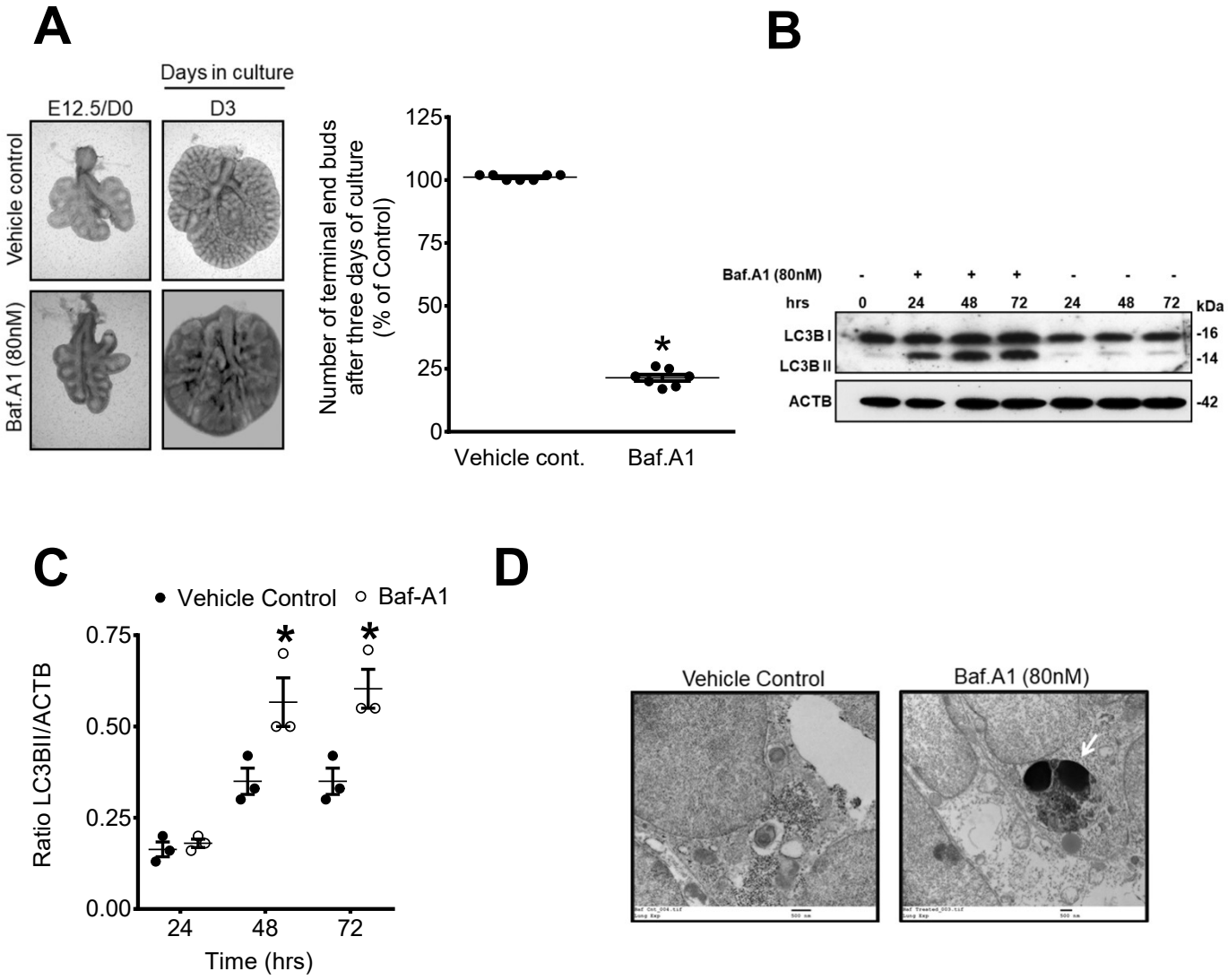
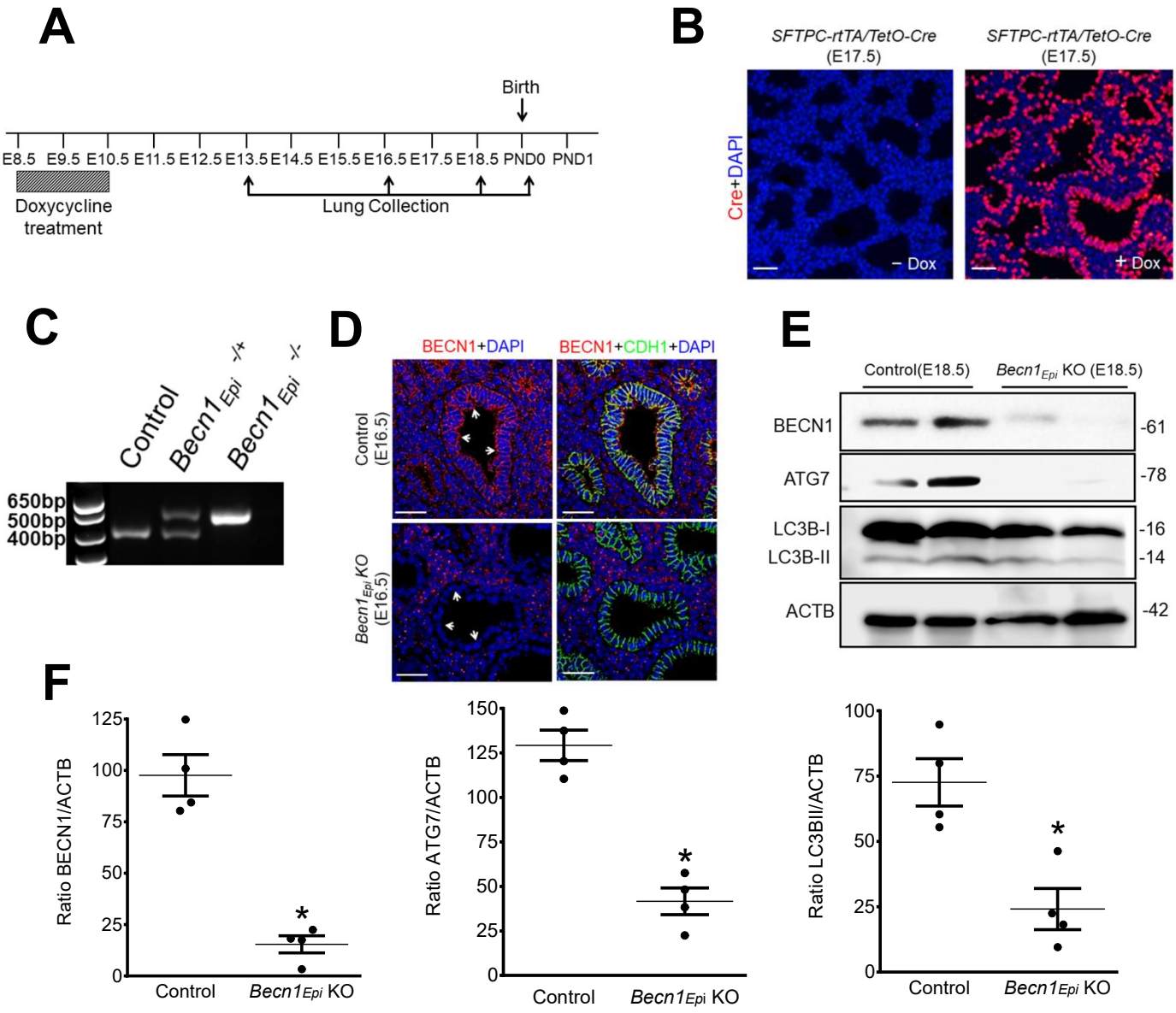
Supplementary Figure 1



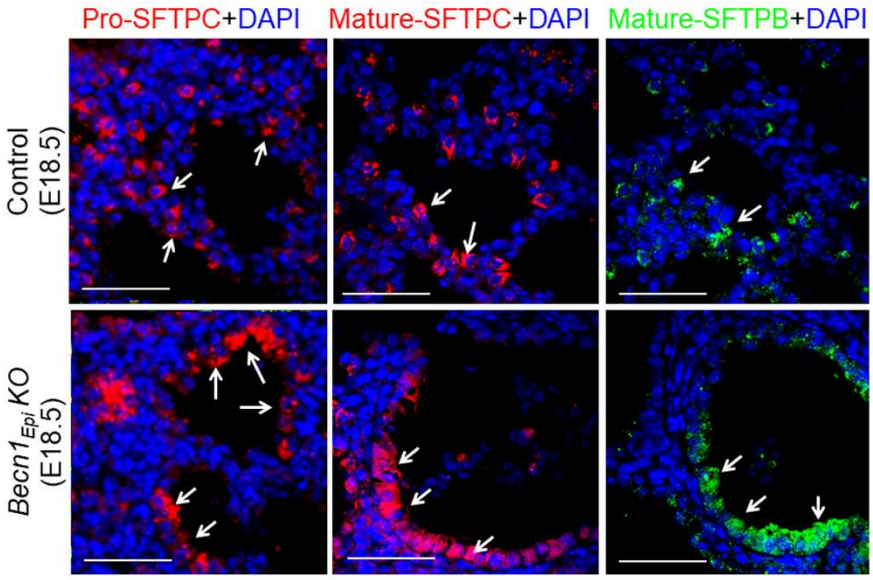
Supplemental Figure 1: Autophagy flux inhibition reduces early lung branching *in vitro.* (**A**-left panel) Representative lung explant micrographs cultured in the presence of autophagy flux inhibitor bafilomycin A_1 (Baf A_1 inhibits autophagosome-lysosome formation. E12.5 lung explants (D0) were treated with 80 nM Baf A1 or vehicle control and terminal buds were counted after 72 hours of culture (D3) for quantitative evaluation of early branching morphogenesis (**A**-right panel). The number of terminal end buds at D3 is expressed as percentage of vehicle control (mean ± SEM, n=7 separate lung explant isolations, *P<0.05 vs. wt control using student-t-test). (**B**) Representative immunoblot for LC3B-II in lysates of lung explants treated with and without 80 nM Baf A_1 for 72 h. (**C**) Densitometric analysis of LC3B-II in lysates of lung explants. Actin (ACTB) was used as protein loading control. Data are expressed as mean ± SEM, n=3 separate lung explant isolations, **P<0.05 vs*. vehicle control using one-way ANOVA followed by Tukey post hoc test. (**D**) Representative TEM images showing accumulation of undigested cargo in autophagolysosomes in Baf A_1 treated explants compared to vehicle-treated explants. Scale bar: 500 nm.

Supplementary Figure 2



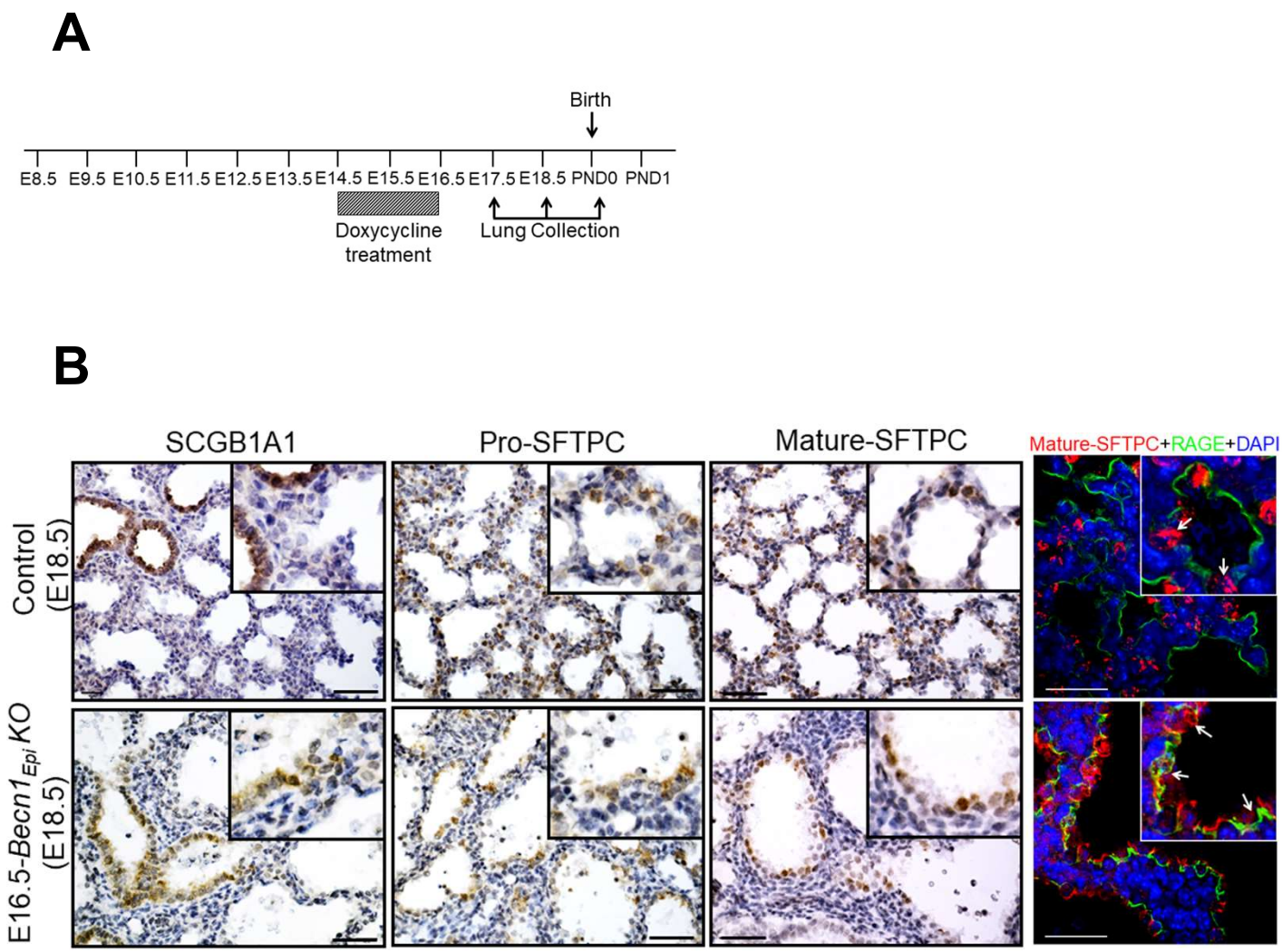
Supplemental Figure 2: Conditional deletion of *Becn1* in mouse lung epithelial cells during fetal lung development. (**A**) Experimental protocol for doxycycline treatment and lung sample collection. (**B**) Representative IF for Cre recombinase in lung tissue sections from E17.5 embryos of *SFTPC-rtTA/TetO-Cre* transgenic mice. Inducible Cre expression in offspring was achieved by feeding doxycycline to the pregnant mice. Scale bar: 20 µm. (**C**) PCR analysis of genomic DNA extracted from *SFTPC-rtTA/TetO-Cre/Becn1*$^{flox/flox}$ and wild-type littermates lungs using specific transgene primers. (**D**) Representative confocal IF microscopy of E16.5 embryos of lung epithelial specific *Becn1* KO (E10.5-*Becn1*$_{Epi}$ KO) and littermate control mice for *Becn1* (red) and CDH1 (green), demonstrate lack of expression of *Becn1* in airway epithelial cells in *Becn1*$_{Epi}$ *KO* lungs. White arrowheads point to airway epithelial cells identified by CDH1 expression. Nuclei were visualized with DAPI (blue). Scale bar: 20 µm. (**E**) Representative Western blots for BECN1, ATG7, LC3B in total lung protein extracts of E18.5 littermate control) and *Becn1*$_{Epi}$ KO mice. Two independent lung samples from both groups are shown. (**F**) Densitometric analysis of BECN1, AtG7 and LC3BII proteins. Actin (ACTB) was used as protein loading control. Data represent mean ± SEM, n=4 independent experiments, *P<0.05 vs. matched wt controls using student-t-test.

Supplementary Figure 3



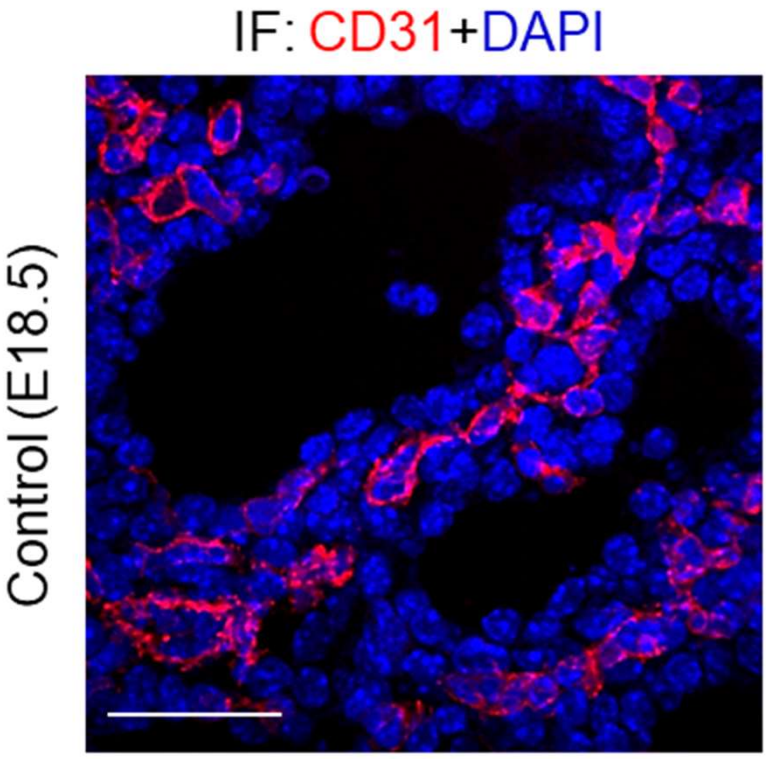
Supplementary Figure 3: Effect of conditional deletion of lung epithelial *Becn1* on distal epithelial differentiation. Representative confocal IF microscopy of E18.5 lung tissue sections from E10.5-*Becn1*$_{Epi}$ KO and littermate control mice stained for alveolar progenitor cell markers, pro-SFTPC (red), mature SFTPC (red) and SFTPB (green). Nuclei were visualized with DAPI.

Supplementary Figure 4



Supplementary Figure 4: Effect of conditional deletion of lung epithelial *Becn1* at E16.5 of lung development on distal epithelial differentiation. (**A**) Experimental protocol for epithelial *Becn1* deletion at canalicular/saccular stages of lung development (E16.5-$Becn1_{Epi}$ KO). Pregnant mice received doxycycline in drinking water (0.4mg/ml) or food for 48h starting at E14.5. Lungs of the embryos were harvested at different stages of development. **(B**-left panel**)** Representative IHC images for Clara cell secretory protein (SCGB1A1), pro- surfactant protein C (Pro-SFTPC) and mature surfactant protein C (SFTPC) in lung tissue sections of E16.5-$Becn1_{Epi}$ KO and littermate control mice at E18.5. Scale bar: 50 um**. (B**-right panel**)** IF microscopy of E18.5 lung tissue sections from E16.5-$Becn1_{Epi}$ KO and littermate control fetuses for mature SFTPC (red) and RAGE (green), an alveolar type I marker. Nuclei were visualized with DAPI. Scale bar: 25 um.

Supplementary Figure 5



Supplementary Figure 5: Endothelial cell marker CD31 staining of embryonic lungs (E18.5).
Confocal IF microscopy of embryonic lungs (E18.5) stained for endothelial cell marker CD31 shows CD31 positivity (red) in a single layer of endothelial cells. Nuclei were visualized with DAPI (blue). Image is captured using 40X objective . Scale bar: 25 um.

Autophagy is required for lung development and morphogenesis

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Supplemental Material

Table 1. List of antibodies and their dilution used in WB and Immuno staining

Target	Host/ Class	Dilution for			Catalog #	Vendor
		WB	IF	IHC		
Primary Antibodies						
Cleaved caspase-3	Rabbit	1:500	-	1:50	9661	Cell Signaling Technology, (Danvers, MA, USA)
Atg5-12	Rabbit	1:1000	-	-	2630	
Atg7 (D12b11)	Rabbit/ Mono	1:1000	-	-	8558	
LC3B	Rabbit	1:1000	-	-	2775	
Beclin-1	Rabbit	1:1000	-	-	3738	
Vimentin	Rabbit/ mAB	-	1:100	-	5741	
CD31 (PECAM-1)	Rabbit/ mAB	1:1000	1:200		77699	
AMPKβ1/2	Rabbit/ mAB	1:1000	-	-	57C12	
P-AMPKβ1 (Ser182)	Rabbit/ mAB	1:1000	-	-	4186	
Cleaved PARP (E51)	Rabbit/ mAB	1:1000	1:200	1:200	ab32064	Abcam, (Cambridge, MA, USA)
TTF1 (NKX2.1)	Rabbit	1:1000	1:200	1:200	ab76013	
Pro-SFTPC	Rabbit	-	1:200	-	Ab40879	
Ki67	Rabbit	-	-	1:50	Ab833	
B-actin	Mouse/ mAB	1:10,000	-	-	A5316	Sigma-Aldrich, (Oakville, ON, Canada)
LC3B	Rabbit	1:3000	-	-	L8918	
Mature SP-B	Rabbit	-	1:800	1:800	WRAB-48604	Seven Hills Bioreagents, (Cincinnati, OH, USA)
Mature SP-C	Rabbit	-	1:800	1:800	WRAB-76694	
LC3B	Rabbit	-	-	1:200	18725-1-AP	Proteintech, (Rosemont, IL, USA)
CDH1	Mouse/ mAB	-	1:50	-	610181	BD Biosciences, (Mississauga, ON, Canada)
Fibronectin	Rabbit	1:200	-	-	Sc-9068	Santa Cruz Biotechnology, Inc. (Mississauga, ON, Canada)
Bax (B-9)	Mouse/ mAB	1:200	-	-	Sc-7480	
CCSP (CC10)	Rabbit	-	-	1:200	Sc-2555	

Secondary Antibodies

Rabbit IgG (HRP-linked)	Goat	1:3,000	-	-	7074	Cell Signaling Technology, (Danvers, MA, USA)
Mouse IgG (HRP-linked)	Horse	1:3,000	-	-	7076	
Rabbit IgG (Biotin-SP)	Goat/ Poly	-	-	1:400	111-065-003	Jackson Immuno Research, (West Grove, PA, USA
Rabbit IgG (H+L) Alexa Fluor 546	Goat/ Poly	-	1:250		A11035	Invitrogen, (Life Technologies Corporation, Burlington, ON, Canada)
Mouse IgG (H+L) Alexa Fluor 488	Goat/ Poly	-	1:250	-	A11034	

WB: Western blot, IF: Immunofluorescent staining, IHC: Immunohistochemistry