Supporting information

The transcription factor HAND2 up-regulates transcription of the *IL15* gene in human endometrial stromal cells

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Running title: *HAND2 regulates IL15 transcription in human ESCs*

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Table S1. Primers

Primers for ChIP-PCR		
Name	Sequence	Target region
hIL15_IVS1_16153F	5'-GGAGATGTACTATATCATTAGGTGTCATTT-3'	IVS1_+16239/+16245
hIL15_IVS1_16346R	5'-TGTATCCTGTAATTTATAAGAGCCATTTTT-3'	
hIL15_IVS1_16002F	5'-CATTTGTGCTTAGTAAAAGATTAGCTGT-3'	IVS1_+16239/+16245
hIL15_IVS1_16253R	5'-ACAAAATACCAGATGAGAATTTGTAATG-3'	
hIL15_-1110F	5'-TTTTGTTAATGGAGACTATCTAGAGTTTGA-3'	-1110/-933
hIL15_-933R	5'-AATTATTCACTTTCCATGTACATAAAGTTC-3'	(negative control)
hIL15_-1937F	5'-AGAACCTACTATAGTTGAAACCCAATAAAC-3'	-1937/-1761
hIL15_-1761R	5'-CATACTTTTAATATATTGAGACCACCAAGA-3'	(negative control)
hIL15_-1687F	5'-CTGAGAGCACTTTCTATAGAGTTTGTGG-3'	HAND2 motif -1628/-1622
hIL15_-1515r	5'-CTCCTTTAATGGATACTGTGTTCTCTG-3'	
hIL15_-1649F	5'-AGAGACAGGATCTGAGATGTGATGATA-3'	HAND2 motif -1628/-1622
hIL15_-1405R	5'-AAGATAGAGAATAGGGATGAACAAGTGTAT-3'	
hIL15_-1211F	5'-AGAAATGGATGATGGCTTCATATTCTTAAA-3'	FOXO1motif -1131/-1137
hIL15_-1052R	5'-TTACTGATGGACTTTTTGATGAGAATTACA-3'	
hIGFBP1-enhancer_F	5'-TTTTCCCCGTGTTAAAAACAAC-3'	IGFBP1 enhancer region
hIGFBP1-enhancer_R	5'-TCACCAGCAGCTGAAAATTG-3'	
hIL15_-2065F	5'-CAACATATTTGGGAGGCAAATAGTAGAG-3'	FOXO1motif -1998/1993
hIL15_-1916R	5'-GTAGGTTCTAGAACAAGATGGACTAAATG-3'	
hIL15_-2051F	5'-GGCAAATAGTAGAGCAAAGCATATCTTATG-3'	FOXO1motif -1998/1993
hIL15_-1907R	5'-TCAACTATAGTAGGTTCTAGAACAAGATGG-3'	

Del & mutant primers		
Name	Sequence	Target
hIL15_-1629R_P	5'-ACATATCATCACATCTCAGATCCTGTCTCT-3'	HAND2 motif -1628/-1622
hIL15_-1621F_forDEL_P	5'-CAGCAGCAATATCTGATATTGAGCTCTGCT-3'	
hIL15_-1628F(ctg>gac)_P	5'-CCTGACGCAGCAGCAATATCTGATATTGAG-3'	
hIL15_-1138R+P	5'-GTTTTTACAACCATTCAAAATTTCACCATA-3'	FOXO1motif -1131/-1137
hIL15_-1130FforDEL+P	5'-TGCATTTTTATACCTCACTTATTTTGTTAA-3'	
hIL15_-1137FforSubsti+P	5'-ACAAAATGCATTTTTATACCTCACTTATTT-3'	

qPCR Primers	
Name	Sequence (5'-3')
hHAND2-Forward	5'-AGAGGAAGAAGGAGCTGAACGA-3'
hHAND2-Reverse	5'-CGTCCGGCCTTTGGTTTT-3'
hIL15-Forward	5'-GTTCACCCCAGTTGCAAAGT-3'
hIL15-Reverse	5'-CCTCCAGTTCCTCACATTC-3'
hEF1A-Forward	5'-TCTGGTTGGAATGGTGACAACATGC -3'
hEF1A-Reverse	5'-AGAGCTTCACTCAAAGCTTCATGG-3'

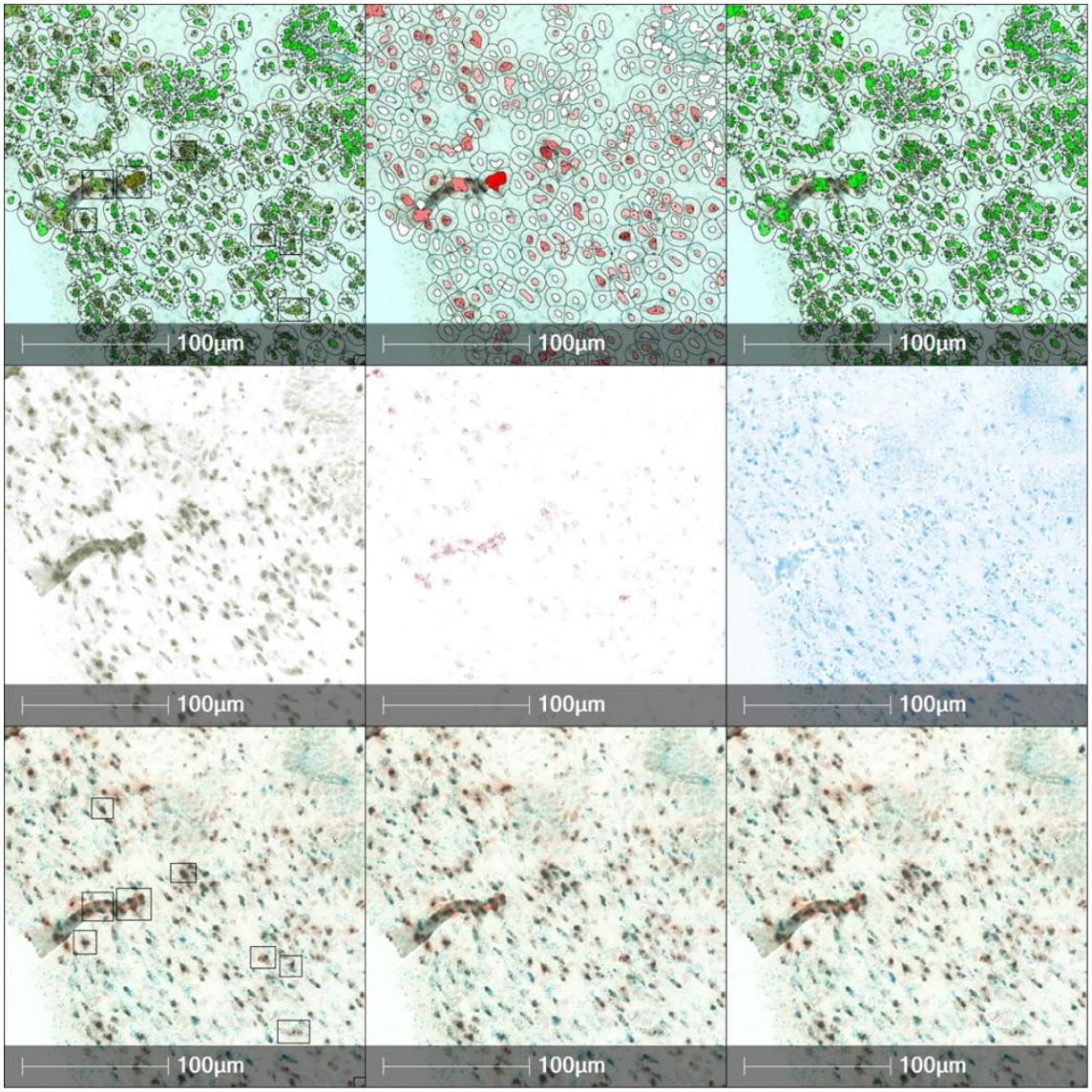


Figure S1. HALO analysis

HAND2 and IL15 represented by FastGreen and FastRed chromogens in RNAscope experiments are highlighted in Green/red (upper left panel), red (upper middle panel) and green (upper right panel) by using ISH ver3.4.3 module in HALO platform (indica labs. Inc., Corrales NM, USA), respectively. ISH ver3.4.3 also shows cell bodies (black lines, upper panels).

The second panels are color extracted images (left: black for nucleus, middle: red for FastRed, right: light blue for FastGreen).

Lower panels are same original images for HALO analysis. Top 10 cells with the highest scores are identified by squares in left lower panel.

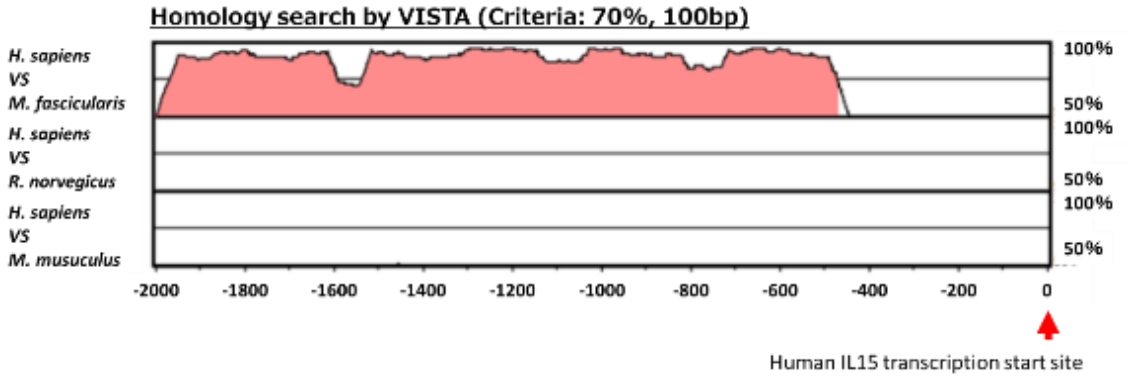


Figure S2.
Sequence homology of *IL15* upstream regions between human and monkey, human and rat, and human and mouse were shown by VISTA.

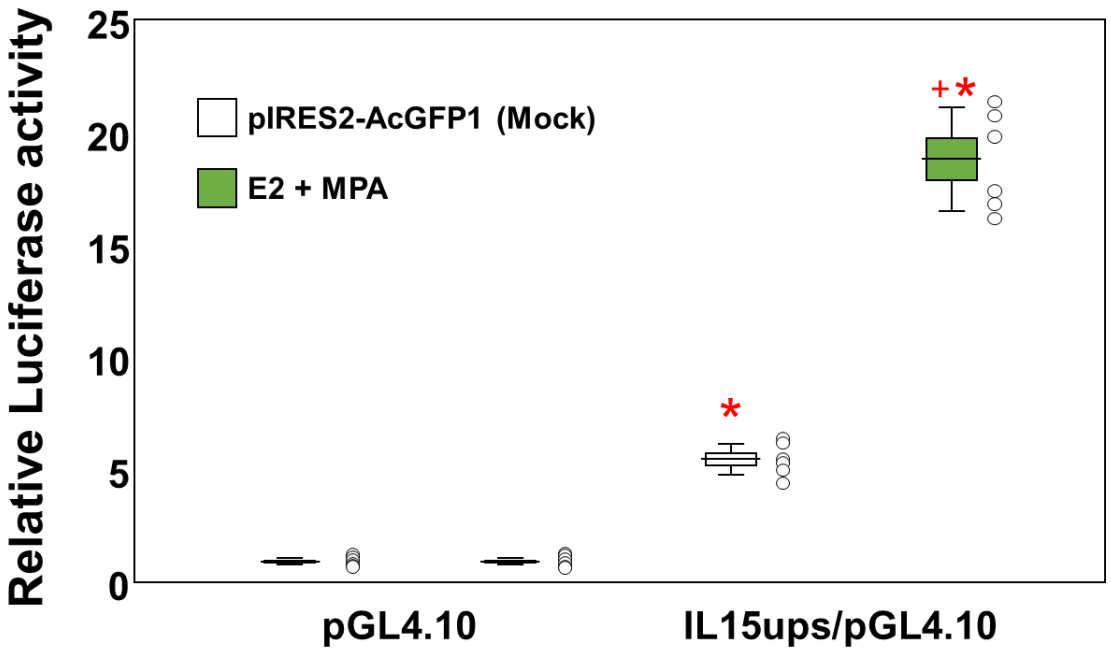
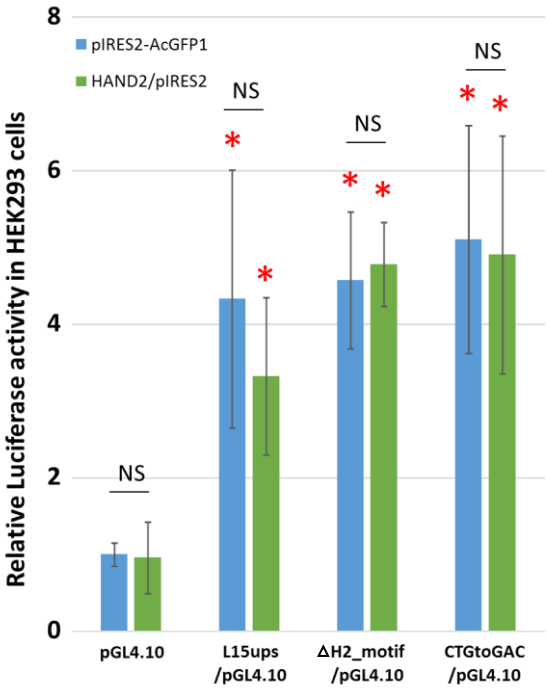


Figure S3.
Luciferase activity in the human *IL15* upstream region of human ESCs with the secretory phase against E_2 and MPA treatment.
ESCs with the secretory phase were treated with E_2 (10^{-8} mol/L) and MPA (10^{-7} mol/L) one day prior to transfection. Two days after transfection, ESCs were lysed and luciferase activity was measured. Multiple comparisons were performed using ANOVA with a Steel-Dwass test, * $p < 0.05$ vs pGL4.10 with Mock; † $p < 0.05$ vs. Mock in each condition. N = 6 in each condition. Horizontal lines and squares indicate the mean ± SEM. Error bars indicate SD. Each dot indicates relative luciferase activity from each well.

Fig S4. Relative Luciferase activity of 1.8k-bp IL15 upstream region in HEK293T cells.
HEK293T (human embryonic kidney) cells were obtained from ATCC (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (GIBCO) at 37 °C and 5% CO_2.

HEK293T cells were seeded into 24-well cell culture plates at a density of 2.5×10^5/well. Two types of luciferase plasmids and one expression vector were co-transfected with LipofectamineTM 3000 Transfection Reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. The following amounts of co-transfected plasmids and vectors were added in each well: 200 ng firefly luciferase-encoding reporter plasmid (pGL4.10, IL15ups/pGL4.10, or IL15ups_mutants/pGL4.10), 20 ng Renilla luciferase-encoding internal control plasmid (pGL4.74), and 200 ng expression vectors (pIRES2-AcGFP1, or HAND2/pIRES2). Approximately 48 h post transfection, HEK293T cells were lysed with lysis buffer (100 µL per well), and 20 µL cell lysate was transferred to an OptiPlate-96 96-well microplate (SUMILON, Tokyo, Japan). Thereafter, Firefly luciferase luminescence (FLU) from the pGL4.10 plasmids and Renilla luciferase luminescence (RLU) from the pGL4.74 plasmids were sequentially measured in duplicate using a PicaGene Dual Sea Pansy Luminescence Kit (TOYO INK CO. Ltd., Chuo-ku, Tokyo, Japan) and the 2030 ARVO X multilabel reader (PerkinElmer Japan Co. Ltd., Yokohama, Japan) according to the manufacturer's protocol. Relative luciferase activity per well was calculated by dividing FLU by RLU. Relative luciferase activity was standardized using the corresponding control condition, which was transfected with pGL4.10 plasmid alone or co-transfected with a pGL4.10 plasmid and a pIRES2-AcGFP1. Activity levels were expressed as the mean of at least 6 independent experiments ± SD.



Normal distribution was determined using the Shapiro-Wilk normality test. We used an ANOVA with a Tukey's multiple comparisons. All values were two-sided with statistical significance set at 0.05 (*). Statistical analyses were performed using IBM SPSS Statistics v. 21.0 (IBM Corp., Armonk, NY, USA).

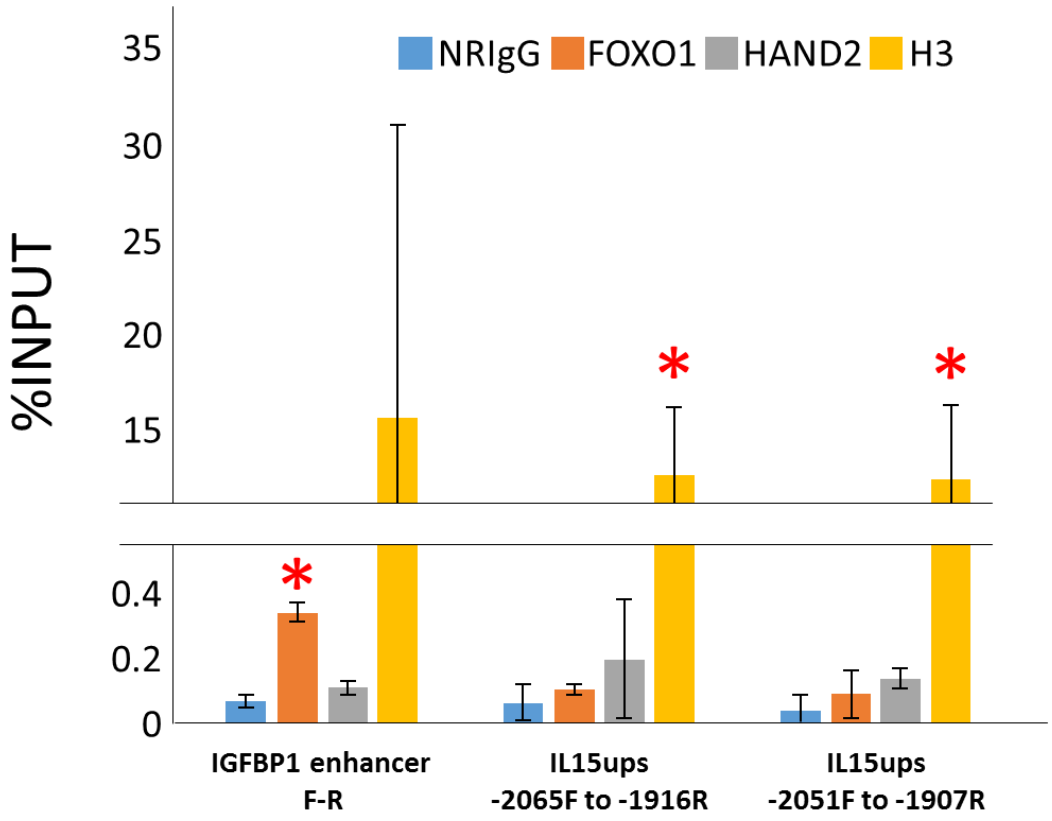


Fig S5. ChIP analysis for FOXO1 motif in IGFBP1 enhancer region.
ESCs were treated with E2 (10^{-8} mol/L) and MPA (10^{-7} mol/L) for 12 days. Chromatin was then immunoprecipitated with corresponding antibodies. The transcription factors HAND2 and FOXO1 to IGFBP1-enhancer and the putative FOXO1 motif at -1993/-1998 were analyzed using ChIP-qPCR assay. Histone H3 antibody (H3) and Normal rabbit IgG (NRIgG) were used as a positive and negative controls, respectively. The relative recruitment levels were analyzed and plotted as the ratio of Immunoprecipitated-DNA to the total INPUT DNA sample (%INPUT). Multiple comparisons were performed using Student T-test with a Bonferroni correction, * $P < 0.0166$ vs Normal rabbit IgG in each region.

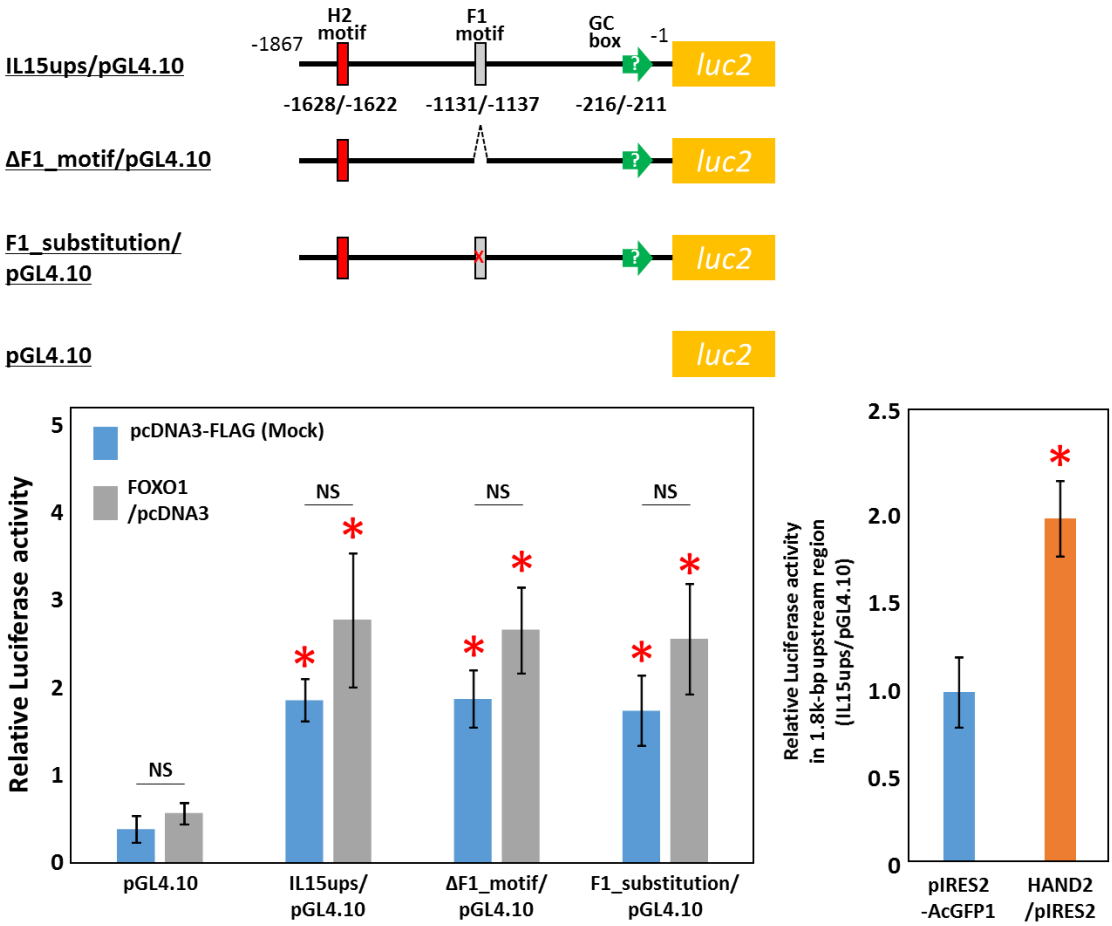


Fig S6. Luciferase activity against FOXO1 expression vector in the 1.8k-bp human IL15 upstream region of human ESCs.

Two days post transfection, ESCs were lysed and luciferase activity was measured. Multiple comparisons were performed using ANOVA with a Tukey test, * $P < 0.05$ vs pGL4.10 with mock; NS, not significance. $N = 4$ in each condition. Student's T-test was conducted between pIRES2-AcGFP1 and HAND2/pIRES2 transfections, * $P < 0.05$ vs pIRES2-AcGFP1.

The deletion mutant ΔF1_motif/pGL4.10 contains an internal deletion at -1131/-1137 within the IL15ups/pGL4.10. Nucleotide substitution reporter F1_substitution/pGL4.10 had all substitutions at -1131/-1137 with TGTTTT to ACAAAA. F1 motif: putative FOXO1 binding site at -1131/-1137 in the 1.8k-bp upstream region of human IL15 gene