

Selenoprotein K deficiency inhibits melanoma by reducing calcium flux required for tumor growth and metastasis

SUPPLEMENTARY MATERIALS

Selenoprotein K immunohistology results

Patients with localized malignant melanoma	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Normal tissue	1	1	1	0	0
Tumor tissue	1	1	0	0	0

Patients with distant or regional metastatic melanoma	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Normal tissue	0	1	0	1	0
Tumor tissue	0	1	0	1	0

Glutathione peroxidase 1 immunohistology results

Patients with localized malignant melanoma	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Normal tissue	2	2	2	1	2
Tumor tissue	2	2	2	2	2

Patients with distant or regional metastatic melanoma	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Normal tissue	2	2	1	1	2
Tumor tissue	2	2	1	1	2

Glutathione peroxidase 4 immunohistology results

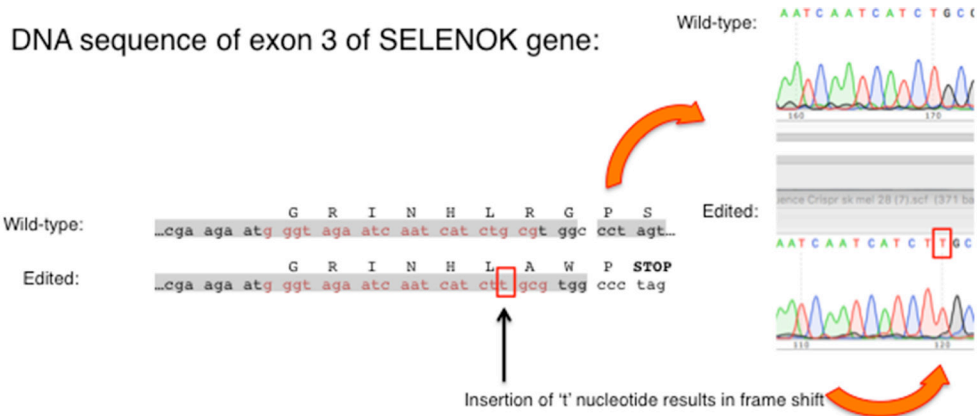
Patients with localized malignant melanoma	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Normal tissue	1	2	1	1	1
Tumor tissue	2	2	1	1	1

Patients with distant or regional metastatic melanoma	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Normal tissue	1	1	1	1	1
Tumor tissue	2	2	1	2	1

Scale

- 0 Negative
- 1 Positive - Weak in at least 25% of the cells
- 2 Positive - Medium in at least 25% of the cells
- 3 Positive - Strong in at least 25% of the cells

Supplementary Figure 1: Results from immunohistochemical (IHC) evaluation of early and late stage melanoma tissues obtained from the University of Hawaii Cancer Center (UHCC). IHC was performed and scored by the UHCC Pathology core for SELENOK along with two other selenoproteins, glutathione peroxidase-1 and -4. Results suggest no changes in SELENOK levels in tumor tissues compared to normal tissue.

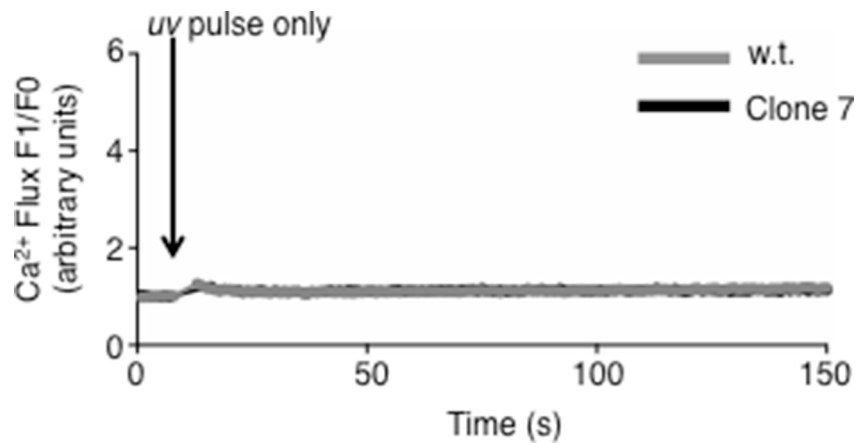


Protein resulting from gene editing:

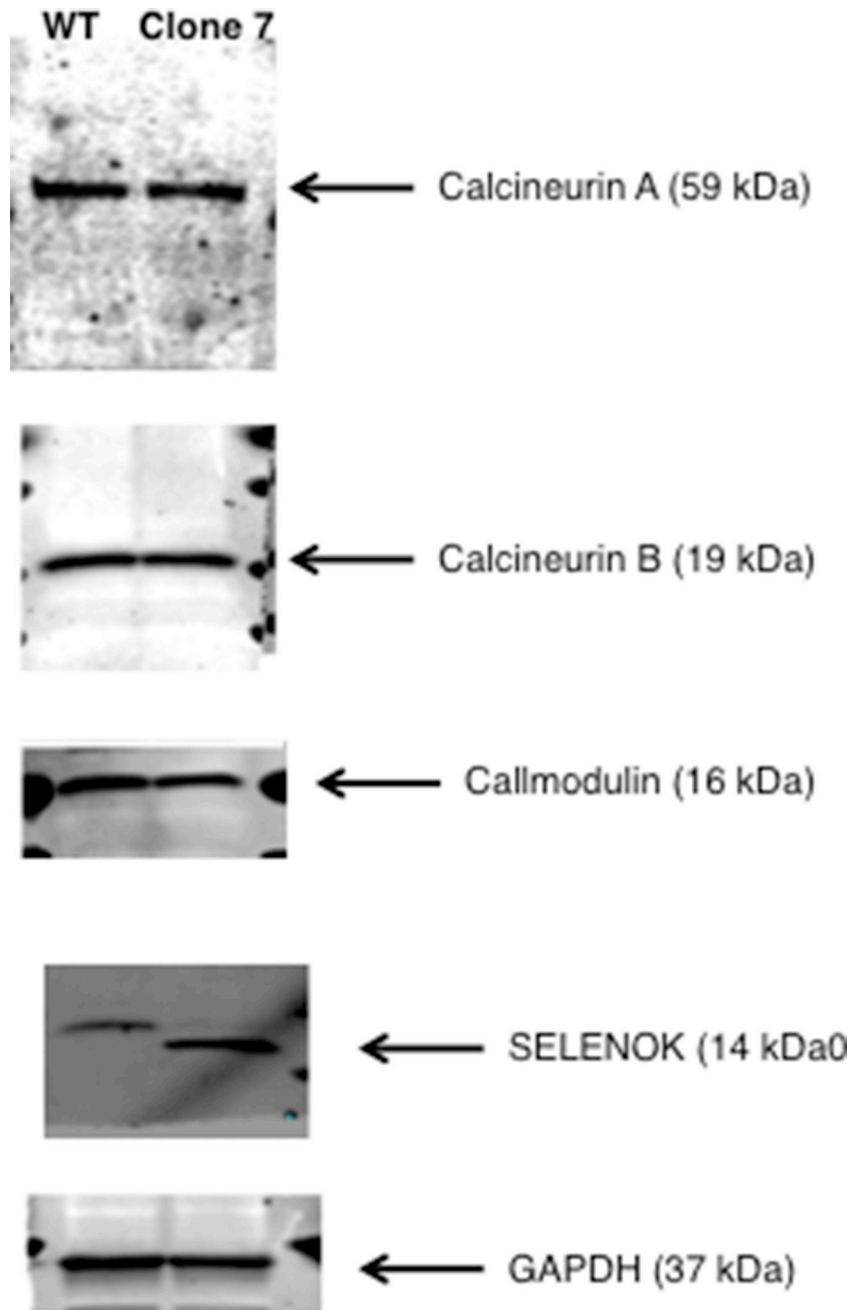
Wild-type: 1 M V Y I S N G Q V L D S R S Q S P W R L S L I T D F F W G I A E F V V L F F K T L L Q Q D V K K R R S Y G N S S D S R Y D D G R G P P G N P P R R M G R I N H L R G P S P P P M A G G U G R 94

Edited: 1 M V Y I S N G Q V L D S R S Q S P W R L S L I T D F F W G I A E F V V L F F K T L L Q Q D V K K R R S Y G N S S D S R Y D D G R G P P G N P P R R M G R I N H L **AWP** 80-83

Supplementary Figure 2: Sanger sequencing results for edited clones compared to w.t. SK-MEL-28 melanoma cells. CRISPR/Cas9 editing led to introduction of a thymidine nucleotide causing a frame shift in DNA. This resulted in altered codons corresponding to amino acids 80–83 followed by a stop codon leading to a truncated SELENOK protein lacking the functional domain.

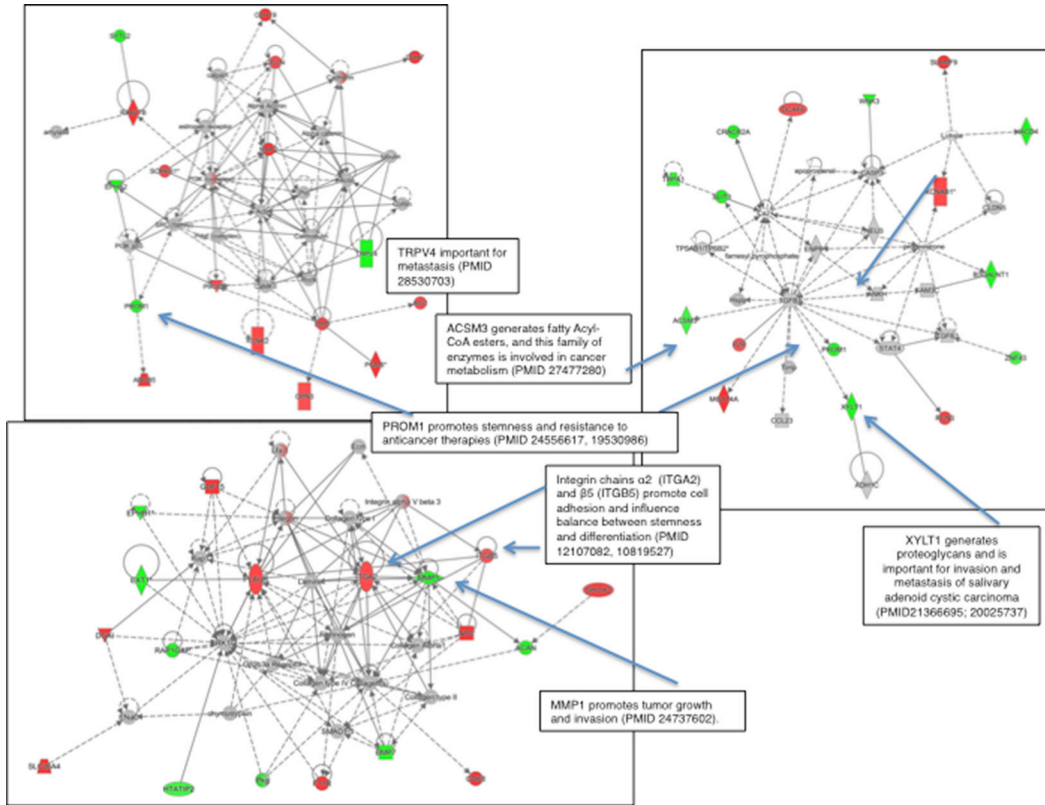


Supplementary Figure 3: SELENOK-deficient cells (Clone 7) compared to wild-type SK-MEL-28 cells were loaded with FuraRed along with no caged IP3 to determine effects of the 2 s uv pulse on Ca²⁺ flux. No caged IP3 showed minimal Ca²⁺ flux, which indicates uv alone does not induce a response.

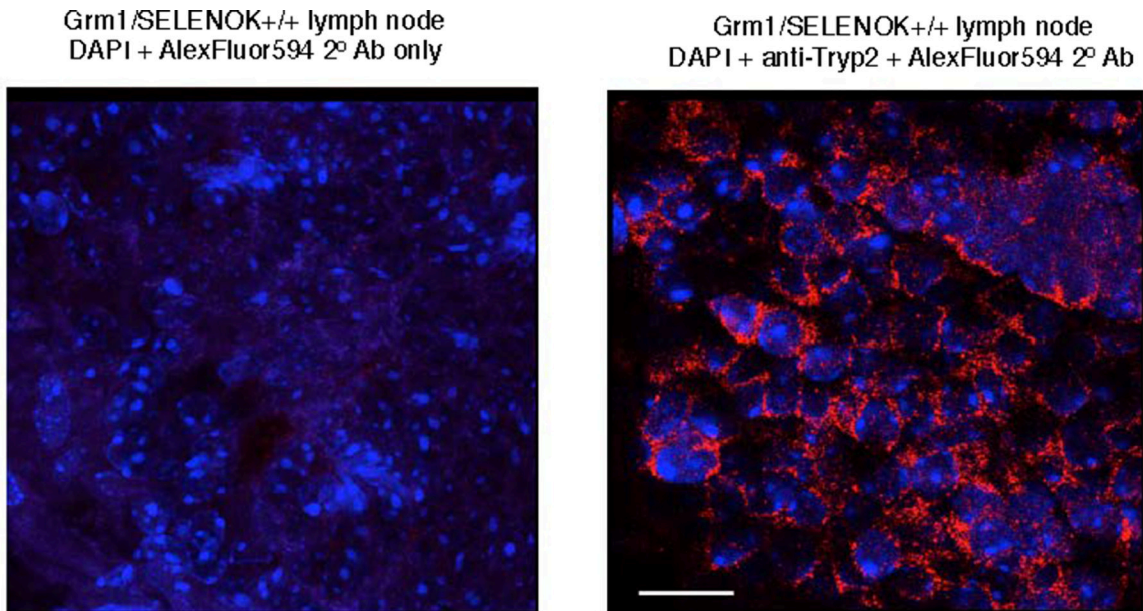


Supplementary Figure 4: Western blotting was performed to determine levels of calcineurin subunits A and B, along with calmodulin in SELENOK-deficient cells (Clone 7) compared to wild-type SK-MEL-28 cells. Results showed no differences. SELENOK was detected in Clone 7 cells as a smaller truncated form as expected and GAPDH was used as a loading control.

GeneSymbol	Fold Change	FDR-adjusted p-value	Average Expression for KO (N=3)	Average Expression for Wild Type (N=3)	GeneSymbol	Fold Change	FDR-adjusted p-value	Average Expression for KO (N=3)	Average Expression for Wild Type (N=3)	GeneSymbol	Fold Change	FDR-adjusted p-value	Average Expression for KO (N=3)	Average Expression for Wild Type (N=3)	GeneSymbol	Fold Change	FDR-adjusted p-value	Average Expression for KO (N=3)	Average Expression for Wild Type (N=3)
ADRL2	2.2807	0.0237	8.2676	7.0781	SPF1	5.7314	0.0072	11.3714	545.25	MALON	0.3461	0.0253	7.7446	5.1014	MEZ10L	-2.9445	0.0363	5.1759	6.7379
DCAF6	2.0594	0.0205	8.9599	8.8184	PPARGC1A	2.1654	0.0297	8.7152	7.6006	NKX3	2.7462	0.03	7.4283	6.1201	SRSF12	-3.0703	0.0108	7.6216	9.24
ATP1B1	2.1094	0.0139	9.9843	8.9074	FGRP2	3.1926	0.0373	8.0571	6.3824	AHNAK2	2.5997	0.0213	8.0619	6.6836	SNF12	-5.562	0.0237	5.4255	7.929
DNM3	2.6882	0.0247	5.9884	4.5618	TUBE	3.8531	0.025	6.7374	4.7914	GABRA5	6.0134	0.018	8.5406	5.9924	AMPH	-2.566	0.0313	6.3068	7.6663
PRPL	2.1109	0.0247	8.1336	7.0528	ITGA2	2.0767	0.0157	8.1925	7.1568	ATP12A	2.399	0.016	8.8294	7.5669	FABP5	-2.317	0.0112	7.9223	9.1346
DIRX3	2.7438	0.0131	8.1398	7.6837	OCN	2.1735	0.0261	7.0974	5.9794	CCPG1/MIR62	2.0943	0.0346	9.1202	8.0676	RMS2	-2.155	0.0007	4.7543	5.862
KONK2	2.1077	0.0217	7.2297	6.148	ERAP2	4.1572	0.0155	8.6709	6.6159	ZDHHC1	3.577	0.043	6.6963	4.7975	EGR3	-2.9975	0.0174	6.9383	8.4921
EPHX1	2.0405	0.0101	10.2105	9.1876	CYBP2	2.248	0.0244	8.008	6.8939	GPRC5B	2.505	0.0298	7.9126	6.5877	TRPA1	-2.2263	0.0461	6.0457	7.2004
UGALS8	2.6707	0.0072	9.0015	8.1843	AMND3	2.8832	0.0225	8.1398	6.6058	GPRC5B	2.505	0.0298	7.9126	6.5877	ECT1/JIP3/wia	-2.8109	0.0386	6.2572	7.4866
NEURL	3.9401	0.0179	7.9407	5.9685	EPHA14A	4.9126	0.0323	5.3885	6.1779	SEPT4	6.2849	0.0022	8.8872	6.2425	HACD4	-3.1423	0.0174	7.7297	8.9255
TNXP	2.3673	0.0101	11.0101	9.7669	NMIS	3.4101	0.0179	5.5883	3.8184	COG8	2.4689	0.0174	12.5952	11.2313	SHB	-3.0477	0.0174	7.5115	9.1825
CTSK	2.4215	0.0155	10.8837	9.6078	ATP6AP1	2.1013	0.0301	9.1171	8.0458	RAB27B	2.7412	0.0173	8.4089	6.9541	MACA	-3.4543	0.0247	5.8037	7.9291
SPRKO	3.8653	0.0072	10.079	8.3224	RG1	2.1416	0.0108	8.619	7.5204	CDH7	2.576	0.0131	7.8469	6.473	TRIO	-4.7452	0.0198	4.2428	6.6499
SPR2B	2.1712	0.094	6.7853	5.6768	NRN1	2.174	0.0369	10.8692	9.2988	CHST9	6.4049	0.0136	6.4623	8.9392	LOHRE3	-13.8236	0.0131	4.8888	8.6244
S100A6	3.9477	0.0165	8.1262	6.1823	GPR63	2.004	0.0192	5.6917	4.6888	CHD19	3.4669	0.0158	7.1682	5.3748	WNK3	-2.5889	0.0138	5.1755	6.5478
SLAMP9	3.1588	0.0252	9.0723	7.4129	FSO1	2.5297	0.0182	9.2712	7.9922	GDF15	4.9795	0.0049	11.7208	9.412	TSKAN7	-2.2004	0.0242	8.0047	9.1625
GPR161	2.0655	0.0363	9.098	8.0497	ABCS5	6.7511	0.0172	11.4479	8.6921	FOXO3/MIR68	6.8627	0.0042	12.6988	9.321	MGMT	-2.4919	0.0205	5.1718	6.4891
ONX3	2.0396	0.0232	7.7807	6.7524	BIS5	2.0751	0.027	9.0265	7.9847	CD22/MIR313	2.2476	0.0438	8.7195	7.5514	DEPDC7	-2.0255	0.0255	4.5146	6.4928
CNH3	5.0007	0.0077	9.7801	7.2944	ADAM22	2.422	0.0239	8.0015	6.7253	KCN3	2.2405	0.0049	10.8491	9.6653	SORCS1	-2.1483	0.0131	7.4842	8.5874
F11R	2.3484	0.0108	10.0395	8.8078	STEAP2	6.1635	0.0217	6.8075	4.8607	SEMA6B	2.4734	0.0215	8.3994	7.0329	ZNF215	-2.725	0.012	6.2579	7.72
INTG2	2.3673	0.0108	9.3963	8.1409	GNGL1	2.3845	0.0072	8.9912	7.9681	OLFML2	2.0216	0.0213	8.3136	7.2981	HITAP2	-2.0274	0.0292	8.344	9.3636
CDK15	3.009	0.0101	6.5941	5.0439	SUC6A4	3.8058	0.0039	7.2559	5.5312	BST2	6.456	0.0131	9.4402	6.7491	DEPDC7	-2.0255	0.0292	6.4523	7.4706
MAP2	2.7741	0.0097	9.473	8.001	MET	3.4631	0.0143	10.0025	8.1514	PLAUR	2.1155	0.0102	10.6004	9.5199	NTRM	-10.4911	0.0012	4.851	8.2421
MGATA4	2.8695	0.0168	8.267	7.4729	CVL	2.5626	0.0072	9.0785	8.1512	ZNF776	2.0375	0.0207	7.6075	6.7808	IFITM3	-4.0026	0.0027	9.2065	11.2289
ZRANB3	2.3676	0.0491	6.1154	5.4741	PCLO	2.134	0.024	9.0218	7.9283	C79103//RPS-5	8.1156	0.005	7.301	6.2615	PHLDA2	-2.2258	0.0166	7.787	8.9141
GPR135	2.7864	0.0197	9.2266	7.7462	SANDR1	2.6725	0.0127	8.8294	7.8298	FT27	2.0889	0.0409	7.9612	6.9182	MPPEO2	-2.6001	0.0312	5.8669	7.4992
TRP	2.0291	0.0294	8.8615	8.8615	DKI	2.3657	0.0174	6.9662	6.9662	ZNF644	2.1811	0.0293	4.2165	3.8095	FABP5	-2.1638	0.011	6.9339	8.0468
HIBCH	2.2895	0.0449	9.0288	7.8338	KAA1147	2.0439	0.0362	8.4954	7.4641	ZNF546	2.3653	0.0303	7.1851	5.9553	SFI	-2.1936	0.0431	6.8595	7.4927
KLF7	2.1725	0.0128	10.2687	9.1493	ZDHHC2	4.5963	0.0257	7.0611	6.0028	ZYG11A	-2.6911	0.0152	4.2347	5.6646	STYL2	-2.4785	0.0072	9.3041	10.1628
EMR	4.1777	0.0072	7.9328	5.8755	MATN2	3.2426	0.0248	7.5443	5.9472	EDARAD	-2.4679	0.037	4.8462	6.1495	MMV1	-13.4407	0.0108	7.9676	11.7892
TTN	2.0928	0.0308	7.8677	6.9223	NOV	2.8049	0.0191	8.6448	7.1569	ERH2	-2.4075	0.0245	5.9842	7.2637	IFITM2	-2.9441	0.0165	7.9113	8.8892
TMEM45A	2.4449	0.0116	9.3116	8.0218	PSCA	3.5805	0.0128	8.0015	7.0015	TMOC4	-2.8551	0.0108	6.617	8.1306	IFITM1	-2.2926	0.0211	6.6046	7.7802
CD96	2.9951	0.0182	5.2436	3.9834	CSGALNACT1	2.0664	0.0414	7.1794	6.1322	RAP1GAP	-3.8761	0.0108	5.2549	7.2035	SPX	-2.4295	0.0232	3.7321	5.0127
EPSEC	2.0621	0.0049	9.3415	8.2534	CLU/MIR6841	2.1107	0.0155	6.1345	5.0568	IAPTM5	-2.352	0.0405	6.2283	7.4702	ONTN1	-2.6048	0.0329	7.9974	9.3785
NEK1	2.255	0.019	6.0446	4.8714	ENPP2	3.251	0.0168	6.8265	6.5534	SNSL1	-2.2867	0.0397	4.3889	6.4996	TRK1D4	-2.366	0.038	6.625	8.9849
PPP2R3A	2.6855	0.0139	9.3027	7.8775	NDRG1	2.6091	0.0077	10.0125	8.6289	HENMT1	-3.776	0.0279	5.7262	7.6431	SLCS8A1	-10.0496	0.0108	3.3034	6.5325
SUCNR1	3.5176	0.0101	5.0813	2.1392	XCR9	2.1392	0.0239	6.1571	5.06	CTSL	-2.0543	0.0143	6.0088	7.0774	TRPV4	-3.3185	0.0101	8.9501	10.6006
WVE	2.1991	0.049	9.9928	8.8559	ROBB	2.5193	0.0113	9.1196	7.7666	RORG	-2.4538	0.0174	8.3152	8.6102	LINC00082	-2.8412	0.0389	8.624	10.1305
KCNMB1	2.1249	0.0174	8.3712	7.2827	NARF1	2.27	0.0155	7.7864	6.5858	///RNF202-ZNF69	-3.0227	0.0108	6.1316	7.861	TRK1D4	-2.366	0.038	6.625	8.9849
GPR160	3.1847	0.0185	7.7971	6.1291	GKAP1	2.3945	0.0108	8.4521	3.6421	KHL29	-4.5666	0.0166	4.8299	7.0205	DNAJC15	-6.8111	0.007	7.3472	10.1151
NLGN1	2.1464	0.0131	8.9607	8.5048	BAAT	10.3718	0.0131	8.7009	4.6563	ARHGAP25	-2.287	0.0367	7.1085	8.302	CRP2	-2.0263	0.0149	7.4465	8.4453
LMN1	2.4709	0.017	9.5689	8.2639	IS	2.4045	0.0252	7.4229	6.1846	POTEE	-7.423	0.0132	3.867	6.8947	FLVCR2	-2.1534	0.0389	5.1418	6.2477
OSBPL10	4.81	0.012	9.3677	7.017	PODS	2.4344	0.0232	7.1669	5.6834	BOSAL1	-2.2867	0.0397	4.3889	6.4996	ENAHM	-2.767	0.0257	6.2322	7.2006
CAFP5	6.2635	0.0072	9.2566	6.6086	HTRC2	2.2822	0.0492	6.4228	5.2324	CERS6	-2.2632	0.0414	7.7863	9.0209	ACAN	-2.7391	0.0465	6.448	7.9593
EP4E3	2.8319	0.0052	7.0262	6.0244	STR26	2.0534	0.0239	8.9742	7.5632	ICOS	-8.8068	0.0058	2.9203	6.0589	LRRK1	-2.1226	0.0007	8.1143	9.2002
CCDC131	2.4902	0.0256	6.592	5.2758	MAGEC1	9.4116	0.0108	9.1958	5.9613	ADAM23	-3.3993	0.0138	8.1925	9.9577	CYP11A1	-2.6169	0.0153	5.8029	7.1908
HRS99//ZNF81	2.39	0.0247	10.1811	8.9241	DMD	2.2421	0.0174	5.5526	4.8878	FAR2P1	-4.155	0.0286	4.7174	6.7723	X11TL	-3.5521	0.0182	6.7721	8.6129
ISAM9	2.4976	0.0221	9.5593	8.2496	RFXDC2	10.3874	0.0106	8.2885	4.8938	POTEF	-11.6344	0.0131	3.8913	7.4315	YFEL7	-2.8449	0.0047	6.7047	7.9942
RABL3	2.1059	0.0152	8.4266	7.3026	DVDC2	2.6771	0.0152	7.9098	6.1891	POTEF	-6.617	0.0217	3.0746	5.7642	AGM38	-2.0116	0.0181	8.9211	6.8296
WDR56	2.1171	0.0108	7.2282	6.086	PNLRP9	6.3812	0.0152	9.1921	6.5183	GRB14	-2.9486	0.0453	4.3949	5.8949	TMEM5	-2.0887	0.0072	9.4301	10.5596
ITGB5	2.041	0.0134	10.3682	9.3689	FAM1189A	2.3601	0.0374	9.1417	7.9151	RFTN1	-2.2929	0.0114	6.5773	7.7392	MUC10	-4.7798	0.0445	4.1346	5.6599
TMCC1	2.0668	0.0239	7.4889	6.4297	SORBS1	2.14	0.0384	8.0507	6.9631										



Supplementary Figure 6: Signaling pathway analyses conducted with QIAGEN's Ingenuity pathway analysis (Ingenuity Systems). Green indicates down-regulated transcripts and red indicates up-regulated transcripts in SELENOK-deficient cells compared to wild-type SK-MEL-28 cells.



Supplementary Figure 7: Specific immunofluorescence staining using anti-Trp2, scalebar = 20 μm. Blue staining are DAPI-positive nuclei and red cytosolic staining is Trp2 antigen. Supplementary video 1 shows mice at 6 months of age that exhibit a lack of melanoma (Grm1-Tg/SELENOK^{-/-}) or high levels of melanoma (Grm1-Tg/SELENOK^{+/-}). As shown in the video, the mice with high tumor burden are not severely affected in terms of movement or healthy behavior. Several cohorts of both sexes were followed out to the age of 8 months and no increased tumor formation were found in the Grm1-Tg/SELENOK^{-/-} mice.

Supplementary Video 1: Video showing tumors in 8 month-old mouse. See Supplementary_Video 1