

## **Supplementary Information**

### **Contents:**

Supplementary Table S1 Correlation between the expressions of NCL and PD-L1 and clinico-pathological data of TNBC patient tissues

Supplementary Table S2 Multivariate analysis by Cox proportional hazard regression model for the evaluation of prognostic factors

Supplementary Fig. S1 NCL and PD-L1 expressions in TNBC samples

Supplementary Fig. S2 SmartDCs-NCL expression and generation

Supplementary Fig. S3 The quantification of LC3, p62 and PD-L1 expressions in response to CIQ treatment by ImageJ

Supplementary Fig. S4 Colony formation assay of MDA-MB-231 and MCF-7 treated with NCL-specific T cells with or without anti-PD-L1 peptide or curcumin or spautin-1

**Supplementary Table S1.** Correlation between the expressions of NCL and PD-L1 and clinicopathological data of TNBC patient tissues demonstrated by immunohistochemical staining ( $n=144$ ).

Variable (no. of patients)	<i>n</i>	NCL IHC score		<i>P</i>	PD-L1 IHC score		<i>P</i>
		Low	High		Low	High	
Age (years)							
≤55	99	23	76		59	40	
>55	45	12	33	0.402	23	22	0.220
pT stage							
1-2	134	31	103		78	56	
3-4	10	4	6	0.201	4	6	0.214
pM stage							
Absence	144	35	109		58	86	
Presence	0	0	0	ns	0	0	ns
LN metastasis							
Negative	93	24	69		58	35	
Positive	51	11	40	0.362	24	27	0.055
Perineural metastasis							
Negative	121	31	90		73	48	
Positive	23	4	19	0.290	9	14	0.050
Tumor size (cm)							
≤5	137	32	105		78	59	
>5	7	3	4	0.225	4	3	0.652

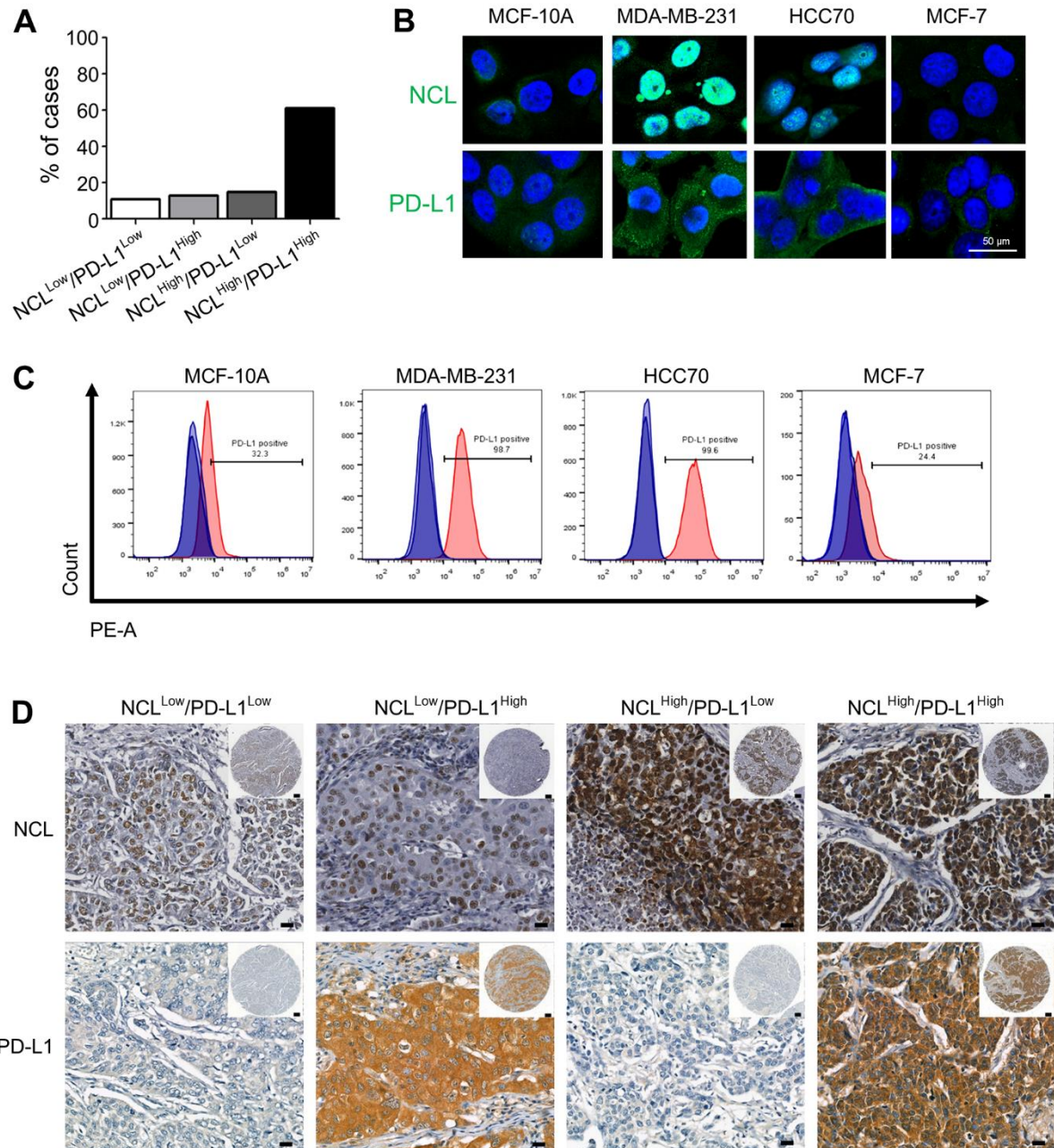
Fisher's exact probability test was used for the comparison of variables that had 2 categories; ns:

No statistics are computed because the number is a constant.

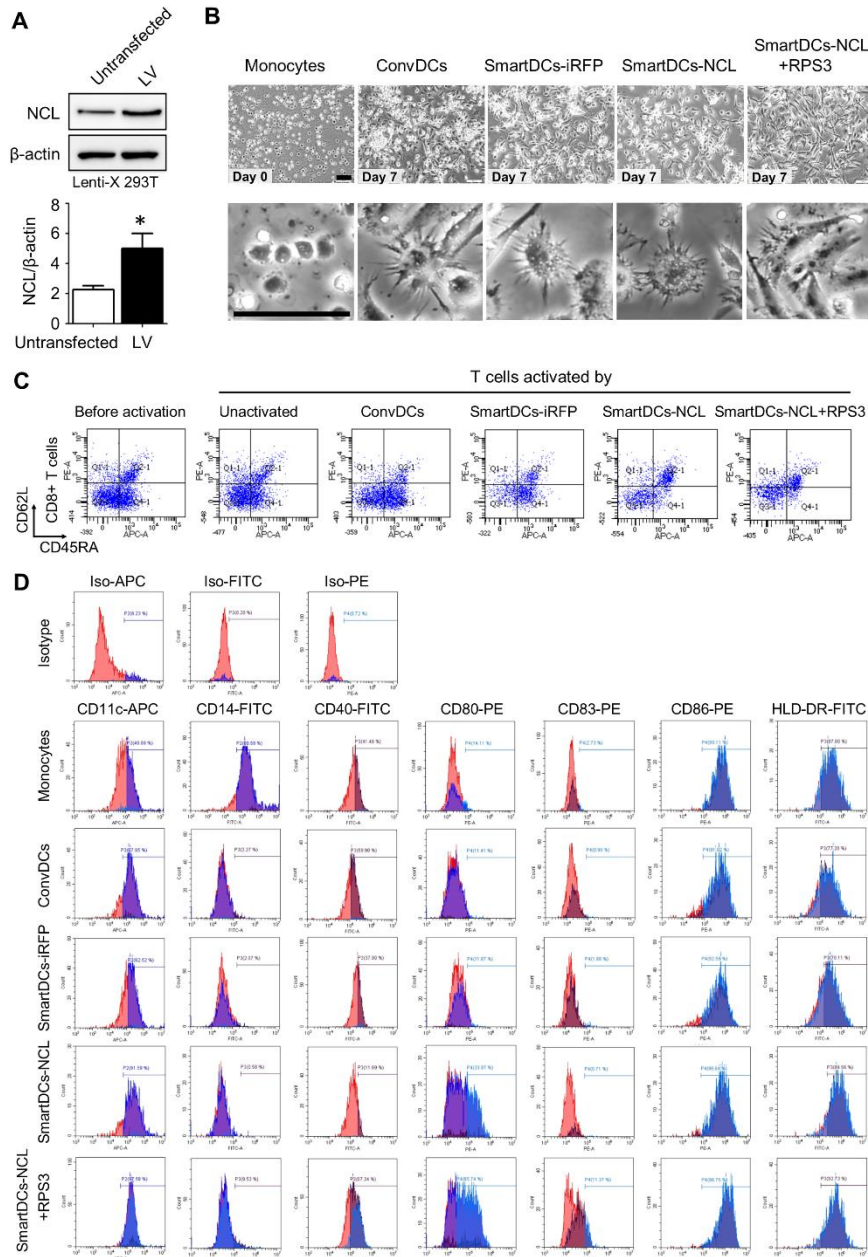
**Supplementary Table S2.** Multivariate analysis by Cox proportional hazard regression model for the evaluation of prognostic factors.

Variable (no. of patients)	Univariate analysis			Multivariate analysis		
	Hazard ratio (HR)	95% Confidence interval (CI)	<i>P</i>	Hazard ratio (HR)	95% Confidence interval (CI)	<i>P</i>
Age (years)						
≤55 (99)	1			1		
>55 (45)	1.185	0.830-1.691	0.351	1.189	0.832-1.698	0.342
pT stage						
1-2 (134)	1			1		
3-4 (10)	1.055	0.538-2.069	0.877	0.880	0.416-1.538	0.503
pN stage						
1-2 (135)	1			1		
3-4 (9)	0.897	0.451-1.786	0.758	1.052	0.534-1.072	0.884
pM stage						
Absence (144)	1			1		
Presence (0)	ns	ns	ns	ns	ns	ns
Clinical staging						
1-2 (117)	1			1		
3-4 (27)	0.854	0.557-1.310	0.471	1.007	0.961-1.065	0.080
LN metastasis						
Negative (93)	1			1		
Positive (51)	1.197	0.847-1.692	0.308	1.577	0.937-2.653	0.086
Perineural metastasis						
Negative (121)	1			1		
Positive (23)	0.993	0.633-1.559	0.976	0.235	0.378-1.270	0.693
Tumor size (cm)						
≤5 (137)	1			1		
>5 (7)	0.924	0.845-1.011	0.084	1.724	0.840-3.537	0.138

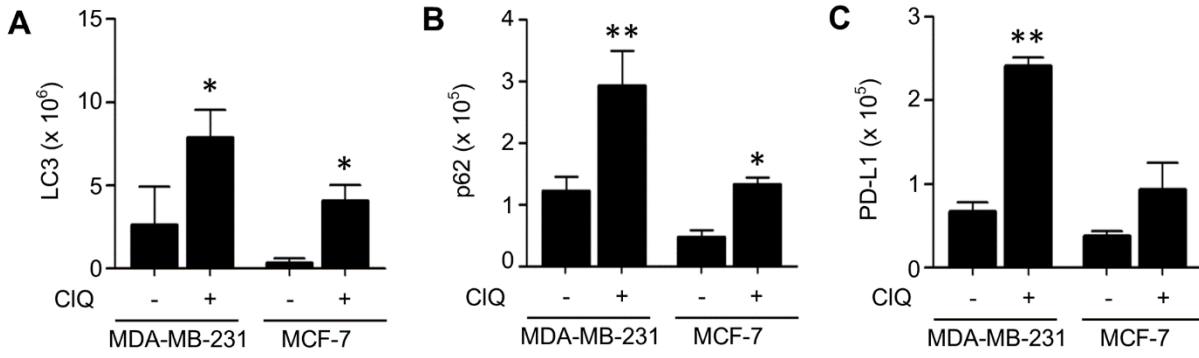
Univariate and multivariate analysis by Cox proportional hazard regression; CI 95% indicates 95% confidence interval; ns: No statistics are computed because the number is a constant.



**Supplementary Figure S1.** NCL and PD-L1 expressions in TNBC samples and cell lines. **(A)** The number of cases in 144 matched pair cases of NCL<sup>Low</sup>/PD-L1<sup>Low</sup>, NCL<sup>Low</sup>/PD-L1<sup>High</sup>, NCL<sup>High</sup>/PD-L1<sup>Low</sup> and NCL<sup>High</sup>/PD-L1<sup>High</sup>. **(B)** Immunofluorescence staining of NCL and PD-L1, and **(C)** flow cytometry of surface PD-L1 content in three breast cancer cell lines, MDA-MB-231, HCC70 and MCF-7 compared to the MCF-10A normal breast line. Scale bar = 50  $\mu$ M; original magnification = 630x. Magnification is 400x. Data are shown as a representative figure of triplicates obtained in three independent experiments. **(D)** Immunohistochemistry staining of NCL and PD-L1 in breast cancer tissues (Scale bar = 200  $\mu$ M; original magnification of 50x and scale bar = 20  $\mu$ M; original magnification of 400x).

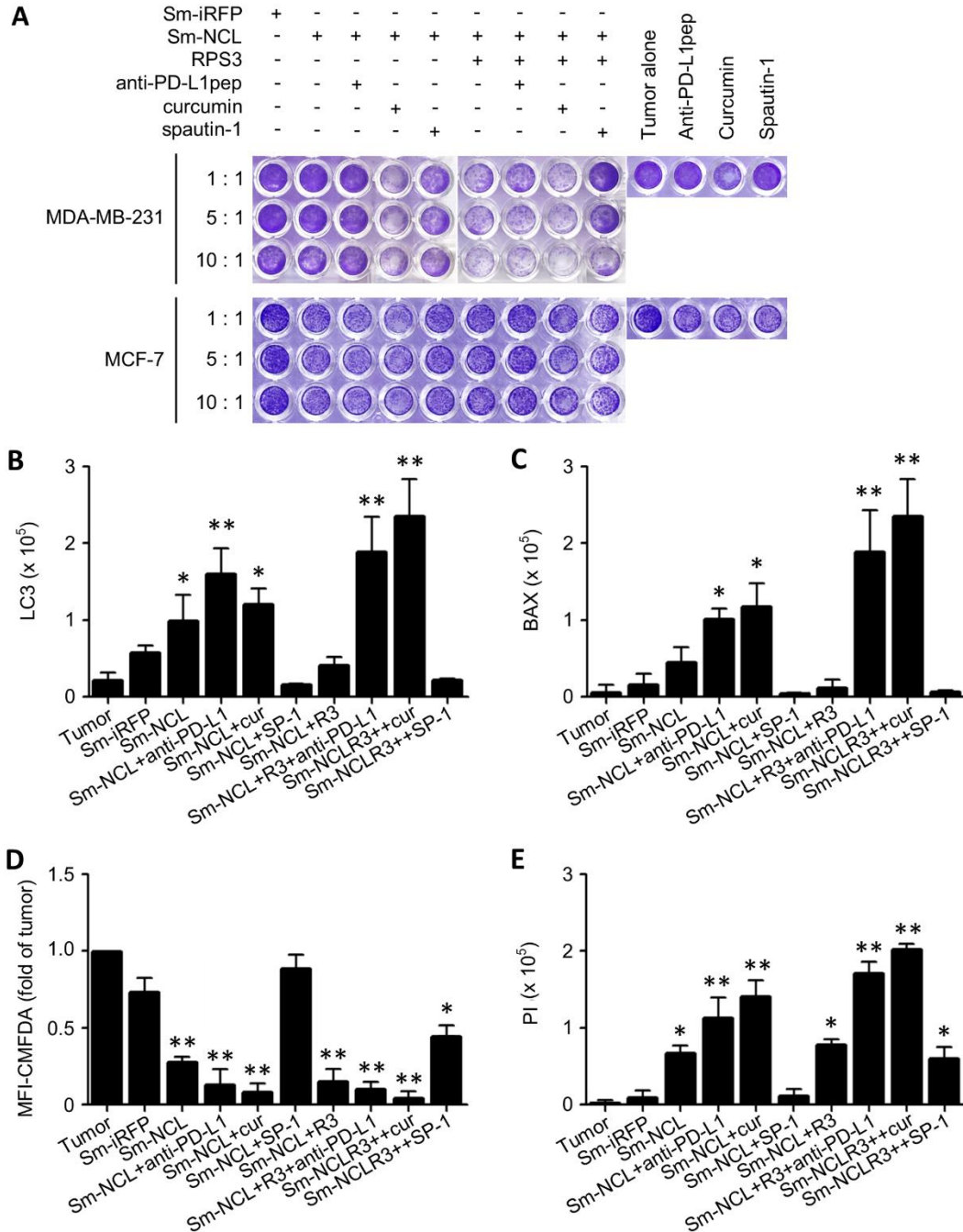


**Supplementary Figure S2. SmartDCs-NCL generation.** (A) Western blot (representatives of three independent experiments) analysis of NCL expression in Lenti-X 293T cells transfected with lentivirus. Data are shown as mean  $\pm$  SD. \*,  $P < 0.05$  compared to untransfected cells. (B) Morphology of monocytes at day 0, cytokine-driven monocyte-derived DCs by conventional method (ConvDCs), SmartDCs-iRFP, SmartDCs-NCL and SmartDCs-NCL+RPS3 at days 7 of culture. Scale bars represent 50  $\mu$ m. (C) Flow cytometry dot plot analysis of T cell subset. Indicated are comparisons of populations of CD3<sup>+</sup>, CD8<sup>+</sup> T cells that are defined as effector memory T cells (T<sub>EM</sub>; CD3<sup>+</sup>/CD8<sup>+</sup>/CD45RA<sup>-</sup>/CD62L<sup>-</sup>), late effector memory T cells re-expressing CD45RA (T<sub>EMRA</sub>; CD3<sup>+</sup>/CD8<sup>+</sup>/CD45RA<sup>+</sup>/CD62L<sup>-</sup>) and central memory T cells (T<sub>CM</sub>; CD3<sup>+</sup>/CD8<sup>+</sup>/CD45RA<sup>-</sup>/CD62L<sup>+</sup>) and naive T cells (T<sub>N</sub>; CD3<sup>+</sup>/CD8<sup>+</sup>/CD45RA<sup>+</sup>/CD62L<sup>+</sup>). (D) Representative histogram of the flow cytometry analysis of co-stimulatory markers of monocytes and DCs. All data shown were reproduced in three separated experiments.



**Supplementary Figure S3.** The quantification of (A) LC3, (B) p62 and (C) PD-L1 expressions in response to CIQ treatment by ImageJ. The data were reproduced in three separated experiments. Y-axis represents the ImageJ quantification of fluorescence signals. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .





**Supplementary Figure S4.** (A) Colony formation (representative of three separate experiments) assay of MDA-MB-231 and MCF-7 in NCL-specific T cells with or without anti-PD-L1 peptide (anti-PD-L1 pep) or curcumin or spautin-1 treatment. (B) The quantification of BAX and (C) LC3 expressions by ImageJ (n=3). (D) Mean fluorescence intensity (MFI) and (E) propidium iodine (PI)-relative to spheroid treated with tumor alone at 24 h after treatment were calculated and plotted at E : T of 10 : 1. Scale bar = 100  $\mu$ M; original magnification of 100x. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  and \*\*\*,  $P < 0.001$ . #,  $P < 0.05$  compared to unactivated T cells conditioned; §,  $P < 0.05$  compared to E : T ratio 1 : 1 of each treatment. Scale bar = 20  $\mu$ M; original magnification = 630x (n=3).