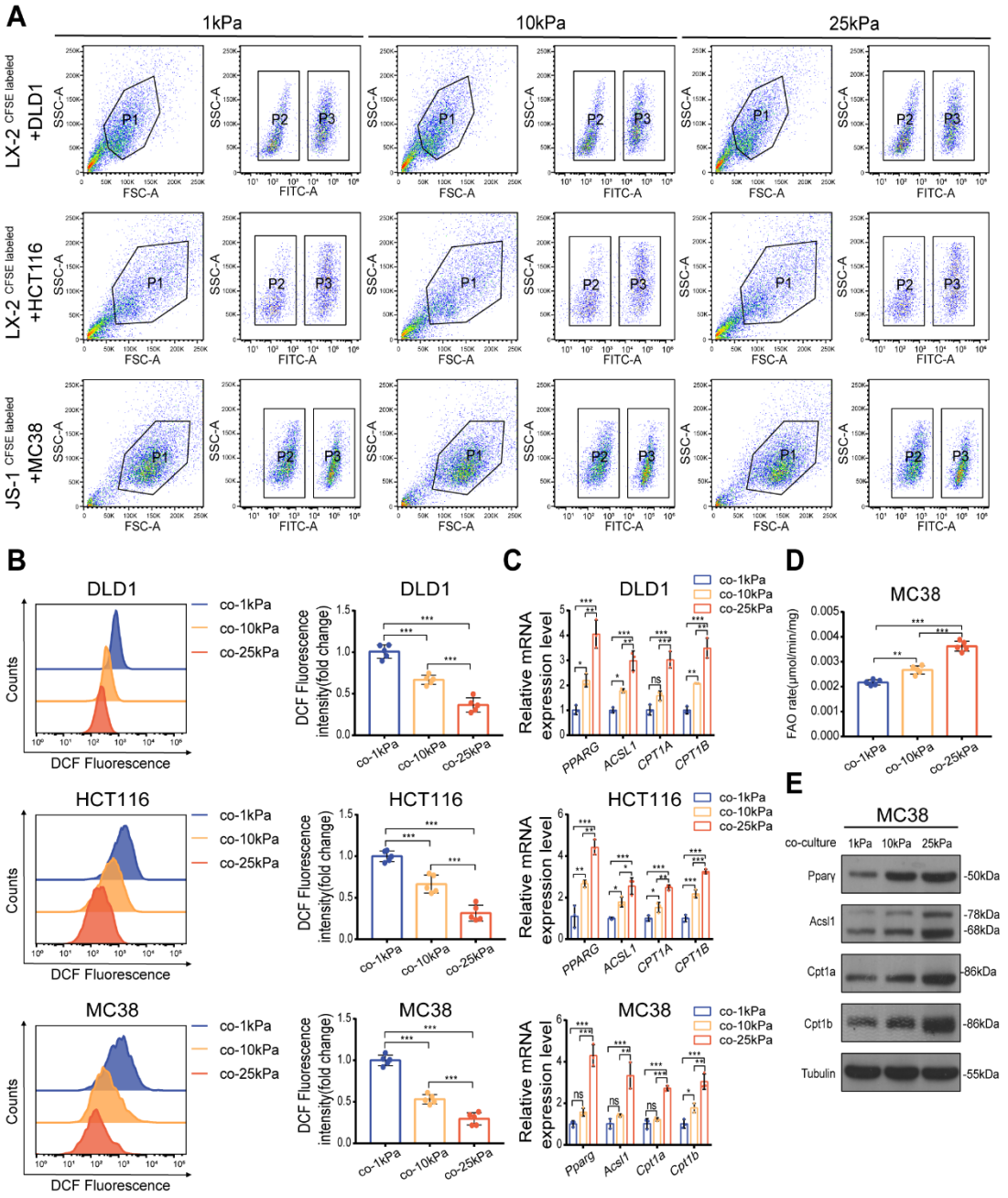
Supplementary Fig. S2



Supplementary Figure.S2 HSCs are essential for matrix stiffness-mediated FAO metabolic reprogramming in colon cancer cells. (A) DLD1 cells or HCT116 cells were mixed co-cultured with CFSE labelled LX-2 cells, MC38 cells were mixed co-cultured with CFSE labelled JS-1 cells on indicated substrates for 48 h. Colon cancer cells and HSCs in co-culture system were separated through flow cytometry. The left panel shows that colon cancer cells and HSCs are found in population 1 (P1), and the separated colon cancer cells (population 2; P2) and HSCs (population 3; P3) were detected with FITC signals. **(B)** ROS content in DLD1, HCT116 and MC38 cells co-cultured with HSCs on 1kPa,10kPa and 25kPa polyacrylamide hydrogels (one-way ANOVA, n=5 independent experiments). **(C)** Relative mRNA levels of FAO related genes in DLD1, HCT116 and MC38 cells co-cultured with HSCs on 1kPa,10kPa

and 25kPa polyacrylamide hydrogels (one-way ANOVA, n=3 independent experiments). **(D, E)** Fatty acid oxidation rate (**D**) and protein levels of FAO related genes (**E**) in MC38 cells co-cultured with JS-1 cells on 1kPa,10kPa and 25kPa polyacrylamide hydrogels (one-way ANOVA, n=5 independent experiments). Data are graphed as the mean ± SD. ns, not significant, $P>0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.