

**Elevated cellular PpIX potentiates sonodynamic therapy in a mouse glioma stem cell-bearing glioma model by downregulating the Akt/NFκB/MDR1 pathway**

Kenji Shono<sup>1</sup>, Izumi Yamaguchi<sup>1</sup>, Yoshifumi Mizobuchi<sup>1</sup>, Kohei Nakajima<sup>1</sup>, Yuri Fujiwara<sup>2</sup>, Toshitaka Fujihara<sup>1</sup>, Keiko Kitazato<sup>1</sup>, Kazuhito Matsuzaki<sup>1</sup>, Yoshihiro Uto<sup>2</sup>, Oltea Ulsula<sup>3</sup>, Hideyuki Saya<sup>3</sup>, Yasushi Takagi<sup>1</sup>

| Supplemental Table1    List of antibodies used in western blotting analysis |                                    |          |         |                                 |
|-----------------------------------------------------------------------------|------------------------------------|----------|---------|---------------------------------|
| Antibodies                                                                  | Manufacturers                      | Dilution | Species | Blocking                        |
| Cleaved Caspase-3 (Asp175),                                                 | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | 5% skim milk                    |
| Cleaved Caspase-8 (Asp387)                                                  | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | 5% skim milk                    |
| Cleaved Caspase-9                                                           | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | 5% skim milk                    |
| Cleaved PARP (Asp214)                                                       | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | 5% skim milk                    |
| phospho NF-κB p65                                                           | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | 2% BSA                          |
| phospho I-κB (CST#2859)                                                     | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | Protein-free<br>(T7132A.Takara) |
| MDR-1 (Ab 170904)                                                           | Abcam, Cambridge, UK               | 1:1000   | Rabbit  | 5% skim milk                    |
| AKT2 (CST#2964)                                                             | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | 2% BSA                          |
| Phospho AKT (CST#4060)                                                      | Cell Signaling Technology, MA, USA | 1:1000   | Rabbit  | 2% BSA                          |
| β-actin                                                                     | Sigma-Aldrich, MO, USA             | 1:5000   | Mouse   | 5% skim milk                    |

Supplemental Table 2 List of antibodies used in immunohistochemistry

| Antibodies                 | Manufacturers                      | Dilution | Species | Blocking            |
|----------------------------|------------------------------------|----------|---------|---------------------|
| Cleaved Caspase-3 (Asp175) | Cell Signaling Technology, MA, USA | 1:100    | Rabbit  | Protein block, DAKO |
| Cleaved Caspase-8 (Asp387) | Cell Signaling Technology, MA, USA | 1:100    | Rabbit  | Protein block, DAKO |
| Cleaved Caspase-9          | Cell Signaling Technology, MA, USA | 1:100    | Rabbit  | Protein block, DAKO |
| Cleaved PARP (Asp214)      | Cell Signaling Technology, MA, USA | 1:100    | Rabbit  | Protein block, DAKO |
| MDR1 (Ab170904)            | Abcam, Cambridge, UK               | 1:100    | Rabbit  | Protein block, DAKO |
| COX2 ( <b>ab15191</b> )    | Abcam, Cambridge, UK               | 1:100    | Rabbit  | Protein block, DAKO |