A SUMOylation-Dependent Transcriptional Subprogram Is Required for Myc-Driven Tumorigenesis

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Supporting Online Material

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Materials and Methods
Figs. S1 to S15
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A SUMOylation-Dependent Transcriptional Subprogram Is Required for Myc-Driven Tumorigenesis

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Myc is an oncogenic transcription factor frequently dysregulated in human cancer. To identify pathways supporting the Myc oncogenic program, we used a genome-wide RNA interference screen to search for Myc–synthetic lethal genes and uncovered a role for the SUMO-activating enzyme (SAE1/2). Loss of SAE1/2 enzymatic activity drives synthetic lethality with Myc. Inactivation of SAE2 leads to mitotic catastrophe and cell death upon Myc hyperactivation. Mechanistically, SAE2 inhibition switches a transcriptional subprogram of Myc from activated to repressed. A subset of these SUMOylation-dependent Myc switchers (SMS genes) is required for mitotic spindle function and to support the Myc oncogenic program. SAE2 is required for growth of Myc-dependent tumors in mice, and gene expression analyses of Myc-high human breast cancers suggest that low SAE1 and SAE2 abundance in the tumors correlates with longer metastasis-free survival of the patients. Thus, inhibition of SUMOylation may merit investigation as a possible therapy for Myc-driven human cancers.

Cancers are driven by genomic alterations that result in the activation of proto-oncogenes and the inactivation of tumor suppressor genes. Removing oncogene function can often reverse the tumorigenic phenotype, a phenomenon referred to as "oncogene addiction" (*1*, *2*), and cancer researchers have focused on exploiting oncogene addiction by discovering and targeting cancer-causing oncogenes (*3*). However, many oncogenes such as *Ras* and *Myc* have proven difficult to inhibit pharmacologically, highlighting the need for complementary approaches. One such approach is based on the concept of non-oncogene addiction (NOA), which postulates that oncogenic mutations confer dependencies on cellular processes that can be exploited therapeutically (*4*–*6*). The genes and signaling pathways underlying such oncogenic support processes are largely unexplored, and because these genes are not themselves oncogenes or otherwise mutated in cancer, they cannot be identified through direct analyses of cancer genomes and epigenomes.

The NOA pathways supporting the classical c-*Myc* oncogene (referred to herein as *Myc*) are poorly understood. The *Myc* gene, which codes for a basic helix-loop-helix zipper transcription factor, is frequently dysregulated in cancer cells by amplification, mutation, overexpression, or protein stabilization (*7*). Amplification or overexpression of Myc occurs in ~25% of breast cancers (*8*–*11*) and are associated with poor prognosis (*12*). Genetic experiments have shown that Myc is required for tumor maintenance and progression in several types of malignancy (*13*, *14*). However, despite three decades of research, there is no effective method to inhibit Myc in human cancer.

Oncogenic activation of Myc promotes a delicate balance in cells, conferring both pro- and anti-tumorigenic properties (*2*, *15*, *16*). This raises the interesting possibility that the balance between these opposing properties could be influenced by inhibiting *Myc* oncogenic support pathways. To

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search for pathways required for cells to tolerate the *Myc* oncogene, we performed a genome-wide genetic screen for Myc–synthetic lethal (MySL) short hairpin RNAs (shRNAs) in human mammary epithelial cells (HMECs) engineered with an inducible c-*Myc*–estrogen receptor fusion transgene (Myc-ER HMECs) (fig. S1A). Induction resulted in increased expression of known Myc targets (fig. S1B) and a modest decrease in HMEC proliferation rate (fig. S1C). HMECs are ER-negative, and in the absence of Myc-ER, do not respond to tamoxifen (fig. S2). Using this system, we screened for shRNAs that alter cell fitness only in the presence of aberrant Myc signaling (screen design in Fig. 1A).

To identify MySL shRNAs, we transduced Myc-ER HMECs with a retroviral library of 74,905 shRNAs targeting 32,293 unique transcripts in three independent replicates. Transduced Myc-ER HMECs were propagated in the absence or presence of Myc-ER induction, and the relative change in shRNA-barcode abundance was measured in both cell states (Myc-off and Myc-on). We identified 403 MySL shRNAs exhibiting more than a twofold decrease in abundance in the Myc-on state (relative to the Myc-off state) ($P < 0.02$; Fig. 1B, fig. S3, and table S1). Content analysis [Gene Ontology (GO)] indicated that these candidates were highly enriched for ion channels and enzymes functioning in ubiquitin-like protein conjugation (including SUMOylation) ($P = 0.002$). Components of the mitotic spindle were also highly enriched among MySL candidates (Fig. 1C). Analysis of the MySL candidates using Human Protein Reference Database (HPRD) revealed a highly connected protein-protein interaction network, with many components of this network playing a role in the mitotic spindle (Fig. 1D; protein labels shown in fig. S4), suggesting that Myc hyperactivation may impose a stress on proper mitotic progression (*17*).

Among MySL candidates were several genes previously implicated in the survival of Myc hyperactivated cells (*GSK3β*, *FBXW7*, and *PTK2*; fig. S5A) (*18*–*20*). In addition, shRNAs targeting MDM2 exhibited Myc synthetic lethality, whereas p53-targeting shRNAs enhanced proliferation in a Myc-selective manner (Fig. 1B and fig. S5B). Myc promotes p53 activation (*2*, *21*–*23*), and these dependencies are consistent with the role of p53 defects in promoting Myc-induced tumorigenesis (*2*, *21*, *22*).

We also identified many candidates with previously unknown roles in Myc biology. To prioritize these, we rank ordered MySL genes using a modified two-way analysis of variance that we developed to summarize the effects of all shRNAs for a given gene. Using this method, we identified eight genes exhibiting Myc synthetic lethality with a $P \leq 0.001$ (table S2). The most significant candidate from both of these analyses was the SUMO-activating enzyme (SAE) subunit 2 (SAE2/UBA2) ($P < 0.00001$), a critical component of the sole SUMO-activating enzyme necessary for SUMO conjugation to proteins (*24*). Multiple SAE2-shRNAs exhibited Myc synthetic lethality in the primary screen (fig. S5C). The primary screen also identified SAE1, the heterodimeric partner of SAE2 (table S2). Thus, we focused on SAE and the potential synthetic lethal interaction between SUMOylation and Myc.

To explore the physiological importance of the Myc-SAE2 synthetic lethal interaction, we transduced Myc-ER HMECs with two independent shRNAs targeting SAE2 or a control shRNA and measured the effect of Myc activation on HMEC proliferation. SAE2-targeting shRNAs depleted SAE2 protein (fig. S6A) and profoundly

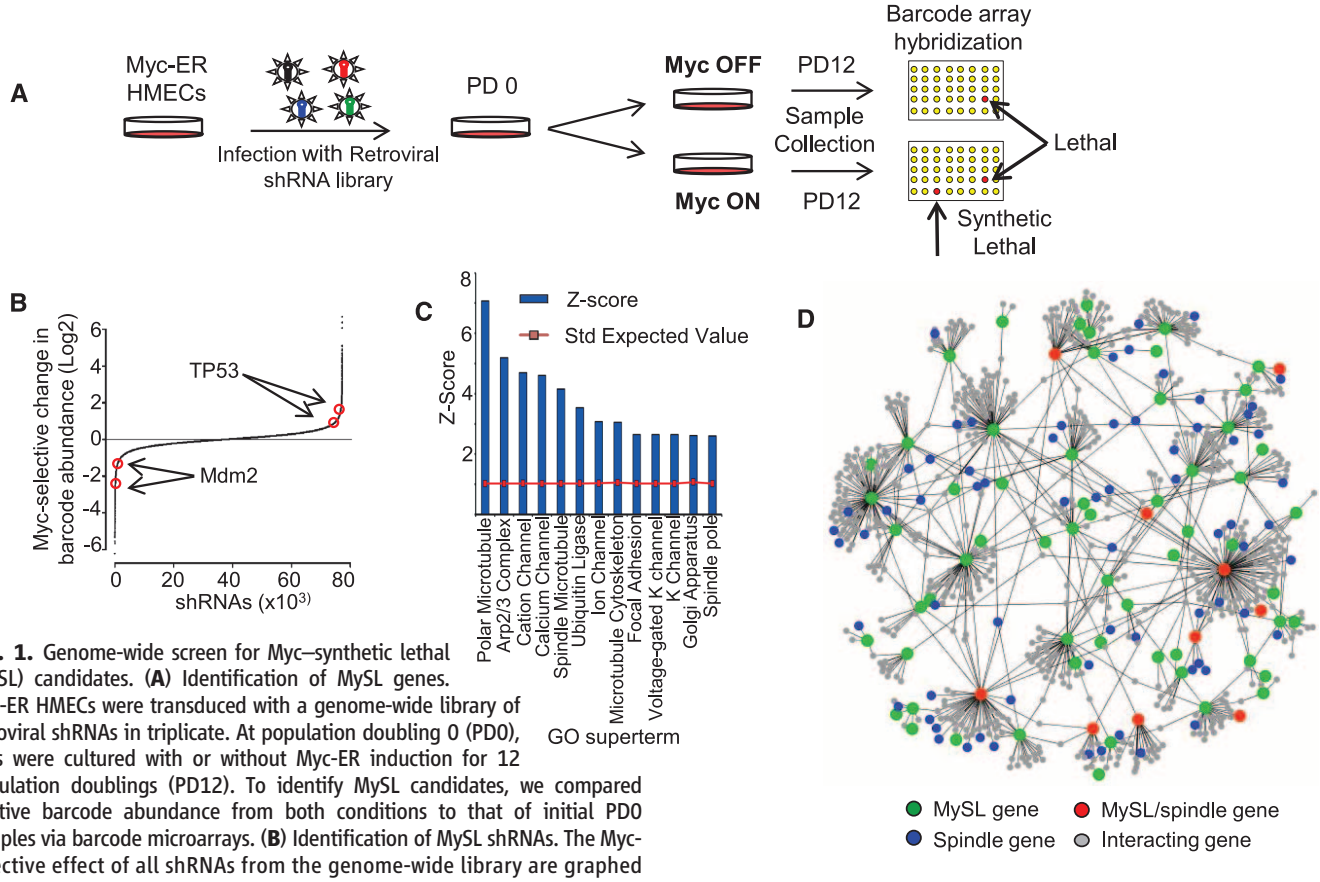

Fig. 1. Genome-wide screen for Myc–synthetic lethal (MySL) candidates. (**A**) Identification of MySL genes. Myc-ER HMECs were transduced with a genome-wide library of retroviral shRNAs in triplicate. At population doubling 0 (PD0), cells were cultured with or without Myc-ER induction for 12 population doublings (PD12). To identify MySL candidates, we compared relative barcode abundance from both conditions to that of initial PD0 samples via barcode microarrays. (**B**) Identification of MySL shRNAs. The Myc-selective effect of all shRNAs from the genome-wide library are graphed [*y* axis represents median difference between Myc-ER-on and Myc-ER-off groups (log 2)], with a ratio < −1.0 indicating a decrease of at least twofold. shRNAs are shown on the *x* axis (rank ordered by MySL effect). (**C**) Multiple cellular processes are required to tolerate the Myc oncogenic state. MySL candidates were analyzed for gene ontology (GO; Z scores for enriched cellular components and processes). (**D**) MySL proteins engage in a highly connected interaction network that regulates the mitotic spindle. Protein-protein interactions between the top 100 MySL proteins were analyzed via HPRD. Green indicates a MySL protein, blue indicates a protein with a known role in mitotic spindle function, red indicates a MySL protein with a known role in spindle function, and gray indicates a protein that interacts with a MySL protein.

increased doubling time upon Myc induction (Fig. 2A, left graph; representative images in right panels). Similar results were observed when a constitutive Myc transgene was expressed together with shSAE2, indicating that these observations are not an artifact of the Myc-ER fusion system (fig. S7). Notably, two independent SAE2 shRNAs elicited a Myc–synthetic lethal phenotype (Fig. 2A), and restoration of SAE2 protein abundance with a SAE2 wild-type cDNA suppressed the MySL phenotype of SAE2 shRNA (Fig. 2, C and D; described below), indicating that the Myc-SAE2 synthetic phenotype is not due to an RNA interference (RNAi) off-target effect. Furthermore, multiple shRNAs targeting SAE1 and the downstream SUMO E2-conjugating enzyme UBE2I (UBC9) (fig. S6, B and C) were also synthetically lethal with Myc hyperactivation (Fig. 2A, middle and right graphs), demonstrating that SUMOylation interference is synthetically lethal with hyperactivated Myc.

We next investigated whether SUMOylation is required for cells to tolerate aberrant Myc activation. Depletion of SAE2 decreased abundance of SUMO1- or SUMO2/3-modified proteins (Fig. 2B), indicating global impairment of SUMOylation in these cells. To determine whether SAE2 enzymatic activity is required to support Myc, we engineered Myc-ER HMECs with an inducible SAE2-shRNA (pINDUCER11-shSAE2) (*25*) together with constitutive shRNA-resistant cDNAs encoding wild-type (WT) SAE2, catalytically inactive SAE2-C173S, or control enhanced green fluorescent protein (eGFP). SAE2 WT and mutant cDNAs restored SAE2 to endogenous SAE2 levels (Fig. 2C). Restoration of WT SAE2 suppressed the MySL phenotype of SAE2 shRNA (Fig. 2D). However, SAE2-C173S failed to suppress the synthetic lethality of SAE2-shRNA (Fig. 2D), indicating that SAE2 enzymatic activity is required to prevent the Myc-SAE2 synthetic lethal interaction. Together, these data suggest that SUMOylation is required for HMECs to tolerate aberrant Myc signaling.

A key question is how SAE2 depletion in the presence of Myc hyperactivation impairs proliferation. This could be due to changes in the cell cycle and/or cell death, so we examined the effects of Myc hyperactivation and SAE2 depletion on these processes. In SAE2-depleted cells, Myc induction increased the number of cells with a G_2/M DNA content (fig. S8A) accompanied by a concomitant accumulation of aberrant (>2N) DNA content (Fig. 3A and fig. S8A). These cell cycle defects were followed by a significant apoptotic response (Fig. 3B and fig. S8, B and C). The increase in G_2/M and >2N DNA content is characteristic of mitotic defects known to cause mitotic catastrophe and apoptosis. A potential mitotic defect is supported by our observation that MySL genes are enriched for genes involved in the mitotic spindle (Fig. 1D), suggesting that Myc hyperactive cells might experience mitotic stress. To explore this possibility, we examined mitotic spindles in Myc hyperactive cells in the presence or absence of SAE2 depletion. As hypothesized, Myc-active, SAE2-inactive HMECs exhibited significantly more spindle defects (defects in >25% of all mitoses) than cells expressing Myc or shSAE2 alone (Fig. 3, C and D; $P = 7 \times 10^{-7}$). These defects, which included abnormal spindle number and lagging chromosomes, might explain the extensive aneuploidy and apoptosis observed. Collectively, these data suggest that the Myc-SAE2 genetic interaction results in dysregulation of the mitotic spindle, which may in turn contribute to synthetic lethality.

We next investigated how Myc hyperactivation and SAE2 depletion result in the mitotic aberrations. Myc hyperactivation induces different cellular consequences (e.g., proliferation, apoptosis, senescence) depending on the genetic and epigenetic context. If this is due to the ability of Myc to regulate distinct transcriptional programs, loss of SAE2 may lead to mitotic dysfunction by altering Myc's transcriptional program. Therefore, we used gene expression profiling to define the transcriptional effects of Myc with or without SAE2 inactivation. Myc activation alone in HMECs led to significant changes in the level

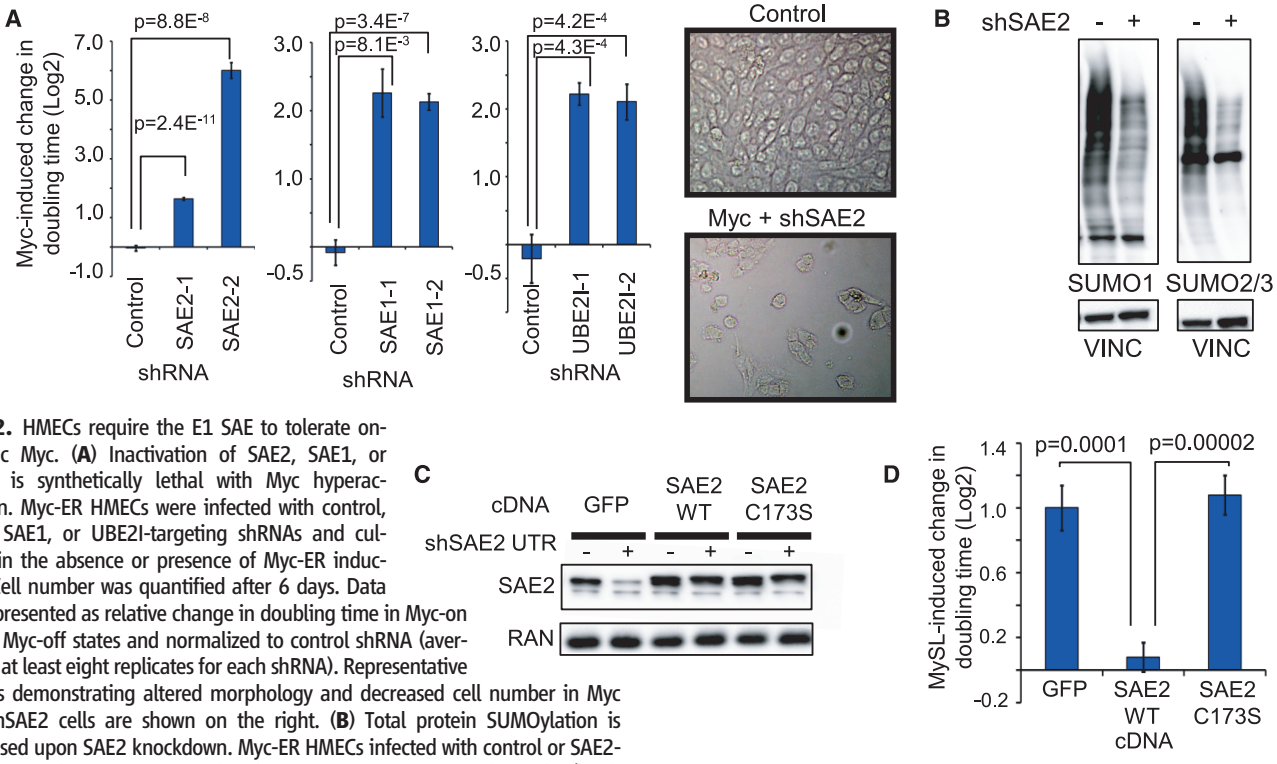

Fig. 2. HMECs require the E1 SAE to tolerate oncogenic Myc. (**A**) Inactivation of SAE2, SAE1, or UBE2I is synthetically lethal with Myc hyperactivation. Myc-ER HMECs were infected with control, SAE2, SAE1, or UBE2I-targeting shRNAs and cultured in the absence or presence of Myc-ER induction. Cell number was quantified after 6 days. Data are represented as relative change in doubling time in Myc-on versus Myc-off states and normalized to control shRNA (average of at least eight replicates for each shRNA). Representative images demonstrating altered morphology and decreased cell number in Myc and shSAE2 cells are shown on the right. (**B**) Total protein SUMOylation is decreased upon SAE2 knockdown. Myc-ER HMECs infected with control or SAE2-shRNA–encoding virus were analyzed for SUMO1, SUMO2/3, and vinculin (loading control) protein abundance. (**C** and **D**) SAE2 catalytic activity is required to tolerate Myc hyperactivation. Myc-ER HMECs transduced with a doxycycline (dox)–inducible shRNA targeting the SAE2 untranslated region (pInducer11-mir-SAE2 UTR-eGFP) were subsequently infected with a virus expressing GFP, SAE2 WT, or SAE2 C173S cDNAs. Western blots were performed to confirm depletion of SAE2 (C). Cells were cultured with or without Dox and in the absence or presence of Myc-ER induction, and cell number was quantified after 8 days. The *y* axis indicates the relative change in growth of shSAE2-expressing cells due to Myc induction in the presence of the indicated transgenes (average of eight replicates) (D). Error bars in (A) and (D) represent the SEM.

of 605 mRNAs ($P < 0.05$; Fig. 3E, left panel). Surprisingly, 22.5% (86/383) of Myc-induced transcripts are not induced or become repressed in response to Myc when SAE2 is depleted (Fig. 3E, right panel), suggesting that a portion of the Myc transcriptional response is "switched" depending on the status of SAE2 function. Because the expression of these genes switches from Myc-induced to Myc-repressed in a SAE2-dependent manner, we termed these genes SUMOylation-dependent Myc switchers (or SMS genes).

MESH analysis revealed that SMS genes were significantly enriched for regulators of the mitotic spindle ($P < 4.9 \times 10^{-12}$) (fig. S9A), and mining of published literature revealed that 17 of 86 SMS genes have been shown genetically to participate in the assembly or integrity of mitotic spindles (*26*–*31*). Each of these spindle-related genes is induced by Myc hyperactivation (Fig. 3F and fig. S9B, blue bars) but exhibits a strong SAE2-dependent switch in their Myc response (Fig. 3F and fig. S9B, red bars). These observations highlight regulation of spindle assembly as a key vulnerability in cells harboring the Myc-active, SAE2-inactive state, and suggest that SMS genes may be linchpins in the Myc-SAE2 synthetic lethal relationship. To test this hypothesis, we examined whether SMS genes known to play a role in the mitotic spindle are synthetically lethal with Myc. We found that three of the top four SMS genes (*CASC5*, *BARD1*, *CDC20*) were synthetically lethal with Myc hyperactivation, with depletion of SMS candidates leading to up to

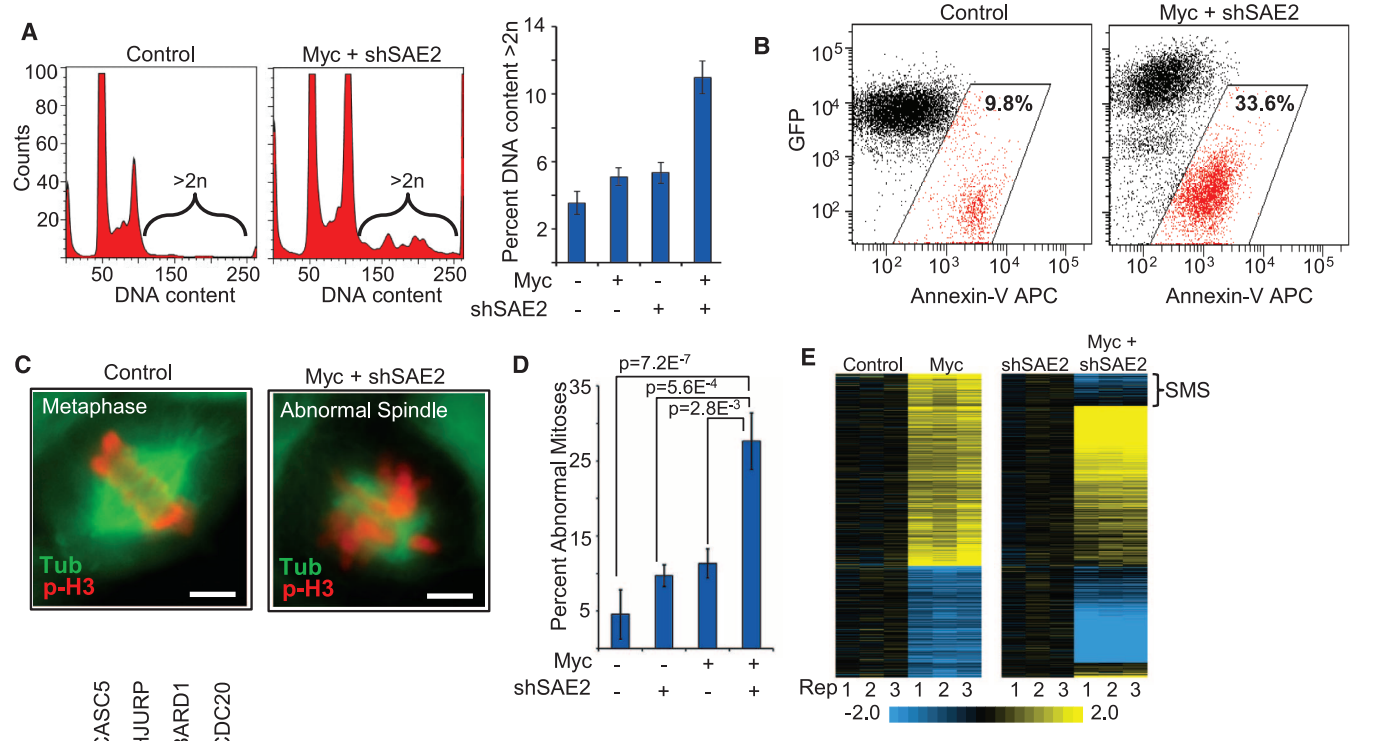

Fig. 3. Inactivation of SAE2 switches the Myc transcriptional program and dysregulates mitotic fidelity and cell viability. (**A**) Ectopic Myc activation and SAE2 inactivation lead to an increase in G_2/M cells and aberrant chromosomal content. Myc-ER HMECs transduced with inducible shSAE2 were cultured in the absence or presence of Myc-ER induction (24 hours) and with or without shSAE2 induction. Cells were analyzed for DNA content by flow cytometry (quantification of cells with >2N DNA, right panel). (**B**) Depletion of SAE2 induces apoptosis in cooperation with Myc hyperactivation. pINDUCER-mir-SAE2-eGFP Myc-ER HMECs were cultured in the absence or presence of Myc-ER-induction and with or without shSAE2 induction (48 hours). The cells were analyzed for apoptosis (Annexin-V) by flow cytometry. (**C** and **D**) Myc-SAE2 genetic interaction leads to defects in the mitotic spindle. Myc-ER HMECs transduced with inducible shRNA-SAE2 were cultured in the absence or presence of Myc-ER induction (16 hours) and with or without shSAE2 induction. Cells were stained for Tubulin (green) and phospho-H3 (red) to visualize mitotic defects. Images from (C) were quantified for both total and abnormal mitotic events (D). Data are represented as the percentage of abnormal mitoses (at least 100 mitotic events were counted per condition; *P* values are from Fisher's exact test). Scale bar, 5 μM. (**E**) Loss of SAE2 alters the transcriptional response to Myc. HMECs expressing Myc-ER and dox-inducible SAE2-shRNA were analyzed by gene expression profiling in the absence or presence of Myc-ER induction and with or without SAE2-shRNA induction. All mRNAs altered by Myc-ER induction ($P < 0.05$, twofold) are shown. The effect of Myc-ER induction on mRNA levels in the absence or presence of shRNA-SAE2 induction are shown (left and right panels, respectively). mRNAs that change their response to Myc in the presence or absence of shSAE2 are termed "sumoylation-dependent Myc switchers," or SMS genes. (**F**) Loss of SAE2 alters Myc control of spindle-regulatory genes. The effect of Myc in the absence or presence of shSAE2 (blue and red bars, respectively) is shown for the top 4 of 17 SMS genes with known roles in spindle integrity and function (see fig. S9B for the list of 17 SMS genes). (**G**) SMS genes are required to tolerate Myc hyperactivation. Myc-ER HMECs transduced with shRNAs targeting the indicated SMS genes were cultured in the absence or presence of Myc-ER induction for 6 days. Cell numbers were counted and analyzed as in Fig. 2A. Error bars in (A), (D), and (G) represent the SEM; error bars in (F) are the SE.

an eightfold Myc-selective increase in cell doubling time (Fig. 3G and fig. S10, A to C). These results suggest the SMS transcriptional subprogram may be required to tolerate the Myc oncogenic state.

The Myc-SAE2 synthetic lethal interaction suggests that Myc-driven cancers may be dependent on SAE2 and SUMOylation to support their tumorigenic phenotypes. To test this hypothesis, we first assessed the dependency of human breast cancer–derived cell lines on Myc function. The cells were transduced with control or Myc-targeting shRNA viruses and tested for clonogenicity. The clonogenicity of SUM159 and MDA-MB-231 breast cancer cells was significantly impaired by Myc depletion, whereas MCF7 and SKBR3 breast cancer cells were unaffected (Fig. 4A). We therefore classified the SUM159 and MDA-MB-231 cells as Myc-dependent and the MCF7 and SKBR3 cells as Myc-independent.

To determine whether Myc-dependent breast cancer cells are similarly dependent on SAE2 function, we transduced each breast cancer cell line with an inducible SAE2-shRNA lentivirus. SAE2 protein was significantly depleted in a doxycycline (dox)–dependent manner in each cell line (fig. S11A). SAE2 depletion decreased clonogenicity of the Myc-dependent breast cancer cells but had no effect on Myc-independent cells (Fig. 4B). Similarly, depletion of SAE2 also reduced the growth rate of Myc-dependent breast cancer cells, as determined by a multicolor competition assay (fig. S11B). By contrast, SAE2-shRNA had no effect or only modest effects on the proliferation of several normal cell types (fig. S12). Collectively, these results suggest that SAE2 is required for the growth and fitness of Myc-dependent breast cancer cells.

To determine if SAE2 is essential for the tumorigenicity of Myc-dependent cancer cells in vivo, we engineered Myc-dependent (SUM159 and MDA-MB-231) and Myc-independent (MCF7) breast cancer cells with a dox-inducible SAE2-shRNA, transplanted the cells into immunocompromised mice, and measured tumor volume over time. To circumvent the effects of SAE2 depletion on in vitro proliferation from confounding the tumorigenicity analyses, we treated the mice with or without dox only after tumor transplantation. SAE2 depletion inhibited tumor growth of Myc-dependent SUM159 and MDA-MB-231 tumors in vivo (Fig. 4C, left and middle panels), but had no significant effect on Myc-independent MCF7 tumors (right panel). SAE2 depletion also increased survival time as compared to the animals that were not treated with dox (fig. S13). Furthermore, the tumors emerging in dox-treated mice contained fewer GFP/shSAE2-expressing cells, consistent with a selection against tumor cells depleted of SAE2 during tumor growth (fig. S14). Together, these data suggest that SAE2 function is required for tumorigenicity of Myc-dependent breast cancers.

The data derived from the above model systems would predict that Myc-high human breast cancers with low expression of the SUMO-activating enzyme may exhibit a less aggressive clinical behavior. To test this hypothesis, we compiled breast cancer data sets (n = 1297 patients) for which there was gene expression data (Affymetrix U133 platform only) and a common endpoint criterion (metastatic recurrence) (*32*–*39*). Tumors were stratified on the basis of Myc expression levels, with 432 and 429 tumors defined as Myc-high and Myc-low, respectively. We then determined if levels of SAE1 and SAE2 were associated with patient outcome (metastasis-free survival) in the Myc-high or Myc-low groups. In patients with Myc-high tumors, those with

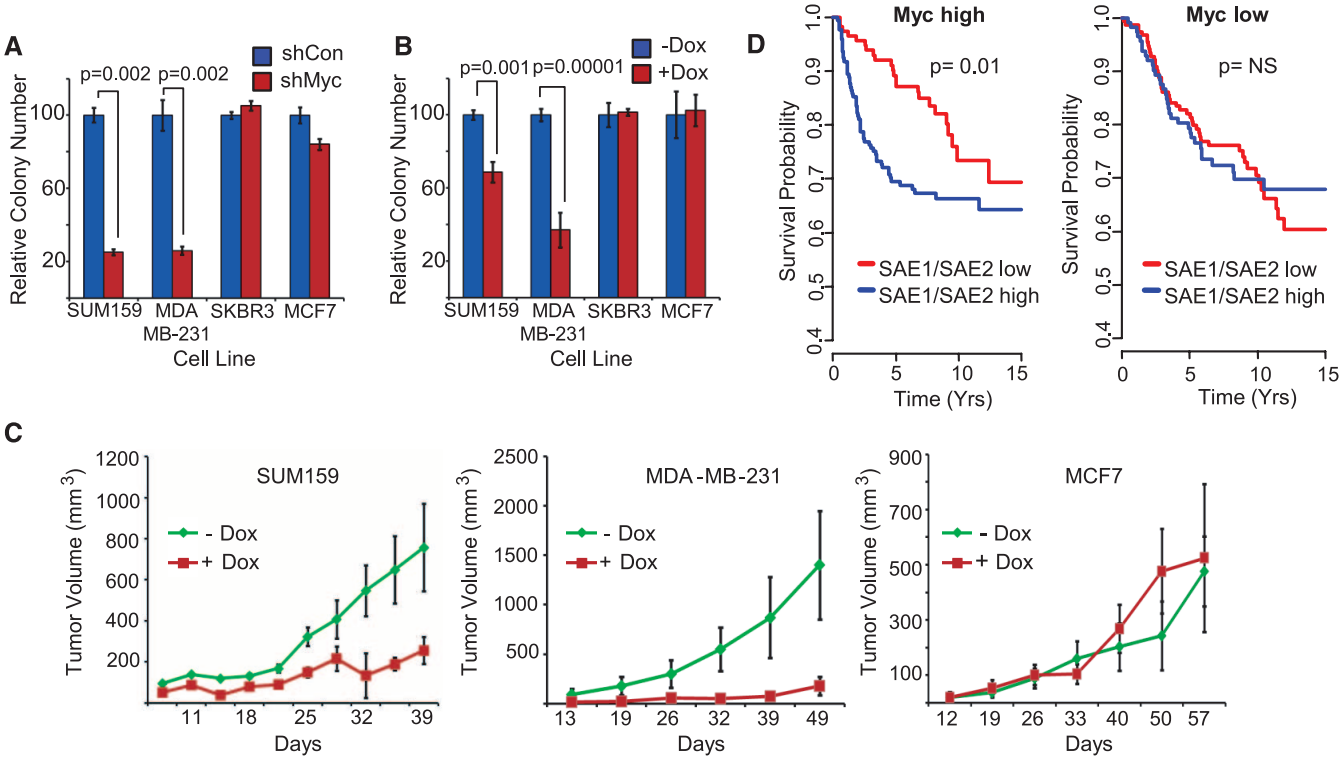

Fig. 4. The E1 SAE enzyme is required to support Myc-dependent human breast cancer cells in vitro and in mice. (**A**) Myc dependency in human breast cancer cells. Breast cancer–derived cell lines infected with control- or Myc-shRNA lentivirus were analyzed for clonogenic growth. Macroscopic colonies were quantified and normalized to control-shRNA–infected cells for each cell line. (**B**) Inactivation of SAE2 inhibits clonogenicity in Myc-dependent breast cancer cells. Breast cancer–derived cell lines infected with dox-inducible control- or SAE2-shRNA lentivirus were analyzed for clonogenic growth in the absence or presence of dox. (**C**) Inactivation of SAE2 inhibits tumorigenicity of Myc-dependent tumors. Myc-dependent (SUM159 and MDA-MB-231; left and middle panels, respectively) or Myc-independent (MCF7, right panel) breast cancer cells infected with dox-inducible SAE2-targeting shRNA lentivirus were transplanted into nude mice. Recipient animals were treated with or without dox, and xenograft volume was measured over time. (**D**) Low SAE gene expression correlates with patient metastasis-free survival selectively in Myc-high breast cancers. The expression of SAE1 and SAE2 is inversely correlated with increased metastasis-free survival in patients with Myc-high tumors (P = 0.01, log-rank test). Tumors with the highest and lowest tertile of Myc mRNA expression were considered "Myc-high" and "Myc-low," respectively. Patients with the highest and lowest tertile of SAE1 and SAE2 mRNA expression are shown as blue and red lines, respectively. Error bars in (A) to (C) represent the SEM.

lower-level expression of SAE1 and SAE2 had significantly better metastasis-free survival than those with higher SAE1 and SAE2 (Fig. 4D, left panel, $P = 0.01$ log-rank test). By contrast, lower-level expression of SAE1 and SAE2 did not correlate with outcome in patients with Myc-low tumors (Fig. 4D, right panel). This suggests that Myc hyperactivation leads to an increased dependency on SAE1 and SAE2 in human breast cancers.

We have shown here that the E1 SAE 1 and 2 enable cells to tolerate Myc hyperactivation. SAE1 and SAE2 represent enzymatic examples of the "non-oncogene addiction" concept, and their discovery illustrates the power of unbiased genetic screens for identifying potential new leads for cancer therapeutics. Loss of SUMOylation leads to substantial mitotic catastrophe and cell death by switching a subprogram of Myc transcriptional targets that support mitotic spindle function. Thus, inactivation of SAE2 mimics the mitotic disruption caused by spindle poisons, but in a genotype-specific way (i.e., selectively in cells that harbor oncogenic Myc activation). Notably, mitotic interference is a mainstay of cancer therapeutics, and agents such as taxanes that disrupt proper spindle function are used to treat a wide variety of cancers. However, a major limitation of this class of therapeutics is their toxicity to nontumor organ systems, thus limiting their therapeutic window. Our observation that inhibition of SUMOylation can mimic spindle poisons selectively in cells expressing hyperactivated Myc raises the possibility that drugs targeting the SUMO pathway may have the antitumor effects of spindle poisons with fewer side effects.

Myc promotes a balance of pro- and antitumorigenic properties, and mutations in Myc can shift this balance in pro- and anti-oncogenic Myc functions, demonstrating that distinct transcriptional (or other biochemical) functions of Myc may be segregated (*15*, *16*). We propose that the Myc transcriptional program can be shifted to favor the anti-oncogenic state. Specifically, our data suggest that the inactivation of SAE2 drives synthetic lethality with the *Myc* oncogene by altering a subprogram of Myc transcriptional targets that supports proper mitosis and thus cell viability, a subprogram we term SUMOylation-dependent Myc switchers, or SMS genes. This SMS program is highly enriched in proteins that control spindle integrity, and the Myc-SAE2 synthetic lethal interaction elicits frequent aberrations in the mitotic spindle and eventual cell death. SUMOylation may thus contribute to Myc-induced oncogenesis at least in part by cooperating with Myc to maintain expression of Myc target genes involved in mitotic fidelity. These observations highlight the idea that altering distinct subprograms of Myc transcription (by SAE2 inactivation or other mechanisms) may be exploited as a therapeutic strategy in Myc-driven cancers, and more broadly, suggest that subverting transcriptional programs may be a general strategy in treating cancers driven by oncogenic transcription factors that are notoriously difficult to target therapeutically.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1212728/DC1
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Locally Synchronized Synaptic Inputs

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Synaptic inputs on dendrites are nonlinearly converted to action potential outputs, yet the spatiotemporal patterns of dendritic activation remain to be elucidated at single-synapse resolution. In rodents, we optically imaged synaptic activities from hundreds of dendritic spines in hippocampal and neocortical pyramidal neurons ex vivo and in vivo. Adjacent spines were frequently synchronized in spontaneously active networks, thereby forming dendritic foci that received locally convergent inputs from presynaptic cell assemblies. This precise subcellular geometry manifested itself during *N*-methyl-D-aspartate receptor–dependent circuit remodeling. Thus, clustered synaptic plasticity is innately programmed to compartmentalize correlated inputs along dendrites and may reify nonlinear synaptic integration.

Cortical microcircuits are nonrandomly intertwined and form cell assemblies that fire in a spatiotemporally orchestrated manner. This patterned activity is decoded by the dendrites of downstream neurons. Dendrites are arborized and electrically active (*1*), which allows them to exhibit local nonlinear membrane potential dynamics (*2*–*4*) and to transform different spatiotemporal sequences of incoming inputs into different output patterns (*5*, *6*). Therefore, knowing whether synaptic inputs are clustered or dispersed over dendrites at a given time (fig. S1) is critical for determining the dendritic computational power (*7*, *8*); however, these dynamics are still poorly understood.