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## Supplemental information

# Cardamonin suppresses pro-tumor function of macrophages by decreasing M2 polarization on ovarian cancer cells via mTOR inhibition

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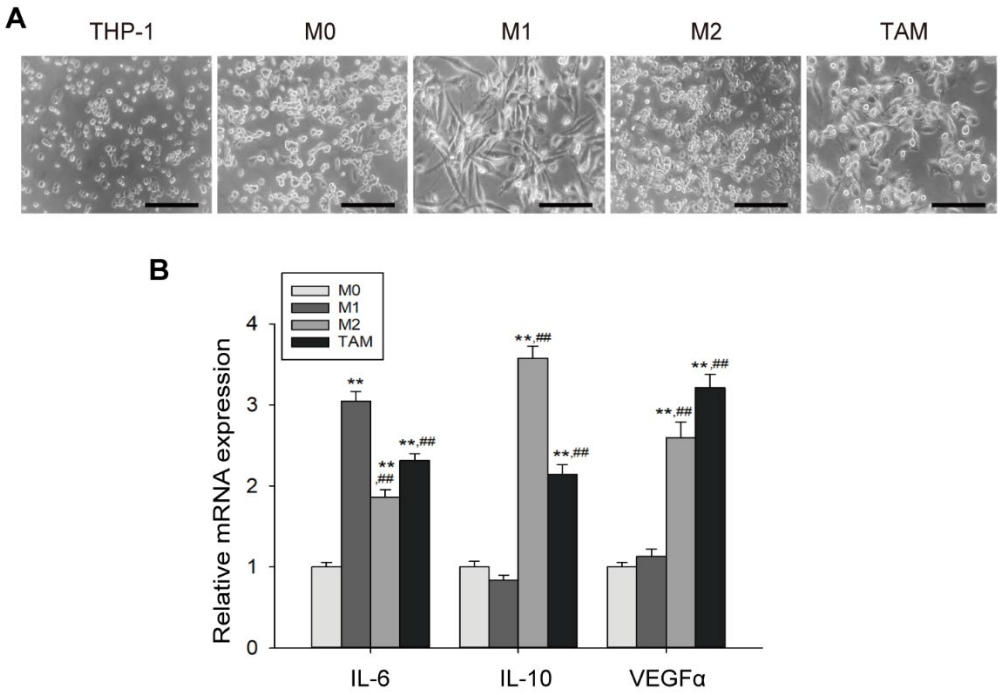


**Figure S1. THP-1 monocytes were induced to different phenotypes of macrophages.** (A) Morphology of THP-1 induced macrophages were captured under an inverted microscope (scale bars, 50 μm). (B) mRNA expression of IL-6, IL-10 and VEGFα was measured by Real-time PCR. Relative mRNA expression was normalized to Actin. Data is presented as the mean ± SD (n=3). $^{**}p < 0.01$ vs. M0. $^{\#\#}p < 0.01$ vs. M1.

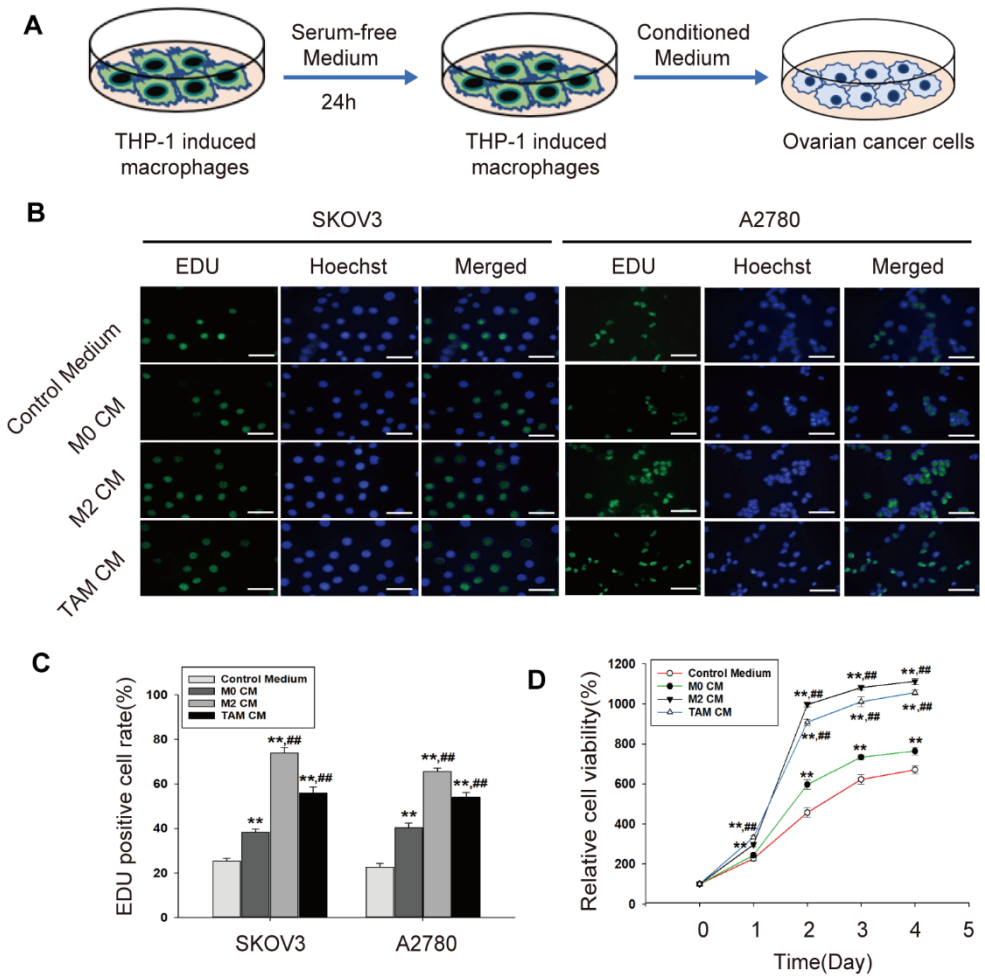


**Figure S2. Conditioned medium from THP-1 induced macrophages promoted the proliferation of SKOV3 cells and A2780 cells.** (A) Schematic diagram of conditioned medium (CM) collection. (B, C) Cell proliferation was measured by EdU test (scale bars, 100 μm). (D) Cell viability was measured with CCK-8 assay. Quantitative is relative to each group of 0 hours, set to 100%. Data is presented as the mean ± SD (n=3). $^{**}p < 0.01$ vs. Control Medium. $^{\#\#}p < 0.01$ vs. M0 CM.

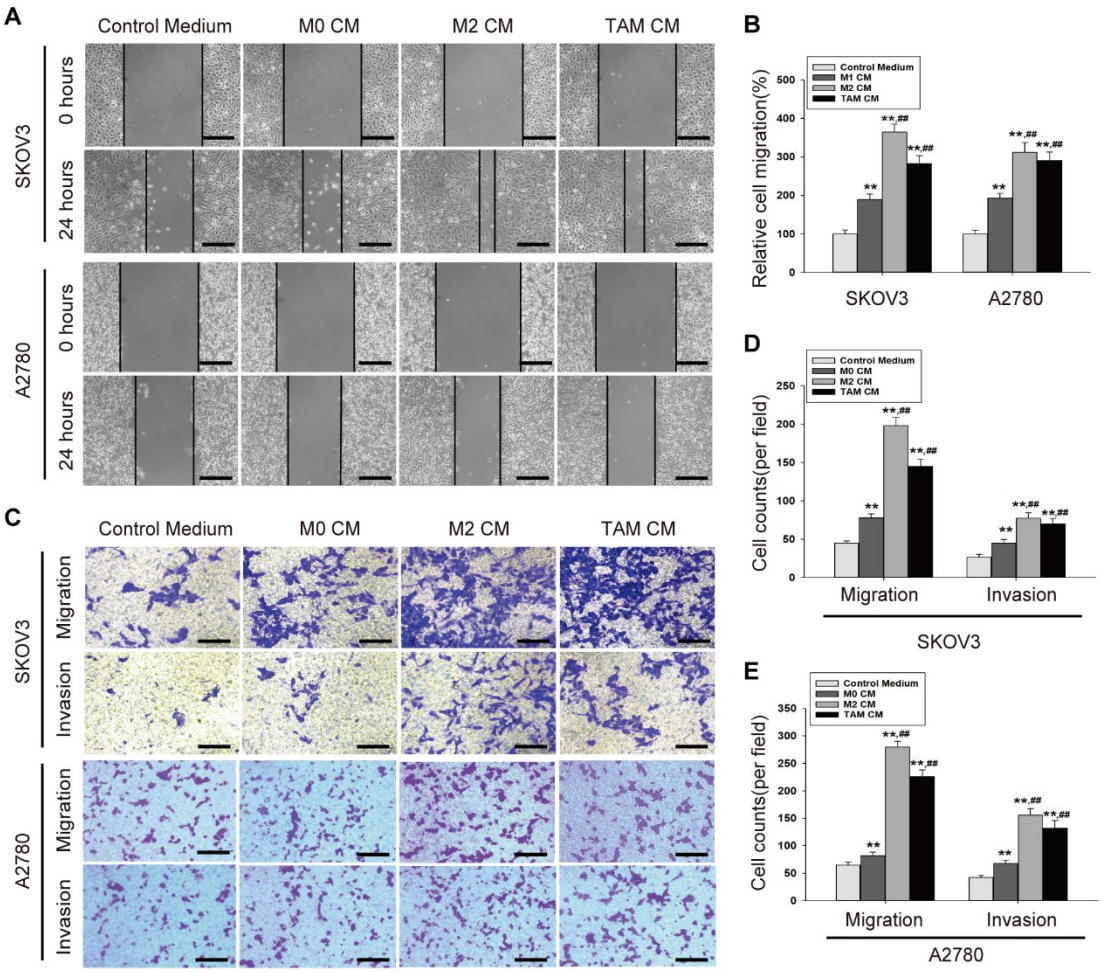


**Figure S3. Conditioned medium from THP-1 induced macrophages promoted the migration and invasion of SKOV3 cells and A2780 cells.** (A, B) Cell migration was measured by Scratch test (scale bars, 100 μm). Quantification was relative to the group cultured with control medium, set as 100%. (C-E) Cell migration and invasion were evaluated by Transwell assays (scale bars, 100 μm). SKOV3 cells and A2780 cells were seeded in the upper chamber, respectively. The specified CM was added to the lower part of the well. Data is presented as the mean ± SD (n=3). $^{**}p < 0.01$ vs. Control Medium. $^{\#\#}p < 0.01$ vs. M0 CM.

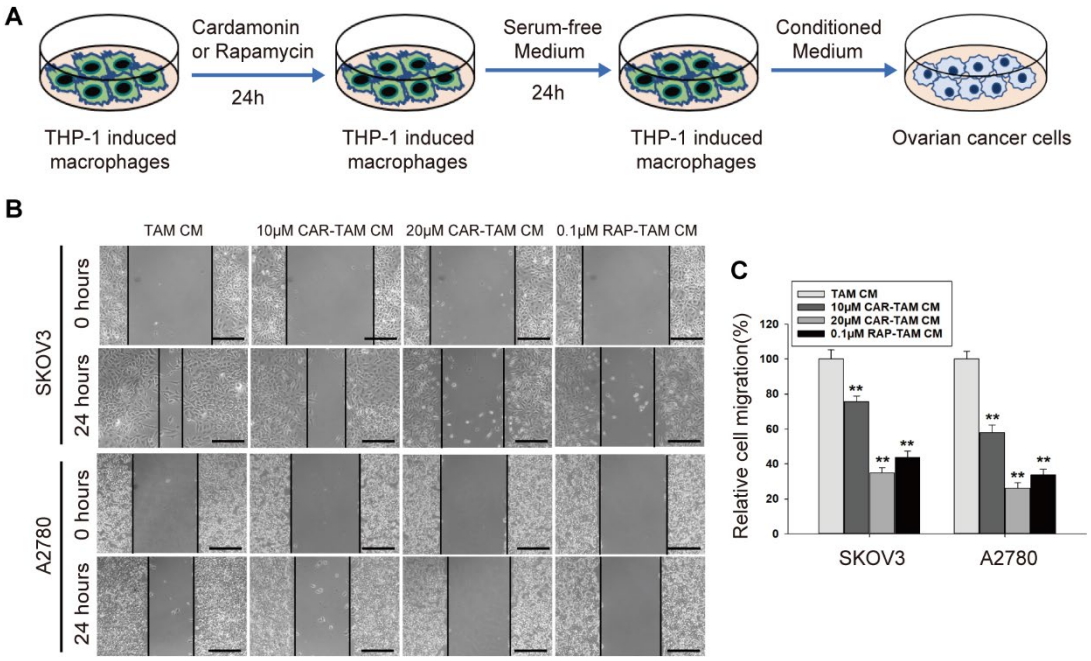


**Figure S4. Conditioned medium from cardamonin pretreated TAMs decreased the migration of SKOV3 cells and A2780 cells.** (A) Schematic diagram of CM collection. (B) Cell migration was measured by Scratch test. Images were captured under an inverted microscope (scale bars, 100 μm) at 0 and 24 h, respectively. Quantification was relative to the group cultured with TAM CM, set as 100%. Data is presented as the mean ± SD (n=3). $^{**}p < 0.01$ vs. TAM CM.

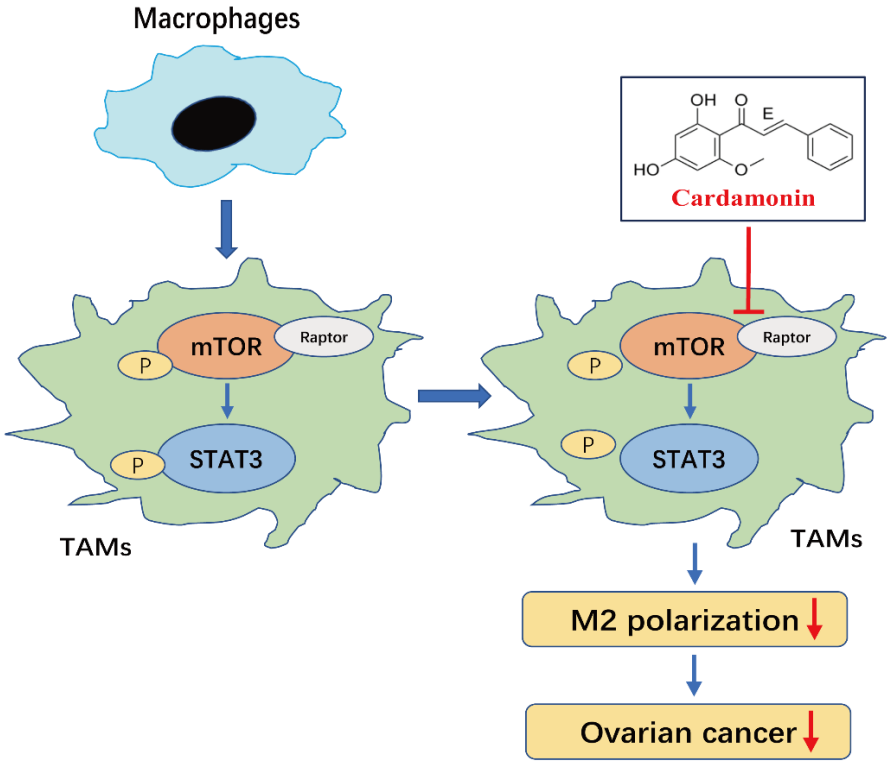


**Figure S5.** Schematic model of TAMs regulation by cardamonin. Macrophages were induced to TAMs via mTOR and STAT3 activation in tumor microenvironment. Cardamonin suppressed Raptor and phosphorylation of mTOR, thus reducing phosphorylation of STAT3, which contributed to M2 polarization inhibition and TAMs pro-tumor function impediment, ultimately leading to a decreased proliferation and migration of ovarian cancer cells. mTOR, mammalian target of rapamycin; Raptor, regulatory associated protein of mTOR; STAT3, signal transducer and activator of transcription 3; TAMs, tumor-associated macrophages.