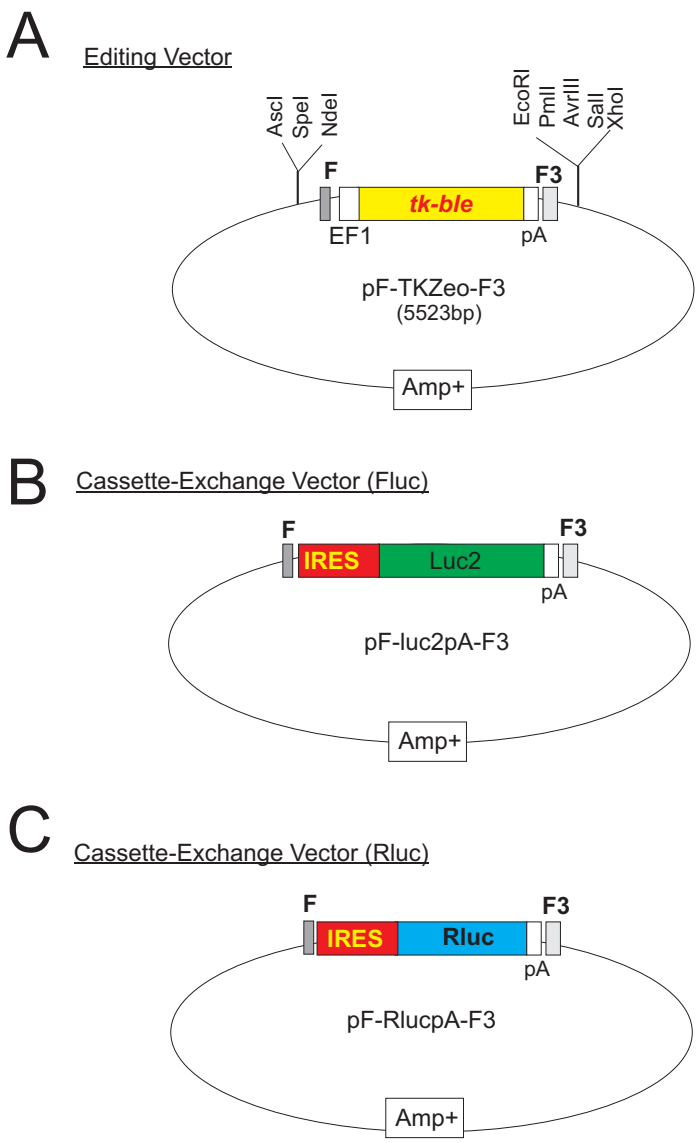

Figure S1: Related to Figure 2**.** Schematic maps of vectors used for generating recombinant cells carrying a bicistronic *in-situ* reporter.

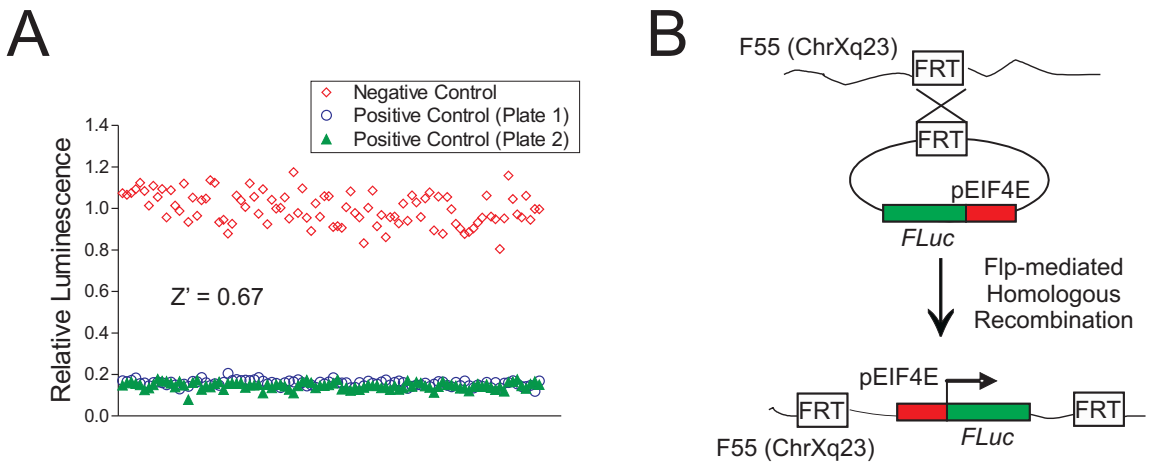

Figure S2: Related to Figure 3.
(A) Reproducibility analysis of the reporter assay for drug screening. Cells in 96-well plates were treated with 1 μM of the general transcription inhibitor, Actinomycin D (Positive Control, two plates), or DMSO (Negative Control, one plate) for 16 h and lysed for luciferase activity assays. The assay results from two positive-control plates were combined and used to calculate Z'.
(B) The schematic shows that Flp-mediated homologous recombination between FRT sites result in integration of the *FLuc* gene driven by a cloned EIF4E promoter into the predefined F55 genomic location. The F55 site is localized in Chromosome X, which is different from the *EIF4E g*ene locus.

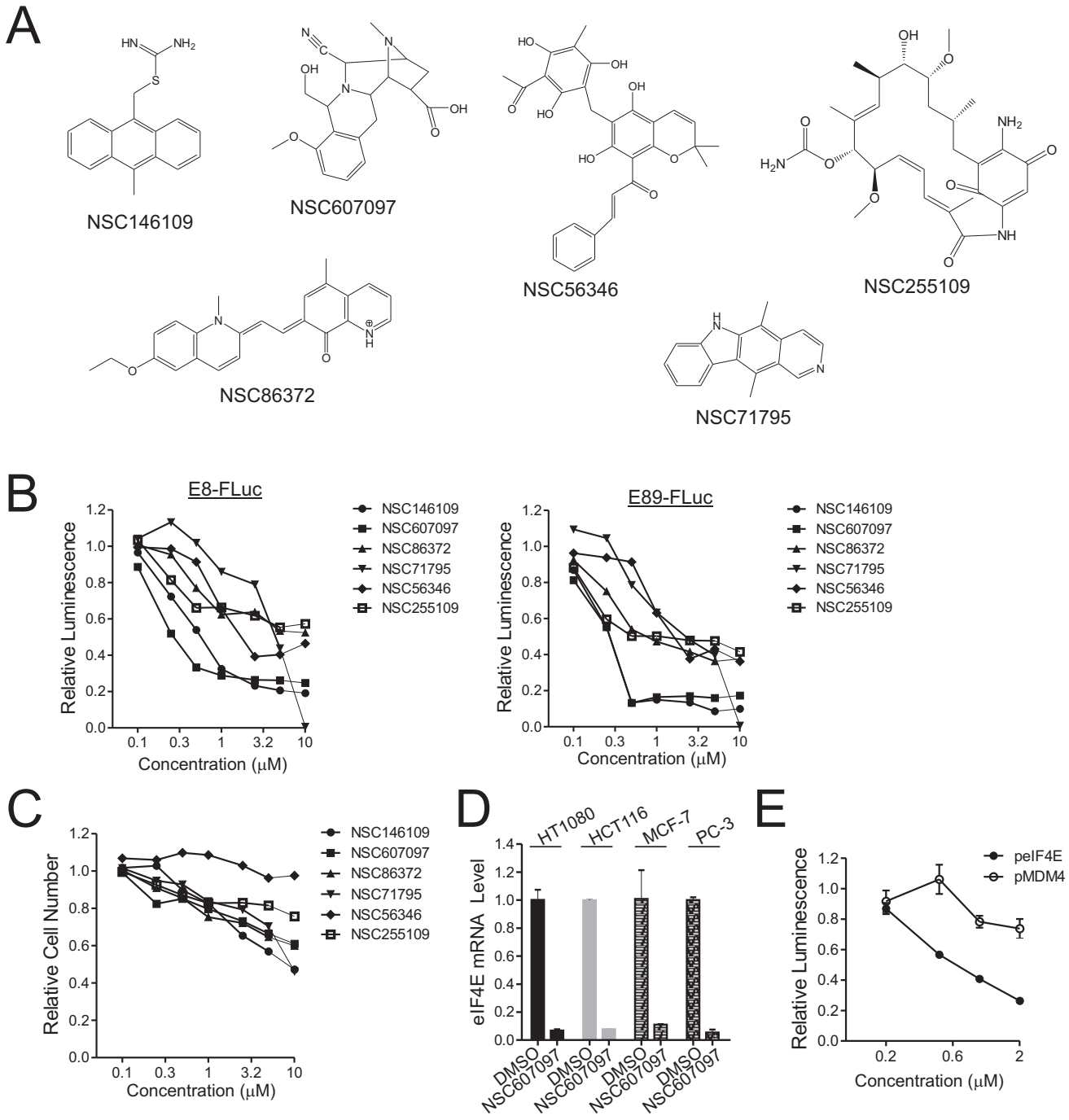

Figure S3: Screening positive hits and their characterization; Related to Figure 3.
A. Chemical structures of the 6 positive screen hits validated by qRT-PCR.
B. Concentration-dependent effects of the screen hits on reporter activity in two independent clones (E8-FLuc, left; E89-FLuc, right) carrying the bicistronic FLuc reporter. These two clones were derived from two independent targeting experiments.
C. MTT assays to evaluate cytotoxicities of indicated compounds. E8-FLuc cells were treated with indicated compounds for 16 h for MTT assays.
D. Inhibition of *EIF4E* expression by NSC607097 in various human cancer cells as measured by qRT-PCR. Error bars represent standard deviation (SD).
E. NSC607097 decreased the eIF4E promoter activity but not the MDM4 promoter activity, demonstrating its specificity on *EIF4E* expression. Error bars represented SD.

Table S2. Known biological activities of screen hits (related to Figure 3)

Compund	Other Name	Reported Activity
NSC 146109	XI-011	inhibits MDMX transcription
NSC 607097	DX-52-1	Inhibits HIF-1 transactivation; binds to microtubules
NSC 86372	CCG-37281	chelate metal ions and inhibit metalloproteinase activity
NSC 71795	ellipticine	DNA intercalator; inhibit topoisomerase II
NSC 56346	rottlerin	potassium channel opener; non-specific PKC inhibitor
NSC 255109	17-AG	Geldanamycin derivative and binds to HSP90

Table S3. Primers used in this study (Related to Experimental Procedure)

Primer	Sequence (5' to 3')
Cloning	
Left homology arm	
EIF4E-LA-For	AGGCGCGCCATTATCCTTTTGACCTCGTG
EIF4E-LA-Rev	GGACTAGTATTGCTTGACGCAGTCTCCT
Right homology arm	
EIF4E-RA-For	GACGAATTCGAGATTTGGGAGCTGAACCA
EIF4E-RA-Rev	CGACCTAGGAGAGACTGCCTTGCAATAAG
PCR Screening	
LA-F	TCTCGATCTCCTGACCTCGT
LA-R	ACCTTCTCTAGGCACCCGTT
RA-F	AGATCcgcGGAAGTTCCTAT
RA-R	TCCCCACCACCTGTACTTTC
RMCE-F	TTATCAGTCCCACGCAGACA
RMCE-R	AGGAACTGCTTCCTTCACGA
sgRNA cloning	
sgEIF4E-For	CACCGtgcgtcaagcaatcgagatt
sgEIF4E-Rev	AAACaatctcgattgcttgacgcaC
Northern blotting probes	
EIF4E-probe-For	aaaggatccaccatggcgactgtcgaacc
EIF4E-probe-Rev	GGCTCGAGttaAACAACAAACCTATTTTTAG
FLuc-probe-1	GGATGGCAGAAGCTATGAAG
FLuc-probe-2	AATGGGAAGTCACGAAGGTG
QRT-PCR	
EIF4E-For	TGGCGACTGTCGAACCG
EIF4E-Rev	AGATTCCGTTTTCTCCTCTTCTGTAG
GAPDH-For	CAGCCTCAAGATCATCAGCA
GAPDH-Rev	TGTGGTCATGAGTCCTTCCA