

Supplementary Materials: ROS Production and Distribution: A New Paradigm to Explain the Differential Effects of X-ray and Carbon Ion Irradiation on Cancer Stem Cell Migration and Invasion

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Table S1. Phosphorylation signals for each protein involved in the Akt/mTOR, STAT3, and MEK/p38/JNK pathways in SQ20B-CSCs. The values (mean $\pm$ SD) were calculated in response to normoxia, chronic hypoxia, and 2 Gy X-ray or C-ion irradiation under both normoxia and hypoxia.

	Normoxia		Normoxia + X-rays		Normoxia + C-ions		Hypoxia		Hypoxia + X-rays		Hypoxia + C-ions	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
p38a	13.615	0.191	21.323	0.342	10.057	0.410	14.358	0.485	18.452	0.699	14.134	0.279
ERK1/2	12.805	0.233	15.001	1.214	9.157	0.319	12.771	0.532	14.254	1.228	12.792	0.162
JNK1/2/3	15.550	0.170	20.474	0.394	17.465	0.621	21.719	0.337	18.579	0.023	18.132	0.404
MSK1/2	15.365	0.276	19.259	2.640	11.391	0.379	15.850	0.378	18.958	3.233	15.943	0.985
c-jun	13.805	0.049	15.993	0.006	13.592	0.334	14.069	0.153	13.972	0.511	14.654	0.369
WNK-1	35.580	2.206	31.568	1.061	34.842	0.539	30.522	0.343	34.541	1.574	31.935	2.472
GSK-3α/β	17.780	0.905	19.483	0.439	16.881	1.170	20.033	0.413	20.298	2.129	16.307	0.603
HSP27	12.810	0.750	11.326	0.187	8.334	0.205	11.729	0.464	12.951	0.023	12.704	0.478
STAT3 Y705	14.455	0.276	14.886	0.056	12.784	0.035	14.625	0.106	13.885	0.802	16.136	0.101
STAT3 S727	18.230	1.365	23.460	0.534	15.338	0.813	16.908	0.337	21.777	1.336	17.718	0.196
β-catenin	14.035	0.064	14.444	0.820	9.434	0.265	13.866	0.907	13.451	0.528	13.416	1.426
Akt1/2/3 S473	13.290	0.757	14.535	0.646	8.850	0.308	13.177	0.140	13.298	0.001	12.709	0.412
Akt1/2/3 T308	20.035	1.379	14.294	0.075	12.909	0.000	15.892	0.118	13.830	0.196	18.303	2.147
mTOR	15.110	0.778	15.749	0.439	12.407	1.345	14.507	0.091	15.331	0.381	14.706	0.029
PRAS40	32.020	1.315	32.046	2.711	31.911	3.112	31.888	1.546	51.658	2.199	27.212	1.096
p70 S6 kinase	16.285	0.445	13.707	0.223	11.667	0.217	14.028	0.112	11.611	0.277	14.998	0.291

Table S2. Phosphorylation signals for each protein involved in the Akt/mTOR, STAT3, and MEK/p38/JNK pathways in SQ20B-CSCs. The values (mean $\pm$ SD) were calculated in response to normoxia, chronic hypoxia, and 10 Gy X-ray or C-ion irradiation under both normoxia and hypoxia.

	Normoxia		Normoxia + X-rays		Normoxia + C-ions		Hypoxia		Hypoxia + X-rays		Hypoxia + C-ions	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
p38a	4.275	0.212	8.060	1.050	0.980	0.170	2.249	1.159	3.120	0.815	0.425	0.085
ERK1/2	2.320	0.297	3.753	0.000	0.350	0.057	2.250	0.028	1.204	0.175	0.130	0.010
JNK1/2/3	4.550	0.099	4.655	0.229	1.005	0.629	4.260	0.099	4.458	1.060	1.325	0.005
MSK1/2	12.625	1.803	16.234	2.396	7.620	1.725	14.262	0.318	10.718	0.630	6.035	0.765
c-jun	1.805	0.219	2.774	0.010	0.795	0.049	1.696	0.410	1.724	0.030	0.730	0.040
WNK-1	19.210	0.679	16.308	1.069	2.815	0.092	17.170	0.148	23.918	0.685	1.660	0.040
GSK-3α/β	6.325	0.163	7.094	0.220	1.195	0.502	6.664	0.240	7.157	1.050	1.575	0.095
HSP27	2.225	0.049	2.653	0.162	0.565	0.276	1.565	0.198	1.075	0.010	0.485	0.075
STAT3 Y705	1.595	0.290	1.613	0.200	0.385	0.120	0.969	0.099	1.131	0.130	0.175	0.125
STAT3 S727	1.675	0.629	1.964	0.449	1.445	0.021	1.350	0.049	1.383	0.375	1.495	0.475
β-catenin	12.855	0.120	10.348	0.506	1.545	0.092	15.303	0.049	16.929	1.105	2.645	0.085
Akt1/2/3 S473	2.265	0.544	3.301	0.353	0.185	0.021	2.385	0.014	2.436	0.715	0.315	0.055
Akt1/2/3 T308	3.000	0.495	3.524	0.477	0.740	0.014	3.011	0.304	3.080	0.650	0.680	0.150
mTOR	1.365	0.064	3.112	0.048	0.235	0.205	0.571	0.035	0.258	0.010	0.335	0.135
PRAS40	40.055	1.421	71.408	7.990	24.125	0.601	54.585	2.560	57.148	1.655	23.230	3.200
p70 S6 kinase	2.645	0.120	2.592	0.286	1.920	0.269	1.297	0.346	1.826	0.310	2.965	0.495

Table S3. Phosphorylation signals for each protein involved in the Akt/mTOR, STAT3, and MEK/p38/JNK pathways in SQ20B^{CD44low}. The values (mean $\pm$ SD) were calculated in response to normoxia, chronic hypoxia, and 10 Gy X-ray or C-ion irradiation under both normoxia and hypoxia.

	Normoxia		Normoxia + X-rays		Normoxia + C-ions		Hypoxia		Hypoxia + X-rays		Hypoxia + C-ions	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
p38a	2.019	0.374	2.058	0.068	1.341	0.442	1.207	0.211	0.784	0.184	1.389	0.646
ERK1/2	1.327	0.041	1.442	0.136	1.115	0.014	0.942	0.095	0.788	0.095	0.736	0.034
JNK1/2/3	1.476	0.061	1.740	0.041	0.851	0.007	1.442	0.177	1.375	0.000	1.481	0.136
MSK1/2	5.591	0.360	5.966	0.388	3.005	0.238	5.279	0.367	4.957	0.483	4.639	0.075
c-jun	1.346	0.082	1.010	0.014	1.462	0.068	0.865	0.109	0.692	0.000	0.462	0.000
WNK-1	5.548	0.163	5.495	0.156	2.582	0.129	4.149	0.129	4.188	0.197	3.957	0.197
GSK-3α/β	2.399	0.061	2.404	0.394	1.577	0.095	2.197	0.170	1.880	0.374	1.990	0.462
HSP27	0.889	0.143	0.933	0.054	0.413	0.190	1.139	0.116	0.909	0.034	0.543	0.184
STAT3 Y705	0.957	0.170	0.380	0.075	1.144	0.320	0.582	0.184	0.620	0.197	0.389	0.197
STAT3 S727	0.976	0.238	0.457	0.197	1.259	0.136	0.404	0.041	0.736	0.007	0.505	0.007
β-catenin	7.490	0.231	7.702	0.258	2.635	0.218	7.779	0.136	7.481	0.163	3.918	0.020
Akt1/2/3 S473	0.788	0.054	0.942	0.027	0.596	0.068	0.875	0.014	0.740	0.054	0.870	0.279
Akt1/2/3 T308	1.635	0.503	1.168	0.197	0.678	0.197	1.043	0.088	1.192	0.027	0.962	0.027
mTOR	0.500	0.000	0.784	0.034	0.538	0.238	0.649	0.061	0.428	0.048	0.476	0.088
PRAS40	8.788	0.639	8.702	0.490	6.231	0.367	8.697	0.469	7.365	1.319	7.135	1.319
p70 S6 kinase	1.135	0.190	0.630	0.020	1.361	0.592	0.457	0.007	0.688	0.007	0.457	0.007

Table S4. Phosphorylation signals for proteins involved in the three signaling pathways in SQ20B-CSCs after DMSO treatment. The values (mean $\pm$ SD) were calculated in response to normoxia and 2 Gy X-ray irradiation under normoxia.

	Normoxia		Normoxia + X-rays	
	Mean	SD	Mean	SD
p38a	7.530	1.159	2.775	0.158
ERK1/2	3.945	0.445	2.158	0.153
JNK1/2/3	8.235	0.120	6.317	0.439
MSK1/2	10.265	1.846	5.202	0.270
c-jun	1.650	0.325	1.732	0.143
WNK-1	3.950	0.353	3.766	0.471
GSK-3α/β	6.03	0.141	4.495	0.085
HSP27	2.77	0.085	1.627	0.069
STAT3 Y705	1.565	0.163	1.245	0.418
STAT3 S727	4.555	0.007	2.44222	0.037
β-catenin	4.165	0.092	2.936	0.100
Akt1/2/3 S473	7.570	0.198	3.961	0.354
Akt1/2/3 T308	1.590	0.014	2.128	0.164
mTOR	4.170	0.254	2.809	0.291
PRAS40	12.820	1.570	9.922	0.048
p70 S6 kinase	1.185	0.0494	1.174	0.159

Table S5. Phosphorylation signals for proteins involved in the three signaling pathways in SQ20B-CSCs after DMSO treatment. The values (mean $\pm$ SD) were calculated in response to normoxia, chronic hypoxia, and 10 Gy X-ray or C-ion irradiation under both normoxia and hypoxia.

	Normoxia		Normoxia + X-rays		Normoxia + C-ions		Hypoxia		Hypoxia + X-rays		Hypoxia + C-ions	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
p38a	4.755	0.092	3.035	0.120	5.035	0.035	3.395	0.106	4.230	0.509	3.475	0.085
ERK1/2	4.205	0.163	2.965	0.134	3.590	0.000	3.555	0.120	3.545	0.021	3.625	0.078
JNK1/2/3	7.945	0.205	8.220	0.014	8.065	0.092	8.325	0.120	8.435	0.573	9.110	0.057
MSK1/2	12.320	0.467	11.355	0.841	11.120	0.834	11.940	0.141	12.860	0.820	10.535	2.029
c-jun	6.590	0.283	3.645	0.007	4.560	0.085	2.350	0.014	2.650	0.000	3.590	0.057
WNK-1	24.755	0.940	22.530	0.198	20.315	0.742	15.965	0.148	27.995	0.672	13.680	0.042
GSK-3α/β	12.340	0.424	8.120	0.042	7.680	0.141	13.480	0.636	10.120	0.085	9.230	0.212
HSP27	4.705	0.247	4.350	0.028	4.075	0.106	4.265	0.389	4.265	0.389	4.470	0.113
STAT3 Y705	3.625	0.134	2.495	0.064	3.160	0.198	3.635	0.389	2.760	0.226	2.730	0.156
STAT3 S727	4.410	0.933	3.055	0.474	2.895	0.290	3.330	0.198	3.055	0.431	2.915	0.276
β-catenin	20.510	0.099	10.815	0.049	6.685	0.191	21.435	0.318	15.030	0.622	5.925	0.049
Akt1/2/3 S473	7.900	0.523	7.500	0.014	5.775	0.106	7.605	0.403	11.200	0.184	6.720	0.170
Akt1/2/3 T308	5.835	1.393	2.945	0.615	3.705	0.516	5.775	2.482	3.485	0.601	3.815	0.870
mTOR	6.390	0.834	5.240	0.240	5.625	0.134	7.630	0.057	5.755	0.177	6.975	0.064
PRAS40	42.730	0.792	41.505	1.322	33.440	1.287	47.260	1.640	32.585	0.460	35.155	2.072
p70 S6 kinase	5.050	0.099	2.545	0.049	3.285	0.191	4.065	0.219	3.410	0.268	3.130	0.028

Table S6. Phosphorylation signals for proteins involved in the three signaling pathways in SQ20B^{CD44low} after DMSO treatment. The values (mean $\pm$ SD) were calculated in response to normoxia, chronic hypoxia, and X-ray and C-ion irradiation under both normoxia and hypoxia.

	Normoxia		Normoxia + X-rays		Normoxia + C-ions		Hypoxia		Hypoxia + X-rays		Hypoxia + C-ions	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
p38a	2.181	0.011	1.237	0.451	0.500	0.087	1.118	0.033	1.530	0.633	0.231	0.065
ERK1/2	1.184	0.151	1.048	0.155	0.179	0.029	1.121	0.013	0.590	0.136	0.071	0.007
JNK1/2/3	2.321	0.051	1.882	0.296	0.513	0.321	2.118	0.259	2.187	0.824	0.720	0.004
MSK1/2	6.441	0.920	5.239	0.087	3.888	0.880	7.092	0.665	5.258	0.490	3.279	0.588
c-jun	0.921	0.111	0.599	0.054	0.406	0.025	0.843	0.213	0.846	0.506	0.397	0.031
WNK-1	9.801	0.346	7.625	0.703	1.436	0.047	8.541	0.825	11.734	0.533	0.902	0.031
GSK-3α/β	3.227	0.083	2.987	0.378	0.610	0.256	3.313	0.184	3.511	0.815	0.556	0.073
HSP27	1.135	0.025	0.770	0.022	0.288	0.141	0.778	0.079	0.528	0.008	0.266	0.058
STAT3 Y705	0.813	0.148	0.862	0.621	0.196	0.061	0.482	0.289	0.555	0.101	0.095	0.096
STAT3 S727	0.854	0.322	0.474	0.072	0.737	0.011	0.672	0.263	0.679	0.291	0.821	0.365
β-catenin	6.558	0.061	5.221	0.415	0.788	0.047	7.609	0.151	8.304	0.859	1.438	0.651
Akt1/2/3 S473	1.156	0.278	1.080	0.223	0.087	0.011	1.071	0.163	0.803	0.556	0.141	0.042
Akt1/2/3 T308	1.536	0.252	1.268	0.292	0.378	0.007	1.497	0.326	1.511	0.505	0.370	0.115
mTOR	0.696	0.032	1.275	0.122	0.120	0.105	0.284	0.008	0.126	0.008	0.182	0.104
PRAS40	28.605	1.122	24.834	0.032	12.309	0.307	27.140	1.515	28.036	1.286	12.625	2.460
p70 S6 kinase	1.349	0.061	0.956	0.063	0.979	0.137	0.645	0.184	0.896	0.241	1.611	0.380

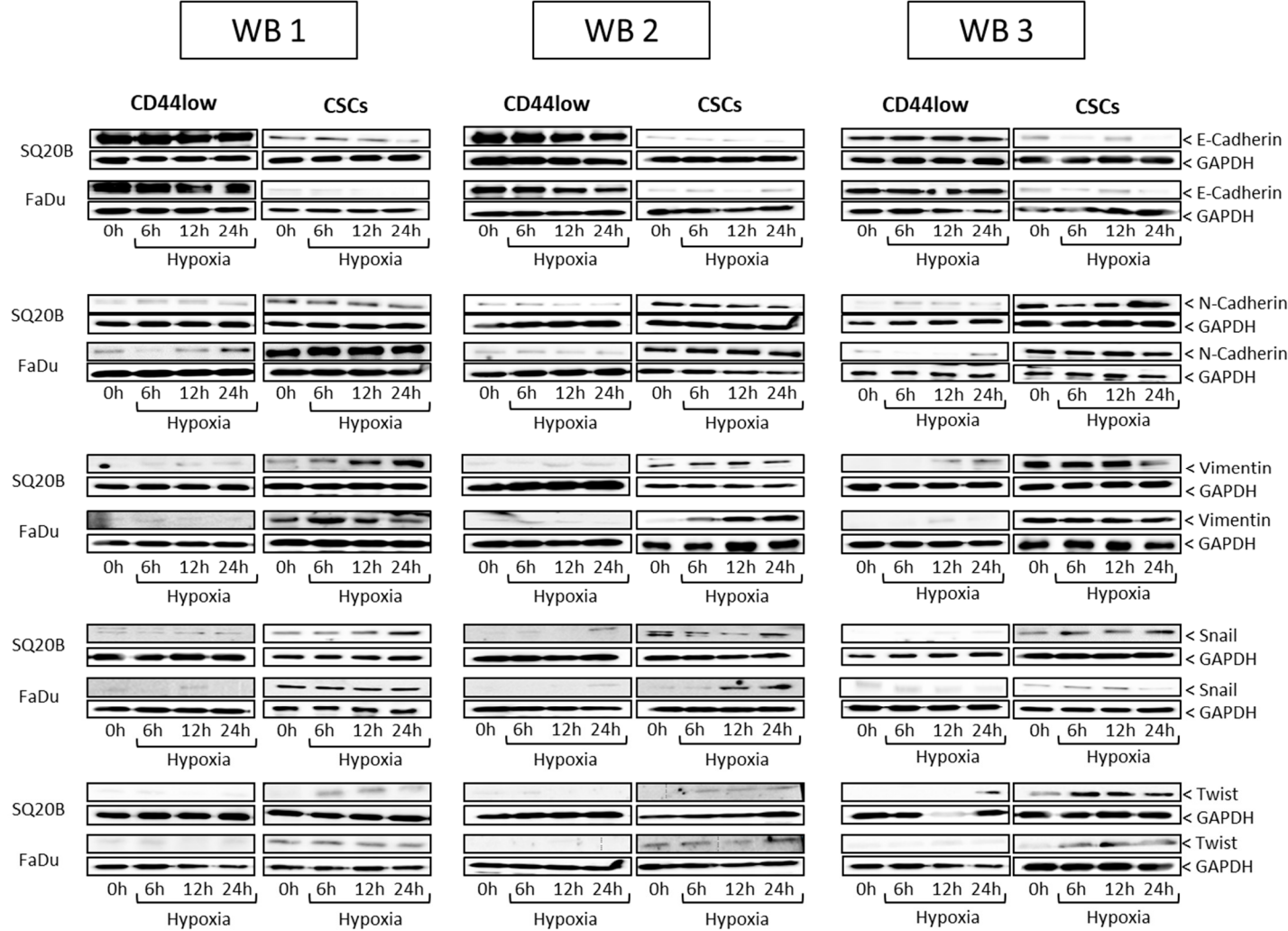


Figure S1. Impact of acute hypoxia on the EMT phenotypes of CSCs and non-CSCs.

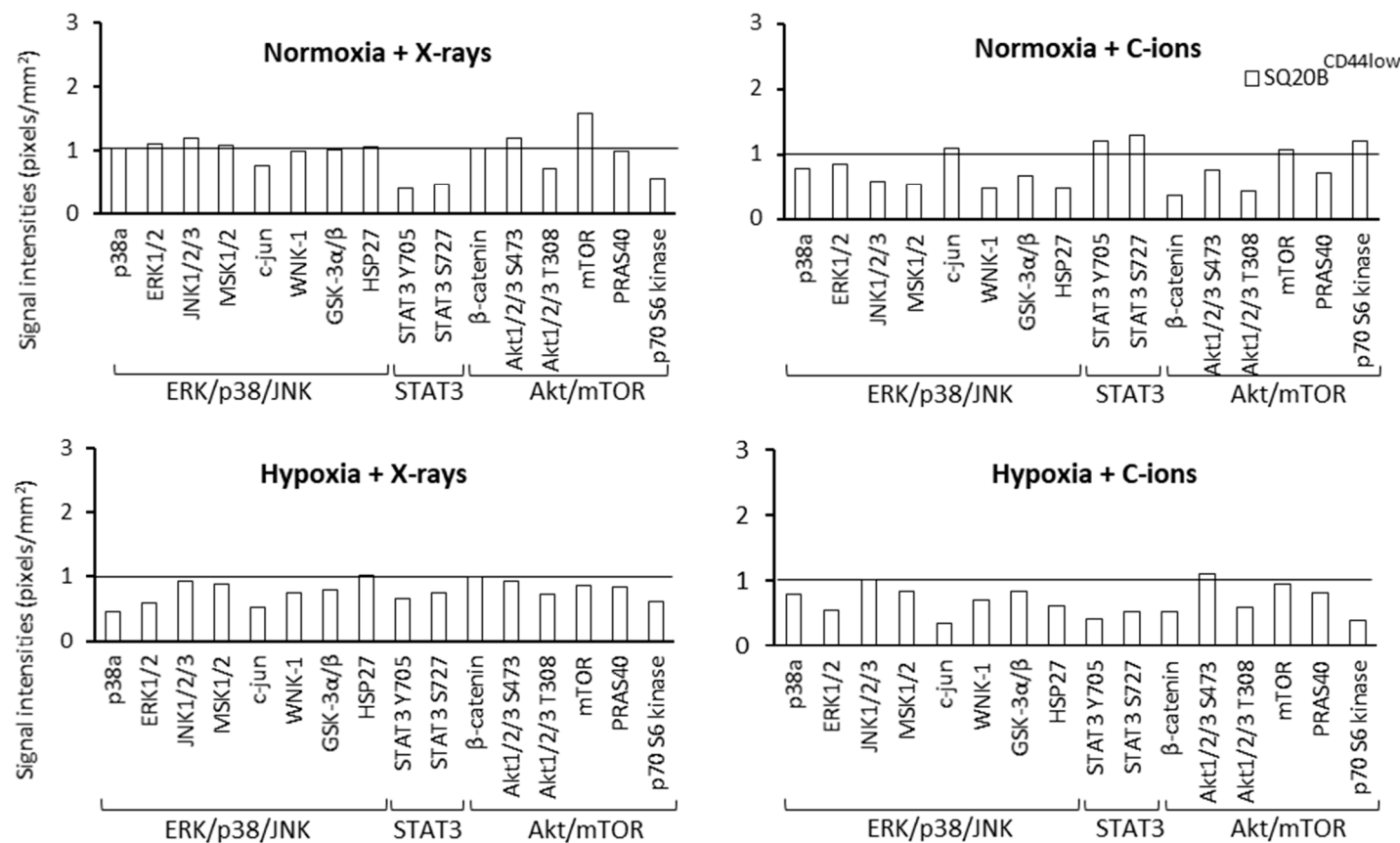


Figure S2. Signaling pathways involved in the migration/invasion processes in SQ20B^{CD44Low}. The phosphorylation levels of the proteins involved in the MEK/p38/JNK, STAT3, and Akt/mTOR pathways were determined in response to 10 Gy X-rays and C-ions ± chronic hypoxia and normalized to the basal conditions for SQ20B-CSCs using the Proteome Profiler Human-Phospho-Kinase Array. A signal > 1 corresponds to an activation of phosphorylation whereas a signal < 1 is associated with inactivation of the kinases ($n \geq 2$ in duplicate).

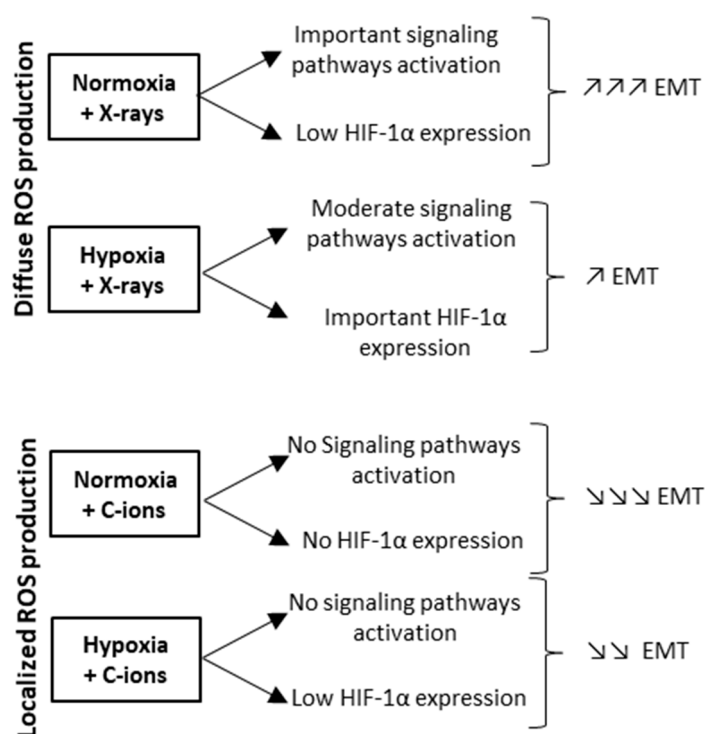


Figure S3. Schematic representation of the mechanisms involved in the EMT in response to 10 Gy X-ray and C-ion irradiation under normoxia and chronic hypoxia.



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