

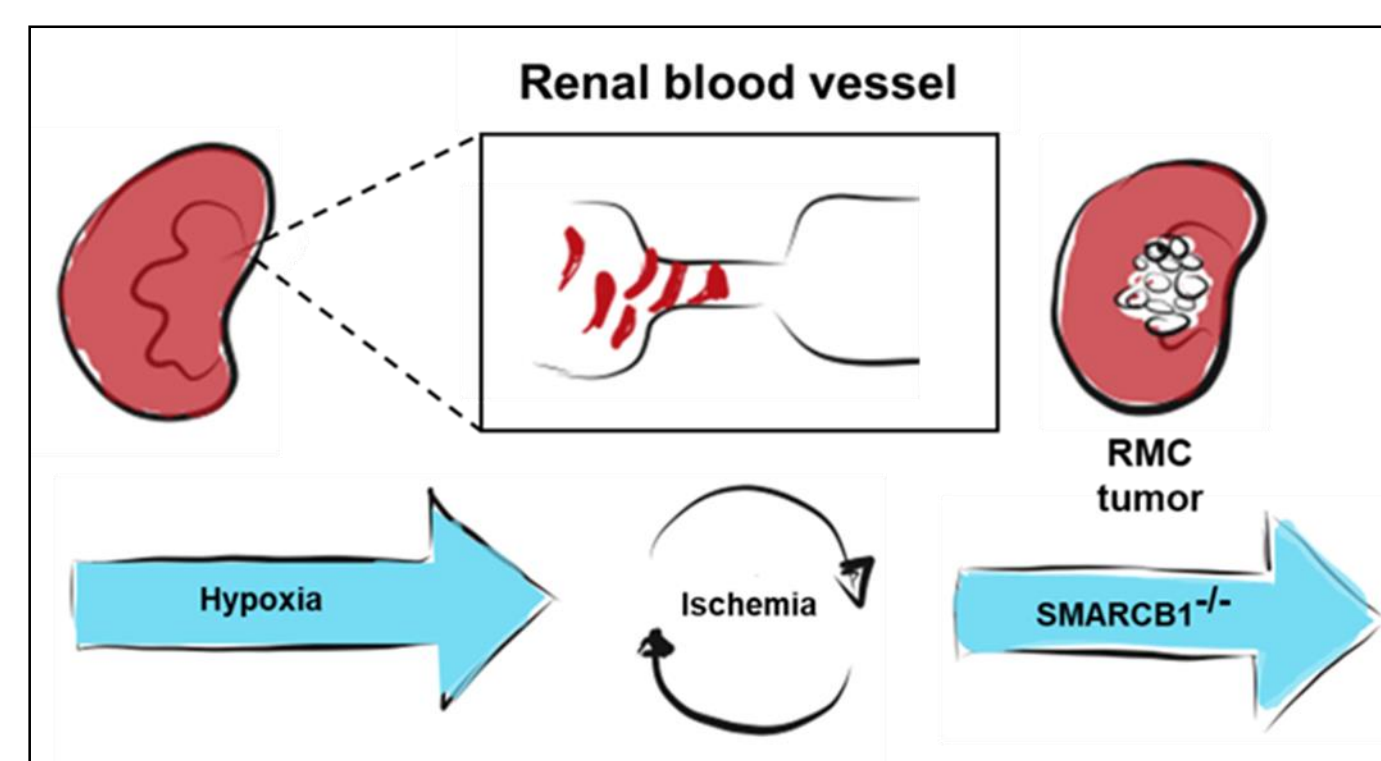
SMARCB1 regulates the hypoxic stress response in sickle cell trait during the pathogenesis of renal medullary carcinoma.

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Introduction

- *SMARCB1* is one of the core subunits of the SWI/SNF complex, an ATP-dependent chromatin remodeling complex.
- Malignancies are characterized by simple genomes and lack of somatic events, suggesting that the dysregulation of the SWI/SNF machinery is sufficient to induce highly malignant states [1].
- Although SWI/SNF dysregulations are related to approximately 20% of human malignancies [2], lack of conditional genetic models of *SMARCB1*-deficient tumors has made it difficult to investigate the molecular bases and dependencies associated with *SMARCB1* loss due to the embryonic lethality associated with SWI/SNF disruption.
- Renal medullary carcinoma (RMC), is an aggressive renal tumor that afflicts primarily young individuals of African descent with **sickle cell trait** [3]. RMC is also characterized by the **complete loss of the *SMARCB1* tumor suppressor** [4], positioning it as an ideal tumor model for studying the role of SWI/SNF dysregulation in tumorigenesis.
- **We hypothesize that sickling red blood cells are promoting a hypoxic microenvironment in the renal medulla that is selecting for the loss of the tumor suppressor *SMARCB1*.**



Main hypothesis: Renal medullary hypoxia promotes chronic hypoxia, creating a selective pressure for tumorigenic *SMARCB1*-deficient cells.

Technique/ Methods

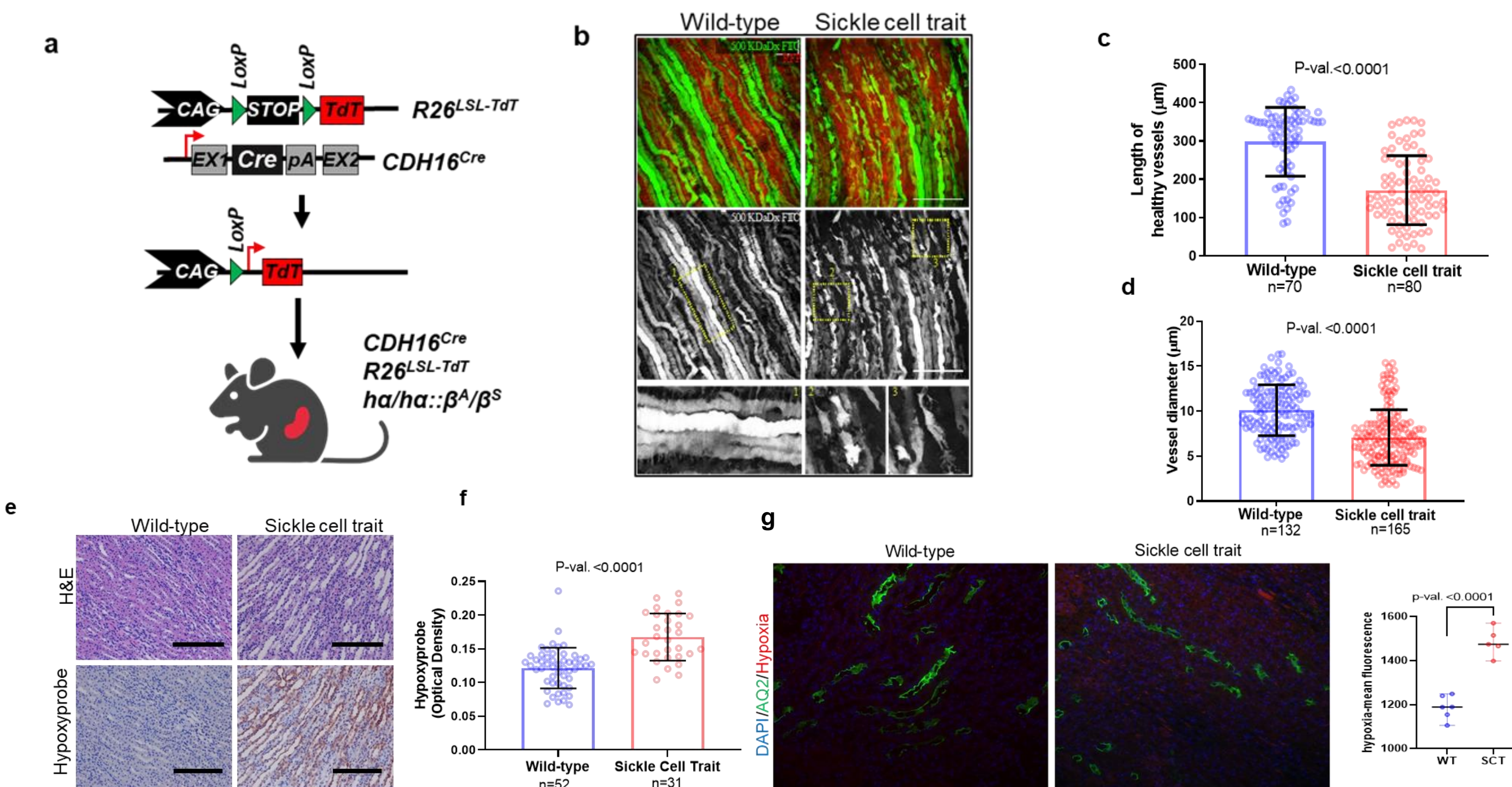


Figure 1. Renal ischemia is associated with chronic hypoxia in sickle cell trait mouse model. (a) Schematic of genetically engineered mouse model (GEMM) of SCT. (b) 3D image reconstruction of renal epithelia (RFP) and FITC-dextran (GFP) in adult mice (n=4-5) with kidney-specific *CDH16^{Cre}* and conditional *R26^{SL-Tet}*. (c, d) Quantification of the diameter (c) and length (d) of the renal blood vessels (10 vessels/image, 3 locations/vessel). (e) IHC of mouse kidneys after injection with Hypoxyprobe. (f) Quantification of the optical density of horseradish peroxidase (HRP) staining for 20x images was done using ImageJ. Data are expressed as mean value ± SD, with P value calculated by student's t test. (g) Immunofluorescence (IF) analysis of hypoxyprobe (red) levels in wild-type mice (n=5) compared to sickle cell trait mice (n=5). Aquaporin 2 (AQ2, green) was used to localize renal medullary region.

Results

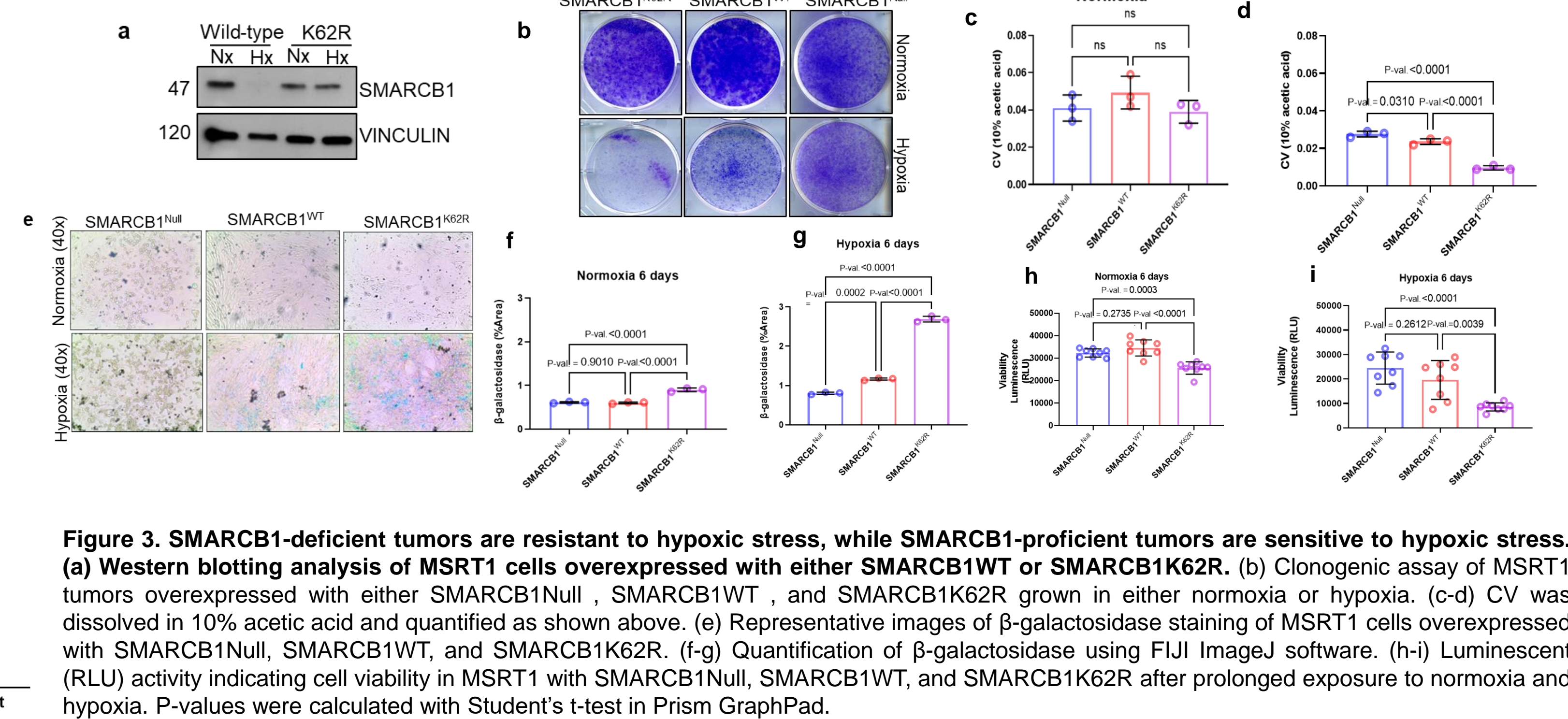
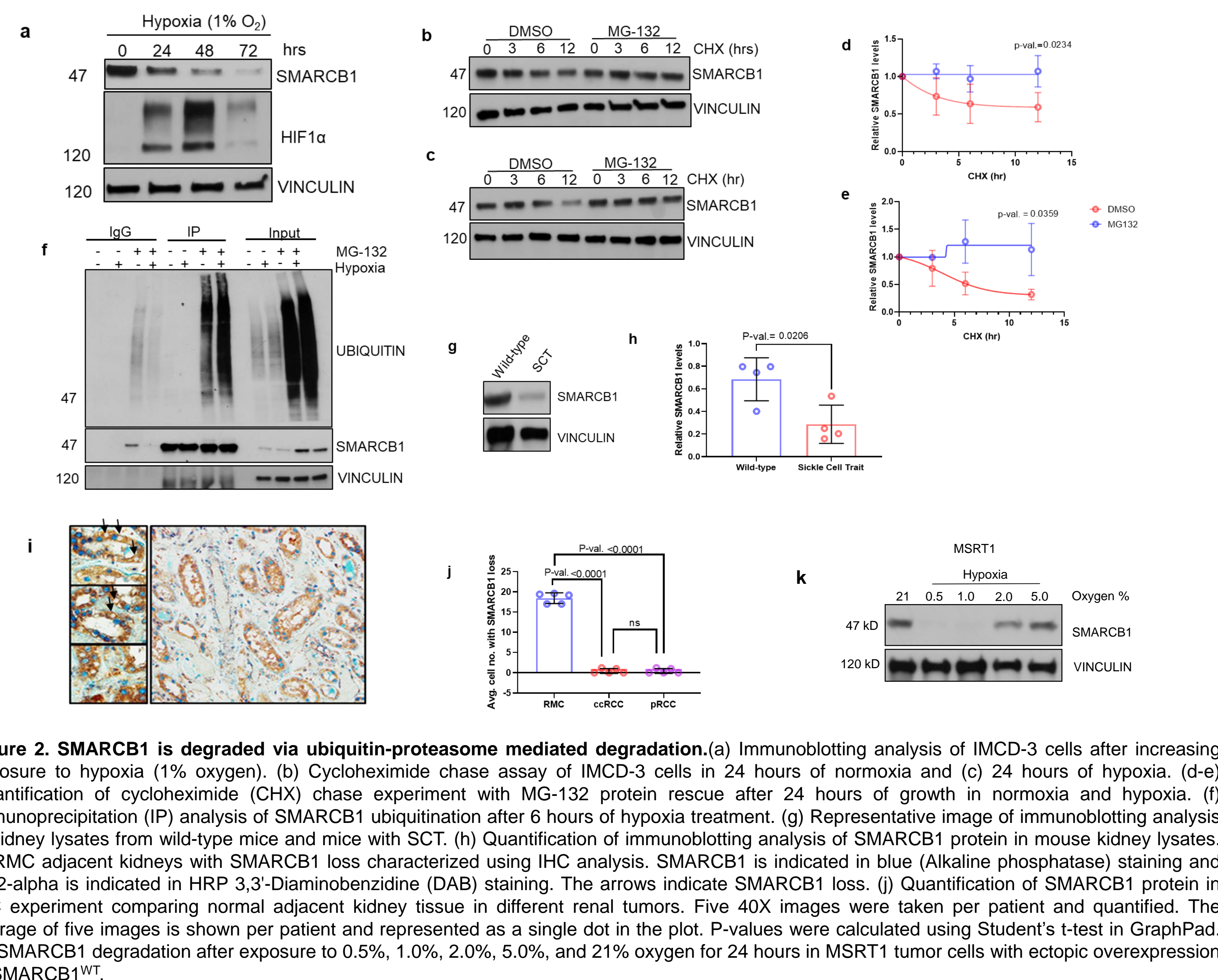


Figure 3. SMARCB1-deficient tumors are resistant to hypoxic stress, while SMARCB1-proficient tumors are sensitive to hypoxic stress. (a) Western blotting analysis of MSRT1 cells overexpressed with either *SMARCB1^{WT}* or *SMARCB1^{K62R}*. (b) Clonogenic assay of MSRT1 tumors overexpressed with either *SMARCB1^{Null}*, *SMARCB1^{WT}*, and *SMARCB1^{K62R}* grown in either normoxia or hypoxia. (c-d) CV was dissolved in 10% acetic acid and quantified as shown above. (e) Representative images of β -galactosidase staining of MSRT1 cells overexpressed with *SMARCB1^{Null}*, *SMARCB1^{WT}*, and *SMARCB1^{K62R}*. (f-g) Quantification of β -galactosidase using FIJI ImageJ software. (h-i) Luminescent (RLU) activity indicating cell viability in MSRT1 with *SMARCB1^{Null}*, *SMARCB1^{WT}*, and *SMARCB1^{K62R}* after prolonged exposure to normoxia and hypoxia. P-values were calculated with Student's t-test in Prism GraphPad.

Results (continued)

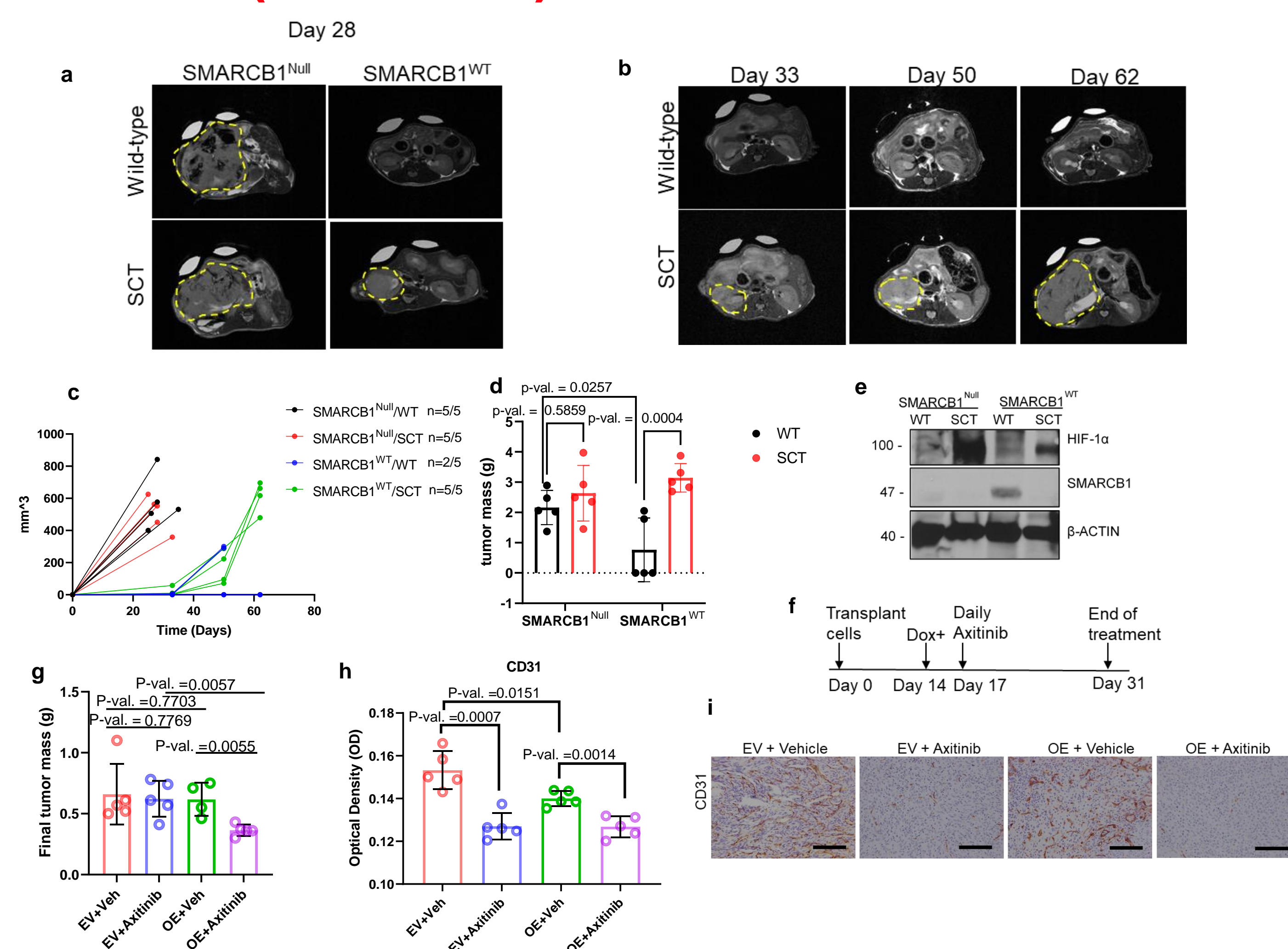
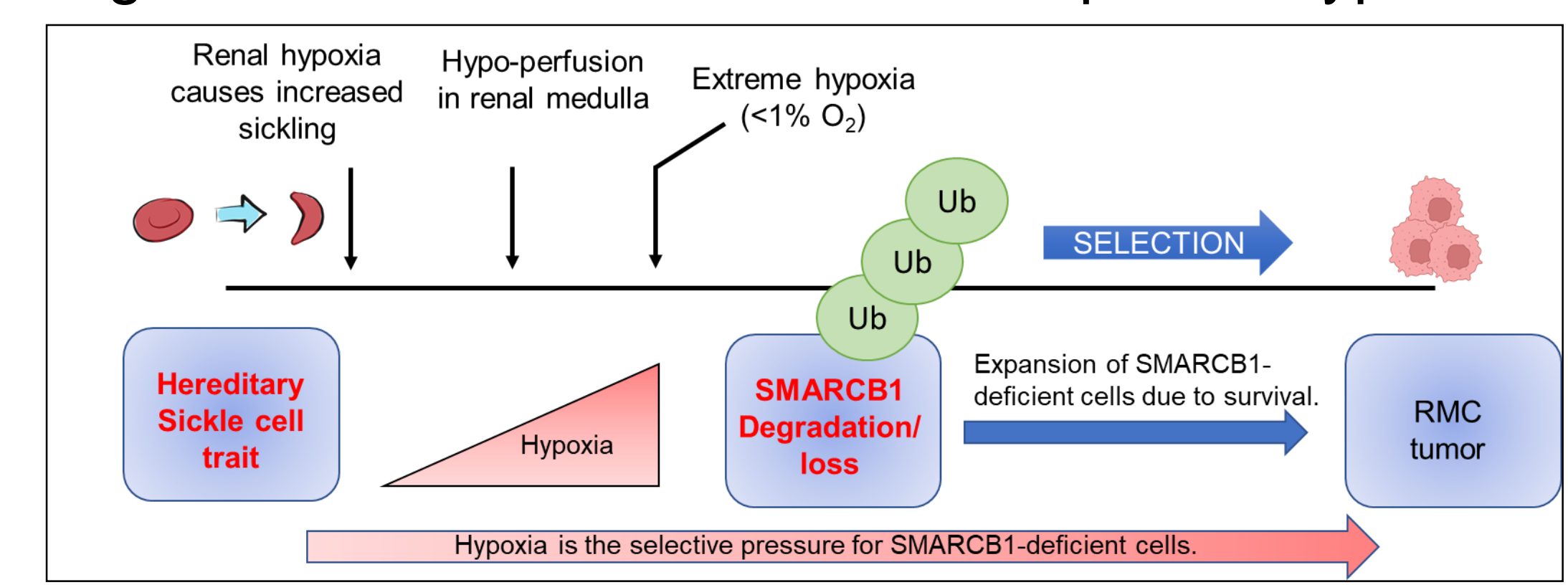


Figure 4. SMARCB1-deficient tumor cells are more resistant to hypoxia and expand under hypoxic conditions. (a) Microscopic images of *SMARCB1^{Null}* mouse renal tumors (GFP) and *SMARCB1^{WT}* cells (GFP/RFP) prior to subcutaneous injection into NSG mice. (b) Flow cytometry evaluation of *SMARCB1^{Null}* and *SMARCB1^{WT}* tumor cells that were mixed in a 1:1 ratio and injected subcutaneously into NSG mice. (c) Immunoblotting analysis of *SMARCB1* protein expression in RMC219-tet-empty vector (*SMARCB1^{Null}*) and RMC219-tet-inducible *SMARCB1^{WT}* and RMC219-tet-inducible *SMARCB1^{K62R}* after 7 days of treatment with 2 μ g of Doxycycline (DOX). (d) Corresponding microscopic images of RMC219-tet-inducible cells showing that cells are healthy prior to subcutaneous injection in NSG mice. (e) Growth curve of subcutaneous tumors in NSG mice. (f) Kaplan-Meier survival curve of RMC219 subcutaneous tumors. For time-to-event event-free survival analysis, 200 mm³ was set as the endpoint. (g) Final tumor mass of RMC219 subcutaneous tumors.

Future Direction

- Elucidate the E3 ubiquitin ligase involved in ubiquitinating *SMARCB1*.
- Investigate the role of the SWI/SNF complex in hypoxia response.



Schematic 1: The pathogenesis of renal medullary carcinoma.

Conclusion

- *SMARCB1* is degraded via ubiquitin-mediated proteasome degradation pathway during extreme hypoxic stress.
- *SMARCB1*-deficient kidney cells are resistant to hypoxia stress and maintain viability and growth compared to *SMARCB1*-proficient counterparts.
- Impairing the degradation of *SMARCB1* with lysine residue mutation K62 decreases cell viability and increases senescence under hypoxia stress, suggesting that *SMARCB1*-deficiency is selected for and required for survival under hypoxia stress.

Reference

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