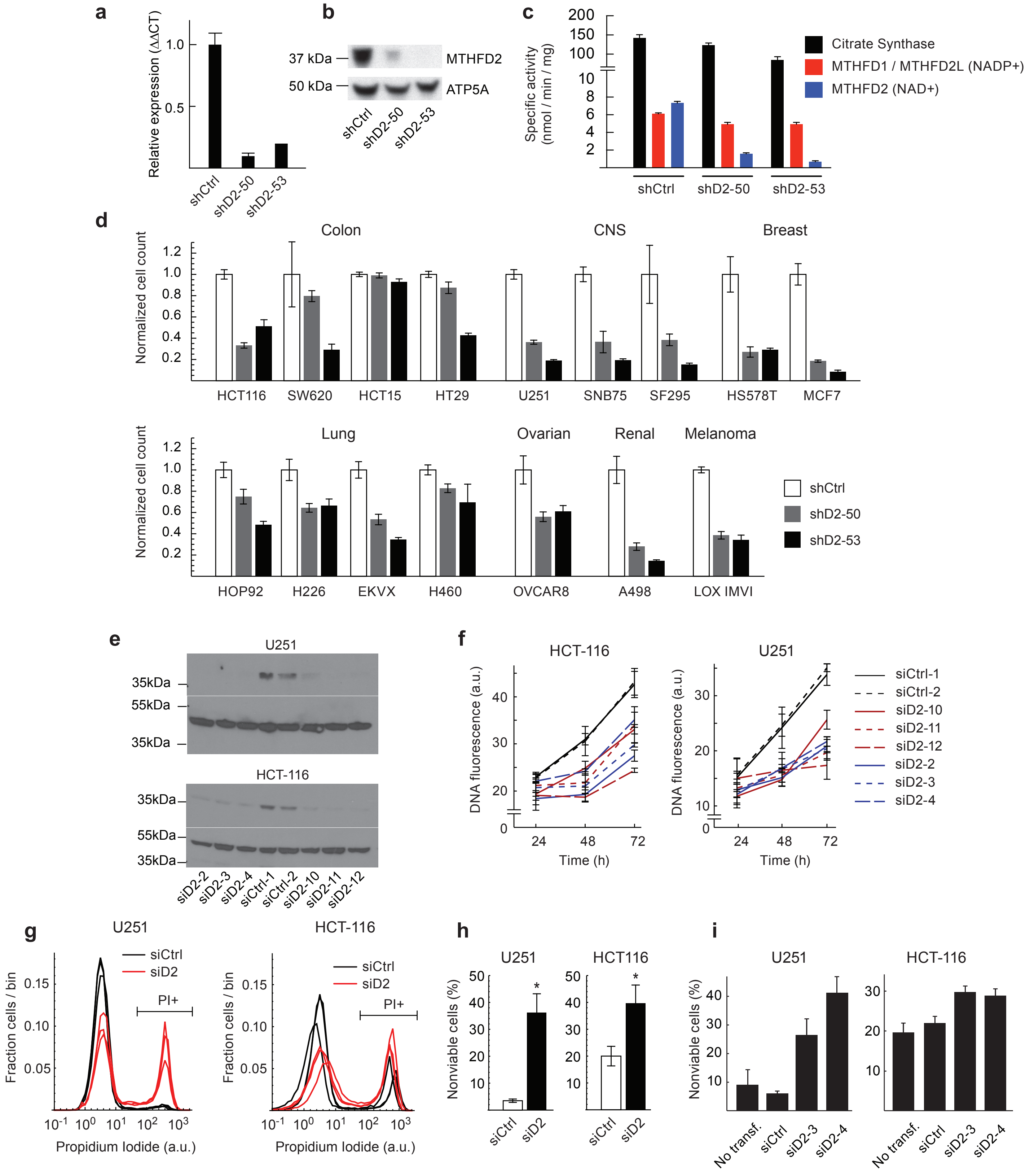
Supplementary Figure 1



RNA interference targeting MTHFD2 causes cancer cell death. **(a)** RT-PCR of MTHFD2 mRNA following shRNA against MTHFD2 (shD2-50 and shD2-53 hairpins) or control shRNA (shCtrl) at day 7 post-transfection, expressed as ΔΔCt values relative to HPRT mRNA (control) and shCtrl values. Error bars denote standard deviation (n = 3). **(b)** Western blot of MTHFD2 and ATP5A (loading control) in knockdown cells, as in (a). **(c)** Enzymatic assay of methylene-THF dehydrogenase activity in knockdown cells, as in (a). Red bars, NADP+ dependent dehydrogenase (MTHFD1/MTHFD2L); blue bars, NAD+ dependent dehydrogenase (MTHFD2); black bars, citrate synthase assay (mitochondrial control). Error bars denote standard deviation (n = 3). **(d)** Cell proliferation in 16 cell knockdown cell lines as in (a), expressed as cell counts at day 7 post-transfection, normalized to shCtrl values. Error bars denote standard deviation (n = 8). **(e)** Western blot of MTHFD2 and ACTB (control) at 48h after transient transfection with siRNA oligos targeting MTHFD2 (siD2-10,11,12,2,3,4) or control oligos (siCtrl-1, siCtrl-2). **(f)** Cell proliferation (DNA content) following transient transfection, as in (e). Error bars denote standard deviation (n = 3). **(g)** Propidium iodide (PI) cell viability assay using flow cytometry at 48h post siRNA transfection, normalized to area-under-curve = 1. PI+ (nonviable) cells were defined as PI > 50, indicated by gate. siD2, pool of oligos siD2-10,11,12. **(h)** Quantification of data in (g), error bars denote standard deviation (n = 3). * $P < 0.02$, two-tailed Student's t-test with Welch's correction for unequal variances. **(i)** PI viability assay as in (g,h) for individual siRNA oligos, control siRNAs, and no transfection. Error bars denote standard deviation (n = 3).

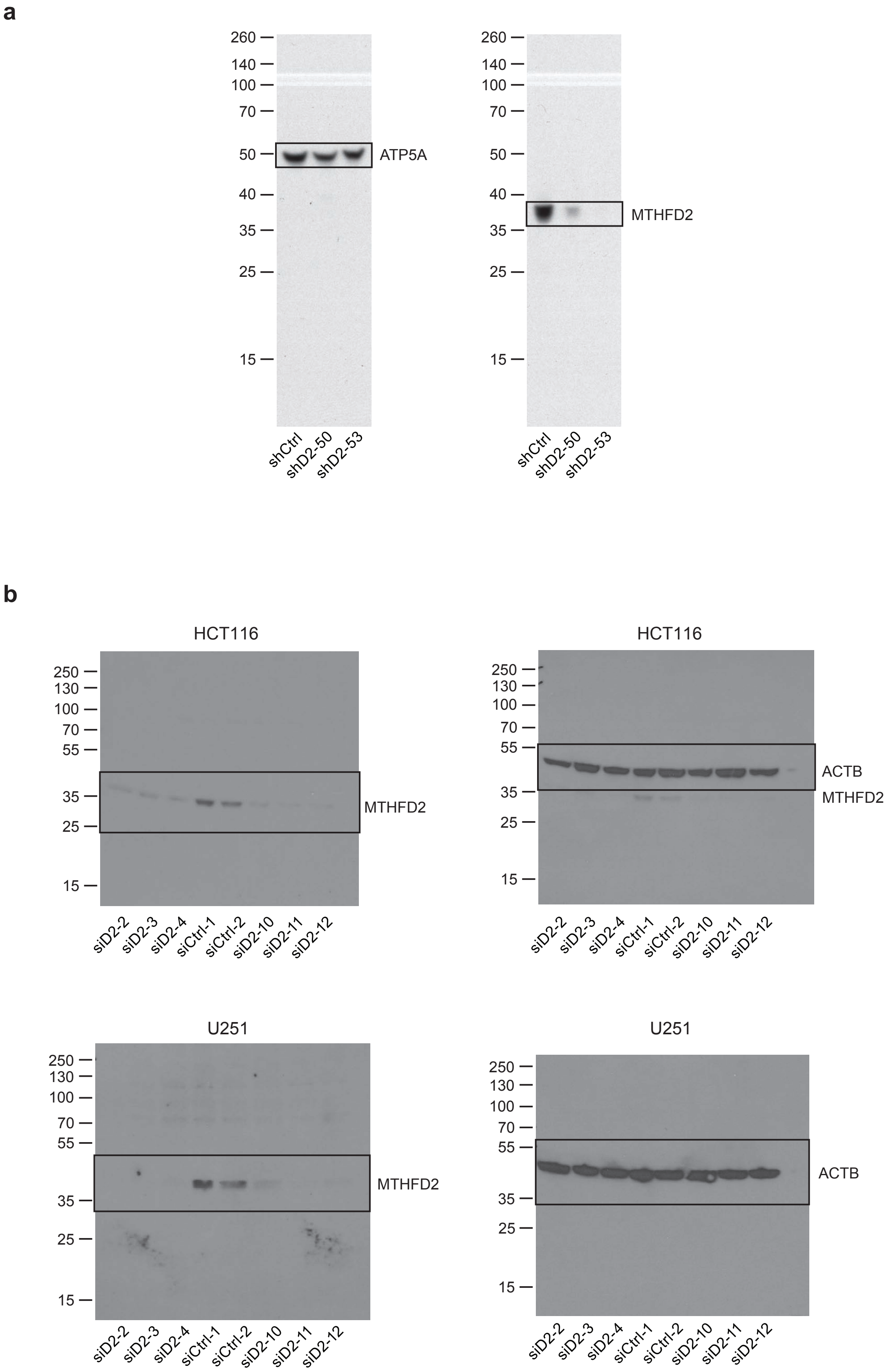
Supplementary Figure 2

ATGGCTGCGACTTCTCTAATGTCTGCTTTGGCTGCCCGGCTGCTGCAGCCCGCGCAC
AGCTGCTCCCTTCGCCTTCGCCCTTTCCACCTCGCGGCAGTTCGAAATGAAGCTGTT
GTCATTTCTGGAAGGAAACTGGCCCAGCAGATCAAGCAGGAAGTGCGGCAGGAGGTA
GAAGAGTGGGTGGCCTCAGGCAACAAACGGCCACACCTGAGTGTGATCCTGGTTG**GC**
GAGAATCCTGCAAGTCACTCCTATGTCCTCAACAAAACCAGGGCAGCTGCAGTTGTG
GGAATCAACAGTGAGACAATTATGAAACCAGCTTCAATTTCAGAGGAAGAATTGTTG
AATTTAATCAATAAACTGAATAATGATGATAATGTAGATGGCCTCCTTGTTCAGTTG
CCTCTTCCAGAGCATATTGATGAGAGAAGGATCTGCAATGCTGTTTCTCCAGACAAG
GATGTTGATGGCTTTCATGTAATTAATGTA**GGACGAATGTGTTTGGATCAGTATTCC**
ATGTTACCGGCTACTCCATGGGGTGTGTGGGAAATAATCAAGCGAACTGGCATTCCA
ACCCTAGGGAAGAATGTGGTTGTGGCTGGAAGGTCAAAAAACGTTGGAATGCCCATT
GCAATGTTACTGCACACAGATGGGGCGCATGAACGTCCCGGAGGTGATGCCACT**GTT**
ACAATATCTCATCGATATACTCCCAAAGA**GCAGTTGAAGAAACATACAAT**TCTTGCA
GATATTGTAATATCTGCTGCAGGTATTCCAAATCTGATCACAGCAGATATGATCAA**G**
GAAGGAGCAGCAGTCATTGATGTGG**GAATAAATAGAGTTCACGA**TCCTGTAACTGCC
AAACCCAAGTTGGTTGGAGATGTGGATTTTGAAGGAGTCAGACAAAAAGCTGGGTAT
ATCACTCCAGTTCCTGGAGGTGTTGGCCCCATGACAGTGGCAATGCTAATGAAGAAT
ACCATTATTGCTGCAAAAAAGGTGCTGAGGCTTGAAGAGCGAGAAGTGCTGAAGTCT
AAAGAGCTTGGGGTAGCCACTAATTAA

shD2-50	**CGAATGTGTTTGGATCAGTAT**
shD2-53	**GCAGTTGAAGAAACATACAAT**
siD2-2	**GGAAGGAGCAGCAGTCATT**
siD2-3	**GGATCAGTATTCCATGTTA**
siD2-4	**GTTACAATATCTCATCGAT**
siD2-10	**GAATAAATAGAGTTCACGA**
siD2-11	**GGACGAATGTGTTTGGATC**
siD2-12	**GCGAGAATCCTGCAAGTCA**

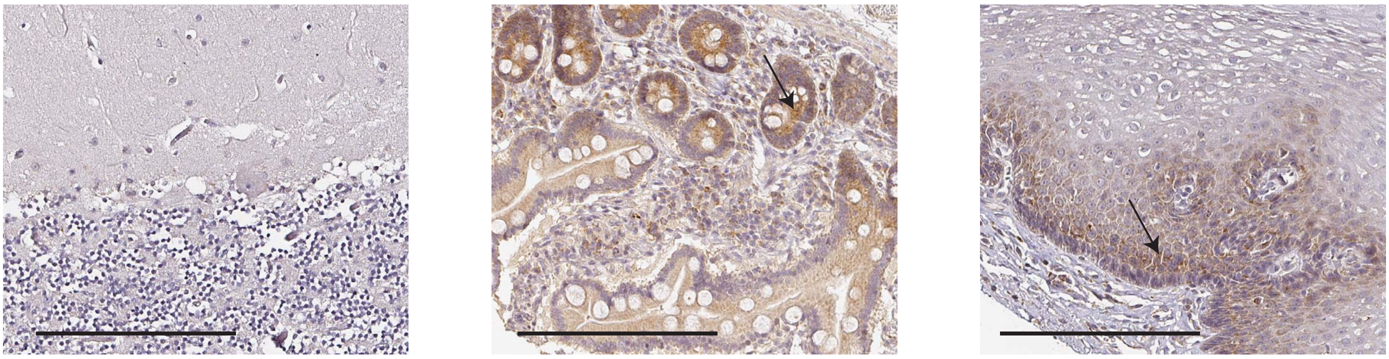
Alignment of RNA interference sequences to the MTHFD2 cDNA. cDNA sequence for the MTHFD2 mRNA, Refseq accession no. NM_006636.3 with siRNA and shRNA target sequences indicated.

Supplementary Figure 3



Full-size images for western blot analyses. (a) Full-size western blots correspondng to Supplementary Figure 1b. A single gel was used for all lanes, samples were loaded in duplicate wells as indicated, and membrane was cut after transfer and separately probed with antibodies against MTHFD2 and ATP5A **(b)** Full-size western blots correspondng to Supplementary Figure 1e. Upper two images are from same gel probed first with antibody against MTHFD2 (left), then with antibody against ACTB (right), and similarly for lower two images. Boxes indicate sections displayed in Supplementary Figure 1.

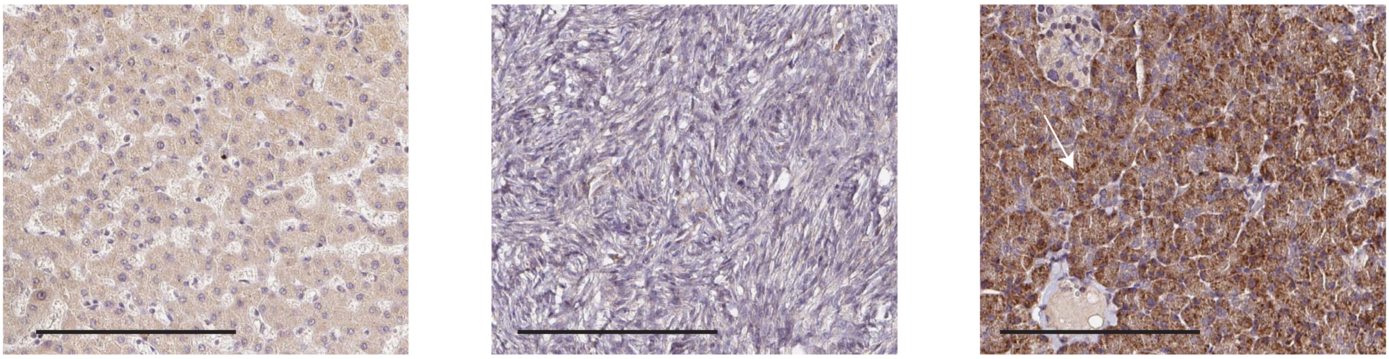
Supplementary Figure 4



Cerebellum

Duodenum

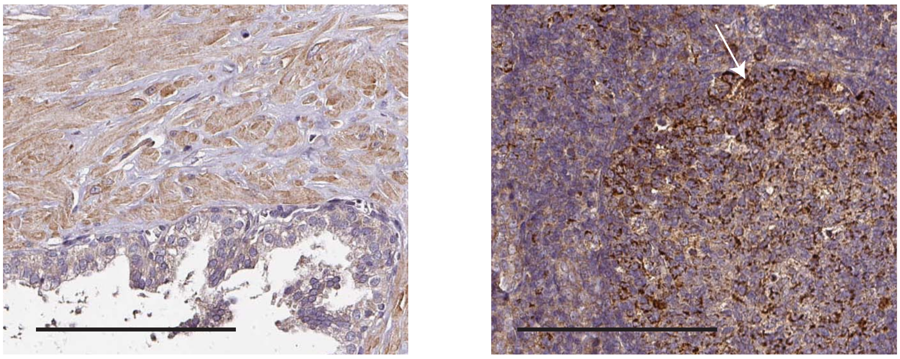
Esophagus



Liver

Ovary

Exocrine pancreas

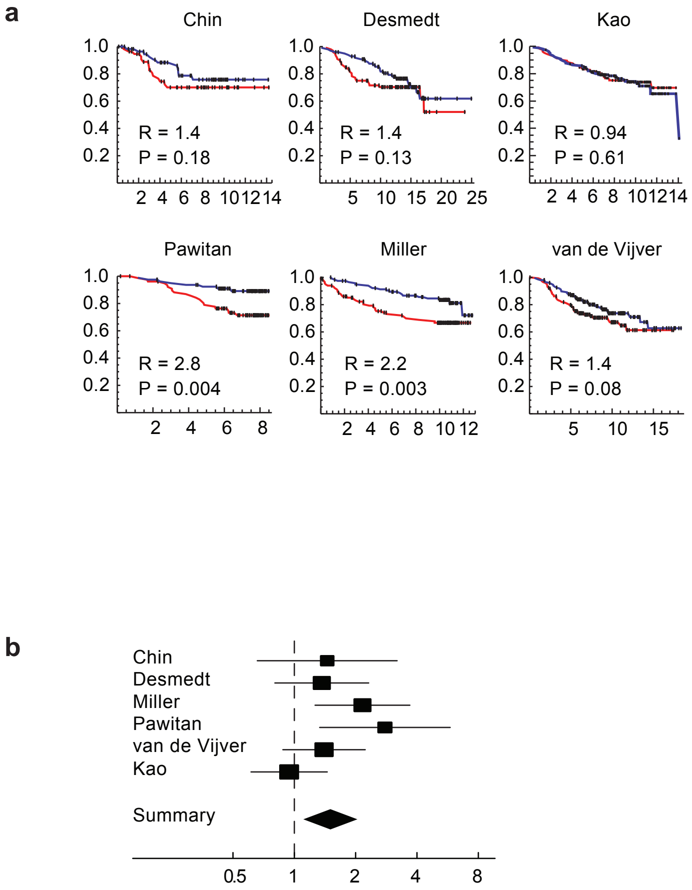


Prostate

Tonsil

MTHFD2 protein expression in normal human tissues. Immunohistochemistry staining for the MTHFD2 protein in samples from eight normal human tissues. Staining is observed in exocrine pancreas, in primarily germinal centres of tonsil, in crypts of the duodenum and in suprabasal layers of the squamous epithelium of esophagus (arrows). Scale bars represent 200 um.

Supplementary Figure 5



Association of MTHFD2 with survival in breast cancer patients. (a) Kaplan-Meier survival curves for six large cohorts of breast cancer patents, stratified by above-median (red) or below-median (blue) expression of MTHFD2. Black bars indicate censored events. R, Cox proportional hazard ratios; P, log rank text P-values. **(b)** Meta-analysis of the six cohorts in (a) by the method of DerSimonian and Laird. Solid lines denote 95% confidence intervals; boxes denote the relative influence of each study over the results (inverse squared standard error). Diamond marks the overall 95% confidence interval.

Tissue / cell type	Classification
AdrenalCortex	Normal/postmitotic
Adrenalgland	Normal/postmitotic
Amygdala	Normal/postmitotic
Appendix	Normal/postmitotic
AtrioventricularNode	Normal/postmitotic
Caudatenucleus	Normal/postmitotic
Cerebellum	Normal/postmitotic
CerebellumPeduncles	Normal/postmitotic
CiliaryGanglion	Normal/postmitotic
CingulateCortex	Normal/postmitotic
DorsalRootGanglion	Normal/postmitotic
GlobusPallidus	Normal/postmitotic
Heart	Normal/postmitotic
Hypothalamus	Normal/postmitotic
Kidney	Normal/postmitotic
Liver	Normal/postmitotic
Lung	Normal/postmitotic
MedullaOblongata	Normal/postmitotic
OccipitalLobe	Normal/postmitotic
OlfactoryBulb	Normal/postmitotic
Pancreas	Normal/postmitotic
PancreaticIslet	Normal/postmitotic
ParietalLobe	Normal/postmitotic
Pituitary	Normal/postmitotic
Pons	Normal/postmitotic
PrefrontalCortex	Normal/postmitotic
Salivarygland	Normal/postmitotic
SkeletalMuscle	Normal/postmitotic
Spinalcord	Normal/postmitotic
SubthalamicNucleus	Normal/postmitotic
SuperiorCervicalGanglion	Normal/postmitotic
TemporalLobe	Normal/postmitotic
TestisSeminiferousTubule	Normal/postmitotic
Thalamus	Normal/postmitotic
Thyroid	Normal/postmitotic
Tongue	Normal/postmitotic
Trachea	Normal/postmitotic
TrigeminalGanglion	Normal/postmitotic
Uterus	Normal/postmitotic
UterusCorpus	Normal/postmitotic
Wholebrain	Normal/postmitotic
pineal_day	Normal/postmitotic
pineal_day	Normal/postmitotic
pineal_day	Normal/postmitotic

pineal_night	Normal/postmitotic
pineal_night	Normal/postmitotic
retina	Normal/postmitotic
retina	Normal/postmitotic
BDCA4+_DentriticCells	Normal/proliferating
Bonemarrow	Normal/proliferating
CD105+_Endothelial	Normal/proliferating
CD14+_Monocytes	Normal/proliferating
CD19+_BCells(neg._sel.)	Normal/proliferating
CD33+_Myeloid	Normal/proliferating
CD34+	Normal/proliferating
CD4+_Tcells	Normal/proliferating
CD56+_NKCells	Normal/proliferating
CD71+_EarlyErythroid	Normal/proliferating
CD8+_Tcells	Normal/proliferating
Lymphnode	Normal/proliferating
Ovary	Normal/proliferating
Placenta	Normal/proliferating
Prostate	Normal/proliferating
Skin	Normal/proliferating
Testis	Normal/proliferating
TestisGermCell	Normal/proliferating
TestisIntersitial	Normal/proliferating
TestisLeydigCell	Normal/proliferating
Thymus	Normal/proliferating
Tonsil	Normal/proliferating
WholeBlood	Normal/proliferating
colon	Normal/proliferating
small_intestine	Normal/proliferating
721_B_lymphoblasts	Transformed
Colorectaladenocarcinoma	Transformed
Leukemia_chronicMyelogenousK-562	Transformed
Leukemia_promyelocytic-HL-60	Transformed
Leukemialymphoblastic(MOLT-4)	Transformed
Lymphoma_burkitts(Daudi)	Transformed
Lymphoma_burkitts(Raji)	Transformed

Supplementary Table 1. Classification of human tissue and cell samples.