Supporting Information for

4-Hydroxy-7-oxo-5-heptenoic Acid (HOHA) Lactone Induces Angiogenesis through Several Different Molecular Pathways

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Figure S1. The pro-angiogenic effect of HOHA-lactone-GSH (-OH) on HUVECs in the wound-healing assay. S3

Figure S2. The pro-angiogenic effect of HOHA-lactone-GSH (=O) on HUVECs in the tube formation assay. S4

Figure S3. Effect of RPE conditioned medium on HUVECs in a tube formation assay in the absence and in the presence of Avastin (20 μg/ml) or AAL-993 (100 nM). S5

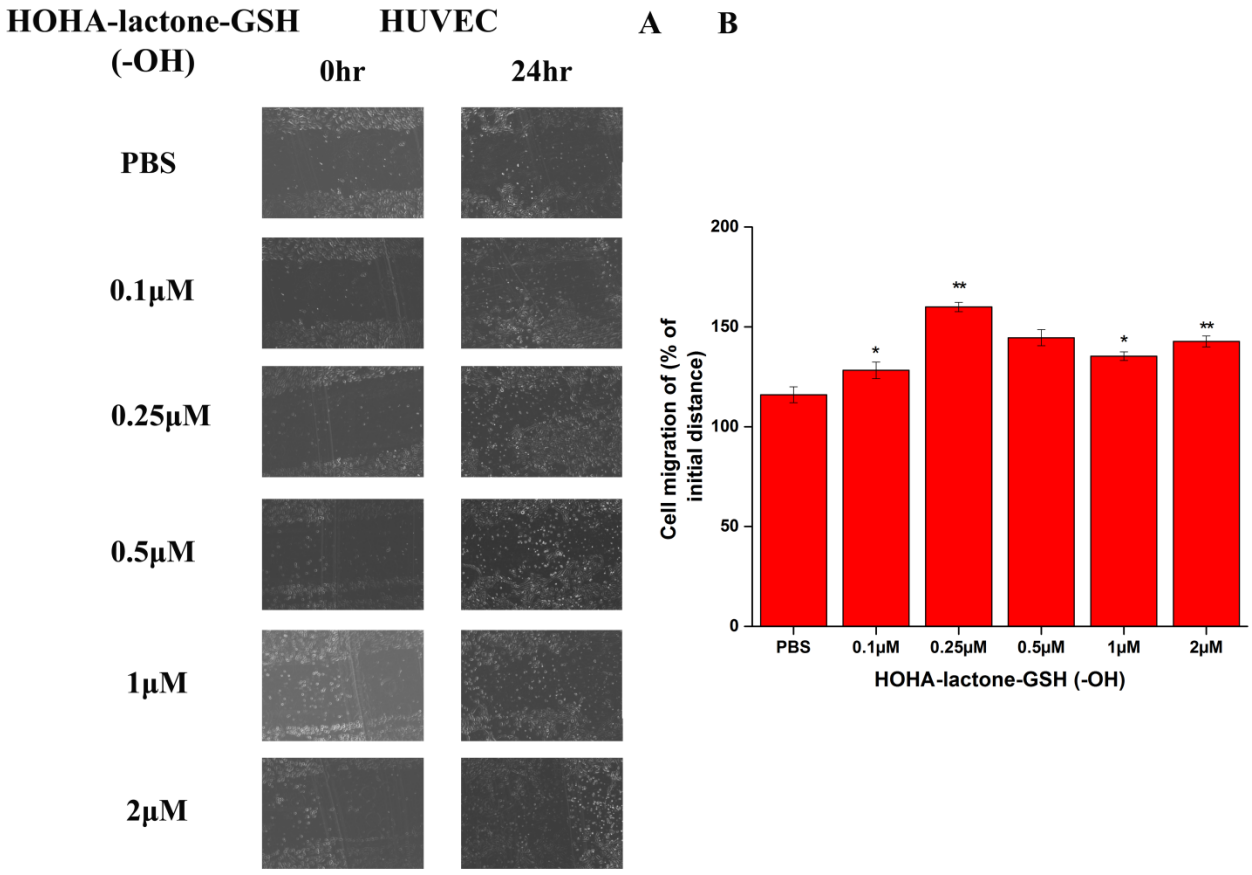

Figure S1. The pro-angiogenic effect of HOHA-lactone-GSH (-OH) on HUVECs in the wound healing assay. A: representative micrographs of cells with different treatment as indicted. B: quantification of wound healing assay. After HUVECs ($1x10^5$ cells in 300 µl of a cell culture medium) formed a monolayer, they were scratched with a 200 µL pipette tip. The cells were left in the medium for 24 h in the presence of various concentrations of HOHA-lactone-GSH (-OH) (0-2.0 µM). The images are representative of four independent experiments showing very similar results. The data in bar graph represents the mean ± SD (n = 4).

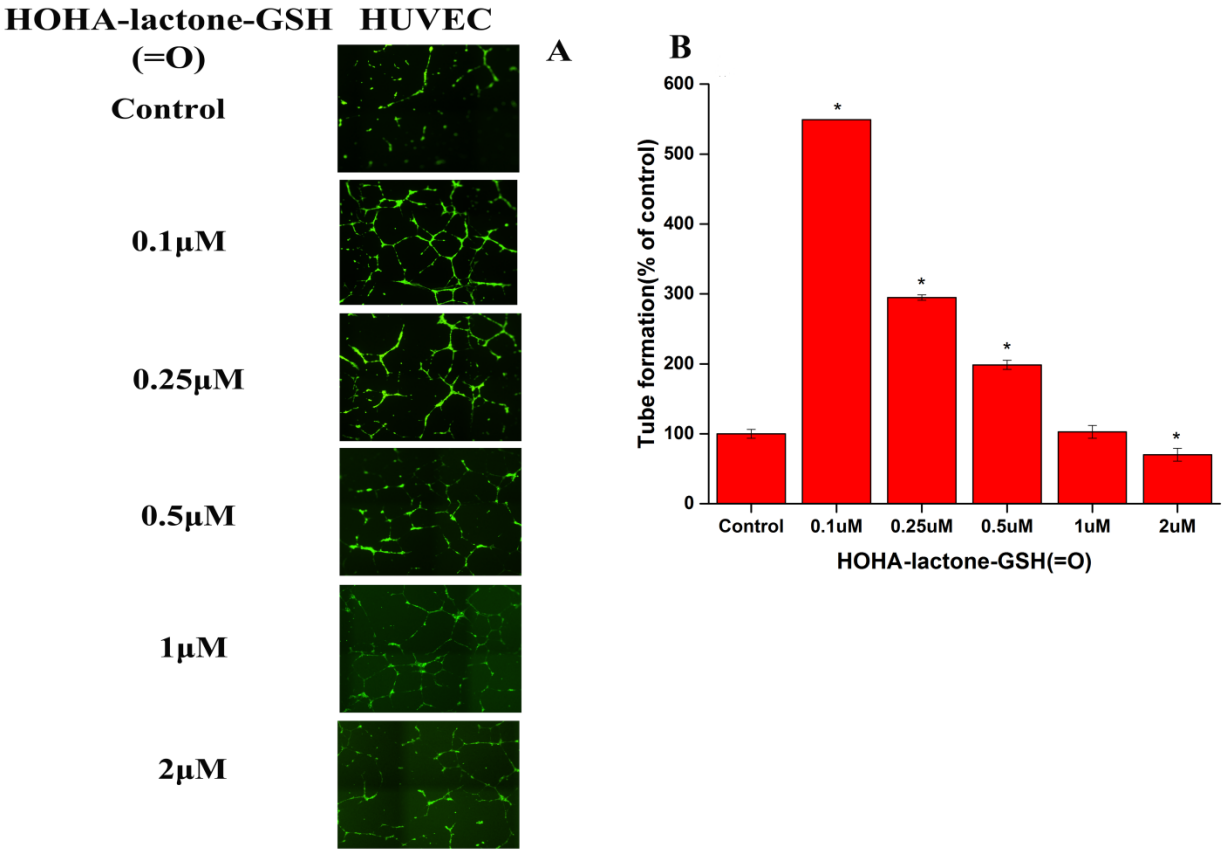

Figure S2. The pro-angiogenic effect of HOHA-lactone-GSH (=O) on HUVECs in the tube formation assay. A: representative micrographs of cells with different treatment as indicted. B: quantification of tube formation assay. HUVECs ($2.5x10^4$ cells/well) in 24-flat bottom tissue culture plate were allowed to grow on matrigel (175 µL) for 4 h in presence of various concentrations of HOHA-lactone-GSH (=O) (0-2.0 µM) for 16 h. The cells were stained with Calcein AM for 1 h at 37 °C in a CO_2 incubator. The images are representative of four experiments showing very similar results. The data in bar graph represents the mean ± SD (n = 4).

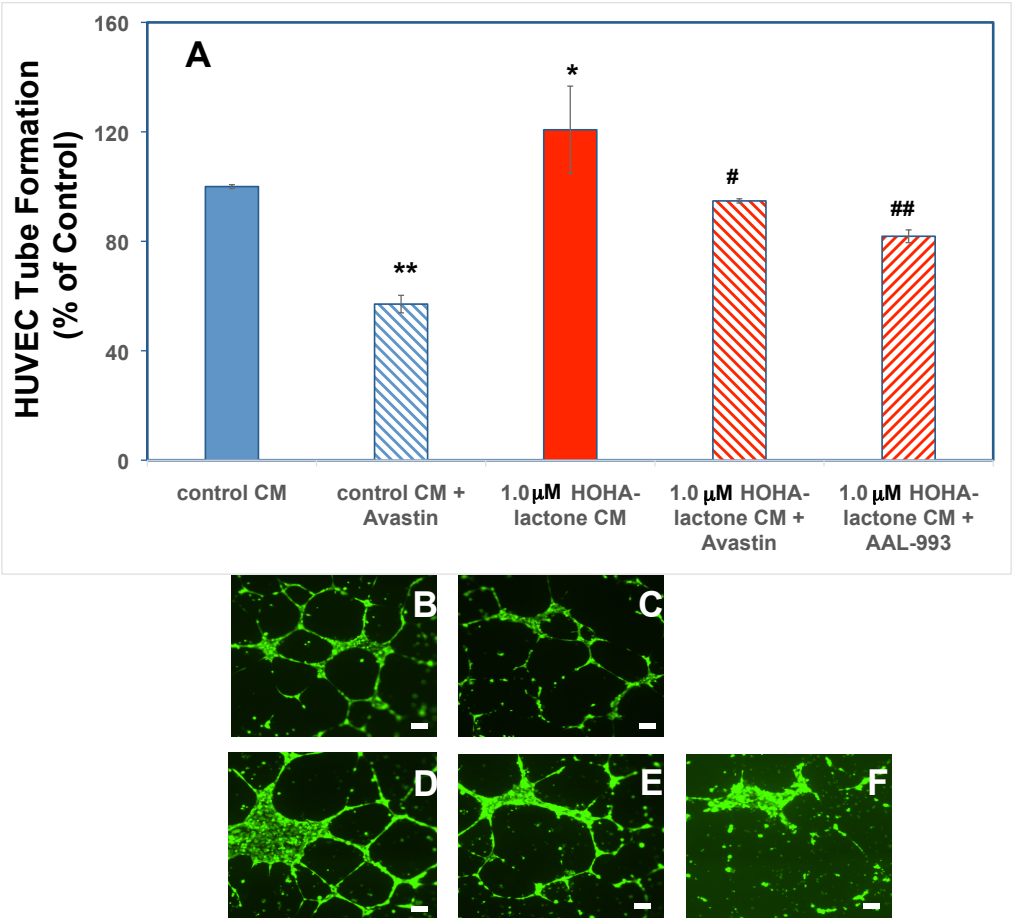

Figure S3. Effect of RPE conditioned medium (CM) on HUVEC in a tube formation assay in the absence and in the presence of Avastin (20 µg/ml) or AAL-993 (100 nM), panel A. Representative fluorescence images of cells with various treatments as indicated. HUVEC (2.0 x10^4 cells/well) in 24-flat bottom tissue culture plate were seeded on RGF-matrigel (175µL, approximately 15 mg/ml protein; 4 h) and then incubated with the CM, from ARPE-19 cells that had been challenged with 0 or 1 µM of HOHA-lactone, that had or had not been pre-treated with Avastin (3h pre-incubation at room temperature) or with AAL-993 VEGFR kinase inhibitor for another 16 h. The cells were stained with Calcein AM for 1 h at 37 °C in a CO_2 incubator. The images are representative of three experiments showing very similar results (B) HUVE cells incubated with control ARPE-19 CM; (C) control ARPE-19 CM pre-treated with Avastin (20 µg/mL); (D) 1 µM HOHA-lactone CM; (E) 1 µM HOHA-lactone CM pre-treated with Avastin; (F) 1 µM HOHA-lactone CM and AAL-993 VEGFR kinase inhibitor).The data in the bar graph represents the mean ± SD (n = 4); "**" $p<0.01$, "#"$p<0.01$, "##" $p<0.001$ (for 1.0 µM HOHA-lactone CM from ARPE-19 cells). Scale bars are 25.0 µm.