**Table S1** Primers used for quantitative real-time RT-PCR (qRT-PCR)

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| --- | --- | --- |
| Gene name | Primer name | Primers sequences (5’-3’) |
| β-actin | Forw  Rev | CACCATTGGCAATGAGCGGTTCC  GTAGTTTCGTGGATGCCACAGG |
| CT45A | Forw  Rev | TCGAAATGCTTGAAGGAGTG  TACATCTTGCTGCTTCCTTGA |
| UEV1A | Forw  Rev | GAGAGGTTCAAGCGTCTTACCTGAA  ACTGTGCCATCTCCTACTCCTTTCT |
| TWIST1 | Forw  Rev | CATCCTCACACCTCTGCATT  TTCCTTTCAGTGGCTGATTG |
| ALDH1A1 | Forw  Rev | GATCCAGGGCCGTACAATAC  CAAATGAGCATAACCAACGG |
| KIT | Forw  Rev | ACAAAGAGCAAATCCATCCC  CAATAATGCACATCATGCCA |
| MAGED4B | Forw  Rev | GGAGAGGGCAAATAAGTTGG  CGTTGCTCGTTCAATGATCT |
| HOXB6 | Forw  Rev | CCAGCTACCGCTCTATTCGT  GCGGGTAATAGGAGGAAGTG |
| HOXD13 | Forw  Rev | ATACGAGCCCTTACCAGCAC  GTGCAGTACACCTGGCTGTT |
| RASGEF1A | Forw  Rev | CCATCATCTCTGGCATGAAC  GGCTGTACGGTAGTTGCAGA |
| SULF2 | Forw  Rev | GACACATCGGTGCTACATCC  AGGGTTTCAATCTCGTGGTC |
| CXCR4 | Forw  Rev | GCCCTAGCTTTCTTCCACTG  TTGGAGAGGATCTTGAGGCT |
| N-cadherin | Forw  Rev | AGCCAACCTTAACTGAGGAGT  GGCAAGTTGATTGGAGGGATG |
| E-cadherin | Forw  Rev | CCCATCAGCTGCCCAGAAAATGAA  CTGTCACCTTCAGCCATCCTGTTT |
| Vimentin | Forw  Rev | GACAATGCGTCTCTGGCACGTCTT  TCCTCCGCCTCCTGCAGGTTCTT |

**Table S2** Upregulated genes in *UEV1A*-overexpressed MDA-MB-231 breast cancer cells (fold change > 5)

|  |  |  |
| --- | --- | --- |
| Gene ID | Fold Change | Gene Description |
| 541465 | 224.6806 | cancer/testis antigen CT45-6 |
| 441521 | 131.8982 | cancer/testis antigen CT45-5 |
| 441519 | 107.5529 | cancer/testis antigen CT45-3 |
| 7032 | 86.1545 | trefoil factor 2 (spasmolytic protein 1) (TFF2) |
| 114798 | 47.0413 | SLIT and NTRK-like family, member 1 (SLITRK1) |
| 728911 | 40.7197 | cancer/testis antigen CT45-2 |
| 8722 | 35.0693 | cathepsin F (CTSF) |
| 90737 | 30.2083 | P antigen family, member 5 (prostate associated) (PAGE5) |
| 6035 | 29.5467 | ribonuclease, RNase A family, 1 (pancreatic) (RNASE1) |
| 9840 | 29.4148 | thymocyte expressed, positive selection associated 1 (TESPA1) |
| 56143 | 29.0436 | protocadherin alpha 5 (PCDHA5) |
| 2577 | 28.2247 | G antigen 5 (GAGE5) |
| 3887 | 27.9001 | keratin, hair, basic, 1 (KRTHB1) |
| 53826 | 26.7176 | FXYD domain containing ion transport regulator 6 (FXYD6) |
| 2576 | 26.147 | G antigen 4 (GAGE4) |
| 8477 | 24.2815 | G protein-coupled receptor 65 (GPR65) |
| 4102 | 23.1769 | melanoma antigen family A, 3 (MAGEA3) |
| 4105 | 22.627 | melanoma antigen family A, 6 (MAGEA6) |
| 2574 | 21.1109 | G antigen 2 (GAGE2) |
| 26749 | 20.8572 | G antigen 2E (GAGE2E) |
| 2578 | 18.1914 | G antigen 6 (GAGE6) |
| 6347 | 16.6346 | chemokine (C-C motif) ligand 2 (CCL2) |
| 2543 | 16.5282 | G antigen 1 (GAGE1) |
| 4642 | 15.9014 | myosin ID (MYO1D) |
| 3889 | 15.615 | keratin, hair, basic, 3 (KRTHB3) |
| 196051 | 13.2882 | phosphatidic acid phosphatase type 2 domain containing 1A (PPAPDC1A) |
| 3641 | 12.7966 | insulin-like 4 (placenta) (INSL4) |
| 57447 | 11.5288 | NDRG family member 2 (NDRG2) |
| 2556 | 11.3839 | gamma-aminobutyric acid (GABA) A receptor, alpha 3 (GABRA3) |

|  |  |  |
| --- | --- | --- |
| 3294 | 9.7785 | hydroxysteroid (17-beta) dehydrogenase 2  (HSD17B2) |
| 623 | 9.6186 | bradykinin receptor B1 (BDKRB1) |
| 945 | 9.1698 | CD33 molecule (CD33) |
| 2331 | 8.8503 | fibromodulin (FMOD) |
| 9535 | 8.3232 | glia maturation factor, gamma (GMFG) |
| 84707 | 8.1616 | brain expressed X-linked 2 (BEX2) |
| 7433 | 8.1194 | vasoactive intestinal peptide receptor 1 (VIRP1) |
| 57407 | 7.8678 | HSCARG protein (HSCARG) |
| 57451 | 7.4394 | odz, odd Oz/ten-m homolog 2 (Drosophila) (ODZ2) |
| 3119 | 7.2527 | major histocompatibility complex, class II, DQ beta 1 (HLA-DQB1) |
| 7078 | 7.0654 | TIMP metallopeptidase inhibitor 3 (Sorsby fundus dystrophy, pseudoinflammatory) (TIMP3) |
| 7745 | 6.9846 | zinc finger protein 192 (ZNF192) |
| 389860 | 6.8923 | P antigen family, member 2B (PAGE2B) |
| 203569 | 6.2991 | P antigen family member 2 (PAGE2) |
| 794 | 6.1328 | calbindin 2, 29kDa (calretinin) (CALB2) |
| 55799 | 5.7369 | calcium channel, voltage-dependent, alpha 2/delta 3 subunit (CACNA2D3) |
| 6695 | 5.4644 | sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1 (SPOCK1) |
| 8862 | 5.2982 | apelin, AGTRL1 ligand |
|  |  |  |

**Supplemental figure legends**

**Figure S1 Cancer/testis antigen family CT45A identity and their expression in *UEV1A*-transfected MDA-MB-231 cells.** (**A**) The altered expression of genes encoding cancer/testis antigens in *UEV1A*-overexpressed MDA-MB-231 cells extracted from microarray data. The red line indicates 25-fold induction threshold. (**B**)The amino acid sequence alignment of 10 *CT45A* family genes.

**Figure S2 The ectopic expression of *UEV1A* and *UEV1A-F38E* in (A)** MDA-MB-231 and (B) MCF7 cells as detected by western blot using an HA-tag antibody.

**Figure S3 Efficacy of depleting UEV1A and CT45A in MDA-MB-231 and MCF7 breast cancer cells.** (**A, B**) MDA-MB-231 (**A**) and MCF7 (**B**) cells were transfected with shRNA against *UEV1A* (sh*UEV1A*) and non-specific target (shCK). The transcript levels of *UEV1A* were determined by qRT-PCR. (**C, D**) MDA-MB-231 (**C**) and MCF7 (**D**) cells were transfected with siRNA against *CT45A* (si*CT45A*) and non-specific target (siCK). The transcript levels of *CT45A* were determined by qRT-PCR. All experiments were performed in at least triplicate and the results are the average with standard deviation. \*\*, *P*<0.01.

**Figure S4 Relative *UEV1A* and *CT45A* mRNA levels in HCT116 colorectal cells.** (**A**)HCT116 cells were transfected with shRNA against *UEV1A* (sh*UEV1A*) and non-specific target (shCK). The *UEV1A* transcript levels were determined by qRT-PCR. (**B,C**) The expression levels of *CT45A* (**B**) and *UEV1A* (**C**) in HCT116 cells overexpressing *UEV1A* and depleting *CT45A* were monitored by qRT-PCR. All experiments were performed in at least triplicate and the results are the average with standard deviation. \*\*, *P*<0.01.

**Figure S5 Inhibition of** **the NF-κB pathway by Bay11-7082 treatment.** (**A,C**) MDA-MB-231 (**A**) and HCT116 (**C**) cells transiently transfected with *UEV1A* were treated with or without 40 μM Bay11-7082 for 4 hours. The nuclear fraction (N) was then prepared for western blot analysis using an anti-p65 antibody to assess the nuclear translocation of p65, while the whole cell extract (WCE) was prepared to monitor Uev1A levels by western blot against an HA-tag antibody. (**B,D**) The *CT45A* mRNA levels in MDA-MB-231 (**B**) and HCT116 (**D**) cells transiently transfected with *UEV1A* were treated with or without 40 μM Bay11-7082 followed by qRT-PCR. All experiments were performed in at least triplicate and the results are the average with standard deviation. \*\*, *P*<0.01.

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Figure S1

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Figure S2

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Figure S4

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Figure S5