Epigenetic Modulation of SPCA2 Reverses Epithelial to Mesenchymal Transition in Breast Cancer Cells

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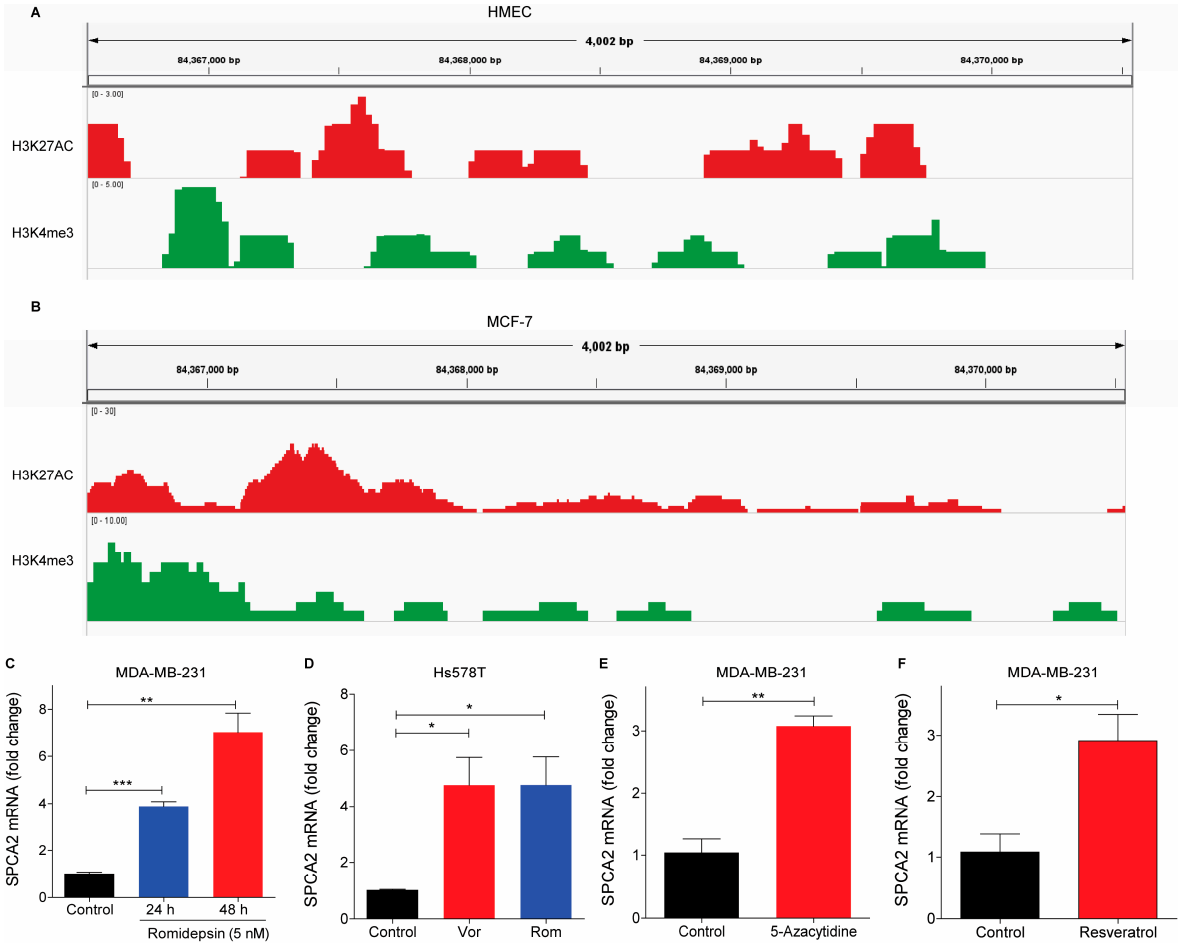

Figure S1. Epigenetic modification in SPCA promoter region. (**A–B**) Epigenetic modification of the promoter region of SPCA2, showing histone acetylation (H3K27AC) and methylation (H3K4me3), in (**A**) HMEC and (B) MCF-7 cells, as described in Methods. Not the scale bar differences, showing increased acetylation and methylation in MCF-7, compared to HMEC. (**C**) Treatment of MDA-MB-231 cells with HDAC inhibitors romidepsin showed a time-dependent increase in SPCA2 expression. $n = 3$. (**D**) Treatment of HS578T cells with HDAC inhibitors vorinostat (2.5 μM) and romidepsin (5 nM) for 24 h significantly increased SPCA2. (**E–F**) Treatment of MDA-MB-231 cells with DNA methyltransferase inhibitor (5-Azacytidine, 10 μM, 48 h) and sirtuin activator (Resveratrol, 2.5 μM, 24 h) significantly increased SPCA2 $n = 3$. Student's *t*-test, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

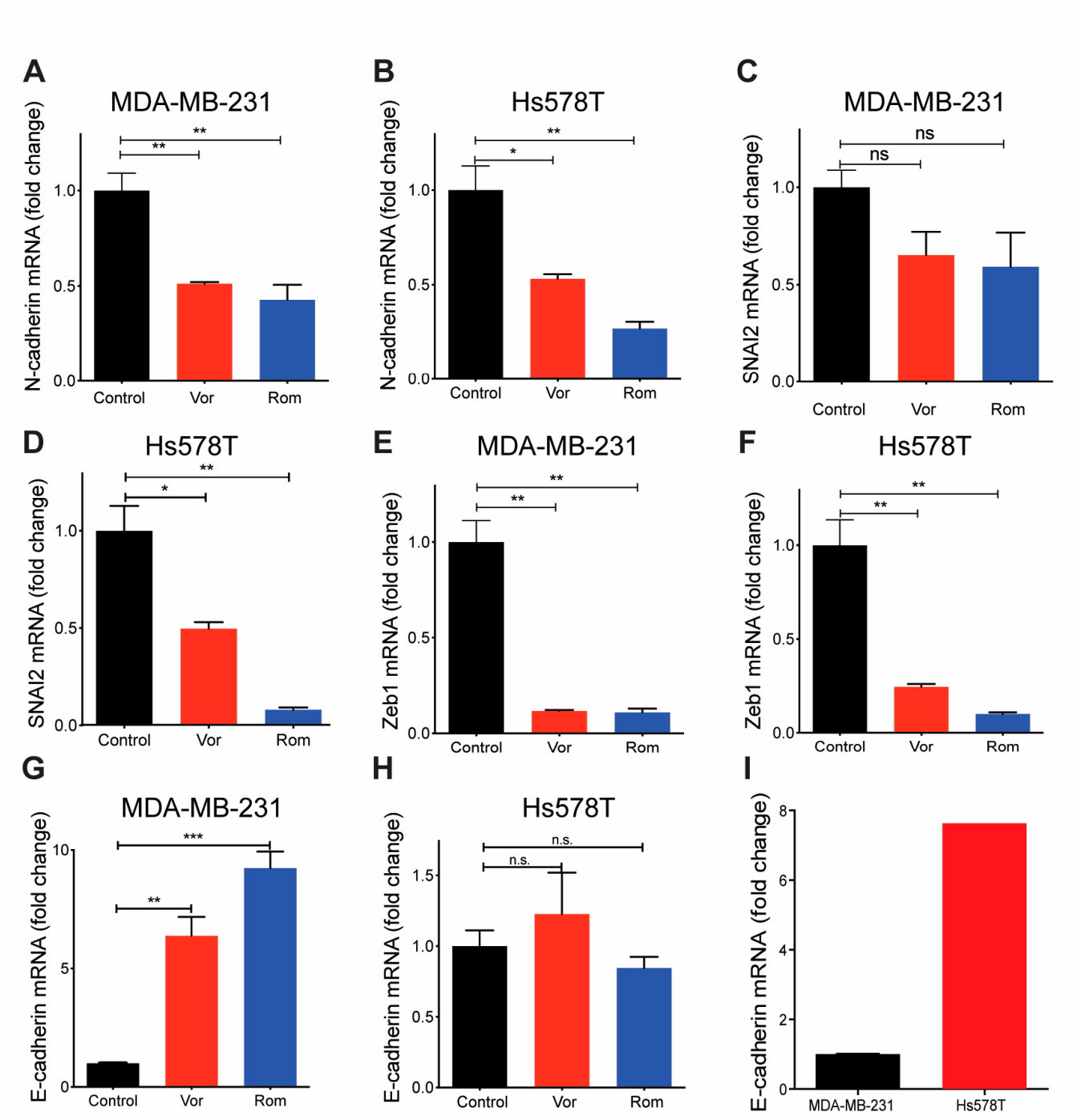

Figure S2. HDAC inhibitors promote MET phenotype TNBC cells. (**A**–**F**) Treatment of MDA-MB-231 and Hs578T cells, as shown, with vorinostat (2.5 μM) and romidepsin (5 nM) for 24 h decreases expression of several mesenchymal gene markers. Transcripts were determined by qPCR, $n = 3$. (**G**–**H**) Treatment with vorinostat (2.5 μM) and romidepsin (5 nM) for 24 h significantly increased transcript of the epithelial gene marker E-cadherin (CDH1) in MDA-MB-231 cells, but not in Hs578T cells; $n = 3$. (**I**) Hs578T has 7-fold higher endogenous CDH1 expression compared to MDA-MB-231, in the absence of HDACi. Student *t*-test, $^{***}p < 0.001$, $^{**}p < 0.01$,$^{*}p < 0.05$, $^{ns}p > 0.05$.

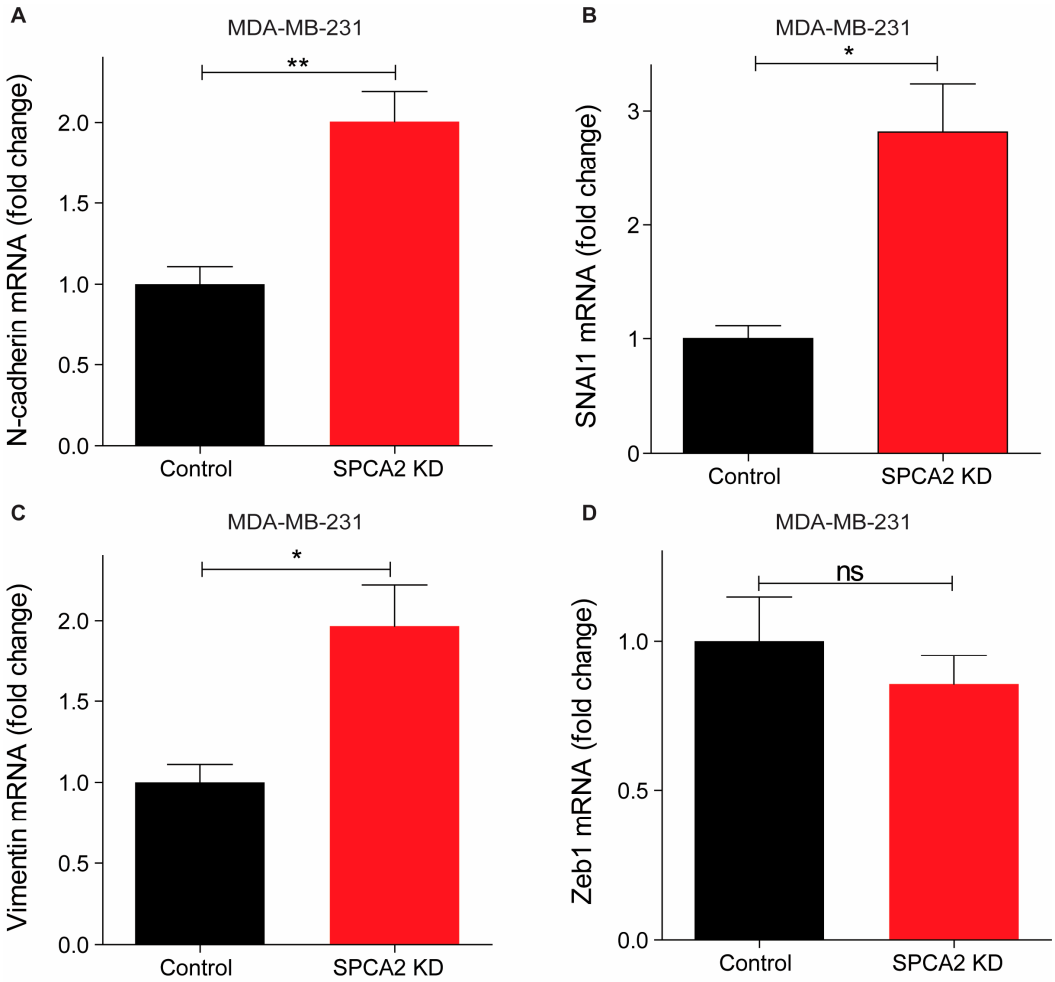

Figure S3. SPCA2 KD induced EMT changes in TNBC cell lines**.** (**A**–**C**) Knockdown of SPCA2 in MDA-MB-231 increased mesenchymal gene markers N-cadherin, SNAI1 and vimentin. (**D**) Knockdown of SPCA2 in MDA-MB-231 did not significantly increase mesenchymal gene marker Zeb1. $n = 3$. $^{**}p < 0.01$,$^{*}p < 0.05$, $^{ns}p > 0.05$.

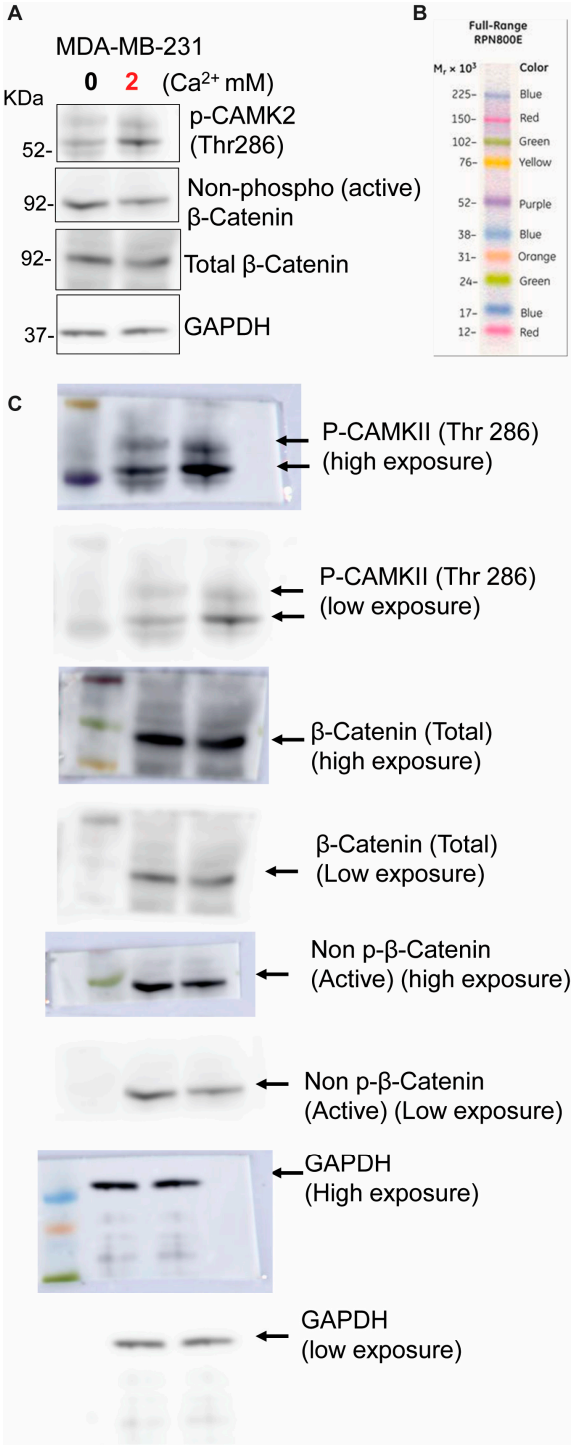

Figure 4. Extracellular Ca entry can also activate non-canonical Wnt/Ca pathway. (**A**) MDA-MD-231 were maintained in DMEM with 0 mM and 2 mM calcium for 42 h. Extracellular Ca entry activated non-canonical Wnt/Ca pathway. (**B**) Amersham ™ ECL ™ Rainbow ™ Marker (RPN800E, Sigma Aldrich) was used for western blots. The figure shows full molecular weight range. (**C**) These blots complement the cropped blots shown in Supplement Figure 4A.

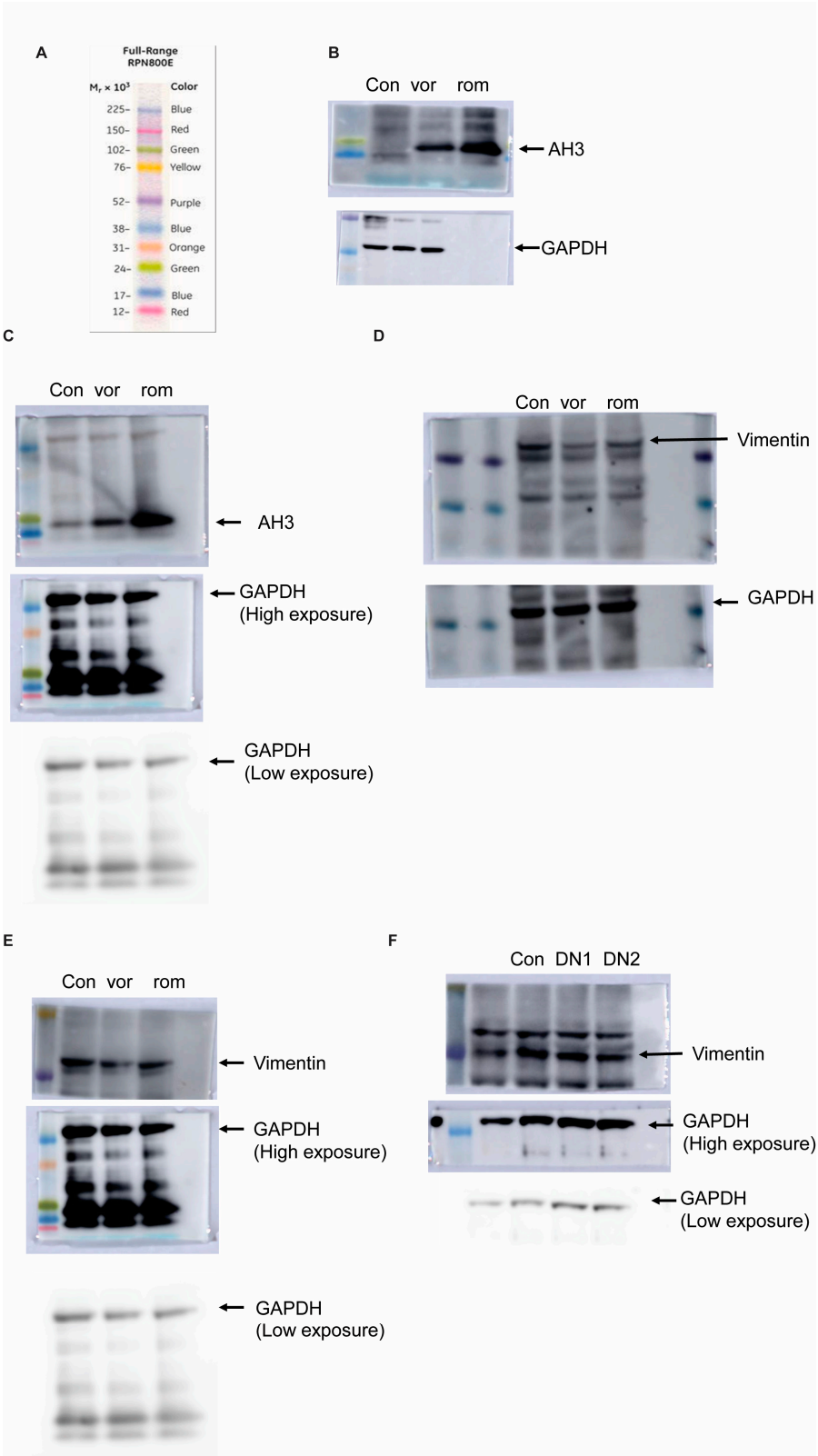

Figure S5. Uncropped western blots for Figures 2, 3 and 5. (**A**) Amersham ™ ECL ™ Rainbow ™ Marker (RPN800E, Sigma Aldrich) was used for western blots. (**B**) These blots complement the cropped blots shown in Figure 2B. (**C**) These blots complement the cropped blots shown in Figure 2B. (**D**) These blots complement the cropped blots shown in Figure 3D. (**E**) These blots complement the cropped blots shown in Figure 3D. This blot was developed using same sample as Figure S5C, so loading control is same as Figure S5C. (**F**) These blots complement the cropped blots shown in Figure 5B.

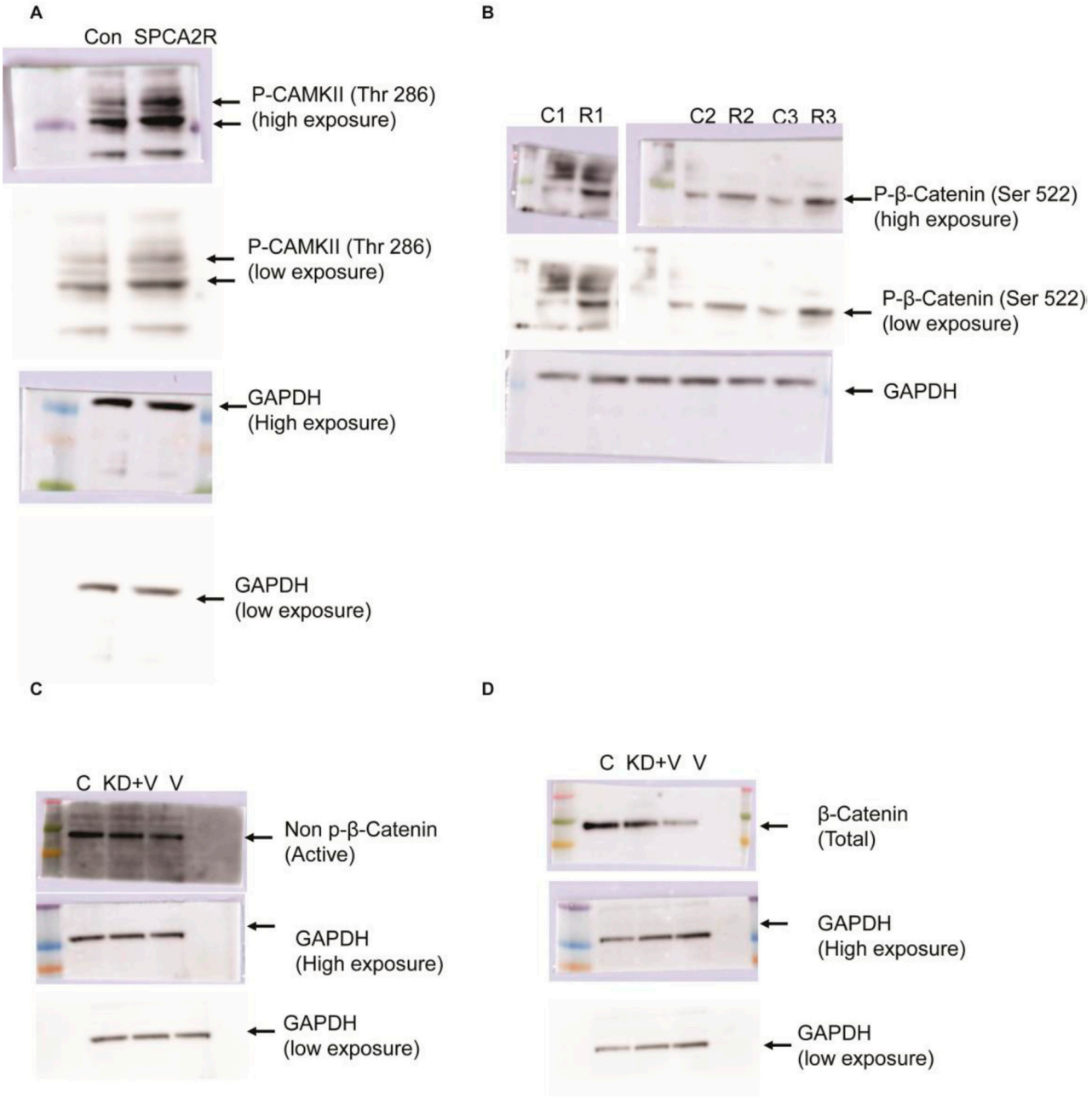

Figure 6. Uncropped western blots for Figure 6. (**A**) These blots complement the cropped blots shown in Figure 6B. (**B**) These blots complement the cropped blots (C3 and R3) shown in Figure 6B. (**C**) These blots complement the cropped blots shown in Figure 6J. (**D**) These blots complement the cropped blots shown in Figure 6J.