**Title:** miR-34c attenuates epithelial-mesenchymal transition and kidney fibrosis with ureteral obstruction

**Names of authors and affiliations:** Ryuji Morizane[1], Shizuka Fujii[1], *Toshiaki Monkawa[1], Ken Hiratsuka[1], Shintaro Yamaguchi[1], Koichiro Homma[1], Hiroshi Itoh[1]

1. Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Supplementary Table S1. miRNAs up-regulated by UUO

| systematic_name | fold change | systematic_name | fold change |
|---|---|---|---|
| mmu-miR-142-5p | 75.0024 | mmu-miR-877* | 3.9418 |
| mmu-miR-466a-3p | 46.2183 | mmu-miR-351 | 3.5247 |
| mmu-miR-212 | 37.5707 | mmu-miR-135a* | 3.4993 |
| mmu-miR-467a | 28.9733 | mmu-miR-28* | 3.4721 |
| mmu-miR-467e | 26.6278 | mmu-miR-714 | 3.3824 |
| mmu-miR-205 | 24.4364 | mmu-miR-425* | 3.3360 |
| mmu-miR-184 | 21.0981 | mmu-miR-672 | 3.3236 |
| mmu-miR-31* | 20.7141 | mmu-miR-466h | 3.2492 |
| mmu-miR-17 | 19.6553 | mmu-miR-132 | 3.2323 |
| mmu-miR-181a-1* | 19.5967 | mmu-miR-300* | 3.1264 |
| mmu-miR-127 | 18.3310 | mmu-miR-546 | 3.0927 |
| mmu-miR-744 | 16.6562 | mmu-miR-770-3p | 3.0723 |
| mmu-miR-467c | 15.5661 | mmu-miR-183* | 3.0623 |
| mmu-miR-342-5p | 15.1527 | mmu-miR-872* | 3.0125 |
| mmu-miR-18a* | 13.1978 | mmu-miR-125a-3p | 2.9349 |
| mmu-miR-505 | 11.9566 | mmu-miR-744* | 2.9254 |
| mmu-miR-379 | 11.7161 | mmu-miR-99b* | 2.9195 |
| mmu-miR-141* | 11.5208 | mmu-miR-685_v14.0 | 2.9088 |
| mmu-miR-214* | 11.0526 | mmu-miR-669h-5p | 2.8887 |
| mmu-miR-296-5p | 10.5931 | mmu-miR-701 | 2.8797 |
| mmu-miR-15b* | 10.3725 | mmu-miR-31 | 2.8371 |
| mmu-miR-677 | 9.3028 | mmu-miR-26b* | 2.8251 |
| mmu-miR-20a* | 8.8458 | mmu-miR-224 | 2.7367 |
| mmu-miR-450a-3p | 8.8068 | mmu-miR-883b-5p | 2.7131 |
| mcmv-miR-m88-1 | 8.7979 | mmu-miR-342-3p | 2.6943 |
| mmu-miR-669l | 8.2181 | mmu-miR-223 | 2.6738 |
| mmu-miR-297a* | 7.6802 | mmu-miR-878-3p | 2.6724 |
| mcmv-miR-M23-1-5p | 6.5483 | mmu-miR-214 | 2.6599 |
| mmu-miR-542-3p | 6.3537 | mmu-miR-678 | 2.5721 |
| mmu-miR-129-3p | 6.0070 | mmu-miR-155 | 2.5038 |
| mmu-miR-1901 | 5.9849 | mmu-miR-1188 | 2.4823 |
| mmu-miR-341 | 5.4161 | mmu-miR-375 | 2.4663 |
| mmu-miR-669b | 5.2025 | mmu-miR-142-3p | 2.4361 |
| mmu-miR-468 | 5.1922 | mmu-miR-190b | 2.4192 |

| | | | |
|---|---|---|---|
| mcmv-miR-M23-1-3p | 5.1037 | mghv-miR-M1-2 | 2.4110 |
| mmu-miR-501-3p | 4.9230 | mmu-miR-467b | 2.3321 |
| mmu-miR-34c* | 4.8129 | mmu-miR-188-3p | 2.2439 |
| mmu-miR-300 | 4.7881 | mmu-miR-421 | 2.2120 |
| mmu-miR-877 | 4.6055 | mmu-miR-680 | 2.1965 |
| mmu-miR-146b | 4.5654 | mmu-miR-199a-5p | 2.1906 |
| mmu-miR-802 | 4.3538 | mmu-miR-1186 | 2.1368 |
| mmu-miR-21* | 4.3344 | mmu-miR-34c | 2.1339 |
| mmu-miR-883a-5p | 4.2978 | mmu-miR-669a | 2.1301 |
| mmu-miR-290-5p | 4.1858 | mmu-miR-210 | 2.1154 |
| mmu-miR-130b | 4.1544 | mmu-miR-292-5p | 2.0997 |
| mmu-miR-106b* | 4.0159 | mmu-miR-186* | 2.0665 |
| mmu-miR-329 | 3.9942 | mmu-miR-489 | 2.0274 |
| mmu-miR-449a | 3.9734 | mmu-miR-199a-3p | 2.0233 |

Supplementary Table S2. miRNAs up-regulated by TGF-β in MCT

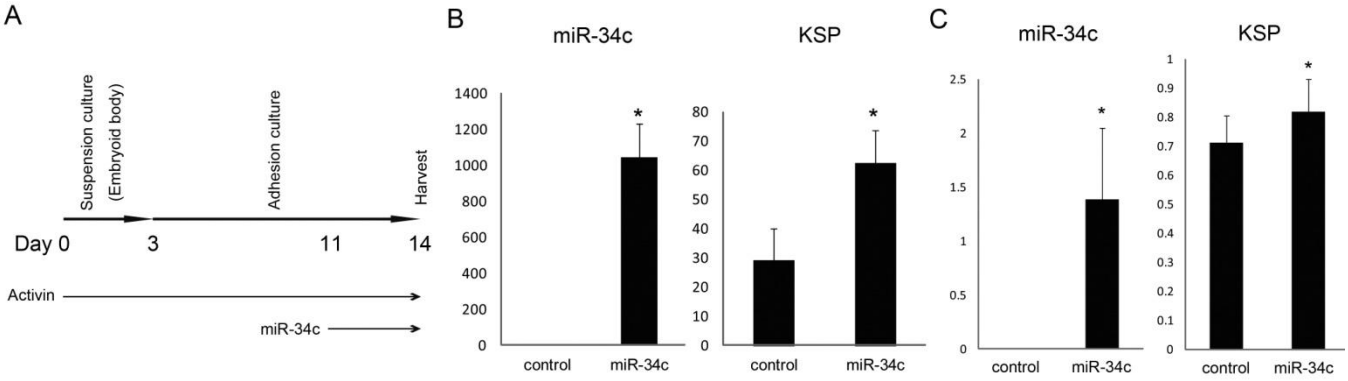
| systematic_name | fold change | systematic_name | fold change |
|---|---|---|---|
| mmu-miR-494 | 273.1822 | mmu-miR-467f | 6.4509 |
| mmu-miR-714 | 99.6733 | mmu-miR-671-5p | 6.0041 |
| mmu-miR-188-5p | 79.7492 | mmu-miR-483 | 5.3729 |
| mmu-miR-574-5p | 78.9182 | mmu-miR-450a-3p | 5.3078 |
| mmu-miR-1906 | 49.7573 | mmu-miR-185 | 5.1866 |
| mmu-miR-689 | 45.1235 | mmu-miR-425 | 4.9647 |
| mmu-miR-135a* | 44.8211 | mmu-miR-1892 | 4.9405 |
| mmu-miR-449a | 34.8543 | mmu-miR-181a | 4.8859 |
| mmu-miR-140* | 30.2912 | mmu-miR-365 | 4.8526 |
| mmu-miR-193 | 29.0345 | mmu-miR-128 | 4.8222 |
| mmu-miR-298 | 28.6193 | mcmv-miR-M87-1 | 4.7572 |
| mmu-miR-721 | 25.1908 | mmu-miR-705 | 4.1271 |
| mmu-miR-872 | 24.3895 | mmu-miR-340-3p | 4.0287 |
| mmu-miR-29b* | 14.9323 | mmu-miR-30c-1* | 3.9218 |
| mmu-miR-1224 | 14.2491 | mmu-miR-681 | 3.7968 |
| mmu-miR-1895 | 11.9413 | mmu-miR-125a-3p | 3.7460 |
| mmu-miR-1187 | 10.4649 | mmu-let-7b* | 3.7248 |
| mcmv-miR-M23-1-5p | 10.3352 | mmu-miR-30c | 3.4835 |

| | | | |
|---|---|---|---|
| mmu-miR-712 | 9.3542 | mmu-miR-181b | 2.5848 |
| mmu-miR-715 | 7.8953 | mmu-miR-34c | 2.5734 |
| mmu-miR-1897-5p | 7.7120 | mmu-miR-125b-5p | 2.5555 |
| mmu-miR-370 | 7.6363 | mmu-miR-34a | 2.3150 |
| mmu-miR-1894-3p | 7.3622 | mmu-miR-34b-5p | 2.2151 |
| mmu-miR-680 | 6.8924 | mmu-miR-30d | 2.1574 |

Supplementary Table S3. miRNAs down-regulated by Activin in mouse ES cells

| systematic_name | fold change | systematic_name | fold change |
|---|---|---|---|
| mmu-miR-467b | -27.73095 | mmu-miR-181b | -5.343746 |
| mmu-miR-19a | -27.163473 | mmu-miR-34b-5p | -5.2363887 |
| mmu-miR-1904 | -26.608042 | mmu-miR-181c | -5.16735 |
| mmu-miR-685_v14.0 | -25.322464 | mmu-miR-30c | -5.1665454 |
| mmu-miR-223 | -25.279774 | mmu-miR-26b | -5.130635 |
| mmu-miR-291a-5p | -18.82812 | mmu-miR-669a | -5.101503 |
| mmu-miR-290-5p | -18.221317 | mmu-miR-483* | -5.075339 |
| mmu-miR-705 | -18.081573 | mmu-miR-181d | -5.057848 |
| mmu-miR-671-5p | -16.059662 | mmu-miR-96 | -5.0199513 |
| mghv-miR-M1-2 | -8.658391 | mmu-miR-141 | -4.9155693 |
| mmu-miR-125a-3p | -8.328011 | mmu-miR-218 | -4.851454 |
| mmu-miR-10a | -8.32481 | mmu-miR-34c | -4.6313596 |
| mmu-miR-100 | -8.0890045 | mmu-miR-494 | -4.620617 |
| mmu-miR-1897-5p | -7.596741 | mmu-miR-367 | -4.581095 |
| mmu-miR-202-3p | -7.5897546 | mmu-miR-423-5p | -4.521743 |
| mmu-miR-1895 | -7.5245156 | mmu-miR-434-3p | -4.2810717 |
| mmu-miR-10b | -6.700873 | mmu-miR-677 | -4.2440267 |
| mmu-miR-301a | -6.578678 | mmu-miR-702 | -4.1883388 |
| mmu-miR-139-3p | -6.2485433 | mmu-miR-483 | -4.1605797 |
| mmu-miR-148a | -6.011732 | mmu-miR-877 | -4.013603 |
| mmu-miR-135a* | -5.9646735 | mmu-miR-712 | -3.9998624 |
| mmu-miR-124 | -5.778936 | mmu-miR-29a | -3.8379686 |
| mmu-miR-429 | -5.696437 | mmu-miR-290-3p | -3.331493 |
| mmu-miR-30d | -5.379591 | mmu-miR-721 | -3.1268754 |

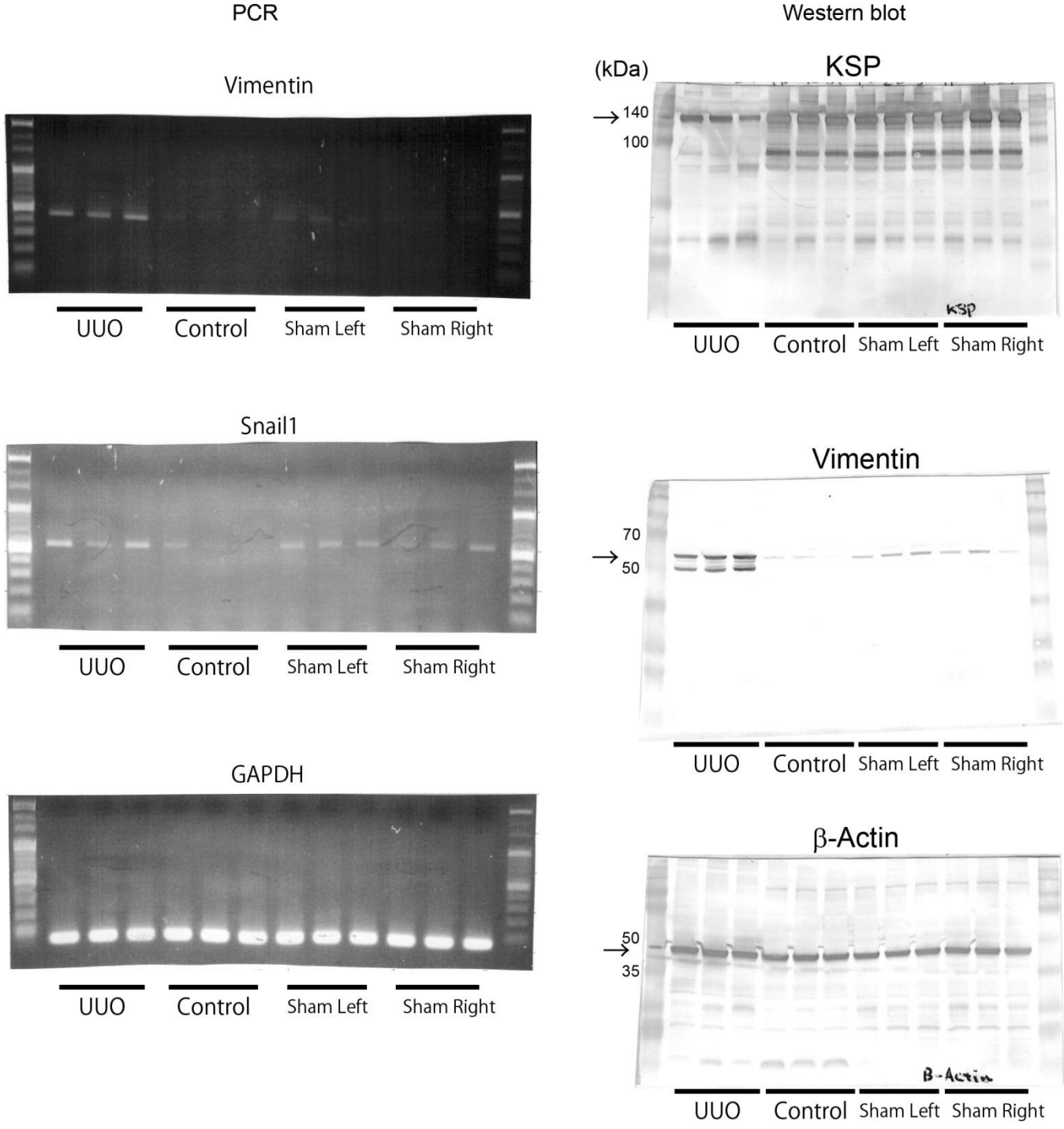
Supplementary Figure S1. miR-34c up-regulated the expression of KSP in mouse ES cells and mouse embryonic kidneys. (A) The protocol of differentiation of mouse ES cells with Activin and miR-34c over-expression. miR-34c was transfected at day 11 of the differentiation. (B) Real-time PCR of miR-34c and KSP at day 14 of the differentiation. (C) Real-time PCR of miR-34c and KSP. E14.5 mouse embryonic kidneys were transfected with precursors of miR-34c, and samples were harvested after 3 days organ culture. Asterisks indicate statistically significant against control.



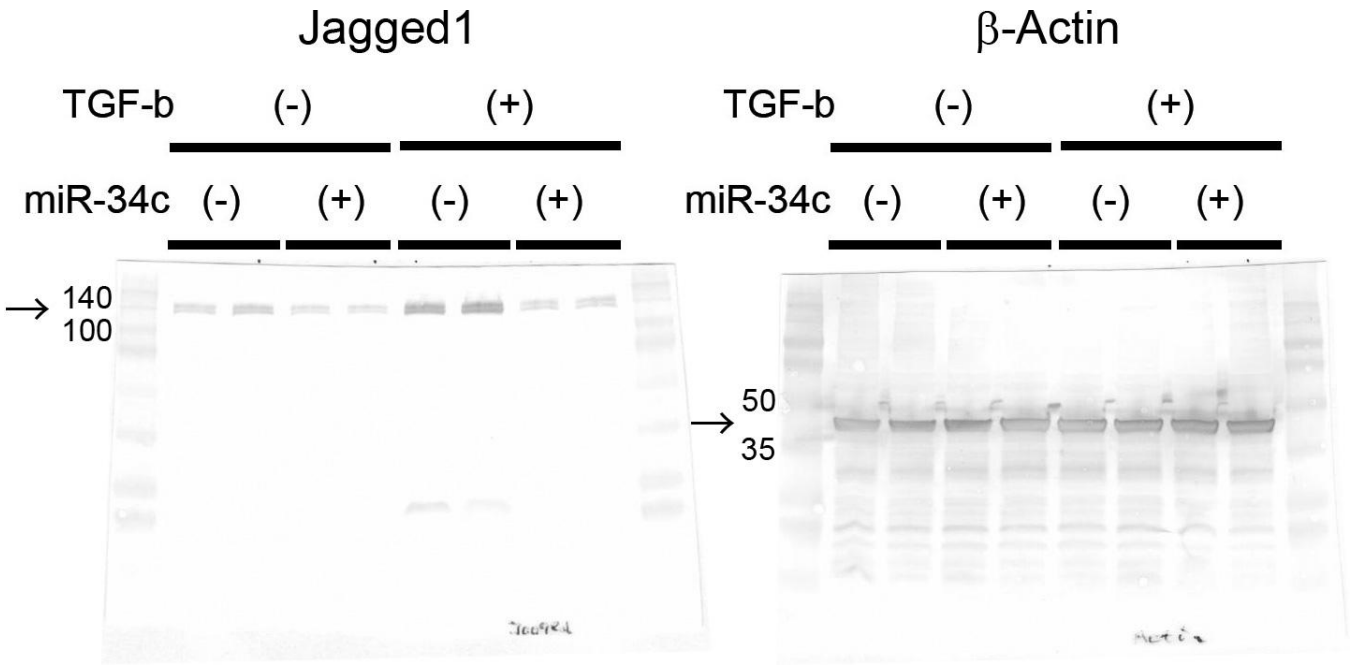
Supplementary Figure S2. The original whole blot pictures of figure 1A, B.

UUO: unilateral ureter obstruction. n=3. Control: contralateral kidneys in UUO mice. n=3.

Sham Left: left kidneys in sham-operated mice. Sham Right: right kidneys in sham-operated mice.

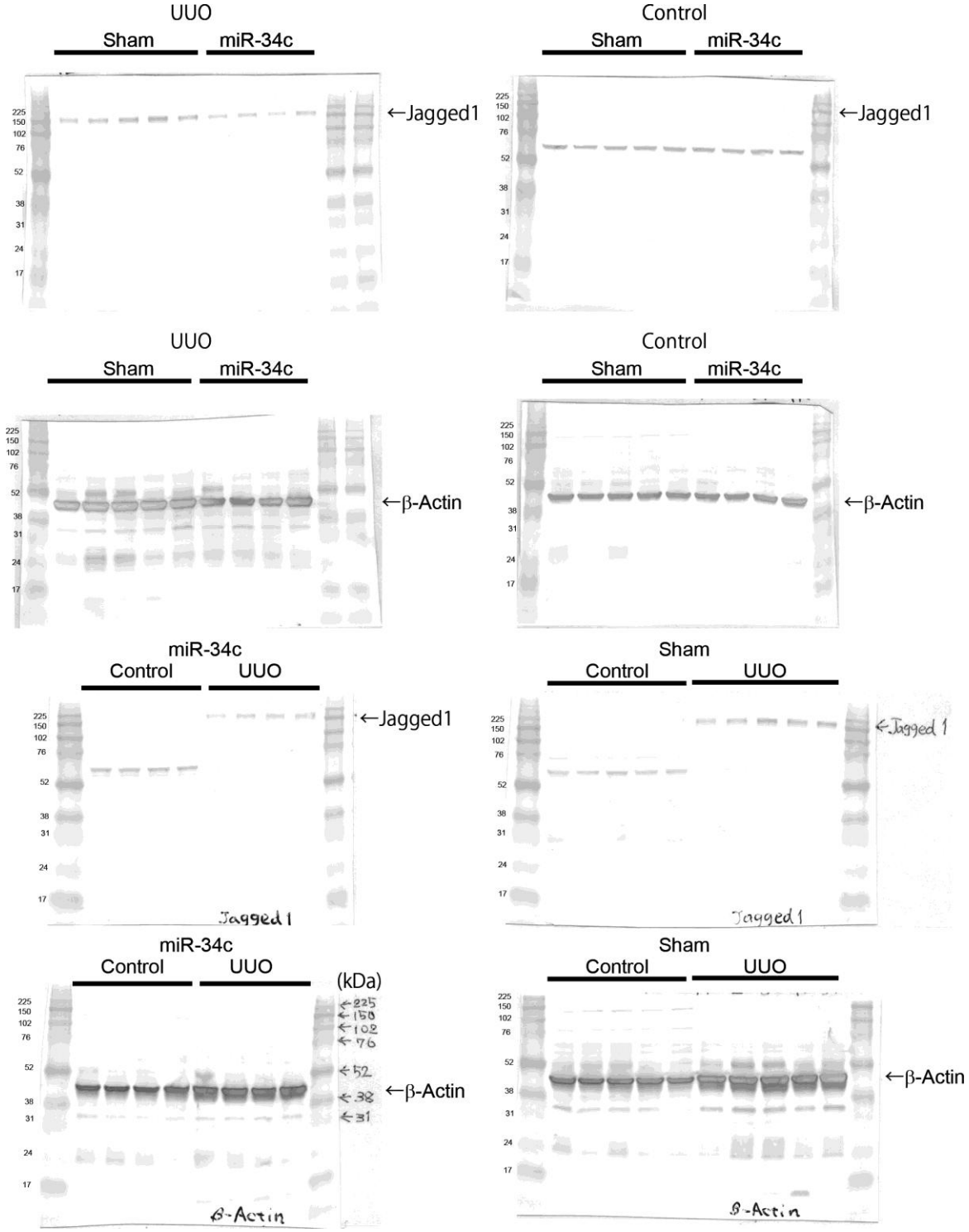


Supplementary Figure S3. The original blot pictures of figure 2F.



Supplementary Figure S4. The original blot pictures of figure 3B.

To compare miR-34c samples with sham samples, those samples were electrophoresed into a same blotting membrane. Moreover, to confirm the difference of Jagged1 between UUO and control kidneys, those samples were electrophoresed into a same blotting membrane. The former results was used for quantification. Sham-injected mice, n=5. miR-34c injected mice, n=4.



Supplementary Figure S5. Representative pictures of Masson trichrome staining.
Quantification of fibrosis area was done by ImageJ using 10 randomly taken pictures at x100. Fibrosis area was calculated by "Color Threshold" using following parameters; Hue 130-180, Saturation 0-255, Brightness 0-255.