PLK1 Inhibition Induces Immunogenic Cell Death and Enhances Immunity Against NSCLC

Fig S1. Negative correlation between PLK1 expression and infiltrating levels of T cells, B lineage, myeloid dendritic cells and endothelial cells in LUAD mined from the TCGA database.

Fig S2. Gating strategies for the detection ratio of CD4⁺/CD8⁺ T cells.

Fig S3. BI2536 could hardly enhance DC function.
A. The percentage of CD11c⁺ BMDCs with engulfed Lewis cells after treatment with
BI2536.

B-D. Surface expression of CD80, CD86 and MHCII on BI2536-treated CD11c⁺ DCs.

**Supplementary Table 1. Multiple Comparisons of the PLK1 expressions at stages 1-4**

| (I) Stage | (J) Stage | Mean difference (I-J) | Std. Error | Sig. | 95% CI |
|-----------|-----------|-----------------------|------------|------|--------|
| 1         | 2         | -0.03918*             | 0.0107     | 0    | -0.0602 to -0.0181 |
| 1         | 3         | -0.04520*             | 0.01236    | 0    | -0.0695 to -0.0209 |
| 1         | 4         | -0.06015*             | 0.01996    | 0.003 | -0.0994 to -0.0209 |
| 2         | 1         | 0.03918*              | 0.0107     | 0    | 0.0181 to 0.0602   |
| 2         | 3         | -0.00602              | 0.01409    | 0.669 | -0.0337 to 0.0217  |
| 2         | 4         | -0.02097              | 0.02107    | 0.32 | -0.0624 to 0.0204  |
| 3         | 1         | 0.04520*              | 0.01236    | 0    | 0.0209 to 0.0695   |
| 3         | 2         | 0.00602               | 0.01409    | 0.669 | -0.0217 to 0.0337  |
| 3         | 4         | -0.01495              | 0.02196    | 0.496 | -0.0581 to 0.0282  |
| 4         | 1         | 0.06015*              | 0.01996    | 0.003 | 0.0209 to 0.0994   |
| 4         | 2         | 0.02097               | 0.02107    | 0.32 | -0.0204 to 0.0624  |
| 4         | 3         | 0.01495               | 0.02196    | 0.496 | -0.0282 to 0.0581  |

* The mean difference is significant at the 0.05 level.

LSD = least significance difference
### Supplementary Table 2. ANOVA analysis of the PLK1 expression at stages 1-4

|                      | Sum of squares | df  | Mean square | F    | Sig. |
|----------------------|----------------|-----|-------------|------|------|
| Between Groups       | 0.246          | 3   | 0.082       | 8.67 | 0    |
| Within Groups        | 4.683          | 495 | 0.009       |      |      |
| Total                | 4.929          | 498 |             |      |      |

### Supplementary Table 3. Multiple Comparisons of the PLK1 expression at stages 2-4

**LSD**

| (I)Stage | (J) Stage | Mean difference (I-J) | Std. Error | Sig. | 95% CI |
|----------|-----------|-----------------------|------------|------|--------|
|          |           |                       |            |      | Lower Bound | Upper Bound |
| 2        | 3         | -0.00602              | 0.01352    | 0.657| -0.0327     | 0.0206       |
|          | 4         | -0.02097              | 0.02022    | 0.301| -0.0608     | 0.0189       |
| 3        | 2         | 0.00602               | 0.01352    | 0.657| -0.0206     | 0.0327       |
|          | 4         | -0.01495              | 0.02107    | 0.479| -0.0565     | 0.0266       |
| 4        | 2         | 0.02097               | 0.02022    | 0.301| -0.0189     | 0.0608       |
|          | 3         | 0.01495               | 0.02107    | 0.479| -0.0266     | 0.0565       |

LSD= least significance difference

### Supplementary Table 4. ANOVA analysis of the PLK1 expression at stages 2-4

|                      | Sum of squares | df  | Mean square | F    | Sig. |
|----------------------|----------------|-----|-------------|------|------|
| Between Groups       | 0.01           | 2   | 0.005       | 0.553| 0.576|
| Within Groups        | 1.925          | 221 | 0.009       |      |      |
| Total                | 1.935          | 223 |             |      |      |
Supplementary Table 5. Correlations between PLK1 and immune checkpoints

| Variable 1 | Variable 2 | Pearson correlation coefficient | P value |
|------------|------------|---------------------------------|---------|
| PLK1       | PD1        | 0.1561                          | 0.0005  |
| PLK1       | PDL1       | 0.2644                          | <0.0001 |
| PLK1       | LAG3       | 0.1779                          | <0.0001 |
| PLK1       | SIGLEC10   | 0.1673                          | 0.0002  |
| PLK1       | IDO1       | 0.1682                          | 0.0002  |
| PD1        | PDL1       | 0.4063                          | <0.0001 |
| PD1        | LAG3       | 0.613                           | <0.0001 |
| PD1        | SIGLEC10   | 0.554                           | <0.0001 |
| PD1        | IDO1       | 0.238                           | <0.0001 |
| PDL1       | LAG3       | 0.2966                          | <0.0001 |
| PDL1       | SIGLEC10   | 0.4036                          | <0.0001 |
| PDL1       | IDO1       | 0.302                           | <0.0001 |
| LAG3       | SIGLEC10   | 0.4968                          | <0.0001 |
| LAG3       | IDO1       | 0.3463                          | <0.0001 |
| SIGLEC10   | IDO1       | 0.2761                          | <0.0001 |