High Molecular Weight Hyaluronan Attenuates Tubulointerstitial Scarring in Kidney Injury

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Supplemental Data

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Lenti-IL-10-GFP treatment upregulates HA production but does not induce pathological changes in normal kidneys.

To confirm the efficacy of lenti-IL-10-GFP treatment and determine whether it would induce any pathological changes in the kidney, we injected lenti-IL-10-GFP in the normal kidney. First, we confirmed that green fluorescent protein is expressed in the renal cortex 3 days after one injection of lenti-IL-10-GFP ($1*10^9$ TU), with immunofluorescent (IF) staining using an antibody specific to GFP. Representative images are shown in Supplementary Figure 1. Next, we performed histology and IHC to evaluate whether lenti-IL-10-GFP caused changes to the normal kidney architecture at 14 days post-injection. PAS staining shows that lenti-IL-10-GFP injected kidney cortex did not have increased intratubular spaces, compared to normal control. Similarly, no significant changes were observed in the deposition of collagen and connective tissue (shown by Masson's trichrome) or α-SMA staining in the lenti-IL-10-GFP injected kidney when compared to normal controls. HABP IHC staining revealed increased HA levels in the lenti-IL-10-GFP treated kidneys compared to control kidneys, as expected based on our previous literature and preliminary data in kidney [1]. No discomfort or undue side effects were observed in lenti-IL-10-GFP injected mice. Our results demonstrated that lenti-IL-10-GFP injection on normal kidneys has no pathological or significant baseline effect, save increased levels of HA.

Supplemental Figure Legends

Supplementary Figure 1. Lenti-IL-10-GFP injected kidneys expressed GFP 3 days after injection, as demonstrated by representative images of immunofluorescent staining of control kidneys and control kidneys treated with 1 x 10^9 TU of lenti-IL-10-GFP 3 days after injection. GFP expression is shown in green, counterstained with DAPI (blue). Scale bar: 50um.

Supplemental Figure 2. Representative images of periodic acid-Schiff stain (PAS) staining, trichrome, and hyaluronan binding protein (HABP) staining of the renal cortex with and without lenti-IL-10 treatment. Scale bars: 75um for PAS, Masson's trichrome and α-SMA staining, 100um for HABP staining for a broader view of the HA changes during the process.

Supplemental Figure 3. Full length of gel electrophoresis and immunoblotting images for Figure 1E and 6D. For gel electrophoresis images, we used two markers that covered a broad range of HA molecular weights. All gels ran in one tank, with exactly the same voltage and time. For anti-HAS1, anti-STAT-3 and associated anti-Beta Actin, we used a marker that was not suitable for chemiluminescent detection in this blotting, therefore an image of marker in the colorimetric mode is shown. Anti-HAS1 and anti-STAT3 are probed on one film, while anti-HAS2 and anti-p-STAT3 are probed on the other one. All bands included in the article are circled with red rectangles.

Supplemental Table 1.
Primer sequences for quantitative PCR validation

Oligonucleotide	Sequence, 5′–3′	Amplicon size (bp)	Intron spanning
Mo Has1 2F	CATGGGCTATGCTACCAAGT	77	3251
Mo Has1 2R	TCAACCAACGAAGGAAGGA		
Mo Has3 1F	CAGTGGACTACATCCAGAGGTG	73	2345
Mo Has3 1R	ACTCGAAGCATCTCAATGGT		
Mo Hyal2 1F	TCTTCACGCGTCCCACATA	79	421
Mo Hyal2 1R	GCACTCTCACCGATGGTAGA		
Mo GAPDH F	GCTGGAGAAGGTTTGTGCG	100	182 and 252
Mo GAPDH R	AGTGATTCTCAAAGTCTTGGTA GGC		

F, forward; R, reverse.

Supplemental References:

1. Balaji S, Wang X, King A, et al. Interleukin-10-mediated regenerative postnatal tissue repair is dependent on regulation of hyaluronan metabolism via fibroblast-specific STAT3 signaling. *Faseb j.* 2017;31(3):868-881.

Fig. S1

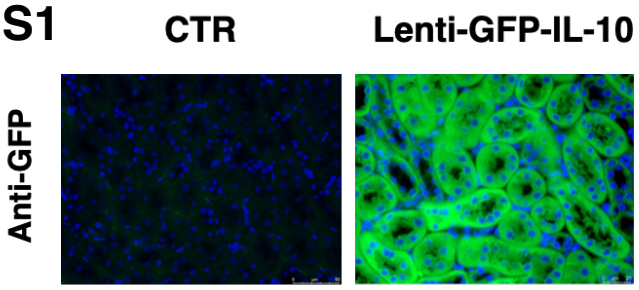

CTR
Lenti-GFP-IL-10
Anti-GFP

Fig. S2

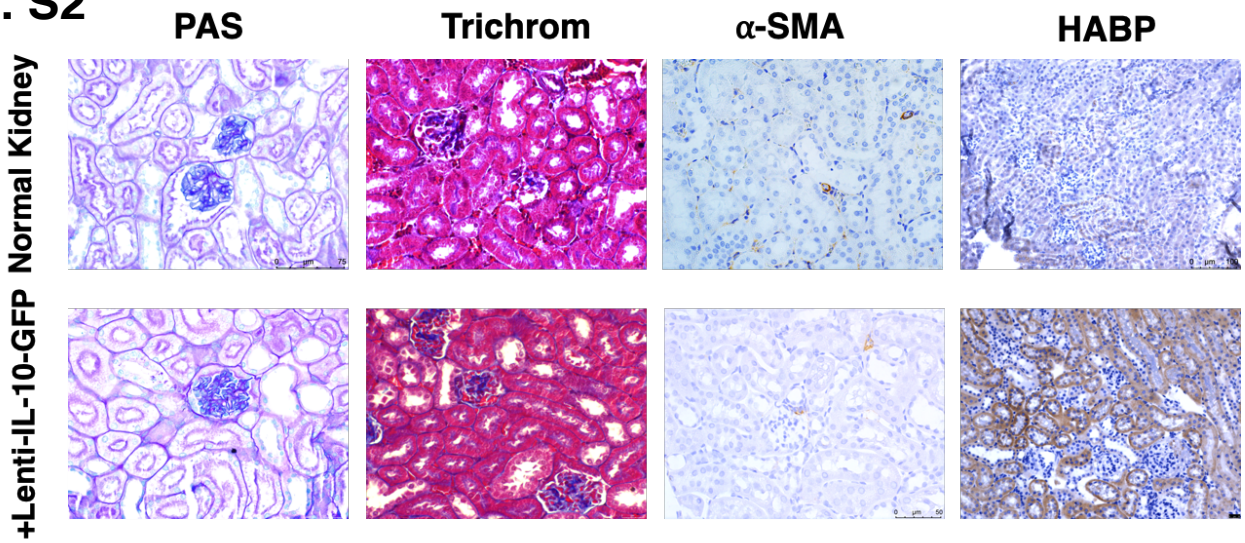
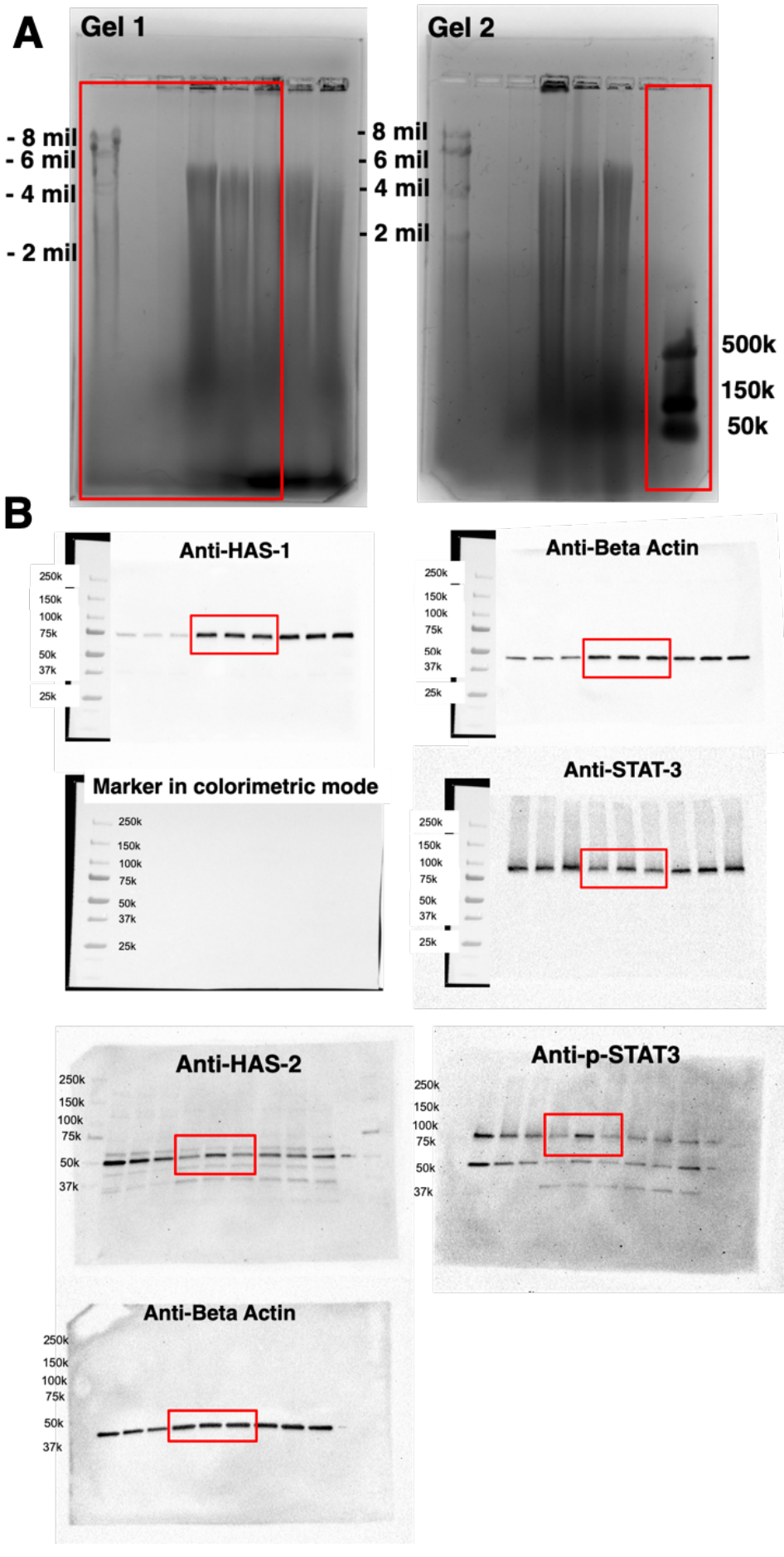

PAS
Trichrom
α-SMA
HABP
Normal Kidney
+Lenti-IL-10-GFP

Fig. S3


A
Gel 1
- 8 mil
- 6 mil
- 4 mil
- 2 mil
Gel 2
- 8 mil
- 6 mil
- 4 mil
- 2 mil
500k
150k
50k
B
Anti-HAS-1
250k
150k
100k
75k
50k
37k
25k
Anti-Beta Actin
250k
150k
100k
75k
50k
37k
25k
Marker in colorimetric mode
250k
150k
100k
75k
50k
37k
25k
Anti-STAT-3
250k
150k
100k
75k
50k
37k
25k
Anti-HAS-2
250k
150k
100k
75k
50k
37k
Anti-p-STAT3
250k
150k
100k
75k
50k
37k
Anti-Beta Actin
250k
150k
100k
75k
50k
37k