# Cytosolic sequestration of the Vitamin D Receptor as a therapeutic option for vitamin D–induced hypercalcemia

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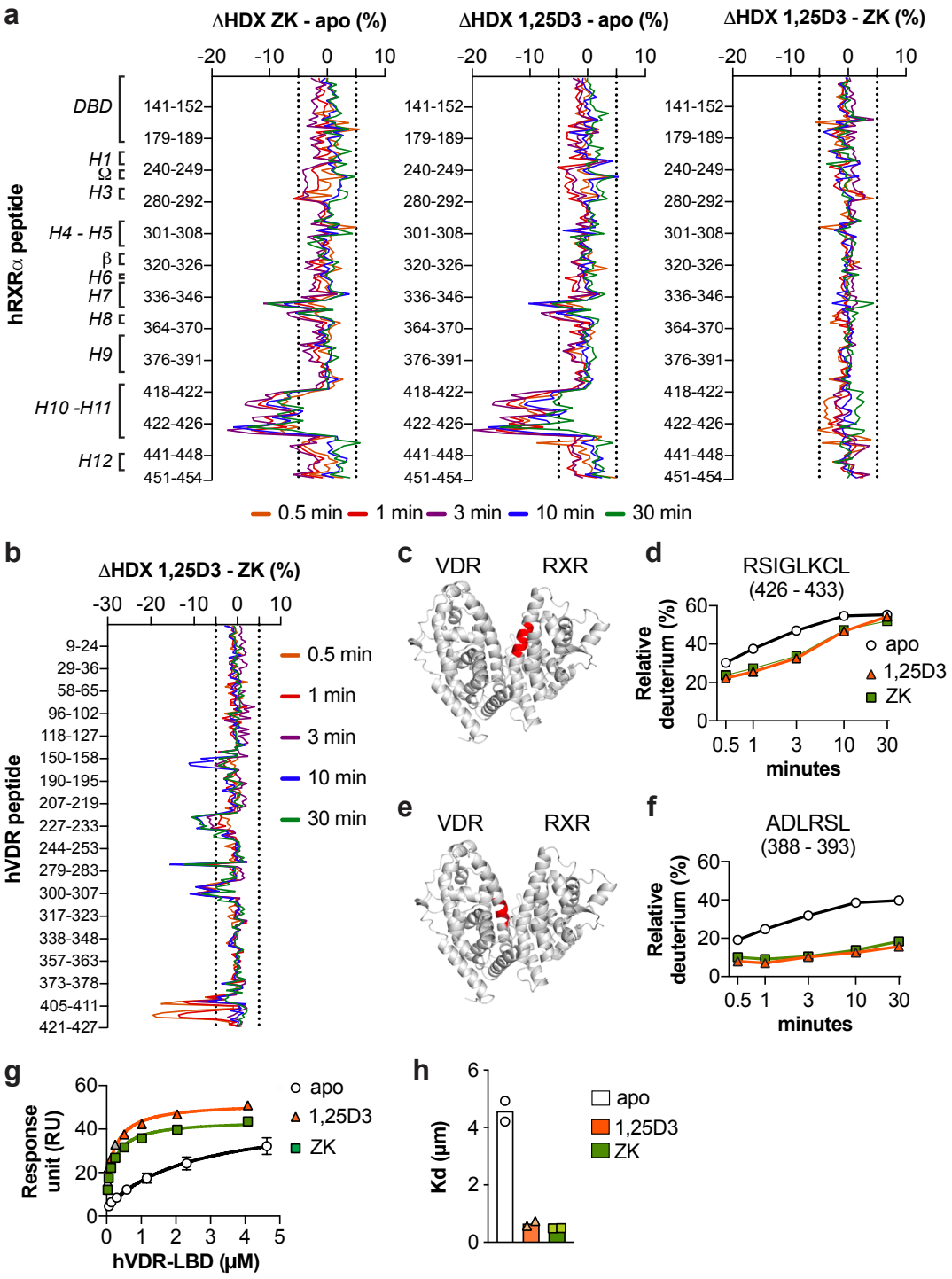
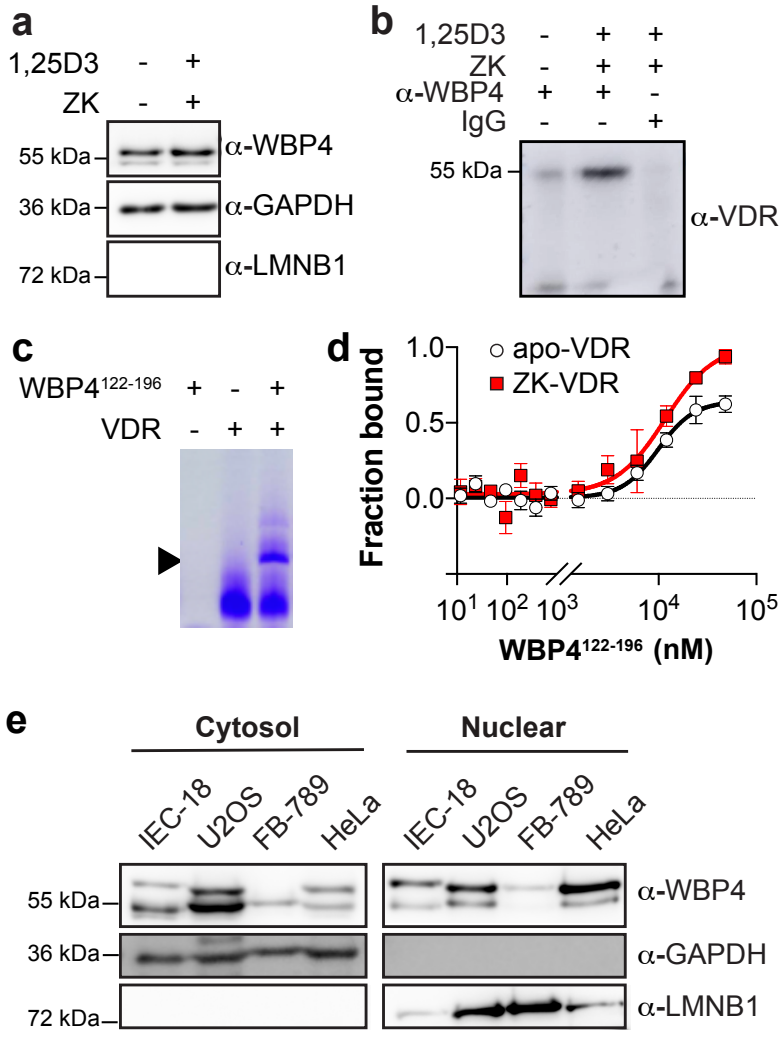
## Supplementary information files

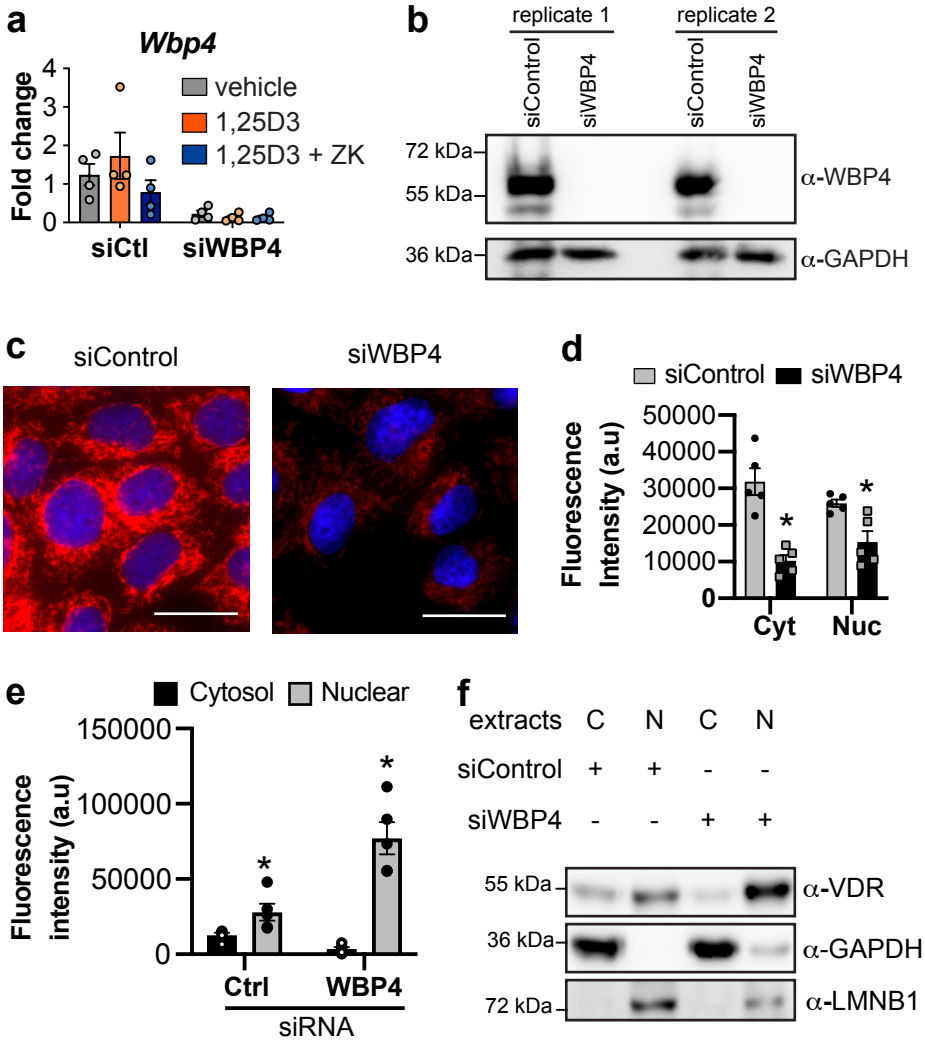
- **Supplementary Figure 1 - 6**
- **Supplementary Table 1 - 4**



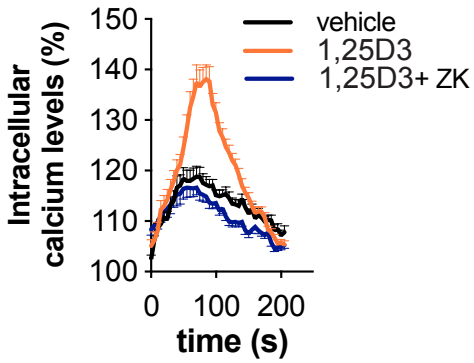

**Supplementary Fig. 1. Effect of ligands on hVDR and hRXRαΔNTD conformations. (a)** Differential deuterium uptake of hRXRα peptides between ZK-bound, 1,25D3-bound and apo hVDR/hRXRαΔNTD heterodimers. **(b)** Differential deuterium uptake of hVDR peptides between ZK-bound and 1,25D3-bound hVDR/hRXRαΔNTD heterodimers. Dashed lines represent a ΔHDX of 5 %. Peptides from H10 of hRXRα (426-RSIGLKCL-433) **(c)** and of hVDR (388-ADLRSL-393) **(e)** mapped onto hVDR/hRXRα LBD (PDB: 1DB1[44]), and differential deuterium uptake plots in ZK-bound, 1,25D3-bound and apo hVDR/hRXRαΔNTD heterodimers of RSIGLKCL (**d**) and ADLRSL (**f**) peptides. n = 3 technical replicates / condition. Analysis of the interactions between hRXRα-LBD and ZK-bound (ZK), 1,25D3-bound (1,25D3) and apo hVDR LBDs by surface plasmon resonance (**g**) and calculated Kd (**h**). n= 2 independent biological replicates / condition.



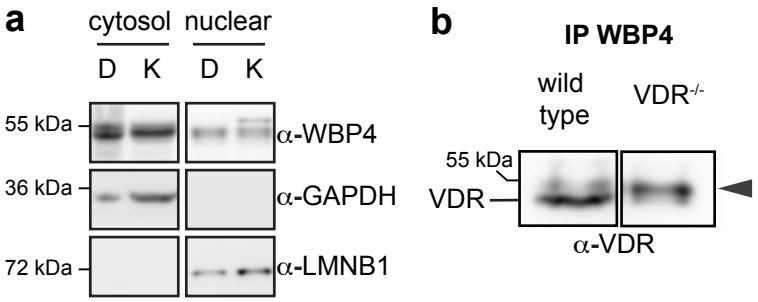

**Supplementary Fig. 2. WBP4 cellular localization and characterization of its interaction with VDR. (a)** Representative WBP4 immunoblot of cytosolic extracts of FB-789 cells treated for 1.5 h with vehicle, or with 100 nM 1,25D3 and 1 µM ZK. Uncropped blots in Source Data. (**b**) Representative VDR immunoblot of WBP4-immunoprecipitated cytosolic extracts of FB-789 cells treated for 1.5 h with vehicle, or 100 nM 1,25D3 and 1 µM ZK. Cytosolic extracts from FB-789 cells treated for 1.5 h with 100 nM 1,25D3 and 1 µM ZK immunoprecipitated with Rabbit IgG were used as a negative control. Uncropped blots in Source Data. **(c)** Native PAGE of hWBP4 amino acids 122 to 196 and ZK-bound hVDR full-length. Analysis by maldi MS of the band indicated by the arrow revealed the presence of the hVDR and hWBP4 polypeptides. Source data are provided as a Source data file. **(d)** WBP4 binding to hVDR measured by microscale thermophoresis. Unlabelled hWBP4 polypeptides (aa 122-196) were titrated into a fixed concentration of labelled recombinant VDR in the presence or absence (apo) of saturating concentrations of ZK. Isotherms were averaged over three consecutive measurements and fitted according to the law of mass action to yield the apparent dissociation constant. Kd=11±4 µM for ZK-bound VDR and Kd > 20 µM for apo-VDR. **(e)** Representative WBP4 immunoblot of cytosolic and nuclear extracts of IEC-18, U2OS, FB-789 and HeLa cells. GAPDH and LMNB1 were used as internal controls. Unprocessed blots in Source Data.



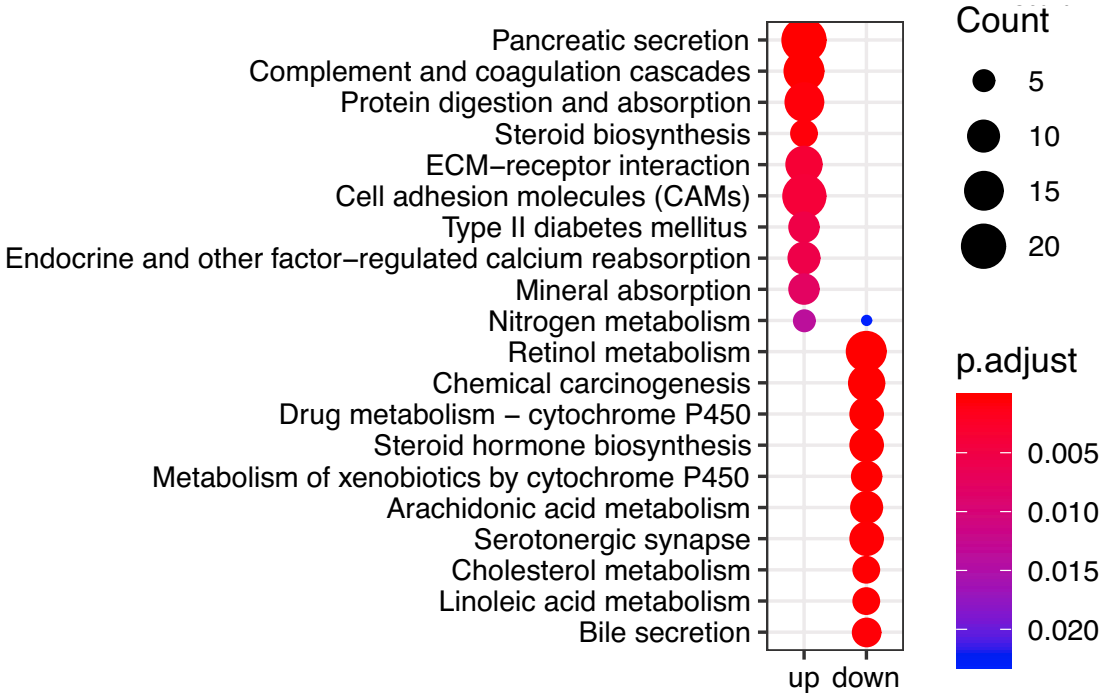

**Supplementary Fig. 3. Effect of WBP4-silencing.** WBP4 transcript **(a)** and protein **(b)** levels, as well as WBP4 immunocytostaining **(c)** of IEC-18 cells transfected with WBP4 siRNA or unrelated siRNA (Control, Ctl). Nuclei are stained with DAPI. Scale bar: 20 µm. Data in **(a)** represent n = 4 independent biological replicates / condition. **(d)** Quantification of the intensity per µm$^2$ of WBP4 immunostaining in WBP4- (siWBP4) or unrelated- (siControl) silenced IEC-18 cells. n = 5 cells / condition. **(e)** Quantification of the intensity per µm$^2$ of VDR immunostaining in WBP4- (siWBP4) or unrelated- (siControl) silenced IEC-18 cells of Fig. 4e. n = 5 cells / condition. **(f)** Representative VDR immunoblot in cytosolic (C) and nuclear (N) extracts from WBP4- (siWBP4) or unrelated- (siControl) silenced IEC-18 cells. GAPDH and LMNB1 were used as internal controls. Unprocessed blots in Source Data. * $p < 0.05$ vs. siControl, two-way ANOVA with Tukey post-hoc test. The exact significant p values are provided in **Supplementary Table 4**.




**Supplementary Fig. 4. Effect of VDR ligands on intracellular calcium levels.** Intracellular calcium flux in IEC-18 cells treated for 48 h with vehicle, 100 nM 1,25D3, or co-treated with 100 nM 1,25D3 and 1 µM ZK. n > 40 cells, 3 independent biological replicates / condition. Data are expressed as % of basal levels.




**Supplementary Fig. 5. WBP4 cellular localization and interaction with VDR in mice. (a)** Representative WBP4 immunoblot of cytosolic and nuclear extracts from duodenum (D) and kidney (K) of a wild type mouse. GAPDH and LMNB1 were used as internal controls. Uncropped blots in Source Data. **(b)** Representative VDR immunoblot in WBP4 immunoprecipitated renal extracts from wild type and VDR-null ($VDR^{-/-}$) mice. The arrowhead represents WBP4 antibody heavy chains. Uncropped blots in Source Data.




**Supplementary Fig. 6. Pathways analyses of genes deregulated by 1,25D3.** KEGG pathway of the genes up and down regulated in the duodenum of mice treated with 1,25D3 for 6 h. Source data are provided as a Source Data file.

| Data Set | hVDR- hRXRαΔNTD |
|---|---|
| HDX reaction details | 50 mM Tris, 200 mM NaCl, 2% glycerol, 1 mM TCEP, pDread = 7.6, 25 °C |
| HDX time course (min) | 0.5, 1, 3, 10, 30 |
| HDX control samples | Maximally-labeled control (zebrafish VDR LBD protein) |
| Back-exchange (mean / IQR) | 48.05% / 9.69% |
| # of Peptides | VDR: 179; RXRα: 127 |
| Sequence coverage | VDR: 92.1%; RXRα: 88.7% |
| Average peptide length / Redundancy | VDR: 7.91 / 3.99; RXRα: 8.33 / 3.98 |
| Replicates (biological or technical) | 3 (technical) |
| Repeatability | 0.049 (average standard deviation) |
| Significant differences in HDX | p-value < 0.05 and ΔHDX > 5% (biological threshold) |

**Supplementary Table 1.** HDX data summary.

| #term ID | term description | gene count | FDR |
|---|---|---|---|
| GO:0043229 | intracellular organelle | 60 | 2.42E-26 |
| GO:0005622 | intracellular | 63 | 4.25E-26 |
| GO:0044422 | organelle part | 52 | 4.25E-26 |
| GO:0044446 | intracellular organelle part | 51 | 5.51E-26 |
| GO:0044424 | intracellular part | 62 | 8.99E-26 |
| GO:0044444 | cytoplasmic part | 53 | 5.59E-25 |
| GO:0044464 | cell part | 64 | 1.47E-24 |
| GO:0005737 | cytoplasm | 56 | 1.24E-22 |
| GO:0043232 | intracellular non-membrane-bounded organelle | 37 | 1.24E-22 |
| GO:0032991 | protein-containing complex | 40 | 1.41E-21 |

**Supplementary Table 2:** Top 10 cellular component terms obtained by Gene Ontology (GO) annotation of the proteins identified by MS and selectively enriched (p value < 0.01) in VDR-immunoprecipitated cytosolic extracts of 1,25D3 and ZK co-treated IEC-18 cells compared to vehicle-treated cells.

## Oligonucleotides

**RT-qPCR (mouse)**

| Gene | Forward primer 5' - 3' | Reverse primer 5' - 3' |
|---|---|---|
| cyp24a1 | GGCGGAAGATGTGAGGAATA | GCCCAGCACTTGGGTATTTA |
| slc30a10 | GGTGATTCCCTGAACACCGA | ACGTGCAAAAGAACACCTCTG |
| slc37a2 | TAGGGCCAGACTAGAGCCA | ACATGCTCATCTCTGCCGAC |
| nkain1 | GGAGTCAAAGCCACCGATGA | CCTAGCTCTGTTCGGCTTCG |
| atp2b1 | CACGTCCCGCGACCC | TGTATACATCTCGCCCGCAG |
| 18 s | AGCTCACTGGCATGGCCTTC | CGCCTGCTTCACCACCTTC |

**qPCR (mouse)**

| genomic region | Forward primer 5' - 3' | Reverse primer 5' - 3' |
|---|---|---|
| cyp24a1_VDRE | CAGTGACTCCCGGATGAACC | TCAGGAAGGCTGCTAAAGGC |
| slc30a10_VDRE | ACAGGGTGCAAAGAAGCCAA | AAGGACCTCCTCCCTACGTC |
| slc37a2_VDRE | TCCTGGGTGTGCCGATTCTA | CCTGGTCTGGTTGTTACCGT |
| nkain1_VDRE | GCCTGTGCTAACCACTTCGT | TCGTTTAACACGTACCAGGCA |
| atp2b1_VDRE | TTAAACCCAGTAACCTGCAATGT | AAGGTCACCTGAACCTCGAAA |

**RT-qPCR (rat)**

| Gene | Forward primer 5' - 3' | Reverse primer 5' - 3' |
|---|---|---|
| Cyp24a1 | TCCATGAGGCTTACCCCAAG | GCGTATTCACCCAGAACCGT |
| Atp2b1 | GTGGAGGTTCCATTCAGGGG | CTCGGATACCCCACAGGAGT |
| S100g | GACAGCAAGCAGCACAGAAAA | TGGACAGCTGGTTTGGATCG |
| Trpv6 | GAGCACAGGTTGTGGCTACT | CCAAGACCATACTCTCGCCC |
| Wbp4 | CCTGGAAAGTTCTGAGGCGA | TACTCGCGGCGCCTGAC |
| Gapdh | AGTGCCAGCCTCGTCTCATA | GGTAACCAGGCGTCCGATAC |

**RT-qPCR (human)**

| Gene | Forward primer 5' - 3' | Reverse primer 5' - 3' |
|---|---|---|
| CYP24A1 | GACATCCAGGCCACAGACAA | ACCACCATCTGAGGCGTATT |
| RPLP0 | CGTCCTCGTGGAAGTGACAT | TAGTTGGACTTCCAGGTCGC |

## Antibodies

| Target | Host species | Supplier | Reference | Dilution |
|---|---|---|---|---|
| VDR | Rabbit | Cell Signaling | D2K6W, #12550 | 1/2000 |
| WBP4 (IP and IF) | Rabbit | Abcam | ab108144 | IP: 3µg IF:1/200 |
| WBP4 (WB) | Rabbit | Abcam | ab272629 | 1/1000 |
| LMNB1 | Rabbit | Abcam | ab16048 | 1/10000 |
| GAPDH | Rabbit | Cell Signaling | 14C10, #2118 | 1/20000 |
| anti-rabbit HRP conjugated | Goat | Jackson Immunoresearch | 111-035-003 | 1/10000 |
| anti-rabbit CY3 conjugated | Goat | Jackson Immunoresearch | 111-165-003 | 1/400 |
| anti-mouse HRP conjugated | Goat | Jackson Immunoresearch | 115-035-003 | 1/10000 |
| anti-rabbit IgG (WB) | Mouse | Cell Signaling | L27A9, #3678 | 1/2000 |
| anti-rabbit IgG (IP) | Rabbit | Diagenode | C15410206 | 3µg |

**Supplementary Table 3.** Summary of the oligonucleotides and antibodies used.

**Supplementary Table 4:** exact p-values

| Figure 1a | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **Cyp24a1** | | | |
| | 1,25D3 vs Vehicle | 1.079 to 2.791 | <0.0001 |
| | ZK vs Vehicle | 0.2528 to 1.965 | 0.0091 |
| | 1,25D3 + ZK vs Vehicle | -0.9378 to 0.9378 | >0.9999 |
| +KTC | 1,25D3 vs Vehicle | 0.2971 to 2.463 | 0.0102 |
| | ZK vs Vehicle | -1.284 to 0.8821 | >0.9999 |
| | 1,25D3 + ZK vs Vehicle | -1.225 to 0.9408 | >0.9999 |
| **s100g** | | | |
| | 1,25D3 vs Vehicle | 0.08666 to 1.276 | 0.0224 |
| | ZK vs Vehicle | 0.04940 to 1.238 | 0.0317 |
| | 1,25D3 + ZK vs Vehicle | -0.8920 to 0.4372 | 0.7593 |
| +KTC | 1,25D3 vs Vehicle | 0.2101 to 1.399 | 0.0069 |
| | ZK vs Vehicle | -0.7141 to 0.4748 | 0.9365 |
| | 1,25D3 + ZK vs Vehicle | -0.5681 to 0.6209 | 0.9992 |
| **Atp2b1** | | | |
| | 1,25D3 vs Vehicle | 0.1642 to 1.189 | 0.0081 |
| | ZK vs Vehicle | 0.03144 to 1.057 | 0.0356 |
| | 1,25D3 + ZK vs Vehicle | -0.8623 to 0.1628 | 0.2465 |
| +KTC | 1,25D3 vs Vehicle | -0.1989 to 0.8262 | 0.0315 |
| | ZK vs Vehicle | -0.6352 to 0.3899 | 0.9014 |
| | 1,25D3 + ZK vs Vehicle | -0.3732 to 0.6520 | 0.8633 |
| **trpv6** | | | |
| | 1,25D3 vs Vehicle | 0.2207 to 1.327 | 0.0053 |
| | ZK vs Vehicle | 0.03403 to 1.141 | 0.0357 |
| | 1,25D3 + ZK vs Vehicle | -0.8910 to 0.3463 | 0.5953 |
| +KTC | 1,25D3 vs Vehicle | 0.09541 to 1.202 | 0.0193 |
| | ZK vs Vehicle | -0.3316 to 0.7751 | 0.6627 |
| | 1,25D3 + ZK vs Vehicle | -0.4452 to 0.6615 | 0.9415 |

| Figure 1b | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **2h** | | | |
| | 1,25D3 vs Vehicle | -1.188 to -0.06841 | 0.0219 |
| **4h** | | | |
| | 1,25D3 vs Vehicle | -1.637 to -0.517 | <0.0001 |
| **6h** | | | |
| | 1,25D3 vs Vehicle | -1.96 to -0.6765 | <0.0001 |

| Figure 1d | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **2h** | | | |
| | 1,25D3 vs Vehicle | -0.2052 to 0.04889 | 0.1227 |
| | ZK vs Vehicle | -0.2661 to -0.1597 | 0.0033 |
| | 1,25D3 + ZK vs Vehicle | -0.2810 to -0.02973 | 0.0332 |
| | ZK vs 1,25D3 | -0.2209 to -0.04853 | 0.0211 |
| | 1,25D3+ZK vs 1,25D3 | -0.3205 to 0.1661 | 0.35 |
| **4h** | | | |
| | 1,25D3 vs Vehicle | -0.1990 to -0.05034 | 0.0183 |
| | ZK vs Vehicle | -0.3393 to -0.1030 | 0.0148 |
| | 1,25D3 + ZK vs Vehicle | -0.2353 to -0.09690 | 0.0092 |
| | ZK vs 1,25D3 | -0.1403 to -0.05269 | 0.0109 |
| | 1,25D3+ZK vs 1,25D3 | -0.1850 to 0.1021 | 0.04061 |
| **6h** | | | |
| | 1,25D3 vs Vehicle | -0.3043 to -0.1897 | 0.0018 |
| | ZK vs Vehicle | -0.3173 to -0.2089 | 0.0009 |
| | 1,25D3 + ZK vs Vehicle | -0.4519 to -0.02152 | 0.0416 |
| | ZK vs 1,25D3 | -0.1277 to 0.09547 | 0.7683 |
| | 1,25D3+ZK vs 1,25D3 | -0.1494 to 0.1700 | 0.9644 |

| Figure 1f | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **FB-789** | | | |
| | 1,25D3 vs Vehicle | -3.312 to -1.031 | 0.0023 |
| | ZK vs Vehicle | -2.467 to -0.1855 | 0.0262 |
| | 1,25D3 + ZK vs Vehicle | -1.987 to 0.5117 | 0.3009 |
| **FB-CYP** | | | |
| | 1,25D3 vs Vehicle | -3.851 to -0.9949 | 0.0042 |
| | ZK vs Vehicle | -1.935 to 1.258 | >0.9999 |
| | 1,25D3 + ZK vs Vehicle | -1.695 to 1.498 | >0.9999 |

| Figure 3b | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **cytosol** | | | |
| | 1,25D3 vs Vehicle | -0.1343 to 0.4303 | 0.3771 |
| | 1,25D3 + ZK vs Vehicle | -0.4475 to 0.1561 | 0.4331 |
| | 1,25D3 vs 1,25D3 + ZK | -0.5760 to -0.01140 | 0.0412 |
| **Nucleus** | | | |
| | 1,25D3 vs Vehicle | -1.215 to -0.4558 | 0.0002 |
| | 1,25D3 + ZK vs Vehicle | -0.6941 to 0.06468 | 0.1092 |
| | 1,25D3 vs 1,25D3 + ZK | 0.1411 to 0.8998 | 0.0085 |

| Figure 3d | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **cytosol** | | | |
| | 1,25D3 vs Vehicle | 0.1179 to 0.4458 | 0.0006 |
| | ZK vs Vehicle | -0.09035 to 0.2376 | 0.732 |
| | 1,25D3 + ZK vs Vehicle | -0.05639 to 0.2715 | 0.3394 |

| | | | |
|---|---|---|---|
| **Nucleus** | | | |
| | 1,25D3 vs Vehicle | -0.5923 to -0.2644 | <0.0001 |
| | ZK vs Vehicle | -0.5879 to -0.2600 | <0.0001 |
| | 1,25D3 + ZK vs Vehicle | -0.3447 to -0.01676 | 0.0266 |
| | 1,25D3 vs 1,25D3 + ZK | 0.5481 to 1.892 | 0.0005 |

| **Figure 3f** | **Tukey's multiple comparisons test** | **95% confidence interval** | **p-value** |
|---|---|---|---|
| **KTC cytosol** | | | |
| | 1,25D3 vs Vehicle | -0.4506 to 0.6408 | 0.9582 |
| | ZK vs Vehicle | -0.7415 to 0.3500 | 0.7369 |
| | 1,25D3 + ZK vs Vehicle | -0.6948 to 0.3967 | 0.8618 |
| **KTC Nucleus** | | | |
| | 1,25D3 vs Vehicle | -1.215 to -0.1232 | 0.0139 |
| | ZK vs Vehicle | -0.4380 to 0.6535 | 0.941 |
| | 1,25D3 + ZK vs Vehicle | -0.3535 to 0.7380 | 0.7471 |
| | 1,25D3 vs ZK | 0.2310 to 1.322 | 0.0044 |
| | 1,25D3 vs 1,25D3 + ZK | 0.3155 to 1.407 | 0.0018 |

| **Figure 4f** | | **Umpaired t test** | **p-value** |
|---|---|---|---|
| | **Cyp24a1** | | |
| | | SiWbp4 vs SiControl | 0.0319 |
| | **s100g** | | |
| | | SiWbp4 vs SiControl | 0.0456 |
| | **Atp2b1** | | |
| | | SiWbp4 vs SiControl | 0.0452 |

| **Figure 4g** | **Tukey's multiple comparisons test** | **95% confidence interval** | **p-value** |
|---|---|---|---|
| **Cyp24a1** | | | |
| SiControl | 1,25D3 vs Vehicle | 1.629 to 10.28 | 0.0087 |
| | 1,25D3 + ZK vs Vehicle | -3.708 to 4.943 | 0.9219 |
| SiWbp4 | 1,25D3 vs Vehicle | 1.170 to 10.84 | 0.0163 |
| | 1,25D3 + ZK vs Vehicle | 0.8583 to 10.53 | 0.022 |
| **s100g** | | | |
| SiControl | 1,25D3 vs Vehicle | 0.6758 to 6.083 | 0.0144 |
| | 1,25D3 + ZK vs Vehicle | -2.686 to 2.721 | 0.9998 |
| SiWbp4 | 1,25D3 vs Vehicle | 0.008517 to 5.369 | 0.0492 |
| | 1,25D3 + ZK vs Vehicle | 0.4399 to 6.170 | 0.0234 |
| **Atp2b1** | | | |
| SiControl | 1,25D3 vs Vehicle | 0.4576 to 4.249 | 0.013 |
| | 1,25D3 + ZK vs Vehicle | -1.604 to 2.187 | >0.9999 |
| SiWbp4 | 1,25D3 vs Vehicle | 0.08553 to 4.324 | 0.0401 |
| | 1,25D3 + ZK vs Vehicle | 0.08012 to 4.319 | 0.0406 |

| Figure 5a | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **Calcium** | | | |
| | 1,25D3 vs Vehicle | 0.03564 to 0.1998 | 0.0057 |
| | 1,25D3+ZK 0.3 ug/kg vs Vehicle | -0.05863 to 0.1055 | 0.8748 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -0.1296 to 0.03460 | 0.3743 |
| | VDR-null vs Vehicle | -0.2323 to -0.06813 | 0.0009 |
| | VDR -/- vs 1,25D3 + ZK 1ug/kg | -0.1848 to -0.02065 | 0.0139 |

| Figure 5c | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **Cyp24a1** | | | |
| | 1,25D3 vs Vehicle | 0.5453 to 2.439 | 0.0208 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -1.393 to 1.276 | 0.9643 |
| **Slc30a10** | | | |
| | 1,25D3 vs Vehicle | 0.6763 to 1.576 | 0.0083 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -0.3523 to 0.9828 | 0.1908 |
| **Slc37a2** | | | |
| | 1,25D3 vs Vehicle | 0.7242 to 2.151 | 0.0128 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -2.331 to 1.650 | 0.6418 |
| **Atp2b1** | | | |
| | 1,25D3 vs Vehicle | 0.6757 to 1.940 | 0.0122 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -1.283 to 1.082 | 0.879 |
| **Nkain1** | | | |
| | 1,25D3 vs Vehicle | 0.2554 to 1.589 | 0.0468 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -2.105 to 3.704 | 0.4094 |

| Figure 5e | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| **Slc30a10** | 1,25D3 WT vs Vehicle WT | -1.574 to -0.2210 | 0.0097 |
| | 1,25D3+ZK VDR null vs WT 1,25D3+ZK | -1.437 to 0.02488 | 0.0593 |
| | 1,25D3 WT vs 1,25D3+ZK WT | 0.2375 to 1.591 | 0.0086 |
| **Cyp24a1** | 1,25D3 WT vs Vehicle WT | -3.806 to -1.383 | 0.0002 |
| | 1,25D3+ZK VDR null vs WT 1,25D3+ZK | 0.3449 to 2.962 | 0.0133 |
| | 1,25D3 WT vs 1,25D3+ZK WT | 1.341 to 3.764 | 0.0003 |
| **Slc37a2** | 1,25D3 WT vs Vehicle WT | -1.707 to -0.4892 | 0.001 |
| | 1,25D3+ZK VDR null vs WT 1,25D3+ZK | 0.8732 to 2.189 | 0.0001 |
| | 1,25D3 WT vs 1,25D3+ZK WT | 0.1775 to 1.396 | 0.0116 |
| **Nkain1** | 1,25D3 WT vs Vehicle WT | -1.621 to 0.1988 | 0.145 |
| | 1,25D3+ZK VDR null vs WT 1,25D3+ZK | -1.817 to 0.1494 | 0.1057 |
| | 1,25D3 WT vs 1,25D3+ZK WT | -0.08013 to 1.740 | 0.0774 |
| **Atp2b1** | 1,25D3 WT vs Vehicle WT | -1.617 to -0.4263 | 0.0032 |
| | 1,25D3+ZK VDR null vs WT 1,25D3+ZK | -0.9715 to 0.3595 | 0.4741 |
| | 1,25D3 WT vs 1,25D3+ZK WT | 0.9209 to 2.252 | 0.0004 |

| Figure 6b | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| Calcium | | | |
| | 1,25D3 vs Vehicle | 0.06159 to 0.6073 | 0.0339 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -0.2255 to 0.4540 | 0.3173 |
| | 1,25D3 vs 1,25D3 + ZK 1ug/kg | -0.3580 to -0.08246 | 0.0202 |

| Figure 6c | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| PTH | | | |
| | 1,25D3 vs Vehicle | -2.167 to -1.326 | 0.0024 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -2.154 to 1.684 | 0.7772 |
| | 1,25D3 vs 1,25D3 + ZK 1ug/kg | 0.004253 to 3.018 | 0.0497 |

| Figure 6d | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| Duodenal | | | |
| | 1,25D3 vs Vehicle | 0.06159 to 0.6073 | 0.0339 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -0.2255 to 0.4540 | 0.3173 |
| | 1,25D3 vs 1,25D3 + ZK 1ug/kg | -0.3580 to -0.08246 | 0.0202 |

| | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| Renal | | | |
| | 1,25D3 vs Vehicle | 0.2821 to 1.279 | 0.0071 |
| | 1,25D3 + ZK 1ug/kg vs Vehicle | -0.6336 to 0.3637 | 0.6995 |
| | 1,25D3 vs 1,25D3 + ZK 1ug/kg | -1.414 to -0.4170 | 0.0032 |

| Sup Fig 3a | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| Wbp4 | | | |
| SiControl | 1,25D3 vs Vehicle | -1.473 to 2.275 | 0.8498 |
| | 1,25D3 + ZK vs Vehicle | -2.795 to 0.9540 | 0.4386 |
| SiWbp4 | 1,25D3 vs Vehicle | -2.809 to 0.9395 | 0.4278 |
| | 1,25D3 + ZK vs Vehicle | -2.662 to 1.087 | 0.5427 |

| Sup Fig 3d | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| Wbp4 | | | |
| | SiWBP4 Cyt vs SiControl Cyt | -0.7014 to -0.3090 | <0.0001 |
| | SiWBP4 Nucl vs SiControl Nucl | -0.5217 to -0.1055 | 0.0038 |

| Sup Fig 3f | Tukey's multiple comparisons test | 95% confidence interval | p-value |
|---|---|---|---|
| VDR | | | |
| | SiWBP4 Cyt vs SiControl Cyt | 0.3739 to 1.368 | 0.041 |
| | SiWBP4 Nucl vs SiControl Nucl | -1.105 to -0.02279 | 0.0014 |