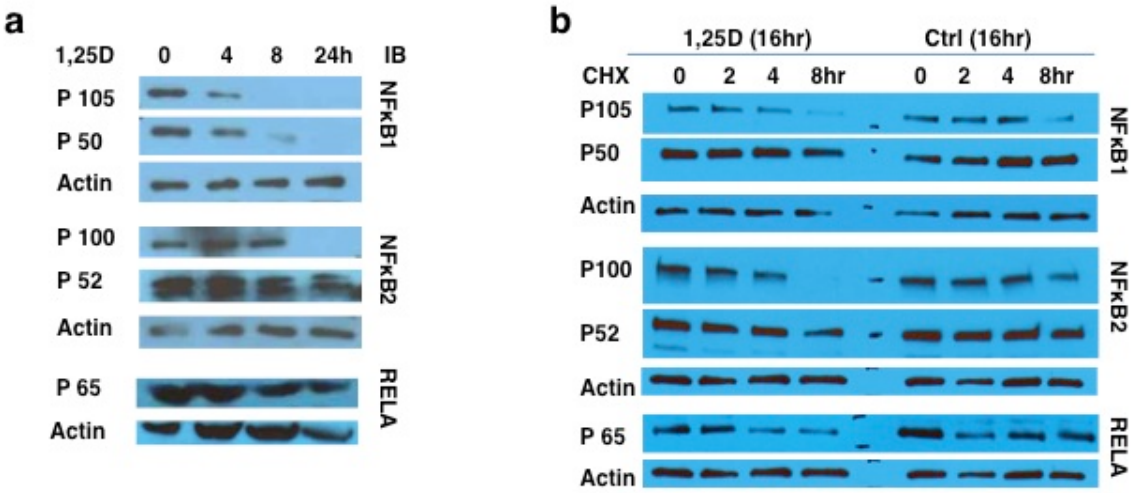
The hormone-bound vitamin D receptor enhances the FBW7-dependent turnover of NF-κB subunits

Fatemeh Fekrmandi[1], Tian-Tian Wang[2], and John H. White[1, 2*]

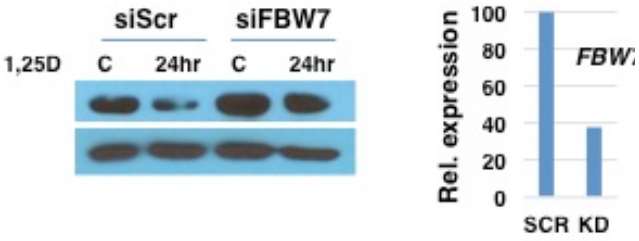
Departments of Medicine[1] and Physiology[2], McGill University, Montreal, Quebec, Canada

*Address correspondence to john.white@mcgill.ca

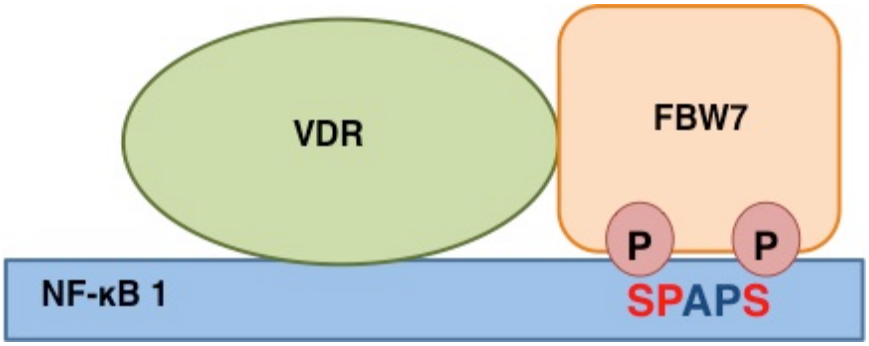
Supplementary Figures.



Supplementary Figure 1. a. Effect of treatment with 1,25D (100nM) on expression of NF-kB subunits in LNCaP cells. **b**. Effect of preincubation (16h) of LNCaP cells with DMSO vehicle (control) or 1,25D (100nM) followed by incubation with cycloheximide on turnover or NF-kB subunits, as indicated.



Supplementary Figure 2. Effect of treatment with 1,25D (100nM) on expression of NF-kb subunit p65 in LNCaP cells transfected with scrambled control siRNA or siRNA targeting *FBW7*. RT/qPCR analysis of *FBW7* expression is shown at right.



Supplementary Figure 3. Schematic illustration of suggested mechanism for formation of a VDR-FBW7-p105 complex to promote NF-κB degradation.

Supplementary Tables.

Table 1: siRNAs	
Gene name	siRNA Sequence
FBW7	5’-CCCTAAAGAGTTGGCACTCTA-3’
Control	5’-CAGGGTATCGACGATTACAAA-3’

Table 2: qPCR primers	
Primer name	Sequence
FBW7- Forward	5’-CAGCAGTCACAGGCAAATGT-3’
FBW7- Reverse	5’-GCATCTCGAGAACCGCTAAC-3’
NFκB1- Forward	GCAGCACTACTTCTTGACCACC
NFκB1- Reverse	TCTGCTCCTGAGCATTGACGTC
NFκB2- Forward	GGCAGACCAGTGTCATTGAGCA
NFκB2- Reverse	CAGCAGAAAGCTCACCACACTC
RELA- Forward	TGAACCGAAACTCTGGCAGCTG
RELA- Reverse	CATCAGCTTGCGAAAAGGAGCC
NFκBIA- Forward	TCCACTCCATCCTGAAGGCTAC
NFκBIA- Reverse	CAAGGACACCAAAAGCTCCACG
MYB- Forward	GGGAACAGATGGGCAGAAATCG
MYB- Reverse	GCTGGCTTTTGAAGACTCCTGC
BCL3- Forward	GAACACCGAGTGCCAAGAAACC
BCL3- Reverse	GCTAAGGCTGTTGTTTTCCACGG
CCND1- Forward	TCTACACCGACAACTCCATCCG
CCND1- Reverse	TCTGGCATTTTGGAGAGGAAGTG
UCP2- Forward	ACAAAGGGTTCATGCCCTCC
UCP2- Reverse	ACGAACATCACCACGTTCCA
GCNT- Forward	CTGGAAACGGAGAGGATGCCAT
GCNT- Reverse	CACGAAGTAGGCACTGCCAGAA
GAPDH- Forward	GTCTCCTCTGACTTCAACAGCG
GAPDH- Reverse	ACCACCCTGTTGCTGTAGCCAA