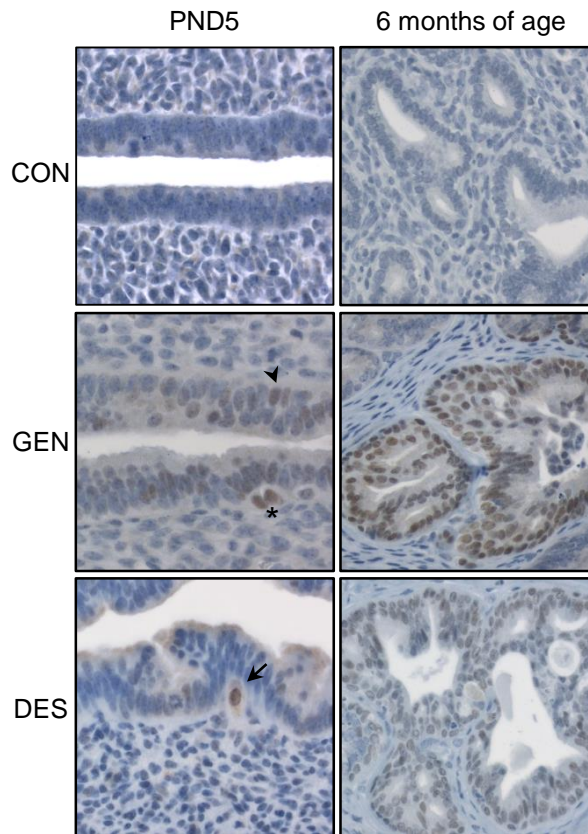
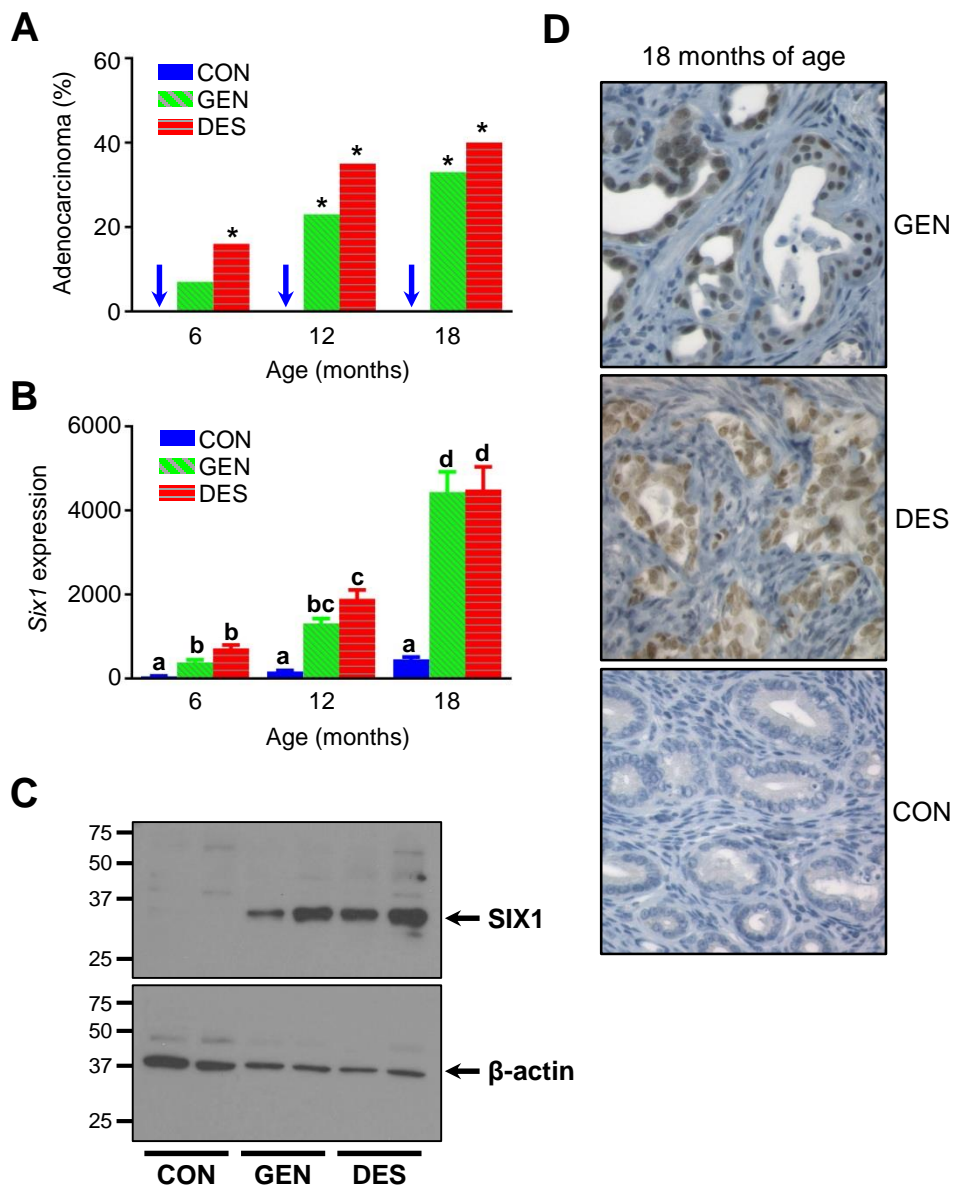


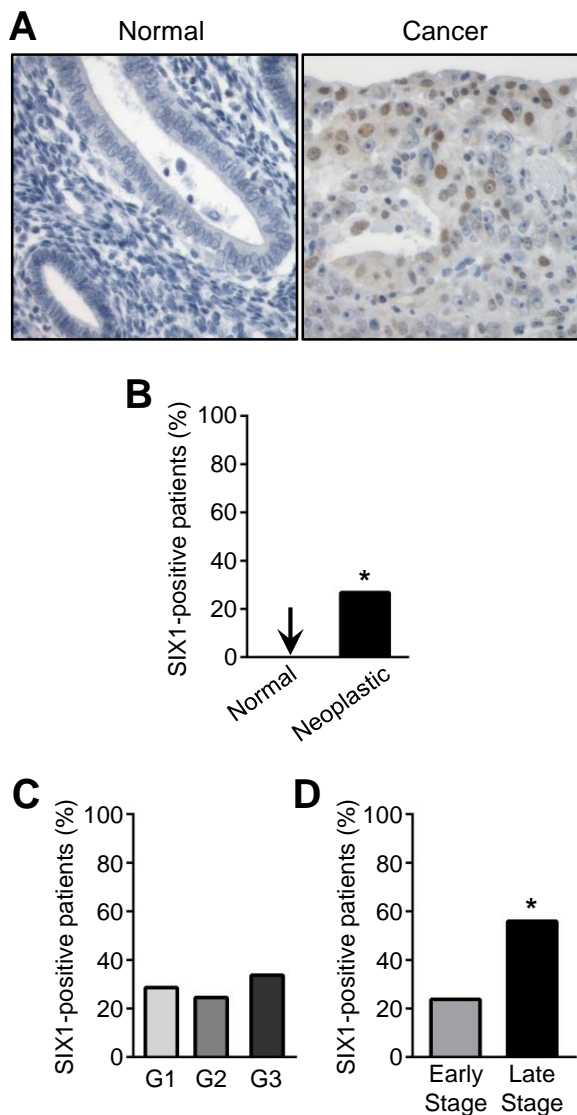
**Figure 1A.** Representative SIX1 immunolabeling in a control adult female mouse reproductive tract at 6 months of age. Arrowhead indicates squamocolumnar junction (SCJ). Images were taken at an objective magnification of 40x.



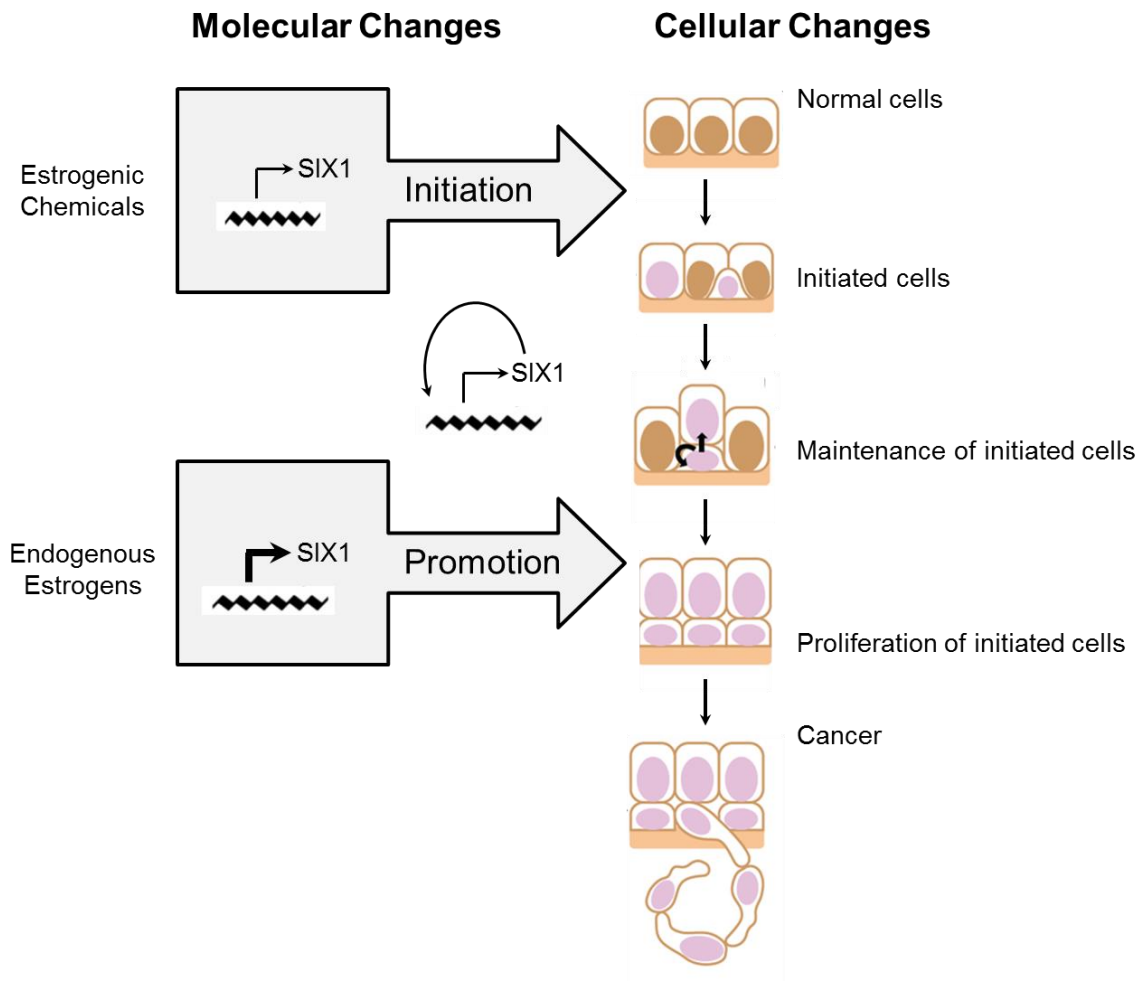
**Figure 1B.** Appearance and expansion of SIX1 immunolabeled cells in mouse endometrium following neonatal GEN or DES exposure at PND5 or 6 months of age. Arrowhead indicates SIX1-positive columnar cells and asterisk indicates SIX1-positive basal-type cells underlying the glandular epithelium. Arrow indicates large SIX1-positive basal-type cell that appears to be traversing the basement membrane. Representative images were taken at an objective magnification of 60x (PND 5) or 40 x (6 months of age) .



**Figure 2.** Association between development of endometrial carcinoma and SIX1 expression in mice neonatally exposed to GEN or DES. A, Incidence of endometrial carcinoma over time in aged CON, GEN, or DES groups.  $n=26-31$  mice per treatment and age group. One-tailed Fisher's Exact Test,  $*p<0.05$  compared to corresponding age-matched CON group. B, *Six1* transcript expression in aged CON, GEN, and DES groups.  $n=27-33$  mice per treatment and age group; mean  $\pm$  s.e.m. is plotted. Two-way ANOVA with Tukey's test for multiple comparison (a-d),  $p=0.0001$ . C, SIX1 immunoblotting of whole uterine horn tissue from two individual CON, GEN, and DES mice at 6 months of age; protein from one mouse per lane;  $n=4$  mice per group in two blots. D, SIX1 immunolabeling in endometrial carcinoma lesions of neonatally GEN- or DES-exposed mice at 18 months of age. Normal endometrium from CON mouse at 18 months of age shown for comparison. Images were taken at an objective magnification of 40x.

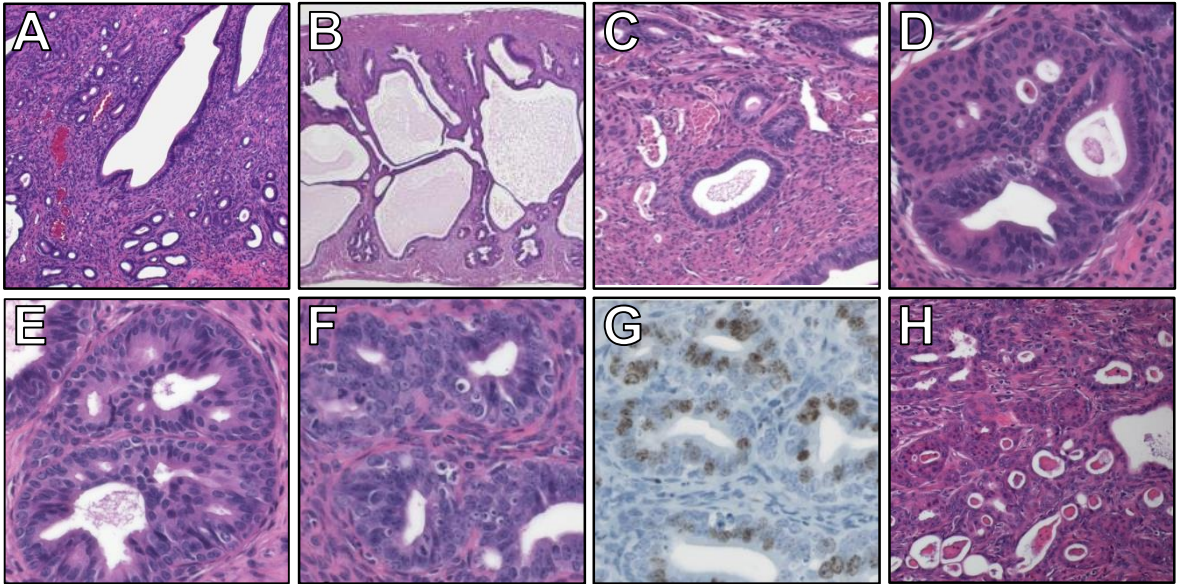


**Figure 3.** SIX1 immunolabeling in human endometrial cancers. A, Normal endometrial tissue (left) and endometrial carcinoma (right). Images were taken at an objective magnification of 40x. B, Percentage of patients with SIX1 immunolabeling;  $n=28$  normal patients and  $n=299$  cancer patients. Two-tailed Fisher's Exact Test,  $*p=0.0025$ . C,D, Percentage of patients with SIX1 immunolabeling separated by C, grade;  $n=84$  for G1,  $n=110$  for G2, and  $n=83$  for G3 and D, stage;  $n=248$  for early and  $n=34$  for late. Two-tailed Fisher's Exact Test,  $*p=0.0003$ .



**Figure 4.** Multi-hit model outlining the developmental origins and progression of estrogen-induced hormonal carcinogenesis. The model is described in detail in the text. Blue nuclei, cells that do not express SIX1; brown nuclei, cells that express SIX1.





**Supplementary Figure S1.** Uterine abnormalities resulting from neonatal exposure to DES or GEN. A, Normal endometrium with regularly spaced glands lined by a single layer of epithelium. B, Cystic change, defined by dilatation of multiple endometrial glands not part of the central lumen. C, Adenomyosis defined by non-neoplastic endometrial glands within the myometrium. D, Squamous metaplasia, defined by a maturation lineage of stratified squamous epithelial cells in place of glandular epithelium. E, Basal cell metaplasia/hyperplasia, defined by a distinct subluminal layer of cuboidal cells with round to oval nuclei and scant cytoplasm (white arrowheads). F, Atypical hyperplasia, defined by increased numbers and layers of luminal cells, often with loss of polarity, increased basophilia, mitoses, anisokaryosis, irregular glandular architecture, and/or squamous differentiation. G, Immunolabeling of hyperplastic glandular epithelial cells for the cell proliferation marker Ki67; and H, endometrial adenocarcinoma, defined by local invasion of endometrial stroma, myometrium, and/or lymphatics by atypical neoplastic glands. Images were taken from control (A) or DES-treated (B-H) mice at 6 months. Representative images were taken at an objective magnification of 10x (A), 4x (B), 40x (C-G) or 20x (H). Slides for all images were stained with H&E (A-F, H) or immunostained for Ki67 antigen (G).