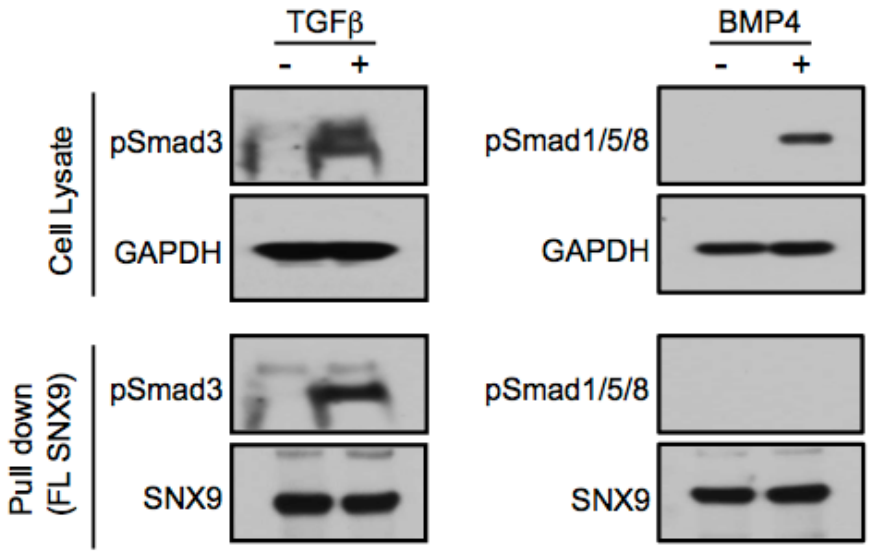


Figure S1. **Sorting nexin 9 (SNX9) specifically binds pSmad3 and not pSmad 1/5/8**. Lysates from AKR-2B cells untreated (-) or stimulated (+) for 45 min with 5 ng/ml TGFβ or 10 ng/ml BMP4 were incubated with full length (FL) SNX9 immobilized on GST beads as in Figure 1A. Bound proteins were eluted and assessed by Western analysis for pSmad3 or pSmad1/5/8. Cell lysate reflects signal obtained from 10 μg total protein. Blots are representative of 3 separate experiments.

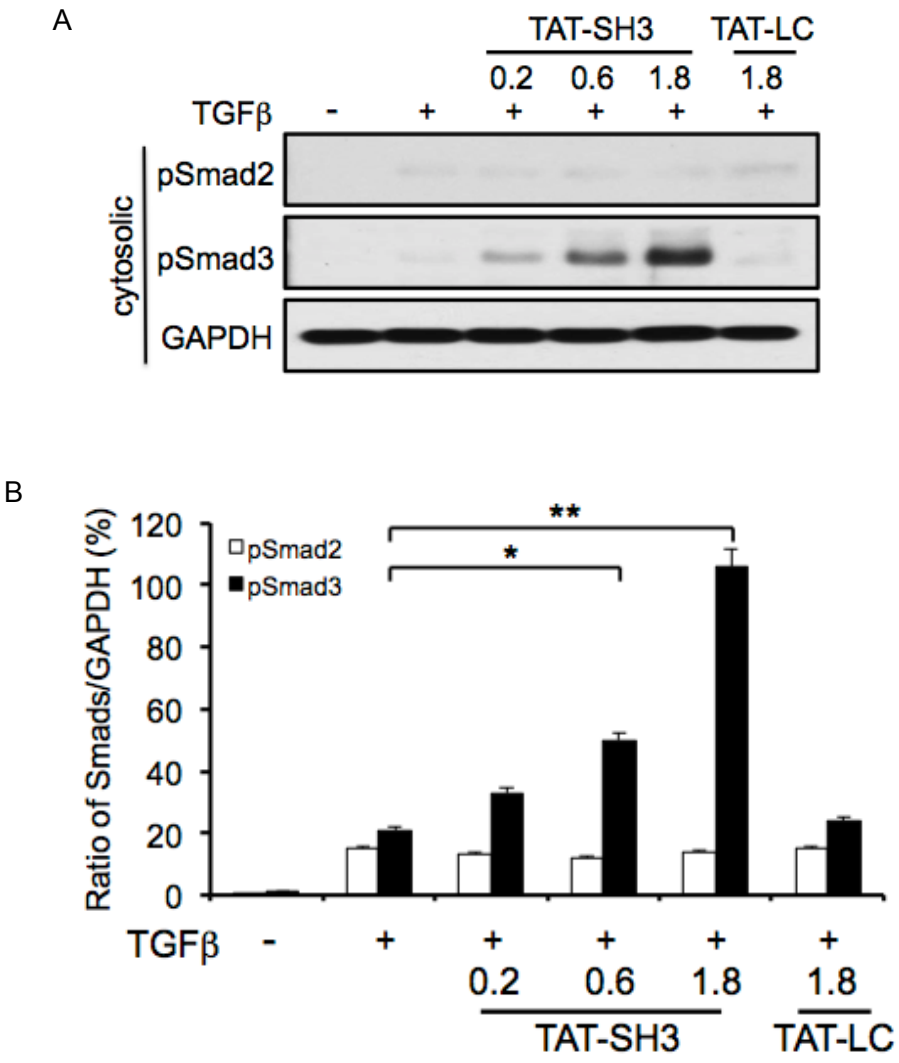
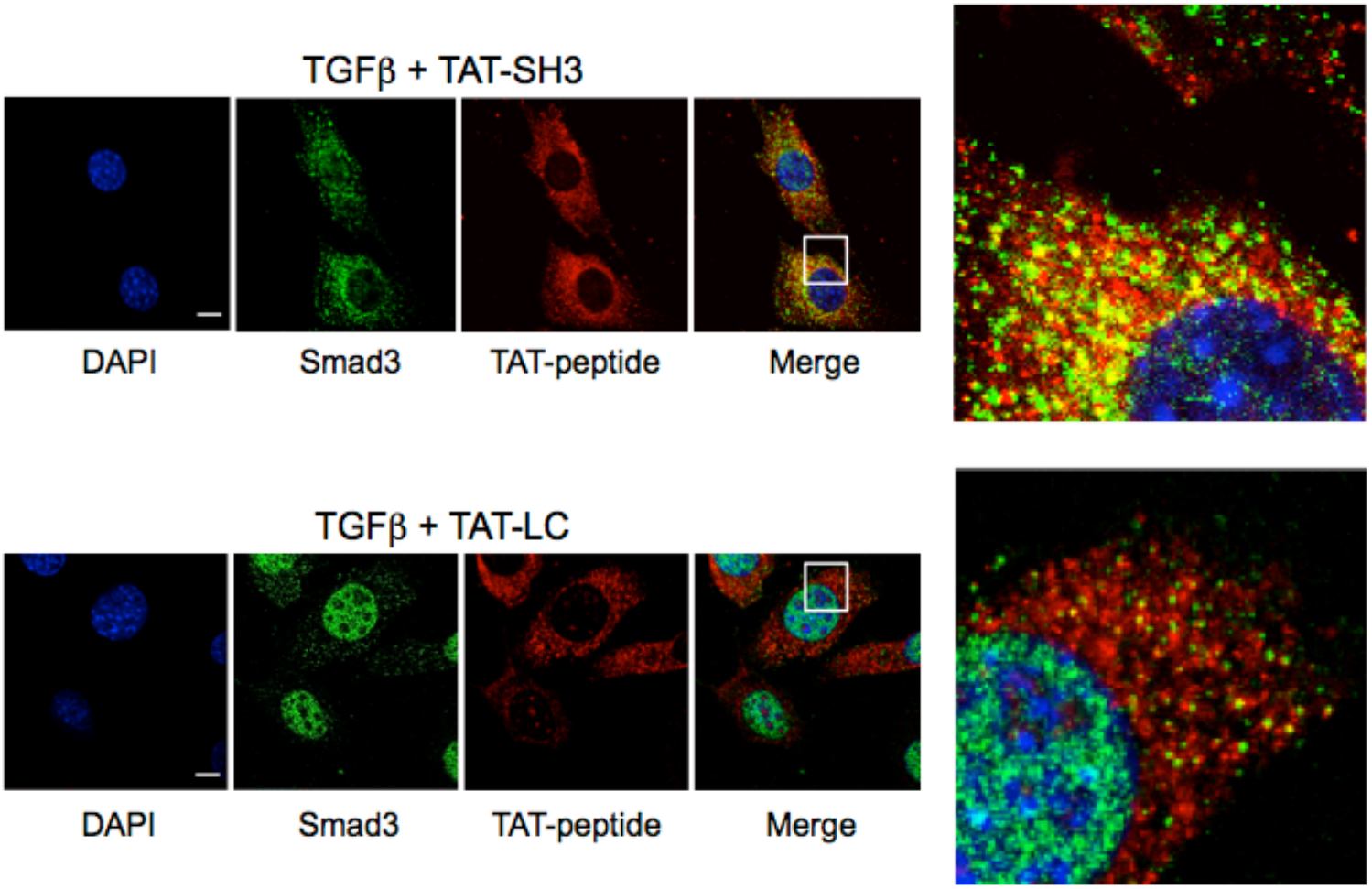


Figure S2. SH3 domain of sorting nexin 9 prevents nuclear pSmad3 import. (**A**) AKR-2B cells were transduced for 90 min with the indicated concentration (μM) of TAT fusion peptide to the SH3 or LC domains of SNX9 as in Figure 1B. Following washing and 1 hr TGFβ (5 ng/ml) treatment cytosolic fractions were isolated and Western blotted for pSmad2, pSmad3, or GAPDH. Blots are representative of 3 separate experiments. (**B**) Quantitation of cytosolic pSmads was performed with Image J software and represents the mean +/- SEM of 3 experiments. Data was analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. *P<0.05, **P<0.005.

A



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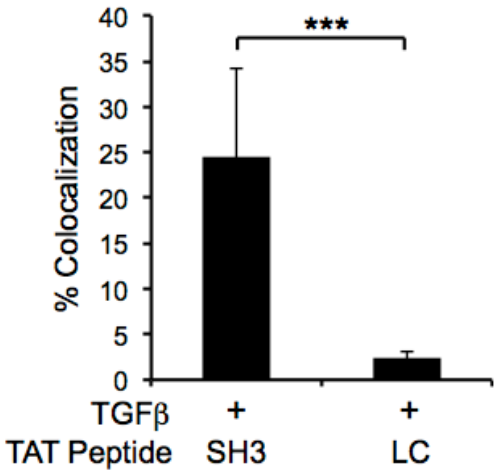


Figure S3. TAT-SH3 co-localizes with pSmad3. (**A**) AKR-2B cells were transduced for 90 min with TAT-SH3 or TAT-LC (1.8 μM). Following washing and 45 min TGFβ (5 ng/ml) treatment immunofluorescence for Smad3 or the HA-tagged TAT peptide was performed as described in Methods. Nuclei were stained with DAPI and right panels reflect higher magnification of boxed areas to visualize Smad3/TAT co-localization (yellow). (**B**). Quantitation of Smad3/TAT co-localization of 20 cells from each of 3 separate experiments using Image J software. Data was analyzed by 2-tailed Student's t-test. ***P<0.0005.

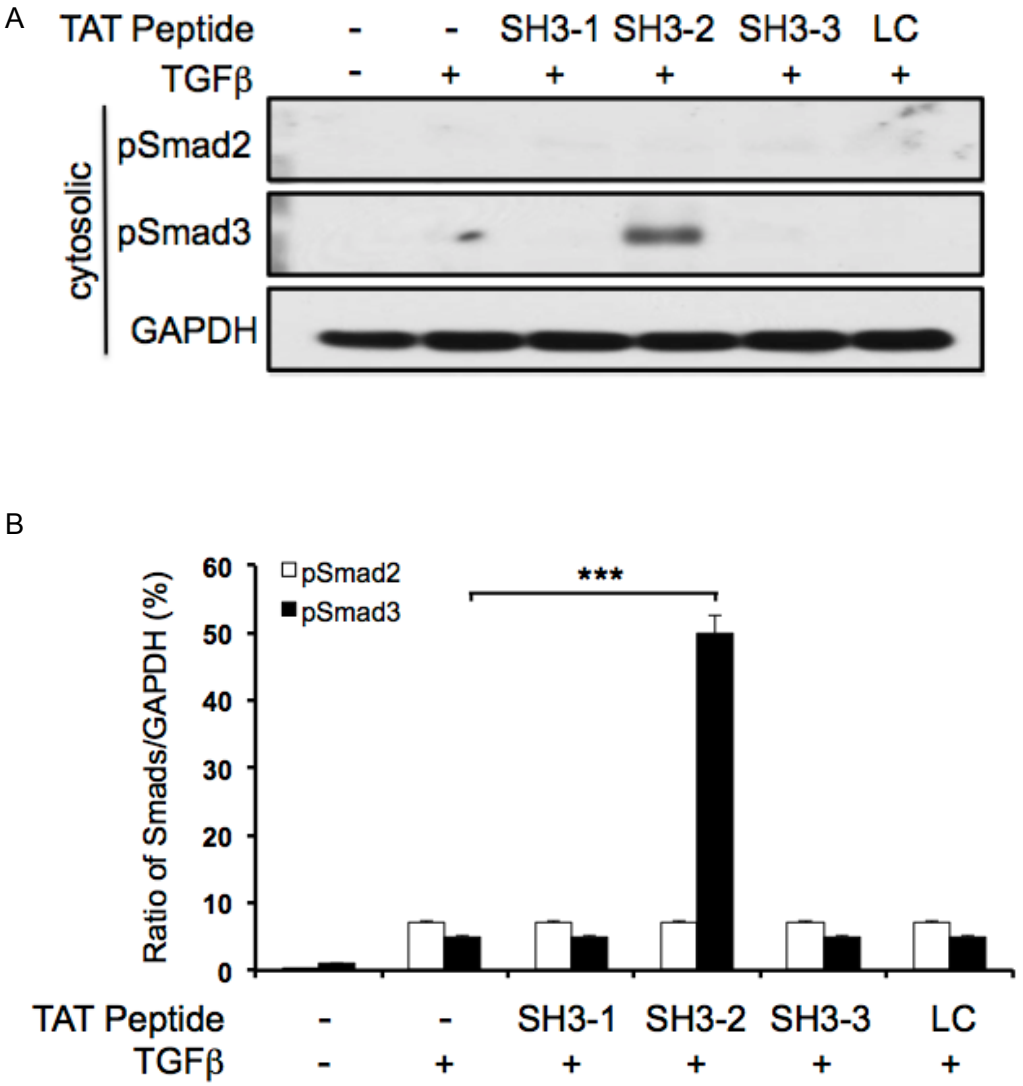


Figure S4. TAT-SH3-2 domain of sorting nexin 9 prevents nuclear pSmad3 import. (**A**) AKR-2B cells were transduced for 90 min with the indicated TAT peptide. Following washing and 1 hr TGFβ (5 ng/ml) treatment cytosolic fractions were isolated as in Figure S2 and Western blotted for pSmad2, pSmad3, or GAPDH. Blots are representative of 3 separate experiments. (**B**) Quantitation (mean +/- SEM) of cytosolic pSmad2 or pSmad3 from 3 experiments using Image J software. Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. ***P<0.0005.

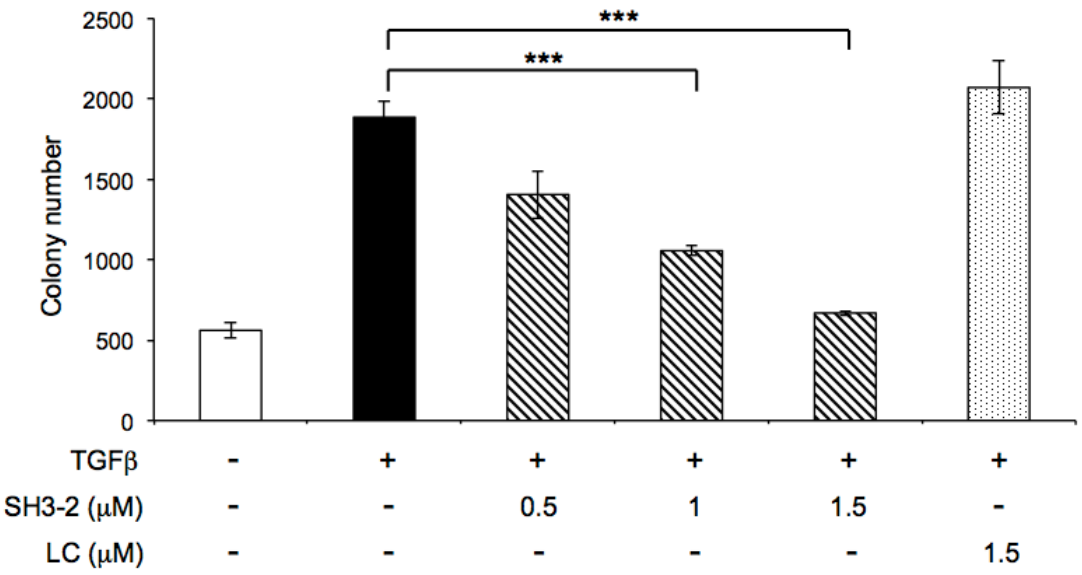


Figure S5. Dose-dependent inhibition of anchorage-independent growth by TAT-SH3-2. Soft agar colony formation was performed as described in Methods. Following 7 days growth in the presence (+) or absence (-) of TGFβ (20 ng/ml) or the indicated TAT peptide, colonies >50 mm were determined. Data reflect the mean +/- SEM of triplicate wells from 3 experiments. Data was analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. ***P<0.0005.

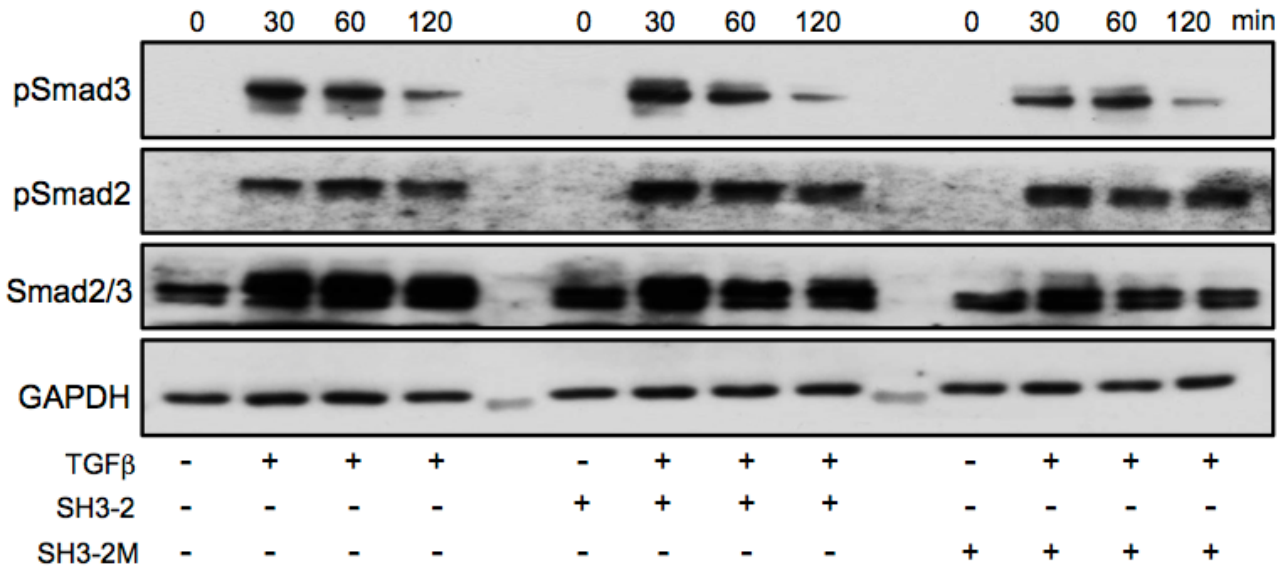
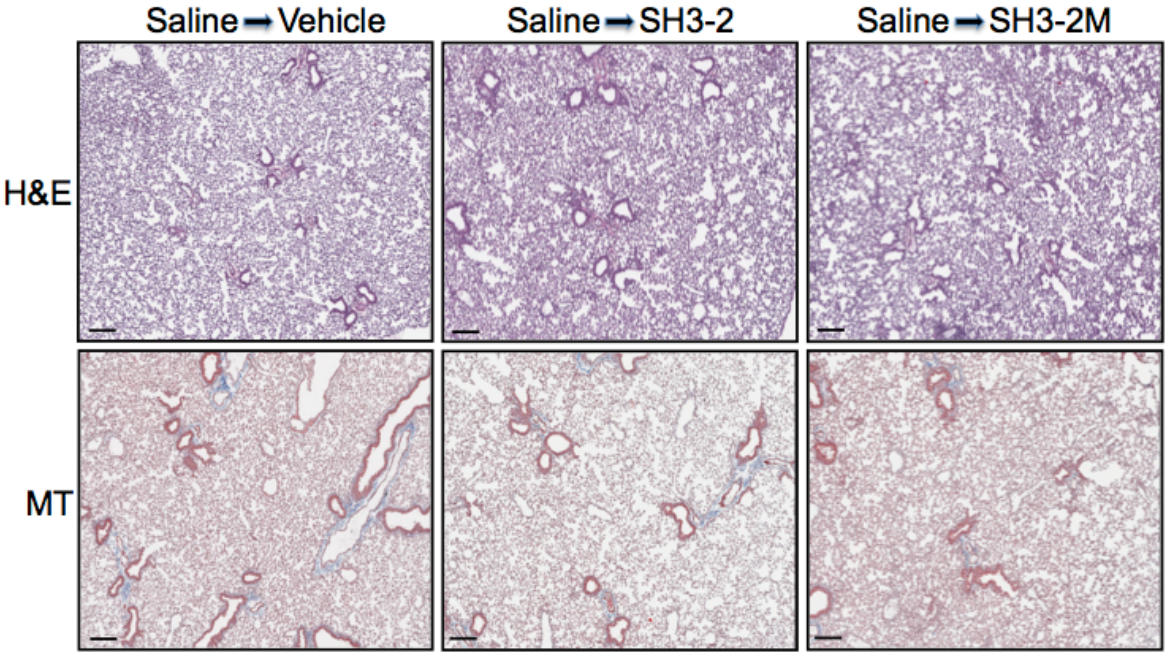


Figure S6. TAT-SH3-2 peptide does not affect Smad2 or Smad3 phosphorylation. AKR-2B cells were transduced for 90 min with 1.5 μM TAT-SH3-2 or TAT-SH3-2M. After transduction, cells were treated (+) with TGFβ (5 ng/ml) for the indicated times and processed by Western analysis for the indicated phosphorylated (p) or total protein. Band running slightly below GAPDH is the 34 kDa marker detected by the GAPDH sera. Blots are representative of 3 separate experiments.

A



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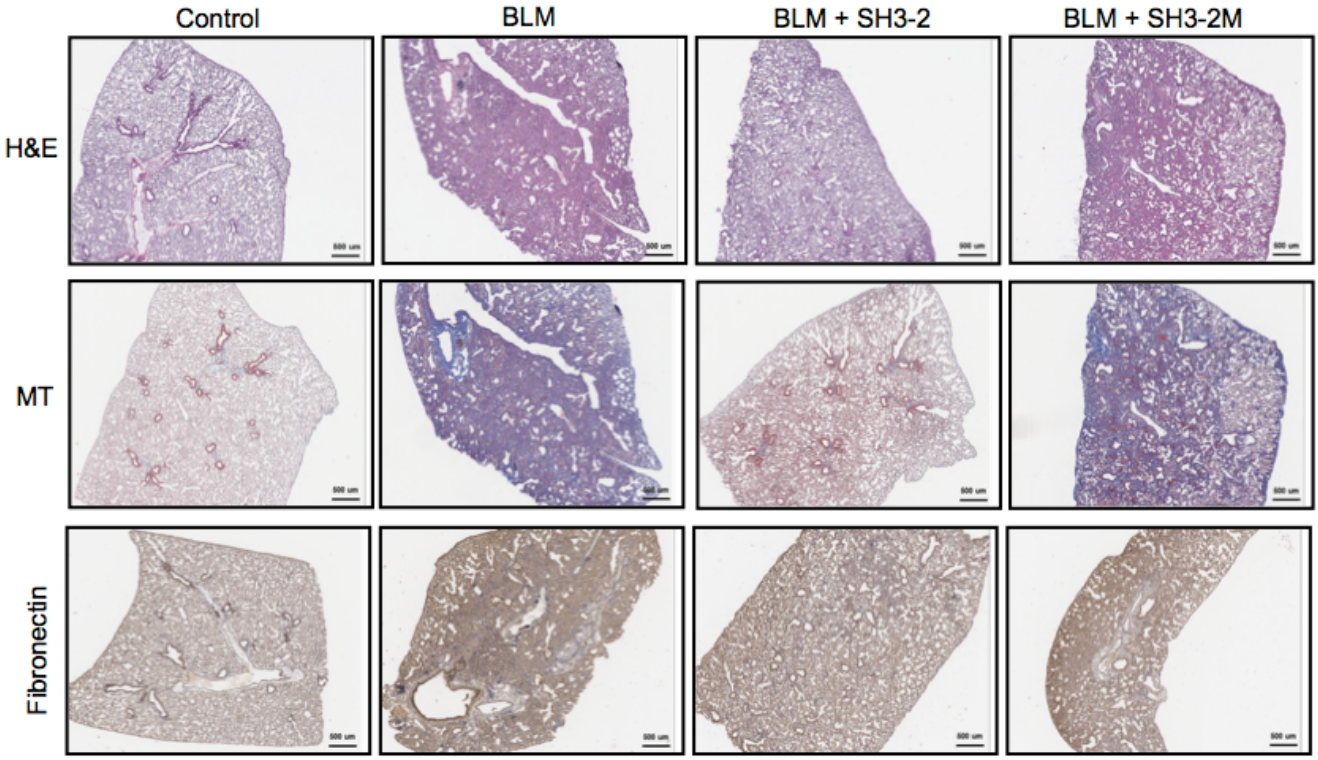
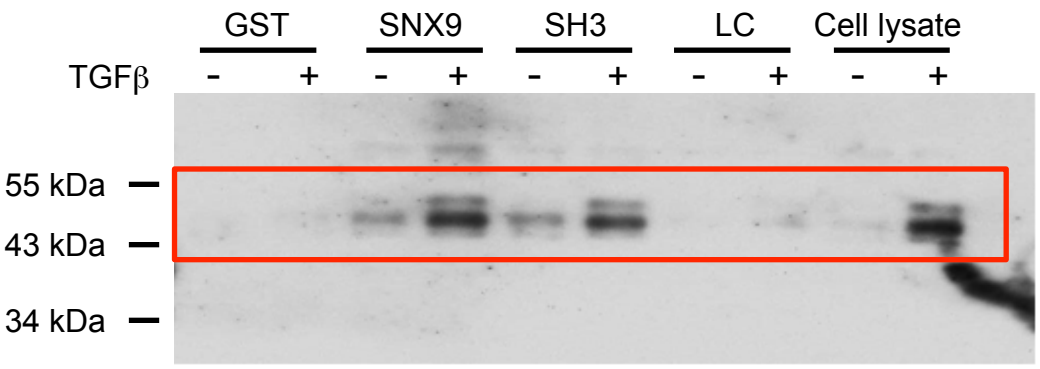
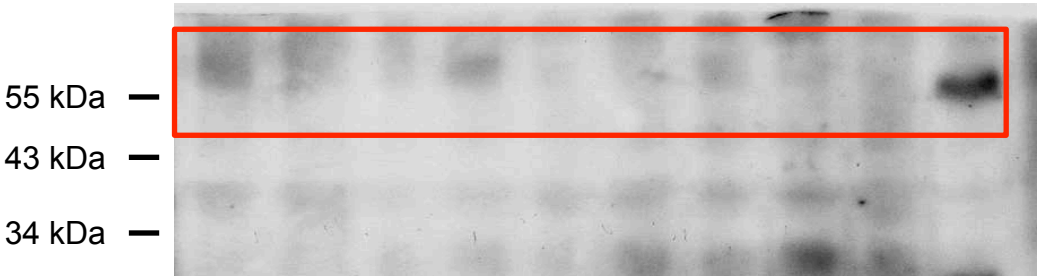


Figure S7. Effect of TAT peptides on mouse lung histopathology. (A) C57BL/6 mice were intratracheally treated with 0.9% normal saline as described in Methods. On day 14 animals were daily-administered vehicle (Methocel/saline) or Methocel/1.0 mg/kg of the indicated TAT peptide (SH3-2 or SH3-2M) intraperitoneally. Lung tissue was harvested at day 28 and subjected to Hematoxylin and Eosin (H&E) staining for histology and Masson's Trichrome (MT) for collagen. Representative images from 5 animals are shown. Scale bar: 200 μm. (**B**) Hematoxylin and Eosin (H&E), Masson's Trichrome (MT), and fibronectin staining on a greater range of lung tissue from Figure 7A. Scale bar: 500 μm.

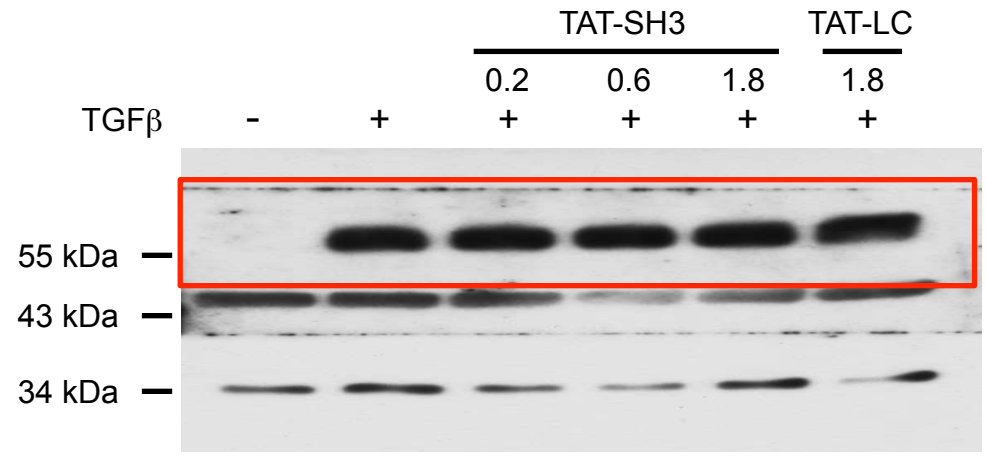


Full unedited gel for Figure 1A pSmad3

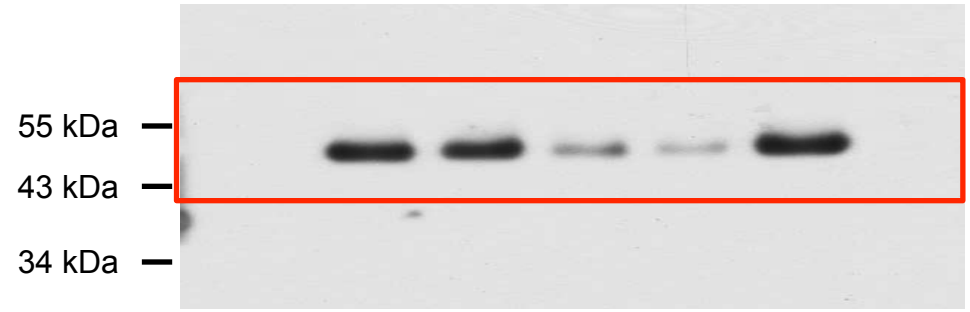


Full unedited gel for Figure 1A pSmad2

Figure 1A



Full unedited gel for Figure 1B pSmad2



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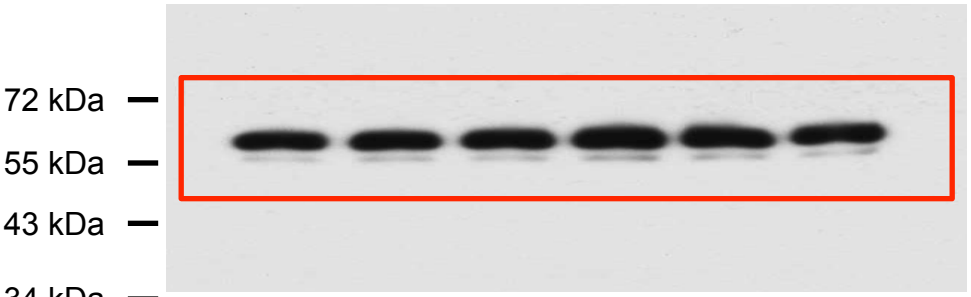
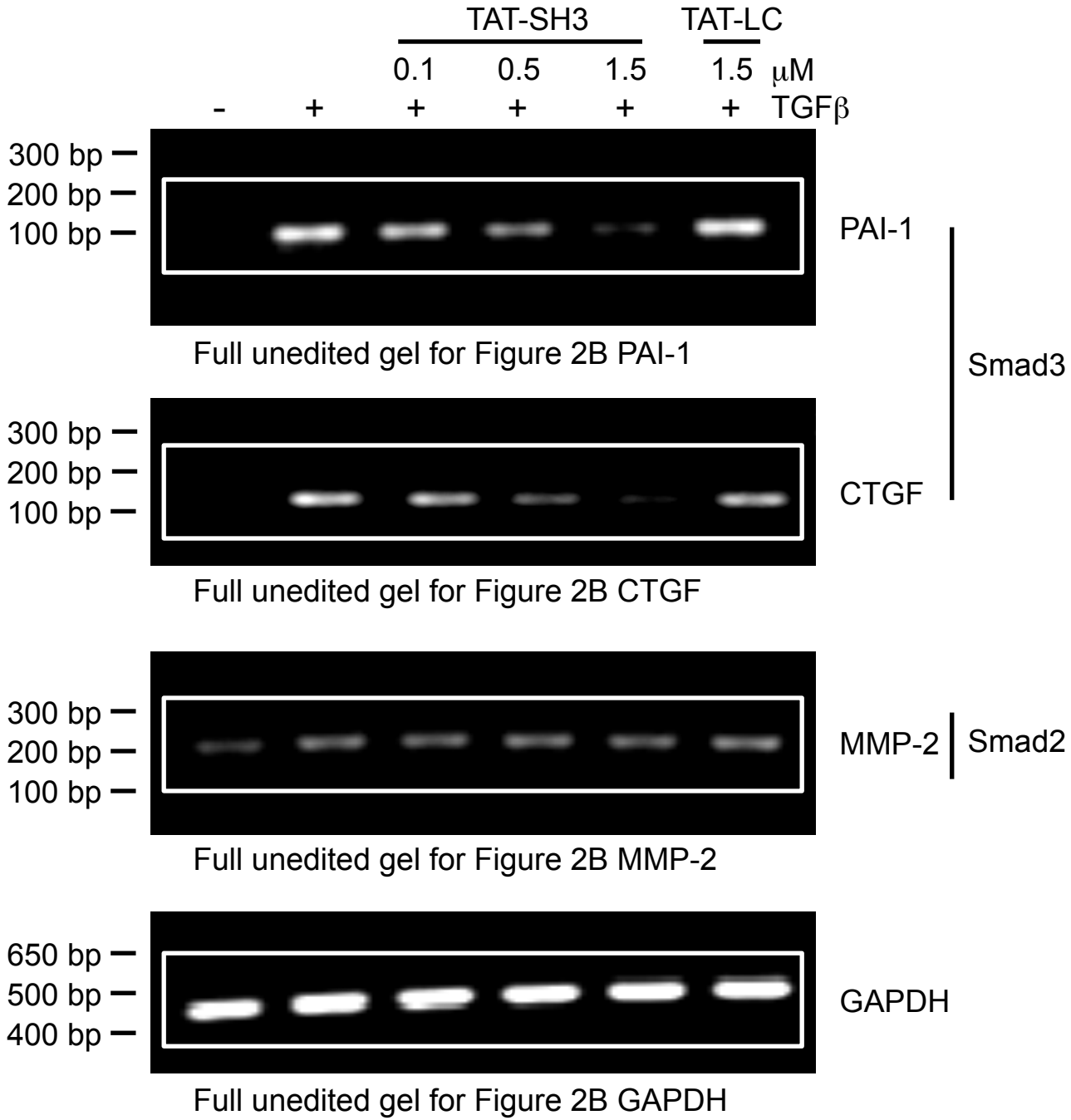


Full unedited gel for Figure 1B HDAC

Figure 1B


TAT-SH3
TAT-LC
0.1
0.5
1.5
1.5 μM
-
+
+
+
+
+
TGFβ
300 bp
200 bp
100 bp
PAI-1
Smad3
Full unedited gel for Figure 2B PAI-1
300 bp
200 bp
100 bp
CTGF
Full unedited gel for Figure 2B CTGF
300 bp
200 bp
100 bp
MMP-2
Smad2
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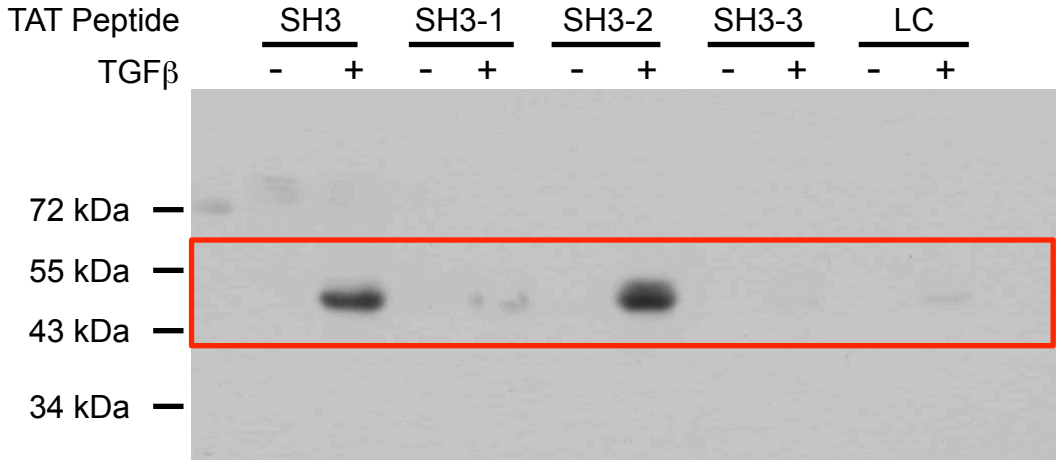
650 bp
500 bp
400 bp
GAPDH
Full unedited gel for Figure 2B GAPDH

Figure 2B



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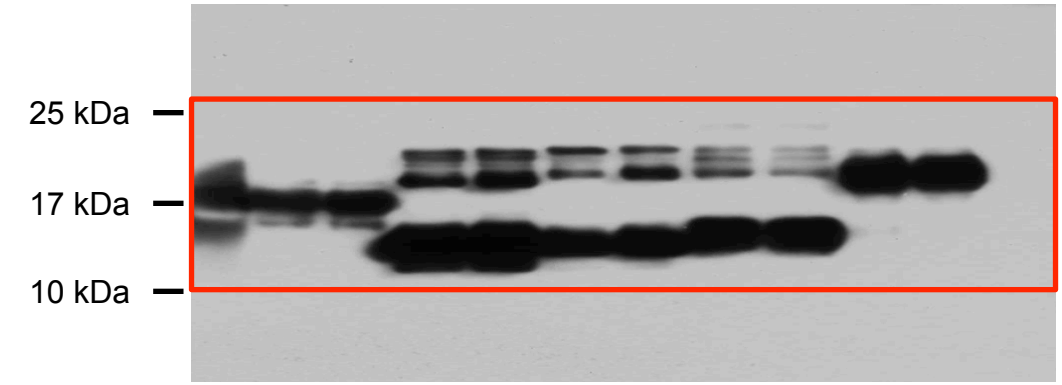
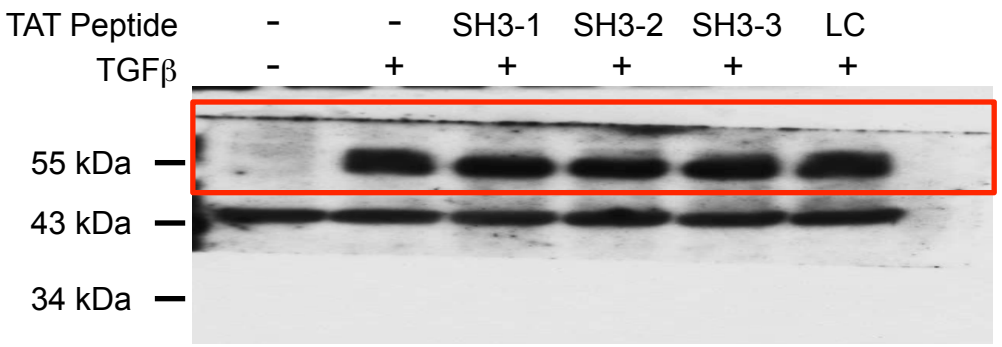


Full unedited gel for Figure 3A His

Figure 3A



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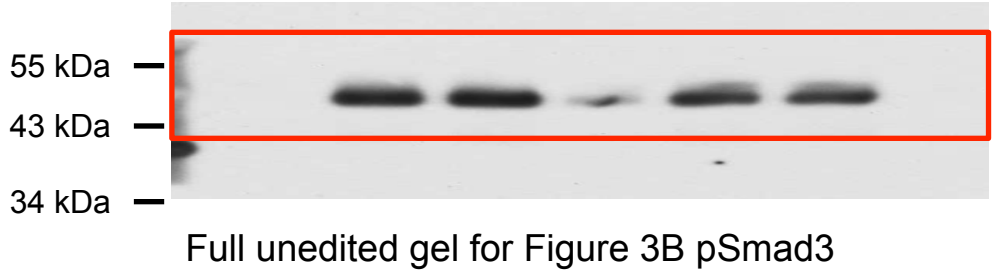

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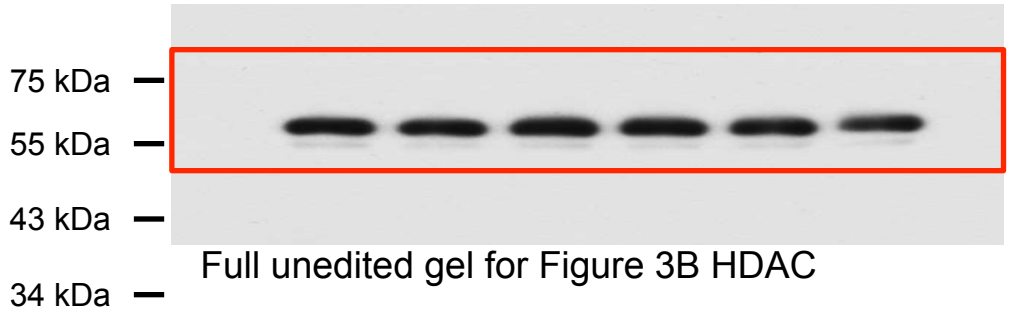
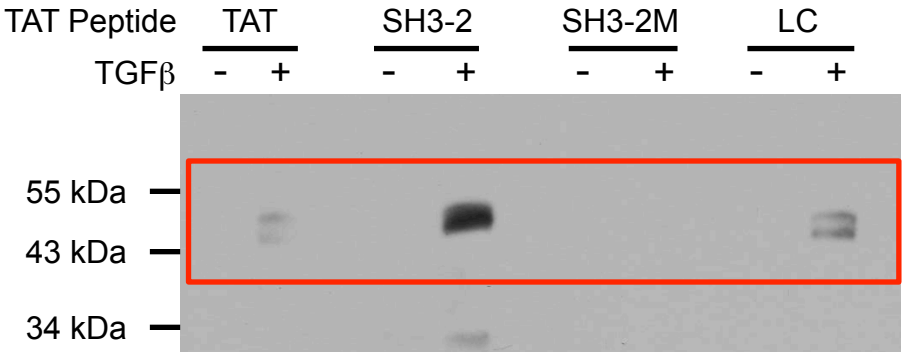


Full unedited gel for Figure 3B HDAC

Figure 3B



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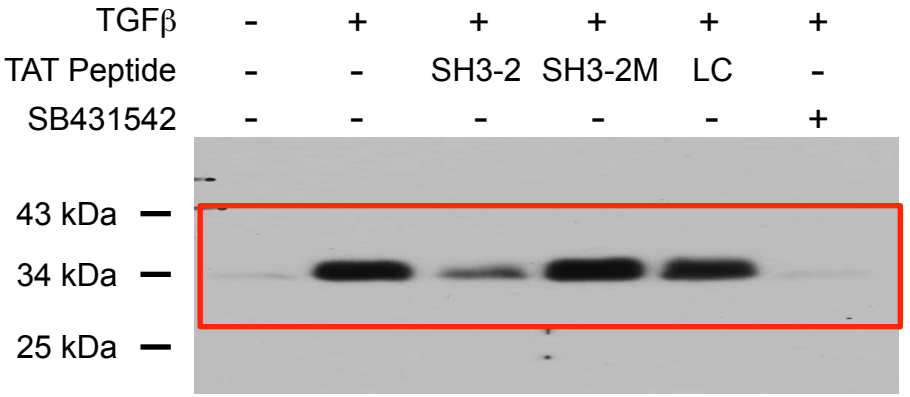
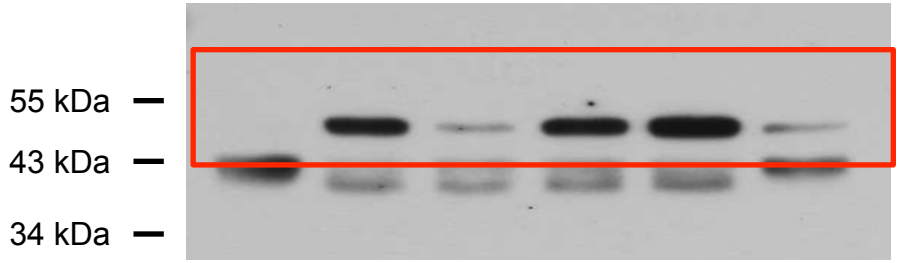
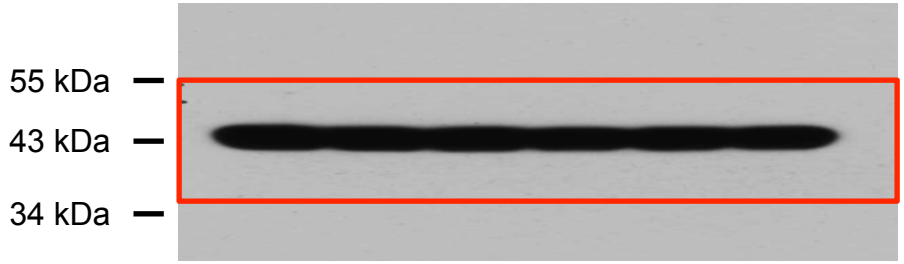


Full unedited gel for Figure 4A CTGF

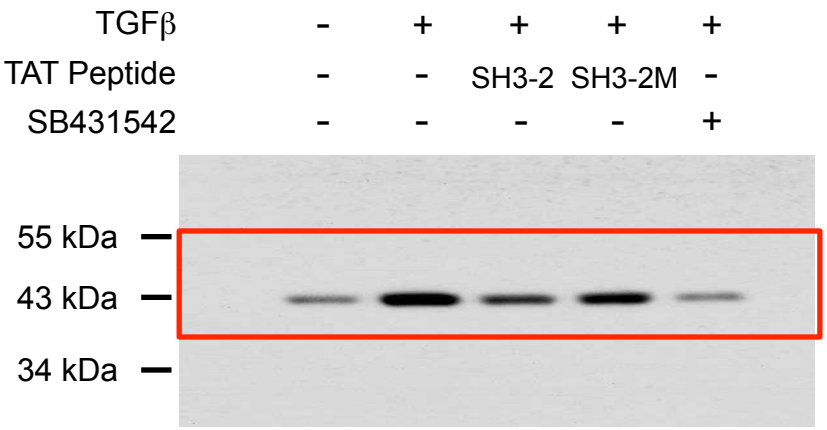


Full unedited gel for Figure 4A PAI-1

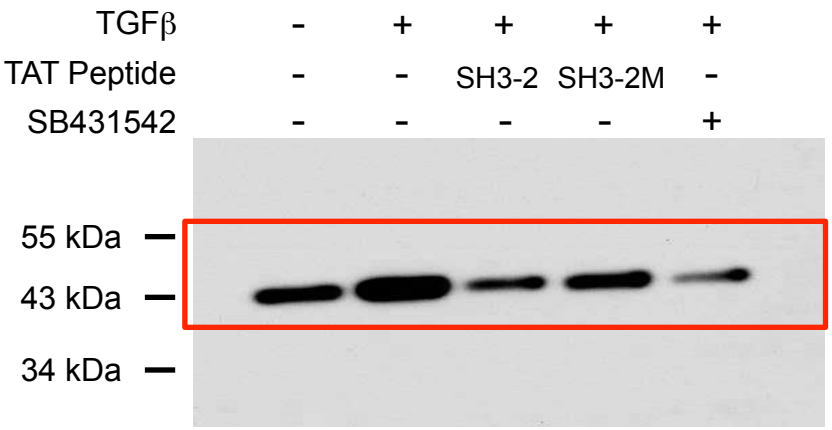


Full unedited gel for Figure 4A β-actin

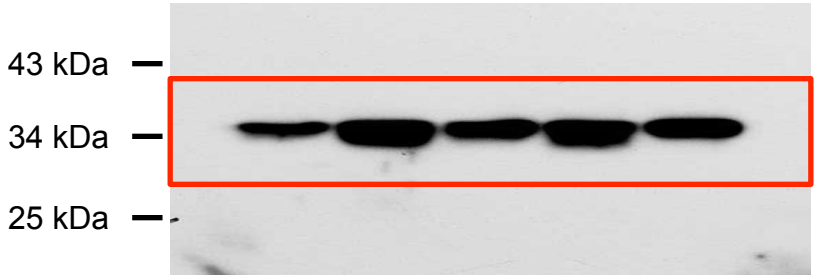
Figure 4A



Full unedited gel for Figure 5B α-SMA on NHLF



Full unedited gel for Figure 5B α-SMA on IPF



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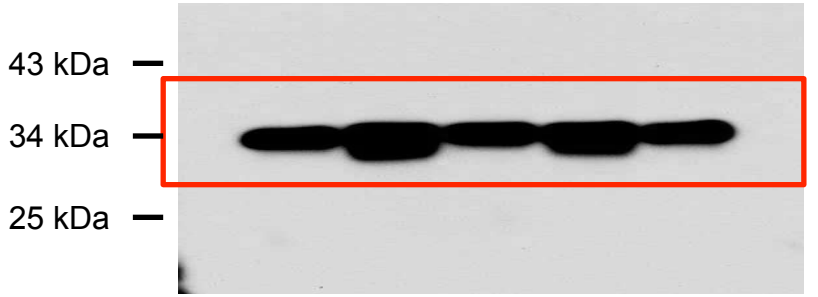

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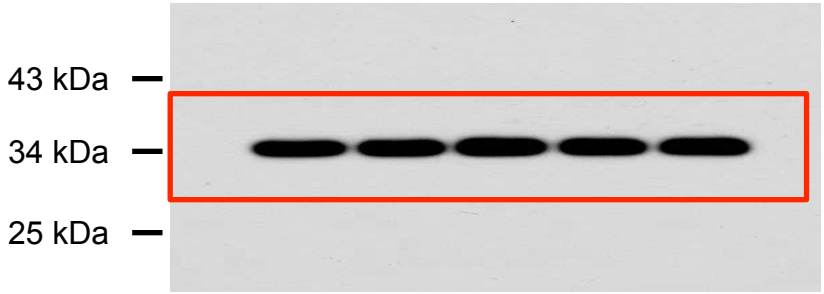

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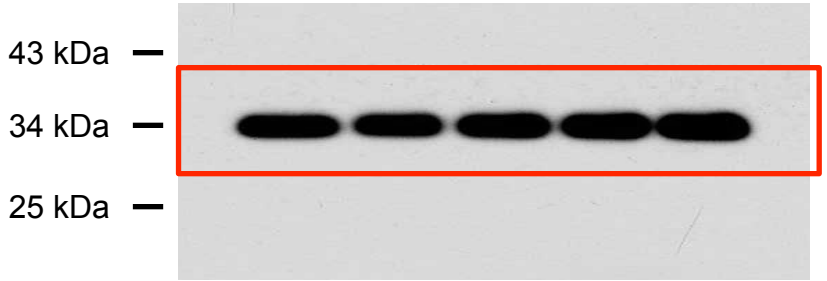

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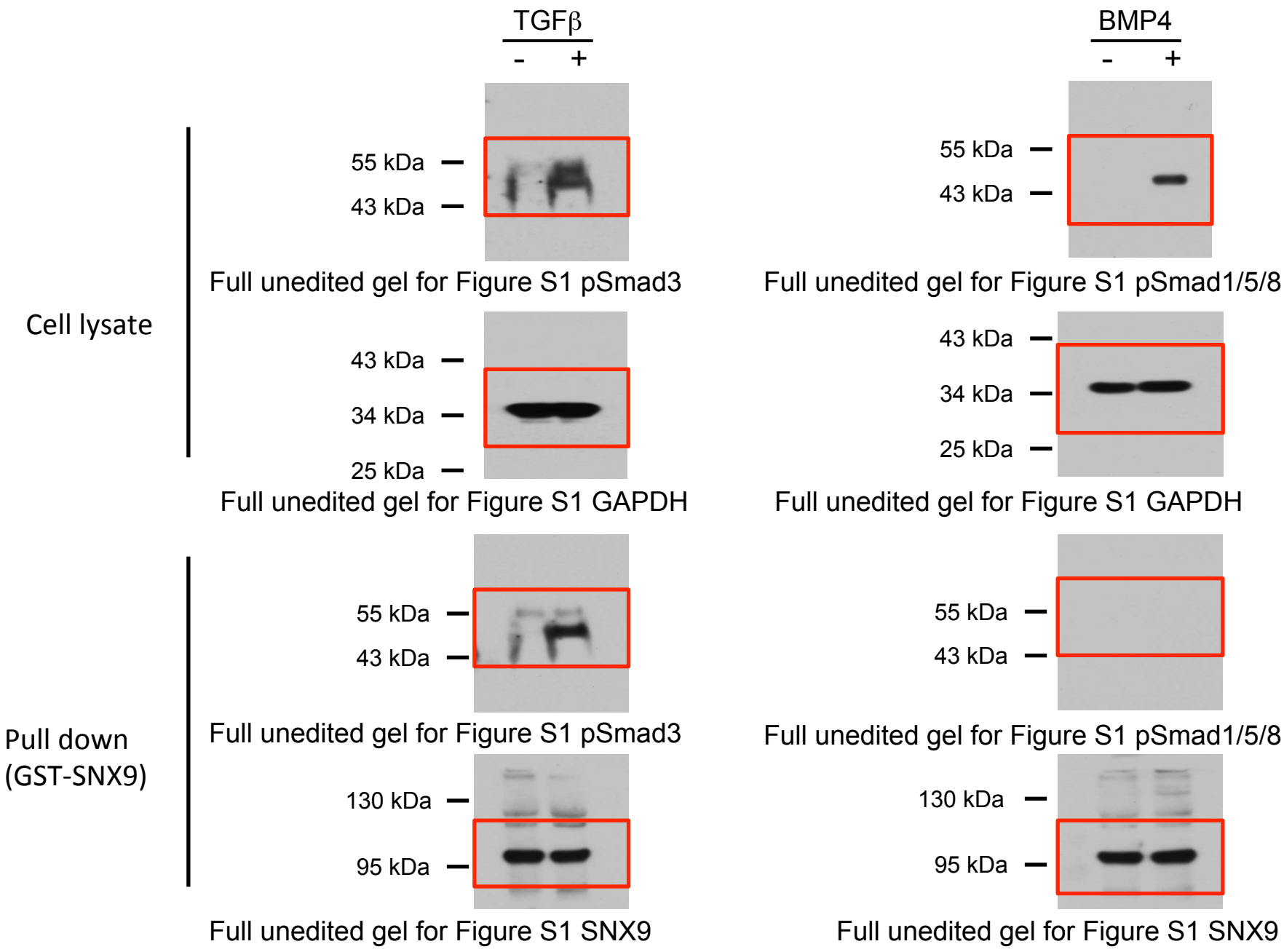
Figure 5B


TGFβ
- +
BMP4
- +
Cell lysate
55 kDa
43 kDa
Full unedited gel for Figure S1 pSmad3
55 kDa
43 kDa
Full unedited gel for Figure S1 pSmad1/5/8
43 kDa
34 kDa
25 kDa
Full unedited gel for Figure S1 GAPDH
43 kDa
34 kDa
25 kDa
Full unedited gel for Figure S1 GAPDH
Pull down
(GST-SNX9)
55 kDa
43 kDa
Full unedited gel for Figure S1 pSmad3
55 kDa
43 kDa
Full unedited gel for Figure S1 pSmad1/5/8
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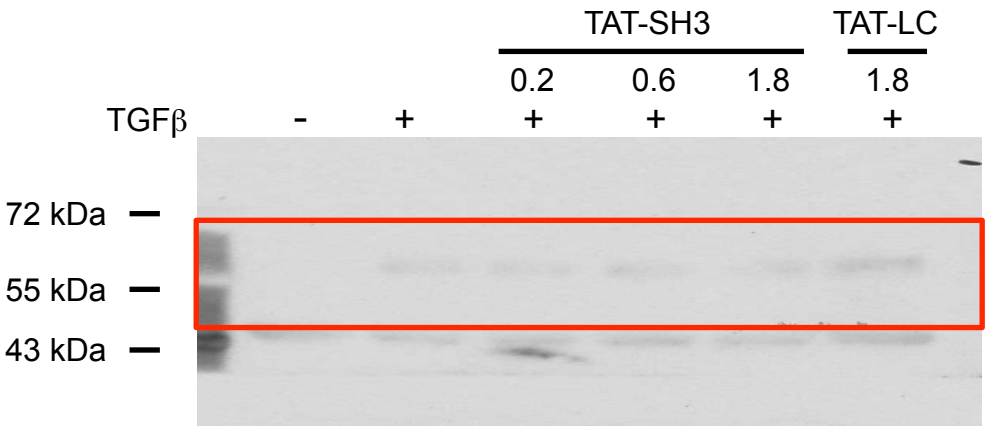
130 kDa
95 kDa
Full unedited gel for Figure S1 SNX9

Figure S1



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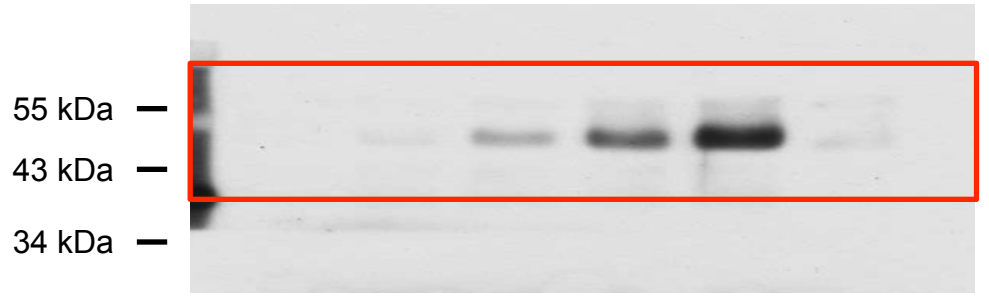

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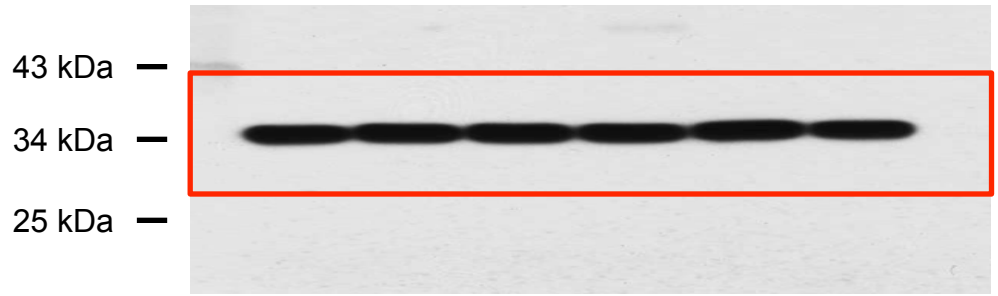
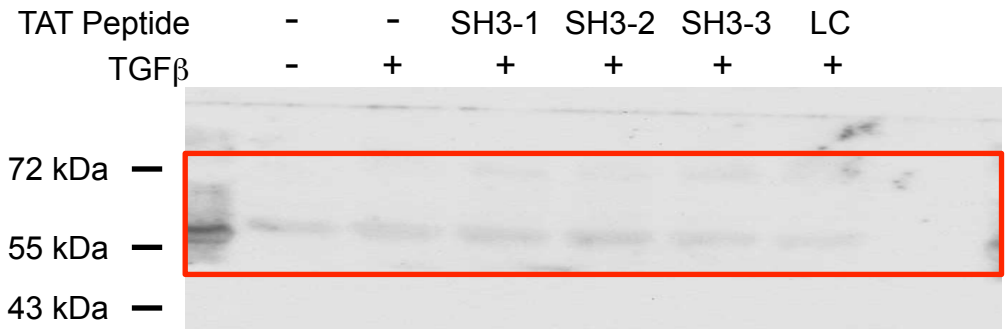


Full unedited gel for Figure S2 GAPDH

Figure S2



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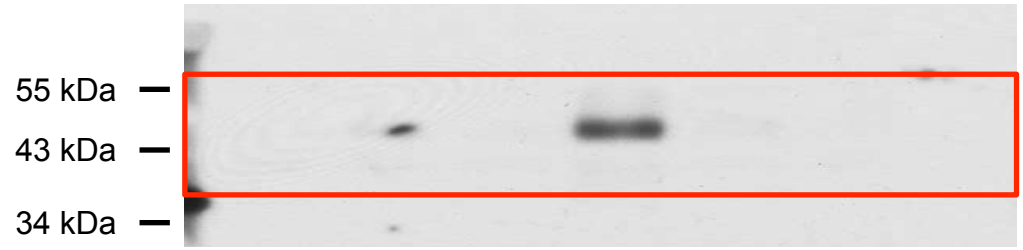

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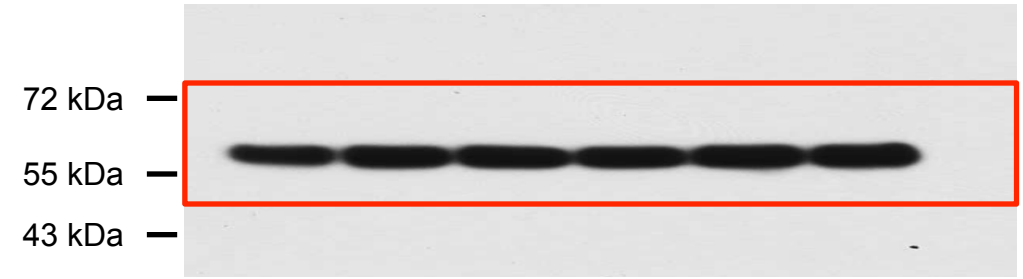
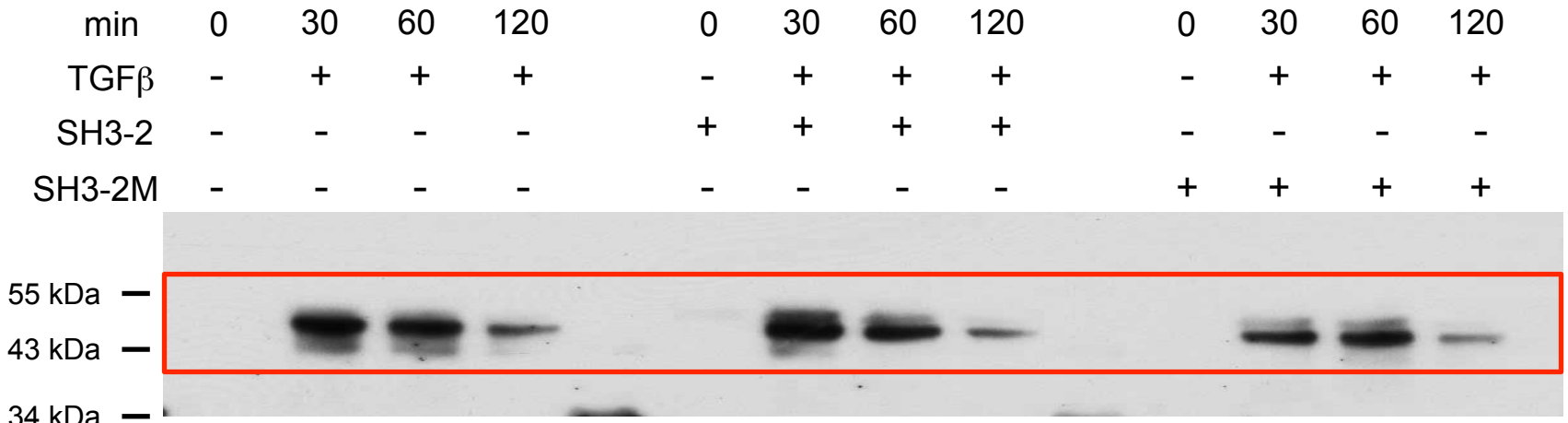


Full unedited gel for Figure S4 GAPDH

Figure S4



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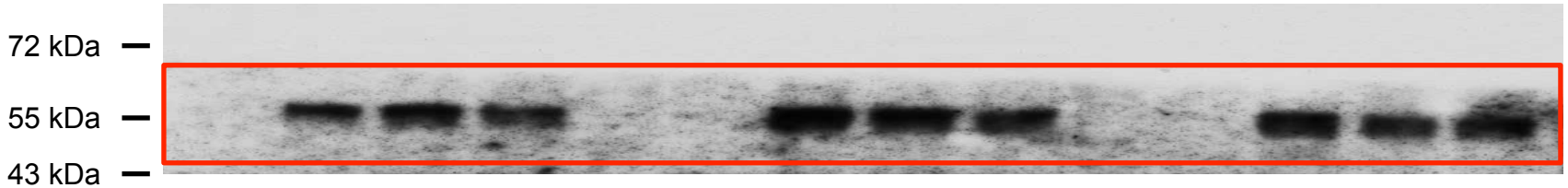
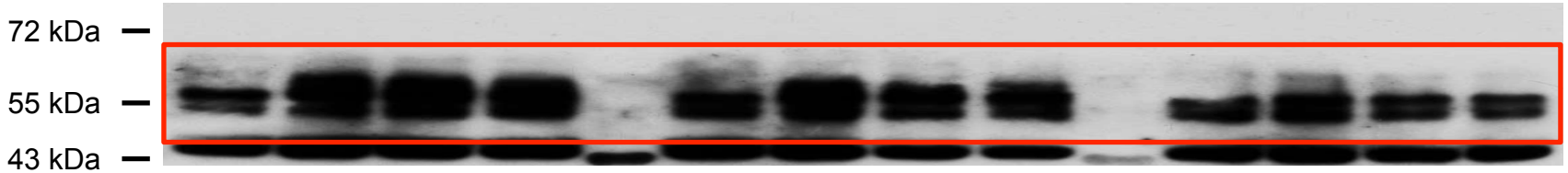
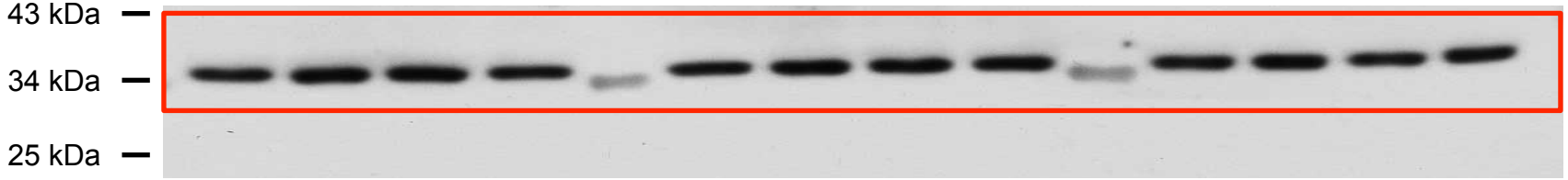


Full unedited gel for Figure S6 pSmad2



Full unedited gel for Figure S6 Smad2/3



Full unedited gel for Figure S6 GAPDH

Figure S6