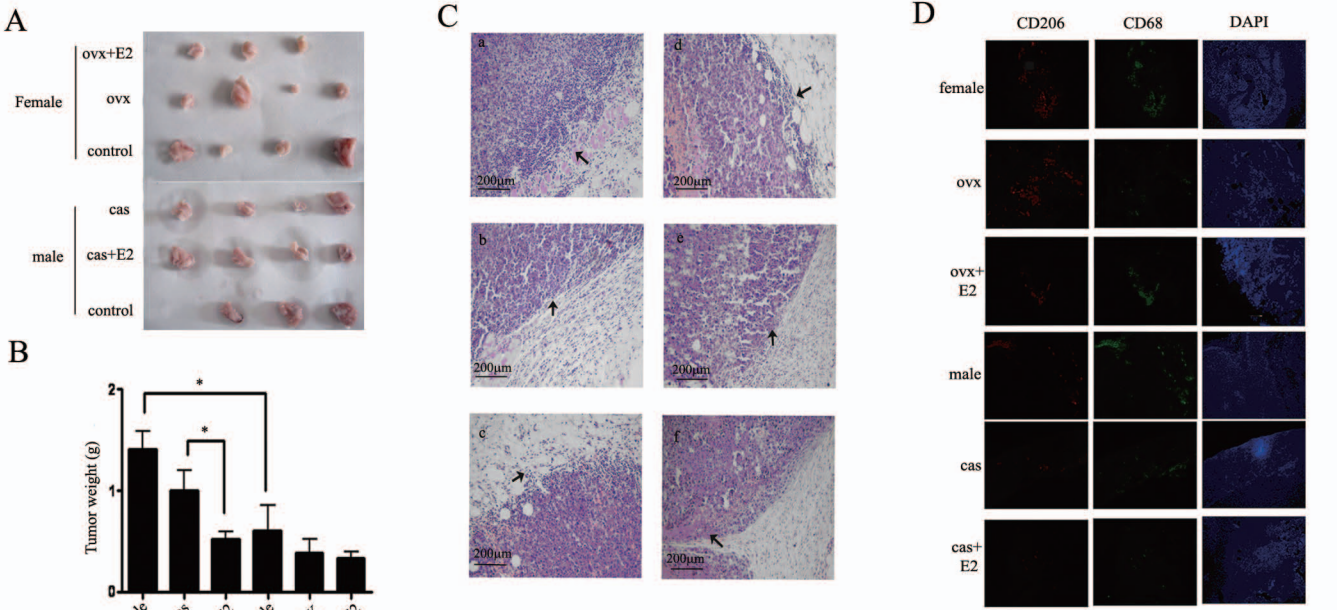

supplementary figure 5

Supplementary Figure 5. **Estrogen inhibits macrophage alternative activation in ectopic mouse liver tumor models.**

(A) The size of stripped tumor from ectopic mouse liver tumor models. Female: female control, ovariectomized female, ovariectomized female with E2 administration (50μg/kg); male: male control, castrated male, castrated male with E2 administration (50μg/kg). The tumor tissue from female group, OVX, and OVX with E2 group had less skin adhesion. However, the tumor tissue from control male groups had relatively large skin adhesion, and more angiogenesis was found within the tumor. Castrated male and castration with E2 treatment group had less skin adhesion, regular shape and no significant angiogenesis internal in comparison to male control group. **(B)** Measurement of stripped tumor weights. Values were expressed as means±SEM. (n=6 * $P<0.05$). **(C)** Histological features of representative xenotopic liver tumors from mice in each group. Female: **(a)** female control, **(b)** underwent ovariotomy, **(c)** E2 (50 μg/kg) administration after ovariotomy; male: **(d)** male control, **(e)** underwent castration, **(f)** E2 (50 μg/kg) administration after castration. Inflammatory cell infiltration was demonstrated (black arrows). In each group, tumor cell displayed a fairly homogeneous distribution behavior. Meanwhile, the tumor tissue had more mononuclear inflammatory cell infiltration than that in orthotopic, but no significant difference among groups. (magnification, 100×). **(D)** The activation state of macrophages in tumor-bearing mice was determined by immunofluorescence staining. Sections of 8-week-old ectopic hepatocarcinoma mouse livers were immunolabeled with anti-mouse CD68 (FITC conjugated, green) to identify macrophage populations. Anti-mouse CD206 (PE conjugated, red) were used to label alternative activated macrophage. DAPI (blue) was used to mark nucleus. (magnification, 100×).

Macrophages within tumor tissue existed as a $CD206^+$ state. The macrophages number in ectopic tumor mouse was more than that in situ tumors mouse, but there was no significant change among each group of ectopic tumor mice. In male mice, male control, castrated male mice and castration with estrogen treatment group, the number of $CD206^+$ macrophage had no significant change. However, administration of estrogen after ovariectomy could reduce the number of $CD206^+$ macrophages.