ABHD2 inhibitor identified by activity-based protein profiling reduces acrosome reaction

Marc P. Baggelaar[a,†,‡], Hans den Dulk[a], Bogdan I. Florea[b], Domenico Fazio[c], Nicola Bernabò[c], Marcello Raspa[d], Antonius P. A. Janssen[a], Ferdinando Scavizzi[d], Barbara Barboni[c], Hermen S. Overkleeft[b], Mauro Maccarrone[e,f,*] & Mario van der Stelt[a,*,‡]

[a]*Department of Molecular Physiology, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands.*

[b]*Department of Bioorganic Synthesis, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands.*

[c]*Unit of Basic and Applied Biosciences, University of Teramo, Via Balzarini 1, 64100 Teramo, Italy.*

[d]*National Research Council (IBCN), CNR-Campus International Development (EMMA INFRAFRONTIER-IMPC), Via E. Ramarini 32, 00015 Monterotondo Scalo, Italy.*

[e]*Department of Medicine, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy.*

[f]*European Centre for Brain Research/IRCCS Santa Lucia Foundation, via del Fosso del Fiorano 65, 00143 Rome, Italy.*

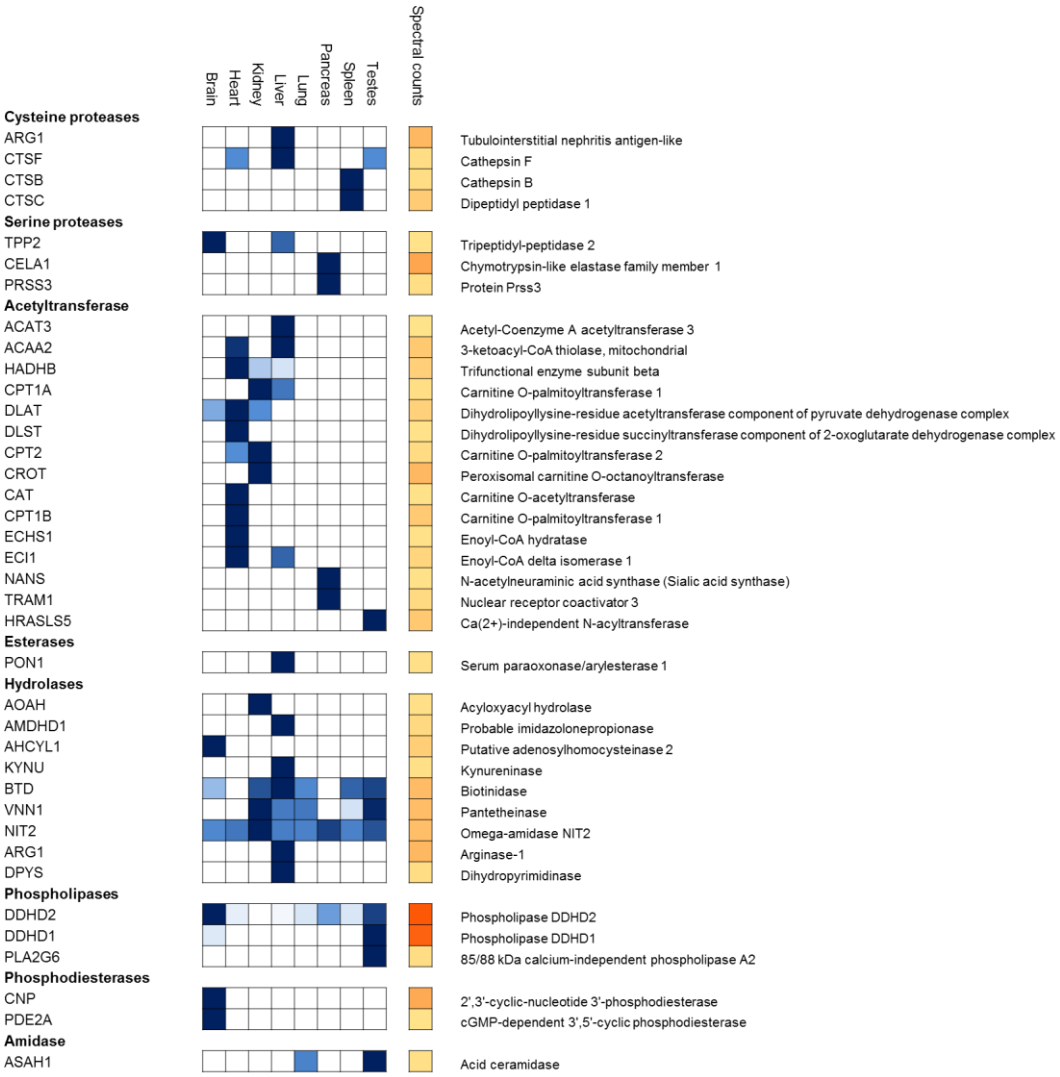
†Current adress, 1Department of Chemistry, Imperial College London, Molecular Sciences Research Hub, White City Campus, London W12 OBZ, UK

‡Corresponding author:m.baggelaar@imperial.ac.uk, m.van.der.stelt@chem.leidenuniv.nl

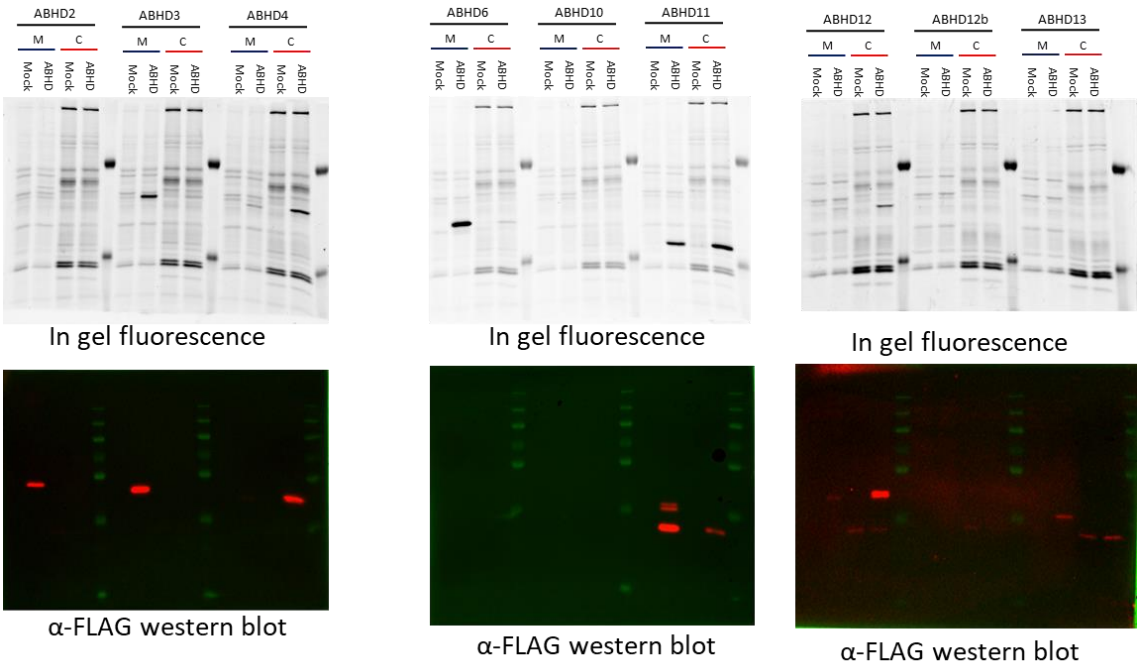
**Equally senior authors.*

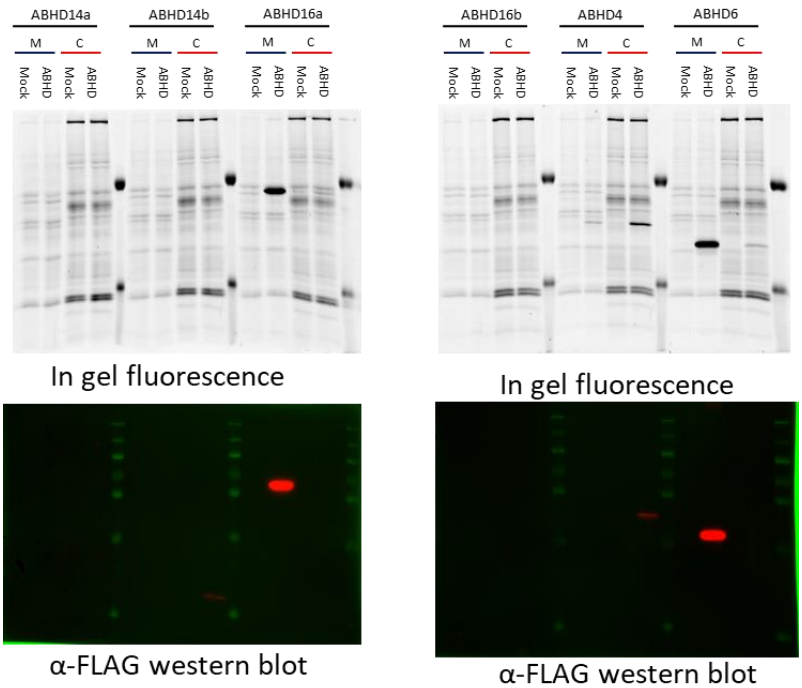
Molecular Cloning.

Full-length human cDNA of ABHD2, ABHD 3, ABHD4, ABHD6, ABHD11, ABHD12, ABHD16A and DAGL-α (Source Bioscience) was cloned into mammalian expression vector pcDNA3.1 (Invitrogen), containing genes for ampicillin and neomycin resistance. The inserts were cloned in frame with a C-terminal FLAG-tag and also for ABHD2 with a C-terminal eGFP-FLAG. Plasmids were isolated from transformed XL-10 Z-competent cells (Maxi Prep kit: Qiagen) and sequenced at the Leiden Genome Technology Center. Sequences were analyzed and verified (CLC Main Workbench). I.M.A.G.E. clone identification numbers[1]:ABHD2: 4826878, ABHD3: 3942164, ABHD4: 5122692, ABHD6: 3641064, ABHD11: 3873594, ABHD12: 3641064, ABHD16a: 4931559, DAGL-α: 40148003

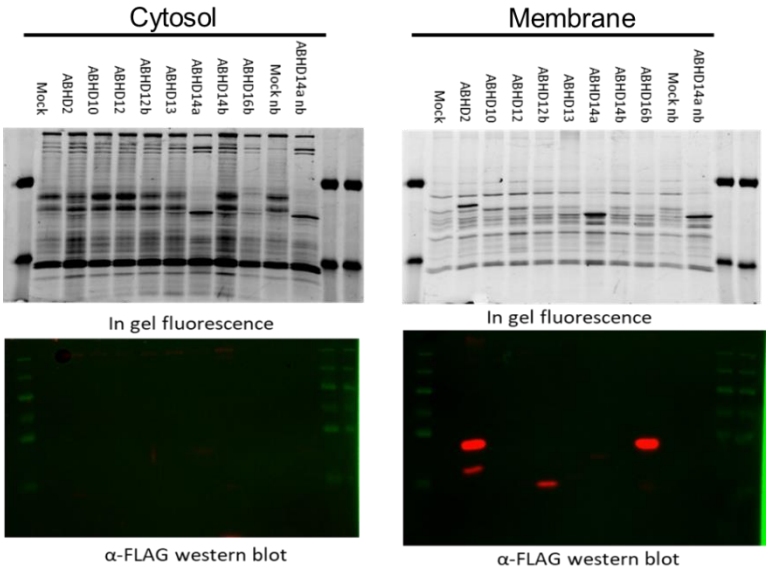


Supporting Figure 1. Non α,β-hydrolase fold proteins with hydrolase activity that were detected by MB108. Hydrolases, proteases, transferases, esterases and amidases as classified by panther gene ontology.

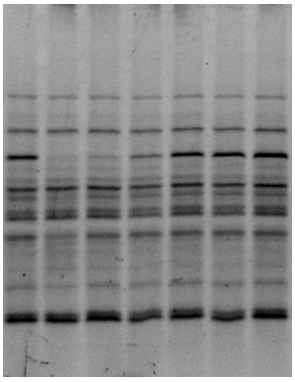




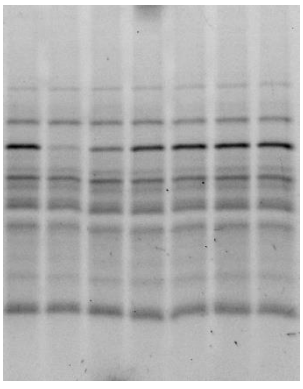
Supporting Figure 2. Full blots and in gel fluorescence images of the gels and blots shown in Figure 1. Additional proteins that were not expressed and/or not labelled are shown here.



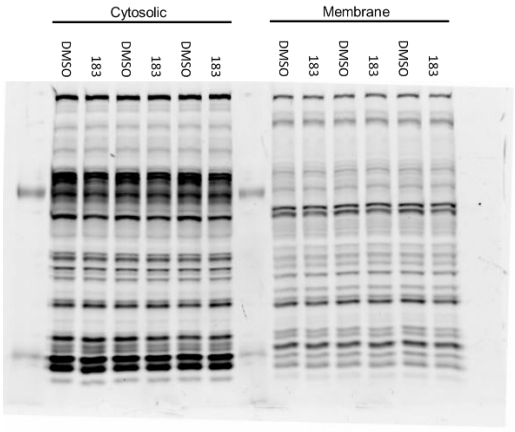
Supporting Figure 3. Full in-gel fluorescence images and western blots showing expression and labelling of ABHD2 in U2OS cells.



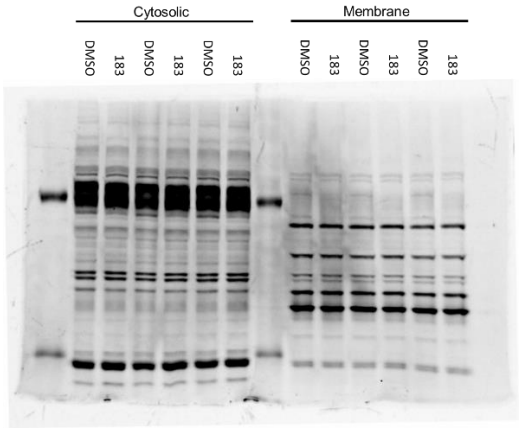
Supporting Figure 4. Representative gel of the full lanes of cut-outs in Figure 4A (**183**).



Supporting Figure 5. Representative gel of the full lanes of cut-outs in Figure 4A (**184**).



Supporting Figure 6. Full gel from Figure 5, gel-based competitive screen between **183** (20 µM) and TAMRA-FP (2 µM) in the mouse testis proteome.



Supporting Figure 7. Full gel from Figure 5, gel-based competitive screen between **183** (20 µM) and MB064 (2 µM) in the mouse testis proteome.

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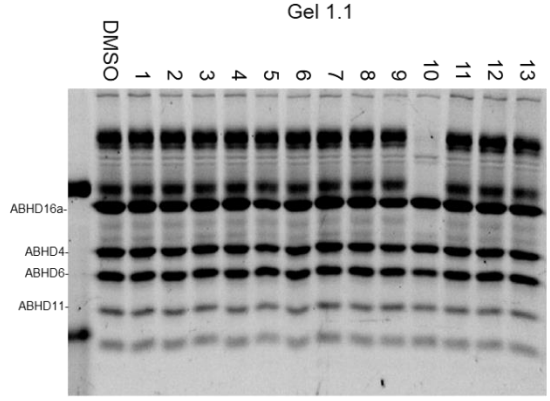

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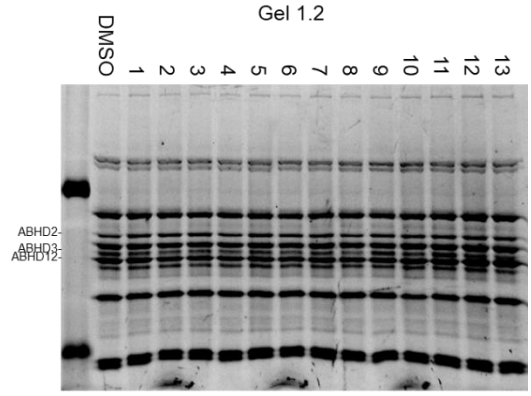

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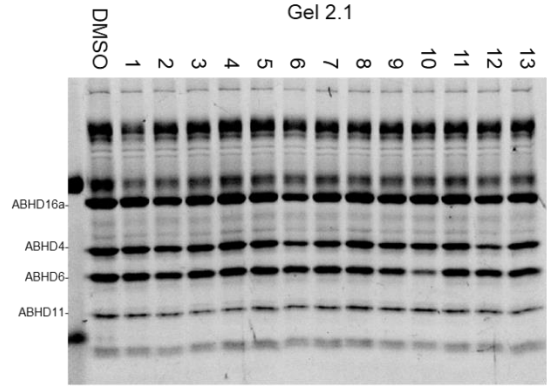

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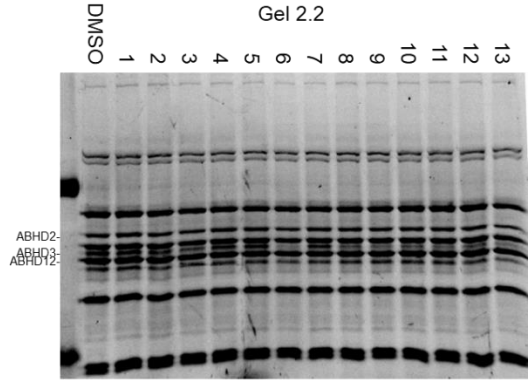

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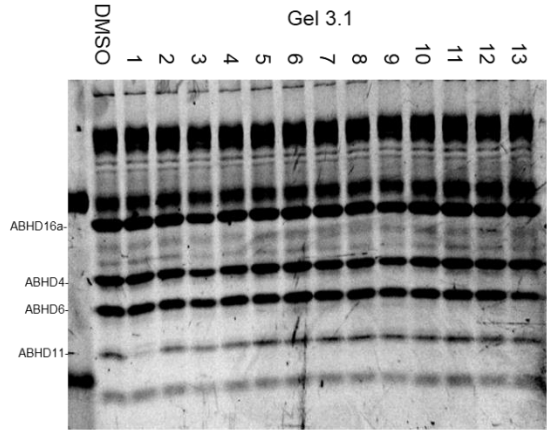

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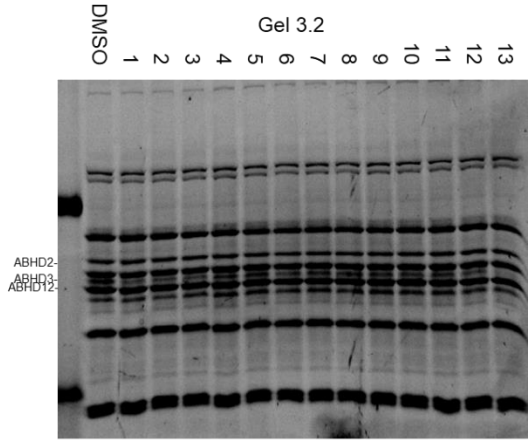

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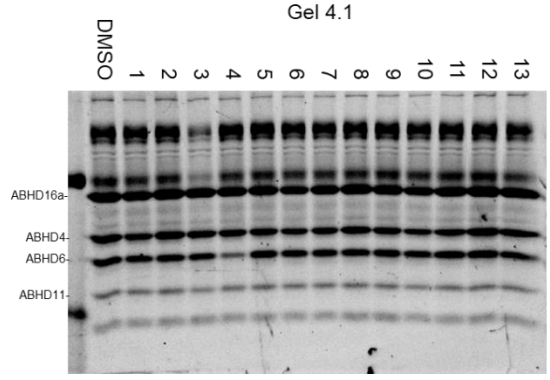

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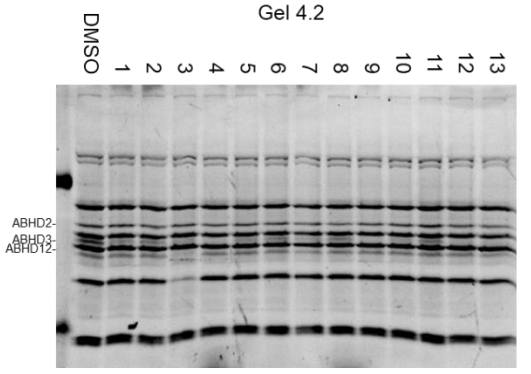

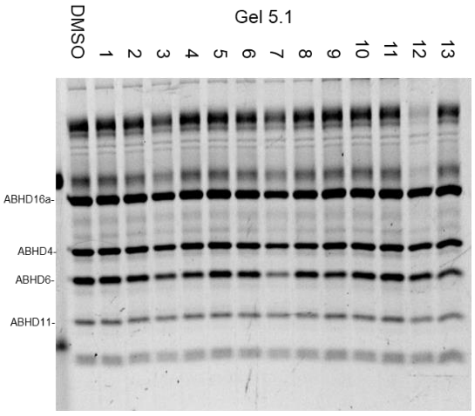
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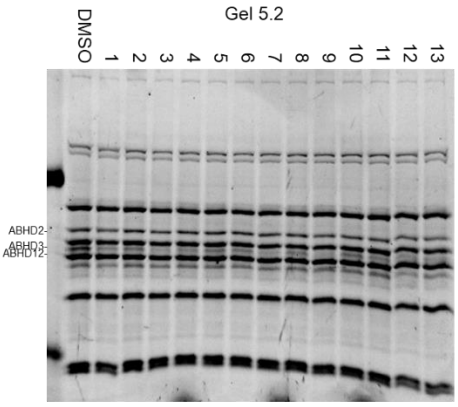
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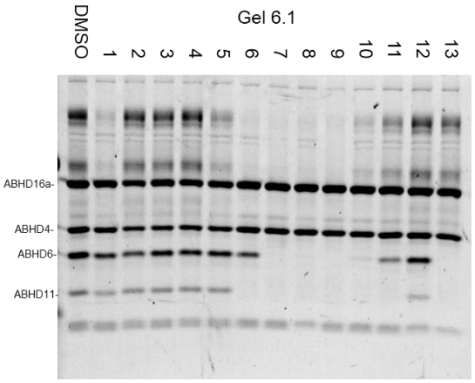
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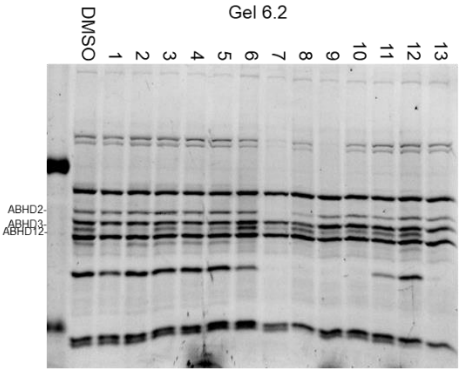
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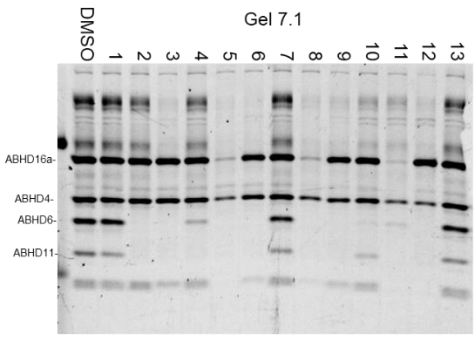
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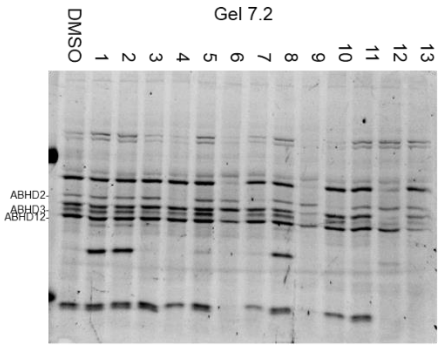
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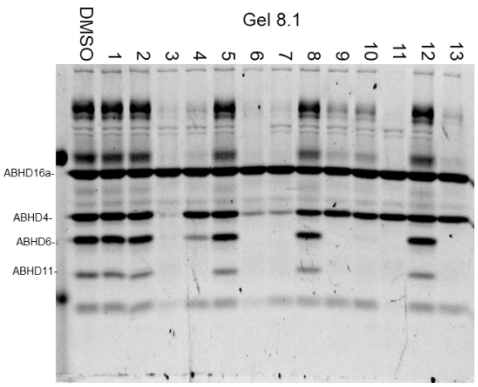
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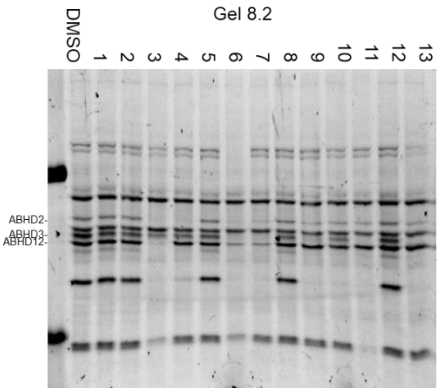
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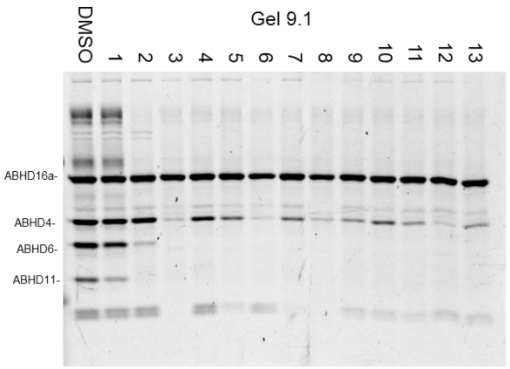

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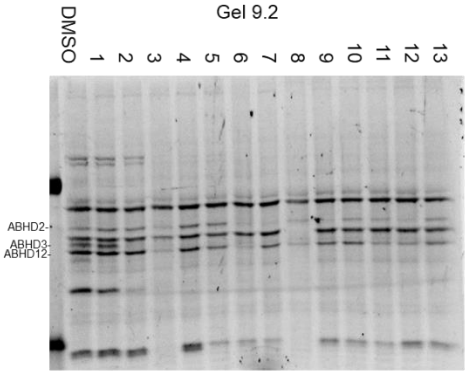

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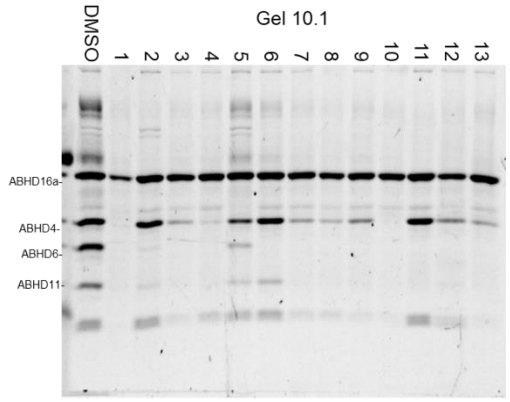

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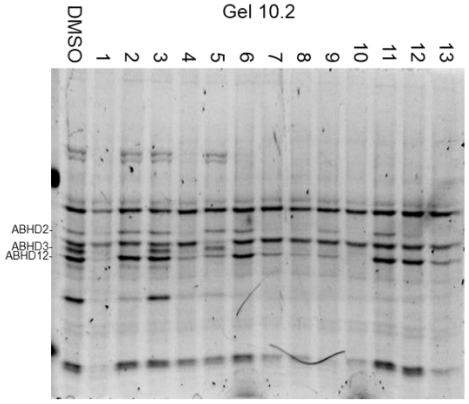

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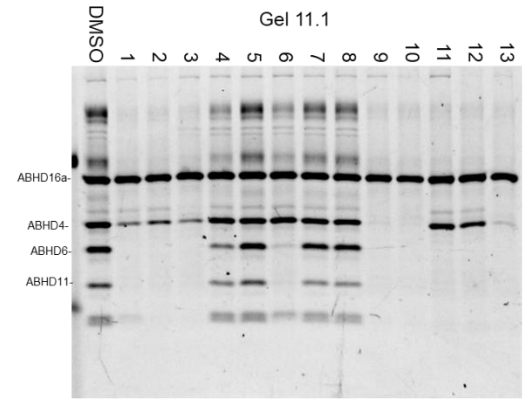

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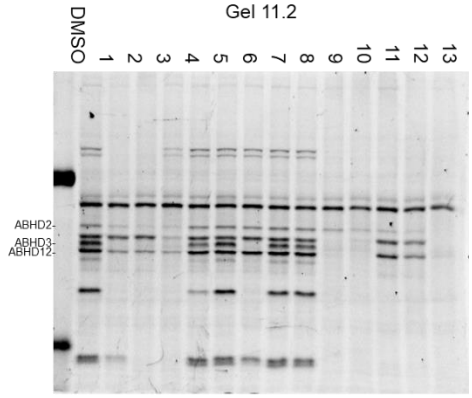

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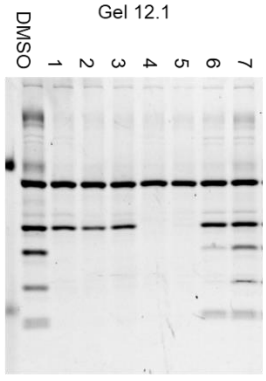

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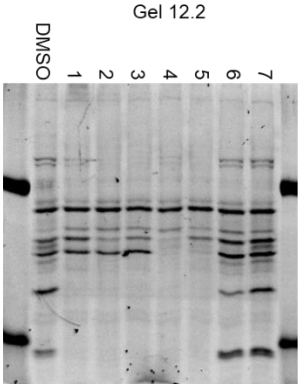

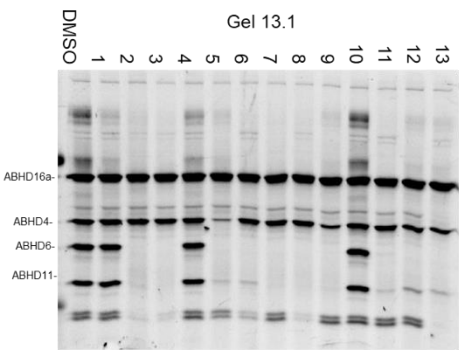
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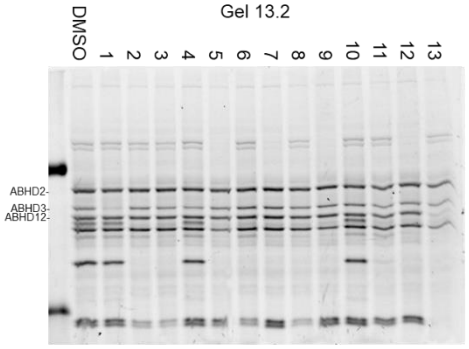
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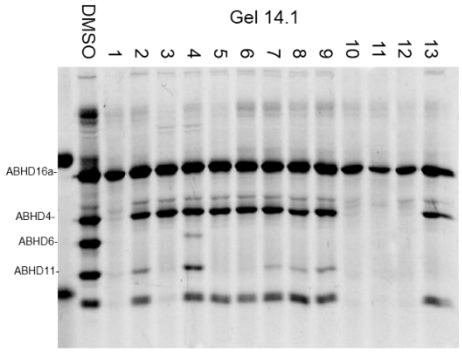
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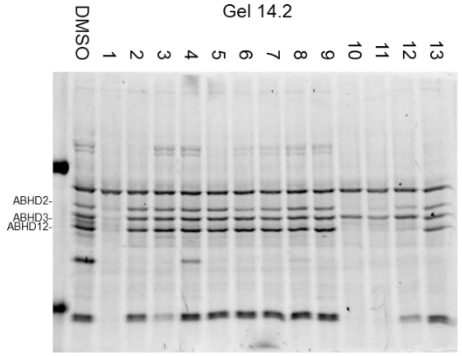
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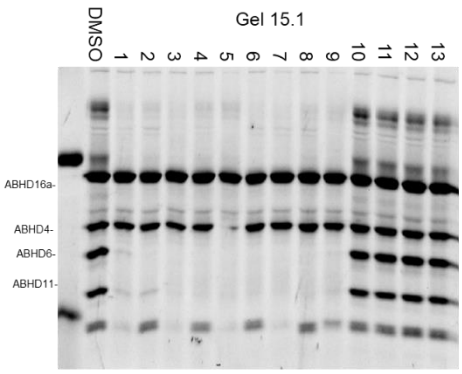
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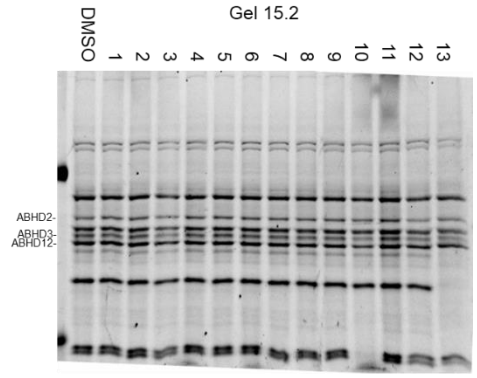
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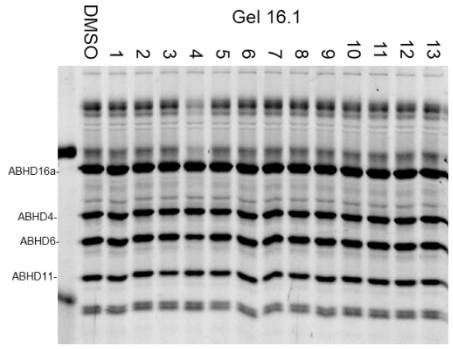
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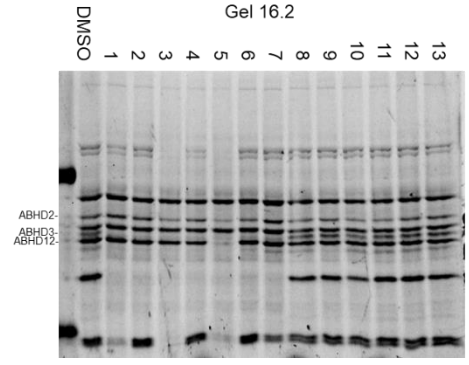
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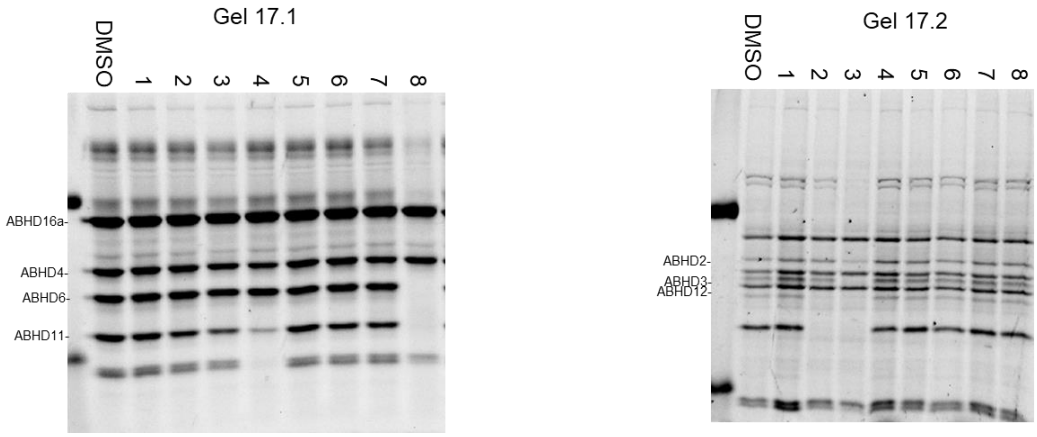
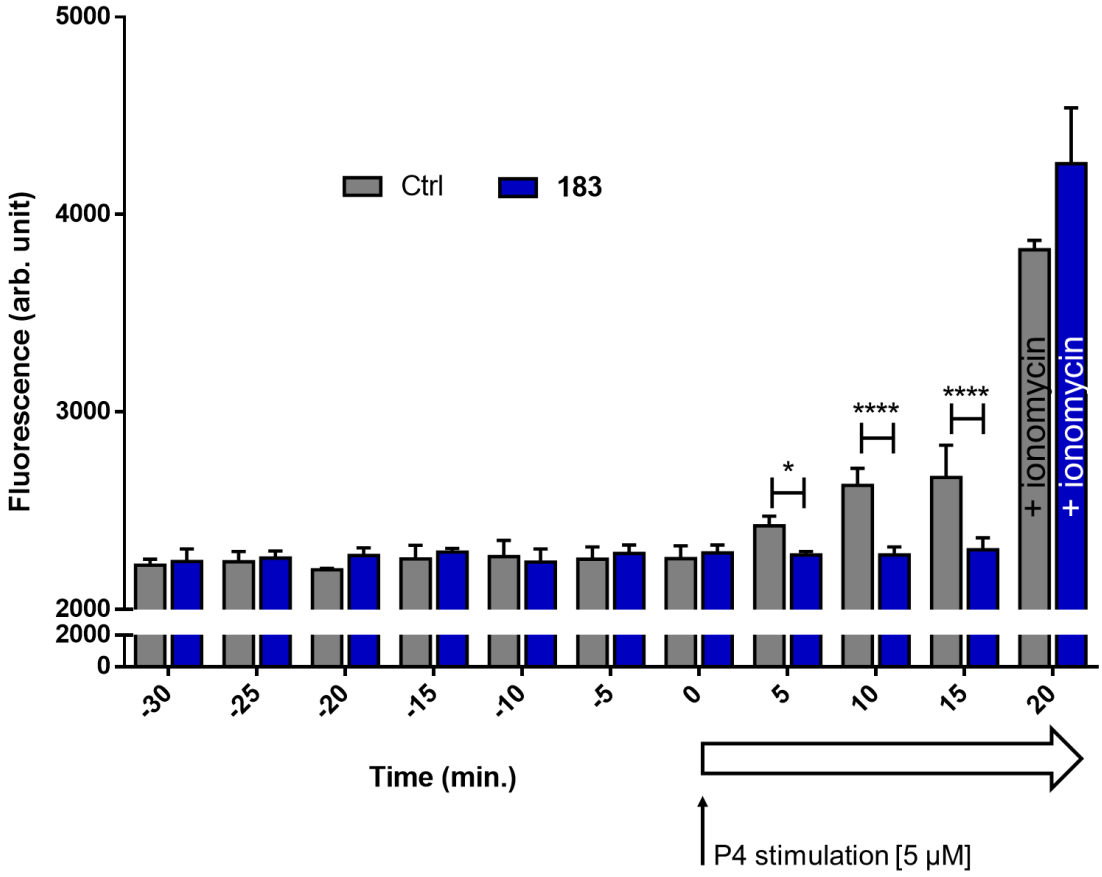


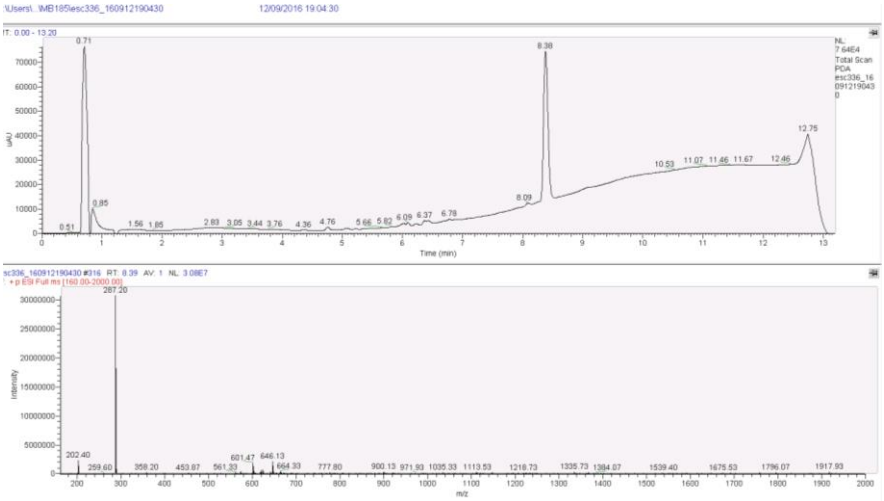
Supporting Figure 8. Gels of the inhibitor screen. Corresponding inhibitors and quantifications are given in Supporting Table 2.

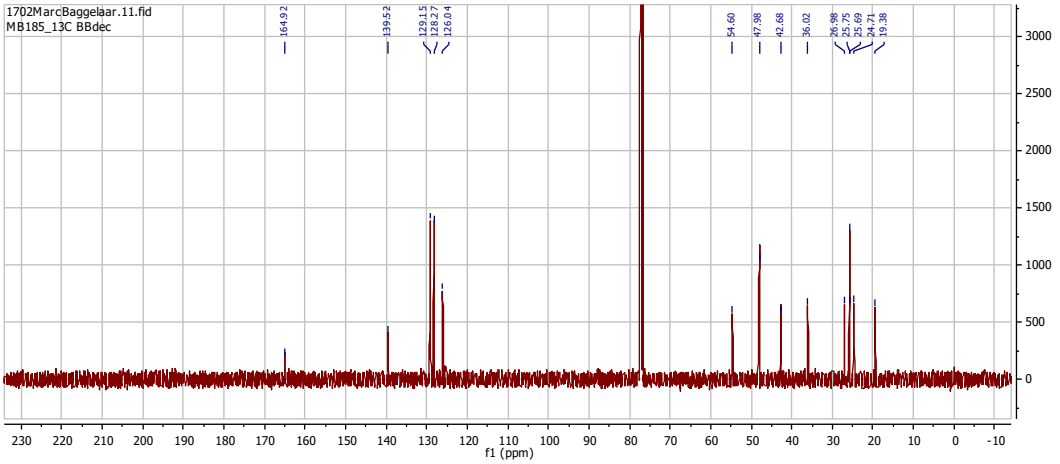


Supporting Figure 9. Full time course of the intracellular calcium measurements and ionomycin positive control. Stimulation with P4 increases intracellular Calcium levels in mouse spermatozoa as measured using Fluo-3 AM. **183** (2 µM) blocks the rise in intracellular calcium levels 5, 10 and 15 minutes after P4 stimulation. Intracellular calcium levels were monitored and stable for 30 minutes before P_4 stimulation. Ionomycin was added as a positive control 20 minutes after P_4 stimulation. The

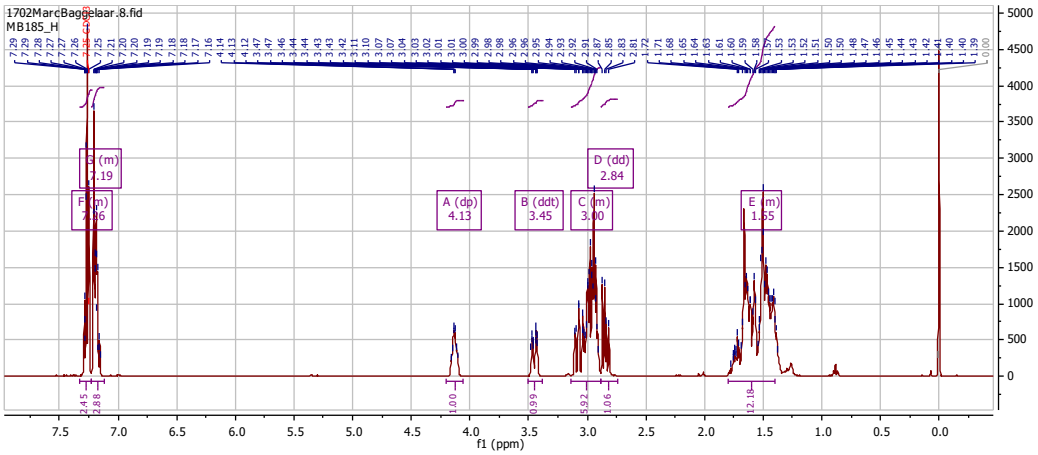
data are expressed as Florescence Arbitrary Units (Arb. Units) and are the mean +/- standard deviation of three independent samples. *P<0.05, ****P<0.0001

Resynthesis of 183: 2-benzylpiperidine (50 mg, 0.29 mmol) was dissolved in 5 mL CH_2Cl_2. Na_2CO_3 (90 mg, 0.85 mmol) was added, and the mixture was cooled to 0 °C. Bis(trichloromethyl)carbonate (69 mg, 0.23 mmol) was added and the mixture was allowed to stir at rt o/n. Piperidine (123 mg, 1.45 mmol) was added and the mixture was stirred for 5h at rt. The reaction mixture was washed with sat aq. $NaHCO_3$, 1 M HCl and brine. The organic layer was dried on $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography using pentane/ethyl acetate. Yielding **183**, 7.0 mg, 0.024 mmol, 8.3%.





^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.92, 139.52, 129.15, 128.27, 126.04, 54.60, 47.98, 42.68, 36.02, 26.98, 25.75, 25.69, 24.71, 19.38.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.22 (m, 2H), 7.22 – 7.11 (m, 3H), 4.13 (dp, *J* = 7.6, 3.4 Hz, 1H), 3.45 (ddt, *J* = 13.5, 3.3, 1.7 Hz, 1H), 3.14 – 2.88 (m, 6H), 2.84 (dd, *J* = 13.4, 8.3 Hz, 1H), 1.80 – 1.40 (m, 12H).

Supporting Figure 9. LC/MS and NMR characterization of compound **183.**

References

1. Lennon, G.G., Auffray, C., Polymeropoulos, M., Soares, M.B. The I.M.A.G.E. Consortium: An Integrated Molecular Analysis of Genomes and their Expression. Genomics **1996**, *33*,151-152.