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Supplemental Data

Mitochondrial p32 Is a Critical Mediator of ARF-Induced Apoptosis

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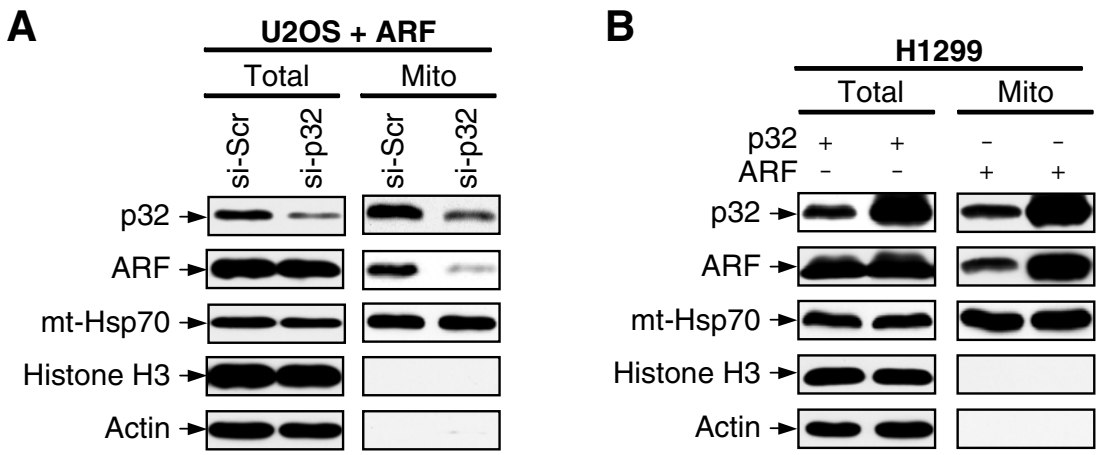

Figure S1. p32 is required for ARF to localize to mitochondria

(**A**) U2OS (ARF-negative) cells were transfected with p32 siRNA for 24 hours and then infected with adenovirus expressing ARF for 2 days. Cells were isolated for total and mitochondrial fractions and analyzed by western blotting with indicated antibodies.

(**B**) Overexpression of p32 promotes ARF mitochondrial localization. H1299 cells were infected with adenoviruses expressing ARF and p32 as indicated. Total SDS lysates and mitochondrial fractions were prepared 2 days after infection and analyzed by western blotting.

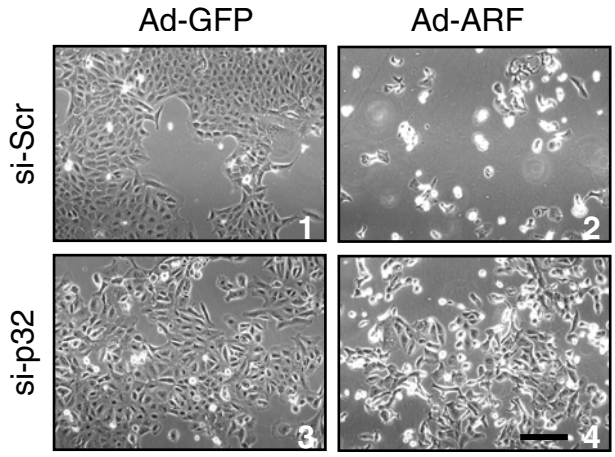

Figure S2. Depletion of p32 causes resistance to ARF-induced apoptosis
U2OS cells were transfected with siRNA for 24 hours and then infected with adenovirus expressing either GFP or ARF for 2 days. Phase-contrast images of representative cells with each treatment are shown. The scale bar represents 200 μm.

RV	GASQS**RR**P**R**PP**R**Q**R**DSSTSGDDSG**R**DSGGP**RRRR**GN**R**G**R**
EBNA1	G**R**G**R**G**R**G**R**GGG**R**PGAPGGS
	GGGG**R**G**R**GGSGG**R**G**R**GGSGG**R**G**R**GGSGG**RR**G**R**G**R**E**R**A**R**GGS**R**E**R**A**R**G**R**G**R**G**R**
REV	T**R**QA**RR**N**RRRR**W**R**E**R**Q**R**
LBR	**R**S**R**S**R**S**R**S**R**S
ORF73	P**RRR**KAK**RRR**
	P**R**K**RRR**KLKP

Figure S3. p32 binding sequences are rich in Arg
Amino acid sequences of reported p32 binding sites from RV (capsid protein of Rubella virus, a.a. 30-69), EBNA1 (Epstein Barr virus, a.a. 40-60 and 325-376), Rev (a.a. 34-50), LBR (lamin B receptor, a.a. 75-84), and ORF73 (Herpes virus Saimiri, a.a. 3-12 and 33-42) are shown, with Arg residues highlighted in bold face.

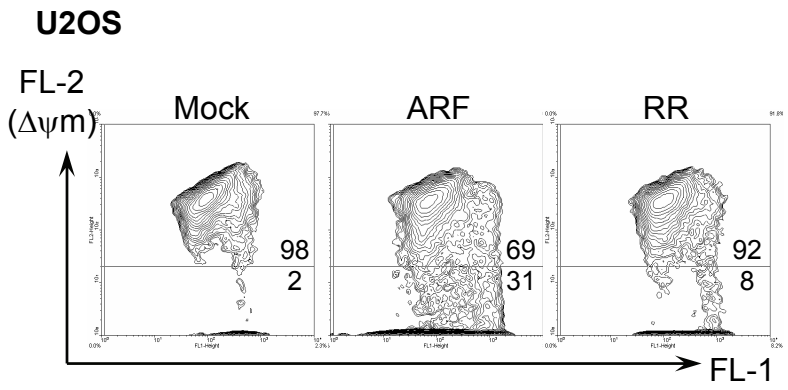

Figure S4. Overexpression of ARF reduces mitochondrial membrane potential
U2OS cells were either uninfected (Mock) or infected with adenovirus expressing wild type ARF (ARF) or a p32 binding-deficient ARF mutant (RR) for 2 days. Loss of $\Delta\psi_m$ was measured by incubating the cells with JC-1 dye followed by flow cytometry analysis. Flow cytometry data plots are shown with percentage of total events indicated. Data are representative of three independent experiments.