Supplementary figure legends

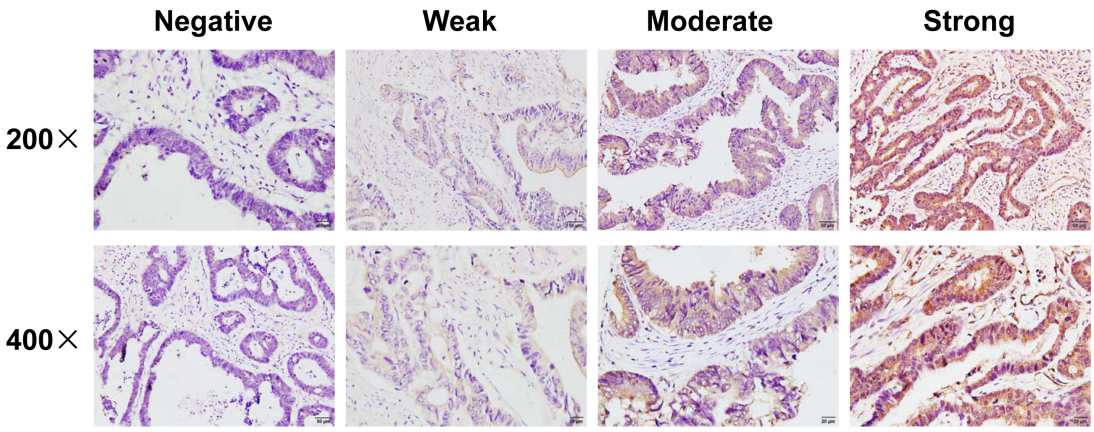

Figure S1. The IHC staining was scored semi-quantitatively based on the staining intensity of positive tumor cells. Negative (–) and weak (+) samples were scored as low DKK2, while moderate (++) and strong (+++) samples were scored as high DKK2.

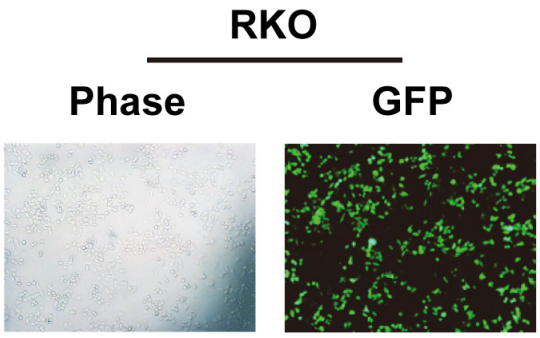

Figure S2. RKO cells were infected with DKK2 overexpressed LV-DKK2 and the transfection efficency was assessed by detecting the expression of GFP under fluorescence microscope.

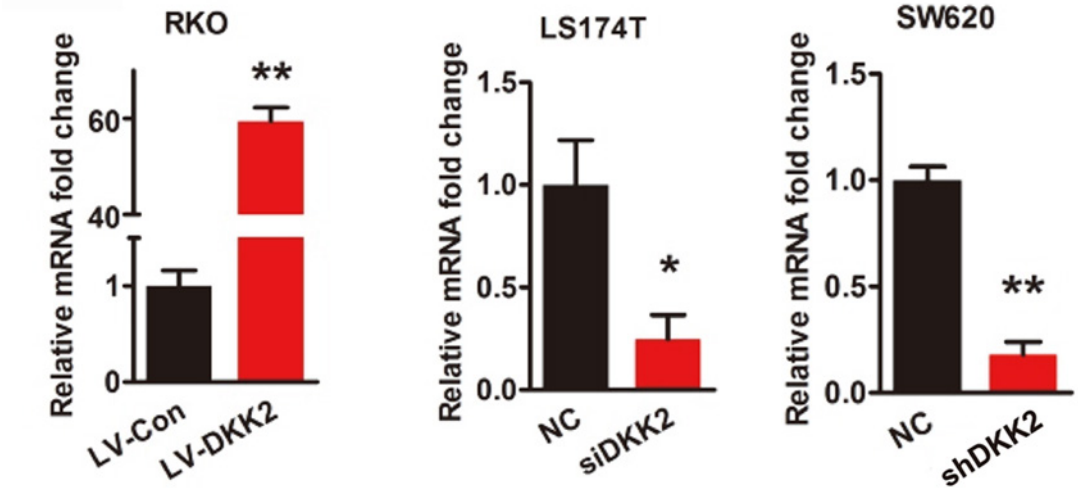

Figure S3. Relative expression of DKK2 was detected by real-time qPCR in indicated cells to confirm the successful construction of DKK2 overexpression and silencing vectors (mean ± SD, n = 3). The asterisk (*) indicates $P < 0.05$. The asterisk (**) indicates $P < 0.01$.

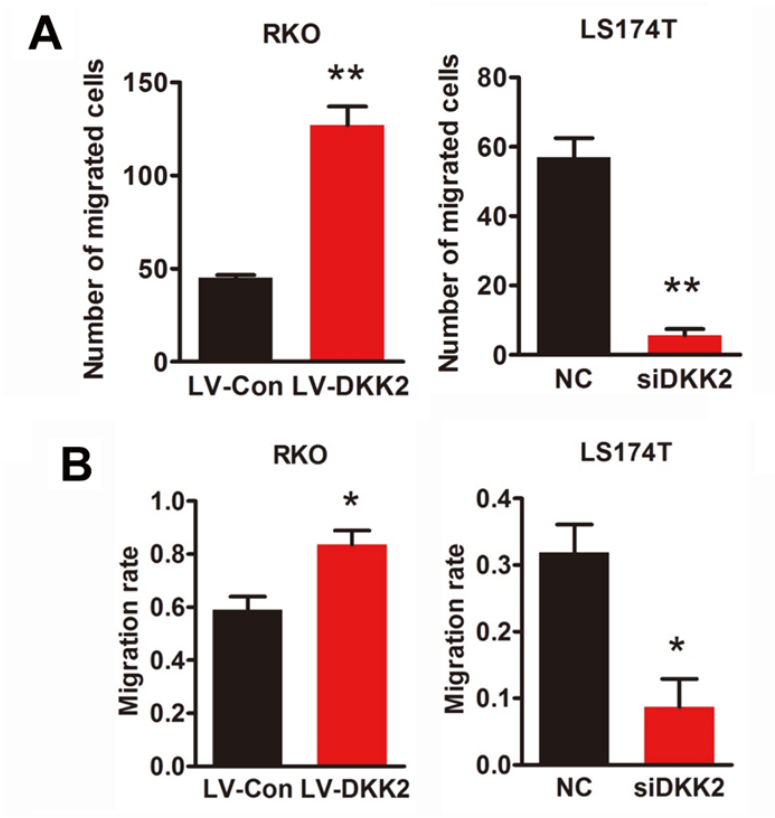

Figure S4. (A) The representative figures of cell migration were measured by transwell migration assay using LV-DKK2 RKO cells and siDKK2 LS174T cells. Bars represent the number of migrated cells. (B) The representative figures of cell migration were measured by wound-healing assay using LV-DKK2 RKO cells and siDKK2 LS174T cells. Bars represent the migration rate.

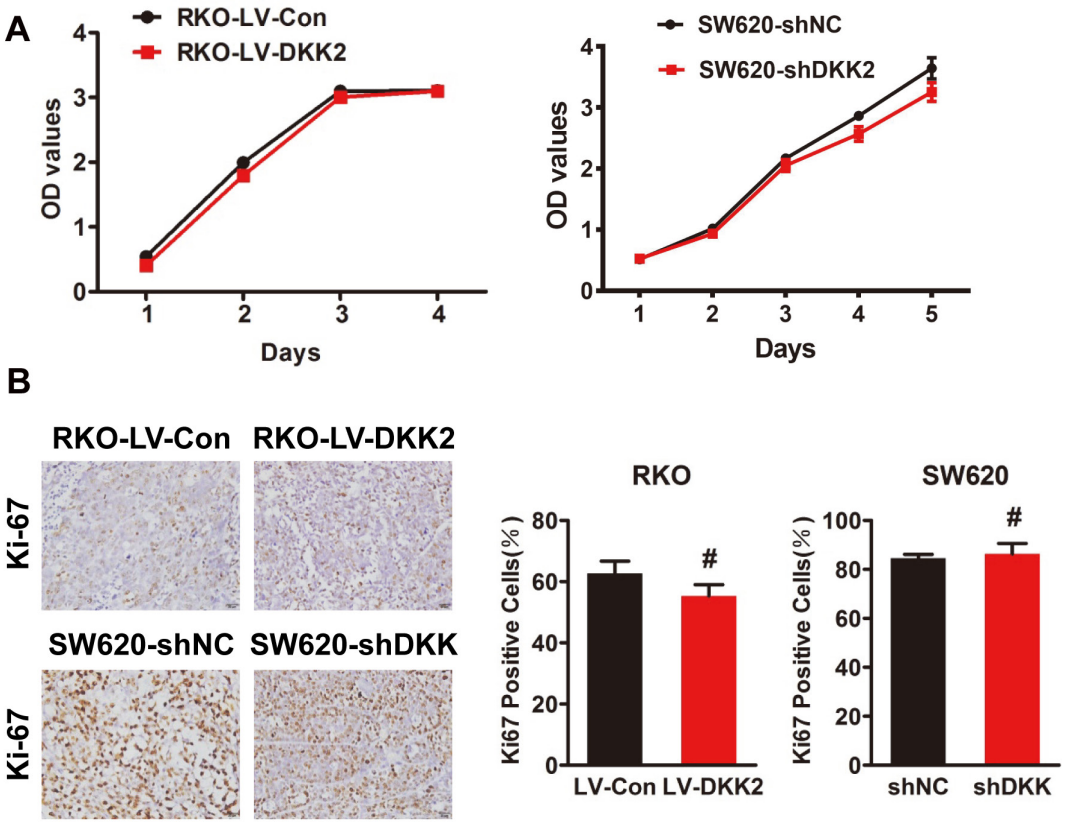

Figure S5. (A) CCK-8 assay was used to detect the effects of DKK2 on CRC cell proliferation (mean ± SD, n = 3). (B) The representative IHC images of Ki-67 index in DKK2 overexpressing or silenicng subcutaneous tumors. (#) indicates P > 0.05. Bars in the right panel represents the rate of Ki-67 positive cells.

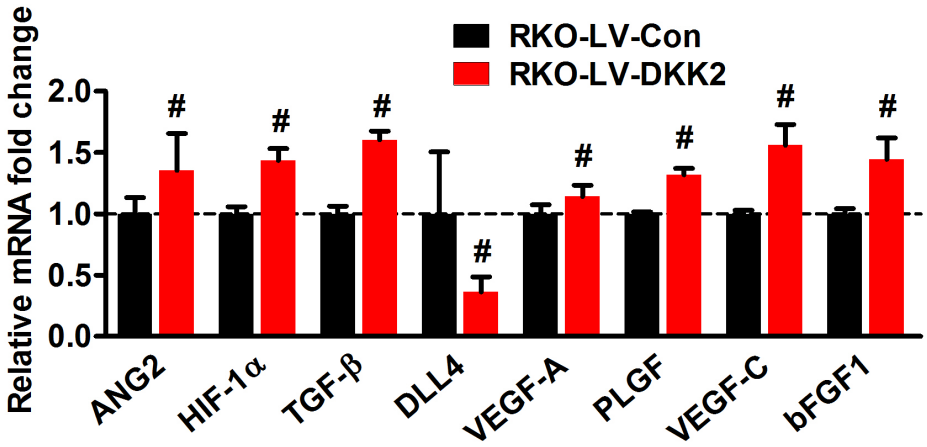

Figure S6. Real-time qPCR detects the effects of DKK2 on the expression of classical angiogenic factors including ANG2, HIF1α, TGFβ, DLL4, VEGF, PLGF and bFGF-1 in indicated cells (mean ± SD, n = 3). (#) indicates $P > 0.05$.

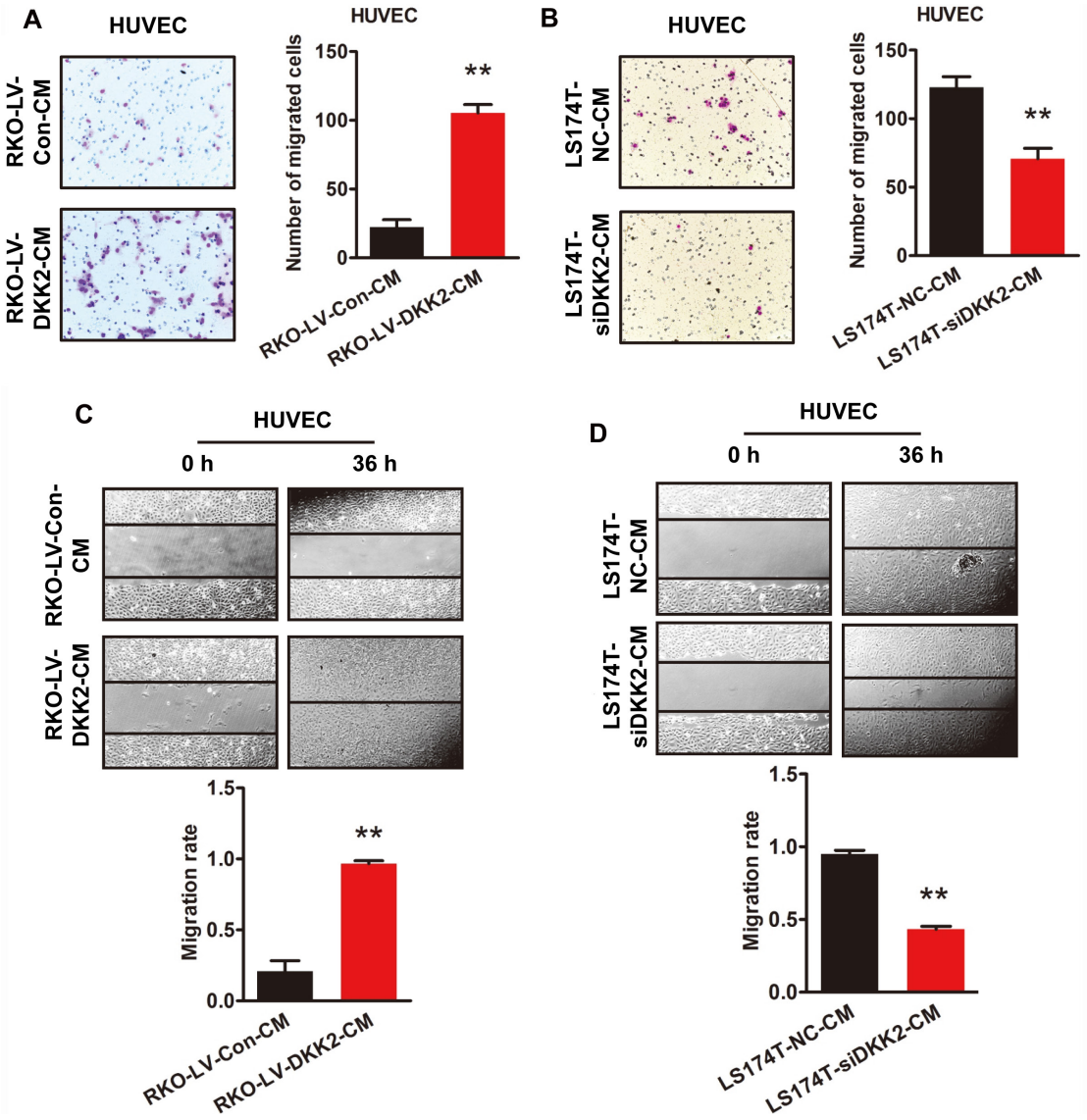

Figure S7. (A) The representative figures of HUVECs migration treated with DKK2 overexpressed RKO culture medium was measured by transwell assay. Bars in the right panel represent the number of migrated cells (mean ± SD, n=3). (B) The representative figures of HUVECs migration treated with DKK2 silencing LS174T culture medium were measured by transwell assay. Bars in the right panel represent the number of migrated cells (mean ± SD, n=3). (C&D) The representative figures of HUVECs migration treated with DKK2 overexpressed RKO (C) and DKK2 silenicng LS174T (D) culture medium were measured by wound-healing assay. Bars in the right panel represent the migration rate (mean ± SD, n=3).

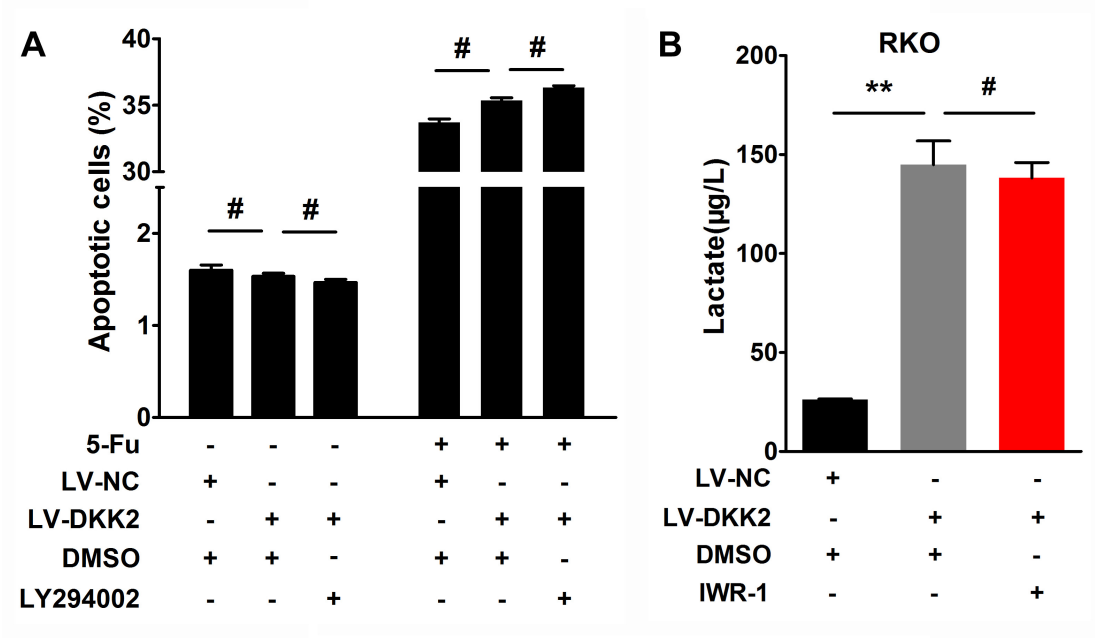

Figure S8. (A) The apoptosis of control and DKK2 overexpressed RKO cells were detected by flow cytometry with or without the absence of 5-Fu and LY294002 treatment (mean ± SD, n = 3), (#) indicates $P > 0.05$. (B) The secretion of lactate in the culture medium of control and DKK2 overexpressed RKO cells with or without the absence of IWR-1 (mean ± SD, n = 3), (**) indicates $P < 0.01$.

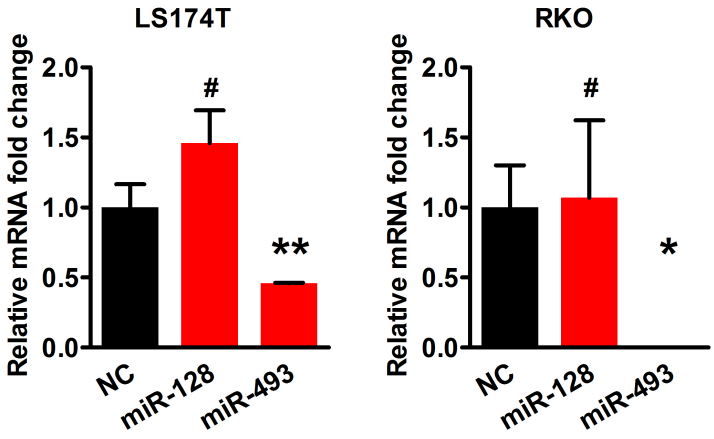

Figure S9. Real-time PCR analysis of DKK2 expression in control, miR-493-5p, and miR-128 transfected LS174T and RKO cells (mean ± SD, n = 3). (*) indicates $P < 0.05$, (**) indicates $P < 0.01$. Only miR-493-5p was verified as an upstream regulator of DKK2.

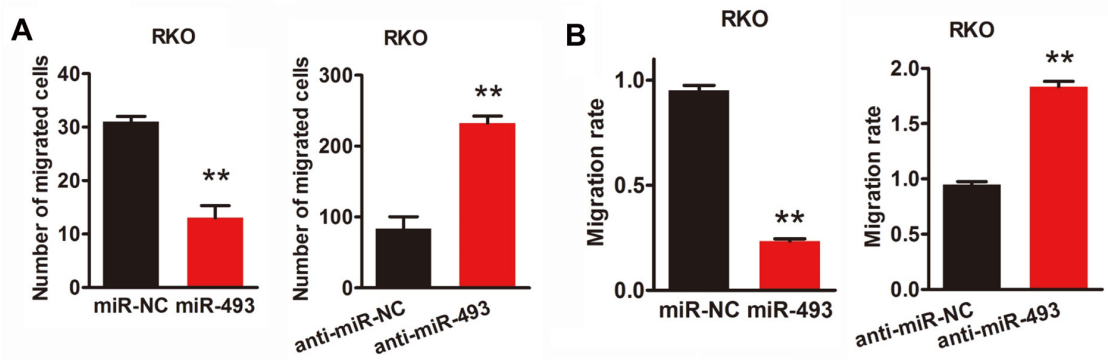

Figure S10. (A) Transwell assay on miR-493-5p overexpressing or silencing RKO cells. Bars of the right panel represent the number of invaded cells (mean ± SD, n = 3). (B) Wound-healing assay on miR-493-5p overexpressing or silencing RKO cells. Bars of the right panel represent the migration rate (mean ± SD, n = 3).

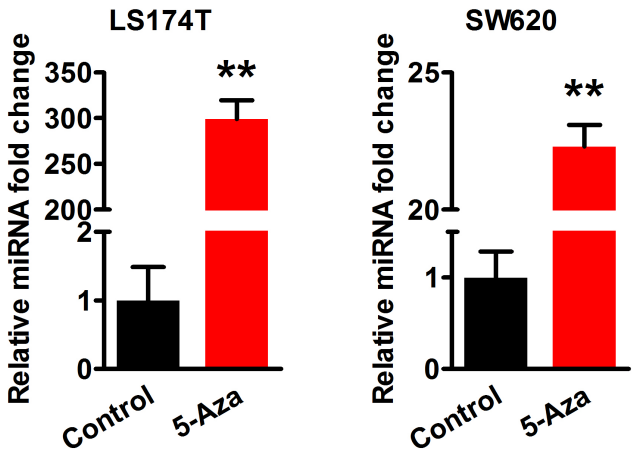

Figure S11. Real-time PCR analysis of miR-493-5p expression in control and 5-Aza treated LS174T and SW620 cells (mean ± SD, n = 3). (**) indicates $P < 0.01$.