

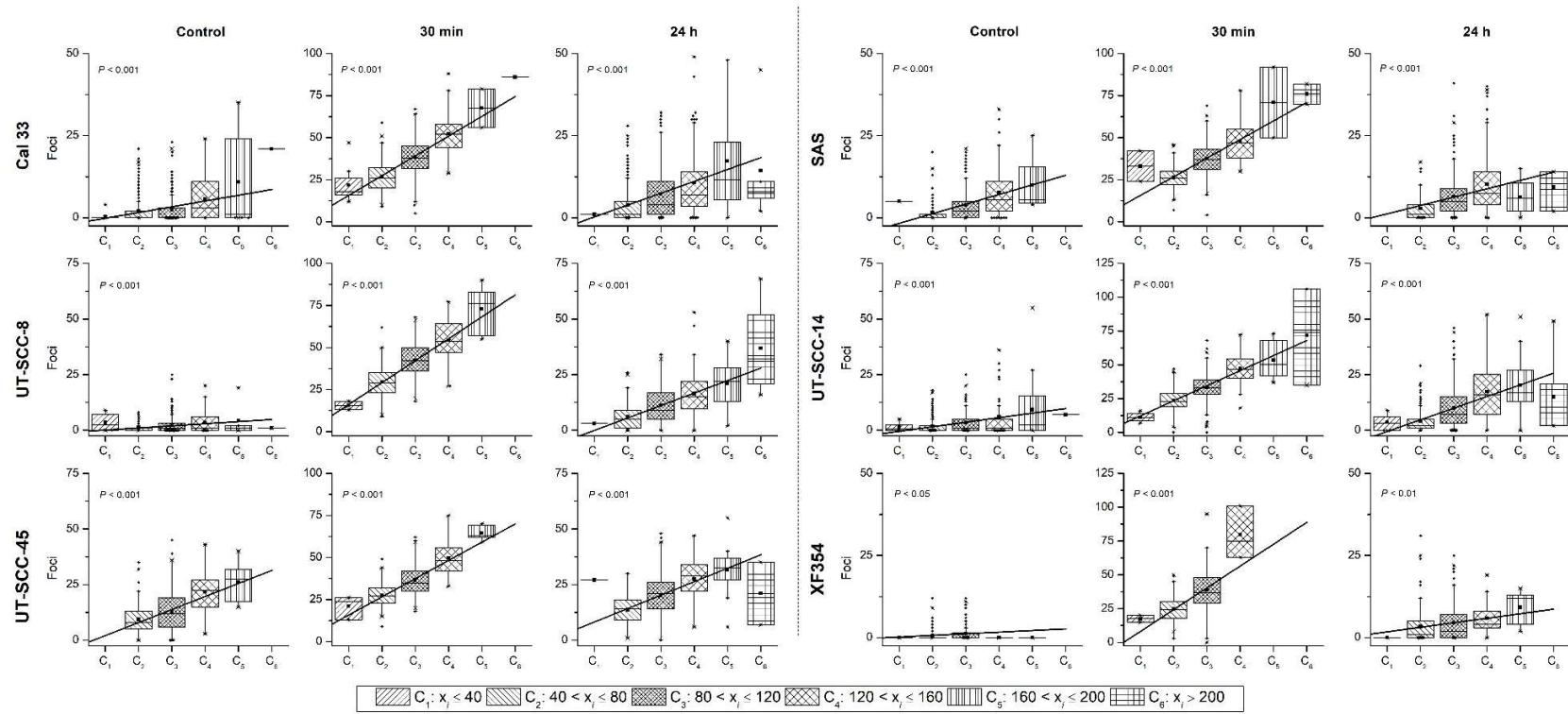


Figure S1 Number of foci linearly increase with the nucleus area (x_i) categories (C). Box plots of foci number of six hHNSCC models were classified into six nucleus area categories with an interval size of $40 \mu\text{m}^2$. Foci number of controls and exposed (4 Gy) tumors, which were fixed 30 min and 24 h post exposure for the *in vivo* set-up are shown. *P* value of linear regression analysis is shown. Linear regression analysis outputs is presented in supplementary Table S4. Please note the different Y-axis for 30 min post-exposure.

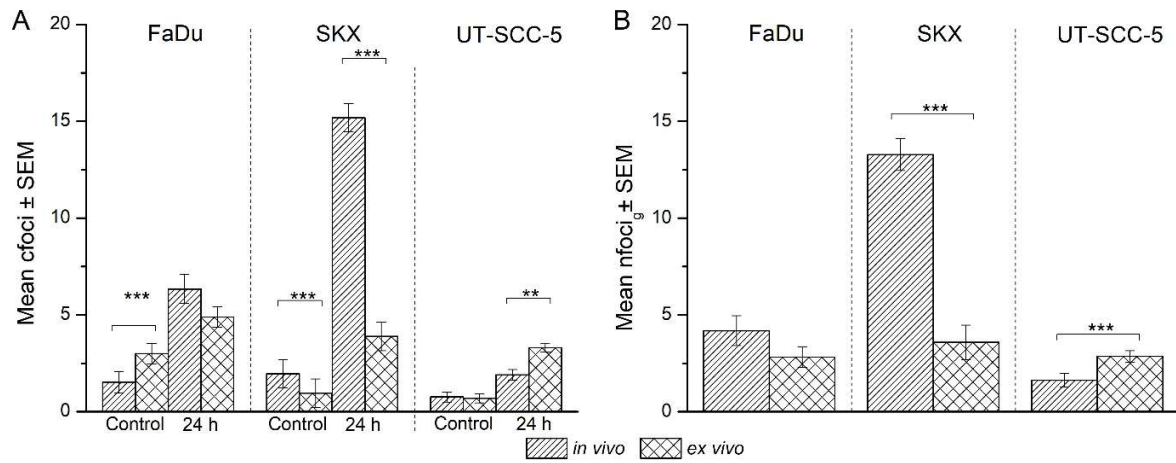


Figure S3. Comparison of mean cfoci ± standard error of mean (SEM) (A) and nfoci_g ± SEM (B) in three tumor models subjected to both experimental settings of the γ H2AX assay. Data were fitted into LMEM where the experimental setting was defined as fixed effect and tumor, specimen, and ROI were defined as random effects. (**: $P < 0.01$, ***: $P < 0.001$). ROI was repeatedly included to generate the missing factor (specimen) for the *in vivo* set-up. Exact P values are presented in Supplementary Table S7.

Table S1. Summary of the input data for the statistical analysis

	Tumor line	Condition	No. of tumors	No. of specimen	No. of ROI	Total data input
<i>in vivo</i>	FaDu	Control	9	-	90	450
		30 min post IR	9	-	90	450
		24 h post IR	8	-	80	400
	SKX	Control	9	-	90	450
		30 min post IR	10	-	100	500
		24 h post IR	9	-	90	450
	UT-SCC-5	Control	8	-	80	400
		30 min post IR	9	-	90	450
		24 h post IR	8	-	80	400
	Cal33	Control	9	-	90	450
		30 min post IR	8	-	80	400
		24 h post IR	9	-	90	450
	SAS	Control	8	-	80	400
		30 min post IR	8	-	80	400
		24 h post IR	8	-	80	400
	UT-SCC-8	Control	8	-	80	400
		30 min post IR	7	-	70	350
		24 h post IR	9	-	90	450
	UT-SCC-14	Control	9	-	90	450
		30 min post IR	10	-	100	500
		24 h post IR	9	-	90	450
	UT-SCC-45	Control	8	-	80	400
		30 min post IR	8	-	80	400
		24 h post IR	9	-	90	450
	XF354	Control	7	-	70	350
		30 min post IR	4	-	40	200
		24 h post IR	7	-	70	350
<i>ex vivo</i>	FaDu	Control	16	60	438	4380
		24 h post IR	16	60	439	4390
	SKX	Control	9	31	217	2170
		24 h post IR	9	31	200	2000
	UT-SCC-5	Control	10	39	253	2530
		24 h post IR	10	40	283	2830

IR: Irradiation

Table S2. Summary of the statistical output of nucleus area analyzed by a LMEM. Bonferroni correction was applied for multiple comparisons.

	Tumor line	Condition	Mean	Standard deviation	P value ^a		
					Control	30 min	24 h
<i>in vivo</i>	FaDu	Control	116.4466	33.34720	-	0.804	1.000
		30 min post IR	122.2892	32.38627	-	-	0.297
		24 h post IR	112.6412	30.59298	-	-	-
	SKX	Control	100.2680	25.86748	-	0.281	0.001
		30 min post IR	107.1704	26.17204	-	-	0.084
		24 h post IR	119.1873	33.93800	-	-	-
	UT-SCC-5	Control	65.2466	15.69384	-	0.701	1.000
		30 min post IR	69.4444	18.16860	-	-	0.739
		24 h post IR	66.1853	19.15192	-	-	-
	Cal33	Control	80.2138	27.38123	-	1.000	0.120
		30 min post IR	81.1026	28.31106	-	-	0.156
		24 h post IR	94.9764	35.76368	-	-	-
	SAS	Control	93.8444	24.26456	-	1.000	0.000
		30 min post IR	93.5702	24.84296	-	-	0.000
		24 h post IR	108.7612	28.34736	-	-	-
	UT-SCC-8	Control	89.5806	28.27604	-	1.000	0.004
		30 min post IR	90.7311	26.55216	-	-	0.017
		24 h post IR	103.3802	33.47841	-	-	-
	UT-SCC-14	Control	85.7874	31.78737	-	1.000	0.787
		30 min post IR	90.5895	29.16455	-	-	1.000
		24 h post IR	94.9591	35.63261	-	-	-
	UT-SCC-45	Control	97.3420	24.88855	-	1.000	0.109
		30 min post IR	93.9249	23.99820	-	-	0.011
		24 h post IR	106.2284	28.73631	-	-	-
	XF354	Control	73.1351	19.25533	-	1.000	0.001
		30 min post IR	73.2460	17.68541	-	-	0.004
		24 h post IR	89.1600	23.93514	-	-	-
<i>ex vivo</i>	FaDu	Control	85.596336	25.218263	-	-	0.001
		24 h post IR	91.355702	26.967857	-	-	-
	SKX	Control	75.658134	30.057774	-	-	0.040
		24 h post IR	79.744765	31.672199	-	-	-
	UT-SCC-5	Control	75.021162	25.737746	-	-	0.522
		24 h post IR	75.603470	26.228616	-	-	-

^a Statistical significance was determined by fitting logarithmic nucleus area into a LMEM with condition as fixed effect and tumor and ROI as random effects. IR: Irradiation

Table S3. Statistical output of the comparison between the experimental settings of logarithmic nucleus area in three tumor lines. Tumor or ROI was assigned as random effects in the LMEM repeatedly to complete the missing factor for *in vivo* set-up.

	Tumor line	Condition	Experimental set-up (I)	Experimental set-up (J)	Mean Difference (I-J)	Standard error of mean	Degree of Freedom	P Value
Repeat tumor	FaDu	Control	<i>in vivo</i>	<i>ex vivo</i>	0.132	0.008	2536.809	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	0.095	0.018	108.437	0.000
	SKX	Control	<i>in vivo</i>	<i>ex vivo</i>	0.137	0.011	738.752	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	0.186	0.019	44.917	0.000
	UT-SCC-5	Control	<i>in vivo</i>	<i>ex vivo</i>	-0.006	0.009	1134.012	0.515
		24 h	<i>in vivo</i>	<i>ex vivo</i>	-0.076	0.028	35.043	0.011
Repeat ROI	FaDu	Control	<i>in vivo</i>	<i>ex vivo</i>	0.123	0.007	2241.822	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	0.092	0.012	41.231	0.000
	SKX	Control	<i>in vivo</i>	<i>ex vivo</i>	0.141	0.010	1121.132	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	0.187	0.017	20.967	0.000
	UT-SCC-5	Control	<i>in vivo</i>	<i>ex vivo</i>	-0.043	0.008	1502.768	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	-0.054	0.024	19.827	0.033

Table S4. Linear regression coefficient and R² of γ H2AX foci number as a function of nucleus area categories from different tumor lines and treatment conditions

	Tumor line	Condition	Slope	Sig. (Slope)	Intercept	Sig. (Intercept)	R Square
<i>in vivo</i>	FaDu	Control	0.836	0.000	-0.231	0.716	0.046
		30 min post IR	8.624	0.000	2.229	0.240	0.383
		24 h post IR	2.582	0.000	-2.038	0.062	0.141
	SKX	Control	1.256	0.000	-1.710	0.007	0.077
		30 min post IR	9.090	0.000	10.581	0.000	0.193
		24 h post IR	4.161	0.000	0.610	0.641	0.227
	UT-SCC-5	Control	0.447	0.063	-0.323	0.533	0.009
		30 min post IR	8.560	0.000	0.546	0.769	0.202
		24 h post IR	2.423	0.000	-3.169	0.000	0.097
	Cal33	Control	1.750	0.000	-1.782	0.026	0.068
		30 min post IR	11.749	0.000	3.815	0.038	0.424
		24 h post IR	3.616	0.000	-3.436	0.010	0.131
	SAS	Control	2.924	0.000	-4.669	0.000	0.127
		30 min post IR	11.078	0.000	4.342	0.027	0.402
		24 h post IR	2.548	0.000	-1.263	0.395	0.076
	UT-SCC-8	Control	0.954	0.000	-0.739	0.241	0.044
		30 min post IR	13.023	0.000	3.265	0.117	0.481
		24 h post IR	5.601	0.000	-5.535	0.000	0.264
	UT-SCC-14	Control	2.046	0.000	-2.374	0.006	0.090
		30 min post IR	11.170	0.000	0.774	0.638	0.432
		24 h post IR	5.242	0.000	-5.701	0.000	0.214
	UT-SCC-45	Control	5.855	0.000	-3.560	0.045	0.200
		30 min post IR	10.828	0.000	4.836	0.007	0.442
		24 h post IR	6.041	0.000	2.127	0.214	0.222
	XF354	Control	0.498	0.010	-0.421	0.360	0.019
		30 min post IR	16.135	0.000	-7.835	0.051	0.320
		24 h post IR	1.376	0.001	0.487	0.665	0.032
<i>ex vivo</i>	FaDu	Control	1.792	0.000	-1.289	0.000	0.051
		24 h post IR	2.652	0.000	-2.570	0.000	0.100
	SKX	Control	0.562	0.000	-0.415	0.005	0.041
		24 h post IR	2.239	0.000	-1.448	0.000	0.136
	UT-SCC-5	Control	0.445	0.000	-0.373	0.028	0.016
		24 h post IR	1.706	0.000	-0.743	0.003	0.091

IR: Irradiation

Table S5. Summary of the statistical analysis output analyzed by a random effect model for cfoci from the *in vivo* and *ex vivo* datasets

	Tumor line	Condition	Mean	Stand ard error of mean	P value			
					Tum or	Tumor x ROI	Tumor x Specime n	Tumor x Specime n x ROI
<i>in vivo</i>	FaDu	Control	2.569	1.335	0.128	0.136	-	-
		30 min post IR	33.216	1.335	0.057	0.000	-	-
		24 h post IR	6.335	1.416	0.144	0.300	-	-
	SKX	Control	1.939	1.303	0.134	^a	-	-
		30 min post IR	39.707	1.236	0.070	0.000	-	-
		24 h post IR	15.186	1.303	0.086	0.009	-	-
	UT- SCC-5	Control	0.599	1.013	0.127	^a	-	-
		30 min post IR	19.984	0.955	0.064	0.001	-	-
		24 h post IR	1.908	1.013	0.155	0.234	-	-
	Cal33	Control	2.473	0.663	0.142	^a	-	-
		30 min post IR	34.516	0.703	0.245	0.003	-	-
		24 h post IR	6.596	0.663	0.382	0.160	-	-
	SAS	Control	3.404	0.848	0.101	0.695	-	-
		30 min post IR	35.748	0.848	0.178	0.004	-	-
		24 h post IR	6.417	0.848	0.108	0.070	-	-
	UT- SCC-8	Control	1.880	1.103	^a	0.213	-	-
		30 min post IR	39.868	1.180	0.107	0.064	-	-
		24 h post IR	11.156	1.040	0.276	0.050	-	-
	UT- SCC- 14	Control	2.847	1.568	0.128	^a	-	-
		30 min post IR	31.766	1.488	0.043	0.000	-	-
		24 h post IR	8.956	1.568	0.074	0.320	-	-
	UT- SCC- 45	Control	13.367	1.231	0.137	0.237	-	-
		30 min post IR	36.032	1.231	0.074	0.051	-	-
		24 h post IR	21.072	1.160	0.108	0.290	-	-
	XF354	Control	0.723	0.576	0.166	0.037	-	-
		30 min post IR	29.431	0.762	0.384	0.131	-	-
		24 h post IR	4.117	0.576	0.559	0.792	-	-
<i>ex vivo</i>	FaDu	Control	3.343	0.672	0.028	-	0.000	0.000
		24 h post IR	4.715	0.817	0.015	-	0.000	0.000
	SKX	Control	0.931	0.216	0.146	-	0.007	0.050
		24 h post IR	4.208	0.680	0.123	-	0.007	0.000
	UT- SCC-5	Control	0.666	0.084	^a	-	0.003	0.070
		24 h post IR	3.348	0.353	0.079	-	0.005	0.000

a: This covariance parameter is redundant. The test statistic and confidence interval cannot be computed.
IR: Irradiation

Table S6. Summary of the statistical analysis output analyzed by a random effect model for nfoci_g from the *in vivo* and *ex vivo* datasets

	Tumor line	Condition	Mean	Stand ard error of mean	P value			
					Tum or	Tumor x ROI	Tumor x Specime n	Tumor x Specime n x ROI
<i>in vivo</i>	FaDu	30 min post IR	30.648	2.330	0.057	0.000	-	-
		24 h post IR	4.191	0.521	0.144	0.284	-	-
	SKX	30 min post IR	37.768	1.944	0.070	0.000	-	-
		24 h post IR	13.286	1.060	0.087	0.009	-	-
	UT-SCC-5	30 min post IR	19.391	1.642	0.064	0.001	-	-
		24 h post IR	1.623	0.361	0.156	0.308	-	-
	Cal33	30 min post IR	32.043	1.093	0.245	0.003	-	-
		24 h post IR	4.839	0.527	0.384	0.181	-	-
	SAS	30 min post IR	32.344	1.092	0.178	0.004	-	-
		24 h post IR	3.800	0.778	0.118	0.045	-	-
	UT-SCC-8	30 min post IR	37.988	2.199	0.107	0.064	-	-
		24 h post IR	9.411	0.607	0.292	0.056	-	-
	UT-SCC-14	30 min post IR	28.750	2.332	0.043	0.001	-	-
		24 h post IR	6.547	1.124	0.078	0.284	-	-
	UT-SCC-45	30 min post IR	22.665	1.791	0.074	0.051	-	-
		24 h post IR	8.619	0.773	0.130	0.410	-	-
	XF354	30 min post IR	28.711	1.669	0.384	0.132	-	-
		24 h post IR	3.611	0.341	0.581	0.872	-	-
<i>ex vivo</i>	FaDu	24 h post IR	2.823	0.642	0.014	-	0.000	0.000
	SKX	24 h post IR	3.598	0.625	0.126	-	0.007	0.000
	UT-SCC-5	24 h post IR	2.862	0.329	0.079	-	0.006	0.000

IR: Irradiation

Table S7A. Summary of the statistical analysis output analyzed by LMEM for cfoci for direct comparison between *in vivo* and *ex vivo* datasets. Tumor or ROI was assigned as random effects in the LMEM repeatedly to complete the missing factor for *in vivo* set-up

	Tumor line	Condition	Experimental set-up (I)	Experimental set-up (J)	Mean Difference (I-J)	Standard error of mean	Degree of Freedom	P Value
Repeat tumor	FaDu	Control	<i>in vivo</i>	<i>ex vivo</i>	-3.235	0.297	2670.483	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	1.988	1.287	50.761	0.129
	SKX	Control	<i>in vivo</i>	<i>ex vivo</i>	1.687	0.278	826.754	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	11.170	0.897	13.885	0.000
	UT-SCC-5	Control	<i>in vivo</i>	<i>ex vivo</i>	-0.325	0.202	1461.697	0.107
		24 h	<i>in vivo</i>	<i>ex vivo</i>	-1.457	0.397	60.007	0.001
Repeat ROI	FaDu	Control	<i>in vivo</i>	<i>ex vivo</i>	-1.465	0.285	2530.096	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	1.444	0.922	28.381	0.128
	SKX	Control	<i>in vivo</i>	<i>ex vivo</i>	1.004	0.265	1795.228	0.000
		24 h	<i>in vivo</i>	<i>ex vivo</i>	11.291	1.037	18.506	0.000
	UT-SCC-5	Control	<i>in vivo</i>	<i>ex vivo</i>	0.069	0.182	1451.606	0.705
		24 h	<i>in vivo</i>	<i>ex vivo</i>	-1.405	0.356	19.670	0.001

Table S7B. Summary of the statistical analysis output analyzed by LMEM for nfoci_g for direct comparison between *in vivo* and *ex vivo* datasets. Tumor or ROI was assigned as random effects in the LMEM repeatedly to complete the missing factor for the *in vivo* set-up.

			P value		
Tumor line			SKX	FaDu	UT-SCC-5
Repeat tumor	Fixed effect	Experimental setting	< 0.0001	0.206	0.022
	Random effect	Inter-tumoral	0.051	0.004	0.038
		Inta-tumoral inter-specimen	0.033	< 0.0001	0.0056
		Inta-tumoral intra-specimen	< 0.0001	< 0.0001	< 0.0001
Repeat ROI	Fixed effect	Experimental setting	< 0.0001	0.159	0.016
	Random effect	Inter-tumoral	0.019	0.003	0.021
		Intra-tumoral inter-specimen	0.000	< 0.0001	0.003
		Intra-tumoral intra-specimen	0.009	< 0.0001	< 0.0001