Cell Reports, Volume *30*

Supplemental Information

MYC Dysregulates Mitosis, Revealing Cancer Vulnerabilities

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Supplemental tables

Table S1. MYC regulates the expression of mitotic spindle genes, related to Figure 3 and Table S2. Twenty-seven mitotic genes expressed in a MYC-dependent manner in different model systems and datasets (**see Figure 3G**). We examined occupancy of their promoters by MYC in three cell lines according to (Walz et al., 2014) and murine B-cell tumors according to (Sabò et al., 2014). CPC – Chromosomal passenger complex. SAC – spindle assembly checkpoint. APC – Anaphase promoting complex.

		MYC bound promoters			
		Walz et al.			Sabo et al.
Gene	Function	T cells	MEFs	Pancreas cells	B-cell tumor
Aurkb	kinase, CPC, SAC, chromatin-induced microtubule stabilization	+	+	+	+
Birc5 (Survivin)	CPC, localizes TPX2 to microtubules	+	+	+	+
Bub1	kinase, SAC	-	-	+	+
Ccnb2	cyclin, activates CDK1	-	+	+	+
Cdc20	activates APC	+	+	+	+
Cdca5	sister chromatid cohesion	na	na	na	+
Cdca8	chromatin-induced microtubule stabilization	+	+	+	+
Cdt1	kinetochore-microtubule attachments	+	+	+	+
Cenpa	centromere protein	+	+	-	+
Cenpf	centromere protein	+	+	+	+
Chek1	kinase, DNA damage checkpoint	+	+	+	+
Kif11 (Eg5)	motor protein	+	+	+	+
Kif14	motor protein	na	na	na	+
Kif15	motor protein	-	-	+	+
Kif18a	motor protein, microtubule depolymerase	+	-	+	+
Kif20a	motor protein	-	+	-	+
Kif2c	motor protein, microtubule depolymerase	+	+	+	+
Kifc1	motor protein	+	+	+	-
Melk	kinase	na	na	na	+
Mybl2	transcription factor	+	+	+	+
Plk1	mitotic kinase	-	+	+	+
Psrc1	microtubule depolymerase recruitment	-	+	-	+
Stmn1	microtubule destabilization	+	+	+	+

Tpx2	acentrosomal microtubule nucleation, AURKA activation	+	+	+	+
Ttk	kinase, centrosome duplication, AURKB activation	+	+	+	+
Ube2c	ubiquitin ligase, APC	+	+	+	+
Ube2s	ubiquitin ligase, APC	+	+	+	+

Supplemental Figures

Figure S1

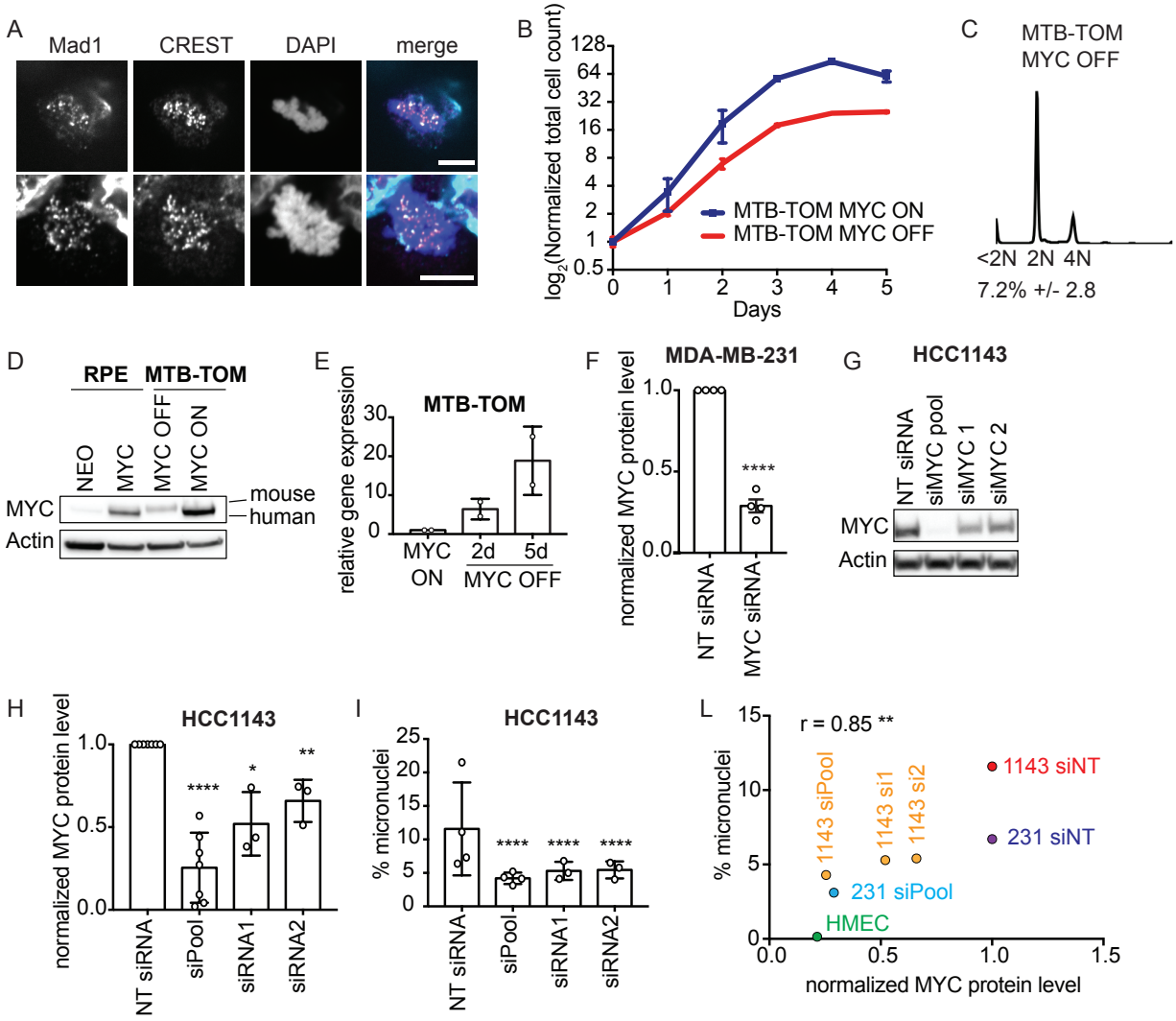

Figure S1. MYC reversibly induces CIN and characterization of the MTB-TOM cell line, related to Figure 1 and STAR Methods. A Mad1 localizes to kinetochores indicating an active SAC. Staining of RPE-MYC cells with anti-Mad1, anti-CREST (kinetochores) and DAPI (DNA). Co-localization of Mad1 and CREST staining is observed. **B** Growth curve of MTB-TOM MYC ON and MYC OFF. Mean +/- S.D., n=3. **C** Cell cycle profile of MTB-TOM MYC OFF for three days. Sub 2N DNA content, mean +- S.D., n=6. **D** Western blot of MYC in human RPE-NEO and RPE-MYC and murine MTB-TOM MYC ON and MYC OFF for three days. The anti-MYC

Y69 antibody recognizes a slightly smaller product for the human protein but has the same affinity for both mouse and human (Lehmann et al., 2012). **E** Relative expression of murine MYC measured by quantitative PCR in MTB-TOM MYC ON and MYC OFF. Mean, S.D., n=2. **F** MYC protein level in MDA-MB-231 cells three days after treatment with non-targeting (NT) siRNA or MYC siRNA normalized to beta-actin. Mean +/- S.D., unpaired t-test, n=4. **G-I** MYC protein level in HCC1143 three days after treatment with non-targeting (NT), two single (siRNA1 and 2) or pooled siRNA (siPool)**. G** Representative western blot of MYC. **H** MYC level normalized to beta-actin. Mean +- S.D., unpaired t-test, n=3-7. **I** Percent of cells with micronuclei. Mean +/- S.D., Fisher's exact test, n=849-1437. **J** Correlation of MYC protein levels and percent of HCC1143 (1143) and MDA-MB-231 (231) cells and HMEC MYC OFF with micronuclei. Correlation coefficient was computed using Pearson correlation.

* $p<0.05$, ** $p< 0.01$, *** $p<0.001$, **** $p<0.0001$.

Figure S2

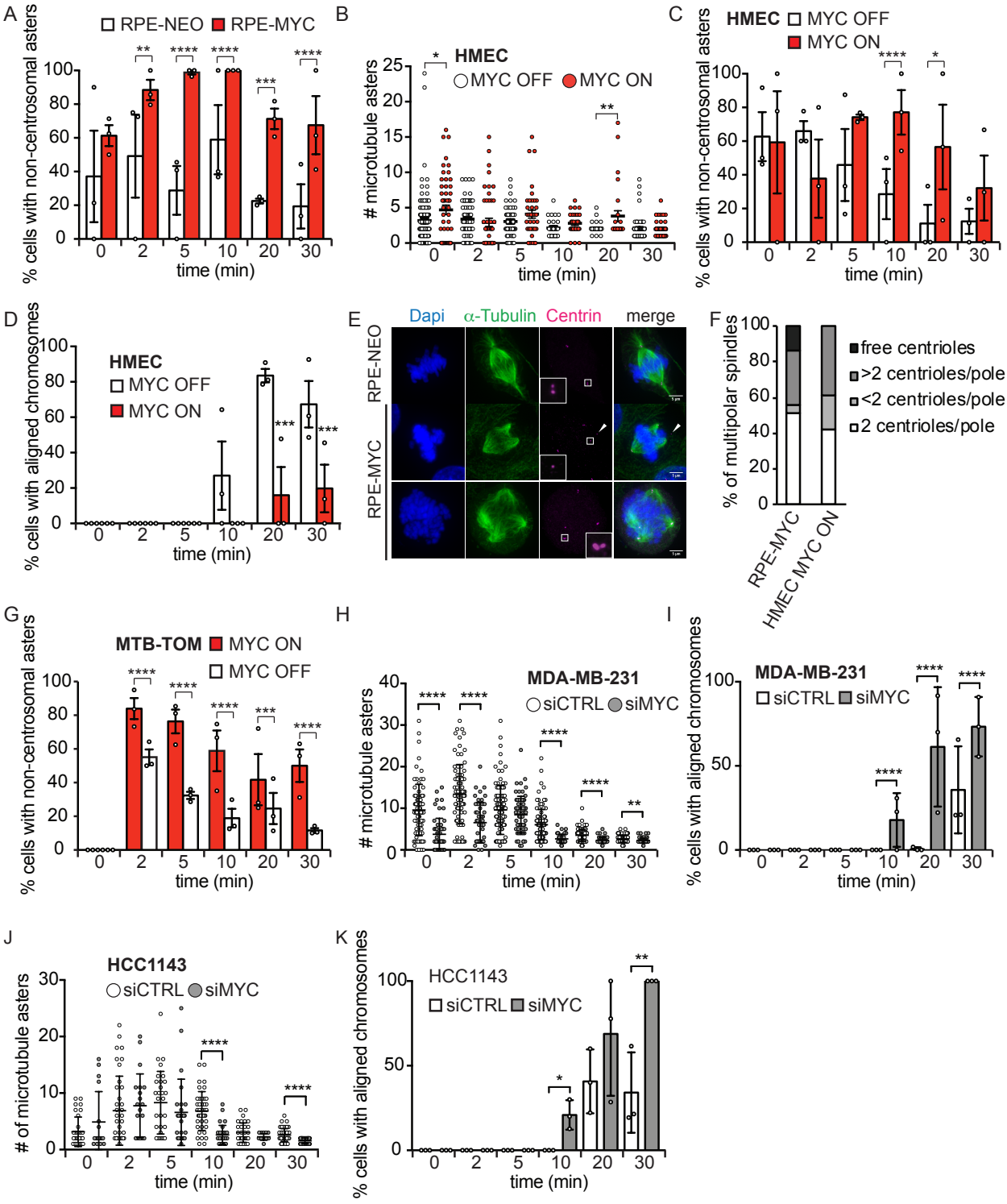

Figure S2. MYC impairs mitotic spindle formation, related to Figure 2. A-D Cells were fixed at the indicated time points after nocodazole washout and stained with anti-α-tubulin, anti-γ-

tubulin, anti-CREST (kinetochores) and DAPI (DNA). **A** Percent RPE-NEO and RPE-MYC cells with non-centrosomal microtubule asters. Mean +/- S.E.M. Fisher's exact test, n=26-99 from three independent experiments. **B** Number of microtubule asters in HMEC MYC OFF and MYC ON. Mean +/- S.E.M. t-test, n=29-101 from three independent experiments. **C** Percent HMEC cells with non-centrosomal microtubule asters. Mean +- S.E.M. Fisher's exact test, n=29-101 from three independent experiments. **D** Percentage of HMEC cells with aligned chromosomes. Mean +- S.E.M. Fisher's exact test, n=29-101 from three independent experiments. At 30 minutes, 17% $\pm$ 8.8 of HMEC MYC OFF cells enter anaphase. **E** Representative confocal micrographs of RPE-NEO and RPE-MYC cells stained with anti-α-tubulin, anti-centrin and DAPI (DNA). Insets show centrosomes with two centrioles (upper row, RPE-NEO), one centriole (middle row, RPE-MYC) and three centrioles (lower row, RPE-MYC). Arrow: free centriole that is not associated with a spindle pole. Scale bar 5 μm. **F** Percent multipolar RPE-MYC and HMEC MYC ON cells with free centrioles, two, more than two and less than two centrioles per pole quantified from confocal micrographs. Mean, n=59-141. **G** Percent MTB-TOM MYC ON and MYC OFF for three days with non-centrosomal microtubule asters. Mean +- S.E.M, Fisher's exact test, n=80-208 from three independent experiments. **H-K** Nocodazole wash-out assay in breast cancer cell lines. **H** Number of microtubule asters in MDA-MB-231 cells. Mean +- S.E.M. t-test, n=47-106 from three independent experiments. **I** Percentage of MDA-MB-231cells with aligned chromosomes. Mean +/- S.D. Fisher's exact test, n=47-106 from three independent experiments. **J** Number of microtubule asters in HCC1143 cells. Mean +/-S.E.M. t-test, n=17-51 from three independent experiments. **K** Percentage of HCC1143 with aligned chromosomes. Mean +/- S.D. Fisher's exact test, n=17-51 from three independent experiments.

* $p<0.05$, ** $p< 0.01$, *** $p<0.001$, **** $p<0.0001$.

Figure S3

A

gene 1	gene 2	p-value	Log Odds Ratio	Association
MYC	TPX2	<0.001	1.485	Tendency towards co-occurence (significant)

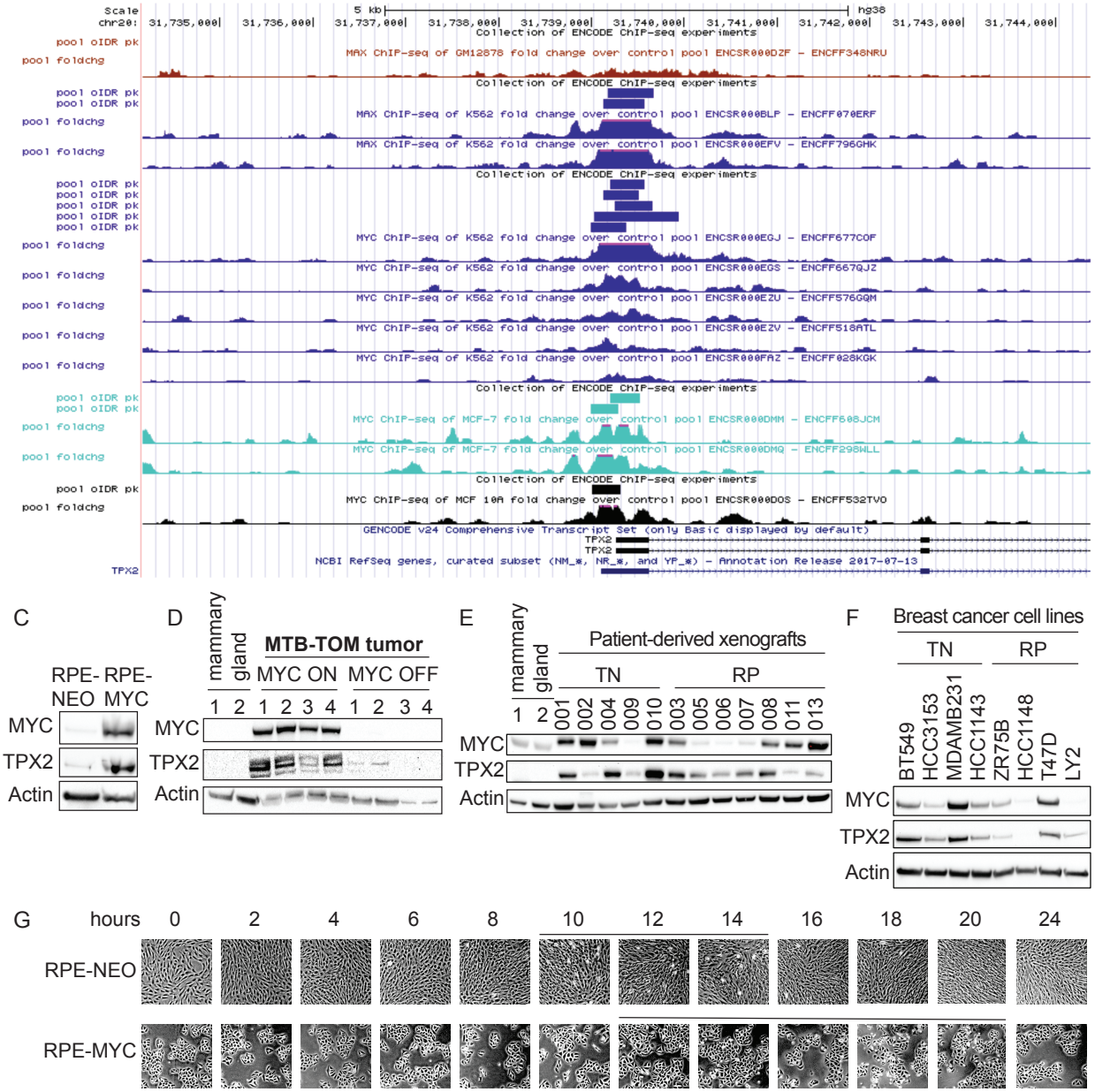

Figure S3. Correlation betweenTPX2 and MYC expression, related to Figure 4. A MYC and TPX2 mRNA correlation in human breast cancer. Data were obtained from cBioportal, Breast Invasive Carcinoma (TCGA, provisional, 960 samples). **B** ChIP sequencing data for MYC and

MAX binding to the promoter region of TPX2 (chr20:31,734,166-31,744,376) from the ENCODE project (human Dec 2013 (GRCh38/hg38) assembly). **C-F** Representative western blots of MYC and TPX2 in **C** RPE-NEO and RPE-MYC, **D** MTB-TOM tumors from four mice on doxycycline diet (MYC ON), four tumors from mice that were off doxycycline for three days (MYC OFF) and two non-tumor mammary glands (N1 and N2), **E** patient-derived xenograft tumors and mouse mammary gland (N1 and N2) and **F** triple-negative (TN) and receptor positive (RP) breast cancer cell lines. **G** Micrographs of RPE-NEO (top) and RPE-MYC (bottom) cells taken every two hours after release from a double thymidine block. The appearance of mitotic cells is indicated with a bar for each cell line.

Figure S4

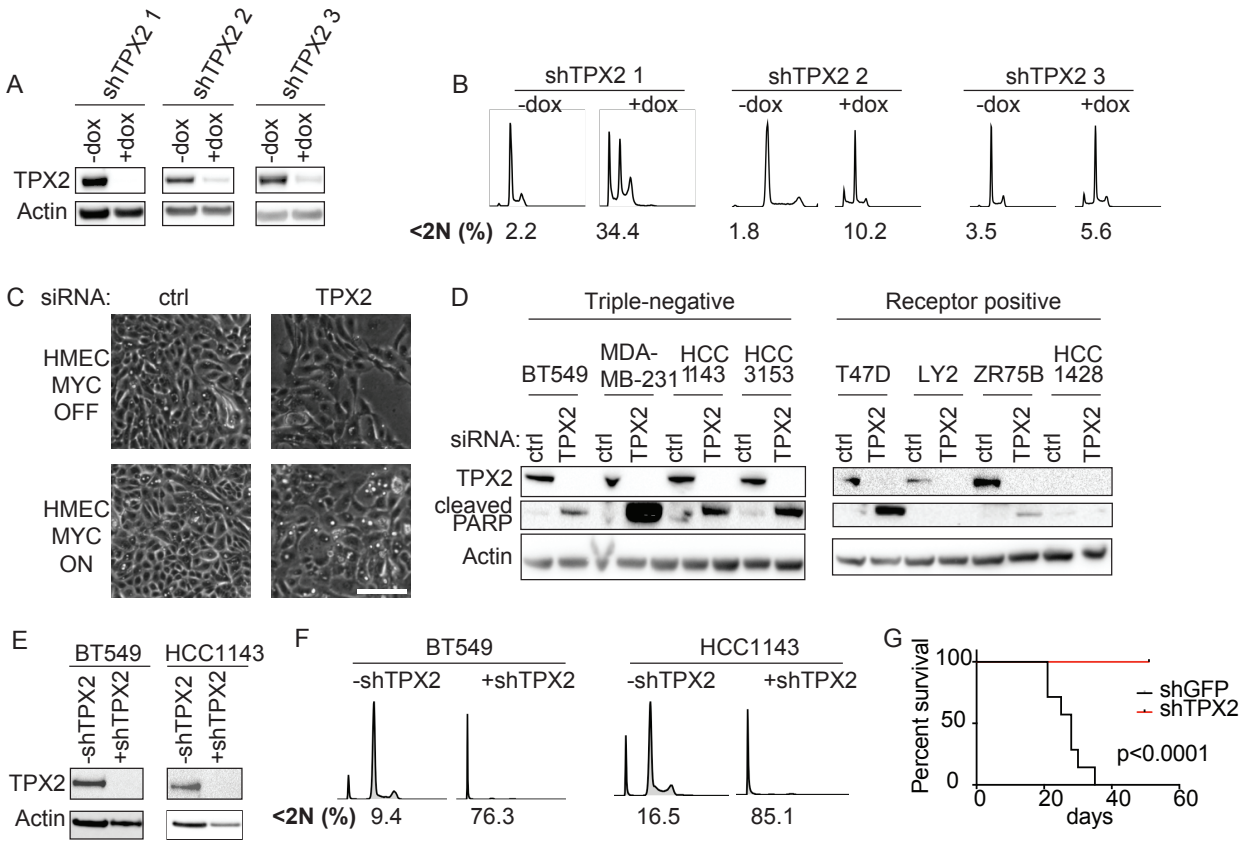

Figure S4. TPX2 is required for the survival of MYC high cells, related to Figure 5.

A and B Western blots (**A**) and cell cycle profiles with sub2N quantification (**B**) of RPE-MYC cells expressing three different doxycycline-inducible shRNAs against TPX2 (shTPX2 1 – 3) three days after adding doxycycline. **C** Micrographs of HMEC MYC OFF and MYC ON for two days, three days after transfection with control (ctrl) or TPX2 siRNA. **D** Western blot of breast cancer cell lines three days after transfection with control (ctrl) or TPX2 siRNA. **E and F** Western blots (**E**) and cell cycle profiles (**F**) of BT549 and HCC1143 cells expressing doxycycline-inducible shTPX2 1 three days after adding doxycycline. **G** Kaplan-Meier survival curve of BT549 xenograft tumor mice following the indicated shRNA expression.

Figure S5

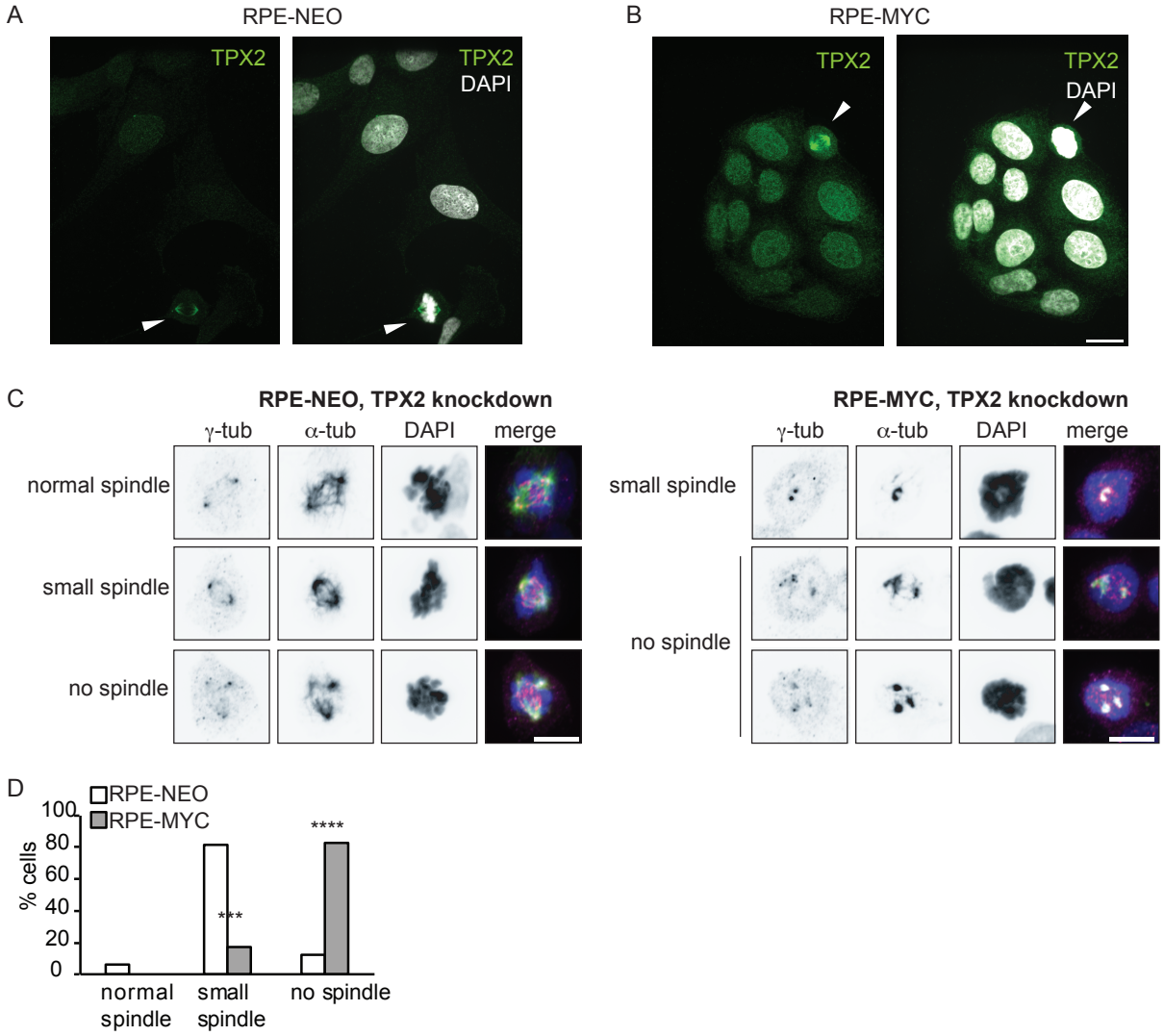

Figure S5. TPX2 protects mitotic spindle function in MYC high cells, related to Figure 6. A and B Confocal micrographs of RPE-NEO (**A**) and RPE-MYC cells (**B**), fixed and stained with an antibody against TPX2 (green) and DAPI (white). **C** Representative confocal micrographs of asynchronously growing RPE-NEO (left) and RPE-MYC (right) 18 hours after transfection with TPX2 siRNA. Cells were fixed and stained with anti-α-tubulin, anti-γ-tubulin and DAPI (DNA). Scale bar, 10 μm. **D** Percent RPE-NEO and RPE-MYC cells with normal, small or no spindles 12 hours after transfection with TPX2 siRNA. Mean, Fisher's exact test, n=16 and 23 from two independent experiments. *** $p<0.001$, **** $p<0.0001$.