Figure S1

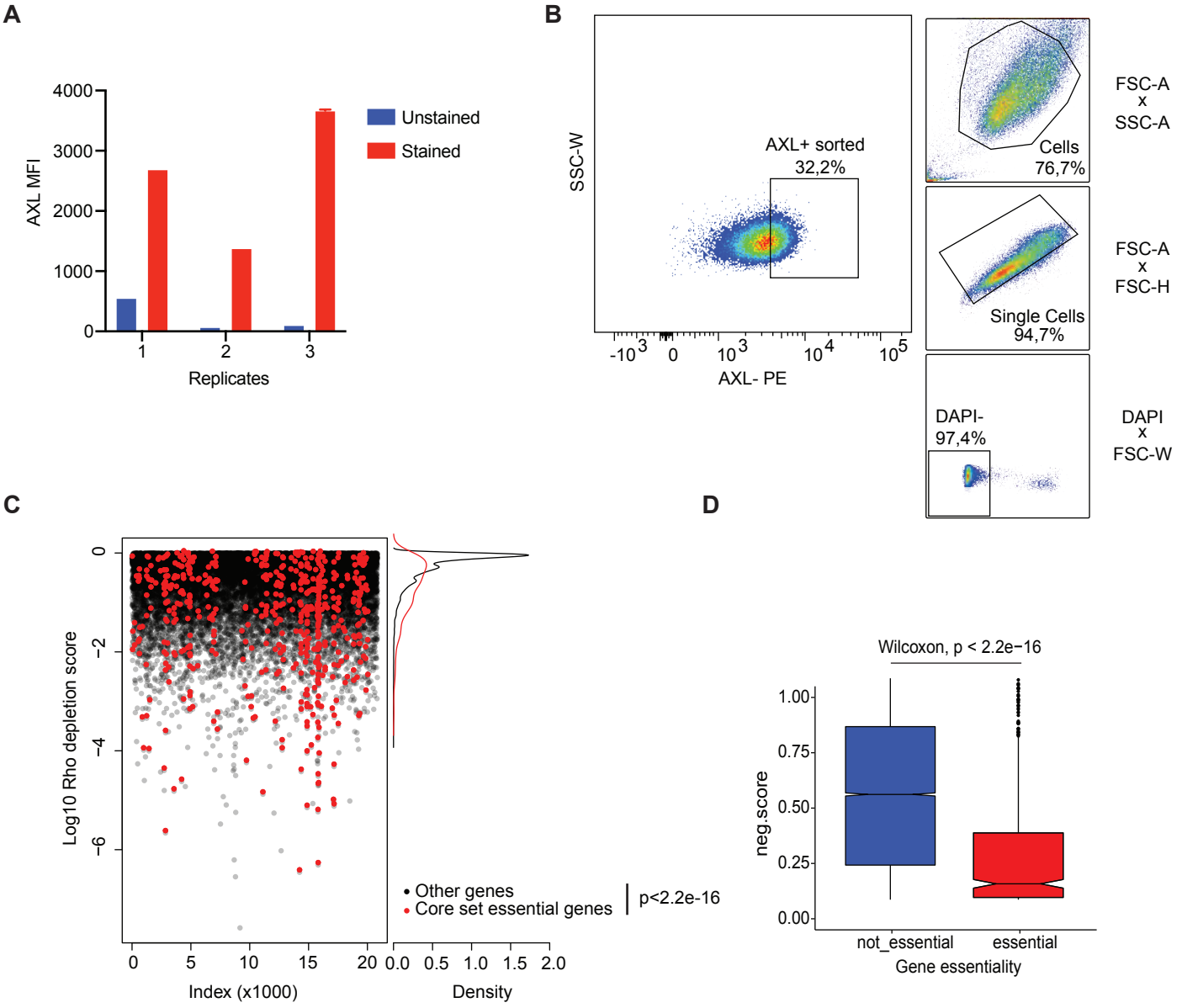

A
AXL MFI
4000
3000
2000
1000
0
1
2
3
Replicates
Unstained
Stained
B
SSC-W
AXL+ sorted
32,2%
-10^3
0
10^3
10^4
10^5
AXL- PE
Cells
76,7%
FSC-A
x
SSC-A
Single Cells
94,7%
FSC-A
x
FSC-H
DAPI-
97,4%
DAPI
x
FSC-W
C
Log10 Rho depletion score
0
−2
−4
−6
0
5
10
15
20
Index (x1000)
0.0
0.5
1.0
1.5
2.0
Density
Other genes
Core set essential genes
p<2.2e-16
D
Wilcoxon, p < 2.2e−16
neg.score
1.00
0.75
0.50
0.25
0.00
not_essential
essential
Gene essentiality

Figure S2

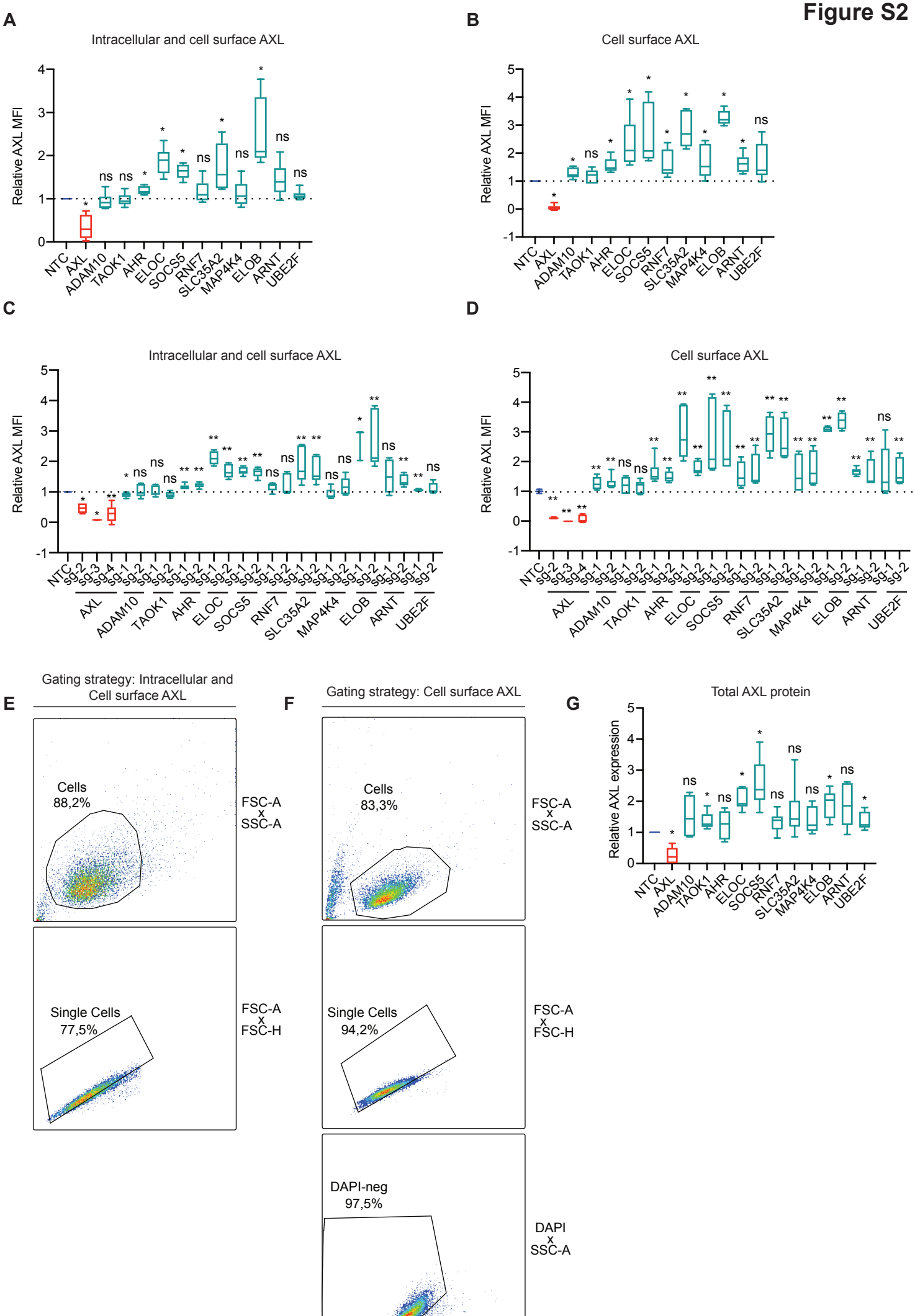

A
Intracellular and cell surface AXL
Relative AXL MFI
NTC
AXL
ADAM10
TAOK1
AHR
ELOC
SOCS5
RNF7
SLC35A2
MAP4K4
ELOB
ARNT
UBE2F
B
Cell surface AXL
C
Intracellular and cell surface AXL
D
Cell surface AXL
E
Gating strategy: Intracellular and Cell surface AXL
Cells
88,2%
FSC-A x SSC-A
Single Cells
77,5%
FSC-A x FSC-H
F
Gating strategy: Cell surface AXL
Cells
83,3%
FSC-A x SSC-A
Single Cells
94,2%
FSC-A x FSC-H
DAPI-neg
97,5%
DAPI x SSC-A
G
Total AXL protein
Relative AXL expression

Figure S3

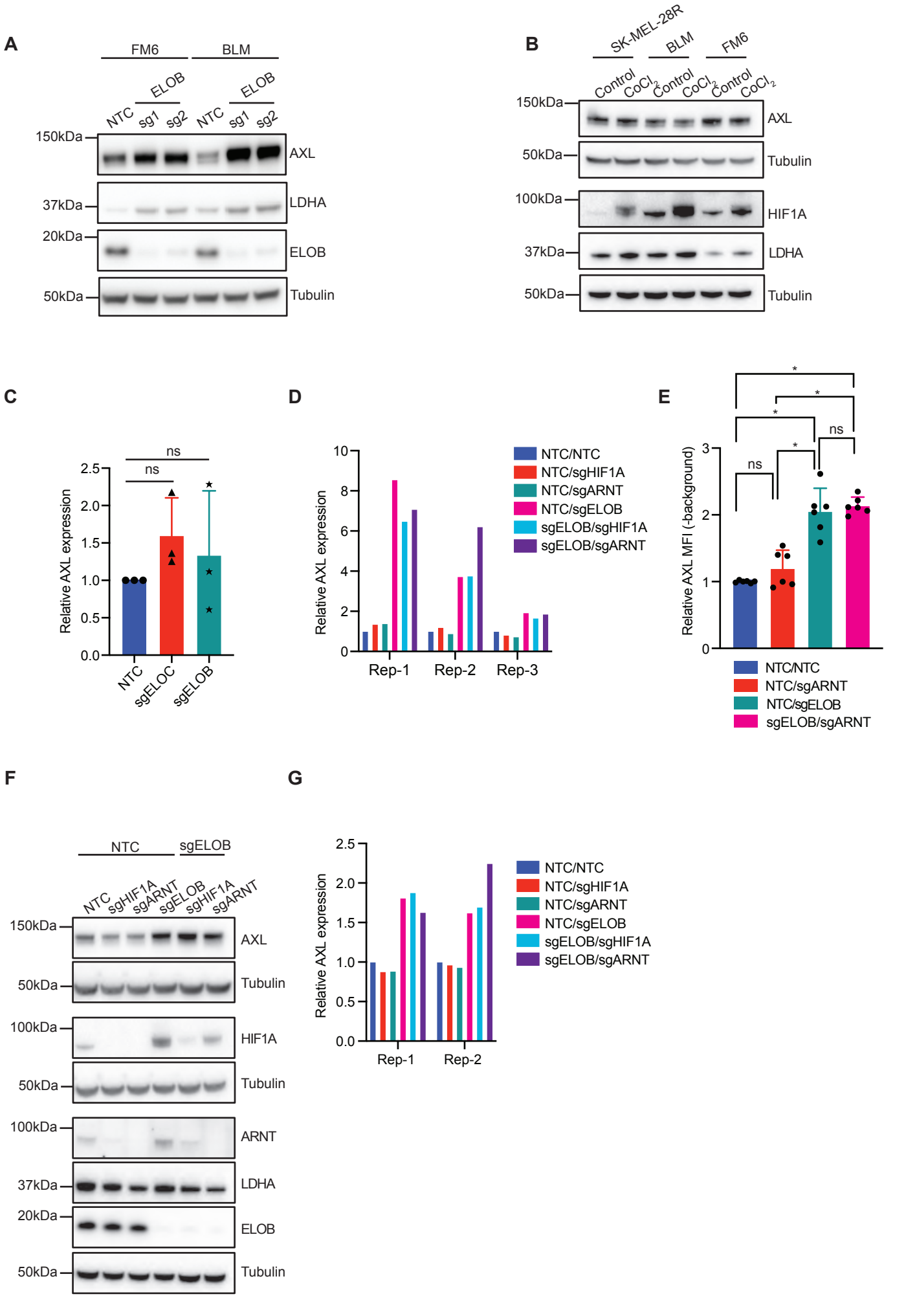

A
FM6
BLM
ELOB
ELOB
NTC
sg1
sg2
NTC
sg1
sg2
150kDa
AXL
37kDa
LDHA
20kDa
ELOB
50kDa
Tubulin
B
SK-MEL-28R
BLM
FM6
Control
CoCl2
Control
CoCl2
Control
CoCl2
150kDa
AXL
50kDa
Tubulin
100kDa
HIF1A
37kDa
LDHA
50kDa
Tubulin
C
ns
ns
Relative AXL expression
2.5
2.0
1.5
1.0
0.5
0.0
NTC
sgELOC
sgELOB
D
Relative AXL expression
10
8
6
4
2
0
Rep-1
Rep-2
Rep-3
NTC/NTC
NTC/sgHIF1A
NTC/sgARNT
NTC/sgELOB
sgELOB/sgHIF1A
sgELOB/sgARNT
E
*
*
*
ns
ns
*
Relative AXL MFI (-background)
3
2
1
0
NTC/NTC
NTC/sgARNT
NTC/sgELOB
sgELOB/sgARNT
F
NTC
sgELOB
NTC
sgHIF1A
sgARNT
sgELOB
sgHIF1A
sgARNT
150kDa
AXL
50kDa
Tubulin
100kDa
HIF1A
50kDa
Tubulin
100kDa
ARNT
37kDa
LDHA
20kDa
ELOB
50kDa
Tubulin
G
Relative AXL expression
2.5
2.0
1.5
1.0
0.5
0.0
Rep-1
Rep-2
NTC/NTC
NTC/sgHIF1A
NTC/sgARNT
NTC/sgELOB
sgELOB/sgHIF1A
sgELOB/sgARNT

Figure S4

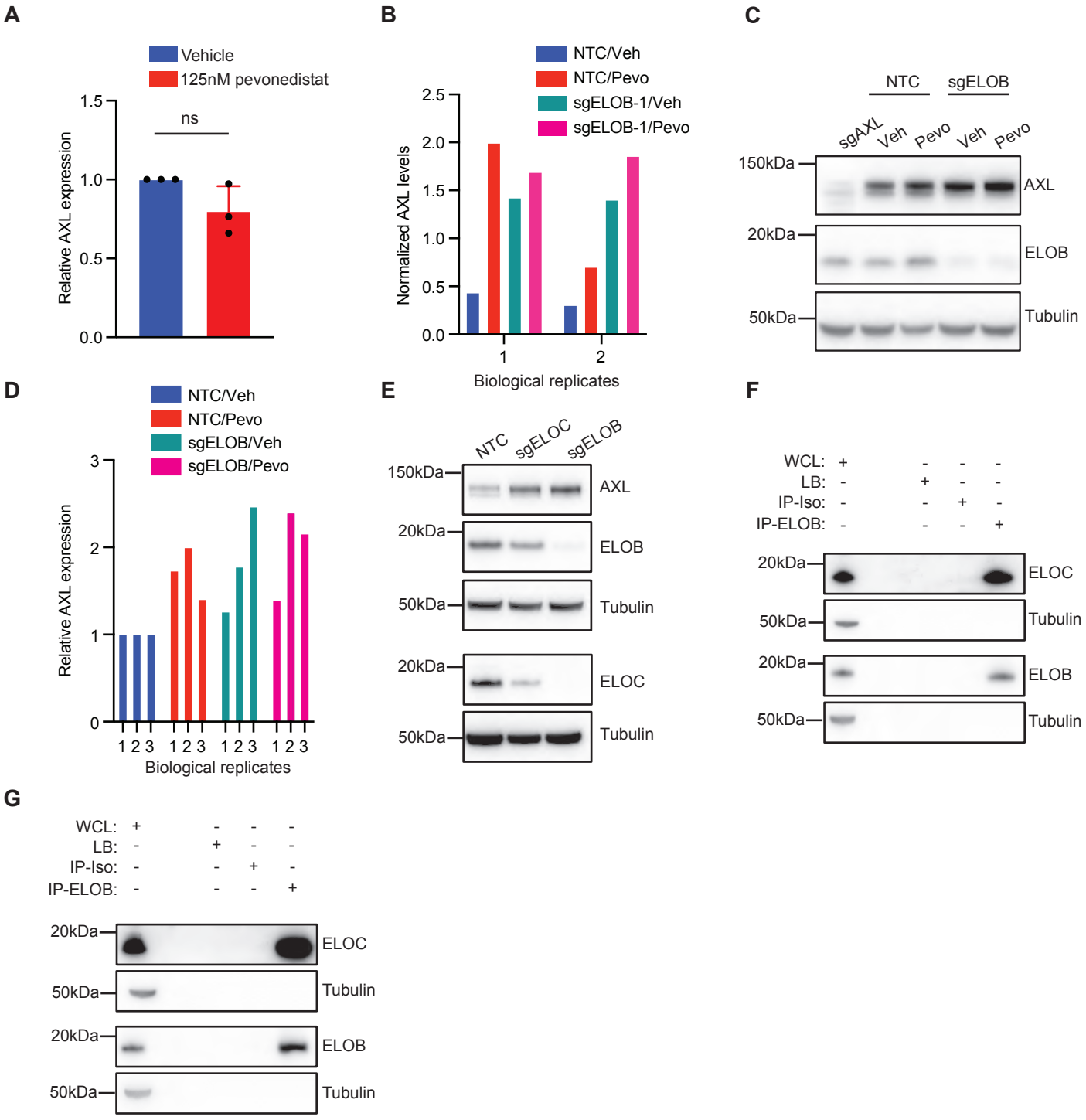
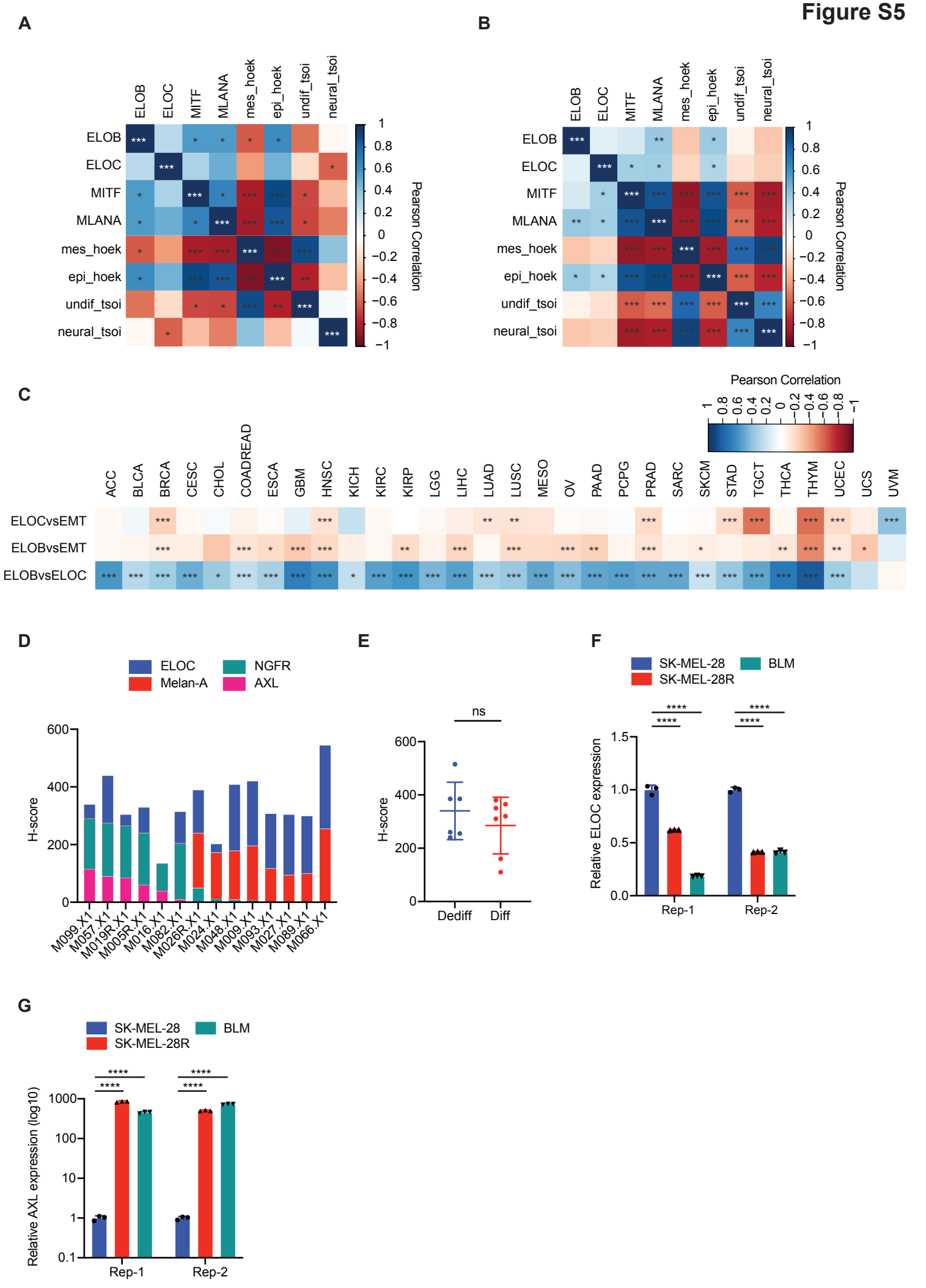

A
Vehicle
125nM pevonedistat
ns
Relative AXL expression
1.5
1.0
0.5
0.0
B
NTC/Veh
NTC/Pevo
sgELOB-1/Veh
sgELOB-1/Pevo
Normalized AXL levels
2.5
2.0
1.5
1.0
0.5
0.0
1
2
Biological replicates
C
NTC
sgELOB
sgAXL
Veh
Pevo
Veh
Pevo
150kDa
AXL
20kDa
ELOB
50kDa
Tubulin
D
NTC/Veh
NTC/Pevo
sgELOB/Veh
sgELOB/Pevo
Relative AXL expression
3
2
1
0
1 2 3 1 2 3 1 2 3 1 2 3
Biological replicates
E
NTC
sgELOC
sgELOB
150kDa
AXL
20kDa
ELOB
50kDa
Tubulin
20kDa
ELOC
50kDa
Tubulin
F
WCL: + - - -
LB: - + - -
IP-Iso: - - + -
IP-ELOB: - - - +
20kDa
ELOC
50kDa
Tubulin
20kDa
ELOB
50kDa
Tubulin
G
WCL: + - - -
LB: - + - -
IP-Iso: - - + -
IP-ELOB: - - - +
20kDa
ELOC
50kDa
Tubulin
20kDa
ELOB
50kDa
Tubulin

Figure S5


A
B
C
D
E
F
G
ELOB
ELOC
MITF
MLANA
mes_hoek
epi_hoek
undif_tsoi
neural_tsoi
Pearson Correlation
ACC
BLCA
BRCA
CESC
CHOL
COADREAD
ESCA
GBM
HNSC
KICH
KIRC
KIRP
LGG
LIHC
LUAD
LUSC
MESO
OV
PAAD
PCPG
PRAD
SARC
SKCM
STAD
TGCT
THCA
THYM
UCEC
UCS
UVM
ELOCvsEMT
ELOBvsEMT
ELOBvsELOC
ELOC
Melan-A
NGFR
AXL
H-score
M099.X1
M057.X1
M019R.X1
M005R.X1
M016.X1
M082.X1
M026R.X1
M024.X1
M048.X1
M009.X1
M093.X1
M027.X1
M089.X1
M066.X1
ns
Dediff
Diff
SK-MEL-28
SK-MEL-28R
BLM
Relative ELOC expression
Rep-1
Rep-2
Relative AXL expression (log10)

Supplementary Figure Legends:

Figure S1: Extended data on Figure 1: Whole genome-wide CRISPR-Cas9 screen identifies several negative regulators of AXL.

A) AXL MFI, which was assessed by flow cytometry. Viable SK-MEL-28R cells were stained with AXL-PE or left unstained. 3 different biological replicates are shown, each with at least 1 technical replicate.
B) Gating strategy of SK-MEL-28R cell sorting for AXL-high cells.
C) Log10-transformed MAGeCK RRA scores of depleted genes comparing bulk library reference sample to T0 in SK-MEL-28R cells. Essential genes were taken from Behan et al., 2019[33]. Red= core essential genes. Grey= other genes. y-axis= log10 Rho depletion score, x-axis= gene index.
D) Essential gene dropout score[33], related to Figure S1C. Statistical analysis is a Wilcoxon rank test.

Figure S2: Extended data on Figure 2: Elongin BC complex members serve as key AXL regulators.

A) Quantification of intracellular and cell surface AXL by flowcytometry on fixed and permeabilized SK-MEL-28R cells expressing the depicted sgRNAs. Error bars are SD of 2 or 3 biological replicates, each with at least 1 technical replicate. Statistics are based on the pooled data of all the sgRNAs targeting their respective genes. Data were normalized within each experiment to the non-targeting control. Min and max are depicted. Statistical analysis by Mann-Whitney, *p<0,05, ns=not significant.
B) Quantification of cell surface AXL by flowcytometry on viable SK-MEL-28R cells expressing the depicted sgRNAs. Error bars represent SD of at least 2 biological replicates with 2 technical replicates each. Statistics are based on the pooled data of all the sgRNAs targeting their respective genes. Data were normalized within each experiment to the non-targeting control. Min and max are depicted. Statistical analysis by Mann-Whitney, *p<0,05, ns=not significant.
C) Quantification of intracellular and cell surface AXL by flowcytometry on fixed and permeabilized SK-MEL-28R cells expressing the depicted sgRNA's. Error bars represent SD of pooled technical replicates of 2 or 3 biological replicates. Data was normalized within each experiment to the non-targeting control. Min and max are depicted. Statistical analysis by Mann-Whitney, *p<0,05, **p<0,01, ns=not significant.
D) Quantification of cell surface AXL by flowcytometry on viable SK-MEL-28R cells expressing the depicted sgRNA's. Error bars represent SD of pooled technical replicates of 2 or 3 biological replicates. Data was normalized within each experiment to the non-targeting control. Min and max are depicted. Statistical analysis by Mann-Whitney, *p<0,05, **p<0,01, ns=not significant.
E) Gating strategy analysis for determining intracellular and cell surface AXL in fixed and permeabilized SK-MEL-28R cells used in the screen validation.
F) Gating strategy analysis for determining cell surface AXL in viable SK-MEL-28R cells used in the screen validation.
G) Quantification of AXL by western blot on SK-MEL-28R cells expressing the depicted sgRNAs. Error bars represent SD and n=2 or 3 biological repeats. Statistics are based on the pooled data of all the sgRNAs targeting their respective genes. Data were normalized within each experiment to the non-targeting control. Min and max are depicted. Statistical analysis by Mann-Whitney, *p<0,05, ns=not significant.

Figure S3: Extended data on Figure 3: Elongin B/C regulate AXL independently of hypoxia.

A) Western blot analysis of *ELOB* sgRNA-expressing FM6 and BLM melanoma cell lines. Tubulin was used as loading control. The markings indicate the closest molecular weight marker. At least 2 biological repeats were performed.
B) Western blot analysis of AXL-high cell lines (SK-MEL-28R, BLM and FM6) exposed to 100 μM $CoCl_2$ for 3 days. Tubulin was used as loading control. The markings indicate the closest molecular weight marker. 2 biological repeats were performed.
C) RT-PCR analysis of *AXL* mRNA in BLM cells harboring *ELOB/C* knockouts. ns=not significant. Statistical analysis by a Kruskal-Wallis test. ns=not significant. Error bars represent SD of three biological replicates with each 3 technical replicates. Data normalized to NTC.
D) Western blot quantification of AXL protein expression depicted in Figure 3H. 3 biological experiments are depicted, each with 1 technical replicate. Data normalized to non-targeting control (NTC/NTC) within each biological replicate.
E) FACS analysis of viable SK-MEL-28R cells harboring sgRNA's targeting *ELOB* and/or *ARNT*. Error bars represent SD of technical replicates of three biological experiments. Statistical analysis is Kruskal-Wallis test. *p<0,05, and ns=not significant. Data normalized to non-targeting control (NTC/NTC) within each biological replicate.
F) Western blot analysis of SK-MEL-28R expressing different combinations of sgRNAs targeting *ARNT*, *HIF1A* or *ELOB*. Non-targeting control (NTC) was used as control guide. Tubulin was used as loading control. The markings indicate the closest molecular weight marker. 2 biological repeats were performed.
G) Quantification of AXL protein expression depicted in F. 2 biological replicates are depicted, each with 1 technical replicate. Data normalized to non-targeting control (NTC/NTC) within each biological replicate.

Figure S4: Extended data on Figure 4: Elongin B/C interact with AXL and loss of Elongin B/C stabilizes AXL protein levels.

A) RT-PCR analysis of *AXL* mRNA in BLM cells treated with 125 nM pevonedistat for 72 hours. Error bars are SD. Average of three biological replicates with each 3 technical replicates is depicted. Data was normalized within each experiment to vehicle condition. Statistical analysis by Mann-Whitney test, ns=not significant.
B) Quantification of Western blot analysis shown in 4D. Two biological replicates are shown. Normalized AXL levels to tubulin are depicted. Both NTC and *ELOB* KO treated and untreated groups were normalized within each experiment to own tubulin. 2 biological replicates were performed, each with 1 technical replicate.
C) Western blot analysis of epistasis experiment of *ELOB* knockout and pevonedistat in SK-MEL-28R cells. Tubulin was used as loading control. Cells were incubated with 125 nM of pevonedistat for 3 days. 3 biological replicates were performed.
D) Quantification of figure C. Independent epistasis experiments of *ELOB* knockout and pevonedistat on SK-MEL-28R cells are depicted. Numbers on the x-axis represent different biological replicates. Data of each experiment was normalized to non-targeting control/vehicle condition. 3 biological replicates were performed, each with 1 technical replicate.
E) Western blot analysis of BLM harboring sgRNAs against *ELOC* and *ELOB*. NTC is non-targeting control. Tubulin was used as loading control. The markings indicate the closest molecular weight marker. 2 biological replicates were performed.
F) Western blot of immuno-precipitated ELOB in FM6. Tubulin was used as loading control. The markings indicate the closest molecular weight marker. WCL=whole cell lysate, LB=lysis buffer, Iso=isotype control. Lanes 2 and 3 are empty. 2 biological replicates were performed.

G) Western blot of immuno-precipitated ELOB in BLM. Tubulin was used as loading control. The markings indicate the closest molecular weight marker. WCL=whole cell lysate, LB=lysis buffer, Iso=isotype control. Lanes 2 and 3 are empty. 2 biological replicates were performed.

Figure S5: Extended data on Figure 5: Low expression of *ELOB/C* is associated with phenotype switching and EMT.

A) Pearson correlation matrix of *ELOB/C* and phenotype switch signatures using Müller et al., 2014 dataset[2]. Blue is positive correlation and red is negative correlation.
B) Pearson correlation matrix of *ELOB/C* and phenotype switch signatures using in house PDX dataset[35]. Blue is positive correlation and red is negative correlation.
C) Pearson correlation matrix of *ELOB/C* and Hallmark EMT signature using pan-cancer TCGA. Blue is positive correlation and red is negative correlation.
D) H-score summary of ELOC, NGFR, Melan-A and AXL in melanoma PDX (Kemper et al., 2016 and Krijgsman et al., 2021)[24, 35] (n=14).
E) H-score analysis of ELOB in dedifferentiated and differentiated PDX. Dedifferentiation was assessed by high NGFR and low Melan-A expression. Error bars are SD. Statistical analysis is unpaired T-test, n=6 and 7. ns=not significant. Dediff stands for dedifferentiation and diff for differentiation.
F) RT-PCR analysis of *ELOC* mRNA in SK-MEL-28, SK-MEL-28R and BLM. Error bars are SD. Two biological experiments with each 3 technical replicates each. Data was normalized within each experiment to SK-MEL-28. Statistical analysis is an ANOVA test. ****p<0,0001.
G) RT-PCR analysis of *AXL* mRNA in SK-MEL-28, SK-MEL-28R and BLM. Error bars are SD. Two biological experiments with each 3 technical replicates. Data was normalized within each experiment to SK-MEL-28. Statistical analysis is an ANOVA test. ****p<0,0001.