

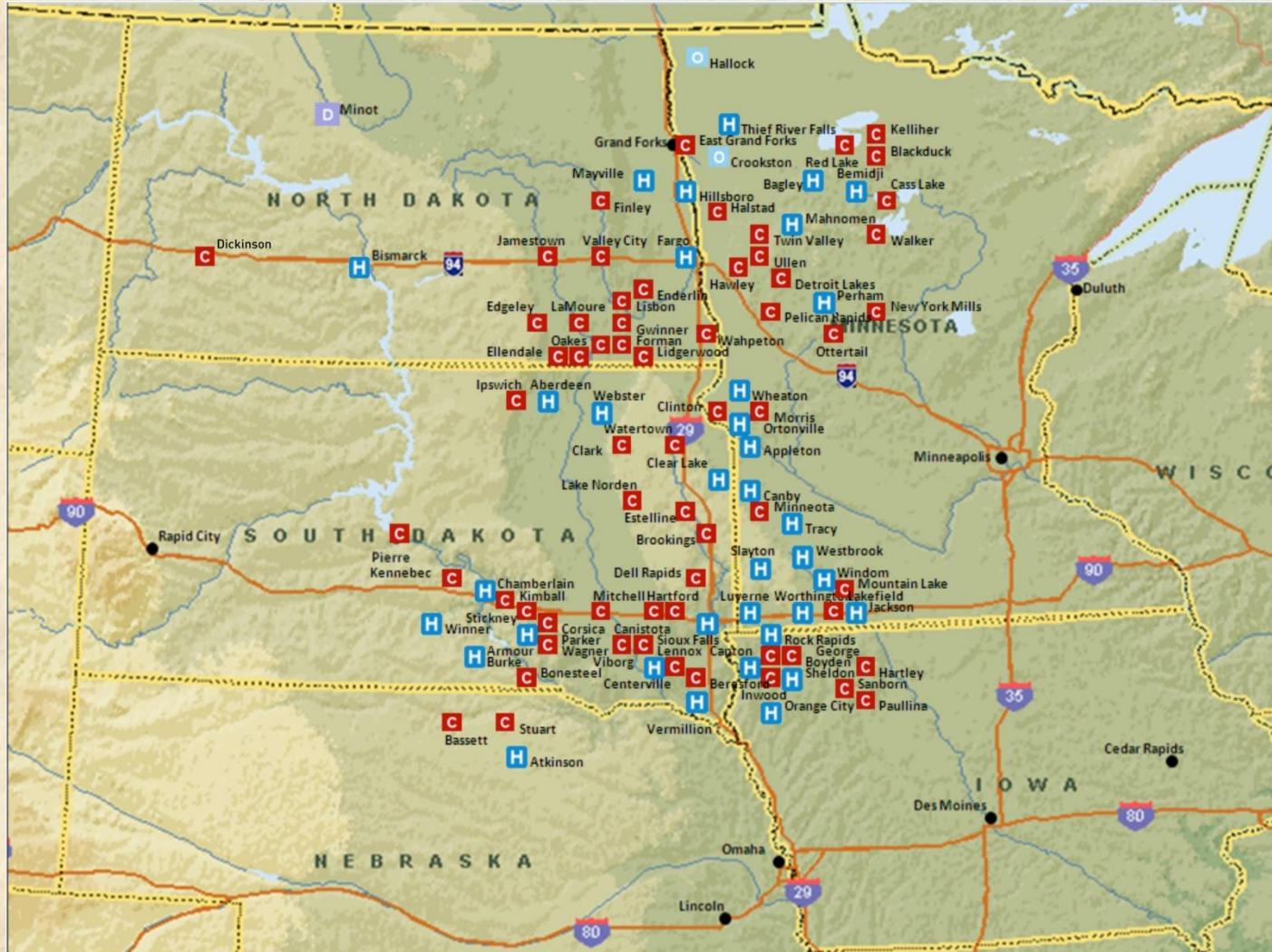
Smoking and cervical cancer health disparity

Subhash C. Chauhan
Sanford Research/USD

May 31, 2013

Over-view:

- Sanford Heath and Sanford Research/USD



Sanford Health and Sanford Research/USD

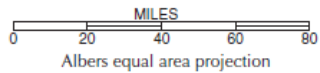
- Sanford Health is an integrated health system headquartered in the Dakotas and is now the largest rural, not-for-profit health care system in the nation with locations in 126 communities in seven states
- Sanford Health includes 35 hospitals, 140 clinic locations and nearly 1,200 physicians in 70 specialty areas of medicine.
- With more than 25,000 employees, Sanford Health is the largest employer in North and South Dakota.
- The system is experiencing dynamic growth and development in conjunction with Denny Sanford's nearly \$700 million in gifts, the largest ever to a health care organization in America.
- These gifts are making possible the implementation of the several initiatives including global children's clinics, multiple research centers and finding a cure for type 1 diabetes and breast cancer.



FEDERAL LANDS AND INDIAN RESERVATIONS

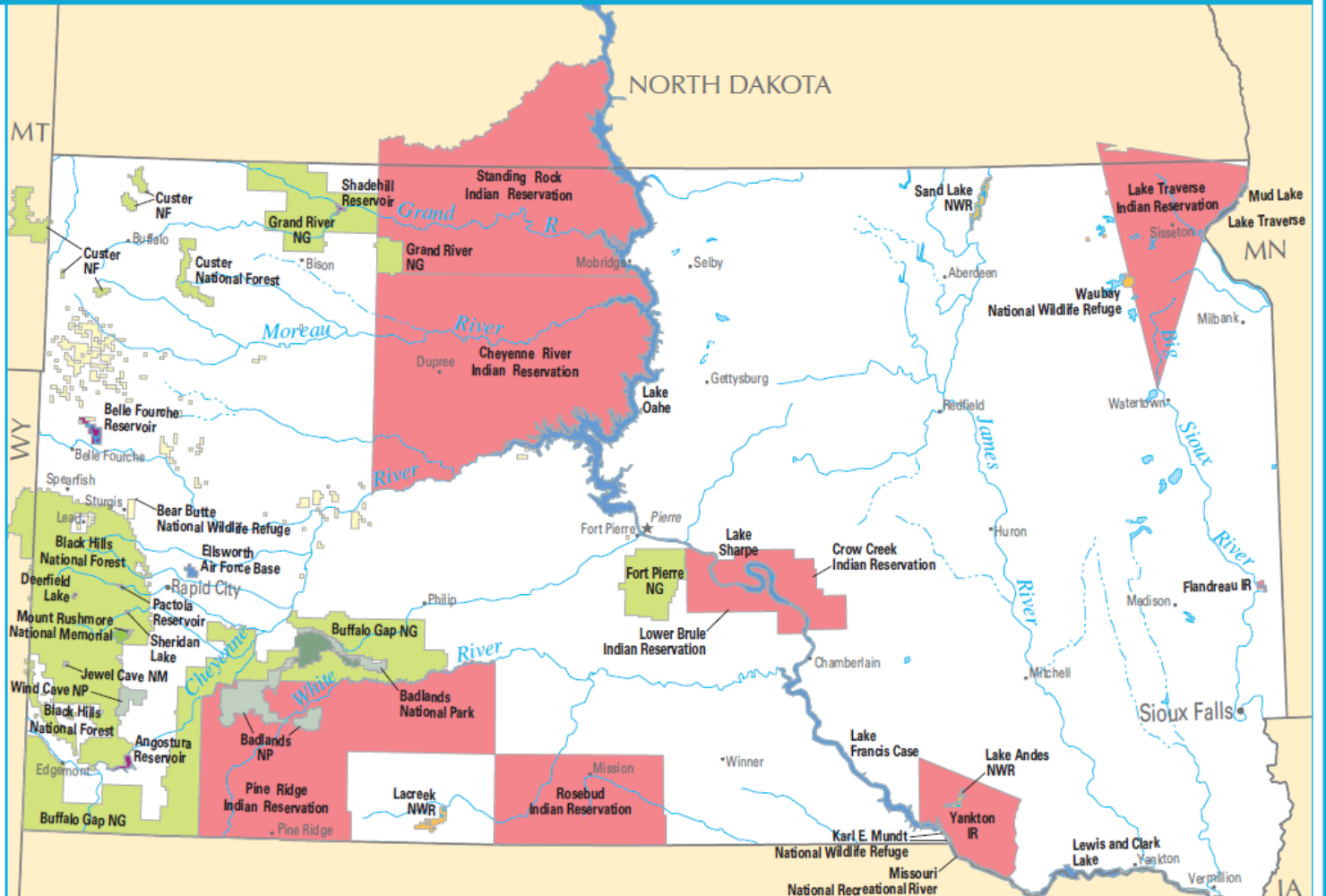
- Bureau of Indian Affairs
- Bureau of Land Management / Wilderness
- Bureau of Reclamation
- Department of Defense (includes Army Corps of Engineers lakes)
- Fish and Wildlife Service / Wilderness
- Forest Service / Wilderness
- National Park Service / Wilderness

Some small sites are not shown, especially in urban areas.



Abbreviations

- IR Indian Reservation
- NF National Forest
- NG National Grassland
- NM National Monument
- NP National Park
- NWR National Wildlife Refuge



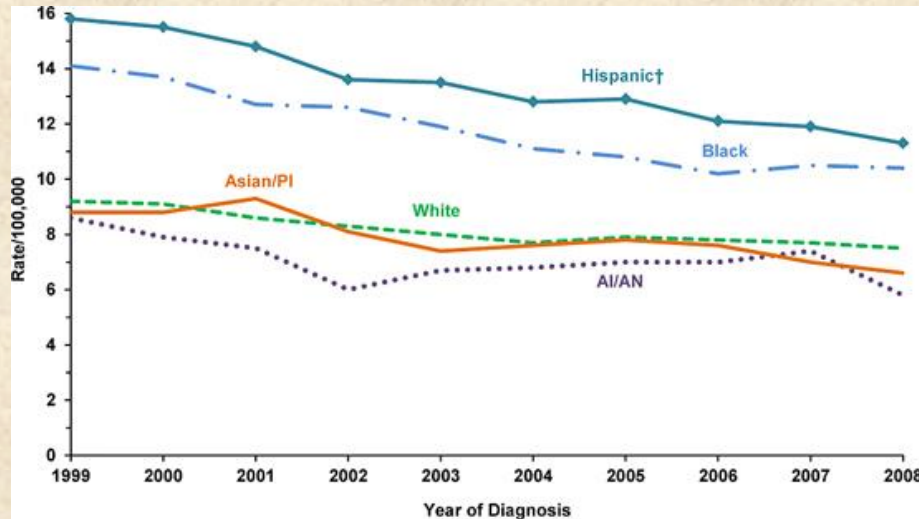
- South Dakota: Population of 824,000
- Native American: 8.9 % (73,300 people)

Cervical Cancer

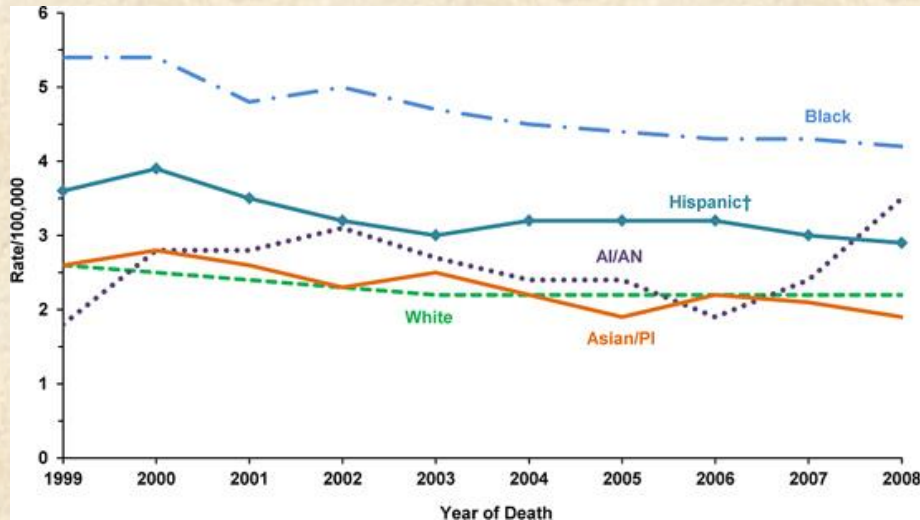
- **Globally: Cervical Cancer is the 2nd most common cancer among women**
 - **Per year, approximately 470,000 new cases, 233,000 deaths**
 - **Majority of cases occur in the developing world**
 - **In many developing countries, cervical cancer is the leading cause of cancer mortality in women**
 - **Screening programs have not been successfully established**
- **In the United States, during 2011**
 - ~ 12,710 cases of invasive cervical cancer were diagnosed
 - ~ 4,290 women in the US died
- **Strong correlation between infection with a high risk genotype of Human Papillomavirus and Cervical Cancer**
- **Development of the GARDASIL HPV vaccine (targeting HPV 16/18 and 11/6) prevents HPV infection and therefore reduces cervical cancer and genital warts caused by these genotypes of HPV**
 - **Promising, however, cervical cancer screening will still be important...**

Cervical Cancer 1999–2008

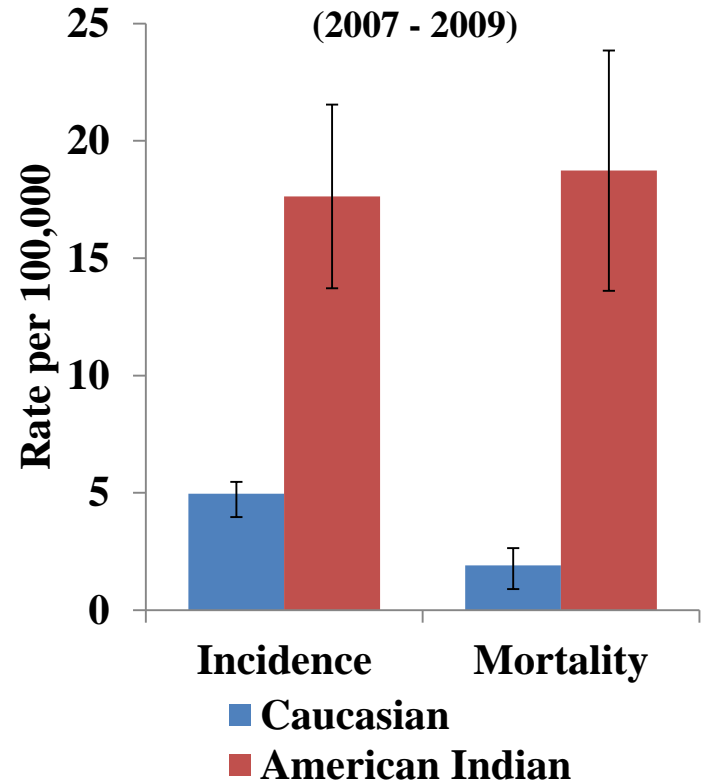
Incidence Rates* by Race and Ethnicity, U.S.,



Mortality Rates* by Race and Ethnicity, U.S.,

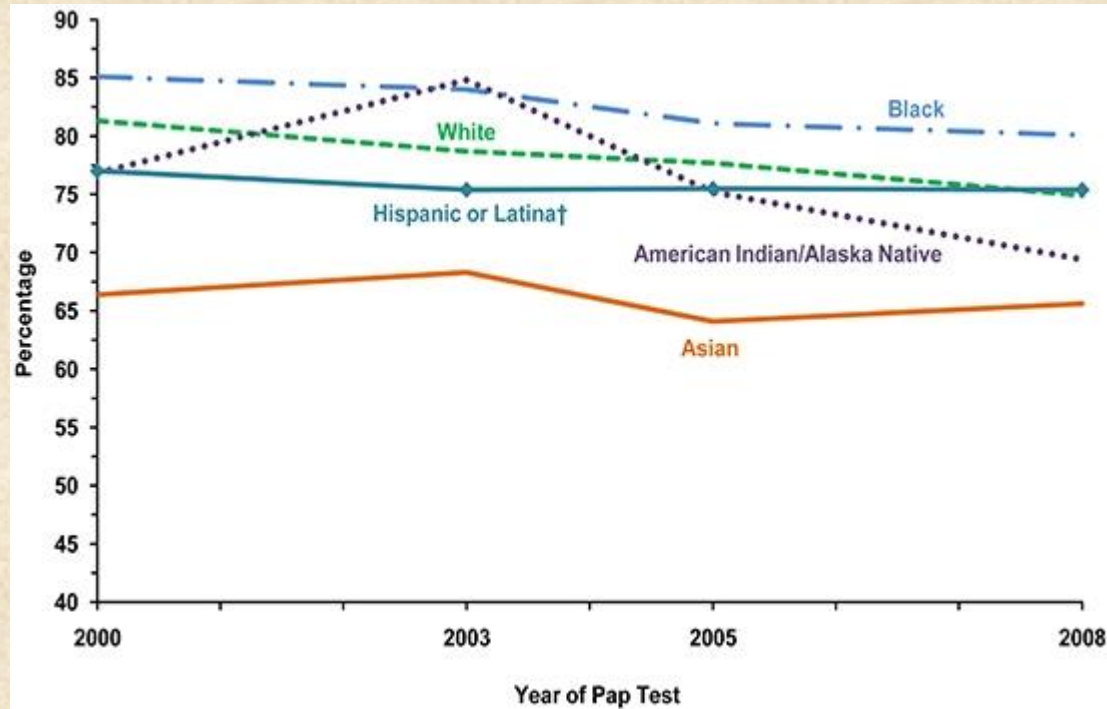


Average Incidence and Mortality of Cervical Cancer in South Dakota (2007 - 2009)



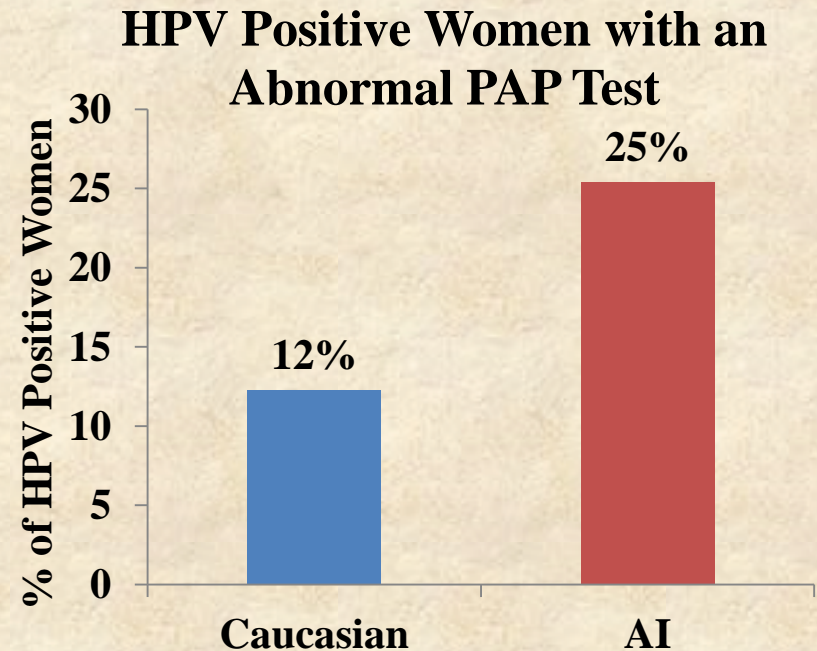
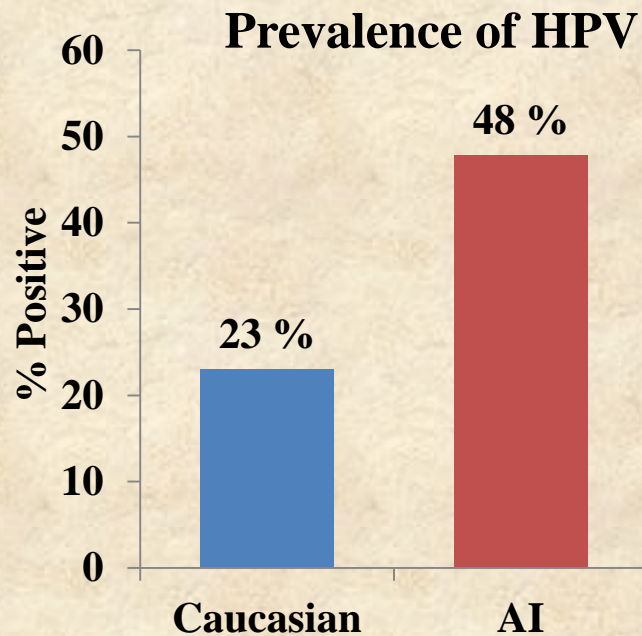
Data from SD Department of Health (2007 to 2009).

Percentage of U.S. Women Aged 18 Years and Older Who Have Had a Pap Test in the Last 3 Years by Race and Ethnicity

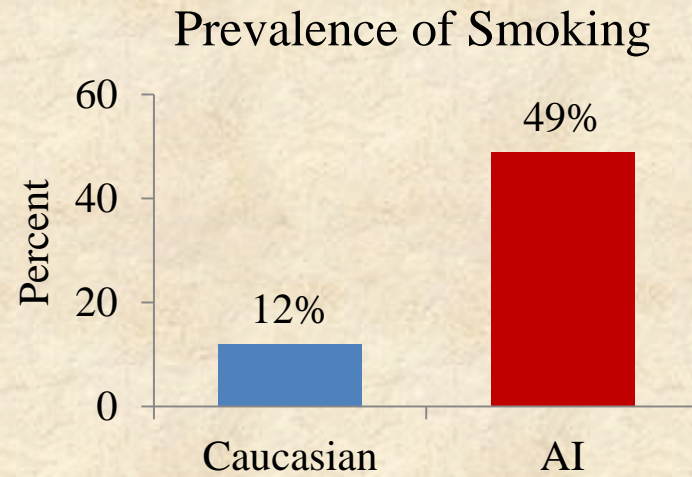


Available data suggests a similar or higher rate of cervical cancer screening is obtained for American Indian women living in South Dakota.

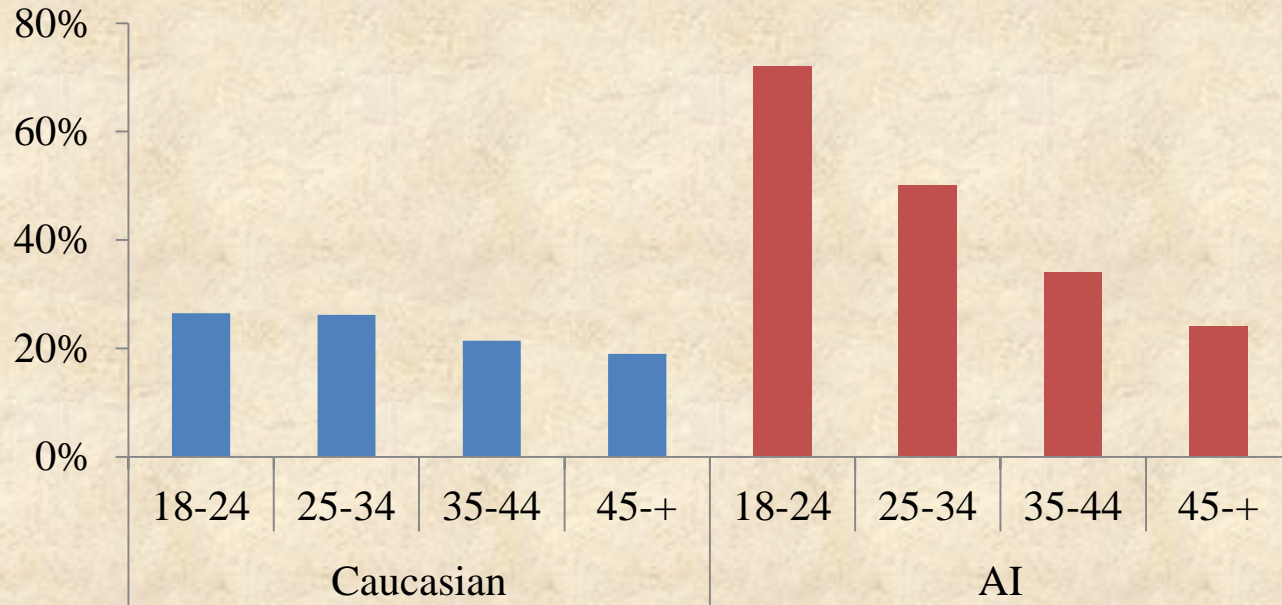
HPV is more commonly detected in AI women and HPV Positive AI women have higher rates of abnormal PAP tests



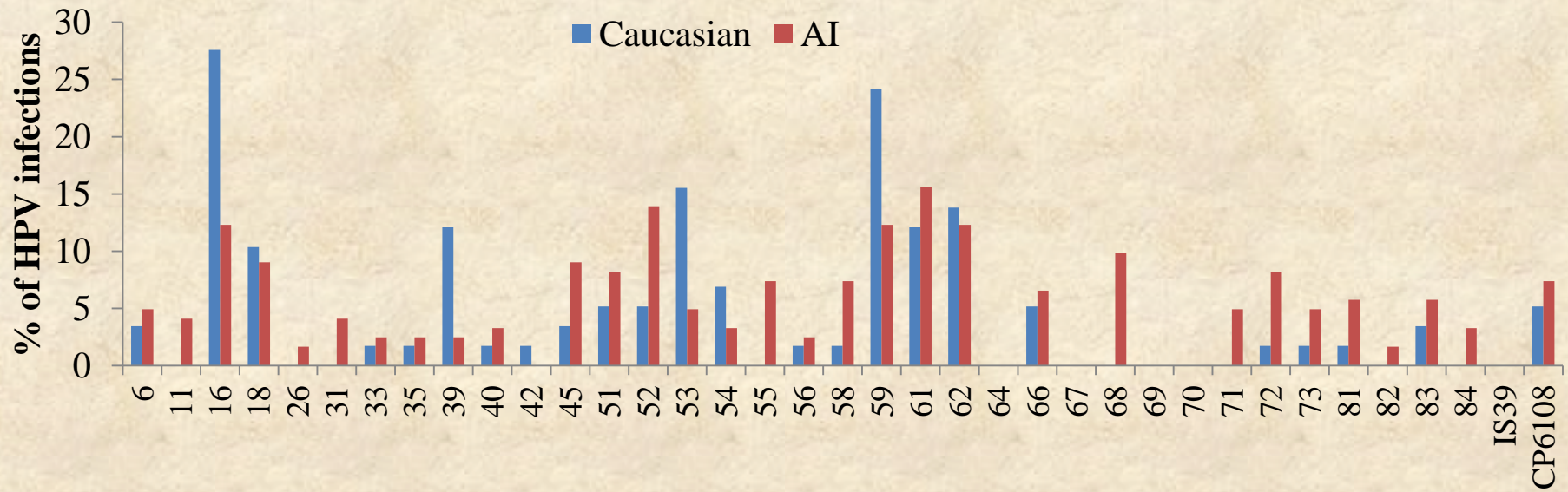
Smoking Rates



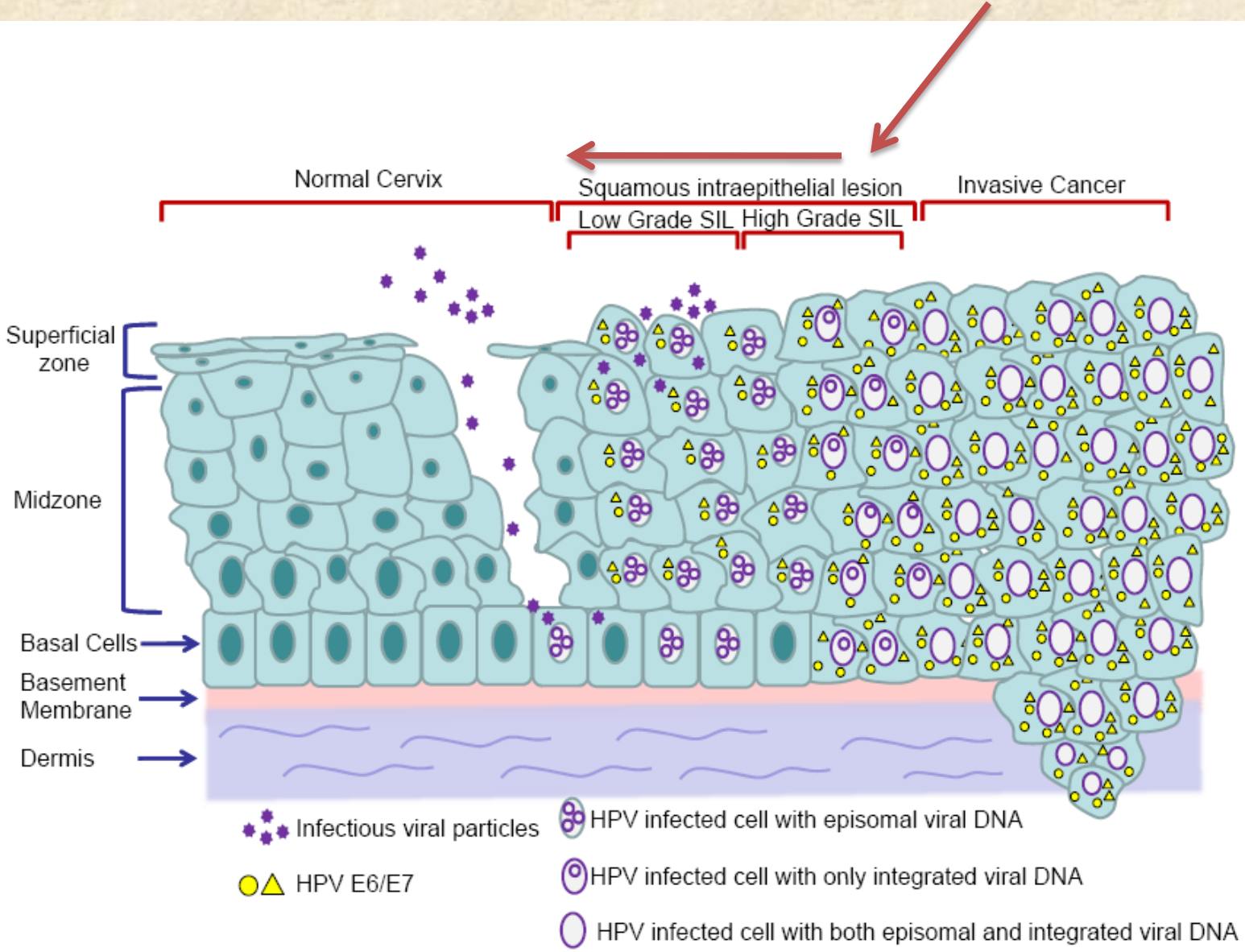
Percent HPV Positive by Age Group



Prevalence of HPV Genotypes (% Based on Positives)



Natural Progression of HPV Infection and a Role for Chemoprevention...



Maher et al, Advances in Gynecological Oncology; 2010
 Control of Human Papillomavirus gene expression by transcription factors and the upstream regulatory region

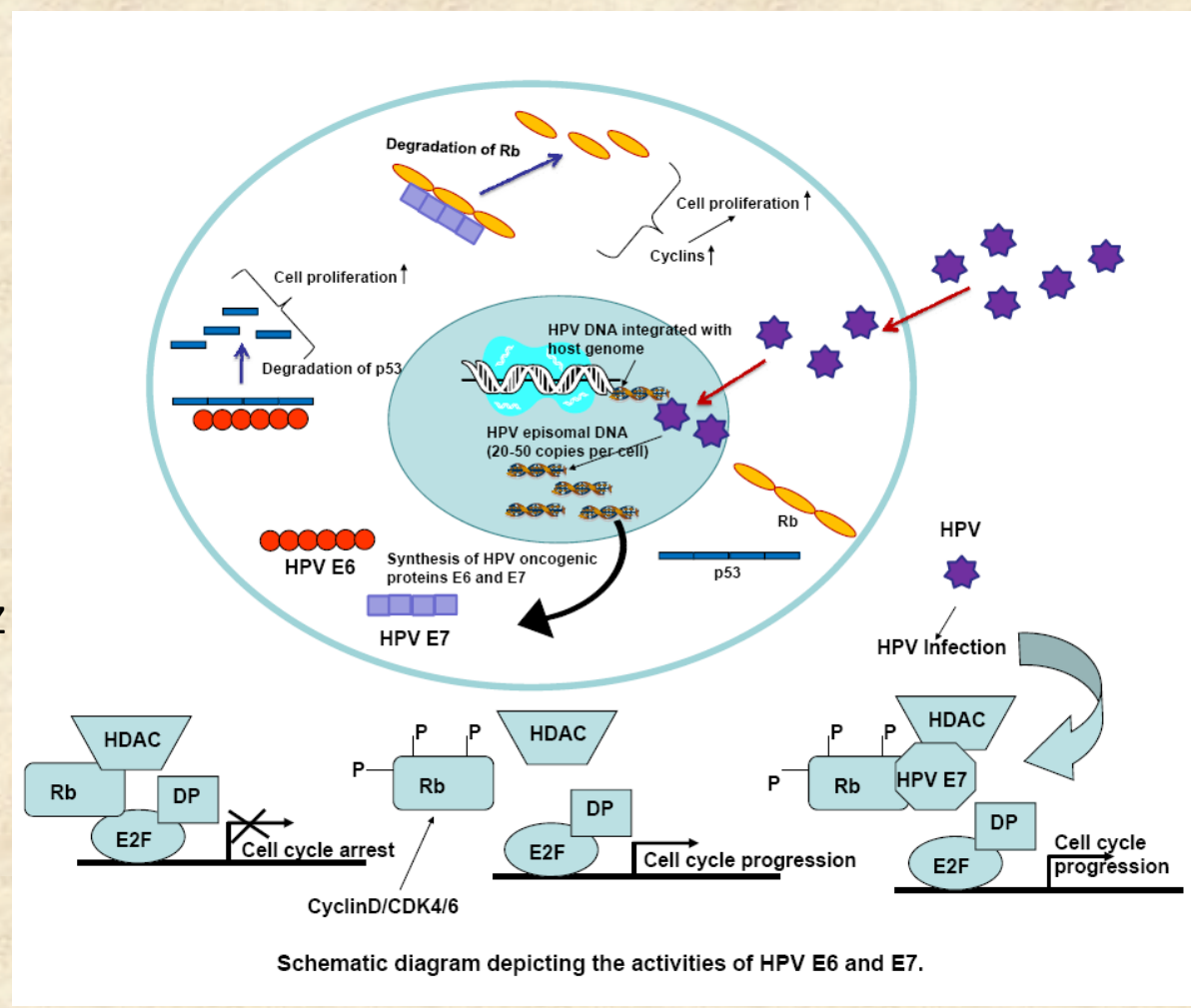
- HPV E6/E7 from high risk HPV genotypes are required for the development of cervical cancer

- **Most famous pathways**

- E6 – degrades p53 (tumor suppressor)
 - E7 – interferes with retinoblastoma protein (tumor suppressor)

- **Other important pathways**

- E6 – activation of telomerase, degradation of proteins with PDZ domains (roles in cell signaling and adhesion)
 - E7 - up-regulation of AKT pathway, and interactions with various cell signaling molecules (cyclin A and E, p27, p21 etc.)



Schematic diagram depicting the activities of HPV E6 and E7.

- Reducing the amount of HPV E6/E7 should be beneficial in interrupting the development of invasive cervical cancer.



Curcumin (diferuloyl methane)



Biological Effects of Turmeric/Curcumin

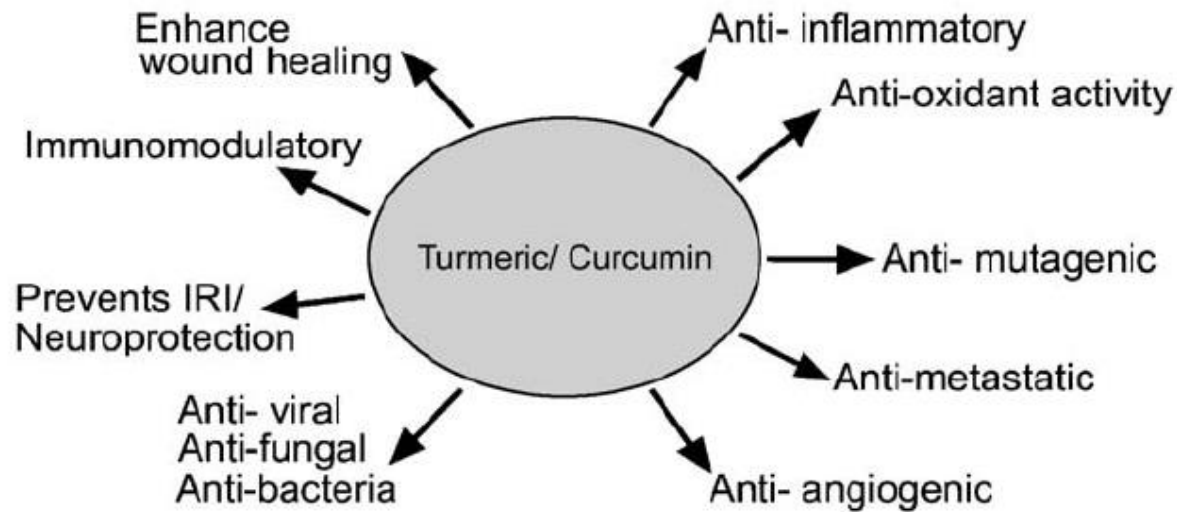


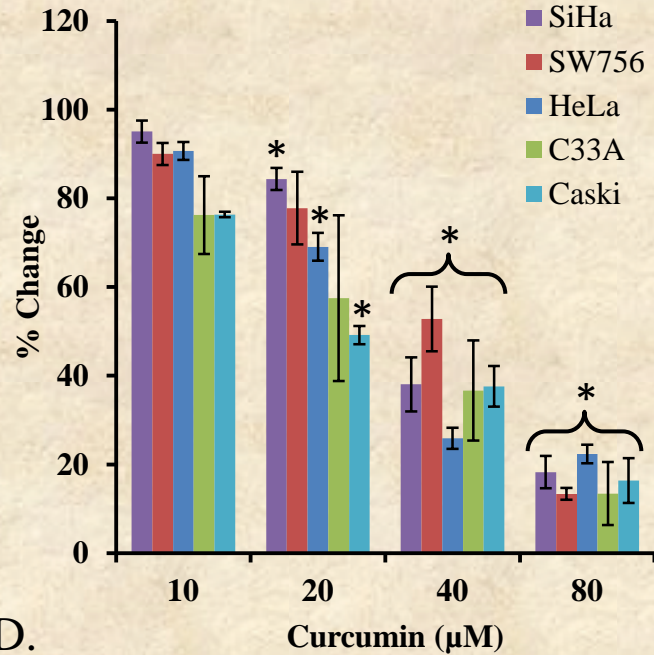
Fig. 2. Schematic showing multiple biological activities of turmeric/curcumin.

Maheshwari, et al. 2006. Life Sciences.

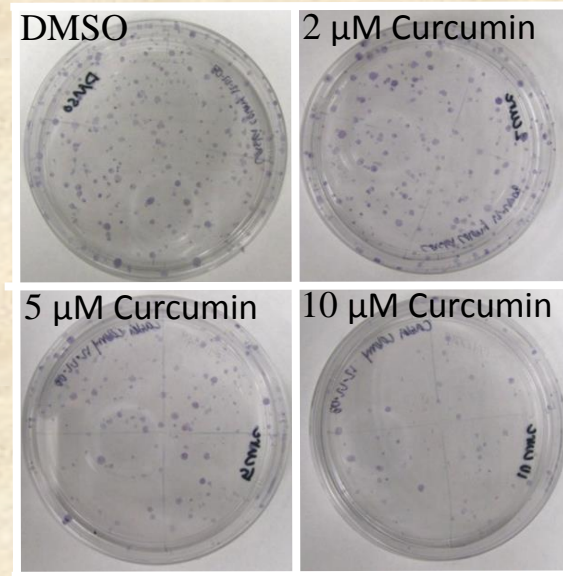


Curcumin treatment suppresses cervical cancer cell growth in monolayer and organotypic raft culture systems

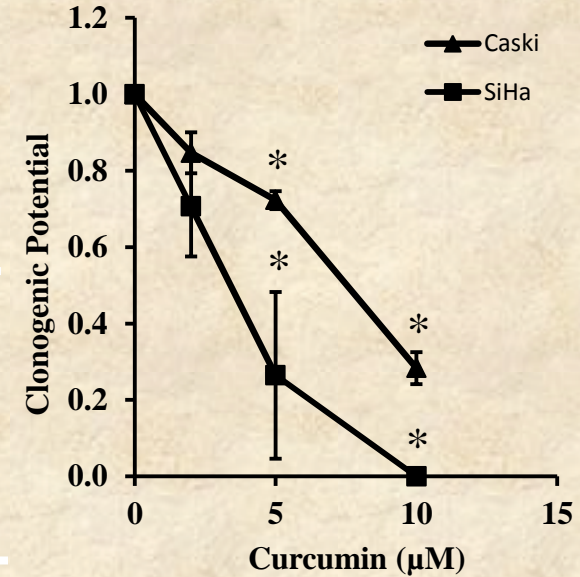
A.



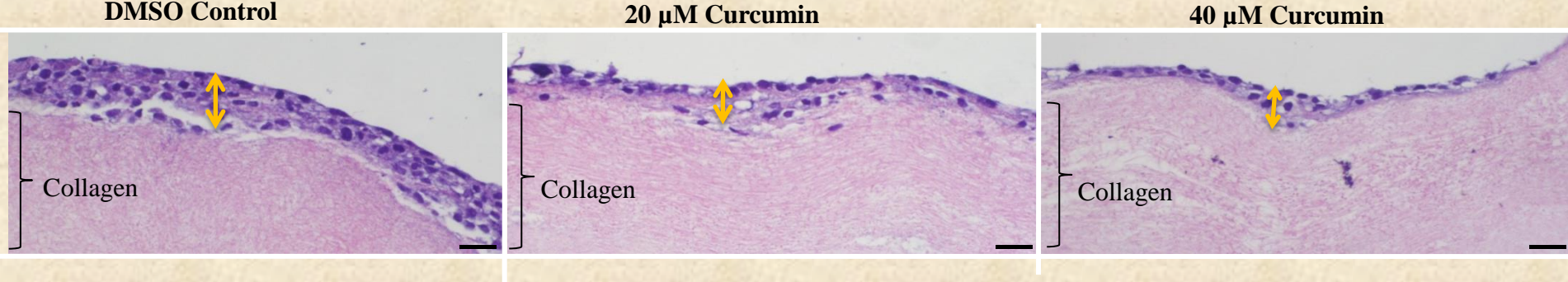
B.



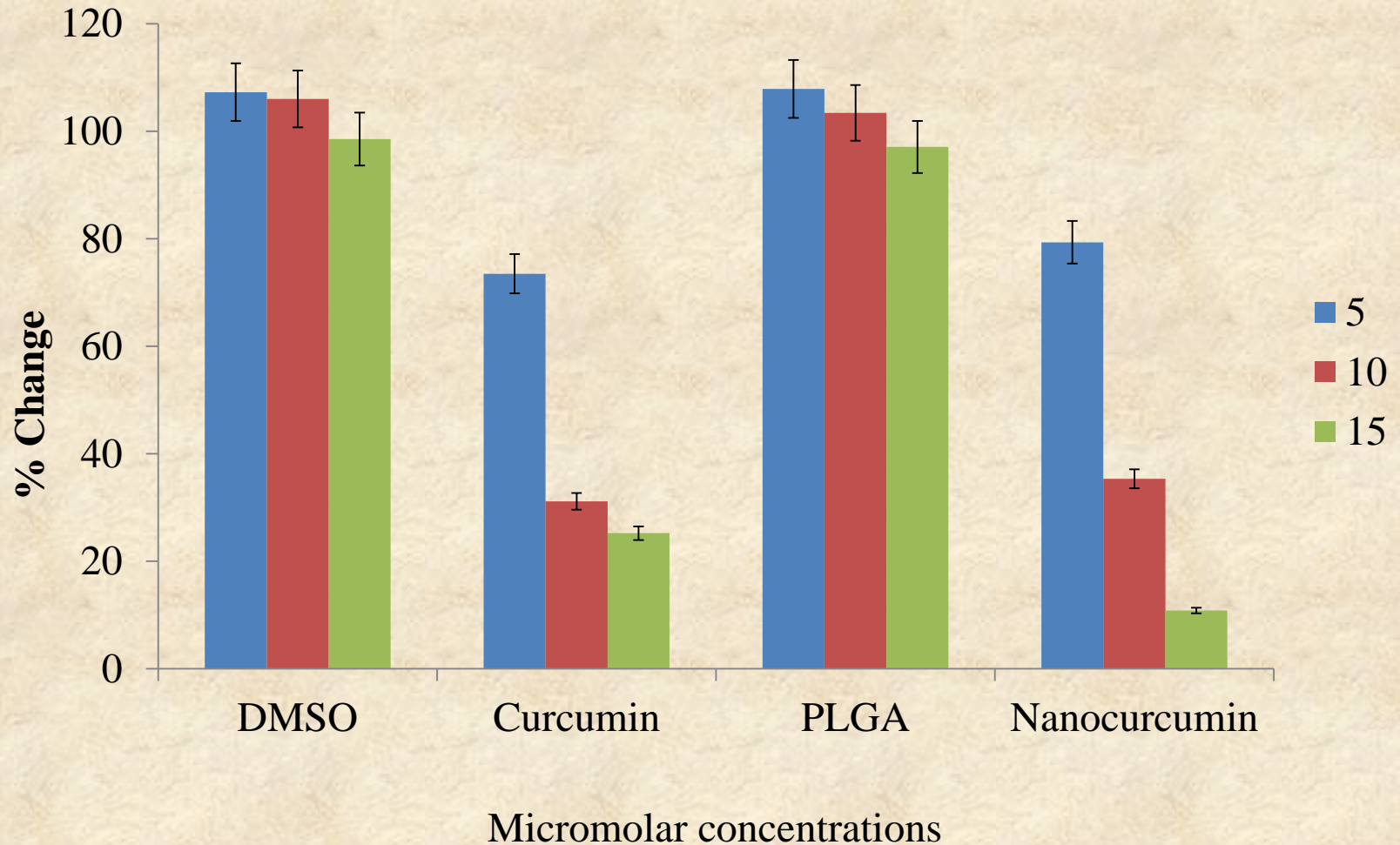
C.



D.

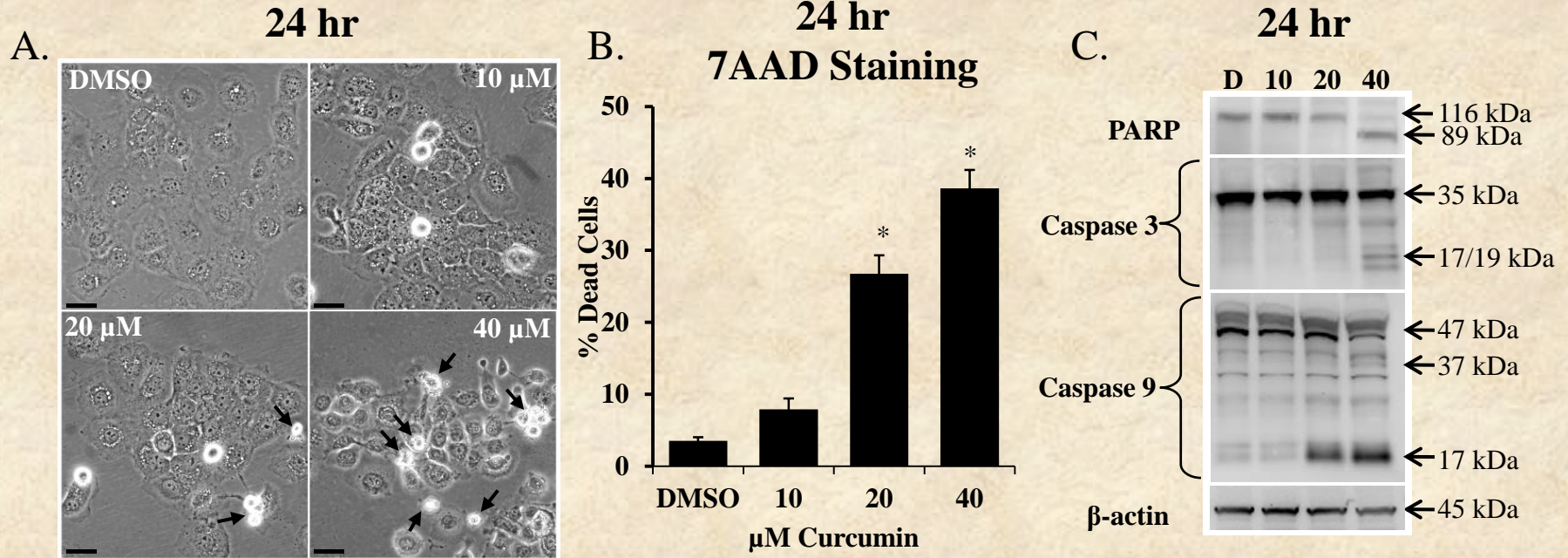


A PLGA nano-formulation of Curcumin effectively suppresses cervical cancer cell growth



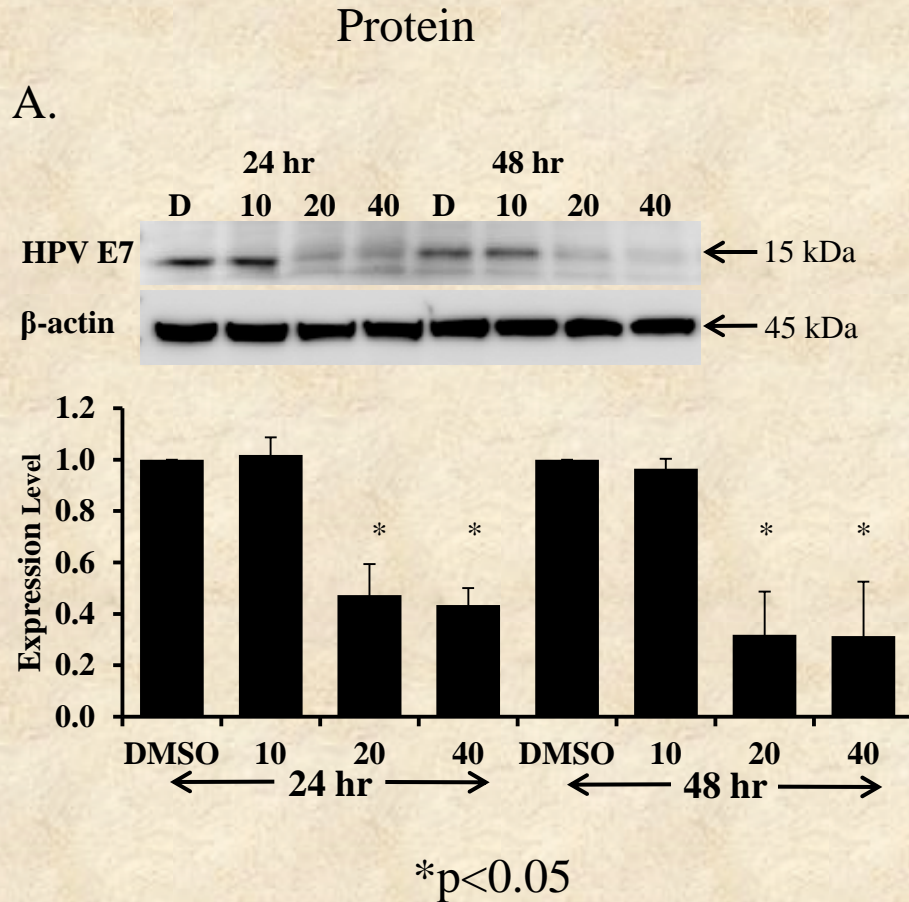
MTS assay 24 hours after treatment

Curcumin treatment induces apoptosis in cervical cancer cells via caspase-mediated signaling.

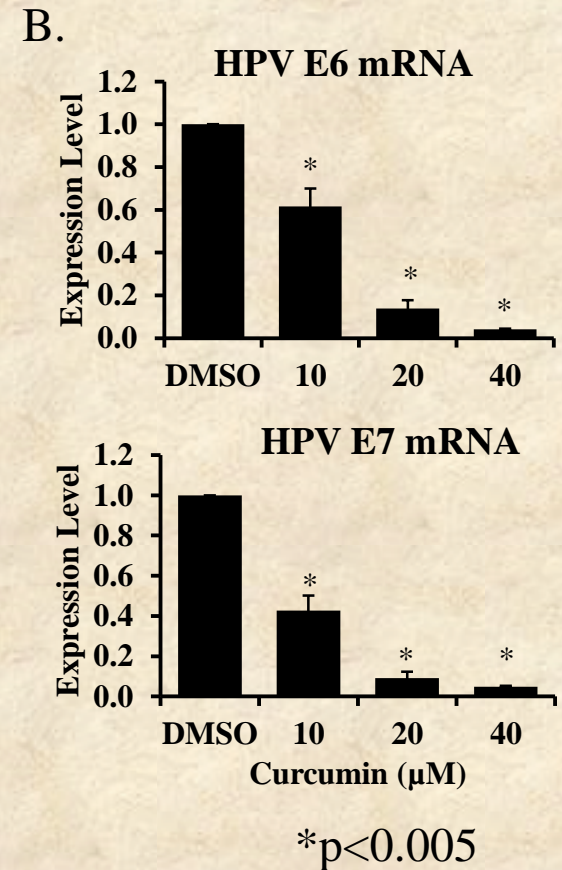


* $p < 0.05$

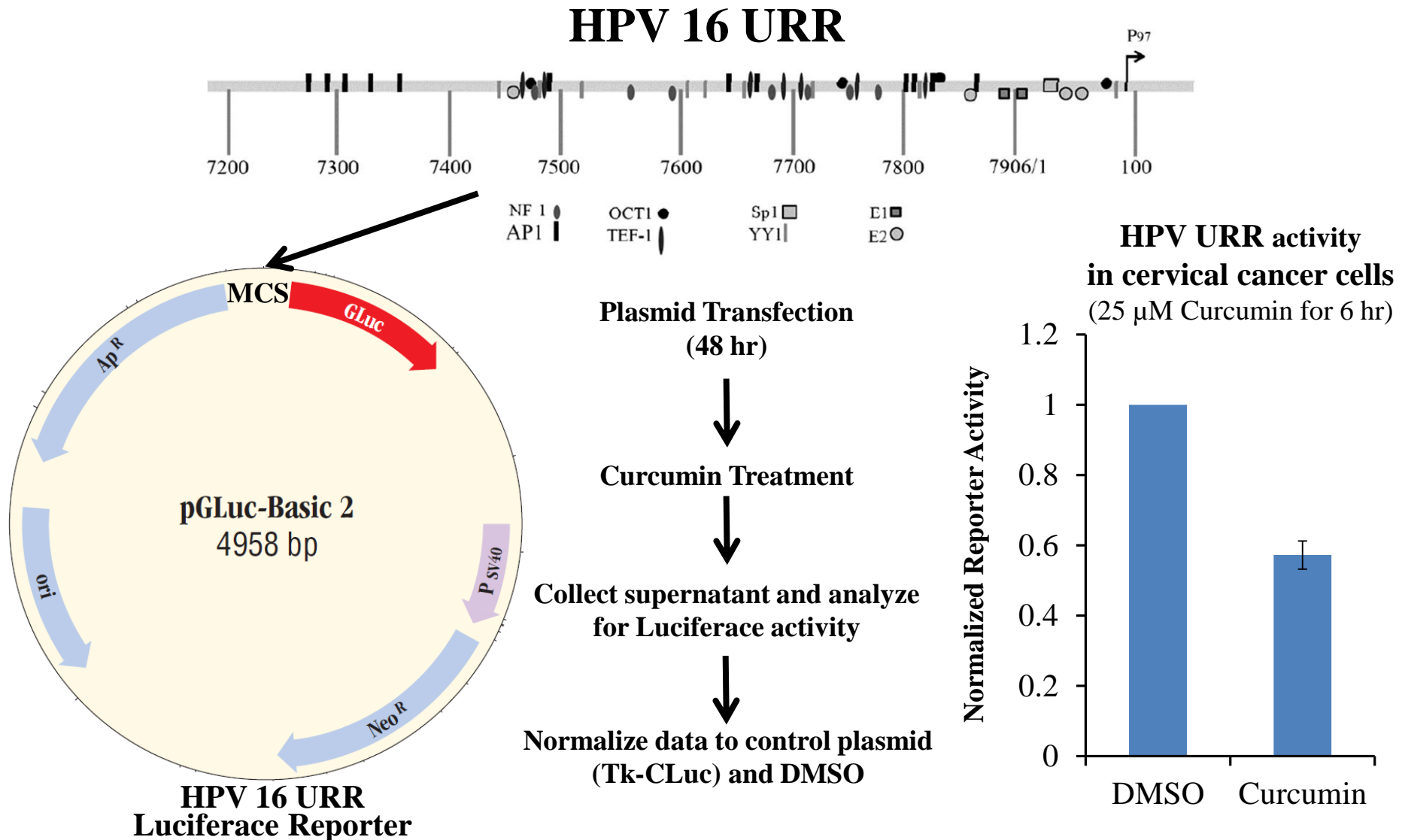
Curcumin treatment represses the expression of HPV oncogenes E6 and E7



RNA: 6 hr after addition of curcumin

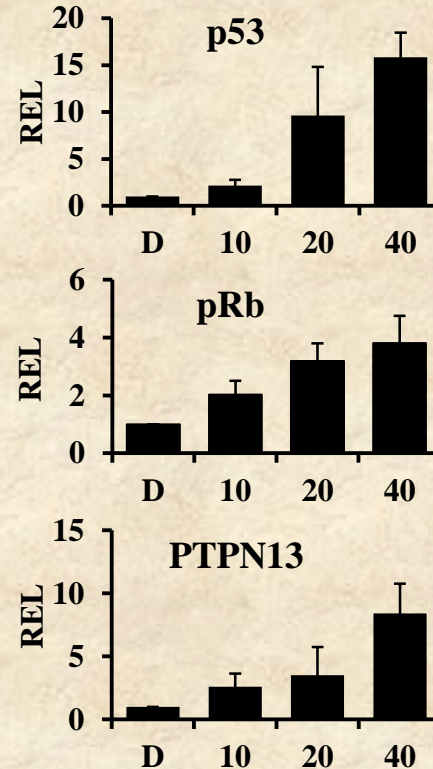
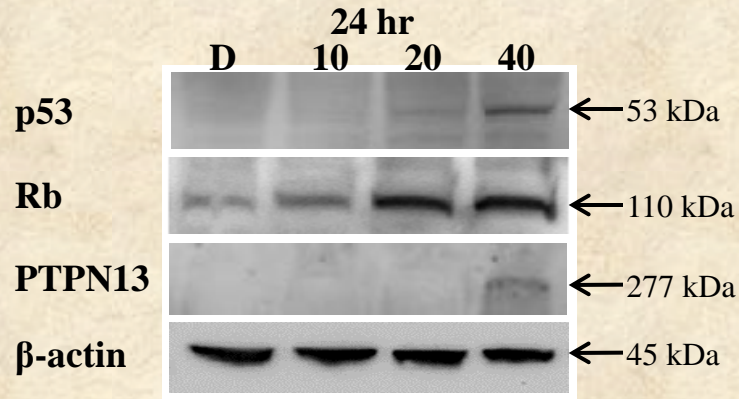


Curcumin treatment represses the activity of the HPV16 URR (upstream regulatory region)



Curcumin restores the expression of tumor suppressor proteins: p53, Rb, PTPN13

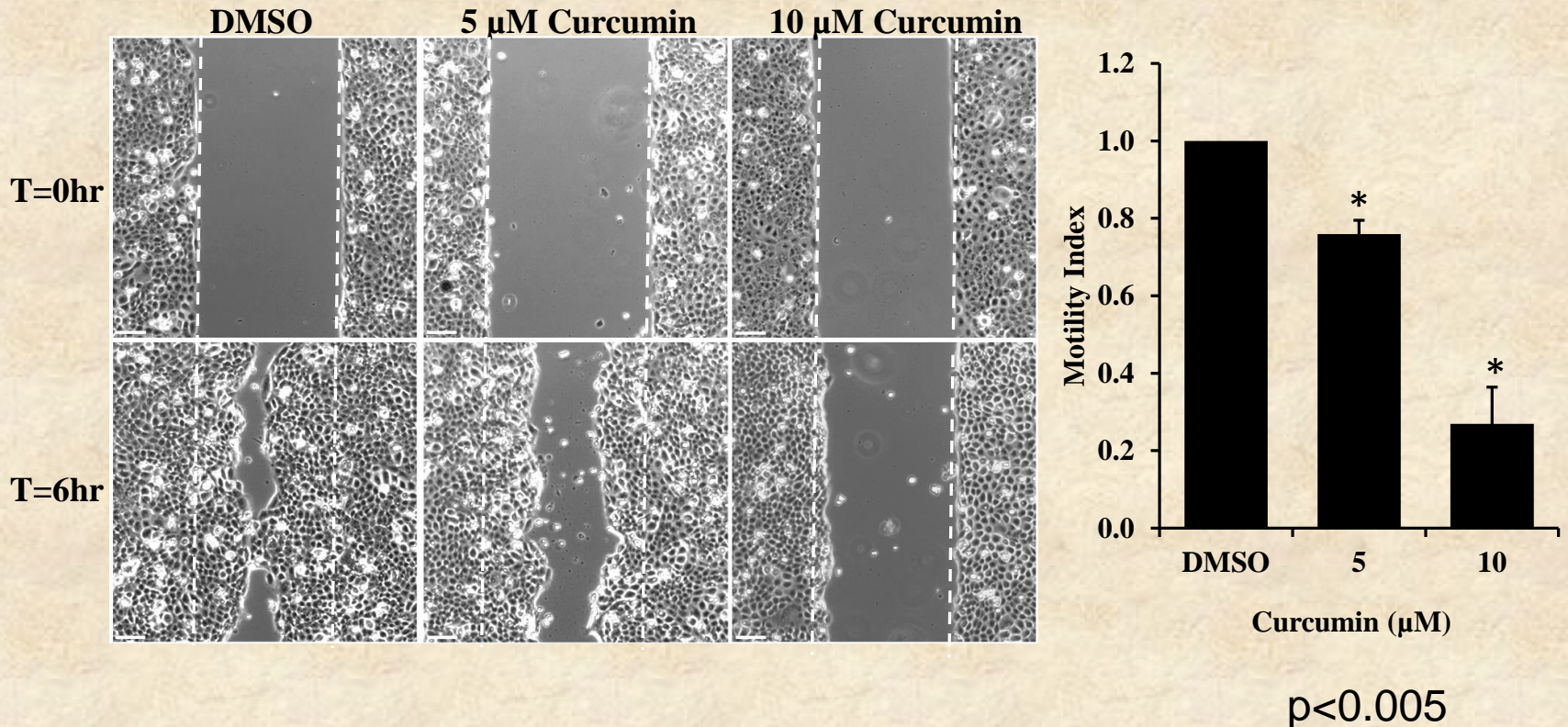
(each known to be degraded when HPV E6 or E7 is expressed)



D = DMSO

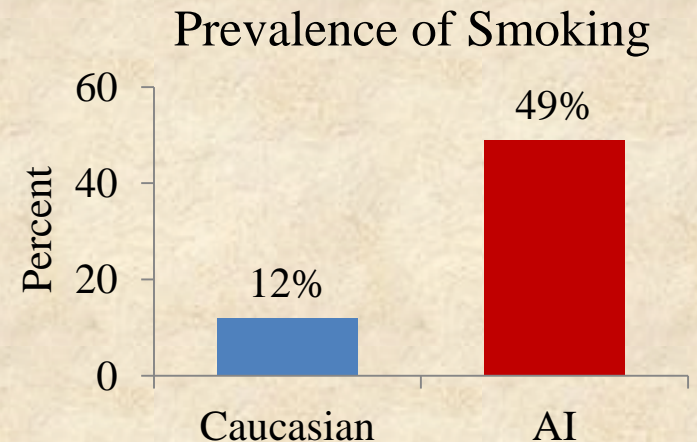
REL: relative expression level

Curcumin treatment inhibits the motility of cervical cancer cells

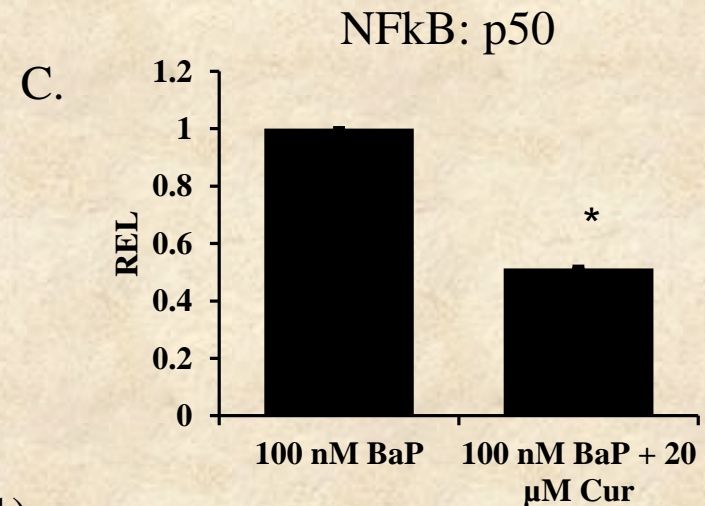
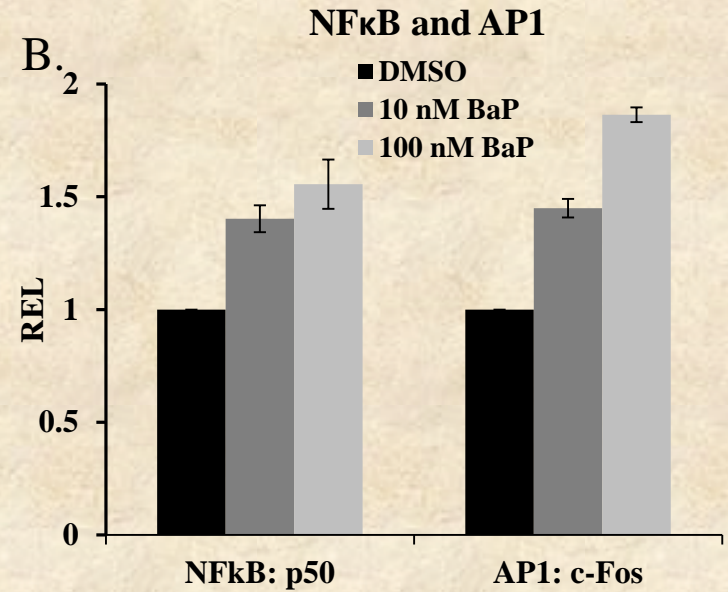
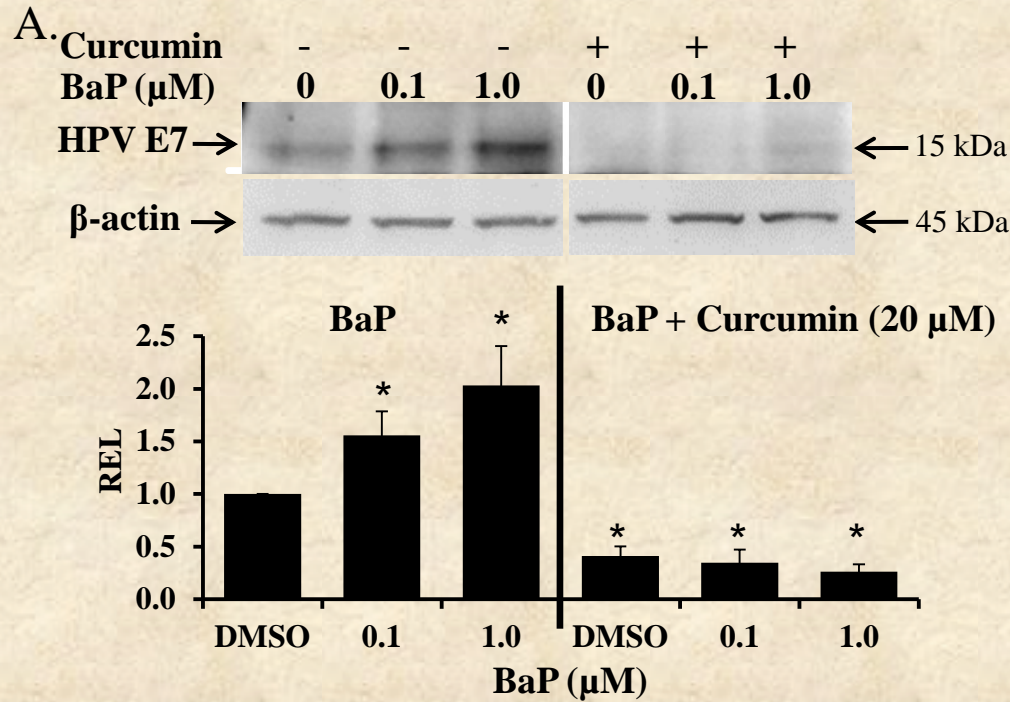


Smoke Carcinogen: Benzo[*a*]pyrene (BaP)

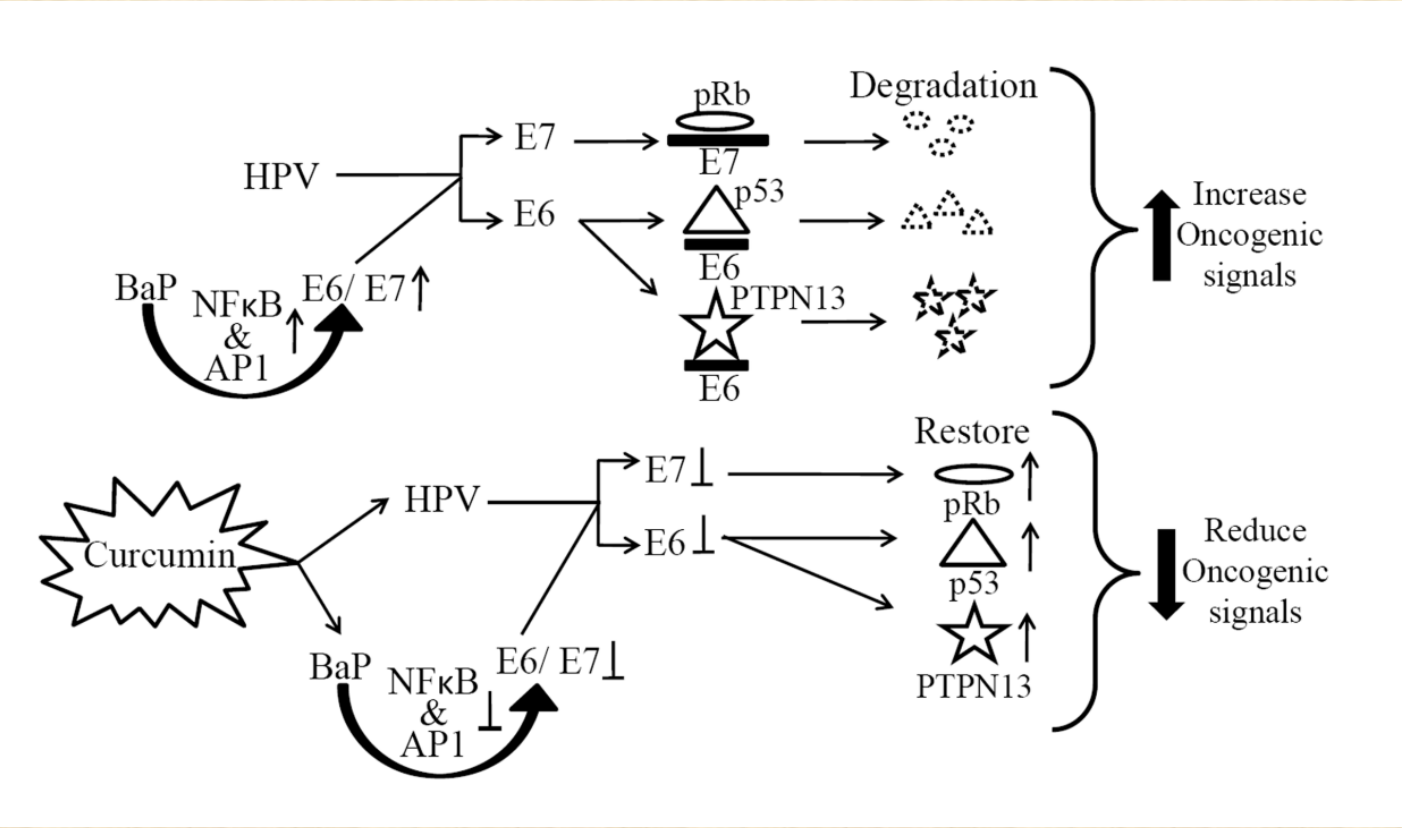
- Cigarette smoking is known to be a risk factor for cervical cancer
 - However, the molecular link between smoking and HPV is unknown
- BaP is a polycyclic hydrocarbon. These compounds are generated by burning carbon containing materials, such as:
 - Tobacco, Charbroiled Meat, Fried Food (especially when the same oil is used repeatedly), Wood
- BaP is detected in cervical mucus of women who smoke
 - Melikian AA, Sun P, Prokopczyk B, et al. Identification of benzo[*a*]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. *Cancer Lett* 1999; 146: 127-34.
 - McCann MF, Irwin DE, Walton LA, Hulka BS, Morton JL, Axelrad CM. Nicotine and cotinine in the cervical mucus of smokers, passive smokers, and nonsmokers. *Cancer Epidemiol Biomarkers Prev* 1992; 1: 125-9.



Tobacco smoke compound BaP up-regulates the expression of HPV oncogenes, NFκB and AP1 but is suppressed by curcumin treatment



Schematic model of increased oncogenic signals *via* HPV E6/E7 oncoproteins and molecular effect of curcumin on HPV associated cellular events.



Top: HPV oncoproteins degrade tumor suppressor proteins, increasing the risk of developing cancer. BaP increases the expression of HPV oncoproteins, potentially increasing oncogenic signals and even in the presence of only a few copies of HPV genome.

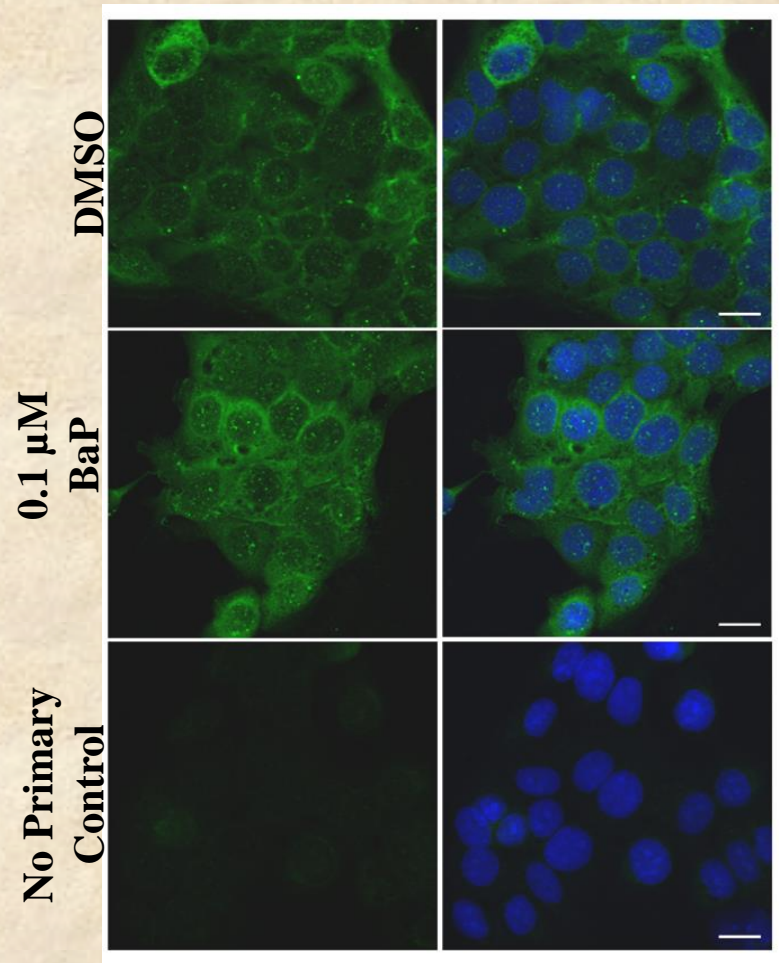
Bottom: Curcumin specifically inhibits the expression of HPV oncoproteins, even in the presence of BaP, thereby reducing the oncogenic signals and potentially inhibiting cancer development.

BaP exposure activates the AhR pathway increasing CYP1A1 expression

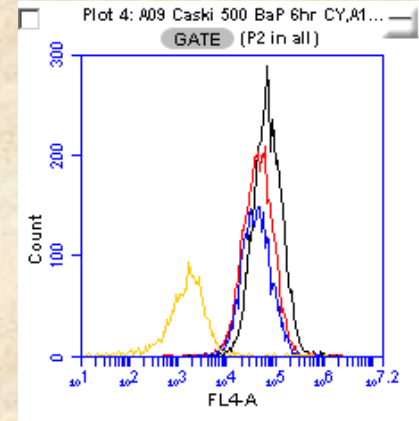
Caski

AhR

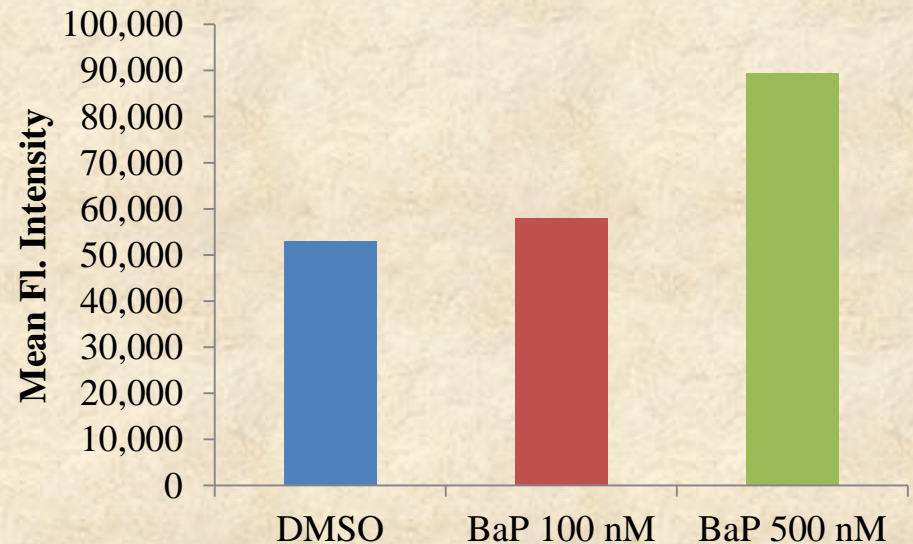
Composite



6 hour

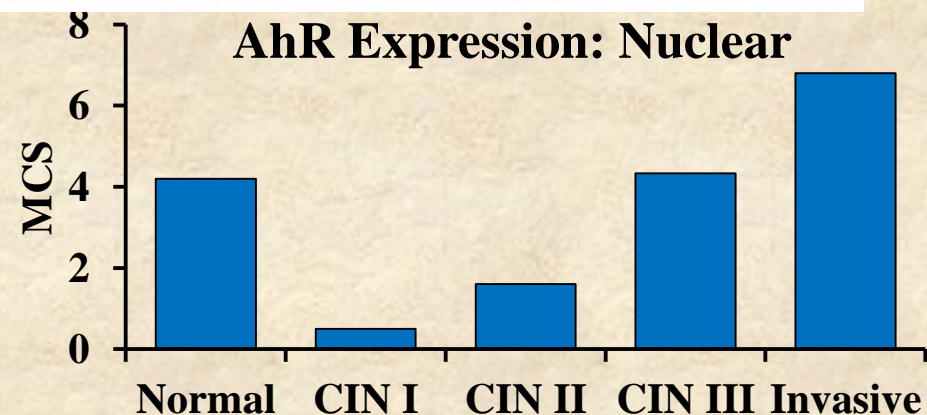
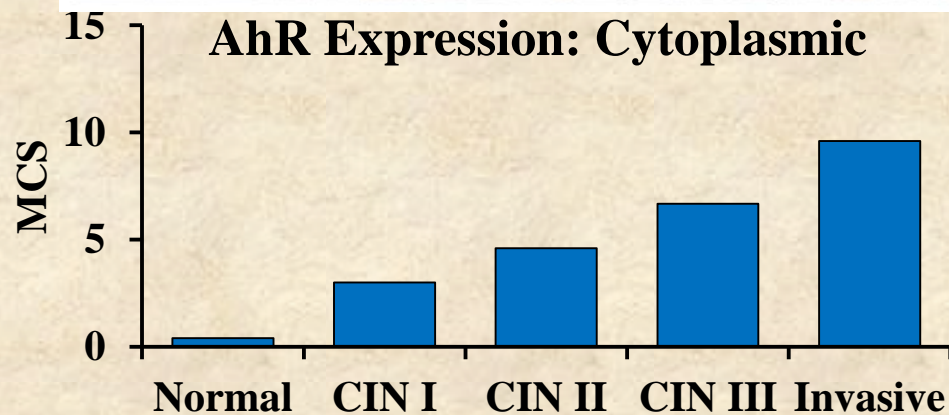
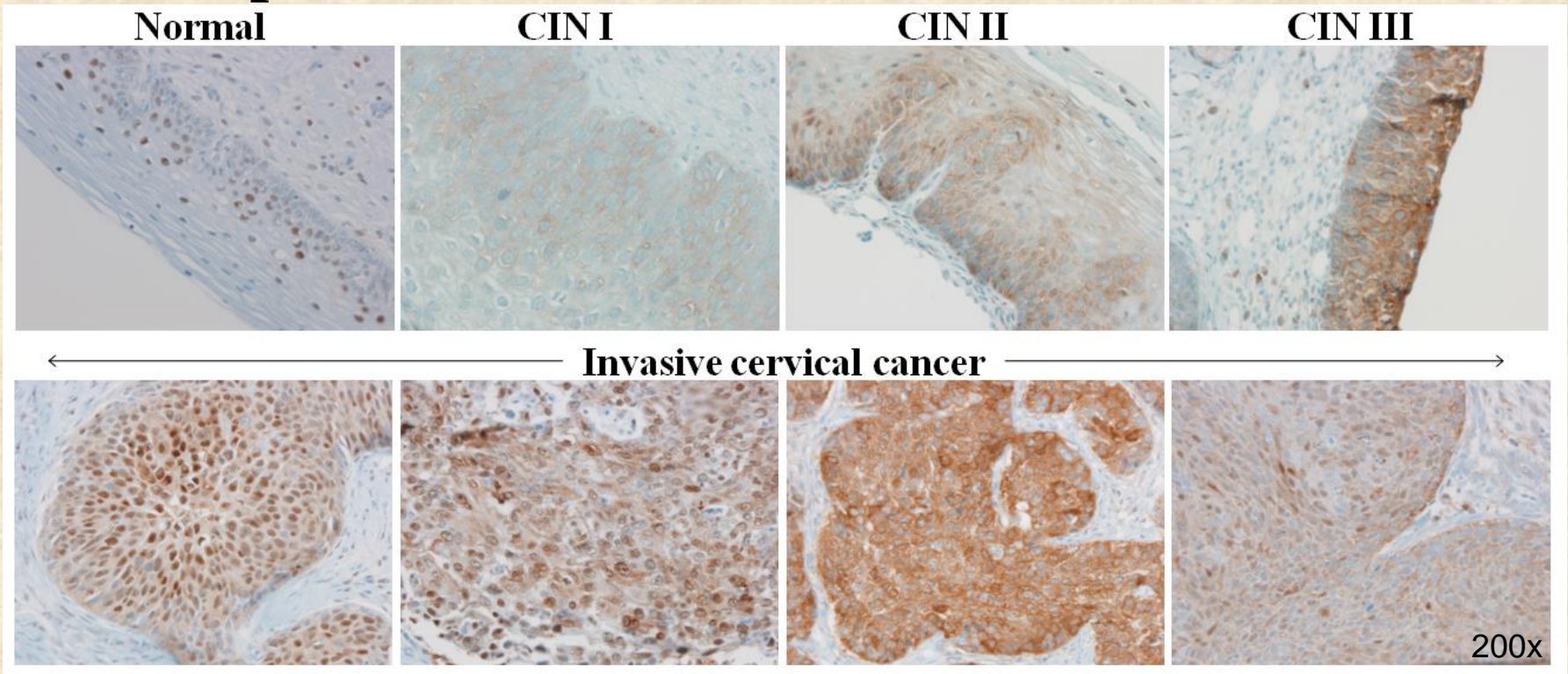


- 500 nM BaP
- 100 nM BaP
- DMSO
- No Primary Control



Representative data from 3 independent experiments

AhR is aberrantly expressed and localized in pre-neoplastic and invasive cervical cancer tissues

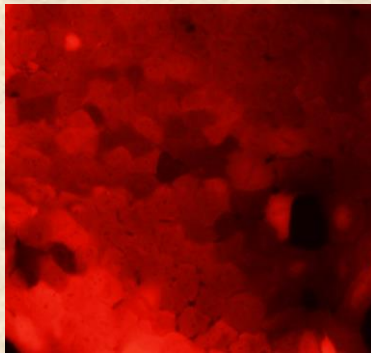


normal (n=10), cervical intraepithelial neoplasia I (CIN I, n=10), CIN II (n=10), CIN III (n=6), and invasive cervical cancer (n=15).

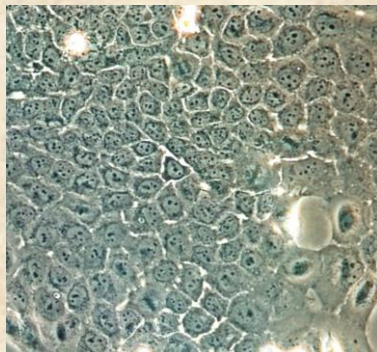
Development of an orthotopic cervical cancer mouse model

A) Stable tdTomato expression in cervical cancer cells

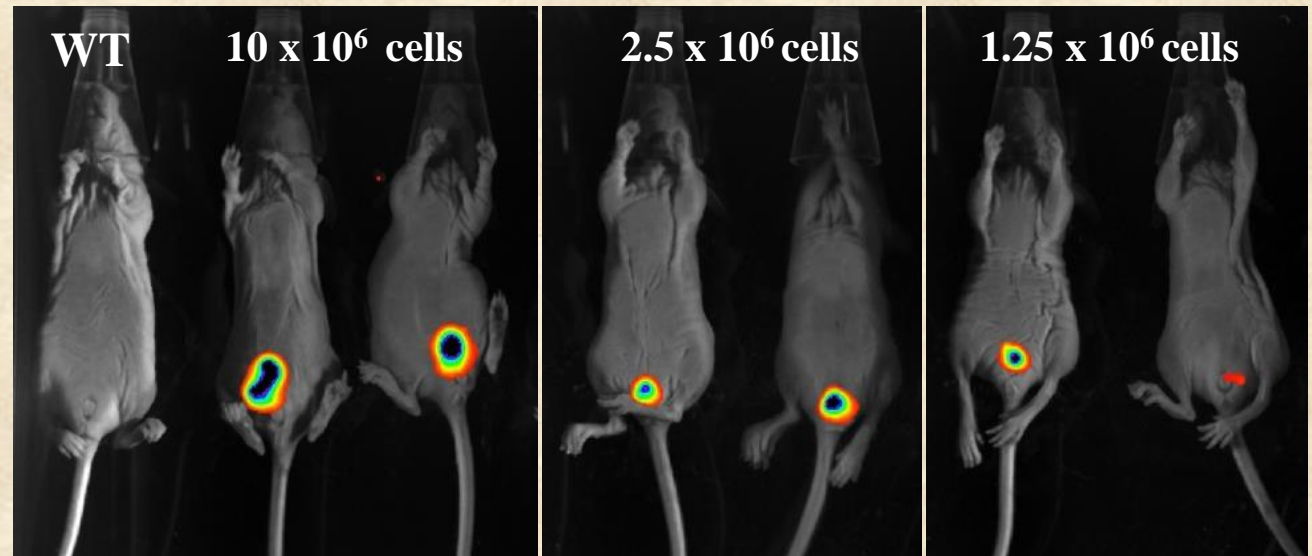
Red Fluorescence



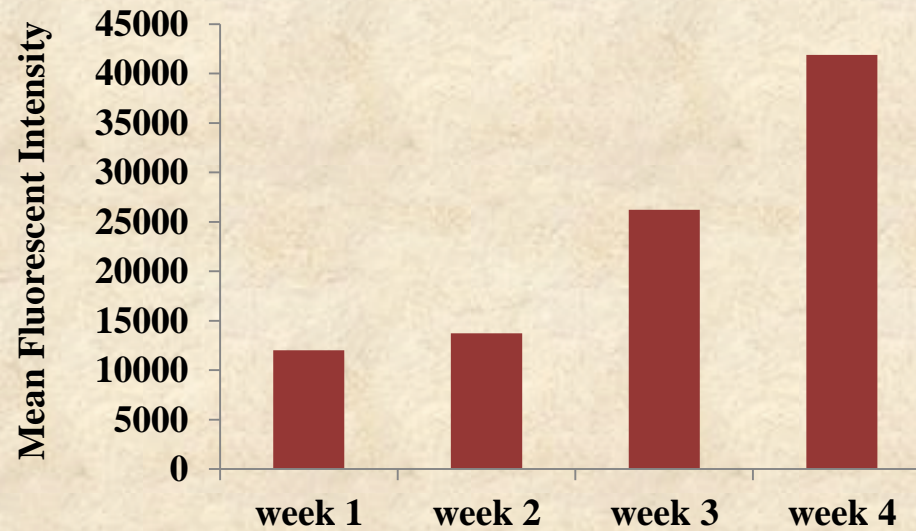
Phase Contrast



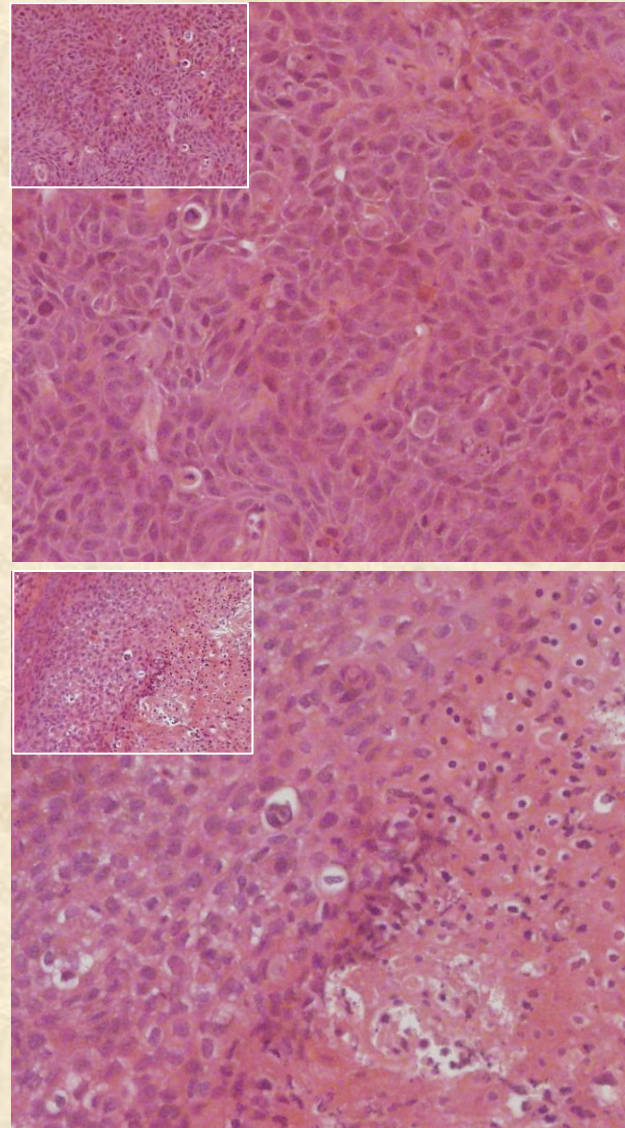
B) *In vivo* detection of tdTomato expressing cervical cancer cells



Growth of orthotopic cervical tumors



Cervical Cancer Orthotopic Mouse Model



Treatment of orthotopic cervical tumors

PBST



Curcumin



PLGA

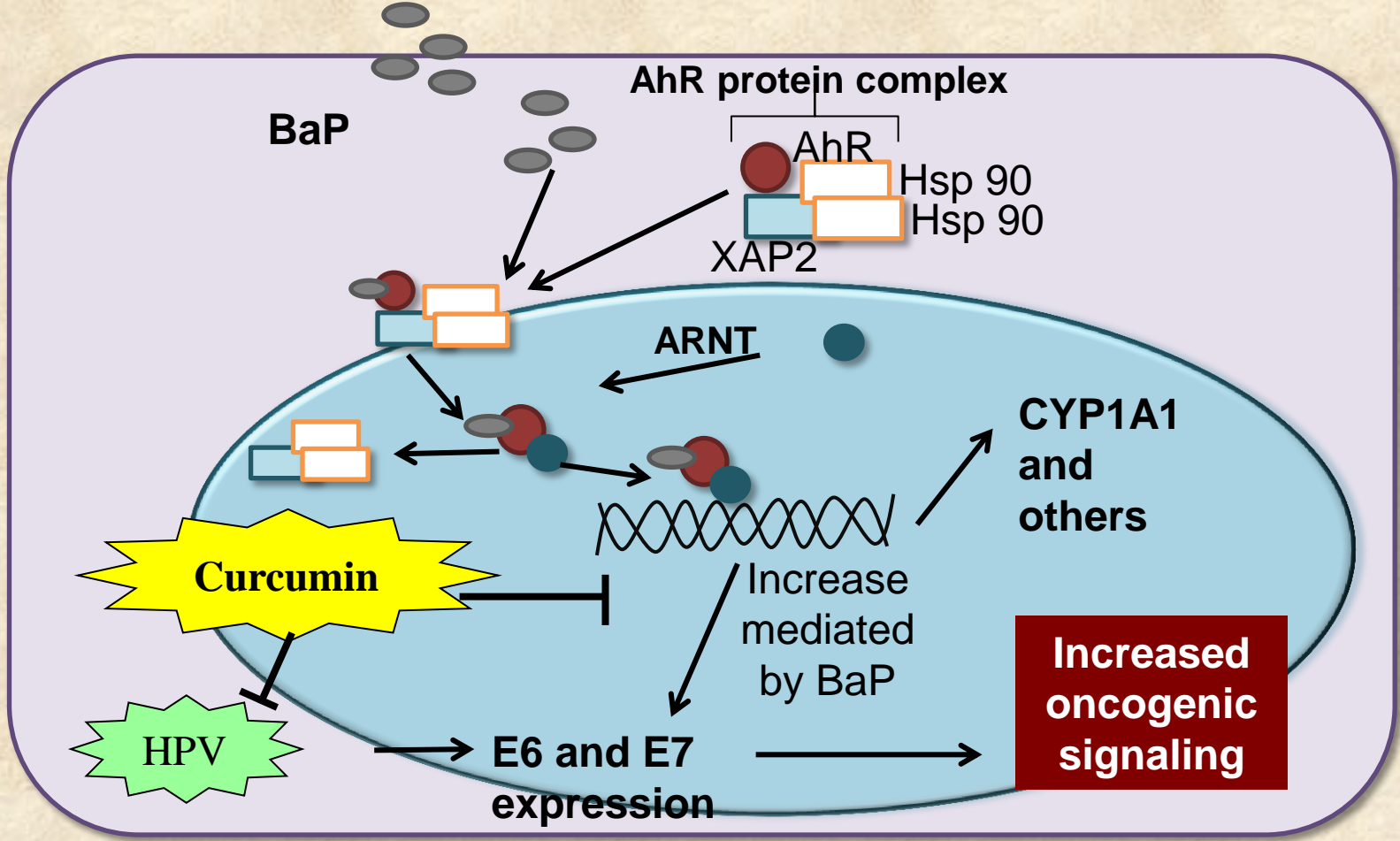


PLGA-curcumin



Mice were treated by intratumoral injection 2x's per week

Smoke Exposure Containing BaP



- BaP increases oncogenic signals from HPV infection
- Curcumin may inhibit this signaling *in vivo*
- Smoke exposure may modulate the local immune response, favoring persistent HPV infection and development of cervical cancer

Acknowledgements

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