Table S1. HITS-CLIP data modified from Loeb *et al.* [25] used to identify putative miR-22 target genes in T cells for qRT-PCR analysis shown in Fig. 2c. Asterisks indicate genes with additional published verification of miR-22 targeting.

Ensembl or Refseq Identifier	Gene Symbol	miRNA	Log_2(Reads)	Start of Peak*	End of Peak*	miRNA seed position
NM_001199296.1	***Acly****	mir-22	3.0719	810	830	818
NM_001038642.1	***Ets1***	mir-22	5.57867	2093	2124	2104
NM_008960.2	***Pten****	mir-22	3.73283	672	703	694
ENSMUST00000063191	***Serpinb9***	mir-22	6.72302	868	907	889
NM_001040400.2	***Tet2****	mir-22	3.04318	1397	1430	1408

Table S2. qRT-PCR primer sequences.

Target mRNA	Direction	Sequence
GW182	sense	CGGGAAGTTGACCACCTCAG
	anti-sense	TGGAGGCACGAATGGATGAC
Tnrc6b	sense	CTGCCAAATCTCCACCGACT
	anti-sense	GGTTGTACCCCCTAGCACAC
Tnrc6c	sense	TCTCCCCTGGACCAGAATCC
	anti-sense	GCTATGGGGAACAGCTACGG
β-Actin	sense	GCGGGCGACGATGCT
	anti-sense	TGCCAGATCTTCTCCATGTCG

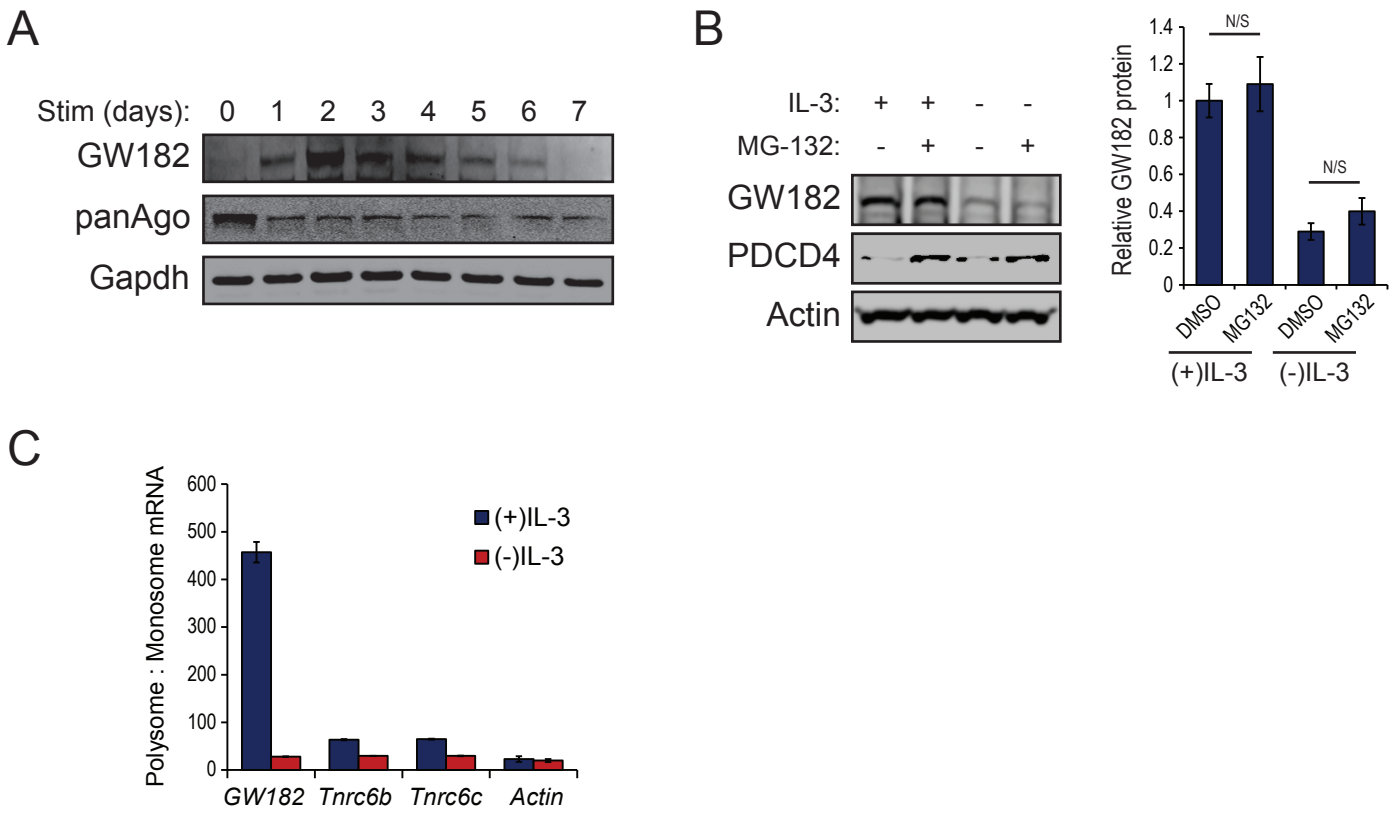

Figure S1 - Supplement to Figure 1. (A) Splenic T cells from C57BL/6 mice were stimulated for 3 days with microbeads coated with αCD3 and αCD28 antibodies in the presence of 25 U/mL recombinant murine interleukin-2 (rIL-2). On day 3 beads were removed and cells were maintained at approximately 1 x 10^6 cells/mL for an additional 4 days. Cells were collected daily and lysates prepared for Western blots using antibodies against GW182, Argonaute1-4 (panAgo) and Gapdh as an endogenous control. (B) (left) Representative Western blot for GW182 using lysates of FL5.12.xL cells cultured in the presence or absence of IL-3 ± 10 μM MG-132 for 24 hours. Pdcd4 serves as a control for MG-132 function and Actin as an endogenous control. (right) Quantification of GW182 protein expression from three independent experiments. Bars represent mean normalized GW182 protein expression ± standard deviation. Actin was used to normalize between lanes. (C) Enrichment of GW182, Tnrc6b, Trnc6c and Actin mRNA in polysome fractions from FL5.12.xL cells cultured in the presence or absence of IL-3 for 24 hours as determined by qRT-PCR. Bars represent the ratio of mRNA detected in polysome fractions to mRNA detected in monosome fractions based on comparison of qRT-PCR Ct values to a standard curve generated from input samples.

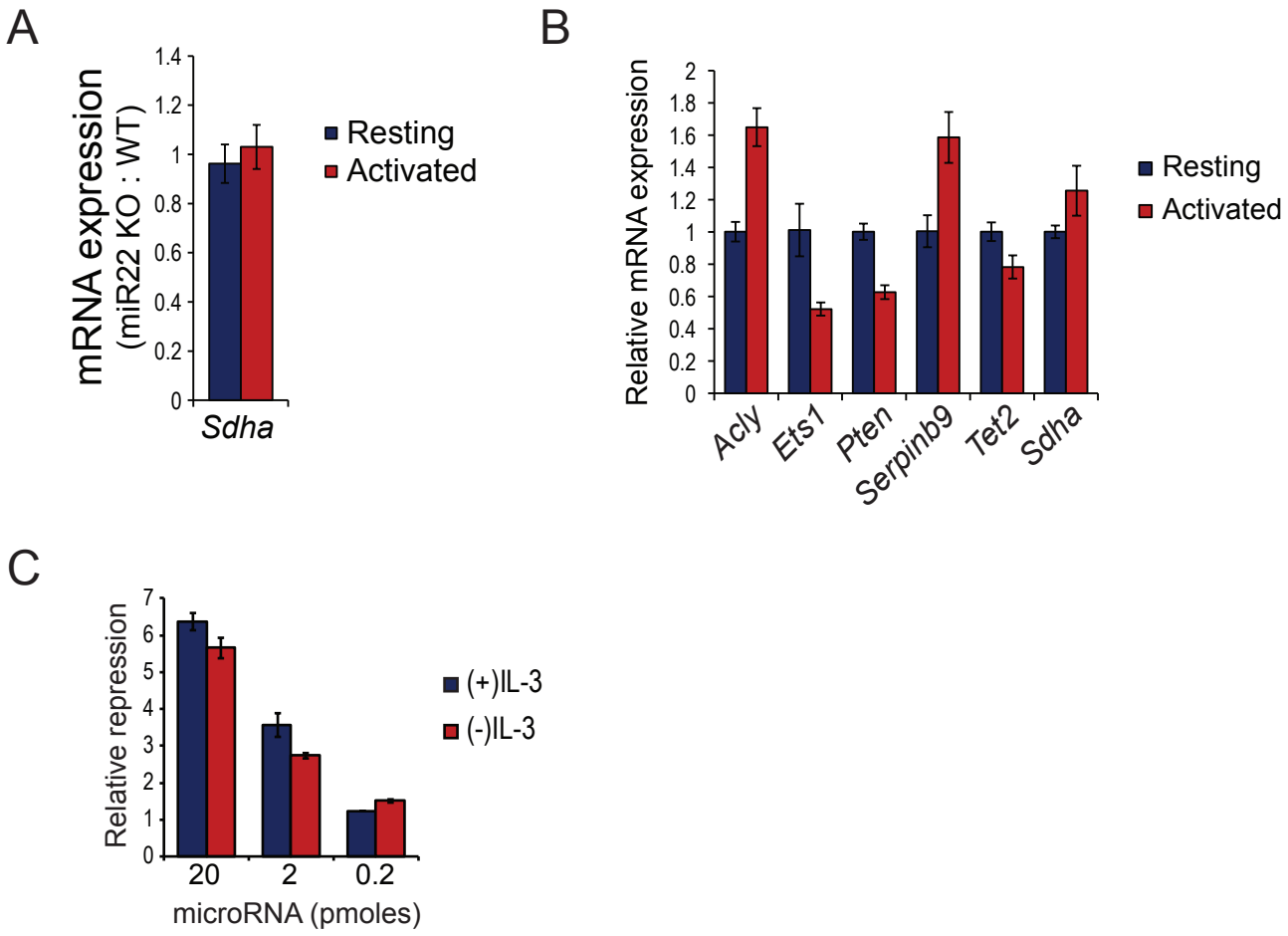

Figure S2 – Supplement to Figure 2. (A) Expression of *Sdha* mRNA lacking miR-22 seed sites in freshly isolated miR-22 knockout T cells (Resting) versus miR-22 knockout T cells activated *ex vivo* for two days as in Fig. 2B. Bars represent mean expression ± standard deviation of indicated transcripts in miR-22 knockout T cells relative to wild-type 129S1/SvImJ T cells as determined by qRT-PCR using the ΔΔCt method with *Tbp* as an endogenous control. (B) Changes in expression of mRNAs from Figures 2B and S2A resulting from *ex vivo* stimulation of wild-type 129S1/SvImJ splenic T cells for 2 days with microbeads coated with αCD3 and αCD28 antibodies in the presence of 25 U/mL recombinant murine interleukin-2 (rIL-2). (C) A renilla luciferase reporter containing a perfectly complementary sequence to the synthetic microRNA used in Figures 2D and 2F or a control reporter lacking the microRNA target sequence were co-transfected with pGL3 and 0, 0.2, 2 or 20 pmoles targeting microRNA per 10^6 cells into FL5.12.xL cells following overnight culture in the presence or absence of IL-3. Bars represent mean reporter repression calculated (see Materials and Methods) from a Dual-luciferase (DLR) experiment performed in quadruplicate ± standard deviation.

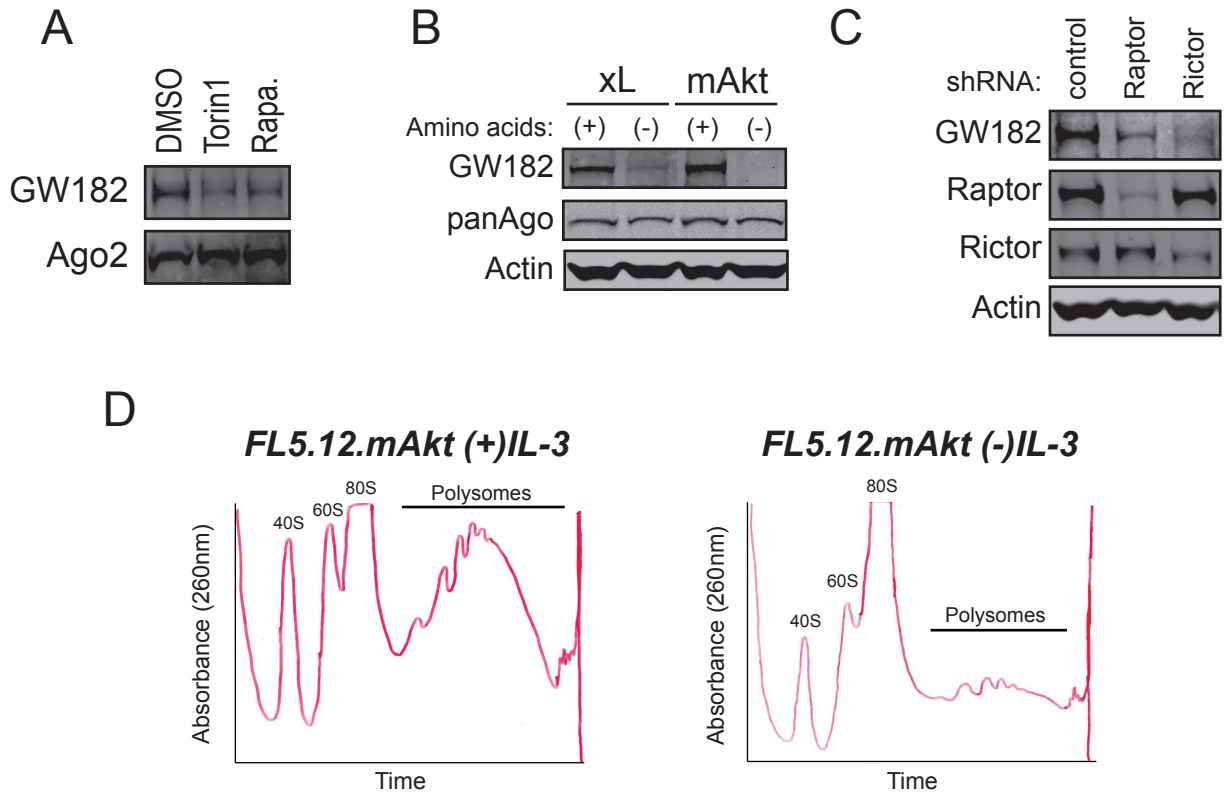

Figure S3 - Supplement to Figure 3. (A) Jurkat T cells engineered to resist apoptosis by over-expression of Bcl-2 [55] were treated with Torin1 (500 nM), Rapamycin (100 nM) or vehicle control (DMSO) for 24 hours. Cells were then lysed and lysates subjected to Western blot for GW182 or Ago2 as an endogenous control. (B) Western blots showing GW182 and Argonaute expression in FL5.12.xL or FL5.12.mAkt cells cultured for 24 hours in IL-3 containing medium with or without amino acids. Actin serves as an endogenous control. (C) Expression of GW182, Raptor or Rictor in FL5.12.mAkt cells following puromycin selection of retrovirally transduced clones expressing shRNAs targeting either Raptor or Rictor [56] or a non-targeting control. Actin serves as an endogenous control. (D) Representative UV absorbance traces recorded during collection of fractions of lysates from FL5.12.mAkt cells cultured overnight in the presence or absence of IL-3 centrifuged through 10 – 50% sucrose gradients. Fractions collected for qRT-PCR comparison of polysome and monosome associated mRNAs are noted.

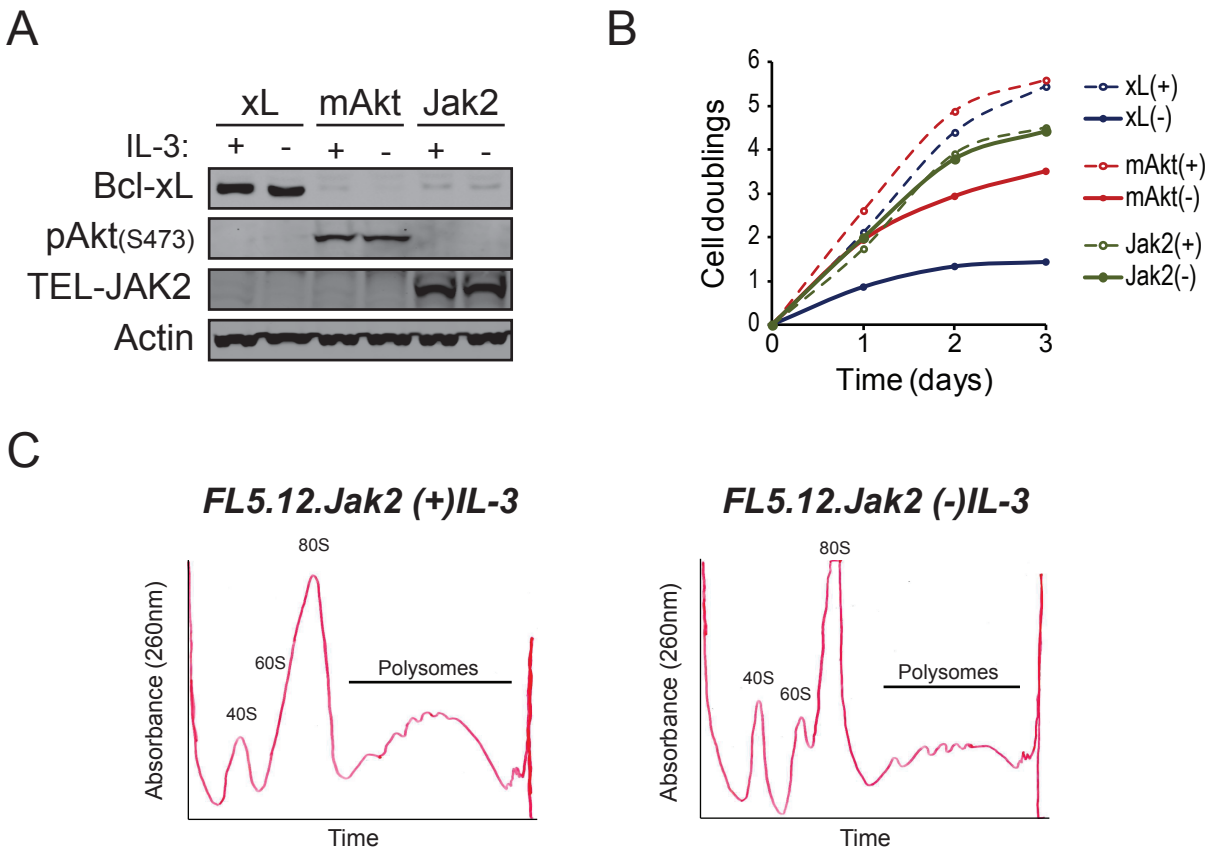

Figure S4 – Supplement to Figure 4. (A) Over-expression of Bcl-xL in FL5.12.xL cells, constitutively phosphorylated Akt in FL5.12.mAkt cells and TEL-JAK2 in FL5.12.Jak2 cells as determined by Western blot. Actin serves as an endogenous control. (B) Proliferation of FL5.12.xL, FL5.12.mAkt and FL5.12.Jak2 cells in the presence or absence of IL-3 determined by daily cell counting. (C) Representative UV absorbance traces recorded during collection of fractions of lysates from FL5.12.Jak2 cells cultured overnight in the presence or absence of IL-3 centrifuged through 10 – 50% sucrose gradients. Fractions collected for qRT-PCR comparison of polysome and monosome associated mRNAs are noted.

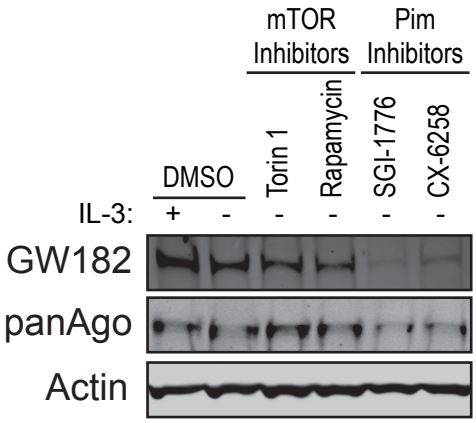

Figure S5 - Supplement to Figure 5. Western blot for expression of GW182 and Argonaute 1 – 4 (panAgo) following 24 hour incubation of FL5.12.Jak2 cells in the presence or absence of IL-3 and either Torin 1 (0.5 μM), Rapamycin (0.1 μM), SGI-1776 (2.5 μM), CX-6258 (1 μM) or vehicle control (DMSO).

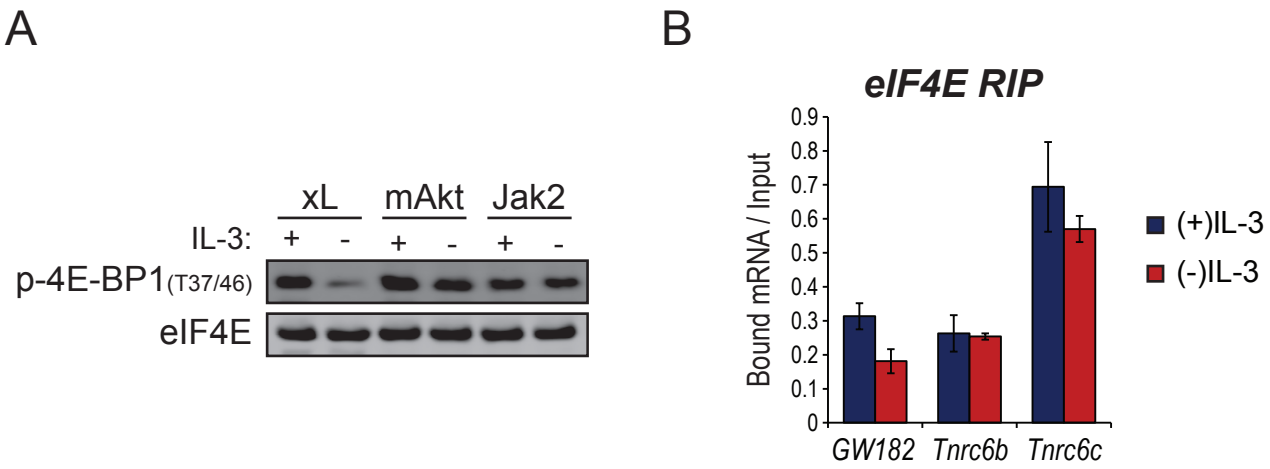

Figure S6. Supplement to Figure 6. (A) Western blots for phosphorylation of 4E-BP1 in IL-3 dependent FL5.12 cells expressing either Bcl-xL (xL), myristolated Akt (mAkt) or the TEL-JAK2 fusion protein (Jak2) following 24 hours of culture in the presence or absence of IL-3. eIF4E serves as an endogenous control. (B) eIF4E associated RNAs were purified by RNA immunoprecipitation (RIP) using antibodies against eIF4E, or control rabbit IgG, from FL5.12.xL cells that were cultured overnight in the presence (blue bars) or absence (red bars) of IL-3. Input and RIP-purified RNA were reverse transcribed and resulting cDNA was amplified by qRT-PCR using TaqMan primer-probe sets to detect the mRNA coding for GW182, Tnrc6b or Tnrc6c. Fraction of bound GW182 mRNA was determined by comparing Ct values from RIPs to a standard curve generated from input RNA. Bars represent mean fraction of bound mRNA ± 95% confidence interval of the mean. Control IgG RIPs could not be graphed because Ct values were not reached for most qRT-PCR reactions despite running 45 cycles of amplification.

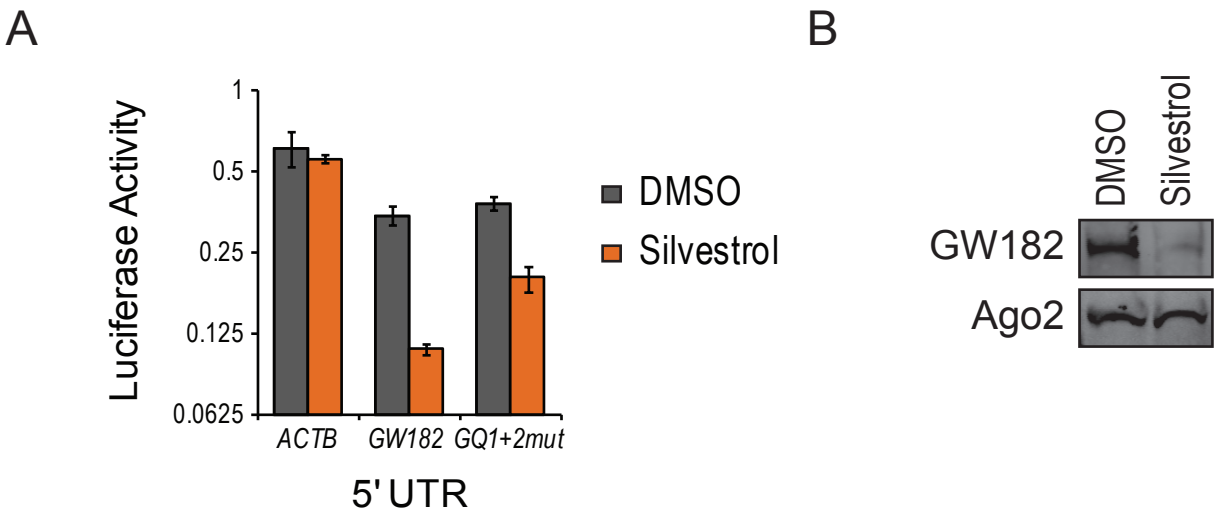

Figure S7 - Supplement to Figure 7. (A) GW182 5' UTR reporter constructs used in Figure 7 were transfected into Jurkat cells along with pGL3 control and Dual-luciferase (DLR) assays were performed following overnight incubation in complete medium in the absence (grey bars) or presence (orange bars) of silvestrol (10 nM). Bars represent mean normalized luciferase activity ± standard deviation from one experiment performed in quadruplicate. (B) Representative Western blots for GW182 and Ago2 as an endogenous control using whole cell lysates from Jurkat cells cultured under indicated conditions for 24 hours.