Supplemental data

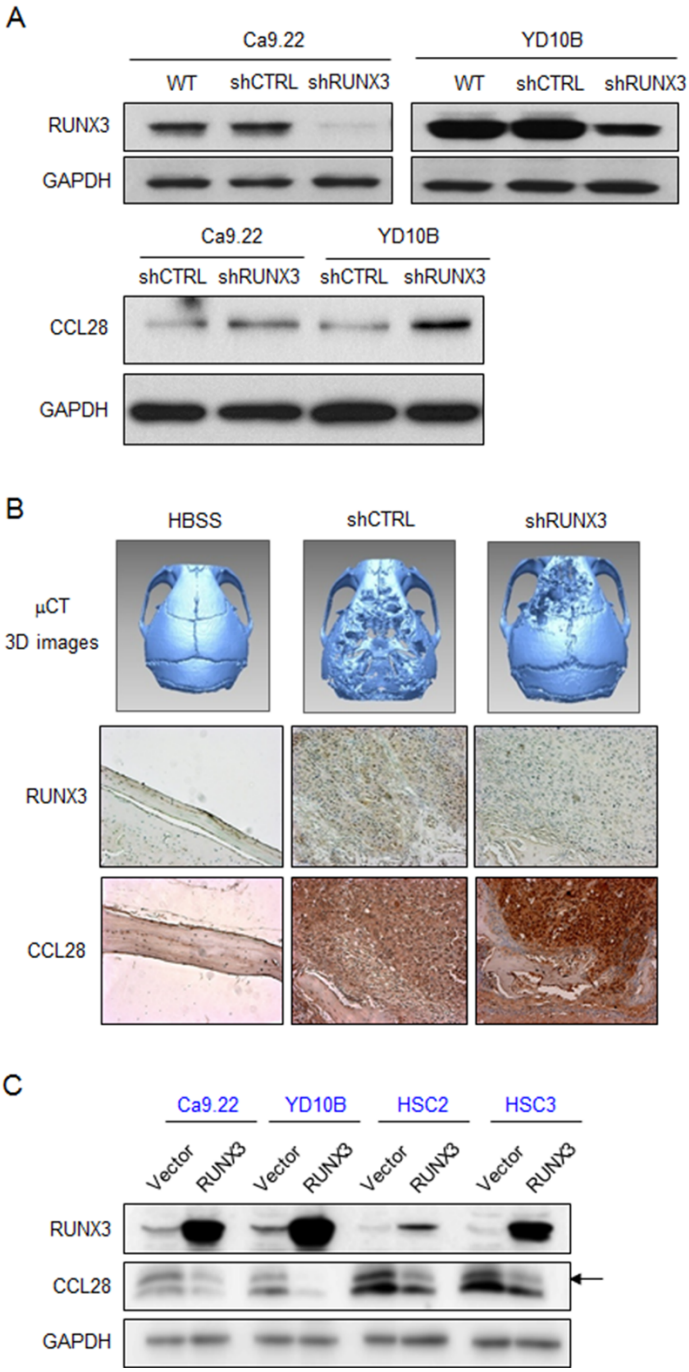

Figure S1. CCL28 expression is increased by RUNX3 knockdown. (**A**) Increased CCL28 levels in RUNX3 knockdown Ca9.22 and YD10B cells. Representative Western blot images. (**B**) Increased CCL28 levels in tumor tissues of RUNX3 knockdown Ca9.22 cell-injected mice (n = 11) obtained from our previous study (27). Representative images of IHC staining for RUNX3 and CCL28. (**C**) Reduced CCL28 levels in RUNX3-overexpressing Ca9.22 or YD10B cells and RUNX3-expressing HSC-2 or HSC-3 cells. Representative Western blot images.

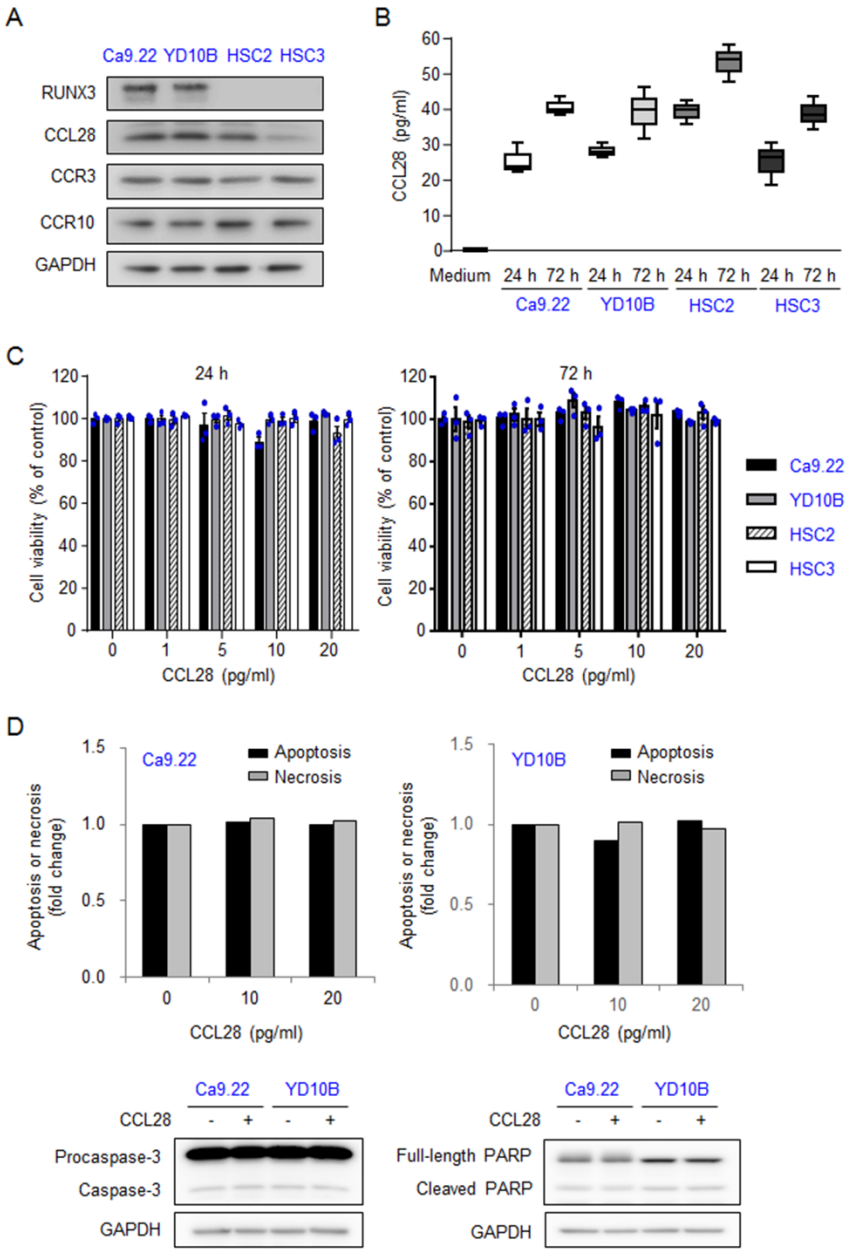

Figure S2. CCL28 is produced in 4 OSCC cell lines but does not affect the viability and death of OSCC cells. (**A**) CCL28, CCR3, and CCR10 expression levels in RUNX3-expressing Ca9.22 and YD10B cells and RUNX3-nonexpressing HSC2 and HSC3 cells. Representative Western blot images. (**B**) Levels of CCL28 secreted by 4 different OSCC cell lines (mean ± SEM, n = 3). (**C**) Viability of 4 OSCC cell lines treated with CCL28 (mean ± SEM, n = 3). (**D**) Induction of apoptosis in Ca9.22 cells and YD10B cells treated with CCL28 for 24 h (mean, n = 2). Apoptotic and necrotic cell death was measured using the Cell Death Detection ELISA Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instruction. Levels of procaspase-3, caspase-3, and full-length and cleaved PARPs in Ca9.22 cells and YD10B cells treated with CCL28 (20 pg/ml) for 24 h. Representative Western blot images.

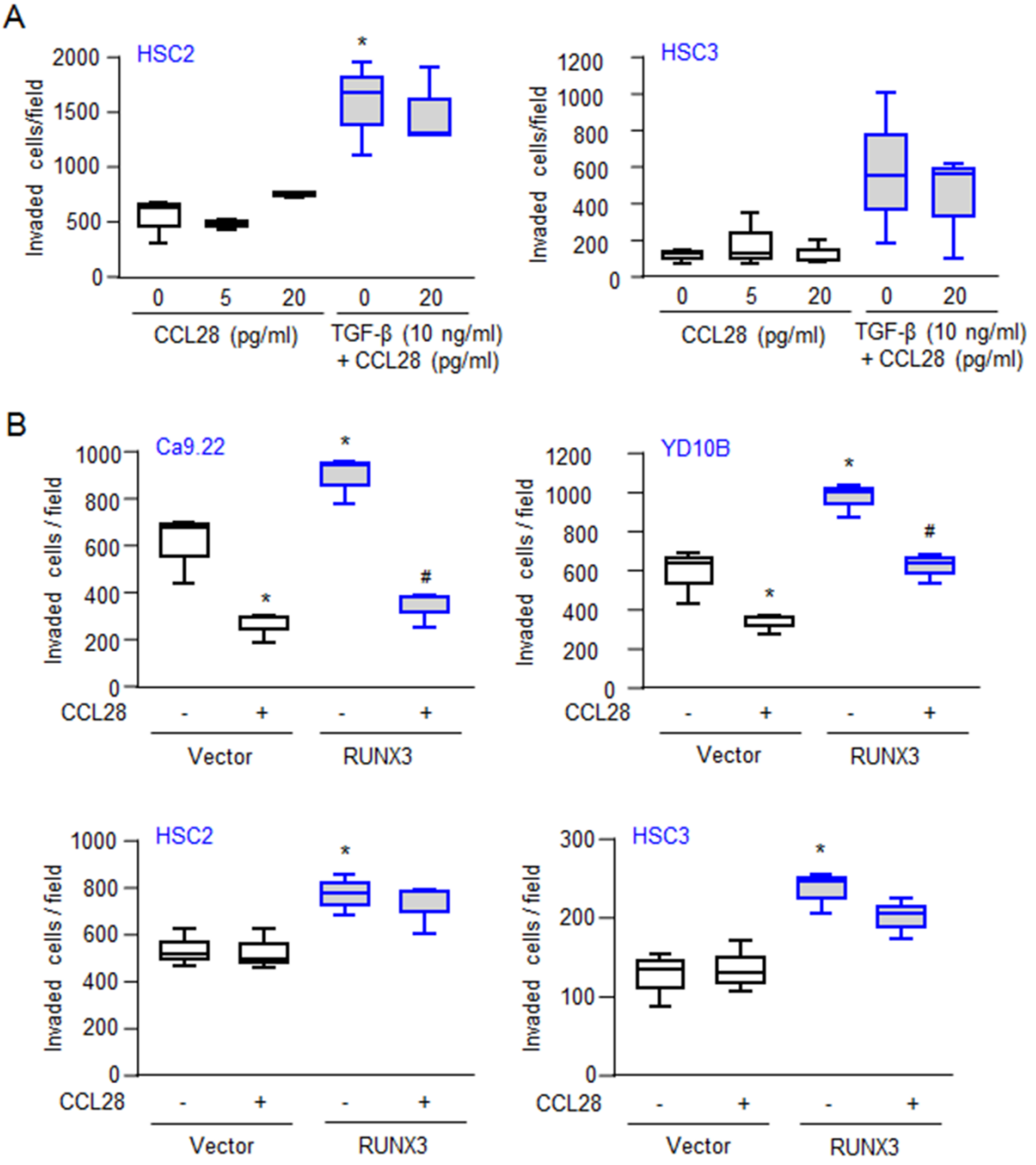

Figure S3. Increased RUNX3 expression promotes invasion of OSCC cells, but CCL28 inhibits invasion of RUNX3-overexpressing Ca9.22 and YD10B OSCC cells. (**A**) Invasion of RUNX3-nonexpressing HSC2 or HSC3 cells treated with CCL28 and/or TGF-β (mean ± SEM, $n = 3$). $^{*}P < 0.05$ versus cells without CCL28 and TGF-β by one-way ANOVA with multiple comparisons test. (B) Invasion of OSCC cells with increased RUNX3 expression in the presence of CCL28 (20 pg/ml) (mean ± SEM, $n = 3$). $^{*}P < 0.05$ versus OSCC/vector cells; $^{\#}P < 0.05$ versus cells with increased RUNX3 expression by one-way ANOVA with multiple comparisons test.

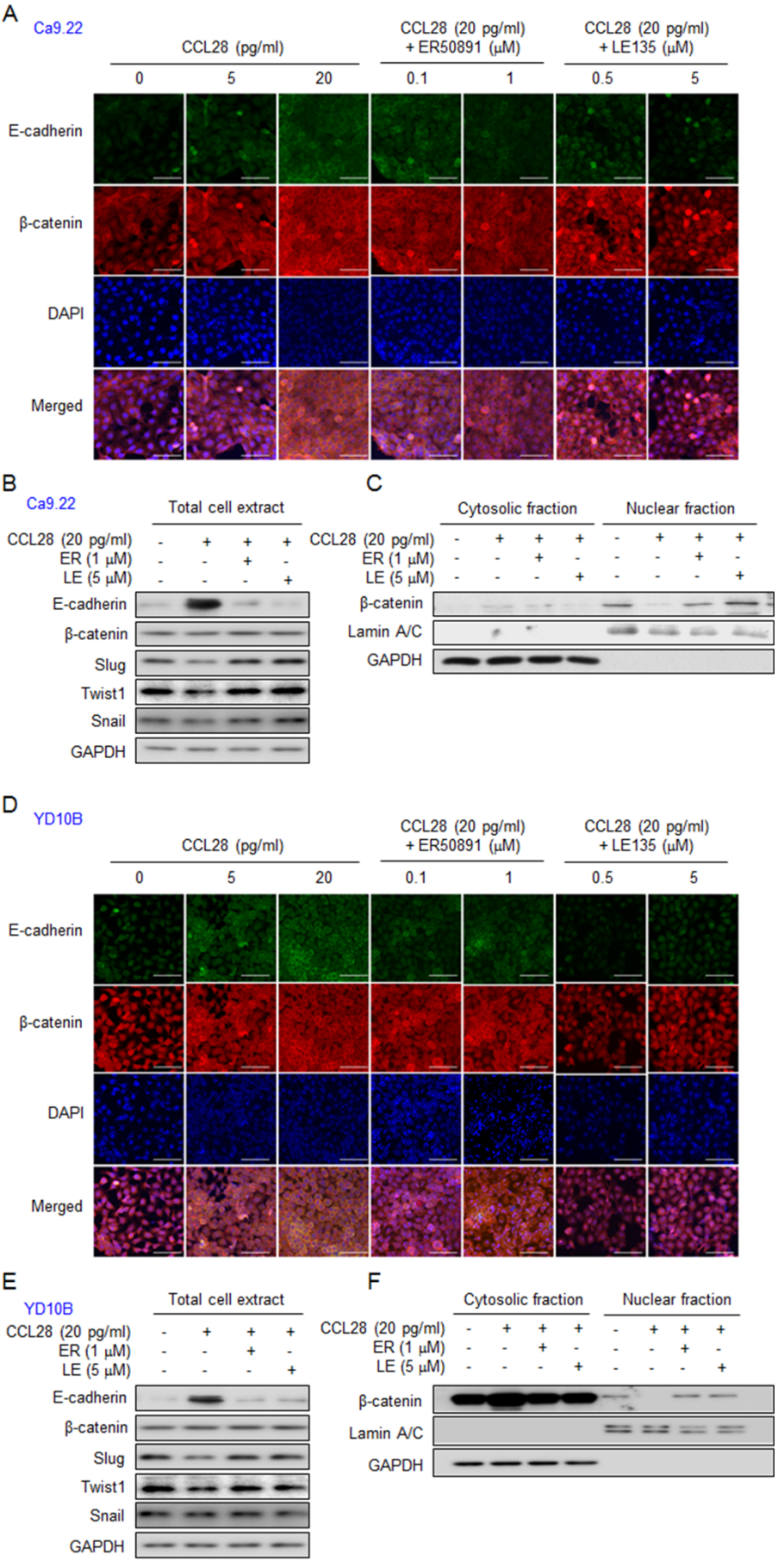
A
Ca9.22
CCL28 (pg/ml)
0
5
20
CCL28 (20 pg/ml) + ER50891 (μM)
0.1
1
CCL28 (20 pg/ml) + LE135 (μM)
0.5
5
E-cadherin
β-catenin
DAPI
Merged
B
Ca9.22
Total cell extract
CCL28 (20 pg/ml)
ER (1 μM)
LE (5 μM)
E-cadherin
β-catenin
Slug
Twist1
Snail
GAPDH
C
Cytosolic fraction
Nuclear fraction
CCL28 (20 pg/ml)
ER (1 μM)
LE (5 μM)
β-catenin
Lamin A/C
GAPDH
D
YD10B
CCL28 (pg/ml)
0
5
20
CCL28 (20 pg/ml) + ER50891 (μM)
0.1
1
CCL28 (20 pg/ml) + LE135 (μM)
0.5
5
E-cadherin
β-catenin
DAPI
Merged
E
YD10B
Total cell extract
CCL28 (20 pg/ml)
ER (1 μM)
LE (5 μM)
E-cadherin
β-catenin
Slug
Twist1
Snail
GAPDH
F
Cytosolic fraction
Nuclear fraction
CCL28 (20 pg/ml)
ER (1 μM)
LE (5 μM)
β-catenin
Lamin A/C
GAPDH

Figure S4. Selective RARα antagonist ER50891 and RARβ antagonist LE135 block the upregulation of E-cadherin and restore the expression levels of EMT-related transcription factors and nuclear β-catenin levels in CCL28-treated OSCC cells. (**A** and **D**) Expression levels and cellular localization of E-cadherin and β-catenin in Ca9.22 and YD10B OSCC cells treated with RARα or RARβ antagonist in the presence of CCL28. Representative immunofluorescence images of cells at ×100 magnification. Scale bar, 50 μm. (**B** and **E**) Expression levels of E-cadherin, β-catenin, and EMT-related transcription factors in Ca9.22 and YD10B OSCC cells treated with RARα or RARβ antagonist in the presence of CCL28. (**C** and **F**) Cytosolic and nuclear β-catenin levels in Ca9.22 and YD10B OSCC cells treated with RARα or RARβ antagonist in the presence of CCL28. (**B**, **C**, **E**, and **F**) Representative Western blot images.

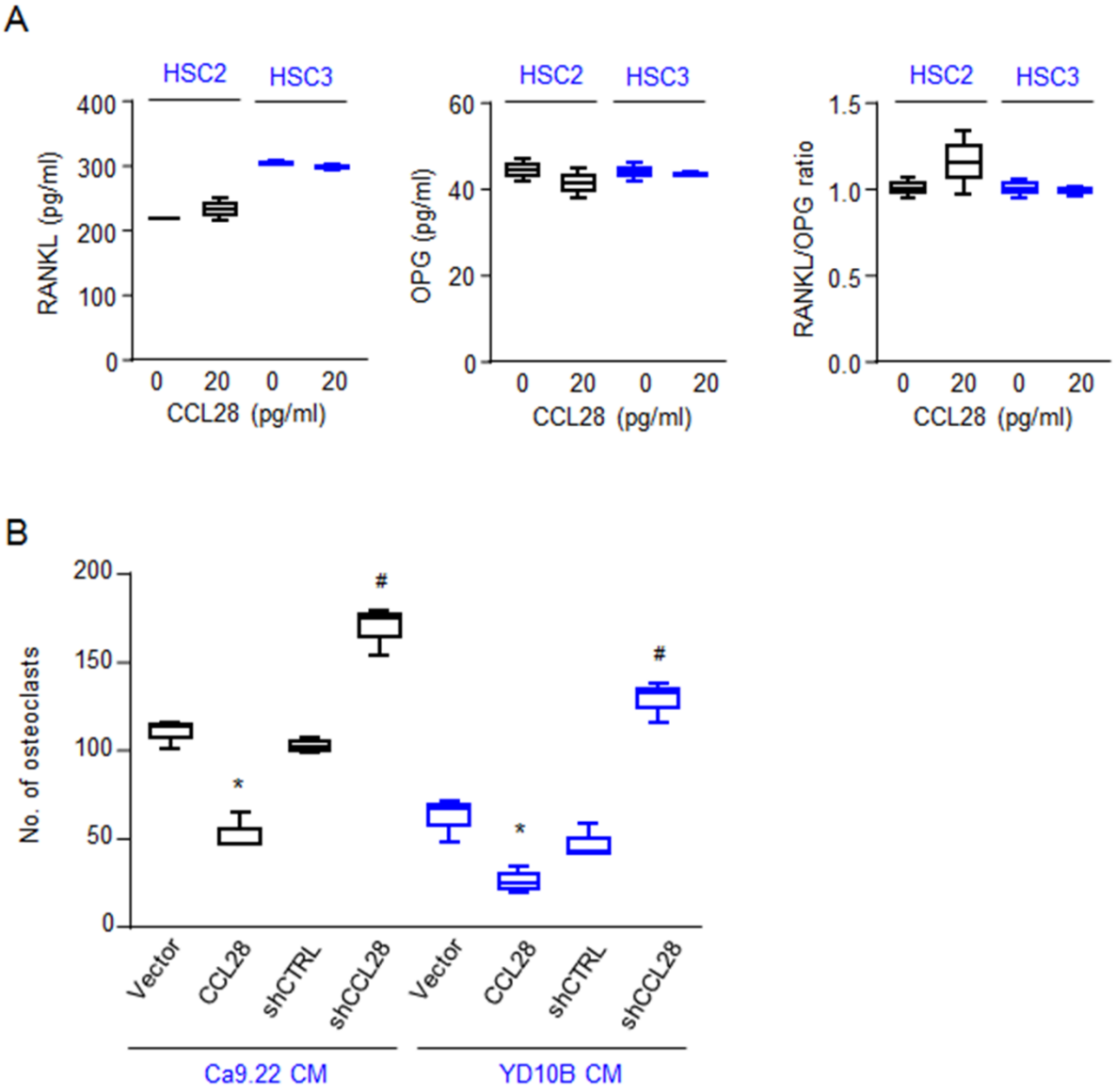

Figure S5. CCL28 treatment does not affect the RANKL/OPG ratio in HSC-2 and HSC-3 cells, and increased CCL28 expression in Ca9.22 and YD10B cells inhibits RANKL-induced osteoclastogenesis. (**A**) RANKL and OPG levels secreted by CCL28-treated HSC-2 and HSC-3 cells into the culture media, and the RANKL/OPG ratio (mean ± SEM, $n = 3$). (**B**) Osteoclast formation in BMMs treated with RANKL and culture media of CCL28-overexpressing or CCL28-knockdown cells (mean ± SEM, $n = 3$). $^{*}P < 0.05$ versus OSCC/vector cells; $^{\#}P < 0.05$ versus OSCC cells with control shRNA by two-tailed Student's *t* test.

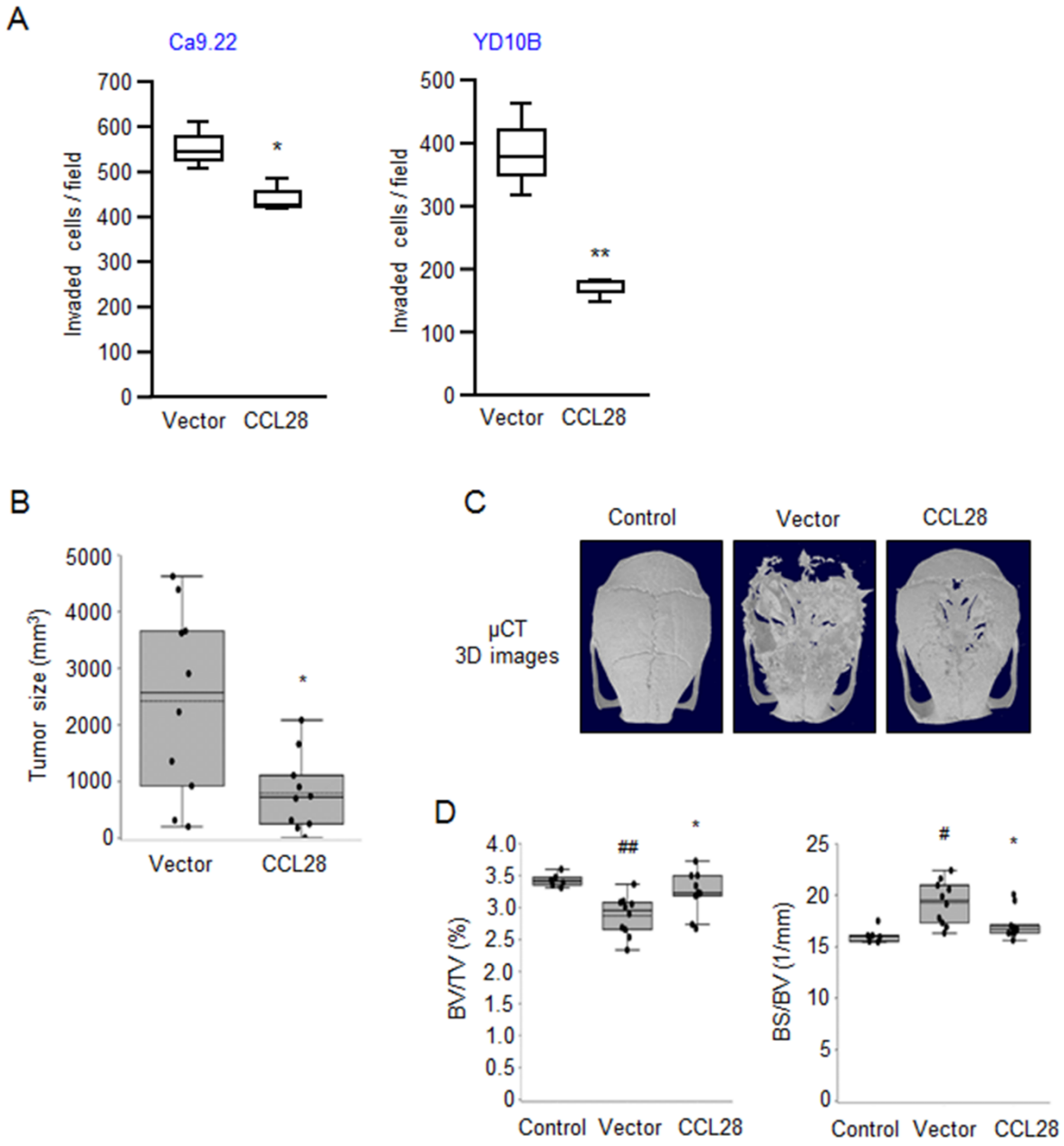

Figure S6. CCL28 overexpression inhibits the invasion of OSCC cells and in vivo tumor growth and osteolysis. (**A**) Invasion of CCL28-overexpressing Ca9.22 and YD10B OSCC cells (mean ± SEM, $n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus OSCC/vector cells by two-tailed Student's t test. (**B-D**) Ca9.22 cells with empty vector or CCL28-overexpressing OSCC cells were inoculated subcutaneously in the mouse calvaria ($n = 6$ for control and $n = 10$ for experimental groups). (**B**) Tumor size (mean ± SEM). $^{*}P <$ 0.01 versus OSCC/vector cell-inoculated mice by two-tailed Student's t test. (**C**) Representative μCT 3D images of calvarial osteolytic lesions. (**D**) Bone morphometric parameters, BV/TV and BS/TV (mean ± SEM). $^{\#}P < 0.005$, $^{\#\#}P < 0.001$ versus control mice; $^{*}P < 0.05$ versus OSCC/vector cell-inoculated mice by one-way ANOVA with multiple comparisons test.

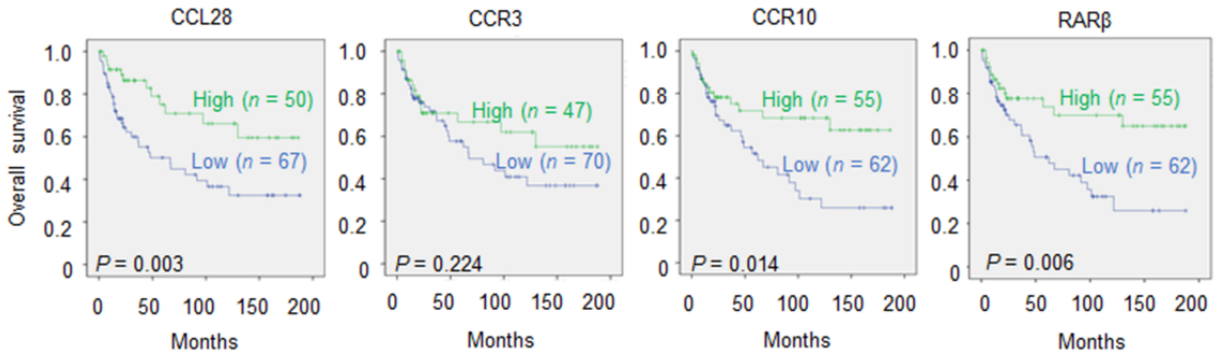

Figure S7. Kaplan-Meier survival curve of OSCC patients stratified based on CCL28, CCR3, CCR10 or RARβ expression by the log-rank test. CCL28, CCR3, CCR10, or RARβ expression was categorized as low or high according to the median value of histoscore. The histoscore median value for CCL28 was 40 (range, 0-240); for CCR3 0 (0-210); CCR10 110 (0-270); RARβ 90 (0-270).

Table S1. Alteration in the gene expression of chemokines and their receptors by RUNX3 knockdown in Ca9.22 OSCC cells

Well	Gene	Fold change	Well	Gene	Fold change
A01	*C5*	2.62*	D10	*CX3CL1*	-7.90
A02	*C5AR1*	1.19	D11	*CX3CR1*	1.17
A03	*CCBP2*	1.79	D12	*CXCL1*	1.31
A04	*CCL1*	-1.20	E01	*CXCL10*	-1.30
A05	*CCL11*	1.34	E02	*CXCL11*	1.28
A06	*CCL13*	1.27	E03	*CXCL12*	1.68
A07	*CCL14*	-1.35	E04	*CXCL13*	1.17
A08	*CCL15*	1.45	E05	*CXCL14*	1.12
A09	*CCL16*	2.09	E06	*CXCL16*	1.67
A10	*CCL17*	-2.04	E07	*CXCL2*	-1.02
A11	*CCL18*	2.07	E08	*CXCL3*	1.00
A12	*CCL19*	2.63	E09	*CXCL5*	-1.05
B01	*CCL2*	1.29	E10	*CXCL6*	1.14
B02	*CCL20*	-1.19	E11	*CXCL9*	1.23
B03	*CCL21*	2.21	E12	*CXCR1*	1.74
B04	*CCL22*	4.06	F01	*CXCR2*	-1.31
B05	*CCL23*	1.68	F02	*CXCR3*	-1.39
B06	*CCL24*	2.93	F03	*CXCR4*	1.40
B07	*CCL25*	-1.70	F04	*CXCR5*	2.02
B08	*CCL26*	-1.28	F05	*CXCR6*	1.77
B09	*CCL27*	1.45	F06	*CXCR7*	-1.29
B10	***CCL28***	4.99*	F07	*DARC*	1.57
B11	*CCL3*	1.86	F08	*FPR1*	1.63
B12	*CCL4*	1.27	F09	*GPR17*	1.82
C01	*CCL5*	3.15	F10	*HIF1A*	1.20
C02	*CCL7*	-1.00	F11	*IL16*	1.05
C03	*CCL8*	1.36	F12	*IL1B*	-1.32
C04	*CCR1*	1.41	G01	*IL4*	1.27
C05	*CCR10*	2.02	G02	*IL8*	1.14
C06	*CCR2*	1.46	G03	*PF4V1*	1.54
C07	*CCR3*	-3.29	G04	*PPBP*	-2.32
C08	*CCR4*	1.62	G05	*SLIT2*	-1.24
C09	*CCR5*	1.34	G06	*TLR2*	1.57
C10	*CCR6*	3.02	G07	*TLR4*	-1.05
C11	*CCR7*	1.29	G08	*TNF*	2.07
C12	*CCR8*	2.00	G09	*TYMP*	1.33
D01	*CCR9*	1.19	G10	*XCL1*	2.04
D02	*CCRL1*	1.41	G11	*XCL2*	1.14
D03	*CCRL2*	1.25	G12	*XCR1*	1.30
D04	*CKLF*	1.20	H01	*ACTB*	1.09
D05	*CMKLR1*	1.55	H02	*B2M*	1.02
D06	*CMTM1*	2.73	H03	*GAPDH*	-1.09
D07	*CMTM2*	2.02	H04	*HPRT1*	1.04
D08	*CMTM3*	1.26	H05	*RPLP0*	-1.06
D09	*CMTM4*	1.30			

[a]The gene expression levels are indicated as fold changes in RUNX3 knockdown (shRUNX3) Ca9.22 cells versus RUNX3-expressing Ca9.22 cells ($n = 3$). *$P < 0.05$ by two-tailed Student's *t*-test.

Table S2. Relationship between CCL28 and RARβ expression in human oral cancer tissues

		RARβ		*P*
		Low	High	
CCL28	Low	59(73.8)	21(26.3)	< 0.001
	High	5(13.5)	32(86.5)	