Fig. S1.

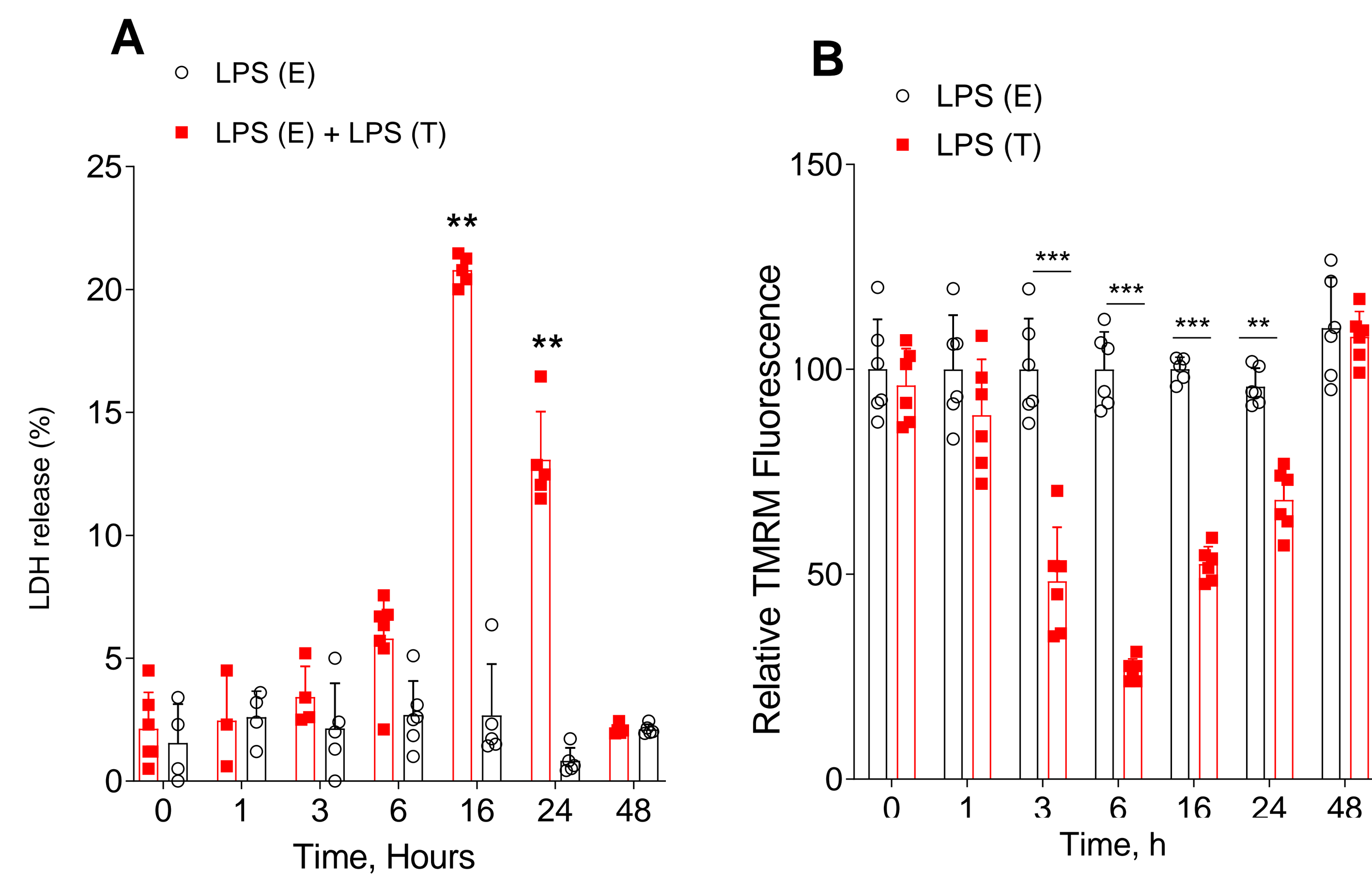

Fig. S1. related to Figure 1. Time course of intracellular LPS-induced endothelial pyroptotic cell death and decreased mitochondrial membrane potential. (A) hLMVECs were primed with extracellular LPS (LPS (E), 1 μg/ml, 3h), followed by medium change (0.01% FBS) and transfection with LPS (LPS (T), 0.5 μg/ml, 0-48 h) to increase intracellular LPS levels. The LDH levels in the medium were analyzed by an LDH assay kit, n = 4-6. (B) hLMVECs were primed with LPS (LPS (E), 1 μg/ml, 3h), followed by medium change and transfection with LPS (LPS (T), 0.5 μg/ml, 0-48 h). The mitochondrial membrane potential (MMP) in hLMVECs was analyzed by staining with the potentiometric mitochondrial dye TMRM, n = 5-6. * * $P < 0.01$, * * * $P < 0.001$, two-tailed *t*-test. Values are mean ± SD. Data were obtained from three independent experiments.

Fig. S2.

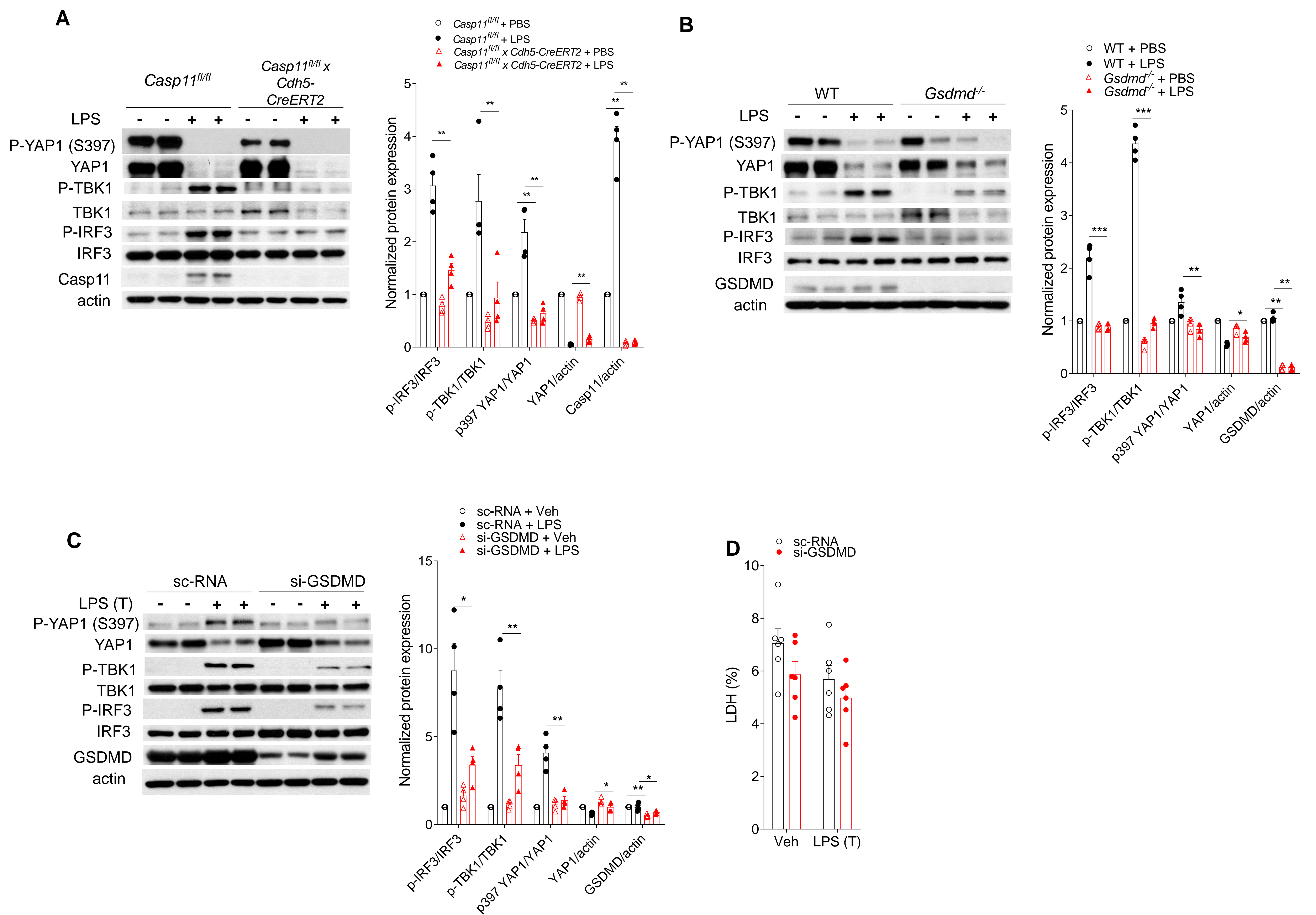

Fig. S2. related to Figure 3 and 5. LPS induced cGAS-YAP1 signaling is dependent on Casp11-GSDMD pathway. (A) Primary mouse lung endothelial cells were isolated from *Casp11*$^{fl/fl}$ and *Casp11*^{ECKO} mouse post-LPS (10 mg/kg, *i.p*, 6 h) challenge. The expression of proteins was analyzed by immunoblotting, quantified and normalized against actin, n = 4. (B) Primary mouse lung endothelial cells were isolated from WT and *GSDMD*$^{-/-}$ mouse post-LPS (10 mg/kg, *i.p*, 6 h) challenge. The expression of proteins was analyzed by immunoblotting, quantified and normalized against actin, n = 4. (C) hLMVECs were transfected with 300 nM of negative control si-RNA (sc-RNA) and si-RNA target to human GSDMD (si-GSDMD) for 48 h. Then, cells were primed with LPS (LPS (E), 0.2 μg/ml, 3h), followed with transfection with/without LPS (T) (0.5 μg/ml; 6h) treatment. The expression of proteins was analyzed by immunoblotting, quantified and normalized against actin, n = 4. (D) LDH level from hLMVECs with siRNA and LPS (T) treatment was assayed by using LDH assay kit, n = 6. * $P < 0.05$, * * $P < 0.01$, * * * $P < 0.001$, two-tailed *t*-test. Values are mean ± SD. Data were obtained from three independent experiments.

Fig. S3.

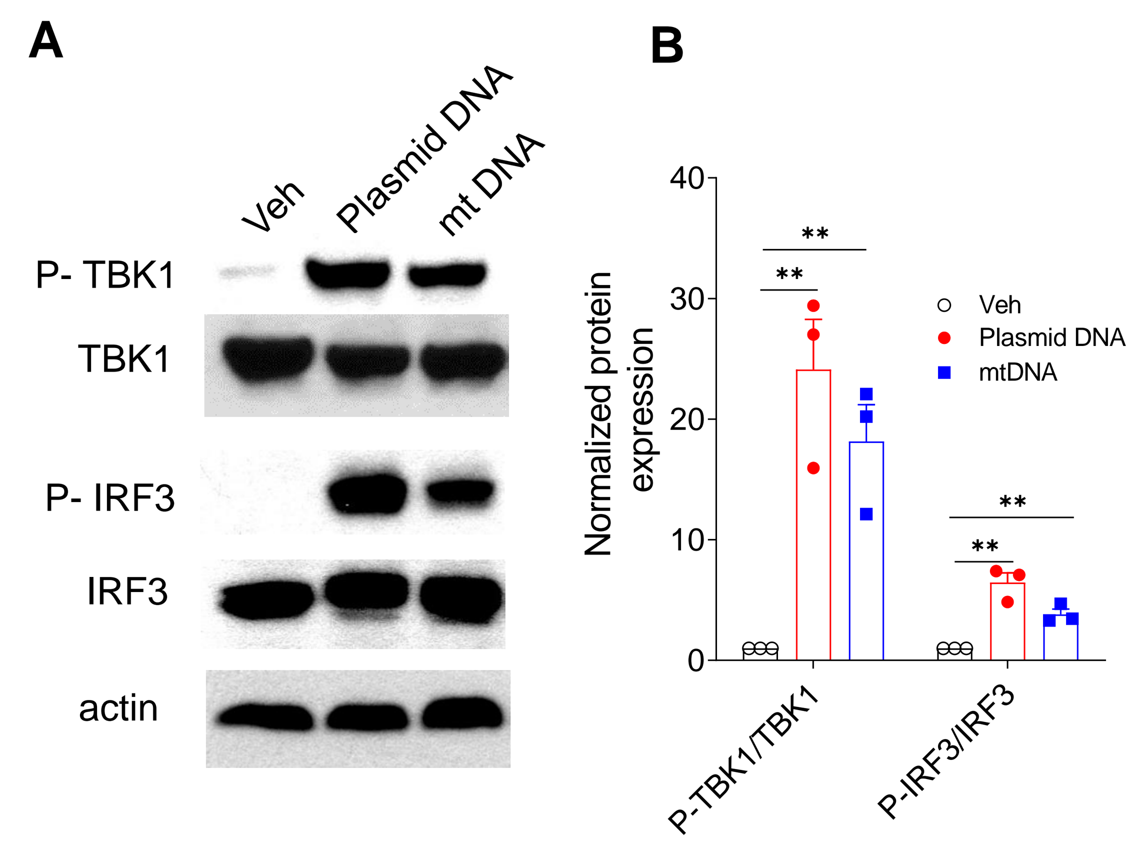

Fig. S3. related to Figure 3. Plasmid DNA and mtDNA induce activation of cGAS signaling in human endothelial cells (hLMVECs). (A) hLMVECs were transfected with plasmid DNA or mtDNA (3 µg/ml). 6 h post transfection, the expression of proteins in hLMVECs was analyzed by immunoblotting. (B) Quantification of protein expression at 6 h post transfection of plasmid DNA or mtDNA. Values are mean ± SD. Data were obtained from three independent experiments. * * $P < 0.01$, two-tailed *t*-test.

Fig. S4.

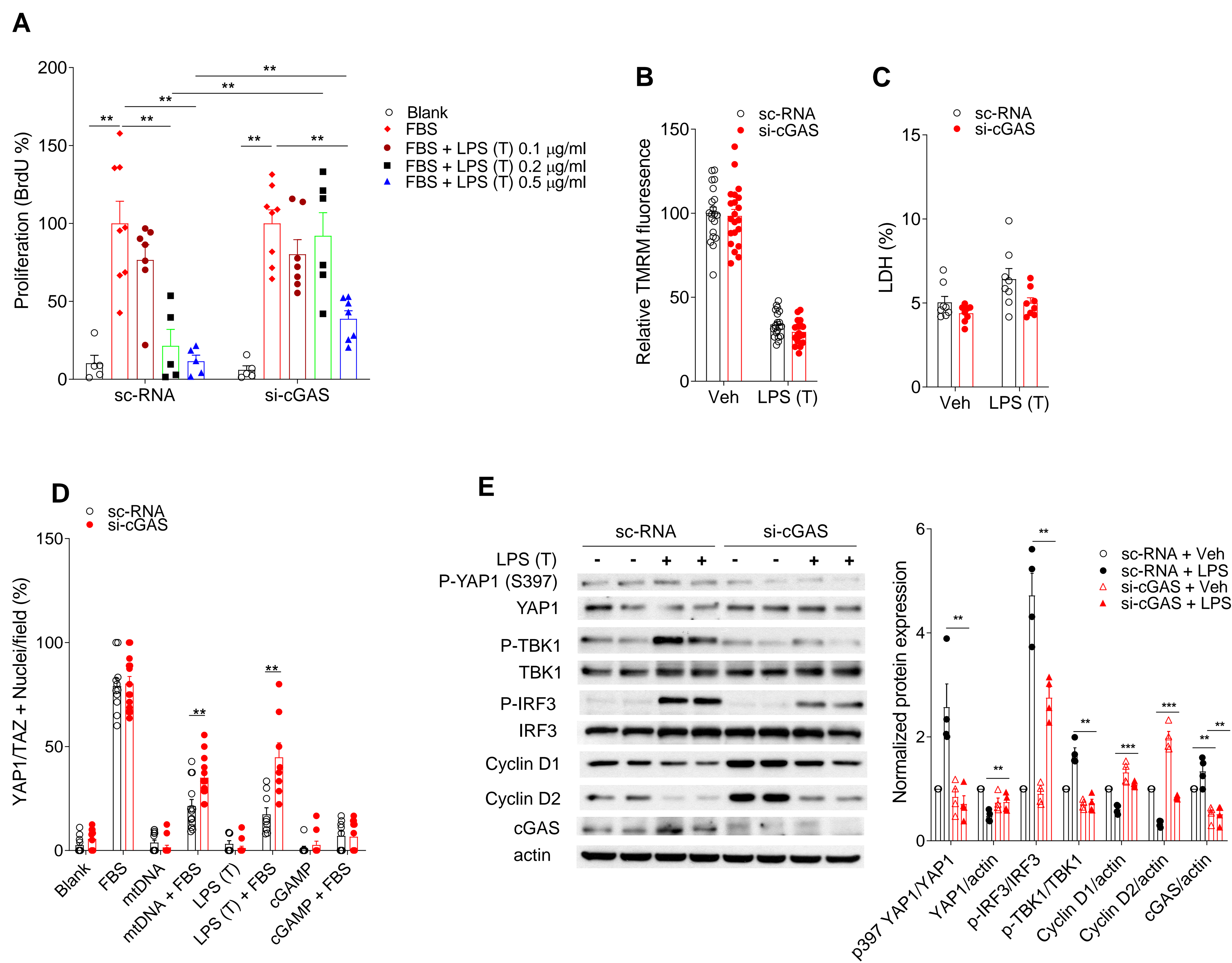

Fig. S4. related to Figure 4 & 5. Intracellular LPS regulates cell proliferation via cGAS-YAP1 pathway. hLMVECs were transfected with 300 nM of negative control si-RNA (sc-RNA) and si-RNA target to human cGAS (si-cGAS) for 48 h. (A) siRNA transfected cells were primed with LPS (LPS (E), 0.2 μg/ml, 3h), followed with LPS (T) (0, 0.1, 0.2, 0.5 μg/ml) with/without 10%FBS for 6 h. And the cell proliferation was analyzed by using the proliferation assay kit, n = 5-8. (B) Mitochondrial membrane potential (MMP) in siRNA transfected hLMVECs with LPS (T) (0, 0.5 μg/ml, 6 h) was analyzed by TMRM staining. Values are shown as mean ± SD; data were obtained from three independent experiments, n = 18-21. (C) The LDH levels in the medium of siRNA transfected hLMVECs exposed to LPS (T) (0, 0.5 μg/ml, 6 h) were analyzed using LDH assay kit. Values are shown as mean ± SD; data were obtained from three independent experiments, n = 8. (D) siRNA transfected cells were transfected with mtDNA or cGAMP (0, 3 μg/ml; 20h) or LPS (T) (0, 0.5 ug/ml, 6h), and followed with treatment of FBS treatments (10%, 1h). The nuclear translocation of YAP1/TAZ complex was analyzed by immunofluorescence staining. Values are shown as mean ± SD; data were obtained from three independent experiments, n = 8-15. (E) siRNA transfected cells were treated with LPS (T) (0, 0.5 μg/ml, 6h). The expression of proteins was analyzed by immunoblotting, quantified and normalized against actin. Values are mean ± SD obtained from four independent experiments, two-tailed *t*-test. * * $P < 0.01$, * * * $P < 0.001$.

Fig. S5

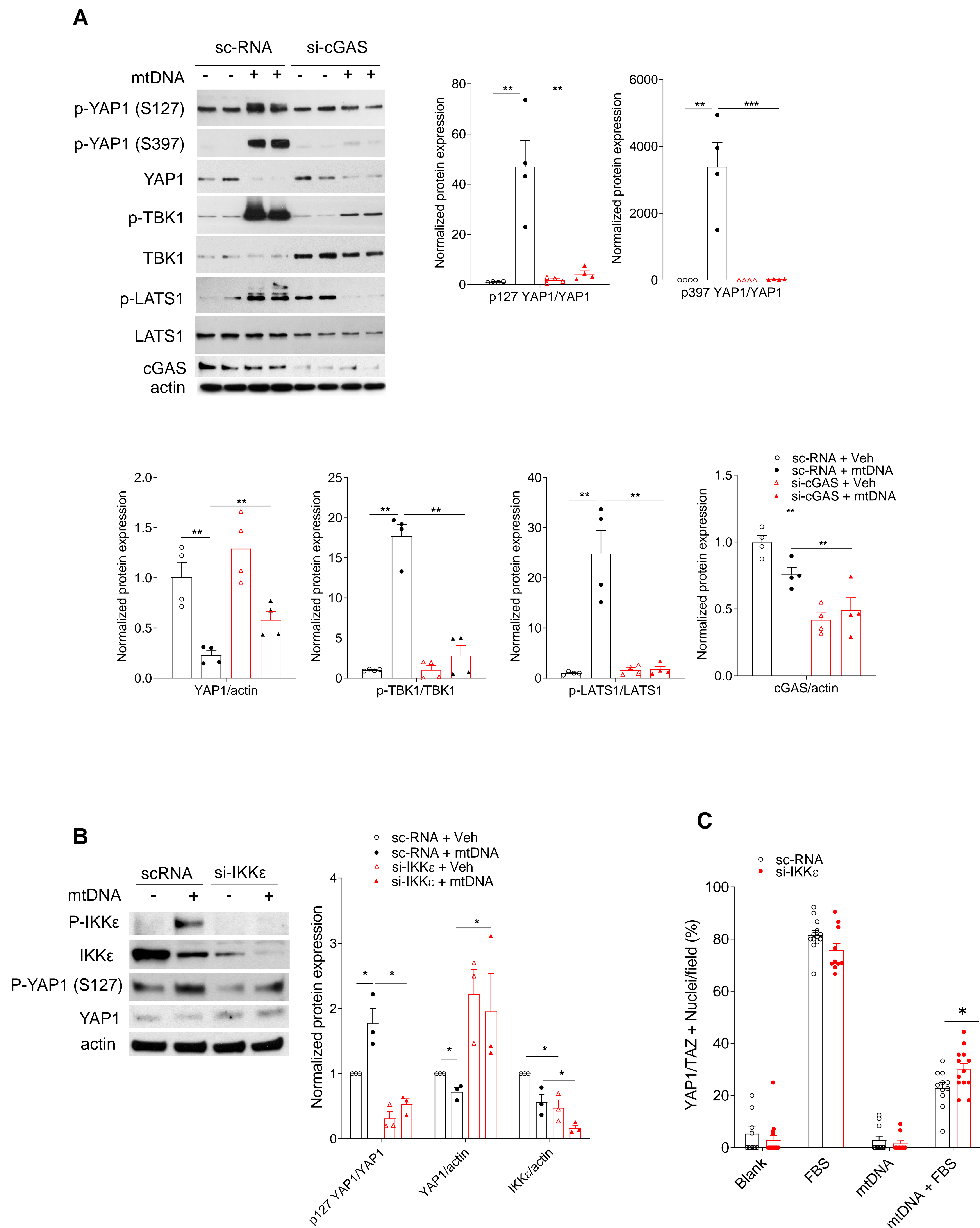

Fig. S5. related to Figure 5. (A) cGAS regulates mtDNA inhibited YAP1 signaling. hLMVECs were transfected with 300 nM of negative control si-RNA (sc-RNA) and si-RNA targeting human cGAS (si-cGAS) for 48 h. Cells were then transfected with mtDNA (0, 3 μg/ml; 6h). The expression of proteins was analyzed by immunoblotting, quantified and normalized against actin. Data were obtained from four independent experiments. * * $P < 0.01$, two-tailed *t*-test. Values are mean ± SD. **(B, C) Intracellular mtDNA regulates cell proliferation partly through IKKε pathway.** hLMVECs were transfected with 300 nM of negative control si-RNA (sc-RNA) and si-RNA targeting human IKKε (si-IKKε) for 48 h. (A) siRNA transfected cells were treated with mtDNA (3 μg/ml) for 6 h. The expression of proteins was analyzed by immunoblotting, quantified and normalized against actin. Values are mean ± SD. Data were obtained from three independent experiments. (B) siRNA transfected cells were subsequently transfected with mtDNA (0, 3 μg/ml; 20h), and followed by treatment of medium containing FBS (10%, 1h). The nuclear translocation of YAP1/TAZ complex were analyzed by immunofluorescence staining. Values are shown as mean ± SD; data were obtained from three independent experiments, n = 9-15, two-tailed *t*-test. * $P < 0.05$, * * $P < 0.01$, * * * $P < 0.001$.