Supplemental Data

Keith et al, Molecular Carcinogenesis--MS# MC-12-0161

Supplemental Table 1. Doubling times of clonal cell lines isolated from $KO^{hVDR\text{-}F}$ parental cells.

Supplemental Table 2. Effect of 1,25D on caspase activity in model cell lines

Supplemental Figure 1. Characterization of clonal cell lines from $KO^{hVDR\text{-}F}$ cell line.

Supplemental Figure 2. Effect of 1,25D on CYP24 protein expression.

Supplemental Figure 3. VDR expression in model cell lines.

Supplemental Figures

Supplemental Figure 1. Characterization of clonal cell lines from KO^{hVDR-F} cell line. A. Western blot of VDR in clones B5 and C5 treated with vehicle or 100nM 1,25D for 48h. B. Relative VDR mRNA expression (measured by real-time PCR) in clones B5 and C5 treated with vehicle or 100nM 1,25D for 24h. C. Relative luciferase activity of an SV-40 driven reporter plasmid transiently transfected into clones B5 and C5. Cells were treated with vehicle or 100nM 1,25D for 24h and data was normalized and calculated as for Figure 1B.

Supplemental Figure 2. Effect of 1,25D on CYP24 protein expression. WT145, KO240 and KO^{EV} cells were treated with vehicle or 100nM 1,25D for 48h and processed for western blot of CYP24 as described for Figure 1C.

Supplemental Figure 3. VDR expression in model cell lines. WT145, KO240, KO^{hVDR-C} KO^{hVDR-E}, KO^{hVDR-F} and KO^{G46D} cells were treated with vehicle or 100nM 1,25D for 48h and processed for western blot of VDR and actin as described for Figure 1A. rVDR, recombinant human VDR included as positive control.

Supplemental Table 1. Doubling times of clonal cell lines isolated from KO^{hVDR-F} parental cells.

	WT145	KO^{hVDR-F}	Clone B5	Clone C5
Control	48.6	36.0	42.8	42.6
1,25D	112.1	77.1	100.3	83.7

Data represent culture doubling time in hours for the WT145 cell line, the KO^{hVDR-F} parental cultures and two lines (B5 and C5) which were cloned from KO^{hVDR-F}. Assays were conducted in the presence and absence of vehicle or 100nM 1,25D and calculated as described in the Methods section. The data shown for WT145 cells and KO^{hVDR-F} cells is reproduced from Figure 2 for comparison.

Supplemental Table 2. Effect of 1,25D on caspase activity in model cell lines

	WT145	KO240	hVDR-C	hVDR-F	hVDR-E
Caspase-3	2.9	0.8	1.9	1.4	0.8
Caspase-2	3.1	1.2	1.9	1.7	0.9
Caspase-8	1.2	1.0	1.0	1.0	0.7
Caspase-9	1.3	1.1	1.1	1.2	0.8

Data represent mean fold-change in caspase activity in cells treated with 100nM 125D for 48h compared to vehicle treated cells. Assays were conducted in duplicate.

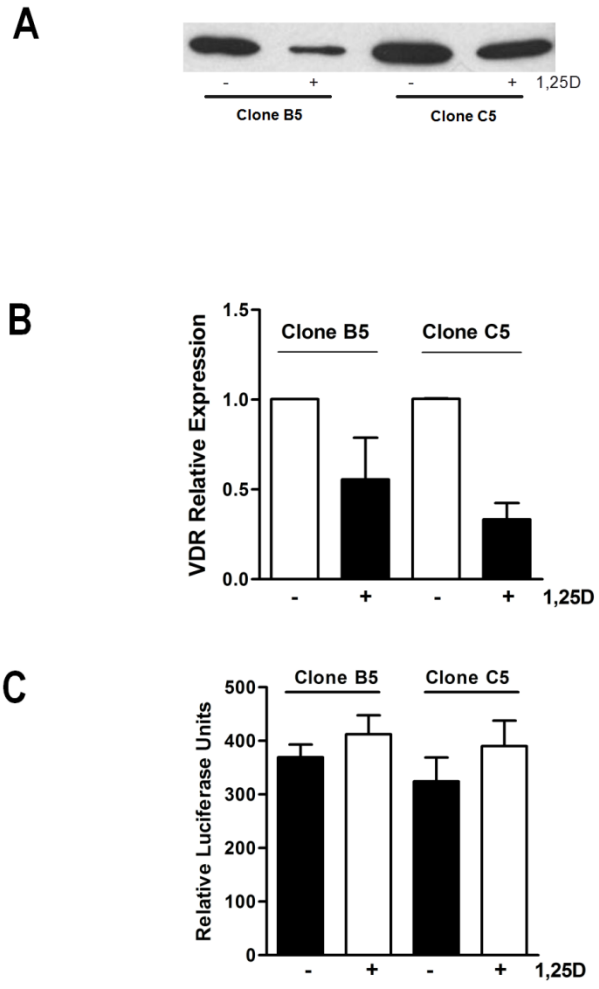
Supplemental Figure Legends

Supplemental Figure 1. Characterization of clonal cell lines from KO^{hVDR-F} cell line. A. Western blot of VDR in clones B5 and C5 treated with vehicle or 100nM 1,25D for 48h. B. Relative VDR mRNA expression (measured by real-time PCR) in clones B5 and C5 treated with vehicle or 100nM 1,25D for 24h. C. Relative luciferase activity of an SV-40 driven reporter plasmid transiently transfected into clones B5 and C5. Cells were treated with vehicle or 100nM 1,25D for 24h and data was normalized and calculated as for Figure 1B.

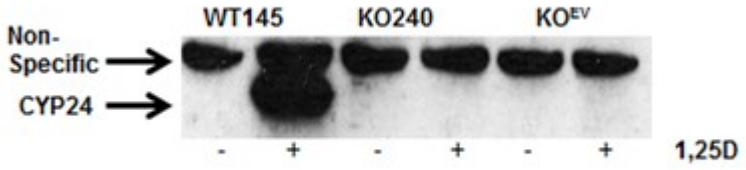
Supplemental Figure 2. Effect of 1,25D on CYP24 protein expression. WT145, KO240 and KO^{EV} cells were treated with vehicle or 100nM 1,25D for 48h and processed for western blot of CYP24 as described for Figure 1C.

Supplemental Figure 3. VDR expression in model cell lines. WT145, KO240, KO^{hVDR-C} KO^{hVDR-E}, KO^{hVDR-F} and KO^{G46D} cells were treated with vehicle or 100nM 1,25D for 48h and processed for western blot of VDR and actin as described for Figure 1A. rVDR, recombinant human VDR included as positive control.

Supplemental Figure 1. Characterization of clonal cell lines from KO$^{hVDR\text{-}F}$ cell line.



Supplemental Figure 2. Effect of 1,25D on CYP24 protein expression.



Supplemental Figure 3. VDR expression in model cell lines.