Supplementary Data

Supplemental Figure S1. Consequences of inhibition of WNT signaling in glioblastoma cells: a less proliferative and less tumorigenic phenotype. DBTRG cells were transfected with two different amounts of β-CATENIN siRNA or a scramble siRNA control. Twenty-four hours later, cells were counted and plated for soft agar assay, and the number of colonies was scored 21 days later (A). Data represent means ± SEM. *P<0.01, **P<0.001 relative to scramble transfected cells (Tukey's Multiple Comparison Test). Protein levels of β-catenin and CyclinD1 were determined by Western blotting three days after siRNA transfections (B). Cell cycle analysis of DBTRG cells 72h after siRNA transfections (C). DBTRG cells were transiently transfected with TCF-Luciferase and TA-Renilla plasmids, alone or in combination with different amount of dnTCF4 plasmid. Results represent the ratio of Luciferase/Renilla activity, measured 24h after transfections (D). Data represent means ± SEM. *P<0.01, **P<0.001 relative to control cells (Tukey's Multiple Comparison Test). Over-expression of AXIN1 in DBTRG cells produced a strong reduction in their ability to grow in anchorage-independent fashion (E). Data represent means ± SEM. *P<0.05 relative to control cells (Student's t test).

Supplemental Figure S2. Effects of different compound AXIN stabilizers on TNKS1 and TNKS2. IC_{50} of SEN461 and XAV939 on *in vitro* auto-PARsylation of TNKS1 and TNKS2 (A). DBTRG cells were treated overnight with different amount of SEN461 and XAV939. Lysates were then analyzed by Western blotting with anti-TNKS (B).

Supplemental Figure S3. Comparative WNT transcriptional activity and growth inhibition with different compound AXIN stabilizers. DBTRG cells stably transfected for TCF-Luciferase and TA-Renilla were exposed to different amount of SEN461, XAV939 and IWR2 and reporter

activity was measured 24h later. The half-maximal inhibitory concentration (IC_{50}) for DBTRG cells is shown, determined from the soft agar assay.

Supplemental Figure S4. SEN461 affects canonical WNT ligands mediated transcription. HEK293 cells, transiently transfected with TCF-Luciferase and TA-Renilla and *WNT3A* and *LRP6* expression plasmids were either treated with DMSO (vehicle) or different amounts of SEN461. The data showed potent inhibition of WNT transcriptional activity, without affecting WNT-independent TA-Renilla activity. The data represent the average of two independent experiments (each data point is done in triplicate) with standard deviations.

Supplemental Figure S5. In vitro response of T98G cells to WNT signaling inhibition. Inhibition of canonical WNT signaling by lentiviral infection with dnTCF4 produced a strong reduction in the ability of T98G cells to grow in anchorage-independent fashion (A). Inducible expression was achieved with 10ng/ml of doxycyclin added fresh on the top layer every two days. Over-expression of AXIN1 (B) phenocopies the pharmacological effect produced by SEN461 in soft agar assay. Lysate from cells transfected with AXIN1 or infected with dnTCF4 were analyzed by Western blotting with anti-AXIN1 or anti-HA respectively to confirm the presence of the exogenous proteins. Soft agar data represent means ± SEM. *P<0.05 relative to control cells (Student's t test).

Supplemental Figure S6. Average body weight graph. Average and SEM of body weights (g) are reported starting from the randomization day. All treatments were well tolerated with no significant body weight loss and signs of toxicity.