Bypassing AMPK Phosphorylation

Benoit Viollet, Marc Foretz, Uwe Schlattner

▶ **To cite this version:**

Benoit Viollet, Marc Foretz, Uwe Schlattner. Bypassing AMPK Phosphorylation. Chemistry and Biology, 2014, 21 (5), pp.567-569. 10.1016/j.chembiol.2014.05.003 . inserm-01171798

HAL Id: inserm-01171798

https://inserm.hal.science/inserm-01171798

Submitted on 6 Jul 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

HAL
archives-ouvertes.fr

Bypassing AMPK Phosphorylation

Benoit Viollet, Marc Foretz, Uwe Schlattner

▶ **To cite this version:**

Benoit Viollet, Marc Foretz, Uwe Schlattner. Bypassing AMPK Phosphorylation. Chemistry and Biology, 2014, 21 (5), pp.567-569. <10.1016/j.chembiol.2014.05.003>. <inserm-01171798>

HAL Id: inserm-01171798

http://www.hal.inserm.fr/inserm-01171798

Submitted on 6 Jul 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Bypassing AMPK phosphorylation

Benoit Viollet[1,2,3,*], Marc Foretz[1,2,3]and Uwe Schlattner[4,5]

[1]INSERM, U1016, Institut Cochin, Paris, France

[2]CNRS, UMR8104, Paris, France

[3]Université Paris Descartes, Sorbonne Paris Cité, France

[4]Univ. Grenoble Alpes, Laboratory of Fundamental and Applied Bioenergetics, Grenoble, France
[5]Inserm, U1055, Grenoble, France

*Correspondence: benoit.viollet@inserm.fr
Phone: + 33 1 44 41 24 01; Fax: +33 1 44 41 24 21

Summary
AMP-activated protein kinase (AMPK) functions as a signaling hub to balance energy supply with demand. Phosphorylation of activation loop Thr172 has been considered as an essential step in AMPK activation. In this issue of *Chemistry & Biology*, Scott and colleagues show that the small molecule direct AMPK activator, A-769662, bypasses this phosphorylation event, and acts synergistically with AMP on naive AMPK.

Main text
Maintaining cellular energy balance is a key process for cell function and survival. Over the recent years, the AMP-activated protein kinase (AMPK) has emerged as a major regulator of cellular energy homeostasis. This serine/threonine kinase,evolutionary conserved in all eukaryotes, integrates multiple extra- and intracellular input signals directly or indirectly linked to limited energy availability to coordinate a wide array of compensatory, protective and energy-sparing responses at the cellular, organ and organism levels. AMPK isubiquitously expressed and exists as aheterotrimericcomplexcomprising a catalytic α-subunit, a scaffolding β-subunit and a regulatory γ-subunit.

The AMPK activation mechanism was so far considered to involve two steps: an obligatory covalent phosphorylation of the α-subunit Thr172 in the so-called activation loop of the kinase domain, and a stimulatory allosteric binding of AMPto cystathionine-beta-synthase (CBS) motifsin the γ-subunit. Covalent activation is complex, since it involves stimulated phosphorylation by several upstream kinases (LKB1, CaMKKβ) and inhibited dephosphorylation by phosphatases, both favored by β-subunit myristoylation and binding of AMP (and to a lesser extend ADP) to the different allosteric activation siteswithin the γ-subunit(Oakhill, et al., 2011; Xiao, et al., 2011).There seems to exist a delicate phosphorylation/dephosphorylation equilibrium, where the dephosphorylation

step is of major regulatory importance. However, not much is known about the nature of the phosphatases as itmay depend on both cell type and the environmental conditions.Allosteric activation of AMPK is even more intricate, since it is orchestrated by various structural elements in different AMPK subunits (Figure 1). Crystal structures of AMPK bound to either AMP or ATP revealed that only three of the four potential nucleotide-binding sitesin the γ-subunit contribute to nucleotide regulation(Chen, et al., 2012; Xiao, et al., 2013; Xiao, et al., 2011). Since the activating ligand is bound on the γ-subunit and the kinase domain is in the α-subunit, intersubunit communication has to occur when switching to fully active states (Figure 1). Valuable insight has been provided by core structures of AMPK heterotrimersshowing AMP induced conformational changes in the AMPK holocomplex and even the γ-subunit (Figure 1) (Chen, et al., 2012). Important regulatory features for this conformational switchareprovided by α-subunit flexible components, the autoinhibitory domain (AID) and the α-RIM/α-hook interacting with the exchangeable nucleotide-binding sites on the γ-subunit, offering a signaling mechanism for nucleotide allosteric regulation(Chen, et al., 2012; Chen, et al., 2013; Xiao, et al., 2013; Xiao, et al., 2011).Given these complex regulatory mechanisms, it is not surprising that despite intense research the AMPK "activation switch" is still not entirely understood.

Because of its critical role in maintaining cellular energy homeostasis, AMPK has attracted broad interest as a prime target for pharmacological intervention in several energy-related pathologies.Thishas led to increased efforts to develop direct AMPK-activating drugs. In this issue of *Chemistry and Biology*, (Scott, et al., 2014) refine the mechanism of action of the first described small molecule direct AMPK activator, the thienopyridone drug A-769662,identified by Abbott laboratories (Cool, et al., 2006). Previous studies have reported that A-769662activates AMPK both allosterically and by inhibiting the dephosphorylation of AMPK on Thr172(Cool, et al., 2006; Sanders, et al., 2007). However, in contrast to adenine nucleotides, A-769662 does not bind the γ-subunit but requires the β1 regulatory subunit and its autophosphorylation at Ser108 within the carbohydrate bindingmodule (CBM) (Figure 1)(Sanders, et al., 2007; Scott, et al., 2008).

Previous studies reported one intriguing observation: A-769662 can trigger robust phosphorylation of the AMPK downstream target acetyl-CoA carboxylase in hepatocytes in absence of α-subunit Thr172 phosphorylation (Foretz, et al., 2010; Goransson, et al., 2007), raising some doubt about the requirement of AMPK activation loop phosphorylation for A-769662 action. (Scott, et al., 2014)nowusecatalytically inactive,bacterially expressed AMPK α1β1γ1, which is not phosphorylated on Thr172, also showing a 65-fold activation with A-769662.However, other studies demonstrated that purified liver AMPK treated with phosphatase was refractory to A-769662 allosteric activation, suggesting that phosphorylation at another site is required for drug activation. Scott and colleagues now reveal that bacterially expressed AMPK α1β1γ1 is strongly phosphorylated at β-subunit Ser108, a phosphosite already linked to A-

769662activation (Sanders, et al., 2007). Indeed, $AMPK_{T172A/S108E}$ and $AMPK_{T172A/S108D}$ mutants lacking phosphorylation at Thr172, but mimicking the one at S108, and treated in addition with λ phosphatase, retained full activation by A-769662, demonstrating that phosphorylation on β-subunit Ser108 alone is sufficient to render AMPK sensitive to A-769662 allosteric activation(Figure 1, bottom).The authors also identify Ser108 phosphorylation as a cis-autophosphorylationevent, without excluding the existence of a specific upstream kinase, but nevertheless raising questionsabout A-769662 effects *in vivo*. Indeed, if β-subunit Ser108 is only autophosphorylated, it is entirely dependent on AMPK activity. This would explain why A-769662 activation is completely blunted in cells lacking LKB1 (Foretz, et al., 2010; Goransson, et al., 2007).

Foretz*et al.*(Foretz, et al., 2010) had previously found that a combined treatment with the two AMPK activators A-769662 and the AMP analog 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR), which act on different binding sites (β-CMB and γ-CBS, respectively), exhibits a synergistic effect on AMPK phosphorylation and its downstream targets. Scott and colleagues next looked to extend their findings and tested whether combiningA-769662 and AMP has synergistic effects on activation of AMPK complexes containing $AMPK_{T172A/S108E}$, $AMPK_{T172A/S108D}$ and $AMPK_{T172A/S108A}$ mutants. Here they employed recombinant AMPK complexes expressed in mammalian cells containing all post-translational modifications (e.g., myristoylation of β-subunit) required to fully activateAMPK. Interestingly, A-769662-activation of $AMPK_{T172A/S108E}$ and $AMPK_{T172A/S108D}$ was further increased two to four-fold when combined with AMP. A surprising finding was the dramatic activation of $AMPK_{T172A/S108A}$mutant following incubation with both A-769662 and AMP. This result is unexpected and novel. The authors demonstrate for the first time that AMPK can be activated in the absence ofphosphorylation at both β-subunit Ser108 and α-subunit Thr172.This synergistic AMPK activation was not mediated by autophosphorylation and occured exclusively via an allosteric mechanism. Another important finding is that the combined action of A769662 and AMP is entirely dependent on β-CBM as $AMPK_{T172A}$ mutants lacking this regulatory domain are insensitive to synergistic allosteric activation. One plausible explanation is that A-769662 binding is critical to induce a conformational change in the AMPK holocomplexleading to the rearrangement and stabilization of the unphosphorylated activation loop to enhance AMPK activation upon AMP binding (Figure 1). This is consistent withthe recent structural studies showing that A-769662 binding facilitates movement in the αC-helix in the kinase domain to interact with the C-interacting helix in the CBM, thus making an important contribution in the allosteric activation of AMPK (Xiao, et al., 2013). Strikingly, ADP, which does not cause AMPK Thr172 phosphorylation (Oakhill, et al., 2011; Xiao, et al., 2011), was unable to activate $AMPK_{T172A/S108A}$ mutant when combined with A-769662.To dig deeper in the mechanism of AMPK conformational changes, Scott and colleagues examined the role of individual CBS domains within the γ-subunit. When γ-subunit residues Asp90 (site 1), Asp245 (site

3) and Asp317 (site 4) were individually exchanged with Ala, synergistic activation induced by the combined action of A-769662 and AMP is severely blunted.

Collectively, Scott and colleagues report how AMPK can be activated via a purely allosteric mechanism, bypassing the requirement of α-subunit Thr172 and β-subunit Ser108 phosphorylation.These findings are highly relevant for the development of specific directly-acting AMPK agonists andthe future direction of AMPK-based therapy in disease-focused research. This study is also critical to our understanding of how to target AMPK pharmacologically, depending on the cellular context (e.g., genetic loss of upstream kinase, expression of β1- and β2-subunits). There have been two recent reports evaluating the functional effects of A-769662 in combination with AICARor indirectAMPK activators (metformin, phenformin, oligomycin and hypoxia) acting via the elevation of cellular AMP levels.Consistent with enhanced AMPK activation by co-treatment, inhibition of hepatic lipogenesis(Ducommun, et al., 2014)and activation of cardiac glucose transport(Timmermans, et al., 2014)were greatly improved compared with A-769662 alone. These results reinforce the view that combinatorial treatments would be of value to enhance AMPK activation. In addition, such treatments could help to reduce the amount of drugs administrated to patients and better balance tolerability and efficacy. Future research will have to elucidate the beneficial effects of metformin in combination with various AMPK activators targeting the A-769662 binding site for patients suffering of type 2 diabetes, insulin resistance, cardiovascular diseases and also cancer.

REFERENCES

Chen, L., Wang, J., Zhang, Y.Y., Yan, S.F., Neumann, D., Schlattner, U., Wang, Z.X., and Wu, J.W. (2012). Nat Struct Mol Biol 19, 716-718.

Chen, L., Xin, F.J., Wang, J., Hu, J., Zhang, Y.Y., Wan, S., Cao, L.S., Lu, C., Li, P., Yan, S.F., et al. (2013). Nature 498, E8-10.

Cool, B., Zinker, B., Chiou, W., Kifle, L., Cao, N., Perham, M., Dickinson, R., Adler, A., Gagne, G., Iyengar, R., et al. (2006). Cell Metab 3, 403-416.

Ducommun, S., Ford, R.J., Bultot, L., Deak, M., Bertrand, L., Kemp, B.E., Steinberg, G.R., and Sakamoto, K. (2014). Am J Physiol Endocrinol Metab 306, E688-696.

Foretz, M., Hebrard, S., Leclerc, J., Zarrinpashneh, E., Soty, M., Mithieux, G., Sakamoto, K., Andreelli, F., and Viollet, B. (2010). The Journal of clinical investigation 120, 2355-2369.

Goransson, O., McBride, A., Hawley, S.A., Ross, F.A., Shpiro, N., Foretz, M., Viollet, B., Hardie, D.G., and Sakamoto, K. (2007).

Oakhill, J.S., Steel, R., Chen, Z.P., Scott, J.W., Ling, N., Tam, S., and Kemp, B.E. (2011). Science 332, 1433-1435.

Sanders, M.J., Ali, Z.S., Hegarty, B.D., Heath, R., Snowden, M.A., and Carling, D. (2007). The Journal of biological chemistry 282, 32539-32548.

Scott, J.W., Ling, N., Issa, S.M., Dite, T.A., O'Brien, M.T., Chen, Z.P., Galic, S., Langendorf, C.G., Steinberg, G.R., Kemp, B.E., et al. (2014). Chem Biol, this issue.

Scott, J.W., van Denderen, B.J., Jorgensen, S.B., Honeyman, J.E., Steinberg, G.R., Oakhill, J.S., Iseli, T.J., Koay, A., Gooley, P.R., Stapleton, D., et al. (2008). Chem Biol 15, 1220-1230.

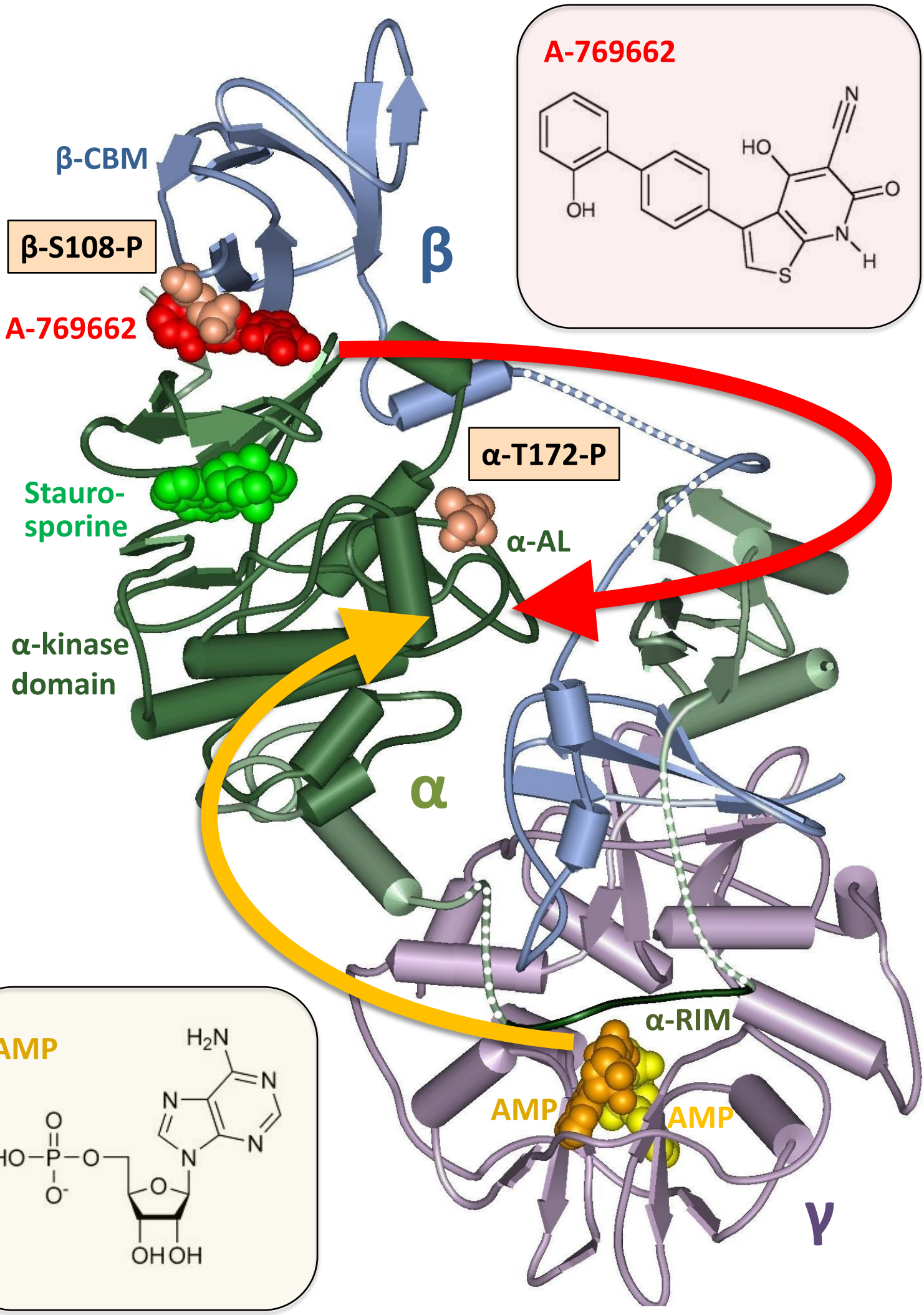
Timmermans, A.D., Balteau, M., Gelinas, R., Renguet, E., Ginion, A., de Meester, C., Sakamoto, K., Balligand, J.L., Bontemps, F., Vanoverschelde, J.L., et al. (2014). Am J Physiol Heart Circ Physiol, in press.

Xiao, B., Sanders, M.J., Carmena, D., Bright, N.J., Haire, L.F., Underwood, E., Patel, B.R., Heath, R.B., Walker, P.A., Hallen, S., et al. (2013). Nat Commun 4, 3017.

Xiao, B., Sanders, M.J., Underwood, E., Heath, R., Mayer, F.V., Carmena, D., Jing, C., Walker, P.A., Eccleston, J.F., Haire, L.F., et al. (2011). Nature 472, 230-233.

FIGURE LEGEND

Figure 1: AMPK activation. Binding of the activating AMPK ligands AMP (γ-subunit) and A-769662 (β-subunit) has to be transduced to the α-subunit kinase domain for activation, involving conformational changes (see arrows). **Top**: AMPK subunits α (green), β (blue) and γ (magenta) (PDB 1CFF; (Xiao, et al., 2013)) with α-subunit kinase domain, activation loop (AL), and regulatory interacting motif (RIM, (Chen, et al., 2013)) indicated (dark green) and β-subunit carbohydrate binding module (CBM) labeled; sequences missing in the structure (dashed) include the α-auto-inhibitory domain (AID). The figure shows (in spacefill representation) the activation-relevant phosphosites (α-T172 in the activation loop, and β-S108 in the CBM; red-brown) and ligands co-crystallized in the structure: allosteric AMPK-activators A-769662 (red) and AMP (orange/yellow), and the kinase inhibitor Staurosporine in the active site (green). **Bottom**: Table summarizing the effect of phosphorylation or mutation of α-subunit T172 and β-subunit S108 on AMPK activation by AMP, A-769662, or both(Scott, et al., 2014).



AMP

AMPK α	T172Ⓟ	T172A	T172A	T172Ⓟ	T172A
β	S108Ⓟ	S108Ⓟ	S108E/D	S108A	S108A
γ					
+ AMP	✔	✗	✗	✗	✗
+ A-769662	✔	✔	✔	✗	✗
+ AMP + A-769662	✔	✔	✔	✔	✔