## Supplementary information

ABCG2 regulates self-renewal and stem cell marker expression but not tumorigenicity or radiation resistance of glioma cells

Boyoung Wee<sup>1,2,11</sup>, Alexander Pietras<sup>3,4,5,11</sup>, Tatsuya Ozawa<sup>3,4</sup>, Elena Bazzoli<sup>1,2</sup>, Ondrej Podlaha<sup>6,7</sup>, Christophe Antczak<sup>8,9</sup>, Bengt Westermark<sup>10</sup>, Sven Nelander<sup>10</sup>, Lene Uhrbom<sup>10</sup>, Karin Forsberg-Nilsson<sup>10</sup>, Hakim Djaballah<sup>8</sup>, Franziska Michor<sup>6,7</sup>, and Eric C. Holland<sup>3,4</sup>

<sup>1</sup>Cancer Biology and Genetics Program, <sup>2</sup>Brain Tumor Center, <sup>8</sup>HTS Core Facility, Memorial Sloan Kettering Cancer Center, New York, NY 10021, USA; <sup>3</sup>Human Biology Division, Solid Tumor and Translational Research, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA; <sup>4</sup>Neurosurgery and Alvord Brain Tumor Center, University of Washington, Seattle, WA 98104, USA; <sup>5</sup>Translational Cancer Research, Department of Laboratory Medicine, Lund University, SE-22363 Lund, Sweden; <sup>6</sup>Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA; <sup>7</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02215, USA; <sup>9</sup>Current address: Novartis Institutes for Biomedical Research, Center for Proteomic Chemistry, 250 Massachusetts Avenue, Cambridge, MA 02139, USA; <sup>10</sup>Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Rudbeck Laboratory, Uppsala University, 751 85 Uppsala, Sweden

<sup>11</sup>Co-first authors

## **Supplementary Information**



**Suppl. Fig. S1. Establishing U87MG cell line with high ABCG2 activity and primary cultures with tet-off-ABCG2.** (A-B) U87MG cell line with high ABCG2 activity was generated by retroviral transduction of pbabe-ABCG2 and double sorting for SP fraction. (C-D) Tet-off-ABCG2 was generated by stably expressing tet-regulatory element and tet-off-ABCG2. As low as 1 ng of doxycycline was enough to reduce significantly reduce ABCG2 protein levels.



**Suppl. Fig. S2. Differentiation and stem cell marker expression following FTC treatment.** Additional high-resolution and higher-magnification images of cells presented and studied in Fig. 1g. Blue=DAPI, red=indicated protein, green=GFP (applies to Tuj1, CNPase, S100b).

## Suppl. Table S1. List of qRT-PCR primers used in the study

Gene	Forward Primer 5´->3´	Reverse Primer 5´->3´
svmbol		
-,		
MEF/ELF	5'-TGGAAGGCAGTTTTTTGCTGA-3'	5'-GACTTCCGCGGTTGACATG-3'
4		
(human)		
SOX2	5'-GCCTGGGCGCCGAGTGGA-3'	5'-GGGCGAGCCGTTCATGTAGGTCTG-3'
(human)		
( )		
POU5F1	5'-GCTCGAGAAGGATGTGGTCC -3'	5'-CGTTGTGCATAGTCGCTGCT -3'
4		
(human)		
HES1	5'-IGGAAAIGACAGIGAAGCACCI-	5'-GTTCATGCACTCGCTGAAGC-3'
(human)	3'	
HEY1	5'-CGAAATCCCAAACTCCGATA-3'	5'-TGGATCACCTGAAAATGCTG-3'
(human)		
(naman)		
HEY2	5'-TTGTCAGTATCAGCCACGTC-3'	5'-AGTTACCGAGCTGCCTTGAA-3'
(human)		
ID1	Purchased from Qiagen	
(human)	(QT00230650)	
ID4	5'-GTGCGATATGAACGACTGCT-3'	5'-CAGGATCTCCACTTTGCTGA-3'
(human)		
(naman)		
185	5'-GTAACCCGTTGAACCCCATT-3'	5'-CCATCCAATCGGTAGTAGCG-3'
(human)		
Mef/Elf4	5'-	5'-ATGGTGCTGCCTTTGCCATC-3'
(mouse)	TCCTGGATGAGAAGCAGATCTTCA	
	-3'	

Sox2	5'-	5'-GAAGTGCAATTGGGATGAAAA-3'
(mouse)	TCCAAAAACTAATCACAACAATCG-	
	3'	
Pou5f1	5'-GTTGGAGAAGGTGGAACCAA-3'	5'-CTCCTTCTGCAGGGCTTTC-3'
(mouse)		
Hes1	5'-TGCCAGCTGATATAATGGAGAA-	5'-CCATGATAGGCTTTGATGACTTT-3'
(mouse)	3'	
Hey1	5'-CATGAAGAGAGCTCACCCAGA-	5'-CGCCGAACTCAAGTTTCC-3'
(mouse)	3'	
ld1	Purchased from Qiagen	
(mouse)	(QT01743756)	
Klt4	5'-	5'-IGCCGTCTGGGCTTCCTTIGCT-3'
(mouse)	GCGGGCTGATGGGCAAGTTTGT-3'	
- 14		5' A00400040700040044 0'
смус	5- GACAGCAGCICGCCCAAAI -3	5 - AGCAGCGAGTCCGAGGAA -3
(mouse)		
Candh	5' TOOCAAAOTOOAOATTOTTOOO	5' AAGATGGTGATGGGGTTGGGG 2'
Gapon	5-IGUCAAAGIGGAGAIIGIIGUU-	5-AAGATGGTGATGGGCTTCCCG-3
(mouse)	3'	