# Supporting Information

## Gibby et al. 10.1073/pnas.0810952106

**A**

```
hBP1        MKICSLTLLSFLLLAAQVLLVEG-KKKVKNGLHS-KVVSEQKDTLGNTQIKQKSRPGN-- 56
chBP1       MWIKNVGLLCVLILVSQMLLASCERQKERRRGKQGIEHGGKKQAESNPEREKGRKPKGGK 60
            * *    **  * *  * **      *              *    *
                          1                2        3
hBP1        ---KGKFVTKDQANCRWAATEQEEGISLKVECTQLDHEFSCVFAGNPTSCLKLKDER-VY 112
chBP1       ASPKGKFKSKENADCSWAVTDMS-AATVHIECRNGDSAFWCEFSGDPSACPHYAANQKSY 119
               ****  *  * * ** *          **   *  * * * * *  *         *
                          4           5
hBP1        WKQVARNLRSQKDICRYSKTAVKTRVCRKDFPESSLKLVSSTLFGNTKPRKEKTEMSPRE 172
chBP1       WKQVSRSLKKQKQICQDPRSILKPKICRKGPRGAHLKLTRSSLLAAVDPAKGHPAHHAAE 179
            **** * *  ** **       *   ***      ***  * *     * *        *
                                    6                7       8
hBP1        HIKGKETTPSSLAVTQTMATKAPECVEDPDMANQRKTALEFCGETWSSLCTFFLSIVQDT 232
chBP1       DAQGPAASET----GKQPEHSPPDCVEDVDYIDQRKVAEEYCPESLLSLCNFFITMVQDK 224
                                  * * ** *   *** * * * *   *** **   ***

hBP1        SC 234
chBP1       KC 237
             *
```

**B**

```
                                                      1
hBP2        MKFVPCLLLVTLSCLGTLGQAPRQKQGSTGEEFHFQTGGRDSCTMRPSSLGQGAGEVWLR 60
chBP2       MKRVALLFLVVICGMGGLGQKLKPKKRSNGEEINFRTKTKDVCTMRIS----GDEEMKAR 56
            ** *  * **     * ***    *  * ***  * *   * **** *    *  *   *
              2        3        4                        5          6
hBP2        VDCRNTDQTYWCEYRGQPSMCQAFAADPKPYWNQALQELRRLHHACQGAPVLRPSVCREA 120
chBP2       IECKGQGKSYWCEYTGMPLLCHPFQNNPKMYWNQITMELRKLPHACESTQMLKASMCQKA 116
              *      ***** * *  *  *   ** ****   *** * ***     *  * *  *

hBP2        GPQAHMQQVTSSLKGSPEPNQQPEAGTPSLRPKATVKLTEATQ-LGKDSMEELGKAKPTT 179
chBP2       PVDALMKQVAAGVE--------PEDGAN--RDKSSQKTSASVRGAGKSSVKKTG--KPAV 164
               * * **             ** *    * *   *        ** *    *  **
                                      7       8
hBP2        RPTAKPTQPGPRPGGNEEAKKKAWEHCWKPFQALCAFLISFFRG 223
chBP2       LPRIKPTQHGQGSENETEAMKLAREHCWESLHEFCSYIIGFFRG 208
             *  **** *       ** * * ****      *   * ****
```

**C**

```
                                    1               2        3        4
chBP1   55  KPKGGKASPKGKFKSKENADCSWAVTDMSAATVHIECRNGDSAFWCEFSGDPSACPHYAA  114
            KPK      +  F++K    C+  ++        IEC+    ++WCE++G P  C  +
chBP2   23  KPKKRSNGEEINFRTKTKDVCTMRISGDEEMKARIECKGQGKSYWCEYTGMPLLCHPFQN  82
                               5           6
chBP1  115  NQKSYWKQVSRSLKKQKQICQDPRSILKPKICRKGPRGAHLKLTRSSLLAAVDPAKG---  171
            N K YW Q++  L+K    C+  + +LK  +C+K P  A +K     + A V+P  G
chBP2   83  NPKMYWNQITMELRKLPHACESTQ-MLKASMCQKAPVDALMK----QVAAGVEPEDGANR  137
                                                                 7
chBP1  172  ----HPAHHAAEDAQGPAASETGK---QPEHSPPDCVEDVDY-IDQRKVAEEYCPESLLS  223
                     +   A   +  +TGK    P   P    +  +   +  K+A E+C ESL
chBP2  138  DKSSQKTSASVRGAGKSSVKKTGKPAVLPRIKPTQHGQGSENETEAMKLAREHCWESLHE  197
             8
chBP1  224  LCNFFI  229
             C++ I
chBP2  198  FCSYII  203
```

**Fig. S1.** Amino acid sequence alignments of human and chicken BPs. (*A*, *B*) Human and *Gallus gallus* BP1 and BP2 alignments. The conserved domain of the FGF-BP protein family is *PFAM06473*. The conserved cysteine spacing pattern in the protein family is C-15/19-C-8-C-8-C-23/24-C-10/11-C-50–90-C-7-C. The respective 8 conserved cysteine positions are numbered from 1 to 8, and identical amino acids are indicated by an asterisk. The C-terminal 42 amino acids in human BP1 contain the FGF-binding domain (10). (*C*) Alignment of *chBP1* and *chBP2*. Twenty-six percent amino acid identity and 44% conservative replacements (+) were calculated by the BLAST algorithm at the National Center for Biotechnology Information. GenBank entry numbers are hBP1, NP_005121; hBP2, NP_114156; chBP1, XP_420773, and chBP2, NP_989778.

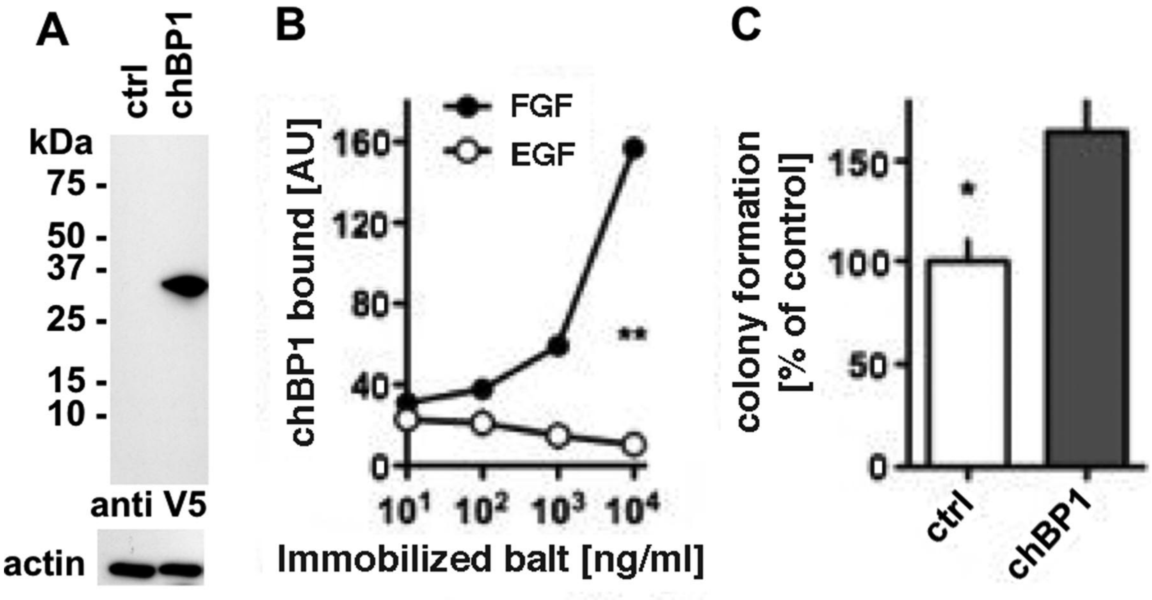

**Fig. S2.** *chBP1* binding to FGF2 and effect on colony formation of SW-13 cells. (*A*) Western blot for VP5-tagged protein harvested from *chBP1*- versus empty vector (control)-transfected cells. Actin served as a loading control. (*B*) Binding of *chBP1* to different concentrations of immobilized FGF2 relative to a negative control (epidermal growth factor, EGF). The bound *chBP1* was quantitated by ELISA. **, $P < 0.01$. (*C*) Colony formation of SW-13 cells in soft agar. Control (empty vector)- and *chBP1*-transfected cells were compared. *, $P < 0.05$.

**Table S1. Tissue expression of *chBP1* and *chBP2***

| Organ | Expression Level (number of samples) | |
|---|---|---|
| | *chBP1* | *chBP2* |
| Brain | + (3) | + (4) |
| Cartilage | +++ (3) | + (4) |
| Eye | ++ (3) | + (2) |
| Heart | 0 (3) | 0 (4) |
| Intestine | +++ (2) | +++ (4) |
| Kidney | + (2) | ++ (4) |
| Liver | 0 (3) | 0 (5) |
| Lung | ++ (2) | ++ (2) |
| Skin | +++ (4) | +++ (5) |

Expression of *chBP1* and *chBP2* mRNA in different tissues. Expression levels were determined by in situ hybridization as described earlier (37). Tissues harvested at the respective peak expression time were analyzed (see Fig. 2). Scoring was done in a blinded fashion.