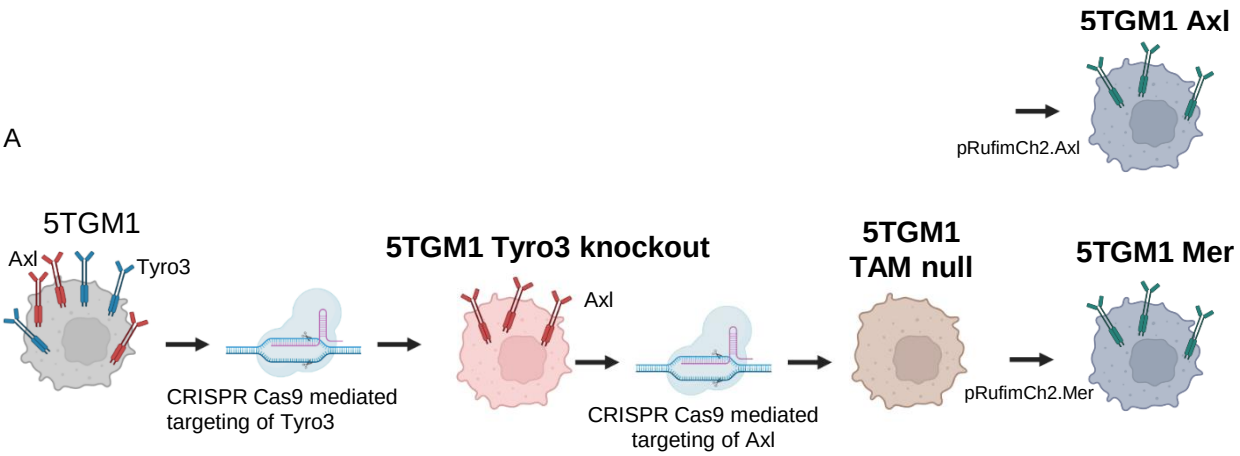
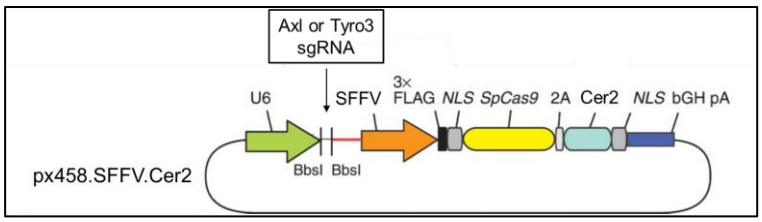
Supplementary figures

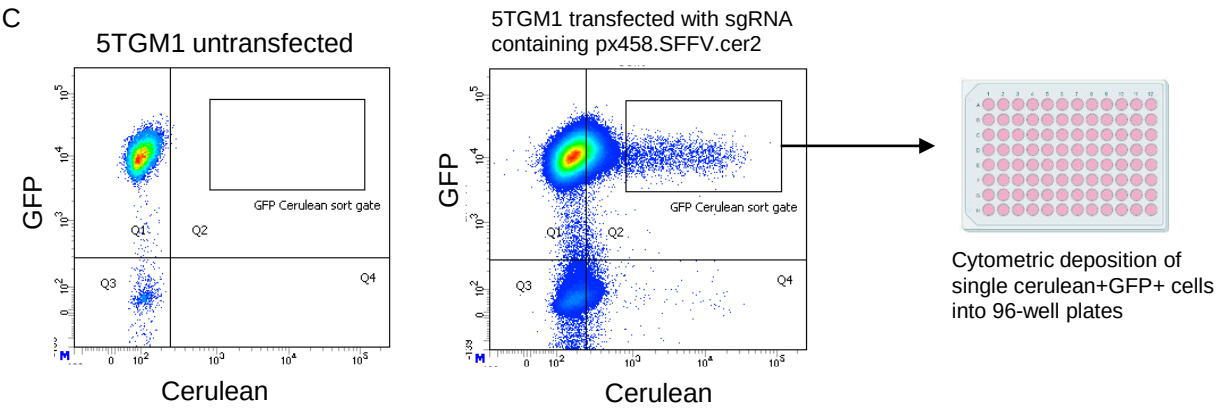
A


5TGM1 Axl
pRufimCh2.Axl
5TGM1
Axl
Tyro3
CRISPR Cas9 mediated
targeting of Tyro3
5TGM1 Tyro3 knockout
Axl
CRISPR Cas9 mediated
targeting of Axl
5TGM1
TAM null
pRufimCh2.Mer
5TGM1 Mer

B


Axl or Tyro3
sgRNA
U6
SFFV
3×
FLAG
NLS
SpCas9
2A
Cer2
NLS
bGH pA
BbsI
BbsI
px458.SFFV.Cer2

C


5TGM1 untransfected
GFP
GFP Cerulean sort gate
Q1
Q2
Q3
Q4
Cerulean
5TGM1 transfected with sgRNA
containing px458.SFFV.cer2
GFP
GFP Cerulean sort gate
Q1
Q2
Q3
Q4
Cerulean
Cytometric deposition of
single cerulean+GFP+ cells
into 96-well plates

Supplementary Figure 1. CRISPR-Cas9 mediated gene targeting and retroviral transduction were utilised to generate 5TGM1 single TAM receptor expressing cell lines. . (A) 5TGM1 cells initially expressing Axl and Tyro3 were initially targeted using Tyro3 sgRNAs to generate 5TGM1 Tyro3 knockout clonal cell lines. A 5TGM1 Tyro3 knockout cell line was then retargeted with Axl sgRNAs to generate a 5TGM1 double knockout (TAM null) cell line that does not express any TAM receptors. 5TGM1 Mer and 5TGM1 Axl cell lines were then generated by retroviral transduction of this 5TGM1 TAM null cell line with pRufimCh2.Mer and pRufimCh2.Axl respectively. **(B)** sgRNA guide sequences were cloned into an expression plasmid bearing a sgRNA scaffold backbone, Cas9, the U6 promoter, the SFFV promoter and the cerulean reporter (Cer2), figure adapted from Ran, *et al.,* 2013 [49]. **(C)** 5TGM1 cells were transiently transfected with the px458.SFFV.cer2 vector encoding Axl or Tyro3 sgRNA (middle) compared to untransfected 5TGM1 cells (left) Single GFP+ cerulean+ cells were deposited into 96-well plates. 5TGM1 clonal knockout cell lines were then expanded from single cells.

A

Tyro3 wild type

PAM

sgRNA binding site

GGATGGCACCGTGGT**CCA**GAATGCAAGCCAGGTGTCCA

Tyro3 KO clone #1

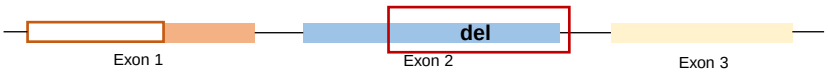
a) GGATGGCACCGTGGTCCAGAATTCAAGCCAGGTGTCCA **(1b.p. ins)**

b) GGATGGCACCGTGGTCCAGAAT--AAGCCAGGTGTCCA **(2b.p. del)**

Tyro3 KO clone #2

AAGCTCAACTGCAGCGTG--(130b.p.)--agcgggaggactg

(110b.p. del of exon 2, 20b.p. del of intron 2)



Tyro3 KO clone #3

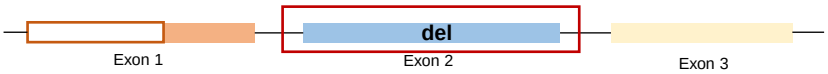
a) GGATGGCACCGTGGTCCAGAAT-CAAGCCAGGTGTCCA **(1b.p. del)**

b) G----------CAAGCCAGGTGTCCA **(22b.p. del)**

Tyro3 KO clone #4

Ggaggttcagctag---581b.p.)---aaaatgaccatgttaaac

(291b.p. del intron 1, 184 b.p. del of whole exon 2, 106b.p. del intron 2)

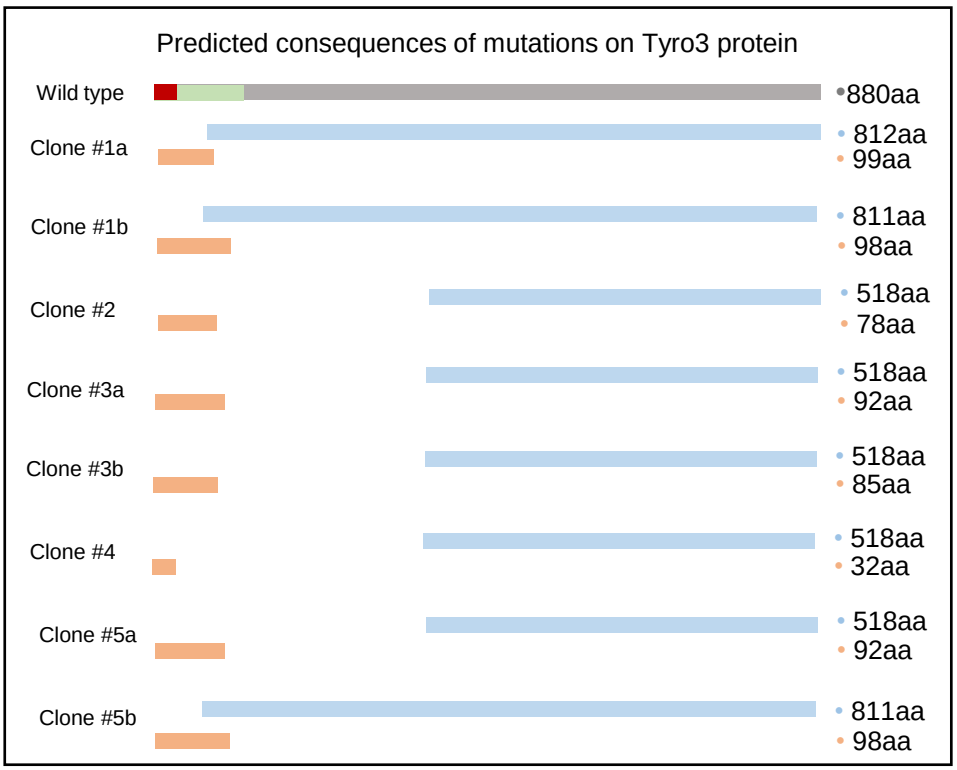


Tyro3 KO clone #5

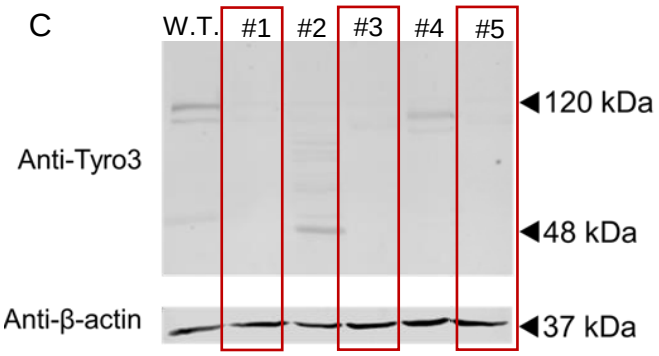
a) GGATGGCACCGTGGTCCAG-ATGCAAGCCAGGTGTCCA **(1b.p. del)**

b) GGATGGCACCGTGGTCCAG--TGCAAGCCAGGTGTCCA **(2 b.p. del)**

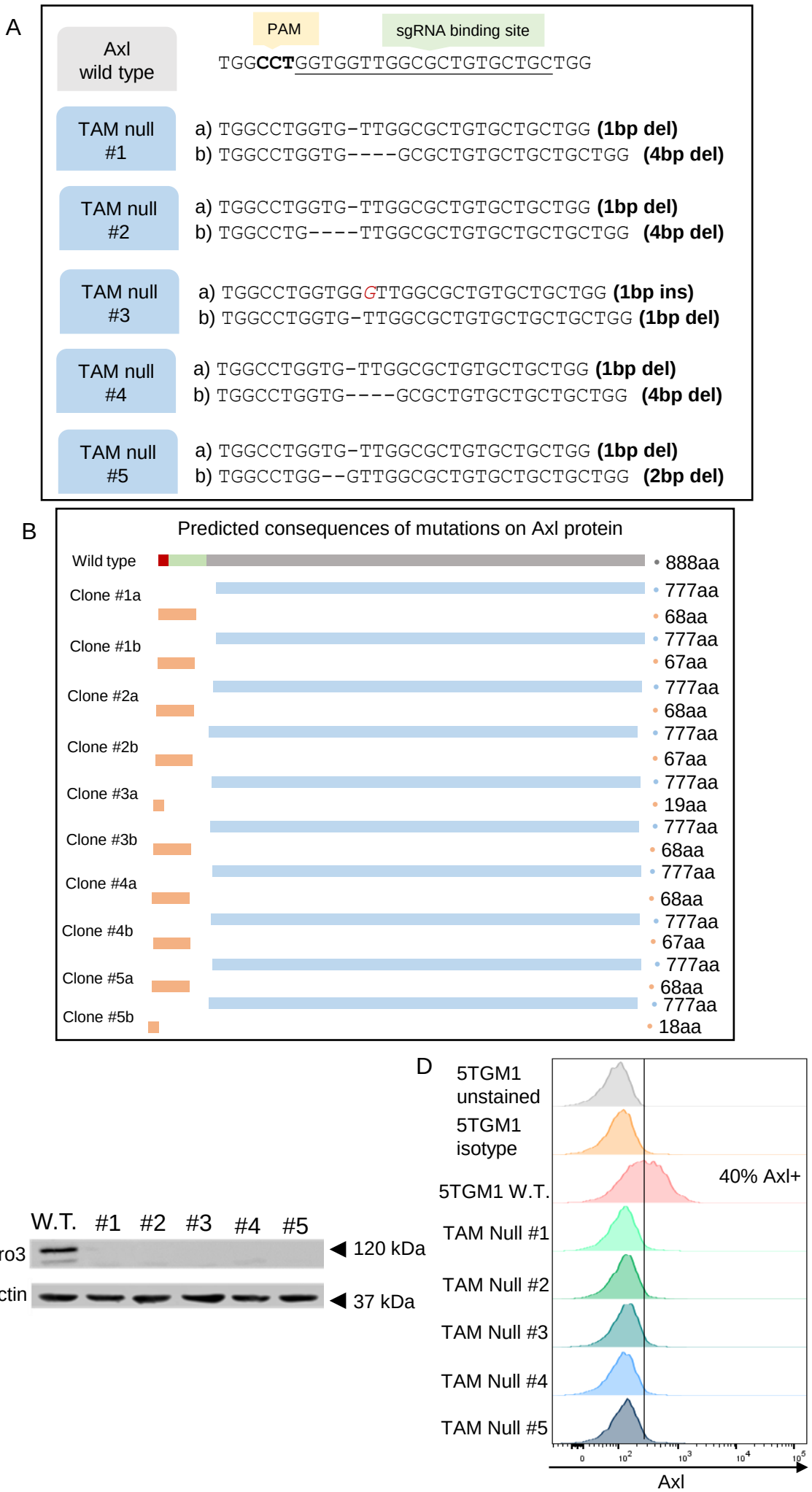
B



C

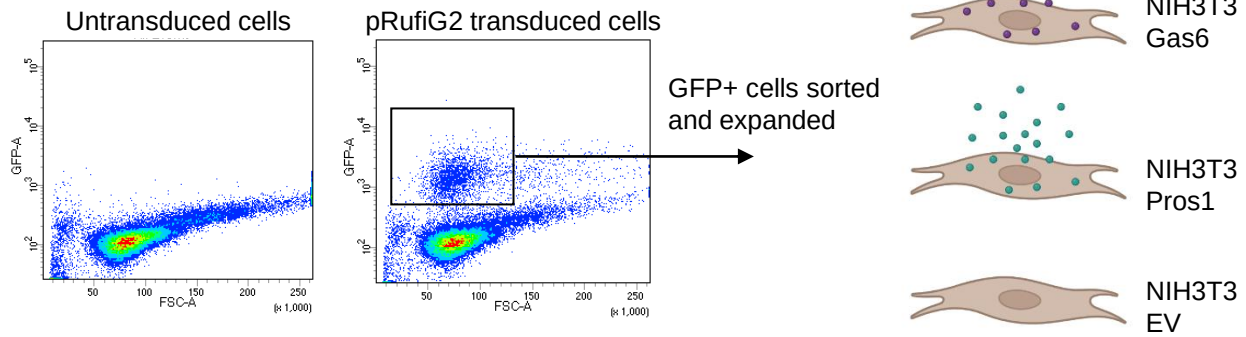


Supplementary figure 2. Confirmation of CRISPR-Cas9 mediated Tyro3 knockout in clonal 5TGM1 cell lines. (A) Sanger sequencing results of PCR products amplifying Tyro3 exon 2 genomic DNA from clonal 5TGM1 cell lines targeted with Tyro3 sgRNA were compared to wild type Tyro3 sequence. The Tyro3 sgRNA binding site (underlined) and 5' PAM sequence (bold) are shown in the wild type Tyro3 sequence. Insertion (in) and deletion (del) mutations in individual clones are shown as red text (N) and dashes (-), respectively. Mutations were either heterozygous (clones 1, 3 and 5) or homozygous (clones 2 and 4). Larger deletions in clones #2 and #4 are depicted below sequences, with deletions shown by red boxes. **(B)** Predicted consequences of mutant Tyro3 alleles on encoded Tyro3 protein. Unmutated, wild type Tyro3 is indicated in grey at the top, with a green band overlain to indicate the Gas6 binding domain and red to indicate the signal peptide. Sizes of potential Tyro3 proteins that could be produced by mutant alleles are indicated. Orange bars start at the established initiating methionine, blue bars start at putative alternative downstream methionines. **(C)** Proteins from clonal Tyro3 knockout cell lines were subjected to western blotting using an anti-Tyro3 primary antibody. β-actin was used as a loading control. Red boxes identify 5TGM1 Tyro3 knockout clones that do not produce any detectable Tyro3 protein.

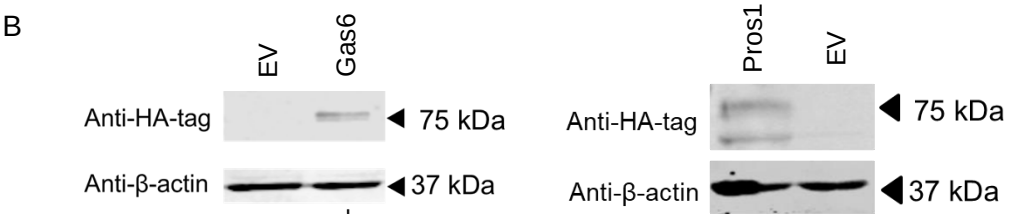

A
Axl wild type
PAM
sgRNA binding site
TGGCCTGGTGGTTGGCGCTGTGCTGCTGG
TAM null #1
a) TGGCCTGGTG-TTGGCGCTGTGCTGCTGG (1bp del)
b) TGGCCTGGTG----GCGCTGTGCTGCTGG (4bp del)
TAM null #2
a) TGGCCTGGTG-TTGGCGCTGTGCTGCTGG (1bp del)
b) TGGCCTG----TTGGCGCTGTGCTGCTGG (4bp del)
TAM null #3
a) TGGCCTGGTGGGTTGGCGCTGTGCTGCTGG (1bp ins)
b) TGGCCTGGTG-TTGGCGCTGTGCTGCTGG (1bp del)
TAM null #4
a) TGGCCTGGTG-TTGGCGCTGTGCTGCTGG (1bp del)
b) TGGCCTGGTG----GCGCTGTGCTGCTGG (4bp del)
TAM null #5
a) TGGCCTGGTG-TTGGCGCTGTGCTGCTGG (1bp del)
b) TGGCCTGG--GTTGGCGCTGTGCTGCTGG (2bp del)
B
Predicted consequences of mutations on Axl protein
Wild type
888aa
Clone #1a
777aa
68aa
Clone #1b
777aa
67aa
Clone #2a
777aa
68aa
Clone #2b
777aa
67aa
Clone #3a
777aa
19aa
Clone #3b
777aa
68aa
Clone #4a
777aa
68aa
Clone #4b
777aa
67aa
Clone #5a
777aa
68aa
Clone #5b
777aa
18aa
C
W.T. #1 #2 #3 #4 #5
Anti-Tyro3
120 kDa
Anti-β-actin
37 kDa
D
5TGM1 unstained
5TGM1 isotype
5TGM1 W.T.
40% Axl+
TAM Null #1
TAM Null #2
TAM Null #3
TAM Null #4
TAM Null #5
0
10^2
10^3
10^4
10^5
Axl

Supplementary Figure 3. Confirmation of CRISPR-Cas9 mediated Axl knockout in clonal 5TGM1 Tyro3 knockout cell line. (A) Sanger sequencing results of PCR products amplifying Axl exon 1 genomic DNA from clonal 5TGM1 Tyro3 knockout cell line #3 targeted with Axl sgRNA1 were compared to wild type Axl sequence. The Axl sgRNA1 binding site (underlined) and 5' PAM sequence (bold) are shown in the wild type Axl sequence. Insertion (In) and deletion (del) mutations in individual mutant alleles are shown as red text (N) and dashes (-) respectively. Mutations were all heterozygous. **(B)** Axl protein produced by unmutated, wild type Axl gene is indicated in grey, with a green band overlayed to indicate the Gas6 binding domain and red to indicate the signal peptides. Sizes of potential Axl proteins that could be produced by mutant alleles are indicated. Orange bars start at the established initiating methionine, blue bars start at a putative alternative downstream methionine. **(C)** Proteins from 5TGM1 TAM null cell lines were subjected to western blotting using an anti-Tyro3 primary antibody. B-actin was used as a loading control. **(D)** 5TGM1 double knockout cell lines were subjected to antibody staining for cell surface Axl expression and analysis by flow cytometry. Axl expression was compared to unaltered 5TGM1 cells and unstained and istotype controls.

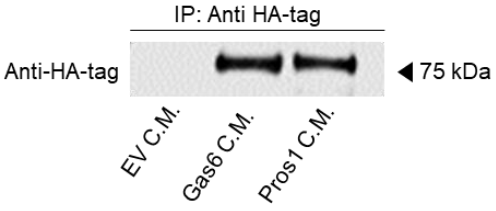
A


Untransduced cells
pRufiG2 transduced cells
GFP+ cells sorted
and expanded
NIH3T3
Gas6
NIH3T3
Pros1
NIH3T3
EV
GFP-A
FSC-A
(x 1,000)

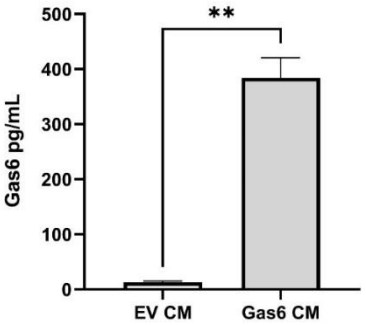
B


EV
Gas6
Anti-HA-tag
75 kDa
Anti-β-actin
37 kDa
Pros1
EV
Anti-HA-tag
75 kDa
Anti-β-actin
37 kDa

C


IP: Anti HA-tag
Anti-HA-tag
75 kDa
EV C.M.
Gas6 C.M.
Pros1 C.M.

D


**
Gas6 pg/mL
500
400
300
200
100
0
EV CM
Gas6 CM

Supplementary Figure 4. Retroviral mediated generation of NIH3T3 cells overexpressing Gas6 or Pros1 transgenes. (A) NIH3T3 cells were transduced with the pRufiG2.Gas6-HA, pRufiG2.Pros1-HA or empty vector retroviruses and GFP+ cells were sorted and expanded. Representative FACS plots with sort gate is shown. **(B)** Protein lysates from NIH3T3 EV compared to NIH3T3 Gas6 (left) and NIH3T3 Pros1 (right) cell lines were subjected to western blot using an anti-HA-tag primary antibody with β-actin used as a loading control. **(C)** Conditioned media was collected from the NIH3T3 cell lines and subjected to immunoprecipitation using an anti-HA-tag antibody. Purified proteins were then subjected to immunoblotting with anti-HA-tag antibody. **(D)** Conditioned media from the NIH3T3 EV and NIH3T3 Gas6 cell lines was used in an ELISA for Gas6, data presented as mean ± SEM, *n*=3, Student's t-test **p<0.01.