

Supplementary information

Supplementary Table S1. List of primers used for qPCR.

Target	Forward / reverse primer (5'–3')
<i>CSF3</i>	CACTCTGGACAGTGCAGGAA/ AGAGAGTGTCCGAGCAGCA
<i>PDPN</i>	CTGCTCTTCGTTTTGGGAAG/ TTATAGCGGTCTTCGCTGGT
<i>IL1A</i>	GTAAGCTATGGCCCACTCCA/ AGGTGCTGACCTAGGCTTGA
<i>IL6</i>	CACAGACAGCCACTCACCTC/ AGTGCCTCTTTGCTGCTTTC
<i>ACTA2</i>	CCTATCCCCGGGACTAAGAC/ ACAATGGATGGGAAAACAGC
<i>CCN2</i>	CCTGGTCCAGACCACAGAGT/ TGGAGATTTTGGGAGTACGG
<i>TAGLN</i>	TCCAGGTCTGGCTGAAGAATGG/ CTGCTCCATCTGCTTGAAGACC
<i>MLY9</i>	GGATGTGATTCGCAACGCCTTTG/ CGGTACATCTCGTCCACTTCT
<i>GADPH</i>	AGCCACATCGCTCAGACAC / GCCCAATACGACCAAATCC

Supplementary Table S2. Average percentages and 95% confidence interval of specific killing of Jeko-1-Luc and NCI-H929-Luc cells in response to CAR19 or CAR138 NK-92 cells in 2D culture.

Conditions			% Specific killing		
			means \pm S.D.	95% CI	
Fig. 2C (left)	Jeko-1-Luc (4 h)	Luciferase	WT NK-92 (1:1)	0.00 \pm 0.00	0.00, 0.00
			CAR19 NK-92 (1:1)	38.75 \pm 5.45***	30.07, 47.43
			WT NK-92 (2:1)	0.00 \pm 0.00	0.00, 0.00
			CAR19 NK-92 (2:1)	64.48 \pm 1.90***	61.46, 67.49
		Annexin V/7-AAD	WT NK-92 (1:1)	5.23 \pm 0.57	3.82, 6.65
			CAR19 NK-92 (1:1)	51.07 \pm 1.96***	46.21, 55.92
			WT NK-92 (2:1)	9.00 \pm 1.22	5.98, 12.02
			CAR19 NK-92 (2:1)	65.40 \pm 1.15***	62.54, 68.26
Fig. 2C (right)	NCI-H929-Luc (4 h)	Luciferase	WT NK-92 (1:10)	3.10 \pm 3.93	-3.16, 9.36
			CAR138 NK-92 (1:10)	49.03 \pm 6.86***	38.12, 59.93
			WT NK-92 (1:5)	18.70 \pm 7.11	7.38, 30.02
			CAR138 NK-92 (1:5)	62.98 \pm 4.64***	55.59, 70.36
			WT NK-92 (1:2)	47.08 \pm 5.45	38.41, 55.74
			CAR138 NK92 (1:2)	93.15 \pm 2.82***	88.66, 97.64
		Annexin V/7-AAD	WT NK-92 (1:10)	10.83 \pm 1.97	7.69, 13.96
			CAR138 NK-92 (1:10)	33.40 \pm 4.85***	25.68, 41.12
			WT NK-92 (1:5)	19.13 \pm 5.13	10.96, 27.29
			CAR138 NK-92 (1:5)	42.28 \pm 4.51***	35.11, 49.44
			WT NK-92 (1:2)	33.80 \pm 5.91	24.40, 43.20
			CAR138 NK-92 (1:2)	59.78 \pm 2.64***	55.57, 63.98

*** $P < 0.001$ (power > 95%) versus WT NK-92 cells at the same concentration; two-sided Student's *t*-test.

Supplementary Table S3. Average percentages and 95% confidence interval of specific killing of NCI-H929-Luc cells in response to CAR138 NK-92 cells in the presence of activated stromal cells in 2D culture.

Conditions		% Specific killing			
		means \pm S.D.	95% CI		
Fig. 4B (upper)	E:T = 1:10 (4 h)	WT NK-92	NCI-H929	0.27 \pm 0.46	-0.88, 1.41
			+ WI-38-40	0.00 \pm 0.00	0.00, 0.00
			+ UE6E7T-3	1.37 \pm 2.37	-4.51, 7.25
		CAR138 NK-92	NCI-H929	40.43 \pm 3.11	32.70, 48.17
			+ WI-38-40	15.30 \pm 6.44 ^{***}	-0.69, 31.29
			+ UE6E7T-3	40.90 \pm 7.18 ^{###}	23.07, 58.73
	E:T = 1:5 (4 h)	WT NK-92	NCI-H929	10.23 \pm 1.30	7.00, 13.47
			+ WI-38-40	0.00 \pm 0.00 [*]	0.00, 0.00
			+ UE6E7T-3	2.60 \pm 4.50	-8.59, 13.79
		CAR138 NK-92	NCI-H929	60.87 \pm 5.83	46.39, 75.35
			+ WI-38-40	38.90 \pm 4.23 ^{***}	25.38, 49.42
			+ UE6E7T-3	56.57 \pm 9.67 ^{###}	54.91, 58.22
	E:T = 1:2 (4 h)	WT NK-92	NCI-H929	39.07 \pm 3.27	30.94, 47.19
			+ WI-38-40	6.17 \pm 2.63 ^{***}	-0.37, 12.70
			+ UE6E7T-3	7.67 \pm 9.62 ^{***}	-16.24, 31.57
CAR138 NK-92		NCI-H929	88.93 \pm 0.51	87.66, 90.21	
		+ WI-38-40	76.47 \pm 0.72 [*]	74.67, 78.26	
		+ UE6E7T-3	83.03 \pm 9.31	82.27, 83.79	
Fig. 4B (lower)	E:T = 1:10 (24 h)	WT NK-92	NCI-H929	0.00 \pm 0.00	0.00, 0.00
			+ WI-38-40	0.00 \pm 0.00	0.00, 0.00
			+ UE6E7T-3	0.00 \pm 0.00	0.00, 0.00
		CAR138 NK-92	NCI-H929	71.90 \pm 1.83	67.37, 76.43
			+ WI-38-40	28.03 \pm 3.86 ^{***}	18.46, 37.61
			+ UE6E7T-3	70.30 \pm 1.54 ^{###}	66.48, 74.12
	E:T = 1:5 (24 h)	WT NK-92	NCI-H929	26.40 \pm 1.65	22.31, 30.49
			+ WI-38-40	0.00 \pm 0.00 ^{***}	0.00, 0.00
			+ UE6E7T-3	6.77 \pm 3.50 ^{***}	-1.93, 15.47
		CAR138 NK-92	NCI-H929	86.73 \pm 0.76	84.84, 88.63
			+ WI-38-40	59.73 \pm 5.20 ^{***}	46.81, 72.66
			+ UE6E7T-3	89.50 \pm 3.31 ^{###}	81.29, 97.71
	E:T = 1:2 (24 h)	WT NK-92	NCI-H929	61.10 \pm 2.69	54.42, 67.78
			+ WI-38-40	11.17 \pm 2.96 ^{***}	3.62, 18.51
			+ UE6E7T-3	36.70 \pm 9.13 ^{***, ###}	13.02, 58.38
CAR138 NK-92		NCI-H929	97.80 \pm 0.95	95.43, 100.2	
		+ WI-38-40	89.93 \pm 1.51	86.07, 93.60	
		+ UE6E7T-3	98.50 \pm 0.26	97.84, 99.16	

^{*} $P < 0.05$, ^{***} $P < 0.001$ (power > 95%) versus 2D culture of NCI-H929-Luc cells alone under the same conditions; ^{###} $P < 0.001$ (power > 78%) versus NCI-H929-Luc with WI-38-40 cells under the same conditions; one-way ANOVA with Tukey's post-test.

Supplementary Table S4. Average percentages and 95% confidence interval of specific killing of NCI-H929-Luc cells in response to CAR138 NK-92 cells in 3D MM spheroids.

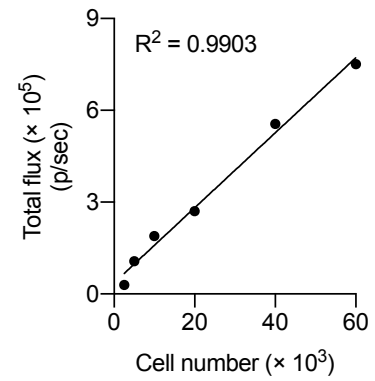
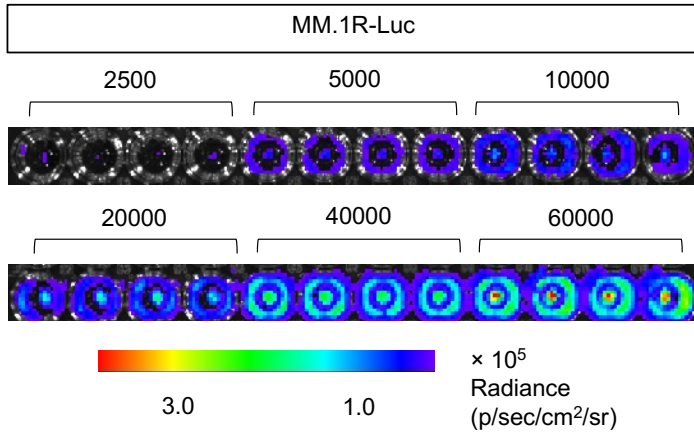
Conditions			% Specific killing	
			means \pm S.D.	95% CI
Fig. 5E	NCI-H929-Luc (4 h)	WT NK-92 (25000)	5.18 \pm 3.47	-0.35, 10.70
		CAR138 NK-92 (25000)	18.23 \pm 2.88**	13.63, 22.82
		WT NK-92 (50000)	17.03 \pm 7.21	5.55, 28.50
		CAR138 NK-92 (50000)	33.83 \pm 5.54*	25.01, 42.64
	NCI-H929-Luc (8 h)	WT NK-92 (25000)	17.73 \pm 2.49	13.77, 21.68
		CAR138 NK-92 (25000)	40.35 \pm 7.40**	28.58, 52.12
		WT NK-92 (50000)	34.73 \pm 4.82	27.05, 42.40
		CAR138 NK-92 (50000)	58.45 \pm 6.62**	47.91, 68.99
	NCI-H929-Luc (24 h)	WT NK-92 (25000)	15.43 \pm 3.23	10.28, 20.57
		CAR138 NK-92 (25000)	62.15 \pm 13.51***	40.65, 83.65
		WT NK-92 (50000)	31.20 \pm 5.90	21.82, 40.58
		CAR138 NK-92 (50000)	78.20 \pm 8.12***	65.28, 91.12

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (power > 95%) versus WT NK-92 cells at the same conditions; two-sided Student's *t*-test.

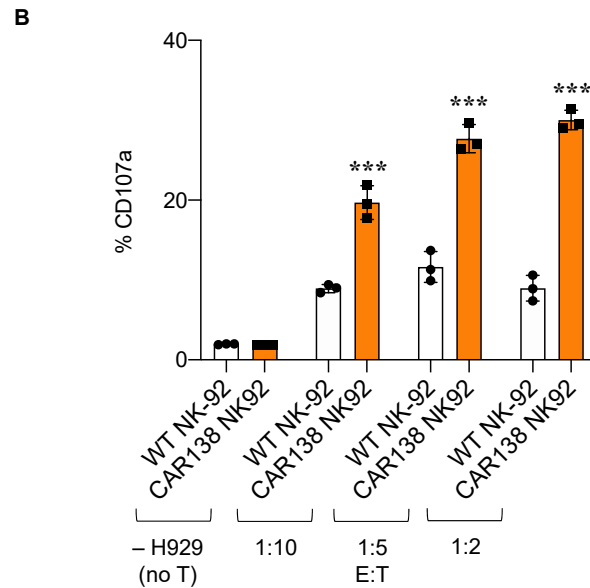
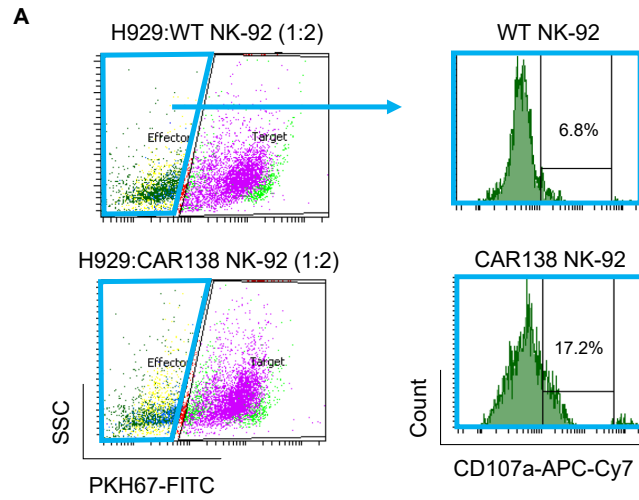
Supplementary Table S5. Average percentages and 95% confidence interval of specific killing of NCI-H929-Luc cells in response to CAR138 NK-92 cells in the presence or absence of activated stromal cells in 3D MM spheroids.

Conditions			% Specific killing		
			means \pm S.D.	95% CI	
Fig. 6D (upper)	NK 25000 (4 h)	WT NK-92	NCI-H929	1.00 \pm 2.45	-1.57, 3.57
			+ WI-38-40	0.82 \pm 2.00	-1.28, 2.91
	CAR138 NK-92	NCI-H929	23.92 \pm 4.89	18.78, 29.05	
		+ WI-38-40	1.23 \pm 3.02 ^{***}	-1.94, 4.40	
	NK 50000 (4 h)	WT NK-92	NCI-H929	25.17 \pm 4.91	20.02, 30.32
			+ WI-38-40	18.50 \pm 2.96 [*]	15.40, 21.60
CAR138 NK-92	NCI-H929	60.05 \pm 5.17	54.62, 65.48		
	+ WI-38-40	41.75 \pm 9.56 ^{**}	31.68, 51.82		
Fig. 6D (lower, left)	NK 25000 (8 h)	WT NK-92	NCI-H929	28.90 \pm 8.10	20.40, 37.40
			+ WI-38-40	14.85 \pm 4.93 ^{**}	9.67, 20.03
	CAR138 NK-92	NCI-H929	56.38 \pm 5.38	50.74, 62.03	
		+ WI-38-40	28.72 \pm 5.47 ^{***}	22.98, 34.46	
	NK 50000 (8 h)	WT NK-92	NCI-H929	57.68 \pm 6.00	51.38, 63.98
			+ WI-38-40	51.58 \pm 15.79	35.02, 68.15
CAR138 NK-92	NCI-H929	86.05 \pm 3.22	82.67, 89.43		
	+ WI-38-40	77.82 \pm 12.08	65.14, 90.49		
Fig. 6D (lower, right)	NK 25000 (24 h)	WT NK-92	NCI-H929	32.10 \pm 5.58	26.24, 37.96
			+ WI-38-40	37.93 \pm 13.30	23.97, 51.89
	CAR138 NK-92	NCI-H929	70.83 \pm 6.46	64.06, 77.61	
		+ WI-38-40	61.55 \pm 6.77 [*] #1	54.45, 68.65	
	NK 50000 (24 h)	WT NK-92	NCI-H929	41.87 \pm 3.51	38.18, 45.55
			+ WI-38-40	53.98 \pm 6.01 ^{**}	47.68, 60.29
CAR138 NK-92	NCI-H929	88.32 \pm 3.73	84.40, 92.23		
	+ WI-38-40	77.03 \pm 2.82 ^{***}	74.07, 79.99		

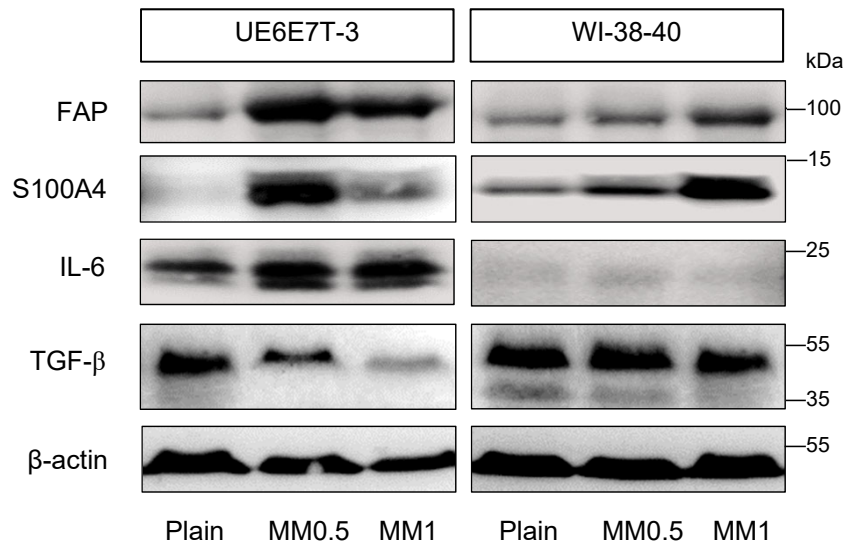
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (power > 80%; except for remark #1 at power ~70%) versus 3D culture of NCI-H929-Luc cells alone under the same conditions; two-sided Student's *t*-test.



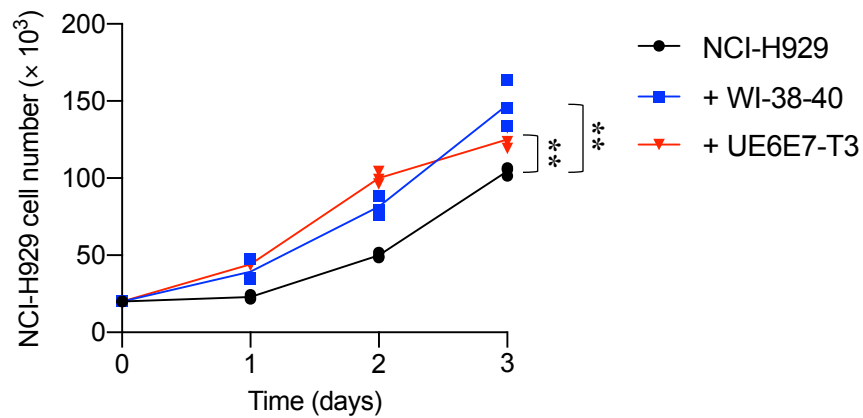
Supplementary Figure S1. Luciferase activity in human MM MM.1R-Luc cells was assessed by IVIS bioluminescence imaging. Data showed a linear correlation between luminescence signal (total flux) and cell number.



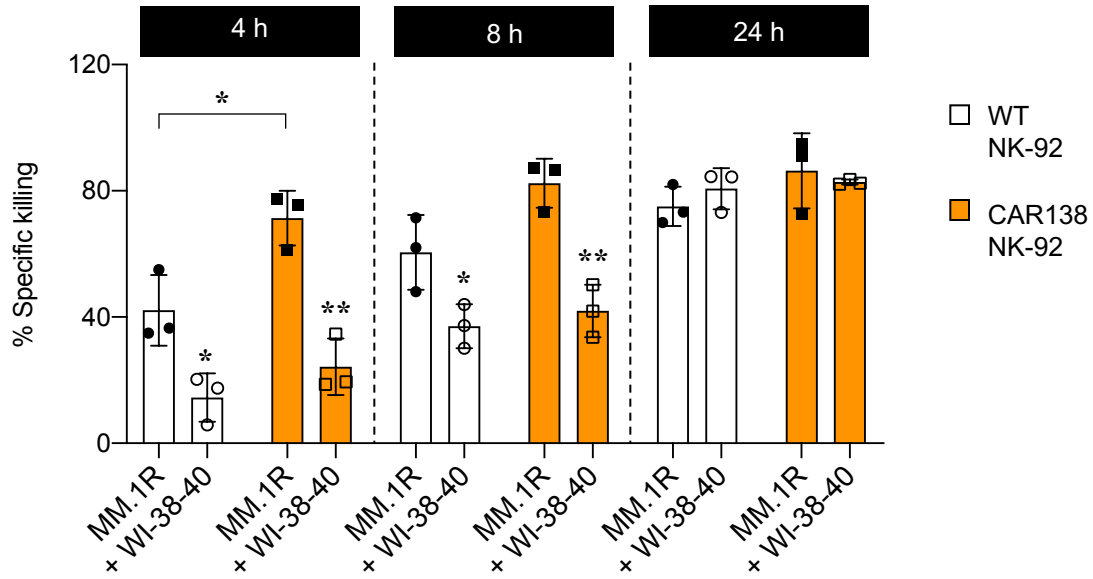
Supplementary Figure S2. Flow cytometric analysis of CD107a in NK cells upon NCI-H929 exposure. WT or CAR138 NK-92 cells were exposed to NCI-H929 cells at various E:T ratios for 4 h. (A) Representative flow cytometric analysis of CD107a, a functional NK marker, in WT or CAR138 NK-92 cells. (B) Percentages of CD107a in WT or CAR138 NK-92 cells at various E:T ratios were shown. *** $P < 0.001$ versus WT NK-92 cells at the same E:T ratio; two-sided Student's t -test.



Supplementary Figure S3. Western blot analysis of common CAF markers in UE6E7T-3 and WI-38-40 cells upon exposure to various concentrations of MM-CM, i.e., MM0.5-CM and MM1-CM, at 48 h.



Supplementary Figure S4. Activated stromal cells promoted MM cell proliferation in 2D culture. NCI-H929-Luc cells were cocultured with WI-38-40 and UE6E7-T3 cells activated by MM-CM, and NCI-H929 cell number was evaluated by luciferase assay. $**P < 0.05$ versus NCI-H929 cells alone at day 3; two-sided Student's *t*-test.



Supplementary Figure S5. Percentages of specific killing of MM.1R-Luc cells in 3D MM/CAF spheroids (formed for 2 days) in response to WT and CAR138 NK-92 cells at 25000 cells at 4–24 h as evaluated by luciferase assay. * $P < 0.05$, ** $P < 0.01$ versus bare 3D MM spheroids without activated stromal cells under the same conditions or as indicated; two-sided Student's *t*-test.