Supporting Information

Figure S1. Peptide data are shown for the HDX experiment described in the Experimental Procedures including APE1 along with APE1 incubated with 1.6 mM E3330. Apo refers to full-length APE1 alone, E3330 to the sample of APE1 incubated with E3330. Peptide coverage for the experiment was greater than 90%. Four peptides were identified with differences in HDX including 68-74 (seen for both the +1/+2 peptides), 266-271, and 267-273.

Figure S2. Peptide data are shown for HDX performed at 25 μM E3330 (A and C) and similar peptides obtained from the experiment performed by using 1.6 mM E3330 (B and D). See Experimental Procedures for description of full-length APE1 experiments. Differential, solution phase HDX experiments were performed to detect the conformational change of Δ40 APE1, an N-terminally truncated form lacking the N-terminal 40 amino acids, induced by E3330 binding as previously described (*1*). Δ40 APE1was incubated with E3330 at a ratio of 1:10 for at least 1 h on ice before HDX analysis. Each exchange reaction was initiated by incubating 2 μL of 25 μM protein (with or without 250 μM E3330) with 18 μL of D_2O protein buffer for a predetermined time (10, 30, 60, 120, 900, and 3600 s) at 4 °C. The exchange reaction was quenched by mixing with 30 μL of 3 M urea, 1% TFA at 1 °C. The mixture was passed across custom-packed pepsin column (2 mm X 2 cm) at 200 μL/min, and the digested peptides were captured on a 2 mm X 1 cm C_8 trap column (Agilent) and desalted (total time for digestion and desalting was 3 min). Peptides were then separated across a 2.1 mm X 5 cm C_{18} column (1.9 μ Hypersil Gold, Thermo Scientific) with a linear gradient of 4%–40% CH_3CN, 0.1% formic acid, over 5 min. Protein digestion and peptide separation were performed on columns immersed in an ice-water bath to reduce D/H back exchange. Mass spectrometric analyses were carried out with capillary

temperature at 225 °C, and data were acquired with a measured mass resolving power of 100,000 at *m/z* 400. Three replicates were performed for each ion-exchange time point. Under the conditions of this experiment accepting a K_d for binding of E3330 to APE1 of 390 μM, we expect approximately 8% complex formation. Peptides were identified and HDX data processed as described in the Experimental Procedures.

Figure S3. Differential scanning fluorimetry analysis of the effect of Mg^{2+} on the melting temperature of APE1. A concentration range of 10 μM – 10 mM $MgCl_2$ was used to determine the effect of Mg^{2+} on the melting temperature of APE1. Concentrations of 10 μM – 5 mM increased the melting temperature of APE1 by 4.3 °C maximally. However, addition of higher concentrations of Mg^{2+} resulted in a lower melting temperature than that obtained for 5 mM.

References

1. Zhang, J., Chalmers, M. J., Stayrook, K. R., Burris, L. L., Garcia-Ordonez, R. D., Pascal, B. D., Burris, T. P., Dodge, J. A., and Griffin, P. R. (2010) Hydrogen/deuterium exchange reveals distinct agonist/partial agonist receptor dynamics within vitamin D receptor/retinoid X receptor heterodimer, *Structure 18*, 1332-1341.