

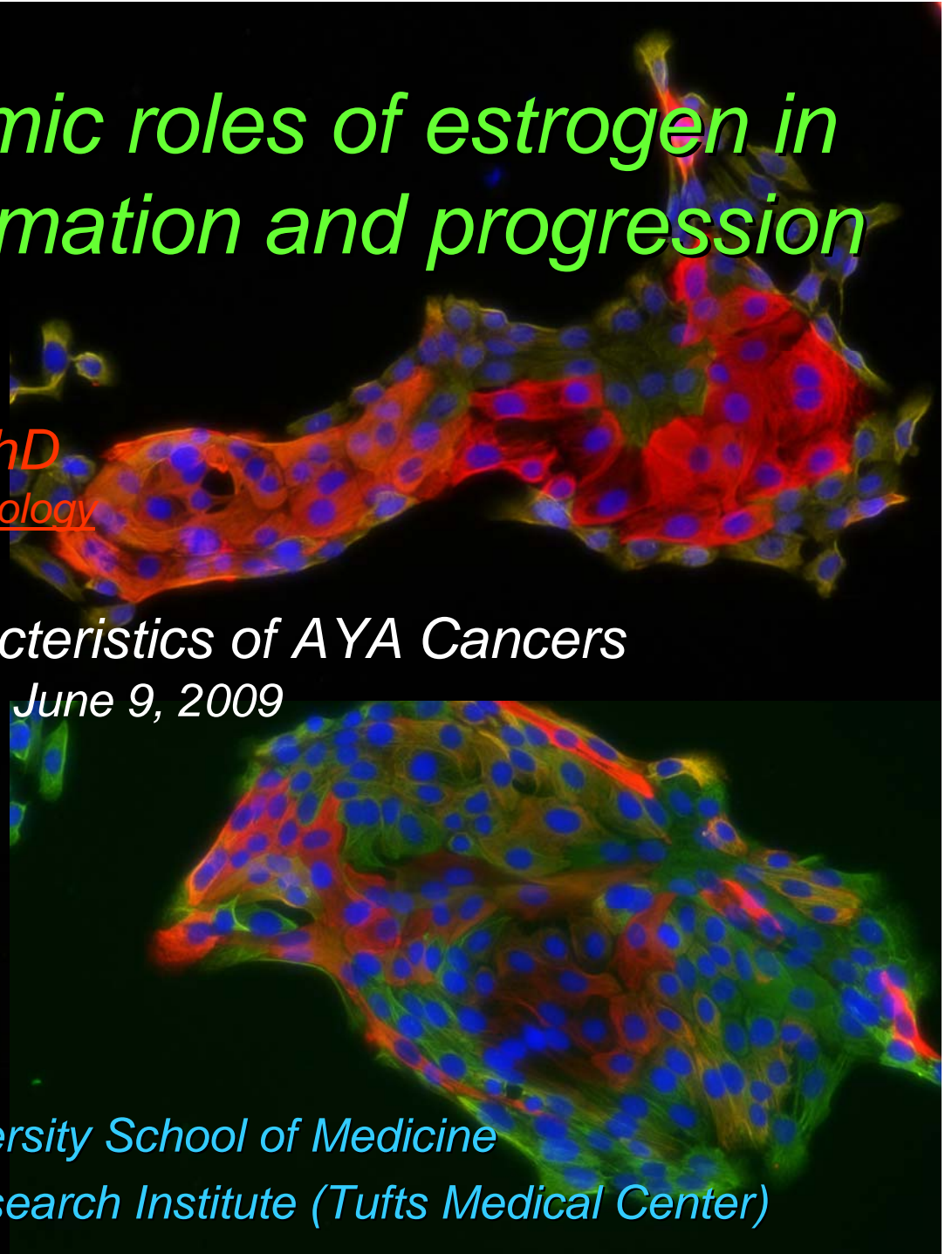
# *Local and systemic roles of estrogen in breast cancer formation and progression*

*Charlotte Kuperwasser, PhD*  
*Department of Anatomy & Cellular Biology*

*Unique Characteristics of AYA Cancers*

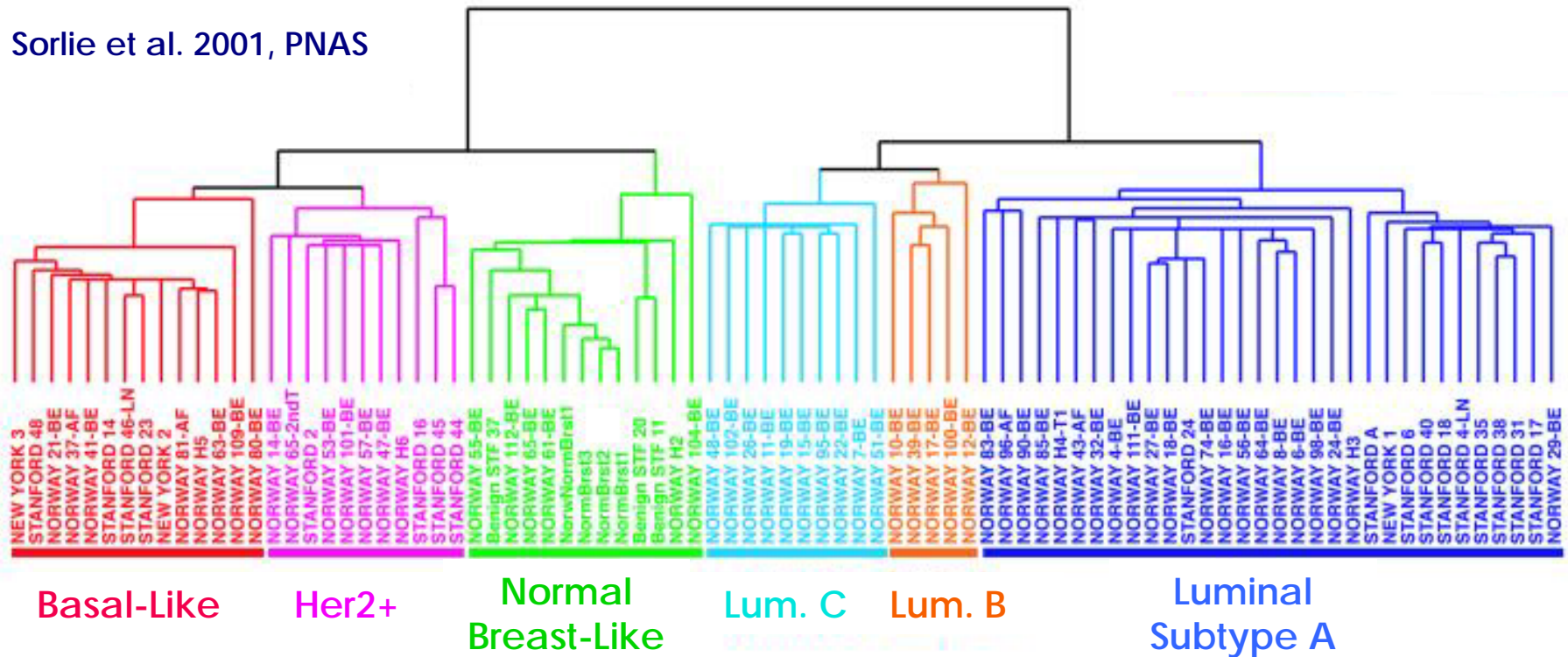
*June 9, 2009*

*Tufts University School of Medicine*  
*Molecular Oncology Research Institute (Tufts Medical Center)*



# Breast Cancer is a Heterogeneous Disease

Sorlie et al. 2001, PNAS



## Basal type:

- ER/PR/Her2 negative
- Express basal epithelial cytokeratins (CK5/6/14/17)
- Often overexpress EGFR and have p53 mutations
- Associated with BRCA-1 mutations/low BRCA-1 expression
- Likely to be high grade and poorly differentiated

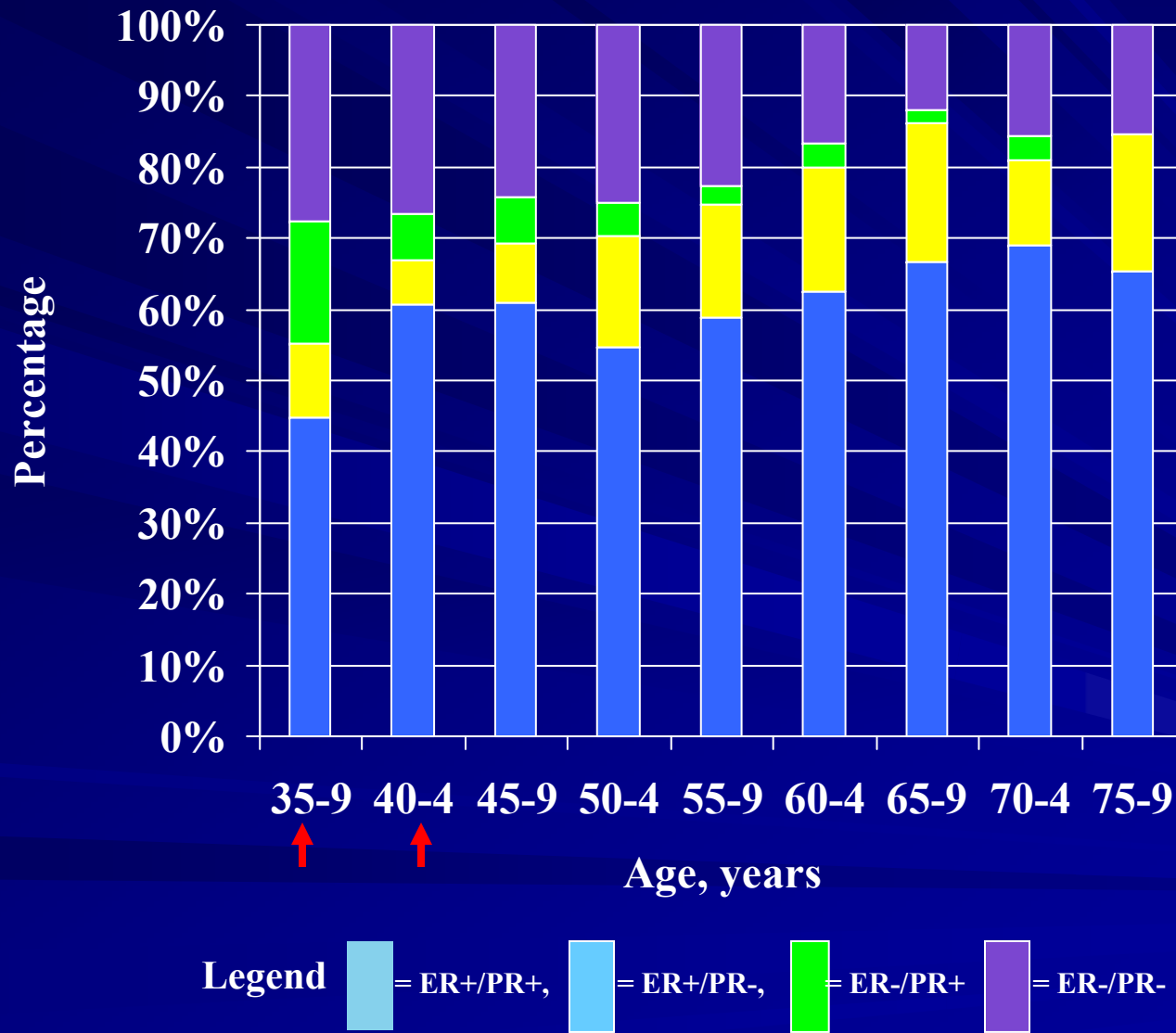
## HER2 type:

- Her2 positive
- Overexpress genes in the Her2 amplicon on chromosome 17q21
- Hormone receptor negative or low
- Higher frequency of p53 mutations
- More likely to be high grade

## Luminal type:

- Most common, usually low grade
- Hormone receptor (ER/PR) positive
- Express luminal cytokeratins (CK8/18)
  - Luminal A has higher expression of ER-related genes and lower proliferative genes vs Luminal B

# Incidence of ER- negative breast cancers is more prevalent in younger women

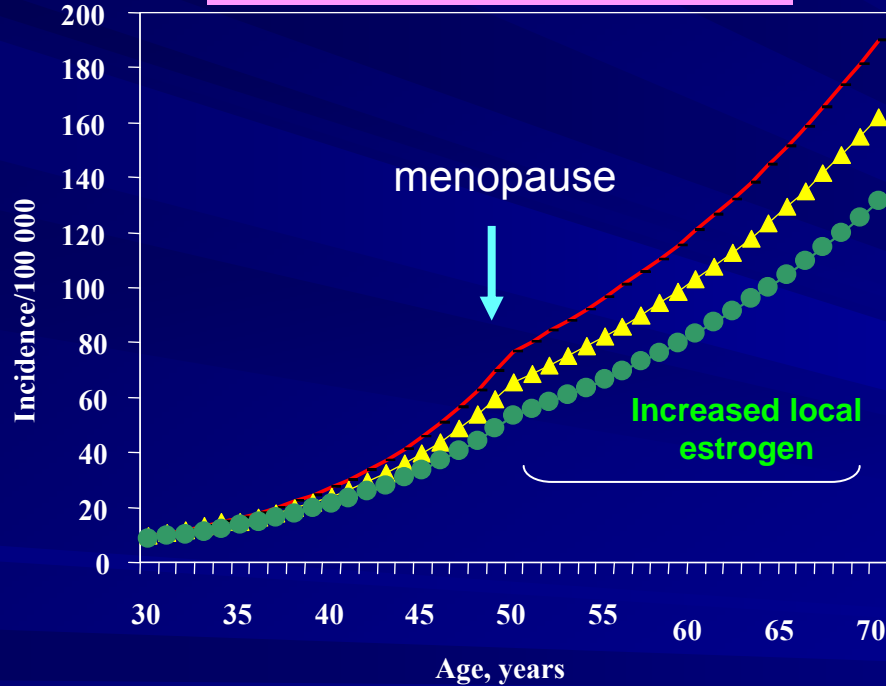


*Curtsey of Graham A. Colditz*

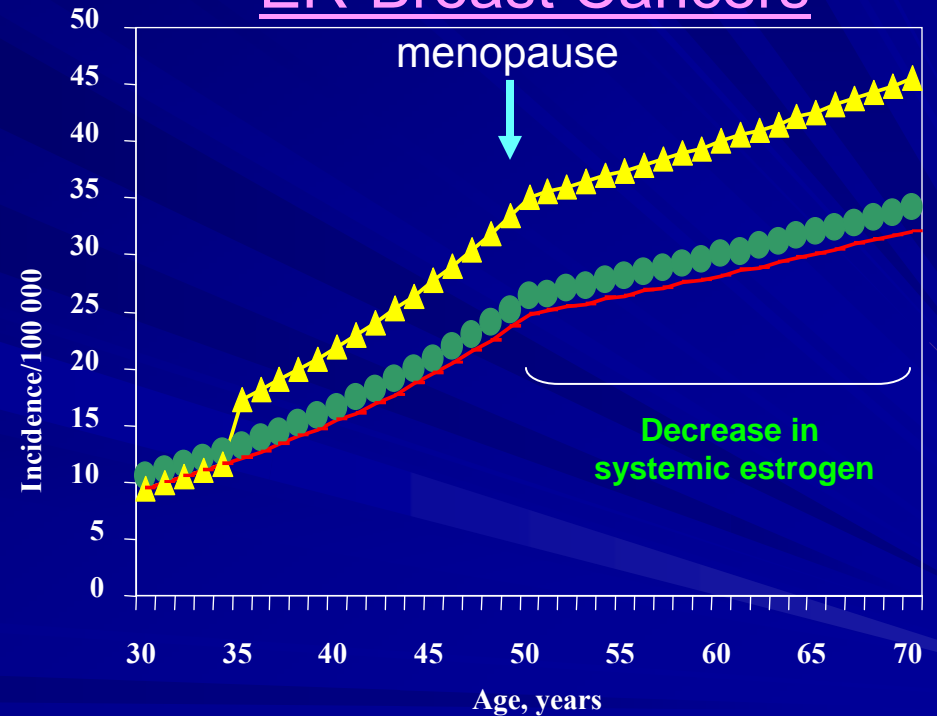
# ER negative breast cancers are sensitive to circulating estrogens

— nulliparous    ▲ age at birth=35    ● age at births =20,23,26,29

## ER+ Breast Cancers

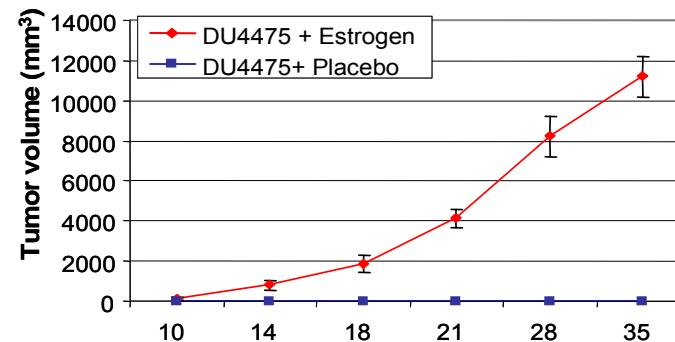
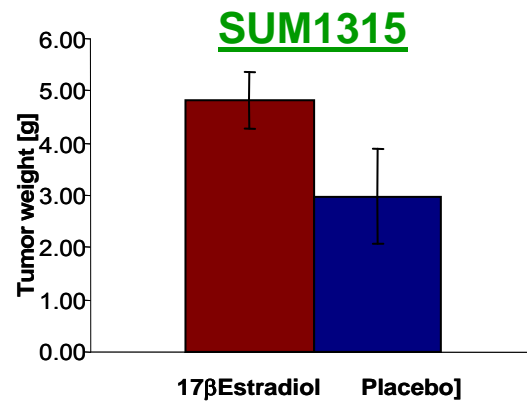
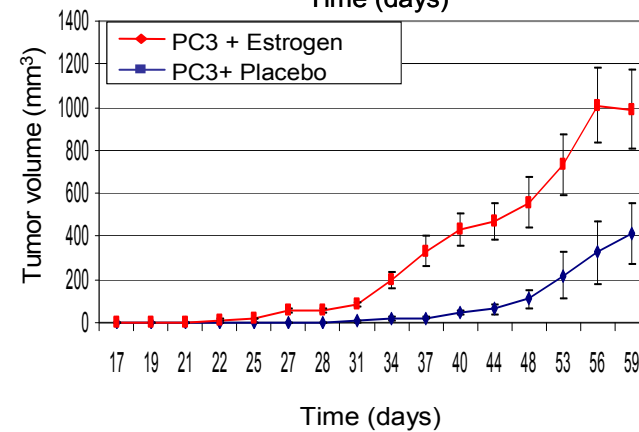
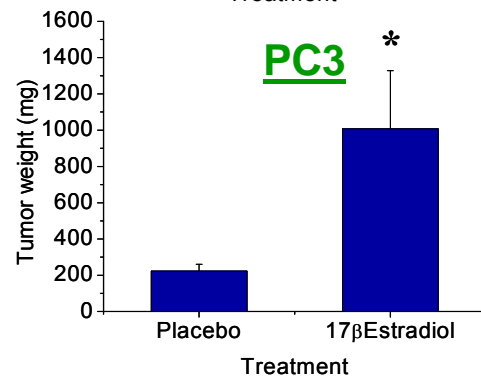
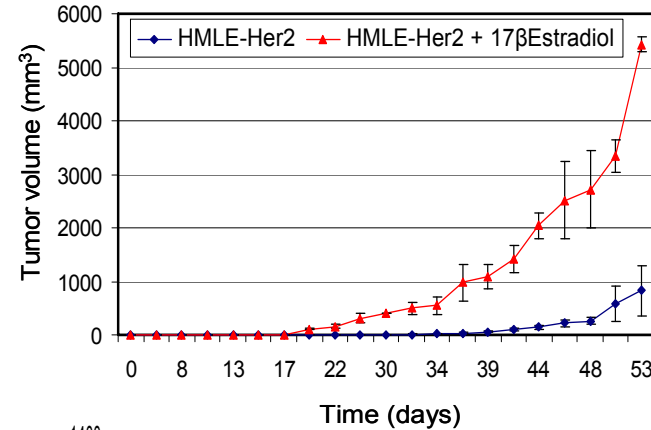
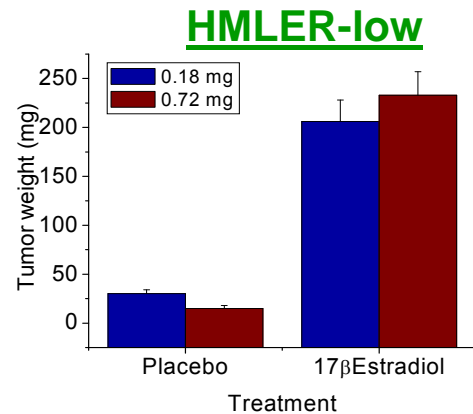


## ER-Breast Cancers



# Estrogen accelerates tumor growth in ER-negative tumor models

Gupta et al, Cancer Res, 2007

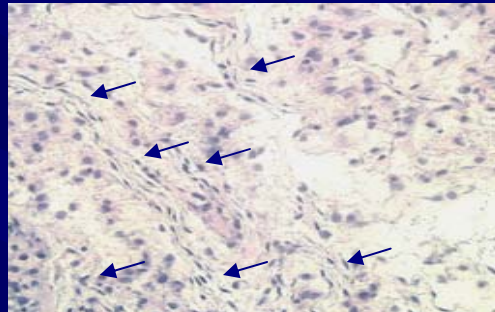
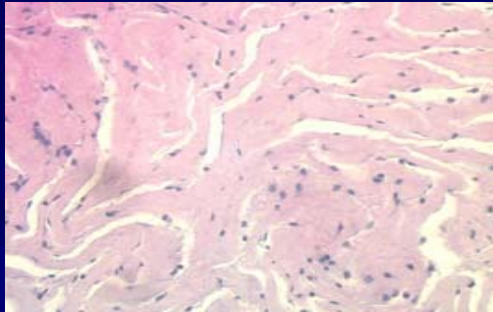


# Estrogen promotes increased cellular recruitment and angiogenesis in the absence of tumor growth

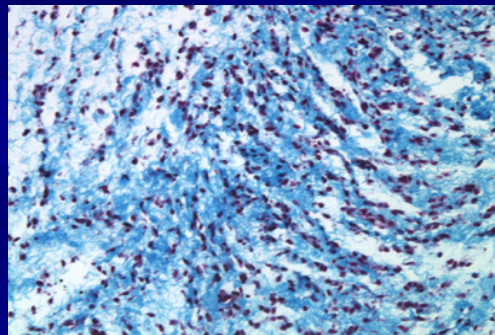
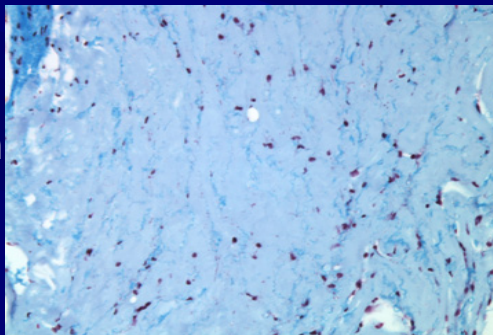
placebo

17 $\beta$ Estradiol

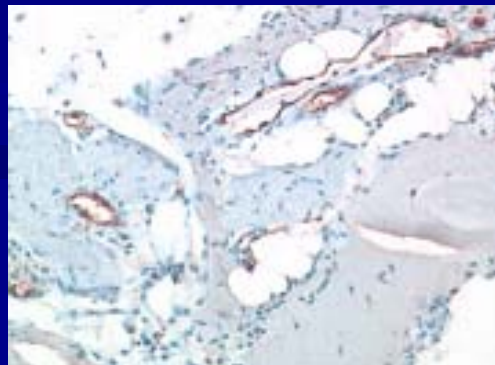
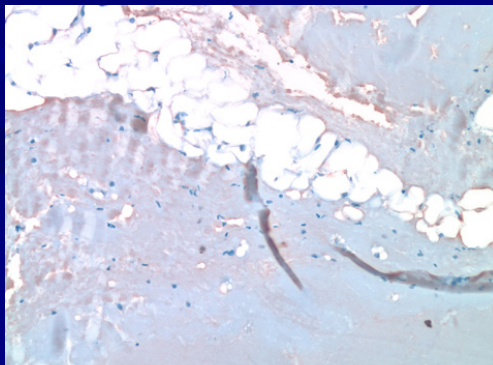
H&E



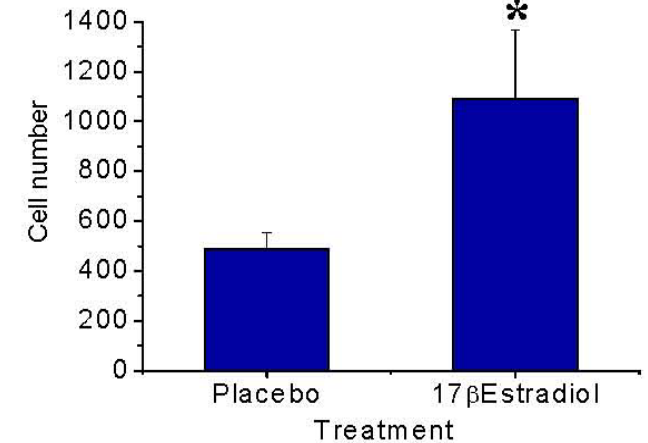
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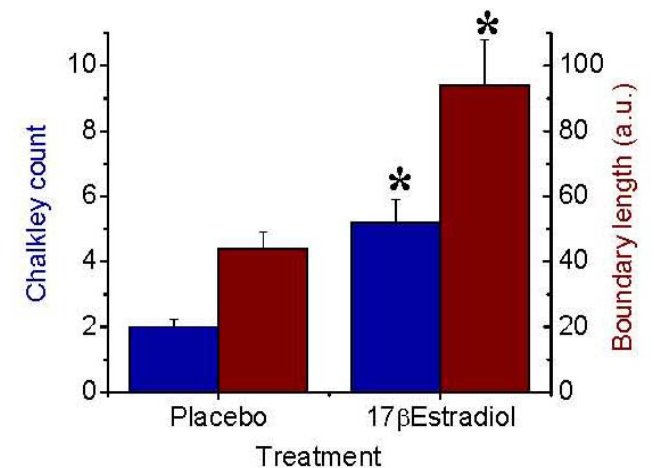
vWF



## Cellularity



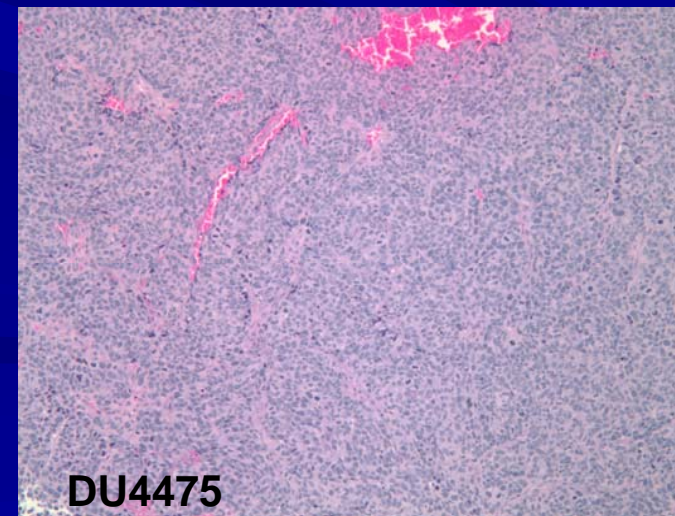
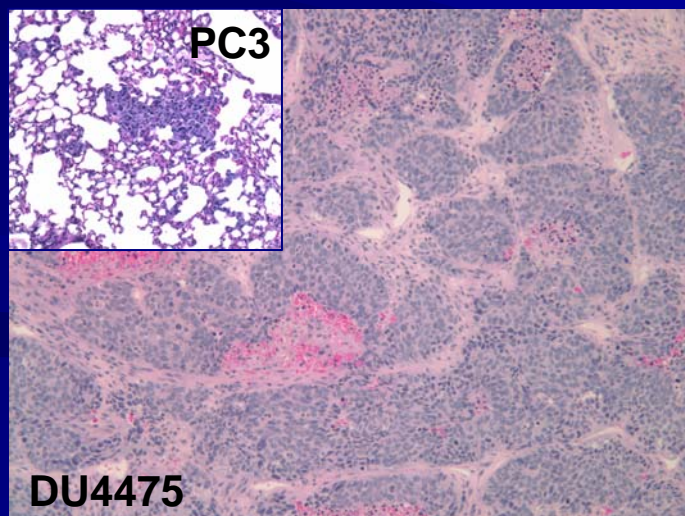
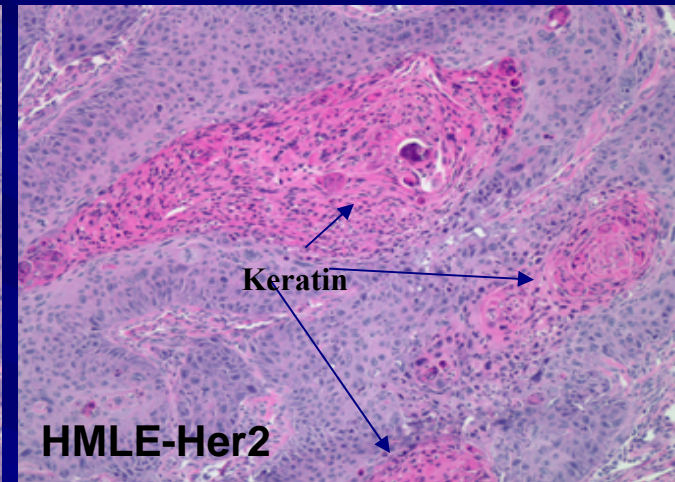
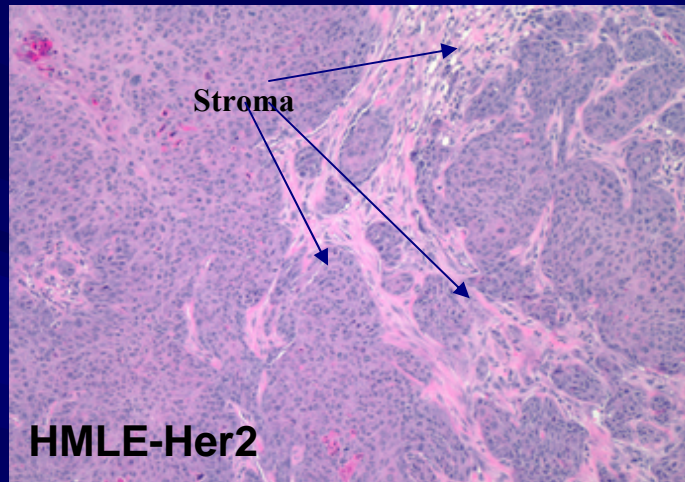
## Angiogenesis



# Increased stromal contribution and metastasis following estrogen-treatment

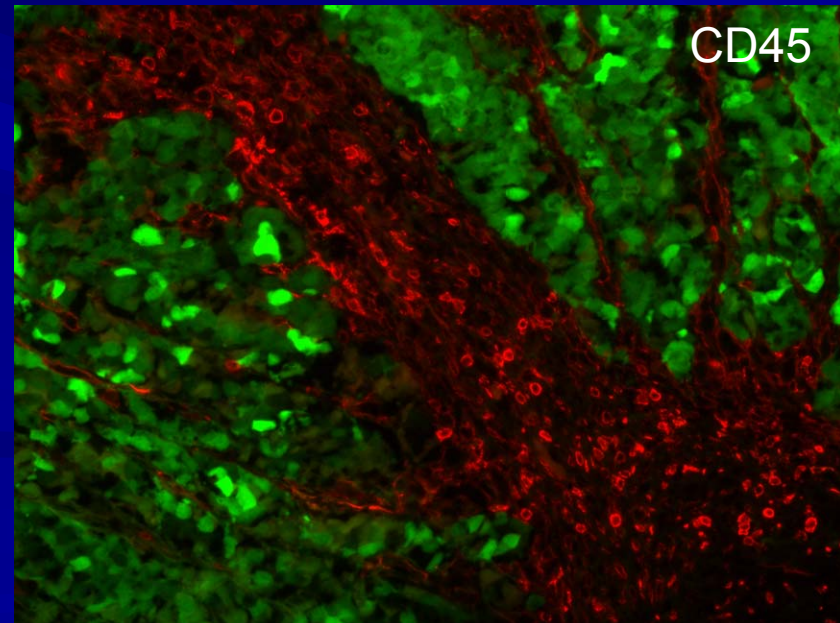
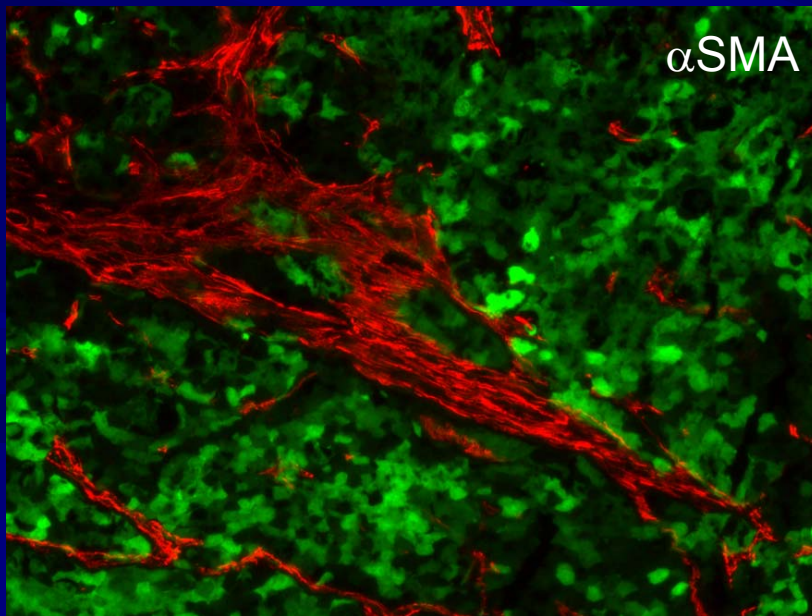
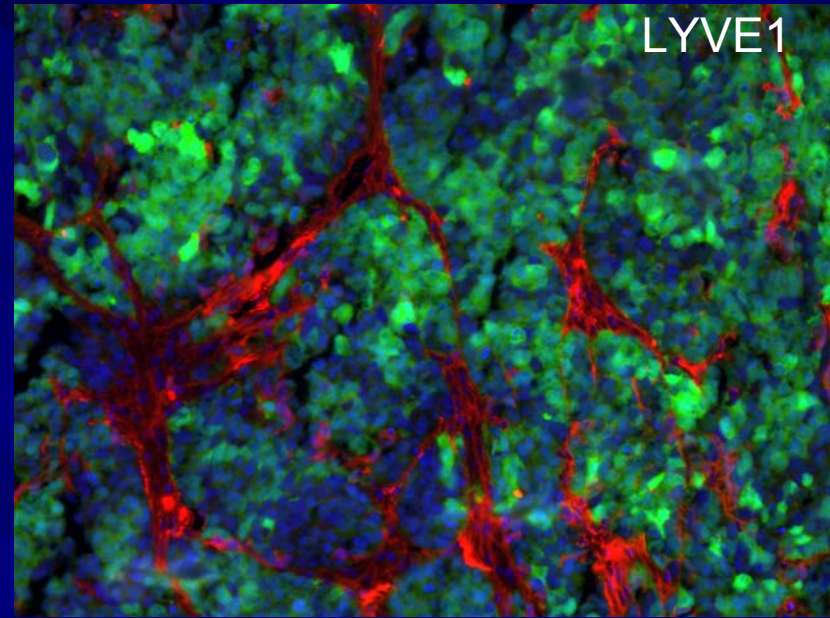
+17 $\beta$ Estradiol

Placebo



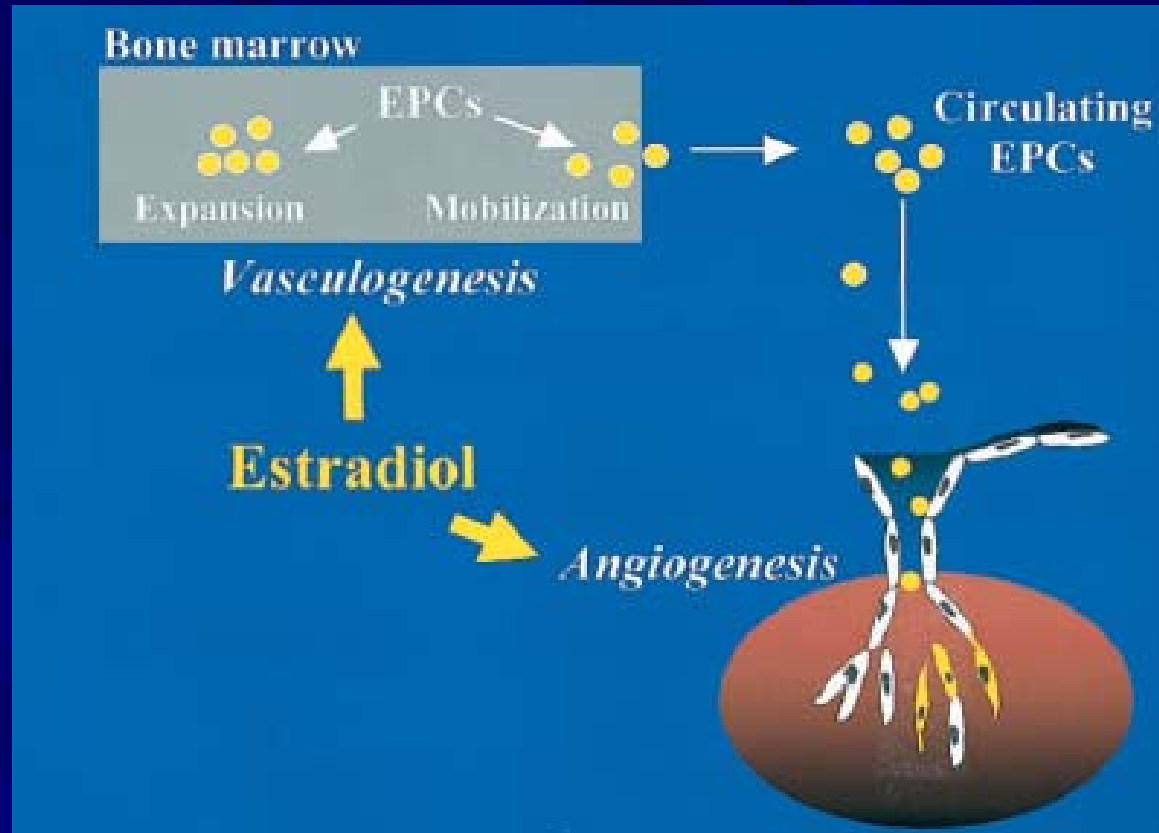
Tumor associated stroma  
following estrogen  
treatment is composed of  
LYVE1+ , CD45+ and  
 $\alpha$ SMA+ cells

GFP: Tumor Cells  
Texas-Red: IHC



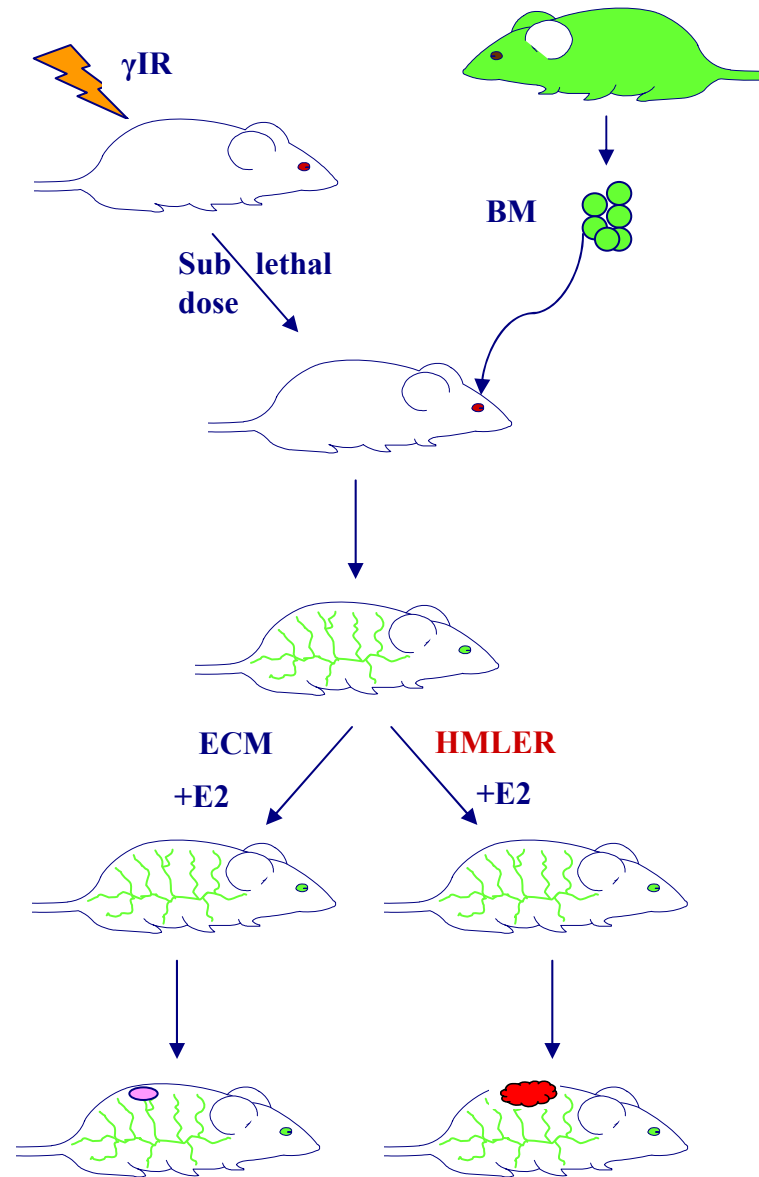


# Where does stromal recruitment come from??

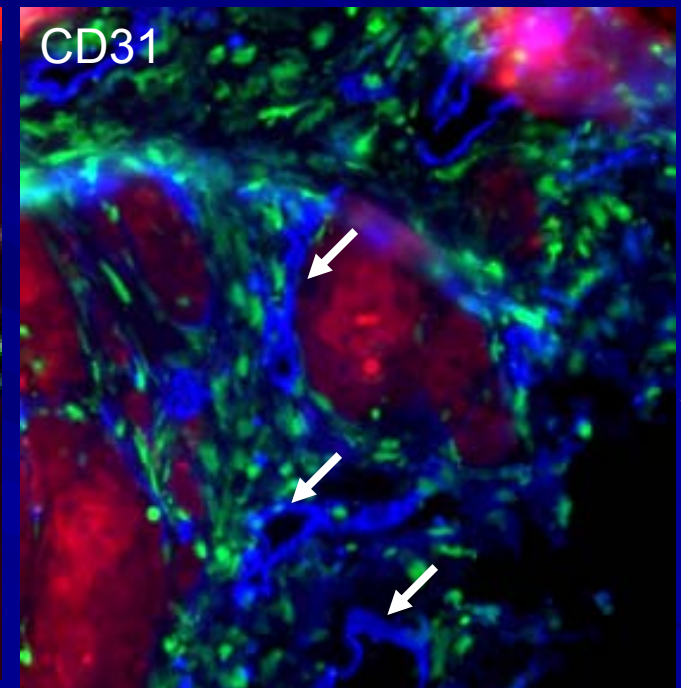
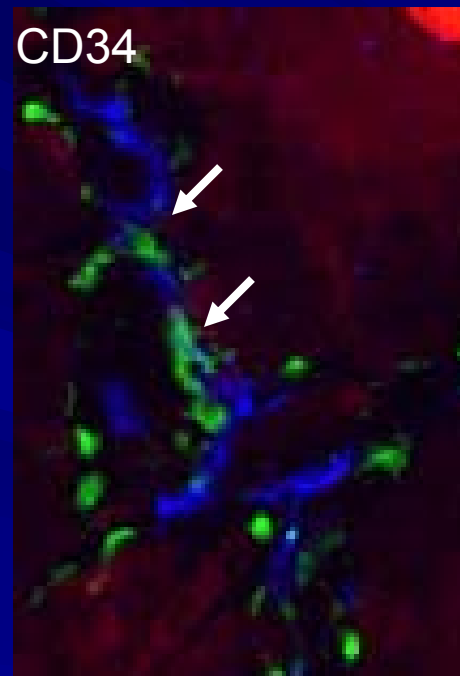
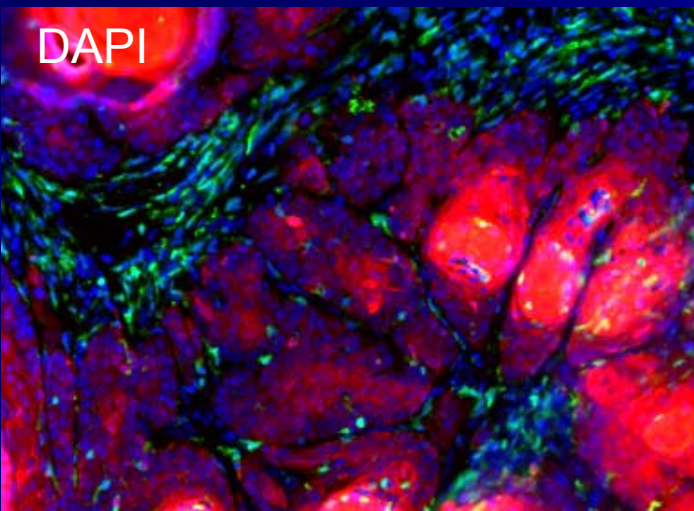
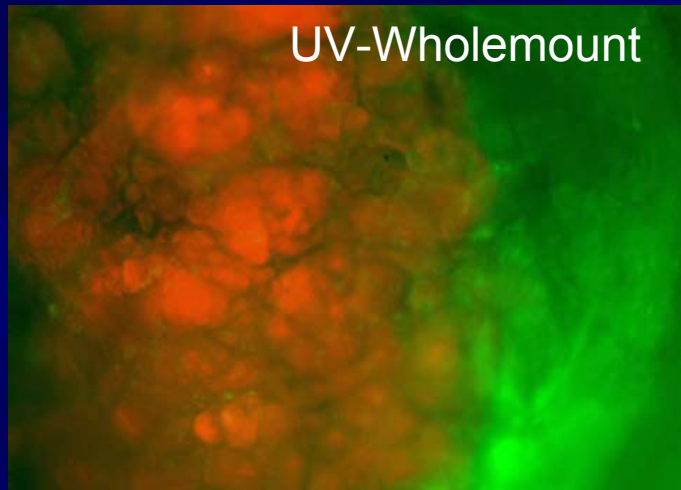


- **Estrogen and angiogenesis** *Arterioscler Thromb Vasc Biol.* 2001 Jan;21(1):6-12.
- **Estrogen increases bone marrow-derived endothelial progenitor cell production.** *Circulation.* 2003 Jun 24;107(24):3059-65.

# Can estrogen promote increased systemic angiogenesis through BM-cell recruitment?

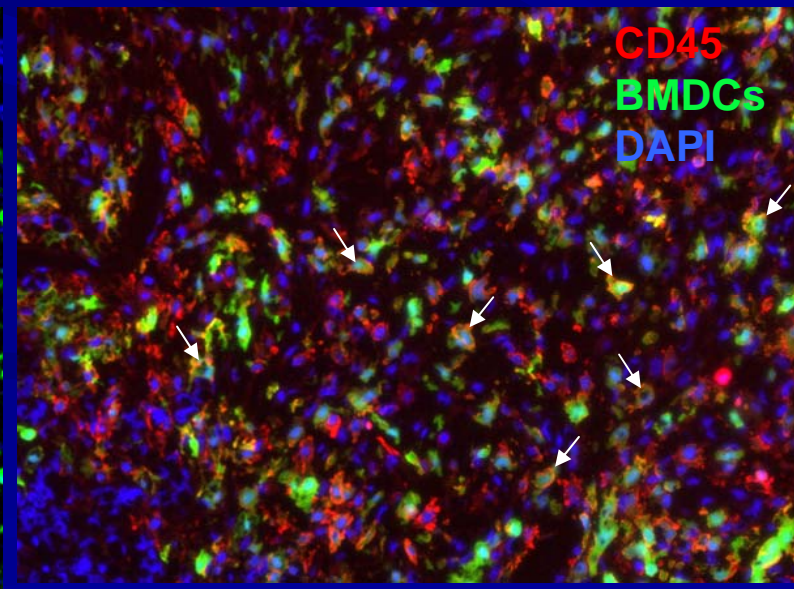
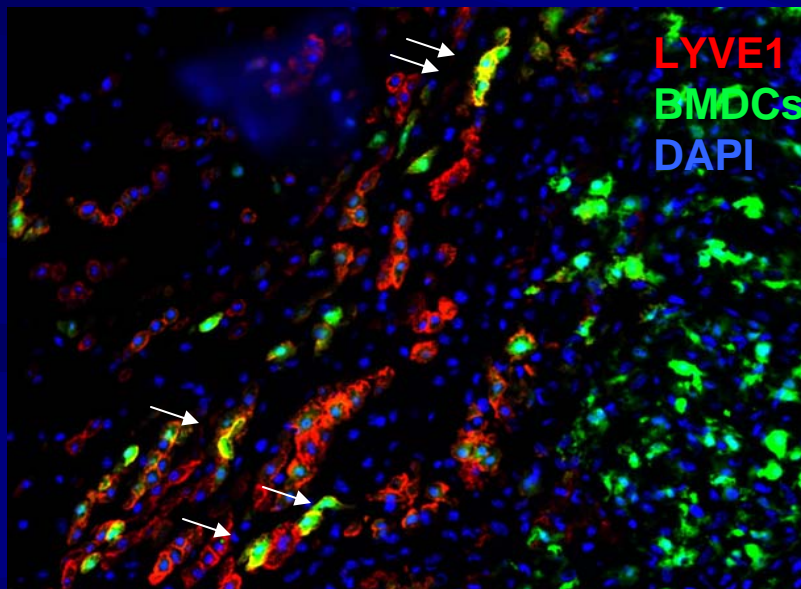
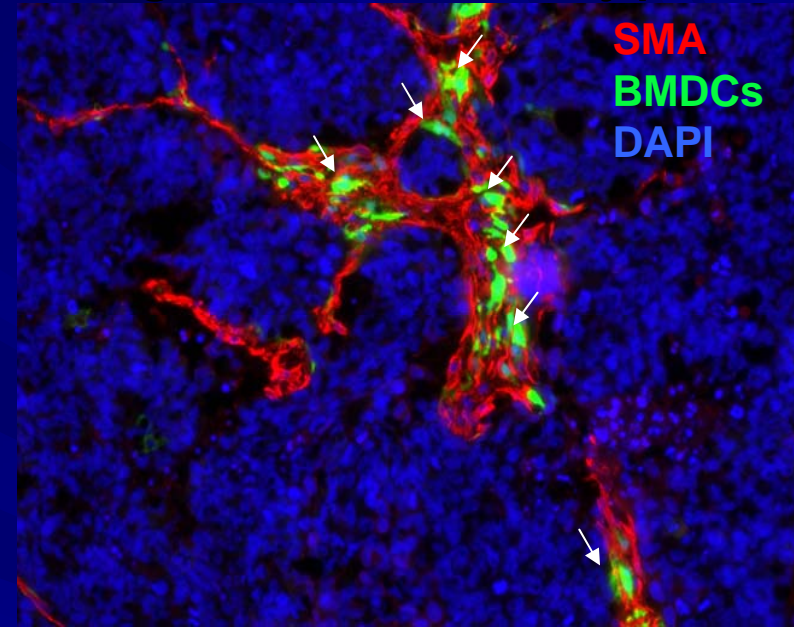
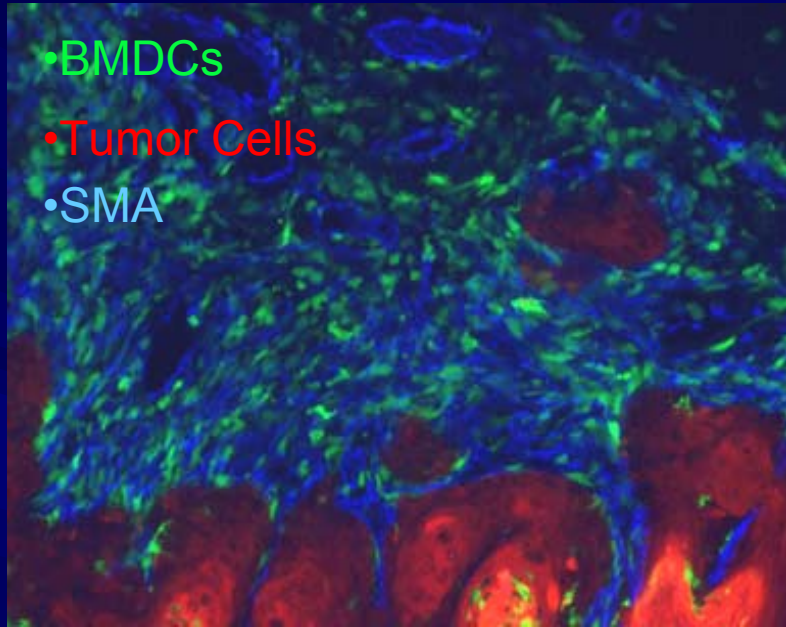


# Tumor-associated stroma is bone marrow derived- but not EPCs or MSCs



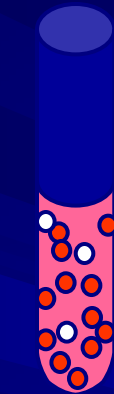
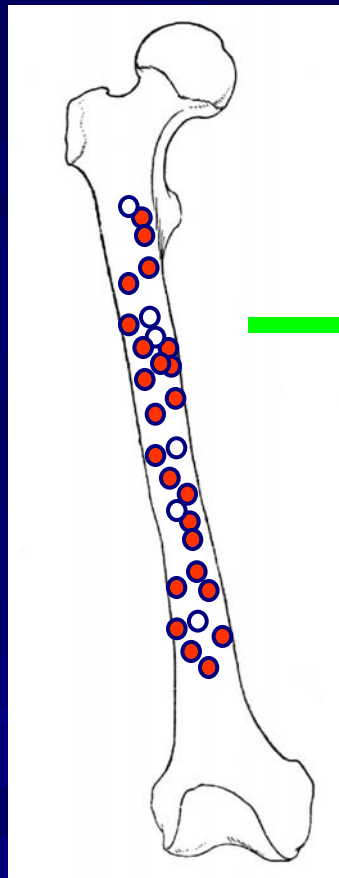
High-ras tumor  
GFP BM cells  
AMCA- IHC

# Bone marrow derived cells in tumors are interspersed within stroma and heterogeneous cell types



# Are bone-marrow derived cells from post-partum or estrogen-treated mice causal in the formation of tumors ??

Harvested Bone Marrow



Bone Marrow Cells

+



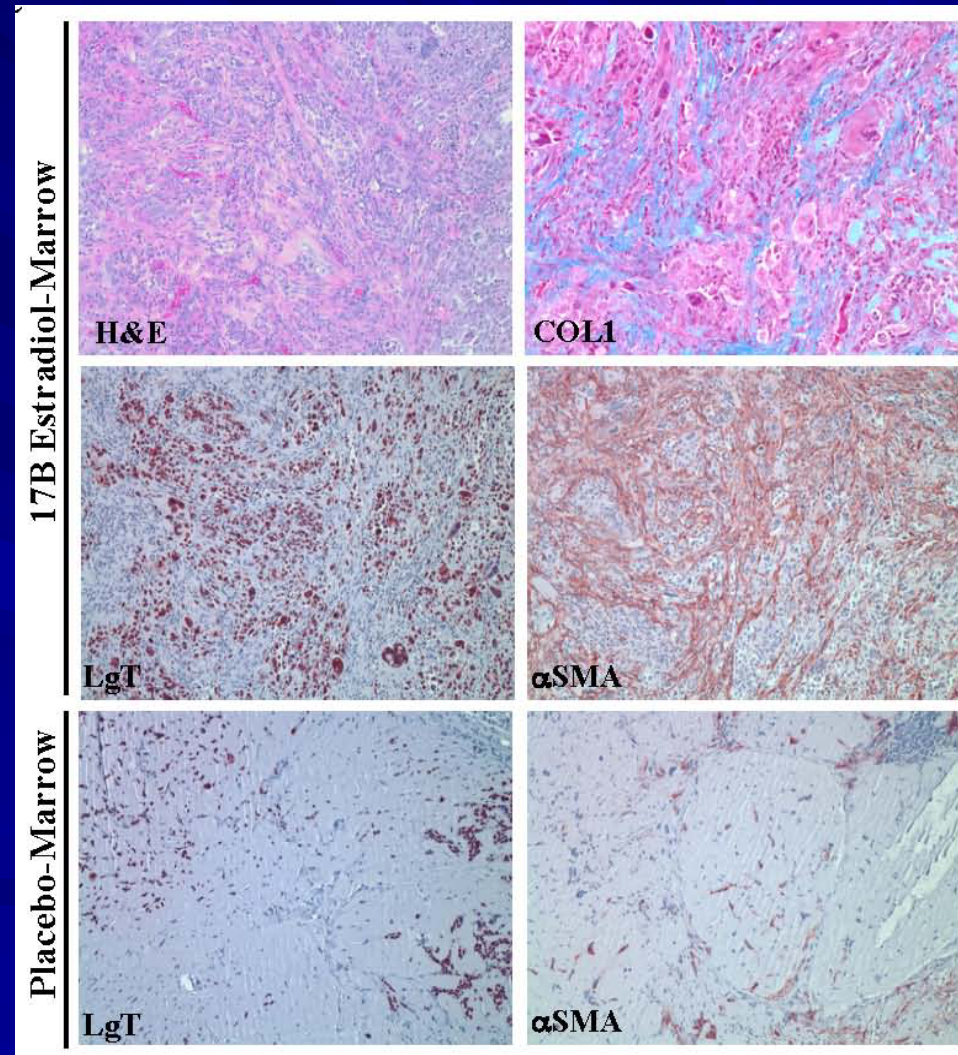
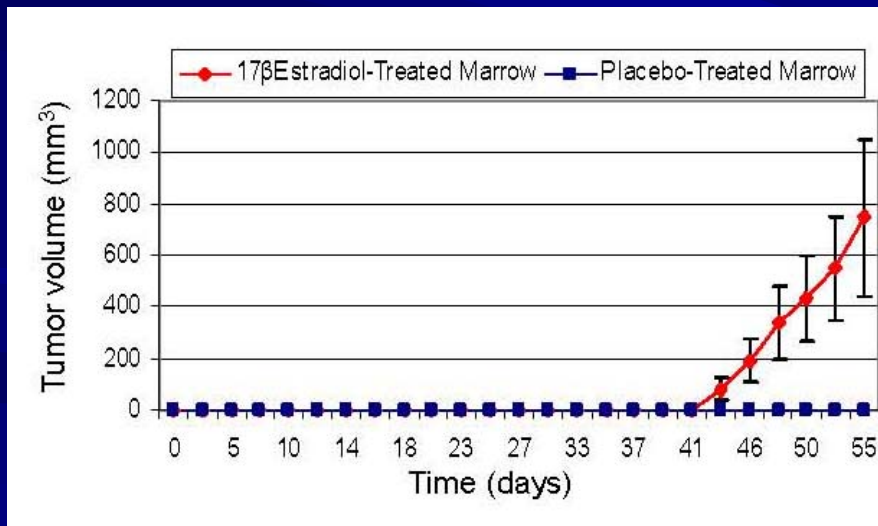
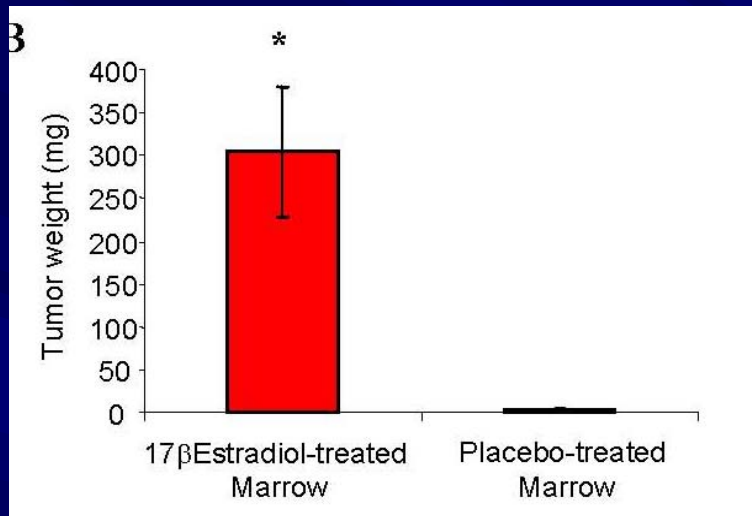
250,000 HMLER Cells

Co-mix and inject into untreated nulliparous mouse

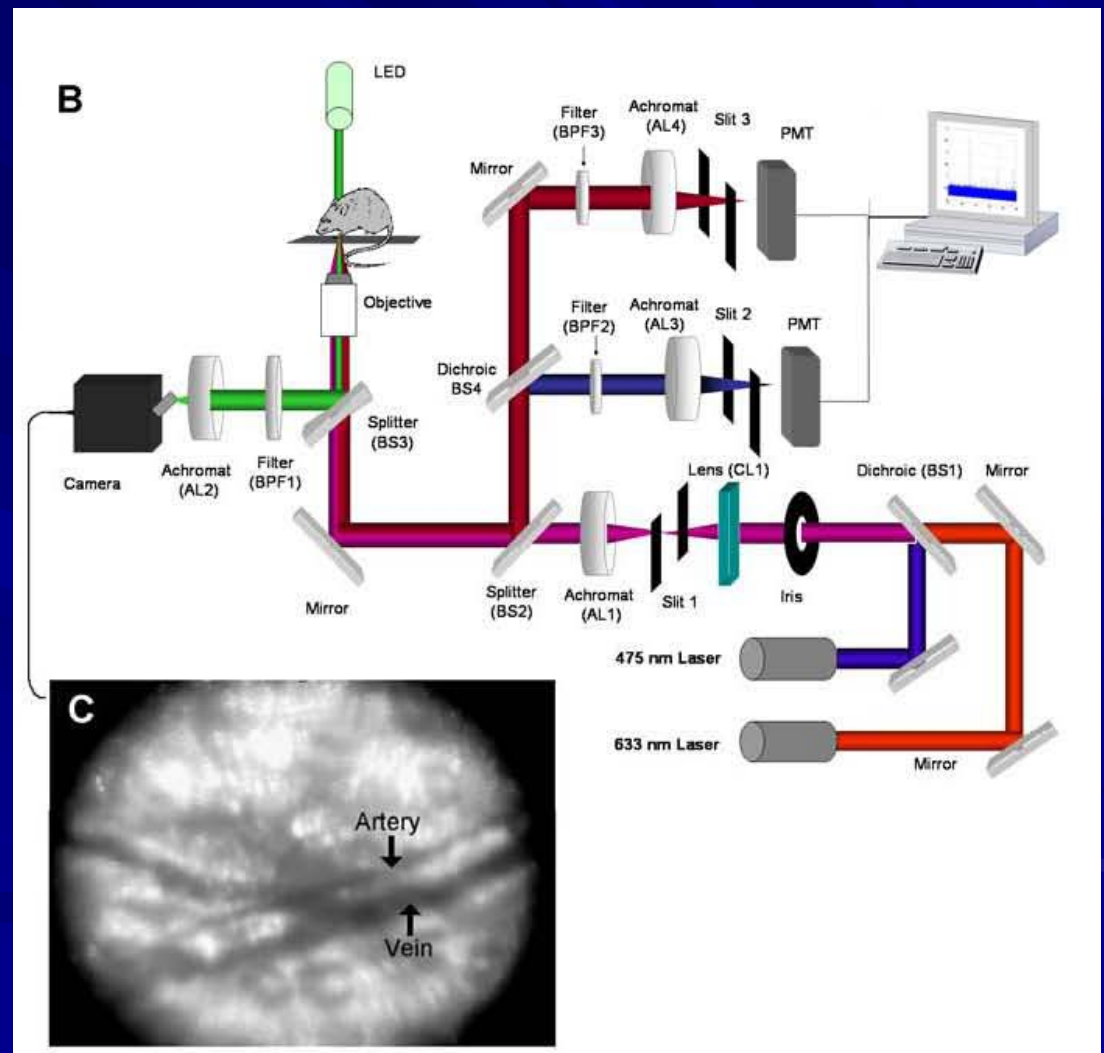
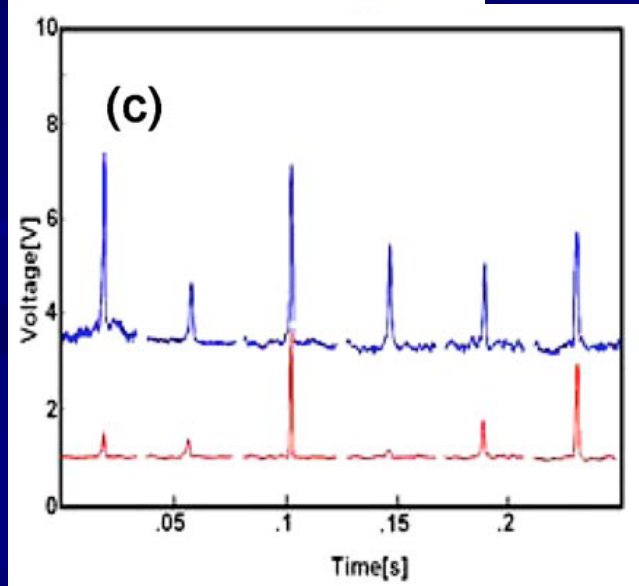
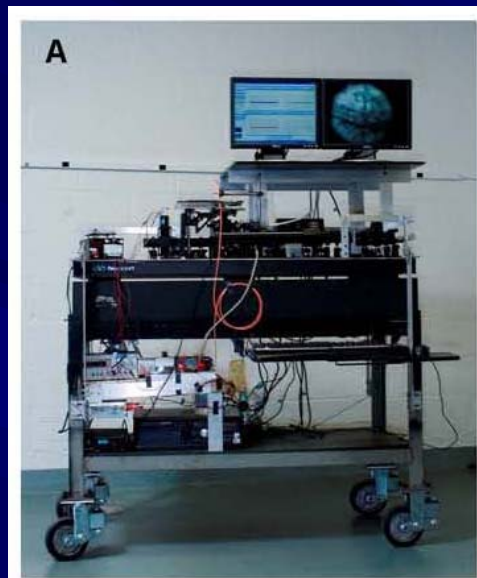


Placebo- vs.  $17\beta$ Estradiol-treated

# Estrogen treated bone marrow is sufficient to promote and phenocopy tumor formation

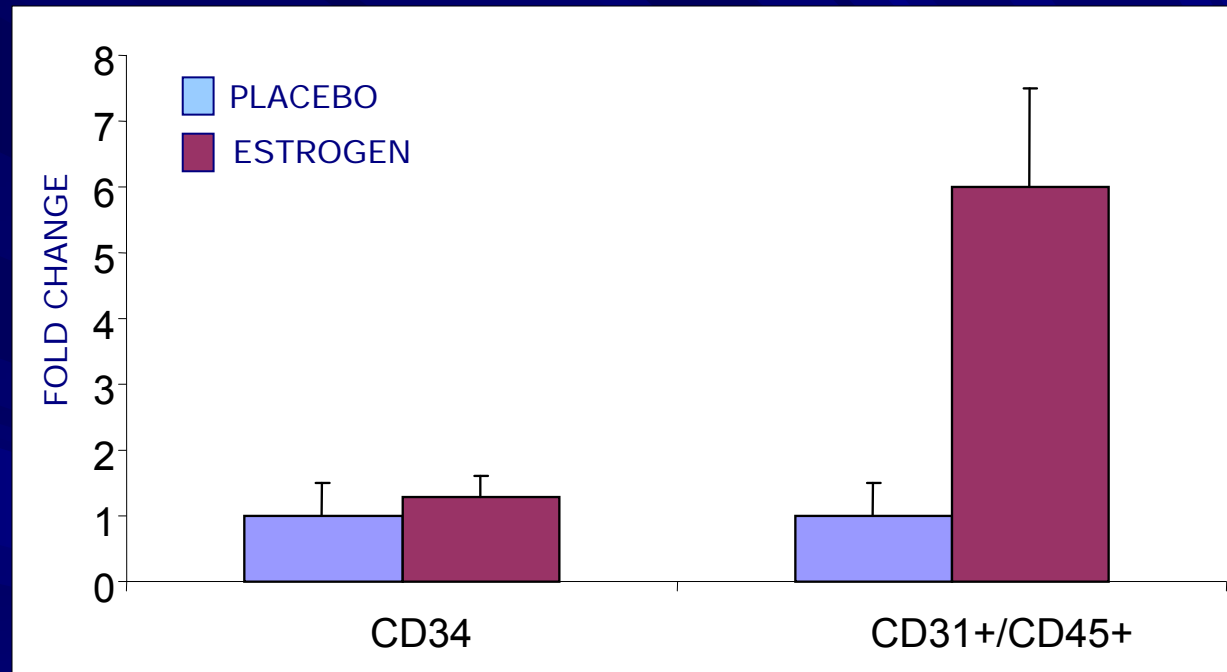


# Portable two-color *in vivo* flow cytometer for real-time detection of fluorescently-labeled circulating cells



# Estrogen mobilizes CD45<sup>+</sup>/CD31<sup>+/low</sup> cells into the circulation not CD34<sup>+</sup> cells

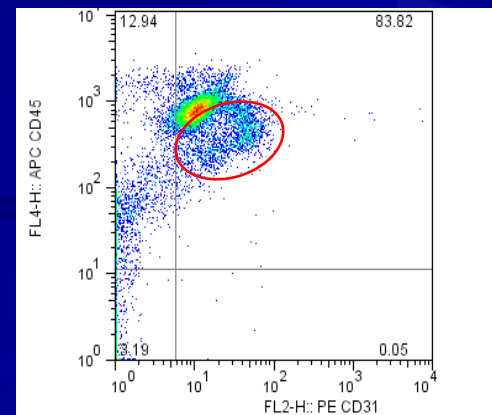
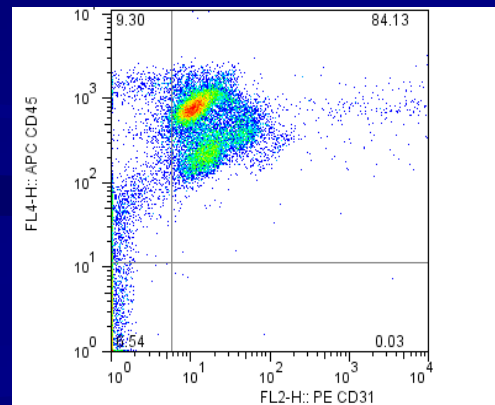
Circulation



Placebo

Estrogen

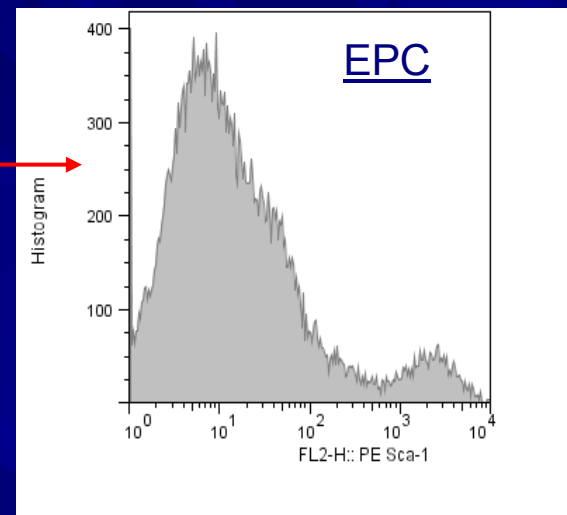
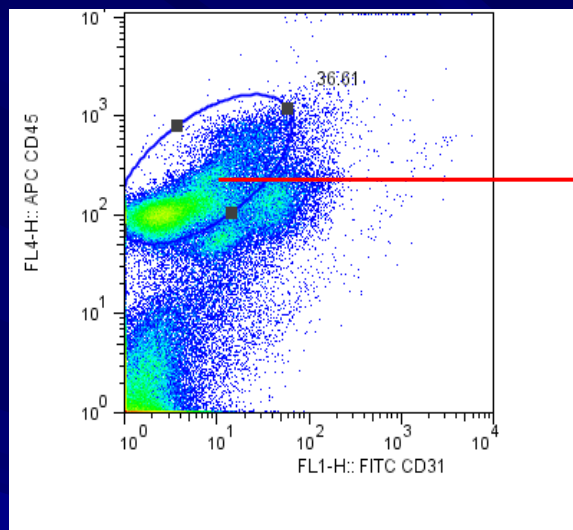
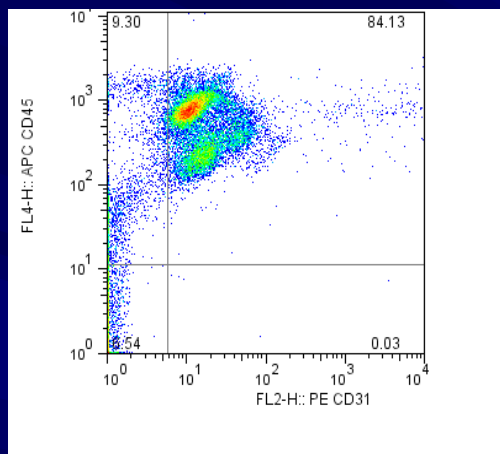
Bone Marrow



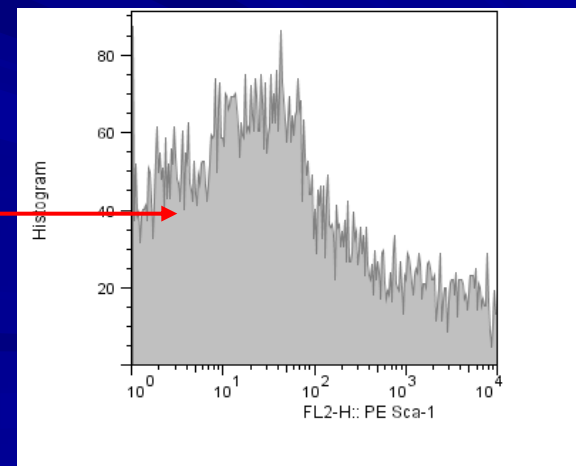
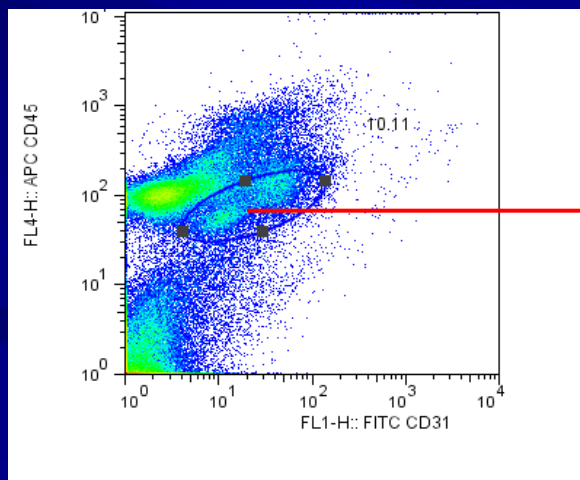
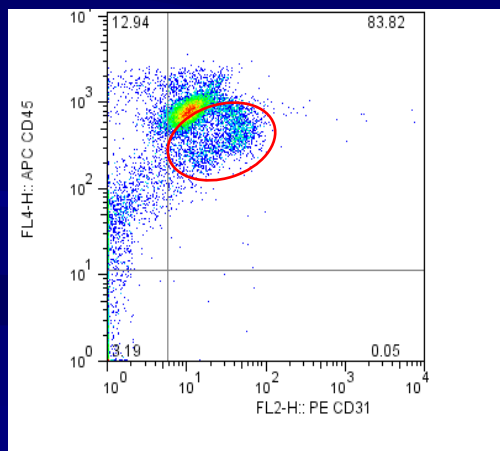


# Estrogen-sensitive CD45<sup>+</sup>/CD31<sup>+/low</sup> cells are not Sca1<sup>+</sup> EPCs

Placebo



Estrogen

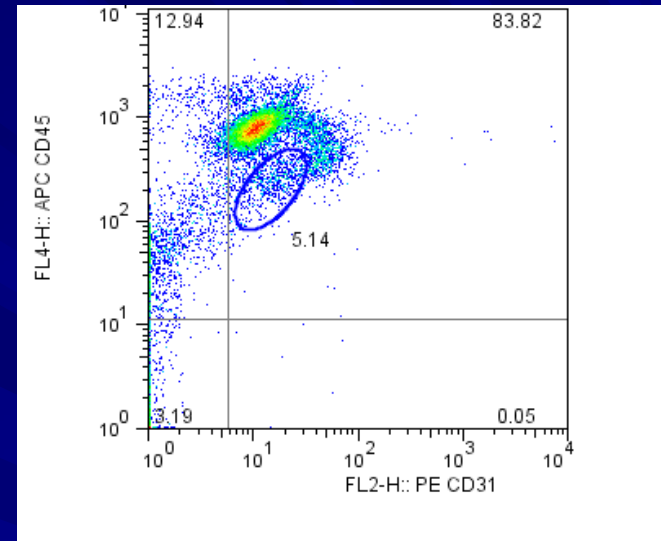
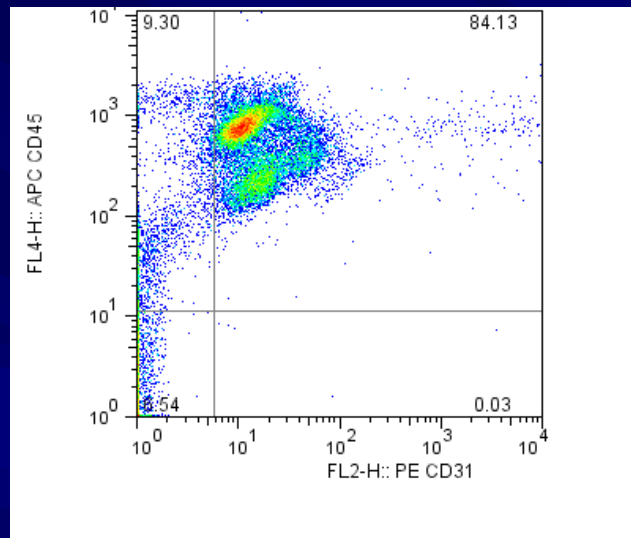


# Estrogen mobilization of CD45<sup>+</sup>/CD31<sup>+/low</sup> cells is ER $\alpha$ -dependent

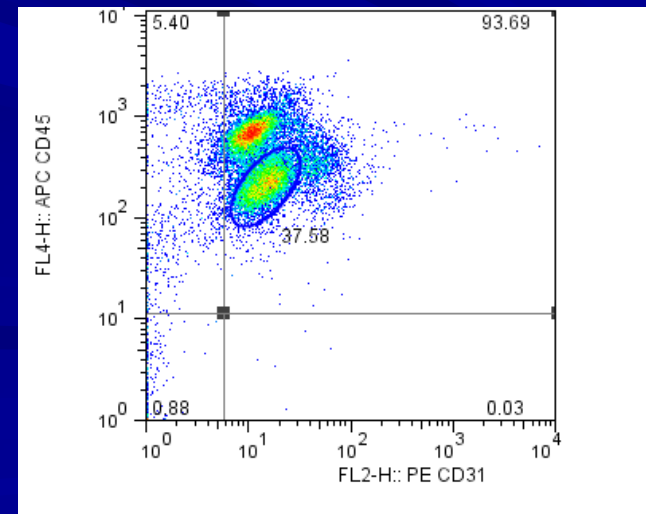
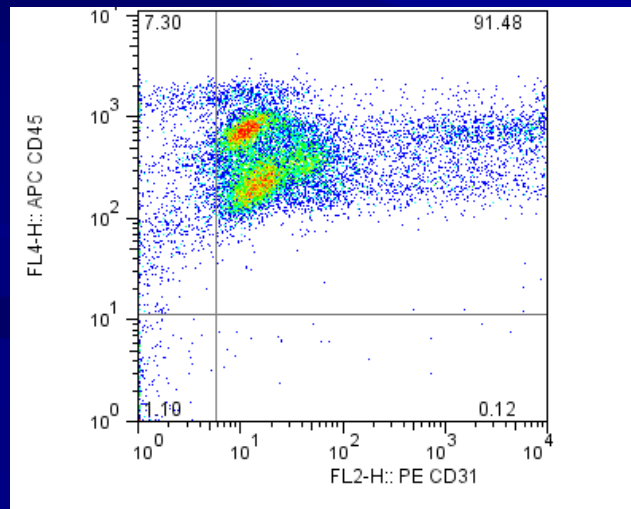
+placebo

+17 $\beta$  estradiol

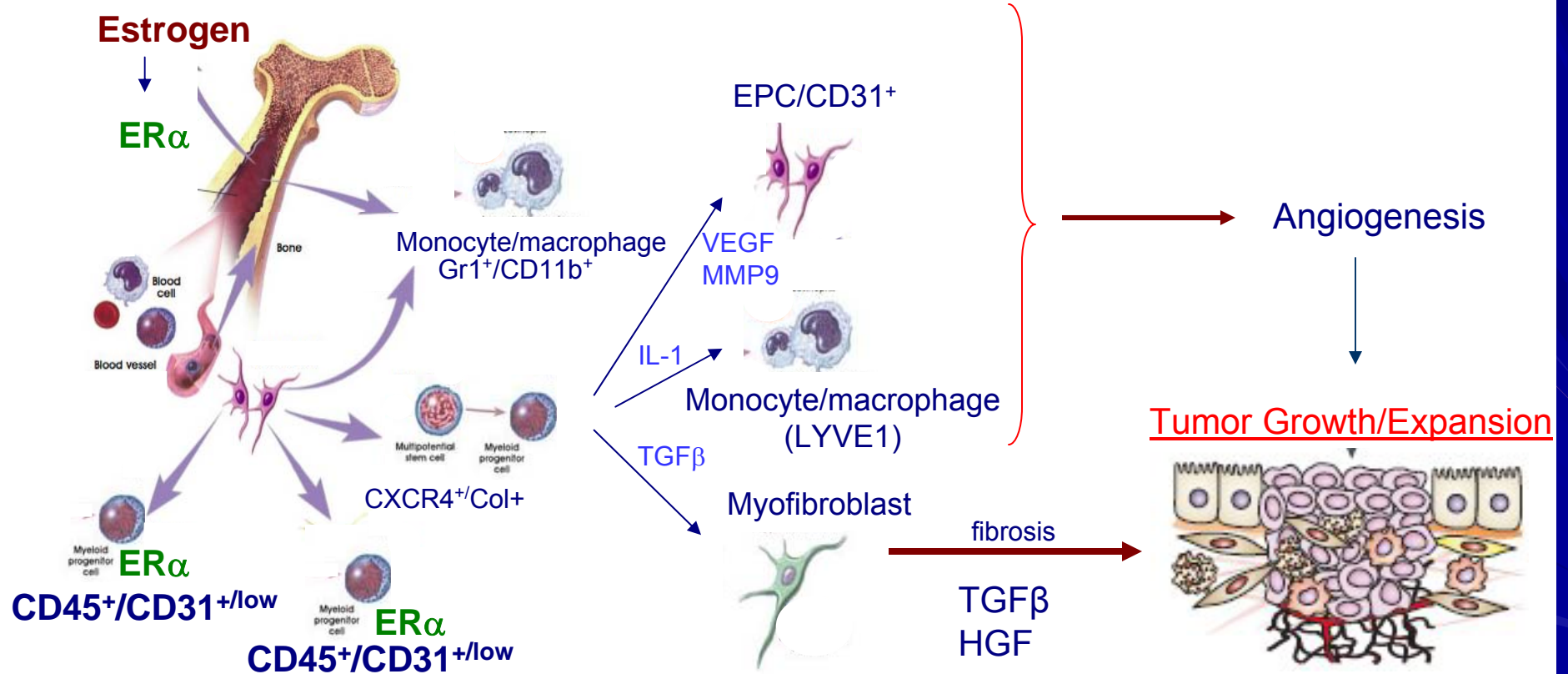
*WT*



*ER $\alpha$ KO*



# Working model of estrogen and stroma contributions in breast cancer growth



# Acknowledgments

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- Derrick Hwu

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- Pushkar Padke, MD/PhD

### Broad/MIT

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