Analysis of Runx3 protein species in mature WT and KO BMDC

Western blot analysis of proteins from mature bone marrow derived DC (BMDC) revealed that in addition to the two bands of ~48 and 46 kDa that correspond to the full-length Runx3 proteins (Levanon *et al*., 1994; Bangsow *et al*., 2001), a third Runx3 protein of ~ 33 kDa was also present (Figure S1). Interestingly, this p33 Runx3 protein was also present in the KO DC, in contrast to the absence of the full-length p48 and p46 proteins (Figure S1) and was also observed the KO thymus (not shown) as well as in WT myeloid cells (Angel Corbi unpublished). RT-PCR and sequencing analysis using KO DC RNA revealed the presence of an mRNA species lacking exon #2, which could encode a 33 kDa protein. A similar mRNA species was also detected in the WT RNA albeit as a minor band (570 bp in Figure S1). This mouse Runx3/p33 is similar to the human RUNX3 p27 cDNA, which was previously isolated from human leukocytes (Bangsow *et al*., 2001). The common intriguing feature of p27 and p33 is that both are alternatively spliced isoforms lacking half the coding region of the runt domain. The cellular signals directing the synthesis of the Runx3/p33 isoform are not known and more experiments are needed to clarify the nature of the mRNA encoding the 33kDa protein and to elucidate its function in DC.

Figure S1

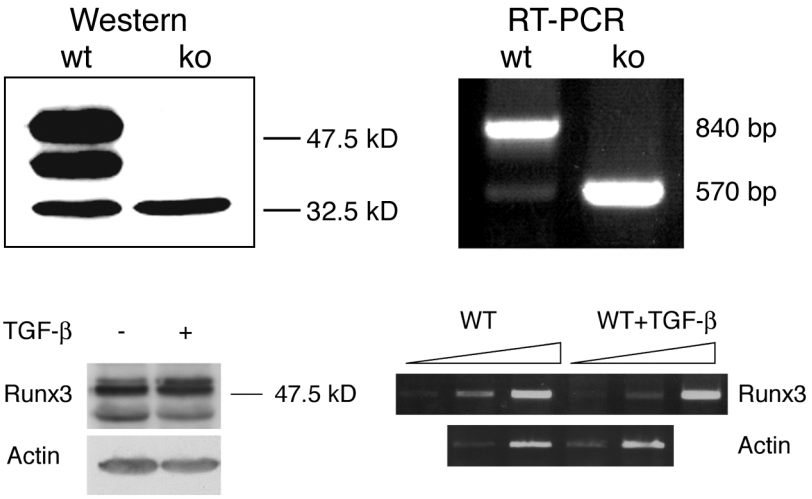
Western
wt
ko
47.5 kD
32.5 kD
RT-PCR
wt
ko
840 bp
570 bp
TGF-β
-
+
Runx3
47.5 kD
Actin
WT
WT+TGF-β
Runx3
Actin

Figure. S1. Expression of Runx3 in mature WT and KO BMDC. **(A)** Western blot analysis (left) of proteins extracted from WT and KO mature LPS treated BMDC using anti Runx3 Ab. WT Runx3 show two bands ~48 and 46 kDa and a smaller ~33 kDa protein. Only the 33 kDa protein is present in the KO DC. RT-PCR (right) using primers derived from exons 1 and 6 of *Runx3* revealed a 840 bp product that corresponds to the full size mRNA and a ~570 bp product that corresponds to a mRNA lacking exon 2. Cells and tissue extracts were obtained and analyzed by Western and RT-PCR as previously described (Levanon *et al*., 1996; Ben Aziz-Aloya *et al*., 1998). The following primers were used for detection of the *Runx3* p33 mRNA by RT-PCR (Bangsow *et al*., 2001): exon1, forward-GCAGTCCTTGCCCACTGTCA, exon 6, reverse-GTGAGGCTCTGCAGCGTAG.
(B) Western blot (left) of proteins extracted from WT day 7 BMDC incubated for 16h with LPS (1μg/ml) and treated or untreated with TGF-β (10ng/ml) for 4h. Blots were reacted with purified anti Runx3 antibodies and with monoclonal anti β Actin clone AC-15 (Sigma USA). RT-PCR (right). *Runx3* was measured by PCR at 23, 25 and 27 cycles with the primers F: GGCAAGATGGGCGAGAACAG; R: CGTAGGGAAGGAGCGGTCAA producing a 654bp fragment and compared to actin at 15 and 16 cycles with primers F: GATGACGATATCGCTGCGCTG; R: GTACGACCAGAGGCATACAGG that produced a fragment of 439bp.