

Corresponding author(s):	Karl Kuchler
Last updated by author(s):	Aug 27, 2019

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

St:	sti	ct	ic

Statistics
For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section. n/a Confirmed The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly The section of the same sample of the sample of the same sample of the sample of the same sample of the sample of the same sample of the same sample of the sample of the sample of the sample of the A description of all covariates tested A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Software and	code
Policy information al	pout <u>availability of computer code</u>
Data collection	No software was used.
Data analysis	GraphPad Prism 5.00, R Studio, FlowJo7, Image Studio Ver 2.1, ZEN, VMD 1.9.2, ClustalX2, GROMACS, PYMOL, PYTHON
	ustom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. de deposition in a community repository (e.g. GitHub). See the Nature Research <u>guidelines for submitting code & software</u> for further information.

Data

- Policy information about availability of data

 All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
 Accession codes, unique identifiers, or web links for publicly available datasets
 A list of figures that have associated raw data
 A description of any restrictions on data availability

Supplementary movies and source data for Figures 2, 3, 4, 5 and 6 are available online.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection. Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Sample preparation	Samples were prepared from HEK293 cells by trypsinization. After incubation with substrate with/without inhibitor, cells were washed and resuspend in cold PBS for measurement. Details are provided in Materials and methods.
Instrument	FACSCalibur
Software	Flowlo 7
Cell population abundance	Cell populations were selected according to the size (forward-scatter) and complexity (side-scatter). Debris were excluded. 10,000 events per data point were collected for analysis.
Gating strategy	Cell populations according to the size (forward-scatter) and complexity (side-scatter) were gated for analysis. Debris were excluded. 10,000 events per data point were collected.
Tick this box to confirm th	hat a figure exemplifying the gating strategy is provided in the Supplementary Information.

Life sciences study design

ine serences study design						
ll studies must dis	close on these points even when the disclosure is negative.					
Sample size	At least three biological independent replicates were performed in all experiments. Each single data point was represented as a grey dot in the graphs. Unpaired t-test was used for statistical analysis.					
Data exclusions	No data excluded.					
Replication	All replicates were successful.					
Randomization	In this study, samples were not from animals or human. Randomization was not applicable.					
Blinding	Blinding was not relevant in this study since samples from animal or human were not used. Blinding was not applicable.					

Reporting for specific materials, systems and methods

We require information from	authors about some typ	es of materials, e	experimental s	ystems and r	methods us	sed in many:	tudies. He	re, indicate	whether	each ma	teria
system or method listed is re-	lovant to your study. If w	us are not sure if	a list item ann	dies to your	rosparch re	ead the anni	nnriate ce	tion hefore	coloctine	a recon	nso

Materials & experimental systems	Methods	
'a Involved in the study	n/a Involved in the study	_
Antibodies	ChIP-seq	
Eukaryotic cell lines	Flow cytometry	
Palaeontology	MRI-based neuroimaging	
Animals and other organisms		
Human research participants		
Clinical data		

Antibodies used	Mouse anti-ABCG2 (BXP-21, Santa Cruz Biotechnology), rabbit anti-β-actin (D6A8, Cell Signaling), IRDye 800CW secondary antibodies against mouse or rabbit (LI-COR Biosciences)
Validation	All commercial antibodies were validated according to the companies. Positive and negative controls were included in every experiment.
entranski silikari	

Eukaryotic cell lines

P	olicy information about cell lines	
	Cell line source(s)	HEK293 (ATCC)
	Authentication	No
	Mycoplasma contamination	Cells were checked and no contamination from Mycoplasma.
	Commonly misidentified lines (See ICLAC register)	No

Flow Cytometry

Conf	 the set	

The axis labels state the marker and fluorochrome used (e.g. CD4-EITC)	

- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.