Supplemental support data

Ubiquitin carboxyl-terminal hydrolase-L5 promotes TGFβ-1 signaling by de-ubiquitinating and stabilizing Smad2/Smad3 in pulmonary fibrosis

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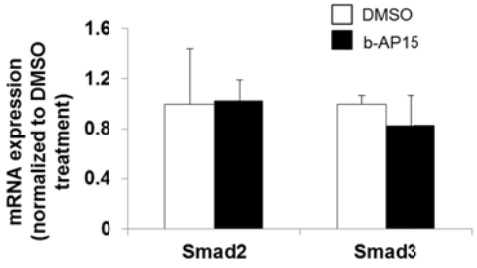
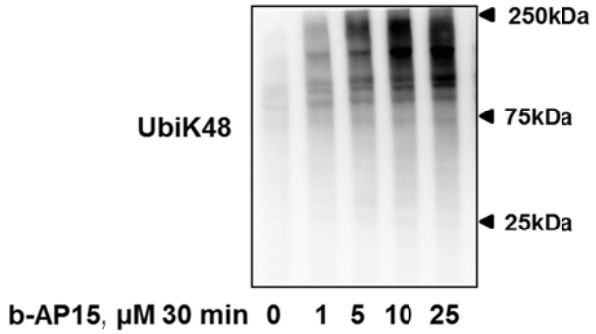
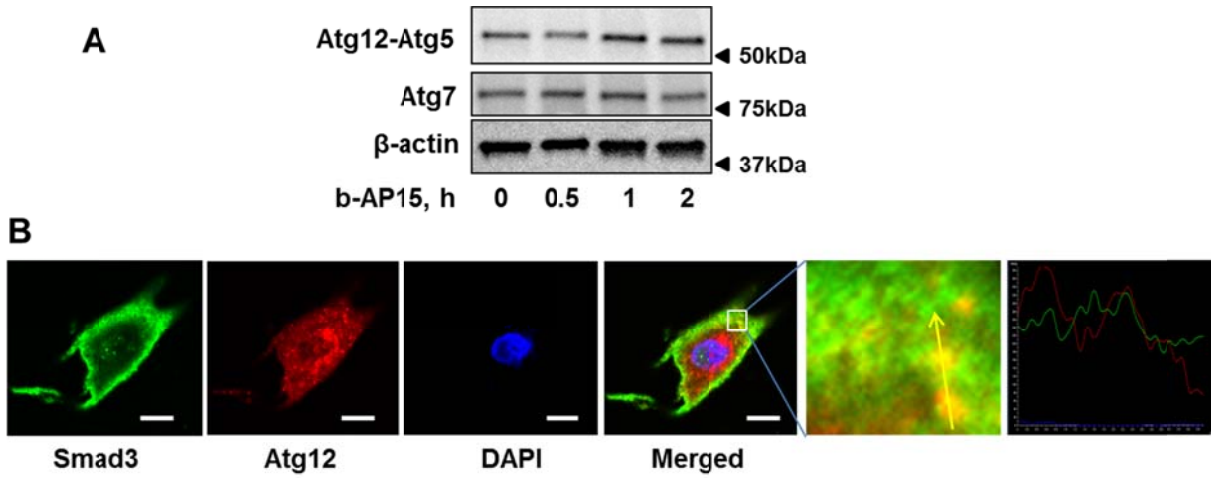


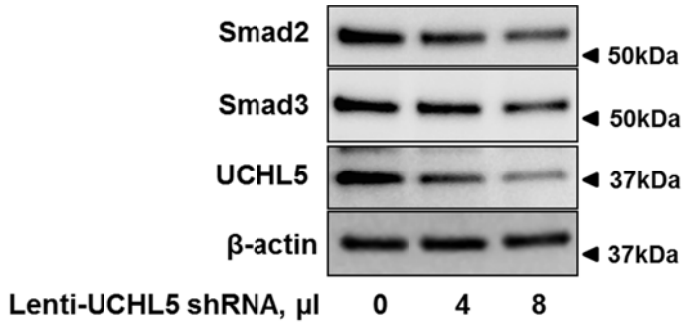
Suppl. Fig. 1. b-AP15 has no effect on mRNA levels of Smad2 and Smad3. HLF cells were treated with b-AP15 (10 μM) for 1 h. Total RNA was isolated and cDNA was synthesized by reverse transcription. Quantitative PCR was performed to assess mRNA expression of Smad2 and Smad3.



Suppl. Fig. 2. b-AP15 causes accumulation of poly-ubiquitination in HLF cells. HLF cells were treated with different doses of b-AP15 (0, 1, 5, 10, 25 μM) for 1 h and then cell lysates were analyzed by immunoblotting with an ubiquitinK48 antibody. Western blot images were cropped to improve the conciseness of the data; samples derived from the same experiment and the blots were processed in parallel. Representative of experiments performed at least 3 independent times.



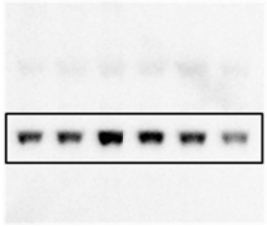
Suppl. Fig. 3. b-AP15 increases Atg12-Atg5 conjugation and co-localization of Smad3 and Atg12. A. Mrc5 cells were treated with b-AP15 (5 μM) for 0-2 h. Cell lysates were analyzed by Atg12, Atg7, and β-actin antibodies. Western blot images were cropped to improve the conciseness of the data; samples derived from the same experiment and the blots were processed in parallel. Representative of experiments performed at least 3 independent times. **B.** HLF cells were treated with bafilomycin A1 (10 μM, 1 h) prior to b-AP15 (5 μM) for 1 h. The localization of Smad3 and Atg12 were detected by immunofluorescence staining. Smad3, green; Atg12, red; nuclei, blue. Scale bar, 10 μm. Co-localization was analyzed by NIS-Elements software. 94% of the cells with double staining show positive co-localization.



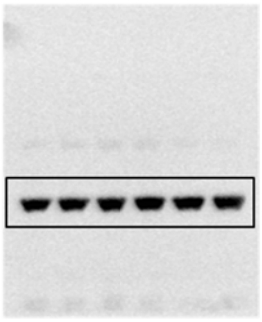
Suppl. Fig. 4. UCHL5 shRNA reduces Smad2/Smad3 levels. HLF cells were infected with different doses of lentivirus vector containing UCHL5 shRNA (0, 4, and 8 μl) and incubated for 72 h. Cell lysates were analyzed by Smad2, Smad3, UCHL5, and β-actin antibodies. Western blot images were cropped to improve the conciseness of the data; samples derived from the same experiment and the blots were processed in parallel. Representative of experiments performed at least 3 independent times.

Fig. 1A

FN

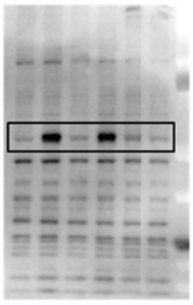


SMA

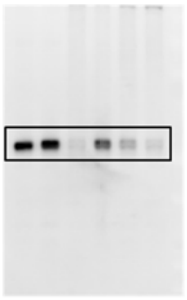


β-actin

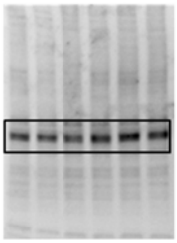
Fig. 1C



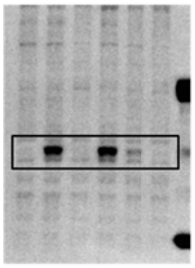
P-Smad2



Smad2



ALK5



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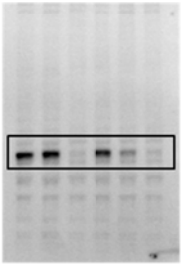

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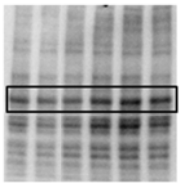
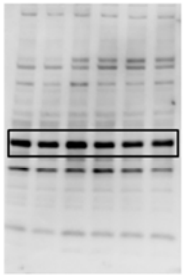
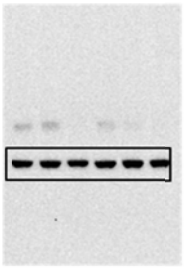


Smad7



UCHL5



β-actin

Fig. 2A

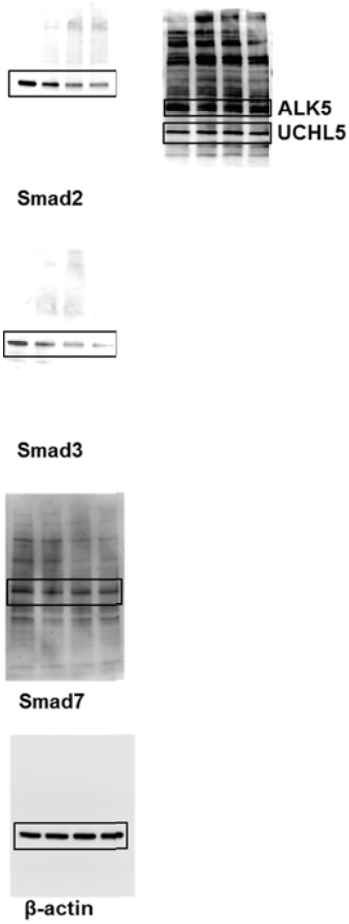


Fig. 2B

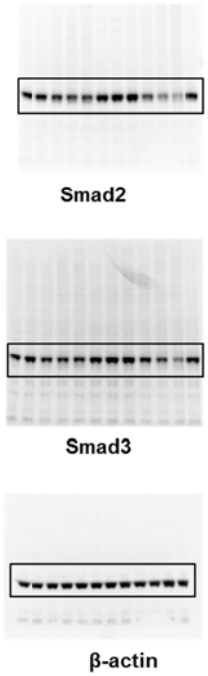


Fig. 2C

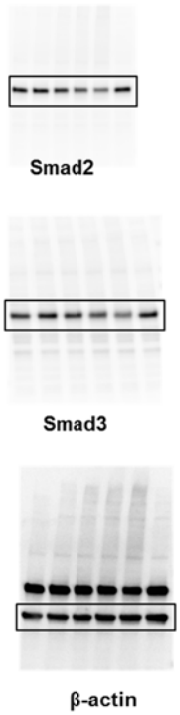


Fig. 2D

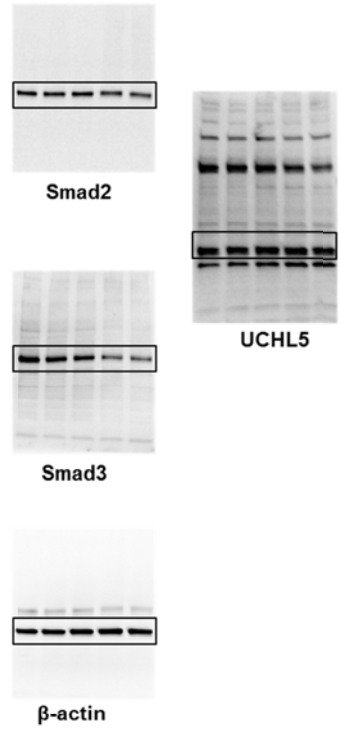
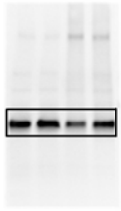
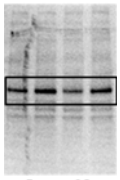


Fig. 3A



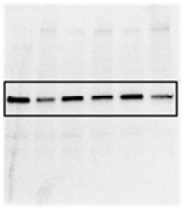
Smad2



Smad3

β-actin

Fig. 3B



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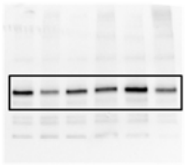
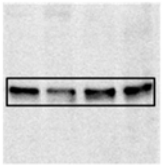


Smad3

β-actin

Fig. 3C

Smad2



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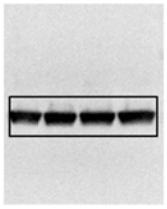
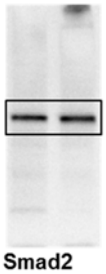


β-actin

Fig. 3E

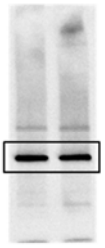
Smad2

Smad3

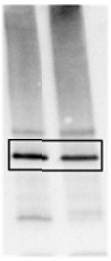


Smad2

Smad3

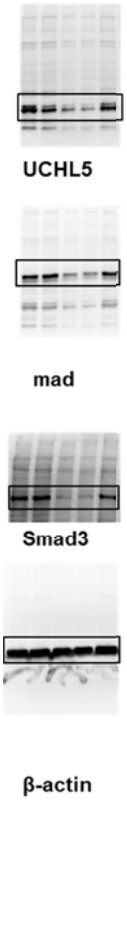


β-actin



β-actin

Fig. 4A



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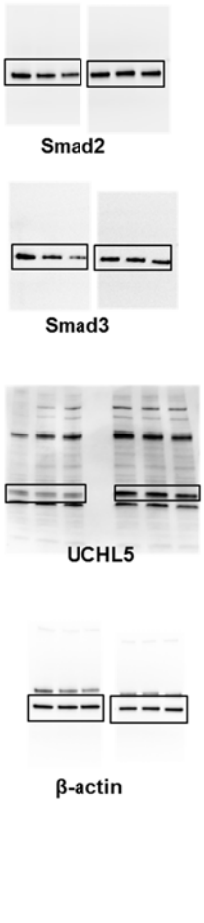
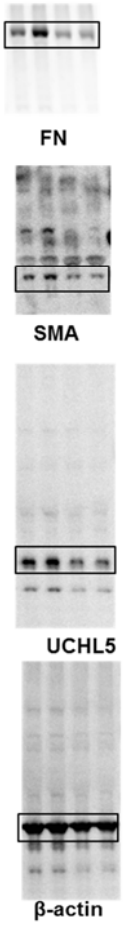


Fig. 4C



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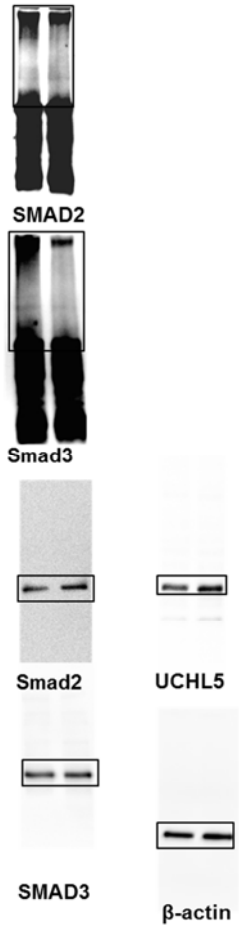


Fig. 4E

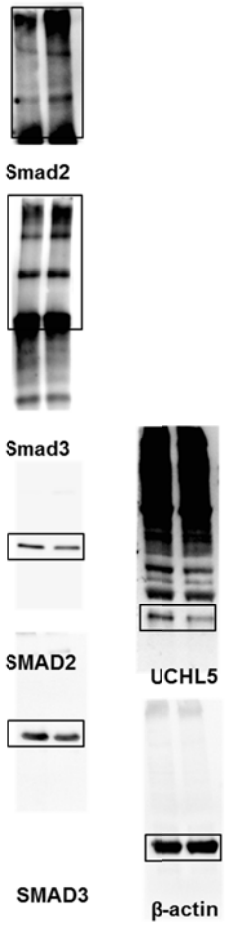
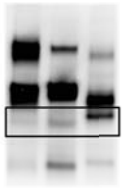


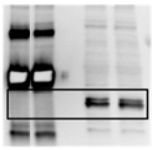
Fig. 5A



UCHL5

Smad2/3

Fig. 5B



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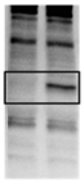
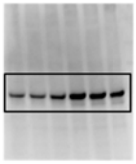
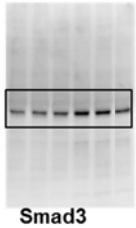


P-Smad3

Fig. 6B



Smad2



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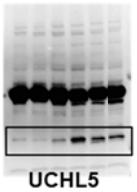

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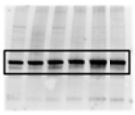
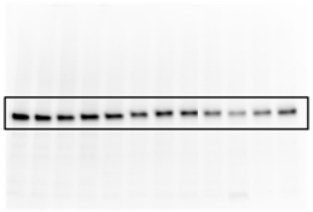


USP14

β-actin

Fig. 7A



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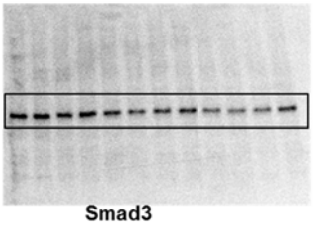
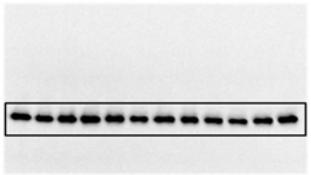


Smad3



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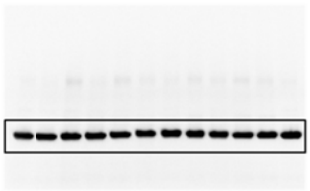
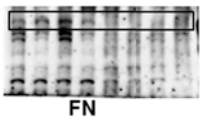


β-actin

Fig. 7C



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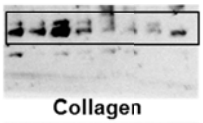
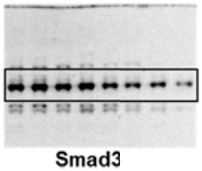
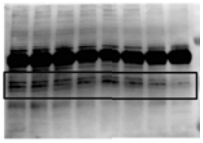


Collagen

Smad2



Smad3



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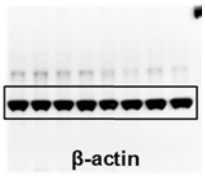
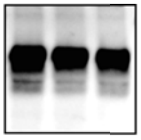


β-actin

Fig. 5D



UCHL5

Flag

β-actin