# Supporting Information

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### SI Materials and Methods

**Plasmids and shRNA.** Expression vectors for transient transfection of tRNA were generated by PCR amplification of Gly(GCC) chr1.tRNA68 (chr1:161493501–161493953), Gly(GCC) chr17.tRNA5 (chr17:8028941–8029251), and a CU1276 predicted genomic precursor (chr19:35115779–35116077) followed by insertion into the multiple cloning site of pcDNA3 expression vector (Invitrogen). CU1276 hairpin-expressing vector was generated by restriction digestion of pcDNA3 vector containing the human miR-26a-1 locus (chr3:38010684–38011069) with NaeI and BtgI to remove the miR-26a-1 hairpin; annealed oligos with sequences 5′-CGTGGCCTCGTCGATTCCCGGCCAATGCACCAAGTGCAGGTCCCAATGTGGT GCATTGTCTGGGAATTCACGGGGACGCGGGCCTGGACGCC-3′ and 5′-GGCGTCCAGGCCCGCGTCCCCGTGAATTCCCAGACAATGCACCACATTGGGACCTGCACTTGGTGCATTGGCCGGGAATCGACGAGGCC-3′ were ligated in its place. An inducible CU1276 hairpin-expressing vector was created by restriction digest of the pcDNA3-CU1276_hairpin vector and subsequent subcloning into the SfiI sites of pRTS1-GLSVP vector (1). Self-ligated pRTS1-GLSVP vector containing no insert was used as an empty vector control. shRNA-expressing plasmids pLKO.1-puro-shCtrl, pLKO.1-puro-shDICER-58, pLKO.1-puro-shDICER-61, and pLKO.1-puro-shDICER-62 were purchased from Open Biosystems. pIRESneo-HA-FLAG-AGO1, -AGO2, -AGO3, -AGO4, and -EGFP were obtained from Addgene (2). The RPA1, WHSC1L1, and STAG2 3′UTR reporter constructs were generated by PCR amplification of human genomic DNA, followed by insertion into the multiple cloning site of the pmiRGLO vector (Promega). RPA1 3′UTR-MUT reporter was generated by site-directed mutagenesis with primers 5′-CAAATAGGCATAATTTCCTATATTTCCTCCCACCTCCG-3′ and 5′-GGAAATATAGGAAATTATGCCTATTTGCAAACTTCTGC-3′; STAG2 3′UTRMUT reporter was generated by site-directed mutagenesis with primers 5′-GCTGTTAGTTGGCTTTTTCCTATATTATTTCATGCTT-3′ and 5′-GAAATAATATAGGAAAAAGCCAACTAACAGCGCATAAATAAAATA-3′.

**qRT-PCR Conditions.** RNA samples were reverse-transcribed with the SuperScript II First Strand Synthesis Kit (Invitrogen) in the presence of 0.2 μM RTFS primer (5′-TGTCAGGCAACCGTATTCACCGTGAGTGGTTGGTGCATTG-3′), random hexamers, or Oligo d(T) primer according to the manufacturer's indications. One-tenth of the cDNA volume was used as a template for PCR amplification in the presence of 70 nM SS primer (5′-CGTCAGATGTCCGAGTAGAGGGGGAACGGCGTCGATTCCCGGC-3′), and 70 nM each of MPF (5′-TGTCAGGCAACCGTATTCACC-3′) and MPR (5′-CGTCAGATGTCCGAGTAGAGG-3′) universal primers, or gene specific primers, as appropriate. The 5s rRNA and RNU66 were detected from cDNA generated by random hexamer reverse transcription, according to gene-specific primers (5srRNAF: 5′-GCCCGATCTCGTCTGATCT-3′, 5srRNAR: 5′-AGCCTACAGCACCCGGTATT-3′, RNU66F: 5′-GGTGATGGAAATGTGTTAGCC-3′, RNU66R: 5′-AGGATAGAAAGAACCACCTCA-3′). RPA1, RPA2, and GAPDH were detected from cDNA generated by Oligo d(T) reverse transcription, according to gene-specific primers (RPA1F: 5′-CTTCACGTCCATCACAGTGG-3′, RPA1R: 5′-TTTCCAGAATGCCAACTTCC-3′, RPA2F: 5′-AGGGAGAGCACCTATCAGCA-3′, RPA2R: 5′-TTCAACCCTTCAGGTCTTGG-3′, GAPDHF: 5′-CTGACTTCAACAGCGACACC-3′, GAPDHR: 5′-CCCTGTTGCTGTAGCCAAAT- 3′).

**Western Blot Conditions and Antibody Information.** All primary antibody dilutions were prepared in PBS with 0.1% Tween 20 and 5% (mass/mass) BSA. Dilutions and incubation conditions were as follows: ACTB (A5441; Sigma), 1:5,000, 1 h at room temperature; DICER (13D6; Abcam), 1:1,000, overnight at 4 °C; GFP (JL-8; Clontech), 1:5,000, 30 min at room temperature; γH2AFX (05-636; Millipore), 1:1,000, overnight 4 °C; H2AFX, total (A300-082A; Bethyl Laboratories), 1:500, overnight at 4 °C; RPA1 (2267; Cell Signaling), 1:1,000, overnight at 4 °C; RPA2 (14692; Santa Cruz), 1:500, overnight at 4 °C. HRP-conjugated secondary antibodies anti-mouse IgG HRP (NA931V; GE Healthcare), anti-goat IgG HRP (2020; Santa Cruz), and anti-rabbit IgG HRP (1238850; Boehringer Mannheim) were diluted at 1:10,000 in PBS with 0.1% Tween 20, 5% (mass/mass) milk, and used for 30 min to 3 h incubations at room temperature. Blots were visualized with ECL substrate or SuperSignal West Dura ECL substrate (Thermo Scientific). Where applicable, blots were quantified by using the ImageJ software suite (3).


1. Bornkamm GW, et al. (2005) Stringent doxycycline-dependent control of gene activities using an episomal one-vector system. *Nucleic Acids Res* 33(16):e137.
2. Meister G, et al. (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol Cell* 15(2):185–197.
3. Abramoff M, Magalhaes P, Ram S (2004) Image Processing with ImageJ. *Biophotonics Int* 11(7):36–42.

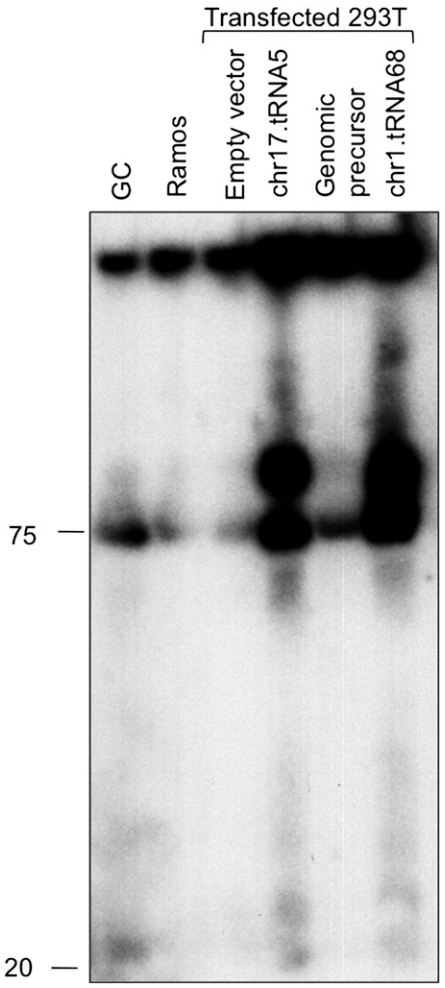


**Fig. S1.** CU1276 is expressed from two independent tRNA loci. Northern blot of total RNA from purified germinal center (GC) B cells, the Burkitt's lymphoma cell line Ramos, and 293T cells transiently transfected with empty vector, a vector encoding for Gly(GCC) chr17.tRNA5, a vector encoding for a predicted genomic precursor for CU1276, or a vector encoding for Gly(GCC) chr1.tRNA68. Both tRNA-encoding vectors were sufficient to express an ~22-nt band that comigrates with endogenous GC-expressed CU1276.

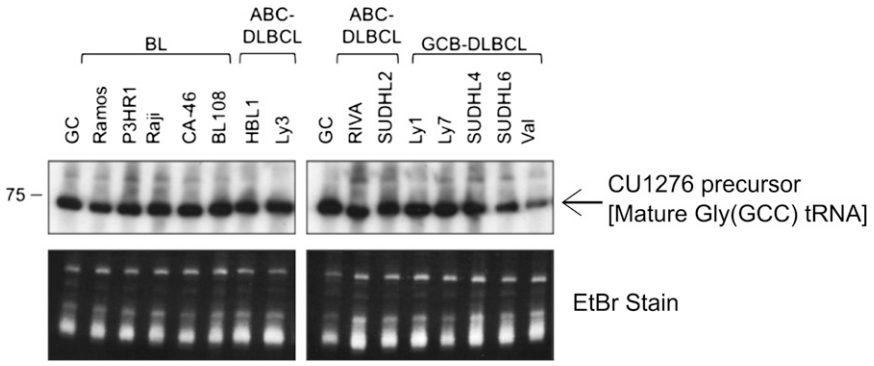


**Fig. S2.** Normal GC B cells and B-cell lymphoma lines express similar levels of Gly(GCC) tRNA. Northern blot analysis of total RNA from purified GC B cells, and from a panel of GC-derived lymphoma cell lines, including Burkitt's lymphoma (BL), activated B cell-like diffuse large B cell lymphoma (ABC-DLBCL), and GC-like diffuse large B cell lymphoma (GCB-DLBCL) subtypes. Membranes were blotted with a radioactive probe complementary to the 3′ end of mature Gly(GCC) tRNA. Ethidium bromide staining was used as loading control. The majority of cell lines express levels of tRNA similar to those observed in GC B cells, suggesting that any deficiency in CU1276 expression is likely due to mechanisms acting downstream of transcriptional regulation.

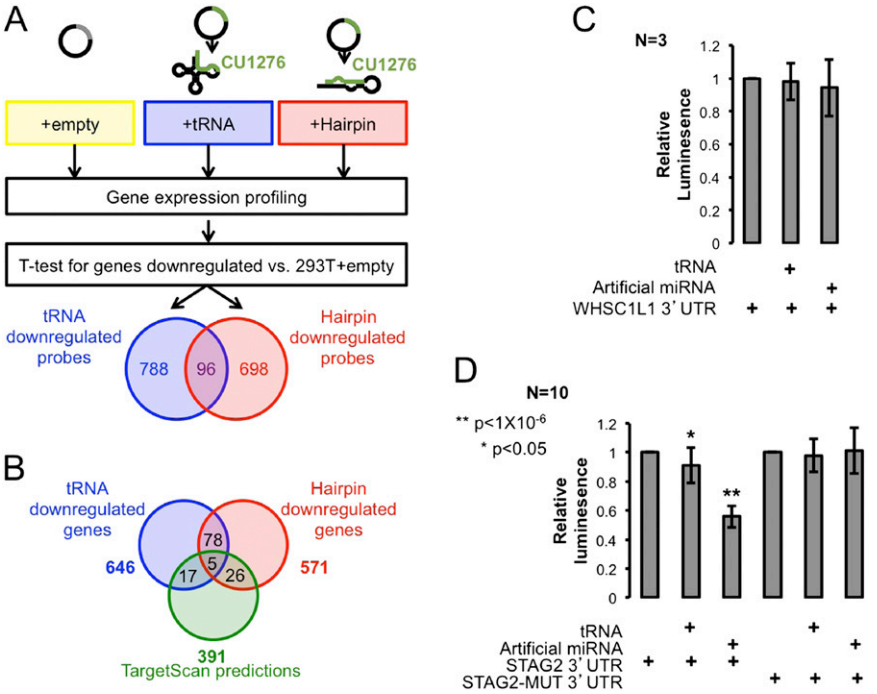


**Fig. S3.** Experimental schematic of CU1276 target candidate identification and validation of CU1276 targets. (*A*) The 293T cells were transiently transfected with empty vector, chr1.tRNA68, or CU1276 hairpin-encoding vectors. Forty-eight hours after transfection, cells were harvested, and their extracted total RNA was used for gene expression profiling with Affymetrix HGU133Plus2.0 arrays. Genes that were significantly down-regulated (threshold $P < 0.05$) in tRNA-, and/or in hairpin-expressing cells relative to empty vector transfected cells, were considered for further analysis. The statistically significant overlap ($P < 1e\text{-}40$) between tRNA–down-regulated and hairpin–down-regulated probes confirms that hairpin delivery of CU1276 recapitulates its physiological activity on at least a subset of transcripts. (*B*) Overlap of genes containing TargetScan-predicted CU1276 binding sites in their 3′UTR with those significantly down-regulated (threshold $P < 0.05$) upon expression of CU1276. Significant enrichment (Hypergeometric test, $P = 8.6e\text{-}8$) was observed for CU1276 TargetScan-predicted targets among the genes down-regulated by tRNA and/or hairpin expression. (*C*) WHSC1L1 3′UTR reporter activity in response to Gly(GCC) chr1.tRNA68-delivered or hairpin-delivered CU1276. The WHSC1L1 3′UTR was not sensitive to repression by either tRNA-mediated or hairpin-mediated expression of CU1276. Mutational analysis of the predicted binding site for CU1276 was not pursued. Firefly luciferase values were normalized to a Renilla luciferase control and plotted relative to reporter activity upon cotransfection of empty vector. Error bars represent the SD of three independent experiments, each performed in duplicate. (*D*) STAG2 3′UTR reporter activity in response to Gly(GCC) chr1.tRNA68- or hairpin-delivered CU1276. The STAG2 3′UTR is strongly repressed by hairpin-mediated delivery of CU1276, with high significance (Student *t* test, $P = 3.8e\text{-}7$), demonstrating that the CU1276 sequence does indeed interact with this 3′UTR. However, the effect of tRNA expression was ambiguous, showing robust repression in some experiments, and no repression in others. Although this repression did eventually reach a minimal threshold of significance (Student's *t* test, $P = 3.6e\text{-}2$), the low magnitude of repression suggests that STAG2 may be only weakly targeted by tRNA-delivered CU1276 in this cellular context. Firefly luciferase values were normalized to a Renilla luciferase control and plotted relative to reporter activity upon cotransfection of empty vector. Error bars represent the SD of 10 independent experiments, each performed in duplicate.

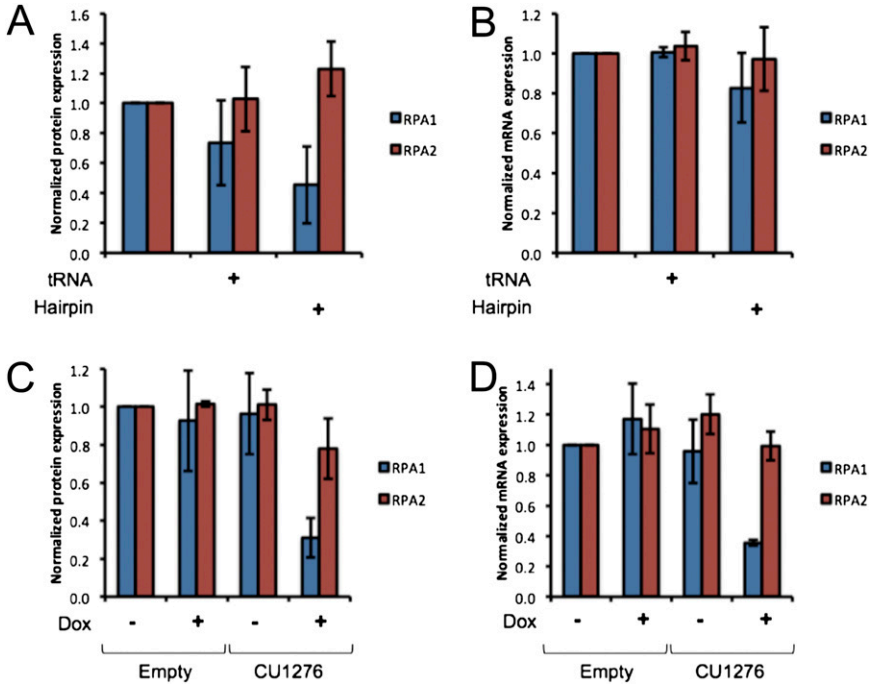


**Fig. S4.** Western Blot quantifications and qRT-PCR of *RPA1* upon CU1276 expression. (*A*) Western Blot quantification of RPA1 and RPA2 from 293T cells transiently transfected with either Gly(GCC) tRNA-expressing (tRNA), or CU1276 hairpin-expressing (Hairpin) vector; values are normalized to ACTB expression; error bars are the SD of four independent experiments. (*B*) qRT-PCR of *RPA1* and *RPA2* mRNA levels in 293T cells transiently transfected with tRNA- or Hairpin-expressing vector; values are normalized to *GAPDH* expression; error bars are the SD of three independent experiments. *RPA1* is not significantly repressed at the mRNA level in this cellular context. (*C*) Western Blot quantification of RPA1 and RPA2 from stable P3HR1 cells that express either GFP alone (Empty) or GFP plus CU1276 hairpin (CU1276) upon doxycycline (Dox) treatment; values are normalized to ACTB expression; error bars are the SD of four independent experiments. (*D*) qRT-PCR of *RPA1* and *RPA2* mRNA levels in stable P3HR1 cells that express either GFP alone (Empty) or GFP plus CU1276 hairpin (CU1276) upon doxycycline (Dox) treatment; values are normalized to *GAPDH* expression; error bars are the SD of four independent experiments. *RPA1* mRNA is significantly repressed by CU1276 expression ($P = 1.3e\text{-}3$).

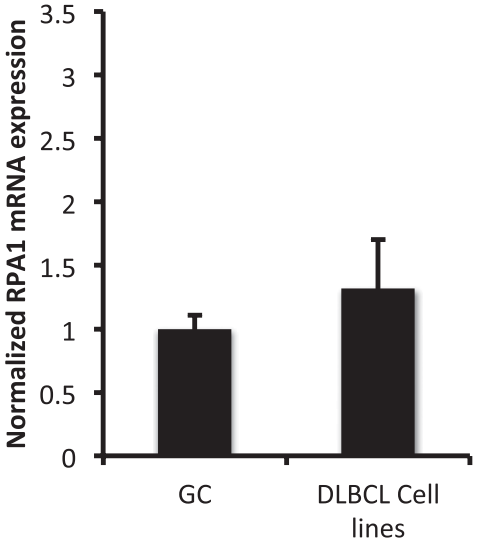


**Fig. S5.** GC cells and diffuse large B cell lymphoma (DLBCL) cell lines express similar levels of RPA1 mRNA. Normalized RPA1 mRNA expression in Germinal Center cells ($n = 5$) and a panel of DLBCL cell lines ($n = 8$; HBL1, Ly3, SUDHL2, RIVA, Ly7, SUDHL4, SUDHL6, and Val). Expression values were measured by Affymetrix HG-U133p2 GeneChip expression arrays. There is no significant difference between the two sample groups.