

---

**Supplementary information**

---

# **A non-canonical vitamin K cycle is a potent ferroptosis suppressor**

---

In the format provided by the  
authors and unedited

---

**Supplementary information**

---

# **A non-canonical vitamin K cycle is a potent ferroptosis suppressor**

---

In the format provided by the  
authors and unedited

## **A non-canonical vitamin K cycle is a potent ferroptosis suppressor**

Eikan Mishima\*, Junya Ito, Zijun Wu, Toshitaka Nakamura, Adam Wahida, Sebastian Doll, Wulf Tonnus, Palina Nepachalovich, Elke Eggenhofer, Maceler Aldrovandi, Bernhard Henkelmann, Ken-ichi Yamada, Jonas Wanninger, Omkar Zilka, Emiko Sato, Regina Feederle, Daniela Hass, Adriano Maida, André Santos Dias Mourão, Andreas Linkermann, Edward K. Geissler, Kiyotaka Nakagawa, Takaaki Abe, Maria Fedorova, Bettina Proneth, Derek A. Pratt & Marcus Conrad\*

**\*Correspondence:** eikan@med.tohoku.ac.jp (E.M.) or marcus.conrad@helmholtz-muenchen.de (M.C.)

### **Table of Contents:**

Supplementary Table 1. Sequences of sgRNA guides used to generate the knockout cell lines.

Supplementary Table 2. Analytical condition of FIA-MS analysis for detection of MK4-H2.

Supplementary Table 3. Summary of the potency of vitamin K for ferroptosis inhibition.

Supplementary Fig 1. Uncropped gel images.

Supplementary Fig 2. NMR spectrums of dimethylmenadione, VK-coumarin and CoQ-coumarin.

Supplementary Fig 3. An example of the gating strategy in Bodipy C-11 assay

Supplementary Video 1. Live imaging of HT1080 cells treated with RSL3 (0 - 8 h after addition of RSL3).

Supplementary Video 2. Live imaging of HT1080 cells cotreated with RSL3 + MK4 (0 - 8 h after addition of RSL3).

**Supplementary Table 1. Sequences of sgRNA guides used to generate the knockout cell lines.**

Nucleotides in italics show the overhangs introduced into oligos that are necessary for cloning in the *BsmBI* restriction sites of lentiCRISPR v2 (LCv2) or *BbsI* site of pKLVU6gRNA(*BbsI*) vectors.

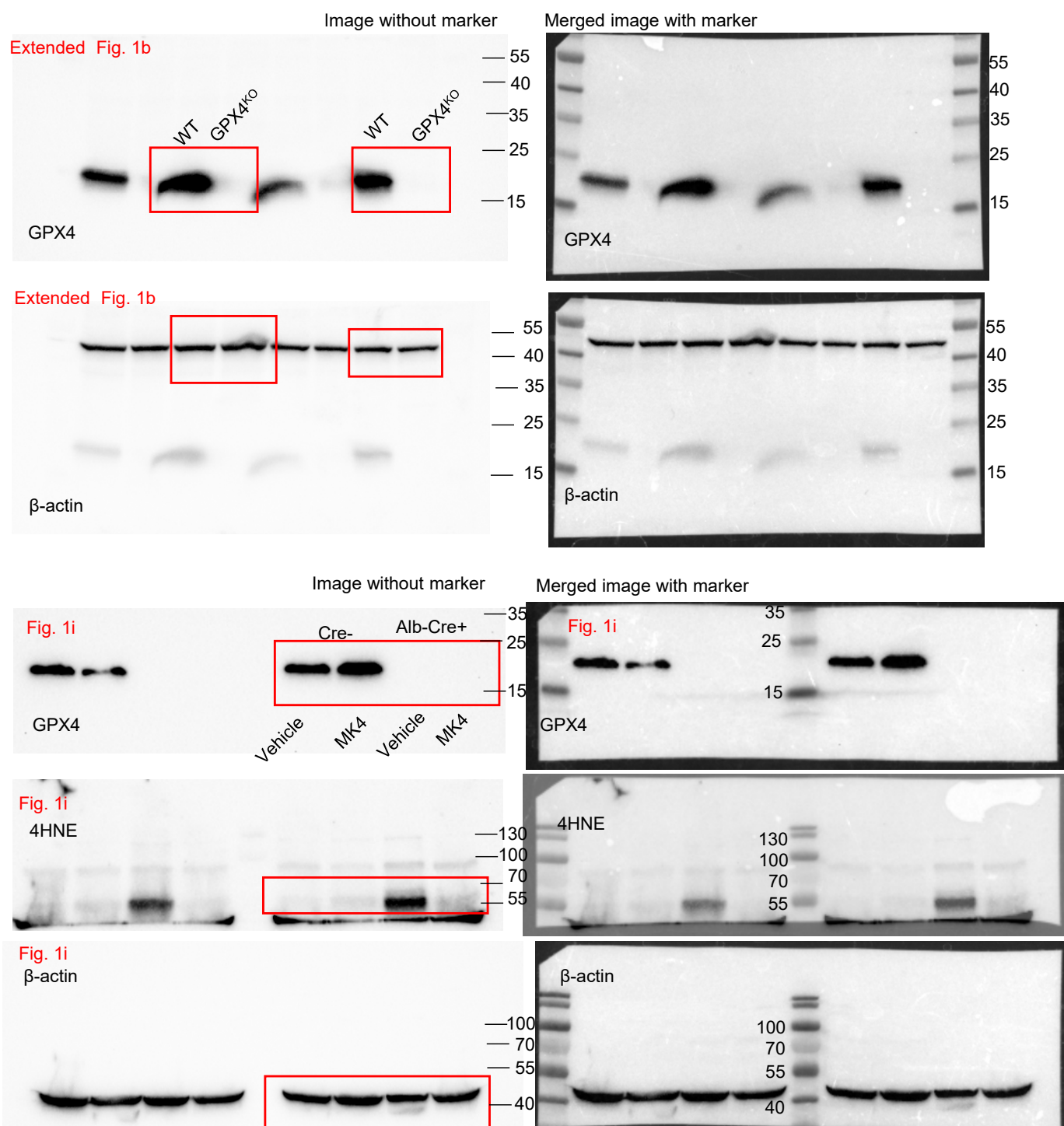
| Parental cell             | Cell name                                     | Backbone vector | Target gene  | sgRNA (5'-)                        | Cas 9         |
|---------------------------|-----------------------------------------------|-----------------|--------------|------------------------------------|---------------|
| A375                      | A375 GPX4 <sup>KO</sup>                       | LCv2_blast      | <i>hGPX4</i> | <i>caccGCGTGTGCATCGTCACCAACG</i>   | Transient     |
|                           |                                               | LCv2_puro       |              | <i>caccGCACGCCCGATACGCTGAGTG</i>   |               |
| 786-O                     | 786-O GPX4 <sup>KO</sup>                      | LCv2_blast      | <i>hGPX4</i> | <i>caccGCGTGTGCATCGTCACCAACG</i>   | Stable        |
|                           |                                               | LCv2_puro       |              | <i>caccGCACGCCCGATACGCTGAGTG</i>   |               |
| B16F10                    | B16F10 GPX4 <sup>KO</sup>                     | LCv2_blast      | <i>mGpx4</i> | <i>caccGCATGCCCGATATGCTGAGTG</i>   | Transient     |
|                           |                                               | LCv2_puro       |              | <i>caccGCGTGTGCATCGTCACCAACG</i>   |               |
| B16F10 GPX4 <sup>KO</sup> | B16F10 GPX4 <sup>KO</sup> /FSP1 <sup>KO</sup> | LCv2_blast      | <i>mFsp1</i> | <i>caccGCCGTGCACGTGGTGATCGT</i>    | Transient     |
|                           |                                               | LCv2_puro       |              | <i>caccGCCACGATCACCACGTGCA</i>     |               |
| HepG2                     | HepG2 FSP1 <sup>KO</sup>                      | LCv2_puro       | <i>hFSP1</i> | <i>caccGGGAATCGGGAGCTCTGCACG</i>   | Stable        |
|                           |                                               | LCv2_puro       |              | <i>caccGGGTGCAGAGAATCACCAGGT</i>   |               |
| 786-O _pCW-Cas9-blast     | 786-O FSP1 <sup>KO</sup>                      | pKLV-U6gRNA     | <i>hFSP1</i> | <i>caccGGTCTTTTTGGCGAACCCCTGgt</i> | Dox-inducible |
| HT1080 _pCW-Cas9-blast    | HT1080 FSP1 <sup>KO</sup>                     | pKLV-U6gRNA     | <i>hFSP1</i> | <i>caccGGTCTTTTTGGCGAACCCCTGgt</i> | Dox-inducible |

**Supplementary Table 2. Analytical parameters of FIA-MS analysis for the detection of MK4-H<sub>2</sub>.**

|                                   |                               |
|-----------------------------------|-------------------------------|
| Source                            | Electrospray ionization (ESI) |
| Ion polarity                      | Positive                      |
| Scan type                         | Enhanced MS                   |
| Scan rate (Da/s)                  | 10000                         |
| Mass range (m/z)                  | 200–600                       |
| Declustering potential (V)        | 60.00                         |
| Entrance potential (V)            | 10.00                         |
| Collision energy (V)              | 10.00                         |
| Collision cell exit potential (V) | 0.00                          |
| Curtain gas (psi)                 | 30                            |
| Ion spray voltage (V)             | 5500                          |
| Temperature (oC)                  | 450                           |
| Ion source gas 1 (psi)            | 60                            |
| Ion source gas 2 (psi)            | 60                            |
| Flow rate (mL/min)                | 0.4                           |
| Mobile phase                      | Methanol                      |

**Supplementary Table 3. Summary of the potency of vitamin K for ferroptosis inhibition**

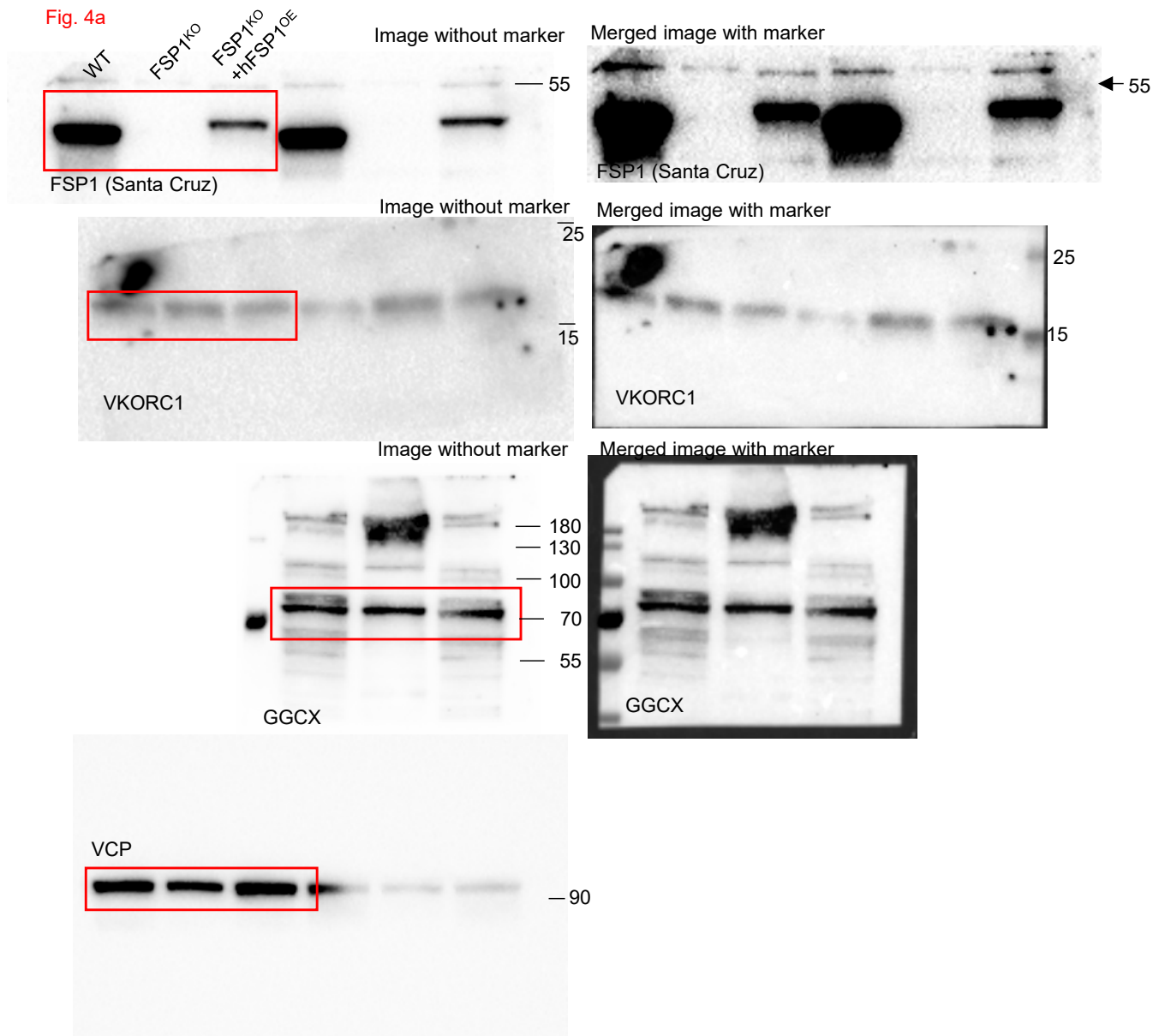
| Cells                                            | Induction of ferroptosis    | IC50 (μM) for inhibiting ferroptosis |       |       |                |
|--------------------------------------------------|-----------------------------|--------------------------------------|-------|-------|----------------|
|                                                  |                             | PK                                   | MK4   | Menad | Dimethyl-menad |
| Pfa1                                             | <i>Gpx4</i> deletion by TAM | 0.18                                 | 0.074 | 1.4   | No inhibition  |
| A375 GPX4 <sup>KO</sup>                          | Lip1 withdrawal             | 0.061                                | 0.016 | 0.47  |                |
| 786O GPX4 <sup>KO</sup>                          | Lip1 withdrawal             | 0.49                                 | 0.078 | 1.3   |                |
| 786O wt                                          | RSL3                        | 0.49                                 | 0.39  | 1.1   |                |
| 786O FSP1 <sup>KO</sup>                          | RSL3                        | 6.6                                  | 5.5   | 3.7   |                |
| 786O FSP1 <sup>KO</sup><br>+ hFSP1 <sup>OE</sup> | RSL3                        | 0.50                                 | 0.56  | 1.5   |                |



### Supplementary fig. 1. Uncropped gel images.

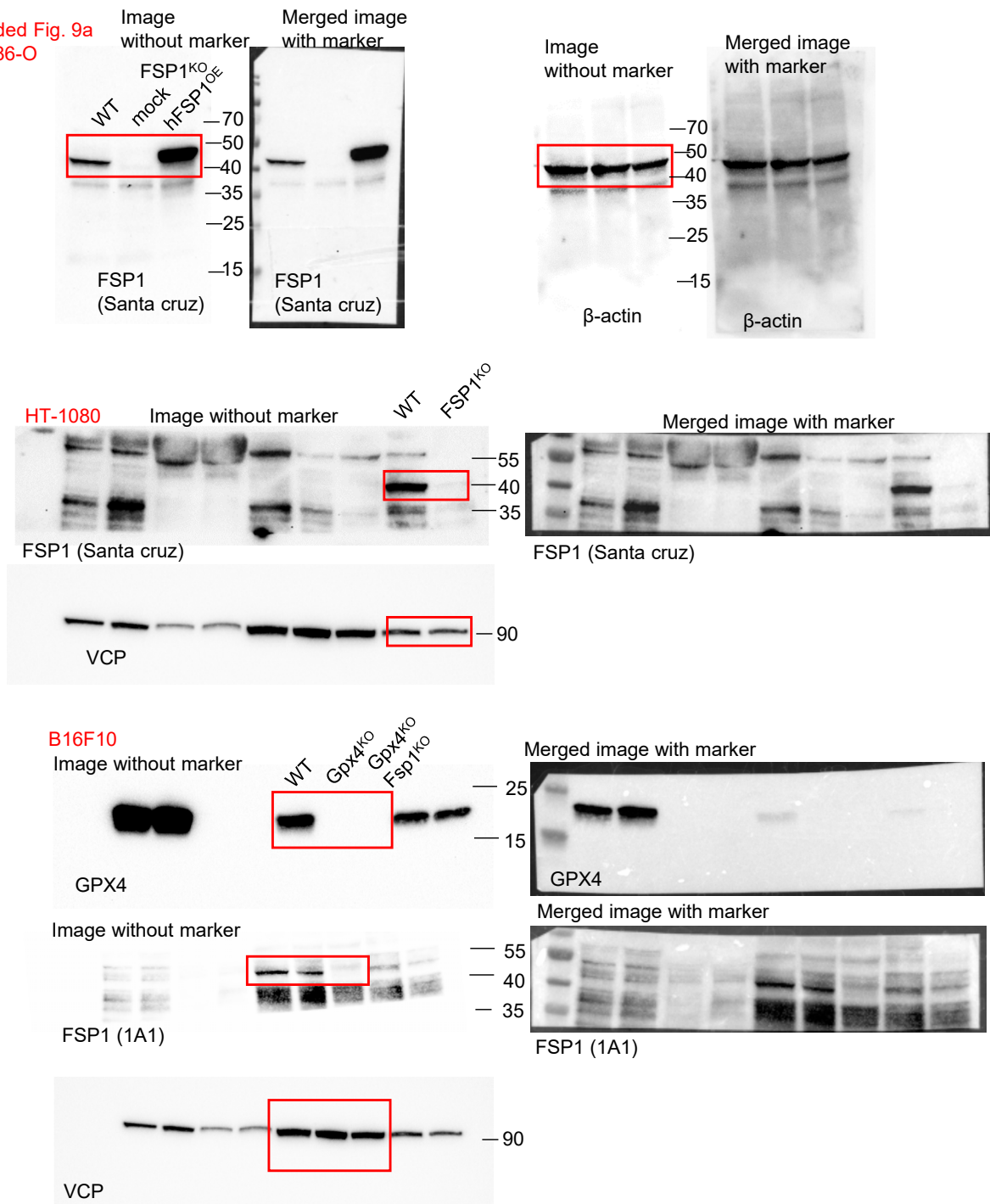
Raw images of immunoblotting and the images merged with a molecular marker image were shown. Images with the VCP antibody were shown as the raw images with the molecular mass information of 90 kD attached at the time of image acquisition. In the images with the VCP antibody, only a clear single band at the predicted molecular mass size (97 kD) can be detected across the whole membrane as shown in the raw image of extended fig. 10b.

Fig. 4a

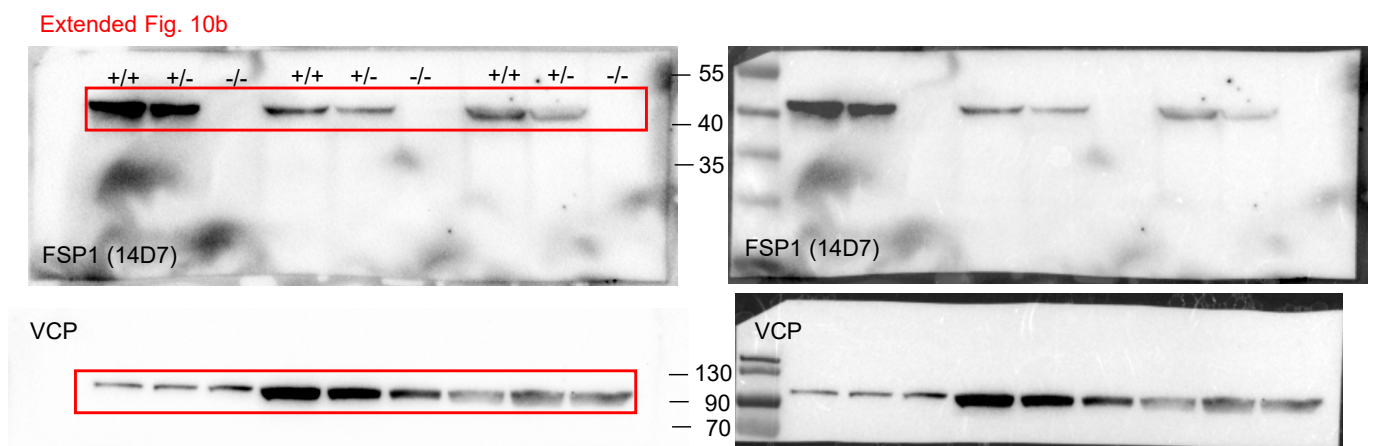
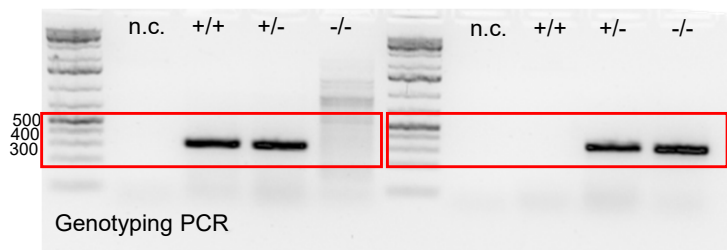
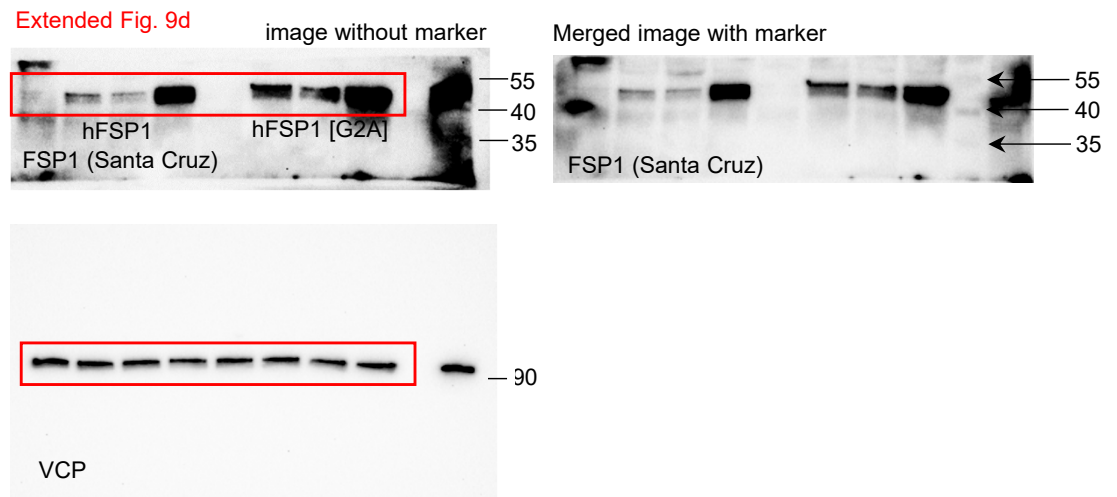
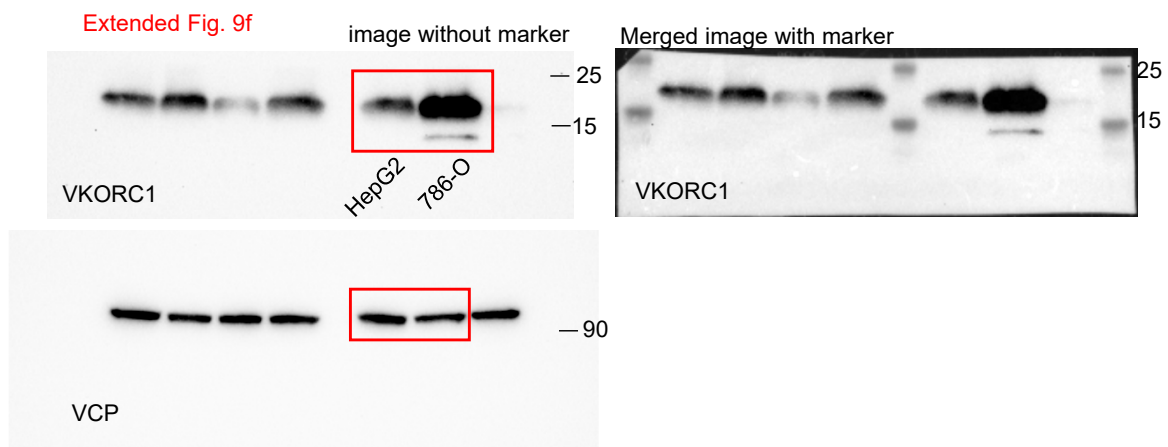


Supplementary fig. 1 (continued)

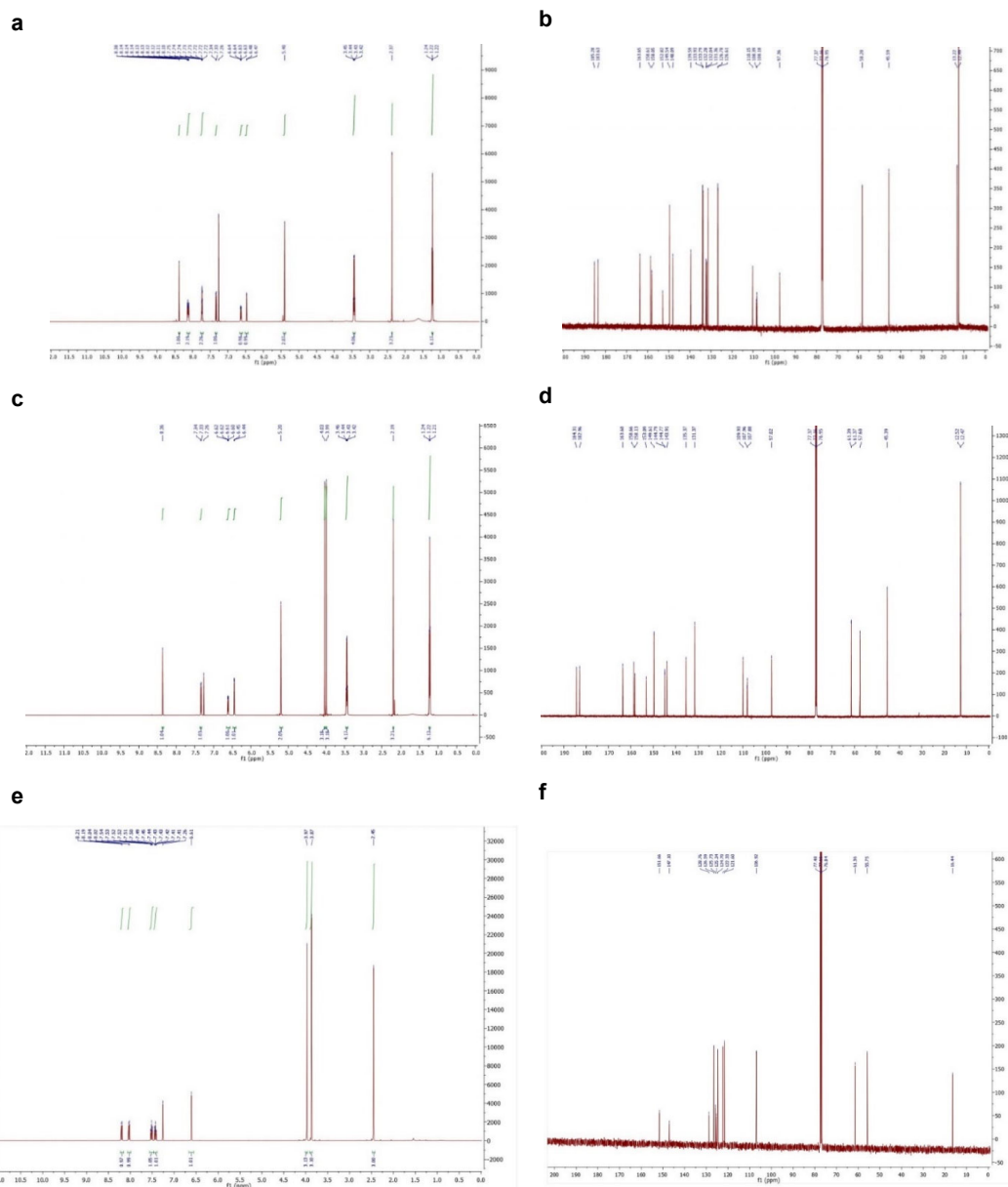
Extended Fig. 9a  
786-O



Supplementary fig. 1 (continued)

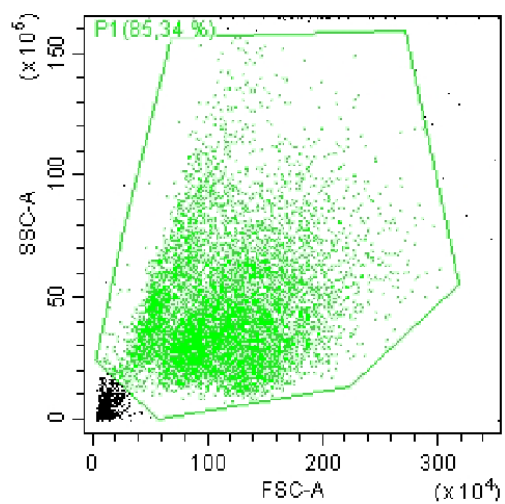


**Supplementary fig. 1 (continued)**



**Supplementary Fig 2. NMR spectra of dimethylmenadione, VK-coumarin and CoQ-coumarin.**

- <sup>1</sup>H NMR spectrum of VK-coumarin in CDCl<sub>3</sub>.
- <sup>13</sup>C NMR spectrum of VK-coumarin in CDCl<sub>3</sub>.
- <sup>1</sup>H NMR spectrum of CoQ-coumarin in CDCl<sub>3</sub>.
- <sup>13</sup>C NMR spectrum of CoQ-coumarin in CDCl<sub>3</sub>.
- <sup>1</sup>H NMR spectrum of 1,4-dimethoxy-2-methylnaphthalene in CDCl<sub>3</sub>.
- <sup>13</sup>C NMR spectrum of 1,4-dimethoxy-2-methylnaphthalene in CDCl<sub>3</sub>.



**Supplementary Fig 3. An example of the gating strategy in Bodipy C-11 assay**