

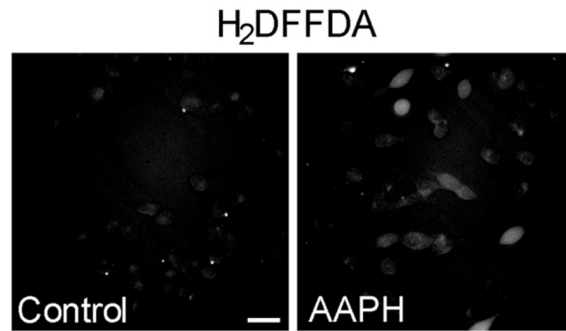
## **Stigmasterol prevents glucolipotoxicity induced defects in glucose-stimulated insulin secretion**

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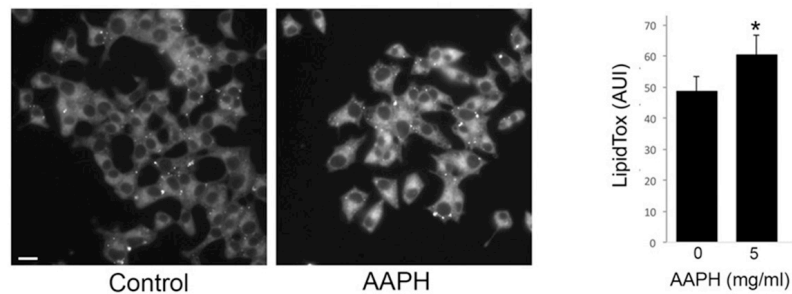
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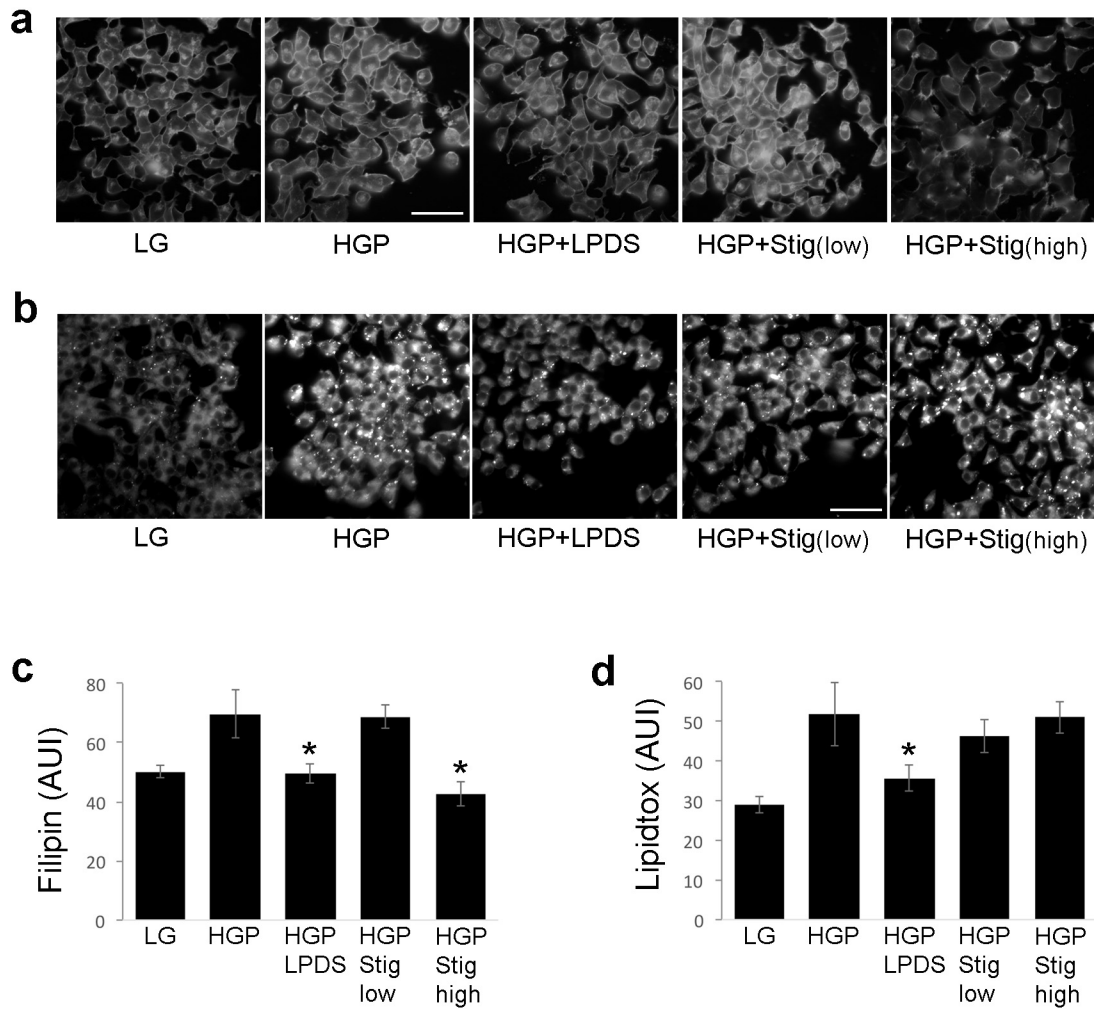
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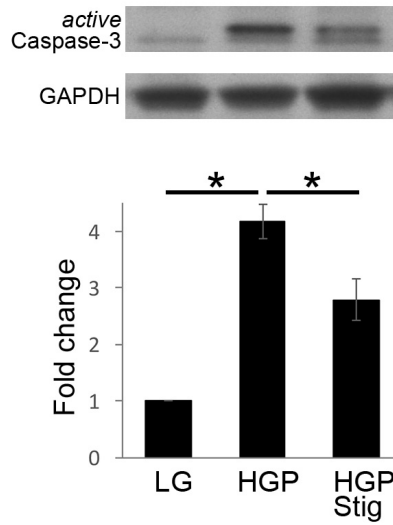
**Supplementary Figure 1. AAPH induces ROS production in INS-1 cells.** INS-1 cells were incubated for 1 h with 5 mg/ml AAPH and imaged using carboxy-H<sub>2</sub>DFFDA. Scale bar, 15  $\mu$ m.



**Supplementary Figure 2. ROS induces neutral lipid accumulation in INS-1 cells.** Lipidtox staining of INS-1 cells incubated for 1 h with 5 mg/ml AAPH in serum free media. Scale bar, 15  $\mu$ m. Lipidtox intensity per cell was quantified. \* $p$ <0.05.



**Supplementary Figure 3. Reversal of HGP-induced cholesterol increase by LPDS and stigmaterol.** INS-1 cells treated for 72 h with LG, HGP, HGP+LPDS or HGP+stigmaterol (Stig) were stained with filipin (a) or lipidtox (b). Low and high stigmaterol concentration refers to 12.5  $\mu\text{g/ml}$  and 50  $\mu\text{g/ml}$ , respectively. Filipin (c) and lipidtox (d) fluorescence was quantified. Scale bars, 50  $\mu\text{m}$ . \* $p < 0.05$  against HGP.



**Supplementary Figure 4. Stigmasterol reduces HGP-mediated apoptosis**

**indicated by decreased active caspase-3.** Cells were treated as indicated, lysed and immunoblotted for active (cleaved) caspase-3. Equal amounts of total protein were loaded and GAPDH was used as loading control. \* $p < 0.05$  against HGP.