Intestinal epithelial HMGB1 inhibits bacterial infection via STAT3 regulation of autophagy

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Supplementary Figure Legends:

Figure S1. NFKB, MAPK/JNK, and TLR signaling pathways in the intestine of *Hmgb1*$^{\Delta IEC}$ mice compared with the *Hmgb1*$^{loxP/loxP}$ mice. (**A**) Western blot analysis of NFKB and MAPK/JNK expression in intestinal epithelial cells of *Hmgb1*$^{loxP/loxP}$ and *Hmgb1*$^{\Delta IEC}$ mouse 8 h post *Salmonella* Typhimurium infection. (**B**) Western blot analysis of TLR in intestine of *Hmgb1*$^{loxP/loxP}$ and *Hmgb1*$^{\Delta IEC}$ mouse 8 h postinfection. VIL1 is used as a marker for epithelial cells and an internal control. ACTB is also used as an internal control. (n = 3).

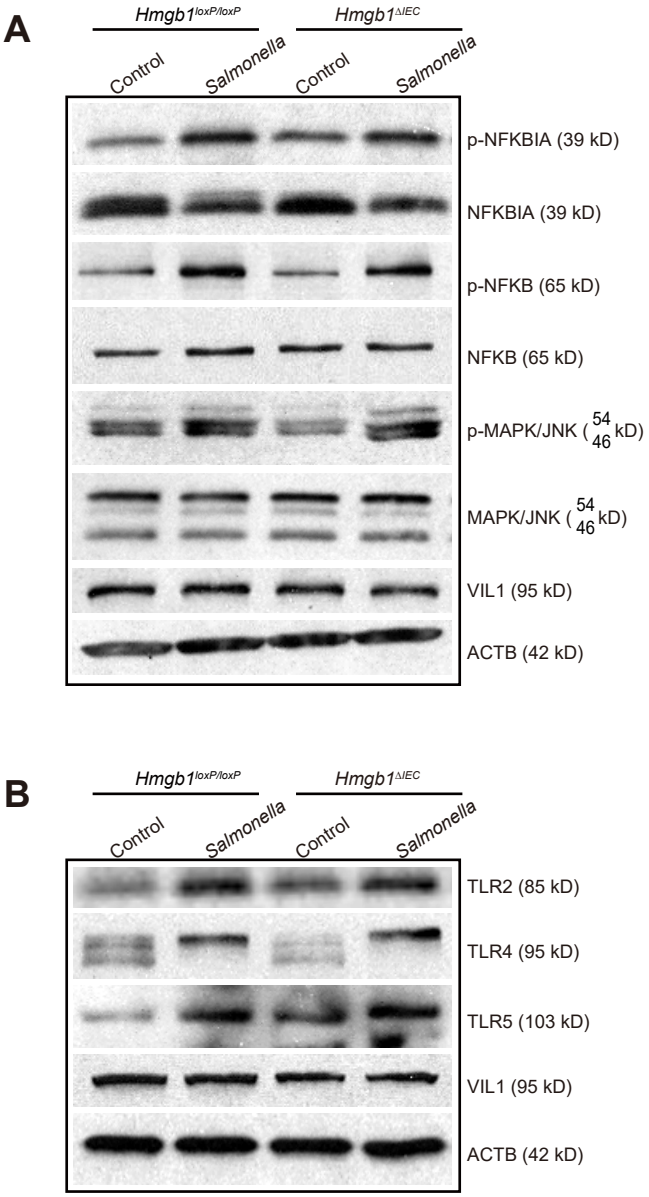
Figure S2. The absence of gene targeted-deletion of intestinal epithelial *Hmgb1* increased p-STAT3 and led to total STAT3 nuclear translocation *in vitro*. (**A**) Western blot analysis of p-STAT3 and STAT3 expression of *Hmgb1*$^{+/+}$ and *hmgb1*$^{-/-}$ MEF cells colonized with wild-type *Salmonella* Typhimurium. (**B**) Western blot analysis of p-STAT3 and STAT3 levels in cytosolic (C) and nuclear (N) extracts isolated from *Hmgb1*$^{+/+}$ and *hmgb1*$^{-/-}$ MEF cells colonized with wild-type *Salmonella* Typhimurium. The nuclear protein SP1, which is absent in the cytosol, served as a nuclear protein loading control. Cytosolic and nuclear protein density expression normalized with total protein. The red arrow marks p-STAT3. (**C**) The relative intensity of cytoplasmic and nuclear STAT3 from *Hmgb1*$^{+/+}$ and *hmgb1*$^{-/-}$ MEF cells colonized with wild-type *Salmonella* Typhimurium (n=3/group. student's t-test, *p<0.05). Cytosolic and nuclear protein density expression normalized with total protein.

Figure S3. HMGB1 could interact with STAT3. (**A**) Detailed information of HMGB1 interaction with STAT3 by using PrePPI software. (**B**) PrePPI-predicted 3 binding domains in HMGB1. (**C**) PrePPI-predicted 5 binding domains in STAT3.

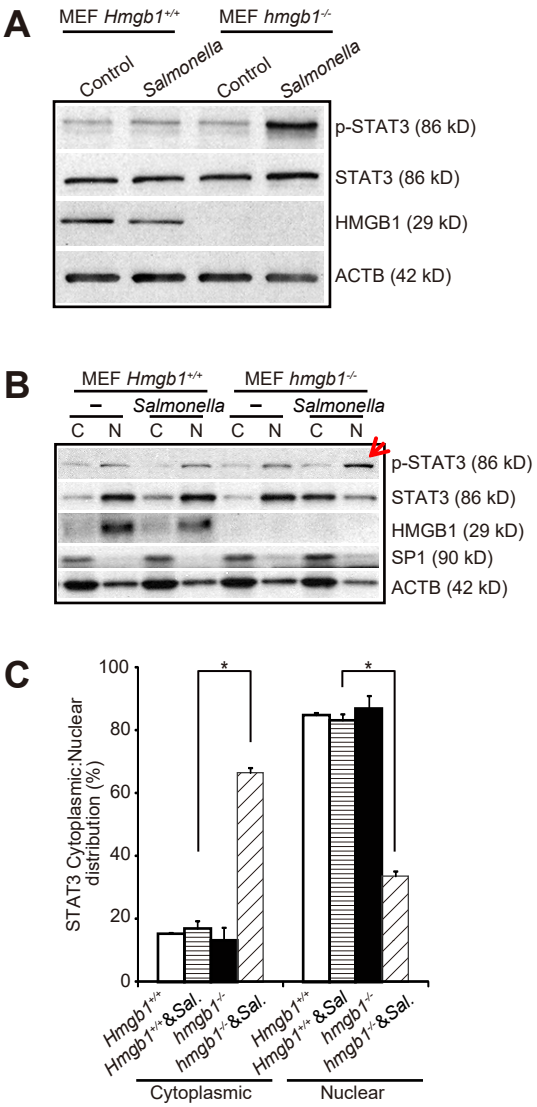
Figure S4. Nuclear STAT3 was decreased and cytosolic STAT3 was increased in *Salmonella*-infected MEF *hmgb1*$^{-/-}$ cells. (**A**) STAT3 and HMGB1 staining of *Hmgb1*$^{+/+}$ and *hmgb1*$^{-/-}$ MEF cells colonized with wild-type *Salmonella* Typhimurium. MEF cells were grown in the Lab-Tek chambered coverglass system. The MEFs were colonized with

Salmonella Typhimurium for 30 min, washed, and incubated in fresh DMEM medium for 30 min. Images for each protein shown represent 3 separate experiments.

Supplemental Figure 1

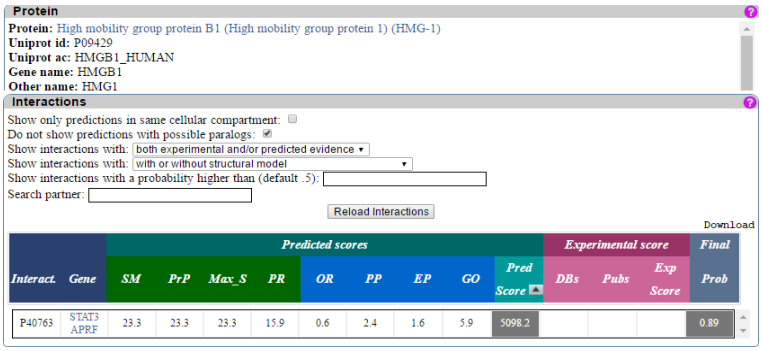


Supplemental Figure 2



Supplemental Figure 3

A



B Predicted binding domains in HMGB1

1: Positions: 13 14 15 16 17 18 20 21 22 24

Amino Acids: M S S Y A F V Q T R

2: Positions: 99 100 101 102 103 104 106 107 108 109 110 111 113

Amino Acids: P S A F F L C S E Y R P I

3: Positions: 122 123 125 126 128 130 131 132 133 136

Amino Acids I G V A K G E M W T

C Predicted binding domains in STAT3

1: Positions: 1 3 4 6 7 9 10 11 12 13

Amino Acids: M Q W Q L Q L D T R

2: Positions: 39 40 41 42 44 45 46

Amino Acids: E S Q D A Y A

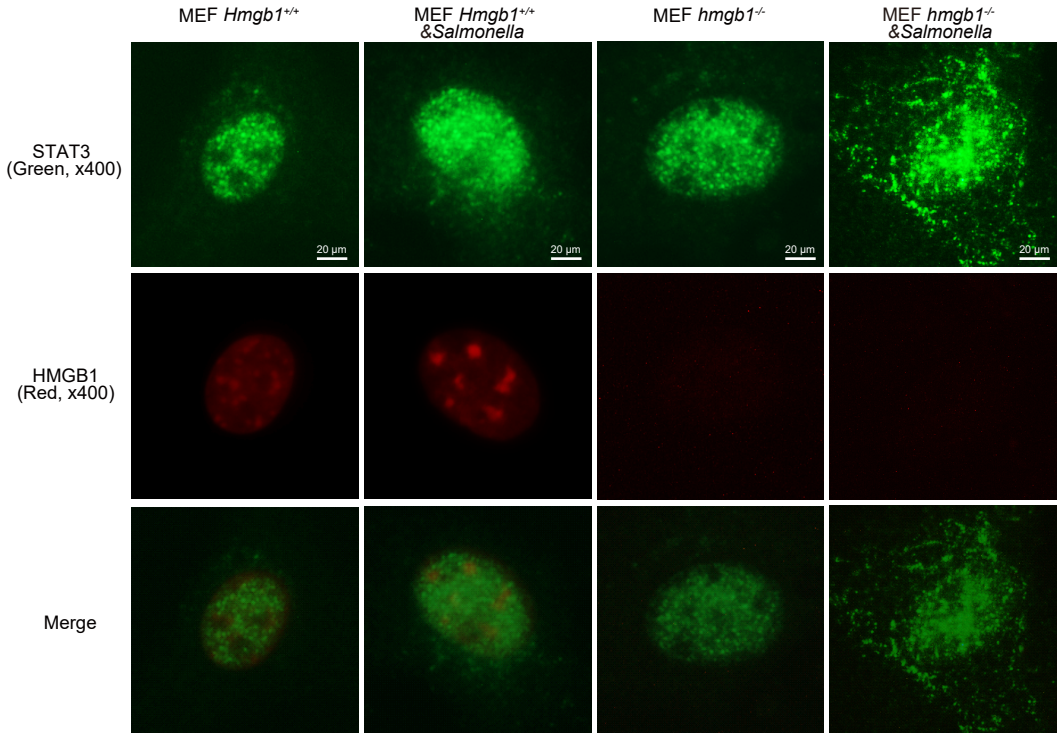
3: Positions: 133 134 135 136 137 138 139

Amino Acids: T A A V V T E

4: Positions: 204 205 207 208 209

Amino Acids: E Q L T A

5: Positions: 251 252 253 254 255 256 257

Amino Acids: C I G G P P N

Supplemental Figure 4