine
isoleucine
glycine
threonine
uric_acid
choline
glutamate
tyrosine
niacinamide
asparagine

Supplementary Figure legends:

Figure S1. Related to Figure 1.

A) Relative mRNA levels of indicated genes in parental Jurkat cells grown in complete (gray), serine-free (blue), cystine-free (green) or asparagine-free (red) media for eight hours (mean ± SD, n=3).
B) Fold change in cell number (log_2) of parental Jurkat cells after growth in media with indicated serine (left) or asparagine (right) concentrations for 5 days (mean ± SD, n=3).
C) Changes in the abundance of individual EHMT2 (left) or ATF4 (right) sgRNAs in complete media (gray) or absence (black) of asparagine (top) or serine (bottom).
D) Fold change in cell number (log_2) of parental (black), ZBTB1 knockout (blue), and ZBTB1 knockout (gray) expressing ZBTB1 cDNA Jurkat cells after growth in HPLM treated with L-asparaginase (0.03125 U/mL) for the indicated number of days (mean ± SD, n=3).
E) Fold change in cell number (log_2) of parental (black), ZBTB1 knockout (blue), and ZBTB1 knockout (gray) expressing ZBTB1 cDNA Jurkat cells after growth in HPLM with the indicated concentration of L-asparaginase for 5 days (mean ± SD, n=3).
F) Fold change in cell number (log_2) of parental (black), ZBTB1 knockout mixed population (blue) Jurkat cells after growth in in media with indicated glutamine and asparagine concentrations for 4 days (mean ± SD, n=3).

Figure S2. Related to Figure 2.

A) Immunoblot analysis of parental, ATF4 knockout, and ATF4 knockout expressing ATF4 cDNA Jurkat cells, as indicated. GAPDH was used as a loading control.
B) Abundance of labeled glutamine in ZBTB1 knockout or ZBTB1 knockout cells expressing ZBTB1 cDNA Jurkat cells cultured for 8 hours in media containing [U-13C]-glutamine (2000 uM) in the absence of asparagine. Colors indicate mass isotopomers, as indicated (mean ± SD, n=3).

Figure S3. Related to Figure 3.

A) Immunoblot analysis of parental, ZBTB1 knockout, and ZBTB1 knockout expressing ZBTB1 or N-FLAG ZBTB1 cDNA Jurkat cells, as indicated. Actin was used as a loading control (top). Fold change in cell number (log_2) of indicated Jurkat cells after growth in media with indicated L-asparaginase concentrations for 5 days (mean ± SD, n=3).
B) Immunofluorescent microscopy images of HeLa cells expressing Flag-ZBTB1 cDNA after growth in 50 uM or 2mM glutamine, as indicated. Cells were stained with DAPI (top), an anti-FLAG antibody and anti-mouse secondary antibody (middle), and the two images were overlaid (bottom).
C) Enrichment of ZBTB1 (red) or ATF4 (blue) at the transcription start site (TSS) of ZBTB1, ATF4 or ZBTB1 and ATF4 overlapping peaks.
D) ChIP-Seq tracks near the PHGDH (top), PSAT1 (middle) and SLC1A5 (bottom) promoters for ATF4 (blue) or ZBTB1 (red) in FLAG-ZBTB1 expressing ZBTB1 knockout cells in the presence (+N) or absence (-N) of asparagine.

E) Peaks for ZBTB1 (left) and ATF4 (right) ranked by p-value, relative to input (ATF4) and FLAG-ZBTB1 relative to FLAG-GFP both relative to input (ZBTB1). Peaks enriched within the promoter of ASNS are indicated in red.
F) Immunoblot analysis of parental Jurkat cells treated with L-asparaginase (0.25 U/mL) for the indicated number of hours (top). Actin was used as a loading control. Immunoblot analysis of parental or ZBTB1 knockout cells expressing indicated cDNA (bottom). GAPDH was used as a loading control.
G) SDS-PAGE of recombinant zinc-finger domain of ZBTB1 expressed in bacteria. Whole cell lysate (WCL), anti-His beads, wash, and elution samples are indicated. Expected purified protein is indicated (arrow).
H) Fold change in cell number (log_2) of parental or ZBTB1 knockout Jurkat cells expressing vector or RUNX1 cDNA after treatment with L-asparaginase (0.03U/mL) or left untreated for 5 days (mean ± SD, n=3, left). RUNX1 transcripts per million (TPM) in parental or ZBTB1 knockout Jurkat cells.
I) ATAC-Seq tracks near the promoter of ASNS in parental, ZBTB1 knockout, and ZBTB1 knockout expressing ZBTB1 cDNA Jurkat cells in the presence (+N) or absence (-N) of asparagine, as indicated.
J) Schematic of the protein structure of ZBTB1 including the broad complex, tramtrack, and bric-a-brac (BTB) domain, zinc fingers 1-7 (ZF), ubiquitin-binding zinc finger domain (UBZ4) (top). Fold change in cell number (log_2) of parental or ZBTB1 knockout Jurkat cells expressing indicated cDNAs after treated with asparaginase (0.25 U/mL, gray) or left untreated (black) for 4 days (mean ± SD, n=3).
K) Fold change in cell number (log_2) of RPMI-8402 (left) or ALL-SIL (right) cells expressing vector or ASNS cDNA after treatment with L-asparaginase (0.25 U/mL, gray) or no treatment (black) for 6 days (mean ± SD, n=3, top). Immunoblot analysis of cells lines with indicated antibodies. GAPDH was used as a loading control.
L) Immunoblot analysis of parental and ZBTB1 knockout Jurkat cells grown in HPLM left untreated or treated with L-asparaginase and expressing vector or ATF4 cDNA, as indicated. Actin was used as a loading control.
M) Immunoblot analysis of parental, ZBTB1 knockout and ZBTB1 knockout expressing ZBTB1 cDNA Jurkat cells grown in HPLM treated with L-asparaginase, as indicated. GAPDH was used as a loading control.

Figure S4. Related to Figure 4.

A) Relative fold change in cell number of indicated cell lines expressing ZBTB1 sgRNA or vector control after growth in HPLM treated with L-asparaginase (0.03125 U/mL) for five days (mean ± SD, n=3).
B) Weight of mice with the indicated engrafted cells and treatment at the indicated day normalized to their initial weight (mean ± SD, n=7).
C) Relative abundance of indicated metabolites in the serum of mice treated with L-asparaginase (1000 U/kg, gray) or left untreated (black).
Statistics: two-tailed unpaired *t*-test. $^{**}P<0.05$, $^{***}P<0.01$, $^{****}P<0.001$