**supplementary file 1a**

**The top candidate interacting proteins of TIPE identified by mass spectrometry**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Accession** | **Protein name** | **Gene name** | **Coverage(%)** | **#Peptides** |
| P35527 | Keratin type I cytoskeletal 9 | KRT9 | 54 | 29 |
| P60709 | Actin cytoplasmic 1 | ACTB | 45 | 12 |
| P02751 | Fibronectin | FN1 | 11 | 22 |
| P68363 | Tubulin alpha-1B chain | TUBA1B | 37 | 10 |
| Q9BQE3 | Tubulin alpha-1C chain | TUBA1C | 33 | 9 |
| P13647 | Keratin type II cytoskeletal 5 | KRT5 | 34 | 24 |
| P35579 | Myosin-9 | MYH9 | 6 | 6 |
| P02533 | Keratin type I cytoskeletal 14 | KRT14 | 36 | 15 |
| P21333 | Filamin-A | FLNA | 5 | 6 |
| O60832 | H/ACA ribonucleoprotein complex subunit DKC1 | DKC1 | 17 | 11 |
| P63267 | Actin gamma-enteric smooth muscle | ACTG2 | 16 | 5 |
| P68032 | Actin alpha cardiac muscle 1 | ACTC1 | 16 | 5 |
| P68133 | Actin alpha skeletal muscle | ACTA1 | 16 | 5 |
| P62736 | Actin aortic smooth muscle | ACTA2 | 16 | 5 |
| Q14498 | RNA-binding protein 39 | RBM39 | 17 | 7 |
| P11387 | DNA topoisomerase 1 | TOP1 | 12 | 8 |
| P09211 | Glutathione S-transferase P | GSTP1 | 29 | 4 |
| P05787 | Keratin type II cytoskeletal 8 | KRT8 | 23 | 14 |
| P08779 | Keratin type I cytoskeletal 16 | KRT16 | 19 | 10 |
| **P14618** | **Pyruvate kinase PKM** | **PKM** | **19** | **5** |
| Q7Z794 | Keratin type II cytoskeletal 1b | KRT77 | 10 | 9 |
| P13646 | Keratin type I cytoskeletal 13 | KRT13 | 10 | 6 |

**supplementary file 1b**

**Genes up-regulated in response to low oxygen levels (hypoxia) in melanoma**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hypoxia score was calculated by single sample GSEA (ssGSEA)** | | | | |
| |  | | --- | | *ACKR3* | | *ADM* | | *ADORA2B* | | *AK4* | | *AKAP12* | | *ALDOA* | | *ALDOB* | | *ALDOC* | | *AMPD3* | | *ANGPTL4* | | *ANKZF1* | | *ANXA2* | | *ATF3* | | *ATP7A* | | *B3GALT6* | | *B4GALNT2* | | *BCAN* | | *BCL2* | | *BGN* | | *BHLHE40* | | *BNIP3L* | | *BRS3* | | *BTG1* | | *CA12* | | *CASP6* | | *CAV1* | | *CAVIN1* | | *CAVIN3* | | *CCN1* | | *CCN2* | | *CCN5* | | *CCNG2* | | *CDKN1A* | | *CDKN1B* | | *CDKN1C* | | *CHST2* | | *CHST3* | | *CITED2* | | *COL5A1* | | *CP* | | |  | | --- | | *CSRP2* | | *CXCR4* | | *DCN* | | *DDIT3* | | *DDIT4* | | *DPYSL4* | | *DTNA* | | *DUSP1* | | *EDN2* | | *EFNA1* | | *EFNA3* | | *EGFR* | | *ENO1* | | *ENO2* | | *ENO3* | | *ERO1A* | | *ERRFI1* | | *ETS1* | | *EXT1* | | *F3* | | *FAM162A* | | *FBP1* | | *FOS* | | *FOSL2* | | *FOXO3* | | *GAA* | | *GALK1* | | *GAPDH* | | *GAPDHS* | | *GBE1* | | *GCK* | | *GCNT2* | | *GLRX* | | *GPC1* | | *GPC3* | | *GPC4* | | *GPI* | | *GRHPR* | | *GYS1* | | *HAS1* | | |  | | --- | | *HDLBP* | | *HEXA* | | *HK1* | | *HK2* | | *HMOX1* | | *HOXB9* | | *HS3ST1* | | *HSPA5* | | *IDS* | | *IER3* | | *IGFBP1* | | *IGFBP3* | | *IL6* | | *ILVBL* | | *INHA* | | *IRS2* | | *ISG20* | | *JMJD6* | | *JUN* | | *KDELR3* | | *KDM3A* | | *KIF5A* | | *KLF6* | | *KLF7* | | *KLHL24* | | *LALBA* | | *LARGE1* | | *LDHA* | | *LDHC* | | *LOX* | | *LXN* | | *MAFF* | | *MAP3K1* | | *MIF* | | *MT1E* | | *MT2A* | | *MXI1* | | *MYH9* | | *NAGK* | | *NCAN* | | |  | | --- | | *NDRG1* | | *NDST1* | | *NDST2* | | *NEDD4L* | | *NFIL3* | | *NOCT* | | *NR3C1* | | *P4HA1* | | *P4HA2* | | *PAM* | | *PCK1* | | *PDGFB* | | *PDK1* | | *PDK3* | | *PFKFB3* | | *PFKL* | | *PFKP* | | *PGAM2* | | *PGF* | | *PGK1* | | *PGM1* | | *PGM2* | | *PHKG1* | | *PIM1* | | *PKLR* | | *PKP1* | | *PLAC8* | | *PLAUR* | | *PLIN2* | | *PNRC1* | | *PPARGC1A* | | *PPFIA4* | | *PPP1R15A* | | *PPP1R3C* | | *PRDX5* | | *PRKCA* | | *PYGM* | | *RBPJ* | | *RORA* | | *RRAGD* | | |  | | --- | | *S100A4* | | *SAP30* | | *SCARB1* | | *SDC2* | | *SDC3* | | *SDC4* | | *SELENBP1* | | *SERPINE1* | | *SIAH2* | | *SLC25A1* | | *SLC2A1* | | *SLC2A3* | | *SLC2A5* | | *SLC37A4* | | *SLC6A6* | | *SRPX* | | *STBD1* | | *STC1* | | *STC2* | | *SULT2B1* | | *TES* | | *TGFB3* | | *TGFBI* | | *TGM2* | | *TIPARP* | | *TKTL1* | | *TMEM45A* | | *TNFAIP3* | | *TPBG* | | *TPD52* | | *TPI1* | | *TPST2* | | *UGP2* | | *VEGFA* | | *VHL* | | *VLDLR* | | *WSB1* | | *XPNPEP1* | | *ZFP36* | | *ZNF292* | |

**supplementary file 1c**

**Limiting dilution data**

|  |  |  |  |
| --- | --- | --- | --- |
| **A375** | **Cell number of injection** | **Ctrl-sh** | **TIPE-sh** |
|  | 10000 | 2/5 | 0/5 |
| **Frequency** | 100000 | 5/5 | 2/5 |
|  | 100000 | 5/5 | 5/5 |

**supplementary file 1d**

**Confidence intervals for 1/ (****stem cell frequency)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **A375** | **Lower** | **Estimate** | **Upper** | ***P* value** |
| **Ctrl-sh** | 60864 | 18441 | 5588 | *P*<0.001 |
| **TIPE-sh** | 624917 | 202749 | 65780 |

**supplementary file 1e** **Primer or siRNA sequences**

|  |  |
| --- | --- |
| Primer sequences | |
| *NANOG* | F: AATACCTCAGCCTCCAGCAGATG  R: TGCGTCACACCATTGCTATTCTTC | |
| *NOTCH1* | F: CCTGAGGGCTTCAAAGTGTC  R: CGGAACTTCTTGGTCTCCAG | |
| *POU5F1* | F: CTTGCTGCAGAAGTGGGTGGAGGAA  R: CGGAACTTCTTGGTCTCCAG | |
| *SOX2* | F: AAATGGGAGGGGTGCAAAAGAGGAG  R: CAGCTGTCATTTGCTGTGGGTGATG | |
| *BMI-1* | F: TGGAGAAGGAATGGTCCACTTC  R: CAGCTGTCATTTGCTGTGGGTGATG | |
| *TIPE* | F: TTCAGGCCTCCCTCTTTAACAATC  R: CGTTCGTGGCAGGGGTTATT | |
| *HIF-1α* | F: CCAGTTAGGTTCCTTCGATCAGT  R: TTTGAGGACTTGCGCTTTCA | |
| *PKM2* | F: TTGCAGCTATTCGAGGAACTCCG  R: CACGATAATGGCCCCACTGC | |
| *18S rRNA* | F: CGGCTACCAC ATCCAAGGAA  R: GCTGGAATTACCGCGGCT | |
| *NES* | F: AGCTGGCGCACCTCAAGATGT  R: CCTGAAAGCTGAGGGAAGTCT | |
| *SOX10* | F: TCATGGTGTGGGCTCAGGCA  R: CGCTTGTCACTTTCGTTCAGC | |
| *LDHA(HRE)* | F: TTGGAGGGCAGCACCTTACTTAGA  R: GCCTTAAGTGGAACAGCTATGCTGAC | |
| *SLC2A1(HRE)* | F: CTGTAATCCCAGCTACTCGG  R: CACGATCTCGGCTCACTGTA | |
| *LDHA* | F: ATCTTGACCTACGTGGCTTGGA  R: CCATACAGGCACACTGGAATCTC | |
| *SLC2A1* | F: CGGGCCAAGAGTGTGCTAAA  R: TGACGATACCGGAGCCAATG | |
| siRNA/shRNA sequence | |
| TIPE-sh1 | gtTTCCATCAGGTGGATTATA | |
| TIPE-sh2 | ccACCTTAATAGACGACACAA | |
| PKM2-sh | Purchased from Santa Cruz Biotechnology, Inc. (#sc-62820-SH) | |

**supplementary file 1f**

**Experimental materials**

|  |  |  |
| --- | --- | --- |
| Reagent or resource | Source | Identifier |
| Antibodies for western blot or immunofluorescence staining | | |
| TIPE | Abcam | ab195810 |
| p-PKM2(Ser37) | Affinity | DF7772 |
| PKM2(Rabbit) | Bioworld | BS6443 |
| PKM2(Mouse) | Invitrogen | MA5-32976 |
| p-PKM2(Tyr105) | Affinity | DF2975 |
| Flag | Bioworld | AP0007 |
| HA | Bioworld | AP0005M |
| His | Zenbio | 350175 |
| CD44, APC | Multisciences | AH04405 |
| LDH | Bioworld | MB63796 |
| HIF-1α(Mouse) | Cloud-Clone | MAA798Hu22 |
| HIF-1α(Rabbit) | Abcam | ab179483 |
| ERK1/2 | CST | #5013 |
| p-ERK1/2 | CST | #4370 |
| Lamin B1 | Proteintech | 12987-1-AP |

**supplementary file 1g**

**The** **clinicopathological characteristics of 48 melanoma specimens**

|  |  |
| --- | --- |
| **Parameters** | **Number of cases (%)** |
| **Gender** |  |
| Female | 25 (52.08%) |
| Male | 23 (47.92%) |
| **Age (years)** |  |
| < 60 | 25 (52.08%) |
| ≥ 60 | 23 (47.92%) |
| **Pathological type** |  |
| Malignant melanoma | 48 (100.00%) |
| **T classification** |  |
| T3 | 4 (8.33%) |
| T4 | 28 (58.33%) |
| Data missing | 16(33.33%) |
| **N classification** |  |
| N0 | 22 (45.83%) |
| N1 | 10 (20.83%) |
| Data missing | 16(33.33%) |
| **Clinical stage** |  |
| II | 27 (56.25%) |
| III | 1 (2.08%) |
| IV | 4 (8.33%) |
| Data missing | 16(33.33%) |