

## **Supplemental Material to:**

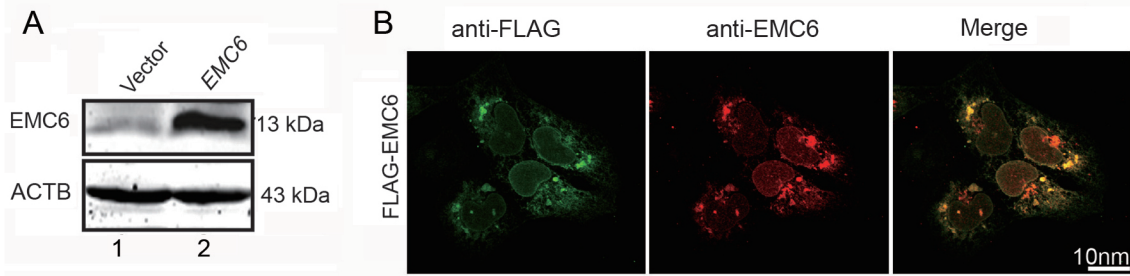
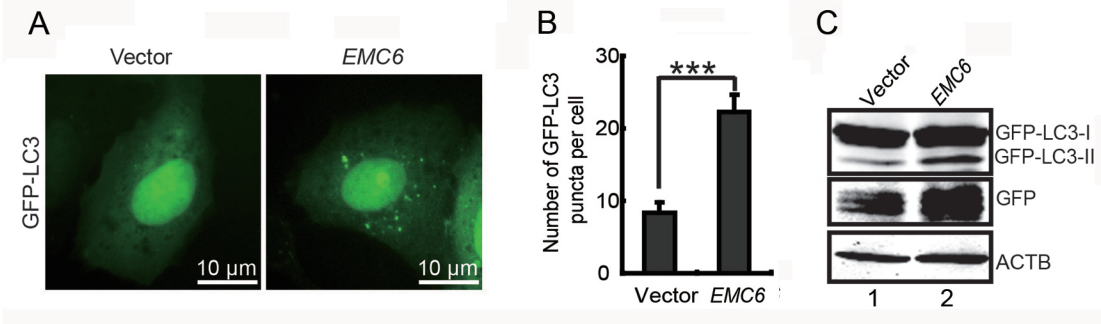
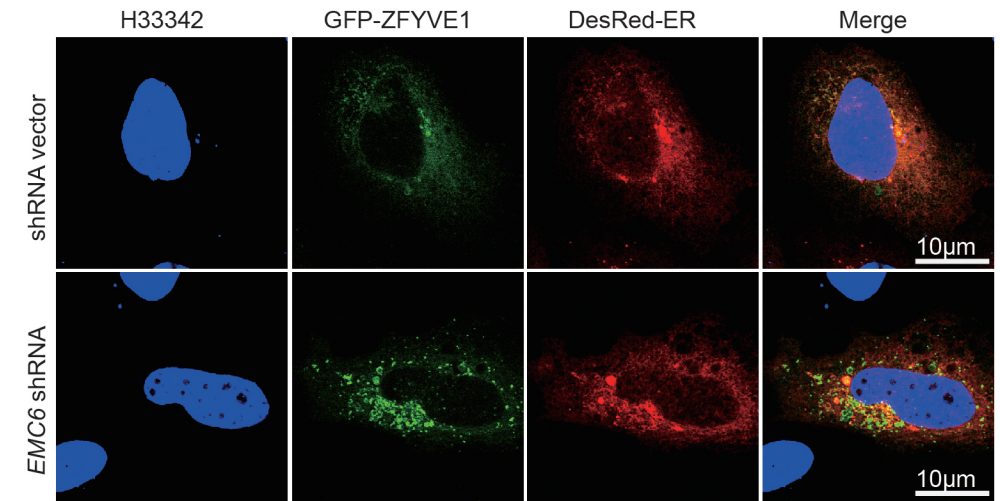
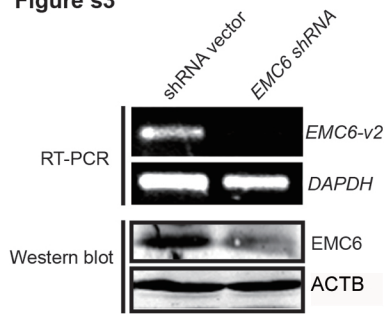
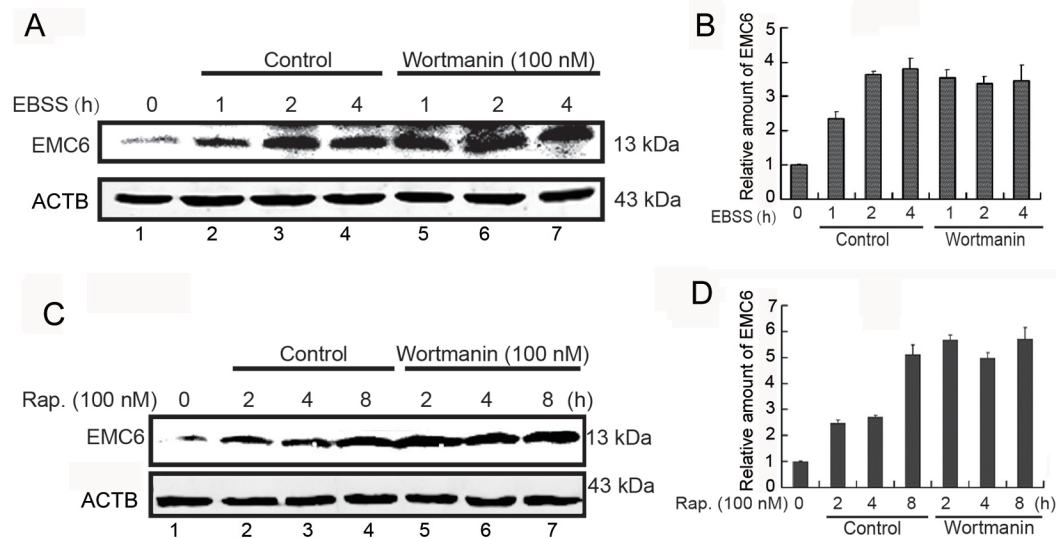
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**A novel ER-localized transmembrane protein, EMC6,  
interacts with RAB5A and regulates cell autophagy**

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**Figure s1****Figure s2****Figure s4****Figure s3****Figure s5**

**Figure S1.** Validation of the rabbit anti-EMC6 specific antibody by western blot and immunofluorescence assay. **(A)** EMC6 protein expression was detected by western blotting using the rabbit anti-EMC6 antibody in U2OS cells, which were transfected with vector (lane 1) or *EMC6* (lane 2). ACTB was detected as the protein loading control. **(B)** Immunofluorescence staining of cells expressing FLAG-EMC6. U2OS cells were transfected with FLAG-EMC6 expression plasmids, cultured for 24 h, immunostained with rabbit anti-EMC6 and mouse anti-FLAG monoclonal antibody and then observed and documented by confocal microscopy.

**Figure S2.** EMC6 overexpression promotes cell autophagy. **(A)** Representative fluorescence microscopy images obtained from U2OS cells cotransfected with plasmids expressing GFP-LC3 and vector or *EMC6* at a ratio of 1:3 cultured for 24 h and observed under fluorescence microscopy. **(B)** Quantification of GFP-LC3 dots in control or EMC6-overexpressing cells. Data are means  $\pm$  SD of at least 100 cells scored (\*\**p* < 0.001). **(C)** Western blot analysis of GFP-LC3-II and free GFP fragments in U2OS cells treated as in **(A)**.

**Figure S3.** Validation of *EMC6* shRNA by RT-PCR and western blot assay. U2OS cells were transfected with either *EMC6* shRNA or shRNA vectors for 24 h. *EMC6* mRNA and protein levels were detected by RT-PCR and western blot, respectively.

**Figure S4.** Localization of GFP-ZFYVE1 in *EMC6*-silenced cells. U2OS cells were cotransfected with plasmids expressing GFP-ZFYVE1, DsRed-ER and shRNA vector or

*EMC6* shRNA at a ratio of 1:1:3, cultured for 24 h, and then observed under a confocal microscope.

**Figure S5.** EMC6 protein is upregulated in cell autophagy. **(A)** U2OS cells were incubated in EBSS containing 0.01% DMSO (control) or 100 nM of wortmannin for the indicated time, and EMC6 was detected by western blot. **(B)** Quantification of the amounts of EMC6 relative to ACTB treated as in **(A)**. The average value in the cells without DMSO or wortmannin treatment was normalized as 1. Data are the means  $\pm$  SD of results from three experiments. **(C)** U2OS cells were incubated in DMEM containing 10% FBS, 100 nM of rapamycin (Rap.) and 0.01% DMSO (control) or 100 nM of wortmannin for the indicated time, and EMC6 level was detected by western blot. **(D)** Quantification of the amounts of EMC6 relative to ACTB treated as in **(C)**. The average value in the cells without DMSO or wortmannin treatment was normalized as 1. Data are the means  $\pm$  SD of results from three experiments.