# Supplementary Materials: Depletion of Fumarate Hydratase, an Essential TCA Cycle Enzyme, Drives Proliferation in a Two-Step Model

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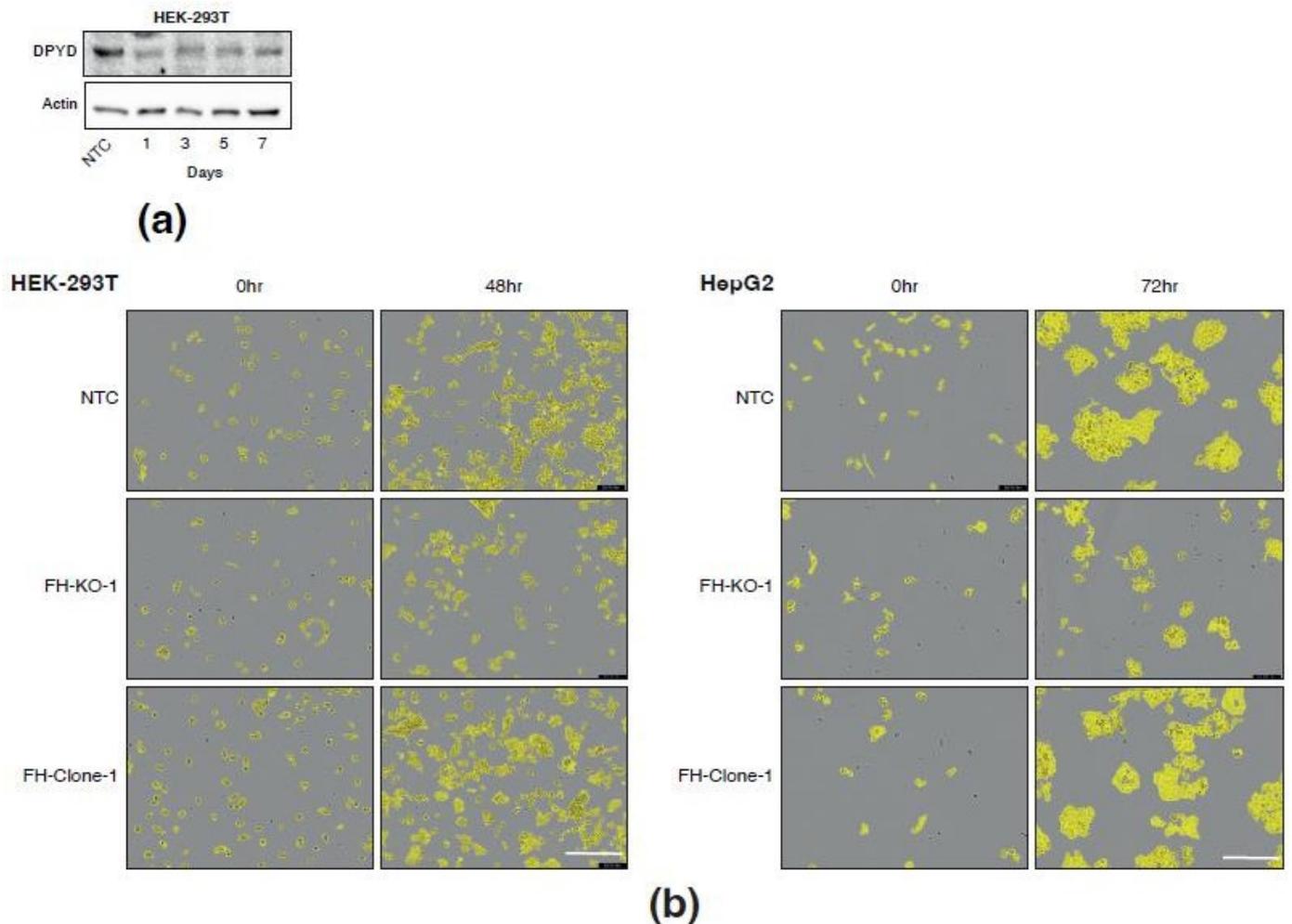

Figure S1. Short-term FH loss inhibits cell proliferation: (**a**) DPYD was silenced in HEK-293T using the CRISPR-Cas9 system and then subjected to 24 hours of puromycin selection. At the indicated timespost-selection (short-term), the cells were lysed and subjected to immunoblotting using the indi- cated antibodies. NTC is a nontargeted control. (**b**) Representative pictures of the proliferation assayas taken by the IncuCyte. Images representing the number of cells during 0 and 48 hours in HEK-293T and 0 and 70 hours in HepG2. Bar = 400 μm.
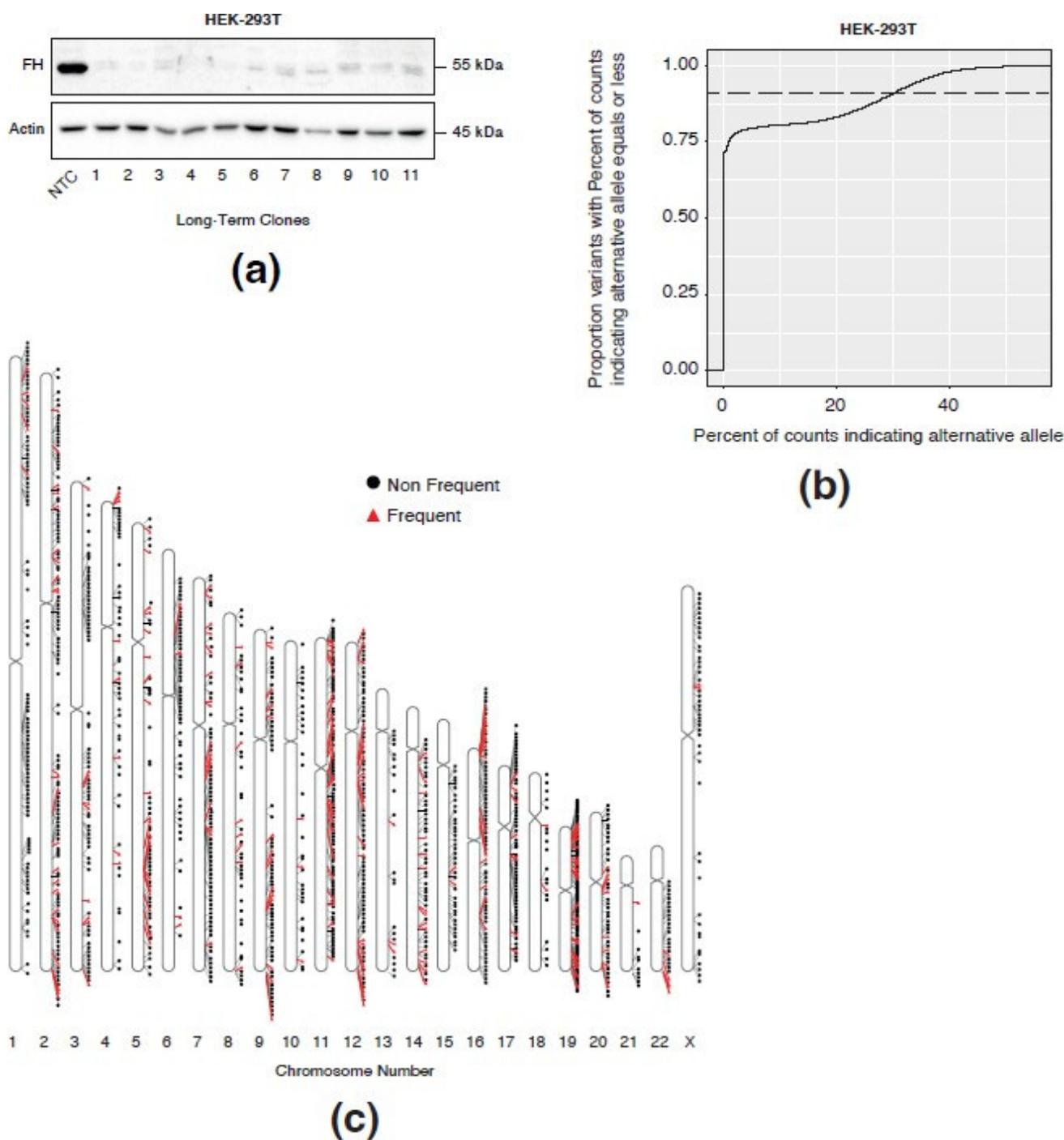
Figure S2. Distribution of the different variants: (**a**) Western blot indicating FH level in the 11 differentFH-KO long-term stable clones. The cells were lysed and subjected to immunoblotting using the indicated antibodies. NTC is a nontargeted control. (**b**) The cumulative distribution plot of the percent of counts indicates an alternative allele in the HEK-293T cell line. (**c**) The synonymous mutation distribution pattern is represented in the different chromosomes. The mutation that occurred in more than 4 clones, is considered a frequent mutation and marked as a red triangle.

(a)

(b)

(c)
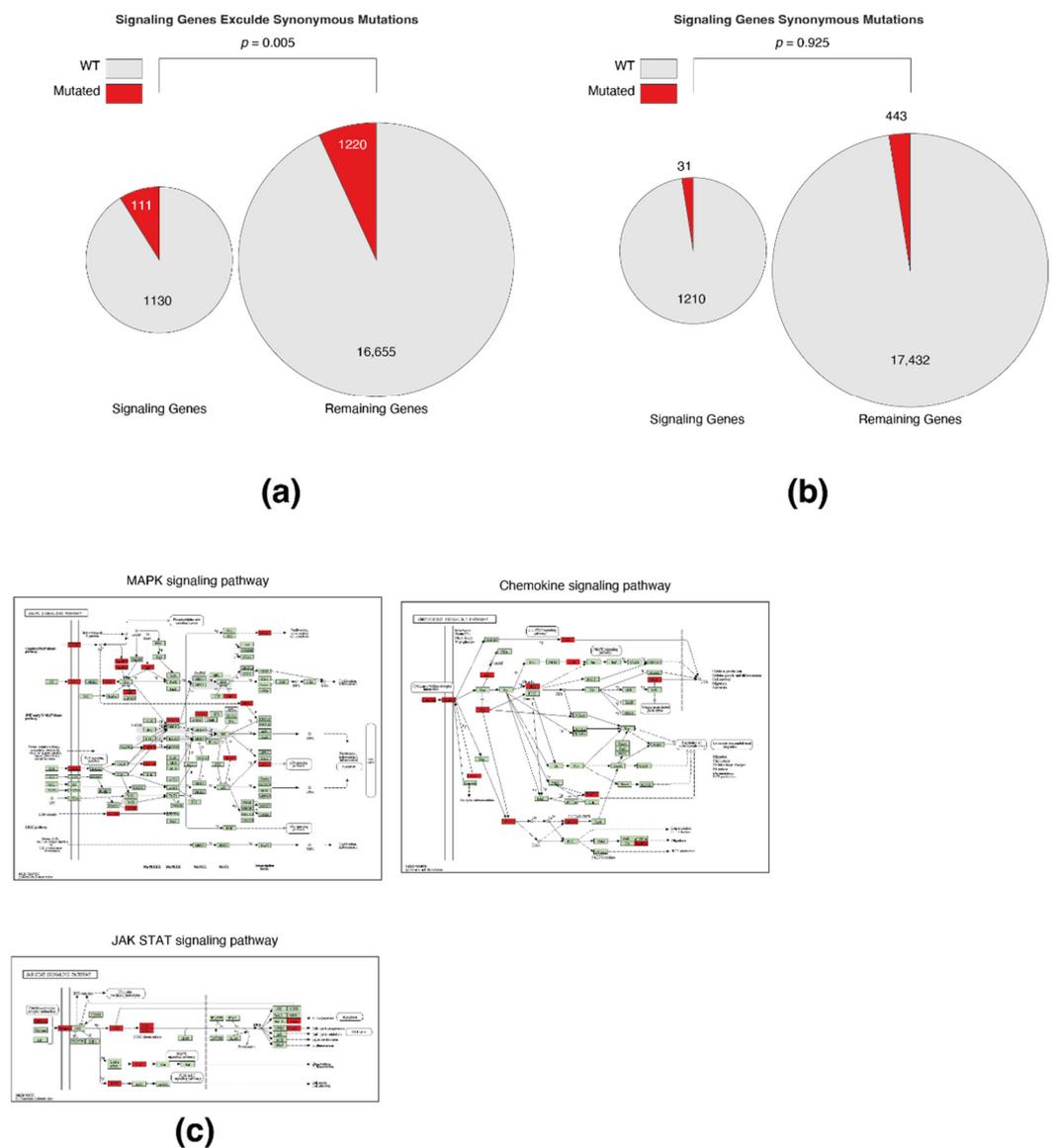
Figure S3. FH loss-dependent mutations are enriched in signaling genes: (**a**) Genes encoding for signalingmolecules are enriched with mutations caused by FH knockout. All of the signaling genes were defined based on the KEGG pathway database (https://www.genome.jp/kegg/pathway.html). The number of mutations (excluding synonymous) in the signaling and non-signaling genes were de- termined. A pie chart representing the proportion of mutations in the signaling genes versus the remaining genes. Pvalue is calculated by Fisher's exact test using R. (**b**) Same analysis as A but for synonymous mutations. (**c**) A selected set of genes found to be mutated in FH long-term clones are highlighted in the corresponding signaling cascade. Specifically, the genes were labeled using the KEGG color website (https://www.genome.jp/kegg/pathway.html) [8], which displays the distribution of the mutated genes (marked in red) in the different signaling pathways.
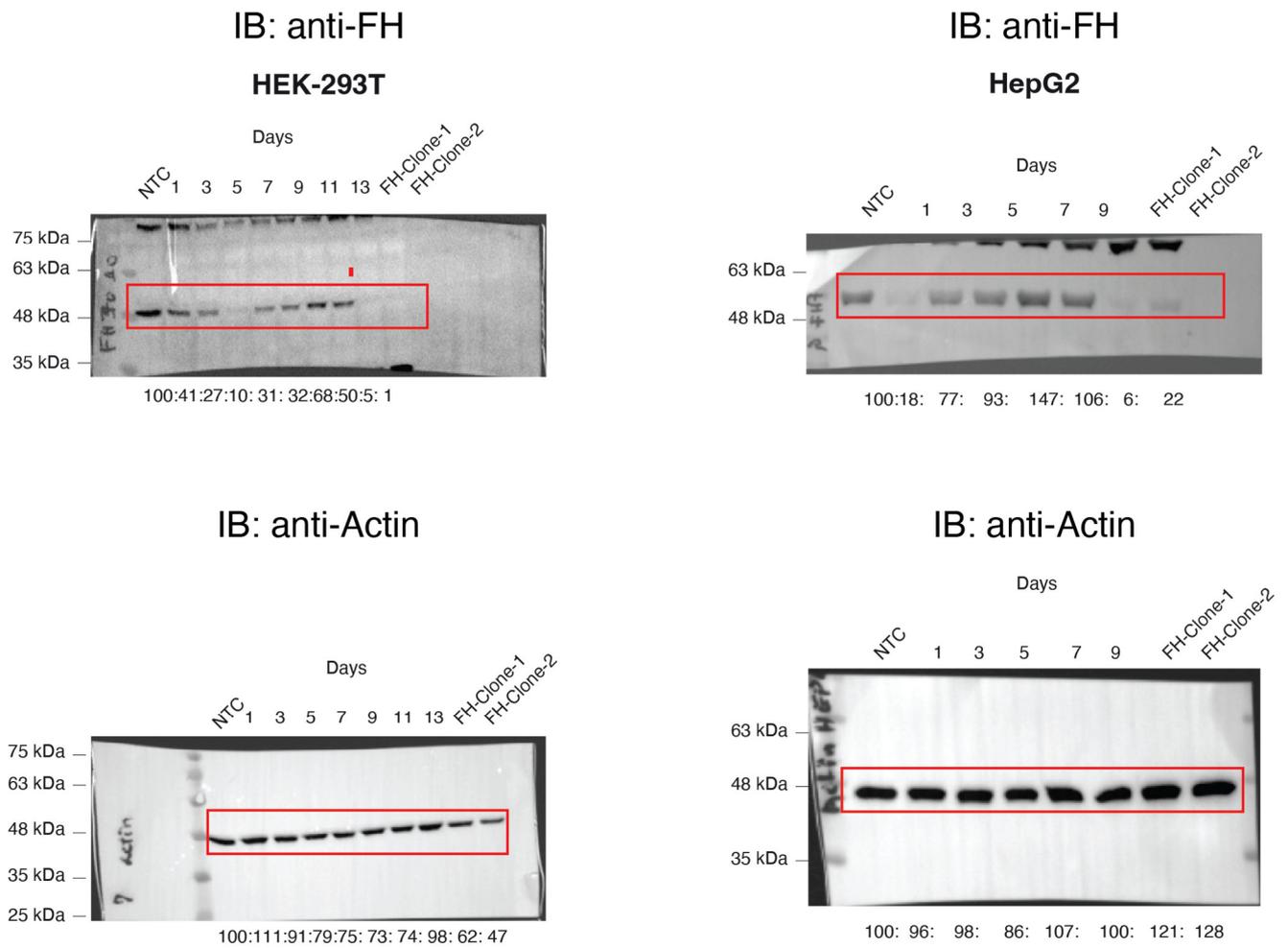
**Figure S4.** Uncropped Western Blots of blots shown in Figure 1.
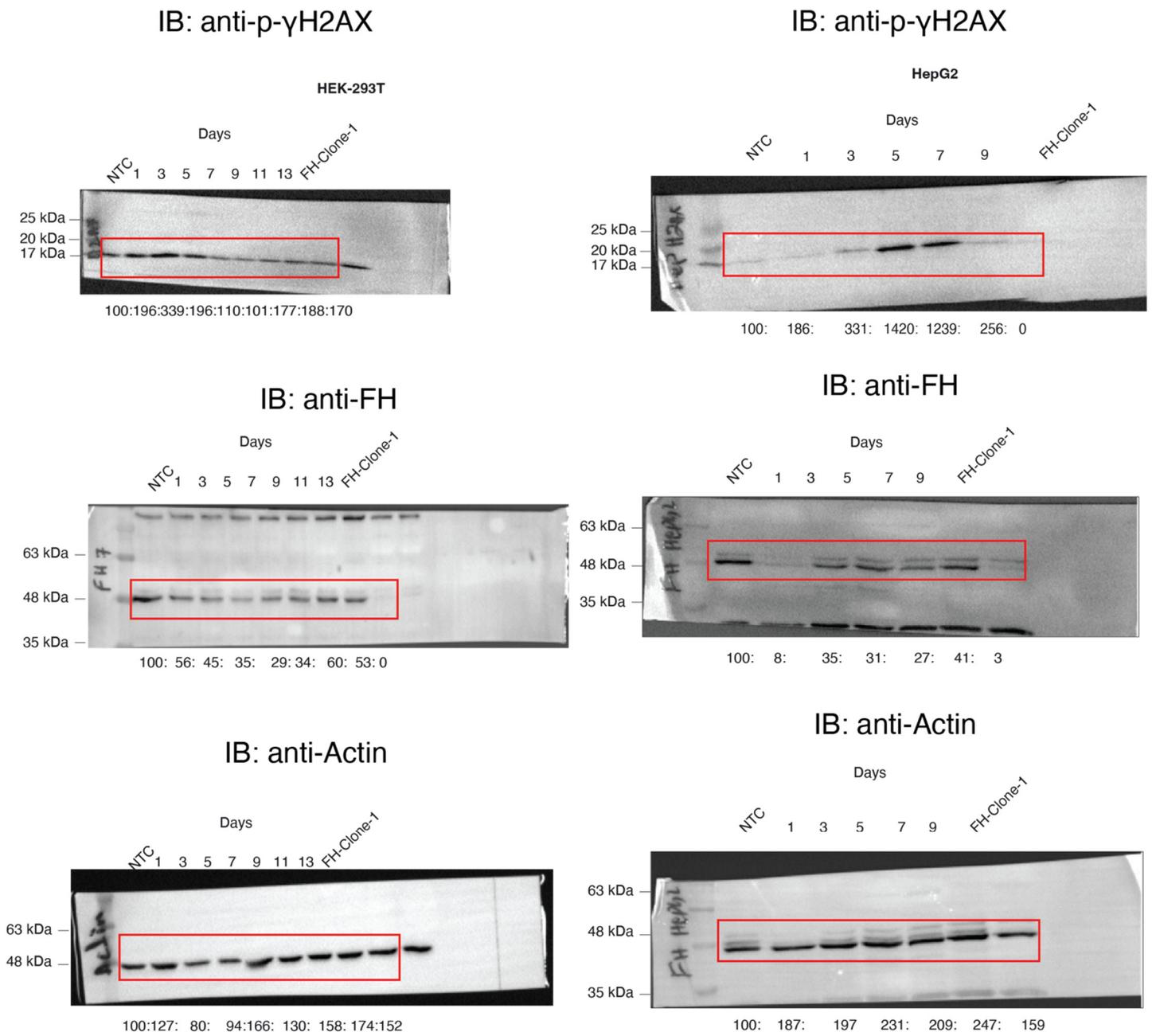
**Figure S5.** Uncropped Western Blots of blots shown in Figure 2.

IB: anti-pSTAT3 (Y705)　　IB: anti-STAT3　　IB: anti-FH　　IB: anti-actin

(b)

IB: anti-pSTAT3 (Y705)　　IB: anti-STAT3　　IB: anti-actin

IB: anti-pSTAT3 (Y705)　　IB: anti-STAT3　　IB: anti-actin
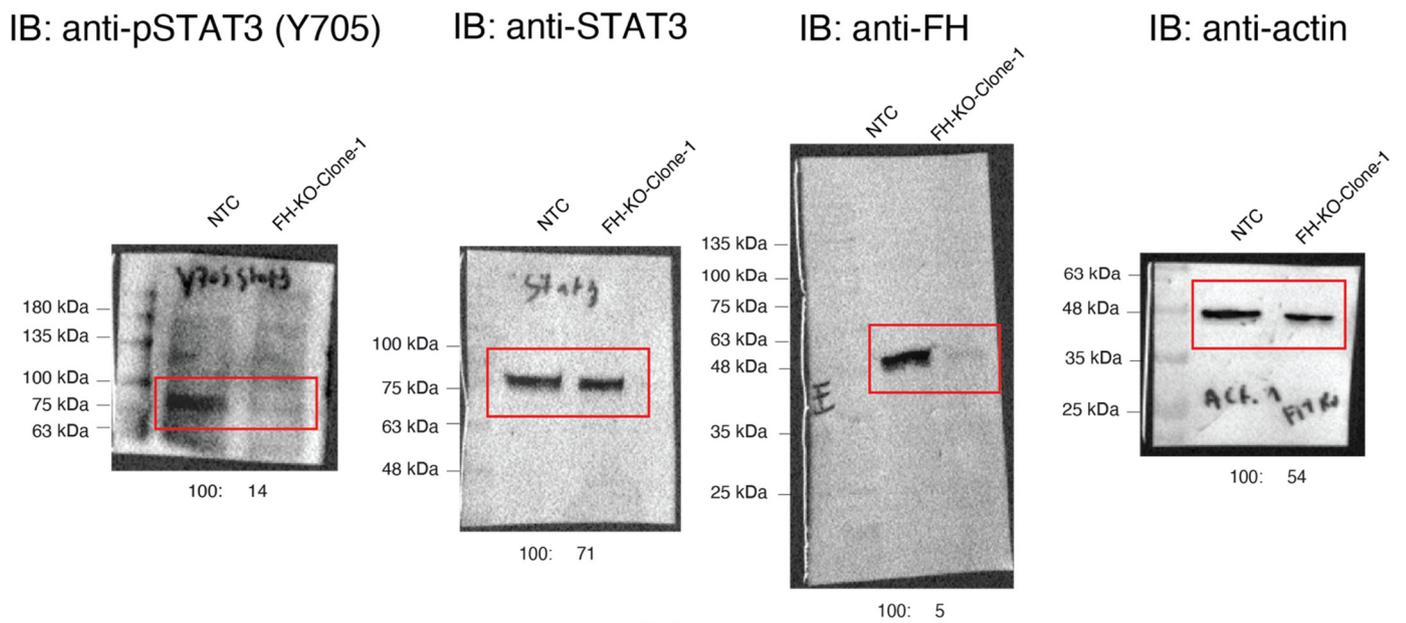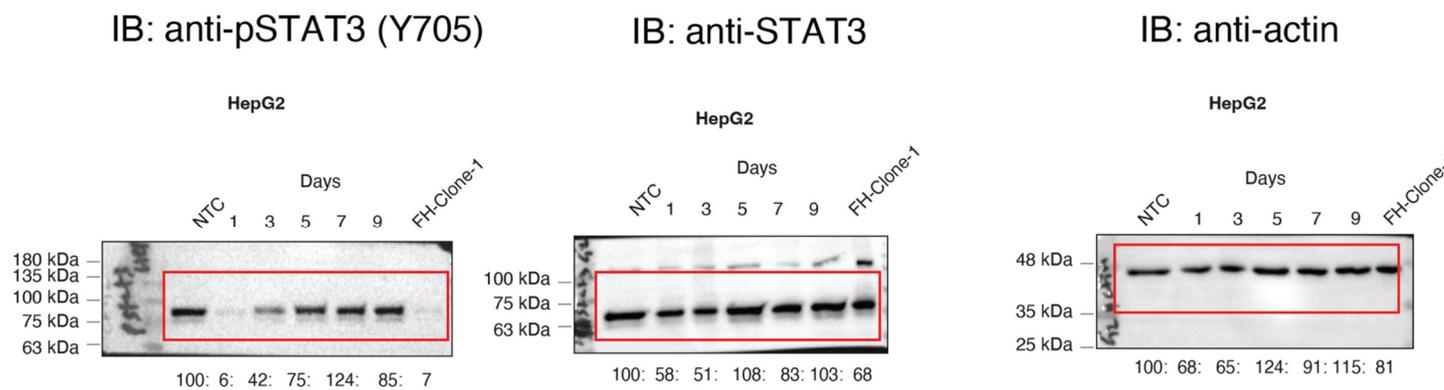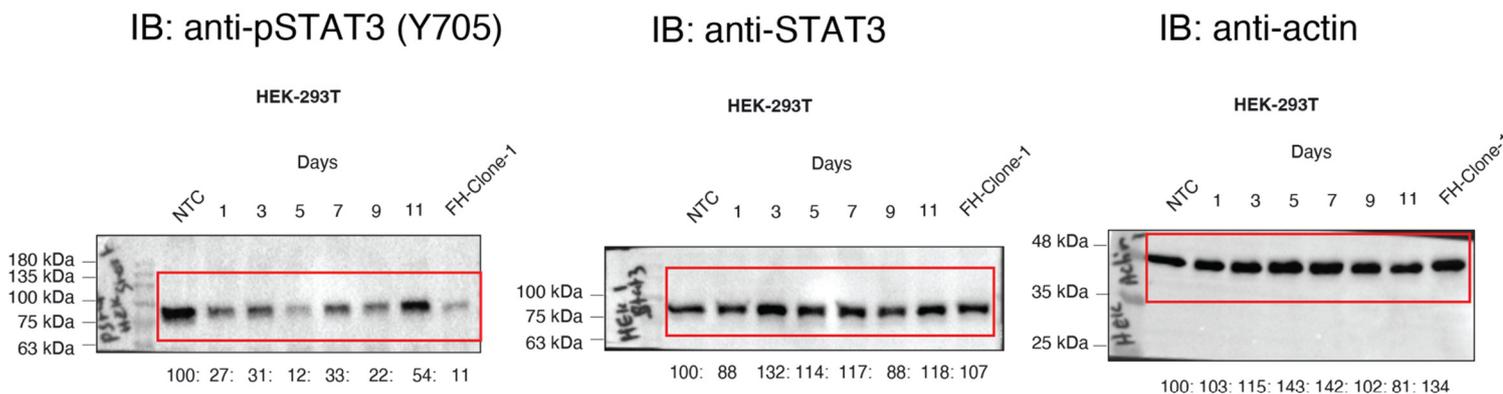
(d)

Figure S6. Uncropped Western Blots of blots shown in Figure 5.

IB: anti-FH

NTC 1 2 3 4 5 6 7 8 9 10 11

75 kDa —
63 kDa —
48 kDa —
35 kDa —

100: 1: 1: 1: 1: 1: 3: 6: 3: 1: 1: 1

IB: anti-actin

NTC 1 2 3 4 5 6 7 8 9 10 11

63 kDa —
48 kDa —
35 kDa —

100:92:98: 56: 72: 82: 111:117: 37: 97: 72: 125
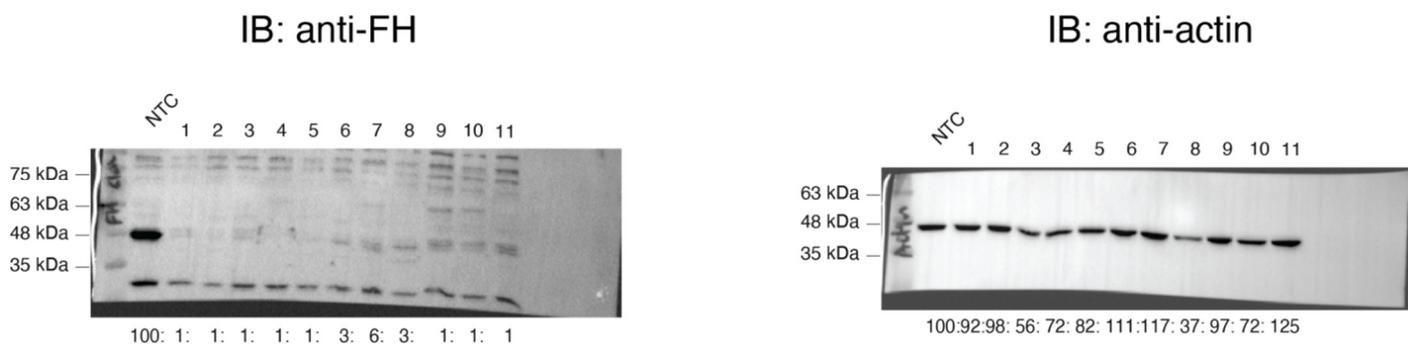
Figure S7. Uncropped Western Blots of blots shown in Figure S1 and S2.