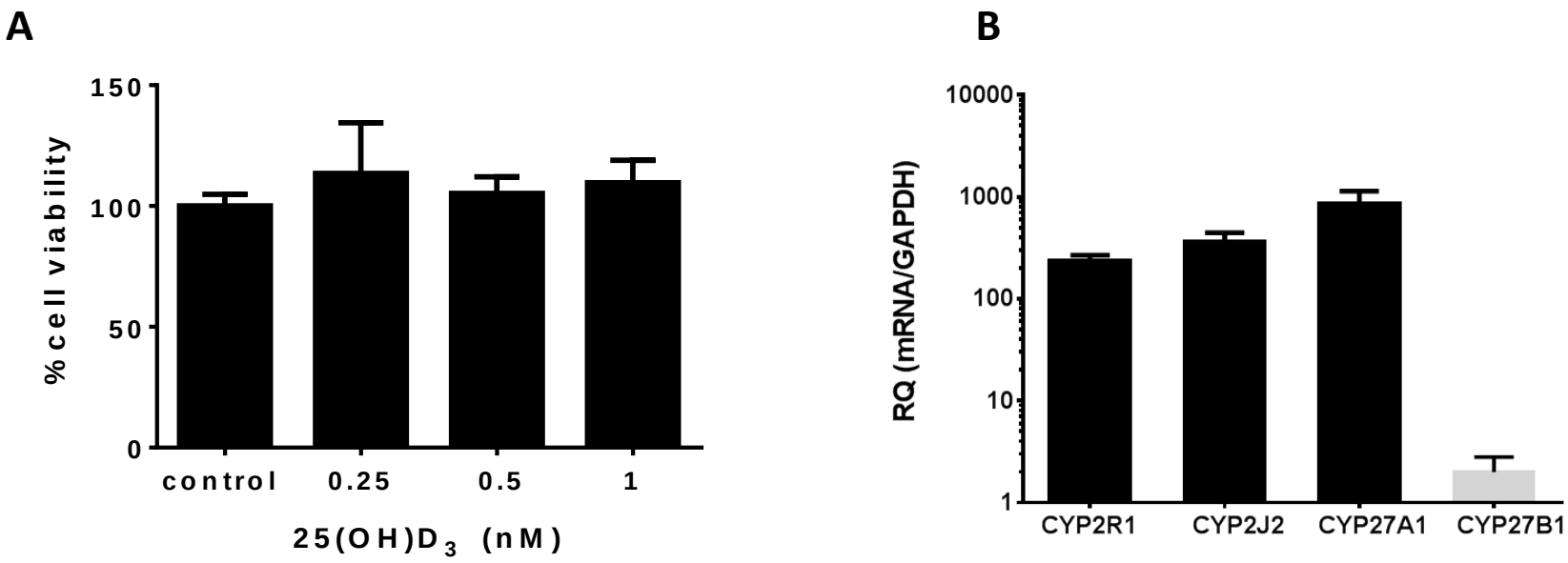
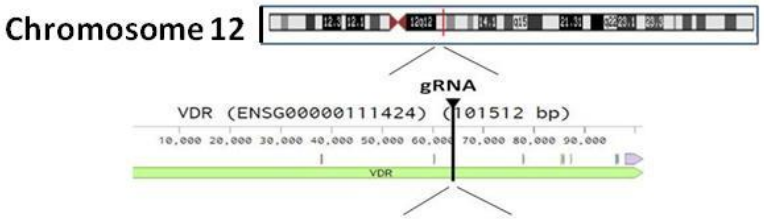


Figure S1. Effect of 25(OH)D_3 on Huh7.5 cell viability.

Huh7.5 cells treated with different concentration of 25(OH)D3 (12.5-1000nM) for 24h. Cell viability was determined by AalamarBlue Cell Viability Reagent measuring fluorescence intensity in culture supernatants. Percent of inhibition and percent of viable cells was determined compared to non-treated cells set as 100%. (B) Real-time PCR analysis of vitamin D 25-hydroxylases (25(OH)ases RNA expression levels in Huh7.5 cells. Results are presented as relative quantity of the target gene normalized to GAPDH mRNA values. CYP27B1 was assigned a value of 1 and results are normalized to it. Results are shown as means relative quantity ± SD.

A)

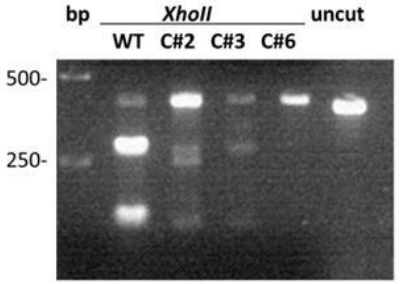


Target genomic locus

5'-CGGAACGTGCCCCGGATCTG TGG-3'

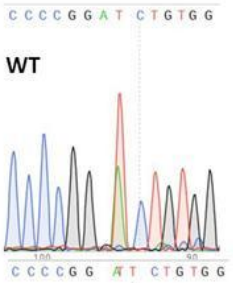
3'-CCCACAGATCCGGGGCACGT TCC-5'

B)

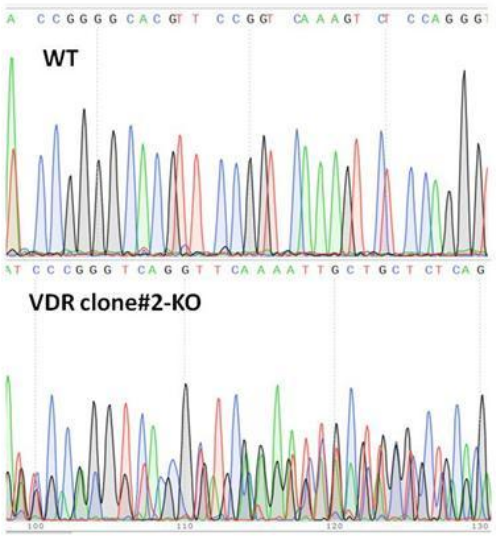


C)

a.



b.



c.

WT MEAMAASTSLPDPGDFDRNVPRICGVCGDRATGFHFNAMTCEGCKGFFRRSMKRKALFTC

VDR-KO-Clone #6 MEAMAASTSLPDPGDFDRNVPRILWGVWRPSHWLSLQCYDL*RLQRLLQAKHEAEGTIHL

D)

a.

gRNA ----------CGGAACGTGCCCCGGATCTG----------

WT GAGACTTTGACCGGAACGTGCCCCGGATCTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGGA-CTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGGG-CTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGGA-TTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGGT-CTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGT--CTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGG--CTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCCT----GTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGG----GTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGG----ATGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGG----ATGGTGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGG-----ATGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCTCTG-----TGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGG------ATGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCTGT------GGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCTGTG--------GGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCG--------GGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCC---------GGATGTGTGGAGA

GAGACTTTGACCGGAACGTGTC--------TGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGT-----------GGGGTGTGTGGAGA

GAGACTTTGACCGGAACGTG------------GGGTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGGAT-------GTGTGTGGAGA

GAGACTTTGACCGGAACGTGCCCCGGA--TGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGT---------CTGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAAC------------TGTGGGGTGTGTGGAGA

GAGACTTTGACCGGAACGT----------GTGTGTGGAGA

GAGACTTTGACCGGAAC----------GTGTGGAGA

GAGACTTTA----------TCTGTGGGGTGTGTGGAGA

GAGACTTTGACCG----------TCTGTGGGGTGTGTGGAGA

b.

gRNA CCGGAACGTGCCCCGGAT---CTG

WT CCGGAACGTGCCCCGGAT---CTGTGGGGTGTG

CCGGAACGTGCCCCGGATT--CTGTGGGGTGTG

CCGGAACGTGCCCCGGAAT--CTGTGGGGTGTG

CCGGACCGTGCCCCGGATT--CTGTGGGGTGTG

CCGGAACGTGCCCCGGATC--CTGTGGGGTGTG

CCGGAACGTGCCCCGGATAT-CTGTGGGGTGTG

CCGGAACGTGCCCCGGATTT-CTGTGGGGTGTG

CCGGAACGTGCCCCGGATGATCTGTGGGGTGTG

Figure S2. Outline of the generation of VDR-knockout (KO) Huh7.5.cells

(A) Schematic presentation of the target genomic locus of the human VDR gene. A boxed sequence indicates the site targeted by the guide RNA designed in this study. PAM; protospacer adjacent motif depicted in green (B) Gel electrophoresis of VDR target specific PCR of WT and selected clones (2,3 and 6) after digestion with MflI restriction enzyme. (C) DNA sequencing histogram of a. VDR #6 mutant and WT gene, the red arrows indicated the insertion positions; b. VDR #2 mutant and WT gene. c. Putative protein translation of the WT and the mutated seq. (VDR #6). (D) Sequence alignment of target regions of VDR gene sequences in mutated cell pool based on deep sequencing and analysis with the Cas-Analyzer analysis, gRNA position is indicated, multiple sequence alignment was performed using MULTALIN program (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_multalinan.html) a) deletions b) insertions