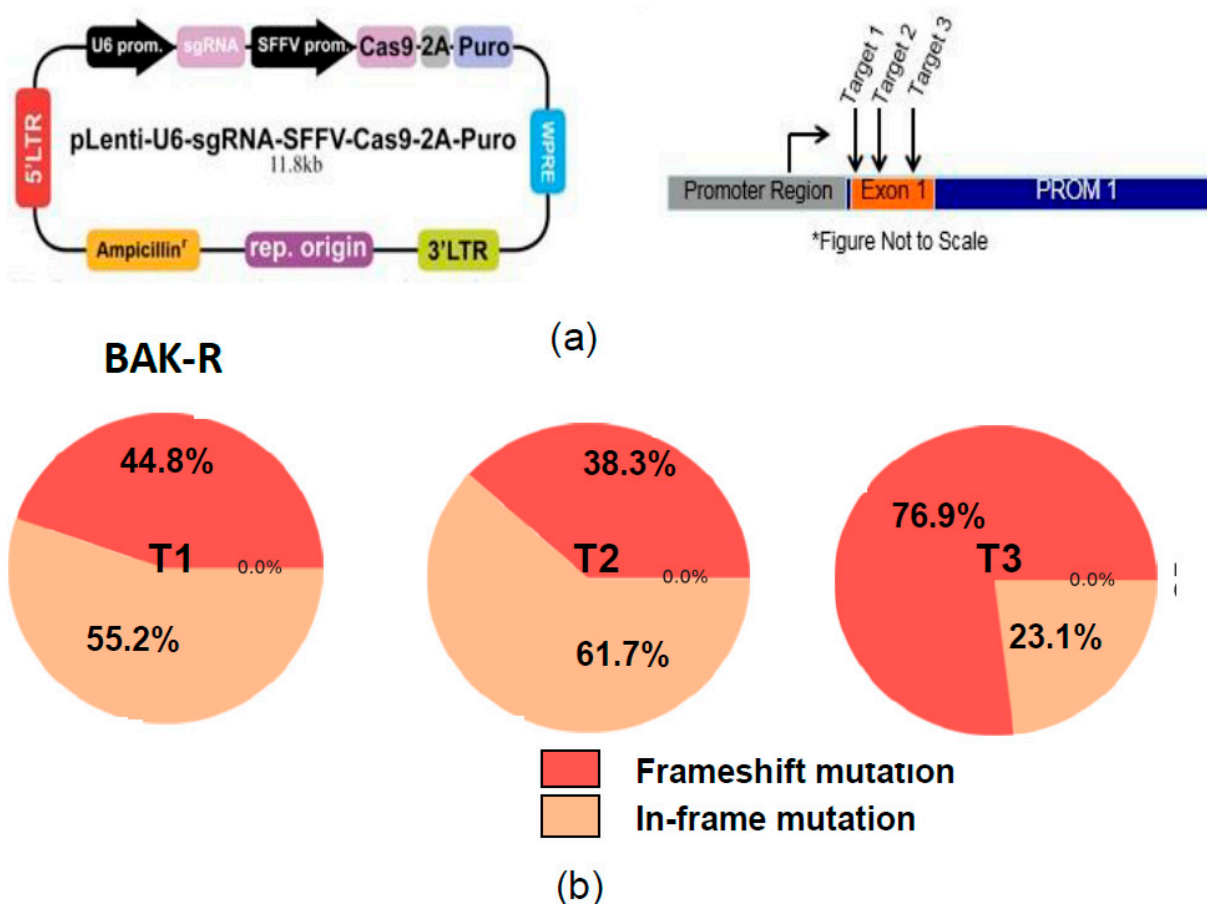
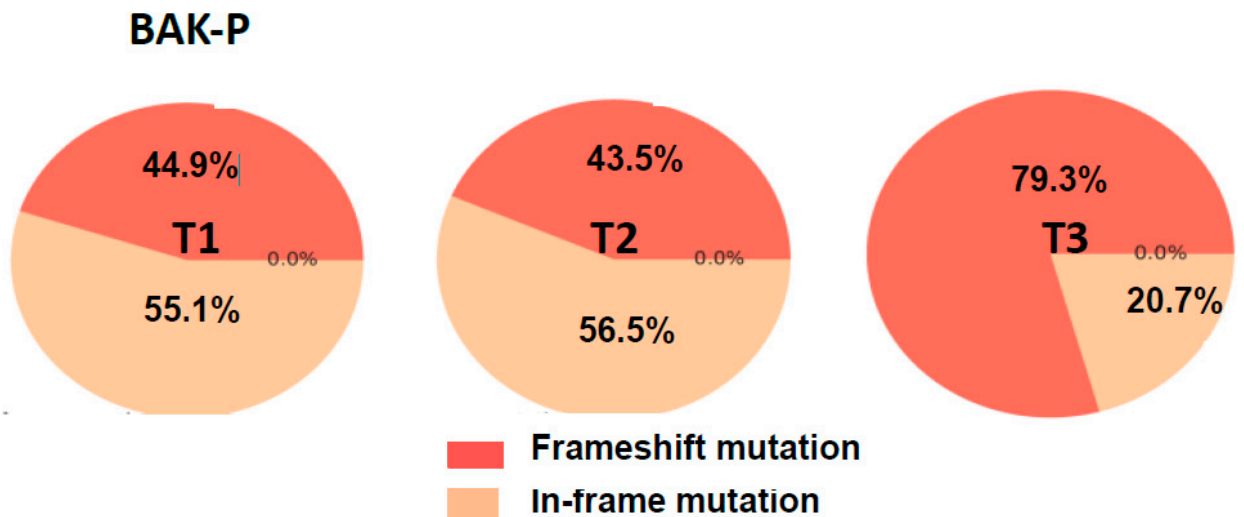


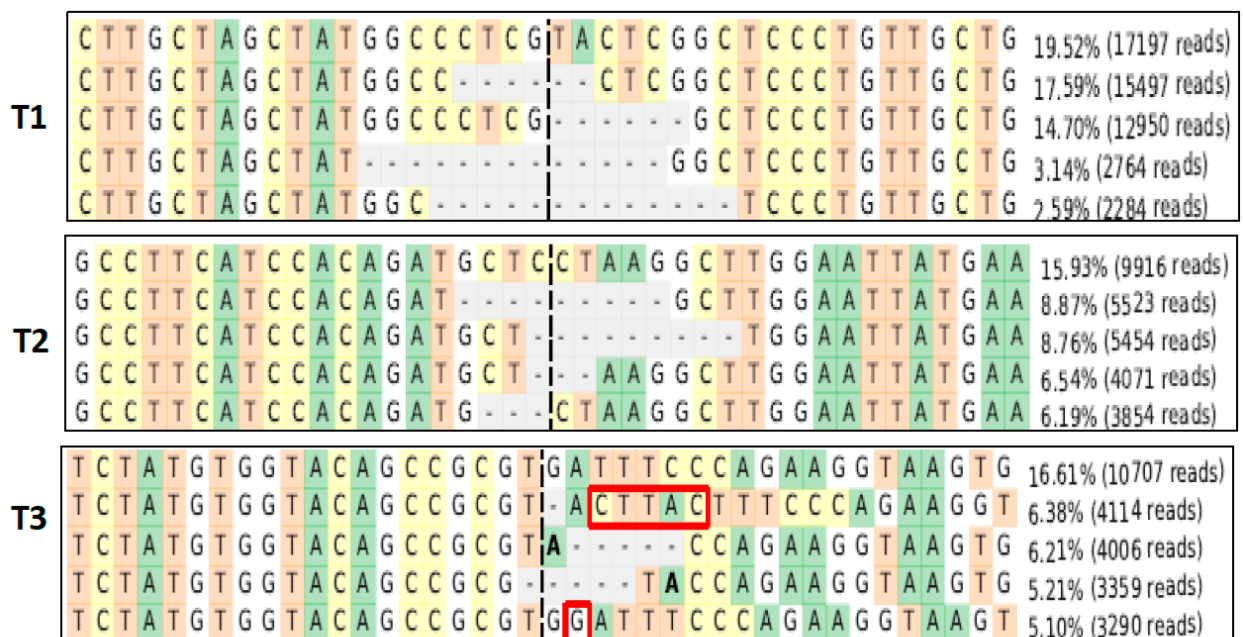
Supplementary Materials: CRISPR-Cas9 Knockdown and Induced Expression of CD133 Reveal Essential Roles in Melanoma Invasion and Metastasis



FigureS1. (a) CRISPR Cas9 pLenti plasmid from ABM and Gene Targets. pLenti plasmid (left) expresses Cas9 endonuclease and a puromycin resistance gene under control of the constitutively expressed spleen focus-forming virus (SFFV) promoter, and sgRNAs targeting three loci within exon 1 of the PROM1 gene (right) or scrambled sgRNA with no genomic targets, under control of the U6 promoter. (b) Frameshift mutation analysis from Next Generation Sequencing of BAK-R Scrambled control (SC), and BAK-R T1, T2, and T3 pooled clones.



(a)



(b)

Figure S2. (a) Frameshift mutation analysis from Next Generation Sequencing and (b) visualization of allelic mutations at CRISPR target sites.

Supplementary Material S3: Scans of whole gel western blots showing molecular weight sizes of relevant proteins (indicated in arrows)

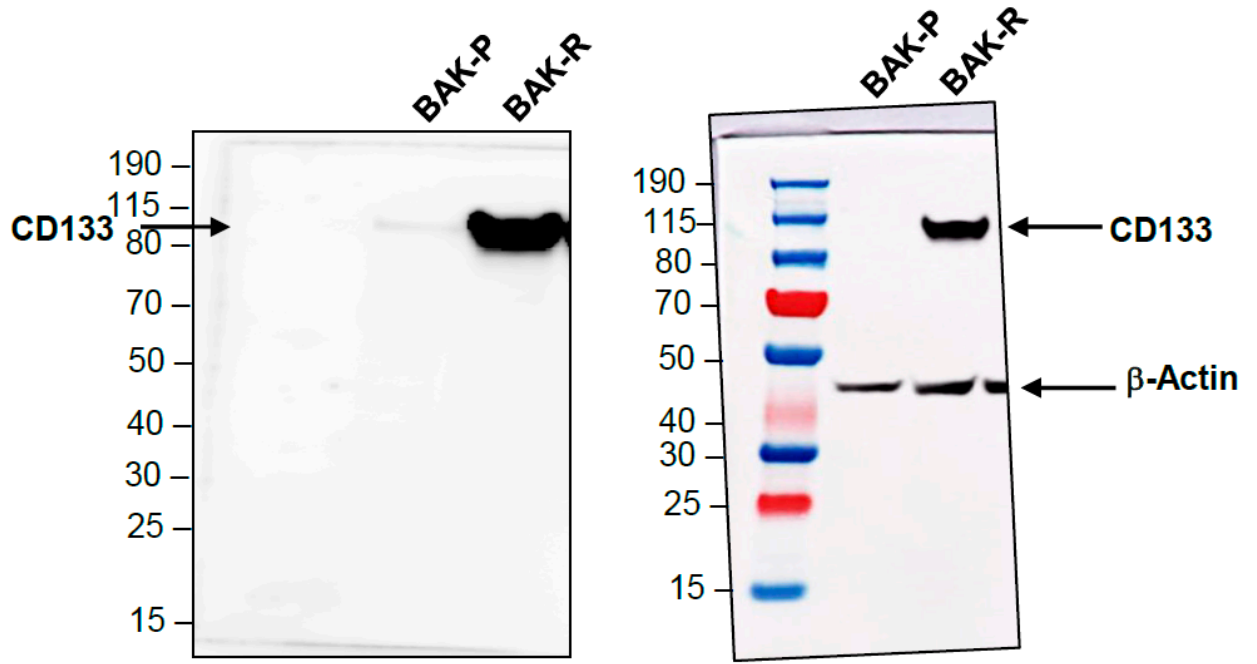
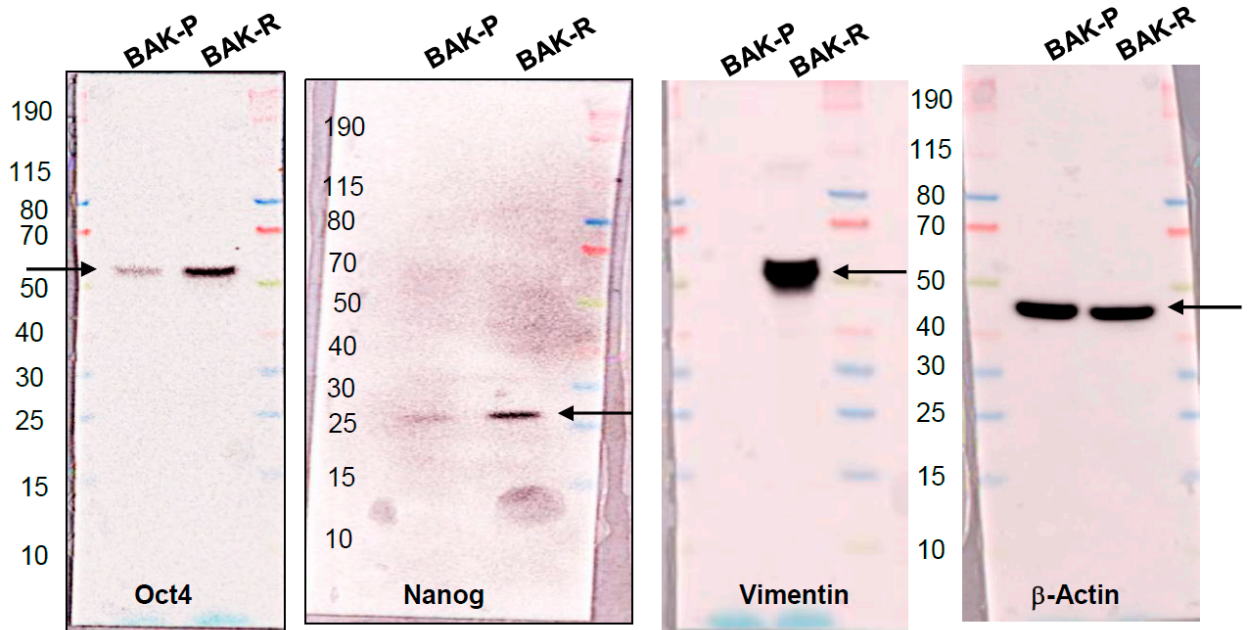


Fig. 1f whole gels



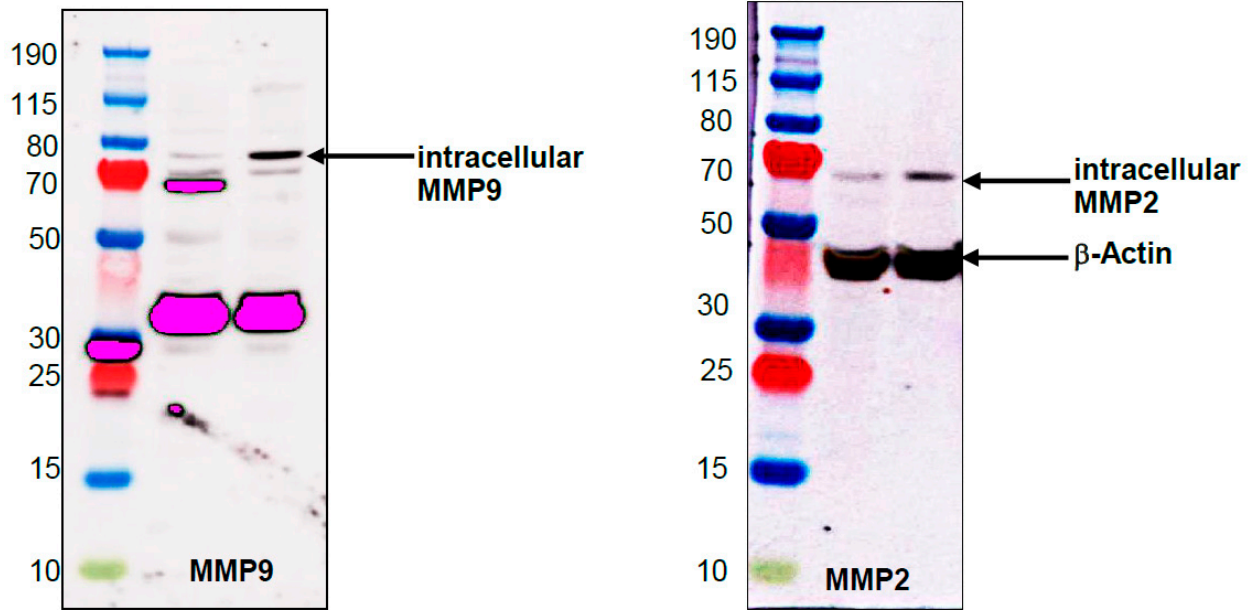


Fig. 2a whole gels

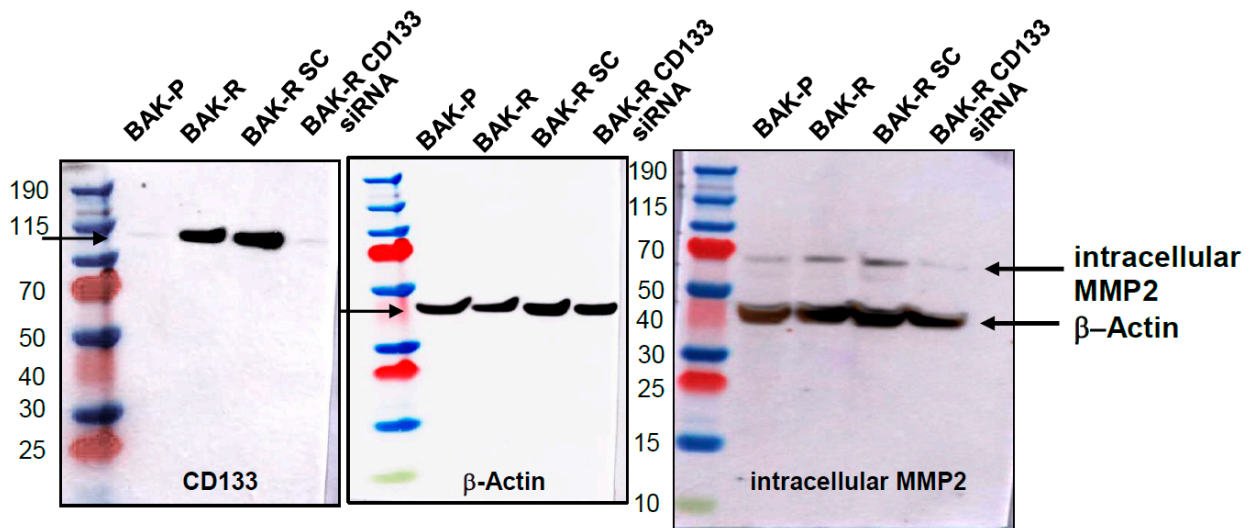


Fig. 3a whole gels

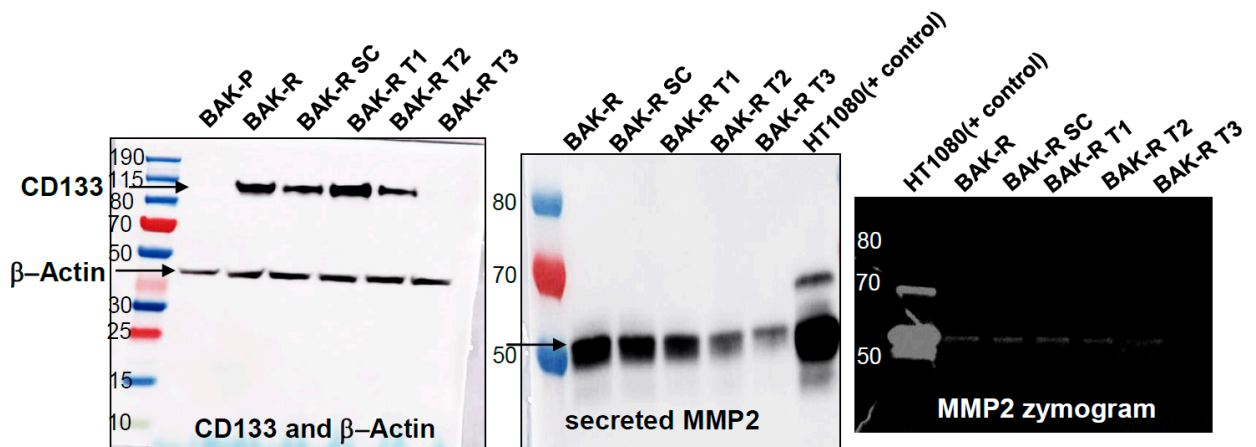


Fig. 4c whole gels

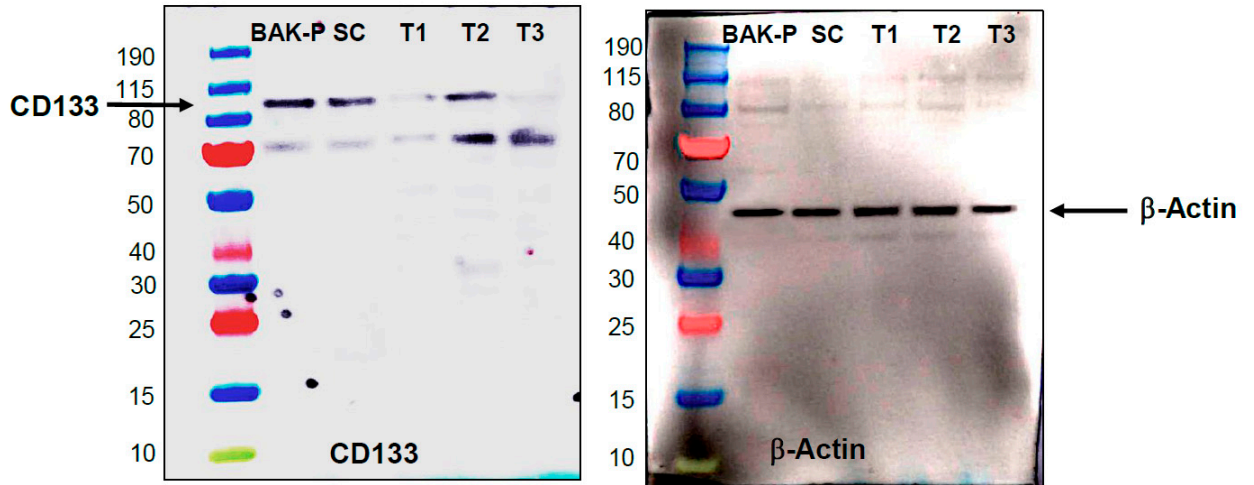


Fig. 5a whole gels

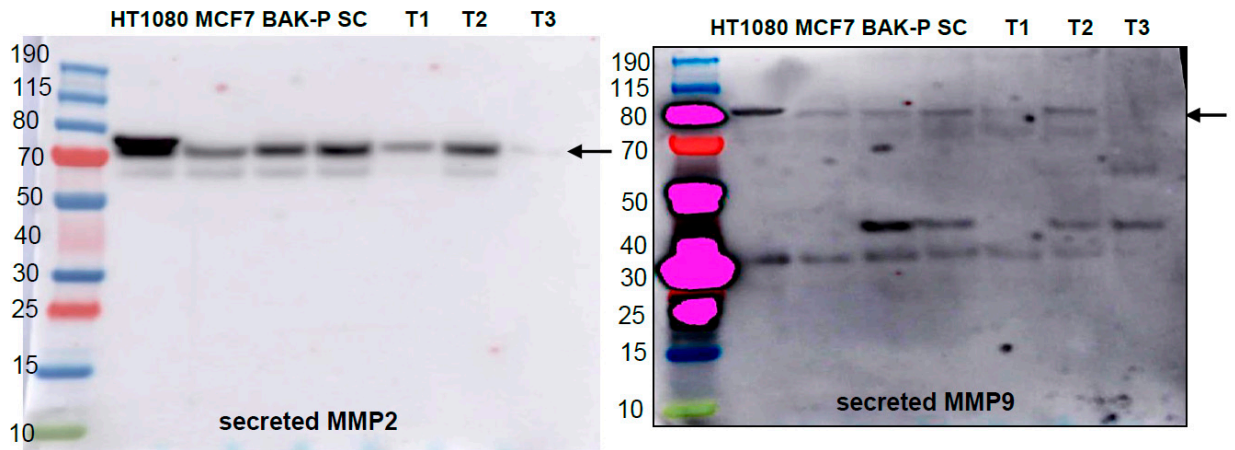


Fig. 5a whole gels

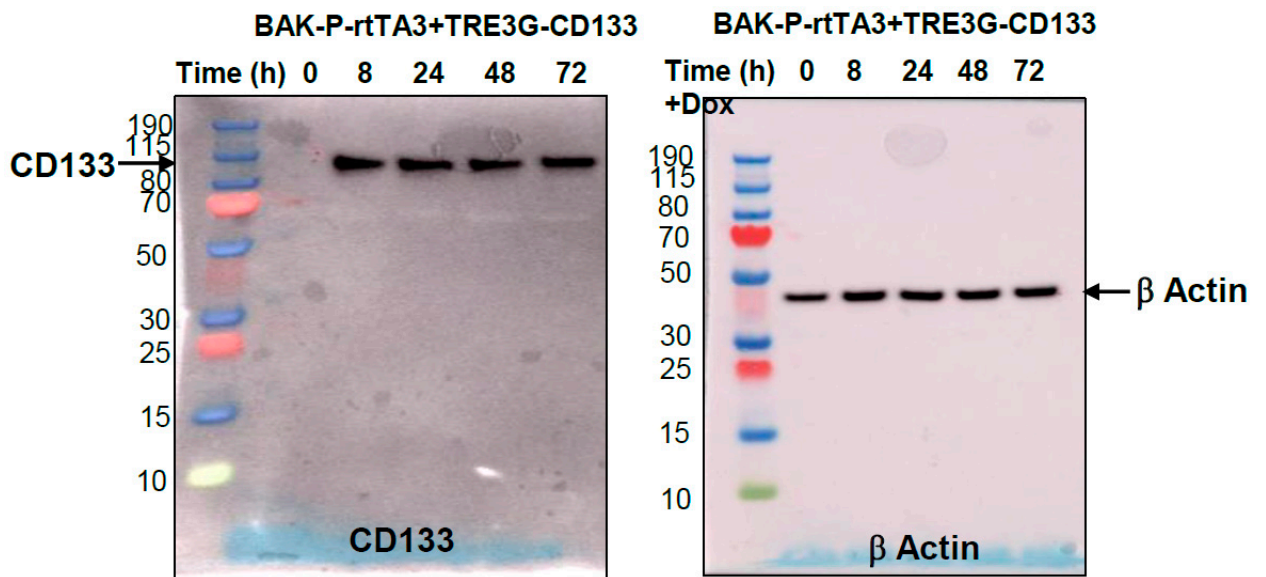
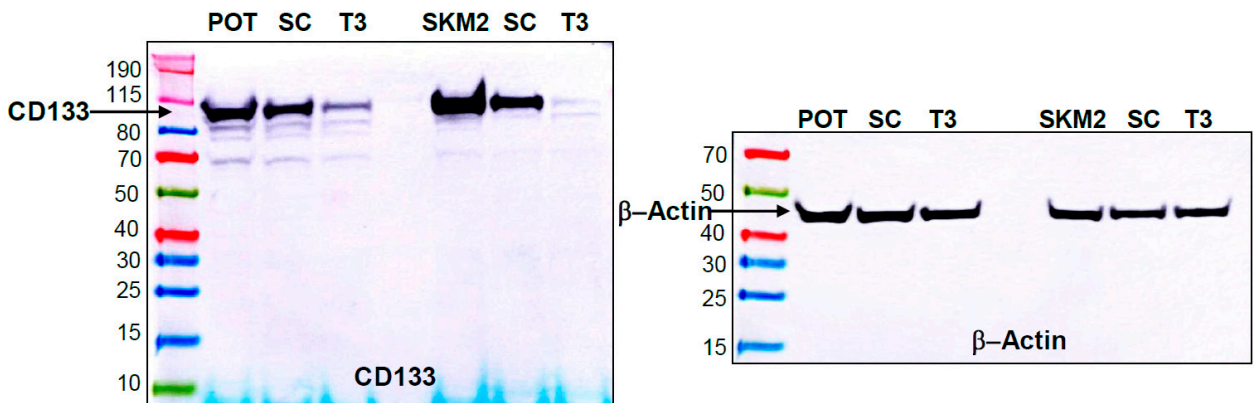
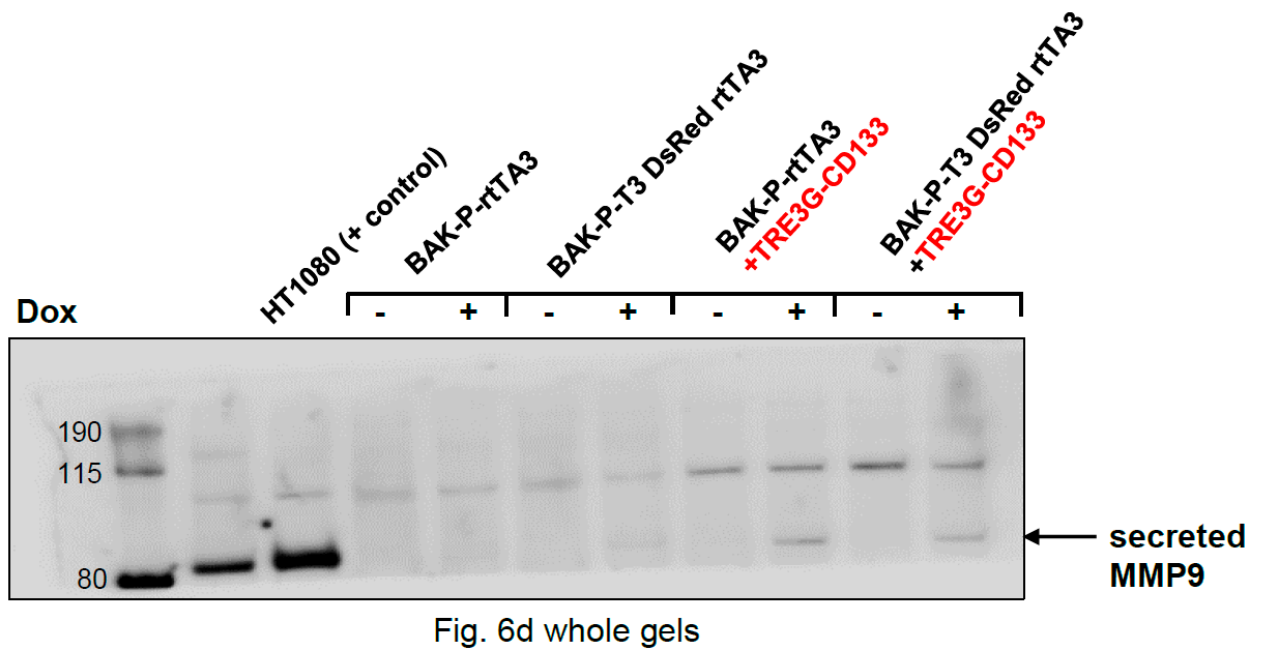
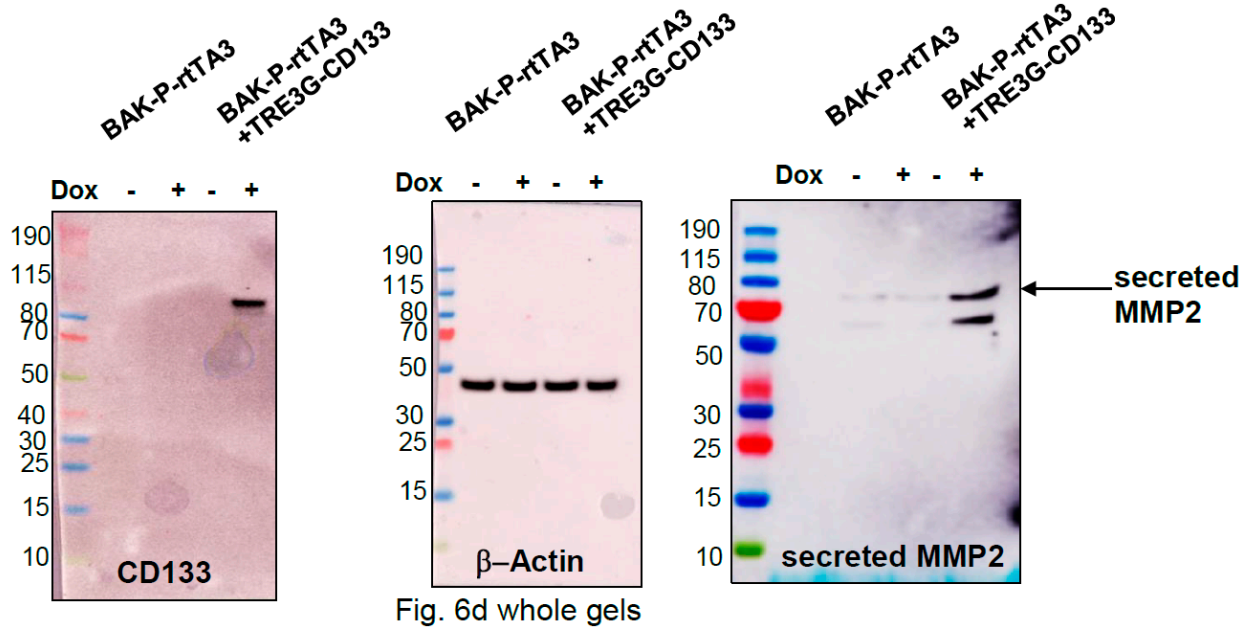


Fig. 6b whole gels



Supplementary Figure S3. Whole gel images for Figures 1f, 2a, 3a, 4c, 5a, 6b, 6d, 6f, and 6g.

Supplementary Materials S4: Densitometry analysis of western blots (band intensity normalized to loading control β -Actin; intensity ratio of each band relative to control are shown in tables)

Figure 1f BAK-P BAK-R

CD133	1.0	14.3
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Figure 2a	BAK-P BAK-R	
CD133	1.0	14.3
Oct4	1.0	7.0
Nanog	1.0	1.9
Vimentin	1.0	184.1
Intracellular MMP9	1.0	13.3
Intracellular MMP2	1.0	3.6

Figure 3a	BAK-P BAK-R	BAK-R SC BAK-R	CD133 siRNA	
CD133	1.0	29.6	20.6	0.7
Intracellular MMP2	1.0	2.0	1.6	0.1

Figure 4c	BAK-R	BAK-R SC BAK-R	T1 BAK-R	T2 BAK-R	T3
CD133	1.0	0.7	0.9	0.5	0.1
Secreted MMP2	1.0	0.8	0.6	0.3	0.1
MMP2 Zymogram	1.0	0.7	0.7	0.5	0.5

Figure 5a	BAK-P BAK-P	SC BAK-P	T1 BAK-P	T2 BAK-P	T3
CD133	1.0	0.8	0.1	0.8	0.1
Secreted MMP2	1.0	1.4	0.4	1.3	0.0
Secreted MMP9	1.0	3.1	0.4	2.5	0.0

Figure 6b BAK-P-rtTA3+TRE3G-CD133

Dox Induction (h)	0	8	24	48	72
CD133	1.0	268.0	194.4	175.6	310.4

Figure 6d	BAK-P-rtTA3	BAK-P-rtTA3	BAK-P-rtTA3	BAK-P-rtTA3
	(- Dox)	(+ Dox)	+TRE3G-CD133 (- Dox)	+TRE3G-CD133 (+ Dox)
CD133	1.0	1.0	1.1	815.7
Secreted MMP2	1.0	59.2	52.6	1238.5
Secreted MMP9	1.0	1.2	1.2	6.7

Figure 6f POT POT SC POT T3

CD133	1.0	0.6	0.2
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Figure 6g SK-MEL 2 SK-MEL 2 SC SK-MEL 2 T3

CD133	1.0	0.8	0.0
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