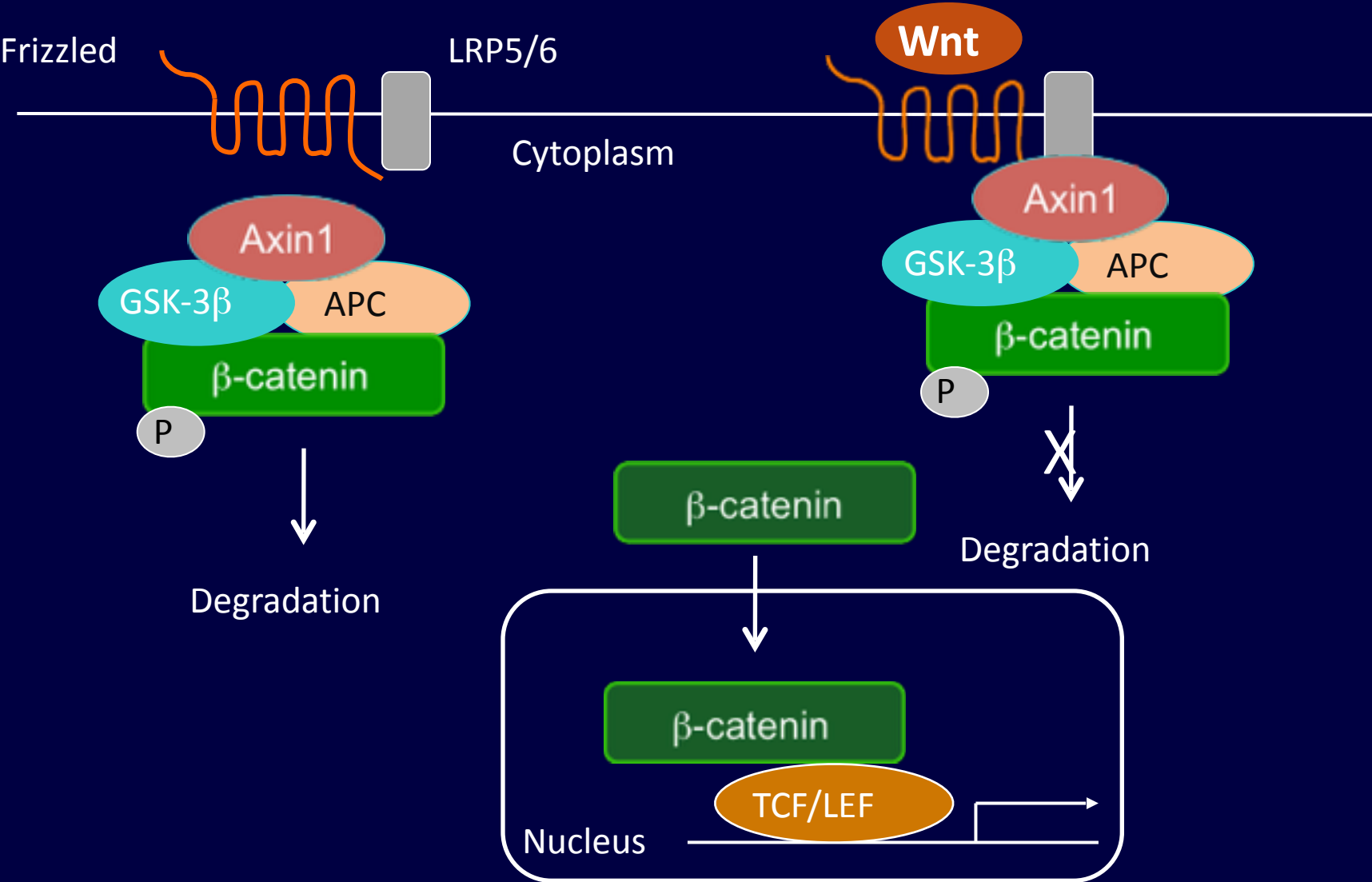


Basic Properties of Wnt Ligands (Wingless + Intron-1)

- 19 in mammals
-
- 40 kDa Lipid-modified glycoproteins
 - Palmitoylation (activity)
- N-linked glycosylation (regulates secretion)
- Wnt ligands regulate fundamental and specialized cellular processes (development, differentiation, proliferation, apoptosis, *etc.*)

(23,925 papers in Pubmed, over 1,218 this year, 10 per day)

Wnt/ β -catenin (canonical) signaling pathway

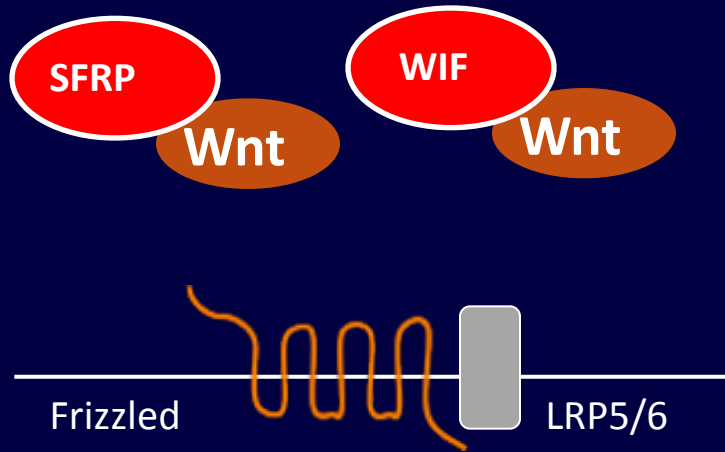


Canonical Wnt signaling in cancer

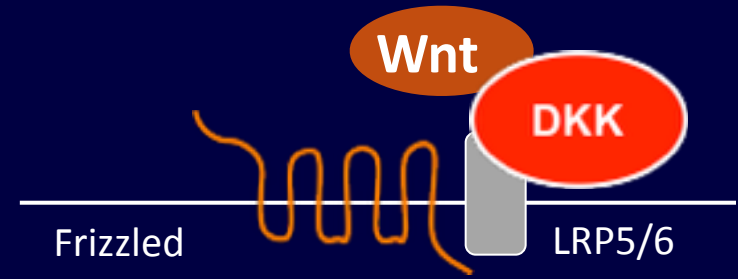
- **Wnt-1** cloned based on integration of mouse mammary tumor virus
- APC (Adenomatous polyposis coli) mutated in 80% colon cancer
- CTNNB1 (beta-catenin) mutated in...
 - 18% liver and uterine endometrial cancer
 - 5% prostate cancer
- No mutations found in AML
- Pathway is activated independent of mutations
 - Epigenetic regulation of pathway factors

Activating Wnt signaling in AML

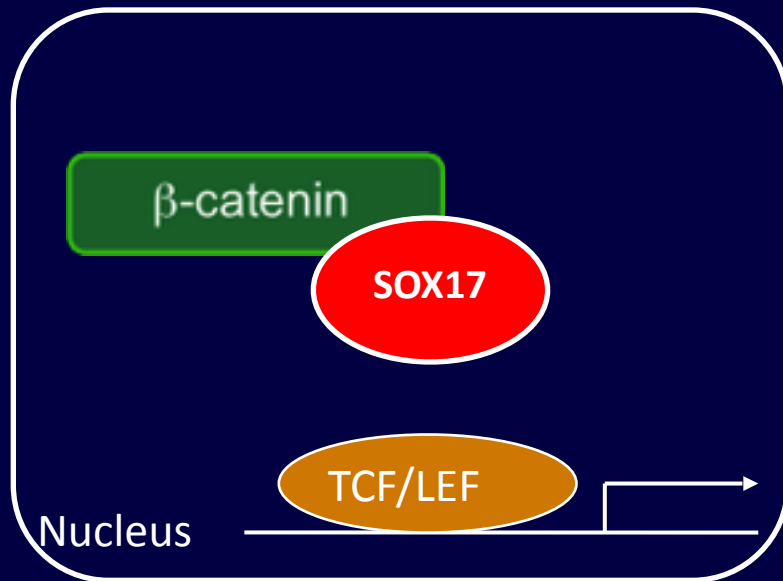
A



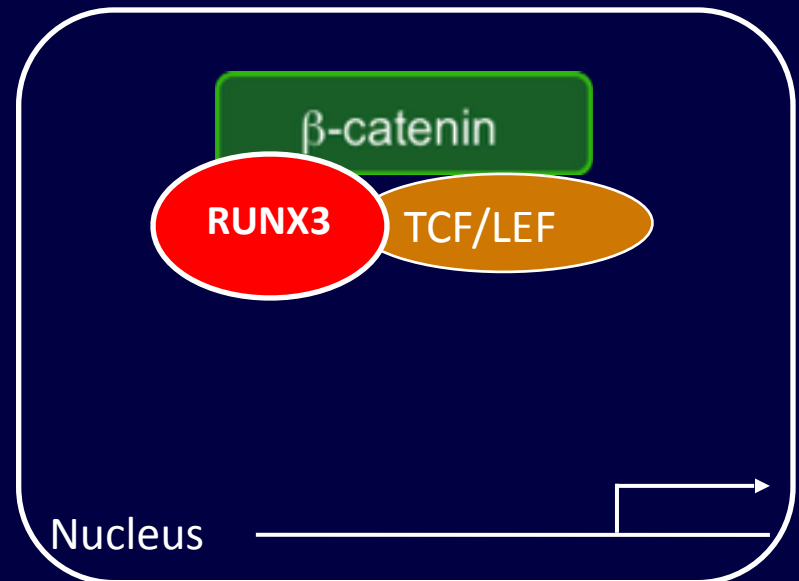
B



C

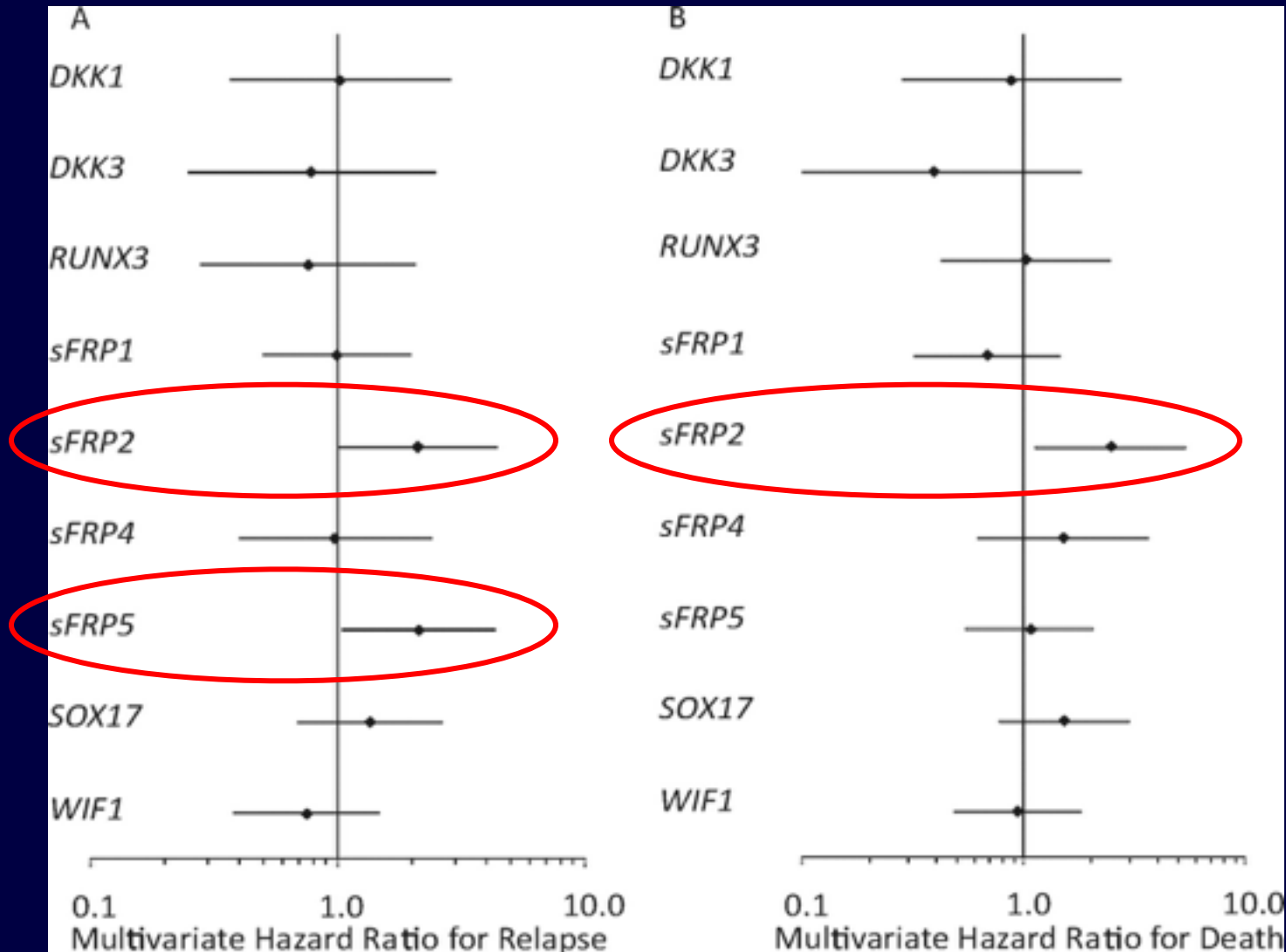


D



Epigenetic activation of Wnt signaling in AML

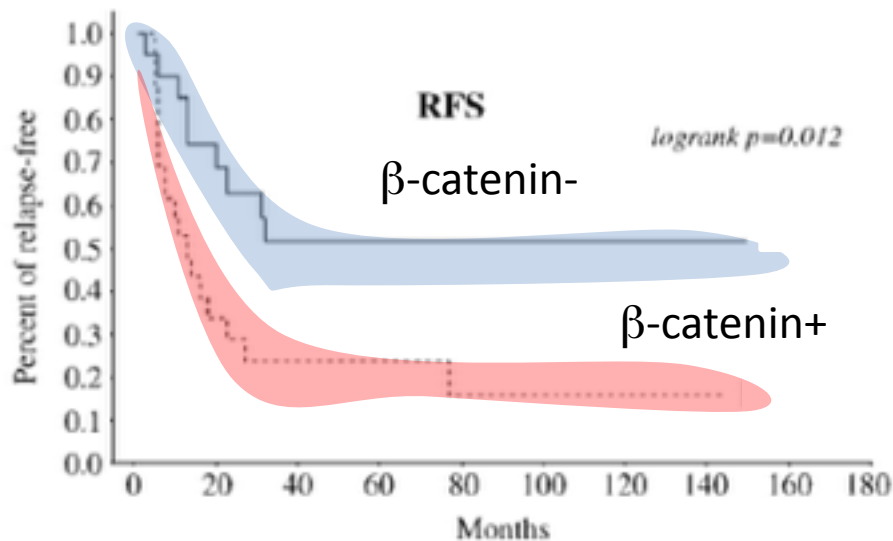
72 NK AML Patients, uniformly treated (Griffiths, et al., 2010)



Canonical Wnt signaling in AML

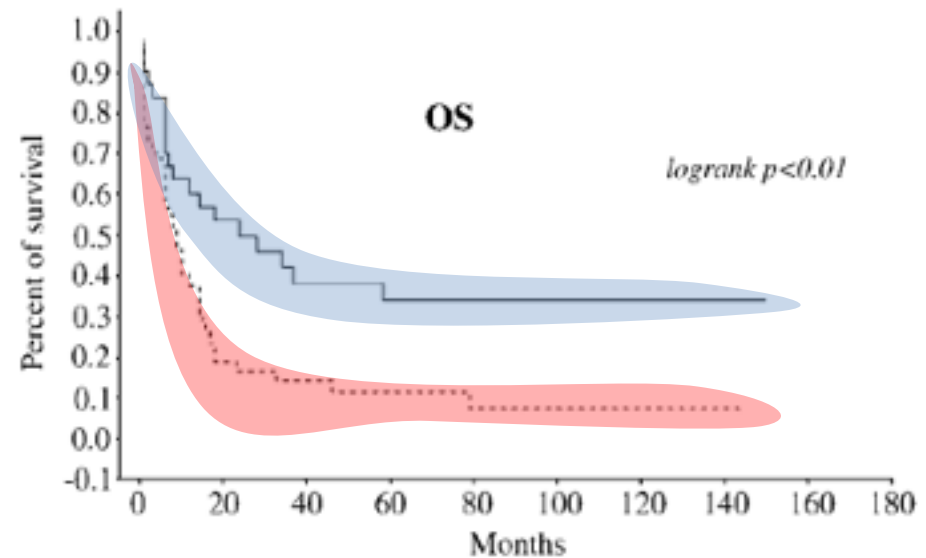
- Dispensable for normal hematopoiesis
- Indispensable for AML (mouse genetics)
- β -catenin levels correlate with increased relapse rate

Relapse Free Survival



N=49

Overall Survival



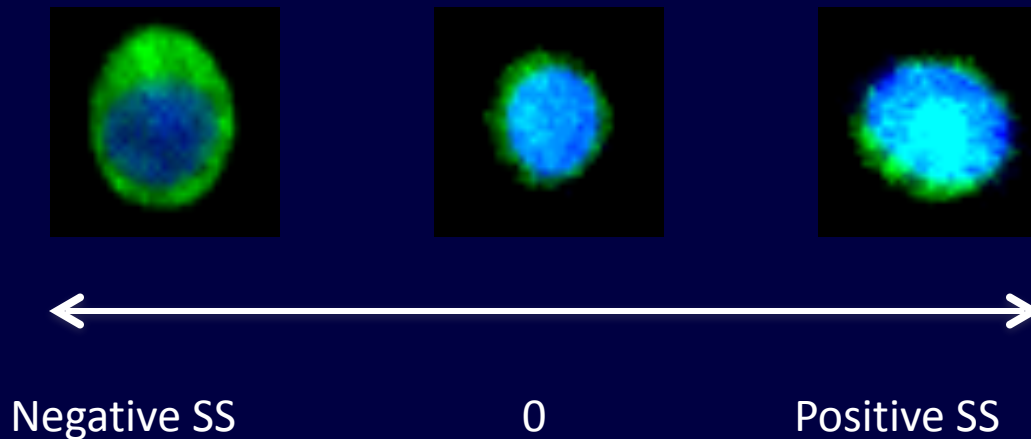
N=82

Canonical Wnt signaling in AML

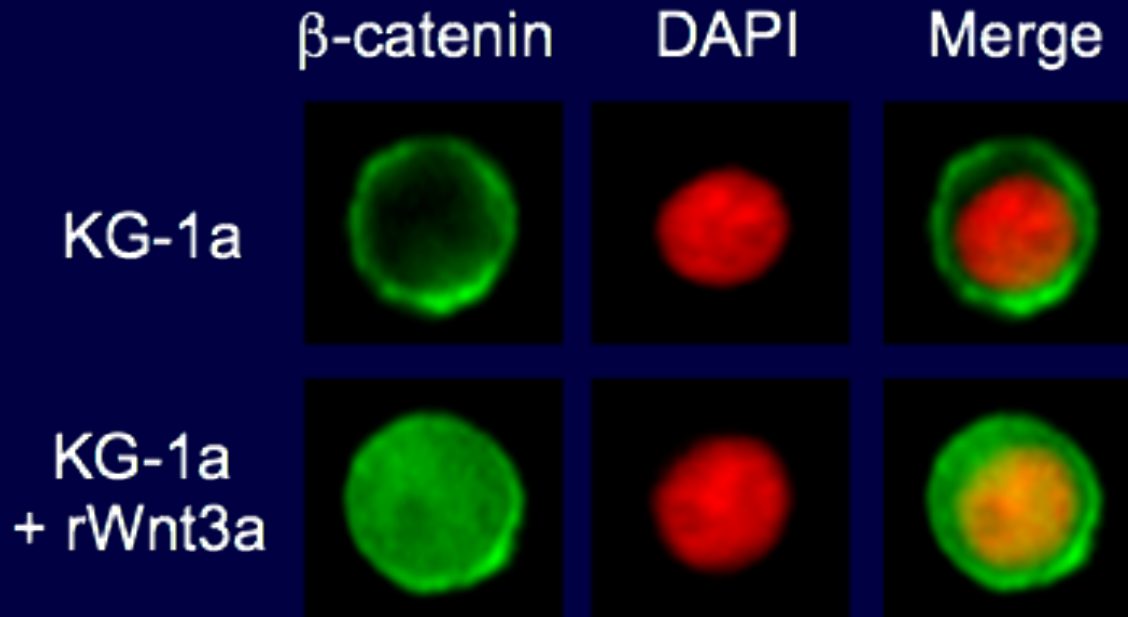
- β -catenin knock-out models: prevent development of AML
- Knock-down of β -catenin in human AML cell lines suppresses proliferation and *in vivo* growth
- Knock-down of β -catenin in primary human AML has variable effect
- Identify stage/type of AML where targeting oncogenic Wnt signaling will have the most benefit
 - ◇ Therapeutic inhibitors of Wnt signaling progressing towards clinic

ImageStream Analysis

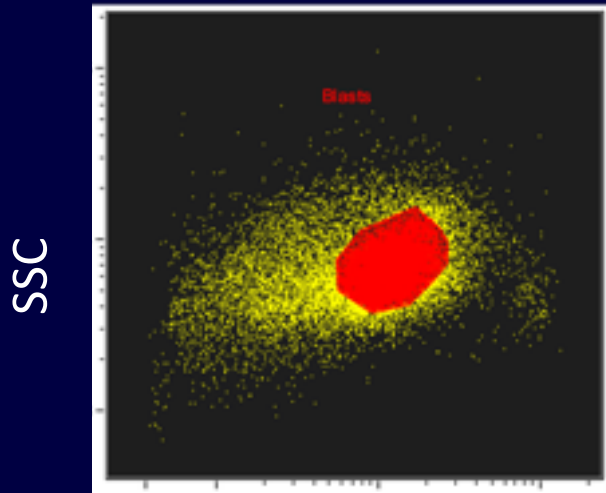
- Flow cytometer that combines basic flow parameters with the addition of visual images (as in microscopy)
- Can detect cellular localization in defined populations
- Similarity score – *quantifies* co-localization between beta-catenin (**AlexaFluor 488**) and nuclear stain (**DAPI**)



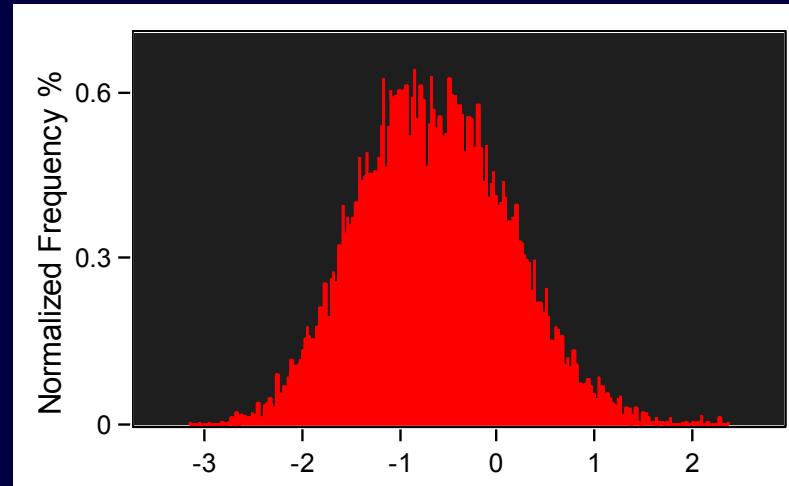
ImageStream Analysis of Wnt Signaling



ImageStream Analysis of AML Samples



CD45



Median Similarity Score = -0.67

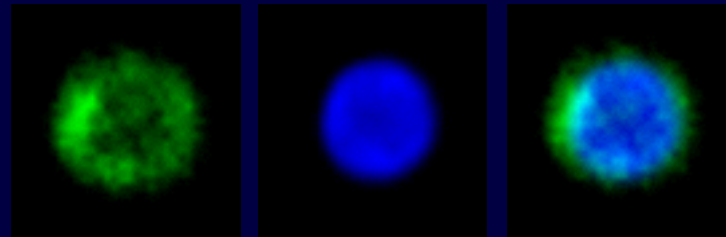
AML Blasts

Low Side Scatter
Low CD45

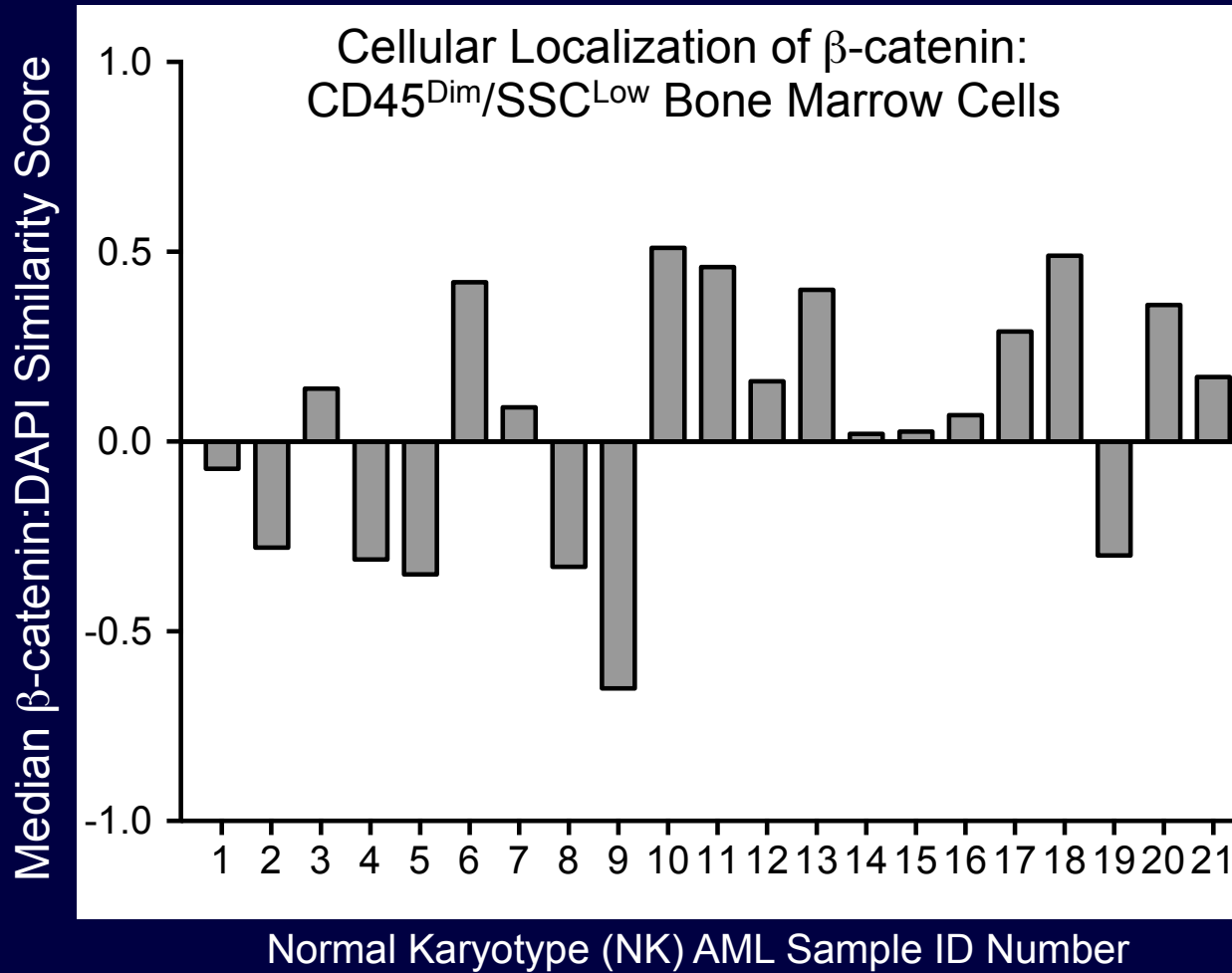
β -catenin

DAPI

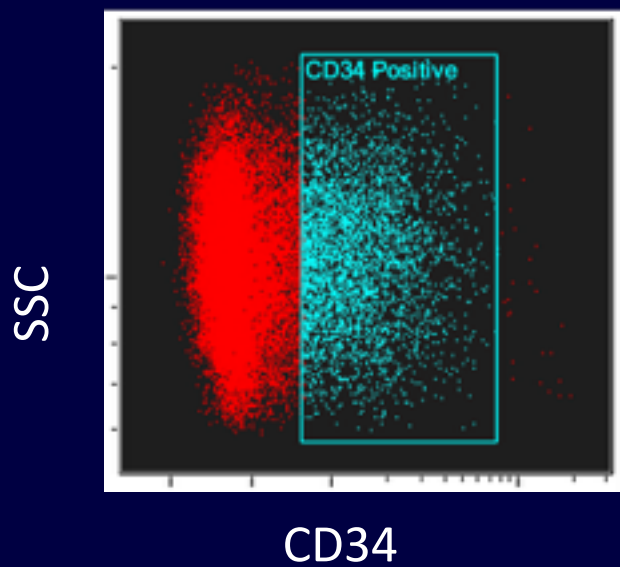
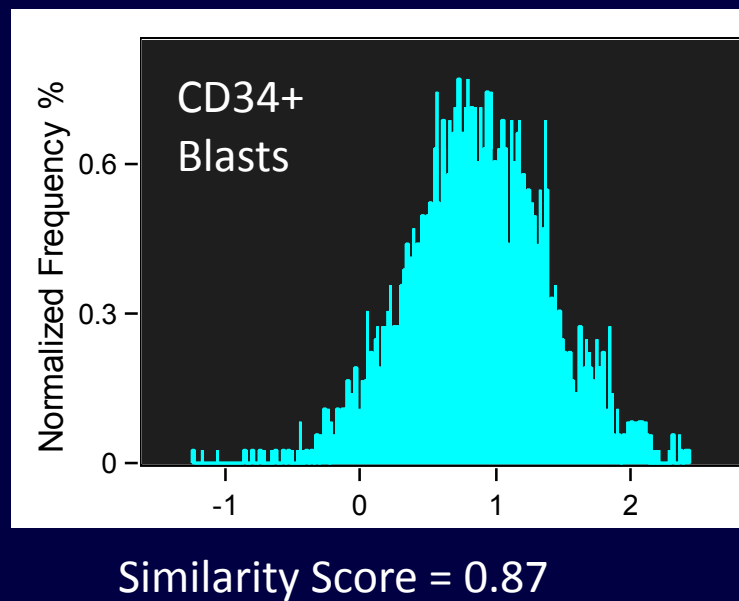
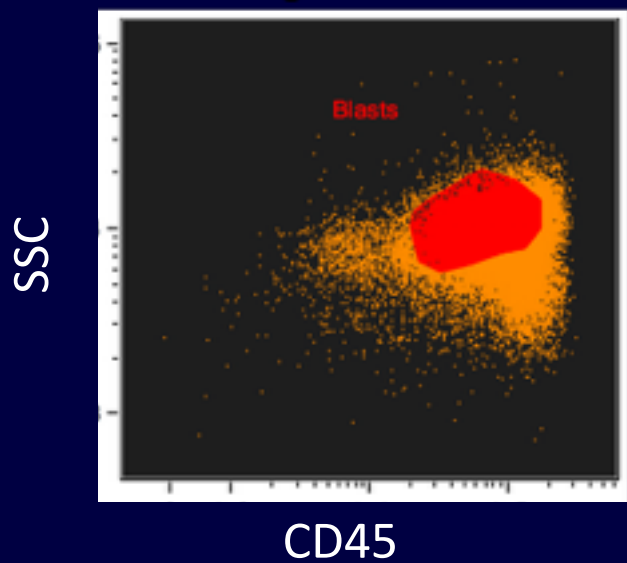
Merge



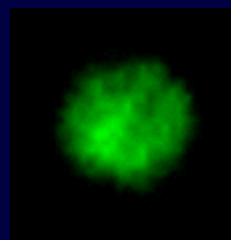
ImageStream Analysis of AML Samples



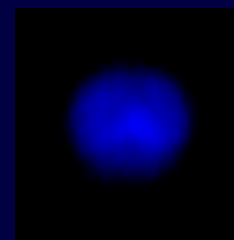
ImageStream Analysis of AML Samples



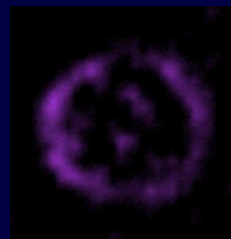
β -catenin



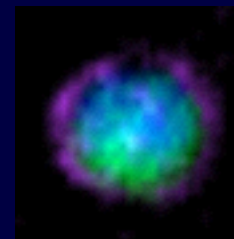
DAPI



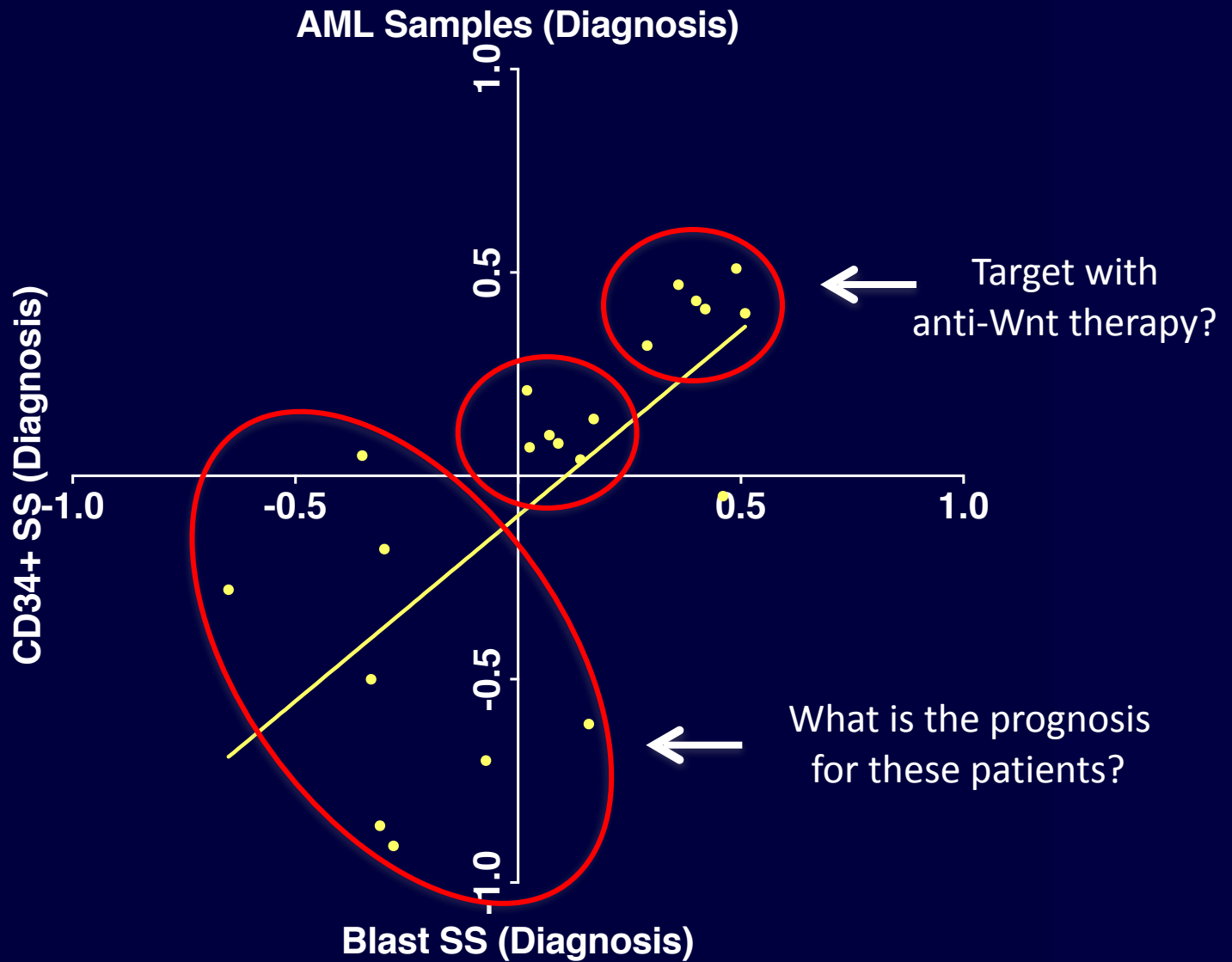
CD34



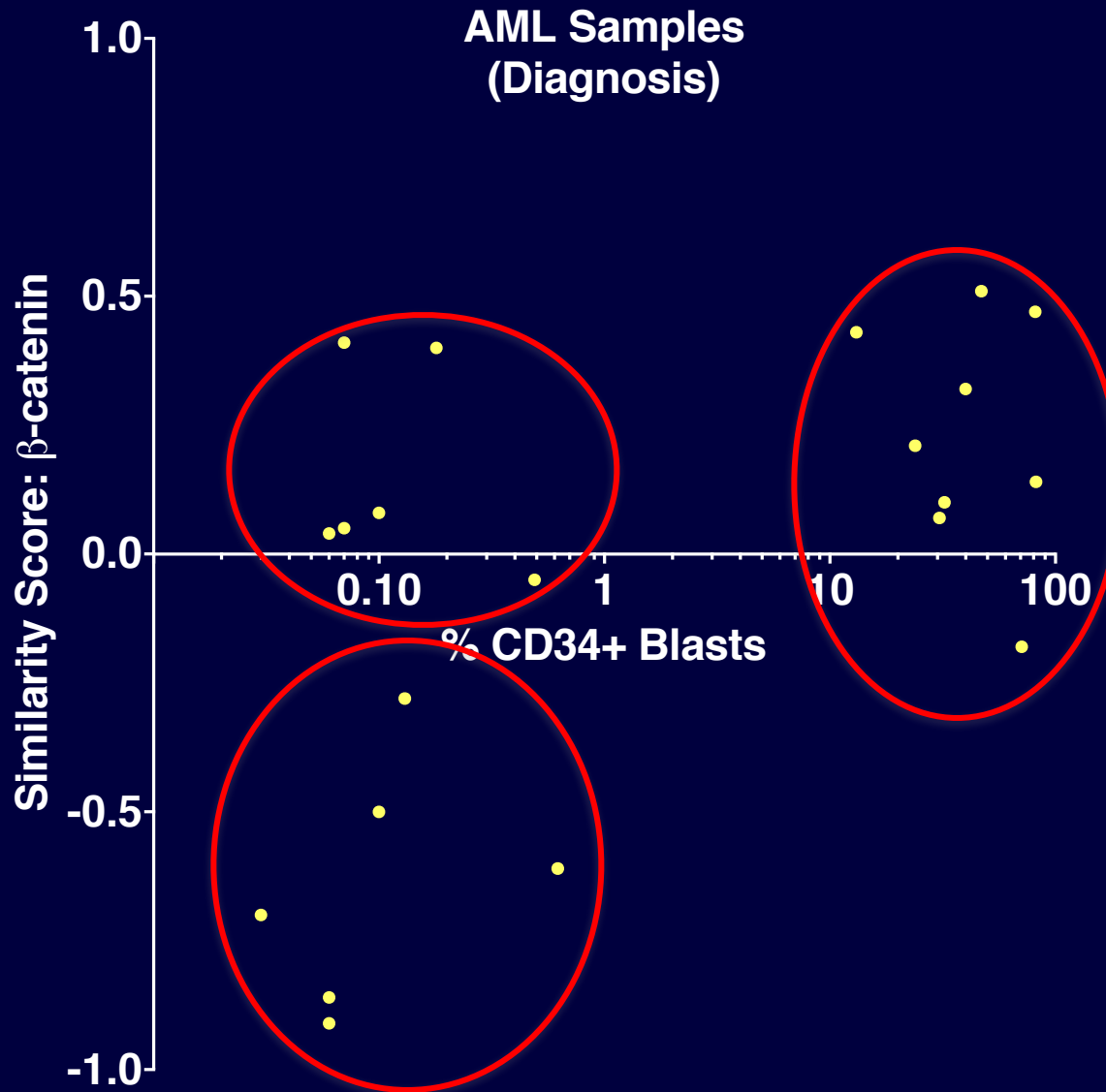
Merge



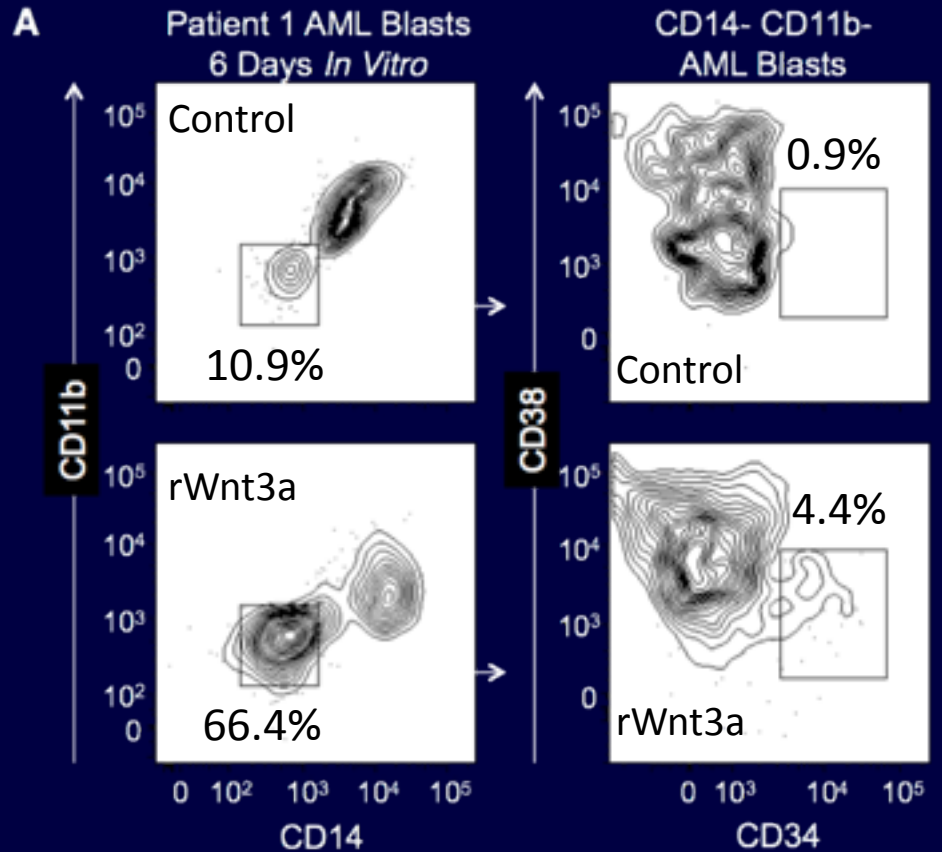
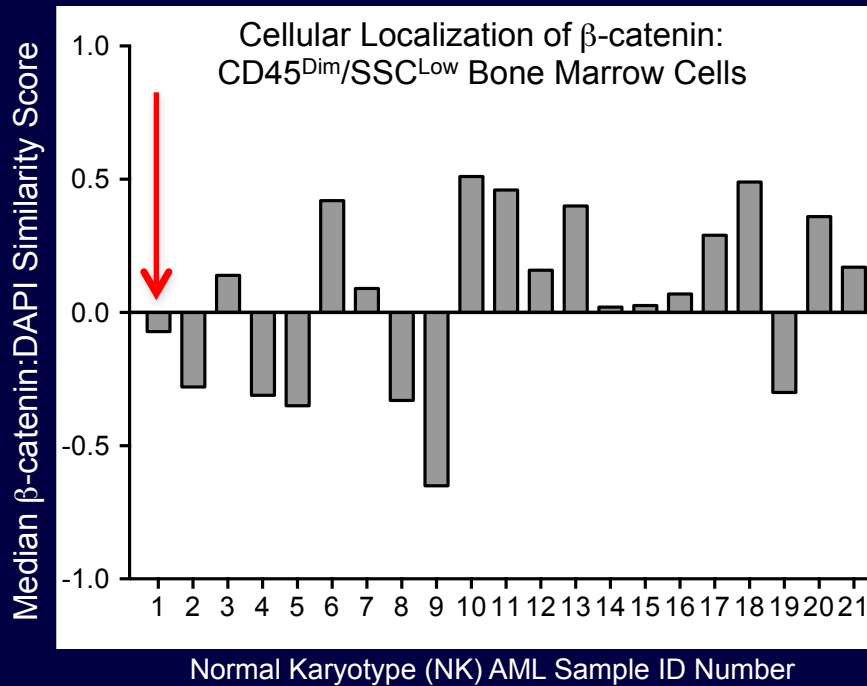
ImageStream Analysis of AML Samples



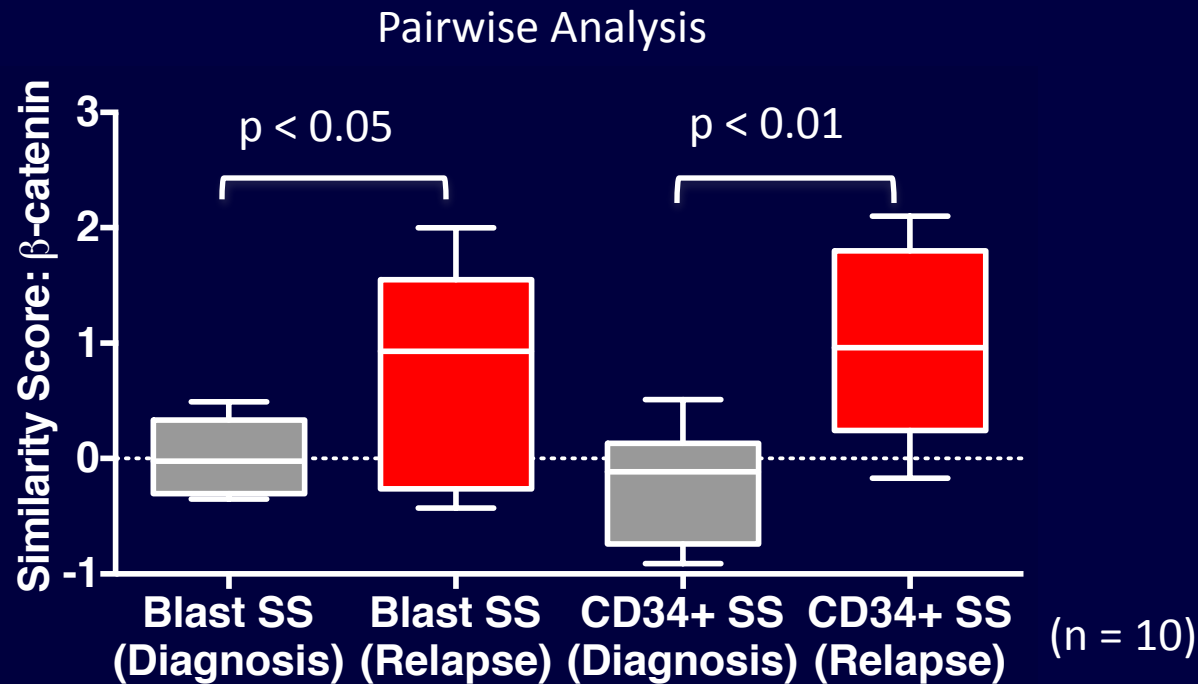
Increased numbers of CD34+ blasts are associated with increased nuclear localization of β -catenin



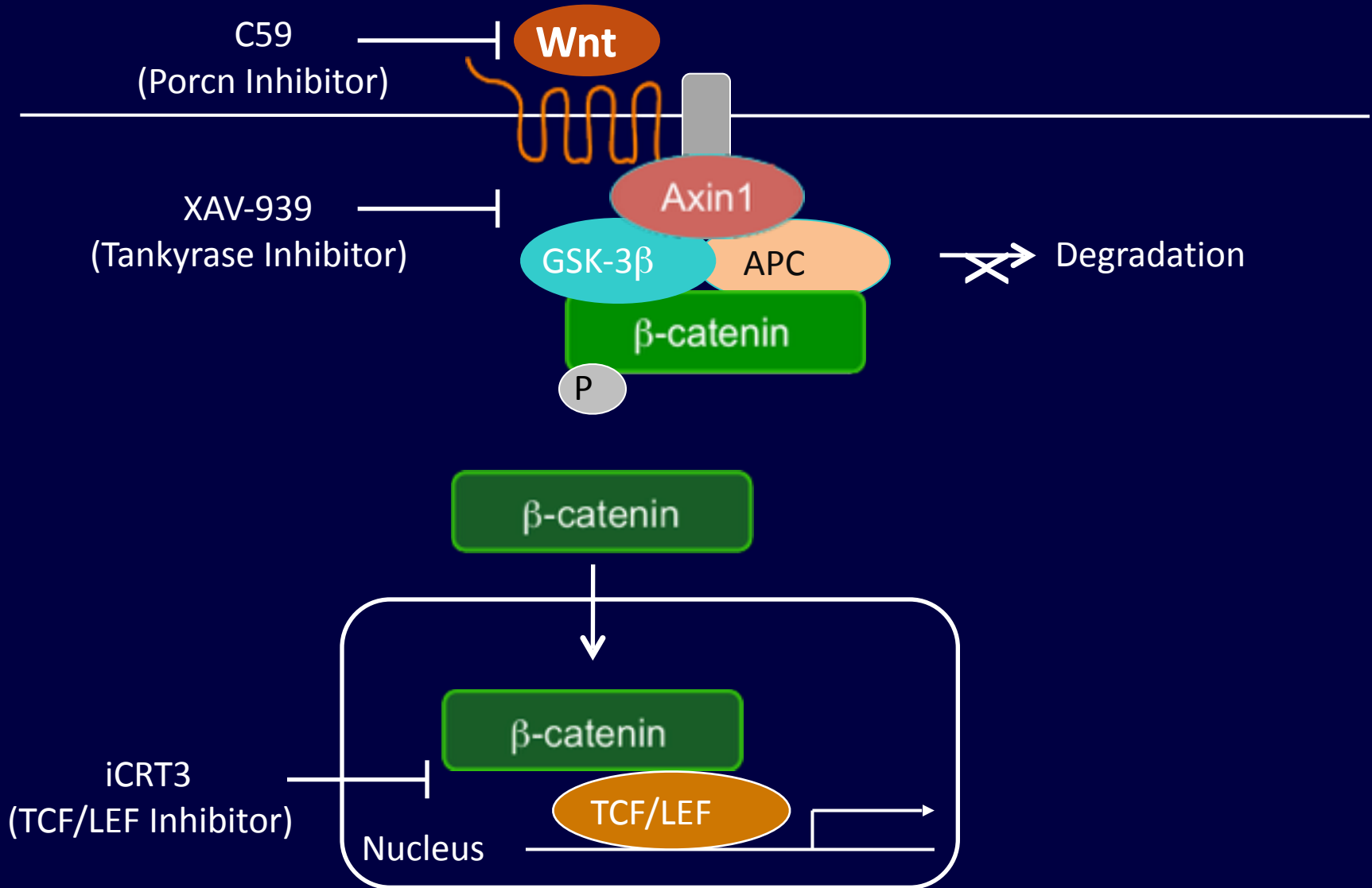
Wnt pathway is intact in AML cells

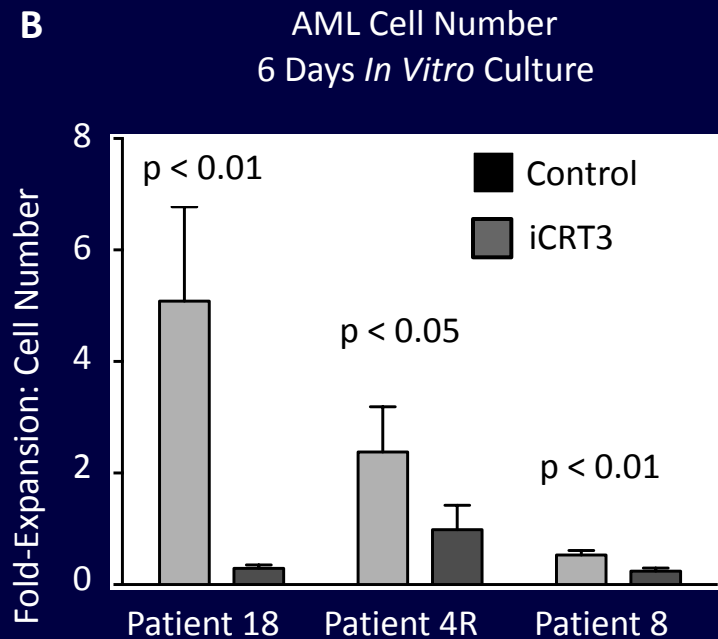
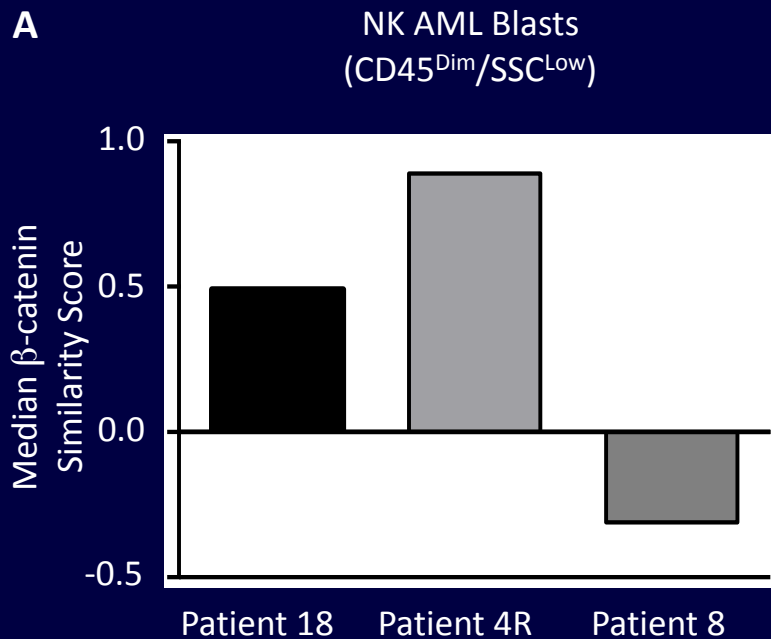


Relapsed AML has increased nuclear localization of β -catenin

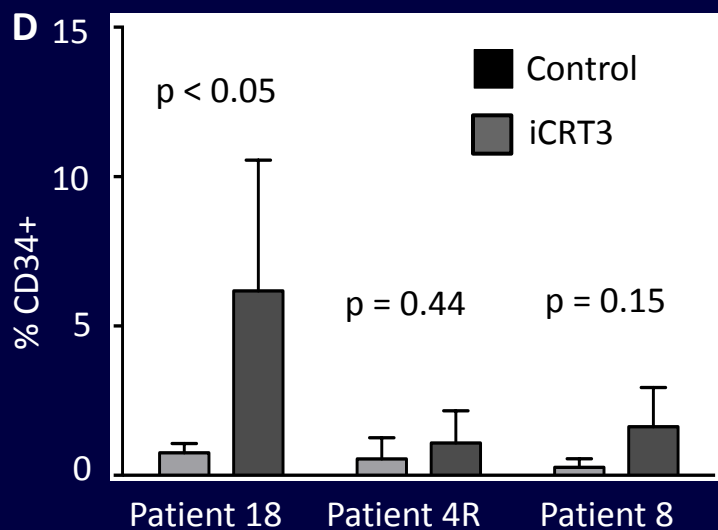
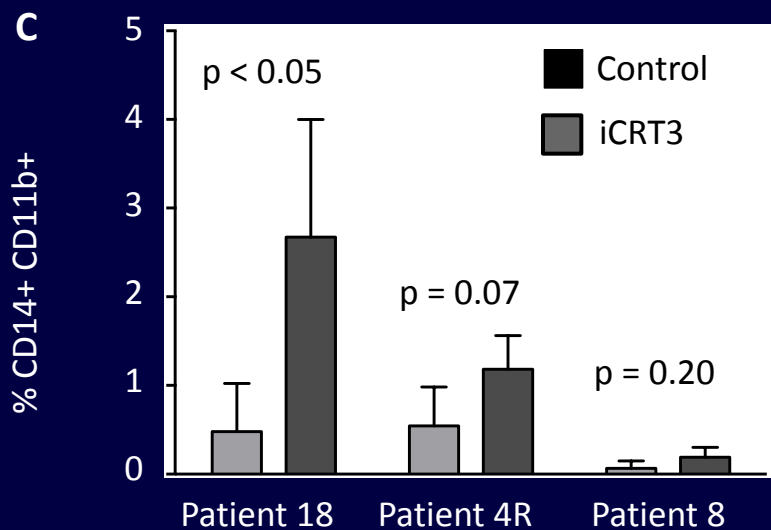


Targeting Wnt/ β -catenin signaling





AML Blast (CD45^{Dim}/SSC^{Low}) Phenotype 6 Days *In Vitro* culture



Canonical Wnt signaling in AML

- Treatment of primary human AML cells has variable effect
- Determine effect on paired diagnostic *versus* relapse sample
- Identify potential for resistant sub-populations
- Test alternate inhibitors (e.g. C59 and XAV-939)