

CLAIMS

1. A method for identifying a chemical compound or agent able to induce ubiquitination of a protein of interest, the method comprising:
 - (i) contacting a chemical compound or agent of interest with a eukaryotic cell comprising and/or expressing the protein of interest and manipulating said cell to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity;
 - (ii) determining the level of at least one member of an E3 ligase complex present in the cell;

wherein said chemical compound or agent is identified to induce ubiquitination of the protein of interest if the level of the at least one member of the E3 ligase complex is increased compared to the level determined in the absence of said chemical compound or agent of interest.
2. The method of claim 1, wherein in step (i) said cullin-RING ubiquitin ligase (CRL) activity is enhanced compared to the cullin-RING ubiquitin ligase (CRL) activity in a control cell that is not manipulated to comprise enhanced CRL activity.
3. The method of claim 1 or 2, wherein said cell manipulated to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity is a cell manipulated to comprise constantly neddylated cullin-RING ubiquitin ligases/CRLs and/or that is manipulated to comprise constant neddylation of cullin-RING ubiquitin ligases/CRLs.

- 4.. The method of anyone of claims 1 to 3, wherein the cell manipulated to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity and/or to comprise constantly neddylated cullin-RING ubiquitin ligases/CRLs is manipulated by inactivation of a target protein like a Cullin-associated NEDD8-dissociated protein selected from the group consisting of CAND1, CAND2 or CAND1 and CAND2, by inactivation of (a) target protein(s) being at least one member of the COP9 signalosome (CSN) and/or by inactivation of the COP9 signalosome (CSN).
5. The method of claim 4, wherein said inactivation of the COP9 signalosome (CSN) comprises the inactivation of the COPS5 subunit, the COPS8 subunit and/or of the COPS7B subunit.
6. The method of claim 4 or 5, wherein said inactivation comprises an inactivation by recombinant means and/or a hypomorphic mutation leading to a decrease of activity and/or abundance of the corresponding target protein or comprises the inactivation via a chemical compound/chemical inhibitor.
7. The method of claim 6, wherein said target protein is selected from the group consisting of CAND1, CAND2 or a protein member of the COP9 signalosome (CSN).
8. The method of any one of claims 3 to 7, wherein said inactivation comprises the step of contacting the cell to be manipulated to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity and/or to comprise constantly neddylated cullin-RING ubiquitin ligases/CRLs with an inhibitor of the COP9 signalosome (CSN).
9. The method of claim 8, wherein said inhibitor of the COP9 signalosome (CSN) is an inhibitor of the COPS5 subunit, preferably CSN5i-3.
10. The method of anyone of claims 1 to 3, wherein the cell manipulated to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity and/or to comprise constantly neddylated cullin-RING ubiquitin ligases/CRLs is

manipulated by overexpressing at least one protein able to induce neddylation of the cullin-RING ubiquitin ligases/CRLs.

11. The method of claim 10, wherein said at least one protein to be overexpressed are selected from the group consisting of (i) a cullin/cullin backbone protein/culling homolog, (ii) an adaptor protein, (iii) a ligase substrate receptor, (iv) an E1 activating enzyme and (v) an E2 conjugating enzyme.
12. The method of claim 10 or 11, whereby said at least one protein to be overexpressed is at least one member of the E3 ligase complex and whereby said
 - (i) cullin/cullin backbone protein/cullin homolog is selected from the group consisting of CUL4A, CUL4B, CUL1, CUL2, CUL3, CUL5, CUL6 and CUL7; preferably selected from human CUL4A, human CUL4B, human CUL1, human CUL2, human CUL3, human CUL5, and human CUL7, more preferably selected from the group consisting of human CUL4A and human CUL4B;
 - (ii) adaptor protein is selected from the group consisting of DBB1, SKP1, preferably DDB1;
 - (iii) ligase substrate receptor is selected from the group consisting of cereblon (CRBN), DCAF15, DCAF16, DCAF1, DCAF5, DCAF8, DET1, FBXO7, FBXO22, KDM2A, KDM2B, preferably cereblon (CRBN) or DCAF15;
 - (iv) E1 activating enzyme is NAE1, UBA3 subunit or a heterodimer of NAE1 and UBA3 subunit; and
 - (v) E2 conjugating enzyme is EBc12 or UBE2M, preferably UBE2M.
13. The method of any one of claims 10 to 12, wherein said overexpression of at least one protein able to induce neddylation is an ectopical overexpression.
14. The method of anyone of claims 3 to 13, wherein said constantly neddylated cullin-RING ubiquitin ligases/CRLs and/or said constant neddylation of cullin-RING ubiquitin ligases/CRLs leads to destabilization and/or auto-degradation of at least one member of said CRL.

15. The method of claim 14, wherein said constant neddylation leads to destabilization and/or auto-degradation of a substrate receptor of the CRL.
16. The method of any of claims 1 to 15, wherein the cell has been manipulated to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity prior to contacting a chemical compound or agent with the cell.
17. The method of any of claim 1 to 15, wherein the cell is manipulated to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity after contacting a chemical compound or agent with the cell.
18. The method of any one of claims 1 to 17, wherein said protein of interest to be ubiquitinated is endogenously expressed by the cell.
19. The method of any one of claims 1 to 18, wherein the protein of interest is a protein encoded by an oncogene-addiction (OA) gene or a non-oncogene addiction (NOA) gene.
20. The method of claim any one of claims 1 to 19, wherein the protein of interest is selected from the group consisting DNA-binding proteins including transcription factors, RNA binding proteins, scaffolding proteins, GTPases, solute carriers, kinases, phosphatases, bromodomain- and chromodomain containing proteins, and G-protein coupled receptors.
21. The method of any of the preceding claims, wherein cullin-RING ubiquitin ligase (CRL) or the cullin-RING ubiquitin ligase complex comprises CUL4A, CUL4B, CUL1, CUL2, CUL3, CUL5, CUL6 and/or CUL7, preferably human CUL4A, human CUL4B, human CUL1, human CUL2, human CUL3, human CUL5 and/or human CUL7, more preferably human CUL4A and/or human CUL4B.
- 22.. The method of any of the preceding claims, wherein the method further comprises determining the level of the at least one member of the E3 ligase complex in the absence of said chemical compound or agent.

23. The method of any of the preceding claims, wherein the level of the at least one member of the E3 ligase complex is determined by addition of a binding agent specific for said at least one member of the E3 ligase complex.
24. The method of any of the preceding claims, wherein the at least one member of the E3 ligase complex comprises a modification allowing its direct or indirect detection.
25. The method of claim 24, wherein the modification is the addition of tag and/or a label, preferably a fluorescent tag, a split-fluorescent tag, a luminescent tag, a luciferase, or a peptide binding a reporter enzyme.
26. The method according to any of the preceding claims, wherein said level of the at least one member of the E3 ligase complex is determined using an immunoassay, a bioassay and/or a report assay, wherein preferably said immunoassay is selected from the group consisting of immunoprecipitation, enzyme immunoassay (EIA), radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassay, a chemiluminescent assay, an agglutination assay, nephelometric assay, turbidimetric assay, a Western Blot, a competitive immunoassay, a noncompetitive immunoassay, a homogeneous immunoassay a heterogeneous immunoassay, and wherein preferably said reporter assay is a luminescence assay, like a luciferase assay or luminex.
27. The method of any of the preceding claims, wherein the chemical compound or agent to be identified is a chemical compound or agent that has been predetermined to bind to the protein of interest or to bind to the protein of interest if the chemical compound or agent simultaneously binds to the protein of interest and a second protein, wherein the second protein preferably comprises a member of the E3 ligase complex.

28. The method of any of the preceding claims, wherein the chemical compound or agent to be identified is a peptide, a small molecule, or a molecule having a size between 0.5 and 2 kDa, preferably between 0.8 and 1.5 kDa.
29. The method of claim 28, wherein the small molecule or molecule having a size between 0.5 and 2 kDa, preferably between 0.8 and 1.5 kDa comprises a first moiety binding the protein of interest and a second binding moiety, wherein the first and the second binding moiety are optionally connected by a linker
30. The method of any of the preceding claims, wherein the chemical compound or agent is part of a library of compounds or agents.
31. The method of claims 30, wherein the ability to induce ubiquitination of the protein of interest is determined for at least two compounds or agents comprised in the library.
32. The method of claim 30 or 31, wherein the compounds or agents of the library comprise a first moiety binding to the protein of interest, a second binding moiety and a linker connecting the first and the second binding moiety; and wherein at least one of the compounds or agents comprises the second binding moiety of a second compound or agent of the library and/or a linker from the second or a third compound or agent of the library.
- ~~33. A chemical compound or agent identified by any of the methods of claim 1 to 32.~~
- ~~34. The chemical compound or agent of claim 33 for use in medicine.~~
- ~~35. The chemical compound or agent of claim 33 or 34 for use in the treatment of cancer, a metabolic disease/disorder, an infectious disease/disorder or a neurological disease/disorder.~~
- ~~36. A method for treating cancer, a metabolic disease/ disorder, an infectious disease/disorder or a neurological disease/disorder comprising administering~~

- ~~the chemical compound or agent of any one of claims 33 to 35 to a patient suffering from said cancer, said metabolic disease/disorder, said infectious disease/disorder or said neurological disease/disorder.~~
- ~~37. A eukaryotic cell manipulated to comprise an enhanced cullin-RING ubiquitin ligase (CRL) activity.~~
- ~~38. The cell of claim 37, wherein said cell manipulated to comprise enhanced cullin-RING ubiquitin ligase (CRL) activity is a cell manipulated to comprise constantly neddylated cullin RING ubiquitin ligases/CRLs and/or that is manipulated to comprise constant neddylation of cullin-RING ubiquitin ligases/CRLs.~~
- ~~39. The cell of claim 38 or 39, wherein the cell manipulated to comprise enhanced CRL activity is manipulated by inactivation of a Cullin-associated NEDD8-dissociated protein selected from the group consisting of CAND1, CAND2 or CAND1 and CAND2 or by inactivation of at least one member of the COP9 signalosome (CSN).~~
- ~~40. The cell of claim 39, wherein said inactivation of the COP9 signalosome (CSN) comprises the inactivation of the COPS5 subunit, the COPS8 subunit and/or of the COPS7B subunit.~~
- ~~41. The cell of claim 39 or 40, wherein said inactivation comprises an inactivation by recombinant means and/or a hypomorphic mutation leading to a decrease of activity and/or abundance of the corresponding target protein or comprises the inactivation via a chemical compound/chemical inhibitor.~~
- ~~42. The cell of any one of claim 41, wherein said target protein is selected from the group consisting of CAND1, CAND2 or a protein member of the COP9 signalosome (CSN).~~
- ~~43. The cell of any one of claims 39 to 42, wherein said inactivation comprises the step of contacting the cell to be manipulated to comprise enhanced cullin-~~

- ~~RING ubiquitin ligase (CRL) activity and/or to comprise constantly neddylated cullin RING ubiquitin ligases/CRLs with an inhibitor of the COP9 signalosome (CSN).~~
44. ~~The cell of claim 43, wherein said inhibitor of the COP9 signalosome (CSN) is an inhibitor of the COPS5 subunit, preferably CSN5i-3.~~
45. ~~The cell of claim 37 or 38, wherein the cell manipulated to comprise enhanced CRL activity is manipulated by overexpressing at least one protein able to induce neddylation of the CRLs.~~
46. ~~The cell of claim 45, wherein said overexpressed at least one protein is selected from the group consisting of (i) a cullin/cullin backbone protein/culling homolog, (ii) an adaptor protein, (iii) a ligase substrate receptor, (iv) an E1 activating enzyme (NAE; a heterodimer of NAE1 and UBA3 subunit), and (v) an E2 conjugating enzyme (Ubc12, UBE2M).~~
47. ~~The cell of claim 46, whereby said overexpressed at least one protein is at least one member of the E3 ligase complex and whereby said~~
~~—— (i) cullin/cullin backbone protein/cullin homolog is selected from the group consisting of CUL4A, CUL4B, CUL1, CUL2, CUL3, CUL5, CUL6 and CUL7; preferably selected from the group consisting of human CUL4A, human CUL4B, human CUL1, human CUL2, human CUL3, human CUL5 and/or human CUL7, more preferably human CUL4A and/or human CUL4B;~~
~~—— (ii) adaptor protein is selected from the group consisting of DBB1, SKP1, preferably DDB1;~~
~~—— (iii) ligase substrate receptor is selected from the group consisting of cereblon (CRBN), DCAF15, DCAF16, DCAF1, DCAF5, DCAF8, DET1, FBXO7, FBXO22, KDM2A, KDM2B, preferably cereblon (CRBN) or DCAF15;~~
~~—— (iv) E1 activating enzyme is NAE1, UBA3 subunit or a heterodimer of NAE1 and UBA3 subunit; and~~
~~—— (v) E2 conjugating enzyme is Ebc12 or UBE2M, preferably UBE2M.~~
48. ~~The cell of any of claim 37 to 47, wherein the cell expresses an oncogene or is~~

~~a cancer cell such as a lung cancer cell, a gastric cancer cell, a melanoma cell, a sarcoma cell, a leukemia cancer cell, a colon cancer cell or a neuroblastoma cell.~~

~~49. The cell of any of claim 37 to 48 for use in the method as characterized in any one of claims 1 to 32.~~