SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. U2AF1 S34F mutant cells show altered DNA damage response and inflammatory cytokine secretion.

A. Scatter plot of gene expression changes (TPM, Transcripts per million) in wt/wt and wt/S34F cells at steady state determined by RNA sequencing. The red and blue points represent genes that showed either increased (up > = 1.5 fold), or decreased (down > = 1.5 fold) expression respectively in wt/S34F cells.
B. Quantitative immunostaing of macroH2A1 showing the S34F mutant induced altered splicing events results in altered protein isoform levels of macroH2A1.1 and macroH2A1.2 in the same nucleus of individual wt/wt cells. Scatter plots analysis of normalized fluorescence intensity in individual nuclei immunostained for both macroH2A1.1 and macroH2A1.2 from wt/wt, wt/S34F and wt/S34F- cells
C. Representative bright field images of wt/wt, wt/-, wt/S34F and wt/S34F- cells irradiated with x-rays (20Gy), fixed and stained for β-galactosidase (stained black) after indicated times.
D. Cytokine levels measured by ELISA in media collected at indicated times from wt/wt, wt/S34F, wt/- and wt/S34F- mutant cells after irradiation with x-rays (20Gy). IL1α secreted into media is presented as pg/mL (average of 3 independent experiments +/- s.d.).

Supplementary Figure2. U2AF1 binds RNA in the cytoplasm.

A. RT-qPCR analysis of IL8 expression in wt/wt, wt/S34F and wt/S34f- cells (3 biological replicates +/- s.d.)
B. Immunoprecipitation of U2AF1 from nuclear and cytoplasmic fractions from wt/wt cells (2 fold excess of secondary antibody bound beads) followed by western blotting analysis of U2AF1 in nuclear and cytoplasmic fractions (T = total nuclear fraction, IP = immunoprecipitate and S = supernatant).
C. RT-PCR analysis for detection of Myc and Fn1 pre-mRNA in cytoplasmic fraction isolated from wt/wt, wt/S34F and wt/S34F- cells (Ex = exonic primers; In = intronic primers).
D. Detection of IL1α, IL8, DNA polH and RPB3 polyadenylated mRNA in U2AF1 bound cytoplasmic RIP

from wt/wt, wt/S34F and wt/S34F- cells (IP = immunoprecipitate; S = IP supernatant).

Supplementary Figure 3. U2AF1 functions as a translational repressor in the cytoplasm.

A. Polysome profiles from wt/wt (black), wt/S34F (green) and wt/S34F- (red) cells after cycloheximide treatment. Inset: profile showing collapse of polysomes into monosomes after RNAse A/T1 (blue) treatment.

B. Semi-quantitative RT-PCR profiles of indicated mRNA in polysomes from wt/wt, wt/S34F and wt/S34F- cells. Limited cycle (18 cycles) PCR reaction products fractionated on 15% agarose gels.

Supplementary Figure 4. U2AF1 directly represses translation of hundreds of genes.

The targets of this translational repression pathway are enriched in messages which themselves code for translation machinery, potentially resulting in direct and indirect changes in translation. EIF4A-regulated messages show increased translation in wt/S34F cells, despite not being direct binding targets. This result, however, is consistent with the change in EIF4A2 translation (Extended Data Table 3, 4).

A. Polysome profiles of indicated genes showing their enhanced distribution in heavy polysomes from wt/S34F cells. Normalization to unit area for each sample.

B. Average polysome profile from EIF4A-sensitive transcripts (Extended Data Table 2): polysome profiles from wt/wt (black), wt/S34F (green) and wt/S34F- (red). n=69 transcripts; bootstrap error.

C. Polysome/monosome ratio for several different groups of transcripts. Each group contains three boxes reflecting the distribution of polysome/monosome ratios (from left to right): wt/wt, wt/S34F, wt/S34F-. The transcript groups, from left to right, are: decile 1 of changes in RIP/input from wt/wt to wt/S34F, decile 10, mTOR-sensitive transcripts, and EIF4A-sensitive transcripts. ** = $p<0.001$. * = $p<0.05$.

D. mRNA length distribution within the Δ RIP deciles. There is no systematic difference in binding

efficiency based on transcript length.

E. GO terms for transcripts in decile 1 of changes in RIP/input from wt/wt to wt/S34F.

F. Box plot analysis of mRNA enriched in RIP over input in the top decile of RIP, mTOR sensitive mRNA, EIF4A sensitive mRNA from wt/wt (gray), wt/S34F (green) and wt/S34F- (red) cells.

Supplementary Figure 5. Direct association of U2AF at start codons of translationally regulated mRNAs.

A. Photocrosslinked U2AF1 immunoprecipitated with anti-FLAG antibodies from cytoplasmic or chromatin fractions, nuclease digested, 5'-32P labeled, 3'-adapter ligated and fractionated on SDS-PAGE.

B. PAR-CLIP sequence enrichment peaks of WT U2AF along the entire NPM1 gene (Top panel). The top two tracks are PAR-CLIP of U2AF1 and U2AF2 from the cytoplasm and the chromatin fractions respectively. The third track represents U2AF1 PAR-CLIP from the chromatin fraction. The region from the 5'-UTR to downstream exons is enlarged to show specific interactions of U2AF at the ATG codon and the 5'-UTR immediately upstream in the cytoplasmic fraction and no association along the gene body. PABPC1 (Bottom panel), U2AF shows no association with the 5'-UTR in the cytoplasm of S34F (top track) and WT cells (middle track), and chromatin of WT cells (bottom track).

Supplementary Figure 6. U2AF1 controls translation of IL8 via its 5'-UTR.

A. FACS of wt/wt or wt/S34F cells expressing wt- or mt-5'-UTR IL8-2A-GFP reporter. wt/wt cells served as control for autofluorescence and the wt/wt and wt/S34F cells expressing the wt- or mt-IL8-GFP reporter were gated above the auto fluorescence from wt/wt control cells.

B. Representative images of wt/wt or wt/S34F cells expressing wt- or mutant 5'-UTR reporter.

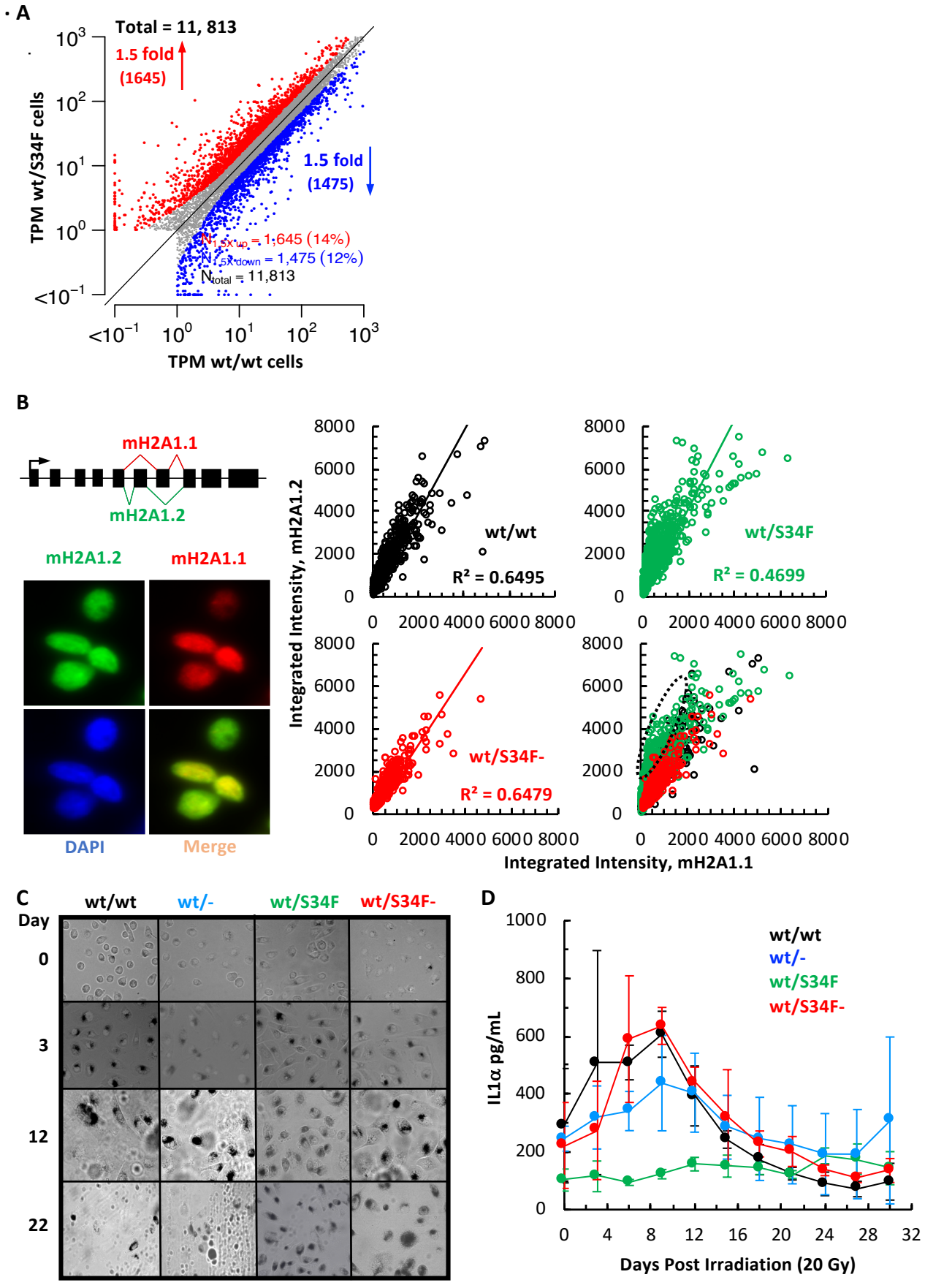
Supplementary Figure 7. IL8 can induce EMT, inflammation and tumor progression.

Serum IL8 levels (gray bar) and the number of macroscopic lung nodules (black circles) from NOG mice injected (tail vein) with H441 isogenic cells, and treated with either isotype control or anti-IL8 antibodies for 10 weeks (n = 3 for each treatment group) .

Supplementary Table 1

	Gene Ontology Term	Support	fdr
GO:0006271	DNA strand elongation involved in DNA replication	0.025	3.00E-20
GO:0032201	telomere maintenance via semi-conservative replication	0.018	7.00E-14
GO:0000722	telomere maintenance via recombination	0.018	8.00E-13
GO:0006270	DNA replication initiation	0.018	9.00E-12
GO:0006297	nucleotide-excision repair, DNA gap filling	0.012	4.00E-08
GO:0032508	DNA duplex unwinding	0.018	4.00E-06
GO:0000083	regulation of transcription involved in G1/S transition of mitotic cell cycle	0.011	2.00E-05
GO:0000076	DNA replication checkpoint	0.0075	4.00E-05
GO:0006298	mismatch repair	0.01	6.00E-05
GO:0006284	base-excision repair	0.014	3.00E-04

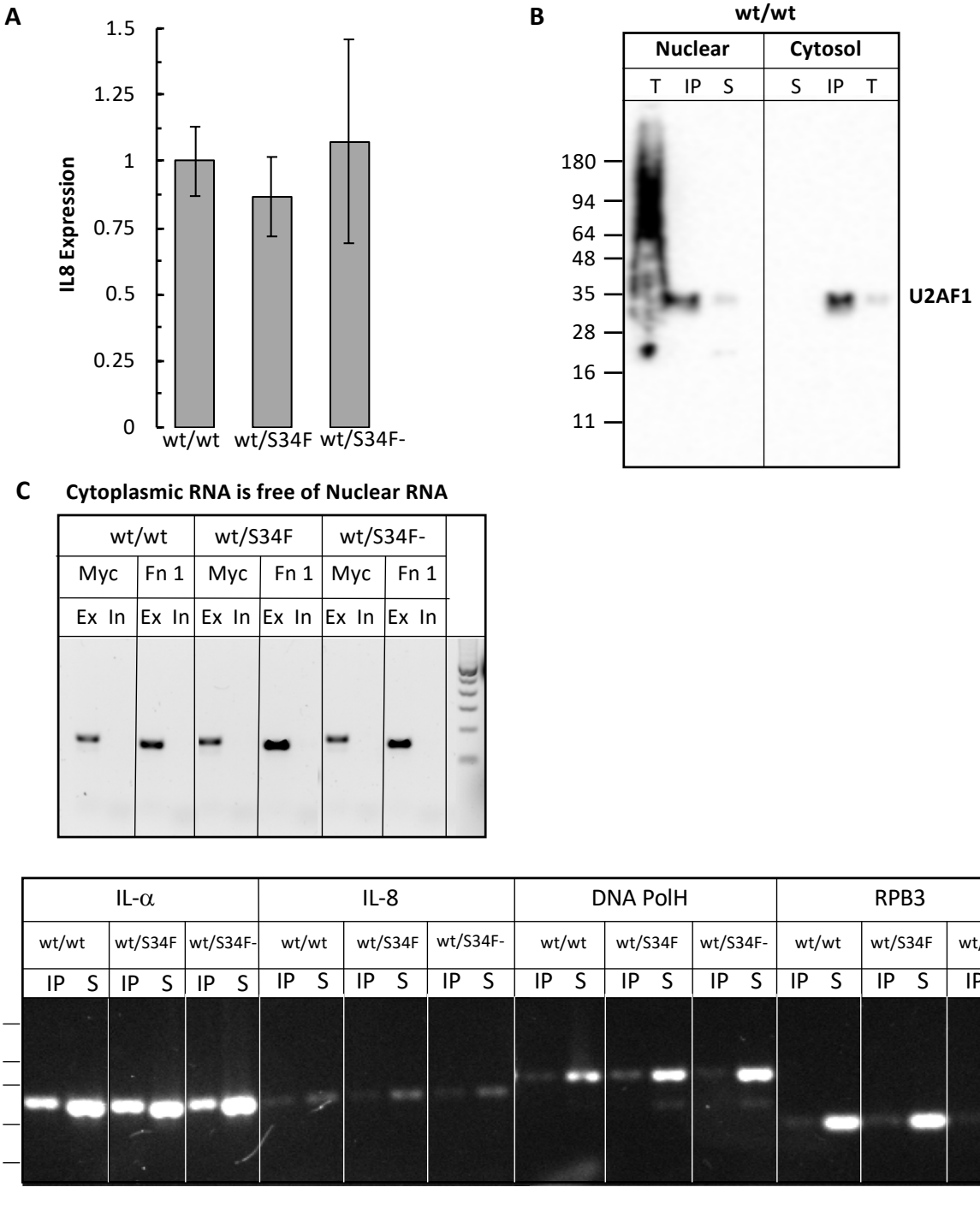
Supplementary_Figure_1



Supplementary_Figure_1. U2AF1 S34F mutant cells show altered DNA damage response and inflammatory cytokine secretion.

A. Scatter plot of gene expression changes (TPM, Transcripts per million) in wt/wt and wt/S34F cells at steady state determined by RNA sequencing. The red and blue points represent genes that showed either increased (up > = 1.5 fold), or decreased (down > = 1.5 fold) expression respectively in wt/S34F cells. B. Quantitative immunostaing of macroH2A1 showing the S34F mutant induced altered splicing events results in altered protein isoform levels of macroH2A1.1 and macroH2A1.2 in the same nucleus of individual wt/wt cells. Scatter plots analysis of normalized fluorescence intensity in individual nuclei immunostained for both macroH2A1.1 and macroH2A1.2 from wt/wt, wt/S34F and wt/S34F- cells. C. Representative bright field images of wt/wt, wt/-, wt/S34F and wt/S34F- cells irradiated with x-rays (20Gy), fixed and stained for b-galactosidase (stained black) after indicated times. D. Cytokine levels measured by ELISA in media collected at indicated times from wt/wt, wt/S34F, wt/- and wt/S34F- mutant cells after irradiation with x-rays (20Gy). IL1a secreted into media is presented as pg/mL (average of 3 independent experiments +/- s.d.).

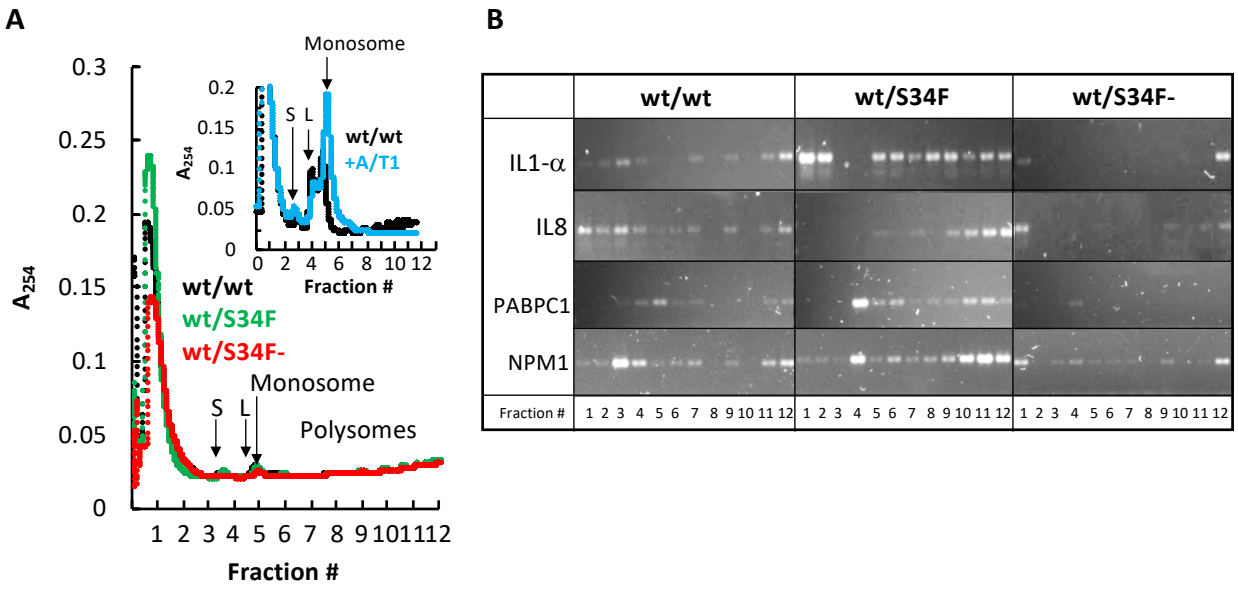
Supplementary_Figure_2. U2AF1 binds RNA in the cytoplasm.



Supplementary_Figure_2. U2AF1 binds RNA in the cytoplasm.

A. RT-qPCR analysis of IL8 expression in wt/wt, wt/S34F and wt/S34f- cells (3 biological replicates +/- s.d.). B. Immunoprecipitation of U2AF1 from nuclear and cytoplasmic fractions from wt/wt cells (2 fold excess of secondary antibody bound beads) followed by western blotting analysis of U2AF1 in nuclear and cytoplasmic fractions (T = total nuclear fraction, IP = immunoprecipitate and S = supernatant). C. RT-PCR analysis for detection of Myc and Fn1 pre-mRNA in cytoplasmic fraction isolated from wt/wt, wt/S34F and wt/S34F- cells (Ex = exonic primers; In = intronic primers). D. Detection of IL1a, IL8, DNA polH and RPB3 polyadenylated mRNA in U2AF1 bound cytoplasmic RIP from wt/wt, wt/S34F and wt/S34F- cells (IP = immunoprecipitate; S = IP supernatant).

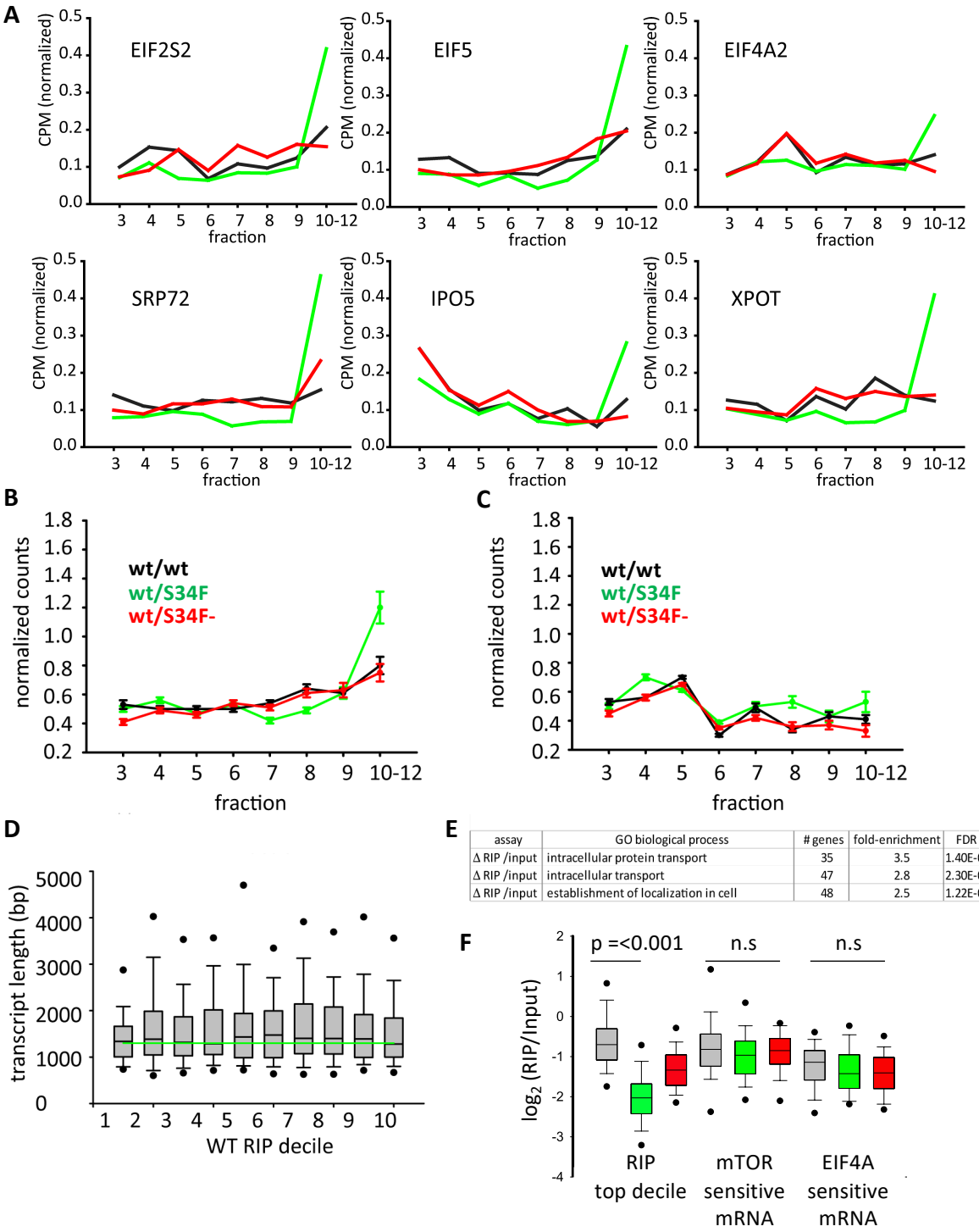
Supplementary_Figure_3. U2AF1 functions as a translational repressor in the cytoplasm.



Supplementary_Figure_3. U2AF1 functions as a translational repressor in the cytoplasm.

A. Polysome profiles from wt/wt (black), wt/S34F (green) and wt/S34F- (red) cells after cycloheximide treatment. Inset: profile showing collapse of polysomes into monosomes after RNAse A/T1 (blue) treatment. B. Semi-quantitative RT-PCR profiles of indicated mRNA in polysomes from wt/wt, wt/S34F and wt/S34F- cells. Limited cycle (18 cycles) PCR reaction products fractionated on 1.5% agarose gels.

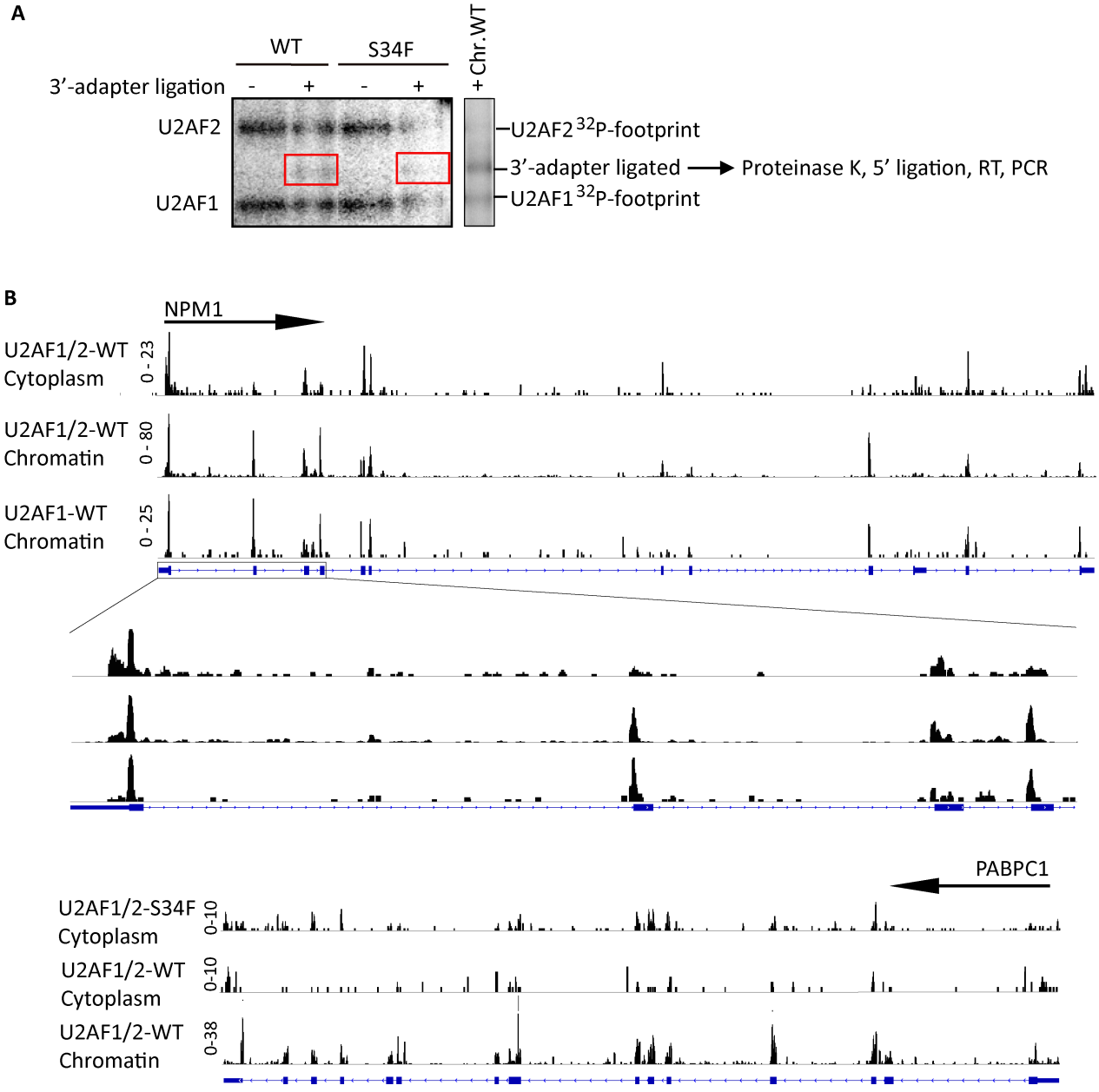
Supplementary_Figure_4. U2AF1 directly represses translation of hundreds of genes.



assay	GO biological process	# genes	fold-enrichment	FDR
Δ RIP /input	intracellular protein transport	35	3.5	1.40E-06
Δ RIP /input	intracellular transport	47	2.8	2.30E-06
Δ RIP /input	establishment of localization in cell	48	2.5	1.22E-05

Supplementary_Figure_4. U2AF1 directly represses translation of hundreds of genes.
The targets of this translational repression pathway are enriched in messages which themselves code for translation machinery, potentially resulting in direct and indirect changes in translation. EIF4A-regulated messages show increased translation in wt/S34F cells, despite not being direct binding targets. This result, however, is consistent with the change in EIF4A2 translation (Extended Data Table 3, 4). A. Polysome profiles of indicated genes showing their enhanced distribution in heavy polysomes from wt/S34F cells. Normalization to unit area for each sample. B. Average polysome profile from EIF4A-sensitive transcripts (Extended Data Table 2): polysome profiles from wt/wt (black), wt/S34F (green) and wt/S34F- (red). n=69 transcripts; bootstrap error. C. Average polysoome profile from mTOR-sensitive transcripts (Extended Data Table 2): polysome profiles from wt/wt (black), wt/S34F (green) and wt/S34F- (red). n=77 transcripts; bootstrap error. D. mRNA length distribution within the Δ RIP deciles. There is no systematic difference in binding efficiency based on transcript length. E. GO terms for transcripts in decile 1 of changes in RIP/input from wt/wt to wt/S34F. F. Box plot analysis of mRNA enriched on RIP over input in the top decile of RIP, mTOR sensitive mRNA and EIF4A sensitive mRNA from wt/wt (gray), wt/S34F (green) and wt/S34F- (red) cells.

Supplementary_Figure-5. Direct association of U2AF to ATG codons of translationally regulated mRNAs.

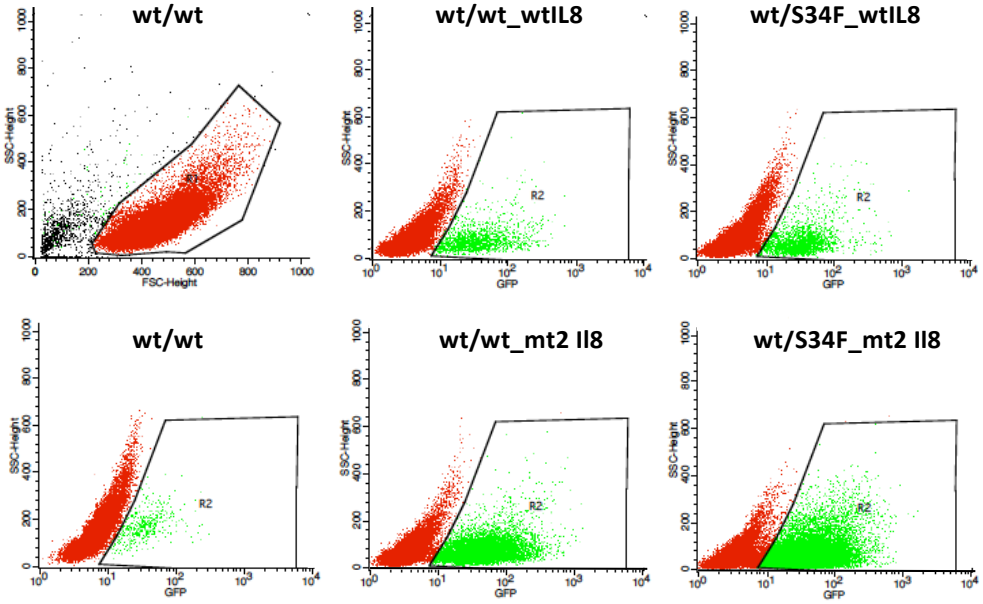


Supplementary_Figure_5. Direct association of U2AF to ATG codons of translationally regulated mRNAs.

A. Photocrosslinked U2AF1 immunoprecipitated with anti-FLAG antibodies from cytoplasmic or chromatin fractions, nuclease digested, 5'-32P labeled, 3'-adapter ligated and fractionated on SDS-PAGE. B. PAR-CLIP sequence enrichment peaks of WT - U2AF along the entire NPM1 gene (Top panel). The top two tracks are PAR-CLIP of U2AF1 and U2AF2 from the cytoplasm and the chromatin fractions respectively. The third track represents U2AF1 PAR-CLIP from the chromatin fraction. The region from the 5'-UTR to downstream exons is enlarged to show specific interactions of U2AF at the ATG codon and the 5'-UTR immediately upstream in the cytoplasmic fraction and no association along the gene body. PABPC1 (Bottom panel), U2AF shows no association with the 5'-UTR in the cytoplasm of S34F (top track) and WT cells (middle track), and chromatin of WT cells (bottom track).

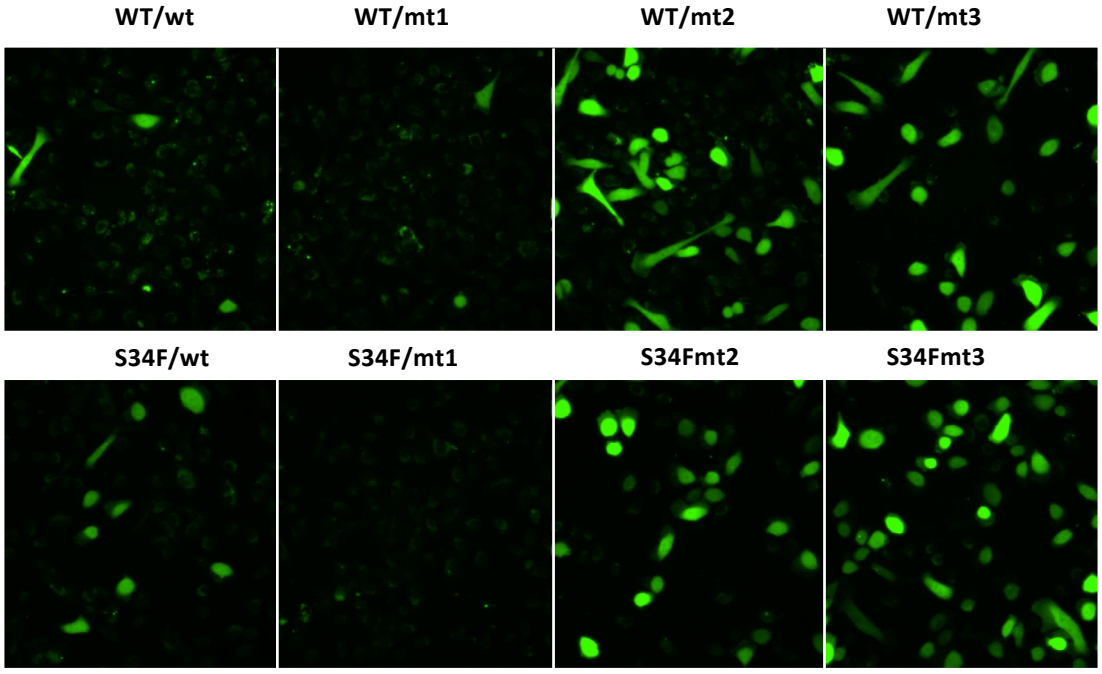
Supplementary_Figure_6. U2AF1 controls translation of IL8 via its 5’-UTR.

A

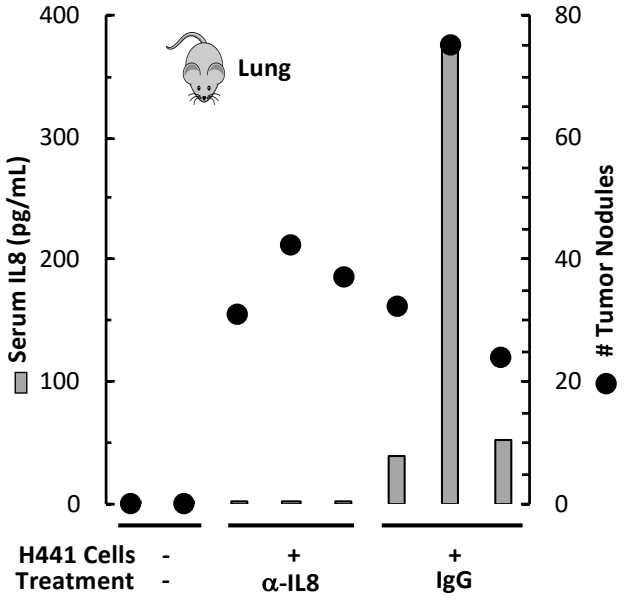


B IL8 5’-UTR mutants

```
5’-UTR wt    GCACAAACTTTCAGAG…
5’-UTR mt1   GCAGAGTTTCAAACAG…
5’-UTR mt2   GCACAAACTTTCATAG…
5’-UTR mt3   GCACTAACAATGAAAG…
```



Supplementary_Figure_6. U2AF1 controls translation of IL8 via its 5'-UTR.
A. FACS of wt/wt or wt/S34F cells expressing wt- or 5'-UTR mt2 IL8-2A-GFP reporter. wt/wt cells served as control for autofluorescence and the wt/wt and wt/S34F cells expressing the wt- or mt-IL8-GFP reporter were gated above the auto fluorescence from wt/wt control cells. B. Representative images of wt/wt or wt/S34F cells expressing wt- or mutant 5'-UTR reporter.



Supplementary_Figure_7. U2AF1 S34F mutant cells show altered DNA damage response and inflammatory cytokine secretion.

Supplementary_Figure_7. U2AF1 S34F mutant cells show altered DNA damage response and inflammatory cytokine secretion.

A. Serum IL8 levels (gray bar) and the number of macroscopic lung nodules (black circles) from NOG mice injected (tail vein) with H441 isogenic cells, and treated with either isotype control or anti-IL8 antibodies for 10 weeks (n = 3 for each treatment group) .