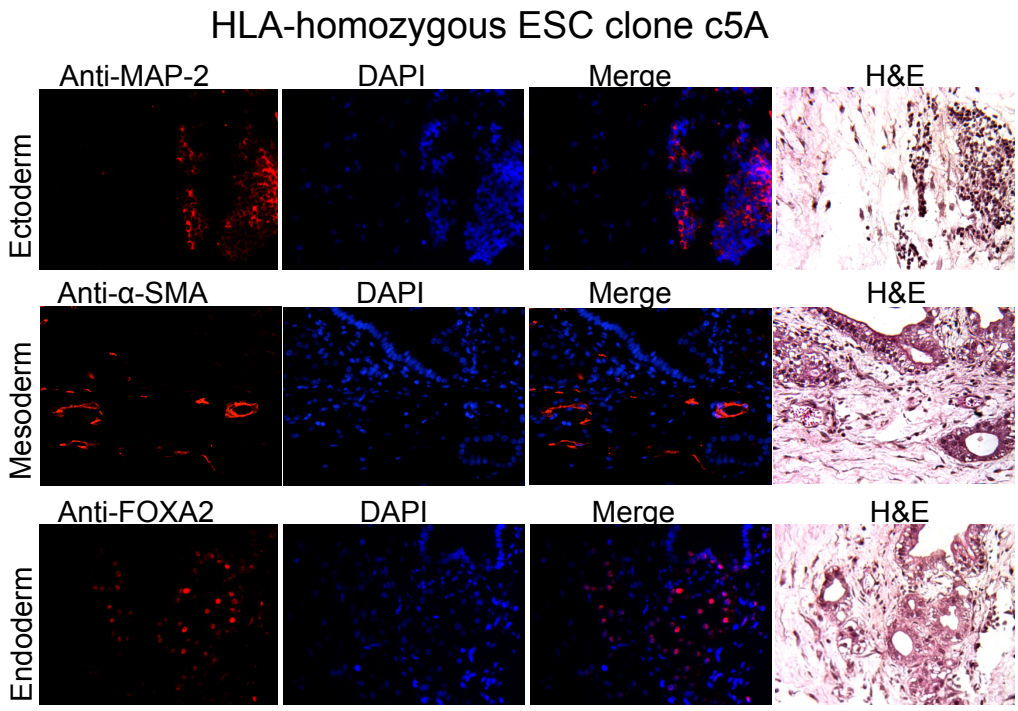
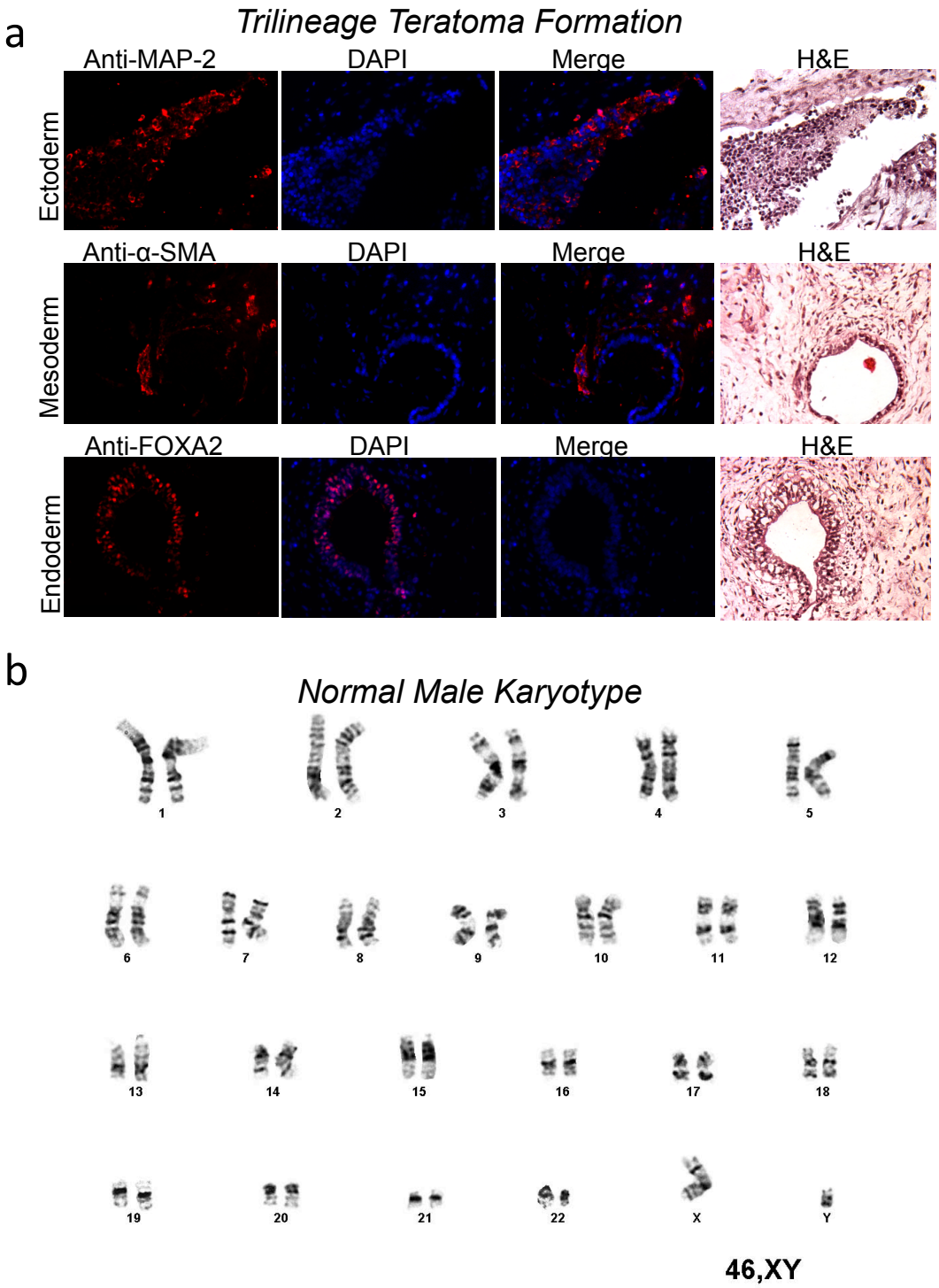


Supplemental Figure 1. Trilineage teratoma formation in HLA-homozygous ESC clone c5A. Teratomas derived from clone c5A were stained with the indicated antibodies or DAPI and examined by fluorescence microscopy. Serial sections are shown stained with hematoxylin and eosin (H&E). MAP-2, Microtubule Associated Protein-2. α-SMA (alpha-Smooth Muscle Actin). FOXA2, Forkhead Box Protein A2. Scale bars = 100 microns.



Supplemental Figure 2. Trilineage teratoma formation and normal karyotype of *B2M*$^{loxP/loxP}$ cells. (a) Teratoma produced by Cre'd out *B2M*$^{loxP/loxP}$ HLA class I-negative clone c1 stained with the indicated antibodies or DAPI and examined by fluorescence microscopy. Serial sections are shown stained with hematoxylin and eosin (H&E). MAP-2, Microtubule Associated Protein-2. α-SMA (alpha-Smooth Muscle Actin). FOXA2, Forkhead Box Protein A2. Scale bars = 100 microns. **(b)** Karyotype of the same clone. Five out of five spreads examined were normal male cells.

Supplemental Table 1. *HMGA1* targeting and GCV-resistant clone analysis.

Cell line	Hyg-R clones	Targeted clones	GCV-R subclones	*HMGA1* probe	HyTK probe	HLA type	Presumed basis for GCV resistance
H1	5	5	c1A-L	WT	Missing	Hetero	Gene conversion
			c2A	Targeted	Targeted	NA	HyTK mutation
			c4A,B	Targeted	New band	NA	HyTK rearrangement
			c4C-H	Targeted	Targeted	NA	HyTK point mutation
			c5A	WT	Missing	Homo	Mitotic recombination
H7	5	4	NA				
BG01	3	3	NA				
	pc1	100%	pc1A-N	WT	Missing	Hetero	Gene conversion
			pc1O,P	Targeted	Targeted	NA	HyTK mutation
	pc2	100%	pc2A-D	WT	New band	NA	HyTK rearrangement
	pc3	58%	pc3A,B	WT	New band	NA	HyTK rearrangement
	pc4	100%	pc4A,B	WT	Missing	Hetero	Gene conversion
	pc5	100%	pc5A-C	WT	Missing	Hetero	Gene conversion
	pc6	100%	NA				
BG02	pc1	100%	pc1A-J	WT	Missing	Hetero	Gene conversion
	pc2	100%	pc1A	WT	New band	NA	HyTK rearrangement
	pc3	38%	NA				
	pc4	100%	NA				
	pc5	100%	NA				
	pc6	100%	pc1A	WT	Missing	Hetero	Gene conversion
	pc7	100%	NA				
BG03	14	14	c1A,B	Targeted	Targeted	NA	HyTK mutation

Supplemental Table 2. *B2M* targeting experiments.

Cell line	Allele 1 vector	MOI	Total CFU	# $G418^R$ CFU	% $G418^R$ CFU	$G418^R$ CFU/ infected cell	# $G418^R$ CFU screened	# $G418^R$ CFU targeted	% $G418^R$ CFU targeted	% Total CFU targeted	Targeted CFU/ infected cell
H1	AAV2-B2M-ETKNpA	20,000	1575	291 345 336 298	18.5 21.9 21.3 18.9	1.9E-04 2.3E-04 2.2E-04 2.0E-04	30	9	30.0	6.1	6.35E-05
H1	none	0	1575	0	<0.06	0					
Cell line	**Allele 2 vector**	**MOI**	**Total CFU**	**Hyg^R CFU**	**% Hyg^R CFU**	**Hyg^R CFU/ infected cell**	**# Hyg^R CFU screened**	**# Hyg^R CFU targeted**	**% Hyg^R CFU targeted**	**% Total CFU targeted**	**Targeted CFU/ infected cell**
C23-2	AAV3-B2M-EHyTKpA	2,000	450	7 8 10 11 16 10 8 14 19 14	1.6 1.8 2.2 2.4 3.6 2.2 1.8 3.1 4.2 3.1	1.4E-05 1.6E-05 2.0E-05 2.2E-05 3.2E-05 2.0E-05 1.6E-05 2.8E-05 3.8E-05 2.8E-05	60	6	10.0	0.26	2.34E-06
C23-2	none	0	450	0	<0.2	0					

Abbreviations: CFU, colony-forming unit; Hyg, Hygromycin; MOI, multiplicity of infection.