**Table ST1. List of oligonucleotides and siRNAs used in this study.**

| Purpose | Sequence (5'-3') |
|---|---|
| OTUD6B-1 cloning | Fwd-GGGGCTAGCATGGAGCCCCGGGTGAG<br>Rev-GGGGCTAGCTTAGCTGCAATTTTGAC<br>Rev2-<br>CCCCTCGAGTTAATGGTGGTGGTGATGATGTCCAGCGTAATCTGGAA<br>CATCGTATGGGTATCCTGGTCCTGGGCTGCAATTTTCAGTAAC |
| OTUD6B-2 cloning | Fwd-GGGGCTAGCATGATATCTAAGGAAAAG<br>Rev-GGGGCTAGCTTAGCTGCAATTTTCAGTA |
| OTUD6B-3 cloning | Fwd-GGGGCTAGCATGGAGGCGGTATTGAC<br>Rev-GGGGCTAGCATGGAGGCGGTATTGAC |
| OTUD6B-1 qPCR | Fwd-AAGAGACGGGAAAAGAAAGC<br>Rev-ATTAGGGTTTGTTAAAAATGGCAGA |
| OTUD6B-2 qPCR | Fwd-TGAGGGGTTTTGGATTAGATG<br>Rev-AATGGCAGAAAGTCTTCCACA |
| OTUD6B-3 qPCR | Fwd-ATTGTAAACACAGCTGCATGG<br>Rev-TAAGAAACAACCCCAACTGG |
| b-Tubulin qPCR | Fwd-ACCTTCAGTGTGGTGCCTTC<br>Rev-GTGGCTGAGACAAGGTGGTT |
| b-Actin qPCR | Fwd-TCCCTGGAGAAGAGCTACGA<br>Rev-AGCACTGTGTTGGCGTACAG |
| RPL13 qPCR | Fwd-CATAGGAAGCTGGGAGCAAG<br>Rev-ACAAGATAGGGCCCTCCAAT |
| c-Myc qPCR | Fwd-CTCCTGGCAAAAGGTCAGAG<br>Rev-TCGGTTGTTGCTGATCTGTC |
| Cyclin-D1 qPCR | Fwd-GGGGGCGTAGCATCATAGTA<br>Rev-TGTGAGCTGGCTTCATTGAG |
| OTUD6A qPCR | Fwd-TTCTTCAGCAACCCCGAGAC<br>Rev-CATAGCGCAGGTAGACCAGG<br>Fwd-AGAGTGAACAGCAGCGCATA<br>Rev- TTCGTCTTGTCGGTCTTGGG |
| OTUD6B-2 exon skipping oligos | mAmUmCmCmAmGmGmCmAmCmCmUmGmAmAmUmCmAmGmA<br>mGmAmAmUmAmAmGmCmAmCmCmUmUmAmGmAmUmAmUmC |
| Inactive siRNA to OTUD6B-2 | GGAAAUCUUUCUGCGUGCUGUUUCC |
| Effective siRNA to OTUD6B-2 | GUUUUGGAUUAGAUGAUAUCUAAGG<br>GGGGUUUUGGAUUAGAUGAUAUCUA |
| SI04185034 | Hs_OTUD6B_3 FlexiTube siRNA (Qiagen); sequence (top strand):<br>AAGGAGCGAGAAGAACGGATA |
| SI04233915 | Hs_OTUD6B_4 FlexiTube siRNA; sequence (top strand):<br>CAGACCGCTGAGTATATGCAA |
| SI02809590 | Hs_YOD1_4 FlexiTube siRNA |
| SI04303754 | Hs_OTUD5_3 FlexiTube siRNA |

**Table ST2. List of antibodies used in this study.**

| Antibody | Specificity | Company | Catalog# |
|---|---|---|---|
| Rabbit polyclonal | Human, Mouse, Rat, **OTUD6B** | Novus Biologicals | NBP1-85652 |
| Rabbit IgG | Human, Mouse, Rat, **4E-BP1** | Cell Signaling | 9644 |
| Rabbit IgG | Human, Mouse, **phospho-Akt (S473)** | Cell Signaling | 3787 |
| Rabbit IgG | Human, Mouse, Rat, **Akt** | Cell Signaling | 4691S |
| Mouse IgG1 | Human, Mouse, Rat, **ribosomal protein S6** | Cell Signaling | 2317 |
| Rabbit IgG | Human, Mouse, Rat, **eIF4A** | Cell Signaling | 2013 |
| Mouse IgG2b | Human, **eIF4B** | Cell Signaling | 13088 |
| Rabbit IgG | Human, Mouse, Rat, **eIF4E** | Cell Signaling | 2067 |
| Rabbit IgG | Human, Mouse, Rat, **eIF4G** | Cell Signaling | 8701 |
| Mouse IgG2b | Human **eIF4G** | Santa Cruz Biotech. | Sc-133155 |
| Mouse IgG1 | All, **Ubiquitin** | Cell Signaling | 3936 |
| Rabbit polyclonal | Human, Mouse, Rat, **p70 S6 Kinase** | Cell Signaling | 9202 |
| Rabbit polyclonal | Human, Mouse, Rat, **Phospho-p70 S6 Kinase (Thr389)** | Cell Signaling | 9205 |
| Mouse IgG1 | Human, Mouse, **c-Myc** | ED-Millipore | OP31 |
| Rabbit IgG | Human, Mouse, Rat, **cyclin-D1** | Cell Signaling | 2978 |
| Mouse monoclonal IgG1 | Human, glyceraldheyde-3-phosphate dehydrogenase | Millipore | MAB374 |
| Rabbit polyclonal | Anti-DDDDK tag | Abcam | ab1162 |
| Rabbit polyclonal | Simian Virus 40 VP-1 antigen (Irrelevant IgG) | Abcam | ab53977 |
| Goat polyclonal, HRP conjugated | Rabbit IgG (H+L) | ThermoFisher Scientific | 31460 |
| Goat polyclonal, HRP conjugated | Mouse IgG (H+L) | ThermoFisher Scientific | 31430 |

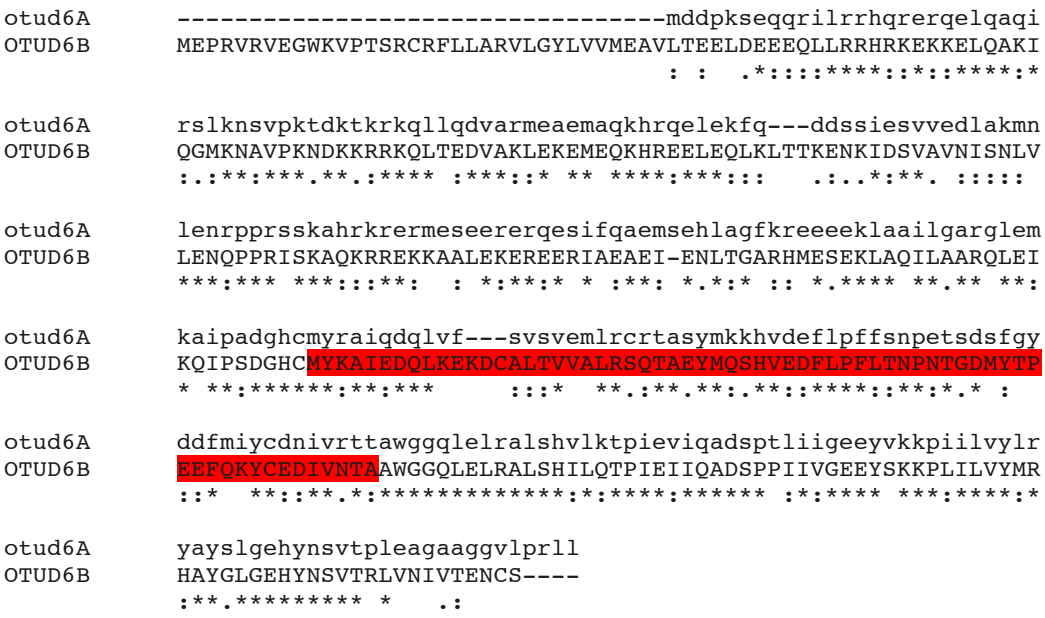



**Figure S1**
Sequence alignment of OTUD6A and OTUD6B (isoform 1) performed using Clustal omega software (available at http://www.ebi.ac.uk/Tools/msa/clustalo/). The sequence highlighted in red corresponds to the protein sequence used to raise the anti-OTUD6B antibody.

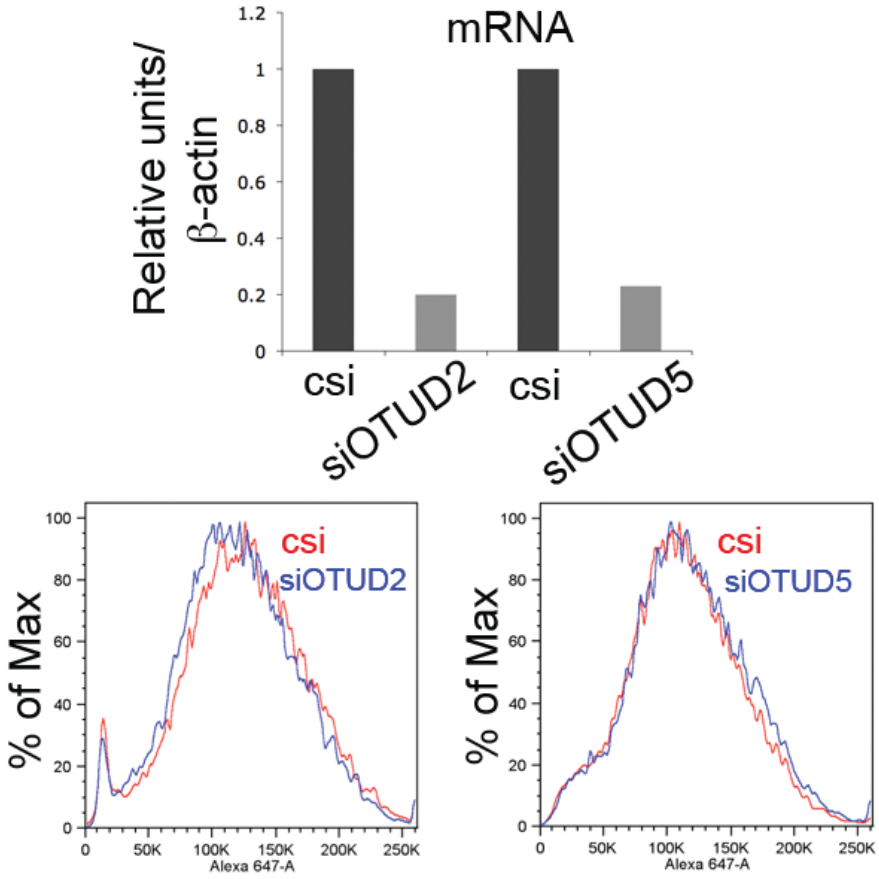



**Figure S2.**
RNA silencing to OTUD2 and OTUD5 in NSCLC cells does not affect the protein synthesis rate.

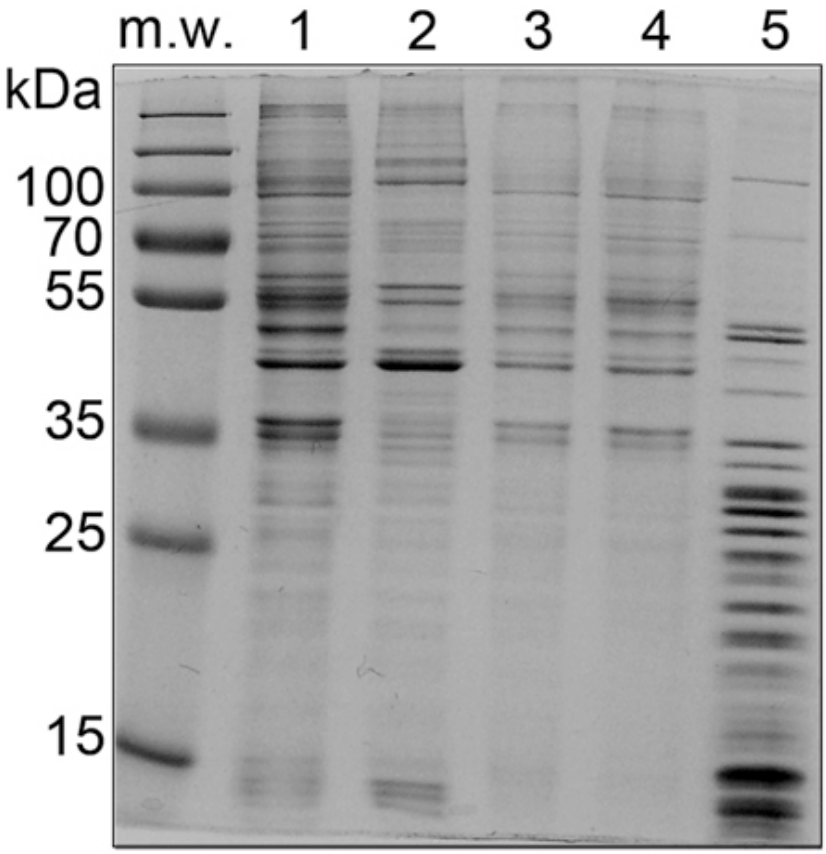



**Figure S3.**

Comassie blue staining of a representative SDS PAGE gel loaded with 40 µg of proteins obtained from total cell lysate (1), nuclear fraction (2), postmitochondrial fraction (3), postribosomal fraction (4) and ribosomes (5). Note the typical enrichment in low molecular weigh bands (ribosomal proteins) of the ribosomal fraction.

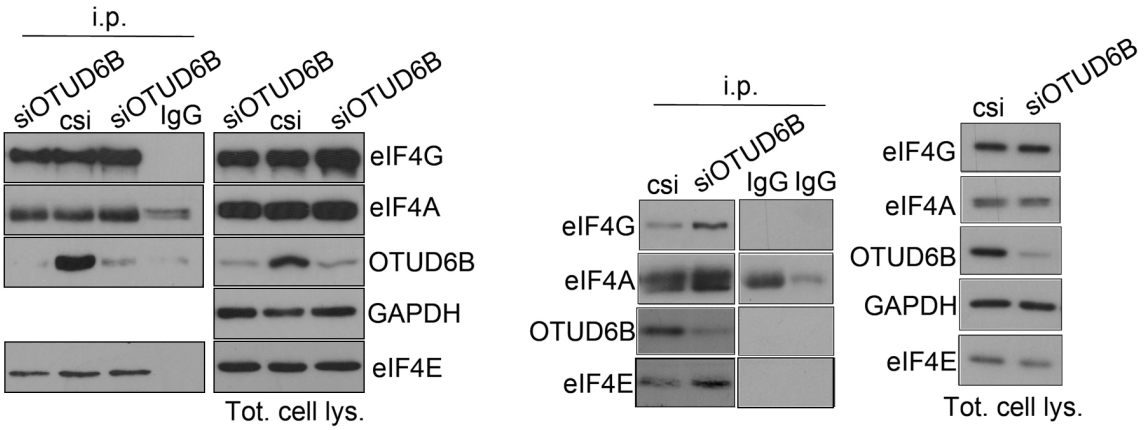
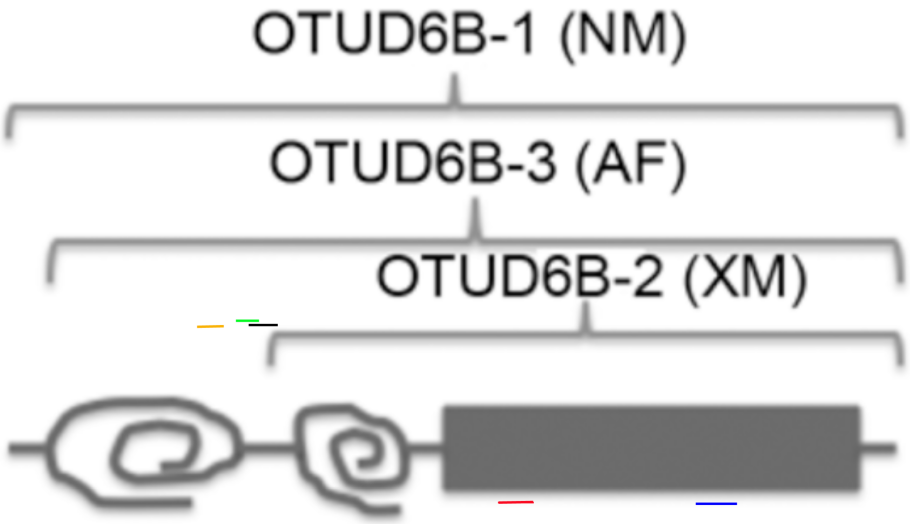



**Figure S4.**

OTUD6B coimmunoprecipitates with eIF4G and other eIF4F components. Two experiments in H1299 cells. The irrelevant IgG here (anti FLAG tag) retained some non-specific bands reactive to the eIF4A antibody. For this reason, the anti SV40, irrelevant IgG was used for the immunoprecipitations in Figure 3 (Supplementary Table ST2).

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OTUD6B-1      MEPRVRVEGWKVPTSRCRFLLARVLGYLVVMEAVLTEELDEEEQLLRRHRKEKKELQAKI
OTUD6B-3      ------------------------------MEAVLTEELDEEEQLLRRHRKEKKELQAKI
OTUD6B-2      ------------------------------------------------------------


OTUD6B-1      QGMKNAVPKNDKKRRKQLTEDVAKLEKEMEQKHREELEQLKLTTKENKIDSVAVNISNLV
OTUD6B-3      QGMKNAVPKNDKKRRKQLTEDVAKLEKEMEQKHREELEQLKLTTKENKIDSVAVNISNLV
OTUD6B-2      ------------------------------------------------------------


OTUD6B-1      LENQPPRISKAQKRREKKAALEKEREERIAEAEIENLTGARHMESEKLAQILAARQLEIK
OTUD6B-3      LENQPPRISKAQKRREKKAALEKEREERIAEAEIENLTGARHMESEKLAQILAARQLEIK
OTUD6B-2      -----------MISKEKKAALEKEREERIAEAEIENLTGARHMESEKLAQILAARQLEIK
                         :*******************************************

OTUD6B-1      QIPSDGHCMYKAIEDQLKEKDCALTVVALRSQTAEYMQSHVEDFLPFLTNPNTGDMYTPE
OTUD6B-3      QIPSDGHCMYKAIEDQLKEKDCALTVVALRSQTAEYMQSHVEDFLPFLTNPNTGDMYTPE
OTUD6B-2      QIPSDGHCMYKAIEDQLKEKDCALTVVALRSQTAEYMQSHVEDFLPFLTNPNTGDMYTPE
              ******************************************************

OTUD6B-1      EFQKYCEDIVNTAAWGGQLELRALSHILQTPIEIIQADSPPIIVGEEYSKKPLILVYMRH
OTUD6B-3      EFQKYCEDIVNTAAWGGQLELRALSHILQTPIEIIQADSPPIIVGEEYSKKPLILVYMRH
OTUD6B-2      EFQKYCEDIVNTAAWGGQLELRALSHILQTPIEIIQADSPPIIVGEEYSKKPLILVYMRH
              ************************************************************

OTUD6B-1      AYGLGEHYNSVTRLVNIVTENCS
OTUD6B-3      AYGLGEHYNSVTRLVNIVTENCS
OTUD6B-2      AYGLGEHYNSVTRLVNIVTENCS
              ***********************
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**Figure S5**

Schematic (Top) and sequence alignment (Bottom) of OTUD6B isoforms. Large spiral: coiled coil domain. In our laboratory the OTUD6B isoforms have been named NM, XM, and AF, respectively. The catalytic triad is highlighted in red and underlined. Colored lines in schematic: positions of the siRNAs used in this study; siRNA3; siRNA4; siRNA9, siRNA6, siRNA1. Note that siRNA1, 6 and 9 target a region of the 5'-untranslated region of the OTUD6B-2 mRNA.

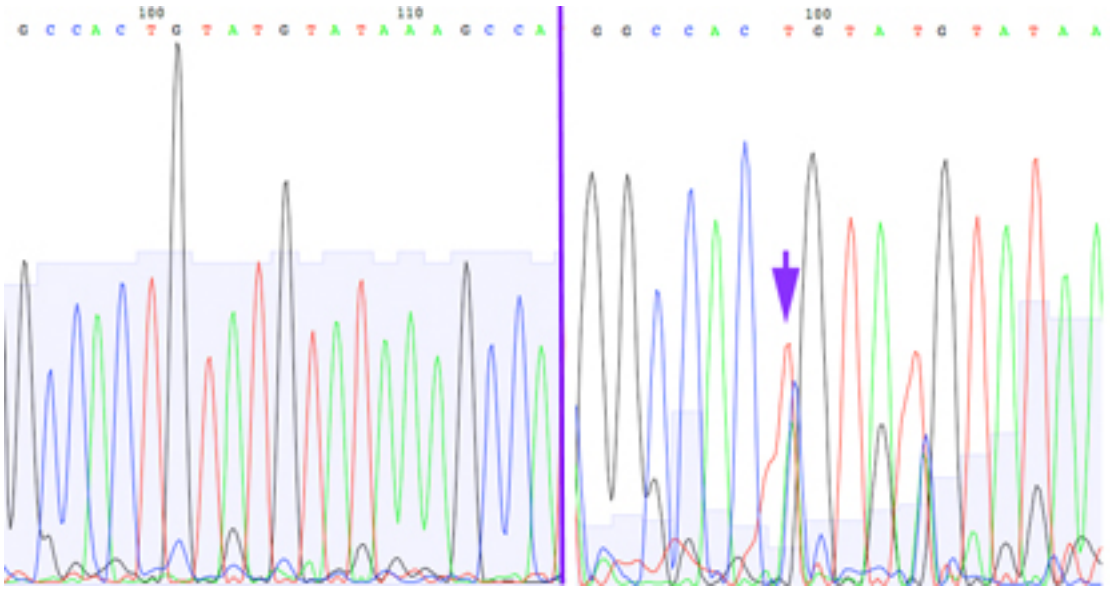

**Figure S6**

Evidence of allelic mutation (arrow) of the OTUD6B catalytic core in HCC827. Such mutation would result in a Cys to Arg missense mutation. Left: sequence of NSCLC cell line H1299. Right: sequence of NSCLC cell line HCC827. Note that the HCC827 appears noisy. Because we have repeated this sequence using different primers, it is possible that this sequence is a result of gene duplication. Chromosome 8 amplifications are quite common in NSCLC (Pei J, Balsara BR, Li W, Litwin S, Gabrielson E, Feder M, et al. Genomic imbalances in human lung adenocarcinomas and squamous cell carcinomas. Genes Chromosomes Cancer 2001;31:282-7).

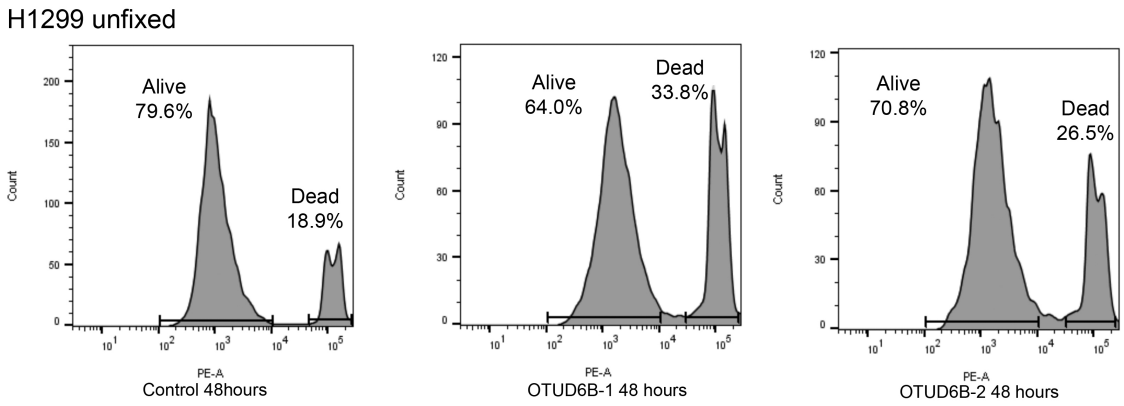


**Figure S7.**

OTUD6B-1 and OTUD6B-2 cause cell death when overexpressed in NSCLC cells. Representative propidium iodide staining of non-permeabilized H1299 cells 48 hours after transfection with the indicated plasmids. Similar results were obtained in A549 and H1437 cells.

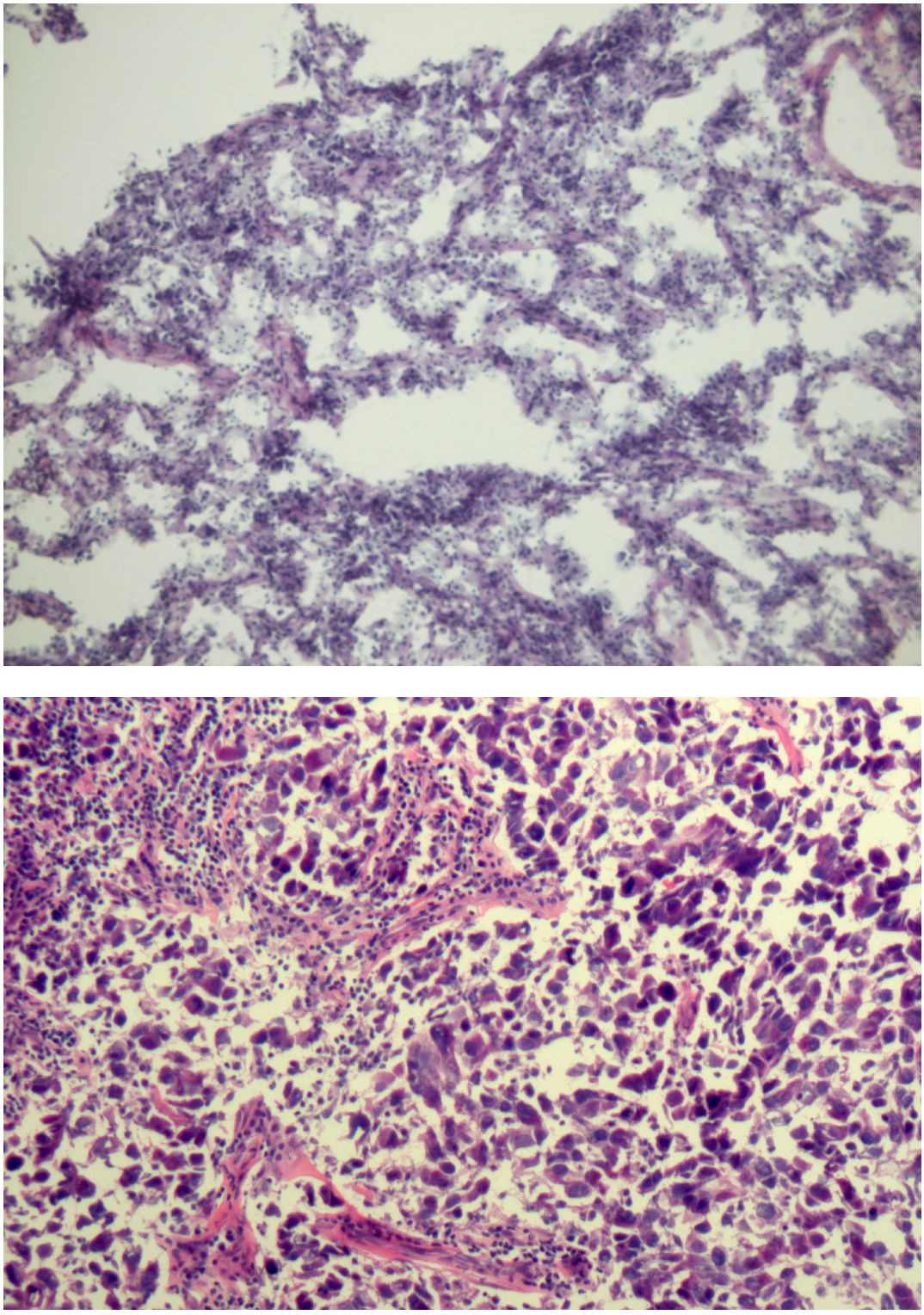

**Figure S8**
Hematoxylin/Eosin staining of 8μm-thick, frozen slides of representative normal lung (Top) and NSCLC (Bottom). Original magnification 20X (Top), 40X (Bottom).