Supplementary Material

Sharpening up tumor microenvironment to enhance the efficacy of immune checkpoint blockade on head and neck cancer using a CpG-oligodeoxynucleotide

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### Supplementary table 1 PCR primers for human genes

| Gene   | Forward Primer  | Reverse Primer       |
|--------|-----------------|----------------------|
| β-Actin | CTCTTCCAGCCTTCCCTTCCCT | TGTTGGCGTACAGGTCTTTG |
| β-Actin Rev | GAAGGTTGAAGGTGGAGGAGTCA | AATGAAAGGGGTATTGATGG |
| GAPDH  | GAAGGTGAAGGTCGGAGTG | AATGAAGGGTGATTGATGG |
| IL-1β  | GGACAAGCTGAGGGAAGTG | TCGTTATCCCATGTGTCGAA |
| IL-1β Rev | AGACAGCCACTCACTCTTTTTGAGT | TCTGCCAATGCTCTCTTGTG |
| IL-6   | TTCTGCCAGTGCACTCTTGTG | GACATCTGCACTTTTCACTCCAG |
| IL-12A | CAATCTCTTCAGAAATGGAAGG | CATTTTTTCGGCAGATGAGCCCGT |
| IL-12A Rev | GCCCTTCCAACTTCCCTACAAACC | GGACTTTCAGTTCAGGCTCCAG |
| IL-12B | GACATTCTGCGTCCAGGTTGCAAGG | CATTTTTTCGGCAGATGAGCCCGT |
| IFNα   | AACCTCTTCTCTGCTCCATCAA | TTCTGCCAAGTGATGAGCCCGT |
| IFNα Rev | GGAAGACCCCTCCCATGATAG | TGGTGGTCTGATCTGCCTCAAC |
| IFNα2  | TGGGCTGATGCTTGCACTCAAC | CAGCCTTTTGGAACTTGTTG |
| IFNα2 Rev | GACATCTGCGTCCAGGTTGCAAGG | CATTTTTTCGGCAGATGAGCCCGT |
| IFNβ   | TTCTGCACTGCTCTGGTCAAGG | TGGTGGTCTGATCTGCCTCAAC |
| IFNβ Rev | GACATCTGCGTCCAGGTTGCAAGG | CATTTTTTCGGCAGATGAGCCCGT |
| IFNγ   | TTCAGCTCTCGCATCGTGGTGAAGA | TGGTGGTCTGATCTGCCTCAAC |
| IFNγ Rev | GACATCTGCGTCCAGGTTGCAAGG | CATTTTTTCGGCAGATGAGCCCGT |
**Supplementary table 2** PCR Primers for mouse genes

| Gene     | Sequence                        |
|----------|---------------------------------|
| β-Actin For | GCTACAGCTTCACCACCACA           |
| β-Actin Rev | AAGGAAGGGCTGGAAAGAGC           |
| GAPDH For  | AGAACATCATCCCTGCATCC           |
| GAPDH Rev  | CACATTGGGGGTAGGAACAC           |
| IL-1β For  | AGAGCTTCAGGCAGGCAGTA           |
| IL-1β Rev  | AGGTGCTCATGTTCCTCATCC         |
| IL-6 For   | CCGGAGAGGAGACTTCACAG           |
| IL-12A For | ACGGGCAGAGAAACTGAA            |
| IL-12A Rev | CTACCAAGGCACAGGGTCAT           |
| IL-12B For | CACGCCTGAAGAGATGACA           |
| IL-12B Rev | AGTCCCCTTTGTCAGTGTTG         |
| TNFα For   | ACGGCATGGATCTCAAGAC           |
| TNFα Rev   | GTGGGTTGGAGAGCAGTAG           |
| IFNα2 For  | ATCCAGAAGGCTCAAGGCCATCC       |
| IFNα2 Rev  | GGAGGGTTGTATTCCAGACAG          |
| IFNβ For   | GCCTTTGCCATCAGAGATGC         |
| IFNβ Rev   | ACACTGTCTGTGTTGAGGTTC         |
| IFNγ For   | CAGCAACAGCAAGGCGAAAAAGG       |
| IFNγ Rev   | TTTCCGCTTCGAGGCTGGAT         |
| CD3 For    | CTGCTACACACAGCTCAA           |
| CD3 Rev    | GCCTTGGCCTCTATTCCTT          |
| CD4 For    | CTGATGTTGGAGAGGCGAGAGAG      |
| CD4 Rev    | GAGACCTGGGGGTATCTTGAGG       |
| CD8 For    | TATGGCTTCATCCACACACA         |
| CD8 Rev    | GACTGGCAGCAGACAAGACTGA       |
| CD45 For   | TCAGCAAGTTTCCCATCAAAG       |
| CD45 Rev   | CCCCTGTGCAGAATGTATT          |
| CD20 For   | GTCTTCGCACCCATCTGTTT       |
| CD20 Rev   | AATGGCAGAAGAGAGCTTA         |
| CD86 For   | TCAGTGATCGCCAACCTCAG        |
| CD86 Rev   | TTAGGTTTCGGTGACCTTG         |
| F4/80 For  | CTCTGTGGTCCACCTTCATCAT      |
| F4/80 Rev  | GATGGCAGAAGGATCTGAA        |
| CCR7 For   | AACGGGCTGGTGATACTGAC      |
| CCR7 Rev   | TAGGCAGAAGGAGGAAT          |
| CD206 For  | TTTGAGTGAGTGATGGAACC       |
| CD206 Rev  | ACAGCATGGCTTTGATACC     |
| Gene   | Forward Primer          | Reverse Primer          |
|--------|-------------------------|-------------------------|
| iNOS   | CACCTTGGAGTTCACCCAGT    | ACCACTCGTACTTGGGATGC    |
| ARG1   | ACAGGGCTCCTTTCAAGGACTA  | AGTCAGTCCTGGCTTTATGGT   |
| BST2   | CAATCTACTTCGCGCTACGA   | TCTCTCCAGGGACTCTCTGA    |
| NKp46  | ACTGTGGCTTGGGCTATGTC    | CTCGATGGCTTTGGTCTCTC    |
| TLR9   | TTCTCTTCATGGACGGGAAC    | GGCACCTTTGTGAGGTTTGT    |
Supplementary Fig. 1 CpG-2722 augments suppressive effect of intravenous injected immune checkpoint inhibitor on growth of head and neck squamous cell carcinomas. a C57BL/6J mice were orthotopically injected with $2 \times 10^6$ NHRI-HN1 cells to grow HNSCC. Thirteen days later, when the tumors reached approx. 100 mm$^3$, the mice were intratumorally injected with the control vehicle or 50 μg CpG-2722 every four days in combination with or without the intravenous injection of 10 μg anti-PD-1 antibody every eight days as illustrated. b Tumor sizes were measured (each group contained five mice and five tumors). c Endpoint of the tumor growths are represented as indicated. d Tumor samples were visualized by H&E staining (left upper panel). Scale bar represents 100 μm. Leukocyte infiltrations in the 20x magnification areas were counted by using Image J software (Right panel). Data represent mean ± SEM. Asterisk *, **, and *** represent the statistically significant difference p < 0.05, p < 0.01, and p < 0.001, respectively, compared to the control.