Supporting Information for

Original article

UBE2G2 inhibits vasculogenic mimicry and metastasis of uveal melanoma by promoting ubiquitination of LGALS3BP

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Supplemental Figures

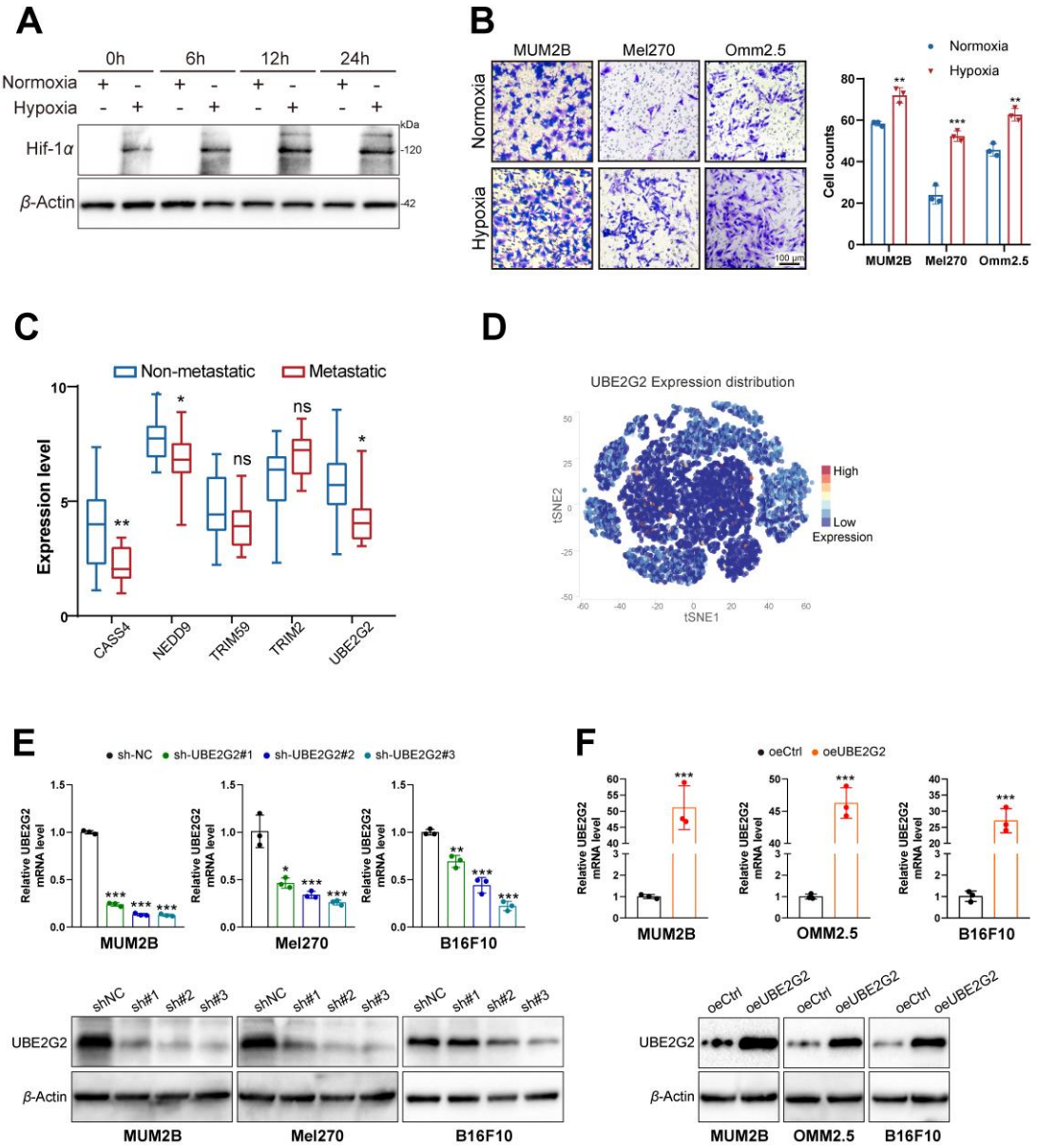

Figure S1. related to Figure 1. UBE2G2 is downregulated by hypoxia and correlated with favorable clinicopathology of uveal melanoma (UM).

(**A**) The protein level of Hif-1α in MUM2B under hypoxia for 0, 6, 12 and 24 h. (**B**) Transwell migration of four melanoma cell lines (MUM2B, Mel270, Omm2.5, B16F10) under normoxia or hypoxia for 24 h. (**C**) Relative expression of five identified genes in non-metastatic and metastatic UM patients from the GSE22138 cohort. (**D**) Expression distribution of UBE2G2 in UM cells in the CancerSEA database (GSE139829). (**E, F**) qRT-PCR and Western blotting were used to measure the knockdown and

overexpression efficiency of UBE2G2 in UM cell lines. All data are presented as the mean ± SD of three independent experiments. $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

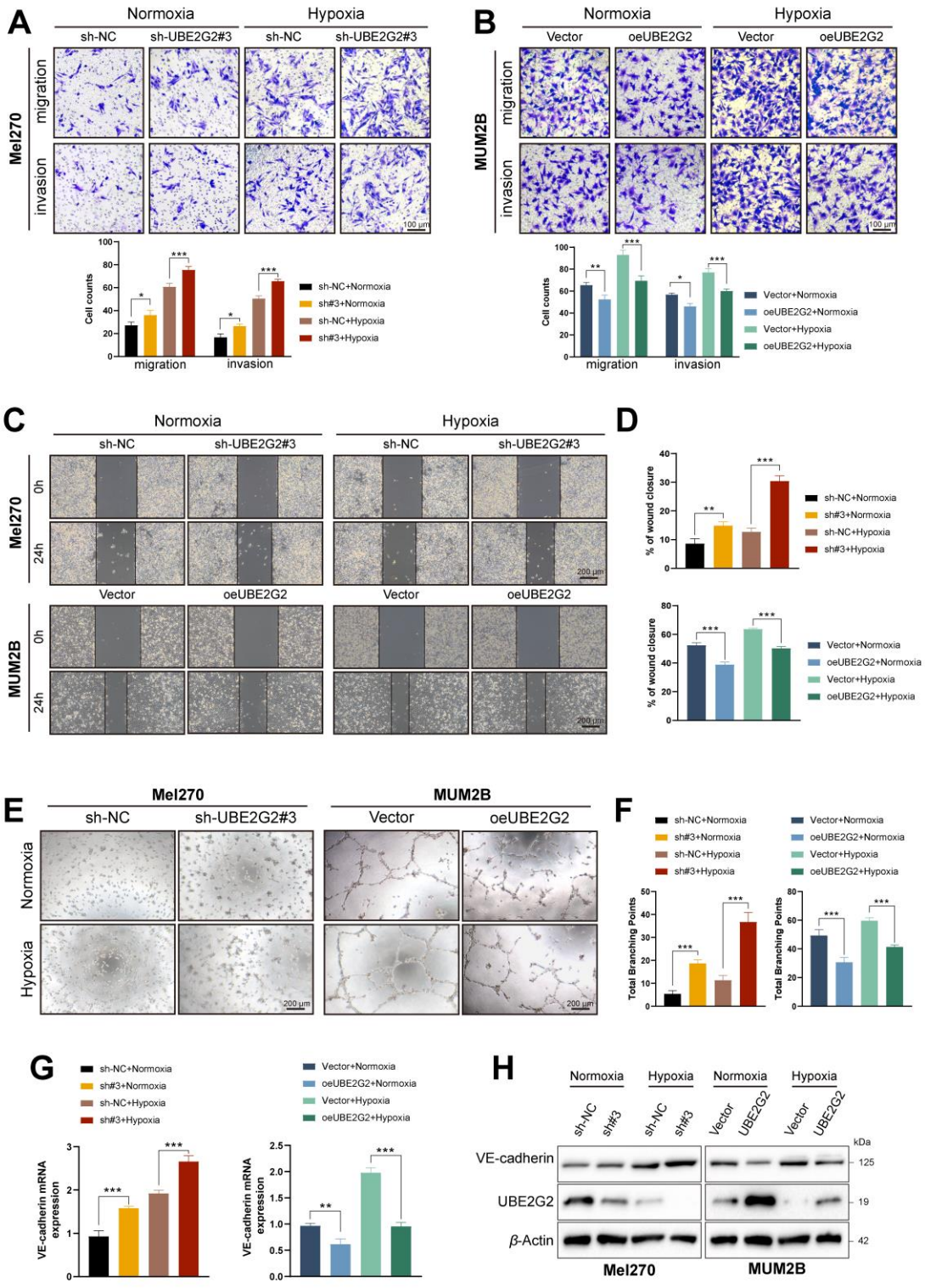

Figure S2. related to Figure 2. UBE2G2 inhibits UM cell metastasis and vasculogenic mimicry (VM) *in vitro*.

(**A, B**) Transwell assays were performed to evaluate the migration and invasion abilities of UM cells. (**C, D**) Wound healing assays were used to assess cell migration ability.

(**E, F**) Matrigel tube formation was performed to evaluate the vasculogenic mimicry abilities of UM cells. (**G, H**) The mRNA and protein level of VE-cadherin (a marker of VM) were used to assess cell vasculogenic mimicry ability. All data are presented as the mean ± SD of three independent experiments. $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

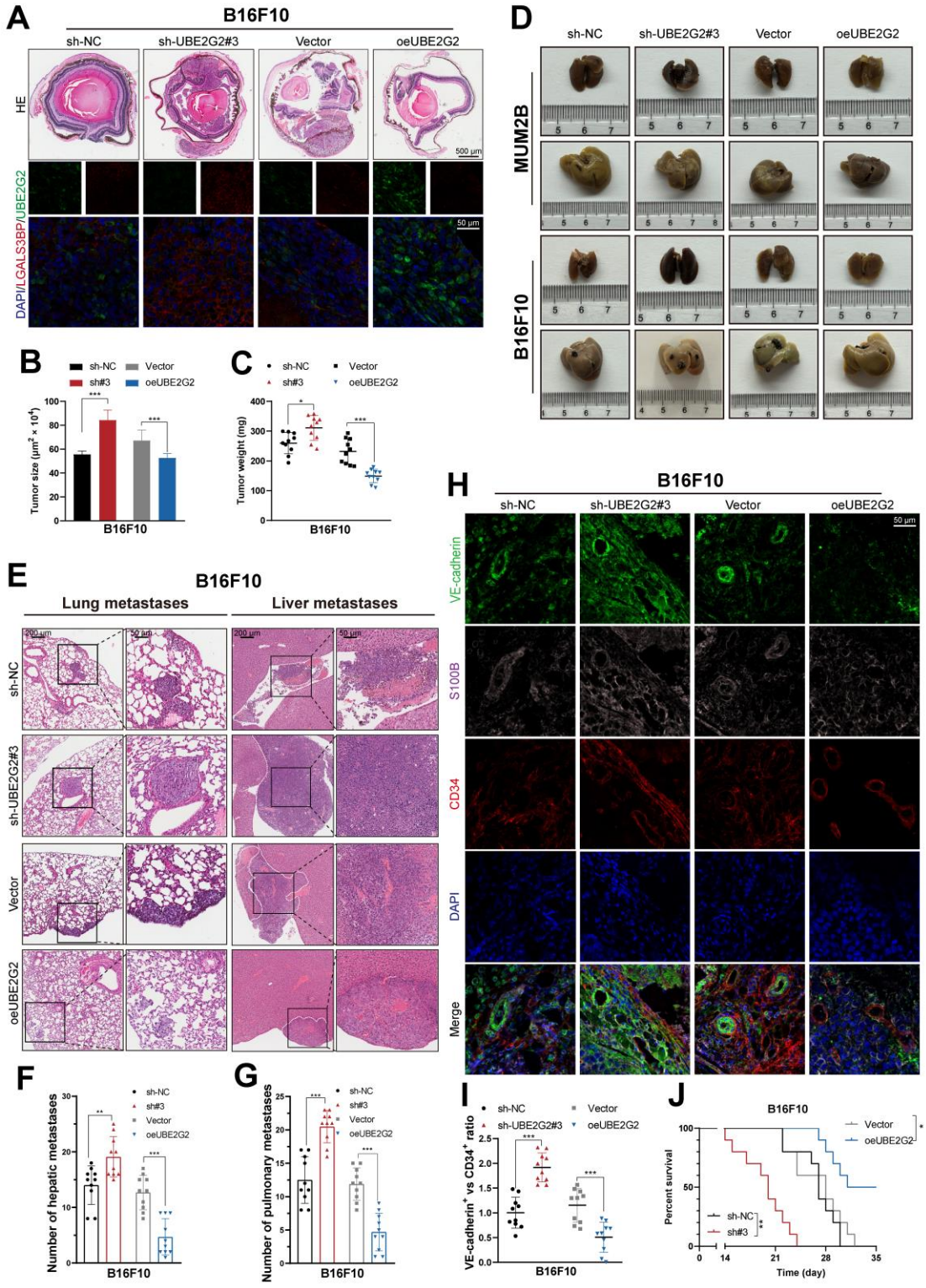

Figure S3. related to Figure 3. UBE2G2 inhibits UM cell metastasis and VM *in vivo.*

(**A**–**C**) B16F10 mouse melanoma cells (1×10^6/eye) were injected in the sub-uveal area of C57BL/6 mice eyes, where they form melanomas in the uvea. On Day 7, tumor-

bearing eyes were enucleated, fixed, and stained to evaluate tumor burden. Representative images of hematoxylin and eosin staining and co-immuno staining of tumor-bearing eyes (**B**), along with quantification of eye tumor size (**C**) and tumor weight (**C**). (**D**–**G**) Representative photographs (**D**) and hematoxylin and eosin stainings (**E**) of liver and lung metastases. The number of pulmonary metastasis (**F**) and hepatic metastasis (**G**). (**H**) B16F10-derived tumor sections were co-immuno stained for CD34, VE-cadherin, and S100B. VM channels: $CD34^{-}/VE^{-}cadherin^{+}/S100B^{+}$, endothelial vessels: $CD34^{+}/VE^{-}cadherin^{+}/S100B^{-}$. (**I**) Statistical analysis of VE^{-} cadherin/CD34 expression radio in UM orthotopic xenografts. (**J**) After eye enucleation, mice were euthanized when reaching the IACUC endpoint criteria. Kaplan–Meier analysis was used to measure the survival of mice burdened with metastatic melanoma. All data are presented as the mean ± SD of independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

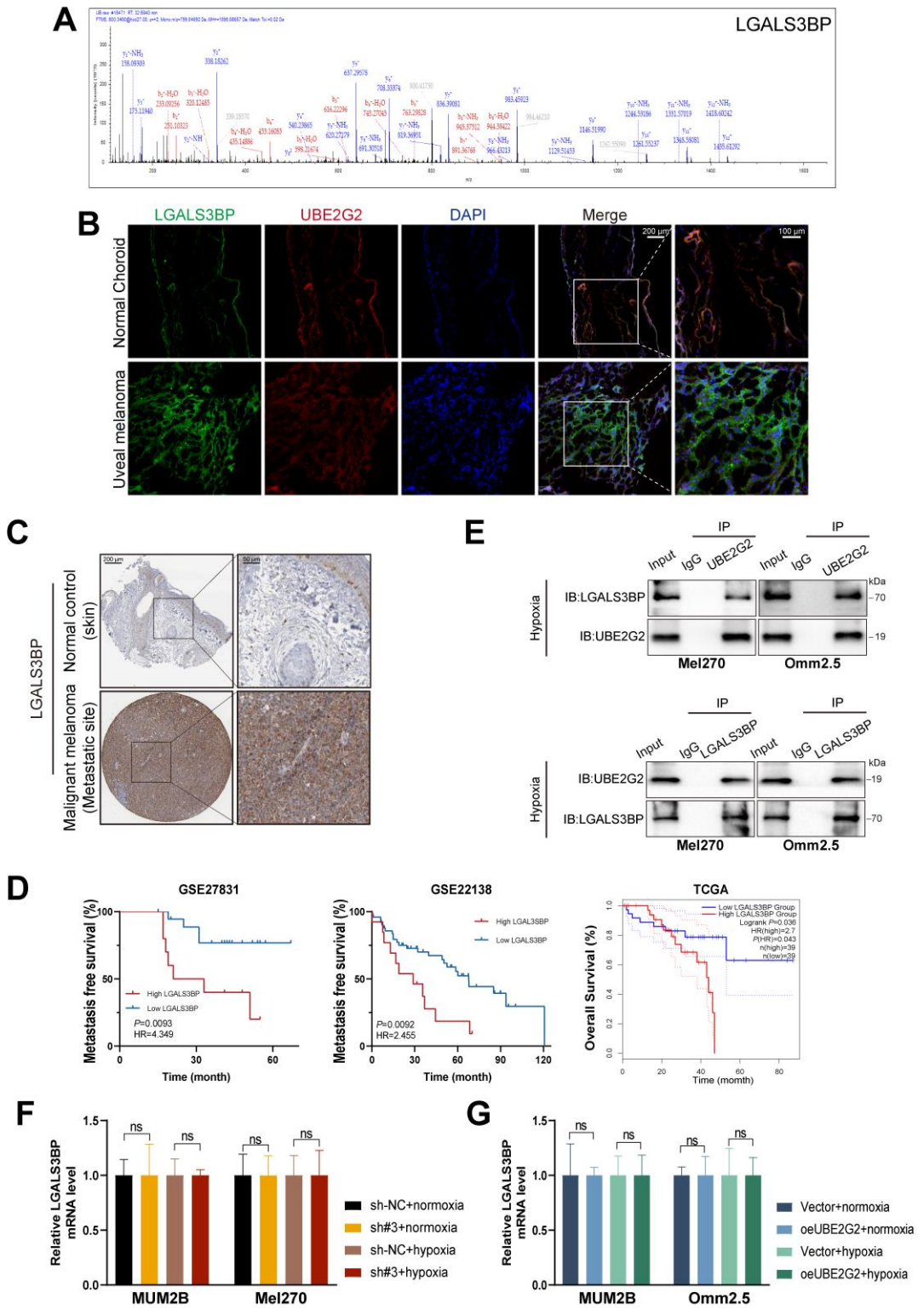

Figure S4. related to Figure 4. LGALS3BP is associated with poor prognosis in UM.

(**A**) The result of mass spectrometry (MS) followed by Co-Immunoprecipitation (co-IP) assay indicated that LGALS3BP specifically interacted with UBE2G2. (**B**)

Representative images of the LGALS3BP and UBE2G2 expression in UM tissues and normal control. (**C**) The distribution and relative abundance of LGALS3BP in malignant melanoma tissues and normal human tissues from the Human Protein Atlas. (**D**) Kaplan–Meier Metastasis-free survival curves of UM patients with high and low expression of LGALS3BP genes from GSE22138 and GSE27831 UM cohorts, and overall survival curves of UM patients with high and low expression of LGALS3BP genes from TCGA UM cohort. (**E**) Mel270 and Omm2.5 cells were cultured under hypoxia for 24 h and treated with MG132 (10 μmol/L) before harvesting. Cell lysates were analyzed by co-IP followed by Western blotting. (**F, G**) The mRNA level of LGALS3BP in UM cells with knockdown (**F**) or ectopic (**G**) UBE2G2 cultured under normoxia and hypoxia was detected by qPCR. All data are presented as the mean ± SD of three independent experiments. $^{ns}P > 0.05$.

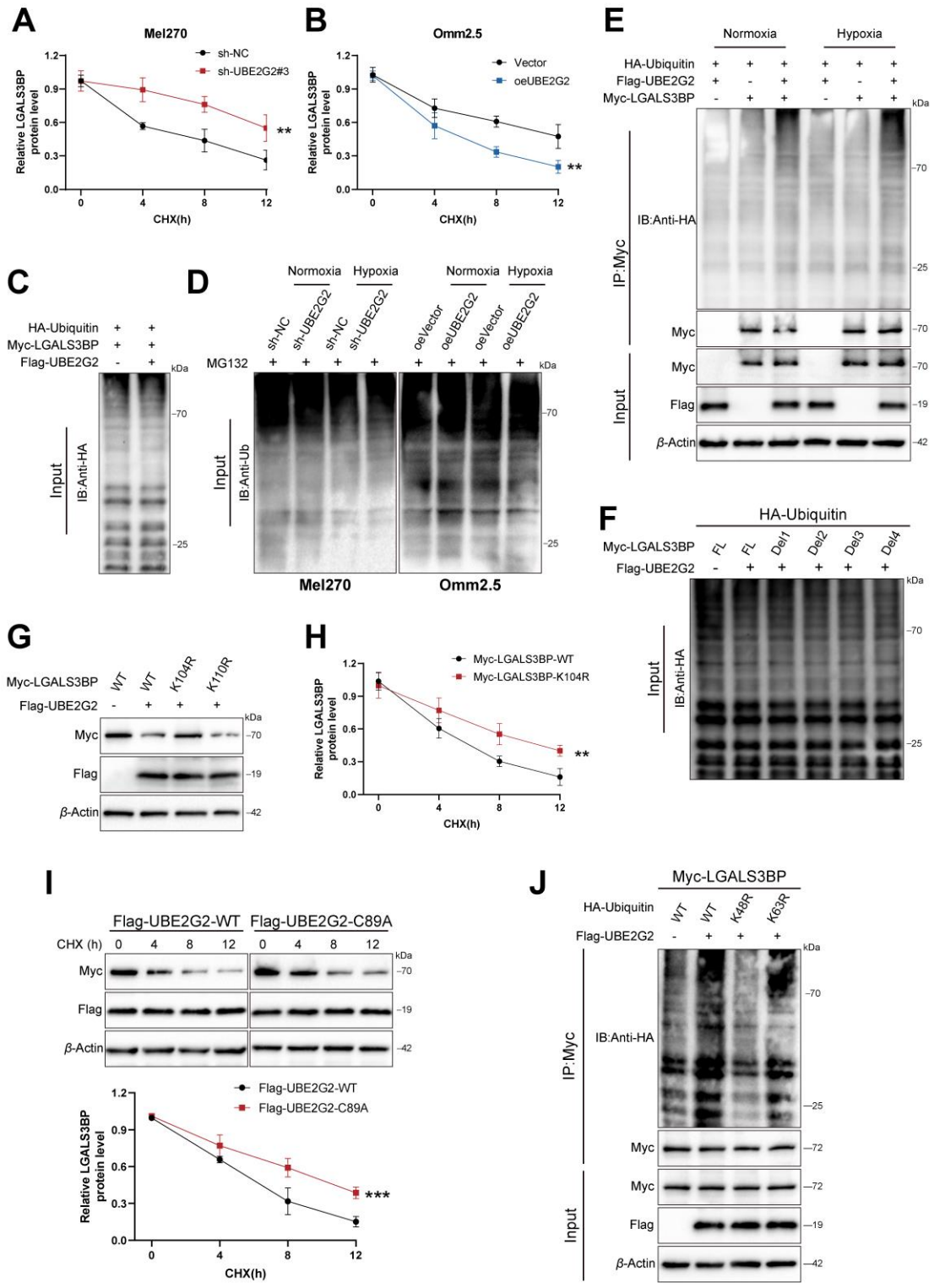

Figure S5. related to Figure 5. UBE2G2 promotes the polyubiquitination and degradation of LGALS3BP at K104 residue.

(**A, B**) Relative LGALS3BP protein levels in UM cells treated with CHX for the indicated times. (**C, D**) Ubiquitin control in whole cell lysate (WCL)/input of Figure

5D (**C**) and Figure 5E (**D**). (**E**) HEK-293T cells were transiently transfected with plasmids expressing LGALS3BP and UBE2G2, along with HA-tagged ubiquitin. Ubiquitination of LGALS3BP in HA-tagged ubiquitin transfected cells under normoxia and hypoxia. (**F**) Ubiquitin control in WCL/input of Figure 5G. (**G**) HEK-293T cells were transiently transfected with plasmids expressing Myc-tagged of indicated mutant LGALS3BP and plasmids expressing UBE2G2. The protein level of LGALS3BP of transfected cells. (**H**) HEK-293T cells were transfected with Myc-LGALS3BP-WT or Myc-LGALS3BP-K104R plasmid and then treated with CHX for the indicated times before harvesting. The protein level of LGALS3BP in transfected cells. (**I**) HEK-293T cells were transfected with plasmids expressing Myc-tagged LGALS3BP and Flag-UBE2G2-WT or Flag-UBE2G2-C89A plasmid and then treated with CHX for the indicated times before harvesting (Top). The protein level of LGALS3BP in transfected cells (Bottom). (**J**) HEK-293T cells were transfected with UBE2G2 plasmid and HA-tagged of indicated mutant ubiquitin plasmids, along with plasmids expressing Myc-tagged LGALS3BP. Cell lysates were analyzed by immunoblotting with indicated antibodies. All data are presented as the mean ± SD of three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$.

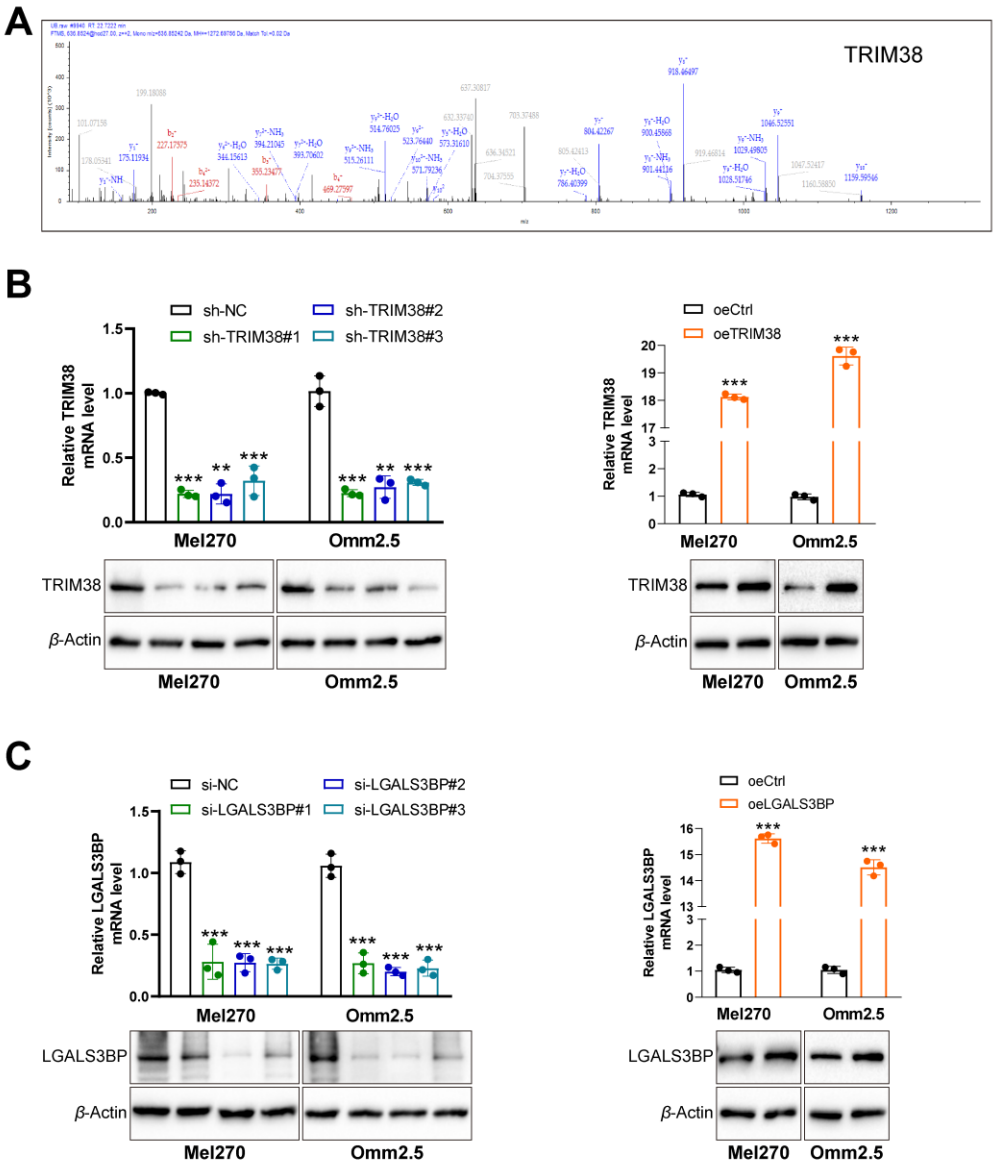

Figure S6. related to Figure 6. TRIM38 cooperates with UBE2G2.

(**A**) The result of MS followed by co-IP assay indicated that TRIM38 specifically interacted with UBE2G2. (**B, C**) qRT-PCR and Western blotting were used to measure the knockdown and overexpression efficiency of TRIM38 (**B**) and LGALS3BP (**C**) in UM cell lines. All data are presented as the mean ± SD of three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$.

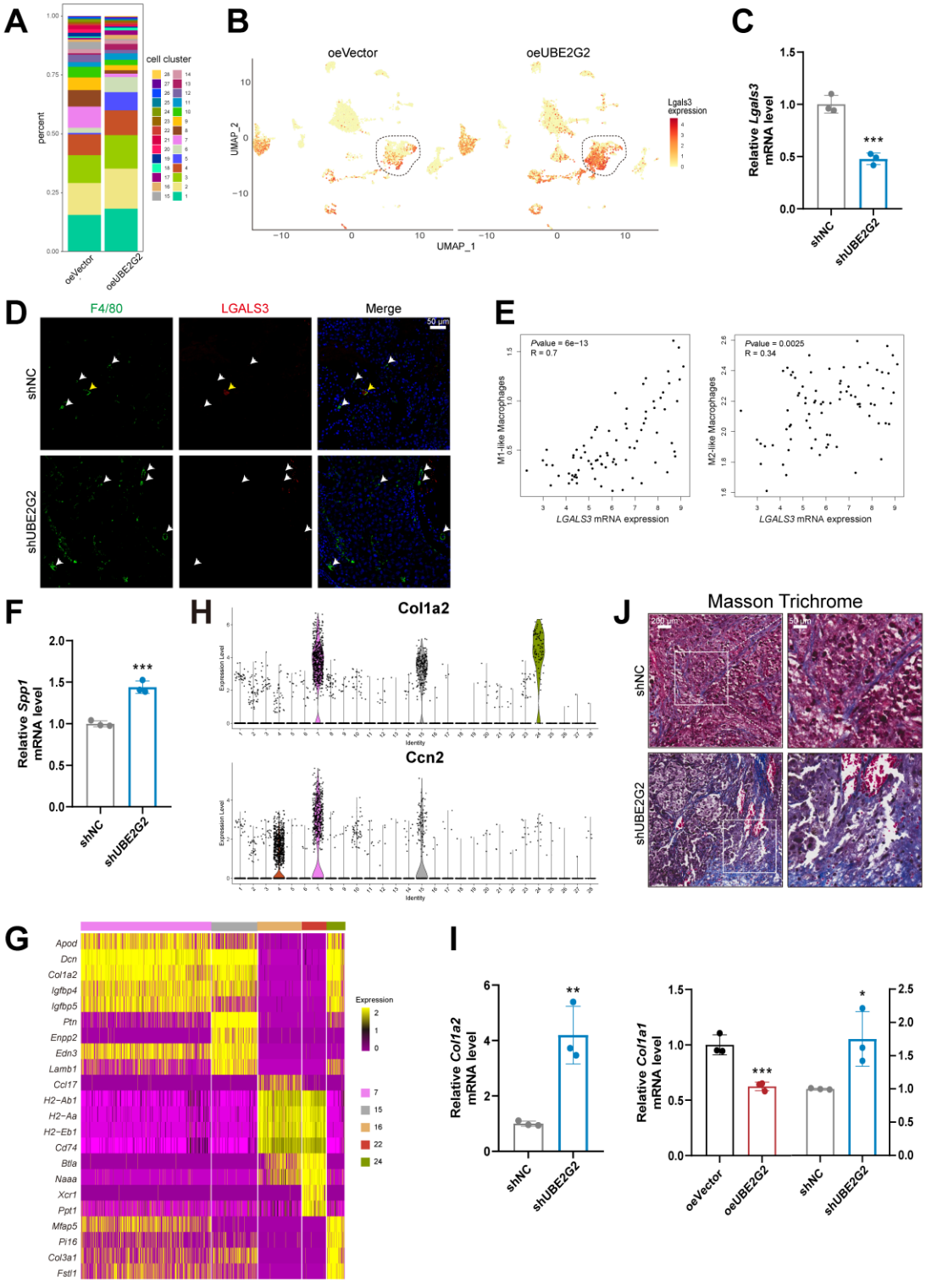

Figure S7. related to Figure 7. Identification of Ube2g2-induced transcriptional signature in macrophages and fibroblasts.

(**A**) Stacked bar plots showing the percentage of major cellular compartments in each cluster. (**B**) The distribution of *Lgals3* in cellular compartments of the indicated groups.

(**C**) The relative expression of *Lgals3* in macrophages of the indicated group. (**D**) Representative Immunofluorescence image showing F4/80 (a marker of macrophage) and LGALS3 staining in sections from orthotopic tumors from indicated groups. yellow arrow: LGALS3^{+}; white arrow: LGALS3^{-}. (**E**) Correlation between *LGALS3* mRNA expression and M1/M2-like macrophage signature in TCGA UM cohort. (**F**) The relative expression of *Spp1* in macrophages of the indicated group. (**G**) Heatmap of the top-5 genes for each cluster of fibroblasts. (**H**) Violin plots showed the expression of *Col1a2* and *Ccn2* in each cluster of fibroblasts. (**I**) The relative expression of *Col1a2* and *Col1a1* in fibroblasts of the indicated group. (**J**) Masson Trichrome staining in sections from orthotopic tumors from indicated groups. All data are presented as the mean ± SD of three independent experiments. $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

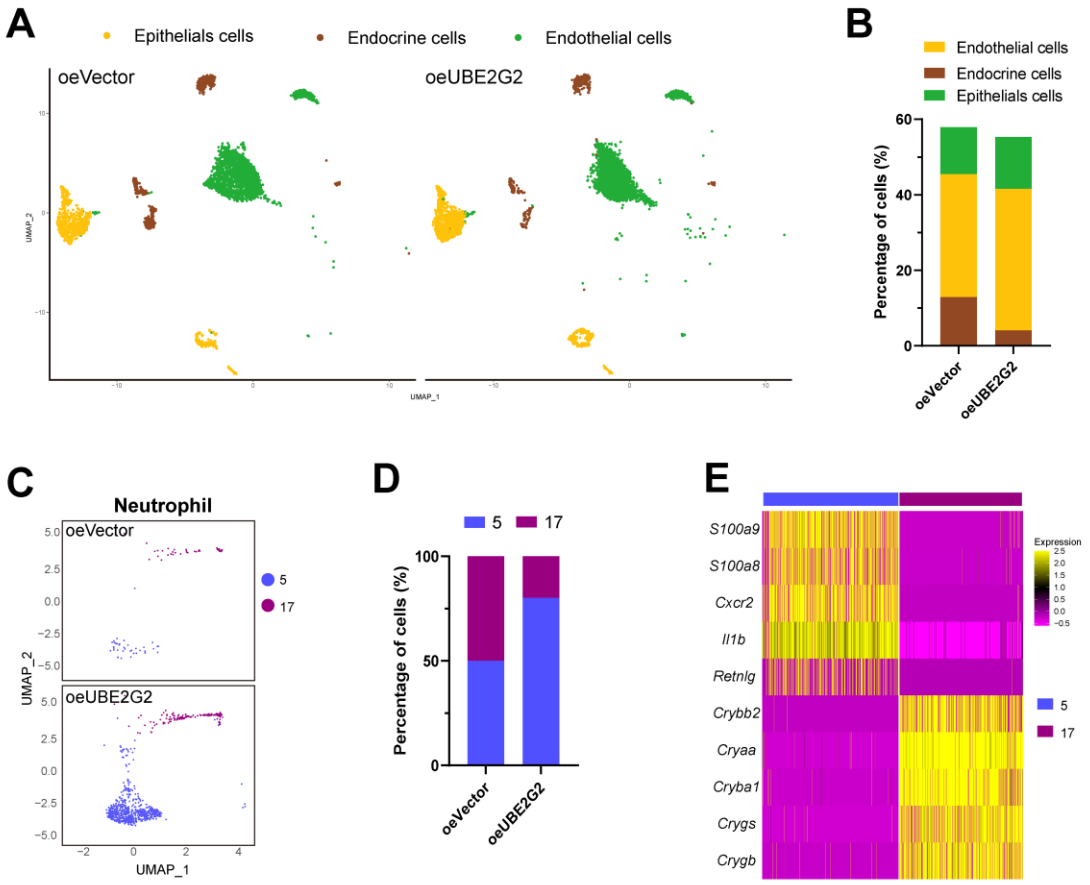

Figure S8. related to Figure 8. UBE2G2 reprograms the immune microenvironment *in vivo.*

(**A**) UMAP plot of the epithelial compartment from orthotopic tumors colored by Seurat clusters. (**B**) Stacked bar plots of the percentage of cells in the epithelial compartment. (**C**) UMAP plot with reclustering of neutrophils, split by sample. (**D**) Stacked bar plots showing the percentage of neutrophils in each cluster. (**E**) Heatmap of the top-5 genes for each cluster in neutrophils.

Supplemental Tables

Table S1. The clinical characteristics of uveal melanoma patients.

Patient No.	Sex (Female=0, Male=1)	Age	Stage (American Joint Committee on Cancer 9th)
1	1	48	T1
2	1	50	T2
3	1	36	T1
4	1	46	T2
5	0	55	T1
6	0	54	T2
7	0	72	T1

Table S2. Primer sequences used in the study.

Primer name		**Primer sequences**
UBE2G2	Forward Primer	ATCTACCCTGATGGGAGAGTCT
	Reverse Primer	CTCCACTTTCGTCATTGGGC
CASS4	Forward Primer	GCCAGGGCACTTTATGACAAC
	Reverse Primer	CTTCCACCAACCCTCGCTT
NEDD9	Forward Primer	ATGGCAAGGGCCTTATATGACA
	Reverse Primer	TTCTGCTCTATGACGGTCAGG
TRIM59	Forward Primer	AAGATCCTCGTGTACTGCCAT
	Reverse Primer	CAATGCCAGTTGGAGCAATTTC
TRIM2	Forward Primer	TGCGCCAGATTGACAAGCA
	Reverse Primer	GCACCTCTCGCAGAAAGTG
ACTB	Forward Primer	CATGTACGTTGCTATCCAGGC
	Reverse Primer	CTCCTTAATGTCACGCACGAT
CDH5	Forward Primer	TTGGAACCAGATGCACATTGAT
	Reverse Primer	TCTTGCGACTCACGCTTGAC
LGALS3BP	Forward Primer	AGGTACTTCTACTCCCGAAGGA
	Reverse Primer	GGCCACTGCATAGGCATACA
TRIM38	Forward Primer	GAGCCTGATGACGAACCCAG
	Reverse Primer	TCTTGATCCGTCTCTTTGAGGG
Lgals3	Forward Primer	TGGTTCCAGGGACTCAAGGTA
	Reverse Primer	CCACCGGCCTCTGTAGAAGA
Ube2g2	Forward Primer	TGGCCGAGTATAAGCAATTAACC
	Reverse Primer	GGCTCAAGGGGTAGTCAAGT
Spp1	Forward Primer	ATCTCACCATTCGGATGAGTCT
	Reverse Primer	TGTAGGGACGATTGGAGTGAAA
Col1a2	Forward Primer	TCGTGCCTAGCAACATGCC
	Reverse Primer	TTTGTCAGAATACTGAGCAGCAA
Col1a1	Forward Primer	GCTCCTCTTAGGGGCCACT
	Reverse Primer	ATTGGGGACCCTTAGGCCAT

Table S3. List of primary antibodies.

Name	Manufacturer	Catalog	Application	Dilution
Anti-HIF-1 alpha antibody	Abcam	ab179483	Western blot	1:1000
Anti-UBE2G2 antibody	Abcam	ab174296	Western blot	1:2000
			Immunofluorescence	1:200
			Immunoprecipitation	2 μg/test
Anti-β-actin antibody	Proteintech	81115-1-RR	Western blot	1:5000
Anti-VE-cadherin antibody	SANTA CRUZ	sc-9989	Western blot	1:1000
			Immunofluorescence	1:200
Anti-LGALS3BP antibody	Proteintech	10281-1-AP	Western blot	1:1000
			Immunofluorescence	1:200
			Immunoprecipitation	2 μg/test
Anti-S100B antibody	SANTA CRUZ	sc-58839	Immunofluorescence	1:200
Anti-CD34 antibody	Abcam	ab81289	Immunofluorescence	1:150
Anti-Myc-tag antibody	Proteintech	16286-1-AP	Western blot	1:1000
			Immunoprecipitation	2 μg/test
Anti-FLAG-tag antibody	Proteintech	20543-1-AP	Western blot	1:20000
			Immunoprecipitation	2 μg/test
Anti-GST-tag antibody	CST	#2622	Western blot	1:1000
			Immunoprecipitation	2 μg/test
Anti-Ubiquitin antibody	Proteintech	10201-2-AP	Western blot	1:1000
Anti-TRIM38 antibody	Proteintech	13405-1-AP	Western blot	1:500
			Immunoprecipitation	2 μg/test
Anti-His-tag antibody	Proteintech	66005-1-Ig	Western blot	1:5000
			Immunoprecipitation	2 μg/test
Anti-HA-tag antibody	Proteintech	51064-2-AP	Western blot	1:5000
Anti-ERK antibody	Abcam	ab50011	Western blot	1:10000
Anti-p-ERK antibody	Abcam	ab184699	Western blot	1:10000
Anti-JNK antibody	CST	#9252S	Western blot	1:1000
Anti-p-JNK antibody	CST	#9251S	Western blot	1:1000
Anti-AKT antibody	Proteintech	60203-2-Ig	Western blot	1:5000
Anti-p-AKT antibody	Proteintech	66444-1-Ig	Western blot	1:2000
Anti-PI3K antibody	Proteintech	20584-1-AP	Western blot	1:1000
Anti-p-PI3K antibody	Proteintech	#13857	Western blot	1:1000
Anti-F4/80 antibody	Proteintech	28463-1-AP	Immunofluorescence	1:200
Anti-LGALS3 antibody	Proteintech	60207-1-Ig	Immunofluorescence	1:400
Anti-rabbit secondary antibody	Proteintech	RGAR001	Western blot	1:10000
Anti-mouse secondary antibody	Proteintech	SA00001-1	Western blot	1:10000

Table S4. List of differently expressed proteins by label-free proteomic analysis.
Table S5. List of differently expressed proteins identifeid by Co-IP–MS.
See Excel files.