Supplemental Information

Real Time Visualization of Cancer Cell Death, Survival and Proliferation Using Fluorochrome-Transfected Cells in an IncuCyte® Imaging System

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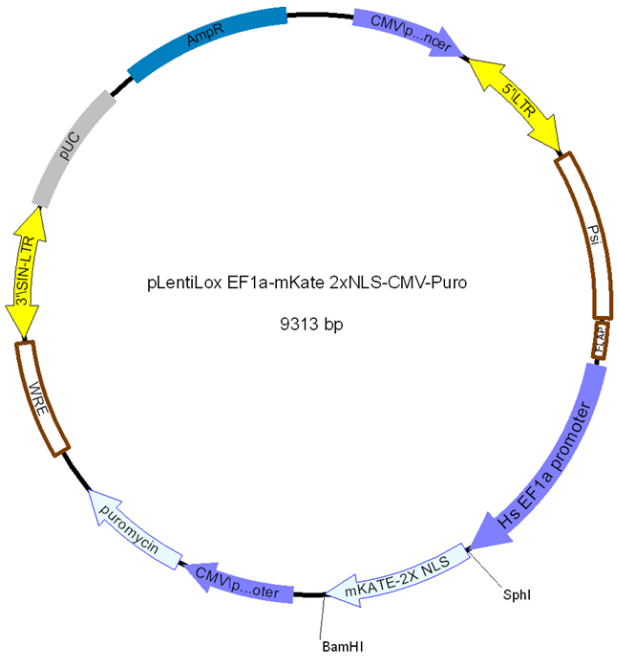

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9240  CATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAA
9300  AGTGCCACCTGAC
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Primer Name	Sequence
mKate F SphI	5'- AAT *GCA TGC* GCC ACC ATG GTG AGC GAG CTG ATT AAG GAG
2XNLS R BamHI	5'- TAG A*GG ATC C*TT ACT TCT ACC TTT CTC TTC TTT TTT GGA TCT ACC TTT CTC TTC TTT TTT GGA TCA GCT CGA GAT CTT CCT CTG TGC CCC AGT TTG CTA GGG AGG

Figure S1. Construct map, sequence and primer design for pLentiLox EF1a-mKate 2xNLS-CMV-Puro. Plasmid map **(A)** and sequence **(B)** for full pLentiLox EF1a-mKate 2xNLS-CMV-Puro construct. **C.** PCR primer design for mKate used in the construction of pLentiLox EF1a-mKate 2xNLS-CMV-Puro. The primer sequence in blue are specific to mKate CDS (mKate F SphI is the 5' end of mKate CDS and 2xNLS R BamHI is the 3' end of mKate CDS). The underlined sequence is the nuclear localization signal from SV40 large T cell antigen repeated twice. The sequence in red are the restriction sites followed by either 3 or 4 bases to facilitate restriction digestion of the PCR product. The stop codon is in the pLentilox EF1a vector after the BamHI site.

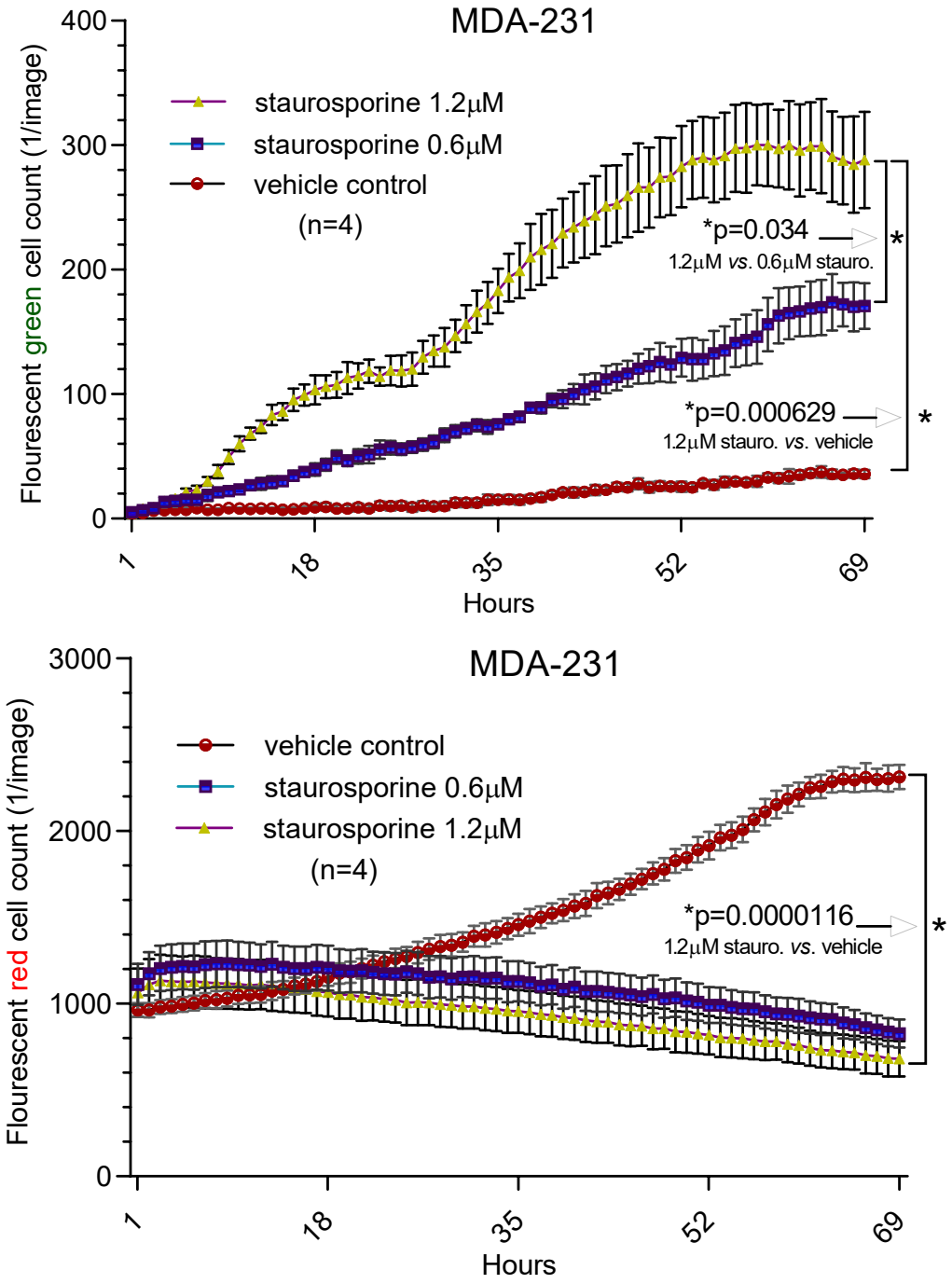

Figure S2. MDA 231 breast cancer cells treated with apoptosis inducing staurosporine. Staurosporine induced apoptosis in MDA-231 human breast cancer cells at concentrations of 1.2μM and 0.6μM. As shown, the higher concentration of staurosporine induced more apoptosis in tumor cells showing the sensitivity of MDA-231 HBCCs to an apoptotic stimulus. On the bottom panel, we correspondingly show that survival of MDA-231 HBCCs is reduced with staurosporine treatment.

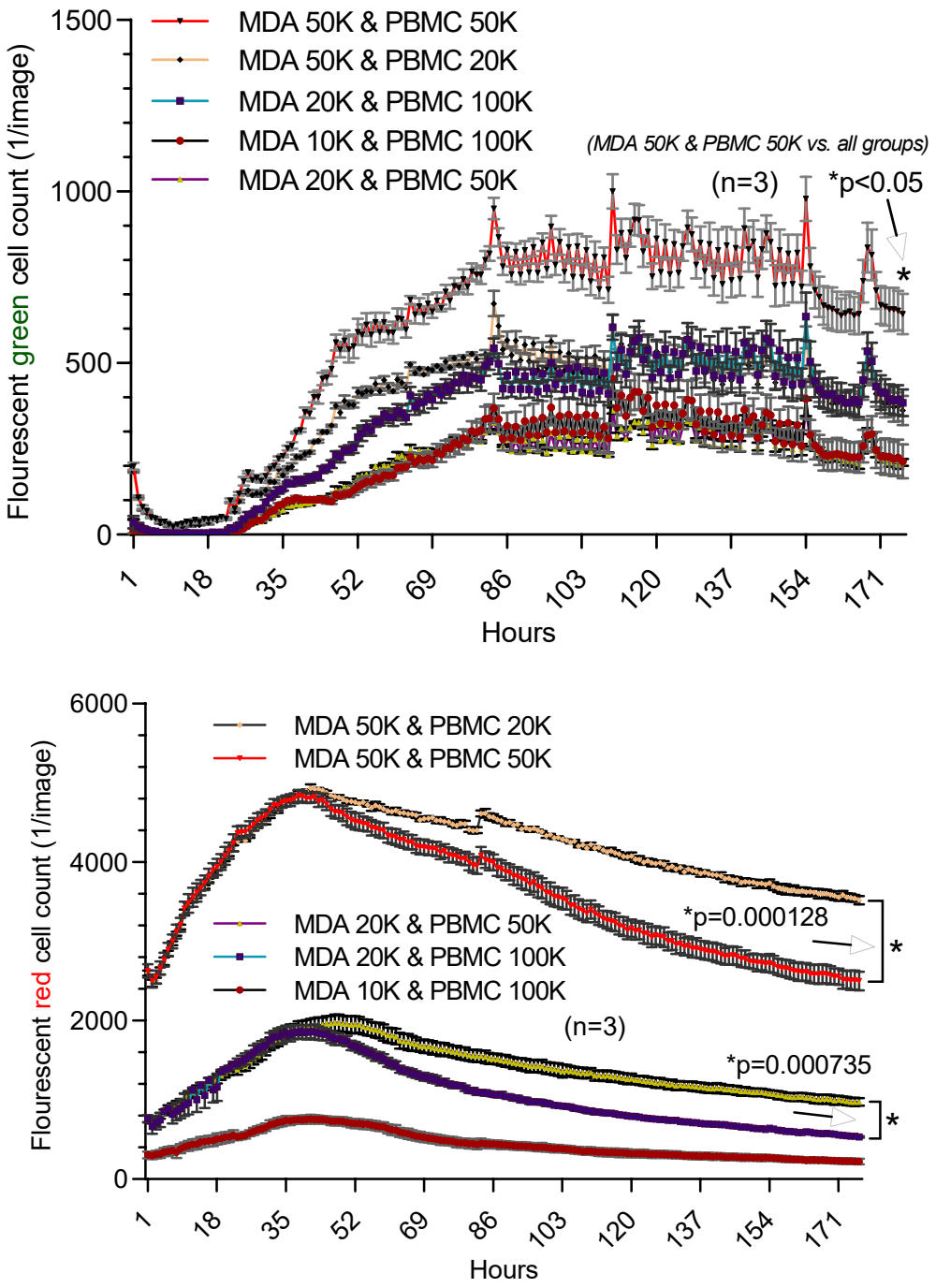

Figure S3. Multiple plating densities for MDA 231 breast cancer cells in co-culture with PBMCs. As shown, less MDA 231 human breast cancer cells with more PBMCs show more apoptosing cells (upper panel). The number of surviving human breast cancer cells (HBCCs) decreases when co-cultured with more PBMCs (lower panel). When the ratio of MDA cells decreases with the amount of PBMCs – tumor cell survival is reduced.

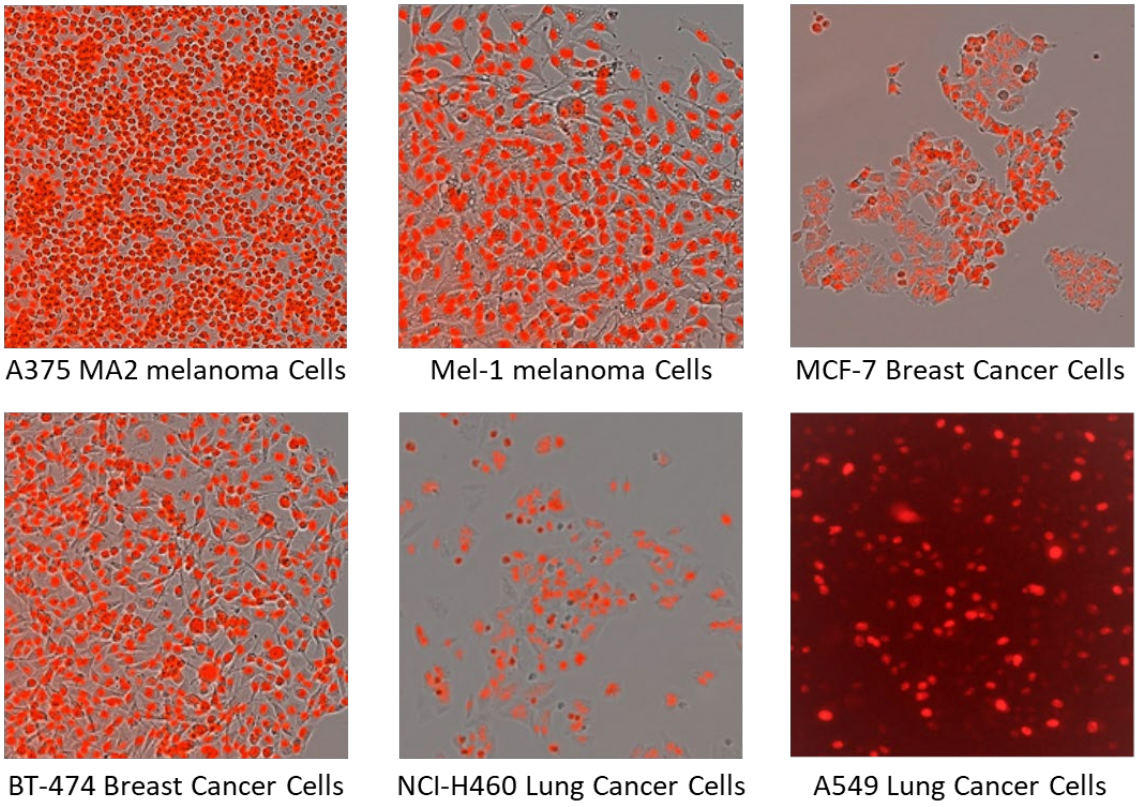

Figure S4. Additional cell lines transduced with lentivirus mKate2 representing melanoma, breast and lung cancers. The cell lines were clonally isolated for the strongest expressing cells and images taken on the IncuCyte system except the A549 cells, which shows a transduced population of cells and the image was taken on Olympic inverted fluorescent microscope. The A375 MA2 cells were grown in mice, extracted and plated in culture for analysis.

Cell Type	Supplier (Depositor)	Catalogue #	Culture Conditions
HEK 293T	ATCC (Rockefeller University)	CRL-11268	10% FBS, DMEM media, 5% CO_2
A549	ATCC (M. Lieber)	CCL-185	10% FBS, F-12K media, 5% CO_2
MDA 231	Sartorius	HTB-26	10% FBS, RPMI media, 5% CO_2
MDA 231	ATCC (R. Cailleau)	HTB-26	10% FBS, RPMI media, 5% CO_2
MDA 436	ATCC (R. Cailleau)	HTB-130	10% FBS, RPMI media, 5% CO_2
T47D	ATCC (I. Keydar)	HTB-133	10% FBS, RPMI media, 5% CO_2
A375	ATCC (D.J. Giard)	CRL-1619	10% FBS, RPMI media, 5% CO_2
LNCAP	ATCC (J.S. Horoszewicz)	CRL-1740	10% FBS, RPMI media, 5% CO_2
A375 MA2	ATCC (R. Hynes)	CRL-3223	10% FBS, RPMI media, 5% CO_2
Mel-1	ATCC (G. Trempe, L.J. Old)	HTB-67	10% FBS, RPMI media, 5% CO_2
MCF-7	ATCC (C.M. McGrath)	HTB-22	10% FBS, RPMI media, 5% CO_2
BT-474	ATCC (E.Y. Lasfargues)	HTB-20	10% FBS, RPMI media, 5% CO_2
NCI-H460	ATCC (A.F. Gazdar, J.D. Minna)	HTB-177	10% FBS, RPMI media, 5% CO_2

Table S1. Cell lines used to generate red fluorescent lines using lenti-mKate2.