Supplementary figures

Protocadherin 19 (PCDH19) interacts with paraspeckle protein NONO to co-regulate gene expression with estrogen receptor alpha (ERα)

Duyen H. Pham[1,2], Chuan Tan[1], Claire C. Homan[3], Kristy Kolc[1,2], Mark Corbett[1,2], Dale McAninch[3], Archa Fox[4], Paul Thomas[3], Raman Kumar[1,2], Jozef Gecz[1,2,3,5]

[1] School of Medicine, The University of Adelaide, Adelaide 5000, Australia.

[2] Robinson Research Institute, The University of Adelaide, Adelaide 5006, Australia.

[3] School of Biological Sciences, The University of Adelaide, Adelaide 5000, Australia.

[4] Harry Perkins Institute of Medical Research and Centre for Medical Research, University of Western Australia, Crawley, Western Australia 6009, Australia.

[5] South Australian Health and Medical Research Institute, Adelaide 5000, Australia.

To whom the correspondence should be addressed at: School of Medicine and The Robinson Research Institute, The University of Adelaide, Adelaide, SA 5000, Australia. Tel: +61 883133245; Fax: +61 881617342; Email: jozef.gecz@adelaide.edu.au

Figure S1. Spectrum of PCDH19 mutations. Schematic diagram showing spectrum of *PCDH19* mutations: missense (squares), nonsense and CNV (triangles) and those examined in this study (black-squares and a triangle).

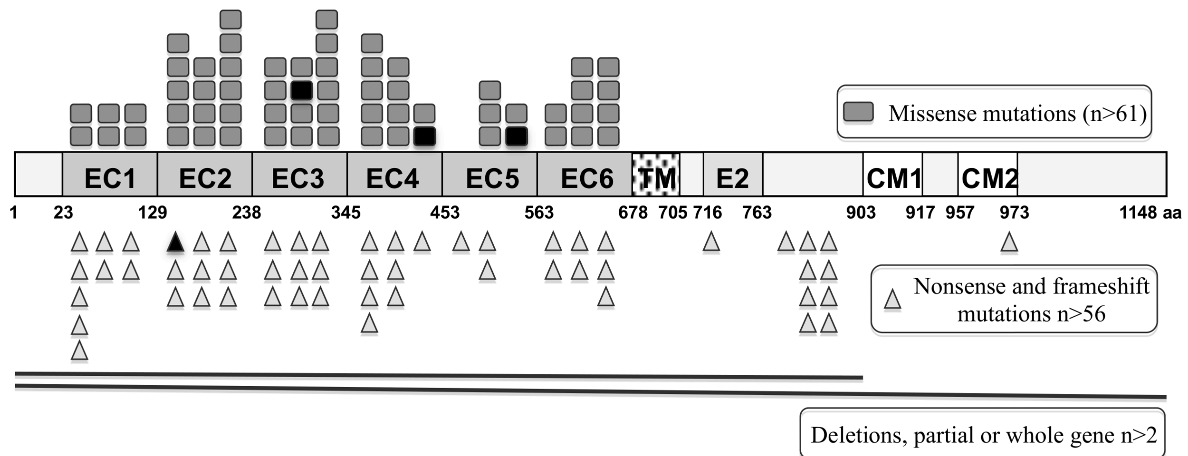
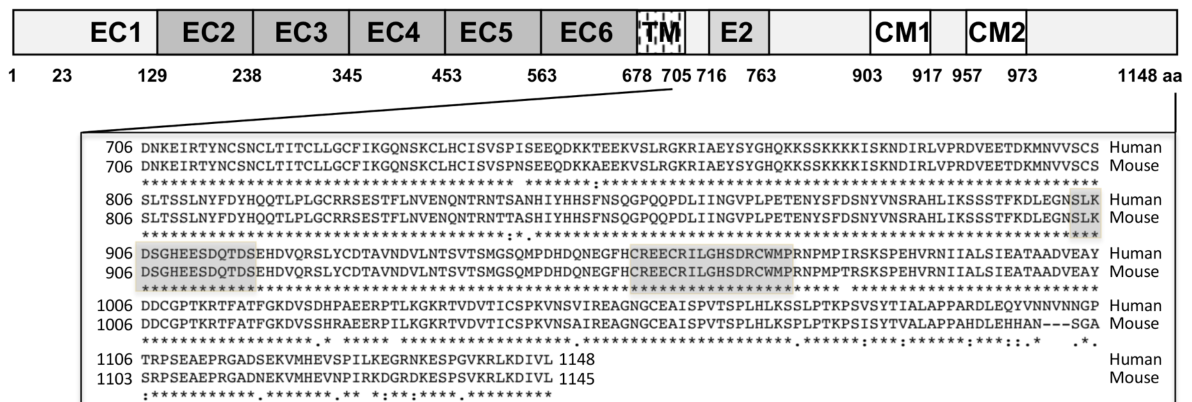


Figure S2. Schematic diagram of PCDH19 protein. (**A**) Alignment of C-terminal region of human PCDH19 and mouse Pcdh19. Shaded amino acids represent CM1 and CM2, respectively. (**B**) Putative nuclear localization signals (NLSs) in the C-terminal region of PCDH19.

A



B

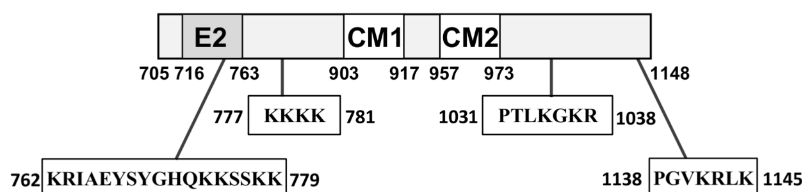


Figure S3. Cellular localization of Myc-PCDH19-FLAG. HeLa cells expressing the double-tagged PCDH19 expression construct with N-terminal Myc and C-terminal FLAG tags (Myc-PCDH19-FLAG) showing predominantly perinuclear and nuclear localization of PCDH19 visualized by staining with anti-Myc (green) and anti-FLAG (red) antibodies. Cell nuclei were visualized by staining with DAPI (blue). Scale bars = 5 µm. Images are representative of >200 cells examined.

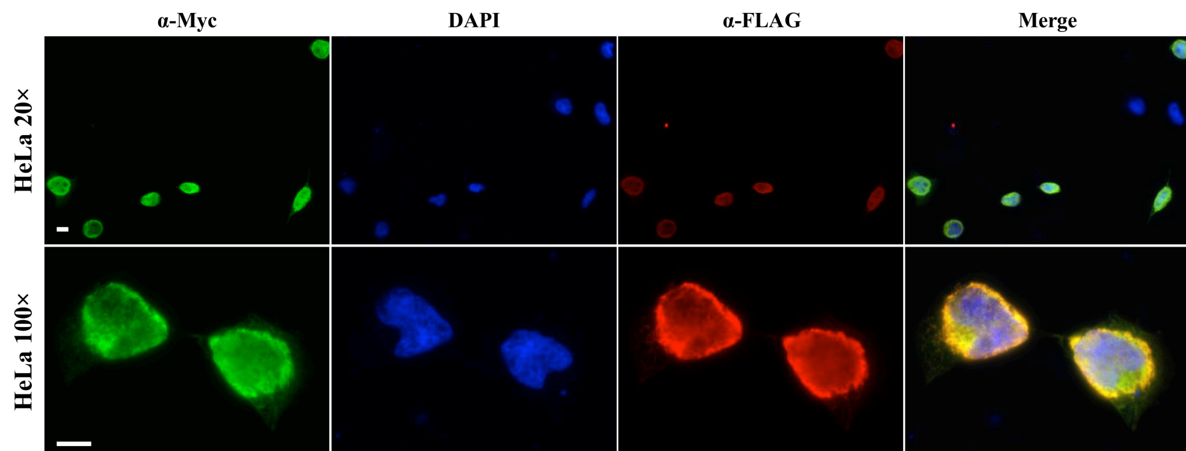


Figure S4. PCDH19, NONO and ERα are present predominantly in the MCF-7 nucleus. Western blot of subcellular fractions from Myc-PCDH19 transfected MCF-7 cells was probed with anti-Myc antibody and endogenous protein with anti-PCDH19 antibody. Endogenous NONO and ERα were also visualised. Histone H3, HSP90 and KCNT1 were used as nuclear, cytoplasmic and membrane markers, respectively.

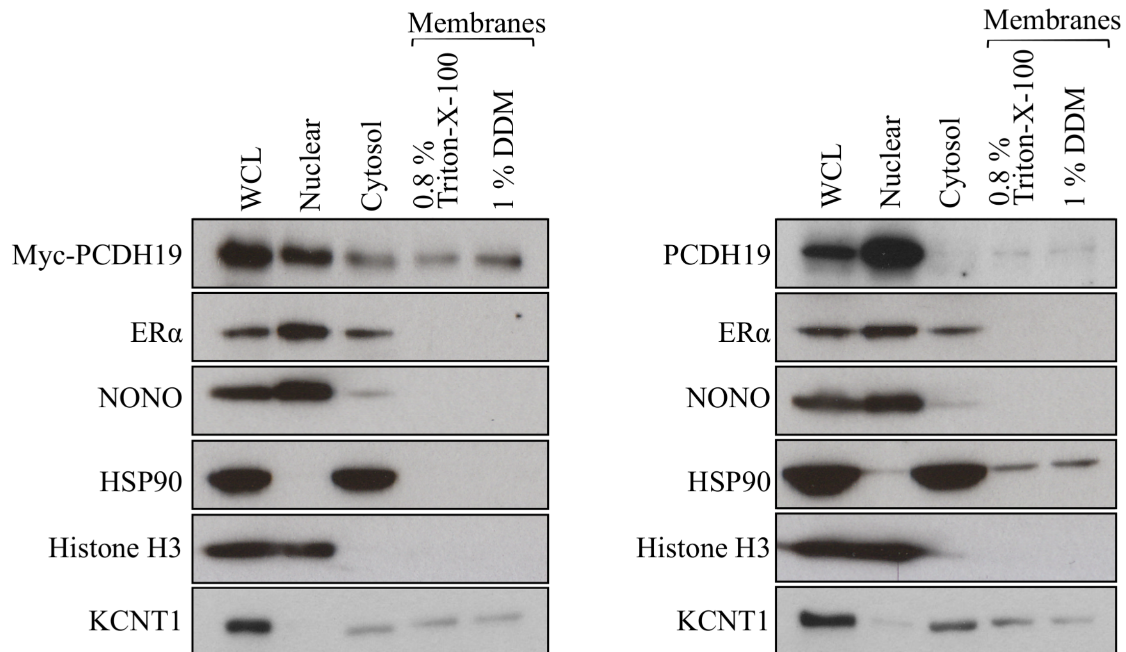


Figure S5. PCDH19 and steroid hormone receptor mRNA levels in a panel of breast cancer cell lines. Steroid hormone receptors ERα, PGR, AR and PCDH19 mRNA was assayed in five cancer and one normal (MCF-10A) breast cell lines by RT-qPCR. MCF-7 cell line has high levels of expression of endogenous PCDH19 and all three steroid hormone receptors (boxed).

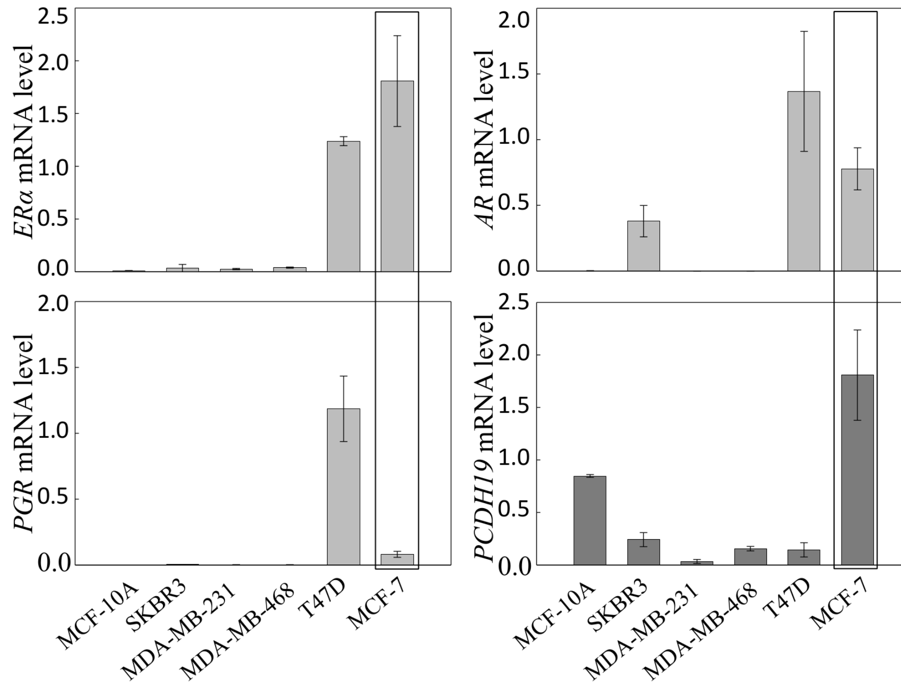


Figure S6. PCDH19 regulates ERα transcriptional activity. (**A**) MCF-7 cells were transfected with PCDH19 or control siRNA (SCR) for 24h and subsequently transfected with 3× ERE TATA luc reporter plasmid. Cells were cultured in charcoal-stripped medium for 16 h and then for 6 h in the presence or absence of 10 nM E2. Data are expressed as relative luciferase activity ± SD from three independent experiments. * $p < 0.05$ comparing +E2 SCR vs –E2 SCR, **[1] $p < 0.01$ comparing –E2 SCR vs –E2 PCDH19 siRNA, **[2] $p < 0.01$ comparing +E2 SCR vs +E2 PCDH19 siRNA cells using Bonferroni adjusted planned comparisons. (**B**) PCDH19 knockdown was confirmed by western blotting the MCF-7 cell extracts with anti-PCDH19 antibody. β-Actin was used as a loading control.

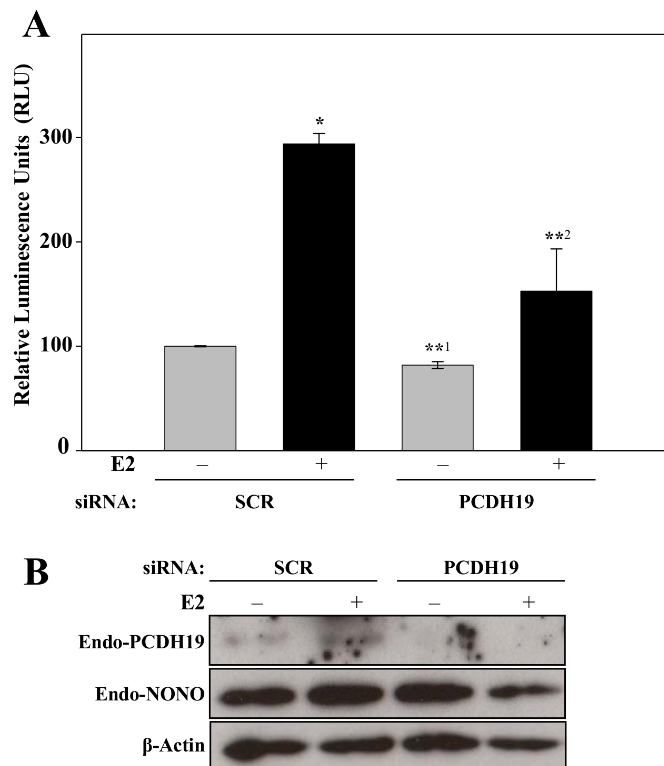


Figure S7. ERα-target gene expression is increased in presence of wild-type but is suppressed in presence of mutant Myc-PCDH19. Aldo-keto reducatase 3 (*AKR1C3*), apolipoprotein D (*APOD*), ectodermal neural cortex 1 (*ENC1*) and Oxytoxin receptor (*OXTR*) mRNA was assayed by RT-qPCR in MCF-7 cells transfected with vector, wild-type or Asn557Lys Myc-PCDH19 expression vectors and cultured in the presence of 10 nM E2. The mRNA levels are expressed relative to HPRT. Data are the mean ± range from two independent experiments. Each sample was analyzed in triplicate.

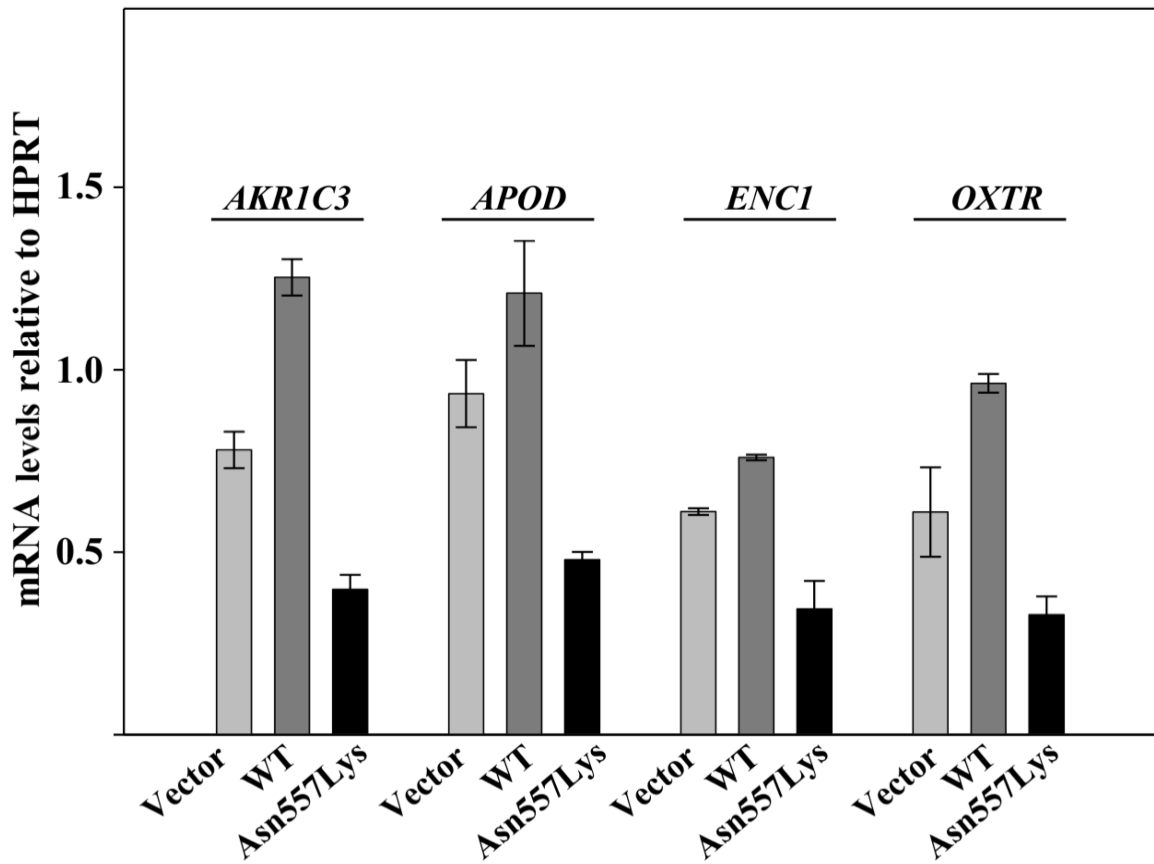


Figure S8. Wild-type or mutant Myc-PCDH19 does not regulate ERα promoter via ERE in ERα- cells. (**A**) Luciferase activity was assayed in MCF-7 (ERα+) or MDA-MB-231 (ERα-) cells transfected with reporter containing 3× ERE TATA luc and control, wild-type or mutant Myc-PCDH19 expression vectors. Cells were initially culture in charcoal-stripped medium for 16 h and then for 6 h in the presence or absence of 10 nM E2. Data are expressed as relative luciferase activity ± SD from four independent experiments. * $p < 0.05$ comparing +E2 WT vs +E2 vector control cells. (**B**) Levels of Myc-PCDH19 and endogenous-NONO protein were determined by western blotting cell extracts expressing wild-type or mutant Myc-PCDH19 with anti-Myc and anti-NONO antibody, respectively. β-Actin was used as a loading control.

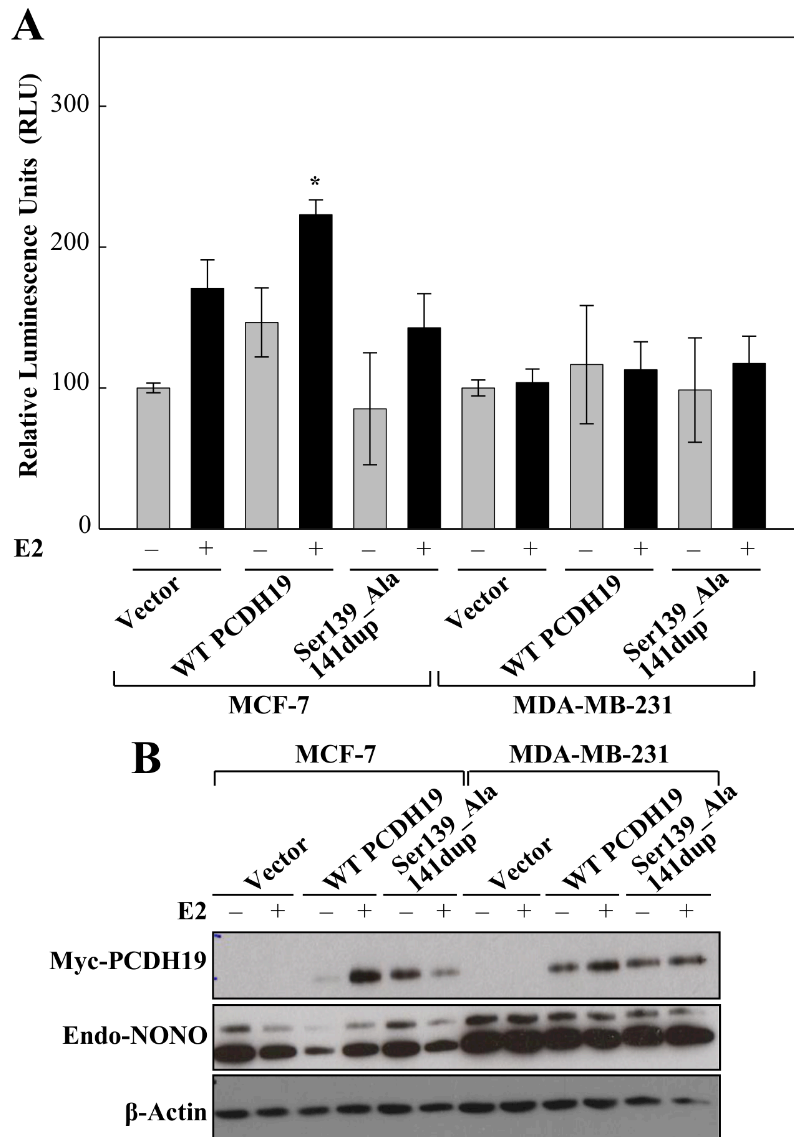


Figure S9. PCDH19-mediated ERα transcriptional activity is ERα-dependent. MCF-7 cells were transfected with ERα or control siRNA for 24h and subsequently co-transfected with control or wild-type Myc-PCDH19 plasmids in conjunction with 3× ERE TATA luc reporter plasmid. Cells were cultured in charcoal-stripped medium for 16 h and then in for 6 h in the presence or absence of 10 nM E2. Data are expressed as relative luciferase activity ± SD from three independent experiments. * $p < 0.05$ comparing +E2 vs –E2 SCR vector control, ** $p < 0.01$ comparing –E2 ERα siRNA vector control vs –E2 SCR vector control, **[1] $p < 0.01$ comparing +E2 ERα siRNA vector control vs +E2 SCR vector control, **[2] $p < 0.01$ comparing –E2 SCR WT vs –E2 SCR vector control, **[3] $p < 0.01$ comparing +E2 SCR WT vs +E2 SCR vector control, *[4] $p < 0.05$ comparing –E2 ERα siRNA WT vs –E2 SCR WT, **[5] $p < 0.01$ comparing +E2 ERα siRNA WT vs +E2 SCR WT cells using Bonferroni adjusted planned comparisons. Western blot of MCF-7 cell extracts expressing wild-type Myc-PCDH19 was probed with anti-Myc antibody. Endogenous ERα was detected using anti-ERα antibody and β-Tubulin was used as a loading control.

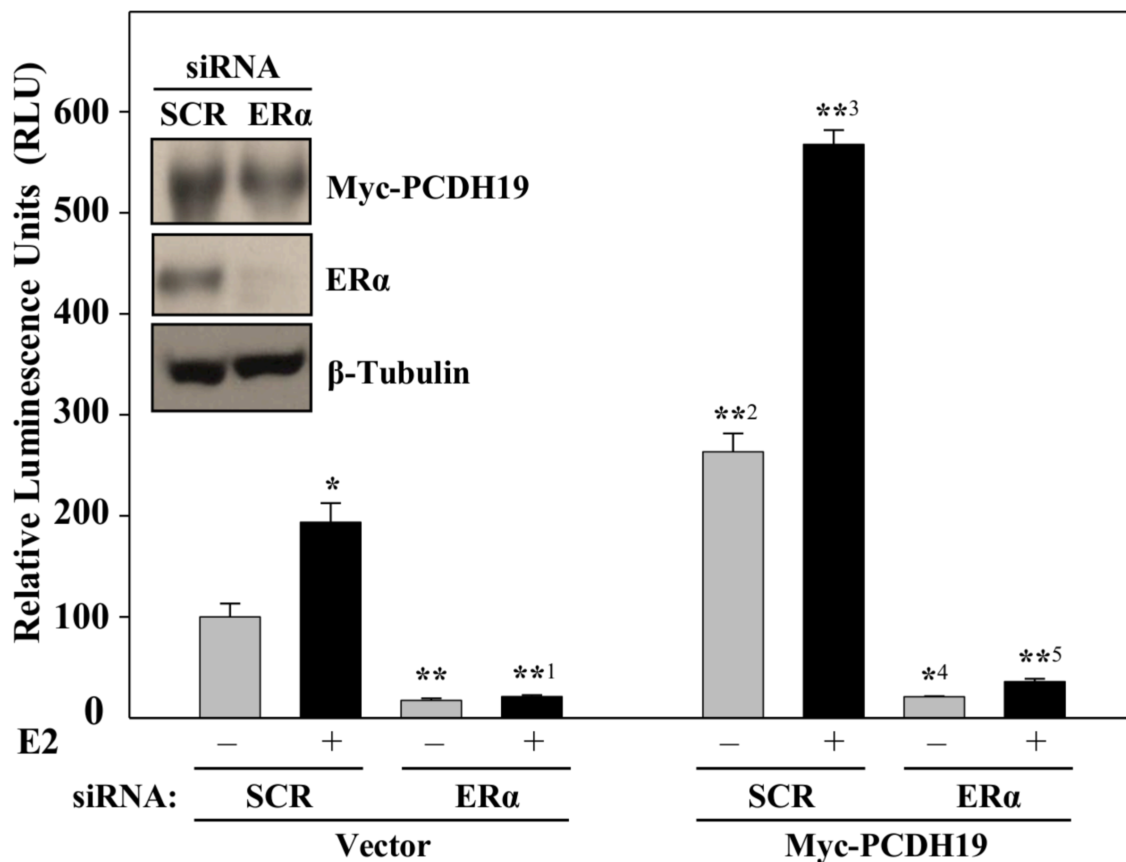
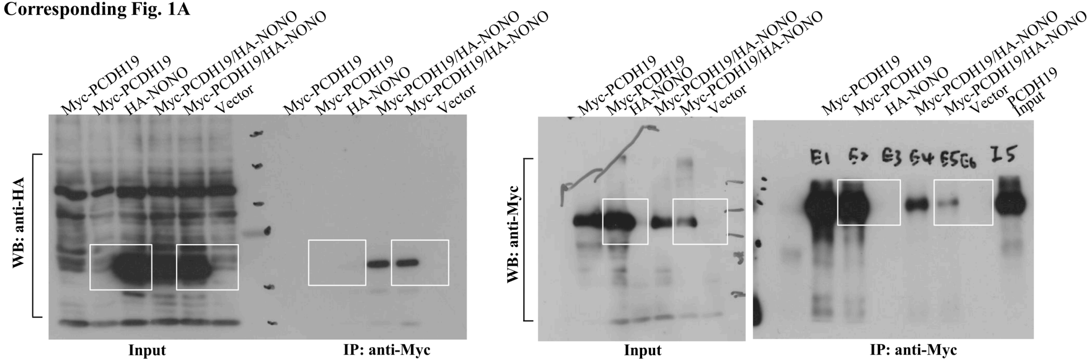
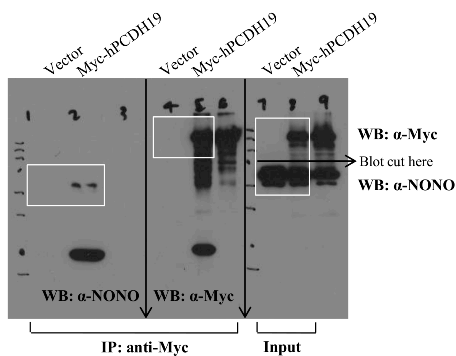


Figure S10. Full-length western blots. Original full-length western blots before cropping.

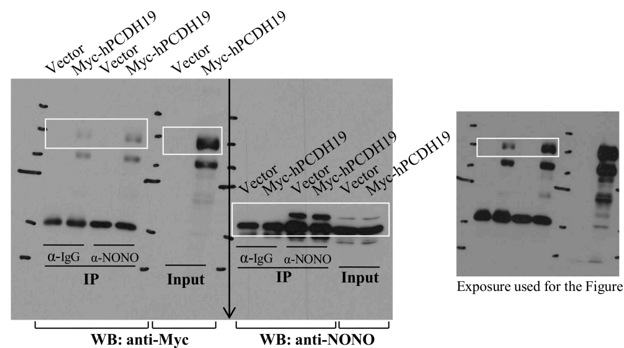
Corresponding Fig. 1A



Corresponding Fig. 1B



Corresponding Fig. 1C



Corresponding Fig. 5B