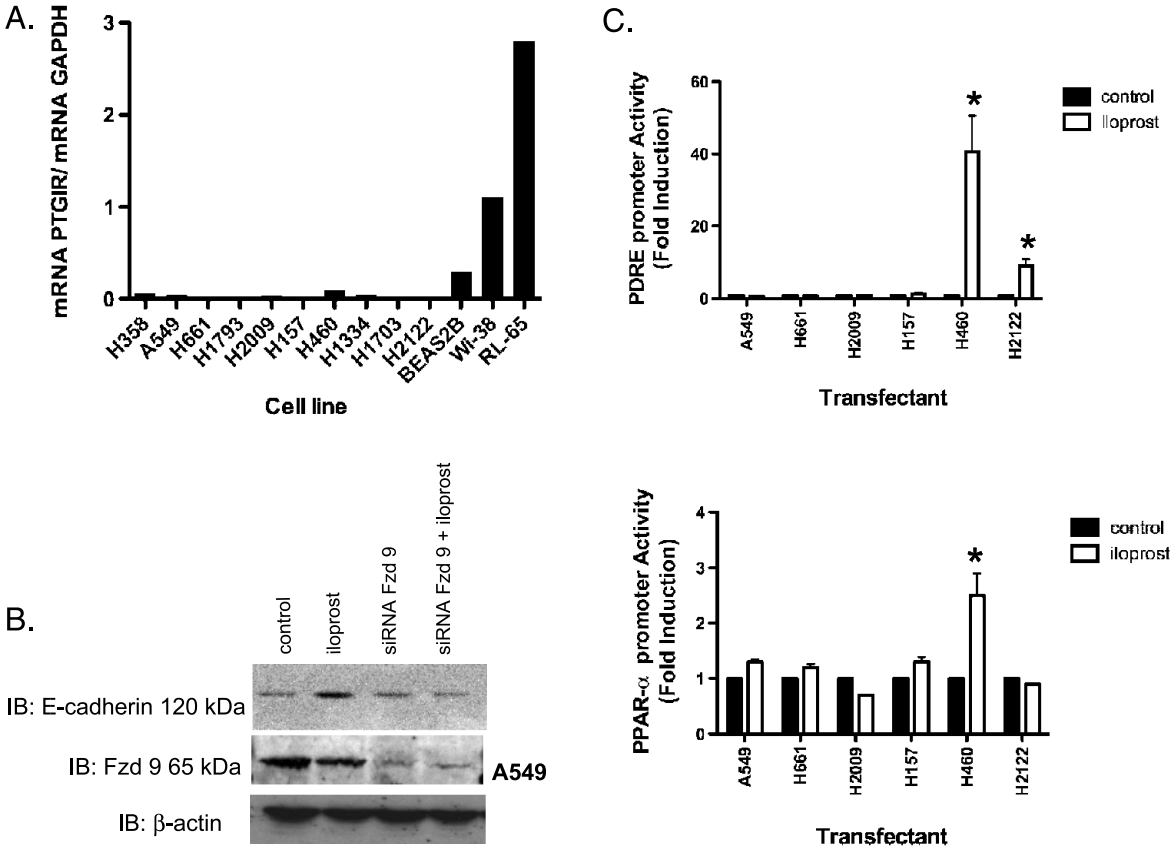

Figure W1. siRNA reduces E-cadherin expression and PTGIR is absent in NSCLC. (A) Total RNA purified from the indicated NSCLC cell lines were submitted to quantitative RT-PCR using primers specific for PTGIR as described under Materials and Methods. The relative mRNA abundance for PTGIR in the different samples was normalized to human GAPDH measured by RT-PCR in the same samples. (B) E-cadherin protein expression by iloprost and Fzd 9 are reduced by Fzd 9 siRNA knockdown. Aliquots of extracts containing equal protein as measured by the Bradford assay from the indicated cells were resolved by SDS-PAGE and immunoblotted with antibodies to E-cadherin (125 kDa; BD Transduction Laboratories) and Fzd 9 (100 kDa; Aviva Systems Biology). The filters were stripped and reimmunoblotted for β-actin (47 kDa; Abcam) as a loading control. (C) The indicated NSCLC cell lines were transiently transfected with PDRE or PPARα-luc, along with CMV-β-gal to normalize for transfection efficiency. After an overnight incubation, cells were exposed for 48 hours with 10 μM iloprost. Extracts were prepared, and promoter activity was determined as luciferase units normalized to CMV-β-gal. Results represent the mean of three independent experiments with the SEM indicated.

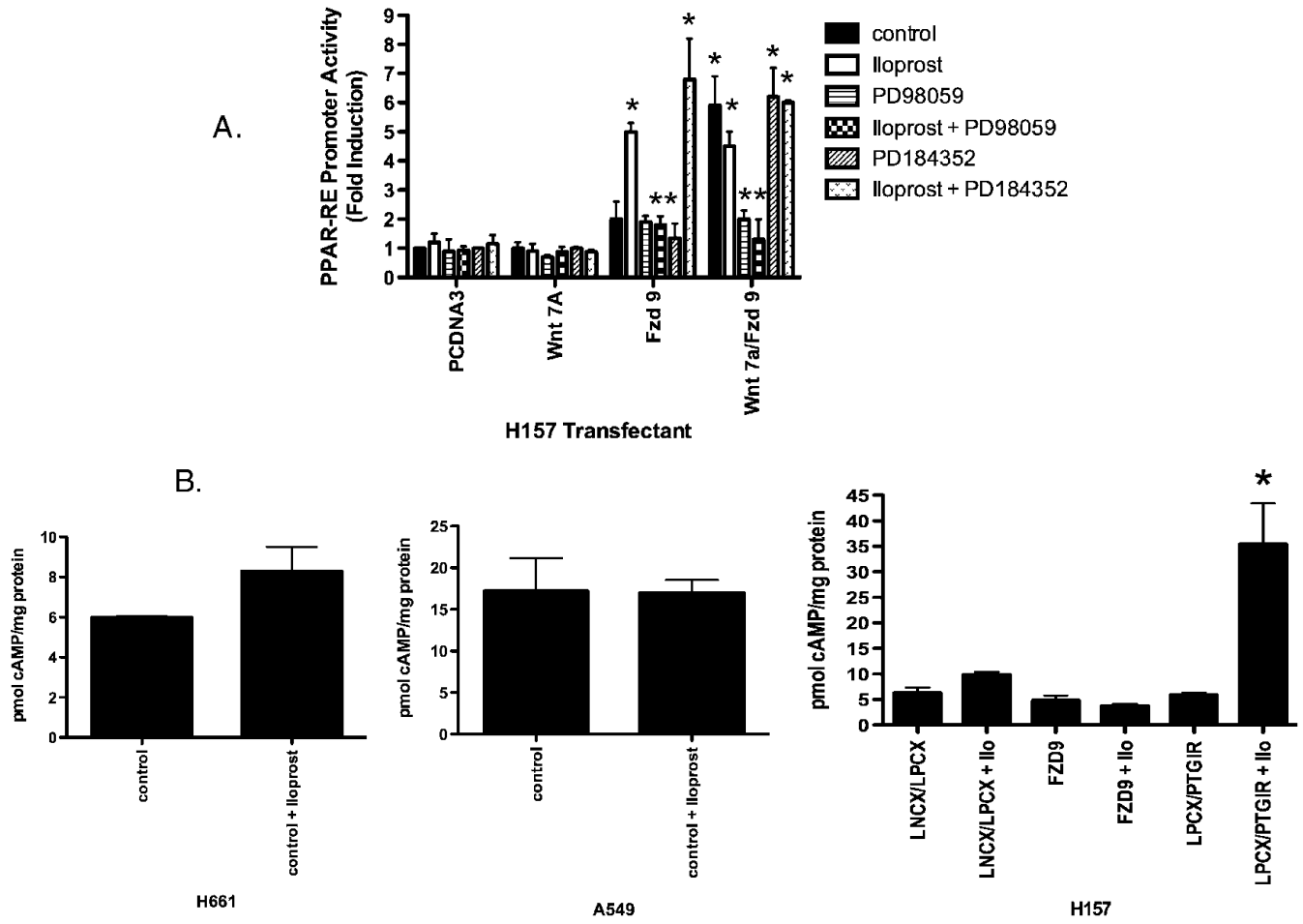

Figure W2. Iloprost and Fzd 9 do not activate PPARγ through activation of cAMP and PD98059 blocks its effect. (A) The ERK pathway inhibitor PD98059 significantly reduces PPAR-RE activity induced by both iloprost and Fzd 9 or Wnt 7a/Fzd 9, but the MEK1/2 inhibitor PD184352 has no affect the PPAR-RE activity induced by either iloprost and Fzd 9 or Wnt 7a/Fzd 9. The H157 cell line was transiently transfected with PPAR-RE, empty vector pCDNA3, Wnt 7a, Fzd 9, or both Wnt 7a/Fzd 9, along with CMV-β-gal to normalize for transfection efficiency. After an overnight incubation, cells were exposed for 48 hours with either 25 μM PD98059, 5 μM PD184352, and/or iloprost 10 μM. (B) The indicated cell lines were exposed to 10 μM of iloprost, and then cellular cAMP content was measured using the direct cAMP kit from Assay Designs. Results are reported as picomole of cAMP per milligram of protein. The H157 cell line encoding empty vector LPCX, LPCX-Fzd 9, or LPCX-PTGIR were exposed to iloprost 5 μM and measured for direct cAMP kit from Assay Designs. Results are reported as picomole of cAMP per milligram of protein.