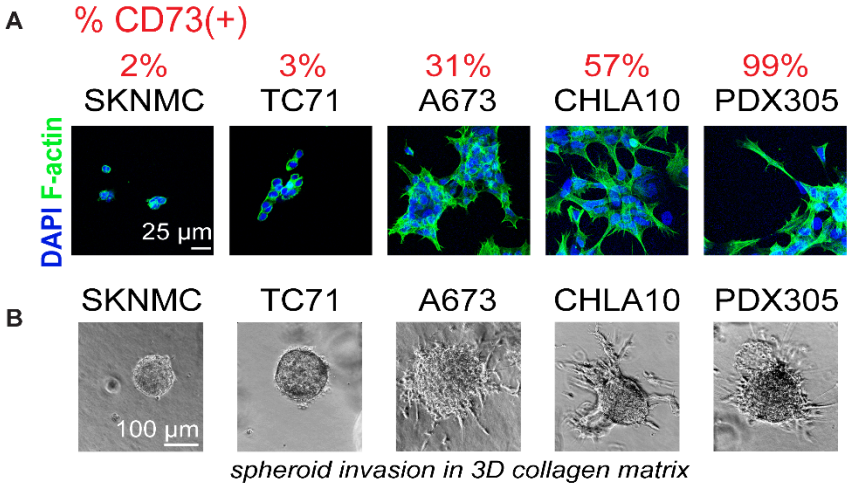
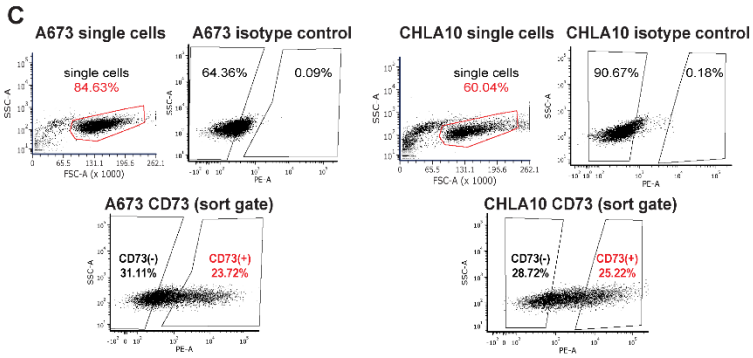
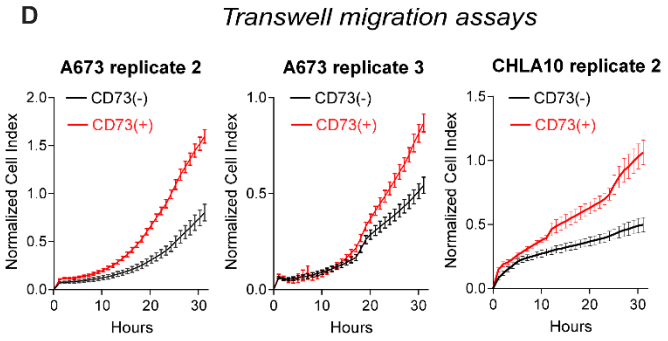
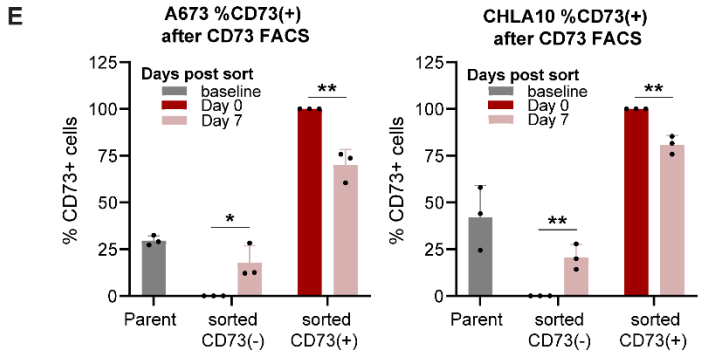
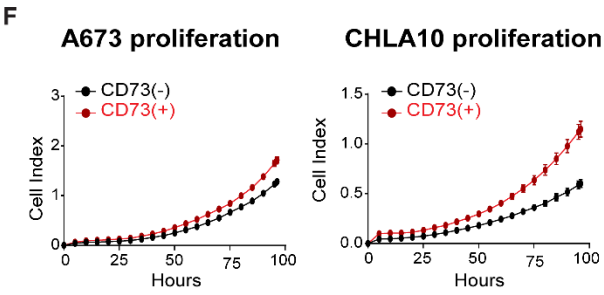
Figure S1

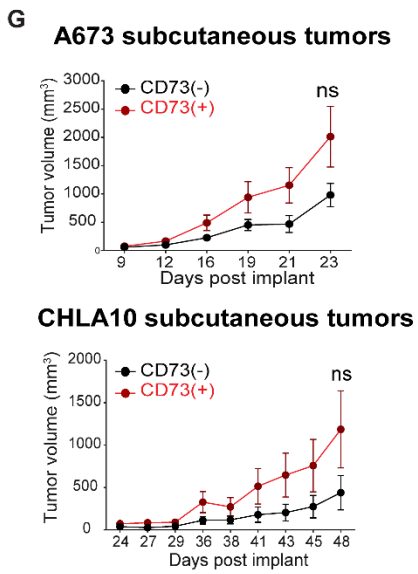

A
% CD73(+)
2%
3%
31%
57%
99%
SKNMC
TC71
A673
CHLA10
PDX305
DAPI F-actin
25 µm
B
SKNMC
TC71
A673
CHLA10
PDX305
100 µm
spheroid invasion in 3D collagen matrix

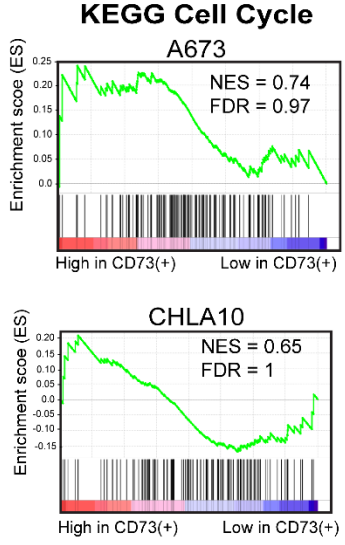

C
A673 single cells
A673 isotype control
CHLA10 single cells
CHLA10 isotype control
single cells
84.63%
64.36%
0.09%
single cells
60.04%
90.67%
0.18%
A673 CD73 (sort gate)
CHLA10 CD73 (sort gate)
CD73(-)
31.11%
CD73(+)
23.72%
CD73(-)
28.72%
CD73(+)
25.22%

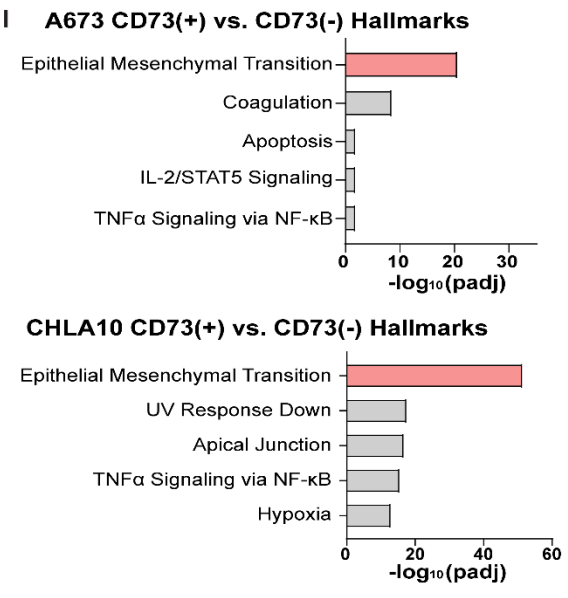

D
Transwell migration assays
A673 replicate 2
A673 replicate 3
CHLA10 replicate 2
CD73(-)
CD73(+)
Normalized Cell Index
Hours


E
A673 %CD73(+) after CD73 FACS
CHLA10 %CD73(+) after CD73 FACS
Days post sort
baseline
Day 0
Day 7
% CD73+ cells
Parent
sorted CD73(-)
sorted CD73(+)


F
A673 proliferation
CHLA10 proliferation
CD73(-)
CD73(+)
Cell Index
Hours


G
A673 subcutaneous tumors
CD73(-)
CD73(+)
ns
Tumor volume (mm³)
Days post implant
CHLA10 subcutaneous tumors
CD73(-)
CD73(+)
ns
Tumor volume (mm³)
Days post implant


H
KEGG Cell Cycle
A673
NES = 0.74
FDR = 0.97
Enrichment scoe (ES)
High in CD73(+)
Low in CD73(+)
CHLA10
NES = 0.65
FDR = 1
Enrichment scoe (ES)
High in CD73(+)
Low in CD73(+)


I
A673 CD73(+) vs. CD73(-) Hallmarks
Epithelial Mesenchymal Transition
Coagulation
Apoptosis
IL-2/STAT5 Signaling
TNFα Signaling via NF-κB
-log10(padj)
CHLA10 CD73(+) vs. CD73(-) Hallmarks
Epithelial Mesenchymal Transition
UV Response Down
Apical Junction
TNFα Signaling via NF-κB
Hypoxia
-log10(padj)

Supplemental Figure S1. CD73+ EwS cells retain proliferative and tumorigenic capacity. Related to Figure 1. A) DAPI and F-actin staining of EwS cell lines in 2D culture. Panels ordered left to right based on $CD73^+$ cell frequency. B) EwS cell spheroids from the same cell lines after 4 days in 3D spheroid culture in rat tail collagen. C) Flow cytometry gating strategy for CD73 FACS. D) Real-time proliferation assay of $CD73^-$ vs. $CD73^+$ sorted cells (n=1). Error bars = SEM of technical replicates. E) After FACS (using CD73-PE antibody) sorted $CD73^-$ or $CD73^+$ A673 or CHLA10 cells were cultured normally. 7 days later, cell surface CD73 was measured again by flow cytometry (CD73-BB515 antibody, in case of any residual CD73-PE signal). n=3, *p*-values = unpaired t-tests. F) Left, real-time transwell cell migration assay of isogenic $CD73^-$ and $CD73^+$ cells (additional biological replicates, see Figure 1E). G) Subcutaneous tumor growth of $CD73^-$ and $CD73^+$ sorted cells implanted in NSG mice (n=5). *p*-values = unpaired t-tests. Error bars = SEM. H) GSEA on RNA-seq data from $CD73^+$ vs. $CD73^-$ cells for cell-cycle related genes (KEGG) (n=3). I) Gene ontology (Enrichr) of RNA-seq data for Hallmark gene sets in $CD73^+$ vs. $CD73^-$ cells.