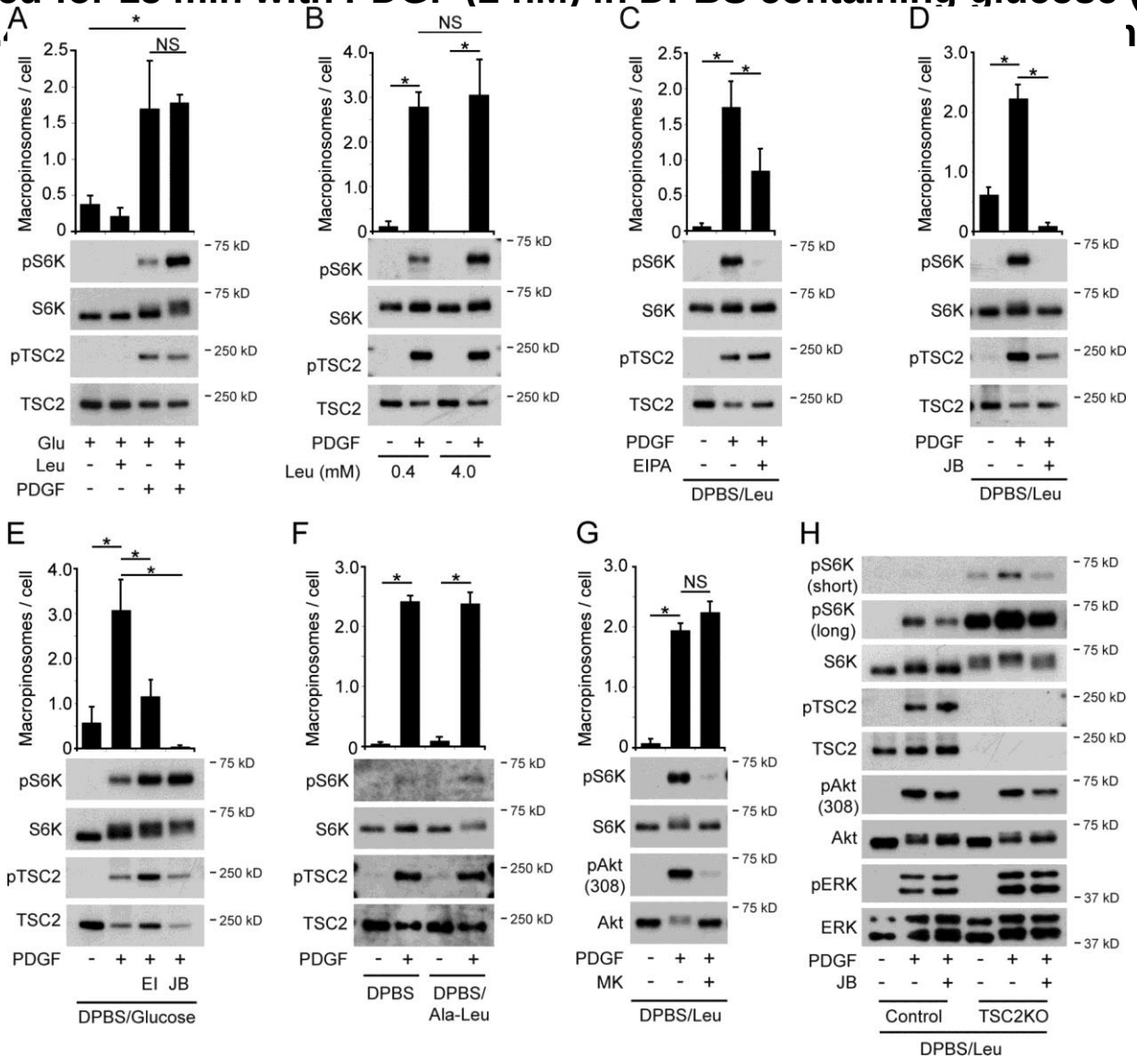
**Macropinocytosis is required for leucine-dependent activation of mTORC1 by PDGF in MEFs. (A) Amino acid–dependent activation of mTORC1 by PDGF. MEFs were incubated 30 min in DPBS and then stimulated for 15 min with PDGF (2 nM) in DPBS containing glucose (Glu; 5.6 mM) and leucine (Leu; 0.4 mM). Macropinocytosis and mTORC1 activity**

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**Macropinocytosis is required for leucine-dependent activation of mTORC1 by PDGF in MEFs.** (A) Amino acid–dependent activation of mTORC1 by PDGF. MEFs were incubated 30 min in DPBS and then stimulated for 15 min with PDGF (2 nM) in DPBS containing glucose (Glu; 5.6 mM) and leucine (Leu; 0.4 mM) and scored for macropinosome formation (top) and mTORC1 activity (bottom). (B) Effects of leucine concentration on macropinocytosis (top) and mTORC1 activity (bottom). (C and D) Stimulation of macropinocytosis and mTORC1 by PDGF and 0.4 mM leucine were inhibited by EIPA (C) and JB (D). (E) Stimulation of mTORC1 by PDGF and glucose (5.6 mM) was not inhibited by EIPA (EI) or JB. (F) Activation of mTORC1 after 30 min in PDGF and Ala-Leu. MEFs were incubated in DPBS for 30 min, followed by 30 min in DPBS, with or without PDGF, with or without 4 mM Ala-Leu. (G) Effects of Akt inhibitor MK2206 (MK; 2 μM for 30 min) on macropinocytosis (top), Akt activity, and mTORC1 (bottom). Macropinocytosis measurements of A–G show the means ± SEM from three independent experiments, with >25 cells scored per condition. *, $P < 0.05$, one-tailed *t* test. (H) Stimulation of mTORC1 in TSC2-deficient (TSC2KO) and WT MEFs. mTORC1 activity in TSC2KO MEFs was increased by PDGF and inhibited by JB.