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Supplementary Materials for

TACE Activation by MAPK-Mediated Regulation of Cell Surface Dimerization and TIMP3 Association

Pinglong Xu, Jianming Liu, Masayo Sakaki-Yumoto, Rik Derynck*

*To whom correspondence should be addressed. E-mail: rik.derynck@ucsf.edu

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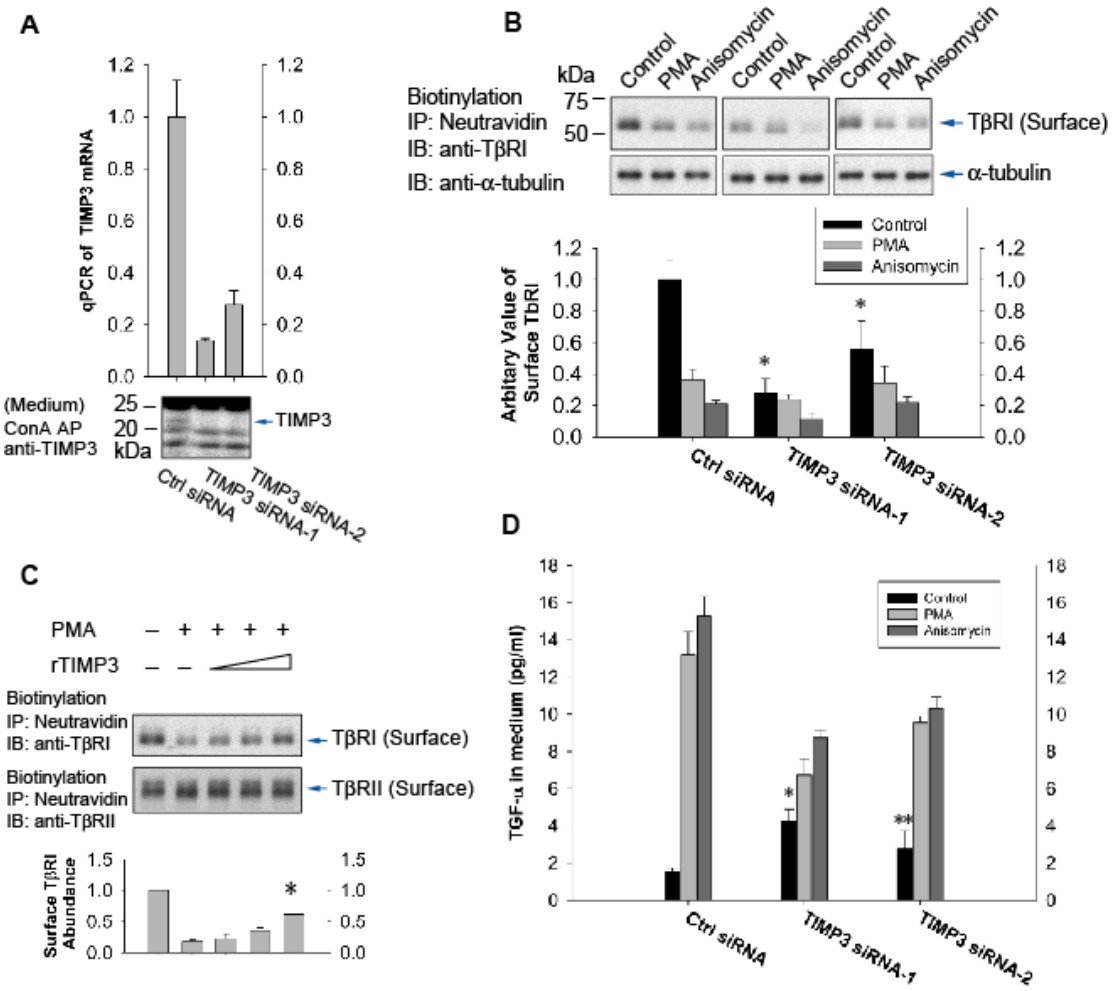
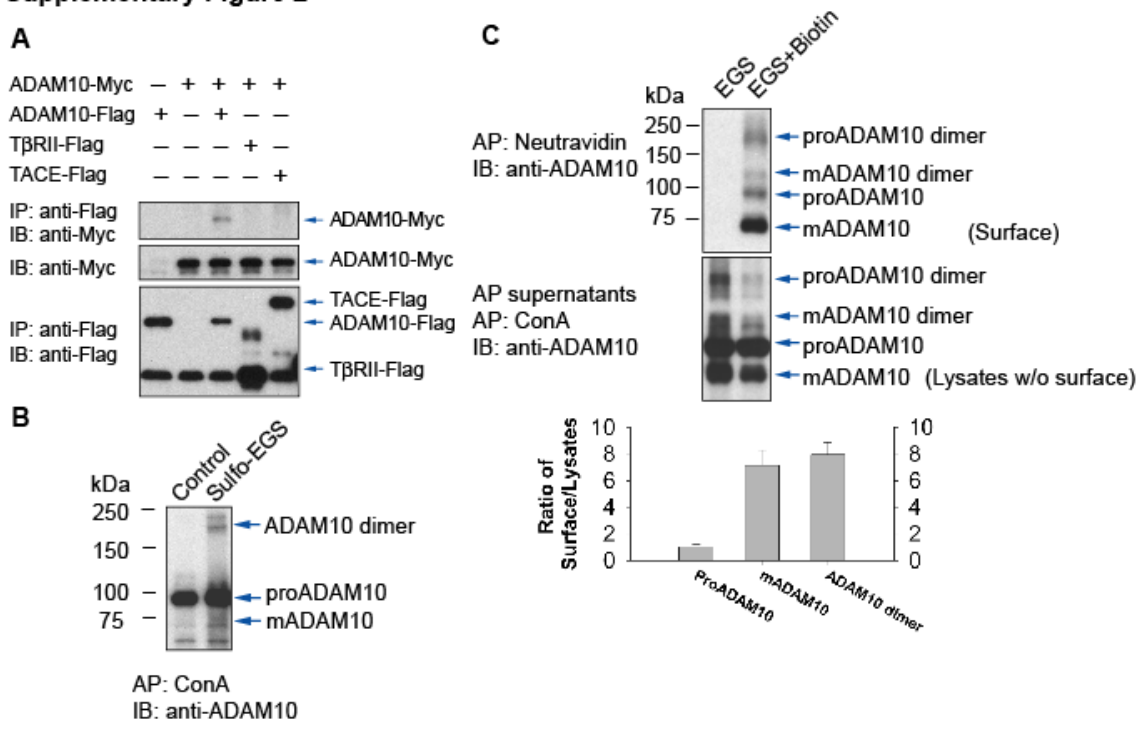
The PDF file includes:

Supplementary Figure 1



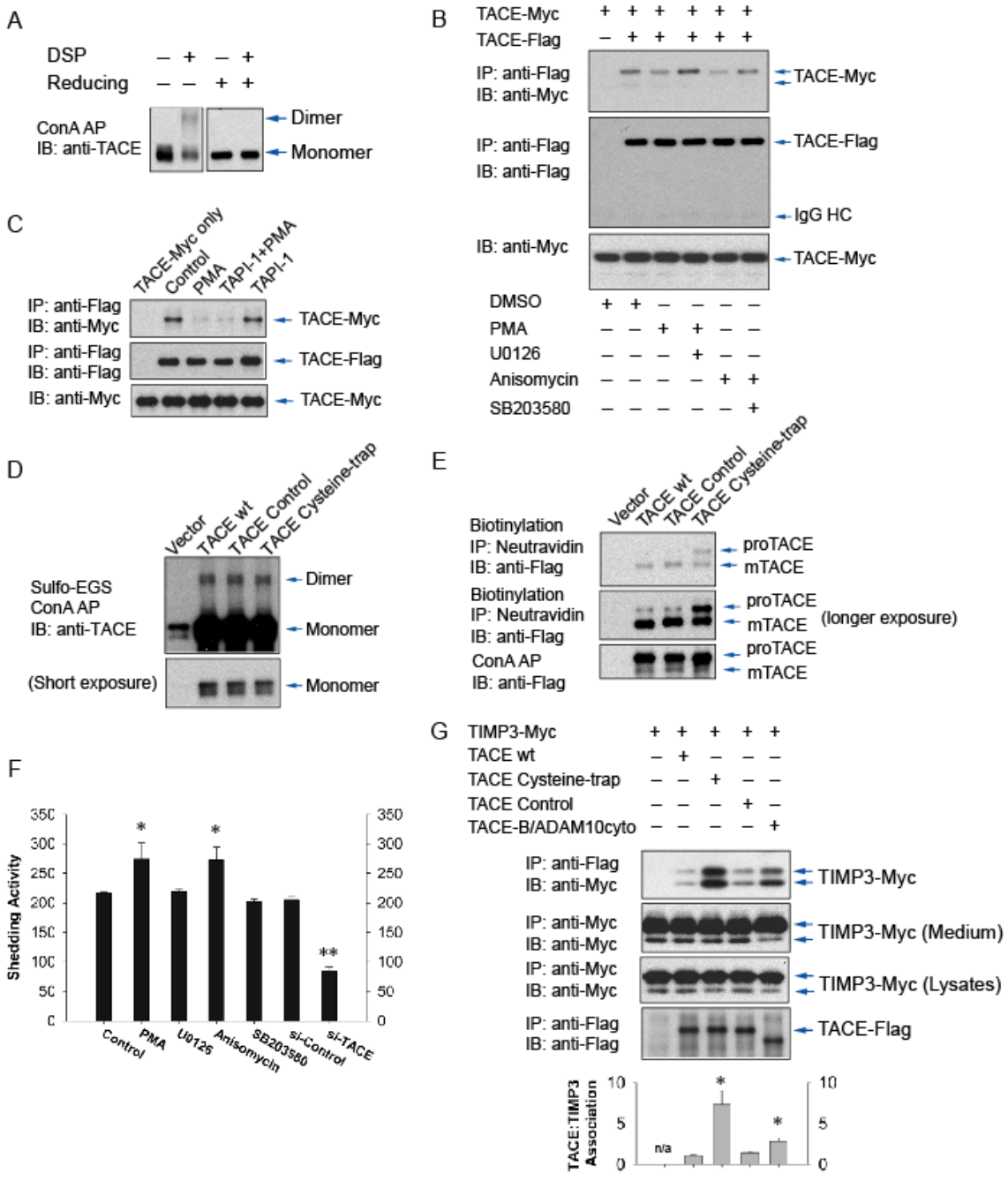
Supplementary Figure 1. TIMP3 regulates TACE-mediated shedding of endogenous substrates. T4-2 cells were transfected with control or TIMP3 siRNA. **(A)** Endogenous TIMP3 mRNA level (upper panel) and secreted TIMP3 (lower panel) were decreased upon transfection of TIMP3 siRNA. TIMP3 mRNA was measured by qRT-PCR (upper panel), and endogenous TIMP3 in medium was assessed by concanavalin A affinity precipitation and anti-TIMP3 immunoblotting. Only the lower MW form (22-23 kD) of TIMP3 is apparent, because the higher MW form is covered by the IgG light chain band. **(B)** Endogenous TβRI at the cell surface, revealed by cell surface biotinylation, Neutravidin precipitation, and immunoblotting. Relative TβRI abundance at the cell surface was quantified by density scanning (lower panel). N=3 blots; t-tests were performed. *, $P < 0.05$, compared with control siRNA. **(C)** Effects of increasing amounts of TIMP3, added for 30 min, on PMA-induced shedding of endogenous TβRI, revealed as in (B). Endogenous cell surface TβRII, which is not a substrate of TACE (1), is shown as control. Relative TβRI abundance at the cell surface was quantified by density scanning (lower panel). N=4 blots; t-tests were performed. *, $P < 0.01$, compared with PMA treatment without TIMP3. **(D)** Endogenous TGF-α secreted into in medium, released after 60 min treatment of cells with PMA or anisomycin. Soluble TGF-α was quantified by ELISA. N=3 ELISA assays; t-tests were performed. *, $P < 0.01$, compared with control siRNA.

Supplementary Figure 2



Supplementary Figure 2. ADAM10 forms dimers at the cell surface. (A) Cα cells expressing Myc-tagged ADAM10 and Flag-tagged ADAM10, TβRII or TACE were analyzed by anti-Flag immunoprecipitation and anti-Myc immunoblotting, revealing association of Myc-tagged with Flag-tagged ADAM10. **(B)** Cα cells were treated with sulfo-EGS or control solvent, and endogenous ADAM10 was visualized by immunoblotting of concanavalin A affinity-purified proteins, showing monomers and presumed dimers. **(C)** Cα cells were treated simultaneously with sulfo-EGS and EZ-link sulfo-NHS-LC-biotin. Biotinylated cell surface proteins were adsorbed to Neutravidin beads, and endogenous ADAM10 was visualized by immunoblotting (upper panel). The remaining cell lysate was subjected to concanavalin A affinity purification and anti-ADAM10 immunoblotting (middle panel). Relative dimer and monomer abundance of TACE at the cell surface versus the supernatant (lane 2) were quantified by density scanning (lower panel), N=3 blots.

Supplementary Figure 3



Supplementary Figure 3. **TACE dimerization, surface TACE abundance, TACE activity, and TACE:TIMP3 association. (A)** The TACE dimer, which was detected following treatment of the cells with the cross-linker DSP, was absent after disulfide reduction. Cα cells expressing TACE were treated with the membrane-permeable DSP or control solvent, prior to cell lysis and concanavalin A affinity precipitation, thus enriching glycosylated proteins, including TACE. TACE was visualized by immunoblotting after reducing or non-reducing SDS-PAGE, showing TACE monomers and TACE dimers in cells treated with DSP, but no dimers upon reduction. **(B)** HepG2 cells expressing

Myc-tagged and Flag-tagged TACE were treated with PMA or anisomycin without or with the MEK inhibitor U0126 or p38 MAPK inhibitor SB203580. Anti-Flag immunoprecipitated proteins were immunoblotted with anti-Myc or anti-Flag to visualize dimerization of Myc-tagged with Flag-tagged TACE. The abundance of Myc-tagged TACE was shown by immunoblotting of cell lysates. **(C)** HepG2 cells expressing Myc-tagged and Flag-tagged TACE were treated with PMA without or with the matrix metalloprotease inhibitor TAPI-1. Anti-Flag immunoprecipitated proteins were immunoblotted with anti-Myc or anti-Flag to visualize Myc-tagged TACE association with Flag-tagged TACE. Immunoblotting of cell lysates revealed the expression of Myc-tagged TACE. **(D)** Dimerization of wild-type, cysteine-trap and control mutant TACE. Cα cells expressing wild-type TACE, the cysteine-trap mutant, or its control mutant were subjected to cross-linking with sulfo-EGS. Glycosylated proteins including TACE were enriched by concanavalin A beads, and immunblotted using anti-TACE antibody (upper panel). A short exposure of the same experiment shows the abundance of wild-type and mutant TACE (lower panel). **(E)** Cell surface amounts of wild-type, cysteine-trap and control mutant TACE. Cα cells expressing TACE wild-type, cysteine-trap or control mutant TACE were subjected to cell surface biotinylation. Biotinylated proteins were adsorbed to Neutravidin beads, and immunoblotted using anti-Flag antibody (upper panel). The supernatants were adsorbed to concanavalin A beads, and analyzed by immunoblotting using anti-Flag antibody, to evaluate total TACE abundance (lower panel). **(F)** HepG2 cells were transfected with control or TACE siRNA, and treated with PMA or anisomycin for 30 min, or the MAPK inhibitors U0126 or SB203580 for 3 hours, as indicated, and their in vitro shedding activity was assessed by anti-TACE immunopurification and ELISA. N=3 ELISA assays; t-tests were performed. *, P <0.05, ** P<0.01, compared with control siRNA and solvent treatment. **(G)** Cα cells expresssing Flag-tagged wild-type, cysteine-trap, control mutant TACE, or TACE-B/ADAM10cyto chimera, with Myc-tagged TIMP3 were lyzed and subjected to anti-Flag immunoprecipitation, followed by anti-Myc immunoblotting. The abundance of TACE and TIMP3 in cell lysates, and TIMP3 in the conditioned media, were shown by immunoblotting. Ratios of TACE:TIMP3 association were obtained by density scanning. N=3 experiments; t-tests were performed. *, $P < 0.01$, compared with wild-type TACE.