

Supplementary Materials

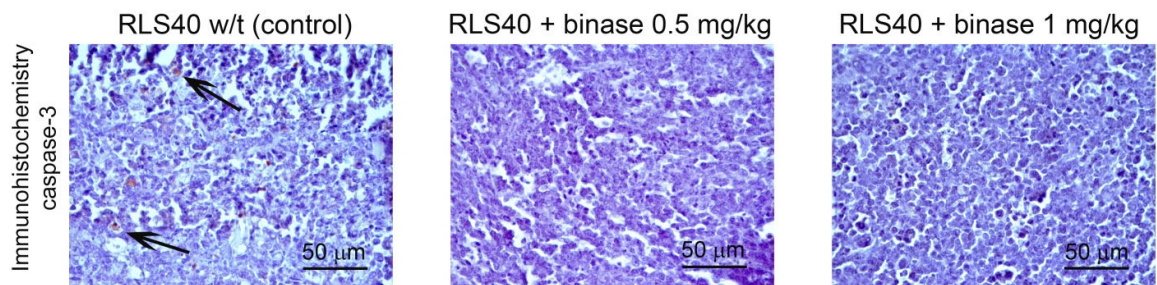


Figure S1. Caspase-3-positive cells in RLS₄₀ tumor tissues after binase administration. Representative histological images of tumor sections. Immunohistochemical staining with anti-caspase-3 monoclonal antibodies. Caspase-3-positive cells are indicated by black arrows. Magnification: $\times 400$. Scale bar corresponds to 50 μm .

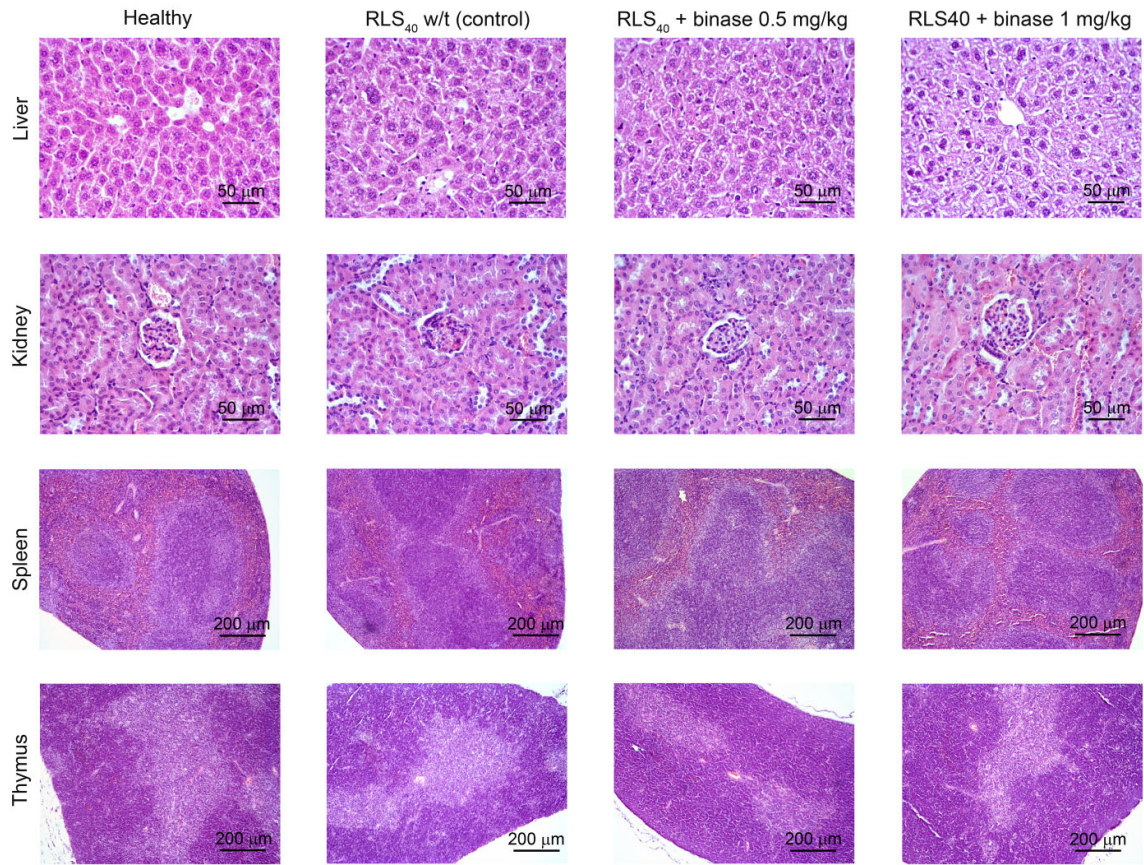


Figure S2. Representative histological images of liver, kidney, spleen and thymus sections. Hematoxylin and eosin staining. Magnification: $\times 400$. Bar corresponds to 50 μm (liver and kidney) and 200 μm (spleen and thymus).

Table S1. The primer sequence for the reverse transcription (RT) of miRNAs.

Name	Sequences of the RT primer, 5' → 3'
RT mir-31	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGCTATGCC
RT mir-21	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAACATCAG
RT mir-145a	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGATTCT
RT mir-155	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGGATACGAC
RT mir-10b	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACAAATTCG
RT let7-g	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACTGTACAA
RT U6	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAATATGGAACG

Table S2. Sequences of the forward and reverse miRNA-specific primers for qPCR.

Name	Sequences of the Specific Primer, 5' → 3'
mir-31-F	AGGCAAGATGCTGGCA
mir-21-F	AGACTAGCTTATCAGACTGA
mir-145a-F	AGGTCCAGTTTTCCAGGA
mir-155-F	ACTTAATGCTAATTGTGATAGG
mir-10b-F	TACCCTGTAGAACCGAA
let7-g-F	AACGCTGAGGTAGTAGTTTGT
U6-F	CTCGCTTCGGCAGCACA
Universal-R	GTGCAGGGTCCGAGGT

Table S3. Blood biochemistry of healthy mice and RLS₄₀-bearing mice treated with a saline buffer and binase at the doses of 0.5 and 1 mg/kg.

Groups	Biochemical Parameters					
	Liver				Kidneys	
	ALT (U/L)	AST (U/L)	ALK (U/L)	Total Protein (g/L)	Creatinin (mmol/L)	BUN (mmol/L)
Healthy	42 ± 1.6	67.3 ± 1.5	68.1 ± 5.5	55.4 ± 2.1	32.9 ± 1.3	9.9 ± 0.1
Control	39.4 ± 3.4	114.2 ± 13 *	79.8 ± 20	53.4 ± 2.3	39.3 ± 1.3 *	8.4 ± 0.2 *
Binase 0.5 mg/kg	35.6 ± 1.7 *	116.5 ± 14.4 *	130.5 ± 13.5*#	62.4 ± 2.1 *#	31.2 ± 5.1 #	8.8 ± 0.5 *
Binase 1 mg/kg	60.9 ± 10.3 *#	211.4 ± 49.8 *#	134.5 ± 14.8 *#	61.9 ± 3.2 *#	39.2 ± 1.2*	9.7 ± 1.2

Control – RLS₄₀-bearing mice treated with saline buffer.

*The differences from the healthy mice were significant at $p \leq 0.05$.

#The differences from the control mice were significant at $p \leq 0.05$.

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALK: alkaline phosphatase, BUN: blood urea nitrogen.

Table S4. Morphological parameters of the spleen and thymus of healthy mice and RLS₄₀-bearing mice treated with a saline buffer and binase at the doses of 0.5 and 1 mg/kg.

Morphological Parameters	Healthy	Control	Binase 0.5 mg/kg	Binase 1 mg/kg
Spleen				
Red pulp, Vv (%)	57.3 ± 3.1	49.4 ± 2.9*	31.2 ± 1.1*#	30.4 ± 0.8*#
White pulp, Vv (%)	42.6 ± 1.9	50.4 ± 2.9*	68.5 ± 1.1*#	69.5 ± 0.8*#
Diameter of follicles (µm)	229.9 ± 13.5	270.2 ± 10.1*	322.3 ± 7.6*#	325 ± 11.8*#
Thymus				
Cortex, Vv (%)	39.9 ± 1.6	35.1 ± 3.8	40.5 ± 1.1	40.4 ± 2.2
Medulla, Vv (%)	55 ± 1.9	64.8 ± 3.8	59.5 ± 1.1	59.5 ± 2.2
Cortex/medulla index	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.03	0.7 ± 0.08

Control – RLS₄₀-bearing mice treated with a saline buffer.

*The differences from the healthy mice were significant at $p \leq 0.05$.

#The differences from the control mice were significant at $p \leq 0.05$.

Table S5. Gene targets for mmu-miR-21a participating in pro-oncogenic events and pathways.

	Target Gene	Description	Signaling Pathway or Event	Function
1	<i>PDCD10</i>	Programmed Cell Death 10	- Validated targets of C-MYC transcriptional activation	- Promotes cell proliferation - Modulates apoptotic pathways - Modulates cell migration - Modulates angiogenesis, vasculogenesis and hematopoiesis
2	<i>PTEN</i>	Phosphatase and Tensin Homolog	- IL-2 pathway - PI3K/Akt signaling pathway - p53 signaling - MicroRNAs in cancer - T-cell receptor signaling pathway - Pathways in cancer - B-Cell receptor signaling pathway - MAPK signaling; - RhoA signaling pathway	- Tumor suppressor; acts as a dual-specificity protein phosphatase
3	<i>RC3H1</i>	Ring Finger and CCCH-Type Domains 1	- STRING interaction network	- Regulation microRNA homeostasis - Post-transcriptional repressor of mRNAs
4	<i>PDCD4</i>	Programmed Cell Death 4	- Apoptosis and autophagy - MAPK signaling	- Inhibitor of translation initiation and cap-dependent translation - Inhibits of events important in driving invasion, and consequent JUN-dependent transcription - Plays a role in apoptosis - Inhibits tumor promoter-induced neoplastic transformation
5	<i>TGFBI</i>	Transforming Growth Factor Beta Induced	- Adhesion	- Cell adhesion.
6	<i>TIMP3</i>	TIMP Metallopeptidase Inhibitor 3	- Matrix metallo-proteinases - Angiogenesis - Cell adhesion, ECM remodeling - MicroRNAs in cancer - VEGF signaling	- Irreversibly inactivates metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13, MMP-14 and MMP-15).
7	<i>MMP9</i>	Matrix Metallopeptidase 9	- Transcriptional misregulation in cancer - Regulation of Wnt-mediated beta catenin signaling and target gene transcription	- Plays an essential role in local proteolysis of the extracellular matrix and in leukocyte migration - Exhibits identical protein binding and metallopeptidase activity

Table S6. Gene targets for mmu-miR-10b participating in pro-oncogenic events and pathways.

	Target Gene	Description	Signaling Pathway or Event	Function
1	<i>RAB11B</i>	RAB11B, Member RAS Oncogene Family	<ul style="list-style-type: none"> - Vesicle-mediated transport - Autophagy pathway - Sertoli–Sertoli cell junction dynamics - Remodeling of the adherens junctions 	<ul style="list-style-type: none"> - Key regulator of intracellular membrane trafficking
2	<i>MCC</i>	MCC Regulator of WNT Signaling Pathway	<ul style="list-style-type: none"> - Wnt pathway 	<ul style="list-style-type: none"> - Suppresses cell proliferation and the Wnt/b-catenin pathway in colorectal cancer cells - Involved in cell migration
3	<i>VAMP3</i>	Vesicle Associated Membrane Protein 3	<ul style="list-style-type: none"> - Vesicle-mediated transport - Sertoli–Sertoli cell junction dynamics 	<ul style="list-style-type: none"> - Involved in vesicular transport from the late endosomes to the trans-Golgi network
4	<i>LATS1</i>	Large Tumor Suppressor Kinase 1	<ul style="list-style-type: none"> - DNA damage - Wnt/Hedgehog/Notch 	<ul style="list-style-type: none"> - Plays a pivotal role in organ size control and tumor suppression by restricting proliferation and promoting apoptosis - Regulates cellular genes important for cell proliferation, cell death, and cell migration - Involves in the control of p53 expression
5	<i>TCF25</i>	Transcription Factor 25	<ul style="list-style-type: none"> - Non-canonical Wnt pathway - Regulation of activated PAK-2p34 by proteasome-mediated degradation 	<ul style="list-style-type: none"> - Play a role in cell death control - Acts as a transcriptional repressor
6	<i>PTPN2</i>	Protein Tyrosine Phosphatase Non-Receptor Type 2	<ul style="list-style-type: none"> - JAK/STAT signaling pathway 	<ul style="list-style-type: none"> - Negatively regulates numerous signaling pathways and biological processes like hematopoiesis, inflammatory response, cell proliferation and differentiation and glucose homeostasis
7	<i>CDK7</i>	Cyclin Dependent Kinase 7	<ul style="list-style-type: none"> - Cyclins and cell cycle regulation - Mitotic G1-G1/S phases - Cell cycle, mitotic - Cell cycle control of chromosomal replication 	<ul style="list-style-type: none"> - Involves in cell cycle control and in RNA-polymerase-II-mediated RNA transcription.
8	<i>PTK6</i>	Protein Tyrosine Kinase 6	<ul style="list-style-type: none"> - Cell cycle, mitotic - Mitotic G1-G1/S phases - Signaling by PTK6 	<ul style="list-style-type: none"> - Implicates in the regulation of a variety of signaling pathways that control the differentiation and maintenance of normal epithelia, as well as tumor growth - Promotes proliferation, migration and invasion. - In cytoplasmic form, it might activate oncogenic signaling pathways
9	<i>CDKN1A</i>	Cyclin Dependent Kinase Inhibitor 1A	<ul style="list-style-type: none"> - Cyclins and cell cycle regulation - p53 signaling 	<ul style="list-style-type: none"> - Involved in p53/TP53-mediated inhibition of cellular proliferation in response to DNA damage

			<ul style="list-style-type: none"> - Cell cycle, mitotic - JAK/STAT signaling pathway - Apoptosis modulation and signaling - Cell cycle checkpoints 	<ul style="list-style-type: none"> - Plays an important role in controlling cell cycle progression and DNA damage-induced G2 arrest
10	<i>PRF1</i>	Perforin 1	<ul style="list-style-type: none"> - Apoptosis modulation and signaling - Apoptosis and survival caspase cascade 	<ul style="list-style-type: none"> - Plays a key role in secretory granule-dependent cell death - Promotes cytolysis and apoptosis of target cells
11	<i>DNASE2</i>	Deoxyribonuclease 2, Lysosomal	<ul style="list-style-type: none"> - Vesicle-mediated transport - Lysosome 	<ul style="list-style-type: none"> - Plays a major role in the degradation of nuclear DNA in cellular apoptosis during development
12	<i>PSMB11</i>	Proteasome Subunit Beta 11	<ul style="list-style-type: none"> - Cell cycle, mitotic - Cell cycle checkpoints - Mitotic metaphase and anaphase - Apoptotic execution phase 	<ul style="list-style-type: none"> - Plays a pivotal role in the development of CD8-positive T cells - Cleaves peptides with Arg, Phe, Tyr, Leu and Glu

Table S7. Gene targets for mmu-miR-31 participating in pro-oncogenic events and pathways.

	Target Gene	Description	Signaling Pathway or Event	Function
1	<i>PDGFB</i>	Platelet-Derived Growth Factor Subunit B	<ul style="list-style-type: none"> - MAPK signaling pathway - Ras signaling pathway - JAK/STAT signaling pathway - MicroRNAs in cancer - Pathways in cancer - PI3K/Akt signaling pathway 	<ul style="list-style-type: none"> - Growth factor that plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis - Plays an important role in wound healing
2	<i>TBC1D2</i>	TBC1 Domain Family Member 2	<ul style="list-style-type: none"> - Vesicle-mediated transport TBC/RABGAPs 	<ul style="list-style-type: none"> - Signal effector participating in the inhibition of cadherin degradation and reduced cell–cell adhesion
3	<i>FGF10</i>	Fibroblast Growth Factor 10	<ul style="list-style-type: none"> - Ras signaling pathway - MAPK signaling pathway - PI3K/Akt signaling pathway - Pathways in cancer 	<ul style="list-style-type: none"> - Plays an important role in the regulation of embryonic development, cell proliferation and cell differentiation

Table S8. Gene targets for mmu-miR-145 participating in pro-oncogenic events and pathways.

	Target Gene	Description	Signaling Pathway or Event	Function
1	<i>ACVR1B</i>	Activin A Receptor Type 1B	- Apoptosis pathway - MAPK signaling pathway	- Regulates pathological processes including wound healing, extracellular matrix production, immunosuppression and carcinogenesis
2	<i>CDC14B</i>	Cell Division Cycle 14B	- Cell cycle	- Dual-specificity phosphatase involved in the DNA damage response - Important regulator of the G2 DNA damage checkpoint following DNA damage

Table S9. Genes-targets for mmu-miR-155 participating in pro-oncogenic events and pathways.

	Target Gene	Description	Signaling Pathway or Event	Function
1	<i>CISH</i>	Cytokine-Inducible SH2-Containing protein	- IL-2 signaling pathway - JAK/STAT signaling pathway - Toll-like receptor signaling pathway	- Involved in the negative regulation of cytokines that signal through the JAK/STAT5 pathway
2	<i>RHOD</i>	Ras Homolog Family Member D	- IL-2 pathway - Mitotic prometaphase - Apoptotic pathways in synovial fibroblasts - Molecular mechanisms of cancer - Ras pathway	- May coordinate membrane transport with the function of the cytoskeleton - Modulates the reorganisation of the actin cytoskeleton and focal adhesion dissolution
3	<i>TSPAN14</i>	Tetraspanin 14	- Innate immune system	- Regulates the maturation and trafficking of the transmembrane metalloprotease ADAM10 - Negatively regulates ADAM10-mediated cleavage of GP6 - Promotes the ADAM10-mediated cleavage of CDH5
4	<i>CSNK1A1</i>	Casein Kinase 1 Alpha 1	- Wnt signaling pathway - PI3K/Akt signaling pathway - p53 signaling	- Regulates epithelial cell migration - Plays a role in segregating chromosomes during mitosis - Participates in Wnt signaling; - Phosphorylates a large number of proteins
5	<i>TAB2</i>	TGF- β -Activated Kinase 1 (MAP3K7) Binding Protein 2	- MAP kinase signaling - IL-1 signaling pathway - Death receptor signaling - Molecular mechanisms of cancer	- Promotes MAP3K7 activation in the IL-1 signaling pathway
6	<i>PATJ</i>	PATJ Crumbs Cell Polarity Complex Component	- Cell junction organization - PI3K/Akt signaling pathway - Tight junction	- Regulates protein targeting, cell polarity and the integrity of tight junctions
7	<i>AGTRAP</i>	Angiotensin II Receptor Associated Protein	- Oncogenic MAPK signaling	- Negative regulator of type-1 angiotensin II receptor-mediated signaling, as well as a receptor-desensitisation mechanism, such as phosphorylation - Induces a decrease in cell proliferation and angiotensin II-stimulated transcriptional activity
8	<i>ETV3</i>	ETS Variant Transcription Factor 3	- Macrophage differentiation and growth	- Transcriptional repressor that contributes to growth arrest during terminal macrophage differentiation - Represses MMP1 promoter activity
9	<i>TP53INP1</i>	Tumor Protein P53 Inducible Nuclear Protein 1	- Regulation of TP53 activity mediating the transcription of cell death genes	- Antiproliferative and proapoptotic protein involved in the cell stress response, which acts as a dual regulator of transcription and autophagy

				<ul style="list-style-type: none"> - Possesses both a p53/TP53-independent intracellular reactive oxygen species (ROS) regulatory function and a p53/TP53-dependent transcription regulatory function - Acts as a tumor suppressor by inducing cell death by an autophagy and caspase-dependent mechanism.
10	<i>RIPK1</i>	Receptor-Interacting Serine/Threonine Kinase 1	<ul style="list-style-type: none"> - Caspase activation via extrinsic apoptotic signalling pathway - Apoptosis modulation and signaling - Apoptosis and Autophagy 	<ul style="list-style-type: none"> - Key regulator of TNF-mediated apoptosis, necroptosis and inflammatory pathways
11	<i>SIPR1</i>	Sphingosine-1-Phosphate Receptor 1	<ul style="list-style-type: none"> - Sphingolipid signaling pathway - IL-4 and IL-13 signaling 	<ul style="list-style-type: none"> - Plays an important role in cell migration - Plays an important role in the regulation of sprouting angiogenesis and vascular maturation
12	<i>PMAIP1</i>	Phorbol-12-Myristate-13-Acetate-Induced Protein 1	<ul style="list-style-type: none"> - Apoptosis modulation and signaling - Apoptosis and autophagy - Pathways in cancer - Molecular mechanisms of cancer - TP53 network 	<ul style="list-style-type: none"> - Promotes the activation of caspases and apoptosis; promotes mitochondrial membrane changes and the efflux of apoptogenic proteins from the mitochondria
13	<i>SGK3</i>	Serum/Glucocorticoid Regulated Kinase Family Member 3	<ul style="list-style-type: none"> - PI3K/Akt signaling 	<ul style="list-style-type: none"> - Involved in the regulation of a wide variety of ion channels, membrane transporters, cell growth, proliferation, survival and migration
14	<i>GPR65</i>	G Protein-Coupled Receptor 65	<ul style="list-style-type: none"> - Gastrin-CREB signaling pathway via PKC and MAPK 	<ul style="list-style-type: none"> - Plays a role in activation-induced cell death or differentiation of T-cells
15	<i>FGF7</i>	Fibroblast Growth Factor 7	<ul style="list-style-type: none"> - Akt signaling - Ras signaling pathway - MAPK signaling pathway - Pathways in cancer 	<ul style="list-style-type: none"> - Plays an important role in the regulation of embryonic development, cell proliferation and cell differentiation
16	<i>MYB</i>	MYB Proto-Oncogene, Transcription Factor	<ul style="list-style-type: none"> - PI3K/Akt signaling pathway - IL-4-mediated signaling events 	<ul style="list-style-type: none"> - Plays an important role in the control of proliferation and differentiation of hematopoietic progenitor cells

Table S10. Gene targets for mmu-let-7-g participating in pro-oncogenic events and pathways.

	Target Gene	Description	Signaling Pathway or Event	Function
1	<i>APC2</i>	APC Regulator of WNT Signaling Pathway 2	<ul style="list-style-type: none">- PI3K/Akt signaling pathway- MicroRNAs in cancer- Wnt signaling pathway- Pathways in cancer	<ul style="list-style-type: none">- May function in Wnt signaling by promoting the rapid degradation of CTNNB1