Acquired Dependence Of Acute Myeloid Leukemia On The DEAD-BOX RNA Helicase DDX5

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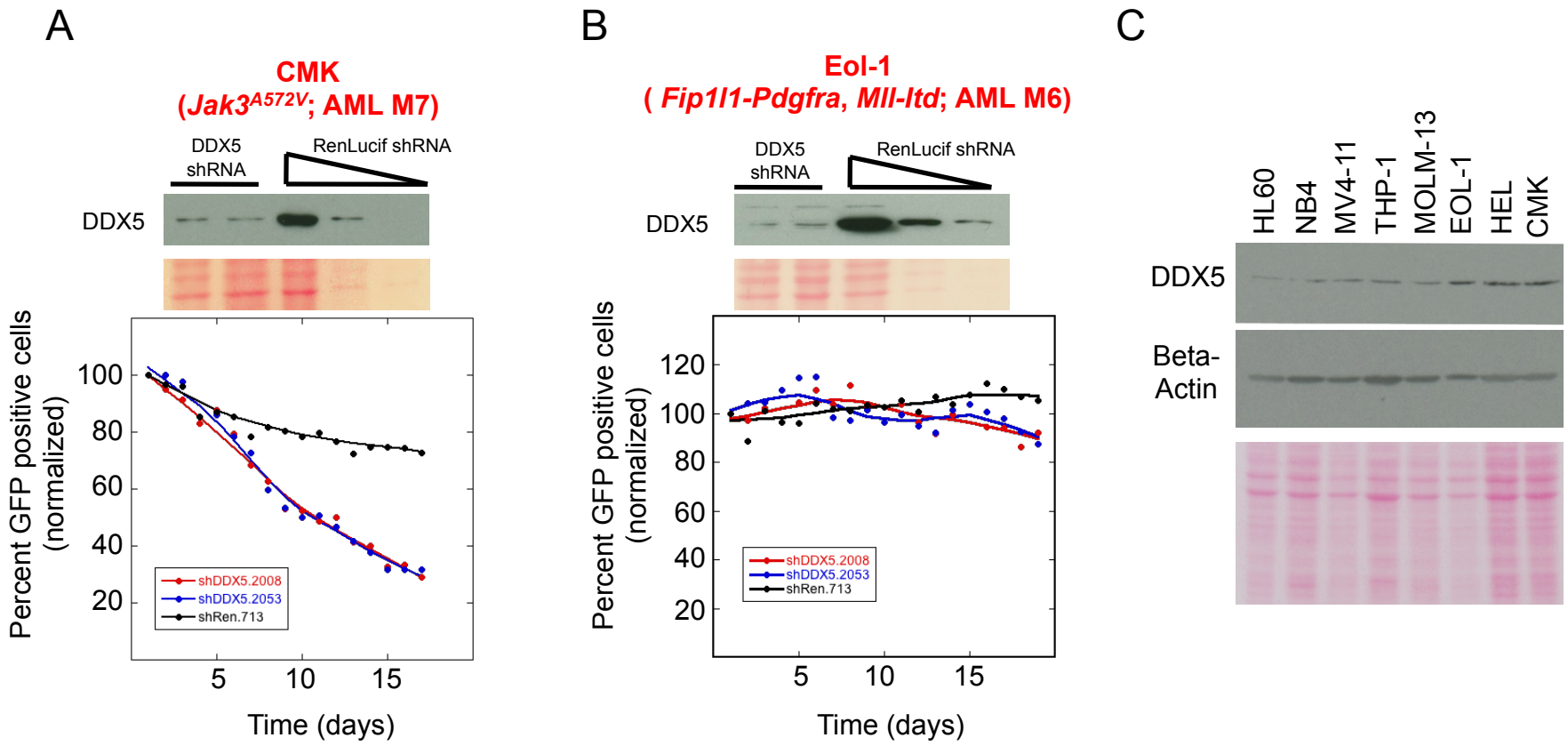


Figure S1, related to Figure 1. Sensitivity of AML cell proliferation to DDX5 inhibition.

(A) and (B) The indicated AML cell lines were infected with retrovirus encoding GFP expression as well as either of two different DDX5 shRNAs (shDDX5.2008 or shDDX5.2053) or a control shRNA targeting Renilla Luciferase (shRen.713). Immuno-blot analysis and cell proliferation assays were performed as described in Figure 1 and Experimental Procedures.

(C) Immuno-blot analysis of DDX5 protein abundance in whole cell extracts (WCEs) prepared from the indicated AML cell lines. WCEs prepared from 50,000 cells for each cell line are loaded in each lane for the immuno-blot analysis.

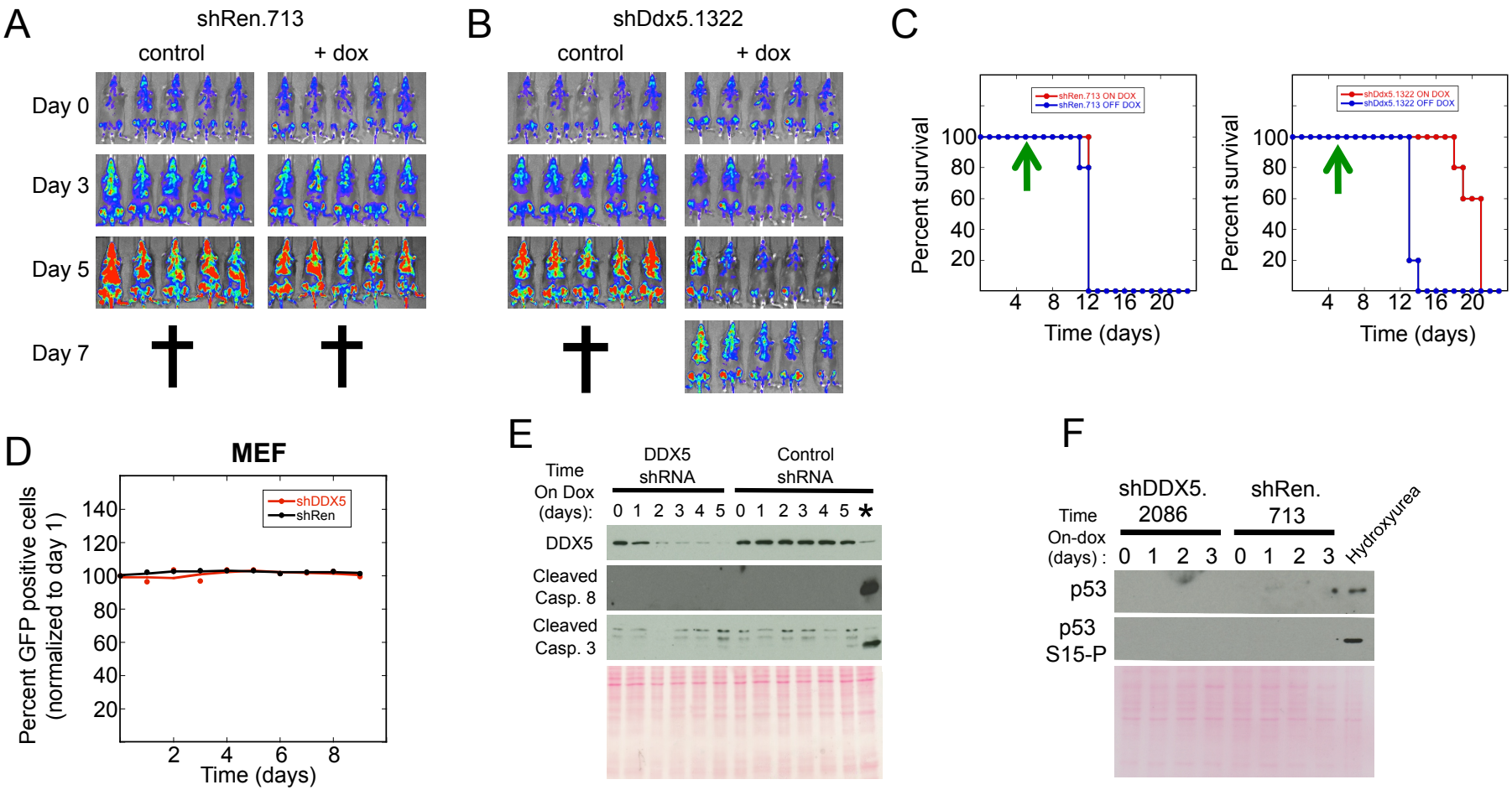


Figure S2, related to Figures 2 and 3. DDX5 is required for AML progression *in vivo* but not MEF proliferation and does not induce p53.

(A) and (B) RN2 AML cells selected for either doxycycline-inducible shDDX5.1322 or shRen.713 shRNA expression were transplanted by tail vein injection from leukemic spleens into secondary recipient mice, given 5 days to establish AML, then shRNAs were induced in the transplanted AML cells with doxycycline as described in the Experimental Procedures. AML progression was monitored over time.

(C) Kaplan-Meier curves showing survival of mice transplanted with AML cells induced (blue line) or not induced (red line) to express either the control shRen.713 (left) or experimental shDDX5.1322 (right) shRNAs. The green arrow indicates the timepoint 5 days post-transplantation when mice in the doxycycline treatment groups were started on doxycycline.

(D) Proliferation of immortalized MEF cells infected with retrovirus encoding GFP expression as well as either shDDX5.2086 (red line) or shRen.713 (black line). Competition cell proliferation assay was performed as described in Figure 1 and Experimental Procedures.

(E) Immuno-blot analysis of WCEs prepared from MEF cells at the indicated timepoints after doxycycline-induced expression of either shDDX5.2086 or shRen.713. The asterisk indicates the lane on the blot that was loaded with WCE from RN2 AML cells induced to express shDDX5.2086 for 3 days to knockdown DDX5. This WCE was used as a positive control for cleaved Caspase 3 and Caspase 8 on the immuno-blot.

(F) Immuno-blot analysis of p53 and p53 phosphorylated on serine 15 in WCEs obtained from RN2 AML cells at the indicated timepoints after inducing expression of either shDDX5.2086 or shRen.713 with doxycycline. WCE from RN2 AML cells treated for 8hrs with 20mM hydroxyurea (HU) was loaded as a positive control.

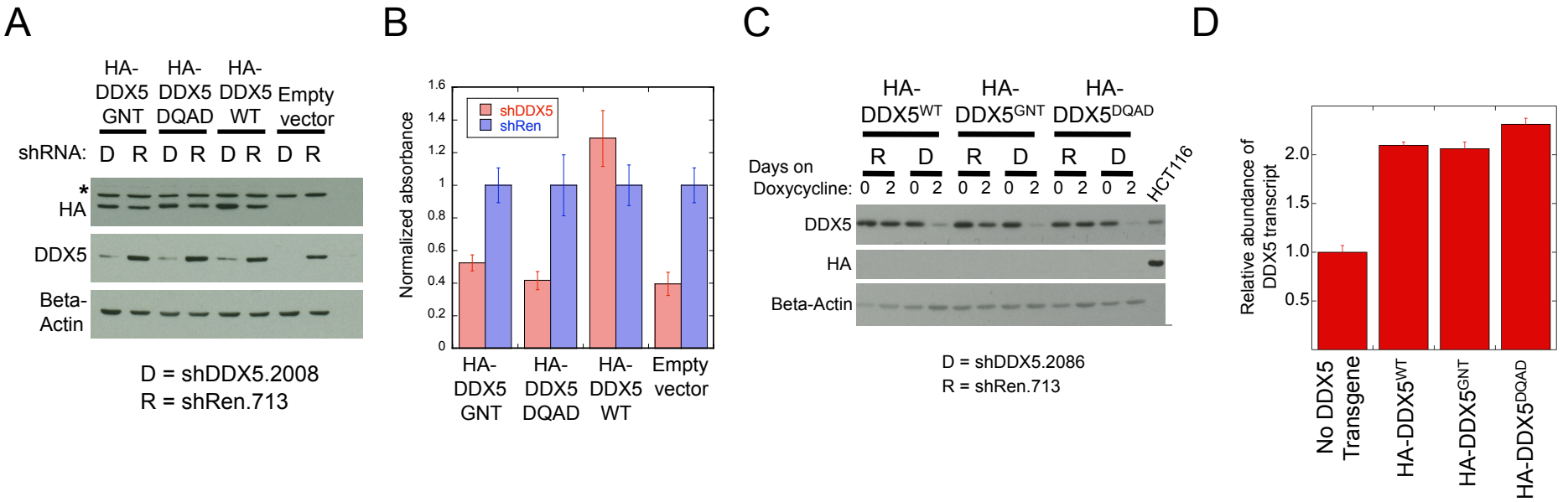


Figure S3, related to Figure 3. DDX5 expression is tightly regulated in AML cells.

(A) Immuno-blot analysis of the indicated proteins in WCEs obtained from HCT116 cells expressing the indicated RNAi resistant *HA-Ddx5* transgenes (wild-type, GNT mutant, or DQAD mutant) with or without endogenous DDX5 knockdown (D = cells transduce with shDDX5.2008 and R = cells transduced with the negative control shRen.713 shRNA). Beta-actin was blotted as a loading control for the immuno-blot. Note the equivalent expression of RNAi resistant DDX5 wild-type or mutant proteins after knockdown of endogenous DDX5 for the WCEs obtained from HCT116 cells expressing the transgenes compared to HCT116 cells infected with empty vector lacking an RNAi resistant *Ddx5* transgene. The asterisk marks the upper band on the HA immuno-blot which is a non-specific cross-hybridizing band detected by this antibody.

(B) Proliferation assay results for HCT116 cells expressing the different RNAi resistant *Ddx5* transgenes with or without endogenous *Ddx5* knockdown. In this assay, following infection of HCT116 cells with virus encoding either of the different shRNAs the HCT116 cells were seeded at low density in tissue culture plates and growth media to maintain selection for transgene and shRNA expression. After allowing 8 days for the cultures to expand they were stained with crystal violet as previously reported (Mazurek et al., 2012). The cultures were then destained and the released stain was quantified using a spectrophotometer. The amount stain released from each culture expressing the indicated RNAi resistant *Ddx5* transgene with DDX5 knockdown was then normalized to the stain released from the culture expressing the same RNAi resistant *Ddx5* transgene but without DDX5 knockdown (shRen.713 control cultures). The mean from triplicate cultures per condition are plotted and the standard deviations are shown. Note that proliferation of HCT116 cells not expressing an RNAi resistant *Ddx5* transgene (Empty vector control) is impaired by endogenous DDX5 knockdown however, expression of RNAi resistant wild-type *Ddx5* transgene rescues HCT116 proliferation. Neither the GNT nor DQAD mutant *Ddx5* transgenes rescue HCT116 proliferation after endogenous DDX5 knockdown.

(C) Immuno-blot analysis of the indicated proteins obtained from RN2 AML cells expressing the indicated RNAi resistant *HA-Ddx5* transgenes. The derivative RN2 cell lines tested were those having doxycycline-inducible expression of either the experimental shDDX5.2086 or control shRen.713 shRNAs. WCEs were prepared from the cultures that were either not induced with doxycycline (0 day) or after 2 days of doxycycline treatment to induce endogenous DDX5 knockdown. A WCE obtained from HCT116 cells stably expressing wild-type HA-DDX5 was loaded as a positive control for the HA immuno-blot.

(D) Q-PCR analysis of *Ddx5* transcript in puromycin resistant RN2 AML cells transduced with the indicated *HA-Ddx5* transgenes using primers that amplify both the endogenous and ectopically expressed *Ddx5* transcripts. Results are normalized to the abundance of endogenous *Ddx5* transcript measured in RN2 cells transduced with an empty vector that did not encode a *Ddx5* transgene. The average from duplicate samples are presented along with standard deviation.

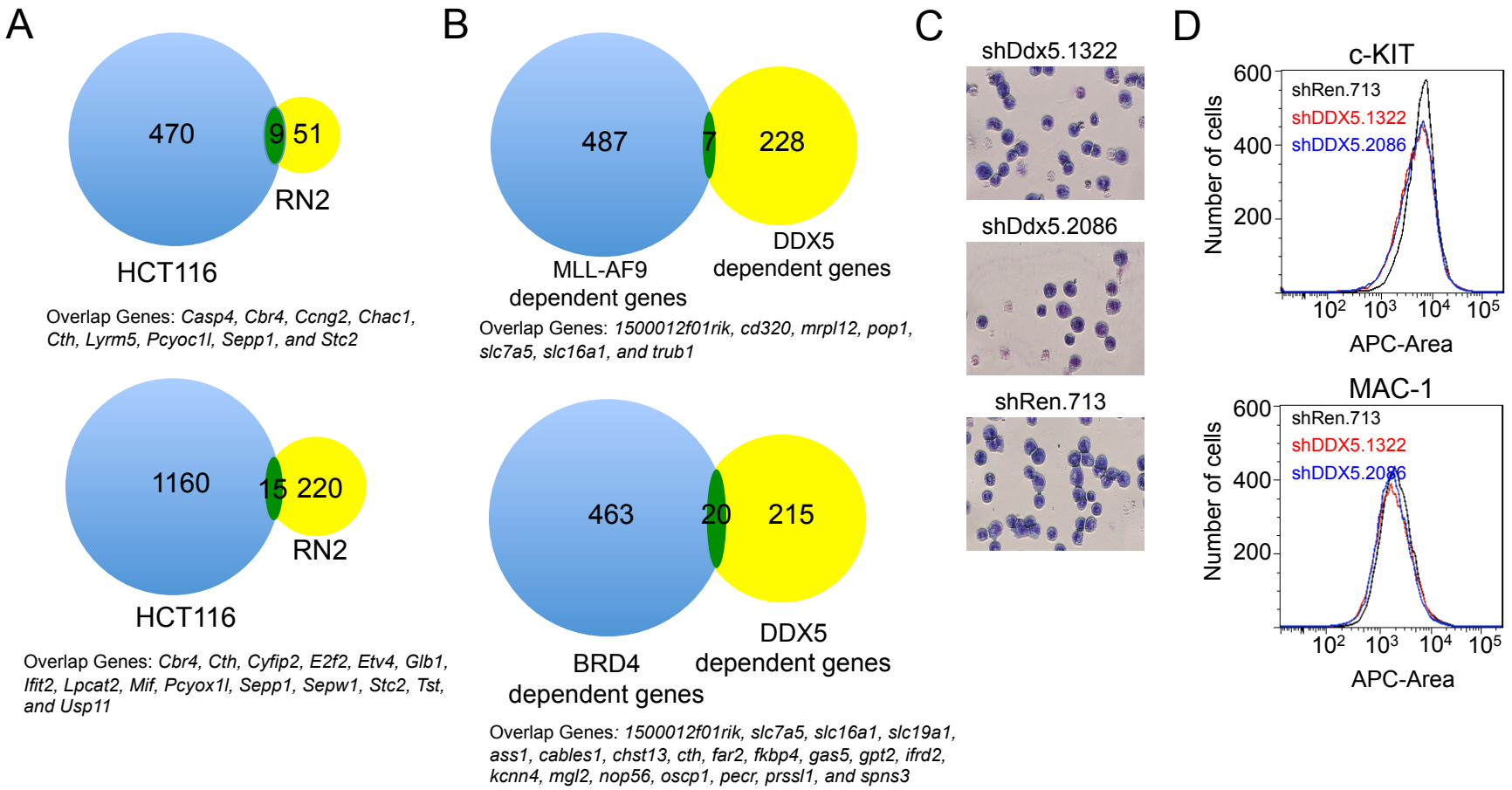


Figure S4, related to Figure 4. DDX5 knockdown affects the expression of different genes in different cancer cell contexts and does not induce differentiation in AML cells.

(A) Venn diagram showing overlap in differentially expressed genes at 24hrs (top) and 48hrs (bottom) after DDX5 knockdown in HCT116 cells compared to RN2 AML cells. Shared differentially expressed genes at this timepoint are shown.

(B) Venn diagram showing overlap in differentially expressed genes at 48hrs after DDX5 knockdown in RN2 AML cells compared to RN2 AML cells in which the expression of MLL-AF9 was turned off (top) (Zuber et al., 2011a) or genes that are downregulated after BRD4 knockdown (bottom) Zuber et al., 2011b).

(C) Images of RN2 AML cells after 4 days of DDX5 knockdown with either of the noted DDX5 shRNAs compared to the control shRen.713 shRNA. Apoptotic bodies are evident in the shDDX5.1322 and shDDX5.2086 cultures but morphology of live cells is very similar to that of the live shRen.713 control cells and does not indicate differentiation.

(D) Flow cyotmetry analysis of c-KIT and MAC-1 surface markers of differentiation on RN2 AML cells after 4 days of DDX5 knockdown with the indicated shRNAs compared to the shRen.713 control shRNA. APC-conjugated antibodies were used to detect these differentiation markers.

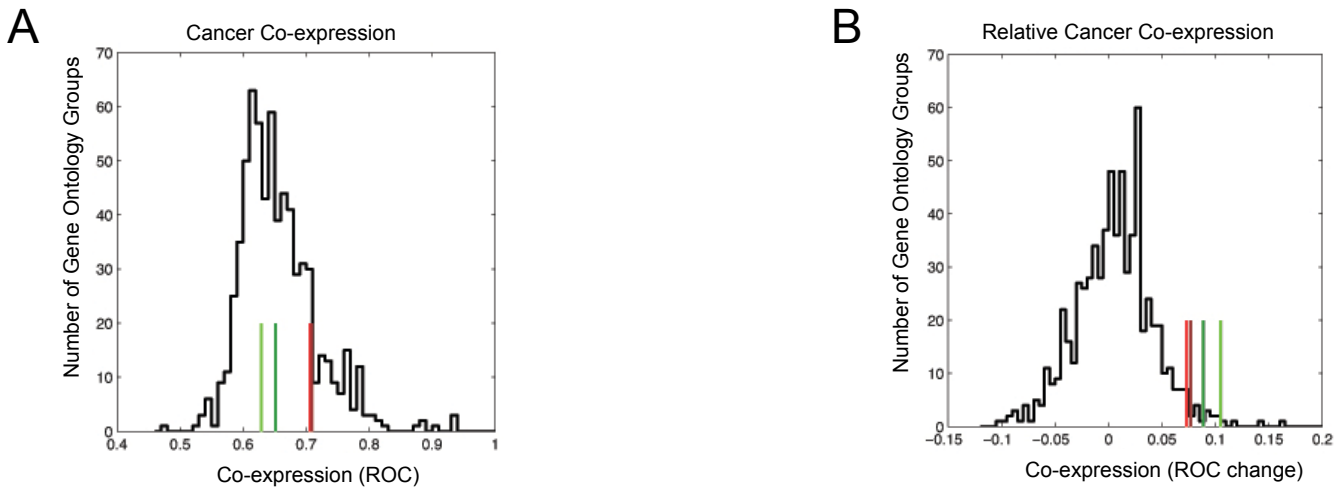


Figure S5, related to Figure 4. Co-expression network analysis reveals that differentially expressed genes resulting from DDX5 knockdown in AML cells preferentially cluster with cancer based co-expression networks compared to normal cell based co-expression networks.

(A) Results of cancer co-expression gene network analysis performed with either gene ontology processes or genes affected by DDX5 knockdown by either of two different shRNA's (shDDX5.1322 or shDDX5.2086) compared to the control shRNA (shRen.713) at either 24hrs or 48hrs after DDX5 knockdown in AML cells. The cancer co-expression network was constructed from meta-analysis of 23 human cancer studies that used a total of 1081 microarrays (see Experimental Procedures). A five-fold cross-validation was applied through voting of neighboring genes was applied to estimate functional network connectivity. Essentially we attempted to determine with the cancer co-expression network we constructed from meta-analysis whether knowing four-fifths of genes annotated to a particular GO process or in the genes affected by DDX5 knockdown ($q<0.05$) would enable accurate prediction of the remaining one-fifth of genes in these gene sets. A receiver operator characteristic curve (ROC) score of 1.0 (x-axis) indicates that providing the cancer co-expression network enabled perfect prediction of the remaining one-fifth of genes and an ROC score of 0.5 indicates that our predictions were no better than random. The dark green vertical line on the plot shows the "learnability" from our cancer co-expression network of the differentially expressed genes by shDDX5.2086 vs. shRen.713 at 24hrs after DDX5 knockdown, the light green line on the plot shows the learnability for shDDX5.1322 vs. shRen.713 differentially expressed genes at 24hrs after DDX5 knockdown, the dark red line shows the learnability for shDDX5.2086 vs. shRen.713 differentially expressed genes at 48hrs after DDX5 knockdown, and the light red line shows the learnability for shDDX5.1322 vs. shRen.713 differentially expressed genes at 48hrs. The black curve shows the results for the learnability of gene ontology processes by the cancer co-expression network. Highly significant results were obtained using our cancer co-expression network particularly when differentially expressed genes observed 48hrs after DDX5 knockdown were tested(ROC ~0.7 for both shDDX5.1322 and shDDX5.2086) indicating that DDX5 knockdown is altering the expression of genes significantly learnable by the cancer co-expression network (FDR ~ 1×10^{-15}).

(B) Results after subtracting ROC scores from the cancer and brain co-expression gene network analysis observed for gene ontology processes and genes affected by DDX5 knockdown by either of the two DDX5 shRNAs vs. control at either 24hrs or 48hrs after knockdown. Color-coding of the DDX5 RNAi vs. control differentially expressed gene sets at 24hrs and 48hrs after DDX5 knockdown as well as the gene ontology processes are the same as in (A). Note a value of 0 on the x-axis indicates that dropout genes for the GO process were predicted equally well from the cancer co-expression gene network as by the brain co-expression gene network. A positive value on the x-axis means that the dropout genes from the GO process or DDX5-dependent gene set were predicted better by the cancer co-expression gene network than by the brain co-expression gene network. Importantly, when the ROC scores obtained from the cancer and brain-based co-expression networks were subtracted this revealed that the differentially expressed genes resulting from DDX5 knockdown at both 24hrs and 48hrs after knockdown were very specifically learnable from the cancer co-expression networks compared to the brain-based co-expression networks (Figure 4C, ROC difference >0.05) indicating that DDX5 is regulating the expression of genes much more specific for cancer than our control brain-based gene networks. Note that gene ontology processes are mostly equally learnable by the cancer co-expression network as by the brain-based co-expression network (most processes have ROC difference < 0.05). Thus the differentially expressed genes resulting from DDX5 knockdown in AML cells preferentially cluster in this analysis with the cancer gene networks than non-cancer gene networks. Genes in the tail-end distribution of the ROC differences (ROC difference > 0.05) are enriched for those that mediate "monosaccharide metabolic process" and "hexose metabolic process".

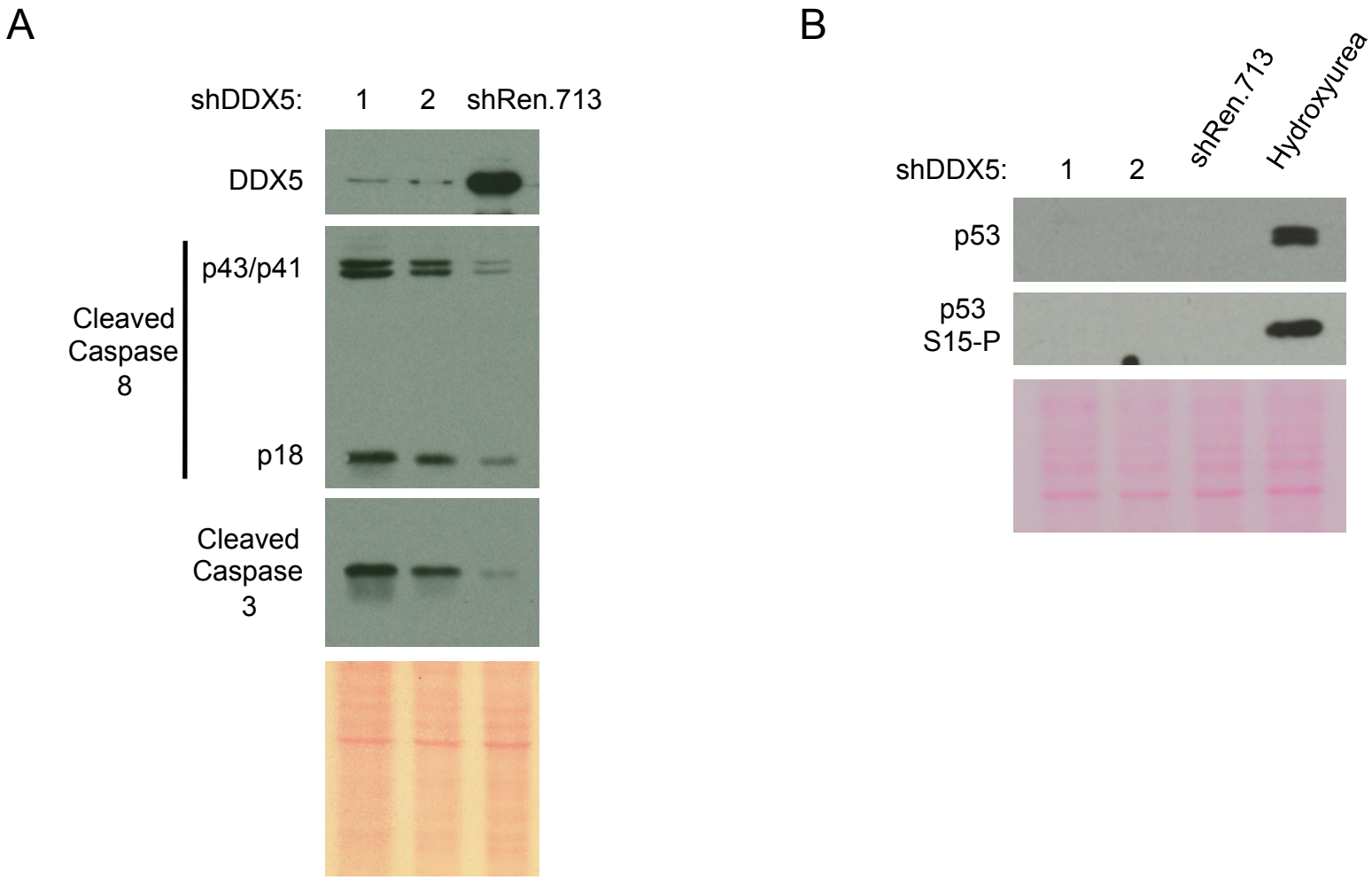


Figure S6, related to Figure 5. DDX5 inhibition induces apoptosis but not p53 stabilization in MV4-11 AML cells.
(A) Western blot analysis of MV4-11 WCEs with DDX5 knockdown by either shDDX5.2008 (lane 1) or shDDX5.2053 (lane 2) compared to MV4-11 cells expressing the control shRen.713 shRNA (lane 3).
(B) Western blot analysis of MV4-11 WCEs from cells expressing the same shRNAs as in (A). Cells were treated with 20mM hydroxyurea (HU) for 8 hours to stabilize p53 and are loaded as a positive control on this western blot.

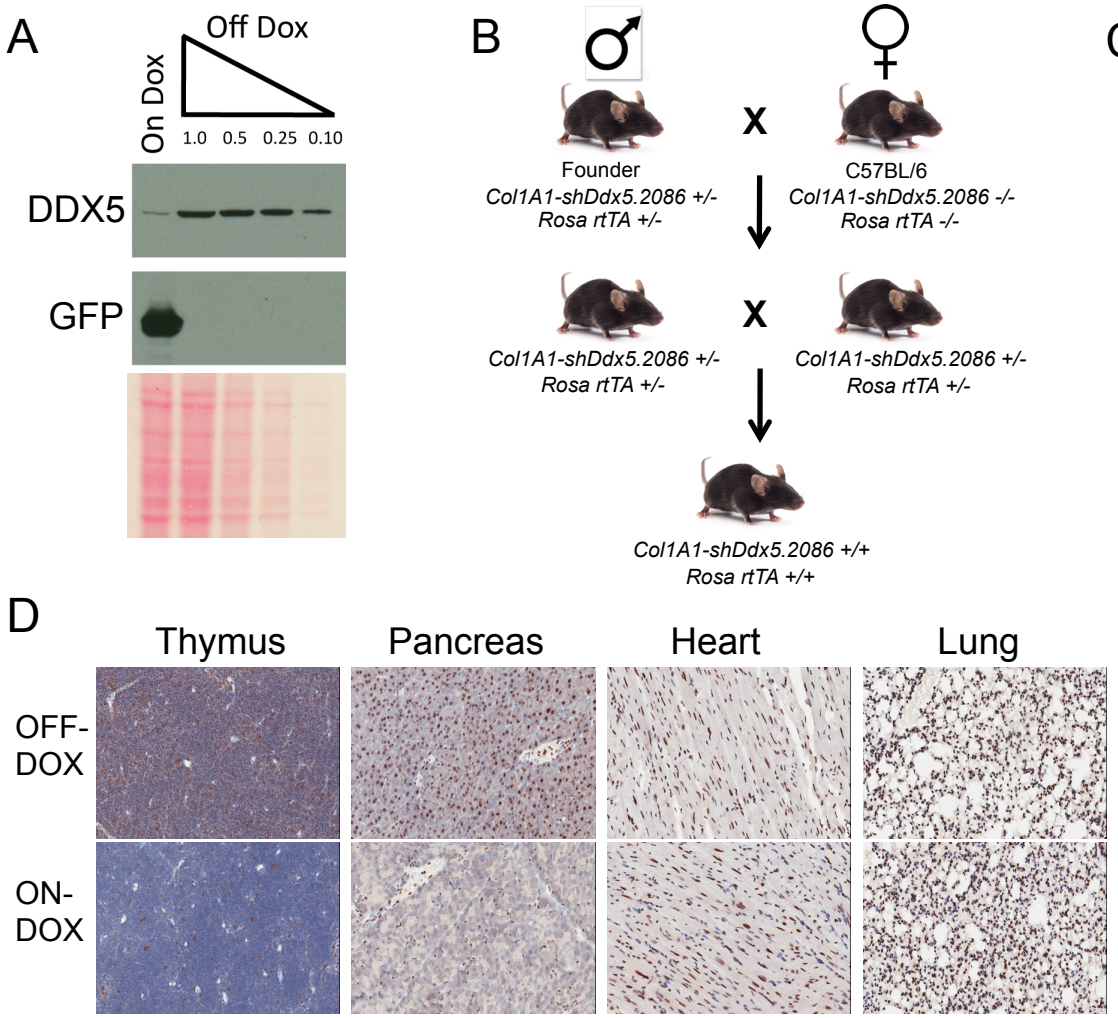


Figure S7, related to Figure 6. Generation of shDDX5.2086 transgenic mice and DDX5 knockdown in tissues from double homozygote shDDX5.2086 transgenic mice given doxycycline for 2 weeks.

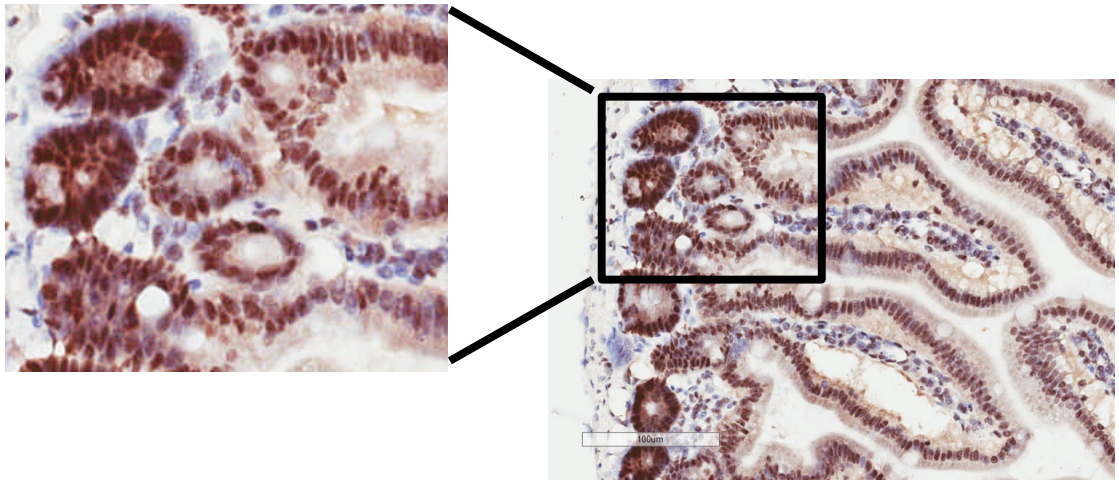
(A) Western blot analysis of the KH2 ES cell clone WCE used to generate shDDX5.2086 transgenic mice. WCE prepared from cells not treated with doxycycline is loaded onto the western blot at either equal total protein loading (lane 2 indicated as "1.0") as the WCE from doxycycline-treated cells (lane 1), or was diluted 1-to-2 (lane 3 indicated as "0.5"), or was diluted 1-to-4 (lane 4 indicate as "0.25"), or was diluted 1-to-10 (lane 5 indicated as "0.10").

(B) Breeding used to acquire double homozygote transgenic mice for experiments.

(C) Western blot analysis of DDX5 in WCEs prepared from the indicated tissues dissected from mice for two different mice given doxycycline (lanes 1 and 2) and 2 different mice not given doxycycline (lanes 3 and 4). WCE prepared from the indicated tissues of the fourth mouse that was not given doxycycline was loaded onto the western blot at either equivalent total protein as WCEs from the other mice (lane 4 indicated as "1.0") or was diluted either 1-to-2 (lane 5 indicated as "0.5), diluted 1-to-4 (lane 6 indicated as "0.25"), or was diluted 1-to-10 (lane 7 indicated as "0.10").

(D) Immunohistochemical analysis of DDX5 protein in the indicated tissues dissected from mice +/- 2 weeks of doxycycline.

A



B

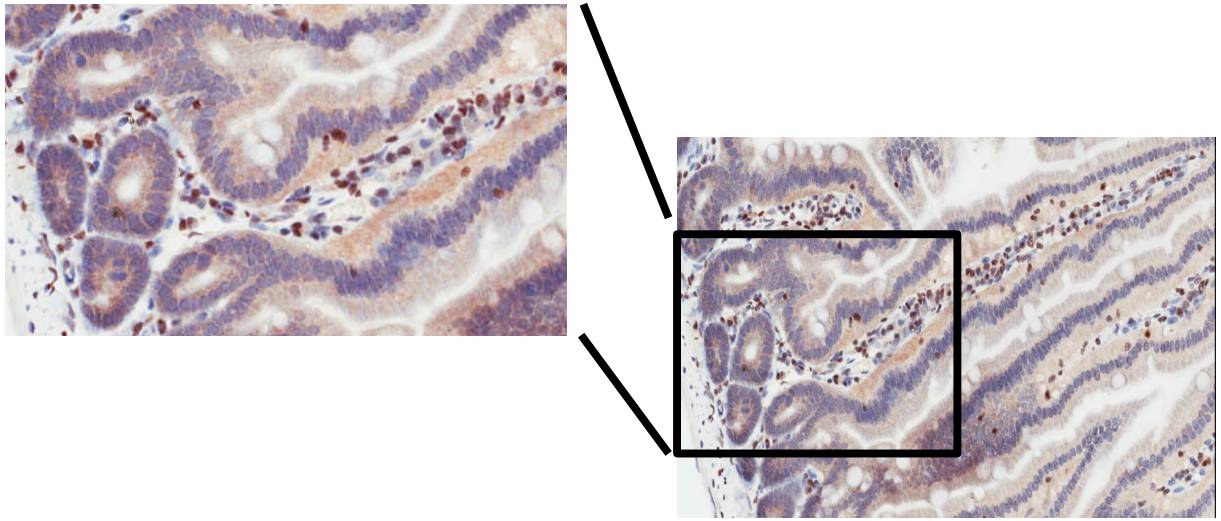


Figure S8, related to Figure 6. DDX5 depletion in intestine.
(A) Immunohistochemical analysis of DDX5 protein in the small intestine of a mouse not given doxycycline.
(B) Immunohistochemical analysis of DDX5 protein in small intestine of a mouse given doxycycline for 2 weeks.

Table S1

Common differentially expressed genes at 24hrs after DDX5 knockdown in RN2 AML cells for comparisons shDDX5.1322 vs. shRen.713 and shDDX5.2086 vs. shRen.713.
FC = Log2 Fold change difference in expression FDR = False discovery rate (q-value) Average RPKM values per transcript from 3 experiments per RNAi condition are shown

gene	locus	RPKM shRen.713	RPKM shDDX5.1322	RPKM shDDX5.2086	FC shRen vs. shDDX5.1322	FC shRen vs. shDDX5.2086	FDR shRen vs. shDDX5.1322	FDR shRen vs. shDDX5.2086
cth	chr3:157557211-157588	7.18488	1.20293	1.03478	-2.57841	-2.79564	0	0
cox6a2	chr7:135317459-135349	4.54659	0.803592	0.92317	-2.50025	-2.30012	0	1.51E-10
stc2	chr11:31259440-312700	7.35743	3.48201	3.37299	-1.07928	-1.12517	8.51E-08	1.43E-06
gpt2	chr8:88016515-880514	8.08409	3.76234	3.8454	-1.10346	-1.07195	4.36E-10	2.32E-07
chac1	chr2:119176977-119180	5.97686	2.7582	3.17104	-1.11566	-0.91443	5.56E-07	0.00100142
asns	chr6:7625170-7643182	91.9083	48.4989	49.4	-0.922245	-0.895685	0.000523334	0.00435257
hist1h2ao	chr13:21924930-219254	175.789	118.356	96.7979	-0.570715	-0.8608	0.000123594	2.36E-08
ass1	chr2:31325789-313761	3.88093	2.33193	2.23112	-0.734878	-0.798632	0.00924957	0.0161866
slc4a7	chr14:15535538-156324	6.17658	3.71297	3.59603	-0.734233	-0.780404	0.000169591	0.000690041
hip1r	chr5:124423636-12445	3.06866	1.73374	1.95017	-0.823716	-0.654004	0.000124349	0.024104
gstcd	chr3:132645514-132754	9.99819	6.41627	6.4289	-0.639931	-0.637094	0.00283554	0.0155306
mri1	chr8:86774474-8678122	12.8271	7.72459	8.34446	-0.731667	-0.620308	0.00021647	0.0173714
cbr4	chr8:63966530-639822	9.49769	6.36497	6.20505	-0.577423	-0.614134	0.030852	0.0656214
gpr171	chr3:58810899-591481	5.47125	2.8475	3.59221	-0.942173	-0.606999	9.67E-06	0.0570594
tars2	chr3:95543896-955589	21.31	14.808	14.6459	-0.525159	-0.541037	0.000833948	0.00682617
rftn1	chr17:50132631-50329	11.8963	7.48387	8.31558	-0.66866	-0.516628	0.000953277	0.0877303
ifit2	chr19:34625183-34651	3.65594	5.54318	5.22272	0.600471	0.51456	0.00519474	0.0996513
olfm4	chr14:80400108-80421	7.86097	11.3457	11.3269	0.529372	0.526971	0.0267704	0.0933873
cd9	chr6:125410283-12544	13.8896	20.4143	20.0818	0.555574	0.531881	0.0109243	0.0639747
lyrm5	chr6:145159653-14516	9.1748	14.1137	13.3643	0.621345	0.542639	0.000523334	0.0188249
upf3b	chrX:34631828-346503	4.10931	7.01029	6.0308	0.77058	0.553456	0.000201918	0.0834936
fam33a	chr11:86922762-86936	16.8884	26.2986	24.8657	0.638955	0.558128	0.0011767	0.0381583
saal1	chr7:53941477-539660	5.20288	7.50487	7.66156	0.528518	0.558329	0.0169899	0.0421354
klhdc4	chr8:124320207-12435	13.7387	19.7227	20.3274	0.521608	0.565177	0.0175648	0.0349449
snapc5	chr9:64027103-64030	12.5263	21.3564	18.6865	0.769707	0.577031	0.000192756	0.0570295
nt5c2	chr19:46961320-47098	14.2284	22.6582	21.3105	0.671266	0.582796	1.87E-07	0.000672954
fkbp2	chr19:7052228-705495	14.4902	22.2764	21.7137	0.620435	0.583524	8.16E-08	4.66E-05
rgs2	chr1:145846467-14585	10.0456	14.6287	15.0584	0.542241	0.584002	0.010889	0.0255814
cdkn2c	chr4:109333480-10933	18.5377	26.8401	27.8155	0.533929	0.585424	0.0174423	0.0271461
fam50a	chrX:71558371-71565	5.6342	8.48563	8.46447	0.59081	0.587208	0.0173717	0.0615455
slc20a1	chr2:129024508-12903	11.5454	17.4045	17.3978	0.592142	0.591581	0.000243486	0.00381379
zfp414	chr17:33766036-33768	8.49844	12.9283	12.8998	0.605257	0.602078	0	0
slfn9	chr11:82793804-82805	4.14003	5.94597	6.32994	0.522269	0.61255	0.0219318	0.0173714
safb2	chr17:56702364-56724	4.46963	6.68965	6.8405	0.581775	0.613947	0.00653945	0.0173714
gm12942	chr4:126803289-12680	5.30561	7.60488	8.16981	0.519409	0.622785	0.0984623	0.0688023
angpt1	chr15:42256270-42508	8.68492	12.6423	13.3795	0.54167	0.62344	0.0143632	0.0155615
hspa2	chr12:77505162-77507	11.9851	18.3599	18.5487	0.615315	0.630074	0.000428159	0.00247542
scly	chr1:93194914-93217	18.6336	27.2435	28.8406	0.548004	0.630192	0.0145168	0.0161544
sepp1	chr15:3220766-32305	4.97766	7.62143	7.70643	0.614593	0.630593	1.18E-06	0.000623342
pigl	chr11:62271961-62327	3.53116	5.14704	5.52032	0.543604	0.644613	0.0697773	0.0507967
aldh3b1	chr19:3913490-39297	30.032	43.1191	47.0793	0.521829	0.648593	0.0311998	0.0190147
sec11c	chr18:65960231-65977	113.722	171.133	179.93	0.589605	0.661927	0.0208647	0.0346571
dpp7	chr2:25207809-25211	10.0508	14.3783	16.2225	0.516586	0.690695	0.028952	0.00426006
mns1	chr9:72286335-72306	3.22067	5.82939	5.20594	0.855984	0.692797	0.000111016	0.0190147
2810030e01	chr2:17965713-17970	3.33965	5.11735	5.41091	0.615697	0.696173	0.00434062	0.00435257
casp4	chr9:5308859-5336791	7.02436	10.8433	11.467	0.626359	0.70705	0.00613153	0.00618559
scarna6	chr1:89652645-89688	55.6509	83.4859	91.1305	0.585128	0.711529	0.0103761	0.00396441
txn2	chr15:77745480-77759	12.1277	18.0031	19.9255	0.569942	0.716312	0.00808045	0.00223699
ccng2	chr5:93696598-93705	9.06306	13.686	14.9287	0.594632	0.720021	0.00362633	0.00183643
xkr5	chr8:18932728-18950	3.14346	4.86498	5.19231	0.630081	0.724024	0	0
herpud2	chr9:24912573-24956	10.248	16.0406	17.1073	0.646385	0.739268	0.00127787	0.0015724

alox5	chr6:116360088-116411	38.1001	58.6348	64.7041	0.62196	0.764061	0.0253344	0.0184701
isoc2b	chr7:4796561-4817781	7.69525	11.3692	13.1206	0.563087	0.769795	0.0330163	0.00311569
cx3cr1	chr9:119957810-119977	4.41481	7.06182	7.80404	0.677688	0.821868	0.000652197	0.000169093
ttll3	chr6:113328106-113364	3.56747	5.76569	6.40824	0.692594	0.84503	5.42E-05	1.87E-06
mgl2	chr11:69943858-699510	4.44466	8.24525	8.07123	0.891491	0.860717	2.98E-05	0.000781713
4930515g01	chr5:115223741-115224	4.78496	7.83299	8.77613	0.711057	0.87508	0.00457189	0.00100142
cyp4f13	chr17:33061632-330843	3.46038	6.11558	6.77466	0.821559	0.969218	0.000414705	9.09E-05
pcyox1l	chr18:61856490-618672	12.8299	28.2866	30.3813	1.14061	1.24367	3.29E-12	5.95E-11
rps16	chr7:29135707-2913771	59.466	135.019	148.677	1.18302	1.32205	6.11E-11	2.59E-11
dynlt1e	chr17:6851323-686065	4.46855	11.3147	12.5983	1.34032	1.49535	2.26E-09	1.28E-10

Table S2

Common differentially expressed genes at 48hrs after DDX5 knockdown in RN2 cells for comparisons shDDX5.1322 vs. shRen.713 and shDDX5.2086 vs. shRen.713.

Table S3

Common GO biological processes enriched in genes differentially expressed between shDDX5.1322 vs. shRen.713 and shDDX5.2086 vs. shRen.713 samples at 48hrs of DDX5 knockdown. All GO biological processes are enriched with FDR < 0.05.

Biological Process:	max FDR
monosaccharide metabolic process	0.008
hexose metabolic process	0.008
hexose catabolic process	0.008
single-organism carbohydrate catabolic process	0.008
monosaccharide catabolic process	0.008
carbohydrate catabolic process	0.009
transferase activity, transferring nitrogenous groups	0.011
glucose metabolic process	0.01
transaminase activity	0.031
glycolysis	0.035
neuromuscular junction development	0.035
translational initiation	0.036
carbohydrate kinase activity	0.042
glucose catabolic process	0.042
aminoacyl-tRNA ligase activity	0.044
ligase activity, forming carbon-oxygen bonds	0.044
ligase activity, forming aminoacyl-tRNA and related	0.044
alpha-amino acid metabolic process	0.049
eye development	0.05

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell lines, plasmids, antibodies, and shRNAs:

All human and mouse AML cell lines as well as immortalized MEF cells were obtained as described (Zuber et al., 2011b). The pLMN and pTRIN (pTRMPV-Neo) plasmids used to prepare retrovirus for transduction of either constitutive or doxycycline-inducible shRNA expression into human and mouse cell lines were generated as described (Zuber et al., 2011b). pMSCVneomycin^R-BCL2 was provided by Dr. Christopher Vakoc and the BCL2 transgene was cloned into the pMSCVpuromycin^R plasmid (Clontech) for experiments. The plasmid encoding HA-DDX5 was generously provided by Ralf Janknecht (Shin et al., 2007) and the pLPC plasmid was generously provided by Scott Lowe. HA-DDX5 was subcloned into the pLPC plasmid using PCR. Overlapping PCR was applied to introduce base substitutions into the HA-DDX5 transgene for the K144N (GNT) and E249Q (DQAD) amino acid substitutions. Antibodies used for immuno-blot analysis included: anti-DDX5 (Bethyl, A300-523A), anti-Beta Actin (Sigma, A5316), anti-Cleaved Caspase 8 – Mouse Specific (Cell Signaling, #9429), anti-Cleaved Caspase 8 – Human Specific (Cell Signaling, #9496), anti-Cleaved Caspase 3 (Cell Signaling, #9661), anti-BCL2 – Mouse Specific (BioLegend, 633501), anti-BCL2 – Human Specific (Cell Signaling, #4223), anti-MCL1 (Cell Signaling, #5453), anti-BCL-XL (Cell Signaling, 2764), anti-p53 clone DO-1 – Mouse Specific (Santa Cruz, sc-126), anti-p53 clone IMX25 (Vector labs, VP-P952), anti-p53 Phospho-Serine 15 specific (Cell Signaling, #9284), anti-p21 (Santa Cruz, sc-397), and anti-GFP (Sigma, G1544). Antibodies used for immunohistochemical analysis included: anti-DDX5 (Bethyl, A300-523A), anti-p21 (Abcam, ab2961), and anti-Cleaved Caspase 3 (Cell Signaling, #9661).

Antibodies used for flow cytometry analysis included: APC/Cy7 conjugated anti-CD45 (BioLegend, 103116), PE/Cy5 conjugated anti-CD11b (BioLegend, 101210), PE conjugated anti-Ly-6G/Ly-6C (BioLegend, 108408), PE/Cy5 conjugated anti-CD45R/B220 (BioLegend, 103210), PE conjugated anti-IgM (Pharmingen, 553409), PE/Cy5 conjugated anti-CD4 (BioLegend, 100409), PE conjugated anti-CD8a (BioLegend, 100708), and Pacific Blue conjugated mouse anti-CD90.2 (BioLegend, 105324). The following sequences encoding the indicated shRNAs were cloned into the XhoI/EcoRI sites of either pLMN or pTRIN for knockdown studies (antisense sequences directing knockdown are underlined):

shDDX5.1322:CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCTGGGTTCTAAATGAATTCAAATAGTGAAGCCACAGATGTATTTGAATTCATTTAGAACCCAATGCCTACTGCCTCGGAATTC

shDDX5.2008:CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCCCAATAAGACTTTAGAAGTATAGTGAAGCCACAGATGTATACTTCTAAAGTCTTATTGGGATGCCTACTG CCTCGGAATTC

shDDX5.2053:CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCGCTCTTTATATTGTGTGTTATTAGTGAAGCCACAGATGTAATAACACACAATATAAAGAGCATGCCTACTG CCTCGGAATTC

shDDX5.2086:CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCAGGAATTATAATGCTTATCTATAGTGAAGCCACAGATGTATAGATAAGCATTATAATTCCTATGCCTACTGCCTCGGAATTC

shRen.713:CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCAGGAATTATAA TGCTTATCTATAGTGAAGCCACAGATGTATAGATAAGCATTATAATTCCTATG CCTACTGCCTCGGAATTC

Preparation of cell lines with either constitutive or inducible shRNA expression:

Cell lines with either constitutive or inducible shRNA expression were prepared using retroviral infection. For constitutive shRNA expression either Phoenix-Ecotrophic (infection of mouse cell lines) or Phoenix-Amphotrophic (infection of human cell lines) retroviral packaging cells were plated 24hrs prior to transfection into 6-well tissue culture plates at 1.4 million cells per well in 2mL DMEM + 10% fetal bovine serum (FBS). Each packaging cell culture was then transfected with either 3µg Ecotrophic or 1.4µg Amphotrophic helper plasmid + 3µg (Phoenix-Ecotrophic transfection) or 2µg (Phoenix-Amphotrophic transfection) of either pLMN-shRNA vector (constitutive shRNA expression) or pTRIN-shRNA (doxycycline-inducible shRNA expression) using 20µL (Phoenix-Ecotrophic transfection) or 10µL (Phoenix-Amphotrophic transfection) Lipofectamine 2000 (Invitrogen catalog # 11668-027) and the manufacturer's protocol. For Phoenix-Amphotrophic cell transfections 0.6µg pVSVG plasmid was also included in the transfection with the pHelper-Amphotrophic and shRNA expression plasmid for a total of 4µg of transfected plasmids. A final volume of 0.5mL transfection mix containing the plasmids and lipofectamine was added to each packaging cell culture containing cells in 2mL DMEM + 10% FBS. Roughly 24hrs after transfection the transfection media was aspirated from each packaging cell culture and discarded. 2mL of RPMI + 10% FBS was then added to each packaging culture. At 36hrs and 48hrs post-transfection the media

containing virus was collected from each packaging culture and filtered through a 0.45μm filter (Corning catalog #431220) and supplemented with 8μg/mL Hexadimethrine Bromide (Sigma catalog # H9268). 2mL RPMI + 10% FBS was returned onto each packaging culture. Human or mouse AML cells were pelleted from log phase 3.5cm cultures by centrifugation for 5 minutes at 1,700rpm in a Beckman Coulter Allegra 6R centrifuge. Supernatants containing growth media were aspirate from the cell pellets and each cell pellet was suspended in 4mL viral supernatant. Virus in each supernatant encodes either constitutive or inducible expression of one shRNA. Infecting cells were then centrifuged for 30 minutes at 1,700rpm in the Beckman Coulter Allegra 6R centrifuge at room temperature. Following centrifugation the pelleted cells were resuspended in the viral supernatant and then seeded into new wells of 6-well tissue plates and returned to the tissue culture incubator. 24hrs after the second round infection with 48hr post-transfection viral supernatant the cells in the infected cultures were pelleted by centrifugation at 1,700rpm for 5 minutes and the viral supernatants were aspirated and discarded. Each cell pellet was suspended in 4mL RPMI + 10% FBS and seeded into new 6-well tissue culture plates. For selection of infected cells the cultures were allowed another 24hrs to recover from infection then split 1-to-2 into new 6-well tissue culture plates in 4mL RPMI + 10% FBS + 1mg/mL G418 (Gibco catalog #11811-031). For cells infected with MSCVpuromycin^{R}-BCL2 virus for BCL2 overexpression the cells were selected with 2μg/mL puromycin (Sigma catalog #P7255-25MG).

Cell Proliferation Assay (For Figure S5B):

In Figure S5B, cell proliferation was measured using crystal violet staining as previously described (Mazurek et al., 2012). Briefly, HCT116 cells stably expressing either HA-DDX5^{WT}, HA-DDX5^{GNT}, or HA-DDX5^{DQAD} were infected with retrovirus encoding either shDDX5.2008 or shRen.713. Following infection and selection for cells expressing the shRNAs the cells were seeded at 10,000 cells per well into 12-well tissue culture plates. After allowing 8 days for the cultures to expand the cells were stained with crystal violet. Each wells was then destained with 10% glacial acetic acid and the absorbance of the released stain was quantitated at OD600 on an Eppendorf BioPhotometer.

In vivo mouse experiments:

All mouse experiments were approved by the Cold Spring Harbor Laboratory animal care and use committee. For the in vivo AML experiments these were performed as described (Zuber et al., 2011b). The shRNAs targeting the DDX5 transcript were cloned into the 3' untranslated region of a *dsRed* transgene as described (Zuber et al., 2011a). This enabled dsRed fluorescence to be analyzed as a surrogate marker of shRNA expression following treatment of the cells with doxycycline. In general, treating the RN2 cell line derivatives transduced with the different shRNAs with doxycycline resulted in activation of dsRed expression in 60-to-80% of cells in the cultures. This indicated a sizeable fraction of cells in these cultures in which shRNA expression was not induced by doxycycline. Doxycycline thus would not activate DDX5 knockdown in these dsRed negative AML cells and this would yield a false negative result for in vivo experiment. Therefore, RN2 clones with relatively uniform doxycycline induced dsRed expression ($x \geq 99\%$ of the cells) were selected for each derivative RN2 cell line (shDDX5.1322, shDDX5.2086, and

shRen.713). After confirming doxycycline induced DDX5 knockdown in these clones they were transplanted into the tail veins of sub-lethally irradiated primary recipient mice. To remove artifacts of tissue culture the expression of the DDX5 or Renilla luciferase shRNAs were not induced in these mice, but instead a frank leukemia was allowed to develop to terminal stage after which the leukemic ladened spleens were collected from these primary transplant recipients. In addition to doxycycline inducible expression of dsRed-shRNA, RN2 cells also have constitutive expression of GFP driven by the PGK promoter. Therefore in the absence of doxycycline, the density of RN2 cells in cell suspensions prepared from the leukemic ladened spleens was measured using flow cytometry analysis of GFP positive cells. Spleen suspensions containing a total of 1,000,000 RN2 cells that encoded either of the DDX5 shRNAs or the shRen.713 control shRNA were then transplanted by tail vein injection into sub-lethally irradiated secondary recipient mice. RN2 cells constitutively express firefly luciferase enabling bioluminescence imaging of leukemia onset and progression in mice following transplantation. Doxycycline was provided to mice as described (Zuber et al., 2011b) on day 5 following tail-vein transplantation of primary leukemia cells into the mice since leukemia was clearly detected in the mice at this timepoint. This timepoint 5 days post-transplantation when the mice were started on doxycycline-containing food and water is indicated as day 0 in Figure 2B and Figure S2A and S2B.

Transgenic DDX5 shRNA mice were developed as described in Dow et al. (Dow et al., 2012) that included targeting vector preparation, targeting vector transfection into KH2 ES cells, ES cell clone selection, and tetraploid embryo complementation. For targeting

vector preparation the shDDX5.2086 shRNA was cloned using XhoI/EcoRI restriction sites into the cTGM plasmid. Breeding of founder mice was performed as described in Figure S10B to generate *Rosa-rtTA +/+ ; Col1A1-shDDX5.2086 +/+* mice for experiments. Doxycycline was provided to induce shRNA expression in the mice through drinking water containing 2mg/mL doxycycline (Sigma, D-9891) with 2% Sucrose and in food (625mg/kg, Harlan Laboratories). Mice were given doxycycline-containing food and water for 2-4 weeks to induce DDX5 knockdown then doxycycline food with doxycycline-free water thereafter to maintain DDX5 knockdown.

ROS measurements in RN2 cells:

200,000 RN2 cells were seeded per well of 6-well tissue culture plates on day 1 of the experiment in 4mL RPMI + 10% FBS + 1mg/mL G418 + 1µg/mL doxycycline per culture. Following 3 days of doxycycline induced shRNA expression cell number in each culture was determined using hemacytometer. 2 x 10E6 cells per suspension were transferred to new wells of 6-well tissue culture plates each in 2mL RPMI + 10% FBS to which 4µL of CellROX Deep Red Reagent (LifeTechnologies catalog #C10422) was added. The cultures were returned to the tissue culture incubator maintained at 37 degrees C and 5% CO_2 for 30 minutes. After 30 minutes the cells were processed for flow cytometry analysis using the manufacturer's protocol. Analysis was performed on the LSRII flow cytometer and viable cells in each suspension were gated for analysis of CellROX fluorescence.

RNA-Seq Experiment:

DDX5 was knocked down in RN2 AML cells by doxycycline-induced expression of either shDDX5.1322 or shDDX5.2086. For control, expression of the shRen.713 shRNA was similarly induced in RN2 AML cells. At 24hrs and 48hrs RNA was isolated using the Qiagen RNeasy MiniKit (catalog # 74104) that included on-column DNase digestion as per the manufacturer's protocol. Each RNA sample for RNA-Seq was prepared from triplicate cell cultures expressing each shRNA (triplicate biological replicates x 3 different shRNAs x 2 time-points resulted in 18 samples different RNA samples total). Barcoded cDNA libraries were prepared from 0.7µg of each RNA sample using the Illumina TruSeq Stranded Total RNA kit (RS-122-2201) as per manufacturer's instructions. The quality of resulting barcoded cDNA libraries were checked on an Agilent Bioanalyzer 2100 and confirmed to migrate at the expected average size of 310 bases. The 18 barcoded cDNA libraries were pooled into 3 pools of 6 cDNA libraries each and then each pool was loaded onto 2 lanes of an Illumina HiSeq 2000 sequencer on which paired-end 101 sequencing runs were performed. Sequence reads from each lane were obtained and the first and last 25 bases of each read were trimmed. The trimmed reads were mapped to the mouse genome using TopHat and the Mmusculus.UCSC.mm9 reference genome. CuffDiff was used to compare the RPKM values per transcript in each DDX5 shRNA sample at each time-point of knockdown to the RPKM values per transcript in the shRen.713 control RNAi samples at the respective time-point. Differentially expressed genes called in each DDX5 shRNA triplicate compared to the shRen.713 control triplicate at each time-point were those that were expressed with a RPKM value of 3 or greater and differed between the experimental triplicates and control triplicates at each time-point with an FDR ≤ 0.1. The data discussed in this publication

have ben deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE53599 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53599)

Co-expression network analysis:

Coexpression networks were constructed through meta-analysis of 23 human cancer studies that used a total of 1081 microarrays as previously described (Gillis and Pavlidis, 2011). While statistical analysis can serve to determine the null expectation, we wanted a more targeted control dataset and so a brain-based co-expression network was also constructed from meta-analysis of 20 studies that used a total of 1851 microarrays (aggregated from the Gemma database) (Zoubarev et al., 2012). Briefly, we included only large expression studies (minimally 20 samples each) conducted on the GPL570 array. For each expression experiment, the absolute value of the correlation between gene pairs across samples was calculated. The correlations were then standardized to a uniform distribution between 0 and 1 and summed across experiments. This largely non-parametric approach decreases the potential role of any confound related to single experiments (such as batch effects), since each experiment has a minimal individual impact on the final coexpression data. The networks constructed are thus fully connected between all genes with varying weights reflecting the degree to which significant correlation was seen between the given pair of genes across many experiments. Each experiment was subject to a variety of quality control measures and these, as well as the underlying experimental data can be accessed at:

http://www.chibi.ubc.ca/Gemma/expresionExperiment/showExpressionExperiment.html?id=###

where ### is replaced by an experimental identifier.

For the cancer data, the experimental identifiers (###) were:

263 487 533 536 549 569 642 647 662 932 981 1007 1041 1063 1110 1150 1160 1180 1216 1237 1243 1382 1394

For the brain data, the experimental identifiers were:

4 263 271 275 549 738 987 1381 1881 1882 738 1885 1779 1902 842 843 701 312 946

A five-fold cross-validation was applied through voting of neighboring genes to estimate functional network connectivity(Gillis and Pavlidis, 2011). Essentially we attempted to determine whether knowing four-fifths of genes annotated to a particular GO process or in the genes affected by DDX5 knockdown ($q<0.05$) would enable accurate prediction of the remaining one-fifth of genes in these gene sets. A receiver operator characteristic curve (ROC) score of 1.0 indicates that providing the cancer co-expression or brain-based co-expression networks enabled perfect prediction of the remaining one-fifth of genes and an ROC score of 0.5 indicates that our predictions were no better than random.

Q-PCR analysis of DDX5

RNA from RN2 AML cells transduced with transgenes encoding either HA-DDX5^{WT}, HA-DDX5^{GNT}, or HA-DDX5^{DQAD} was isolated from cells using the Qiagen RNeasy Mini

Kit (catalog # 74104) that included the recommended on-column DNase digestion step. cDNA was prepared from 1µg of each RNA sample using TaqMan Reverse Transcription Reagents (Applied Biosystems catalog # N808-0234) with random hexamer priming and the manufacturer's protocol. Q-PCR analysis of endogenous and ectopically expressed DDX5 was performed on a Roche LightCycler 480 instrument using the LightCycler 480 SYBR Green I Master Mix (Roche catalog # 04887352001) and the following primers:

DDX5-1686F: tccaacagggacttaccaga

DDX5-1776R: tgcctgttggttcataccat

Beta-Actin-Ex8F: ccttccttcttgggtatgga

Beta-Actin-Ex8R: acggatgtcaacgtcacact

For analysis of Q-PCR results, DDX5 transcript signal was normalized to the Beta-Actin transcript signal for each sample to account for variability in sample input.

Whole cell extract preparation from mouse organs:

Whole cell extract was prepared from intestinal epithelial cells as described (McJunkin et al., 2011). Whole cell extracts were prepared by collecting bone marrow aspirates from mouse femur into PBS, washing cell pellets 5 minutes in ACK red blood cell lysis buffer (150mM NH_4Cl, 10mM $KHCO_3$, 0.1mM EDTA), then lysing cells in 2x SDS loading buffer (125mM Tris pH 6.8, 20% glycerol, 2% SDS, 2% Beta-mercaptoethanol, 0.1% Bromophenol blue). For other organs, the after dissection the organs were minced using a scalpel then homogenized in a modified RIPA buffer (Phosphate buffered saline solution

containing 1% Triton X-100, 0.1% SDS, 150mM NaCl, 0.5% Sodium deoxycholate, 1mM EDTA, and Complete EDTA-free Protease Inhibitor cocktail – Roche catalog # 11873580001) using a dounce homogenizer and B-pestle. The homogenates were then centrifuged at maximum speed for 10 minutes at 4 degrees C in an Eppendorf Microcentrifuge (Centrifuge 5417C). Supernatants were then diluted wit 2x SDS loading buffer for gel analysis.

Immunohistochemical Analysis

The indicated organs were dissected from mice and fixed overnight in 4% paraformaldehyde. Organs were then washed in PBS. Samples were then processed in a Shandon Excelsior Tissue Processor and embedded in paraffin. Six micron sections were then cut and mounted onto VWR Superfrost Plus slides. The slides were then heated for 10 minutes at 60 degrees C to melt the paraffin. Deparaffinization was performed with two 10 minute washes with Xylene solution followed by two 5 minute incubations in 100% ethanol, two 2 minute incubations in 95% ethanol, and two 2 minute incubations in 75% ethanol to rehydrate the tissue sections. After washing 2x1min in distilled water slides were incubated for 10 minute in 3% H_2O_2/H_2O to block endogenous peroxidase. The slides were washed three times for 1 minute each in distilled water. Antigen retrieval was performed in Citrate buffer pH6.0 (Vector, H-3300) that included a 15 minute heated incubation in a Cuisinart Electric Pressure Cooker and 20 minute room temperature incubation. The slides were then rinsed in distilled water and washed two times for 5 minutes each in TBS. The slides were blocked in TBS + 1% BSA + 5% NHS for 1hr at room temperature followed by incubation overnight with primary antibody at 4 degrees

C. The next day the slides were rinsed three times in TBT for 10 minutes each. Slides were incubated with anti-rabbit (Vector, MP-7401) or anti-mouse (Vector, MP-7402) secondary antibodies for 30 minutes at room temperature followed by another three TBS rinses for 10 minutes each. The slides were incubated with peroxidase substrate (Vector, ImmPACT DAB, SK-4105) for 3 minutes then rinsed in distilled water and counterstained with Hematoxylin. Finally the slides were rinsed in distilled water, dried, and protected with a coverslip using Surgipath mounting medium for analysis. Histochemical analysis was performed in the Cold Spring Harbor Laboratory histochemical shared resource laboratory.

References for Supplemental Experimental Procedures

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