Supplementary information, Data S1

Materials and Methods

Quantitative fluorescent EMSA

Quantitative fluorescent EMSA (FEMSAs) was performed to determine the dissociation constants between the wild-type and mutant WOPR domain and 20-bp dsDNA fragments of different sequences [1]. The single-stranded probe labeled by 5'-FAM (5'-aagaagttaaacttttttga-3') corresponding to the template strand and the complementary strand (No label) were synthesized by Sangon Biotech (Shanghai). The dsDNAs were prepared by annealing of the template and complementary strands from 95 °C to 22 °C over a period of 6 h. Protein concentrations were determined by the Nanodrop (Thermo Fisher Scientific, Inc.). In the assays, 3 nM labeled dsDNA was used. DNA-binding reactions (20 µl) were carried out for 1 h at 4 °C in the binding buffer (20 mM Tris-HCl, pH 8.0, and 30 mM NaCl) with the protein of 14 gradient concentrations (0 nM, 20 nM, 40 nM, 60 nM, 80 nM, 100 nM, 140 nM, 180 nM, 220 nM, 260 nM, 300 nM, 400 nM, 500 nM, and 1000 nM). After addition of 3 µl of the gel loading buffer (50% glycerol and 0.02% bromophenol blue), the reaction mixtures were run on gel containing 6% acrylamide, 0.5× TBE (45 mM Tris-borate, pH 8.0, and 1 mM EDTA), and 2.5% glycerol at 40 V for 120 min. The gels were imaged using Typhoon™ FLA 9000 (GE Healthcare, Ltd.) at the fluorescence excitation wavelength of 485 nm. Data were analyzed using the FujiFilm imaging analyzer. According to the known protein concentration and the fraction of the DNA probe, the curve was fit by the GraphPad Prism.

1 Pagano JM, Clingman CC, Ryder SP. Quantitative approaches to monitor protein-nucleic acid interactions using fluorescent probes. *RNA* 2011; **17**:14-20.