



**Figure S1. *F. nucleatum* Induces Cancer Metastasis via the Autophagy Pathway**

(A) 16S rDNA analysis of the relative abundance of *Fusobacterium* in CRC tissues from patients without metastasis (n = 7) and with metastasis

(n = 9).

(B-D) Transwell assays were conducted to examine the migratory and invasive abilities of SW480 cells cocultured with *F. nucleatum* (F01), CQ or PBS control. The indicated migrated and invaded cells were quantified in five randomly selected fields, and the data are presented as the means  $\pm$  SDs (\*\* $P < 0.01$ ; unpaired Student's *t*-test).

(E) Relative fusobacterial gDNA abundance ( $2^{-\Delta C_t}$ ) in tumor tissues from APC<sup>Min/+</sup> mice with *F. nucleatum* (F01), CQ or PBS. (n = 5-6 mice/group; the error bars indicate the means  $\pm$  SDs; \*\*\* $P < 0.001$ , Mann-Whitney U test).

(F-I) Representative data for small intestinal tumors (red arrows) and HE staining of tumor tissues from the indicated groups of APC<sup>Min/+</sup> mice (F). Statistical analysis of the small intestinal tumor numbers (G), whole intestinal tumor numbers (H) and tumor sizes (I) in different groups of mice (n = 5-6 mice/group; \* $P < 0.05$ , and \*\*\* $P < 0.001$ ; nonparametric Mann-Whitney test).

(J) Western blot analysis was performed with HCT116 cells transfected with non-targeting (NC) or CARD3-targeting (si001, si002, si003) siRNA.

**Supplementary Table 1**

Primers		Sequence 5'-3'	Purpose
<i>F. nucleatum</i>	Forward	5'- CAACCATTACTTTAACTCTACCATGTTCA -3'	qPCR
	Reverse	5'- GTTGACTTTACAGAAGGAGATTATGTAAAAATC -3'	
PGT	Forward	5'- ATCCCCAAAGCACCTGGTTT -3'	qPCR
	Reverse	5'- AGAGGCCAAGATAGTCCTGGTAA -3'	
H-GAPDH	Forward	5'- CATCATCCCTGCCTCTACTGG -3'	qPCR
	Reverse	5'- GTGGGTGTCGCTGTTGAAGTC -3'	
H-E-cadherin	Forward	5'- TTCTTCGGAGGAGAGCGG -3'	qPCR
	Reverse	5'- CAATTCATCGGGATTGGC -3'	
H-CARD3	Forward	5'- GCTGCATTCTGTGATCACAAGAC -3'	qPCR
	Reverse	5'- GACAGAAGGGCATCTAGCGAC -3'	
H-Vimentin	Forward	5'- AGGCAAAGCAGGAGTCCACT -3'	qPCR
	Reverse	5'- CGTTCCAGGGACTCATTGGT -3'	
H-ATG5	Forward	5'- TGTTTATTCGTCGGTTCATTTTG -3'	qPCR
	Reverse	5'- CAGCTTAGTGTTCCCTGCATTC -3'	
H-ATG12	Forward	5'- CTAATTCAATTGCTGCTGGAGG -3'	qPCR
	Reverse	5'- GCCAGCAGGTTCCCTCTGTTC -3'	
H-ATG16L1	Forward	5'- GCTAAGTTCCTGCTGGACAATG -3'	qPCR
	Reverse	5'- GGCAGTAATCTTTCCCAACAGC -3'	
H-ATG7	Forward	5'- TTCCTCCTCTTGACATTTGCAG -3'	qPCR
	Reverse	5'- TATCTTCGTCCTTTGACCTTGG -3'	
H-beclin1	Forward	5'- GGCACAATCAATAACTTCAGGC -3'	qPCR

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Reverse

5'-GGCAGCTCCTTAGATTTGTCTG-3'

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