SUPPLEMENTAL FIGURE LEGENDS

Figure S1. **Mtbp induces proliferation.** Flag-tagged Mtbp or vector control was overexpressed in immortalized human retinal epithelial cells, immortalized human mammary epithelial cells, rat fibroblasts, and wild-type mouse embryonic fibroblasts. Whole cell lysates were Western blotted for Flag or as a control, β-actin. Proliferation was monitored at intervals by MTT (*$p < 0.001$, student's t-test). Error bars represent standard deviation.

Figure S2. **MTBP bound proteins TIP48 and TIP49 identified by mass spectrometry.** CHiPS data of TIP48 and TIP49 amino acid coverage. Amino acid sequences identified by the mass spectrometry analysis of the silver-stained protein bands in Figure 2A are indicated in red.

Figure S3. **Mtbp increases the pro-proliferative and transforming function of Myc.** A) Immortalized human mammary epithelial cells were infected with lentiviruses encoding YFP and RFP, Mtbp and YFP, Myc and RFP, or Mtbp/YFP and Myc/RFP. Whole cell protein lysates were Western blotted. Proliferation was measured by MTT assay (*$p=0.005$ RFP + YFP vs. Myc, **$p<0.03$ RFP + YFP vs. Mtbp, ***$p<0.0001$ RFP + YFP vs. Myc + Mtbp, $p<0.03$ Myc vs. Myc+Mtbp; student's t-tests). Soft agar assays were performed and colonies counted after 30 days (p values from student's t-tests). B) Representative cell cycle histograms of NIH3T3 cells infected with bicistronic retroviruses encoding YFP and RFP, Myc and RFP, Mtbp and YFP or Myc/RFP and Mtbp/YFP. DNA content of live cells cultured in media with 10% serum was analyzed by flow cytometry, following addition of propidium iodide to fluorescently label DNA. Dean-Jett-Fox analyses (blue line) of cell cycle profiles were performed. The percentage of cells in each phase of the cell cycle is indicated.

Figure S4. Increased MTBP and MYC expression correlates with poor colon and lung cancer prognosis. Kaplan Meier survival curves from RNA-Seq mRNA expression data from the TCGA database of colon (A) and lung (B) cancers with high *MYC* mRNA expression divided into low or high *MTBP mRNA* expression. The number (n) of patients in each group is indicated. Log rank tests determined p values. Due to many patients still early in follow up, statistical significance was not reached for both cancers.

Figure S5. Location of *MTBP* and *MYC* on 8q24. Diagram of human chromosome 8 with the red box encompassing 8q24.12 to 8q24.30. The genes encoded and their location in this region are in the black box. *MTBP* and *MYC* are boxed in yellow and are 7.2 Mb apart. (Adapted from http://genome.ucsc.edu/index.html.)

Figure S6. Myc-mediated transcription is inhibited with Mtbp knockdown. MycER expressing NIH3T3 cells were transfected with non-targeting control siRNA or *Mtbp* siRNA. Western blots of whole cell lysates were performed. MycER was activated with 4-OHT for 0 or 8 hrs in cells in media containing 10% serum. qRT-PCR was performed for the indicated Myc target genes. All samples were normalized to *β-actin.* Error bars represent standard deviation, *p=0.0113 and **p=0.0005, student's t-test.

Figure S7. MTBP knockdown inhibits proliferation. A, B) H1299 lung adenocarcinoma (A) and HEK293T (B) cells were transfected with vectors encoding non-targeting (NT) control shRNA or one of two different *MTBP*-specific shRNA (shRNA-1 or shRNA-2). Western blots of whole cell lysates were performed. Proliferation was monitored by MTT at intervals (*p<0.0001

NT vs. shRNA-1, #p<0.0003 NT vs. shRNA-2, p<0.0001 shRNA-1 vs. shRNA-2 24-96 hours for H1299 cells; *p<0.0001 for NT vs. shRNA-1 in HEK293T cells). C) NIH3T3 cells were transfected with non-targeting control siRNA or *Mtbp* siRNA. Western blots of whole cell lysates were performed. Proliferation was monitored by MTT at intervals (*p<0.001). Error bars represent standard deviation and p-values calculated by student's t-test.

Figure S8. **The C-terminal Mtbp mutant inhibits breast cancer cell expansion.** MDA-MB-231 cells expressing YFP alone (vector) or YFP with Flag-tagged central or C-terminal Mtbp mutants were subjected to MTT assays at 24 hour intervals (*p < 0.001 Vector vs. C-term, student's t-test). Error bars represent standard deviation. Western blot of whole cell lysates shown.

Figure S9. Working model of MTBP function. MTBP binds TIP48 and TIP49, which bind MYC at promoters of genes MYC transcriptionally activates that are pro-proliferative (CAD, CCDN2, NCL, and ODC). MTBP also associates with MYC at promoters of genes that MYC transcriptionally represses that are anti-proliferative (p15, p21, p27). The consequences of both of these actions are that MYC induces proliferation, transformation, and tumor development. MYC also transcriptionally upregulates MTBP (1), causing more MTBP to be available to regulate MYC-mediated transcription (feed-forward regulatory loop). When there are elevated levels of MTBP, as is the case in many human cancers (Tables 1 and S1), MYC has increased proliferative and transforming abilities and cells are less susceptible to MYC-induced apoptosis.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Expression vectors and si/shRNA

Vectors encoding Flag-tagged full-length Myc and Myc deletion mutants (Δ20-48 and Δ118-152) were kindly provided by Dr. Michael Cole (2). The MSCV-MycER-IRES-GFP bicistronic retrovirus was previously published (3, 4). A pCMV-Tag2B vector encoding Flag-tagged full length Mtbp was generously provided by Dr. Tomoo Iwakuma (KU). Flag-Mtbp mutants encoding amino acids 1-298, 299-596, or 597-894 were generated by PCR using the pCMV-Tag2B-Flag-Mtbp vector and cloned back into the pCMV-Tag2B vector. Flag-Mtbp and Mtbp mutants were cloned into the MSCV-IRES-YFP bicistronic retrovirus and Flag-Myc was cloned into the MSCV-IRES-RFP bicistronic retrovirus. Human MTBP, TIP48 and TIP49 were cloned by RT-PCR, tagged with HA or Flag, and cloned into the pCEP vector; plasmids were sequenced to verify wild-type sequence for each. Glutathione S-transferase-tagged TIP48 and TIP49 were subcloned into the pGEX vector for protein production. Human MTBP and yeast protein MGA2 were cloned into pYES-BBV for *in vitro* transcription and translation. Murine *Mtbp* siRNA was purchased (SMARTpool ON-TARGETplus, Thermo-Scientific, Pittsburgh, PA). Human *MTBP* shRNA (shRNA1 GGAGAGTGTTCTAGCTATT or shRNA2 GAAACACAGTATTACCGAG) and non-targeting control (GACTTACGAGATCAGAAAG) were used in pSuper constitutive expression constructs (Oligoengine, Seattle, WA).

Mass spectrometry analysis

LC-MS-MS analysis of the peptides was performed using a Thermo LTQ ion trap mass spectrometer equipped with a Thermo MicroAS autosampler, Thermo Surveyor HPLC pump, and nanospray source. Utilizing a "vented column" setup

(https://gygi.med.harvard.edu/index.html/node/10), the peptides were first trapped on a 100 μm x 4 cm reversed phase column (Jupiter C_{18}, 5 μm, 300 Å, Phenomonex, Torrance, CA), and then resolved using an aqueous to acentonitrile gradient on a 15 cm column analytical column pack directly into a pulled capillary emitter tip. A flow split was employed to allow for approximately 700 nl/min of flow across the columns. Peptide MS/MS spectra were acquired in a data dependent manner utilizing dynamic exclusion to minimize acquisition of redundant spectra. These spectra were queried against the protein database using Sequest (http://pubs.acs.org/doi/abs/10.1021/ac00104a020) and the resulting identifications filtered and collated into protein identifications using CHiPs.

Primers for quantitative RT-PCR

Primers for gene expression:

CAD-F – AACTGCGTAGGCTTCGACCATACA

CAD-R – AATCAATGCGGGTGAGCTCGTAGA

ODC-F – GCATGTGGGTGATTGGATGCTGTT

ODC-R – TTGCCACATTGGCCGTGACATTAC

NCL-F – ACTGGAAAGACCAGCACTTGGAGT

NCL-R – CCCTTTAGGTTTGCCATGTGGGTT.

Primers for ChIP:

CAD-F – AGTCTCTGCTGCTGCCGCCAA (5)

CAD-R – GAGAGGCGCATCACAGAGTGGGATAA (5)

NCL-F – TTTTGCGACGCGTACGAG (6)

NCL-R – ACTAGGGCCGATACCGCC (6)

ODC-F – ATTTCCCTTTTCCGCTCTCG

ODC-R – TGAACGGCAGAGCCTGTAGC

ODC Upstream-F – TTTCAGCCAGTCCAACCACC

ODC Upstream-R – CTCACCTAAGTTCTGGGACCAA

Cyclin D2-F – CATCAGGGCGCTGGTCTCT

Cyclin D2-R – TGGCGTTTCTTCACCTCCT

p15-F – TCCTAGGAAGGAGAGAGTGC

p15-R – CGCTGGCCAGACCCTCATC

p21-F – ACCGGCTGGCCTGCTGGAACT (7)

p21-R – TCTGCCGCCGCTCTCTCACCT (7)

p21 Downstream-F – ATGTTAGGCAAGTTACTTAACTTA (7)

p21 Downstream-R – CTCTTGGTAACTTCACACCAAGTT (7)

p27-F – AGCAGGTTTGTTGGCAGCA

p27-R - GAAAATGATTGACACGGCGAG

SUPPLEMENTAL REFERENCES

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