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SUPPLEMENTARY INFORMATION

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# *SAMMSON* fosters cancer cell fitness by concertedly enhancing mitochondrial and cytosolic translation

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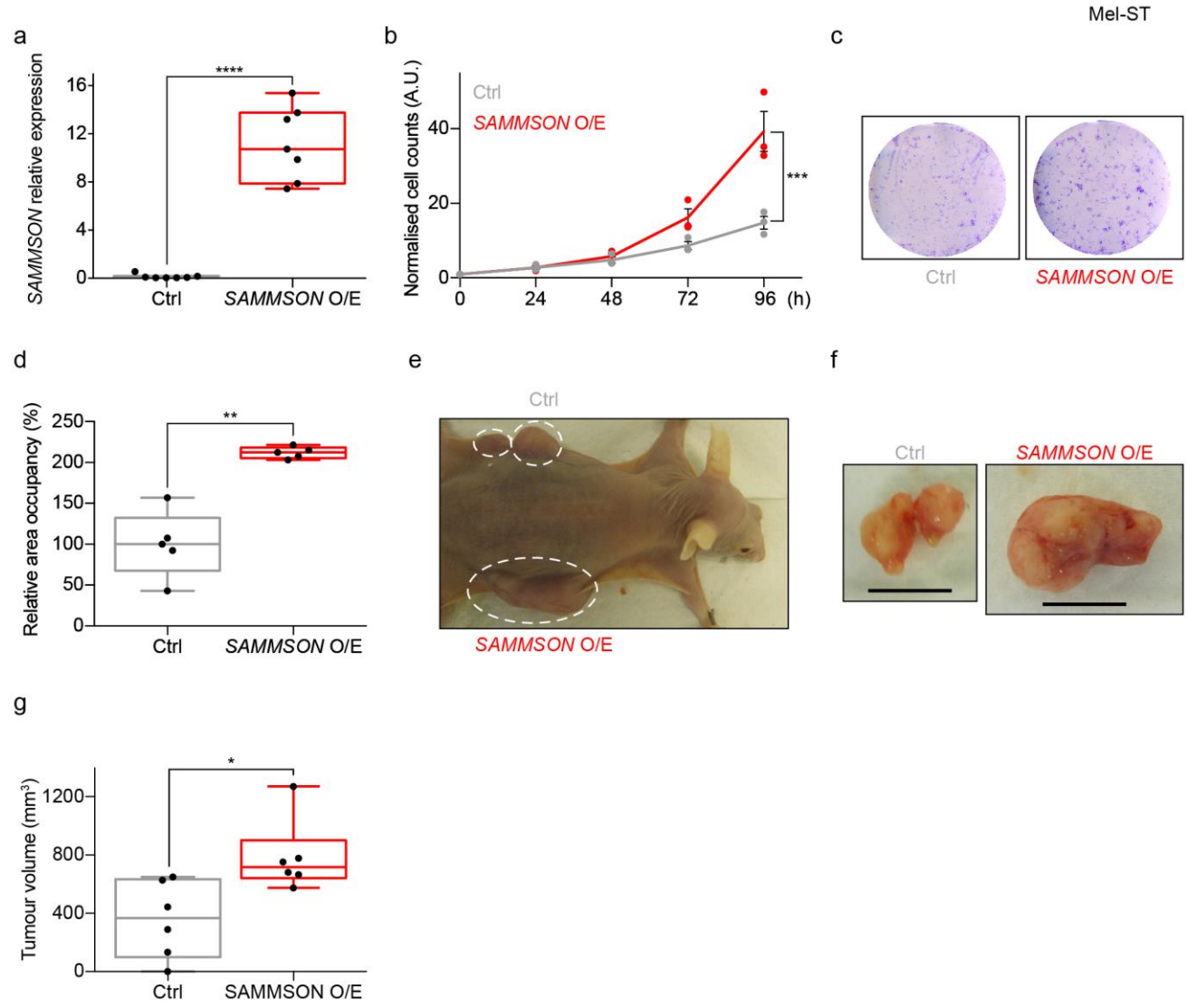
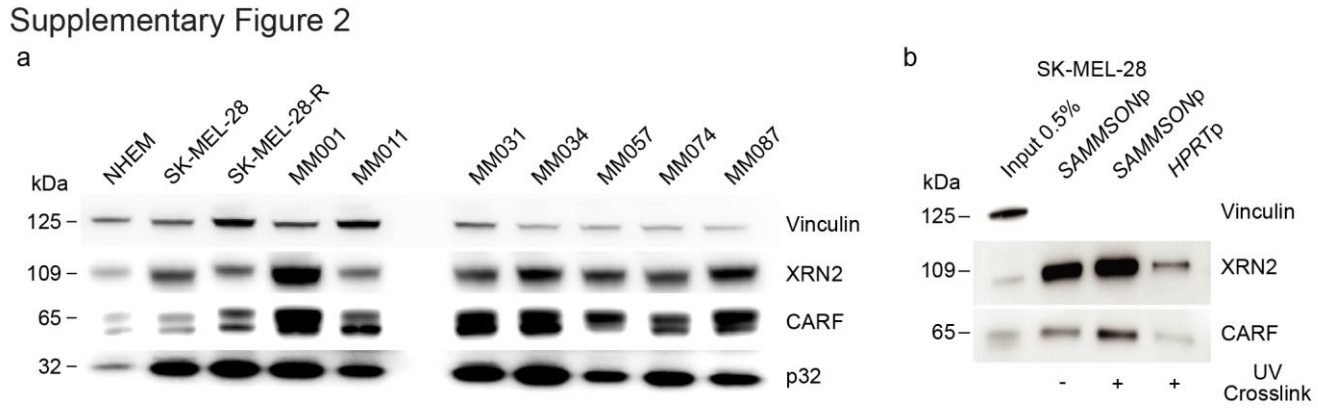
Supplementary Figure 1




**Supplementary Figure 1**

***SAMMSON* actively participates to malignant transformation.**

(**a**) *SAMMSON* relative expression measured by RT-qPCR in Mel-ST cells infected with an empty (Ctrl) or a *SAMMSON*-encoding (*SAMMSON* O/E) expression vector; n=7. (**b**) Cell proliferation assays in Mel-ST cells described in **a**. Error bars represent mean ± s.e.m.; n=3. (**c**) Colony formation assays 5 days after seeding 1 x $10^3$ Mel-ST cells as described in **a**. The violet colour is given by crystal violet, a compound that binds intracellular DNA and protein thus highlighting the cells in the plate. Representative image of five independent experiments. (**d**) Quantification of colony formation assays of Mel-ST cells as described in **a** and **c** presented as the mean density (% of area occupancy); n=5. (**e**) Representative picture of xenograft tumors (encircled by the white dashed line) grown in nude mice derived from subcutaneous injection of 5 x $10^3$ Mel-ST cells as described in **a**. (**f**) Representative picture of resected xenograft tumors as described in **e**. Scale bar, 1 cm. (**g**) Tumor volume of xenografts as described in **e**. Error bars represent mean ± s.e.m.; n=6. Box boundaries represent 25th and 75th percentiles; center line represents the median; whiskers, last data point within ±1.5 interquartile range. *P* values were calculated by paired two-tailed Student's t-test. * *P*<0.05; ** *P*<0.01; *** *P*<0.001; **** *P*<0.0001.



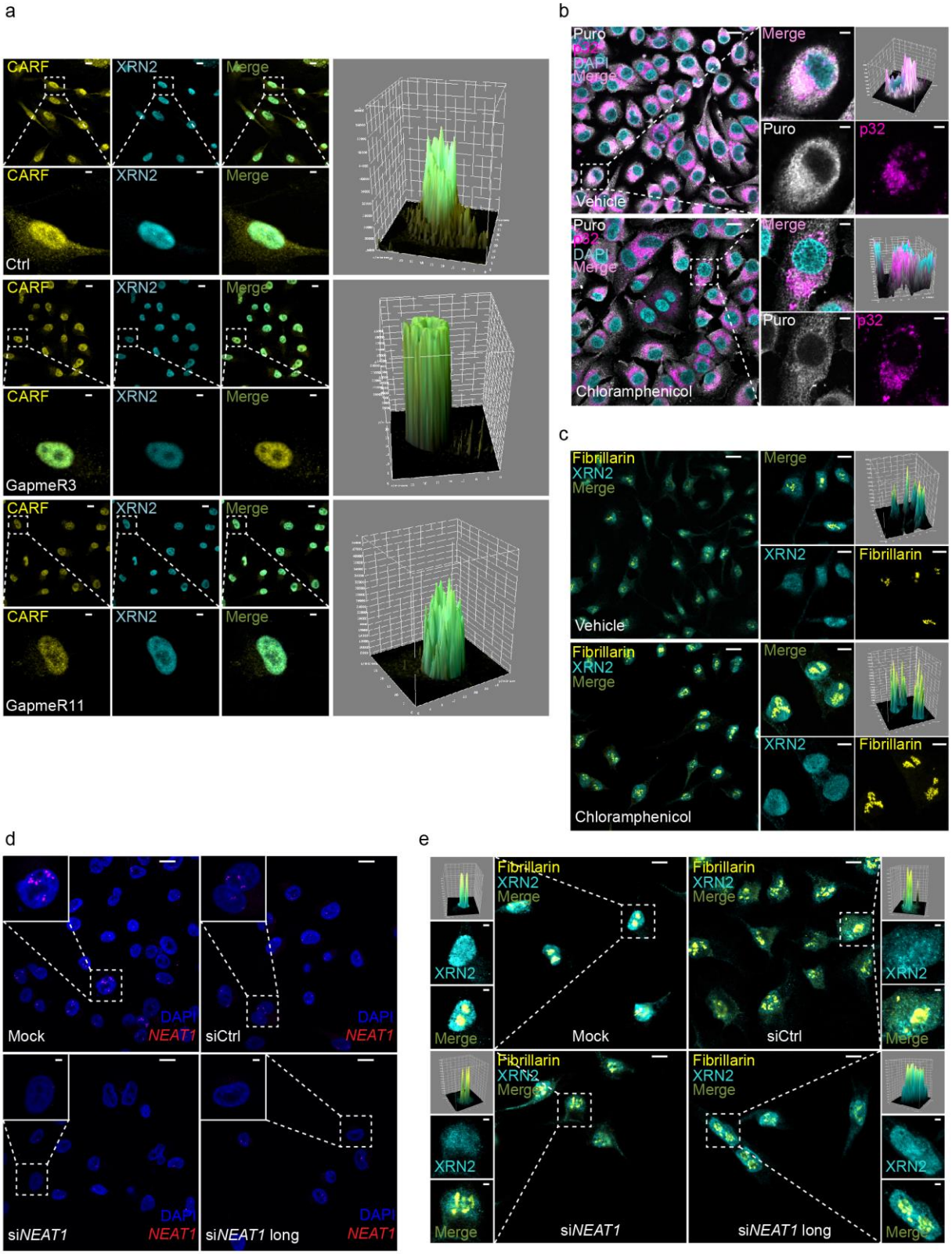

**Supplementary Figure 2**

**XRN2, CARF and p32 levels are increased in melanoma.**

(**a**) Western blot for XRN2, CARF and p32 in Normal Human Epidermal Melanocytes (NHEM), in SK-MEL-28 WT or BRAFi-resistant (SK-MEL-28-R) and in a panel of short-term melanoma cultures (MM-lines, with different mutational backgrounds and phenotype). (**b**) *SAMMSON* and *HRPT* pulldown in native (-) and UV crosslinking (+) conditions (using two sets of 48 biotinylated probes recognising mature transcripts, *p*) and western blotting in SK- MEL-28 cells. Representative image of three independent experiments. Uncropped gel images are shown in Supplementary Data Set 1.

Supplementary Figure 3

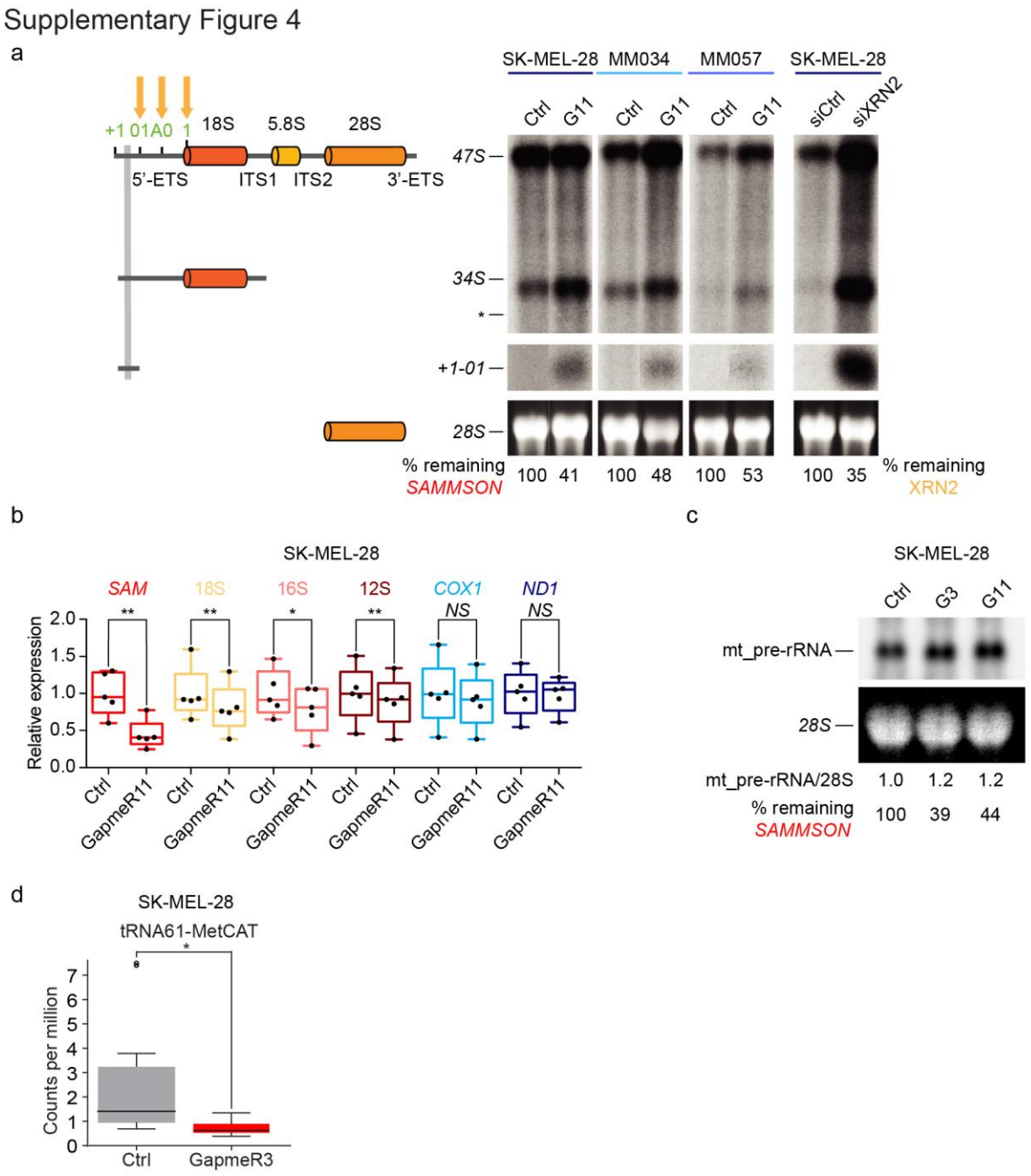
SK-MEL-28



**Supplementary Figure 3**

**CARF and XRN2 localization are specifically affected by *SAMMSON* knockdown and not by other stressors.**

(**a**) XRN2 (cyan) and CARF (yellow) IF in SK-MEL-28 cells 30 hours after transfection with a non-targeting GapmeR (Ctrl), GapmeR3 or GapmeR11. Scale bar low magnification, 10 μm; high magnification, 2 μm. Representative image of three independent experiments. (**b**) Puromycin (Puro, white) or p32 (magenta) IF in SK-MEL-28 cells treated for 48 hours with 200 μg $mL^{-1}$ chloramphenicol or vehicle (EtOH). Cell nuclei are stained with DAPI (cyan). Scale bar low magnification, 10 μm; high magnification, 2 μm. Representative image of three independent experiments. (**c**) XRN2 (cyan) and fibrillarin (yellow) IF in SK-MEL-28 cells treated as in **b**. Scale bar low magnification, 10 μm; high magnification, 7 μm. Representative image of three independent experiments. (**d**) *NEAT1* (red) RNA fluorescence *in situ* hybridization (FISH) in untreated SK-MEL-28 cells (Mock) or in cells 72 hours after transfection with a control siRNA pool (siCtrl) or pools targeting *NEAT1* (si*NEAT1*) or si*NEAT1* long form only (si*NEAT1* long). Cell nuclei are stained with DAPI (blue). Scale bar low magnification, 10 μm; high magnification, 2 μm. Representative image of three independent experiments. (**e**) XRN2 (cyan) and fibrillarin (yellow) IF in SK- MEL-28 cells treated as described in **d**. Scale bar low magnification, 10 μm; high magnification, 2 μm. Representative image of three independent experiments.



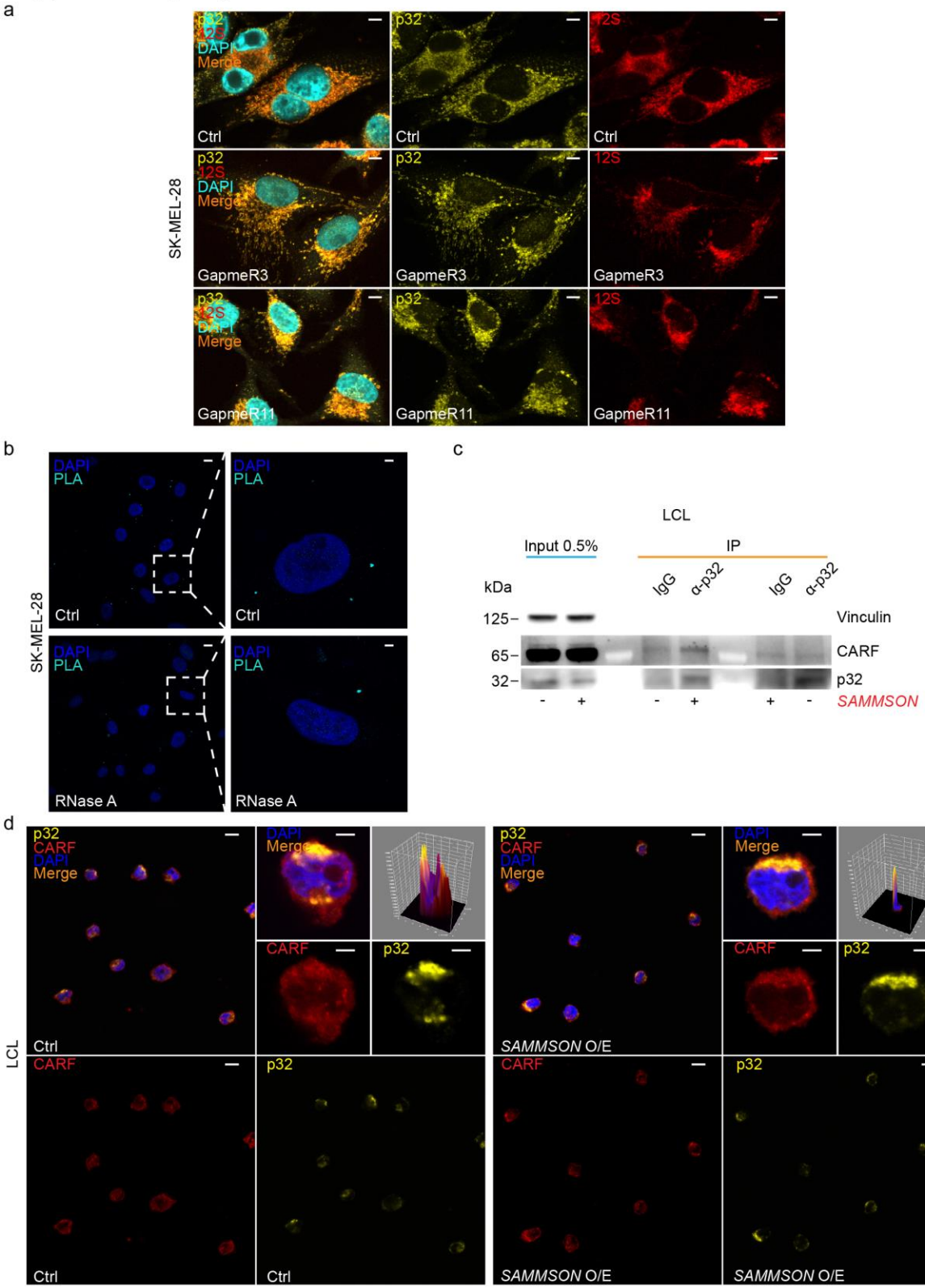

**Supplementary Figure 4**

**XRN2 functions are specifically affected by *SAMMSON* knockdown.**

**(a)** Left, schematic representation of the pre-rRNAs and the mature rRNAs detected by northern blotting, the orange arrows indicate the sites of pre-rRNA processing inhibition in the 5'-ETS (01, A0, and 1). The probe used is highlighted in grey. ETS: external transcribed spacers; ITS: internal transcribed spacers. The aberrant 34S RNA is produced when cleavage occurs in ITS1 prior to 5'-ETS. *, truncated form of the 34S RNA. Right, northern blot hybridization analysis of pre-rRNA isolated from three melanoma cell lines (with different mutational backgrounds and phenotype) transfected with a non-targeting GapmeR (Ctrl) or GapmeR11 (G11) or of SK-MEL-28 transfected with a XRN2-targeting (siXRN2) or control (siCtrl) siRNA. KD efficiency is shown for both *SAMMSON* KD and XRN2 KD. Representative image of three independent experiments. (**b**) *SAMMSON* (*SAM*), 18S, 16S, 12S, Cyclooxygenase 1 (*COX1*) and

NADH-ubiquinone oxidoreductase chain 1 (*ND1*) relative expression measured by RT-qPCR in SK-MEL-28 cells 30 hours after transfection with a non- targeting GapmeR (Ctrl) or with GapmeR11; n=5. **(c)** Northern blot hybridization analysis of mitochondrial pre-rRNA (mt_pre-rRNA) isolated from SK-MEL-28 cells transfected with a non-targeting GapmeR (Ctrl), GapmeR3 (G3) or a GapmeR11 (G11). Efficiency of *SAMMSON* KD and ratios of mt_pre-rRNA over 28S (the mature 28S is visualised by methylene blue staining of the denaturing agarose gel) rRNA are shown below the gel. Representative image of three independent experiments. **(d)** tRNA61-MetCAT expression levels in SK-MEL-28 cells treated with a non-targeting GapmeR (Ctrl) and GapmeR3; n=3. Box boundaries represent 25th and 75th percentiles; centre line represents the median; whiskers, last data point within ±1.5 interquartile range. *P* values were calculated by paired two-tailed Student's t-test. * $P<0.05$; ** $P<0.01$; *NS*, not significant. Uncropped gel images are shown in Supplementary Data Set 1.

# Supplementary Figure 5

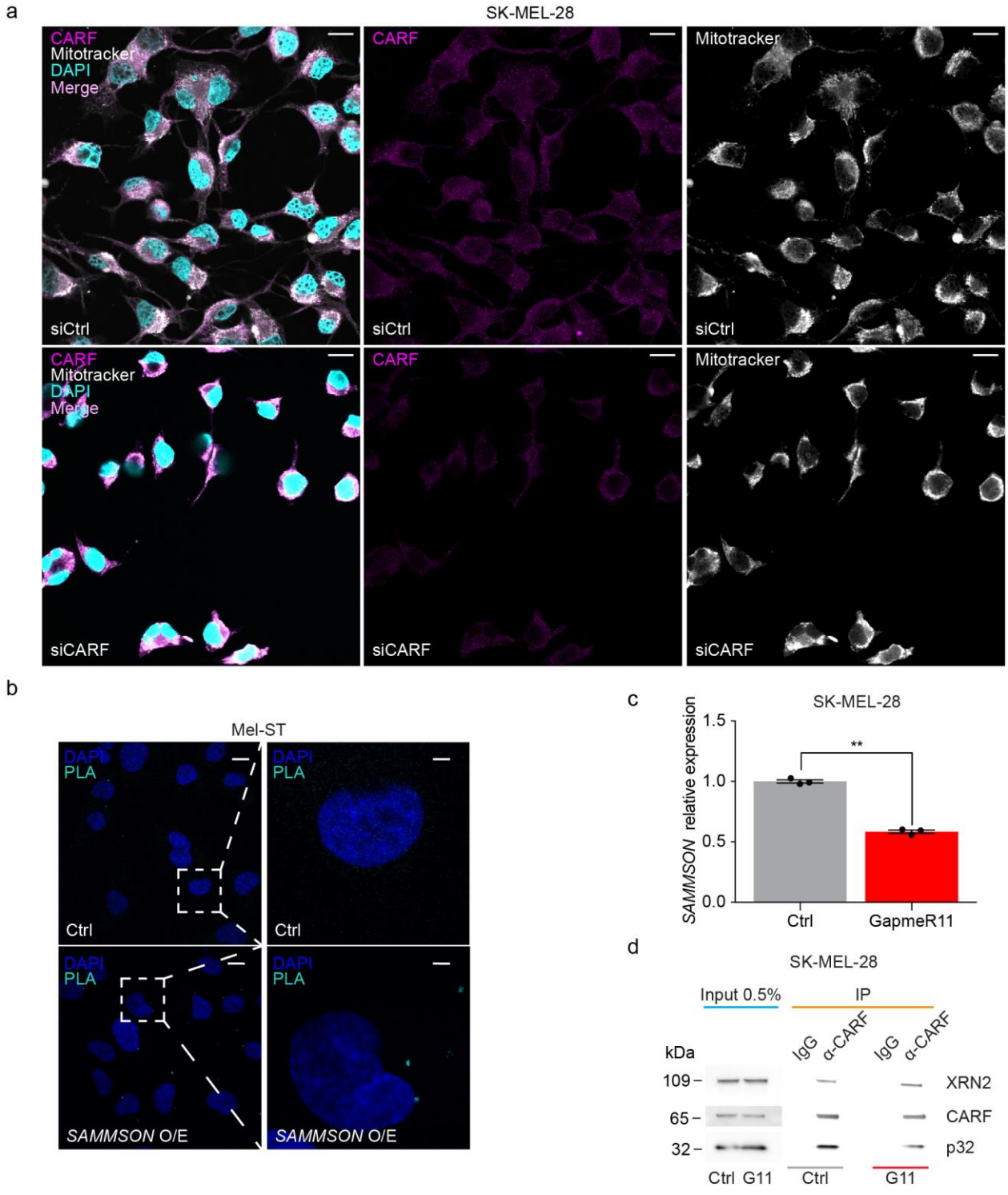


**Supplementary Figure 5**

**CARF localization and its interaction with p32 are RNA and *SAMMSON* dependent.**

(**a**) p32 (yellow) IF combined with 12S (red) RNA fluorescence *in situ* hybridization (FISH) in SK-MEL-28 cells 30 hours after transfection with a non-targeting GapmeR (Ctrl), GapmeR3 or GapmeR11. Cell nuclei are stained with DAPI (cyan). Scale bar, 4 μm. Representative image of three independent experiments. **(b)** Proximity Ligation Assay (PLA, cyan) using antibodies against CARF and p32 in SK-MEL-28 cells in normal conditions (Ctrl) or after addition of RNase A. Cell nuclei are stained with DAPI (blue). Scale bar low magnification, 10 μm; high magnification, 2 μm. Representative image of three independent experiments. (**c**) p32 RIP in LCL cells infected with an empty (-) or a *SAMMSON*-encoding (+) expression vector and western blotting. Representative image of three independent experiments. (**d**) CARF (red) and p32 (yellow) IF in LCL cells as described in **c**. Cell nuclei are stained with DAPI (blue). Scale bar low magnification, 10 μm, Scale bar high magnification, 2 μm. Representative image of three independent experiments. Uncropped gel images are shown in Supplementary Data Set 1.

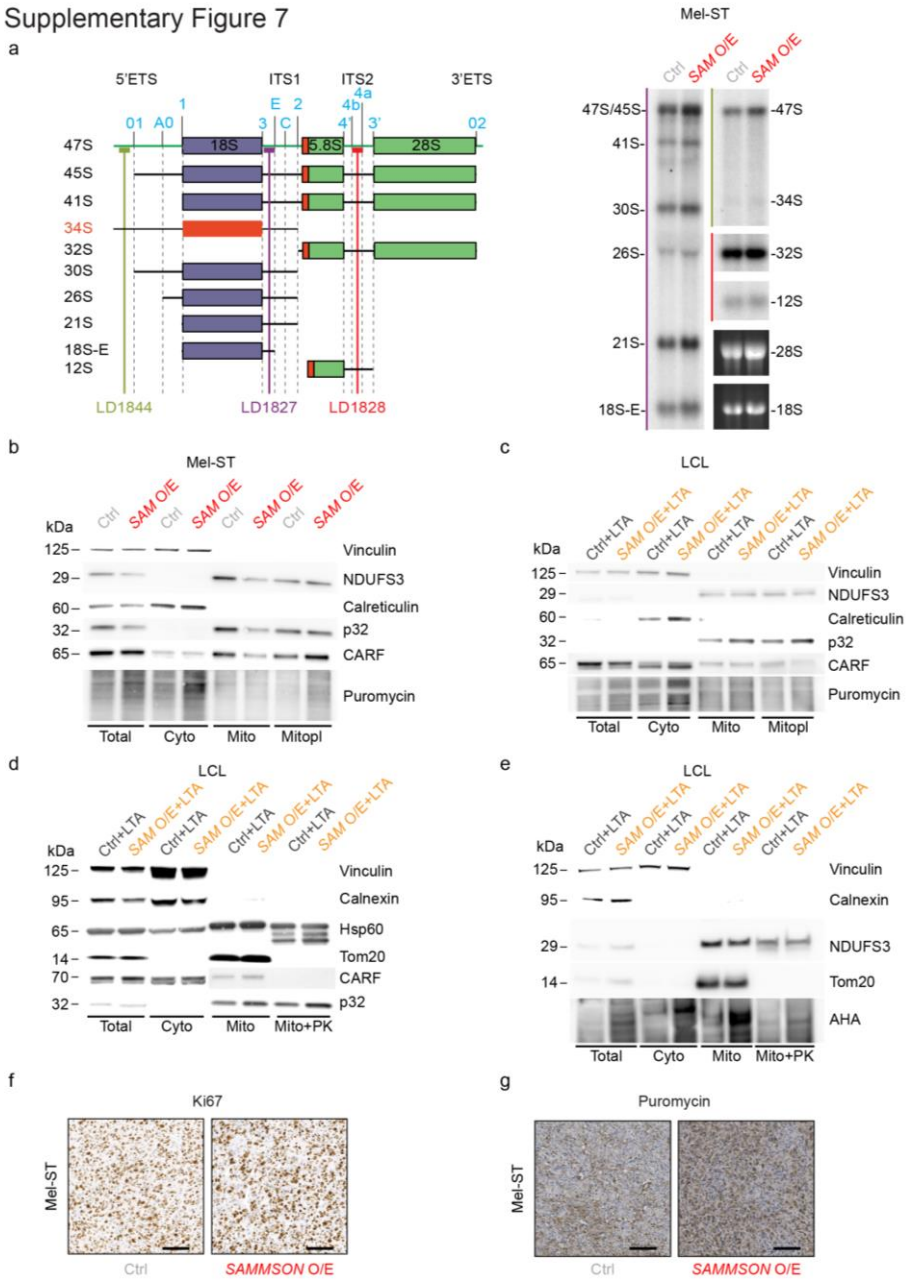
Supplementary Figure 6




**Supplementary Figure 6**

***SAMMSON* regulates the interaction between XRN2, CARF and p32.**

(**a**) CARF (magenta) and mitotracker (white) IF in SK-MEL-28 cells 72 hours after transfection with a control siRNA pool (siCtrl) or an siRNA pool targeting CARF (siCARF). Cell nuclei are stained with DAPI (cyan). Scale bar, 10 μm. Representative image of three independent experiments. (**b**) PLA (cyan) assay using antibodies against CARF and p32 in Mel-ST cells described in **Supplementary Figure 1a**. Cell nuclei are stained with DAPI (blue). Scale bar low magnification, 10 μm; high magnification, 2 μm. (**c**) *SAMMSON* relative expression measured by RT-qPCR in SK-MEL-28 cells 30 hours after transfection with a non-targeting GapmeR (Ctrl) or GapmeR11 (G11). Error bars represent mean ± s.e.m.; n=3. (**d**) CARF IP in SK-MEL-28 cells treated as described in **c** and western blotting. Representative image of three independent experiments. *P* values were calculated by paired two-tailed Student's t-test. ** *P*<0.01. Uncropped gel images are shown in Supplementary Data Set 1.




**Supplementary Figure 7**

***SAMMSON* regulates rRNA biogenesis and protein synthesis.**

(**a**) Pre-rRNA processing analysis in Mel-ST cells infected with an empty (Ctrl) or a *SAMMSON*-encoding (*SAM* O/E) expression vector. Left, structure of the pre-rRNAs detected and probes used. The aberrant 34S RNA observed after *SAMMSON* depletion (see **Figure 5b**) is highlighted in red. Right, northern blot hybridizations. The mature rRNAs are visualized by ethidium-bromide staining of the denaturing agarose gel. Representative image of three independent experiments. (**b**) Western blotting after a 10-minute pulse with puromycin and subsequent cytosol(Cyto)/mitochondria (Mito)/mitoplast(Mitopl) fractionation in Mel-ST cells infected with an empty (Ctrl) or a *SAMMSON*-encoding (*SAM* O/E). Representative image of four (Total and Cyto) and three (Mito and Mitopl) independent experiments. (**c**) Western blotting after a 10-minute pulse with puromycin and subsequent cytosol(Cyto)/mito-

chondria(Mito)/mitoplast(Mitopl) fractionation in LCL cells described in **a**. Representative image of five independent experiments. **(d)** Western blotting after cytosol(Cyto)/mito- chondria(Mito)/proteinase K-treated mitochondria(Mito+PK) fractionation in LCL cells described in **Figure 1a**. Representative image of four independent experiments. **(e)** Western blotting after a 3-hours pulse with AHA, followed by cytosol(Cyto)/mito- chondria(Mito)/proteinase K-treated mitochondria(Mito+PK) fractionation and subsequent Click-iT alkyne reaction in LCL cells described in **Figure 1a**. (**f**) IHC Ki67 staining of xenograft tumors as described in **Supplementary Figure 1e-g**. Scale bar, 100 μm. Representative image of eight independent experiments. (**g**) IHC puromycin staining of tumors as described in **Supplementary Figure 1e-g**. Scale bar, 100 μm. Representative image of eight independent experiments. Uncropped gel images are shown in Supplementary Data Set 1.

| Primers | Sequence (5' to 3') | Note |
|---|---|---|
| *SAMMSON* forward | CCTCTAGATGTGTAAGGGTAGT | qPCR primer |
| *SAMMSON* reverse | TTGAGTTGCATAGTTGAGGAA | qPCR primer |
| *TERRA* forward | CCCTAACCCTAACCCTAACCCTAACCCTA | qPCR primer |
| *TERRA* reverse | GAATCCACGGAATGCTTTGTGTACTT | qPCR primer |
| *MALAT1* forward | GGATTCCAGGAAGGAGCGAG | qPCR primer |
| *MALAT1* reverse | ATTGCCGACCTCACGGATTT | qPCR primer |
| *LINC00698* forward | CTGGCAATTGGGACATCTAT | qPCR primer |
| *LINC00698* reverse | GGCTTCTTTGTCAGCTTCTA | qPCR primer |
| *p32* forward | ACACCGACGGAGACAAAG | qPCR primer |
| *p32* reverse | GGGATGCTGTTGTTAATGTTG | qPCR primer |
| *COX1* forward | CTGCTATAGTGGAGGCCGGA | qPCR primer |
| *COX1* reverse | GGGTGGGAGTAGTTCCCTGC | qPCR primer |
| *ND1* forward | CGAGCAGTAGCCCAAACAAT | qPCR primer |
| *ND1* reverse | CGGTTGGTCTCTGCTAGTGT | qPCR primer |
| *18S* forward | TTCGGAACTGAGGCCATG | qPCR primer |
| *18S* reverse | TTTCGCTCTGGTCCGTCT | qPCR primer |
| *16S* forward | CTCGATGTTGGATCAGGACA | qPCR primer |
| *16S* reverse | CCTGGACTCCGGTCTGA | qPCR primer |
| *12S* forward | ACTGCTCGCCAGAACACTAC | qPCR primer |
| *12S* reverse | GGTGAGGTTGATCGGGGTTT | qPCR primer |
| *HPRT* forward | AGCCAGACTTTGTTGGATTTG | qPCR primer |
| *HPRT* reverse | TTTACTGGCGATGTCAATAAG | qPCR primer |
| *TBP* forward | CGGCTGTTTAACTTCGCTTC | qPCR primer |
| *TBP* reverse | CACACGCCAAGAAACAGTGA | qPCR primer |
| *UBC* forward | ATTTGGGTCGCGGTTCTTG | qPCR primer |
| *UBC* reverse | TGCCTTGACATTCTCGATGGT | qPCR primer |
| HIW885-F-BamHI-CMV-*SAMMSON*-BGH | GAAGGATCCCTGAAGTCGCTAGACATTTGAG | For SAMMSON sub-cloning, Deletion Construction |
| HIW886-R-XhoI-CMV-*SAMMSON*-BGH | CAACTCGAGTTTGTTGGTTTGGTTTTTTTTGAGACG | For SAMMSON sub-cloning, Deletion Construction |
| HIW890-*SAMMSON*-Seq1 | AGAGGTGTGGCTAGATCCAAC | For SAMMSON sequencing |
| HIW891-*SAMMSON*-Seq2 | CAAAACCATTACCTTTAGCCAAG | For SAMMSON sequencing |
| HIW897-XhoI-RAT-For | GGACTCGAGTAAGGAGTTTATATGGAAACC | For SAMMSON 3'RAT construction |
| HIW898-RAT-ApaI-Rev | CAAGGGCCCGGCACGAGTGTAGCTAAACCTC | For SAMMSON 3'RAT construction |
| HIW899-R-XhoI-*SAMMSON*-del1 | CAACTCGAGTTACTCCATTGGAAGGCAGATTATG | For Deletion Construction |
| HIW900-F-BamHI-*SAMMSON*-del2 | GAAGGATCCGAATGTCTGGACTCTTTCCTTCAC | For Deletion Construction |
| HIW901-F-BamHI-*SAMMSON*-del3 | GAAGGATCCGTGTATGATATTGCATGAGTTGTC | For Deletion Construction |

| LNAs | Sequence (5' to 3') | |
|---|---|---|
| GapmeR3 | GTGTGAACTTGGCT | |
| GapmeR11 | TTTGAGAGTTGGAGGA | |
| Non-targeting GapmeR | TCATACTATATGACAG | |

| siRNAs | Sequence (5' to 3') | |
|---|---|---|
| siXRN2#1 sense | CAUCGUUAGAGAUUAGGGA | |
| siXRN2#1 antisense | UCCCUAAUCUCUAACGAUG | |
| siXRN2#2 sense | GAGUACAGAUGAUCAUGUU | |
| siXRN2#2 antisense | AACAUGAUCAUCUGUACUC | |
| siCtrl sense | UGGUUUACAUGUCGACUAATT | |
| siCtrl antisense | CGUACGCGGAAUACUUCGATT | |
| siCtrl_northern blot | Scramble sequence, undisclosed | |
| siXRN2_northern blot | GGAAAGUUGUGCAGUCGUATT | |
| siNEAT1 | Pool of siRNAs from siTOOLS | |
| siNEAT1_long | Pool of siRNAs from siTOOLS | |
| siCARF | Pool of siRNAs from Dharmacom | |

| Northern blot probes | Sequence (5' to 3') |
|---|---|
| mt_pre-rRNA | GGGTAAATGGTTTGGCTAAGGTTGTCTGGT |
| 5'-ETS (LD1844) | CGGAGGCCCAACCTCTCCGACGACAGGTCGCCAGAGGACAGCGTGTCAGC |
| 5'-ITS1 (LD1827) | CCTCGCCCTCCGGGCTCCGTTAATGATC |
| ITS-2 (LD1828) | CTGCGAGGGAACCCCCAGCCGCGCA |
| RAT Tag | [Biotin]ACGTCTAAGGGTTTCCATATAAACTCCTT |
| U2-#1 probe (1-28 nt) | GATCTTAGCCAAAAGGCCGAGAAGCGAT[Biotin] |
| *SAMMSON* (909-933 nt) | GTCACAGGTCACTAGGTGTGGGAAG[Biotin] |