Appendix

Modulation of intrinsic inhibitory checkpoints using nanocarriers to unleash NK cell activity

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Appendix Table S1

Exact statistical test results for indicated figures.

| Figure 1A | comparison           | test                  | pval    | padj  |
|------------|----------------------|-----------------------|---------|-------|
| Ctrl - SHP-1 | 1-sample t-test      | 0.0156               | 0.0156  |
| Ctrl - Cbl-b | 1-sample t-test      | 0.0097               | 0.0145  |
| Ctrl - c-Cbl | 1-sample t-test      | 0.0076               | 0.0145  |

| Figure 1C | comparison           | test                  | pval    | padj  |
|------------|----------------------|-----------------------|---------|-------|
| MOCK _CW4_MOCK _CW7    | 1-sample t-test      | 0.0005               | 0.0016  |
| MOCK _CW4_NS_CW4       | 1-sample t-test      | 0.4916               | 0.4916  |
| MOCK _CW4_SHP-1 and Cbls siRNA _CW4  | 1-sample t-test      | 0.027               | 0.0404  |
| ALL                  | 1-way ANOVA          | 0.0099               | NA      |
| MOCK _CW7-SHP-1 and Cbls siRNA _CW4  | Tukey                | NA                  | 0.987   |
| NS_CW4-SHP-1 and Cbls siRNA _CW4   | Tukey                | NA                  | 0.0177  |
| NS_CW4-SHP-1 and Cbls siRNA _CW7   | Tukey                | NA                  | 0.0181  |

| Figure 1D | comparison           | test                  | pval    | padj  |
|------------|----------------------|-----------------------|---------|-------|
| ALL                  | 1-way ANOVA          | 0.0039               | NA      |
| Mock_CW4-SHP-1 and Cbls siRNA _CW4  | Tukey                | NA                  | 0.0222  |
| Mock_CW7-SHP-1 and Cbls siRNA _CW4  | Tukey                | NA                  | 0.9321  |
| NS_CW4-SHP-1 and Cbls siRNA _CW4   | Tukey                | NA                  | 0.0349  |
| Mock_CW7-Mock _CW4   | Tukey                | NA                  | 0.0133  |
| NS_CW4-Mock _CW4     | Tukey                | NA                  | 0.9503  |
| NS_CW4-Mock _CW7     | Tukey                | NA                  | 0.02    |

| Figure 1E | comparison           | test                  | pval    | padj  |
|------------|----------------------|-----------------------|---------|-------|
| ALL                  | 1-way ANOVA          | 0.0002               | NA      |
| Mock_CW4-SHP-1 and Cbls siRNA _CW4  | Tukey                | NA                  | 0.0119  |
| Mock_CW7-SHP-1 and Cbls siRNA _CW4  | Tukey                | NA                  | 0.9106  |
| N.S._CW4-SHP-1 and Cbls siRNA _CW4  | Tukey                | NA                  | 0.0101  |
| Mock_CW7-Mock _CW4   | Tukey                | NA                  | 0.0035  |
| N.S._CW4-Mock _CW4   | Tukey                | NA                  | 0.9991  |
| N.S._CW4-Mock _CW7   | Tukey                | NA                  | 0.003   |

Figure 4A YTS
### Figure 4A pNK

| comparison          | test            | pval     | padj  |
|---------------------|-----------------|----------|-------|
| control-SHP-1       | 1-sample t-test | 0.0024   | 0.0049|
| control-Cbl-b       | 1-sample t-test | 0.0032   | 0.0049|
| control-c-Cbl       | 1-sample t-test | 0.0104   | 0.0104|

### Figure 4C

| comparison          | test            | pval     | padj  |
|---------------------|-----------------|----------|-------|
| control-SHP-1       | 1-sample t-test | 0.0024   | 0.0049|
| control-Cbl-b       | 1-sample t-test | 0.0032   | 0.0049|
| control-c-Cbl       | 1-sample t-test | 0.0104   | 0.0104|

### Figure 4D

| comparison          | test            | pval     | padj  |
|---------------------|-----------------|----------|-------|
| ALL                 | 1-way ANOVA     | 0.0001   | NA    |
| NS_Cw4-Mix_Cw4      | Tukey           | NA       | 0.0022|
| NS_Cw4-NS_Cw7       | Tukey           | NA       | 0.0031|
| Mix_Cw4-NS_Cw7      | Tukey           | NA       | 0.9996|

### Figure 4E

| comparison          | test            | pval     | padj  |
|---------------------|-----------------|----------|-------|
| ALL                 | 1-way ANOVA     | 0.0308   | NA    |
| NS_CW4-SHP-1 and Cbls siRNA_CW4 | Tukey | NA | 0.0321|
| NS_CW7-SHP-1 and Cbls siRNA_CW4 | Tukey | NA | 0.7625|
| NS_CW7-NS_CW4       | Tukey           | NA       | 0.0775|

### Figure 4F

| comparison          | test            | pval     | padj  |
|---------------------|-----------------|----------|-------|
| NS_CW4_SHP-1 and Cbls siRNA_CW4 | 1-sample t-test | 0.0169 | 0.0169|
| NS_CW4_NS_CW7       | 1-sample t-test | 0.0052   | 0.0102|
| NS_CW4-SHP-1 and Cbls siRNA_CW7 | 1-sample t-test | 0.0068 | 0.0102|
| ALL                 | 1-way ANOVA     | 0.0991   | NA    |
| SHP-1 and Cbls siRNA_CW7-SHP-1 and Cbls siRNA_CW4 | Tukey | NA | 0.4977|
| NS_CW7-SHP-1 and Cbls siRNA_CW4 | Tukey | NA | 0.4674|
| NS_CW7-SHP-1 and Cbls siRNA_SHP-1 and Cbls siRNA | Tukey | NA | 0.0831|

### Figure 5B IL-10

| comparison          | test            | pval     | padj  |
|---------------------|-----------------|----------|-------|
| ALL                 | 1-way ANOVA     | 0.0004   | NA    |
| comparison | test       | pval | padj |
|------------|------------|------|------|
| NPs only-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.9772 |
| Positive ctrl (PHA)-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.0013 |
| Untreated-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.9877 |
| Positive ctrl (PHA)-NPs only | Tukey | NA   | 0.0025 |
| Untreated-NPs only | Tukey | NA   | 0.8853 |
| Untreated-Positive ctrl (PHA) | Tukey | NA   | 0.0008 |

**Figure 5B IFN-γ**

| comparison | test       | pval | padj |
|------------|------------|------|------|
| ALL        | 1-way ANOVA | 0.0001 | NA |
| NPs only-NPs-siRNA | Tukey | NA   | 0.9954 |
| Positive ctrl (PHA)-NPs-siRNA | Tukey | NA   | 0.0004 |
| Untreated-NPs-siRNA | Tukey | NA   | 1 |
| Positive ctrl (PHA)-NPs only | Tukey | NA   | 0.0007 |
| Untreated-NPs only | Tukey | NA   | 0.9919 |
| Untreated-Positive ctrl (PHA) | Tukey | NA   | 0.0003 |

**Figure 5B IL-6**

| comparison | test       | pval | padj |
|------------|------------|------|------|
| ALL        | 1-way ANOVA | 0    | NA |
| NPs only-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.9962 |
| Positive ctrl (PHA)-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0 |
| Untreated-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.8422 |
| Positive ctrl (PHA)-NPs only | Tukey | NA   | 0 |
| Untreated-NPs only | Tukey | NA   | 0.9288 |
| Untreated-Positive ctrl (PHA) | Tukey | NA   | 0 |

**Figure 5B TNF-α**

| comparison | test       | pval | padj |
|------------|------------|------|------|
| ALL        | 1-way ANOVA | 0.0004 | NA |
| NPs only-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.9689 |
| Positive ctrl (PHA)-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.0032 |
| Untreated-NPs-SHP-1 and Cbls siRNA | Tukey | NA   | 0.8981 |
| Positive ctrl (PHA)-NPs only | Tukey | NA   | 0.0013 |
| Untreated-NPs only | Tukey | NA   | 0.9947 |
| Untreated-Positive ctrl (PHA) | Tukey | NA   | 0.0008 |

**Figure 6B**

| 0         | First injection | independent samples t-test | 0.7623 |
|   |   | independent samples t-test |   |
|---|---|-----------------------------|---|
| 1 |   | 0.6300                      |   |
| 2 |   | 0.3484                      |   |
| 3 | Second injection | 0.0517                      |   |
| 4 |   | 0.0068                      |   |
| 5 |   | 0.0024                      |   |
| 6 | Third injection | 0.0009                      |   |
| 7 |   | 0.0010                      |   |
| 8 |   | 0.0007                      |   |
| 9 | Forth injection | 0.0005                      |   |
|10 |   | 0.0006                      |   |
|11 |   | 0.0007                      |   |
|12 | Fifth injection | 0.0008                      |   |
|13 |   | 0.0006                      |   |
|14 |   | 0.0005                      |   |
|15 | Sixth injection | 0.0003                      |   |
|16 |   | 0.0004                      |   |

**Figure 6C**

| comparison | test | pval |
|------------|------|------|
| NPs N.S siRNA - NPs SHP-1 Cbls siRNAs | independent samples t-test | 0.001 |

**Figure 6E**

| comparison | test | pval |
|------------|------|------|
| N.S siRNA - SHP-1 and Cbls siRNA | 1-sample t-test | 0.0092 |

**Figure 6F**

| condition | N  | Observed | Expected | (O-E)^2/E | (O-E)^2/V |
|-----------|----|----------|----------|-----------|-----------|
| MIX       | 18 | 18       | 26.5     | 2.73      | 15.3      |
| N.S       | 16 | 16       | 7.5      | 9.62      | 15.3      |
Chisq= 15.3 on 1 degrees of freedom, p=0.00009

| comparison | test | pval | padj |
|------------|------|------|------|
| Mock - 250pmol Cbl-b siRNA | 1-sample t-test | 0.0071 | 0.0212 |
| Mock - 500pmol Cbl-b siRNA | 1-sample t-test | 0.02 | 0.03 |
| 250pmol Cbl-b siRNA - 500pmol Cbl-b siRNA | independent samples t-test | 0.0513 | 0.0513 |

| comparison | test | pval | padj |
|------------|------|------|------|
| Mock - 250pmol c-Cbl siRNA | 1-sample t-test | 0.0151 | 0.0226 |
| Mock - 500pmol c-Cbl siRNA | 1-sample t-test | 0.0052 | 0.0155 |
| 250pmol c-Cbl siRNA - 500pmol c-Cbl siRNA | independent samples t-test | 0.0314 | 0.0314 |

| comparison | test | pval | padj |
|------------|------|------|------|
| Mock - 250pmol SHP-1 siRNA | 1-sample t-test | 0.0104 | 0.0256 |
| Mock - 500pmol SHP-1 siRNA | 1-sample t-test | 0.0171 | 0.0256 |
| 250pmol SHP-1 siRNA - 500pmol SHP-1 siRNA | independent samples t-test | 0.8872 | 0.8872 |

| comparison | test | pval | padj |
|------------|------|------|------|
| YTS NS siRNA - YTS SHP-1 siRNA | 1-sample t-test | 0.0157 | 0.0391 |
| YTS NS siRNA - YTS Cbl-b siRNA | 1-sample t-test | 0.0356 | 0.0593 |
| YTS NS siRNA - YTS c-Cbl siRNA | 1-sample t-test | 0.0629 | 0.0629 |
| YTS NS siRNA - YTS Cbl-b+c-Cbl siRNA | 1-sample t-test | 0.0479 | 0.0598 |
| YTS NS siRNA - YTS SHP-1+Cbls siRNA | 1-sample t-test | 0.0015 | 0.0073 |

| comparison | test | pval |
|------------|------|------|
| Caspase 3_NS - Caspase 3_CBL SHP1 | independent samples t-test | 0.00000 |

| comparison | test | pval |
|------------|------|------|
| N.S - SHP-1 and Cbls siRNA | independent samples t-test | 0.9037 |
## Appendix Table S2

Antibody details of all antibodies used in this research.

| Antigen /marker | Fluorochrome | Species/ host | Clone | Dilution | Vendor | Catalog no | Application |
|-----------------|--------------|---------------|-------|----------|--------|------------|-------------|
| SHP-1           | -            | Rabbit        | C-19  | 1/1000   | Santa Cruz | SC-287     | WB          |
| GAPDH           | -            | Rabbit        | FL-335| 1/1000   | Santa Cruz | SC-25778   | WB          |
| Cbl- b          | -            | Mouse         | G-1   | 1/250    | Santa Cruz | SC-8006    | WB          |
| Cbl             | -            | Mouse         | A-9   | 1/250    | Santa Cruz | SC-1651    | WB          |
| CD107a          | -            | Mouse         | H4A3  | 1/20     | Bio Legend |            | FC          |
| CD107a          | FITC         | Mouse         | H4A3  | 1/20     | Bio Legend | 328606     | FC          |
| Alexa Fluor 647- conjugated Goat anti-Mouse | Alexa Fluor 647 | Goat | - | 1/2500 | Invitrogen | A21235 | FC |
| Alexa Fluor 594- conjugated Goat anti-Rabbit | Alexa Fluor 594 | Goat | - | 1/2000 | Invitrogen | | FC |
| KIR2DL1/S1      | -            | Mouse         | HP-MA4| 1/100   | Bio Legend | 339502 | FC |
| KIR2DL1/S1      | PE           | Mouse         |       | 1/100   | Miltenyi  | 130099209 | FC          |
| Caspase 3       | -            | Rabbit        | 5AIE  | 1/1000  | Bio Legend | 9664      | IHC         |
| CD56            | FITC         | Mouse         | HCD56 | 1/50    | Bio Legend | 318303 | FC |
| CD3             | PE-Cy5       | Mouse         |       | 1/100   | BD        | 555334 | FC |