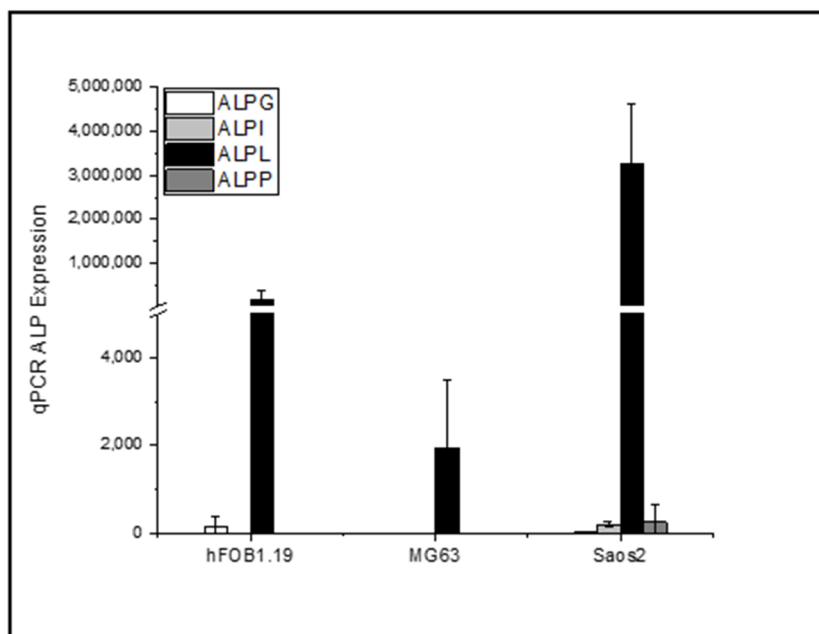
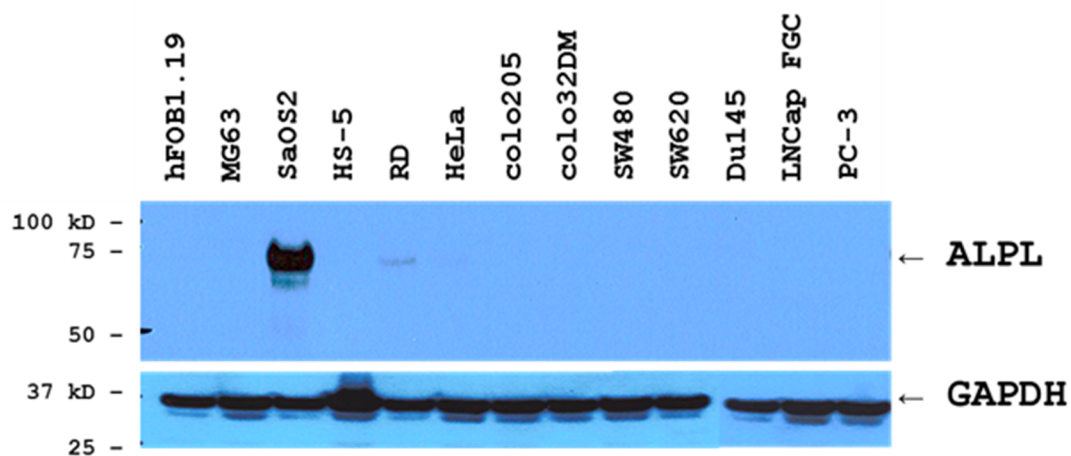


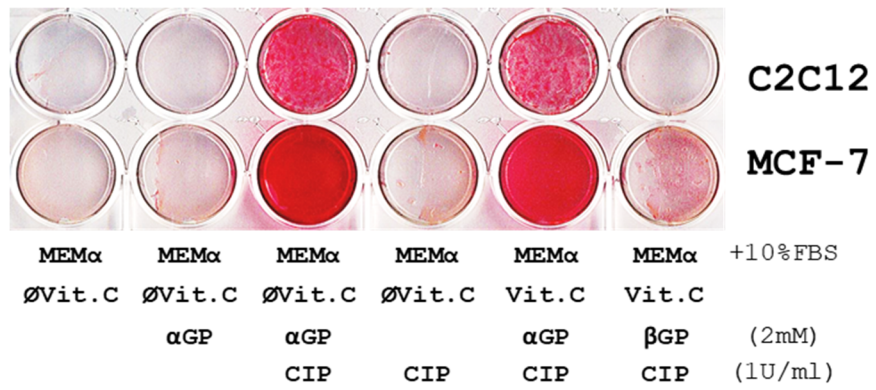
A



B

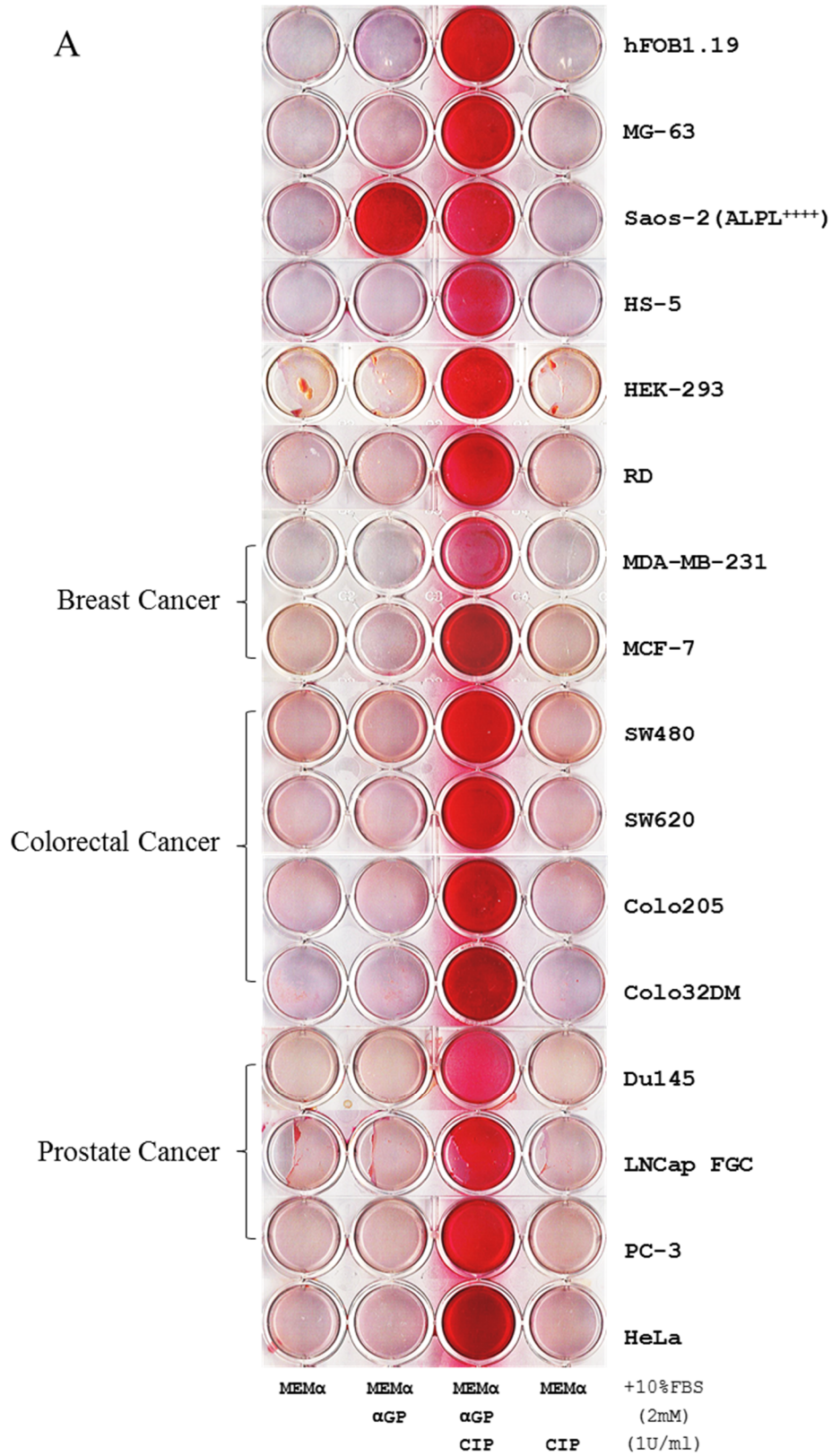


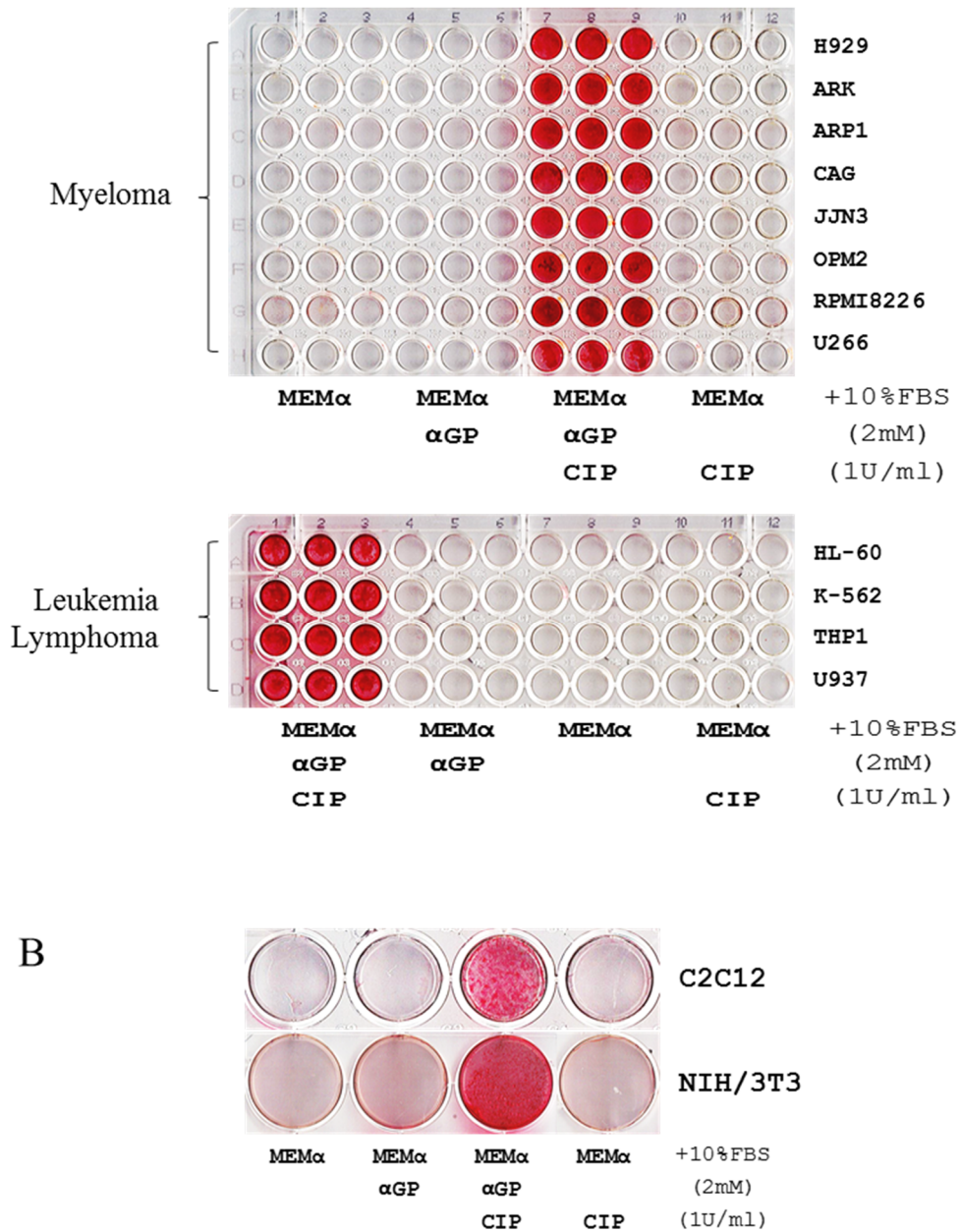
**Figure. S1.** Gene expression profiling and western blot detection of human alkaline phosphatase (ALP) in cell lines. (A) TaqMan quantitative RT-PCR of human ALPs (ALPG, ALPI, ALPL, and ALPP) in hFOB1.19, MG63, and Saos-2 cell lines. Error bars indicate the standard deviations of three individual RT-PCR assays in triplicate. (B) Western blot of ALPL and GAPDH in 13 human cell lines (20  $\mu$ g of total protein from cell lysate per lane).



**Figure. S2.** Biomineralization of C2C12 and MCF-7 cells was dependent on  $\alpha$ GP (2 mM) and CIP (1 U/ml) supplemented MEM $\alpha$ /10% FBS with ascorbic acid (Vit. C) or without ascorbic acid ( $\emptyset$ Vit. C). The cells were cultured for 7 days (the media were changed on day 4) and then stained with Alizarin Red S.

A





**Figure. S3.** Biom mineralization of human and mouse cells without induction of cellular differentiation. Alizarin Red S staining revealed mineralization in (A) human cell lines (n=28) and (B) mouse cell lines (n=2) after 7 days of culture in MEMα/10% FBS supplemented with αGP (2 mM) and CIP (1 U/ml), the media were changed on day 4. The reaction did not occur if any one of the three elements was missing.

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ALPL      1  MISPLVLAIGTCLTNSLVPEKEKDPKYWRDQAQETLKYALELQKLN  48
CIP       1  MQGACVLLLLLGLHLQLSLGLVPVEEEDPAFWNRQAAQALDVAKKLQPIQT  50
SAP       1  MIGRTTFIALFVKVLTIWSTFKGED-CVWDNDVDYP-----EYPPLIL  42
          . . . . . * *
          . . . . .

ALPL     49  NVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVALS  98
CIP     51  -AAKNVILFLGDGMGVPTVTATRILKGQMNGKLGPEPLAMDQFPYVALS  99
SAP     43  DSSFQLVLPVLEGD-----QRITSVQS----GSELILACPGREISALG  81
          . . . . . * * * * * * * * * * * *
          . . . . .

ALPL     99  KTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVT  148
CIP    100  KTYNVDRQVPDSAGTATAYLCGVKGNRYRTIGVSAARYNQCKTTRGNEVT  149
SAP     82  ---SEDAQATCLGG----KLVEVDGKEWNIVELGCTKMASETIHR-----  119
          * * * * * . . . . .

ALPL    149  SILRWAKDAGKSVGIVTTTTRVNHATPSAAYAHSDRDWYSDNEMPPEALS  198
CIP    150  SVMNRACKKAGKSVGVVTTTTRVQHASPAGAYAHTVNRNWYSDADLPADAQM  199
SAP    120  -NLGQCQDQ--DLGIYEVIGFDLPTTGHFY-----ELI  149
          . . . . . *
          . . . . .

ALPL    199  QGCKDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARGTRLD  248
CIP    200  NGCQDIAAQLVNNM-DIDVILGGGRKYMFPVGTDPPEYRDDASVNGVRKR  248
SAP    150  RVCFDPANETTIFS--ENIVHGAS----IAAKDIDPGRPSFKTSTGFFSV  193
          * * * . . * * * * * * * * * * *
          . . . . .

ALPL    249  GLDLVDTWKSFKPRYKHSFIWNRELLTLDPHN-VDYLLGLFEPGDMQY  297
CIP    249  KQNLVQAWQ---AKHQGAQYVWNRTALLQAADDSSVTHLMGLFEPADMKY  295
SAP    194  SMISVYSQR-----NQLELMKNLLGD-DELAATIIDPSKQFY  229
          * . . . . * * * * * * * * * * *
          . . . . .

ALPL    298  ELNRNNTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQ  347
CIP    296  NVQQDHTKDPTLQEMTEVALRVVSRNPRGFYLFVEGGRIDHGHHDDKAYM  345
SAP    230  FAKGHMAPDADFVTVEQDATYYYINALPQWQAFNNG---NWKYLEYDTR  276
          * . . . . * * * * * * *
          . . . . .

ALPL    348  ALHEAVEMDRAIGQAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFG  397
CIP    346  ALTEAGMFDNAIAKANELTSSELDLILVTADHSHVFSFGGYTLRGTSIFG  395
SAP    277  DLAEKHGTDLTVYSGG-----WGVLELEDINGNPVEIYLGLAQDKK----  317
          * * * * * . . . . * * * * * * *
          . . . . .

ALPL    398  LAPMLSDTDKPFTAILYNGPGYKVVGGERENVMVDYAHNNYQAQSAV  447
CIP    396  LAPSKALDSKS-YTSILYNGPGYALGGGSRPDVNDSTSEDPSYQQAAV  444
SAP    318  VVPAPALTWKVIYEKDTN---RAAAIVGINNPHITTAP-----E  353
          . * . . * . . . . *
          . . . . .

ALPL    448  PLRHETHGGEDVAVFSKGPMAHLLHGVHEQNYVPHVMAYAACIGANLGH  497
CIP    445  PQASETHGGEDVAVFARGPQAHLVHGVEEETFVAHIMAFAGCPEPYTDCN  494
SAP    354  PLCTDICSSLTWLDFDFG---DLVHGTYCCSVADLRAAIPNVPDLGDVD  400
          * . . . . * * * * * * * * * * *
          . . . . .

ALPL    498  APASS-----AGSLAAGPLLLALALYPLSVLF*  525
CIP    495  LPAPTATSIPDAAHLAASPPPLALLAGAMLLLLLAPTLY  533
SAP    401  ILDE  404

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**Figure. S4.** Alignment of amino acid sequences of human tissue-nonspecific ALP (ALPL), calf intestinal ALP (CIP), and shrimp hepatopancreas ALP (SAP). ALPs from three distant species across Kingdom Animalia demonstrate substantial differences; 48 residues are conserved (\*).