Figure S3

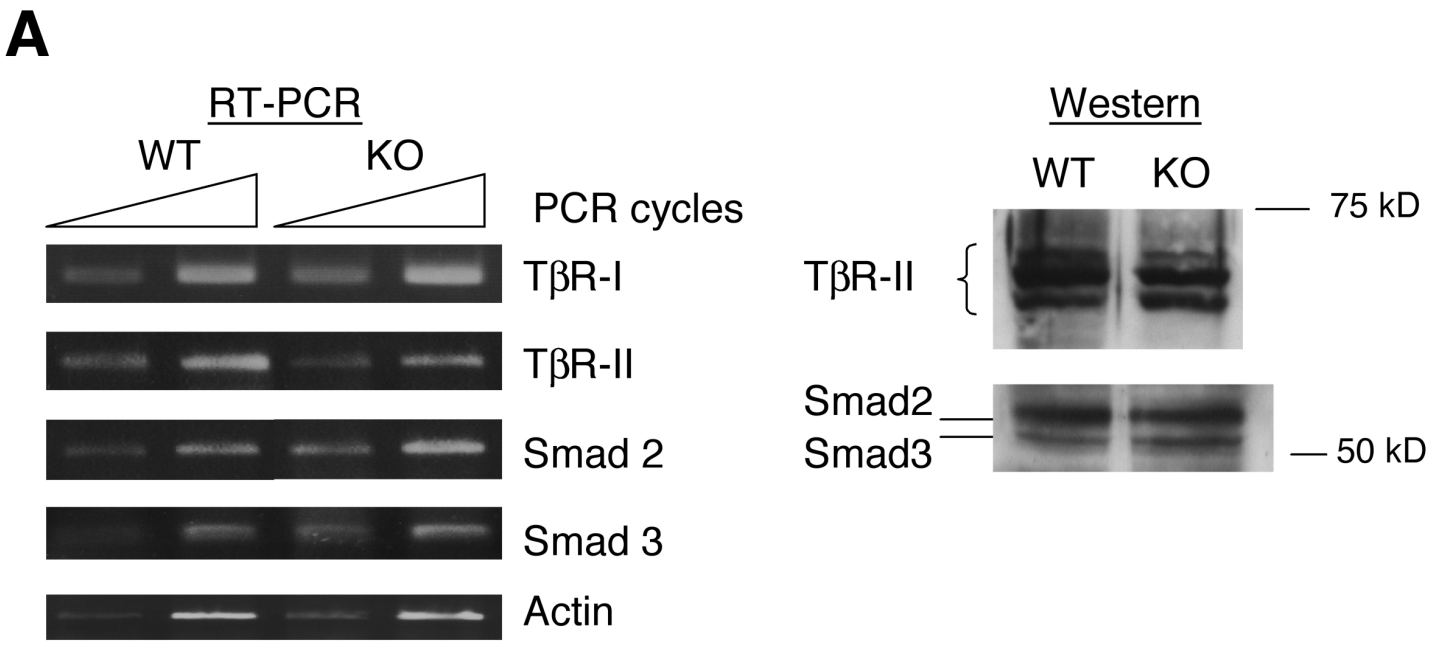
A
RT-PCR
WT
KO
PCR cycles
TβR-I
TβR-II
Smad 2
Smad 3
Actin
Western
WT
KO
75 kD
TβR-II
Smad2
Smad3
50 kD
B
WT
KO
TGF-β
-
+
-
+
Smad2-P
50 kD
Actin

Figure S3. Expression of TGF-β receptors (TβR-I and TβR-II) and signal transducers (Smad2 and Smad3) in WT and KO BMDC. **(A)** RT-PCR (left). Day 7 WT and KO BMDC were treated with LPS (1μg/ml), for 16h before RNA was extracted and analyzed by semiquantitative RT-PCR. Equal amounts of cDNA (compared to Actin) were used for PCR and # of cycles was pre-optimized for each pair of primers. The following primers were used at the indicated # of PCR cycles:

TGFβR1: F: CGAAGGCATTACAGTGTTTCTG; R: GAAAGGGCGATCTAGTGATGG, 29, 31 cycles yielding a 430bp fragment.
TGFβR2: F: AAGTCCTGCATGAGCAACTGC; R: ACACGGTAGCAGTAGAAGATG; 23, 25 cycles yielding a 380bp fragment.
Smad2: F: AAAATGTCGTCCATCTTGCC; R: GAGCTCATGATGGCTGTGAA, 23, 25 cycles yielding a 434bp fragment.
Smad3: F: ACAAGGTCCTCACCCAGATG; R: TGGCGATACACCACCTGTTA, 28, 30 cycles yielding a 319bp fragment.
Actin: F: GATGACGATATCGCTGCGCTG; R: GTACGACCAGAGGCATACAGG, 15, 16 cycles yielding a 439bp fragment.

Western (right). Day 7 WT and KO BMDC were treated with LPS (1μg/ml), for 16h before extraction of protein for blot analysis with anti TβR-II antibodies (L-21) and anti Smad2/3 antibodies (E-20) (Santa Cruz Biotechnology, CA USA).

(B) TGF-β dependent phosphorylation of Smad2 in BMDC. Western blot of proteins extracted from day 7 WT and KO BMDC incubated for 16h with LPS (1μg/ml) and treated or untreated with TGF-β (10ng/ml) for 4h before protein extraction. Blots were reacted with Smad2-P antibodies (anti phospho-Smad2 antibodies specific for the phosphorylated C-terminal tail in Smad2 (Persson *et al.*, 1998), a gift of Peter ten Dijke The Netherlands Cancer Institute, Amsterdam) and with monoclonal anti β Actin clone AC-15 (Sigma, USA).