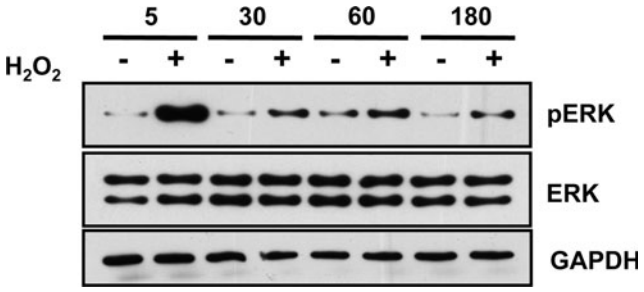
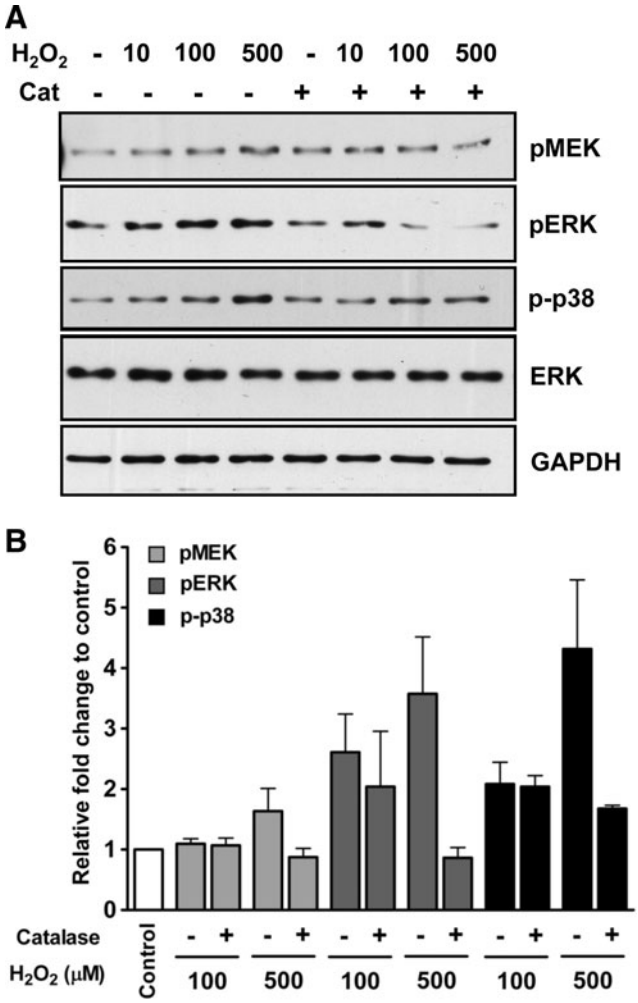
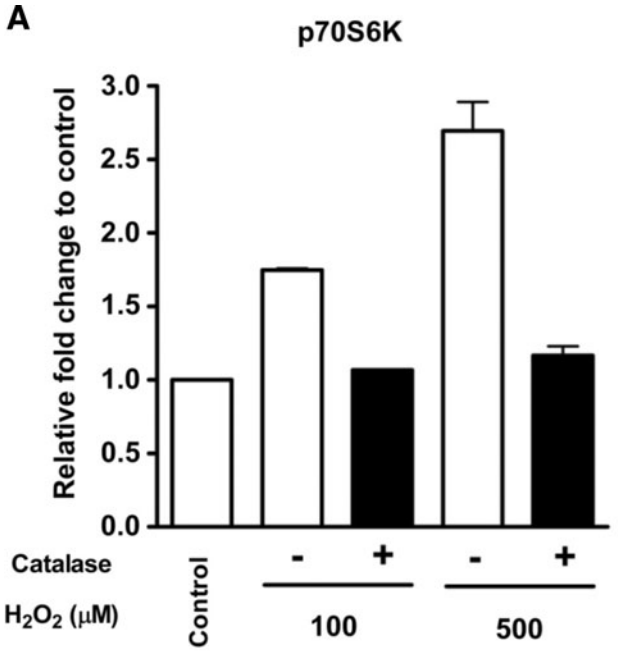
Supplementary Data

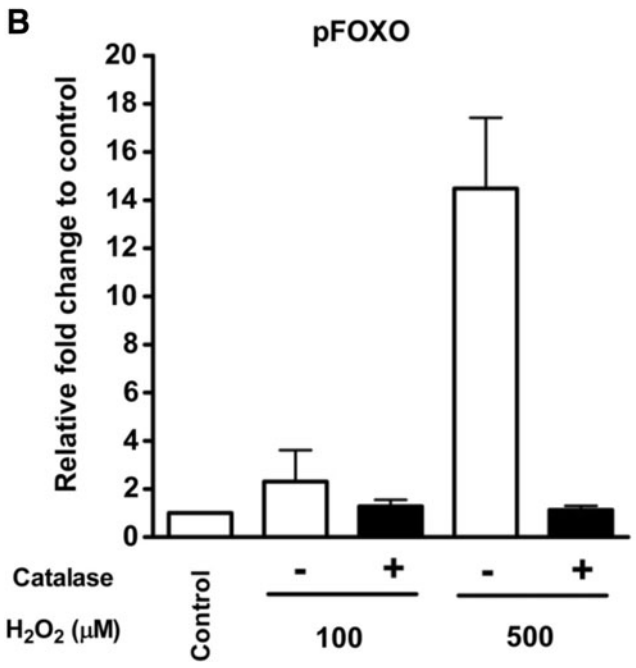


SUPPLEMENTARY FIG. S1. Exogenous H_2O_2 induced rapid activation of ERK in *Anopheles stephensi* cells. ASE cells were treated with PBS or with 500 μM H_2O_2 for 5, 30, 60, and 180 min. Protein samples were collected at indicated times for western blot analysis. Blots are representative of three independent experiments. ERK, extracellular signal-regulated kinase; H_2O_2, hydrogen peroxide; PBS, phosphate-buffered saline.

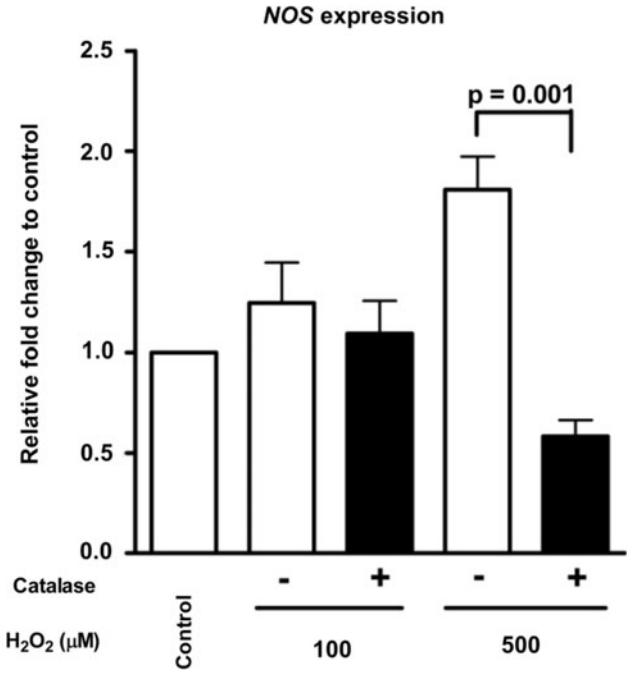


SUPPLEMENTARY FIG. S2. Exogenous H_2O_2 activated MEK, ERK, and p38 mitogen-activated protein kinase phosphorylation in *Anopheles gambiae* 4a3B cells. (A) H_2O_2 dose dependently induced MEK, ERK, and p38 phosphorylation in 4a3B cells. Cells were treated with PBS or with increasing concentrations of H_2O_2 for 15 min. For catalase treatment, cells were pretreated with catalase at 200 units/ml for 40 min before H_2O_2. Blots are representative of two independent experiments. **(B)** Relative fold increases of pMEK, pERK, and p-p38 were normalized to total ERK (for pERK only) or GAPDH and are shown relative to PBS controls. MEK, mitogen activated protein kinase kinase.

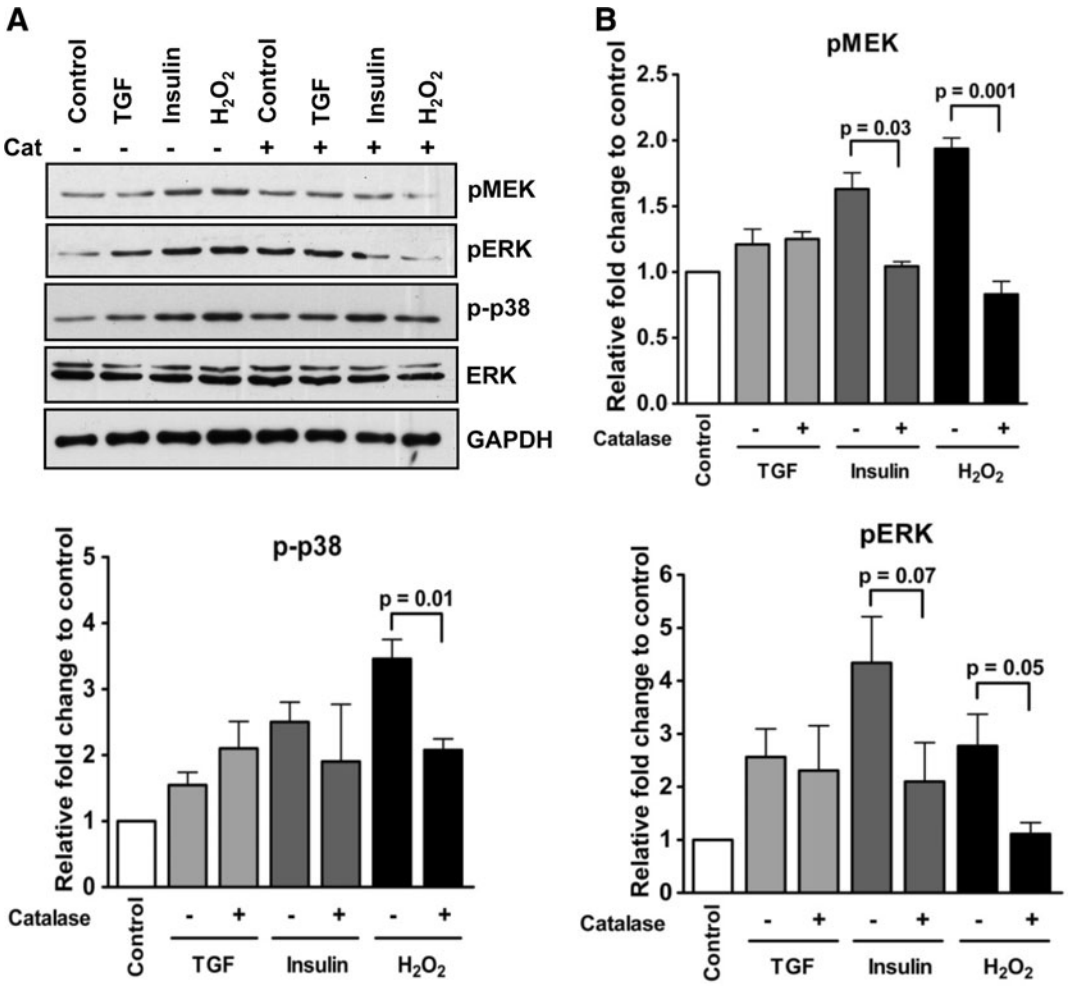




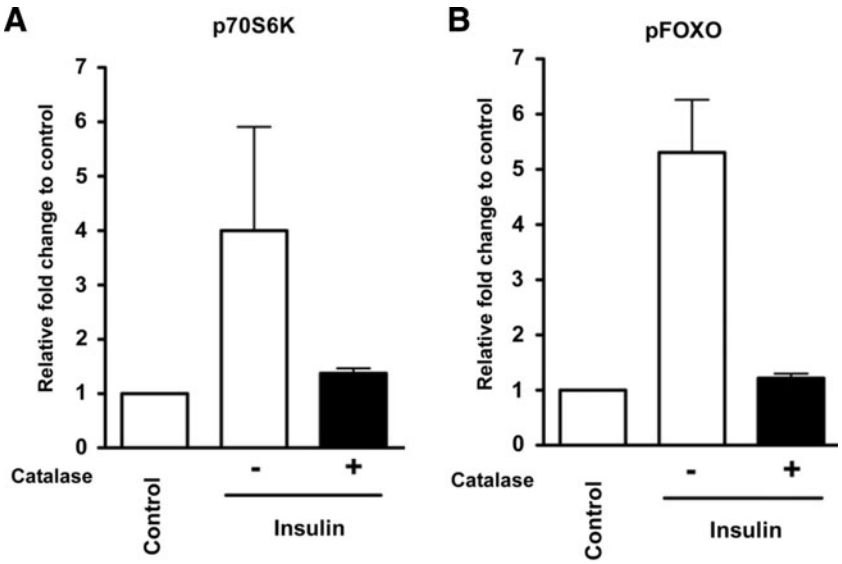
SUPPLEMENTARY FIG. S3. H_2O_2 induced FOXO and p70S6K phosphorylation in ASE cells. Increasing doses of H_2O_2 activated FOXO and p70S6K phosphorylation in ASE cells and pretreatment with catalase at 200 units/ml for 40 min before H_2O_2 treatment reduced this phosphorylation. Fold increases of pFOXO **(A)** and p-p70S6K **(B)** from Figure 5E and an additional replicate were normalized to GAPDH and are shown relative to PBS treated control cells. Data are represented as means ± SEMs from two independent experiments. FOXO, forkhead box O1; SEM, standard error of the mean.



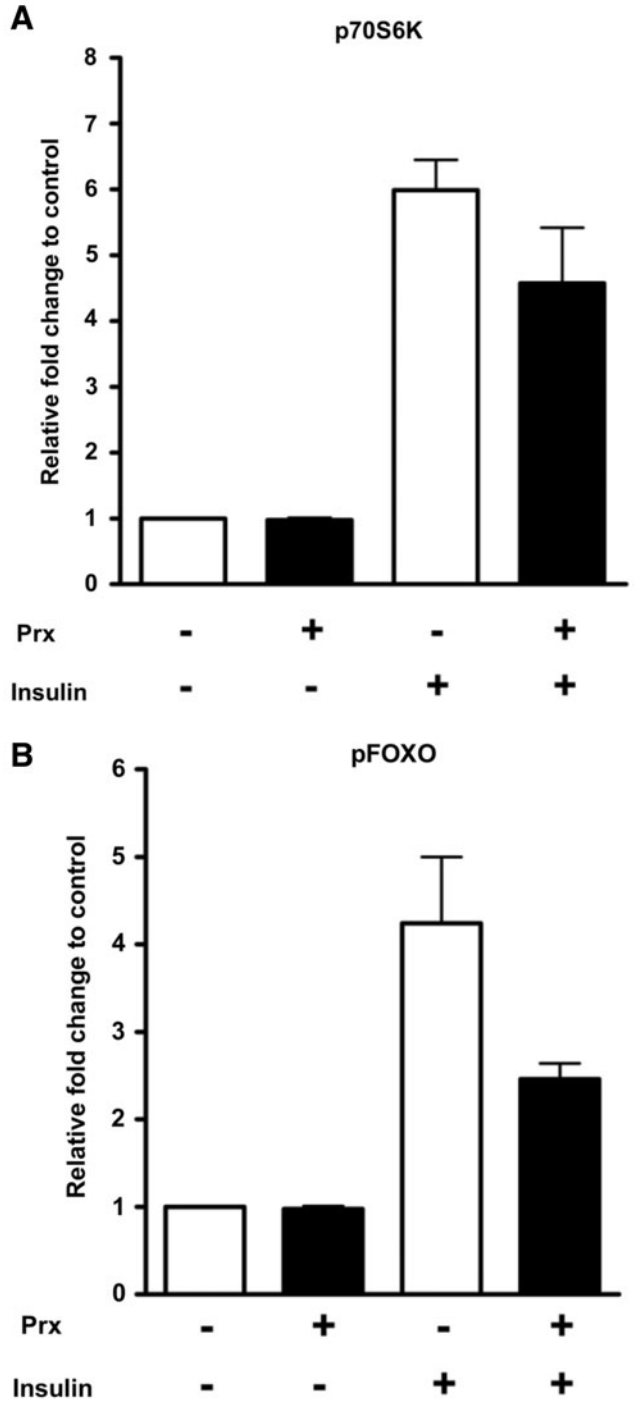
SUPPLEMENTARY FIG. S4. H_2O_2 induced *NOS* expression in *A. gambiae* cells. 4a3B cells were treated with PBS as a control, with 100 or 500 μM H_2O_2 alone or pretreated with 200 units/ml catalase for 40 min before H_2O_2 treatment. *NOS* gene expression was analyzed by quantitative reverse transcriptase–polymerase chain reaction at 24 h post-treatment. Fold inductions of *NOS* relative to control from three independent experiments are represented as mean ± SEMs. Pairwise comparisons of treatments were analyzed by Student's *t*-test ($\alpha = 0.05$). Significant differences within treatment pairs are indicated. NOS, nitric oxide synthase.



SUPPLEMENTARY FIG. S5. ROS regulated the signaling responses of *A. gambiae* 4a3B cells to human insulin. (A) 4a3B cells were stimulated with 6000 pg/ml human TGF- beta1, 1.7 μM human insulin, or 500 μM H_2O_2 for 15 min. To determine the effect of ROS on signaling protein activation, cells were pretreated with 200 units/ml catalase for 40 min before each treatment. Blots are representative of three independent experiments. **(B)** Relative fold increases of pMEK, pERK, and p-p38 were normalized to total ERK (for pERK only) or GAPDH and are shown relative to PBS controls. Data are represented as means $\pm$ SEMs from three independent experiments. Pairwise comparisons of treatments were analyzed by Student's *t*-test ($\alpha = 0.05$). Significant differences within treatment pairs are indicated. ROS, reactive oxygen species; TGF, transforming growth factor.



SUPPLEMENTARY FIG. S6. ROS regulated insulin-induced FOXO and p70S6K phosphorylation in *A. stephensi* cells. ASE cells were stimulated with 1.7 μM human insulin with or without pretreatment with 200 units/ml catalase for 40 min before stimulation. Relative fold increases of **(A)** p70S6K and **(B)** pFOXO from Figure 6B and from one additional replicate were normalized to GAPDH and are shown relative to PBS controls. Data are represented as means $\pm$ SEMs from two independent experiments.



SUPPLEMENTARY FIG. S7. Overexpression of *A. stephensi* 2-cys peroxiredoxin (*As*Prx) reduced insulin signaling in ASE cells. ASE cells were transfected with plasmid TLP-58 encoding a constitutively expressed *As*Prx and were stimulated 1.7 μM human insulin for 5 min (48). Cells overexpressing *As*Prx are indicated as "Prx+," and cells transfected with the negative control plasmid TLP-55 are indicated as "Prx−." Relative fold increases of **(A)** p70S6K and **(B)** pFOXO from Figure 7D and from one additional replicate were normalized to GAPDH and are shown relative to PBS-treated control cells from Figure 7D. Data are represented as means ± SEMs from two independent experiments.