**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**

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| --- |
| **Hypoxia score was calculated by single sample GSEA (ssGSEA)** |
|

|  |
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| *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* |

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| *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* |

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|  |
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| *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* |

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| *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* |

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| *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* |

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**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: AATACCTCAGCCTCCAGCAGATGR: TGCGTCACACCATTGCTATTCTTC |
| *NOTCH1* | F: CCTGAGGGCTTCAAAGTGTCR: CGGAACTTCTTGGTCTCCAG |
| *POU5F1* | F: CTTGCTGCAGAAGTGGGTGGAGGAAR: CGGAACTTCTTGGTCTCCAG |
| *SOX2* | F: AAATGGGAGGGGTGCAAAAGAGGAGR: CAGCTGTCATTTGCTGTGGGTGATG |
| *BMI-1* | F: TGGAGAAGGAATGGTCCACTTCR: CAGCTGTCATTTGCTGTGGGTGATG |
| *TIPE* | F: TTCAGGCCTCCCTCTTTAACAATCR: CGTTCGTGGCAGGGGTTATT |
| *HIF-1α* | F: CCAGTTAGGTTCCTTCGATCAGTR: TTTGAGGACTTGCGCTTTCA |
| *PKM2* | F: TTGCAGCTATTCGAGGAACTCCGR: CACGATAATGGCCCCACTGC |
| *18S rRNA* | F: CGGCTACCAC ATCCAAGGAAR: GCTGGAATTACCGCGGCT |
| *NES* | F: AGCTGGCGCACCTCAAGATGTR: CCTGAAAGCTGAGGGAAGTCT |
| *SOX10* | F: TCATGGTGTGGGCTCAGGCAR: CGCTTGTCACTTTCGTTCAGC |
| *LDHA(HRE)* | F: TTGGAGGGCAGCACCTTACTTAGAR: GCCTTAAGTGGAACAGCTATGCTGAC |
| *SLC2A1(HRE)* | F: CTGTAATCCCAGCTACTCGGR: CACGATCTCGGCTCACTGTA |
| *LDHA* | F: ATCTTGACCTACGTGGCTTGGAR: CCATACAGGCACACTGGAATCTC |
| *SLC2A1* | F: CGGGCCAAGAGTGTGCTAAAR: 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%) |