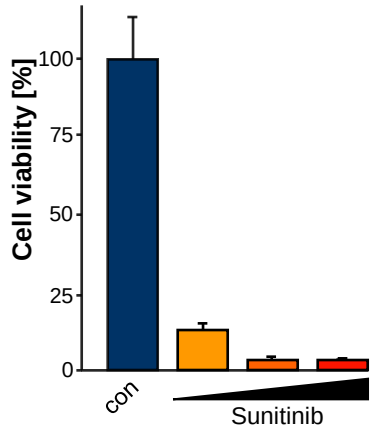
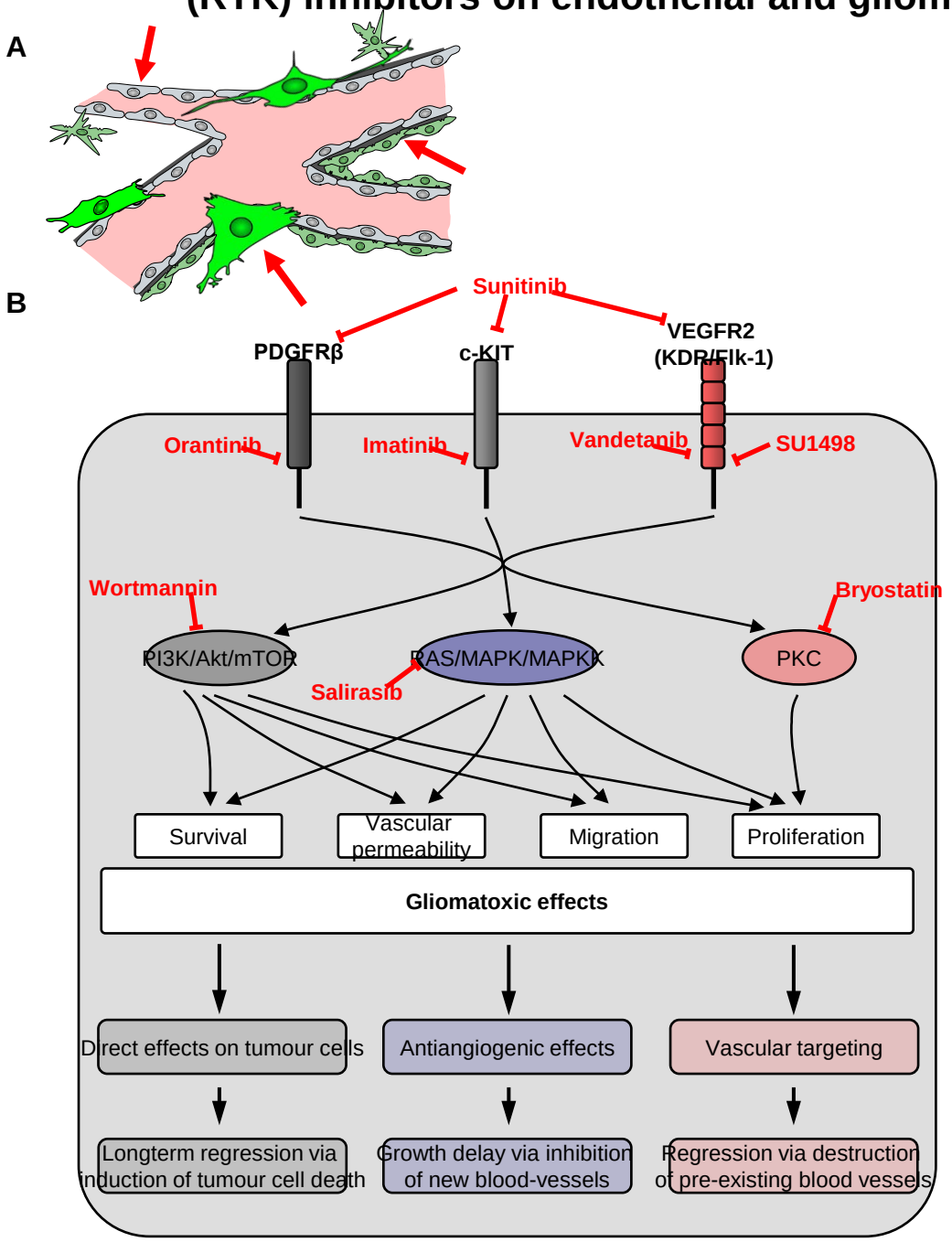
Sunitinib operates gliomatoxic on primary glioblastoma cells



Suppl. Figure 1:
Sunitinib operates gliomatoxic on primary glioblastoma cells.
Sunitinib induces cell death in primary human glioblastomas. Cell survival was monitored in human GBM tissue. Quantification of cell survival after Sunitinib treatment. Sunitinib acts in a dose-dependent manner on glioma cells. Control (blue column), 5 µM Sunitinib (dark yellow column), 10 µM Sunitinib (orange column), 20 µM Sunitinib (red column). Quantification is given for at least n = 10 per group. Values are given as mean ± S.D. with controls set as 100%. Differences were considered statistically significant with $p < 0.05$ (asterisks, two-sided Student's *t*-test).

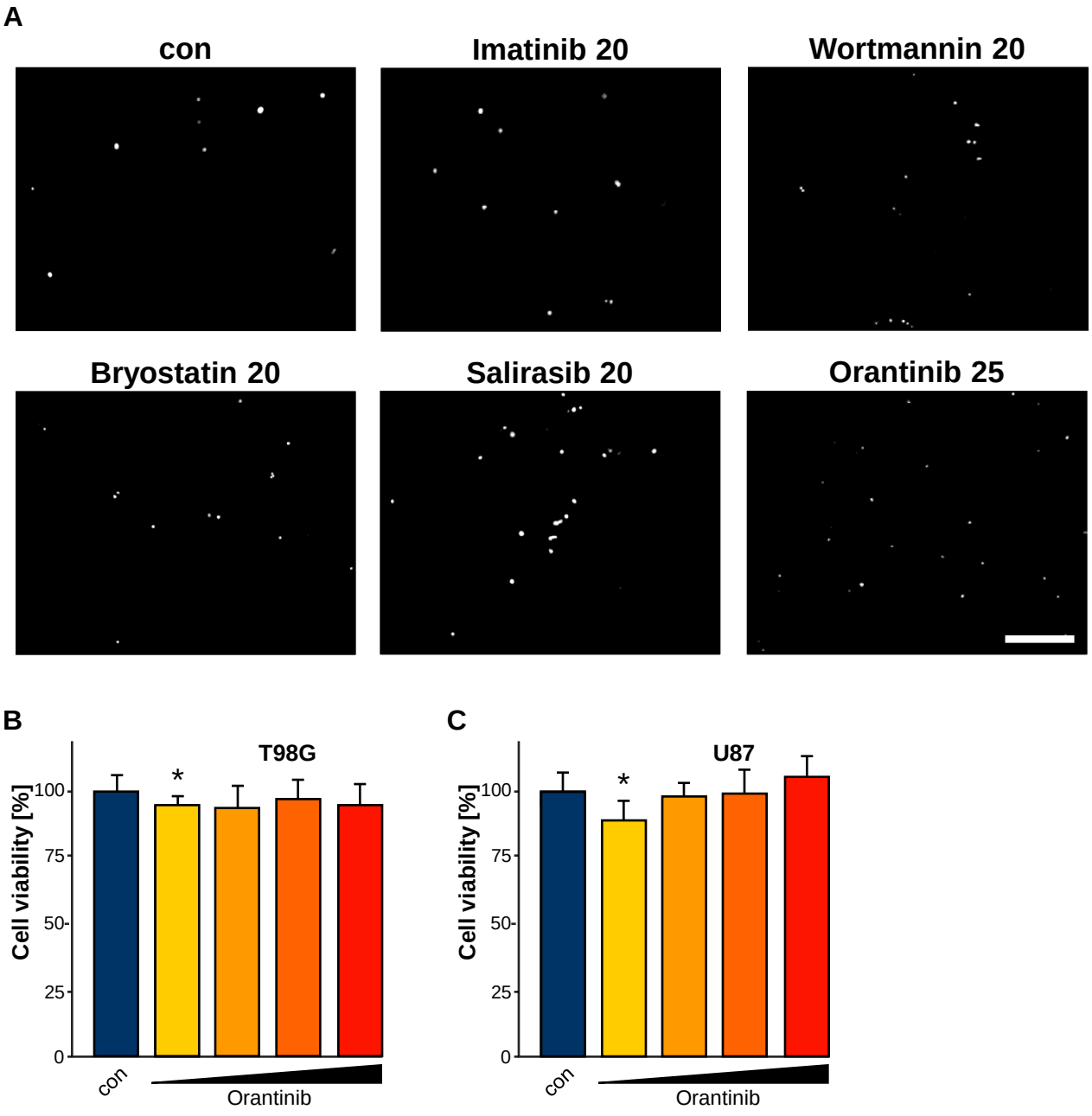
Molecular targets of sunitinib and other receptor tyrosine kinase (RTK) inhibitors on endothelial and glioma cells



Suppl. Figure 2:

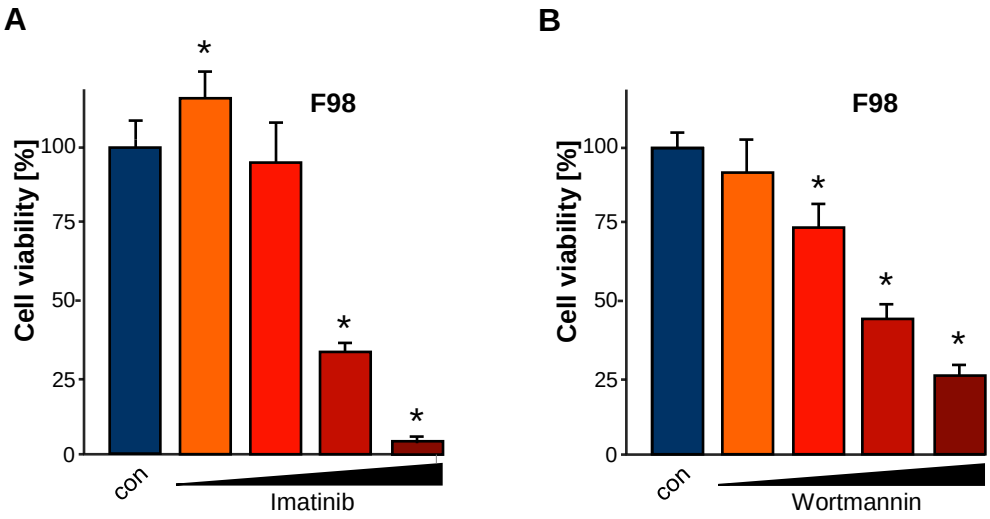
Molecular targets of sunitinib and other receptor tyrosine kinase (RTK) inhibitors on endothelial and glioma cells. A, Anti-angiogenic actions of pan-RTK inhibitor sunitinib and its cellular targets. In principle, sunitinib can operate on tumor endothelial cells, tumor cells and pericytes (marked with red arrows). **B**, The small molecule receptor tyrosine kinase inhibitor sunitinib has been shown to be active against a number of tumor types. The anti-angiogenic effects of sunitinib is proposed to be mediated by targeting of pro-angiogenic receptor such as the VEGF receptor 2 (VEGFR2, KDR, Flk-1), PDGF receptor β and c-Kit. Furthermore, receptor and kinase-specific small molecule inhibitors are displayed: Oranitinib is directed against PDGFR β, Imatinib is targeted against c-kit, and SU1498 is specifically directed against VEGFR2. In principle, interference with the common pathways PI3K/Akt/mTor, Ras/MAPK/MAPKK and PKC can initiate blood vessel formation as well as glioma cell death and tumor regression (survival, proliferation and migration).

Toxicity profiles of various kinase inhibitors on malignant gliomas



Suppl. Figure 3:
Toxicity profiles of various kinase inhibitors on malignant gliomas. A, Molecular targets of sunitinib-induced gliomatoxicity have been tested with specific inhibitors. Representative images of cell death in malignant glioma cells under control conditions (con, untreated), and treatment with 20 µM imatinib, 20 µM wortmannin, 20 µM bryostatin, 20 µM salirasib and 25 µM orantinib. **B**, Validation of dose-responses of Orantinib in human gliomas. Orantinib, a PDGFR β inhibitor does not affect glioma cell proliferation at concentrations of 1 µM, 5 µM, 10 µM, and 20 µM. Left, orantinib dose-response curve is given for T98G glioma cells. Right, orantinib dose-response curve is given for U87 glioma cells. Quantification is given for at least n = 12 per group. Values are given as mean ± S.D. with controls set as 100%. Differences were considered statistically significant with $p < 0.05$ (asterisks, two-sided Student's *t*-test).

Extended dose-response analysis in glioma cells



Suppl. Figure 4:
Extended dose-response analysis in glioma cells. Glioma cells were treated with increasing concentrations of specific kinase inhibitors and tumor cell viability was evaluated by the MTT assay. **A**, Imatinib is an inhibitor of the c-Kit pathway and affects glioma cell survival solely at higher concentrations. Imatinib was tested at 10 µM, 20 µM, 50 µM, and 100 µM. **B**, Wortmannin, an inhibitor of PI3 kinase, inhibits glioma cell proliferation in a concentration-dependent manner at higher concentrations. Wortmannin was tested at 10 µM, 20 µM, 50 µM, and 100 µM. Quantification is given for at least n = 12 per group. Values are given as mean ± S.D. with controls set as 100%. Differences were considered statistically significant with $p < 0.05$ (asterisks, two-sided Student's *t*-test).

Details and comparison of various kinase inhibitors used in this study

Inhibitor	Target	MW	Cytotoxicity IC_{90}
SUNI (Sunitinib)	pan RTK	532	10 µM
Bryostatin	PKC	905	15 nM
Imatinib	c-kit	589	1 µM
Salirasib	Ras	358	100 µM
SU1498	VEGFR2	390	
Orantinib	PDGFRß	310	
Wortmannin	PI3K	428	250 nM

Suppl. Table 1:
Details and comparison of various kinase inhibitors used in this study. Kinase inhibitors and their alternative names and target structures are given. Note, that beside bryostatin all inhibitors have comparable molecular weights, which does not require dosage adaptions in comparative studies. The reported IC90 are below the levels used in this study, recruiting sufficient compound levels. References quoted in the table: Hood et al., Anticancer Drug Des. 2001, Fruehauf et al., Anticancer Drugs 2006, Santen et al., Anticancer Drugs. 2006, Kinsellaa et al., Exp Cell Res. 2012, Van et al., Respirology. 2012 and Xie et al., J Physiol. 1999.