**Supplementary Information**

# Ribosome biogenesis during cell cycle arrest fuels EMT in development and disease

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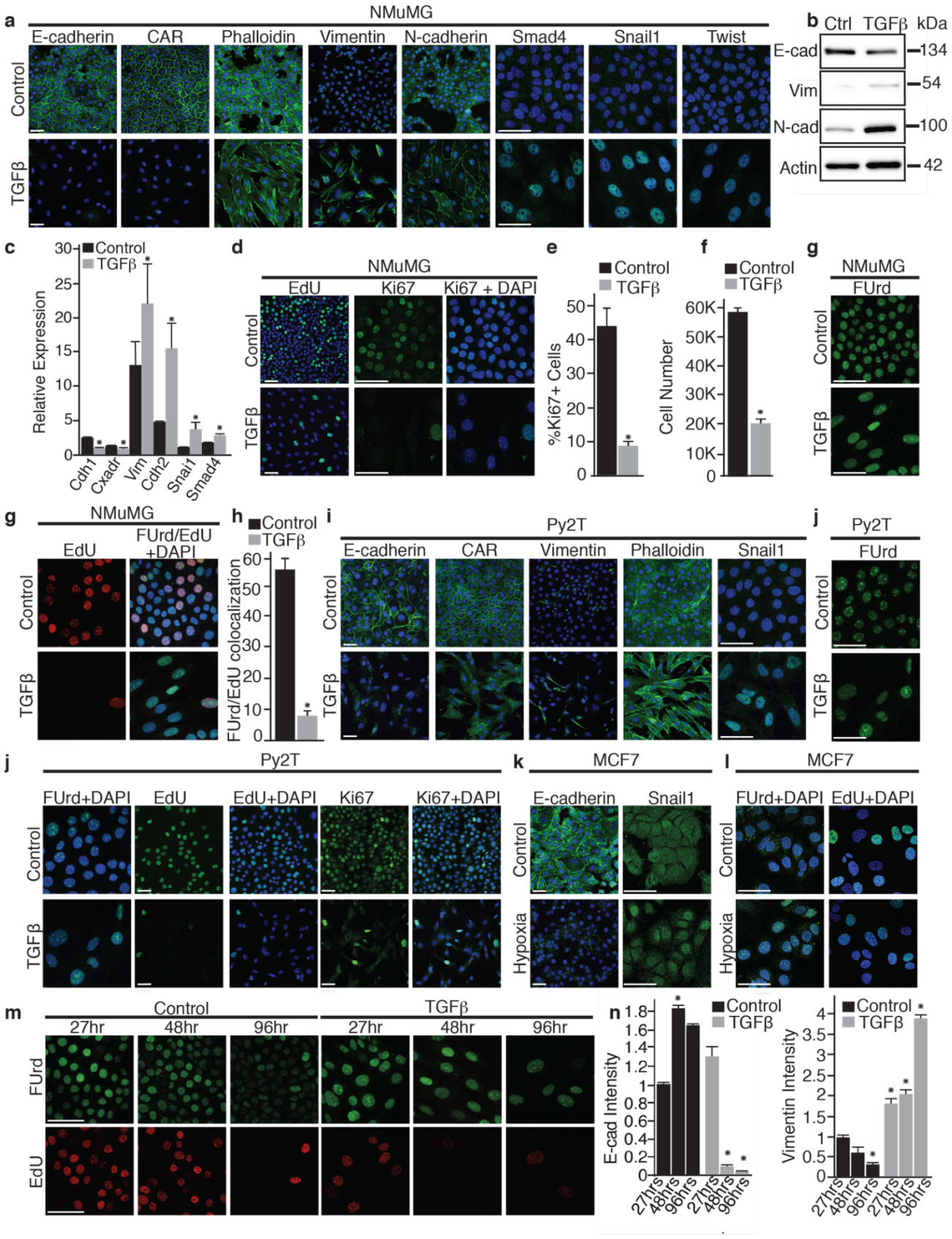
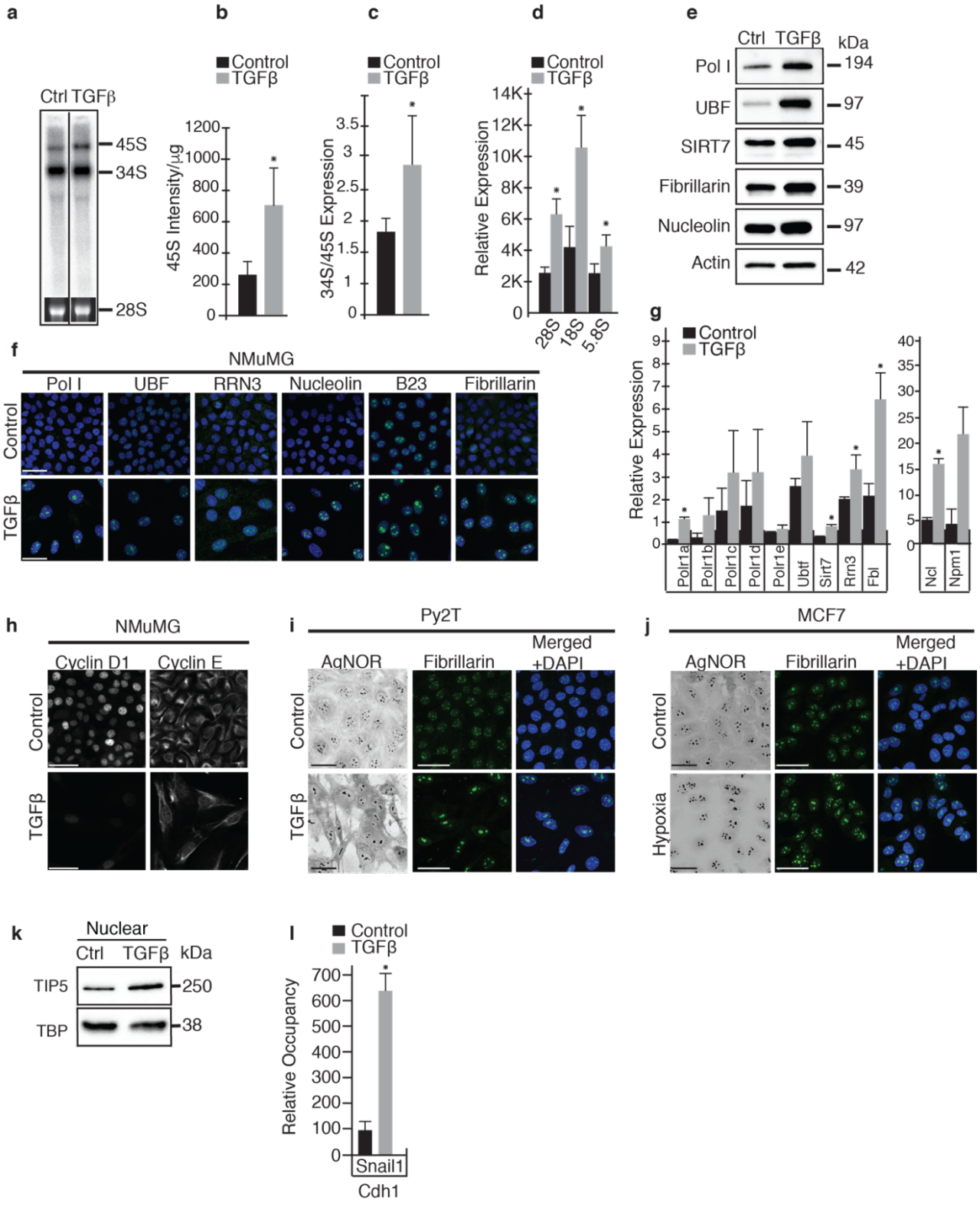


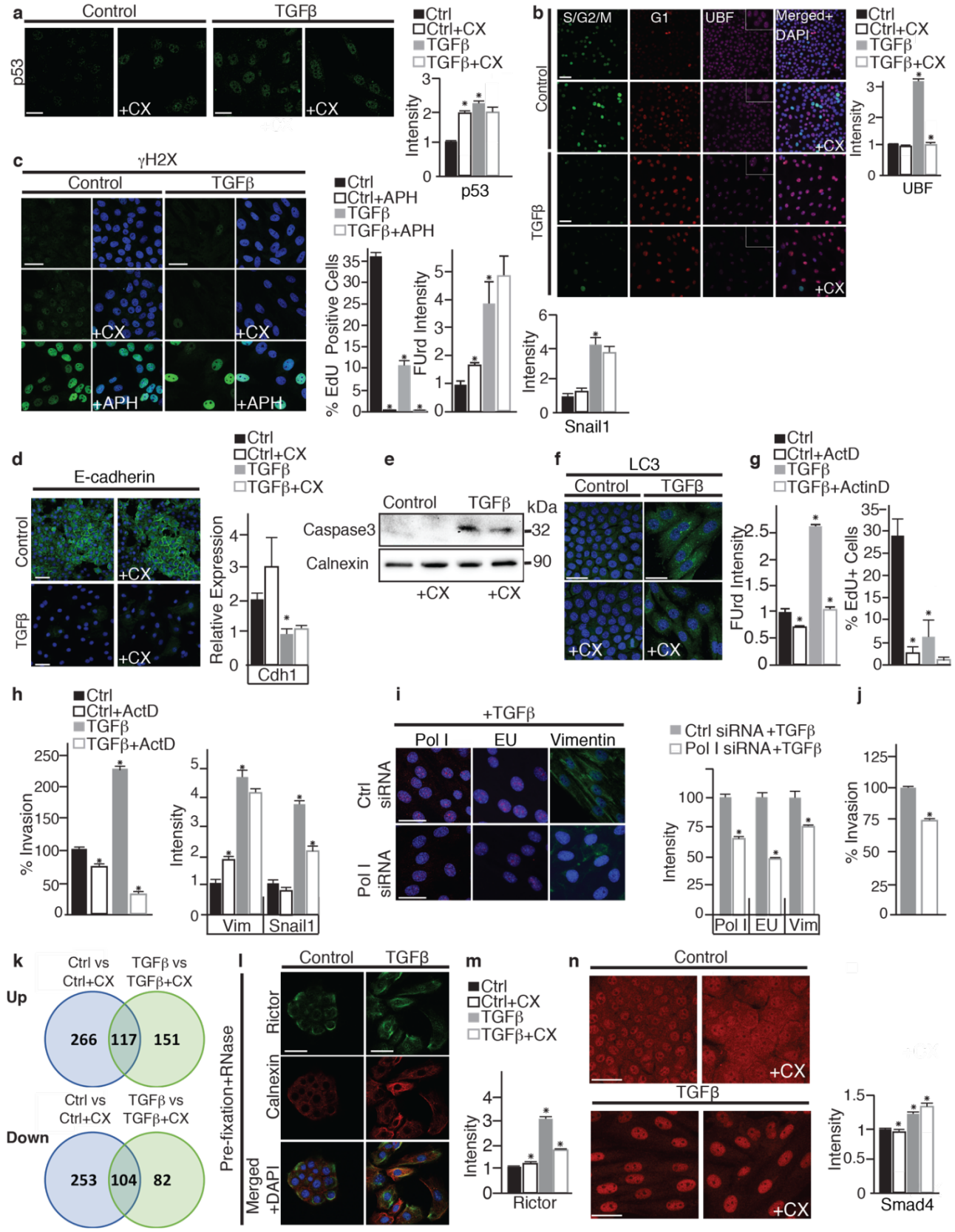

**Supplementary Figure 1. Enhanced rRNA synthesis in EMT is independent of cell proliferation. a**,

Immunostaining of E-cadherin, CAR, Phalloidin, Vimentin, N-cadherin, Smad4, Snail1, and Twist (green) in proliferating (Control) cells and after 48 hours of TGFβ treatment (TGFβ), merged with DAPI (blue). **b**, E-cadherin (E-cad), Vimentin (Vim) and N-cadherin (N-cad) western blot ± TGFβ in NMuMG cells. Actin serves as a loading control. **c**, qRT-PCR of E-cadherin (Cdh1), CAR (Cxadr), Vimentin (Vim), N-cadherin (Cdh2), Snail1 (Snai1) and Smad4 ± TGFβ in NMuMG cells, $P<0.02$. Error bars ± SD n = 3. **d**, Immunostaining of EdU (green) and Ki67 (green) ± TGFβ, NMuMG cells, DAPI (blue). **e**, Quantification of $Ki67^+$ cells, $P<0.001$. Error bars ± SE, n= 3. **f**, Quantification of NMuMG cell number ± 48 hour TGFβ treatment, $P<0.001$. Error bars ± SD, n= 3 **g**, Co-localization of FUrd, (green) and $EdU^+$, (red) ± TGFβ, merged with DAPI (blue). **h**, Quantification of FUrd/EdU co-localization in (**g**), $P<0.001$. Error bars ± SE, n=3. **i**, Immunostaining of E-cadherin, CAR, Vimentin, Phalloidin, and Snail1 (all green) in proliferating (Control) and TGFβ-treated (TGFβ) Py2T cells merged with DAPI (blue). **j**, FUrd, EdU and Ki67 (green) in Py2T, alone and merged with DAPI (blue). **k**, Immunostaining of E-cadherin (green) and Snail1 (green) in MCF7 cells under normoxia (Control) or 48-hour hypoxia (Hypoxia) merged with DAPI (blue). **l**, Immunostaining of rRNA synthesis (FUrd, green) and DNA synthesis (EdU, green) in MCF7 cells under normoxia (Control) or 48-hour hypoxia (Hypoxia) merged with DAPI (blue). **m**, Immunostaining of rRNA synthesis (FUrd, green) and DNA synthesis (EdU, red) over 27, 48, and 96 hours in proliferating (Control) and TGFβ-treated (TGFβ) NMuMG cells. **n**, Quantification of E-cadherin (Ecad) and Vimentin time course images from (**m**) in proliferating (Control) and TGFβ– (TGFβ) NMuMG cells. $P<0.05$, $P<0.02$. Asterisk denotes t-test significance. Error bars ± SE, n=3. Scale bars = 50 μm.




**Supplementary Figure 2. Characteristic features of the EMT-associated ribosome biogenesis program. a**, Representative Northern blot of NMuMG cells ± TGFβ. **b**, Quantification of Northern blot

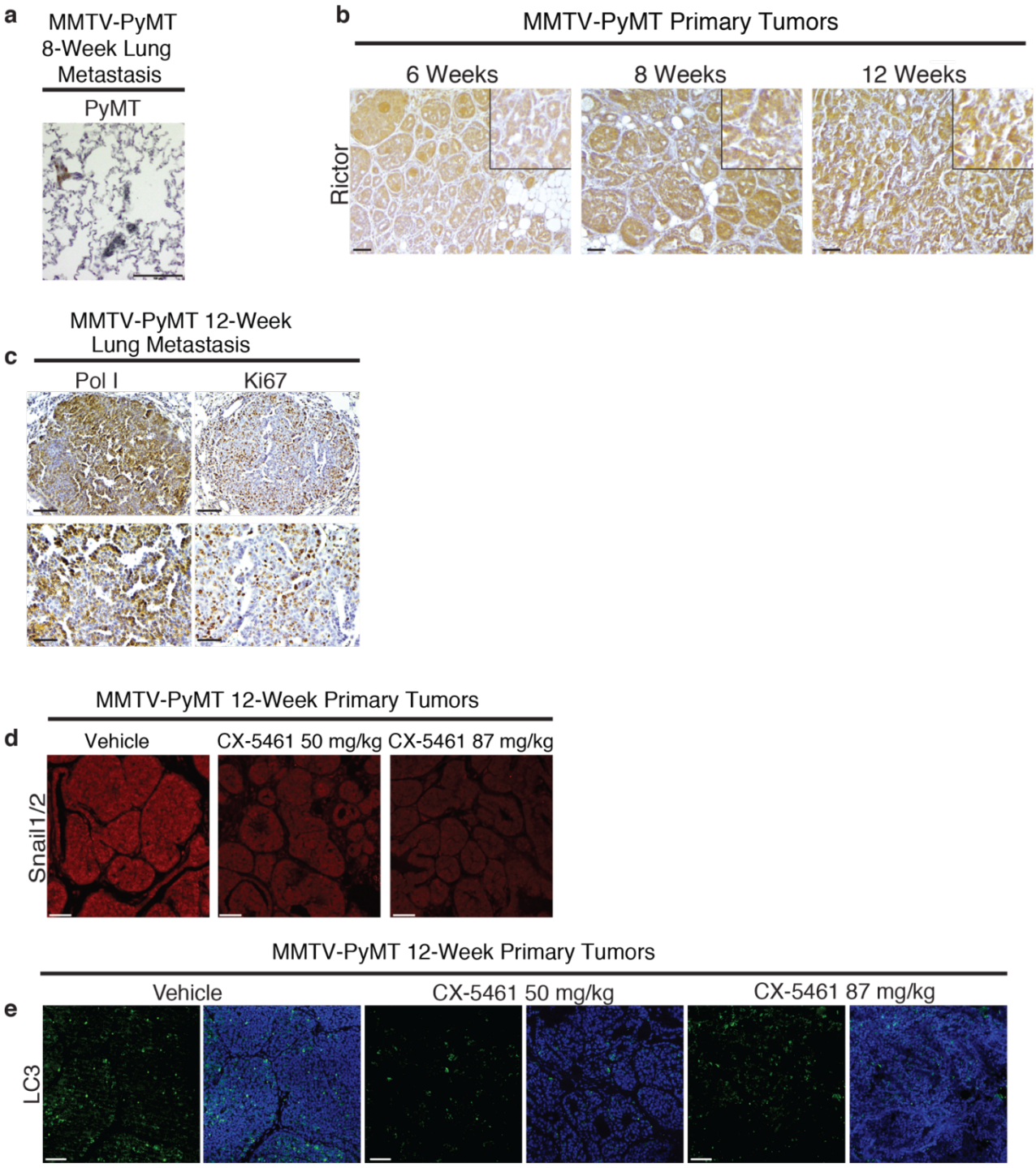
analysis of 45S expression in NMuMG cells ± TGFβ. P<0.03. **c**, Quantification of Northern blot analysis of 34S/45S expression in NMuMG cells ± TGFβ. P<0.04. **d**, qRT-PCR of 28S, 18S and 5.8S rRNA transcripts. P<0.01. **e**, Western Blot of Pol I, UBF, SIRT7, Fibrillarin and Nucleolin in NMuMG cells ± TGFβ. Actin serves as a loading control. **f**. Pol I, UBF, RRN3, Nucleolin, B23, and Fibrillarin (all green) immunostaining in NMuMG ± TGFβ, merged with DAPI (blue). **g**, qRT-PCR analysis of Pol I subunits (Polr1a-e), UBF (Ubtf), Sirt7, Rrn3, Fibrillarin (Fbl), Nucleolin (Ncl), and B23 (Npm1) ± TGFβ, NMuMG cells, P<0.01 for Polr1a, Sirt7, Rrn3, Fbl, and Ncl. Error bars ± SD, n=3 biological replicates. **h,** Immunostaining of Cyclin D1 and E (white) ± TGFβ, NMuMG cells. **i**, Silver staining of nucleolar organizer regions (NORs) and Fibrillarin expression (green) ± TGFβ, merged with DAPI (blue) in Py2T cells. **j**, Silver staining of nucleolar organizer regions (NORs), immunostaining of Fibrillarin (green) in MCF7 cells ± hypoxia, merged with DAPI (blue). **k,** Western blot of TIP5 nuclear expression levels in NMuMG cells ± TGFβ. TBP serves as a loading control. **l**, Snail1 binding to the E-cadherin (Cdh1) promoter, P<0.0002. Error bars ± SE, n= 3. Asterisk denotes t-test significance. Scale bars = 50 μm.




**Supplementary Figure 3. Inhibition of Pol I initiation at rDNA operons impairs the EMT program.**

All cell culture experiments performed in NMuMG, TGFβ, CX-5461, Actinomycin D (ActD) and

Aphidicolin (APH) labeled in each panel. **a**, p53 expression. Quantification of p53, Control/TGFβ ± CX-5461 treatment, P<0.0001 **b**, Cell cycle analysis using FUCCI system, S/G2/M (geminin, green), G1 (Cdt1, red), UBF (magenta) and DAPI (blue), merged. Quantification of UBF, Control/TGFβ ± CX-5461 treatment, P<0.0001 **c**, γH2X staining (green) with DAPI (blue) ± APH/CX-5461 treatment (blue). Quantification of EdU ± APH treatment, P<0.002. Quantification of FUrd and Snail ± APH treatment, P<0.01. **d**, E-cadherin (green) immunostaining with treatment, merged with DAPI (blue). qRT-PCR of E-cadherin (Cdh1) mRNA expression changes with treatment, P<0.02. **e,** Cleaved Caspase-3 Western Blot. Calnexin serves as a loading control. **f**, LC3 (green) immunostaining merged with DAPI (blue). **g**, Quantification of FUrd and EdU post ActD treatment, P<0.02, P<0.001. **h**, Relative percent invasion from Boyden chamber invasion assay with ActD treatment. P<Control/control+ActD, P<0.003, Control/TGFβ+ActD, P<0.001 Quantification of ActD treated Vimentin and Snail1 (green) immunofluorescence. P<0.03. **i**, Immunostaining of Pol I, EU and Vimentin transfected with Pol I siRNA or Ctrl siRNA in the presence of TGFβ. Quantification of Pol I, EU and Vimentin (Vim) intensity with Pol siRNA or Ctrl siRNA in the presence of TGFβ. P<0.0002. **j**, Relative percent invasion from Boyden chamber invasion assay, with Pol I RNAi or Ctrl siRNA in the presence of TGFβ, P<0.0001. **k**, Venn diagram depicting the overlap of genes upregulated by CX-5461 in proliferating (Control) or TGFβ-treated NMuMG cells. Venn diagram depicting the overlap of genes downregulated by CX-5461 in proliferating (Control) or TGFβ-treated NMuMG cells. **l,** Immunostaining of Rictor (green) and Calnexin (green) ± RNase A (RNase). **m,** Quantification of Rictor in NMuMG cells ± TGFβ± CX-5461 P<0.0001 control compared to (TGFβ and TGFβ compared to (TGFβ+CX-5461). **n,** Immunostaining of Smad4 (green). Quantification of Smad4, Control/TGFβ ± CX-5461 treatment, P<0.0001. For all quantifications: asterisk denotes t-test significance, error bars ± SE, n = 3. Scale bars = 50 μm.




**Supplementary Figure 4. Human breast tumors exhibit high-levels of Pol I expression.** **a**, IHC staining for PyMT lung micro-metastasis, 8 weeks. **b**, IHC staining of Rictor expression in primary tumor for at 6, 8 and 12 weeks. **c**, IHC staining of 12-week lung metastasis for Pol I and Ki67 expression. **d**, Immunostaining of Snail1/2 (red) in vehicle, 50 mg/kg, or 87 mg/kg CX-5461-treated tumor. **e**, Immunostaining of LC3 (green) in vehicle, 50 mg/kg or 87 mg/kg CX-5461 treated tumors, DAPI (blue).

**Supplementary Table 1**
**Antibody List**

| Antibody | Order information | Fixation Solution/Time Immunoflourescence | IF/IHC Dilution | Western Blot Dilution | ChIP Dilution |
|---|---|---|---|---|---|
| B23 | Santa Cruz SC-271737 | 4% Paraformaldehyde, 15 minutes | 1:25 | | |
| Beta Actin | Abcam ab1801 | | | 1:1000 | |
| BrdU 405 | Novusbio NBP2-34784AF405 | | 1:1000 | | |
| BrdU | Sigma Aldrich B2531 Clone BU33 | 4% Paraformaldehyde, 15 minutes | 1:1000 | | |
| Calnexin | Abcam ab75801 | | | 1:1000 | |
| Calnexin | Abcam ab22595 | 4% Paraformaldehyde, 15 minutes | 1:150 | | |
| CAR1 | gift of Jonas Fuxe Lab | Ethanol, 10 minutes | 1:1000 | | |
| Cleaved Caspase 3 | Cell Signalling 9661 | | | 1:1000 | |
| Cytokeratin 8+18 | Abcam ab53280 | | 1:100 | | |
| E-cadherin | BD Transduction Labs 610182 | Ethanol, 10 minutes | 1:100 | 1:300 | |
| Estrogen Receptor alpha | Abcam ab32063 | | 1:250 | | |
| Fibrillarin | Abcam ab5821 | 4% Paraformaldehyde, 15 minutes | 1:1000 | 1:1000 | |
| H3K4Me3 | Abcam ab8580 | | | | 1:40 |
| H3k27AC | Abcam ab4729 | | | | 1:40 |
| IgG for ChIP | Abcam ab46540 | | | | 1:400 |
| Ki67 | Thermo Scientific RM-9106S1 | 4% Paraformaldehyde, 15 minutes | 1:1000 | | |
| LC3 | MBL International | 4% Paraformaldehyde, 15 minutes | 1:1000 | 1:1000 | |
| N-cadherin | Abcam ab18203 | Methanol, 20 seconds | 1:1000 | | |
| N-cadherin | Abcam ab12221 | | | 1:200 | |
| Nucleolin | Abcam ab22758 | 4% Paraformaldehyde, 15 minutes | 1:200 | 1:300 | |
| Phalloidin | Life technologies A22287 | 4% Paraformaldehyde, 15 minutes | 1:40 | | |
| Pol 1_serum | gift of Piergiorgio Percipalle Lab | | | 1:300 | 1:500 |
| Polr1e | Atlas Antibodies HPA022527 | | IHC 1:500 | | |
| p53 | Santa Cruz SC-126 | 4% Paraformaldehyde, 15 minutes | 1:50 | | |
| RICTOR antibody [7B3] | Abcam ab104838 | 4% Paraformaldehyde, 15 minutes | 1:150 | | |
| RPA194 | Abcam ab48385 | 4% Paraformaldehyde, 15 minutes | 1:25 | | |
| RRN3 | Abcam ab169511 | 4% Paraformaldehyde, 15 minutes | 1:25 | | |
| Sirt7 | Abcam ab62748 | | | 1:200 | 1:40 |
| Smad 4 | Santa Cruz B-8 SC-81417 | 4% Paraformaldehyde, 15 minutes | 1:25 | 1:50 | |
| Snail 1 | gift from Garcia de Herrero lab | 4% Paraformaldehyde, 15 minutes | undilluted | | |
| Snail1/2 | Abcam ab85936 | 4% Paraformaldehyde, chick 1hr | 1:100 | | |
| SNAI 1 (H-130) | Santa Cruz SC-28199 | | | | 1:27 |
| Sox10 | Novusbio AF2864 | | 1:500 | | |
| TBP | Abcam ab51841 | | | 1:1000 | |
| TIP5 | Life technologies 49-1037 | | | 1:1000 | 1:25 |
| TIP5 | Diagenode C15310090 | | | 1:1000 | |
| UBF | Santa Cruz F-9 13125 | 4% Paraformaldehyde, 15 minutes | 1:100 | 1:300 | |
| UBF | Santa Cruz H-300, 9131 | | | | 1:40 |
| Vimentin | Abcam ab45939 | Methanol, 20 seconds | 1:1000 | 1:200 | |

**Supplementary Table 2**
**Primer List**

| RT-PCR | | | |
|---|---|---|---|
| **protein** | **gene** | **Forward** | **Reverse** |
| **Car** | Cxadr | ccctggggttgcaaataag | gatccatccacgaagcatct |
| **E-cadherin** | Cdh1 | atcctcgccctgctgatt | accaccgttctcctccgta |
| **Fibrillarin** | Fbl | aggaggatgcccttgtcac | ggttccaggctctgtactcaa |
| **Gapdh** | Gapdh | gtatgactccactcacggcaaa | ggtctcgctcctggaagatg |
| **N-cadherin** | Cdh2 | gccatcatcgctatccttct | ccgtttcatccataccacaaa |
| **Nucleolin** | Ncl | catggtgaagctcgcaaag | tcactatcctcttccacctcctt |
| **Nucleophosmin** | Npm1 | gaaaaaggcggttctcttcc | tttcctccactgccagagat |
| **Vimentin** | Vim | tgcgccagcagtatgaaa | gcctcagagaggtcagcaaa |
| **PolI A** | Polr1a | cgttcatcgaggactaccagt | tcttcatcagtgggagcttca |
| **PolI B** | Polr1b | atggcgacccttactacagc | gttgtccacaacacagttttcttta |
| **PolIC** | Polr1c | cggtaactacgccggttatg | atttgcaccacgtccacac |
| **PolID** | Polr1d | agcgccacctgaggattt | ttgaggtcttcaatcctgatatttt |
| **PolIE** | Polr1e | gaaaggtcacatcggaagaca | gggcagagacttcagcattt |
| **Rrn3** | rrn3 | gcctcctgccatgtacagtt | caaaaatgcttctgcaaatcc |
| **Sirt7** | Sirt7 | tgtcatgcaactcctcatgaat | ggtcgccaaggagaagatt |
| **Smad4** | Smad4 | gagaacattggatggacgact | cacagacgggcatagatcac |
| **Snail** | Snai1 | cttgtgtctgcacgacctgt | caggagaatggcttctcacc |
| **Ubf** | Ubtf | tcactagcacccacttgctc | gcttctccgttcattctcca |
| **Rictor** | Rictor | tgcgatattggccatagtga | acccggctgctcttacttct |
| | **rRNA** | **Forward** | **Reverse** |
| | **45S rRNA (pre-rRNA)** | gcttgtttctcccgattgc | cgcgaacaactgagaaaagt |
| | **28S rRNA** | agtcgggttgcttgggaatgc | cccttacggtacttgttgact |
| | **18S rRNA** | cggctaccacatccaagg | tacagggcctcgaaagagtc |
| | **5.8S rRNA** | actcttagcggtggatcactc | aagcgacgctcagacagg |
| **Mouse Metastasis Studies** | | **Forward** | **Reverse** |
| | **mCherry** | ctcccacaacgacgactaca | cttgtacagctcgtccatgc |
| | **Beta-Actin** | tcctggcctcactgtccac | gcaagtgcttctaggcggac |
| **ChIP** | | **Forward** | **Reverse** |
| | **rDNA promoter** | gacctgtcggtcttatcagttc | ccggacctcaaaggaacaac |
| | **18S** | cggctaccacatccaagg | tacagggcctcgaaagagtc |
| | **28S** | agtcgggttgcttgggaatgc | cccttacggtacttgttgact |
| | **E-cadherin promoter** | agacaggggtggaggaagtt | accagtgagcagcgcagag |