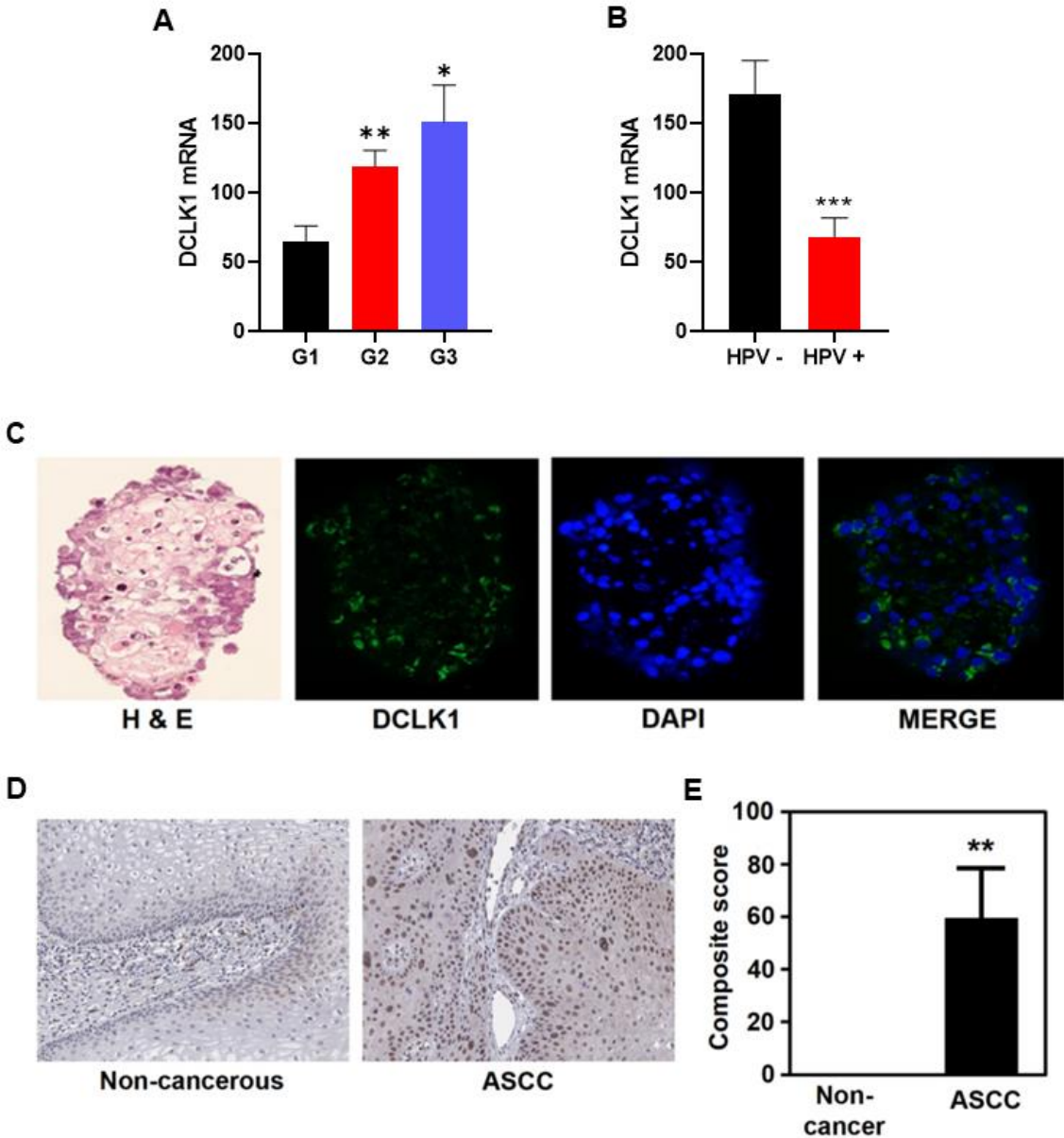


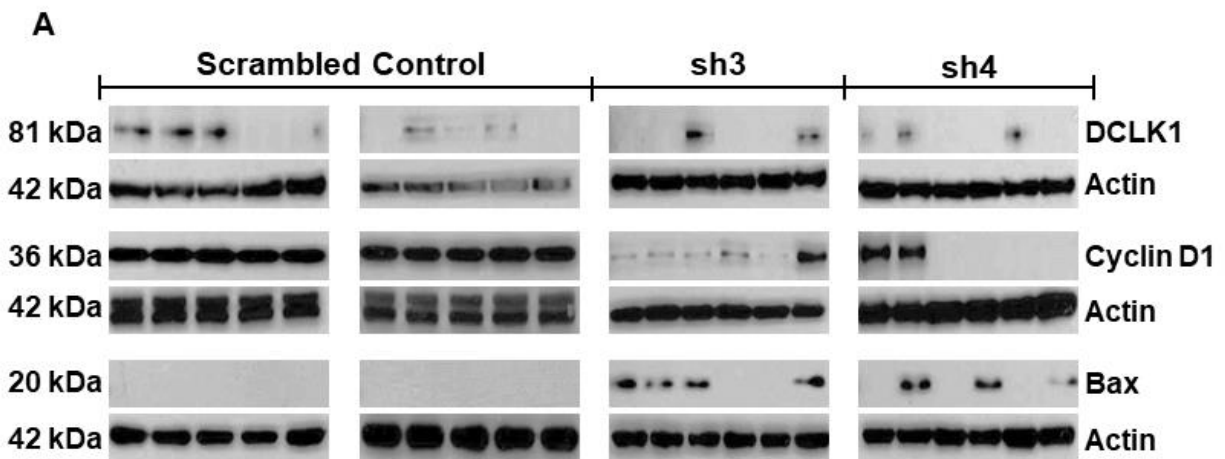
Supplementary Figure 1



939 **Supplementary Figure 1.** (A) DCLK1 mRNA expression by histological tumor grade
 940 represented as fragments per kilobase of transcript per million (FPKM) mapped reads,
 941 obtained through TCGA analysis (G1, n=64; G2, n=312; G3, n=125) (B) DCLK1 mRNA
 942 expression in HPV negative (n=74) and HPV positive tumors (n=42) represented as
 943

944 fragments per kilobase of transcript per million (FPKM) mapped reads, obtained
 945 through TCGA analysis (C) HN5 spheroids stained with H&E, or DCLK1 (green) and
 946 DAPI (blue). (D) Representative images of DCLK1 staining in non- cancerous anal
 947 mucosa (n=3) and ASCC (n=14). (E) Cumulative results of composite score (intensity x
 948 % positivity) of DCLK1 staining in anal mucosa samples. *p<0.05, **p<0.01, ***p<0.001
 949

Supplementary Figure 2



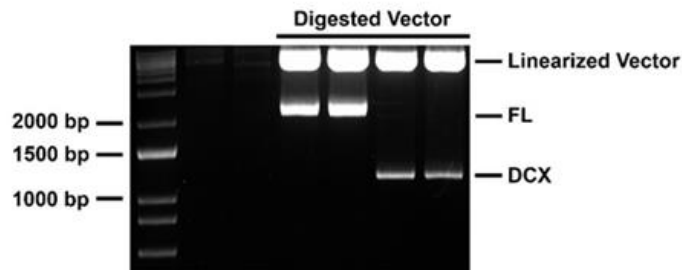
950
 951 **Supplementary Figure 2.** (A) Western blots from **xenograft** tumor lysates. 1×10^6 **FaDu**
 952 cells stably transfected with shRNA targeting DCLK1-3 (sh3), DCLK1-4 (sh4) or
 953 scrambled control shRNA were injected subcutaneously into NSG mice.

Supplementary Figure 3

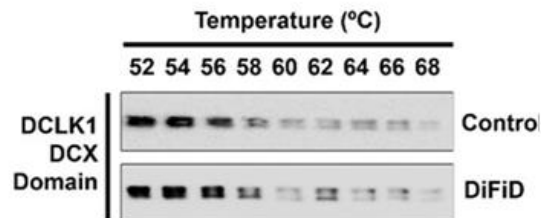
A



B



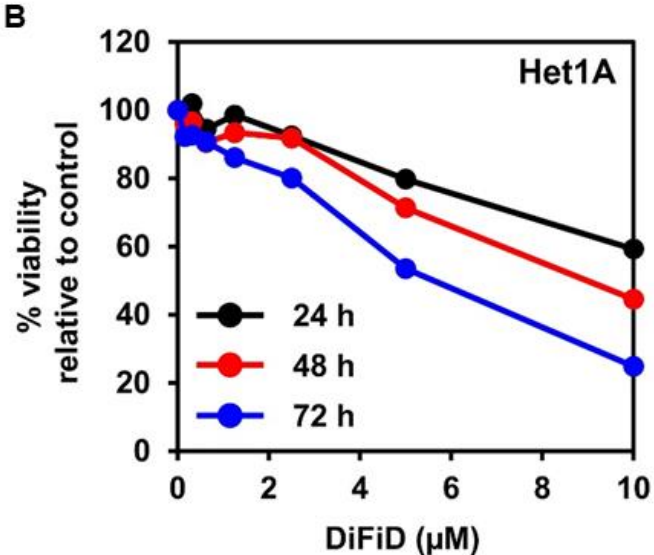
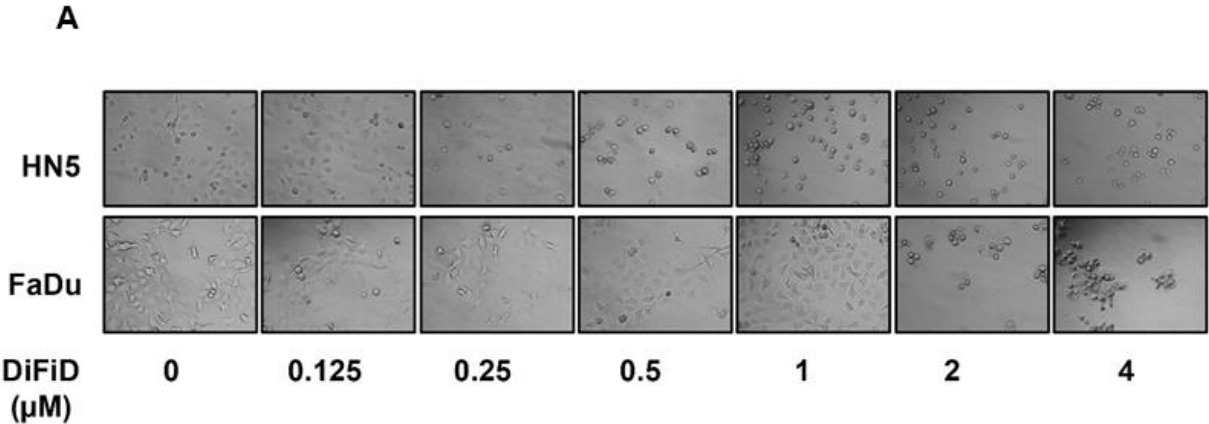
C



954

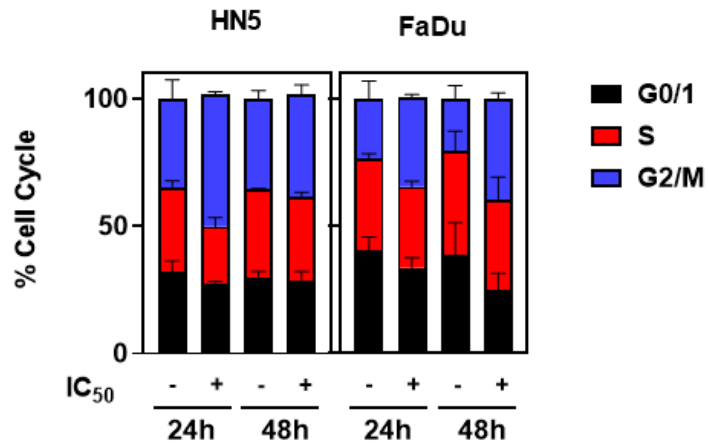
955 **Supplementary Figure 3.** (A) Diagram of DCLK1 full length and DCX domain
 956 fragments. (B) Restriction digested plasmid constructs expressing either full length (FL)
 957 or DCX domain (DCX) fragments. (C) Western blot analysis of CETSA samples from
 958 DCX domain expressing FaDu cells treated with DMSO or DiFiD.

Supplementary Figure 4



959
960 **Supplementary Figure 4.** (A) Bright field images of HN5 and FaDu cells at 100X
961 magnification treated with increasing doses of DiFiD for 48 h. (B) Hexosaminidase
962 assay for Het1A cells treated with DiFiD for up to 72 hours.

Supplementary Figure 5

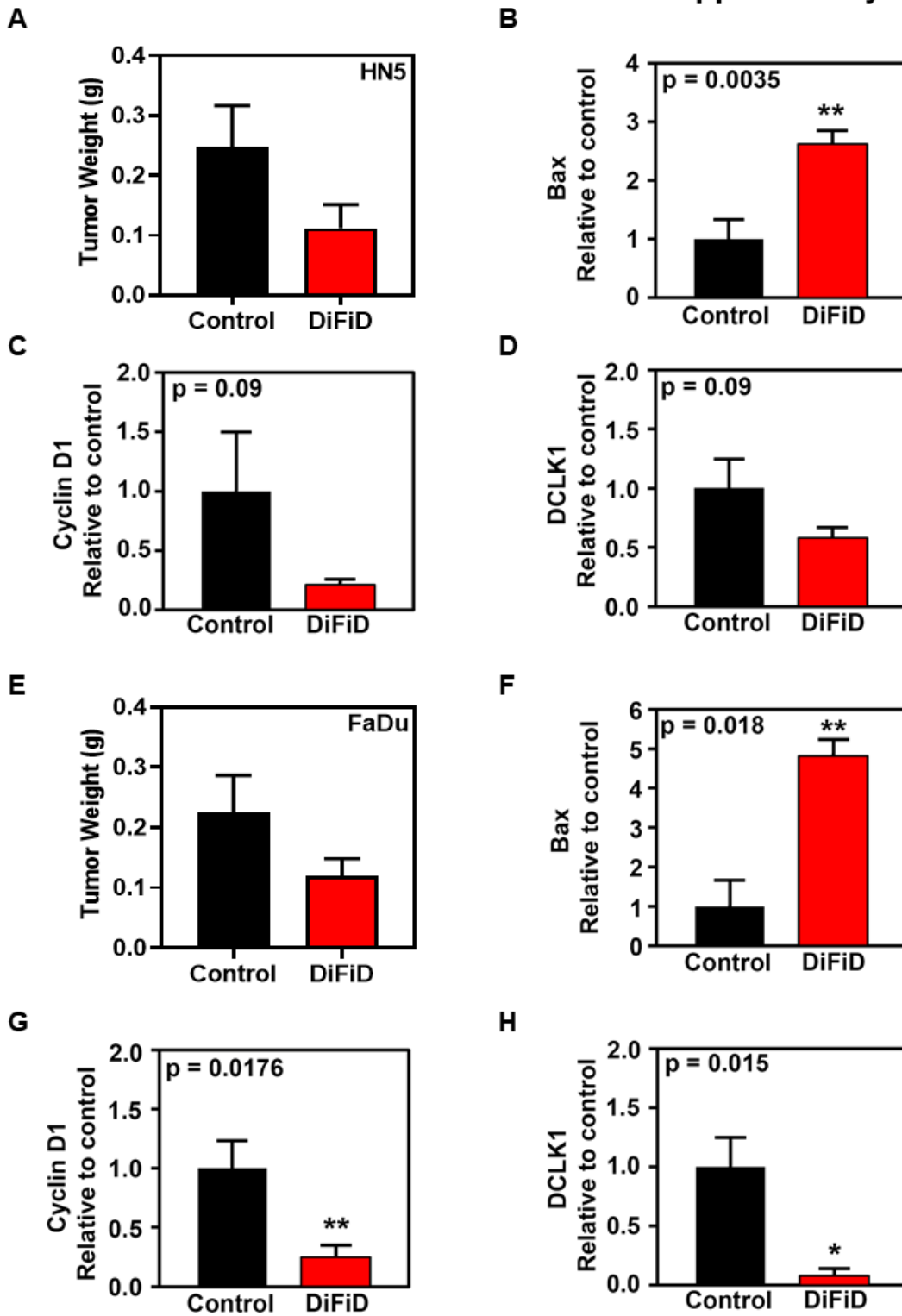


963

964 **Supplementary Figure 5.** Quantification of cell cycle data from three independent

965 experiments with HN5 and FaDu cells treated with DiFiD for 24 h or 48 h.

Supplementary Figure 6



966

967 Supplemental Figure 6.

968 HN5 or FaDu (1×10^6) cells were inoculated subcutaneously into the right flank of
969 athymic *Foxn1^{nu/nu}* mice. DiFiD (2 mg/kg) or vehicle control (DMSO) was administered
970 by intraperitoneal injection once daily for 15 days. HN5 Tumor weight (A) (n=7
971 mice/group), are depicted. HN5 xenograft tumors were subjected to biomarker
972 analyses. Densitometric analysis of HN5 xenograft tumors immunoblot signals for (B)
973 Bax (C) Cyclin D1 (D) DCLK1. (E) FaDu tumor weight (n=10 mice/group).
974 Densitometric analyses of FaDu tumor signals for (F) Bax (G) Cyclin D1 (H) DCLK1 are
975 depicted graphically, including \pm SEM, *p<0.05.

976