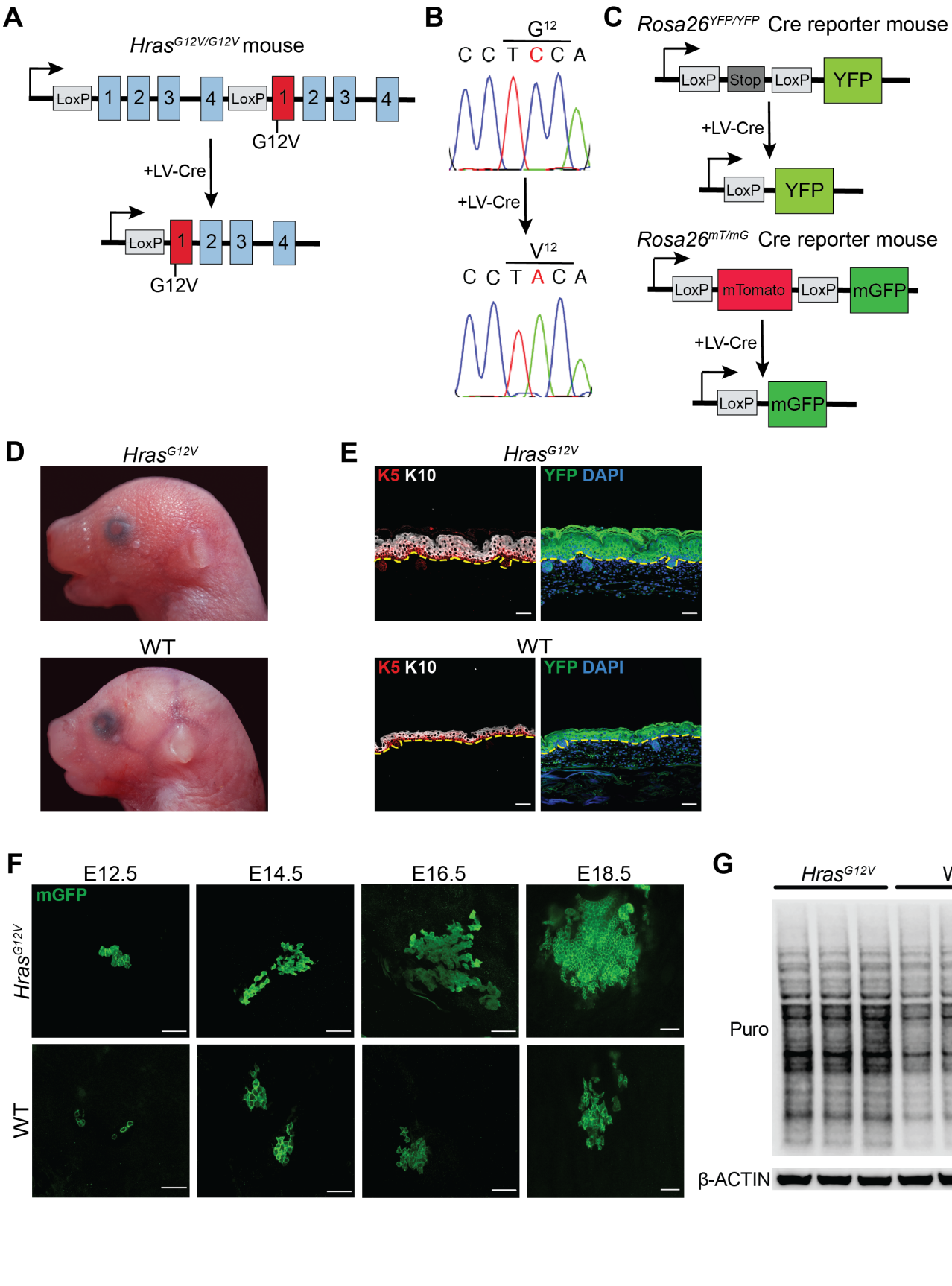
A
Hras^G12V/G12V mouse
LoxP
1
2
3
4
LoxP
1
2
3
4
G12V
+LV-Cre
LoxP
1
2
3
4
G12V
B
G12
C C T C C A
+LV-Cre
V12
C C T A C A
C
Rosa26^YFP/YFP Cre reporter mouse
LoxP
Stop
LoxP
YFP
+LV-Cre
LoxP
YFP
Rosa26^mT/mG Cre reporter mouse
LoxP
mTomato
LoxP
mGFP
+LV-Cre
LoxP
mGFP
D
Hras^G12V
WT
E
Hras^G12V
K5 K10
YFP DAPI
WT
K5 K10
YFP DAPI
F
E12.5
E14.5
E16.5
E18.5
mGFP
Hras^G12V
WT
G
Hras^G12V
WT
Puro
β-ACTIN

Figure S1. RAS activation in mouse epidermis induces accelerated tissue growth, Related to Figure 1

A) Physiological knock-in model for Cre-inducible expression of oncogenic *Hras*^{G12V}. Cre recombinase is expressed from a lentivirus that can co-express shRNA or ORF.

B) Sequencing traces of the *Hras1* cDNA in epidermal basal cells following LV-Cre transduction demonstrate efficient activation of *Hras*^{G12V} expression.

C) *Rosa26*^{YFP} Cre reporter mouse exchanges stop codon for YFP following Cre expression. *Rosa26*$^{mT/mG}$ Cre reporter mouse exchanges membrane-associated red (mTomato) fluorophore with green (mGFP) following Cre expression. Cre recombinase is expressed from a lentivirus that can co-express shRNA or ORF.

D) Gross skin morphology of E18.5 *Hras*^{G12V} and WT mice transduced with LV-Cre along with control shRNA.

E) Representative immunofluorescence staining of keratin 5 (K5, red) and keratin 10 (K10, white) in WT and *Hras*^{G12V} E18.5 epidermal cross-sections. *Hras*^{G12V} and WT mice are transduced with control shRNA. Cre reporter YFP (green) marks the LV-Cre transduced epidermis and DAPI (blue) marks nuclei. Dashed line indicates basement membrane. Scale bars, 50 μm.

F) Representative immunofluorescence staining of clones in E12.5, E14.5, E16.5, and E18.5 WT and *Hras*^{G12V} epidermis transduced with control shRNA at E9.5. Transduced cells are marked by mGFP. Scale bars, 50 μm.

G) Western blot of *Hras*^{G12V} and WT primary keratinocytes for puromycin incorporation (Puro) following 30 min of puromycin incubation. Keratinocytes are transduced with control shRNA. 3 biological replicates are shown.

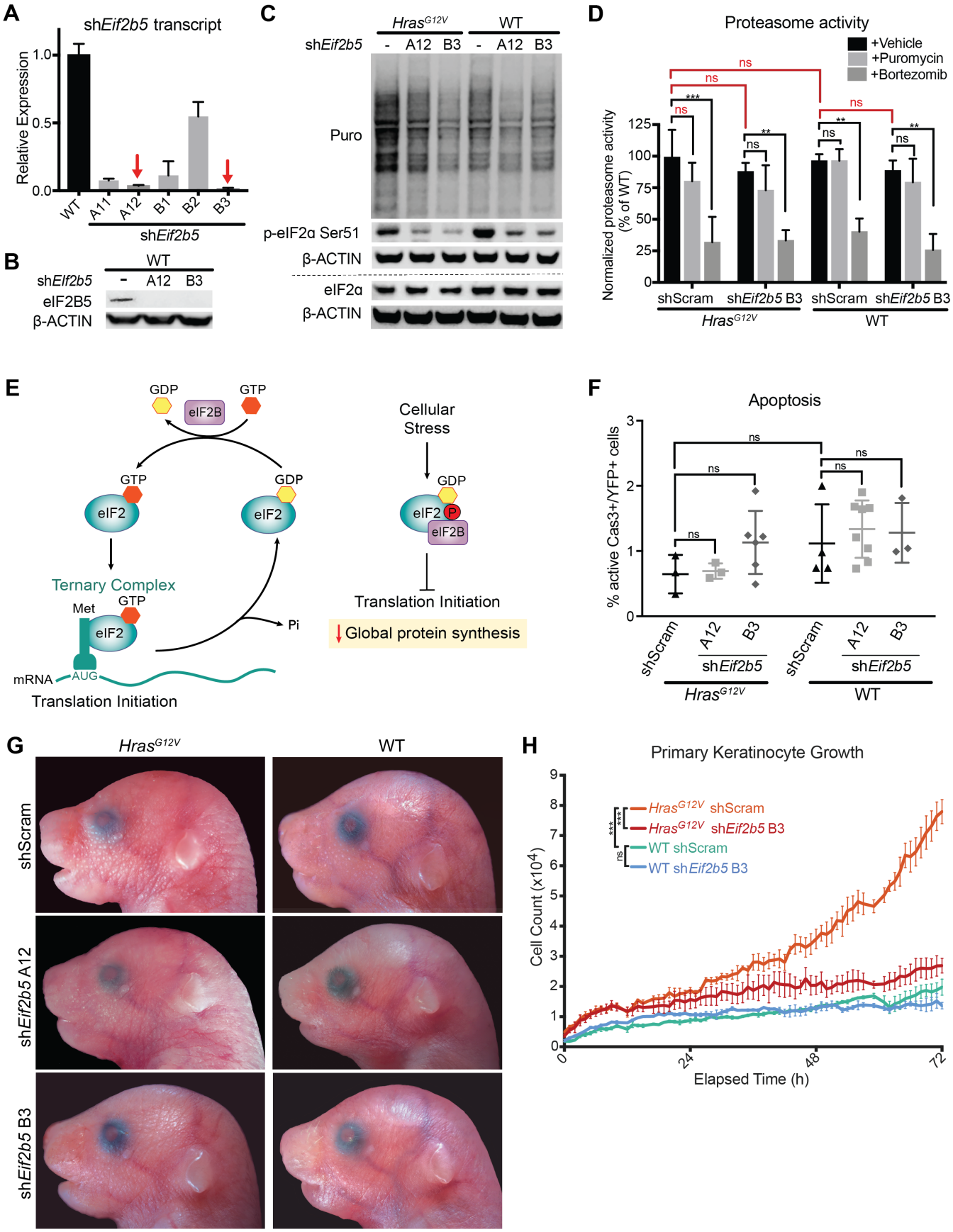
A
shEif2b5 transcript
Relative Expression
1.0
0.5
0.0
WT
A11
A12
B1
B2
B3
shEif2b5
B
WT
shElf2b5
-
A12
B3
eIF2B5
β-ACTIN
C
Hras^G12V
WT
shEif2b5
-
A12
B3
-
A12
B3
Puro
p-eIF2α Ser51
β-ACTIN
eIF2α
β-ACTIN
D
Proteasome activity
+Vehicle
+Puromycin
+Bortezomib
Normalized proteasome activity (% of WT)
125
100
75
50
25
0
ns
***
**
shScram
shEif2b5 B3
shScram
shEif2b5 B3
Hras^G12V
WT
E
GDP
GTP
eIF2B
GTP
GDP
eIF2
eIF2
Ternary Complex
GTP
Met
eIF2
Pi
mRNA
AUG
Translation Initiation
Cellular Stress
GDP
eIF2
P
eIF2B
Translation Initiation
Global protein synthesis
F
Apoptosis
% active Cas3+/YFP+ cells
3
2
1
0
ns
shScram
A12
B3
shScram
A12
B3
shEif2b5
shEif2b5
Hras^G12V
WT
G
Hras^G12V
WT
shScram
shEif2b5 A12
shEif2b5 B3
H
Primary Keratinocyte Growth
Cell Count (x10^4)
9
8
7
6
5
4
3
2
1
0
Hras^G12V shScram
Hras^G12V shEif2b5 B3
WT shScram
WT shEif2b5 B3
***
***
ns
0
24
48
72
Elapsed Time (h)

Figure S2. eIF2B5 is necessary for RAS-induced epidermal overgrowth, Related to Figure 2

A) mRNA expression of *Eif2b5* in WT keratinocytes transduced with control or sh*Eif2b5* shRNAs. Two shRNAs that induced the strongest transcript depletion (red arrows) were used for *in vivo* functional studies. n=3 biological replicates.

B) Representative western blot of eIF2B5 in WT keratinocytes transduced with two most efficient shRNAs targeting *Eif2b5.*

C) Representative western blot of puromycin incorporation (Puro), total eIF2α, and phospho-eIF2α Ser51 in primary keratinocytes transduced with control shRNA or sh*Eif2b5*.

D) Proteasome activity was evaluated by measuring chymotrypsin-like activity in primary keratinocytes following vehicle (DMSO), puromycin, or bortezomib treatment. Activity is expressed as percentage of vehicle-treated WT activity. n=3 biological replicates. Key comparisons shown in red.

E) Schematic of the eIF2B complex in translation initiation and inhibitory regulation of eIF2B by eIF2α phosphorylation.

F) Quantification of apoptosis based on Caspase3+ basal cells using flow cytometry in E18.5 epidermis transduced with control shRNA or sh*Eif2b5*. n=3 $Hras^{G12V}$+shScram, 3 $Hras^{G12V}$+sh*Eif2b5* A12, 6 $Hras^{G12V}$+sh*Eif2b5* B3, 4 WT+shScram, 8 WT+sh*Eif2b5* A12, and 3 WT+sh*Eif2b5* B3 animals.

G) Gross skin morphology of E18.5 $Hras^{G12V}$ and WT mice transduced with control shRNA or sh*Eif2b5*.

H) Growth of transduced primary keratinocytes. n=3 biological replicates.

Mean and SD are shown. ANOVA with Tukey's range test for multiple comparisons used to determine statistical differences. **:P-value<0.01; ***:P-value<0.001; ns=not significant.

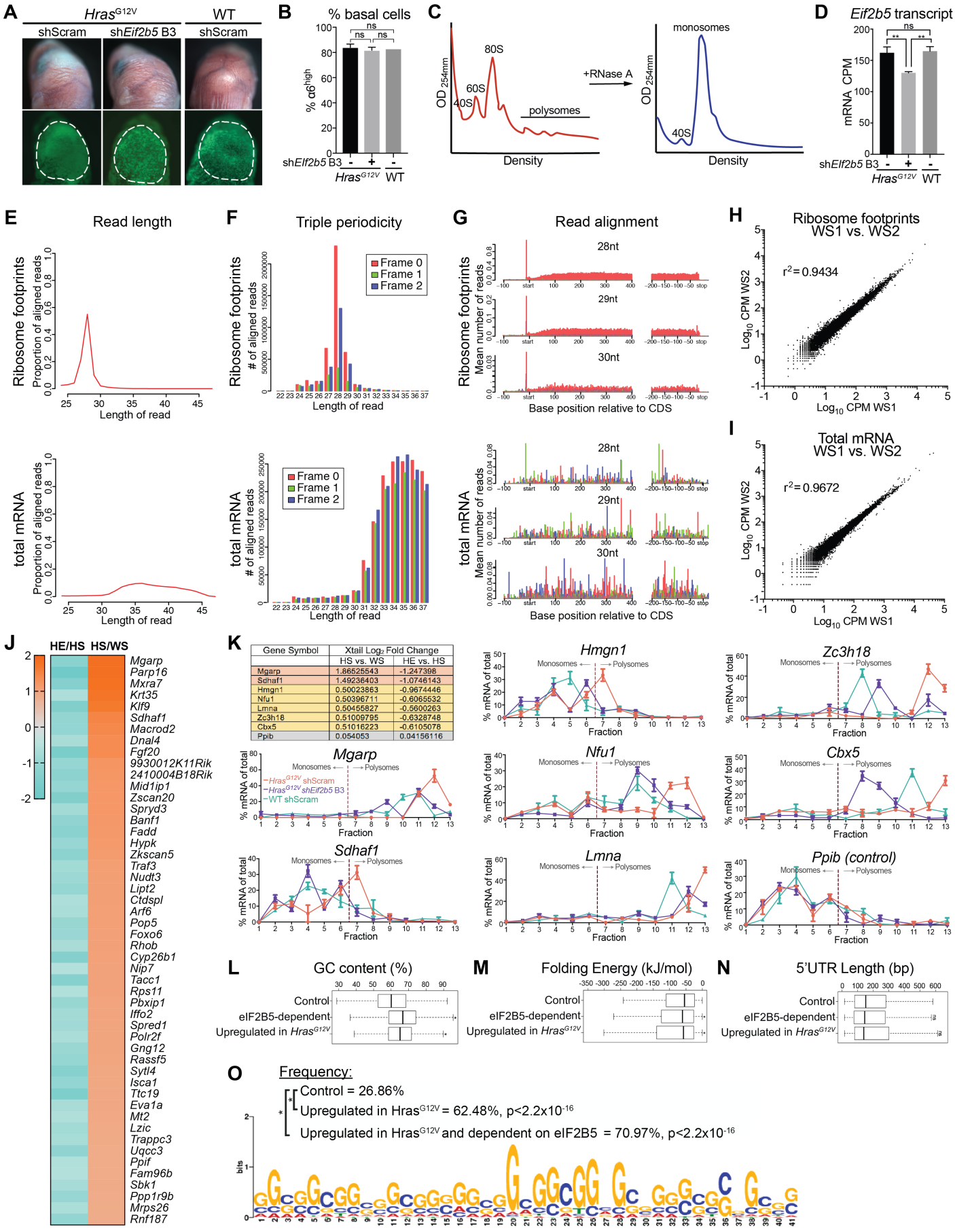
A
Hras^G12V
WT
shScram
shEif2b5 B3
shScram
B
% basal cells
% q6^high
ns
shEif2b5 B3
Hras^G12V
WT
C
80S
60S
40S
polysomes
OD 254mm
Density
+RNase A
monosomes
40S
D
Eif2b5 transcript
mRNA CPM
**
shEif2b5 B3
Hras^G12V
WT
E
Read length
Ribosome footprints
Proportion of aligned reads
Length of read
total mRNA
F
Triple periodicity
Ribosome footprints
# of aligned reads
Frame 0
Frame 1
Frame 2
Length of read
total mRNA
G
Read alignment
Ribosome footprints
Mean number of reads
28nt
29nt
30nt
start
stop
Base position relative to CDS
total mRNA
H
Ribosome footprints
WS1 vs. WS2
r^2 = 0.9434
Log10 CPM WS2
Log10 CPM WS1
I
Total mRNA
WS1 vs. WS2
r^2 = 0.9672
Log10 CPM WS2
Log10 CPM WS1
J
HE/HS HS/WS
Mgarp
Parp16
Mxra7
Krt35
Klf9
Sdhaf1
Macrod2
Dnal4
Fgf20
9930012K11Rik
2410004B18Rik
Mid1ip1
Zscan20
Spryd3
Banf1
Fadd
Hypk
Zkscan5
Traf3
Nudt3
Lipt2
Ctdspl
Arf6
Pop5
Foxo6
Rhob
Cyp26b1
Nip7
Tacc1
Rps11
Pbxip1
Iffo2
Spred1
Polr2f
Gng12
Rassf5
Sytl4
Isca1
Ttc19
Eva1a
Mt2
Lzic
Trappc3
Uqcc3
Ppif
Fam96b
Sbk1
Ppp1r9b
Mrps26
Rnf187
K
Gene Symbol | Xtail Log2 Fold Change | HS vs. WS | HE vs. HS
Mgarp | 1.86525543 | -1.247398
Sdhaf1 | 1.49236403 | -1.0746143
Hmgn1 | 0.50023863 | -0.9674446
Nfu1 | 0.50396711 | -0.6065532
Lmna | 0.50455827 | -0.5600263
Zc3h18 | 0.51009795 | -0.6328748
Cbx5 | 0.51016223 | -0.6105078
Ppib | 0.054053 | 0.04156116
Hmgn1
Zc3h18
Mgarp
Nfu1
Cbx5
Sdhaf1
Lmna
Ppib (control)
% mRNA of total
Monosomes
Polysomes
Fraction
Hras^G12V shScram
Hras^G12V shEif2b5 B3
WT shScram
L
GC content (%)
Control
eIF2B5-dependent
Upregulated in Hras^G12V
M
Folding Energy (kJ/mol)
N
5'UTR Length (bp)
O
Frequency:
Control = 26.86%
Upregulated in Hras^G12V = 62.48%, p<2.2x10^-16
Upregulated in Hras^G12V and dependent on eIF2B5 = 70.97%, p<2.2x10^-16
bits

Figure S3. *Hras*^{G12V} and eIF2B5 specific translation is associated with a functional guanine-rich *cis*-regulatory element, Related to Figure 3

A) Fluorescence and bright field imaging of transduced (marked by GFP) E18.5 headskin. Dashed line marks area processed by enzymatic digestion to isolate basal cells.

B) Flow cytometry quantification of basal cells, marked by high α6 integrin expression, after enzymatic digestion of headskin. n=3 biological replicates.

C) Enzymatic digestion strategy preserves polysomes in WT basal cells. RNase A digestion successfully isolates monosomes.

D) *Eif2b5* transcript reduction is observed in total mRNA libraries from basal cells transduced with sh*Eif2b5*. n=3 biological replicates.

E-G) Representative **(E)** read length, **(F)** reading frame, and **(G)** read alignment distributions for ribosome footprint and total RNA libraries. Ribosome footprint libraries are enriched for 28nt reads which are in frame with the start codon (Frame 0, in red) and distributed along the entire length of the coding sequence (CDS).

H-I) Representative plots of CPM correlation in **(H)** ribosome footprint and (**I)** total mRNA libraries for independent biological replicates. *In vivo* basal cell ribosome profiling strategy results in highly reproducible quantifications. Pearson's χ^2 test was used to evaluate replicate correlation.

J) Heatmap of top 50 translation efficiency (TE) upregulated genes in *Hras*^{G12V}+shScram vs. WT+shScram (HS/WS) comparison and corresponding TE change in *Hras*^{G12V}+sh*Eif2b5* vs. *Hras*^{G12V}+shScram (HE/HS) comparison.

K) Validation of several significant genes from ribosome profiling analysis that are translationally upregulated in *Hras*^{G12V} and dependent on eIF2B5**.** Polysome fractionation of primary keratinocytes transduced with control shRNA or sh*Eif2b5* and qPCR analysis reveals transcript enrichment in heavier polysome fractions in *Hras*^{G12V} keratinocytes (orange), which is rescued by *Eif2b5* knockdown (purple). n=3 biological replicates.

L-N) (L) GC content, **(M)** folding energy, and **(N)** length of 5'UTRs of mRNAs translationally upregulated by *Hras*^{G12V} activation or dependent on eIF2B5, in comparison to the entire mouse genome (control). Wilcoxon two-sided test used to analyze significant differences

O) The guanine-rich translational element (GRTE) consensus sequence. FIMO analysis reveals that the GRTE is significantly enriched in 5'UTRs of mRNAs that are translationally upregulated in *Hras*^{G12V} and dependent on eIF2B5, in comparison to the entire mouse genome (control).

Mean and SD are shown. ANOVA with Tukey's range test for multiple comparisons used to determine statistical differences. *p<0.05; **p:-value<0.01; ns=not significant.

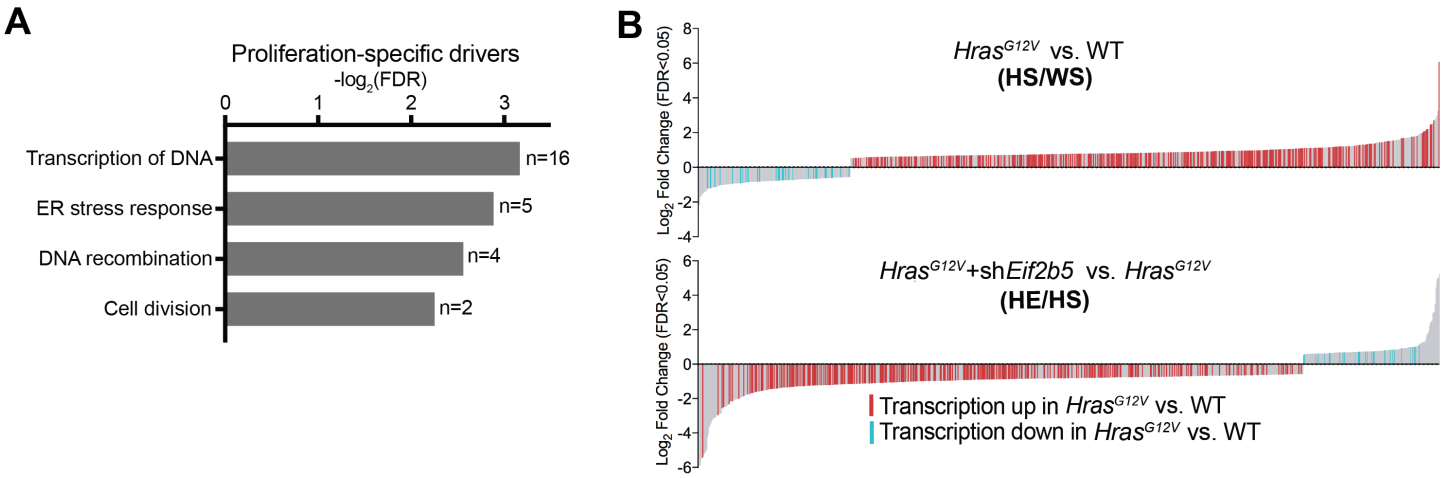

Figure S4. eIF2B5 promotes proliferation in *Hras^{G12V}* basal cells via translation of transcription regulators, Related to Figure 4

A) Ingenuity pathway analysis of eIF2B5-dependent pro-proliferation genes.

B) Needle plots of significantly altered transcripts ($Log_2FC>0.5$, FDR<0.05) from EdgeR analysis of total mRNA libraries. Genes that are transcriptionally upregulated (red) or downregulated (blue) in *Hras^{G12V}* compared to WT basal cells are rescued by *Eif2b5* depletion. n= 3 biological replicates.

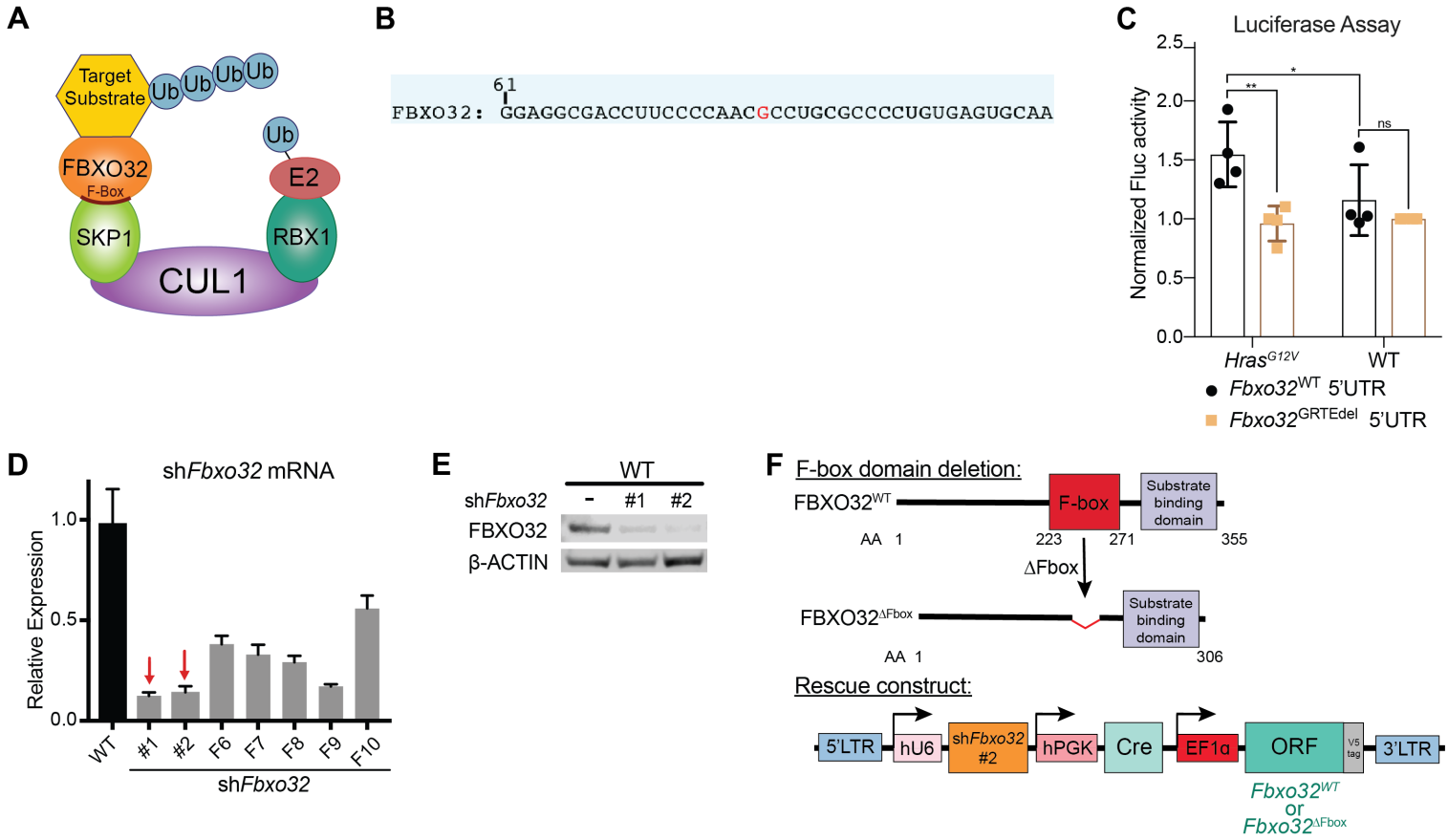

Figure S5. FBXO32 is translationally regulated via its 5'UTR GRTE and functions as a ubiquitin ligase, Related to Figure 5 and Figure 6

A) FBXO32 is the substrate recognition component of the Skp1-Cullin-Fbox (SCF) E3 ubiquitin ligase complex. SKP1 interacts with the F-box domain of FBXO32 to acquire substrate specificity.

B) GRTE in the 5'UTR of *Fbxo32*, as identified by FIMO analysis (Grant et al., 2011).

C) Luciferase reporter assay in primary keratinocytes to test translation initiation using the full length *Fbxo32* 5'UTR (*Fbxo32*^{WT}) or a GRTE deletion mutant (*Fbxo32*^{GRTEdel}). Translation level is quantified by normalizing luciferase RLU to mRNA level. n=4 biological replicates.

D) mRNA expression of *Fbxo32* in WT keratinocytes transduced with control or sh*Fbxo32* shRNAs. Two shRNAs that induced the strongest transcript depletion (red arrows) were used for *in vivo* functional studies. n=3 biological replicates.

E) Representative western blot of FBXO32 in WT keratinocytes transduced with the two most efficient shRNAs targeting *Fbxo32.*

F) The Skp1-Cullin-Fbox (SCF) E3 ubiquitin ligase complex requires interaction of SKP1 with the F-box domain of FBXO32 to acquire substrate specificity. Deletion of the 49aa F-box domain in *Fbxo32*^{WT} generates *Fbxo32*$^{\Delta Fbox}$ ORF. Lentivirus rescue construct co-expresses Cre to activate oncogenic *Hras*^{G12V}, sh*Fbxo32 #2* to deplete endogenous *Fbxo32*, and V5-tagged rescue ORF (*Fbxo32*^{WT} or *Fbxo32* $^{\Delta Fbox}$) driven by strong constitutive promoter EF1α.

G) mRNA expression of endogenous and total *Fbxo32* transcript in WT keratinocytes transduced with sh*Fbxo32 #2* + *Fbxo32*^{WT} or *Fbxo32*$^{\Delta Fbox}$ ORF. Endogenous transcript is detected using primers against the 3'UTR, and total transcript is detected using primers against the CDS between exons 3 and 5. n=3 biological replicates.

H) Representative western blot of wild type (40kDa) or mutant (33kDa) FBXO32 and V5 tag in WT keratinocytes. Endogenous *Fbxo32* is depleted with sh*Fbxo32 #2* and replaced by expression of *Fbxo32*^{WT} or *Fbxo32*$^{\Delta Fbox}$ ORF.

I) mRNA expression of *Fbxo32* and *Eif2b5* in WT keratinocytes transduced with sh*Eif2b5* alone or in combination with rescue *Fbxo32*^{WT} ORF. n=3 biological replicates. Mean and SD are shown.

J) Representative western blot of FBXO32 and eIF2B5 in WT keratinocytes.

Mean and SD are shown. ANOVA with Tukey's range test for multiple comparisons used to determine statistical differences. *:P-value<0.05; **:P-value<0.01; ns=not significant.

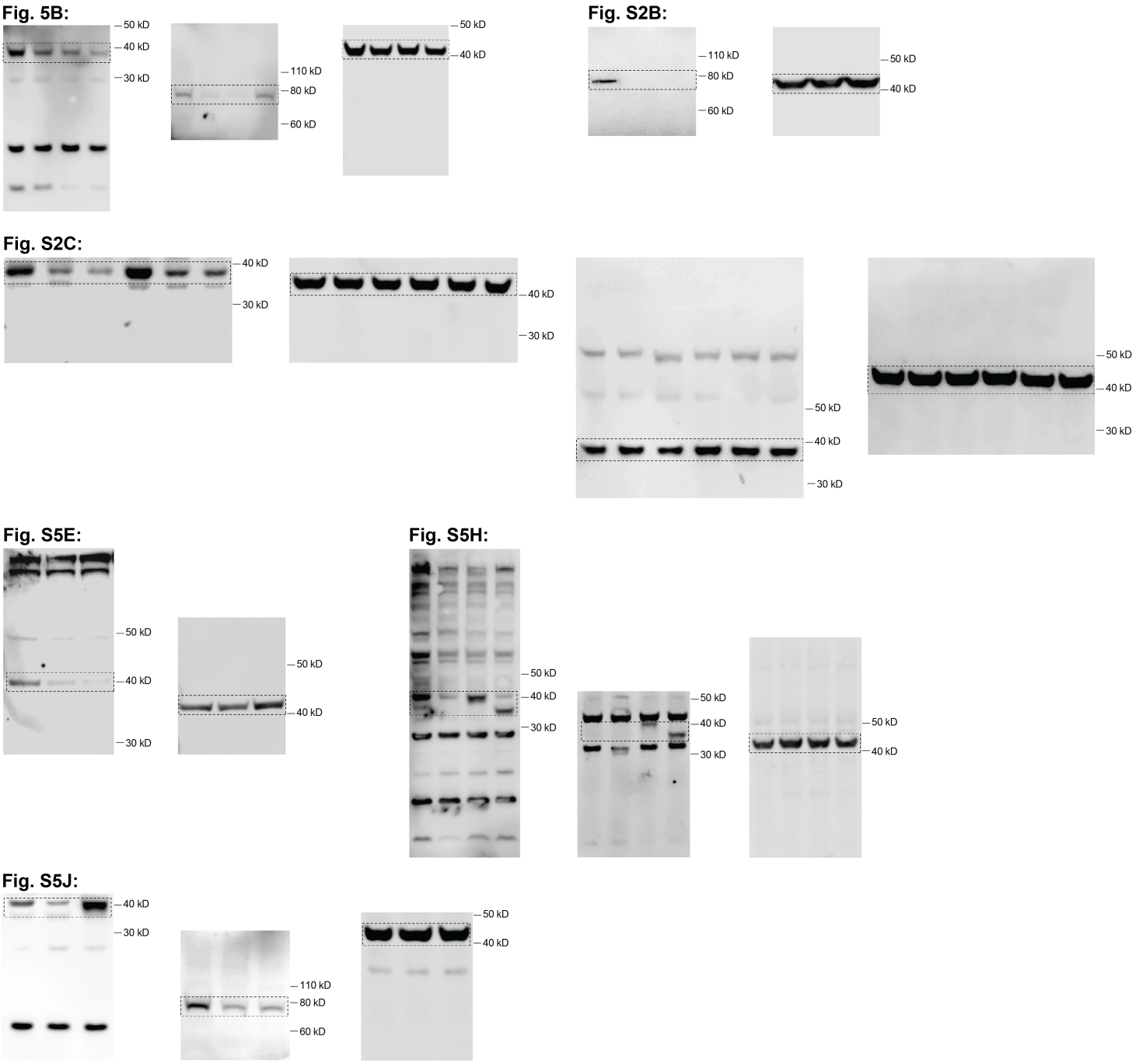

Figure S6. Uncropped western blots presented in this study, Related to STAR Methods