1. **Supplementary of Fig2: details of construction of anticalin library for in vitro selection.**

The process of amplification of BBP1: 3min at 94℃, 30s at 94℃, 30s at 45℃, 1min at 72℃, 5cylcles; 30s at 94℃, 30s at 60℃, 1min at 72℃, 25cylcles; 5min at 72℃. The PCR performed using PfuUltra^{TM} Ⅱ fusion HS DNA Polymerase and the primers Fbbp1and Rbbp1.

The process of amplification of BBP2: 3min at 94℃, 30s at 94℃, 30s at 45℃, 1min at 72℃, 5cylcles; 30s at 94℃, 30s at 60℃, 1min at 72℃, 25cylcles; 5min at 72℃. The PCR performed using PfuUltra^{TM} Ⅱ fusion HS DNA Polymerase and the primers F3437 and R5860.

The process of amplification of BBP3: 3min at 94℃, 30s at 94℃, 30s at 45℃, 1min at 72℃, 5cylcles; 30s at 94℃, 30s at 60℃, 1min at 72℃, 25cylcles; 5min at 72℃. The PCR performed using PfuUltra^{TM} Ⅱ fusion HS DNA Polymerase and the primers F8797 and R8797.

The process of amplification of BBP4: 3min at 94℃, 30s at 94℃, 30s at 45℃, 1min at 72℃, 5cylcles; 30s at 94℃, 30s at 60℃, 1min at 72℃, 25cylcles; 5min at 72℃. The PCR performed using PfuUltra^{TM} Ⅱ fusion HS DNA Polymerase and the primers F1416 and R2527.

The process of amplification of BBP5: 3min at 94℃, 30s at 94℃, 30s at 45℃, 1min at 72℃, 5cylcles; 30s at 94℃, 30s at 60℃, 1min at 72℃, 25cylcles; 5min at 72℃. The PCR performed using PfuUltra^{TM} Ⅱ fusion HS DNA Polymerase and the primers Ftail and Rtail.

BBP1 and BBP2 fragment were assembled. In the SOE experiment, the reaction mixture (50ng BBP1 fragment, 50ng BBP2 fragment) was subjected to an initial denaturation step at 94℃ for 3 min followed by 5 cycles of 94℃ for 30 s,40℃ for 30 s , 72℃ for 1 min and then followed by 25 cycles of 94℃ for 30 s, 57.5℃ for 30 s, 72℃ for 1 min and a final extension step at 72℃ for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers Fbbp1 and R69 were added.

BBP3 and BBP4 fragment were assembled. In the SOE experiment, the reaction mixture (50ng BBP3 fragment, 50ng BBP4 fragment) was subjected to an initial denaturation step at 94℃ for 3 min followed by 10 cycles of 94℃ for 30 s, 53℃ for 30 s , 72℃ for 1 min and then followed by 25 cycles of 94℃ for 30 s, 57.5℃ for 30 s, 72℃ for 1 min and a final extension step at 72℃ for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers F88971 and R25272 were added.

BBP library were assembled. In the SOE experiment, the reaction mixture (35ng BBP1+BBP2 fragment, 35ng BBP3+BBP4 fragment, 35ng BBP5 fragment) was subjected to an initial denaturation step at 94℃ for 3 min followed by 5 cycles of 94℃ for 30 s, 40℃ for 30 s , 72℃ for 1 min and then followed by 25 cycles of 94℃ for 30 s, 57.5℃ for 30 s, 72℃ for 1 min and a final extension step at 72℃ for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers Fbbp1 and Rtail were added.

The process of amplification of T fragment:the PCR protocol consisted of an initial denaturation step at 94℃ for 3 min followed by 15 cycles of 94℃ for 30 s, 40℃ for 30 s,

72℃ for 1min and then followed by 20 cycles of 94℃ for 30 s, 45℃ for 30 s, 72℃ for 1min a final extension step at 72℃ for 5 min.

The process of amplification of P fragment:the PCR protocol consisted of an initial denaturation step at 94℃for 3 min followed by 35 cycles of 94℃ for 30 s, 53℃ for 30 s, 70℃ for 1 min and a final extension step at 72℃ for 5 min.

T fragment and BBP library were assembled. In the SOE experiment, the reaction mixture (50ng BBP library, 50ng T fragment) was subjected to an initial denaturation step at 94℃ for 3 min followed by 15 cycles of 94℃ for 30 s, 42℃ for 30 s , 72℃ for 1 min and followed by 8 cycles of 94℃ for 30 s, 64℃ for 30 s, 72℃ for 1 min and then followed by 15 cycles of 94℃ for 30 s, 45℃ for 30 s, 72℃ for 1 min a final extension step at 72℃ for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers FT7 and Rtail were added.

Anticalin library were assembled. In the SOE experiment, the reaction mixture (50ng T+BBP library, 50ng P fragment) was subjected to an initial denaturation step at 94℃ for 3 min followed by 10 cycles of 94℃ for 30 s, 55℃ for 30 s , 72℃ for 1 min , 25pmol of each of the two primers FT7 and PRDR were added at cycle 11 and then followed by 25 cycles of 94℃ for 30 s, 45℃ for 30 s, 72℃ for 1 min a final extension step at 72℃ for 5 min. To amplify the assembled fragment.

2. Supplementary of sequences of 20 clones.

After 5^{th} and 8^{th} cycle of selection, 20 BBP clones which is equal to the fifth and eighth round was sequenced and the result indicated the ratio of mutation to death for the BBP library of the fifth and eighth round were 30% and 40%, respectively(Fig

S1,FigS2).

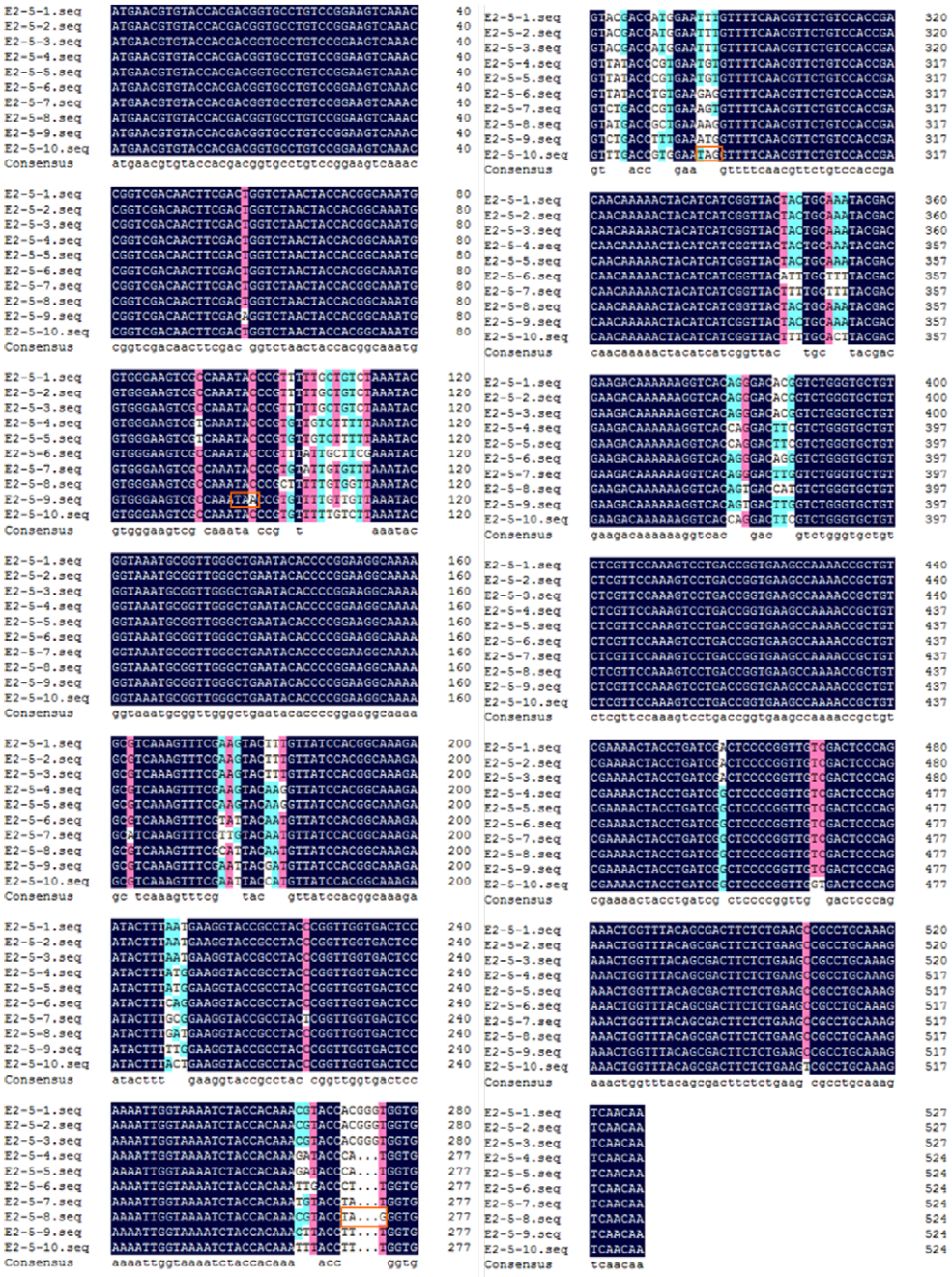


Fig S1 Supplementary of sequences of 10 clones obtained from fifth round. (Color region represents the designed random mutation sites. Orange frame represents fatal mutations. The sequences of E_2-5-1, E_2-5-2 and E_2-5-3 are fully identical and we named them E_2-A. The sequences of E_2-5-4 and E_2-5-5 are fully identical and we named them E_2-B.

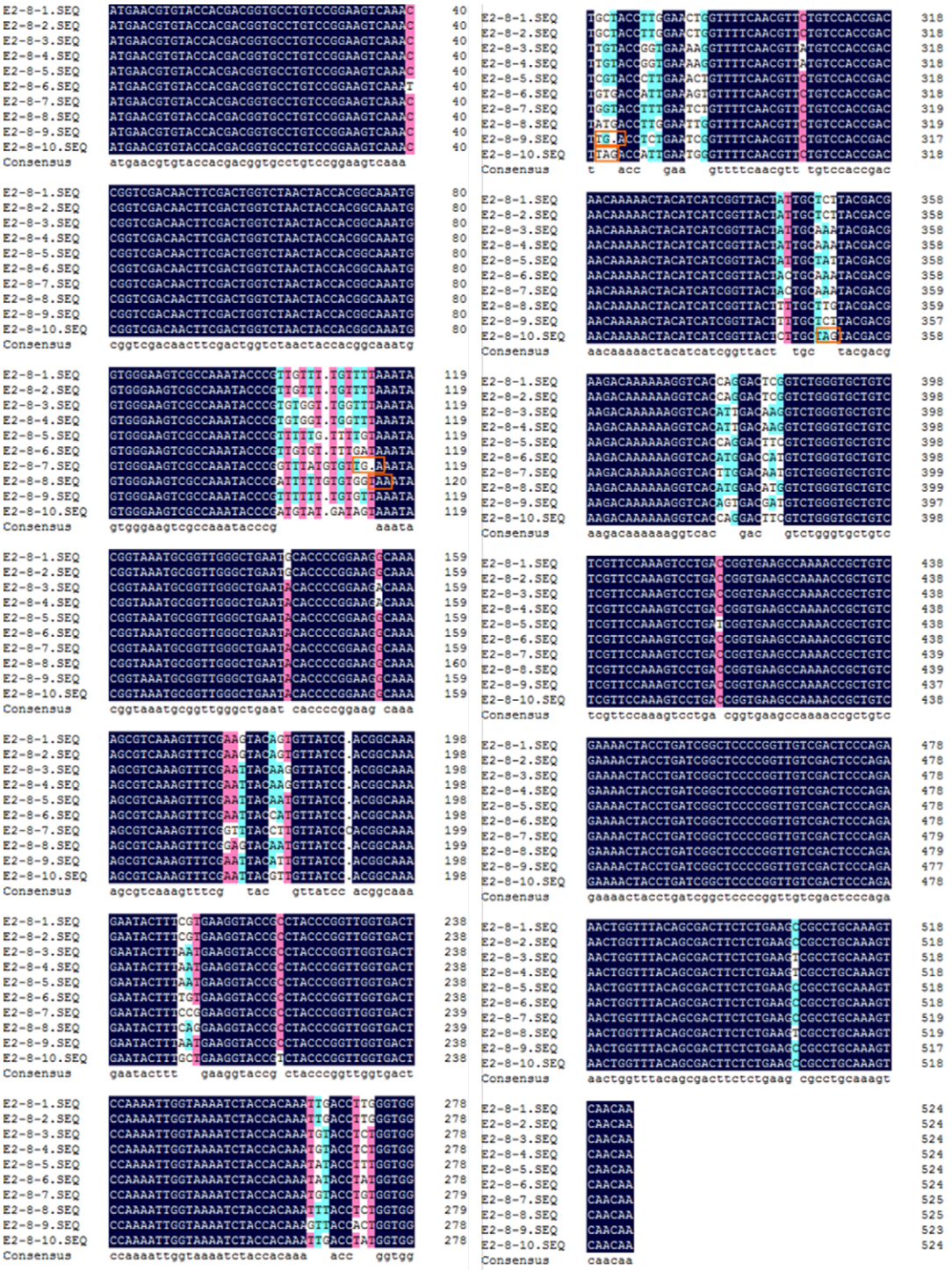


Fig S2 Supplementary of sequences of 10 clones obtained from fifth round. (Color region represents the designed random mutation sites. Orange frame represents fatal mutations. The sequences of E_2-8-1 and E_2-8-2 are fully identical and we named them E_2-C. The sequences of E_2-8-3 and E_2-8-4 are fully identical and we named them E_2-D.

3. Supplementary of sequences of BBP template for designing 17 oligodeoxynucleotides (Table 1).

AACGTGTACCACGACGGTGCCTGTCCGGAAGTCAAACCGGTCGACAACTTCGACTGGTCT

AACTACCACGGCAAATGGTGGGAAGTCGCCAAATACCCGAACAGCGTTGAAAAATACGG

TAAATGCGGTTGGGCTGAATACACCCCGGAAGGCAAAAGCGTCAAAGTTTCGAACTACCA

CGTTATCCACGGCAAAGAATACTTTATTGAAGGTACCGCCTACCCGGTTGGTGACTCCAAA

ATTGGTAAAATCTACCACAAACTGACCTACGGTGGTGTCACCAAAGAAAACGTTTTCAAC

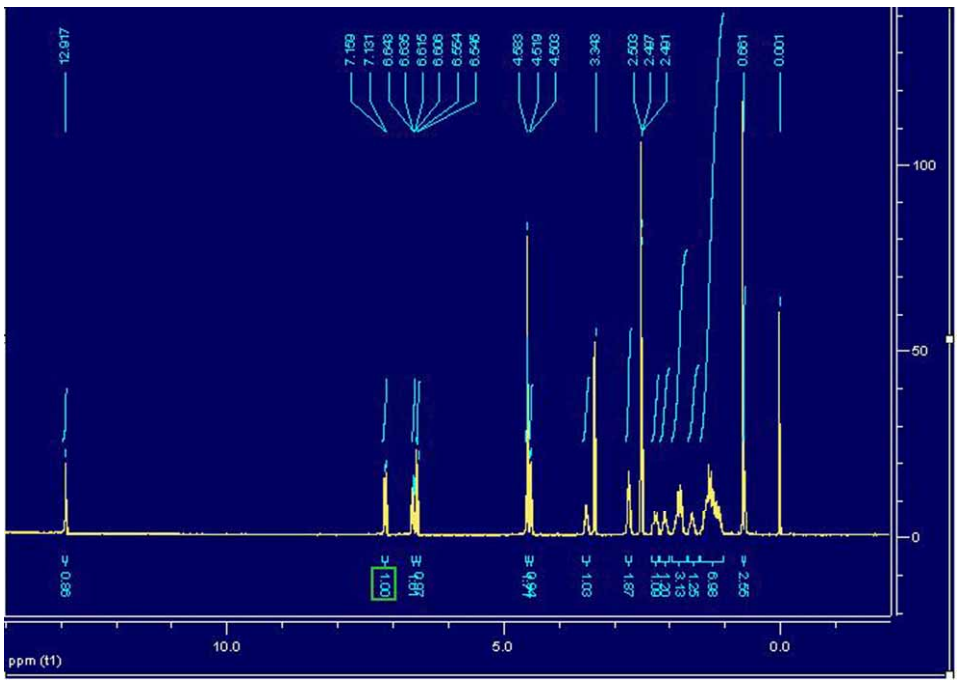
GTTCTGTCCACCGACAACAAAAACTACATCATCGGTTACTACTGCAAATACGACGAAGAC

AAAAAAGGTCACCAGGACTTCGTCTGGGTGCTGTCTCGTTCCAAAGTCCTGACCGGTGAA

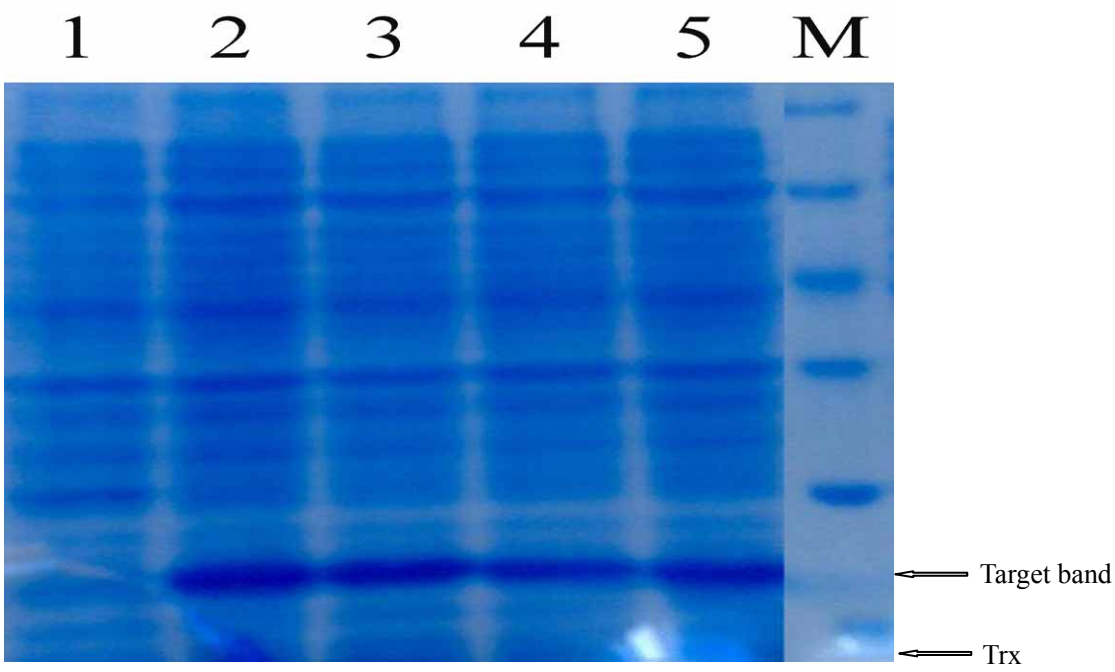
GCCAAAACCGCTGTCGAAAACTACCTGATCGGCTCCCCGGTTGTCGACTCCCAGAAACTG

GTTTACAGCGACTTCTCTGAAGCCGCCTGCAAAGTCAACAAT

4. Supplementary of ^{1}H NMR of estradiol-3-carboxymethy ether (E_2-3-CME).



5. Supplementary of SDS-PAGE assay of four random selected soluble anti-E_2 (approximately 20kDa) (Fig. S3)



Lane M: protein standards; lane 1: induced plasmid pTIG-trx; lane 2: the supernatant of cellular lysate of induced E_2-A; lane 3: the supernatant of cellular lysate of induced E_2-B; lane 4: the supernatant of cellular lysate of induced E_2-C; lane 5: the supernatant of cellular lysate of induced E_2-D;