The spliceosome is a therapeutic vulnerability in MYC-driven cancer

Tiffany Y.-T. Hsu1,2,3,4, Lukas M. Simon5, Nicholas J. Neill1,4, Richard Marcocci5, Azin Sayad6, Christopher S. Bland1,4, Gloria V. Echeverria6,7,8, Tingting Sun1,4, Sarah J. Kurley1,4, Siddhartha Tyagi1,4, Kristen L. Karlin1,4, Rocio Domínguez-Vidaña1,2,4, Jessica D. Hartman4,9, Alexander Renwick4, Kathleen Scorsone8, Ronald J. Bernardi5, Samuel O. Skinner1,10,11, Antrix Jain1, Mayra Orellana1,4, Chandralah Lagisetti11, Ido Golding10,11, Sung Y. Jung1, Joel R. Neilson10,9, Xiang H.-F. Zhang10, Thomas A. Cooper7,8, Thomas R. Webb11, Benjamin G. Neel7,11, Chad A. Shaw4 & Thomas F. Westbrook1,2,4

MYC (also known as c-MYC) overexpression or hyperactivation is one of the most common drivers of human cancer. Despite intensive study, the MYC oncogene remains recalcitrant to therapeutic inhibition. MYC is a transcription factor, and many of its protumorigenic functions have been attributed to its ability to regulate gene expression programs1–3. Notably, oncogenic MYC activation has also been shown to increase total RNA and protein production in many tissue and disease contexts4–7. While such increases in RNA and protein production may endow cancer cells with pro-tumour hallmarks, this increase in synthesis may also generate new or heightened burden on MYC-driven cancer cells to process these macromolecules properly8. Here we discover that the spliceosome is a new target of oncogenic stress in MYC-driven cancers. We identify BUD31 as a MYC-selective therapeutic vulnerability, and that BUD31 is present at several stages of spliceosomal assembly. Core spliceosomal factors (such as SF3B1 and U2AF1) associated with spliceosome required for its assembly and catalytic activity. Core spliceosomal protein (BUD31) to tolerate dysregulated MYC. Notably, MYC hyperactivation induces an increase in total precursor messenger RNA synthesis, suggesting an increased burden on the core spliceosome to process pre-mRNA. In contrast to normal cells, partial inhibition of the spliceosome in MYC hyperactivated cells leads to global intron retention, widespread defects in pre-mRNA maturation, and deregulation of many essential cell processes. Notably, genetic or pharmacological inhibition of the spliceosome in vivo impairs survival, tumorigenicity and metastatic proclivity of MYC-dependent breast cancers. Collectively, these data suggest that oncogenic MYC confers a collateral stress on splicing, and that components of the spliceosome may be therapeutic entry points for aggressive MYC-driven cancers.

To discover genes and cellular processes required to tolerate oncogenic MYC expression, we previously performed a genome-wide MYC-selective synthetic lethal screen in human mammary epithelial cells (HMECs) engineered with an inducible MYC and oestrogen receptor fusion protein (MYC-ER) for candidates affecting cell viability in an MYC-selective manner9. This screen nominated BUD31 shRNA (Fig. 1c and Extended Data Fig. 1d), indicating that the phenotype is an RNA interference (RNAi) on-target effect.

BUD31 has been linked to the spliceosome in yeast10, but its function in mammalian systems has not been determined. To uncover the molecular function(s) of BUD31, we identified BUD31-interacting proteins by Flag-tagged BUD31 immunoprecipitation from cells with or without RNase A (which eliminates protein–protein interactions mediated by RNA tethering), followed by mass spectrometry. Remarkably, 79 out of 134 core spliceosomal components were associated with BUD31 (Extended Data Fig. 2a), suggesting a strong association between BUD31 and the spliceosome in human cells.

The spliceosome is a dynamic molecular machine consisting of several nuclear protein complexes that cycle on and off of pre-mRNA during intronic splicing11. Co-immunoprecipitation experiments confirmed that BUD31 associates with several subcomplexes of the spliceosome, including the Prp19–CDC5L subcomplex (PRPF19), the U2 small nuclear ribonucleoprotein particles (snRNPs, SF3B1 and SF3A1), U2-related factors (U2AF1), the U5 snRNP (EFTUD2) and Sm proteins (SNRPF) (Fig. 1d and Extended Data Fig. 2c), but interaction with non-spliceosomal proteins was not detected (Extended Data Fig. 2d, e). To test more broadly the association of BUD31 with subcomplexes of the spliceosome, we performed bimolecular fluorescence complementation (BiFC) between BUD31 and proteins from each major spliceosomal subcomplex. BiFC analysis indicated that BUD31 associates with components of the major snRNPs (U1, U2, U4/U6 and U5) as well as Sm proteins (Fig. 1e and Extended Data Fig. 2b), indicating that BUD31 is present at several stages of spliceosomal assembly.

To examine more directly whether BUD31 has a role in pre-mRNA splicing, we tested in vitro splicing efficiency using nuclear extracts with or without BUD31 knockdown. BUD31 loss significantly inhibited pre-mRNA splicing (Extended Data Fig. 2f–i). In addition, knockdown of BUD31 led to defects in early spliceosome assembly, as indicated by impaired formation of complex A (Extended Data Fig. 2h, i). Collectively, these data indicate that HMECs require a core spliceosomal protein (BUD31) to tolerate dysregulated MYC.

We proposed that cells with oncogenic MYC required BUD31 for cell survival because of its role in the spliceosome. To test this hypothesis, we generated a BUD31 mutant deficient in binding core spliceosomal proteins by mutating a highly conserved region spanning a C2-C2 zinc-finger. Mutation of this region abrogated BUD31 interaction with spliceosomal proteins (Extended Data Fig. 2j). To determine whether this region is also necessary for cells to tolerate MYC hyperactivation, we performed an in vitro competition assay. Green

1Verna & Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030, USA. 2Interdepartmental Program in Molecular and Biomedical Sciences, Baylor College of Medicine, Houston, Texas 77030, USA. 3Medical Scientist Training Program, Baylor College of Medicine, Houston, Texas 77030, USA. 4Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA. 5Princess Margaret Cancer Centre, University Health Network, Toronto M5G 2C4, Canada. 6Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030, USA. 7Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas 77030, USA. 8Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA. 9Department of Medical Biophysics, Baylor College of Medicine, Houston, Texas 77030, USA. 10Department of Medical Biophysics, University of Toronto, Toronto M5S 2J7, Canada. 11Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030, USA. 12Center for Chemical Biology, Bioscience Division, SRI International, Menlo Park, California 94025, USA. 13The Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas 77030, USA. 14Department of Physics, University of Illinois, Urbana, Illinois 61801, USA. 15Center for Chemical Biology, Bioscience Division, SRI International, Menlo Park, California 94025, USA. 16The Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas 77030, USA. 17Department of Medical Biophysics, University of Toronto, Toronto M5S 2J7, Canada. Present address: Humacyte, Merriville, North Carolina 27560, USA.
The spliceosome is required for cells to tolerate oncogenic MYC hyperactivation. a, BUD31 is a MYC-synthetic lethal gene. BUD31 shRNA (shBUD31) barcode abundances with/without MYC-ER hyperactivation (mean ± s.e.m., n = 3 biological replicates). c, Relative number of MYC-ER HMECs with dox-inducible shRNA targeting the 3′ untranslated region (UTR) of BUD31, and constitutive shRNA-resistant Flag–GFP or Flag–BUD31 expression (mean ± s.e.m., n = 4 technical replicates). d, Flag–BUD31 co-immunoprecipitation for core spliceosomal factors. e, Interaction between BUD31 and spliceosomal proteins assessed by BiFC (mean ± s.e.m., n = 3 technical replicates). f, GFP+ MYC-dependent cells with inducible shBUD31-UTR and constitutive wild-type, mutant BUD31, or negative control cDNA expression were mixed with GFP+ cells and passaged (mean ± s.e.m., n = 4 technical replicates, two-tailed Student’s t-test). g. Change in MYC-ER HMEC clonogenicity after SD6 treatment (mean ± s.e.m., n = 4 technical replicates, two-tailed Student’s t-test). h-k, Relative number of MYC-ER HMECs after partial depletion of core spliceosomal proteins (mean ± s.e.m., n = 4 technical replicates, one-way analysis of variance (ANOVA)). ***P < 0.01, ****P < 0.001.

Fluorescent protein (GFP)-expressing MYC-driven breast cancer cells encoding inducible BUD31 shRNA were transduced with shRNA-resistant wild-type or mutant BUD31 complementary DNA, and these cells were mixed with non-transduced, GFP-negative cells. BUD31 knockdown significantly inhibited the proliferation of MYC-driven cancer cells. Proliferation was fully rescued by wild-type BUD31 cDNA but not by a BUD31 mutant deficient in spliceosomal binding (Fig. 1f), suggesting that BUD31 association with the spliceosome is required to support the survival of MYC-hyperactivated cells. More broadly, these results indicate that oncogenic MYC may increase cellular dependency on spliceosome function. By contrast, ectopic expression of the oncogenes HER2 (also known as ERBB2) and EGFR did not enhance the effects of BUD31 depletion (Extended Data Fig. 3a, b), suggesting that the stress imposed by MYC on spliceosomal function is not a universal feature of the oncogenic state.

To test whether one or more subcomplexes of the spliceosome are required to tolerate aberrant MYC activity, we examined additional components of spliceosome assembly and catalysis including SF3B1 (U2 snRNP), U2AF1 (U2-related splicing factor), EFTUD2 (U5 snRNP) and SNRPF (core Sm protein found in every snRNP complex). Notably, partial depletion of each spliceosomal component led to loss of cell viability (Fig. 1h–k and Extended Data Fig. 4a–d) and increased apoptosis (Extended Data Fig. 4e–h) in MYC-hyperactivated cells. This suggests that several subcomplexes of the core spliceosome are required for cells to tolerate oncogenic MYC, and that MYC-hyperactivated cells are sensitive to modest perturbations in spliceosome function.

Next, we investigated whether pharmacological inhibition of the spliceosome is also synthetic lethal with MYC. Several pharmacological agents (for example, FR901464, pladienolides and their derivatives) have been characterized to bind the core SF3b spliceosomal complex components and inhibit spliceosome function12. However, most of these inhibitors are not amenable for in vivo delivery. We developed a new small molecule inhibitor of SF3B1, known as SD6, that impairs spliceosome function and is bioavailable in mammals13. Consistent with our genetic data, low SD6 concentrations significantly suppressed colony formation (Fig. 1g) and induced apoptosis (Extended Data Fig. 4i) in a MYC-selective manner. The synthetic-lethal interaction between MYC hyperactivation and core spliceosome perturbation suggests that pre-mRNA splicing is necessary to tolerate oncogenic MYC.

In many different cell lineages and experimental systems, oncogenic MYC activation has been shown to amplify the synthesis of cellular mRNA through direct or indirect mechanisms4,5,14,15. In agreement, MYC hyperactivation in HMECs increased total cellular mRNA synthesis and mRNA steady-state levels (Fig. 2a) without an increase in cellular growth rate (Extended Data Fig. 3c). In contrast to a recent report in B-cell compartments16, MYC hyperactivation did not affect the levels of spliceosome proteins in HMECs (data not shown), suggesting that increased pre-mRNA dosage is not compensated for by higher spliceosome levels. Thus, we proposed that the MYC-induced increase in global mRNA synthesis confers increased pressure on the spliceosome to process pre-mRNAs, and partial perturbation of the spliceosome would lead to widespread defects in the splicing of pre-mRNA introns in the MYC-hyperactive state. To test this hypothesis, we compared intron retention (IR) after BUD31 knockdown in MYC-normal or MYC-hyperactivated cells. We performed RNA-sequencing (RNA-seq) from cells in each state (normal, BUD31 knockdown, MYC-hyperactive, and MYC-hyperactive with BUD31 knockdown) and determined the pre-mRNA splicing efficiency by calculating IR at junctions across the genome (Fig. 2b). Because the analysis of intronic reads may be influenced by the presence of stable RNAs within introns and/or spliced lariats, we restricted the analysis to reads directly spanning exon–intron or exon–exon junction sequences (75,623 junctions in 6,861 genes) (see Methods).
To examine the effects of spliceosome perturbation in the normal and oncogenic MYC states, we compared the effect of BUD31 knockdown on junction IR coefficients in wild-type and MYC-hyperactivated cells. Notably, BUD31 depletion caused significantly more IR in the MYC-hyperactive state than in the MYC-normal state (Fig. 2c, P < 10^{-32}). Similar results were observed when junction coefficients were computed on a gene level (Fig. 2d, P < 10^{-189}). The increase in IR conferred by aberrant MYC activation and BUD31 shRNA was validated on individual exon–intron junctions via quantitative reverse transcriptase PCR (qRT–PCR) (examples in Fig. 2e–j). IR was not limited to a few discrete genes. Instead, BUD31 knockdown in the MYC-hyperactive state led to significantly increased IR in 42% of genes analysed (2,848 of 6,861, P < 0.05). These data indicate that the combination of oncogenic MYC activation and partial spliceosome inhibition leads to a widespread increase in IR. This is consistent with the hypothesis that the MYC-induced increase in pre-mRNA synthesis enhances cellular dependency on optimal spliceosome function by raising the level of pre-mRNA substrates for spliceosomal processing.

Intron-retaining pre-mRNAs often fail to complete mRNA maturation and are commonly degraded via quality control mechanisms. Because the combination of MYC hyperactivation and spliceosome perturbation leads to a global increase in intron retention (Fig. 2c, d), we propose that these cells may contain widespread defects in pre-mRNA maturation and stability (Fig. 3a). To test this hypothesis, we measured the levels of cellular poly(A)^+ RNA in each of the four states (with/without MYC hyperactivation, with/without BUD31 shRNA) before and after treatment with the transcriptional inhibitor actinomycin D. After actinomycin D treatment, cellular poly(A)^+ RNA decreased by comparable levels (~16–19%) in control cells with or without BUD31 knockdown (Fig. 3b). Notably, MYC-hyperactivated cells exhibited enhanced mRNA stability, perhaps resulting from increased polysomal loading of mRNA during MYC-induced translation. By contrast, cells containing MYC hyperactivation and BUD31-depletion exhibited a substantially greater loss (38%) of poly(A)^+ RNA after actinomycin D treatment, suggesting a defect in pre-mRNA maturation and/or stability in the combined MYC-hyperactivated and BUD31-shRNA state. Similarly, fluorescence in situ hybridization (FISH) measurements...

**Figure 2** In MYC-hyperactivated cells, perturbation of the spliceosome leads to global intron retention. **a**, Left, total poly(A)^+ RNA per cell (10^{-4} ng). Right, newly synthesized 4-sU-labelled poly(A)^- RNA per cell (10^{-5} ng). Data are mean ± s.e.m., n = 4 technical replicates for both assays, two-tailed Student’s t-test. **b**, Schematic of IR analysis. **c, d**, Empirical cumulative distribution of IR coefficients for 75,623 exon–intron junctions (c) or 6,861 genes (d). Curves represent IR differences after BUD31 depletion in MYC-normal and MYC-hyperactive states. A rightward shift in the MYC-hyperactive curve indicates increased IR (Kolmogorov–Smirnov test). **e-g**, log2-fold changes in junction IR relative to untreated by RNA-seq of representative genes (mean ± s.e.m., n = 3 biological replicates, two-tailed Student’s t-test). h-j, qRT–PCR validation showing fold change in junction IR relative to untreated (mean ± s.d., n = 3 biological replicates, two-tailed Student’s t-test). *P < 0.05, **P < 0.01, ***P < 0.001.

**Figure 3** Combined spliceosomal perturbation and MYC hyperactivation inhibits pre-mRNA maturation. **a**, Model of MYC-spliceosome synthetic lethality. **b**, Difference in cellular poly(A)^+ RNA in HMECs after actinomycin D (AD) treatment (n = 3 biological replicates, two-tailed Student’s t-test). **c**, Steady-state poly(A)^+ RNA levels per cell (10^{-4} ng) (n = 4 biological replicates, two-tailed Student’s t-test). **d**, Gene Ontology (GO) enrichment of intron-retained genes in the MYC-hyperactive and BUD31-depleted state. Dashed line indicates P = 0.05. **e, f**, In MYC-hyperactive BUD31 shRNA cells, representative genes display increased IR (e) and decreased steady-state RNA levels (f) after BUD31 knockdown in MYC-hyperactivated cells. Bar colours represent GO terms, see legend in Extended Data Fig. 6. Data are mean ± s.e.m. **P < 0.01, ***P < 0.001. NS, not significant.
Hybridization of poly(A)⁺ RNA revealed that the combination of MYC hyperactivation and BUD31 knockdown led to a substantially greater decrease (60%) in poly(A)⁺ RNA after actinomycin D treatment (Extended Data Fig. 5a). Similar trends were observed in nuclear RNA pools, consistent with defects in nuclear pre-mRNA maturation (Extended Data Fig. 5b). Consistent with this decrease in pre-mRNA maturation and stability, cells containing oncogenic MYC and BUD31 knockdown exhibited significantly lower (54%) steady-state levels of poly(A)⁺ and stability, consistent with defects in nuclear pre-mRNA maturation (Extended Data Fig. 3c). Collectively, these results indicate that MYC hyperactivation increases cellular pre-mRNA synthesis, and inhibition of the spliceosome reduces the cellular capacity to process this pre-mRNA burden. The result of this MYC-hyperactivated and spliceosome-hypomorphic state is enhanced intron retention, decreased mRNA maturation and stability, and a significant loss of steady-state cellular mRNA.

Gene Ontology analysis of genes with the most significant intron retention in the combined MYC-hyperactive and BUD31-depleted state (2,848 out of 6,816 genes analysed for IR) suggests that many essential processes and subcellular structures were affected, including gene expression, DNA replication and repair, the mitotic spindle, unfolded protein response, and RNA splicing (Fig. 3d). Many genes participating in these essential cell processes exhibited increased IR in the combined MYC-hyperactive and BUD31-knockdown state (representative genes in Fig. 3e) and a concomitant decrease in RNA levels, consistent with a defect in maturation and stability of IR-containing transcripts (Fig. 3f). Consistent with their role in crucial cellular processes, knockdown of these genes reduced cell number by 0.7–4.2-fold (as quantified by barcode-tag abundance, Extended Data Fig. 6). Together, these data are consistent with the hypothesis that the combination of oncogenic MYC and spliceosome inhibition leads to widespread loss of mRNA integrity, resulting in the deregulation of many essential genes and processes instead of a single pathway.

Because oncogenic MYC significantly increases the sensitivity of HMECs to inhibition of the spliceosome, we proposed that MYC-driven cancers may be hyperdependent on core spliceosomal function to support their survival. We queried whether MYC-driven breast cancer cell lines exhibit increased sensitivity to knockdown of core spliceosomal genes. Recently, we conducted genome-wide RNAi screens in a panel of 72 breast cancer and immortalized cell lines for genes affecting cell viability (Fig. 4a) (R.M., A.S. and B.G.N., manuscript in preparation). From this data set, we tested for a correlation between MYC-dependency (as indicated by sensitivity to MYC shRNAs) and dependency on the spliceosome (as indicated by sensitivity to shRNAs targeting spliceosome components in the shRNA library), or on 100,000 randomly drawn gene sets. Notably, MYC-dependent breast cancer cell lines were significantly more sensitive to shRNAs targeting the core spliceosome (Fig. 4b, P = 0.005). The correlation between MYC-dependency and spliceosome-dependency was significantly pronounced in the basal breast cancer lines (Fig. 4c, P < 0.00001), an aggressive molecular subtype of breast cancer frequently driven by MYC.

Triple-negative breast cancers are commonly driven by MYC, and exhibit an aggressive, highly metastatic clinical course. To determine whether MYC-driven triple-negative breast cancers are dependent on spliceosomal integrity for their tumorigenic and metastatic proclivity, we tested the effects of genetic and pharmacological inhibition of the spliceosome on MYC-dependent and metastatic triple-negative breast cancer (TNBC) models. Inducible BUD31 shRNA reduced cell viability and increased apoptosis in MYC-dependent TNBC cell lines in vitro (Fig. 4d, e and Extended Data Fig. 7a, b). Similar to MYC-ER HMECs, MYC protein levels remained unchanged during BUD31 depletion in these MYC-dependent cancer cell lines (Extended Data Fig. 8a, b), suggesting that the apoptotic response was not due to loss of the driver oncogene (MYC). To assess the effect of spliceosomal perturbation on tumour growth, we established a pooled competition assay that uses shRNA-associated barcodes to detect changes in tumour cell fitness (Extended Data Fig. 9). In the metastatic TNBC cell line MDA-MB-231-LM2 (LM2)50, inducible MYC-shRNA-expressing cells dropped out of the tumour population, confirming the MYC dependency of this TNBC model (Fig. 4f). Similarly, tumour cells containing BUD31 or SF3B1 shRNA dropped out of the tumour population (Fig. 4g). Tumorogenicity of another MYC-dependent TNBC model (SUM159) was similarly impaired by BUD31 depletion (Extended Data Fig. 7c, d). These data suggest that the loss of BUD31 or other core spliceosomal factors inhibits MYC-dependent breast cancer growth in vivo.

Because MYC-driven breast cancers are prone to metastasize to visceral organs including the lungs50, we tested whether perturbation of spliceosome function affected metastatic expansion of MYC-dependent LM2 cells. As shown in Fig. 4g, metastatic cells with BUD31 knockdown were significantly depleted from the population numbers (d, top) (mean ± s.e.m., n = 8 technical replicates, two-tailed Student’s t-test), and increased caspase-3 cleavage (e, bottom) and caspase-3/7 luminescence (e, top) (mean ± s.e.m., n = 3 technical replicates, two-tailed Student’s t-test). f, g, Barcode-shRNA abundance of LM2 cells within primary tumours (f) or pulmonary metastases (g). Mean barcode abundance in each tumour or lung is normalized to the injected cell population (n = 3 technical replicates, two-tailed Student’s t-test). h, Change in LM2 tumour growth after 2 weeks of vehicle (n = 13) or SD6 (n = 10) infusion. Bars indicate mean values (two-tailed Student’s t-test), i, Pulmonary LM2 bioluminescence after 10-day infusion with vehicle (n = 7) or SD6 (n = 6). Bars indicate median values (Mann–Whitney test). *P < 0.05, **P < 0.01, ***P < 0.001.
May be a target of both oncogene addiction and oncogenic stress.

Next, we tested whether pharmacological inhibition of the spliceosome also impaired tumorigenic and metastatic potential of MYC-dependent TNBC cells. Compared to MYC-normal cell lines (half-some also impaired tumorigenic and metastatic potential of MYC-dependent breast tumorigenicity and metastatic expansion

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Author Information RNA-seq data sets have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE65618. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to T.F.W. (thomasw@bcm.edu).

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METHODS

Vectors and virus production. Commercially available pGIPZ shRNAs targeting BUD31 (V2LHS_4771 and V2LHS_4770), EFTUD2 (V2LHS_28167), SNRF (V3LHS_39782), and U2AF1 (V2LHS_8467) were obtained from Open Biosystems. shRNAs targeting the 3′ UTR of BUD31 were designed using the BiopredikSI and RNAi Codex algorithms (shRNA sequence 5′-TGGTCTGAGATGGGCGGCTGTTCGATCTGTTAGTGAAGCCACAGATGTAAATCACAGCTGATAGACAGCGATG-3′). For inducible RNAi experiments, shRNAs were sub-cloned into the pINDUCER dox-inducible lentiviral expression system31.

Lentiviruses and retroviruses were produced by transiently transfecting shRNA or cDNA constructs using Muris Bio TransIT transfection protocols into 293T cells and collecting viral supernatants 48 h after transfection.

Cell culture. HMECs expressing hTERT and inducible MYC-ER (MYC-ER HMECs), F7 epithelial cells and human mammary epithelial HME1 cells were cultured in mammary epithelial growth medium (MEGM, Lonza), 293T cells, HeLa cells and MDA-MB-231-LM2 human breast cancer cells were cultured in DMEM (Gibco) supplemented with 10% FBS. SUM159 human breast cancer cells were cultured in F12 (Gibco) media supplemented with 5% FBS, 10 mM HEPES (Gibco), 5 μg ml−1 insulin (Invitrogen), and 1 μg ml−1 streptomycin and 1 μg ml−1 penicillin. The P493-6 human B-cell lymphoma cell line was cultured in RPMI-1640 supplemented with 10% FBS (Clontech) and 1% GlutaMAX (Invitrogen). All cell lines were incubated at 37°C and 5% CO2. Cell lines were obtained from ATCC, and all cell lines are tested yearly for mycoplasma contamination. Stable cell lines expressing shRNAs or cDNAs were generated by lentiviral or retroviral transduction in the lines described previously33.

For immunoprecipitations, nuclear and whole-cell extracts were ultracentrifuged at 100,000 g for 4 days.

Immunoprecipitations. BUD31 mutagenesis. Wild-type and mutant BUD31 cDNAs were generated by gene synthesis (IDT DNA) and recombined into the pQXIN-N-YFP fusion vector. Mutant BUD31 consisted of substituting human BUD31 amino acid residues 105–114 with an equivalent number of glycine residues (codon GGA). In vitro competition assay. MYC-dependent SUM159 breast cancer cells with pINDUCER-shBUD31-3′ UTR were transduced with viruses containing wild-type or mutant BUD31 or negative control cDNA recombined into pQXIN-N-YFP vectors. Infected, GFP+ cells were mixed at an 80:20 ratio with non-transduced, GFP− parental cells and seeded into 96-well plates and treated either with or without dox (1 μg ml−1). At confluence, cells were passaged 1:10 and processed for flow cytometry. The in vitro competition assay was continued for two passages.

Immunoblotting. Cells were lysed in 1× SD sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 0.5% β-mercaptoethanol) and heated at 95°C for 12 min. The following antibodies were used for western blotting: Flag (Sigma, A8592), BUD31 (ProteinTech, 11798-1-AP, SF31 (Bethyl, A300-996A), Pp19 (Bethyl, A300-101A), U2AF1 (Bethyl, A302-079A), SF3A1 (Bethyl, A301-603A), EFTUD2 (Bethyl, A300-957A), SNRPW (Abcam, ab154870), HER2 (Millipore, 06-562), EGFR (Cell Signaling, 2232), cleaved caspase-3 (Cell Signaling, 9664), RPS8 (Assay Biotechnology, R12-3466), EIF2S1 (Abgent, AP13469s), E3F1 (Proteintech, 646701) and c-Myc (DB412) (Cell Signaling, 5605). Vinculin (Sigma, V9131) and Ran (BD Biosciences, 610340) were used as loading controls.

In vitro transcription. Uniformly ³²P-UTP radiolabelled MINX pre-mRNA was in vitro transcribed from a BamHI-digested plasmid44, DNASel (Ambion) treated and gel-isolated on a 8 M urea 6% polyacrylamide gel.

In vitro splicing. HeLa nuclear extracts used for in vitro splicing assays were made as described previously35 from HeLa cells transduced with an inducible BUD31-targeting shRNA and grown in the presence or absence of 1 μg ml−1 dox. Splicing reactions of 15 μl contained: 8 nM RNA substrate, 0.8 nM DTT, 1.7 mM magnesium acetate, 1.7 mM ATP, 17 mM phospho-creatine, 20 mM glycerine, 1 μl−1 RNasin Plus (Promega), 3.7% PVA and 50 μg of HeLa nuclear extracts. Splicing reactions were incubated for indicated time points at 30°C and stopped by digestion with proteinase K (Ambion) for 30 min at 45°C followed by RNA purification. RNA purified from splicing reactions was electrophoresed on 8 M urea 8% polyacrylamide gels, then exposed to a phosphorimager screen (Typhoon Trio phosphorimager, GE Healthcare). Alternatively, RNA purified from in vitro splicing reactions was added to RT–PCR reactions as previously described46 with the following antibodies: β-actin (Abcam, ab562), EGFR (Cell Signaling, 2232), cleaved caspase-3 (Cell Signaling, 9664), RPS8 (Assay Biotechnology, R12-3466), EIF2S1 (Abgent, AP13469s), E3F1 (Proteintech, 646701) and c-Myc (DB412) (Cell Signaling, 5605). Vinculin (Sigma, V9131) and Ran (BD Biosciences, 610340) were used as loading controls.

Spliceosome complex formation assay. In vitro splicing reactions were carried out as described above, placed on ice, and heparin was added to a final concentration of 2 μg ml−1. Reactions were incubated in the presence of heparin at 30°C for 5 min and immediately loaded onto 0.75-mm non-denaturing 4% acrylamide–0.4% agarose composite gels. Gels were run at 250 V at room temperature in 1× tris-glycine running buffer for 3 h, then placed on Whatman paper and exposed to a phosphorimager screen.

Spliceosomal RNA isolation and qRT–PCR. RNA isolation was performed with the RNeasy Mini Kit (Qiagen). Reverse transcription was performed using the High Capacity RNA-to-cDNA Master Mix (Applied Biosystems), and qPCR was performed using SYBR Green Master Mix (Applied Biosystems). The following primers were used: BUD31 forward 5′-ACCAACTTCTCGGAGACAACTG-3′, reverse 5′-CAGGCACTTCCAGGATTT-3′; EFTUD2 forward 5′-CCTCGTGTCCGTCGAGAGT-3′, reverse 5′-TGGTTGAGTGTTTAGTGTAAG-3′; SF3A1 forward 5′-GGATGAGTCTAGCAACATTG-3′, reverse 5′-GGAGGCAATGAGTGAGGAT-3′; SNRF forward 5′-GGGAAAGGGATGAGGGGAC-3′, reverse 5′-GGATGAGTCTAGCAACATTG-3′; SF3A1 forward 5′-GGATGAGTCTAGCAACATTG-3′, reverse 5′-GGAGGCAATGAGTGAGGAT-3′; U2AF1 forward 5′-GGGAAAGGGATGAGGGGAC-3′, reverse 5′-GGAGGCAATGAGTGAGGAT-3′. qPCR products were run on 6% non-denaturing polyacrylamide gels and visualized after staining with etidium bromide.

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tamoxifen in triplicates. Total RNA was isolated using the RNeasy kit (Qiagen). RNA samples were rRNA depleted, and NGS libraries were constructed and sequenced as 75 bp paired-end reads by Illumina HiSeq 2000.

Quality assessment of RNA-seq. RNA-seq NGS reads quality was evaluated using FastQC application (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Alignment of RNA-seq data. RNA-seq NGS reads were mapped using STAR RNASeq aligner (version 2.3.1). To improve mapping accuracy, the database file of splice junctions (http://it-collabo01.cshl.edu/shares/gingerslab/www-data/dobin/STAR/STARgenomes/GENCODE/ Old/genencode.v14.annotation.gtf.sjdb) was supplied at the genome index generation step with command line option sjdbOverhang 7, together with http://it-collabo01.cshl.edu/shares/gingerslab/www-data/dobin/STAR/STARgenomes/GENCODE/ Old/hg19.Genencode1.4overhang75/ and default parameters. Duplicate reads were marked with the MarkDuplicates function of the Picard-tools software package (http://picard.sourceforge.net; version 1.107) using default settings.

Intron–exon junction definition. To prevent confounding effects in our analysis of IR within HMECs, we confined our analyses to exons in non-overlapping genes that are included within all isoforms of a given gene (75,623 junctions in 6,861 genes).

Intron–exon junctions were obtained using the University of California Santa Cruz Genome Browser ‘KnownGene’ table (downloaded 4 June 2014). Constitutive junctions were defined as junctions that (1) appear in each transcript annotated to a given gene symbol, (2) do not overlap with any transcript annotated to a different gene symbol, and (3) do not mark the start or stop of a transcript.

Junction IR calculation. Because analysis of intronic reads may be influenced by the presence of stable RNAs within introns and/or spliced lariats, we calculated junction IR as the ratio of exon–intron reads to exon–exon reads, restricting the analysis to reads directly spanning exon–intron or exon–exon junction sequences. We used R together with the Rsamtools package to calculate IR. In brief, for each intron–exon junction, we extracted all non-duplicate reads overlapping this junction. Next, we assigned these reads into two categories: (1) ‘intronic’ if the read mapped to at least the first base of the intron, (2) ‘exonic’ if none of the bases of the read mapped to the first base of the intron and at least one base mapped to a subsequent exon. We counted the total number of reads assigned into each category for each junction. IR was calculated as:

\[ IR_j = \frac{I_{j,\text{intronic}}}{I_{j,\text{total}}} \]

in which IR\(_j\) represents the IR score for junction \(j\) in sample \(i\), and \(I_{j,\text{intronic}}\) and \(I_{j,\text{total}}\) refer to the count of reads classified as intronic and exonic for junction \(j\) in sample \(i\), respectively. To avoid ratios with 0 in the denominator, we added 1 to each of these counts. The scripts used to conduct this calculation are available on request.

We restricted all following analyses to intron–exon junctions with an average of at least 25 total (intronic and exonic) reads in the control and MYC hyperactivated samples.

Gene IR calculation. For the cumulative distribution analyses, the mean IR score for all junctions in a gene was averaged.

Gene annotation. A custom gene annotation file was generated to correspond to the set of intron–exon junctions considered in the IR analysis. In brief, exons were defined as: (1) an exon flanked by two junctions annotated to the same symbol, and (2) an exonic region flanked by one junction and conserved across all transcripts annotated to the same symbol.

Statistical analysis of RNA-seq. Statistical analyses were performed using the open source statistical programming environment ‘R’. Empirical cumulative distributions (ECDF) of IR scores were compared using two-sided Kolmogorov–Smirnov test and default parameters. Duplicate reads were marked with the MarkDuplicates function of the Picard-tools software package (http://picard.sourceforge.net; version 1.107) using default settings.

Permutation-based test of significance. The significance of the difference of empirical cumulative distributions of junction-level IR scores was evaluated using a permutation-based approach. The null hypothesis was that splicing perturbations had no effect on IR changes in the MYC-normal and MYC-hyperactivated states. To model this null hypothesis, the treatment information was blinded to the assignments of MYC activity. We generated a third control sample by randomly selecting half of the junctions from samples 1-control and 2-control. Control and MYC samples were grouped as 6 ‘normal’ samples, and LowBUD31 and Myc_LowBUD31 samples were grouped as ‘splicing perturbed’ samples. Next, we comprehensively generated all possible normal and splicing perturbed contrasts by subtracting the average of each junction IR score of three normal samples from that of the splicing perturbed group. The empirical distribution of all possible double differences was generated and used to assign significance to the original observations. An analogous approach was used to evaluate the difference of empirical cumulative distributions of gene-level IR scores.
Zyla 4.2 sCMOS camera. For each treatment condition and actinomycin time point, ≥150 cells were analysed for mean FITC intensity. Cellular FITC values were adjusted for background fluorescence by subtracting the mean extra-cellular pixel value. Image analysis was performed using Nikon Elements.

**Luminescent apoptosis assays.** Caspase-3/7 activity was assessed in MYC-ER HMECs and breast cancer cell lines by incubating Caspase-Glo 3/7 Reagent with cells in triple-well plates of a 96-well plate and measuring luminescence with a plate reader ( Molecular Devices). Luminescence was normalized using cell numbers determined by TrypanBlue staining of a duplicate plate, followed by nuclei counting using the Celse Imaging Cell Cytometer (Brooks).

**Tumorigenicity and metastasis assays.** SUM159 breast cancer cells were transduced with pLNCUER11-shBUD31-3 UTR virus and analysed by flow cytometry to confirm ≥98% transduction. In total 8×10⁶ transduced cells were injected with matrigel (BD Biosciences) subcutaneously into the flank of four-week-old female athymic nude Foxn1-nu mice (Harlan Labs). Tumour volume was measured using calipers, and once tumours achieved 150 mm³, mice were randomized onto and maintained on sucrose water (−dox) or sucrose water with dox (+dox).

For mixed population experiments, MDA-MB-231-LM2 breast cancer cells were individually transduced with pLNCUER11-shRNAs targeting the indicated genes at an MOI appropriate to transduce all cells (1.3–1.5). The individual pooled shRNA screens in breast cancer cell lines. Essentially as described. In brief, cell lines were infected with a lentiviral shRNA library at a MOI of 0.3, and passed under standard conditions. At 4 and 8 doublings, respectively, DNA is isolated and hybridized to a custom chip to assess shRNA dropout. A detailed description of the results of these screens will be published separately (R.M., A. S. and B.G.N., manuscript in preparation).

**Correlation between MYC dependency and spliceosome dependency.** First, to calculate MYC dependency scores using probe on-target hairpins, we used assay observations associated with 3 hairpins incorporated into the first ATARIS solution for MYC. MYC dependency scores were generated using a hierarchical linear model, with pooled shRNA screen observations as the independent variable and two regression covariates: initial signal intensity (with coefficient β₁) and linear time-course dropout trend (with coefficient β₂). The dropout trend is calculated for each cell line separately, resulting in a per-cell-line MYC dropout score (the value of coefficient β₂).

Second, the MYC dependency score was used in a hierarchical linear model to search for associations with the essentiality of other genes (such as spliceosome encoding genes). This model uses pooled shRNA screen observations as the independent variable and three regression covariates: initial assay signal intensity (with coefficient β₁), linear time-course dropout trend (with coefficient β₂), and an interaction term between dropout trend and MYC dependency score (with coefficient β₃). The P-value associated with the interaction term β₃ is used to determine whether a significant association exists. A detailed description of this approach will be published elsewhere (A.S., R.M. and B.G.N., manuscript in preparation).

A summary statistic using results from the single-gene analyses was used to test the significance of the association between MYC dependency and the essentiality of a gene set. For a gene set containing genes g, we calculate the gene set summary statistic as

$$ \sum_{g \in \text{gene set}} \text{sign}(\beta_g) \log_{10}(P \text{ value}(\beta_g)) $$

in which sign(β₃) and P value(β₃) indicate the values associated with the regression coefficient β₃. The resulting metric, termed a siMEM (mixed-effect model) score, indicates the significance and correlation between sensitivity to MYC shRNAs and sensitivity to a group of shRNAs targeting a gene set (such as those targeting the spliceosome). A gene set (for example, spliceosome genes) for which a substantial number of genes are significantly associated with MYC dependency, and all with the same direction (sign) of association, will have a large positive score.

When calculated for the gene set consisting of the core spliceosome, this value summarizes the direction and strength of the significance observed across genes in the spliceosome. To determine whether this observation is significant, the same statistic is calculated for 100,000 randomly drawn gene sets of the same size as the core spliceosome, yielding the null distributions of gene set summary statistics in Fig. 4b, c.

**Statistical analysis.** All experiments were performed on biological replicates unless otherwise specified. Sample size for each experimental group/condition is reported in the appropriate figure legends and methods. For cell culture experiments, sample size was not predetermined, and all samples were included in analyses. For significance testing, analyses were chosen if data met the assumptions of the tests. Data was checked for comparable variance before statistical analysis. Statistically significant differences between control and experimental groups were determined using two-tailed unpaired Student’s t-test, one-way ANOVA with Tukey-Kramer minimum significant difference test, Mann–Whitney test, Kolmogorov–Smirnov test, Wilcoxon test, permutation-based test of significance, and log-rank test as indicated in the appropriate figure legend and methods text.

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Extended Data Figure 1 | Validation of BUD31 as a MYC-synthetic lethal gene in HMECs. 

a, qRT–PCR analysis of BUD31 mRNA level (mean ± s.d., n = 3 biological replicates). 

b, Clonogenicity of MYC-ER HMECs with or without MYC hyperactivation or BUD31 depletion (mean ± s.e.m., n = 4 biological replicates, **P < 0.01, two-tailed Student’s t-test).

c, Caspase-3/7 activation by caspase luminescence assay (mean ± s.e.m., n = 3, ***P < 0.001, one-way ANOVA).

d, Flag-tagged protein levels in MYC-ER HMECs in which vinculin was used as a loading control.
Extended Data Figure 2 | BUD31 interacts with core spliceosomal factors and is required for spliceosomal assembly and pre-mRNA splicing. a, 134 core spliceosomal proteins are listed. Proteins in red are shown to interact with BUD31, as discovered by Flag–BUD31 immunoprecipitation mass spectrometry and BUD31 BiFC. b, Heat map of BUD31-interacting spliceosomal proteins, organized by spliceosome sub-complexes. A black-green colour scale depicts normalized BiFC interaction values between spliceosomal proteins and negative control protein (technical replicates in two left lanes) and BUD31 (technical replicates in two right lanes). c, Spliceosomal snRNPs (coloured circles) interact in a stepwise manner to excise intronic sequences from pre-mRNA. snRNPs with proteins identified from the BUD31 immunoprecipitation and mass spectrometry are noted (blue outline) to be BUD31-associated. d, Co-immunoprecipitation of Flag–BUD31 for non-spliceosomal proteins. Input and immunoprecipitation blots probed by EIF2S1 and EIF3I were taken at different exposures to minimize background signal. e, Interaction between N-YFP-tagged BUD31 and C-YFP-tagged spliceosomal (DDX46) or cytoplasmic proteins (TRIM9, SOCS2 and EPHA8) was assessed by cellular fluorescence (mean ± s.e.m., n = 3 technical replicates). f, Nuclear extracts with or without BUD31 knockdown were incubated with pre-mRNA substrate, and RT–PCR of unspliced RNA (top) and spliced RNA (bottom) was performed, using primers at the indicated arrows (left). BUD31 protein levels in the nuclear extracts were normalized to vinculin expression (middle) and quantified (right). g, Radioactively labelled pre-mRNA (MINX) was incubated with nuclear extracts with or without BUD31 depletion. RNA purified from the splicing reaction was run on a denaturing gel and imaged by autoradiography. The identities of prominent bands are based on size. Asterisk denotes putative intron-lariat band. h, After in vitro splicing was performed as described previously, products were electrophoresed on native gel, and spliceosome complexes were visualized by autoradiography. Complex A and nonspecific H complexes are labelled. i, Phosphorimager quantification of the ratio of RNA in complex A compared to that in complex H. j, Interaction between N-YFP-tagged wild-type (WT) or mutant BUD31 and C-YFP-tagged splicing factors was assessed by cellular fluorescence (mean ± s.e.m., n = 2 technical replicates, ***P < 0.001, two-tailed Student’s t-test).
Extended Data Figure 3 | HMECs with oncogenic activation of HER2 and EGFR do not require BUD31. a, Cell number changes in HMECs with inducible shBUD31 and constitutive HER2 or EGFR expression (mean ± s.e.m.; n = 4 technical replicates; *P < 0.05, two-tailed Student’s t-test). HER2 and EGFR protein is normalized to vinculin (right). b, MYC protein levels in HMECs with constitutive HER2 or EGFR expression. c, MYC induction by tamoxifen in MYC-ER HMECs does not increase cell proliferation over time (mean ± s.e.m., n = 8 technical replicates).
Extended Data Figure 4 | Partial knockdown of core splicing factors is MYC-synthetic lethal in HMECs. a–d, mRNA levels for core splicing factors SF3B1 (a), U2AF1 (b), EFTUD2 (c) and SNRPF (d) were evaluated by qRT–PCR (mean ± s.d., n = 3 technical replicates). e–i, Caspase-3/7 luminescence in MYC-ER HMECs with partial suppression of core spliceosomal proteins (e–h) or spliceosome inhibitor SD6 (i) (mean ± s.e.m., n = 3 technical replicates, ***P < 0.001, one-way ANOVA).
Extended Data Figure 5 | BUD31 loss in MYC-hyperactivated cells destabilizes mRNA. a, b, MYC-ER HMECs with inducible shBUD31 treated with actinomycin D for 5 h were labelled with oligo(dT)25 LNA probes via fluorescence in situ hybridization. Cellular FITC intensity was assessed within cellular (a) and nuclear (DAPI+) (b) regions. Data are represented as the difference in cellular FITC intensity between 0 and 5 h of actinomycin D treatment in each cell state (mean ± s.e.m., n = 150, ***P < 0.001, two-tailed Student’s t-test).
Extended Data Figure 6 | BUD31 depletion in MYC-hyperactivated cells enhances intron retention and decreases expression of cell-essential genes.

In MYC-hyperactive cells, 17 representative genes display increased IR and decreased steady-state RNA levels after BUD31 knockdown. Depletion of these genes by shRNA decreased cell viability (mean barcode abundance ± s.e.m.). Twofold decrease in barcode abundance is noted by the dashed red line. All values are reflective of three biological replicates, and genes are colour-coded based on their Gene Ontology term annotation.
Extended Data Figure 7 | MYC-dependent breast cancer cells require BUD31 for in vitro and in vivo growth. a, Relative cell number of SUM159 cells with doxycycline-inducible shBUD31 in vitro (mean ± s.e.m., n = 8 technical replicates, ***P < 0.001, two-tailed Student’s t-test). b, Caspase-3/7 luminescence in BUD31-depleted SUM159 cells (mean ± s.e.m., n = 3 technical replicates, ***P < 0.001, two-tailed Student’s t-test). c, d, SUM159 cells engineered with dox-inducible shBUD31 were subcutaneously transplanted into mice and randomized onto dox treatment (−dox n = 10, + dox n = 9). Loss of BUD31 in SUM159 xenografts inhibits tumour growth (mean ± s.e.m., ***P < 0.001 at day 21, two-tailed Student’s t-test) (c) and prolongs progression-free survival (d) in nude mice (P-value, log-rank test).
Extended Data Figure 8 | BUD31 depletion does not affect levels of MYC protein. a, MYC protein levels in MYC-ER HMECs with inducible shBUD31 expression normalized to vinculin expression. To confirm specificity of MYC antibody, HMECs without the MYC-ER construct were engineered to express inducible MYC shRNA. b, MYC protein levels in SUM159 and LM2 cells with inducible shBUD31 normalized to vinculin expression. To confirm specificity of MYC antibody, SUM159 cells were engineered to express inducible MYC shRNA.
Extended Data Figure 9 | Schematic for in vivo barcode-based competition assay. LM2 cells transduced with inducible shRNAs targeting negative control genes or candidate genes were mixed at an equal ratio. This mixed population was transplanted into mice, and tumours were allowed to form in the presence or absence of dox. At the experimental endpoint, genomic DNA was isolated for comparisons of relative barcode (shRNA) abundance in tumour genomic DNA.
Extended Data Figure 10 | Spliceosome inhibitor SD6 inhibits MYC-dependent cancer cells in vitro and in vivo. 

a, MYC-dependent breast cancer cells (SUM159 and LM2) and MYC-normal immortalized epithelial cells (F7 and HME1) were cultured with SD6 at low density and analysed for clonogenic growth.
b, MYC-repressible human B-cell line P493-6 was treated with or without 100 nM SD6 in the absence or presence of MYC hyperactivation for four days, and cells were counted for relative cell number changes (mean ± s.e.m., n = 3 biological replicates, ***P < 0.001, one-way ANOVA). 
c, Kaplan–Meier survival analysis of nude mice with pulmonary seeding of LM2 cells treated with or without SD6 for 10 days (vehicle n = 7, SD6 n = 6, P-value by log-rank test).