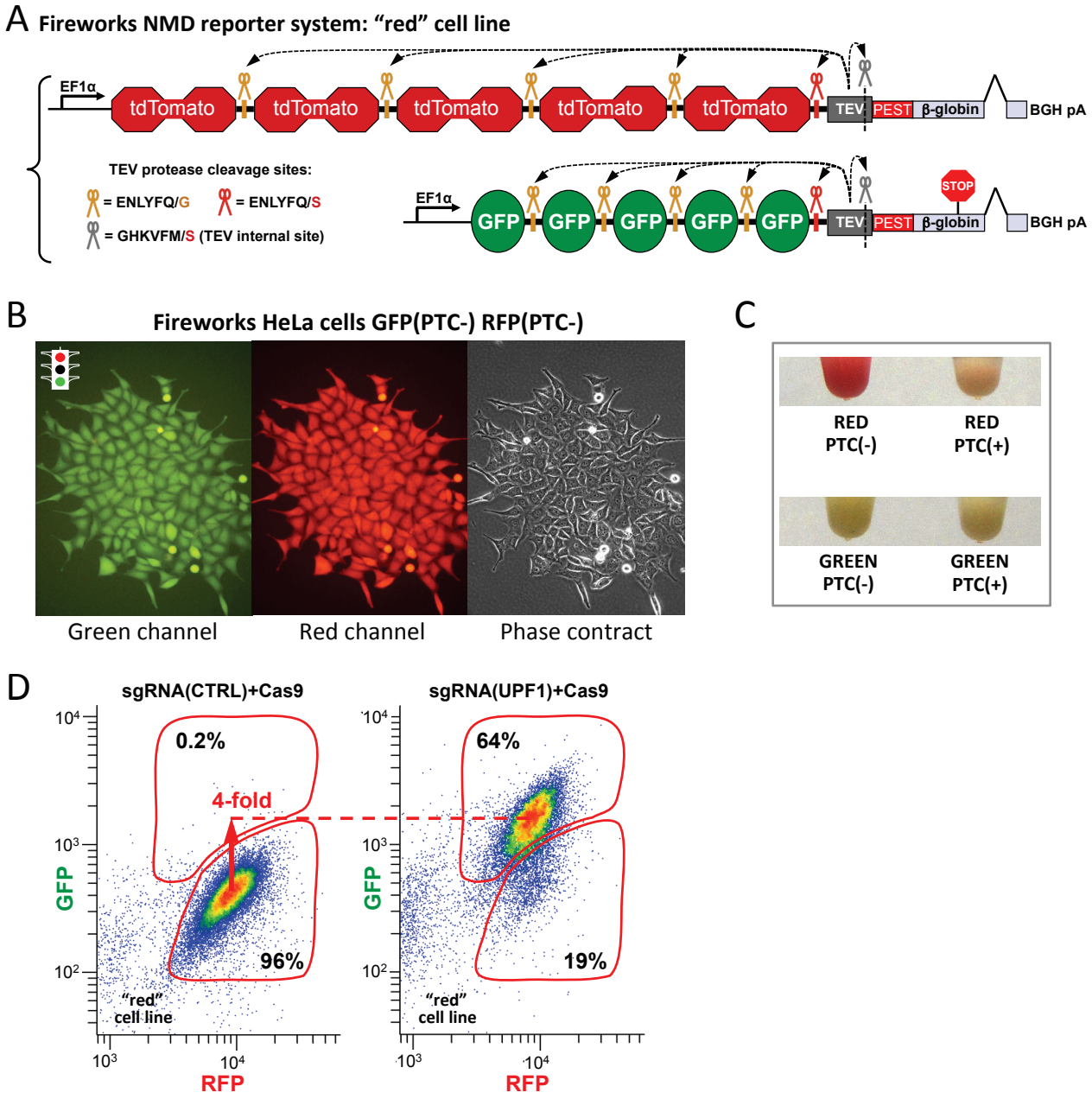

Figure S1, related to Figure 1. Fireworks in vivo NMD reporter system: **(A)** Schematics of the orthogonal "red" Fireworks cell line. In this cell line, mRNA transcribed from the PTC-containing GFP reporter is destabilized by NMD, whereas the PTC-lacking RFP reporter serves as an expression control. Otherwise, the reporters are identical to those shown for "green" Fireworks cell line in **Figure 1A**. Together, the "green" and "red" Fireworks cell lines provide controls for cell line- and fluorescent protein-specific effects. **(B)** There is little observable cell-to-cell variation in the fluorescence of Fireworks cells. Magnified view of a single colony of the HeLa Fireworks cell line in which both RFP and GFP reporters lack PTCs. **(C)** Pellets of HeLa cells carrying individual genome-integrated Fireworks reporters (shown at ambient lighting) exhibit high levels of fluorescent protein expression. **(D)** NMD inhibition via expression of Cas9 and UPF1-targeting sgRNA results in a 4-fold increase in green fluorescence of the "red" Fireworks cell line (shown in **Figure S1A** and the right panel of **Figure 1C**).

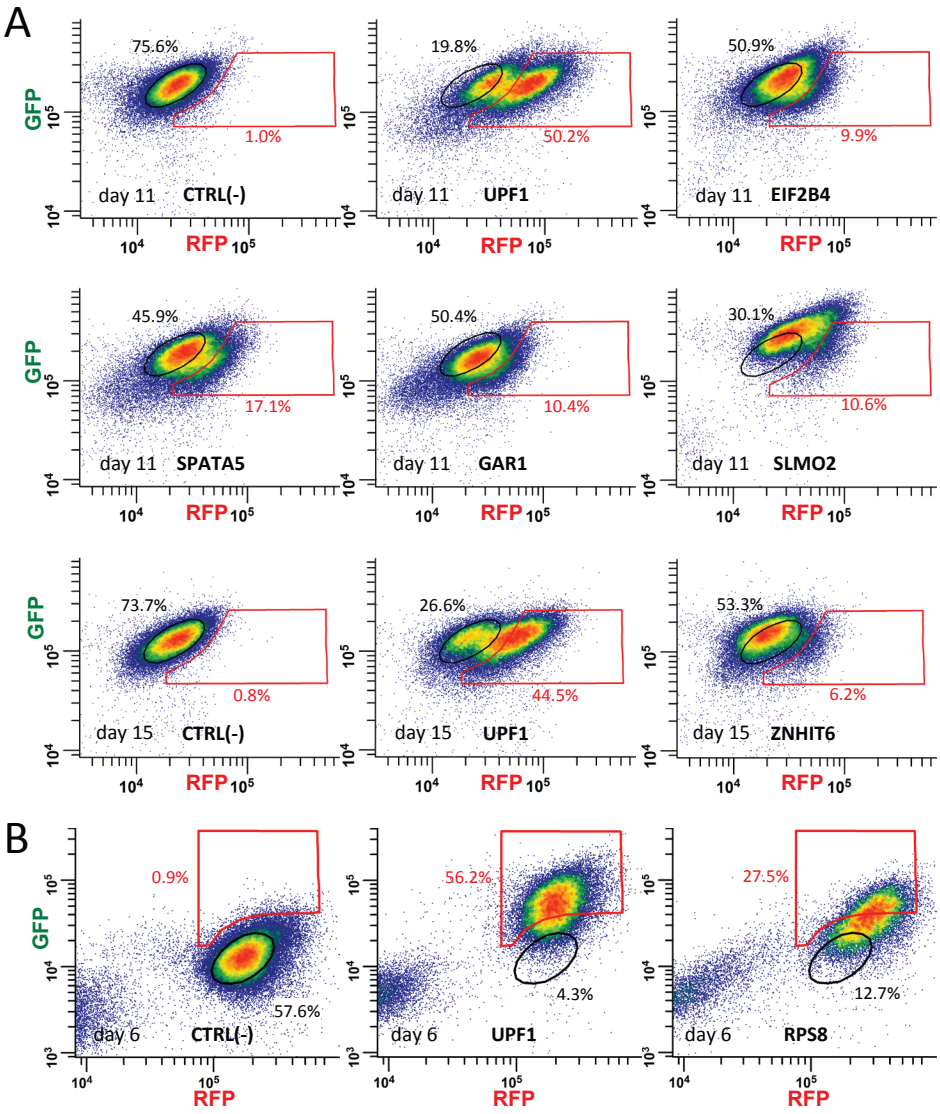

Figure S2, related to Figure 3. FACS analysis of the fluorescence shift produced by individual sgRNAs identified by Fireworks screening of the GeCKO-LtCRISPR sgRNA library for factors affecting human NMD. (A) FACS analysis of "green" Fireworks cells transduced with individual sgRNAs obtained from the genome-wide screen (**Table 1**) was conducted as described in **Figure 3A** and Experimental Procedures. **(B)** Transduction of RPS8-targeting shRNA into the "red" Fireworks cell line results in an increase in green fluorescence. Populations of cells with increased green fluorescence are seen in the red gate.

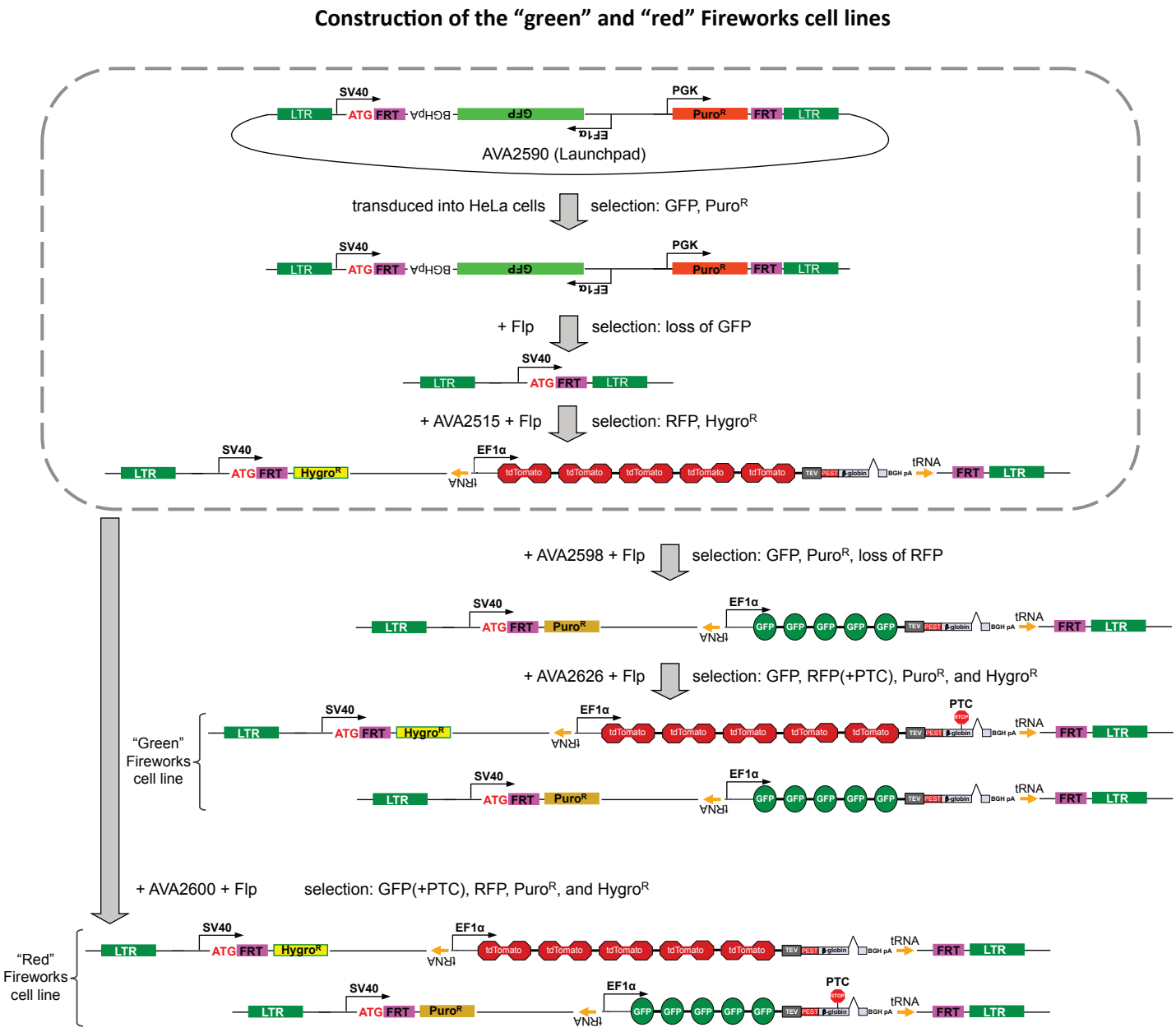

Figure S3, related to Experimental Procedures. Schematics of the construction of the orthogonal "green" and "red" HeLa Fireworks cell lines. Genomic integration of FRT sites was achieved via transduction of HeLa cells with the lentiviral vector AVA2590 (Launchpad) and the resulting pool of cells was FACS-selected for cells stably expressing Launchpad's GFP. FRT-flanked sequence of the integrated Launchpad was excised using transient transfection of Flp recombinase and the cells were FACS-selected for loss of GFP. PTC-lacking red Fireworks reporter (AVA2515) was FRT-integrated using Flp recombinase and the cells were FACS-selected for stable expression of the red Fireworks reporter. Subsequent genomic integration of the other Fireworks reporters, AVA2600 [GFP(PTC+)], AVA2598 [GFP(PTC-)], and AVA2626 [RFP(PTC+)], was achieved via Flp-mediated cassette integration/exchange and antibiotic selection, as shown; cells, concurrently expressing both green and red reporters were isolated using FACS sorting. Final "green" and "red" HeLa Fireworks cell lines were obtained by isolating individual colonies that displayed minimal, if any, silencing of integrated reporters after long-term propagation.

Supplemental Data File S1, related to Experimental Procedures. Supplemental vector sequences.