Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

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Supporting information

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Discrimination of cellular developmental states focusing on glycan transformation and membrane dynamics by using BODIPY-tagged lactosyl ceramide

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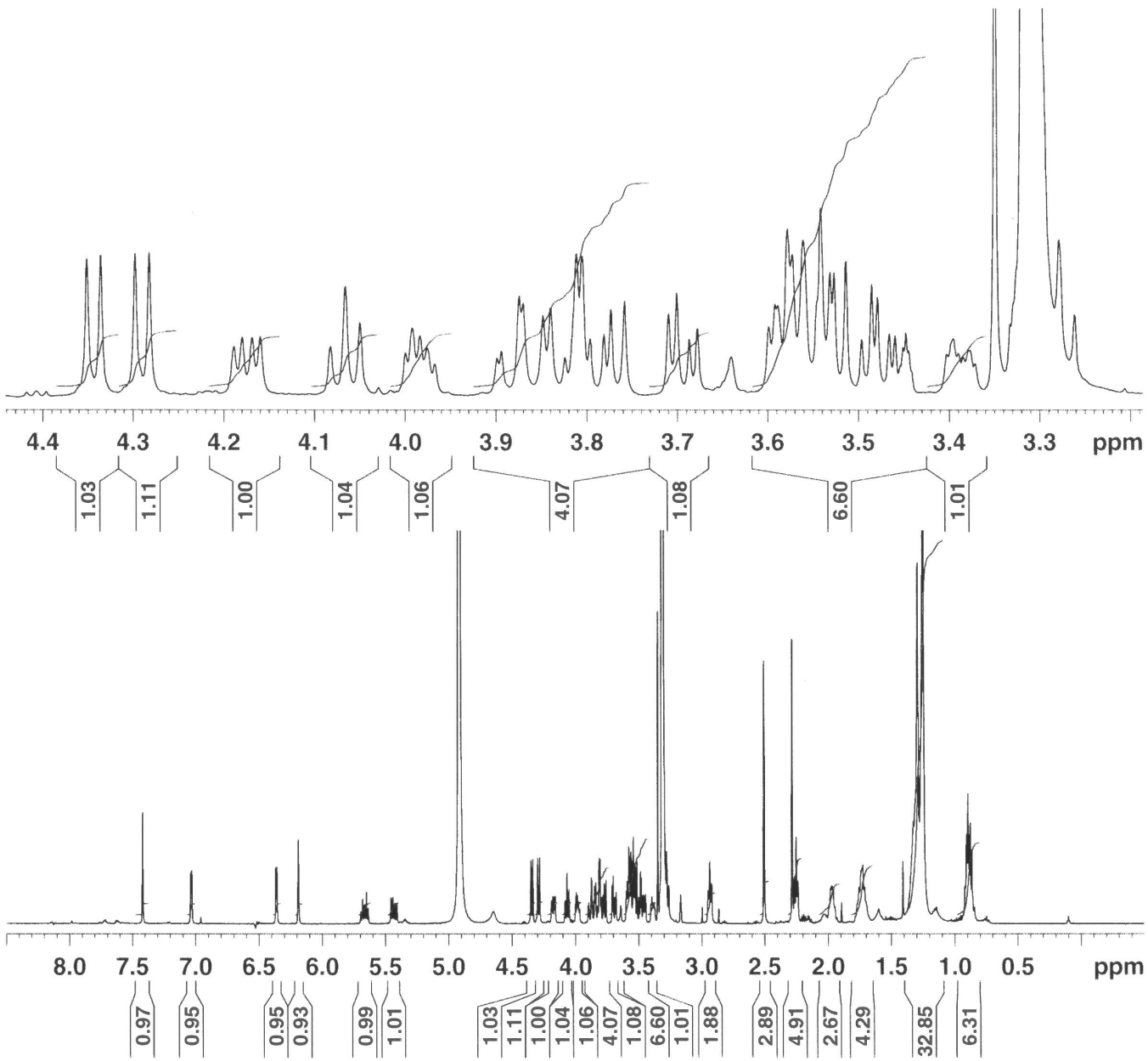
We carried out several experiments that support our findings.

Preparation of a vector pCX4 bsr/hygro LifeAct-eGFP.

The plasmid expressing LifeAct-eGFP (pEGFP-N1-LifeAct) was obtained from S. Fukuhara (National Cerebral and Cardiovascular Center Research Institute, Japan). The plasmid used for preparing the retrovirus vector expressing LifeAct-eGFP (pCX4 bsr/hygro LifeAct-eGFP) was constructed by introducing the LifeAct-eGFP fragment (amplified by polymerase chain reaction from pEGFP-N1-LifeAct) into the *Hpa*I site of pCX4 bsr or pCX4 hygro using the In-Fusion HD cloning kit (TaKaRa Bio, Otsu, Japan). The primers used are listed in Supplementary Table S1.

Table S1

295 pEGFP pCX4 Hpa F
GCGGTAACAATTGTTTCAGATCCGCTAGCGCTACCGGTC
296 pEGFP pCX4 Hpa R
TAGCTTAAGTTAGTTGTAAAACCTCTACAAATGTGGTATGG



BRUKER

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Current Data Parameters
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EXPNO                       20
PROCNO                       1

F2 - Acquisition Parameters
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Time                   21.02
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PULPROG                 zg30
TD                     65536
SOLVENT                 MeOD
NS                       128
DS                         2
SWH               10330.578 Hz
FIDRES             0.157632 Hz
AQ                 3.1720407 sec
RG                        16
DW                   48.400 usec
DE                    6.00 usec
TE                   298.0 K
D1                1.00000000 sec
MCREST            0.00000000 sec
MCWRK             0.01500000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     8.25 usec
PL1                   -5.70 dB
SFO1          500.1330885 MHz

F2 - Processing parameters
SI                     32768
SF            500.1300160 MHz
WDW                       EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                      1.00
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Fig. S1. ^{1}H NMR spectrum of compound 1.

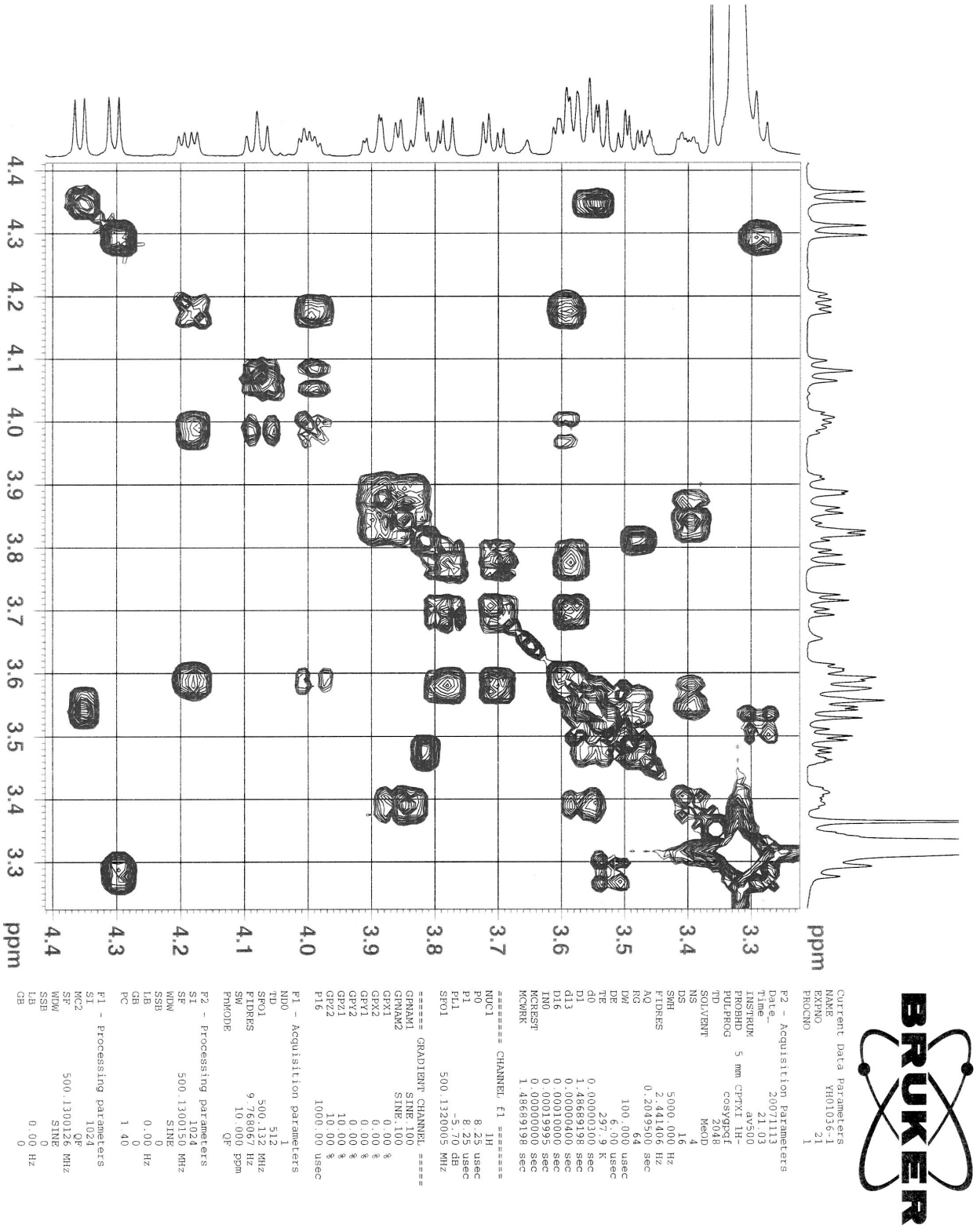

Fig. S2. ^{1}H-^{1}H COSY NMR spectrum of compound 1.

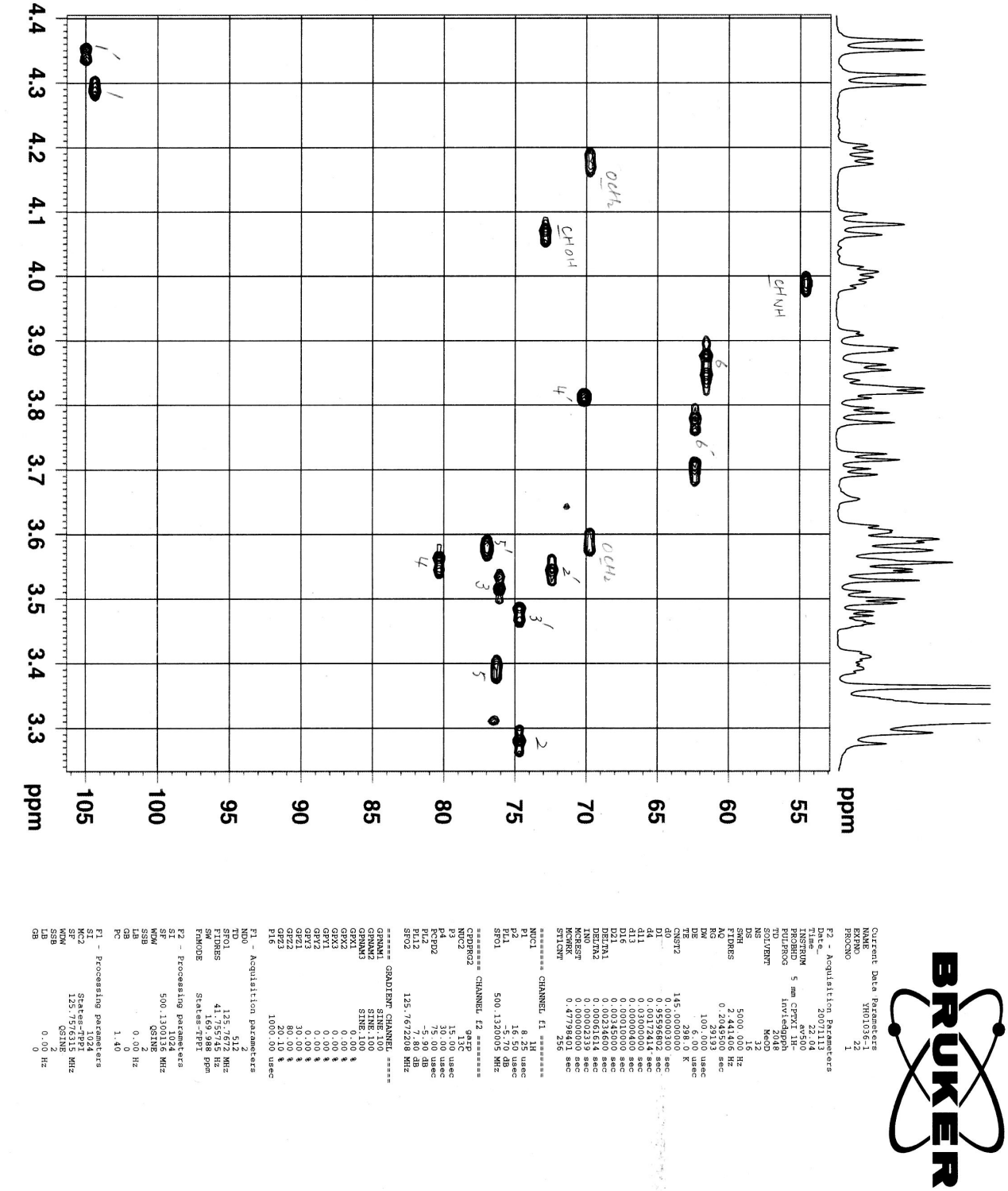

Fig. S3. HSQC NMR spectrum of compound 1.

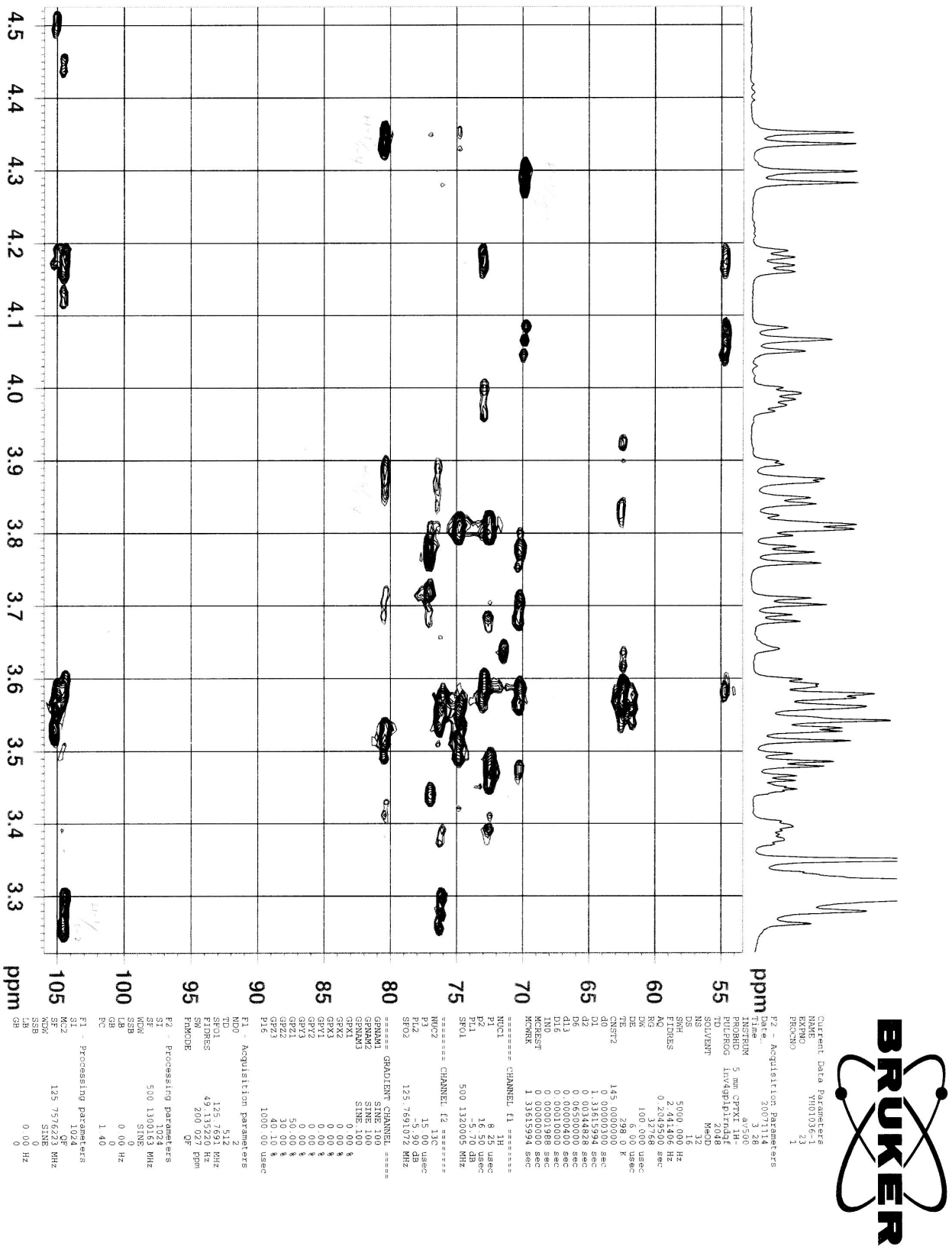

Fig. S4. HMBC NMR spectrum of compound 1.

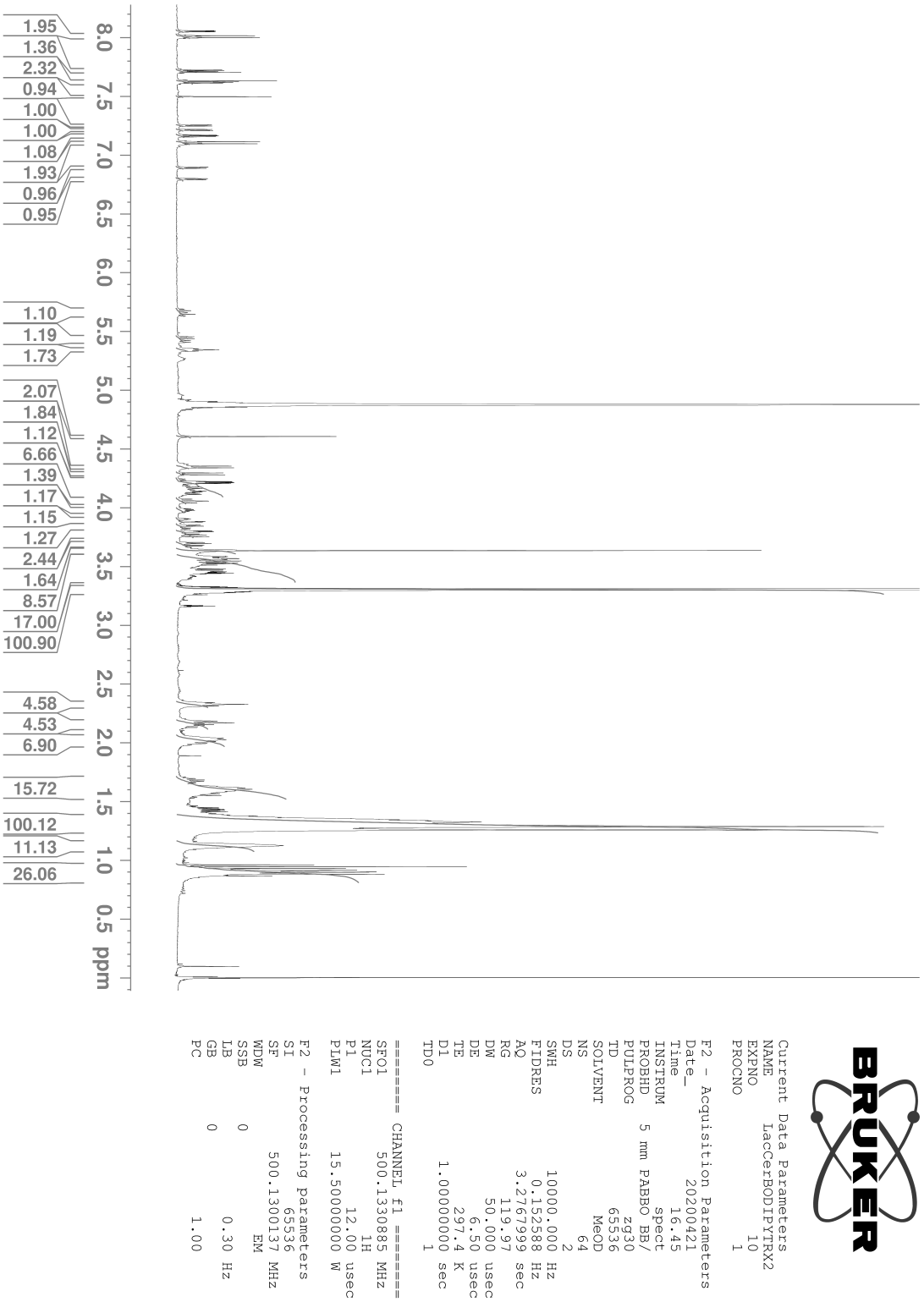

Fig. S5. ^{1}H NMR spectrum of compound 2.

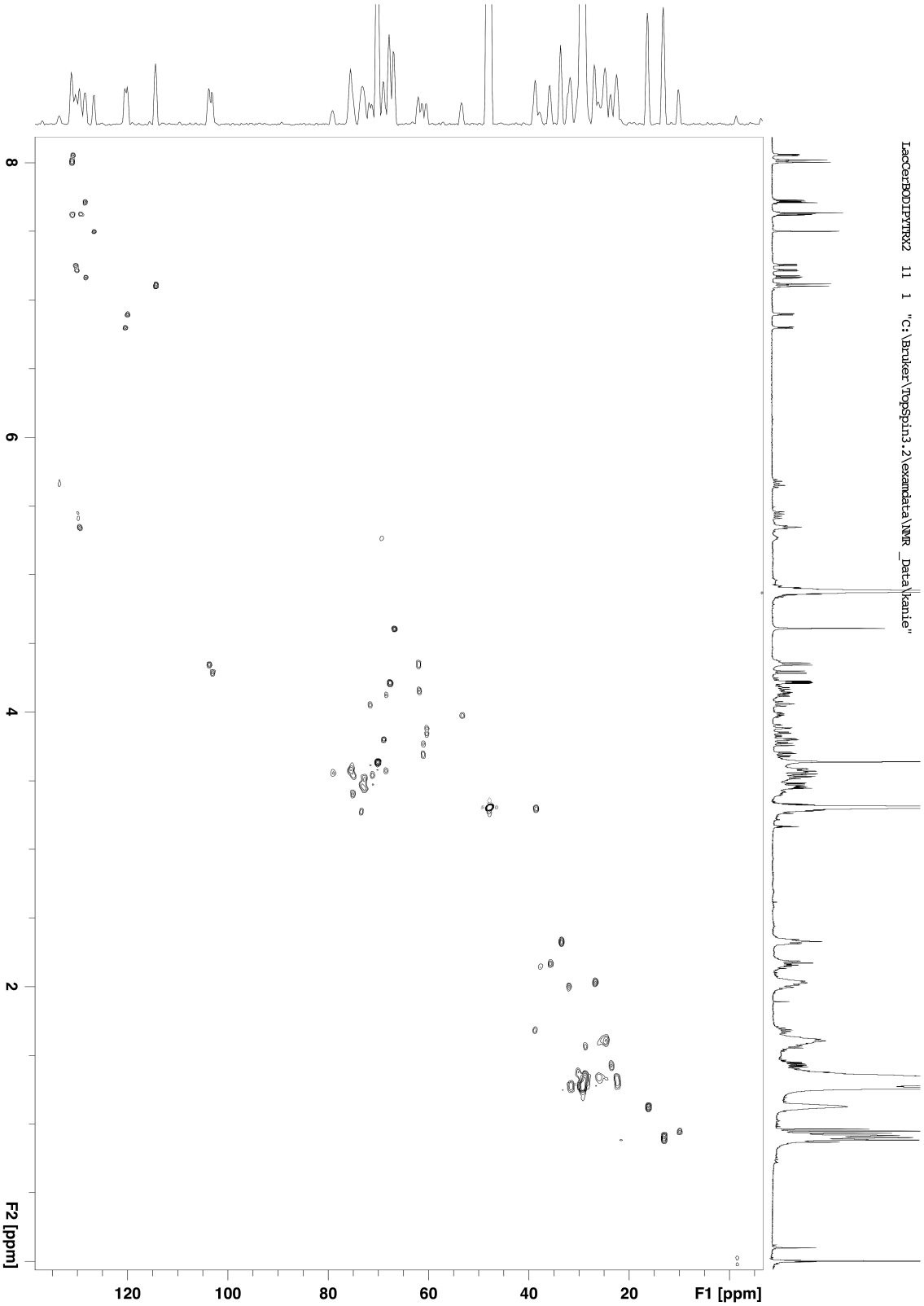

Fig. S6. HSQC NMR spectrum of compound 2.

Fig. S7. ^{1}H NMR spectrum of compound 3.

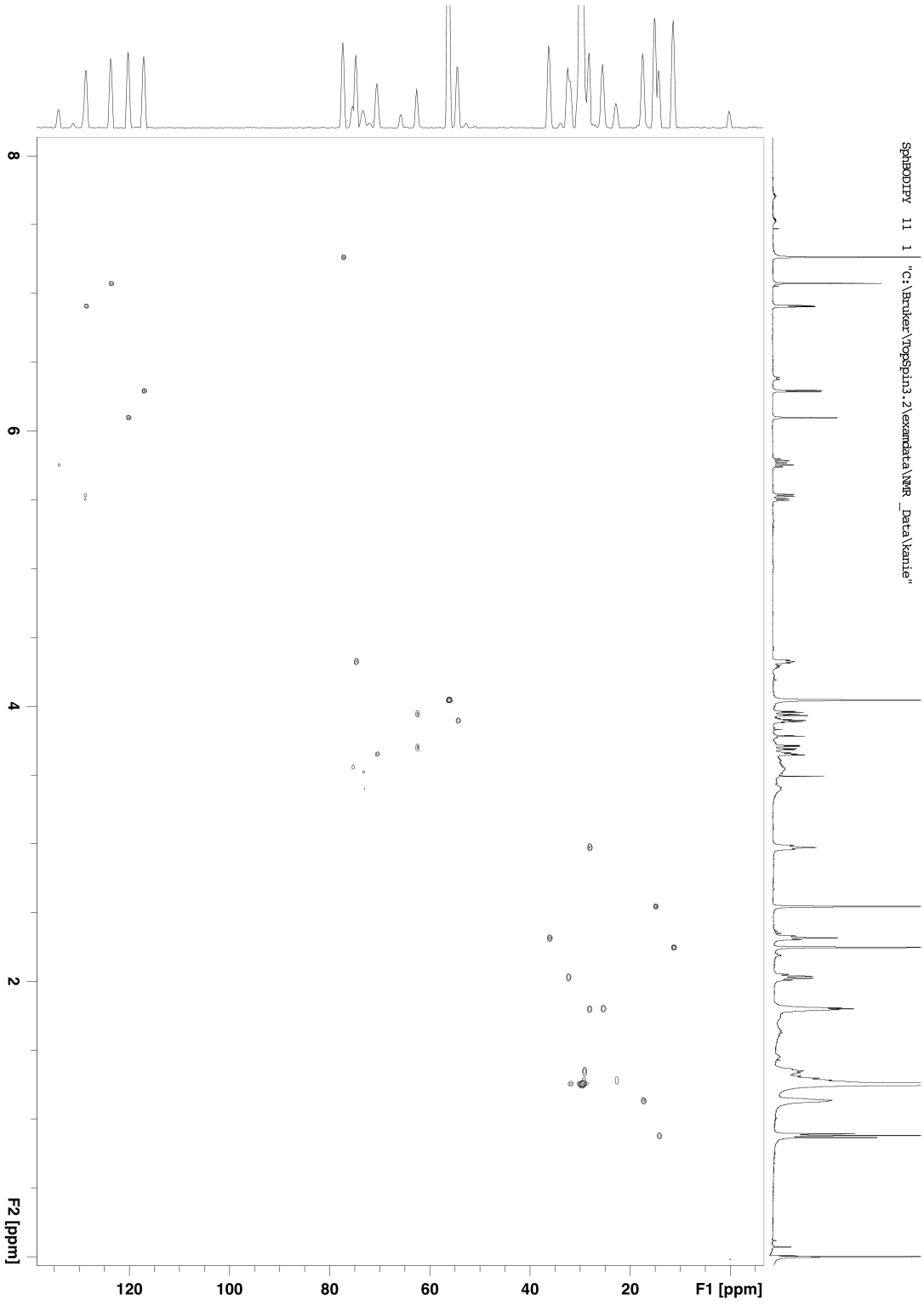

Fig. S8. HSQC NMR spectrum of compound 3.

Time study showing the internalisation speed difference depending on differentiation states.

Fluorescence derived from compound **1** could be monitored over a day. Here we show the localisation changes from pulse introduction of compound **1** over approximately three hours. The fluorescence of plasma membranes in undifferentiated PC12D cells almost disappeared after 15 minutes (Fig. S1A). In differentiated cells, the fluorescence of plasma membranes disappeared after approximately 60 minutes (Fig. S1B). The difference in internalisation rates indicates that the fluorescent probe density on the plasma membrane changed during the FRAP experiment over time.

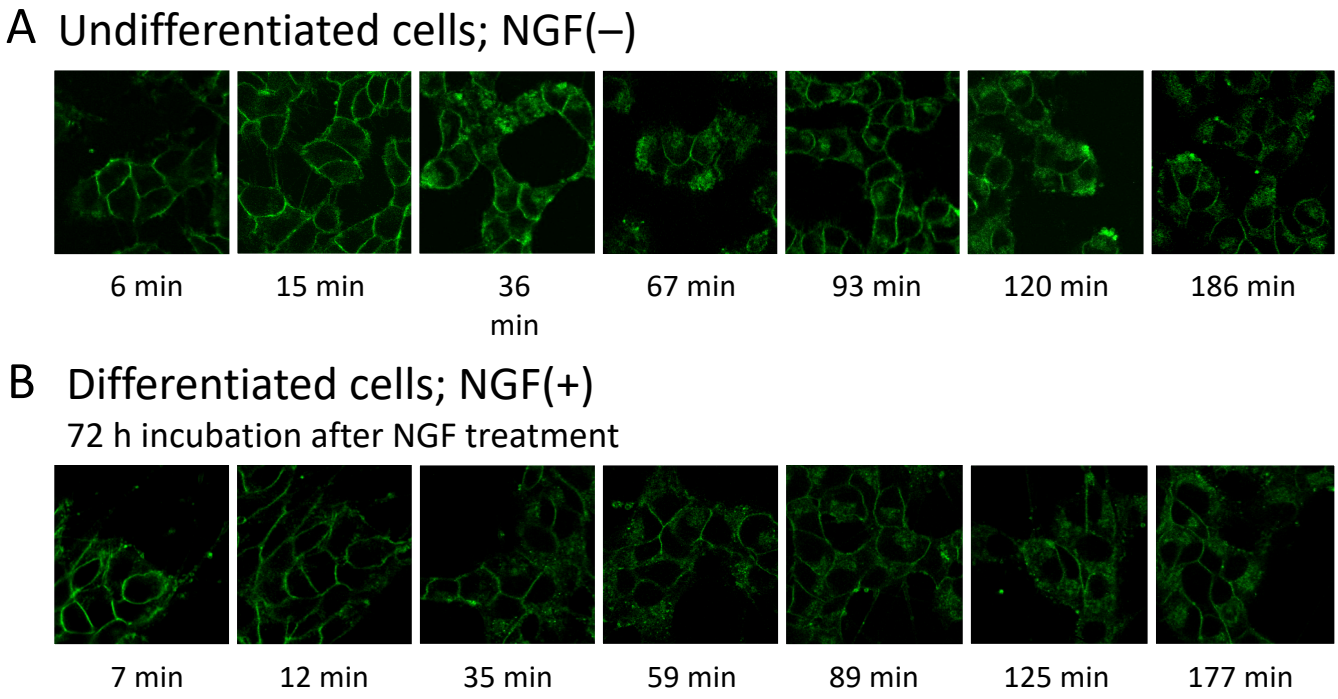

Fig. S9. Visualisation of the recycling process of the fluorescent probe.

Time course study of nerve-like projection extension on NGF treatment

PC12D neurite outgrowth was induced by NGF stimulation and the process was observed over 48 hours. Neurite length in differential images was analysed by using Image J with Neuron J plug-in. A remarkable neurite outgrowth was observed after NGF stimulation and the length of the neurite was increased in a time dependent manner (Fig. S1).

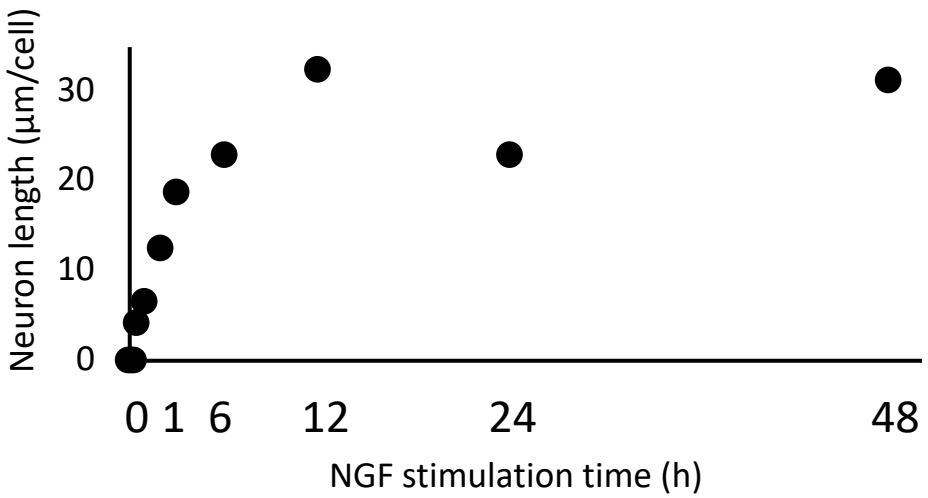

Fig. S10. The average length of neuron-like cells after NGF stimulation.