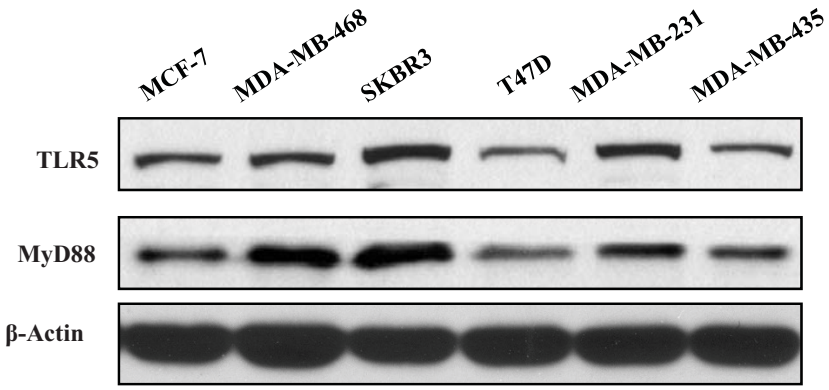
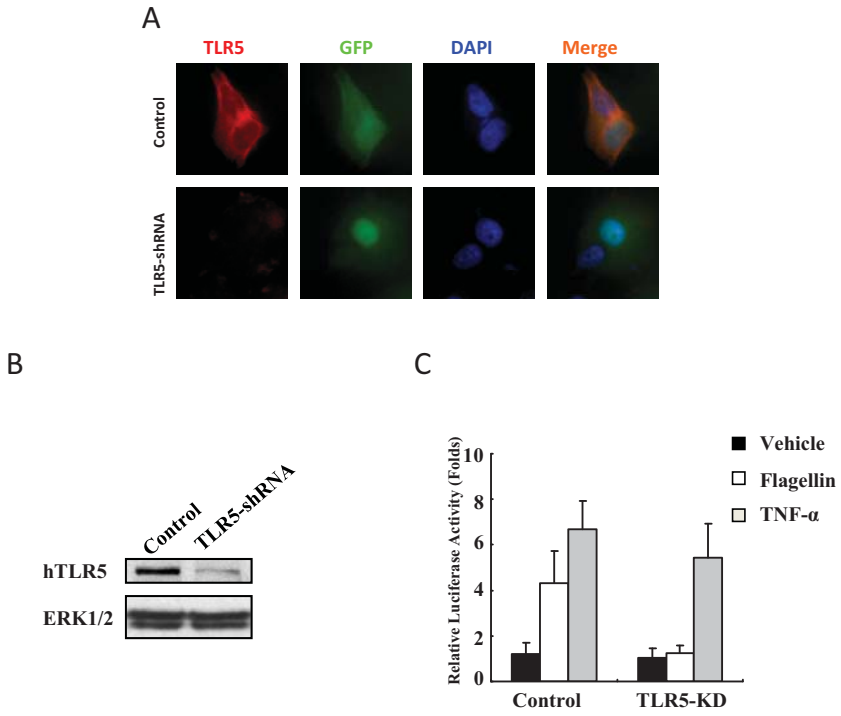
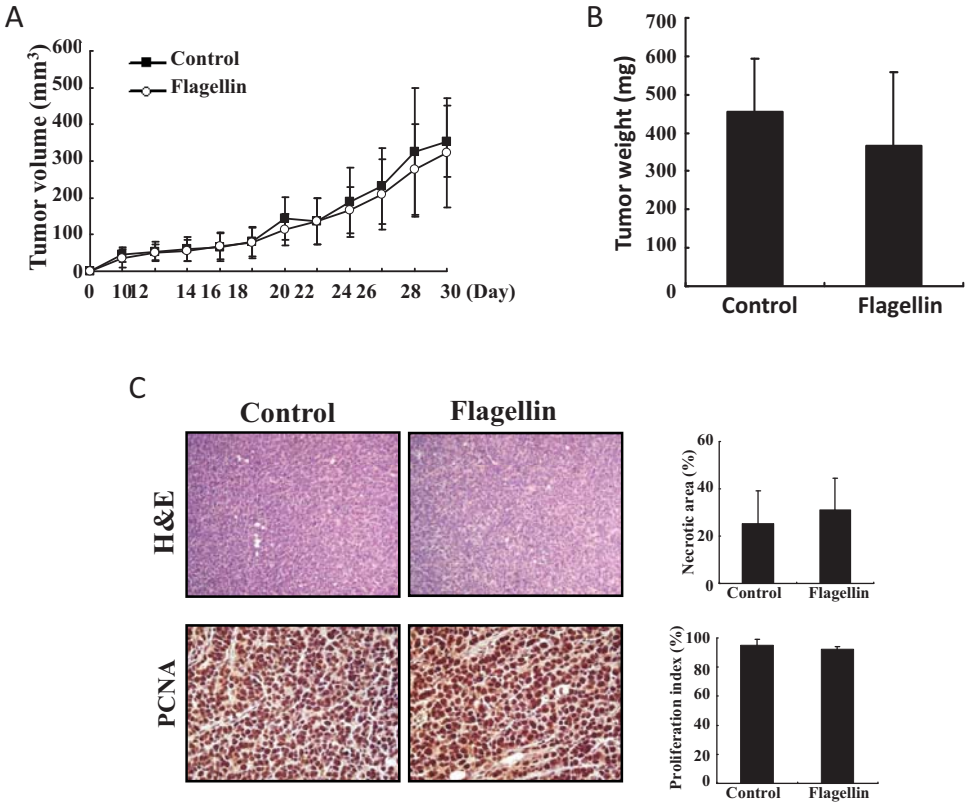


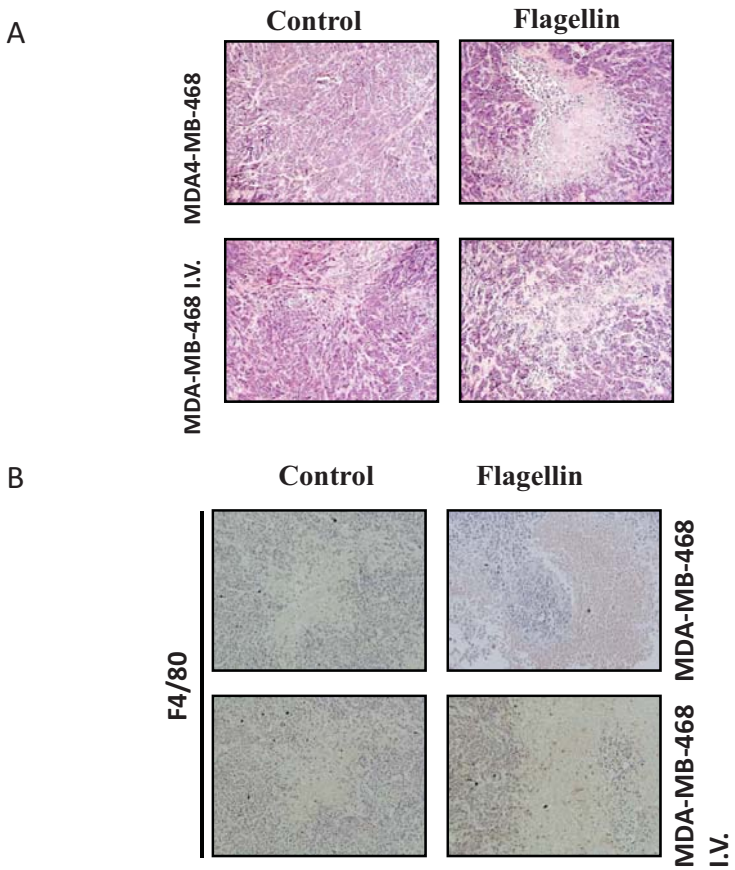
Supplementary Figure 1. Expression of TLR5 and MyD88 in breast cancer cells. With western blotting analysis, the expression of TLR5 and its adaptor MyD88 was detected in MCF-7, MDA-MB-468, SKBR3, T47D, MDA-MB-231 and MDA-MB-435 cells.



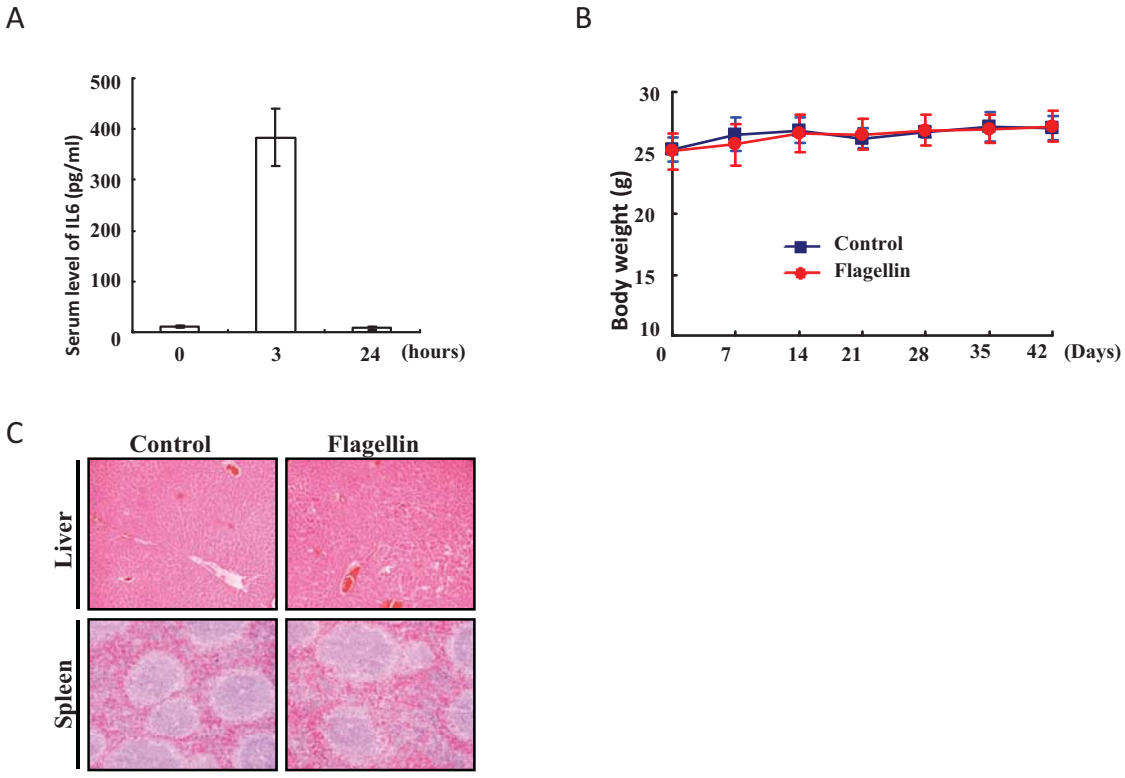
Supplementary Figure 2. Generating TLR5 shRNA knocked down cells. A, Detection of TLR5 expression in MCF-7 control and knocked down cells by immunoflorescence with the same exposure time. Because these vectors encode a GFP protein, stably transfected cells were identified by GFP expression. B, TLR5 protein expression was examined by Western blot in MCF-7 control and knocked down cells. And total ERK1/2 level as a loading control. C, MCF-7 knocked down cells and scrabble control were transfected with NFκB reporter plasmid and analyzed for luciferase activity induced by flagellin (0.1 μg/ml) and TNF-α (100U/ml).



Supplementary Figure 3. Flagellin treatment did not alter the growth of breast cancer xenograft mouse model generated from MCF-7- TLR5-shRNA cells. A, tumor growth curves of control. B, tumor weight of control or flagellin-treated groups were measured. C H&E staining of tumor sections (*Top panel*). The percentage necrosis area was calculated from five sections per tumor. Mean ± s.d. values of data from five tumors are shown (*Top right*). Cell proliferation marker PCNA staining of tumor sections (*Bottom panel*). The ratio of PCNA-positive to total tumor cells was calculated from five sections per tumor. Mean ± s.d. values of data from five tumors are shown (*Bottom right*). Magnification: H&E, 10X; PCNA, 40X.



Supplementary Figure 4. Histology study of tumor sections from MDA-MB-468 xenografts. A, H&E staining of MDA-MB-468 xenograft tumor sections showed more prevalent necrosis and leucocytes infiltration in tumors either by peritumoral and i.v. flagellin treatment. Magnification: 10X. B, Tumor sections from MDA-MB-468 xenografts were stained by neutrophil-specific marker Gr-1. Representative images from tumor sections with flagellin peritumoral or i.v. treatment are shown. Red indicates positive staining. Magnification: 10X.



Supplementary Figure 5. Toxicity assessment following intravenous delivery of flagellin in nude mice. A, Serum level of IL-6 in mice (n=6) following flagellin (2 μg per mouse) administration at baseline (0 hour), 3 and 24 hours. B, Body weight curves of mice in control and flagellin-treated groups during the period of i.v. injection. C, H&E staining of liver and spleen sections in control and flagellin-treated groups. Magnification: 10X.

Supplementary Table 1. Clinicopathologic features of TLR5 in patients with breast cancer *

	TLR5	
	Positive	**Negative**
	N=60 80%)	**N=15 (20%)**
Age		
< 50	32 (42.7%)	11(14.7%)
≥ 50	28 (37.3%)	4 (5.3%)
Tumor type		
Invasive ductal carcinoma	53(70.7%)	8(10.7%)
Ductal carcinoma *in situ*	1 (1.3%)	1(1.3%)
Medullary carcinoma	2 (2.7%)	0
Mucinous carcinoma	0	3(4%)
Invasive lobular carcinoma	1(1.3%)	2(2.7%)
Mixed lobular and ductal carcinoma	1(1.3%)	0
Ductal papillary carcinoma	1(1.3%)	0
Invasive papillary carcinoma	1(1.3%)	0
Neuroendocrine carcinoma	0	1(1.3%)
Tumor grade		
I	2(2.7%)	0
I- II	2(2.7%)	3(4%)
II	12(16%)	5(6.7%)
II- III	27(36%)	6(8%)
III	17(22.7%)	1(1.3%)
Tumor size		
T1	8(10.7%)	0
T2	36(48%)	15(20%)
T3	12(16%)	0
T4	3(4%)	0
Tis (Carcinoma *in situ*)	1(1.3%)	0
Lymph node metastasis		
N0	42(56%)	14(18.7%)
N1	16(21.3%)	1(1.3%)
N2	3(4%)	0
Distant metastasis		
M0	58(77.3%)	15(20%)
M1	2(2.7%)	0

*** One adenosquamous carcinoma sample and one squamous cell carcinoma sample were not counted in this study.**

Supplemental Table 2 Primers used in this study

Oligonucleotide	Sequence (5'→3')	Purpose
Human IL-1β-F	GACACATGGGATAACGAGGC	RT-PCR
Human IL-1β-R	ACGCAGGACAGGTACAGATT	RT-PCR
Human IL-6-F	GGAGACTTGCCTGGTGAA	RT-PCR
Human IL-6-R	GCATTTGTGGTTGGGTCA	RT-PCR
Human IL-8-F	TTGGCAGCCTTCCTGATTTC	RT-PCR
Human IL-8-R	AACTTCTCCACAACCCTCTG	RT-PCR
Human IL-12-F	TGGAGTGCCAGGAGGACAGT	RT-PCR
Human IL-12-R	GATGATGTCCCTGATGAAGAAGC	RT-PCR
Human TNF-α-F	CGAGTGACAAGCCTGTAGC	RT-PCR
Human TNF-α-R	GGTGTGGGTGAGGAGCACAT	RT-PCR
Human β-actin F	TCCCTGGAGAAGAGCTACG	RT-PCR
Human β-actin R	GTAGTTTCGTGGATGCCACA	RT-PCR