SUPPLEMENTARY DATA AND METHODS

Supplementary Methods

siRNA and shRNAs. Lincode SMARTpool siRNA targeting *YIYA* (R-189445), Lincode non-targeting control siRNAs (D-001320), and ON-TARGETplus SMARTpool siRNA targeting *CDK6* (L-003240), and *FBXW7* (L-004264) from GE Healthcare Dharmacon were used in this study. The knockdown efficiency and specificity of all siRNAs were validated either by RT-qPCR or immunoblotting. The oligonucleotides for shRNAs targeting *YIYA* were designed based on Lincode SMARTpool siRNA sequence and cloned into pLKO.1-Puro vector (Sigma), two shRNAs producing the best knockdown efficiency were used in the following functional studies. Detailed shRNA sequences were listed in **Oligonucleotide Sequences, Probes and Primers** section.

Antibodies. The following antibodies were used for immunoprecipitation and immunoblotting: anti-His tag (2365), Skp1 (2156S), C-Jun (60A8), C-myc (D84C12), CDK6(DCS83), Cyclin D3 (DCS22), PFKFB3 (D7H4Q) and Cyclin E1 (HE12) from Cell Signaling Technology; anti-phosphoserine (1553609A) and anti-phosphothreoine (1347464A) from Invitrogen; FBXW7 (ab109617), STK38 (AB56977), anti-thiophosphate ester antibody (ab92570) and RabMab (ab92570) from Abcam; GAPDH (6C5) from Santa Cruz Biotechnology; Myc-tag (4A6) from Millipore. anti-His tag (2365) from Cell Signaling Technology; anti-FLAG tag (F3165) from Sigma-Aldrich.

Plasmids and constructs. Human YIYA sequence was synthesized by GenScript and cloned into pGEM-3Z vector (Promega) for in vitro transcription. Mammalian expression vector for full-

length YIYA was constructed by sub-cloning the gene sequence into pcDNA3.1 (+) (Life technologies) or pBABE-puro (Addgene) backbones. The full-length cDNA of CDK6, FBXW7, SKP1, PFKFB3, PRMT5, and STK38 were obtained from the shRNA and ORF core of MD Anderson Cancer Center and subsequent sub-cloned into an SFB-tagged (kindly provided by J. Chen, MD Anderson Cancer Center) or Myc-tagged expression vectors (Life Technologies) using the Gateway system (Life Technologies) or pET-28a backbone (Novagen). All point or domain deletion mutants were generated by using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies).

Biotinylated RNA preparation. The *YIYA* lncRNA sequence was cloned into pGEM-3Z vector (Promega) for *in vitro* transcription using Biotin RNA Labeling Mix (Roche) and MEGAscript® Transcription Kit (Life Technologies). Biotinylated RNAs were purified by RNA Clean & Concentrator™-5 (Zymo Research).

RNA isolation, cytoplasmic/nuclear RNA Purification, and quantitative real-time PCR. Total RNA was isolated from cultured cells using the RNeasy Plus Mini Kit with QIAshredder columns (Qiagen). Cytoplasmic and nuclear RNAs were purified from 1×10^6 MDA-MB-231 cells using SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit (Fisher Scientific). The expression of YIYA and β-actin were analyzed by qRT-PCR. cDNA was prepared using the iScript Reverse Transcription Supermix (Bio-Rad). RT-qPCR was performed with iTaq™ universal SYBR® Green supermix (Bio-Rad) and detected on a CFX Connect Real-Time PCR Detection System (Bio-Rad).

Protein recombination and purification. Recombinant proteins His-CDK6, His-FBXW7, His-SKP1, and His-CCND3 wide type and their corresponding mutants were expressed in E.coli strain BL21-CodonPlus (DE3)-RIPL (Agilent Technologies) and purified using HisPur Cobalt Resin Kit (Piece). Recombinant full-length human active CDK6 + CCND3 complex was purchased from Abcam (ab84557). Recombinant proteins His-PFKFB3 (13230-H20B-50) and His-PRMT5 (11074-H18H-20) were purchased from Sino Biological Inc., GST-STK38 (H00011329-P01) was from Novus Bioligicals.

Experimental setting of mass spectrometry analysis. Tryptic peptide mixtures were analyzed using nanoflow HPLC (Proxeon Biosystems) and a linear ion trap instrument 6460 Triple Quadrupole LC/MS System (Agilent Technologies). Peptides were eluted from a 75 μm analytical column (Reprosil C18, Dr. Maisch GmbH) on a linear gradient running from 5–35% acetonitrile in 60 min and sprayed directly into the LTQ-Orbitrap mass spectrometer. Proteins were identified by tandem mass spectrometry via information-dependent acquisition of fragmentation spectra of multiple-charged peptides.

Spectra were searched against a non-redundant human protein data-base using the Mascot algorithm (version 2.2.0; Matrix Science). The following search parameters were applied: Trypsin as cleaving enzyme, peptide mass tolerance 10 ppm, MS/MS tolerance 0.8 Da, one missed cleavage allowed. Carbamidomethylation of cysteine was set as a fixed modification, and phosphorylation of serine, threonine, and tyrosine were chosen as variable modifications. Only peptides with a length of more than five amino acids were considered.

Separate LC-MS runs were performed for YIYA pulldown and CDK6/FBXW7 affinity pulldown. For each protein, ion intensities of all observed charge states of peptides belonging to

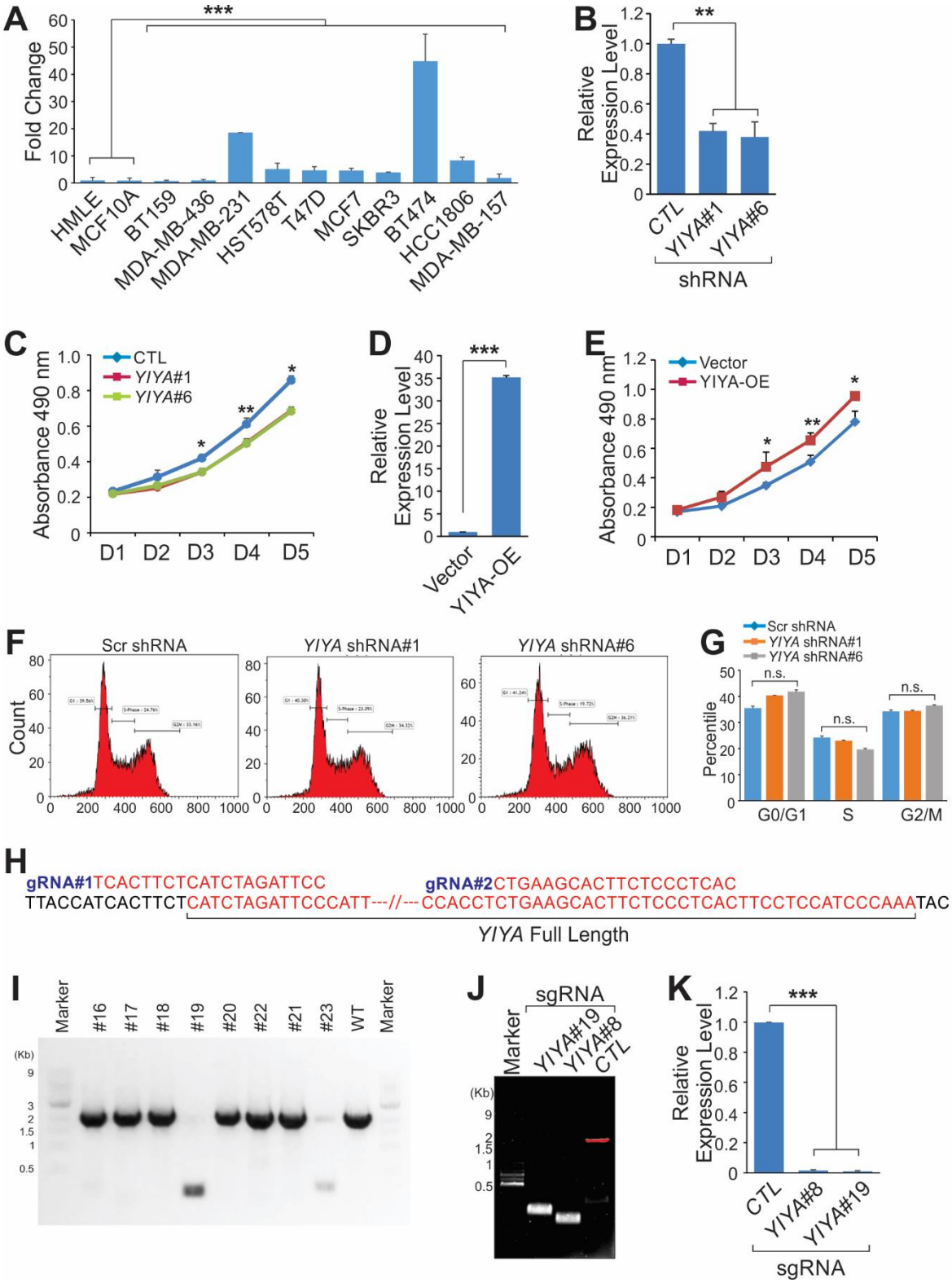
that protein were normalized to total ion intensities per run. Ratios were calculated for each peptide separately based on normalized ion intensities, and peptide ratios were subsequently averaged to obtain protein ratios. Using this information, a multiple testing correction was applied to the whole data set using the false discovery rate (FDR). Reported proteins correspond to a cut-off FDR of 5%.

Oligonucleotide Sequences (5'-3') and primers (forward and reverse)

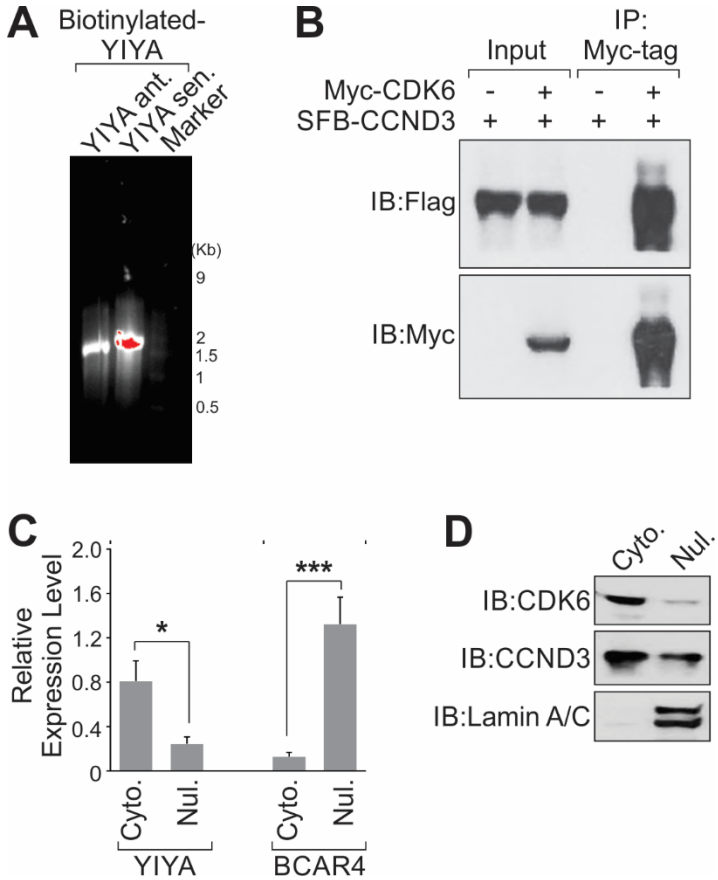
qPCR primers gene expression and RIP: YIYA (forward, CAC AAA CCC CTT GCT GCT TC; reverse, CTT GGC TGA GAG GAT CCG AC), B2M (forward, AGA TGA GTA TGC CTG CCG TG; reverse, TCA TCC AAT CCA AAT GCG GC). siRNA: YIYA SMARTpool (CCA AAU AAU AGG GUA AGU A; ACA AAU GGG AUC CGC UGA A; CAU AUA GUU UCU AGA ACG U; UGA GAG UGU UUG AGU CGG A).

shRNA: YIYA #1 (CAG GCA GAG AGA TAA TAA A); YIYA #2 (CTG CCA GCA AAG CAC TAA T); YIYA #3 (CCC AGG CAG AGA GAT AAT A); YIYA #4 (GAG AGG ACT TCG ACT TCA C). YIYA #5 (GGA AGG AAT GTT ACC CTT A); YIYA #6(TAG ATC AGG TAG ACA CGT C); YIYA #7 (GTG GCA GAT GGA GAA GTT G); YIYA #8 (CAG TCA GTT GAT AGA TGA T). MISSION® pLKO.1-puro Non-Target shRNA Control (targeting no known genes from any species) was purchased from Sigma-Aldrich. The two CRISPR guide sequences targeting the YIYA are: gRNA1: GGAATCTAGATGAGAAGTGA and gRNA2: GTGAGGGAGAAGTGCTTCAG. PCR using primers that flank the gRNA target sites (F1: CTTGAGTGCATCCACTTGTCTC; R1: TCTCTGGCACATAGTCAGCACT).

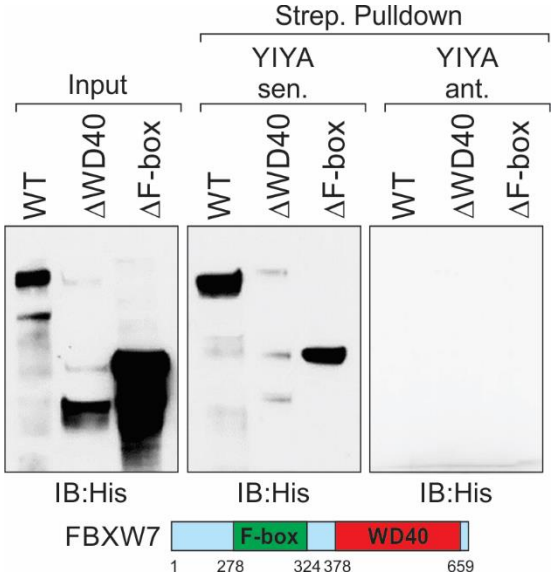
Supplementary Figures and Figure Legends



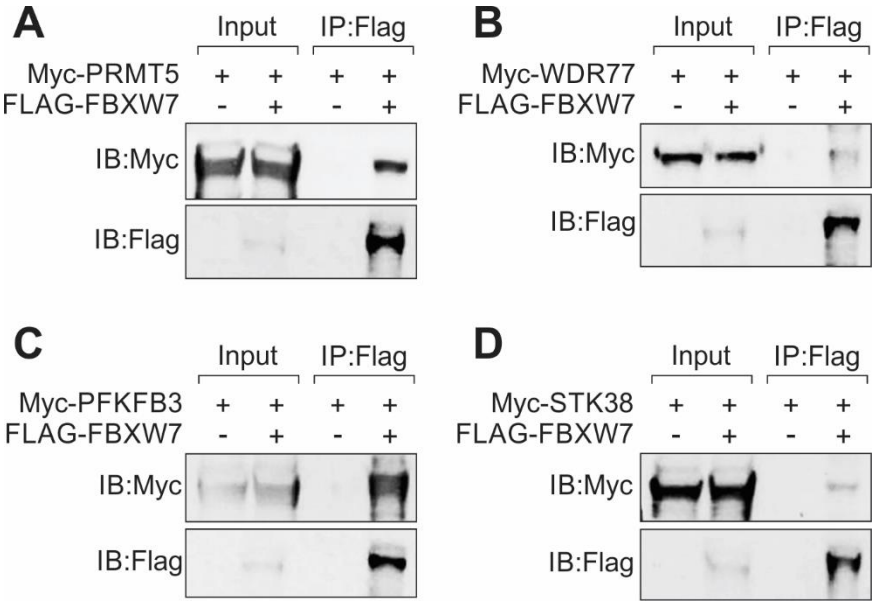
Supplementary Figure 1. YIYA promotes breast cancer growth. **A**, RT-qPCR determination of YIYA expression in a panel of breast cancer cell lines. **B**, RT-qPCR determination of YIYA expression in MDA-MB-231 cells harbors indicated shRNAs. **C**, OD490 absorbance of MDA-MB-231 cells harbors indicated shRNAs. **D**, RT-qPCR determination of YIYA expression in MDA-MB-231 cells with YIYA overexpression (OE). **E**, OD490 absorbance of MDA-MB-231 cells expressing YIYA or blank vectors. **F** and **G**, Percentage of G1, S and G2/M phase of MDA-MB-231 cells harboring indicated shRNAs. **H**, sgRNAs design for YIYA gene. **I,** PCR of *YIYA* genes in indicated single cell clones harboring YIYA sgRNAs. **J**, PCR of *YIYA* genes using chromatin extracted from YIYA parental or indicated KO cells. **K,** RT-qPCR determination of YIYA expression in MDA-MB-231 cells harbors indicated sgRNAs. Error bar, SEM, n=three independent experiments (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, student t-test).



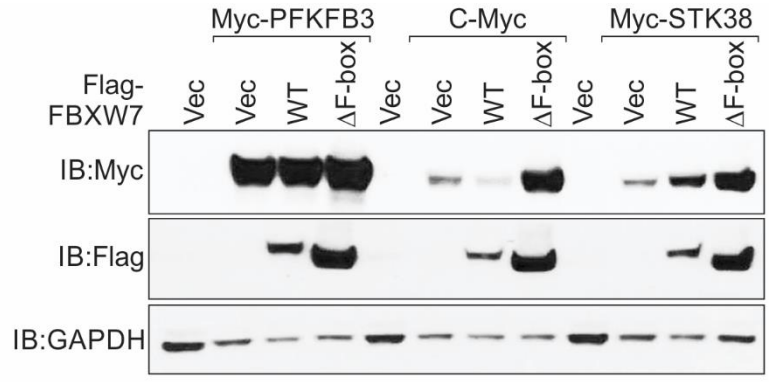
Supplementary Figure 2. YIYA associates with CDK6 and SCF complex. **A**, RNA agarose gel electrophoresis of YIYA sense (sen.) and anti-sense (ant.) transcripts. **B**, Immunoprecipitation followed by immunoblotting detection using indicated antibodies in MDA-MB-231 cells expressing indicated constructs. **C,** RT-QPCR detection of YIYA expression level in cytosol (Cyto.) and nuclear (Nul.) fractionation of MDA-MB-231 cells. **D,** Immunoblotting detection using indicated antibodies in cytosol (Cyto.) and nuclear (Nul.) fractionation of MDA-MB-231 cells. Error bar, SEM, n=three independent experiments (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, student t-test).



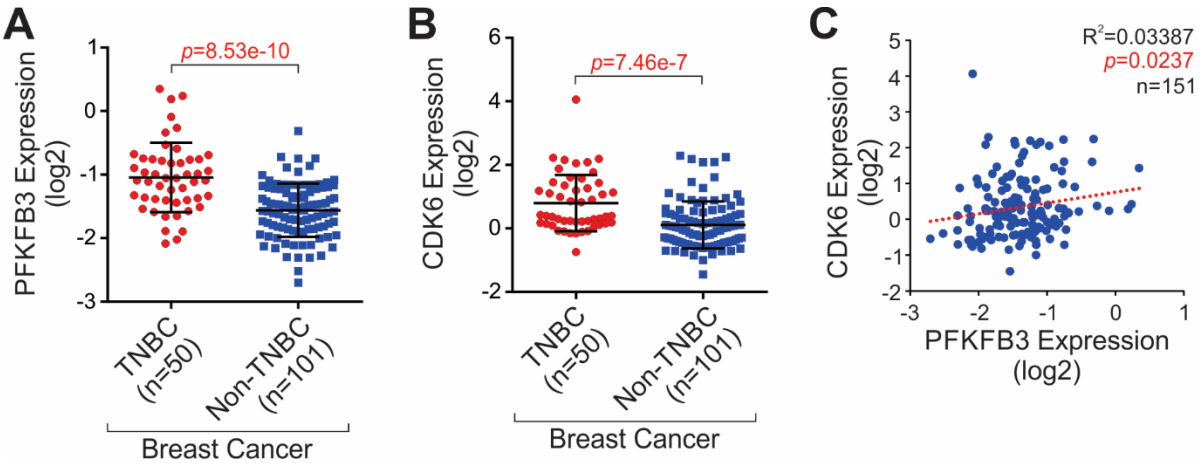
Supplementary Figure 3. YIYA associates with FBXW7. Streptavidin (Strep.) pull-down followed by immunoblotting detection using recombinant FBXW7 WT, ΔWD40, or ΔF-box in the presence of biotinylated YIYA sense or anti-sense. Bottom panel: graphic illustration of domain structure of FBXW7.



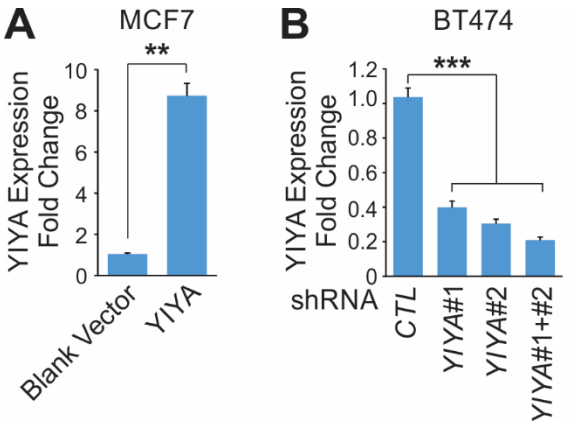
Supplementary Figure 4. FBXW7 associates with PFKFB3 and STK38. **A-D,** Immunoprecipitation followed by immunoblotting detection using indicated antibodies in MDA-MB-231 cells expressing FLAG-tagged FBXW7 and indicated constructs.



Supplementary Figure 5. PFKFB3 is not subjected to SCF complex-mediated protein degradation. Immunoblotting detection using indicated antibodies in MDA-MB-231 cells expressing indicated constructs.



Supplementary Figure 6. Correlation between CDK6 and PFKFB3 in breast cancer. **A** and **B,** Expression status of PFKFB3 (A) or CDK6 (B) in Gluke Breast, Oncomine. **C,** Pearson's correlation between the expression of PFKFB3 and CDK6 in breast cancer tissues, n=151 tissues.



Supplementary Figure 7. YIYA regulates breast cancer cell glycolysis. **A** and **B,** RT-QPCR detection of YIYA in MCF7 cells expressing indicated constructs (A) or BT474 cells harboring indicated shRNAs (B). Error bar, SEM, n=three independent experiments (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, student t-test).