

Expression of mammalian transgenes enriched in rare codons is enhanced by the MAPK pathway

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Supplementary Figure 1. cDNAs.

Supplementary Figure 2. A reporter to characterize the expression of rare-codon enriched transcripts.

Supplementary Figure 3. MAPK pathway enhances the expression of rare-codon enriched transcripts.

Supplementary Figure 4. MAPK pathway enhances the translation of rare-codon enriched transcripts.

Supplementary Figure 5. Uncropped immunoblots shown in figures.

Supplementary Table 1. Summary of the Cancer Toolkit (CTK) lentiviral library screen.

a

cDNA: GFP^{rare}

CAI: 0.729

Source: gene synthesis

5'ATGGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGCGATGTTAATG
GGCAAAAATTCTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACCTTACCCTTAAATTTATTTGC
ACTACTGGGAAGCTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTATGGTGTTCATGCTTTTCAAGAT
ACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATA
TTTTACAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAAT
CGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAAATGGAATACAACATAACTCAC
ATAATGTATACATCATGGCAGACAAACCAAAGAATGGAATCAAAGTTAACTTCAAATTAGACACAACATTA
GATGGAAGCGTTCAATTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGAC
AACCATTACCTGTCCACACAATCTGCCCTTCCAAAGATCCCAACGAAAAGAGAGATCACATGATCCTTCTTGAG
TTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA

b

cDNA: GFP^{com}

CAI: 0.962

Source: pEGFP-c3; Clontech

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTA
GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCT
GCACCACCGGCAAGCTGCCCGTGCCTGGCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGC
CGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCAC
CATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAAC
CGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACA
ACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAAC
ATCGAGGACGGCAGCGTGAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGC
TGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC
CTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

c

cDNA: mCherry^{com}

CAI: 0.965

Source: mCherry2-C1 plasmid; Addgene 54563

5'ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGA
GGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCAGAC
CGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCTGGGACATCCTGTCCCCTCAGTTCATGTACG
GCTCCAAGGCCTACGTGAAGCACCCCGCGACATCCCCGACTACTTGAAGCTGTCTTCCCCGAGGGCTTCAATT
GGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCG
AGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTCCCCTCCGACGGCCCCGTAATGCAGTGTCTGATCATG
GGCTGGGAGGCTCCACTGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTG
AAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGC
CCGGCGCTACAACGTGACATCAAGTTGGACATCCTTTCCACAACGAGGACTACACCATCGTGGAAACAGTAC
GAACGCGCCGAGGGCCGCACTCCACCGCGGCATGGACGAGCTGTACAAGTAA

d

cDNA: mCherry^{rare}

CAI: 0.731

Source: gene synthesis

5'ATGGTCTCAAAGGGTGAAGAAGATAACATGGCAATTATTAAGAGTTTATGCGTTTCAAGGTGCATATGGAG
GGATCTGTCAATGGGCATGAGTTTGAATTGAAGGTGAAGGAGAAGGCCGACCATATGAGGGAACACAAACC
GCAAACTAAAGGTAACATAAGGCGGACCATTACCATTCGCCTGGGACATCCTCTCTCCACAGTTCATGTATGGA
AGTAAAGCTTATGTTAAACATCCGGCAGATATAACCAGATTATTTGAACTTTTCATCCCGGAGGGTTTTAAGTGG
GAACGCGTAATGAATTTTGAAGACGGAGGAGTTGTTACAGTGACGCAAGACTCAAGCCTCCAAGATGGAGAAT
TTATTTATAAGTCAAACCTTCGAGGAACGAATTTCCCTCGGATGGACCTGTTATGCAGAAGAAGACTATGGGA
TGGGAAGCTTCAAGTAAAAGAATGTACCCTGAAGACGGTCTCTTAAGGGAGAGATTAACAACGTCTTAAATT
GAAAGATGGAGGACATTACGATGCTGAGGTGAAGACAACCTACAAAGCCAAAAAACCAGTTTCAGCTGCCAGGA
GCGTACAATGTTAATATTAACCTGGATATCACCTCCCACAACGAGGATTACACTATCGTTGAGCAATATGAAAGA
GCTGAAGGGCGGCACTCGACAGGTGGCATGGATGAATTGTATAAGTAA

e

cDNA: GFP^{opt20}

CAI: 0.806

Source: gene synthesis, rare codons for all leucine and glycine converted to common, converted nucleotides are marked by lower case

5'ATGGTGAGTAAAGGcGAAGAACTgTTCACTGGgGTTGTCCCAATTCTgGTTGAActgGATGGCGATGTTAATGG
cAAAAAATTCTCTGTGCTGAGTGGcGAGGGcGAAGGcGATGCAACATACGGcAAACTgACCCTgAAATTTATTTGCACT
ACTGGcAAGCTgCCTGTTCCATGGCCAACACTcGTCACTACTCTGACCTATGGcGTTCAATGCTTTTCAAGATAACC
CAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCCAAGGcTATGTACAGGAAAGAACTATATTTT
ACAAAGATGACGGcAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGcGATACCCTgGTTAATAGAATCGAG
ctgAAAGGcATTGATTTTAAAGAAGATGGcAACATTCTgGGgCACAAAATGGAATACAACATAACTCACATAATG
TATACATCATGGCAGACAAACCAAGAATGGcATCAAAGTAACTTCAAATTAGACACAACATTAAGATGGc
AGCGTTCAAActcGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCTgctgCCAGACAACCATTA
CCTGTCCACACAATCTGCCCTgTCCAAAGATCCCAACGAAAAGAGAGATCACATGATCCTgCTgGAGTTTGTAA
AGCTGCTGGGATTACACATGGCATGGATGAACTgTACAAATAA

f

cDNA: GFP^{opt40}

CAI: 0.858

Source: gene synthesis, rare codons for all leucine, glycine, valine and lysine converted to common, converted nucleotides are marked by lower case

5'ATGGTGAGTAAgGGcGAAGAACTgTTCACTGGgGTgGTgCCAATTCTgGTcGAActgGATGGCGATGTaAATGGc
CAAAAgTTCTCTGTgAGTGGcGAGGGcGAAGGcGATGCAACATACGGcAAgCTgACCCTgAAgTTTATTTGCACTA
CTGGcAAGCTgCCTGTgCCATGGCCAACACTcGTgACTACTCTGACCTATGGcGTgCAATGCTTTTCAAGATACCCA
GATCATATGAAgCAGCATGACTTTTTCAAGAGTGCCATGCCCCAAGGcTATGTcCAGGAAAGAACTATATTTTAC
AAgGATGACGGcAACTACAAGACACGTGCTGAAGTgAAGTTTGAAGGcGATACCCTgGTgAATAGAATCGAGctg
AAgGGcATTGATTTTAAgGAAGATGGcAACATTCTgGGgCACAAgATGGAATACAACATAACTCACATAATGTcT
ACATCATGGCAGACAAGcCAAAGAATGGcATCAAAGTgAACTTCAAgATTAGACACAACATTAAGATGGcAGCG
TgCAAActcGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTgCTgctgCCAGACAACCATTAACCTG

TCCACACAATCTGCCCTgTCCAAAGATCCCAACGAAAAGAGAGATCACATGATCCTgCTgGAGTTTGTgACAGCT
GCTGGGATTACACATGGCATGGATGAACTgTACAAGTAA

g

cDNA: GFP^{opt60}

CAI: 0.888

Source: gene synthesis, rare codons for all leucine, glycine, valine, lysine, threonine, aspartic acid, and glutamic acid converted to common, converted nucleotides are marked by lower case

5'ATGGTGAGTAAgGGcGAgGAgCTgTTCACcGGgGTgGTgCCAATTCTgGTcGAgctgGAcGGCGAcGTaAATGGcC
AAAAGTTCTCTGTgAGTGGcGAGGGcGAgGGcGATGCAACcTACGGcAAgCTgACCCTgAAgTTTATTTGCACcACc
GGcAAGCTgCCTGTgCCATGGCCAACcCTcGTgACcACcCTGACCTATGGcGTgCAATGCTTTTCAAGATACCCAGA
cCATATGAAgCAGCATGACTTTTTCAAGAGTGCCATGCCCCAAGGcTATGTcCAGGAgAGAACcATATTTTACAAG
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GcATTGAcTTTAAgGAgGAcGGcAACATTCTgGGgCACAAGATGGAgTACAACATAACTCACATAATGTcTACATC
ATGGCAGACAAGCCAAAGAATGGcATCAAgGTgAACTTCAAgATTAGACACAACATTAAGAcGGcAGCGTgCAA
ctcGCAGACCATTATCAACAAAATACcCCAATTGGCGAcGGCCCTGTgCTgctgCCAGACAACCATTACCTGTCCACc
CAATCTGCCCTgTCCAAAGAcCCCAACGAgAAGAGAGATCACATGATCCTgCTgGAGTTTGTgACcGCTGCTGGGA
TTAcTcATGGCATGGAcGAgCTgTACAAGTAA

h

cDNA: GFP^{opt80}

CAI: 0.912

Source: gene synthesis, rare codons for all leucine, glycine, valine, lysine, threonine, aspartic acid, glutamic acid, isoleucine, serine and proline converted to common, converted nucleotides are marked by lower case

5'ATGGTGAGcAAgGGcGAgGAgCTgTTCACcGGgGTgGTgCCcATcCTgGTcGAgctgGAcGGCGAcGTaAATGGcC
AAAAGTTCagcGTgtccGGcGAGGGcGAgGGcGATGCAACcTACGGcAAgCTgACCCTgAAgTTTATcTGCACcACcG
GcAAGCTgCCcGTgCCcTGGCCcACcCTcGTgACcACcCTGACCTATGGcGTgCAATGCTTTAgcAGATAACCCGAcCA
TATGAAgCAGCATGACTTTTTCAAGtccGCCATGCCCCAAGGcTATGTcCAGGAgAGAACcATcTTTTACAAGGAcG
ACGGcAACTACAAGACcCGTGCTGAgGTgAAGTTTGAgGGcGAcACCCTgGTgAATAGAATCGAGctgAAgGGcAT
cGAcTTTAAgGAgGAcGGcAACATcCTgGGgCACAAGATGGAgTACAACATAACagcCATAATGTcTACATCATGG
CAGACAAGCCAAAGAATGGcATCAAgGTgAACTTCAAgATcAGACACAACATcAAAGAcGGcAGCGTgCAActcGC
AGACCATTATCAACAAAATACcCCcATcGGCGAcGGCCcGTgCTgctgCCcGACAACCATTACCTGagCACcCAATC
cGCCCTgagCAAAGAcCCCAACGAgAAGAGAGATCACATGATCCTgCTgGAGTTTGTgACcGCTGCTGGGATcActC
ATGGCATGGAcGAgCTgTACAAGTAA

i

cDNA: GFP^{opt100}

CAI: 0.960

Source: gene synthesis, rare codons for all leucine, glycine, valine, lysine, threonine, aspartic acid, glutamic acid, isoleucine, serine, proline, phenylalanine, alanine, glutamine, histidine, asparagine, tyrosine and arginine converted to common, converted nucleotides are marked by lower case

5'ATGGTGAGcAAgGGcGAgGAgCTgTTCACcGGgGTgGTgCCcATcCTgGTcGAgctgGAcGGCGAcGTaAAcGGcC
AAAAGTTCagcGTgtccGGcGAGGGcGAgGGcGATGcACcTACGGcAAgCTgACCCTgAAgTTcATcTGCACcACcG

GcAAGCTgCCcGTgCCcTGGCCcACcCTcGTgACcACcCTGACCTAcGGcGTgCAGTGCTTcagccgcTACCCcGAcCac
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GGcAACTACAAGAcCcGcGcGAgGTgAAGTTcGAgGGcGAcACCCTgGTgAAccGcATCGAGctgAAgGGcATcGA
cTTcAAgGAgGAcGGcAACATcCTgGGgCACAAgATGGAgTACAACtAcAACagcCAcAAcGTcTAtATCATGGCcGA
CAAgCCAAAGAAcGGcATCAAgGTgAACTTCAAgATccGcCACAAcATcAAAGAcGGcAGCGTgCagctcGcGACC
AcTAcCAGcAgAAcCcCCcATcGGCGAcGGCCcGTgCTgctgCCcGACAACCcTACCTGagCACcAgTCcGCCCTg
agCAAAGAcCCCAACGAgAAGcGcGATCACATGATCCTgCTgGAGTTcGTgACcGcGcGGGATcActCATGGCAT
GGAcGAgCTgTACAAGTAA

j

cDNA: FLAG-GAG
CAI: 0.759
Source: pGAG-EGFP

ATGGACTACAAGGACGACGATGACAAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGA
TGGGAAAAAATTCGGTTAAGGCCAGGGGAAAGAAGAAGTACAAGCTAAAGCACATCGTATGGGCAAGCAGG
GAGCTAGAACGATTCGAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCT
ACAACCATCCCTTCAGACAGGATCAGAGGAGCTTCGATCACTATACAACACAGTAGCAACCCTCTATTGTGTGCA
CCAGCGGATCGAGATCAAGGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAACAAGTCCAAGAA
GAAGGCCAGCAGGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAAC
ATCCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGA
AGGCTTTCAGCCAGAAGTGATACCCATGTTTTAGCATTATCAGAAGGAGCCACCCACAGGACCTGAACACG
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AATGGGATAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTG
ACATAGCAGGAATACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTAGGA
GAGATCTACAAGAGGTGGATAATCCTGGGATTGAACAAGATCGTGAGGATGTATAGCCCTACCAGCATTCTGG
ACATAAGACAAGGACCAAAAAGAACCCTTTAGAGACTATGTAGACCGGTTCTATAAAACTCTAAGAGCTGAGCAA
GCTTCACAGGAGGTAATAAATTGGATGACAGAAACCTTGTGGTCCAAAATGCGAACCCAGATTGTAAGACCAT
CCTGAAGGCTCTCGGCCAGCGGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTAGGAGGACCCGGC
CATAAGGCAAGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATTCAGCTACCATAATGATGCAGAGAGGCA
ATTTTAGGAACCAAGAAAGATTGTTAAGTGTTCATTGTGGCAAAGAAGGGCACACAGCCAGAAATTGCAG
GGCCCTAGGAAAAGGGCTGTTGGAAATGTGGAAAGGAAGGACACCAATGAAAGATTGTACTGAGAGACA
GGCTAATTTTTAGGGAAGATCTGGCCTTCTACAAGGGAAGGCCAGGGAATTTCTTCAGAGCAGACCAGAGC
CAACAGCCCCACCAGAAGAGAGCTTCAGGTCTGGGGTAGAGACAACAACCTCCCCCTCAGAAGCAGGAGCCGAT
AGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTACAATAA

k

cDNA: FLAG-GAG^{com}
CAI: 0.995
Source: gene synthesis, based on p96ZM651.8 Gag sequence (NIH AIDS Reagent Program; 8675) with
codon-optimized amino acid substitutions to match the exact amino acid sequence of Gag^{rare}.

ATGGACTACAAGGACGACGATGACAAGATGGGCGCCCGCGCCAGCGTGCTGAGCGGGCGGAGCTGGACCGG
TGGGAGAAGATCCGCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGCCAGCCGC
GAGCTGGAGCGCTTCGCCGTGAACCCCGCCCTGCTGGAGACCAGCGAGGGCTGCCGGCAGATCCTGGCCAG
CTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGCAGCCTGTACAACACCGTGGCCACCCTGTACTGCG
TGCACCAGCGGATCGAGATCAAGGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGAGCA

AGAAGAAGGCCAGCAGGCCGCCGCCGACACCGGCCACAGCAACCAGGTGAGCCAGAACTACCCCATCGTGCA
GAACATCCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCGCACCCTGAACGCCTGGGTGAAGGTGGTGGAG
GAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGAGCGAGGGCGCCACCCCCAGGACCTGA
ACACCATGCTGAACACCGTGGGCGGCCACCAGGCCGCATGCAGATGCTGAAGGAGACCATCAACGAGGAGG
CCGCCGAGTGGGACCGCGTGCACCCCGTGCACGCCGCCCATCGCCCCGCCAGATGCGCGAGCCCCGCGG
CAGCGACATCGCCGGCACCACCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCG
TGGGCGAGATCTACAAGCGCTGGATCATCCTGGGCCTGAACAAGATCGTGCGCATGTACAGCCCCACCAGCATC
CTGGACATCCGGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGCTTCTACAAGACCCTGCGCGCCGA
GCAGGCCAGCCAGGAGGTGAAGAACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAA
GACCATCTGAAGGCCCTGGGCCCGCCGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGC
CCCGGCCACAAGGCCCGCTGCTGGCCGAGGCCATGAGCCAGGTGACCAACAGCGCCACCATCATGATGCAGC
GGGGCAACTTCCGGAACCAGCGGAAGATCGTGAAGTGCTTCAACTGCGGCAAGGAGGGCCACACCGCCCGCA
ACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGCAGGCAAGGAGGGCCACCAGATGAAGGACTGCACCG
AGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCAGCTACAAGGGCCGCCCGGCAACTTCTGCGAGAGCCG
CCCCGAGCCCACCGCCCCCCCCGAGGAGAGCTTCCGACGCGCGTGGAGACCACCACCCCCCCCCAGAAGCAG
GAGCCATCGACAAGGAGCTGTACCCCTGACCAGCCTGCGGAGCCTGTTTCGGCAACGACCCAGCAGCCAGT
AA

I

cDNA: FLAG-KRAS

CAI: 0.752

Source: Cloned from previously described pBabePuro-KRAS construct²².

ATGGACTACAAAGACGATGACGACAAGACTGAATATAAATTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGA
GTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGGATTCCTACAGG
AAGCAAGTAGTAATTGATGGAGAACTGTCTCTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTACAGTGC
AATGAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGATTTGCCATAAATAACTAAATCATTTG
AAGATATTCACCATTATAGAGAACAATTAAGAGTAAAGGACTCTGAAGATGTACCTATGGTCTAGTAGGA
AATAAATGTGATTTGCCTTCTAGAACAGTAGACACAAAACAGGCTCAGGACTTAGCAAGAAGTTATGGAATTC
TTTTATTGAAACATCAGCAAAGACAAGACAGAGAGTGGAGGATGCTTTTTATACATTGGTGAGAGAGATCCGAC
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TAA

m

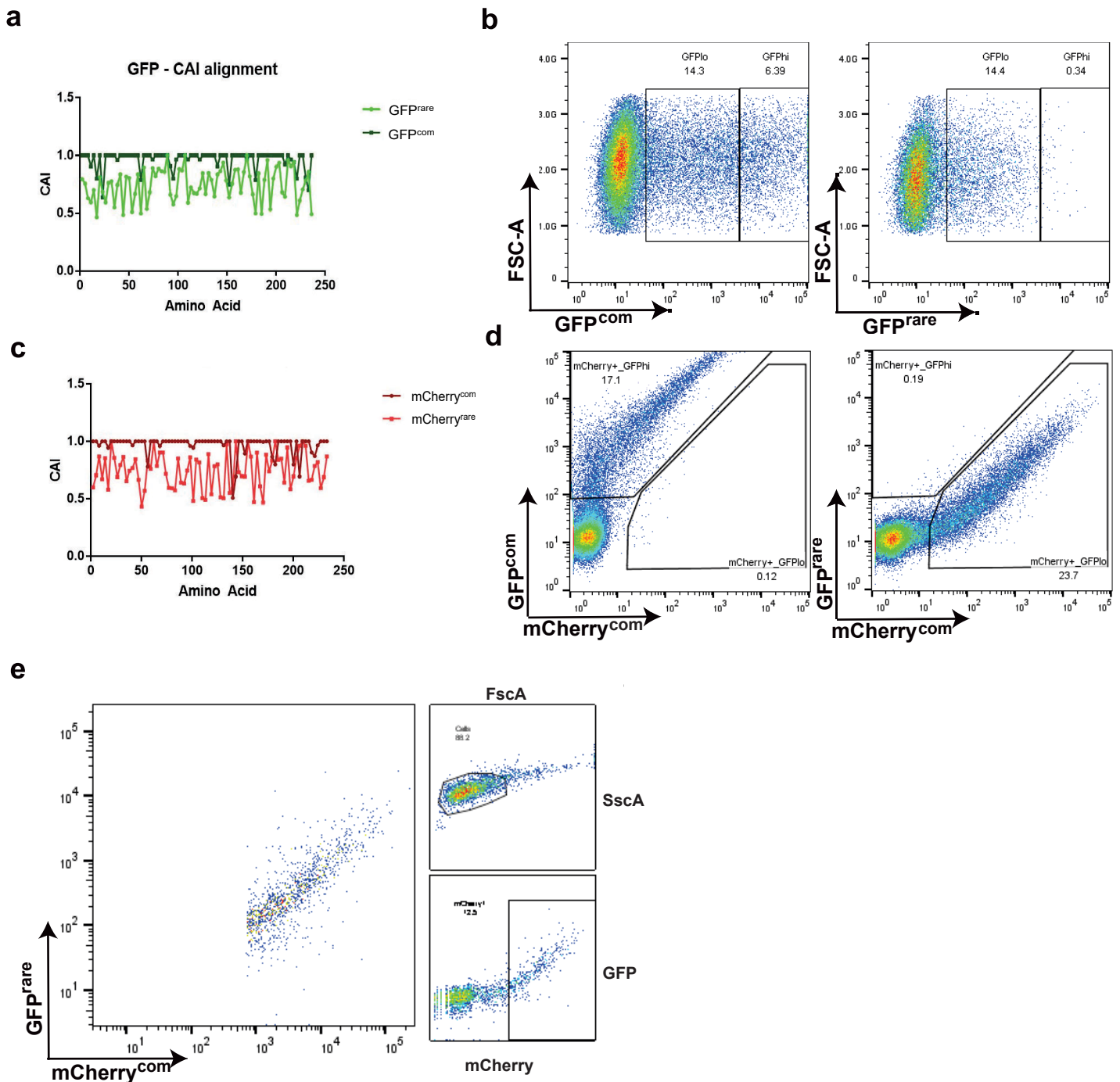
cDNA: FLAG-HRAS

CAI: 0.90

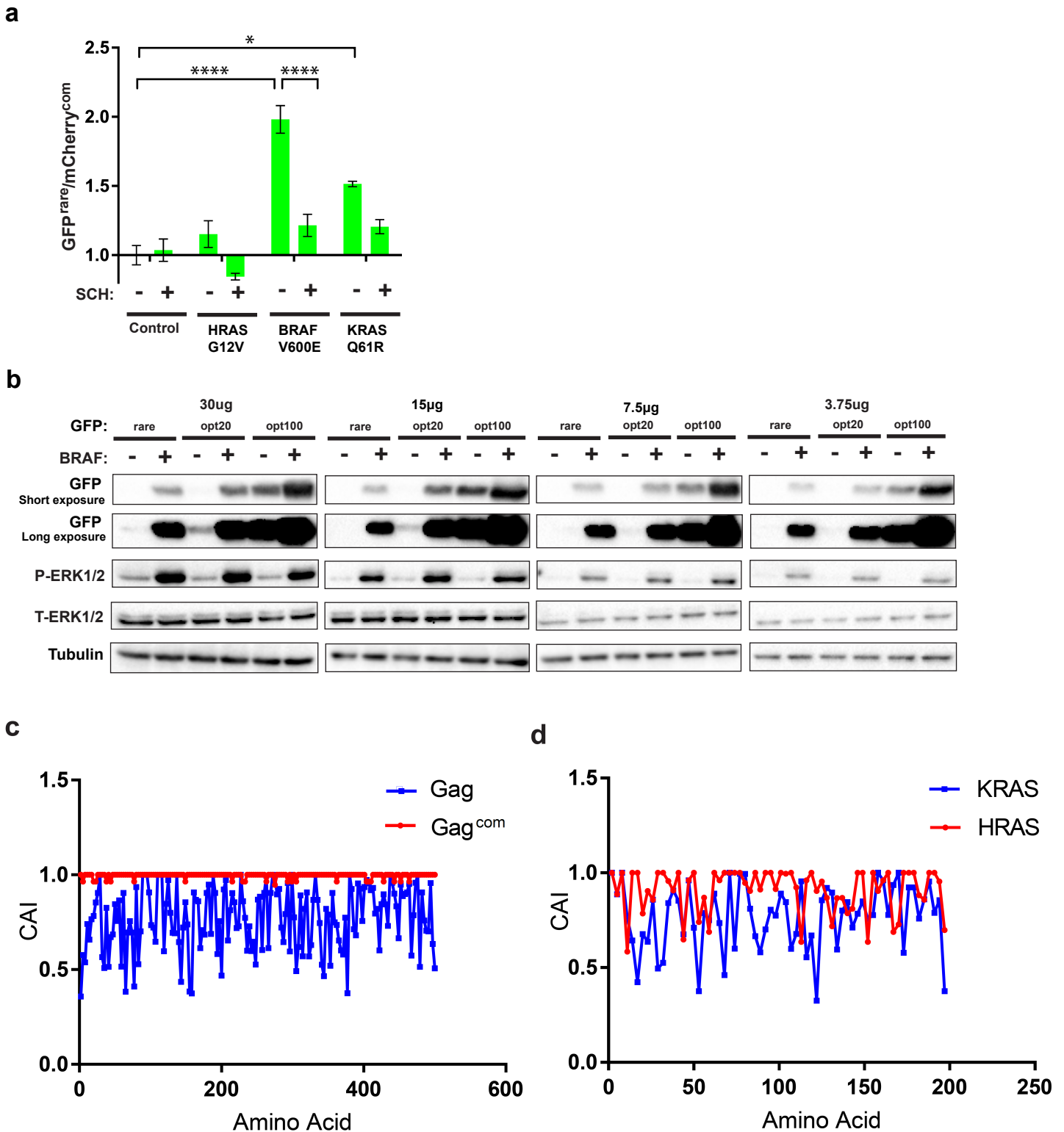
Source: Cloned from previously described pBabePuro-HRAS construct²².

ATGGACTACAAAGACGATGACGACAAGACGGAATATAAGCTGGTGGTGGTGGGCGCTGGAGGCGTGGGAAAG
AGTGCCCTGACCATCCAGCTGATCCAGAACCACTTTGTGGACGAATACGACCCCACTATAGAGGATTCCTACCG
GAAGCAGGTGGTCATTGATGGGGAGACGTGCCTGTTGGACATCCTGGATACCGCCGCCAGGAGGAGTACAG
CGCCATGCGGGACCAGTACATGCGCACCGGGGAGGGCTTCTGTGTGTGTTTGCCATCAACAACACCAAGTCTT
TTGAGGACATCCACCAGTACAGGGAGCAGATCAAACGGGTGAAGGACTCGGATGACGTGCCCATGGTGTGGT
GGGGAACAAGTGTGACCTGGCTGCACGCACTGTGGAATCTCGGCAGGCTCAGGACCTCGCCGAAGCTACGGC
ATCCCCTACATCGAGACCTCGGCCAAGACCCGGCAGGGAGTGGAGGATGCCTTCTACACGTTGGTGCCTGAGA
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GCTCTCCTGA

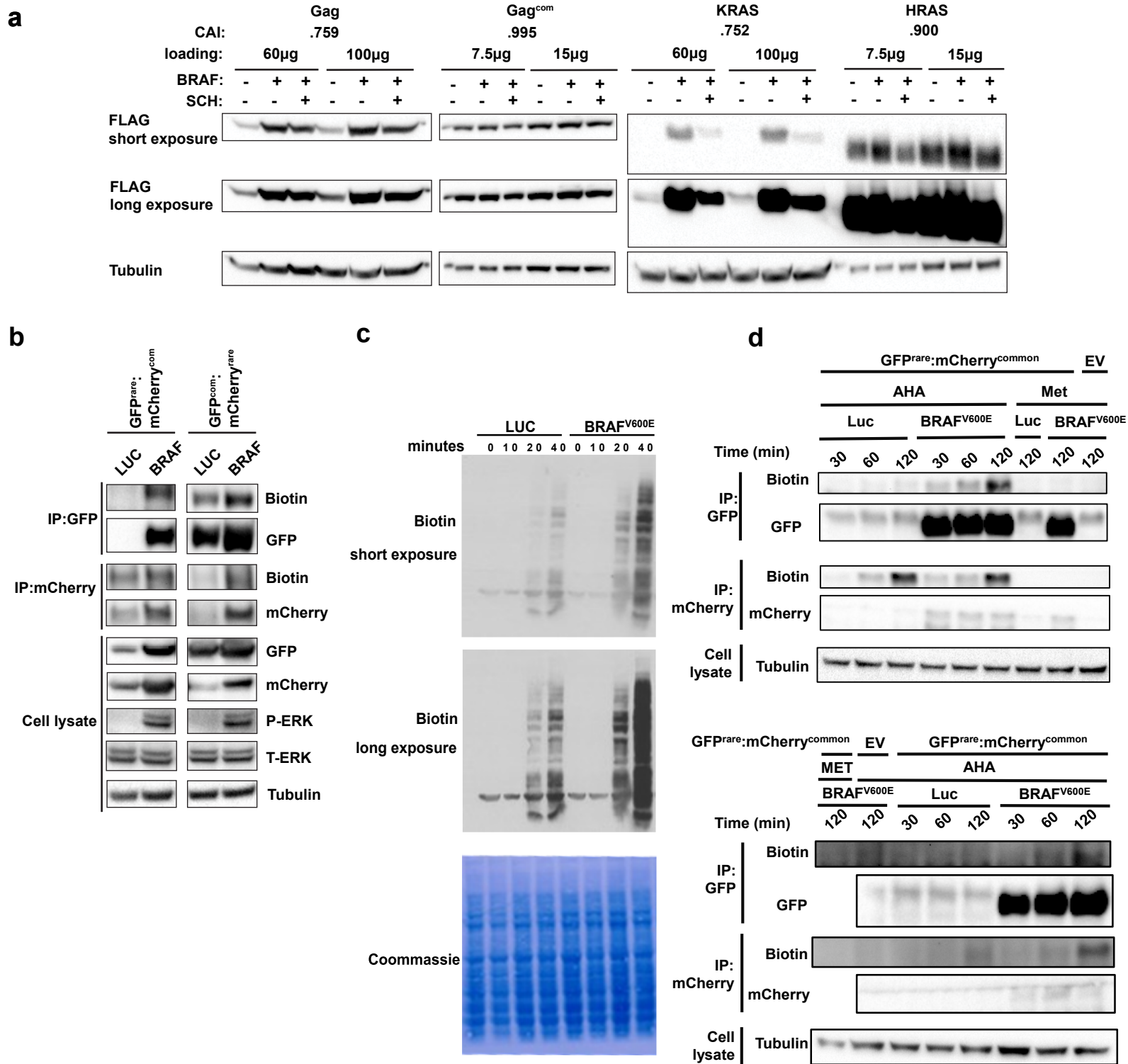
Supplementary Figure 1. cDNAs. (a-m) Gene name, CAI, source, and nucleotide sequence of cDNAs used as reporters.



Supplementary Figure 2. A reporter to characterize the expression of rare-codon enriched transcripts. (a,c) Codon Adaptation Index (CAI) plotted as a function of amino acid residue for the indicated reporters. **(b,d)** Flow cytometry plots of single **(b)** and dual color **(d)** reporters with gates drawn to illustrate differences in the ability of each respective assay to exclude events from reporters using different codon usages. X axis: GFP intensity; Y axis: forward scatter area; GFP^{lo}: GFP low; GFP^{hi}: GFP high; Number indicates the percentage of highlighted population. **(e)** Flow cytometry plots demonstrating common gating scheme used to generate mean fluorescence intensities in this work; cells were gated according to forward and side scatter (FSC and SSC, respectively) and the MFI of positively transfected cells was calculated for each fluorescent reporter.



Supplementary Figure 3. MAPK pathway enhances the expression of rare-codon enriched transcripts. (a) Mean \pm SEM of the ratio of GFP^{rare} to mCherry^{com} fluorescence of 293T cells stably infected with lentivirus encoding the indicated transgenes normalized to the fluorescence of parallel luciferase control (Ctrl) cells in the absence and presence of DMSO vehicle (-) or the SCH779284 ERK inhibitor (+) from three technical replicates. Two-way ANOVA with Sidak's multiple comparisons test: * $p < 0.05$ and **** $p < 0.0001$. (b) Expanded immunoblot for Fig. 3b. Blots separated by white space are from different parts of the same gel or from different gels at the same exposure in the same experiment. Specifically, 30 μ g and 15 μ g samples were loaded on one gel while 7.5 μ g and 3.75 μ g samples were loaded on a separate gel. (c,d) Codon Adaptation Index (CAI) plotted as a function of amino acid residue for the indicated reporters.



Supplementary Figure 4. MAPK pathway enhances the translation of rare-codon enriched transcripts. (a) Immunoblot analysis of FLAG-tagged Gag, Gag^{com}, KRAS, HRAS, and tubulin from 293 cells co-transfected with a vector encoding either luciferase control (-) or BRAF^{V600E} (+) and the indicated FLAG-tagged proteins in the absence (-) or presence (+) of the ERK1/2 inhibitor SCH772984 (SCH). The lysate is the same as that used for figure 3c with variations in protein loading amount as indicated. Blots separated by white space are from different gels. (b) Biological replicate of figure 3d. Left and right blots (separated by white space) are from different parts of the same gel at different exposures, except for cell lysate: mCherry, P-ERK, T-ERK and Tubulin, which are different parts of the same gel at the same exposure. (c) Immunoblot analysis of whole cell lysates derived from 293T cells transiently expressing BRAF^{V600E} (BRAF) or control luciferase (Luc) and labeled with AHA for the indicated times. Coomassie stain: control for protein loading. One of two biological replicates. (d) Replicate experiments of immunoblot analysis of AHA-biotin labeled mCherry or GFP in 293T cells transiently expressing mCherry^{com} and GFP^{rare} or mCherry^{rare} and GFP^{com} in the presence of BRAF^{V600E} (BRAF) or control luciferase (Luc) and labeled with AHA for the indicated times. EV: empty vector, AHA: L-Azidohomoalanine, Met: methionine.

Fig 2b

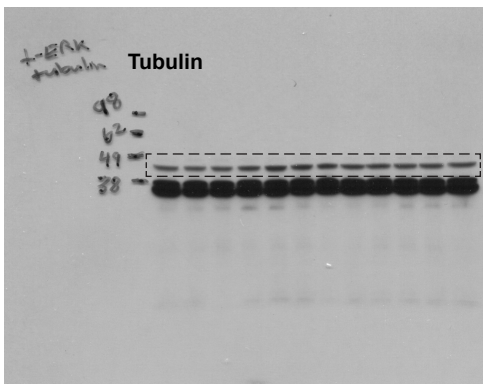
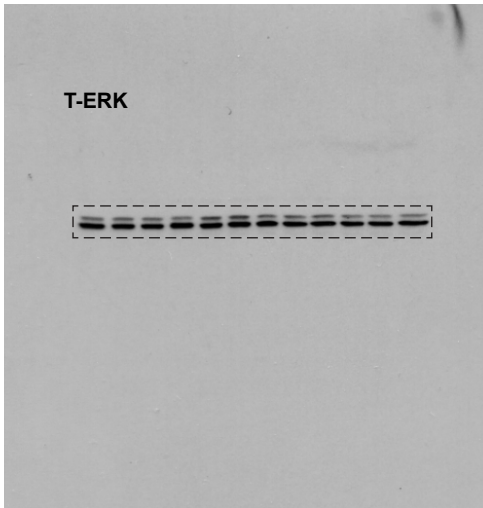
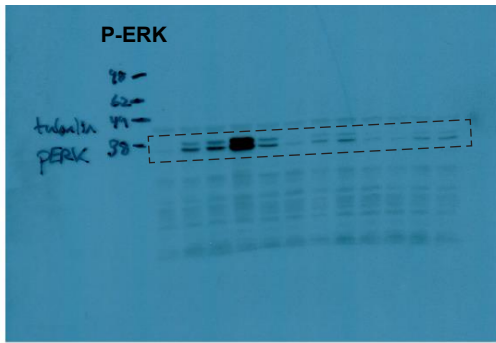


Fig 2c

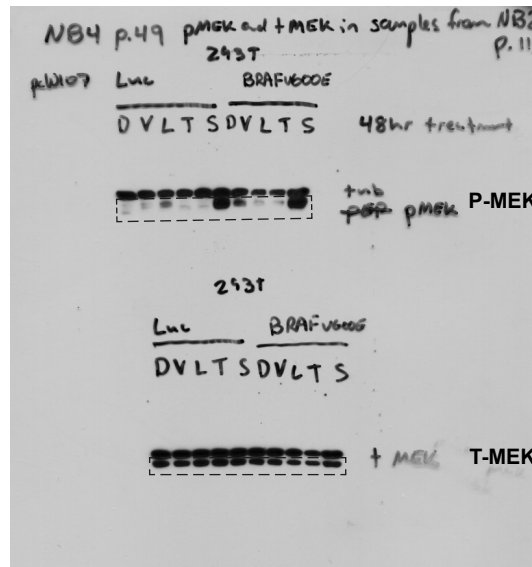
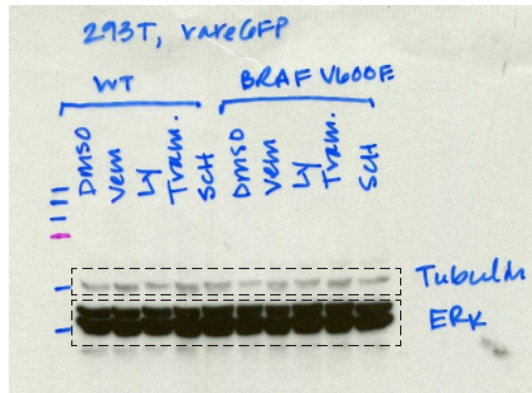
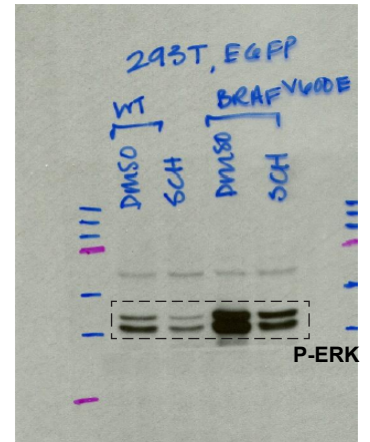
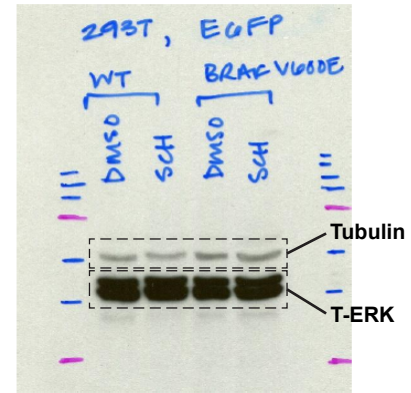


Fig 2d



Supplementary Figure 5. Uncropped blots.

Figure 3a

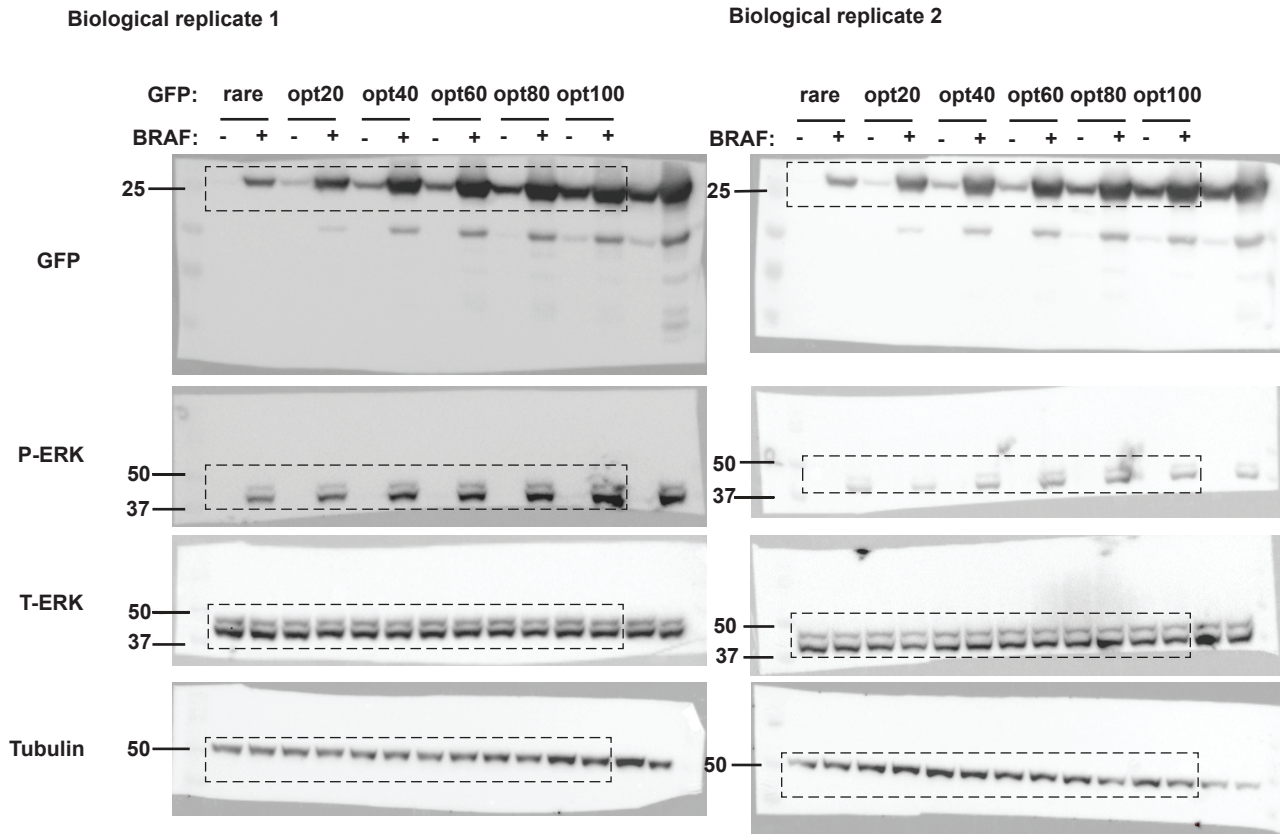
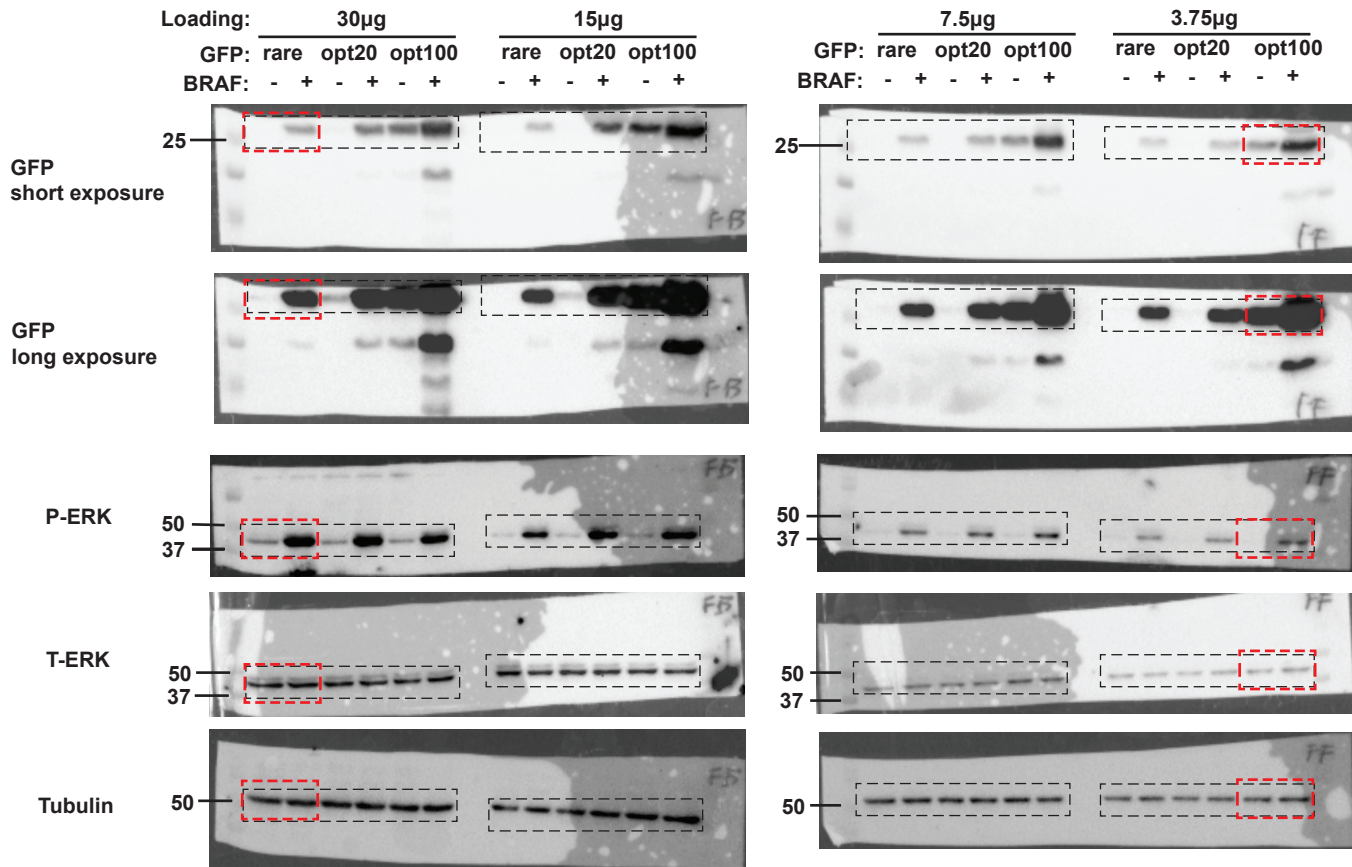
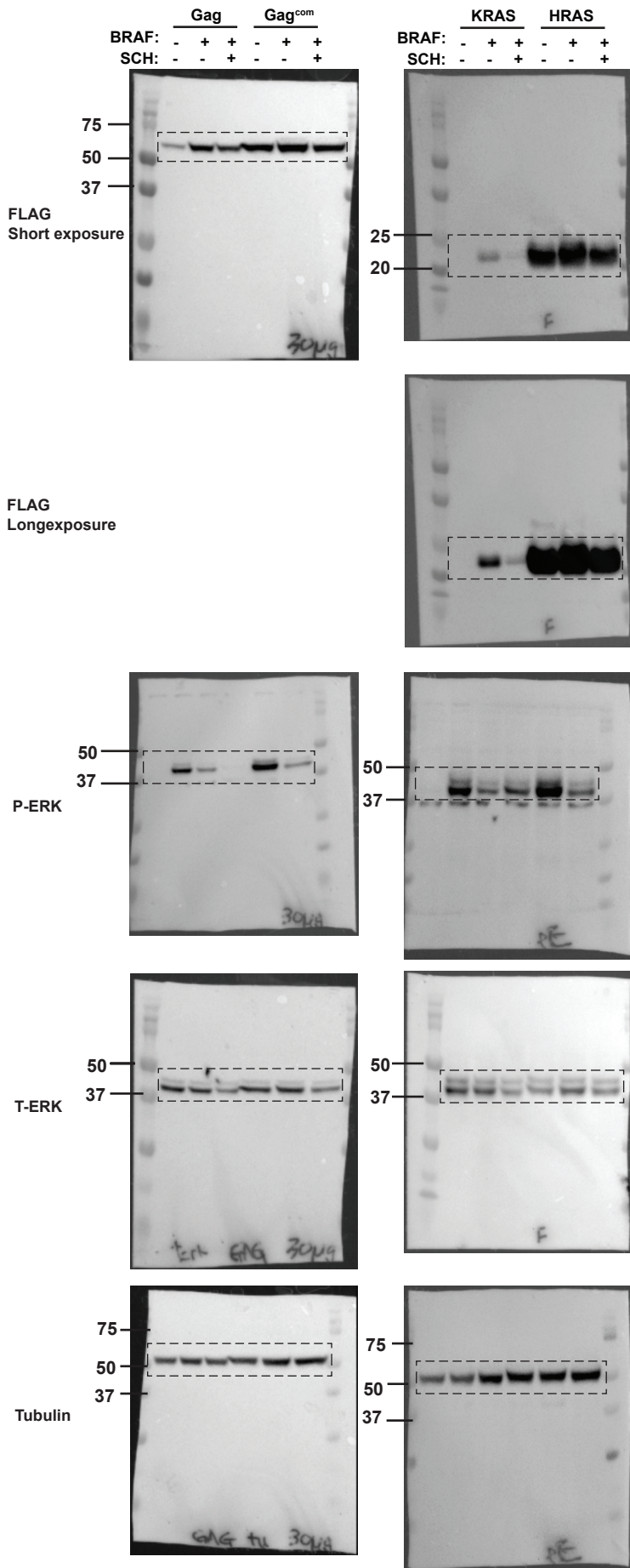


Figure 3b (red box) and Supplementary Figure 3b (black box)



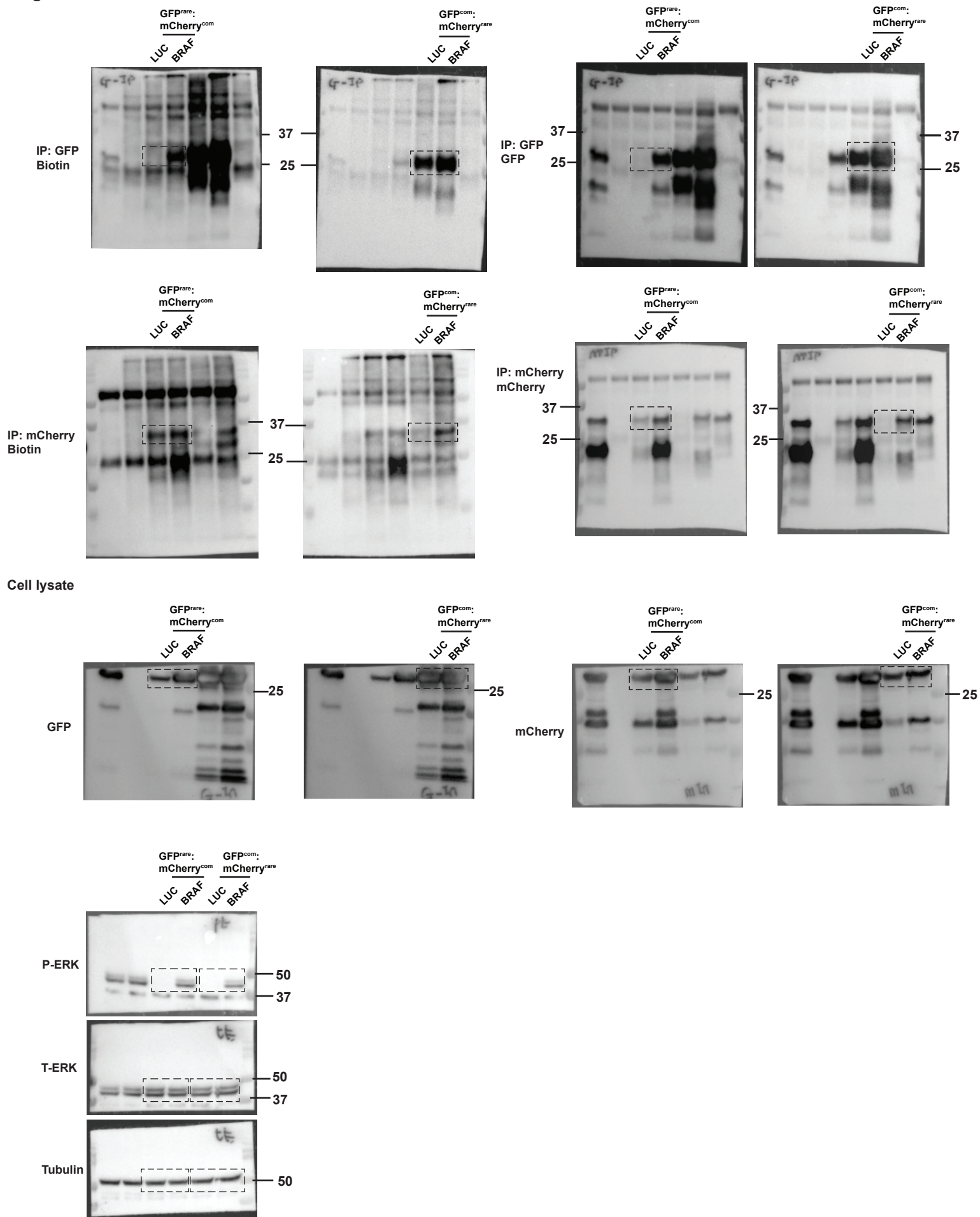
Supplementary Figure 5, continued. Uncropped blots.

Figure 3c



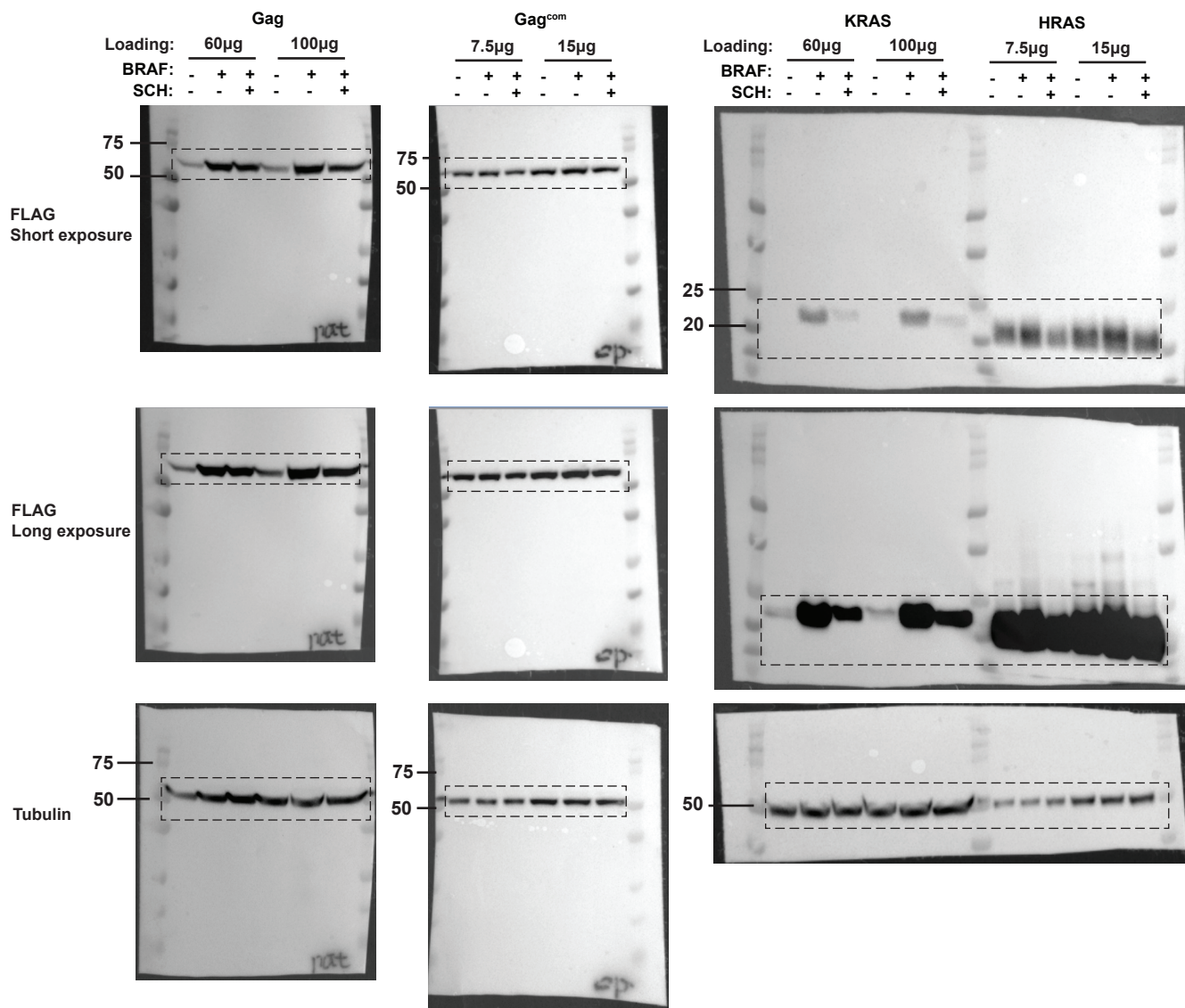
Supplementary Figure 5, continued. Uncropped blots.

Figure 3d



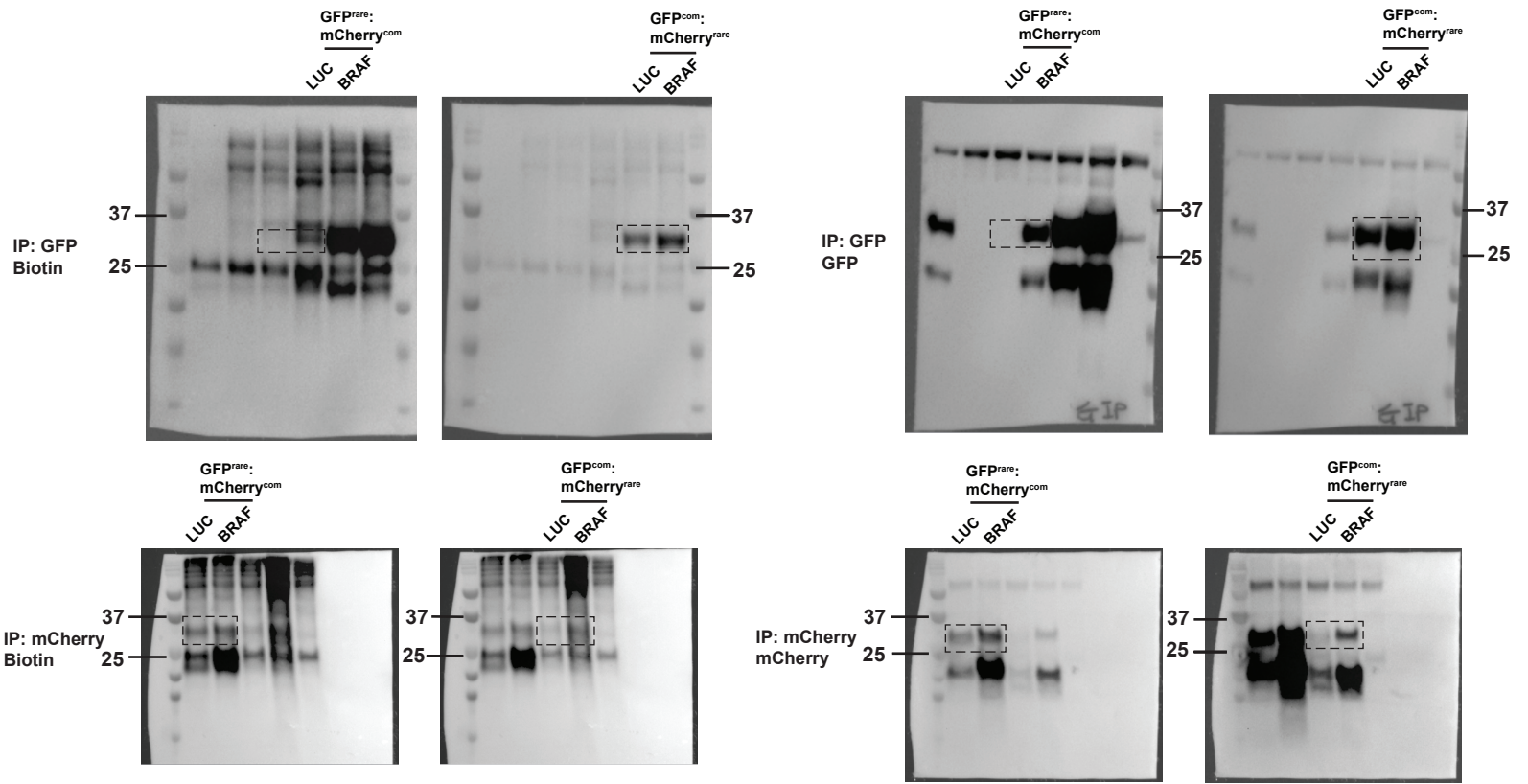
Supplementary Figure 5, continued. Uncropped blots.

Supplementary Figure 4a

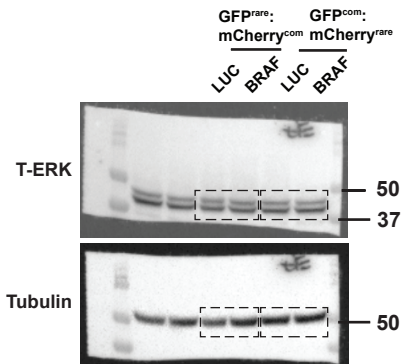
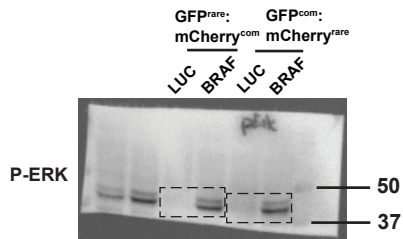
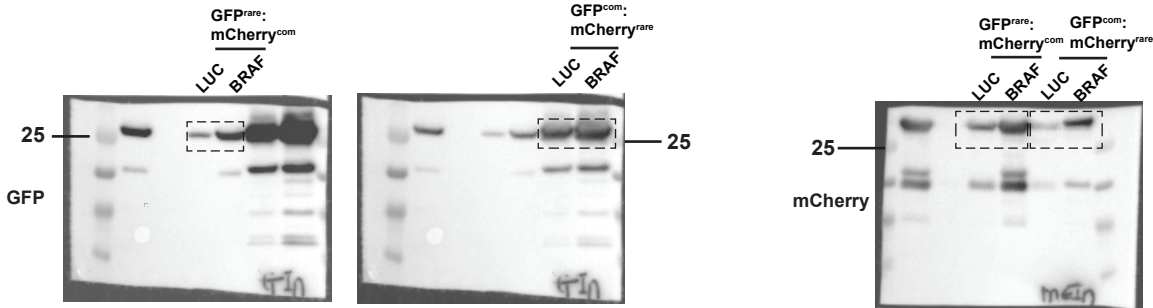


Supplementary Figure 5, continued. Uncropped blots.

Supplementary Figure 4b

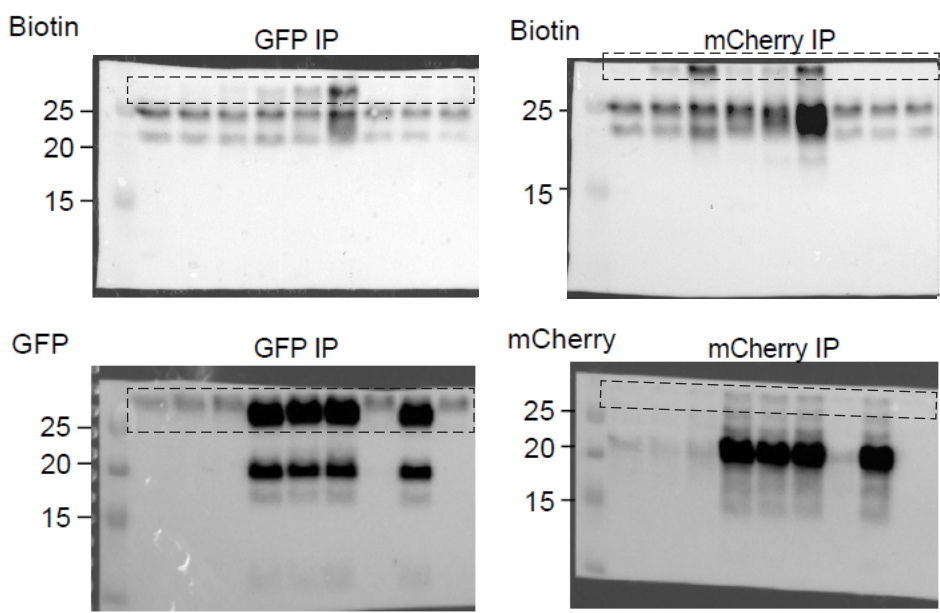


Cell lysate

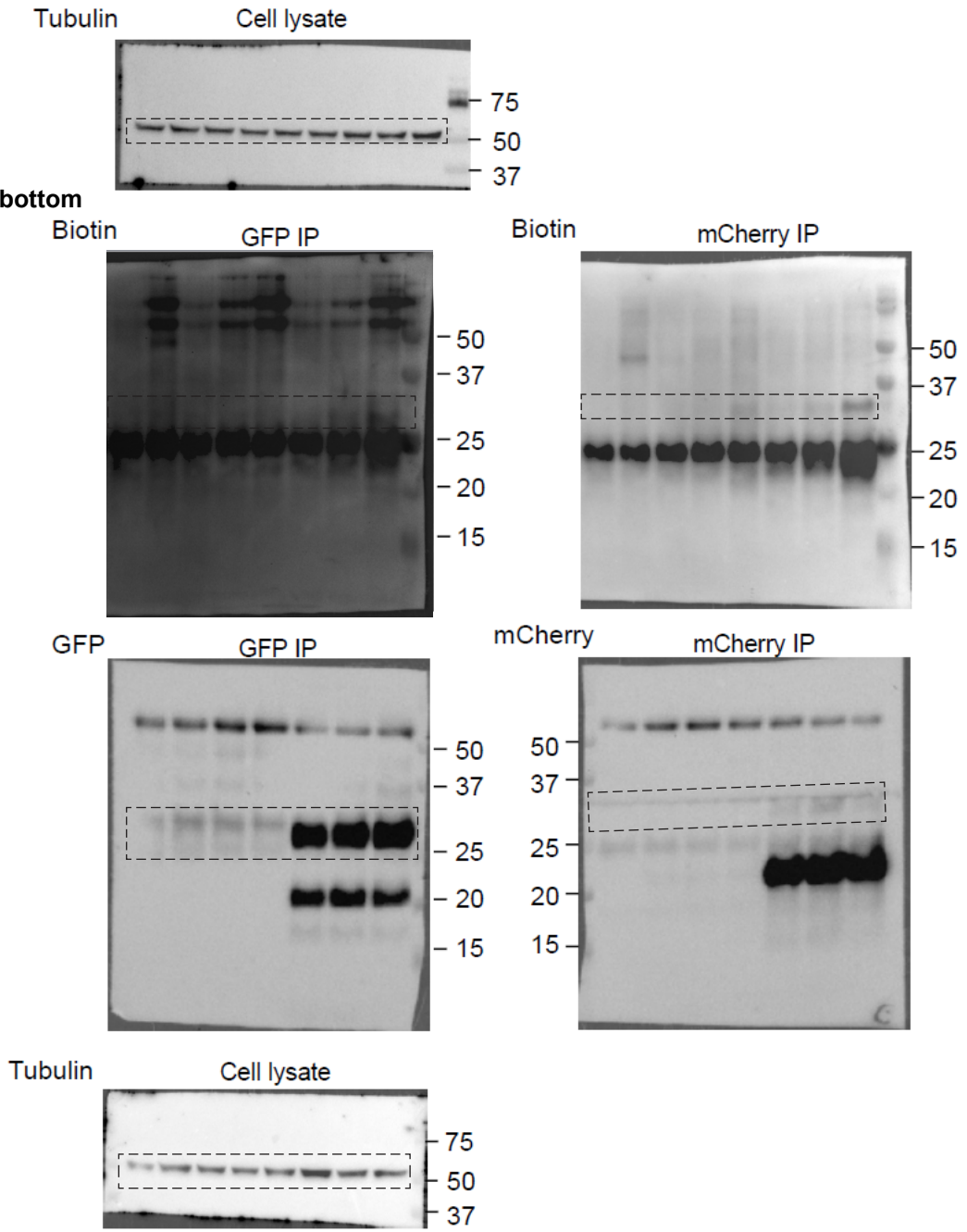


Supplementary Figure 4c Uncropped immunoblots shown in figures.

Supplementary Figure 4d top



Supplementary Figure 4d bottom



Supplementary Figure 5, continued. Uncropped blots.

Entry Clone	Gene	Mutation	RefSeq	mCherry	GFP	GFP/mCherry	Fold over control
R780-E002	Luciferase	wt					
R780-E003	MAP2K1	wt	NM_002755.3	5175	28972	0.178620737	0.91381
R780-E004	KRAS	G12V	NM_004985.3	7751	40558	0.191109029	0.9777
R780-E005	HRAS	G12V	NM_005343.2	14831	53747	0.275940983	1.41169
R780-E006	MAP2K1	S281D/S222D	NM_002755.3	6582	33176	0.198396431	1.01498
R780-E009	RHEB	Q64L	NM_005614.3	5319	29544	0.180036556	0.92105
R780-E010	CTNNB1	S22A/S37A/ T41A/S45A	NM_001165902 (Mm)	6453	35132	0.183678697	0.93968
R780-E011	GSK3B	K85A	NM_001146156.1	5985	31659	0.189045769	0.96714
R780-E012	CTNNB1	S33Y	NM_001904.3	5178	26662	0.194208987	0.99356
R780-E013	TGFBR1	wt	NM_004612.2	5102	27405	0.186170407	0.95243
R780-E014	BCL2	wt	NM_000633.2	4833	25068	0.192795596	0.98633
R780-E015	BCL2L1	wt	NM_138578.1	4637	23489	0.197411554	1.00994
R780-E016	CASP8	C360A	NM_033355.3	5313	26864	0.197773973	1.01179
R780-E017	CASP3	C163A	NM_032991.2	4848	25714	0.188535428	0.96453
R780-E018	TP53	R175H	NM_001126114.2	4484	25023	0.17919514	0.91675
R780-E019	AKT1	E17K	NM_005163.2	6795	34778	0.195382138	0.99956
R780-E020	BRAF	V600E	NM_004333.4	13523	40449	0.334322233	1.71036
R780-E021	CCND1	P287S	NM_053056.2	1093	6644	0.164509332	1.054727
R780-E022	EGFR	L858R	NM_005228.3	820	286	0.348780488	1.074195
R780-E024	FGFR3	S249C	NM_000142.4	1255	6934	0.180992212	1.160405
R780-E025	FLT3	D835Y	NM_004119	1710	6003	0.284857571	1.826322
R780-E026	MAP2K1	P124L	NM_002755.3	1049	335	0.319351764	0.983559
R780-E027	MTOR	S2215Y	NM_004958.3	9149	764	0.083506394	1.032618
R780-E029	NFE2L2	E79Q	NM_006164.4	1363	7302	0.186661189	1.19675
R780-E030	NRAS	G12D	NM_002524.4	2771	16194	0.171112758	1.097064
R780-E031	NRAS	Q61K	NM_002524.4	10620	47027	0.225827716	1.371798
R780-E032	PDGFRA	E229K	NM_006206.4	817	282	0.345165239	1.063061
R780-E033	PIK3CA	E545K	NM_006218.2	7832	675	0.086184883	1.06574
R780-E034	PIK3CA	H1047R	NM_006218.2	1215	7030	0.172830725	1.108078
R780-E037	PTEN	R130Q	NM_000314.4	11298	809	0.071605594	0.885456
R780-E039	STK11	D194N	NM_000455	1192	6673	0.178630301	1.145262
R780-E040	KRAS	G12C	NM_004985.3	6067	36722	0.165214313	1.0036
R780-E041	KRAS	G12D	NM_004985.3	2661	16727	0.159084116	1.019944
R780-E042	KRAS	G13D	NM_004985.3	2512	6380	0.393730408	0.961762
R780-E043	KRAS	Q61L	NM_004985.3	960	409	0.426041667	1.312149
R780-E044	KRAS	Q61R	NM_004985.3	2362	6329	0.373202718	1.564193
R780-E045	CTNNB1	S37C	NM_001165902.1	6889	676	0.09812745	1.213419
R780-E046	BAP1	E31A	NM_004656.3	2034	8222	0.247385064	1.036857

R780-E047	CDH1	D254Y	NM_004360.4	5959	38643	0.154206454	0.93673
R780-E048	EZH2	Y646N	NM_004456	1278	4726	0.270418959	1.1334
R780-E049	GATA2	H460P	NM_032638.4	1138	5454	0.208654199	0.87453
R780-E051	IDH1	R132H	NM_005896	1905	5498	0.346489633	1.45223
R780-E053	MLH1	R217C	NM_000249.3	1307	7381	0.177076277	1.1353
R780-E054	MSH2	Q419K	NM_000251.2	5787	32458	0.178291947	1.08304
R780-E055	MYD88	L265P	NM_002468.4	1749	7158	0.244341995	1.56656
R780-E056	RET	M918T	NM_020630.4	1323	8093	0.163474608	1.04809
R780-E057	STAG2	R370W	NM_006603.4	1327	5547	0.239228412	1.00267
R780-E058	U2AF1	S34F	NM_006758	1351	7413	0.182247403	1.16845
R780-E059	SMAD4	R361H	NM_005359.5	1219	4638	0.262828806	1.10159
R780-E060	TET2	S268A	NM_001127208.2	9423	691	0.073331211	0.90679
R780-E061	JAK3	L527R	NM_000215.3	1308	7665	0.170645793	1.09407
R780-E062	MAP2K4	R134W	NM_003010.3	1074	6413	0.167472322	1.07372
R780-E063	RUNX1	D198G	NM_001754.4	1209	6073	0.199077886	0.83439
R780-E065	VHL	N78D	NM_000551.3	1689	8860	0.190632054	1.22221
R780-E066	KLF4	K409Q	NM_004235.5	1780	8469	0.210178297	1.34753
R780-E069	CYLD	G160R	NM_001042412.1	7036	729	0.103610006	1.28121
R780-E070	SMAD2	R321Q	NM_005901.5	6067	34426	0.17623308	1.07053
R780-E071	ACVR1B	R484H	NM_004302.4	1292	7308	0.176792556	1.13348
R780-E072	HGF	E199K	NM_000601.5	4065	641	0.157687577	1.02042
R780-E073	BCL2	R98H	NM_000633.2	4350	700	0.16091954	1.04134
R780-E075	CHEK2	K373E	NM_007194.3	4284	671	0.156629318	1.01358
R780-E076	KEAP1	R470C	NM_203500.1	4827	700	0.145017609	0.93844
R780-E077	FOXA1	I176M	NM_004496.3	3701	563	0.152121048	0.9844
R780-E079	EIF4A2	G336W	NM_001967.3	4525	688	0.152044199	0.98391
R780-E080	ELF3	S133F	NM_004433.4	5328	33130	0.160821008	0.97691
R780-E081	HIST1H1C	K109N	NM_005319.3	4192	665	0.158635496	1.02656
R780-E082	CDKN1B	D63E	NM_004064.4	2160	5027	0.429679729	1.04957
R780-E083	CBFB	R151H	NM_001755.2	5540	34261	0.161699892	0.98225
R780-E084	CDKN1A	W49L	NM_000389.4	4200	670	0.15952381	1.03231
R780-E085	H3F3B	G35R	NM_005324.4	4139	617	0.149069824	0.96466
R780-E086	GNAS	R201H	NM_000516.5	12413	76364	0.162550416	0.98742
R780-E087	B4GALT3	H194Y	NM_003779.3	5821	37704	0.154386802	0.93783
R780-E088	SPOP	F133L	NM_003563.3	5344	32796	0.162946701	0.98982
R780-E089	TSC1	R786Q	NM_000368	21888	1617	0.073876096	0.91353

Supplementary Table 1. Summary of the Cancer Toolkit (CTK) lentiviral library screen.