

An engineered T7 RNA polymerase that produces mRNA free of immunostimulatory byproducts

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SUPPLEMENTARY INFORMATION

An engineered T7 RNA polymerase that produces mRNA free of immunostimulatory byproducts

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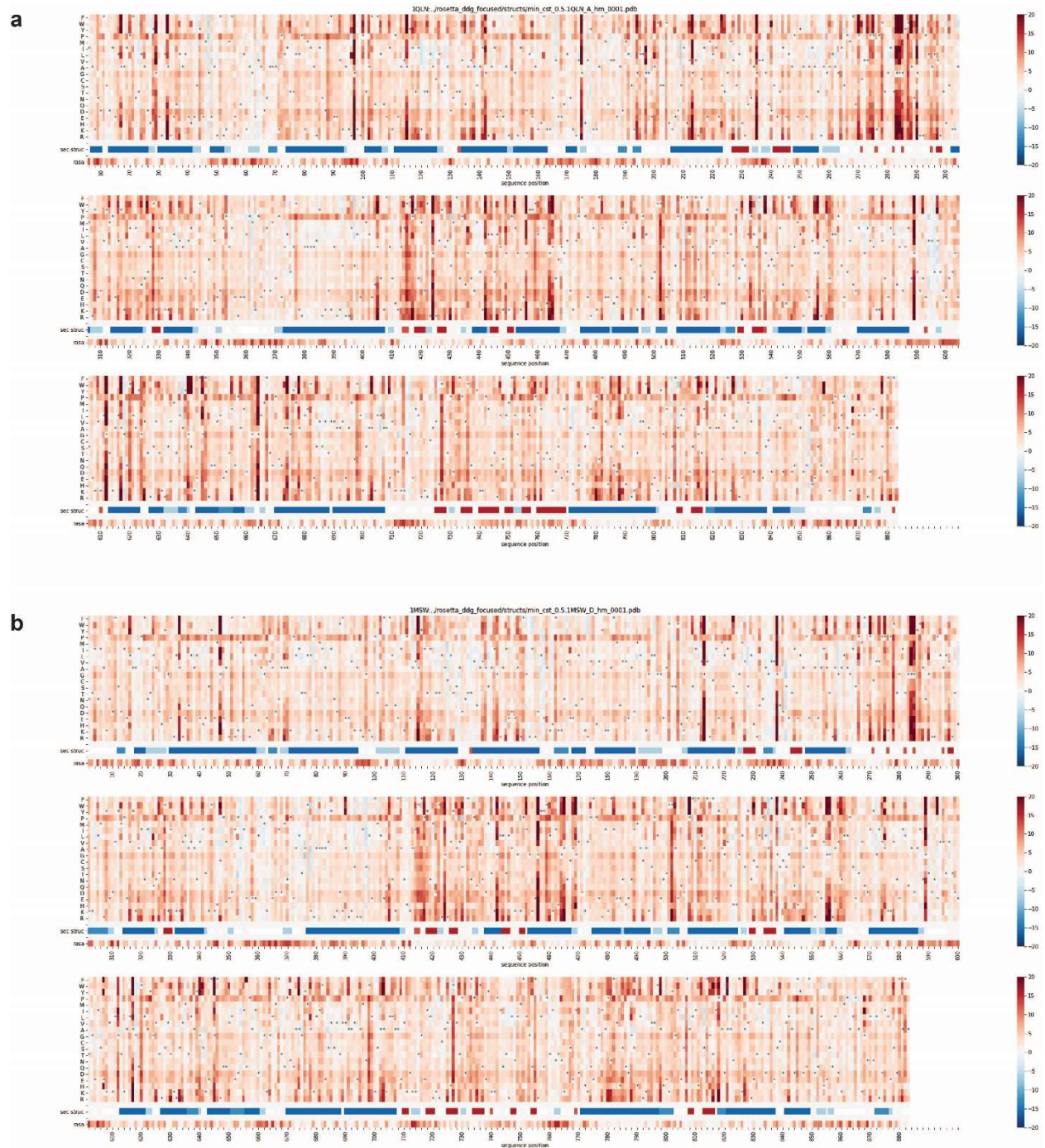


Figure S1. a) $\Delta\Delta E_{\text{mut}}$ heatmap for structure of initiation complex (pdb 1QIN). The columns correspond to amino acid sequence positions (1-883), and the rows correspond to amino acid

identities. The amino acids are grouped and ordered as per Kowalsky.¹ Asterisks (‘*’) denote the wildtype amino acid at each position. The $\Delta\Delta E_{\text{mut}}$ values are in Rosetta energy units (REUs), and the heatmap spans -20 REUs (blue) to 20 REUs (red). The bottom two rows correspond to secondary structure (blue: alpha helix, light blue: helix turn, red: beta strand) and relative solvent accessible surface area. **b)** $\Delta\Delta E_{\text{mut}}$ heatmap for structure of elongation complex (pdb 1MSW). The columns correspond to amino acid sequence positions (1-883), and the rows correspond to amino acid identities. The amino acids are grouped and ordered as per Kowalsky.¹ Asterisks (‘*’) denote the wildtype amino acid at each position. The $\Delta\Delta E_{\text{mut}}$ values are in Rosetta energy units (REUs), and the heatmap spans -20 REUs (blue) to 20 REUs (red). The bottom two rows correspond to secondary structure (blue: alpha helix, light blue: helix turn, red: beta strand) and relative solvent accessible surface area.

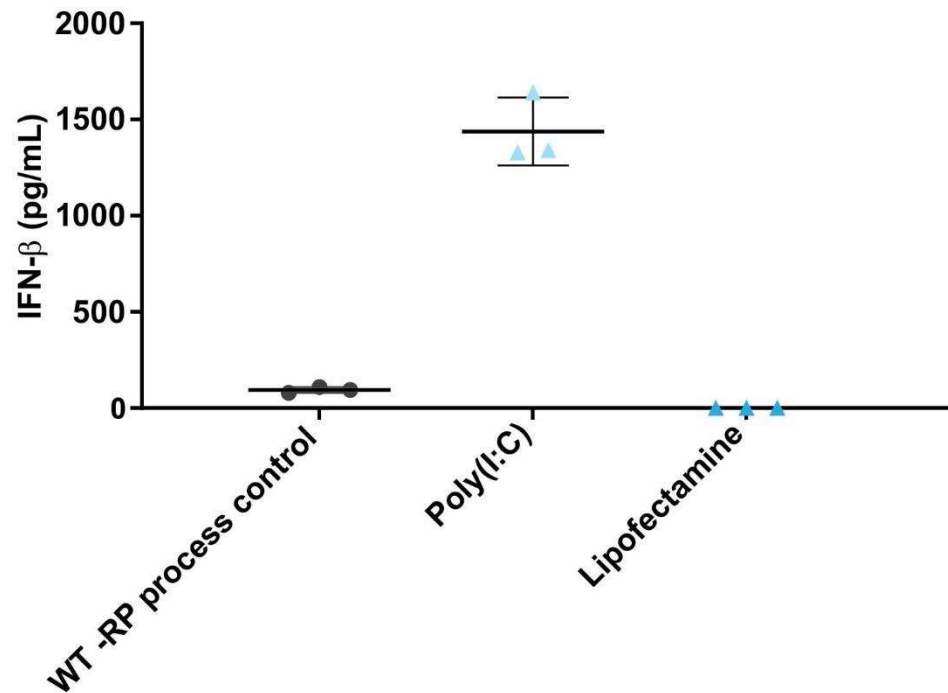


Figure S2: IFN- β response for the commercially available known RIG-I agonist, Poly(I:C) (InvivoGen), was tested in BJ Fibroblasts using n=3 technical replicates (error bars represent standard deviation). Cells were transfected with 250 ng/ μ L mRNA (WT -RP process control) or 10 ng/ μ L Poly(I:C) using Lipofectamine 2000 (Thermo Fisher Scientific). Cell culture supernatants were harvested 48 hours after transfection for IFN- β analysis.

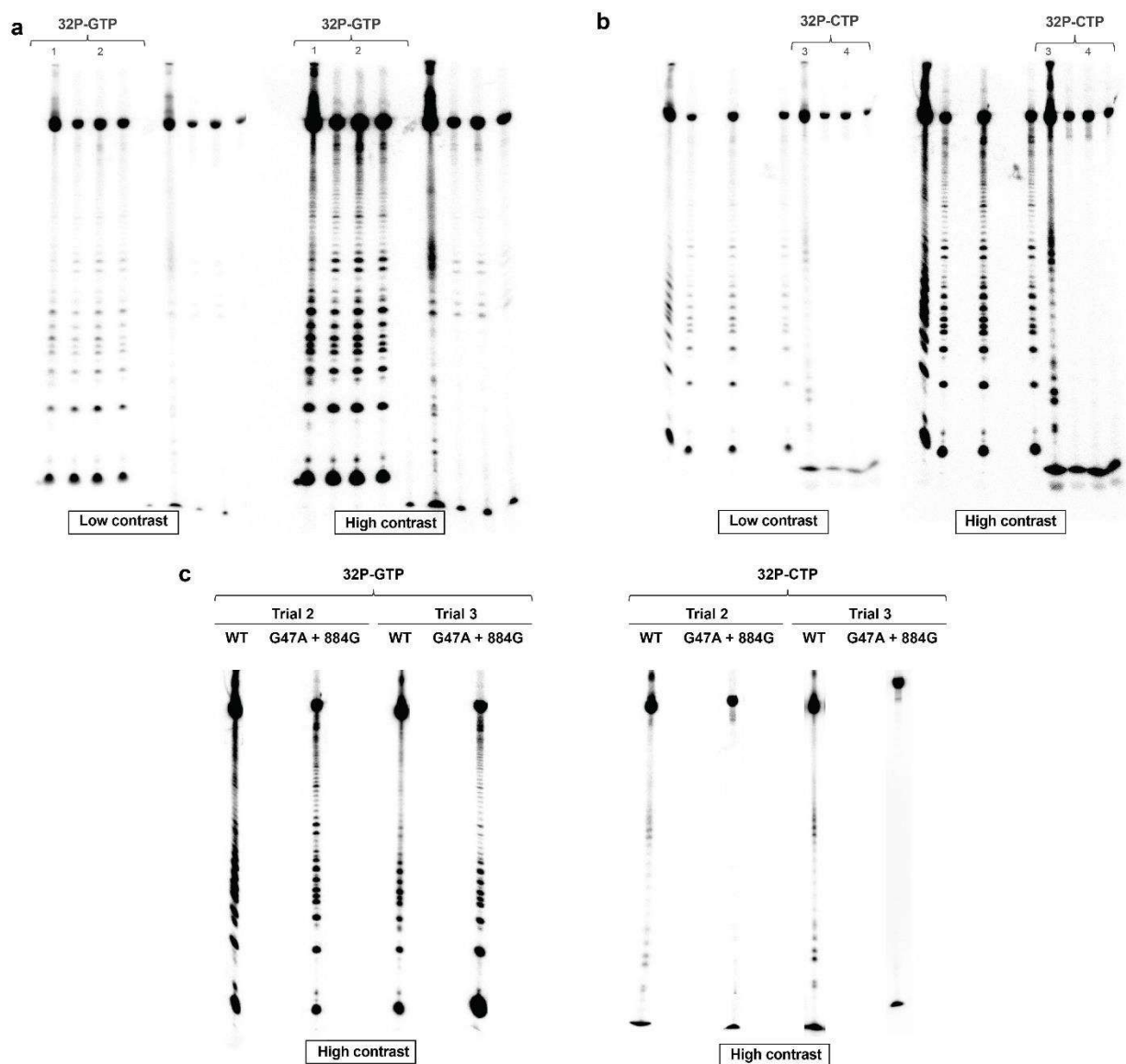


Figure S3: a and c) Original radioactive gels of IVT products generated by T7 RNAP WT and G47A+884G mutant. Only high contrast figures are displayed in the manuscript. **a)** Low and high contrast uncropped gel for ^{32}P -GTP; lanes marked 1 and 2 correspond to WT and G47A+884G, respectively in Figure 4A (blue panels). **b)** Low and high contrast uncropped gels for ^{32}P -GTP; lanes marked 3 and 4 correspond to WT and G47A+884G, respectively in Figure 4A (red panels). **c)** Two additional independent trials of IVT and cropped radioactive sequencing

gels (only high contrast cropped gels are shown) to monitor abortive (^{32}P -GTP) and reverse complements (^{32}P -CTP).

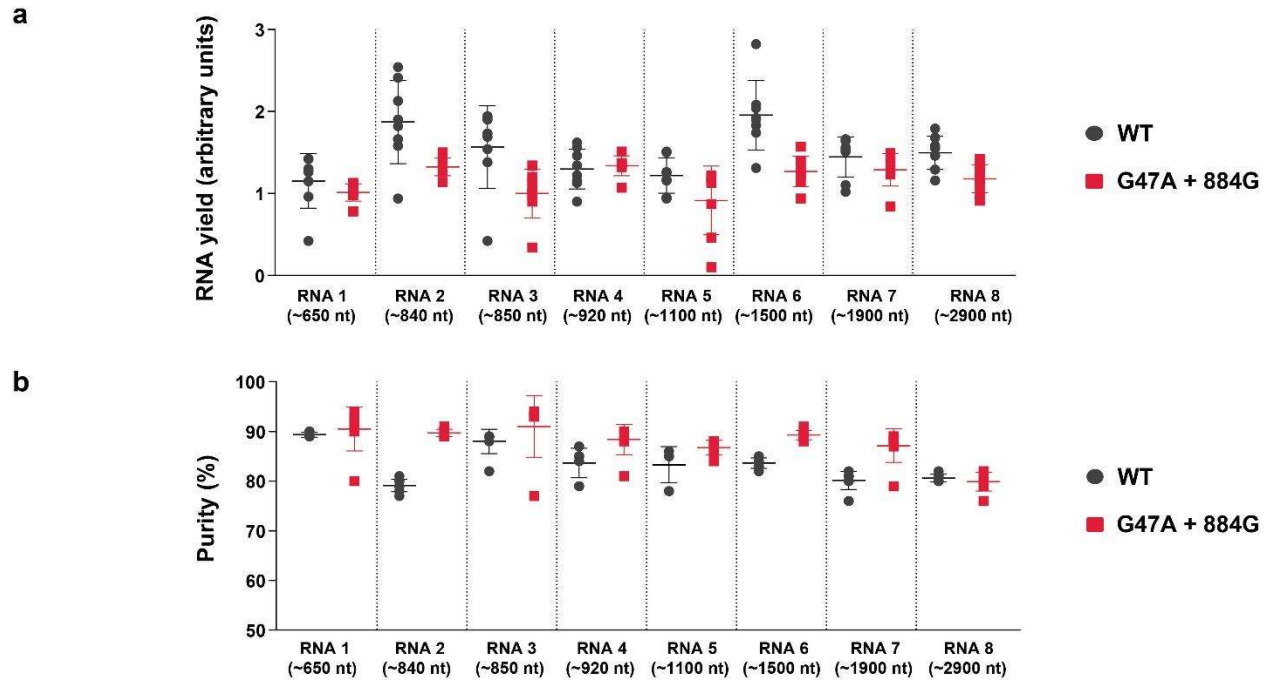


Figure S4: a) RNA yield and **b)** purity were evaluated for the 8 mRNAs with varying lengths and sequence composition presented in **Figure 4b-d** (N=8 mRNA lots; error bars represent standard deviation). RNA yield was measured by UV using A260 nm and RNA purity was measured by capillary electrophoresis via Fragment Analyzer.

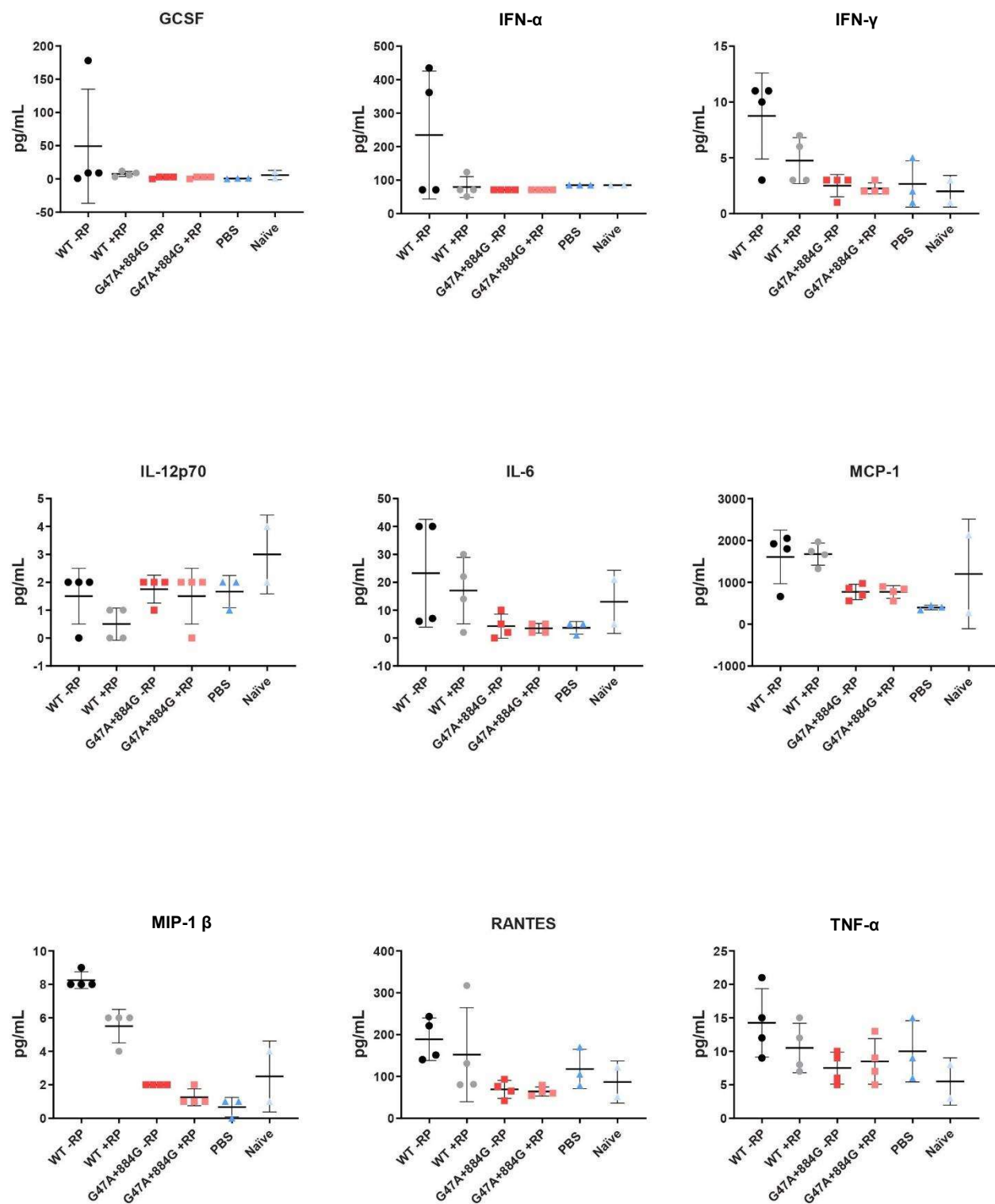


Figure S5 *In vivo* analysis of hEPO mRNA evaluated serum cytokine and chemokines using an innate immune response Luminex panel. Data are presented as mean $\pm$ standard deviation,

N=4 for all treatment groups, N=3 for PBS and naive controls. GCSF, granulocyte-colony stimulating factor; hEPO, human erythropoietin; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MIP-1, macrophage inflammatory protein-1; mRNA, messenger RNA; PBS, phosphate-buffered saline; RP, reverse phase; TNF, tumor necrosis factor; WT, wild-type.

Table S1. Flags used with RosettaCM: partial threading.

-in:file:template_pdb 1MSW_D2A_renumber.pdb
-ignore unrecognized_res
-in:file:fasta target.fasta
-in:file:alignment target_template_aligned.aln
-relax:constrain relax to start coords

Table S2. Target-template alignments (target_template_aligned.aln).

<pre>## sp P00573 RPOL_BPT7 1QLN_GTP_renumber.pdb_thread # scores from program: 0 0 NTINIAKNDFSIELAAIPFNTLADHYGERLAREQLALEHESYEMGEARFRKMFERQLKAGEVADNAAAKPLITTLPLPKMIARINDW FEEVKA KRGRPTAFQFLQEIKPEAVAYITIKTLACLTSADNTTVQAVASAIGRAIEDEARFGRIRDLEAKHFKNVEEQLNKRVGH VYKKA FMQVVEADMLSKGLLGGEAWSSWHKEDSIHVGVRCEIEMIESTGMVSLHRQNAGVVGQDSEITELAPEYAEAIATRAGAL AGISPMFQPCVVPKPWTGITGGGYWANGRRPLALVRTHSKKALMRYEDVYMPEVYKAINIAQNTAWKINKKVLAVANVITKWK HCPVEDIPAIEREELPMKPEDIDMNPEALTAWKRAAAAVYRKDKARKSRRISLEFMLEQANKFANHKAIWFPYNMDWRGRVYAVS MFNPQGNDMTKGLLTLAKGKPIGKEGYWVKIHGANCAGVDKVPFPERIKFIEENHENIMACAKSPLENTWWAEQDSPFCFLAFCF EYAGVQHHLGSLYNCSLPLAFDGS CSGIHFSA MLRDEVGGRVAVNLLPSETVQDIYGIVAKKVNEILQADAINGTDNEVVTVDENT GEISEKVKLGTKALAGQWLA YGVTRSVTKRSVMTLAYGSKEFGFRQQVLEDTIQPAIDSGKGLMFTQPNQAAGYMAKLIWESVSV TVVAAVEAMNWLKSAAKLLAAEVKDKKTGEILRKRCVHVWTPDGFVPVWQEYKKPIQTRLNLMFLGGQFRLQPTINTNKDSEIDAH KQESGIAPNFVHSQDGSHLRKT VVWAHEKYGIESFALIHDSFGTIPADAANLFKAVRETMVDITYESCDVLADFYDQFADQLHESQL DKMPALPAKG NNLNRDILESDFafa 0 ----IAKNDFSIELAAIPFNTLADHYGERLAREQLALEHESYEMGEARFRKMF----- PLITTLPLPKMIARINDWFEEVKA KRGRPTAFQFLQEIKPEAVAYITIKTLACLTSADNTTVQAVASAIGRAIEDEARFGRIRDLEAK HFKNVEEQLNKRVGHVYKKA FMQVVEADMLSKGLLGGEAWSSWHKEDSIHVGVRCEIEMIESTGMVSLHRQNAGVVGQDSEITEL APEYAEAIATRAGALAGISPMFQPCVVPKPWTGITGGGYWANGRRPLALVRTHSKKALMRYEDVYMPEVYKAINIAQNTAWK INKKVLAVANVITKWKHCPVEDIPAIEREELPMKPEDIDMNPEALTAWKRAAAAVYRKDKARKSRRISLEFMLEQANKFANHKAIW FPYNMDWRGRVYAVSMFNPQGNDMTKGLLTLAKGKPIGKEGYWVKIHGANCAGVDKVPFPERIKFIEENHENIMACAKSPLENT WWAEQDSPFCFLAFCFEYAGVQHHLGSLYNCSLPLAFDGS CSGIHFSA MLRDEVGGRVAVNLLPSETVQDIYGIVAKKVNEILQADAI NGTDNEVVTVDENTGEISEKVKLGTKALAGQWLA YGVTRSVTKRSVMTLAYGSKEFGFRQQVLEDTIQPAIDSGKGLMFTQPNQ AAGYMAKLIWESVSVTVVAAVEAMNWLKSAAKLLAAEVKDKKTGEILRKRCVHVWTPDGFVPVWQEYKKPIQTRLNLMFLGGQF RLQPTINTNKDSEIDAHKQESGIAPNFVHSQDGSHLRKT VVWAHEKYGIESFALIHDSFGTIPADAANLFKAVRETMVDITYESCDVL ADFYDQFADQLHESQLDKMPALPAKG NNLNRDILESDFafa</pre>
<pre>## sp P00573 RPOL_BPT7 1MSW_D2A_renumber.pdb_thread # scores from program: 0 0 NTINIAKNDFSIELAAIPFNTLADHYGERLAREQLALEHESYEMGEARFRKMFERQLKAGEVADNAAAKPLITTLPLPKMIARINDW FEEVKA KRGRPTAFQFLQEIKPEAVAYITIKTLACLTSADNTTVQAVASAIGRAIEDEARFGRIRDLEAKHFKNVEEQLNKRVGH VYKKA FMQVVEADMLSKGLLGGEAWSSWHKEDSIHVGVRCEIEMIESTGMVSLHRQNAGVVGQDSEITELAPEYAEAIATRAGAL AGISPMFQPCVVPKPWTGITGGGYWANGRRPLALVRTHSKKALMRYEDVYMPEVYKAINIAQNTAWKINKKVLAVANVITKWK HCPVEDIPAIEREELPMKPEDIDMNPEALTAWKRAAAAVYRKDKARKSRRISLEFMLEQANKFANHKAIWFPYNMDWRGRVYAVS MFNPQGNDMTKGLLTLAKGKPIGKEGYWVKIHGANCAGVDKVPFPERIKFIEENHENIMACAKSPLENTWWAEQDSPFCFLAFCF EYAGVQHHLGSLYNCSLPLAFDGS CSGIHFSA MLRDEVGGRVAVNLLPSETVQDIYGIVAKKVNEILQADAINGTDNEVVTVDENT GEISEKVKLGTKALAGQWLA YGVTRSVTKRSVMTLAYGSKEFGFRQQVLEDTIQPAIDSGKGLMFTQPNQAAGYMAKLIWESVSV TVVAAVEAMNWLKSAAKLLAAEVKDKKTGEILRKRCVHVWTPDGFVPVWQEYKKPIQTRLNLMFLGGQFRLQPTINTNKDSEIDAH KQESGIAPNFVHSQDGSHLRKT VVWAHEKYGIESFALIHDSFGTIPADAANLFKAVRETMVDITYESCDVLADFYDQFADQLHESQL DKMPALPAKG NNLNRDILESDFafa 0 NTINIAKNDFSIELAAIPFNTLADHYGERLAREQLALEHESYEMGEARFRKMFERQLKAGEVADNAAAKPLITTLPLPKMIARINDW FEEVKA KRGRPTAFQFLQEIKPEAVAYITIKTLACLTSADNTTVQAVASAIGRAIEDEARFGRIRDLEAKHFKNVEEQLNKRVGH VYKKA FMQVVEADMLSKGLLGGEAWSSWHKEDSIHVGVRCEIEMIESTGMVSLHRQ----- SETIELAPEYAEAIATRAGALAGISPMFQPCVVPKPWTGITGGGYWANGRRPLALVRTHSKKALMRYEDVYMPEVYKAINIAQNT AWKINKKVLAVANVITKWKHCPVEDIPAIEREELPMK----- TAWKRAAAAVYRKDKARKSRRISLEFMLEQANKFANHKAIWFPYNMDWRGRVYAVSMFNPQGNDMTKGLLTLAKGKPIGKEGY WVKIHGANCAGVDKVPFPERIKFIEENHENIMACAKSPLENTWWAEQDSPFCFLAFCFEYAGVQHHLGSLYNCSLPLAFDGS CSGI HFSAMLRDEVGGRVAVNLLPSETVQDIYGIVAKKVNEILQADAINGTDNEVVTVDENTGEISEKVKLGTKALAGQWLA YGVTRSVT KRSVMTLAYGSKEFGFRQQVLEDTIQPAIDSGKGLMFTQPNQAAGYMAKLIWESVSVTVVAAVEAMNWLKSAAKLLAAEVKDKK TGEILRKRCVHVWTPDGFVPVWQEYKKPIQTRLNLMFLGGQFRLQPTINTNKDSEIDAHKQESGIAPNFVHSQDGSHLRKT VVWAHE KYGIESFALIHDSFGTIPADAANLFKAVRETMVDITYESCDVLADFYDQFADQLHESQLDKMPALPAKG NNLNRDILESDFafa</pre>

Table S3. Flags used with RosettaCM: hybridize

```
-in:file:fasta target.fasta
-parser:protocol hybridize.xml
-default_max_cycles 200
-dualspace
-nstruct 5
```

Table S4. Hybridize XML script (hybridize.xml)

```
<ROSETTASCRIPTS>
<TASKOPERATIONS>
</TASKOPERATIONS>
<SCOREFXNS>
  <ScoreFunction name="stage1" weights="score3" symmetric="0">
    <Reweight scoretype="cart_bonded" weight="0.5"/>
    <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
  </ScoreFunction>
  <ScoreFunction name="stage2" weights="score4_smooth_cart" symmetric="0">
    <Reweight scoretype="cart_bonded" weight="0.5"/>
    <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
  </ScoreFunction>
  <ScoreFunction name="fullatom" weights="ref2015" symmetric="0">
    <Reweight scoretype="pro_close" weight="0.0"/>
    <Reweight scoretype="cart_bonded" weight="0.5"/>
    <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
  </ScoreFunction>
</SCOREFXNS>
<MOVERS>
  <Hybridize name="hybridize" stage1_scorefxn="stage1" stage2_scorefxn="stage2" fa_scorefxn="fullatom" batch="1"
stage1_increase_cycles="1.0" stage2_increase_cycles="1.0" linmin_only="1" add_hetatm="1">
    <Template pdb="1MSW_D2A_renumber.pdb_thread.pdb" cst_file="AUTO" weight="1.000" />
  </Hybridize>
  <FastRelax name="relax" scorefxn="fullatom" />
</MOVERS>
<PROTOCOLS>
  <Add mover="hybridize" />
  <Add mover="relax" />
</PROTOCOLS>
<OUTPUT scorefxn="fullatom" />
</ROSETTASCRIPTS>
```

Table S5. Flags used with min_with_cst

```
-in:file:1 pdblast.txt
-in:file:fullatom
-ignore_unrecognized_res
-ex1
-ex2
-ex1aro
-ex2aro
-use_input_sc
-fa_max_dis 9.0
-ddg::harmonic_ca_tether 0.5
-ddg::constraint_weight 1.0
-ddg::sc_min_only false
-ddg::out_pdb_prefix min_cst 0.5
```

Table S6. Flags used with ddg_monomer

```
-in:file:s <pdfile>
-in:file:fullatom
-ddg:mut_file <mutfile>
```

```

-ignore_unrecognized_res
-ex1
-ex2
-ex1aro
-ex2aro
-use_input_sc
-ddg:mean false
-ddg:min true
-ddg:weight_file ddg.wts
-ddg:min_cst false
-ddg:sc_min_only false
-ddg:iterations 5
-ddg:output_silent true
-ddg:dump_pdb false
-ddg:suppress_checkpointing true
-delete_old_poses

```

Table S7. Example mutfile for ddg_monomer (min_cst_0.5.model-3_0001.119.mutfile)

```

total 20
1
S 119 A
1
S 119 C
1
S 119 D
1
S 119 E
1
S 119 F
1
S 119 G
1
S 119 H
1
S 119 I
1
S 119 K
1
S 119 L
1
S 119 M
1
S 119 N
1
S 119 P
1
S 119 Q
1
S 119 R
1
S 119 S
1
S 119 T
1
S 119 V
1
S 119 W
1
S 119 Y

```

REFERENCES

1. Kowalsky, C.A., et al. Rapid fine conformational epitope mapping using comprehensive mutagenesis and deep sequencing. *J Biol Chem* **290**, 26457-26470 (2015)