

Figure S1. The synthesis of the vitamin D hydroxyderivatives used in the present study. (A) hydroxyl groups were added to the vitamin D3 starting material enzymatically using recombinant cytochromes P450 (CYP, in red) in a reaction initiated by CYP11A1 with subsequent involvement of CYP27A1, CYP24A1 and/or CYP27B1, as shown by the blue arrows. Some derivatives were also synthesized chemically (green arrows). (B) hydroxyl groups were added to vitamin D2 enzymatically by the action of CYP11A1.

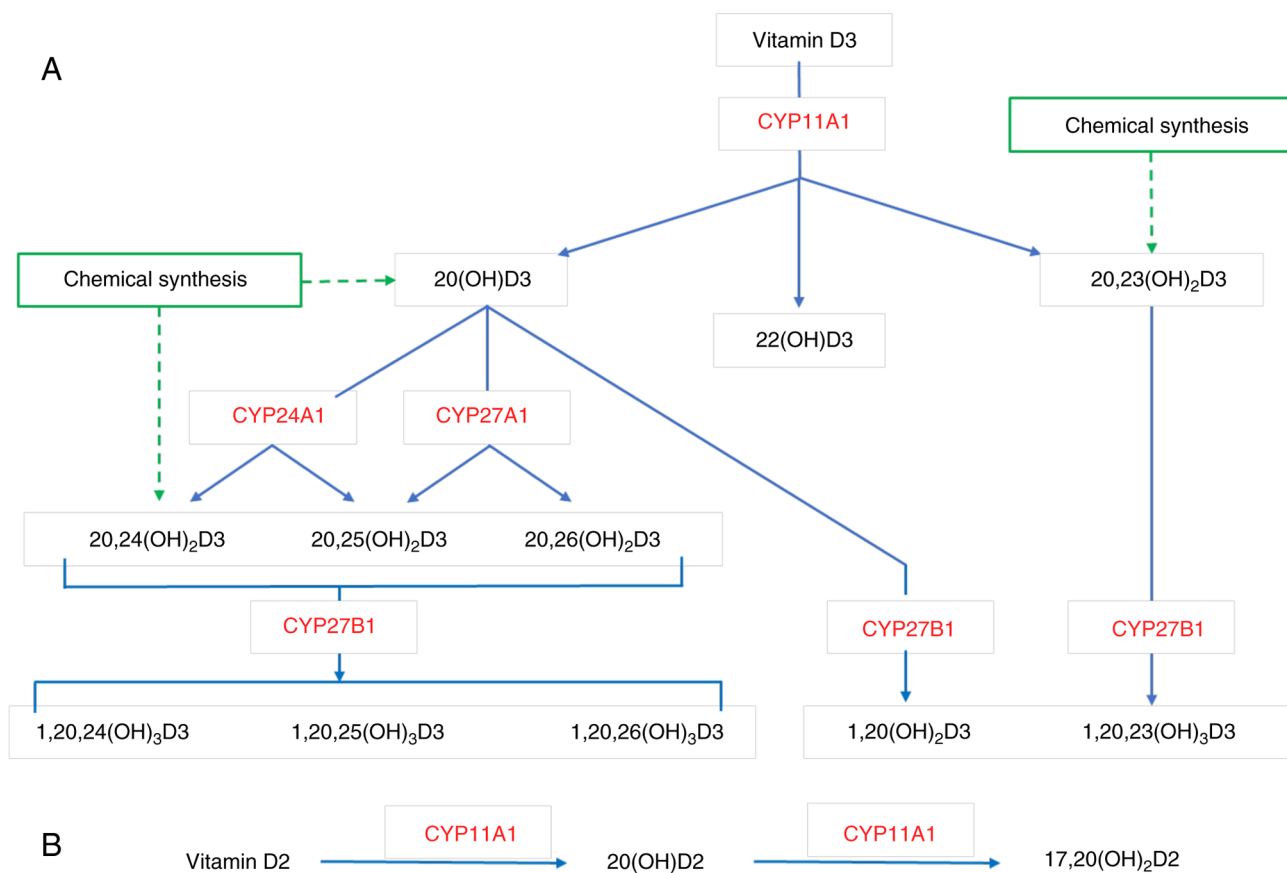
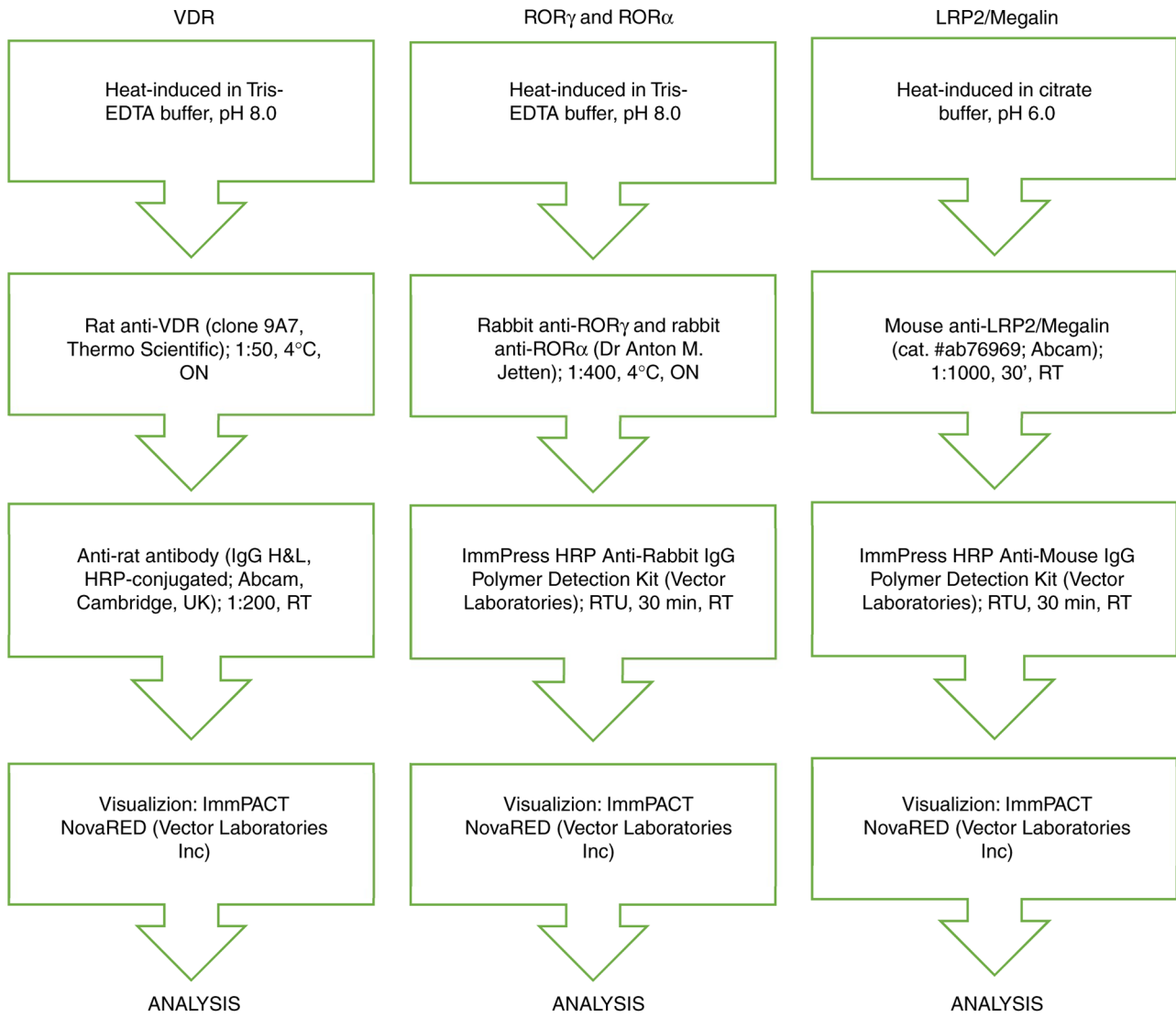


Figure S2. Scheme for immunohistochemistry and characteristic of reagents used.



ON – overnight; RT – room temperature; RTU – ready-to-use

Figure S3. A431 cells were treated with 100 nM 1,20(OH)<sub>2</sub>D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, 20(OH)D<sub>3</sub>, or ethanol (vehicle). After 24 h of incubation, cells were fixed in 2% PFA for 1 h and cell pellet washed with PBS. Permeabilized cells were stained with primary antibody against involucrin or with isotype control antibody (IgG<sub>1</sub>; used as controls) overnight, washed with PBS, and incubated with sheep anti-mouse FITC-conjugated secondary antibody for 3 h, subsequently. Acquisition and analysis was performed on a FACSCalibur flow cytometer. Number of events (positive cells), FL-1 signal is collected from 10,000 events in side scatter/forward scatter window after debris exclusion. Forward (FSC; relative to cell size) and side (SSC; relative to cell granularity) scatter were also recorded. Representative flow chart for each treatment.

