

## SUPPLEMENTAL MATERIAL

Cao et al., <https://doi.org/10.1084/jem.20170957>

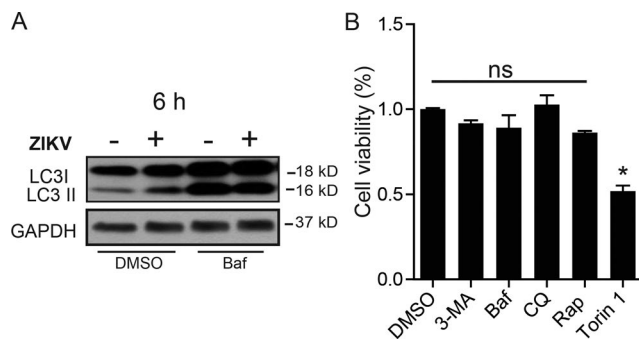


Figure S1. **ZIKV infection in human trophoblasts.** (A) Western blots for LC3-I/II show enhanced autophagy flux upon ZIKV infection at 6 hpi. ZIKV-infected JEG-3 (multiplicity of infection 0.1, 2 h) were cultured for 6 h and harvested. Baf A1 was applied for 30 min before harvesting to monitor autophagic flux. Images represent data from four independent experiments. (B) Cell viability assays of uninfected JEG-3 cells cultured in medium supplemented with indicated autophagy modulators (at same dosage as described in Fig. 1 F) for 48 h;  $n = 4$  for each group. Data depict mean  $\pm$  SEM. \*,  $P < 0.05$ ; ns, not significant (ANOVA with a Dunn's multiple-comparison test). Rap, rapamycin.

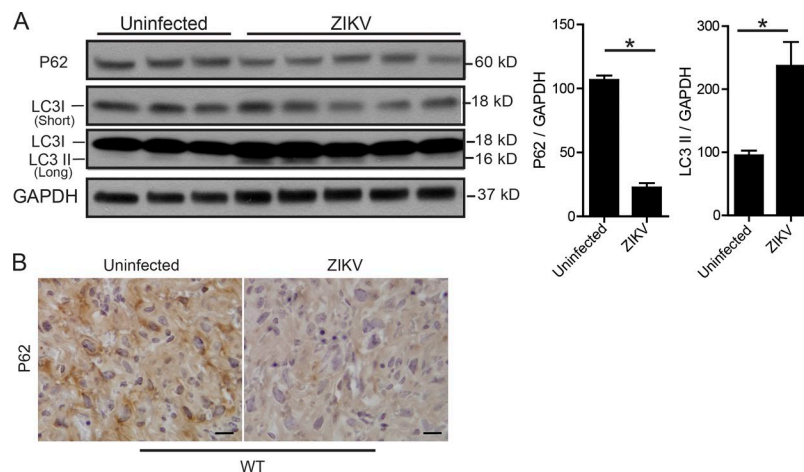


Figure S2. **ZIKV infection induces autophagic activity in mouse placentas.** (A) Immunoblot and quantification of LC3 and p62 show increased autophagic activity in mouse placentas (at E14.5) infected with ZIKV compared with uninfected controls. GAPDH, a loading control;  $n = 3-5$ . Data depict mean  $\pm$  SEM. \*,  $P < 0.05$ , Mann-Whitney test. (B) Representative immunohistochemical staining of p62 in WT placentas with or without ZIKV infection at E14.5. Images represent data from five independent dams. Bars, 100  $\mu$ m.

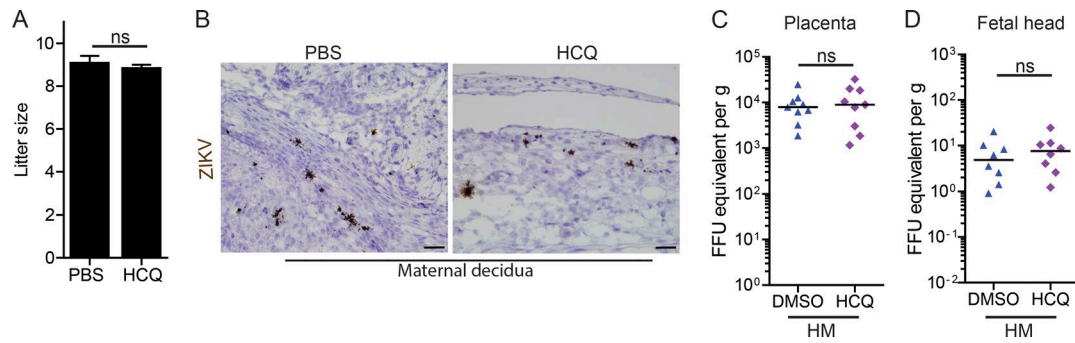


Figure S3. **HCQ treatment reduces placental and fetal ZIKV infection specifically via autophagy.** (A) Litter size at E14.5 with or without HCQ treatment;  $n = 6$  per group. Results represent mean  $\pm$  SEM, three independent experiments. ns, not significant, Mann-Whitney test. (B) Representative ISH images of ZIKV RNA in indicated maternal uterine decidua. Bars, 100  $\mu$ m. (C and D) Pregnant HM female mice were treated with HCQ (40 mg/kg/day via intraperitoneal route) or DMSO as a mock control from day +1 post-ZIKV infection (E10.5) to E14.5. Viral burden of ZIKV in placentas (C) and fetal head (D) were measured by quantitative RT-PCR;  $n = 8$  from three independent dams. ns, not significant, Mann-Whitney test.