**Fig s1**

**A**

- TIME
  - 10 Weeks
  - 15 Weeks
  - 20 Weeks
  - 25 Weeks

**B**

1 Week
- DOX ON
- 3 Days
- DOX OFF
- 1 Day
- DOX ON

**C**

- 6 Months
- CR
- CRS

**D**

- 6 Months
- Tumors Per Mouse
- CR
- CRS

**E**

- Axial
- Coronal
- H&E
- SNAI1

- 38 Weeks
- DOX ON
- 1 Week
- DOX OFF

**F**

- CR
- CRS
- % pERK

**G**

- Senescence Gene Sets
- MARTINEZ_TP53_TARGETS_DN
- CHICAS_RB1_TARGETS_SENESCENT

**H**

- CMV-rtTA/SNAI1-6SA-tetO7-luc MEFs
- SNAI1
- ACTIN
- BLI

**I**

- Relative Cell Count
- Absorbance (560nm)

**J**

- DOX OFF
- DOX ON
- % SmoGal Positive
- ns
- ***
Fig. s1. Characterizing a Kras$^{G12D}$ driven EMT mouse model of NSCLC. A, 25 week time course of CRS mouse model with inducible SNAI1 expression monitored by gross anatomy, cone-beam computed tomography (CBCT) scans, and hematoxylin and eosin (H&E) staining. B, Doxycycline (DOX) inducible expression of SNAI1 was verified with bioluminescence imaging (BLI) and SNAI Western blotting. C, D, CR and CRS mouse tumor CBCT scans (C) and quantification (D) at 6 months. E, Tumor regression of CRS mice 38 weeks post-removal of DOX from mice drinking water as seen by CBCT, H&E, and SNAI1 immunohistochemistry (IHC) staining. F, IHC staining of phosphorylated ERK (pERK) IHC in CRS mouse tumors compared to CR mouse tumors. G, Gene Set Enrichment Analysis (GSEA) enrichment plots (data sets obtained from the Molecular Signatures Database) were generated using differentially expressed genes between CR and CRS mice and identified significantly matching gene sets involving senescence. H-J, Inducible SNAI1 expressing MEFs overcome HRAS$^{G12D}$ induced OIS. Validation of the DOX inducible SNAI1 expressing MEFs by Western blotting and luciferase reporter (visualized by BLI, H). I, J, Inducible SNAI1 MEFs were transduced with HRAS$^{G12V}$ or vector control with or without SNAI1 expression (± 2μg/mL DOX in cell culture media) and relative change in cell number (I; P<0.001 by two-way ANOVA followed by Holm-Sidak’s multiple comparisons test), SA-βGal staining (J) were measured. Error bars, mean ± standard deviation (s.d.). P values were derived from an unpaired, two-tailed Student’s t-test (*P<0.05; ***P<0.001; ****P<0.0001).
**Fig s2**

### A

#### Glycolysis
- **A549**
  - **Vector**
  - **Twist1 WT**
  - **Twist1 Mut**

#### Glycolytic Capacity
- **A549**
  - **Vector**
  - **Twist1 WT**
  - **Twist1 Mut**

#### Glycolytic Reserve
- **A549**
  - **Vector**
  - **Twist1 WT**
  - **Twist1 Mut**

### B

#### Glycolysis
- **PC9**
  - **Vector**
  - **Twist1 WT**
  - **Twist1 Mut**

#### Glycolytic Capacity
- **PC9**
  - **Vector**
  - **Twist1 WT**
  - **Twist1 Mut**

#### Glycolytic Reserve
- **PC9**
  - **Vector**
  - **Twist1 WT**

### C

- **GNPNAT1**
  - Spearman: 0.185
  - Spearman: 0.260
  - Spearman: 0.278

- **PGM1**
  - Spearman: 0.016
  - Spearman: 0.268
  - Spearman: 0.032

- **PGM2**
  - Spearman: 0.260
  - Spearman: 0.278
  - Spearman: 0.095

- **UAP1**
  - Spearman: 0.169
  - Spearman: 0.278
  - Spearman: 0.314

### D

#### GFPT2
- Spearman: 0.185
- Spearman: 0.260
- Spearman: 0.032

#### UAP1
- Spearman: 0.032
- Spearman: 0.095

### E

- **H358**
  - **Vector**
  - **shSNAI1 1**
  - **shSNAI1 2**

### F

- **Relative mRNA Expression**
  - **GFPT1**
  - **GFPT2**
  - **GNPNAT1**
  - **PGM1**
  - **PGM2**
  - **UAP1**

- **Relative mRNA Expression**
  - **GFPT1**
  - **GFPT2**
  - **GNPNAT1**
  - **PGM1**
  - **PGM2**
  - **UAP1**
Fig. s2. The EMT increase glycolysis and induce the HBP gene expression in human NSCLC. A, B, Seahorse analysis of glycolytic flux of EMT NSCLC cells. Glycolytic activity, capacity, and reserve was determined in real-time using the Seahorse extracellular flux analyzer for vector, Twist1, and loss of function Twist1 in A549 (A) and PC9 (B) cells. C, Human tissue gene expression from Medisapiens In Silico Transcriptomics (IST) from 60 healthy tissues; significant gene expression of GFPT2 and UAP1 were found in mesenchymal stem cells (purple arrows). D, E, mRNA expression of the HBP genes by qPCR in vitro with knocking down Snai1 in NSCLC cell lines H358 (D), and in vivo autochthonous lung tumor mouse models with and without DOX inducible expression of SNAI1/KrasG12D (E). Error bars, mean ± s.d. P values were derived from an unpaired, two-tailed Student’s t-test. (ns P≥0.05; *P<0.05; **P<0.01).
Fig. s3. The HBP is critical for senescence of normal lung cells. A-D, Genetic inhibition of the HBP with knockdown of GFPT2 and UAP1 as shown by mRNA (A) and protein levels (B) and resulting cell number change per field of view (FOV; C) and SA-βGal staining (D) (statistical analysis by One-way ANOVA with Dunnett’s multiple comparison test). E-G, qPCR of senescence gene expression in HBECs treated with high concentration (50 µM) of DON for 48 hours (E) and metabolic rescue by supplementation with glucosamine (GlcN; F) or N-acetylglucosamine (GlcNAc; G) (statistical analysis by One-way ANOVA with Dunnett’s multiple comparison test). H, I, Bright field images of SA-βGal staining using genetic siRNA (H) and pharmacological DON (I) inhibition of the HBP.
Fig s4

A

| CS LSL | LSL-KrasG12D | CT LSL |
| CCSP-rtTA / SNAI1-tetO7-luc | | CCSP-rtTA / Twist1-tetO7-luc |

B

CS-LSL

ON

OFF

C

CS-LSL

ON

OFF

D

Relative mRNA Expression

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<th>SNAI1</th>
<th>p53</th>
<th>GFPT2</th>
<th>UAP1</th>
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E

PRE

POST

ON

OFF
Fig. s4. Characterizing a novel Kras$^{G12D}$ driven EMT mouse model of NSCLC. A, Breeding schematic of the Lox-Stop-Lox Kras$^{G12D}$ (LSL) model with DOX inducible lung specific expressing SNAI1 (CS) and Twist1 (CT) mouse models to generate DOX inducible EMT models with conditional expression of Kras$^{G12D}$ upon intranasal administration of Ad-CMV-iCre (AdCre) virus. ON and OFF indicate animals with or without DOX, respectively in their drinking water. B-E, Representative BLI (B), H&E (C), qPCR (D) and CT (E), of CS-LSL mice with and without inactivation of SNAI1 expression by withdrawing DOX from mouse drinking water, while maintaining expression of Kras$^{G12D}$ expression. (E) PRE and POST represents CT scans obtained prior to and after DOX withdrawal, respectively. Unless stated, error bars, mean ± s.d. P values were derived from an unpaired, two-tailed Student’s t-test (**P<0.001; ****P<0.0001).
Fig. s5. Inhibition of the HBP reduces cell viability and abrogates tumor growth and promotes senescence phenotypes. A, Genetic knockdown in A549 and H358 cells with siRNA targeting GFPT2 and UAP1 confirmed on the protein level by Western blot. B, Clonogenicity measured by crystal violet stained colonies on 10 cm dishes of A549 cells after 48 hours of pharmacological inhibition of the HBP with DON treatment. C, Senescence morphology. D-F, qPCR (D) Western blot (E, F) for markers of senescence and apoptosis (cleaved caspase 3) in 3 NSCLC cell lines upon 48 hour treatment of DON. The actin blot in (E) was from a parallel, contemporaneously run blot. G-I, H358 NSCLC cells (8x10^6) were injected into ~5 week old athymic nude female mice. Within the first week (visible tumors of ~50 mm3), mice were treated with 20 mg/kg/week DON and were assessed for tumor growth ($P<0.01$ by long-rank test; G), tumor mass (H), and with no noticeable toxicities to the mice measured by body mass (I) ($P>0.05$ by Two-way ANOVA) over a 5 week period. J-M, Likewise, A549 NSCLC cells (1x10^6) were implanted and treated with 20 mg/kg/week DON and were assessed for tumor size (J), tumor volume (n≥12 tumors per arm; $P<0.0001$ by two-way ANOVA followed by Holm-Sidak’s multiple comparisons test; K), tumor growth ($P<0.0001$ by log-rank test; L), and markers of senescence (M) over a 3 week period. N, CS-LSL mouse mass with and without DON treatment over 4 weeks ($P>0.05$ by Two-way ANOVA). and H&E staining (O) at 5 weeks post-AdCre.Unless stated. Error bars, mean ± s.d. P values were derived from an unpaired, two-tailed Student’s t-test (*$P<0.05$; ***$P<0.001$).
Fig. s6. Inhibiting HBP by *GFPT2* knockdown resulted in more senescent cells and decreased clonogenic ability of lung cancer cells. A, SA-β-Gal staining of lung cancer cell lines after *GFPT2* knockdown (**p<0.001, ***p<0.0001). B-C, Clonogenic assays of lung cancer cell lines with *GFPT2* (B) knockdown or (C) GFPT2 overexpression.
Fig s7

A

A549 Vector

SNAI1

DAPI SNAI1 O-GlcNAc MERGE

B

MEF SNAI1-6SA-tetO-luc

-DOX +DOX

DAPI SNAI1 O-GlcNAc MERGE

C

H358

shSNAI1

shSCR

DAPI SNAI1 O-GlcNAc MERGE
Fig. s7. SNAI1 expression correlates with O-GlcNAcylation. A-C, Immununofluoresence (IF) of SNAI1 and O-GlcNAc co-expressing A549 NSCLC cells (with elevated O-GlcNAc cytoplasmic immunopositive foci) (A), 48 hour DOX inducible SNAI1 expressing MEFs (B), and stable knockdown of SNAI1 in H358 NSCLC cells (C) (blue – DAPI; red – SNAI1; green – O-GlcNAc [RL2]).
Fig. s8. N-Linked glycosylation and O-GlcNAcylation in lung cancer cell line and novel EMT mouse models of lung cancer. A, Effects on total N-glycosylation and O-GlcNAcylation with 48 hour SNAI1 transient expression in A549 cells. B-D, LSL mouse tumors were compared to CS-LSL (B) and CT-LSL (C) mouse tumors for O-GlcNAcylation by Western blot. (D) IHC staining of O-GlcNAc and Snai1 on CR, CRS mouse tumors. E, CR, CRS, and CRT mouse tumors were assessed for N-glycosylation with the terminal glycosyl/mannosyl binding lectin (concanavalin A [conA]) and specificity of the conA lectin was assessed by blotting with 200 mM Mannose
Fig S9

A. Cell Number

B. Clonogenicity

C. SA-β-gal Positive Cell (%)

D. O-GlcNAc

E. Fold Change in Gene Expression
Fig. s9. OGT and cell viability. A, Cell proliferation of MEF cells with inducible OGT knockout by tamoxifen (4-OHT) (p<0.0001 by Two-way ANOVA). B, SA-beta-Galactosidase staining 8 days after AdCre treatment on primary type II pneumocytes isolated from LSL-Kras\textsuperscript{G12D} (LSL) and LSL-Kras\textsuperscript{G12D}/OGT\textsuperscript{fl/Y} (LSL-OGT) mice, with or without OGA inhibitor (Thiamet-G (TMG), 50uM) treatment (*p<0.01, ***p<0.0001). C, Clonogenic assay of A549 cells with Ogt knockdown by siRNA (p<0.0001 by t test). D, Overexpression of OGA in A549 cell line lead to cellular senescence. Western-blots and quantification of total O-GlcNAc (left two panels), (AdCre vs AdOGA, n=3, t-test p=0.014), quantification of SA-β-Gal staining after OGA overexpression (*** p<0.0001) and RT-PCR for p16 and OGA (** p<0.001). E, TMG treatment resulted in elevated protein O-GlcNAcylation and decreased expression level of several genes associated with senescence in the lung tissue of LSL-Kras\textsuperscript{G12D} mice (LSL) (*p<0.01, **p<0.001).
Untreated 5 µM DON 10 µM DON
50% DMSO 0.5 µg/µL TUNI 1 µg/µL TUNI

N-Glycosylation

N-Glycosylation + 200 mM Mannose

50% DMSO 0.5 µg/µL TUNI 1 µg/µL TUNI

Fig s10
Fig. s10. Inhibition of N-glycosylation with tunicamycin does not induce senescent phenotypes, unlike inhibition of the HBP with DON. A-E, Normal (A) and NSCLC cells (B-D) were treated with either tunicamycin (TUNI, 0.5 or 1 μg/μL) or DON (5 or 10 μM) and blotted with concanavalin A (ConA) to assess N-link glycosylation occupancy; ConA was assessed for specificity by competitive blotting with 200 mM mannose. E-H, Senescent morphology was seen in DON (5 or 10 μM) treated NSCLC cells, but not with TUNI treatment (0.5 or 1 μg/μL).
Fig s11

Control

LSL-Kras$^{G12D}$ + AdCre

OGT$^{fl/Y}$ + AdCre

LSL-Kras$^{G12D}$/OGT$^{fl/Y}$ + AdCre
Fig. s11. Morphology of LSL-Kras$^{G12D}$/OGT$^{III/Y}$ mouse lung tissue 8 days after AdCre. No relative increased cell death was observed in the lung tissue of OGT knockout mice either with or without concomitant activation of $Kras^{G12D}$. LSL-Kras$^{G12D}$ (LSL) and LSL-Kras$^{G12D}$/OGT$^{III/Y}$ (LSL-OGT$^{-/Y}$) mouse lungs demonstrated early atypical hyperplasia, but there was not an obvious difference at this early time point. Adenoviral-Cre intranasal infection demonstrated general signs of inflammation equally across the mouse strains.
Fig. s12. O-GlcNAc staining of LSL-Kras<sub>G12D/OGT<sup>fl/fl</sup>/Y</sub> mouse lung tissue 8 days after AdCre. Blue arrows indicate cells with low or no O-GlcNAc staining. Original Magnification: x200
Fig s13
Fig. s13. The HBP and Myc in novel EMT mouse models of lung cancer. A-B, LSL mouse tumors were compared to CS-LSL (A) and CT-LSL (B) mouse tumors for HBP enzymes Gftp2 and Uap1, Myc, and SNAI1/TWIST by Western blot.