SUPPLEMENTAL MATERIALS

Supplemental Methods

Gene Expression:

Total RNA was extracted following homogenization using TRI-Reagent (Molecular Research Center, Cincinnati, OH) and treated with RNase-free DNase (Qiagen, Valencia, CA). First strand cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA), with 1 µg of RNA used for each reverse-transcriptase reaction. PCR reactions contained 100ng cDNA, 300nM of each primer, and 1X iQTM SYBR® Green Supermix (Bio-Rad) in 50µl. The threshold cycle (Ct) of each gene product was normalized to the Ct for hypoxanthine guanine phosphoribosyl transferase (HPRT) for all qRT-PCR experiments. Gene primer sequences used for qPCR analysis were as follows:

mouse *HPRT* forward: 5'- TGATAGATCCATTCCTATGACTGTAGA-3'
mouse *HPRT* reverse: 5'-AAGACATTCTTTCCAGTTAAAGTTGAG-3'

mouse *Spp1* forward: 5'-GGAGGAAACCAGCCAAGG-3'
mouse *Spp1* reverse: 5'-TGCCAGAATCAGTCACTT TCA-3'

mouse *Tnfα* forward: 5'-CCCTCACACTCAGATCATCTTCT-3'
mouse *Tnfα* reverse: 5'-GCTACGACGTGGGCTACAG-3'

mouse *Il-1β* forward: 5'-GCCACCTTTTGACAGTGATGAG-3'
mouse *Il-1β* reverse: 5'-GACAGCCCAGGTCAAAGGTT-3'

mouse *Col1α1* forward: 5'- CATGTTCAGCTTTGT GGACCT-3'
mouse *Col1α1* reverse: 5'-GCAGCTGACTTCAGGGATGT-3'

mouse *Adgre1* (F4/80) forward: 5'-CTTTGGCTATGGGCTTCCAGTC -3'

mouse *Adgre1* (F4/80) reverse: 5'-GCAAGGAGGACAGAGTTTATCGTG-3'

human *HPRT* forward: 5'-TGATAGATCCATTCCTATGACTGTAGA'

human *HPRT* reverse: 5'-CAAGACATTCTTTCCAGTTAAAGTTG-3'

human *Spp1* forward: 5'-GAGGGCTTGGTTGTCAGC-3'

human Spp1 reverse: 5'CAATTCTCATGGTAGTGAG TTTTCC-3'.

Immunohistochemistry staining for macrophages:

For CD68 immunohistochemistry (IHC), sections were de-paraffinized and steamed in 0.01M citrate buffer (pH=6.0) for 20 minutes then incubated in 3% H_2O_2 for 10 minutes followed by incubation in horse serum for 1 hour at room temperature. Sections were then incubated with anti-CD68 (#ab125212, Abcam; Waltham, MA) overnight at 4°C. An ImmPRESS® HRP goat anti-rabbit IgG (#MP-7451, Vector Laboratories, Burlingame, CA) was applied for 1 hour at room temperature followed by the incubation with DAB substrate and hematoxylin counterstaining.