

Supplementary Material

Title:

Proline-rich tyrosine kinase 2 mediates transforming growth factor-beta-induced hepatic stellate cell activation and liver fibrosis

Authors:

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Supplementary Material

Supplementary Table S1. Target gene-specific Taqman primers for qPCR.

Gene	Species	Cat #
<i>Acta2</i>	mouse	Mm01546133_m1
<i>Col1a1</i>	mouse	Mm00801666_g1
<i>Tgfb1</i>	mouse	Mm03024053_m1
<i>Ctgf</i>	mouse	Mm01192933_g1
<i>Cyr61</i>	mouse	Mm00487498_m1
<i>Ankrd1</i>	mouse	Mm00496512_m1
<i>Gapdh</i>	mouse	Mm99999915_g1
<i>ACTA1</i>	human	Hs00559403_m1
<i>COL1A1</i>	human	Hs00164004_m1
<i>TGFB1</i>	human	Hs00998133_m1
<i>CTGF</i>	human	Hs01026927_g1
<i>SMAD7</i>	human	Hs00998193_m1
<i>CYR61</i>	human	Hs00155479_m1
<i>ANKRD1</i>	human	Hs00173317_m1
<i>18S</i>	human	Hs99999901_s1

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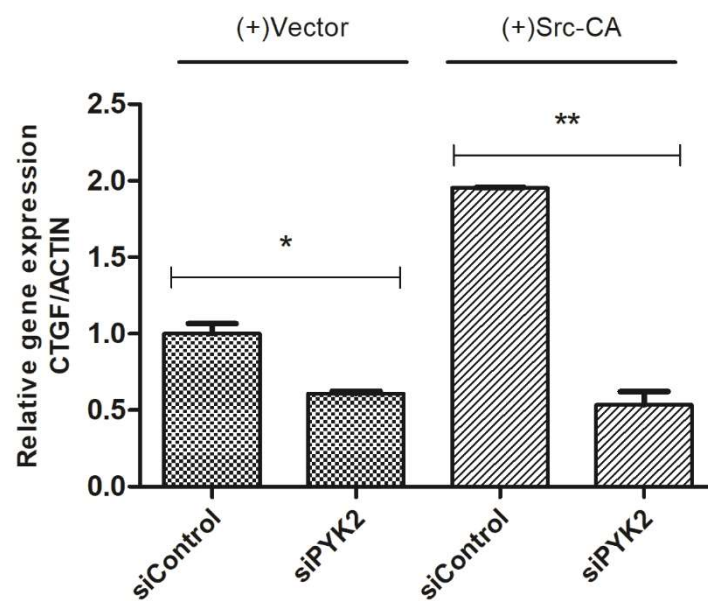
Supplementary Table S2. siRNA sequences.

Gene	Species	Sequence (5' – 3')
<i>FAK</i>	human	GGACAGCGUGAGAGAGAAA
<i>PYK2</i>	human	CCAUGGAGCAAGAGAGGAA
<i>SRC</i>	human	AAGCAACUUGCCCAGCUAUGA
<i>SMAD2</i>	human	GUCCCAUGAAAAGACUUA
<i>SMAD3</i>	human	1) GGAGAAAUGGUGCGAGAAG 2) CUCUGACGCUUGUGACAGU
<i>YAP</i>	human	AAGAAGUAUCUCUGACCAG
<i>TAZ</i>	human	AGGUACUUCCUCAAUCACA

Supplementary Material

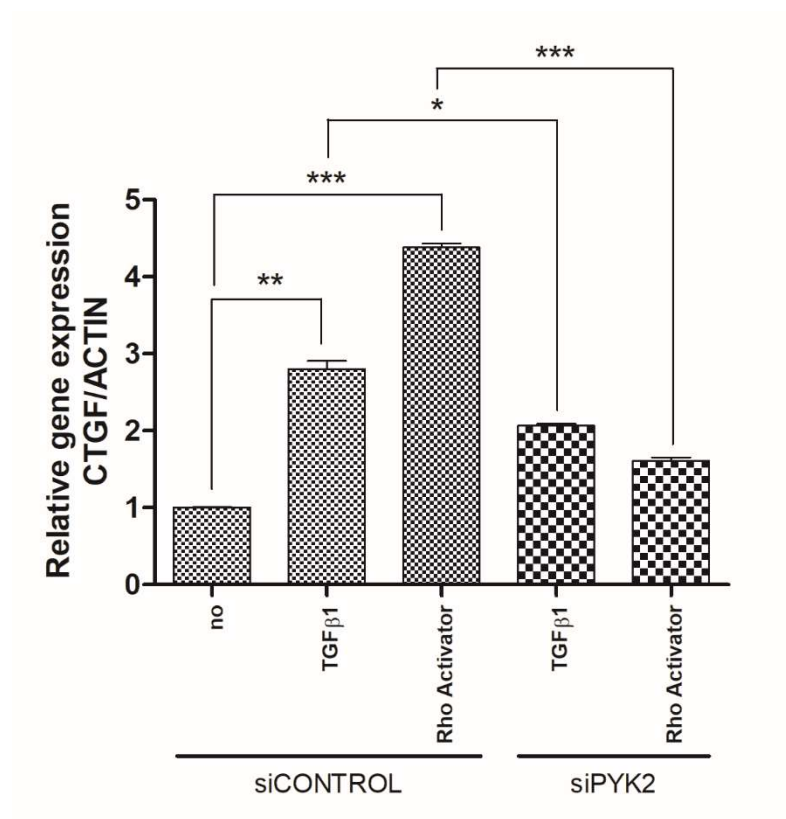
Supplementary Figure S1. Knock-down of PYK2 suppresses CTGF expression induced by ectopic

expression of constitutively active Src protein. LX2 cell lines was transfected with either siRNA of control or PYK2, and then followed by 2nd round transfection with either Vector (pcDNA 3.1) or Src-CA (Y527F, a gift from David Shalloway (Addgene, #17675)). After 48 hr post transfection, CTGF expression was monitored by RT-qPCR (n=2). Student's t-test; *p<0.05, **p<0.01, and ***p<0.001.



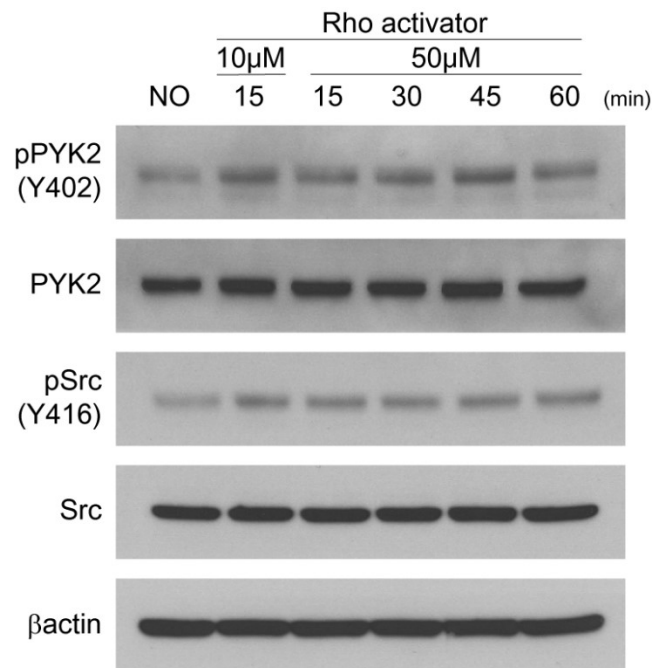
Supplementary Material

Supplementary Figure S2. Knock-down of PYK2 suppresses CTGF induction on treatment with known Rho activator. LX2 cells were transfected with either siRNA of control or PYK2, and then followed by 2hr treatment of Rho activator (50uM of Calpeptin, a major component of CN-01), along with TGF- β 1 (5ug/ml). CTGF expression was monitored by RT-qPCR (n=2). Student's t-test; *p<0.05, **p<0.01, and ***p<0.001.



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Supplementary Figure S3. Rho activator treatment alone was sufficient to increase Pyk2 auto-phosphorylation as well as Src activation. LX2 was treated with 10uM or 50uM of Rho activator (Calpeptin) for 15, 30, 45, or 60 min. Western blot was performed to monitor the phosphorylated forms of Pyk2 and Src.



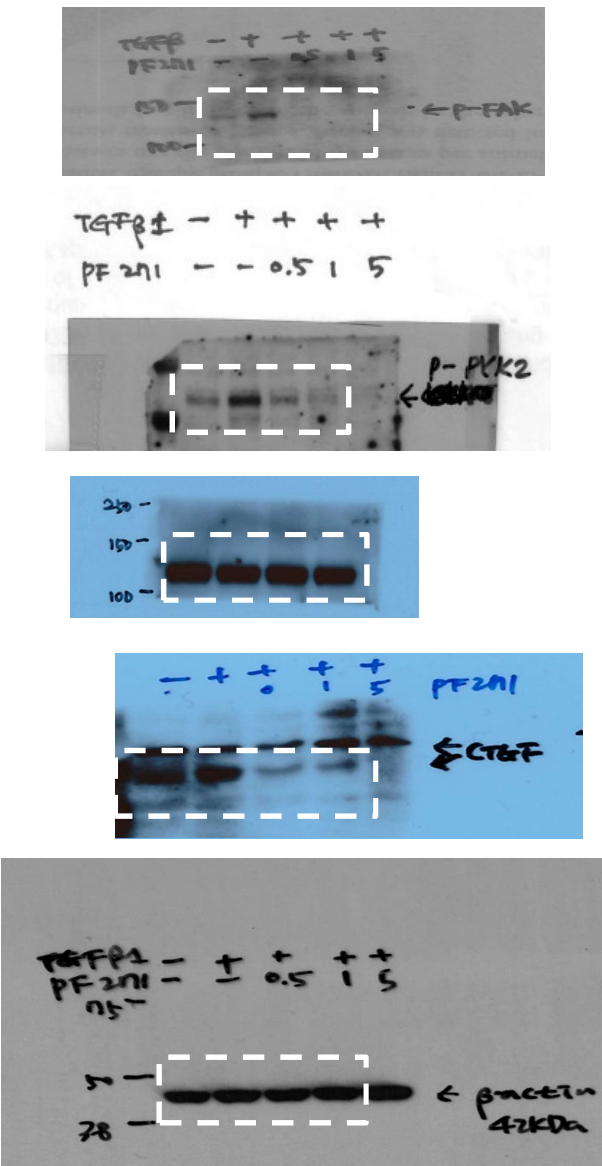
Supplementary Material

Gel scans from the main figures

Figure 2. C



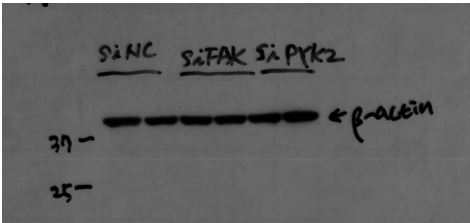
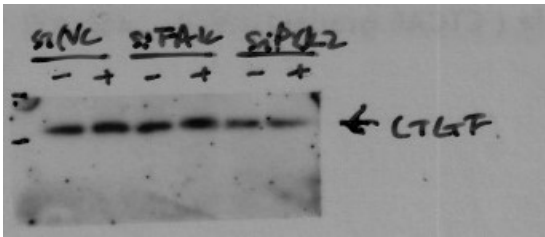
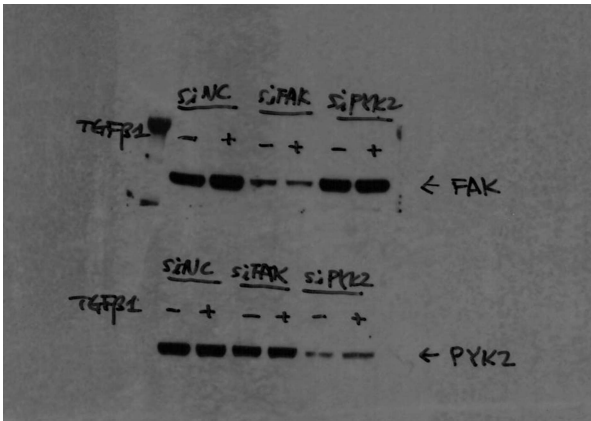
Figure 2. D



Supplementary Material

Gel scans from the main figures

Figure 2. G



Supplementary Material

Gel scans from the main figures

Figure 3. A

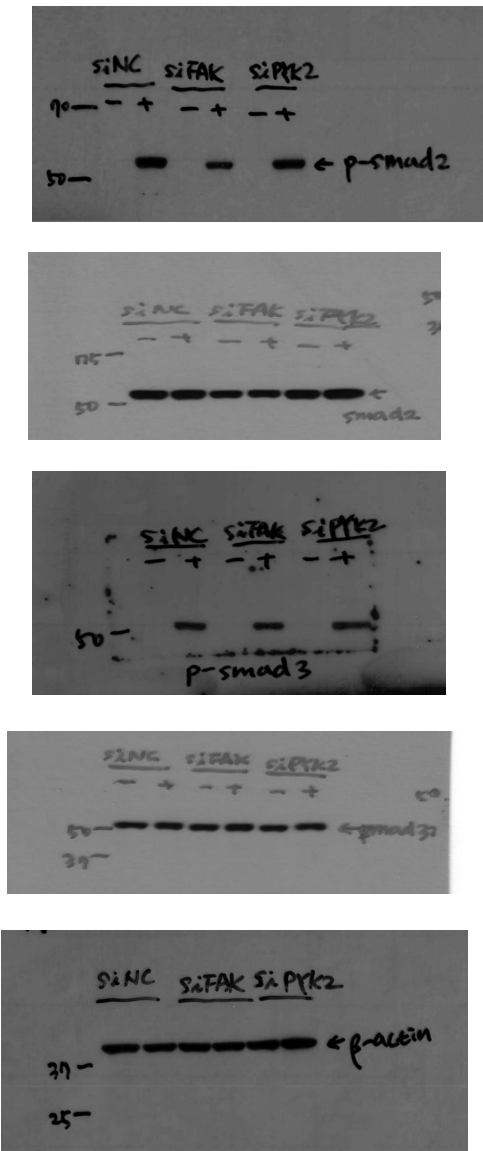
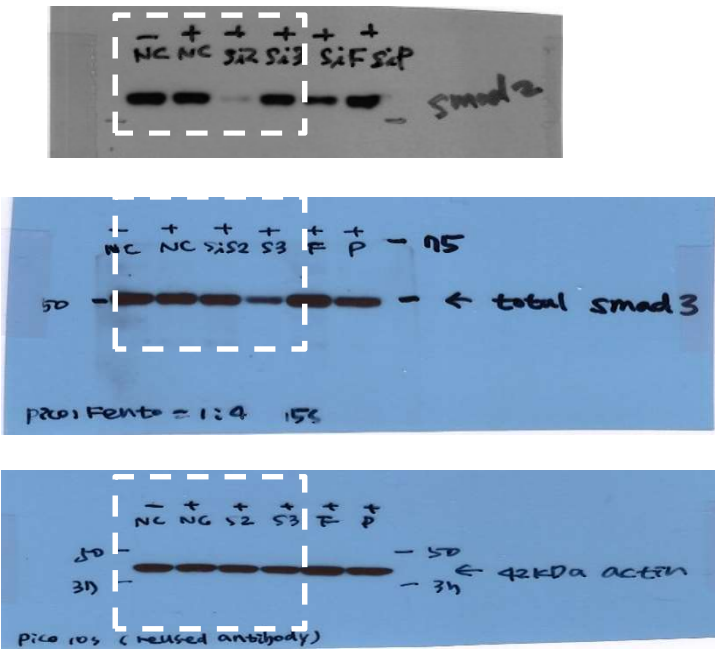


Figure 3. C



Supplementary Material

Gel scans from the main figures

Figure 4. A

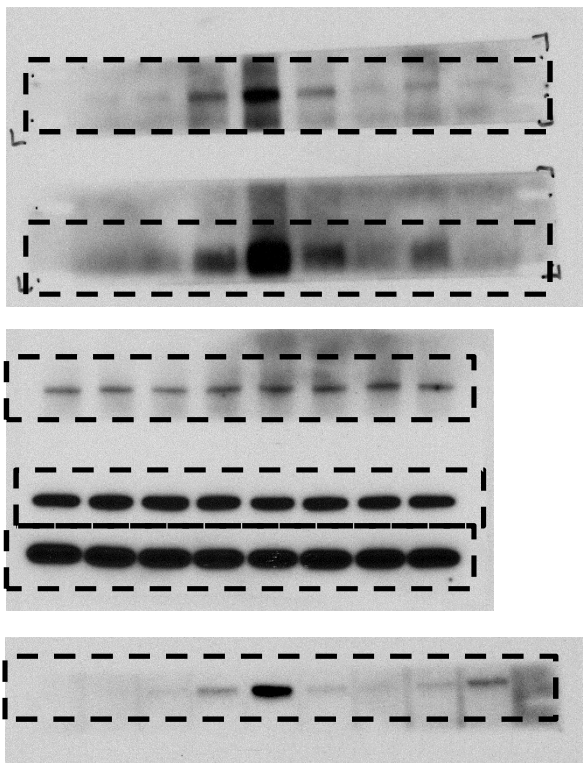
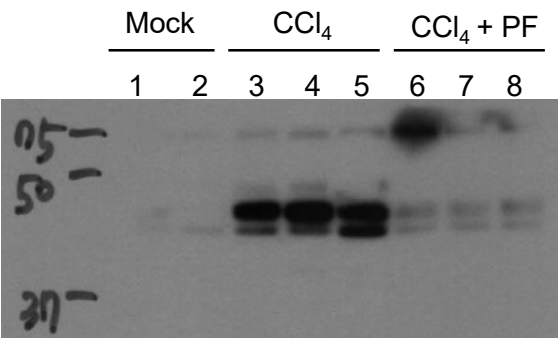
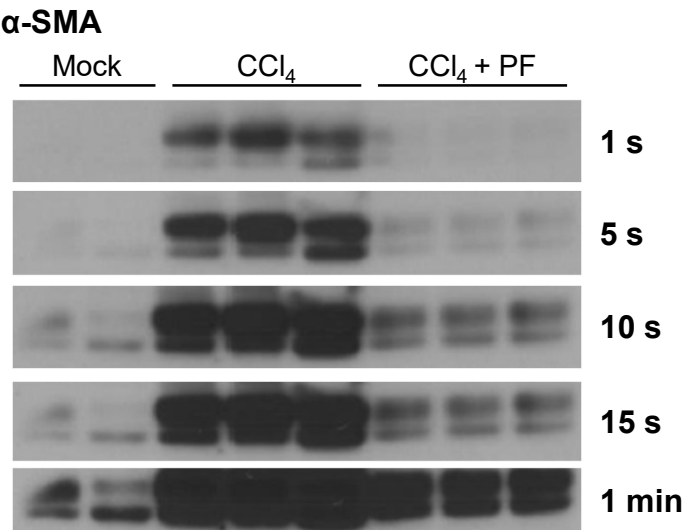
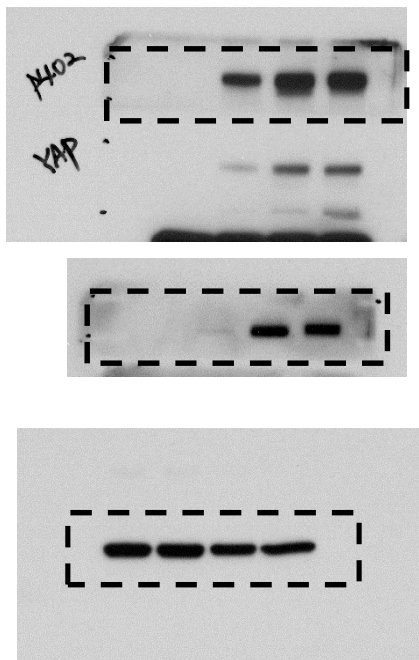


Figure 4. C



Supplementary Material

Gel scans from the main figures

Figure 5. A

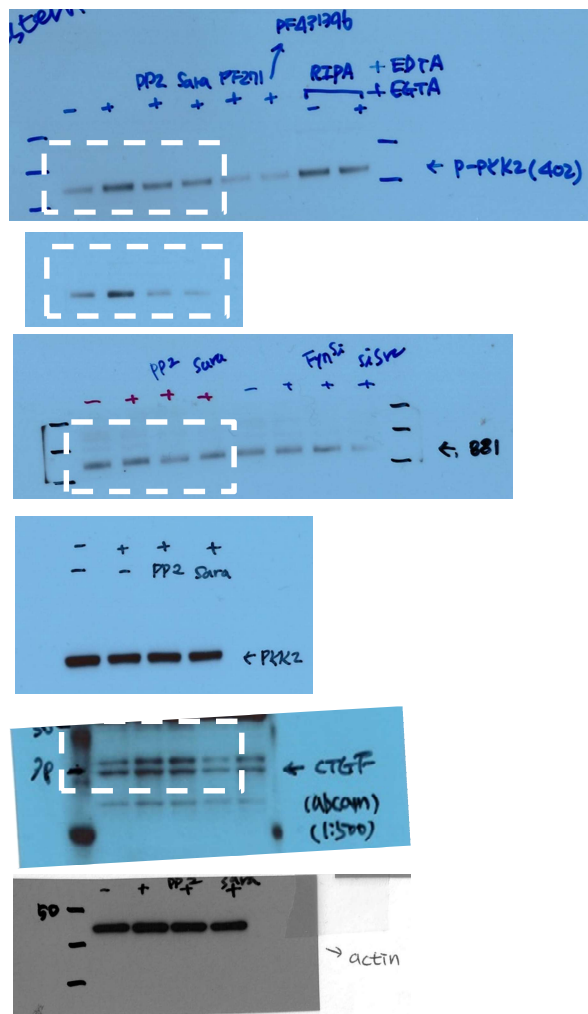
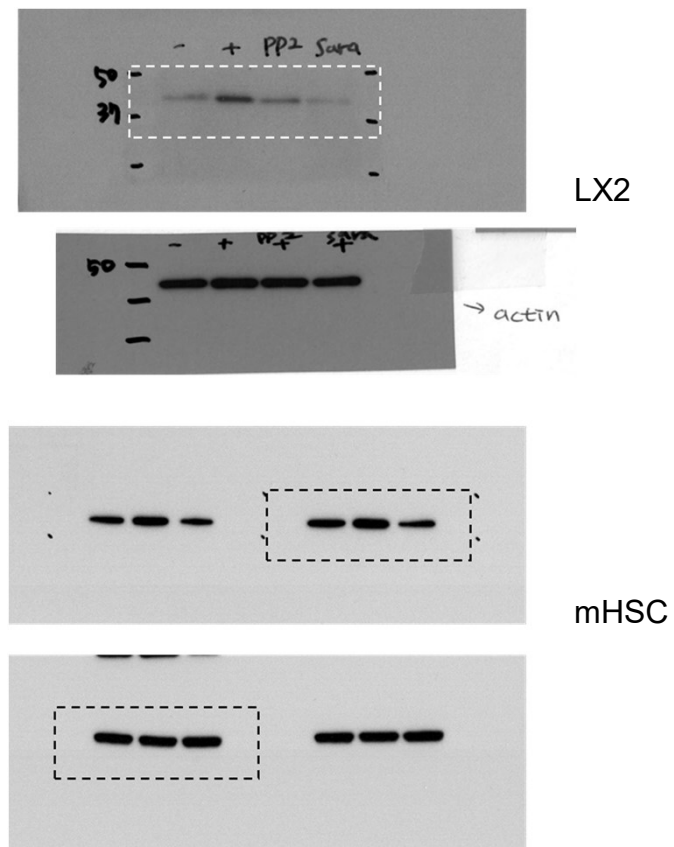


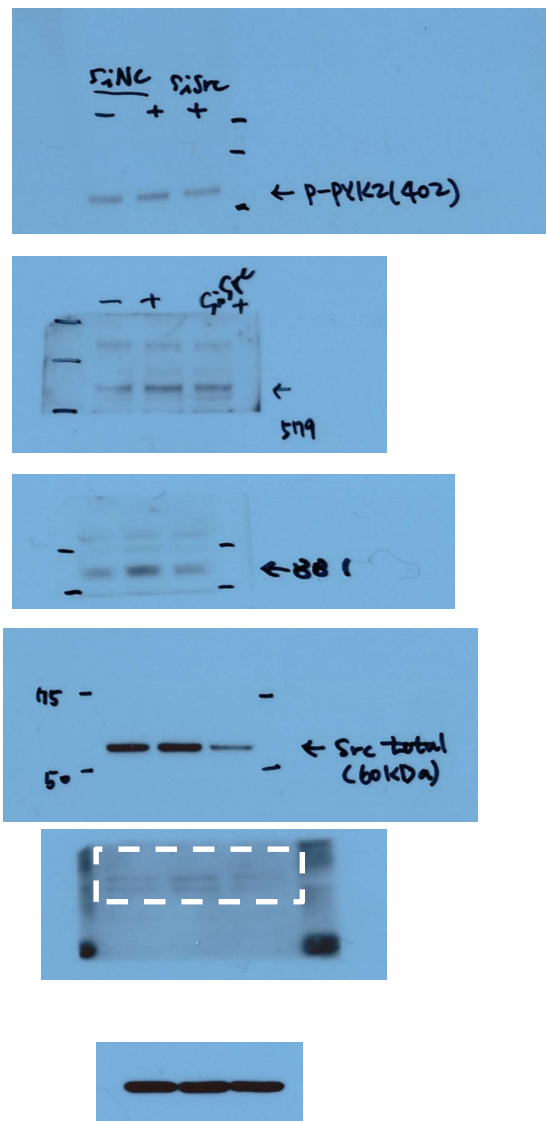
Figure 5. C



Supplementary Material

Gel scans from the main figures

Figure 5. D



Supplementary Material

Gel scans from the main figures

Figure 6. C

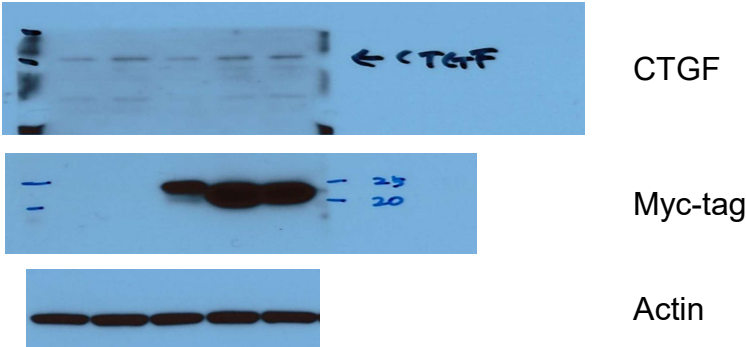
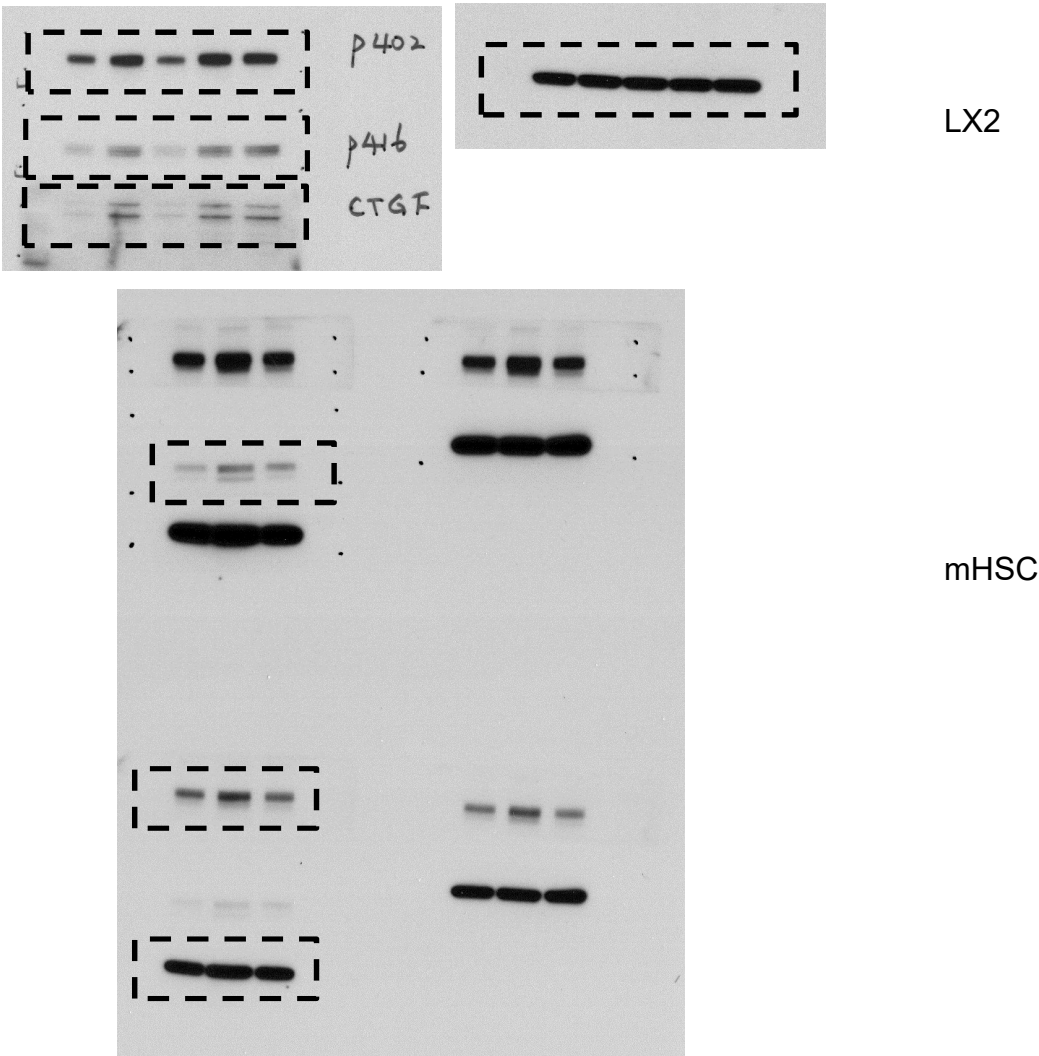


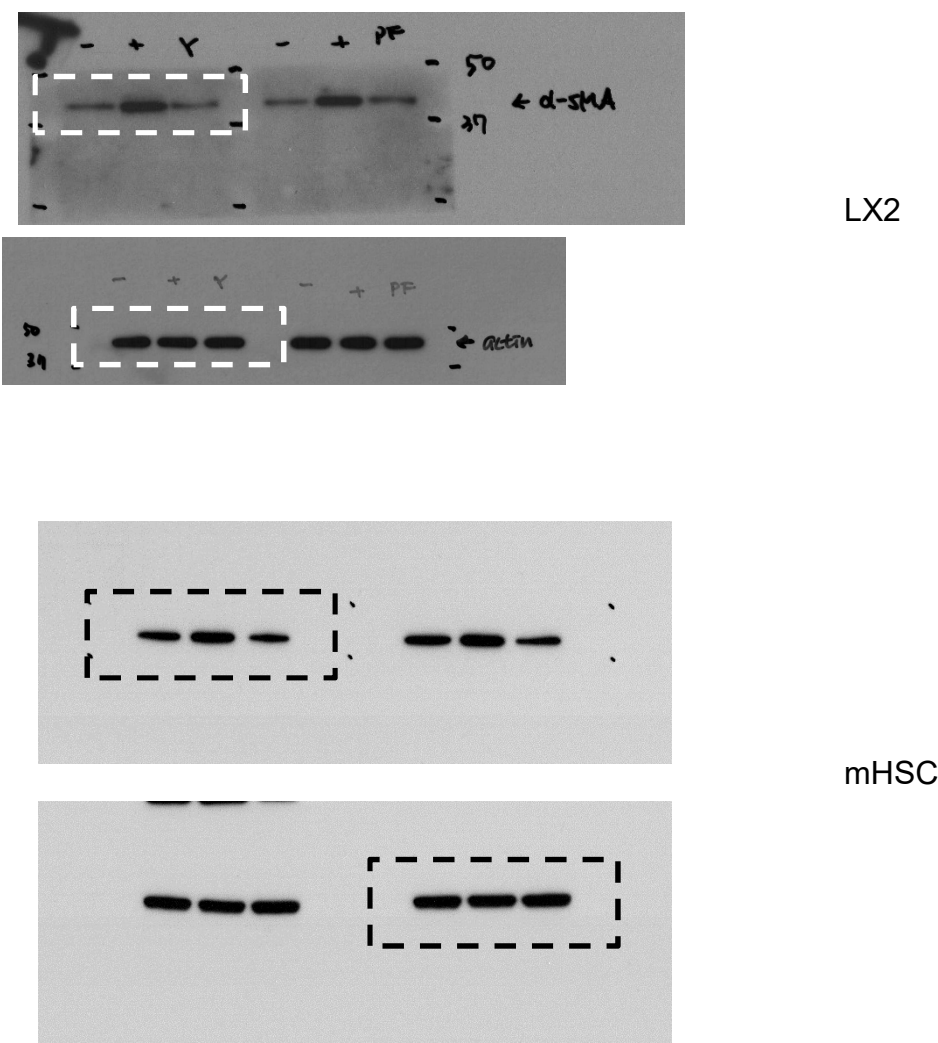
Figure 6. E



Supplementary Material

Gel scans from the main figures

Figure 6. G



Supplementary Material

Gel scans from the main figures

Figure 7. B

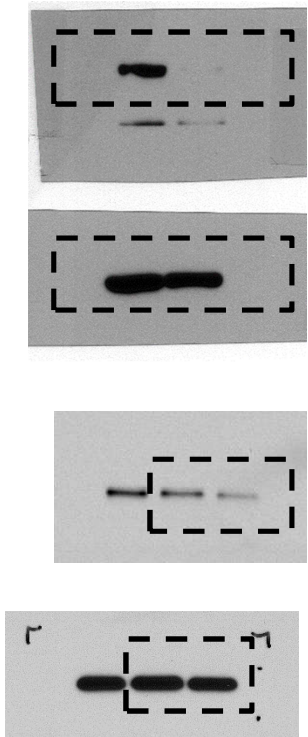


Figure 7. G

