SUPPLEMENTAL MATERIAL

Schmitz et al., http://www.jem.org/cgi/content/full/jem.20090528/DC1

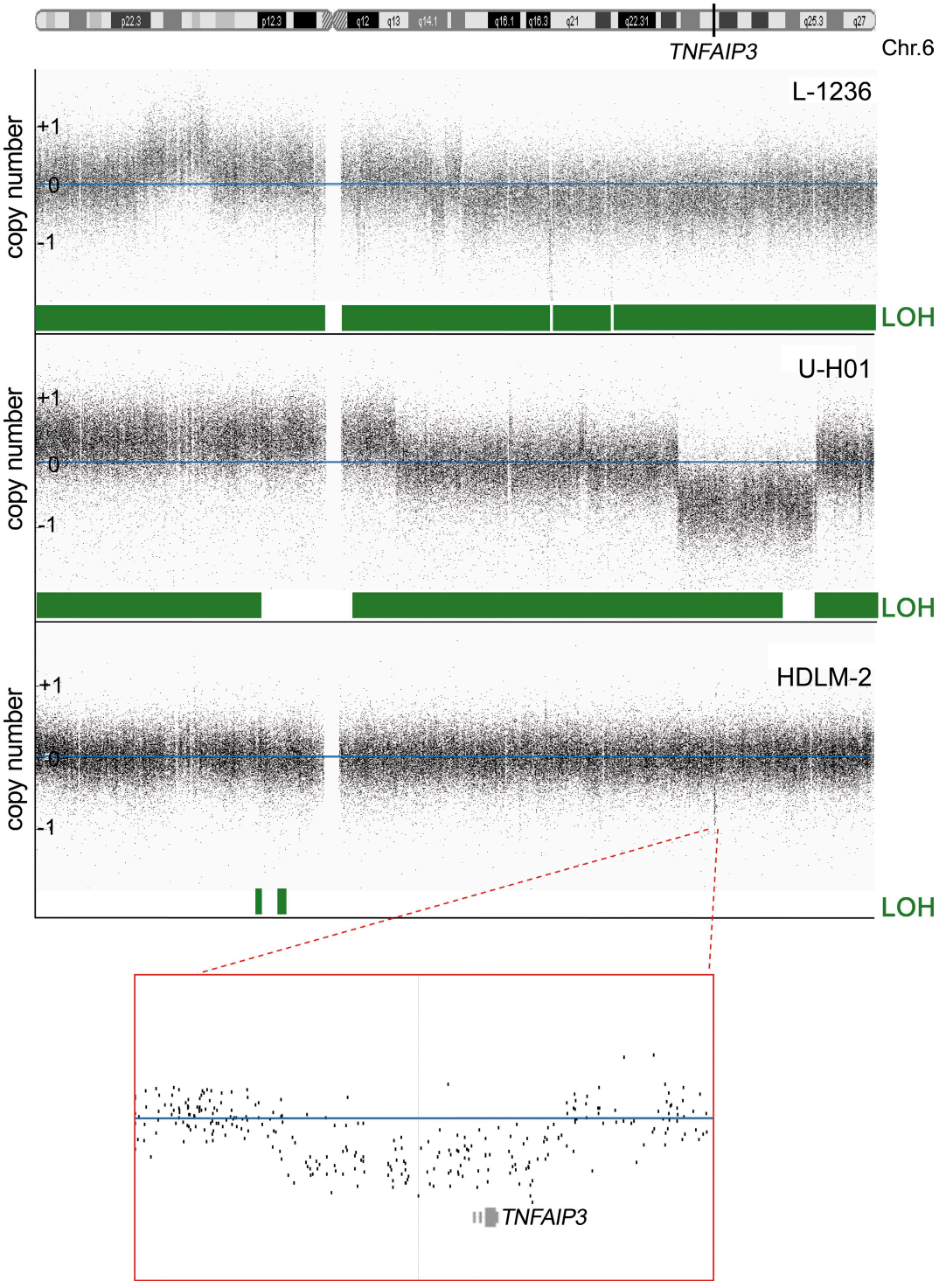


Figure S1. **Genome-wide human SNP Array 6.0 analyses of cHL cell lines revealing deletions and LOH at the *TNFAIP3* locus.** Copy number and LOH analysis of chromosome 6 in the cHL cell lines L-1236, U-HO1, and HDLM-2 using the genome-wide human SNP Array 6.0. Copy number analysis, LOH analysis, and segmentation were calculated using Genotyping Console software (version 3.0). Segments with aberrant copy numbers were considered as copy number aberration only if they consisted of at least 20 consecutive SNPs and comprised a minimal size of 100 kb. "0" indicates a balanced status at the given ploidy of the respective cell line. Gains and losses are indicated by positive and negative values, respectively, but because of the changes in ploidy in some of the cell lines; values need not necessarily reflect the true changes in absolute copy numbers. Regions of LOH are indicated by green bars. Deletions encompassing the *TNFAIP3* locus were detected in all three cell lines. In cell line L-1236, which is nearly triploid, the nondeleted copies additionally showed LOH. The minimal deleted region comprises ~200 kb and involves the *TNFAIP3* gene (bottom).

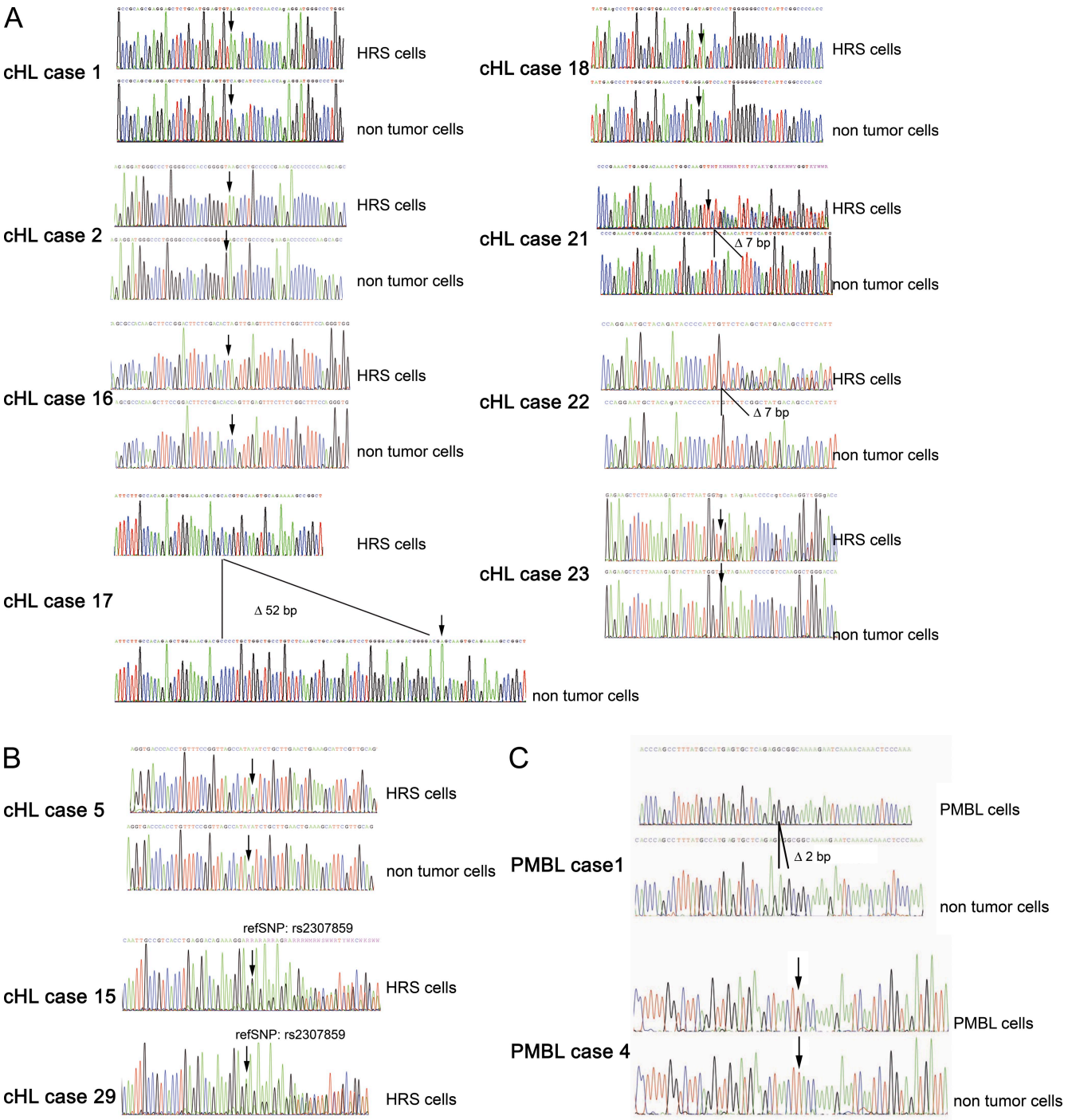


Figure S2. *TNFAIP3* sequences of primary HRS cells, PMBLs, and corresponding nontumor cells. (A) Representative sequence electropherograms of eight HL cases showing mutations of *TNFAIP3* in HRS cells and their absence in non-HRS cells. Detection of only the mutated alleles in cases 1, 2, 16, 17, and 18 indicates loss of the second allele. Positions of mutations are indicated by arrows or lines. Δ, deletion. (B) Sequence electropherograms of three HL cases. Case 5 shows a sequence variant (arrow), presumably a polymorphism, in HRS and non-HRS cells. SNP rs2307859 (indicated by arrows) was used in cases 15 and 29 to determine the presence of both alleles in HRS cells. (C) Sequence electropherograms of PMBL cases 1 and 4 showing somatic mutations in PMBLs. Positions of mutations are indicated by arrows or lines.

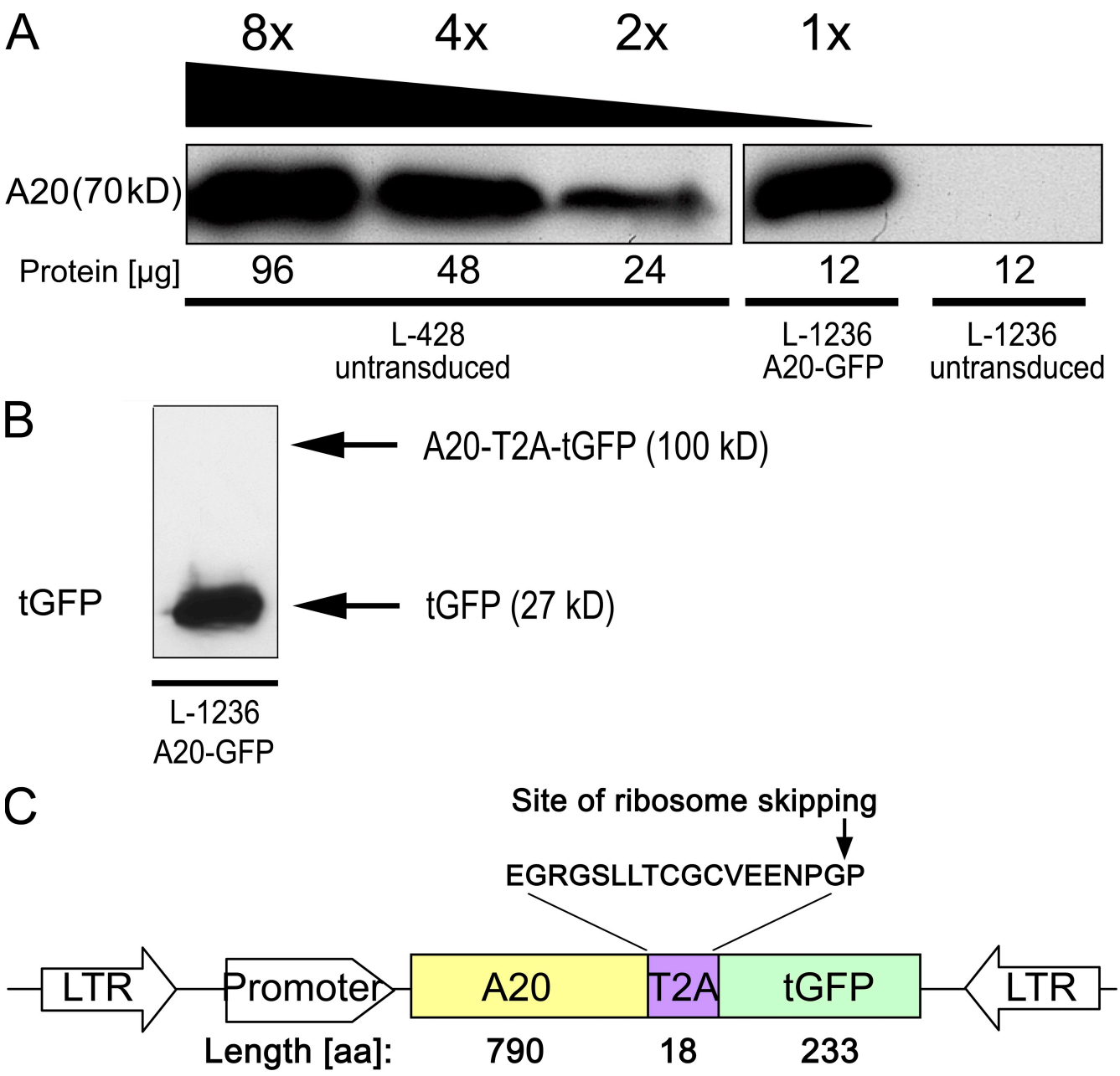
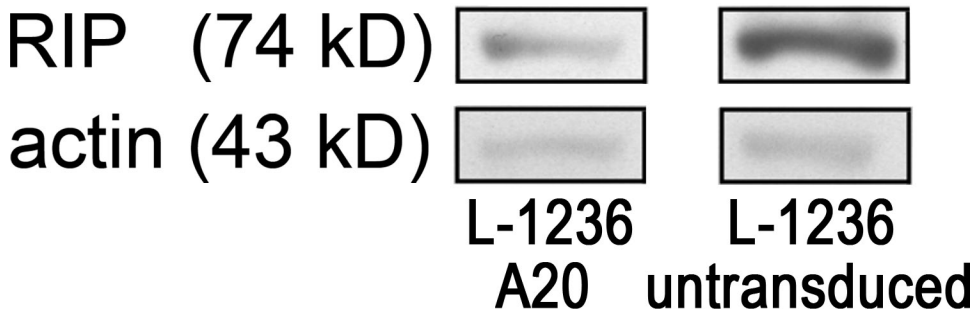


Figure S3. Expression and function of the A20-T2A-GFP lentiviral construct. (A) A20 protein expression level from the lentiviral construct in FACS-sorted GFP$^+$ L-1236 cells, in comparison to different amounts of cell lysates from L-428 cells, expressing endogenous A20. The transduced L-1236 cells express approximately fourfold more A20 than the L-428 cells. (B) Western blot analysis for GFP expression in L-1236 cells transduced with the lentiviral vector encoding A20-T2A-GFP. Only turboGFP (tGFP) of 27 kD is visible, but not the fusion protein of A20 with GFP, demonstrating efficient ribosome skipping mediated by the T2A sequence. Shown is a representative result of two Western blot analyses performed. (C) Structure of the A20-T2A-GFP lentiviral construct. Coding sequences of *TNFAIP3*, T2A, and *GFP* were cloned in frame behind a promoter derived from the CMV. LTR, long terminal repeat.



xFigure S4. Reduction of RIP protein levels upon A20 reexpression in L-1236 cells. Untransduced L-1236 cells and L-1236 cells transduced with a lentiviral vector encoding A20 were analyzed by immunoblotting for expression of RIP protein, revealing a strongly reduced protein level in A20-expressing L-1236 cells. Blotting with an antibody for β-actin confirms equal protein loading.

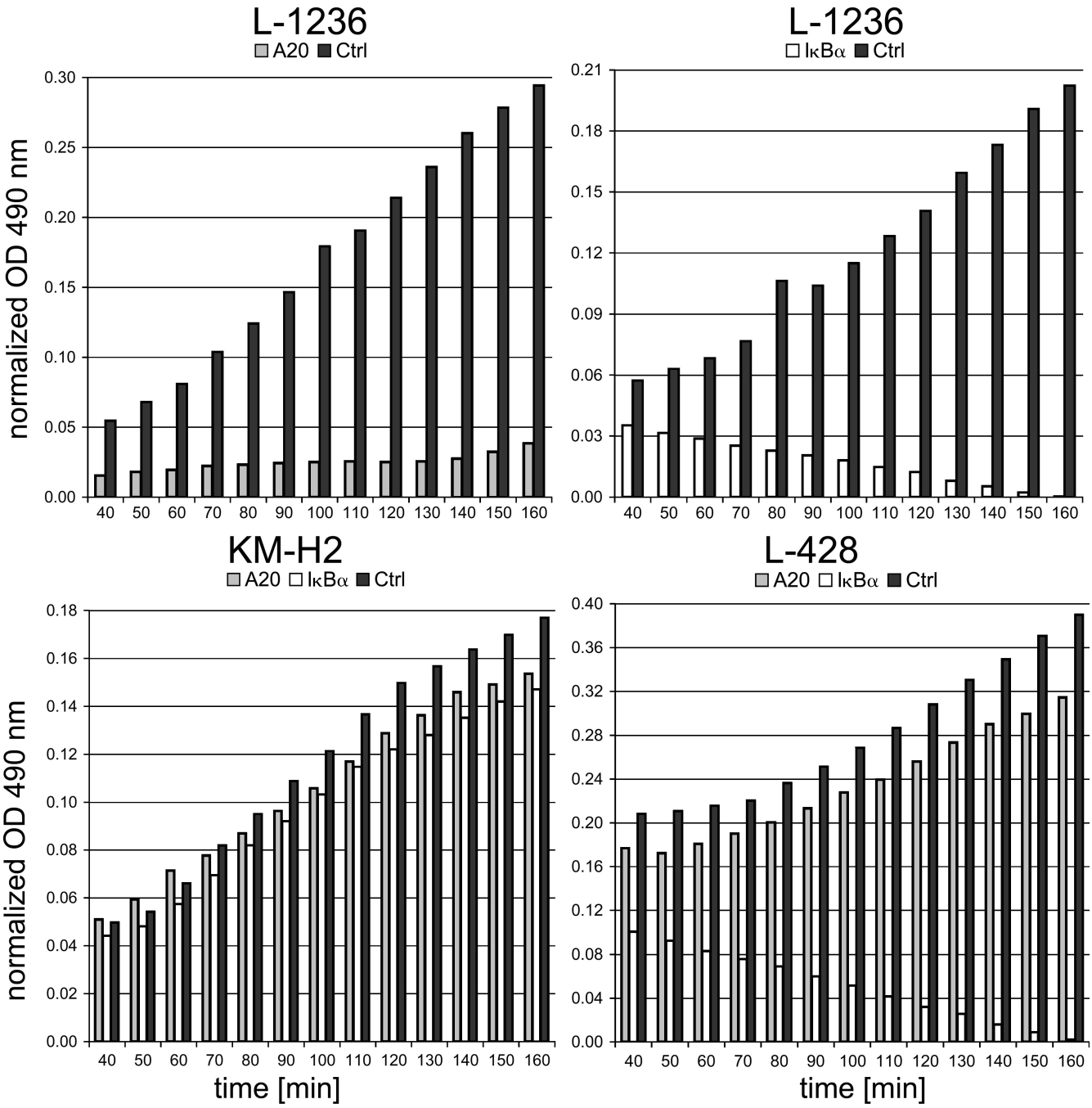


Figure S5. A20 reconstitution in cHL cell lines harboring inactivating *TNFAIP3* mutations. GFP$^+$ cells were FACS sorted, cultured for 48 h, and analyzed by MTS assay for metabolic activity. Mean OD values, reflecting conversion of MTS into a formazan product, are normalized to the empty medium control. The quantity of the formazan product as measured by the amount of 490-nm absorbance is directly proportional to the number of living cells in culture. Infection and MTS assay of L-1236 cells transduced with A20 or IκBα was split to two separate experiments. Each value is based on four measurements.

Table S2. Primer sequences used for seminested two-round genomic DNA amplification of *TNFAIP3*

Primer name	Sequence (5′ to 3′)	Amplification of
A20E2exF	TGCCTACAGATCAGGGTAATGACAAG	exon 2
A20E2intF	GTTTCCTGCAGGCAGCTATAGAGG	exon 2
A20E2R	AGCTTCATGAATGGGGATCCAGCAG	exon 2
A20E3exF	ACCATTCAGTCCCCTAGAATAGCAG	exon 3
A20E3intF	ACCTTTGCTGGGTCTTACATGCAG	exon 3
A20E3R	TATGCCCACCATGGAGCTCTGTTAG	exon 3
A20E4-5exF	TGAATAATTGTAGAGTGATGTCAGAATGAC	exon 4/5
A20E4-5intF	TACAGGGAGTACAGGATACATTCAAGC	exon 4/5
A20E4-5R	GGAAAACCCTGATGTTTCAGTGTCTAG	exon 4/5
A20E6exR	AATCACTCTACTGTTGAGCTTCAGG	exon 6
A20E6F	TGAGATCTACTTACCTATGGCCTTG	exon 6
A20E6intR	TCAGGTGGCTGAGGTTAAAGACAG	exon 6
A20E7.1exF	GGTTCTACAATTCTTGCCATAATCCAC	exon 7
A20E7.1intF	GAGCTAATGATGTAAAATCTTGTGTGTG	exon 7
A20E7.1R	CAAAATCCGTTGTGCTGCACATTCAG	exon 7
A20E7.2exR	CAGTTCTGCCTGACTGCCTACATG	exon 7
A20E7.2F	CTCTCGGGGAGAAGCCTATGAGC	exon 7
A20E7.2intR	GAACAAAACCCCTTCTGGACAGCAG	exon 7
A20E8exR	ATGAGGAGACAGAACCTGGCAGAG	exon 8
A20E8F	ACTGTCAGCATCTCTGTATCGGTG	exon 8
A20E8intR	TGTCACTGTCGGTAGAAAACGCTC	exon 8
A20E9exF	GTAGACTCCACACTCTCCAATGAG	exon 9
A20E9intF	GTGCTCTCCCTAAGAAATGTGAGC	exon 9
A20E9R	GGGTTACCAAACCTGAGCATCGTGC	exon 9