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Technical Data Sheet: 293[HEK-293] dCas9-KRAB Cell Line

ATCC® Number	CRL-1573dCas9-KRAB™
Organism	*Homo sapiens,* human
Tissue/Disease Source	Embryonic kidney, normal
Product Description	293[HEK-293] dCas9-KRAB cell line was created by knocking-in a KRAB-dCas9 (from *S. pyogenes*) expression cassette into the safe harbor AAVS1 locus using CRISPR/ Cas9 gene editing technology. This cell line stably expresses KRAB-dCas9, RFP.
Application	Functional evaluation of CRL1573dCas9-KRAB shows greater than 50% gene repression can be achieved for p53 and SETD9 genes when their respective gRNAs were delivered into the cells. 293[HEK-293] dCas9-KRAB cell line can be used as a tool for loss-of-function genetic studies.

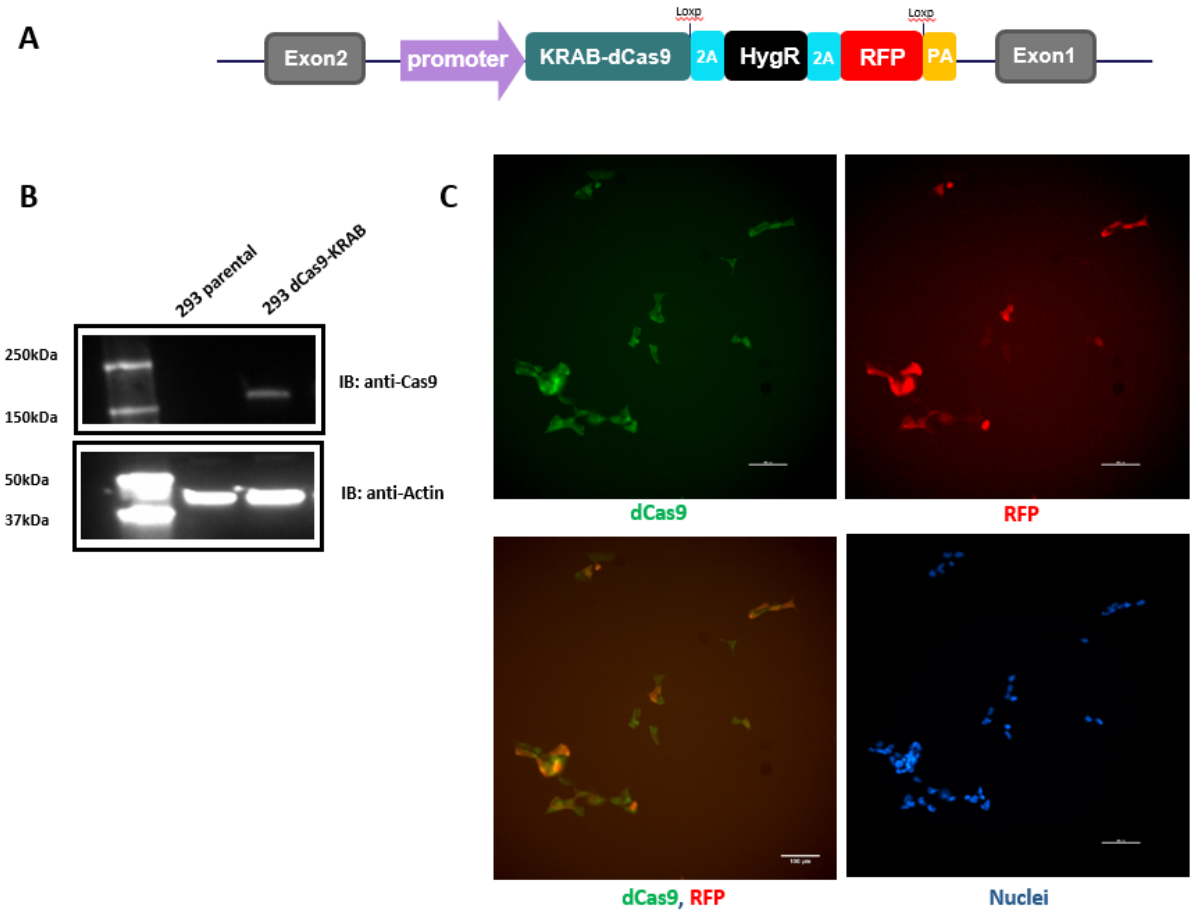

Figure 1. Generation of 293 dCas9-KRAB (ATCC® HTB-22dCas9-KRAB™). (A) Schematic of AAVS1 dCas9-KRAB expression knock-in cassette, showing RFP gene and hygromycin selection marker. (B) Detection of dCas9 protein expression by Western blotting in 293[HEK-293] dCas9-KRAB cells, but not in parental cells. (C) Co-localization of dCas9 protein (green; top left) and RFP protein (red; top right) in 293[HEK-293] dCas9-KRAB cells. The overlay image (bottom left) indicates dCas9 and RFP are expressed in the same cells. The nuclei of cells were stained with DAPI (blue, bottom right).

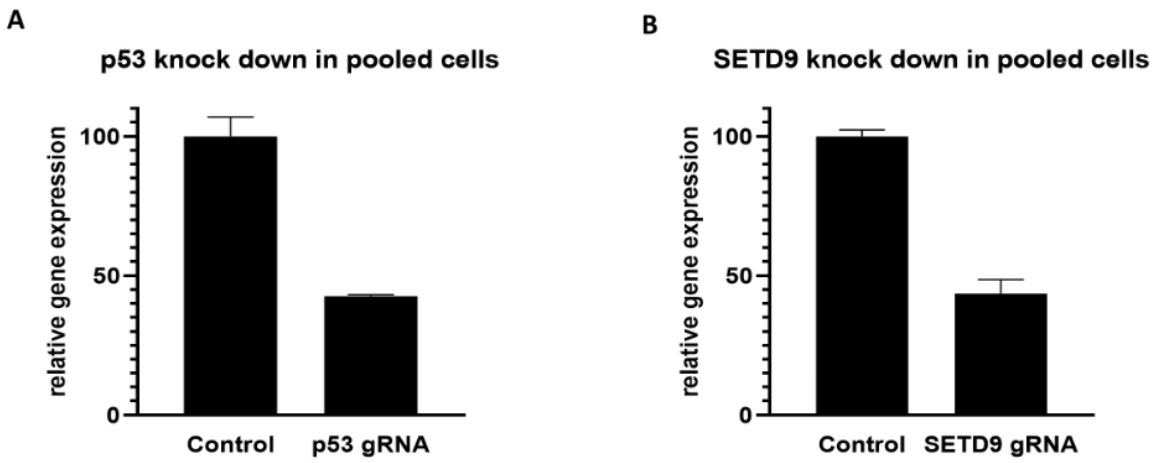

Figure 2. Validation of gene expression knockdown in 293[HEK-293] dCas9-KRAB cells (ATCC® CRL-1573dCas9-KRAB™). Repression of p53 and SETD9 gene expression. Lentivirus expressing gRNAs targeting p53 and SETD9 gene were used individually to infect 293[HEK-293] dCas9-KRAB cells. Lentivirus without gRNA expression was used as the control. 24 hours after infection, antibiotics was added to the culture media to enrich antibiotics resistance cells. Cell pellets were collected after 5 days selection and subject to ddPCR gene expression quantification analysis. The expression of p53 gene (left), and SETD9 gene (right) was significantly repressed in cells infected with gRNAs.