

Supplementary Figures

Figure S1

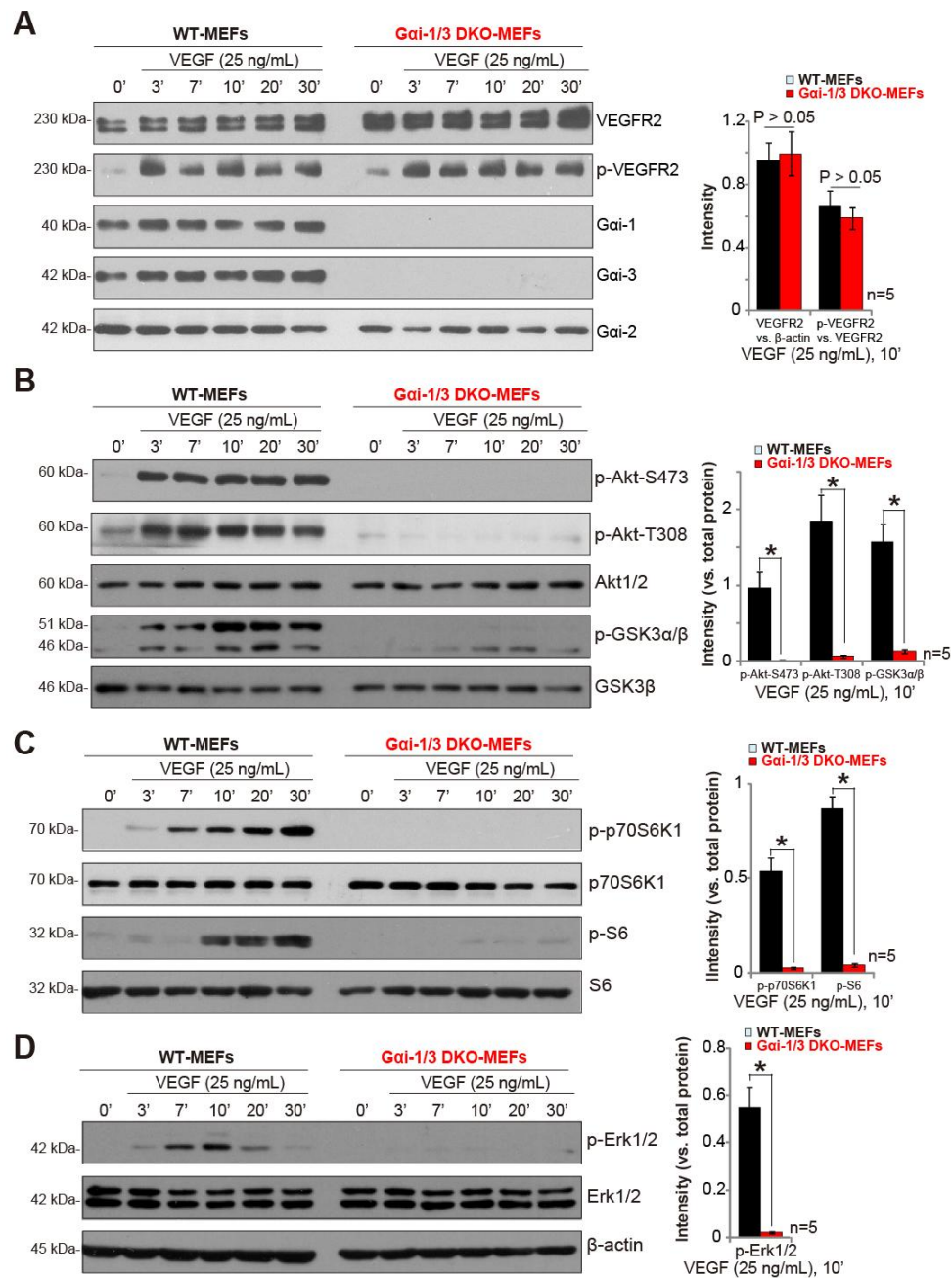


Figure S1. Gai1 and Gai3 double knockout abolishes VEGF-induced Akt-mTORC1 and Erk activation in mouse embryonic fibroblasts. (A-D) The wild-type (WT) or Gai1 and Gai3 double knockout (DKO) mouse embryonic fibroblasts (MEFs) were treated with VEGF ("A", at applied concentration for indicate time), and were tested by Western blotting assay of listed proteins. For all the Western blotting assay, each lane was loaded with exact same amount of quantified protein lysates (30 μ g per treatment). $*P < 0.05$.

Figure S2

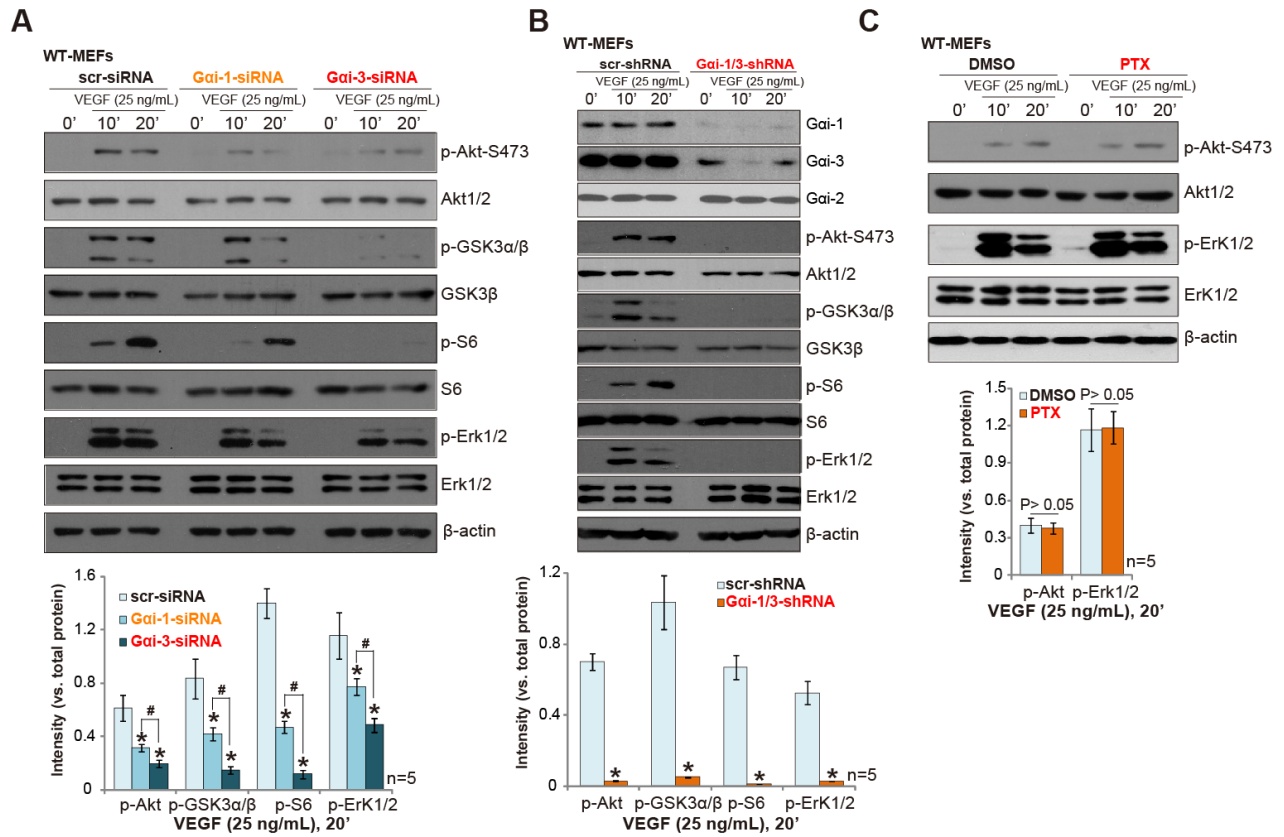


Figure S2. Knockdown of Gai1/3 inhibits VEGF-induced Akt-mTORC1 and Erk activation in mouse embryonic fibroblasts. (A) WT mouse embryonic fibroblasts (MEFs) were transfected with 100 nM of scramble control siRNA (scr-siRNA), Gai1 or Gai3 siRNA (24 hours per round, 2 rounds), cells were then treated with VEGF ("A", 25 ng/mL) for indicated time, and were tested by Western blotting assay of listed proteins. **(B)** The puromycin-selected stable MEFs, expressing Gai1 and Gai3 shRNA or the scramble control shRNA ("scr-shRNA"), were treated with VEGF ("A", 25 ng/mL) for indicated time, and were tested by Western blotting assay of listed proteins. **(C)** WT MEFs were pretreated overnight with pertussis toxin (PTX, 100 ng/mL), followed by indicated VEGF treatment, listed proteins were tested. *P < 0.05 vs. "scr-siRNA" (A). *P < 0.05 vs. "scr-shRNA" (B). #P < 0.05 (A).

Figure S3

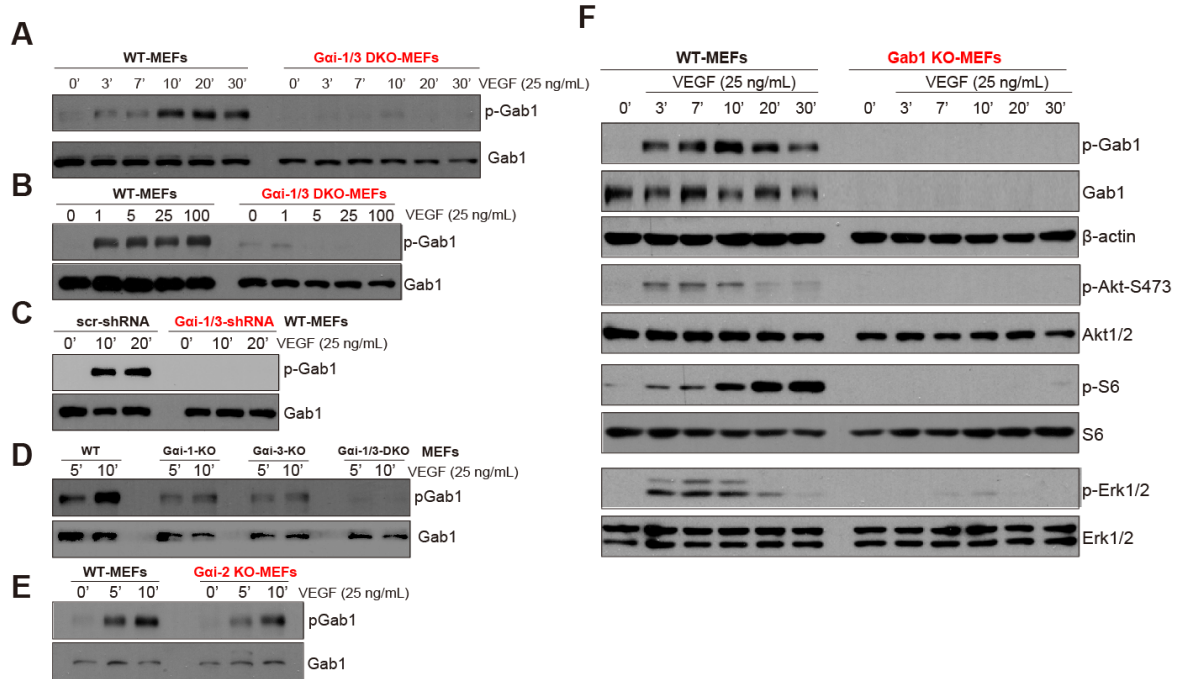


Figure S3. Gab1 lies downstream of *Gai1/3* in VEGFR2 signaling in mouse embryonic fibroblasts. (A-B) WT and DKO MEFs were treated with VEGF (“-A”, at tested concentration) for indicated time, p-Gab1 and total Gab1 in total cell lysates were tested by Western blotting assay. (C) The puromycin-selected stable MEFs, expressing lentiviral *Gai1* and *Gai3* shRNA or the scramble control shRNA (“scr-shRNA”), were treated with VEGF (“-A”, 25 ng/mL), p-Gab1 and total Gab1 were shown. (D-E) WT MEFs, *Gai1* or *Gai3* single knockout (SKO) MEFs, *Gai2* SKO MEFs, and *Gai1/3* double knockout (DKO) MEFs were treated with VEGF (“-A”, 25 ng/mL), p-Gab1 and total Gab1 expression were shown. (F) WT MEFs or *Gab1* knockout MEFs were treated with VEGF (“-A”, 25 ng/mL), and were tested by Western blotting assay of listed proteins.

Figure S4

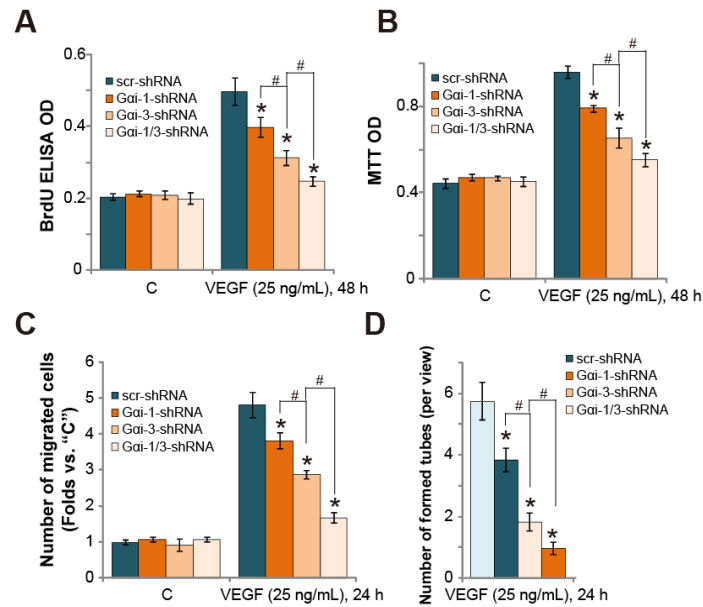


Figure S4. Gai1 shRNA or Gai3 shRNA inhibits VEGF-induced proliferation, migration and vessel-like tube formation in HUVECs. Stable HUVECs, expressing scramble control shRNA (“scr-shRNA”), lentiviral Gai1 and/or Gai3 shRNA, were treated with/without VEGF (“-A”, 25 ng/mL) for indicated time, cell proliferation was tested by BrdU ELISA assay (**A**) and MTT OD assay (**B**); Cell migration was tested by “Transwell” assay (**C**); Vessel-like tube formation results were also quantified (**D**). For the “Transwell” assay, ten random views (1: 200) of each condition were included to calculate average number of invaded cells (**C**). For the vessel-like tube formation assay, 25 random views (1: 200) were included to calculate average the number of formed-vessels (**D**). * $P < 0.05$ vs. HUVECs with “scr-shRNA”. # $P < 0.05$.