SUPPORTING INFORMATION

Electrostatic and Hydrophobic Interactions Mediate Single-Stranded DNA Recognition and *Acta2* Repression by Purine-Rich Element Binding Protein B

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SUPPLEMENTAL MATERIALS AND METHODS

Epitope Screening ELISA. Purified recombinant NHis-Purβ proteins were diluted to a concentration of 100 nM in coating buffer consisting of 20 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$-$6H_2O$ pH 7.5 (HBSM pH 7.5) supplemented with 0.5 mM DTT and 5.0 μg/ml BSA. Coating solutions were applied at 100 μL per well to an EIA/RIA plate (Costar® 96 Well Easy Wash™, Certified High Binding, Corning, Inc.) and the plate was incubated overnight at 4°C. All subsequent steps were carried out at room temperature. Protein-coated wells were rinsed twice with 300 μL of wash buffer consisting of HBSM pH 7.5 with 0.05% (v/v) Tween 20 and then blocked for 2 h with 300 μL of 2% (w/v) BSA in HBSM pH 7.5. Blocking buffer was removed and 100 μL of primary rabbit anti-Purβ 210-229 antibody diluted in antibody buffer consisting of 0.2% (w/v) BSA, 0.05% (v/v) Tween 20 in HBSM pH 7.5 was applied to the wells. Following a 1 h incubation with primary antibody, wells were washed three times and 100 μL of a 1:4000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Inc.) was applied to each well. Following a 1 h incubation with secondary antibody, wells were washed four times before addition of 100 μL ABTS peroxidase substrate solution (Millipore). The reaction was quenched by the addition of 100 μL of 1% (w/v) SDS. Following a short 5 min stabilization period, the absorbance value of each well at 405 nm was obtained using a Vmax plate reader operated by SoftMax Pro Version 6.4.1 software (Molecular Devices). Absorbance values were corrected for nonspecific binding by subtracting background absorbance generated in BSA only-coated wells. Data points were plotted and fit to a hyperbola using Prism 6 (GraphPad Software, Inc.).

Expression and purification of NHis-Purβ point mutants. A total of 4 L of Miller's LB broth (Fisher Scientific) containing 100 μg/mL ampicillin was inoculated with a 1:16 dilution of starter culture (pQE30-NHis-Purβ WT or mutated plasmid transformed into *E. coli* strain JM109)

and incubated in a shaker-incubator at 250 rpm and 37°C. Once the culture reached a density of 0.6 absorbance units at 600 nm, isopropyl-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM in the case of Purβ R267A and the culture was incubated for an additional 5 h at ambient temperature. The expression of all other NHis-Purβ point mutants was achieved using 1.0 mM IPTG for 4 h at 37°C. The cells were collected by centrifugation at 6,540 × *g* for 16 min at 4°C and then frozen at −80°C.

Cell pellets were treated with lysozyme and then sonicated in lysis buffer consisting of 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole pH 8.0, 10 mM β-mercaptoethanol (BME), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 1.0 μg/mL each of pepstatin A, leupeptin, and aprotinin as previously described [1, 2]. Cleared cell lysate was combined with 3-4 mL of HIS-Select® Nickel Affinity Gel (Sigma-Aldrich) and incubated on a rocker overnight at 4°C. The resin was transferred into a small chromatography column and washed with lysis buffer until the absorbance at 280 nm (A_{280}) of the eluate was less than 0.1 units. In the case of NHis-Purβ R267A mutant, the resin was then washed with imidazole-free lysis buffer containing 2 M NaCl until A_{280} of the eluate was less than 0.02 units. In the case of the double (R159A/R267A) and triple (K82/R159A/R267A) point mutants, 10 mM imidazole was retained in the 2 M NaCl wash buffer. After re-equilibrating the column with complete lysis buffer, His-tagged protein was eluted from the resin using a gradient of 10 to 500 mM imidazole in lysis buffer. Fractions were monitored for the presence of protein by A_{280} measurement in conjunction with SDS-PAGE. In the case of the double and triple point mutants, fractions enriched in NHis-Purβ protein were pooled, concentrated, and then subjected to calibrated size exclusion chromatography (SEC) in buffer consisting of 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole pH 8.0, 10 mM BME. In the case of NHis-Purβ R267A, an additional purification step was required prior to SEC due to the

presence of degradation products and other contaminants. Specifically, R267A-enriched fractions from the Ni-affinity column were pooled, diluted 2-fold, and dialyzed into low salt heparin affinity buffer consisting of 20 mM Tris-Cl pH 7.4, 100 mM NaCl, 0.5 mM EDTA, pH 7.4, 10 mM BME, and 0.5 mM PMSF. The dialyzed protein was applied to a heparin agarose column and washed with low salt buffer until the A_{280} reached a constant baseline value. Bound protein was eluted with a 0.1-2 M NaCl gradient in heparin affinity buffer. Fractions enriched in NHis-Purβ R267A were pooled, dialyzed against SEC buffer, and concentrated to ~2 mL using a 10,000 MWCO Amicon Ultra Centrifugal filter device (Millipore). The concentrated protein was then applied to a 1.5 × 120 cm Sephacryl S200-HR and resolved at a flow rate of ~1.5 mL/min. Fractions enriched in NHis-Purβ were pooled and the final protein concentration was determined from A_{280} and A_{320} measurements using the Rayleigh correction factor for light scattering and a theoretical molar extinction coefficient of 20,400 M^{-1} cm^{-1}.

Table S1

Primers used to generate expression plasmids encoding mouse Purβ point mutants

Primer Name	Primer Sequence (5′ to 3′)
R267A sense	GTGAAGCCGTCCTACGCCAATGCCATCACCGT
R267A antisense	ACGGTGATGGCATTGGCGTAGGACGGCTTCAC
K82A sense	GTGGGCGCGGGCGGCTCCGCGAGCCGCCTCACGCTGTCGATG
K82A antisense	CATCGACAGCGTGAGGCGGCTCGCGGAGCCGCCCGCGCCCAC
R159A sense	GCTTCCTGCGCATCGCCCAGACGGTGAACC
R159A antisense	GGTTCACCGTCTGGGCGATGCGCAGGAAGC

Figure S1

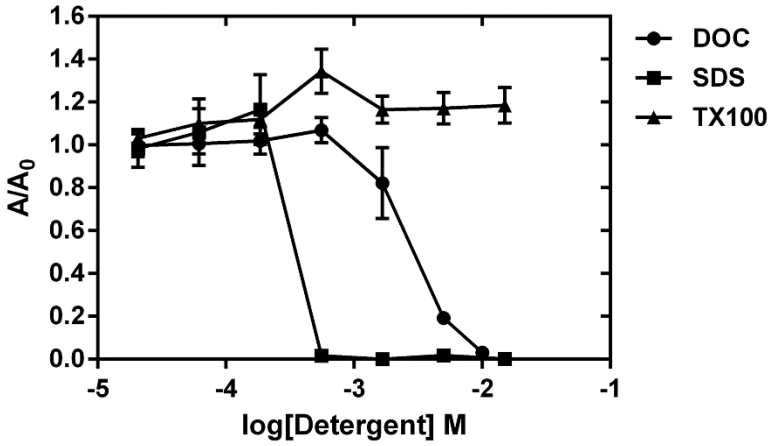

Figure S1. Effect of various detergents on the interaction of Purβ with *Acta2* ssDNA. The binding of 1.0 nM full-length Purβ to 0.5 nM PE32-bF ssDNA was assessed in assay buffer containing varying concentrations of sodium deoxycholate (DOC), sodium dodecyl sulfate (SDS), or Triton X-100 (TX100). Solid-phase Purβ-PE32-bF complexes were detected by ELISA using rabbit anti-mouse Purβ 210-229 as the primary antibody. Nonspecific background absorbance at 405 nm in control wells without any DNA was subtracted from the signal generated in PE32-bF-coated wells. Data points show absorbance values at 405 nm normalized to the mean absorbance value determined in wells without added detergent (mean ± SD, n = 3).

Figure S2

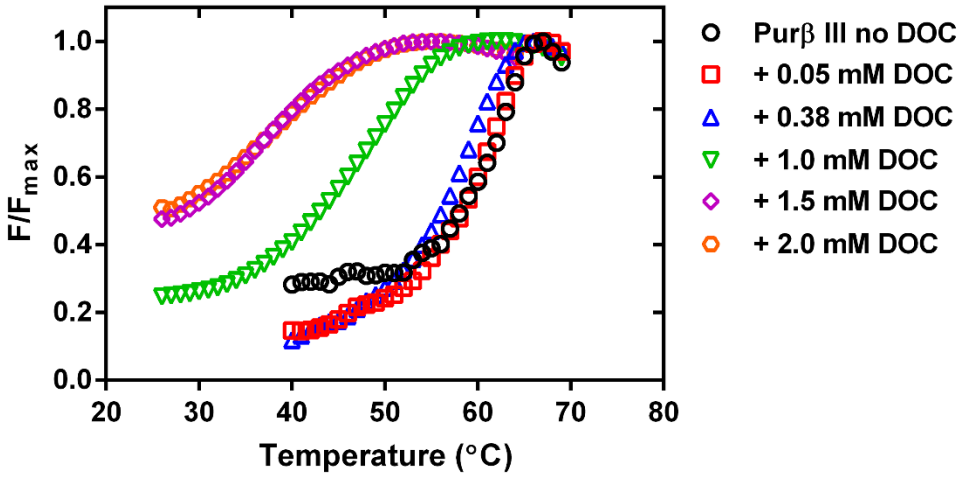

Figure S2. Relative thermostability of the isolated Purβ III intermolecular subdomain in the presence of deoxycholate. The unfolding of the Purβ III dimerization domain was evaulated by thermal shift assay at a protein concentration of 100 μg/mL in buffer composed of 20 mM HEPES, 150 mM NaCl, 10 mM β-mercaptoethanol pH 7.5 supplemented with increasing concentrations of sodium deoxycholate (DOC). The leftward shift of the curves as a function of increasing DOC concentration from 0.05 to 2.0 mM is indicative of some loss in the structural integrity of this subdomain.

Figure S3

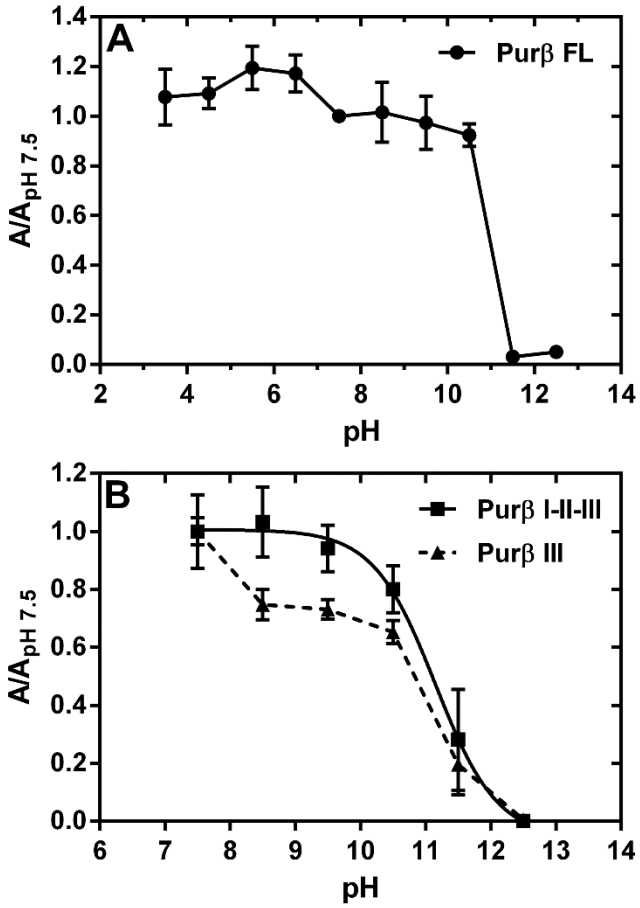

Figure S3. Effect of solution pH on the interaction of Purβ truncation proteins with *Acta2* ssDNA. (A) The binding of 1.0 nM full-length Purβ (Purβ FL) to 0.5 nM biotinylated PE32-bF ssDNA was assessed in complete binding buffer at pH ranging from 3.5 to 12.5. Solid-phase Purβ-PE32-bF complexes were detected by ELISA using rabbit anti-mouse Purβ 210-229 as the primary antibody. Nonspecific background absorbance at 405 nm in control wells without any DNA was subtracted from the signal generated in PE32-bF-coated wells. Background corrected A405 values measured at each pH were normalized by dividing by the mean A405 value determined at pH 7.5. (B) The interaction of Purβ I-II-III (1.0 nM) and Purβ III (5.0 nM) with 0.5 nM PE32-bF ssDNA was assessed by ELISA as in (A) in binding buffer without $MgCl_2$ at pH ranging from 7.5 to 12.5.

Figure S4


sp|O35295|PURB_MOUSE --MADGDSGSERG----GGGGGGGGPGGFQPAPRGGGGGGGGPGG----- 39
sp|Q96QR8|PURB_HUMAN --MADGDSGSERG----GGGG----PCGFQPASRGGG------------ 27
sp|P42669|PURA_MOUSE --MADRDSGSEQGGAALGSGGSLGHPGSGSGSGGGGGGGGGGGGGSGGGGG 48
sp|Q00577|PURA_HUMAN --MADRDSGSEQGGAALGSGGSLGHPGSGSGSGGGGGGGGGGGGGSGGGGG 48
sp|Q8R4E6|PURG_MOUSE MERARRRGGGGSGGGRGRGGKNVGGPGLSKSRLYPQAQHSHYPHYSASAT 50
sp|Q9UJV8|PURG_HUMAN MERARRRGGGG---GRGRGGKNVGGSGLSKSRLYPQAQHSHYPHYAASAT 47
tr|O16860|O16860_DROME --MSDLGSGDD------GISGSKYNVANMEGSSSRNDFDSSAKGR----- 37
sp|O35295|PURB_MOUSE ------EQETQELASKRLDIQNKRFYLDVKQNAKGRFLKIAEVGAGGS-- 81
sp|Q96QR8|PURB_HUMAN ------EQETQELASKRLDIQNKRFYLDVKQNAKGRFLKIAEVGAGGS-- 69
sp|P42669|PURA_MOUSE -APGGLQHETQELASKRVDIQNKRFYLDVKQNAKGRFLKIAEVGAGGN-- 95
sp|Q00577|PURA_HUMAN GAPGGLQHETQELASKRVDIQNKRFYLDVKQNAKGRFLKIAEVGAGGN-- 96
sp|Q8R4E6|PURG_MOUSE PNQSGGTSEIQELASKRVDIQKKRFYLDVKQSSRGRFLKIAEVWIGRGRQ 100
sp|Q9UJV8|PURG_HUMAN PNQAGGAAEIQELASKRVDIQKKRFYLDVKQSSRGRFLKIAEVWIGRGRQ 97
tr|O16860|O16860_DROME ------SGVEQELATKMLQIQSKRFYLDVKQNRRSRFIKVAEIGADGR-- 79
sp|O35295|PURB_MOUSE ----KSRLTLSMAVAAEFRDSLGDFIEHYAQLGP---------------- 111
sp|Q96QR8|PURB_HUMAN ----KSRLTLSMAVAAEFRDSLGDFIEHYAQLGP---------------- 99
sp|P42669|PURA_MOUSE ----KSRLTLSMSVAVEFRDYLGDFIEHYAQLGP---------------- 125
sp|Q00577|PURA_HUMAN ----KSRLTLSMSVAVEFRDYLGDFIEHYAQLGP---------------- 126
sp|Q8R4E6|PURG_MOUSE DNIRKSKLTLSLSVAAELKDCLGDFIEHYAHLGLKGHRQEHGQSKEQVSR 150
sp|Q9UJV8|PURG_HUMAN DNIRKSKLTLSLSVAAELKDCLGDFIEHYAHLGLKGHRQEHGHSKEQGSR 147
tr|O16860|O16860_DROME -----RNPILGSFTAAEFRDHLSSFSDYYASLSP---------------- 108
sp|O35295|PURB_MOUSE -----SSP-EQLAAGAEEGGGPRRALKSEFLVRENRKYYLDLKENQRGRF 155
sp|Q96QR8|PURB_HUMAN -----SSP-EQLAAGAEEGGGPRRALKSEFLVRENRKYYLDLKENQRGRF 143
sp|P42669|PURA_MOUSE -----SQP-PDLAQAQDE---PRRALKSEFLVRENRKYYMDLKENQRGRF 166
sp|Q00577|PURA_HUMAN -----SQP-PDLAQAQDE---PRRALKSEFLVRENRKYYMDLKENQRGRF 167
sp|Q8R4E6|PURG_MOUSE RRQKHSAPSPPVSVGSEE--HPHSVLKTDYIERDNRKYYLDLKENQRGRF 198
sp|Q9UJV8|PURG_HUMAN RRQKHSAPSPPVSVGSEE--HPHSVLKTDYIERDNRKYYLDLKENQRGRF 195
tr|O16860|O16860_DROME ---------QNTDNLPED-----GKLKSEMMIKDYRRYYLDLKENARGRF 144
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sp|Q96QR8|PURB_HUMAN LRIRQTVNRGGGGFGAGPGPGGLQSGQTIALPAQGLIEFRDALAKLIDDY 193
sp|P42669|PURA_MOUSE LRIRQTVNRG-------PGLGSTQ-GQTIALPAQGLIEFRDALAKLIDDY 208
sp|Q00577|PURA_HUMAN LRIRQTVNRG-------PGLGSTQ-GQTIALPAQGLIEFRDALAKLIDDY 209
sp|Q8R4E6|PURG_MOUSE LRIRQTMMRGTGMIG-YFGHSLGQ-DQTIVLPAQGMIEFRDALVQLIEDY 246
sp|Q9UJV8|PURG_HUMAN LRIRQTMMRGTGMIG-YFGHSLGQ-EQTIVLPAQGMIEFRDALVQLIEDY 243
tr|O16860|O16860_DROME LRVSQTITRG--------GPRS-----QIALPAQGMIEFRDALTDLLEEF 181
sp|O35295|PURB_MOUSE GGDEDELAGGPGGGAGGPGGGLYGELPEGTSITVDSKRFFFDVGCNKYGV 255
sp|Q96QR8|PURB_HUMAN GGEDDELAGGPGGGAGGPGGGLYGELPEGTSITVDSKRFFFDVGCNKYGV 243
sp|P42669|PURA_MOUSE GVEEE------------P-----AELPEGTSLTVDNKRFFFDVGSNKYGV 241
sp|Q00577|PURA_HUMAN GVEEE------------P-----AELPEGTSLTVDNKRFFFDVGSNKYGV 242
sp|Q8R4E6|PURG_MOUSE GEGDIE---ERRCGDDDP-----LELPEGTSFRVDNKRFYFDVGSNKYGI 288
sp|Q9UJV8|PURG_HUMAN GEGDIE---ERRGGDDDP-----LELPEGTSFRVDNKRFYFDVGSNKYGI 285
tr|O16860|O16860_DROME GANDGG--------------RFKGDLPEERHMKVDNKNFYFDIGQNNRGV 217
sp|O35295|PURB_MOUSE FLRVSEVKPSYRNAITVPFKAWGKFGGAFCRYADEMKEIQERQRDK---- 301
sp|Q96QR8|PURB_HUMAN FLRVSEVKPSYRNAITVPFKAWGKFGGAFCRYADEMKEIQERQRDK---- 289
sp|P42669|PURA_MOUSE FMRVSEVKPTYRNSITVPYKVWAKFGHTFCKYSEEMKKIQEKQREKRAAC 291
sp|Q00577|PURA_HUMAN FMRVSEVKPTYRNSITVPYKVWAKFGHTFCKYSEEMKKIQEKQREKRAAC 292
sp|Q8R4E6|PURG_MOUSE FLKVSEVRPPYRNTITVPFKAWTRFGENFIKYEEEMRKICNSHKEK---- 334
sp|Q9UJV8|PURG_HUMAN FLKVSEVRPPYRNTITVPFKAWTRFGENFIKYEEEMRKICNSHKEK---- 331
tr|O16860|O16860_DROME YMRISEVKNNFRTSITIPEKCWIRFRDIFNDYCEKMKKSSDSITAE---- 263

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sp|Q96QR8|PURB_HUMAN        --LYERRGGGSGGGEES----EGEEVDED- 312
sp|P42669|PURA_MOUSE        EQLHQQQQQQQEETTAATLLLQGEEEGEED 321
sp|Q00577|PURA_HUMAN        EQLHQQQQQQQEETAAATLLLQGEEEGEED 322
sp|Q8R4E6|PURG_MOUSE        ---------RMDGRRAS-----GEEQECLD 350
sp|Q9UJV8|PURG_HUMAN        ---------RMDGRKAS-----GEEQECLD 347
tr|O16860|O16860_DROME      ----------INLPTSS------NSLK--- 274
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Figure S4. CLUSTAL 2.1 multiple sequence alignment of mouse and human Purα, Purβ, and Purγ proteins in comparison to *Dm* Purα. Residues selected for mutagenesis in Purβ are highlighted in yellow or light blue. The peptide epitopes of the rabbit anti-mouse Purβ antibodies (B210-229 and B302-324) used in this study are underlined.

Figure S5

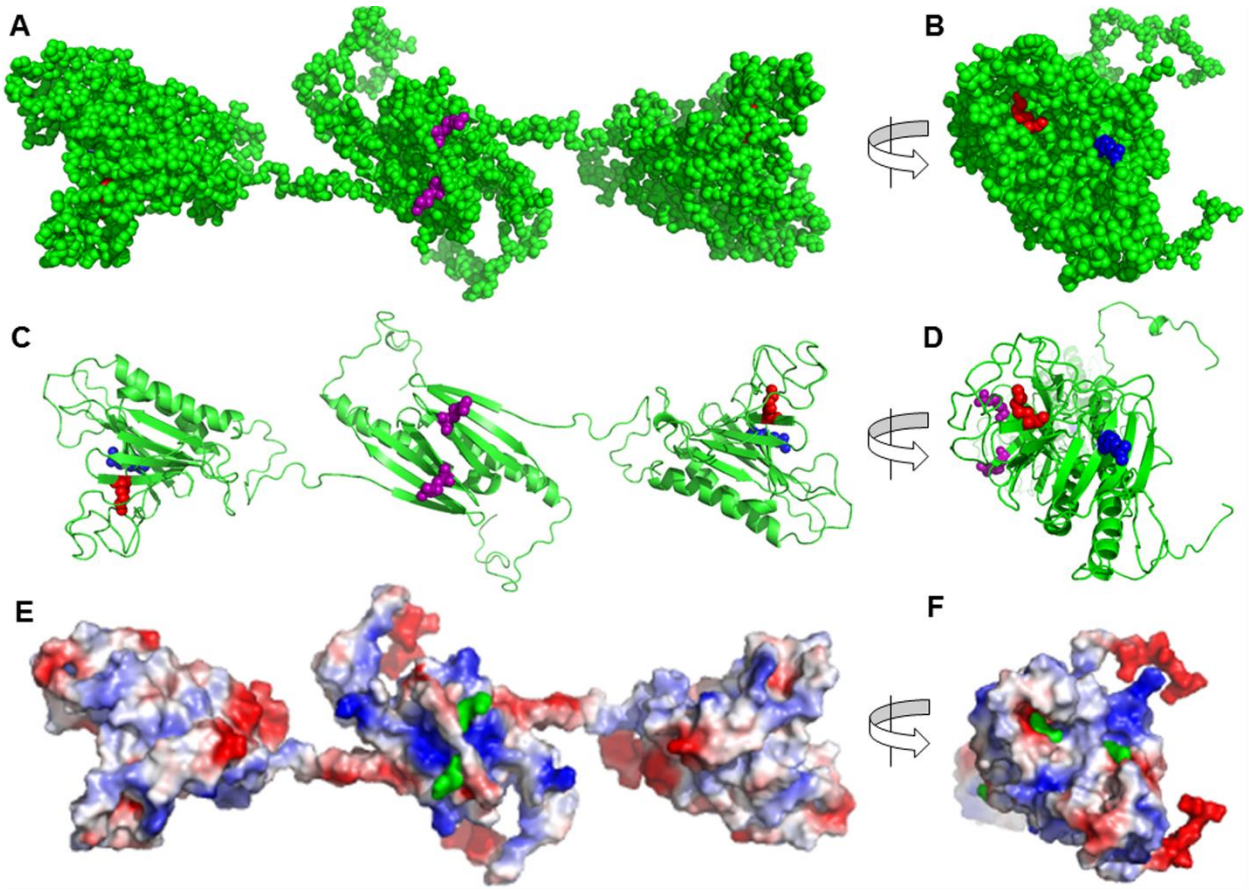

Figure S5. Homology model of *Mm* Purβ highlighting basic residues implicated in ssDNA-binding. (A) Space-filling model of the *Mm* Purβ dimer depicting surface exposure of K82 (red), R159 (blue), and R267 (purple). (B) Model in (A) rotated 90° around the vertical axis clearly shows the location of K82 and R159. (C) A 3D ribbon model of the *Mm* Purβ dimer highlighting the location of the selected basic residues. (D) Model in (C) rotated 90° around the vertical axis. (E) Electrostatic surface maps of the *Mm* Purβ dimer showing distinct regions of charged amino acids. All selected basic amino acids are located within positively charged clusters (blue areas). (F) Model in (E) rotated 90° around the vertical axis to depict a distinct positively charged channel.

Figure S6

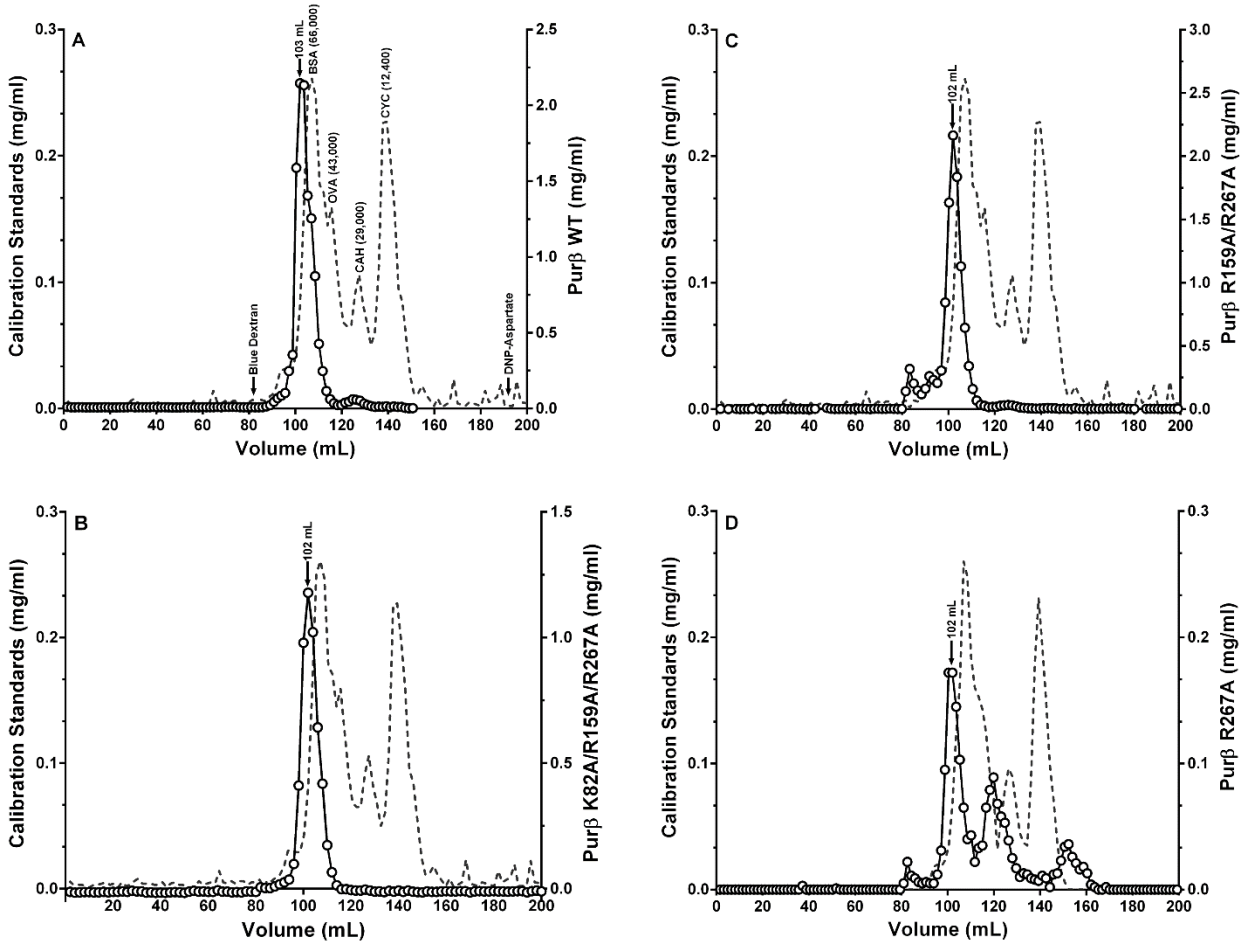

Figure S6. Quaternary structure of Purβ point mutants. Calibrated SEC was conducted on preparations of Purβ WT (A), Purβ K82A/R159A/R267A (B), Purβ R159A/R267A (C), and Purβ R267A (D) at loading concentrations in excess of 10 μM (open circles). The elution profile of a mixture of molecular weight standards is shown for comparison (dash lines). Numbers in (A) indicate the apparent molecular weights of the four globular protein standards used to calibrate the 1.5 × 120 cm column packed with Sephacryl S200. Arrows in (A) indicate the elution positions of Blue Dextran and DNP-asparate, respectively. The minor protein peaks eluting after the homodimer in (D) correspond to contaminants and/or degradation products. WT, wild-type, BSA, bovine serum albumin, OVA, ovalbumin, CAH, carbonic anhydrase, CYC, cytochrome C.

Figure S7

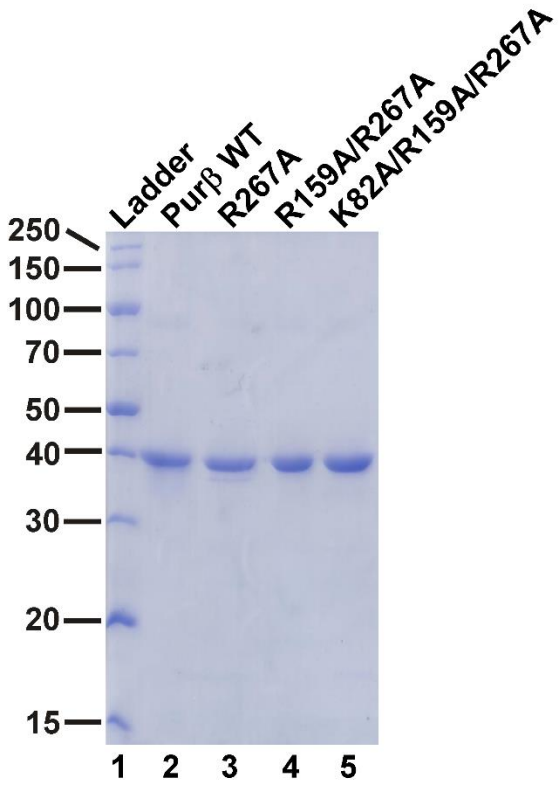

Figure S7. SDS-PAGE analysis of Purβ point mutants. The indicated proteins (1.5 μg) were resolved on a 12% mini-gel and stained with Coomassie Brilliant Blue. Numbers on the left indicate molecular mass in kilodaltons of each standard protein in the ladder in lane 1.

Figure S8

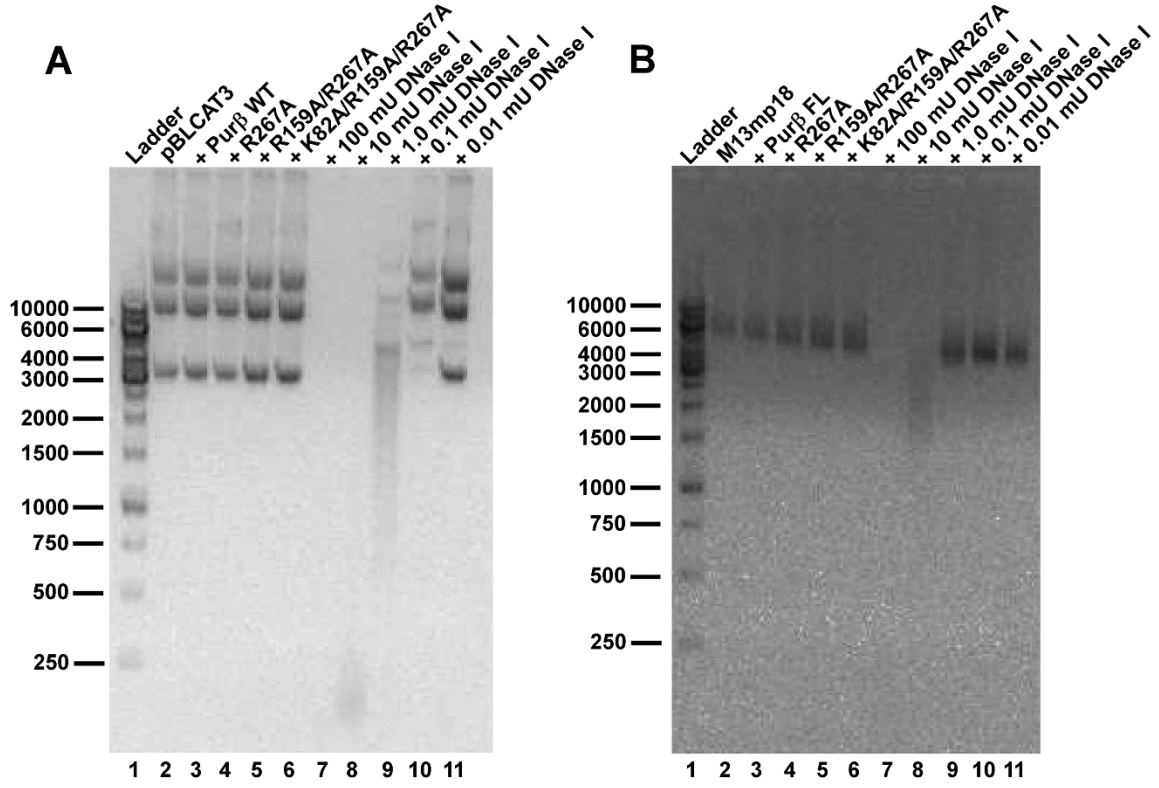

Figure S8. Absence of detectable nuclease activity in purified preparations of recombinant Purβ. (A, B) Solutions containing 1.0 μM of the indicated NHis-Purβ proteins plus 2.0 μg of either double-stranded pBLCAT3 plasmid (A) or single-stranded M13mp18 DNA (B) were incubated for 1 h at 37°C. Solvent conditions were identical to those used in nucleoprotein binding assays with the exception that DTT and the dT32 oligonucleotide were left out of the buffer. As a positive control for the presence of nuclease activity, DNA substrates were incubated in parallel with 100 to 0.01 mUnits of DNase I. The DNA in each reaction mixture was extracted, precipitated, and resolved (~1 μg per lane) on a 1% w/v agarose gel in the presence of ethidium bromide. Fluorescent DNA bands were visualized by UV illumination. Numbers on the left indicate the size of bands (in bp) present in the 1 kb DNA ladder (lane 1). WT, wild-type.

Figure S9

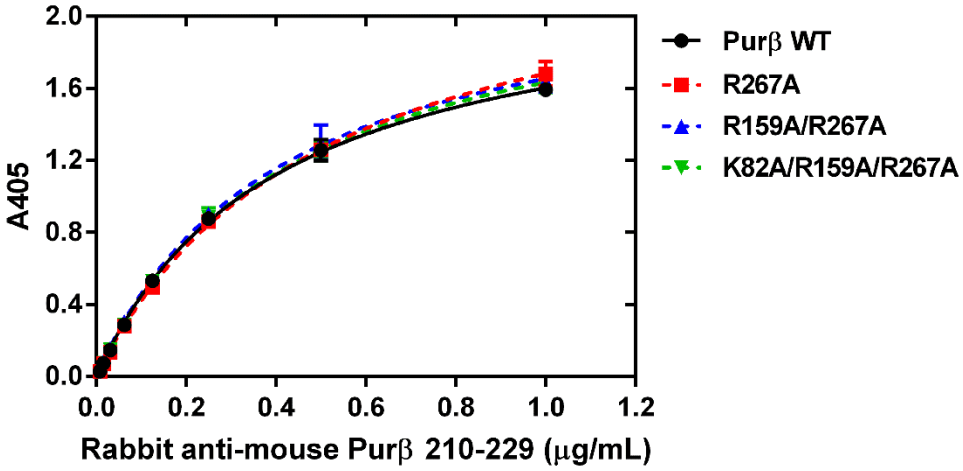

Figure S9. Screening of a rabbit anti-mouse Purβ antibody raised against residues 210-229 for reactivity with wild-type Purβ (Purβ WT) and single (R267A), double (R159A/R267A), or triple (K82A/R159A/R267A) Purβ point mutants. Microtiter wells coated with 100 nM of the indicated Purβ proteins were incubated with varying concentrations of peptide affinity-purified rabbit anti-mouse Purβ 210-229 antibody. Complex formation was detected by ELISA. Binding curves were generated by fitting absorbance values at 405 nm to the equation for a hyperbola. As shown, the resultant lines are virtually superimposable.

Figure S10

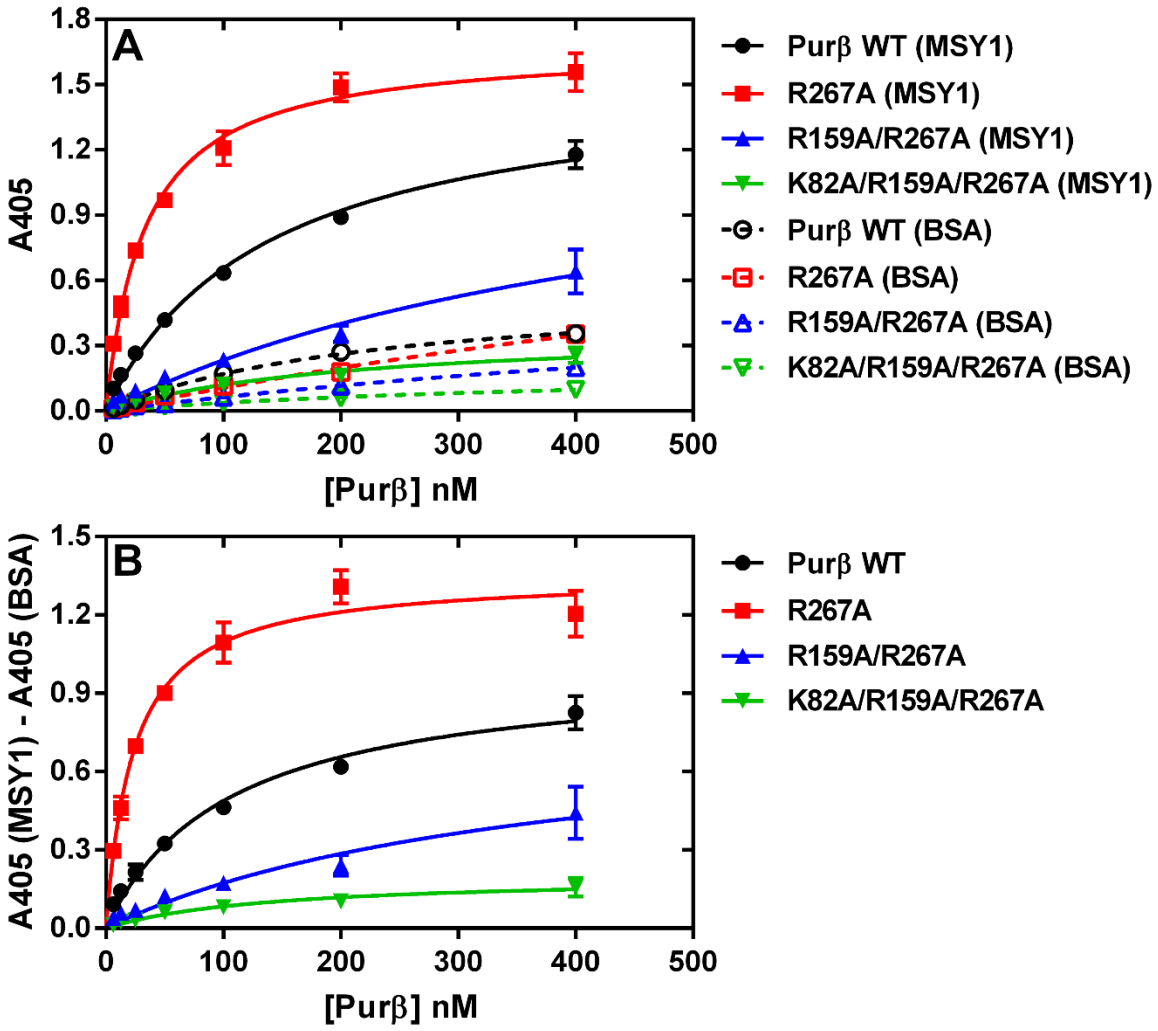

Figure S10. Effect of R/K point mutations on the interaction of Purβ with MSY1. (A) The binding of purified Purβ wild-type (WT) protein or the indicated single (R267A), double (R159A/R267A), or triple (K82A/R159A/R267A) point mutants to immobilized MSY1 (filled symbols, solid lines) or BSA (open symbols, dashed lines) was evaluated by ELISA. Purβ-MSY1 complexes were detected using rabbit anti-Purβ 302-324 as the primary antibody. Binding isotherms were generated by fitting data points to the equation for a rectangular hyperbola. (B) Binding curves generated after subtracting out the nonspecific absorbance measured in BSA only-coated wells from the absorbance obtained in MSY1-coated wells.

SUPPLEMENTAL REFERENCES

[1] Knapp, A. M., Ramsey, J. E., Wang, S. X., Godburn, K. E., Strauch, A. R., and Kelm, R. J., Jr. (2006) Nucleoprotein interactions governing cell type-dependent repression of the mouse smooth muscle alpha-actin promoter by single-stranded DNA-binding proteins Pur alpha and Pur beta, *J Biol Chem 281*, 7907-7918.

[2] Ramsey, J. E., Daugherty, M. A., and Kelm, R. J., Jr. (2007) Hydrodynamic studies on the quaternary structure of recombinant mouse Purbeta, *J Biol Chem 282*, 1552-1560.