

## Supporting Information

### Matrix-immobilized yeast for large scale production of recombinant human lactoferrin

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Human LF QEKFGKDKSPKFQFLFGSPSGQKDLLFKDSAIGFSRVPPRIDSGLYLGSY 344  
Simian LF QEKFGKDKSPKFQFLFGSPSGQKDLLFKDSAIGFLRVPLRIDSGLYLGSY 344  
Goat LF QEKFGKDKSPKFQFLFGSPSGQKDLLFKDSALGFVRIPSKVDSALYLSRY 349  
Sheep LF QEKFGKDKSPKFQFLFGSPSGQKDLLFKDSALGFVRIPSKVDSALYLSRY 343  
Porcine LF QKKFGKSNPQEFQFLFGSPSGQKDLLFRDATIGFLKIPSKIDSKLYLGLPY 339  
Bat LF QEKFGKDKSAFQFLFSSPRGQKDLLFKDNALGFLRIPSKIDAGLYLGSNY 299  
Rodent LF QEKFGKDKSAFQFLFASPSGQKDLLFKESAIGFVRVPPQKVDVGLYLTFSY 342  
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Human LF FTAIQNLRKSEEEVAARRARVVWCAVGEQELRKCNQWSSGLSEGSVTCSSA 394  
Simian LF FTAIQNLRKSEEEVAARRARVVWCAVGEQELRKCNQWSSGLSEGSVTCSSA 394  
Goat LF LTALKNLRETAEEVKARCTRVVWCAVGPPEEQSKCQQWSEQSGQVTCATA 399  
Sheep LF LTALKNLRETAEEVKARCTRVVWCAVGPPEEHSKCCQQWSEQSGQVTCATA 393  
Porcine LF LTAIQGLRETAEEVEARQAVVWCAVGPPEELRKCRQWSSQSSQNLNCSLA 389  
Bat LF ITALKGLKETSAEEVARRARVVWCAVGPPEEQKCCQQWSSQNSQNGRTVACATA 349  
Rodent LF TTSIQNLNKKQDVIASKARVTWCAVGESEKRRKCDOWNRASRGRVTCISF 392  
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Human LF STTEICIALVLKGEADAMSLDEGYVYTAGKCGLVPLAENYKSQQSSDPD 444  
Simian LF STTEICIALVLKGEADAMSLDGGYVYTAGKCGLVPLAENYKSQQSSDPD 444  
Goat LF STTDICIALVLKGEADALSDGGYIYTAGKCGLVPMVAENRKSYSKSSLD 449  
Sheep LF STTDICIALVLKGEADALSDGGYIYTAGKCGLVPMVAENRKSYSKSSLD 443  
Porcine LF STTEICIVQLKGEADAMSLDGGFIYTAGKCGLVPLAENQKSRQSSSD 439  
Bat LF ATTEICIALVLKGEADAMSLDGGFIYTAGKCGLVPLAESQKSESGNSD 399  
Rodent LF PTTEICIAAIMKGDADAMSLDGGYIYTAGKCGLVPLAENQKSSKSNGLD 442  
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Human LF PNCVDRPVEGILAVAVVRRSDTLTWNVSKGKKSCHTAVDRTAGWNI PMG 494  
Simian LF PNCVDRPVEGILAVAVVRRSDTLTWNVSKGKKSCHTAVDRTAGWNI PMG 494  
Goat LF --CVLRPTEGILAVAVVKKANEGLTWNVSKGKKSCHTAVDRTAGWNI PMG 497  
Sheep LF --CVLRPTEGILAVAVVKKANEGLTWNVSKGKKSCHTAVDRTAGWNI PMG 491  
Porcine LF --CVHRPTQGI FAVAVVRKANGGITWNSVRGTGKKSCHTAVDRTAGWNI PMG 487  
Bat LF --CVNRPVEGILAVAVVKKSNAGFTWNSLRGKKSCHTAVGRTAGWNI PMG 447  
Rodent LF --CVNRPVEGILAVAAVRREDAGFTWNSLRGKKSCHTAVDRTAGWNI PMG 490  
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Human LF LLFNQTGSCKFDEYFSQSCAPGSDPRSNLALCALCIGDEQGENKCVPSNERN 544  
Simian LF LLFNQTGSCKFDEYFSQSCAPGSDPRSNLALCALCIGDEQGENKCVPSNERN 544  
Goat LF LIANQTGSCAFDEFFSQSCAPGADPKSSLALCAGDDQGLDKCVPSNERSKEK 547  
Sheep LF LIANQTGSCAFDEFFSQSCAPGADPKSSLALCAGDDQGLDKCVPSNERSKEK 541  
Porcine LF LLVNTGSCKFDEFFSQSCAPGSPGNSLALCVGNDQGVDKCVPSNERN 537  
Bat LF LLFNQTGSCKFDEFFSQSCAPGSDPNSNLCTLCIGNEARQNKCAPSNERN 497  
Rodent LF LLANQTRSCKFNEFFSQSCAPGADPKSNLALCALCIGDEKGENKCAPNSKER 540  
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Human LF YGYTGAFRCLAENAGDVAFVKDVTVLQNTDGNNEAWAKDLKLDLDFALL 594  
Simian LF YGYTGAFRCLAENAGDVAFVKDVTVLQNTDGNNEAWAKDLKLDLDFVLL 594  
Goat LF YGYTGAFRCLAEDVGDVAFVKNDT VVENTNGESSADWAKNLNREDFRLL 597  
Sheep LF YGYTGAFRCLAEDVGDVAFVKNDT VVENTNGESSADWAKNLNREDFRLL 591  
Porcine LF YGYTGAFRCLAENAGDVAFVKDVTVLDNTNGQNTTEWARELRSDDFELL 587  
Bat LF YGYTGAFRCLAENAGDVAFVKDATVLENTNGKGTEDWAKDLRLEDLDFELL 547  
Rodent LF YQGYTGALRCLAEKAGNVAFKDSITVLQNTDGNTEEWARNLKLDFELL 590  
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Human LF CLDGKRPVTEARSCHLAMAPNIAVVSRRMDKVERLKQVLLHQQAKFGRNG 644  
Simian LF CLDGKQKPVTEARSCHLAMAPNIAVVSRTDKVERLKQVLLHQQAKFGRNG 644  
Goat LF CLDGTTPKPVTEAQSCYLAVAPNIAVVSRSRAAHVEQVLLHQQALFGKNG 647  
Sheep LF CLDGTTPKPVTEAQSCYLAVAPNIAVVSRSRAAHVEQVLLHQQALFGKNG 641  
Porcine LF CLDGTTRKPVTEAQSNCHLAVAPNIAVVSRRKEKAAQVEQVLLTEQAQFGRNG 637  
Bat LF CLNGTRKPVTEAKNSCYLAKAPNIAVVSRRDKVEHLEQVLLDQQARFGRNG 597  
Rodent LF CLDDTRKPVTEAKNCHLAIAPNIAVVSRTDKVEVLQVLLDQQARFGRNG 640  
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Human LF SDCPKDFCLFQSETKNLLFNDNTECLARLHGKTTYEKYLGPOYVAGITNL 694  
Simian LF SDCPKDFCLFRSETKNLLFNDNTECLARLHGKTTYEKYLGPOYVTAITNL 694  
Goat LF KNCPDQFCLFKSETKNLLFNDNTECLAKLGGRPITYEKYLGTEYVTAIANL 697  
Sheep LF KNCPDQFCLFKSETKNLLFNDNTECLVKGGRPITYEKYLGTEYVTAIANL 691  
Porcine LF KDCPKDFCLFRSETKNLLFNDNTECLAQLQKTTYEKYLGSEYVTAIANL 687  
Bat LF RDCPGTFCLFQSETKNLLFNDNTECLANLQKTTYEKYLGAEYVMAVGNL 647  
Rodent LF QRCPEGFCLFQSKTKNLLFNDNTECLAKIPGKTTYEKYLGKEYVIATERL 690  
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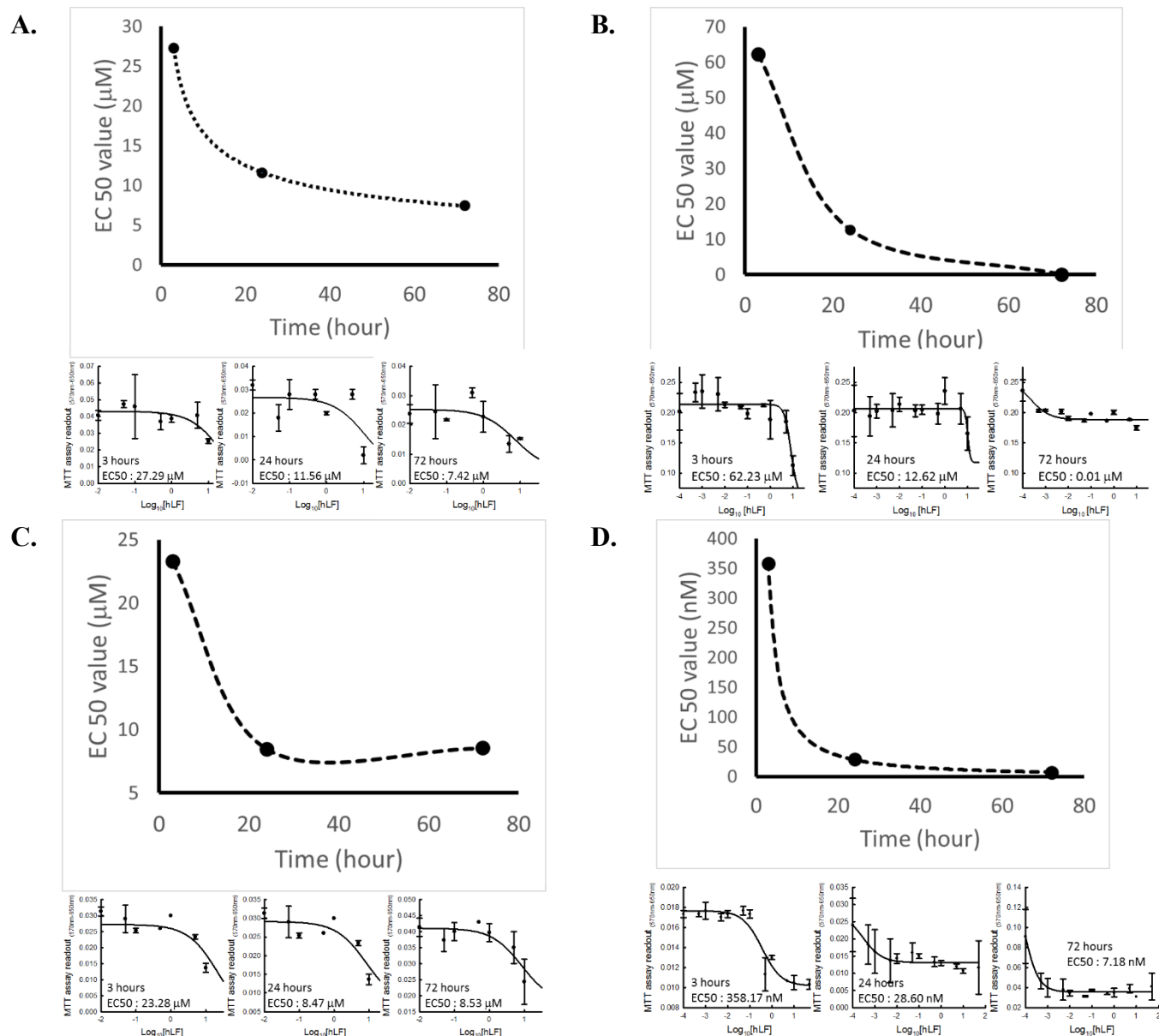
Human LF          KKCSTSPLEACEFLRK 711
Simian LF         KKCSTSPLEACEFLRK 711
Goat LF           KKCSTSPLEACAFLTR 714
Sheep LF          KKCSTSPLEACAFLTR 708
Porcine LF        KQCSVSPLEACAFMMR 704
Bat LF            RQCSTSPLEACAFLRK 664
Rodent LF         KQCSSSPLEACAFL-- 705
                  ::** ***** *:

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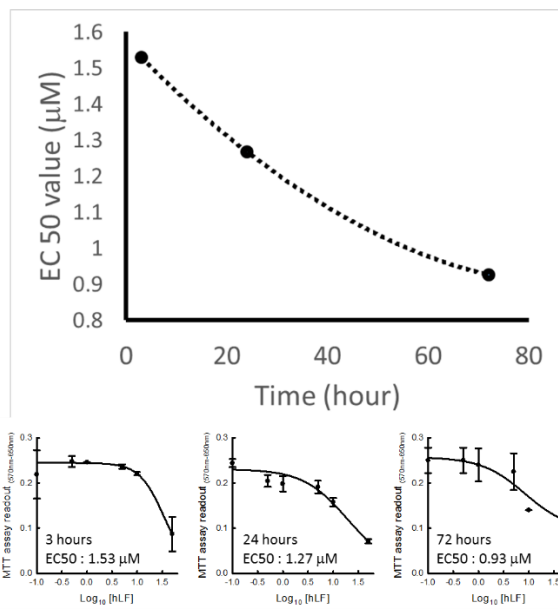
**Supplementary Figure S1.** Sequence Alignment of lactoferrin from various sources. Ferric ion interacting residues are indicated in red, the conserved region for conferred anti-fungal activity is shown in green.

## 2. Anti-cancer inhibition curves of lactoferrin against breast, gastric and colorectal cancer cell line

The purified lactoferrin was tested against breast cancer cell line MCF7, gastric cancer cell line AGS, colorectal cancer cell lines LoVo, CT26 and HCT116. The assay was measured at 3, 24 and 72 hours interval and the EC50 curve used to evaluate the duration for the lactoferrin to take effect on the various cancer cell lines.

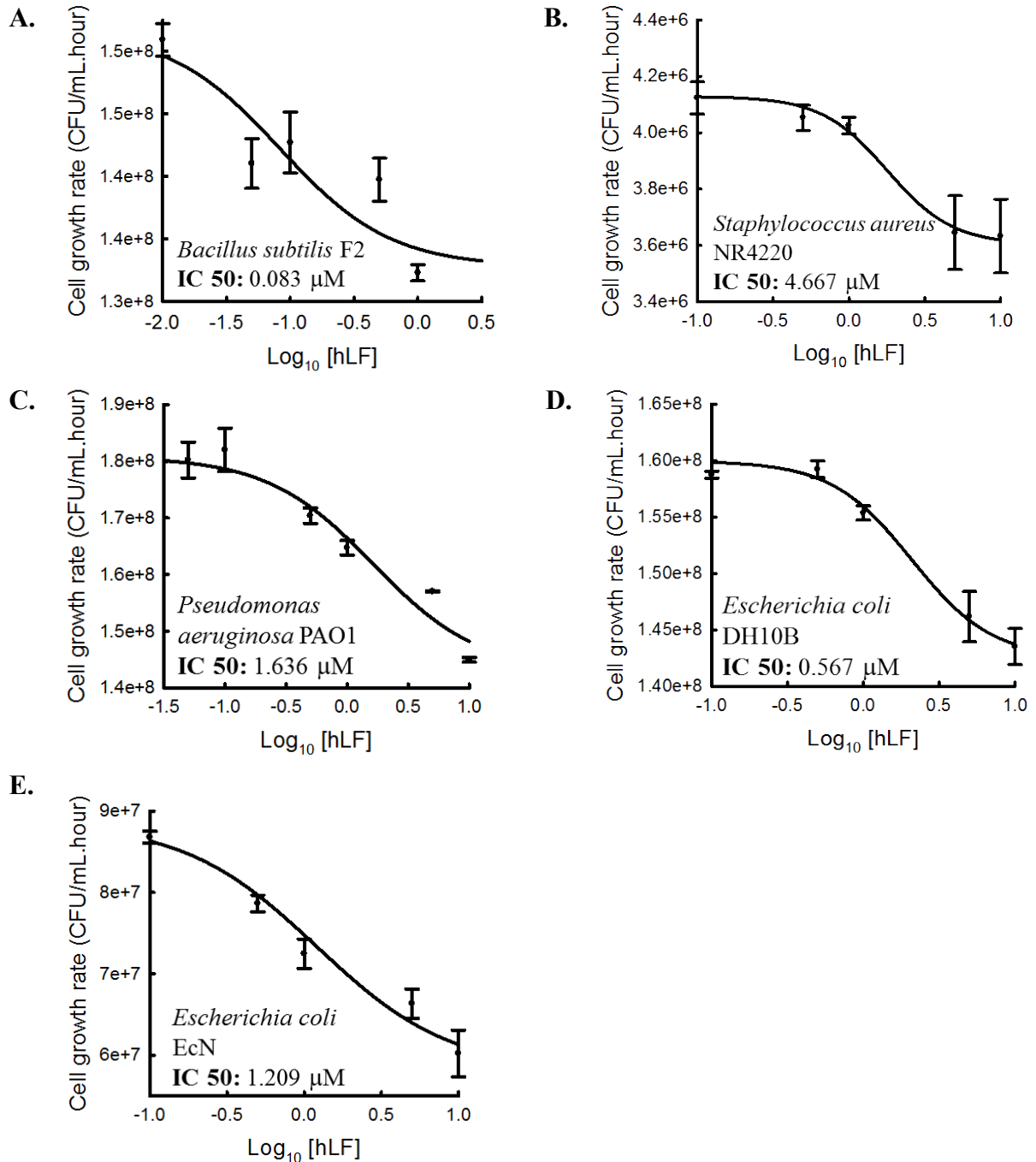


**E.**



**Supplementary Figure S2.** The anti-cancer assay using various cancer cell lines observed over the period of 3, 24 and 72 hours (Inset figures: The EC50 curve of 3, 24 and 72 hour treatment). **A.** Breast cancer cell line, MCF7 **B.** Gastric cancer cell line, AGS **C.** Colorectal cancer cell line, LoVo **D.** Colorectal cancer cell line, CT26 **E.** Colorectal cancer cell line, HCT116

### 3. Anti-bacterial inhibition curves of lactoferrin against gram-positive and gram-negative microbial



**Supplementary Figure S2.** The lactoferrin anti-bacterial assay tested against gram-positive bacteria (A. *Bacillus subtilis* F2 B. *Staphylococcus aureus* NR4220) and gram-negative bacteria (C. *Pseudomonas aeruginosa* PAO1 D. *Escherichia coli* DH10B E. *Escherichia coli* EcN).