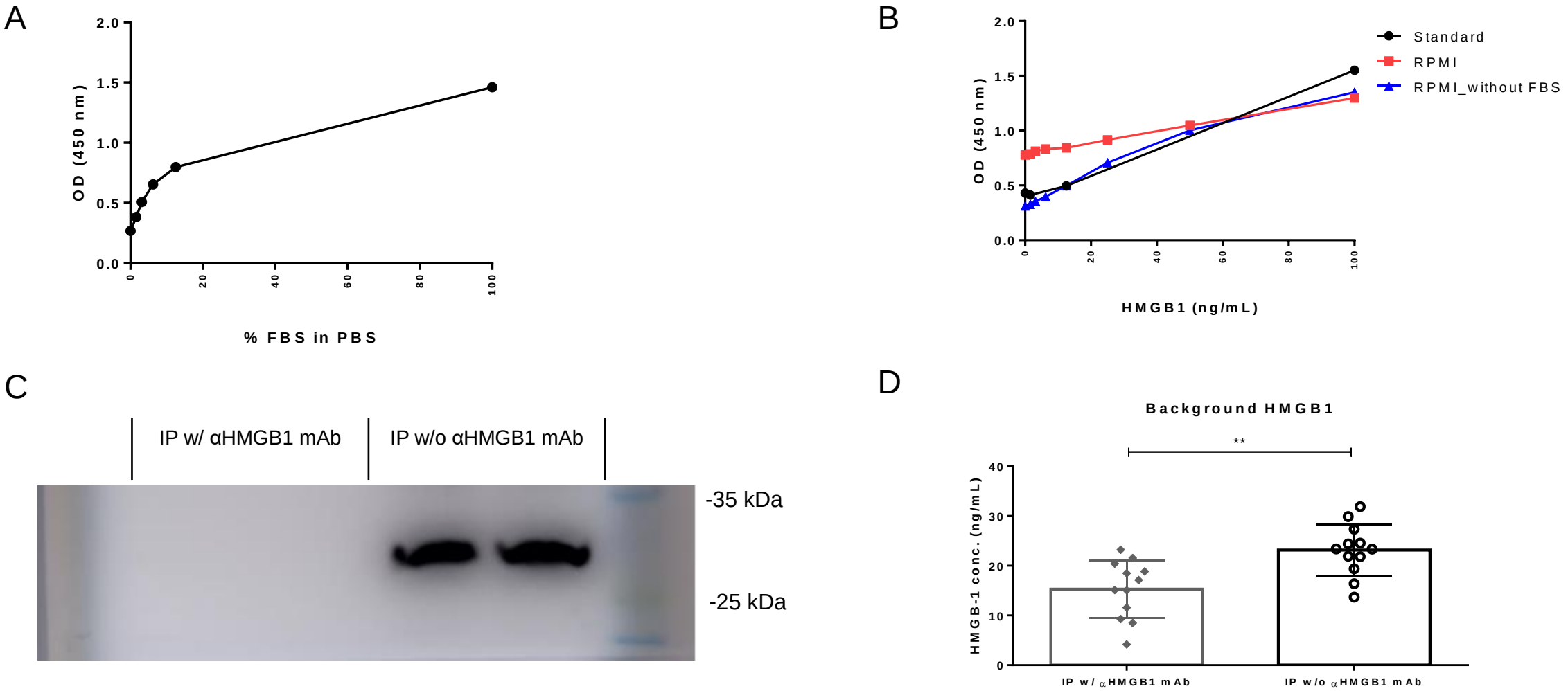
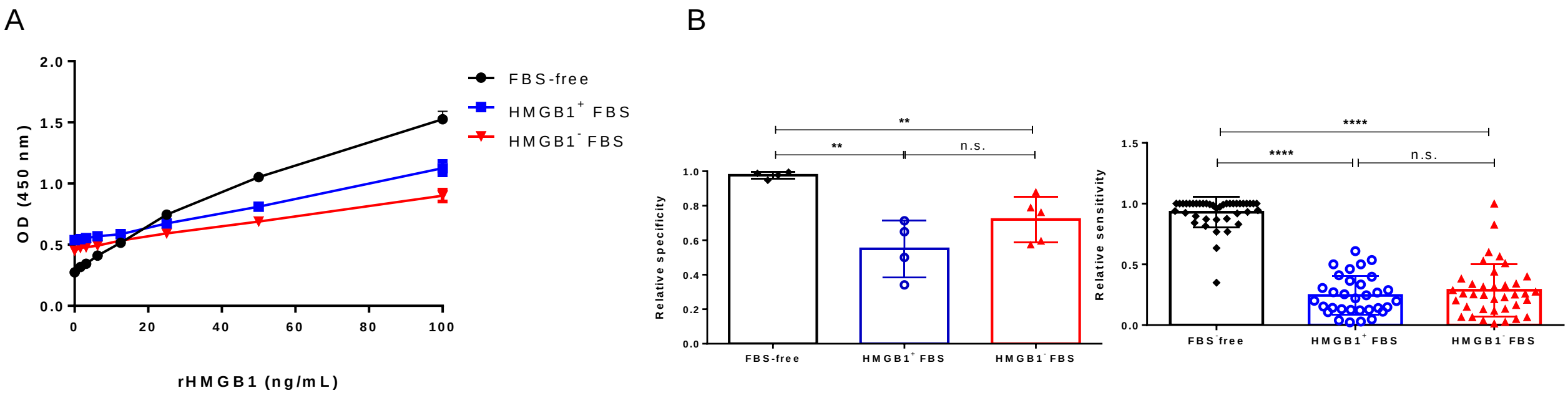
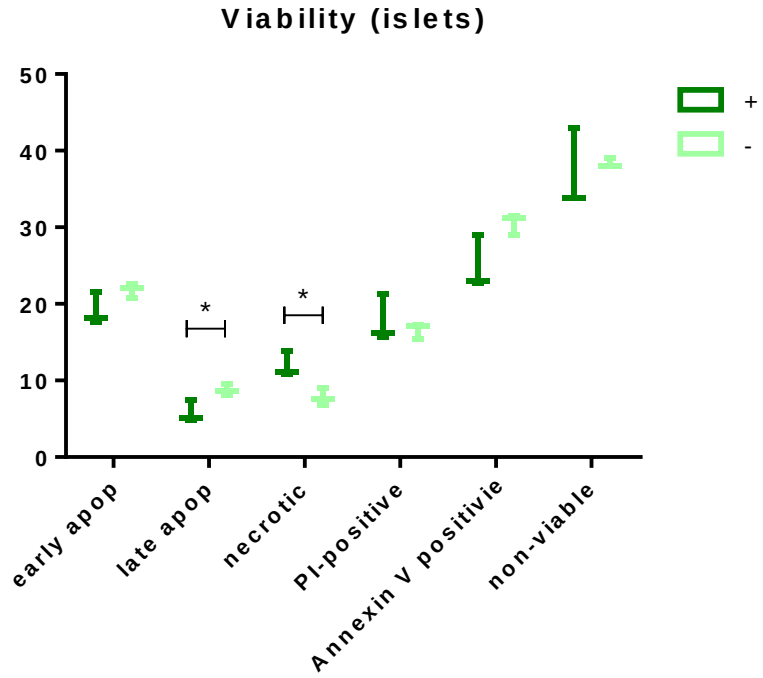


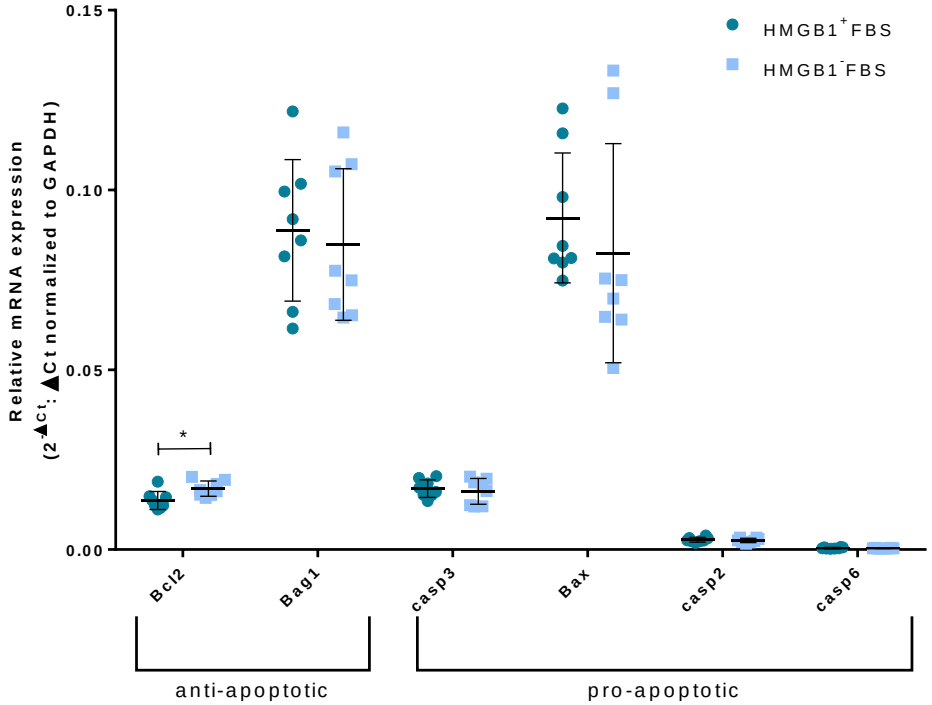
Supplementary Figure 1. Tests against FBS interference on sandwich ELISA signals **(A)** Signals generated with ten-fold dilutions of commercial FBS by our sandwich ELISA. **(B)** Murine rHMGB1 signals in sample dilution buffer, 10% FBS-supplemented RPMI1640 media, and RPMI1640 without FBS. The reaction volume was 50 µL. © To confirm absolute removal of HMGB1 from the FBS, SDS-PAGE and western blot analysis was performed on the FBS after immunoprecipitation with or without anti-HMGB1 mAb. Immunoprecipitation resulted in the depletion of pre-existing HMGB1 from FBS. Two separate samples of FBS were run by SDS-PAGE. **(D)** The background HMGB1 signal difference between 10% RPMI 1640 supplemented with FBS after immunoprecipitation with or without anti-HMGB1 mAb. Data are from 5 independent experiments. Data are presented as mean ± SD. **, P<0.01.



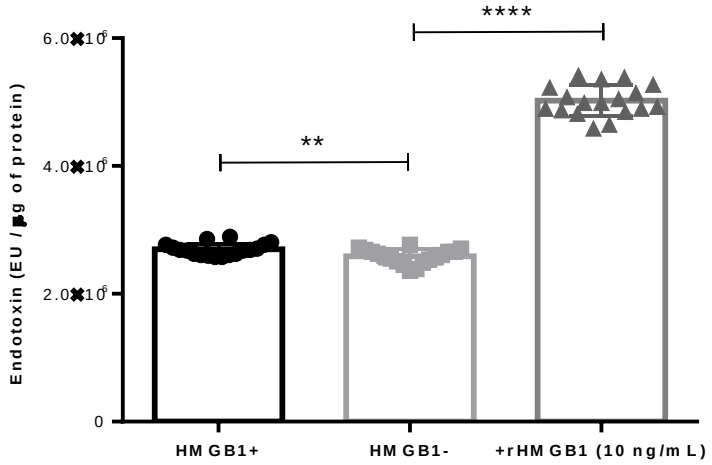
Supplementary Figure 2. HMGB1 ELISA signal interference by FBS with or without pre-existing HMGB1 removal. (A) Standard curves of rHMGB1 were generated with various sample matrices. The curves are representative of 3 independent experiments. FBS-free, RPMI 1640 without FBS supplement; HMGB1^{+}FBS, 10% FBS-supplemented RPMI 1640; HMGB1^{-}FBS, 10% of HMGB1-removed FBS-supplemented RPMI 1640. (B) The specificity (left) and sensitivity (right) of ELISA signals generated with known amount rHMGB1 in culture media without FBS (black), with FBS (blue), and with HMGB1-removed FBS (red). Data are presented as mean ± SD. **, P<0.01 as calculated by unpaired t-test; ****, P<0.0001 as calculated by unpaired t-test; n.s., not significant.



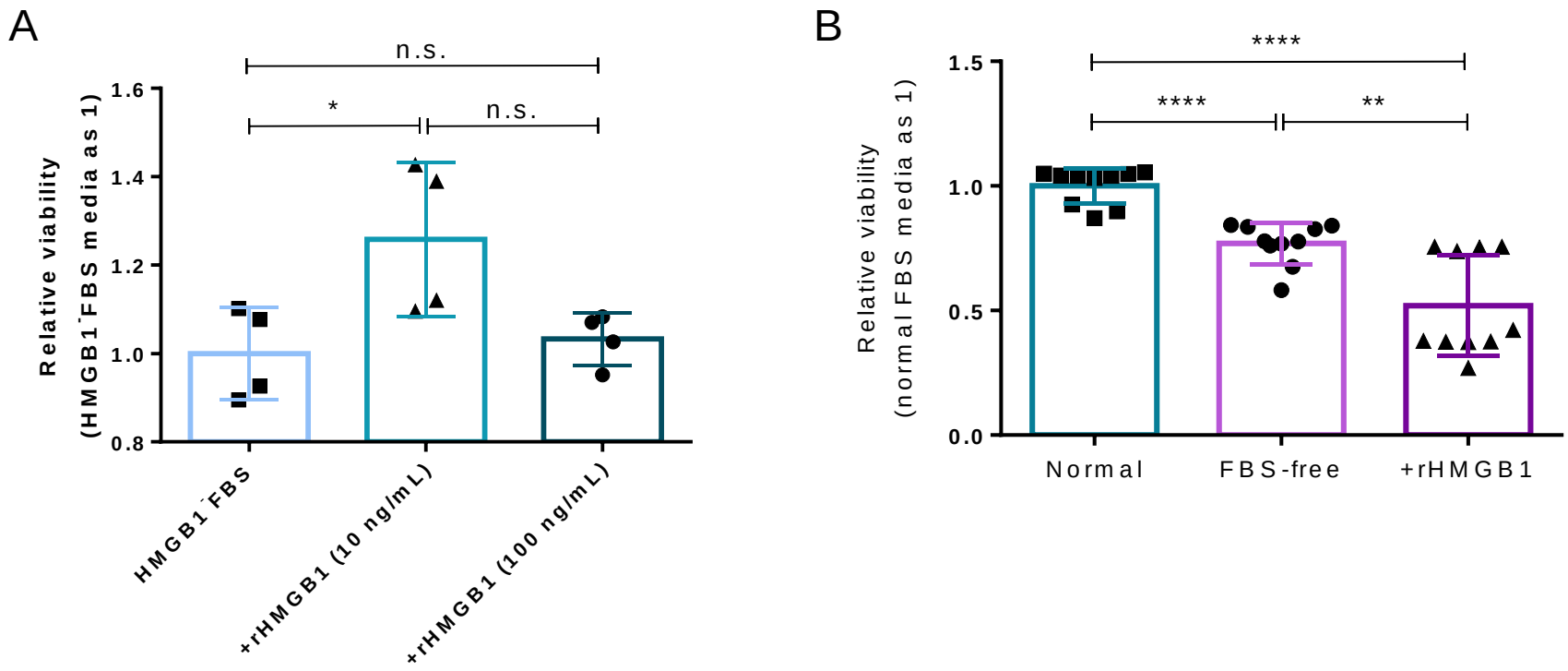
Supplementary Figure 3. Viability of primary islet single cells (3 x 10^4 cells) determined by Annexin V and PI staining. *, P<0.05 as calculated by unpaired t-test.



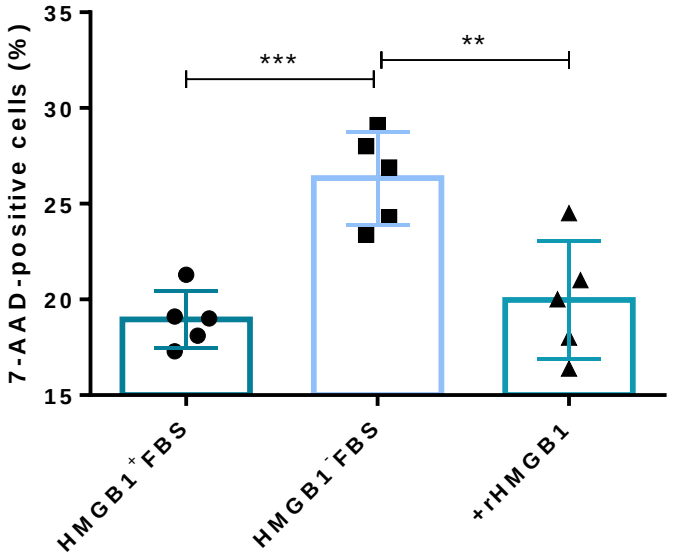
Supplementary Figure 4. qRT-PCR analysis of apoptosis-related genes in MIN6 cells. After the culture experiments, MIN6 cells were washed with PBS twice and total RNA was extracted with TRIZOL (Thermo Fisher Scientific) as the manufacturer's recommendations. The primers used were: gapdh F, 5′-GGA GAG TGT TTC CTC GTC CC-3′ and R, 5′-ATG AAG GGG TCG TTG ATG GC-3′; bcl2 F, 5′-TTC GCA GAG ATG TCC AGT CA-3′ and R, 5′-TTC AGA GAC AGC CAG GAG AA-3′; bag1 F, 5′-GAA ACA CCG TTG TCA GCA CT-3′ and R, 5′-GCT CCA CTG TGT CAC ACT C-3′; bax F, 5′-GGC TGG ACA CTG GAC TTC CT-3′ and R, 5′-GGT GAG GAC TCC AGC CAC AA-3′; casp2 F, 5′-GGC TAC AAT GTC CAT GTG CT-3′ and R, 5′-CCA CTA CGC AGG AGT CTG TG-3′; casp3 F, 5′-CAA GTC AGT GGA CTC TGG GA-3′ and R, 5′-CGA GAT GAC ATT CCA GTG CT-3′; casp6 F, 5′-TCA GGG CTA GGA CAC CG-3′ and R, 5′-TTG AAG ATG AGG GCA ACT CC-3′. Data are presented as mean ± SD. *, $P<0.05$ as calculated by unpaired t-test.



Supplementary Figure 5. Endotoxin level in various types of 10% FBS-supplemented RPMI 1640 media. Data are presented as mean ± SD. **, P<0.01 as calculated by unpaired t-test; ****, P<0.0001 as calculated by unpaired t-test; n.s., not significant.



Supplementary Figure 6. Viability of MIN6 cells in different culture conditions determined by CCK-8 assay. (A) MIN6 cells were cultured in HMGB1-depleted FBS-supplemented DMEM or the same media plus 10 ng/mL or 100 ng/mL of rHMGB1 for 48 hours. *, P<0.05 as calculated by unpaired t-test; n.s., not significant. (B) MIN6 cells were cultured in FBS-supplemented DMEM, FBS-free DMEM, or FBS-free DMEM plus 10 ng/mL of rHMGB1 for 48 hours. Data are presented as mean ± SD. ***, P<0.001 as calculated by unpaired t-test; **, P<0.01 as calculated by unpaired t-test.



Supplementary Figure 7. Viability of MIN6 cells determined by 7-AAD staining after rHMGB1 re-addition. Data are presented as mean ± SD. ***, P<0.001 as calculated by unpaired t-test. **, P<0.01 as calculated by unpaired t-test.