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Supplementary Material

Co-Chaperone Bag-1 Plays a Role in the Autophagy-Dependent Cell Survival Through Beclin-1 Interaction

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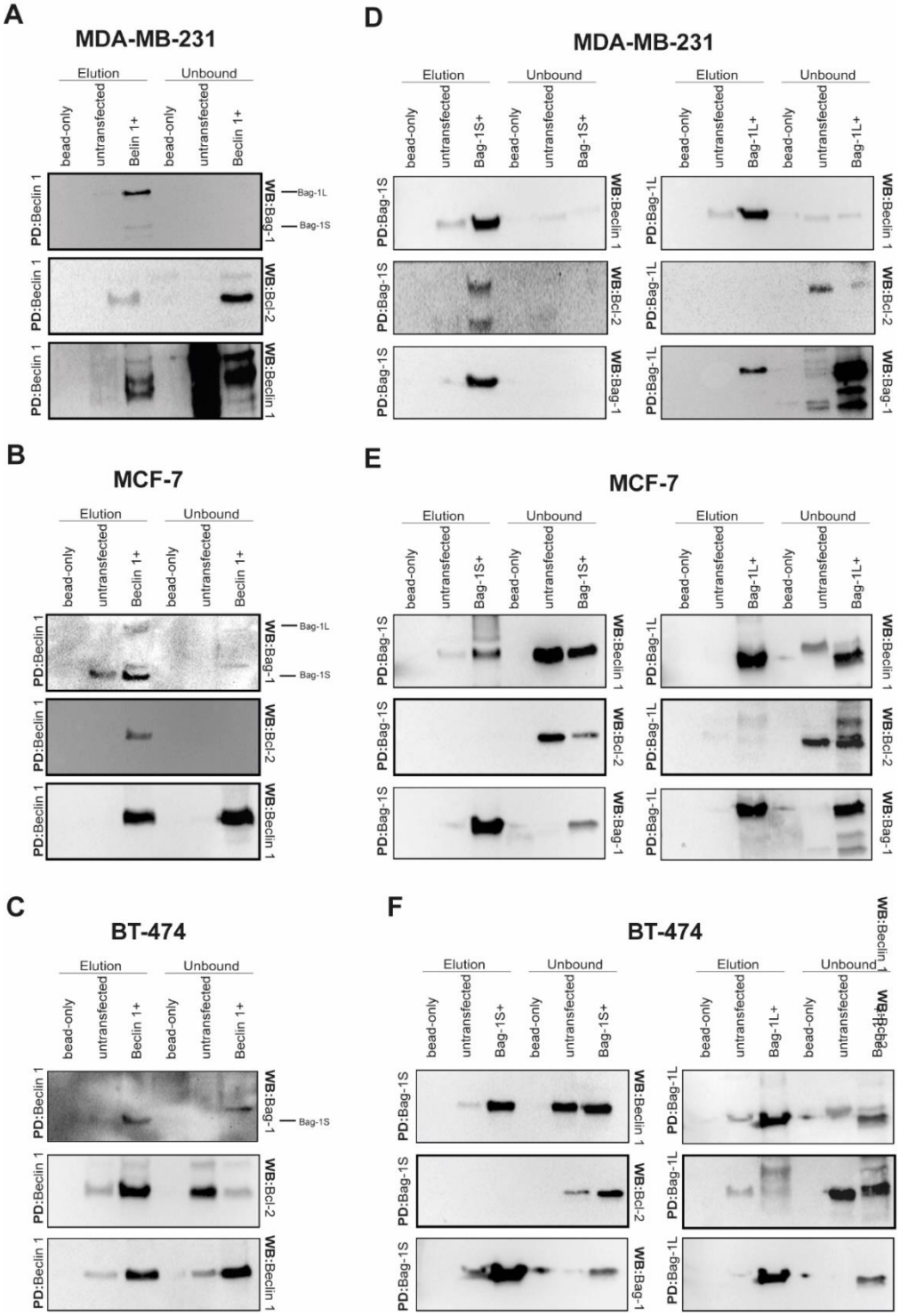

Figure S1. Beclin 1 interacts with both Bag-1S and Bag-1L isoforms. A-C) His_6Beclin 1 was his-pulled down by Ni-NTA beads from (A) MDA-MB-231, (B) MCF-7, and (C) BT-474 cell lysates. After wash, bound Beclin 1-related complexes were released by imidazole. As a control, precipitations were also carried out in parallel experiments with untransfected cell lysate and empty Ni-NTA beads. Elution and unbound fractions were subjected to western blot analysis using anti-Bag-1, anti-Bcl-2, anti- Beclin 1 antibodies. D-F) Both His_6Bag-1S and His_6Bag-1L were his-pulled down in (D) MDA-MB-231, (E) MCF-7 and (F) BT-474 cells as reciprocal experiments and analyzed by western blotting. Bag-1 and Beclin 1 were co-precipitated in both directions.

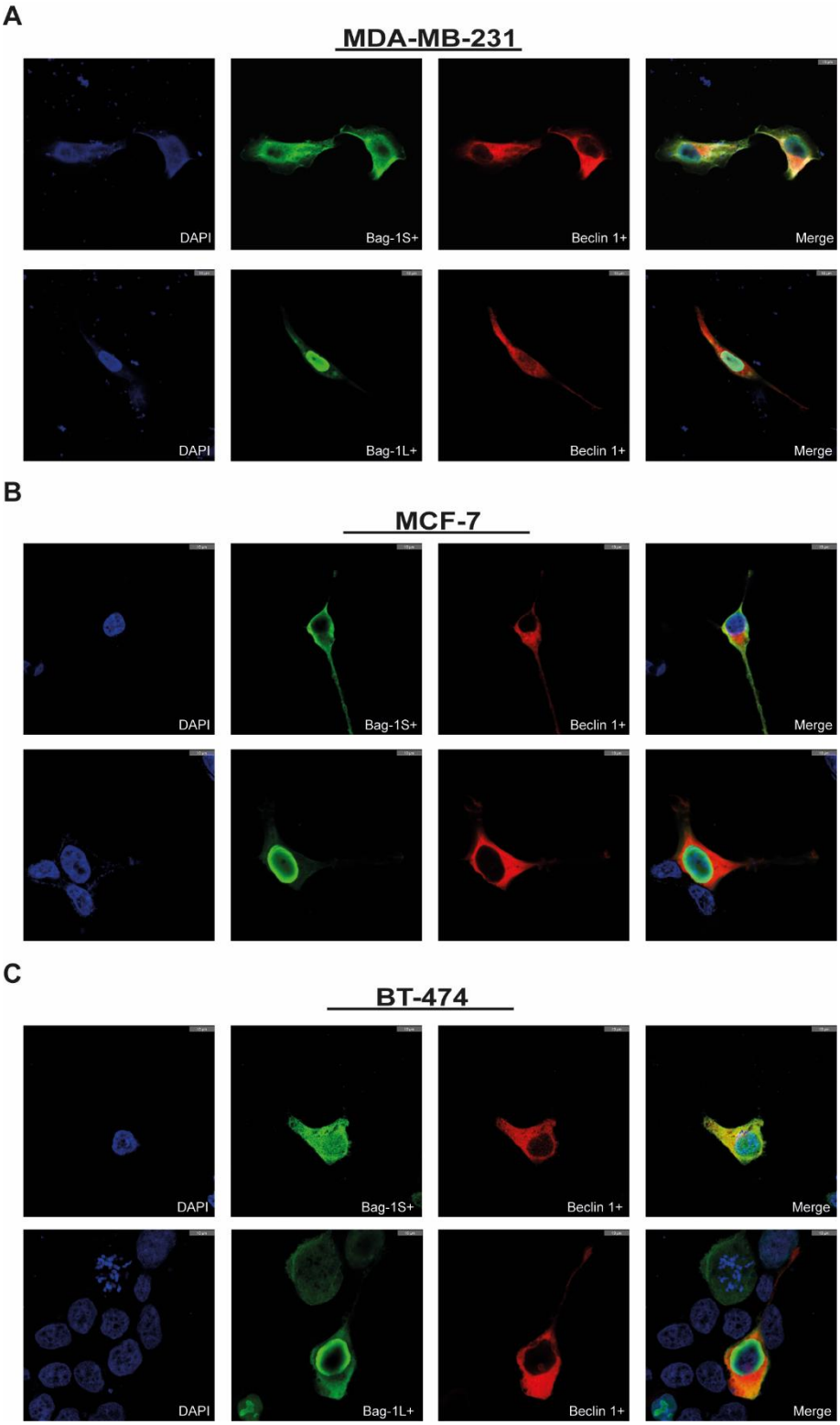

Figure S2. Interaction of Bag-1S and Beclin 1 is revealed in situ by immunocytochemistry assay. Interaction of Bag-1S and Beclin 1 is revealed in situ by immunocytochemistry assay. (A) MDA-MB-231, (B) MCF-7, and (C) BT-474 cells were co-transfected with His_6Beclin 1 and His_6Bag-1S or His_6Bag-1L and their co-localization is investigated via immunocytochemistry. Bag-1S and Bag-1L is independently stained green and Beclin 1 was stained as red while nucleus was shown in blue. Co-localized areas were represented in yellow. Scale bar equals to 10μm. In all cell lines, Bag-1S and Beclin 1 was observed in cytoplasm while Bag-1L is located inner side of nuclear membrane. Only small isoform of Bag-1 is observed co-localized to Beclin 1.

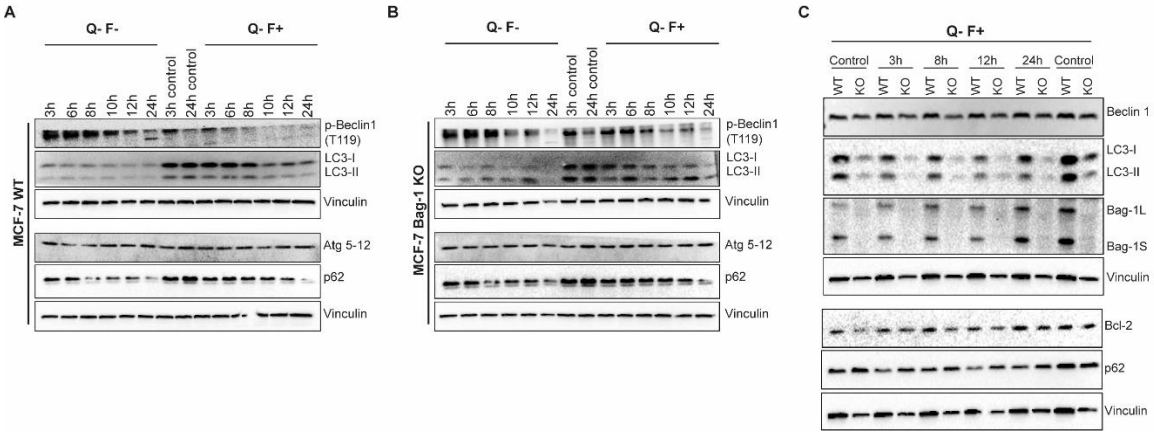

Figure S3. Bag-1 participates in autophagic regulation in breast cancer cells. (A,B) Immunoblotting of cell lysates from wild-type (WT) (A) and Bag-1 knock-out (KO) (B) MCF-7 cells that were treated in only glutamine-free (Q-F+) or serum and glutamine-free (Q-F-) conditions for 3, 6, 8, 10, 12, and 24 hours (h). Controls include lysates from cells treated with standard DMEM for 3 (left) and 24 hours (right). Expression of some autophagy markers including p-Beclin 1 (T119), LC3, p62, and Atg5-12 were detected. Vinculin was used as endogenous control. (C) Immunoblotting of cell lysates from wild-type (WT) and Bag-1 knock-out (KO) MCF-7 cells treated in glutamine-free conditions for 3, 8, 12 and 24 hours. Controls include lysates from cells treated with standard DMEM for 3 hours (left) and 24 hours (right). Bag-1 expression together with some autophagy markers including Beclin 1, LC3, p62, and Bcl-2 were detected. GAPDH was used as endogenous control. 20 μg protein samples were loaded to each well in both panels.

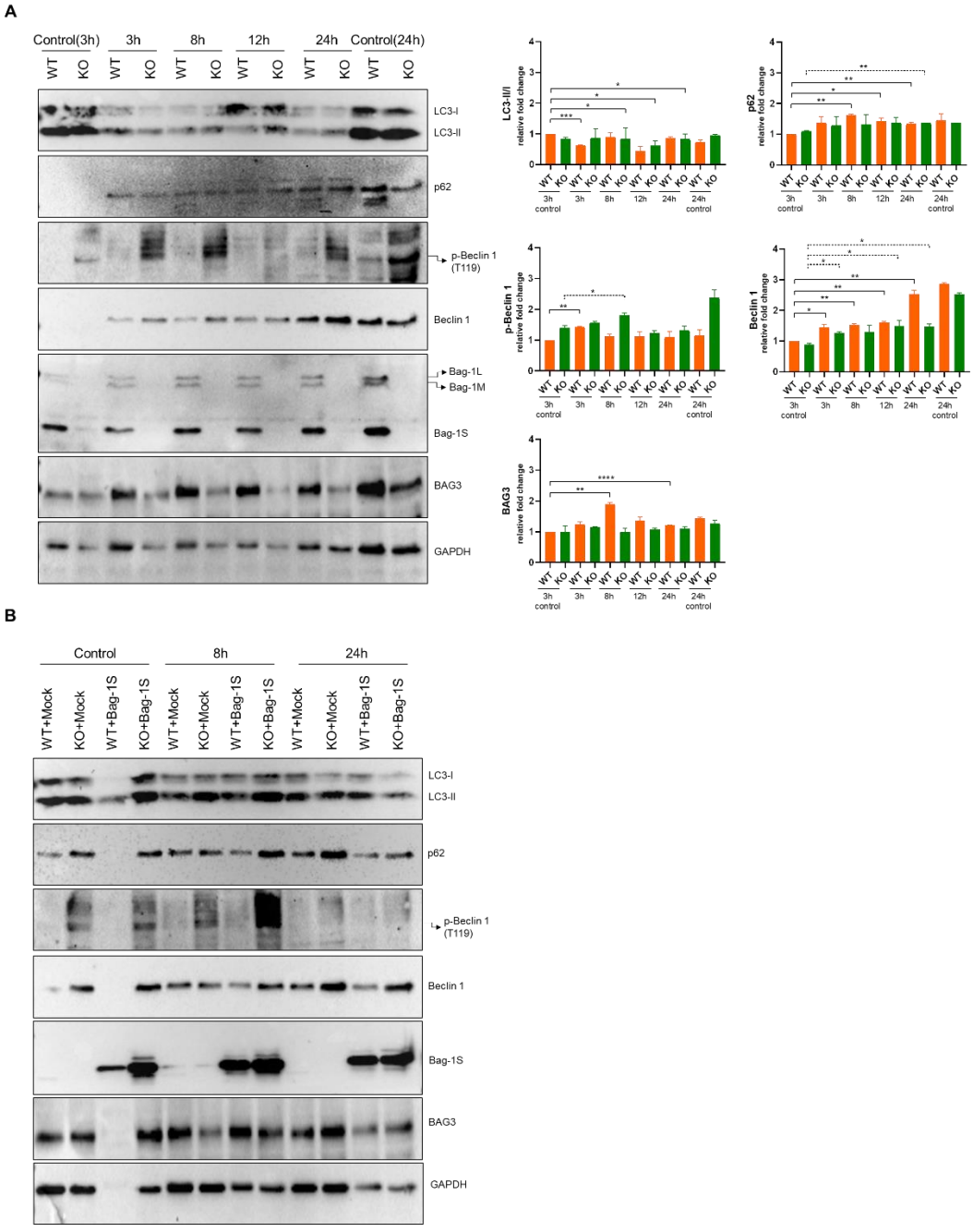

Figure S4. Bag-1 participates in autophagic regulation in breast cancer cells. (A) Immunoblotting of cell lysates from wild-type (WT) and Bag-1 knock-out (KO) MCF-7 cells that were treated in serum and glutamine-free conditions for 3, 8, 12, and 24 hours (h). Controls include lysates from cells treated with standard DMEM for 3 (left) and 24 hours (right). Bag-1 expression together with some autophagy markers including LC3, p62, p-Beclin 1 (T119), and Beclin 1 were detected. BAG3 levels were also measured to screen Bag-1/BAG3 ratio. GAPDH was used as endogenous control. (B) Immunoblotting of cell lysates from wild-type (WT) and Bag-1 knock-out (KO) MCF-7 cells that were transfected with *Mock* or *Bag-1S* vectors and treated in serum-free and glutamine-free conditions for 8 and 24 hours. Controls include lysates from cells treated with standard DMEM for 24 hours. Bag-1 expression together with some autophagy markers including LC3, p62, p-Beclin 1 (T119), Beclin 1 were detected. BAG3 levels were also measured to screen Bag-1/BAG3 ratio. GAPDH was used as endogenous control. 20 μg protein samples were loaded to each well in both panels.