Supplementary information
LOXL2 drives epithelial-mesenchymal transition via activation of IRE1-XBP1 signalling pathway.

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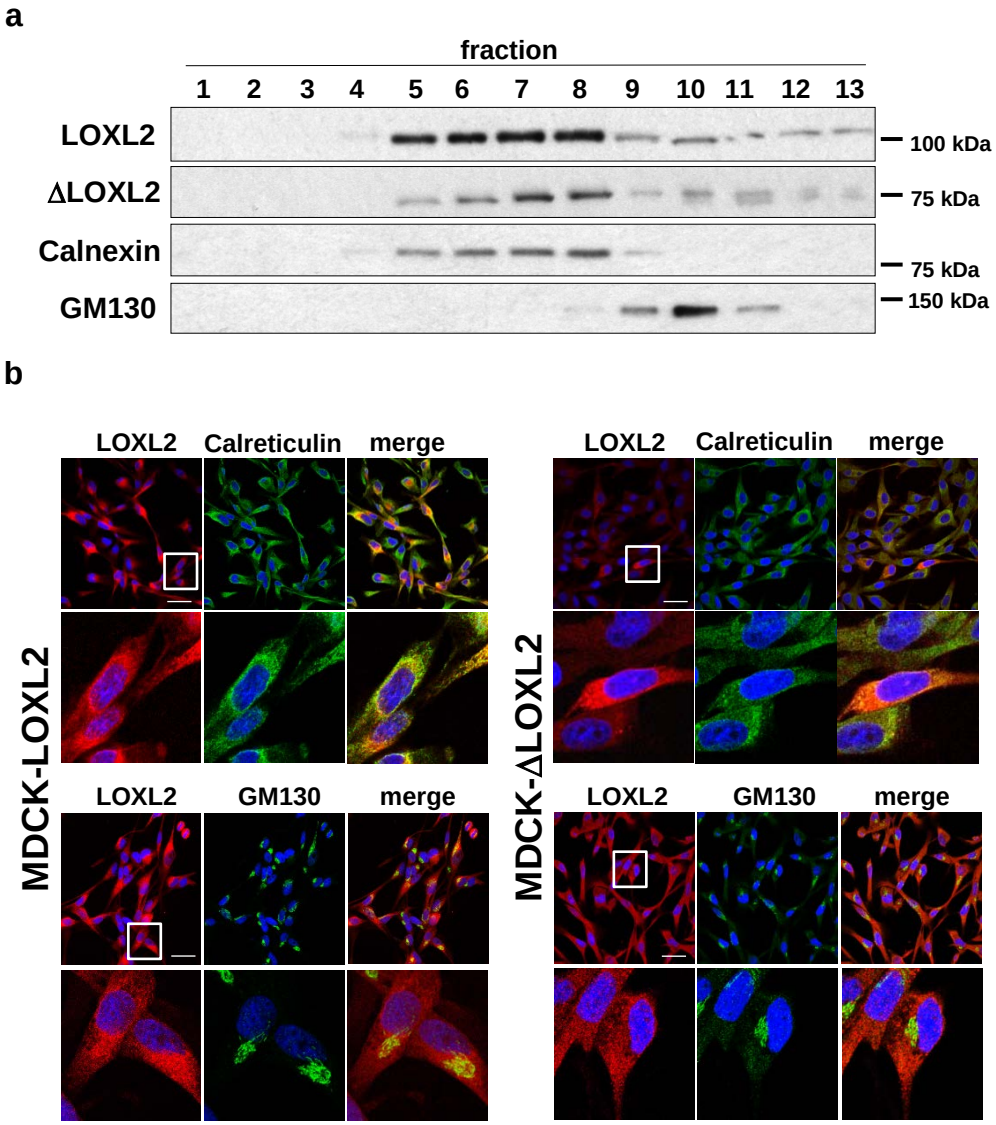
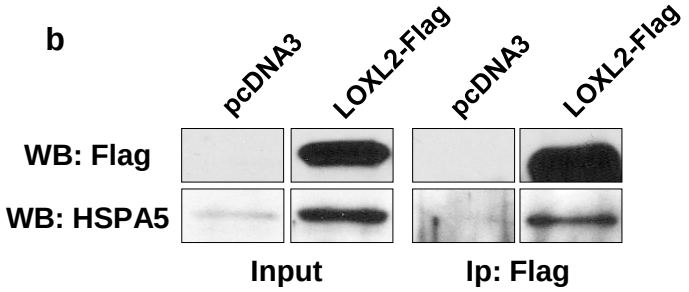
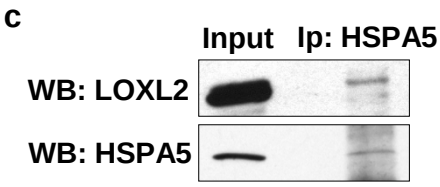


Supplementary Figure 1: Ectopic expression of LOXL2 and ΔLOXL2 provokes its retention in the ER. (**a**) MDCK cells stably expressing LOXL2 and ΔLOXL2 and fractionated on linear Optiprep gradient; fractions were analyzed by immunoblotting using Calnexin and GM130 as ER and cis-Golgi markers, respectively. (**b**) Confocal immunofluorescence images for ectopically expressed LOXL2 variants. Calreticulin and GM130 were used as markers of ER and cis-Golgi respectively. Amplified (detail) images are shown as indicated. Nuclei were counterstained with DAPI (blue); scale bars, 50 µm.

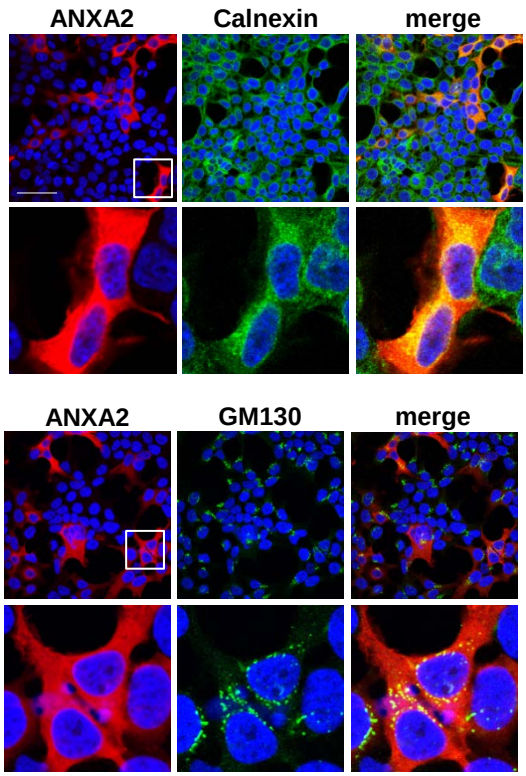
a

Accession	Description	Score	Matches
Q9Y4K0	LOXL2	591	16
P83731	RPL24	197	4
P11021	HSPA5	179	5
P02538	KRT6A	165	3
P62241	RPS8	140	3
P26373	RPL13	137	5
P30050	RPL12	106	2
P36578	RPL4	104	5
P61313	RPL15	99	3
P07437	TUBB	91	4
P15880	RPS2	74	4
P13639	EEF2	71	2
P62910	RPL32	71	2
P49327	FASN	59	2
Q02878	RPL6	55	2
P39023	RPL3	49	2
Q02543	RPL18A	46	2
O14654	IRS4	42	2
P62854	RPS26	29	2
P61247	RPS3A	22	2

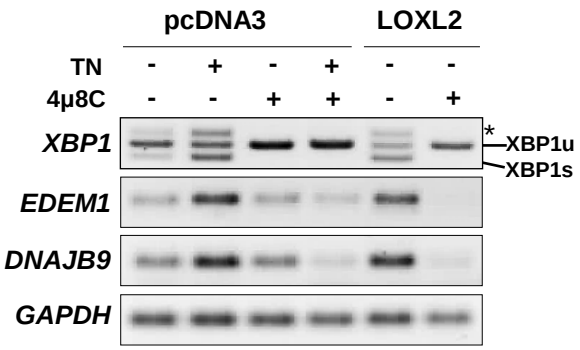




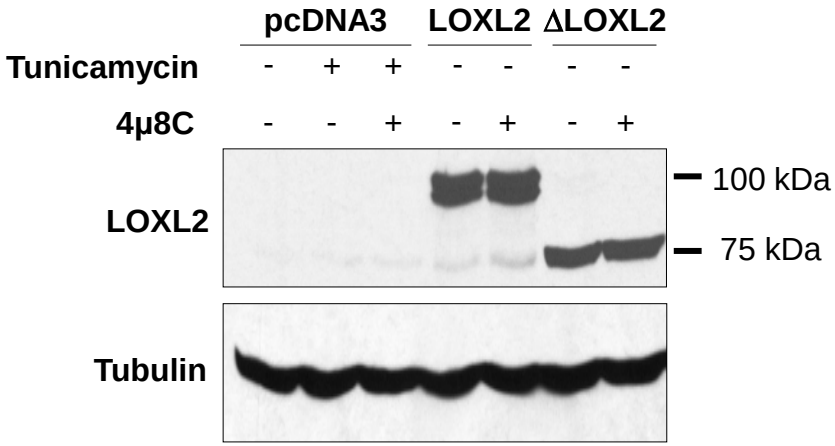
Supplementary Figure 2: LOXL2 interacts with HSPA5. (**a**) Mass spectrometry data of proteins immunoprecipitated with LOXL2. (**b**) Whole cell extracts from HEK293T cells transiently transfected with a Flag-tagged version of LOXL2 were immunoprecipitated with anti-Flag M2 affinity gel and analyzed by WB with anti-Flag or anti-HSPA5 antibodies. (**c**) Crude membranes fraction from Hs578T cells were immunoprecipitated with anti-HSPA5 antibody and analyzed by WB with anti-LOXL2 (Origene) or anti-HSPA5 antibodies.



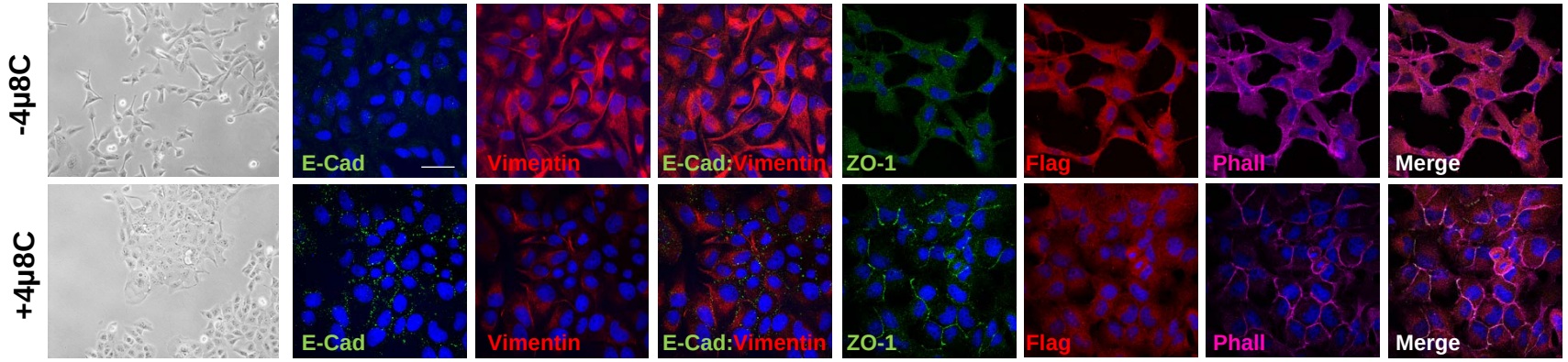
Supplementary Figure 3: Immunofluorescence analysis of ANXA2 localization. Confocal immunofluorescence images of HEK293T cells ectopically expressing ANXA2 (red). Calnexin and GM130 (green) were used as markers of ER and cis-Golgi respectively. Merged images are shown on the right panels. Amplified (detail) images are shown as indicated. Nuclei were counterstained with DAPI (blue); scale bars, 50 μm.



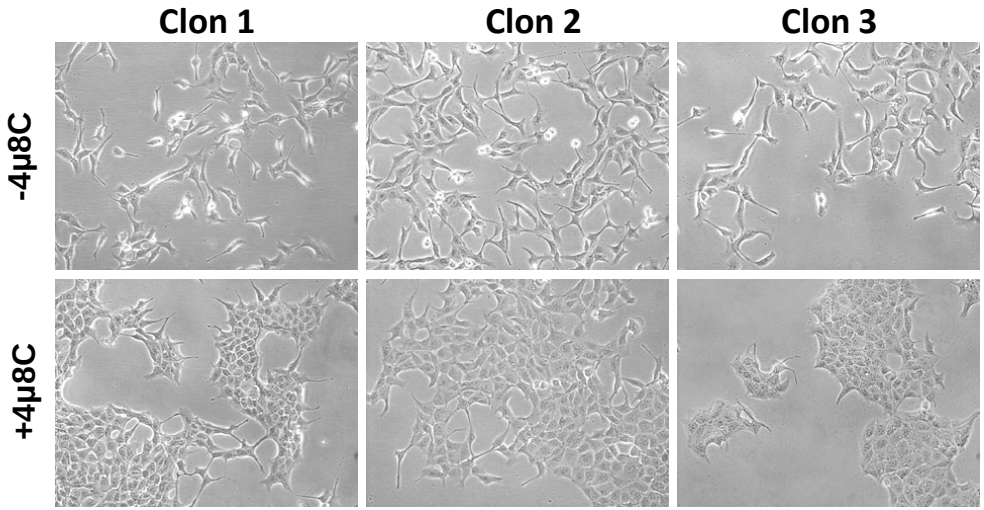
Supplementary Figure 4. **XBP1 splicing is induced by LOXL2.** RT-PCR analysis of XBP1 splicing in HEK293T cells transiently transfected with LOXL2. As control, XBP1 splicing was analysed in cells transfected with empty plasmid (pcDNA3) and treated with tunicamycin (TN) for 24 h. GAPDH levels serve as loading control. Unspliced (XBP1u) and spliced (XBP1s) forms of XBP1 are indicated. (*) XBP1 hybrid band [69]. One representative RT-PCR analysis of fourth independent experiments is shown.



Supplementary Figure 5: HEK293T cells transiently transfected with either empty vector pcDNA3 or pcDNA3 carrying *LOXL2*-HA or Δ*LOXL2*-FLAG genes were treated with tunicamycin (lanes 2 and 3) and/or 4μ8C inhibitor (lanes 3, 5 and 7) for 24 h and processed for WB. Anti-HA and anti-Flag were used as primary antibodies.



Supplementary Figure 6. The IRE1-XBP1 branch of the UPR mediates the ability of LOXL2 to induce EMT. MDCK-II cells with inducible expression of LOXL2-Flag were treated with doxycycline and after that with the IRE1 inhibitor 4μ8C for two weeks. Representative images of confocal immunofluorescence analyses of control and 4μ8C treated cells with antibodies against LOXL2 (anti-Flag, red), E-cadherin (E-cad, green), or ZO-1 (green), and vimentin (red) are shown. F-actin was detected with phalloidin stain (Phall, magenta). Nuclei were stained with DAPI. Merge images of LOXL2, F-actin and DAPI are shown on the right panels. Scale bars 50 μm.



Supplementary Figure 7. IRE1 inhibitor 4μ8C impedes the EMT induced by LOXL2. MDCK-II cells were transfected with LOXL2 and grown in presence and absence of 4μ8C. Phase contrast images of three independent MDCK-LOXL2 clones after 4 weeks of inhibitor treatment (bottom) compared to control untreated cells (upper).

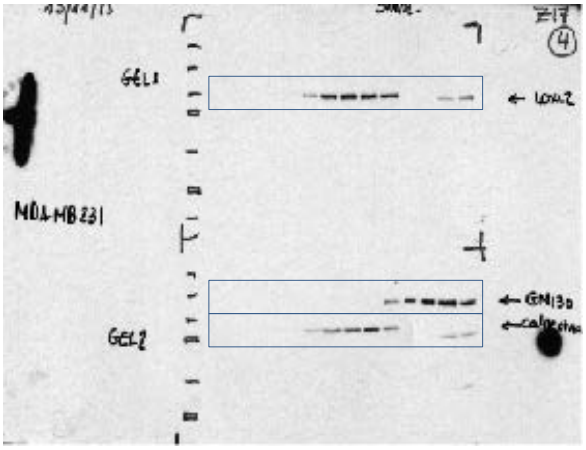


Fig. 1a

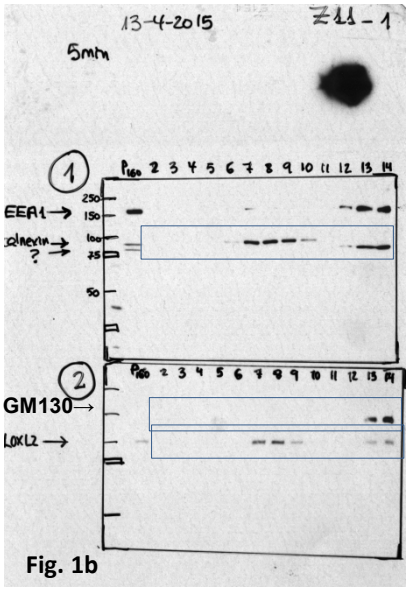
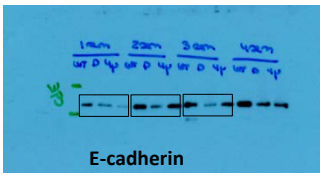
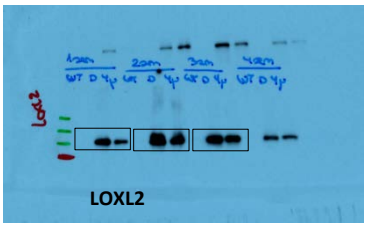
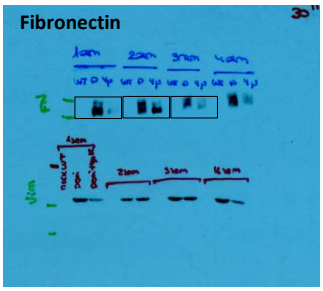
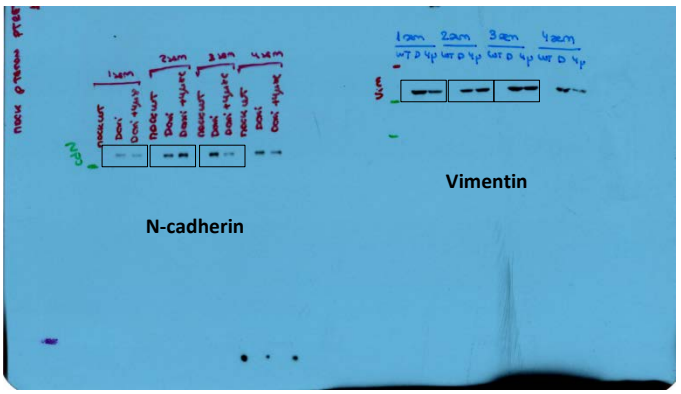


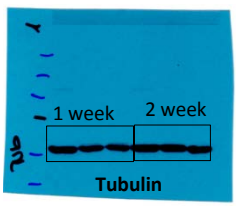
Fig. 1b











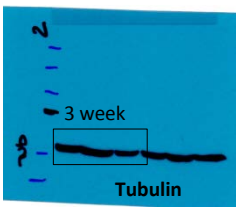


Fig. 5a

Supplementary Figure 8: Uncropped scans of western blot displayed in Fig. 1 and Fig. 5

Supplementary Table 1. Primers used in ChIP experiments

Gene	Forward	Reverse
SNAI1	GTCTCCCTCACTGGACCAGA	GAAGCGAGGAAAGGGACAC
SNAI2	GTGTGGGCTTGTCCAACGTG	CCATGCAAAGAAAACCCCGCA
ZEB2	ACCTGCTGCCTTGTTTCCTC	GTGGCTCCATCCAACCTCTC
TCF3	GACTCCGGCTGCCACACTAT	CCCATGGGTCTGAGGGTGTC
HSPA5	GTGAACGTTAGAAACGAATAGCAGCCA	GTCGACCTCACCGTCGCCTA

Supplementary Table 2. Primers used in RT-PCR analysis

Gene	Forward	Reverse
CDH1	CAAGCTATCCTTGCACCTCAG	GCATCAGAGAACTCCTATCTTG
EDEM1	CAATGAAGGAGAAGGAGAC	CAATGTGTCCCTCTGTTGTG
DNAJB9	AAAATAAGAGCCCGGATGCT	CGCTTCTTGGATCCAGTGTT
GAPDH	ACGGATTTGGTCGTATTGG	TTGACGGTGCCATGGAATT
LOXL2/ΔLOXL2	GGCACCGTGTGCGATGACGA	GCTGCAAGGGTCGCCTCGTT
SNAI1	GCGAGCTGCAGGACTCTAATC	AGGACAGAGTCCCAGATGAGC
SNAI2	CGCTCCTTCCTGGTCAAGA	TTGCGTCACTCAGTGTGC
TWIST1	GGAGTCCGCAGTCTTACGAG	TCTGGAGGACCTGGTAGAG
TCF3	GAGGAGAAAGACCTGAGGGACC	ACCTGACACCTTTTCCTCTTCTC
XBP1	TTACGAGAGAAAACTCATGGCC	GGGTCCAAGTTGTCCAGAATGC
ZEB1	GCCAATAAGCAAACGATTC	TTTGGCTGGATCACTTTCA
ZEB2	GGCGCAAACAAGCCAATCC	TTCACTGGACCATCTACAG

Supplementary Table 3. Primers used in quantitative real-time PCR analysis

Gene	Forward	Reverse
DDIT3	GGAGCATCAGTCCCCCACTT	TGTGGGATTGAGGGTCACATC
EDEM1	GCTACGACAACTACATGGCTC	GACTTGGACGGTGGAATCTTT
DNAJB9	TCGGCATCAGAGCGCCAAATCA	ACCACTAGTAAAAGCACTGTGTCCAAG
SEL1L	ATCTCCAAAAGGCAGCAAGC	TGGGAGAGCCTTCCTCAGTC
GAPDH	CTTCACCACCATGGAGGAGGC	GGCATGGACTGTGGTCATGAG

Supplementary Table 4. Antibodies used in the analysis.

A. Primary antibodies

Marker	Antibody	Supplier	Dilution (WB/IF)
ATF6	Rabbit pAb	Origene	1:1000/na
Calnexin	Rabbit pAb	Abcam	1:5000/1:100
Calreticulin	PA3-900	ThermoFisher	na/1:100
E-cadherin	ECCD-2	M Takeichi[1]	1:200/1:100
N-cadherin	3B9	Zymed	1:500/1:500
EIF2a	Rabbit pAb	Cell Signaling	1:1000/na
pEIF2a (Ser51)	Rabbit mAb	Cell Signaling	1:1000/na
F-Actin	Alexa Fluor 647 Phalloidin	Amersham	na/1:1000
Flag	M2	Sigma	1:1000/1:100
Fibronectin	IST-4	Sigma	1:5000/1:400
GM130	EP892Y	Abcam	1:1000/1:100
HA	3F10	Sigma	1:1000/1:100
HSPA5	Rabbit pAb	Abcam	1:1000/na
LOXL2	4H3	Origene	1:2000/1:100
LOXL2	Rabbit pAb	K. Csiszar[2]	1:1000/na
Tubulin	(DM1A)	Sigma	1:10000/na
Vimentin	V9D	Dako	1:1000/1:100
ZO-1	Rabbit pAb	Zymed	na/1:100

[1] Navarro *et al.*, J. Cell Biol (1991) 115(2):517-33.

[2]Fong SF *et al*., Genes, Chromosomes and Cancer (2007) 46: 644-655

na. not apply

B. Secondary antibodies

Antibody	Catalog	Supplier	Immunological Procedure [a]	Dilution
Anti-Rat HPRT conjugated	31470	Thermo Fisher	WB	1:10000
Anti-mouse HPRT conjugated	NXA931	GE Heathcare	WB	1:10000
Anti-rabbit HPRT conjugated	NA934	GE Heathcare	WB	1:10000
Anti-Rat Alexa 488	A-11006	Molecular Probes	IF	1:1000
Anti-Rat Alexa 594	A-11007	Molecular Probes	IF	1:1000
Anti-Mouse Alexa 488	A-11001	Molecular Probes	IF	1:1000
Anti-Mouse Alexa 594	A-11005	Molecular Probes	IF	1:1000
Anti-Rabbit Alexa 488	A-11008	Molecular Probes	IF	1:1000
Anti-Rabbit Alexa 594	A-11012	Molecular Probes	IF	1:1000

WB. Western blot; IF: immunofluorescence.