Supplementary Figure 1. Cooperation of CBFB LOF and mutant PIK3CA in human breast tumorigenesis. 

(A) Percentage of transformed cells estimated by the anchorage-independent assay using MCF10A cells with indicated genotypes. (B) Colony size estimated by the anchorage-independent assay.
A

![Survival curve graph](image)

B

![Histological image](image)

C

![Survival curve graph](image)

D

![Pie charts](image)

E

![Western blots](image)

F

![Histological images](image)

G

![Gel image](image)

H

<table>
<thead>
<tr>
<th>TCGA sample</th>
<th>Symbol</th>
<th>amino_acid_change</th>
<th>tumor_vaf (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCGA-A8-A09W-01</td>
<td>CBFB</td>
<td>p.W73*</td>
<td>76.19</td>
</tr>
<tr>
<td>TCGA-BH-A1FL-01</td>
<td>CBFB</td>
<td>p.I102M</td>
<td>75</td>
</tr>
</tbody>
</table>
Supplementary Figure 2. The CP mouse model recapitulates the functional interaction between CBFB and PIK3CA mutations in humans. (A) Kaplan-Meier survival curve of different GEMMs with indicated genotypes. p-values are from the log-rank (Mantel-Cox) test. (B) H&E staining of a representative tumor from a MMTV-Cre;Cbfβ-/- mouse. (C) Kaplan-Meier survival curve of nulliparous CP and P models. CP, MMTV-Cre;Cbfβfl/+;Pik3caH1047R/+. P, MMTV-Cre;Cbfβ+/+;Pik3caH1047R/+. MMTV-Cre: MMTV-driven Cre. Cbfβfl/+ or Cbfβ+/+: heterozygous or wild type Cbfβ allele, respectively. Pik3caH1047R/+: heterozygous for the Pik3caH1047R transgene in the ROSA26 locus. p-values are from the log-rank (Mantel-Cox) test. (D) Tumor spectra of nulliparous CP and P models. MGT, mammary gland tumors; ET, eye tumors; TT/ST, tail tumors or skin tumors; L/T, lymphoma or thymoma; Other, no obvious. (E) Immunoblotting of ERα, CBFB, PIK3CA, and β-actin in MCF10A cells with indicated genotypes. MCF7 cells were used as a positive control for ERα. (F) H&E staining showing histology of multiparous P and CP tumors. The scale bar is 200 µm. (G) Loss of heterozygosity (LOH) of the Cbfβ gene in mouse mammary gland tumors. The Cbfβ+ allele, a 214bp band; the Cbfβ- (deleted) allele, a 300bp band. Shown on the left part is the PCR result of genomic DNA using passage-3 tumor cells derived from tumors. 124825, 124848, 125386, and 126851 are mouse IDs from which tumors were derived. The genotypes of the mice are shown on the top. Positive controls are PCR results of genomic DNA from mouse tails. (H) LOH of the CBFB gene in human breast tumors in TCGA. CBFB mutated allele frequencies (tumor_vaf) in human breast tumors in TCGA. Tumor_vaf around 75% suggest that about half of the tumor cells have LOH. Because the analysis requires SNV, copy number, and tumor purity data, these were the only two tumors that have these data and can be used to estimate LOH.
Supplementary Figure 3. CBFB and hnRNPK bind to mt-mRNAs. (A and B) RIP followed by real-time PCR to detect the association of CBFB (A) and hnRNPK (B) with mt-mRNAs in MCF10A cells. (C) Immunoblot showing lower endogenous protein levels of CBFB in MCF7 cells compared to MCF10A cells. (D) RIP followed by real-time PCR showing the effect of CBFB overexpression (OE) on binding of CBFB and hnRNPK to mt-mRNAs in MCF7 cells. p-values are from the Mann-Whitney test. (E) RIP followed by real-time PCR showing the effect of CBFB overexpression (OE) on binding of CBFB and hnRNPK to mt-mRNAs in BT474 cells. p-values are from the Mann-Whitney test. (F) Western blot showing the knockdown of CBFB in MDA-MB-361 cells. (G) RIP followed by real-time PCR showing the effect of CBFB knockdown (KD) on the binding of CBFB and hnRNPK to mt-mRNAs in MDA-MB-361 cells. p-values are from the Mann-Whitney test.
$\alpha$-CBFB and $\alpha$-TUFM

**A**

$\alpha$-CBFB and $\alpha$-TUFM

**B**

PIK3CA(H1047R)_OE CBFB_KO

**C**

$\alpha$-CBFB and $\alpha$-TUFM

**D**

P tumor
CP tumor

**E**

PIK3CA
CBFB
DAPI
MERGE

CP tumor
P tumor

**F**

CBFB
MT-CO1

**G**

WT
KO
KO 751

TUFM
CBFB
$\beta$-Actin

$R^2 = 0.8773$
Supplementary Figure 4. CBFB and TUFM interact in vivo. (A) PLA detecting the interaction between CBFB and TUFM in formalin-fixed paraffin embedded (FFPE) slides made from a KRAS-driven MCF10A xenograft tumor. Left, PLA with both CBFB and TUFM antibodies; right, PLA with only CBFB antibody. (B) PLA detecting the interaction between CBFB and TUFM in formalin-fixed paraffin embedded (FFPE) slides made from PIK3CA(H1047R) overexpressing (OE) and CBFB_KO driven MCF10A xenografts. (C) PLA of an FFPE slide of normal human breast tissue (US Biomax, Cat#BRN801b) using both CBFB and TUFM antibodies. (D) PLA showing the co-localization of CBFB and TUFM in the P and CP tumors. (E) Immunofluorescence showing the expression of CBFB and PIK3CA in the P and CP tumors. (F) Correlation of CBFB and MT-CO1 immunostaining signal in a P tumor. Left, an image with an area for calculating correlation; middle, histogram showing the correlation in the area in left; right, R² showing the strength of the correlation. (G) Effect of CBFB deletion on TUFM protein level in MCF10A cells. (H) Immunofluorescence showing the expression of TUFM in the P and CP tumors. (I) WB showing the effect of CBFB overexpression (OE) on the steady-state levels of mt-proteins in MCF7 and BT474 cells. (J) In situ imaging of mitochondrial translation in MCF7 cells transduced with empty vector (EV) or CBFB-expressing lentiviruses.
Supplementary Figure 5. Mitochondrial translation dysregulation renders cells dependent on elevated autophagy and mitophagy for survival. (A) Flow cytometry showing mCherry and EGFP signals in WT and CBFB_KO, clones 12 and 751 MCF10A cells. (B) Quantitative analysis of mitophagy index (yellow signal versus green signal) in Figure 6E. (C) Examples of machine learning to estimate mitophagy based on immunostaining images. (D) Electron microscopy images of WT and CBFB_KO MCF10A cells. Mt, mitochondria; M, mitophagy. (E) Live-cell propidium iodide staining to measure the effect of HCQ on cell survival of WT MCF10A cells and CBFB_KO clones (12 and 751). Left, flow cytometry; right, quantitative analysis of left. (F) Luminescence signal at Day 0 and Day 28 after HCQ treatment on CBFB_KO MCF10A (carrying a luciferase reporter) cell-derived xenografts. p-values are from the Mann-Whitney test.
Supplementary Figure 6. PIK3CA(H1047R) mutant does not alter CBFB-regulated mitochondrial translation and the downstream metabolic changes. (A) *In situ* mitochondrial translation assays using MCF10A cells with indicated genotypes. (B) Quantitative analysis of (A). p-values are from the Mann-Whitney test. NS, not significant. (C) Mitochondrial Stress test using MCF10A cells with indicated genotype. (D) Glycolysis Stress test. (E) Immunoblotting showing the effect of PIK3CA(H0147R) mutant on autophagy upregulation (ratio of LC-II to LC-I) caused by CBFB deletion. (F) IHC showing CBFB, PIK3CA, TUFM, MT-CO1 and LC3 A/B staining of FFPE slides of MCF10A xenografts driven by PIK3CA(H1047R) overexpression (OE), CBFB knockout (KO) or CBFB KO plus Pkl3CA(H1047R) OE.
Supplementary Figure 7. Mutant PIK3CA enhances the dependence to autophagy for survival. (A and B) Immunoblotting to detect the effect of PIK3CA(H1047R) mutant on autophagy inhibitors-induced apoptosis using MCF10A-derived cells. Cells were treated with 1 uM MRT68921 (A) and 10 uM SBI-0206965 (B) for 24 hours.
Supplementary Figure 8. Synergistic killing of breast cancer cells by autophagy and PIK3CA inhibitors in vitro. (A) Cell viability of MCF10A, CBFB_KO, PIK3CA(H1047R) cells treated with HCQ or BYL719 alone or in combination at the indicated concentration (in µm) for 72 hours. CI, combination index. Values less than 1 show synergy. (B) Heatmaps showing cell viability and synergy score using Bliss, HAS, and Loewe algorithms. Values greater than 20 show synergy.
Supplementary Figure 9. The synergistic effect of autophagy inhibitor and PIK3CA inhibitor on breast tumors. (A) Schematic design of HCQ and BYL719 single or combo treatment of CBFB_KO+PIK3CA(H1047R) MCF10A xenografts. (B-D), Image (B), tumor weight (C), and tumor size (D) of xenografts from CBFB_KO+PIK3CA(H1047R) MCF10A cells treated with indicated drugs. p-values are from the Mann-Whitney test. NS, not significant.