

uPAR controls vasculogenic mimicry ability expressed by drug-resistant melanoma cells

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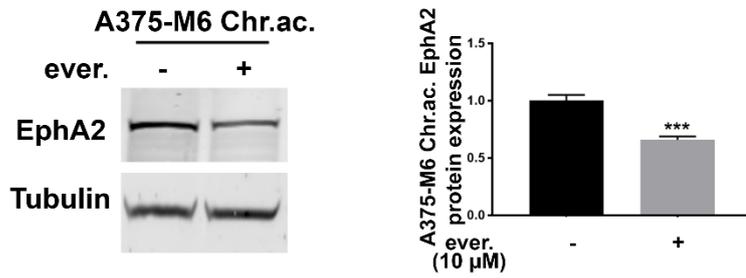
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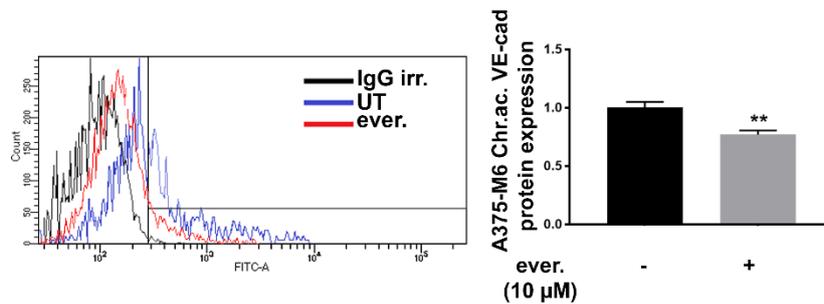
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Supplementary Figure S1

a

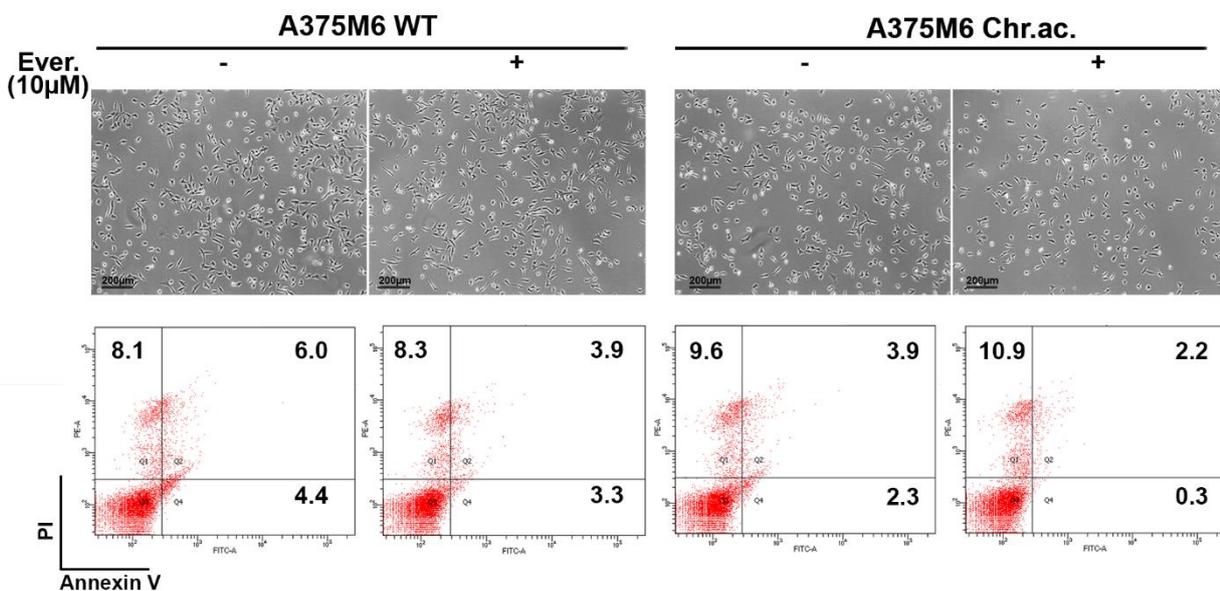


b



Supplementary Figure S1. Everolimus inhibits VM of acid-adapted A375-M6 cells. a) Western blot analysis and relative quantification chart of EphA2 in acid-adapted A375-M6 cells treated or not for 24 h with 10 μ M everolimus. T-test, GraphPad Prism. b) Flow cytometer analysis and relative quantification chart of VE-cadherin expression in acid-adapted A375-M6 melanoma cells treated or not for 24 h with 10 μ M everolimus. T-test, GraphPad Prism. ** $p < 0.01$, *** $p < 0.001$.

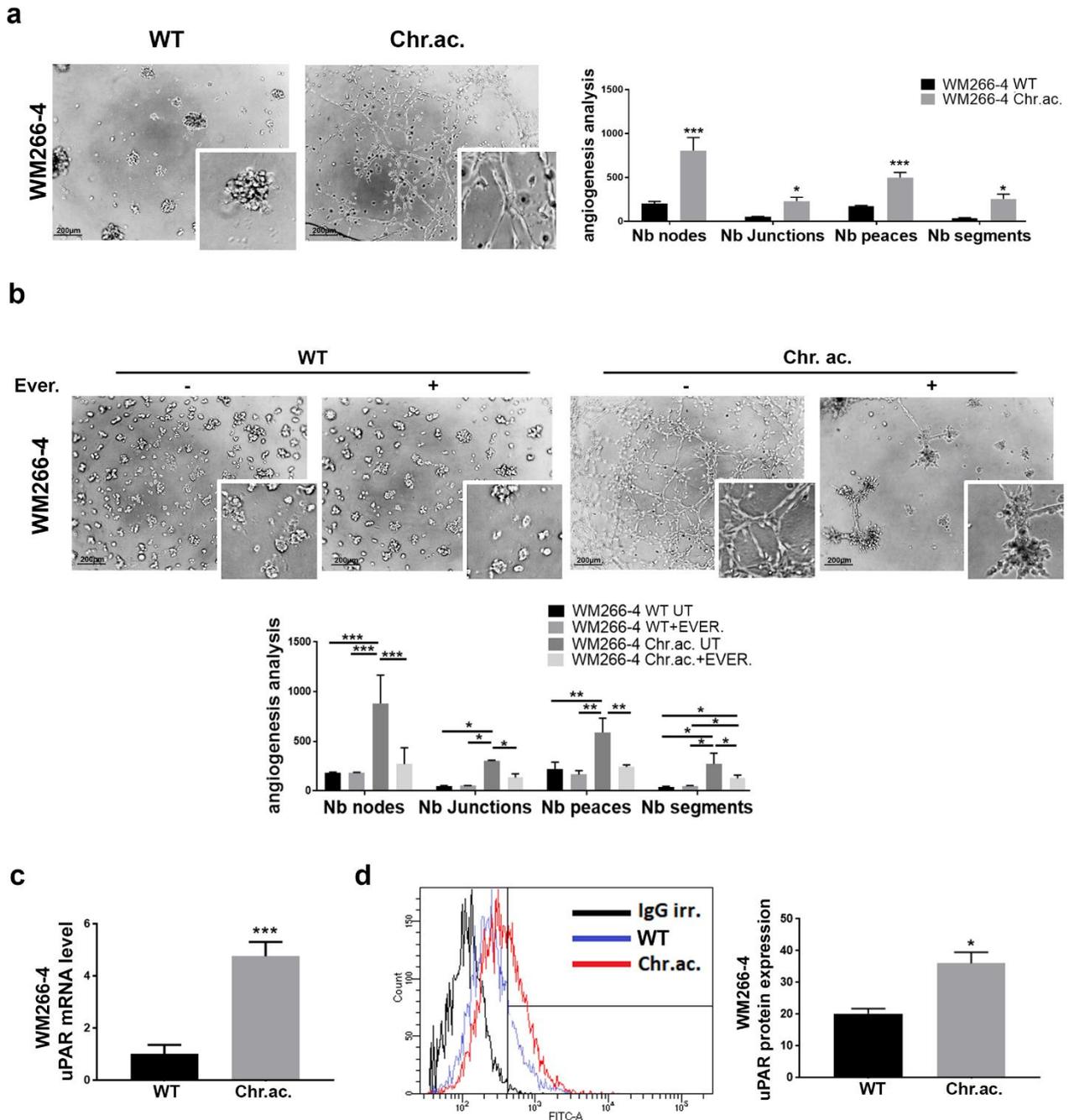
Supplementary Figure S2



Supplementary Figure S2. VM-inhibiting everolimus dose does not impair A375-M6 cell viability.

Representative pictures of morphology (upper, scale bar 200 μ m) and Annexin/PI flow cytometer plots (lower) of WT or acid-adapted (Chr.ac.) A375-M6 cells treated or not for 24 h with 10 μ M everolimus. Two-way ANOVA, GraphPad Prism.

Supplementary Figure S3

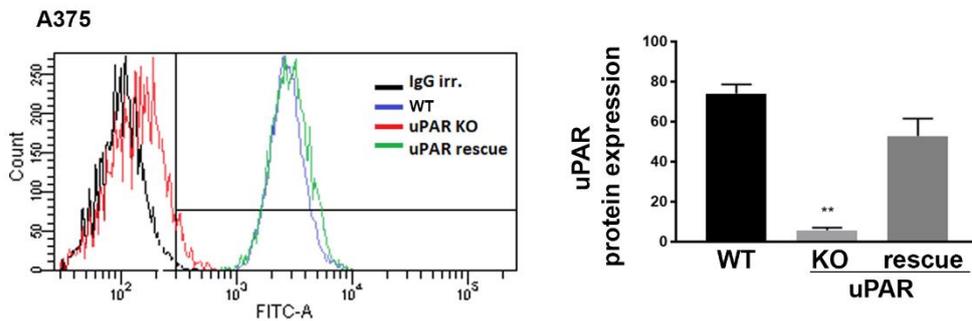


Supplementary Figure S3. Acidosis-induced VM in WM266-4 melanoma cells. (a) Representative pictures (scale bar 200 μ m) and relative quantification chart of morphogenesis capillary assay of WT and acid-adapted

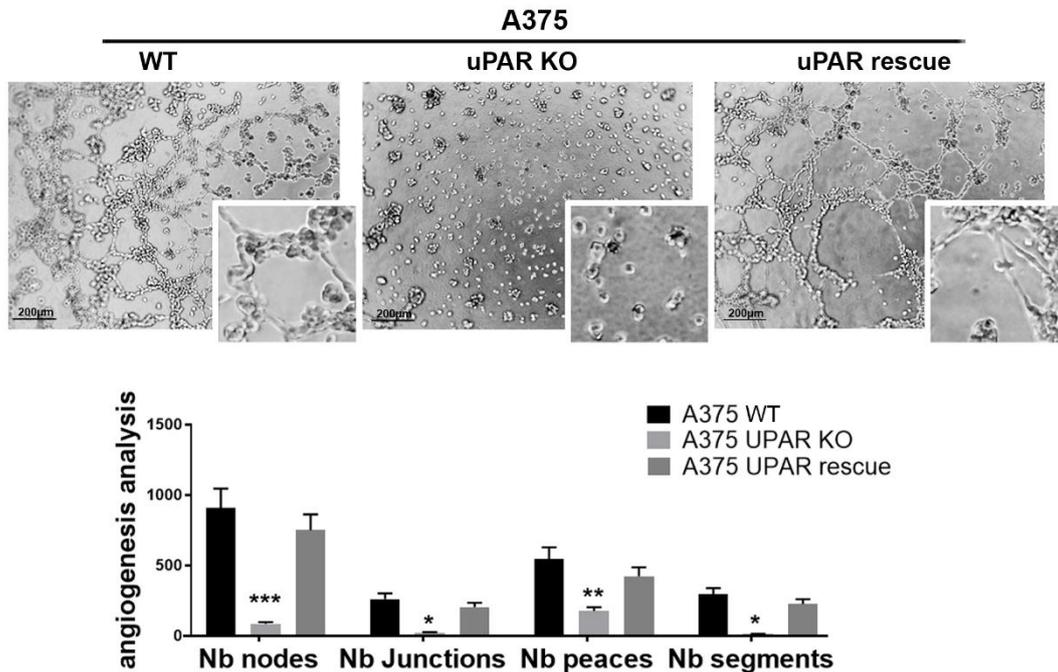
(Chr.ac.) WM266-4 melanoma cells. **(b)** Representative pictures (scale bar 200 μm) and relative quantification chart of morphogenesis capillary assay of WT and acid-adapted (Chr.ac.) WM266-4 melanoma cells in the presence or absence of 10 μM everolimus. Two-way ANOVA, GraphPad Prism. **(c)** qPCR and **(d)** flow cytometer analysis of uPAR level in WT and acid-adapted WM266-4 melanoma cells, T-test, GraphPad Prism. * $p < 0.05$, *** $p < 0.001$.

Supplementary Figure S4

a



b



Supplementary Figure S4. uPAR expression is required for VM in A375 melanoma cells. **(a)** uPAR flow cytometer analysis and relative quantification chart of WT, uPAR KO, and uPAR rescue A375 cells. One-way ANOVA, GraphPad Prism. **(b)** Representative pictures of capillary morphogenesis assay (scale bar 200 μm) and relative quantification chart of WT, uPAR KO, and uPAR rescue A375 cells. Two-way ANOVA, GraphPad Prism. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.