Supplemental Data

Impact of Divalent Metal Ions on Regulation of Adenylyl Cyclase Isoforms by Forskolin Analogs

Miriam Erdorf, Tung-Chung Mou, and Roland Seifert

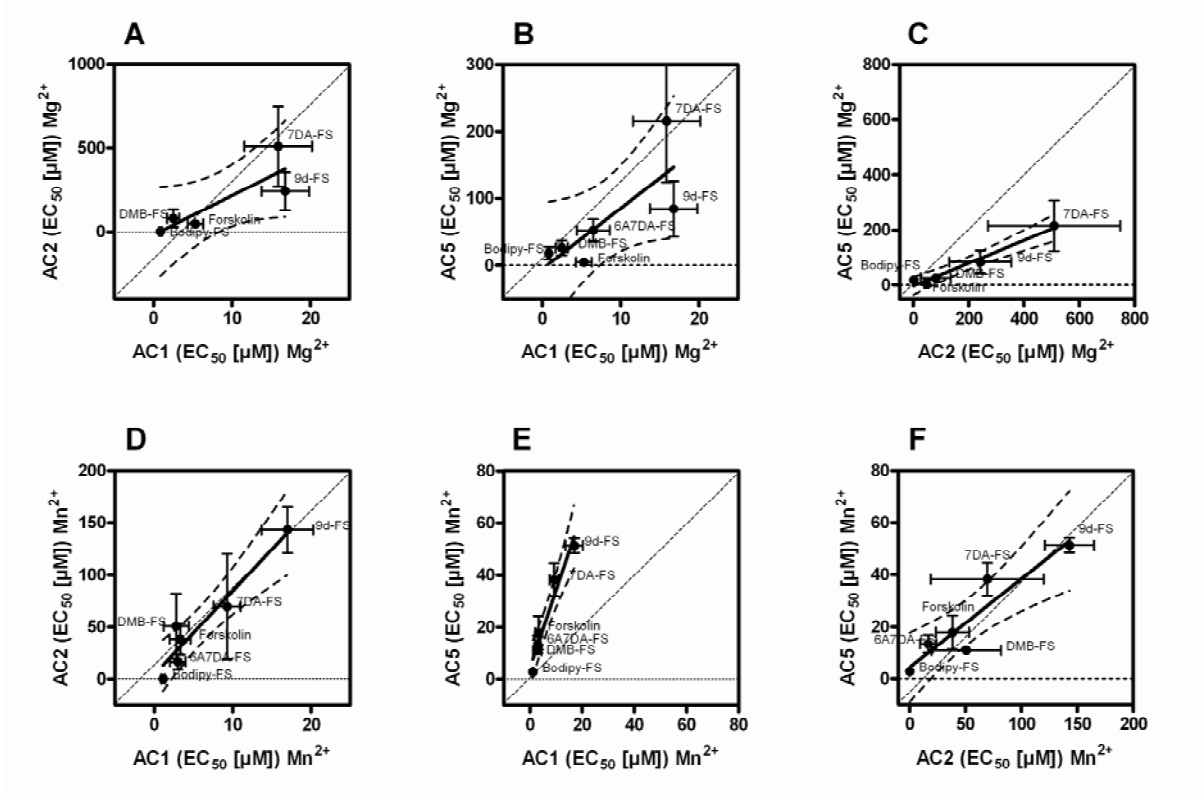

Fig. S1. Correlation of the potencies of FS analogs on the different AC isoforms. **A**, correlation of AC1 *vs.* AC2 in presence of Mg^{2+} (r^2 = 0.73; slope = 23.4 ± 8.3; p = 0.07). **B**, correlation of AC1 *vs.* AC5 under Mg^{2+} conditions (r^2 = 0.62; slope = 9.04 ± 3.5; p = 0.06). **C**, correlation of AC2 with AC5 in presence of Mg^{2+} (r^2 = 0.97; slope = 0.41 ± 0.04; p = 0.002). **D**, correlation of AC1 *vs.* AC2 under Mn^{2+} conditions (r^2 = 0.92; slope = 8.00 ± 1.2; p = 0.003). **E**, correlation of AC1 with AC5 in the presence of Mn^{2+} (r^2 = 0.94; slope = 3.0 ± 0.36; p = 0.001). **F**, correlation of AC2 and AC5 under Mn^{2+} conditions (r^2 = 0.86; slope = 0.34 ± 0.07; p = 0.008). Note the different scales of the x- and y-axes in **A**, **B**, **D** and **F**. Comparisons were analyzed by linear regression; the dashed lines indicate 95% confidence intervals. The diagonal dotted line has a slope of 1.0 and represents a theoretical curve for identical values.

In the presence of Mg^{2+}, correlations of the EC_{50}-values on ACs 1, 2 and 5 with each other revealed differences in their pharmacological profiles. As shown in Figs. S1A and B, stimulatory potencies on AC1 compared to AC2 and AC5 resulted in very steep slopes of 23.4 ± 8.3 and 9.04 ± 3.5, respectively and the corresponding correlation coefficients (r^2) of 0.73 and 0.62 differ considerably from unity. This indicates remarkable differences in the diterpene profile with lower potencies of FS analogs for AC5 as compared to AC1 and very low potencies at AC2. The correlation of AC2 and

AC5 shown in Fig. S1C, revealed a slope of 0.41 ± 0.04 with an r^2 of 0.97 indicating that FS and the six FS analogs bind only with less than half of the affinity to AC2 than to AC5 in presence of Mg^{2+}. The influence of Mn^{2+} revealed isoform-specific patterns of EC_{50}-values determined for FS and FS analogs (Figs. S1D-F). Consistently for all diterpenes (r^2 ~ 1), the stimulatory potencies on AC1 compared to AC2 and AC5 were considerably higher, expressed by slopes of 8.0 ± 1.2 for AC1 *vs*. AC2 and 3.0 ± 0.36 for AC1 *vs.* AC5.

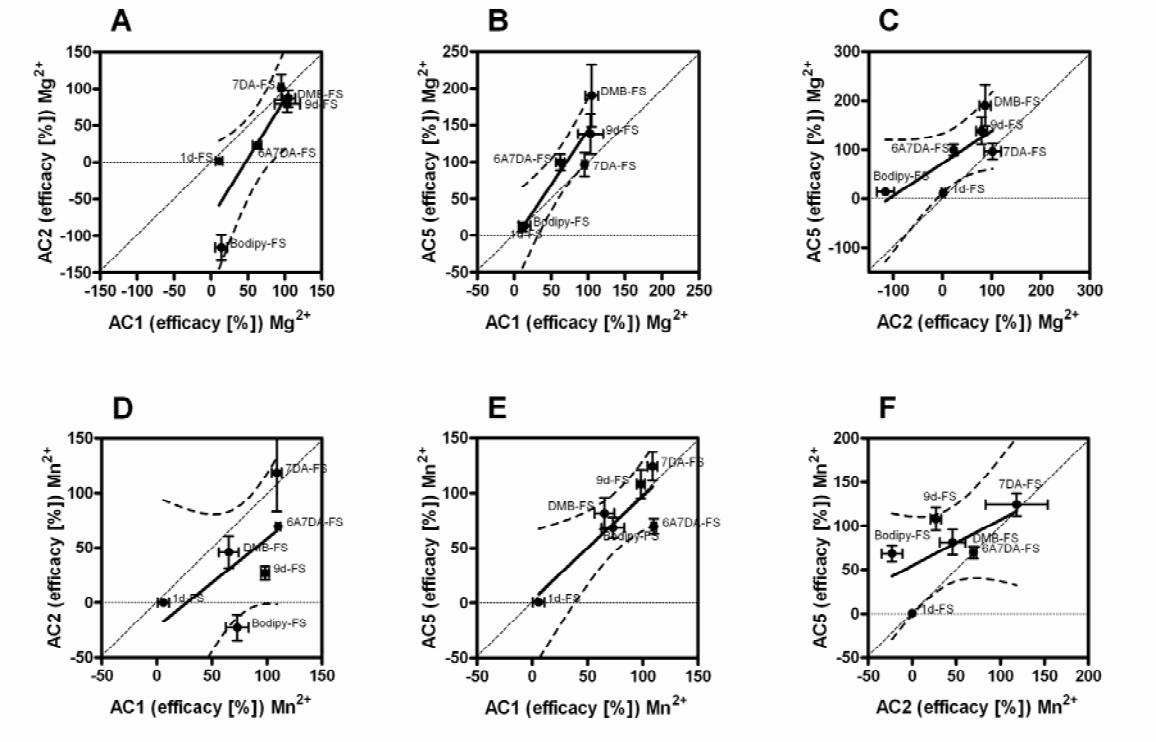

Fig. S2. Correlation of the efficacies of the diterpenes on each recombinant AC isoform compared to each other. A, correlation of AC1 *vs.* AC2 in presence of Mg^{2+} (r^2 = 0.75; slope = 1.61 ± 0.5; p = 0.026). **B**, correlation of AC1 *vs.* AC5 under Mg^{2+} conditions (r^2 = 0.86; slope = 1.49 ± 0.3; p = 0.008). **C**, correlation of AC2 with AC5 in presence of Mg^{2+} (r^2 = 0.59; slope = 0.66 ± 0.28; p = 0.074). **D**, correlation of AC1 *vs.* AC2 under Mn^{2+} conditions (r^2 = 0.39; slope = 0.80 ± 0.50; p = 0.19). **E**, correlation of AC1 with AC5 in the presence of Mn^{2+} (r^2 = 0.76; slope = 0.94 ± 0.27; p = 0.02). **F**, correlation of AC2 and AC5 under Mn^{2+} conditions (r^2 = 0.38; slope = 0.52 ± 0.33; p = 0.19). Comparisons were analyzed by linear regression; the dashed lines indicate 95% confidence intervals. The diagonal dotted line has a slope of 1.0 and represents a theoretical curve for identical values.

Focusing on the comparison of the efficacies of diterpenes on ACs 1, 2 and 5, no similarities were found under Mg^{2+} conditions (Figs. S2A-C). Independently of the divalent metal ion, the maximum stimulation of the different ACs by FS analogs did not yield a uniform picture. Some compounds like 7DA-FS or DMB-FS stimulated ACs 1, 2 or 5 more effectively, whereas other compounds, e.g. 6A7DA-FS or BODIPY-FS, yield lower AC activity in some cases, reflected by data points outside the 95% confidence interval. Under Mn^{2+} conditions, correlations of efficacies of FS analogs on AC1 with AC2 or AC5, respectively, are characterized by slopes close to 1.0, but the correlation coefficients are very low (r^2 = 0.39, r^2 = 0.76) (Figs. S2D and E).

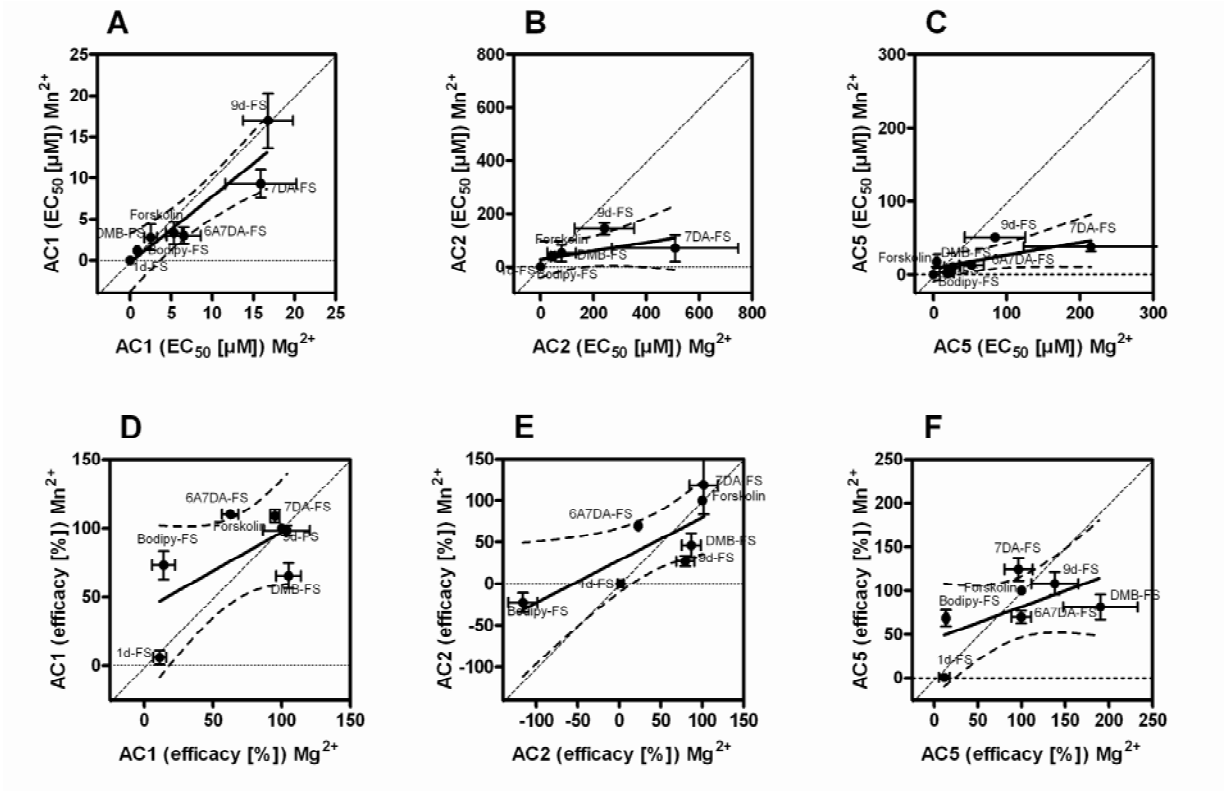

Fig. S3. Correlation of potencies and efficacies of FS and FS analogs under Mg^{2+} conditions *vs.* under Mn^{2+} conditions. A-C, correlation of isoform-specific EC_{50}-values determined in presence of Mg^{2+} *vs.* in presence of Mn^{2+}. **D-F**, correlation of the efficacies on ACs 1, 2 and 5, respectively, Mg^{2+} conditions *vs.* Mn^{2+} conditions. **A**, r^2 = 0.86; slope = 0.80 ± 0.2; p = 0.0028. **B**, r^2 = 0.36; slope = 0.16 ± 0.11; p = 0.21. **C**, r^2 = 0.49; slope = 0.17 ± 0.08; p = 0.08. **D**, r^2 = 0.41; slope = 0.56 ± 0.18; p = 0.12. **E**, r^2 = 0.61; slope = 0.51 ± 0.18; p = 0.04. **F**, r^2 = 0.34; slope = 0.37 ± 0.23; p = 0.17. Data were analyzed by linear regression; the dashed lines indicate 95% confidence intervals. The diagonal dotted line has a slope of 1.0 and represents a theoretical curve for identical values.

The comparison of the pharmacological parameters of each AC isoform in the presence of Mg^{2+} with those in presence of Mn^{2+} showed different AC-sensitivity to divalent cations. Striking effects on the diterpene profile depending on whether Mg^{2+} or Mn^{2+} serves the role of cation cofactor were observed for potencies at AC2 and AC5 (Figs. S3B and C). The lowest impact of the cations was determined for the potencies of AC1 (r^2 = 0.86; slope = 0.80 ± 0.2) (Fig. S3A). Correlations of efficacies in the presence of Mg^{2+} *versus* Mn^{2+} deviated from identity (slope 1.00) as well (Figs. S3D, E and F).

AC1 C1 domain

```
AC1_C1_Human  TERSQRKAFLQARSCIEDRLRLEDENEKQERLLMSLLPRNVAMEMKEDFLKPPERIFHKI 294
AC1_C1_Mouse  T.S.........N.................................................. 293
AC1_C1_Bovine A.R.........N.................................................. 295

AC1_C1_Human  YIQRHDNVSILFADIVGFTGLASQCTAQELVKLLNELFGKFDELATENHCRRIKILGDCY 354
AC1_C1_Mouse  ............................................................... 353
AC1_C1_Bovine ............................................................... 357

AC1_C1_Human  YCVSGLTQPKTDHAHCCVEMGLDMIDTITSVAEATEVDLNMRVGLHTGRVLCGVLGLRKW 414
AC1_C1_Mouse  ............................................................... 413
AC1_C1_Bovine ............................................................... 417

AC1_C1_Human  QYDVWSNDVTLANVMEAAGLPGKVHITKTTLACLNGDYEVEPGYGHERNSFLKTHNIETF 474
AC1_C1_Mouse  .........................................H.....T..R....... 473
AC1_C1_Bovine .........................................H.....S..K....... 477

AC1_C1_Human  FIVPSHRRKIFPGLILSDIKPAKRMKFKTVCYLLVQLMHCRKMFKAEIPFSNVMTCEDDD 534
AC1_C1_Mouse  ............................................................... 533
AC1_C1_Bovine ............................................................... 537

AC1_C1_Human  KRRALRTASEKLRNRSSFSTNVVYTTPGTRVNRYISRLLEARQTELEMADLNFFTLKYKH 594
AC1_C1_Mouse  ......................Y...........S.......T..............H 593
AC1_C1_Bovine ......................Q...........G.......M.............. Q 597

AC1_C1_Human  VEREQKYHQLQDEYFT 610
AC1_C1_Mouse  V...Q........... 609
AC1_C1_Bovine A...R........... 612
```

AC1 C2 domain

```
AC1_C2_Human  SCALALHARQVDIRLRLDYLWAAQAEEEREDMEKVKLDNRRILFNLLPAHVAQHFLMSNP 854
AC1_C1_Mouse  ..T.........VR..............D...R.....K..................... 853
AC1_C1_Bovine ..T.........VK..............D...K.....K..................... 857

AC1_C2_Human  RNMDLYYQSYSQVGVMFASIPNFNDFYIELDGNNMGVECLRLLNEIIADFDELMEKDFYK 914
AC1_C1_Mouse  ...........................................................D..... 913
AC1_C1_Bovine ...........................................................D..... 917

AC1_C2_Human  DIEKIKTIGSTYMAAVGLAPTSGTKAKKSISSHLSTLADFAIEMFDVLDEINYQSYNDFV 974
AC1_C1_Mouse  ......................A..R...S.....C.......D.................. 973
AC1_C1_Bovine ......................A..K...C.....S.......E.................. 977

AC1_C2_Human  LRVGINVGPVVAGVIGARRPQYDIWGNTVNVASRMDSTGVQGRIQVTEEVHRLLRRCPYH 1034
AC1_C1_Mouse  ...................................................K.CS.Q 1033
AC1_C1_Bovine ...................................................R.GS.R 1037

AC1_C2_Human  FVCRGKVSVKGKGEMLTYFLEGRTDGNGSQIRSLGLDRKMCPFGRAGLQGR----RPPVC 1094
AC1_C1_Mouse  ............................S.HG.TFRLE.R.C.Y..G.G.A.----R..LC 1093
AC1_C1_Bovine ............................G.QT.SLNSE.K.Y.F..A.L.T.LAAGH..VP 1097

AC1_C2_Human  PMPGVSVRAG--------LPPHSPGQYLPSAAAGKEA 1119
AC1_C1_Mouse  .AA.PP.RP.---------.P.APTS.Y.SST.A.... 1118
AC1_C1_Bovine .AA.LP.GA.PGALQGSG.A.GPPG.H.PPG.S.... 1134
```

AC2 C1 domain

```
AC2_C1_Human HKHLMELALQQTYQDTCNCIKSRIKLEFEKRQQERLLLSLLPAHIAMEMKAEIIQRLQGP 267
AC2_C1_Mouse .............R.......................................... 266
AC2_C1_Rat   .............R.......................................... 266

AC2_C1_Human KAGQMENTNNFHNLYVKRHTNVSILYADIVGFTRLASDCSPGELVHMLNELFGKFDQIAK 327
AC2_C1_Mouse ............................................................ 326
AC2_C1_Rat   ............................................................ 326

AC2_C1_Human ENECMRIKILGDCYYCVSGLPISLPNHAKNCVKMGLDMCEAIKKVRDATGVDINMRVGVH 387
AC2_C1_Mouse ............................................................ 386
AC2_C1_Rat   ............................................................ 386

AC2_C1_Human SGNVLCGVIGLQKWQYDVWSHDVTLANHMEAGGVPGRVHISSVTLEHLNGAYKVEEGDGD 447
AC2_C1_Mouse ...........................................................E 446
AC2_C1_Rat   ...........................................................E 446

AC2_C1_Human IRDPYLKQHLVKTYFVINPKGERRSPQHLFRPRHTLDGAKMRASVRMTRYLESWGAAKPF 507
AC2_C1_Mouse ............................................................ 506
AC2_C1_Rat   ............................................................ 506

AC2_C1_Human AHLHHRDSMTTENGKISTTDVPMGQHNFQNRTLRTKSQKKRFEEELNERMIQAIDGINAQ 567
AC2_C1_Mouse ............................................................ 566
AC2_C1_Rat   ............................................................ 566

AC2_C1_Human KQWLKSEDIQRISLLFYNKVLEKEYRATALPAFK 601
AC2_C1_Mouse ..............F....NI.............. 600
AC2_C1_Rat   ..............L....NI.............. 600
```

AC2 C2 domain

```
AC2_C2_Human RQNEYYCRLDFLWKNKFKKEREEIETMENLNRVLLENVLPAHVAEHFLARSLKNEELYHQ 881
AC2_C2_Mouse ..S......................................................... 880
AC2_C2_Rat   ..S......................................................... 880

AC2_C2_Human SYDCVCVMFASIPDFKEFYTESDVNKEGLECLRLLNEIIADFDDLLSKPKFSGVEKIKTI 941
AC2_C2_Mouse ............................................................ 940
AC2_C2_Rat   ............................................................ 940

AC2_C2_Human GSTYMAATGLSAVPSQEHSQEPERQYMHIGTMVEFAFALVGKLDAINKHSFNDFKLRVGI 1001
AC2_C2_Mouse ............V.....A................Y..................... 1000
AC2_C2_Rat   ............I.....A................Y..................... 1000

AC2_C2_Human NHGPVIAGVIGAQKPQYDIWGNTVNVASRMDSTGVLDKIQVTEETSLVLQTLGYTCTCRG 1061
AC2_C2_Mouse ...........................................I............ 1060
AC2_C2_Rat   ...........................................I............ 1060

AC2_C2_Human IINVKGKGDLKTYFVNTEMSRSLSQSNVAS 1091
AC2_C2_Mouse ........................L.. 1090
AC2_C2_Rat   ........................L.. 1090
```

AC5 C1 domain

```
AC5_C1_Human  CTHYPAEVSQRQAFQETRECIQARLHSQRENQQQERLLLSVLPRHVAMEMKADINAKQED 455
AC5_C1_Mouse  C.............................................................. 455
AC5_C1_Rat    C.............................................................. 457
AC5_C1_Dog    C.............................................................. 454(376)
AC5_C1_Rabbit -.............................................................. 457

AC5_C1_Human  MMFHKIYIQKHDNVSILFADIEGFTSLASQCTAQELVMTLNELFARFDKLAAENHCLRIK 515
AC5_C1_Mouse  ............................................................... 515
AC5_C1_Rat    ............................................................... 517
AC5_C1_Dog    ............................................................... 514(436)
AC5_C1_Rabbit ............................................................... 517

AC5_C1_Human  ILGDCYYCVSGLPEARADHAHCCVEMGMDMIEAISLVREVTGVNVNMRVGIHSGRVHCGV 575
AC5_C1_Mouse  .................................L............................. 574
AC5_C1_Rat    .................................S............................. 575
AC5_C1_Dog    .................................L............................. 577(496)
AC5_C1_Rabbit .................................L............................. 577

AC5_C1_Human  LGLRKWQFDVWSNDVTLANHMEAGGKAGRIHITKATLNYLNGDYEVEPGCGGDRNAYLKE 635
AC5_C1_Mouse  .................................N.............E....... 635
AC5_C1_Rat    .................................N.............E....... 634
AC5_C1_Dog    .................................S.............E....... 637(556)
AC5_C1_Rabbit .................................N.............E....... 637

AC5_C1_Human  HSIETFLILSCTQKRKEEKAMIAKMNRQRTNSIGHNPPHWGAERPFYNHLGGNQVSKEMK 695
AC5_C1_Mouse  .........R..................................................... 695
AC5_C1_Rat    .........R..................................................... 697
AC5_C1_Dog    .........R..................................................... 694(616)
AC5_C1_Rabbit .........R..................................................... 697

AC5_C1_Human  RMGFEDPKDKNAQESANPEDEVDEFLGRAIDARSIDRLRSEHVRKFLLTFREPDLEKKYS 755
AC5_C1_Mouse  ..............A........................K.............. 755
AC5_C1_Rat    ..............A........................K.............. 757
AC5_C1_Dog    ..............A........................K.............. 754(676)
AC5_C1_Rabbit ..............T........................R.............. 757

AC5_C1_Human  KQVDDRFG 763
AC5_C1_Mouse  ........ 763
AC5_C1_Rat    ........ 765
AC5_C1_Dog    ........ 762(684)
AC5_C1_Rabbit ........ 765
```

AC5 C2 domain

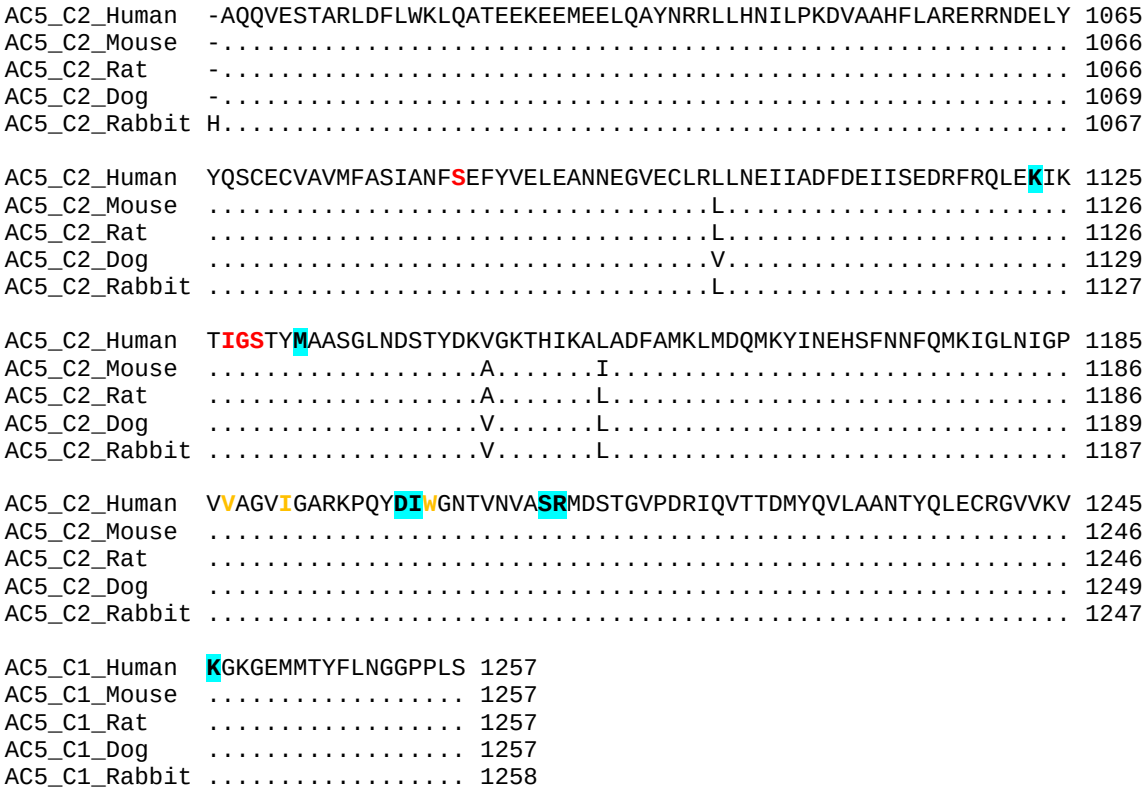

Fig. S4. Multiple sequence alignment of the amino acid sequences of the C1 and C3 domains of ACs 1, 2 and 5 from various species. The amino acid position of each AC is shown at the end of each line, and the residue numbers for the C1 domain of AC5 are indicated in parentheses. *Red* amino acid symbols indicate residues that directly interact with forskolin. *Orange* amino acid symbols represent the region interacting with the N-methylanthraniloyl group of AC inhibitors. Amino acids underlined in *blue* correspond to residues interacting with metal ions and the substrate ATP.

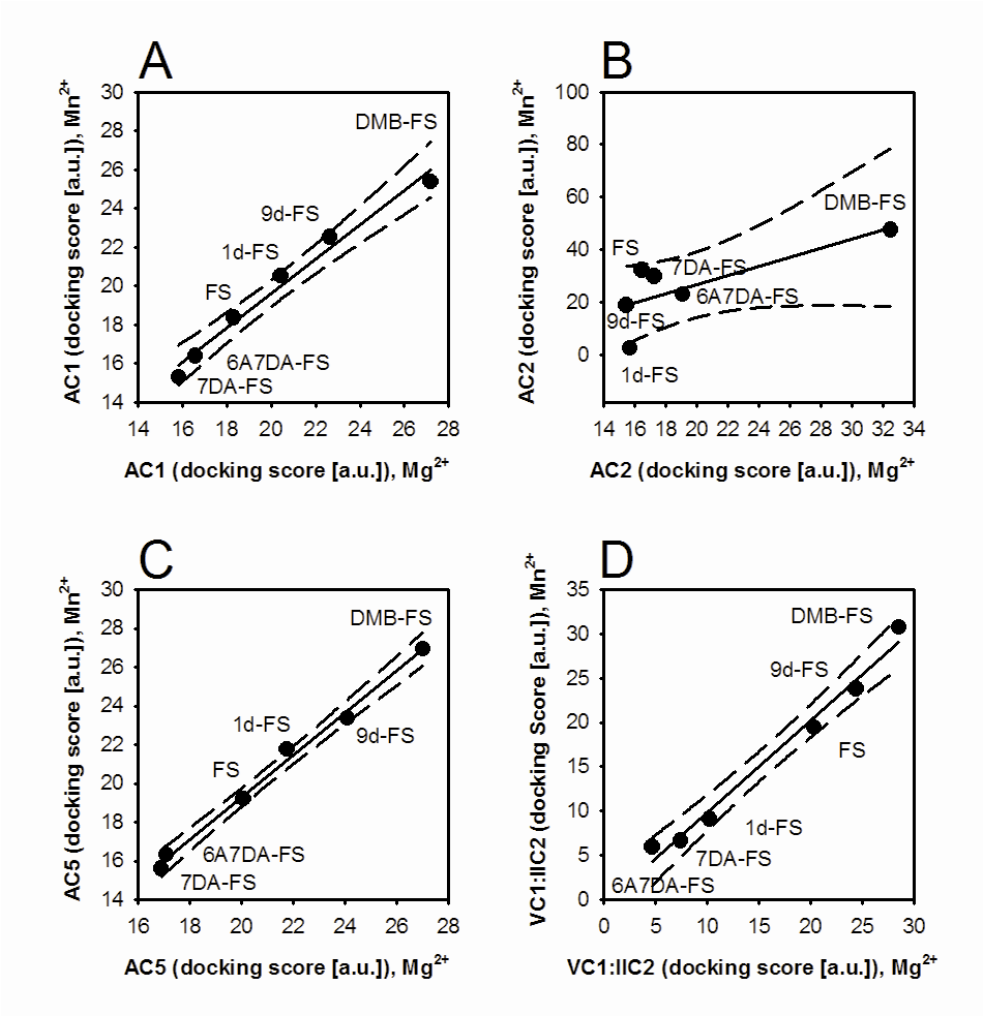

Fig. S5. Correlation of the docking profiles of diterpenes to mAC isoforms and VC1:IIC2 under Mg^{2+} conditions *vs.* Mn^{2+} conditions. A-C, correlation of docking scores for mAC isoform models 1, 2 and 5 in the presence of Mg^{2+} *vs.* Mn^{2+}. **D**, correlation of docking scores for crystal structure of VC1:IIC2 complex in the presence of Mg^{2+} *vs.* Mn^{2+}. **A**, r^2 = 0.98; slope = 0.89 ± 0.06. **B**, r^2 = 0.57; slope = 1.74 ± 0.8. **C**, r^2 = 0.99; slope = 1.09 ± 0.04. **D**, r^2 = 0.98; slope = 1.03 ± 0.07. Data were analyzed by linear regression; the dashed lines indicate 95% confidence intervals. a.u.; arbitrary unit.