Figure S1. Sequencing traces for ATLL FERM domain mutations L156P. A) Direct Sanger sequencing of a PCR product from one of our patients showed a low peak for G under a higher peak for A. The trace shows the complement of the codon so that the variant is a T to C substitution. Thus, the codon for Lys (CTC) was mutated to Pro (CCC). B) Direct PCR sequence trace for control genomic DNA which is pooled from over 50 donors of the local Nashville Red Cross. No significant background is present. C) We found the L156P variant in 10% of clones of PCR products in pGEM-T vector. A sequence representative of the positive clones is shown.

Figure S2. Frequency of Tax approximates the frequency of mutations in one ATLL patient. One patient was identified as having a JAK3 L156P mutation at a frequency of approximately 10% as estimated by number of subclones containing the mutations. Genomic DNA for this patient was quantified for tax copies (blue diamonds) using quantitative PCR (qPCR). Tax levels are roughly 8.7% of the control Chromosome 11 short tandem sequence (STS) (red squares) levels. Tax frequency was calculated as the difference between mean Ct for tax (1 copy) and the mean Ct for STS (2 copies), which was 1:32. Tax quantification approximates the frequency of leukemic cells in the buffy coat sample. We conclude that the leukemic cell frequency is approximately the same as the frequency of L156P mutation.

Figure S3. Mutant JAK3s show binding to γ_c comparable with that of WT JAK3. JAK3 (106 kDa) and γ_c (48 kDa) co-immunoprecipitation (IP) and Western blot (WB) analysis are shown. BaF3 cells were transduced with WT JAK3 or mutant JAK3s and then subjected to IP. Blots were performed with infrared dye labeled secondary antibodies and were quantified. The quantification is shown in bar graphs. The proteins precipitated were quantified an normalized to the comparable band seen for WT JAK3. IP data represents two independent experiments.

Figure S4. Quantification of JAK3 and pSTAT5 for HuT-102 and MT-2 from experiment in Fig. 3. Protein lysates from the experiments similar to those shown in Figure 4 were blotted quantitatively for JAK3, STAT5, pSTAT5 and tubulin. JAK3 and pSTAT5 protein quantity was normalized to that seen in cells treated with DMSO (vehicle) alone.

Figure S5. Cell growth with inhibition of PI3K/mTor. BEZ-235 binds the ATP-binding clefts of PI3K and mTOR inhibiting their activity. MTT experiments were performed at concentrations of 0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000nM. Goodness of fit as represented by R^2 values are shown in red and 95% confidence intervals for IC50s are shown in blue. Graphed data is a compilation of two experiments done in quadruplicate error bars represent SEM.

Figure S6. Cell growth with inhibition of PI3K. BKM -120 is a pan-PI3K inhibitor PI3K. MTT experiments were performed at concentrations of 0, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 50, and 100μM. Goodness of fit as represented by R^2 values are shown in red and 95% confidence intervals for IC50s are shown in blue. Graphed data is a compilation of two experiments done in quadruplicate error bars represent SEM.

Figure S7. Cell growth with inhibition of ERK1/2. CI-1040 is a non-competitive inhibitor of MEK1/2 as measured but activation of ERK1/2. MTT experiments were performed at concentrations of 0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000nM. Goodness of fit as represented by R^2 values are shown in red and 95% confidence intervals for IC50s are shown in blue. Graphed data is a compilation of two experiments done in quadruplicate error bars represent SEM.

Table S1. JAK3 FERM domain mutations and SNPs identified in ATLL patients. A) Three DNA point mutations were identified in JAK3 exon 4 for 4 out of the 36 ATLL patients sampled. B) Of two are nonsynonymous SNPs listed in the NCBI Entrez database only P132T was identified in 1 of our 36 ATLL patient samples. Five Single Nucleotide Polymorphysims (SNPs) are listed for JAK3 FERM domain in NCBI Entrez database. Additionally, multiple SNP varients within introns 2-5 were identified for 3 ATLL patients (data not shown).

Table S2. Primer list for REVEAL PCR and sequencing of 36 ATLL and 24 ethnic matched control samples

Table S3. Primer list for site directed mutagenesis of JAK3 to introduce ATLL mutations

Table S4. Growth media and conditions for all cell lines

Table S5. Origins of ethnically matched control samples

Table S1

Cluster id	Region of JAK3	# Patients	dbSNP Allele	Protein Residue
rs3212711	Intron 1	2 +HUT102	C to T	NA
rs3212712	Intron 1	2 +HUT102	G to A	NA
rs3212713	Intron 1	2 +HUT102	G to A	NA
rs3212716	Exon 3	1	C356G	Leu99 synonymous
rs3212718	Intron 3	1	A to G	NA
rs3212719	Intron 3	1	A to G	NA
rs3212723	Exon 4	1	C453A	Pro132Thr
novel mutation	Exon 4	1	T525C	Leu156Pro
novel mutation	Exon 4	1	A606G	Glu183Gly
novel mutation	Exon 4	2	G573A	Arg172Gln

Table S2

	Sense	Antisense	Product size
Exon2:	CAGAAGTCCAATCCCCTCTG	AGCTTCCAATCTTGGCCC	321
Exon3:	GATCTGGACGGTTGGGTATG	CAAGTGACCCACCCTCTCTG	356
Exon4-5:	CCCCACCATAATGTCACTCC	AGCCCACGTTGCTCACTC	592
Exon6:	TTTGTGTGTGTCCCCGC	TACCACTCTCCGGCCCC	435
Exon7-8:	ATAGGGAGTGGATGGTGTGG	TGAGGCATAGAGAAGGGGAG	537
Exon9:	TACCTGAATTTGAGCCCAGG	ATTCAAACGTCACCTCCTCC	248
Exon10:	CTGGAATGAGTTCATGGTGC	GGCTTTAATGAGCAAGTGGC	976
Exon11-12:	TTGGGGACTTTTCACCTCTG	TTTCTCTGCATCCACGACC	583
Exon13:	TTGGGATTATTGGAGTGGAAG	AGAGGTGGGAAGAACAGCC	211
Exon14:	GCAGAACCTCCTCAACACAAG	AATTCCTCTTCCACCCAGAG	261
Exon15-16:	GCCAAACAGACTCTTCATTCATC	GACTGGATGTCAGTCTGCCC	496
Exon17:	AGATTGGGGTGGGTCTATTG	ACCACGCTCCTTCCACTG	275
Exon18-19:	TCAACTCAGGAGTGGGGC	GCAGGAGGGTAAGAATGTGC	547
Exon20:	GCAAAACTGAGGTCGAGAGG	ACCCCAAACCACTCCTCAG	330
Exon21:	GTCACGCTTGGGGTACCTG	CTGGGGAGCAAAGCAGC	337
Exon22:	CCCCTCTTCCTGTCCTTTC	CAGGCGCAGACAGGTTG	253
Exon23:	ATCACAGATGGCCCCTACC	TGAAAGTGCTCGACTTGCC	382
Exon4	GTAAAACGACGGCCAGTGGGGTCAGCCCAGGATTG	CAGGAAACAGCTATGACCGCCCTGGGTCATAGGAACAC	~400
Exon16	GTAAAACGACGGCCAGTACCCCCACTTTGACAGAAGG	CAGGAAACAGCTATGACCCAACCTCACCAGACACACAGG	~400

Table S3

	Sense	Antisense	RE Site Introduced
L156P	GGGCGCCTCCCCGTGGGCCCCAGTCTCAAGGAGCAGGG	CCCGCGGAGGGGCACCCGGGGTCAGAGTTCCTCGTCCC	ApaI
E183G	GCGAGAGCAGGCCCAGCGGCCGGGAGGGCTGCTGAAGACTGTCAGC	CGCTCTCGTCCGGGTCGCCGGCCCTCCCGACGACTTCTGACAGTCG	BglI
R172Q	TTGGACCTGGCCCAGATGGCGCGAGAG	CTCTCGCGCCATCTGGGCCAGGTCCAA	Bst51
P132T	GCTATCCTTGACTTAACAGTCCTGGAGCACC	GGTGCTCCAGGACTGTTAAGTCAAGGATAGC	MseI
K855A	GGTGGCCGTGGCACAGCTGCAGC	GCTGCAGCTGTGCCACGGCCACC	BtgI
Y100C	CCCAAGTCCTGCTGTGCAGGATTCG	CGAATCCTGCACAGCAGGACTTGGG	BsgI

Table S4

	Cell Culture Media
BaF3	IMDM 10% FBS, 1% Penicillin/Streptomycin, 2% WeHi conditioned media
WEHI-3B	IMDM 10% FBS, 1% Penicillin/Streptomycin
OP9-DL1	alphaMEM, 20% FBS, 1% Penicillin/Streptomycin
OP9-GFP	alphaMEM, 20% FBS, 1% Penicillin/Streptomycin
HuT-102	RPMI, 10% FBS, 1% Penicillin/Streptomycin, 100U/ml hIL-2
MT-2	RPMI, 10% FBS, 1% Penicillin/Streptomycin
HEK 293T	DMEM, 10% FBS, 1% Penicillin/Streptomycin
Phoenix	DMEM, 10% FBS, 1% Penicillin/Streptomycin

Table S5

	Specimen ID	Country of Origin
1	V280	Haiti
2	V289	Haiti
3	V290	Jamaica
4	V291	Haiti
5	V292	Haiti
6	V293	Jamaica
7	V295	Jamaica
8	V281	Jamaica
9	V296	Haiti
10	V297	Jamaica
11	V298	Haiti
12	V299	Haiti
13	V304	Haiti
14	V307	Haiti
15	V312	Jamaica
16	V314	Jamaica
17	V318	Haiti
18	V319	Jamaica
19	V321	Jamaica
20	V322	Jamaica
21	V325	Jamaica
22	V326	Jamaica
23	V327	Jamaica

Figure S1

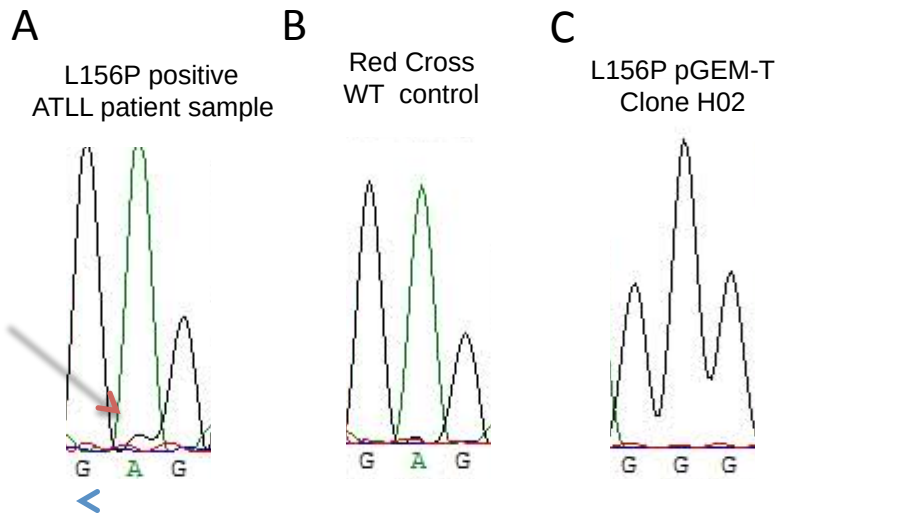

A
L156P positive
ATLL patient sample
G A G
B
Red Cross
WT control
G A G
C
L156P pGEM-T
Clone H02
G G G
Reverse Complement

Figure S2

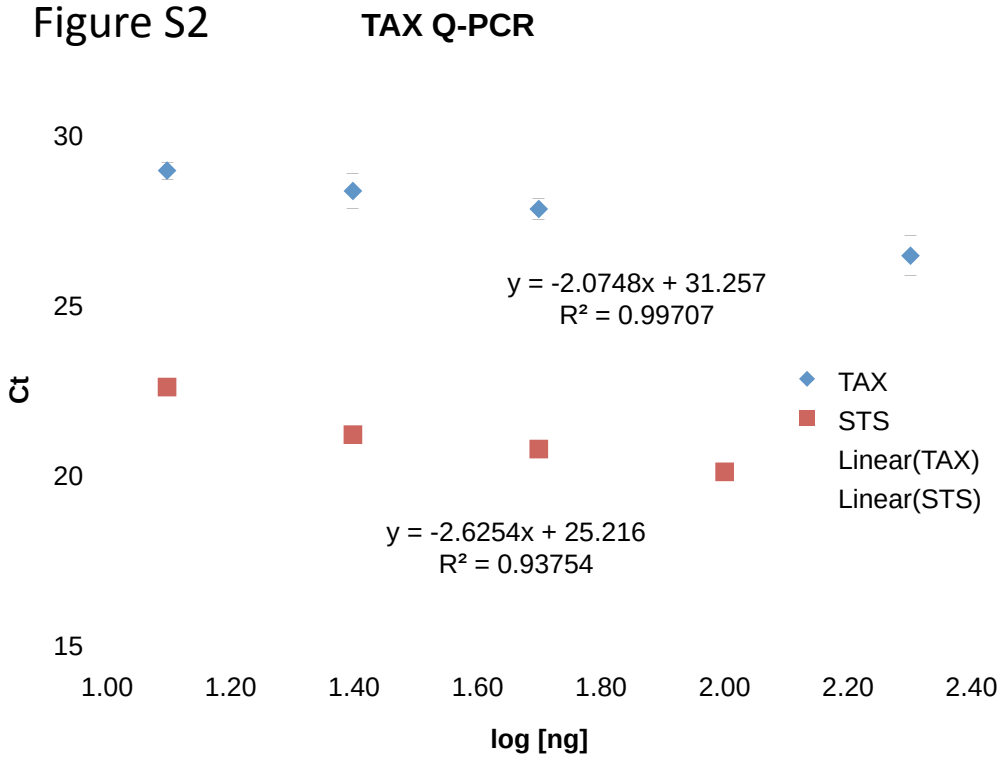

TAX Q-PCR
30
25
20
15
Ct
y = -2.0748x + 31.257
R² = 0.99707
y = -2.6254x + 25.216
R² = 0.93754
TAX
STS
Linear(TAX)
Linear(STS)
1.00
1.20
1.40
1.60
1.80
2.00
2.20
2.40
log [ng]

Figure S3

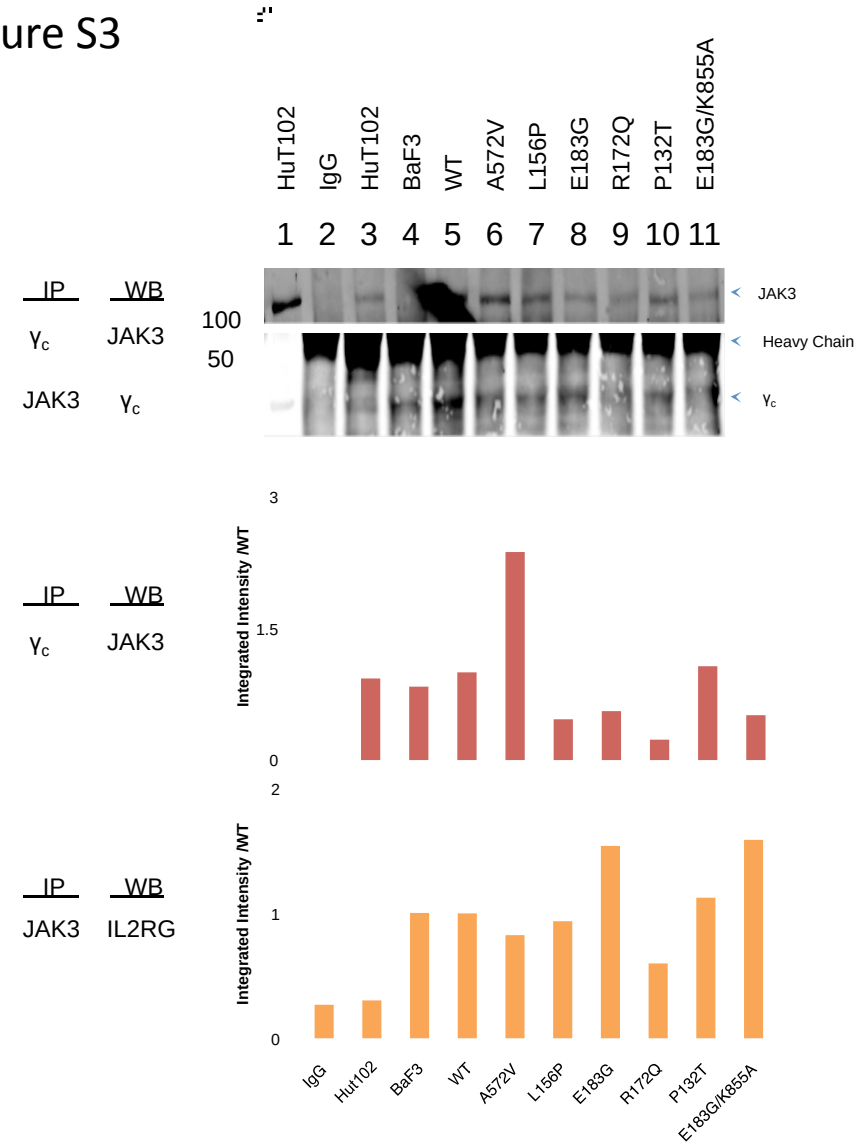

HuT102
IgG
HuT102
BaF3
WT
A572V
L156P
E183G
R172Q
P132T
E183G/K855A
1 2 3 4 5 6 7 8 9 10 11
IP WB
γc JAK3
JAK3 γc
100
50
JAK3
Heavy Chain
γc
IP WB
γc JAK3
Integrated Intensity /WT
3
1.5
0
IP WB
JAK3 IL2RG
Integrated Intensity /WT
2
1
0
IgG
Hut102
BaF3
WT
A572V
L156P
E183G
R172Q
P132T
E183G/K855A

Figure S4

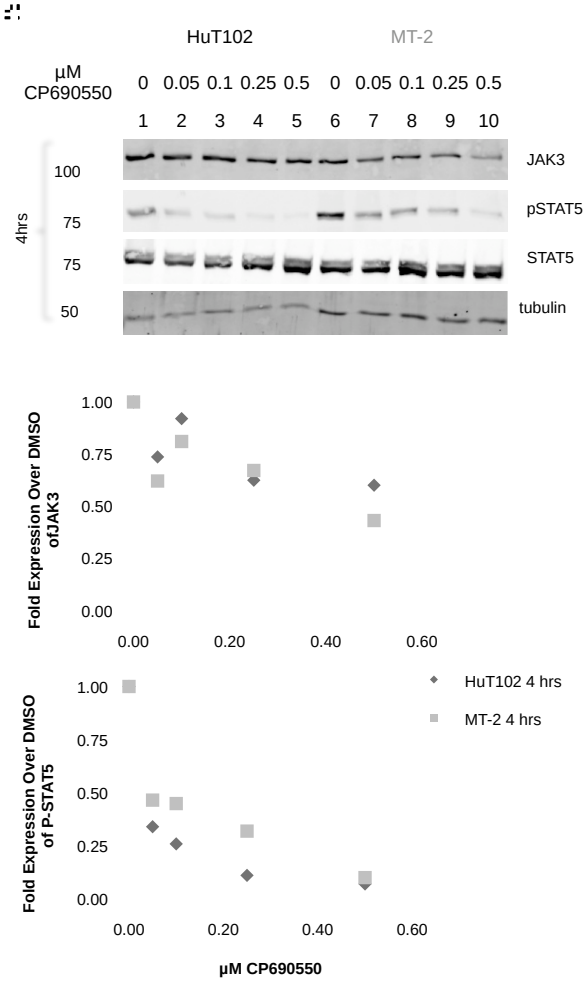
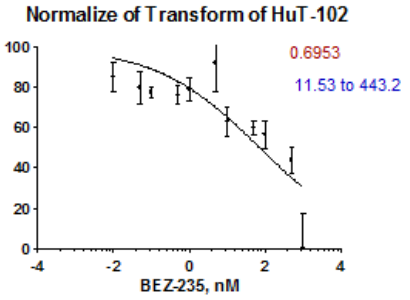
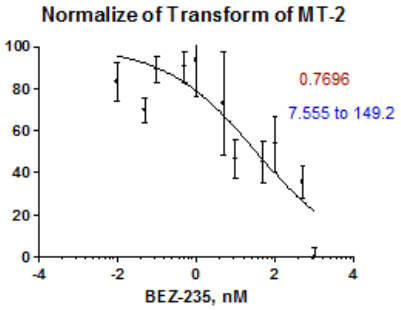
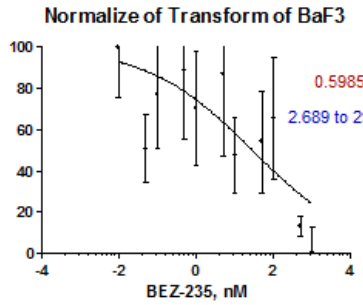

HuT102
MT-2
μM CP690550
0 0.05 0.1 0.25 0.5 0 0.05 0.1 0.25 0.5
1 2 3 4 5 6 7 8 9 10
4hrs
100
75
75
50
JAK3
pSTAT5
STAT5
tubulin
Fold Expression Over DMSO ofJAK3
1.00
0.75
0.50
0.25
0.00
0.00 0.20 0.40 0.60
HuT102 4 hrs
MT-2 4 hrs
Fold Expression Over DMSO of P-STAT5
μM CP690550

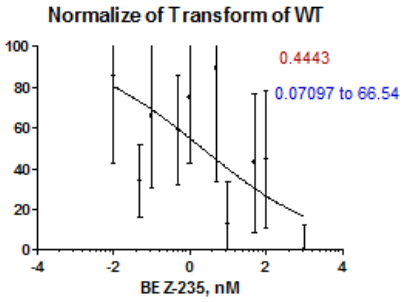
Figure S5

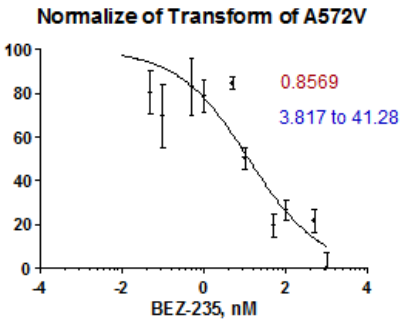
BEZ-235 (PI3K/mTOR)

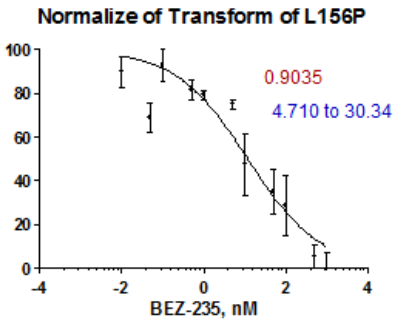


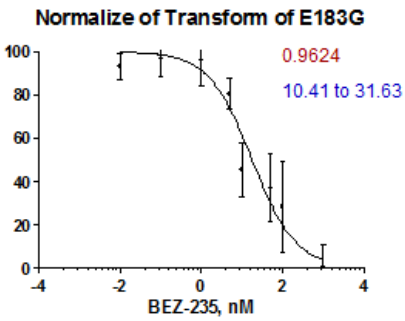












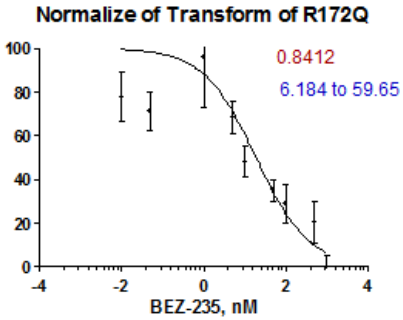


Figure S6

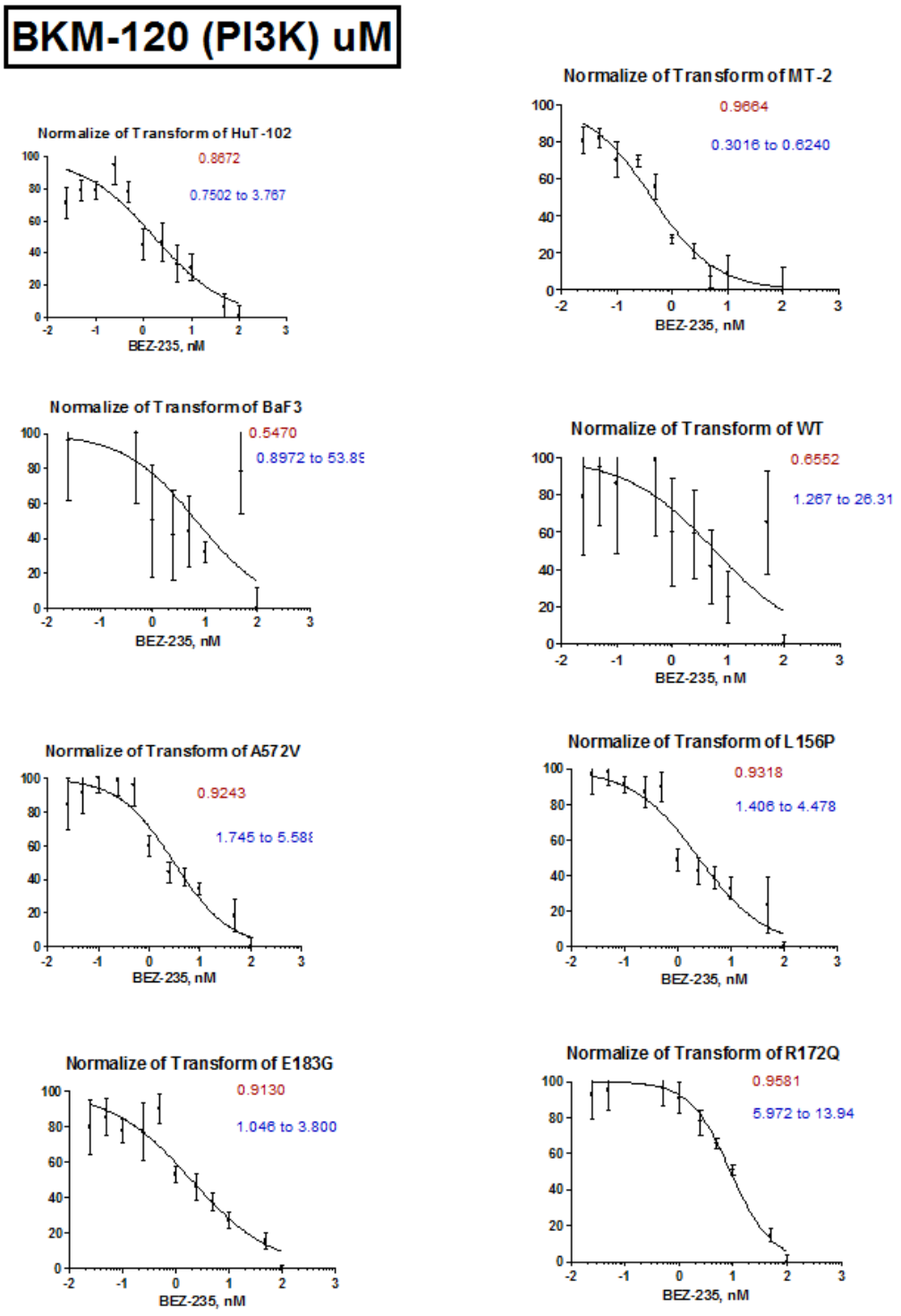
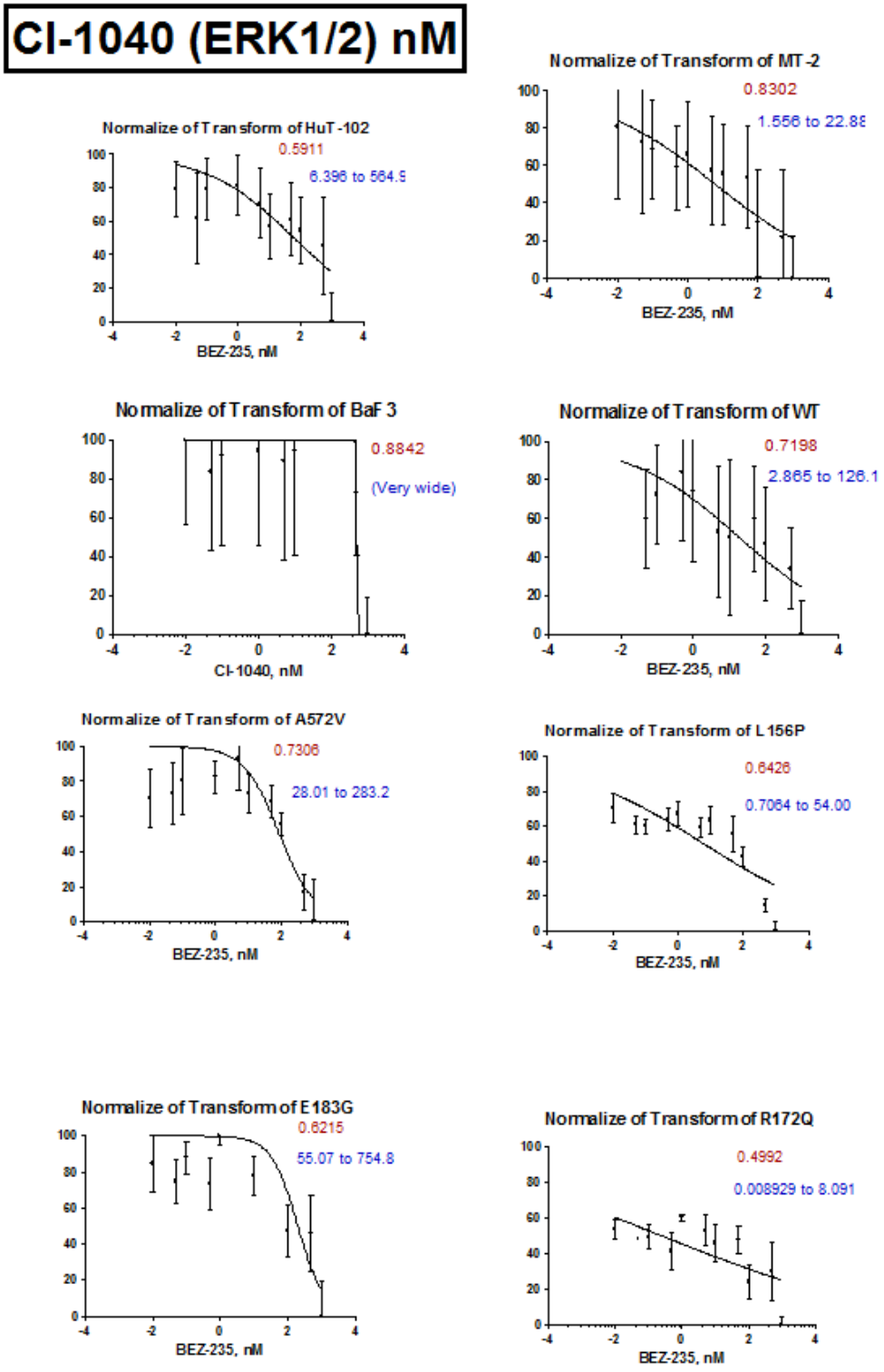

BKM-120 (PI3K) uM
Normalize of Transform of HuT-102
0.8672
0.7502 to 3.767
BEZ-235, nM
Normalize of Transform of MT-2
0.9664
0.3016 to 0.6240
BEZ-235, nM
Normalize of Transform of BaF3
0.5470
0.8972 to 53.85
BEZ-235, nM
Normalize of Transform of WT
0.6552
1.267 to 26.31
BEZ-235, nM
Normalize of Transform of A572V
0.9243
1.745 to 5.588
BEZ-235, nM
Normalize of Transform of L156P
0.9318
1.406 to 4.478
BEZ-235, nM
Normalize of Transform of E183G
0.9130
1.046 to 3.800
BEZ-235, nM
Normalize of Transform of R172Q
0.9581
5.972 to 13.94
BEZ-235, nM

Figure S7


CI-1040 (ERK1/2) nM
Normalize of Transform of HuT-102
0.5911
6.396 to 564.9
BEZ-235, nM
Normalize of Transform of MT-2
0.8302
1.556 to 22.88
BEZ-235, nM
Normalize of Transform of BaF 3
0.8842
(Very wide)
CI-1040, nM
Normalize of Transform of WT
0.7198
2.865 to 126.1
BEZ-235, nM
Normalize of Transform of A572V
0.7306
28.01 to 283.2
BEZ-235, nM
Normalize of Transform of L156P
0.6426
0.7064 to 54.00
BEZ-235, nM
Normalize of Transform of E183G
0.6215
55.07 to 754.8
BEZ-235, nM
Normalize of Transform of R172Q
0.4992
0.008929 to 8.091
BEZ-235, nM