

(51) International Patent Classification:
C40B 30/02 (2006.01)(21) International Application Number:
PCT/US2014/035035(22) International Filing Date:
22 April 2014 (22.04.2014)

(25) Filing Language: English

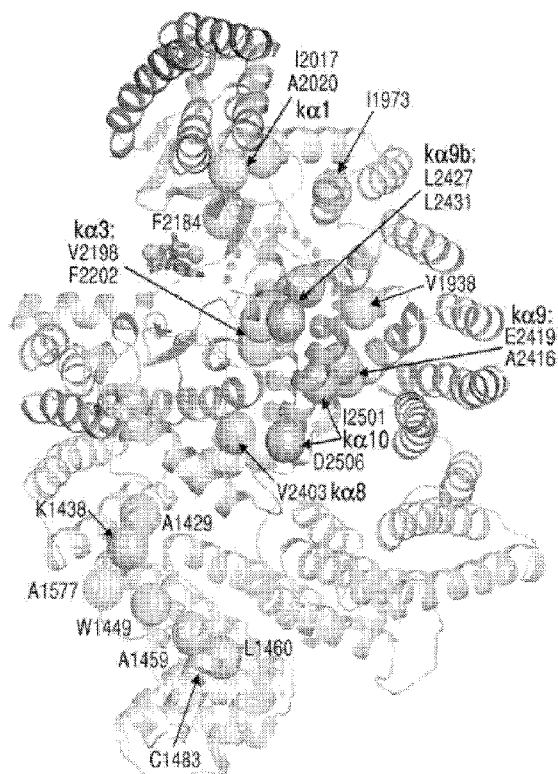
(26) Publication Language: English

(30) Priority Data:
61/814,800 22 April 2013 (22.04.2013) US(71) Applicant: MEMORIAL SLOAN KETTERING CAN-
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Massachusetts 02110 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM,
ZW.

[Continued on next page]

(54) Title: mTOR CRYSTALS AND MODULATORS

FIGURE 5

(57) Abstract: The present invention provides mTOR crystals. The
present invention also provides mTOR modulators that interact with
sites present in and/or defined by such crystals. The present inven-
tion also provides methods of making and using such crystals and
modulators. Other aspects and/or features of the present invention
will be apparent to those skilled in the art, reading the present spe-
cification.



(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (*Art. 21(3)*)
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (*Rule 48.2(h)*)

mTOR CRYSTALS AND MODULATORS

Cross-Reference to Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 61/814,800, filed April 22, 2013, the contents of which are hereby incorporated herein in its entirety.

Background

[0002] The mammalian target of rapamycin (mTOR), also known as mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1 (FRAP1), is a protein that in humans is encoded by the *FRAP1* gene (Brown EJ *et al.*, *Nature*, 1994 Jun 30;369(6483):756-8; Moore PA *et al.*, *Genomics*,) 1996 Apr 15;33(2):331-2). There has been considerable interest in the mTOR signaling pathway because of its role in many processes that have been implicated in both proliferative and metabolic diseases.

Summary

[0003] The present disclosure provides insights into mTOR structure and function that together define agents useful as modulators of mTOR.

[0004] Among other things, the present invention identifies the source of one or more problems with prior efforts to crystallize mTOR, and provides solutions to such problems. The present invention also identifies the source of one or more problems with prior efforts to define or determine mTOR structure, or relevant aspects of mTOR-interacting agents, based on comparisons with other kinases. The present invention provides solutions to such problems. Thus, among other things, the present invention provides mTOR polypeptides, mTOR crystal (and/or crystallizable) compositions, mTOR binding agents, systems that tangibly embody or

otherwise contain or utilize information about such polypeptides, compositions, and/or binding agents, and methods of generating and/or using each of these.

[0005] The present invention provides, among other things, A designed mTOR inhibitor having a structure comprising one or more of the following features:

$$\text{B} \text{---} \text{A} \text{---} \text{C}$$

$$\begin{array}{cc} | & | \end{array}$$

$$\text{D} \text{ or } \text{D}$$

wherein:

A is or comprises a moiety that fits in the mTOR adenosine (ATP) binding site and optionally may make one or two hydrogen bonds to main chain groups of the “hinge” extending from Gly2238 to Val2240;

B is or comprises a moiety that stacks with tryptophan (Trp2239);

C is or comprises a moiety that binds to an inner hydrophobic pocket formed by mTOR residues Tyr2225, Val2227, Met2199, Ile2237, Ile2356, Phe2358, Leu2192, Asp2195, Asp2357 and Gly2359; and

D is or comprises a moiety that binds to the N-lobe hydrophobic pocket (Ile2163, Pro2169 and Leu2185).

[0006] In some embodiments, the present invention provides methods of designing or characterizing mTOR modulator comprising providing an image of an mTOR crystal that includes at least one potential interaction site, docking in the image at least one moiety that is a potential mTOR modulator structural element; and assessing one or more features of a potential moiety-interaction site interaction.

[0007] In some embodiments, the present invention provides a system comprising a computer or computer readable medium in which an mTOR crystal structure, or coordinates thereof, is embedded and/or displayed.

[0008] One feature of the present invention is that it permits assessment of potential and/or actual mTOR modulators based on both structural and functional attributes. For example, in some embodiments, the present invention provides methods of designing and/or characterizing an mTOR modulator, which methods comprise steps of (i) using a system comprising a computer or computer readable medium in which an mTOR crystal structure, or coordinates thereof, is embedded and/or displayed to assess one or more structural features of the mTOR modulator; and (ii) performing one or more *in vitro*, *in vivo* or cell-based assays to characterize the mTOR modulator.

[0009] In some embodiments, the present invention provides an inhibitor of mTOR characterized in that it binds in the binding pocket having a three-dimensional structure characterized by the structure coordinates of any one of Tables 1, 2, 3, 4, 5, and/or 6.

[0010] In some embodiments, the present invention provides a designed mTOR inhibitor, comprising the crystallography coordinates of any one of Tables 1, 2, 3, 4, 5 and/or 6, wherein the crystallography coordinates are within about a root mean square deviation of not more than about 1.5 Å from the backbone atoms of the amino acids according to Tables 1, 2, 3, 4, 5 and/or 6.

[0011] In some embodiments, the present invention provides a computer system containing a set of information to perform a design or characterization of an mTOR inhibitor having a user interface comprising a display unit, the set of information comprising:

- (i) logic for inputting an information regarding a binding of a mTOR protein to a moiety known to bind mTOR protein;
- (ii) logic for design a candidate mTOR inhibitor based on the binding of the mTOR protein to the moiety known to bind mTOR protein;

- (iii) logic for determining an information regarding a binding of the mTOR protein to the candidate mTOR inhibitor; and
- (iv) logic for making a conclusion regarding a mTOR inhibitory properties of the candidate mTOR inhibitor based on the determination of step (iii).

[0012] In some embodiments, the present invention provides a computer-readable storage medium containing a set of information for a general purpose computer having a user interface comprising, a display unit, the set of information comprising:

- (i) logic for inputting an information regarding a binding of a mTOR protein to a chemical known to binding mTOR protein;
- (ii) logic for design a candidate mTOR inhibitor based on the binding of the mTOR protein to the chemical known to bind mTOR protein;
- (iii) logic for determining an information regarding a binding of the mTOR protein to the candidate mTOR inhibitor; and
- (iv) logic for making a conclusion regarding a mTOR inhibitory properties of the candidate mTOR inhibitor based on the determination step of step (iii).

[0013] In some embodiments, the present invention provides an electronic signal or carrier wave that is propagated over the internet between computers comprising a set of information for a general purpose computer having a user interface comprising a display unit, the set of information comprising a computer-readable storage medium containing a set of information for a general purpose computer having a user interface comprising a display unit, the set of information comprising:

- (i) logic for inputting an information regarding a binding of a mTOR protein to a chemical known to bind mTOR protein;

- (ii) logic for designing a candidate mTOR inhibitor based on the binding of the mTOR protein to the chemical known to bind mTOR protein;
- (iii) logic for determining an information regarding a binding of the mTOR protein to the candidate mTOR inhibitor; and
- (iv) logic for making a conclusion regarding a mTOR inhibitory properties of the candidate mTOR inhibitor based on the determination of step (iii).

[0014] In some embodiments, the present invention provides a crystalline or crystallizable composition comprising or consisting of an mTOR polypeptide.

Brief Description of the Drawing

[0015] The Figures described below, that together make up the Drawing, are for illustration purposes only, not for limitation.

[0016] **Figure 1** depicts an exemplary structure of the mTOR^{ΔN}-mLST8 complex. **FIG. 1A** depicts an exemplary structure of the mTOR^{ΔN}-mLST8-ATPγS-Mg complex looking down one end of the catalytic cleft. mTOR is colored according to its domains, summarized in the linear representation, and mLST8 is colored green. ATP is shown as sticks, and Mg²⁺ ions as spheres. Dotted loops indicate disordered regions. **FIG. 1B** depicts an exemplary view looking down the vertical axis of FIG. 1A.

[0017] **Figure 2** depicts an exemplary representation of an mTOR kinase domain and active site conformation. **FIG. 2A** depicts an exemplary representation of a superposition of the kinase domains of mTOR and the type 3 PIK3C3 (PDB 3IHY) in two views rotated by ~180°. The left view is related to that of FIG. 1A, and right view is rotated by ~180°. The mTOR insertions are colored dark blue, except for the FRB, which is colored red. PIK3C3 is colored green. Black dashed line delineates the LBE, and the blue dotted loop indicates the 55-residue disordered region between kα9b and kα10. The FATC N-terminal half (kα11) is not colored

blue, as a corresponding helix is present in PI3Ks. **FIG. 2B** depicts an exemplary representation of the 3.5 Å *Fo-Fc* electron density, contoured at 2.5 σ , of the mTOR TS complex before ADP-MgF₃-Mg₂ was built. Active site residues shown are labeled in FIG. 2C. **FIG. 2C** depicts an exemplary superposition of the mTOR and CDK2 TS (PDB 3QHW) complexes. CDK2 and its nucleotide are colored green, and mTOR is colored as in FIG. 1A. Residues discussed in the text are labeled (CDK2 residue labels are in parentheses). **FIG. 2D** depicts an exemplary molecular surface representation of the C lobe portion of the mTOR catalytic cleft, colored according to conservation entropy (red invariant in 22 orthologs, orange in 21, yellow-orange in 20, and yellow invariant either in 19 or in 18 with one conservative substitutions). Dashed lines indicate the boundaries of mLST8 and of the structural elements discussed in the text. A CDK2-bound substrate peptide (light blue) was docked using the mTOR-CDK2 superposition in (c). Its threonine phosphorylation site is shown in sticks, and the C β atoms of the rest of its side chains as spheres, with the +1 position indicated by a white arrow.

[0018] **Figure 3** depicts an exemplary structure of the mTOR kinase active site, which is recessed at the bottom of a deep cleft. **FIG. 3A** depicts an exemplary surface representation of the mTOR^{ΔN}-mLST8-ADP-MgF₃-Mg₂ structure in two orthogonal views. mTOR is colored according to its domains, summarized in the linear representation, and mLST8 is colored green. ATP is shown as sticks, and Mg²⁺ ions as spheres. Dotted loops indicate disordered regions. The mTOR^{ΔN} N-terminus is labeled. A docked substrate peptide from FIG. 2D is shown in ribbon representation (positions -2 to +2) and its phosphorylation site (position 0) in sticks (dark blue). Pink dotted loop indicates the disordered region between α 9b and α 10. **FIG. 3B** depicts an exemplary surface representation of a model of the mTOR^{ΔN}-mLST8-rapamycin-FKBP12 complex constructed by superposing the FRB domains of mTOR^{ΔN} and the FRBrapamycin-FKBP12 complex (PDB 1FAP). Rapamycin (cyan) and FKBP12 (blue) are labeled.

[0019] **Figure 4** depicts an exemplary representation of the rapamycin-binding site of the FRB and recruitment of S6K1 into the catalytic cleft. **FIG. 4A** depicts an exemplary surface representation of the FRB and portion of the catalytic cleft, colored by conservation as in FIG. 2D. The active site-proximal face of the FRB (left) but not the opposite face (right) contains a

hotspot of conserved residues. The docked substrate peptide from FIG. 2D is shown as a blue ribbon (positions -1 to +1), with its threonine side chain in sticks and its hydroxyl group labelled. ADP-MgF₃-Mg₂ is in cyan with the TS mimic MgF₃ group labelled as “ATP γP”. The black dashed line indicates the boundary between the FRB and KD. **FIG. 4B** depicts an exemplary result showing the phosphorylation of S6K1^{ki} (10 μM) by mTOR^{ΔN}-mLST8 (20 nM), measured by ³²P incorporation (top panel) and by immunoblotting with a phosphoThr389-specific antibody (lower panel), in the presence of the indicated micromolar concentrations of rapamycin or FK506. The average and standard deviation of three independent repetitions is plotted as a percentage of the zero macrolide concentration reaction for each set (right panel). **FIG. 4C** depicts an exemplary result showing phosphorylation of S6K1^{ki} (10 μM) by mTOR^{ΔN}-mLST8 (20 nM) in the presence of the indicated micromolar concentrations of the wild type or S20351 mutant FRB. Reactions were repeated twice, and their quantitation plotted as in FIG. 4B. **FIG. 4D** depicts an exemplary result showing phosphorylation of GST-tagged polypeptides (10 μM), corresponding to residues 367-398 and 367-392 of the C-terminal tail of S6K1, by mTOR^{ΔN}-mLST8 (20 nM) in the presence of the indicated concentrations of rapamycin. Two different exposures (15 sec. and 3 min.) of the phosphoThr389-specific immunoblot are shown. The quantitation of the 15 sec. immunoblot is plotted on the right.

[0020] **Figure 5** depicts an exemplary representation of mTOR activating mutations that map to structural elements involved in restricting active site access. Activating mutations⁴⁵⁻⁴⁷ reported for mTOR, yeast Tor2 and Tor1p are shown as large spheres (labeled), colored according to the mTOR domain they are located in. The structural elements of the KD mutations are also labeled.

[0021] **Figure 6** depicts exemplary structures of the inhibitors Torin2, PP242 and PI-103 bound to the mTOR catalytic cleft. **FIG. 6A** depicts an exemplary stick representation of Torin2 (in cyan, F atoms in green) and of mTOR residues that are within 4 Å of Torin2 (except for D2195 and D2357 discussed in the text). The mTOR cleft is shown in transparent surface representation, with the N lobe in yellow and C lobe in pink. Green dotted line indicates atoms within hydrogen-bonding distance and geometry. **FIG. 6B** depicts an exemplary PP242-mTOR

structure, represented as in FIG. 6A. **FIG. 6C** depicts an exemplary representation of the conformational change in the inner hydrophobic pocket of mTOR on PP242 (cyan) binding. Arrows indicate side chain rotations and main chain shifts in PP242-bound mTOR compared to the ATP γ S-bound mTOR (gray). View is approximately looking down the vertical axis of FIG. 6B. **FIG. 6D** depicts an exemplary PI-103-mTOR structure, represented as in FIG. 6A.

[0022] **Figure 7** depicts exemplary results for N-terminally truncated human mTOR (residues 1376 to 2549; thereafter mTOR ^{Δ N}) bound to full-length human mLST8, including exemplary data collection, phasing and refinement statistics. mTOR ^{Δ N}-mLST8 complex was co-crystallized with ADP, Mg²⁺ and MgF₃⁻, a mimic of the γ -phosphate group of ATP in the transition state (TS)³⁶.

[0023] **Figure 8** depicts exemplary superpositions of mTOR and PI3K kinase domains (rmsd in Ca positions in Å).

[0024] **Figure 9** depicts exemplary results for mTOR ^{Δ N}-mLST8 bound to Torin2 and PP242, including exemplary inhibitor data collection and refinement statistics.

[0025] **Figure 10** depicts exemplary results showing phosphorylation of S6K1 and 4EBP1 by the mTOR ^{Δ N}-mLST8 and mTORC1 complexes. **FIG. 10A** depicts exemplary results showing phosphorylation of the indicated concentrations of full-length S6K1 harboring a kinase inactivating mutation (K100R, thereafter S6K1^{ki}) by mTOR ^{Δ N}-mLST8 and mTORC1 (each at 100 nM concentration). Top panels are ³²P autoradiograms and lower panels are immunoblots using an S6K1 phosphoThr389-specific antibody. Reactions were performed as described in methods. Graph shows quantitation of the ³²P autoradiograms (blue circles for mTOR ^{Δ N}-mLST8, and red triangles for mTORC1). **FIG. 10B** depicts exemplary results showing phosphorylation of the indicated concentrations of full-length 4EBP1 by mTOR ^{Δ N}-mLST8 and mTORC1. Assays were performed as in FIG. 10A, except for immunoblotting with an antibody specific for 4EBP1 phosphorylated at Thr37 and Thr46.

[0026] **Figure 11** depicts exemplary secondary structure, sequence conservation and temperature factors of human mTOR ^{Δ N}. **FIG. 11A** depicts exemplary sequence alignments of

human, fish, fly, budding yeast, fission yeast, plant and worm orthologs. Helices are indicated as rounded cylinders, β strands as arrows, segments lacking regular secondary structure as solid lines, and disordered regions as dashed lines. The various domains are colored as in FIG. 1A. The boundaries between the three TRD and one HRD domains are indicated by arrows. Segments discussed in text are indicated by horizontal brackets and are labeled. Active site residues are marked by asterisks. The structure-based sequence alignment of the human PIK3C3, a type 3 PI3K (PDB 3IHY), is given as the last sequence extending across the kinase domain. The PIK3C3 residues that superimpose on mTOR are underlined, and dots represent insertions in the mTOR KD relative to PIK3C3. **FIG. 11B** depicts an exemplary plot of the temperature factors (vertical axis of each panel in $\text{\AA}^2$) of the mTOR^{ΔN} Cα atoms from one of the two mTOR^{ΔN}-mLST8 complexes in the asymmetric unit of the apo crystals. The overall temperature factor of this entire mTOR^{ΔN} protomer is 75.47 $\text{\AA}^2$ (92.60 $\text{\AA}^2$ for the second protomer), and of the kinase domain excluding the FRB is 50.44 $\text{\AA}^2$ (61.37 $\text{\AA}^2$ for the second protomer).

[0027] **Figure 12** depicts an exemplary comparison of the mTOR kinase domain to other PIKKs. **FIG. 12A** depicts an exemplary PIKK sequence alignment of the KD region based on the mTOR structure. The FRB-like insertions in SMG1, DNAPKcs and TRRAP are predicted to be alpha helical by the JPRED server (www.compbio.dundee.ac.uk/www-jpred/). ATM and ATR appear to lack an insertion comparable to the FRB. The LBE-like insertions in all six PIKKs are predicted to have two helices, except for the longer insertion in SMG1. Secondary structure restraints from the mTOR structure were included in the alignment with the program Indonesia (xray.bmc.uu.se/dennis/). Annotations as in FIG. 11A. Residues with over 50 % sequence similarity are indicated in yellow. **FIG. 12B** is an exemplary model showing an FRB-like domain is present in DNAPKcs. Top panel shows a stereo view of two-fold ncs averaged *2Fo-Fc* electron density for the FRB region using the 6.6 $\text{\AA}$ native data of DNAPKcs^{Supp.1} and phases from our rebuilding of the deposited model (PDB 3KGV). The deposited model, which lacks helices in the FRB region, is shown in green. Middle panel shows the superimposed mTOR structure, indicating that the FRB-like domain of DNAPKcs is tilted towards the C lobe. Bottom panel shows a manual fitting of the mTOR FRB (red) by optimizing the fit of helices *fa1* and *fa2*

in the electron density. Helices $\alpha 3$ and $\alpha 4$ appear to have different orientations compared to those of mTOR, which is expected given the sequence divergence between the two PIKKs. Figures were prepared with Pymol (The PyMOL Molecular Graphics System, Schrödinger, LLC). Applicants rebuilt the DNAPKcs model by first replacing the PI3K-based kinase domain in the deposited model with that of mTOR using molecular replacement and omitting the FRB, LBE and $\alpha 9b$ to avoid model bias. Inspection of the resulting electron density maps revealed electron density consistent with the presence of all three omitted segments. In addition, the maps indicated that DNAPKcs should have essentially all 28 helices of the mTOR FAT domain, even though the deposited model contained only 17 helices in this region. The mTOR FAT domain was subsequently fitted into electron density that was calculated without any of the FAT helices, first as four rigid bodies corresponding to the TRD1, TRD2, TRD3 and HRD domains, then by adjustment of individual helical repeats (see Fig. 22 for DNAPKcs FAT domain electron density and discussion). The resulting model was used to phase the *Fo-Fc* electron density shown.

[0028] **Figure 13** depicts exemplary results showing the mTOR catalytic residues and reaction mechanism are closely related to canonical protein kinases. **FIG. 13A** depicts an exemplary stereo view of the spine of interactions (side chains within ~ 4 Å distance) extending from the LBE (magenta) to the FATC (blue), activation loop (A.L. orange) and $\alpha 9b$ (purple) elements in the ATP γ S crystals. The catalytic loop (C.L.) is in cyan, and the remaining elements are colored as in FIG. 1A (light blue for FAT, yellow for N lobe, light pink for C lobe, and green for ATP γ S). Purple dotted line indicates disordered loop between $\alpha 9b$ and $\alpha 10$. **FIG. 13B** depicts an exemplary stereo view of the *Fo-Fc* electron density of the ATP γ S crystals, calculated at 3.3 Å and contoured at 2.5 σ , is shown in blue mesh. Superimposed as red mesh is the anomalous fourier map of apocrystals soaked in AMPPNP and manganese (FIG. 7), after the map was skewed to the unit cell of the ATP γ S crystals. **FIG. 13C** depicts an exemplary structure-based sequence alignment of the catalytic and activation loop portions of mTOR and CDK2 that are superimposed in FIG. 2C, with uppercase CDK2 residues having their $C\alpha$ atoms within 1.8 Å of the corresponding ones in mTOR (lowercase residues are displaced by 2.8 to 4.6 Å). The catalytic residues as discussed herein are labeled and in red. Applicants note that the art-

proposed mechanism for PI3K postulated that a histidine (corresponding to mTOR His2340) acts as the catalytic base, and an arginine (corresponding to mTOR Arg2339) serves to neutralize the charge of the TS state. However, in mTOR the Arg2339 guanidinium group is ~ 7 Å away from γ -phosphate TS mimic, compared to less than ~ 3 Å for His2340. Without wishing to be bound by any particular theory, the present invention proposes that mTOR's His2340, rather than Arg2339, acts as the catalytic base. The present disclosure further proposes that, particularly given that the relative arrangement of the Asp2338 catalytic base and the γ -phosphate TS mimic in CDK2 is nearly identical in mTOR, the residue corresponding to mTOR Arg2339 may also act as the catalytic base in other PIKK and/or PI3K family kinases. For example, the present invention proposes that Arg127 in CDK2 may act as the catalytic base in this kinase. **FIG. 13D** depicts exemplary results showing 4EBP1 phosphorylation by wild type, D2338A and H2340A FLAG-mTOR Δ N-mLST8, each at 100 nM concentration. Top panel is an immunoblot using an antibody specific for 4EBP1 phosphorylated at Thr37 and Thr46 and exposed for 15 seconds. Middle panel is the same immunoblot exposed for 180 seconds. Unphosphorylated 4EBP1 (no mTOR added) was run as a background control for the nonspecific signal from the antibody at long exposure times and at 100 μ M concentration of 4EBP1. From quantitation of multiple exposures the mutations reduce 4EBP1 phosphorylation by a factor of ~ 3000 to ~ 9500 . The bottom panel is an immunoblot with an anti-FLAG antibody.

[0029] **Figure 14** depicts an exemplary model showing putative substrate-binding grooves on the C lobe portion of the mTOR catalytic cleft. **FIG. 14A** is an exemplary stick representation of FIG. 2D, showing residues that line the putative substrate-binding groove on the C lobe portion of the catalytic cleft, colored by conservation as in FIG. 2D (+1 position is labeled). In the (+) direction, the groove is lined by Phe2371, Tyr2542 and Trp2545, consistent with a positional scanning peptide array² showing preference for hydrophobic and aromatic residues at +1. In the (-) direction, there are multiple local pockets that extend farther away from the catalytic center. In addition to hydrophobic residues, there are several polar residues that appear to be uninvolved in stabilizing the structure but are invariant in either all 22 orthologs (His2247) or in all but one (Asn2262 and His2265). Without wishing to be bound by any

particular theory, the present disclosure proposes that such pockets could underlie the low-level of sequence preference at the -4 and -5 positions suggested by the positional scanning peptide array^{Supp.2}. **FIG. 14B** depicts exemplary sequence alignments of major phosphorylation sites (red asterisk) in the 4EBP1 and AGC kinase substrates of mTOR. Known mTOR substrates appear to lack a strong consensus motif at their phosphorylation sites. The four main phosphorylation sites of 4EBP1 have essentially no sequence homology flanking the Ser/Thr-Pro sites. While the ~7 residue HM motifs of the S6K1, AKT, PKC α and SGK1 AGC family kinases are conserved, they bear little or no relationship to the 4EBP1 sites. In addition, HM motif conservation is due, at least in part, to its role in mediating the activation of the kinase through phosphorylation-induced intra-molecular interactions^{Supp.3}.

[0030] **Figure 15** depicts exemplary results of a comparison of the kinase activity of mTOR^{ΔN}-mLST8, full-length mTOR-mLST8, mTOR-mLST8-RAPTOR, and mTOR^{ΔNΔ2443-2486}-mLST8 complexes. **FIG. 15A** depicts exemplary results showing phosphorylation (³²P autoradiogram) of the indicated concentrations of full-length S6K1^{ki} with a mutated TOS motif (FDID to AAAA; S6K1^{ki/TOS-}) by mTOR^{ΔNΔ2443-2486}-mLST8 (red circles) mTOR^{ΔN}-mLST8 (blue squares), mTOR-mLST8 (purple diamonds) and mTOR-mLST8-RAPTOR (green triangles), each at 100 nM concentration. Reactions were performed as described in the Exemplification section herein. **FIG. 15B** depicts exemplary results showing phosphorylation (³²P autoradiogram) of the indicated concentrations of full-length 4EBP1 with a mutated TOS motif (FEMDI to AAAAA; 4EBP1^{TOS-}) by mTOR^{ΔNΔ2443-2486}-mLST8 (red circles) mTOR^{ΔN}-mLST8 (blue squares), mTOR-mLST8 (purple diamonds) and mTOR-mLST8-RAPTOR (green triangles), each at 100 nM concentration. Reactions were performed as described in the Exemplification section herein.

[0031] **Figure 16** depicts an exemplary model showing the rapamycin-binding site mapped to the FRB surface closest to the active site, in the midst of a hotspot of highly conserved residues. **FIG. 16A** depicts an exemplary sequence alignment showing sequence conservation of the FRB in 22 mTOR orthologs and homologs (Tor2 in yeast), with 22/22 identity highlighted in red, and 19/22 in yellow. Residues that make up the conservation hotspot

in and around the rapamycin binding site are marked by asterisks, and are shown in FIG. 16B.

FIG. 16B (left panel) depicts an exemplary stick representation of FIG. 4A, showing the conserved residues in and around the rapamycin binding site. **FIG. 16B** (right panel) depicts an exemplary isolated FRB (light blue) bound to rapamycin (green) from the FRB-rapamycin-FKBP12 structure^{Supp.11}.

[0032] **Figure 17** depicts exemplary results illustrating mapping of the S6K1 tail sequences involved in FRB-mediated recruitment to mTOR active site. **FIG. 17A** depicts exemplary results showing inhibition, by the indicated concentrations of free rapamycin, of mTOR^{ΔN}-mLST8 (100 nM) phosphorylation of full-length S6K1^{ki} (blue squares) and a GST-tagged S6K1 polypeptide of residues 351-415 from the C-terminal tail (GST-S6K1³⁵¹⁻⁴¹⁵, red diamonds), each present at 2 μM concentration in the same reaction. Reactions were performed as in the Exemplification section herein. The S6K1 C-terminal tail, which starts with residue 351 immediately after the kinase domain structure^{Supp.12} and ends at residue 502, is likely unstructured based on secondary-structure prediction programs (not shown). The reduced ³²P incorporation by GST-S6K1³⁵¹⁻⁴¹⁵ compared to full length S6K1 is due, at least in part, to the presence of multiple in vitro phosphorylation sites after residue 416. **FIG. 17B** depicts exemplary results showing phosphorylation of the indicated GST-S6K1 tail polypeptides (2 μM) by mTOR^{ΔN}-mLST8 (100 nM) and inhibition by free rapamycin (20 μM; lanes marked with +). Top panel is the ³²P autoradiogram, middle panel immunoblots using an S6K1 phosphoThr389-specific antibody, and bottom is ³²P quantitation. Reactions were performed as set forth in the Exemplification section herein. C-terminal truncation of residues 403-410 reduces ³²P incorporation ~3-fold, while a further truncation of 399-402 results in an additional ~5-fold reduction, although phosphorylation remains rapamycin-sensitive. While N-terminal truncations of residues 367-381 reduce phosphorylation, this reduction is apparently caused by GST interference, presumably due to its proximity to the HM motif when fused to residue 382, because the reduction is not observed after cleavage of the GST tag (not shown). An alternative explanation for inhibition by free rapamycin and the dominant negative effect of the isolated FRB is that the FRB site mediates dimerization that may be important for substrate

phosphorylation. Mammalian but not yeast TORC1 can form dimers, although this is dependent on RAPTOR, which is absent in the assays. Nevertheless, Applicant found no evidence of mTOR^{ΔN}-mLST8 dimerization or FRB binding to mTOR^{ΔN}-mLST8. A hypothetical dimer whose inhibition by rapamycin or the isolated FRB would explain reduced S6K1 phosphorylation should be readily detectable, as it would have to form at mTOR^{ΔN}-mLST8 concentrations as low as 20 nM used in the kinase assays of FIG. 4B, C and D.

[0033] **Figure 18** depicts an exemplary model showing that FAT forms a C-shaped α solenoid and clamps onto the kinase domain. **FIG. 18A** depicts an exemplary model illustrating an overall view of the mTOR^{ΔN}-mLST8-ATP γ S-Mg complex looking down the vertical axis of FIG. 1, highlighting the C-shaped FAT domain clamping onto the KD. Colored as in FIG. 1. **FIG. 18B** depicts an exemplary model illustrating an overall view of mTOR^{ΔN} with the FAT domain colored according to its three TPR domains and one HEAT repeat domain. TRD1 (light green) has 1.5 repeats, TRD2 (light cyan) has 4.5 repeats, TRD3 (light purple) has 5 repeats, and HRD (light blue) has 3 repeats. Based on the DALI server, TRD1 is structurally most similar to TPR repeats of Rab geranylgeranyltransferase- α (1LTX, Z-score 9.1); TRD2 to a synthetic consensus TPR protein (2HYZ; Z-score 13.5); TRD3 to the TPR-containing protein MamA (3ASH; Z-score 12.2), and HRD to the HEAT repeats of the microtubule binding protein XMAP215 (2QK2; Z-score 9.3) and the HEAT repeats of the PI3K p110 γ (2V4L; Z-score 9.1). The non-canonical TRD1-TRD2 packing involves a lateral shift in the helical arrangement compared to regular TPRs, and it is stabilized by the TRD1 α 2 packing with the TRD2 α 11- α 12 loop. The TRD2-TRD3 packing is more divergent, being mediated by the second helix of TRD3 (α 14) packing inside the concave surface of TRD2 (α 6, α 8, α 10 and α 12). This is associated with a reversal of curvature between the two segments, and gives the TRD2-TRD3 portion of the α solenoid a corkscrew-like twist. The HRD C-terminus forms a nearly continuous transition to the kinase N lobe, with only 2 amino acids intervening between the last HRD helix and the first KD helix. **FIG. 18C** depicts a model showing the interface between TRD1 (green) and the KD C lobe (pink). **FIG. 18D** depicts a model showing the interface between the HRD (light blue) and the KD C lobe (pink) and N lobe (yellow). The red letter “M” indicates two contact residues

(Ala2416 and Glu2419) which were isolated as activating mutations of fission yeast Tor2p in the Rheb-independent growth screen^{Supp.15}. **FIG. 18E** depicts an exemplary model showing the superposition of the three HEAT repeats of the PI3K p110 α (PDB 2RDO) with the HRD (light blue) of mTOR. The p110 α HRD domain is in red and the rest of the protein in green. mTOR is colored as in FIG. 18B, except the FRB is in brown. The mTOR HRD-KD contacts are also analogous to the p110 α HEAT repeat-KD contacts. For example, the mTOR Gln1941-Gln2200 interaction (FIG. 18D) is also present in p110 α (Gln634-Gln815), and these residues are conserved in all PI3K family members. The rest of the FAT domain N-terminal to the HRD is unrelated to PI3Ks, which typically have at most two helical repeats capping the N-terminus of their HEAT-repeat domain.

[0034] **Figure 19** depicts an exemplary model of the mTOR-mLST8 interface. **FIG. 19A** shows mLST8 (green) consists exclusively of seven WD40 repeats. They fold into a cononical β propeller structure, except for a permutation of the N- and C-terminal strands. The seven blades are numbered 1 through 7, and the strands of each blade are labeled “a” to “d”. Whereas in canonical WD40 domains the N-terminal strand corresponds to strand “d” of a blade (blade 7), the N-terminal strand of mLST8 corresponds to strand “b” (blade 1). Only residues 1-7 and 325-326 of the full-length mLST8 in the crystals are disordered. LBE (residues 2258-2296) is in pink. **FIG. 19B** depicts an exemplary model showing the mTOR-mLST8 interface involves all but one of the mLST8 WD40 repeats, and both helices and intervening loop of the LBE, as well as a single residue from the FATC α 12. View is rotated $\sim 90^\circ$ along x from that of FIG. 19A. In the absence of mLST8, there would be only two hydrophobic residues (Met2271 and Met2281) that would be solvent exposed on the LBE. These are unlikely to account for the reduced solubility (not shown) and heat-shock protein association¹⁶ of mTOR overexpressed in the absence of mLST8.

[0035] **Figure 20** depicts an exemplary close-up stereo view of activating mutations in the vicinity of α 9b. Activating mutation residues at α 9b or at the structural elements that pack with it (α 3, α 9 and α 10) are shown in red, and the residues in their immediate vicinity are shown in yellow (N lobe), pink (C lobe) or cyan (FAT HRD). Pink dotted line indicates the

disordered loop between $\alpha 9b$ and $\alpha 10$. The hyperactivating mutations were isolated in three separate screens for either bypassing the requirement for Rheb for growth¹⁵, suppressing the temperature-sensitive (ts) growth phenotype of an Lst8ts mutant¹⁷, or resistance to the ATP-competitive inhibitor caffeine. FIG. 5 excludes three mutations that map to screen-specific structural elements. They are the A2290V and L2302Q mutations that map to the LBE and were isolated from the Lst8ts screen (discussed in the mLST8 section of the main text), and the ATP-site W2239R from the caffeine screen. The LBE A2290V and L2302Q mutations have not been assessed biochemically for increased mTOR phosphorylation activity, and the W2239R mutant is unlikely to be a general activating mutation; it has a temperature sensitive phenotype and the structure suggests it would have reduced affinity for both ATP and caffeine. Of the remaining mutations, only a subset has been assessed biochemically. However, the fact that Val2198 mutations were isolated in both the Rheb-null and Lst8ts screens is supportive of the notion that mutations that target the $\alpha 9b$ -centered structural framework have a common effect in increasing mTOR kinase activity. Applicants also note that while one of the mutant alleles from the Lst8ts screen (named SL1) has three mutations, we presume Val2198 is key for the hyperactive phenotype because the other two residues are not evolutionarily conserved.

[0036] **Figure 21** depicts an exemplary model of mTOR complexes with Torin2, PP242 and PI-103. **FIG. 21A** shows exemplary modeling of the 3.5 Å resolution *Fo-Fc* electron density of the Torin2-mTOR ΔN -mLST8 crystals, calculated with model phases before the inhibitor was built; density is shown in stereo as a blue mesh contoured at 2.2 σ . View approximately looking down the vertical axis of FIG. 6A. The N lobe portion of the cleft is above the plane of the figure, and it is not shown as it would obstruct the view. Green dotted line indicates atoms that are within hydrogen-bonding distance and geometry. **FIG. 21B** shows the 3.45 Å resolution *Fo-Fc* electron density of the PP242-mTOR ΔN -mLST8 crystals, calculated as in FIG. 21A, and contoured at 2.2 σ . **FIG. 21C** depicts an exemplary omit map of the two polypeptide segments from the N lobe (residues 2223-2226; orange) and C lobe (residues 2353-2355; pink) of the PP242-mTOR ΔN -mLST8 crystals that undergo a conformational change. The structure of the ATP γ S-bound mTOR is in gray, and PP242 in light cyan. The 3.45 Å resolution

Fo-Fc map was calculated with phases after the model with the omitted segments was subjected to simulated annealing refinement to remove model bias. Contoured at 2.0σ . **FIG. 21D** shows the $3.6\text{ \AA}$ resolution *Fo-Fc* electron density of the PI-103–mTOR Δ N–mLST8 crystals, calculated as in FIG. 21A, and contoured at 2.2σ .

[0037] **Figure 22** depicts an exemplary model showing DNAPKcs contains a FAT clamp that interacts with the KD domain as in the mTOR structure. **FIG. 22A** depicts an exemplary stereo view of two-fold ncs averaged *Fo-Fc* electron density for the FAT region using the $6.6\text{ \AA}$ native data of DNAPKcs1 and phases from our mTOR-based rebuilding (omitting the FAT) of the deposited model (PDB 3KGV). The deposited model, shown in red, contains only 17 of the 28 main mTOR FAT helices (2/3 helices in TRD1, 6/9 in TRD2, 7/10 in TRD3, and 2/6 in HRD). All but one ($\alpha 20$ of TRD3) of the missing helices are evident at the 2.5σ contour level shown. **FIG. 22B** depicts an exemplary stereo view of the DNAPKcs FAT model constructed by fitting the mTOR TRD1, TRD2, TRD3 and HRD domains as rigid bodies into the density (colored as in FIG. 17A). TRD1 and HRD require minimal adjustment consistent with their interactions with the KD. TRD2 and TRD3 are shifted and rotated substantially, consistent with their minimal sequence conservation among PIKKs. In addition, individual repeats of TRD2 and TRD3 and the last repeat of the HRD require additional shifts for fitting the electron density (not shown), and they also appear to differ in helix lengths compared to mTOR. **FIG. 22C** depicts an exemplary mTOR Δ N structure superimposed on the rigid-body domain fitted FAT model of DNAPKcs, underscoring the similar arrangement of TRD1 and HRD relative to the KD, but divergent arrangements of TRD2 and TRD3.

[0038] **Figure 23** depicts an exemplary sequence alignment of mTOR amino acid residues from human (SEQ ID NO: ()), rat (SEQ ID NO: ()), mouse (SEQ ID NO: ()), sheep (SEQ ID NO: ()), zebrafish (SEQ ID NO: ()), rhesus monkey (SEQ ID NO: ()), goat (SEQ ID NO: ()), fruit fly (SEQ ID NO: ()) and chimpanzee (SEQ ID NO: ()). Protein sequences were aligned using Clustal Omega, a multiple sequence alignment program for proteins. The CLUSTAL OMEGA software is available for download here (www.clustal.org/omega/).

[0039] **Figure 24** depicts an exemplary block diagram of a computing device and a mobile computing device.

[0040] **Figure 25** depicts an exemplary block diagram of a network environment for establishing a multi-channel context aware communication environment.

Definitions

[0041] *Administration:* As used herein, the term “administration” refers to the administration of a composition to a subject. Administration may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and vitreal.

[0042] *Amino acid:* As used herein, the term “amino acid,” in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with the general structure above. For example, in some embodiments, an amino acid may be modified by methylation, amidation, acetylation, and/or substitution as compared with the general structure. In some embodiments, such modification may, for example, alter the

circulating half life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in some embodiments, the term “amino acid” is used to refer to a free amino acid; in some embodiments it is used to refer to an amino acid residue of a polypeptide.

[0043] *Animal:* As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In some embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0044] *Approximately:* As used herein, the term “approximately” and “about” is intended to encompass normal statistical variation as would be understood by those of ordinary skill in the art as appropriate to the relevant context. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0045] *Associated with:* Two events or entities are “associated” with one another, as that term is used herein, if the presence, level and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility of the disease, disorder, or condition (e.g., across a relevant

population). In some embodiments, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are and remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0046] *Characteristic sequence element:* As used herein, the phrase “characteristic sequence element” refers to a sequence element found in a polymer (e.g., in a polypeptide or nucleic acid) that represents a characteristic portion of that polymer. In some embodiments, presence of a characteristic sequence element correlates with presence or level of a particular activity or property of the polymer. In some embodiments, presence (or absence) of a characteristic sequence element defines a particular polymer as a member (or not a member) of a particular family or group of such polymers. A characteristic sequence element typically comprises at least two monomers (e.g., amino acids or nucleotides). In some embodiments, a characteristic sequence element includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, or more monomers (e.g., contiguously linked monomers). In some embodiments, a characteristic sequence element includes at least first and second stretches of contiguous monomers spaced apart by one or more spacer regions whose length may or may not vary across polymers that share the sequence element. In certain embodiments, particular characteristic sequence elements may be referred to as “motifs”.

[0047] *Combination therapy:* As used herein, the term “combination therapy” refers to those situations in which a subject is simultaneously exposed to two or more therapeutic agents. In some embodiments, such agents are administered simultaneously; in some embodiments, such agents are administered sequentially; in some embodiments, such agents are administered in overlapping regimens.

[0048] *Comparable:* The term “comparable”, as used herein, refers to two or more agents, entities, situations, sets of conditions, etc that may not be identical to one another but that

are sufficiently similar to permit comparison therebetween so that conclusions may reasonably be drawn based on differences or similarities observed. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc to be considered comparable.

[0049] *Computer-readable medium:* The term “computer-readable medium”, as used herein, refers to non-volatile (i.e. secondary storage) computer data storage and/or memory to retain digital data even when not powered. Examples of computer-readable medium include, but are not limited to hard disk, floppy disk, flash memory(i.e. solid state memory), Ferroelectric RAM (F-RAM), Magnetoresistive RAM (MRAM), optical disc, standalone RAM disks, ZIP drives, magnetic tape and holographic memory.

[0050] *Computer system:* The term “computer system” or “computer”, as used herein, refers to a computing device that can be used to implement the techniques described in this disclosure. An exemplary computing device 2500 and a mobile computing device are shown in FIG. 24.

[0051] *Corresponding to:* As used herein, the term “corresponding to” is often used to designate the position/identity of a residue in a polymer, such as an amino acid residue in a polypeptide or a nucleotide residue in a nucleic acid. Those of ordinary skill will appreciate that, for purposes of simplicity, residues in such a polymer are often designated using a canonical numbering system based on a reference related polymer, so that a residue in a first polymer “corresponding to” a residue at position 190 in the reference polymer, for example, need not actually be the 190th residue in the first polymer but rather corresponds to the residue found at the 190th position in the reference polymer; those of ordinary skill in the art readily appreciate how to identify “corresponding” amino acids, including through use of one or more commercially-available algorithms specifically designed for polymer sequence comparisons.

[0052] *Crystal structure:* As used herein, the term “crystal structure” of a composition shall mean a computer readable medium in which is stored a representation of three dimensional positional information (i.e. coordinates) for atoms of the composition.

[0053] *Derivative:* As used herein, the term “derivative” refers to a structural analogue of a reference substance. That is, a “derivative” is a substance that shows significant structural similarity with the reference substance, for example sharing a core or consensus structure, but also differs in certain discrete ways. In some embodiments, a derivative is a substance that can be generated from the reference substance by chemical manipulation. In some embodiments, a derivative is a substance that can be generated through performance of a synthetic process substantially similar to (e.g., sharing a plurality of steps with) one that generates the reference substance.

[0054] *Docking:* As used herein, the term “docking” refers to orienting, rotating, translating a chemical entity in the binding pocket, domain, molecule or molecular complex or portion thereof based on distance geometry or energy. Docking may be performed by distance geometry methods that find sets of atoms of a chemical entity that match sets of sphere centers of the binding pocket, domain, molecule or molecular complex or portion thereof. See Meng et al. J. Comp. Chem. 4: 505-524 (1992). Sphere centers are generated by providing an extra radius of given length from the atoms (excluding hydrogen atoms) in the binding pocket, domain, molecule or molecular complex or portion thereof. Real-time interaction energy calculations, energy minimizations or rigid-body minimizations (Gschwend et al., J. Mol. Recognition 9:175-186 (1996)) can be performed while orienting the chemical entity to facilitate docking. For example, interactive docking experiments can be designed to follow the path of least resistance. If the user in an interactive docking experiment makes a move to increase the energy, the system will resist that move. However, if that user makes a move to decrease energy, the system will favor that move by increased responsiveness. (Cohen et al., J. Med. Chem. 33:889-894 (1990)). Docking can also be performed by combining a Monte Carlo search technique with rapid energy evaluation using molecular affinity potentials. See Goodsell and Olson, Proteins: Structure, Function and Genetics 8:195-202 (1990). Software programs that carry out docking functions include but are not limited to MATCHMOL (Cory et al., J. Mol. Graphics 2: 39 (1984); MOLFIT (Redington, Comput. Chem. 16: 217 (1992)) and DOCK (Meng et al., supra).

[0055] *Dosage form:* As used herein, the term “dosage form” refers to a physically discrete unit of a therapeutic agent for administration to a subject. Each unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen).

[0056] *Designed:* As used herein, the term “designed” refers to an agent (i) whose structure is or was selected by the hand of man; (ii) that is produced by a process requiring the hand of man; and/or (iii) that is distinct from natural substances and other known agents.

[0057] *Dosing regimen:* As used herein, the term “dosing regimen” refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (i.e., is a therapeutic dosing regimen).

[0058] *Engineered:* In general, the term “engineered” refers to the aspect of having been manipulated by the hand of man. For example, a polynucleotide is considered to be “engineered” when two or more sequences, that are not linked together in that order in nature, are manipulated by the hand of man to be directly linked to one another in the engineered polynucleotide. For example, in some embodiments of the present invention, an engineered polynucleotide comprises a regulatory sequence that is found in nature in operative association with a first coding sequence but not in operative association with a second coding sequence, is linked by the hand of man so that it is operatively associated with the second coding sequence. Comparably, a cell or organism is considered to be “engineered” if it has been manipulated so that its genetic information is altered (e.g., new genetic material not previously present has been

introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols). As is common practice and is understood by those in the art, progeny of an engineered polynucleotide or cell are typically still referred to as “engineered” even though the actual manipulation was performed on a prior entity.

[0059] *Fragment:* A “fragment” of a material or entity as described herein has a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a polymer fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (e.g., residues) as found in the whole polymer. In some embodiments, a polymer fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of the monomeric units (e.g., residues) found in the whole polymer. The whole material or entity may in some embodiments be referred to as the “parent” of the whole.

[0060] *Homology:* As used herein, the term “homology” refers to the overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% similar (e.g., containing residues with related chemical properties at corresponding positions). For example, as is well known by those of ordinary skill

in the art, certain amino acids are typically classified as similar to one another as “hydrophobic” or “hydrophilic” amino acids, and/or as having “polar” or “non-polar” side chains. Substitution of one amino acid for another of the same type may often be considered a “homologous” substitution. Typical amino acid categorizations are summarized below:

<u>Alanine</u>	Ala	A	nonpolar	neutral	1.8
<u>Arginine</u>	Arg	R	polar	positive	-4.5
<u>Asparagine</u>	Asn	N	polar	neutral	-3.5
<u>Aspartic acid</u>	Asp	D	polar	negative	-3.5
<u>Cysteine</u>	Cys	C	nonpolar	neutral	2.5
<u>Glutamic acid</u>	Glu	E	polar	negative	-3.5
<u>Glutamine</u>	Gln	Q	polar	neutral	-3.5
<u>Glycine</u>	Gly	G	nonpolar	neutral	-0.4
<u>Histidine</u>	His	H	polar	positive	-3.2
<u>Isoleucine</u>	Ile	I	nonpolar	neutral	4.5
<u>Leucine</u>	Leu	L	nonpolar	neutral	3.8
<u>Lysine</u>	Lys	K	polar	positive	-3.9
<u>Methionine</u>	Met	M	nonpolar	neutral	1.9
<u>Phenylalanine</u>	Phe	F	nonpolar	neutral	2.8
<u>Proline</u>	Pro	P	nonpolar	neutral	-1.6
<u>Serine</u>	Ser	S	polar	neutral	-0.8
<u>Threonine</u>	Thr	T	polar	neutral	-0.7
<u>Tryptophan</u>	Trp	W	nonpolar	neutral	-0.9

<u>Tyrosine</u>	Tyr	Y	polar	neutral	-1.3
<u>Valine</u>	Val	V	nonpolar	neutral	4.2

Ambiguous Amino Acids	3-Letter	1-Letter
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z
Leucine or Isoleucine	Xle	J
Unspecified or unknown amino acid	Xaa	X

[0061] As will be understood by those skilled in the art, a variety of algorithms are available that permit comparison of sequences in order to determine their degree of homology, including by permitting gaps of designated length in one sequence relative to another when considering which residues “correspond” to one another in different sequences. Calculation of the percent homology between two nucleic acid sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-corresponding sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position; when a position in the first sequence is occupied by a similar nucleotide as the corresponding position in the second sequence, then the molecules are similar at that position. The percent homology between the two sequences is a function of the number of identical and similar positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences.

Representative algorithms and computer programs useful in determining the percent homology between two nucleotide sequences include, for example, the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent homology between two nucleotide sequences can, alternatively, be determined for example using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0062] *Homology model:* As used herein, the term “homology model” refers to a set of coordinates derived from known three-dimensional structure used as template. Generation of the homology model, termed “homology modeling”, involves sequence alignment, residue replacement and residue conformation adjustment through energy minimization.

[0063] *Human:* In some embodiments, a human is an embryo, a fetus, an infant, a child, a teenager, an adult, or a senior citizen.

[0064] *Hydrophilic:* As used herein, the term “hydrophilic” and/or “polar” refers to a tendency to mix with, or dissolve easily in, water.

[0065] *Hydrophobic:* As used herein, the term “hydrophobic” and/or “non-polar”, refers to a tendency to repel, *not* combine with, or an inability to dissolve easily in, water.

[0066] *Identity:* As used herein, the term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “substantially identical” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. As will be understood by those skilled in the art, a variety of algorithms are available that permit comparison of sequences in order to determine their degree of homology, including by permitting gaps of designated length in one sequence relative to another when considering which residues “correspond” to one another in different sequences. Calculation of the percent identity between two nucleic acid sequences, for example, can be performed by

aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-corresponding sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. Representative algorithms and computer programs useful in determining the percent identity between two nucleotide sequences include, for example, the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined for example using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0067] *Inhibition model:* As used herein, the term “inhibition model” refers to a region or regions of proteins that can associate with another chemical entity or compound. Such regions are of significant utility in fields such as drug discovery. These regions are formed by amino acid residues key for ligand binding or may be residues that are spatially related and define a three-dimensional shape of the binding pocket. In some embodiments, the amino acid residues may be contiguous or non-contiguous in primary sequence. In some embodiments, the region or regions may be embodied as a dataset (e.g. an array of structure coordinates) recorded on computer readable media.

[0068] *Isolated:* As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2)

designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered “isolated” or even “pure”, after having been combined with certain other components such as, for example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. In some embodiments, isolation involves or requires disruption of covalent bonds (e.g., to isolate a polypeptide domain from a longer polypeptide and/or to isolate a nucleotide sequence element from a longer oligonucleotide or nucleic acid).

[0069] *Modulator:* The term “modulator” is used to refer to an entity whose presence in a system in which an activity of interest is observed correlates with a change in level and/or nature of that activity as compared with that observed under otherwise comparable conditions when the modulator is absent. In some embodiments, a modulator is an activator, in that activity is increased in its presence as compared with that observed under otherwise comparable conditions when the modulator is absent. In some embodiments, a modulator is an inhibitor, in that activity is reduced in its presence as compared with otherwise comparable conditions when the modulator is absent. In some embodiments, a modulator interacts directly with a target entity whose activity is of interest. In some embodiments, a modulator interacts indirectly (i.e., directly with an intermediate agent that interacts with the target entity) with a target entity whose activity is of interest. In some embodiments, a modulator affects level of a target entity of interest; alternatively or additionally, in some embodiments, a modulator affects activity of a target entity of interest without affecting level of the target entity. In some embodiments, a modulator affects

both level and activity of a target entity of interest, so that an observed difference in activity is not entirely explained by or commensurate with an observed difference in level.

[0070] *Nucleic acid:* As used herein, the term “nucleic acid,” in its broadest sense, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some embodiments, “nucleic acid” refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides); in some embodiments, “nucleic acid” refers to an oligonucleotide chain comprising individual nucleic acid residues. In some embodiments, a “nucleic acid” is or comprises RNA; in some embodiments, a “nucleic acid” is or comprises DNA. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a nucleic acid analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. For example, in some embodiments, a nucleic acid is, comprises, or consists of one or more “peptide nucleic acids”, which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. Alternatively or additionally, in some embodiments, a nucleic acid has one or more phosphorothioate and/or 5’-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine). In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a nucleic acid comprises one or more modified

sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a nucleic acid includes one or more introns. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long.

[0071] *Patient:* As used herein, the term “patient” or “subject” refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate) to whom therapy is administered. In many embodiments, a patient is a human being. In some embodiments, a patient is a human presenting to a medical provider for diagnosis or treatment of a disease, disorder or condition. In some embodiments, a patient displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a patient does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a patient is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition.

[0072] *Pharmaceutically acceptable:* The term “pharmaceutically acceptable” as used herein, refers to agents that, within the scope of sound medical judgment, are suitable for use in contact with tissues of human beings and/or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0073] *Polypeptide:* The term “polypeptide”, as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids, linked to one another by peptide bonds. In some embodiments, the term is used to refer to specific functional classes of polypeptides, such as, for example, autoantigen polypeptides, nicotinic acetylcholine receptor

polypeptides, alloantigen polypeptides, etc. For each such class, the present specification provides several examples of amino acid sequences of known exemplary polypeptides within the class; in some embodiments, such known polypeptides are reference polypeptides for the class. In such embodiments, the term “polypeptide” refers to any member of the class that shows significant sequence homology or identity with a relevant reference polypeptide. In many embodiments, such member also shares significant activity with the reference polypeptide. For example, in some embodiments, a member polypeptide shows an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40%, and is often greater than about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (i.e., a conserved region, often including a characteristic sequence element) that shows very high sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and often up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. In some embodiments, a useful polypeptide as described herein may comprise or consist of a fragment of a parent polypeptide. In some embodiments, a useful polypeptide as described herein may comprise or consist of a plurality of fragments, each of which is found in the same parent polypeptide in a different spatial arrangement relative to one another than is found in the polypeptide of interest (e.g., fragments that are directly linked in the parent may be spatially separated in the polypeptide of interest or vice versa, and/or fragments may be present in a different order in the polypeptide of interest than in the parent), so that the polypeptide of interest is a derivative of its parent polypeptide.

[0074] *Protein:* As used herein, the term “protein” refers to a polypeptide (i.e., a string of at least two amino acids linked to one another by peptide bonds). Proteins may include moieties other than amino acids (e.g., may be glycoproteins, proteoglycans, etc.) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a “protein” can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a characteristic portion thereof. Those of ordinary skill will appreciate that

a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term “peptide” is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[0075] *Reference:* The term “reference” is often used herein to describe a standard or control agent or value against which an agent or value of interest is compared. In some embodiments, a reference agent is tested and/or a reference value is determined substantially simultaneously with the testing or determination of the agent or value of interest. In some embodiments, a reference agent or value is a historical reference, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference agent or value is determined or characterized under conditions comparable to those utilized to determine or characterize the agent or value of interest.

[0076] *Small molecule:* As used herein, the term “small molecule” means a low molecular weight organic compound that may serve as an enzyme substrate or regulator of biological processes. In general, a “small molecule” is a molecule that is less than about 5 kilodaltons (kD) in size. In some embodiments, provided nanoparticles further include one or more small molecules. In some embodiments, the small molecule is less than about 4 kD, 3 kD, about 2 kD, or about 1 kD. In some embodiments, the small molecule is less than about 800 daltons (D), about 600 D, about 500 D, about 400 D, about 300 D, about 200 D, or about 100 D. In some embodiments, a small molecule is less than about 2000 g/mol, less than about 1500 g/mol, less than about 1000 g/mol, less than about 800 g/mol, or less than about 500 g/mol. In some embodiments, one or more small molecules are encapsulated within the nanoparticle. In

some embodiments, small molecules are non-polymeric. In some embodiments, in accordance with the present invention, small molecules are not proteins, polypeptides, oligopeptides, peptides, polynucleotides, oligonucleotides, polysaccharides, glycoproteins, proteoglycans, *etc.* In some embodiments, a small molecule is a therapeutic. In some embodiments, a small molecule is an adjuvant. In some embodiments, a small molecule is a drug.

[0077] *Storage environment:* As used herein, the term “storage environment” comprises any environment comprising secondary storage, i.e. long-term persistent storage. In some embodiments, a storage environment comprises computer-readable medium. In some embodiments, a storage environment comprises a network environment for establishing a multi-channel context aware communication environment (i.e. cloud computing). For example, FIG. 25 is a block diagram of a network environment for establishing a multi-channel context aware communication environment.

[0078] *Subject:* As used herein, the term “subject” refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre and post natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0079] *Substantially:* As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0080] *Suffering from:* An individual who is “suffering from” a disease, disorder, or condition has been diagnosed with and/or exhibits or has exhibited one or more symptoms or characteristics of the disease, disorder, or condition.

[0081] *Susceptible to:* An individual who is “susceptible to” a disease, disorder, or condition is at risk for developing the disease, disorder, or condition. In some embodiments, an individual who is susceptible to a disease, disorder, or condition does not display any symptoms of the disease, disorder, or condition. In some embodiments, an individual who is susceptible to a disease, disorder, or condition has not been diagnosed with the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, or condition is an individual who has been exposed to conditions associated with development of the disease, disorder, or condition. In some embodiments, a risk of developing a disease, disorder, and/or condition is a population-based risk (e.g., family members of individuals suffering from allergy, etc.).

[0082] *Symptoms are reduced:* According to the present invention, “symptoms are reduced” when one or more symptoms of a particular disease, disorder or condition is reduced in magnitude (e.g., intensity, severity, etc.) and/or frequency. For purposes of clarity, a delay in the onset of a particular symptom is considered one form of reducing the frequency of that symptom.

[0083] *Therapeutic agent:* As used herein, the phrase “therapeutic agent” refers to any agent that has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect, when administered to a subject. In some embodiments, an agent is considered to be a therapeutic agent if its administration to a relevant population is statistically correlated with a desired or beneficial therapeutic outcome in the population, whether or not a particular subject to whom the agent is administered experiences the desired or beneficial therapeutic outcome.

[0084] *Therapeutically effective amount:* As used herein, the term “therapeutically effective amount” means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, and/or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, and/or condition (e.g., allergy). In some embodiments, a therapeutically effective amount is one that reduces the incidence and/or severity of, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that the term “therapeutically effective amount” does not in fact require successful treatment be achieved in a particular

individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. It is specifically understood that particular subjects may, in fact, be “refractory” to a “therapeutically effective amount.” To give but one example, a refractory subject may have a low bioavailability such that clinical efficacy is not obtainable. In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine, etc). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective agent may be formulated and/or administered in a single dose. In some embodiments, a therapeutically effective agent may be formulated and/or administered in a plurality of doses, for example, as part of a dosing regimen.

[0085] *Therapeutic regimen:* A “therapeutic regimen”, as that term is used herein, refers to a dosing regimen whose administration across a relevant population is correlated with a desired or beneficial therapeutic outcome.

[0086] *Treatment:* As used herein, the term “treatment” (also “treat” or “treating”) refers to any administration of a substance that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces frequency, incidence or severity of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

[0087] *Three dimensional representation:* As used herein, the term “three dimensional representation” refers to converting the lists of structure coordinates into structural models or graphical representation in three-dimensional space. In some embodiments, the three dimensional structure may be displayed or used to performing computer modeling or fitting operations. In some embodiments, the structure coordinates themselves, without the displayed model, may be used to perform computer-based modeling and fitting operations.

Detailed Description of Certain Embodiments

mTor

[0088] The mTOR pathway controls cell growth in response to energy, nutrients, growth factors and other environmental cues, and it figures prominently in cancer^{1,2}. Central to the pathway is the mammalian target of rapamycin (mTOR) protein that belongs to the phosphoinositide 3-kinase (PI3K)-related protein kinase (PIKK) family³.

[0089] mTOR assembles into two different complexes within cells with distinct inputs and downstream effects. mTOR Complex 1 (mTORC1) is defined by its RAPTOR subunit⁴⁻⁶, which is replaced by RICTOR in mTORC2^{6,7}. Both complexes also contain the requisite mLST8 subunit^{8,9}, but they differ in a number of other subunits that interact with RAPTOR or RICTOR1.

[0090] mTORC1 regulates cell growth by promoting translation, ribosome biogenesis and autophagy^{1,4,5}. Its activation requires nutrients and amino acids, which result in the RAPTOR-mediated recruitment of mTORC1 to lysosomes and late endosomes^{10,11}, and co-localization with its activator, the small GTPase RHEB^{12,13}. Proposed mechanisms of RHEB action include binding to and activating the mTOR kinase domain¹⁴, and the displacement of the mTORC1 inhibitor PRAS40 from RAPTOR^{15,16}. RHEB in turn is negatively-regulated by the GTPase-activating domain of the TSC2 tumor suppressor, which relays signals from multiple growth factor and stress pathways¹⁷. mTORC1 substrates include the eIF4E-binding protein 1 (4EBP1) and ribosomal S6 kinases (S6K), which control cap-dependent translation initiation and elongation, respectively¹⁸. Phosphorylation of 4EBP1 and S6K1 is dependent on their Tor

Signaling Sequence (TOS) motif^{19,20} that binds to RAPTOR, and which is also present in the negative regulator PRAS40^{21,22}. Table 7 presents representative sequences produced by engineered expression constructs for certain such associated mTOR proteins:

Table 7: Representative sequences produced by engineered expression constructs

Protein	Residues	Description	SEQ ID NO.
RAPTOR	1335 aa	Full-length NP_065812	SEQ ID NO: ()
FLAG-tagged RAPTOR	1343 aa	FLAG-tagged RAPTOR	SEQ ID NO: ()
mLST8	326 aa	Full-length NP_071767	SEQ ID NO: ()
FLAG-tagged mLST8	333 aa	FLAG-tagged mLST8	SEQ ID NO: ()
S6K1 ^{ki}	502 aa	Full-length NP_001258989	SEQ ID NO: ()
FLAG-tagged S6K1 ^{ki}	512 aa	FLAG-tagged S6K1 ^{ki}	SEQ ID NO: ()
S6K1 ^{ki/TOS-}	501 aa	mutated TOS motif	SEQ ID NO: ()
FLAG-tagged S6K1 ^{ki/TOS-}	512 aa	FLAG-tagged S6K1 ^{ki/TOS-}	SEQ ID NO: ()
4EBP1	118 aa	Full-length NP_004086	SEQ ID NO: ()
GST-tagged 4EBP1	348 aa	GST-tagged 4EBP1	SEQ ID NO: ()

[0091] mTORC2 responds primarily to growth factors, promoting cell-cycle entry, cell survival, actin cytoskeleton polarization, and anabolic output^{6,7,23}. Its substrates include the Ser/Thr protein kinases AKT, SGK and PKC, which share the hydrophobic motif (HM) phosphorylation site with S6K1^{1,2}.

mTOR Structure

[0092] mTOR has a characteristic multidomain structure comprising a kinase domain, FRB (FKBP12-Rapamycin Binding) domain, a putative auto-inhibitory domain (Repressor domain) near the C-terminus and up to 20 tandemly repeated HEAT motifs at the amino terminus, as well as FAT (FRAP-ATM-TRRAP) and FATC (FAT C-terminus) domains. mTOR proteins have been identified in a variety of species, and have been sequenced. Table 8 presents exemplary sequences of certain such mTOR proteins:

Table 8: Exemplary mTOR and related sequences

Protein	Species	Residues	Accession/Identifier	SEQ ID NO.
mTOR	Homo sapiens (Human)	2549 aa	GI:4826730 NP_004949	SEQ ID NO: ()
mTOR ^{ΔN}	Homo sapiens (Human)	1174 aa	Residues 1376-2549 of full-length mTOR (NP_004949)	SEQ ID NO: ()
FLAG-tagged mTOR ^{ΔN}	Homo sapiens (Human)	1204 aa	FLAG tag plus residues 1376-2549 of full-length mTOR	SEQ ID NO: ()
mTOR ^{ΔNΔ2443-2486}	Homo sapiens (Human)	1130 aa	Internally deleted mTOR ^{ΔN}	SEQ ID NO: ()
FLAG-tagged mTOR ^{ΔNΔ2443-2486}	Homo sapiens (Human)	1160 aa	FLAG-tagged mTOR ^{ΔN}	SEQ ID NO: ()
mTOR	Rattus norvegicus (Rat)	2549 aa	GI:9845251 NP_063971	SEQ ID NO: ()
mTOR	Mus musculus (Mouse)	2549 aa	GI:227330586 NP_064393	SEQ ID NO: ()
mTOR	Ovis aries (Sheep)	2550 aa	GI:224451145 NP_001138927	SEQ ID NO: ()
mTOR	Danio rerio (Zebrafish)	2515 aa	GI:118601079 NP_001070679	SEQ ID NO: ()
mTOR	Macaca mulatta (Rhesus macaque)	2549 aa	GI:383417207 AFH31817	SEQ ID NO: ()
mTOR	Capra hircus (Goat)	2549 aa	GI:297613711 ADI48287	SEQ ID NO: ()
mTOR, isoform B	Drosophila melanogaster (Fruit fly)	2471 aa	GI:442627689 NP_001260427	SEQ ID NO: ()
mTOR, isoform A	Drosophila melanogaster (Fruit fly)	2470 aa	GI:17864562 NP_524891	SEQ ID NO: ()
mTOR	Pan troglodytes (chimpanzee)	2549 aa	GI:410334665 JAA36279	SEQ ID NO: ()

[0093] Figures 11A, 12A and 23 present or include various sequence alignments illustrating conservation of certain sequence elements within mTOR proteins.

[0094] As can be seen, and as is known by those of ordinary skill in the art, various conserved sequence elements are found within mTOR protein domains, and in certain

embodiments can be considered to be “characteristic” of mTOR polypeptides. For example, Table 9 presents certain representative sequence elements that may be found in exemplary mTOR polypeptides.

Table 9: Representative sequences characteristic of mTOR polypeptides

Protein	Sequence Element	Residues	Description	SEQ ID NO.
mTOR	FKBP12-Rapamycin-Binding Domain (FRB)	97 aa	mTOR residues 2022-2118	SEQ ID NO: ()
mTOR	mLST8-binding element (LBE)	38 aa	mTOR residues 2259-2296	SEQ ID NO: ()
mTOR	kaAL helix in activation loop	11 aa	mTOR residues 2360-2370	SEQ ID NO: ()

[0095] As discussed herein, mTOR is generally considered to be a member of the PIKK kinase family, which is related to the PI3K kinase family. Figure 12 shows sequence alignments of certain mTOR polypeptides with one another and with certain mammalian PIKKs. The six mammalian PIKKs regulate diverse cellular processes²⁷. They share three regions of homology consisting of a ~600 residue FAT domain (FRAP, ATM, TRRAP), a ~300 residue PI3K-related protein kinase catalytic domain, and a ~35 residue FATC domain at the Cterminus²⁸. In mTOR, the art-accepted location of the ~100 residue FRB domain was thought to occur in-between the FAT and catalytic domains (i.e. the structure proposed and accepted in the art prior to the present disclosure has the FRB domain occurring *prior* to the kinase domain), and the region N-terminal to the FAT domain is required for binding to RAPTOR and RICTOR1. One aspect of the present invention is the surprising and unexpected result that, contrary to the art-proposed structure, the FRB occurs *within* the kinase domain.

[0096] In general, PI3K proteins also typically include kinase, ATP-binding, and catalytic loop and activation loop motifs. Table 10 includes various sequences found in PI3K and/or PIKK proteins.

Table 10: Representative sequences present in PI3K and/or PIKK families

Family	Sequence Element	Residues	Description	SEQ ID NO.
PI3K/PIKK	ATP-binding loop	9 aa	mTOR residues 2164 - 2172	SEQ ID NO: ()
PI3K/PIKK	Catalytic loop	15 aa	mTOR residues 2332 - 2346	SEQ ID NO: ()
PI3K/PIKK	Activation loop	23 aa	mTOR residues 2357 - 2379	SEQ ID NO: ()

[0097] In some embodiments, an mTOR polypeptide as described herein is one that shares an overall level of sequence identity and/or one or more characteristic sequence elements with a reference mTOR protein (e.g., as set forth in Table 8) or a corresponding fragment thereof. In some embodiments, an mTOR polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with a wild-type mTOR protein or a corresponding fragment thereof.

[0098] In some embodiments, an mTOR polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with an mTOR protein from any species, including but not limited to, *Homo sapiens* (NP_004949; SEQ ID NO: ()), *Rattus norvegicus* (NP_063971; SEQ ID NO: ()), *Mus musculus* (NP_064393; SEQ ID NO: ()), *Ovis aries* (NP_001138927; SEQ ID NO: ()), *Danio rerio* (NP_001070679; SEQ ID NO: ()), *Macaca mulatta* (AFH31817; SEQ ID NO: ()), *Capra hircus* (ADI48287; SEQ ID NO: ()), *Drosophila melanogaster* (NP_001260427; NP_524891, SEQ ID NOS: () and ()), *Pan troglodytes* (JAA36279; SEQ ID NO: ()) or a corresponding fragment thereof.

[0099] In some embodiments, an mTOR polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with truncated and/or internally deleted mTOR protein or a corresponding fragment thereof. In some embodiments, an mTOR polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with human mTOR^{ΔN} protein (SEQ ID NO: ()) or a corresponding fragment thereof, wherein the N-terminus has been deleted. In some embodiments, an mTOR

polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with mTOR^{ΔNΔ2443-2486} (SEQ ID NO: ()) or a corresponding fragment thereof, wherein the N-terminus has been deleted and residues 2443-2486 have been internally deleted.

[0100] In some embodiments, an mTOR polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with an mTOR protein that includes a sequence tag, including but not limited to FLAG and GST tags, or a corresponding fragment thereof. In some embodiments, an mTOR polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with FLAG-tagged mTOR^{ΔN} (SEQ ID NO: ()) or a corresponding fragment thereof. In some embodiments, an mTOR polypeptide shares an overall level of sequence identity and/or one or more characteristic sequence elements with FLAG-tagged mTOR^{ΔNΔ2443-2486} (SEQ ID NO: ()) or a corresponding fragment thereof.

[0101] Alternatively or additionally, in some embodiments, an mTOR polypeptide is one that shares an overall level of sequence identity and/or one or more characteristic sequence elements with a reference PI3K or PIKK protein (e.g., as set forth in Table 11), or a corresponding fragment thereof. In some embodiments, an mTOR polypeptide shares an overall degree s as described herein are shared between the mTOR polypeptide and one or more PI3K and/or PIKK kinases. In some embodiments, an mTOR polypeptide of interest is one that shares the relevant overall sequence identity and/or sequence element with a reference mTOR polypeptide but not with a reference PI3K or PIKK polypeptide.

Table 11: Representative PI3K and PIKK sequences

Gene	Protein	Aliases	Accession/Identifier	SEQ ID NO.
PIK3C2A	PI3K, class 2, alpha polypeptide	PI3K-C2α	GI:157671929 NP_002636	SEQ ID NO: ()
PIK3C2B	PI3K, class 2, beta polypeptide	PI3K-C2β	GI:194097347 NP_002637	SEQ ID NO: ()
PIK3C2G	PI3K, class 2, gamma polypeptide	PI3K-C2γ	GI:194353959 NP_004561	SEQ ID NO: ()
PIK3C3	PI3K, class 3	Vps34	GI:34761064	SEQ ID NO:

Gene	Protein	Aliases	Accession/Identifier	SEQ ID NO.
			NP_002638	()
PIK3CA	PI3K, catalytic, alpha polypeptide	p110- α	GI:54792082 NP_006209	SEQ ID NO: ()
PIK3CB	PI3K, catalytic, beta polypeptide	p110- β	GI:5453894 NP_006210	SEQ ID NO: ()
PIK3CG	PI3K, catalytic, gamma polypeptide	p110- γ	GI:21237725 NP_002640	SEQ ID NO: ()
PIK3CD	PI3K, catalytic, delta polypeptide	p110- δ	GI:156564405 NP_005017	SEQ ID NO: ()
PIK3R1	PI3K, regulatory subunit 1 (alpha)	p85- α	GI:32455248 NP_852664	SEQ ID NO: ()
PIK3R2	PI3K, regulatory subunit 2 (beta)	p85- β	GI:4826908 NP_005018	SEQ ID NO: ()
PIK3R3	PI3K, regulatory subunit 3 (gamma)	p55- γ	GI:166795245 NP_003620	SEQ ID NO: ()
PIK3R4	PI3K, regulatory subunit 4	p150	GI:23943912 NP_055417	SEQ ID NO: ()
PIK3R5	PI3K, regulatory subunit 5	p101	GI:217330622 NP_055123	SEQ ID NO: ()
PIK3R6	PI3K, regulatory subunit 6	p87	GI:58082081 NP_001010855	SEQ ID NO: ()
ATM	serine-protein kinase ATM	AT1; ATA; ATC; ATD; ATE; ATDC; TEL1; TELO1	GI:71902540 NP_000042	SEQ ID NO: ()
ATR	serine/threonine-protein kinase ATR	FCTCS, FRP1, MEC1, SCKL, SCKL1	GI:157266317 NP_001175	SEQ ID NO: ()
PRKDC	DNA-dependent protein kinase catalytic subunit isoform 1	DNA-PKcs, DNAPK, DNP1, HYRC, HYRC1, XRCC7, p350	GI:13654237 NP_008835	SEQ ID NO: ()
PRKDC	DNA-dependent protein kinase catalytic subunit	DNA-PKcs, DNAPK,	GI:126032350 NP_001075109	SEQ ID NO: ()

Gene	Protein	Aliases	Accession/Identifier	SEQ ID NO.
	isoform 2	DNPK1, HYRC, HYRC1, XRCC7, p350		
SMG1	serine/threonine-protein kinase SMG1	ATX; LIP; 61E3.4	GI:62243658 NP_055907	SEQ ID NO: ()
TRRAP	transformation/transcription domain-associated protein isoform 1	Tra1; TR-AP; PAF400; STAF40; PAF350/400	GI:347360922 NP_001231509	SEQ ID NO: ()
TRRAP	transformation/transcription domain-associated protein isoform 2	Tra1; TR-AP; PAF400; STAF40; PAF350/400	GI:4507691 NP_003487	SEQ ID NO: ()

[0102] In many embodiments, an mTOR polypeptide includes one or more HEAT sequence elements, which are repeat-like α -helical structures which mediate protein-protein interactions.

[0103] In some embodiments, an mTOR polypeptide of interest may show three-dimensional similarity with one or more reference mTOR polypeptides and/or PI3K and/or PIKK polypeptides, but may or may not show a particular level of sequence identity or homology overall, or with respect to the relevant portion showing the three-dimensional similarity. For example, the catalytic domain of mTOR (SEQ ID NO: ()) and PIK3C3 (SEQ ID NO: ()) only share 24% sequence identity, but share a high degree of three-dimensional similarity, including the arrangement of key active site residues, when superpositioned bound to the same ATP TS mimic (For example, see Example 1, FIG. 2C, FIG. 13C). In some embodiments, an mTOR polypeptide of interest shows three-dimensional similarity with one or more reference mTOR polypeptides with respect to at least one feature that is not shared with a reference PI3K and/or with a PIKK polypeptide; in some embodiments, the at least one feature is not shared with any known and/or wild type PI3K and/or PIKK polypeptide.

[0104] Among other things, the present invention demonstrates that superimposition of provided mTOR structures can identify and/or characterize key residues and/or side chains in unknown structures. For example, superimposition of provided mTOR structures with known structures of CDK2 (SEQ ID NO: (____)) reveals that the arrangement of key active site residues is conserved in mTOR (see FIG. 2C, FIG. 13C) including Asn2342 and Asp2357, which serve as ligands for the two metal ions and Lys 2187, which coordinates the ATP phosphate group. mTOR Asp2338, which is a key catalytic residue that helps orient and activate the substrate hydroxyl group for nucleophilic attack, superimposed with CDK2 Asp127. mTOR His2340, which interacts with both the substrate hydroxyl group and the γ -phosphate TS mimic to stabilize the buildup of charge at the transition state, superimposed with CDK2 lys129 side chains (see FIG. 2C).

[0105] As described herein, among other things, the present invention defines certain mTOR structural elements that interact with modulators of interest. For example, in accordance with the present invention, one or more moieties within the mTOR ATP binding domain may mediate interaction with modulators of interest. Alternatively or additionally, in some embodiments, moieties at or near the mTOR catalytic loop and/or activation loop may mediate interaction with such modulators. In some embodiments, in accordance with the present invention, mTOR polypeptides are provided that include moieties corresponding to those defined herein as mediating interaction with modulators of interest. To give but a few examples, in some embodiments, provided mTOR polypeptides include residues corresponding to mTOR residues within a relevant portion of the mTOR ATP binding domain, catalytic loop domain, activation loop domain, the kaAL helix in the activation loop domain, FKBP12-rapamycin-binding domain (FRB), mLST8-binding element (LBE) domain, and/or combinations thereof. In some embodiments, provided mTOR polypeptides include residues corresponding to mTOR residues SEQ ID NO: (____) 2022-2118, 2259-2296, 2360-2370, 2164 -2172, 2332 -2346, 2357 -2379, 1376-2549, 1376-2443, 2443-2549, 1-2549, and/or combinations thereof.

mTOR Funtion

[0106] It is generally believed that mTOR plays a role in the development and function of the immune system, chromosome maintenance and repair, cell-cycle control, and tumor suppression. mTOR has been reported to have a variety of biological activities including, for example, promoting cell growth (e.g. promoting translation, ribosome biogenesis, autophagy), responding to growth factors, promoting cell-cycle entry, cell survival, actin cytoskeleton polarization, and anabolic output. According to some reports, mTOR is thought to integrate the input from multiple upstream pathways, including insulin, growth factor, and amino acids. mTOR may be involved in sensing cellular nutrient, oxygen, and/or energy levels. mTOR is known to act via interaction with one or more binding partners. mTOR is the catalytic subunit of two multi-protein complexes, mTORC1 and mTORC2. It has been reported that mTORC1 and mTORC2 regulate processes that control cell growth and proliferation, including protein synthesis, autophagy, and metabolism. mTORC1 phosphorylates the translational regulators S6 Kinase 1 (S6K1) and the eIF-4E binding proteins (4EBP1 and 4EBP2) while mTORC2 activates Akt and serum/glucocorticoid regulated kinase 1 (SGK1) and is part of the growth factor-stimulated phosphoinositide-3-kinase (PI3K) pathway.

[0107] A variety of assays are known or have been developed that detect and/or characterize mTOR activities (Torai-Barza L, Zhang WG, Lamison C, Larocque J, Gibbons J, Yu K. *Biochem Biophys Res Commun*. 2005 Jun 24;332(1):304-10; Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. *Cell* 2002 Jul 26;110(2):163-75; Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. *Science*. 2005 Feb 18;307(5712):1098-101). In some embodiments, mTOR activity is or comprises interacting with a particular binding partner or partners. In some embodiments, mTOR activity is or comprises discriminating between potential partners. In some embodiments, mTOR activity is or comprises mTOR1 activity. In some embodiments, mTOR activity is or comprises mTOR2 activity.

[0108] In some embodiments, mTOR activity is or comprises phosphorylation of a particular substrate or substrates. Despite the diverse process controlled by mTOR, few substrates are known. Known mTOR substrates include the translational regulators S6 Kinase 1

(S6K1), the eIF-4E binding proteins (4EBP1 and 4EBP2), Akt (Protein Kinase B; PKB) and serum/glucocorticoid regulated kinase 1 (SGK1).

[0109] In some embodiments, an mTOR polypeptide shares at least one of these activities with a reference mTOR polypeptide, such as a wild type mTOR. In some embodiments, an mTOR polypeptide is considered to “share” an activity with a reference if it shows an activity that would be recognized by those skilled in the art as comparable to, or not significantly different from, that of a relevant reference in an appropriate assay that detects and/or characterizes the activity. While the kinase activity of an mTOR polypeptide compared to a reference may be significantly lower, it can be recognized by its ability to phosphorylate mTOR-specific sites in bona-fide mTOR substrates. One example of such site is the S6 Kinase 1 Thr389 site, which is not known to be phosphorylated substantially by any other kinase.

mTOR Crystal Structure

[0110] Among other things, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide. In some embodiments, such a provided composition consists of or consists essentially of the mTOR polypeptide. In some embodiments, a composition is considered to “consist of” mTOR polypeptide if it includes only the polypeptide, one or more solvents, and optionally salts and/or metals. In some embodiments, such a provided composition includes one or more other agents such as one or more other polypeptides (e.g., one or more potential or actual mTOR binding partner polypeptides) and/or one or more interacting agents (e.g., small molecules).

[0111] In some embodiments, such a provided composition comprises a wild-type mTOR polypeptide. Exemplary wild-type mTOR polypeptides include, but are not limited to, polypeptides listed in Table 8. In some embodiments, such a provided composition comprises an mTOR polypeptide comprising an N-terminal truncation (mTOR^{ΔN}). In some embodiments, an mTOR polypeptide comprises one or more internal deletions. In some embodiments, an mTOR polypeptide comprises one or more internal deletions comprising residues 2443-2486

(mTOR^{Δ2443-2486}). In some embodiments, an mTOR polypeptide comprises both an N-terminal truncation *and* one or more internal deletions. In some embodiments, an mTOR polypeptide comprises both an N-terminal truncation *and* one or more internal deletions comprising residues 2443-2486 (mTOR^{ΔNΔ2443-2486}).

[0112] In some embodiments, such a provided composition comprises an mTOR polypeptide bound to one or more binding partners. In some embodiments, such a provided composition comprises an mTOR polypeptide bound to mLST8 (mTOR-mLST8 complex). In some embodiments, provided composition comprises an N-terminally truncated mTOR polypeptide bound to mLST8 (mTOR^{ΔN}-mLST8 complex). In some embodiments, provided composition comprises an N-terminally truncated and internally deleted mTOR polypeptide bound to mLST8 (mTOR^{ΔNΔ2443-2486}-mLST8 complex).

[0113] In some embodiments, such a provided composition comprises an mTOR polypeptide bound to two or more binding partners. In some embodiments, such a provided composition comprise an mTOR polypeptide bound to mLST8 and RAPTOR (mTOR-mLST8-RAPTOR complex). In some embodiments, provided composition comprises an N-terminally truncated mTOR polypeptide bound to mLST8 and to RAPTOR (mTOR^{ΔN}-mLST8-RAPTOR complex). In some embodiments, provided composition comprises an N-terminally truncated and internally deleted mTOR polypeptide bound to mLST8 and to RAPTOR (mTOR^{ΔN Δ2443-2486}-mLST8-RAPTOR complex).

[0114] In some embodiments, such a provided composition comprises an mTOR polypeptide bound to one or more interacting agents (e.g., small molecules). In some embodiments, interacting agents comprise mimics of the γ -phosphate group of ATP in the transition state. In some embodiments, a γ -phosphate group mimic comprise ADP, Mg²⁺ or MgF₃⁻, and/or combinations thereof. In some embodiments, such a provided composition comprise an mTOR polypeptide bound to ADP. In some embodiments, such a provided composition comprise an mTOR polypeptide bound to Mg²⁺. In some embodiments, such a provided composition comprise an mTOR polypeptide bound to MgF₃⁻.

[0115] In some embodiments, such a provided composition comprises an mTOR polypeptide bound to one or more mTOR modulators. In some embodiments, provided composition comprises an mTOR polypeptide bound to one or more exemplary mTOR modulators listed in Table 12. In some embodiments, provided composition includes, but it not limited to, an mTOR modulator consisting of the group rapamycin, PI 103 HCL, AZD8055, AZD2014, CH5132799, XL765 (SAR245409), GDC-0980 (RG7422), GSK1059615, GSK2126458, Torin1, Torin2, INK128, KU0063794, BEZ235 (NVP-BEZ235), NVP-BGT226, OSI-027, Palomid 529, PF-05212384 (PKI-587), PKI-179, WAY-600, WYE-125132 (WYE-132), WYE-23, WYE-28, WYE-354, WYE-687, PF-04691502, PP-121, PP242, PP30 and/or combinations therein.

[0116] In some embodiments, such a provided composition comprises an mTOR polypeptide bound to one or more binding partners and bound to one or more interacting agents (e.g., small molecules). In some embodiments, provided composition comprise an mTOR-mLST8 complex bound to an interacting agent. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to an interacting agent. In some embodiments, provided composition comprise an mTOR^{ΔNΔ2443-2486}-mLST8 complex bound to an interacting agent. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to a mimic of the γ-phosphate group of ATP in the transition state. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8-ADP complex. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8- Mg²⁺ complex. In some embodiments, provided composition comprises mTOR^{ΔN}-mLST8- MgF₃⁻.

[0117] In some embodiments, such a provided composition comprises an mTOR polypeptide bound to one or more binding partners and bound to one or more mTOR modulators (e.g. exemplary modulators listed in Table 12). In some embodiments, provided composition comprise an mTOR-mLST8 complex bound to an mTOR modulator. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to an mTOR modulator. In some embodiments, provided composition comprise an mTOR^{ΔNΔ2443-2486}-mLST8 complex bound to an mTOR modulator. In some embodiments, provided composition comprise an

mTOR^{ΔN}-mLST8 complex bound to Torin2. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to PP242. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to PI 103.

[0118] The present invention also provides structural information and/or analyses of mTOR polypeptide crystals and/or sets thereof. In some embodiments, such structural information includes, but is not limited to, diffraction patterns, and/or coordinates, as well as any data sets, images, models, and/or graphical representations thereof or generated therefrom. In some embodiments, such graphical representations may include, for example, space-filling models, molecular surface representations, shell or boundary models, ribbon models, stick models; and/or combinations thereof.

[0119] In some embodiments, provided information is or comprises differences observed between or among structures that differ from one another in the presence or absence of one or more binding partners and/or interacting agents. In some embodiments, provided information is or comprises differences observed between or among structures that differ from one another in the presence or absence of one or more binding partners and/or one or more modulators.

[0120] In some embodiments, such structural information and/or analyses may be embodied in a tangible medium (e.g., a computer-readable medium) or a storage environment. Thus, the present invention provides tangible embodiments of mTOR polypeptide crystal structure information, as well as its use, for example, by or with a computer system, in any of a variety of applications. For example, in some embodiments, such structural information and/or analyses may be accessed by, transported to or from, and/or otherwise utilized by a computer system or program running thereon.

[0121] Among other things, the present disclosure embodies a recognition of the source of a problem with providing mTOR polypeptide crystals. mTOR has long known to be a protein of significant biological and pharmacological interest. Significant efforts have been made to obtain mTOR crystals, among other things in order to permit or inform effective drug discovery efforts. However, prior to the present disclosure, useful mTOR crystals have not been obtained, and crystal structure information has not been provided. Those interested in considering mTOR

protein structure, for example in design and/or development of candidate mTOR modulators, have often been relegated to consulting crystal structures of related kinases such as PI3K kinases (there are no defined PIKK kinase structures; one report of DNAPK electron density at 6.6 Å resolution, which is not high enough resolution to define a structure). Commonly employed PI3K kinase structures include PI3K gamma (Liu Q, Wang J, Kang SA, Thoreen CC, Hur W, Ahmed T, Sabatini DM, Gray NS. *J Med Chem.* 2011 Mar 10;54(5):1473-80; Finlay MR, Buttar D, Critchlow SE, Dishington AP, Fillery SM, Fisher E, Glossop SC, Graham MA, Johnson T, Lamont GM, Mutton S, Perkins P, Pike KG, Slater AM. *Bioorg Med Chem Lett.* 2012 Jun 15;22(12):4163-8; Xie H, Lee MH, Zhu F, Reddy K, Huang Z, Kim DJ, Li Y, Peng C, Lim DY, Kang S, Jung SK, Li X, Li H, Ma W, Lubet RA, Ding J, Bode AM, Dong Z. *Mol Cancer Ther.* 2013 Mar 27; Liu Q, Kang SA, Thoreen CC, Hur W, Wang J, Chang JW, Markhard A, Zhang J, Sim T, Sabatini DM, Gray NS. *Methods Mol Biol.* 2012;821:447-60.; Tsou HR, MacEwan G, Birnberg G, Grosu G, Bursavich MG, Bard J, Brooijmans N, Toral-Barza L, Hollander I, Mansour TS, Ayril-Kaloustian S, Yu K. *Bioorg Med Chem Lett.* 2010 Apr 1;20(7):2321-5.).

[0122] As described herein, the present disclosure recognizes that one problem with prior efforts to provide mTOR polypeptide crystals is that the utilized mTOR polypeptides were not amenable to crystallization. Among other things, the present invention provides crystallizable mTOR polypeptides, systems for producing them, methods of utilizing them, and crystals thereof.

[0123] For example, the present invention provides truncated and/or internally deleted mTOR polypeptides that are amenable to crystallization. In some embodiments, the present invention provides an mTOR polypeptide, wherein the N-terminus has been deleted. In some embodiments, provided mTOR polypeptides comprise both an N-terminus deletion *and* an internal deletion. In some embodiments, provided mTOR polypeptides comprise both an N-terminus deletion *and* an internal deletion of residues 2443-2486. Without wishing to be bound by any particular theory, truncation and/or internal deletion of the mTOR polypeptide favors crystallization because it can reduce flexibility, can result in more compact shape, and eliminate

undesirable events like aggregation, if for example, the deleted region has a hydrophobic binding site for a protein-binding partner.

[0124] The present disclosure also recognizes that one problem associated with prior efforts to provide mTOR polypeptide crystals is that the utilized expression systems often did not effectively produce crystallizable mTOR polypeptides. Preparing protein crystals appropriate for X-ray diffraction usually requires a considerable amount of highly purified protein. Polypeptides for crystallization are often expressed in well-established microbial systems such as *Escherichia coli*. Polypeptide expression in microbial systems is well-established, fast, simple and usually provides high yields. However, microbial systems lack the chaperones required for folding complex eukaryotic proteins are not able to perform specific eukaryotic post-translational modifications. Alternatively, in some cases polypeptide expression in insect baculovirus systems is used. Insect baculovirus systems are of an evolutionary higher order, and have fewer problems associated with producing the protein in its native state, however expressing large amounts of material in an easy-to-purify form becomes more difficult.

[0125] The present invention encompasses the recognition that neither microbial or baculovirus expression systems may be optimal for expression of mTOR polypeptides, and particularly for expression of crystallizable mTOR polypeptides.

[0126] Among other things, the present invention provides mammalian cells, and particularly human cells, engineered to express an mTOR polypeptide. In some embodiments, provided mammalian cells are human HEK293-F cells.

[0127] In some embodiments, crystallizable or crystallized mTOR polypeptides provided by the present invention are recombinant polypeptides in that they are produced by cells engineered to express them.

[0128] In some embodiments, crystallizable or crystallized mTOR polypeptides provided by the present invention are purified recombinant polypeptides.

[0129] mTOR polypeptide crystals, for example as provided by the present invention, reveal various interesting attributes of the mTOR polypeptide and/or its interactions with other

agents. Provided information includes, for example, the insight that mTOR-mLST8 complex is intrinsically active in the absence of additional regulatory subunits and that PI3Ks employ the same catalytic mechanism as canonical protein kinases, in contrast to art-accepted mechanisms³².

[0130] The present invention surprisingly demonstrates that crystals can be obtained of mTOR polypeptides that diffract with a resolution *higher than* about 4 Å. The present invention provides such crystals. In some embodiments, for example, the present invention provides mTOR polypeptide crystals that diffract to a resolution within about 3.0 to about 3.5 Å. In some embodiments, the present invention provides mTOR polypeptide crystals that diffract to a resolution higher than about 3.5 Å, about 3.4 Å, about 3.3 Å, about 3.2 Å, about 3.1 Å, about 3.0 Å, or higher.

Structure-Based Drug Design

[0131] The mTOR kinase is a master regulator of cell growth, and is the focus of anticancer drug discovery efforts. Findings described herein permit design of modulators with specified characteristics that can interact with mTOR polypeptides. For example, the information provided by mTOR crystal structures described herein permits molecular modeling and/or design of potential binding agents that can interact with sites of interest within an mTOR polypeptide.

[0132] Among other things, the present disclosure identifies the source of a problem with prior efforts to identify, characterize, and/or develop mTOR modulators. Currently FDA-approved mTOR inhibitors are based on the natural compound rapamycin, which was thought to be an allosteric inhibitor. The present invention provides, among other things, the identification of a source of a problem with understanding of rapamycin, its mechanism, and its effectiveness as an mTOR inhibitors. As described herein, we have demonstrated that rapamycin blocks a substrate-binding site on the mTOR kinase.

[0133] Rapamycin, which forms a ternary complex with the FK506-binding protein12 (FKBP12) and the FRB (FKBP12-Rapamycin-Binding) domain of mTOR, has been thought to be an allosteric inhibitor^{24,25}. Rapamycin-FKBP12 inhibits mTORC1 to a variable extent that is substrate and phosphorylation-site dependent²⁵, and it does not bind to mTORC2²³. To overcome these limitations, ATP-competitive inhibitors that potently and uniformly inhibit both mTORC1 and mTORC2 are being developed as anti-cancer agents²⁶.

[0134] Several groups have programs to develop mTOR inhibitors that target the ATP-binding site of the kinase, and many of these are in clinical trials. The present invention encompasses the recognition that such discovery efforts could be materially enhanced if a crystal structure for mTOR, or even for one or more mTOR-related kinases were available. As noted above, mTOR belongs to the PIKK (Phosphatidyl-Inositol 3 Kinase (PI3K)-like protein Kinase) family of kinases; no structures are available for kinases in this family. The closest kinase of a known structure is the phosphatidyl-inositol 3 kinase (PI3K) itself, but it has not been a good model for understanding the potency and specificity of mTOR inhibitors.

[0135] Unfortunately, many available mTOR inhibitors have been found to have limited clinical efficacy. Without wishing to be bound by any particular theory, the present invention suggests that such limited clinical efficacy may result from incomplete and/or substrate-dependent inhibition.

[0136] The present invention recognizes that, in many embodiments, effective mTOR modulators interact with at least one other site within an mTOR complex in addition to the mTOR ATP-binding site.

[0137] In some embodiments, the present disclosure provides systems for identifying and/or characterizing mTOR inhibitors.

[0138] Representative known mTOR modulators are presented in Table 12; the present disclosure does not encompass such known mTOR modulating agents. However, the present invention does encompass use of agents whose structure or identity was previously known (and was known to be within the scope of modulator structure(s) as defined herein), but whose activity modulating mTOR was not previously known.

Computer Systems

[0139] As will be appreciated by those skilled in the art, reading the present disclosure, in some aspects, the present invention is ideally suited for use in computer-implemented inventions. FIG. 24 shows but one example of a computing device 2500 and a mobile computing device 2550 that can be used to implement certain techniques described in this disclosure. The computing device 2500 depicted in FIG. 24 is intended to represent any of a variety of forms of digital computers, such as laptops, desktops, workstations, personal digital assistants, servers, blade servers, mainframes, and other appropriate computers. The depicted mobile computing device 2550 is intended to represent any appropriate form of mobile devices, such as personal digital assistants, cellular telephones, smart-phones, tablet computers, and other similar computing devices. Moreover, the components shown in FIG. 24 and elsewhere in the Figures, their connections and relationships, and their functions, are meant to be examples only, and are not meant to be limiting.

[0140] The computing device depicted in FIG. 24 2500 includes a processor 2502, a memory 2504, a storage device 2506, a high-speed interface 2508 connecting to the memory 2504 and multiple high-speed expansion ports 2510, and a low-speed interface 2512 connecting to a low-speed expansion port 2514 and the storage device 2506. Each of the processor 2502, the memory 2504, the storage device 2506, the high-speed interface 2508, the high-speed expansion ports 2510, and the low-speed interface 2512, are interconnected using various busses, and may be mounted on a common motherboard or in other manners as appropriate. The processor 2502 can process instructions for execution within the computing device 2500, including instructions stored in the memory 2504 or on the storage device 2506 to display graphical information for a GUI on an external input/output device, such as a display 2516 coupled to the high-speed interface 2508. In some implementations, multiple processors and/or multiple buses may be used, as appropriate, along with multiple memories and types of memory. Also, multiple computing devices may be connected, with each device providing portions of the necessary operations (e.g., as a server bank, a group of blade servers, or a multi-processor system).

[0141] The memory 2504 depicted in FIG. 24 stores information within the computing device 2500. In some implementations, the memory 2504 is a volatile memory unit or units. In some implementations, the memory 2504 is a non-volatile memory unit or units. The memory 2504 may also be another form of computer-readable medium, such as a magnetic or optical disk.

[0142] The storage device 2506 depicted in FIG. 24 is capable of providing mass storage for the computing device 2500. In some implementations, the storage device 2506 may be or contain a computer-readable medium, such as a floppy disk device, a hard disk device, an optical disk device, or a tape device, a flash memory or other similar solid state memory device, or an array of devices, including devices in a storage area network or other configurations. Instructions can be stored in an information carrier. The instructions, when executed by one or more processing devices (for example, processor 2502), perform one or more methods, such as those described above. The instructions can also be stored by one or more storage devices such

as computer- or machine-readable mediums (for example, the memory 2504, the storage device 2506, or memory on the processor 2502).

[0143] The high-speed interface 2508 manages bandwidth-intensive operations for the computing device 2500, while the low-speed interface 2512 manages lower bandwidth-intensive operations. Such allocation of functions is an example only. In some implementations, the high-speed interface 2508 is coupled to the memory 2504, the display 2516 (e.g., through a graphics processor or accelerator), and to the high-speed expansion ports 2510, which may accept various expansion cards (not shown). In the implementation, the low-speed interface 2512 is coupled to the storage device 2506 and the low-speed expansion port 2514. The low-speed expansion port 2514, which may include various communication ports (e.g., USB, Bluetooth®, Ethernet, wireless Ethernet) may be coupled to one or more input/output devices, such as a keyboard, a pointing device, a scanner, or a networking device such as a switch or router, e.g., through a network adapter.

[0144] The computing device 2500 may be implemented in a number of different forms, as shown in the figure. For example, it may be implemented as a standard server 2520, or multiple times in a group of such servers. In addition, it may be implemented in a personal computer such as a laptop computer 2522. It may also be implemented as part of a rack server system 2524. Alternatively, components from the computing device 2500 may be combined with other components in a mobile device (not shown), such as a mobile computing device 2550. Each of such devices may contain one or more of the computing device 2500 and the mobile computing device 2550, and an entire system may be made up of multiple computing devices communicating with each other.

[0145] The mobile computing device 2550 depicted in FIG. 24 includes a processor 2552, a memory 2564, an input/output device such as a display 2554, a communication interface 2566, and a transceiver 2568, among other components. The mobile computing device 2550 may also be provided with a storage device, such as a micro-drive or other device, to provide additional storage. Each of the processor 2552, the memory 2564, the display 2554, the communication interface 2566, and the transceiver 2568, are interconnected using various buses,

and several of the components may be mounted on a common motherboard or in other manners as appropriate.

[0146] The processor 2552 depicted in FIG. 24 can execute instructions within the mobile computing device 2550, including instructions stored in the memory 2564. The processor 2552 may be implemented as a chipset of chips that include separate and multiple analog and digital processors. The processor 2552 may provide, for example, for coordination of the other components of the mobile computing device 2550, such as control of user interfaces, applications run by the mobile computing device 2550, and wireless communication by the mobile computing device 2550.

[0147] The processor 2552 may communicate with a user through a control interface 2558 and a display interface 2556 coupled to the display 2554. The display 2554 depicted in FIG. 24 may be, for example, a TFT (Thin-Film-Transistor Liquid Crystal Display) display or an OLED (Organic Light Emitting Diode) display, or other appropriate display technology. The display interface 2556 may comprise appropriate circuitry for driving the display 2554 to present graphical and other information to a user. The control interface 2558 may receive commands from a user and convert them for submission to the processor 2552. In addition, an external interface 2562 may provide communication with the processor 2552, so as to enable near area communication of the mobile computing device 2550 with other devices. The external interface 2562 may provide, for example, for wired communication in some implementations, or for wireless communication in other implementations, and multiple interfaces may also be used.

[0148] The memory 2564 depicted in FIG. 24 stores information within the mobile computing device 2550. The memory 2564 can be implemented as one or more of a computer-readable medium or media, a volatile memory unit or units, or a non-volatile memory unit or units. An expansion memory 2574 may also be provided and connected to the mobile computing device 2550 through an expansion interface 2572, which may include, for example, a SIMM (Single In Line Memory Module) card interface. The expansion memory 2574 may provide extra storage space for the mobile computing device 2550, or may also store applications or other information for the mobile computing device 2550. Specifically, the expansion memory 2574

may include instructions to carry out or supplement the processes described above, and may include secure information also. Thus, for example, the expansion memory 2574 may be provide as a security module for the mobile computing device 2550, and may be programmed with instructions that permit secure use of the mobile computing device 2550. In addition, secure applications may be provided via the SIMM cards, along with additional information, such as placing identifying information on the SIMM card in a non-hackable manner.

[0149] The memory may include, for example, flash memory and/or NVRAM memory (non-volatile random access memory), as discussed below. In some implementations, instructions are stored in an information carrier. that the instructions, when executed by one or more processing devices (for example, processor 2552), perform one or more methods, such as those described above. The instructions can also be stored by one or more storage devices, such as one or more computer- or machine-readable mediums (for example, the memory 2564, the expansion memory 2574, or memory on the processor 2552). In some implementations, the instructions can be received in a propagated signal, for example, over the transceiver 2568 or the external interface 2562.

[0150] The mobile computing device 2550 depicted in FIG. 24 may communicate wirelessly through the communication interface 2566, which may include digital signal processing circuitry where necessary. The communication interface 2566 may provide for communications under various modes or protocols, such as GSM voice calls (Global System for Mobile communications), SMS (Short Message Service), EMS (Enhanced Messaging Service), or MMS messaging (Multimedia Messaging Service), CDMA (code division multiple access), TDMA (time division multiple access), PDC (Personal Digital Cellular), WCDMA (Wideband Code Division Multiple Access), CDMA2000, or GPRS (General Packet Radio Service), among others. Such communication may occur, for example, through the transceiver 2568 using a radio-frequency. In addition, short-range communication may occur, such as using a Bluetooth®, Wi-Fi™, or other such transceiver (not shown). In addition, a GPS (Global Positioning System) receiver module 2570 may provide additional navigation- and location-

related wireless data to the mobile computing device 2550, which may be used as appropriate by applications running on the mobile computing device 2550.

[0151] The mobile computing device 2550 depicted in FIG. 24 may also communicate audibly using an audio codec 2560, which may receive spoken information from a user and convert it to usable digital information. The audio codec 2560 may likewise generate audible sound for a user, such as through a speaker, e.g., in a handset of the mobile computing device 2550. Such sound may include sound from voice telephone calls, may include recorded sound (e.g., voice messages, music files, etc.) and may also include sound generated by applications operating on the mobile computing device 2550.

[0152] The mobile computing device 2550 may be implemented in a number of different forms, as shown in FIG. 24. For example, it may be implemented as a cellular telephone 2580. It may also be implemented as part of a smart-phone 2582, personal digital assistant, or other similar mobile device.

[0153] Various implementations of the systems and techniques described here can be realized in digital electronic circuitry, integrated circuitry, specially designed ASICs (application specific integrated circuits), computer hardware, firmware, software, and/or combinations thereof. These various implementations can include implementation in one or more computer programs that are executable and/or interpretable on a programmable system including at least one programmable processor, which may be special or general purpose, coupled to receive data and instructions from, and to transmit data and instructions to, a storage system, at least one input device, and at least one output device.

[0154] These computer programs (also known as programs, software, software applications or code) include machine instructions for a programmable processor, and can be implemented in a high-level procedural and/or object-oriented programming language, and/or in assembly/machine language. As used herein, the terms machine-readable medium and computer-readable medium refer to any computer program product, apparatus and/or device (e.g., magnetic discs, optical disks, memory, Programmable Logic Devices (PLDs)) used to provide machine instructions and/or data to a programmable processor, including a machine-

readable medium that receives machine instructions as a machine-readable signal. The term machine-readable signal refers to any signal used to provide machine instructions and/or data to a programmable processor.

[0155] To provide for interaction with a user, the systems and techniques described here can be implemented on a computer having a display device (e.g., a CRT (cathode ray tube) or LCD (liquid crystal display) monitor) for displaying information to the user and a keyboard and a pointing device (e.g., a mouse or a trackball) by which the user can provide input to the computer. Other kinds of devices can be used to provide for interaction with a user as well; for example, feedback provided to the user can be any form of sensory feedback (e.g., visual feedback, auditory feedback, or tactile feedback); and input from the user can be received in any form, including acoustic, speech, or tactile input.

[0156] The systems and techniques described here can be implemented in a computing system that includes a back end component (e.g., as a data server), or that includes a middleware component (e.g., an application server), or that includes a front end component (e.g., a client computer having a graphical user interface or a Web browser through which a user can interact with an implementation of the systems and techniques described here), or any combination of such back end, middleware, or front end components. The components of the system can be interconnected by any form or medium of digital data communication (e.g., a communication network). Examples of communication networks include a local area network (LAN), a wide area network (WAN), and the Internet.

[0157] The computing system can include clients and servers. A client and server are generally remote from each other and typically interact through a communication network. The relationship of client and server arises by virtue of computer programs running on the respective computers and having a client-server relationship to each other.

[0158] In some embodiments, there is provided an implementation of an exemplary cloud computing environment for subscription management in a multi-channel context aware communication environment. As shown in FIG. 25, the cloud computing environment 2400 may include one or more resource providers 2402a, 2402b, 2402c (collectively, 2402). Each resource

provider 2402 may include computing resources. In some implementations, computing resources may include any hardware and/or software used to process data. For example, computing resources may include hardware and/or software capable of executing algorithms, computer programs, and/or computer applications. In some implementations, exemplary computing resources may include application servers and/or databases with storage and retrieval capabilities. Each resource provider 2402 may be connected to any other resource provider 2402 in the cloud computing environment 2400. In some implementations, the resource providers 2402 may be connected over a computer network 2408. Each resource provider 2402 may be connected to one or more computing device 2404a, 2404b, 2404c (collectively, 2404), over the computer network 2408.

[0159] The cloud computing environment 2400 depicted in FIG. 25 may include a resource manager 2406. The resource manager 2406 may be connected to the resource providers 2402 and the computing devices 2404 over the computer network 2408. In some implementations, the resource manager 2406 may facilitate the provision of computing resources by one or more resource providers 2402 to one or more computing devices 2404. The resource manager 2406 may receive a request for a computing resource from a particular computing device 2404. The resource manager 2406 may identify one or more resource providers 2402 capable of providing the computing resource requested by the computing device 2404. The resource manager 2406 may select a resource provider 2402 to provide the computing resource. The resource manager 2406 may facilitate a connection between the resource provider 2402 and a particular computing device 2404. In some implementations, the resource manager 2406 may establish a connection between a particular resource provider 2402 and a particular computing device 2404. In some implementations, the resource manager 2406 may redirect a particular computing device 2404 to a particular resource provider 2402 with the requested computing resource.

[0160] As will be appreciated by those skilled in the art, reading the present disclosure in some embodiments, the present invention provides a computer system comprising one or more of (a) atomic coordinate data as disclosed herein [$\pm$ a root mean square deviation from the Ca

atoms of note more than 1.5 Å (or 1.0 Å or 0.5 Å)]; (b) structure factor data (where a structure factor comprises the amplitude and phase of the diffracted wave) for mTOR, said structure factor data being derivable from the atomic coordinate data of Tables 1-6 +/- a root mean square deviation from the Ca atoms of note more than 1.5 Å (or 1.0 Å or 0.5 Å); (c) atomic coordinate data of a mTOR inhibitor protein generated by homology modeling of the target based on the data disclosed herein +/- a root mean square deviation from the Ca atoms of not more than 1.5 Å (or 1.0 Å or 0.5 Å); (d) atomic coordinate data of an mTOR inhibitor protein generated by interpreting X-ray crystallographic data or NMR data by reference to the data disclosed herein +/- a root mean square deviation from the Ca atoms of note more than 1.5 Å (or 1.0 Å or 0.5 Å); or (e) structure factor data a derivable from the atomic coordinate data of (c) or (d). In certain embodiments, a computer system comprises: a computer-readable data storage medium comprising data storage material encoded with the computer-readable data; (a) a working memory for storing instructions for processing said computer-readable data; and (b) a central-processing unit coupled to said working memory and to said computer-readable data storage medium for processing said computer-readable data and thereby generating structures, characterizing structures and/or performing rational drug design.

[0161] In some embodiments, there is provided a machine-readable data storage medium, comprising a data storage material encoded with machine-readable data, wherein said data comprises all or part of an mTOR^{ΔN}-mLST8 complex defined by structure coordinates of mTOR amino acids (SEQ ID NO.:(__)), according Table 1; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex defined by structure coordinates of corresponding amino acids that are identical to said mTOR amino acids, wherein the root mean square deviation of the backbone atoms between said corresponding amino acids and said mTOR amino acids is not more than about 3.0 Å, 2.5 Å, 2.0 Å, 1.5 Å, 1.0 Å; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex defined by structure coordinates of a set of corresponding amino acids, wherein the root mean square deviation of the backbone atoms between said set of corresponding amino acids and said mTOR amino acids is not more

than about 1.1, 0.9, 0.7, or 0.5 Å, and wherein at least one of said corresponding amino acids is not identical to the mTOR amino acid to which it corresponds.

[0162] In some embodiments, there is provided a machine-readable data storage medium, comprising a data storage material encoded with machine-readable data, wherein said data comprises all or part of an mTOR^{ΔN}-mLST8-ADP-MgF₃-Mg²⁺ complex defined by structure coordinates of mTOR amino acids (SEQ ID NO.:()), according to Table 2; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8-ADP-MgF₃-Mg²⁺ complex defined by structure coordinates of corresponding amino acids that are identical to said mTOR amino acids, wherein the root mean square deviation of the backbone atoms between said corresponding amino acids and said mTOR amino acids is not more than about 3.0 Å, 2.5 Å, 2.0 Å, 1.5 Å, 1.0 Å; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8-ADP-MgF₃-Mg²⁺ complex defined by structure coordinates of a set of corresponding amino acids, wherein the root mean square deviation of the backbone atoms between said set of corresponding amino acids and said mTOR amino acids is not more than about 1.1, 0.9, 0.7, or 0.5 Å, and wherein at least one of said corresponding amino acids is not identical to the mTOR amino acid to which it corresponds.

[0163] In some embodiments, there is provided a machine-readable data storage medium, comprising a data storage material encoded with machine-readable data, wherein said data comprises all or part of an mTOR^{ΔN}-mLST8-ATPγS-Mg complex defined by structure coordinates of mTOR amino acids (SEQ ID NO.:()), according to Table 3; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8-ATPγS-Mg complex defined by structure coordinates of corresponding amino acids that are identical to said mTOR amino acids, wherein the root mean square deviation of the backbone atoms between said corresponding amino acids and said mTOR amino acids is not more than about 3.0 Å, 2.5 Å, 2.0 Å, 1.5 Å, 1.0 Å; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8-ATPγS-Mg complex defined by structure coordinates of a set of corresponding amino acids, wherein the root mean square deviation of the backbone atoms between said set of corresponding amino acids and said mTOR amino acids is not more than about 1.1, 0.9, 0.7, or

0.5 Å, and wherein at least one of said corresponding amino acids is not identical to the mTOR amino acid to which it corresponds.

[0164] In some embodiments, there is provided a machine-readable data storage medium, comprising a data storage material encoded with machine-readable data, wherein said data comprises all or part of an mTOR^{ΔN}-mLST8 complex bound with Torin2 defined by structure coordinates of mTOR amino acids (SEQ ID NO.:()), according to Table 4; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex bound with Torin2 defined by structure coordinates of corresponding amino acids that are identical to said mTOR amino acids, wherein the root mean square deviation of the backbone atoms between said corresponding amino acids and said mTOR amino acids is not more than about 3.0 Å, 2.5 Å, 2.0 Å, 1.5 Å, 1.0 Å; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex bound with Torin2 defined by structure coordinates of a set of corresponding amino acids, wherein the root mean square deviation of the backbone atoms between said set of corresponding amino acids and said mTOR amino acids is not more than about 1.1, 0.9, 0.7, or 0.5 Å, and wherein at least one of said corresponding amino acids is not identical to the mTOR amino acid to which it corresponds.

[0165] In some embodiments, there is provided a machine-readable data storage medium, comprising a data storage material encoded with machine-readable data, wherein said data comprises all or part of an mTOR^{ΔN}-mLST8 complex bound with PP242 defined by structure coordinates of mTOR amino acids (SEQ ID NO.:()), according to Table 5; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex bound with PP242 defined by structure coordinates of corresponding amino acids that are identical to said mTOR amino acids, wherein the root mean square deviation of the backbone atoms between said corresponding amino acids and said mTOR amino acids is not more than about 3.0 Å, 2.5 Å, 2.0 Å, 1.5 Å, 1.0 Å; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex bound with PP242 defined by structure coordinates of a set of corresponding amino acids, wherein the root mean square deviation of the backbone atoms between said set of corresponding amino acids and said mTOR amino acids is not more than about 1.1, 0.9, 0.7, or

0.5 Å, and wherein at least one of said corresponding amino acids is not identical to the mTOR amino acid to which it corresponds.

[0166] In some embodiments, there is provided a machine-readable data storage medium, comprising a data storage material encoded with machine-readable data, wherein said data comprises all or part of an mTOR^{ΔN}-mLST8 complex bound with PI-103 defined by structure coordinates of mTOR amino acids (SEQ ID NO.:()), according to Table 6; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex bound with PI-103 defined by structure coordinates of corresponding amino acids that are identical to said mTOR amino acids, wherein the root mean square deviation of the backbone atoms between said corresponding amino acids and said mTOR amino acids is not more than about 3.0 Å, 2.5 Å, 2.0 Å, 1.5 Å, 1.0 Å; or a molecule or molecular complex comprising all or part of an mTOR^{ΔN}-mLST8 complex bound with PI-103 defined by structure coordinates of a set of corresponding amino acids, wherein the root mean square deviation of the backbone atoms between said set of corresponding amino acids and said mTOR amino acids is not more than about 1.1, 0.9, 0.7, or 0.5 Å, and wherein at least one of said corresponding amino acids is not identical to the mTOR amino acid to which it corresponds.

[0167] In some embodiments, the data storage material is encoded with machine-readable data comprising all or part of a superposition of an mTOR complex with one or more additional molecules and/or complexes. In some embodiments, data storage material is encoded with machine-readable data comprising all or part of a superposition of the mTOR and CDK2 TS (PDB 3QHW) complexes. In some embodiments, one or more domain(s) of mTOR are superposed with one or more molecules and/or complexes. In some embodiments, the FRB domains of mTOR and the FRBrapamycin-FKBP12 complex (PDB 1FAP) are superpositioned.

[0168] In some embodiments, the present invention provides a computer system comprising executable code for computer-aided and/or structure-based drug design of mTOR modulators. In some embodiments, the present invention provides a computer system comprising executable code for docking TOR modulators in at least one potential mTOR interaction site. In some embodiments, said executable code comprises instructions for

providing an image of an mTOR crystal that includes at least one potential interaction site; docking in the image at least one moiety that is a potential mTOR modulator structural element; and assessing one or more features of a potential moiety-interaction site interaction.

[0169] In some embodiments, said executable code comprises a determining step, wherein said determining step comprises a prediction of 1.) whether said moiety will bind to at least one potential mTOR interaction site; 2.) calculates the strength of binding affinity; and 3.) calculates moiety specificity. In some embodiments, the determining step further comprises predicting the conformation of the moiety when bound to at least one potential interaction site. In some embodiments, the determining step further comprises one or more predictions of conformational changes in an mTOR polypeptide when said moiety binds at least one potential interaction site.

[0170] In some embodiments, provided computer system comprise executable code for superimposing all or part of an mTOR crystal or crystallizable composition with another crystal or crystallizable composition.

[0171] In some embodiments, provided computer systems comprise executable code for modeling interactions with an an mTOR crystal or crystallizable composition.

mTor modulators

[0172] As described herein, the present disclosure provides methodologies, technologies, and systems for designing and/or characterizing mTOR polypeptide modulators. The macrolide rapamycin is a selective and allosteric inhibitor of the mTORC1 complex but *not* the mTORC2 complex. There is therefore a need to develop small molecule modulators that target *both* mTORC1 and mTORC2 complexes.

[0173] In some embodiments, provided modulators have a structure comprising one or more of the following features:

[0174] A) a moiety that fits in the mTOR adenosine (ATP) binding site and may make one or two hydrogen bonds to main chain groups of the “hinge” extending from Gly2238 to Val2240. In some embodiments, moieties can be any one of well-characterized pharmacophores like pyrazolopyrimidines that have been developed as adenosine-site mimics for Ser/Thr/Tyr and PI3 kinases.

[0175] B) a moiety that stacks with tryptophan (Trp2239). In some embodiments, moieties can be a ring structure that is fused to the ring structure of (A), or it can be attached to (A) in other ways.

[0176] C) a moiety that binds to the “inner hydrophobic pocket” formed by mTOR residues Tyr2225, Val2227, Met2199, Ile2237, Ile2356, Phe2358, Leu2192, Asp2195, Asp2357 and Gly2359. In some embodiments, one or more of these residues have at least two conformations shown by the structure of mTORDn-mLST8 bound to PP242 inhibitor, and either conformation can be used in modulator design and/or characterization.

D) a moiety that binds to the N-lobe hydrophobic pocket (Ile2163, Pro2169 and Leu2185). In some embodiments, when B and C are both present, the modulator is arranged and constructed such that, when it is bound to mTOR, B and C are arranged in three dimensional space relative to one another on opposite sides of A. When D is present, it will extend from A or from B orthogonal to the B-A-C line.

B-----A-----C

| |

D or D

[0177] In some embodiments, provided modulators are not known modulators. For example, in some embodiments, provided modulators are not any of the modulators set forth in Table 12.

[0178] In some embodiments, provided modulators are characterized by desirable mTOR modulating activity. For example, in some embodiments, provided modulators are characterized in that, when the modulator is contacted with an assay system that contains or is capable of producing an mTOR polypeptide and permits detection and/or quantitation of level and/or activity of the mTOR polypeptide, the detected such level or activity is different when the modulator is present as compared with when it is absent.

[0179] In some embodiments, the assay system comprises an isolated mTOR polypeptide. In some embodiments, the assay system comprises one or more cells that can or do produce an mTOR polypeptide. In some embodiments, the assay systems include, but are not limited to any microbial cell, insect cell, mammalian cell, and/or combinations thereof, that can or do produce an mTOR polypeptide. Assay systems useful in detecting and/or characterizing mTOR modulator activity as described herein include *in vitro* kinase assays using S6K1, 4EBP1 or other mTOR substrates, or cell-based assays using immunoblotting with antibodies specific to phosphosylated forms of S6K1, 4EBP1 or other mTOR substrates, or other mTOR assays such as cell growth and proliferation commonly reported in the literature.

[0180] In some embodiments, an mTOR modulator as described herein is an agent whose presence correlates with a level of mTOR activity that is at least about 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, or 2 fold higher or lower in a given assay than that observed under otherwise comparable conditions than that observed absent the modulator.

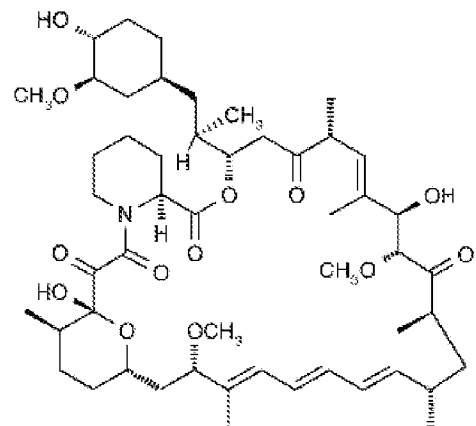
[0181] In some embodiments, an mTOR modulator as described herein impacts mTOR activity in a way or to a degree comparable to or greater than that of a reference mTOR modulator. In some embodiments, a provided mTOR modulator correlates with an increase or decrease of mTOR activity that is within 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,

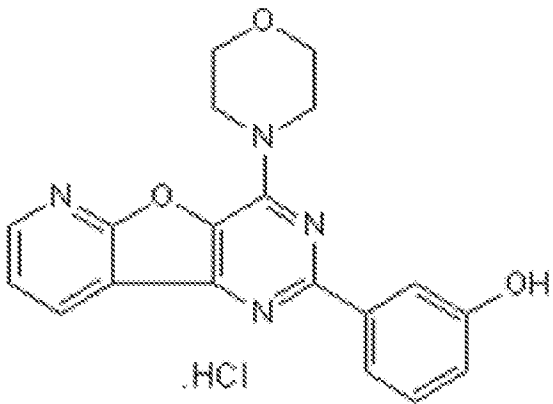
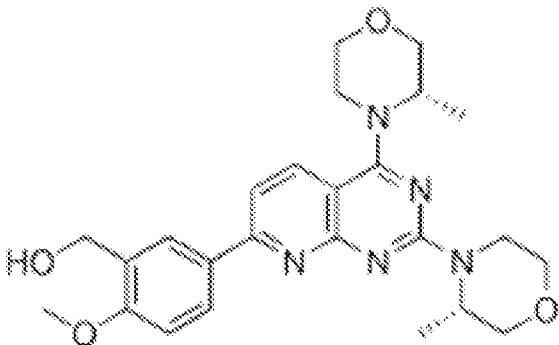
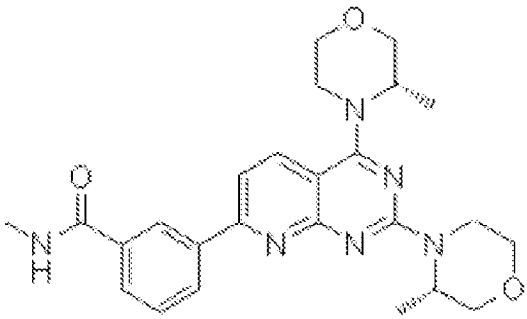
100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more of that observed with such reference mTOR modulator.

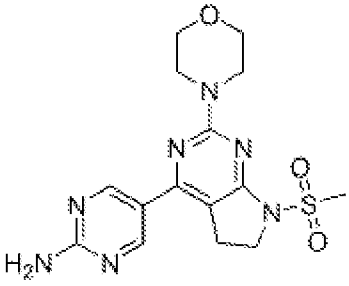
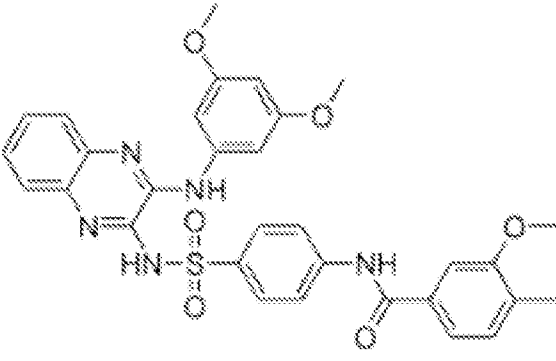
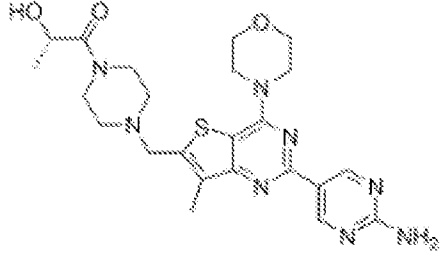
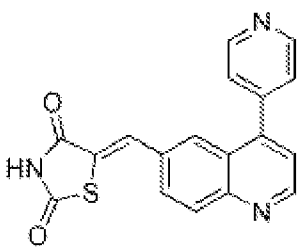
[0182] In some embodiments, provided modulators are ATP-competitive modulators.

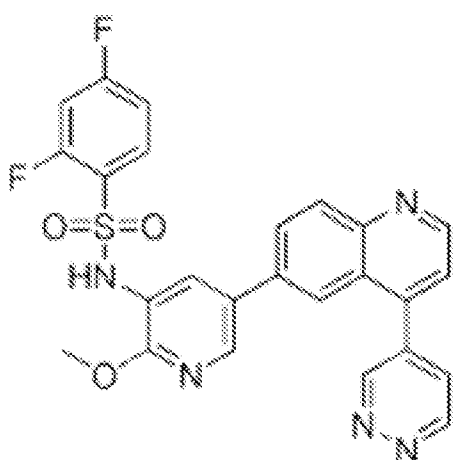
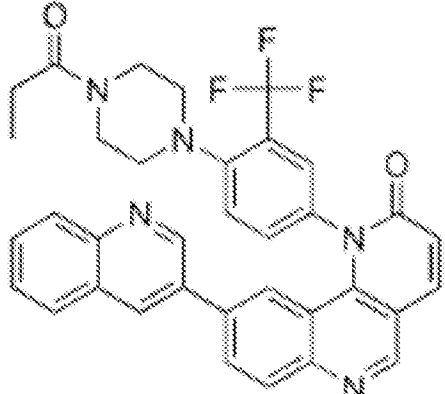
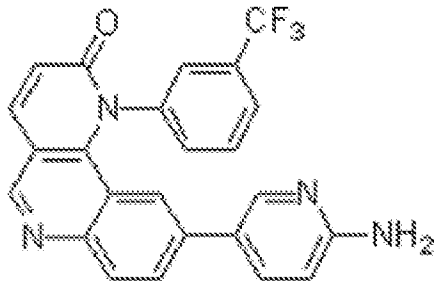
[0183] In some embodiments, a reference mTOR modulator is one included in Table 12. In some embodiments, a reference mTOR modulator includes, but is not limited to, rapamycin, PI 103 HCL, AZD8055, AZD2014, CH5132799, XL765 (SAR245409), GDC-0980 (RG7422), GSK1059615, GSK2126458, Torin1, Torin2, INK128, KU0063794, BEZ235 (NVP-BEZ235), NVP-BGT226, OSI-027, Palomid 529, PF-05212384 (PKI-587), PKI-179, WAY-600, WYE-125132 (WYE-132), WYE-23, WYE-28, WYE-354, WYE-687, PF-04691502, PP-121, PP242, and/or PP30.

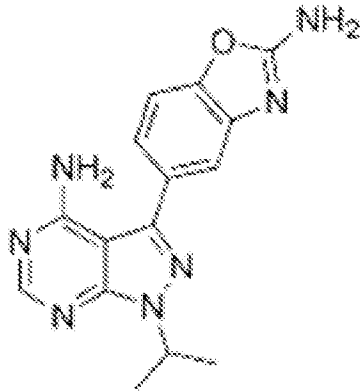
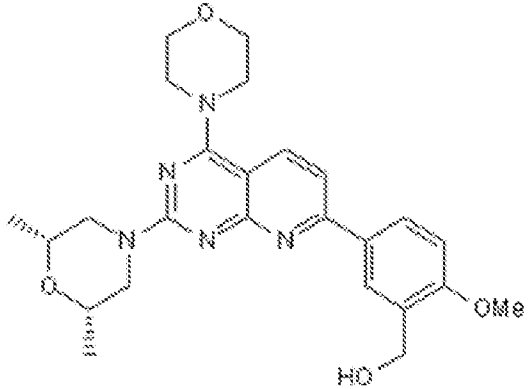
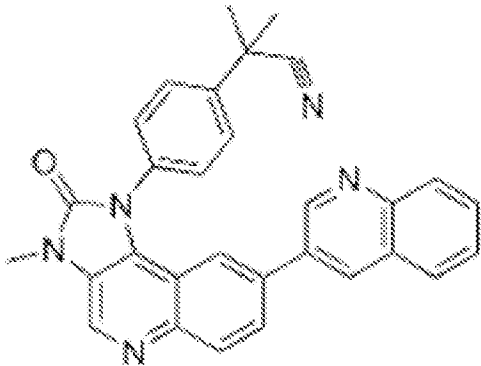
Table 12: Representative mTOR modulators

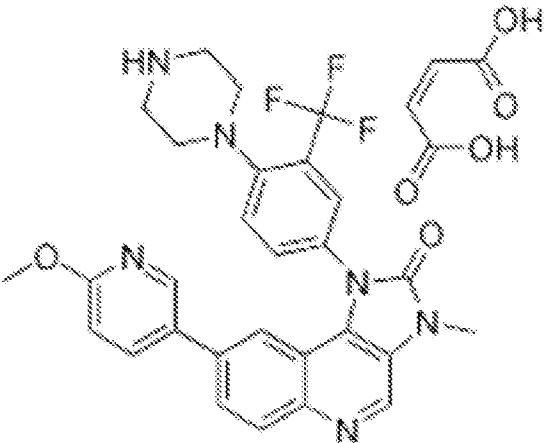
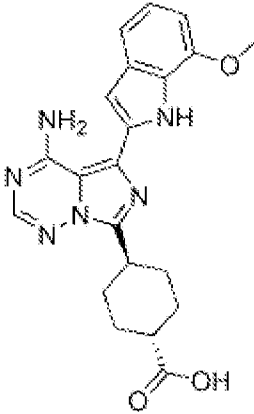
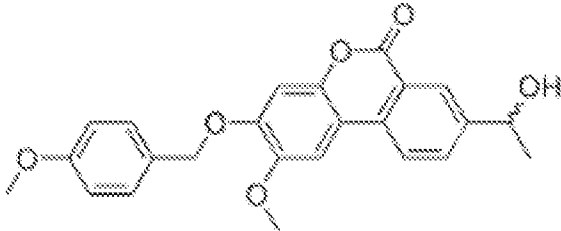
mTOR modulator	Chemical Name / Formula	Structure
Rapamycin	(7E,15E,17E,19E)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34aS-Hexadecahydro-9R,27-dihydroxy-3S-[(1R)-2-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10R,21S-dimethoxy-6R,8,12R,14S,20,26R-hexamethyl-23S,27R-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclohentriacontine-1,5,11,28,29(4H,6H,31H)-pentone $C_{51}H_{79}NO_{13}$	

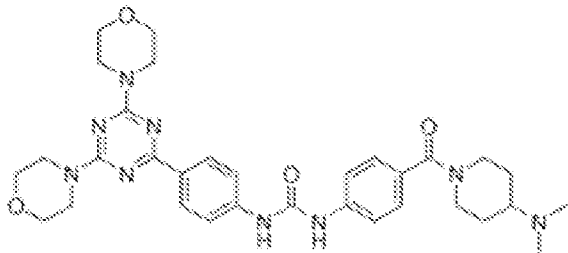
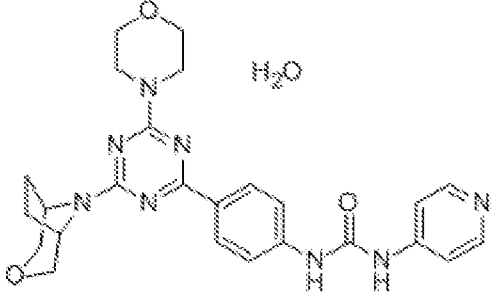
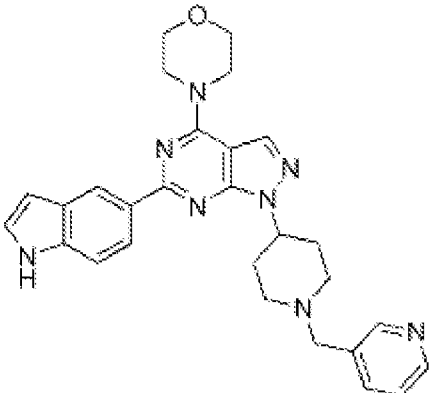
mTOR modulator	Chemical Name / Formula	Structure
PI 103 HCL	3-[4-(4-Morpholinylpyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl)]phenol hydrochloride $C_{19}H_{16}N_4O_3$	
AZD8055	(5-(2,4-bis((S)-3-methylmorpholino)pyrido[2,3-d]pyrimidin-7-yl)-2-methoxyphenyl)methanol $C_{25}H_{31}N_5O_4$	
AZD2014	3-(2,4-bis((S)-3-methylmorpholino)pyrido[2,3-d]pyrimidin-7-yl)-N-methylbenzamide $C_{25}H_{30}N_6O_3$	

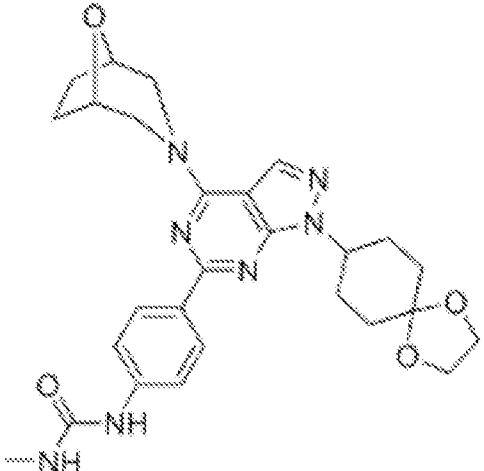
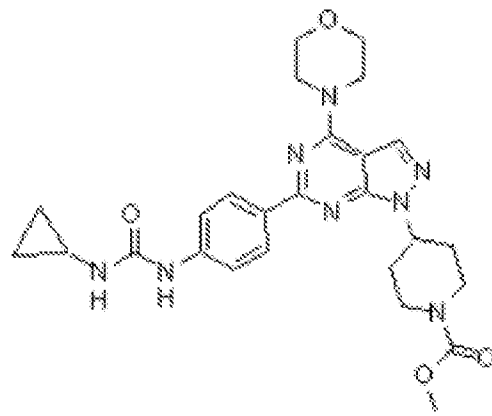
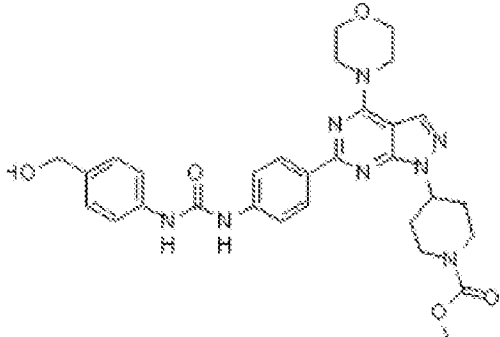
mTOR modulator	Chemical Name / Formula	Structure
CH5132799	5-(7-(methylsulfonyl)-2-morpholino-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrimidin-2-amine $C_{15}H_{19}N_7O_3S$	
XL765 (SAR245409)	Benzamide, N-[4-[[[3-[(3,5-dimethoxyphenyl)amino]-2-quinoxaliny]amino]sulfonyl]phenyl]-3-methoxy-4-methyl- $C_{31}H_{29}N_5O_6S$	
GDC-0980 (RG7422)	(S)-1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxypropan-1-one $C_{23}H_{30}N_8O_3S$	
GSK1059615	5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidenedione $C_{18}H_{11}N_3O_2S$	

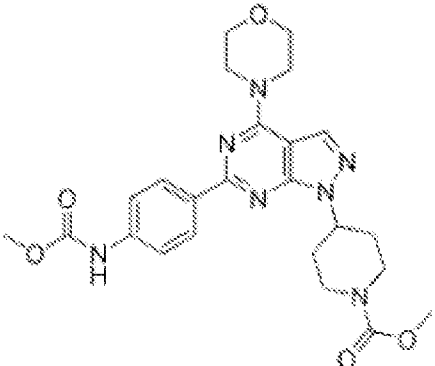
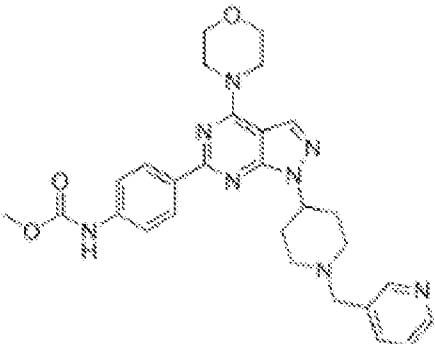
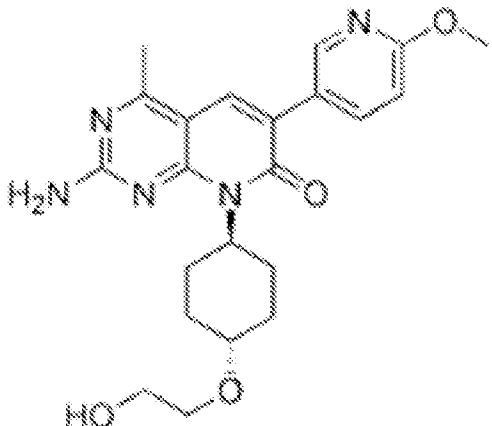
mTOR modulator	Chemical Name / Formula	Structure
GSK2126458	2,4-difluoro-N-(2-methoxy-5-(4-(pyridazin-4-yl)quinolin-6-yl)pyridin-3-yl)benzenesulfonamide $C_{25}H_{17}F_2N_5O_3S$	
Torin1	Benzo[h]-1,6-naphthyridin-2(1H)-one, 1-[4-[4-(1-oxopropyl)-1-piperazinyl]-3-(trifluoromethyl)phenyl]-9-(3-quinolinyl)- $C_{35}H_{28}F_3N_5O_2$	
Torin2	9-(6-Amino-3-pyridinyl)-1-[3-(trifluoromethyl)phenyl]-benzo[h]-1,6-naphthyridin-2(1H)-one $C_{24}H_{15}F_3N_4O$	

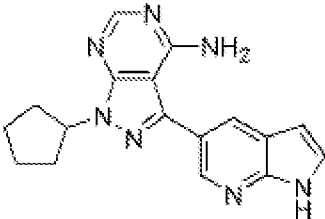
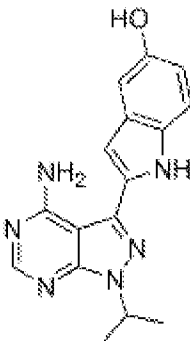
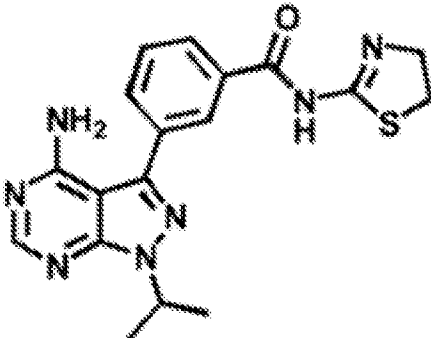
mTOR modulator	Chemical Name / Formula	Structure
INK128	3-(2-aminobenzo[d]oxazol-5-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine $C_{15}H_{15}N_7O$	
KU0063794	rel-5-[2-[(2R,6S)-2,6-dimethyl-4-morpholinyl]-4-(4-morpholinyl)pyrido[2,3-d]pyrimidin-7-yl]-2-methoxybenzenemethanol $C_{25}H_{31}N_5O_4$	
BEZ235 (NVP-BEZ235)	2-methyl-2-(4-(3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl)phenyl)propanenitrile $C_{30}H_{23}N_5O$	

mTOR modulator	Chemical Name / Formula	Structure
NVP-BGT226	8-(6-methoxypyridin-3-yl)-3-methyl-1-(4-(piperazin-1-yl)-3-(trifluoromethyl)phenyl)-1H-imidazo[4,5-c]quinolin-2(3H)-one Maleic acid $C_{28}H_{25}F_3N_6O_2 \cdot C_4H_4O_4$	
OSI-027	(1r,4r)-4-(4-amino-5-(7-methoxy-1H-indol-2-yl)imidazo[1,5-f][1,2,4]triazin-7-yl)cyclohexanecarboxylic acid $C_{21}H_{22}N_6O_3$	
Palomid 529	3-(4-methoxybenzyloxy)-8-(1-hydroxyethyl)-2-methoxy-6H-benzo[c]chromen-6-one $C_{24}H_{22}O_6$	

mTOR modulator	Chemical Name / Formula	Structure
PF-05212384 (PKI-587)	1-(4-(4-(dimethylamino)piperidine-1-carbonyl)phenyl)-3-(4-(4,6-dimorpholino-1,3,5-triazin-2-yl)phenyl)urea $C_{32}H_{41}N_9O_4$	
PKI-179	1-(4-(4-(3-Oxa-8-azabicyclo[3.2.1]octan-8-yl)-6-morpholino-1,3,5-triazin-2-yl)-phenyl)-3-(pyridin-4-yl)urea, hydrate, N-(4-(4-(Morpholinyl)-6-(3-oxa-8-azabicyclo[3.2.1]oct-8-yl)-1,3,5-triazin-2-yl)phenyl)-N'-4-pyridinylurea, hydrate $C_{25}H_{28}N_8O_3 \cdot H_2O$	
WAY-600	6-(1H-indol-5-yl)-4-morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidine $C_{28}H_{30}N_8O$	

mTOR modulator	Chemical Name / Formula	Structure
WYE-125132 (WYE-132)	Urea, N-[4-[1-(1,4-dioxaspiro[4.5]dec-8-yl)-4-(8-oxa-3-azabicyclo[3.2.1]oct-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl]-N'-methyl- $C_{27}H_{33}N_7O_4$	
WYE-23	4-{6-[4-(3-Cyclopropylureido)-phenyl]-4-morpholin-4-yl-pyrazolo[3,4-d]pyrimidin-1-yl}-piperidine-1-carboxylic acid methyl ester $C_{26}H_{32}N_8O_4$	
WYE-28	4-(6-{4-[3-(4-Hydroxymethylphenyl)-ureido]-phenyl}-4-morpholin-4-yl-pyrazolo[3,4-d]pyrimidin-1-yl)-piperidine-1-carboxylic acid methyl ester $C_{30}H_{34}N_8O_5$	

mTOR modulator	Chemical Name / Formula	Structure
WYE-354	4-[6-[4-[(methoxycarbonyl)amino]phenyl]-4-(4-morpholinyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-methyl ester-1-piperidinecarboxylic acid $C_{24}H_{29}N_7O_5$	
WYE-687	methyl 4-(4-morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenylcarbamate $C_{28}H_{32}N_8O_3$	
PF-04691502	2-amino-8-((1r,4r)-4-(2-hydroxyethoxy)cyclohexyl)-6-(6-methoxypyridin-3-yl)-4-methylpyrido[2,3-d]pyrimidin-7(8H)-one $C_{22}H_{27}N_5O_4$	

mTOR modulator	Chemical Name / Formula	Structure
PP-121	1-cyclopentyl-3-(1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine $C_{17}H_{17}N_7$	
PP242	2-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-1H-indol-5-ol $C_{16}H_{16}N_6O$	
PP30		

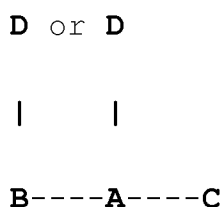
In an assay system that detects and/or quantifies an activity of the mTOR polypeptide, the modulator increases or decreases detected activity of the mTOR polypeptide, for example by increasing or decreasing mTOR polypeptide level and/or activity.

[0184] One aspect of the present invention surprisingly establishes that many theories about interaction of mTOR modulators (e.g., inhibitors) with mTOR have been mistaken. For example, the high affinity of Torin2 for mTOR was proposed, based on a PI3Kgamma-derived

mTOR model, to be due to a pair of hydrogen bonds that are not there in the mTORDn-mLST8-Torin2 structure. Among other things, the present invention establishes that the high affinity and specificity is due primarily to the extensive stacking of the tricyclic benzonaphththyridine ring of Torin2 with the indole group of Trp2239 of mTOR, and secondarily the packing of the Torin2 trifluoromethyl group into the aforementioned N lobe hydrophobic pocket (Figure 6A). In another example, the high affinity and specificity of PP242 for mTOR was not understood, as the paper reporting it noted. The mTORDN-mLST8-PP242 structure showed that the inhibitor causes a conformational change in the inner hydrophobic pocket that is coupled to the binding of the PP242 hydroxyindole group there.

[0185] The present invention defines interaction sites within mTOR polypeptides that, when targeted, can achieve potent and/or selective modulation of mTOR activity. Among other things, the present invention establishes the arrangement and positions of chemical groups that make up the mTOR active site, and how they can be targeted for interactions by inhibitors.

[0186] The present invention therefore defines important structural features of mTor modulating agents. For example, according to the present invention, certain desirable modulating agents have a chemical structure of the form



wherein:

A is a moiety that fits in the mTOR adenosine (ATP) binding site and may make one or two hydrogen bonds to main chain groups of the “hinge” extending from Gly2238 to Val2240;

B is a moiety that stacks with tryptophan (Trp2239) and optionally comprises a ring structure that is fused to the ring structure of (A), and/or B can be attached to (A) in other ways;

C is a moiety that binds to the “inner hydrophobic pocket” formed by mTOR residues Tyr2225, Val2227, Met2199, Ile2237, Ile2356, Phe2358, Leu2192, Asp2195, Asp2357 and Gly2359; in some embodiments, one or more of these residues have at least two conformations shown by the structure of mTOR^{Dn}-mLST8 bound to PP242 inhibitor; and

D is a moiety that binds to the N-lobe hydrophobic pocket (Ile2163, Pro2169 and Leu2185); in some embodiments, when B and C are both present, the modulator is arranged and constructed such that, when it is bound to mTOR, B and C are arranged in three dimensional space relative to one another on opposite sides of A. When D is present, it will extend from A or from B orthogonal to the B-A-C line.

Characterizing mTOR Modulators

[0187] A variety of assays and systems for evaluating mTOR level and/or activity are known in the art (see, for example references 3, 14, 16, 20, 28, 29, 31, 32, 34, 38, 40, 41, 42, 46, 49, Supp. 3, Supp. 4, Supp. 12 and Supp. 14). Those of ordinary skill in the art will readily be able to identify and utilize appropriate such assays for the identification and/or characterization of mTOR modulators of interest as described herein.

[0188] Alternatively or additionally, those skilled in the art will readily be able to identify and utilize appropriate assays for identifying and/or characterizing combinations of mTOR modulators of interest as described herein with one another and/or with other agents (e.g., therapeutic and/or diagnostic agents).

Pharmaceutical Compositions

[0189] The pharmaceutical compositions can be in a variety of forms including oral dosage forms, topic creams, topical patches, iontophoresis forms, suppository, nasal spray and inhaler, eye drops, intraocular injection forms, depot forms, as well as injectable and infusible solutions. Methods for preparing pharmaceutical composition are well known in the art.

[0190] Pharmaceutical compositions typically contain the active agent described herein (e.g. mTOR modulators) in an amount effective to achieve the desired therapeutic effect while avoiding or minimizing adverse side effects. Pharmaceutically acceptable preparations and salts of the active agent are provided herein and are well known in the art. For the administration of mTOR modulators and the like, the amount administered desirably is chosen that is therapeutically effective with few to no adverse side effects. The amount of the therapeutic or pharmaceutical composition which is effective in the treatment of a particular disease, disorder or condition depends on the nature and severity of the disease, the target site of action, the subject's weight, special diets being followed by the subject, concurrent medications being used, the administration route and other factors that are recognized by those skilled in the art. The dosage can be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the subject. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems (e.g., as described by the U.S. Department of Health and Human Services, Food and Drug Administration, and Center for Drug Evaluation and Research in "Guidance for Industry: Estimating Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers", *Pharmacology and Toxicology*, July 2005, the entire contents of which are incorporated herein by reference).

[0191] Various delivery systems are known and can be used to administer active agent described herein (e.g. mTOR modulators) or a pharmaceutical composition comprising the same. The pharmaceutical compositions described herein can be administered by any suitable route including, intravenous or intramuscular injection, intraventricular or intrathecal injection (for central nervous system administration), orally, topically, subcutaneously, intrapulmonary (e.g.,

inhalation), subconjunctivally, intraocularly, or via intranasal, intradermal, sublingual, vaginal, rectal or epidural routes.

[0192] Other delivery systems well known in the art can be used for delivery of the pharmaceutical compositions described herein, for example via aqueous solutions, encapsulation in microparticles, or microcapsules. The pharmaceutical compositions of the present invention can also be delivered in a controlled release system. For example, a polymeric material can be used (see, e.g., Smolen and Ball, *Controlled Drug Bioavailability, Drug product design and performance*, 1984, John Wiley & Sons; Ranade and Hollinger, *Drug Delivery Systems, pharmacology and toxicology series*, 2003, 2nd edition, CRC Press). Alternatively, a pump may be used (Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). The compositions described herein may also be coupled to a class of biodegradable polymers useful in achieving controlled release of the drug, for example, polylactic acid, polyorthoesters, cross-linked amphipathic block copolymers and hydrogels, polyhydroxy butyric acid, and polydihydropyrans.

[0193] As described above, pharmaceutical compositions desirably include a pharmaceutically acceptable carrier. The term carrier refers to diluents, adjuvants, excipients or vehicles with which mTOR modulators are administered. Such pharmaceutical carriers include sterile liquids such as water and oils including mineral oil, vegetable oil (e.g., soybean oil or corn oil), animal oil or oil of synthetic origin. Aqueous glycerol and dextrose solutions as well as saline solutions may also be employed as liquid carriers of the pharmaceutical compositions of the present invention. The choice of the carrier depends on factors well recognized in the art, such as the nature of the peptide, peptide derivative or peptidomimetic, its solubility and other physiological properties as well as the target site of delivery and application. Examples of suitable pharmaceutical carriers are described in Remington: The Science and Practice of Pharmacy by Alfonso R. Gennaro, 2003, 21th edition, Mack Publishing Company. Moreover, suitable carriers for oral administration are known in the art and are described, for example, in U.S. Patent Nos. 6,086,918, 6,673,574, 6,960,355, and 7,351,741 and in WO2007/131286, the disclosures of which are hereby incorporated by reference.

[0194] Further pharmaceutically suitable materials that may be incorporated in pharmaceutical preparations include absorption enhancers including those intended to increase paracellular absorption, pH regulators and buffers, osmolarity adjusters, preservatives, stabilizers, antioxidants, surfactants, thickeners, emollient, dispersing agents, flavoring agents, coloring agents, and wetting agents.

[0195] Examples of suitable pharmaceutical excipients include, water, glucose, sucrose, lactose, glycol, ethanol, glycerol monostearate, gelatin, starch flour (e.g., rice flour), chalk, sodium stearate, malt, sodium chloride, and the like. The pharmaceutical compositions comprising mTOR modulators can take the form of solutions, capsules, tablets, creams, gels, powders sustained release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides (see Remington: The Science and Practice of Pharmacy by Alfonso R. Gennaro, 2003, 21th edition, Mack Publishing Company). Such compositions contain a therapeutically effective amount of the therapeutic composition, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulations are designed to suit the mode of administration and the target site of action (e.g., a particular organ or cell type).

[0196] The pharmaceutical compositions comprising the active agent described herein (e.g. mTOR modulators) also include compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those that form with free amino groups and those that react with free carboxyl groups. Non-toxic alkali metal, alkaline earth metal, and ammonium salts commonly used in the pharmaceutical industry include sodium, potassium, lithium, calcium, magnesium, barium, ammonium, and protamine zinc salts, which are prepared by methods well known in the art. Also included are non-toxic acid addition salts, which are generally prepared by reacting the compounds of the present invention with suitable organic or inorganic acid. Representative salts include the hydrobromide, hydrochloride, valerate, oxalate, oleate, laureate, borate, benzoate, sulfate, bisulfate, acetate, phosphate, tylosate, citrate, maleate, fumarate, tartrate, succinate, napsylate salts, and the like.

[0197] Examples of fillers or binders that may be used in accordance with the present invention include acacia, alginic acid, calcium phosphate (dibasic), carboxymethylcellulose, carboxymethylcellulose sodium, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, dextrin, dextrans, sucrose, tylose, pregelatinized starch, calcium sulfate, amylose, glycine, bentonite, maltose, sorbitol, ethylcellulose, disodium hydrogen phosphate, disodium phosphate, disodium pyrosulfite, polyvinyl alcohol, gelatin, glucose, guar gum, liquid glucose, compressible sugar, magnesium aluminum silicate, maltodextrin, polyethylene oxide, polymethacrylates, povidone, sodium alginate, tragacanth microcrystalline cellulose, starch, and zein. In certain embodiments, a filler or binder is microcrystalline cellulose.

[0198] Examples of disintegrating agents that may be used include alginic acid, carboxymethylcellulose, carboxymethylcellulose sodium, hydroxypropylcellulose (low substituted), microcrystalline cellulose, powdered cellulose, colloidal silicon dioxide, sodium croscarmellose, crospovidone, methylcellulose, polacrillin potassium, povidone, sodium alginate, sodium starch glycolate, starch, disodium disulfite, disodium edathamil, disodium edetate, disodiummethylenediaminetetraacetate (EDTA) crosslinked polyvinylpyrrolidones, pregelatinized starch, carboxymethyl starch, sodium carboxymethyl starch, microcrystalline cellulose.

[0199] Examples of lubricants include calcium stearate, canola oil, glyceryl palmitostearate, hydrogenated vegetable oil (type I), magnesium oxide, magnesium stearate, mineral oil, poloxamer, polyethylene glycol, sodium lauryl sulfate, sodium stearate fumarate, stearic acid, talc and, zinc stearate, glyceryl behapate, magnesium lauryl sulfate, boric acid, sodium benzoate, sodium acetate, sodium benzoate/sodium acetate (in combination), DL-leucine.

[0200] Examples of silica flow conditioners include colloidal silicon dioxide, magnesium aluminum silicate and guar gum. Another most preferred silica flow conditioner consists of silicon dioxide.

[0201] Examples of stabilizing agents include acacia, albumin, polyvinyl alcohol, alginic acid, bentonite, dicalcium phosphate, carboxymethylcellulose, hydroxypropylcellulose, colloidal silicon dioxide, cyclodextrins, glyceryl monostearate, hydroxypropyl methylcellulose,

magnesium trisilicate, magnesium aluminum silicate, propylene glycol, propylene glycol alginate, sodium alginate, carnauba wax, xanthan gum, starch, stearate(s), stearic acid, stearic monoglyceride and stearyl alcohol.

[0202] In some embodiments, the present invention contemplates oral formulations containing the active agent described herein (e.g. mTOR modulators). For example, pharmaceutical compositions described herein may include a cyclodextrin or cyclodextrin derivative. Cyclodextrins are generally made up of five or more α -D-glycopyranoside units linked 1->4. Typically, cyclodextrins contain a number of glucose monomers ranging from six to eight units in a ring, creating a cone shape (α -cyclodextrin: six membered sugar ring molecule, β -cyclodextrin: seven sugar ring molecule, γ -cyclodextrin: eight sugar ring molecule). Exemplary cyclodextrins and cyclodextrin derivatives are disclosed in U.S. Patent No. 7,723,304, U.S. Publication No. 2010/0196452, and U.S. Publication No. 2010/0144624, the entire contents of each of which are incorporated herein by reference. For example, in some embodiments, a cyclodextrin in accordance with the present invention is an alkylated cyclodextrin, hydroxyalkylated cyclodextrin, or acylated cyclodextrin. In some embodiments, a cyclodextrin is a hydroxypropyl β -cyclodextrin. Exemplary cyclodextrin derivatives are disclosed in Szejtli, J. Chem Rev, (1998), 98, 1743-1753; and Szente, L and Szejtli, J., Advance Drug Delivery Reviews, 36 (1999) 17-28, the entire contents of each of which are hereby incorporated by reference. Examples of cyclodextrin derivatives include methylated cyclodextrins (e.g., RAMEB; randomly methylated β -cyclodextrin); hydroxyalkylated cyclodextrins (hydroxypropyl- β -cyclodextrin and hydroxypropyl γ -cyclodextrin); acetylated cyclodextrins (acetyl- γ -cyclodextrin); reactive cyclodextrins (chlorotriazinyl β -cyclodextrin); and branched cyclodextrins (glucosyl- and maltosyl β -cyclodextrin); acetyl- γ -cyclodextrin; acetyl- β -cyclodextrin, sulfobutyl- β cyclodextrin, sulfated α -, β - and γ -cyclodextrins; sulfoalkylated cyclodextrins; and hydroxypropyl β -cyclodextrin.

Dosing

[0203] Typically, active agent described herein (e.g. mTOR modulators) in an amount ranging from 0.001 to 100 mg/kg/day is administered to the subject. For example, in some

embodiments, about 0.01 mg/kg/day to about 25 mg/kg/day, about 1 mg/kg/day to about 20 mg/kg/day, 0.2 mg/kg/day to about 10 mg/kg/day, about 0.02 mg/kg/day to about 0.1 mg/kg/day, or about 1 mg/kg/day to about 100 mg/kg/day is administered to the subject. In some embodiments, active agent described herein (e.g. mTOR modulators) in an amount of about 10 µg/kg/day, 50 µg/kg/day, 100 µg/kg/day, 200 µg/kg/day, 300 µg/kg/day, 400 µg/kg/day, 500 µg/kg/day, 600 µg/kg/day, 700 µg/kg/day, 800 µg/kg/day, 900 µg/kg/day, or 1000 µg/kg/day is administered to the subject.

[0204] In some embodiments, the mTOR modulator is administered at an effective dose ranging from about 1-1,000 µg/kg/day (e.g., ranging from about 1-900 µg/kg/day, 1-800 µg/kg/day, 1-700 µg/kg/day, 1-600 µg/kg/day, 1-500 µg/kg/day, 1-400 µg/kg/day, 1-300 µg/kg/day, 1-200 µg/kg/day, 1-100 µg/kg/day, 1-90 µg/kg/day, 1-80 µg/kg/day, 1-70 µg/kg/day, 1-60 µg/kg/day, 1-50 µg/kg/day, 1-40 µg/kg/day, 1-30 µg/kg/day, 1-20 µg/kg/day, 1-10 µg/kg/day). In some embodiments, the mTOR modulator is administered at an effective dose ranging from about 1-500 µg/kg/day. In some embodiments, the mTOR modulator is administered at an effective dose ranging from about 1-100 µg/kg/day. In some embodiments, the mTOR modulator is administered at an effective dose ranging from about 1-60 µg/kg/day. In some embodiments, the mTOR modulator is administered at an effective dose selected from about 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1,000 µg/kg/day.

[0205] In some embodiments, a therapeutically effective amount of an mTOR modulator may be an amount ranging from about 10-1,000 mg (e.g., about 20 mg – 1,000 mg, 30 mg – 1,000 mg, 40 mg – 1,000 mg, 50 mg – 1,000 mg, 60 mg – 1,000 mg, 70 mg – 1,000 mg, 80 mg – 1,000 mg, 90 mg – 1,000 mg, about 10-900 mg, 10-800 mg, 10-700 mg, 10-600 mg, 10-500 mg, 100-1000 mg, 100-900 mg, 100-800 mg, 100-700 mg, 100-600 mg, 100-500 mg, 100-400 mg, 100-300 mg, 200-1000 mg, 200-900 mg, 200-800 mg, 200-700 mg, 200-600 mg, 200-500 mg, 200-400 mg, 300-1000 mg, 300-900 mg, 300-800 mg, 300-700 mg, 300-600 mg, 300-500 mg, 400 mg – 1,000 mg, 500 mg – 1,000 mg, 100 mg - 900 mg, 200 mg – 800 mg, 300 mg – 700 mg, 400 mg – 700 mg, and 500 mg – 600 mg). In some embodiments, an mTOR modulator is

present in an amount of or greater than about 10 mg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg. In some embodiments, an mTOR modulator is present in an amount of or less than about 1000 mg, 950 mg, 900 mg, 850 mg, 800 mg, 750 mg, 700 mg, 650 mg, 600 mg, 550 mg, 500 mg, 450 mg, 400 mg, 350 mg, 300 mg, 250 mg, 200 mg, 150 mg, or 100 mg. In some embodiments, the therapeutically effective amount described herein is provided in one dose. In some embodiments, the therapeutically effective amount described herein is provided in one day.

[0206] In other embodiments, a therapeutically effective amount may be, for example, about 0.001 mg/kg weight to 500 mg/kg weight, e.g., from about 0.001 mg/kg weight to 400 mg/kg weight, from about 0.001 mg/kg weight to 300 mg/kg weight, from about 0.001 mg/kg weight to 200 mg/kg weight, from about 0.001 mg/kg weight to 100 mg/kg weight, from about 0.001 mg/kg weight to 90 mg/kg weight, from about 0.001 mg/kg weight to 80 mg/kg weight, from about 0.001 mg/kg weight to 70 mg/kg weight, from about 0.001 mg/kg weight to 60 mg/kg weight, from about 0.001 mg/kg weight to 50 mg/kg weight, from about 0.001 mg/kg weight to 40 mg/kg weight, from about 0.001 mg/kg weight to 30 mg/kg weight, from about 0.001 mg/kg weight to 25 mg/kg weight, from about 0.001 mg/kg weight to 20 mg/kg weight, from about 0.001 mg/kg weight to 15 mg/kg weight, from about 0.001 mg/kg weight to 10 mg/kg weight. In some embodiments, the therapeutically effective amount described herein is provided in one dose. In some embodiments, the therapeutically effective amount described herein is provided in one day.

[0207] In still other embodiments, a therapeutically effective amount may be, for example, about 0.0001 mg/kg weight to 0.1 mg/kg weight, e.g. from about 0.0001 mg/kg weight to 0.09 mg/kg weight, from about 0.0001 mg/kg weight to 0.08 mg/kg weight, from about 0.0001 mg/kg weight to 0.07 mg/kg weight, from about 0.0001 mg/kg weight to 0.06 mg/kg weight, from about 0.0001 mg/kg weight to 0.05 mg/kg weight, from about 0.0001 mg/kg weight to about 0.04 mg/kg weight, from about 0.0001 mg/kg weight to 0.03 mg/kg weight, from about 0.0001 mg/kg weight to 0.02 mg/kg weight, from about 0.0001 mg/kg weight to 0.019 mg/kg weight, from about 0.0001 mg/kg weight to 0.018 mg/kg weight, from about 0.0001 mg/kg

weight to 0.017 mg/kg weight, from about 0.0001 mg/kg weight to 0.016 mg/kg weight, from about 0.0001 mg/kg weight to 0.015 mg/kg weight, from about 0.0001 mg/kg weight to 0.014 mg/kg weight, from about 0.0001 mg/kg weight to 0.013 mg/kg weight, from about 0.0001 mg/kg weight to 0.012 mg/kg weight, from about 0.0001 mg/kg weight to 0.011 mg/kg weight, from about 0.0001 mg/kg weight to 0.01 mg/kg weight, from about 0.0001 mg/kg weight to 0.009 mg/kg weight, from about 0.0001 mg/kg weight to 0.008 mg/kg weight, from about 0.0001 mg/kg weight to 0.007 mg/kg weight, from about 0.0001 mg/kg weight to 0.006 mg/kg weight, from about 0.0001 mg/kg weight to 0.005 mg/kg weight, from about 0.0001 mg/kg weight to 0.004 mg/kg weight, from about 0.0001 mg/kg weight to 0.003 mg/kg weight, from about 0.0001 mg/kg weight to 0.002 mg/kg weight. In some embodiments, the therapeutically effective dose may be 0.0001 mg/kg weight, 0.0002 mg/kg weight, 0.0003 mg/kg weight, 0.0004 mg/kg weight, 0.0005 mg/kg weight, 0.0006 mg/kg weight, 0.0007 mg/kg weight, 0.0008 mg/kg weight, 0.0009 mg/kg weight, 0.001 mg/kg weight, 0.002 mg/kg weight, 0.003 mg/kg weight, 0.004 mg/kg weight, 0.005 mg/kg weight, 0.006 mg/kg weight, 0.007 mg/kg weight, 0.008 mg/kg weight, 0.009 mg/kg weight, 0.01 mg/kg weight, 0.02 mg/kg weight, 0.03 mg/kg weight, 0.04 mg/kg weight, 0.05 mg/kg weight, 0.06 mg/kg weight, 0.07 mg/kg weight, 0.08 mg/kg weight, 0.09 mg/kg weight, or 0.1 mg/kg weight. The effective dose for a particular individual can be varied (e.g., increased or decreased) over time, depending on the needs of the individual. In some embodiments, the therapeutically effective amount described herein is provided in one dose. In some embodiments, the therapeutically effective amount described herein is provided in one day.

[0208] In some embodiments, a provided composition is provided as a pharmaceutical formulation. In some embodiments, a pharmaceutical formulation is or comprises a unit dose amount for administration in accordance with a dosing regimen correlated with achievement of the reduced incidence or risk of Cancer. In some embodiments, a pharmaceutical formulation is or comprises a unit dose amount for administration in accordance with a dosing regimen correlated with achievement of the reduced incidence or risk of Diabetes.

[0209] In some embodiments, a formulation comprising an mTOR modulator as described herein administered as a single dose. In some embodiments, a formulation comprising an mTOR modulator as described herein is administered at regular intervals. Administration at an “interval,” as used herein, indicates that the therapeutically effective amount is administered periodically (as distinguished from a one-time dose). The interval can be determined by standard clinical techniques. In some embodiments, a formulation comprising an mTOR modulator as described herein is administered bimonthly, monthly, twice monthly, triweekly, biweekly, weekly, twice weekly, thrice weekly, daily, twice daily, or every six hours. The administration interval for a single individual need not be a fixed interval, but can be varied over time, depending on the needs of the individual.

[0210] As used herein, the term “bimonthly” means administration once per two months (*i.e.*, once every two months); the term “monthly” means administration once per month; the term “triweekly” means administration once per three weeks (*i.e.*, once every three weeks); the term “biweekly” means administration once per two weeks (*i.e.*, once every two weeks); the term “weekly” means administration once per week; and the term “daily” means administration once per day.

[0211] In some embodiments, a formulation comprising an mTOR modulator as described herein is administered at regular intervals indefinitely. In some embodiments, a formulation comprising an mTOR modulator as described herein is administered at regular intervals for a defined period. In some embodiments, a formulation comprising an mTOR modulator as described herein is administered at regular intervals for 5 years, 4, years, 3, years, 2, years, 1 year, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, a month, 3 weeks, 2, weeks, a week, 6 days, 5 days, 4 days, 3 days, 2 days or a day.

Combination Therapy

[0212] In some embodiments, the present invention provides mTOR modulator agents for use in combination with one or more additional therapeutic and/or diagnostic agents and/or modalities. In some embodiments, provided agents are useful in combination with one or more

other therapeutic agents or modalities known to be useful in the treatment or prevention of one or more mTOR-associated diseases, disorders, or conditions, and/or with the relief of one or more symptoms of such diseases, disorders, or conditions. For example, in some embodiments, provided agents are useful in combination with one or more chemotherapeutic, anti-diabetic medications and/or palliative agents. In some embodiments, provided agents are useful in combination with one or more other agents or modalities that is or are approved by the United States Food and Drug Administration or one or more other non-US.

[0213] In some embodiments, agents utilized in combination may be included in a single pharmaceutical compositions. More commonly, however, agents utilized in combination are administered in accordance with overlapping regimens so that a subject is simultaneously exposed to both (or all) agents, and/or is exposed to individual agents in a predetermined order and/or with a predetermined timing.

[0214] For purposes of the present disclosure, the term “palliative” refers to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, in some embodiments, palliative treatment encompasses painkillers and antinausea medications. Alternatively or additionally, in some embodiments, chemotherapy, radiotherapy, and surgery can all be used palliatively (that is, to reduce symptoms without going for cure; e.g., for shrinking tumors and reducing pressure, bleeding, pain, and/or other symptoms or signs of cancer).

[0215] In some embodiments, an mTOR modulator is administered in combination with one or more known therapeutic agents (e.g., anti-diabetic medications) currently used for Diabetes prophylaxis and treatment.

[0216] In some embodiments, the known therapeutic agent(s) is/are administered according to its standard or approved dosing regimen and/or schedule. In some embodiments, the known therapeutic agent(s) is/are administered according to a regimen that is altered as compared with its standard or approved dosing regimen and/or schedule. In some embodiments, such an altered regimen differs from the standard or approved dosing regimen in that one or more unit doses is altered (e.g., reduced or increased) in amount, and/or in that dosing is altered

in frequency (e.g., in that one or more intervals between unit doses is expanded, resulting in lower frequency, or is reduced, resulting in higher frequency).

Applications for mTOR Modulators

[0217] In some embodiments, the present invention provides methods and compositions useful in the detection, characterization, and/or treatment of a disease, disorder or condition that benefits from modulation of mTOR (e.g., from increase or decrease in level, form, and/or activity of mTOR). Methods for the treatment of such diseases comprise administering to a subject in need thereof a therapeutically effective amount of a composition or medicament comprising an mTOR modulator as described herein. In some embodiments, the disease, disorder or condition is a proliferative disease (e.g., cancer). In some embodiments, the disease, disorder or condition is a metabolic disease (e.g. diabetes and obesity). In some embodiments, the disease, disorder or condition is a neurodegenerative disease (e.g. Alzheimer's Disease). In some embodiments, the disease, disorder or condition is an age-associated disease. In certain embodiments, modulating mTOR activity is desirable to slow and/or reduce cellular and tissue aging.

[0218] In some embodiments, a subject is any multicellular organism. In certain embodiments, treated subject is a mammal. In some embodiments, a subject is a mouse, rat, dog, non-human primate or other animal commonly used for laboratory experiments. In some embodiments, a subject is an individual. In certain embodiments, the subject being treated is a human. In some embodiments, a subject has or is susceptible to an mTor-associated disease, disorder, or condition.

[0219] In some embodiments, a subject has or is susceptible to proliferative disease, disorder or condition. In some embodiments, a proliferative disease, disorder, or condition is or comprises an infection; in some embodiments, a proliferative disease, disorder, or condition is or comprises cancer, an immune system disease, disorder, or condition (e.g., transplant rejection,

graft vs. host disease, immune reaction to gene therapy, autoimmune diseases, pathogen-induced immune dysregulation, etc), angiogenesis or macular degeneration.

[0220] The present disclosure provides methods and compositions relating to treatment of cell proliferative disease, disorder or condition. In general, cell proliferative disorders, diseases or conditions include a variety of conditions characterized by aberrant cell growth, preferably abnormally increased cellular proliferation. For example, cell proliferative disorders, diseases, or conditions include, but are not limited to, cancer, immune-mediated responses and diseases (e.g., transplant rejection, graft vs. host disease, immune reaction to gene therapy, autoimmune diseases, pathogen-induced immune dysregulation, etc.), certain circulatory diseases, certain infections, and certain neurodegenerative diseases. In some embodiments, a subject has or is susceptible to metabolic disease, disorder or condition. In some embodiments, a subject has or is susceptible to neurodegenerative disease, disorder or condition. In some embodiments, a subject has or is susceptible to an age-associated disease, disorder or condition. In some embodiments, a subject has or is susceptible to an immune disease, disorder or condition.

[0221] In some embodiments, the present invention provides methods of treating proliferative disease, disorder, or conditions including administering to a subject who is suffering from or susceptible to a proliferative disease, disorder or condition an mTOR modulator. Exemplary proliferative disease, disorder, or conditions include bone cancer, brain cancer, blood cancer, breast cancer, pancreatic cancer, lung cancer, kidney cancer, stomach cancer, ovarian cancer, prostate cancer, colorectal cancer, endometrial cancer, bladder cancer, non-Hodgkin lymphoma, leukemia, neuroblastoma, lymphomas, rhabdomyosarcoma, Wilms' tumor, osteosarcoma, and Ewing's sarcoma. In some embodiments, the cancer is a solid tumor.

[0222] In certain embodiments of the present invention a "therapeutically effective amount" of the inventive compound or pharmaceutical composition is that amount effective for reducing or inhibiting the growth of tumor cells and/or killing tumor cells.

[0223] Other additional anticancer agents that are useful in the compositions and methods of the present invention include, but are not limited to: acivicin; aclarubicin; acodazole

hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminogluthethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefmgol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; interleukin-2 (including recombinant interleukin-2, or rIL2), interferon alfa-2 α ; interferon alfa-2 β ; interferon alfa-n1 ; interferon alfa-n3; interferon beta-1 α ; interferon gamma-1 β ; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedpa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamyciii; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprime; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone

hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; tricirbine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; and zorubicin hydrochloride.

[0224] Further anticancer drugs that are useful in the methods and compositions of the invention include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D₃; 5- ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ ABL antagonists; benzochlorins; benzoylstauroporine; beta Lactam Derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermme; bisnafide; bistratene A; bizelesin; breflate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; carboxamide- amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorlms; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin Analogue; conagenin; crambescidin 816; crisnatol; cryptoplycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplata; cypemycin; cytarabine ocfosphate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone;

dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemniin B; didox; diethylnorspermine;
 dihydro-5-acytidine; dihydrotaxol; dioxamycin; diphenyl spiromustine; docetaxel; docosanol;
 dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine;
 edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine
 analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane;
 fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone;
 fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine;
 gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors;
 gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide;
 hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat;
 imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor
 inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol,
 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide;
 kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate;
 leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon;
 leuprolide+estrogen+ progesterone; leuprorelin; levamisole; liarozole; linear polyamine
 Analogue; lipophilic disaccharide peptide; lipophilic platinum complexes; lissoclinamide 7;
 lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan;
 lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat;
 masoprocyl; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril;
 merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine;
 mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin Analogues;
 mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene;
 molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid
 A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple
 tumor suppressor 1 -based . therapy; mustard anticancer agents; mycaperoxide B; mycobacterial
 cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip;
 naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic
 acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide

antioxidant; nitrullin; 06-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel Analogues; paclitaxel derivatives; palauamiiiie; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum complexes; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inliibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; raboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1 ; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfm; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrigan; thyroid stimulating hormone; tin ethyl etiopurpurirt; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex;

urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; ver amine; verdins; verteporfm; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[0225] The mTOR modulator can be administered to a subject that has undergone or is currently undergoing one or more additional anticancer therapies including, but not limited to, surgery, radiation therapy, or immunotherapy, such as cancer vaccines.

[0226] In some embodiments, the invention provides methods for treating or preventing cancer comprising administering to a subject in need thereof an effective amount of an mTOR modulator to treat or prevent cancer and another anticancer therapy including, but not limited to, surgery, radiation therapy, or immunotherapy, such as a cancer vaccine.

[0227] In some embodiments, the other anticancer therapy is radiation therapy. In another embodiment, the other anticancer therapy is surgery. In still another embodiment, the other anticancer therapy is immunotherapy.

[0228] In some embodiments, the present methods for treating or preventing cancer comprise administering an effective amount of an mTOR modulator and radiation therapy. The radiation therapy can be administered concurrently with, prior to, or subsequent to the mTOR modulator. In some embodiments, the radiation therapy can be administered at least an hour, five hours, 12 hours, a day, a week, a month, in another embodiment several months (e.g., up to three months), prior or subsequent to administration of the mTOR modulator. Where the other anticancer therapy is radiation therapy, any radiation therapy protocol can be administered depending upon the type of cancer to be treated.

[0229] In some embodiments, the present invention provides methods of treating an immune system disease, disorder or condition including administering to a subject who is suffering from or susceptible to an immune system disease, disorder or condition an mTOR modulator. In general, immune-mediated responses, disorders, diseases or conditions include a variety of conditions characterized by and/or relating to treatment of rejection following

transplantation of synthetic or organic grafting materials (e.g., cells, organs, or tissue to replace all or part of the function of tissues, such as heart, kidney, liver, bone marrow, skin, cornea, vessels, lung, pancreas, intestine, limb, muscle, nerve tissue, duodenum, small-bowel, pancreatic-islet-cell, including xeno-transplants, *etc.*), treatment of graft-versus-host disease; autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type I diabetes, juvenile-onset or recent-onset diabetes mellitus, uveitis, Graves' disease, psoriasis, atopic dermatitis, Crohn's disease, ulcerative colitis, vasculitis, auto-antibody mediated diseases, aplastic anemia, Evan's syndrome, autoimmune hemolytic anemia, and the like.

[0230] In some embodiments, the disclosure relates to treatment of an immune response associated with a gene therapy treatment, such as the introduction of foreign genes into autologous cells and expression of the encoded product. In some embodiments, the disclosure relates to treatment of circulatory diseases, such as arteriosclerosis, atherosclerosis, vasculitis, polyarteritis nodosa and/or myocarditis.

[0231] In some embodiments, the present invention provides methods for treating infection including administering to a subject who is suffering from or susceptible to an infection an mTOR modulator. In general, infections can be caused by the growth of a microorganism such as a fungus, protozoa, virus, bacteria (Gram-positive or Gram-negative), or parasite.

[0232] For example, bacterial infections include, but are not limited to growth of Actinomycosis; Acute prostatitis; Aeromonas hydrophila; Annual ryegrass toxicity; Anthrax; Bacteremia; Bacterial meningitis; Bacterial pneumonia; Brazilian purpuric fever; Brodie's abscess; Bubonic plague; Brucellosis; Burkholderia cepacia complex; Buruli ulcer; Campylobacteriosis; Capnocytophaga canimorsus; Caries; Carrion's disease; Chlamydia; Cholera; Diphtheria; Diphtheritic stomatitis; Donovanosis; Erythema migrans; Fitz-Hugh-Curtis syndrome; Fournier gangrene; Group A streptococcal infection; Human granulocytic ehrlichiosis; Impetigo; Late congenital syphilis; Late congenital syphilitic ophthalmopathy; Legionella; Lemierre's syndrome; Leprosy; Leptospirosis; Listeriosis; Ludwig's angina; Lyme

disease; Melioidosis; Meningococemia; Methicillin-resistant *Staphylococcus aureus*; Miliary tuberculosis; *Mycobacterium*; *Mycobacterium avium* complex; Necrotizing fasciitis; Nontuberculous mycobacteria; Omphalitis; Orbital cellulitis; Osteomyelitis; Paratyphoid fever; *Pasteurella multocida*; Periorbital cellulitis; Peritonsillar abscess; Pertussis; Pott's disease; Pseudomembranous colitis; Psittacosis; Pyomyositis; Q fever; Rheumatic fever; *Rickettsia prowazekii*; *Rickettsialpox*; Salmonellosis; Scarlet fever; Scrub typhus; Spondylitis; Staphylococcal infection; Strep throat; Syphilis; Syphilitic aortitis; Tetanus; Tuberculosis; Tularemia; Typhoid fever; or Typhus.

[0233] Exemplary viral infections include, but are not limited to, Acquired Immunodeficiency Syndrome; Adenoviridae Infections; Alphavirus Infections; Arbovirus Infections; Borna Disease ; Bunyaviridae Infections; Caliciviridae Infections; Chickenpox; Condyloma Acuminata; Coronaviridae Infections; Coxsackievirus Infections; Cytomegalovirus Infections; Dengue; DNA Virus Infections; Ecthyma; Encephalitis; Arbovirus; Epstein-Barr Virus Infections; Erythema Infectiosum; Hantavirus Infections; Hemorrhagic Fevers; Hepatitis; Herpes; Herpesviridae Infections; Infectious Mononucleosis; Influenza in birds; Influenza in humans; Lassa Fever; Measles; Molluscum Contagiosum; Mumps; Paramyxoviridae Infections; Phlebotomus Fever; Polyomavirus Infections; Rabies; Respiratory Syncytial Virus Infections; Rift Valley Fever; RNA Virus Infections; Rubella; Slow Virus Diseases; Smallpox; Subacute Sclerosing Panencephalitis; Tumor Virus Infections; Warts; West Nile Fever; Yellow Fever.

[0234] Exemplary parasitical infections include, but are not limited to Amebiasis; Anisakiasis; Ascariasis; Babesiosis; Blastocystis hominis infections; Cestode Infections; Chagas Disease; Cryptosporidiosis; Cyclosporiasis; Cysticercosis; Dientamoebiasis; Diphyllbothriasis; Dracunculiasis; Echinococcosis; Ectoparasitic Infestations; Filariasis; Giardiasis; Helminthiasis; Hookworm Infections; Intestinal Diseases, Parasitic; Larva Migrans; Leishmaniasis; Lice Infestations; Loiasis; Malaria; Mite Infestations; Myiasis; Neurocysticercosis; Onchocerciasis; Protozoan Infections; Scabies; Schistosomiasis; Skin Diseases, Parasitic; Strongyloidiasis; Taeniasis; Toxocariasis; Toxoplasmosis; Trichinosis; Trichomonas Infections; Trypanosomiasis; or Whipworm Infections.

[0235] Exemplary fungal infections include, but are not limited to Ascomycota, Neoelectomycetes, Pneumocystidomycetes, Schizosaccharomycetes, Taphrinomycetes, Pezizomycotina, Arthoniomycetes, Dothideomycetes, Geoglossomycetes, Eurotiomycetes, Laboulbeniomycetes, Lecanoromycetes, Acarosporomycetidae, superficial mycoses, Dermatormycosis furfuracea, Pityriasis versicolor, Tinea versicolor, cutaneous mycoses, subcutaneous mycoses, Trichophyton rubrum, Candida albicans, Trichophyton mentagrophytes, Ephidermophyton floccosum, tinea pedis, Lecanoromycetidae, Ostropomycetidae, Leotiomycetes, Lichinomycetes, Orbiliomycetes, Pezizomycetes, Sordariomycetes, Hypocreomycetidae, Sordariomycetidae, Xylariomycetidae, Saccharomycotina, Saccharomycetes, Basidiomycota, Agaricomycotina, Agaricomycetes, Dacrymycetes, Tremellomycetes, Pucciniomycotina, Agaricostilbomycetes, Attractiellomycetes, Classiculomycetes, Cryptomycocolacomycetes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes, Pucciniomycetes, Ustilaginiomycotina, Ustilaginomycetes, Exobasidiomycetes, Chytridiomycota, Glomeromycota, Glomeromycetes, Zygomycota, Trichomycetes, or Zygomycetes.

[0236] In some embodiments, the present invention provides methods of treating a neurodegenerative disease, disorder or condition including administering to a subject who is suffering from or susceptible to a neurodegenerative disease, disorder or condition an mTOR modulator. In general, neurodegenerative disease, disorder or conditions include, but are not limited to:

I. Disorders characterized by progressive dementia in the absence of other prominent neurologic signs, such as Alzheimer's disease; Senile dementia of the Alzheimer type; and Pick's disease (lobar atrophy);

II. Syndromes combining progressive dementia with other prominent neurologic abnormalities such as A) syndromes appearing mainly in adults (*e.g.*, Huntington's disease, Multiple system atrophy combining dementia with ataxia and/or manifestations of Parkinson's disease, Progressive supranuclear palsy (Steel-Richardson-Olszewski), diffuse Lewy body

disease, and corticodentatonigral degeneration); and B) syndromes appearing mainly in children or young adults (*e.g.*, Hallervorden-Spatz disease and progressive familial myoclonic epilepsy);

III. Syndromes of gradually developing abnormalities of posture and movement such as paralysis agitans (Parkinson's disease), striatonigral degeneration, progressive supranuclear palsy, torsion dystonia (torsion spasm; dystonia musculorum deformans), spasmodic torticollis and other dyskinesia, familial tremor, and Gilles de la Tourette syndrome;

IV. Syndromes of progressive ataxia such as cerebellar degenerations (*e.g.*, cerebellar cortical degeneration and olivopontocerebellar atrophy (OPCA)); and spinocerebellar degeneration (Friedreich's ataxia and related disorders);

V. Syndromes of central autonomic nervous system failure (Shy-Drager syndrome);

VI. Syndromes of muscular weakness and wasting without sensory changes (motoneuron disease such as amyotrophic lateral sclerosis, spinal muscular atrophy (*e.g.*, infantile spinal muscular atrophy (Werdnig-Hoffman), juvenile spinal muscular atrophy (Wohlfart-Kugelberg-Welander) and other forms of familial spinal muscular atrophy), primary lateral sclerosis, and hereditary spastic paraplegia;

VII. Syndromes combining muscular weakness and wasting with sensory changes (progressive neural muscular atrophy; chronic familial polyneuropathies) such as peroneal muscular atrophy (Charcot-Marie-Tooth), hypertrophic interstitial polyneuropathy (Dejerine-Sottas), and miscellaneous forms of chronic progressive neuropathy;

VIII. Syndromes of progressive visual loss such as pigmentary degeneration of the retina (retinitis pigmentosa), and hereditary optic atrophy (Leber's disease).

[0237] In some embodiments, the neurodegenerative disease is Alzheimer's disease, Parkinson's disease, and/or Huntington's disease.

[0238] In some embodiments, the present invention provides methods of treating metabolic disease, disorder or conditions including administering to a subject who is suffering from or susceptible to a metabolic disease, disorder or condition an mTOR modulator. For

example, metabolic disease, disorder or conditions include, but are not limited to, metabolic syndrome, obesity, hyperthyroidism, hypothyroidism, , Diabetes mellitus (type I and type II), dyslipidemia, lipolipidemia, galactosemia, or phenylketonuria. In some embodiments, a metabolic disease, disorder or condition comprises an elevated blood sugar level. In some embodiments, a metabolic disease, disorder or condition comprises metabolic syndrome (triglycerides greater than 150 mg/dL (1.7mmol/L); HDL cholesterol less than 40 mg/dL (1.03 mmol/L) in males; HDL cholesterol less than 50 mg/dL (1.29 mmol/L) in females; raised blood pressure; and raised fasting plasma glucose levels; and/or hypertension), impaired glucose tolerance, insulin resistance, hyperinsulinemia and lipodystrophic disorders (e.g., Berardinelli-Seip congenital lipodystrophy, Dunnigan familial partial lipodystrophy, HIV-related lipodystrophy).

[0239] In some embodiments, the present invention provides methods of treating an age-associated disease including administering to a subject who is suffering from or susceptible to an age-associated disease an mTOR modulator.

Exemplification

Example 1: Expression and Crystallization of an mTOR Polypeptide, mTOR^{ΔN}

[0240] The mammalian target of rapamycin (mTOR), a phosphoinositide 3-kinase related protein kinase (PIKK), controls cell growth in response to nutrients and growth factors and is frequently deregulated in cancer. The present Example describes development of an expression system that permits crystallization of a truncated mTOR polypeptide, containing all regions common to PIKKs, and further permits determination of the structure of crystals of the mTOR polypeptide bound to the activating mLST8 subunit and to an ATP transition state mimic. The determined structures reveal an intrinsically active kinase conformation, with catalytic residues and mechanism remarkably similar to canonical protein kinases. The active site is highly recessed due to the FKBP12-Rapamycin binding (FRB) domain and an inhibitory helix protruding from the catalytic cleft. mTOR activating mutations map to the structural framework that holds these elements in place, indicating that the kinase is controlled by restricted access. In vitro biochemistry indicates that the FRB domain acts as a gatekeeper, with its rapamycin-binding site interacting with substrates to grant them access to the restricted active site. The FKBP12-rapamycin complex inhibits by directly blocking substrate recruitment and by further restricting active site access.

[0241] Specifically, the present Example presents the 3.2 Å crystal structure of an ~1500 amino acid mTOR polypeptide-mLST8 complex containing the FAT, FRB, kinase and FATC domains, as well as the structures of this complex bound to an ATP transition state analog and to ATP-competitive inhibitors. We discuss their implications for understanding mTOR function, regulation and inhibition by rapamycin and ATP-competitive compounds.

Overall structure of mTOR^{ΔN}-mLST8

[0242] Crystals were grown using an N-terminally truncated human mTOR (residues 1376 to 2549; thereafter mTOR^{ΔN}) bound to full-length human mLST8 (FIG. 7). The complex was produced in an HEK293-F cell line that was stably-transfected sequentially by FLAG-tagged mLST8 and FLAG-tagged mTOR^{ΔN} vectors. The kinase activity of mTOR^{ΔN}-mLST8 is overall comparable to that of mTORC1 (FIG. 10). mTORC1 is more active towards lowmicromolar concentrations of S6K1^{ki} (kinase-inactive mutant) and 4EBP1, consistent with RAPTOR recruiting these substrates through their TOS motifs, whereas mTOR^{ΔN}-mLST8 is more active at higher substrate concentrations.

[0243] The mTOR^{ΔN}-mLST8 structure has a compact shape (FIG. 1A). The FAT domain, which consists of α - α helical repeats, forms a “C” shaped a solenoid that wraps halfway around the kinase domain and clamps on it (FIG. 1B). mLST8 and the FRB domain protrude from the kinase domain, on opposite sides of the catalytic cleft. The FATC is integral to the kinase domain structure.

[0244] The ~550-residue mTOR kinase domain (KD) adopts the two-lobe structure that is characteristic of both the PI3K and canonical protein kinase families²⁹. It consists of an N-terminal lobe (N lobe) rich in β sheet, a larger C-terminal lobe (C lobe), and a cleft in-between the two that binds to ATP. It shares a core structure with PI3K KD fold, but with substantial differences. While the KD structures of the five PI3Ks superimpose on each other essentially across their entire ~350 residues, only ~250 residues of the mTOR KD align with PI3Ks (FIG. 8). In addition, the mTOR KD contains ~200 additional residues in unique structural elements that decorate the common fold. The largest of these is the FRB domain (residues 2021 to 2118) that is inserted within the kinase N lobe (FIG. 1A). There is also a ~40 residue insertion in the C lobe that forms the binding site for mLST8 (residues 2258 to 2296; thereafter LBE).

Kinase domain structure

[0245] The mTOR KD structure starts prior to the FRB domain, with the long $\kappa\alpha 1$ helix that is present in PI3Ks as well (FIG. 2A and FIG. 11A). The $\kappa\alpha 1$ helix is integral to the structure

of the N lobe, as it packs in the concave surface of the 5-stranded N lobe β sheet in both mTOR and PI3Ks²⁹. The FRB insertion occurs immediately after $\alpha 1$, in an area where PI3Ks contain a short crossover loop to a $\alpha 2$ helix that packs with $\alpha 1$. In mTOR, the PI3K $\alpha 2$ is replaced by a β strand and by two short helices that pack with the base of the FRB. Thereafter, the mTOR and PI3K N lobes share a similar structure across the $\beta 1$ - $\beta 7$ sheet and the intervening $\alpha 3$ helix.

[0246] The mTOR structure indicates that the $\alpha 1$ helix is present in the remainder of PIKKs as well (FIG. 12A). The SMG1, DNAPKcs and TRRAP PIKKs also appear to have an FRB-like domain, as they contain insertions of 128, 95 and 128 residues, respectively, that are predicted to be alpha-helical. This is supported by the 6.6 Å x-ray diffraction data of DNAPKcs reported recently³⁰. Although the deposited model, which contains a PI3K-derived kinase domain, lacks the FRB insertion, a reinterpretation of the data using the mTOR structure reveals strong *Fo-Fc* electron density indicative of an FRB-like four-helix bundle (FIG. 12B). ATM and ATR appear to lack a comparable insertion.

[0247] In the C lobe, the vicinity of the catalytic cleft contains four structural insertions compared to PI3Ks (LBE, αAL , $\alpha 9b$ and FATC; FIG. 2A). These form a spine of interactions centered on the activation loop, which is a ~30 residue segment with a central role in the function and regulation of canonical protein kinases. It forms part of the polypeptide binding site, provides an active site residue, and also undergoes a conformational change, typically disorder-to-order transition, upon kinase activation³¹. The activation loop is thought to have an analogous role in PI3Ks, among which only in the class 3 PIK3C3/Vps34 structures it is ordered³². In the mTOR structure the entire activation loop is well-ordered (FIG. 11B), and its middle portion contains the αAL helix insertion. One side of the activation loop packs with the $\alpha 9b$ insertion, and the other side with the FATC (FIG. 11A). The FATC is not unique to PIKKs in its entirety, as its N-terminal half forms a helix ($\alpha 11$) that is also present in PI3K structures^{29,32}. Its C-terminal half, which is absent from PI3Ks, forms three short helices that pack with the activation loop on one side and the LBE on the other (Fig. 2a).

[0248] The interactions the FATC makes with the activation loop suggest that it may have a role in stabilizing the activation loop structure, and the LBE, through its interactions with

the FATC, may contribute to this indirectly (FIG. 13A). Consistent with this notion, the FATC and activation loop sequences are highly conserved among PIKKs. While the LBE is not conserved, all PIKK family members contain an LBE-like insertion that may similarly pack with FATC (FIG. 12A).

[0249] The $\kappa\alpha 9b$ insertion (residues 2425 to 2436) plugs one end of the catalytic cleft (FIG. 11A). It partially overlaps with a segment, termed negative regulatory domain (residues 2430 to 2450), whose deletion activates mTOR in vitro³³ and in vivo^{34,35}. After $\kappa\alpha 9b$, there is a 55 residue unstructured segment (2437 to 2491) that is not conserved and has variable length in mTOR orthologs (FIG. 11A).

Active site conformation

[0250] To assess the activation state of the KD structure and investigate the mechanism of phosphotransfer, we co-crystallized mTOR ^{ΔN} -mLST8 with ADP, Mg^{2+} and MgF_3^- , a mimic of the γ -phosphate group of ATP in the transition state (TS)³⁶ (FIG. 7). The 3.5 Å Fo-Fc map of these crystals shows strong electron density extending -from the β -phosphate group of ADP, consistent with a bound MgF_3^- group (FIG. 2B). The presence of two additional Mg^{2+} ions is supported by the anomalous dispersion maps of apo-crystals soaked in AMPPNP- Mn^{2+} (FIG. 13B).

[0251] A superposition with the canonical protein kinase CDK2 bound to the same ATP TS mimic³⁶ reveals that the arrangement of key active site residues is conserved in mTOR (FIG. 2C and FIG. 13C). These include the C lobe Asn2343 and Asp2357, which serve as ligands for the two metal ions, and the N lobe Lys2187, which coordinates the ATP phosphate groups. In addition, Asp2338 superimposes remarkably well with the CDK2 Asp127, which is a key catalytic residue that helps orient and activate the substrate hydroxyl group for nucleophilic attack^{36,37}. The mTOR-CDK2 superposition also reveals a coincidence of the mTOR His2340 and CDK2 Lys129 side chains (FIG. 2C). In protein kinases, a basic residue at this position interacts with both the substrate hydroxyl group and the γ -phosphate TS mimic, and is postulated to stabilize the buildup of charge at the transition state^{36,37}. Consistent with a similar role, we

find that the kinase activity of the H2340A mTOR^{ΔN}-mLST8 mutant is barely detectable, and comparable to the D2338A mutant previously shown to be inactive³⁸ (FIG. 13D).

[0252] These findings strongly suggest that the crystallized mTOR^{ΔN}-mLST8 complex is intrinsically active in the absence of any additional regulatory subunits. They also indicate that PIKKs, and most likely PI3Ks, employ the same catalytic mechanism as canonical protein kinases, in contrast to a recently-proposed PI3K mechanism³² (see legend of FIG. 13C).

Substrate-binding site

[0253] The ATP TS γ -phosphate group mimic points to an extended C lobe groove that is highly conserved and is the likely site of substrate binding. FIG. 2D illustrates this with a CDK2-bound substrate peptide docked by superposing the mTOR and CDK2 TS³⁶ complexes. The groove consists of the activation loop, as in canonical protein kinases, but also of portions of the FATC and LBE.

[0254] In the (+) direction (C-terminal to phosphorylation site), the groove extends only to the +1 position, abruptly terminating with the α 9b helix that plugs this end of the cleft (FIG. 2D). Thereafter, the peptide can exit following the surface of the C lobe towards mLST8, or along the N lobe and towards the FRB. The +1 portion of the groove has a pronounced pocket lined with three aromatic residues, consistent with the two classes of mTOR substrates, exemplified by 4EBP1 and S6K1, having a proline and tyrosine residue, respectively, at +1 (FIG. 15A and FIG. 15B). The groove extends substantially more in the (-) direction, and it may account for the low-level sequence preference at -4 and -5 suggested by a positional scanning peptide array³⁹ (FIG. 2D).

Restricted access to the active site

[0255] The four-helix bundle FRB substantially extends the N lobe side of the catalytic cleft, running roughly parallel to the C lobe platform and mLST8 on the other side of the cleft. This gives the cleft a deep, “V”-shaped cross-section, restricting access to the substrate-binding site at the bottom of the cleft (FIG. 3A). Substrate access is further hindered by one end of the cleft being plugged by α 9b and the following 55-residue unstructured segment. The deletion that defined the negative regulatory region³³⁻³⁵ (residues 2430 to 2450) encompasses 7 of 12 α 9b residues and 14 of 55 unstructured-segment residues. We find that deleting the bulk of the unstructured segment (44 residues; 2443-2486) does not activate mTOR (FIG. 15), suggesting that α 9b is the key element within the negative regulatory domain.

[0256] While the end of the cleft opposite from α 9b is unencumbered in the mTOR ^{Δ N}-mLST8 structure, it is near the FAT N-terminus, and the region deleted from the crystallized

mTOR^{ΔN} may restrict cleft access directly and/or through its associated RAPTOR or RICTOR proteins. In support, we find that towards TOS-mutant 4EBP1 and S6K1^{ki} substrates, mTOR^{ΔN}-mLST8 is more active than mTOR-mLST8, which in turn is more active than mTOR-mLST8-RAPTOR (FIG. 15). A restricted active site would reduce the probability of substrates hitting the active site by random diffusion, and could thus be important in allowing for the regulation of phosphorylation by substrate-recruitment.

Inhibition by rapamycin-FKBP12

[0257] In a model constructed by superposing the FRB domains of the FRB-rapamycin-FKBP1224 and mTOR^{ΔN}-mLST8 structures, FKBP12 extends from the FRB on the N lobe towards mLST8 on the C lobe, nearly capping the catalytic cleft (FIG. 3B). At their closest approach, FKBP12 and mLST8 are only 8 Å apart, with the catalytic center recessed by 37 Å. The model suggests that rapamycin-FKBP12 inhibits in part by drastically reducing the accessibility of the already constricted catalytic cleft. This is consistent with the extent of mTORC1 inhibition being substrate and phosphorylation site dependent²⁵.

[0258] In mTORC2, which is not inhibited by nanomolar rapamycin, there may not be enough space for rapamycin-FKBP12 to access the binding site on the FRB due to the proximity of other mTORC2 components. Consistent with the model that it is the FKBP12 component of rapamycin-FKBP12 that precludes binding to mTORC2, rapamycin can inhibit mTORC2 at micromolar concentrations, when it can bind to the FRB in the absence of FKBP12^{Supp.4}. Also consistent with this model is the reduction in mTORC2 levels upon prolonged incubation with nanomolar rapamycin^{Supp.5}, presumably because the de novo assembly of RICTOR with mTOR, which will now be bound to FKBP12-rapamycin, is blocked.

[0259] Prolonged rapamycin exposure can also destabilize the association of mTOR with RAPTOR^{Supp.6, Supp.7}, as well as a dimeric arrangement of mTORC1^{Supp.8}, although neither of these effects are observed with yeast TORC1^{Supp.9, Supp.10}. It is conceivable that RAPTOR is close enough to the active site that there is barely enough space for FKBP12-rapamycin to fit in. Any residual steric hindrance may destabilize mTOR-RAPTOR during the binding equilibrium of the complex.

FRB recruits S6K1 to active site

[0260] The rapamycin-binding site maps to the FRB surface closest to the active site, in the midst of a hotspot of highly conserved residues (FIG. 4A). Four of these residues are invariant in 22 mTOR orthologs from yeast to man (Ser2035, Phe2039, Trp2102, Phe2108), and another four in 21 (Arg2036, Gln2099, Asp2102, Tyr2105), in stark contrast to the FRB surface opposite from the active site (FIG. 4A and FIG. 16). This raises the possibility that the rapamycin-binding site interacts with substrates to facilitate their entry to the recessed active site.

[0261] This model is supported by the observation that mutation of Ser2035, a rapamycin contact at the centre of the conservation hotspot^{38,40}, reduces phosphorylation of S6K1 and 4EBP133. It can also explain inhibition of mTORC1 and mTORC2 by rapamycin in the absence of FKBP12, albeit at ~100-fold higher micromolar concentrations reflecting the weaker rapamycin-FRB binary interaction⁴¹. However, these observations can also be explained by the rapamycin-binding site interacting with other parts of mTOR, or with associated proteins, especially since rapamycin was thought to be an allosteric inhibitor²⁵. To rule this out, we assayed mTOR^{ΔN}-mLST8, and found that rapamycin but not the closely-related FK506 macrolide inhibits S6K1^{ki} overall phosphorylation, as measured by ³²P incorporation (FIG. 4B). Inhibition reaches ~45 % at the 20 μM solubility limit of rapamycin, close to the free rapamycin-FRB K_d. Phosphorylation of the key Thr389 site of the S6K1 HM motif, measured by a phosphoT389-specific antibody, is inhibited by ~80 % (FIG. 4B). The ~150-residue C-terminal tail of S6K1 contains additional in vitro phosphorylation sites, although most are of unknown significance^{33,42}.

[0262] In a complementary approach, we tested whether the isolated FRB domain inhibits phosphorylation in *trans*. FIG. 4C shows that wild type but not the S2035I FRB mutant^{38,40} inhibits overall S6K1^{ki} phosphorylation by ~50 % and Thr389 phosphorylation by ~75 % at the highest concentration tested. Half-maximal inhibition occurs at ~100-200 μM FRB. This may underestimate the FRB-S6K1 affinity, however, as the isolated FRB-S6K1 association would have to compete with the bivalent binding of both the FRB and active site of mTOR^{ΔN} to S6K1.

[0263] To map the region of S6K1 involved in FRB interactions, we reasoned that its deletion should reduce Thr389 phosphorylation and also render any residual phosphorylation insensitive to rapamycin. By testing successively truncated polypeptides from the S6K1 tail, we found that both criteria are met by residues 393 to 398 (FIG. 4D). In addition, residues 399 to 402 and 403 to 410 likely contribute to FRB interactions, as their truncation progressively reduces phosphorylation (FIG. 17). N-terminal truncations, up to residue 381, have no significant effect.

[0264] Together, these data indicate that the FRB provides a secondary substrate-recruitment site near the entrance of the catalytic cleft. We presume that while the TOS and possibly other motifs are the primary means of substrate recruitment and specificity, the secondary site facilitates substrate entry into the otherwise restricted active site, and also provides an additional level of specificity.

[0265] A bi-partite substrate-recruitment mechanism is analogous to what has been proposed for the calcineurin phosphatase, the target of FK506-FKBP12. Most calcineurin substrates require both a primary and a secondary recruitment motif for efficient dephosphorylation, and FK506FKBP12 inhibits by binding to the secondary recruitment motif site⁴³. The secondary motif is rather degenerate, and its calcineurin affinity and importance vary substantially among different substrates⁴⁴, and this too may be analogous in mTOR substrates.

FAT domain

[0266] The FAT domain contains 28 α helices arranged as α - α helical repeats. Helices α 1 to α 22 belong to the TPR repeat family and form three discontinuous domains (TRD1, TRD2 and TRD3). Helices α 23 to α 28 belong to the HEAT family and form a single domain (HRD). The four domains pack sequentially to form an a solenoid that is shaped like a C-clamp (FIG. 18A). TRD1, at the N-terminal tip of the clamp, interacts with the C lobe on one side of the kinase domain. After TRD2-TRD3 traverse to the other side of the kinase domain, the HRD at the C-terminal part of the clamp interacts with both the N and C lobes (FIG. 18B and FIG. 18C).

[0267] Both the TRD1-KD and HRD-KD contacts involve residues highly conserved in mTOR orthologs, consistent with the two interfaces having an important role in the structure and function of mTOR. For example, a set of buried hydrogen bond contacts at the TRD1-KD interface (between Glu1401 and Arg2317) and at the HRD-KD interface (Arg1905-Glu2419, and Gln1941Gln2200) involve residues invariant in the 22 mTOR orthologs mentioned above (FIG. 18C).

[0268] The TRD1 and HRD segments correspond to the FAT segments best conserved among PIKK family members²⁸, suggesting that the FAT domain clamping onto the KD is a common feature of most PIKKs (FIG. 12). In addition, PI3Ks contain a helical repeat domain analogous to the HRD in its structure and interactions with the KD, although they lack the rest of the FAT domain (FIG. 18D).

mLST8

[0269] mLST8 consists of seven WD40 repeats. At the narrow end of the mLST8 β propeller structure, a surface that extends across six WD40 repeats binds to both helices and intervening loop of the helix-loop-helix LBE. The interface involves mostly polar LBE residues and polar/aromatic mLST8 residues, and it is dominated by hydrogen bonds (FIG. 19).

[0270] mLST8 is thought to be a requisite activating subunit of mTOR complexes^{8,9}. The structure suggests that the extended interaction surface of mLST8 may stabilize the helix-loop helix structure of the LBE directly, and the organization of the active site indirectly, through the LBE-FATC-catalytic loop spine of interactions discussed above (FIG. 13A). In support, we note that lack of mLST8 results in the association of mTOR with heat shock proteins⁹, and we find that the solubility of overexpressed mTOR is highly dependent on mLST8 co-overexpression (not shown). These effects are unlikely to be caused by just two LBE hydrophobic residues (Met2271 and Met2281) that would be solvent exposed without mLST8 (FIG. 19B). In addition, the growth-suppression phenotype of a temperature-sensitive yeast Lst8 allele can be rescued by mutations⁴⁵ either at the LBE hydrophobic core (A2290V) or at the following $\alpha 5$ helix (L2302Q), and the structure is consistent with these mutations stabilizing the local LBE structure.

Control of mTOR kinase activity

[0271] The concept that active site restriction is a key aspect of mTOR regulation is supported by hyper-activating mutations⁴⁵⁻⁴⁷, most of which map to structural elements involved in limiting active site access. A large number of mutations cluster at the end of the catalytic cleft that is plugged by $\alpha 9b$ (FIG. 5 and FIG. 20). They map to the $\alpha 3$, $\alpha 9$, $\alpha 9b$ and $\alpha 10$ helices that pack extensively with each other, and to the FAT HRD portion that packs with $\alpha 3$ and $\alpha 9$. These mutations are likely to loosen the $\alpha 9b$ -centered structural framework that restricts access from this end of the cleft, presumably without destabilizing the entire active site structure. The role of the FAT clamp in stabilizing this structural framework is highlighted by the widely studied E2419K activating mutation, which eliminates the Glu2419-Arg1905 salt bridge at the $\alpha 9$ -HRD interface (FIG. 18C and FIG. 20).

[0272] Two other mutations shown to increase TOR kinase activity map to a portion of the $\alpha 1$ helix that is sandwiched between the FRB base and the N lobe β sheet^{45,46}. These mutations (I2017V and A2020V) are unlikely to unfold the FRB, because an FRB hydrophobic core mutation (W2027F) abolishes kinase activity⁴⁰. The structure suggests that these mutations may loosen the rigid coupling of the FRB the catalytic cleft, increasing access to the active site.

[0273] A third cluster of mutations⁴⁵ maps to the N-terminal portion of the FAT domain, to structural residues of the TRD1 and of the TRD1-proximal portion of TRD2. They likely interfere with the ability of the FAT domain to clamp onto the C lobe of the kinase, with two possible effects. First, the HRD-KD interactions at the C-terminal end of the FAT solenoid may be indirectly destabilized, as the two FAT ends are structurally, and likely thermodynamically, coupled. Second, the disposition of the N-terminal mTOR segment and its associated RAPTOR, which likely further restrict active site access, could change or become more flexible relative to the KD.

Inhibitors of the ATP-binding site

[0274] To explore the determinants of inhibitor potency and specificity, we determined the structures of mTOR^{ΔN}-mLST8 bound to Torin2 and PP242, which are highly specific for mTOR, and to PI-103, which has dual specificity for mTOR and Class I PI3Ks (FIG. 9).

[0275] The overall orientation of Torin2 in the mTOR cleft is as predicted⁴⁸ (FIG. 6A and FIG. 21A). The tricyclic benzonaphththyridine ring binds to the adenine site and makes a hydrogen bond to the N-C lobe “hinge”, analogous to one of two backbone hydrogen bonds made by ATP and diverse PI3/protein kinase inhibitors. The amino-pyridine group reaches into the “inner hydrophobic pocket”, an area in the back of the cleft that many kinase inhibitors contact. However, it does not make the three predicted hydrogen bonds (to Asp2195, Asp2357 and Tyr2225)⁴⁸. Rather, the structure reveals that a key interaction is the extensive stacking of the tricyclic benzonaphththyridine ring with the indole group of Trp2239 from the “hinge” (FIG. 6A). In the ATPγS complex, Trp2239 stacks partially with a three-atom portion of the adenine. Torin2 takes full advantage of Trp2239, with a ten-atom portion of its tricyclic ring stacking with the indole group. This likely makes a substantial contribution to the sub-nanomolar potency⁴⁸ of Torin2. In addition, because Trp2239 is not present in canonical protein kinases or in PI3Ks, it would also contribute to Torin2’s ~800-fold specificity for mTOR over PI3Ks⁴⁸. The structure also reveals the packing of the Torin2 trifluoromethyl group packs into an N lobe pocket (Ile2163, Pro2169 and Leu2185; FIG. 6A). These contacts are less extensive than those made to Trp2239, and only Leu2185 is variable across the PIKK and PI3K families. However, this portion of the N lobe β sheet exhibits considerable variation in its relative orientation in PI3K structures, and it may thus contribute to Torin2-mTOR specificity.

[0276] PP242 consists of the adenine-mimetic pyrazolopyrimidine scaffold common to PI3/tyrosine kinase inhibitors, with a hydroxyindole substituent at a position that often points to the inner hydrophobic pocket⁴⁹. In mTOR, this pocket is lined with residues conserved across the PI3K and PIKK families, and the selectivity of PP242 for mTOR was unexpected⁴⁹. The structure reveals that while these two PP242 groups are generally positioned as predicted⁴⁹, mTOR undergoes a conformational change that expands and deepens its inner hydrophobic pocket, with the hydroxyindole group reaching deep into the new space (FIG. 6B and FIG. 21B).

This involves the side chain of Tyr2225 at the back of the pocket swinging ($\Delta\chi_1 = 108^\circ$) out of the way of the hydroxyindole group (FIG. 6C and FIG. 21C). Because Tyr2225 is part of the local hydrophobic core between the N and C lobes, this necessitates additional concerted changes. The Leu2354 side chain rotates ($\Delta\chi_1 = 166^\circ$) out of the way of Tyr2225, the Gln2223 side chain moves in to plug a resulting gap, and a 3-residue backbone stretch (residues 2223 to 2225) shifts by ~ 1 Å. In PI3Ks, Leu2354 is replaced by a conserve phenylalanine that cannot undergo a similar conformational change without an even more extensive repacking of the local hydrophobic core. Leu2354 is thus likely to be a major, though indirect, determinant of the selectivity of PP242 for mTOR over PI3Ks and other kinases.

[0277] The multi-targeted PI-103⁵⁰ does not take advantage of any of the aforementioned mTOR-specific features. The morpholine ring binds to the adenine pocket and makes two hydrogen bonds to the “hinge”, while the m-phenol group binds to the inner pocket and makes two hydrogen bonds to the Tyr2225 and Asp2195 side chains at the back of the cleft (FIG. 6D and FIG. 21D). These hydrogen bonds are likely to be important for the high affinity of PI-103 for mTOR and, presumably, for Class I PI3Ks where the relevant protein groups are similarly positioned. In support, we note that the ~ 500 -fold weaker affinity⁵⁰ Class III PI3K–PI-103 complex lacks both m-phenol hydrogen bonds and one of the “hinge” hydrogen bonds³².

Discussion

[0278] The mTOR^{ΔN} structure reveals an intrinsically active but otherwise highly restricted catalytic centre, pointing to substrate recruitment as a major mechanism controlling the kinase activity. Our data indicate that in addition to a primary recruitment motif, a secondary recruitment motif proximal to the substrate phosphorylation site is needed for efficient phosphorylation. The FRB acts as a gatekeeper by preventing inappropriate access to the active site, while also granting privileged substrates access by providing a binding site for the secondary motif.

[0279] As demonstrated by this Example, among other things, the present invention encompasses the recognition that there remains a long-felt need for mTOR crystal structure suitable for X-ray diffraction and high resolution structure determination.

[0280] The present invention provides, among other things, mTOR polypeptides, mTOR crystals and methods for producing the same, suitable for X-ray diffraction and high resolution structure determination. As described in the Examples section below, the present invention is, in part, based on the surprising discovery that co-expression of mLST8 with mTOR results in non-aggregated mTOR complexes; co-expression of mTOR and mLST8 solved the long-felt problem of producing non-aggregated mTOR polypeptide suitable for crystallization. Prior to the present invention, no crystals of mTOR have ever been reported.

[0281] In some aspects, the present invention provides mTOR polypeptides suitable for crystallization. In some embodiments, the present invention provides mTOR polypeptides comprising wild-type mTOR as described in Table 8 and SEQ ID NOS.: __ - __. In some embodiments, an mTOR polypeptide is a full-length wild-type human mTOR (NP_004949; 2549 aa) (SEQ ID NO: ____).

[0282] In some embodiments, the present invention provides recombinant mTOR polypeptides. In some embodiments, the present invention provides mTOR polypeptides comprising one or more truncations. In some embodiments, provided mTOR polypeptides comprise an N-terminal truncation. In some embodiments, mTOR polypeptides comprise an N-terminal truncation of residues 1-1375(mTOR^{ΔN}) (SEQ ID NO: __ and __). In some embodiments, mTOR polypeptides comprise one or more internal deletions. In some embodiments, mTOR polypeptides comprise one or more internal deletions comprising residues 2443-2486 (mTOR^{Δ2443-2486}). In some embodiments, mTOR polypeptides comprise both an N-terminal truncation *and* one or more internal deletions comprising residues 2443-2486 (mTOR^{ΔNΔ2443-2486}) (SEQ ID NO: __ and __).

[0283] In some embodiments, , the present invention provides mTOR polypeptides which additionally comprise a protein tag. In some embodiments, a protein tag is a FLAG-tag. In some embodiments, , a protein tag is a GST-tag. In some embodiments, provided mTOR polypeptides is a FLAG-tagged mTOR^{ΔN} polypeptide (SEQ ID NO: __). In some embodiments, provided mTOR polypeptides is a FLAG-tagged mTOR^{Δ2443-2486} polypeptide. In some embodiments,

provided mTOR polypeptides is a FLAG-tagged mTOR^{ΔNA2443-2486} polypeptide (SEQ ID NO: ____). In some embodiments, mLST8 and mTOR are both protein tagged (i.e. FLAG or GST tag).

[0284] In some aspects, the present invention provides methods for producing and/or expressing non-aggregated mTOR polypeptides suitable for crystallization. Without wishing to be bound by any particular theory, the present invention proposes that co-expression of mTOR and mLST8 allows for stable mTOR complex formation *without* aggregation, providing an unexpected solution to the the long-standing and established problem of mTOR aggregation and binding of heat shock proteins.

[0285] In some embodiments, the present invention provides methods for co-expression of an mTOR polypeptide as described in Table 8 together with an mLST8 polypeptide as described in Table 7. In some embodiments, provided methods comprise stable transfection of a stable cell line with a FLAG-tag version of the polypeptides. In some embodiments, provided methods comprise generation of a cell line that was stably-transfected sequentially by DNA vectors encoding mLST8 and mTOR. In some embodiments, a cell line is a human cell line. In some embodiments, a cell line is an HEK 293-F cell line. In some embodiments, a cell line is an insect cell line line. In some embodiments, a cell line is a baculovirus system. In some embodiments, a cell line is a microbial cell line. In some embodiments, a cell line is *E. coli*.

[0286] In some embodiments, the present invention provides methods for purifying recombinant mTOR and mLST8 polypeptides (together referred to as an “mTOR complex”). Exemplary purification methods of an mTOR complex include, but are not limited to ion exchange (MonoQ), gel-filtration chromatography, affinity chromatography with anti-FLAG M2 agarose beads, glutathione affinity chromatography, and/or combinations thereof.

[0287] In some aspects, the present invention provides methods for producing crystals comprising an mTOR polypeptide. In some embodiments, the present invention provides methods for growing mTOR crystals comprising a hanging-drop vapour diffusion method at 4 °C.

[0288] In some embodiments, the present invention provides methods for growing mTOR crystals, wherein a step for growing said crystals includes a stabilization buffer

comprising 100 mM Tris-Cl, 6-8% (w/v) polyethylene glycol (PEG) 8000, 500 mM NaCl, 10% (v/v) glycerol, 10 mM DTT, pH 8.5. In some embodiments, said stabilization buffer further comprises 10 mM MgCl₂, 3 mM ADP, and 20 mM NaF.

[0289] In some embodiments, said stabilization buffer comprises 50 mM Tris-Cl, pH 8.5, 10 mM Tris-Cl, 8.0, 10% PEG8000, 0.1 M NaCl, 6% glycerol. In some embodiments, the stabilization buffer further comprises 5 mM MgCl₂ and 1 mM ATP γ S. In some embodiments, said stabilization step includes a buffer comprising 50 mM Tris-Cl, pH 7.5, 10 mM Tris-Cl, 8.0, 10% PEG8000, 0.1 M NaCl, 6% glycerol. In some embodiments, the stabilization buffer further comprises 1 mM AMPPNP and 2 mM MnCl₂.

[0290] In some embodiments, the present invention provides methods for producing crystals comprising an mTOR polypeptide, wherein a step comprises soaking crystals for one hour in a stabilization buffer. In some embodiments, provided methods comprise a step of soaking crystals for two and one-half (2.5) hours in a stabilization buffer. In some embodiments, said stabilization buffer further comprises an mTOR modulator listed in Table 12. In some embodiments, said stabilization buffer further comprises a cofactor including, but not limited to ADP-MgF₃-Mg₂, ATP γ S-Mg₂, and/or combinations thereof. In some embodiments, said mTOR modulator and/or cofactor is present at 1 mM. In some embodiments, said mTOR modulator and/or cofactor is present at 0.2 mM. In some embodiments, said mTOR modulator and/or cofactor is present at 0.1 mM.

[0291] In some embodiments, the present invention provides methods for harvesting crystals comprising an mTOR polypeptide, comprising a step of harvesting said crystal in stabilization buffer and transferring said crystal to 50 mM Tris-Cl, pH 8.5, 10 mM Tris-Cl, pH 8.0, 0.1 M NaCl, 14% (w/v) PEG8000, 22% (v/v) glycerol. In some embodiments, provided methods comprise a further step of flash-freezing said crystal. In some embodiments, crystals are flash-frozen in liquid nitrogen.

[0292] In some embodiments, the present invention provides methods for collecting X-ray diffraction data from crystals comprising an mTOR polypeptide, wherein said method

comprises a step of collecting diffraction data at -170 °C at the ID24C and ID24E beamlines of the Advanced Photon Source.

[0293] In some aspects, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide. In some embodiments, such a provided composition consists of or consists essentially of the mTOR polypeptide. In some embodiments, a composition is considered to “consist of” mTOR polypeptide if it includes only the polypeptide, one or more solvents, and optionally salts and/or metals. In some embodiments, such a provided composition includes one or more other agents such as one or more other polypeptides (e.g., one or more potential or actual mTOR binding partner polypeptides) and/or one or more interacting agents (e.g., small molecules).

[0294] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an wild-type mTOR polypeptide. Exemplary wild-type mTOR polypeptides include, but are not limited to, polypeptides listed in Table 8. In some embodiments, such a provided composition comprises an mTOR polypeptide comprising an N-terminal truncation (mTOR^{ΔN}). In some embodiments, an mTOR polypeptide comprises one or more internal deletions. In some embodiments, an mTOR polypeptide comprises one or more internal deletions comprising residues 2443-2486 (mTOR^{Δ2443-2486}). In some embodiments, an mTOR polypeptide comprises both an N-terminal truncation *and* one or more internal deletions. In some embodiments, an mTOR polypeptide comprises both an N-terminal truncation *and* one or more internal deletions comprising residues 2443-2486 (mTOR^{ΔNΔ2443-2486}).

[0295] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide bound to one or more binding partners. In some embodiments, such a provided composition comprises an mTOR polypeptide bound to mLST8 (mTOR-mLST8 complex). In some embodiments, provided composition comprises an N-terminally truncated mTOR polypeptide bound to mLST8 (mTOR^{ΔN}-mLST8 complex). In some embodiments, provided composition

comprises an N-terminally truncated and internally deleted mTOR polypeptide bound to mLST8 (mTOR^{ΔNΔ2443-2486}-mLST8 complex).

[0296] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide bound to two or more binding partners. In some embodiments, such a provided composition comprise an mTOR polypeptide bound to mLST8 and RAPTOR (mTOR-mLST8-RAPTOR complex). In some embodiments, provided composition comprises an N-terminally truncated mTOR polypeptide bound to mLST8 and to RAPTOR (mTOR^{ΔN}-mLST8-RAPTOR complex). In some embodiments, provided composition comprises an N-terminally truncated and internally deleted mTOR polypeptide bound to mLST8 and to RAPTOR (mTOR^{ΔN Δ2443-2486}-mLST8-RAPTOR complex).

[0297] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide bound to one or more interacting agents (e.g., small molecules). In some embodiments, interacting agents comprise mimics of the γ -phosphate group of ATP in the transition state. In some embodiments, a γ -phosphate group mimic comprise ADP, Mg^{2+} or MgF_3^- , and/or combinations thereof. In some embodiments, such a provided composition comprise an mTOR polypeptide bound to ADP. In some embodiments, such a provided composition comprise an mTOR polypeptide bound to Mg^{2+} . In some embodiments, such a provided composition comprise an mTOR polypeptide bound to MgF_3^- .

[0298] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide bound to one or more mTOR modulators. In some embodiments, provided composition comprises an mTOR polypeptide bound to one or more exemplary mTOR modulators listed in Table 12. In some embodiments, provided composition includes, but it not limited to, an mTOR modulator consisting of the group rapamycin, PI 103 HCL, AZD8055, AZD2014, CH5132799, XL765 (SAR245409), GDC-0980 (RG7422), GSK1059615, GSK2126458, Torin1, Torin2, INK128, KU0063794, BEZ235 (NVP-BEZ235), NVP-BGT226, OSI-027, Palomid 529, PF-

05212384 (PKI-587), PKI-179, WAY-600, WYE-125132 (WYE-132), WYE-23, WYE-28, WYE-354, WYE-687, PF-04691502, PP-121, PP242, PP30 and/or combinations therein.

[0299] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide bound to one or more binding partners and bound to one or more interacting agents (e.g., small molecules). In some embodiments, provided composition comprise an mTOR-mLST8 complex bound to an interacting agent. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to an interacting agent. In some embodiments, provided composition comprise an mTOR^{ΔNΔ2443-2486}-mLST8 complex bound to an interacting agent. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to a mimic of the γ-phosphate group of ATP in the transition state. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8-ADP complex. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8- Mg²⁺ complex. In some embodiments, provided composition comprises mTOR^{ΔN}-mLST8- MgF₃⁻.

[0300] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide bound to one or more binding partners and bound to one or more mTOR modulators (e.g. exemplary modulators listed in Table 12). In some embodiments, provided composition comprise an mTOR-mLST8 complex bound to an mTOR modulator. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to an mTOR modulator. In some embodiments, provided composition comprise an mTOR^{ΔN Δ2443-2486}-mLST8 complex bound to an mTOR modulator. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to Torin2. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to PP242. In some embodiments, provided composition comprise an mTOR^{ΔN}-mLST8 complex bound to PI 103.

[0301] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide that comprises the same or essentially the same crystallographic parameters (i.e. +/- 10% in unit

cell dimensions; space groups) as an mTOR^{ΔN}-mLST8 complex as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0302] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide that comprises the same or essentially the same crystallographic parameters (i.e. +/- 10% in unit cell dimensions; space groups) as an mTOR^{ΔN}-mLST8 complex bound with ADP, Mg²⁺ and MgF₃⁻ as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0303] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide that comprises the same or essentially the same crystallographic parameters (i.e. +/- 10% in unit cell dimensions; space groups) as an mTOR^{ΔN}-mLST8 complex bound with ATPγS as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0304] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide that comprises the same or essentially the same crystallographic parameters (i.e. +/- 10% in unit cell dimensions; space groups) as an mTOR^{ΔN}-mLST8 complex bound with Torin2 as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0305] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide that comprises the same or essentially the same crystallographic parameters (i.e. +/- 10% in unit cell dimensions; space groups) as an mTOR^{ΔN}-mLST8 complex bound with PP242 as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0306] In some embodiments, the present invention provides a crystalline (i.e., containing at least one crystal) or crystallizable composition comprising an mTOR polypeptide that comprises the same or essentially the same crystallographic parameters (i.e. +/- 10% in unit

cell dimensions; space groups) as an mTOR^{ΔN}-mLST8 complex bound with PI-103 as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0307] In some aspects, the present invention also provides structural information and/or analyses of mTOR polypeptide crystals and/or sets thereof. In some embodiments, such structural information includes, but is not limited to, diffraction patterns, and/or coordinates, as well as any data sets, images, models, and/or graphical representations thereof or generated therefrom. In some embodiments, such graphical representations may include, for example, space-filling models, molecular surface representations, shell or boundary models, ribbon models, stick models; and/or combinations thereof.

[0308] In some embodiments, the present invention provides atomic structure coordinates for an mTOR^{ΔN}-mLST8 complex as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0309] In some embodiments, the present invention provides atomic structure coordinates for an mTOR^{ΔN}-mLST8 complex bound with ADP, Mg²⁺ and MgF₃⁻ as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0310] In some embodiments, the present invention provides atomic structure coordinates for an mTOR^{ΔN}-mLST8 complex bound with ATPγS as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0311] In some embodiments, the present invention provides atomic structure coordinates for an mTOR^{ΔN}-mLST8 complex bound with Torin2 as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0312] In some embodiments, the present invention provides atomic structure coordinates for an mTOR^{ΔN}-mLST8 complex bound with PP242 as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0313] In some embodiments, the present invention provides atomic structure coordinates for an mTOR^{ΔN}-mLST8 complex bound with PI-103 as described herein (see also Yang et al., Nature, 497:217, May 9, 2013, including supplementary materials).

[0314] In some embodiments, provided information is or comprises differences observed between or among structures that differ from one another in the presence or absence of one or more binding partners and/or interacting agents. In some embodiments, provided information is or comprises differences observed between or among structures that differ from one another in the presence or absence of one or more binding partners and/or one or more modulators.

[0315] In some embodiments, such structural information and/or analyses may be embodied in a tangible medium (e.g., a computer-readable medium) or a storage environment. Thus, the present invention provides tangible embodiments of mTOR polypeptide crystal structure information, as well as its use, for example, by or with a computer system, in any of a variety of applications. For example, in some embodiments, such structural information and/or analyses may be accessed by, transported to or from, and/or otherwise utilized by a computer system or program running thereon.

[0316] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

Methods Summary

Protein expression and purification

[0317] The boundary of the crystallized human mTOR^{ΔN} (residues 1376-2549) was identified by screening successive N-terminal truncations of mTOR, produced by transient transfection in HEK293-F cells, for expression levels, solubility and size exclusion chromatography profile (not shown). For large-scale production of mTOR^{ΔN}-mLST8, we generated an HEK 293-F cell line that was stably-transfected sequentially by modified pcDNA3.1 vectors encoding FLAG-tagged mLST8 and FLAG-tagged mTOR^{ΔN}. In the absence of mLST8 co-expression, the level of soluble mTOR^{ΔN} was substantially lower, and it co-

purified with endogenous mLST8 (not shown). The mTOR^{ΔN2443-2486}-mLST8, full-length human mTORMLST8 and human mTORC1 (mTOR-mLST8-RAPTOR) complexes were similarly produced in HEK 293-F cell lines that were stably transfected with the FLAG-tagged versions of the proteins. Cells were grown as monolayers in 15 centimeter plates. They were lysed in 50mM Tris-Cl, pH 8.0, 100 mM KCl, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 % (v/v) glycerol, 2 mM dithiothreitol (DTT) and protease inhibitors using French Press. After centrifugation, the FLAG-tagged proteins were affinity purified using anti-FLAG M2 agarose beads (Sigma). Following cleavage of the FLAG-tags by tobacco etch virus (TEV) protease, they were purified by ion exchange (MonoQ) and gel-filtration chromatography. The purified complex was concentrated to 5 mg ml⁻¹ by ultrafiltration in 20 mM Tris-Cl, 500 mM NaCl, 10 mM DTT, pH 8.0 and was used for crystallization immediately. For kinase assays, 10% (v/v) glycerol was added to protein aliquots for storage at -80 °C.

[0318] Human S6K1αII^{ki} protein, which contains the kinase-inactivating K100R mutation, was produced by infecting High Five insect cells with a pFastBac1 baculovirus expressing the FLAG-tagged protein. It was purified by affinity chromatography with anti-FLAG M2 agarose beads. The protein was concentrated to 8 mg ml⁻¹ in 50 mM Tris-Cl, 400 mM NaCl, 1mM EDTA, 1mM EGTA, 5% (v/v) glycerol, 10 mM DTT, 1 mM Pefabloc, pH 8.0, and it was stored at -80 °C.

[0319] Human 4EBP1 was overexpressed in the *E. coli* strain BL21(DE3) from a modified pGEX4T3 vector, and was purified by glutathione affinity chromatography, cleavage of the GST tag with TEV protease, fractionation on MonoQ column, removal of free GST by glutathione affinity, and finally gel filtration (Superdex75) chromatography. The peak fractions were concentrated to 20 mg ml⁻¹ in 20 mM Tris-Cl, 200 mM NaCl, 5% (v/v) glycerol, 10 mM DTT, pH 8.0.

[0320] The mutant and wild type (for direct comparison in FIG. 13) mTOR complexes were produced by transiently transfecting 293-F cells with modified pcDNA 3.1 (+) vectors encoding FLAG-tagged wild type, D2338A or H2340A mTOR^{ΔN} and untagged mLST8. After two days, the cells were harvested and the proteins were purified using anti-FLAG M2 agarose

beads as described above. After extensive washing of the beads, proteins were eluted with 0.1 mg ml⁻¹ FLAG peptide in 20 mM Tris-HCl, pH 8.0, 400 mM NaCl, 2 mM DTT, 10% glycerol, and concentrated. Protein concentrations were determined by A₂₈₀ and confirmed by immunoblotting with anti-FLAG antibody.

Crystallization and data collection

[0321] Crystals were grown by the hanging-drop vapour diffusion method at 4 °C. Apo-crystals of the mTOR^{ΔN}-mLST8 complex were grown from 100 mM Tris-Cl, 6-8% (w/v) polyethylene glycol (PEG) 8000, 500 mM NaCl, 10% (v/v) glycerol, 10 mM DTT, pH 8.5. Crystals of the mTOR^{ΔN}-mLST8 bound to ADP-MgF₃-Mg₂ were grown similarly, except the well-buffer contained 10 mM MgCl₂, 3 mM ADP, and 20 mM NaF. The mTOR^{ΔN}-mLST8ATPγS-Mg₂ complex was prepared by soaking apo-crystals for one hour in a stabilization buffer of 50 mM Tris-Cl, pH 8.5, 10 mM Tris-Cl, 8.0, 10% PEG8000, 0.1 M NaCl, 6% glycerol, supplemented with 5 mM MgCl₂ and 1 mM ATPγS. The mTOR^{ΔN}-mLST8-AMPPNP-Mn₂ complex was prepared by soaking apo-crystals similarly, except the stabilization buffer had a pH of 7.5 and it was supplemented with 1 mM AMPPNP and 2 mM MnCl₂, and the data was collected at the Manganese absorption edge. Crystals of mTOR^{ΔN}-mLST8-Torin2 and mTOR^{ΔN}-mLST8-PI-103 were prepared by mixing 1 mM of the inhibitors with the protein. Co-crystals appeared from the same condition as the apo-crystals. Crystals of mTOR^{ΔN}-mLST8-PP242 were prepared by soaking apo-crystals for 2.5 hours in the stabilization buffer supplemented with 0.2 mM PP242. Apo-crystals were harvested in stabilization buffer, transferred to 50 mM Tris-Cl, pH 8.5, 10 mM Tris-Cl, pH 8.0, 0.1 M NaCl, 14% (w/v) PEG8000, 22% (v/v) glycerol, and were flash-frozen in liquid nitrogen. Crystals with ADP-MgF₃-Mg₂, ATPγS-Mg₂, Torin2, PI-103 and PP242 were flash-frozen similarly, except for the presence of the corresponding cofactors or inhibitors (0.1 mM) in the buffers. Diffraction data were collected at -170 °C at the ID24C and ID24E beamlines of the Advanced Photon Source, and they were processed with the HKL suite⁵¹.

Structure determination and refinement

[0322] Initial phases were obtained from isomorphous and anomalous differences of two heavy atom derivatives, prepared by soaking crystals in stabilization buffer lacking DTT and supplemented with 1 mM uranyl acetate (1 hour), or with 0.4 mM potassium-gold cyanide (75 min.) at 4 ° C. Initial phases, calculated with the program SHARP52 had a mean figure of merit of 0.35 (35.0 to 4.5 Å). The uranyl derivative had a dispersive phasing power (Pp) of 0.89 and anomalous Pp of 0.60, with a dispersive R_{cullis} of 0.79 and anomalous R_{cullis} of 0.95. The gold derivative had a dispersive Pp of 0.80 and anomalous Pp of 0.29, with a dispersive R_{cullis} of 0.66 and anomalous R_{cullis} of 0.98. The phases were improved using solvent flattening and two-fold ncs averaging with multiple masks with the program DM⁵³. The model was built using O⁵⁴ and refined first with REFMAC5⁵³ and then with PHENIX⁵⁵, using tight ncs restraints on atom positions. The final model contains residues 1385 to 2549 of human mTOR, and 8 to 324 of human mLST8- mTOR^{ΔN} residues 1376-1384 at the N-terminus, residues 1815-1866 in the FAT domain, and residues 2437-2491 between $\alpha 9b$ and $\alpha 10$ in the KD are disordered. mLST8 residues 1-7 and 325-326 from the N- and C-termini are disordered. The Ramachandran plot, calculated by PROCHECK, has 88.5, 11.0 and 0.5 % of the residues in the most favored, additionally allowed, and generously allowed regions, respectively. There are no residues in disallowed regions. The R_{free} test set of the Native data contains 1699 reflections.

In vitro kinase assays

[0323] All kinase assays were performed in a buffer of 25 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl₂, 2 mM DTT, 3 % (v/v) glycerol, for 30 minutes at 30 ° C, in a final volume of 15 μ l. For a typical reaction, the indicated amount of enzymes, substrates, and competitors were incubated together for 10 minutes on ice in the kinase buffer in a final volume of 13.5 μ l. Reactions were started by the addition of 1.5 μ l of cold ATP (0.5 mM final concentration) including 2 μ Ci of [γ -³²P] ATP (6000 Ci/mmol, Perkin-Elmer). In assays with rapamycin, all reactions in a titration contained a constant level of 3.3 % DMSO. Reactions were stopped by the addition of 15 μ l of 2X NuPAGE LDS sample buffer and boiling for 3 minutes, and were resolved in a 4-12 % NuPAGE Bis-Tris gel. Where applicable, each reaction was split into two

for analysis by phosphorimaging and immunoblotting. All antibodies were obtained from Cell Signaling, except for the anti-FLAG antibody which was obtained from Sigma.

Example 2: In vitro assay for screening activity of mTOR modulators

Primary Screening Assay

[0324] Cancer stem cells (i.e. tumor-initiating cells) are isolated as previously described⁵⁶⁻⁵⁹ and cultured as spheres in serum-free cell media. Screening methodologies known⁶ in the art are performed as follows. Cancer stem cells are dissociated into single cells and seeded at 3,000 cells per well in 100 uL medium in 96-well plates. mTOR modulator compounds are dissolved in DMSO, re-aliquoted in daughter plates as 1 and 0.2 mmol/L solutions, and added using a pin tool to achieve final concentrations of 1 or 0.2 umol/L. mTOR modulator effects are compared with cells optimally proliferating in 0.1% DMSO alone, whereas wells filled with media served as the background. alamarBlue (10 uL) is added after 72 hours to evaluate cellular health, and fluorescence intensity is measured after 24 hours on a microplate reader equipped with a λ_{540} excitation/ λ_{590} emission filter. Hits are defined as compounds that cause a signal decrease of at least 45% as compared with controls. Confirmatory tests are performed using 8-point, 3-fold serial dilutions of mTOR modulators. Cells are seeded, treated with serially diluted mTOR modulators, and test for alamar Blue reduction.

Sphere Formation Assay

[0325] Cancer stem cells are seeded in triplicate in untreated 96-well plates at a density of 3,000 cells per well in 50 uL per well, containing 30% SKP condition media. mTOR modulators are diluted in medium (1:1000) and immediately added to the cells in a volume of 50 uL (final concentration of DMSO 0.05%). Cells are retreated 72 hours postplating with drugs and fixed after 6 days with 4% paraformaldehyde. Sphere number is determined by manual counting, and the results are expressed as the mean sphere number of treated wells as compared with DMSO-treated wells x 100.

Kinase Assay

[0326] Cancer stem cells are dissociated into single cells and treated in the presence of 0.1% DMSO or 100 nM of mTOR modulator for 3 hours. Cells are solubilized in NP-40 lysis buffer, the lysates normalized for protein content, and then incubated with mTOR monoclonal antibody. Immune complexes are recovered with protein A-Sepharose beads and incubated with 5 μ Ci of [γ -³²P] ATP (6000 Ci/mmol, Perkin-Elmer) at 30 ° C for 20 minutes and 10 μ g of dephosphorylated 4EBP1 in 30 uL of mTOR kinase buffer (25 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl₂, 2 mM DTT, 3 % (v/v) glycerol). The reactions are stopped by adding 20 uL of Laemmli buffer. Samples are separated by SDS-PAGE, and gels are visualized by autoradiography.

Example 3: Xenograft assay for mTOR modulator toxicity

[0327] Cancer stem cells are resuspended in PBS, mixed 1:3 with Matrigel and injected in 30-uL volume into the dermis of 4-5 week old NOD/SCID (nonobese diabetic/severe combined immunodeficient) mice. mTOR modulator treatment begins when tumor size reaches approximately 50 to 100 mm³. Mice are injected intravenously on 2 consecutive days each week with either mTOR modulator or vehicle for a total of 2 to 3 cycles. Blood samples are collected

24 hours after the last mTOR modulator dose and analyzed to determine the differences in red blood cell/white blood cell counts.

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Sequences

Representative mTOR and related sequences

Human mTOR (2549 aa) | GI:4826730 | NP_004949 | SEQ ID NO: ()

MLGTGPAAATTAATTSSNVSVLQQFASGLKSRNEETRAKAAKELQHYVTMELREMSQEESTRFYDQLNHHIFELVSS
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WLGADRNEGRRHAAVLVLR LAISVPTFFFQOVQPFDDNIFVAVWDPKQAI REGAVAALRA CLILTTQREP KEMQKP
QWYRHTFEEAEKGFD ETLAKEKGMNRDDRIHGALLILNELVRIS SMEGERLREEMEEITQQQLVHDKYCKDLMGFGT
KPRHITPFTSFQAVQPQQSNALVGLLGYSSHQGLMGFGTSPSPAKSTLVE SRCCRDLMEEKFDQVCQWVLKCRNSKN
SLIQMTILNLLPRLAAFRPSAFTDTQYLQDTMNHVLS CVKKEKERTAAAFQALGLLSVAVRSEFKVYLPRVLDIIRAA
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LKMLS LVL MHKPLRHPGMPKGLAHQLASPLGTTLP EASDVGSITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHK
EIRMEAARTCSRLLTPSIHLISGHAHVVSQTAVQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQ AENL
QALFVALNDQVFEIRELAICTVGR LSSMNP AFVMPF LRKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRP
YMEPI LKALILKLKDPDPDPNPGVINNVLATIGELAQVSGLEMRKVVDELFIIMDM LQDSSLLAKRQVALWTLGQL
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IHPIVRTLDQSP ELRSTAMDTLSSLVFQLGKKYQIFIPMVNKVLVRHRINHQR YDVLICRIVKGYTLADEEEDPLIY
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EVTGLDGNYRITCHTVMEVLREHKDSVM AVLEAFVYDPLLNWRLMDTNTKGNKRSRTRTDSY SAGQSVEILDGVELG
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YIGWCPFW

Human mTOR^{ΔN} | SEQ ID NO: ()

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FLAG-tagged human mTOR^{AN} (lower case indicates tag sequence) | SEQ ID NO: ()

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Human mTOR^{ANA2443-2486} | SEQ ID NO: ()

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 QRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDL
 AKLLWLKSPSPSEVWFDRRTNYTRSLAVMSMVGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIP
 FRLTRMLTNAMEVTGLDGNIRITCHTVMEVLREHKDSVMAVLEAFVYDPLLNWRLMDTNTKGNKRSVKPEALNKKAI
 IQIINRVRDKLTGRDFS HDDDTLDVPTQVELLIKQATSHENLCQCYIGWCPFW

FLAG-tagged human mTOR^{ΔNΔ2443-2486} (lower case indicates tag sequence) | SEQ ID NO: ()

mdykdddddhhhhhhhhhhhhhhhenlyfqgtgDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPTPAILESLSINNKLQOPEAAAGVLEYAMKHFGELEIQATWYEKLHEWEDALVAYDKKMDTNKDDPELMLGRMRCLEALGEWGQLHQQCCEKWTLVNDETQAKMARMAAAAWGLGQWDSMEEYTCMI PRDTHDGAFYRAVLALHQDLFSLAQQCIDKARDLLDAELTAMAGESYSRAYGAMVSCHMLSELEEVIQYKLVPERREIIRQIWWERLQGCQRIVEDWQKILMVRSLVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDPSRQLDHLPLPTVHPQVTYAYMKNMWKSARKIDAFQHMQHVFVQTMQOQAQHAIAITEDQQHKQELHKL MARCFLKLGEWQLNLQGINESTIPKVLQYYSAATEHDRSWYKAHAWAVMNFEAVLHYKHQNQARDEKKKLRHASGANITNATTAATTAATATTTASTEGSNSESEAESTENSPTPSPLQKKVTEDL SKTLLMYTVPAVQGFFRSISLSRGNLQDTLRVLTLLWFDYGHWPDVNEALVEGVKAIQIDTWLQVIPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHSNTLVQQAMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEQEWCRKYMKSGNVKDLTQAWDLYYHVFRRI SKQLPQLTSLELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSIAPSLQVITSKQRPRKLTLMGSGNGHEFVFLKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSEVWFDRRTNYTRSLAVMSMGYILGLGDRHPSNMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAMEVTGLDGNYRITCHTVMEVLRHKKDSVMVLEAFVYDPLLNRWLRMDTNTKGNKRSVLKPEALNKKAIQIINRVRDKLTGRDFSDDTLDVPTQVELLIKQATSHENLCQCYIGWCPFW

Rat mTOR (2549 aa) | GI: 9845251 | NP_063971.1 | SEQ ID NO: ()

MLGTGPATATAGAATSSNVSVLQQFASGLKSRNEETRAKAAKELQHYVTMELREMSQEESTRFYDQLNHHIFELVSSDANERKGGILAIASLIGVEGGNSTRIGRFANYLRNLLPSSDPVVMEMASKAIGRLAMAGDTFTA EYVEFEVKRALEWLGADRNEGRRHA AVLVLRELAI SVPTFFFQVQVFFDNIFVAVWDPKQAI REGAVAALRACLILTTQREPKEMQKPQWYRHTFEEAEKGFDETLAKEKGMNRDDRIHGALLILNELVRISSMEGERLREEMEEITQQQLVHDKYCKDLMGFGTKPRHITPFTSFQAVQPQQSNALVGLLGYSSSHQGLMGFGASPSPTKSTLVESRCCRDLMEEFQVQVWLKCRSSKNSLIQMTILNLLPRLAAFRPSAFTDQYQLQDTMNHVLSVCVKEKERTAAAFQALGLLSVAVRSEFKVYLPRVLDIIRAA LPPKDFAHKRQKTQV DATVFTCISMLARAMGPGIQDDIKELLEPMLAVGLSPALTAVLYDLRSRQIPQLKKDIQDGLLKMLSLVLMHKPLRHPGMPKGLAHQLASPLTTLPEASDVASITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHKEIRMEAARTCSRLLTPSIHLISGHAHVVSQTAVQVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQAE NLQALFVALNDQVFEIRELAICTVGRLLSSMNP AFVMPFLRKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRPYMEPILKALILKLKDPDPDPNPGVINNVLATIGELAQVSGLEMRKWVDEL FVIIMDMLQDSSLLAKRQVALWTLGQLVASTGYVVEPYRKYPTLLEVLLNFKLTEQNQGTREAIRVLGLLGALDPYKHKVNIGMIDQSRDASAVSLSESKSSQDSSDYSTSEMLVNMGNLPLDEFYPAVSMVALMRIFRDQSLSHHHTMVVQAITFI FKSGLKCVQFLPQVMPTFLNVI RVCDAIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFWVMNTSIQSTIILLIEQIVVALGGEFKLYLPQLIPHMLRVFMHDNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVKLFDAPVPLPSRKAAL ETVDRLTESLDFTDYASRI IHPIVRTLDQSPELRSTAMDTLSSSLVFQLGKKYQIFIPMVNKLVRHRINHQRVDVICRIVKGYTLADEEEDPLIYQHRMLRSSQGDALASGPVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRRLSLELLKDSSSPSLRSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSI ELALTSQDIAEVTQTLNLAEFMEHSDKGPLPLRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPTPAILESLSINNKLQOPEAASGVLEYAMKHFGELEIQATWYEKLHEWEDALVAYDKKMDTNKDDPELMLGRMRCLEALGEWGQLHQQCCEKWTLVNDETQAKMARMAAAAWGLGQWDSMEEYTCMI PRDTHDGAFYRAVLALHQDLFSLAQQCIDKARDLLDAELTAMAGESYSRAYGAMVSCHMLSELEEVIQYKLVPERREIIRQIWWERLQGCQRIVEDWQKILMVRSLVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDPSRQLDHLPLPTVHPQVTYAYMKNMWKSARKIDAFQHMQHVFVQTMQOQAQHAIAITEDQQHKQELHKL MARCFLKLGEWQLNLQGINESTIPKVLQYYSAATEHDRSWYKAHAWAVMNFEAVLHYKHQNQARDEKKKLRHASGANITNATTTATTAASAAAATSTEGSNSESEAESNESSPTPSPLQKKVTEDL SKTLLLYTVPAVQGFFRSISLSRGNLQDTLRVLTLLWFDYGHWPDVNEALVEGVKAIQIDTWLQVIPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHSNTLVQQAMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEQEWCRKYMKSGNVKDLTQAWDLYYHVFRRI SKQLPQLTSLELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSIAPSLQVITSKQRPRKLTLMGSGNGHEFVFLKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTN

SGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSS
 EVWFDRTNYTRSLAVMSMVGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAM
 EVTGLDGNRYRTTCHTVMLEVLREHKDSVMAVLEAFVYDPLLNWRLMDTNAKGNKRSRTRTDSYSAGQSVEILDGVELG
 EPAHKKTGTTVPESIHSGIFDGLVKPEALNKKAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQC
 YIGWCPFW

Mouse mTOR (2549 aa) | GI:227330586 | NP_064393.2 | SEQ ID NO: ()

MLGTGPAVATASAAATSSNVSVLQQFASGLKSRNEETRAKAAKELQHYVTMELREMSQEESTRFYDQNLNHHIFELVSS
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 WLGAADRNEGRRHA AVLVLRELAI SVPTFFFQVQVPPFDNIFVAVWDPKQAI REGAVAALRACLILTTQREPKEMQKP
 QWYRHTFEEAEKGFDETLAKEKGMNRDDRIHGALLILNELVRISSEMEGERLREEMEEITQQQLVHDKYCKDLMGFGT
 KPRHITPFTSFQAVQPQQPNALVGLLGYS SPQGLMGFGTSPSPAKSTLVESRCCRDLMEEKFDQVCQVWLKCRSSKN
 SLIQMTILNLLPRLAAFRPSAFTDTQYQLQDTMNHVLSVCVKKEKERTAAFQALGLLSVAVRSEFKVYLPRVLDIIRAA
 LPPKDFAHKRQKTQVQV DATVFTCISMLARAMGPGIQQDIKELLEPMLAVGLSPALTAVLYDLRSQIPQLKKDIQDGL
 LKMLSLVLMHKPLRHPGMPKGLAHQLASPLGTTLP EASDVASITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHK
 EIRMEAARTCSRLLTPSIHLISGHAHVVSQTAVQVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENL
 QALFVALNDQVFEIRELAICTVGRLLSSMNPFAVMPFRLKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRP
 YMEPILKALILKLKDPDPDPNPGVINNVLATIGELAQVSGLEMRKWVDELFIIMDMLQDSSLLAKRQVALWTLGQL
 VASTGYVVEPYRKYPTLLEVLNLFKTEQNQGTREAIRVLGLLGALDPYKHKVNIGMIDQSRDASAVSLSESKSSQ
 DSSDYSTSEMLVNMGNLPLDEFYPAVSMVALMRIFRDQSLSHHHTMVVQAITFIFKSLGLKCVQFLPQVMPFTFLNVI
 RVCDGAIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFWVMNTSIQSTIILLIEQIVVALGGEFKLYLPQLIPHM
 LRVFMHDNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVKLFDAPVPLPSRKAAL ETVDRLTESLDFTDYASRI
 IHPIVRTLDQSPELRSTAMDTLSSLVFQLGKKYQIFIPMVNKLVRHRINHQRYDVLICRIVKGYTLADEEEDPLIY
 QHRMLRSSQGDALASGPVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLRLSLELLKDSSSPSLRSCWALAQA
 YNPMARDLNFNAAFVSCWSELNEDQQDELIRSI ELALTSQDIAEVTQTLNLAEFMEHSDKGPLPLRDDNGIVLLGER
 AAKCRAYAKALHYKELEFQKGPTPAILESLSINNKLQQPEAASGVLEYAMKHFGELEIQATWYEKLHEWEDALVAY
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 IWWERLQGCQRIVEDWQKILMVRSLVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDPSRQLDHPPLPTAHP
 QVTYAYMKNMWKSARKIDAFQHMQH FVQTMQQQAQHA IATEDQQHKQELHKL MARCFLKLGEWQLNLQGINESTIPK
 VLQYYSAAATEHDRSWYKAHAWAVMNF EAVLHYKHQONQARDEKKKLRHASGANITNATTAATTAASAAAATSTEGSN
 SESEAESNENSPTPSPLOKKVTEDL SKTLLLYTVPVAVQGFFRSISLSRGNNLQDTLRVLT LWFDYGHWPDVNEALVE
 GVKAIQIDTWLQVIPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHS
 NTLVQQAMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLM
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 APSLQVITSKQRPRKLTLMGSGNHEFVFLKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTN
 SGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSS
 EVWFDRTNYTRSLAVMSMVGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAM
 EVTGLDGNRYRTTCHTVMLEVLREHKDSVMAVLEAFVYDPLLNWRLMDTNTKGNKRSRTRTDSYSAGQSVEILDGVELG
 EPAHKKAGTTVPESIHSGIFDGLVKPEALNKKAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQC
 YIGWCPFW

Sheep mTOR (2550 aa) | GI:224451145 | NP_001138927 | SEQ ID NO: ()

MLGTGPAAATTAATTSSNVSVLQQFASGLKSRNEETRAKAAKELQHYVTMELREMSQEESTRFYDQNLNHHIFELVSS
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WLGADRNEGRRHA AVLVLRELAI SVPTFFFQQVQPFDFNIFVAVWDPKQAI REGAVAALRACLILTTQREPKEMQKP
 QWYRHTFEEAEKGFDETLAKEKGMNRDDRIHGALLILNELVRIS SMEGERLREEMEEITQQQLVHDKYCKDLMGFGT
 KPRHITPFTSFQAVQPQQSNALVGLLGYSSSHQGLMGFGASPSPAKSTLVESRCCDLMEEFQDQVCQWVLKCRNSKN
 SLIQMTILNLLPRLAAFRPSAFTDTQYLQDTMNHVLS CVKKEKERTAAAFQALGLLSVAVRSEFKVYLPRVLDIIRAA
 LPPKDFAHKRQKAMQVDATVFTCISMLARAMGPGIQQDIKELLEPMLAVGLSPALTAVLYDLRSQIPQLKKDIQDGL
 LKMLS LVL MHKPLRHPGMPKGLAHQLASPLGTTLP EASDVGSITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHK
 EIRMEAARTCSRLLTPSVHLISGHAVVVSQTAVQVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQAE NL
 QALFVALNDQVFEIRELAICTVGR LSSMNP AFVMPF LKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRP
 YMEPILKALILKLKDPDPDPNPGVINNVLATIGELAQVSGLEMRKWVDELFIIMDMLQDSSLLAKRQVALWTLGQL
 VASTGYVVEPYRKYPTLLEVLLNFKTEQNQGTRREAIRVLGLLGALDPYKHKVNIGMIDQSRDASAVSLSESKSSQ
 DSSDYSTSEMLVNMGNLPLDEFYPAVSMVALMRIFRDQSLSHHHTMVVQAITFIFRSLGLKCVQFLPQVMPTFLNVI
 RVCDGAIREFLFQQGLMLVSFVKSHIRPYMDEIVTLMREFWVMNTSIOSTIILLIEQIVVALGGEFKLYLPQLIPHM
 LRVFMHDNSSGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVKLFDAP EAPLPSRKALETVDRLTESLDFTDYASRI
 IHPIVRTLDQSP ELRSTAMDTLSSLVFQLGKKYQIFIPMVNVKLVVRHRINHQRYDVLICRIVKGYTLADEEEDPLVY
 QHRMLRSGQGDALASGPVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRRLSLELLKSSPSLRSCWALQA
 YNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLLNLAEFMEHSDKGPLPLRDDNGIVLLGER
 AAKCRAYAKALHYKELEFQKGPTPAILES LISINNKLQQPEAAAGVLEYAMKHFGELEIQATWYEKLHEWEDALVAY
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 IWWERLQGCQRIVEDWQKILMVRSLVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDP SRQLDHP LPTVHP
 QVTYAYMKNMWKSARKIDAFQHMQH FVQTMQQQAQHA IATEDQOQKQELHKL MARCFLKLG EWQLNLQGINESTIPK
 VLQYYSAAATEHDRSWYKAHAWAVMNF EAVLHYKHQ NQARDEKKKLRHASGANITNAATAATTAATATATATSTEGS
 NSESEAESTENSPTPSPLQKKVTEDLSKTL LMYTVP AVQGFFRSISLSRGNNLQDTRLRVLT LWFDYGHWP DVNEALV
 EGVKAIQIDTWLQVIPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEH
 SNTLVQQAMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKG MFEVLEPLHAMMERGPQTLKETSFNQAYGRDL
 MEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRI SKQLPQLTSLELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQS
 IAPSLQVITSKQRPRLKTLMG SNGHEFVFL LKGHEDLRQDERVMQLFGLVNTLLANDPTS LRKNLSIQRYAVIPLST
 NSGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPS
 SEVWFDRRTNYTRSLAVMSMGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNA
 MEVTGLDGNYRITCHTVMEVLREHKDSVM AVLEAFVYDPLLNWRLMDTNTKGNKRSRTRTDSYSAGQSVEILDGVEL
 GEPAHKKTGTTPESIH SFIGYGLVKPEALNKKAIQI INVRDKLTGRDFSHDETLDVPTQVELLIKQATSHENLCQ
 CYIGWCPFW

Zebrafish mTOR (2515 aa) | GI:118601079 | NP_001070679 | SEQ ID NO: ()

MSGTATVLQQFVSGLKSRNEDTRAKAAKDLQHYVTTELRELSQDEATTFFYDELNHHIFELVSSSDVNEKKGGILAIIV
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 VLVRELAVSAPTFFFQQVQPFDFNIFYAVWDSKQAI REGAVSALRACLILTTQRETKEMQKPQWYKQTFEEAEKGF
 DETLAKEKGMNKDDR VHGALLILNELVRIS SMEGERMREEMEEITQQQLVHDKYCKELMGFSTKPRHITPFTSFQSV
 QPQQSNALLGGLYSTPQGFLSFGAAPVPSKSTLVESRYCRELMEERFDQVCRVWLKYRTSKNPLIQMTILNLLPRL
 AAFQPHTFTDQYLQDTMGHLLGCLKKEKERTAAAFQALGLLVAVRADIQPYLSKILEIIKAALPPKDFAHKRQKTMQ
 VDATVFTCISMLSRAMGPSIQQDVKELLEPMLAVGLSPALTAVLYDLRSQIPQLKKDIQDGLLKMLS LVL MHKPLRH
 PGMPKGLAYQLSSPSLTNIPEASDVGSITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHKEIRMEAARTCSRLLT
 PSIHLLSGHGHVVSQTAVQVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQAE NLQALFVALNDEVFEIR
 ELAICTIGRLSSMNP AFVMPF WPKMLIQILTELEHSGVGRNKEQSARMLGHLVSNAPRLIRPYMEPILKALILKLK
 PDPNPGVICV LATIGELAQVSGLEMRKWMDLFPIMDMLQDSSSLAKRQVALWTLGQQVASTGYVVEPYRKYPSL
 LEVLLNFKTEQNQGI RREAIRVLGLLGALDPYKHKVNIGMIDQSRDASAVSLSESKSSQSDADYSTSEMLVNMGNL
 PLDEFYP AVAIVTLMRILRDP SLNHH TMVVQAVTFIFKSLGLKCVQFLPQVMPTFLNVI RVC DASIREFLFQQMGM
 VCVFVKIHIRPYMDDIFTLIREYWTPNNPMQNTIILLIEQIVVALGGEFKLYLPQLIPHMLRVFMHDM SVGRNVTIK
 LLMAIQLFGANLDDYLHLLLPVVKLFDAPDVPLQARKVALETDLRLTESLDFTDYASRIIHPIVRTLDVTPELRNS

SMDTLSSLVFQLGKKYQIFIPMVNKMVLMKLRINHQRYDVLICRIVKGYTLAEEEEEDPLIFQHRQLRSSQSDTLVSGP
VESGPMKKLHVSTTALQKAWGAARKVSKDDWLEWLRRLSVVLLKESSPALRSCWSLAQTYIPLARDLFNAFLSCW
SELSAQDELIRSIELALTSQDIAEVTQTLNLAEFMEHSDKGPLPLRDDNGIVLLGERAAKCRAYAKALHYKELE
FQKGASPLILESLISINNKLQOPEAASGVLEYAMKHFGELEIQATWYEKLHEWEDALVAYDKKIDMNKDDPELILGR
MRCLEALGEWGQLHQQCCEEWTLVSEETQAKMARMAAAAAGWGLGHWDSEEEYTCMI PRDTHDGAFYRAVLALHQDLF
SLAQQCIDKARDLLDAELTAMAGESYSRAYGAMVSCQMLSELEEVIQYKLVPERRDI IRETWWERLQGCQRIVEDWQ
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DAFQHMQHVFQGMQQAQHAIAAEDQQHKLELHKLMARCFKLKGEWQLSLQGINESTIPKVLQYYSHSTEHDNRWYK
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PAVQGFRRSISLSRGNNLQDTRLVLTWLFWDYGHWPVEVNEALVEGIKTIQIDTWLQVLPQLIARIDTPRALVGRLIHQ
LLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHCNTLVQQAIMVSEELIRVAILWHEMWHEGLEEASR
LYFGERNVKGMPFAVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQDWCRKYMRSNGVNDLTQAWDLYYHVFRISK
QLPQLTSLELQYVSPKLLMCRDLELAVPGTYDPNQSIIRIQSIAPSLQVITSKQRPRLTIMGSNGHEFMFLKGE
DLRQDERVMQFLGLVNTLLANDPASLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKKILLNIEHRI
MLRMAPDYDHLTLMEKVEFEHAVNNTAGDDLAKLLWLKSPSEVWFDRRTNYTRSLAVMSVGYILGLGDRHPSNL
MLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAMEVTGLDGNRYITCHTVMEVLRHRDSVMVLEAF
VYDPLLNRWLMDDTNTKGNKRSRTRTDSYTAGQSVEAMEGIDLGETTHKKPGTTVPESIHSGFDGLVQPEALNKKAI
QIINVRDKLTGRDFSHDETLDVPTQVELLIKQATSHENLCQCYIGWCPFW

Rhesus macaque mTOR (2549 aa) | GI:383417207 | AFH31817 | SEQ ID NO: ()

MLGTGPAAATTAATTSSNVSVLQQFASGLKSNEETRAKAAKELQHYVTMELREMSQEESTRFYDQLNHHIFELVSS
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WLGADRNEGRRAAVLVRELAISVPTFFFQVQVPPFDNIFVAVWDPKQAIREGAVAALRACLILTTQREPKEKQK
QWYRHTFEEAEKGFDETLAKEKGMNRDDRIHGALLILNELVRISMEGERLREEMEEITQQQLVHDKYCKDLMGFGT
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SLIQMTILNLLPRLAAFRPSAFTDTQYLQDTMNHVLSVCVKEKERTAAAFQALGLLSVAVRSEFKVYLPRVLDIIRAA
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LKMLSIVLMHKPLRHPGMPKGLAHQLASPLTTLPEASDVGSITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHK
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QALFVALNDQVFEIRELAICTVGRLLSSMNPFAVMPFLRKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRP
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VASTGYVVEPYRKYPTLLEVLLNFKLTEQNQGTREAIRVLGGLGALDPYKHKVNIGMIDQSRDASAVSLSEKSSQ
DSSDYSTSEMLVNMGNLPLDEFYPAVSMVALMRIFRDQSLSHHHTMVVQAITFIFKSLGLKCVQFLPQVMPFTLNVI
RVCDGAIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFWVMNTSIQSTIILLIEQIVVALGGEFKLYLPQLIPHM
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IHPIVRTLDQSPELRSTAMDTLSSLVFQLGKKYQIFIPMVNKMVLRHRINHQRYDVLICRIVKGYTLADEEEDPLIY
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AAKCRAYAKALHYKELEFQKGPTPAILESLSINNKLQOPEAAAGVLEYAMKHFGELEIQATWYEKLHEWEDALVAY
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IWWERLQGCQRIVEDWQKILMVRLVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDPKQLDHPHTAHPHV
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VLQYYSAATEHDSWYKAWHAWAVMNFEAVLHYKHQNGARDEKKKKLRHASGANITNTTTAATTAATATTASTEGSN
SESEAESTENSPTSPPLQKKVTEDLSTLLMYTVPVAVQGFRRSISLSRGNNLQDTRLVLTWLFWDYGHWPVNEALVE
GVKAIQIDTWLQVLPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHS
NTLVQQAMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKGMPFEVLEPLHAMMERGPQTLKETSFNQAYGRDLM
EAQEWCRKYMKSGNVKDLTQAWDLYYHVFRISKQLPQLTSLELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSI
APSLQVITSKQRPRLTIMGSNGHEFVFLKGHEDLRQDERVMQFLGLVNTLLANDPTSLRKNLSIQRYAVIPLSTN

SGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSS
 EVWFDRRTNYTRSLAVMSMGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAM
 EVTGLDGNRYRITCHTVMVLEHREKDSVMAVLEAFVYDPLLNWRLMDTNTKGNKRSRTRTDSYSAGQSVEILDGVELG
 EPAHKKTGTTVPESIHSGIFDGLVKPEALNKKAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQC
 YIGWCPFW

Goat mTOR (2549 aa) | GI:297613711 | ADI48287 | SEQ ID NO: ()

MLGTGPAAATTAATTSSNVSVLQQFASGLKSRNEETRAKAAKEPQHYVTMELREMSQEESTRFYDQNLNHHIFELVSS
 SDANERKGGILAIASLIGVEGGDATRIGRFANYLRNLLPSNDPVVMEMASKAIGRLAMAGDTFTAAYVEFEVKRALE
 WLGAADRNEGRRHA AVLVLRELAI SVPTFFFQQVQPFDFNIFVAVWDPKQAI REGAVAALRACLILTTQREPKEMLKP
 QWYRHTFEEAEKGFDETLAKEKGMNRDDRIHGALLILNELVRISSEMEGERLREGMEEITQQQLVHDKYCKDLMGFGT
 KPRHITPFTSFQAVQPQLSNALVGLLGYSSSHQGLMGFGTSPSPAKSTLVESRCCRDLMEEKFDQVCQVWLKCRNSKN
 SLIQMTILNLLPRLAAFRPSAFTDTQYLQDTMNHVLSVCVKEKERTAAAFQALGLLSVAVRSEFKVYLPRVLDIIRAA
 LPPKDFAHKRQKAMQVDATVFTCISMLARAMGPGIQQDIKELLEPMLAVGLSPALTAVLYDLRSQIPQLKKDIQDGL
 LKMPSLVLMHKPLQHPGMPKGLAHQLASPLGTTPEASDVGSITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHK
 EIRMEATRTCSRLLTPSVHLISGHAHVVSQTAVRVVADVLSKLLVVGITDPPDIRYCVLASLNERFDAHLAQENL
 QALFVALNDQVFETRELAICTVGRLLSSMNPFAFVMPFLRKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRP
 YMEPILKALILKLKDPDPDPNPGVINNVLATIGELTQVSGLEMRKWVDELFIIMDMLQDSSLLAKRQVALWTLGQL
 VASTGYVVEPYRKYPTLLEVLNLFKTEQNQGTREAIRVLGLLGALDPYKHKVNIGMIDQSRDASAVSLSGSKSSQ
 DSSDYSTSEMLVNMGNLPLDEFYPVAVSMVALMRIFRDQSLSHHHTMVVQAITFIFKSLGLKCVQFLPQVMPFTFLNVI
 RVCDGAIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFWVMNTSIQSTIILLIEQIVVALGGEFKLYLPQLIPHM
 LRVFMHDNSSGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVKLFDAPLPSRKALETVDRLTESLDFTDYASRI
 IHPIVRTLDQSPELRSTAMDTLSSLVFQLGKKYQIFIPMVNKLVRHRINHQRYDVLICRIVKGYTLADEEEDPLVY
 QHRMLRSGQGDALASGPVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLRLSLELLKDSSSPSLRSCWALAQA
 YNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNLAEFMEHSDKGPLPLRDDNGIVLLGER
 AAKCRAYAKALHYKELEFQKGPTPAILESLSINNKLQQPEAAAGVLEYAMKHFGELEIQATWYEKLHEWEDALVAY
 DKKMDTNKDDPELMLGRMRCLEALGEWGQLHQQCCEKWTLVNDETQAKMARMAAAAWALGQWDSMEEYTCMI PRDT
 HDGAFYRAVLALHQDLFSLAQQCIDKARDLLDAELTAMAGESYSRAYGAMV SCHMLSELEEVIQYKLVPERREIRQ
 IWWERLQGCQRIVEDWQKILMVRSLVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDPSRQLDHPPLTPVHP
 QVTYAYMKNMWKSARKIDAFQHMQHFFVQTMQQQAQHA IATEDQQHKLHKL MARCFLKLGEWQLNLQGINESTIPK
 VLQYYSAAATEHDRSWYKAHAWAVMNF EAVLHYKHQONARDEKKKLRHASGANITNAATAATATATASTEGSN
 SESEAESTENSPTPSPLQKKVTEDL SKTLLMYTVPVAVQGFFRSISLSRGNNLQDTLRVLT LWFDYGHWPDVNEALVE
 GVKAIQIDTWLQVIPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHS
 NTLVQQAMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLM
 EAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRI SKQLPQLTSLELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSI
 APSLQVITSKQRPRKLTLMGSGNGHEFVFLKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTN
 SGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSS
 EVWFDRRTNYTRSLAVMSMGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAM
 EVTGLDGNRYRITCHTVMVLEHREKDSVMAVLEAFVYDPLLNWRLMDTNTKGNKRSRTRTDSYSAGQSVEILDGVELG
 EPAHKKTGTTVPESIHSGIFDGLVKPEALNKKAIQIINRVRDKLTGRDFSHDETLDVPTQVELLIKQATSHENLCQC
 YLGWCPFW

Fruit fly mTOR, isoform B (2471 aa) | GI:442627689 | NP_001260427 | SEQ ID NO: ()

MSTTSVVQQFVNGLSRNRNVQNKATQDLLFYVKTELREMSQEELAQFFDEFDHHIFTMVNATDINEKKGGALAMKC
 LINEGSLTARKGISPYLNRLRDLILLINDVSMIEAARSLVKLANMPTSKGADSFDFDIKKA FEVL RGERQEYRRHS

AVFILRELAIALPTYFYQHILTTFFEVIFNAIFDPKPAIRSAGEALRAALIVTAQRESTKQSSEPPQWYRICYDEANG
 SFNADLGSSKDQKGVTRDDRIHGGLVVFNEFRCANATWERRYTSKTLFPKTOHNFLEASSSSSMGSQLNTLVPR
 LKVPFIDKLGSTQTHLGEGEHHKGVAKFASQHNVLESAYAQEILQEHYTSICDNVLEQRTSKSPYVQQALLQILPRL
 AAFNRAVFVEKYLQTCVSHLMQILRGKEKDRTVAYITIGYMAVAVQSAIEVHLSSIMTSVKVALPSKDLTSKRKVPV
 DPAVFACITLLAHAVKSEIADDDVKDILEQMFYTGSLPALTVCLRELSENVPQLKSAITEGLIGILSQVLMNKAAILP
 YTALPTIAIDGSLMQNGDGATTVLALKTLGTFNFEEQNMLDFVQRCADYFIVHEQQEIRLEAVQTCTRLKLAVQSS
 ESMENSKTSLDSTVSHVIERLLMVAITDMDCNVRIRILRSLDETFDGKLAQPESLNSLFITLHDEIFEIRELAMVTIG
 RLSSINPAYVMPKLRTTMIELITDLKYSGMSRNKEQSAKMLDHLVISTPRLISSYMNPIKALVPKLHEPESNPGVI
 LNVLRITIGDLAEVNGGSDEMELWADDLLSILLEMLGDAGSPDKRGVALWTLGQLISATGRVVTPYHKYPVLIDILIN
 FLKTEQRRSIRRETIRVLGGLGAMPYKHKMNKGLIDSQKDNVLIAYS DGKVDDESQDISTAELLVNMGNALDEYYPA
 VAIAALMRILRDPTLSTRHTSVVQAVTFIFQSLGIKCPYLAQVLPNLLDNVRTADNNLREFLFQQLAILVAFVKLH
 IISYMGDIFKLIKEFWTINTPLQNTLINLIEQIAVALGCEFRDYLAELIPQILRVLQHDNSKDRMVTRRLQALQKF
 GSTLGYLPLILPPIVKLFDSPYVPQQVSMVALETINNACQLDFTDFSSRIIHPLVRVLDAPPELRDQAMTTLRSL
 AKQLGKKYLVFVPMVQRTLDNKHRIVDPEYEEELLSKIKSCSTLADSYGAGESELRPSRFKNNPEFVTDNRNKNKLQV
 TTNELRTAWQVTRRVSKDDWVEWLKRLSIGLLKESPSHALRACRSYLAQEYDTLLRDLFNAAFISCWTELSPDILKNE
 TQSLIQALQVTDMPFITQITILNLAEFMEHCDRDPPIETKLLGTRAMACRAYAKALRYKEEEFLLREDSQVFESLIL
 INNKLQOREAAEGLLTRYRNAANELNVQGRWYEKLHNWDEALEHYERNLKTDSSEDLRLGHMRCLEALGDWSELSN
 VTKHEWENFGTEAKSRAGPLAAVAAGLQDWEAMREYVRCIPEDTQDGSYRAVLAVHHDDFETAQRLIDETRDLDD
 TELTSMAGESYERAYGAMVCVQMLAELEEVIQYKLIPERREPLKTMWWKRLQGGQRLVEDWRRIIQVHSLVVKPHED
 IHTWLKYASLCRSGSLHLSHKTLMVLLGTDPKLNPNQPLPCNQPVTYAYTKYMAANNQLOEAYEQLTHTFVSTYSQ
 ELSCLPPEALKQQDQRLMARCYLRMATWQNKLODSIRPDALQGALECFEKATSYDPNWKAWHLWAYMNFVQVQAQK
 SALDKQQPPGASMGMTMGSGLDSDLMIQRYAVPAVQGGFRSISLIKGNLQDTRLRLTLWFDYGNHAEVYEALLSG
 MKLIEINTWLQVLPOLIARIDTHRQLVGQLIHQLLMDIGKNHPQALVYPLTVASKSASLARRNAAFKILDSMRKHSP
 TLVEQAVMCSEELIRVAILWHEQWHEGLEEASRLYFGDRNVKGMFEILEPLHAMLERGPQTLKETSFQAYGRELTE
 AYEWSQRYKTSAVVMDLDRAWDIYYHVQKISRQLPQLTSLELPYVSPKLMTCCKDLELAVPGSYNPGQELIRISIK
 TNLQVITSKQRPRLCIRGSNGKDYMYLLKGHEDLRQDERVMQLFSLVNTLLDDPDFTFRNLAIQRYAVIPLSTNS
 GLIGWVPHCDTLHTLIRDYRDKKKVPLNQEHRTMLNFAPDYDHLTLMQKVEVFEHALGQTQGGDLAKLLWLKSPSSE
 LWFERRNNYTRSLAVMSMGYILGLGDRHPSNLMLDRMSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLIKAME
 VTGIEGTYRRTCESVMLVLRNKNDSLMAVLEAFVYDPLLNWRLLDVDDKKGNDAVAGAGAPGGRGGSGMQDSLNSNVE
 DSLPMAKSKPYDPTLQGGGLHNNVADETNSKASQVIKRVKCKLTGTDFQTEKSVNEQSQVELLIQQATNNENLCQCY
 IGWCPFW

Fruit fly mTOR, isoform A (2470 aa) | GI:17864562 | NP_524891 | SEQ ID NO: ()

MSTTSVVQQFVNGLSRNRNVQNKATQDLLFYVKTELREMSQEELAQFFDEFDHHIFTMVNATDINEKKGGALAMKC
 LINCEGSLTARKGISPYLNRLRDLILLINDVSVMEIAARSLVKLANMPTSGGADSFDFDIKKAFEVLRGERQEYRRHS
 AVFILRELAIALPTYFYQHILTTFFEVIFNAIFDPKPAIRSAGEALRAALIVTAQRESTKQSSEPPQWYRICYDEANG
 SFNADLGSSKDQKGVTRDDRIHGGLVVFNEFRCANATWERRYTSKTLFPKTOHNFLEASSSSSMGSQLNTLVPR
 LKVPFIDKLGSTQTHLGEGEHHKGVAKFASHNVLESAYAQEILQEHYTSICDNVLEQRTSKSPYVQQALLQILPRLA
 AFNRAVFVEKYLQTCVSHLMQILRGKEKDRTVAYITIGYMAVAVQSAIEVHLSSIMTSVKVALPSKDLTSKRKVPVD
 PAVFACITLLAHAVKSEIADDDVKDILEQMFYTGSLPALTVCLRELSENVPQLKSAITEGLIGILSQVLMNKAAILPY
 TALPTIAIDGSLMQNGDGATTVLALKTLGTFNFEEQNMLDFVQRCADYFIVHEQQEIRLEAVQTCTRLKLAVQSS
 SMENSKTSLDSTVSHVIERLLMVAITDMDCNVRIRILRSLDETFDGKLAQPESLNSLFITLHDEIFEIRELAMVTIGR
 LSSINPAYVMPKLRTTMIELITDLKYSGMSRNKEQSAKMLDHLVISTPRLISSYMNPIKALVPKLHEPESNPGVIL
 NVLRITIGDLAEVNGGSDEMELWADDLLSILLEMLGDAGSPDKRGVALWTLGQLISATGRVVTPYHKYPVLIDILIN
 LKTEQRRSIRRETIRVLGGLGAMPYKHKMNKGLIDSQKDNVLIAYS DGKVDDESQDISTAELLVNMGNALDEYYPAV
 AIAALMRILRDPTLSTRHTSVVQAVTFIFQSLGIKCPYLAQVLPNLLDNVRTADNNLREFLFQQLAILVAFVKLHI
 ISYMGDIFKLIKEFWTINTPLQNTLINLIEQIAVALGCEFRDYLAELIPQILRVLQHDNSKDRMVTRRLQALQKFG
 STLGYLPLILPPIVKLFDSPYVPQQVSMVALETINNACQLDFTDFSSRIIHPLVRVLDAPPELRDQAMTTLRSLA

KQLGKKYLVFVPMVQRTLNKHRIVDPEYEELLSKIKSCSTLADSYGAGESELRPSRFKNNEPFVTDNRNSNNKNLQVT
 TNELRTAWQVTRRVSKDDWVEWLKRLSIGLLKESPSHALRACRSLAQEYDTLLRDLFNAAFISCWTELSPLDKNELT
 QSLIQALQVTDMPFITQTILNLAEFMEHCDRDPPIPIETKLLGTRAMACRAYAKALRYKEEEFLLREDSQVFESLILI
 NNKLQQREAAEGLLTRYRNAANELNVQGRWYEKLHNWDEALEHYERNLKTDSSDLEARLGHMRCLEALGDWSELSNV
 TKHEWENFGTEAKSRAGPLAAVAAGLQDWEAMREYVRCIPEDTQDGSYYRAVLAVHHDDFETAQRLIDETRDLLDT
 ELTSMAGESYERAYGAMVCVQMLAELEEVIQYKLIIPERREPLKTMWWKRLQGGQRLVEDWRRRIQVHSLVVKPHEDI
 HTWLKYASLCRKSGSLHLSHKTLVMLLGTDPKLNPNQPLPCNQPVITYAYTKYMAANNQLQEAYEQLTHFVSTYSQE
 LSCLPPEALKQQDQRLMARCYLRLMATWQNKLDQSIQGALECFEKATSYDPNWKAWHLWAYMNFKVQQAQKS
 ALDKQQPPGASMGMTMGSLDSDLMIIQRYAVPAVQGFRRSISLIKGNLQDTRLRLTLWFDYGNHAEVYEALLSGM
 KLIEINTWLQVIPQLIARIDTHRQLVGQLIHQLLMDIGKNHPQALVYPLTVASKSASLARRNAAFKILDSMRKHSPT
 LVEQAVMCSEELIRVAILWHEQWHEGLEEASRLYFGDRNVKGMFEILEPLHAMLERGPQTLKETSFQAYGRELTEA
 YEWSQRYKTSAVVMDLDRAWDIYYHVFKIKSRQLPQLTSLELPYVSPKLMTCCKDLELAVPGSYNPGQELIRISIKT
 NLQVITSKQRPRLKICIRSGNGKDYMYLLKGHEDLRQDERVMQFLSLVNTLLDDPDFTFRNLAIQRYAVIPLSTNSG
 LIGWVPNCDTLHTLIRDYRDKKVPLNQEHRTMLNFAPDYDHLTLQKVEVFEHALGQTQGGDLAKLLWLKSPSEL
 WFERRNNYTRSLAVSMVGVIILGLDRHPSNLMLDRMSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLIKAMEV
 TGIEGTYRRTCESVMLVLRNKDSLMAVLEAFVYDPLLNWRLLDVDDKKGNDAVAGAGAPGGRGGSGMQDSLNSVED
 SLPMAKSKPYDPTLQQGGLHNNVADETNSKASQVIKRVKCKLTGTDFTQTEKSVNEQSQVELLIQQATNNENLCQCYI
 GWCPFW

Chimpanzee mTOR (2549 aa) | GI:410334665 | JAA36279 | SEQ ID NO: (___)

MLGTGPAAATTAATTSSNVSVLQQFASGLKSRNEETRAKAAKELQHYVTMELREMSQEESTRFYDQLNHHIFELVSS
 SDANERKGGILAIASLIGVEGGNATRIGRFANYLRNLLPSNDPVMEMASKAIGRLAMAGDTFTAEEYVEFEVKRALE
 WLGADRNEGRRHA AVLVLRELAI SVPTFFFQVQVFFDNIFVAVWDPKQAI REGAVAALRACLILTQREPKEKQK
 QWYRHTFEEAEKGFDETLAKEKGMNRDDRIHGALLILNELVRISMEGERLREEMEEITQQQLVHDKYCKDLMGFGT
 KPRHITPFTSFQAVQPQQSNALVGLLGYSSSHQGLMGFGTSPSPAKSTLVESSRCCDLMEEFKQVQVWLKCRNSKN
 SLIQMTILNLLPRLAAFRPSAFTDTQYLQDTMNHVLSVCVKKEKERTAAFQALGLLSVAVRSEFKVYLPRVLDIIRAA
 LPPKDFAHKRQKAMQVDATVFTCISMLARAMGPGIQQDIKELLEPLAVGLSPALTAVLYDLRSQIPQLKKDIQDGL
 LKMLSIVLMHKPLRHPGMPKGLAHQLASPLTTLPEASDVGSITLALRTLGSFEFEGHSLTQFVRHCADHFLNSEHK
 EIRMEAARTCSRLLTPSIHLISGHAVVSQTAVQVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQAEENL
 QALFVALNDQVFEIRELAICTVGRLLSSMNPFAVMPFLRKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRP
 YMEPILKALILKLKDPDPDPNPGVINNVLATIGELAQVSGLEMRKWVDELFIIMDMQLQDSSLLAKRQVALWTLGQL
 VASTGYVVEPYRKYPTLLEVLLNFKLTEQNQGTREAIRVLGGLGALDPYKHKVNIGMIDQSRDASAVSLSEKSSQ
 DSSDYSTSEMLVNMGNLPLDEFYPVASMVALMRIFRDQSLSHHHTMVVQAITFIFKSLGLKCVQFLPQVMPFTLNVI
 RVCDGAIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFWVMNTSIQSTIILLIEQIVVALGGEFKLYLPQLIPHM
 LRVFMHDNSPGRIVSIKLLAAIQFLGANLDDYLHLLLPPIVKLFDAPLPSRKALETVDRLTESLDFTDYASRI
 IHPIVRTLDQSPELRSTAMDTLSSLVFQLGKKYQIFIPMVNKLVRHRINHQRYDVLCRIVKGYTLADEEEDPLIY
 QHRMLRSGQDALASGPVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRRLSLELLKSSSPSLRSCWALAQA
 YNPMARDLFAAFAVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNLAEFMEHSDKGPLPLRDDNGIVLLGER
 AAKCRAYAKALHYKELEFQKGPTPAILESLSINNKLQPEAAAGVLEYAMKHFGLEIQTWYEKLHEWEDALVAY
 DKKMDTNKDDPELMLGRMRCLEALGEWGQLHQCCCEKWTLVNDETQAKMARMAAAAGLQWDSMEEYTCMIPRDT
 HDGAFYRAVLALHQDLFLSLAQQCIDKARDLLDAELTAMAGESYSRAYGAMV SCHMLSELEEVIQYKLVPERREIRQ
 IWWERLQGCQRIVEDWQKILMVRSLVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDPQRDLHPLPTVHP
 QVTYAYMKNMWKSARKIDAFQHMQHVFQTMQQQAQHA IATEDQHQKQELHKL MARCFLKLG EWQLNLQGINESTIPK
 VLQYYSAAATEHDRSWYKAWHAWVMNFEAVLHYKHQONARDEKKKLRHASGANITNATTAATTAATATTASTEGSN
 SESEAESTENSPTPSPLQKKVTEDL SKTLLMYTVPVAVQGFRRSISLSRGNNLQDTRLRVTLWFDYGHWPDVNEALVE
 GVKAIQIDTWLQVIPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHS
 NTLVQQAMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKGMEFEVLEPLHAMMERGPQTLKETSFNQAYGRDLM
 EAQEWCRKYMKSGNVKDLTQAWDLYYHVFRISKQLPQLTSLELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSI
 APSLQVITSKQRPRLKLTLMGSGNGHEFVLLKGHEDLRQDERVMQFLGLVNTLLANDPTSLRKNLSIQRYAVIPLSTN

SGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSS
EVWFDRRTNYTRSLAVMSMVGYYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAM
EVTGLDGNRYRITCHTVMEVLREHKDSVMAVLEAFVYDPLLNWRLMDTNTKGNKRSRTRTDSYSAGQSVEILDGVELG
EPAHKKTGTTVPESIHSGFIGDGLVKPEALNKKAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQC
YIGWCPFW

Representative expression constructs for mTOR-associated polypeptides

Human RAPTOR | SEQ ID NO: ()

MESEMLQSPLLGLGEEDEADLTDWNLPPLAFMKKRHCEKIEGSKSLAQSWRMKDRMKTVSVALVLCINVGVDPPDVVK
 TTPCARLECWIDPLSMGPQKALETIGANLQKQYENWQPRARYKQSLDPTVDEVKKLCTSLRRNAKEERVLFHYNGHG
 VPRPTVNGEVWVFNKNYTQYIPLSIYDLQTMWGSPSIFVYDCSNAGLIVKSFKQFALQREQELEVAAINPNHPLAQM
 PLPPSMKNCIQLAACEATELLPMIPDLPADLFTSCLTTPIKIALRWFCMQKCVSLVPGVTLDLIEKIPGRINDRRTP
 LGELNWIFTAITDTIAWNVLPRLDFQKLFQDQLLVASLFRNFLAERIMRSYNCTPVSSPRLPPTMHAMWQAWDLA
 VDICLSQLPTIIIEGTAFRHSPFFAEQLTAFQVWLTMGVENRNPPEQLPIVLQVLLSQVHRLRALDLLGRFLDLGPW
 AVSLALSVGIFPYVLKLLQSSARELRPLLVIWAKILAVDSSCQADLVKDNGHKYFLSVLADPYMPAEHRTMTAFIL
 AVIVNSYHTGQEACLGQNLIAICLEQLNDPHPLLQWVAICLGRIWQNFDSARWCGVRDSAHEKLYSLLSDPIPEVR
 CAAVFALGTFVGNASERTDHSTTIDHNVAMMLAQLVSDGSPMVRKELVVALSHLVVQYESNFCVALQFIEEEKNYA
 LPSPATTEGGSLTPVRDSPCTPRLRSVSSYGNIRAVATARS LNKS LQNL SLTEESGGAVAFSPGNLSTSSASSTLG
 SPENEEHILSFETIDKMRRASSYSSLSNLIGVSFNVS YTQIWRVLLHLAADPYEVSDVAMKVLNSIAYKATVNARP
 QRVLDTSsItqsapasptnkgvhihqaggspasstssssltndvakqpvsrdlpgrpgtgpagaqytpshqfp
 rtrkMFDKGPEQTADDADDAAGHKSFISATVQTGFCDWSARYFAQPVMIKIP EHDLESQIRKEREWRLNSRVRRQ
 AQQVIQKGITRLDDQIFLNRNPGVPSVVKFHPFTPCIAVADKDSICFWDWEKGEKLDYFHNGNPRYTRVTAMEYLNG
 QDCSLLL TATDDGAIRVWKNFADLEKNPEMVTAWQGLSDMLPTTRGAGMVVDWEQETGLLMSSGDVRIVR IWDTDRE
 MKVQDIPTGADSCVTSLSCDSHRSLIVAGLGDSIRVYDRRMALSECVRMTYREHTAWVVKASLQKRPDGHIVSVSV
 NGDVRI FDPMPESVNVLQIVKGLTALDIHPQADLIACGSVNQFTAIYNSSGELINNIKYDGFMGQRVGAISCLAF
 HPHWPHLAVGSNDYYISVYSVEKRV

FLAG-tagged human RAPTOR (lower case indicates tag sequence) | SEQ ID NO: ()

mdykdddkESEMLQSPLLGLGEEDEADLTDWNLPPLAFMKKRHCEKIEGSKSLAQSWRMKDRMKTVSVALVLCINVG
 VDPPDVVKTTTPCARLECWIDPLSMGPQKALETIGANLQKQYENWQPRARYKQSLDPTVDEVKKLCTSLRRNAKEERV
 LFHYNGHGVPRPTVNGEVWVFNKNYTQYIPLSIYDLQTMWGSPSIFVYDCSNAGLIVKSFKQFALQREQELEVAAIN
 PNHPLAQMPLPPSMKNCIQLAACEATELLPMIPDLPADLFTSCLTTPIKIALRWFCMQKCVSLVPGVTLDLIEKIPG
 RLNDRRTPLGELNWIFTAITDTIAWNVLPRLDFQKLFQDQLLVASLFRNFLAERIMRSYNCTPVSSPRLPPTMH
 MWQAWDLAVDCLSLPTIIIEGTAFRHSPFFAEQLTAFQVWLTMGVENRNPPEQLPIVLQVLLSQVHRLRALDLLG
 RFLDLGPWAVSLALSVGIFPYVLKLLQSSARELRPLLVIWAKILAVDSSCQADLVKDNGHKYFLSVLADPYMPAEH
 RTMTAFILAVIVNSYHTGQEACLGQNLIAICLEQLNDPHPLLQWVAICLGRIWQNFDSARWCGVRDSAHEKLYSLL
 SDPIPEVRCAA VFALGTFVGNASERTDHSTTIDHNVAMMLAQLVSDGSPMVRKELVVALSHLVVQYESNFCVALQF
 IIEEKNYALPSPATTEGGSLTPVRDSPCTPRLRSVSSYGNIRAVATARS LNKS LQNL SLTEESGGAVAFSPGNLST
 SSASSTLGSPENEEHILSFETIDKMRRASSYSSLSNLIGVSFNVS YTQIWRVLLHLAADPYEVSDVAMKVLNSIAY
 KATVNARPQRVLDTSsItqsapasptnkgvhihqaggspasstssssltndvakqpvsrdlpgrpgtgpagaqy
 TPHSHQFPTRKMFMDKGPEQTADDADDAAGHKSFISATVQTGFCDWSARYFAQPVMIKIP EHDLESQIRKEREWRL
 RNSRVRRQAQQVIQKGITRLDDQIFLNRNPGVPSVVKFHPFTPCIAVADKDSICFWDWEKGEKLDYFHNGNPRYTRV
 TAMEYLNGQDCSLLL TATDDGAIRVWKNFADLEKNPEMVTAWQGLSDMLPTTRGAGMVVDWEQETGLLMSSGDVRI
 RIWDTDREMKVQDIPTGADSCVTSLSCDSHRSLIVAGLGDSIRVYDRRMALSECVRMTYREHTAWVVKASLQKRPD
 GHIVSVSVNGDVRI FDPMPESVNVLQIVKGLTALDIHPQADLIACGSVNQFTAIYNSSGELINNIKYDGFMGQRV
 GAISCLAFHPHWPHLAVGSNDYYISVYSVEKRV

Human mLST8 | SEQ ID NO: ()

MNTSPGTVGSDPVILATAGYDHTVRFWQAHSGICTRTVQHQDSQVNALEVTPDRSMIAAAGYQHIRMVYDLNSNNPNP
 IISYDGVNKNIASVGFHEDGRWMTGGEDCTARIWDLRSRNLQCQRIFQVNAPINCVCLHPNQAEILVGDQSGAIHI
 WDLKTDHNEQLIPEPEVSITSAHIDPDASYMAAVNSTGNCYVWNLTTGGIGDEVTLQIPKTKIPAHTRYALQCRFSPD
 STLLATCSADQTCIKWRTSNFSLMTELSIKSGNPGESSRGWMWGCAFSGDSQYIVTASSDNLARLWCVETGEIKREY
 GGHQKAVVCLAFNDSVLG

FLAG-tagged human mLST8 (lower case indicates tag sequence) | SEQ ID NO: ()

mdykdddkNTSPGTVGSDPVILATAGYDHTVRFWQAHSGICTRTVQHQDSQVNALEVTPDRSMIAAAGYQHIRMVYDL
 NSNNPNPIISYDGVNKNIASVGFHEDGRWMTGGEDCTARIWDLRSRNLQCQRIFQVNAPINCVCLHPNQAEILVGD
 QSGAIHIWDLKTDHNEQLIPEPEVSITSAHIDPDASYMAAVNSTGNCYVWNLTTGGIGDEVTLQIPKTKIPAHTRYAL
 QCRFSPDSTLLATCSADQTCIKWRTSNFSLMTELSIKSGNPGESSRGWMWGCAFSGDSQYIVTASSDNLARLWCVET
 GEIKREYGGHQAQAVVCLAFNDSVLG

Human S6K1^{ki} | SEQ ID NO: ()

MAGVFDIDLDPEDAGSEDELEEGGQLNESMDHGGVGPYELGMEHCEKFEISETSVNRGPEKIRPECFELLRLVLGKG
 GYGKVFQVRKVTGANTGKIFAMRVLKKAMIVRNAKDTAHTKAERNILEEVKHPFIVDLIYAFQTGGKLYLILEYLSG
 GELFMQLEREGIFMEDTACFYLAESMALGHLHQKGIYRDLKPENIMLNHQGHVKLTDFGLCKESIHDGTVTHTFC
 GTIEYMAPEILMRSGHNRAVDWWSLALMYDMLTGAPPFTGENRKKTIDKILKCKLNLPPYLTQEARDLLKKLLKRN
 AASRLGAGPGDAGEVQAHPFFRHINWEELLARKVEPPFKPLLQSEEDVSQFDSKFTRQTPVDSPDDSTLSESANQVF
 LGFTYVAPSVLESVKEKFSFEPKIRSPRRFIGSPRTPVSPVKFSPGDFWGRGASASTANPQTPVEYPMETSGIEQMD
 VTMSGEASAPLPIRQPNSSGPYKKQAFPMISKREHLRMNL

FLAG-tagged human S6K1^{ki} (lower case indicates tag sequence) | SEQ ID NO: ()

mdykdddkkgsAGVFDIDLDPEDAGSEDELEEGGQLNESMDHGGVGPYELGMEHCEKFEISETSVNRGPEKIRPEC
 FELLRLVLGKGYGKVFQVRKVTGANTGKIFAMRVLKKAMIVRNAKDTAHTKAERNILEEVKHPFIVDLIYAFQTGGK
 LYLILEYLSGGELFMQLEREGIFMEDTACFYLAESMALGHLHQKGIYRDLKPENIMLNHQGHVKLTDFGLCKESI
 HDGTVTHTFCGTIEYMAPEILMRSGHNRAVDWWSLALMYDMLTGAPPFTGENRKKTIDKILKCKLNLPPYLTQEARD
 DLLKKLLKRNAASRLGAGPGDAGEVQAHPFFRHINWEELLARKVEPPFKPLLQSEEDVSQFDSKFTRQTPVDSPDDSTL
 SESANQVFLGFTYVAPSVLESVKEKFSFEPKIRSPRRFIGSPRTPVSPVKFSPGDFWGRGASASTANPQTPVEYPMETSGIEQMD
 VTMSGEASAPLPIRQPNSSGPYKKQAFPMISKREHLRMNL

Human S6K1^{ki/TOS-} | SEQ ID NO: ()

AGVAAAALDPEDAGSEDELEEGGQLNESMDHGGVGPYELGMEHCEKFEISETSVNRGPEKIRPECFELLRLVLGKG
 YGKVFQVRKVTGANTGKIFAMrVLKKAMIVRNAKDTAHTKAERNILEEVKHPFIVDLIYAFQTGGKLYLILEYLSGG
 ELFMQLEREGIFMEDTACFYLAESMALGHLHQKGIYRDLKPENIMLNHQGHVKLTDFGLCKESIHDGTVTHTFCG
 TIEYMAPEILMRSGHNRAVDWWSLALMYDMLTGAPPFTGENRKKTIDKILKCKLNLPPYLTQEARDLLKKLLKRNA
 ASRLGAGPGDAGEVQAHPFFRHINWEELLARKVEPPFKPLLQSEEDVSQFDSKFTRQTPVDSPDDSTLSESANQVFL
 GFTYVAPSVLESVKEKFSFEPKIRSPRRFIGSPRTPVSPVKFSPGDFWGRGASASTANPQTPVEYPMETSGIEQMDV
 TMSGEASAPLPIRQPNSSGPYKKQAFPMISKREHLRMNL

FLAG-tagged human S6K1^{ki/TOS-} (lower case indicates tag sequence) | SEQ ID NO: (___)

mdykdddddkgSAGVAAAALDQPEDAGSEDELEEGQLNESMDHGGVGPYELGMEHCEKFEISETSVNRGPEKIRPEC
FELLRLVGKGGYGKVFQVRKVTGANTGKIFAMrVLKKAMIVRNAKDTAHTKAERNILEEVKHPFIVDLIYAFQTGGK
LYLILEYLSGGELFMQLEREGIFMEDTACFYLAEISMA LGHLHQGIIYRDLKPENIMLNHQGHVKLTDFGLCKESI
HDGTVTHTFCGTIEYMAPEILMRSGHNRAVDWWSLGALMYDMLTGAPPFTGENRKKTIDKILKCKLNLPPYLTQEAR
DLLKKLLKRNAASRLGAGPGDAGEVQAHPFFRHINWEELLARKVEPPFKPLLQSEEDVSQFDSKFTRQTPVDSRDD
TLSESANQVFLGFTYVAPSVLESVKEKFSFEPKIRSPRRFIGSPRTPVSPVKFSPGDFWGRGASASTANPQTPVEYP
METSGIEQMDVTMSGEASAPLPIRQPN SGPYKKQAFPMISKRPEHLRMNL

Human 4EBP1 | SEQ ID NO: (___)

MSGSSCSQTPSRAIPATRRVVLGDGVQLPPGDYSTTPGGTLFSTTPGGTRI IYDRKFLMECRNSPVTKTPPRDLPT
IPGVTSPSSDEPPMEASQSHLRNSPEDKRAGGEESQFEMDI

GST-tagged human 4EBP1 (lower case indicates tag sequence) | SEQ ID NO: (___)

mspilgywkikglvqp trllleyleekyeehlyerdegdkwrnk k felglefnp lpyyidgdvkl tqsmairiyiad
khnmlggcpkeraeismlegavldirygvsriayskdfetlkvdflsklpemlkmfedrlchkt ylngdhvthpdfm
lydal dvvlymdpmcldafpklvckkrieaipqidkylksskyiawplqg wqatfgggdhppksdenlyfqqsg rM
SGGSSCSQTPSRAIPATRRVVLGDGVQLPPGDYSTTPGGTLFSTTPGGTRI IYDRKFLMECRNSPVTKTPPRDLPTI
PGVTSPSSDEPPMEASQSHLRNSPEDKRAGGEESQFEMDI

Representative PI3K and PIKK sequences

PI3K, class 2, alpha polypeptide | GI:157671929 | NP_002636 | SEQ ID NO: ()

MAQISSNSGFKECPSSHPEPTRAKDVDKEEALQMEAEALAKLQKDRQVTDNQRGFELSSSTRKKAQVYNKQDYDLMV
 FPESDSQKRALDIDVEKLTQAELEKLLDDSFETKKTPLVLPVTPILSPSFSQAQLYFRPTIQRGQWPPGLPGPSTYAL
 PSIYPSTYSKQAAFQNGFNPRMPTFPSTETPIYLSLPGQSPYFSYPLTPATPFHPQGSPLIYRPVVSTDMAKLFDKIA
 STSEFLKNGKARTDLEITDSKVSNLQVSPKSEDISKFDWLDLDPKPKVDNVEVLDHEEEKNVSSLLAKDPWDAVL
 LEERSTANCHLERKVNKSLSVATVTRSQSLNIRTTQLAKAQGHISQKDPNGTSSLPTGSSLLQEEVEVQNEEMAFC
 RSITKLKTKFPYTNHRTNPGYLLSPVTAQRNICGENASVKVSIIDIEGFQLPVTFTCDVSSTVEIIIMQALCWVHDDL
 NQVDVGSYVLKVCQEEVLQNNHCLGSHEHIQNCRKWDTEIRLQLLTFSAMCQNLARTAEDDETPVDLNKHLYQIEK
 PCKEAMTRHPVEELLDSYHNQVELALQIENQHRAVDQVIKAVRKICSALDGVETLAITESVKKLKRAVNLPRSKTAD
 VTSLFGGEDTSRSSTRGSLNPNENPVQVSINQLTAAIYDLRLHANSRSPDCAQSSKSVEAWTTTEQLQFTIFAA
 HGISSNWVSNYEKYLYICSLSHNGKDLFKPIQSKKVGTYKNFFYLIKWDELIIFPIQISQLPLESVLHLTLFGILNQ
 SSGSSPDSNKQRKGPEALGKVSPLPLDFKRFSLTCTGKLLYLWTSSTNSVPGTVTKKGYVMERIVLQVDFPSPAFDI
 IYTTPOVDRSIIQQHNLETLENDIKGKLLDILHKDSSLGLSKEDKAFLWEKRYCYCFKHPNCLPKILASAPNWKWVNL
 AKTYSLLHQWPALYPLIALELLDSKFADQEVRS LAVTWIEAISDDELTDLLPQFVQALKYEIYLNSSLVQFLLSRAL
 GNIQIAHNLYWLLKDALHVDVQFSTRYEHVLGALLSVGGKRLREELLKQTKLVQLLGGVAEKVRQASGSARQVVLQRS
 MERVQSFFQKNKCRPLPKPSLVAKELNIKSCSFFSSNAVPLKVTMVNADPMGEEINVMFKVGEDLRQDMLALQMIKI
 MDKIWLKEGLDLRMVIFKCLSTGRDRGMVELVPASDTLRKIQVEYGVTSFKDKPLAEWLRYKNPSEEEYEKASENF
 IYSCAGCCVATYVLGICDRHNDNIMLRSTGHMFHIDFGKFLGHAQMFGSFKRDRAPFVLTSDMAYVINGGEKPTIRF
 QLFVDLCCQAYNLIRKQTNLFLNLLSLMIPSGLPELTSIQDLKYVRDALQPQTDAEATIFFTRLIESSLSGIATKF
 NFFIHNLAQLRFSGLPSNDEPILSFSPKTYSFRQDGRIKEVSVFTYHKKYNPDKHYIYVVRILREGQIEPSFVFRTF
 DEFQELHNKLSIIFPLWKLPGFPMRMVLGRTHIKDVAARKIELNSYLOSLMNASTDVAECDLVCTFFHPLLRDEKA
 EGIARSADAGSFSPTPGQIGGAVKLSISYRNGTLFIMVMHIKDLVTEGDADPNPYVKTYLLPDNHKTSKRRTKISRK
 TRNPTFNEMLVISGYSKETLRQRELQLSVLSAESLRENFFLGGVTLPLKDFNLKETVKWYQLTAATYL

PI3K, class 2, beta polypeptide | GI:194097347 | NP_002637 | SEQ ID NO: ()

MSSTQNGEHWKSLESVGISRKELAMAEALQMEYDALSRRLRHDKEENRAKQNAADPSLISWDEPGVDIFYSKPAGRRTD
 LKLLRGLSGSDPTLNYNLSLSPQEGPPNHSTSQGPQPGSDPWPKGSLSGDYLYIFDGSDDGVSSSPGPGDIEGSCKKL
 SPPPLPPRASIWDTPLPPRKGSPSSKISQPSDINTFSLVEQLPGKLLHRILEEEVVLGGGGQGRLLGSVDYDGI
 NDAITRLNLKSTYDAEMLRDATRGWKEGRGPLDFSKDTSGBKPVARSKTMPPQVPPRTYASRYGNRKNATPGKNRRIS
 AAPVGSRPHTVANGHELFEVSEERDEEVAACHMLDILRSGSDIQDYFLTGYVWSAVTPSPEHLGDEVNLKVTVLCD
 RLQEALTFTCNCSSTVDLLIYQTLCTHDDLNRVDVGDFVLKPCGLEEFQNKHALGSHEYIYQCRKFDIDIRLQLM
 EQKVVRSDLARTVNDDQSPSTLNLYLVHLQERPVKQTI SRQALSLLFDTYHNEVDALLADGDFPLKADRVVQSVKAI
 CNALAAVETPEITSALNQLPPCPSRMQPKIQKDPVSLAVRENREKVVEALTAAILDLVELYCNFTNADFQTAVPGSR
 KHDLVQEACHFARSLAFTVYATHRIPIIWATSYEDFYLSCLSHGGKELCSPLQTRRAHFSKYLFHLIVWDQQICFP
 VQVNRLPRETLLCATLYALPIPPGSSSEANKQRRVPEALGWVTTPLFNFRQVLTGCRKLLGLWPATQENPSARWSA
 PNFHQPDSVILQIDFPTSADFIDKFTSPPGDKFSRYEFGSLREEDQRKLKDIMQKESLYWLTADKKRLWEKRYCH
 SEVSSLPLVLASAPSEWACLPDIYVLLKQWTHMNHQDALGLLHATFPDQEVRRMAVQWIGSLSDAELLDYLPQLVQ
 ALKYECYLDSPVRFLLKRAVSDLRVTHYFFWLLKDGKDSQFSIRYQYLLAALLCCCGKGLREEFNRCWLNVNALA
 KLAQQVREAAAPSARQGILRTGLEEVKQFFALNGSCLPLSPSLLVKGIVPRDCSYFNSNAVPLKLSFQNVDPGENI
 RVIFKCGDDLQDMLTLQIMIRIMSKIWVQEGLDMMVIFRCFSTGRGRGMVEMIPNAETLRKIQVEHGVTSFKDRP
 LADWLQKHNPGEDEYEKAVERNFIYSCAGCCVATYVLGICDRHNDNIMLKTTHGMFHIDFGRFLGHAQMFGNIKRDR
 PFVFTSDMAYVINGGDKPSSRFHDFVDLCCQAYNLIRKHTLFLNLLGLMLSCGIPELSDLEDLKYVVDALRPQDTE
 ANATTYFTRLIESSLSGVATKLNFFIHNLAQMKFTGSDDRLTSLFASRTHTLKSSGRISDVFLCRHEKIFHPNKGYI

YVVKVMRENTHEATYIQRTFEFQELHNKLRLFLPSSHLPSFSPSRFVIGRSRGEAVAERRREELNGYIWHLIHAPPE
 VAECDLVYTFHFPLPRDEKAMGTSPAPKSSDGTWARPVGKVGGEVKLSISYKNNKLFIMVMHIRGLQLLQDGNPD
 YVKIYLLPDPQKTTKRKTQVARKTCNPTYNEMLVYDGIPKGDLOQRELQLSVLSEQGFWENVLLGEVNIRLRELDLA
 QEKTGWFAFGSRSHGTL

PI3K, class 2, gamma polypeptide | GI:194353959 | NP_004561 | SEQ ID NO: ()

MAYSWQTDPNPNESHEKQYEHQEFQELFNQPHSSSQVSLGFDQIVDEISGKIPHYESEIDENTFFVPTAPKWDSTGHS
 LNEAHQISLNEFTSKSRELSWHQVSKAPAI GFSPSVLPKPQNTNKECSWGSPIGKHHGADDSRFSILAPSFTSLDKI
 NLEKELENENHNYHIGFESSIPTNSSFSDDFMPKEENKRSQGHVNIVEPSLMMLKGSQPGMWESTWQKNIESIGCS
 IQLVEVPQSSNTSLASFCKVKKIRERYHAADVNFNSGKIWSTTTAFPYQLFSKTKFNIHIFIDNSTQPLHFMPCAN
 YLVKDIAEILHFCTNDQLLPKDHILSVCGSEEFQNDHCLGSHKMFQKDKSVIQLHLQKSREAPGKLSRKHEEDHS
 QFYLNQLLEFMHIWKVSRQCLLTIRKYDFHLKYLLKTQENVYNIIEEVKKICSVLGCVETKQITDAVNELSLILQR
 KGENFYQSSETSAKGLIEKVTTTELSTSIYQLINVCNSFYADFQPVNVPRCTSYLNPGLPSHLSFTVYAAHNIPETW
 VHRINFPLEIKSLPRESMLTVKLFGIACATNNANLLAWTCLPLFPKEKSI LGSMLFSMTLQSEPPVEMITPGVWDVS
 QPSPVTLQIDFPATGWEYMKPDSEENRSNLEEPLKECIKHIA RL SQKQTPLLLSEEKKRYLWFYRFYCNNENC SLPL
 VLGSAPGWDERTVSEMHTILRRWTF SQPLEALGLLTSSFPDQEI R K VAVQQLDNLNDELLEYLPQLVQAVKFEWNL
 ESPLVQLLLHRSLSIQVAHRLYWLLKNAENEAYFKSWYQKLLAALQFCAGKALNDEF SKEQKLIKILGDIGERVKS
 ASDHQRQEV LKKEIGRLEEFFQDVNTCHLPLNPALCIKIDHDACS YFTSNALPLKITFINANPMGKNISII FKAGD
 DLRQDMLVLQLIQVMDNIWLQEGLDQMIIYRCLSTGKDQGLVQMV PDAVTLAKIHRHSGLIGPLKENTIKKWF SQH
 NHLKADYEKALRNFFYSCAGWCVVTFILGVC DRHNDNIMLT KSGHMFHIDFGKFLGHAQTGGIKRDRAPFIFTSEM
 EYFITEGGKNPQHFDQDFVELCCRAYNIIRKHSQ LLLN LEMMLYAGLP ELSGIQDLKYVYNNLRPQD TDLEATSHFT
 KKIKESLECFPVKLNLIHTLAQMSAISPAKSTSQTFPQESCLLSTTRS IERATILGFSKSSNLYLIQVTHSNNET
 SLTEKSFEQFSKLHSQ LQKQFASLTLP EFPHWWHL PFTNSDHRFRDLNHYMEQILNVSHEVTNSDCVLSFFLSEAV
 QQTVEESSPVYLGEKFPDKPKVQLVISYEDVKLTILVKHMKNIHL PDGSAPS AHVEFYLLPY PSEVRRRKTKSVPK
 CTDPTYNEIVVYDEVTELQGHVLM L I V K S K T V F V G A I N I R L C S V P L D K E K W Y P L G N S I I

PI3K, class 3 | GI:34761064 | NP_002638 | SEQ ID NO: ()

MGEAEKFHYIYSCDL D I N V Q L K I G S L E G K R E Q K S Y K A V L E D P M L K F S G L Y Q E T C S D L Y V T C Q V F A E G K P L A L P V R T S
 YKAFSTRWNWNEWLKL PVKYPDLPRNAQVALTIWDVYGPGKAVPVGGTTVSLFGKYGMFRQGMHDLKVWPNVEADGS
 EPTKTPGRTSS TLSEDQMSRLAKLTKAHRQGHMV KVDWLDRLTFREIEMINESEKRSSNFMYLMVEFRVCVKDDKEY
 GIVYYEKDGEDESSPILTSFELVKVPDPQMSMENLVESKHHKLARSLSRSGPSDHDLPNAATRDQLNIIVSYPPTKQL
 TYEEQDLVWKFRYYLTNQEKALTKFLKCVNWDLPQEAQALELLGKWKPM DVEDSLELLSSHYTNPTVRRYAVARLR
 QADDEDLLMYLLQLVQALKYENFDDIKNGLEPTKKDSQSSVSENVSN SGINSAEIDSSQIITSPLPSVSSPPPASKT
 KEVPDGENLEQDLCTFLISRACKNSTLANLYWYVIVECEDQDTQQRDPKTHEMYLNMRRFSQALLKGDKSVRVMR
 SLLAAQQT FVDRLVHLMKAVQRESGNRKKKNERLQALLGDNEKMNLSDVELIPLPLEPQVKIRGIIPETATLFKSAL
 MPAQLFFKTEDGGKYPVIFKHGDDLQDQLILQIISLMDKLLRKENLDLKLTPYKVLATSTKHGFMQFIQSV PVAEV
 LDTEGSIQNFFRKYAPSENGPNGISAEVMDTYVKSCAGYCVITYILGVGDRHLDNLLLTKTGKLFHIDFGYILGRDP
 KPLPPPMLKNKEMVEGMGGTQSEYQEFKQCYTAFLHLRRYSNLILNLFSLMVDANIPDIALEPDKTVKKVQDKFR
 LDLSDEEAVHYMQSLIDESVHALFAAVVEQIHKFAQYWRK

PI3K, catalytic, alpha polypeptide | GI:54792082 | NP_006209 | SEQ ID NO: ()

MPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLITIKHELFKEARKYPLHQLLQDESSYIFVSVTQEA
 EREEFFDETRRLCDLRLFPFLKVIEPVGNREEKILNREIGFAIGMPVCEFDMDVKDPEVQDFRNLNVCKEAVDLR
 DLNSPHSRAMYVPPNVESSELPKHIYNKLDKGQIIIVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIKKKTRS
 MLLSSEQLKLCVLEYQGYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNMLMAKESLYSQLPMDCFMTPSYS
 RRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRDIDKIYVRTGIYHGGEPLCDNVNTQRVPCSNPRWNE
 WLNYDIYIPDLPRARLCLCSVKGKRGAKKEHCPLAWGNINLFDYTDTLVSGKMALNLWPVPHGLEDDLNPIGVT
 GSNPNKETPCLELEFDWFSSVVKFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKAISTRDP
 LSEITEQEKFDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPKPEQAMELLDCNYPDPMVRGFAV
 RCLEKYLTDDKLSQYLIQVLQVVKYEQYLDNLLVRFLLKKALTNQRIGHFFFWHLKSEMHNKTVSQRFGLLLESYCR
 ACGMYLKHLNRQVEAMEKLINLTDILKQEKKDETQKVQMKFLVEQMRRPDFMDALQGFLSPLNPAHQGLNLRLEECR
 IMSSAKRPLWLNWENPDIMSELLFQNNIEIFKNGDDLQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLSIGDCVGL
 IEVVRNSHTIMQIQCKGGLKQALQFNSHTLHQLKDKNKGEIYDAIDLFTTRSCAGYCVATFILGIGDRHNSNIMVK
 DDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLTQDFLIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINL
 FSMMLGSGMPQLQSFDDIAYIRKTLALDKTEQEALEYFMKQMNDAHHGGWTTKMDWIFHTIKQHALLN

PI3K, catalytic, beta polypeptide | GI:5453894 | NP_006210 | SEQ ID NO: ()

MCFSFIMPPAMADILDIWAVDSQIASDGSIPVDFLLPTGTIYIQLEVPREATISYIKQMLWKQVHNYPMFNLLMDIDS
 YMFACVNQTAVYEELEDETRRLCDVRPFLPVLKLVTRSCDPGEKLDISKIGVLIGKGLHEFDSLKDPEVNEFRKMRK
 FSEKILSLVGLSWMDWLKQTYPPHEPSIPENLEDKLYGGKLIVAVHFENCQDVFSFQVSPNMNPIKVNELAIQKR
 LTIHGKEDEVSPYDYVLQVSGRVEYVFGDHPHQFYIRNCVMNRALPHFILVECKIKKMYEQEMIAIEAANRNS
 SNLPLPLPPKKTRIIISHVWENNNPFQIVLVKGNKLNTEETVKVHVRAGLFHGTELLCKTIVSSEVSGKNDHIWNEPL
 EFDINICDLPRMARLCFAVYAVLDKVKTKKSTKTINPSKYQTIRKAGKVHYPVAWVNTMVFDFKGQLRTGDIILHSW
 SSFPDELEMLNPMGTQVQTNPYTENATALHVKFPENKKQPYYPFFDKIIEKAAEIASSDSANVSSRGKKFLPVLK
 EILDRDPLSQLCENEMDLIWTLRQDCREIFPQSLPKLLLSIKWNKLEDVAQLQALLQIWPKLPPREALELLDFNYPD
 QYVREYAVGCLRQMSDEELSQYLLQVLQVVKYEPFLDCALSRFLLEALGNRRIGQFLFWHLRSEVHIPAVSVQFGV
 ILEAYCRGSGVHMKVLSKQVEALNKLKTLNLSIKLNAVKLNRAGKKEAMHTCLKQSAYREALSDLQSPNPCVILSE
 LYVEKCKYMDSKMKPLWLNVNNKVFGEDEVGVIFKNGDDLQDMLTLQMLRLMDLLWKEAGLDLRMLPYGCLATGDR
 SGLIEVVSTSETIADIQLNSSNVAAAAAFNKDALLNWLKEYNSGDDLDRAIEEFTLSCAGYCVASYVLGIGDRHSDN
 IMVKKTGQLFHIDFGHILGNFKSKFGIKRERVPFILTDFIHIYQQGKTGNTEKFGFRQCCEDAYLILRRHGNLFI
 TLFALMLTAGLPELTSVKDIQYLKDSLALGKSEEEALKQFKQKFDALRESWTTKVNWMAHTVRKDYRS

PI3K, catalytic, gamma polypeptide | GI:21237725 | NP_002640 | SEQ ID NO: ()

MELNYKQPVVLRDNCRRRRRMKPRSAAASLSMELIPIEFVLPTSQRKCKSPETALLHVAGHGNVEQMAQVWLR
 ALETSAADFYHRLGPHHFLLLYQKKGQWYIEYDKYQVVQTLDCRLRYWKATHRSPGQIHLVQRHPPSEESQAFQRQL
 TALIGYDVTDVSNVHDELEFTRRGLVTPRMAEVASRDPKLYAMHPWVTSKPLPEYLWKKIANNCIFIVIHRTSTSQ
 TIKVSPDDTPGAILQSFFTKMAKKKSLMDIPESQSEQDFVLRVCGRDEYLVGETPIKNFQWVRHCLKNGEEIHVVLD
 TPPDPALDEVKKEEWPLVDDCTGVTGYHEQLTIHGKDHEVFTVSLWDCDRKFRVKIRGIDIPVLPRNTDLTVFVEA
 NIQHGQQVLCQRRTSPKPFTEEVLWNVWLEFSIKIKDLPKGALLNLQIYCGKAPALSSKASAESPSSESKGKVQLLY
 YVNLILLIDHRFLLRRGEYVLHMWQISGKGEDQGSFNADKLTSATNPDKENSMSISILLDNYCHPIALPKHQPTPDPE
 GDRVRAEMPQNQLRKQLEAIATDPLNPLTAEDKELLWHFRYESLKHPKAYPKLFSVVKWQQEIVAKTYQLLARREV
 WDQSALDVGLTMQLLDCNFSDENVRAIAVQKLESLEDDDVLYHLLQLVQAVKFEPYHDSALARFLLKRGRLNRKIGH
 FLFWFLRSEIAQSRHYQQRFAVILEAYLRGCGTAMLDHFTQQVQVIEMLQKVTLTDIKSLSAEKYDVSSQVISQLKQK
 LENLQNSQLPESFRVPYDPGLKAGALAEKCKVMASKKKPLWLEFKCADPTALSNETIGIIFKHGDDLQDMLILQI
 LRIMESIWETESLDLCLLPYGCISTGDKIGMIEIVKDATTIAKIQQSTVGNTGAFKDEVLNHWLKEKSPTEEFQAA
 VERFVYSCAGYCVATFVLGIGDRHNDNIMITETGNLFHIDFGHILGNYSFLGINKERVFPVLTLPDFLFVMTSGKK

TSPHFQKFQDICVKAYLALRHHTNLLIILFSMMLMTGMPQLTSKEDIEYIRDALTVGKNEEDAKKYFLDQIEVCRDK
GWTVQFNWFLHLVLGIKQGEKHS

PI3K, catalytic, delta polypeptide | GI:156564405 | NP_005017 | SEQ ID NO: ()

MPPGVDCPMEFWTKEENQSVVVDLFLPTGVYLNFPVSRNANLSTIKQLLWHRAQYEPLFHMLSGPEAYVFTCINQTA
EQQELEDEQRRLCDVQPFPLVLRVAREGDRVKKLINSQISLLIGKGLHEFDSLCDPEVNDFRAKMCQFCEEAAARR
QQLGWEAWLQYSFPLQLEPSAQWTGPGTLRLPNRALLVNVKFEGSEESFTFQVSTKDVPLALMACALRKATVFRQP
LVEQPEDYTLQVNGRHEYLYGSYPLCQFYICSLHSGLTPLHLMVHSSSILAMRDEQSNPAPQVQKPRAKPPPIPA
KKPSSVSLWSLEQPFRIELIQGSKVNADERMMLVQAGLFHGNEMLCCTVSSEVSVCSEPVWKQRLEFDINICDLP
RMARLCFALYAVIEKAKKARSTKKKSKKADCPICAWANLMLFDYKDKLKTGERCLYMWPSVPDEKGEMLNPTGTVRSN
PNTDSAAALLICLPEVAPHPVYYPALEKILELGRHSECVHVTEEEQLQLREILERRGSGELYEHEKDLVWKL RHEVQ
EHFPEALARLLLVTKNKHEDVAQMLYLLCSWPELPVLSALELLDFSFPDCHVGSFAIKSLRKLTDDELFOYLLQLV
QVLKYESYLDCELTCKFLLDRLANRKIGHFLFWHLRSEMHPVSVALRFLGLEAYCRGSTHHMKVLMKQGEALSKLK
ALNDFVKLSSQKTPKPKQTKELMHLCMRQEAYLEALSHLQSPDPLSTLLAEVCVEQCTFMDSKMKPLWIMYSNEEAGS
GGSGVGIIFKNGDDLQDMLTLQMIQLMDVLWKQEGDLRMTYPGCLPTGDRGTGLIEVVLRSDTIANIQLNKS NMAAT
AAFNKDALLNWLKSKNPGEALDRAIEEFTLSCAGYCVATYVLGIGDRHSDNIMIRESGQLFHIDFGHFLGNFKTKFG
INRERVPFILTYDFVHVIQQKTNNSEKFERFRGYCERAYTILRRHGLLFLHLFALMRAAGLPELSCSKDIQYLKDS
LALGKTEEEALKHFRVKFNEALRESWKTKVNWLAHNVSKDNRQ

PI3K, regulatory subunit 1 (alpha) | GI:32455248 | NP_852664 | SEQ ID NO: ()

MSAEGYQYRALYDYKKEREEDIDLHLGDILT VNKGS LVALGFS DQGEARPEEIGWLN GYNETTGERGDFPGTYVEYI
GRKKIS PPTPKPRPRPLPVAPGSSKTEADVEQQALTLPDLAEQFAPPDIA PPLLIKLV EAEIEKKGLECSTLYRTQS
SSNLAE LRQLLD CDTPSVDLEMIDVHVLADAFKRYLLDLPNPVIPA AVYSEMI SLAPEVQSSEYIQLLKKLIRSPS
IPHQYWLTLQYLLKHFFKLSQTSSKNLLNARVLSEIFSPMLFRFSAASSDNTENLIKVIEILISTEWNERQPAPALP
PKPPKPTTVANNGMNNNMSLQDAEWYWGDISREEVNEKL RDTADGTFLVRDASTKM HGDYTLTLRKGGNNKLIKIFH
RDGKYGFS DPLTFSSVVELINHYRNESLAQYNPKLDVKLLYPVSKYQQDQVVKEDNIEAVGKKLHEYNTQFQEK SRE
YDRLYEYTRTSQEI QMKRTAIEAFNETIKIFEEQCQTQERY SKEYIEKFKREGNEKEIQRIMHNYDKLSRISEII
DSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTQKGV RQKKLNEWLGNENTEDQYSLVEDDEDL
PHHDEKTWNVGSSNRNKAENLLRGKRDGTFLVRESSKQGCYACSVVVDGEVKHC VINKTATGYGFAEPYNLYSSLKE
LVLHYQHTSLVQHNDSLNVTLAYPVYAQQRR

PI3K, regulatory subunit 2 (beta) | GI:4826908 | NP_005018 | SEQ ID NO: ()

MAGPEGFYRALYPFRRRERPEDLELLPGDVLVVSRAALQALGVAEGGERCPQSVGWMPGLNERTRQRGDFPGTYVEF
LGPVALARPGPRPRGPRPLPARPRDGAPEGLTLPDLPEQFSPPDVAPPLLVKLVEA IERTGLDSESHYRPELPAPR
TDWSLSDVDQWDTAALADGIKSFLALAPLVTPEASAEARRALREAAGPVGPAL EPPTLPLHRALT LRFLQLHGR
VARRAPALGPAVRALGATFGPLLLRAPPPSSPPPGGAPDGSESPDFPALLVEKLLQEHLEE QEVAPPALPPKPPK
AKPAPTVLANGGSPPSLQDAEWYWGDISREEVNEKL RDTPDGTFLVRDASSKIQGEYTLTLRKGGNNKLIKVFHRDG
HYGFSEPLTFCSVVDLINHYRHESLAQYNAKLDTRLLYPVSKYQQDQIVKEDSVEAVGAQLKVYHQYQDKSREYDQ
LYEYTRTSQELQMKRTAIEAFNETIKIFEEQCQTQEKCSKEYLERFRREGNEKEMQRILLNSERLKSRIAEIHESR
TKLEQQLRAQASDNREIDKRMNSLKPDLMLQRLKIRDQYLVWLTQKGARQKKINEWLGIKNETEDQYALMEDEDDLPH
HEERTWYVGKINRTQAEEMLSGKRDGTFLIRESSQRCYACSVVVDGDTKHCVIYRTATGFGFAEPYNLYGSLKELV
LHYQHASLVQHNDALTVTLAHPVRAPGPGPPPAAR

PI3K, regulatory subunit 3 (gamma) | GI:166795245 | NP_003620 | SEQ ID NO: ()

MYNTVWSMDRDDADWREVMMPYSTELIFYIEMDPPALPPKPPKPMTSAPVNGMKDSSVSLQDAEWYWGDISREEVND
KLRDMPDGTFLVRDASTKMQGDYTLTLRKGGNNKLIKIYHRDGKYGFSPLTFNSVVELINHYHHESLAQYNPKLDV
KLMYPVSRYQQDQLVKEDNIDAVGKKLQEYHSQYQEKSEYDRLYEYTRTSQEIOMKRTAIEAFNETIKIFEEQCH
TQEQHSKEYIERFRREGNEKEIERIMMNYDKLSRLGEIHDSKMRLEQDLKNQALDNREIDKKMNSIKPDLIQLRKI
RDQHLVWLNHKGVRQKRLNVWLGKKNEDADENYFINEEDENLPHYDEKTFWVEDINRVQAEDLLYGKPDGAFLIRES
SKKGCYACSVVADGEVHKCVIYSTARGYGFAEPYNLYSSLKELVLHYQQTSLVQHNDSLNVRLAYPVHAQMPSLCR

PI3K, regulatory subunit 4 | GI:23943912 | NP_055417 | SEQ ID NO: ()

MGNQLAGIAPSQILSVESYFSDIHDFEYDKSLGSTRFFKVARAKHREGLVVVKVFAIQDPTLPLTSYKQELEELKIR
LNSAQNCLPFQKASEKASEKAAMLFRQYVRDNLVDRISTRPFLNNIEKRWIAFQILTAVDQAHKSGVRHGDITENV
MVTSWNWVLLTDFASFKPTYLPEDNPADFNFFDTSRRRTCYIAPERFVDGGMFATELEYMRDPSTPLVDLNSNQRT
RGELKRAMDIFSAGCVIAELFTEGVPLFDLSQLLAYRNGHFFPEQVLNKKIEDHSIRELVTQMIHREPDKRLEAEDYL
KQQRGNAPFEIFYTFLQPYMAQFAKETFLSADERILVIRKDLGNIHNLGCHDLPEKAEGEPKENGVLVILSVITSC
LQTLKYCDSKLALELILHLAPRLSVEILLDRITPYLLHFSNDSVPRVRAEALRTLTKVLALVKEVPRNDINIYPEY
ILPGIAHLAQDDATIVRLAYAENIALLAETALRFLELVQLKNLNMENDPNNEEIDEVTHPNGNYDTLQALHEMVQQ
KVVTLLSDPENIVKQTLMEGITRLCVFFGRQKANDVLLSHMITFLNDKNDWHLRGAFFDSIVGVAAYVGWQSSSIL
KPLLQQLGSDAEFVIVKALYALTCMCQLGLLQKPHVYEFASDIAPFLCHPNLWIRYGAVGFITVVARQISTADVYC
KLMPLYLDPYITQPIIQIERKLVLSSLKEPVRSIFDYALRSKDITSLFRHLHMRQKKRNGSLPDCPPPEDPAIAQL
LKKLLSQGMTEEEEDKLLALKDFMMKSNKAKANIVDQSHLHDSSQKGVIDLALGITGRQVDLVKTKQEPDDKRARK
HVKQDSNVNEEWKSMFGSLDPPNMPQALPKGSDQEVITQTKPPRSESSAGICVPLSTSSQVPEVTTVQNKKPVIPVL
SSTILPSTYQIRITTCKTELQQLIQKKREQCNAERIAKQMMENAEWESKPPPPGWRPKGLLV AHLHEHKS AVNRI RV
SDEHSLFATCSNDGTVKIWNSSQKMEGKTTTTRSILTYSRIGGRVKTTLTFCQGSHYLAIASDNGAVQLLGIEASKLPK
SPKIHPLQSRILDQKEDGCVDMHFNHSGAQSVALAYATVNGSLVGWDLRSSSNATLKHDLKSGLITSFAVDIHQCW
LCIGTSSGTMACWDMRFQLPISSHCHPSRARIRRLSMHPLYQSWVIAAVQGNNEVSMWDMETGDRRFTLWASSAPPL
SELQPSPHSVHGIYCS PADGNPILLTAGSDMKIRFWDLAYPERSYVAGSTSSPSVSYRKKIEGTEVVQEI QNKQK
VGPSDDTPRRGPESLPVGHDDIITDVATFQTQTQGFIVTASRDGIVKVWK

PI3K, regulatory subunit 5 | GI:217330622 | NP_055123 | SEQ ID NO: ()

MQPGATTCTEDRIQHALLERCLHGLSLSRSTWSAGLCLNCWSLQELVSRDPGHFLILLEQILQKTREVQEKGTYDL
LTPALLFYSTVLCTPHFPDSDLLKAASTYHRFLTWPVPYCSICQELLTFIDAEKAPGISYQRLVRAEQGLPIR
SHRSSTVTVLLNPVEVQAEFLAVANKLSTPGHSPHSAYTTLLHAFQATFGAHC DVPLGHLQAKTLAELEDIFT
ETAEAQELASGIGDAEARRWLRTKLQAVGEKAGFPVGLDTAKPGKLHTIPIPVARCYTYSWSQDSFDILQEILLKE
QELLQPGILGDDEEEEEEEEEVEEDLETDGHCAERDSLSTSSSLASHDSTLSLASSQASGPALSRHLLTSFVSGLS
GMDSGYVEDSEESSSEWPWRRGSQERRGHRPGQKFIRIYKLFKSTS QLVLRDRSRSLEGSSDTALPLRRAGSLCSP
LDEPVSPPSRAQRSRLPQPKLGTQLPSWLLAPASRPQRRRPFLSGDEDPKASTLRVVVFGSDRISGKVARAYSNL
RLNNRPLLTRFFKLQFFYVPVKRSHGTSPGACPPPRSQTSPPTDSPRHASPGELGTPWEESTNDISHYLGMLDP
WYERNVLGLMHLPPPEVLCCQSLKAEQALEGSPTQLPILADMLLYYCRFAARPVLLQVYQTELTFITGEKTTEIFIH
SLELGHSAATRAIKASGPGSKRLGIDGDREAVPLTLQIIYSKGAISGRSRWSNLEKVCTSVNLNKA CRKQEELDSSM
EALTNLNTEVVKRQNSKSKKGFNQISTSQIKVDKQIIGSNSCPFAVCLDQDERKILQSVVRCEVSPCYKPEKSDLS
SPQTPPDLPAQAAPDLCSLLCLPIMTFSGALP

PI3K, regulatory subunit 6 | GI:58082081 | NP_001010855 | SEQ ID NO: ()

MESSDVELDLQRSVQAVLRELSTQAPALQSNQGMWRWSLHKKVERDPGKSPVLVRILLRELEKAESQDLRHVIIPLL
HTVMYVLTAKTGITEELYQRIYAFCTRLTLPTPYCTVALDCAIRLKTEMVPGTLYQRMVIAEQNLNELYPYQER
VFLFVDPELVASVCSALLLEIEAAQAQQTTPETCMRHVVSHALQAALGEACHAGALHRKLQASPRRTLEHYFHAVVA
ALEQMASEASPSREGHVERLEEIYCSLLGPAAGRCGGDLVQERPPSIPLPSPIYITFHLWTGEEQLWKELVFLRPRS
QLRLSADLEVLDLQGLRPDRELARVSVLSTDSGIERDLPTGADELPAAGSPEMERAGLQKGGIKKRAWPLDFLMPG
SWDGPPGLHRRGTGRPSGDGEMLPGVSRRLHTARVLVLGDDRLGLRLAQAYHRLRKRETQKFCCLTPRLSLQLYYIPVLA
PEKPAASRQPELGELATFLGRVDPWYQSNVNTLCPAIIHKLAEPPSLDTSRTVDPFILDVITYYIRMGTQPIYFYQIY
TVKIFFSDLSQDPTEDI FLIELKVKIQDSKFPKDGFSPPRRRGVAEGPGAELSLCYQKALLSHRPREVTVSLRATGLI
LKAI PASDTEVSGSSHCPLPAAPVTDHTCLNVNVTEVVKSSNLAKGSFSTVTNTFRTNNIQIQSRDQRLTLTSLDKD
DQRTFRDVRFEVAPCEPCSGAQKSKAPWLNHLHGQVEVEAIKAKPKPLMPINTFSGIVQ

serine-protein kinase ATM | GI:71902540 | NP_000042 | SEQ ID NO: ()

MSLVLNDDLICCRQLEHDRATERKKEVEKFKRLIRDPETIKHLDRHSDSKQGKYLNWDAVFRFLQKYIQKETECRLI
AKPNVSASTQASRQKKMQEISSLVKYFIKCANRRAPRLKCQELLNYIMDTVKDSNGAIYGADCSNILLKDILSVRK
YWCEISQQQWLELFSVYFRLLYKPSQDVHRVLVARIHAVTKGCCSQTDLNSKFLDFFSKAIQCARQEKSSSSGLNH
ILAAALTIFLKTAVNFRIRVCELGDDEILPTLLYIWTQHRLNDSLKEVIELFQLQIYIHHPKGAKTQEKGAYESTKW
RSILYNLYDLLVNEISHIGSRGKYSSGFRNIAVKENLIELMADICHQVFNEEDTRSLEISQSYTTTQRESSDYSVPCK
RKKIELGWEVIKDHLOKSQNDFDLVPWLQIATQLISKYPASLPNCELSPLLMILSOLLPOQRHGERTPYVLRCLTEV
ALCQDKRSNLESSQKSDLLKLWNKIWCITFRGISSEIQIQAENFGLLGAI IQGSLVEVDREFWKLFTGSACRPSCPAV
CCLTLALTTSIVPGTVKMGIEQNMCEVNRSFSLKESIMKWLLFYQLEGDLNSTEVPPILHSNPHLVLEKILVSLT
MKNCKAAMNFFQSVPECEHHQKDKEELSFSEVEELFLQTTDFDKMDFLTIVRECIEKHQSSIGFSVHQNLKESLDR
LLGLSEQLLNYSSEITNSETLVRCRSLLVGLVLCYCYMGVIAEEEAAYKSELFQKAKSLMQCAGESITLTKNKTNEE
FRIGSLRNMMLCTRCLSNCTKKSPNKIASGFFLRLLTSKLMNDIADICKSLASFICKPFDRGEVESMEDDTNGNLM
EVEDQSSMNLNFDNDYDSSVSDANEPGESQSTIGAINPLAEYLSKQDLLFLDMLKFLCLCVTTAQTNTVSFRAADIR
RKLLMLIDSSTLEPTKSLHLHMYLMLLKELPGEYPLMEDVLELLKPLSNVCSLYRRDQDVCKTILNHVLHVKNL
GQSNMDSENTRDAQGQFLTIVIGAFWHLTKERKYIFSVMALVNCLKTLLLEADPYSKWAILNVMGKDFPVNEVFTQFL
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TLIAVVLSCSPICEKQALFALCKSVKENGLEPHLVKKVLEKVSETFGYRRLEDFMASHLDYLVLEWNLQDTEYNLS
SFPFILLNYTNIEDFYRSCYKVLIPHILVIRSHFDEVKSIANQIQEDWKSLLTDCFPKILVNILPYFAYEGTRDSGMA
QQRETATKVYDMLKSENLLGKQIDHLFISNLPEIVVELLMTLHEPANSSASQSTDLCDFSGDLDPAPNPPHFP SHVI
KATFAYISNCHKTKLKSILEILSKSPDSYQKILLAIQEAETNNVYKKHRILKIYHLFVSLLLKDIKSGLGGAFAF
VLRDVIYTLIHYINQRPSCIMDVSLRSFSLCCDLLSQVCQTAVTYCKDALENHLHVIVGTIPLVYEQVEVQKQVLD
LLKYLVIDNKDNENLYITIKLLDPFDPDHVVKDLRITQKIKYSRGPFSLLEEINHLFSLSVSYDALPLTRLEGLKDL
RRQLELHKDQMDIMRASQDNPDGIMVKLVNLLQLSKMAINHTGEKEVLEAVGSCLEVGPIDFSTIAIQHSDA
SYTKALKLFEDKELQWTFIMLYLNNTLVEDCVKVRSAAVTCLKNILATKTGHSFWEIYKMTTDPMLAYLQPFRTSR
KKFLEVPRFDKENPFEGLDINLWIPLENHDIWIKTLTCAFLDSGGTKCEILQLLKPMCEVKTDFCQTVLPYLIHD
ILLQDNTNESWRNLLSTHVQGFFTSCLRHFSQTSRSTTPANLDSSEHFFRCCLDKKSQRTMLAVVDYMRQKRPSSG
TIFNDAFWLDLNYLEVAKVQAQSCAAHFTALLYAEIYADKKSMDDQEKRSALAFEEGSQSTTISSLSEKSKEETGISLQ
DLLLEIYRSIGEPDSLYGCGGGKMLQPITRLRTEHEAMWGKALVTDLETAIPSSSTRQAGIIQALQNLGLCHILSV
YLGKLDYENKDWCPLEELHYQAAWRNMQWDHCTSUSKEVEGTSYHESLYNALQSLRDEFSTFYESLKYARVKEVE
EMCKRSLESVYSLYPTLSRLQAIGELESIGELFSRSVTHRQLSEVYIKWQKHSQLLKDSDFSQEPIMALRTVILEI
LMEKEMDNSQRECIKDILT KHLVELSILARTFKNTQLPERAIFQIKQYNSVSCGVSEWQLEEAQVFWAKKEQSLALS
ILKQMIKKLDASCAANNPSLKLTYTECLRVCGNWLAETCLENPAVIMQTYLEKAVEVAGNYDGESSDELNRGKMKAF
LSLARFSDTQYQRIENYMKSSFEFENKQALLKRAKEEVGLLREHKIQTNRYTVKVQRELELDELALRALKEDRKRFLC
KAVENYINCLLSGEEHDMWVFRCLSLWLENSGVSEVNGMMKRDGMKIPTYKFLPLMYQLAARMGTMKGGLGFHEVL

NNLISRISMDHPHHTLFIIILALANANRDEFLTKEVARRSRITKNVPKQSSQLDEDRTEAANRIICTIRSRPQMVR
SVEALCDAYIIILANLDATQWKTQRKGINIPADQPITKLKNEDEVVPTMEIKVDHTGEYGNLVTIQSFKAEFRLAGG
VNLPKIIDCVGSDGKERRQLVKGRDDLQDAVMQOVFQMCNTLLQNTETRKRKLITICTYKVVPLSQRSQVLEWCTG
TVPIGEFLVNNEDGAHKRYRPNDFAFQCQKKMMEVQKKSFEKEYEVFMDVCQNFQPVFRYFCMEKFLDPAIWFEKR
LAYTRSVATSSIVGYILGLDRHVQNILINEQSAELVHIDLGVAFEQGGKILPTPETVFPFRLTRDIVDGMGITGVEGV
FRRCEKTMEVMRNSQETLLTIVEVLLYDPLFDWTMNPALKALYLQQRPEDETELHPTLNADDQECKRNLSDIDQSFN
KVAERVLMLRQEKLGVEEGTVLSVGGQVNLIIQQAIDPKNLSRLFPGWKAWV

serine/threonine-protein kinase ATR | GI:157266317 | NP_001175 | SEQ ID NO: ()

MGEHGLELASMI PALRELGSATPEEYNTVVQKPRQILCQFIDRIILTDVNVVAVELVKKTDQSQPTSVMLLDFIQHIMK
SSPLMFVNVSGSHEAKGSCIEFSNWIITRLLRIAATPSCHLLHKKICEVICSLFLFKSKSPAIFGVLTKEELLQLF
DLVYLHRRNVMGHAVEVPVMSRFLSQLDEHMGYLSAPLQLMQMNFIEVTLMLVLTRIIAIVFFRRQELLLWQ
IGCVLLEYGSPKIKSLAISFLTELFQGLGPAQPASTFFSSFLELLKHLVEMDQDLKLYEEPLSKLIKTLFPFEAE
AYRNIEPVYLNMLLEKLCVMFEDGVLMLRLKSDLLKAALCHLLQYFLKFVPAGYESALQVRKVYVRNICKALLDVLGI
EVDAYELGPLYAALKMESMEIEEIQCQTQENLSSNSDGISPKRRRLSSSLNPSKRAPKQTEEIKHVDNMNQSIL
WSALKQKAESLQISLEYSGLKNPVIEMLEGIAVVLQLTALCTVHC SHQNMNCRFTKDCQHKSKKKPSVITWMSLDF
YTKVLKSCRSLLESVQKLDLEATIDKVVKIYDALIYMQVNSSFEDHILEDLCGMLSLPWYSHSDDGCLKLTFAAN
LLTLSCRISDSYSPQAQSRVFLTLFPFRRIFLEWRTAVYNWALQSSHEVIRASCVSGFFILLQQQNSCNRPVKILI
DKVKDDSDIVKKEFASILGQLVCTLHGMFYLTSSLTEPFSEHGHVDLFCRNLKATSQHECSSLKASVCKPFLFLL
KKKIPSPVKLAFIDNLHHLCKHLDFREDETVDKAVLGTLLNLMEPDKDVRAVAFSGNIKHILESLDSEDGFIKELFV
LRMKEAYTHAQISRNNELKDTLILTGTGIGRAAKGDLVPFALLHLLHCLLSKSASVSGAAYTEIRALVAAKSVKLQS
FFSQYKKPICQFLVESLHSSQMTALPNTPCQNADVRKQDVAHQREMAINTLSEIANVDFDFDLNRFLTRTLQVLLPD
LAAKASPAASALIRTLGKQLNVNRREILINNFYIFSHLVCSCSKDELERALHYLKNETEIELGSLLRQDFQGLHNE
LLLRIGEHYQQVFNGLSILASFASDDPYQGRDIIISPELMADYLQPKLLGILAFFNMQLLSSSVGIEDKKMALNSL
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IFHYLI IENRDAVQDFLHEIYFLPDHPELKKIKAVLQEYRKETSESTDLQTTQLSMKAIQHENVDVRIHALTSKE
TLYKNQEKLIKAYATDSETVEPIISQLVTVLLKGCQDANSQARLLCGECLGELGAIDPGRLDFSTTETQKDFTFVTG
VEDSSFAYGLLMELTRAYLAYADNSRAQDSAAYAIQELL SIYDCREMETNGPGHQLWRRFPEHVREILEPHLNTRYK
SSQKSTDWSGVKKPIYLSKLGSNFAEWSASWAGYLITKVRHDLASKIFTCCSIMMKHDFKVTIYLLPHILVYVLLGC
NQEDQQEVYAEIMAVLKHDDQHTINTQDIASDLCLSTQTVFSMLDHLTQWARHKKFQALKAECPHSKSNRNKVD SM
VSTVDYEDYQSVTRFLDLIPQDTLAVASFRSKAYTRAVMHFESFITEKKQNIQEHLGFLQKLYAAMHEPDGVAGVSA
IRKAEP SLKEQILEHESLGLLRDATAICYDRAIQLEPDQIIHYHGVVKSMLGLGQLSTVITQVNGVHANRSEWTDELN
TYRVEAAWKLSQWDLVENYLAADGKSTTWSVRLGQLLSAKKRDI TAFYDSLKLVRAEQIVPLSAASFERSYQRGY
EYIVRLHMLCELEHSIKPLFQHSPGDSSQEDSLNWVARLEMTQNSYRAKEPIIALRRALLSLNKRPDYNEMVGECWL
QSARVARKAGHHQTAYNALLNAGESRLAELYVERAKWLWSKGDVHQALIVLQKGVELCFPENETPPEGKNMLIHGRA
MLLVGRFMEETANFESNAIMKKYKDVTA CLPEWEDGHFYLAKEYDKLMPMVTDNKMEKQGD LIRYIVLHFGSLQYG
NQFIYQSMRMLTLWLDYGTAKAYEWEKAGRS DRVQMRNDLGKINKVITEHTNYLAPYQFLTAFSQLISRICSHDEV
FVVLMEIIAKVFLAYPQQAMWMMTAVSKSSYPMRVNRCKEILNKA IHMKKSLEKFVGDATRLTDKLLLELCNKPVDGS
SSTLSMSTHFKMLKKLVEEATFSEILIPLQSVMIPTLPSILGTHANHASHEPFPGHWAYIAGFDDMVEILASLQKPK
KISLKGSDGKFYIMMCKPKDDLKDCRLMEFNSLINKLRKDAESRRRELHIRTAVIPLNDECGIIEWVNNTAGLR
PILTKLYKEKGVYMTGKELRQCMLPKSAALSEKLKVFREFLLPRHPPIFHEWFLRTFPDPTSWYSSRSAYCRSTAVM
SMVGYILGLDRHGENILFDSLGTGECVHVDNFCLFNKGETFEVPEIVPFRLTHNMVNGMGMGTGLFRACEVTMR
LMRDQREPLMSVLKTF LHDPLVEWSKPVKGH SKAPLNETGEVVNEKAKTHVLDIEQRLQGVIKTRNRVTGLPLSIEG
HVHYLIQEATDENLLCQMYLGWTPYM

DNA-dependent protein kinase catalytic subunit isoform 1 | GI:13654237 | NP_008835 | SEQ ID NO: ()

MAGSGAGVRCSSLRLQETLSAADRCGAALAGHQLIRGLGQECVLSSSPAVLALQTSLVFSRDFGLLVFVRKSLNSIE
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KFYGELALKKKIPDTVLEKVEYELLGGLGEVHPSEMINNAENLFRAFLGELKTQMTSAVREP KLPVLAGCLKGLSSLL
CNFTKSMEEDPQTSREIFNFVLKAIRPQIDLKRYAVPSAGLRLFALHASQFSTCLLDNYVSLFEVLLKWC AHTNVEL
KKAALSALSF LKQVSNMVAKNAEMHKNKLQYFMEQFYGIIRNVDSSNNKELSAIRGYGLFAGPCKVINAKD VDFMY
VELIQRCQMF LTQTDGDDRVYQMP SF LQSVASVLLYLDTPVEVYTPVLEHLVVMQIDSFPQYSPKMQLVCCRAIV
KVFLALAAKG PVL RNCISTVVHQGLIRICSKPVLPKGPESSEDHRASGEVRTGKWKVPTYKYVDLFRHLLSSDQ
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SPEDPEKYS CFALFVKFGKEVAVKMKQYKDELLASCLTFLLSLPHNIIELDVRAYVPALQMAFKLGLSYTP LAEVGL
NALEEWSIYIDRHVMQPYKDI L PCLDGYLKTSALSDETKNWEVSALSRAAQKGFNKVVLKHLKKTKNLSSNEAIS
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QTKVAACELLHSMVMFMLGKATQMPEGGQGAPPMYQLYKRTFPVLLRLACDQVDQVTRQLYEPLVMQLIHWF TNNKKF
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GFPPSASLCLLDLVKWL LAHCGRPQTECRHKSIELFYKFVPLLPGNRSPNLWLKDV LKEEGVSFLINTFEGGGCGQP
SGILAQPTLLYL RGPFSLQATLCWLDLLLAAL E CYN T F I G E R T V G A L Q V L G T E A Q S S L L K A V A F F L E S I A M H D I I A A
EKCFGTGAAGNR TSPQEGERYNYSKCTVVVRIMEFTTLLNTSPEGWKLLKKDL CNTHLMRVLVQTLCEPASIGFNI
GDVQVMAHLPDVCVNLKALKMSPYKDILETHLREKITAQSIEELCAVNLYGPDAQVDRSRLAAVVSACKQLHRAGL
LHNILPSQSTDLHHSVGT ELLSLVYKGIAPGDERQCLPSLDLSCQQLASGLLELAFAGGLCERLVSLLNPAVLST
ASLGSSQGSVIHFSGEYFYSLFSETINTELLKNLDLAVLELMQSSVDNTKMVSAVLNGMLDQSFRE RANQKHQGLK
LATTILQHWKKCDSSWAKDSPLETKMAVLALLAKILQIDSSVSFNTSHGSFPEVF TTYISLLADTKLDLHLKGQAVT
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HVMEELFQSSFRRIARRGSCVTQVGLLESVYEMFRKDDPRLSFTRQSFVDRSLLTLLWHCSLDALREFFSTIVVD AI
DVLKS RFTKLN ESTFDTQITKKMGYYKILDVMYSRLPKDDVHAKESKINQVFHGCITEGNETKT LIKLCYDAFTE
NMAGENQLLERRR LYHCAAYNCAISVICCVFNELKFYQGFLFSEKPEKNLLIFENLIDLKRRYNFPVEVEVPMERKK
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VLELEMDELNRHECMAPLTALVKHMRSLGPPQGEEDSVPRDLPSWMKFLHGKLGNP I VPLNIRLFLAKLVINTEEV
FRPYAKHWLSPLLQLAASENNGGEGIH YMVVEIVATILSWTGLATPTGPVKDEVLANRLNFMKHFVHPKRAVFRH
NLEI IKT LVECKWDCLSIPYRLIFEKFSGKDPNSKDNSVG IQLLGIVMANDLPYDPQCGIQSSEYFQALVNNM SFV
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DNA-dependent protein kinase catalytic subunit isoform 2 | GI:126032350 | NP_001075109 |
 SEQ ID NO: ()

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serine/threonine-protein kinase SMG1 | GI:62243658 | NP_055907 | SEQ ID NO: ()

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transformation/transcription domain-associated protein isoform 1 | GI:347360922 |
NP_001231509 | SEQ ID NO: ()

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transformation/transcription domain-associated protein isoform 2 | GI:4507691 | NP_003487 |
 SEQ ID NO: ()

MAFVATQGATVVDQTTLMKKYLQFVAALTDVNTPDETKLKMMQEVSENFENVTSSPQYSTFLEHII PRFLTFLQDGE
 VQFLQEKPAQQLRKLVLLEIIHRIPTNEHLRPHTKNVLSVMFRFLETENEENVLICLR I IELHKQFRPPITQEIHHF
 LDFVKQIYKELPKVNVRYFENPQVIPENTVPPPEMVGMITTIAVKVNEREDSETRTHSII PRGSLSLKVLAE LPII
 VVLMYQLYKLN IHNVAEFVPLIMNTIAIQVSAQARQHKLKYNKELYADFI AAQIKTLSFLAYIIRIYQELVTKYSQQ
 MVKGMQLLSNCPAETAHLRKELLIAAKHILTTLELRNQFI PCMDKLFDESILIGSGYTARETLRPLAYSTLADLVHH
 VRQHLPLSDLSLAVQLFAKNIDDES LPSIIQTMCKLLLNLDVCIRSKSEQESGNGRDVLMRMLEVFVLKFHTIARY
 QLSAIFKKCKPQSELGAVEAALPGVPTAPAAPGPAPSPAPVPAPPPPPPPPPATPVTPAPVPPFEKQGEKDKEDKQ
 TFQVTD CRSLVKTLVCGVKTITWGITSCAPGEAQFI PNKQLQPKETQIYIKLVKYAMQALDIYQVQIAGNGQTYIR
 VANCQTVRMKEEKEVLEHFAGVFTMMNPLTFKEIFQTTVPYMERISKNYALQIVANSFLANPTTSALFATILVEYL
 LDRLPEMGSNVELSNLYLKLFLKLVFGSVSLFAAENEQMLKPHLHKIVNSSMELAQTAKEPYNYFLLLRLALFRSIGGG
 SHDLLYQEFPLPLPNLLQGLNMLQSGHLKQHMKDLFVELCLTVPVRLSSLLPYLPMLMDPLVSALNGSQTIVSQQGLR
 TLELCVDNLQPDFLYDHIQPVRAELMQALWRTLNPADSISHVAYRVLGKFGGSNRKMLKESQKLHYVVTEVQGPSI
 TVEFSDCKASLQLPMEKA IETALDCLKSANTEPYRRQAWVEIKCFLVAMMSLEDNKHALYQLLAHPNFTEKTIPNV
 IISHRYKAQDTPARKTFEQALTGAFMSAVIKDLRPSALPFVASLIRHYTMVAVAQQCGPFLPCYQVGSQPSTAMFH
 SEENGSKGMDPLVLIDAI AICMAYEEKELCKIGEVALAVIFDVASII LGSKERACQLPLFSYIVERLCACCYEQAWY
 AKLGGVSIKFLMERLPLTWVLQNQQTFLKALLFVMMDLTGEVSN GAVAMAKTTLEQLLMRCATPLKDEERAEEIVA
 AQEKS FHHVTHDLVREVTSPNSTVRKQAMHSLQVLAQVTGKSVTVIMEPHKEVLQDMVPPKKHLLRHQPANAQIGLM
 EGNTFCTTLQPRFLTMDLNVVEHKVFYTELLNLCEAEDSALTCLPCYKSLPSLVPLRIAALNALAACNYLPQSREKI
 IAALFKALNSTNSELQEAGEACMRKFLEGATIEVDQIHTHMRPLLMMLGDYRSLTLNVNRLTSVTRLPNSFNDKF
 CDQMMQHLRKWMEVVVITHKGGQSRSDGNEMKICSAIINLFHLIPAAPQTLVKPLLEVMKTERAMLIEAGSPFREPL
 IKFLTRHPSQTVELFMMEATLNDPQWSRMFMSFLKHKDARPLRDVLAANPNR FITLLLPGGAQTAVRPGSPSTSTMR
 LDLQFQAIIKIIISIVKNDDSWLASQHSLSVQLRRVWVSENFQERHRKENMAATNWKEPKLLAYCLLNYCKRNYGDIE
 LLFQLLRAFTGRFLCNMTFLKEYMEEIIPKNYSIAQKRALFFRFVDFNDPNFGDELKAKVLQHILNPAFLYSFEKGE
 GEQLLGPPNPEGDNPE SITSVFITKVLDPKQADMLDSLRIYLLQYATLLVEHAPHHIHDNNKNRNSKLRLMTFAW
 PCLLSKACVDPACKYSGHLLLAHIIAKFAIHKKIVLQVFHSLKKAHAMEARAIVRQAMAILTPAVPARMEDGHQMLT
 HWTRKIIIVEEGHTVPQLVHILHLIVQHFKVYYPVRHHLVQHMVSAMQRLGFTPSVTIEQRRLAVDLSEVVIKWELQR
 IKDQQPDSMDPNSSGEGVNSVSSSIKRGLSVDSAQEVKRFRTATGAISAVFGRSQSLPGADSLAKPIDKQHTDTV

VNFLIRVACQVNDNTNTAGSPGEVLSRRCVNLLKTALRPDMWPKSELKLQWFDKLLMTVEQPNQVNYGNICTGLEVL
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 EGLTNYEKATNANPSQLFGTLMILKSACSNPSYIDRLISVFMRSLOKMOVREHLNPQAASGSTEATSGTSELVMLSL
 ELVKTRLAVMSMEMRKNFIAILTSLEKSPDAKILRAVVKIVEEWVKNNSPMAANQTPTLREKSILLVKMMTYIEK
 RFPEDLELNAQFLDLVNYVYRDETLGSELTALEPAFLSGLRCAQPLIRAKFFEVDNSMKRRVYERLLYVTCSQN
 WEAMGNHFWIKQCIELLLAVCEKSTPIGTSCQGAMLPISITNVINLADSHDRAAFAMVTHVKQEPRERENSESKEEDV
 EIDIELAPGDQTSTPKTKELSEKDIGNQLHMLTNRHDKFLDTLREVKTGALLSAFVQLCHISTTLAEKTVVQLFPRL
 WKILSDRQQHALAGEISPFLCSGSHQVQRDCQPSALNCFVEAMSQCVPPIPIRCPVLKYLKGTHTNLWFRSTLMLEHQ
 AFEKGLSLQIKPKQTTEFYEQESITPPQQEILDSLAELYSLQEEEDMWAGLWQKCKYSETATAIAYEQHGFFEQAQ
 ESYEKAMDKAKKEHERSNASPAIFPEYQLWEDHWIRCSKELNQWEALTEYGQSKGHINPYLVLECAWRVSNWTAMKE
 ALVQVEVSCPKEMAWKVNMYRGYLAICHPEEQQLSFIERLVEMASSLAIREWRRLPHVSVSHVHTPLLQAAQQIIELQ
 EAAQINAGLQPTNLGRNNSLHDMKTVVKTWRNRLPIVSDDLSHWSSIFMWRQHYYQAIVTAYENSSQHDPPSSNNAML
 GVHASASAI IQYGKIARKQGLVNLVALDILSRIHTIPTVPIVDCFOKIRQQVKCYLQLAGVMGKNECMQGLEVIESTN
 LKYFTKEMTAEFYALKGMFLAQINKSEEANKAFSAVQMHDVLVKAWAMWGDYLENIFVKERQLHLGVSAITCYLHA
 CRHQNESKSRKYLAKVLWLLSFDDDKNTLADAVDKYCIGVPPIQWLAWIPQLLTCLVSGEGLLLNLISQVGRVYPQ
 AVYFPIRTLYLTLKIEQRERYKSDPGPIRATAPMWRCRIMHMQRELHPTLLSSLEGIVDQMVWFRENWHEEVLRQL
 QQGLAKCYSVAFEKSGAVSDAKITPHTLNFVKKLVSTFGVGLENVSNVSTMTFSSAASESLARRAQATAQDPVFQKLK
 GQFTTDFDFSVP GSMKLHNLISKLLKWKILEAKTKQLPKFFLIEEKCRLSNFSAQTAEVEIPGEFLMPKPTHYYI
 KIARFMPRVEIVQKHNTAARRLYIRGHNGKIYPYLVMNDACTESRREERVQLLRLLNPNCLEKRKETTKRHLFFTV
 PRVAVSPQMRLVEDNPSSLSLVEIYKQRCAKKGIEHDNPISRYYDRLATVQARGTQASHQVLRDILKEVQSNMVPR
 SMLKEWALHTFPNATDYWTFRKMFTIQLALIGFAEFVLHLNRLNPEMLQIAQDTGKLNVAIFRFDINDATGDLNANR
 PVPFRLTPNISEFLTIGVSGPLTASMIAVARCFAPNFKVDGILKTVLRDEIIAWHKKQTQEDTSSPLSAAGQPENM
 DSQQVLVSLVQAVTAIMTRLHNLAQFEGGESKVNTLVAAANSLDNLCRMDPAWHPWL

FKBP12-Rapamycin-Binding Domain (FRB) of mTOR (97 aa) | SEQ ID NO: ()

LWHEMWHEGLEEASRLYFGERNVKGMEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLT
 QAWDLYYHVFRRIKQLPQL

mLST8-binding element (LBE) of mTOR (38 aa) | SEQ ID NO: ()

ILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAG

kaAL helix in activation loop of mTOR (100 aa) | GI:158429121 | 2NPU_A | SEQ ID NO: ()

DCFVAMTREK

ATP-binding loop of mTOR (9 aa) | | SEQ ID NO: ()

TSKQRPRL

Catalytic loop of mTOR (15 aa) || SEQ ID NO: (___)

YILGLGDRHPSNLML

Activation loop of mTOR (23 aa) || SEQ ID NO: (___)

DFGDCFEVAMTREKFPEKIPFRL

Equivalents

[0328] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

We claim:

1. A designed mTOR inhibitor having a structure comprising one or more of the following features:

B-----A-----C

| |

D or D

wherein:

A is or comprises a moiety that fits in the mTOR adenosine (ATP) binding site and optionally may make one or two hydrogen bonds to main chain groups of the “hinge” extending from Gly2238 to Val2240;

B is or comprises a moiety that stacks with tryptophan (Trp2239);

C is or comprises a moiety that binds to an inner hydrophobic pocket formed by mTOR residues Tyr2225, Val2227, Met2199, Ile2237, Ile2356, Phe2358, Leu2192, Asp2195, Asp2357 and Gly2359; and

D is or comprises a moiety that binds to the N-lobe hydrophobic pocket (Ile2163, Pro2169 and Leu2185).

2. Methods of designing or characterizing mTOR modulator:

providing an image of an mTOR crystal that includes at least one potential interaction site;

docking in the image at least one moiety that is a potential mTOR modulator structural element;
and
assessing one or more features of a potential moiety-interaction site interaction.

3. The method of claim 2, wherein the at least one potential interaction site includes a site selected from the group consisting of the mTOR ATP binding site, Trp2239, a hydrophobic surface formed by mTOR residues Tyr2225, Val2227, Met2199, Ile2237, Ile2356, Phe2358, Leu2192, Asp2195, Asp2357 and Gly2359, and combinations thereof.
4. The method of claim 2 or claim 3, wherein the one or more features include at least one feature selected from the group consisting of: spatial separation between the moiety and the potential interaction site; energy of the potential moiety-interaction site interaction, and/or combinations thereof.
5. The method of any one of claims 2-5, further comprising a step of providing an image of a potential mTOR modulator comprising the moiety docked with the image of the mTOR crystal.
6. The method of claim 5, further comprising a step of comparing the image with that of an mTOR crystal including a bound known modulator.

7. A system comprising a computer or computer readable medium in which an mTOR crystal structure, or coordinates thereof, is embedded and/or displayed.
8. A method of designing and/or characterizing an mTOR modulator, which method comprises steps of:
 - (i) using the system of claim 7 to assess one or more structural features of the mTOR modulator; and
 - (ii) performing one or more *in vitro*, *in vivo* or cell-based assays to characterize the mTOR modulator.
9. An inhibitor of mTOR characterized in that it binds in the binding pocket having a three-dimensional structure characterized by the structure coordinates of any one of Tables 1, 2, 3, 4, 5, and/or 6.
10. The method of claim 9, wherein the method further comprises the step:
 - (i) defining the three-dimensional shape of the inhibitor.
11. A designed mTOR inhibitor, comprising the crystallography coordinates of any one of Tables 1, 2, 3, 4, 5 and/or 6, wherein the crystallography coordinates are within about a root mean square deviation of not more than about 1.5 Å from the backbone atoms of the amino acids according to Tables 1, 2, 3, 4, 5 and/or 6.

12. A computer system containing a set of information to perform a design or characterization of an mTOR inhibitor having a user interface comprising a display unit, the set of information comprising:
- (i) logic for inputting an information regarding a binding of a mTOR protein to a moiety known to bind mTOR protein;
 - (ii) logic for design a candidate mTOR inhibitor based on the binding of the mTOR protein to the moiety known to bind mTOR protein;
 - (iii) logic for determining an information regarding a binding of the mTOR protein to the candidate mTOR inhibitor; and
 - (iv) logic for making a conclusion regarding a mTOR inhibitory properties of the candidate mTOR inhibitor based on the determination of step (iii).
13. A computer-readable storage medium containing a set of information for a general purpose computer having a user interface comprising, a display unit, the set of information comprising:
- (i) logic for inputting an information regarding a binding of a mTOR protein to a chemical known to binding mTOR protein;
 - (ii) logic for design a candidate mTOR inhibitor based on the binding of the mTOR protein to the chemical known to bind mTOR protein;
 - (iii) logic for determining an information regarding a binding of the mTOR protein to the candidate mTOR inhibitor; and
 - (iv) logic for making a conclusion regarding a mTOR inhibitory properties of the candidate mTOR inhibitor based on the determination step of step (iii).

14. An electronic signal or carrier wave that is propagated over the internet between computers comprising a set of information for a general purpose computer having a user interface comprising a display unit, the set of information comprising a computer-readable storage medium containing a set of information for a general purpose computer having a user interface comprising a display unit, the set of information comprising:

- (i) logic for inputting an information regarding a binding of a mTOR protein to a chemical known to bind mTOR protein;
- (ii) logic for designing a candidate mTOR inhibitor based on the binding of the mTOR protein to the chemical known to bind mTOR protein;
- (iii) logic for determining an information regarding a binding of the mTOR protein to the candidate mTOR inhibitor; and
- (iv) logic for making a conclusion regarding a mTOR inhibitory properties of the candidate mTOR inhibitor based on the determination of step (iii).

15. A method of modulating activity of an mTOR polypeptide, the method comprising steps of:
contacting the mTOR polypeptide with a modulating agent of claim 1 which modulating agent is not a known modulator.

16. The method of claim 10, wherein the known modulator is a modulator as set forth in Table 12.

17. A crystalline or crystallizable composition comprising or consisting of an mTOR polypeptide.
18. A method of making the composition of claim 17.
19. A method of using the composition of claim 17.

FIGURE 1

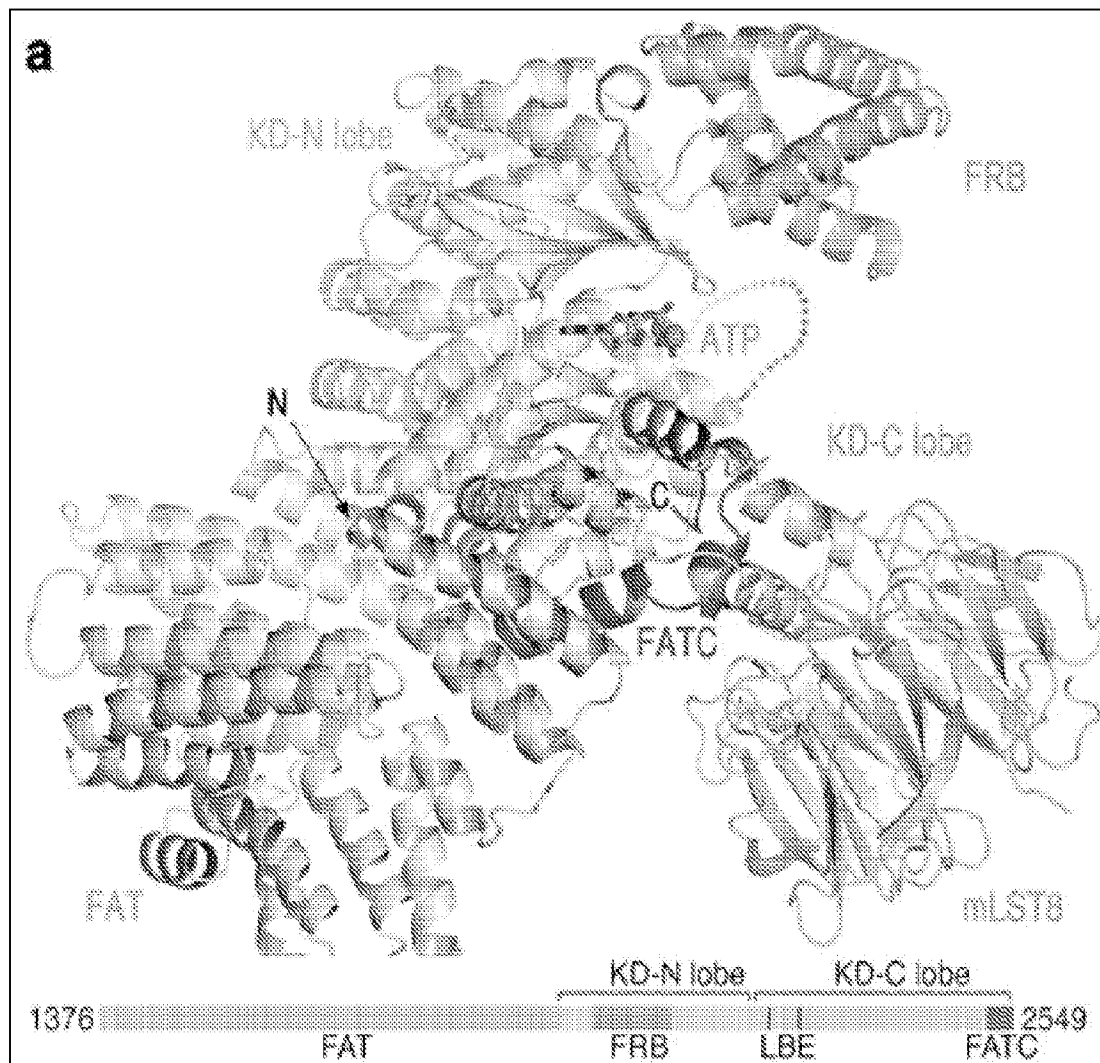


FIG. 1 - continued

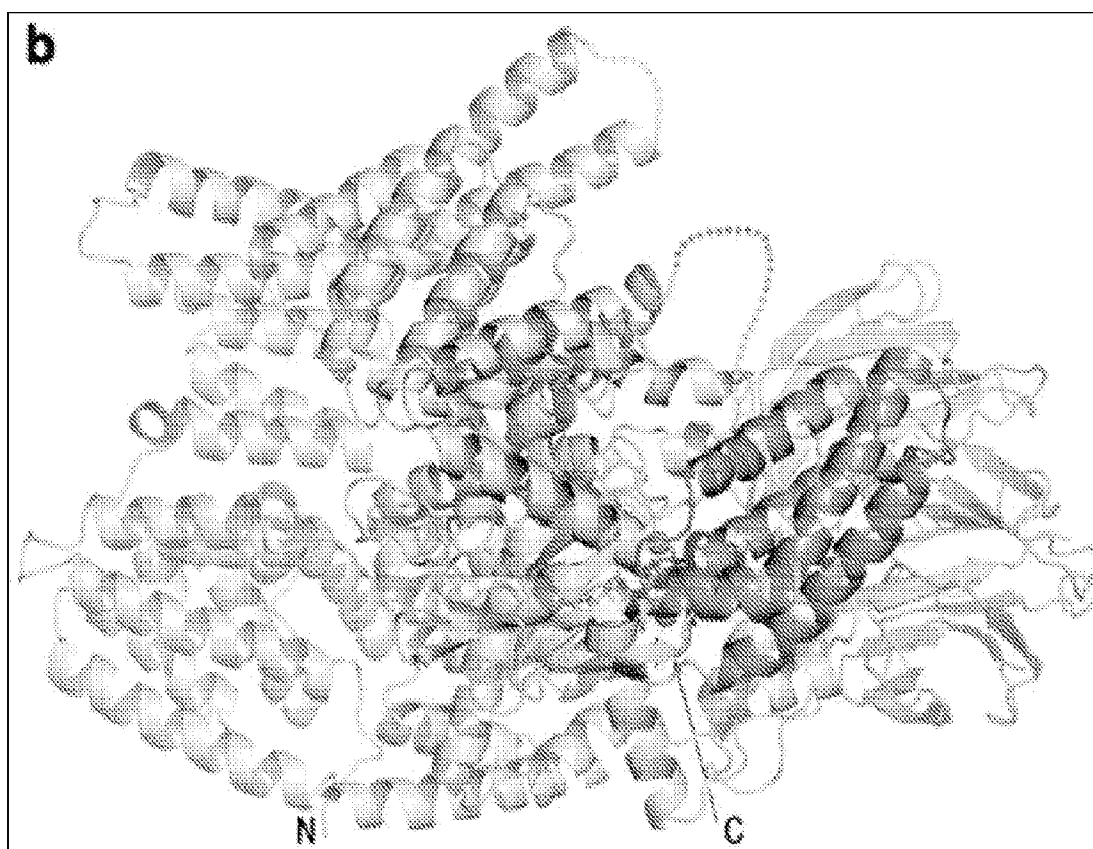


FIGURE 2

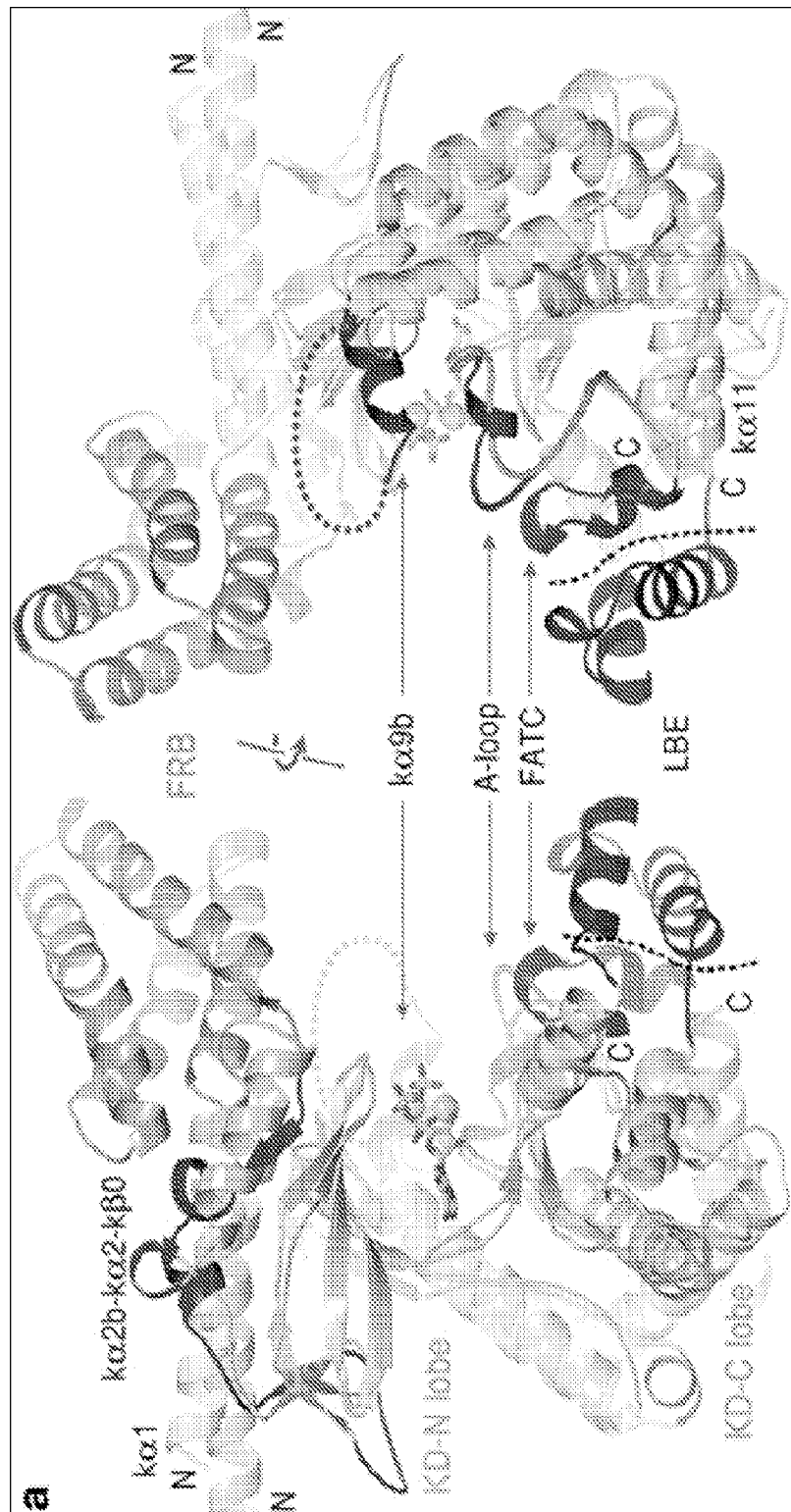


FIG. 2 - continued

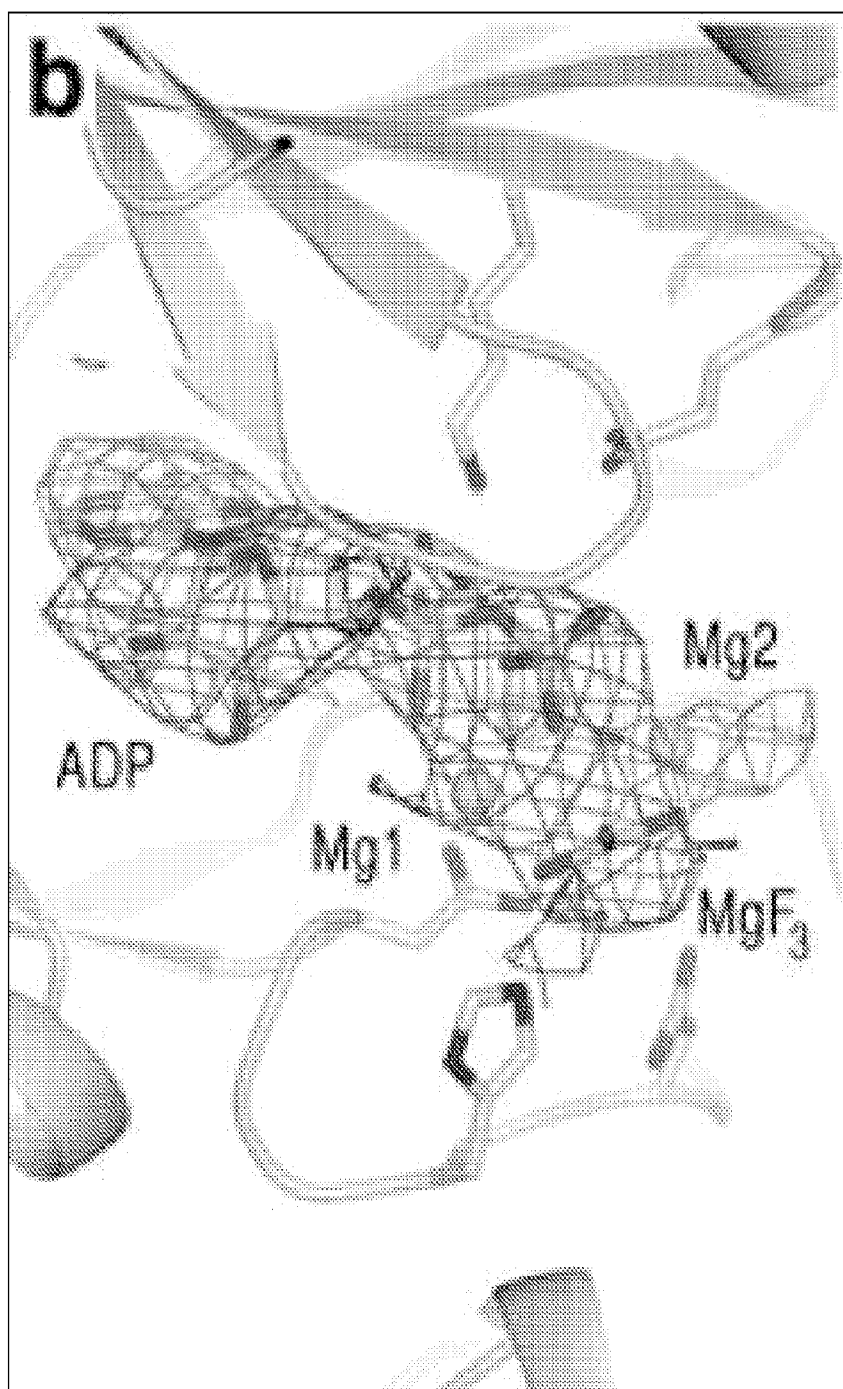


FIG. 2 – continued

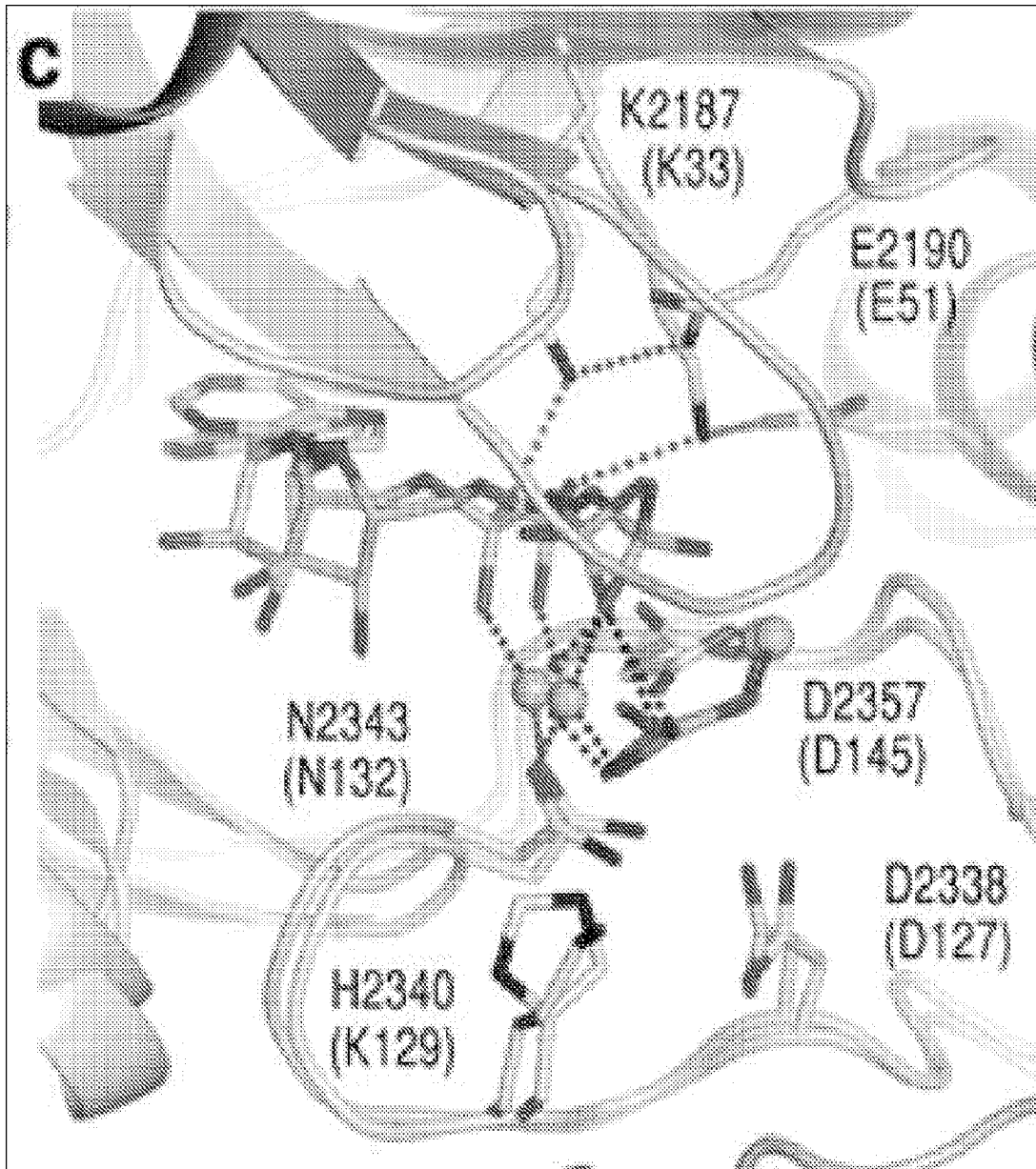


FIG. 2 - continued

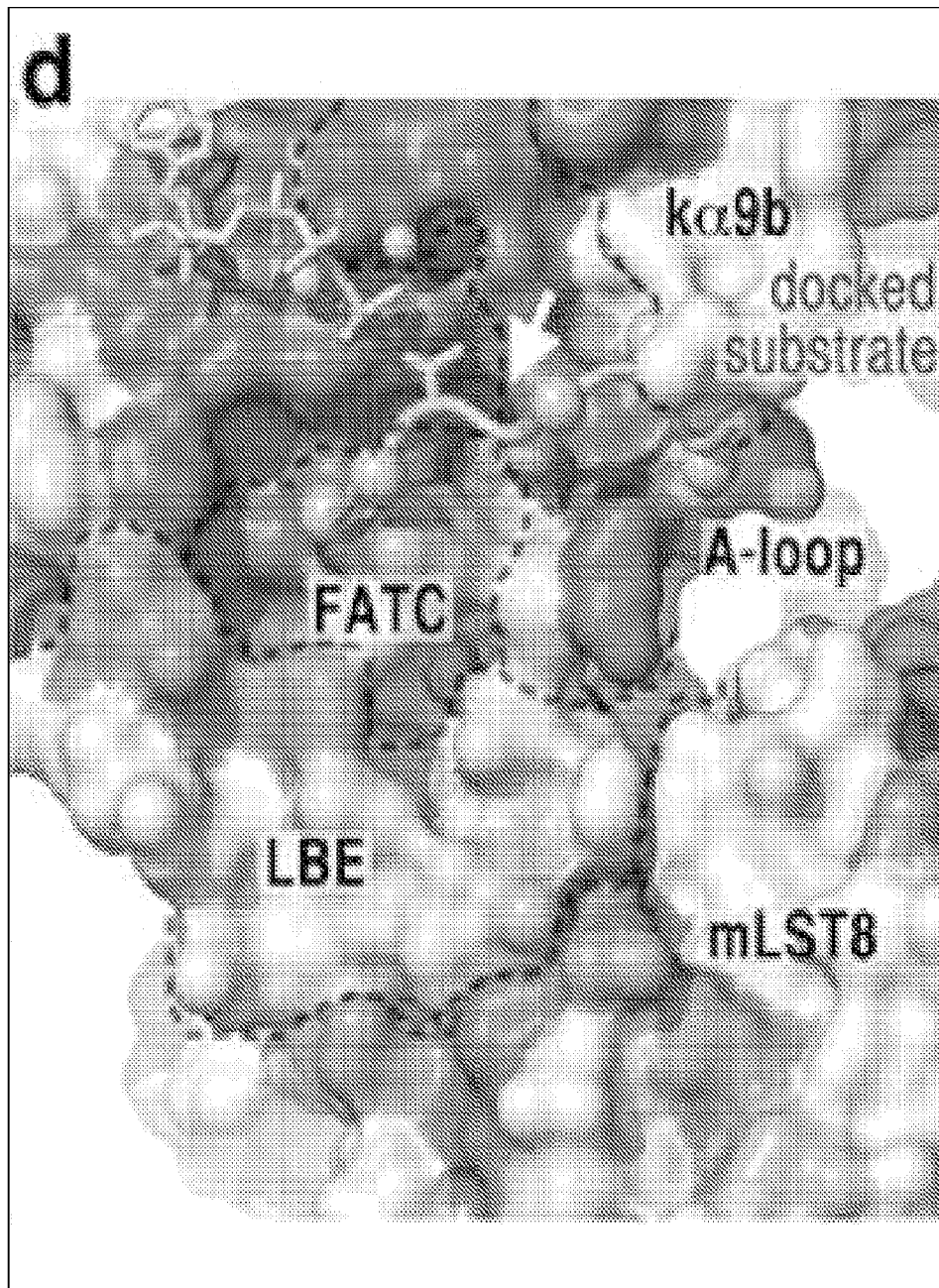


FIGURE 3

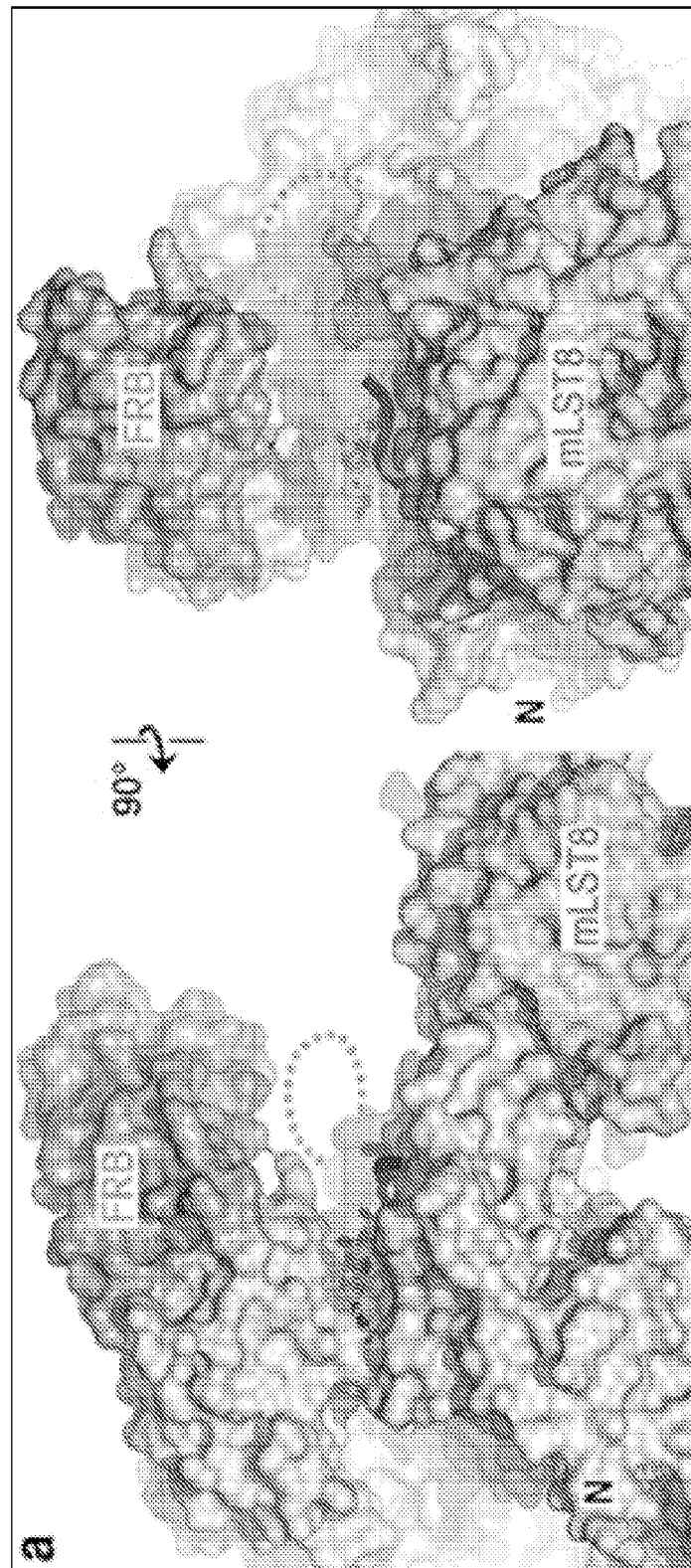
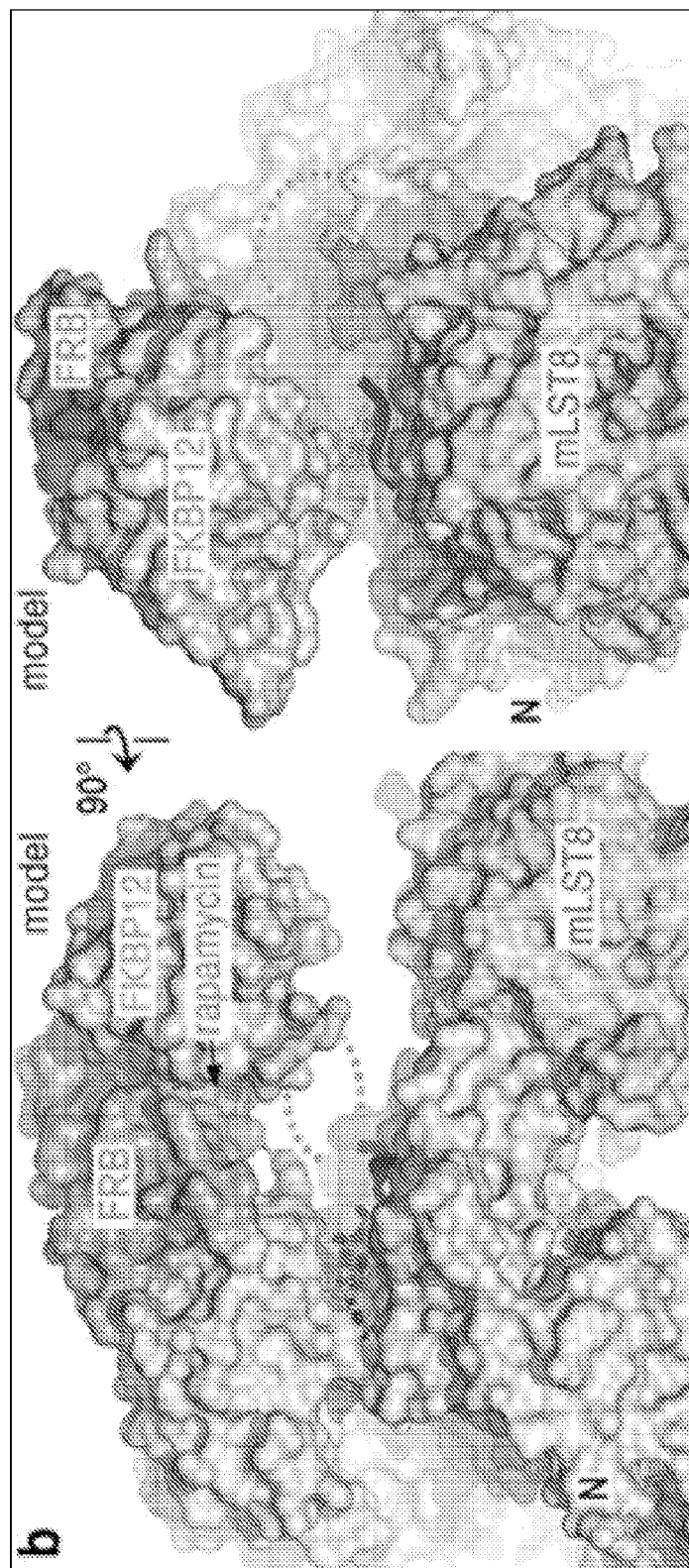
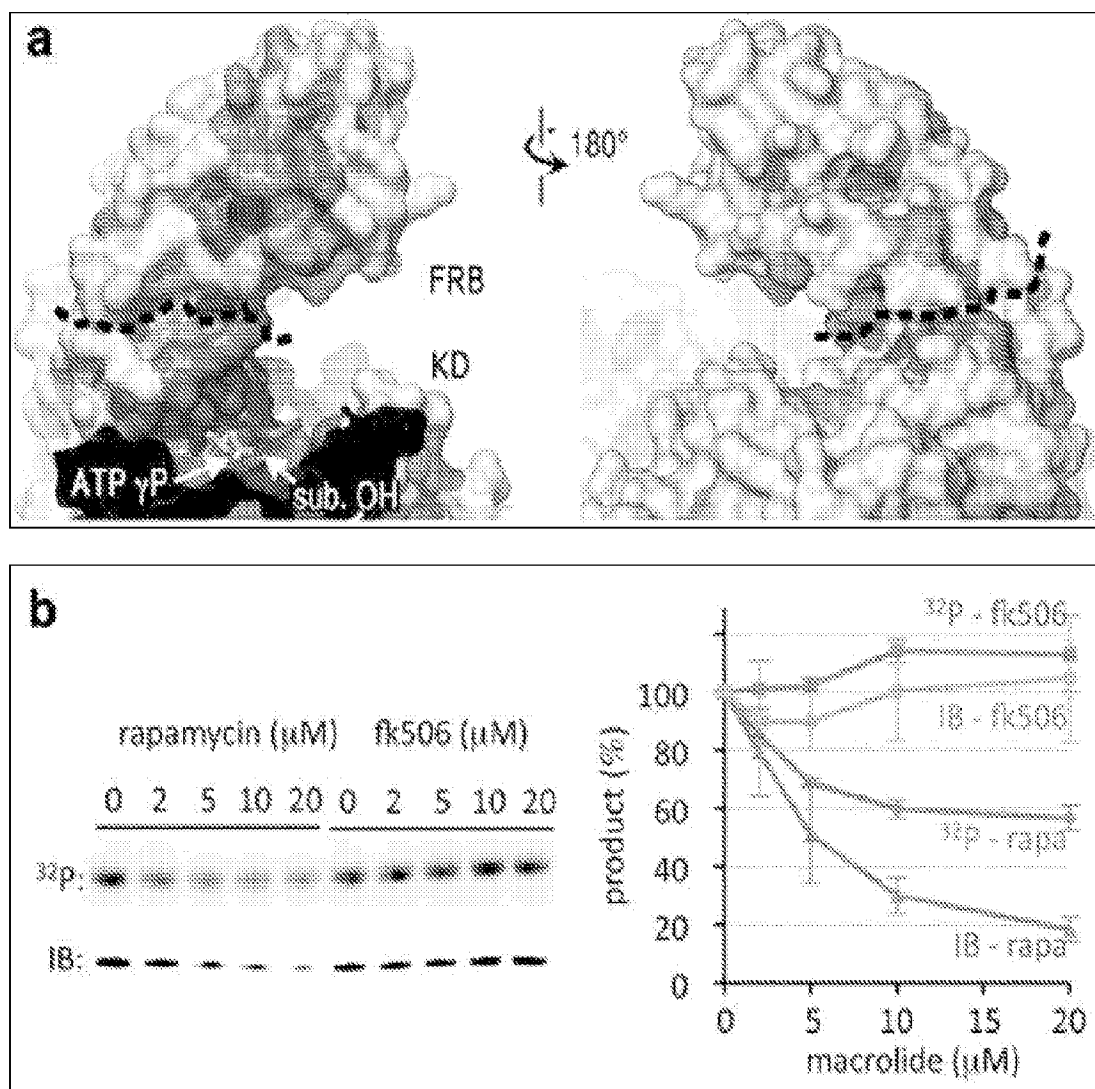


FIG. 3 - continued



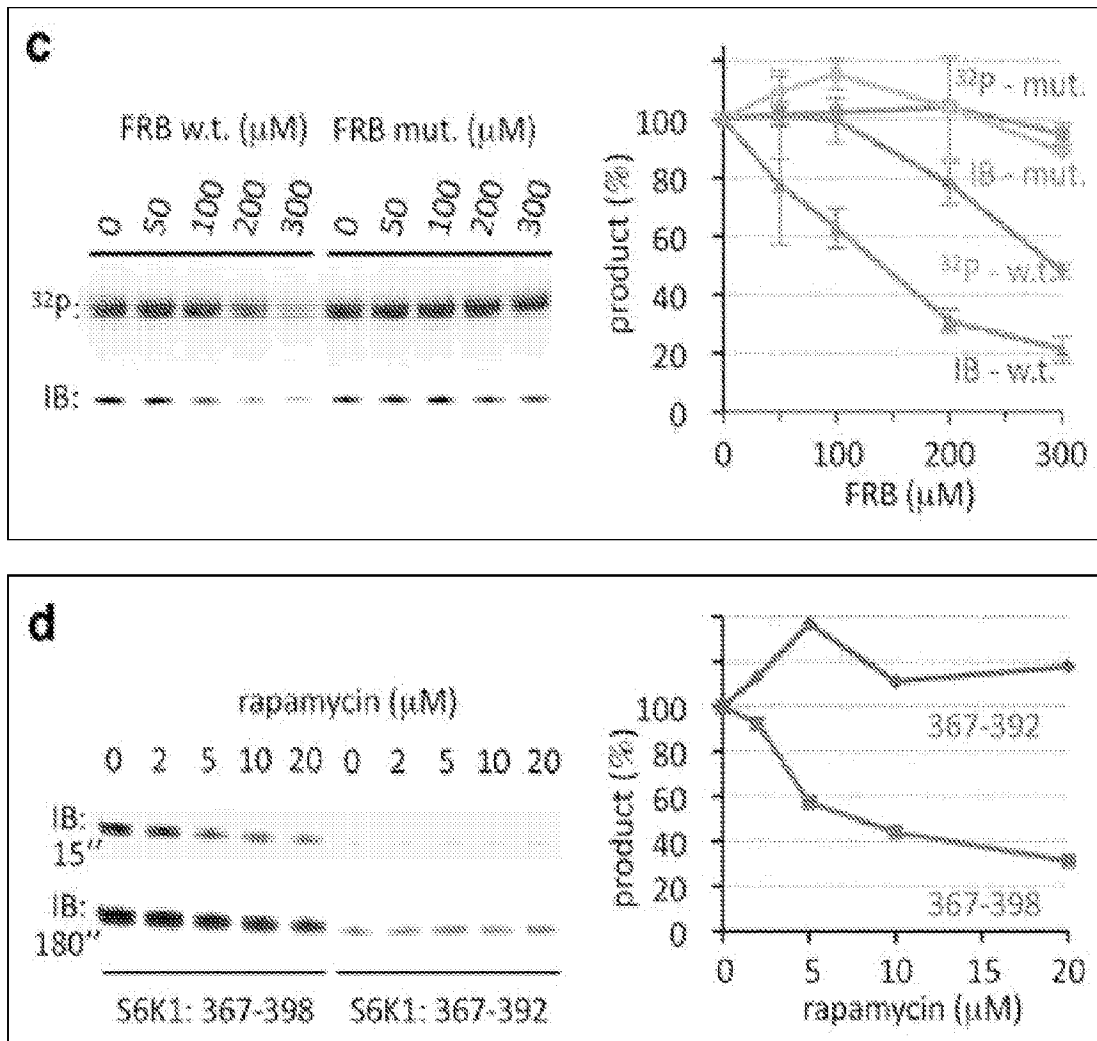
9/57

FIGURE 4



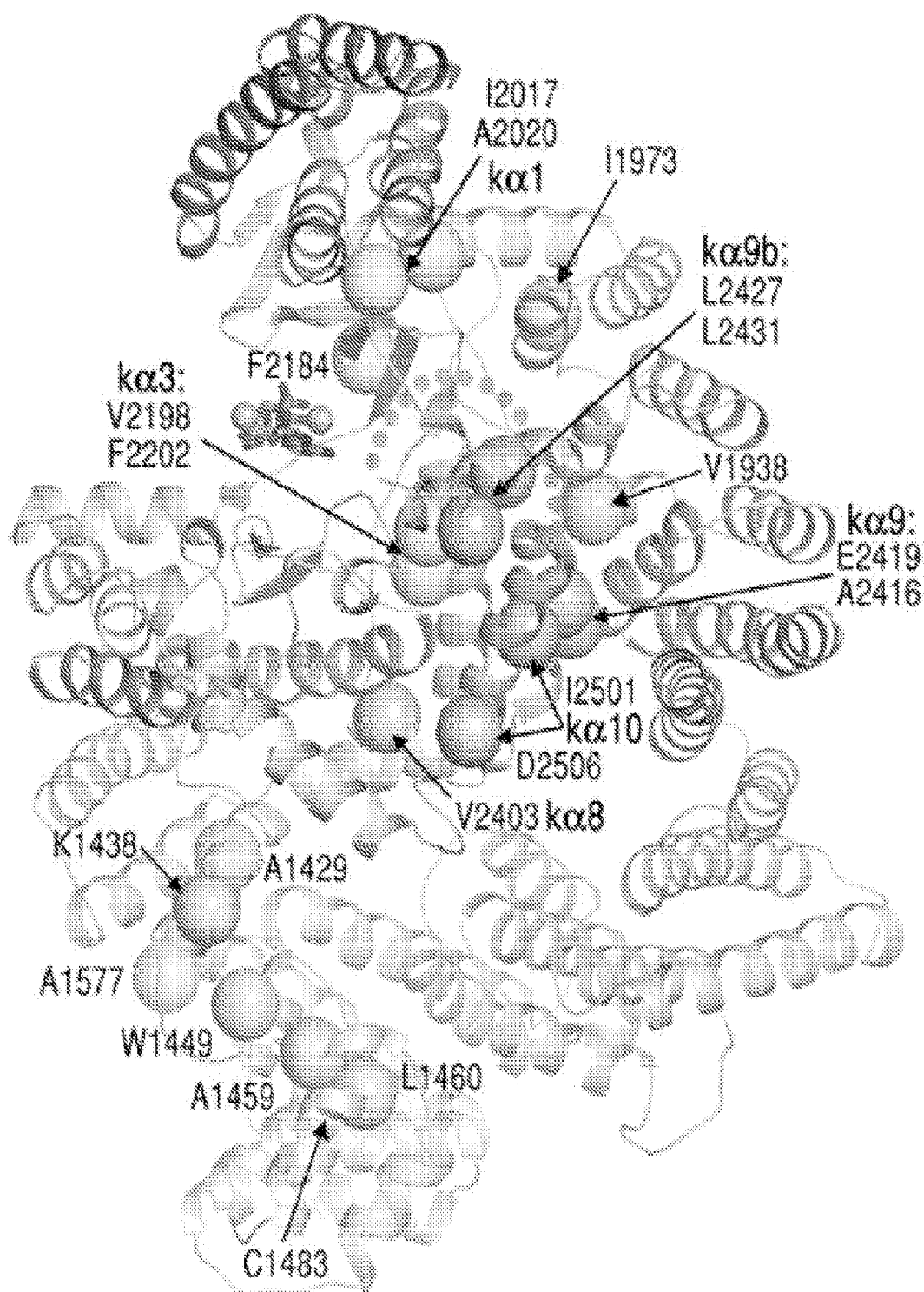
10/57

FIG. 4 - continued



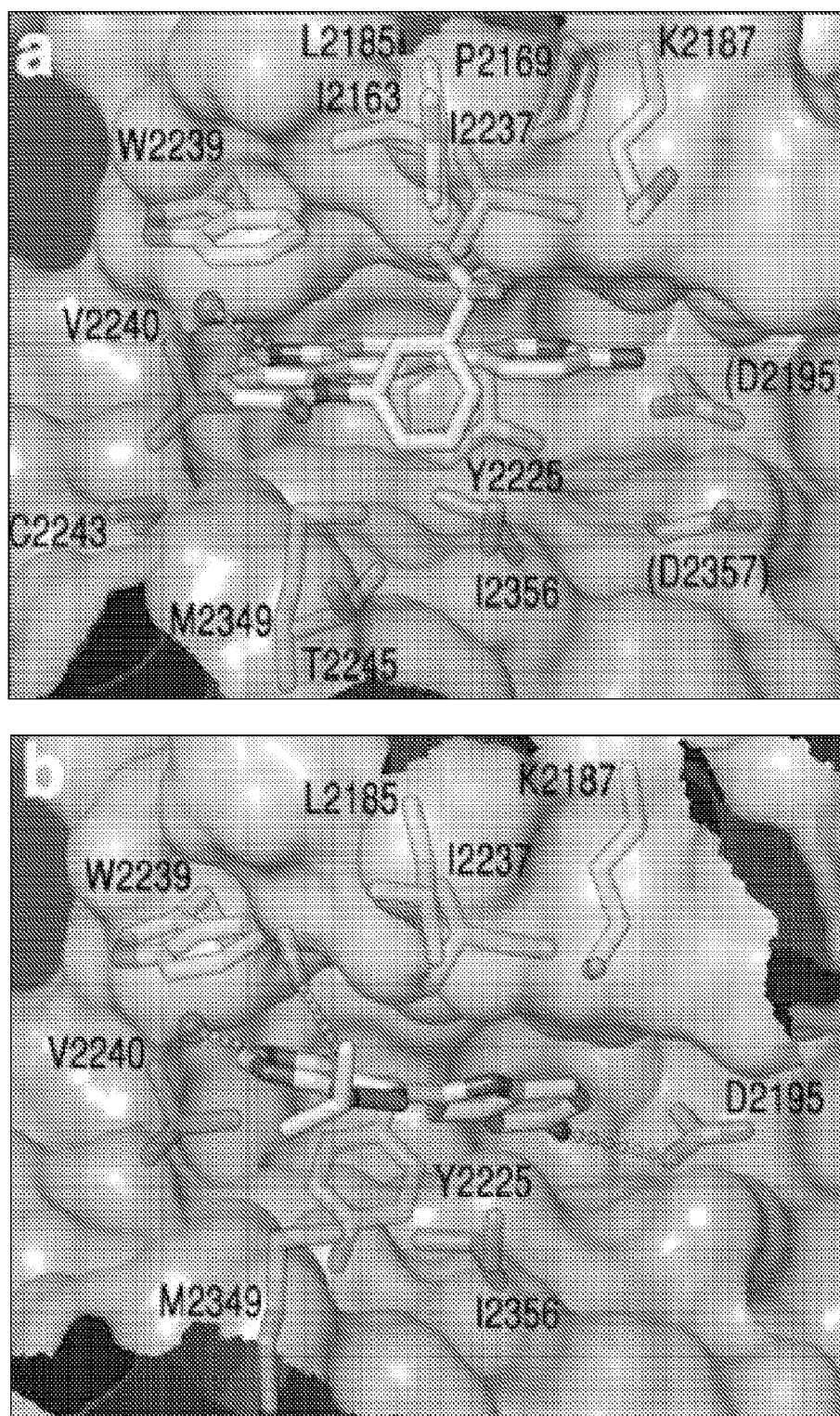
11/57

FIGURE 5



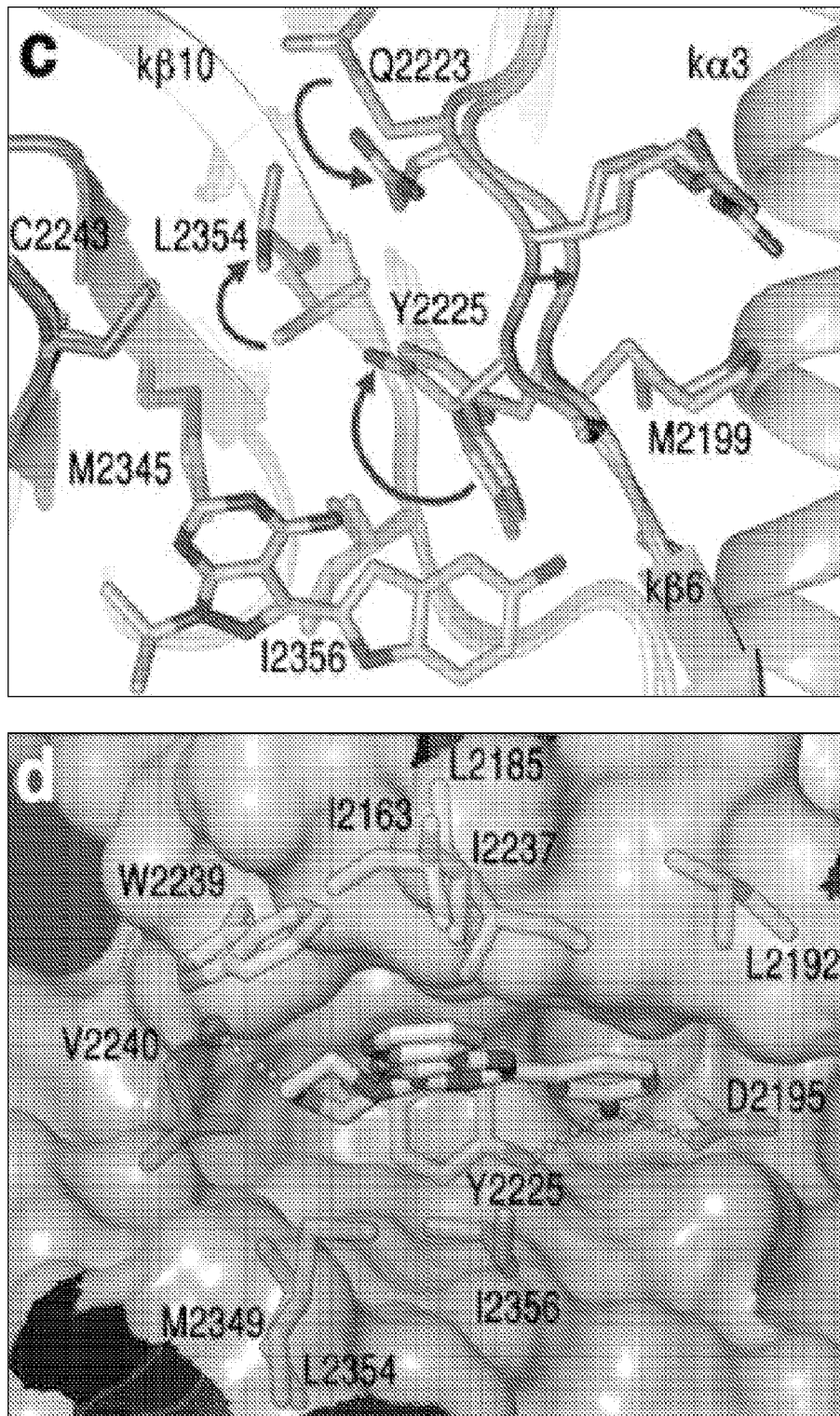
12/57

FIGURE 6



13/57

FIG. 6 – continued



14/57

FIGURE 7

	Native	ATP _γ S-Mg	ADP-Mg-F	AMPPNP-Mn	Au	U
Data collection						
Space group	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2
Cell dimensions						
<i>a</i> , <i>b</i> , <i>c</i> (Å)	163.2, 207.8, 139.4	162.8, 203.7, 138.7	163.8, 208.5, 138.5	162.9, 207.2, 140.0	163.3, 209.2, 139.7	162.8, 207.6, 139.0
α , β , γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	30.0-3.2 (3.31-3.2)*	100.0-3.3 (3.42-3.3)	100.0-3.5 (3.63-3.5)	100.0-3.7 (3.83-3.7)	35.0-4.1 (4.25-4.1)	30.0-4.0 (4.14-4.0)
<i>R</i> _{sym}	0.107 (0.538)	0.149 (0.612)	0.185 (0.665)	0.178 (0.642)	0.197 (0.615)	0.186 (0.704)
<i>I</i> / σ <i>I</i>	11.3 (1.8)	7.8 (1.4)	5.9 (1.4)	11.7 (2.3)	9.2 (2.8)	9.6 (2.3)
Completeness (%)	95.8 (79.4)	98.5 (95.3)	94.6 (83.6)	99.6 (98.1)	98.4 (99.1)	88.9 (89.1)
Redundancy	3.7 (2.3)	4.4 (2.5)	3.6 (2.4)	6.4 (5.5)	6.6 (6.4)	6.2 (5.6)
Refinement						
Resolution (Å)	30.0-3.2	100.0-3.3	100.0-3.5			
No. reflections	66653	63147	49387			
<i>R</i> _{work} / <i>R</i> _{free}	22.2/25.6	23.3/26.8	23.1/25.1			
No. atoms						
Protein	22126	22126	22126			
Ligand	0	66	66			
B-factors						
Protein	77.2	70.8	74.5			
Ligand/ion	-	64.4	64.3			
R.m.s deviations						
Bond lengths (Å)	0.009	0.007	0.006			
Bond angles (°)	1.418	1.253	1.133			

*Highest resolution shell is shown in parenthesis.

FIGURE 8

Kinase domain	mTOR	PI3K C3	p110 α	p110 β	p110 γ	p110 δ
mTOR (493)*	-	1.7 (252)#	1.9 (256)	1.7 (221)	1.7 (249)	1.8 (243)
PI3K C3 (3IHY)†			1.8 (289)	1.6 (291)	1.8 (294)	1.4 (292)
PI3K p110 α (2RDO) (344)				1.6 (310)	1.7 (288)	1.4 (299)
PI3K p110 β (2Y3A) (360)					1.5 (288)	1.1 (332)
PI3K p110 γ (1E8X) (352)						1.5 (288)
PI3K p110 δ (2W XF) (352)						-

*Number of ordered residues in the structure of the specified kinase domain.
#Number of residues that superimpose at the indicated r.m.s.d.
†PDB id.

FIGURE 9

	Torin2	PP242	PI-103
Data collection			
Space group	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2
Cell dimensions			
<i>a, b, c</i> (Å)	164.3, 208.3, 138.8	163.8, 207.0, 139.3	163.6, 207.7, 138.3
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	40.0-3.55 (3.68-3.55)*	40.0-3.45 (3.57-3.45)	40.0-3.6 (3.73-3.60)
R _{sym}	0.187 (0.669)	0.176 (0.629)	0.226 (0.650)
I/σI	7.3 (1.7)	5.8 (1.3)	4.7 (1.5)
Completeness (%)	95.6 (71.1)	97.0 (91.2)	89.0 (76.9)
Redundancy	3.5 (2.0)	3.8 (2.7)	3.3 (2.5)
Refinement			
Resolution (Å)	40.0 – 3.5	40.0 – 3.45	40.0 – 3.6
No. reflections	49412	52634	40902
R _{work} / R _{free}	22.8 / 25.9	23.5 / 27.1	24.0 / 27.5
No. atoms			
Protein	22126	22126	22126
Inhibitor	64	46	52
B-factors			
Protein	76.1	68.9	65.7
Ligand/ion	34.3	36.1	30.1
R.m.s deviations			
Bond lengths (Å)	0.007	0.007	0.006
Bond angles (°)	1.279	1.277	1.219

*Highest resolution shell is shown in parenthesis.

FIGURE 10

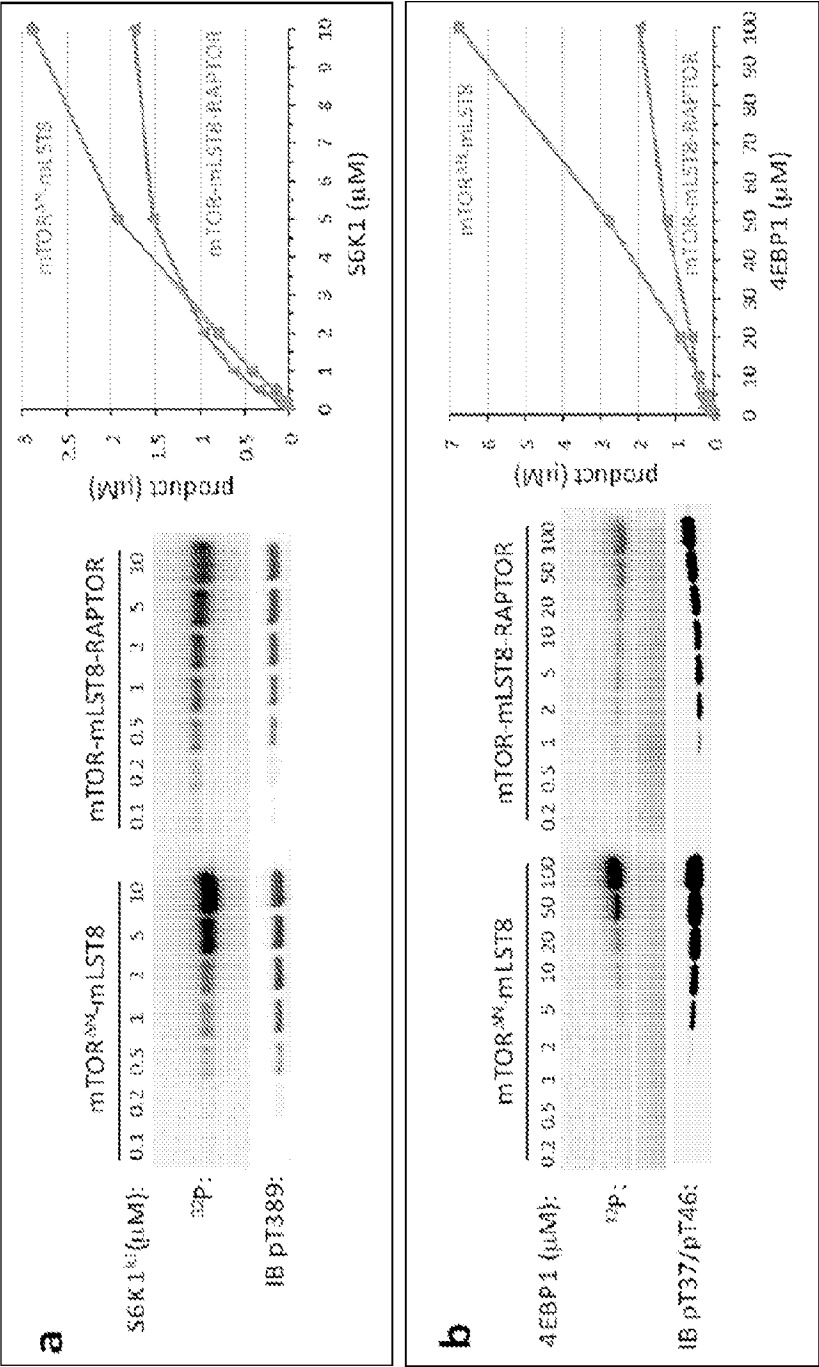


FIG. 11A - continued

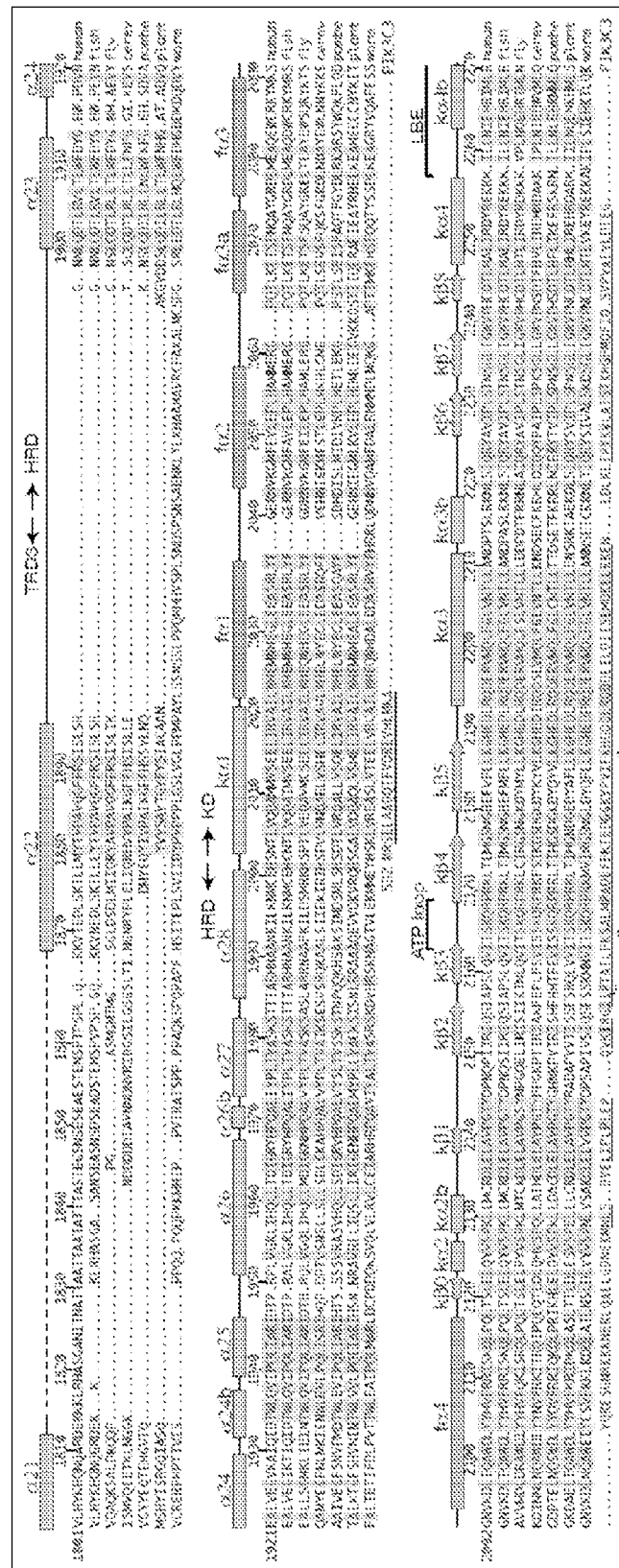


FIG. 11A - continued

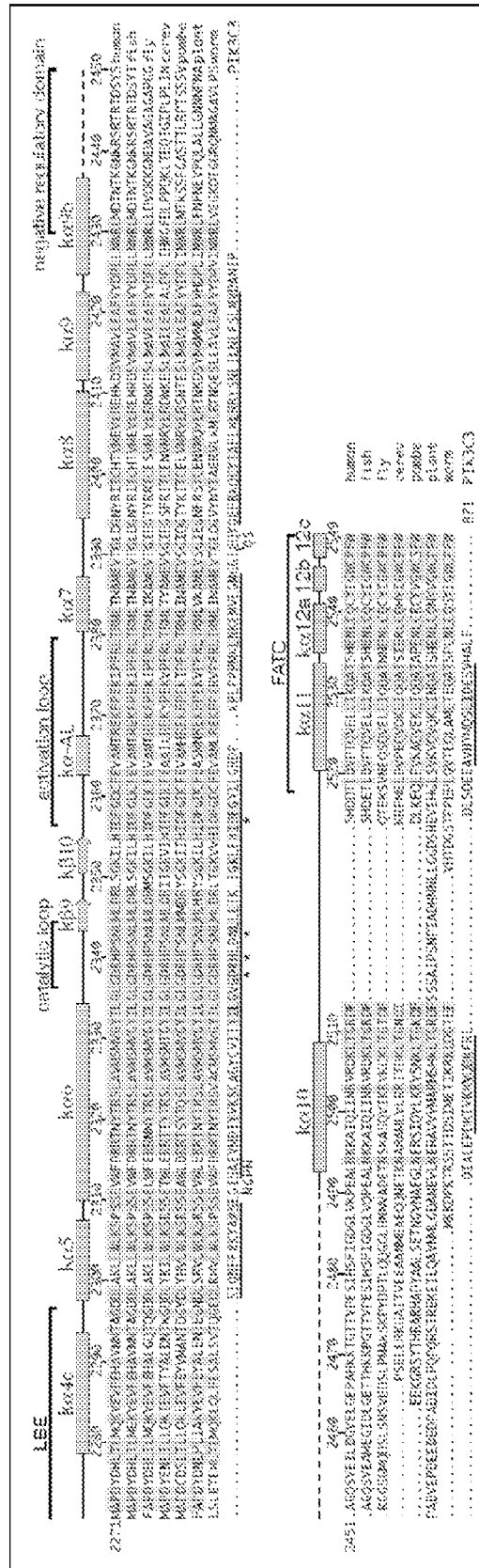


FIG. 11 - continued

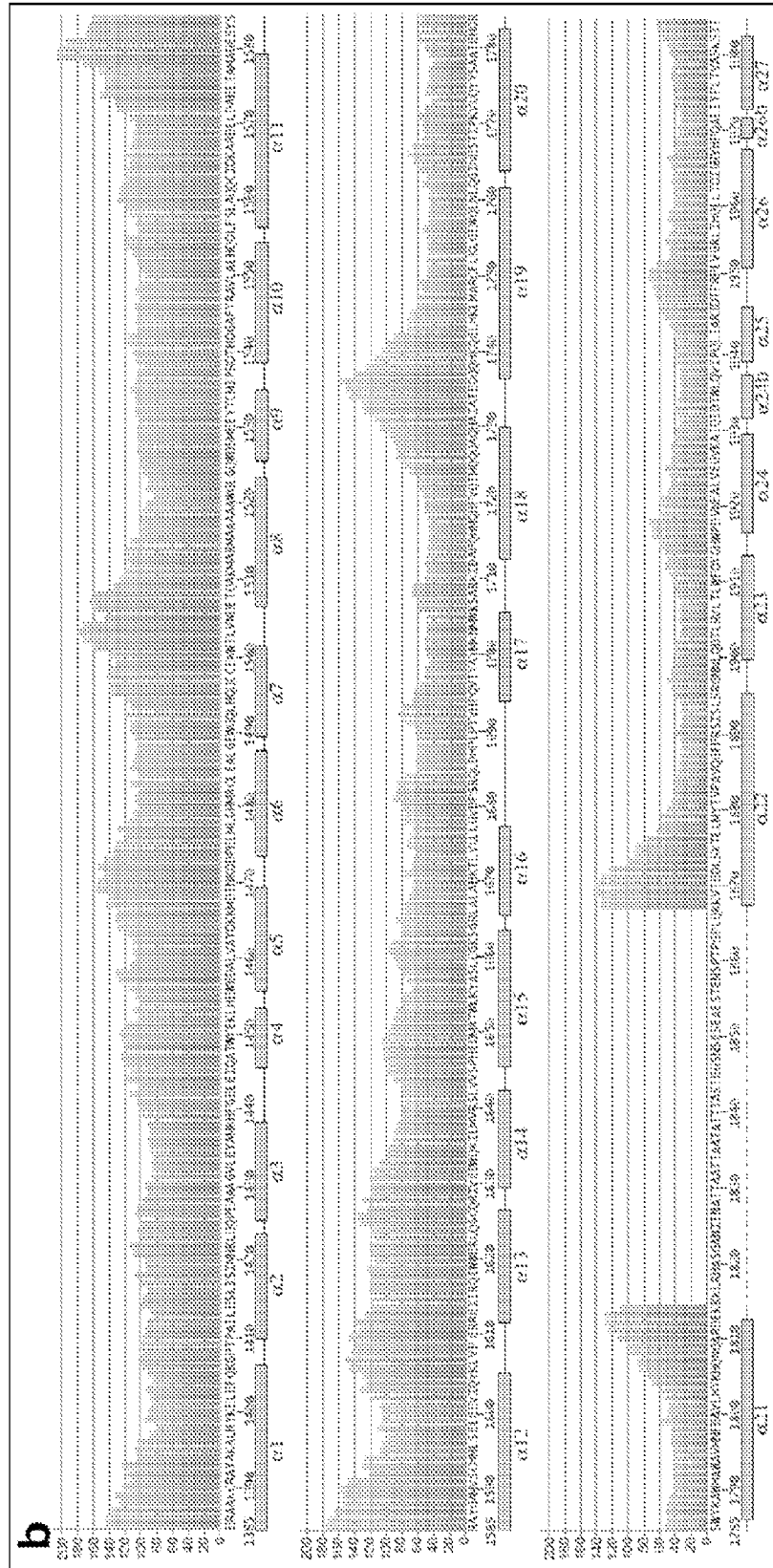
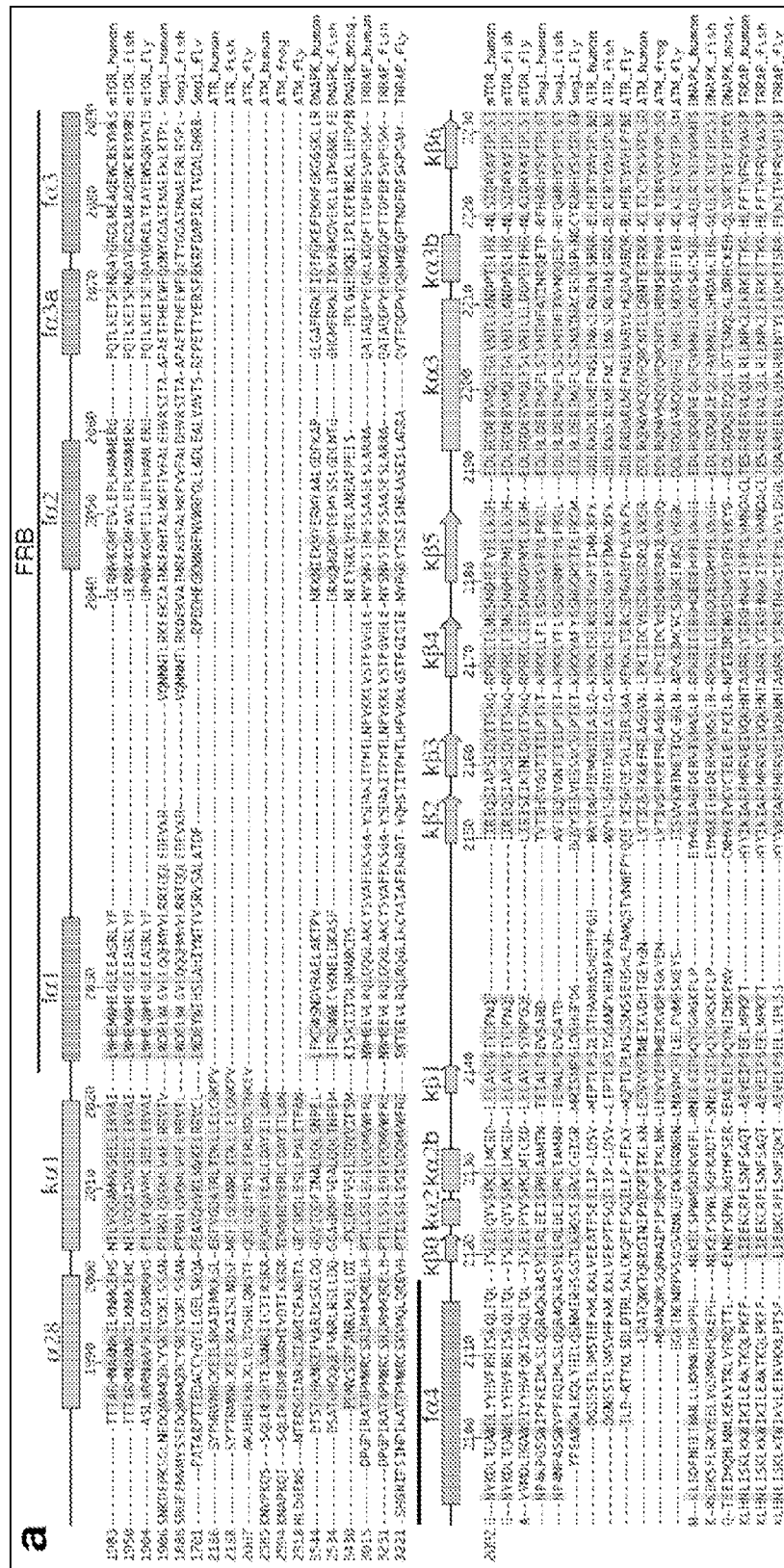


FIGURE 12



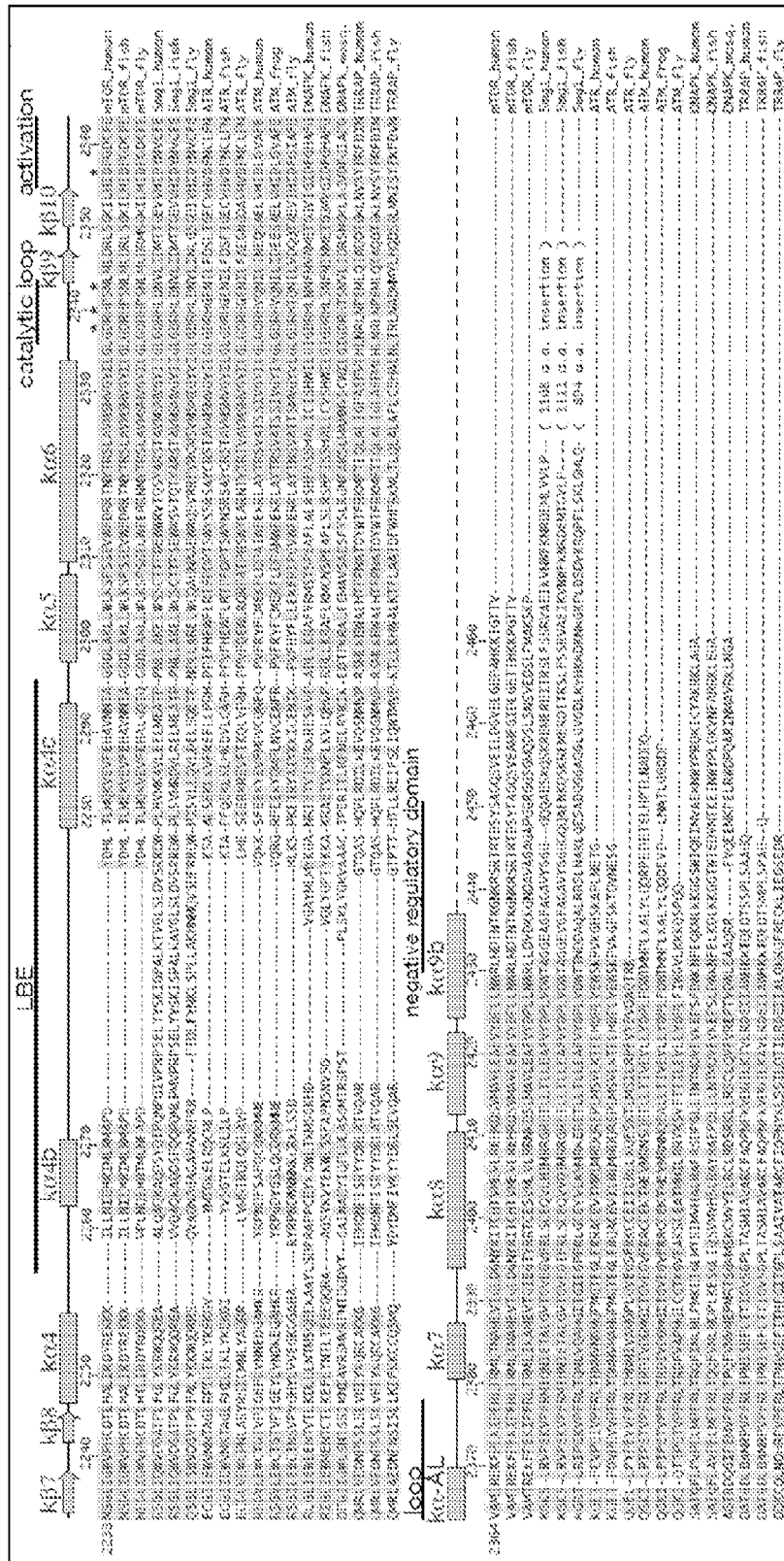
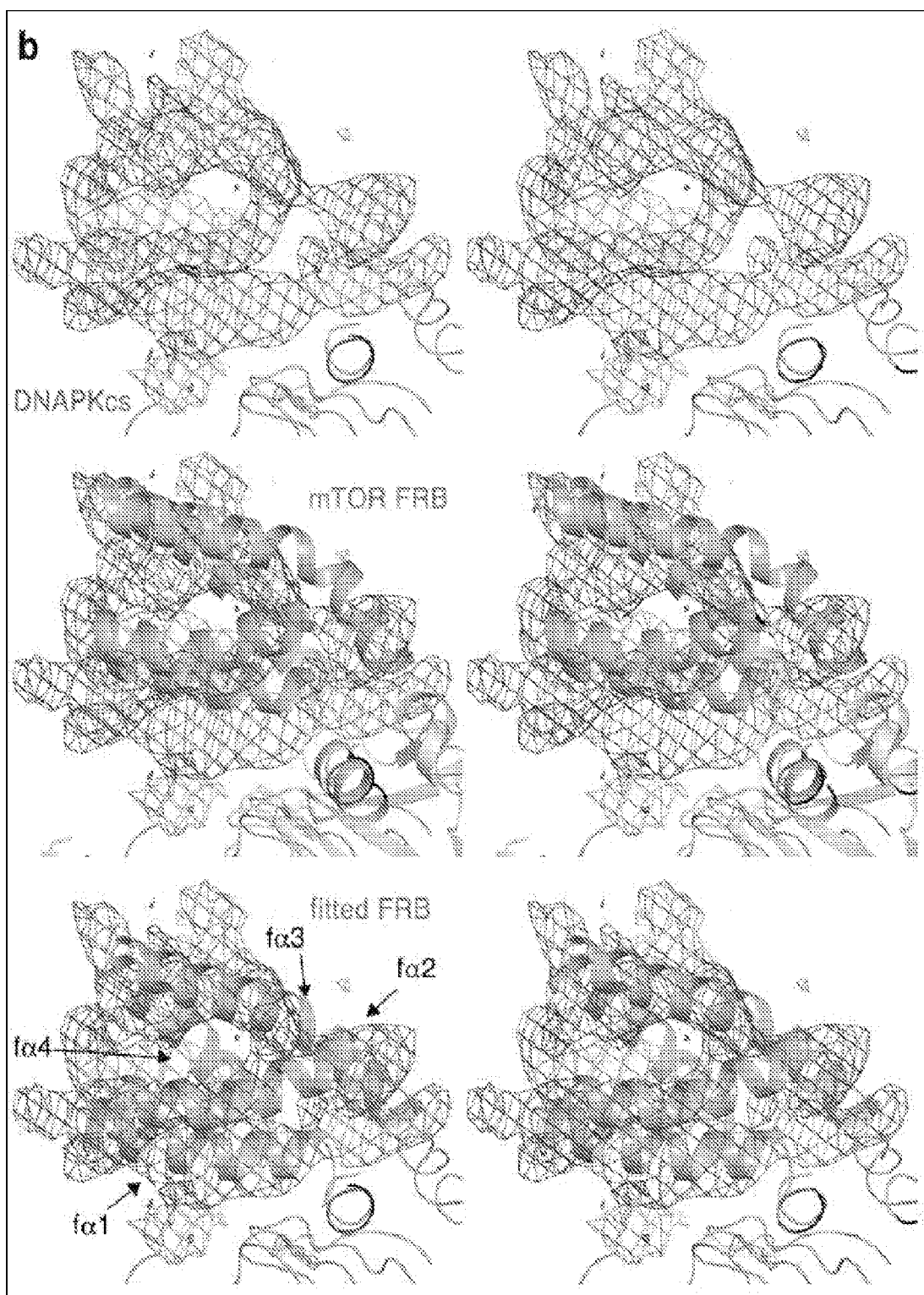
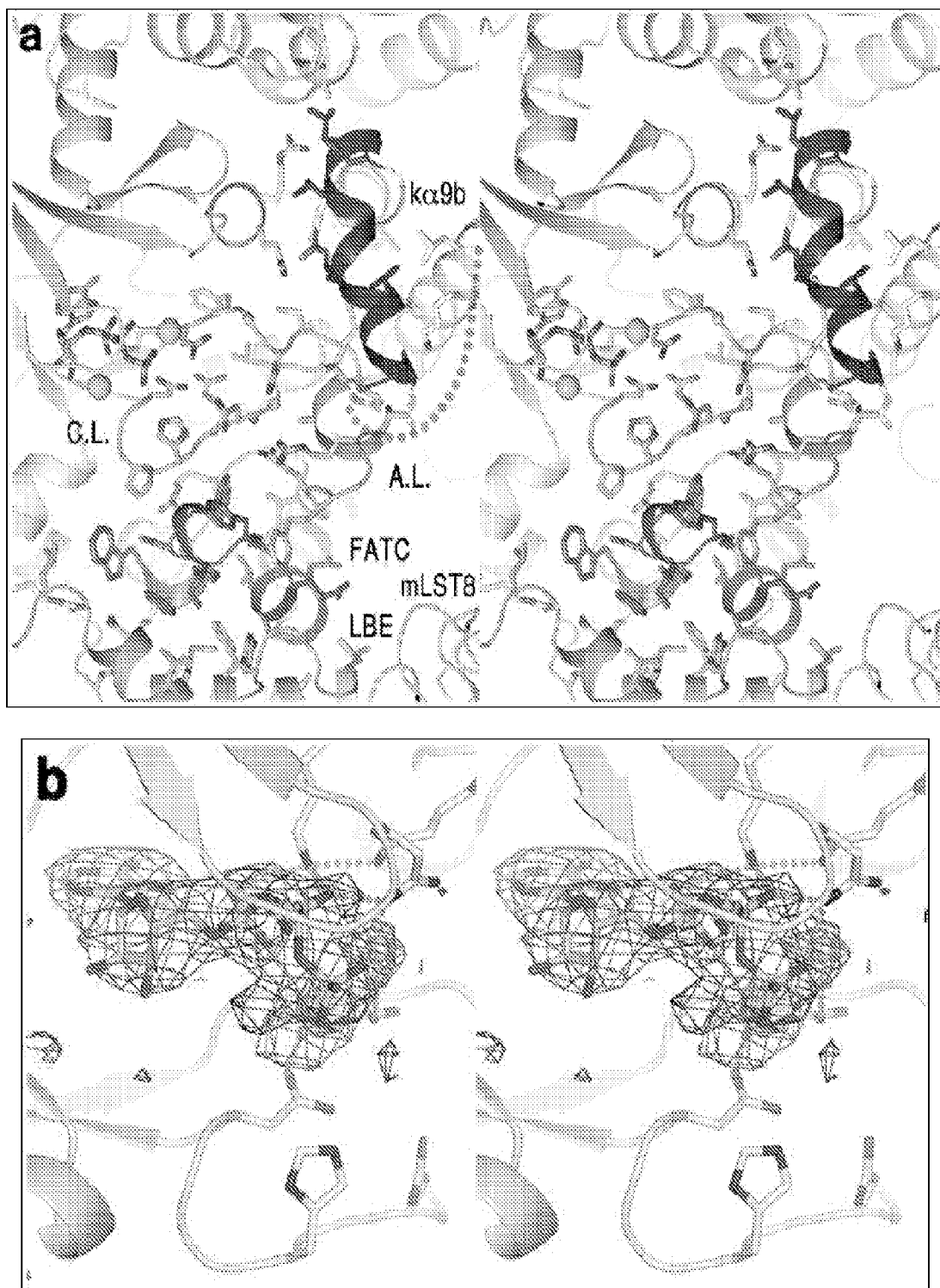


FIG. 12 – continued



27/57

FIGURE 13



258

FIG. 13 – continued

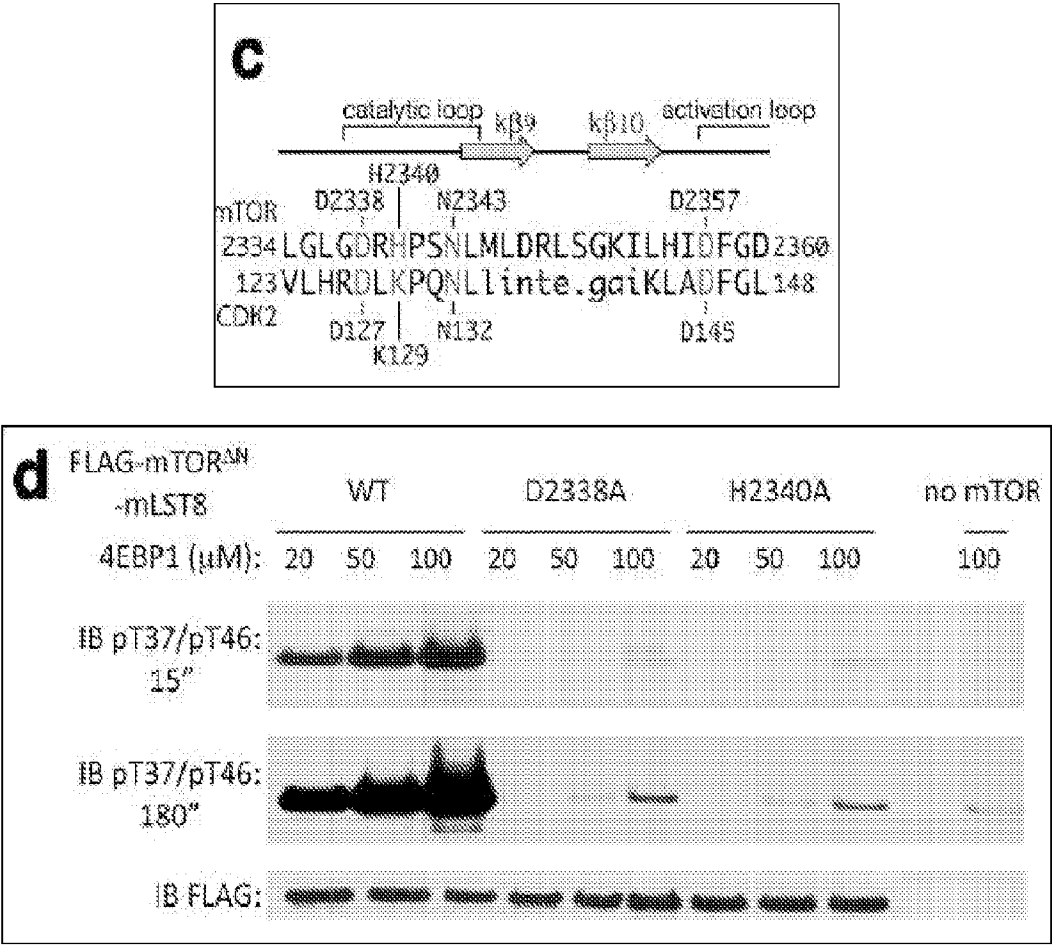
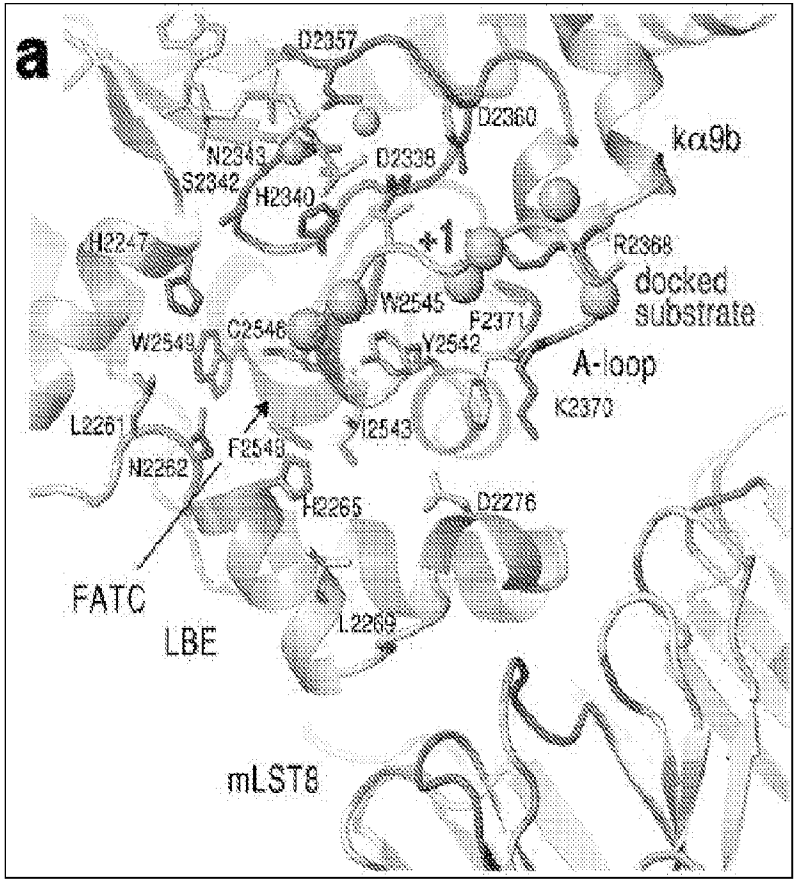


FIGURE 14



b

22	VLGDGVQLPPGDYSTTPGGTLFSTTPGGTRI	4EBP1-T37
31	PGDYSTTPGGTLFSTTPGGTRIIYDRKFLME	4EBP1-T46
50	TRIIYDRKFLMECRNSPVTKTPPRDLPTIPG	4EBP1-S65
55	DRKFLMECRNSPVTKTPPRDLPTIPGVTSPS	4EBP1-T70
374	DSTLSESANQVELGFTYVAPSVLESVKEKFS	S6K1
722	ATPLSPAMQAKFAGFTFVDESAIDENVNNR	sch9_yeast
642	QLVIANIDQSDIEGFSYVNPQFVHPILQSAV	PKC α
458	MECVDSERRPHFPQFSYSASSTA	AKT1
407	VTASVKEAAEFLGFSYAPPTDSFL	SGK1

FIG. 16 - continued

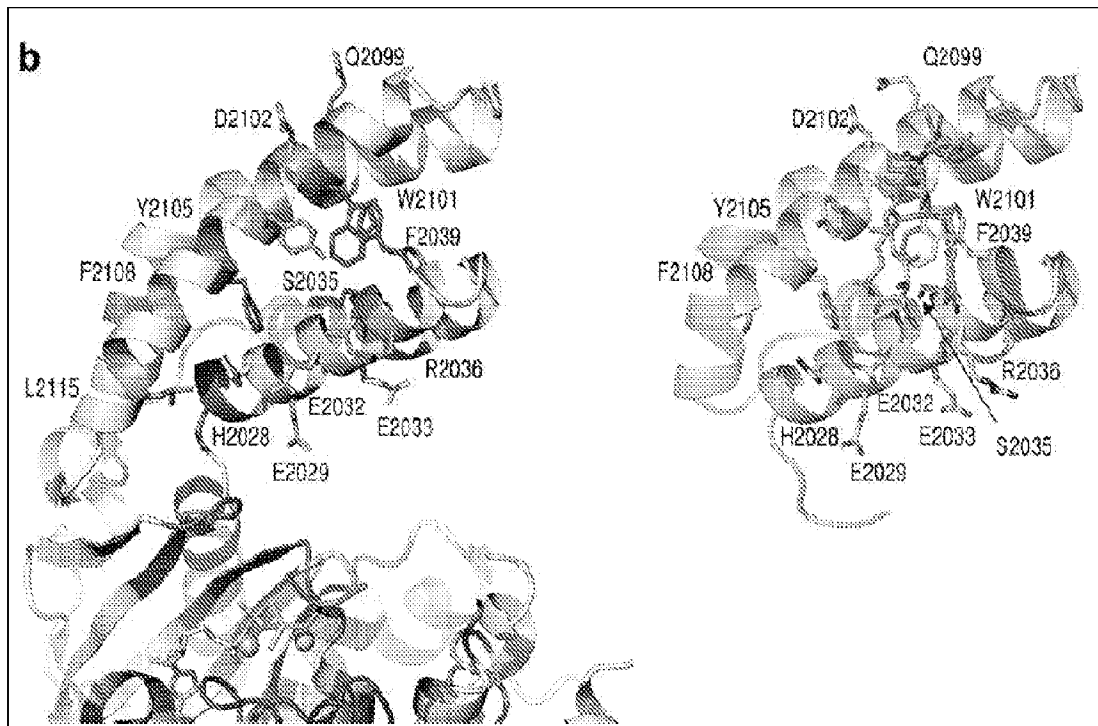


FIGURE 17

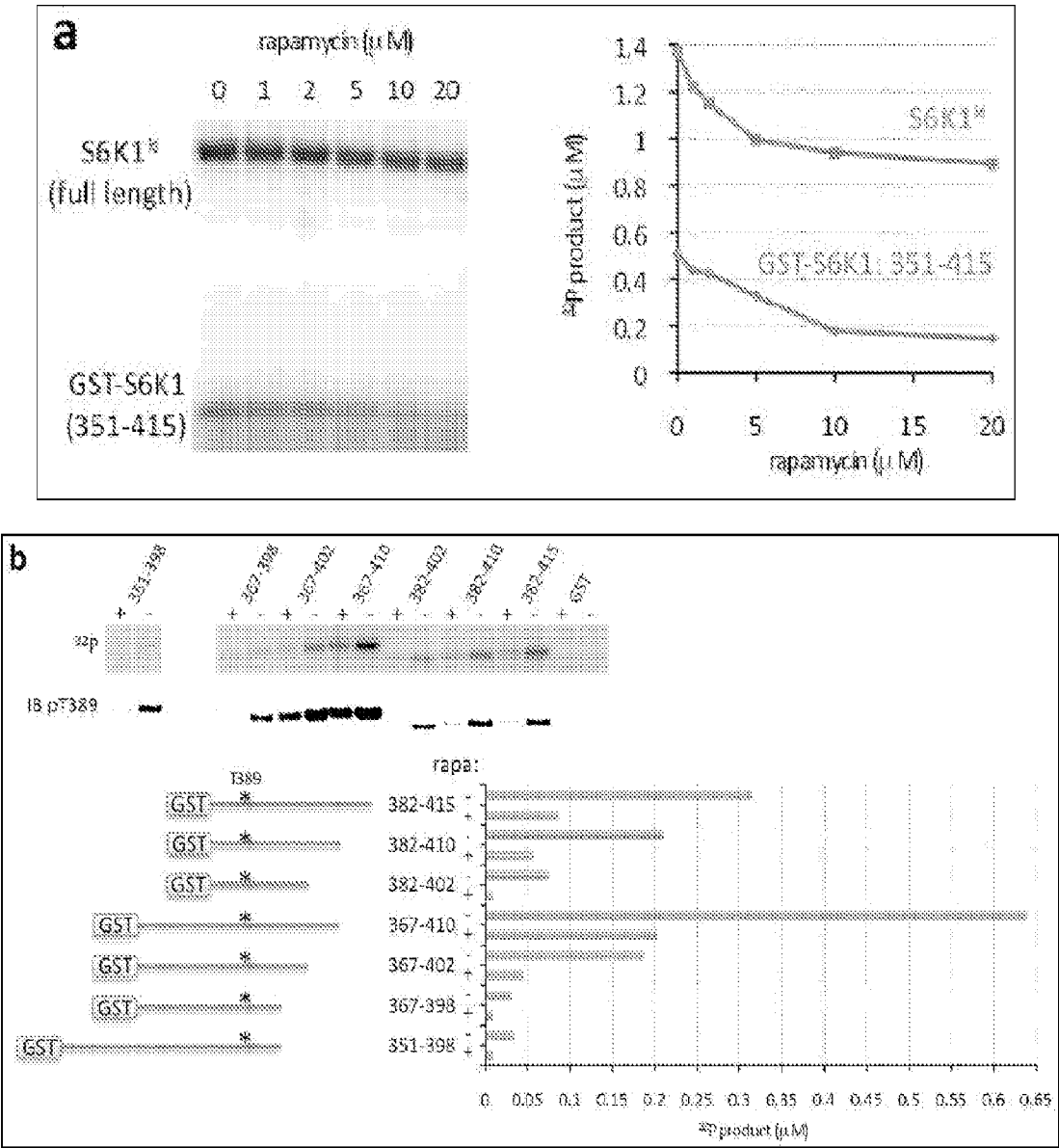


FIGURE 18

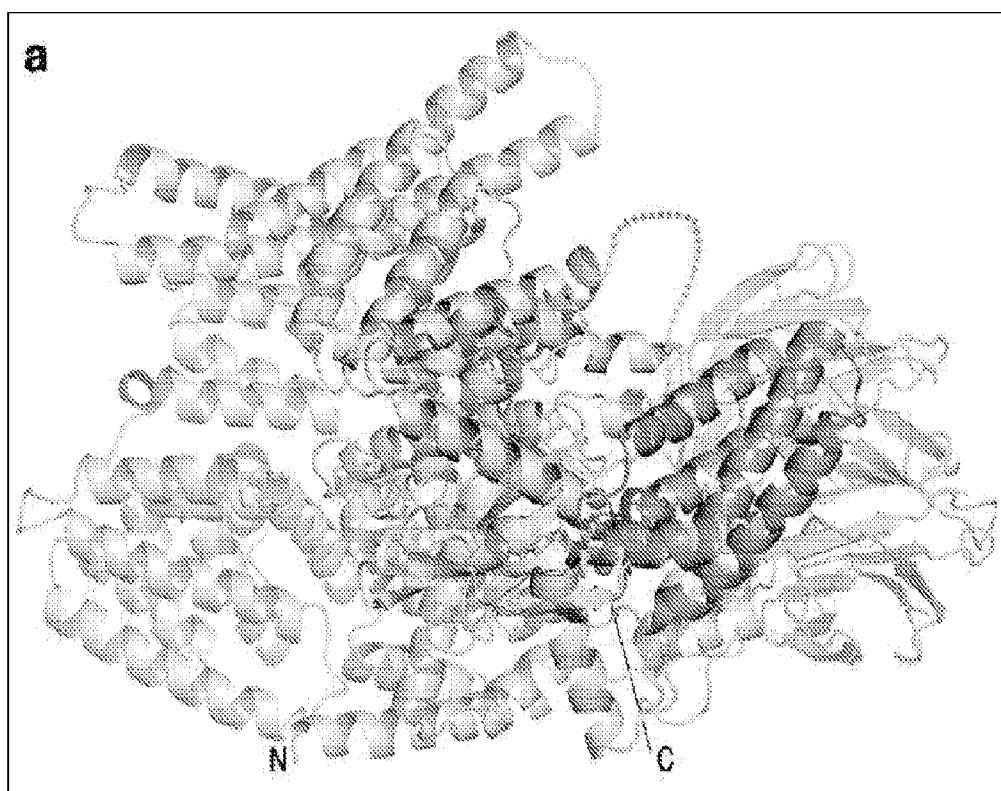


FIG. 18 - continued

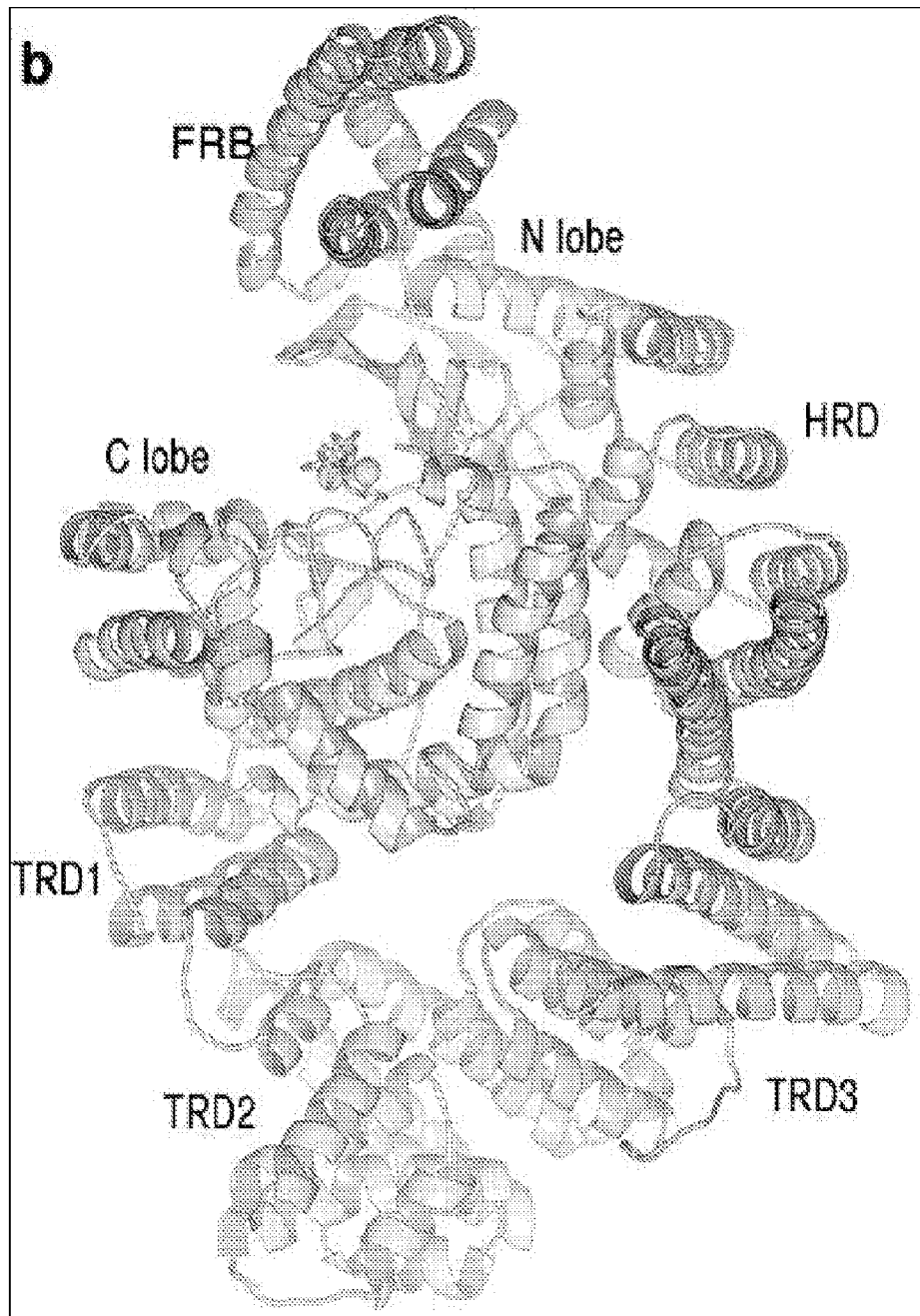
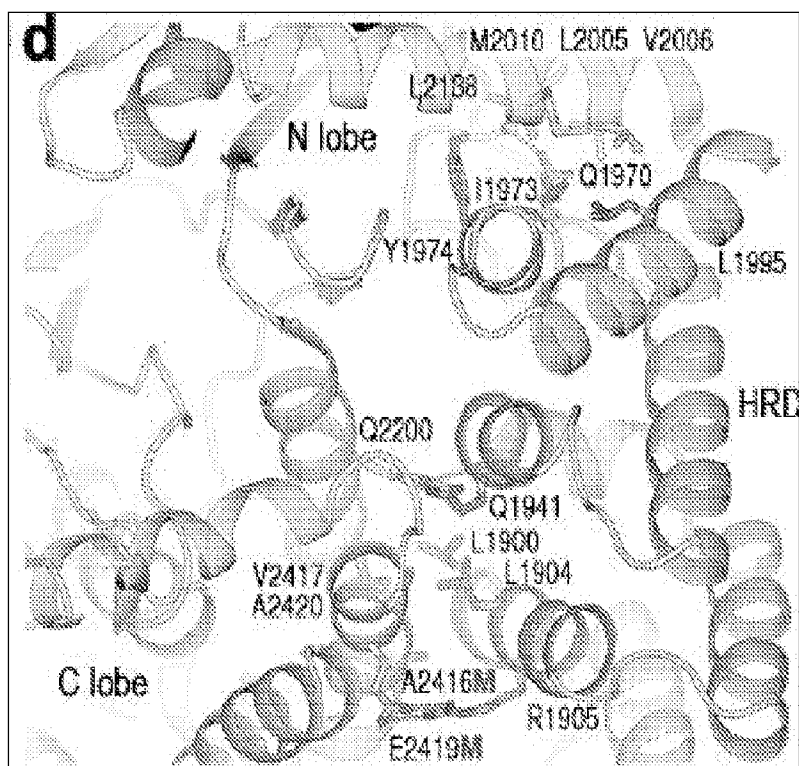
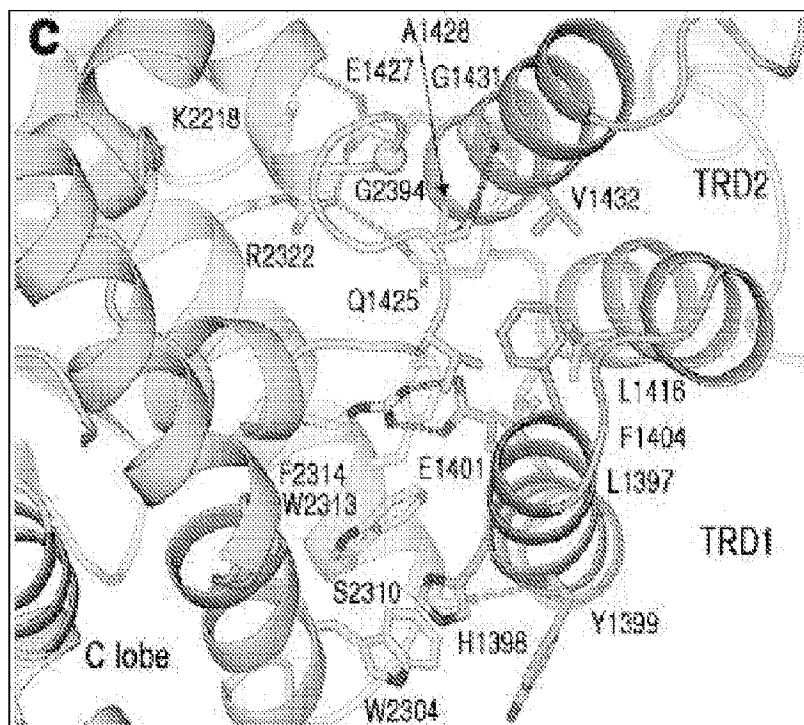
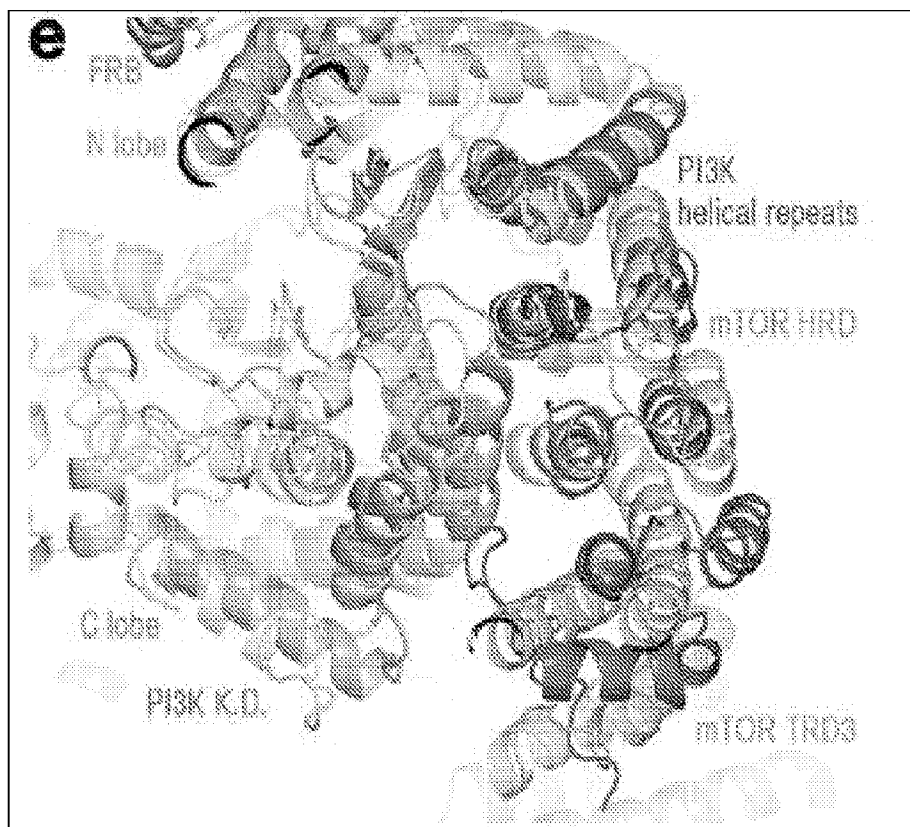


FIG. 18 - continued



37/57

FIG. 18 - continued



268

FIGURE 19

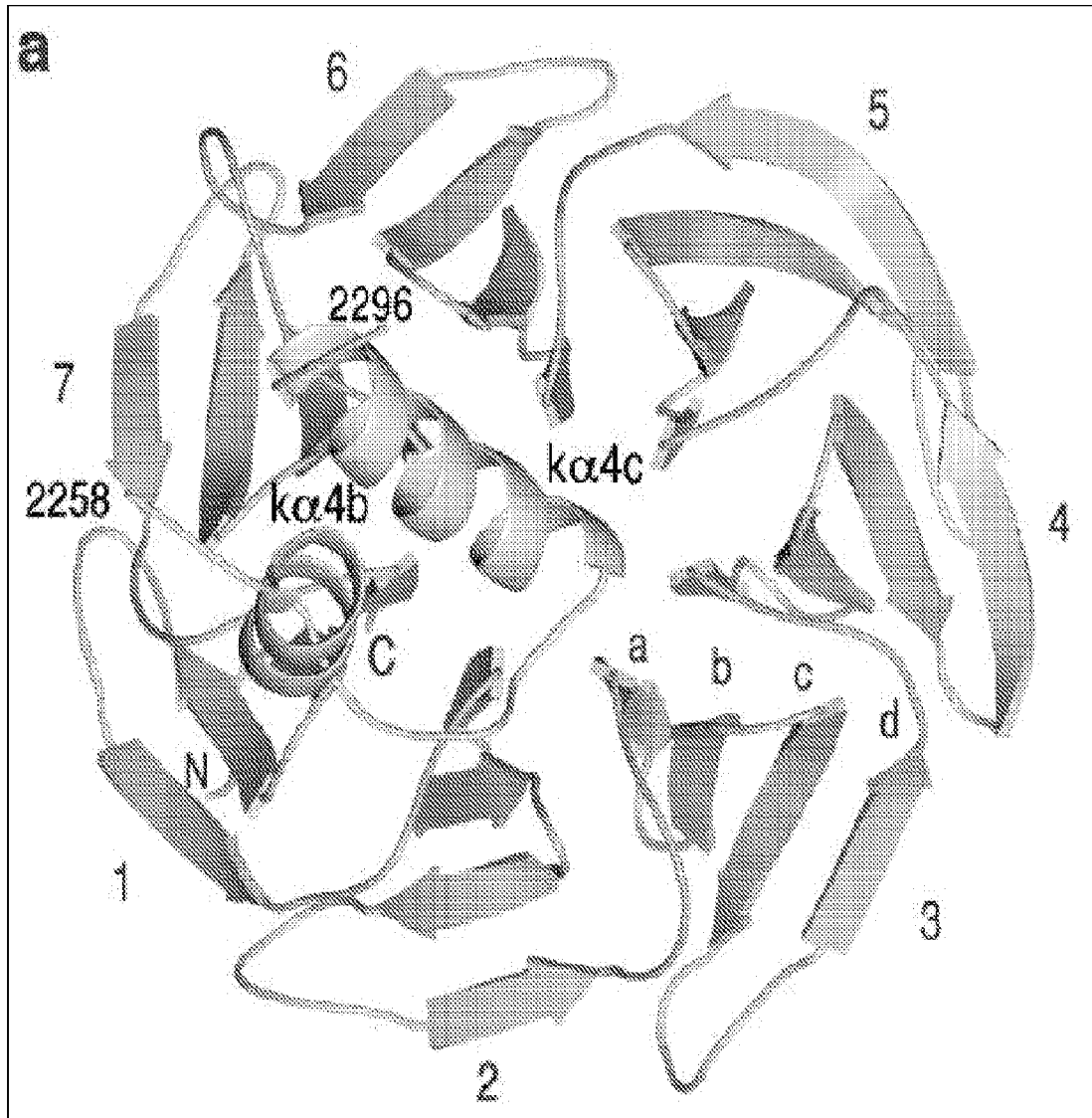


FIG. 19 - continued

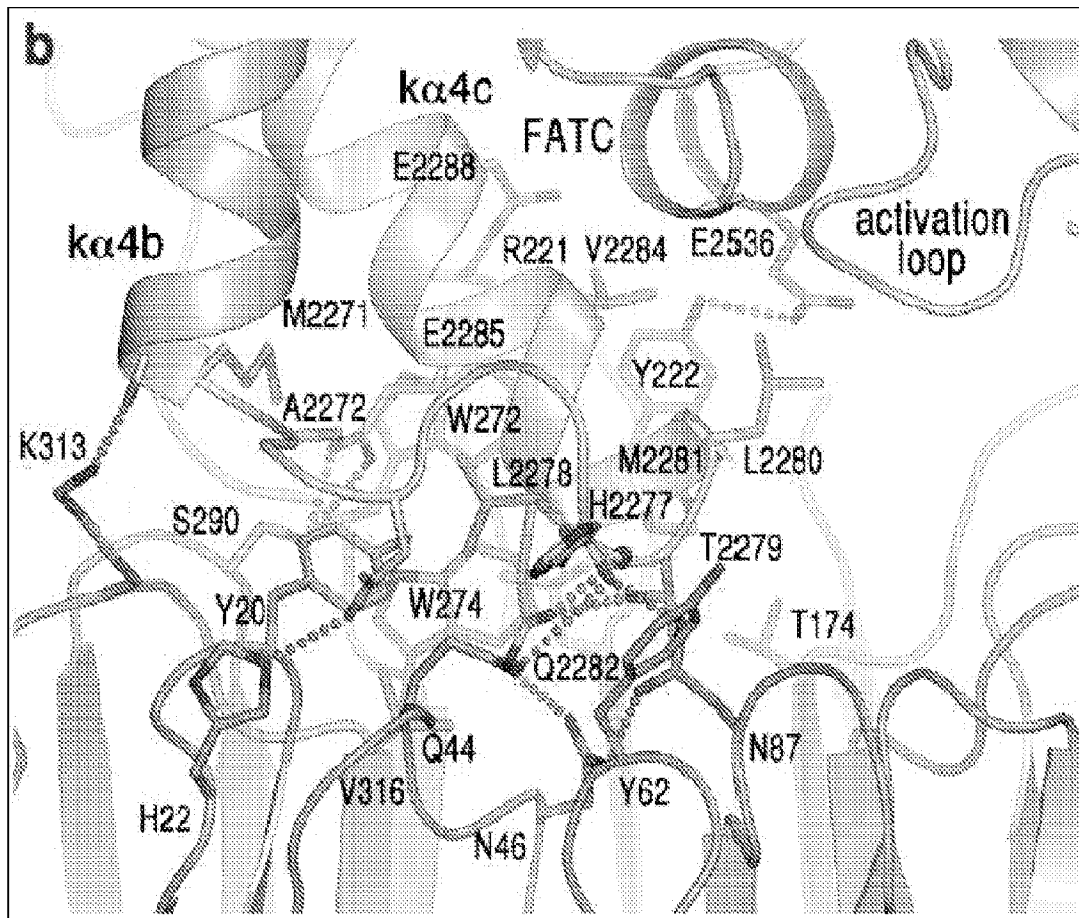


FIGURE 20

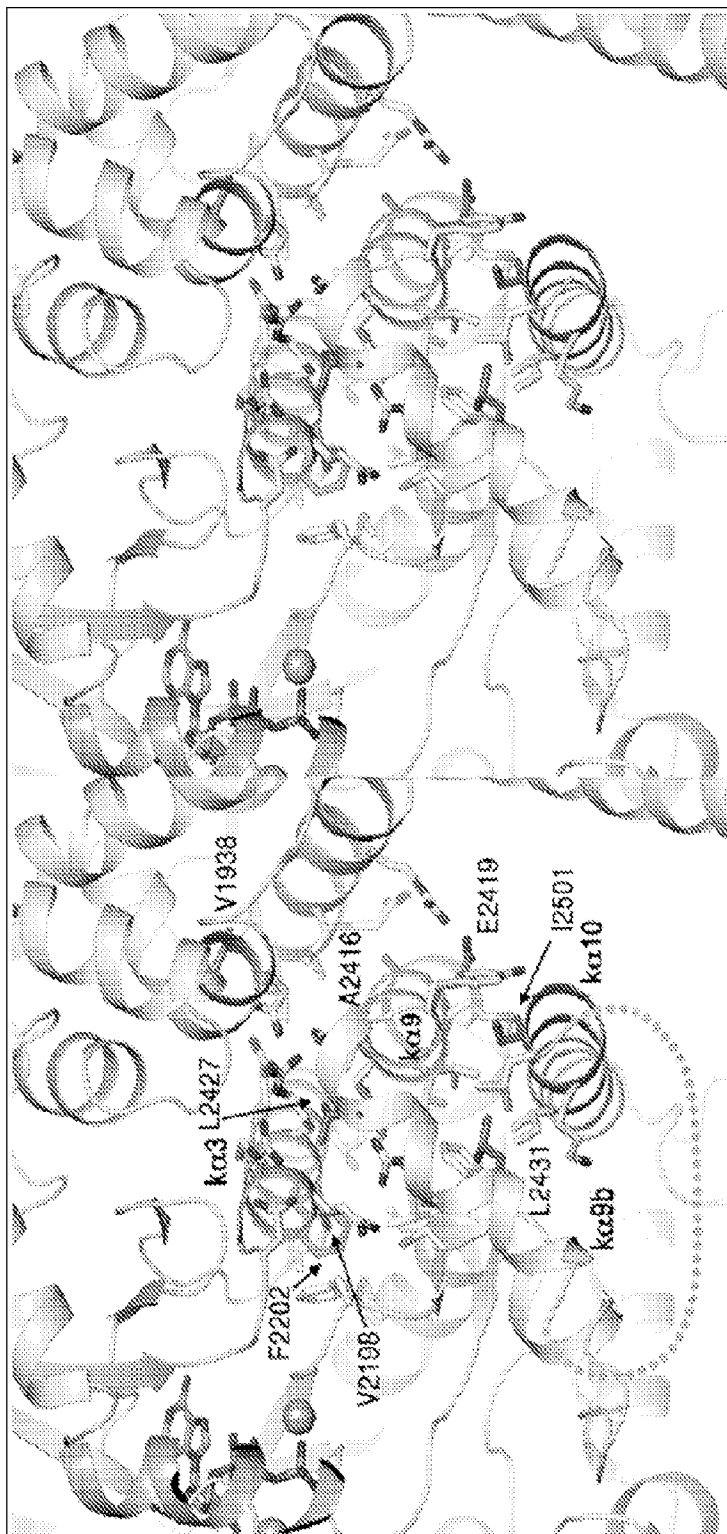


FIGURE 21

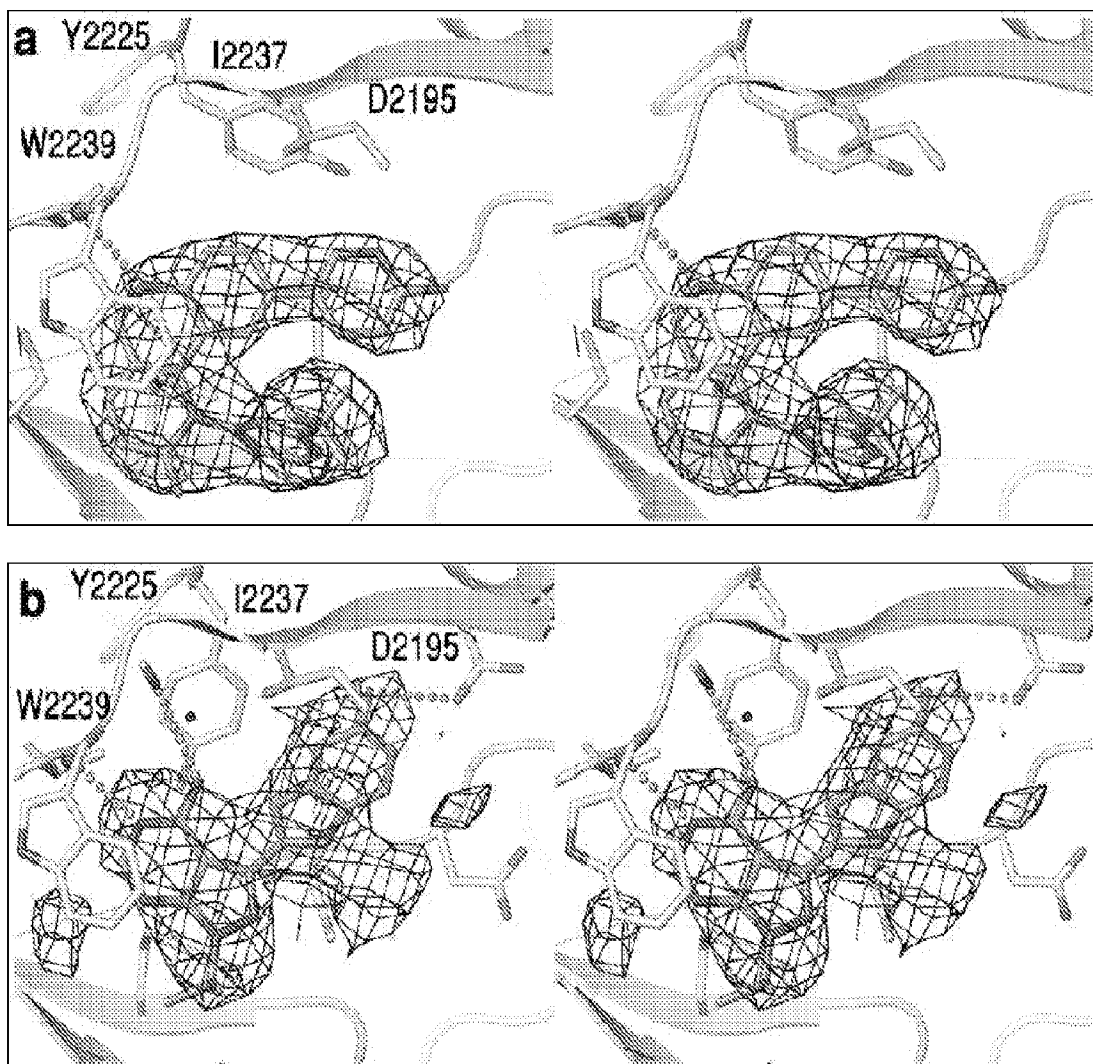


FIG. 21 – continued

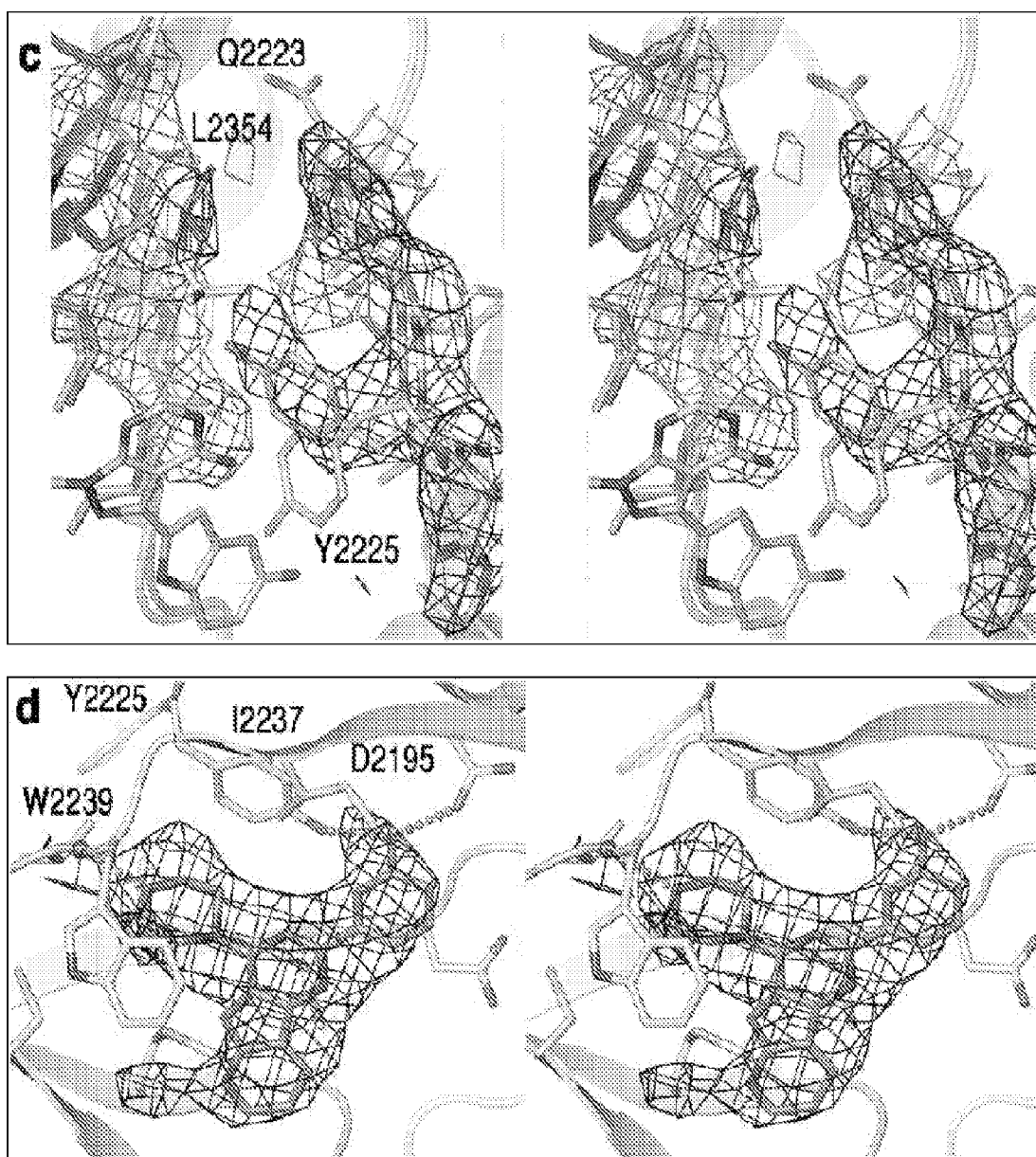


FIGURE 22

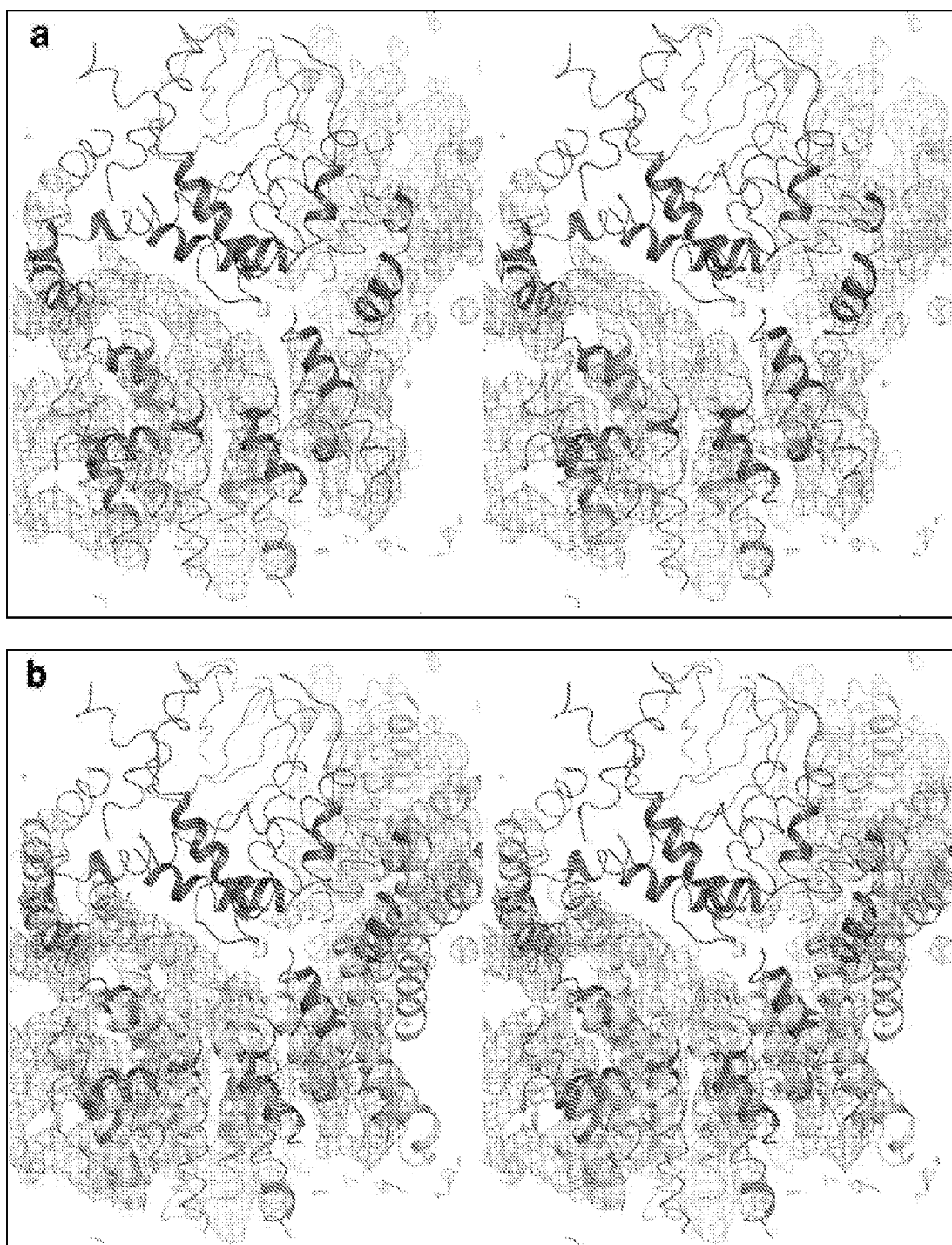
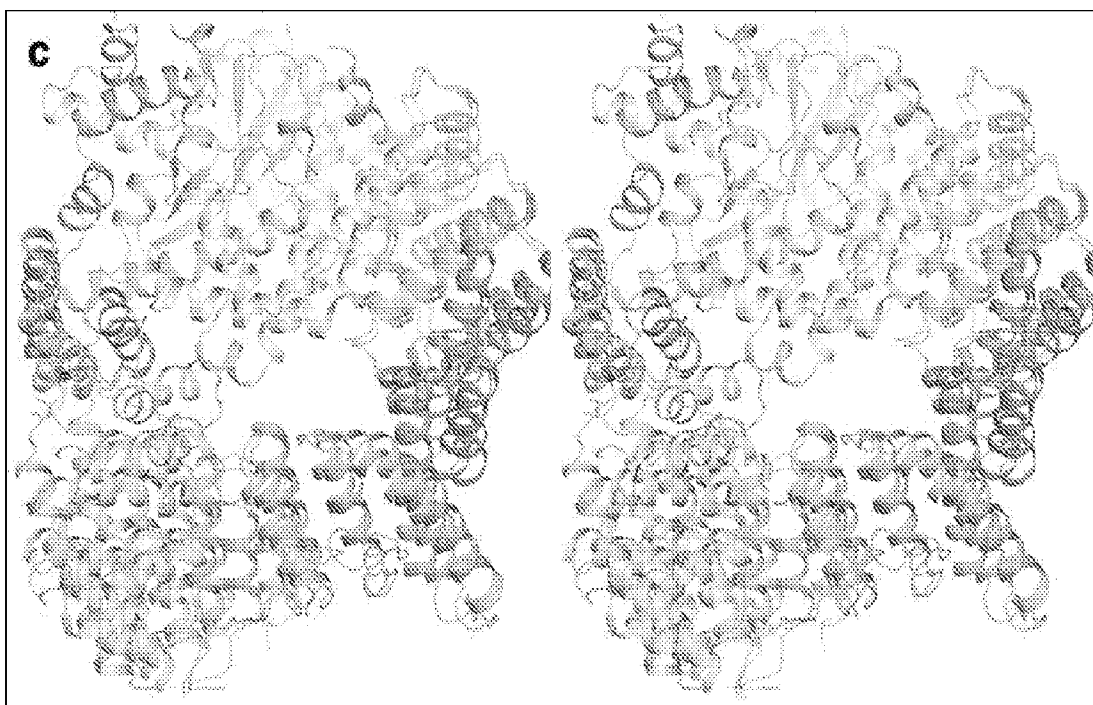


FIG. 22 – continued



SUBSTITUTE SHEET (RULE 26)

277

47/57

FIG. 23- continued

human	AHKRQKAMQVDATVFTCIISMLARAMGPGIQDDIKELLEPMPLAVGLSPALTAVLYDLSRQI
rat	AHKRQKTVQVDATVFTCIISMLARAMGPGIQDDIKELLEPMPLAVGLSPALTAVLYDLSRQI
mouse	AHKRQKTVQVDATVFTCIISMLARAMGPGIQDDIKELLEPMPLAVGLSPALTAVLYDLSRQI
sheep	AHKRQKAMQVDATVFTCIISMLARAMGPGIQDDIKELLEPMPLAVGLSPALTAVLYDLSRQI
zebrafish	AHKRQKIMQVDATVFTCIISMLSRAMGPGSIQDDVKEELLEPMPLAVGLSPALTAVLYDLSRQI
rhesus	AHKRQKAMQVDATVFTCIISMLARAMGPGSIQDDIKELLEPMPLAVGLSPALTAVLYDLSRQI
goat	AHKRQKAMQVDATVFTCIISMLARAMGPGIQDDIKELLEPMPLAVGLSPALTAVLYDLSRQI
fruit_fly_(B)	TSKRK--VPVDFAVFACITLLAHAVKSEIADDVKEILEQMFTTGLSPALTAVLYDLSRQI
fruit_fly_(A)	TSKRK--VPVDFAVFACITLLAHAVKSEIADDVKEILEQMFTTGLSPALTAVLYDLSRQI
chimpanzee	AHKRQKAMQVDATVFTCIISMLARAMGPGIQDDIKELLEPMPLAVGLSPALTAVLYDLSRQI : ** : ** :***:***:***: * :***:*** : ,***** , * ,**,:
human	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAHQLASPGITTLPEASDVGSITLALRT
rat	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAHQLASPGITTLPEASDVASITLALRT
mouse	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAHQLASPGITTLPEASDVASITLALRT
sheep	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAHQLASPGITTLPEASDVGSITLALRT
zebrafish	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAYQLSPSITNIPASDVGSITLALRT
rhesus	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAHQLASPGITTLPEASDVGSITLALRT
goat	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAHQLASPGITTLPEASDVGSITLALRT
fruit_fly_(B)	PQLKSAITEGLIGILSCVLMHMKRAILPYTALPT---IAI---DGLMONGDGCATTVLALKT
fruit_fly_(A)	PQLKSAITEGLIGILSCVLMHMKRAILPYTALPT---IAI---DGLMONGDGCATTVLALKT
chimpanzee	PQLKKDIQDGLLKMMLSLVLMHMKPLRHPGMPKGLAHQLASPGITTLPEASDVGSITLALRT **** , * :** : * *** , * : : : , * : ,***:
human	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSITHLISCHAHVVSQTA
rat	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSITHLISCHAHVVSQTA
mouse	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSITHLISCHAHVVSQTA
sheep	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSVHLISCHAHVVSQTA
zebrafish	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSITHLISCHAHVVSQTA
rhesus	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSITHLISCHAHVVSQTA
goat	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSVHLISCHAHVVSQTA
fruit_fly_(B)	LGTFFNFEEQNMMLDFVORCASYFIVHEQQEIRLEAVQTCTRLIKLAVQSE--SNENSKTL
fruit_fly_(A)	LGTFFNFEEQNMMLDFVORCASYFIVHEQQEIRLEAVQTCTRLIKLAVQSE--SNENSKTL
chimpanzee	LGSFEFECHSLTQFVRHCADHFLNSENKEIRMEAAARTCSRLLTPSITHLISCHAHVVSQTA **:*:** : : :**:*:**:* : * :***:*:**:*:** , : : : , * :
human	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR
rat	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR
mouse	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR
sheep	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR
zebrafish	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR
rhesus	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR
goat	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR
fruit_fly_(B)	SDTVSHVIERLLMVAITDMDCNVIRIRILASLDETFDGKLAQESLNSLPITLHDEIPEIR
fruit_fly_(A)	SDTVSHVIERLLMVAITDMDCNVIRIRILASLDETFDGKLAQESLNSLPITLHDEIPEIR
chimpanzee	VQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQENLQALFVALNDQVFETR ,*:*:**:*:**,*:** * : : * : * : * : * : * : * : * : * : * : * : * : *

279

49/57

FIG. 23- continued

human	YPAVSMVALMRIFRDQSLSHHNTMVVQAITFIFKSLGLKCVQFLPQVMPTFLNVIKVCBG
rat	YPAVSMVALMRIFRDQSLSHHNTMVVQAITFIFKSLGLKCVQFLPQVMPTFLNVIKVCBG
mouse	YPAVSMVALMRIFRDQSLSHHNTMVVQAITFIFKSLGLKCVQFLPQVMPTFLNVIKVCBG
sheep	YPAVSMVALMRIFRDQSLSHHNTMVVQAITFIFRSLGLKCVQFLPQVMPTFLNVIKVCBG
zebrafish	YPAVAIVTLMRIIRDPSLSHNTMVVQAVTFIFKSLGLKCVQFLPQVMPTFLNVIKVCDA
rhesus	YPAVSMVALMRIFRDQSLSHHNTMVVQAITFIFKSLGLKCVQFLPQVMPTFLNVIKVCBG
goat	YPAVSMVALMRIFRDQSLSHHNTMVVQAITFIFKSLGLKCVQFLPQVMPTFLNVIKVCBG
fruit_fly_(B)	YPAVAIAALMRIIRDPSLSTNTSTSVVQAVTFIFQSLGIKCVFYLAQVLPNLLDNVSTADN
fruit_fly_(A)	YPAVAIAALMRIIRDPSLSTNTSTSVVQAVTFIFQSLGIKCVFYLAQVLPNLLDNVSTADN
chimpanzee	YPAVSMVALMRIFRDQSLSHHNTMVVQAITFIFKSLGLKCVQFLPQVMPTFLNVIKVCBG ****;.;:****;* * ;** ***:****;****;*** * **;.;*:; *;.;* *
human	AIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFVWMNTSIQSTIILLIEQIVVALGGE
rat	AIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFVWMNTSIQSTIILLIEQIVVALGGE
mouse	AIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFVWMNTSIQSTIILLIEQIVVALGGE
sheep	AIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFVWMNTSIQSTIILLIEQIVVALGGE
zebrafish	SIREFLFQQLGMLVSFVKSHIRPYMDDIPTLIREYWTNNPMMQNTIILLIEQIVVALGGE
rhesus	AIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFVWMNTSIQSTIILLIEQIVVALGGE
goat	AIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFVWMNTSIQSTIILLIEQIVVALGGE
fruit_fly_(B)	NLREFLFQQLAILVAFVKLHIIISYMGDIFELIKEFWTINTFLQNTLINLIEQIIVALGGE
fruit_fly_(A)	NLREFLFQQLAILVAFVKLHIIISYMGDIFELIKEFWTINTFLQNTLINLIEQIIVALGGE
chimpanzee	AIREFLFQQLGMLVSFVKSHIRPYMDEIVTLMREFVWMNTSIQSTIILLIEQIVVALGGE :*****;.;:*.*** ** ** *;.;*:;*. *;.;* **;.;* *****;**** *
human	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVLFDAPE
rat	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVLFDAPE
mouse	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVLFDAPE
sheep	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVLFDAPE
zebrafish	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLMAIQLFGANLDDYLHLLLPPIVLFDAPE
rhesus	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVLFDAPE
goat	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVLFDAPE
fruit_fly_(B)	FRDYLAEIIPQILAVLQHNSKDRMVTALLQALQKFCSTLGYYLPLILPPIVLFDSFY
fruit_fly_(A)	FRDYLAEIIPQILAVLQHNSKDRMVTALLQALQKFCSTLGYYLPLILPPIVLFDSFY
chimpanzee	EKLYLPQLIPHMLRVFMHNSQGRIVSIKLLAAIQLFGANLDDYLHLLLPPIVLFDAPE *: ** ;***;***; ** * * *; ;** *;* **;.* ** *;***;****;*
human	APLPSRKALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK
rat	VPLPSRKALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK
mouse	VPLPSRKALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK
sheep	APLPSRKALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK
zebrafish	VPLCARVALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK
rhesus	APLPSRKALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK
goat	APLPSRKALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK
fruit_fly_(B)	VPOQVSMVALETINNLAQQLDFTDFSSRIINHPLVRLDAEPRLDQAMTTLRLAKQLGK
fruit_fly_(A)	VPOQVSMVALETINNLAQQLDFTDFSSRIINHPLVRLDAEPRLDQAMTTLRLAKQLGK
chimpanzee	APLPSRKALETVDRLTESLDFTDYASRIINHPIVRTLQSPPELRSTAMDTLSSLVFQLGK .* .*****;.;: .*****;*****;****;**** * ** *; * * *; ****

50/57

FIG. 23- continued

human	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
rat	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
mouse	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
sheep	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
zebrafish	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
rhesus	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
goat	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
fruit_fly_(B)	KYLVFVPMVQRTLNKHRIVDPFEYEELLSKIKSCSTLADSYGAGESELPSPRFKN-----
fruit_fly_(A)	KYLVFVPMVQRTLNKHRIVDPFEYEELLSKIKSCSTLADSYGAGESELPSPRFKN-----
chimpanzee	KYQIFIPMVNKKVLVKKRINNGRYDVLICRIVNGYTLADE-----EEDPLIYQHRMLRSG
	** :*:***: : : :*** : :*: * : :* : :*** : : * * : :..
human	QCDALASGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
rat	QCDALASGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
mouse	QCDALASGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
sheep	QCDALASGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
zebrafish	QSDTLVSGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
rhesus	QCDALASGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
goat	QCDALASGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
fruit_fly_(B)	--EPPVY--DRNSNNKHLQVTTWEELRTANQVTRVSKDDWVWLRLSIGLLKESPSHAL
fruit_fly_(A)	--EPPVY--DRNSNNKHLQVTTWEELRTANQVTRVSKDDWVWLRLSIGLLKESPSHAL
chimpanzee	QCDALASGPFVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRLSLELLKDGSSPSL
	: : : : : :*:*: * : :* : :*:*****:***:***: ***: * * : *
human	RSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
rat	RSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
mouse	RSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
sheep	RSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
zebrafish	RSCWBLAQTYIPLARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
rhesus	RSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
goat	RSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
fruit_fly_(B)	RACRSLAQEYDTLLRDLFNAAFVSCWTELSPDLNELTQSLIQALQVTDMPETDQTLNL
fruit_fly_(A)	RACRSLAQEYDTLLRDLFNAAFVSCWTELSPDLNELTQSLIQALQVTDMPETDQTLNL
chimpanzee	RSCWALAQAYNPMARDLFNAAFVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLNL
	*: * :*** * : :*****:***:***: * : :*: * : : * : * :*:***:***
human	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
rat	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
mouse	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
sheep	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
zebrafish	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
rhesus	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
goat	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
fruit_fly_(B)	AETMEHCDKDDPTFIET----KLLGTRAMACRAYAKALHYKELEFQKGPPTAILESILISIN
fruit_fly_(A)	AETMEHCDKDDPTFIET----KLLGTRAMACRAYAKALHYKELEFQKGPPTAILESILISIN
chimpanzee	AETMEHSDKGPLELRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPPTAILESILISIN
	*****: * : * : : : * * * * * :*****:***: * : :**** *

52/57

FIG. 23- continued

human	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
rat	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
mouse	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
sheep	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
zebrafish	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
rhesus	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
goat	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
fruit_fly_(B)	RKSGSLHLSHNTLVMLLGTDPKLNPNQPLPCNQBPQVTTYAYTKYMAANNQLQEAYEQLTHF
fruit_fly_(A)	RKSGSLHLSHNTLVMLLGTDPKLNPNQPLPCNQBPQVTTYAYTKYMAANNQLQEAYEQLTHF
chimpanzee	GKSGRLALAHKTLVLLLGVDPSRQLDNHFLPTVHPQVTTYAYMKNMWKSARKIDAFQHMCHF
	*** * *,*****,**,** , : ,*** ,*,***** * * , : ,*::: **
human	VQTMQQQAQHAIAIATEDQQHQQLLHLMARCFLELGEWQLNLQG- INESTIPFVLQYYSAA
rat	VQTMQQQAQHAIAIATEDQQHQQLLHLMARCFLELGEWQLNLQG- INESTIPFVLQYYSAA
mouse	VQTMQQQAQHAIAIATEDQQHQQLLHLMARCFLELGEWQLNLQG- INESTIPFVLQYYSAA
sheep	VQTMQQQAQHAIAIATEDQQHQQLLHLMARCFLELGEWQLNLQG- INESTIPFVLQYYSAA
zebrafish	VQGMQQQAQHAIAAEDQQHQLLHLMARCFLELGEWQLSLQG- INESTIPFVLQYYSAA
rhesus	VQTMQQQAQHAIAIATEDQQHQQLLHLMARCFLELGEWQLNLQG- INESTIPFVLQYYSAA
goat	VQTMQQQAQHAIAIATEDQQHQQLLHLMARCFLELGEWQLNLQG- INESTIPFVLQYYSAA
fruit_fly_(B)	VSTYSQELSLCL---PPEALNQDQRLMARCYLRMATWQNRQLQDSIRPDALQGALECFEKA
fruit_fly_(A)	VSTYSQELSLCL---PPEALNQDQRLMARCYLRMATWQNRQLQDSIRPDALQGALECFEKA
chimpanzee	VQTMQQQAQHAIAIATEDQQHQQLLHLMARCFLELGEWQLNLQG- INESTIPFVLQYYSAA
	, ., , : * : ,*****,*::, ** ,** *, ,*, * , : , :
human	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATAT
rat	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAASA-
mouse	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAASA-
sheep	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAATAT
zebrafish	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAATAT
rhesus	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAATAT
goat	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAATAT
fruit_fly_(B)	TSYDENWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAATAT
fruit_fly_(A)	TSYDENWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAATAT
chimpanzee	TEHDRSWYKAWHAWVMNFPAVLHYKHQNGARDENKELRHASGANITNAATTAATTAATAT
	*, : * ,***** ** ,** : , * : : ** ,
human	T-TASTESENSESEAEESTENSPTPEPLQKKVTEDELSKTLMLYTVPAVQGFERSISLSRGN
rat	AAATSETESENSESEAESENESPTPEPLQKKVTEDELSKTLMLYTVPAVQGFERSISLSRGN
mouse	AAATSETESENSESEAESENESPTPEPLQKKVTEDELSKTLMLYTVPAVQGFERSISLSRGN
sheep	ATATSETESENSESEAEESTENSPTPEPLQKKVTEDELSKTLMLYTVPAVQGFERSISLSRGN
zebrafish	----NSEASNSDSEADSTERSPVPSPGQKKVNEDELSKTLMLYTVPAVQGFERSISLSRGN
rhesus	T-TASTESENSESEAEESTENSPTPEPLQKKVTEDELSKTLMLYTVPAVQGFERSISLSRGN
goat	AT-ASTESENSESEAEESTENSPTPEPLQKKVTEDELSKTLMLYTVPAVQGFERSISLSRGN
fruit_fly_(B)	----MTNGS-----GLDS-DLMI IQRYAVPAVQGFERSISLIGN
fruit_fly_(A)	----MTNGS-----GLDS-DLMI IQRYAVPAVQGFERSISLIGN
chimpanzee	T-TASTESENSESEAEESTENSPTPEPLQKKVTEDELSKTLMLYTVPAVQGFERSISLSRGN
	: , * : , : * ,***** ,**

53/57

FIG. 23- continued

human	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
rat	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
mouse	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
sheep	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
zebrafish	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
rhesus	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
goat	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
fruit_fly_(B)	SLQDTLRLLTLWFDYGNHAEVYEALLSGMKLIEINTWLOVI PQLIARIIDTHROLVGOLIH
fruit_fly_(A)	SLQDTLRLLTLWFDYGNHAEVYEALLSGMKLIEINTWLOVI PQLIARIIDTHROLVGOLIH
chimpanzee	NLQDTLRVLTTLWFDYGHWFEDVNEALVEGVKAIQIDTWLQVI PQLIARIIDTPRPLVGRLIH
	*****:*****. :* ***:.* * *:***** * ***:***
human	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
rat	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
mouse	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
sheep	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
zebrafish	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
rhesus	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
goat	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
fruit_fly_(B)	QLLMDIGKNNHPQALVYPLTVASKSASLARRNAAPFKILDSMKKHEPTLVEQAVMCSEELIR
fruit_fly_(A)	QLLMDIGKNNHPQALVYPLTVASKSASLARRNAAPFKILDSMKKHEPTLVEQAVMCSEELIR
chimpanzee	QLLTDIGRYHPQALIYPLTVASKESTTTARHNAANKILKNMCEHSGNTLVQQAMMVSEELIR
	*** ***: *****:*****.: **:*** ***. * :*. ****:*** *****
human	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
rat	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
mouse	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
sheep	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
zebrafish	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
rhesus	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
goat	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
fruit_fly_(B)	VAILWHEQWHEGLEEASRLYFGDRNVKGMEFVLEPLHARMLERCGPQTLKETSFSQAYGREL
fruit_fly_(A)	VAILWHEQWHEGLEEASRLYFGDRNVKGMEFVLEPLHARMLERCGPQTLKETSFSQAYGREL
chimpanzee	VAILWHEMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPGQTLKETSFNQAYGRDL
	***** *****:*****. (*****:*****. *****: *
human	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
rat	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
mouse	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
sheep	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
zebrafish	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
rhesus	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
goat	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
fruit_fly_(B)	TEAYEWSQRYKTSAVVMDLDRWDIXYHVFXISRLPQLTSLELQYVSEKLLMCRDLEL
fruit_fly_(A)	TEAYEWSQRYKTSAVVMDLDRWDIXYHVFXISRLPQLTSLELQYVSEKLLMCRDLEL
chimpanzee	MEAQEWCRKYMKSGNVKDLTQAWDLVYHVFRRI SKQLPQLTSLELQYVSEKLLMCRDLEL
	** :*. :* * . * ** :***:*****: **;***** *****: *;****

54/57

FIG. 23- continued

human	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
rat	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
mouse	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
sheep	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
zebrafish	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
rhesus	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
goat	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
fruit_fly_(B)	AVPGSYNPGQELIRISIIKTNLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
fruit_fly_(A)	AVPGSYNPGQELIRISIIKTNLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
chimpanzee	AVPGTYDPNQPIIRIQSIAPSLQVITSKQRPFRKLFLMGSGNGHEFVFLKKGHEDLRODERV
	****.* * * ****. * .***** : ****.***:*****
human	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
rat	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
mouse	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
sheep	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
zebrafish	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
rhesus	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
goat	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
fruit_fly_(B)	NQLFGLVNTLLDDPDTFRNLAIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
fruit_fly_(A)	NQLFGLVNTLLDDPDTFRNLAIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
chimpanzee	NQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKK
	.** .** .*:*.**:*****:*****:*****.***
human	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
rat	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
mouse	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
sheep	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
zebrafish	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
rhesus	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
goat	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
fruit_fly_(B)	VPLNQEHRTMLNFAPDYDELTLMQKVEVFEHALGOTQGGDLAKLLWLKSPSEELWFERRN
fruit_fly_(A)	VPLNQEHRTMLNFAPDYDELTLMQKVEVFEHALGOTQGGDLAKLLWLKSPSEELWFERRN
chimpanzee	ILLNIEHRIMLRMAPDYDELTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRT
	: ** ** * *.*****:*****: .* *****:***.***
human	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
rat	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
mouse	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
sheep	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
zebrafish	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
rhesus	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
goat	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
fruit_fly_(B)	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
fruit_fly_(A)	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
chimpanzee	NYTRSLAVMSMVGYYILGLGDRHPSNLMMLDRLSGKILHIDFGDCFEVAMTREKFFPKIPFR
	*****:*****:*****

55/57

FIG. 23- continued

human	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
rat	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
mouse	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
sheep	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
zebrafish	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
rhesus	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
goat	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
fruit_fly_(B)	LTRMLIKAMEVTGIECTYRTCSVMIVLRERHDSLMVLEAFVYDPLLNWRRLMDTNTKG
fruit_fly_(A)	LTRMLIKAMEVTGIECTYRTCSVMIVLRERHDSLMVLEAFVYDPLLNWRRLMDTNTKG
chimpanzee	LTRMLTNAMSVTGLDGNVIRITCHTVMEVLRERHDSVMVLEAFVYDPLLNWRRLMDTNTKG
	***** :*****;:*,* **,:** ****,:**:******:*,* : **
human	NKRSRTRTDSYSAGQS-V--EILDGVELGEPANHTTGTTPESIHSGFICDGLVKPEALNK
rat	NKRSRTRTDSYSAGQS-V--EILDGVELGEPANHTTGTTPESIHSGFICDGLVKPEALNK
mouse	NKRSRTRTDSYSAGQS-V--EILDGVELGEPANHTTGTTPESIHSGFICDGLVKPEALNK
sheep	NKRSRTRTDSYSAGQS-V--EILDGVELGEPANHTTGTTPESIHSGFICDGLVKPEALNK
zebrafish	NKRSRTRTDSYSAGQS-V--EAMEGIDLGETTHKKPGTTPESIHSGFICDGLVQSEALNK
rhesus	NKRSRTRTDSYSAGQS-V--EILDGVELGEPANHTTGTTPESIHSGFICDGLVKPEALNK
goat	NKRSRTRTDSYSAGQS-V--EILDGVELGEPANHTTGTTPESIHSGFICDGLVKPEALNK
fruit_fly_(B)	NDAVAGAGAPGCGGSGMQDLSNSVEDSLPMAS-KPYDPT--LQQGGLHNNVADETNS
fruit_fly_(A)	NDAVAGAGAPGCGGSGMQDLSNSVEDSLPMAS-KPYDPT--LQQGGLHNNVADETNS
chimpanzee	NKRSRTRTDSYSAGQS-V--EILDGVELGEPANHTTGTTPESIHSGFICDGLVKPEALNK
	*_ * * : _ . : : _ *_ * _ * : *
human	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
rat	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
mouse	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
sheep	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
zebrafish	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
rhesus	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
goat	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
fruit_fly_(B)	KASQVIKRVKCKLTGTDFTQTEKSVNEQSQVELLIQATNNENLCQCYIGWCPPW
fruit_fly_(A)	KASQVIKRVKCKLTGTDFTQTEKSVNEQSQVELLIQATNNENLCQCYIGWCPPW
chimpanzee	KAIQIINRVRDKLTGRDFSHDDTLDVPTQVELLIKQATSHENLCQCYIGWCPPW
	** *,*:*,* : **** ** : : : : :*****:***_******:*****

FIGURE 24

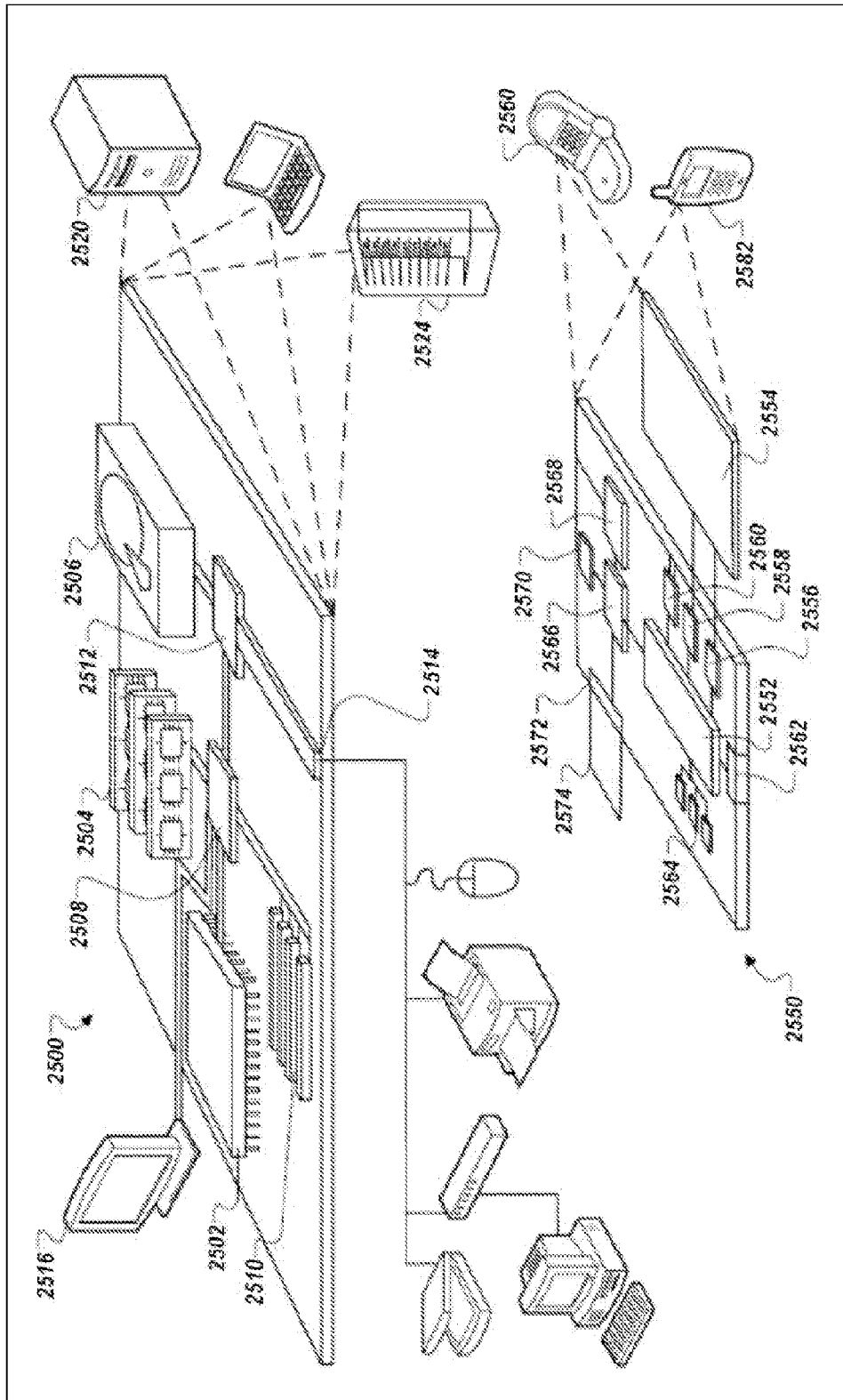
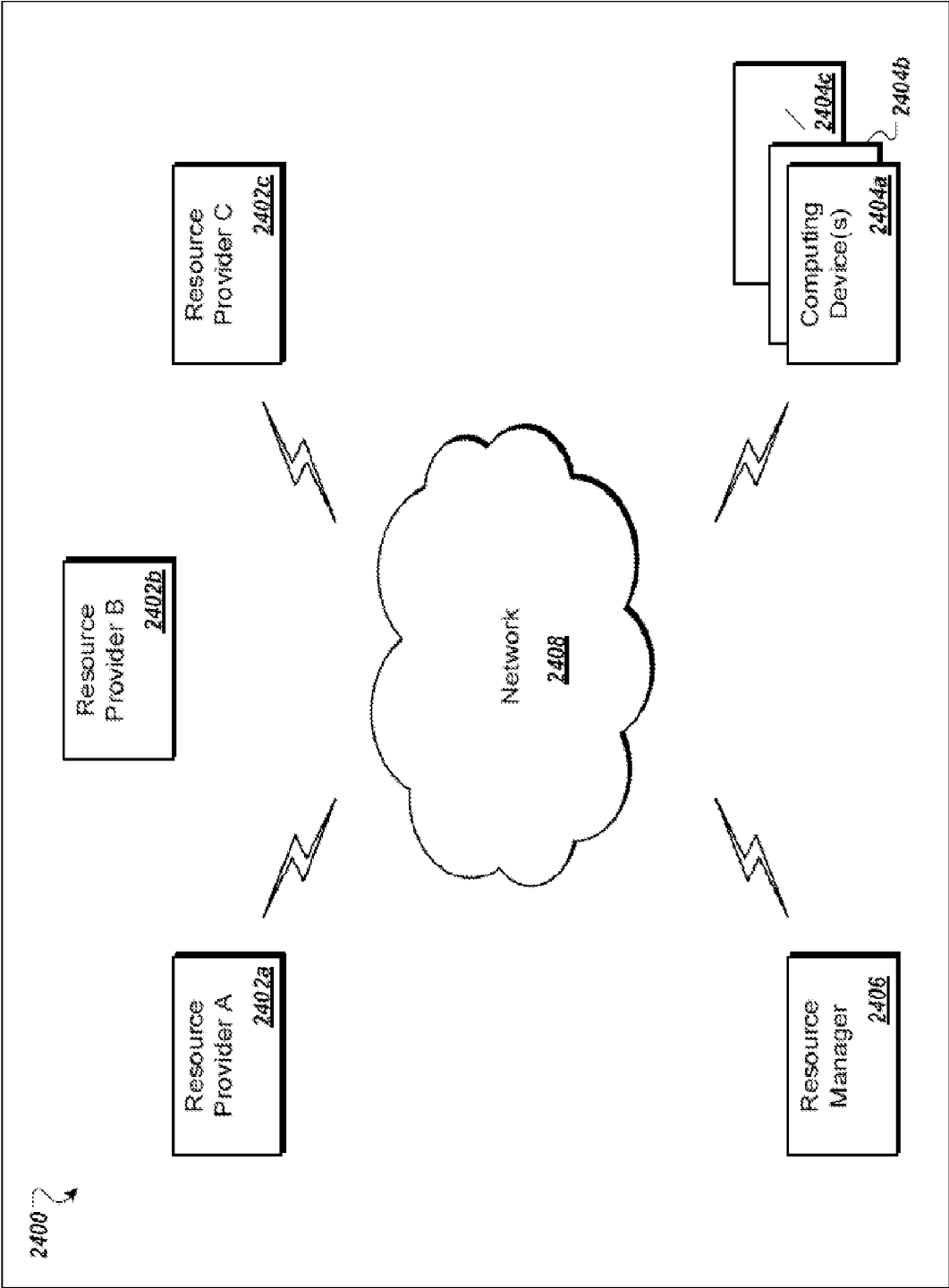


FIGURE 25



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/035035

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☐

in the international application as filed

☐

together with the international application in electronic form

☒

subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/035035

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 9-11, 16
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 9-11 and 16 reference Tables 1, 2, 3, 4, 5, or 6 that were not provided in the instant application and are being held as unsearchable.

3. ☒ Claims Nos.: 5, 6
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/035035

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C40B 30/02 (2014.01)

CPC - C40B 30/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C40B 30/02, 30/04, 30/10, 60/02 (2014.01)

USPC - 506/8, 9, 10, 12, 24, 34

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C40B 30/02, 30/04, 30/10, 60/02 (2014.06)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	TANNEERU et al. "Ligand-based 3-D pharmacophore generation and molecular docking of mTOR kinase inhibitors," J. Mol. Model, Vol. 18, Pgs. 1611-1624. 30 July 2011 (30.07.2011) entire document	1-4, 7, 15, 17-19 ----- 8
X -- Y	TOBAK, ANNE. "Construction of the 3D Structure of the mTOR Kinase-Domain and Discovery of Novel mTOR Inhibitors," PhD Dissertation. Pgs. 1-107. 2007. Article retrieved from the internet. URL: <https://rucore.libraries.rutgers.edu/rutgers-lib/21340/>	12, 13 ----- 14
Y	LIU et al. "Kinome-wide Selectivity Profiling of ATP-competitive Mammalian Target of Rapamycin (mTOR) Inhibitors and Characterization of Their Binding Kinetics," The Journal of Biological Chemistry, Vol. 287, No. 13, Pgs. 9742-9752. 23 March 2012 (23.03.2012) entire document	8
Y	US 2010/0131666 A1 (FEINBERG et al) 27 May 2010 (27.05.2010) entire document	14
P, X	YANG et al. "mTOR kinase structure, mechanism and regulation," Nature, 09 May 2013 (09.05.2013), Vol. 497, Pgs. 217-224 and Supplemental Material, Pgs. 1-25. entire document	1-4, 7, 8, 12-15, 17-19

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 September 2014

Date of mailing of the international search report

02 OCT 2014

Name and mailing address of the ISA/US

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