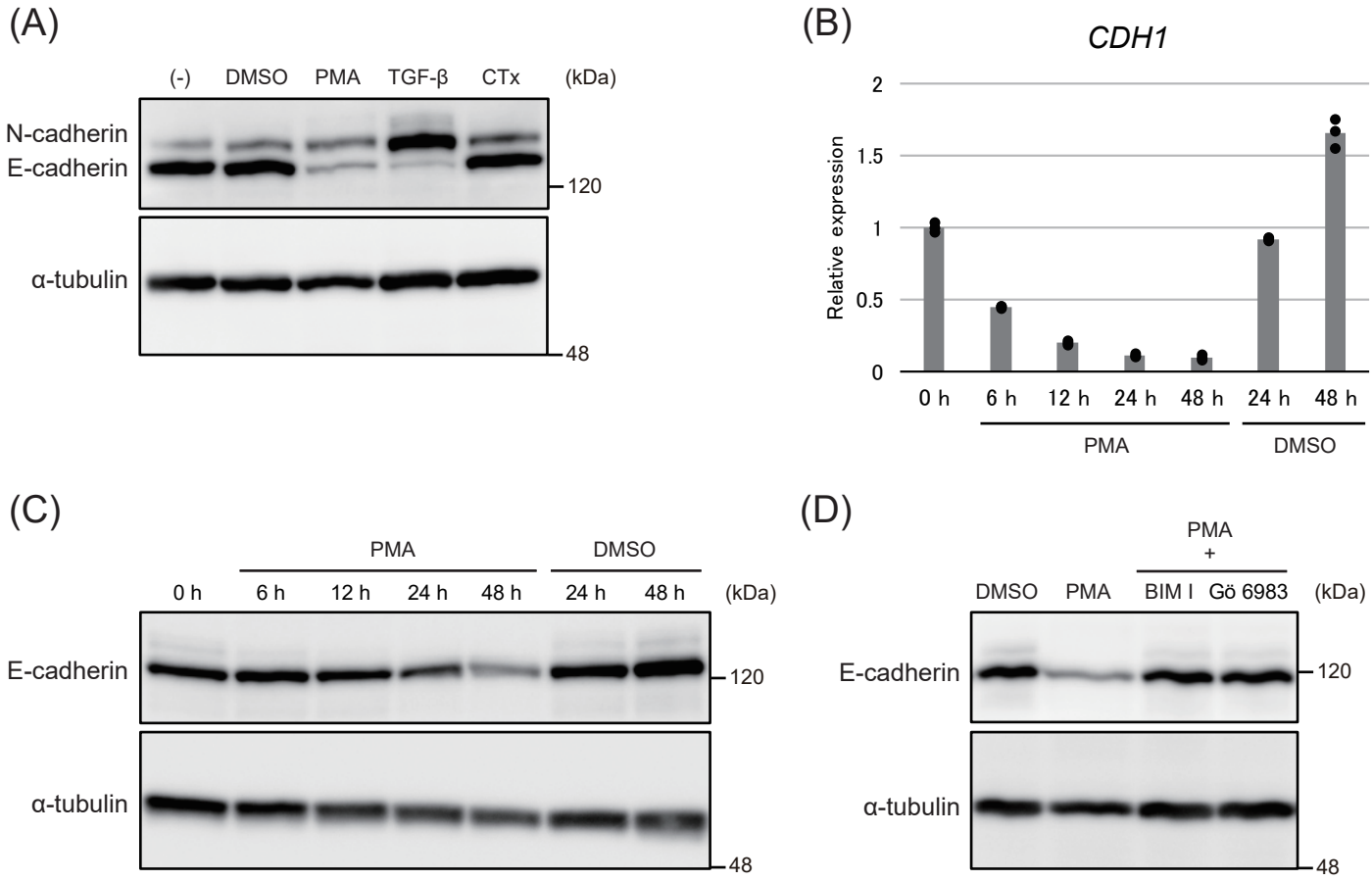

Figure S1. PKC signaling downregulates E-cadherin expression in A549 cells

(A) A549 cells were treated with PMA, TGF-β, and CTx (100 ng/ml) for 48 h. Expressions of E-cadherin and N-cadherin were analyzed by immunoblotting. (B, C) Time course of PMA-induced E-cadherin downregulation. A549 cells were treated with PMA for 6–48 h. (B) Expression of E-cadherin mRNA (*CDH1* mRNA) was analyzed by quantitative RT-PCR (triplicate determination). The relative expression level of each mRNA was normalized against the level of *HPRT1* mRNA. The value at 0 h is shown as "1." (C) Expression of E-cadherin protein was analyzed by immunoblotting. (D) A549 cells were treated with PMA with or without the PKC inhibitors BIM I and Gö6983 (1 μM). Expression of E-cadherin was analyzed by immunoblotting.

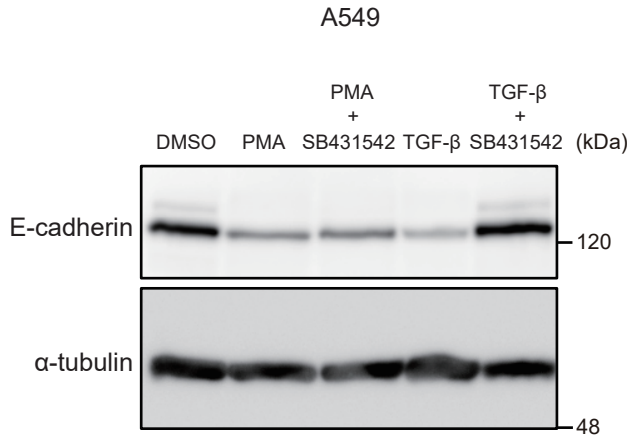

Figure S2. PMA-induced E-cadherin downregulation is not mediated by TGF-β signaling A549 cells were treated with PMA and TGF-β with or without SB431542 (10 μM) for 48 h. Expression of E-cadherin was analyzed by immunoblotting.

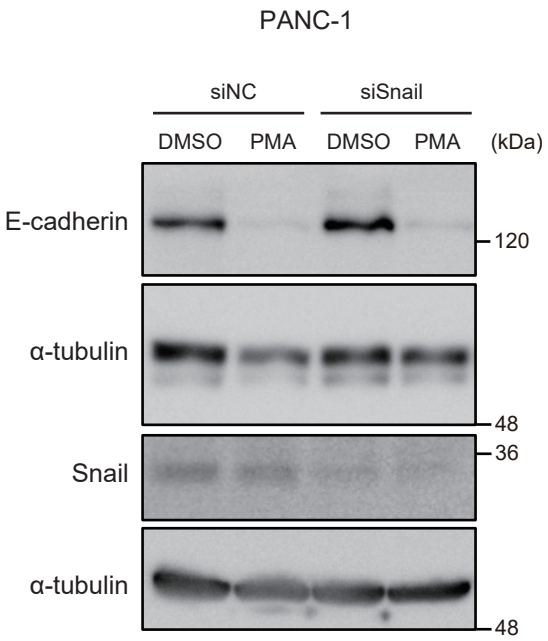

Figure S3. Snail is dispensable for E-cadherin downregulation by PKC signaling PANC-1 cells were transfected with siRNA against Snail or nonspecific control siRNA (NC). At 24 h after transfection, cells were treated with PMA (10 nM) for 48 h. Expressions of E-cadherin and Snail were analyzed by immunoblotting.

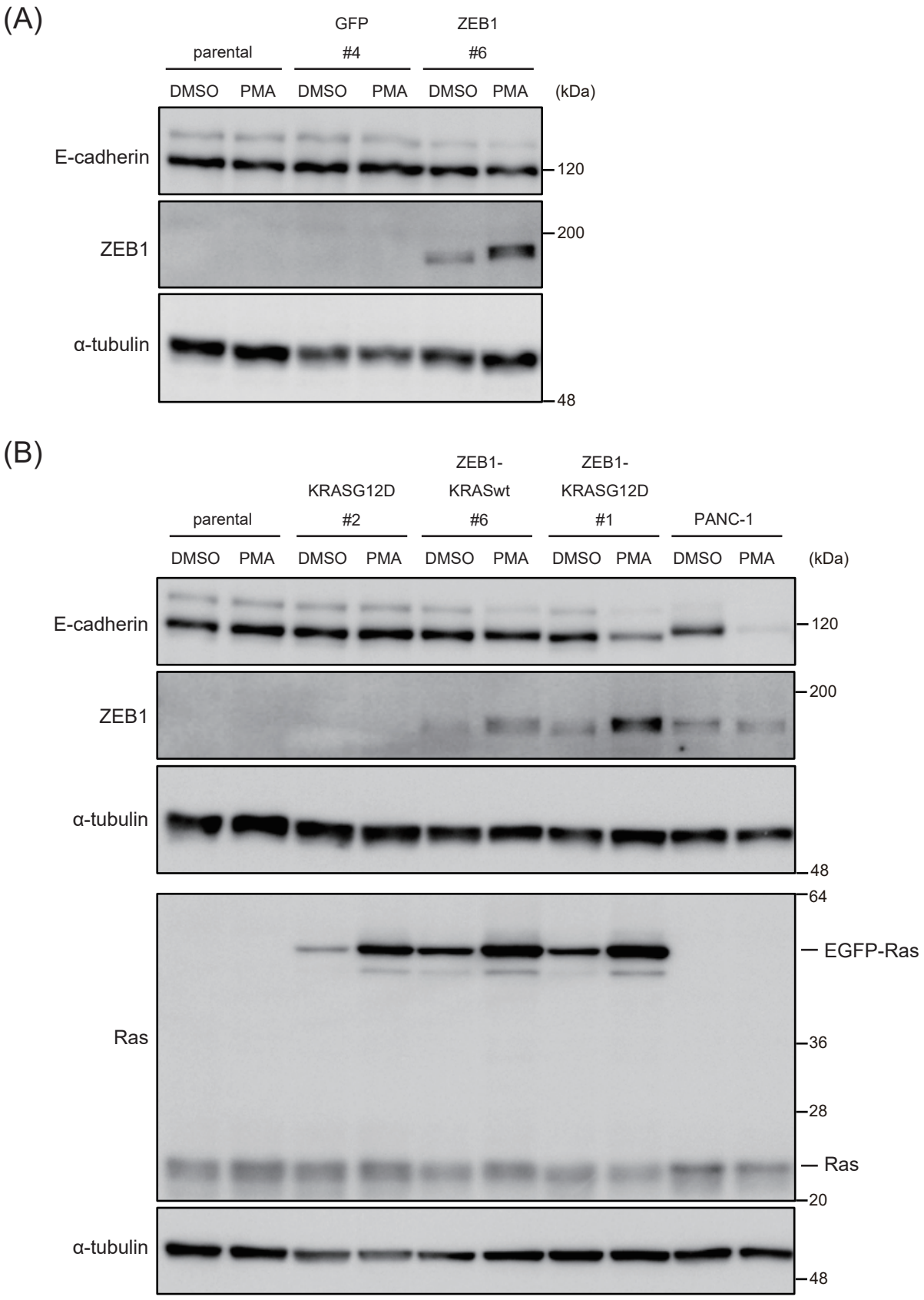

Figure S4. Ectopic expression of ZEB1 and oncogenic K-Ras permits E-cadherin downregulation by PKC in MDCK-I cells

(A) MDCK-I cells and those stably expressing ZEB1 (MDCK-ZEB1) or GFP (MDCK-GFP) were treated with PMA for 48 h. Expressions of E-cadherin and ZEB1 were analyzed by immunoblotting.

(B) MDCK-I cells and those stably expressing K-Ras (G12D) (MDCK-KRASG12D), ZEB1 and wild-type K-Ras (MDCK-ZEB1-KRASwt), or ZEB1 and K-Ras (G12D) (MDCK-ZEB1-KRASG12D) were treated with PMA (10 nM) for 48 h. PANC-1 cells were used as a control. Expressions of E-cadherin, ZEB1, and Ras were analyzed by immunoblotting.

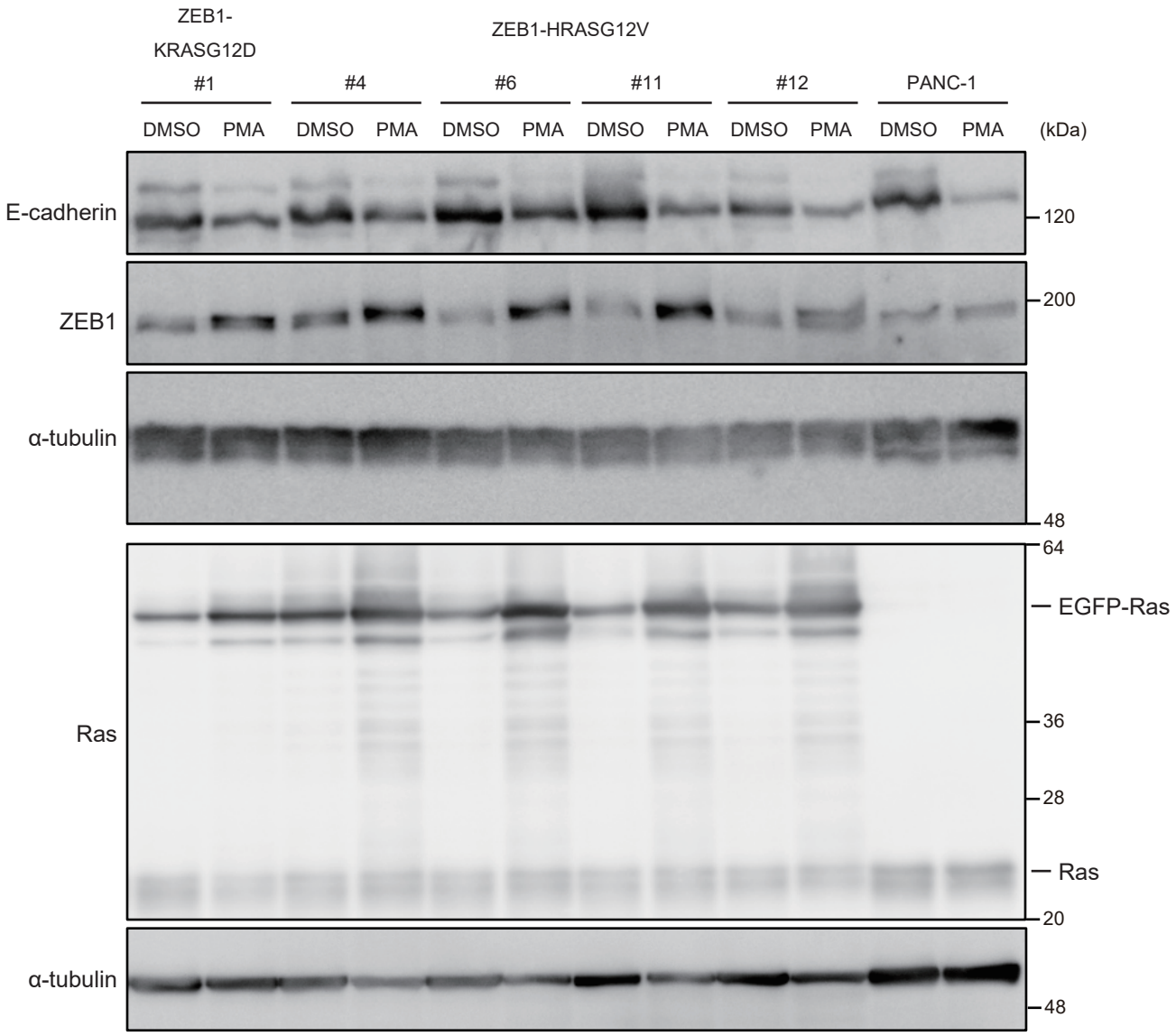

Figure S5. Ectopic expression of oncogenic H-Ras permits E-cadherin downregulation by PKC in MDCK-ZEB1 cells

MDCK-ZEB1 cells stably expressing H-Ras (G12V) (MDCK-ZEB1-HRASG12V) were treated with PMA (10 nM) for 48 h. MDCK-ZEB1-KRASG12D and PANC-1 cells were used as control. Expressions of E-cadherin, ZEB1, and Ras were analyzed by immunoblotting.

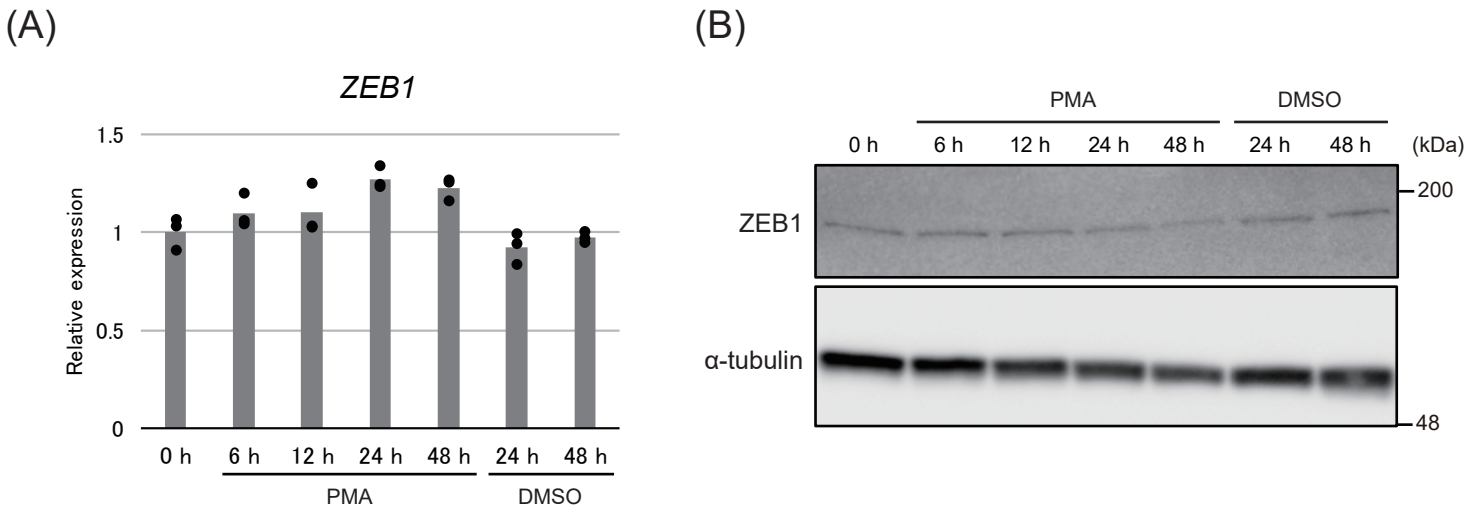

Figure S6. ZEB1 is not upregulated by PMA stimulation in A549 cells

(A, B) Time course of ZEB1 expression during PMA stimulation. A549 cells were treated with PMA for 6–48 h. (A) Expression of *ZEB1* mRNA was analyzed by quantitative RT-PCR (triplicate determination). The relative expression level of each mRNA was normalized against the level of *HPRT1* mRNA. The value at 0 h is shown as "1." (B) Expression of ZEB1 protein was analyzed by immunoblotting.

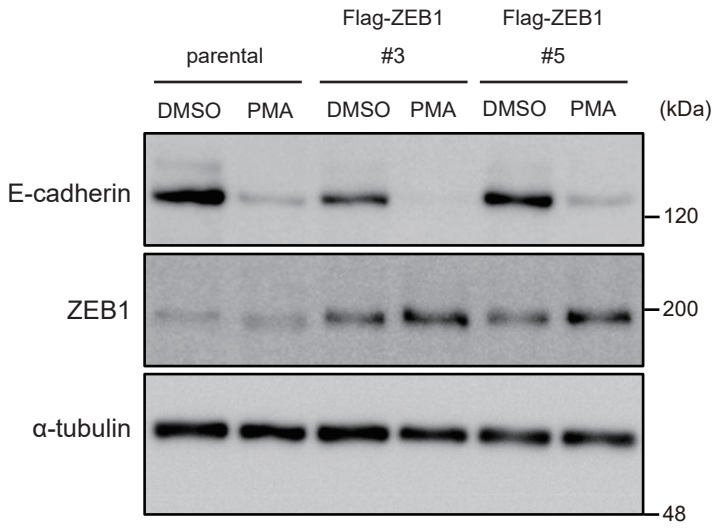

Figure S7. Overexpression of ZEB1 is not sufficient for E-cadherin downregulation PANC-1 cells and those stably expressing Flag-tagged ZEB1 were treated with PMA (10 nM) for 48 h. Expressions of E-cadherin and ZEB1 were analyzed by immunoblotting.

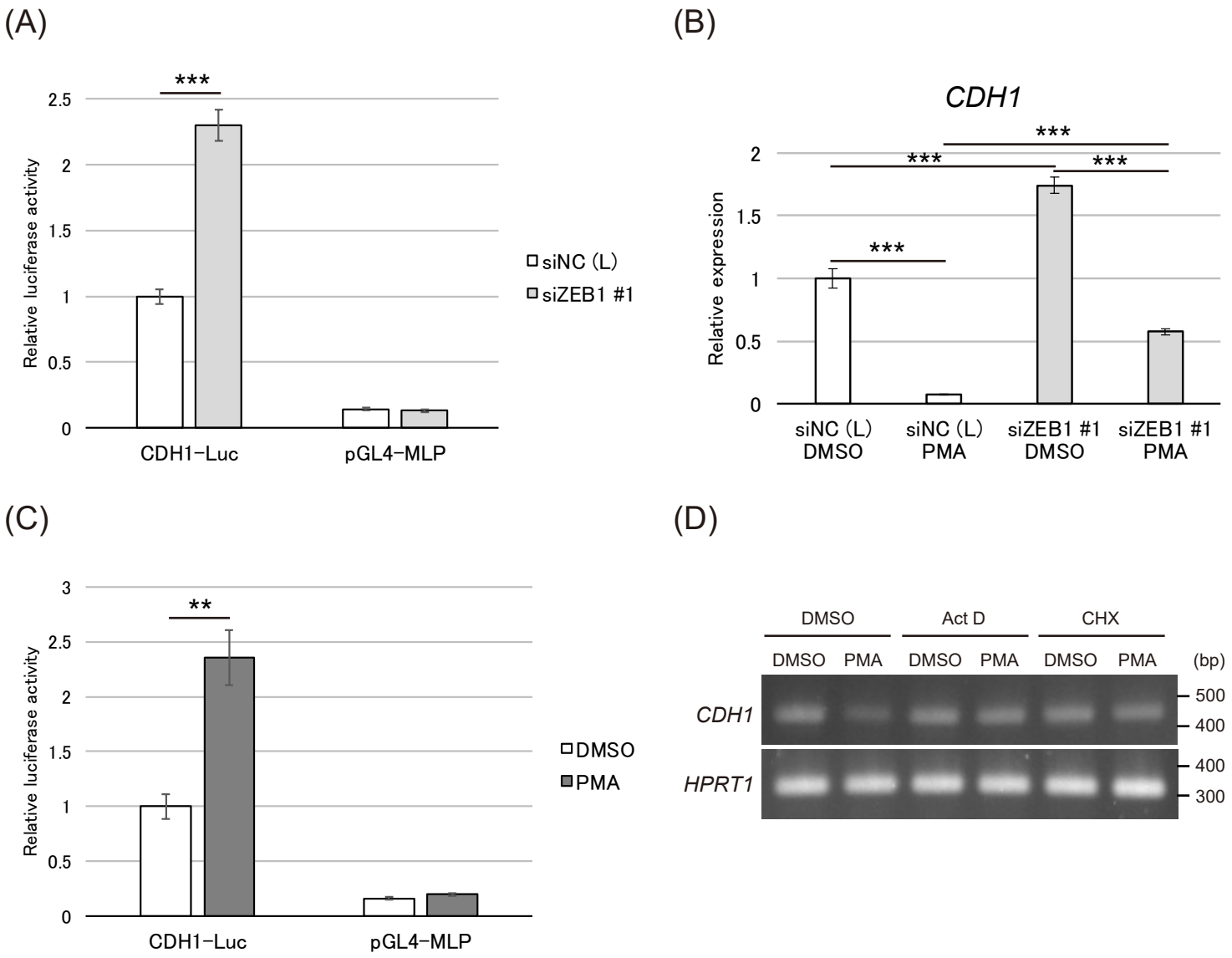

Figure S8. ZEB1-Ras regulatory switch downregulates E-cadherin expression through a novel mechanism in A549 cells

(A) A549 cells were transfected with siRNA against ZEB1 or nonspecific control siRNA (NC). After 24 h of siRNA transfection, cells were transfected with a *CDH1* promoter-reporter construct (CDH1-Luc) or control vector pGL4-MLP. Luciferase activity was measured at 27 h after reporter transfection. (B) A549 cells were transfected with siRNA against ZEB1 or nonspecific control siRNA (NC). After 24 h of transfection, cells were treated with PMA (10 nM) for 48 h. Expression of *CDH1* mRNA was analyzed by quantitative RT-PCR (tripricate determination). The value in DMSO control is shown as "1." (C) A549 cells were transfected with CDH1-Luc or pGL4-MLP. After 9 h of transfection, cells were treated with PMA (10 nM) for 18 h, and then luciferase activity was measured. (D) A549 cells were treated with PMA with or without actinomycin D (1 μg/ml) or cycloheximide (50 μg/ml) for 24 h. Expression of *CDH1* mRNA was analyzed by RT-PCR. Data are presented as mean ± SD. **, $p<0.01$; ***, $p<0.001$.

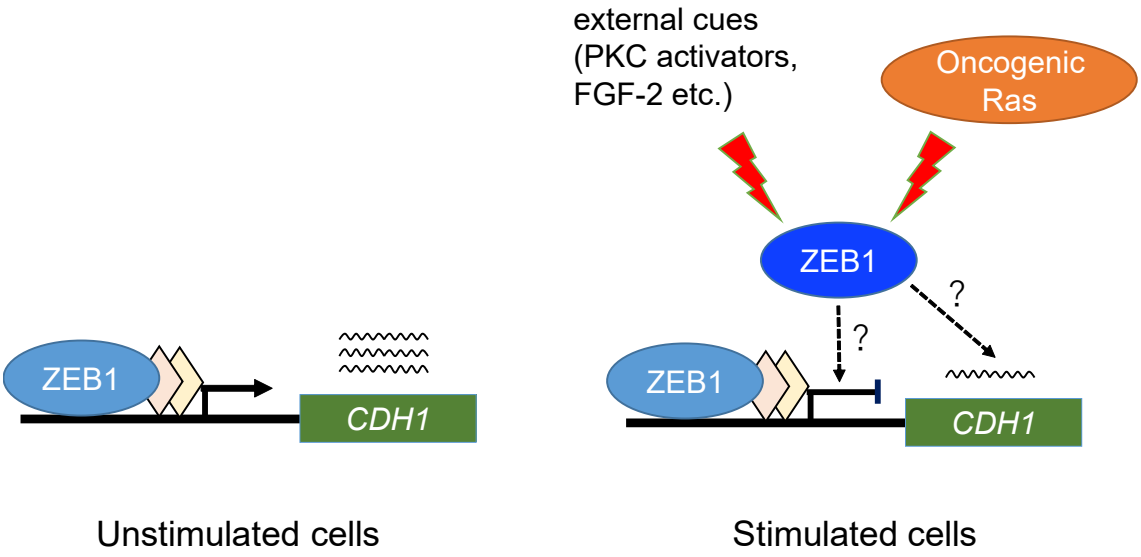

Figure S9. Model of functional modulation of ZEB1 by oncogenic Ras and external cues

In unstimulated cells, ZEB1 can repress E-cadherin expression moderately through its association with the *CDH1* promoter. When cells are stimulated by external cues including PKC activators and FGF-2, ZEB1 can extensively downregulate E-cadherin expression possibly by transcriptional repression through a genomic region distinct from the promoter or decrease in mRNA stability. How ZEB1 function is modulated by oncogenic Ras and external cues remains to be elucidated.