Supplemental materials

A small-sized protein binder specific for human PD-1 effectively suppresses the tumor growth in tumor mouse model

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Figure S1. Binding affinity of selected repebodies for hPD-1 through ITC. Binding affinity of repebodies for hPD-1 was determined using a MicroCal iTC200 (Malvern, UK). 0.1 mM repebody was titrated with 0.01 mM hPD-1 at 25℃. Protein solution was injected 20 times with time interval of 120 s. The data were fitted and analyzed using the Origin program (OriginLab). (A) The binding affinity of r\_A1 was estimated to be 617 nM. (B) r\_G9 showed KD of 17.6 nM for hPD-1, which corresponds to a 35-fold increase compared to r\_A1. (C) Extracellular domain of hPD-1 was expressed from *E.coli* to obtain non-glycosylated form. KD value of r\_G9 for non-glycosylated hPD-1 was 28.9 nM.



**Figure S2. Binding property of the selected repebody for serum albumins from difference species.** Binding activity of the repebody binds to a serum albumin was determined using ELISA. Albumins from mouse, rat, rabbit, and human serum were coated on a 96-well ELISA plate. hPD-1 was used as a positive control. Myc-tagged repebody (r\_Off and r\_G9) was added to a plate followed by washing, and signals were detected by HRP-conjugated anti-c-myc antibody (Santa Cruz Biotechnology). Data represent the means ± standard deviations (n = 3).



Figure S3. Construction of hPD-1-expressing CHO-K1 cells by transient transfection. 1 × 105 CHO-K1 cells were transfected with 3μg of hPD-1/pCMV3 plasmid DNA by lipofectamine transfection reagent. After incubation 48 hrs post transfection, cells were labeled with anti-hPD-1 antibody conjugated with PE (phycoerythrin) (R&D systems) and analyzed by flow cytometry. Transfection efficiency was estimated to be 65.6 %.



**Figure S4. Blood half-life of the repebody.** Male balb/c mice with 4-5 weeks of age were intravenously administered with the anti-hPD-1 repebody (r\_G9) (10 mg/kg, 100 μL). Serum samples were obtained at time intervals, and serum concentration of the repebody was determined by sandwich ELISA. The initial and terminal half-lives of the repebody were determined using GraphPad Prism software. Data represent the mean standard deviation (n = 3).