Supporting Information

Means and Passarelli 10.1073/pnas.0913582107

SI Materials and Methods

Viruses and Insects. Bacmids AcBAC and AcBAC–vfgfKO have been described (1). Viruses were titered in TN-368 cells (2) by TCID50. *T. ni* larvae were purchased from Benzon Research and reared at 27 °C with a 12-h light/dark cycle.

Inoculation of *T. ni* Larvae. *T. ni* were starved for 8 h, and within 2 h after molting into the fourth instar, infected with 500 occlusions (1 μL) of AcBAC or AcBAC–vfgfKO in a 10% sucrose solution. Early fifth instar *T. ni* were infected intrahemocoelically by injecting 10 plaque-forming units (PFU) of AcBAC or AcBAC–vfgfKO BV between the first pair of prolegs at the third abdominal segment. After infection, each larva was separated, and at various times p.i. cohorts of 10–12 insects were killed and processed.

Preparation of Larvae for Immunostaining. Larvae were infected and dissected in PBS (3). Midguts and fat body were removed and fixed overnight in 4% paraformaldehyde at 4 °C. Samples were then placed in fresh fixative for 1 h at room temperature. After fixation, tissues were washed 4× in PBS and permeabilized for 30 min with PBS and 0.1% Triton X-100. Samples were washed (4 × 15 min) and incubated with PBS containing 10% FBS overnight at 4 °C to block nonspecific antibody binding, followed by 1 h at room temperature in fresh blocking solution. Tissues were then immunostained as described below.

Immunostaining of Tissues and Immunoblotting of Lysates. Antibody or antisera was incubated with tissues in PBS containing 10% FBS overnight at 4 °C. The next day, samples were washed (4 × 15 min) in PBS and 0.1% Triton X-100 and incubated with secondary antibodies for 2 h at room temperature in the dark. Samples were then washed with PBS for 30 min, followed by 4 × 15 min additional washes. After washing, tissues were mounted with Fluoromount G (Electron Microscopy Sciences) on slides and allowed to dry for 30 min in the dark, before imaging using a Zeiss LSM 5 PASCAL (laser scanning confocal microscope). The images shown are representative of the population of cells or tissues from independent experiments.

The following pairs of antibodies were used: polyclonal anti-laminin (Sigma) at 1:100, followed by secondary anti-rabbit ribulose-5-phosphate-3-epimerase (RPE) (Sigma) at 1:2,000; polyclonal anti-active DrICE (4) at 1:100, followed by secondary anti-rabbit RPE at 1:2,000; polyclonal anti-collagen type I (Abcam) at 1:1,000, followed by secondary anti-rabbit RPE at 1:2,000; monoclonal anti-GFP primary antibody (Clontech) (1:100), followed by secondary anti-mouse (1:2,000) FITC conjugated (Sigma).

Double-antibody labeling was performed as described for single-antibody stains but using two antibodies, followed by mouse and rabbit secondary antibodies coincubated 2 h at room temperature. Other antibodies used include monoclonal antiphosphotyrosine PY20 at 1:1,000 (Southern Biotechnology Associates, Inc.), anti-CM1 at 1:100 (Cell Signaling), and secondary antibodies anti-rabbit IgG-RPE, anti-mouse IgG-RPE (Southern Biotechnology Associates, Inc.), and anti-mouse IgG-FITC, at 1:5,000 dilution (Sigma). For experiments using two antibodies (i.e., active DrICE and GFP) and different visualization, one of the primary antibodies was FITC conjugated using the FITC antibody labeling kit (Thermo Scientific) as described by the manufacturer.

For immunoblotting, cohorts of 4–5 *T. ni* were killed at various times p.i., and midguts were dissected and placed in 100 μL SDS Laemmli buffer. Samples were then homogenized and sonicated for 15 s with 5-s bursts/10-s breaks and centrifuged at 17,900 × *g* for 15 min. Supernatant (80 μL) was then transferred to a fresh 1.5-mL tube, and 40 μL were analyzed by immunoblotting. Proteins were resolved by SDS/PAGE (15%). The resolved proteins were transferred to a PVDF membrane (Pierce) and immunodetected accordingly. Virus-infected cells were determined with anti-GFP monoclonal at 1:100 and anti-mouse IgG-HRP (horseradish peroxidase) (1:2,000). Type IV collagen was detected with anti-type IV collagen polyclonal at 1:100 and anti-rabbit IgG-HRP at 1:2,000. Laminin was detected with an anti-laminin polyclonal at 1:100 and anti-rabbit IgG-HRP (1:2,000). GAPDH was detected using a polyclonal anti-GAPDH (Abcam) at 1:1,000, followed by anti-rabbit IgG-HRP (1:2,000). Immunoreactive proteins were visualized with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific).

To determine direct cleavage of laminin and type IV collagen by caspases, recombinant human laminin (Abcam) or type IV collagen (Abcam) was mixed with active effector caspase DrICE (5) or active initiator caspase DRONC (5) in 100 μL caspase buffer and incubated at 37 °C for 4 h. The caspase inhibitor zVAD-fmk (100 μM) was used to confirm caspase-dependent cleavage. Samples were then analyzed by immunoblotting.

Processing Tissues for Caspase and MMP Activity Assays. To measure midgut caspase or MMP activity, 4–5 *T. ni* midguts and associated trachea were dissected and placed in 100 μL caspase buffer [50 mM Hepes (pH 7.5), 100 mM NaCl, 1 mM EDTA, 0.1% CHAPS, 10% sucrose, 5 mM DTT]. Samples were processed as described for immunoblotting. Protein (10 μg/100 μL caspase buffer) was mixed with 0.2 μM Ac-DEVD-afc. In some assays, 0.2 mg of procaspase-3 was mixed with 0.4 mg of active MMP-2 or MMP-9 (Calbiochem) in 100 μL caspase buffer and incubated at 37 °C for 4 h before measuring caspase activity.

When caspase or MMP inhibitors were used, larvae were fed 100 μM of zVADfmk (MP Biomedicals) or GM6001 (Calbiochem), the broad spectrum caspase or MMP inhibitor, respectively, in a 10% sucrose solution with or without virus. After feeding, larvae were processed as described previously, and cohorts of four or five were used for activity assays.

Transmission Electron Microscope Sample Preparation. At various times p.i., *T. ni* were killed, and midguts dissected and placed in fixative solution [2% paraformaldehyde, 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2)] overnight at 4 °C with gentle rocking. Samples were then transferred to fresh fixative for an additional 2 h with gentle rocking at room temperature. After fixing, samples were washed 3 × 5 min in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature. Samples were then postfixed in 1% osmiun tetroxide (Ted Pella, Inc.) in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature for 2 h. After postfixing, samples were washed 3 × 5 min in 0.1 M sodium cacodylate (pH 7.2) at room temperature and *en bloc* stained in 2% aqueous uranyl acetate at 4 °C overnight in the dark. The following day, samples were rinsed (3 × 15 min) with water. Samples were then dehydrated (3 × 5 min) with increasing concentrations of ethanol (30–100%) at room temperature and treated with acetone (2 × 5 min). Samples were then infiltrated with EMBED 812/Araldite resin (Ted Pella, Inc.) at room temperature as follows: 3:1 acetone:resin for 30 min, 1:1 acetone:resin for 1 h, 1:3 acetone:resin overnight, 100% resin for 8 h, 100% resin overnight. Samples were then transferred to a mold and filled with fresh resin and oriented. Resin-containing samples were then polymerized at 65 °C for 24–48 h. Thin sections were cut with a glass knife and examined under a transmission electron microscope model LEM 2000 (International Scientific Instruments).

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2. Hink WF (1970) Established insect cell line from the cabbage looper, *Trichoplusia ni*. *Nature* 226:466–467.
3. Potter KN, Miller LK (1980) Transfection of two invertebrate cell lines with DNA of *Autographa californica* nuclear polyhedrosis virus. *J Invertebr Pathol* 36:431–432.
4. Yoo SJ, et al. (2002) Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nat Cell Biol* 4:416–424.
5. Means JC, Muro I, Clem RJ (2006) Lack of involvement of mitochondrial factors in caspase activation in a Drosophila cell-free system. *Cell Death Differ* 13:1222–1234.
6. Means JC, Muro I, Clem RJ (2003) Silencing of the baculovirus Op-*iap3* gene by RNA interference reveals that it is required for prevention of apoptosis during *Orgyia pseudotsugata* M nucleopolyhedrovirus infection of Ld652Y cells. *J Virol* 77:4481–4488.

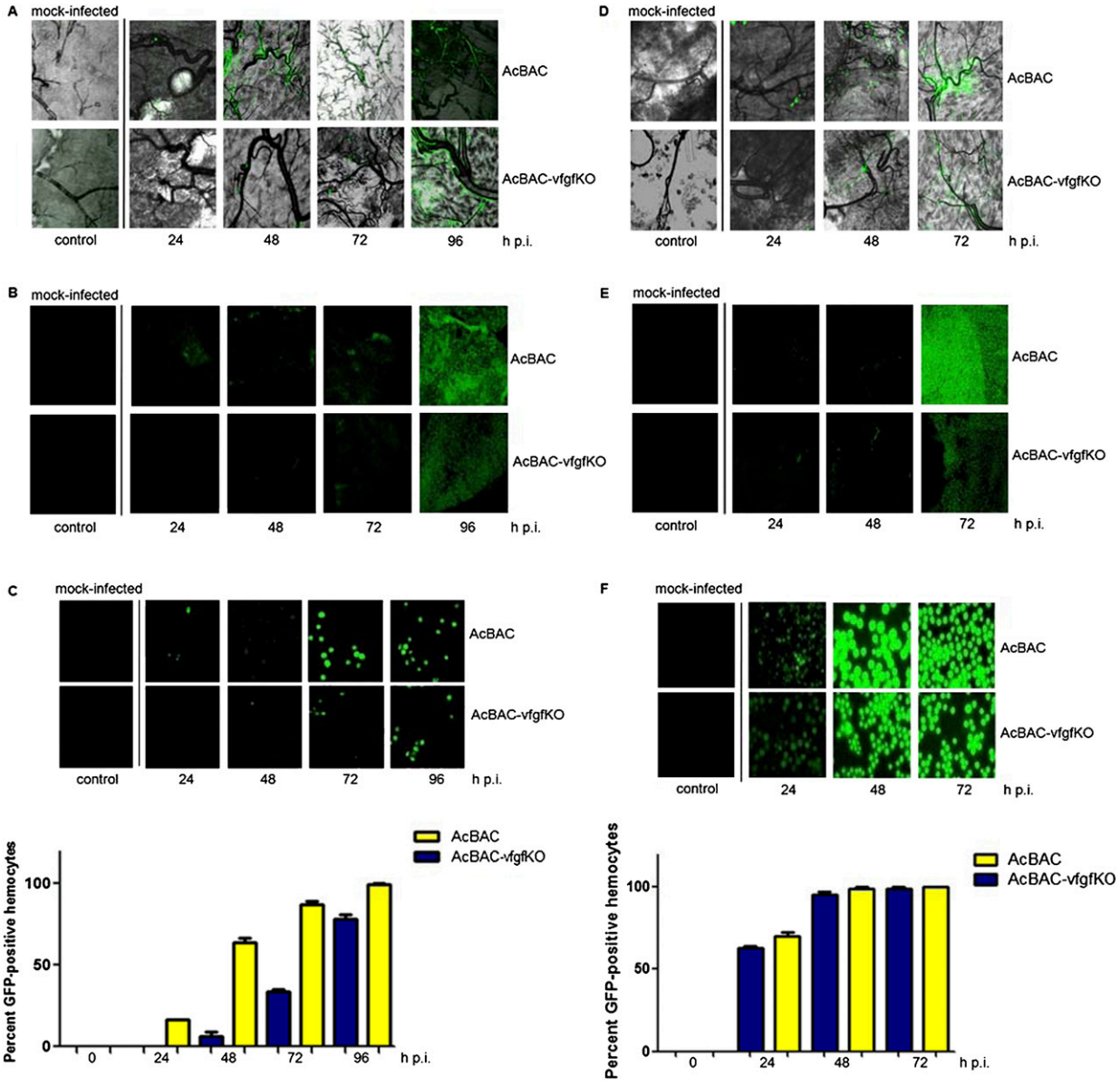

Fig. S1. vFGF is necessary for the timely infection of cells during oral but not intrahemocelic infection. Larvae were orally (*A–C*) or intrahemocelically (*D–F*) infected. Insects were dissected at the indicated hours (h) postinfection (p.i.), and infected cells were detected with GFP antibody (green). (*A–F*) Infected tracheal cells (*A* and *D*), fat body cells (*B* and *E*), and hemocytes (*C* and *F*) detected with GFP antibody. A merge of bright field and eGFP is shown in *A* and *D*. Enumeration of e*gfp*-expressing hemocytes at different hours postinfection in *C* and *F* is shown below each panel.

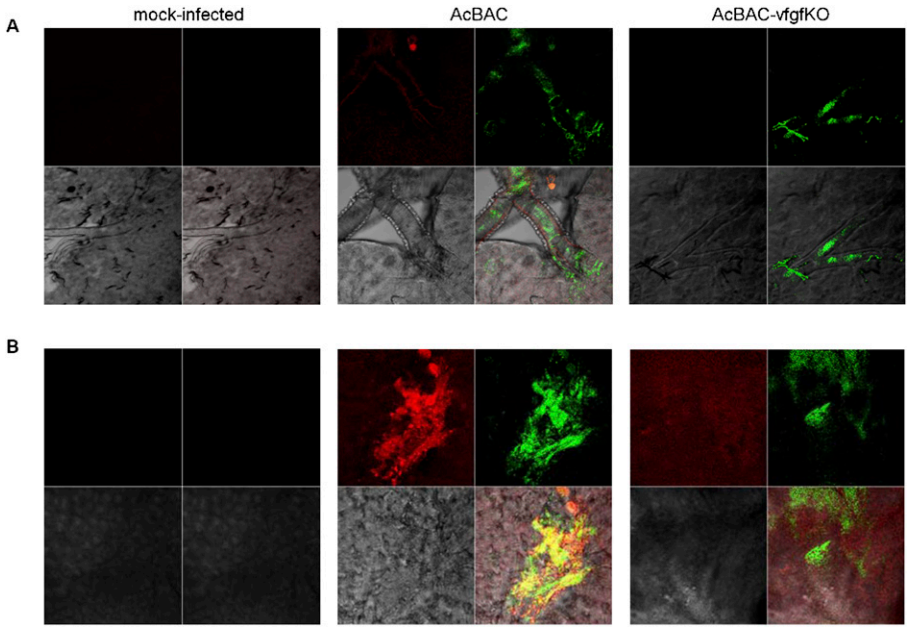

Fig. S2. vFGF induces tyrosine phosphorylation in infected midguts and tracheal epithelial cells. Early fourth instar larvae were orally infected with AcBAC or AcBAC–vfgfKO. Insects were dissected and receptor activation was detected with antiphosphotyrosine antibody. (*Upper Left*) Red, laminin. (*Upper Right*) Green, eGFP. (*Lower Left*) Bright field. (*Lower Right*) Merge. (*A*) Trachea dissected at 48 h p.i. (*B*) Midguts dissected at 12 h p.i.

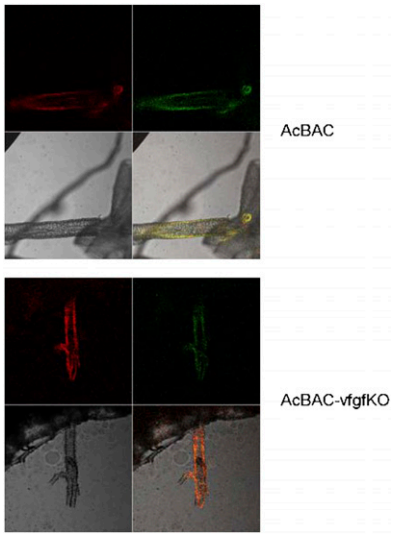

Fig. S3. Basal lamina integrity is observed at late times postinfection. Orally infected larvae were dissected at 96 h p.i., and laminin (red) and infection (green) of trachea were detected using anti-laminin antiserum and anti-GFP antibody, respectively. (*Upper Left*) Red, laminin. (*Upper Right*) Green, eGFP. (*Lower Left*) Bright field. (*Lower Right*) Merge.

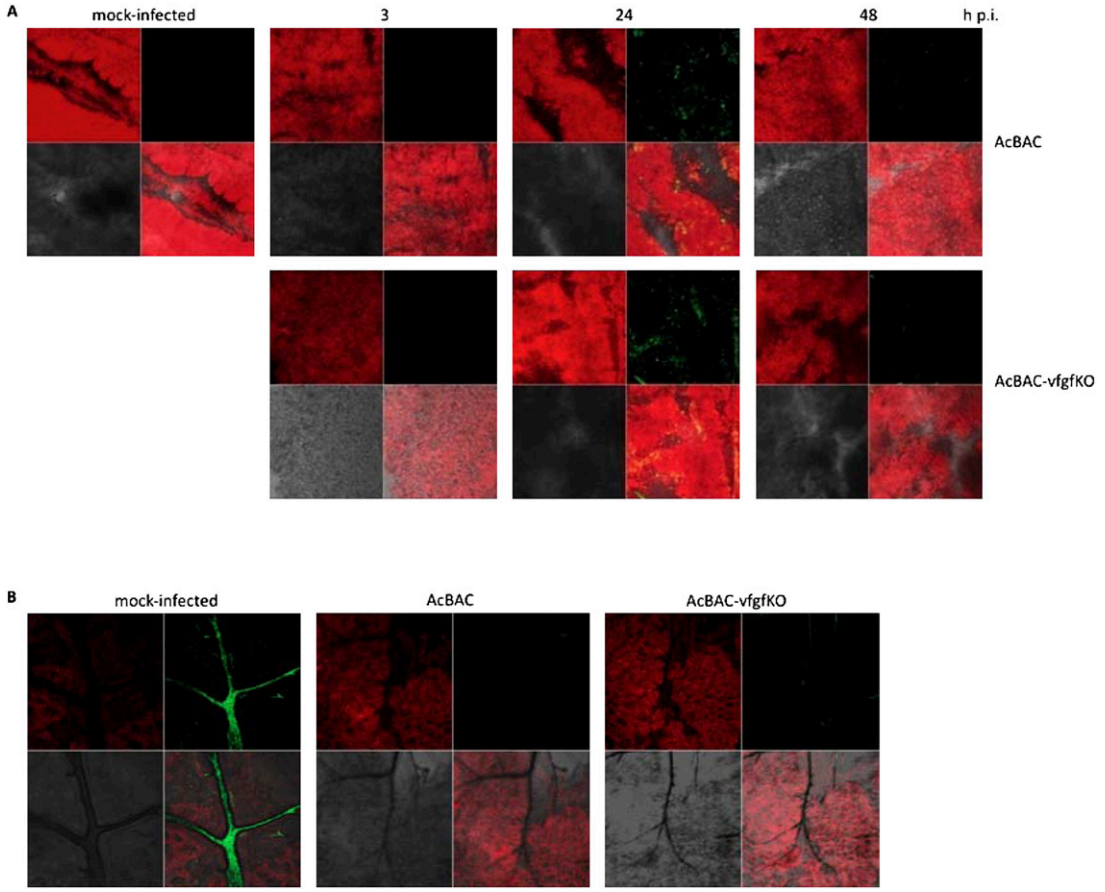

Fig. S4. vFGF does not affect midgut basal lamina composition or collagen I. Larvae were orally infected with AcBAC or AcBAC–vfgfKO, or mock infected. (*A*) Insects were dissected at the indicated hours postinfection, and midgut laminin (red) and infected tissues (green) were detected using anti-laminin antiserum and anti-GFP antibody, respectively. (*Upper Left*) Red, laminin. (*Upper Right*) Green, eGFP. (*Lower Left*) Bright field. (*Lower Right*) Merge. (*B*) Insects were dissected at 24 h p.i., and tracheal laminin (green) and collagen I (red) were detected using anti-laminin and anti-collagen I, respectively. (*Upper Left*) Red, collagen I. (*Upper Right*) Green, laminin. (*Lower Left*) Bright field. (*Lower Right*) Merge.

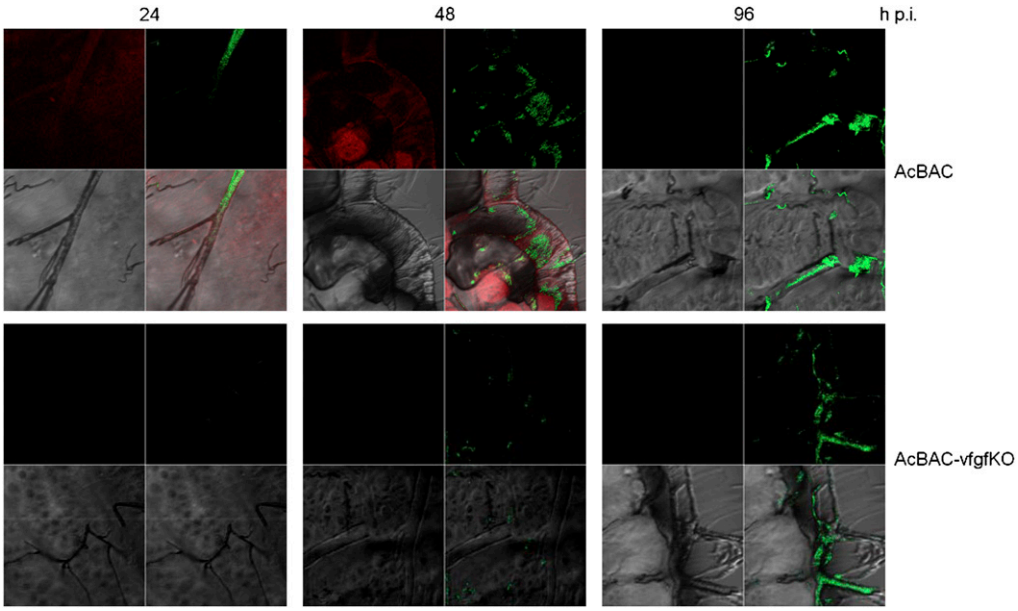

Fig. S5. Caspase activation is not obvious at late times postinfection. Infected larvae were dissected at the indicated times, and caspase activity (red) and infection (eGFP, green) were detected on trachea using anti-DrICE antiserum and anti-GFP antibody, respectively. (*Upper Left*) Red, caspase. (*Upper Right*) Green, eGFP. (*Lower Left*) Bright field. (*Lower Right*) Merge.

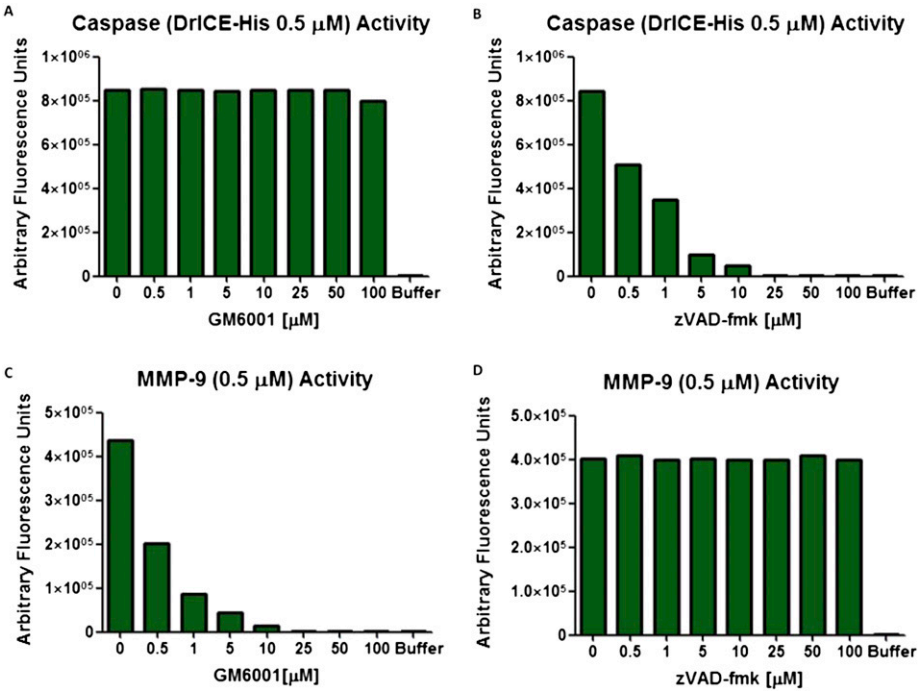

Fig. S6. MMP and caspase inhibitors are specific. Active recombinant caspase DrICE (*A* and *B*) or MMP-9 (*C* and *D*) were incubated with increasing concentrations of the caspase inhibitor zVAD-fmk or the MMP inhibitor GM6001 before determining caspase or MMP activity using fluorescent substrates.

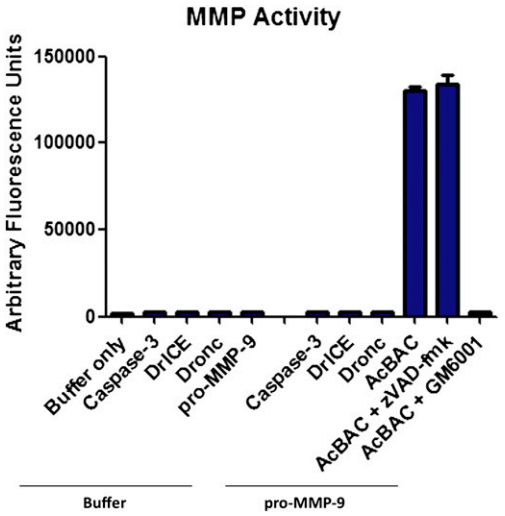

Fig. S7. Pro-MMP-9 is activated by AcBAC. Pro-MMP-9 was incubated with purified caspases, Dronc, DrICE, or caspase 3, or with a lysate from AcBAC-infected midgut and associated trachea in the presence or absence of the caspase inhibitor zVAD-fmk or MMP inhibitor GM6001 and a fluorescent MMP substrate.

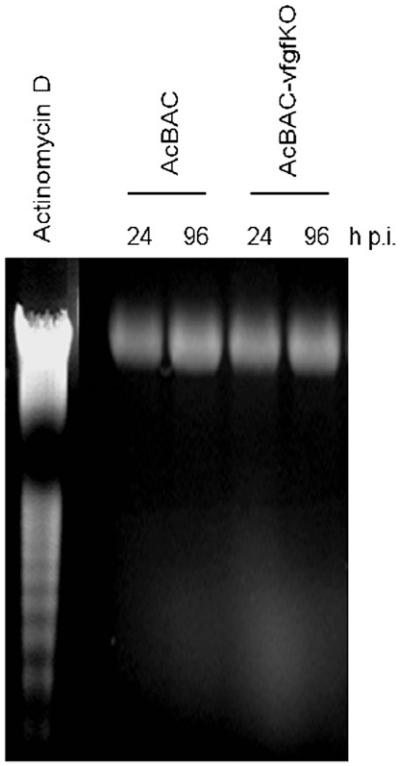

Fig. S8. Lack of DNA fragmentation in AcBAC- or AcBAC–vfgfKO-infected midguts. Larvae were either mock-infected or infected by feeding with either AcBAC or AcBAC–vfgfKO, and midguts were dissected at several times p.i. Mock-infected midguts were treated with actinomycin D (0.5 μg/mL) to induce apoptosis. Midgut DNA from the samples as indicated at the top was resolved by agarose gel electrophoresis and visualized with ethidium bromide as described previously (6).

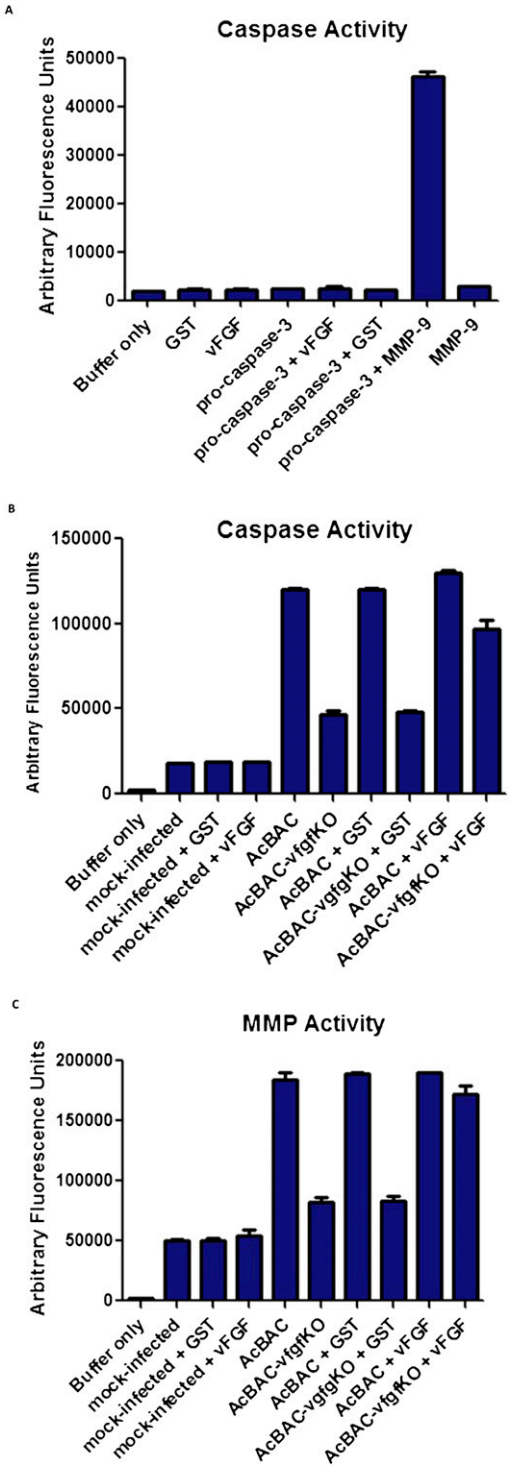

Fig. S9. Virus infection is necessary to activate MMPs and effector caspases. (*A*) Purified MMP-9, partially purified vFGF, or GST was added alone or with procaspase-3, and effector caspase activity was determined. (*B* and *C*) vFGF or GST was added to lysates from mock-, AcBAC-, or AcBAC–vfgfKO-infected midgut, and associated trachea and effector caspase (*B*) or MMP (*C*) activity was determined.