in favour of percentile-based indicators: they are normalized for subject area and time period, they are independent of the skewed distribution of citations and they offer the option of focusing on specific percentile rank classes. Rodríguez-Navarro himself proposes the x-index, a “percentile-based index of the high-citation tail” [2]. He must, therefore, be persuaded by the percentile approach.

Lutz Bornmann has already undertaken several studies into the validity of bibliometric indicators, which are in line with the recommendation from Harnad [3]: “Scientometric predictors of research performance need to be validated by showing that they have a high correlation with the external criterion they are trying to predict.” For example, in references [4,5] the *h*-index is validated with the assessments of experts as an external criterion for quality. Bornmann and Leydesdorff use data from F1000 to validate the percentiles of citation counts externally and show that the correlation of percentiles with F1000 scores is higher than with any other indicator [6].

Rodríguez-Navarro proposes creating quotients from a research indicator and the corresponding research expenditures. The question with these indicators is, of course, can we assume that their validity has already been tested on a broad database? We doubt that. Bibliometricians face great problems in considering research efforts in their studies—for example, by the number of researchers and scope of research resources—particularly when institutions are being compared. These data are usually difficult to obtain, frequently incomplete and always inconsistent. As an external criterion for reviewing the validity of bibliometric indicators, Rodríguez-Navarro proposes using the number of Nobel Prizes. This indicator is also problematic, as the Noble Prize is too infrequent an event; it is often not clear to which institution a Nobel Prize should be awarded; and the prizes are awarded many years after the researcher has delivered the performance in question.

Second, Rodríguez-Navarro criticizes our choice of the proportion and number of top 10% most cited papers. Generally speaking, we have found that when comparing institutions, we get similar results with the top 5% or the top 20%. We used the top 10% most-cited papers because these are equated with highly cited publications [7–10]. Furthermore, this indicator is

already used for the Leiden Ranking [11] and the SCImago Institutions Ranking [12].

Third, Rodríguez-Navarro states that a bibliometric indicator should be able to distinguish between normal and revolutionary science. But what is normal and revolutionary science? Kuhn [13] used examples from history to explain the difference between the two, but fails to shed any light on how it could be determined in modern science. We have already addressed Kuhn’s concept in bibliometric terms. Our results, using the example of the big bang theory [14] and plate tectonics [15], show that regarding the development of research as two phases does not reflect its complexity appropriately. We have therefore proposed the enhancement of Kuhn’s paradigm concept with the Anna Karenina principle [16]: a scientific revolution can only be expected when several key conditions have been fulfilled. For example, solid evidence to answer basic questions must be presented and taken up by colleagues, and must be amenable to verification by means of independent data and methods.

Fourth, Rodríguez-Navarro incorrectly mixes societal and scientific impact measurements of research. The indicators proposed by us measure scientific impact—no more and no less. Indicators of research “that is relevant to society”, have not yet been developed [17,18]. At present, the case study approach, which is very complex, is seen as the best method to measure societal impact. We developed a more simple approach where scientists write assessment reports (such as the IPCC) that explain how their results could be used and/or applied in society [19].

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Rodríguez-Navarro A (2013) *EMBO Rep* **14**: [Epub ahead of print] doi:10.1038/embor.2013.60
2. Rodríguez-Navarro A (2011) *PLoS ONE* **6**: e20510
3. Harnad S (2009) *Scientometrics* **79**: 147–156
4. Bornmann L, Daniel H-D (2005) *Scientometrics* **65**: 391–392
5. Bornmann L, Wallon G, Ledin A (2008) *Res Eval* **17**: 149–156
6. Bornmann L, Leydesdorff L (2013) *J Informetr* **7**: 286–291
7. Bornmann L, de Moya Anegón F, Leydesdorff L (2012) *J Informetr* **6**: 333–335
8. Sahel JA (2011) *Sci Transl Med* **3**: 84cm13
9. Tijssen R, van Leeuwen T (2006) Centres of research ‘excellence’ and science indicators. In *Ninth International Conference on Science and Technology Indicators* (ed. Glänzel W), pp 146–147. Katholieke Universiteit Leuven
10. Tijssen R, Visser M, van Leeuwen T (2002) *Scientometrics* **54**: 381–397
11. Waltman L et al (2012) *J Am Soc Inf Sci Technol* **63**: 2419–2432
12. SCImago Research Group (2012) *SIR World Report 2012*. University of Granada
13. Kuhn TS (1962) *The Structure of Scientific Revolutions*. University of Chicago Press
14. Marx W, Bornmann L (2010) *Scientometrics* **84**: 441–464
15. Marx W, Bornmann L (2013) *Scientometrics* **94**: 595–614
16. Bornmann L, Marx W (2012) *J Am Soc Inf Sci Technol* **63**: 2037–2051
17. Bornmann L (2012) *EMBO Rep* **13**: 673–676
18. Bornmann L (2013) *J Am Soc Inf Sci Technol* **64**: 217–233
19. Bornmann L, Marx W (2013) *Scientometrics* [Epub ahead of print] doi:10.1007/s11192-013-1020-x

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Response: ‘Antibody crossreactivity between the tumour suppressor PHLPP1 and the proto-oncogene β -catenin’

Lobert and co-workers [1] reported a consequential flaw in the immunoreactivity of the antibodies A300-660A and IHC-00382 to PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1) manufactured by Bethyl Laboratories, Inc.

(Montgomery, Texas, USA). The flaw is the crossreactivity of these preparations with β -catenin. This response is to acknowledge the validity of the report of Lobert and co-workers, to report that testing at Bethyl Laboratories has confirmed their findings

and to report production of antibodies to the carboxyl-terminus of PHLPP1 with less than 3% residual crossreactivity with β -catenin when compared with crossreactivity of A300-660A to β -catenin.

The antibodies A300-660A and IHC-00382 had a common means of production in which a peptide (LPDYDTPL) representing the C-terminus of PHLPP1 was conjugated to a carrier protein and used as an antigen for the production of hyperimmune sera in rabbits. Subsequently, antibodies to the peptide were purified by using the peptide conjugated to beads of agarose. The affinity-purified antibodies were tested in immunoblotting and immunoprecipitation. Having found that the preparation of antibodies was useful for immunoblotting and immunoprecipitation, the preparation was offered as part of Bethyl's line of products as product number A300-660A. Subsequent to testing for usefulness in immunohistochemical staining, a portion of A300-660A was reformulated to produce product number IHC-00382. The reformulation consisted of adjusting the concentration of antibody to that which gave positive immunohistochemical staining when IHC-00382 was used at a final working dilution within the range of 1:100–1:500.

When using A300-660A sufficiently concentrated, we observed a more rapidly migrating band (approximately 90 kDa) when immunoblotting whole-cell lysate from HeLa and 293T cells. The band in question was reported by Lobert and co-workers [1] to be β -catenin. Under our conditions for immunoblotting, the intensity of the β -catenin band was considerably less than the intensity of the PHLPP1 bands in HeLa and 293T cells. In addition, when using the antibody sufficiently dilute, PHLPP1 could be readily detected whilst the β -catenin band was not observed. Furthermore, the β -catenin band was not readily observed when using the antibody to perform immunoblot of immunoprecipitates produced using A300-660A. Thus, we had no evidence that the more rapidly migrating band was immunoprecipitated by A300-660A.

On becoming aware of the report by Lobert and co-workers [1], we took three actions. The first was to remove from the market the antibody Bethyl IHC-00382, which had previously been offered with an explicit recommendation for use in immunohistochemistry. Second, we removed the recommendation for use of A300-660A in

immunohistochemistry and stated that the use of A300-660A in immunohistochemistry was strongly discouraged. We chose to continue to offer A300-660A with recommendations for its use in immunoblotting and immunoprecipitation. Third, we initiated testing of the crossreactivity of A300-660A with β -catenin.

We confirmed crossreactivity with β -catenin using A300-660A in immunoblot of immunoprecipitates produced by using a β -catenin antibody (Bethyl product number A302-012A; Fig 1A). In addition, β -catenin was observed by immunoblotting for it (Fig 1B,C) in the immunoprecipitate produced using A300-660A. Notably, β -catenin was not detected when immunoblotting the immunoprecipitates produced by using a different antibody to PHLPP1 (A300-659A, which recognizes an upstream epitope on PHLPP1).

Whilst β -catenin was not listed among the results from Blast analysis of the PHLPP1 peptide LPDYDTPL, alignment of the C-terminal 50 residues of human PHLPP1 (accession number O60346) with the C-terminal 50 residues of β -catenin (accession number P35222) revealed sufficient similarity to presume the C-terminus of β -catenin (LAWFDTDL) to be the epitope bound by A300-660A (Fig 2). We elected to remove antibodies responsible for crossreactivity with the C-terminus of β -catenin by adsorption to a peptide containing the epitope.

The peptide SNQLAWFDTDL was synthesized and conjugated to beads of agarose. The agarose containing the peptide was transferred to a 2.5 $\times$ 20 cm column, and hyperimmune sera from rabbits immunized with the PHLPP1 peptide PDYDTPL were passed through the column to adsorb antibodies that bound to the β -catenin peptide. Adsorption of β -catenin crossreactive antibodies in the sera was repeated multiple times with regeneration of the column between each cycle of adsorption. Subsequently, antibodies to PHLPP1 remaining in the hyperimmune sera were affinity-purified following adsorption to, and elution from, beads of agarose conjugated to the PHLPP1 peptide.

The resulting affinity-purified β -catenin crossreactive antibodies (BL16200) and PHLPP1 antibodies (A304-029A) were tested for crossreactivity with β -catenin. Whereas β -catenin was immunoprecipitated by A300-660A and BL16200,

Fig 1 | Assessment by immunoblot and immunoprecipitation of crossreactivity of antibodies to PHLPP1 with β -catenin. Antibodies to PHLPP1 and β -catenin were used in immunoblotting of the immunoprecipitates produced by using antibodies to PHLPP1 (A300-659A, A300-660A, A304-029A), antibodies to β -catenin (A302-012A) and antibodies purified from PHLPP1 antisera by adsorption to a β -catenin peptide (BL16200). (A) An immunoblot using antibody A300-660A. (B) Short exposure of an immunoblot for β -catenin using antibody A302-012A. (C) Long exposure of an immunoblot for β -catenin using antibody A302-012A. (D) Short exposure of an immunoblot using antibody A304-029A. (E) Long exposure of an immunoblot using antibody A304-029A. PHLPP1, PH domain leucine-rich repeat protein phosphatase 1.

the amount of β -catenin detected in the immunoprecipitate produced by using A304-029A was negligible (Fig 1B,C). Using immunoblot, there was no detectable crossreactivity of A304-029A with β -catenin in whole-cell lysate from HeLa cells or with β -catenin immunoprecipitated from HeLa cells (Fig 1D,E).

PHLPP1 C-terminus	-PELEEEVKIMKHHQEQQQQQPP-PPQLQPQLPRHYQLDQLPDYYDTPL	1717
β -catenin C-terminus	DPMMEHEMGG-HHPGADYPVDGLPDLGHAQDLMDGLPPG-DSNQL-AWFDIDL	781
	* : * . * : : : * . : * ** : : ** : : * *	

Fig 2 | Alignment of the carboxy-terminal 50 residues of PHLPP1 with the corresponding portion of β -catenin. The C-terminal residues of PHLPP1 were aligned with the C-terminal residues of β -catenin. The underlined portion indicates the residues used to generate the antibody A300-660A. Given the antibody A300-660A was shown to crossreact with β -catenin, the similarity of the C-terminus of β -catenin to that of PHLPP1 was sufficient to anticipate that binding of Bethyl A300-660A to β -catenin was at the C-terminus of β -catenin. PHLPP1, PH domain leucine-rich repeat protein phosphatase 1.

Table 1 | Assessment by ELISA of crossreactivity of antibodies to PHLPP1

Peptide	Concentration of antibody (ng/ml)						Antibody
	333.33	100.00	33.33	10.00	3.33	1.00	
PHLPP1	3.18	2.90	2.60	1.52	0.58	0.19	A300-660A
β -catenin	2.58	1.34	0.56	0.23	0.06	0.03	
PHLPP1	3.02	2.69	2.02	1.11	0.38	0.08	A304-029A
β -catenin	0.01	0.04	0.00	0.00	0.00	0.00	

Values are the mean absorbance for duplicate wells after subtraction of background. PHLPP1, PH domain leucine-rich repeat protein phosphatase 1.

When tested for reactivity to the β -catenin peptide by indirect ELISA, reactivity with β -catenin was less than 3% of the reactivity of A300-660A with β -catenin (Table 1). If thoroughly validated in immunohistochemistry, A304-029A might prove useful in determining cellular localization of PHLPP1.

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CONFLICT OF INTEREST
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REFERENCE
1. Lobert VH et al (2012) *EMBO Rep* **14**: 10–11

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