

C-kit induces epithelial–mesenchymal transition and contributes to salivary adenoid cystic cancer progression – Tang et al

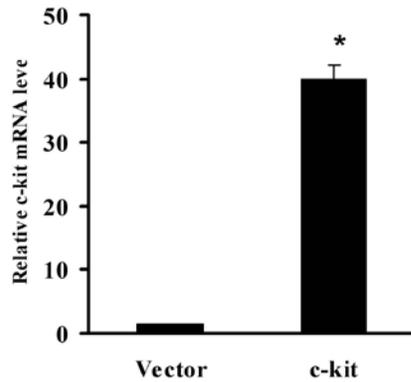


Figure S1: The c-kit mRNA level in ACC-M cells. The c-kit mRNA level was quantified by real-time PCR ($*p<0.001$, compared to the control).

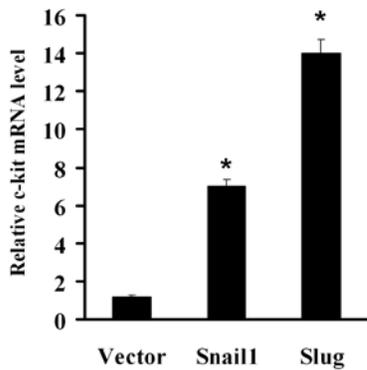


Figure S2: Induction of c-kit by other EMT-inducers. Expression of c-kit mRNA in ACC-M cells ectopically expressing either empty vector or the indicated EMT-inducing genes was assessed by real-time PCR ($*p<0.001$, compared to the control).

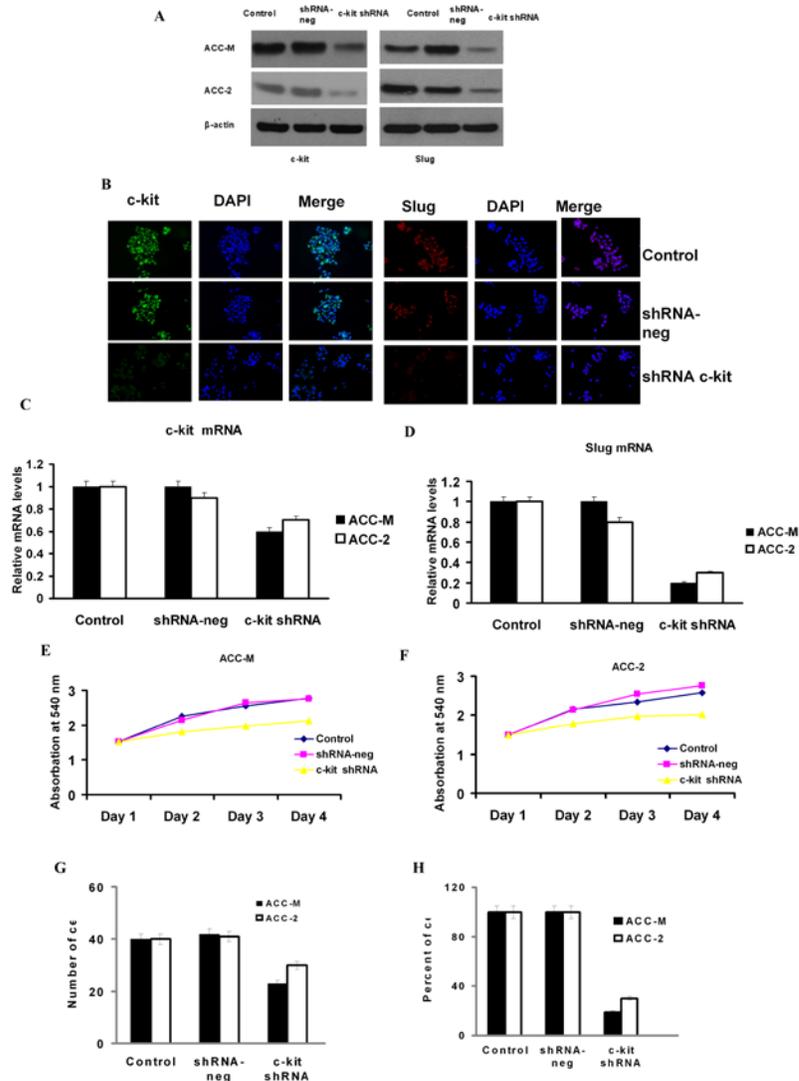


Figure S3: Silencing of c-kit in ACC-M and ACC-2 cells inhibited their invasive ability. A), Expression of c-kit and Slug protein after c-kit silencing in ACC-M and ACC-2 cells. B), Immunofluorescence of c-kit and Slug after c-kit silencing in ACC-M and ACC-2 cells. Scale bar =100 μ m. C and D), Expression of c-kit and Slug mRNA after c-kit silencing in ACC-M and ACC-2 cells. E and F), Proliferation of ACC-M cells (Left) and ACC-2 cells (Right) expressing control, shRNA-neg and shRNA-c-kit were measured using MTT assays. G and H), Invasion assay of ACC-M and ACC-2 cells upon shRNA transfection. The mean was derived from cell counts of five fields, and each experiment was repeated at least three times (* p <0.001, compared to the control).

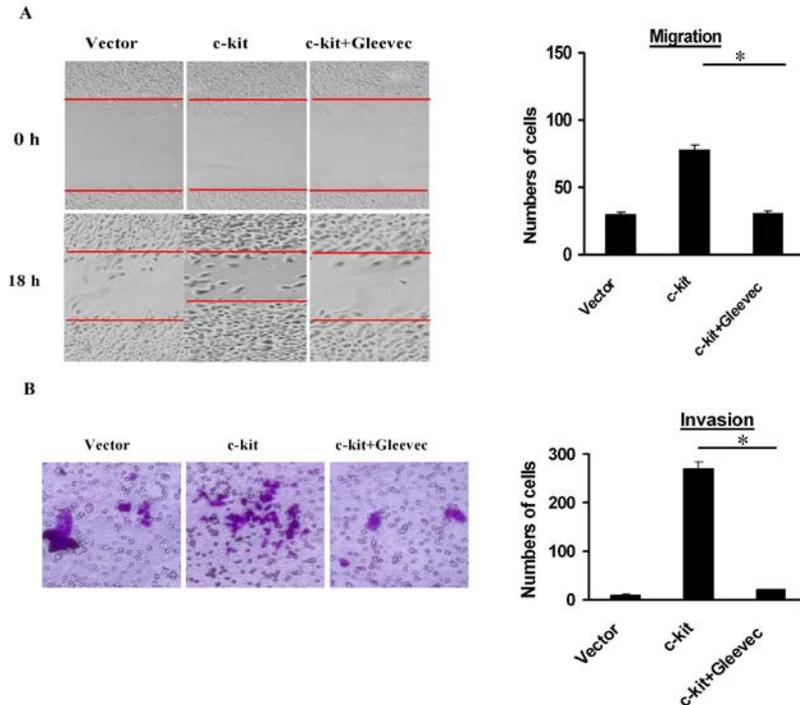


Figure S4: Gleevec had reversal of the enhanced migration and invasion ability of ACC-M cells with c-kit overexpression. A and B), Migration (A) and invasion (B) assays in stable ACC-M cells. The mean was derived from cell counts of 5 fields, and each experiment was repeated 3 times (* $P < 0.001$, compared with the c-kit overexpression group). Representative images of migrated and invaded cells are shown.

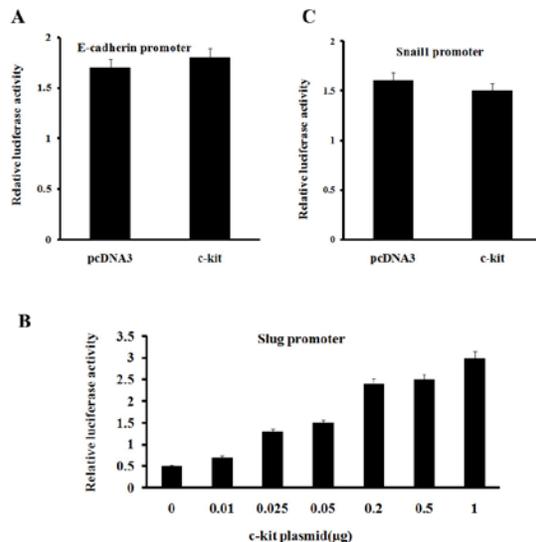


Figure S5: c-kit indirectly regulated E-cadherin transcription. A), 293T cells were transiently co-transfected with 1 μg of c-kit expression construct or 1 μg of pcDNA3, together with E-cadherin promoters. Relative luciferase activity is shown. Error bars represent mean \pm SD of triplicate experiments. B), 293T cells were transiently co-transfected with the indicated amounts of c-kit expression vectors (1 μg of the empty

vector was used in column 1) and Snail1 promoter reporter. Relative luciferase activity is shown. Error bars represent mean \pm SD of triplicate experiments. C) ,293T cells were transiently co-transfected with 1 μ g of c-kit expression construct or 1 μ g of pcDNA3, together with Slug promoter. Relative luciferase activity is shown. Error bars represent mean \pm SD of triplicate experiments.

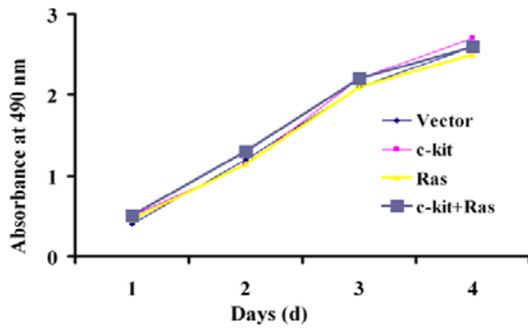


Figure S6: Proliferation of ACC-M cells expressing vector, c-kit, Ras and c-kit +Ras were measured using MTT assays. All the cells exhibited roughly the same growth rates with on statically significant differences.

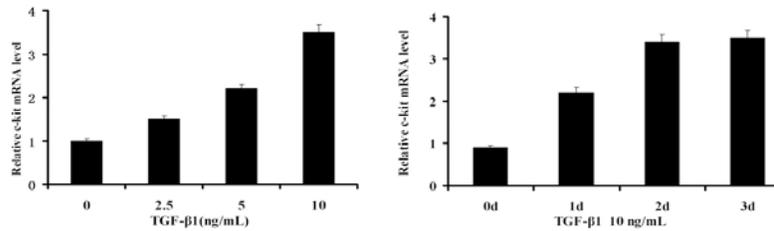


Figure S7: Relative c-kit mRNA expression levels in ACC-M cells treated with activated TGF-β1 at indicated time and concentrations. Error bars represent mean \pm SD of triplicate assays.