

Figure S1. Treatment with IBMX and α -MSH does not affect the cell viability in MNT-1 cells. Cells were treated with vehicle, 100 μ M IBMX, 1 μ M α -MSH or 100 μ M IBMX + 1 μ M α -MSH for 48 h, and then the cell viability was assessed using MTT assay. Data shown are the means \pm SD (n=3). NS, not significant; IBMX, 3-Isobutyl 1-methylxanthine; α -MSH, α -melanocyte-stimulating hormone.

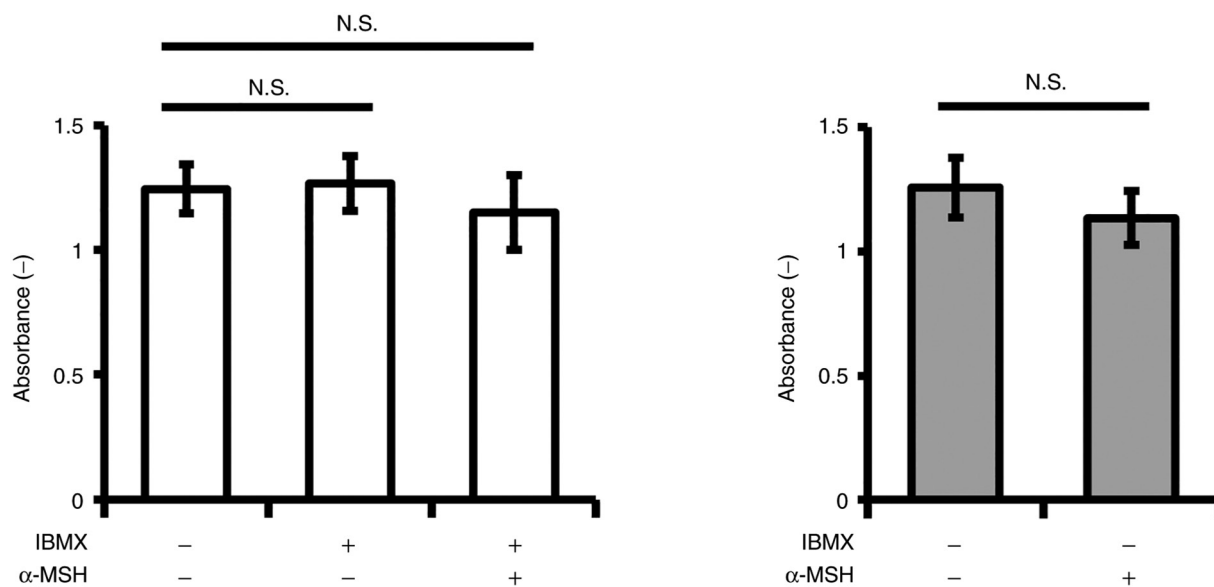


Figure S2. Arbutin does not promote VM in HT1080 cells. (A) Endogenous expression level of TYR was analyzed using western blotting. (B) HT1080 cells were pre-treated with vehicle or 500 μ M arbutin for 48 h, and VM formation assay was performed. (upper) Images of VM were taken 24 h after cell seeding and representative images are shown (scale bars, 200 μ m). (lower) VM in arbutin-treated HT1080 cells were quantified by counting tube numbers. Data are presented as mean \pm SD (n=5). NS, not significant; TYR, tyrosinase; Ctrl, control; VM, vasculogenic mimicry.

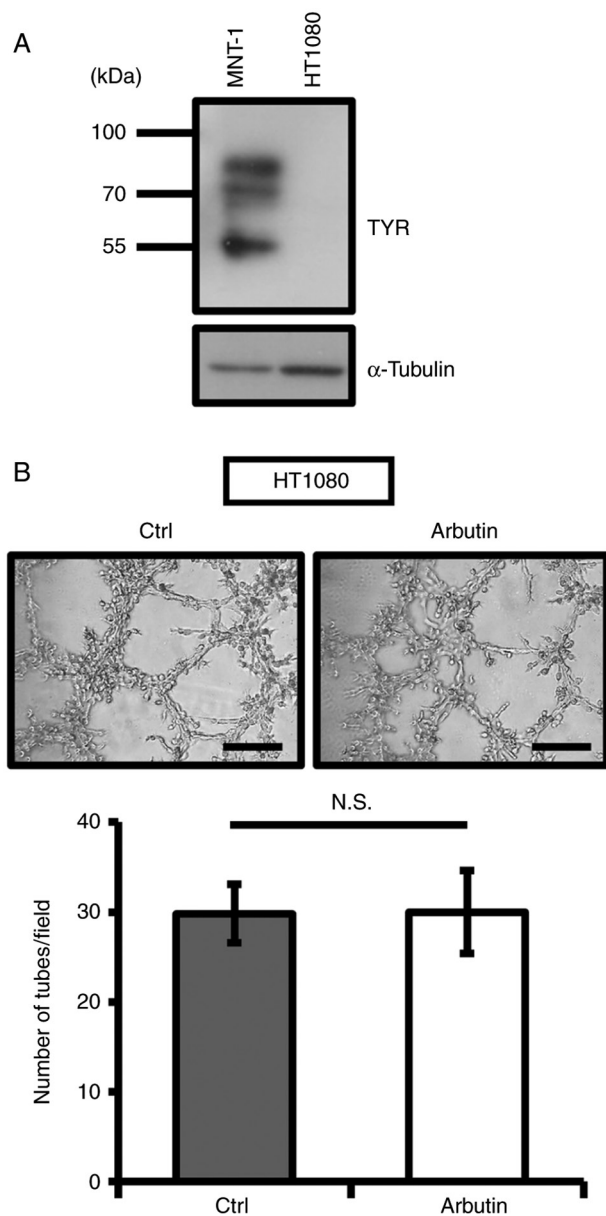


Figure S3. KO of TYR suppresses TYR activity and melanin synthesis in MNT-1 cells. (A) TYR activity assay and (B) melanin content assay was performed in TYR-KO MNT-1 cells. Data are presented as means \pm SD (n=3). *P<0.05, ***P<0.001. TYR, tyrosinase; KO, knockout.

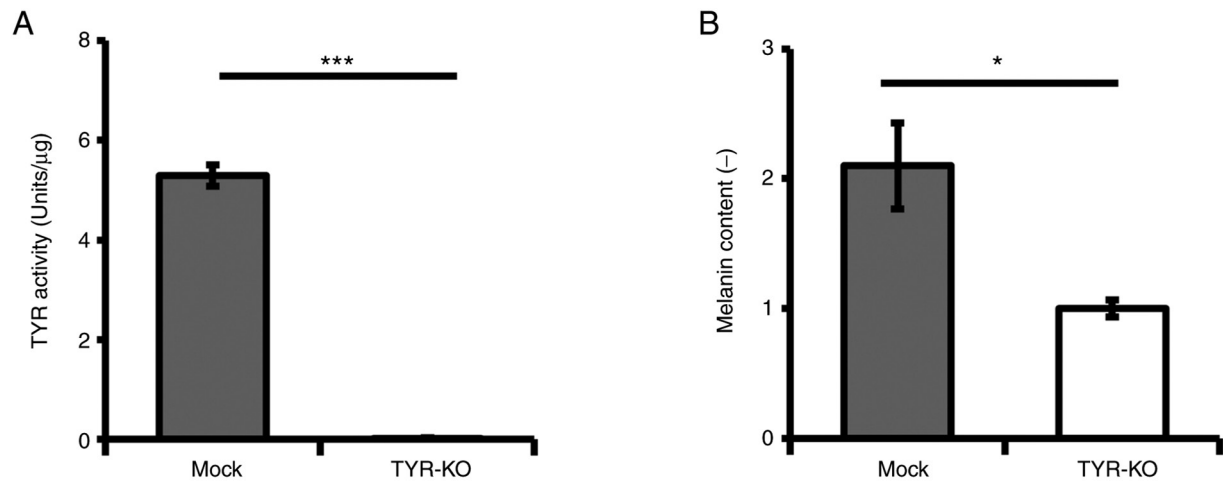


Figure S4. TYR suppresses VM in human amelanotic melanoma cell lines. (A) TYR activity assay was performed in TYR-KO SK-MEL-28 cells. (B) Wild-type TYR was overexpressed in WM266-4 cells, and the stably TYR-overexpressing cells were established. Overexpression of TYR was confirmed by western blotting. CSII-CMV-MCS-IRES2-Bsd-GFP vector was used as a ctrl. (C) TYR activity assay was performed in TYR-overexpressing WM266-4 cells. (D, upper) VM assay of TYR-overexpressing WM266-4 cells; representative images at 24 h after cell seeding (scale bars, 200 μ m). (Lower) VM in TYR-overexpressing WM266-4 cells were quantified by counting tube numbers. Data presented as the means \pm SD (n=5). **P<0.01, ***P<0.001. TYR, tyrosinase; VM, vasculogenic mimicry; KO, knockout; Ctrl, control.

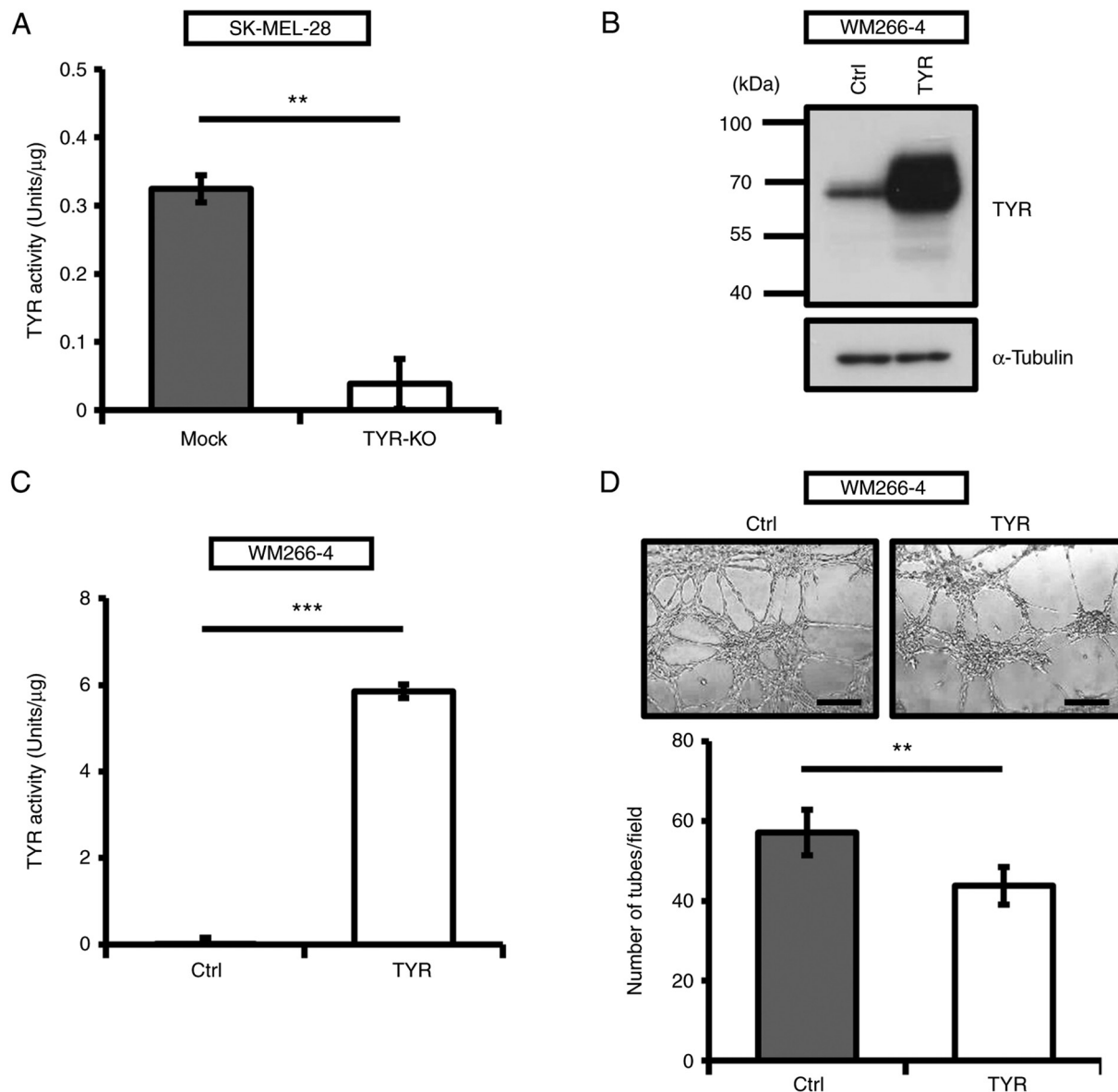


Figure S5. Effect of α -MSH on VM in TYR-KO MNT-1 cells. TYR-KO MNT-1 cells were pretreated with vehicle or 100 μ M α -MSH for 48 h, and VM assay was performed. (Upper) Images of VM were taken 5 h after cell seeding and representative images are shown (scale bars, 200 μ m). (Lower) VM was quantified by counting tube numbers. Data are presented as the means \pm SD (n=5). **P<0.01. α -MSH, α -melanocyte-stimulating hormone; VM, vasculogenic mimicry; TYR, tyrosinase; KO, knockout.

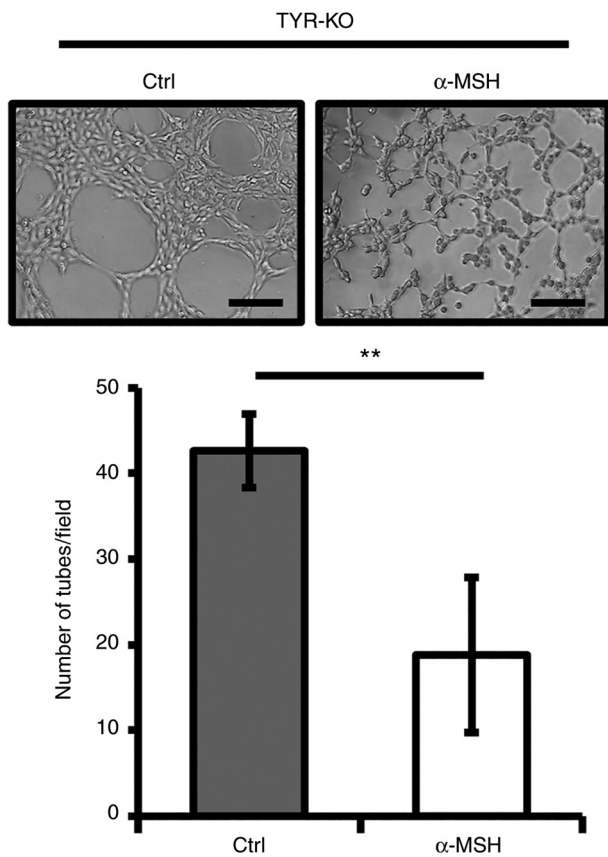


Figure S6. T373K mutation attenuates TYR activity in MNT-1 cells. TYR activity assay was performed in TYR/wt- and TYR/T373K-rescued TYR-KO MNT-1 cells. * $P<0.05$, *** $P<0.001$. TYR, tyrosinase; Wt, wild-type; KO, knockout.

