Supplemental Figure 1. Antigen-specific Nanog up-regulation in tumor cells under immune selection. Human cervical cancer cells from the CaSki line (designated N0, O0, or P0) were retrovirally transduced with the mouse major histocompatibility complex (MHC) class I molecule H2-D^b, pulsed with no peptide, H2-K^b-restricted Ova epitope, or H2-D^b-restricted E7 epitope from human papillomavirus type-16, and mixed with mouse E7-specific $CD8^+$ CTLs. Live tumor cells were recovered and subjected to serial selection 2 more times. Western blot was performed for analysis Nanog expression. β-actin was included as an internal loading control.

Supplemental Figure 2. MHC class I expression and CTL activation capacity by tumor cells are unaffected by Nanog expression. (A) Flow cytometry analysis of MHC class I expression on (H2-D^b) in CaSki-D^b cells transduced with empty vector or Nanog. (B) Empty vector- or Nanog-transduced CaSki-D^b cells pulsed with H2-D^b-restricted E7 epitope were incubated with E7-specific CTLs at a 1:1 effector:target ratio for 16 hours. Cells were then stained for surface CD8 and intracellular IFN-γ to detect CTL activation.

Supplemental Figure 3. Nanog expression in tumor cells does not control surface Fas ligand expression or survival of CTLs. (A) Flow cytometry analysis of surface FasL levels on P0 or P3 CaSki-D^b tumor cells with indicated Nanog expression status. Data are presented as relative mean fluorescence of cells stained with anti-FasL antibody. (B) Empty vector- or Nanog-transduced CaSki-D^b cells were incubated with E7-specific CTLs for 4 hours at a 1:1 effector:target ratio, and the percentage of apoptotic E7-specific CTLs was measured by active caspase-3 staining followed by flow cytometry.

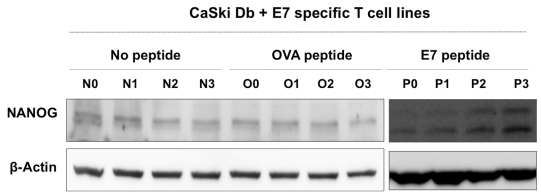
Supplemental Figure 4. Mutations in the CD2 region of the Nanog gene weaken the transcriptional function of its encoded protein. (**A**) Diagram of wild-type (WT) and mutant (MT) Nanog cDNA. The MT form contains 3 point mutations (E264G, E268G, and E272A) in the CD2 region of the Nanog gene. (**B**) Luciferase enzymatic assay of P0 cells transfected with WT or MT Nanog cDNA, together with a luciferase reporter under the control of 5 Nanog-binding elements (pGL3-5×NBE). (**C**) Flow cytometry analysis of the frequency of $Tcl1a^+$ cells among P0 cells transfected with WT or MT Nanog cDNA.

Supplemental Figure 5. HCT116 human colon cancer cells transduced with a single chain trimer of H2-D^b heavy chain linked to β2-microglobulin and the H2-D^b-restricted epitope of E7 (HCT116/SCT-E7) are susceptible to lysis by mouse E7-specific CTLs. (**A**) Diagram of the SCT-E7 construct. (**B**) Western blot analysis of β2-microglobulin expression in HCT116 or HCT116/SCT-E7 cells. β-actin was included as an internal loading control. (**C** and **D**) Flow cytometry analysis of the frequency of apoptotic (active caspase-3^+) cells in HCT116/SCT-E7 cells mixed with E7-specific cytotoxic T lymphocytes at the indicated ratios for 4 hours. (**C**) Representative histograms (grey region: isotype control staining; black line: anti-active capase-3 staining). (**D**) Bar graph summary.

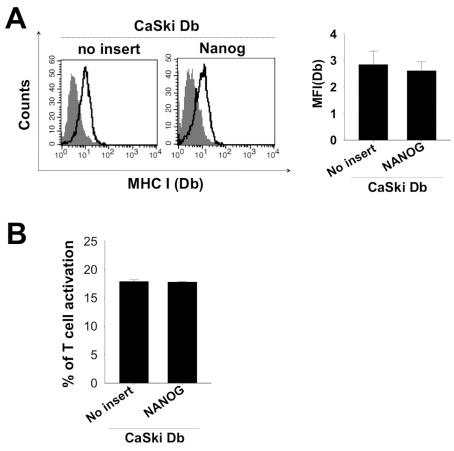
Supplemental Figure 6. The Nanog-Tcl1-Akt axis dictates immune escape and tumorigenicity of tumor cells after immune selection. CaSki-D^b P3 cells were transfected with siRNA targeting GFP (siGFP), Akt (siAkt), Tcl1a (siTcl1), or Nanog (siNanog). (A) Western blot analysis of Akt, Tcl1, and Nanog expression in CaSki-D^b P3 cells transfected

with indicated siRNA constructs. β-actin was used as an internal control. (B) Flow cytometry analysis of frequency of apoptotic tumor cells among siRNA-transfected CaSki-D^b P3 cells mixed with E7-specific $CD8^+$ T cells at a 1:1 ratio for 4 hours. (C) Sphere-forming capacity of siRNA-transfected CaSki-D^b P3 in low-density suspension culture. (D) Tumorigenicity of siRNA-transfected CaSki-D^b P3 cells in NOD/SCID mice. (E) Tumor weight of mice from experiment in (D), measured on day 21 (mean ± SD).

Supplemental Figure 7. Inhibition of the Nanog-Tcl1a-Akt axis renders the tumor vulnerable to immune-mediated control. NOD/SCID mice were injected subcutaneously with 10^6 HCT116/SCT-E7 cells and intravenously administered with chitosan nanoparticles loaded with siRNA targeting GFP (siGFP), Akt (siAkt), Tcl1a (siTcl1), or Nanog (siNanog) after 6, 8, and 10 days. Mice received adoptive transfer of CFSE-labeled E7-specific $CD8^+$ T cells 11 days after tumor inoculation. (A) Tumor volume over time (mean ± SD). (B) Tumor weight 15 days after tumor challenge (top: representative photographs; bottom: bar graph summary). (C) Western blot analysis of Akt, Tcl1a, and Nanog expression in tumor tissue 15 days after tumor challenge. (D) Proliferation of tumor cells derived from tumor-bearing mice treated as indicated. Ki67 was used as a marker of dividing cells. (E) Infiltration of E7-specific CTLs into the tumor after adoptive transfer. (F) Flow cytometry analysis of the frequency of apoptotic tumor cells in tumor-bearing mice treated as indicated.



Suppl. Fig. 1. Noh *et al*



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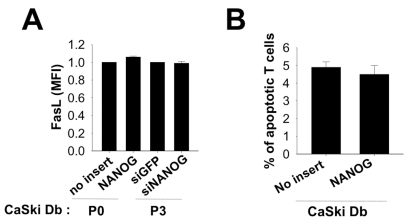

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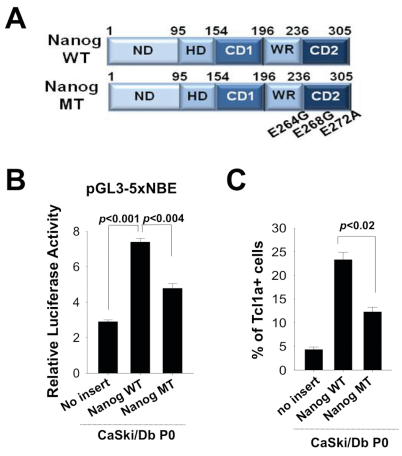

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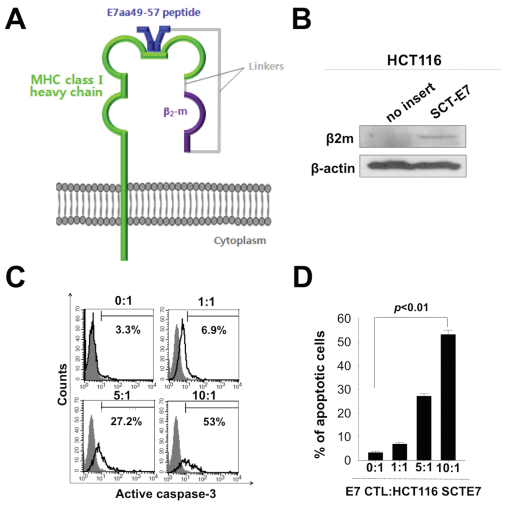

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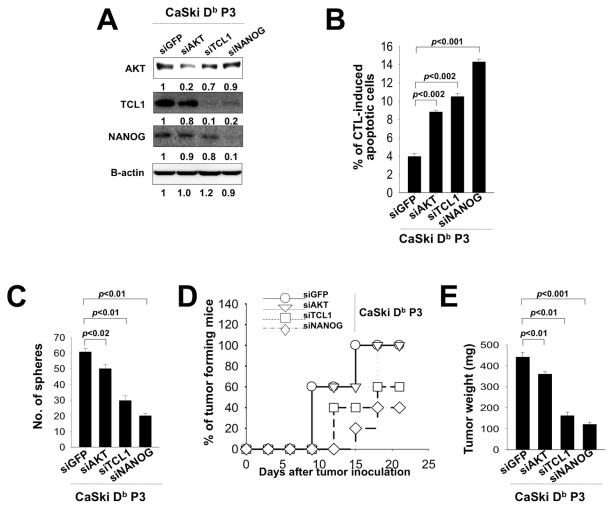
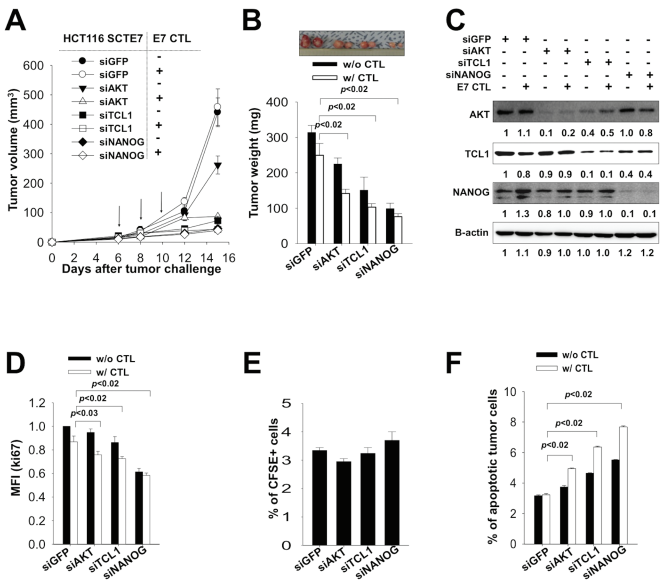


Suppl. Fig. 6. Noh *et al*



Suppl. Fig. 7. Noh *et al*

Supplemental Table 1. Association between Sox2 and Nanog, Tcl1a or pAkt expression in CIN and CIS

	Sox2 expression					
	Low	*%*	High	*%*	N	*p* value
Nanog expression					**151**	***0.007***
Low	25	*27.8*	65	*72.2*	90	
High	6	*9.8*	55	*90.2*	61	
Tcl1a expression					**198**	***0.281***
Low	7	*30.4*	16	*69.6*	23	
High	36	*20.6*	139	*79.4*	175	
pAkt expression					**230**	***0.336***
Low	37	*24.0*	117	*76.0*	154	
High	14	*18.4*	62	*81.6*	76	

Supplemental Table 2. Clinicopathologic significance of Nanog, Tcl1a and pAkt protein expression in human cervical neoplasias

	Nanog		Tcl1a		pAkt	
	No.	Mean (95% CI)	**No.**	**Mean (95% CI)**	**No.**	**Mean (95% CI)**
All study subjects	770	6.0 (5.6-6.3)	**723**	**10.0 (9.7-10.3)**	**812**	**7.8 (7.4-8.1)**
Diagnostic category						
Normal	339	3.9 (3.5-4.3)	**327**	**8.2 (7.8-8.5)**	**342**	**4.8 (4.5-5.1)**
Low grade CIN	68	4.0 (3.1-4.9)	**58**	**10.4 (9.4-11.4)**	**89**	**6.7 (5.9-7.5)**
High grade CIN+CIS	193	7.4 (6.7-8.1)	**184**	**12.3 (11.8-12.9)**	**203**	**9.5 (8.9-10.1)**
Cervical cancer	170	9.3 (8.6-10.0)	**154**	**10.9 (10.2-11.6)**	**178**	**12.0 (11.4-12.6)**
Total	770	6.0 (5.6-6.3)	**723**	**10.0 (9.7-10.3)**	**812**	**7.8 (7.4-8.1)**
p value		*p < 0.001*		***p < 0.001***		***p < 0.001***
FIGO stage						
I	117	9.5 (8.7-10.2)	**109**	**11.4 (10.6-12.2)**	**116**	**11.8 (11.0-12.5)**
II	47	8.7 (7.2-10.2)	**40**	**9.7 (8.3-11.1)**	**53**	**12.5 (11.3-13.7)**
IV	6	11.1 (4.8-17.3)	**5**	**10.1 (7.0-13.1)**	**9**	**12.4 (8.4-16.4)**
Total	170	9.3 (8.6-10.0)	**154**	**10.9 (10.2-11.6)**	**178**	**12.0 (11.4-12.6)**
p value		*p =0.383*		***p = 0.089***		***p = 0.564***
Tumor differentiation						
Well + Moderate	123	9.7 (8.9-10.4)	**100**	**10.8 (9.9-11.6)**	**107**	**11.5 (10.7-12.3)**
Poor	43	8.3 (6.9-9.7)	**50**	**11.0 (9.8-12.2)**	**59**	**13.0 (11.8-14.2)**
Total	166	9.3 (8.6-10.0)	**150**	**10.8 (10.2-11.5)**	**166**	**12.0 (11.3-12.7)**
p value		*p = 0.088*		***p = 0.741***		***p = 0.031***
Cell Type						
Squamous cell carcinoma	136	9.3 (8.5-10.1)	**126**	**11.0 (10.3-11.8)**	**145**	**11.8 (11.1-12.4)**
Other	33	9.1 (8.6-10.0)	**28**	**10.3 (8.7-11.9)**	**33**	**13.1 (11.4-14.8)**
Total	169	9.3 (8.6-10.0)	**154**	**10.9 (10.2-11.6)**	**178**	**12.0 (11.4-12.6)**
p value		*p = 0.843*		***p = 0.412***		***p = 0.093***
Tumor size						
< 4cm	122	9.2 (8.4-10.1)	**112**	**11.2 (10.4-12.0)**	**124**	**12.1 (11.3-12.8)**
≥ 4cm	48	9.5 (8.2-10.8)	**42**	**10.0 (8.7-11.3)**	**54**	**11.9 (10.7-13.1)**
Total	170	9.3 (8.6-10.0)	**154**	**10.9 (10.2-11.6)**	**178**	**12.0 (11.4-12.6)**
p value		*p = 0.769*		***p = 0.116***		***p = 0.812***
Lymphovascular invasion						
Negative	88	9.2 (8.3-10.1)	**81**	**10.9 (9.9-11.9)**	**92**	**12.3 (11.5-13.2)**
Positive	66	9.0 (8.0-10.1)	**61**	**10.8 (9.7-11.9)**	**66**	**11.6 (10.5-12.8)**
Total	154	9.1 (8.4-9.8)	**142**	**10.9 (10.1-11.6)**	**158**	**12.0 (11.4-12.7)**
p value		*p = 0.837*		***p = 0.880***		***p = 0.316***
Lymph node metastasis						
Negative	117	9.0 (8.3-9.8)	**107**	**11.1 (10.3-11.9)**	**121**	**11.9 (11.1-12.6)**
Positive	39	9.6 (8.0-11.1)	**36**	**10.1 (8.7-11.6)**	**39**	**12.7 (11.2-14.2)**
Total	156	9.2 (8.5-9.9)	**143**	**10.9 (10.2-11.6)**	**160**	**12.1 (11.4-12.7)**
p value		*p = 0.507*		***p = 0.246***		***p = 0.292***
Chemoradiation response						
Good	31	8.5 (6.8-10.2)	**27**	**10.0 (8.3-11.7)**	**32**	**11.8 (10.2-13.4)**
Bad	10	12.5 (10.0-14.9)	**7**	**6.9 (2.1-11.6)**	**11**	**12.7 (11.4-13.9)**
Total	41	9.51 (8.0-10.9)	**34**	**9.4 (7.8-10.9)**	**43**	**12.0 (10.8-13.2)**
p value		*p = 0.013*		***p = 0.104***		***p = 0.537***

CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; FIGO, International Federation of Gynecology and Obstetrics.