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Supplemental Information

Reprogramming Bacteriophage Host Range through Structure-Guided Design of Chimeric Receptor Binding Proteins

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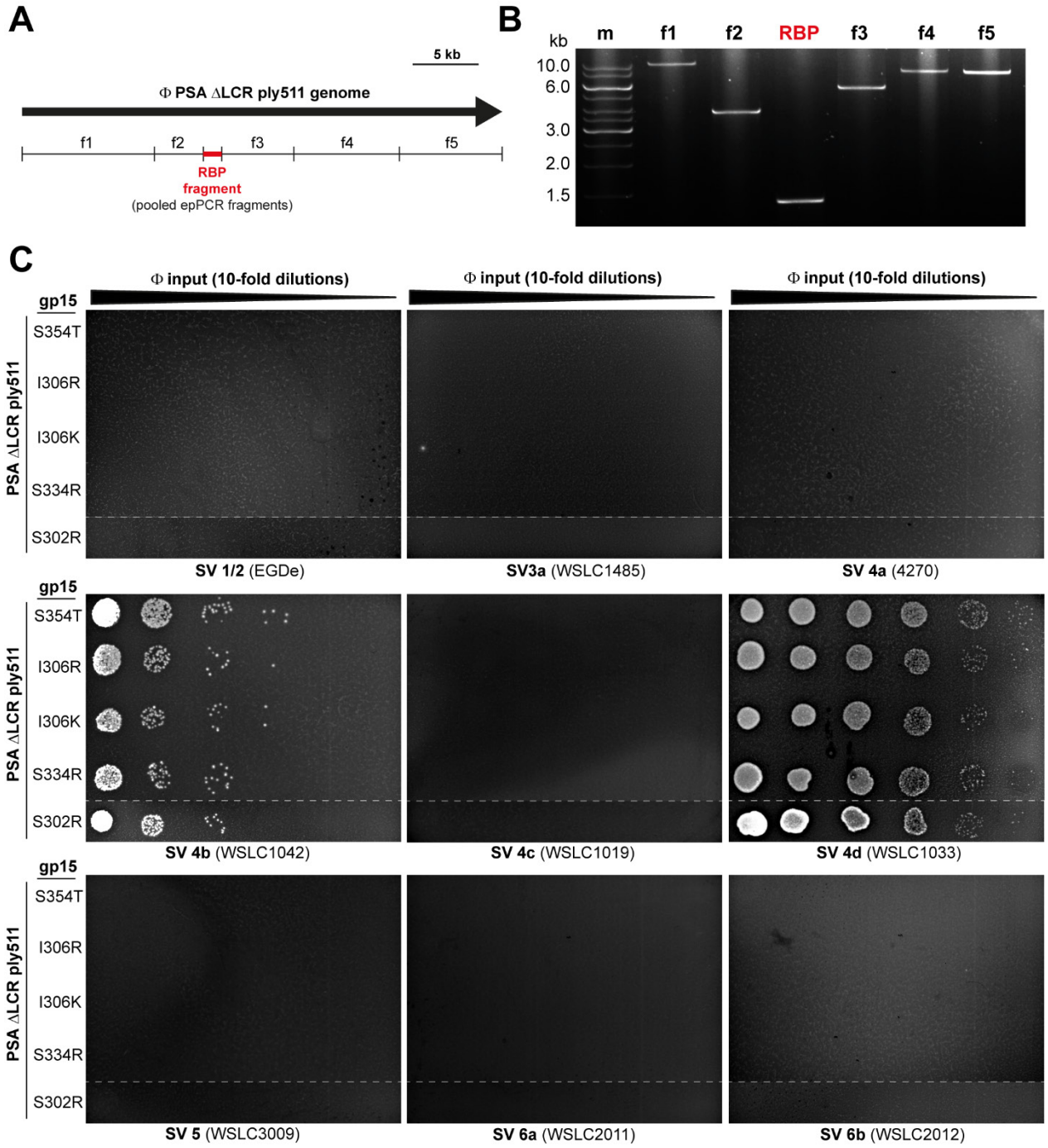

Figure S1, related to Figure 2: (**A-B**) Design and amplification of genome fragments for the construction of randomized RBP phage library. (**A**) Overlapping fragments f1 – f5 were amplified using a high fidelity polymerase, whereas the RBP fragment was amplified by error-prone PCR using primers P11 and P12 (see also **Tables S3 and S4**). (**B**) All fragments were purified and analyzed by agarose gel electrophoresis. (**C**) Host ranges of PSA ΔLCR *ply511*-derived RBP point mutants. Plating efficiencies of all isolated RBP point mutants were determined on representative strains of the major *Listeria* SVs using spot on the lawn assays. Plaque formation was restricted to SVs 4b and 4d.

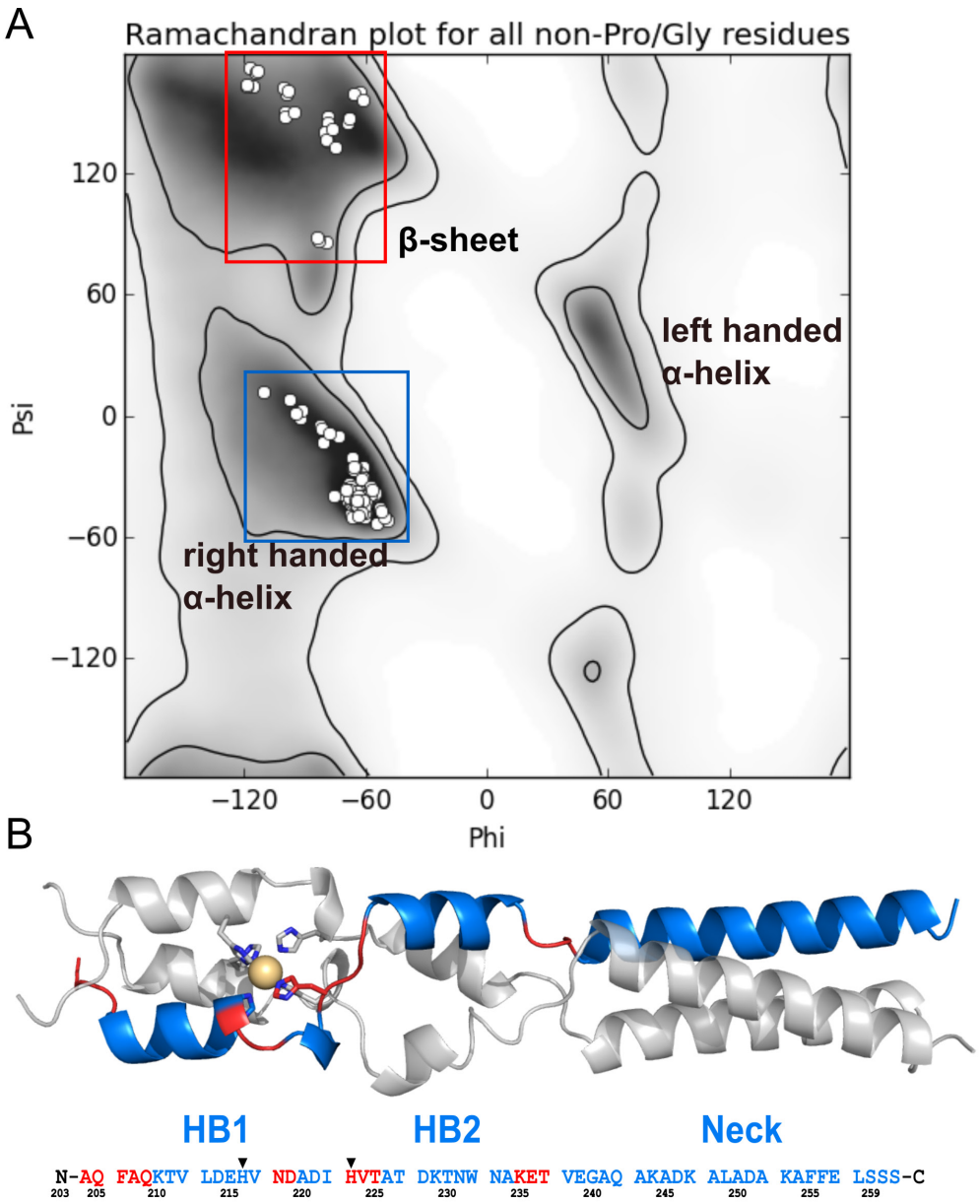

Figure S2, related to Figure 4. Conformational analysis of connecting β-layers and their segmentation of the stem and neck domains. (**A**) Ramachandran plot generated by MolProbity (Chen et al., 2010) of the backbone torsion angles for the stem and neck domains. All residues within helical bundles HB1 and HB2 and 3_{10} helix (blue box) have dihedral angles resembling those of right handed α-helices, whereas all residues within the interconnecting β-layers (red box) have dihedral angles resembling β-sheets (i.e., ϕ = -60 to -120º and ψ = 80 to 180º), as described in detail for this supersecondary structure (Hartmann, 2017). (**B**) Ribbon diagram and corresponding amino acid sequence for the stem and neck domains of gp15 with connecting β-layers and coiled coil α-helices colored red and blue, respectively. Connecting HB1 to HB2 is a 10-residue β-layer featuring the central 3_{10} helix. The shorter 3-residue β-layer connects the end of HB2 to the coiled coil neck domain. At the interface of HB1 and the connecting β-layer is a metal binding site (brown sphere; Cd^{2+}_{stem}) positioned in the center of the three-fold axis of the RBP, octahedrally coordinated by the side-chain Nε2 groups (shown as sticks) of His214 and His221 residues of all three monomers.

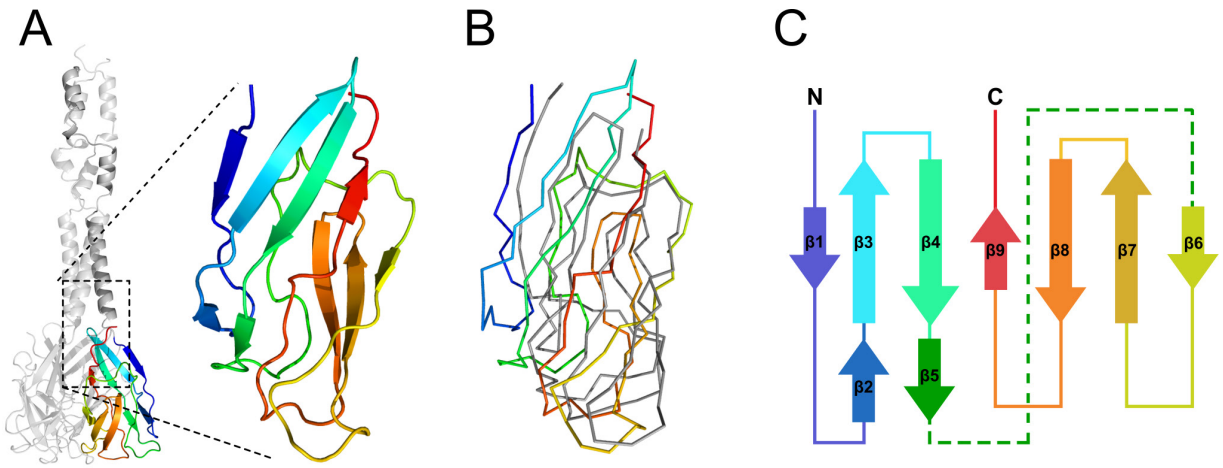

Figure S3, related to Figure 5. (**A**) Ribbon diagram of the $gp15_{CTD}$ homotrimer with a single head domain colored from N-terminus (blue) to C-terminus (red) and highlighted. **B)** Superposition of the gp15 head domain (rainbow) with TP901-1 (PDB ID: 3EJC; shown in grey) and P2 (PDB ID: 2BSD; not shown) yielded rmsd values of 3.2 Å for 130 residues and 3.7 Å for 119 residues, respectively. **C)** Topology diagram with secondary structure β-Strands shown as arrows of a single head domain reveals the quasi-double Greek key motif conserved across various viral head domains (as shown in Fig. 5).

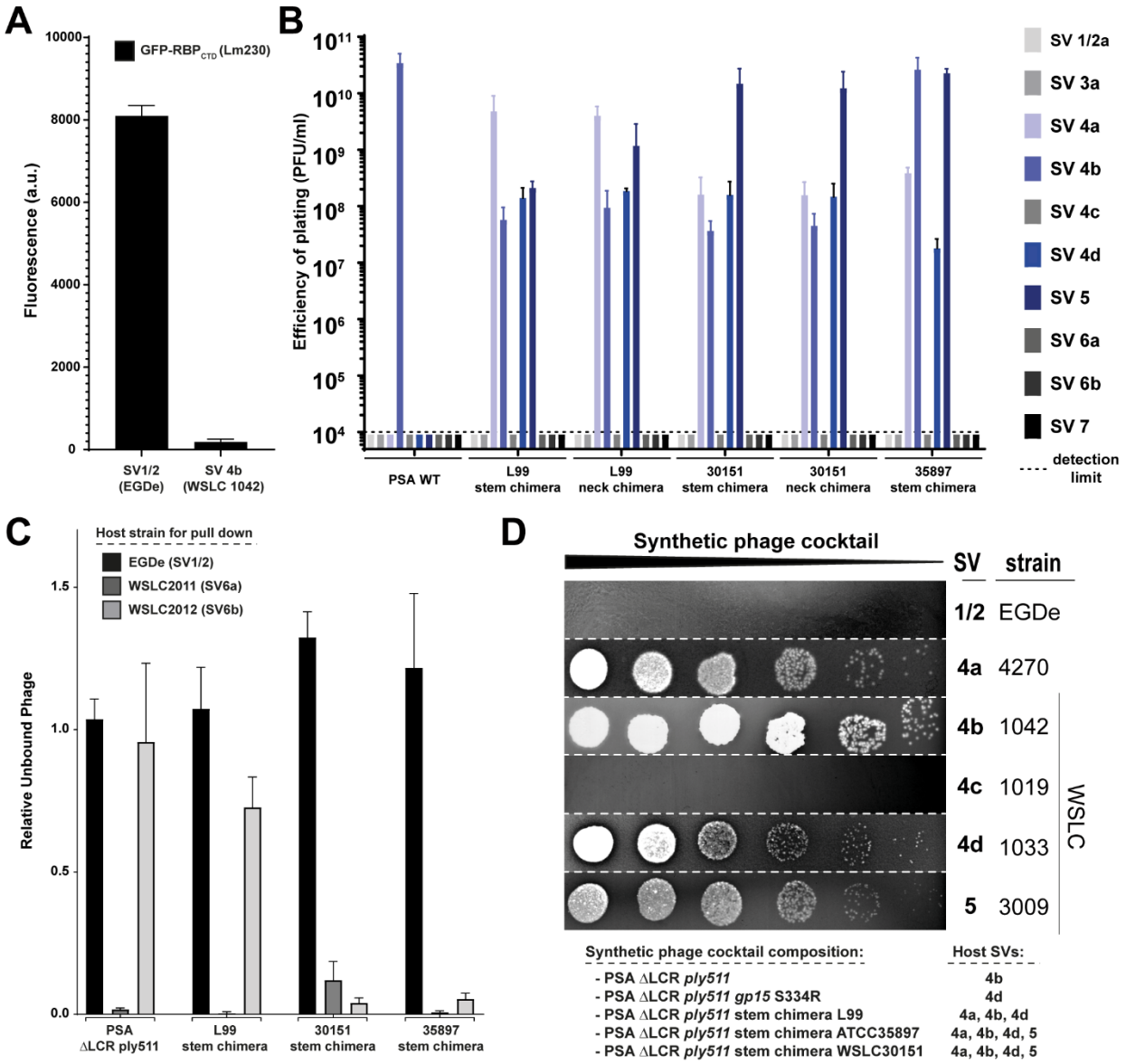

Figure S4, related to Figure 6: (**A**) The RBP candidate identified in strain Lm230 has predicted SV 1/2-specificity: Spectrofluorometric binding assays using a purified GFP-RBP$_{CTD}$ protein derived from the putative Lm230-encoded RBP reveals specific decoration of SV1/2 cells. Even though stem and neck chimeras with Lm230-derived head domains did not produce plaques on any SV, this data proofs the predicted RBP binding-specificity. (**B**) Plating efficiencies of PSA ΔLCR *ply511* and chimeric PSA derivatives on a panel of *Listeria* strains with divergent WTA architectures. Plating efficiencies of PSA ΔLCR *ply511* and the indicated chimeric phages were determined using spot on the lawn assays on *Listeria* strains covering SV 1/2 (strain EGDe), SV 3a (WSLC 1485), SV 4a (4270), SV 4b (WSLC 1042), SV 4c (WSLC 1019), SV 4d (WSLC 1033), SV 5 (WSLC 3009), SV 6a (WSLC 2011), and SV 6b (WSLC 2012). (**C**) Phage binding assays were performed using PSA ΔLCR *ply511* and the indicated PSA ΔLCR *ply511*-derived chimeric phages to quantify adsorption to SV 1/2 (strain EGDe, black bars), SV 6a (WSLC 2011, dark grey), and SV 6b cells (WSLC 2012, light grey), on which no plaque formation was observed. SVs 6b and SV 5 share identical RboP repeating units. SV 6a and SV 4b both feature galactosylated GlcNAc at the RboP C2 position, which is the receptor epitope of WT Gp15. Graph shows unbound phage particles relative to a phage only control. (**D**) PSA-based phage cocktail controls most *Listeria* serovars that feature typeII WTA. A phage cocktail was prepared containing equal titers of the indicated PSA-derivatives. 10-fold dilutions of the resulting phage cocktail were spotted on representative *Listeria* serovars covering both typeI (EGDe) and typeII WTAs (all other). All data is mean ± SD from three independent experiments.