**Supplementary Methods**

**1. Bacterial Transformation**

1.1 Add 3 µL ligation or TOPO reaction mix to 40 µL of chemically competent *E. coli* cells (see **Table of Materials**) and leave on ice for 30 min.

1.2 Heat shock the bacteria at 42 °C for 45 s, then leave on ice for 2 min.

1.3 Add 250 µL of Super Optimal broth with Catabolite repression (SOC) media and recover the bacteria in a 37 °C incubator-shaker for 1 h.

1.4 Spread 80 µL transformed bacterial cells on a pre-warmed Luria broth (LB) agar plate with the appropriate antibiotics. Incubate the plate at 37 °C overnight.

NOTE: Check which antibiotic-resistant cassette is present in the cloning plasmid. Ampicillin is commonly used, but some plasmids may rely on kanamycin, gentamycin, chloramphenicol, or spectinomycin.

NOTE: If ampicillin is used, the transformed bacteria may be directly plated onto antibiotic-containing agar plates immediately after heat shock without the 1 h recovery at 37 °C.

**2. Western blot**

2.1 Dissociate the cells as described in steps 5.1‒5.3 of protocol and aspirate the supernatant.

2.2 Wash the pellet once with cold PBS, and spin them down in a bench top centrifuge at maximum speed (18,000 x g) for 5 min. Discard the supernatant and resuspend the cell pellet in cold RIPA buffer. Add protease inhibitor (see **Table of Materials**) to protect the proteins from degradation. Sonicate the cells for 10 cycles (30 s ON, 30 s OFF).

NOTE: Different protease inhibitors can be used, including aprotinin, leupeptin, pepstatin-A, and phenylmethylsulfonyl fluoride (PMSF). Commercially available cocktail tablets may also be used.

2.3 Determine the protein concentration using bovine serum albumin as a standard. Different methods can be used, including the Bradford assay and the bicinchoninic acid (BCA) assay.

2.4 Boil each cell lysate in 4x Laemmli sample buffer at 100 °C for 5 min.

2.5 For each sample, load 10 µg of protein into one empty well of an sodium dodecyl sulfate (SDS)-PAGE gel. Run the gel for 1‒2 h at 100 V using 1x tris-glycine-SDS (TGS) buffer.

2.6 Transfer the electrophoresed proteins onto a nitrocellulose or polyvinylidene fluoride (PVDF) membrane using 1x tris-glycine (TG) buffer with 10% ethanol.

2.7 Stain the membrane with Ponceau S solution to check that the proteins have been properly transferred. Wash off the stain with water. Block the membrane for 1 h at room temperature using 5% skimmed milk (blocking buffer) and then remove the blocking buffer.

2.8 Dilute the primary antibody with blocking buffer and add it to the membrane. Incubate the membrane at 4 °C overnight with gentle shaking. The dilution factor depends on the antibody and may have to be optimized.

2.9 Remove the primary antibody solution. Wash three times with 0.1% polysorbate in tris-buffered Saline (TBST) at 10 min intervals.

2.10 Remove the 0.1% TBST. Dilute the conjugated secondary antibody with blocking buffer based on supplier’s recommendations, then add it to the membrane. Incubate the membrane at room temperature for 2 h with gentle shaking.

2.11 Remove the secondary antibody solution. Wash three times with 0.1% TBST at 10 min intervals. Add a chemiluminescent reagent (see **Table of Materials**) to the membrane according to manufacturer’s instructions and visualize the membrane using an appropriate imaging system.