Supplementary Figure legends

Supplementary Figure 1. Expression of hypoxia-induced metastasis genes in human UM eye tumors and derived cell lines. (A,B) Effect of KCN1 on the expression of hypoxia-induced metastasis genes in human Mel290 UM cells. Mel290 cells were treated with KCN1 or vehicle (1% DMSO) for 1 hr., then placed under normoxia or hypoxia for another 24 hs before RNA extraction and qRT-PCR analysis. Hypoxia-induced metastasis-related gene list is from Rankin & Giaccia (Science, **2016**:175-80), primers used are listed in Suppl. Table 2 and raw data presented in Suppl. Table 3. Heatmaps with expression data are presented using **(A)** relative (colors are based on min/max in each row) or **(B)** absolute (colors are based on the absolute scale indicated) scales using the Morpheus software. *β-actin* was used as internal control and results are expressed as fold change over normoxic control. **(C)** Heatmap showing expression of hypoxia-inducible metastasis-related genes in eye tumors of UM patients that did or did not develop metastases. Combined microarray datasets (GSE27831 and GSE22138) were used (see Methods). **(D)** Predicted hypoxia response elements (HRE) present in the P4HA1 and P4HA2 genes. **(E)** Western blot, using 4-15 % gradient gel, showing KCN1 inhibition of hypoxia (1% O_2)-induced expression of P4HA1 and P4HA2 in melanoma cells. Cells were treated with vehicle (1% DMSO) or KCN1 (10 μM) for 24 h. Signal intensity was quantified by Image J, normalized to β-actin levels, numbers indicate fold change over normoxic control. * indicates non-specific bands. **(F)** Hypoxia-induced expression of P4HA1 and P4HA2 protein in 92.1 cells. For drug treatment, cells were cultured in normoxia or hypoxia (1% O_2) and

treated with vehicle (0.1% DMSO) or KCN1 (10 μM) for 24 h. For RNAi-mediated silencing, cells were transfected with siCtrl, si*HIF1A*, si*EPAS1*, or si*HIF1A*/si*EPAS1* for 24 h, and subsequently cultured under normoxia or hypoxia (1% O_2) for 24 h. Proteins were detected by Western blot (8 % gel), and signal intensity was quantified by Image J, normalized to β-actin levels, and numbers indicate fold change over normoxic control. *β-actin* was used as loading control and the numbers indicate fold change over normoxic control (** $p<0.01$, *** $p<0.001$ by Tukey's test). Representative result shown of 3 independent biological replicates (n=3).

Supplementary Figure 2. Hypoxia-inducible metastasis-related genes that display significantly altered expression between UM patients with non-metastatic (N) versus metastatic (M) disease. Data were generated by analyzing combined microarray datasets GSE27831 and GSE22138. P values are indicated (Mann-Whitney U-test).

Supplementary Figure 3. Overall survival of UM patients with high and low expression of genes identified in Figure 1B. Kaplan-Meier survival curves were generated by analyzing UM cases deposited in the TCGA database. Groups were compared with the log-rank test.

Supplementary Figure 4. KCN1 is efficiently delivered in the eye and liver upon systemic delivery.

A) Pharmacokinetic parameters of KCN1 in the eye and liver. KCN1 (60 mg/kg, in

Cremophor EL/ethanol formulation) was given i.p. to C57Bl/6 mice bearing B16LS9 tumors. C_{max}: maximal concentration; T_{max}: time to reach C_{max}; $AUC_{0\rightarrow\infty}$: area under the curve (integral of the concentration-time curve); $t_{1/2,e}$: elimination half-life; MRT: mean residence time; CL: clearance. Methods were described in our prior study (Wang W. *et al.* PLOS One **2012**;7).

B) Chemical formula of radiolabeled [^{11}C]KCN1 (3,4-dimethoxy-*N*-[(2,2-dimethyl-*2H*-chromen-6-yl) methyl]-*N*-phenylbenzenesulfonamide). * indicates the labeled position.

C) Representative microPET scan shows KCN1 activity in the mouse eye and liver. Nude mice were injected with 2 MBq of radiolabeled [^{11}C]KCN1 *via* a tail vein and scanning was then conducted on a Concord microPET P4 system.

D) The uptake of [^{11}C]KCN1 in mouse liver and eyes was respectively three and two times higher than in the brain at all time points. The regions of interest were manually drawn over liver, right eye and brain on the frames where they were clearly visible. Time activity curves (TACs) of average pixel intensity were obtained and averages with standard deviations plotted.

Supplementary Figure 5. Pathological changes and total metastatic load in mice livers from the 92.1 survival experiment (related to Fig. 3B).

Livers from vehicle- and KCN1-treated mice from Fig.3B (lower panel) were fixed in formalin, paraffin-embedded, sectioned, and H&E stained. Magnification is as indicated.

A) Pathological changes observed in tumor-burdened livers. In vehicle-treated group, macro-metastases, necrosis, and hemorrhage were observed, while in KCN1-treated

group only micro-metastases were observed. Time from tumor cell inoculation is indicated.

B) Quantification of metastatic load in liver. The ratio of the number of tumor cells/total number of cells was established from H&E images using the Aperio ImageScope [v12.3.2.8013] software. Top panel: typical H&E image (left) and transformed image for quantitative analysis (right). Green lines surround areas that were excluded from analysis. Middle panel: Hepatic metastatic load in individual mice that reached IACUC criteria for termination at different time points. Bottom panel: Average hepatic metastatic load in the vehicle and KCN1-treated groups.

Supplementary Figure 6. Metastases in other organs from the 92.1 survival experiment (related to Fig. 3B).

The same mice as in Fig.3B (lower panel) were analyzed for metastasis in kidney, lung, and heart. **A)** Representative images of metastases in kidney (top panel) and lung (bottom panel). **B)** Table summarizing the percent of organs containing metastases in vehicle- and KCN1-treated groups. *, although all livers showed signs of metastasis, there was a significant difference in the metastatic load.

Supplementary Figure 7. Effect of KCN1 treatment on mice weight and organ pathology.

A. Nu/nu mice (10 per group) injected with 92.1 cells were treated ip 5x week with KCN1 (60 mg/kg) or vehicle for 14 weeks, and animal weights were measured until they reached IACUC termination endpoints or until the end of the experiment (week 51).

Results are shown as average weights in each group, +/- standard error. The number of surviving mice in each group at different times is shown near both curves. At termination one mouse was left in the vehicle group, while 6 were alive in the KCN1 group.

B. Four-week-injection of KCN1 was well tolerated by mice harboring B16LS9 tumors. No extraneous signs of toxicity were apparent. Pathological examination of main organs demonstrated no treatment-related changes in the heart, lung, kidney, spleen, and gastrointestinal tract. The only organ where a treatment-related change was observed was the liver. Liver swelling was observed at autopsy and pathology showed that there was an accumulation of liquid within the bile ducts in all groups, yet without evidence for any hepatocyte death.

Supplementary Figure 8 (related to Fig. 1A, lower right panel). Modified Boyden Chamber Matrigel Invasion assays. Representative membranes showing tumor cells (stained with crystal violet) that migrated to lower side of the membrane. The black “dots” are the membrane pores (size 8.0 µm).

Supplementary Figure 9. Collagen mRNA levels in UM patient eye tumors. Data were generated by analyzing RNAseq data from UM cases deposited in the TCGA database with cBioPortal for Cancer Genomics. TPM = normalized transcripts per million.