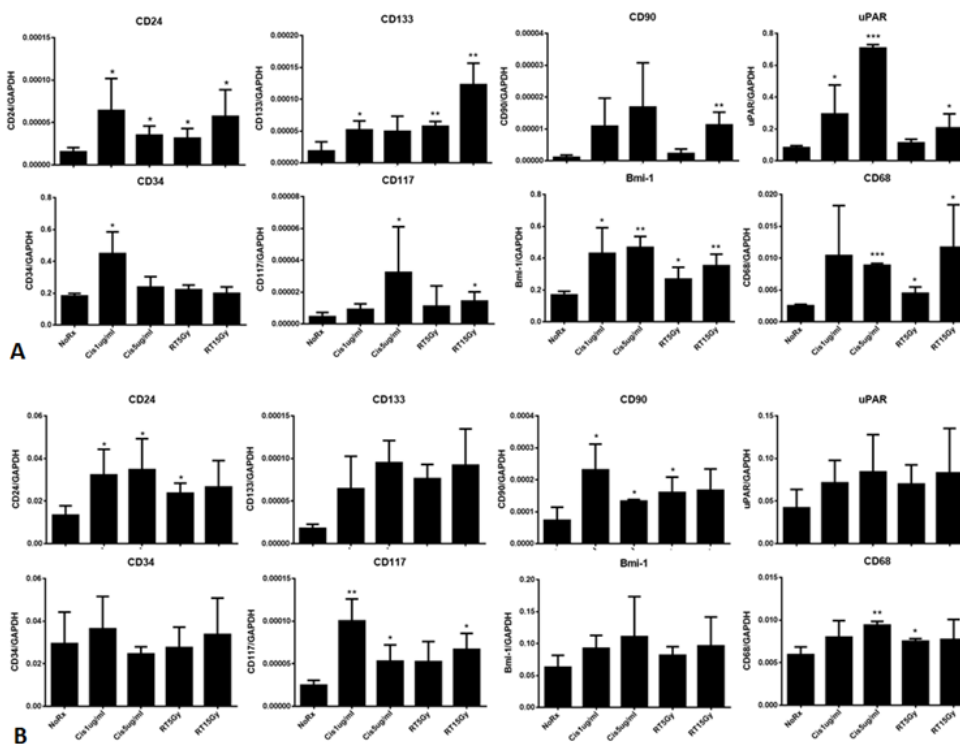
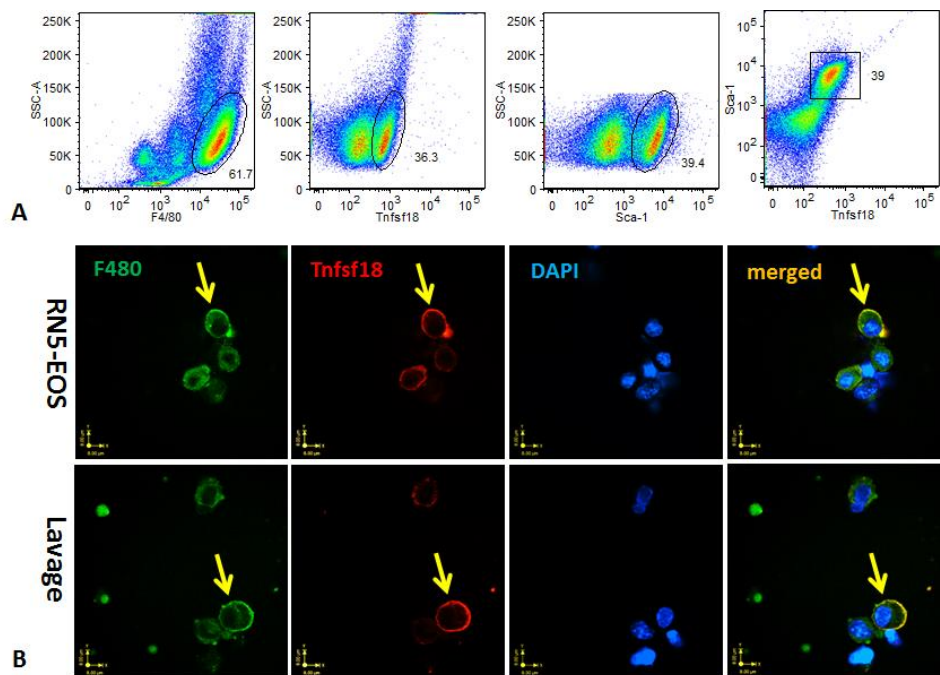


Supplementary data

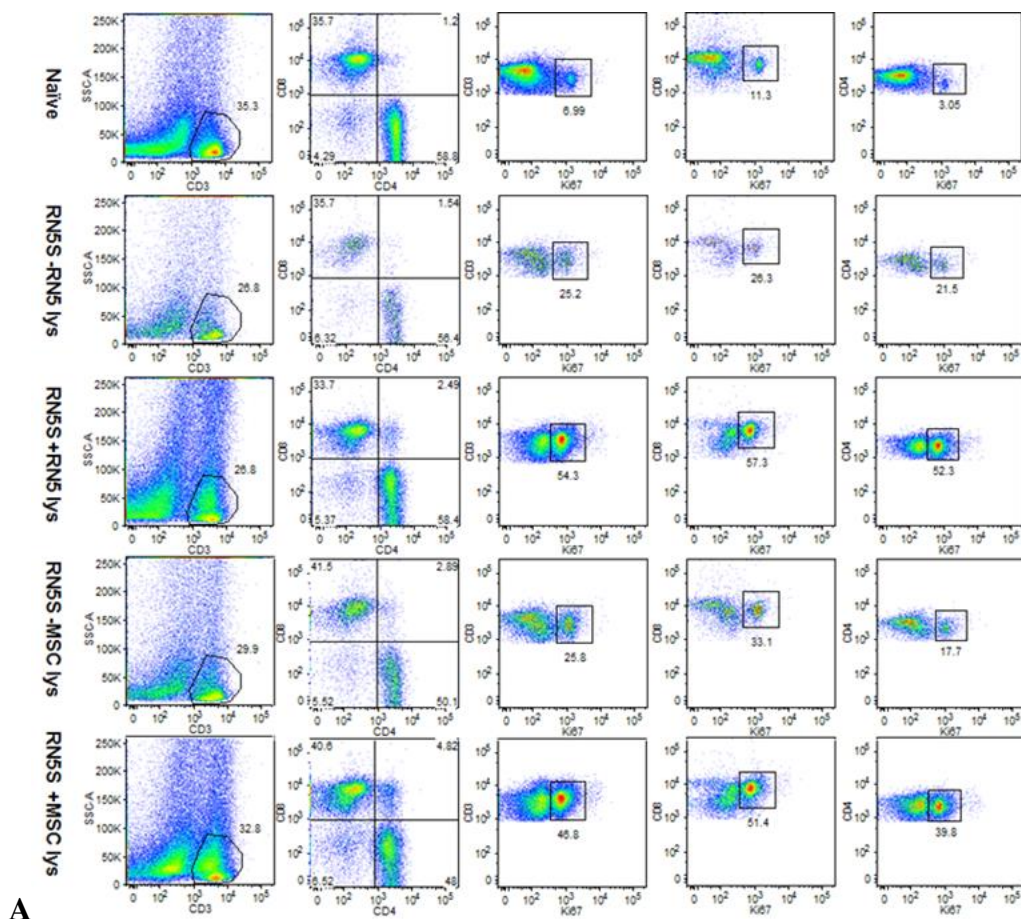
Supplementary figure 1. Expression of CSC-associated genes up-regulated in AB12 and RN5 cells after cisplatin treatment or radiation. Both AB12 and RN5 cells (5×10^6 cells/petri dish) were treated with cisplatin (1 μ g/ml and 5 μ g/ml) or with γ -ray radiation (5 or 15 Gy). Total RNA was isolated for qRT-PCR. Genes of interest were selected based on the literature to determine genes likely associated with mesothelioma stem cells. A) AB12 cells, B) RN5 cells. Data were analyzed from 3 independent experiments. The ratio of each gene to the house-keeping gene GAPDH is reported as mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

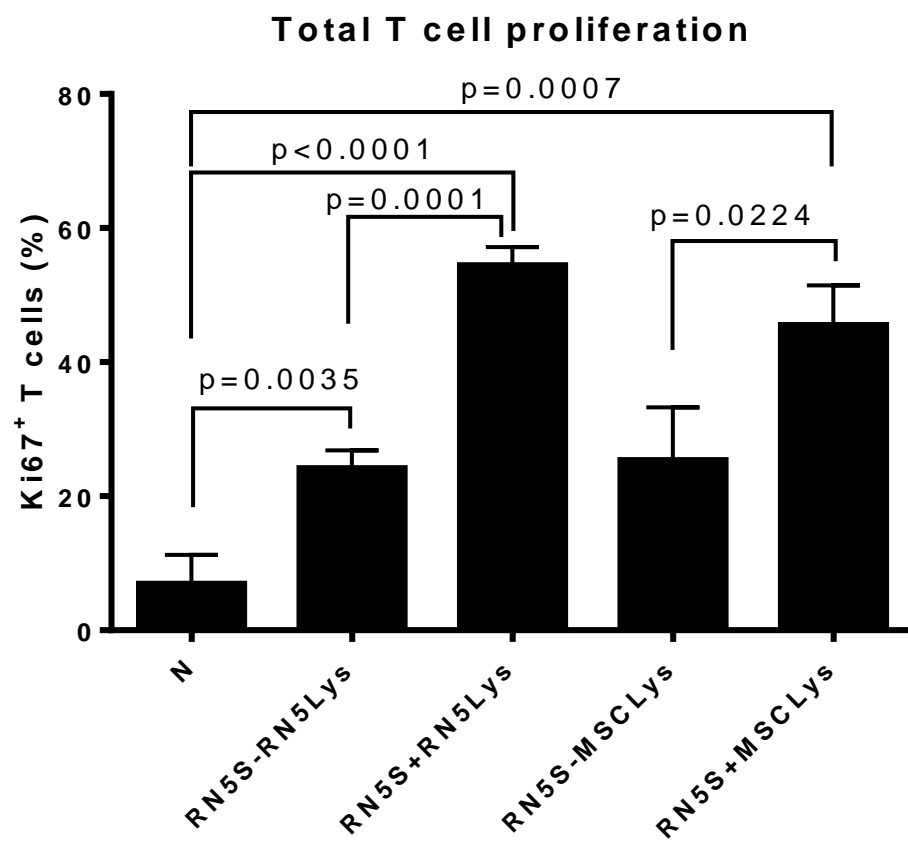


Supplementary figure 2. Tumor-associated macrophages may share the common properties with mesothelioma stem cells. A) Peritoneal lavage cells derived from mice at 4 weeks after i.p. injection of 4×10^6 RN5 cells, express high level of pan macrophage marker F4/80, Tnfsf18 and Sca-1 (Ly6a) determined by flow cytometry; B) Both RN5-EOS-Puro2 cells and peritoneal lavage cells express high levels of F4/80 and Tnfsf18.



Supplementary figure 3. *In vitro* assay of tumor-specific T cell proliferation determined by flow cytometry. Compared with naïve mice, splenocytes derived from RN5 tumor-bearing mice had a significantly higher rate of T cell proliferation (evidenced by Ki67) after overnight treatment with or without pulsation of lysates. Pulsation with RN5-EOS-Puro2 (MSC-enriched) lysates induced similar T cell activation as bulk RN5 cell lysates (A); Pulsation with lysates resulted in significant increase of T cell proliferation compared with the splenocytes of naïve (N) mice (B).





B

Supplementary Table 1 The selected gene list with 2 or more fold change in comparison with untreated RN5 cells

Gene Symbol	RefSeq	p-value	Fold-Change	p-value	Fold-Change	p-value	Fold-Change
		NoRx/Cis	NoRx/Cis	NoRx/RT	NoRx/RT	NoRx/MSC	NoRx/MSC
Nppb	NM_001287348	5.08E-11	3.46728	2.30E-10	2.79787	6.41E-11	3.34607
Serpib9b	NM_011452	2.11E-11	7.67504	4.44E-09	2.83068	5.74E-12	11.0156
A530046M15Rik	NR_046131	3.59E-10	6.20579	1.92E-08	3.0176	4.53E-08	2.69278
Ccl5	NM_013653	4.35E-11	5.94997	3.15E-08	2.17664	4.24E-10	3.81883
Gm6502	XM_006535306	3.84E-07	2.09144	4.12E-08	2.6695	3.77E-07	2.09516
Gm6502	XM_006535306	5.46E-07	2.05338	5.97E-08	2.60074	3.17E-07	2.16338
Fam71f1	NM_001289663	2.13E-10	4.37838	6.99E-08	2.03488	8.71E-09	2.52306
Ly6a	NM_001271416	5.71E-09	2.65776	7.65E-08	2.02012	1.18E-08	2.44015
Tnfrsf18	NM_183391	1.23E-08	2.73531	7.66E-08	2.22059	2.43E-10	5.2116
Csf2	NM_009969	2.97E-09	16.6869	8.47E-08	6.29559	6.88E-06	2.8256
Sprr1a	NM_009264	1.83E-09	5.27875	1.48E-07	2.59303	2.72E-08	3.26421
LOC625240	XM_889788	1.24E-06	2.534	2.17E-07	3.20489	6.67E-07	2.73901
Nr4a1	NM_010444	9.09E-09	-3.09293	2.18E-07	-2.12424	4.73E-10	-5.15002
Gm6509	ENSMUST00000098286	1.23E-06	2.3137	2.45E-07	2.80923	6.85E-07	2.47126
Spp1	NM_001204201	5.81E-09	3.66073	6.17E-07	2.0451	1.66E-07	2.3338
Ngf	NM_013609	2.31E-08	3.53783	6.70E-07	2.27235	3.79E-07	-2.41958
Igfbp5	NM_010518	1.46E-09	-6.62552	7.52E-07	-2.34755	7.72E-07	-2.34047
Ccl17	NM_011332	1.07E-09	12.4757	8.79E-07	2.92424	1.77E-06	2.66352
Pglyrp3	NM_207247	3.09E-08	3.07909	1.13E-06	2.03019	5.24E-08	2.86256
Gm7982	XM_006535314	1.70E-05	2.00772	1.61E-06	2.595	9.86E-06	2.11711
n/a	---	3.01E-05	2.79469	2.65E-06	4.15228	3.61E-06	3.92296
Gm7982	XM_006535314	2.29E-05	2.07099	2.94E-06	2.60858	2.18E-05	2.08136
Gm6502	XM_006535306	1.40E-05	2.6015	3.15E-06	3.20966	1.80E-05	2.51963
Gm26870	ENSMUST00000181957	2.25E-06	5.59193	4.82E-06	4.74211	2.06E-08	23.272
Btg2	NM_007570	2.68E-08	-4.24088	6.20E-06	-2.04276	1.84E-07	-3.09487
Mmp13	NM_008607	2.84E-07	3.24736	9.89E-06	2.09489	1.07E-05	2.07884
Prl2c2	NM_031191	1.03E-05	3.60772	3.07E-05	3.0223	4.98E-08	13.0249
Crabp2	NM_007759	8.57E-07	3.3316	3.54E-05	2.08081	1.37E-06	3.10186
Gm7963	ENSMUST00000165454	0.000327	2.80353	4.50E-05	3.94076	6.01E-05	3.73136
n/a	---	0.000178	-4.2179	0.000103	4.7464	6.51E-06	9.73731
Gm19654	AK051476	5.41E-07	-4.43468	0.00011	-2.07142	1.51E-05	-2.61073
Gm22188	ENSMUST00000179875	0.00026	4.56155	0.000184	4.93439	0.00054	3.89279
LOC102635721	XR_382369	4.68E-06	3.20511	0.000196	2.0036	1.04E-05	2.84972
Prl2c3	NM_011118	5.34E-05	2.39988	0.000227	2.0375	8.57E-08	7.74848
Gm25225	ENSMUST00000158197	0.000108	-3.04738	0.000402	-2.50503	0.00032	-2.58729
Gm24382	ENSMUST00000122698	1.54E-05	-3.76435	0.000611	-2.18732	0.000173	-2.57417
Ly6c2	NM_001099217	2.03E-05	3.70679	0.000625	2.22595	0.000231	2.53427
LOC624931	XM_006536388	0.002918	2.50379	0.000905	3.04692	0.003713	2.41022
Gm23862	ENSMUST00000180342	0.000467	2.79083	0.001033	2.4768	0.00196	2.26291
n/a	---	0.000146	-3.26686	0.001702	-2.25179	9.71E-06	-5.60768
LOC625240	XM_889788	0.006632	2.30924	0.001945	2.832	0.0067	2.30549

Supplementary table 2 Selected genes of potential murine mesothelioma stem cells overlapped with human gene signature profiling (GSEA)

Entrez Gene ID	Gene Symbol	Gene Description
4803	NGF	Nerve growth factor (beta polypeptide)
4879	NPPB	Natriuretic peptide B
6361	CCL17	Chemokine (C-C motif) ligand 17
1437	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
6696	SPP1	Secreted phosphoprotein 1
1382	CRABP2	Cellular retinoic acid binding protein 2
6698	SPRR1A	Small proline-rich protein 1A
6352	CCL5	Chemokine (C-C motif) ligand 5
8995	TNFSF18	Tumor necrosis factor (ligand) superfamily, member 18
4322	MMP13	Matrix metalloproteinase 13 (collagenase 3)
114771	PGLYRP3	Peptidoglycan recognition protein 3
84691	FAM71F1	Family with sequence similarity 71, member F1
84875	PARP10	Poly (ADP-ribose) polymerase family, member 10

Supplementary table 3 GO signaling pathways associated with the selected 41 genes

Pathway	FDR*
GO:0090066_regulation_of_anatomical_structure_size	0.006421
GO:0048588_developmental_cell_growth	0.010192
GO:0009617_response_to_bacterium	0.01004
GO:0032846_positive_regulation_of_homeostatic_process	0.007571
GO:0043069_negative_regulation_of_programmed_cell_death	0.0055
GO:0048545_response_to_steroid_hormone_stimulus	0.005667
GO:0060548_negative_regulation_of_cell_death	0.00815

*FDR: False discovery rate. FDR<0.01