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Supplemental Material to:

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Nucleolar tethering mediates pairing between the *IgH* and *Myc* loci

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SUPPLEMENTAL MATERIAL

Table S1. Morphological characterization of mouse strains			
	C57	**CBA**	**129P3**
Nuclear Diameter (μm)	5.34	5.51	5.02
Mean number of nucleoli*	2.4 (2.6)	1.7 (1.8)	1.5 (1.6)
Percent of cells with:			
1 nucleolus	5.60	45.07	58.27
2 nucleoli	50.40	36.62	30.71
3 nucleoli	44.00	18.31	11.02
Mean total nucleolar volume (μm^3) in cells with:			
1 nucleolus	1.87	1.86	1.78
2 nucleoli	1.62	1.84	2.09
3 nucleoli	1.87	1.92	1.89
Mean per nucleolus volume (μm^3) in cells with:			
1 nucleolus	1.87	1.86	1.78
2 nucleoli	0.81	0.92	1.04
3 nucleoli	0.62	0.64	0.63

*Cells with more than 3 nucleoli were excluded from analysis, however cells with up to 5 nucleoli were identified. The number in parenthese reflects the average number of nucleoli per cell from the full population.

Table S2. Data summary for gene locus to nucleolus measurements					
	C57	**CBA**	**129P3**	**Random without excluded volume**	**Random with excluded volume***
Median distance IgH to nucleolus (μm)	0.55	1.00	0.70		
N (IgH alleles)	239	271	237		
Median distance Myc to nucleolus (μm)	0.35	0.60	1.15		
N (Myc alleles)	235	270	251		
Median distance random gene to nucleolus (μm)	1.31	1.49	1.36	1.17	0.98
N (random alleles)	5000	5680	5080	4000	4000

Simulations which iterated on the true random model by adding an excluded volume featured cells with two nucleoli in fixed positions, and thus differed from the random simulations which were based on the parameters outlined in Table S3.

* Excluded volume is equal to 25% of the total nuclear volume

Table S3. Parameters used for random gene simulations				
	C57	**CBA**	**129P3**	**Iterations on true random model**
Nuclear radius in cells with:				
1 nucleolus	2.74	2.76	2.52	
2 nucleoli	2.64	2.74	2.49	2.64
3 nucleoli	2.71	2.76	2.52	
Nucleolar radius in cells with:				
1 nucleolus	0.76	0.76	0.75	
2 nucleoli	0.58	0.60	0.63	0.58
3 nucleoli	0.53	0.53	0.53	
Number of iterations* for cells with:				
1 nucleolus	70	640	740	
2 nucleoli	630	520	390	1000
3 nucleoli	550	260	140	

*The number of iterations was determined by multiplying the number of cells in each strain with a given number of nucleoli by 10. For example, there were 7 cells with 1 nucleolus and 63 cells with 2 nucleoli in the C57 population.

Table S4. Data summary for pairwise IgH:Myc distance measurements								
	C57	**CBA**	**129P3**	**Random**	**Random no excluded volume* and no tethering****	**Random with excluded volume no tethering**	**Random no excluded volume with tethering**	**Random with excluded volume with tethering**
Median IgH:Myc distance (μm, raw measurements)	2.00	2.20	2.10	2.71	2.66	2.61	2.52	2.46
Median IgH:Myc distance (% of nuclear diameter)	38.60	41.51	40.82	51.22				
N (IgH:Myc pairs)	450	518	469	15760	4000	4000	4000	4000

Simulations which iterated on the true random model by adding an excluded volume and/or tethering featured cells with two nucleoli in fixed positions, and thus differed from the random simulations which were based on the parameters outlined in Table S3.

* Excluded volume is equal to 25% of the total nuclear volume

** When indicated simulated Myc alleles were tethered within 0.5 mm of a nucleolus

Supplemental Figures

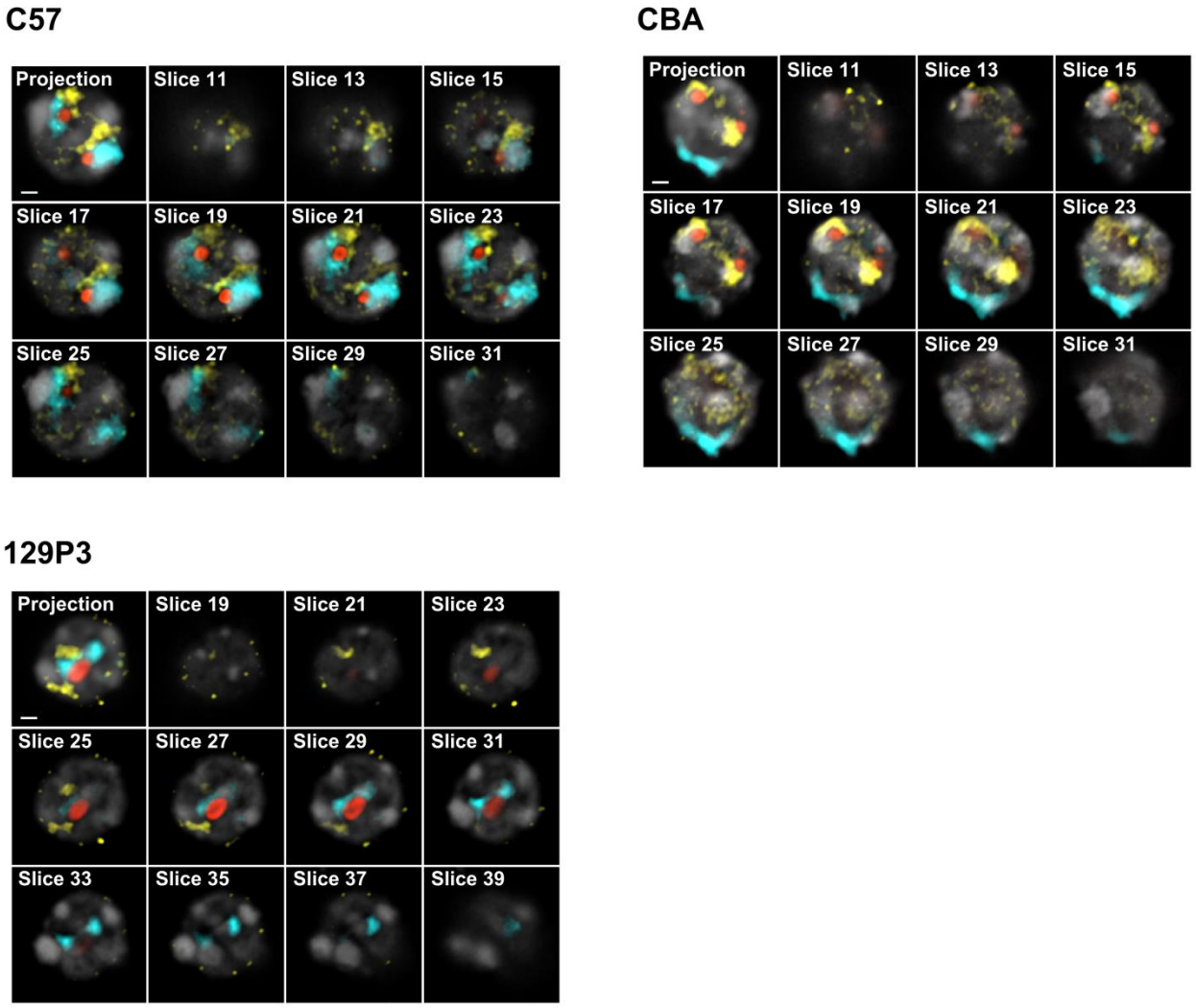

Figure S1. The presence of a NOR modulates chromosome position relative to the nucleolus and periphery. Chr 12 and Chr 15 were labeled with whole chromosome paints and nucleoli were labeled with an antibody to nucleolin in B-cells from each of the three mouse strains used in this study. Average projection and individual z-sections for the reconstructed images depicted in Fig 1B are shown here. Odd numbered z-sections (0.2 µm sections) through each cell are shown. Note that in Fig 1B cells were rotated relative to their original orientation to best highlight the 3D chromosome and nucleolus position. Chr 12 (*cyan*); Chr 15 (*yellow*); nucleoli (*orange*); DAPI (*grey*). Scale bar = 1µm.

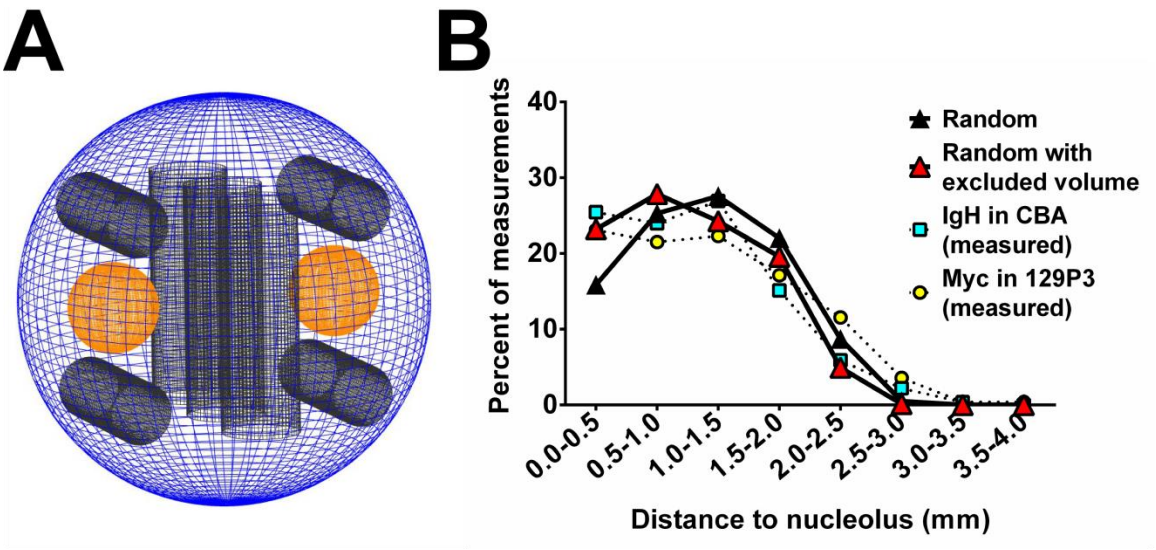

Figure S2. Computer simulations highlight contributions that nuclear topology makes to non-random gene position. (A) Drawing of a simulated cell with cylinders acting as an excluded volume. Nucleus (*blue sphere*); nucleoli (*orange spheres*); excluded volume (*grey cylinders*). (B) Plot of the distribution of distances between a random gene and the closest nucleolus. simulations with excluded volume (*red triangles*); simulations with no excluded volume (*black triangles*). The measured distributions for *IgH* in CBA cells (*cyan squares*) and *Myc* in 129P3 cells (*yellow circles*) are shown as a reference. When an excluded volume is present, simulated genes can be placed anywhere within the nuclear volume except inside the volume defined as excluded volume (grey cylinders). When no excluded volume is present, simulated genes can be placed anywhere within the nuclear volume.

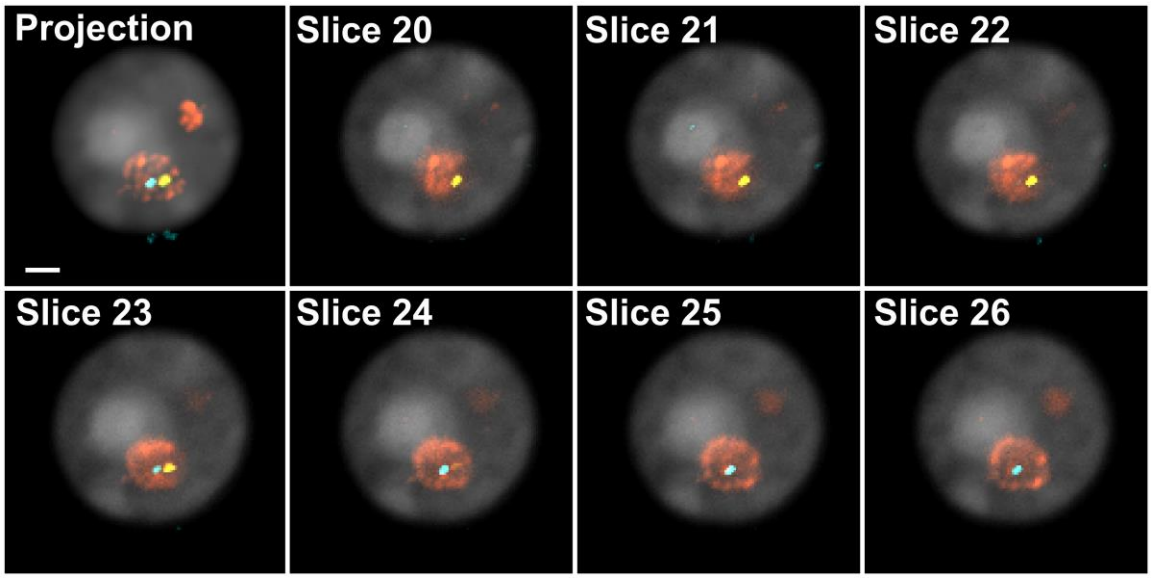

Figure S3. *IgH* and *Myc* genes are expressed in the nucleolar environment. Average projection and a series of z-sections (*slice20-slice26*) through a representative cell processed for primary transcript RNA immuno-FISH. *IgH* (*cyan*) and *Myc* (*yellow*) primary transcripts were labeled together with nucleoli (*orange*); DAPI (*grey*). Note that primary transcripts from both genes are fully within the nucleolus. Scale bar = 1μm. RNA FISH was performed as previously described [1] with the exception that the intronic IgH and Myc sequences were amplified by PCR and cloned into pCR-Blunt II-TOPO or pCR-2.1-TOPO vectors (Life Technologies). These vectors were then used for *in vitro* transcription to generate DIG or DNP labeled probes.[2] The *IgH* probe is specific for the Cμ intron. The cloned sequence was amplified using the following primers: 5'-GGTGGCTTTGAAGGAACAAT-3' and 5'-GCGCATTTTAGAAGCACTCA-3'. The *Myc* probe mix is specific for the 1st and 2nd introns of *Myc*. The cloned sequences were amplified using the following primers: **5'-CTGGTGGTCTTTCCCTGTGT-3'** and **5'-GTTATCCAGCTCTGGTTGGTG-3'**; **5'-CTTCTCCACCACTCATTGGCATTAATTCAATTGGCC-3'** and **5'-GGGAGGAAGTGGAAGATCACAGTTAGCCACGCCTCC-3'**, respectively. The IgH C_l probe has been previously used to label primary transcripts at the IgH locus[3, 4] and we validated that the Myc probe hybridizes adjacent to the Myc gene locus (not shown).

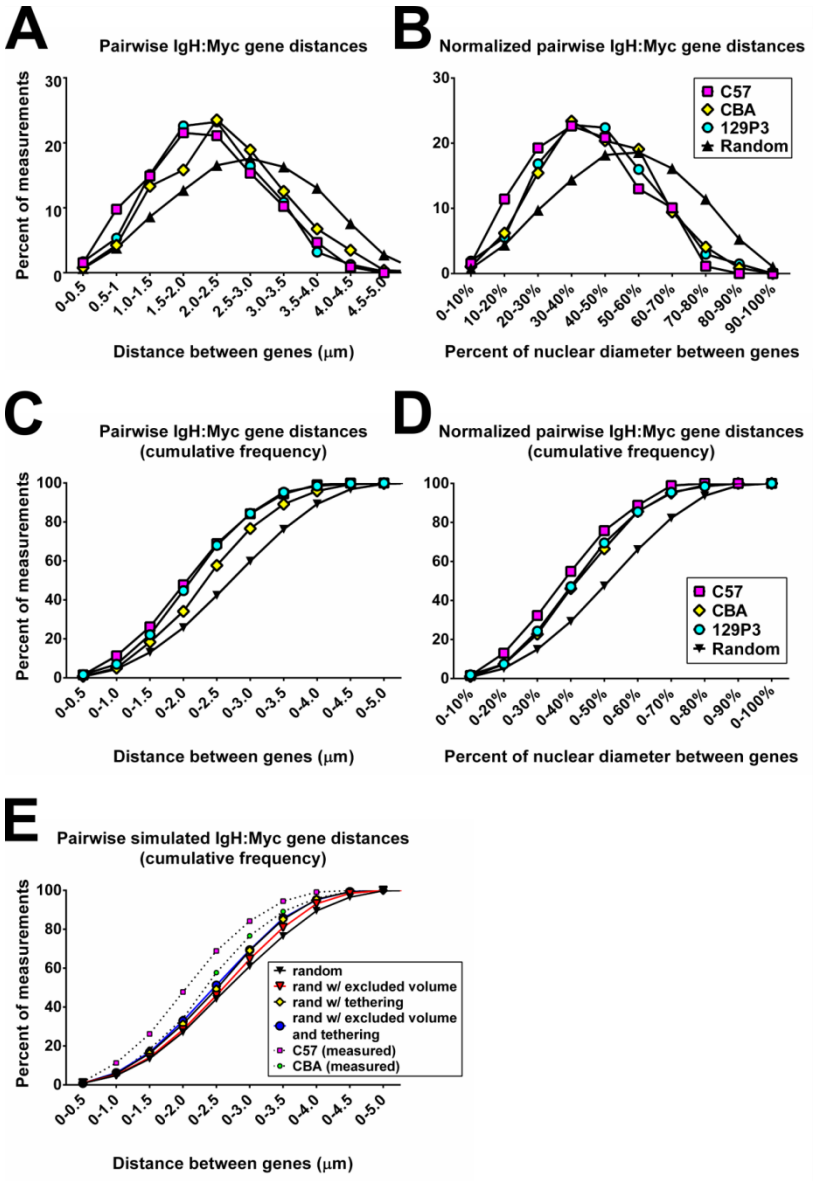

Figure S4. Nucleolar tethering mediates *IgH* and *Myc* gene pairing. (A) Distribution of all pairwise distances between *IgH* and *Myc* loci. (B) Distribution of all pairwise distances between *IgH* and *Myc* loci normalized to nuclear diameter. (C) Cumulative distribution of all pairwise distances between *IgH* and *Myc* loci. (D) Cumulative distribution of all pairwise distances between *IgH* and *Myc* loci normalized to nuclear diameter. (E) Cumulative distribution of pairwise distances between randomly positioned "*IgH*" and "*Myc*" genes with or without an excluded volume and/or tethering of a locus to the nucleolus. The excluded volume is identical to that described in Supplemental figure S2. The tethering condition forced the Myc alleles to be placed within a sphere that is centered on and extends 0.5 μm beyond the outside edge of the simulated nucleolus.

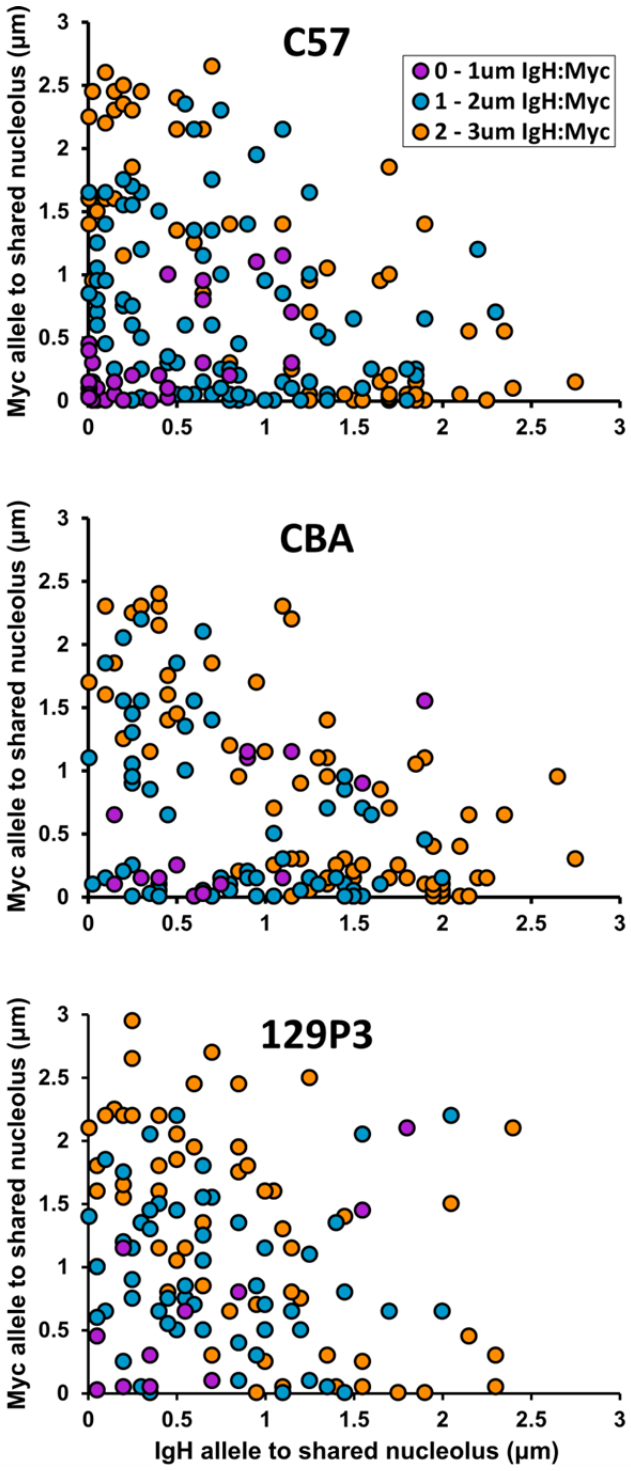

Figure S5. Close *IgH*:*Myc* pairs cluster near the same nucleolus only in C57. The distances from the IgH allele and Myc allele to the closest shared nucleolus (*x* and *y* axes, respectively) are plotted for *IgH*:*Myc* pairs of varying inter-locus distances for C57 (*top*), CBA (*middle*) and 129P3 (*bottom*) cells. 0-1 µm *(violet)*; 1-2 µm *(blue)*; 2-3 µm *(orange)*.

Supplemental References

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