Sainski et al., http://www.jcb.org/cgi/content/full/jcb.201405051/DC1

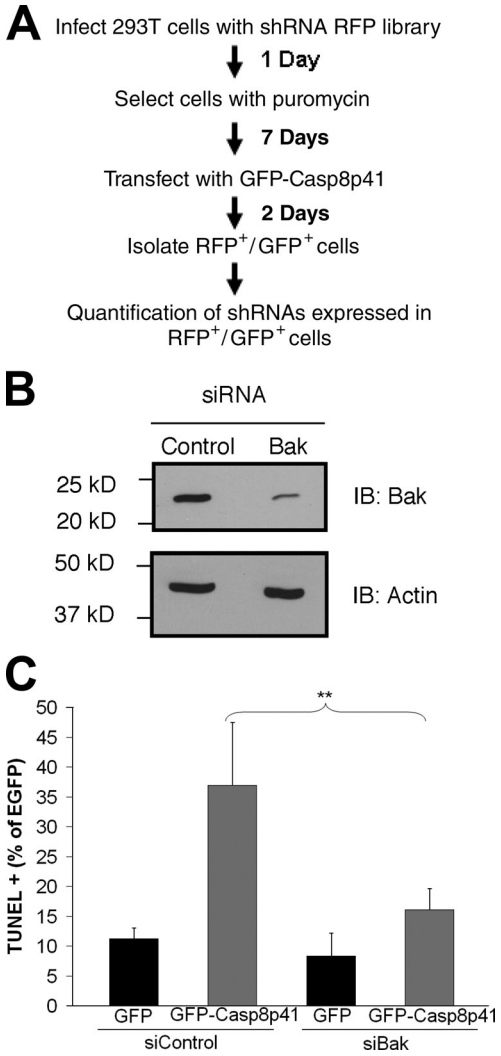

Figure S1. **Casp8p41 requires Bak to induce cell death.** (A) shRNA screen used to identify candidate proteins required for Casp8p41-induced death. (B) I9.2 cells were transfected with siControl or siBak, and Bak expression was measured. (C) After I9.2 cells were transfected with siControl or siBak along with EGFP or EGFP-Casp8p41, death in EGFP+ cells was measured via TUNEL. Summarized results from three independent experiments are shown. **, $P < 0.01$. Error bars indicate SD.

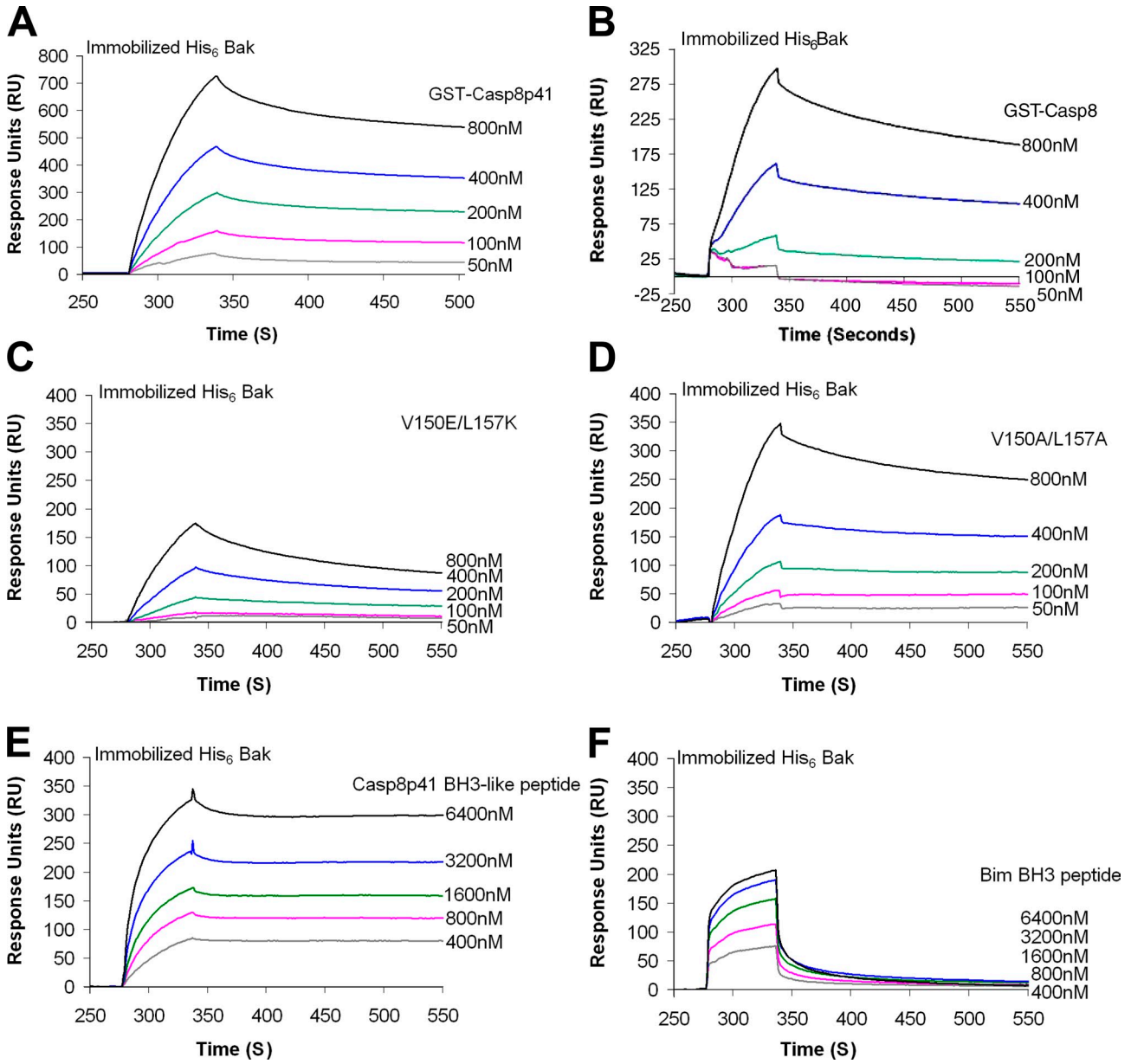

Figure S2. **Ligand binding assays.** (A and B) Binding of 50–800 nM GST-Casp8p41 (A) or GST–procaspase 8 (B) to immobilized His_6-BakΔTM as assessed by SPR. (C and D) Binding of 50–800 nM GST-Casp8p41 V150E/L157K (C) or GST-Casp8p41 V150A/L157A (D) to immobilized His_6-BakΔTM as assessed by SPR. (E and F) Binding of 400–6,400 nM Casp8p41 latent Bak activator peptide (E) or Bim BH3 peptide (F) to immobilized His_6-BakΔTM as assessed by SPR. All experiments are a representative of $n = 3$.

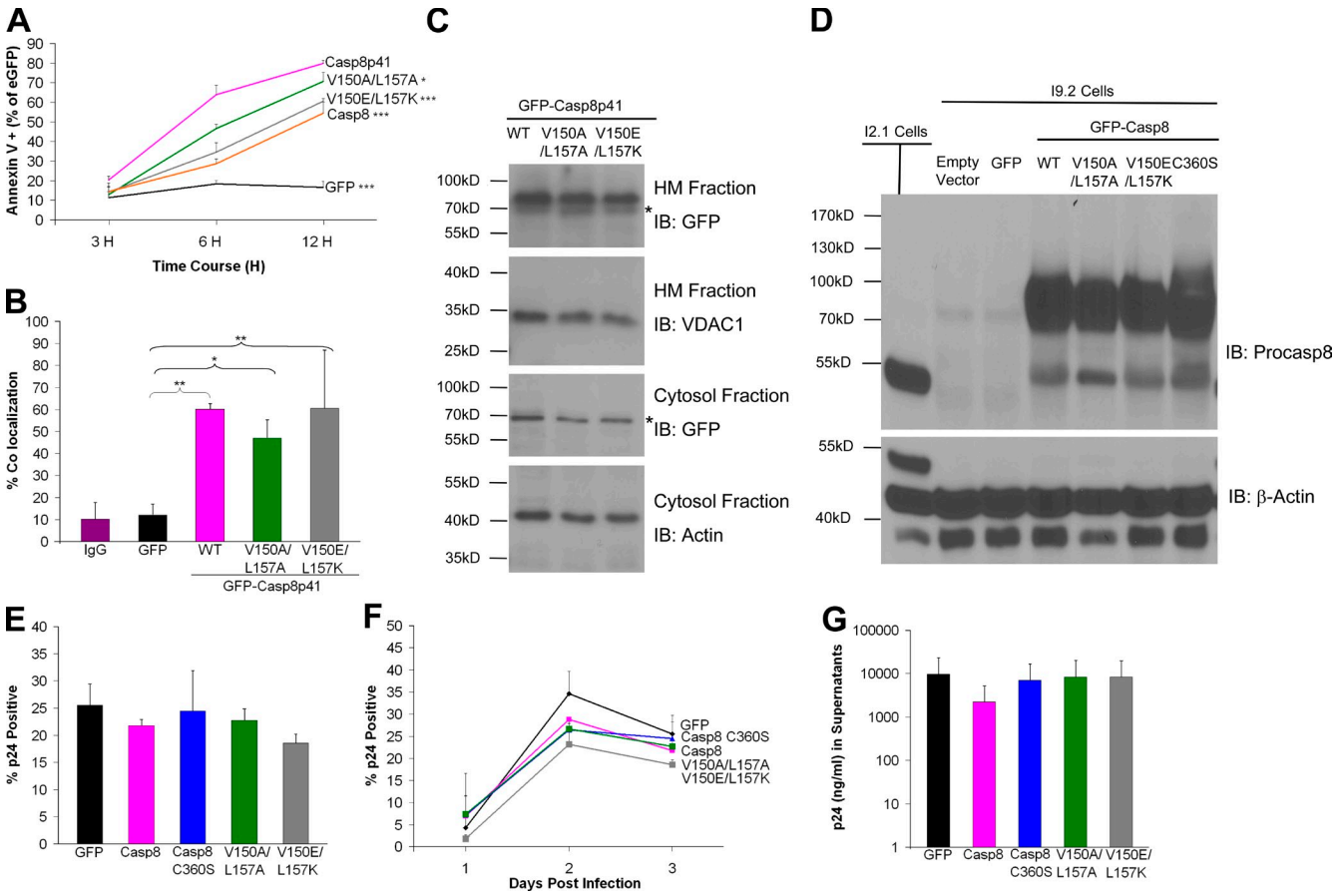

Figure S3. **Caspase 8 mutations do not impair viral production.** (A) Flow cytometry data from Jurkat T cells transfected with empty vector or EGFP-Casp8p41, EGFP-Casp8p41 V150A/L157A, EGFP-Casp8p41 V150E/L157K, or EGFP–procaspase 8 (Procasp8) and stained with Annexin V over a 12-h time course after transfection. (B) Colocalization of GFP and mitochondrial Bak in 293T cells transfected with empty vector, EGFP-Casp8p41, EGFP-Casp8p41 V150A/L157A, or EGFP-Casp8p41 V150E/L157K. Summarized results from three independent images are shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (C) Jurkat cells transfected with empty vector, EGFP-Casp8p41, EGFP-Casp8p41 V150A/L157A, or EGFP-Casp8p41 V150E/L157K were fractionated into heavy membrane (HM) or cytosolic fractions. VDAC1 served as a loading control and proof of fraction purity for heavy membrane fractions. Actin served as a loading control for the cytosol fraction. The asterisk represents a nonspecific band. Representative of $n = 3$. (D–G) I9.2 cells transfected with EGFP, EGFP–procaspase 8 (Procasp8) wt, EGFP-Procasp8 C360S, EGFP-Procasp8 V150A/L157A, or EGFP-Procasp8 V150E/L157K were infected with VSV-G–HIV-1. (D) Procasp8 expression in I9.2 cells reconstituted with EGFP fused to procaspase 8, procaspase 8 V150E/L157K, or procaspase 8 V150A/L157A. (E) Expression of p24-positive cells in the total culture was assessed via flow cytometry on day 3 after infection. (F) Expression of p24-positive cells in the total culture was assessed via flow cytometry over the course of the infections. (G) Expression of p24 in culture supernatants was assessed via ELISA on day 3 after infection. Error bars indicate ±1 SD from three independent experiments. Error bars indicate SD.