

Supplementary Materials

Haploinsufficiency Interactions between RALBP1 and p53 in ERBB2 and PyVT Models of Mouse Mammary Carcinogenesis

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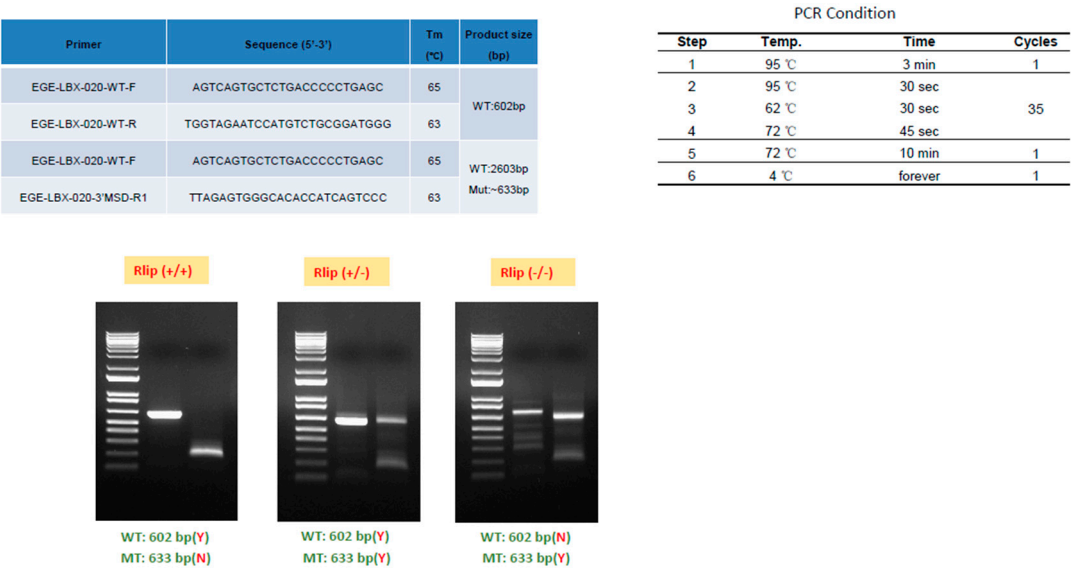


Figure S1. Genotyping details for *Ralpb1* wild-type and knockout genes as optimized by Biocytogen. Panels show the primer sequences, PCR thermal cycling protocol, and representative agarose gel images for each genotype.

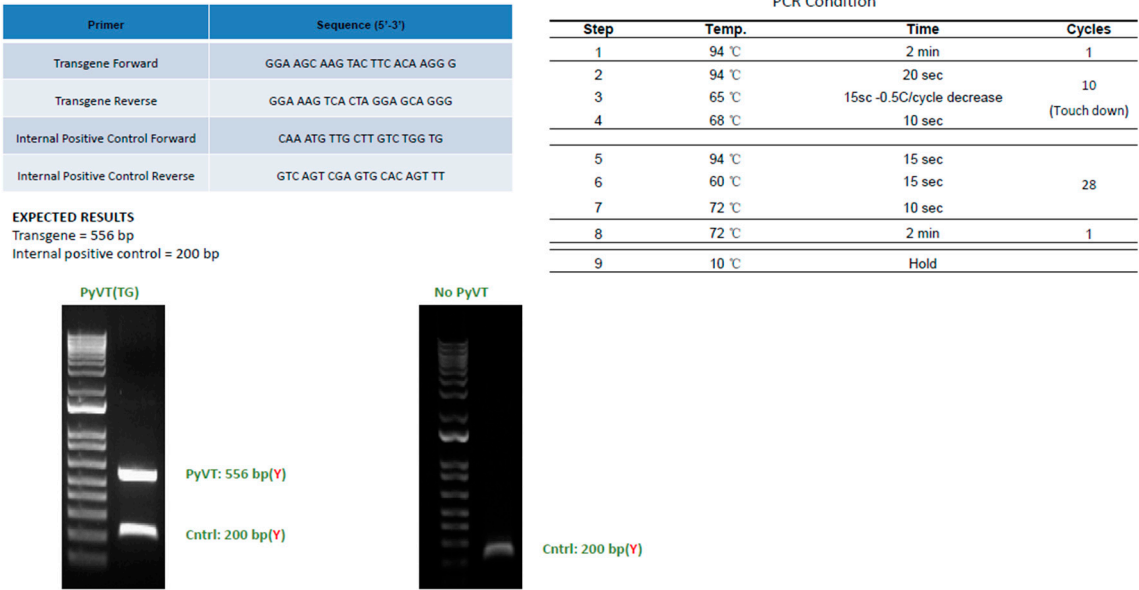


Figure S2. Genotyping details for the PyVT transgene as optimized by Jackson Laboratories. Panels show the primer sequences, PCR thermal cycling protocol, and representative agarose gel images for each genotype.

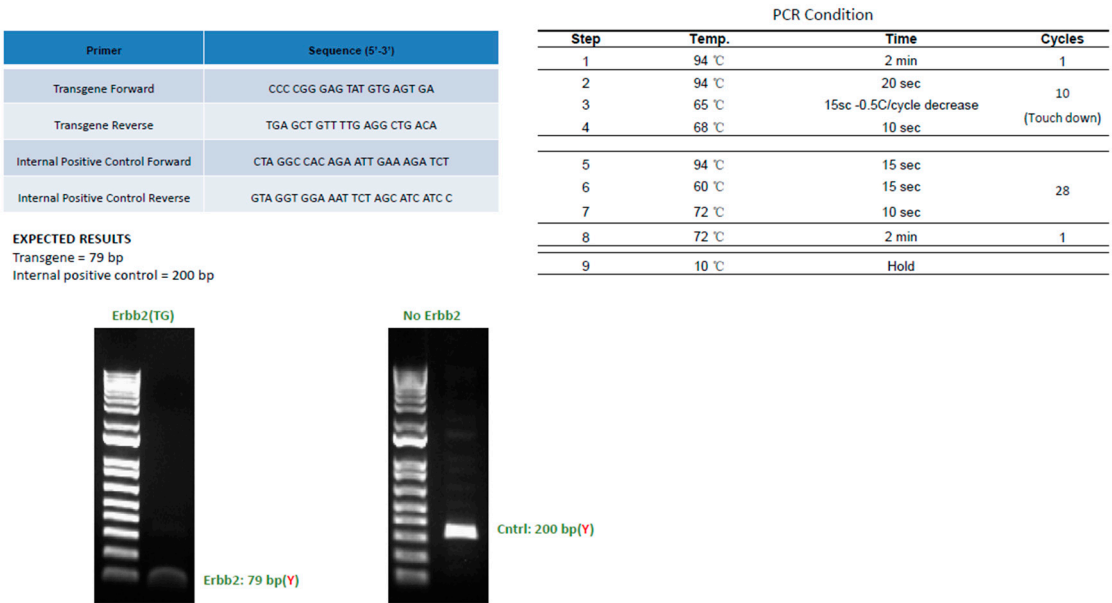


Figure S3. Genotyping details for the Erbb2 transgene as optimized by Jackson Laboratories. Panels show the primer sequences, PCR thermal cycling protocol, and representative agarose gel images for each genotype.

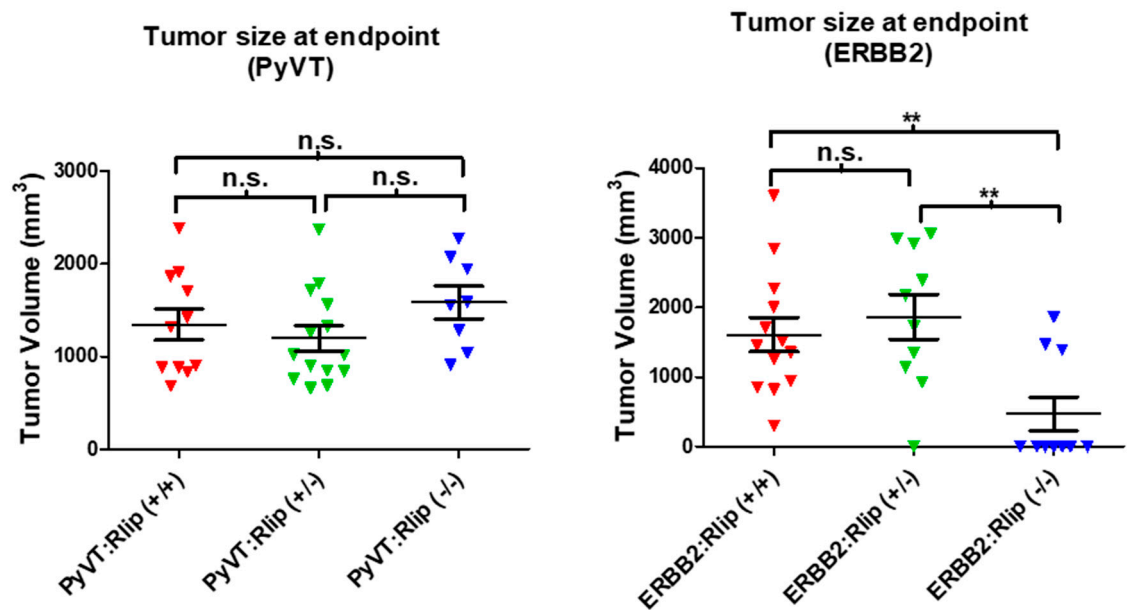


Figure S4. Tumor sizes at endpoint for PyVT and ERBB2 mice with wild-type, heterozygous knockout, or homozygous knockout Rlip genotypes. Note that 7 of 10 ERBB2:Rlip(-/-) mice were tumor-free at endpoint and were euthanized due to morbidities unrelated to tumor growth. The two asterisks (**) indicate $p < 0.01$, and n.s. indicates not significant.

E-Cadherin (4A2) Mouse mAb (Cell signaling, #14472)

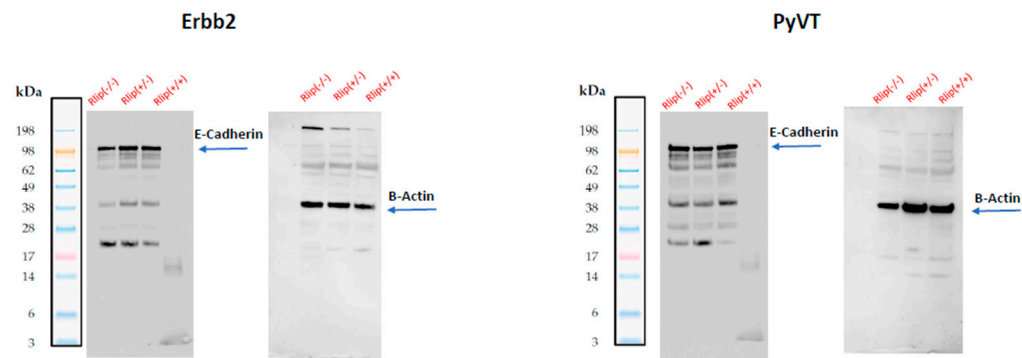


Figure S5. Western blots showing E-Cadherin expression in Erbb2:Rlip^{-/-}, Erbb2:Rlip^{+/-}, and Erbb2:Rlip^{+/+} mice (left panels) and in PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice (right panels).

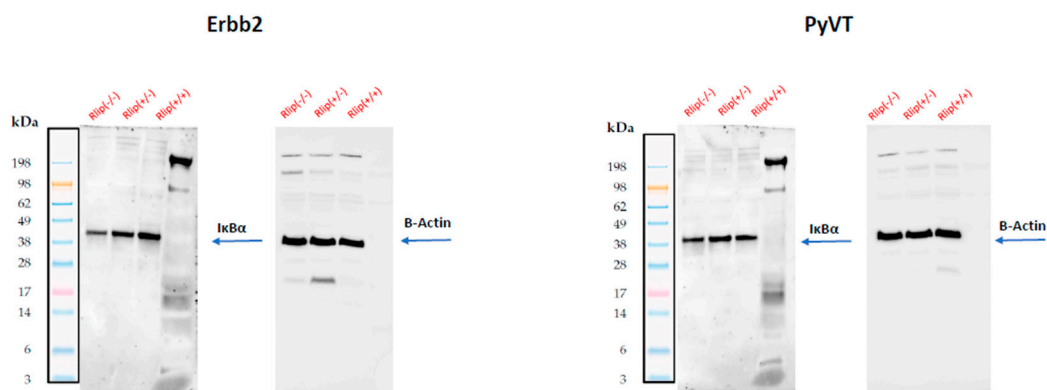
IkB α Antibody (Cell signaling, #9242)

Figure S6. Western blots showing IkB α expression in Erb2:Rlip^{-/-}, Erb2:Rlip^{+/-}, and Erb2:Rlip^{+/+} mice (left panels) and in PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice (right panels).

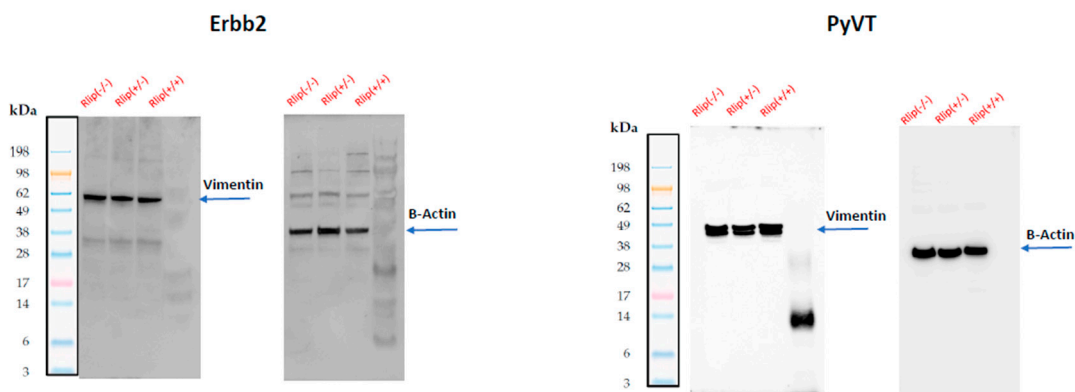
Vimentin [D21H3] XP[®] Rabbit mAb (Cell signaling, #5741)

Figure S7. Western blots showing Vimentin expression in Erb2:Rlip^{-/-}, Erb2:Rlip^{+/-}, and Erb2:Rlip^{+/+} mice (left panels) and in PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice (right panels).

Stat2 [D9J7L] Rabbit mAb(Cell signaling, #72604)

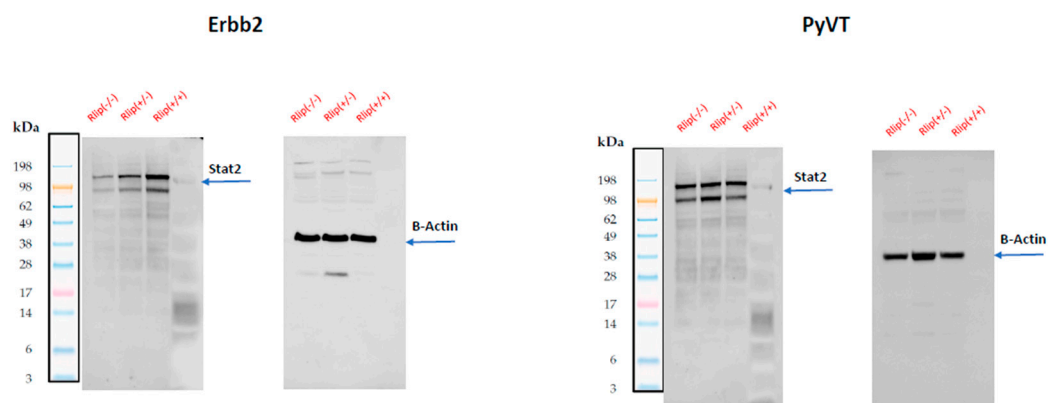


Figure S8. Western blots showing Stat2 expression in ErbB2:Rlip^{-/-}, ErbB2:Rlip^{+/-}, and ErbB2:Rlip^{+/+} mice (left panels) and in PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice (right panels).

p42 MAP Kinase (Erk2) Antibody (Cell signaling, #9108)

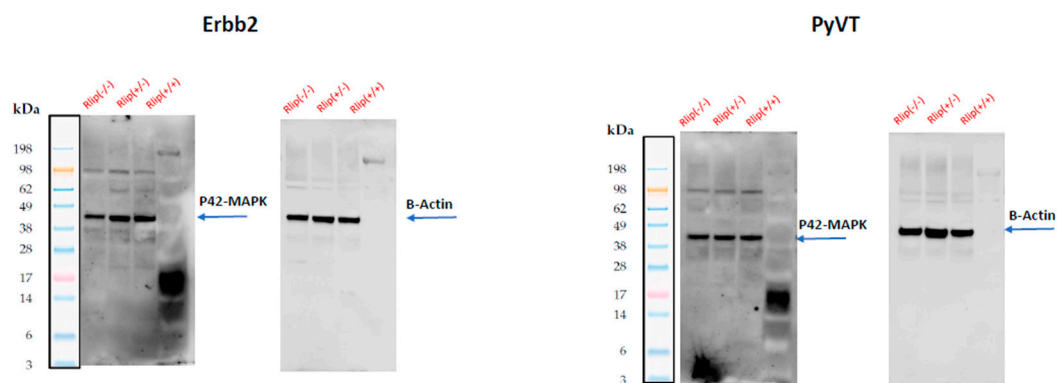


Figure S9. Western blots showing p42 MAP Kinase (Erk2) expression in ErbB2:Rlip^{-/-}, ErbB2:Rlip^{+/-}, and ErbB2:Rlip^{+/+} mice (left panels) and in PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice (right panels).

p70 S6 Kinase Antibody (Cell signaling, #9202)

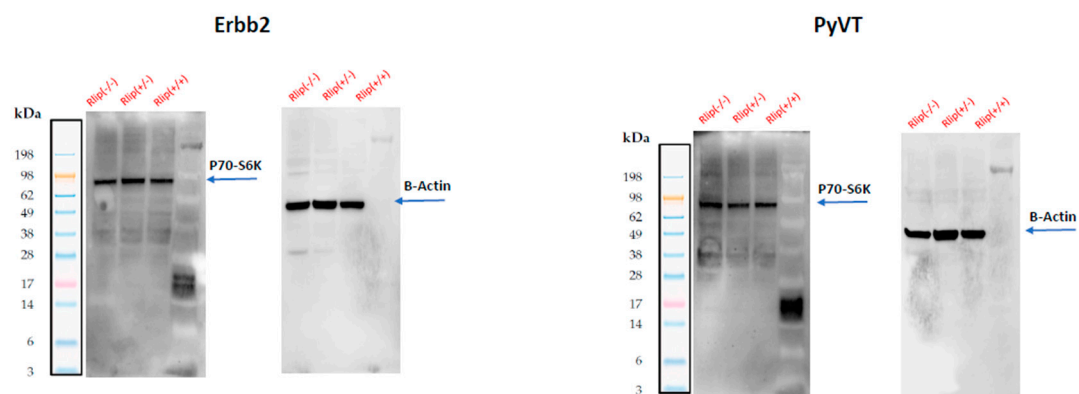


Figure S10. Western blots showing p70 S6 Kinase expression in ErbB2:*Rlip*^{-/-}, ErbB2:*Rlip*^{+/-}, and ErbB2:*Rlip*^{+/+} mice (left panels) and in PyVT:*Rlip*^{-/-}, PyVT:*Rlip*^{+/-}, and PyVT:*Rlip*^{+/+} mice (right panels).

CDK4 Polyclonal Antibody (Invitrogen, PA5-27827)

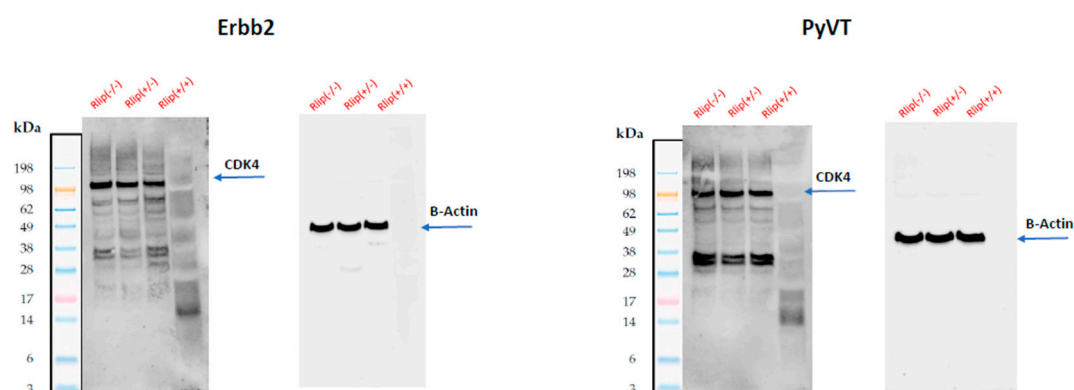


Figure S11. Western blots showing CDK4 expression in ErbB2:*Rlip*^{-/-}, ErbB2:*Rlip*^{+/-}, and ErbB2:*Rlip*^{+/+} mice (left panels) and in PyVT:*Rlip*^{-/-}, PyVT:*Rlip*^{+/-}, and PyVT:*Rlip*^{+/+} mice (right panels).

Bcl-2 Polyclonal Antibody (Invitrogen, PA5-20068)

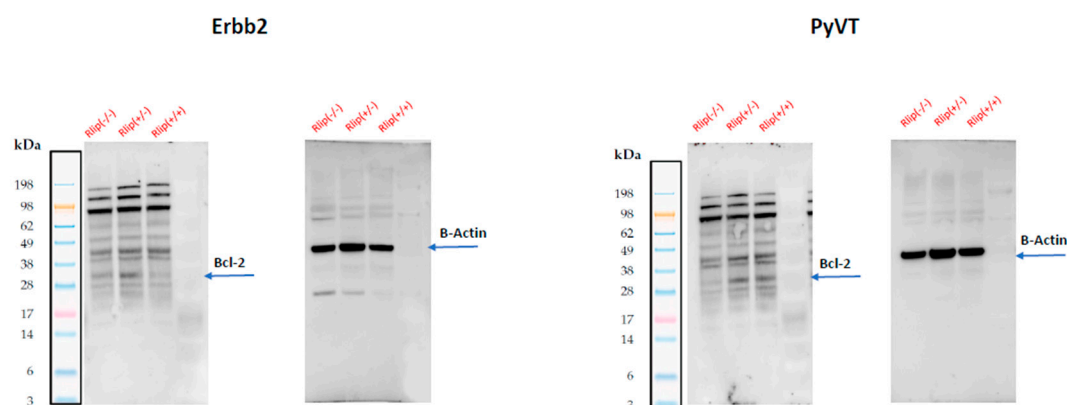


Figure S12. Western blots showing Bcl-2 expression in ErbB2:Rlip^{-/-}, ErbB2:Rlip^{+/-}, and ErbB2:Rlip^{+/+} mice (left panels) and in PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice (right panels).

Cyclin B1 Polyclonal Antibody (Invitrogen, PA5-35245)

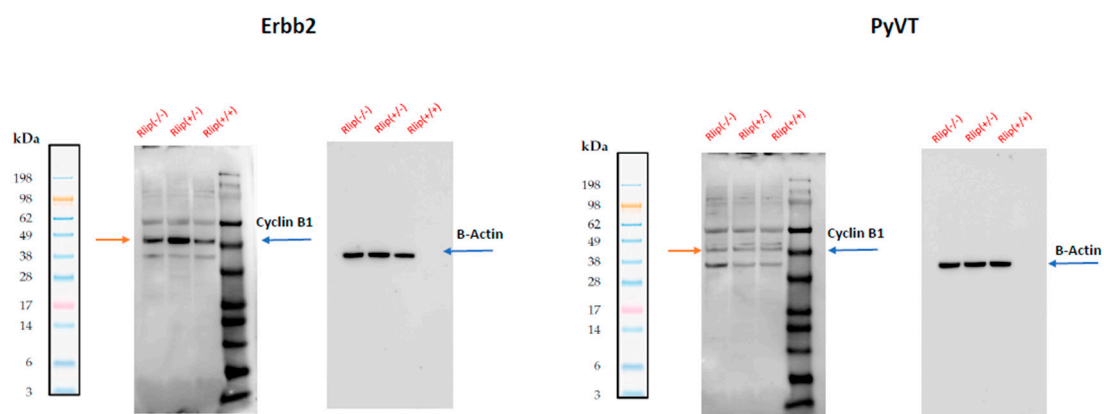


Figure S13. Western blots showing Cyclin B1 expression in ErbB2:Rlip^{-/-}, ErbB2:Rlip^{+/-}, and ErbB2:Rlip^{+/+} mice (left panels) and in PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice (right panels).

PARP1 (cleaved Asp214, Asp215) Polyclonal Antibody (Invitrogen, 44-698G)

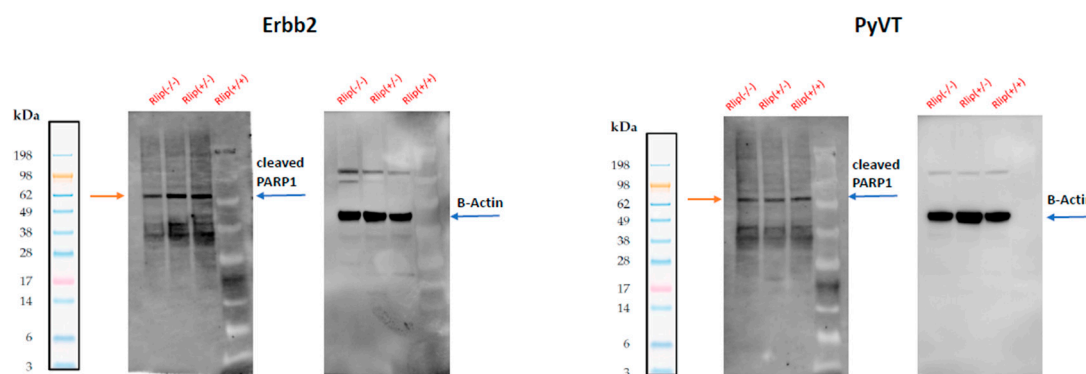


Figure S14. Western blots showing cleaved PARP1 expression in ErbB2:*Rlip*^{-/-}, ErbB2:*Rlip*^{+/-}, and ErbB2:*Rlip*^{+/+} mice (left panels) and in PyVT:*Rlip*^{-/-}, PyVT:*Rlip*^{+/-}, and PyVT:*Rlip*^{+/+} mice (right panels). The indicated band is the 62 kDa fragment.

Bax Polyclonal Antibody (Invitrogen, PA5-11378)

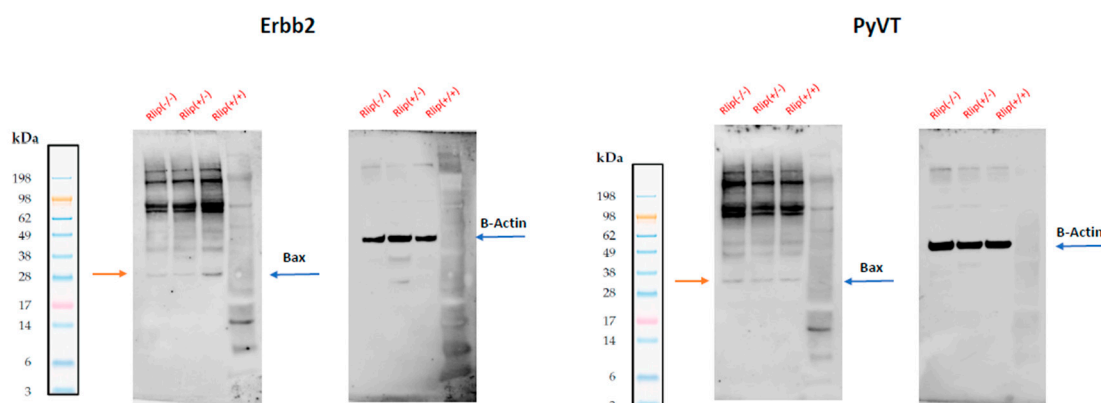


Figure S15. Western blots showing Bax expression in ErbB2:*Rlip*^{-/-}, ErbB2:*Rlip*^{+/-}, and ErbB2:*Rlip*^{+/+} mice (left panels) and in PyVT:*Rlip*^{-/-}, PyVT:*Rlip*^{+/-}, and PyVT:*Rlip*^{+/+} mice (right panels).

Survivin (71G4B7) Rabbit mAb #2808

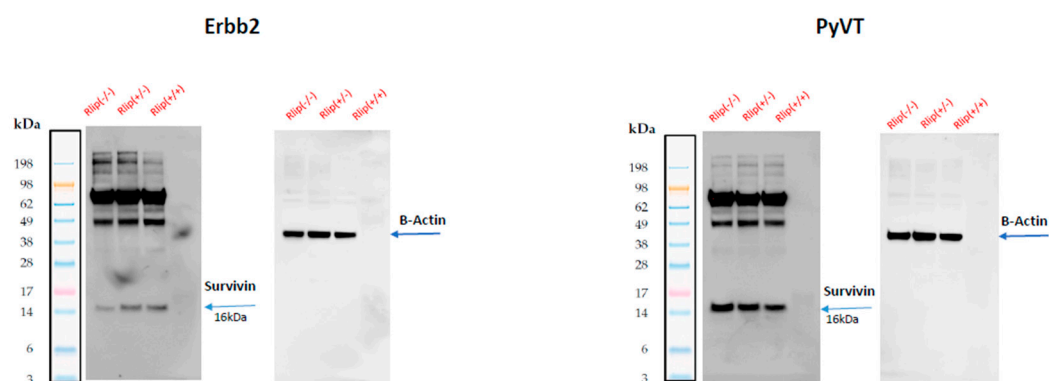


Figure S16. Western blots showing Survivin expression in ErbB2:*Rlip*^{-/-}, ErbB2:*Rlip*^{+/-}, and ErbB2:*Rlip*^{+/+} mice (left panels) and in PyVT:*Rlip*^{-/-}, PyVT:*Rlip*^{+/-}, and PyVT:*Rlip*^{+/+} mice (right panels).

PI3 Kinase Class III Antibody #3811

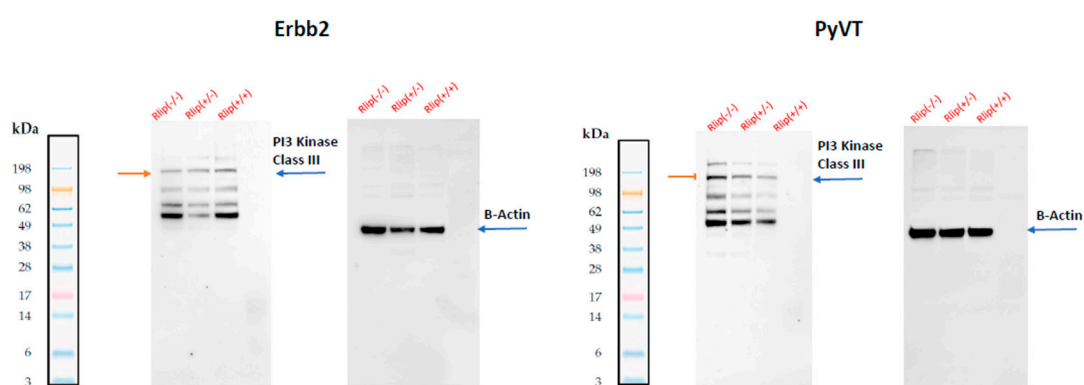


Figure S17. Western blots showing PI3 Kinase Class III expression in ErbB2:*Rlip*^{-/-}, ErbB2:*Rlip*^{+/-}, and ErbB2:*Rlip*^{+/+} mice (left panels) and in PyVT:*Rlip*^{-/-}, PyVT:*Rlip*^{+/-}, and PyVT:*Rlip*^{+/+} mice (right panels).

Table S1. Statistical analyses comparing survival of Erbb2:Rlip^{-/-}, Erbb2:Rlip^{+/-}, and Erbb2:Rlip^{+/+} mice. Output is from GraphPad Prism 9.0 for Windows. Median survival is in days. ** indicates $p < 0.01$; *** indicates $p < 0.001$.

Comparison of Survival Curves Erbb2 Mice			
Log-rank (Mantel-Cox) test (recommended)			
Chi square			17.04
df			2
p value			0.0002
p value summary			***
Are the survival curves sig different?			Yes
Logrank test for trend (recommended)			
Chi square			6.664
df			1
p value			0.0098
p value summary			**
Sig. trend?			Yes
Gehan-Breslow-Wilcoxon test			
Chi square			13.43
df			2
p value			0.0012
p value summary			**
Are the survival curves sig different?			Yes
	Rlip-WT/Erbb2	Rlip-Het/Erbb2	Rlip-Null/Erbb2
Number of rows	36	36	36
# of blank lines	23	26	25
# rows with impossible data	0	0	0
# censored subjects	0	0	0
# deaths/events	13	10	11
Median survival (Days)	236	461.5	326

Table S2. Statistical analyses comparing survival of PyVT:Rlip^{-/-}, PyVT:Rlip^{+/-}, and PyVT:Rlip^{+/+} mice. Output is from GraphPad Prism 9.0 for Windows. Median survival is in days. ns ($p > 0.1$) indicates not significant.

Comparison of Survival Curves of PyVT Mice			
Log-rank (Mantel-Cox) test (recommended)			
Chi square			3.790
df			2
p value			0.1503
p value summary			ns
Are the survival curves sig different?			No
Logrank test for trend (recommended)			
Chi square			0.8180
df			1
p value			0.3658
p value summary			ns
Sig. trend?			No
Gehan-Breslow-Wilcoxon test			
Chi square			2.818
df			2
p value			0.2444
p value summary			ns
Are the survival curves sig different?			No
	Rlip-WT/PyVT	Rlip-Het/PyVT	Rlip-Null/PyVT

Number of rows	40	40	40
# of blank lines	26	28	29
# rows with impossible data	0	0	1
# censored subjects	0	0	0
# deaths/events	14	12	10
Median survival (Days)	133	127	133.5

LT_SV40	1	MDKVLNREESLQMDLLGLERSAWGNIPLMRKAYLKCKKEFHPDKGGDEE	50
MT_POVBG	1	MDRVLSRADKERLLELLKLPQLWGDGFRMQQAYKQQSLLLHPDKGGSHA	50
LT_SV40	51	KMKKMNLTLYKKMEDGVKYAHQPDFGGF-----WDATEIPTYGTD	89
MT_POVBG	51	LMQELNSLWGTFTKTEV--YNLRMNLGGTGFGVRRRLHADGWNLSTKDTFG--	97
LT_SV40	90	EWEQWWNAFNEENLFCSEEMPSSDDE---ATADSQHSTPPKKRKRVEDPK	136
MT_POVBG	98	--DRYYQRFCRMLPTCLVNVKYSSCSCILCLLRKQH-----RELKDKC	138
LT_SV40	137	DFPSELLSFLSHAVFSNRTLACFAI-----YTTKEKAAL-LYKKIMEKY	179
MT_POVBG	139	DARCLVLG-----ECFCLECYMQWFGTPTRDVLNLYADFIASM	176
LT_SV40	180	SVTFISR--HNSYNHNILFFLTPHRHRVSAINNYAQKLCTFSFLICKG--	225
MT_POVBG	177	PIDWLDLDVHSVYN-----PKRR-----SEELRRAATVHYTMTGHS	213
LT_SV40	226	-----VNKEYLMYSALTRDPFVSIEESLPGGLKE	254
MT_POVBG	214	AMEASTSQNGMISSESGETPATSRRLRLPSLLSNPTYSV-----MRS	255
LT_SV40	255	HDFNPEEAEETKQVSWKLVTEYAMETKCDDVLLLLG-----MY	292
MT_POVBG	256	HSYPPTRV--LQIIPHILLEE-----DEILVLLSPMTAYPRTPELly	297
LT_SV40	293	LEFQYSFEMCLKCIKKEQPSHYKYHEKHANAIFADSKNQKTICQQAVD	342
MT_POVBG	298	PE---SDQDQLEPLEEEE-----EEYMPMEDLYLDILPEEQVQLIPP	337
LT_SV40	343	TVLAKKRVDLSQ-LTREQMLTNRFNDLLDRMDIMFGSTGSA-----	382
MT_POVBG	338	PIIPRAGLSPWEGLILRDLQRAHFDPILDASQRMRAHRAALRAHSMQRH	387
LT_SV40	383	--DIEEWMAGVAWLHCLLPKMDSVVYDFLKCMVYNIPKKRYWLFKGPIDS	430
MT_POVBG	388	LRRLGRTLILLVTFLAALLG-----ICLMLFILIKRSRHF-----	421
LT_SV40	431	GKTTLAAALLELCGGKALNVNLPDLRLNFELGVAIDQFLVVFEDVKGTTG	480
MT_POVBG	422	-----	421

Supplemental Data S1. PyVT and LT_SV40 share sequence similarity. From EMBOSS Needle #
Length: 778; # Identity: 89/778 (11.4%); # Similarity: 177/778 (22.8%); # Gaps: 427/778 (54.9%); #Score:
126.0.

