

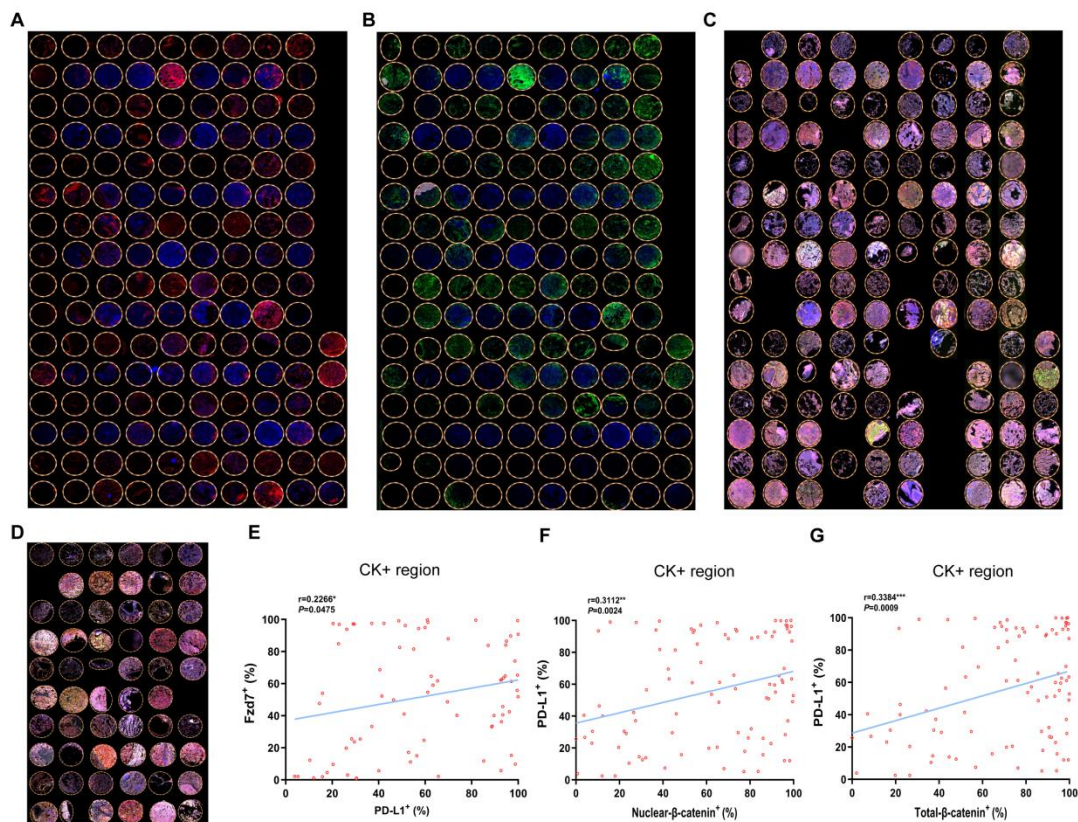


Figure S1 Characterization of NSCLC TMA

A, B RNAscope FISH performed on a human NSCLC TMA (HLugA150CS03) to detect Fzd7 mRNA (red) and PD-L1 mRNA (green) expression. **C, D** TSA mIHC on human NSCLC TMAs (HLugA150CS03, HLugA060PG02). **E** Positive correlation of Fzd7 with PD-L1 in CK⁺ NSCLC tissues, assessed by Pearson correlation analysis ($p = 0.0475$). **F** Positive correlation of nuclear-β-catenin with PD-L1 in CK⁺ NSCLC tissues, assessed by Pearson correlation analysis ($p = 0.0024$). **G** Positive correlation of total-β-catenin with PD-L1 in CK⁺ NSCLC tissues, assessed by Pearson correlation analysis ($p = 0.0009$).

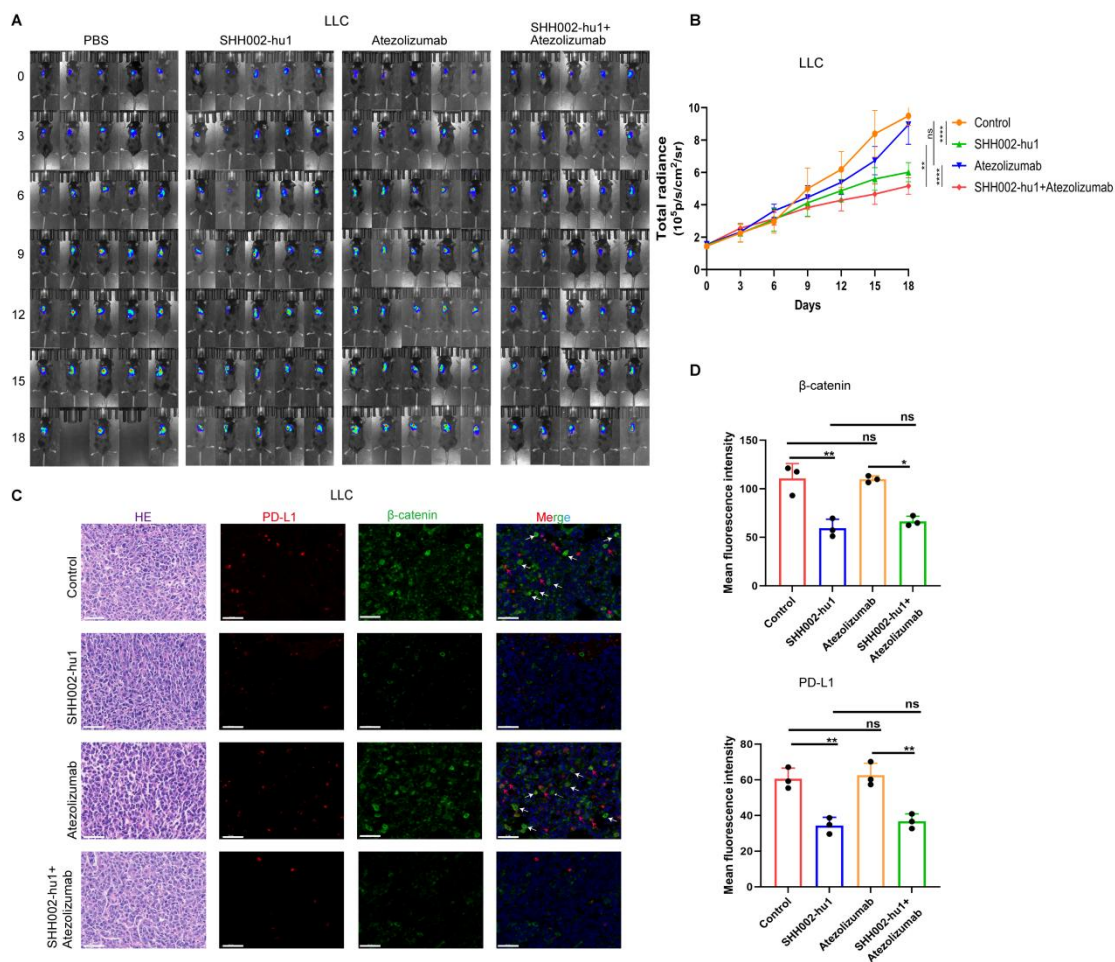


Figure S2 SHH002-hu1 Enhances the Therapeutic Sensitivity of Atezolizumab in LLC

A *In vivo* bioluminescence imaging of orthotopic LLC-luc tumors in C57BL/6 mouse following treatment with PBS (100 μL), SHH002-hu1 (100 nM), Atezolizumab (100 nM), or SHH002-hu1 and Atezolizumab combination ($n = 5$). **B** Tumor growth curves monitored by total radiance ($10^5 \text{ p/s/cm}^2/\text{sr}$) in LLC models across different treatment groups. **C** Expression of PD-L1 and β -catenin in orthotopic LLC xenograft tumors detected by HE and mIHC. Staining showed HE (purple), PD-L1 (red) and β -catenin (green) merged with DAPI-stained nuclei (blue), bar = 20 μm . **D** mIHC analysis of

β -catenin and PD-L1 expression in A549 and H1975 orthotopic tumor tissues across different treatment groups. Results are presented as mean $\pm$ SD. *P* values were determined by ANOVA, **P* < 0.05, ***P* < 0.01, ****P* < 0.0001.

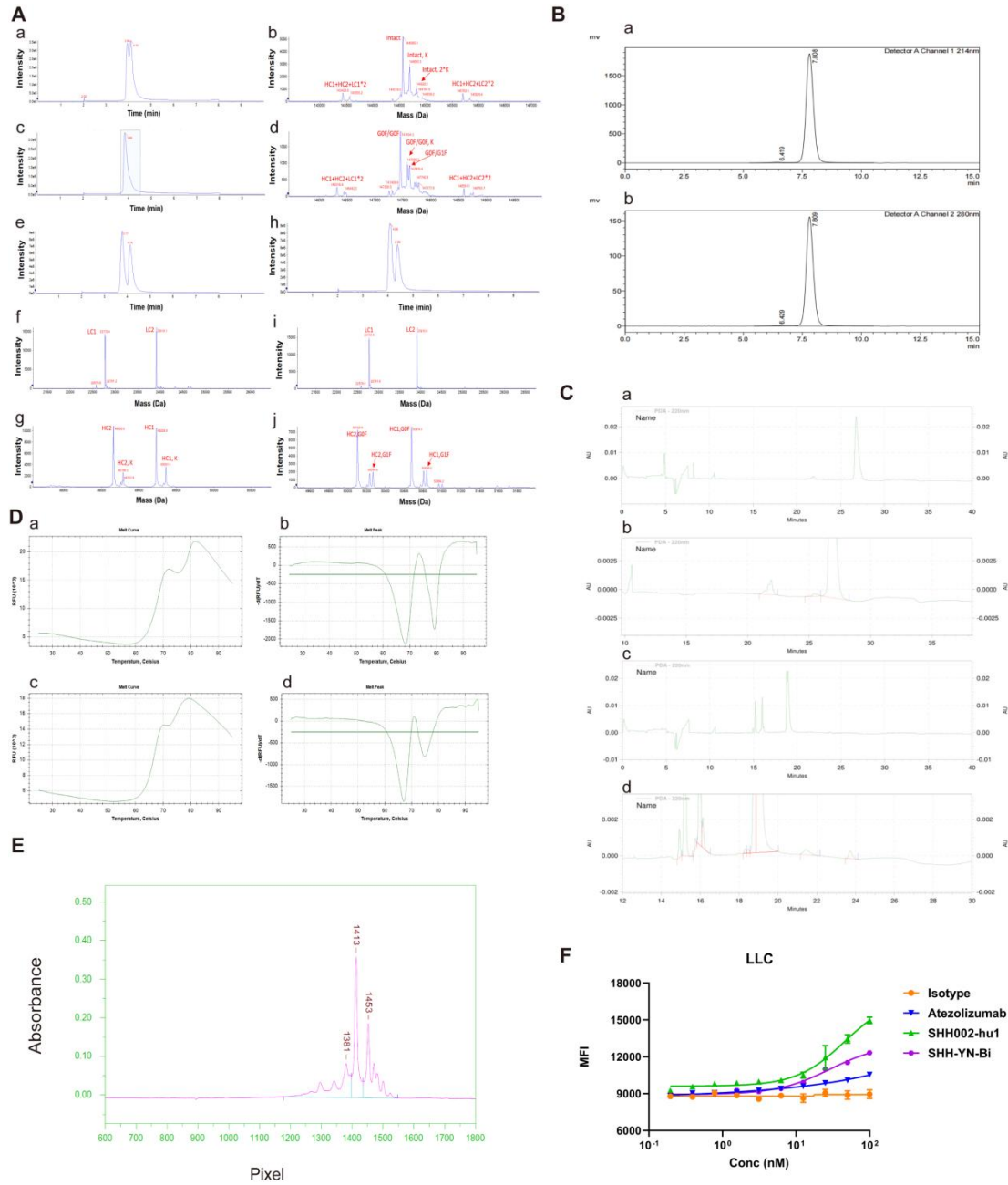


Figure S3 Identification of the SHH-YN-Bi

A Structural characterization of SHH-YN-Bi by HPLC-MS. (a) Total ion chromatogram (TIC) of deglycosylated intact SHH-YN-Bi. (b) Deconvoluted mass spectrum of deglycosylated intact SHH-YN-Bi. (c) TIC of glycosylated intact SHH-YN-Bi. (d) Deconvoluted mass spectrum of glycosylated intact SHH-YN-Bi. (e) TIC of deglycosylated reduced SHH-YN-Bi. (f) Deconvoluted mass spectra of light chain. (g) Deconvoluted mass spectra of heavy chain. (h) TIC of glycosylated reduced SHH-YN-Bi. (i) Deconvoluted mass spectra of light chain. (j) Deconvoluted mass spectra of heavy chain. **B** Purity analysis of SHH-YN-Bi by SEC-HPLC. **C** Purity assessment of SHH-YN-Bi under denaturing conditions by CE-SDS. Analysis under non-reducing and reducing conditions. **D** Thermal stability assessment of SHH-YN-Bi by DSF. **E** Charge heterogeneity analysis of SHH-YN-Bi by cIEF. **F** Binding affinity of SHH002-hu1, Atezolizumab and SHH-YN-Bi to LLC cells.

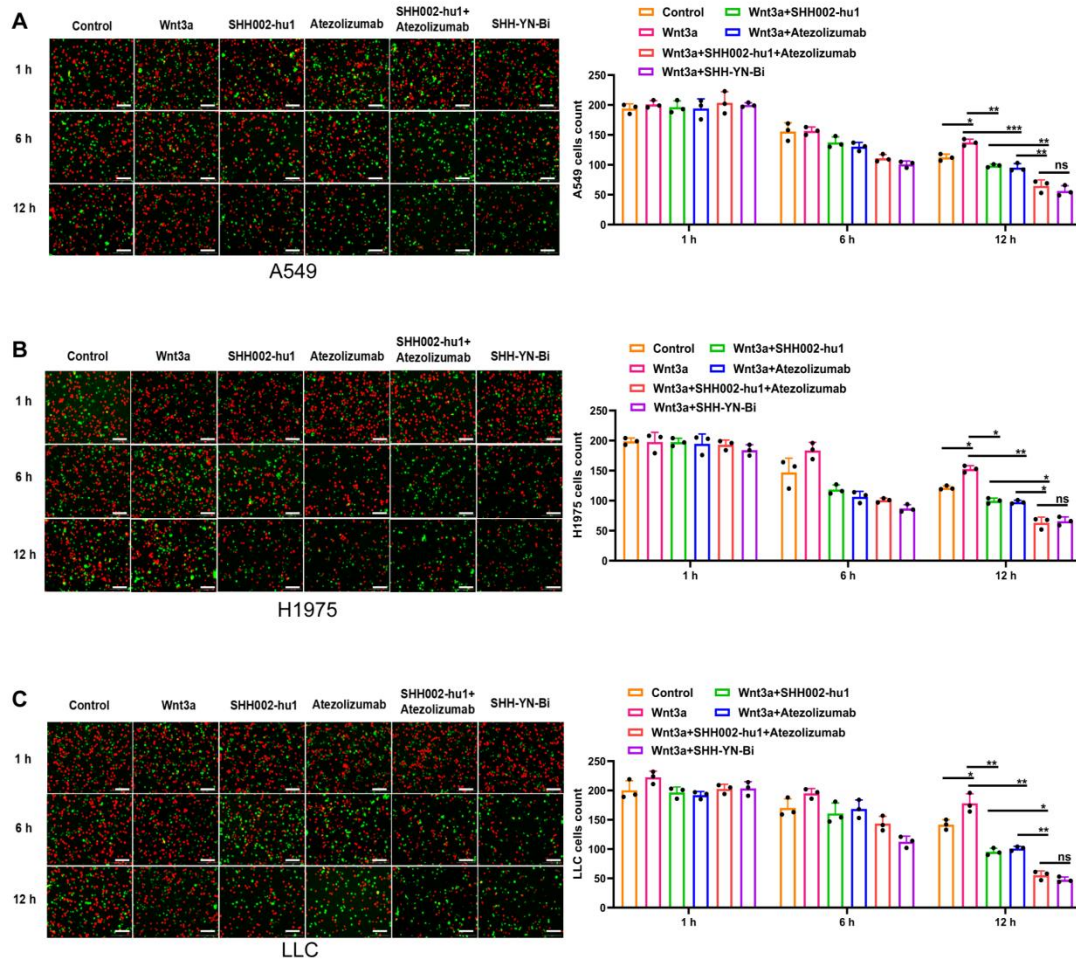


Figure S4 SHH-YN-Bi Enhances Effector T cells Cytotoxicity through simultaneously Blocking PD-1/PD-L1 and Wnt/ β -Catenin axis

A-C Representative fluorescence microscopic images and quantitative analyses of targeting tumor cell survival in co-culture assays. TALL-104/mouse CD8⁺ T-DiO cells (green) were co-incubated with Dil-labeled A549, H1975, LLC tumor cells (red) in the presence of Wnt3a and indicated antibodies. Images were captured at 1h, 6h, and 12h, bar = 100 μ m. Quantification of viable tumor cells were shown as mean $\pm$ SD, n=3, * p < 0.05, ** p < 0.01, *** p < 0.001.

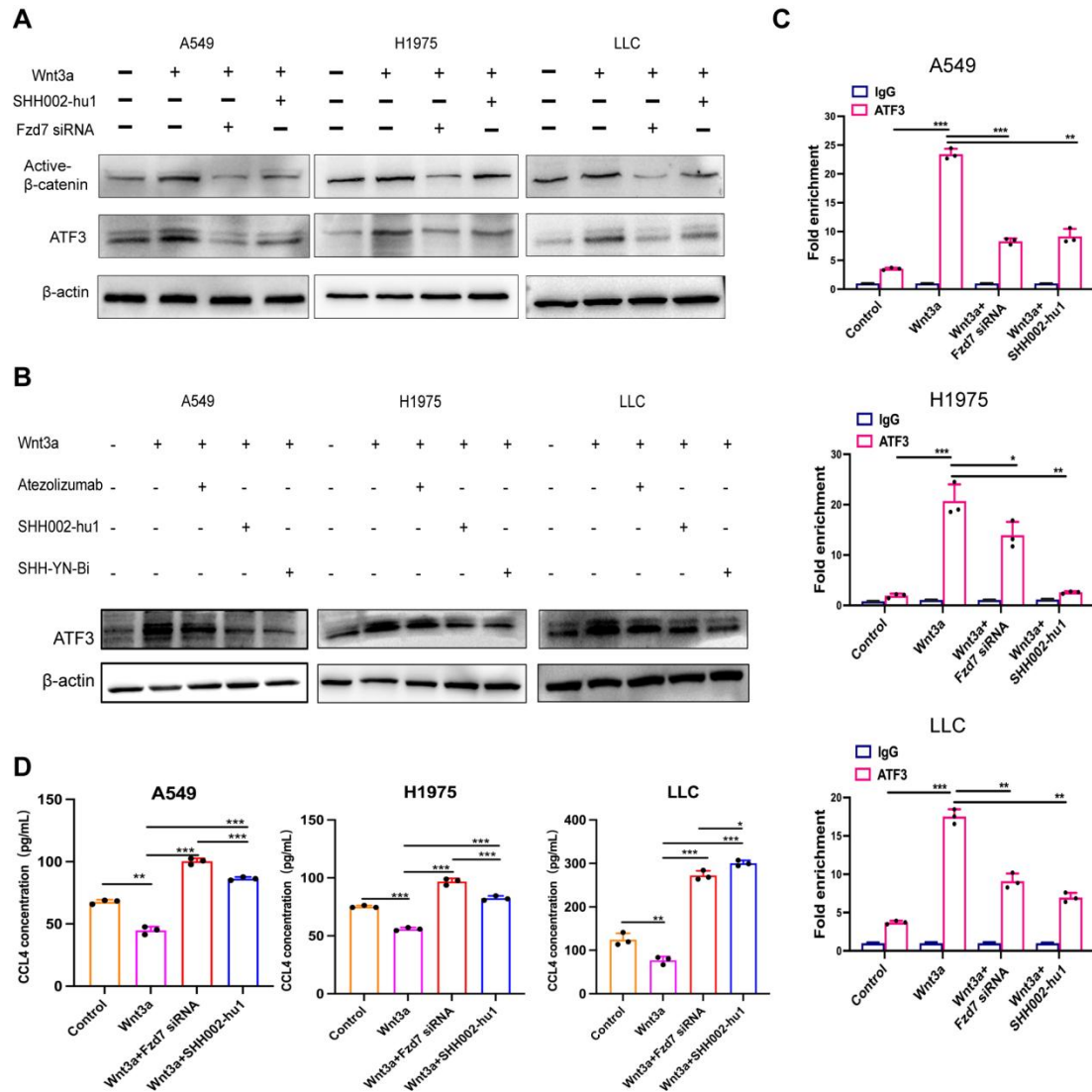


Figure S5 Targeting Fzd7 Increases CCL4 expression by Downregulating ATF3

A Western blot analysis of active-β-catenin and ATF3 in A549, H1975, LLC cells under the indicated treatment. **B** Western blot analysis of ATF3 in A549, H1975, and LLC cells treated with Wnt3a, Atezolizumab, SHH002-hu1, and SHH-YN-Bi. **C** ChIP analysis of ATF3 binding to *Ccl4* promoter region in Wnt3a-stimulated A549, H1975, and LLC cells under Fzd7 siRNA or SHH002-hu1 treatment. qPCR was performed to calculate fold enrichment relative to IgG Control. Data were presented as mean ± SD, n = 3, * $p < 0.05$,

**** $p < 0.01$, *** $p < 0.001$. D** ELISA analysis of CCL4 secretion in NSCLC cells under the the same treatment conditions. Data were presented as mean $\pm$ SD, $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

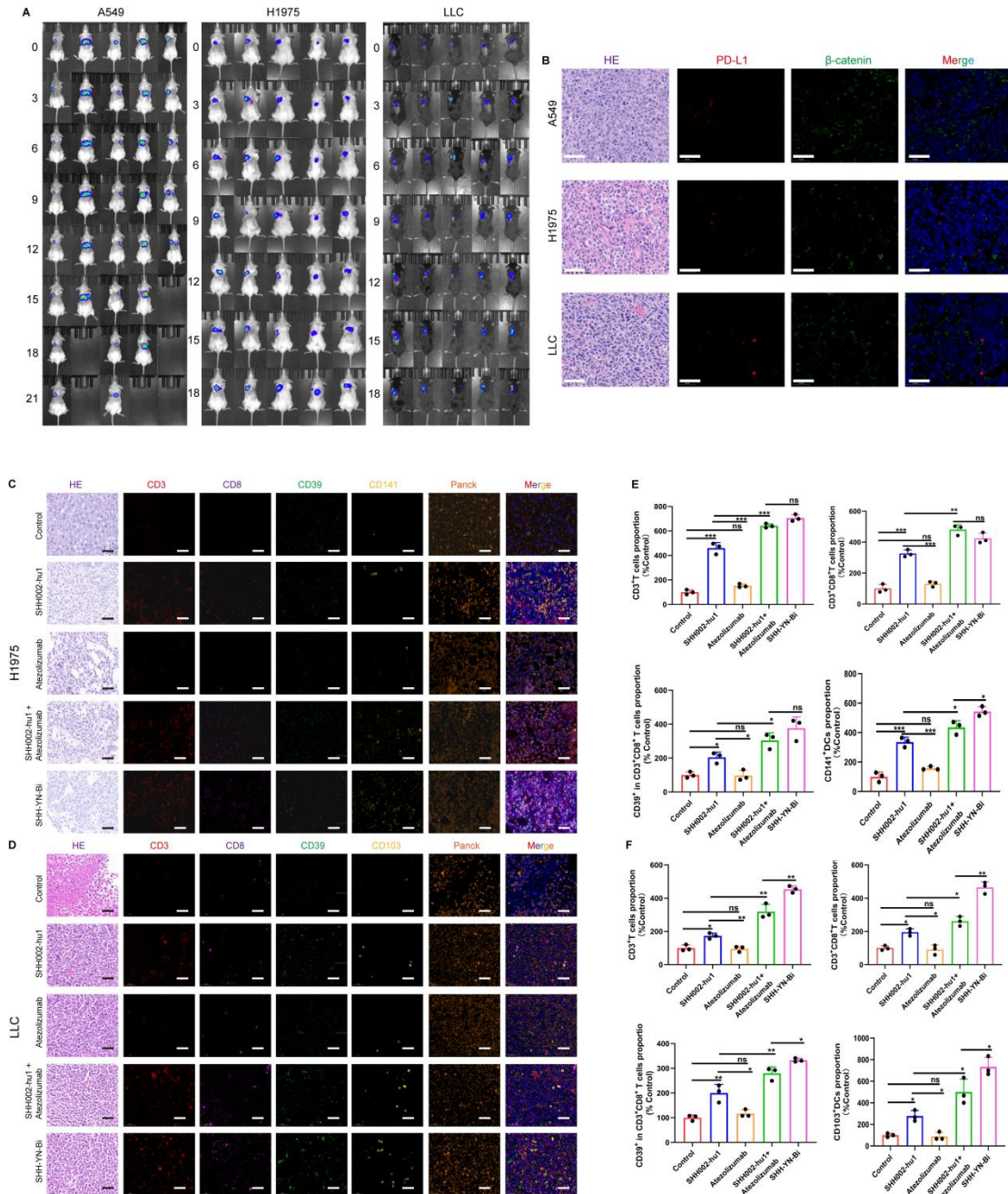


Figure S6 SHH-YN-Bi Suppresses NSCLC *in vivo* by Co-inhibiting Wnt/ β -Catenin

signaling and PD-1/PD-L1 axis

A *In vivo* bioluminescence imaging of orthotopic A549/H1975-luc tumors in CD34⁺ humanized NSG mouse and orthotopic LLC-luc tumors in C57BL/6 mouse following treatment with SHH-YN-Bi (100 nM) (n = 5). **B** Expression of PD-L1 and β -catenin in orthotopic A549/H1975/LLC xenograft tumors detected by HE and mIHC. **C, D** Staining showed HE (purple), PD-L1 (red) and β -catenin (green) merged with DAPI-stained nuclei (blue), bar = 20 μ m. HE and mIHC staining to detect the infiltration of CD8⁺ T cells and CD141⁺/CD103⁺ cDC1s in orthotopic H1975/LLC xenografted tumors. Staining showed HE (purple), CD3 (red), CD8 (purple), CD39 (green), CD141/CD103 (yellow) and PanCK (orange) merged with DAPI-stained nuclei (blue), bar = 20 μ m. **E, F** Quantitative analysis of CD3⁺ cells, CD8⁺ T cells, CD39⁺ CD8⁺ T cells and CD141⁺/CD103⁺ cDC1s relative infiltration rate. Data were presented as the mean $\pm$ SD, n = 3, * P < 0.05, ** P < 0.01, *** p < 0.001.

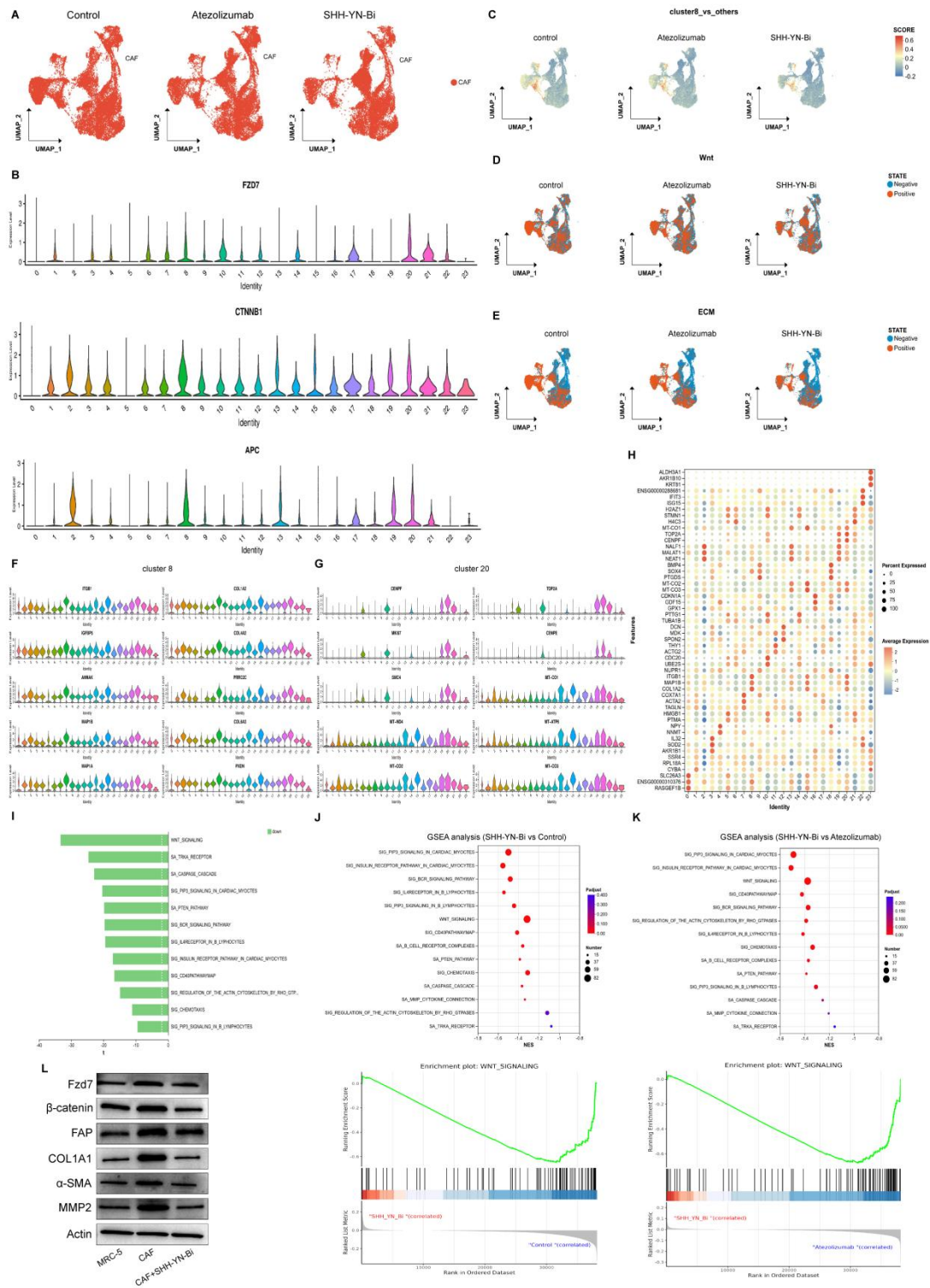


Figure S7 Single-cell Transcriptomic Characterization of the MRC-5-derived CAF

A UMAP visualization of single-cell transcriptomic profiles from MRC-5-derived CAF (*ACTA2*, *FAP*, *COL1A1*, *COL2A1*) **B** Violin plots showing the expression distribution of *Fzd7*, *CTNNB1*, and *APC* across the different clusters. **C** UMAP plots displaying the relationship between cluster 8 and others. **D** Wnt pathway-related cell populations in each clusters. **E** ECM-associated cell populations in each clusters. **F, G** The top 10 genes expression patterns in cluster 8 and cluster 20. **H** Heatmap summarizing the gene expression profiles of distinct cell populations. **I** GSVA reveals pathway suppression following SHH-YN-Bi treatment. **J, K** GSEA enrichment plot of suppressed pathways in SHH-YN-Bi vs. Control/Atezolizumab. **L** Western blot analysis of the CAF associated targets (*Fzd7*, β -catenin, *FAP*, *COL1A1*, α -SMA, and *MMP2*) under the indicated groups.

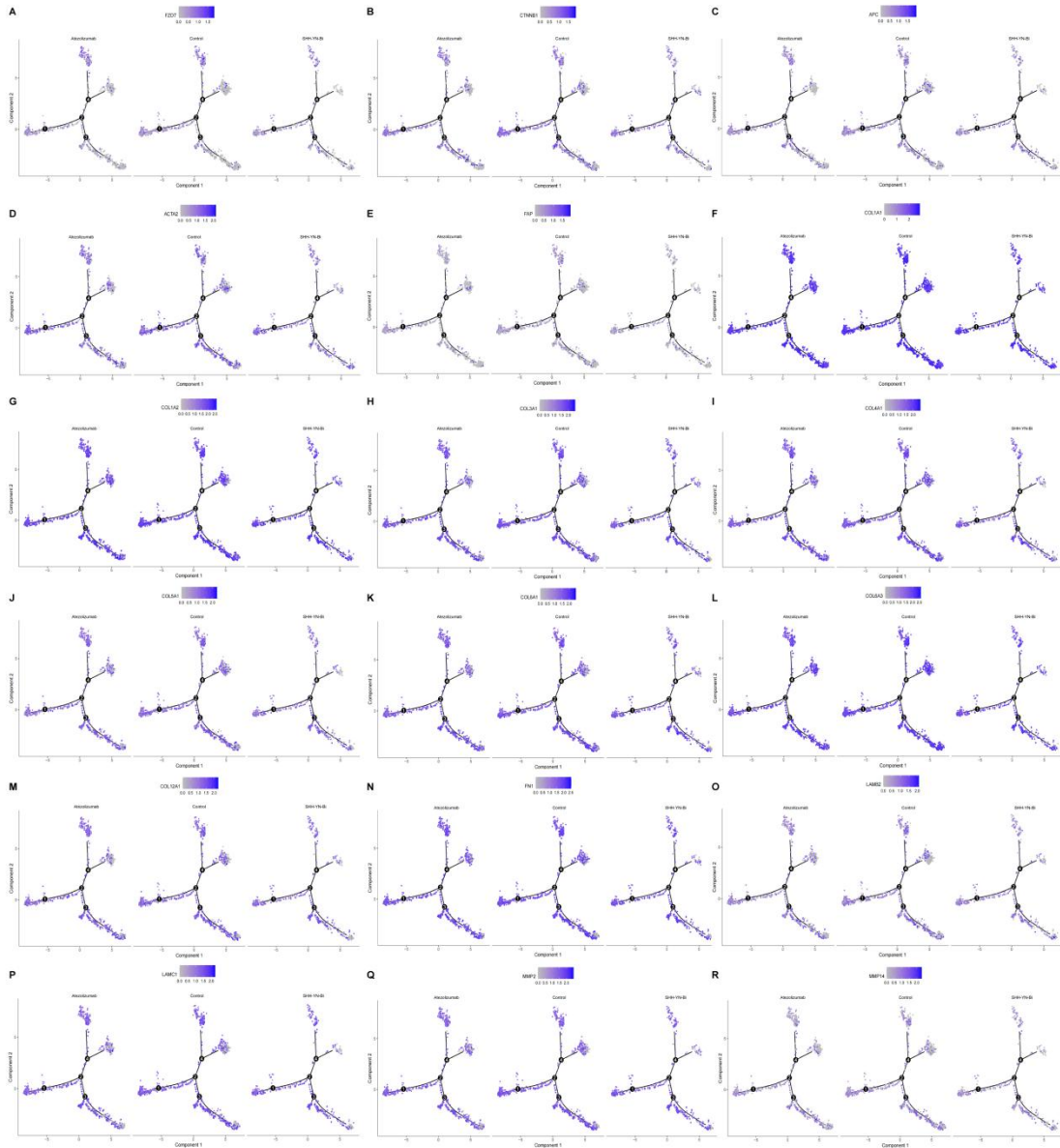


Figure S8 Pseudotime Analysis of CAF-related genes in Response to Different Treatments

Monocle2 pseudotime analysis illustrating the differentiation trajectories of CAF-related genes in MRC-5-derived CAF treated with Control, Atezolizumab, or SHH-YN-Bi. **A-R** correspond to the trajectory dynamics of distinct markers across the three treatment groups

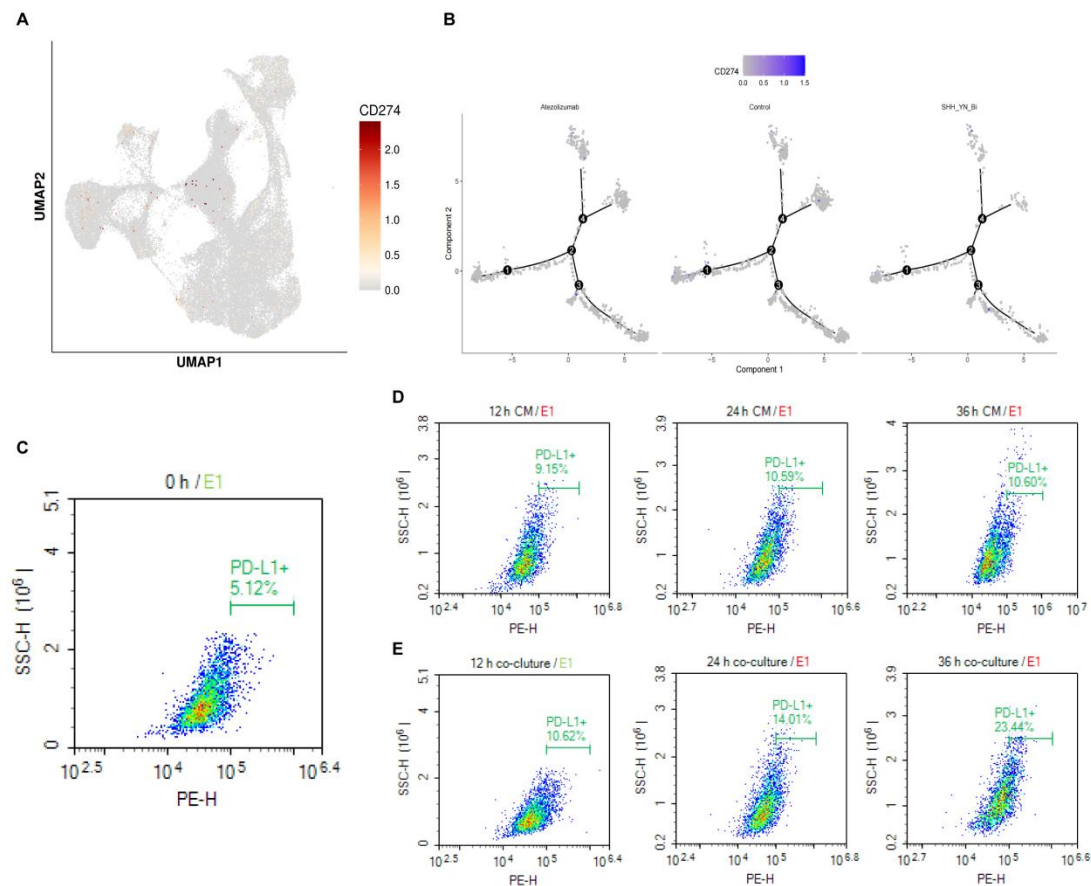
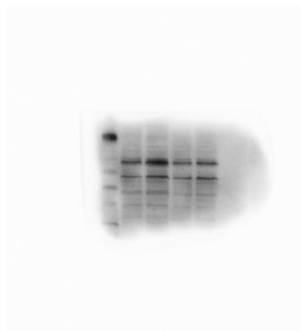


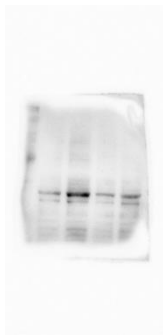
Figure S9 Sustained Non-cell-autonomous Signaling Drives PD-L1 Upregulation in CAFs

A UMAP plot showing the expression level of *CD274* (PD-L1) in single-cell transcriptomic profiles. **B** Pseudotime analysis of *CD274*⁺ cell populations in Control, Atezolizumab, and SHH-YN-Bi treatment groups. **C** FCM plot showing the proportion of PD-L1⁺ cells at 0 h. **D** FCM analysis of PD-L1⁺ cells following 12 h, 24 h, or 36 h of treatment with A549 CM. **E** FCM analysis of PD-L1⁺ cells following 12 h, 24 h, or 36 h of co-culture with A549.

Original western blots



A549-PD-L1



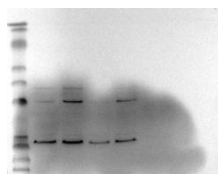
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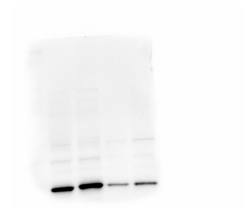
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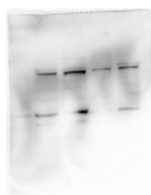
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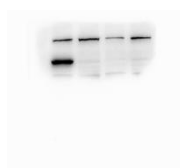
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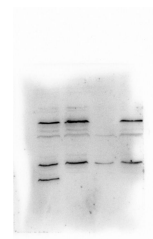
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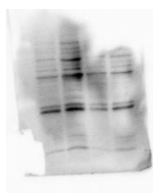
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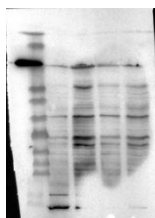
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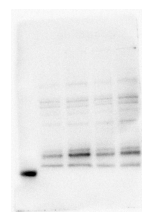
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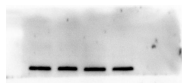
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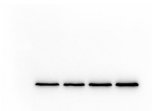
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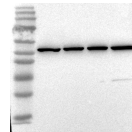
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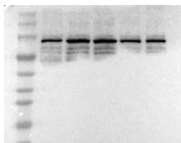
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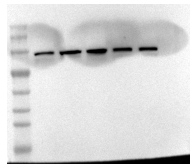
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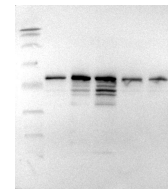
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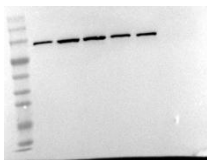
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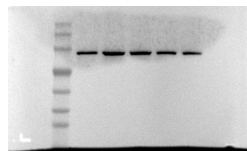
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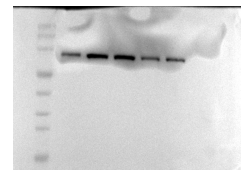
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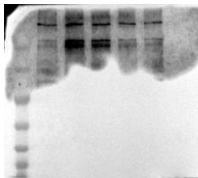
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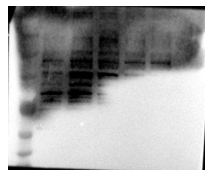
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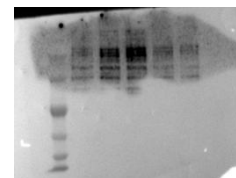
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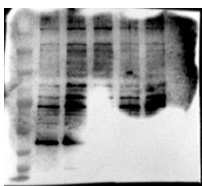
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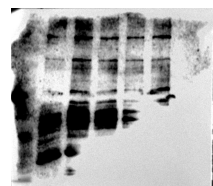
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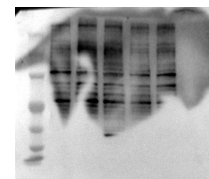
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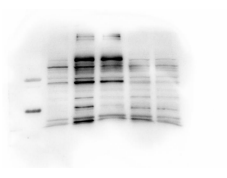
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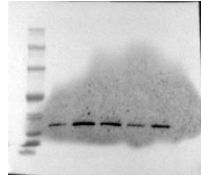
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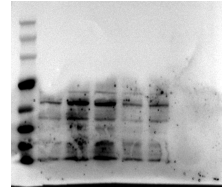
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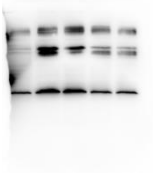
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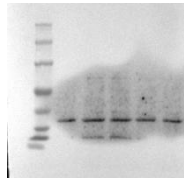
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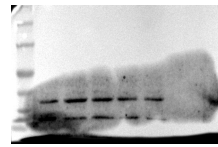
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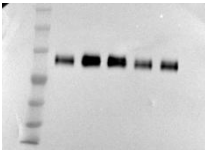
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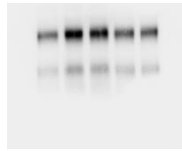
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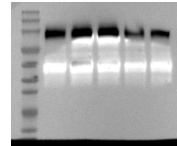
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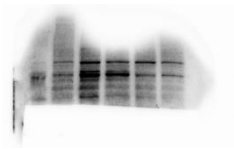
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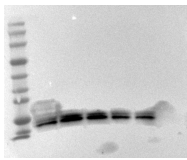
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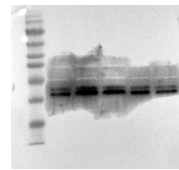
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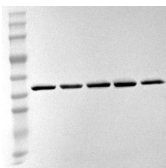
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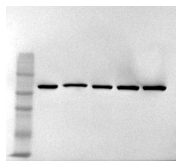
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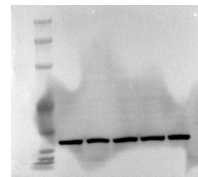
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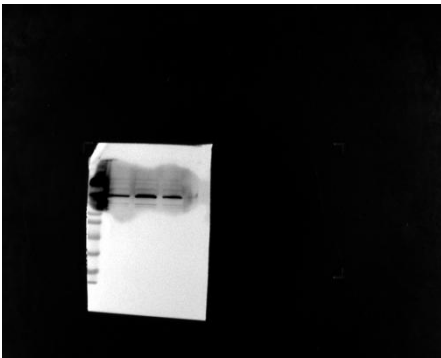
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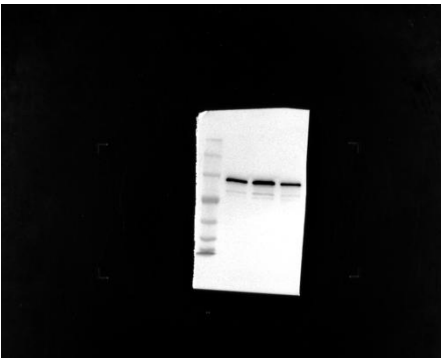
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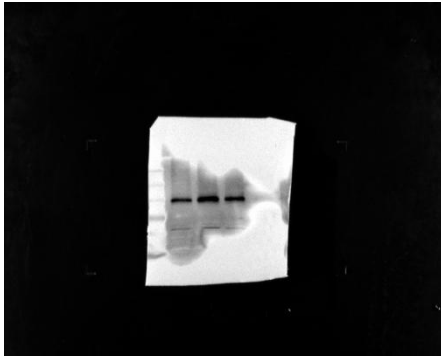
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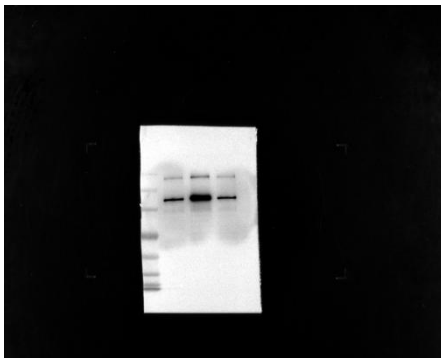
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β-catenin



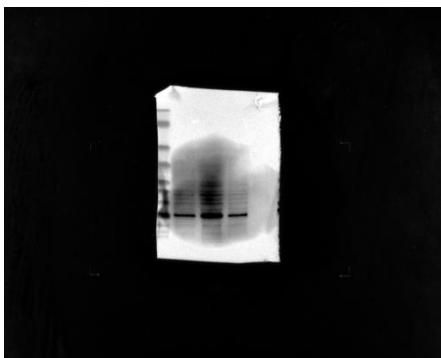
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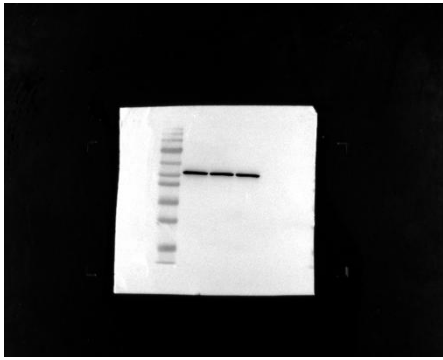
COL1A1



α -SMA



MMP2



Actin