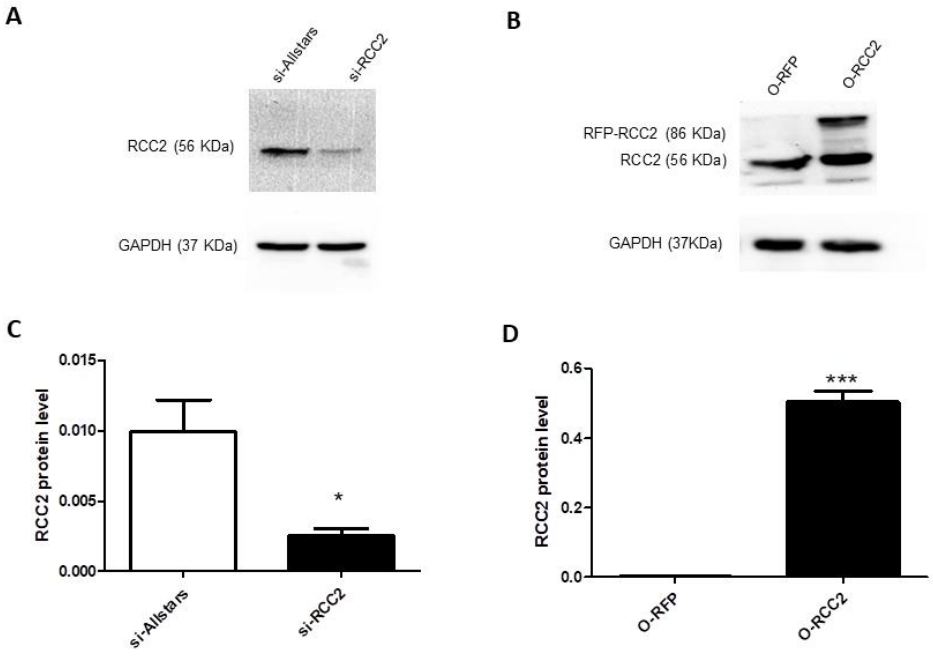
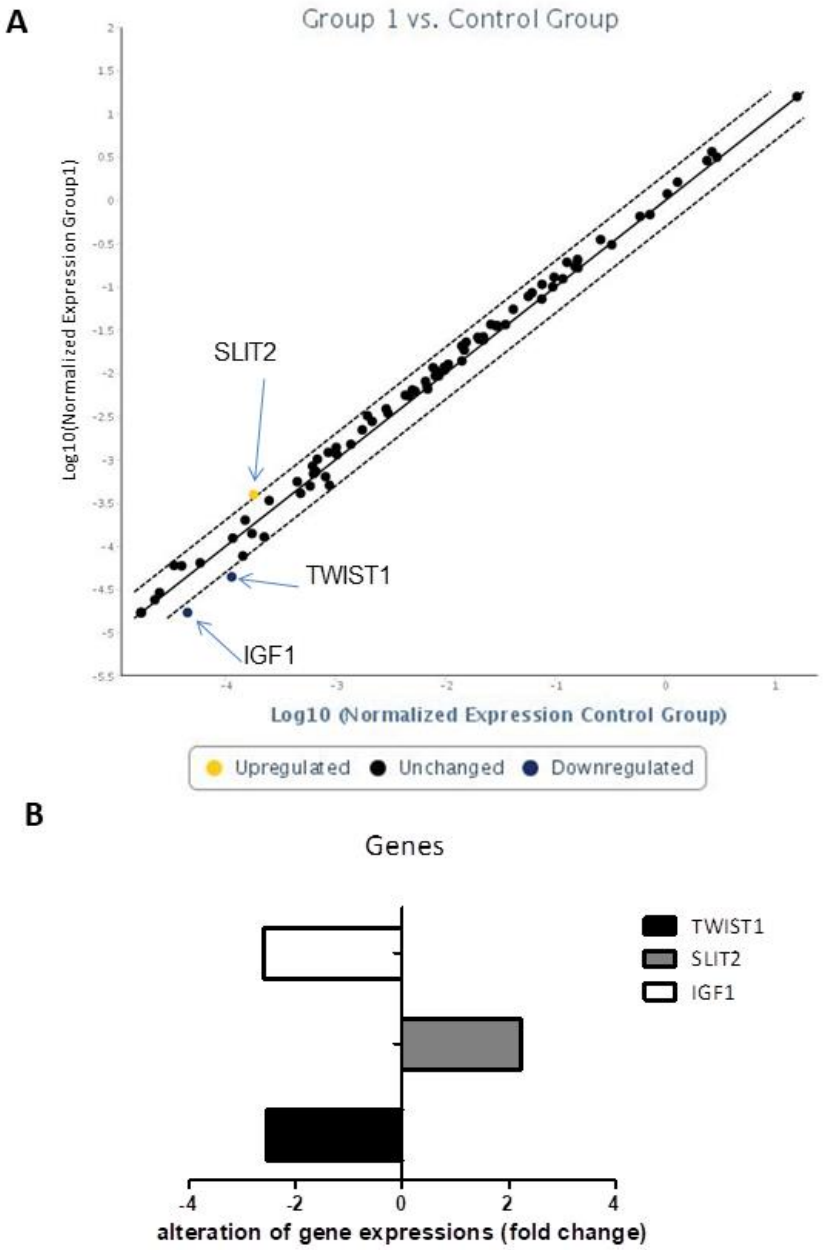
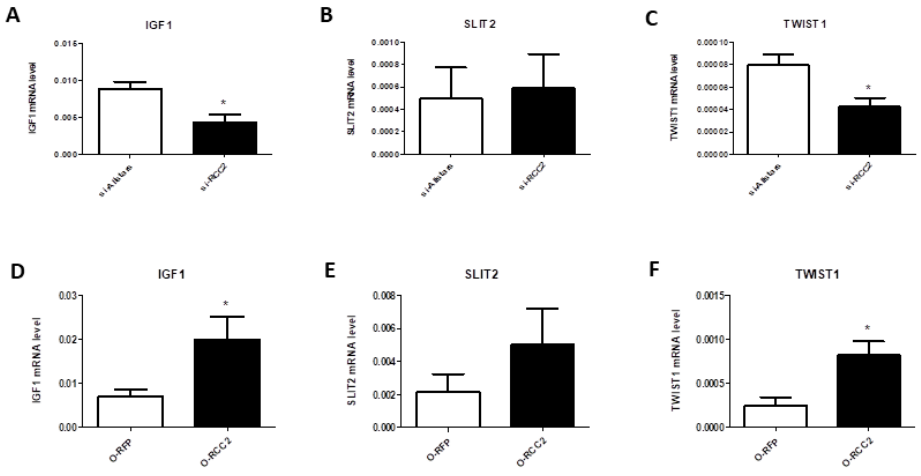


Additional file 1. Expression of RCC2 expression in MCF-7 cells as assessed by Western blot analysis. MCF-7 cells were cultured and transfected with (**A**) anti-RCC2 siRNA (si-RCC2) or (**B**) RCC2-expressing plasmids (O-RCC2). Allstars siRNA (si-Allstars) and RFP-expressing plasmids (O-RFP) were used as the respective controls. Following normalization by GAPDH expression, Western blot analysis detected a significant decrease (**C**) and increase (**D**) in RCC2 expression in MCF-7 cells transfected with anti-RCC2 siRNA or RCC2-expressing plasmid, respectively, compared with the corresponding controls. *$p<0.05$.

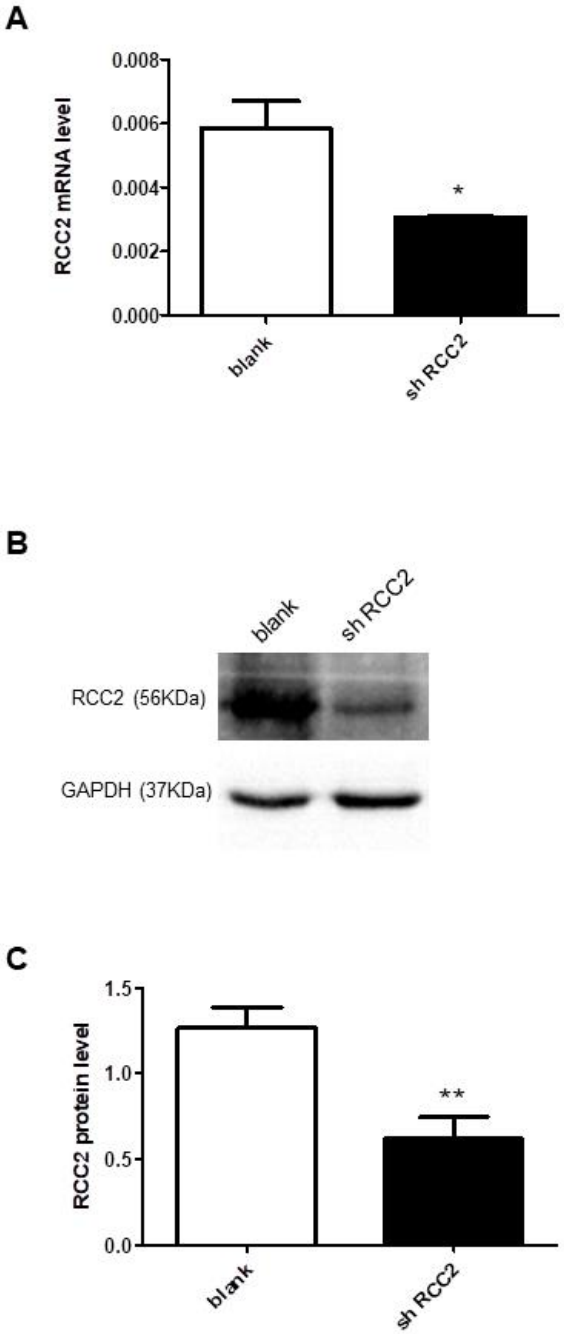


Additional file 2. Determination of the downstream pathway of RCC2 in MCF-7 cells by means of human breast cancer PCR arrays. (**A**) MCF-7 cells were transfected with anti-RCC2 siRNA, and cells transfected with Allstars siRNA were used as a negative control. Fold changes were calculated and expressed as log-normalized ratios

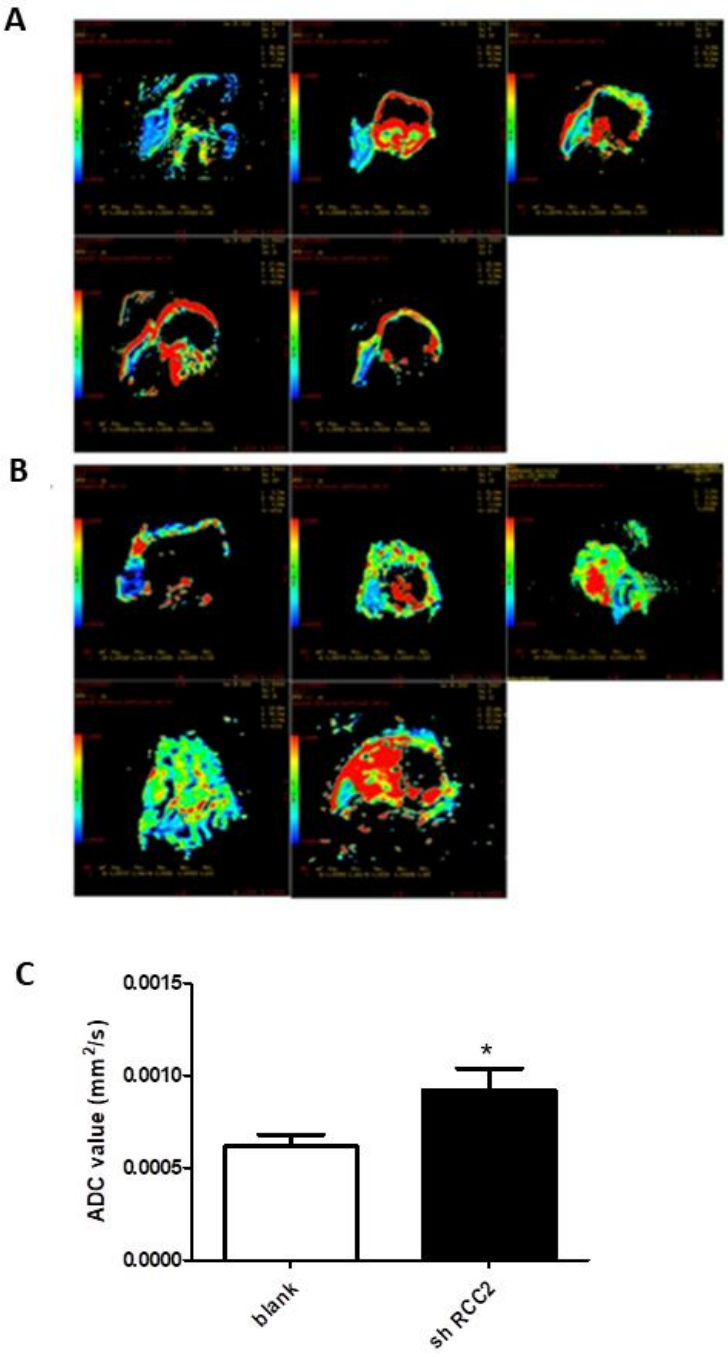
of the siRNA-transfected cells/controls. Genes with at least a 3-fold change in expression were considered to be biologically significant and are shown. (**B**) The result is depicted in a histogram.



Additional file 3. Assessment of the transcription levels of IGF1, SLIT2 and TWIST1 in MCF-7 cells using real-time PCR. MCF-7 cells were transfected with anti-RCC2 siRNA (si-RCC2) (**A, B, C**) or RCC2-expressing plasmids (O-RCC2) (**D, E, F**). Cells transfected with Allstars siRNA (si-Allstars) and RFP-expressing plasmids (O-RFP) were used as the corresponding negative controls. *p<0.05, **p<0.01 and ***p<0.001.

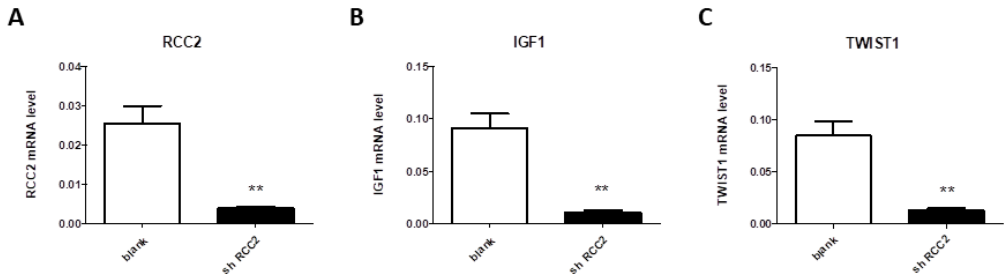


Additional file 4. RCC2 mRNA and protein levels in MCF-7 cells as assessed by real-time PCR and Western blot analysis. MCF-7 cells were infected with anti-RCC2 shRNA lentiviral vector (shRCC2) or empty lentiviral vector (blank). *p<0.05, and **p<0.01.

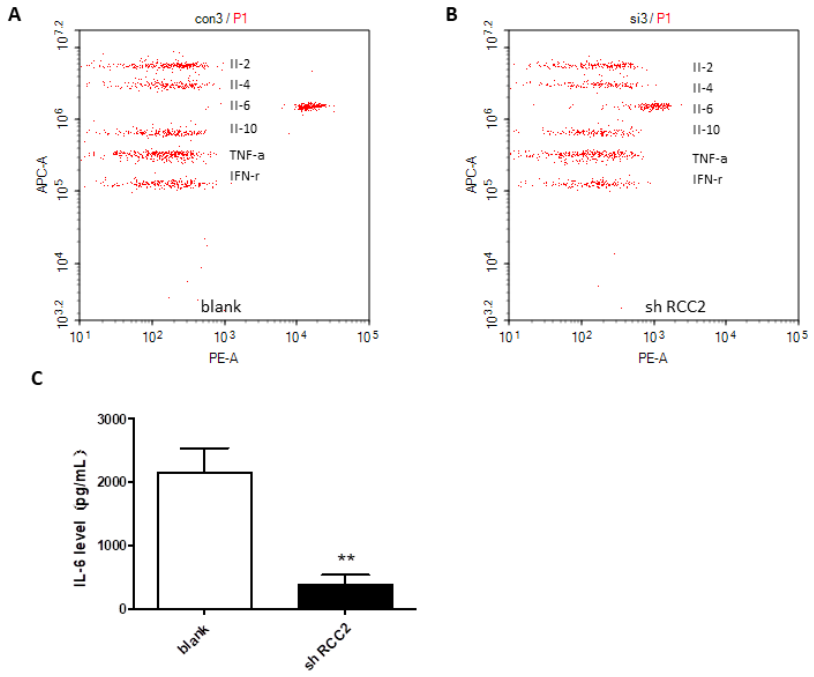


Additional file 5. Effect of RCC2 expression on tumor growth in tumor-bearing mice as assessed by MRI. (**A**) Mice injected with empty vector-infected MCF-7 cells were used as controls (blank). (**B**) MCF-7 cells infected with anti-RCC2 shRNA were injected into nude mice to generate tumor-bearing mice (shRCC2). Sagittal

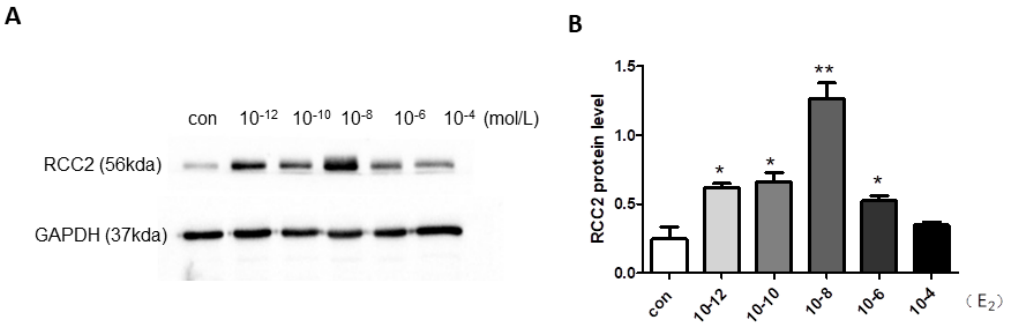
T2-weighted MRI (T2WI) analysis of the tumors was conducted on the 28th day after injection. (**C**) The analytical result is presented in a bar graph. *p<0.05.



Additional file 6. Expression levels of IGF1, RCC2 and TWIST1 in tumors from xenograft mouse models as assessed by real-time PCR. The mice were injected with MCF-7 cells infected with lentivirus containing anti-RCC2 shRNA (shRCC2) or empty vectors (blank). *p<0.05.



Additional file 7. Measurement of cytokine production in sera from tumor-bearing mice. Flow cytometric analysis was used to measure the IL-2, IL-4, Il-6, IL-10, TNF-α and IFN-γ levels in the serum. Serum was obtained from mice bearing tumors originating from MCF-7 cells infected with lentivirus containing anti-RCC2 shRNA (shRCC2) (**A)** or empty vectors (blank) **(B**). (**C**) ELISA was used to measure the IL-6 levels in the serum. **p<0.01.



Additional file 8. Effect of estrogen on RCC2 expression. MCF-7 cells were incubated with estradiol-17β (E_2) at concentrations from 10^{-4} to 10^{-12} mol/L. RCC2 expression was detected using Western blotting (**A**), and the expression was normalized to GAPDH expression (**B**).