Figure S-1. The novel NF-κB activation/TRAF2 binding site is highly conserved. Multiple sequence alignment of TNFR2 intracellular region from different species*: Homo sapiens, Pan troglodytes, Macaca mulata, Mus musculus, Rattus norvegicus, Ailurupoda melanoleuca, Bos Taurus, Sus scrofa, Equus caballus and Canis familiaris.* The residues with 100% identity are shaded in black and those with 80% identity are shaded in grey. Numbers indicate the aminoacid position in the full-length human sequence. The alignment was generated using ClustalW and edited with GENEDOC. The Roman numerals I, II and III refer to the conserved modules found in TNFR2.

Figure S-2. The region comprising aminoacids 343-349 of TNFR2 is responsible of TRAF2 depletion induced by the receptor. *A) Schematic representation of deletions and mutations of the region 343-379 of TNFR2.* Diagrammatic representation of TNFR2 deletions and mutants is as in figure 2. The ability to induce degradation of TRAF2 is also indicated on the right. *B) Analysis of TRAF2 degradation by different TNFR2 variants affecting the region 343-349.* HEK 293 cells were transiently transfected with 0.3 μg de pRK-Myc-TRAF2 and increasing amounts of pR2-BKO-Δ365-378, pR2-BKO-Δ344-356 or pR2-BKO-Δ350-378 (all FLAG tagged) as indicated. 36 h after transfection cells were harvested and lysed. Equal aliquots of the lysates were resolved by SDS-PAGE and Western blotted with anti-TRAF2, anti-FLAG (receptors) or anti-β-actin antibodies.

FIGURE S1

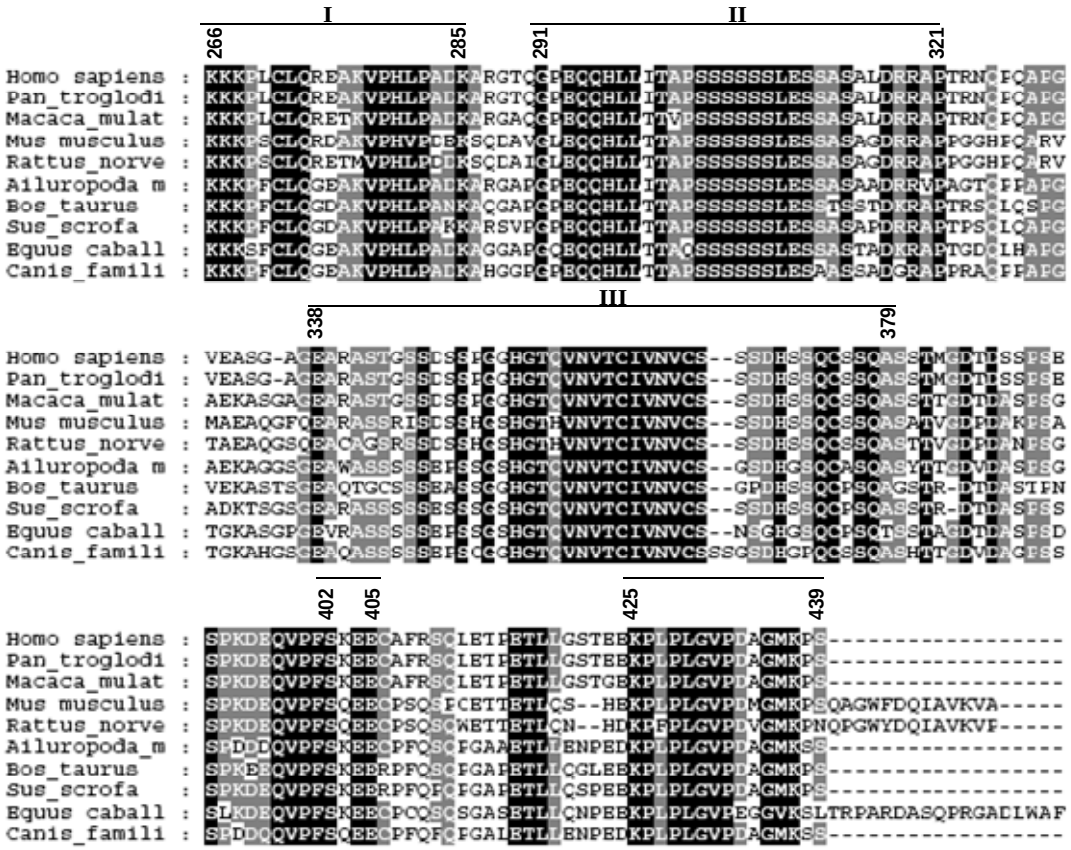
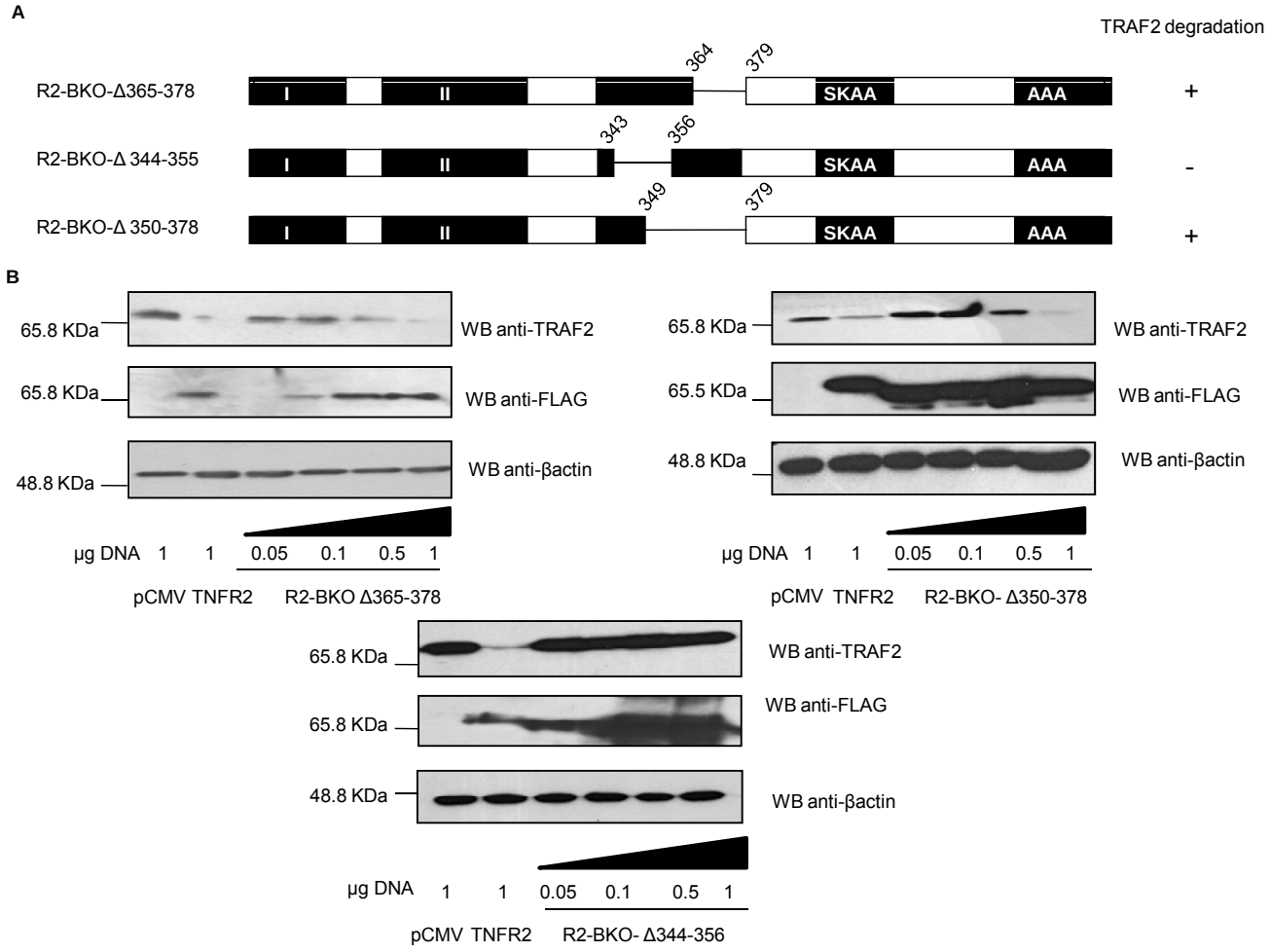

I
II
III
266
285
291
321
338
379
402
405
425
439
Homo sapiens
Pan_troglodi
Macaca_mulat
Mus musculus
Rattus_norve
Ailuropoda m
Bos_taurus
Sus_scrofa
Equus caball
Canis_famili

FIGURE S2


A
TRAF2 degradation
R2-BKO-Δ365-378
364
379
I
II
SKAA
AAA
+
R2-BKO-Δ 344-355
343
356
-
R2-BKO-Δ 350-378
349
379
+
B
65.8 KDa
WB anti-TRAF2
65.8 KDa
WB anti-FLAG
48.8 KDa
WB anti-βactin
µg DNA 1 1 0.05 0.1 0.5 1
pCMV TNFR2 R2-BKO Δ365-378
65.8 KDa
WB anti-TRAF2
65.5 KDa
WB anti-FLAG
48.8 KDa
WB anti-βactin
µg DNA 1 1 0.05 0.1 0.5 1
pCMV TNFR2 R2-BKO- Δ350-378
65.8 KDa
WB anti-TRAF2
65.8 KDa
WB anti-FLAG
48.8 KDa
WB anti-βactin
µg DNA 1 1 0.05 0.1 0.5 1
pCMV TNFR2 R2-BKO- Δ344-356