Heparanase and syndecan-1: Promoters of aggressive myeloma behavior and targets for therapy
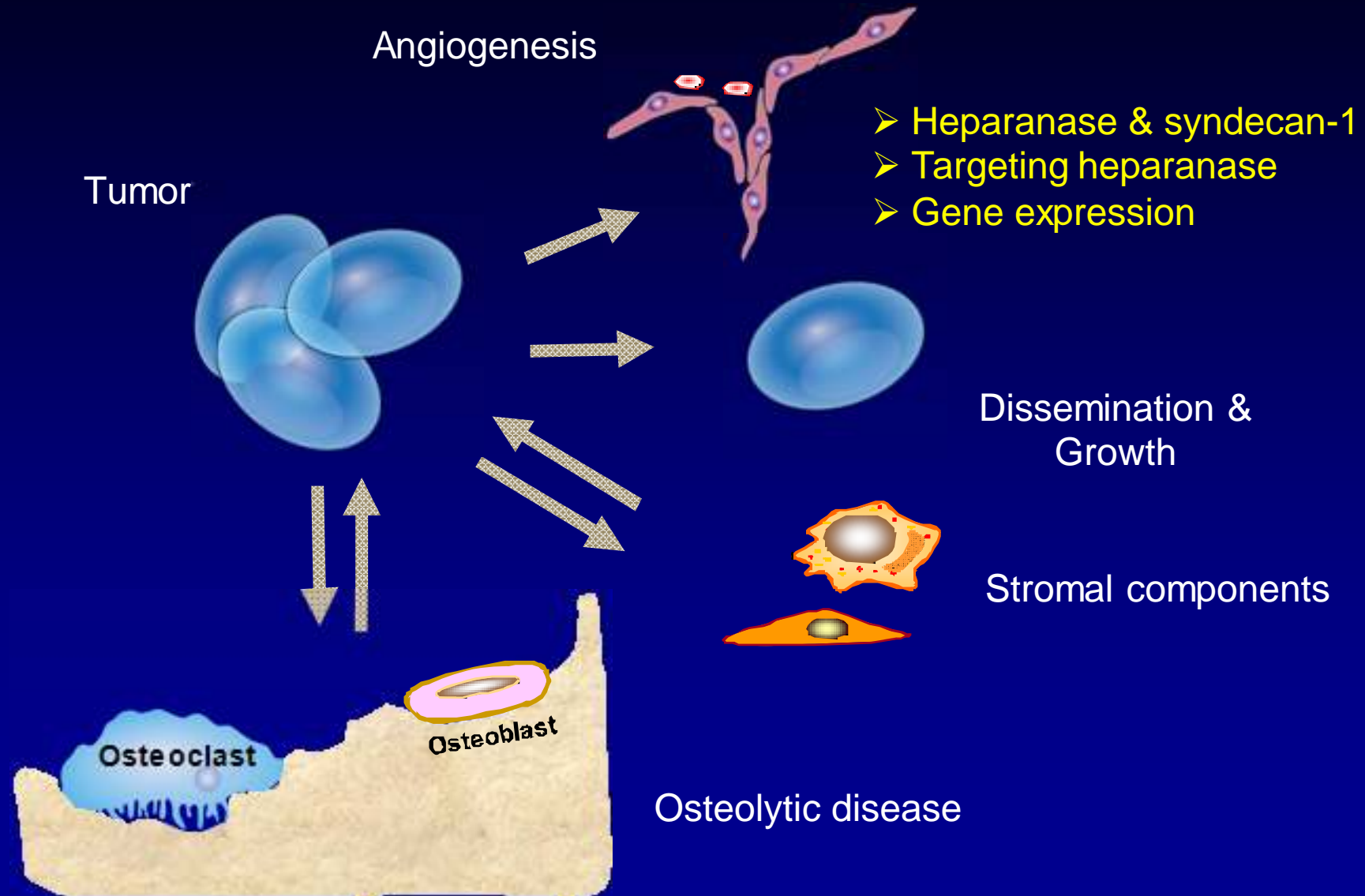
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UAB THE UNIVERSITY OF
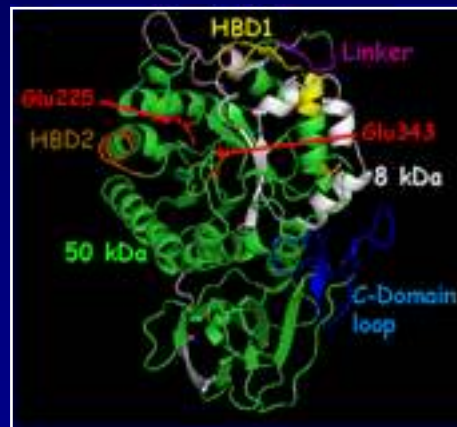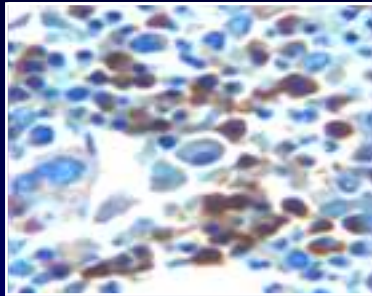ALABAMA AT BIRMINGHAM

Conflict of interest disclosure

Tumor-host crosstalk regulates the microenvironment to promote myeloma progression

Angiogenesis

Tumor

➢ Heparanase & syndecan-1
➢ Targeting heparanase
➢ Gene expression

Dissemination & Growth

Stromal components

Osteoclast

Osteoblast

Osteolytic disease

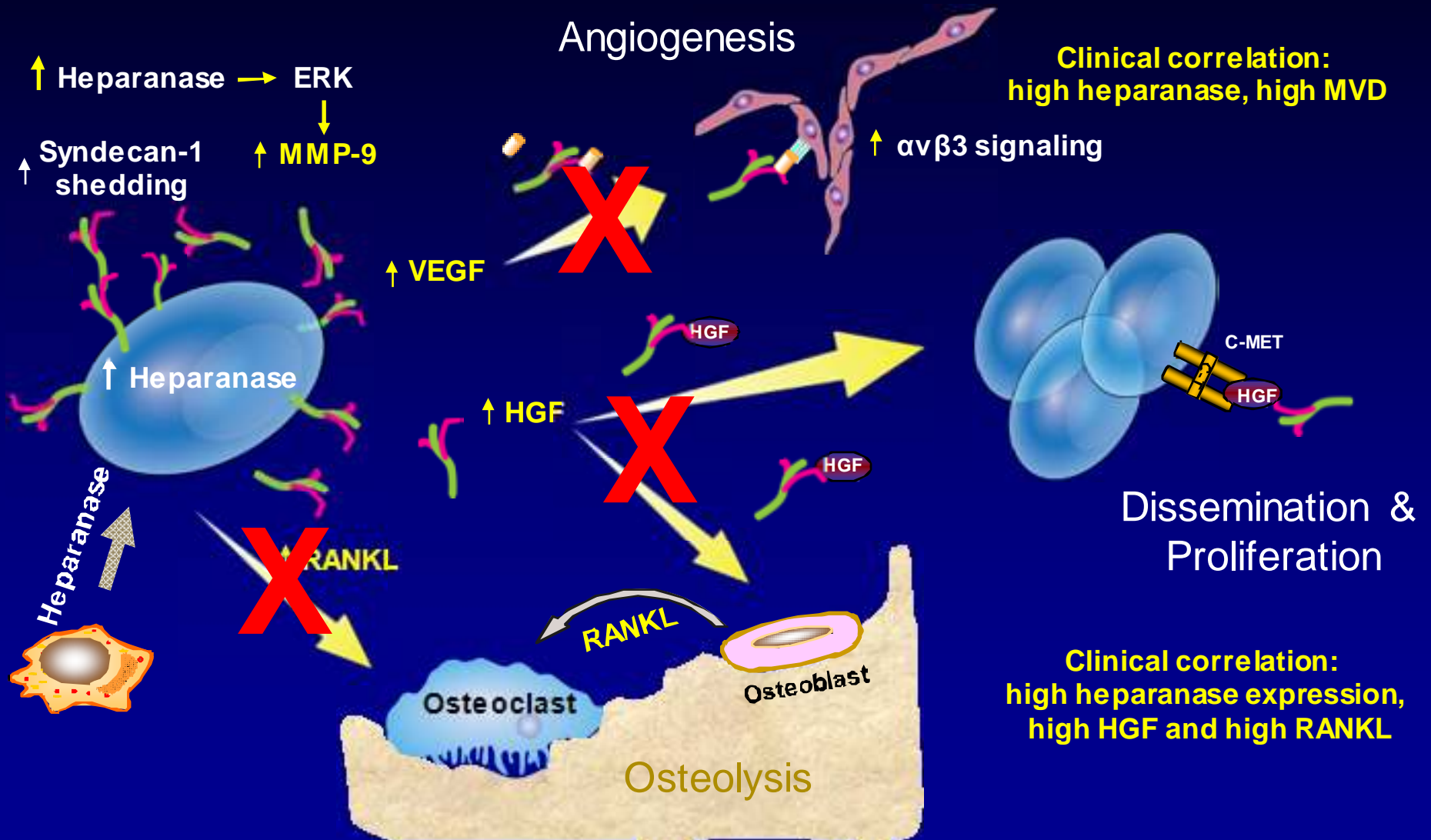# Heparanase and syndecan-1 promote myeloma progression

Heparanase

- High heparanase activity in patient plasma correlates with high microvessel density (Kelly *et al.*, 2003)
- Heparanase promotes myeloma growth and metastasis (Yang *et al.*, 2005)
- High heparanase is an indicator of poor prognosis in myeloma (Mahtouk *et al.*, 2007)

Syndecan-1 (CD138)

Heparanase

Heparan sulfate

Core protein

Sheddase

Cell membrane
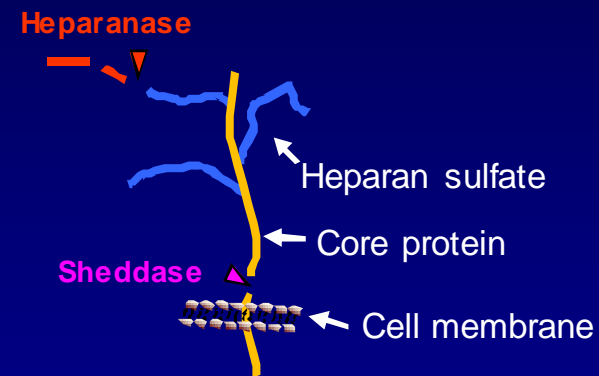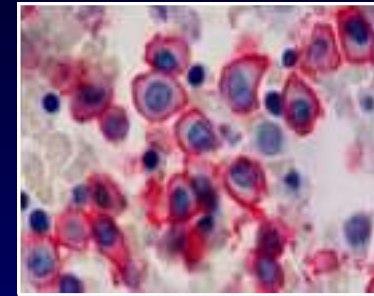
- High syndecan-1 in patients serum correlates with high tumor mass and poor prognosis (Dhodapkar *et al.*, 1997; Seidel *et al.*, 2000)
- Shed syndecan-1 enhances myeloma growth and metastasis in vivo (Yang *et al.*, 2002)
- Knockdown of syndecan-1 or heparan sulfate inhibits growth in vivo (Khotskaya *et al.*, 2009; Reijmers *et al.*, 2010)

Heparanase and syndecan-1 regulate the myeloma microenvironment

Angiogenesis

↑ Heparanase → ERK

↓

Syndecan-1 shedding

↑ MMP-9

Clinical correlation: high heparanase, high MVD

↑ αvβ3 signaling

↑ VEGF

↑ Heparanase

HGF

↑ HGF

HGF

C-MET

HGF

Heparanase

RANKL

RANKL

Osteoclast

Osteoblast

Osteolysis

Dissemination & Proliferation

Clinical correlation: high heparanase expression, high HGF and high RANKL

SST0001: A potent heparanase inhibitor engineered by chemically modifying heparin

Heparanase as a therapeutic target:
- there appears to be a single active heparanase in humans
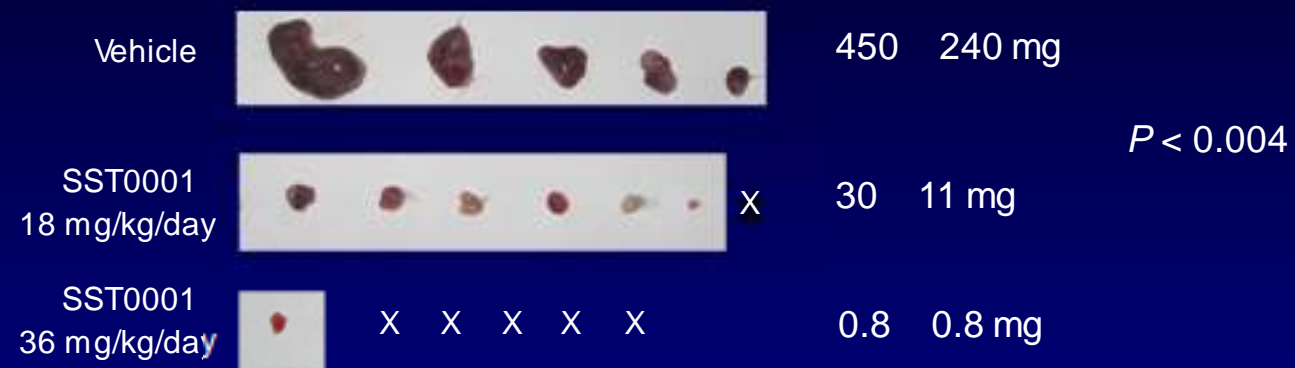- heparanase knockout mice show no obvious deficits
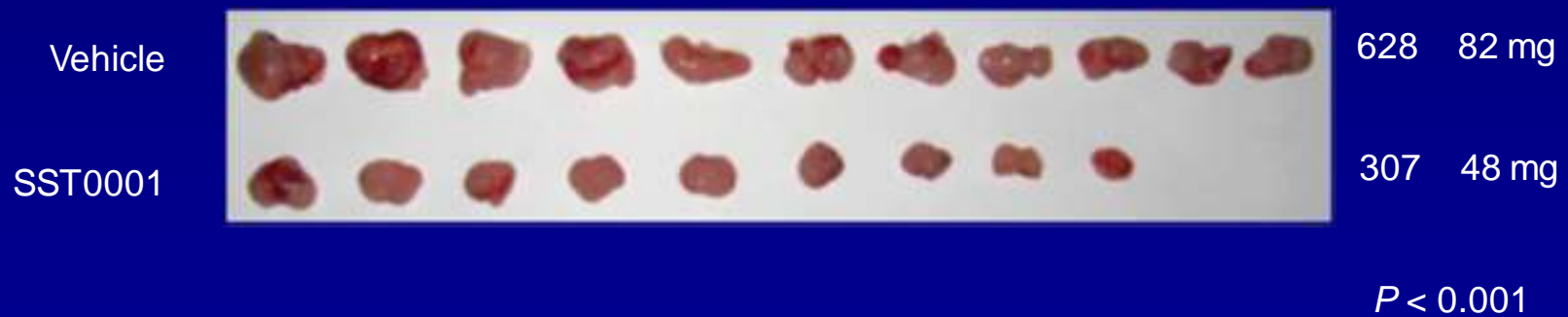

Heparin
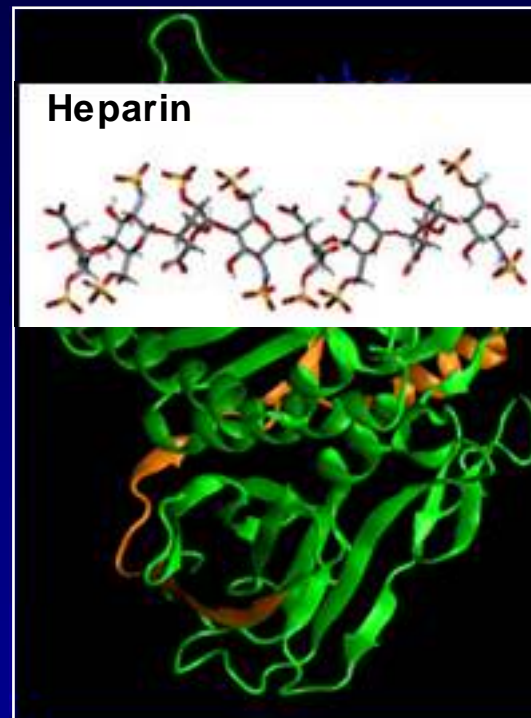
ST0001

SST0001:
- potent inhibitor of heparanase activity
- non-anticoagulant
- not degraded by heparanase

SST0001 inhibits growth of subcutaneous myeloma tumors

CAG tumors

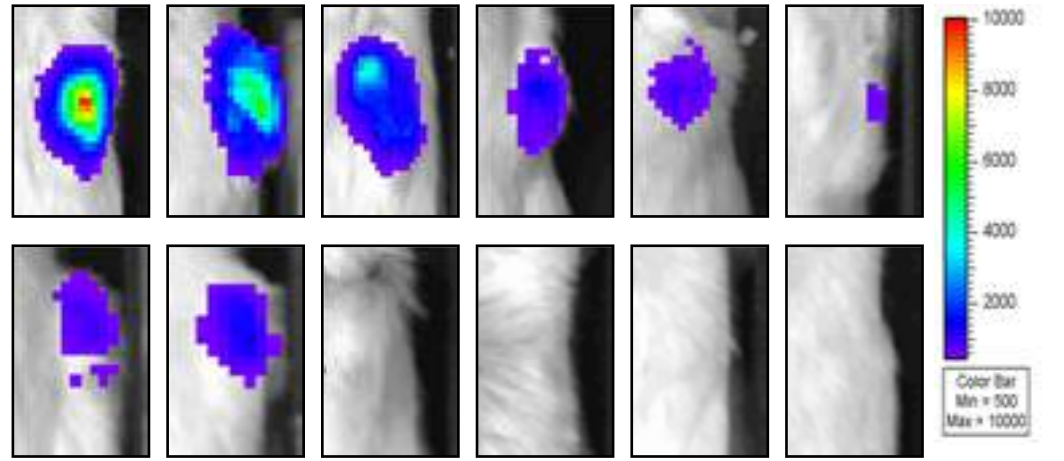|			
Vehicle		450	240 mg
SST0001 18 mg/kg/day	X	30	11 mg
SST0001 36 mg/kg/day	X X X X X	0.8	0.8 mg

P < 0.004

RPMI-8226 tumors

Vehicle		628	82 mg
SST0001		307	48 mg

P < 0.001
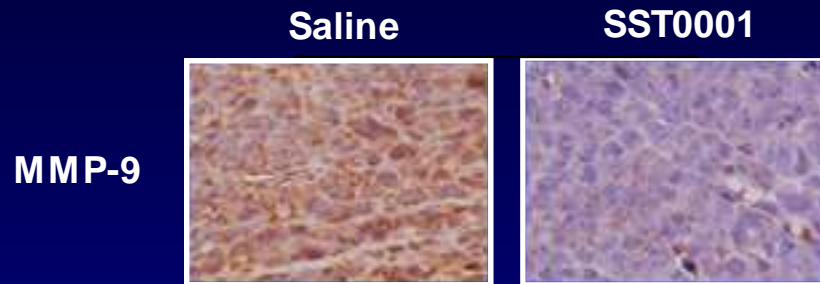
SST0001 blocks CAG myeloma tumor growth in human bones
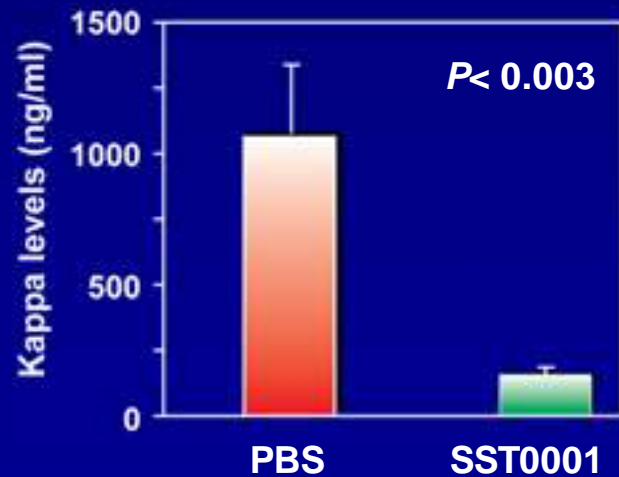
SST0001 inhibits heparanase-enhanced syndecan-1 shedding, angiogenesis and gene expression

SST0001 inhibits heparanase-enhanced syndecan-1 shedding, angiogenesis and gene expression

Saline   SST0001

VEGF

HGF

Subcutaneous tumors
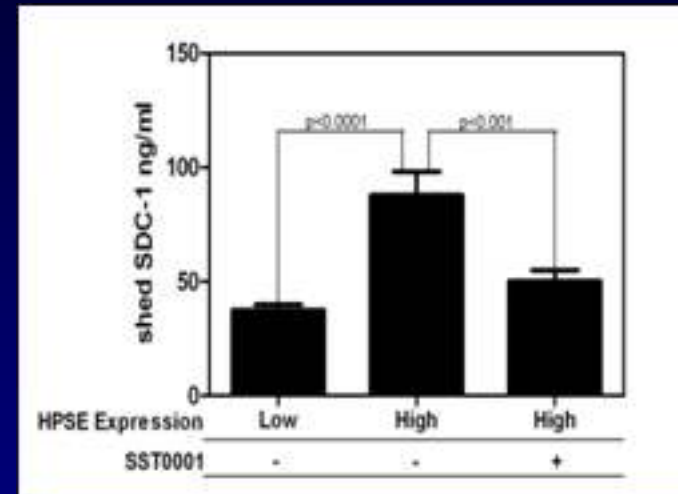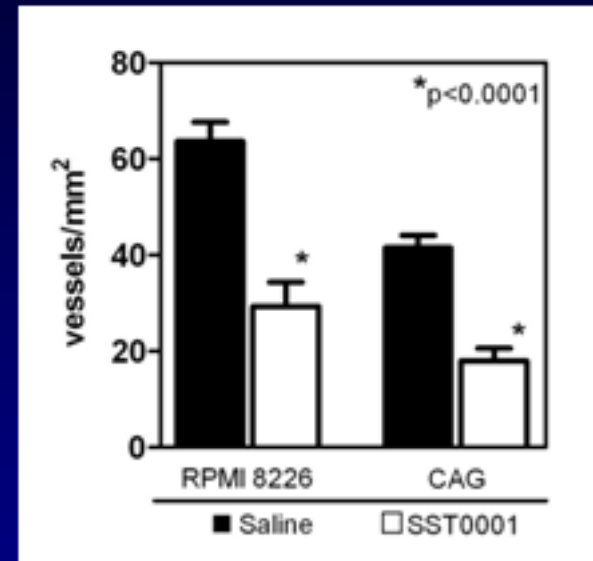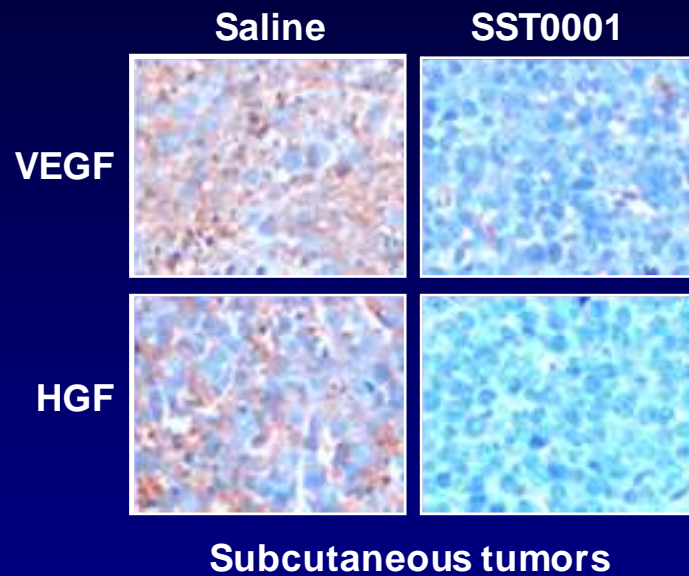
MVD analysis in SCID-hu tumors

SST0001 disrupts the establishment of a microenvironment that supports aggressive tumor growth
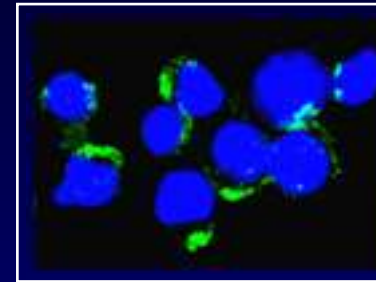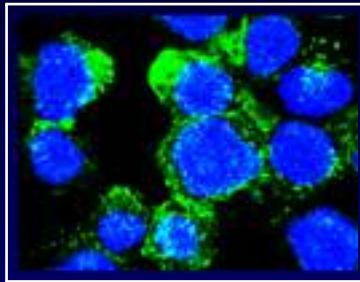
How is heparanase regulating gene expression?
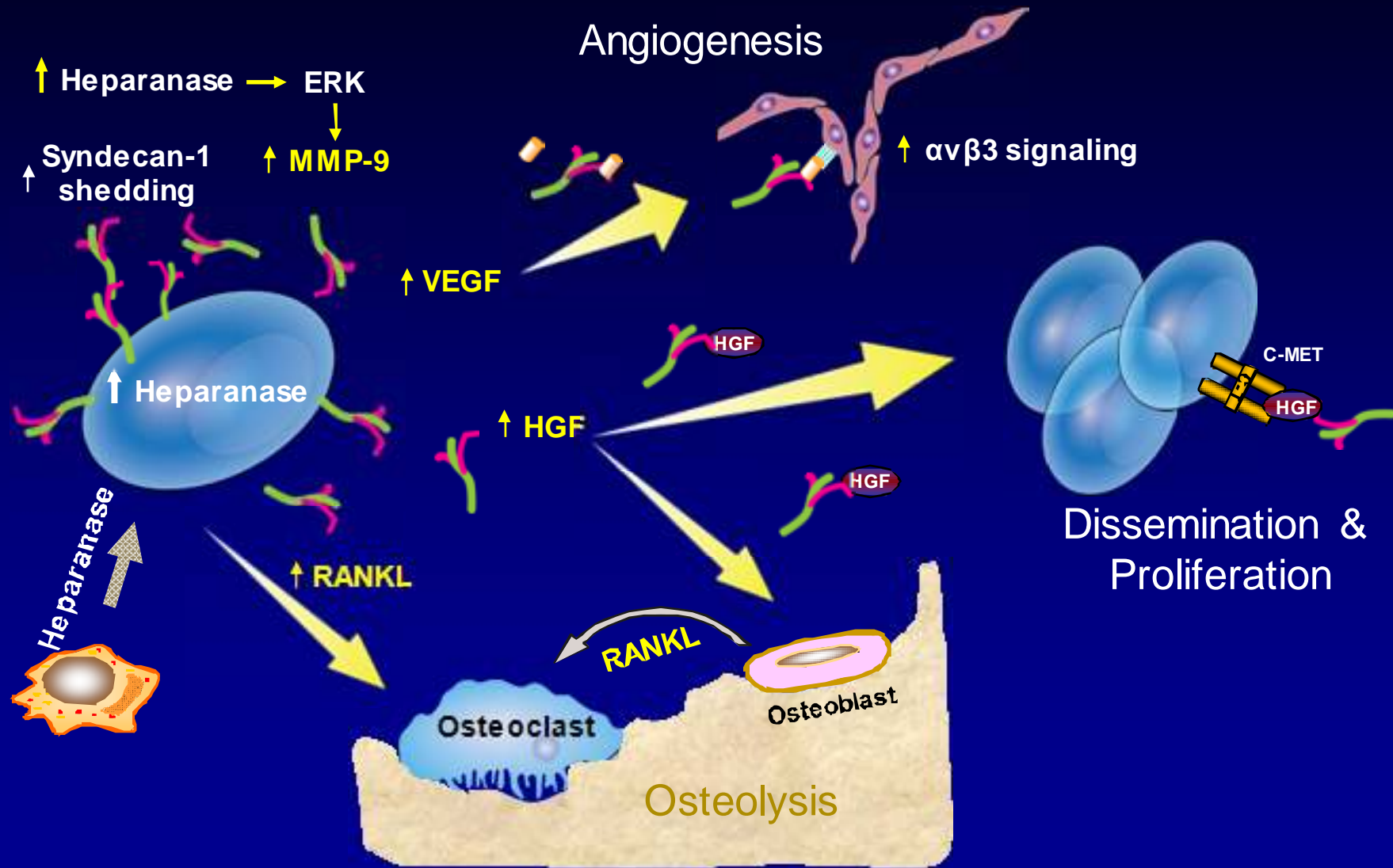
Heparanase decreases the level of nuclear syndecan-1 in myeloma cells

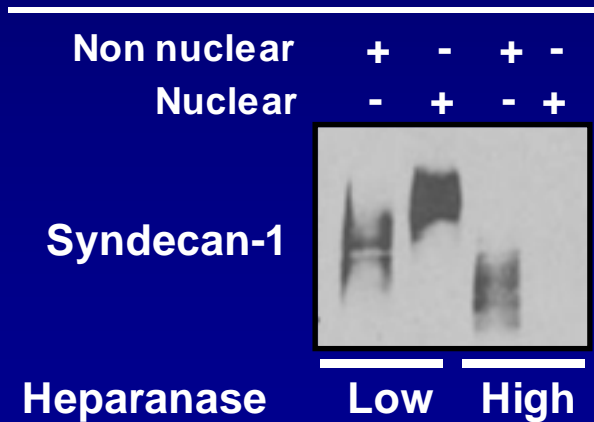**Heparanase expression :**
(CAG myeloma cells)

Low   High
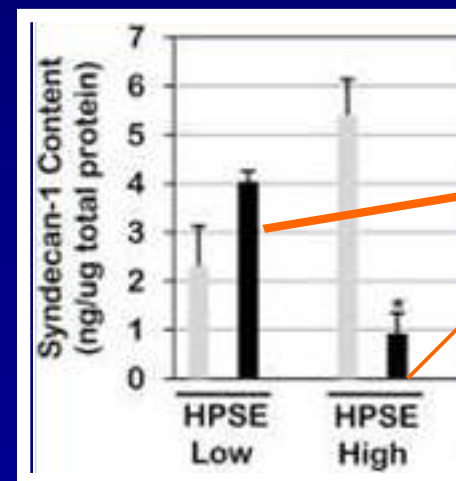

Immunostaining for syndecan-1

Western Blot

Non nuclear	+	-	+	-
Nuclear	-	+	-	+

Syndecan-1

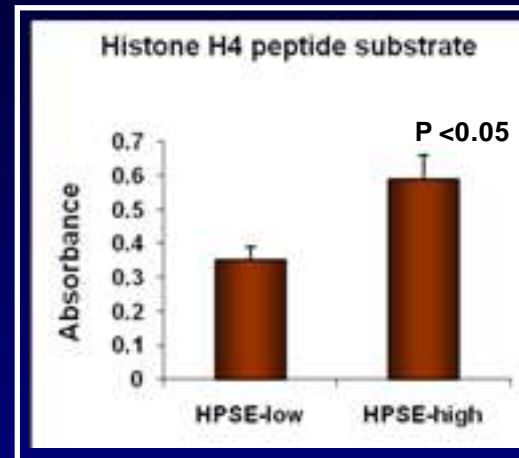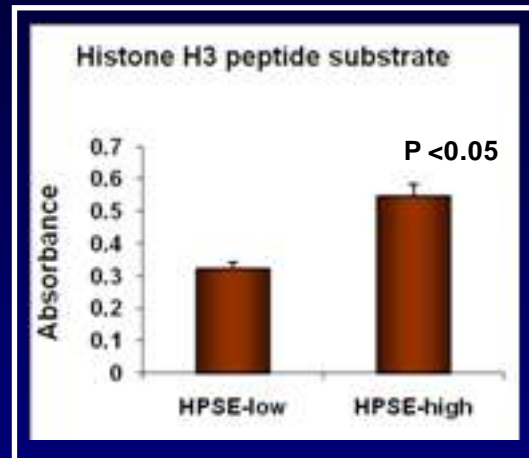**Heparanase**   Low   High

ELISA

Nuclear
Syndecan-1

What is the role of syndecan-1 in the nucleus?

➤ Heparan sulfate/heparin inhibit histone acetyltransferase (HAT) activity (Buczek-Thomas *et al.*)

➤ HATs modify the N-terminal tail region of histones by acetylating key lysines altering DNA-histone and histone-histone contacts to enhance binding of transcriptional complexes to DNA

➤ Abnormal HAT activity is associated with the development of cancer

Heparanase upregulates histone acetyltransferase (HAT) activity in myeloma cells

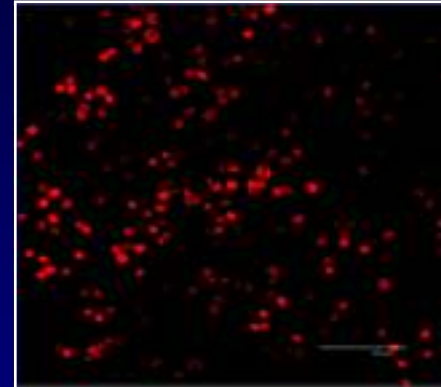**HAT activity assay - nuclear extracts from heparanase low and high CAG cells**
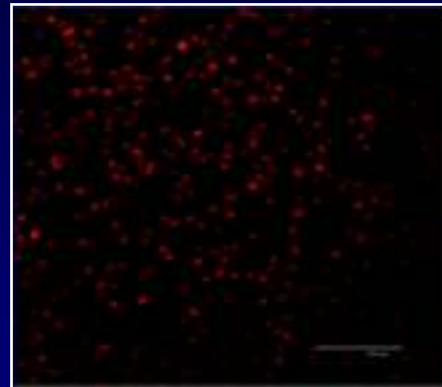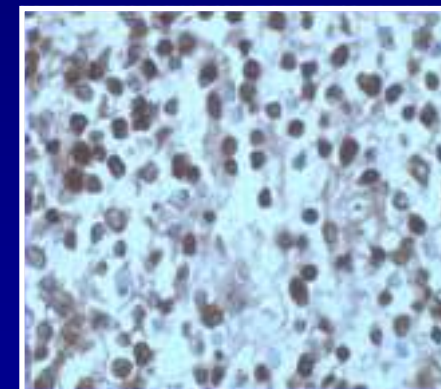
Heparanase upregulates HAT activity
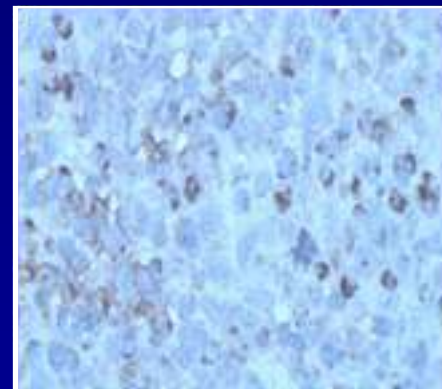

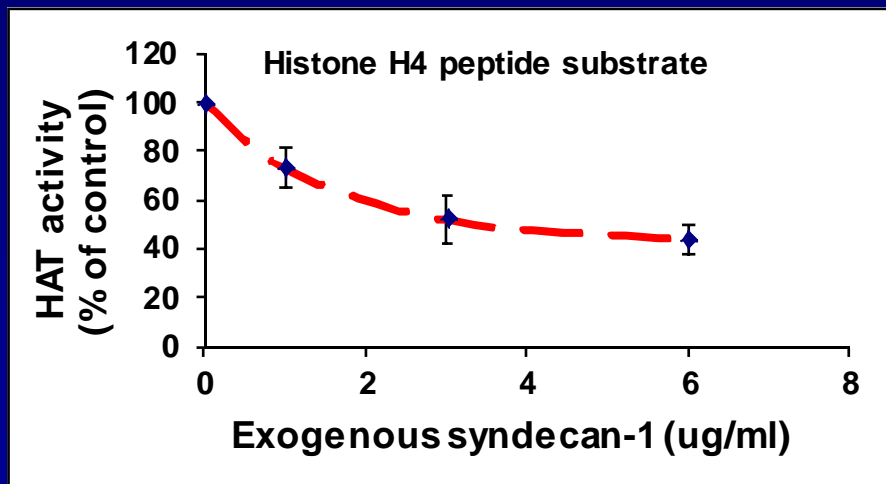Heparanase expression (CAG cells)

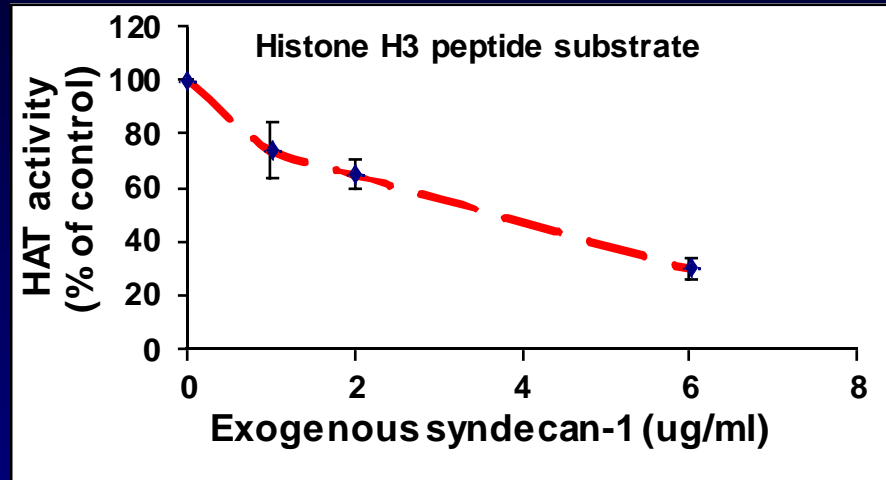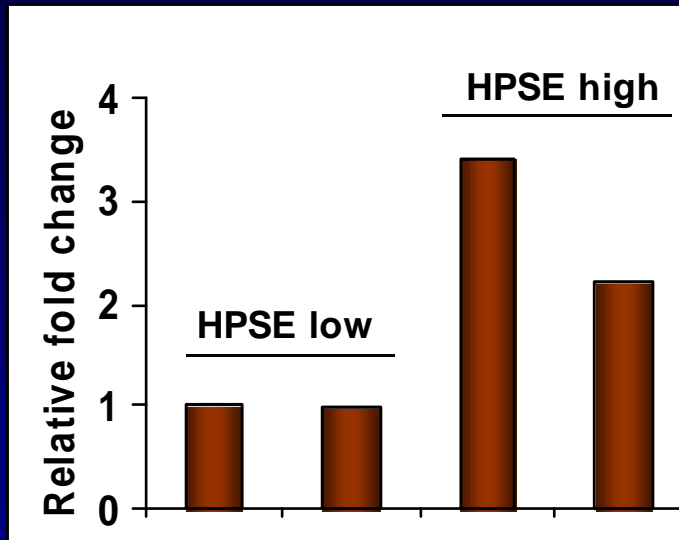Low / High

Cells in culture

Tumors in SCID mice

Immunostaining for acetylated histone H3

Syndecan-1 inhibits HAT activity in heparanase-high cells

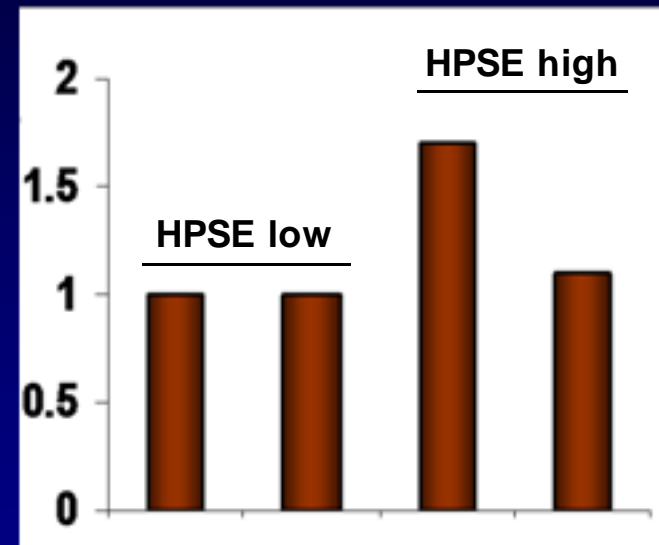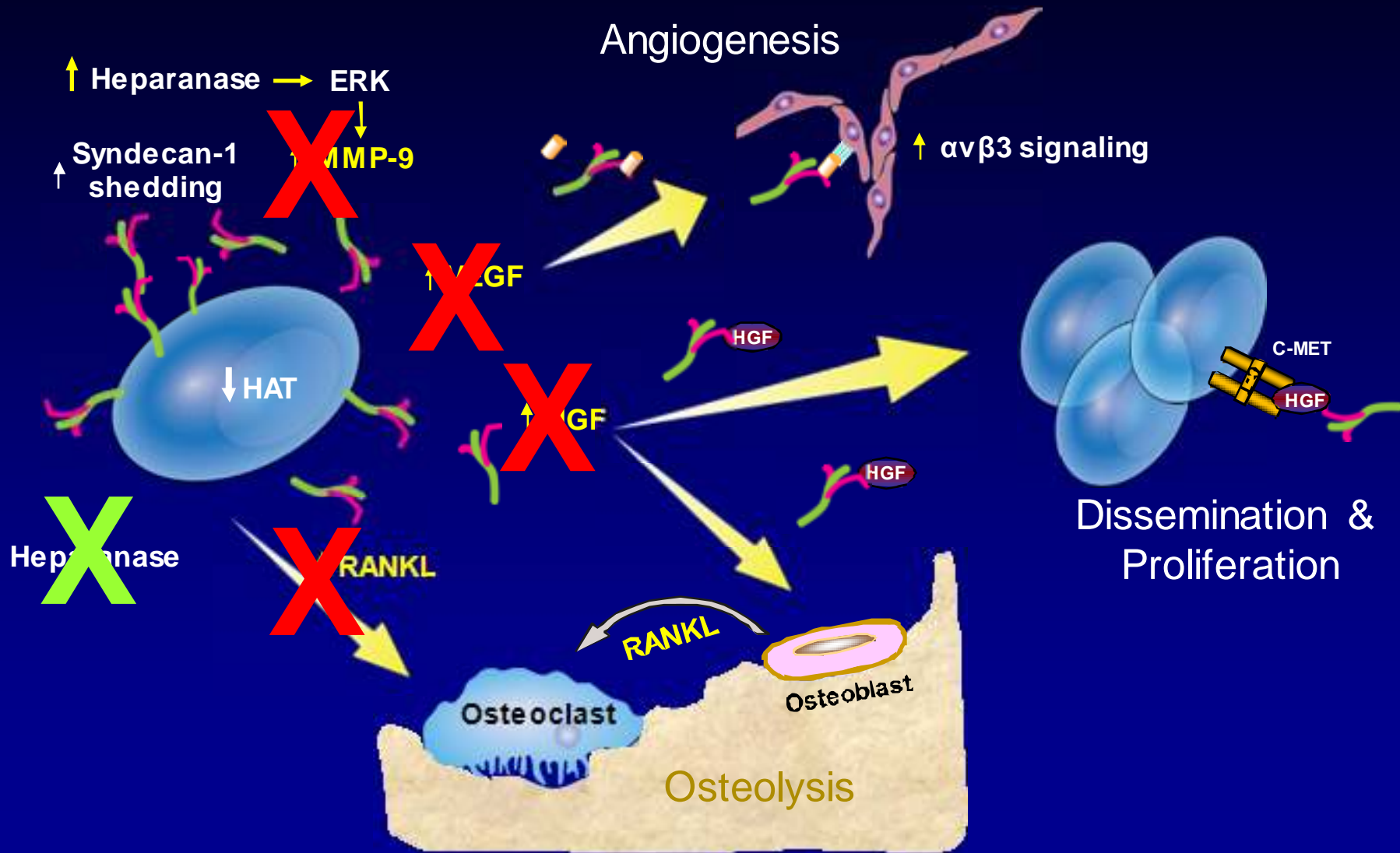# Inhibition of HAT activity inhibits heparanase-mediated upregulation of MMP-9 and VEGF gene expression

Inhibition of heparanase decreases HAT-regulated expression of genes that promote aggressive tumor behavior

Conclusions

➤ **Heparanase and syndecan-1 facilitate tumor-host crosstalk in the microenvironment that enhances myeloma growth, dissemination, angiogenesis and osteolysis**

➤ **Heparanase modifies the tumor microenvironment by:**
  ➤ **Enhancing shedding of syndecan-1**
    ➤ **Shed syndecan-1 binds growth factors and facilitates signaling through growth factor receptors**
    ➤ **Shed syndecan-1 can activate integrins and promote their signaling**
  ➤ **Upregulating tumor cell expression of MMP-9, VEGF, HGF & RANKL**

➤ **The mechanism of regulation of gene expression by heparanase is mediated, at least in part, by disruption of syndecan-1 localization to the nucleus resulting in enhanced histone acetyltransferase activity**

➤ **Inhibitors of heparanase represent a viable therapeutic approach for myeloma and other cancers**

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