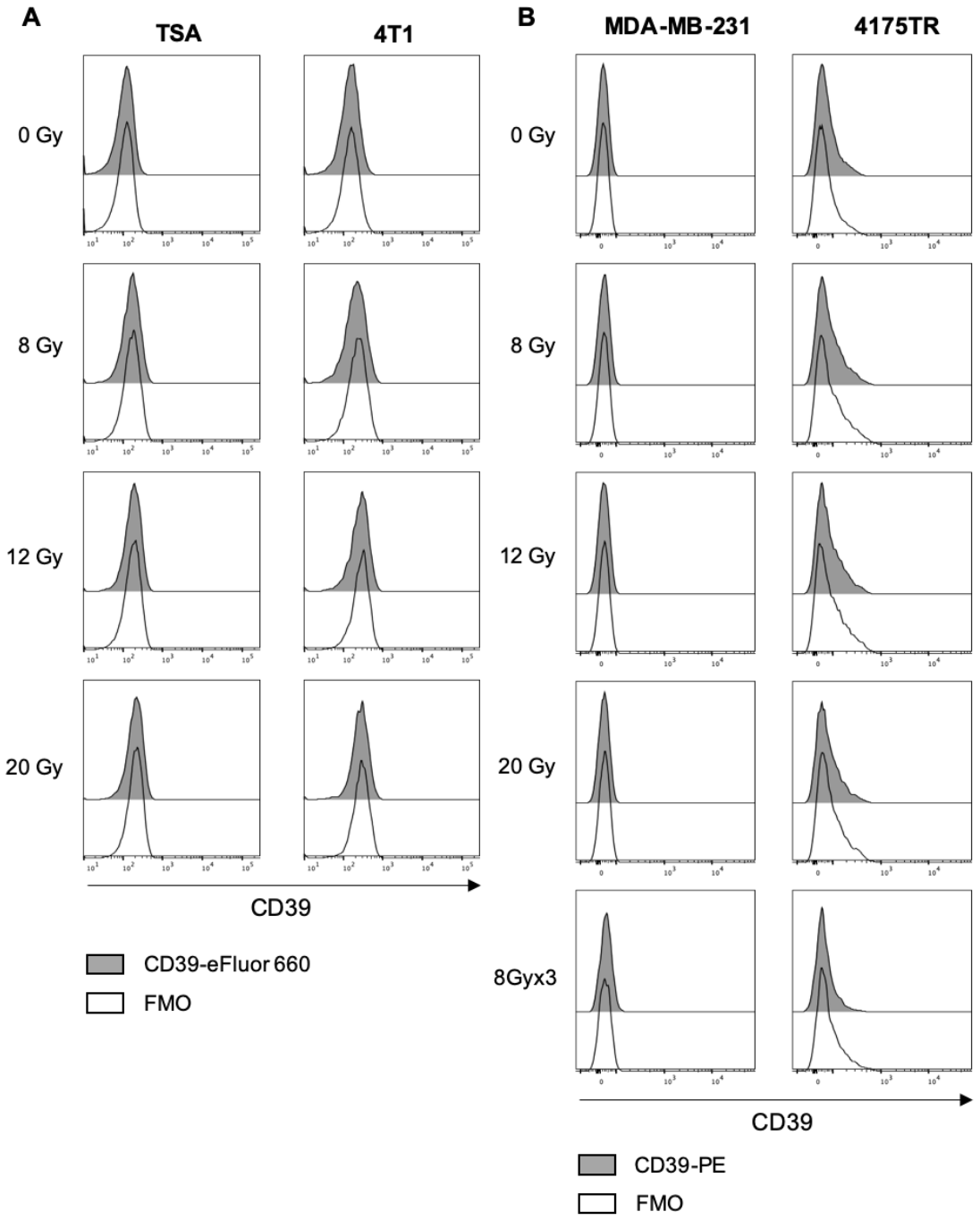
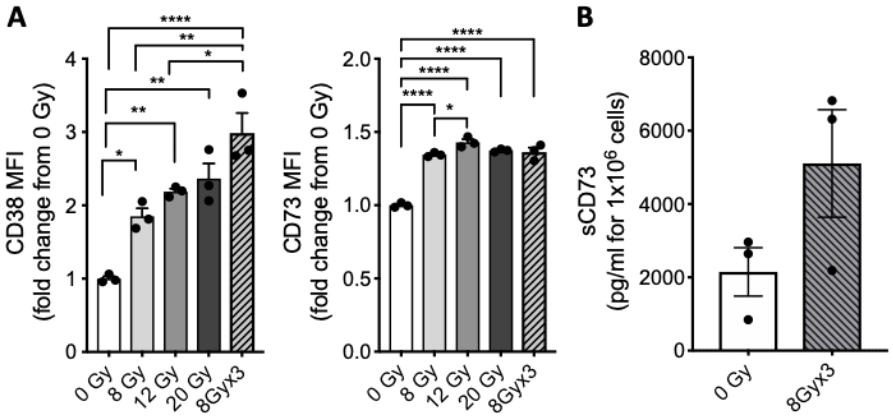
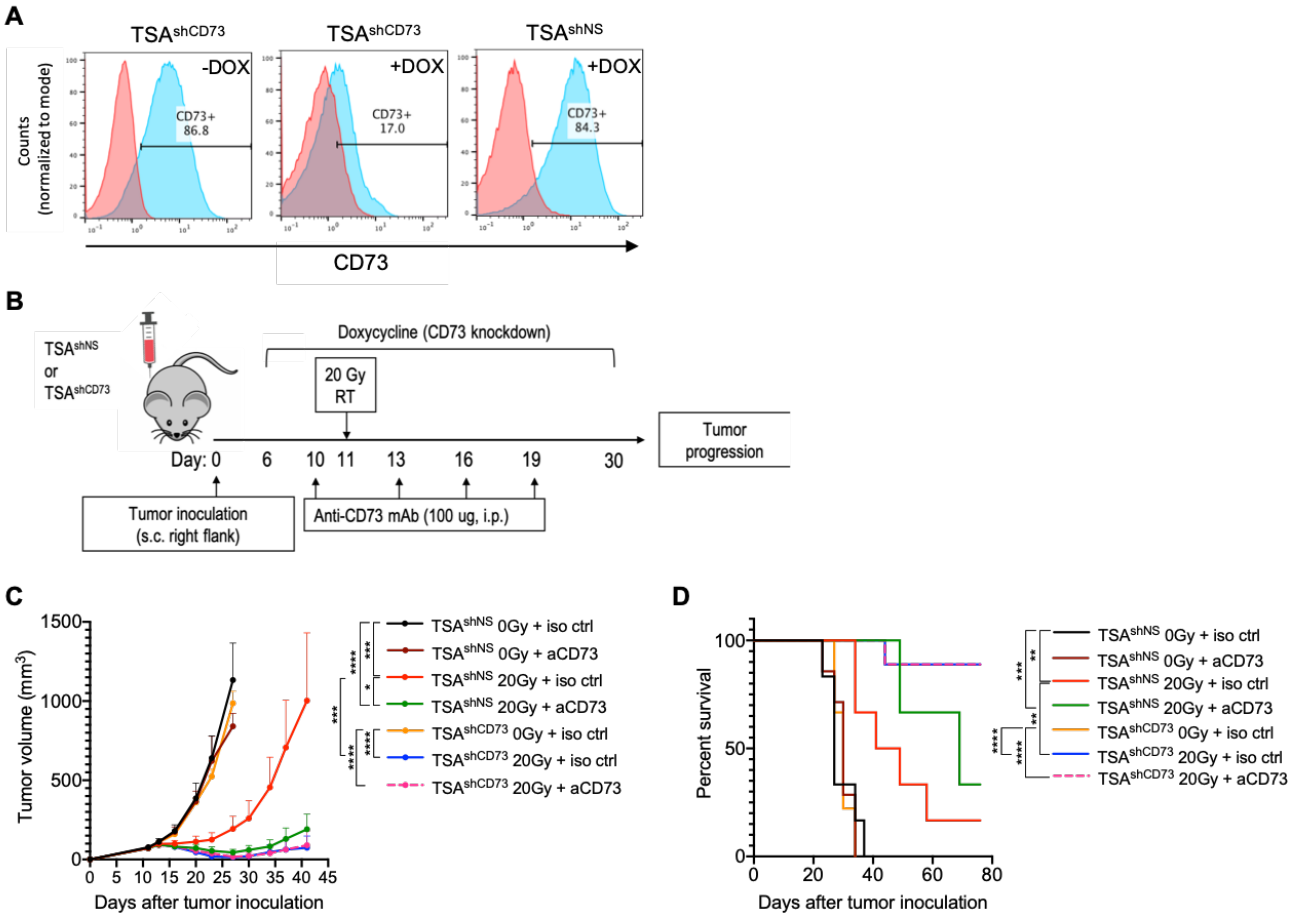
SUPPLEMENTARY MATERIALS:



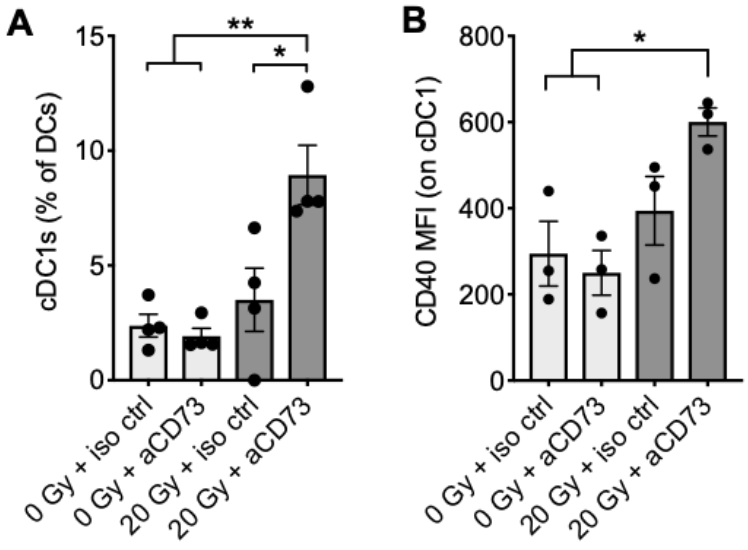
Supplementary figure 1. Representative histograms depicting absence of surface expression of CD39 on mouse TSA and 4T1 mammary carcinoma cells (A) and on human MDA-MB-231 and 4175TR breast carcinoma cells (B), 24 hours following treatment with radiation at the indicated doses.



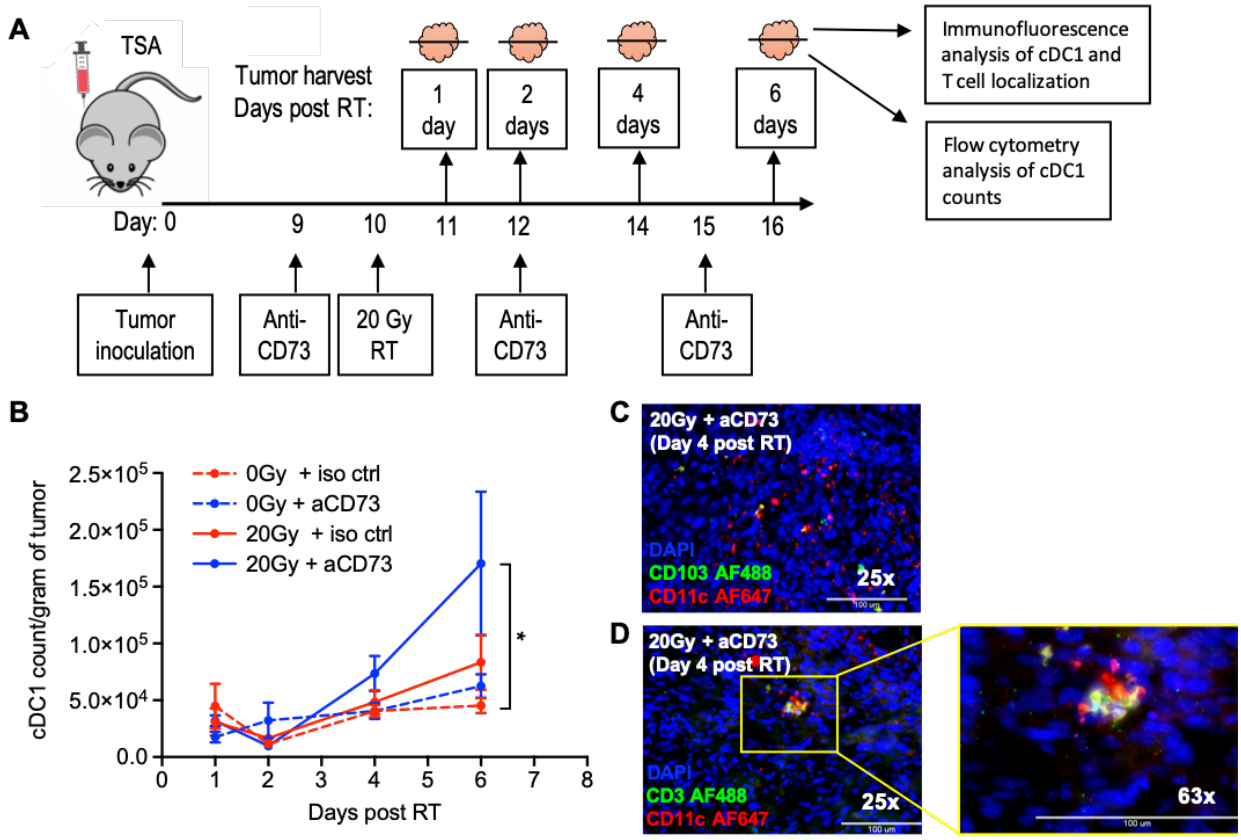
Supplementary figure 2. Radiation-induced upregulation of adenosine-generating ectonucleotidases in human 4175TR cells breast cancer cells. **(A)** Fold change in MFI of CD38 and CD73 compared to mock-treated cells (0 Gy) on 4175TR cells 24 hours following treatment with radiation at the indicated doses (n=3 per group). To correct for increased autofluorescence of irradiated cells, FMO MFI was subtracted from the MFI of each stained sample. ANOVA was used to compare changes in MFI across treatment groups followed by pairwise comparisons using Tukey's method. (**B**) Soluble CD73 (sCD73) in supernatant from 4175TR breast cancer cells treated with 0Gy or 8GyX3 radiation. Cell supernatants were harvested 24 hours after last RT dose and analyzed for concentration of sCD73 by ELISA. Unpaired T-test was used to compare changes in sCD73 levels between treatments groups. *$p<0.05$, ****$p<0.0001$. All data shows mean±s.e.m.



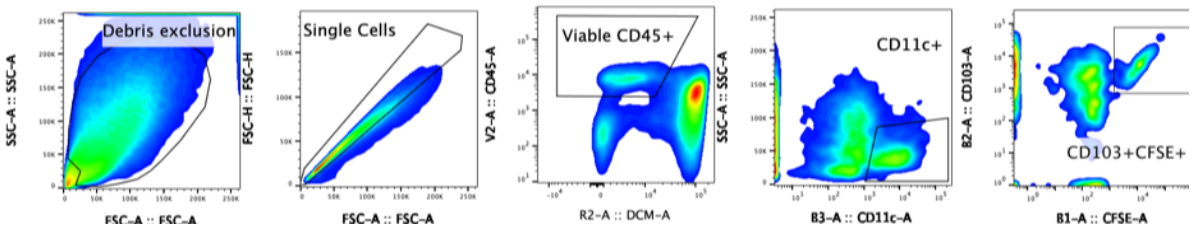
Supplementary figure 3. Targeting CD73 expressed by TSA cells enhances tumor response to 20 Gy RT. (**A**) Surface expression of CD73 on TSA cells transduced with doxycycline-inducible shRNA targeting CD73 (TSA^{shCD73}) or a non-silencing sequence (TSA^{shNS}) 3 days following treatment with doxycycline (+DOX) or without doxycycline (-DOX). (**B**) Experimental schema; $1x10^5$ TSA^{shNS} or TSA^{shCD73} cells were subcutaneously (s.c.) injected in the right flank of BALB/c mice on day 0. Doxycycline was fed to the mice starting on day 6 until day 30. Mice were treated with anti-CD73 (aCD73) or isotype control (iso ctrl) antibody on day 10, 13, 16 and 19. Focal RT was delivered to the tumor in a single dose of 20Gy on day 11. (**C**) Tumor growth over time (n=6-9 mice per group). Statistically significant differences in tumor progression between groups was assessed by repeated measure ANOVA in mock-treated mice (0Gy) between day 10 (treatment start) and day 23 and in RT-treated mice (20Gy) between day 10 and day 41. (**D**) Survival in mice bearing TSA^{shNS} or TSA^{shCD73} tumors. Log-Rank (Mantel-Cox) test was used to calculate statistically significant differences in mouse survival between groups. *<0.05, **<0.01, ***<0.001, ****<0.0001.



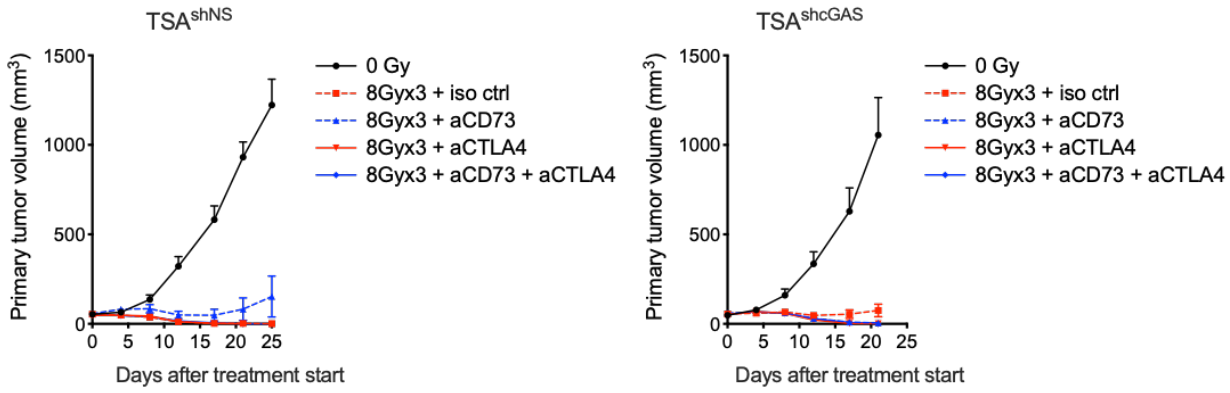
Supplementary figure 4. Analysis of cDC1 infiltrating TSA tumors. Mice bearing TSA tumors were treated with 20Gy RT and/or anti-CD73 antibody (aCD73) or isotype control antibody (iso ctrl) (100 ug, i.p.) as indicated in figure 3A. Mice were sacrificed one day after the third antibody dose for flow cytometric analysis of intratumoral immune cells (n=4). **(A)** Frequency of cDC1 defined by expression of CD103 among intratumoral DCs (n=4 per group). (**B**) Expression of CD40 on cDC1 (n=3 per group). ANOVA was used to calculate statistically significant changes in immune cell infiltration between treatment groups followed by pairwise comparisons using Tukey's method. *$p<0.05$, **$p<0.01$. All data shows mean±s.e.m.



Supplementary Figure 5. **Kinetics and distribution of intratumoral DCs.** (**A**) Experimental schema. BALB/c mice were inoculated with TSA cells in the right flank on day 0 and treated as indicated. Anti-CD73 antibody (TY/23) was given at 100 ug, i.p.. Tumors were harvested on day 1, 2, 4 and 6 post-RT. Half of the tumor was used to prepare a single cell suspension and analyzed by flow cytometry for the number of cDC1 using counting beads.. The other half of the tumor was used to prepare tissue sections for immunofluorescent staining.. (**B**) Number of cDC1 per gram of tumor at various times after treatment with 20 Gy RT (20Gy) or mock-RT treatment (0Gy). ANOVA was used to compare changes in cDC1 density across treatment groups followed by pairwise comparisons using Tukey's method. *$p<0.05$. All data shows mean±s.e.m. (**C-D**) Representative pictures of multicolor immunofluorescence staining of tumors harvested 4 days post RT. Blue, DAPI staining of tumor cell nuclei. (**C**) Tumor localization of cDC1 identified by expression of CD11c (Alexa Fluor 647) and CD103 (Alexa Fluor 488). CD11c+CD103+ cDC1s are identified by the yellow signal observed when the two channels co-localize. (**D**) Cluster containing T cells (CD3 Alexa Fluor 488) and DCs (CD11c Alexa Fluor 647) in close contact. Magnification as indicated, bar=100 μm.



Supplementary figure 6. Representative dot plots depicting the gating strategy for identification of CFSE-labeled cDC1s in mouse tumors.



Supplementary figure 7. Primary tumor growth over time in mice shown in figure 5A-C and bearing TSA tumors expressing the doxycycline-inducible short hairpin RNA (shRNA) directed against cGAS (TSA^{shcGAS}) or a non-silencing sequence (TSA^{shNS}) (n=5-6 per group).

Supplementary Table 1: Dyes and antibodies for flow cytometry and immunofluorescence

Flow Cytometry antibodies

Antibody/dye	Reactivity	Fluorochrome	Clone	Manufacturer	Identifier
CD45	Mouse	V500	30-F11	BD Biosciences	561487
CD11c	Mouse	PerCp-Vio700	N418	Miltenyi	130-103-806
CD11c	Mouse	PerCp-Cy5.5	N418	eBioscience	45-0114-82
MHC class II	Mouse	PE-Vio770	REA528	Miltenyi	130-107-943
MHC class II	Mouse	Pe-Cy7	M5/114.15.2	eBioscience	25-5321-82
CD3e	Mouse	eFluor 450	145-2C11	eBioscience	48-0031-82
CD8a	Mouse	FITC	53-6.7	eBioscience	11-0081-85
CD8b	Mouse	PE-Vio770	H35-17.2	Miltenyi	130-106-389
CD103	Mouse	PE	2E7	eBioscience	12-1030-83
CD103	Mouse	APC	2E7	BioLegend	121413
XCR1	Mouse	PE	ZET	BioLegend	148204
CD86	Mouse	eFluor 450	GL1	eBioscience	48-0862-82
CD4	Mouse	PE-Vio770	GK1.5	Miltenyi	130-102-411
FoxP3	Mouse	Alexa Fluor 488	FJK-16s	eBioscience	53-5773-82
CD25	Mouse	PerCp-Cy5.5	PC61.5	eBioscience	45-0251-82
CD69	Mouse	PerCp-Cy5.5	H1.2F3	eBioscience	45-0691-82
CD39	Mouse	eFluor 660	24DMS1	eBioscience	50-0391-82
CD73	Mouse	PE	eBioTY/11.8	eBioscience	12-0731-82
CD38	Mouse	PE	90	eBioscience	12-0381-82
CD203a	Mouse	APC	REA867	Miltenyi	130-114-268
CD73	Human	PE	AD2	Invitrogen	12-0739-42
CD38	Human	PE	HIT2	Invitrogen	12-0389-42
CD39	Human	PE	eBioA1	Invitrogen	12-0399-42
Viability dye	N/A	eFluor 450	N/A	eBioscience	65-0863-14
Viability dye	N/A	eFluor 780	N/A	eBioscience	65-0865-14

Primary immunofluorescence antibodies

CD3	mouse	non-conjugated	SP7	Abcam	ab16669
CD11c	mouse	non-conjugated	N418	ThermoFisher	MA11C5
CD103	mouse	non-conjugated	EPR22590-27	Abcam	ab224202
CD8	mouse	Alexa Fluor 488	5H10	ThermoFisher	MCD0820

Secondary immunofluorescence antibodies

goat anti-rabbit IgG	rabbit	Alexa Fluor 488	polyclonal	Abcam	ab150077
goat anti-hamster IgG	hamster	Alexa Fluor 647	polyclonal	ThermoFisher	A-21451