Tilak *et. al*. "Adaptor protein ShcD/SHC4 interacts with Tie2 receptor to synergistically promote glioma cell invasion" Molecular Cancer Research 2021

Supplementary Methods

Cell lysis

Cells were washed twice with 5 mL of chilled phosphate-buffered saline (PBS; pH 7.4) and lysed in 650 µL (HEK293T cells) or 350 µL (U87 or T98G cells) of Phospholipase C (PLC) buffer (10% glycerol, 50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10 mM NaPPi, 100 mM NaF, and 1% Triton X-100) supplemented with 10 µg/mL aprotinin, 10 µg/mL leupeptin, 1 mM sodium orthovanadate, and 1 mM phenylmethylsulfonyl (PMSF) (PLC^+). Lysates were centrifuged at 14,000*g* for 15 min and supernatants were stored as whole cell lysates (WCLs) at -20°C.

Immunoprecipitation and GST Pulldown Assays

Immunoprecipitation was performed overnight with rocking at 4ºC using 300-400 µL of WCL, primary antibody and 10% anti-mouse IgG-agarose beads (Sigma-Aldrich; Cat. #A6531) in a total volume of 800 µL PLC^+ buffer. Following removal of supernatant, beads were washed 3x with 800 µL of PLC^+ buffer and protein complexes were eluted in 2x SDS loading buffer by boiling at 100ºC for 5 min. GST-fusion proteins for ShcA-SH2, ShcD-SH2, and its disabled counterpart ShcD-SH2* respectively, were expressed in *Escherichia coli* BL21 cells by overnight induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 37ºC and purified using glutathione-Sepharose™ 4B beads (GE Healthcare; Cat. #GE17-0756-01). Approximately 10 µg of purified GST-fusion proteins was then incubated with lysates from HEK293T cells transiently

transfected with wild-type Tie2 overnight at 4ºC on a nutator and processed as described above for immunoprecipitation.

Western blotting

WCLs from HEK293Ts or stable U87/T98G cells were used to prepare samples for separation with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by diluting 50-80 µL of WCL in 2x or 5x sodium dodecyl sulfate (SDS) loading buffer (50% glycerol, 300 mM Tris, 10% SDS, 25% ß-mercaptoethanol) and boiling at 100°C for 5min. For Western blotting, proteins from WCLs were first resolved on 8% SDS-polyacrylamide gels according to molecular weights (KDa) and then transferred to polyvinylidene fluoride (PVDF) membranes using a semi-dry transfer protocol. Membranes were blocked for 45 min in 1x TBST [10x TBST (0.02 M Tris, 0.15 M NaCl, 0.5% Tween 20) diluted with MiliQ water] containing 5% milk (or 5% BSA for phosphorylation-detecting antibodies) and incubated overnight at 4ºC on a nutator with primary antibody. The next day, membranes were washed 3x for 10 min in 1x TBST and incubated with secondary antibody for 1 hr at room temperature (RT) followed by another set of washes with 1x TBST. Pierce™ ECL Western Blotting Substrate kit (Cat. #32106) was used for blot detection by exposing membranes to film (Pierce™).

WST-1 proliferation assay

U87 or T98G cells stably expressing wild-type or mutant ShcD or wild-type Tie2 or both (as well as Control) were seeded at density of $1.5x10^3$ cells/well in triplicate in a 96-well plate in DMEM containing 2% FBS and allowed to grow for 24 hrs at 37°C and 5% CO_2. After 24 hrs, 10 µL of WST-1 reagent (Roche; Cat. #05-015-944-001) was added and incubated for 30 min ($t = 0$). Absorbance values (540 nm and 630 nm) were measured over 24 hrs to quantify proliferation.