Supplementary Figure S1: The specificity of each HEK293-hTLR-luc cell line was determined by testing its stimulation by every other TLR ligand in a dose response experiment. Each HEK293-hTLR-luc cell line was stimulated with serial dilutions of the TLR ligands FSL-1 (hTLR2, hTLR2/6), Poly[I:C], LPS (hTLR4), Flagellin (hTLR5), R848 (hTLR7, hTLR8) and ODN2006 (hTLR9). Luciferase activity was measured in relative light units (RLU): Each cell line could be stimulated with its respective control ligand. In contrast, poly[I:C] did not only stimulate HEK-hTLR3-luc, but also HEK-hTLR5-luc and HEK-hTLR7-luc. Plain square: HEK-hTLR2-luc, plain triangle: HEK-hTLR3-luc, plain circle: HEK-hTLR4-luc, open square: HEK-hTLR5-luc, inverted plain triangle: HEK-hTLR2/6-luc, plain diamond: HEK-hTLR7-luc, open triangle: HEK-hTLR8-luc, inverted open triangle: HEK-hTLR9-luc.

Specificity was observed for all ligand / HEK-hTLR-luc combinations except for poly[I:C] which stimulated HEK-hTLR3-luc, but also HEK-hTLR5-luc and HEK-hTLR7-luc. We ascribed this to endogenous TLR3 expression. In support of this, stable transfection of HEK-293 cells with the NF-κB-inducible luciferase expression cassette resulted in cellular clones in which luciferase activity was induced with the TLR3 control ligand poly[I:C] (**Supplementary Figure S2**).

Supplementary Figure S2: HEK-293 cells, “naturally” TLR3-positive, were stably transfected with NF-κB-inducible luciferase expression plasmid. Isolated clones were stimulated with poly[I:C]. The fold-induction of luciferase activity in different clones varied greatly.

Supplementary Figure S3: NAB2+Lipofectin, NAB2 or Lipofectin were tested in all HEK-hTLR cell lines in dose response experiments. The highest doses tested were 0.3 µg NAB2 and 1.5µg Lipofectin, mixed or individually (black histogram). Three-fold serial dilutions thereof (1:3, hatched histogram; 1:9, dark grey histogram; 1:27, light grey histogram; 1:8, white histogram) were tested, and fold-inductions of luciferase activity were plotted. NAB2 alone stimulated HEK-TLR3-luc and the cell lines with supposed endogenous TLR3 activity HEK-TLR5-luc and HEK-TLR7-luc, NAB2+Lipofectin stimulated all cell lines. Lipofectin alone had no effect. At high concentrations of NAB2+Lipofectin, fold-inductions in several cell lines regressed, most likely due to toxic effects.

Supplementary Figure S4: RT-PCR on total RNA selected from all HEK-TLR-luc clones and HEK-293 cells. hMDA-5 and hRIG-1-specific RT-PCR was carried out with commercially available primer sets (Invitrogen). (1: HEK-293; 2: HEK-hTLR2-luc; 3: HEK-hTLR3-luc; 4: HEK-hTLR4-luc; 5: HEK-hTLR5-luc; 6: HEK-hTLR6-luc; 7: HEK-hTLR7-luc; 8: HEK-hTLR8-luc; 9: HEK-hTLR9-luc; c: control). In all cell clones, rig-I and mda-5 transcripts can be detected.

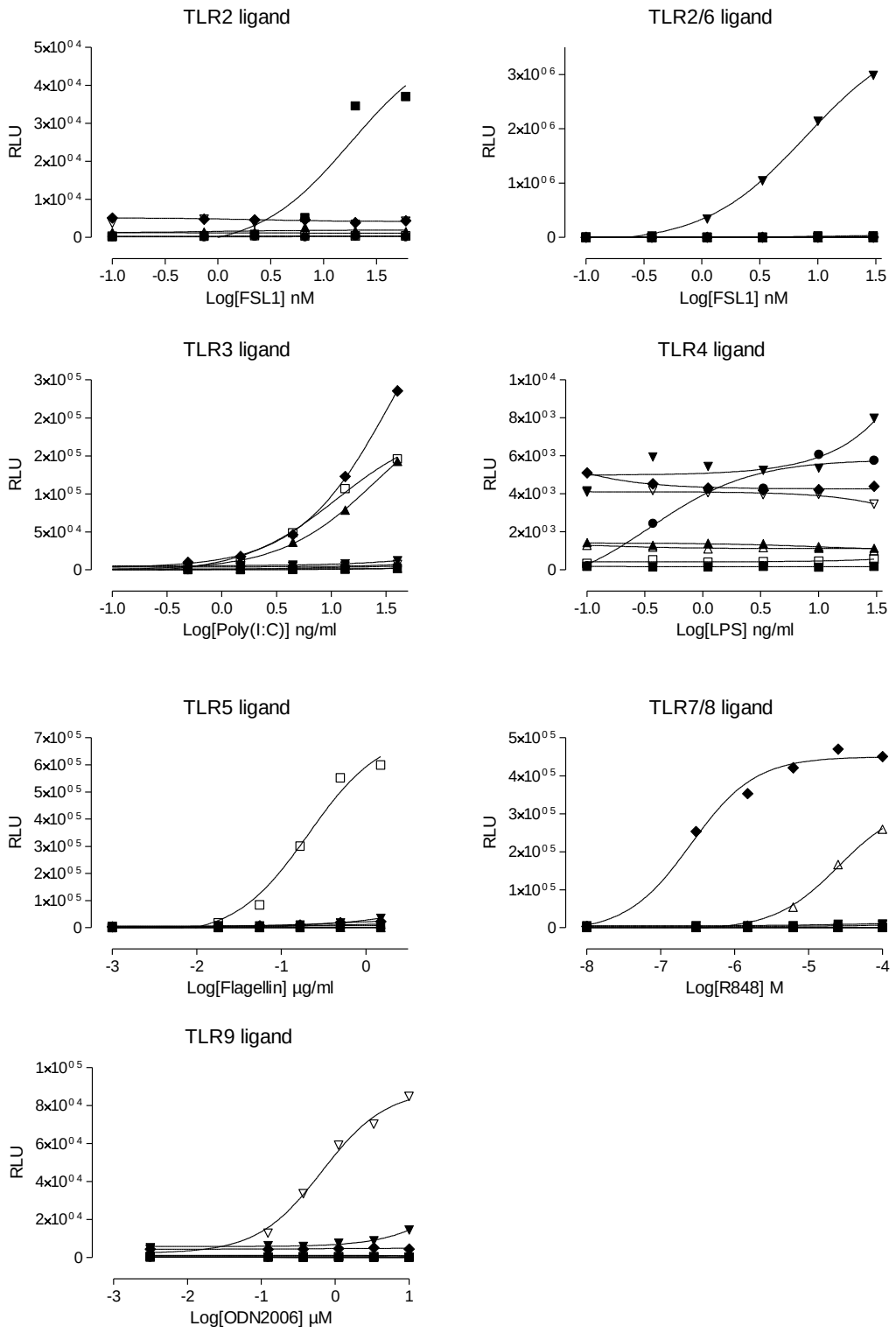
Supplementary Figure S5: NAB2 and NAB2+Lipofectin were tested for their capacity to stimulate human plasmacytoid dendritic cells. pDCs were incubated overnight with the TLR9 ligand ODN2006, the TLR7/8 ligand R848, NAB2, Lipofectin and NAB2+Lipofectin. In this representative experiment, only the TLR7/8 ligand R848 induced the secretion of IFN-α, while the TLR9 ligand ODN2006 induced the secretion of high amounts of IL-6. The secretion of Mip-1β and TNF-α were equally well induced by ODN2006 and R848. In contrast, NAB2, Lipofectin or NAB2+Lipofectin did not induce secretion of any of these cytokines.

Supplementary Figure S6: Determination of suboptimal dose of MVATG9931: Survival in C57BL/6 RMA-MUC1 tumor model was monitored after vaccination with different doses of MVATG9931. Best protection was obtained with MVAT9931 at viral doses of $1x10^5$ and $5x10^5$. The viral dose of $1x10^3$ pfu MVATG9931 was considered as "suboptimal" and chosen to assess the efficacy of adjuvant candidates. An empty viral vector (mock MVA vector) at highest dose ($5x10^7$ pfu) had no effect.

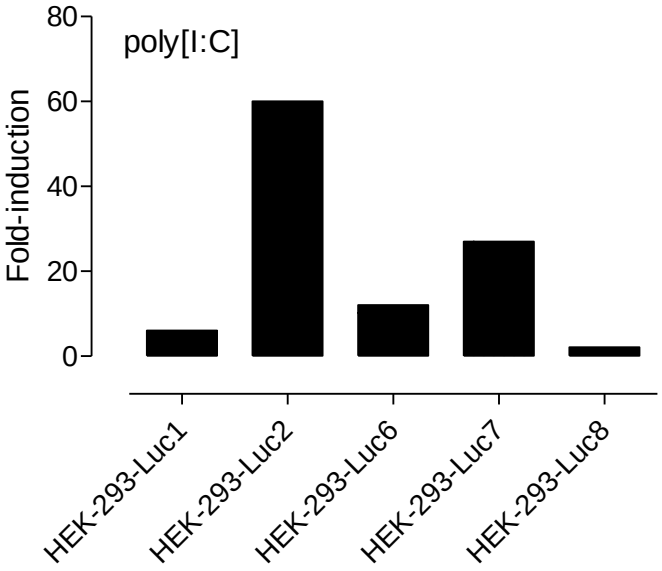
Supplementary Figure S7: Effect of injection schedule: Survival in C57BL/6 RMA-MUC1 tumor model was monitored after vaccination with the suboptimal dose of MVATG9931 ($1x10^3$ pfu) and NAB2+Lipofectin. The mixture of NAB2+Lipofectin was injected at the same site either at the same moment, or timely deferred 6h or 24h after virus injection. To augment vaccination efficiency, NAB2+Lipofectin had to be injected several hours after MVATG9931. No benefit was observed when viral vector NAB2+Lipofectin were co-injected.

Supplementary Figure S8: Extra- and intracellular presence of TLR3 in HEK-hTLR3-luc cells: HEK-TLR3-luc cells were permeabilized or not and labeled with an anti IgG1 isotype control antibody (black line) or anti TLR3 (grey line).

Supplementary Figure S1



Supplementary Figure S2



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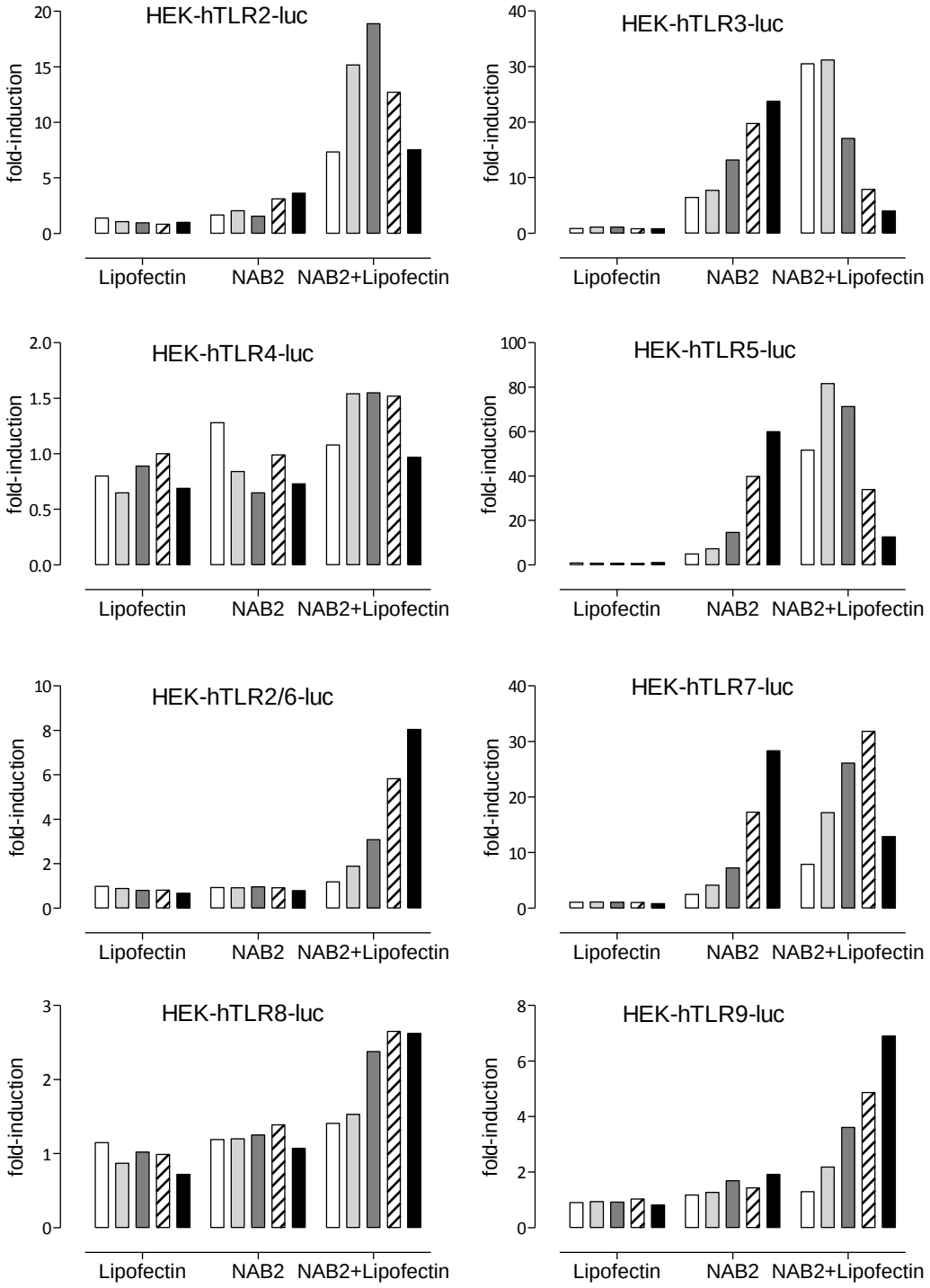

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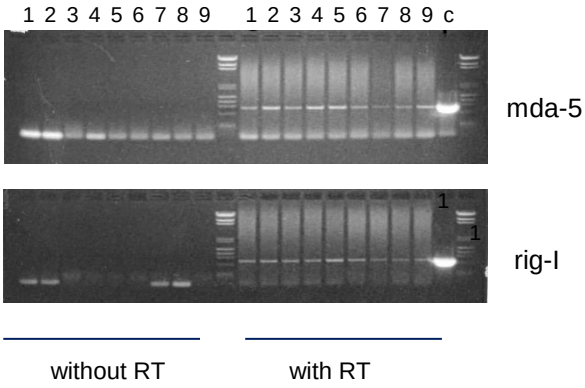

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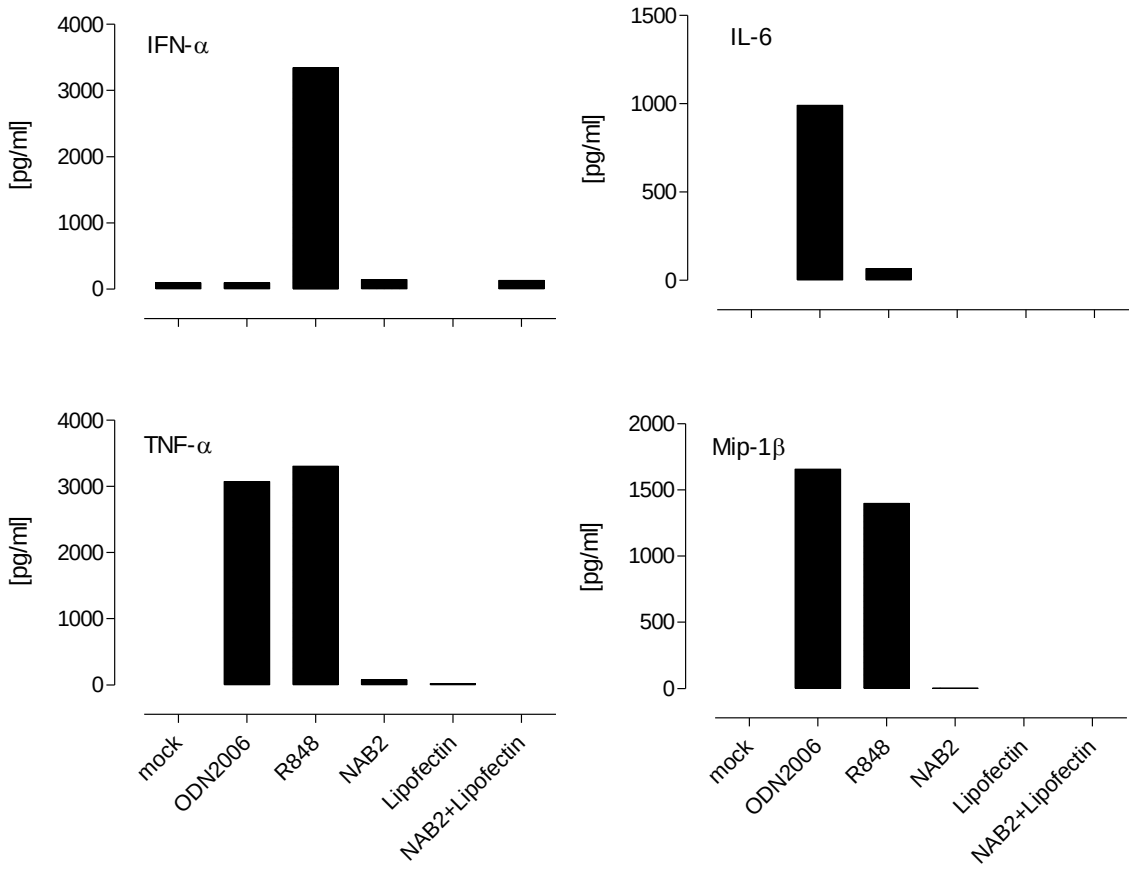

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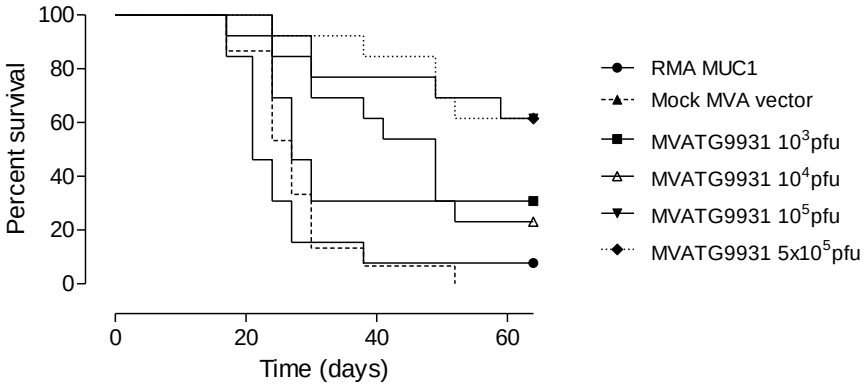

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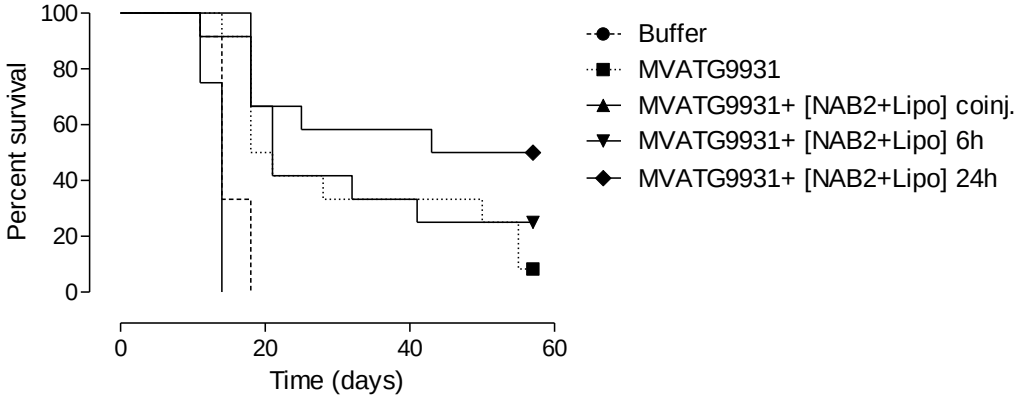

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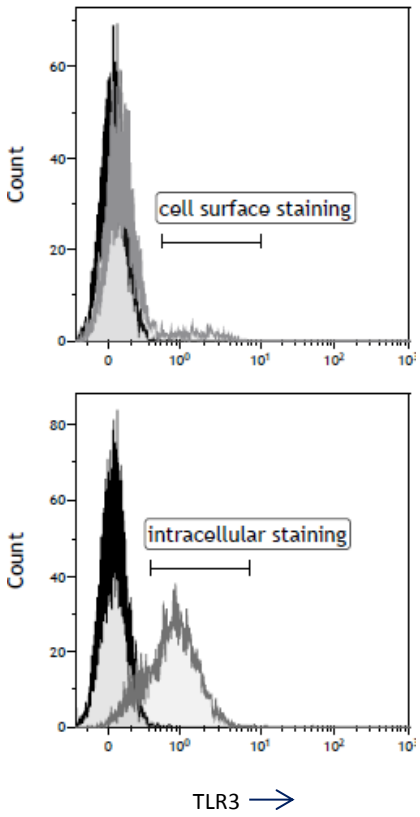

Supplementary data: sequence alignment:

NAB2 derived from Sc JC7 and ScV L-BC (genebank: U01060). 25 bp on 5′ and 3′ extremities correspond to primer sequences.

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3551 CGAAACTCGGTAGATAGAATGGTCGCTCACCAACCTAACACTGGTGAGAC 3600
     |||||||||||||||||||||||||||||||| ||||||||||||||||
3551 cgaaactcggtagatagaatggtcgctcaccaaactaacactggtgagac 3600
                .         .         .         .         .
3601 TTATGATGTTAAAGGGACACTGTTTAGTGGCTGGCGATTAACAACATTTT 3650
     ||||||||||||||||||||||||||||||||||||||||||||||||||
3601 ttatgatgttaaagggacactgtttagtggctggcgattaacaacattta 3650
                .         .         .         .         .
3651 TCAATACGGCGTTGAACTATTGCTACCTGGCTAATGCAGGTATAAACTCA 3700
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3651 tcaatacggcgttgaactattgctacctggctaatgcaggtataaactca 3700
                .         .         .         .         .
```

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3701 CTAGTGCCAACGAGTCTCCATAATGGTGATGATGTTTTTGCAGGGATAAG 3750
     |||||||||||||||||||||||||||||||||| ||||||||||||||
3701 ctagtgccaacgagtctccataatggtgatgatgtctttgcagggataag 3750
               .         .         .         .         .
3751 GACAATAGCTGACGGTATTTCTTTGATCAAAAACGCCGCAGCCACGGGAG 3800
     ||||||||||||||||||||||||||||||||||||||||||||||||||
3751 gacaatagctgacggtatttctttgatcaaaaacgccgcagccacgggag 3800
               .         .         .         .         .
3801 TTCGCGCTAATACAACTAAAATGAACATTGGTACGATAGCAGAGTTTTTG 3850
     ||||||||||||||||||||||||||||||||||||||||||||||||||
3801 ttcgcgctaatacaactaaaatgaacattggtacgatagcagagtttttg 3850
               .         .         .         .         .
3851 AGAGTTGATATGCGTGCAAAAAATAGTACTGGCAGTCAGTATTTAACAAG 3900
     |||||||||||||||||||| ||||||||||||||||||||||||||||
3851 agagttgatatgcgtgcaaaagatagtactggcagtcagtatttaacaag 3900
               .         .         .         .         .
3901 AGGGATTGCTACCTTCACGCACAGTAGGGTTGAGTCTGATGCACCACTGA 3950
     |||||||||||| ||||||||||||||||||||||||||||||||||||
3901 agggattgctactttcacgcacagtagggttgagtctgatgcaccactga 3950
               .         .         .         .         .
3951 CATTGCGCAATCTAGTATCTGCTTACAAAACCAGATATGACGAGATTTTA 4000
     |||||||||| ||||||||||||||||||||||||||||||||||||||
3951 cattgcgcaacctagtatctgcttacaaaaccagatatgacgagatttta 4000
               .         .         .         .         .
4001 GCTCGTGGCGCAAGCATCGATAACATGAAGCCACTCTATCGTAAGCAATT 4050
     ||||||||||||||| ||||||||||||||||||||||||||||||||||
4001 gctcgtggcgcaagcaccgataacatgaagccactctatcgtaagcaatt 4050
               .         .         .         .         .
4051 ATTTTTTGCTAGAAAGTTGTTCAATGTCGAGAAGGACATTGTTGACAATC 4100
     ||||||||||||||||||||||||||||||||||||||||||||||||||
4051 atttttttgctagaaagttgttcaatgtcgagaaggacattgttgacaatc 4100
               .         .         .         .         .
4101 TGATAACGATGGACATATCATGTGGCGGTTTGCAAGAAAAGGGTAGGGTA 4150
     ||||||||||||||||||||||||||||||||||||||||||||||||||
4101 tgataacgatggacatatcatgtggcggtttgcaagaaaagggtagggta 4150
               .         .         .         .         .
4151 TCAGAGATGGTGTTACAGGAGGTTGACATTGAGAATATAGATAGTTATAG 4200
     ||||||||| ||||||| ||||||||||||||||||||||||||||||||
4151 tcagagatagtgttacgggaggttgacattgagaatatagatagttataa 4200
               .         .         .         .         .
4201 GAAGACAAGGATGATCGCCAAACTGATTGACAAGGGGGTTGGCGATTATA 4250
     ||||||||||||||| ||||||||||||||||||||||||||||||||||
4201 gaagacaaggatgattgccaaactgattgacaaggggttggcgattata 4250
               .         .         .         .         .
4251 CTGCATTCCTGAAAACTAACTTTTCCGAGATAGCTGATGCTATCACAAGA 4300
     |||||||||||||||||||||||||||||||||||||||||||||||| |
4251 ctgcattcctgaaaactaacttttccgagatagctgatgctatcacaa.a 4299
               .         .         .         .         .
4301 GAGACACG.TGTAGAGTCAGTGACCAAGGCTTATAATGTTAAGAAGAAAA 4349
     || ||||| |||||||||| ||||||||||||||||||||||||||||||
4300 gaaacacgttgtagagtcattgaccaaggcttataatgttaagaagaaaa 4349
               .         .         .         .         .
4350 CGGTCGTACGCGCGTTTAGGGACCTAAGCGCAGCATATCATGAAAGAGCG 4399
     |||||||| |||||||||||||||||||||||||||||||||||||||||
4350 cggtcgtaagcgcgtttagggacctaagcgcagcatatcatgaaagagcg 4399
               .         .         .         .         .
4400 GTGAGACATGCTTGGAAGGGGATGAGTGGACTACACATAGTCAACAGGAT 4449
     |||||||||||||||||||||||||||||||||||||||||||||||||
4400 gtgagacatgcttggaaggggatgagtggactacacatagtcaacaggat 4449
               .         .         .         .         .
4450 TCGTATGGGAGTGAGCAACTTAGTAATGGTTGTTAGCAAAATCAATCCTG 4499
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     ||||||||| ||||||||||||||||||||||||||||||||||||||||
4450 tcgtatgggtgtgagcaacttagtaatggttgttagcaaaatcaatcctg 4499
          .         .         .         .         .
4500 CAAAAGCTAATGTGCTAGCCAAATCAGGAGATCCTACAAAATGGCTTGCA 4549
     |||||||||||||||||||||||||| ||||||||||||||||||||||
4500 caaaagctaatgtgctagccaaatcaggtgatcctacaaaatggcttgca 4549
          .         .         .         .         .
4550 GTCCTTACATGATATACAGGCAACCACATAAGACCTGAGAACAAAGAGTA 4599
     ||||||||||||||||||||||||||||||||||||||||||||||||||
4550 gtccttacatgatatacaggcaaccacataagacctgagaaCAAAGAGTA 4599
          .
4600 CATACGATACTACGCA 4615
     ||||||||||||||||
4600 CATACGATACTACGCA 4615
```