



Supplemental Material to:

**Fred Lozy, Xiaofeng Cai-McRae, Irina Teplova, Sandy Price,
Anupama Reddy, Gyan Bhanot, Shridar Ganesan,
Alexei Vazquez, and Vassiliki Karantza**

**ERBB2 overexpression suppresses stress-induced
autophagy and renders ERBB2-induced mammary
tumorigenesis independent of monoallelic Becn1 loss**

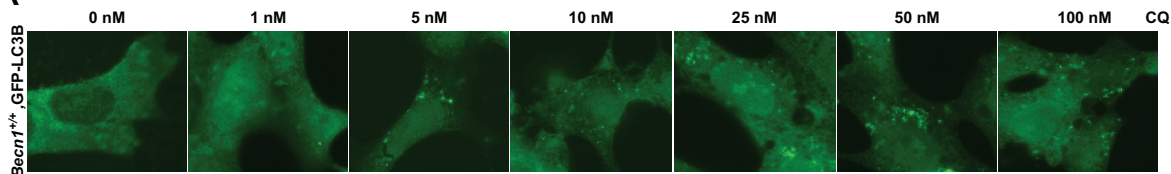
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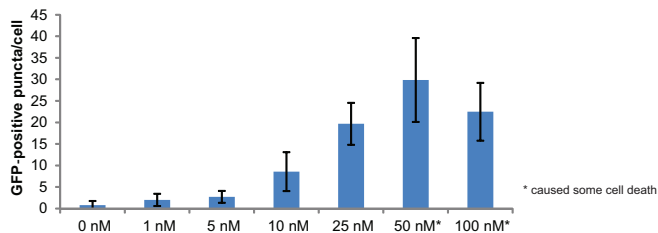
Figure S1

A



B

Bafilomycin A₁ titration



C

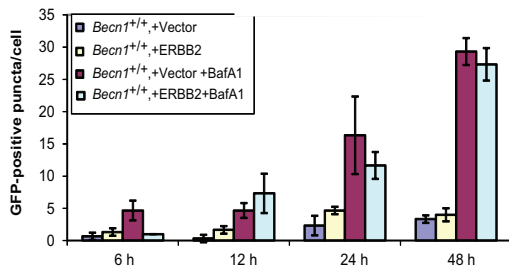


Figure S1. Bafilomycin A₁ titration to saturation of autophagic flux inhibition in *Becn1*^{+/+} iMMECs. **(A)** GFP-fluorescence microscopy of EGFP-LC3B-expressing *Becn1*^{+/+} iMMECs after 3 h of treatment with increasing concentrations of bafilomycin A₁ (0 to 100 nM) in regular culture medium. **(B)** Autophagy quantification of (A) based on number of GFP-fluorescent puncta per cell. Each data point is an average of triplicate experiments ± SD after quantifying puncta in 100 cells per experiment. **(C)** Autophagy quantification in *Becn1*^{+/+} iMMECs maintained in regular culture medium, in the absence or presence of 25 nM BafA₁, at 6 to 48 h post-transfection with a ERBB2-expressing or vector control plasmid.

Figure S2

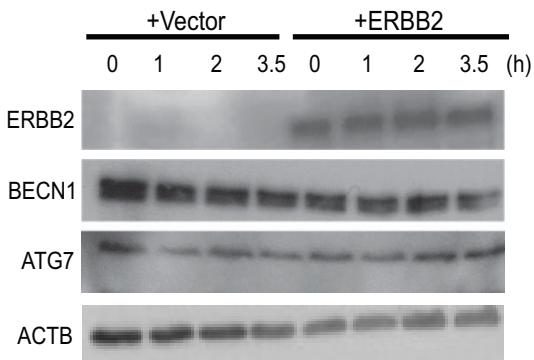


Figure S2. ERBB2 overexpression inhibits stress-induced autophagy without affecting autophagy regulator protein levels. BECN1, ATG7 and ACTB western blots of whole cell protein lysates from *Becn1*^{+/+} iMMECs transiently expressing ERBB2 under nutrient deprivation (Hanks medium) for 0 to 3.5 h.

Figure S3. Gene_Signatures

Gene signature name	Source	Gene list
Autophagy	GO:0006914	LRRK2,MTDH,HTR2B,DAP,BECN1,NPC1,TM9SF1,CTSB,LAMP1,GABARAPL2,WDR45L,DRAM1,MAP1LC3B,RB1CC1,ULK2,VMP1,KIAA1324,CLN3,WIPI1
Fatty acid beta-oxidation	GO:0006635	ACADM,ACADVL,ACADL,ETFDH,ACOX2,EHHADH,HADHB,HSD17B4,PEX7,SLC27A2,MUT,CPT2,CPT1A,HADH,MCEE,BDH2,FABP1,LONP2,ACOX3,ACAA1,SCP2
Glycolysis	GO:0006096	HIF1A,ENO2,ENO1,HK1,HK2,PGK1,PFKFB3,PFKFB4,PFKP,PKM2,HKDC1,GPI,PGAM1,LDHB,LDHA,OGDHL,DLAT,TPI1,PDHA1,DDIT4
Oxidative phosphorylation	GO:0006119	FXN,UQCRH,UQCRB,UQCRC2,ATP5D,UQCRC1,ATP5C1,MSH2,DLD,NDUFA4,NDUFAB1,NDUFA1,NDUFB5,NDUFB3,NDUFC1,NDUFA2,NDUFB6,NDUFA3,NDUFB4,NDUFB2,NDUFA7,NDUFC2,NDUFB10,NDUFB7,NDUFA8,NDUFV3,NDUFA6,NDUFA5,NDUFA9,NDUFAF1,NDUFB9,SDHAF2,UQCR10
Proliferation	http://www.broadinstitute.org/gsea/msigdb/cards/BENPORATH_PROLIFERATION.html	PRC1,CTPS,DBF4,DTYMK,S100A9,SNRPD1,TTK,AURKA,PTTG1,COX5A,COX4NB,APOBEC3B,CDCA7,H2AFV,PRIM2,H2AFZ,CDCA5,ASPM,TMEM14A,GTPBP4,DTL,NUDT5,MND1,TMSB10,MAD2L1,RFC4,SPAG5,HRASLS,CSTB,STMN1,NFE2L3,SNRPG,CTSL2,GGCT,NEK2,PPIL1,ANLN,MYBL2,PDSS1,PSMB4,CSE1L,CDC123,IDH2,PPPDE1,TP53BP2,GINS3,GARS,S100A11,NUF2,ATAD2,NDC80,RAD54L,SUV39H2,GART,HDAC2,CNIH4,PCNA,RIT1,HSPA14,PARP1,PDCD5,CHAF1B,FAM54A,DAP3,FAM3C,EZH2,DEK,CDT1,MAGOHB,FANCE,RANBP1,TOP2A,FANCA,LBR,KDELR2,CDK1,SLC25A5,MRPL9,PBK,MCM2,MC M3,SRPK1,RAD51,ANKRD27,PFDN2,RRM2,GNB4,KPNA2,UBE2T,CKS1B,NDUFB5,NMI,SHFM1,RDX,TYMS,CENPA,NCAPG,BUB1,GPSM2,PTS,EXO1,CENP N,GDI2,CENPM,RAD51AP1,PDCD10,MSH2,CEBPG,CENPF,BIRC5,CENPE,CDKN3,RACGAP1,MLF1IP,CCNB2,ILF2,TFRC,CKS2,LRP8

Figure S3. Gene_Signatures. Gene signature list and source of individual gene signatures.

Figure S4. Figure 9 statistical analysis.

		C	D	E	F	G	H	I	J	K
		Fraction of samples with signature up-regulation within subtype					Enrichment p-value of samples with signature up-regulation within subtype (data for Fig. 9)			
	Signature	ERBB2-/BECN1-	ERBB2-/BECN1+	ERBB2+/BECN1-	ERBB2+/BECN1+		ERBB2-/BECN1-	ERBB2-/BECN1+	ERBB2+/BECN1-	ERBB2+/BECN1+
Decremoux et al 2011	Proliferation	0.64	0.25	0.54	0.59		0.00	1.00	0.16	0.12
	Glycolysis	0.48	0.15	0.37	0.44		0.00	1.00	0.30	0.13
	Oxidative phosphorylation	0.31	0.32	0.28	0.44		0.64	0.58	0.84	0.11
	Fatty acid beta-oxidation	0.14	0.47	0.20	0.41		1.00	0.00	0.98	0.16
	Autophagy	0.16	0.51	0.39	0.67		1.00	0.01	0.62	0.00
	ER-	0.63	0.15	0.48	0.37		0.00	1.00	0.08	0.66
	PR-	0.73	0.41	0.65	0.52		0.00	1.00	0.13	0.80
	Triple-	0.63	0.14	-	-		0.00	1.00	1.00	1.00
Hatzis et al 2011	Signature	ERBB2-/BECN1-	ERBB2-/BECN1+				ERBB2-/BECN1-	ERBB2-/BECN1+		
	Proliferation	0.54	0.35				0.00	1.00		
	Glycolysis	0.47	0.27				0.00	1.00		
	Oxidative phosphorylation	0.36	0.59				1.00	0.00		
	Fatty acid beta-oxidation	0.09	0.49				1.00	0.00		
	Autophagy	0.24	0.48				1.00	0.00		
	ER-	0.57	0.18				0.00	1.00		
	PR-	0.64	0.34				0.00	1.00		
Triple-	0.52	0.16				0.00	1.00			
Servant et al 2011	Signature	ERBB2-/BECN1-	ERBB2-/BECN1+	ERBB2+/BECN1-	ERBB2+/BECN1+		ERBB2-/BECN1-	ERBB2-/BECN1+	ERBB2+/BECN1-	ERBB2+/BECN1+
	Proliferation	0.56	0.31	0.53	0.33		0.00	1.00	0.12	0.84
	Glycolysis	0.37	0.13	0.39	0.47		0.00	1.00	0.04	0.06
	Oxidative phosphorylation	0.24	0.27	0.16	0.13		0.53	0.15	0.94	0.91
	Fatty acid beta-oxidation	0.10	0.40	0.08	0.13		1.00	0.00	1.00	0.91
	Autophagy	0.11	0.28	0.13	0.33		1.00	0.00	0.92	0.16
	ER-	0.43	0.04	0.34	0.13		0.00	1.00	0.05	0.88
	PR-	0.59	0.20	0.63	0.47		0.00	1.00	0.00	0.40
Triple-	0.43	0.03	-	-		0.00	1.00	1.00	1.00	
Sabatier et al 2012	Signature	ERBB2-/BECN1-	ERBB2-/BECN1+	ERBB2+/BECN1-	ERBB2+/BECN1+		ERBB2-/BECN1-	ERBB2-/BECN1+	ERBB2+/BECN1-	ERBB2+/BECN1+
	Proliferation	0.59	0.41	0.50	0.62		0.00	0.98	0.54	0.24
	Glycolysis	0.42	0.25	0.56	0.62		0.04	1.00	0.07	0.05
	Oxidative phosphorylation	0.44	0.50	0.25	0.54		0.69	0.12	0.98	0.37
	Fatty acid beta-oxidation	0.14	0.46	0.13	0.38		1.00	0.00	0.98	0.35
	Autophagy	0.26	0.56	0.38	0.62		1.00	0.00	0.74	0.12
	ER-	0.58	0.26	0.50	0.54		0.00	1.00	0.37	0.30
	PR-	0.55	0.42	0.44	0.31		0.04	0.95	0.73	0.94
Triple-	0.39	0.15	-	-		0.00	0.99	1.00	1.00	

Figure S4. Figure 9 statistical analysis. The fraction of samples manifesting significantly upregulated or downregulated expression for a given gene signature across breast cancer gene expression databases and breast cancer cohorts, defined by ERBB2 and *BECN1* status, was computed with Gene Set Enrichment Analysis (GSEA) (columns C to F). Statistical significance (*P*-value) was calculated by Fisher exact test (columns H to K).