Supplemental Materials

Molecular Biology of the Cell

Elderbroom et al.

Supplemental Table 1: Mutagenesis primers for TβRIII NAAIRS mutants

Mutation:	*Location:*	*Forward Primer Sequence:*	*Reverse Primer Sequence:*
M0	AA 683-688	cccgcaagctgac aatgctgctatacgatcg ctttgtcttcaagc	gcttgaagacaaag cgatcgtatagcagcatt gtcagcttgcggg
M1	AA 689-694	gataagaagcgattc aatgctgctatacgatcg gtcttcaacacc	ggtgttgaagac cgatcgtatagcagcatt gaatcgcttcttatc
M2	AA 695-700	gtcttcaagcct aatgctgctatacgatcg ctctttctacag	ctgtagaaagag cgatcgtatagcagcatt aggcttgaagac
M3	AA 701-706	caacacctcactg aatgctgctatacgatcg ctgacgctgtgtacg	cgtacacagcgtcag cgatcgtatagcagcatt cagtgaggtgttg
M4	AA 707-712	ctacagtgtgag aatgctgctatacgatcg atggagaagcacc	ggtgcttctccat cgatcgtatagcagcatt ctcacactgtag
M5	AA 713-718	gctgtgtacgaag aatgctgctatacgatcg aagttgcctaag	cttaggcaactt cgatcgtatagcagcatt cttcgtacacagc
M6	AA 719-724	gaagcacccccag aatgctgctatacgatcg cctcctgacgaagc	gcttcgtcaggagg cgatcgtatagcagcatt ctgggggtgcttc
M7	AA 725-730	cctaagtgtgtg aatgctgctatacgatcg acctcgctggac	gtccagcgaggt cgatcgtatagcagcatt cacacacttagg
M8	AA 731-736	gacgaagcctgc aatgctgctatacgatcg ataatctgggcc	ggcccagattat cgatcgtatagcagcatt gcaggcttcgtc
M9	AA 737-742	ctggacgcctcg aatgctgctatacgatcg cagaataagaag	cttcttattctg cgatcgtatagcagcatt cgaggcgtccag
M10	AA 743-748	ctgggccatgatg aatgctgctatacgatcg accaagccccttg	caaggggcttggt cgatcgtatagcagcatt catcatggcccag
M11	AA 749-754	gaagacgttc aatgctgctatacgatcg atccaccatgaagc	gcttcatggtggat cgatcgtatagcagcatt gaacgtcttc
M12	AA 755-760	ccccttgctgtg aatgctgctatacgatcg tctaaagaaaaagg	ccttttttctttaga cgatcgtatagcagcatt cacagcaagggg
M13*	AA 761-766	* Made by Mutagenex	
M14	AA 767-772	gaaaaaggtcca aatgctgctatacgatcg ccaatttctccacc	ggtggagaaattgg cgatcgtatagcagcatt tggacctttttc
M15	AA 773-778	gaaggaaccaaat aatgctgctatacgatcg ttccatggtctgg	ccagaccatggaa cgatcgtatagcagcatt atttggttccttc
M16	AA 778-783	ccaatttctccacca aatgctgctatacgatcg accctaaccgtgatg	catcacggttagggt cgatcgtatagcagcatt tggtggagaaattgg

Supplemental Table 2: Mutagenesis primers for TβRIII alanine mutants

Mutation:	*Forward Primer Sequence:*	*Reverse Primer Sequence:*
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s699a	gtcttcaacacc gcc ctgctctttctac	gtagaaagagcag ggc ggtgttgaagac
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t749a	gaagacgttc gcc aagccccttgc	gcaaggggctt ggc gaacgtcttc
k750a	gaagacgttcacc gcc ccccttgctgtg	cacagcaagggg ggc ggtgaacgtcttc
p751a	cgttcaccaag gcc cttgctgtgatc	gatcacagcaag ggc cttggtgaacg
l752a	caccaagccc gcc gctgtgatccac	gtggatcacagc ggc gggcttggtg
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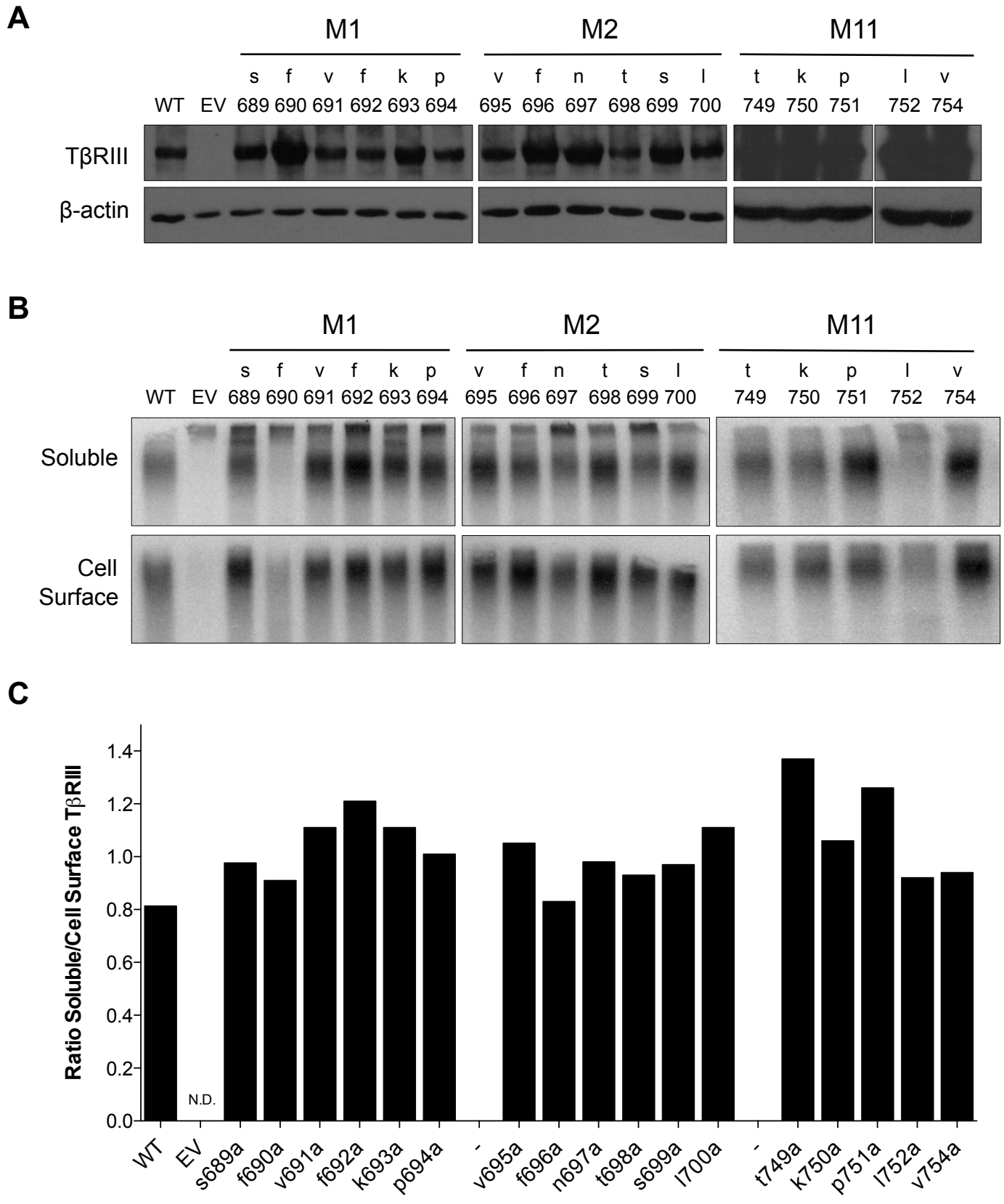
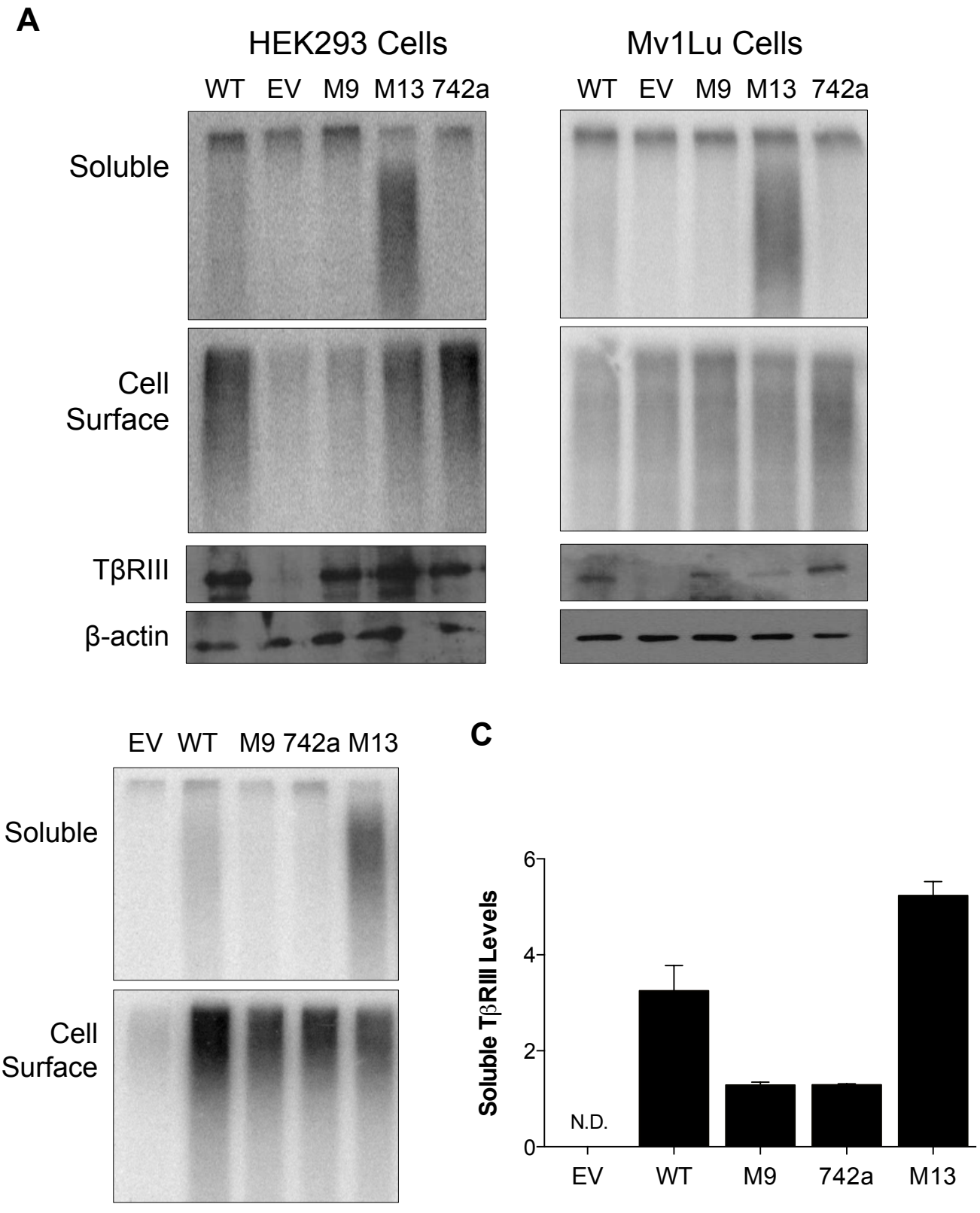
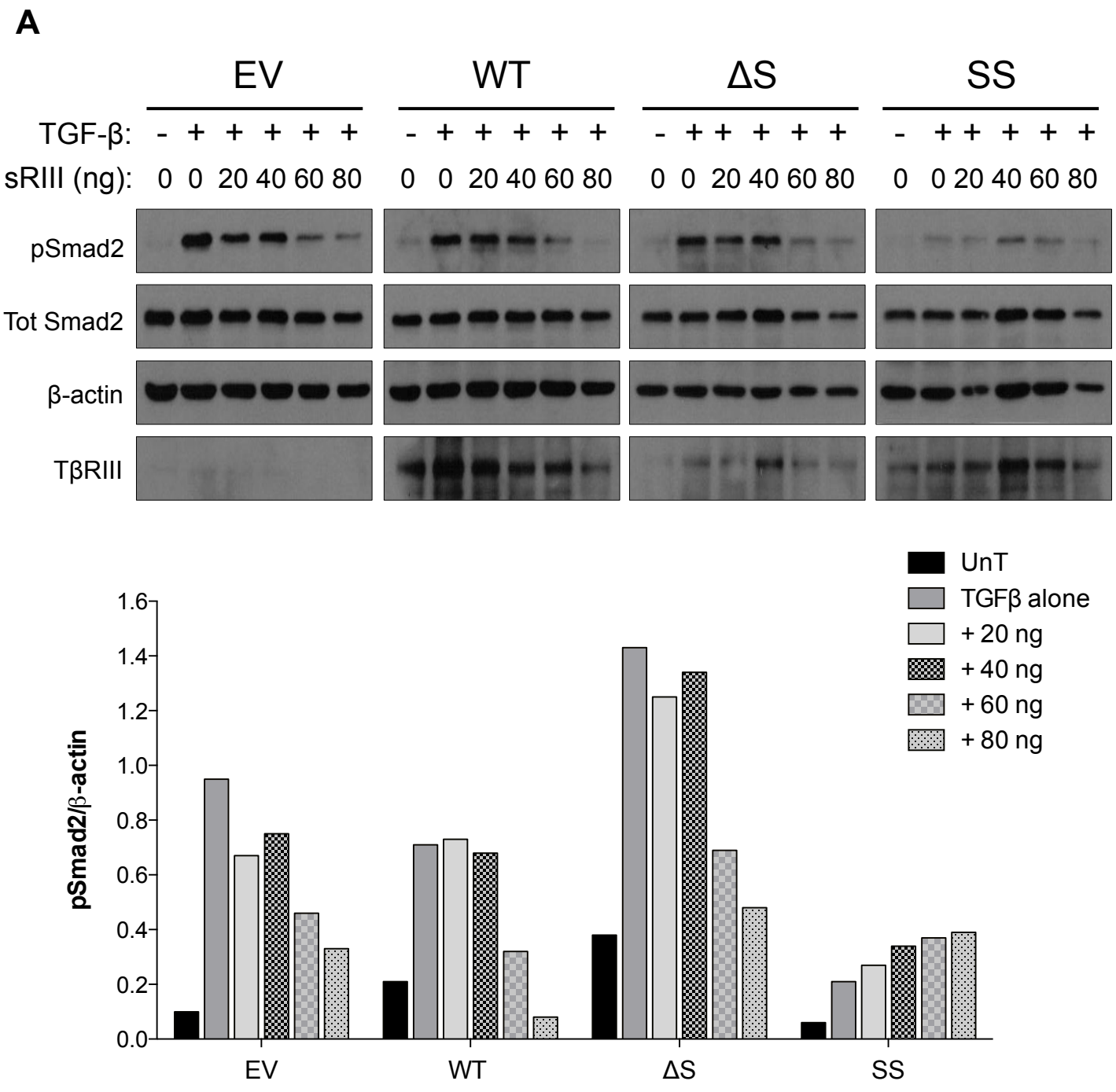


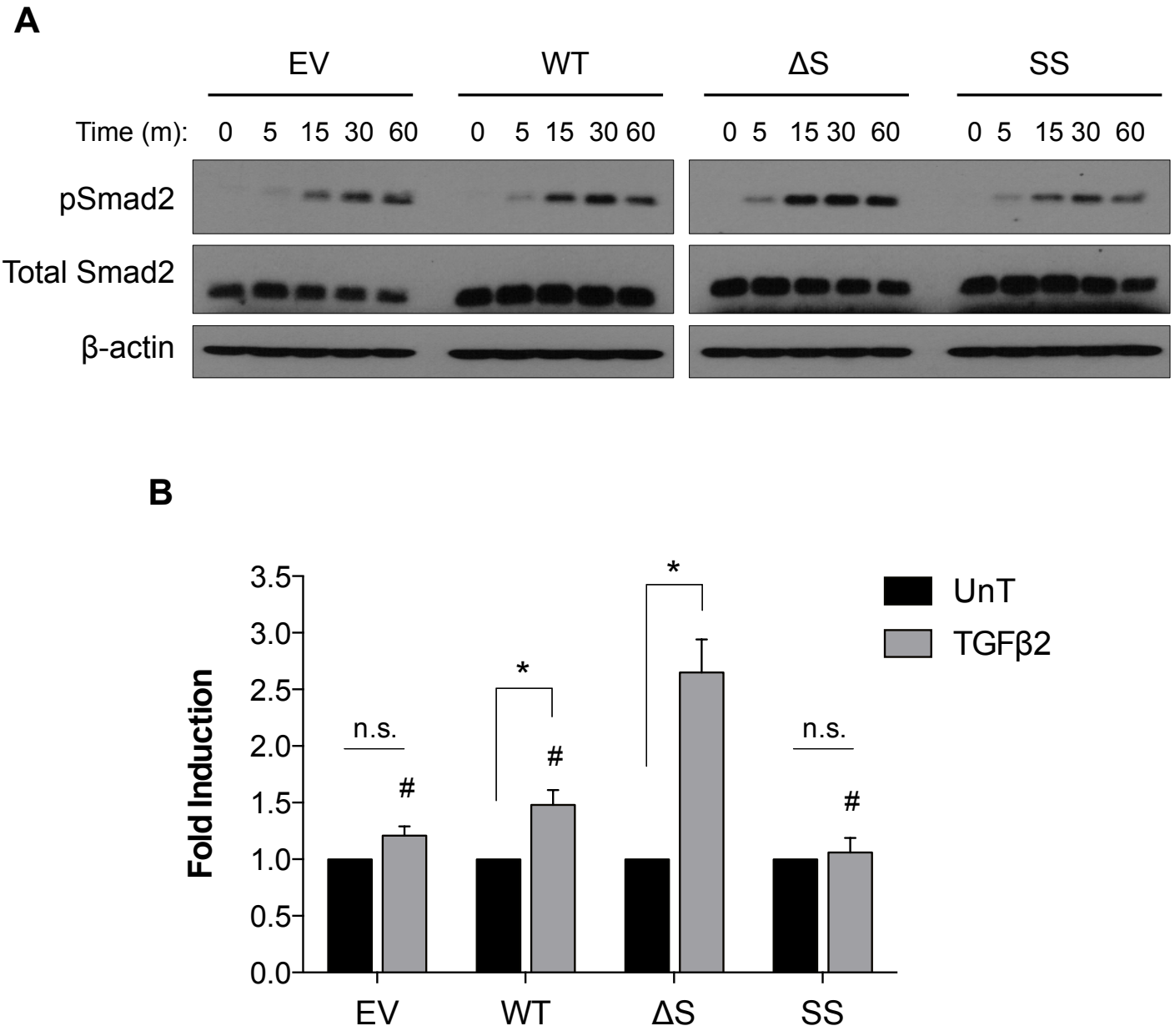
Supplemental Figure 1: *No single point mutations within M1, M2 or M11 significantly altered TβRIII ectodomain shedding.* (A) Western blot of transiently transfected WT-TβRIII or alanine mutants in COS7 cells. β-actin was used as a loading control. (B) Binding and crosslinking of transiently transfected WT-TβRIII or alanine mutants in COS7 cells. 24 h after transfection media was changed to full serum media and allowed to condition overnight. Following ^{125}I-TGF-β1 binding and crosslinking, lysates and conditioned media were immunoprecipitated with an antibody against HA. (C) Quantification of *(B)*. Data shown as densitometry of soluble TβRIII/cell surface TβRIII. Representative image from 2 independent experiments.



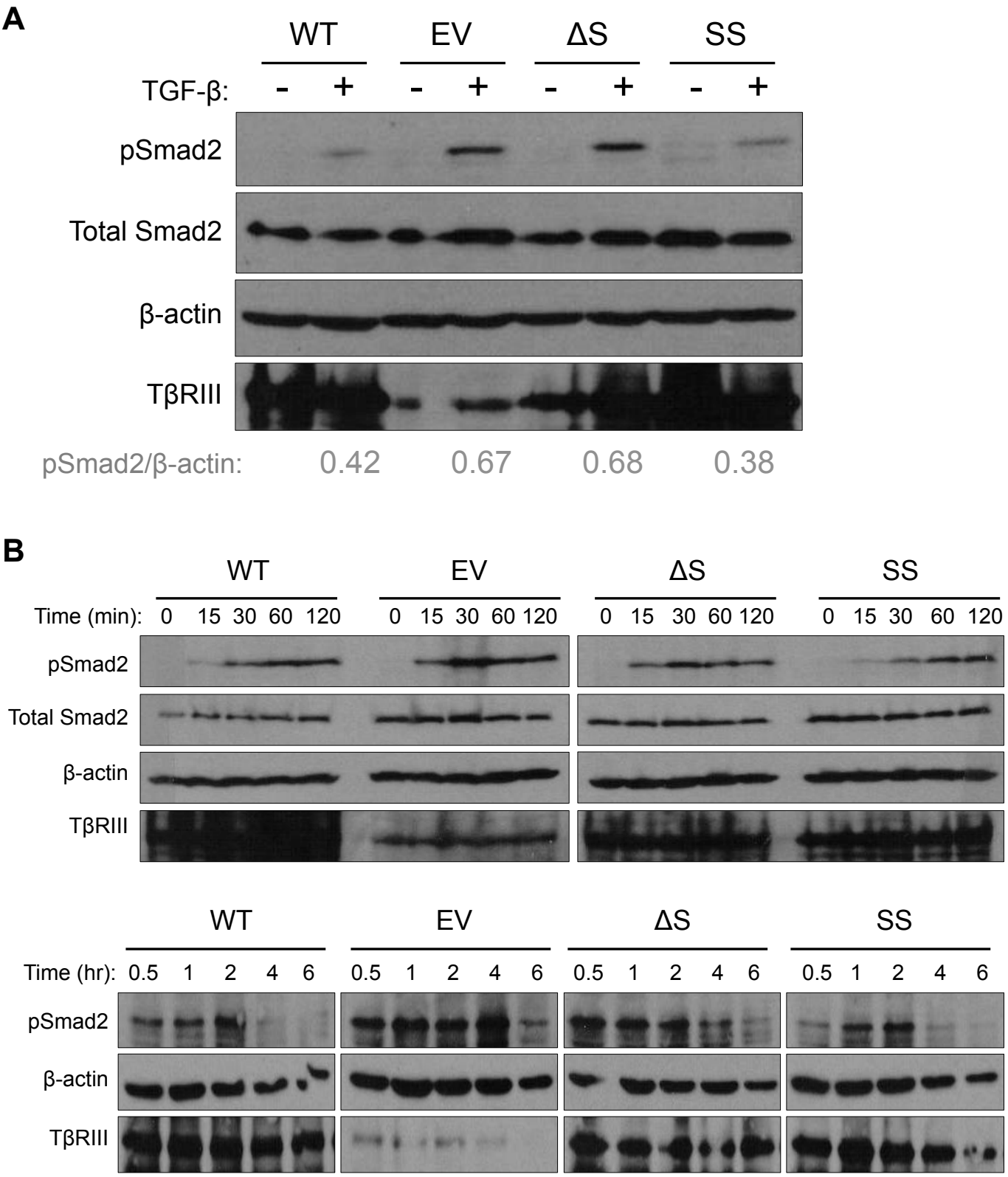
Supplemental Figure 2: *Alterations in TβRIII ectodomain shedding in shedding mutants is maintained in multiple cell lines.* (A) Binding and crosslinking of HEK293 and Mv1Lu cells. Cells were transiently transfected with EV, WT-TβRIII or shedding mutants and 24 h later media was replaced and allowed to condition for 20 h. Following ^{125}I-TGF-β1 binding and crosslinking, lysates and conditioned media were immunoprecipitated with an antibody against TβRIII. TβRIII and β-actin western blots shown as loading controls. Representative images from 2 independent experiments. (B) Binding and crosslinking of final shedding mutants in COS7 cells, performed as in *(A)*. Representative images from 2 independent experiments. (C) ELISA data from media conditioned from transiently transfected COS7 cells for 20 h. Data from 3 independent experiments are shown as levels of soluble TβRIII in the conditioned media normalized to β-actin loading controls from the corresponding cell lysates.



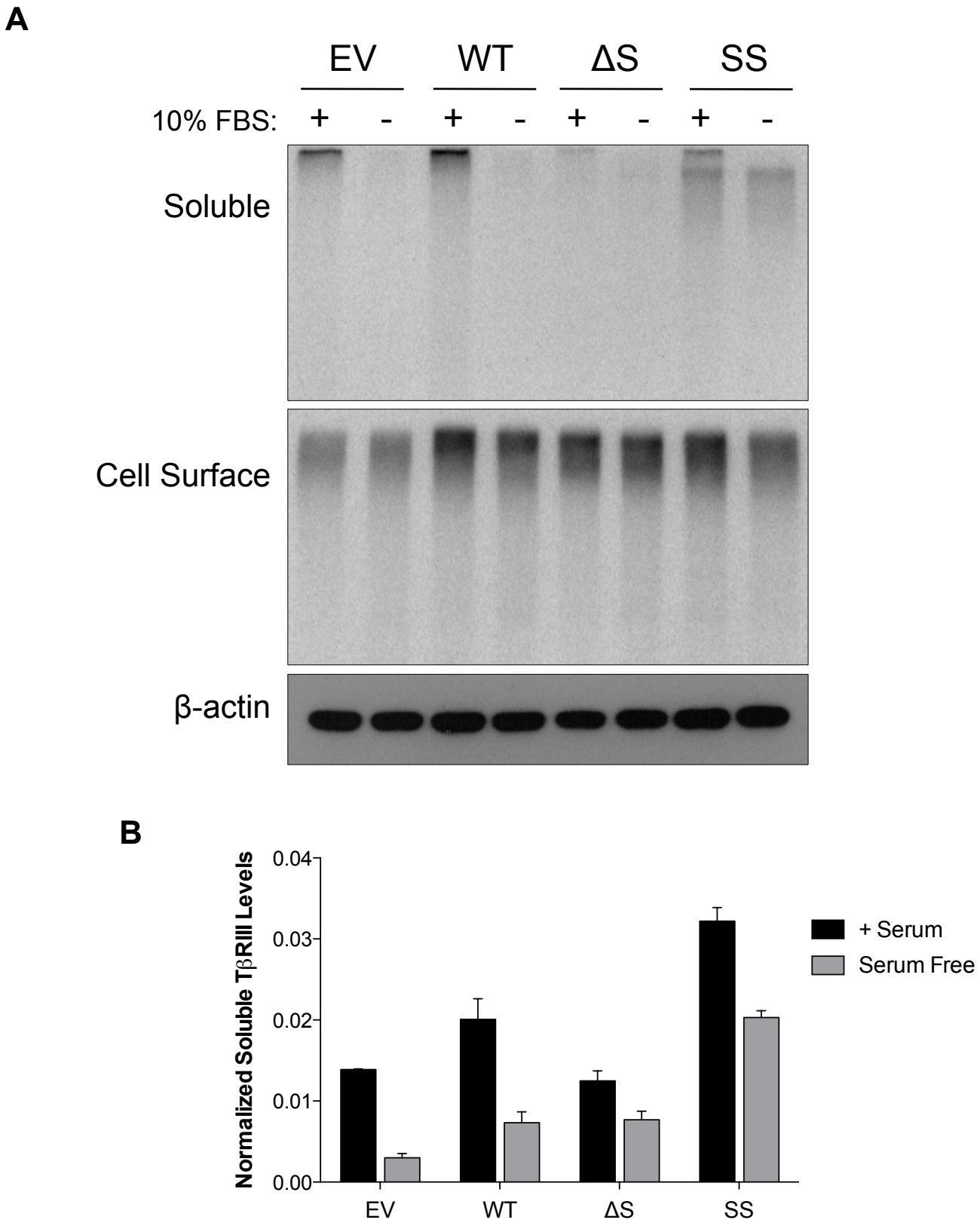
Supplemental Figure 3: *TGF-β signaling is reduced by exogenous soluble TβRIII.* (A) Lentiviral stable MDA-MB-231 cell lines were plated in full serum media and allowed to condition for 20 h before treatment with 50 pM TGF-β1 and indicated amounts of exogenous soluble TβRIII for 30 minutes. Western blot analysis performed with indicated antibodies. TβRIII and β-actin western blots shown as loading controls. Representative image from 2 independent experiments. Densitometric analysis of pSmad2/β-actin control shown below.



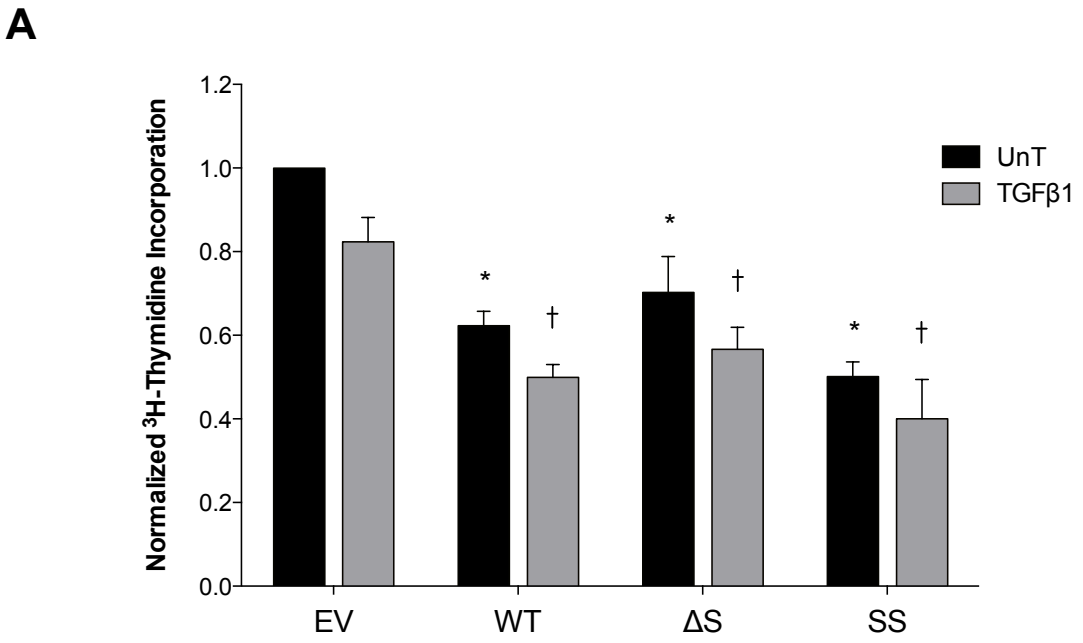
Supplemental Figure 4: *WT-TβRIII and ΔS-TβRIII sensitize MDA-MB-231 cells to TGF-β2.* (A) Lentiviral stable MDA-MB-231 cell lines were plated in full serum media and allowed to condition for 20 h before treatment with 50 pM TGF-β2 for the indicated time periods. Western blot analysis performed with indicated antibodies. Representative images from 2 independent experiments. (B) Stable MDA-MB-231 cell lines were transfected with a pE2.1 responsive luciferase construct and a renilla construct. The following day cells were treated with media that had been pre-conditioned from the corresponding cell line for 24 h and 50 pM TGF-β2. Cells were treated for 24 h. Results from 4 independent experiments are shown as pE2.1/renilla activity, and normalized to ligand untreated condition of each cell line. * One-sample t-test $p < 0.05$ relative to UnT. # Two-tailed *t*-test $p < 0.05$ relative to ΔS + TGF-β2.



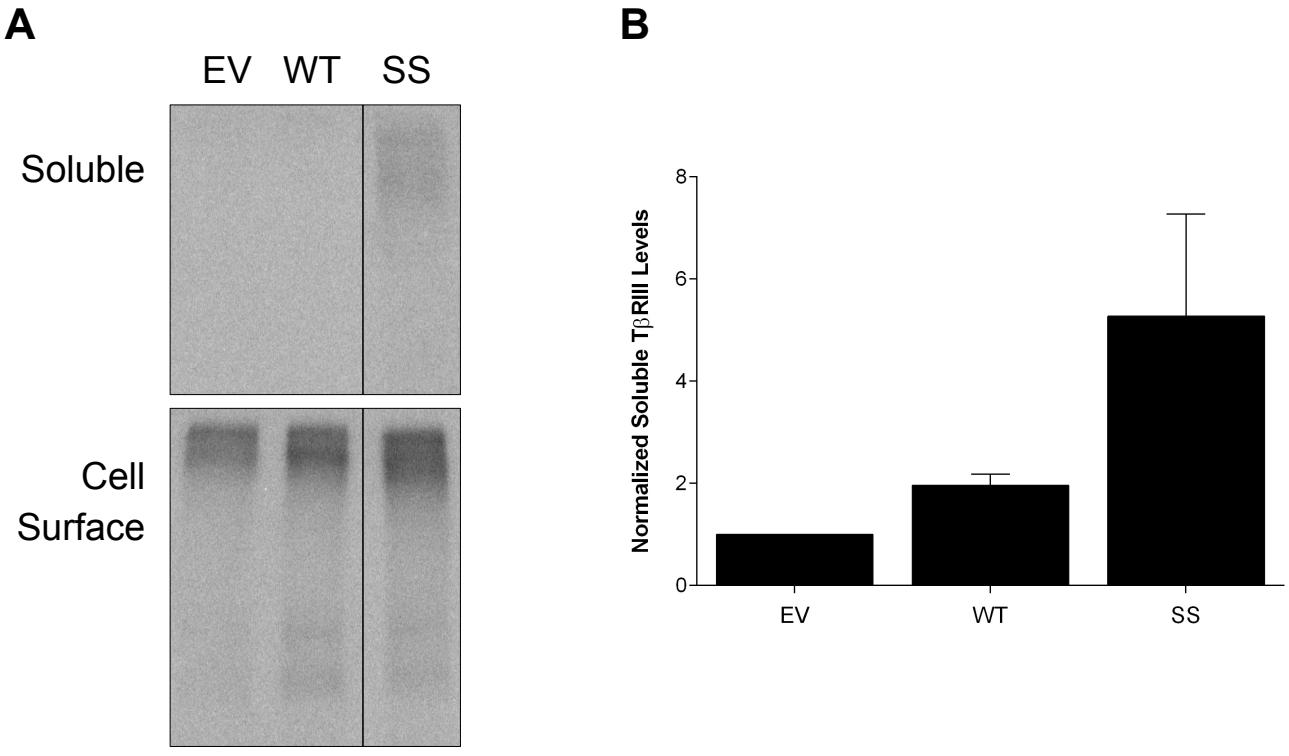
Supplemental Figure 5: *TβRIII ectodomain shedding regulates the kinetics and magnitude of TGF-β signaling in HEK293 cells.* HEK293 cells were transiently transfected with EV, WT-TβRIII or shedding mutants. Following overnight serum starvation, cells were treated with (A) 50 pM TGF-β1 for 30 minutes or (B) 50 pM TGF-β1 for indicated time period. Western blot analysis performed with indicated antibodies. TβRIII and β-actin western blots shown as loading controls. Representative images from 3 independent experiments shown.



Supplemental Figure 6: *TβRIII ectodomain shedding is affected by the presence of serum in MDA-MB-231 cells.* (A) Binding and crosslinking of lentiviral stable MDA-MB-231 cell lines expressing either EV, WT-TβRIII or the shedding mutants. Cells were plated and the following day media was changed to either full serum media (10% FBS) or serum-free media and allowed to condition for 24 h. Following ^{125}I-TGF-β1 binding and crosslinking lysates and conditioned media were immunoprecipitated with an antibody against TβRIII. β-actin western blot shown as a loading control. Representative images from 2 independent experiments shown. (B) ELISA data from media conditioned from lentiviral stable cell lines for 24 h in the absence or presence of 10% FBS. Data from 3 independent experiments are shown as levels of soluble TβRIII in the conditioned media normalized to β-actin loading controls from the corresponding cell lysates. Two-way ANOVA for cell line and treatment $p < 0.001$.



Supplemental Figure 7: *Effects of TβRIII and shedding mutants on proliferation at 24 hours.* (A) MDA-MB-231 lentiviral stable cell lines expressing either EV, WT-TβRIII or the shedding mutants were plated in media pre-conditioned for 24 h from corresponding cells in 96 well plates in triplicate in the absence or presence of 50 pM TGF-β1. 24 hours later proliferation was determined via ^{3}H-Thymidine incorporation. Data from 4 independent experiments are shown as CPM normalized to EV UnT. Two-way ANOVA for cell line $p < 0.002$. * One-sample *t*-test $p < 0.05$ relative to EV UnT. † Two-tailed *t*-test $p < 0.05$ relative to EV + TGF-β1.



Supplemental Figure 8: *Soluble TβRIII production is increased by the SS-TβRIII mutant in MDA-MB-231-4175 cells.* (A) MDA-MB-231-4175 lentiviral stable cell lines expressing either EV, WT-TβRIII or the Super-Shedding TβRIII mutant were plated and 24 h later media was replaced and allowed to condition for 20 h. Following ^{125}I-TGF-β1 binding and crosslinking, lysates and conditioned media were immunoprecipitated with an antibody against TβRIII. Representative images from 2 independent experiments. (B) ELISA data from media conditioned for 20 h. Data from 2 independent experiments are shown as levels of soluble TβRIII in the conditioned media normalized to β-actin loading controls from the corresponding cell lysates.