MUC1-C DRIVES STEMNESS IN PROGRESSION OF COLITIS TO COLORECTAL CANCER

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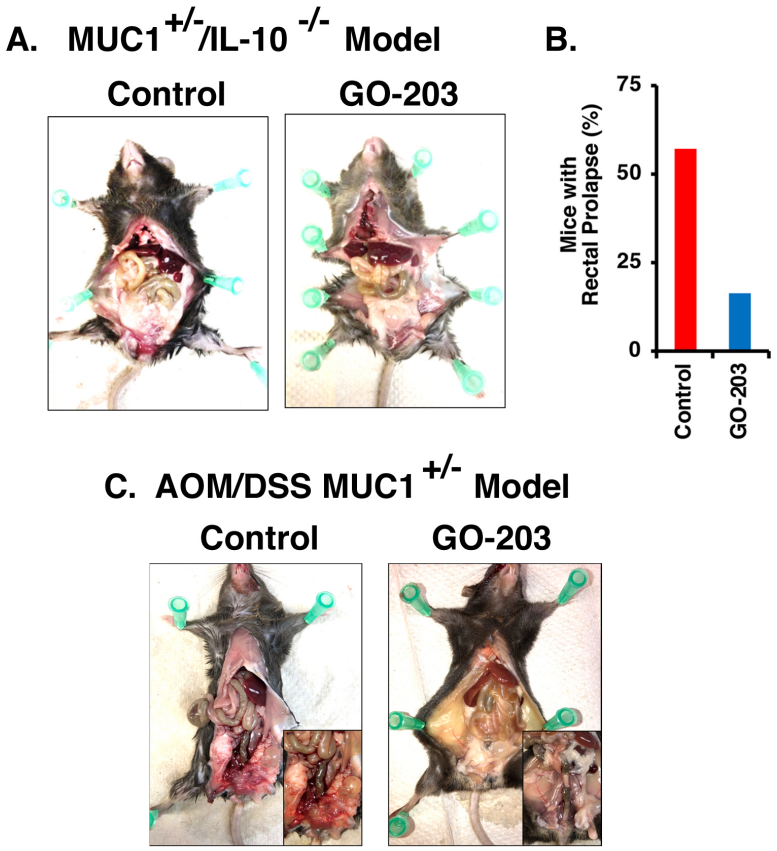
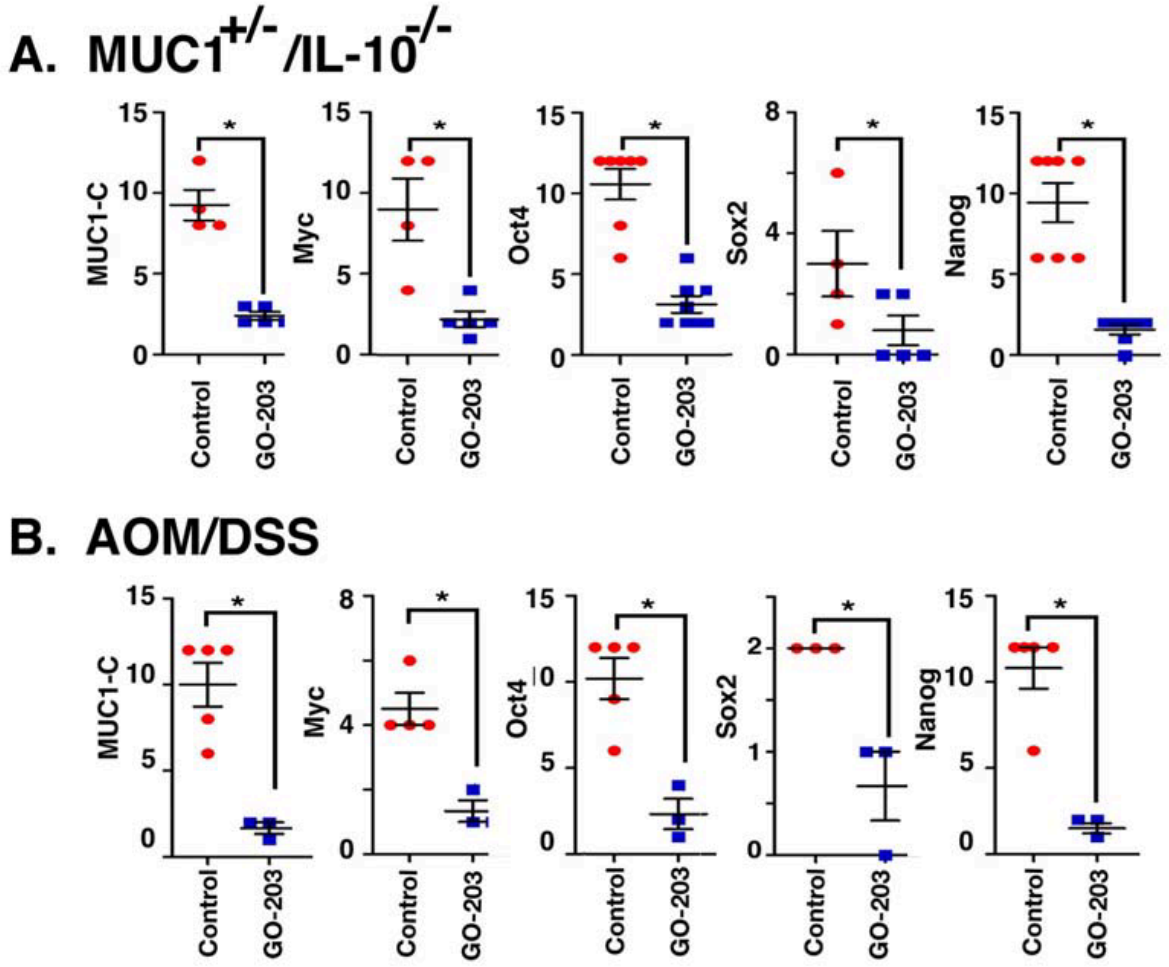
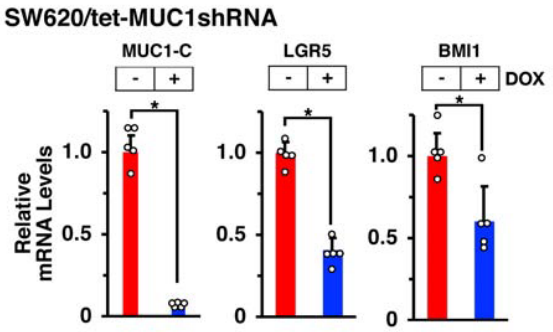


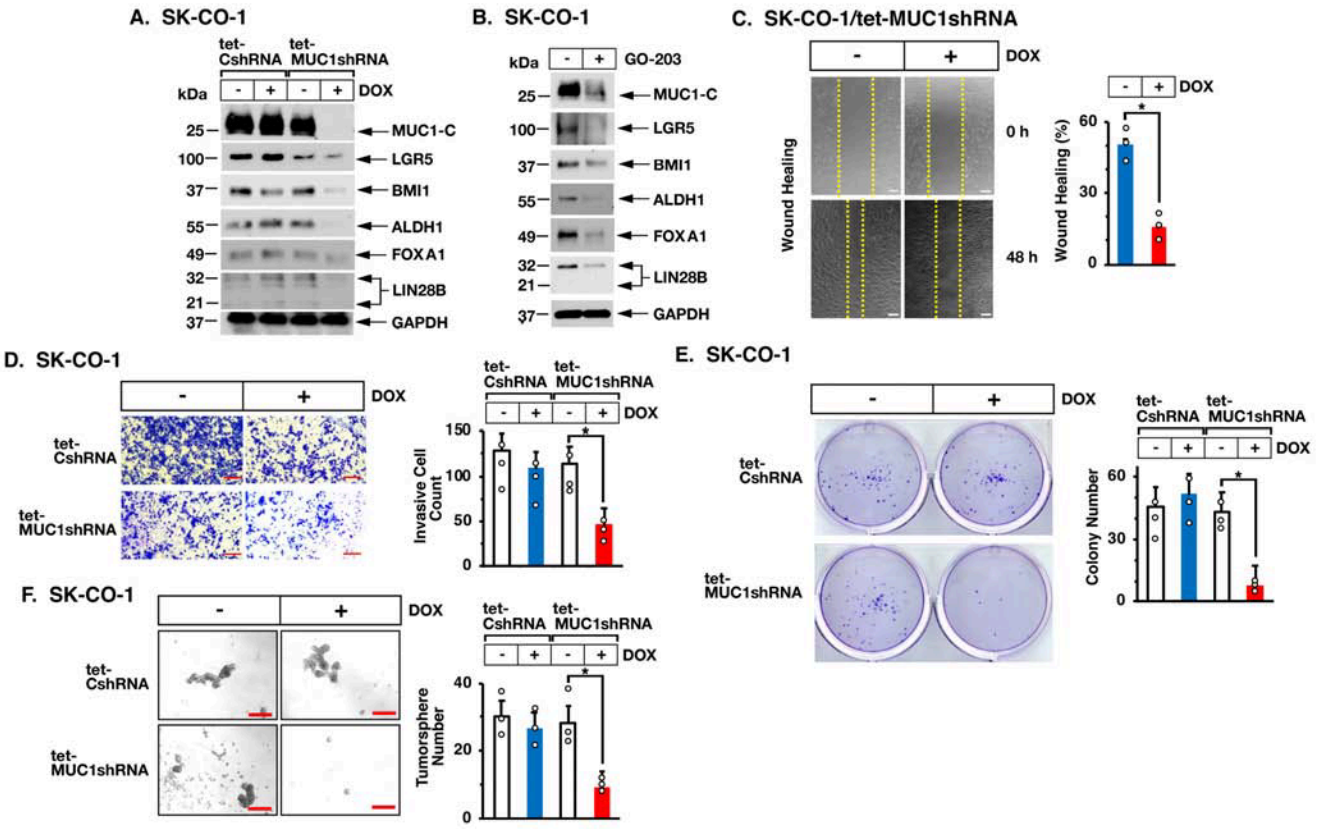
Supplemental Figure S1. GO-203 treatment decreases inflammation and rectal prolapse. A. Images of MUC1$^{+/-}$/IL-10$^{-/-}$ (day 56) mice left untreated (left) and treated with GO-203 (right) showing rectal prolapse. B. Percentage of MUC1$^{+/-}$/IL-10$^{-/-}$ mice left untreated (n=7; blue bar) and treated with GO-203 (n=7; red bar) with rectal prolapse at day 56. Sample size limited detecting a significant difference of proportions between the control and treated groups. C. Images of AOM/DSS-induced MUC1$^{+/-}$ (day 68) mice left untreated (left) and treated with GO-203 (right) showing rectal prolapse.



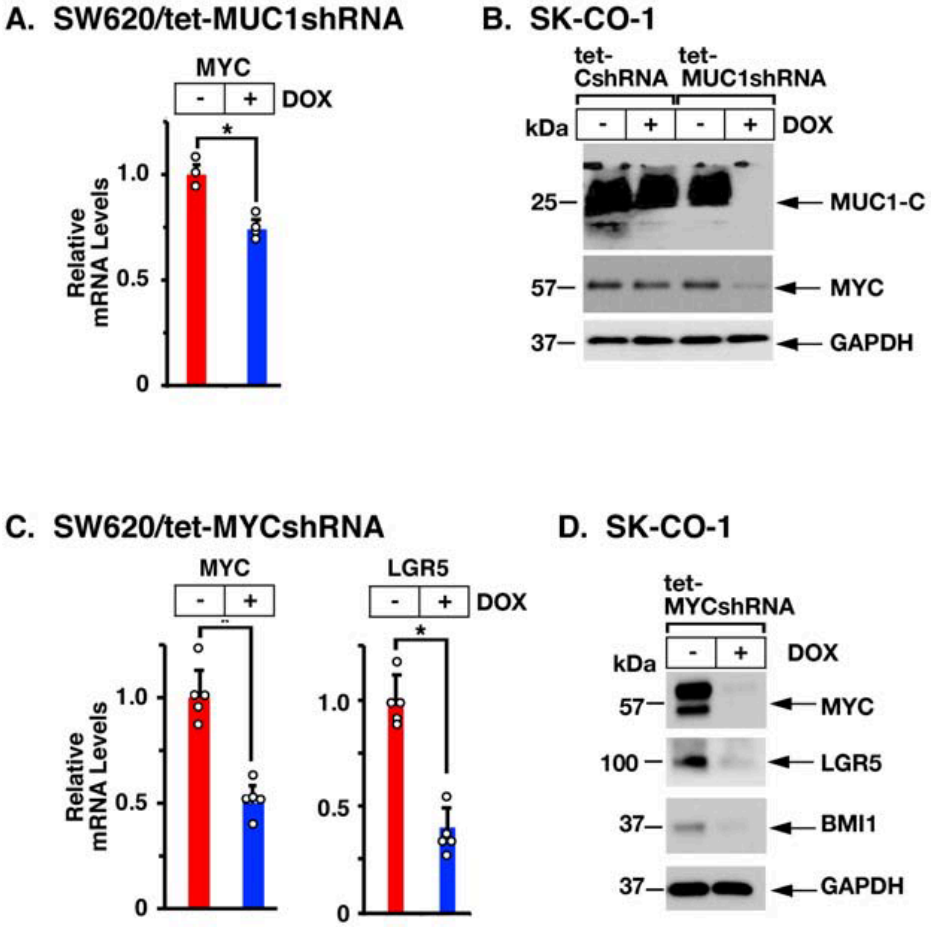
Supplemental Figure S2. IHC histology scores for control and GO-203-treated mouse colitis tissues. A. IHC histology scores of colitis tissues from control and GO-203-treated MUC1$^{+/-}$/IL-10$^{-/-}$ mice stained for Myc, Oct4, Sox2 and Nanog (Supplemental Table S5). B. IHC histology scores of colitis tissues from control and GO-203-treated AOM/DSS-induced MUC1$^{+/-}$ mice stained for Myc, Oct4, Sox2 and Nanog (Supplemental Table S6).



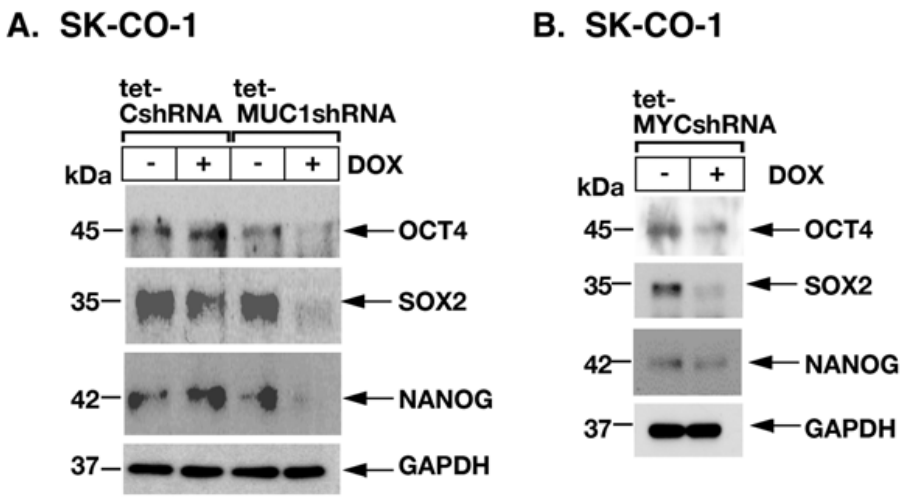
Supplemental Figure S3. Inducible suppression of MUC1-C mRNA levels in SW620 cells. SW620/tet-MUC1shRNA cells treated with vehicle or 500 ng/ml DOX for 7 days were analyzed for the indicated mRNA levels by qRT-PCR using primers listed in Supplemental Table S8. The results (mean±SD) are expressed as relative mRNA levels compared to those obtained for vehicle-treated cells (assigned a value of 1).



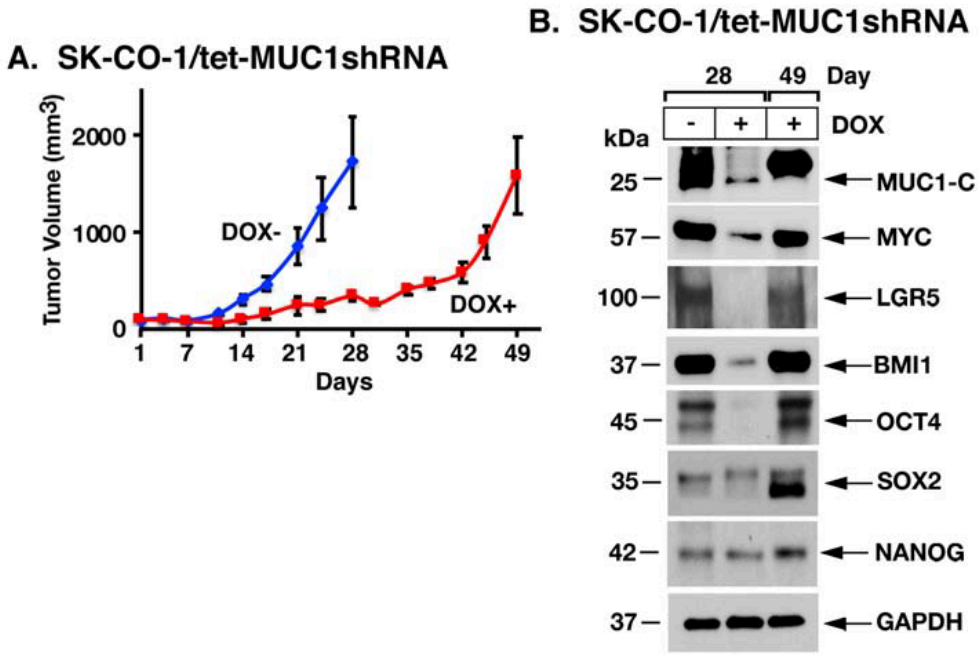
Supplemental Figure S4. Targeting MUC1-C genetically and pharmacologically suppresses stemness of SK-CO-1 cells. A. Human SK-CO-1 colon cancer cells stably expressing a tet-CshRNA or tet-MUC1shRNA were treated with vehicle or 500 ng/ml DOX for 7 days. Lysates were immunoblotted with antibodies against the indicated proteins. B. SK-CO-1 cells were left untreated or treated with 5 μM GO-203 for 48 h. Lysates were immunoblotted with antibodies against the indicated proteins. C. SK-CO-1/tet-MUC1shRNA cells treated with vehicle or 500 ng/ml DOX for 48 h were monitored for wound healing in the scratch assay (left). The results are expressed as a percentage (mean±SD of 3 biologic replicates) of the control at 0 h (right). Scale bars: 100 μm. D. SK-CO-1/tet-CshRNA and SK-CO-1/tet-MUC1shRNA cells were treated with vehicle or 500 ng/ml DOX for 24 h were assayed for invasion (left). The results (mean±SD of 3 biologic replicates) are expressed as the invasive cell number (right). Scale bar: 200 μm. E. SK-CO-1/tet-CshRNA and SK-CO-1/tet-MUC1shRNA cells treated with vehicle or 500 ng/ml DOX for 14 days were assayed for colony formation (left). The results (mean±SD of 3 biologic replicates) are expressed as colony number per field (right). F. SK-CO-1/tet-CshRNA and SK-CO-1/tet-MUC1shRNA cells treated with vehicle or 500 ng/ml DOX for 10 days were assayed for tumorsphere formation (left). The results (mean±SD of 3 biologic replicates) are expressed as tumorsphere number per field (right). Scale bar: 200 μm.



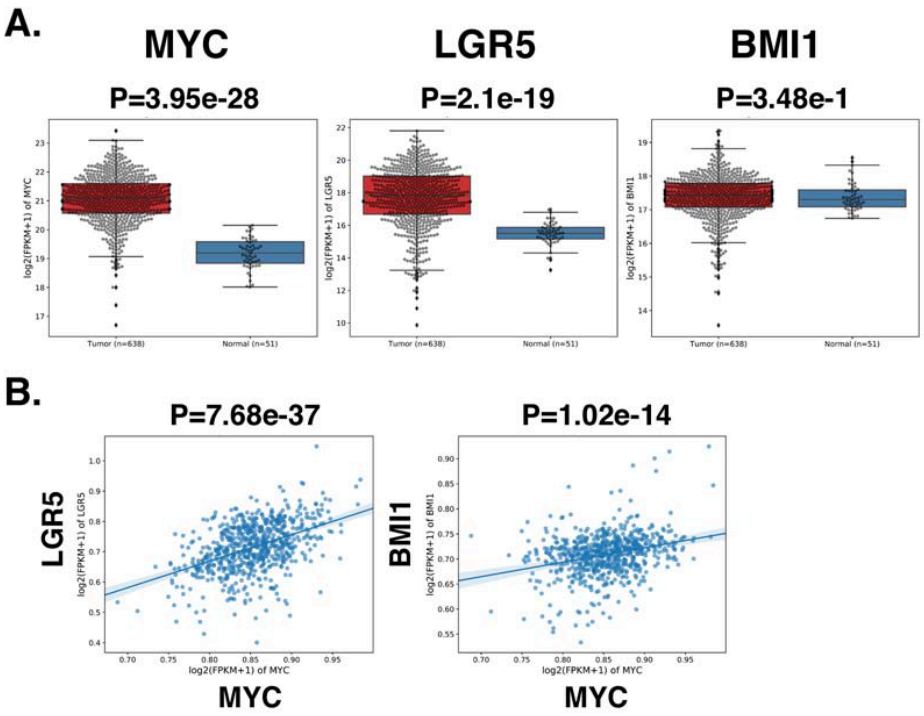
Supplemental Figure S5. Targeting MUC1-C downregulates MYC expression. A. SW620/tet-MUC1shRNA cells treated with vehicle or 500 ng/ml DOX for 7 days were analyzed for MYC mRNA levels. The results (mean±SD) are expressed as relative mRNA levels compared to those obtained for vehicle-treated cells (assigned a value of 1). B. SK-CO-1/tet-CshRNA and SK-CO-1/tet-MUC1shRNA cells were treated with vehicle or 500 ng/ml DOX for 7 days. Lysates were immunoblotted with antibodies against the indicated proteins. C. SW620/tet-MYCshRNA cells treated with vehicle or 500 ng/ml DOX for 7 days were analyzed for MYC and LGR5 mRNA levels. The results (mean±SD) are expressed as relative mRNA levels compared to those obtained for vehicle-treated cells (assigned a value of 1). D. SK-CO-1 cells expressing a tet-MYCshRNA were treated with vehicle or 500 ng/ml DOX for 7 days. Lysates were immunoblotted with antibodies against the indicated proteins.



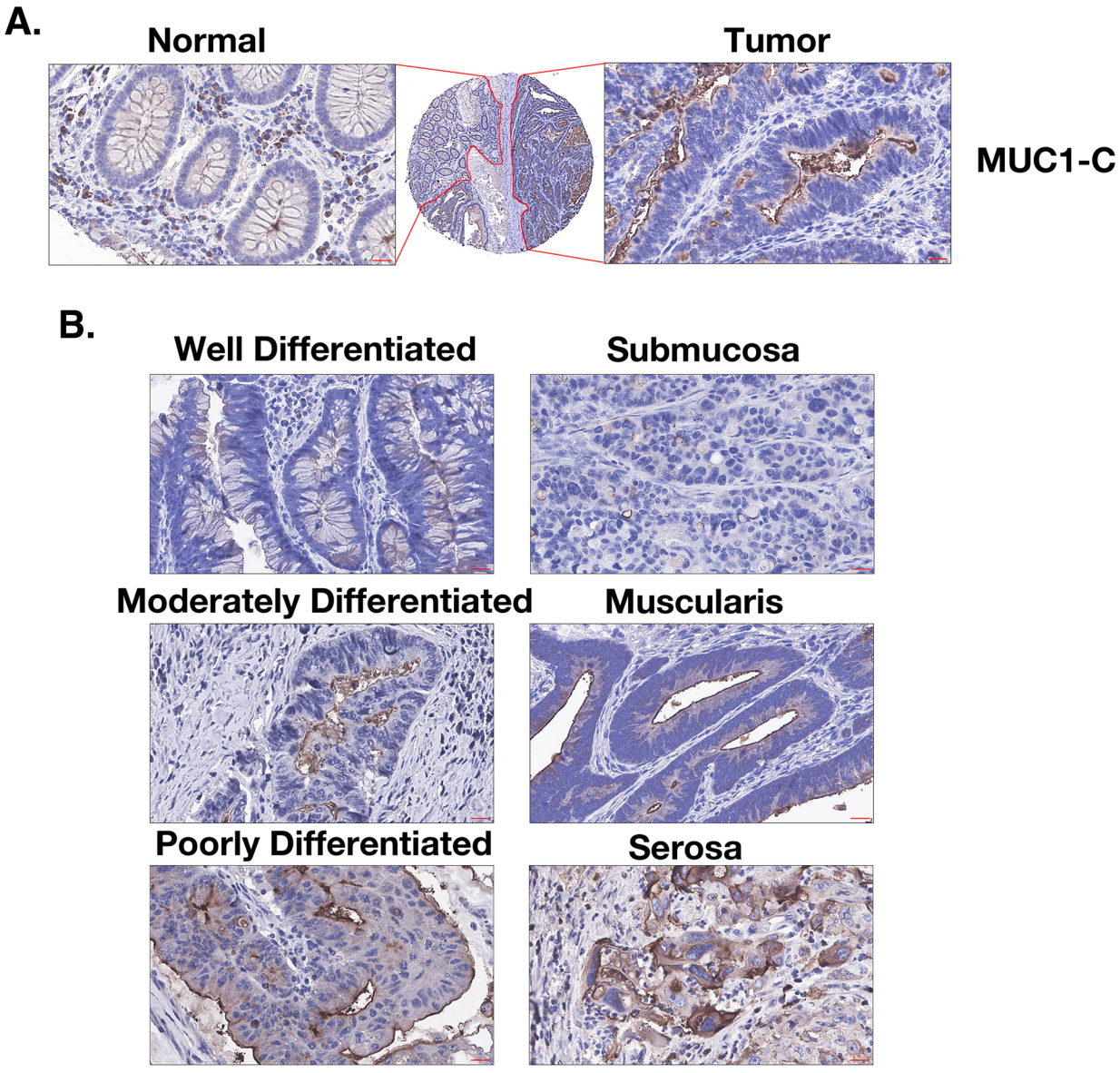
Supplemental Figure S6. Targeting MUC1-C decreases OSN expression. A. Lysates from SK-CO-1/tet-MYCshRNA and SK-CO-1/tet-MUC1shRNA cells treated with vehicle or 500 ng/ml DOX were immunoblotted with antibodies against the indicated proteins. B. Lysates from SK-CO-1/tet-MYCshRNA cells treated with vehicle or 500 ng/ml DOX were immunoblotted with antibodies against the indicated proteins.



Supplemental Figure S7. Silencing MUC1-C inhibits SK-CO-1 tumorigenicity and stemness/pluripotency gene expression. A and B. Six-week old nude male mice were injected subcutaneously in the flank with 3 x 10^6 SK-CO-1/tet-MUC1shRNA cells. Mice were pair-matched into two groups when tumors reached 100-150 mm^3 and were fed without and with DOX. Tumor volumes are expressed as the mean±SD for 6 mice (A). Lysates from tumors obtained on the indicated days were immunoblotted with antibodies against the indicated proteins (B).



Supplemental Figure S8. Expression of MUC1, MYC, LGR5 and BMI1 in normal colonic mucosa and CRC. A. Normal colonic mucosa samples (n=51) were compared to CRC samples (n=638) (Xena gene signature dataset). Multiple probe set IDs for the indicated genes were averaged for each patient sample after normalization to obtain a representative expression value. The center line indicates the median value, bounds of the box denote 25th (lower) and 75th (upper) percentiles, and whiskers indicate minimum (lower) and maximum (upper) values. Student's t-test was used to compared groups. B. MYC, LGR5 (left) and BMI1 (right) gene expression data from the Xena dataset was assessed for correlation using the Pearson's correlation coefficient.



Supplemental Figure S9. IHC staining of MUC1-C expression in CRCs. A. Images of adjacent normal (left) and CRC (right) tissues stained for MUC1-C expression. Scale bars: 20 μm. B. Images of CRCs with the indicated degrees of differentiation stained for MUC1-C expression (left). Images of CRCs with invasion into the submucosa, muscularis and serosa stained for MUC1-C expression (right). Scale bars: 20 μm.

Supplemental Table S1. Analysis of colon tissues from control and GO-203-treated MUC1$^{+/-}$/IL-10$^{-/-}$ mice.

Mouse	Group	Colitis			Dysplasia		Carcinoma
		Mild	Moderate	Severe			
		1	2	3	4	5	6
132-1	Control						√
132-2	Control				√		
149-1	Control			√			
149-2	Control				√		
171-1	Control			√			
171-2	Control				√		
171-3	Control				√		
133-1	GO-203	√					
133-2	GO-203		√				
150-1	GO-203		√				
150-2	GO-203			√			
170-1	GO-203	√					
170-2	GO-203		√				
150-3	GO-203	√					

Frequency of Lesions		
	Control (%)	GO-203 (%)
Mild inflammation	0	42.86
Moderate inflammation	0	42.86
Severe inflammation	28.57	14.29
Dysplasia	57.14	0
Carcinoma	14.28	0

Supplemental Table S2. Epithelial damage score data for control and GO-203-treated MUC1$^{+/-}$/IL-10$^{-/-}$ mice.

Mouse	Epithelial Damage Score	
	Control	**GO-203**
1	6	1
2	4	2
3	3	2
4	4	3
5	3	1
6	4	2
7	4	1

Supplemental Table S3. Analysis of colon tissues from control and GO-203-treated AOM/DSS-induced MUC1$^{+/-}$ mice.

Mouse	Group	Colitis			Dysplasia		Carcinoma
		Mild	Moderate	Severe			
		1	2	3	4	5	6
00057-1	Control					√	
00057-2	Control						√
00057-3	Control						√
00067-2	Control		√				
00067-3	Control						√
00067-4	Control			√			
00067-5	Control					√	
00069-1	Control				√		
00069-2	Control			√			
00069-3	Control				√		
00069-4	Control						√
00058-2	GO-203	√					
00059-1	GO-203	√					
00059-2	GO-203						√
00059-3	GO-203		√				
00059-4	GO-203		√				
00059-5	GO-203		√				
00060-1	GO-203		√				
00060-2	GO-203		√				
00060-3	GO-203			√			

Frequency of Lesions		
	Control (%)	**GO-203 (%)**
Slight inflammation	0	22.2
Moderate inflammation	9.1	55.6
Severe inflammation	18.2	11.1
Slight dysplasia	9.1	0
Moderate dysplasia	9.1	0
Severe dysplasia	9.1	0
Carcinoma	46.0	11.1

Supplemental Table S4. Epithelial damage score data from control and GO-203-treated AOM/DSS-induced MUC1$^{+/-}$ mice.

Mouse	Epithelial Damage Score	
	Control	**GO-203**
1	5	1
2	6	1
3	6	6
4	2	2
5	6	2
6	3	2
7	5	2
8	4	2
9	3	3
10	4	
11	6	

Supplemental Table S5. IHC score data for control and GO-203-treated MUC1$^{+/-}$/IL-10$^{-/-}$ mice.

MUC1-C IHC Score											
Mouse	Group	% Positive Staining Cells					Staining Intensity				Sum Score
		≤10%	11-24%	25-49%	50-74%	≥75%	Negative	Weak	Moderate	Strong	
		0	1	2	3	4	0	1	2	3	
1	Control					√				√	12
2	Control				√					√	9
3	Control					√			√		8
4	Control					√			√		8
5	GO-203			√				√			2
6	GO-203				√			√			3
7	GO-203			√				√			2
8	GO-203				√			√			3
9	GO-203			√				√			2
Myc IHC Score											
1	Control					√			√		8
2	Control					√				√	12
3	Control					√		√			4
4	Control					√				√	12
5	GO-203			√				√			2
6	GO-203			√				√			2
7	GO-203		√					√			1
8	GO-203			√					√		4
9	GO-203			√				√			2
Oct4 IHC Score											
1	Control					√				√	12
2	Control					√				√	12
3	Control					√				√	12
4	Control					√				√	12
5	Control				√				√		6
6	Control					√				√	12
7	Control					√			√		8

8	GO-203			√					√		4
9	GO-203			√				√			2
10	GO-203			√					√		4
11	GO-203				√				√		6
12	GO-203			√				√			2
13	GO-203			√				√			2
14	GO-203			√				√			2
Sox2 IHC Score											
1	Control			√						√	6
2	Control		√							√	3
3	Control		√					√			1
4	Control			√				√			2
5	GO-203		√						√		2
6	GO-203		√				√				0
7	GO-203	√					√				0
8	GO-203		√						√		2
9	GO-203	√					√				0
Nanog IHC Score											
	Control					√				√	12
2	Control					√				√	12
3	Control					√				√	12
4	Control					√				√	12
5	Control				√				√		6
6	Control				√				√		6
7	Control				√				√		6
8	GO-203			√				√			2
9	GO-203			√				√			2
10	GO-203		√				√				0
11	GO-203			√				√			2
12	GO-203			√				√			2
13	GO-203		√					√			1
14	GO-203			√				√			2

Supplemental Table S6. IHC score data for control and GO-203-treated AOM/DSS-induced MUC1$^{+/-}$ mice.

Mouse	Group	% Positive Staining Cells					Staining Intensity				Sum Score
		≤10%	11-24%	25-49%	50-74%	≥75%	Negative	Weak	Moderate	Strong	
		0	1	2	3	4	0	1	2	3	
MUC1-C IHC Score											
1	Control					√			√		8
2	Control					√				√	12
3	Control					√				√	12
4	Control				√				√		6
5	Control					√				√	12
6	GO-203		√					√			1
7	GO-203			√				√			2
8	GO-203			√				√			2
Myc IHC Score											
1	Control					√		√			4
2	Control				√				√		6
3	Control			√					√		4
4	Control			√					√		4
5	GO-203		√					√			1
6	GO-203			√				√			2
7	GO-203		√					√			1
Oct4 IHC Score											
1	Control				√					√	9
2	Control					√				√	12
3	Control					√				√	12
4	Control				√				√		6
5	Control					√			√		12

6	GO-203		√					√			1
7	GO-203			√					√		4
8	GO-203			√				√			2
Sox2 IHC Score											
1	Control			√				√			2
2	Control			√				√			2
3	Control			√				√			2
4	GO-203	√					√				0
5	GO-203		√					√			1
6	GO-203		√					√			1
Nanog IHC Score											
1	Control					√				√	12
2	Control					√				√	12
3	Control					√				√	12
4	Control				√				√		6
5	Control					√				√	12
6	GO-203		√					√			1
7	GO-203			√				√			2
8	GO-203			√				√			2

Supplemental Table S7. MUC1-C expression in patient CRC samples.

Clinicopathological Characteristics		MUC1-C Staining		
		+	-	
	n	n (%)	n (%)	*P*
Gender				
Male	40	37(52.9)	3(4.3)	
Female	30	26(37.1)	4(5.7)	
Age (year)				
＜58	32	31(44.3)	1(1.4)	
≥58	38	32(45.7)	6(8.6)	
Tumor Site				
Colon	52	48(68.6)	4(5.7)	
Rectum	18	15(21.4)	3(4.3)	
Tumor Type				
Adenocarcinoma	61	55(78.6)	6(8.6)	
Mucinous Adenocarcinoma	9	8(11.4)	1(1.4)	
Tumor Size (cm)				
< 5.0	31	28(40.0)	3(4.3)	
≥ 5.0	39	35(50.0)	4(5.7)	
Differentiation				
Well	15	10(14.3)	5(7.14)	0.004*
Moderate + Poor	55	53(75.7)	2(2.9)	
Invasive Depth				
Submucosa + Muscularis	15	10(14.3)	5(7.1)	0.004*
Serosa	55	53(75.7)	2(2.9)	
TNM Stage				
I	12	7(10.0)	5(7.1)	0.026*
II+III+IV	58	51(72.9)	7(10.0)	

Chi-square test, * $p<0.05$

Supplemental Table S8. Primers used for qRT-PCR.

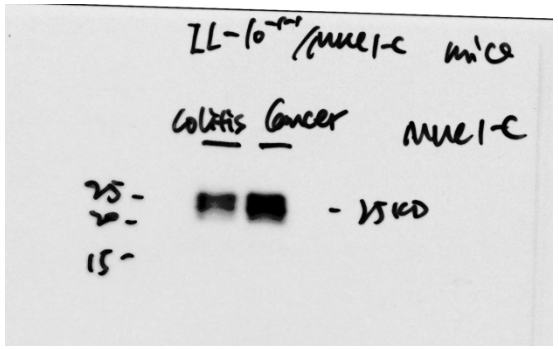
Primer	Forward	Reverse
MUC1-C	AGACGTCAGCGTGAGTGATG	GCCAAGGCAATGAGATAGAC
MYC	TTCGGGTAGTGGAAAACCAG	AGTAGAAATACGGCTGCACC
LGR5	CTGTGCATTTGGAGTGTGTGAG	GGTCTTCCTCAAAGTCAAGCAG
BMI1	TTCATTTTCTGCTGAACGACT	AGGTGGGGATTTAGCTCAGTG
OCT4	AGTCAGTGAACAGGGAATGG	TCGGGATTCAAGAACCTCG
SOX2	GAGAGAAAGAAAGGGAGAGAAG	GAGAGAGGCAAACTGGAATC
NANOG	AGATGCCTCACACGGAGACT	GGACTGGTGGAAGAATCAGG
GAPDH	CCATGGAGAAGGCTGGGG	CAAAGTTGTCATGGATGACC

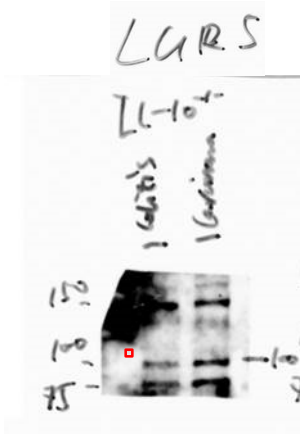
Supplemental Table S9. Primers used for ChIP-PCR.

Primer	Forward	Reverse
LGR5-Promoter	CTGTCACTCTGGCATCGATTTA	CTGCTGCCTTCCTATCTCTTG
BMI1-Promoter	GGCCTGACTACACCGACACT	GCTGAAGGCAGAGTGGAAAC
GAPDH-Promoter	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGAGCGA

Full unedited gel for Figure 3C

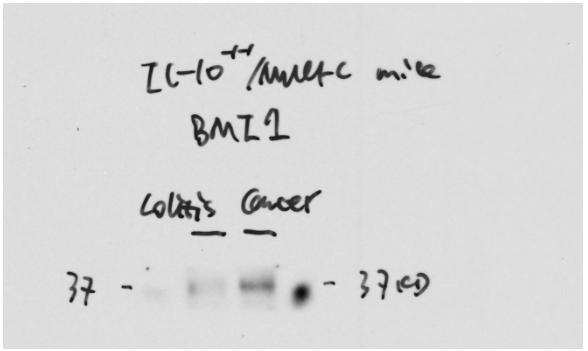
MUC1-C

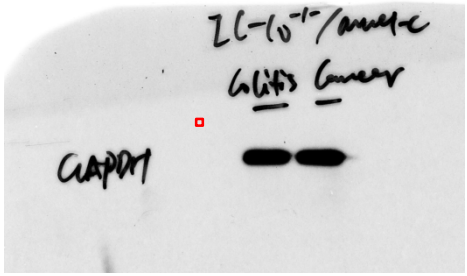




Lgr5

Bmi1

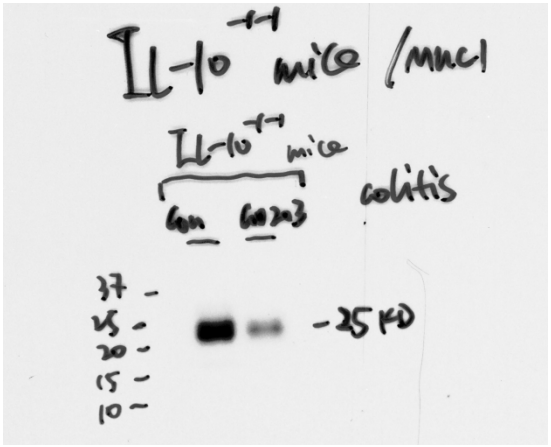


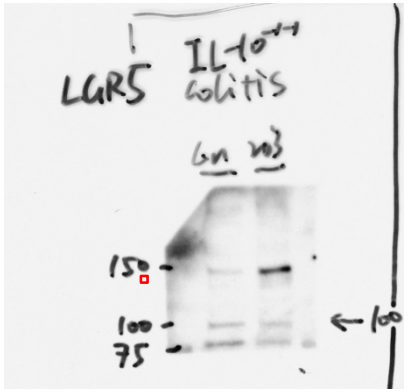


GAPDH

Full unedited gel for Figure 3E

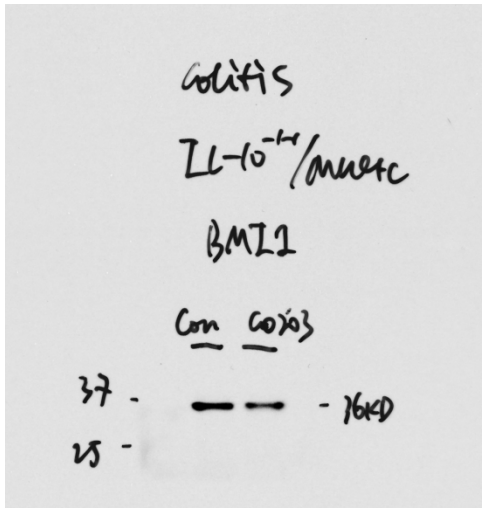
MUC1-C

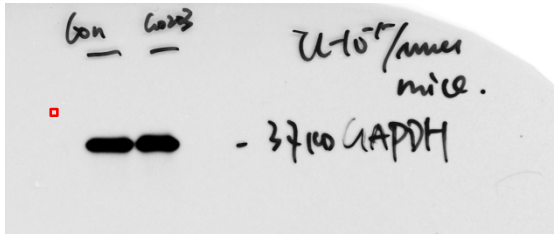




Lgr5

Bmi1

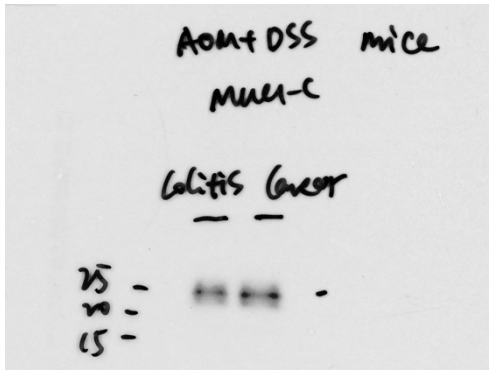


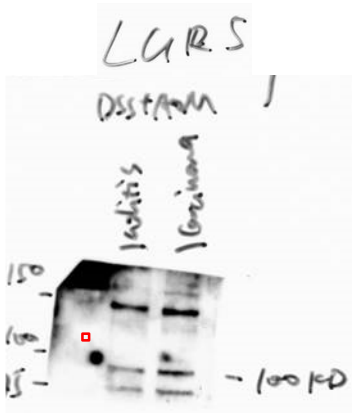


GAPDH

Full unedited gel for Figure 3G

MUC1-C





Lgr5

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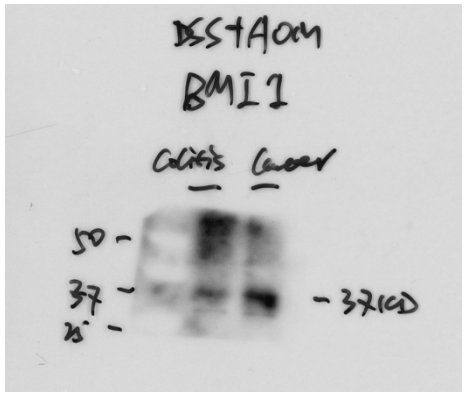
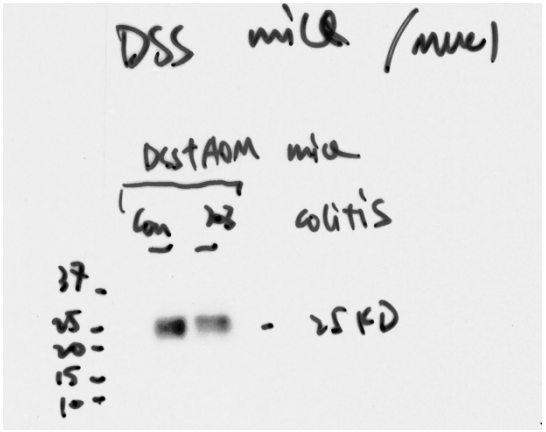
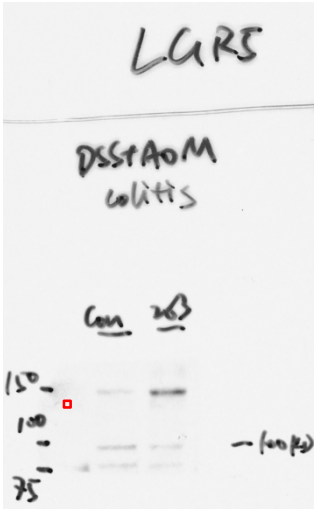


GAPDH

Full unedited gel for Figure 3I

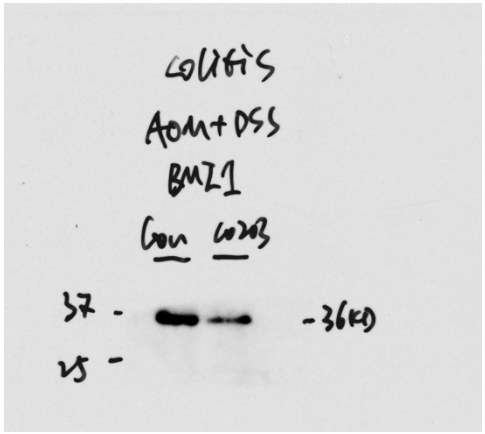
MUC1-C

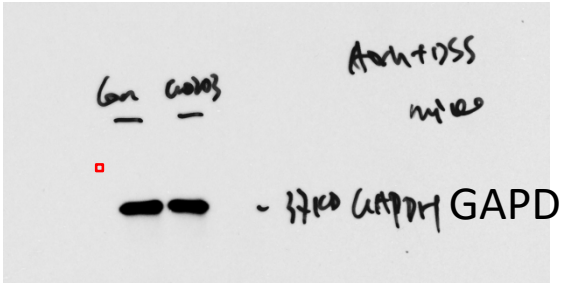




Lgr5

Bmi1

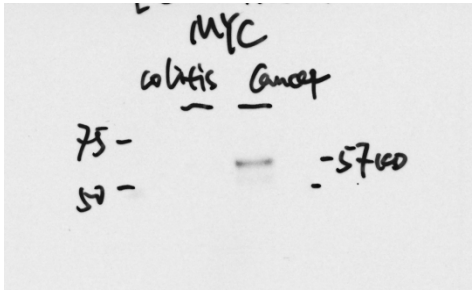


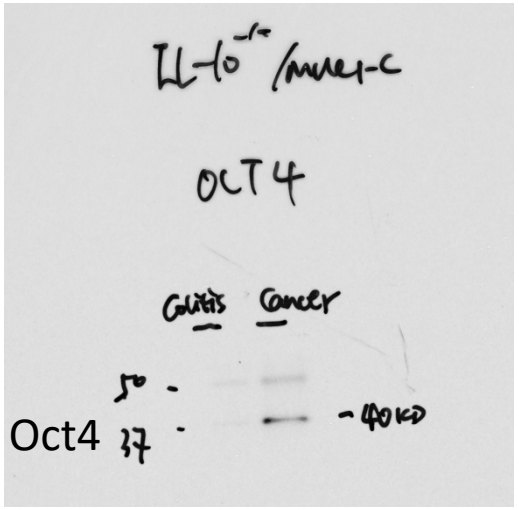


GAPDH

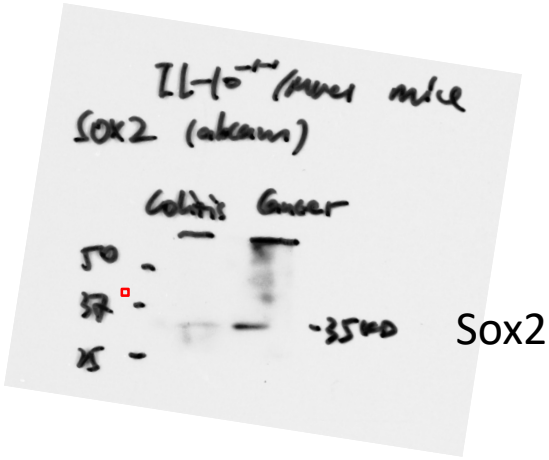
Full unedited gel for Figure 4B

Myc

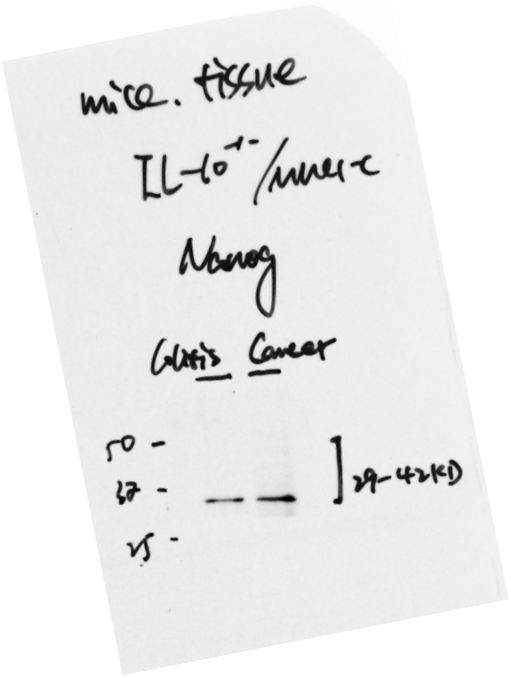




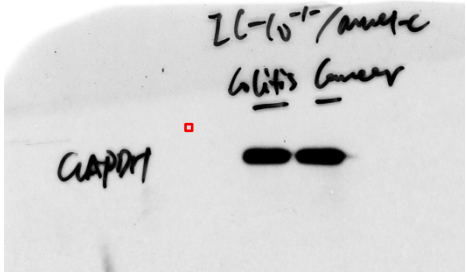
Oct4



Sox2



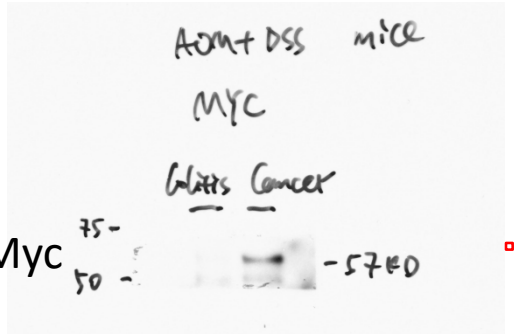
Nanog



GAPDH

Full unedited gel for Figure 4D

Myc



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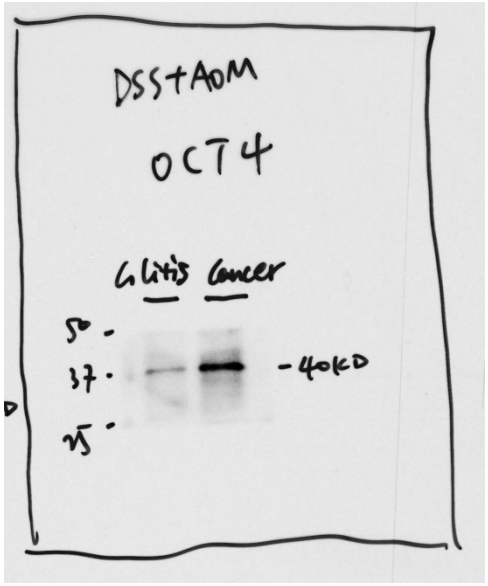
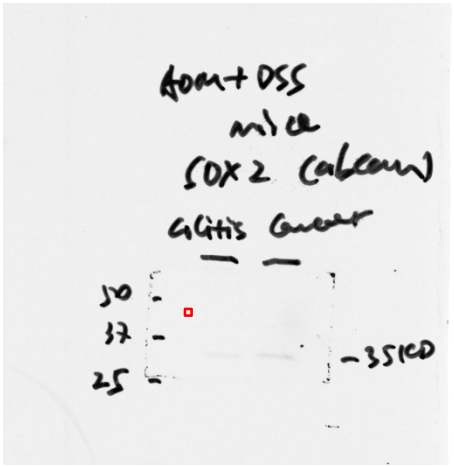
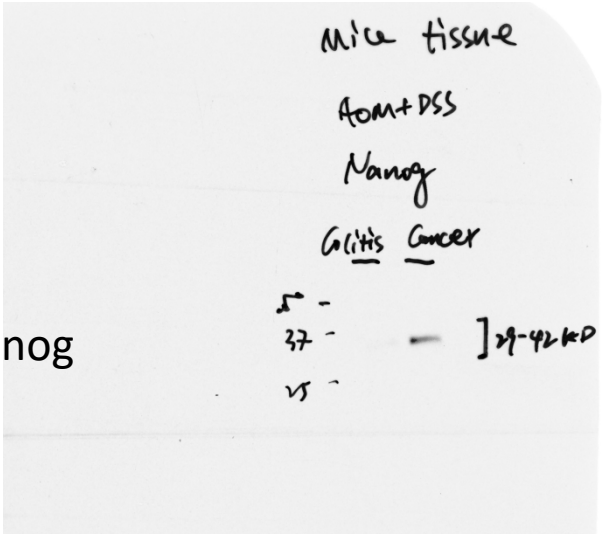




Sox2

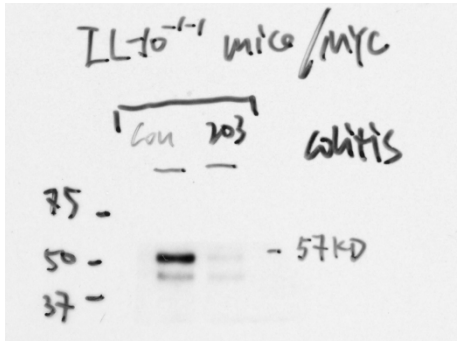
Nanog

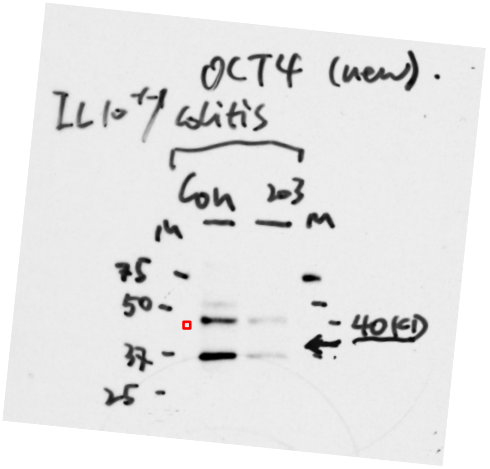


GAPDH

Full unedited gel for Figure 4F

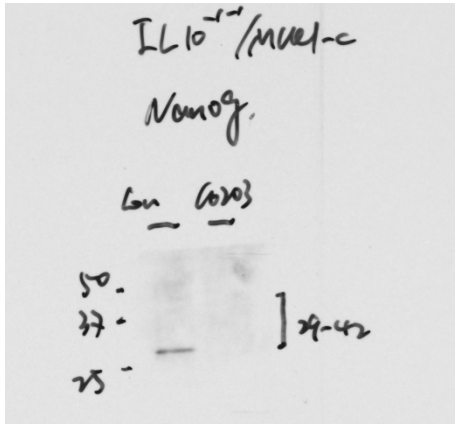
Myc

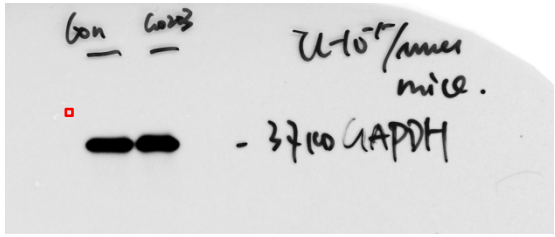




Oct4

Nanog

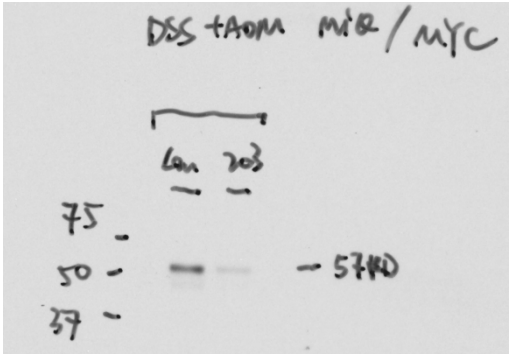


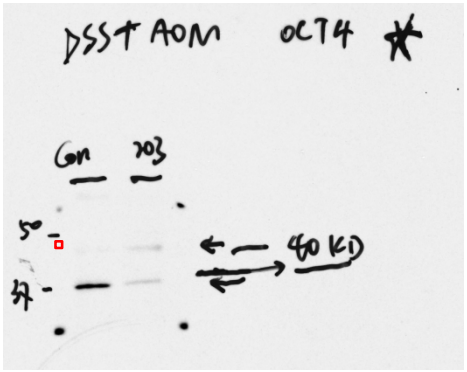


GAPDH

Full unedited gel for Figure 4H

Myc





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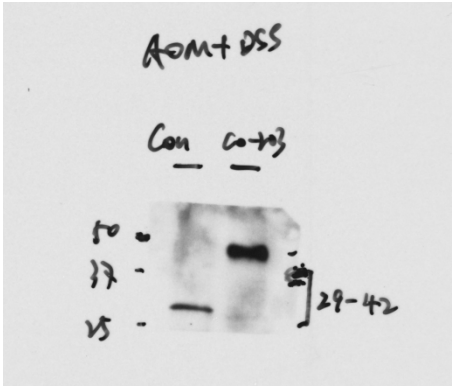
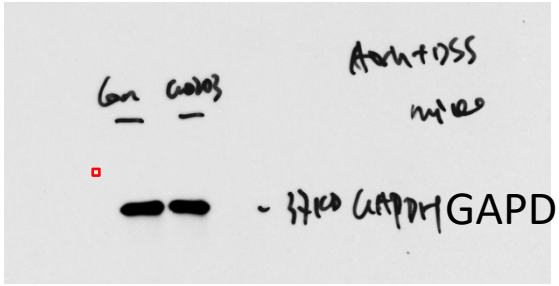
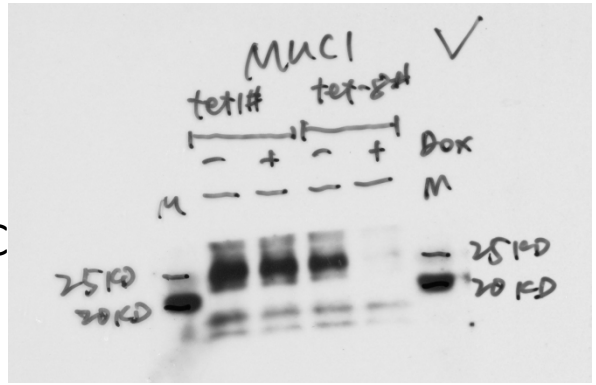




GAPDH

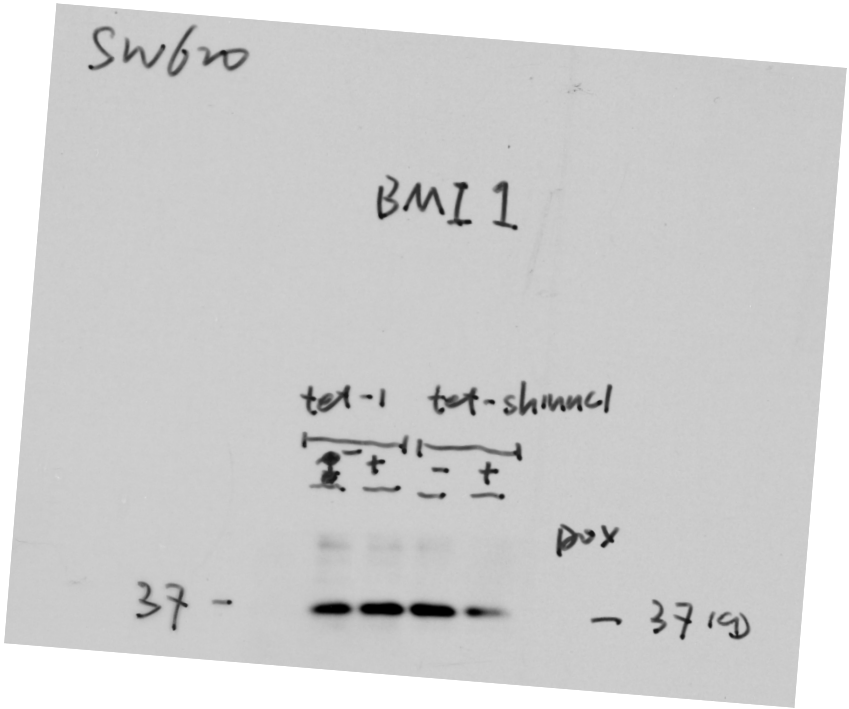
Full unedited gel for Figure 5A

MUC1-C



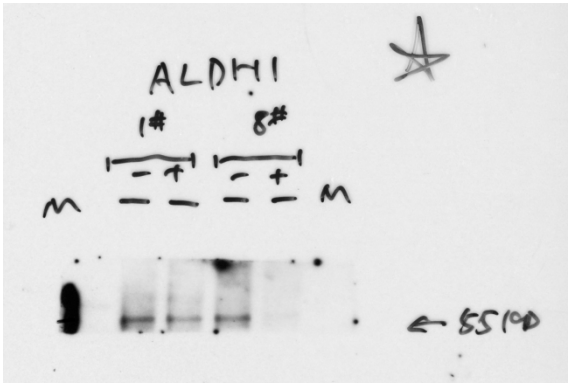
LGR5

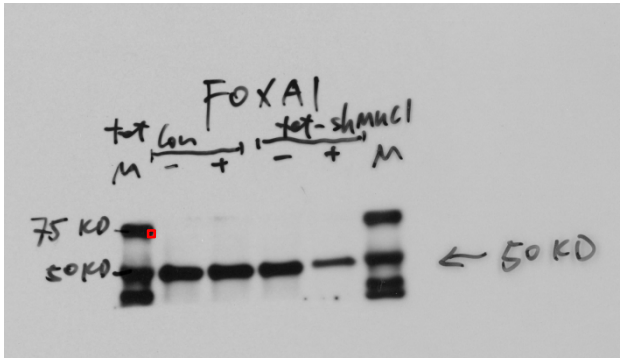
BMI1



Full unedited gel for Figure 5A

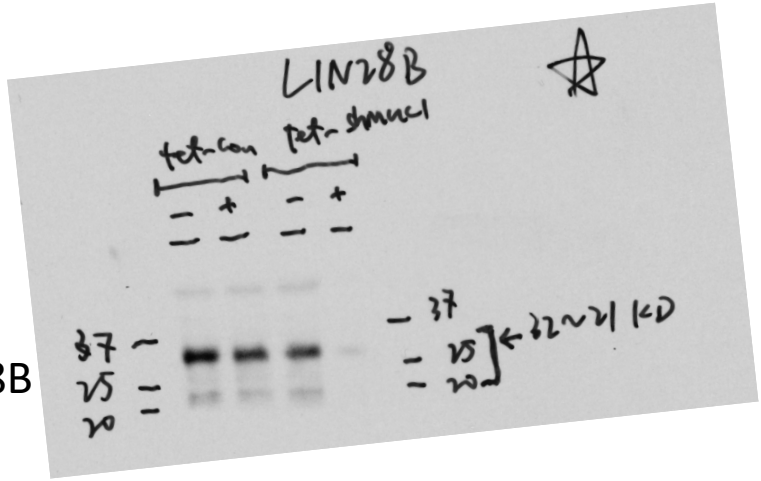
ALDH1

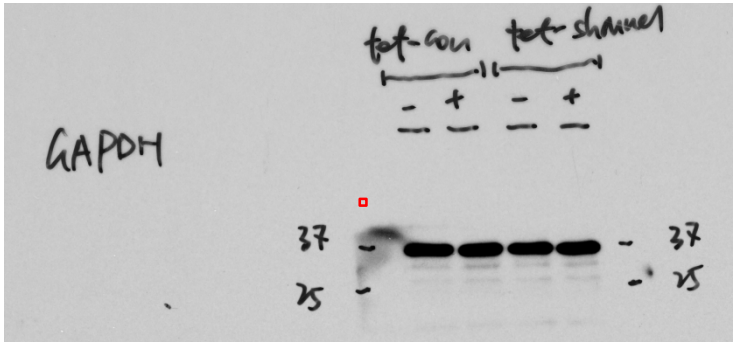




FOXA1

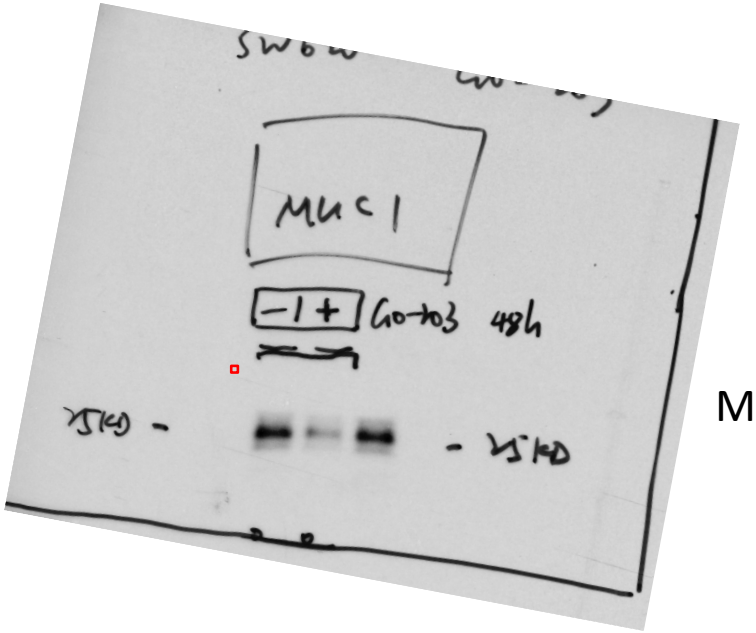
LIN28B



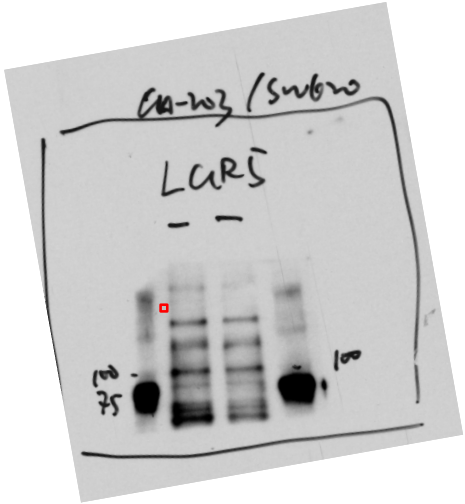


GAPDH

Full unedited gel for Figure 5C

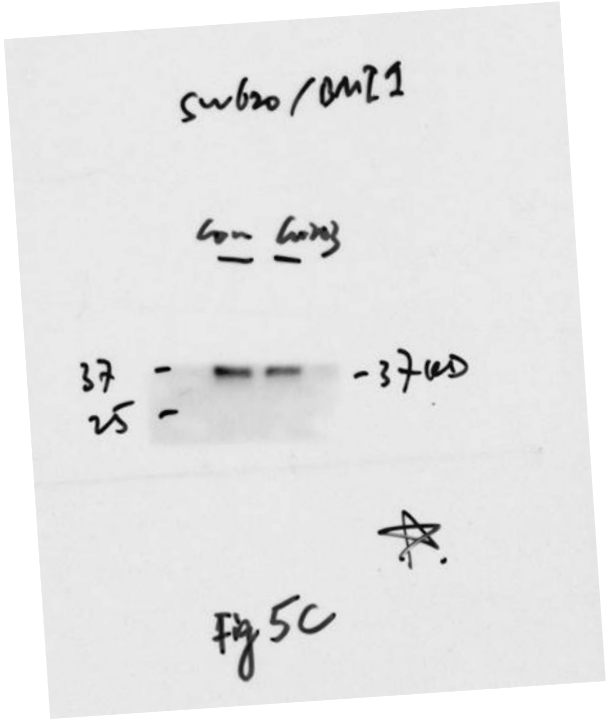


MUC1-C

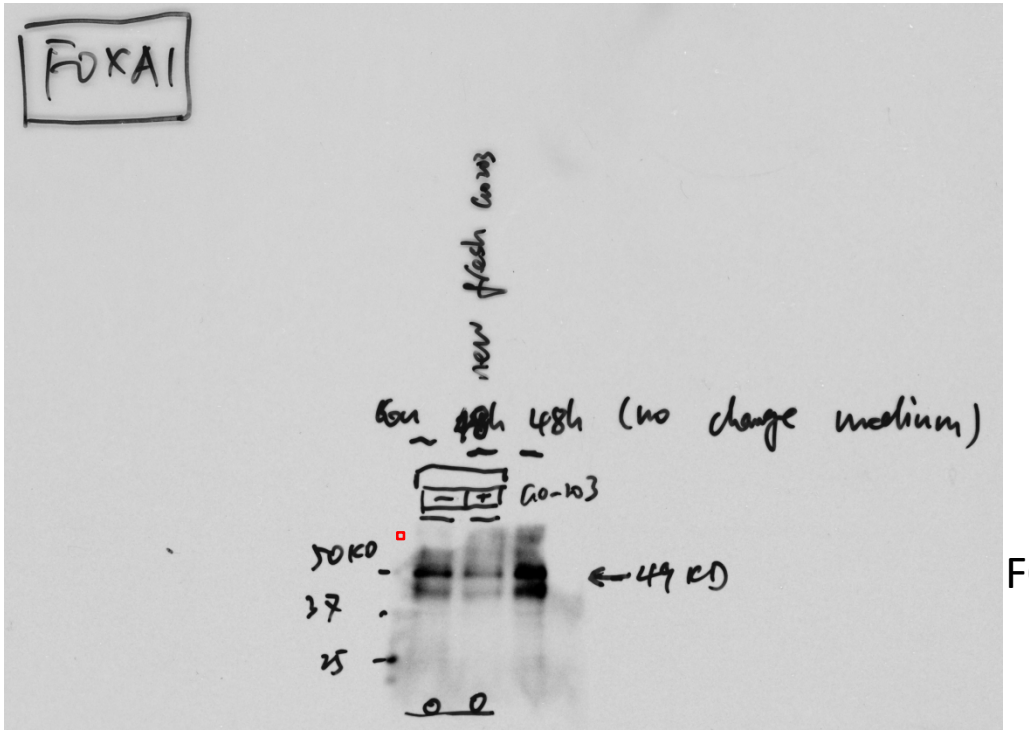


LGR5

BMI1

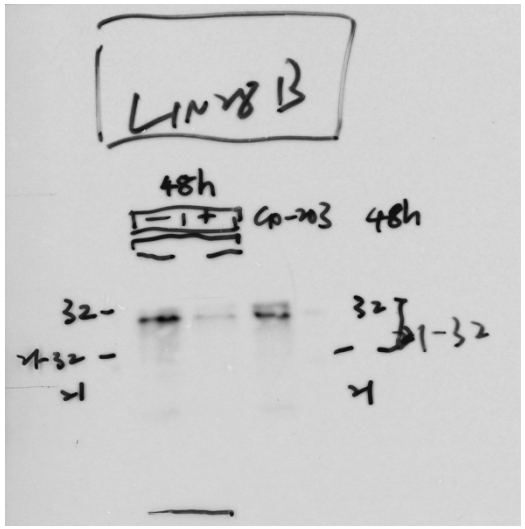


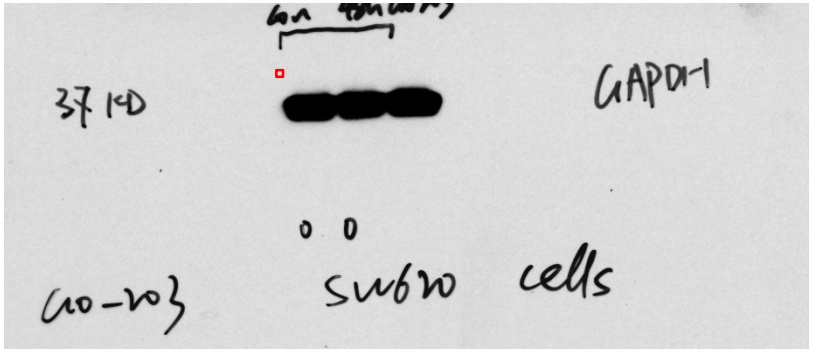
Full unedited gel for Figure 5C



FOXA1

LIN28B

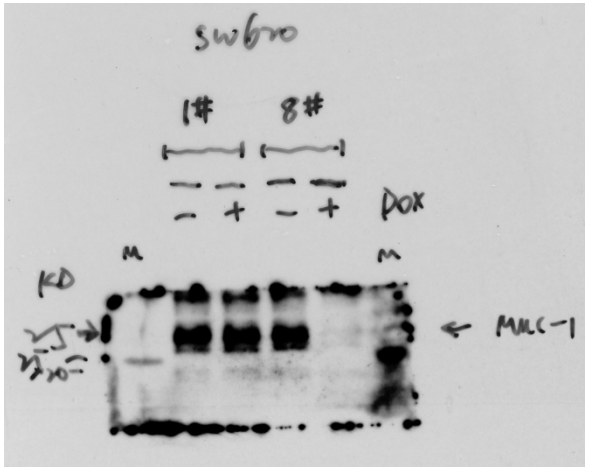


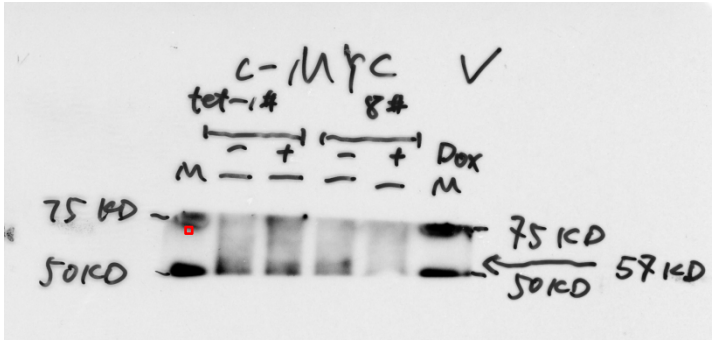


GAPDH

Full unedited gel for Figure 6B

MUC1-C





MYC

GAPDH

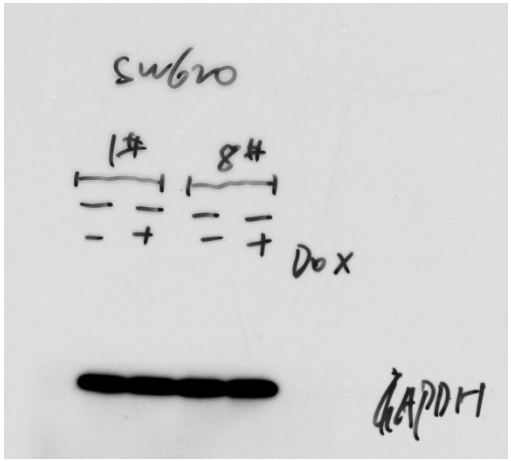
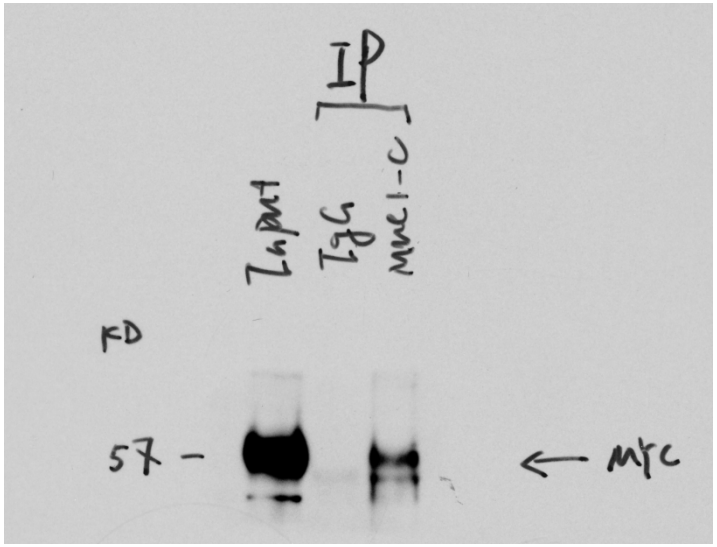
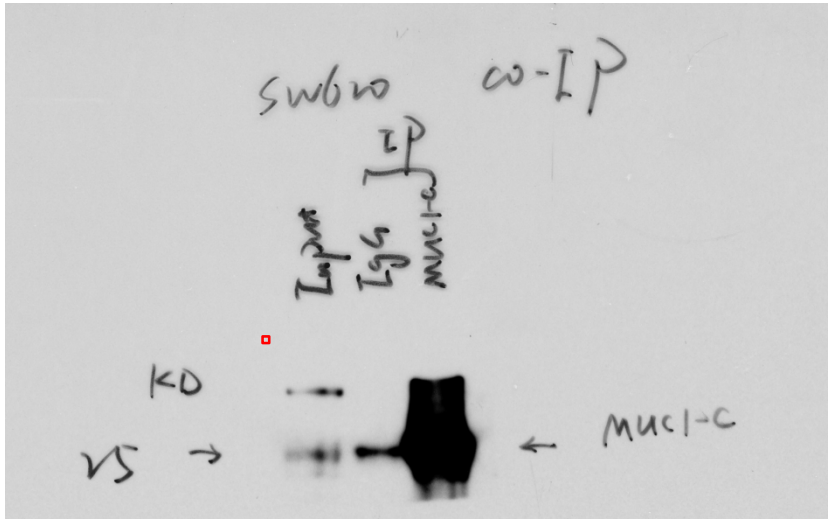


Fig 6C

MYC

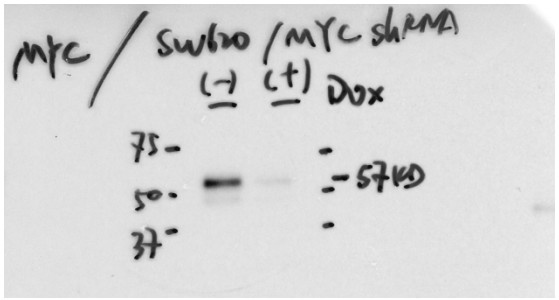


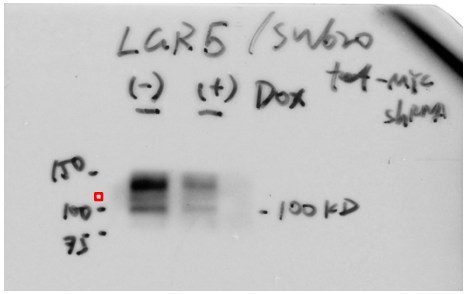


MUC1-C

Full unedited gel for Figure 6D

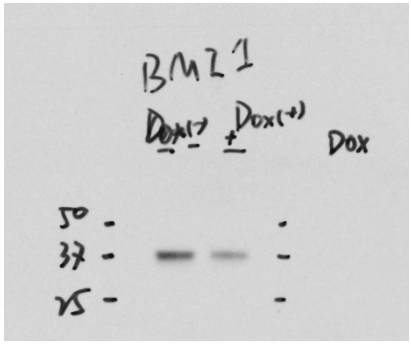
MYC

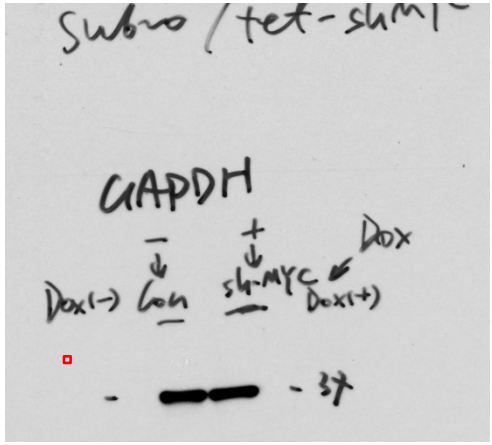




LGR5

BMI1

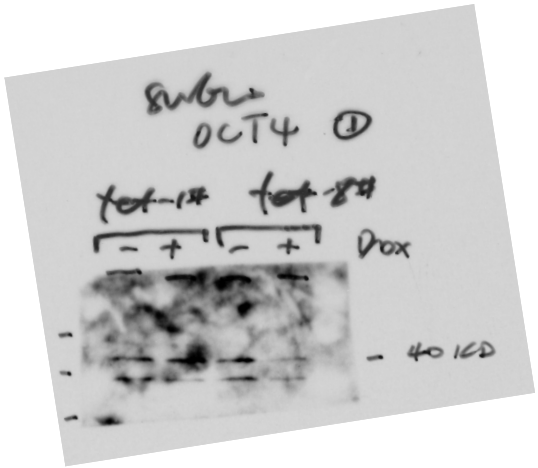


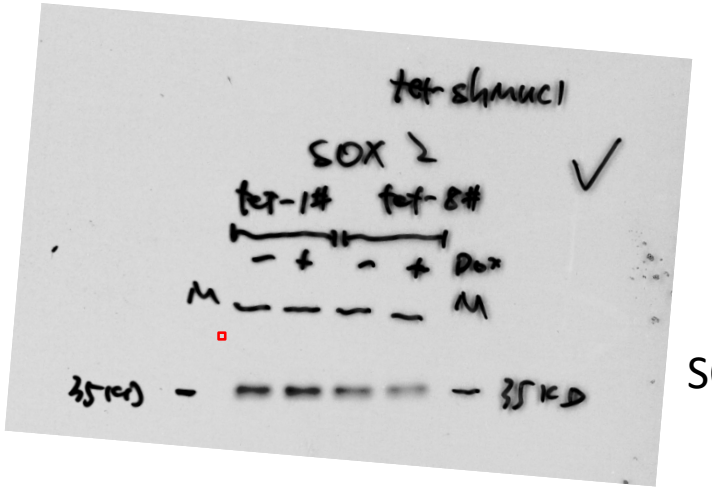


GAPDH

Full unedited gel for Figure 7A

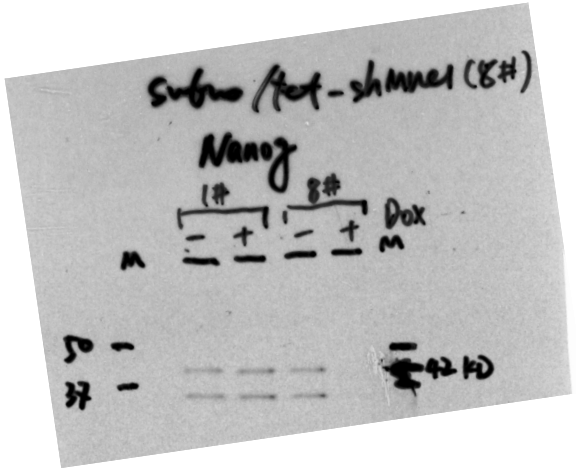
OCT4

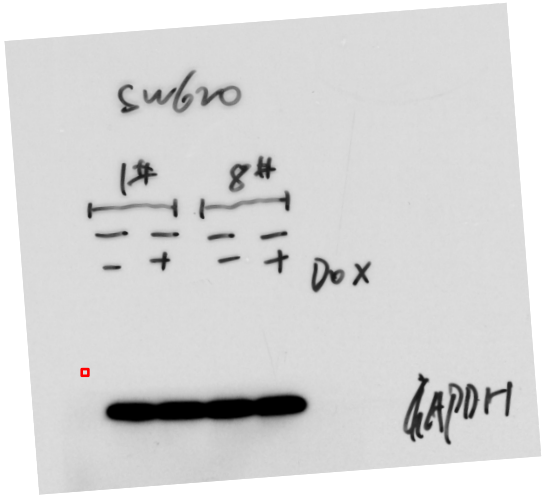




SOX2

NANOG

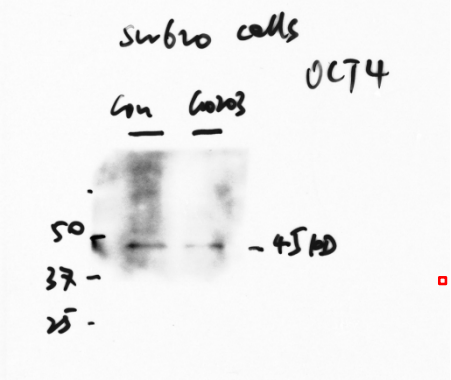




GAPDH

Full unedited gel for Figure 7B

OCT4



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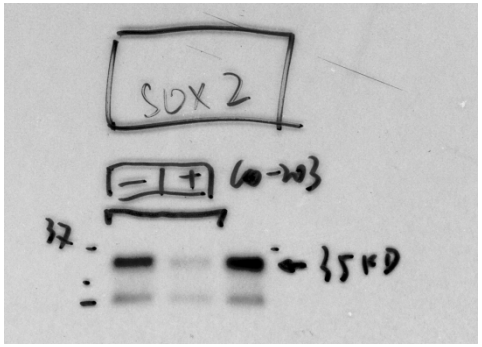

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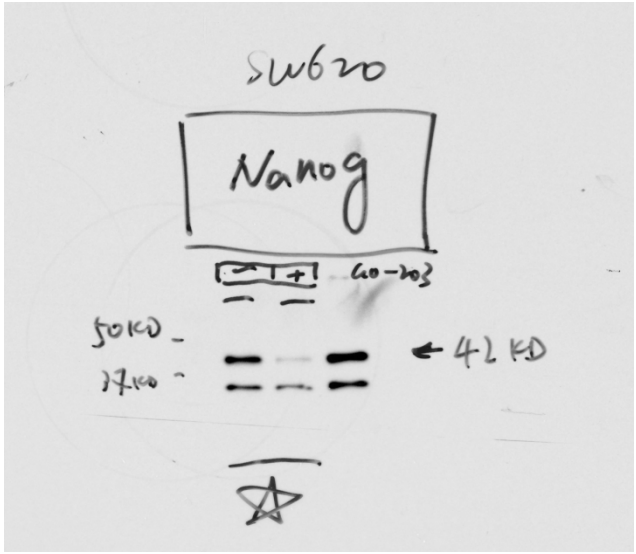
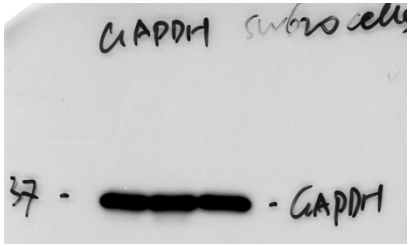
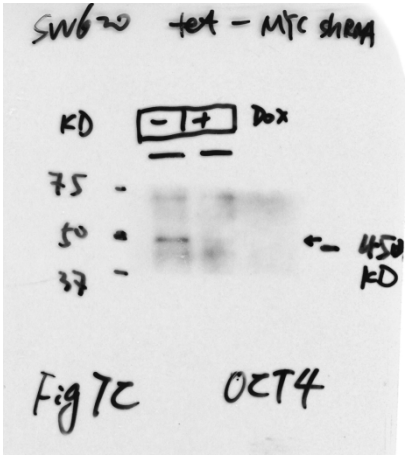


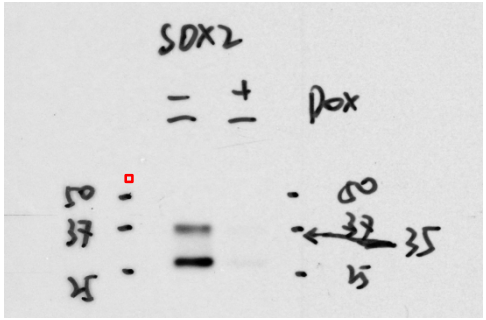
GAPDH



Full unedited gel for Figure 7C

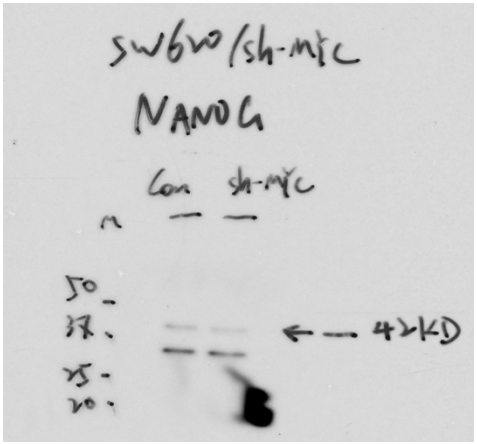
OCT4



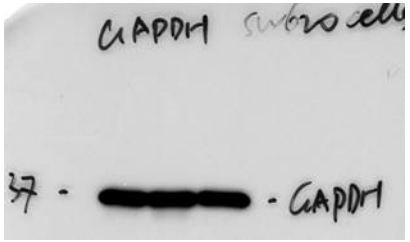


SOX2

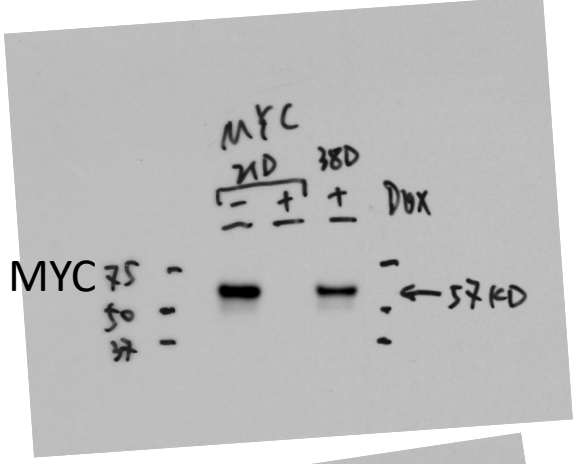
NANOG



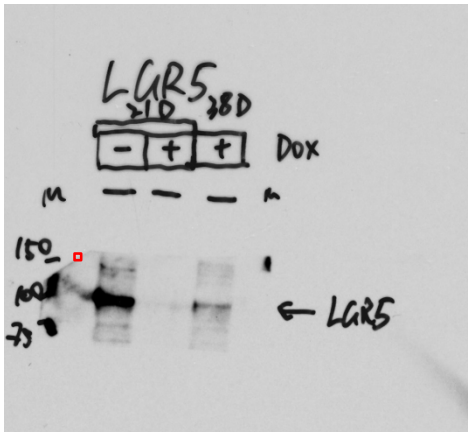
GAPDH



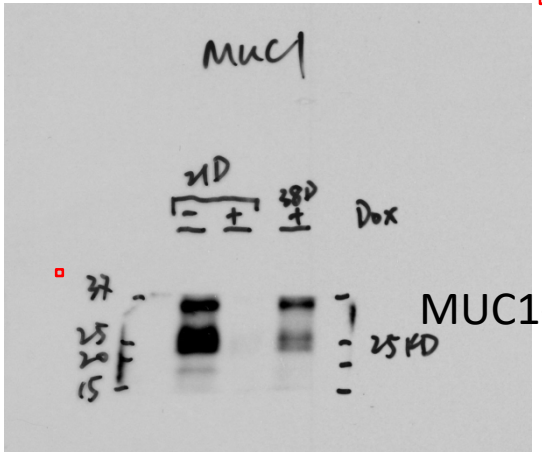
Full unedited gel for Figure 7E



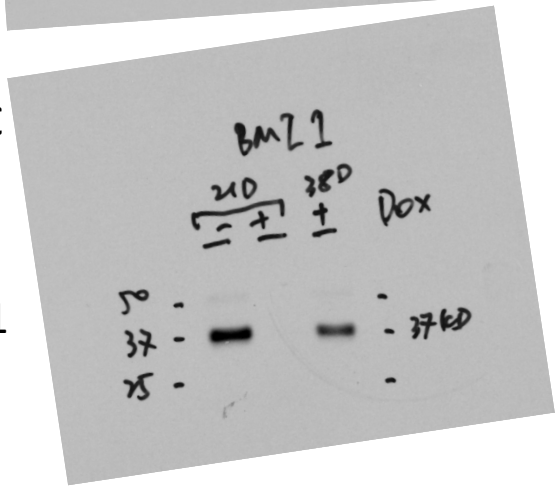
MYC



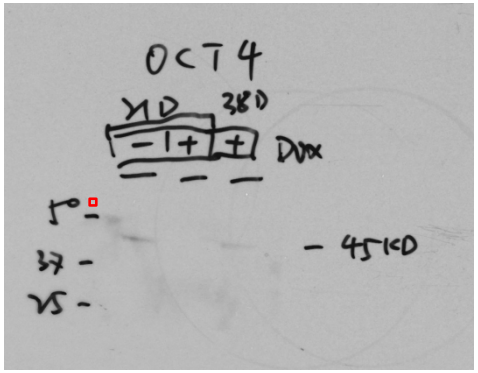
LGR5



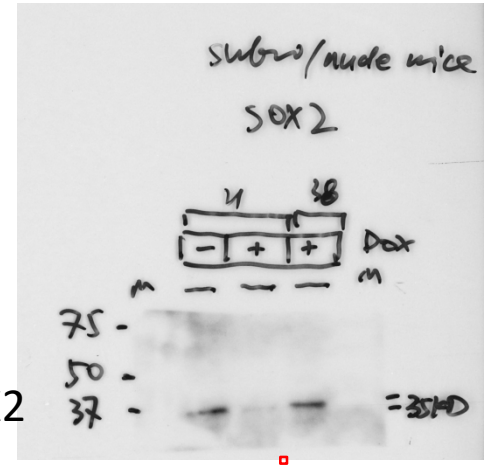
MUC1-C



BMI1

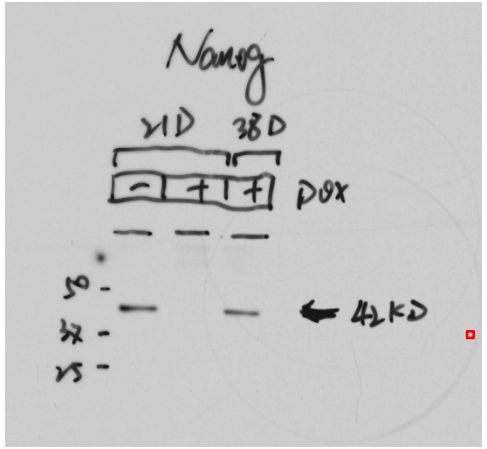


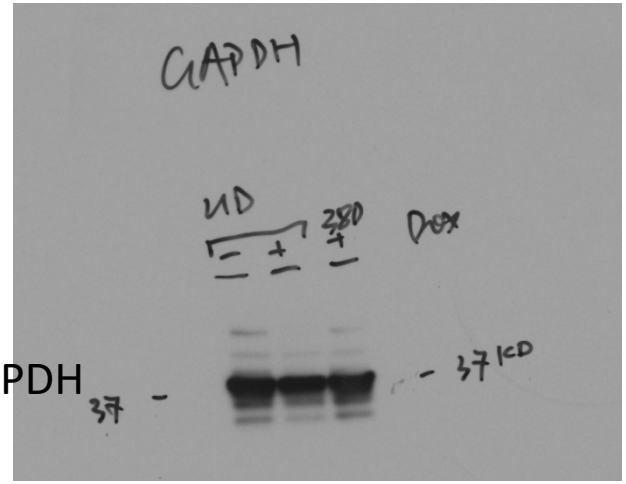
OCT4



SOX2

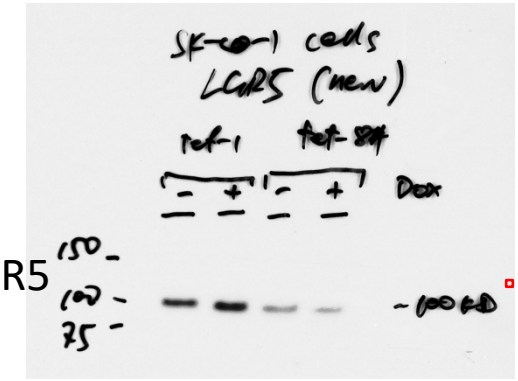
NANOG



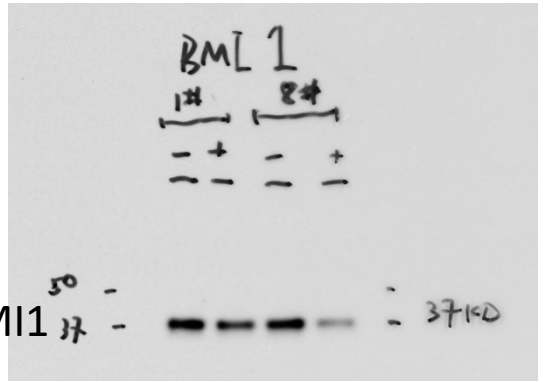


GAPDH

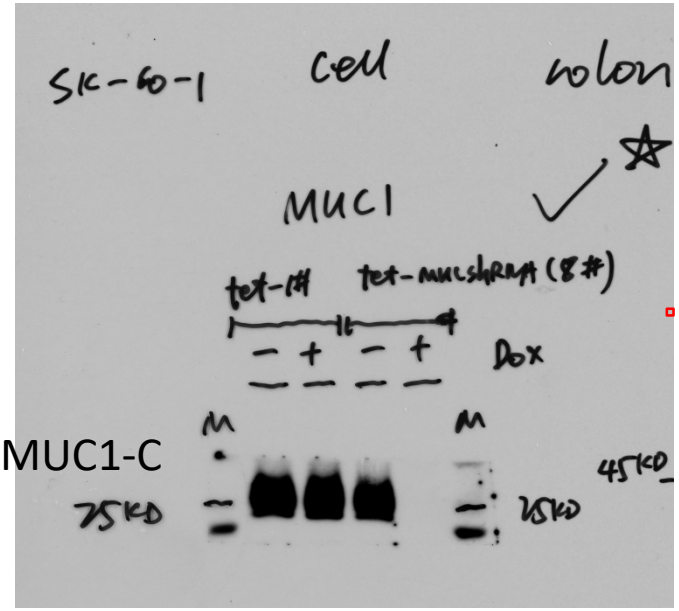
Full unedited gel for SUPP Figure S4A



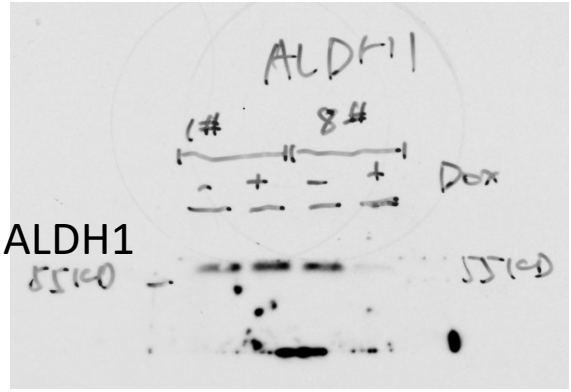
LGR5



BMI1



MUC1-C



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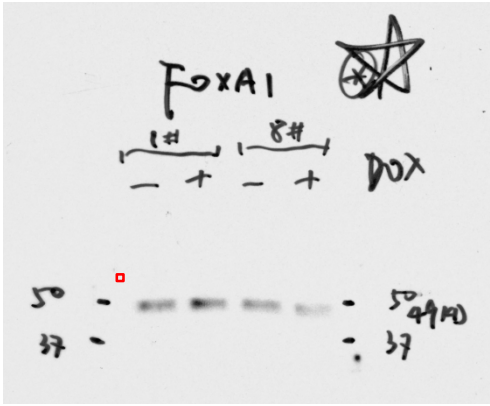

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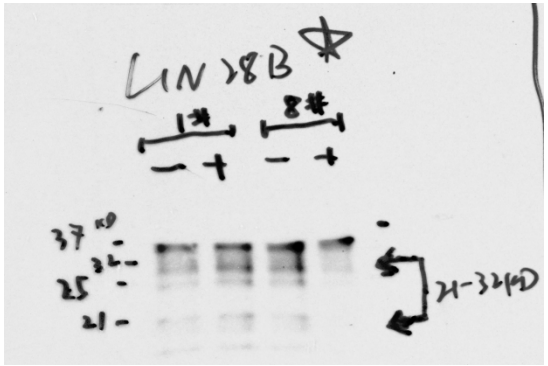

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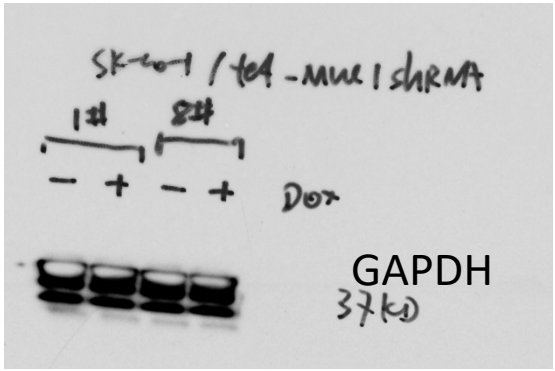
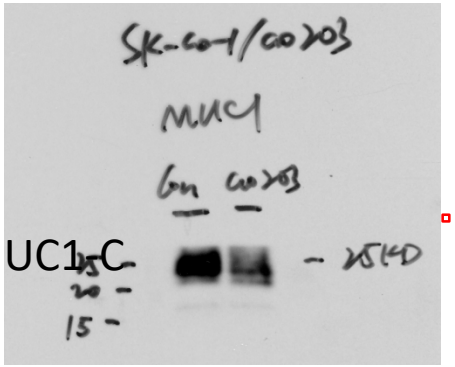
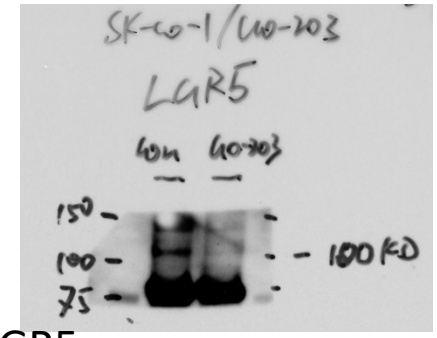


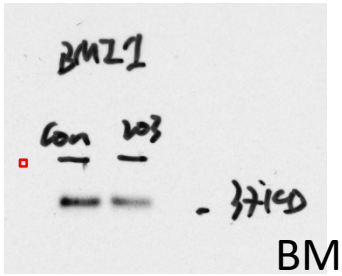
Full unedited gel for SUPP Figure S4B



MUC1-C



LGR5



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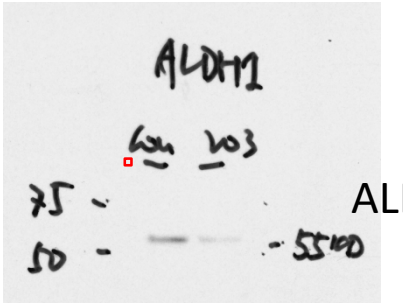
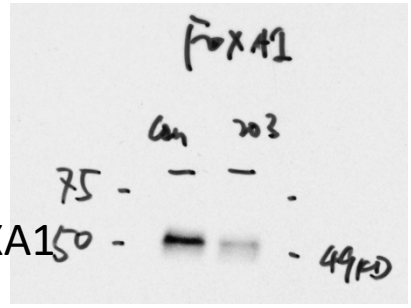


ALDH1



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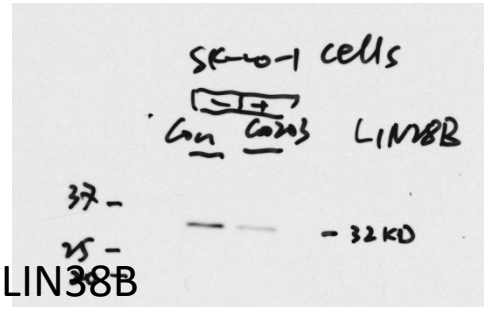

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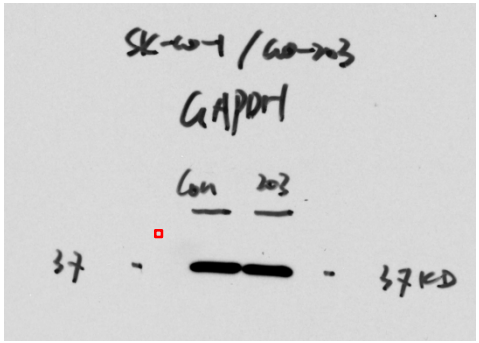
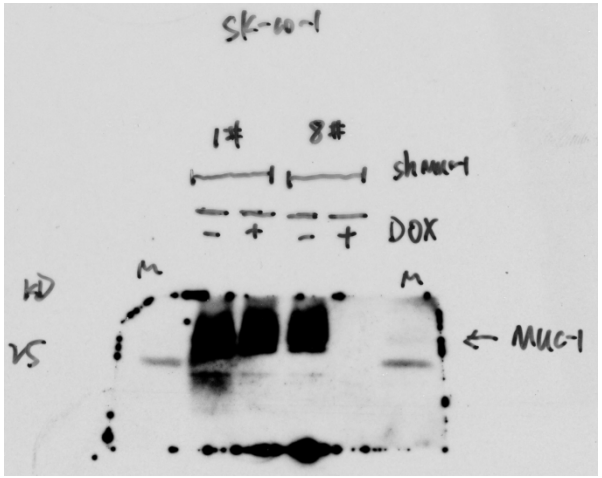


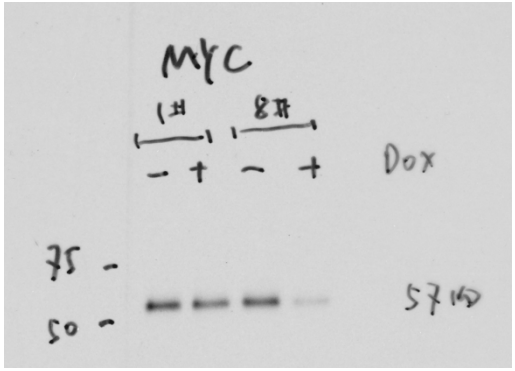
GAPDH

Full unedited gel for SUPP Figure S5B

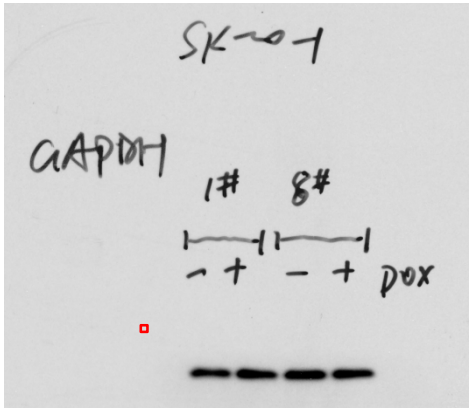
MUC1-C



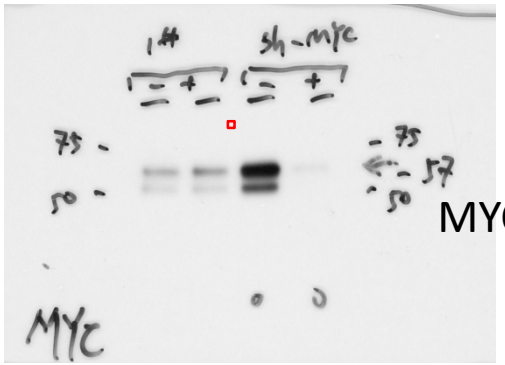
MYC



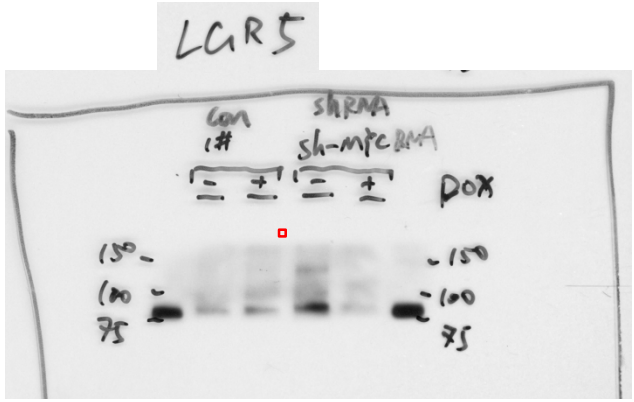
GAPDH



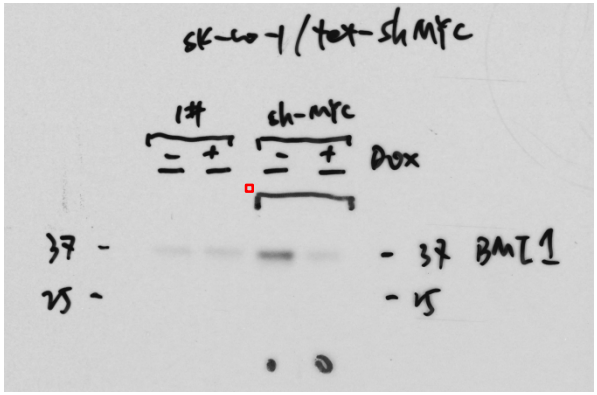
Full unedited gel for SUPP Figure S5D



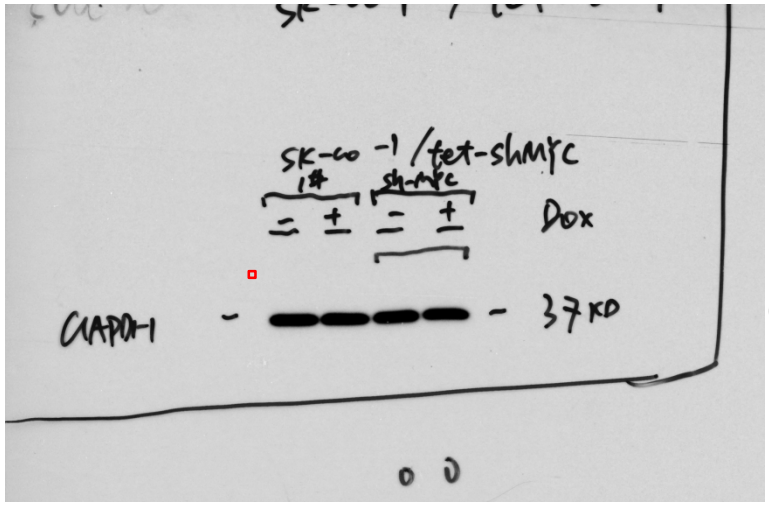
MYC



LGR5

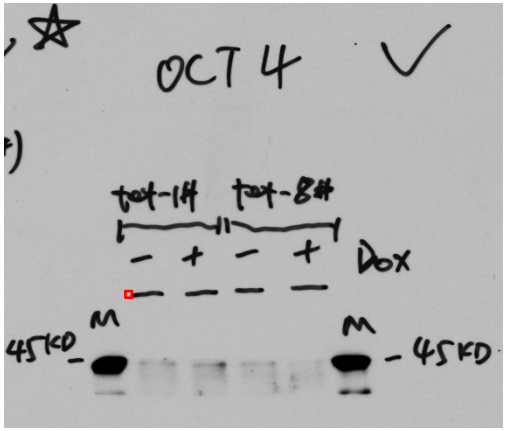


BMI1

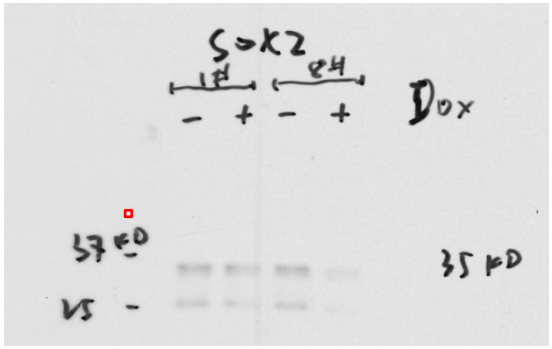


GAPDH

Full unedited gel for SUPP Figure S6A

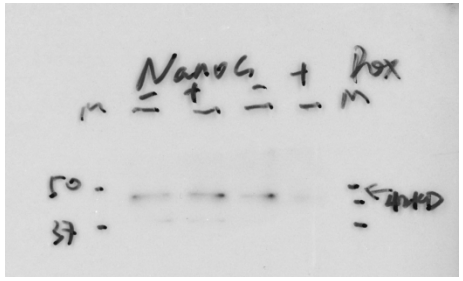


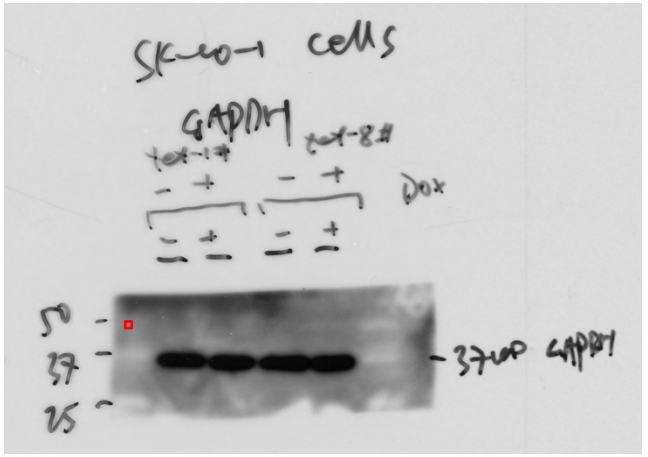
OCT4



SOX2

NANOG

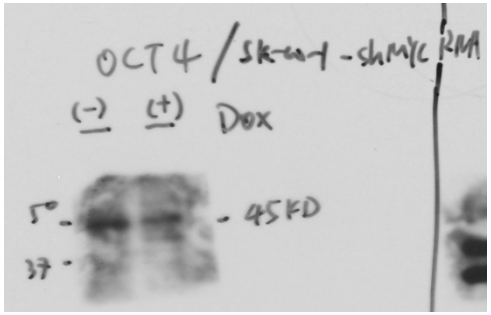


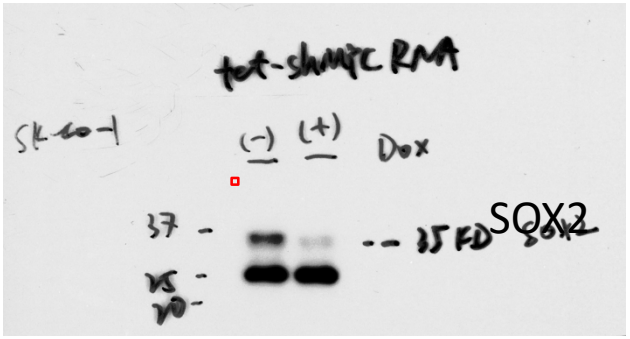


GAPDH

Full unedited gel for SUPP Figure S6B

OCT4





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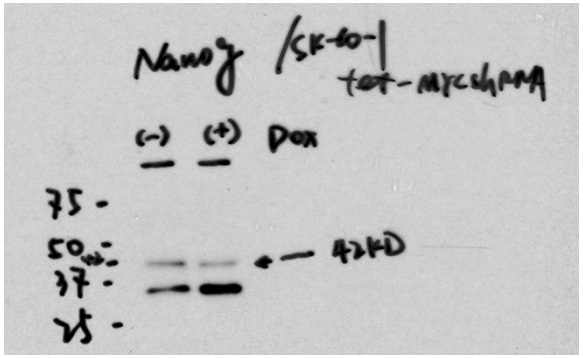
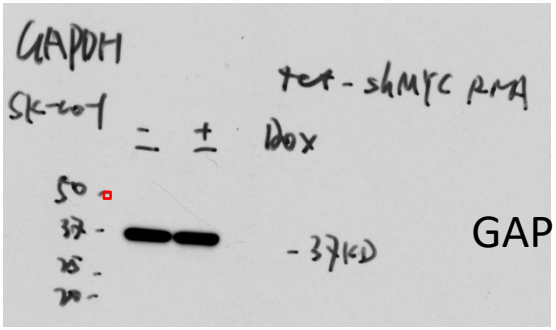
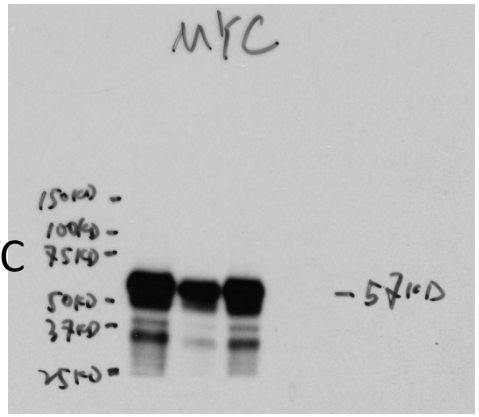


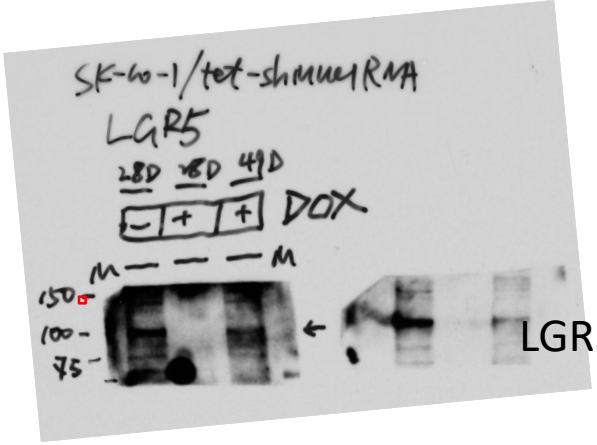


GAPDH

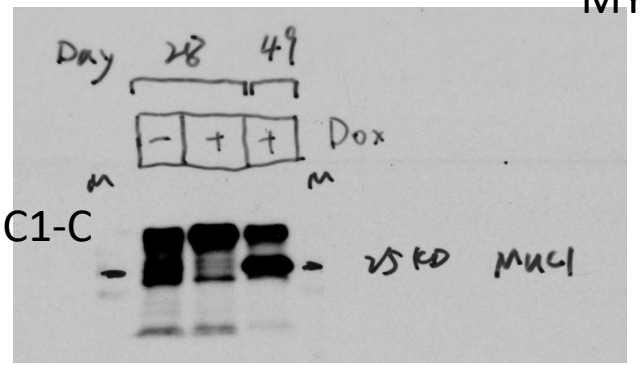
Full unedited gel for SUPP Figure S7B



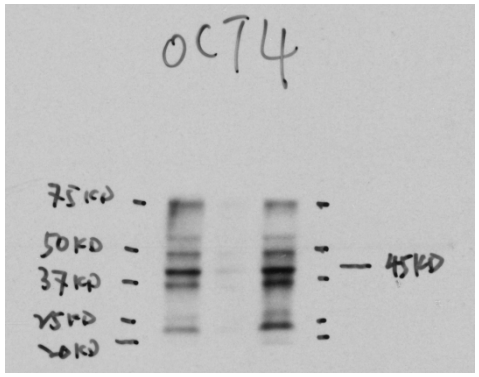
MYC



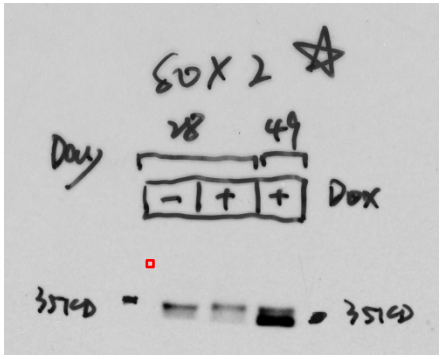
LGR5



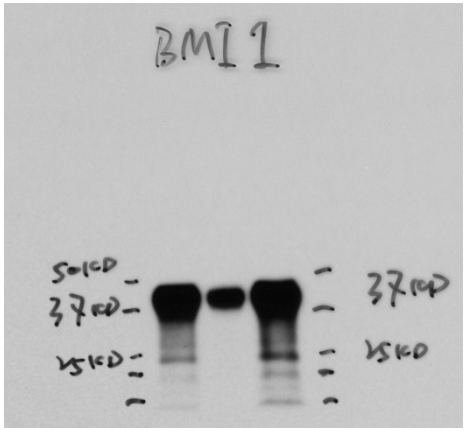
MUC1-C



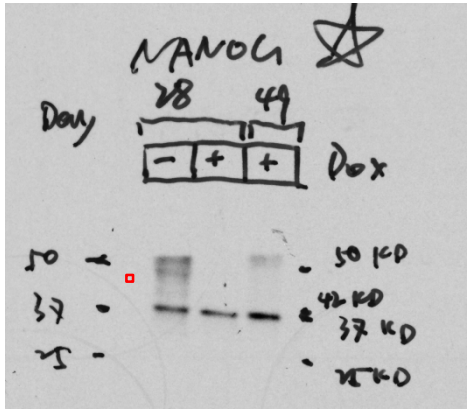
OCT4



SOX2



BMI1



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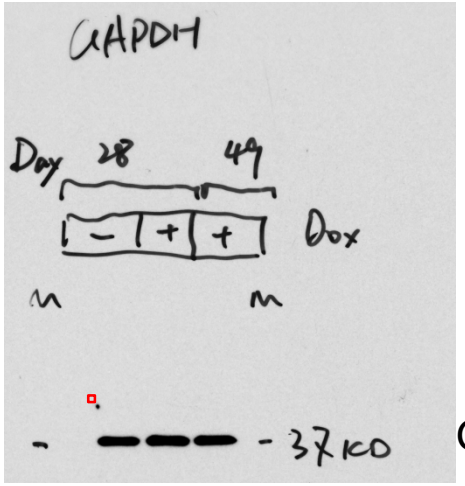

GAPDH