

Figure S1. Dosage effect of glucose concentration on REV production. The colors, blue and red, correspond with the different concentrations of H₂O₂ stimulation: 0.2 and 2 μ M, respectively. Bar plot represents the REV production under (A) 5 mM, (B) 45 mM and (C) 100 mM glucose concentration. The significant increase in REV production were observed in 45 mM and 100 mM glucose concentrations (P=0.0102 and P=0.0248, respectively). However, no significant differences of REV productions were observed at 5 mM glucose cultures using different H₂O₂ concentrations. (D-F) Representative histogram compares annexin V expressing EVs that are used for REV calculations. MFIs of annexin V expressing EVs after stimulation with 0.2 and 2 μ M H₂O₂ for 24 h were compared in (D) 5, (E) 45 and (F) 100 mM of glucose culture. (G-I) Representative histogram of ROS production using different concentrations of H₂O₂ stimulation under different glucose concentrations including (G) 5 mM, (H) 45 mM and (I) 100 mM. Data are presented as the mean $\pm$ standard error of the mean, with individual dots indicating measurements from separate samples. *Indicates statistical significance. REV, red blood cell-derived extracellular vesicle; DM, diabetes mellitus; MFI, mean fluorescence intensity; M, molar; ROS, reactive oxygen species.

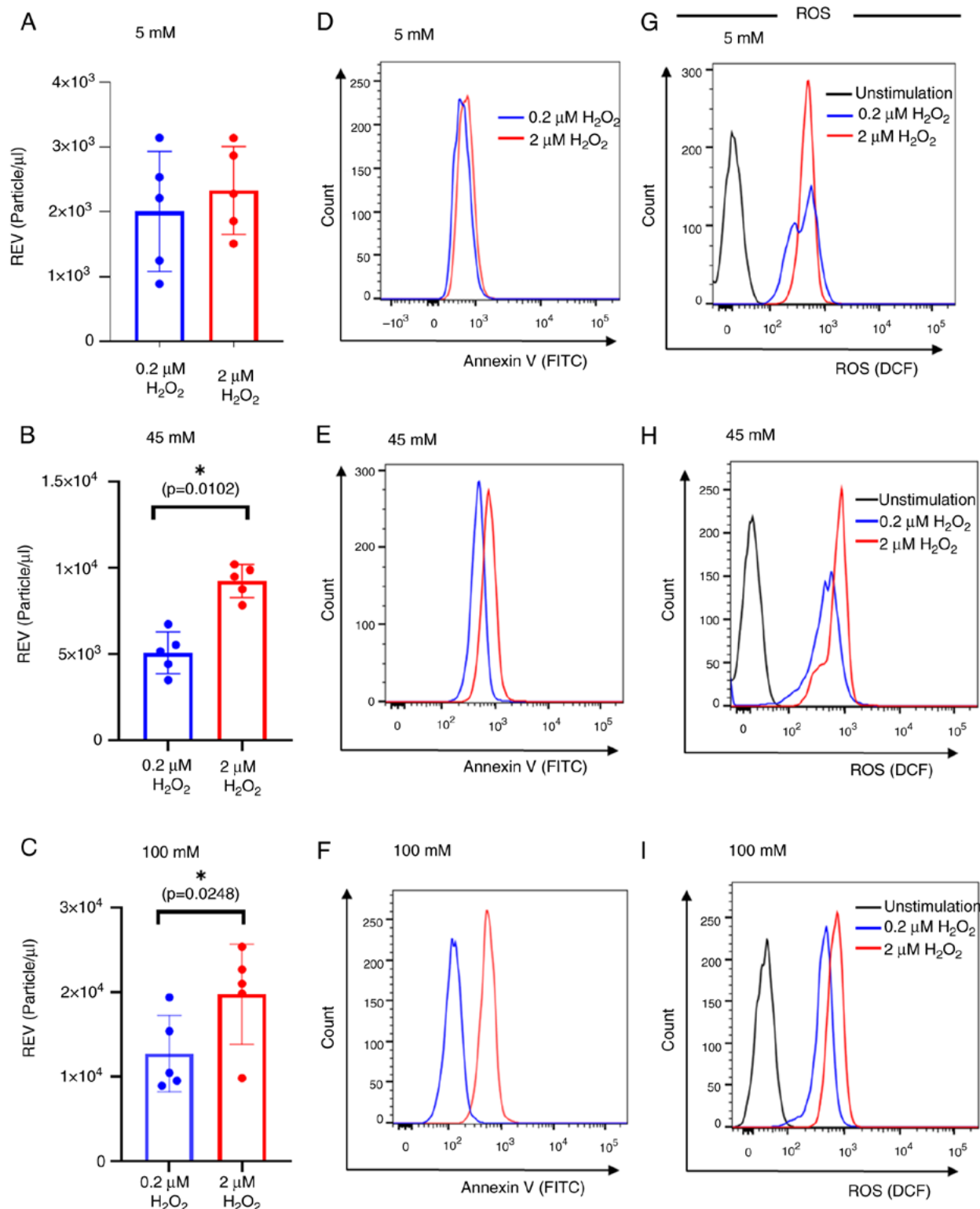


Figure S2. CD235 expression of glucose-treated RBCs. (A) Bar plot comparing the dosage effect of glucose on CD235 expression at 24 and 48 h of incubation. The colors, green, blue and red, correspond with the different treatment groups: Euglycemic control (5 mM), intermediate level (45 mM) and severe hyperglycemia (100 mM), respectively. The significantly decreased CD235 expression was observed at 45-mM glucose concentrations compared with 5 mM after 24 h of treatment ($P=0.0078$). Whereas at 48 h of treatment, the significant decrease of CD235 was observed at 45- and 100-mM glucose concentration. (B) Representative histogram comparing CD235 expression of glucose-treated RBC. MFIs of CD235 in 5-, 45- and 100- mM glucose treatment were compared at 24 h of incubation. (C) Representative histogram comparing CD235 expression of glucose-treated RBC. MFIs of CD235 in 5-, 45- and 100 mM glucose treatment were compared at 48 h of incubation. (D) Bar plots represent CD235 expression among different treatment groups: Euglycemic control (5 mM), intermediate level (45 mM) and severe hyperglycemia (100 mM). The colors, blue and red, correspond with 24 and 48 h of incubation, respectively. CD235 expression significantly downregulated at 100 mM ($P=0.0039$). However, no significant differences in CD47 expression were observed at 5 mM and 45 mM glucose concentration between 24 and 48 h of culture. (E-G) Representative histograms comparing CD235 expression at (E) 5-mM, (F) 45-mM and (G) 100-mM glucose concentrations after 24 and 48 h of incubation. Effect of incubation time on and glucose concentrations on CD235 expression at distinct glucose concentrations, compared using the Wilcoxon sign-rank test. Data are presented as the mean $\pm$ standard error of the mean, with individual dots indicating measurements from separate samples. *Indicates statistical significance. ROS, reactive oxygen species; RBC, red blood cell; MFI, mean fluorescence intensity; M, molar.

