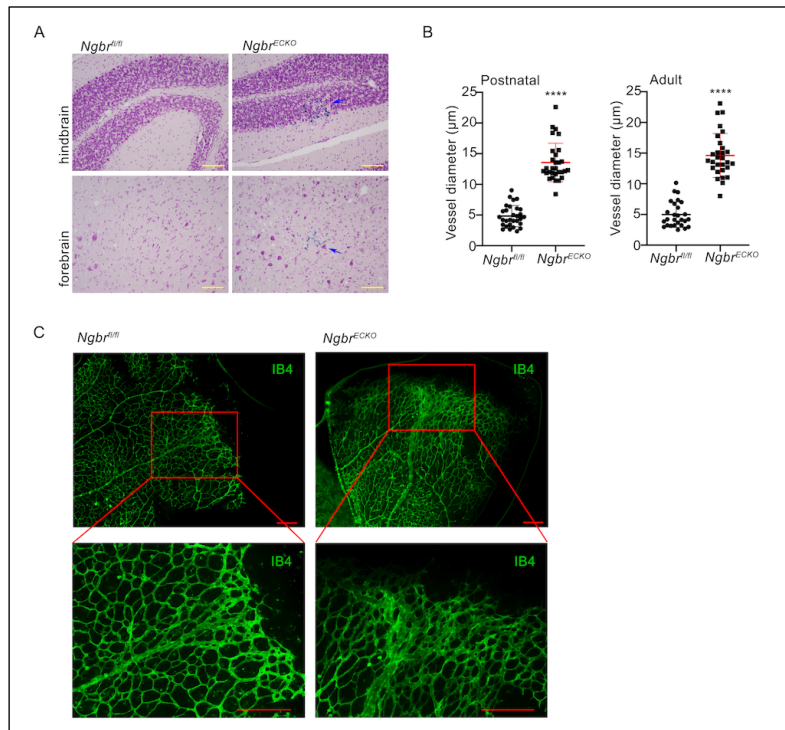
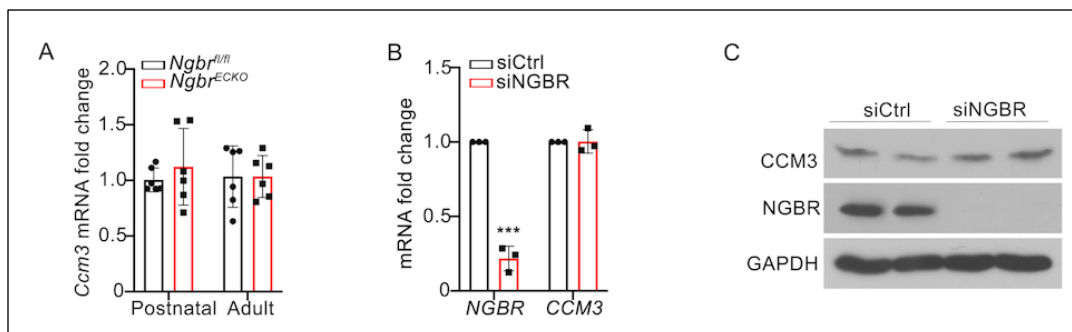


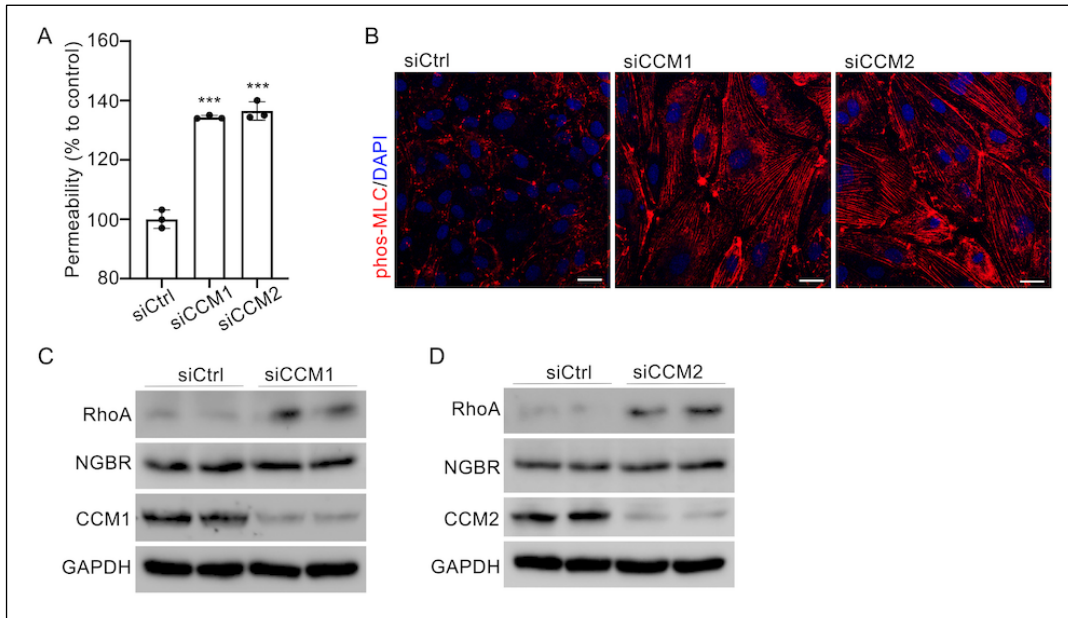
Supplementary figures of manuscript 15138-JCI-RG-RV-2



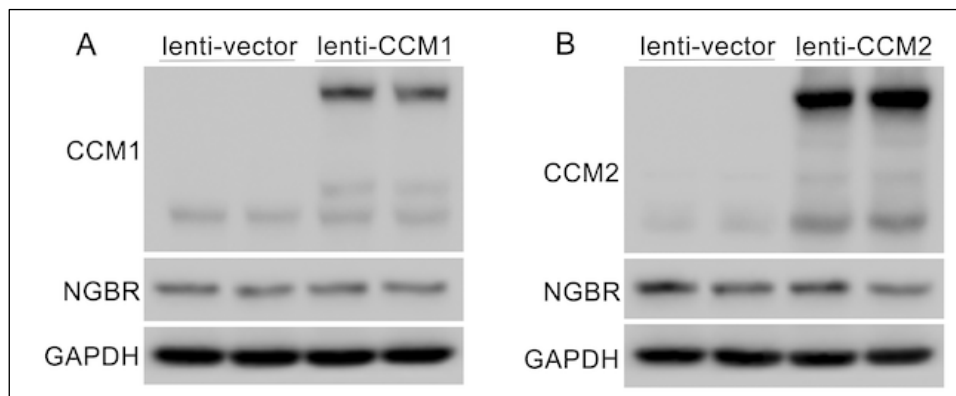
**Figure S1** A. Hemosiderin staining using Iron Stain Kit indicated stale hemorrhage in both forebrain and hindbrain of adult mice. Arrows pointed to the blue-stained positive sites. Scale bar: 50 μm. B. Microvessel diameter was calculated from IB4 staining images of *Ngbr<sup>fl/fl</sup>* and *Ngbr<sup>ECKO</sup>* mice. Results showed microvessel diameter significantly increased in *Ngbr<sup>ECKO</sup>* mice. Images of lesion sites from *Ngbr<sup>ECKO</sup>* group and similar sites from *Ngbr<sup>fl/fl</sup>* group were taken. Microvessels in 6 images randomly selected from 3 mice per group were measured. Data are presented as mean ± SD. Significance was tested by 2-tailed unpaired Student's t-test. \*\*\*\* $P < 0.0001$ . C. Retina were extracted from postnatal (P7) mice of both *Ngbr<sup>fl/fl</sup>* and *Ngbr<sup>ECKO</sup>* groups. ECs were stained with IB4 (green). Results showed that venous vessels in the retina of *Ngbr<sup>ECKO</sup>* group were enlarged. Scale bar: 200 μm.



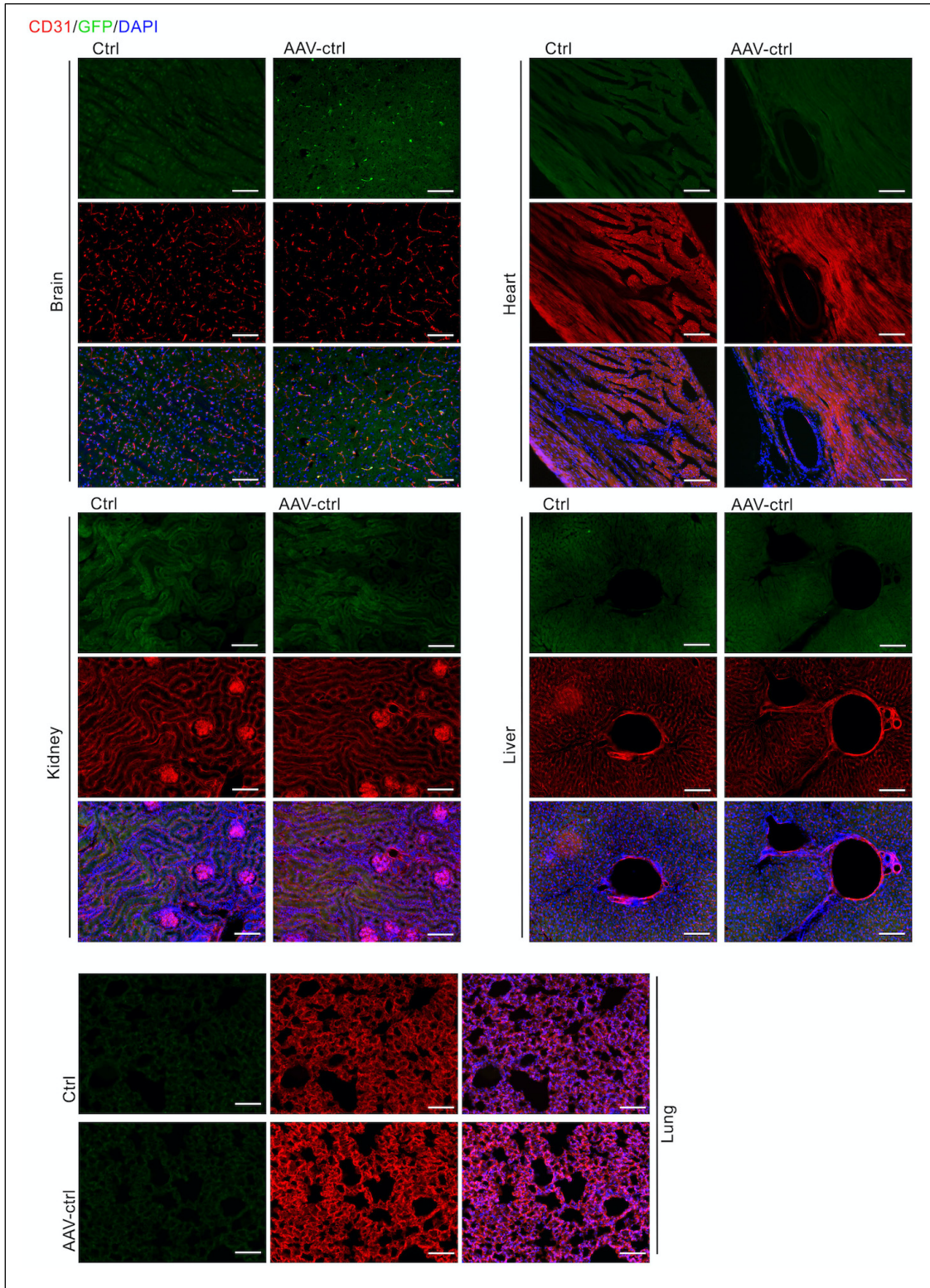
**Figure S2** A. *Ccm3* mRNA levels were detected in MBMVECs isolated from *Ngbr<sup>fl/fl</sup>* and *Ngbr<sup>ECKO</sup>* groups at both postnatal and adult stages. Results showed that the *Ccm3* mRNA level didn't change in *Ngbr<sup>ECKO</sup>* MBMVECs.  $n = 6$  mice per group. B-C. RT-qPCR and western blot showed no significant change of CCM3 expression in *NGBR* deficient HBMVECs. Data are presented as mean ± SD. Significance was tested by 2-tailed unpaired student's t-test. \*\*\* $P < 0.001$ ,  $n = 3$  samples per group.



**Figure S3** A. Knockdown of either *CCM1* or *CCM2* significantly increases endothelial permeability. Data are presented as mean  $\pm$  SD. Significance was tested by 2-tailed unpaired student's t-test. \*\*\* $P < 0.001$  vs. siCtrl group,  $n = 3$  samples per group. B-D. Immunofluorescent staining and western blot showed that knockdown of either *CCM1* or *CCM2* increases RhoA expression and phos-MLC signaling. Phos-MLC and RhoA expression was much higher in *CCM1* or *CCM2* knockdown group compared to siCtrl group. Scale bar: 20  $\mu\text{m}$ .

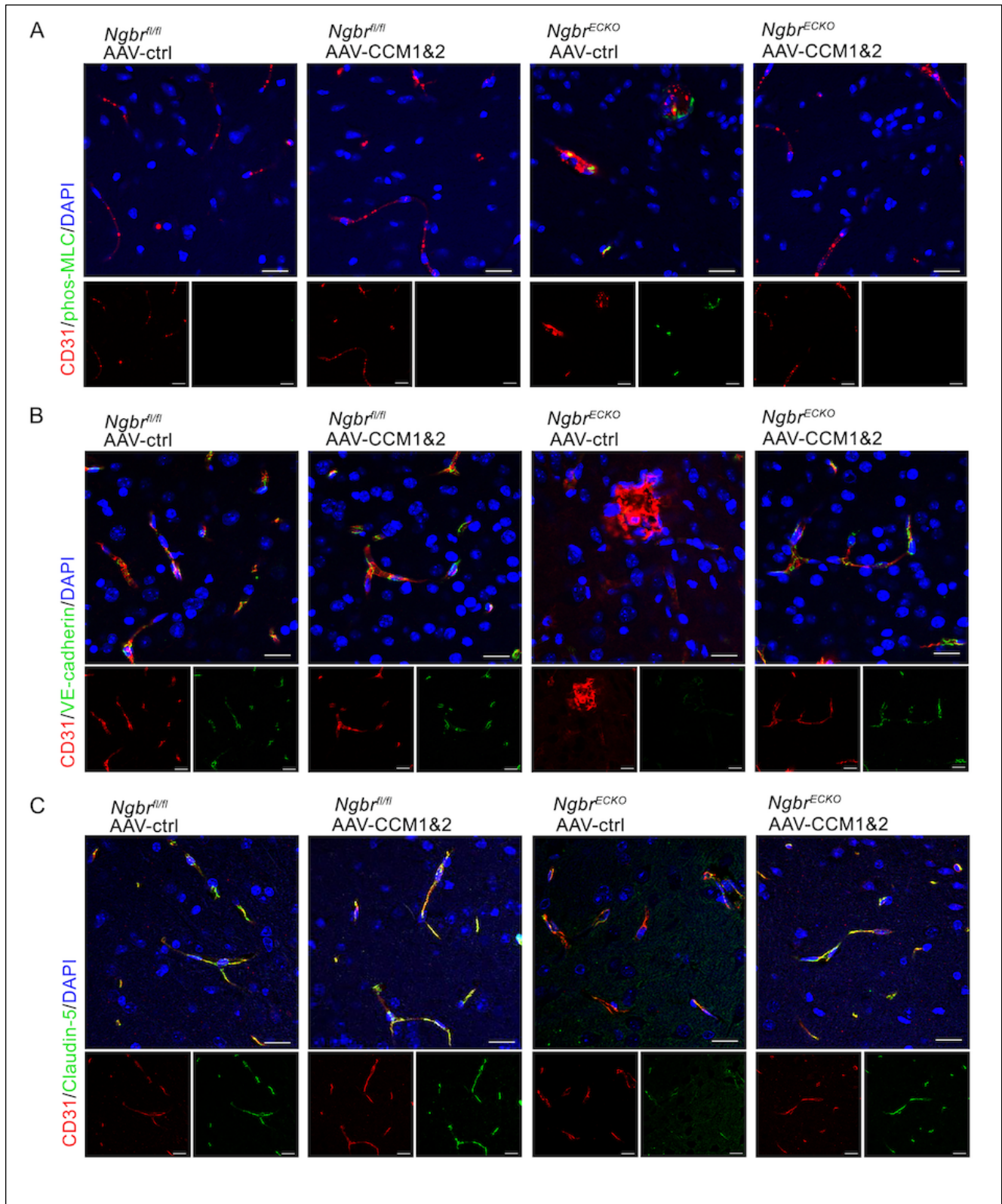


**Figure S4** A-B. Western blot showed that lentivirus transfection efficiency was sufficient. *CCM1* and *CCM2* were overexpressed in HBMVECs when transfected with lentivirus harboring *CCM1* or *CCM2* gene. As noted herein, NGBR protein levels had no change.



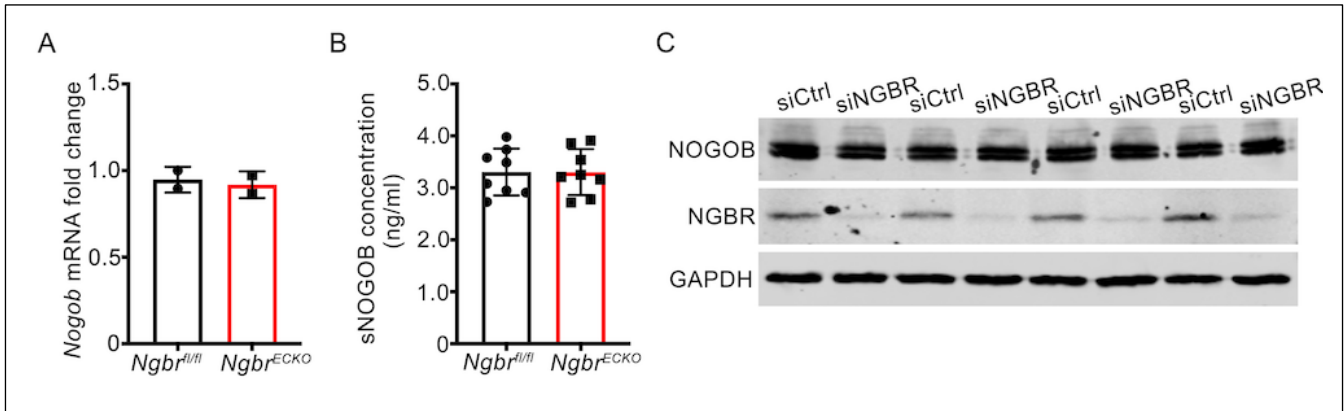
**Figure S5** Mice were injected with AAV-BR1-GFP (AAV-ctrl) and vehicle (Ctrl) via tail vein. Mice were euthanized and perfused by PBS two weeks later. Organs were obtained, fixed, embedded, sectioned, and immunofluorescent stained with CD31, GFP, and DAPI. Results showed that positive GFP staining presented in brain ECs but not in any ECs in the heart, kidney, liver, and lung. Scale bar: 50  $\mu$ m.



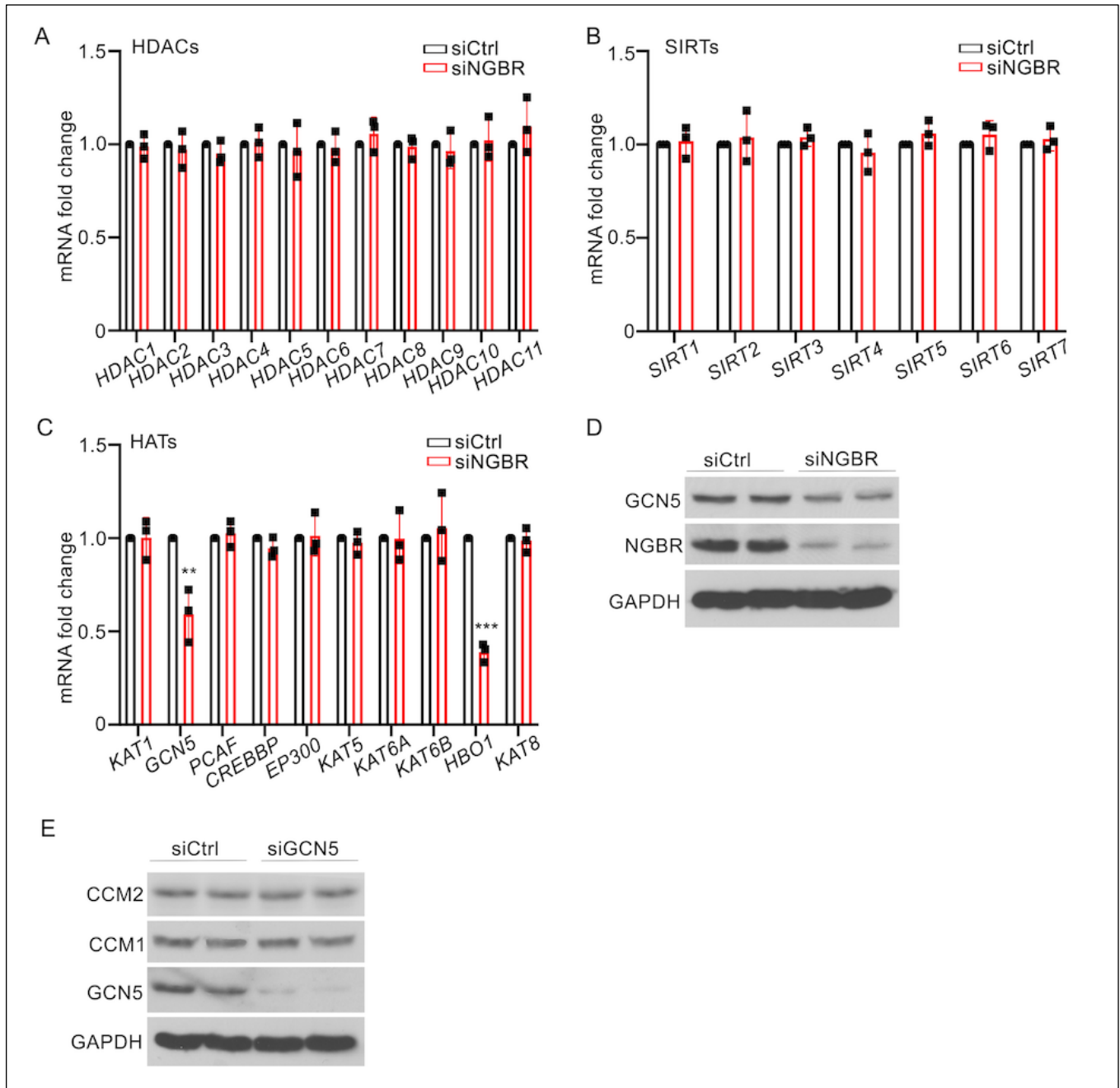


**Figure S6 A-C.** Immunofluorescent staining showed the reduction of phos-MLC (A), as well as restoration of AJs (VE-cadherin, B) and TJs (Claudin-5, C) protein levels in the brain ECs of *Ngbr<sup>ECKO</sup>* mice overexpressing *CCM1* and *CCM2*. Scale bar: 20  $\mu$ m.

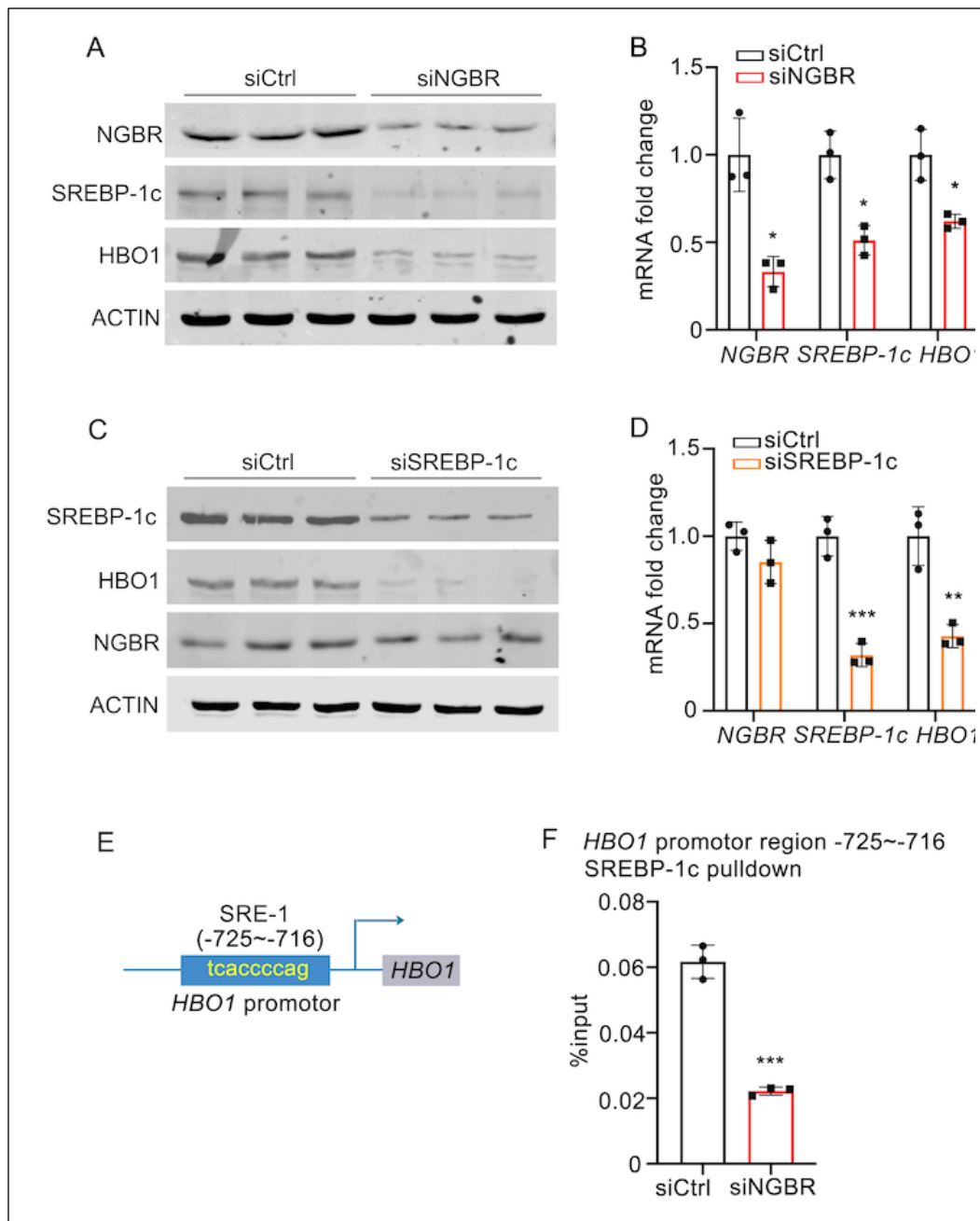




**Figure S7** A. *Nogob* mRNA level did not change in *Ngbr* deficient MBMVECs extracted from *Ngbr*<sup>ECKO</sup> mice. Each point represented an MBMVECs sample from 4 mouse brains. B. The concentration of sNOGOB was detected by ELISA. The levels of NOGOB in serum from *Ngbr*<sup>ECKO</sup> mice are the same as that of *Ngbr*<sup>fl/fl</sup> mice. Data are presented as mean  $\pm$  SD. Significance was tested by 2-tailed unpaired student's t-test. No significant differences were detected. C. NOGOB expression in HBMVECs was detected by western blot. NOGOB expression remained the same under *NGBR* siRNA treatment.

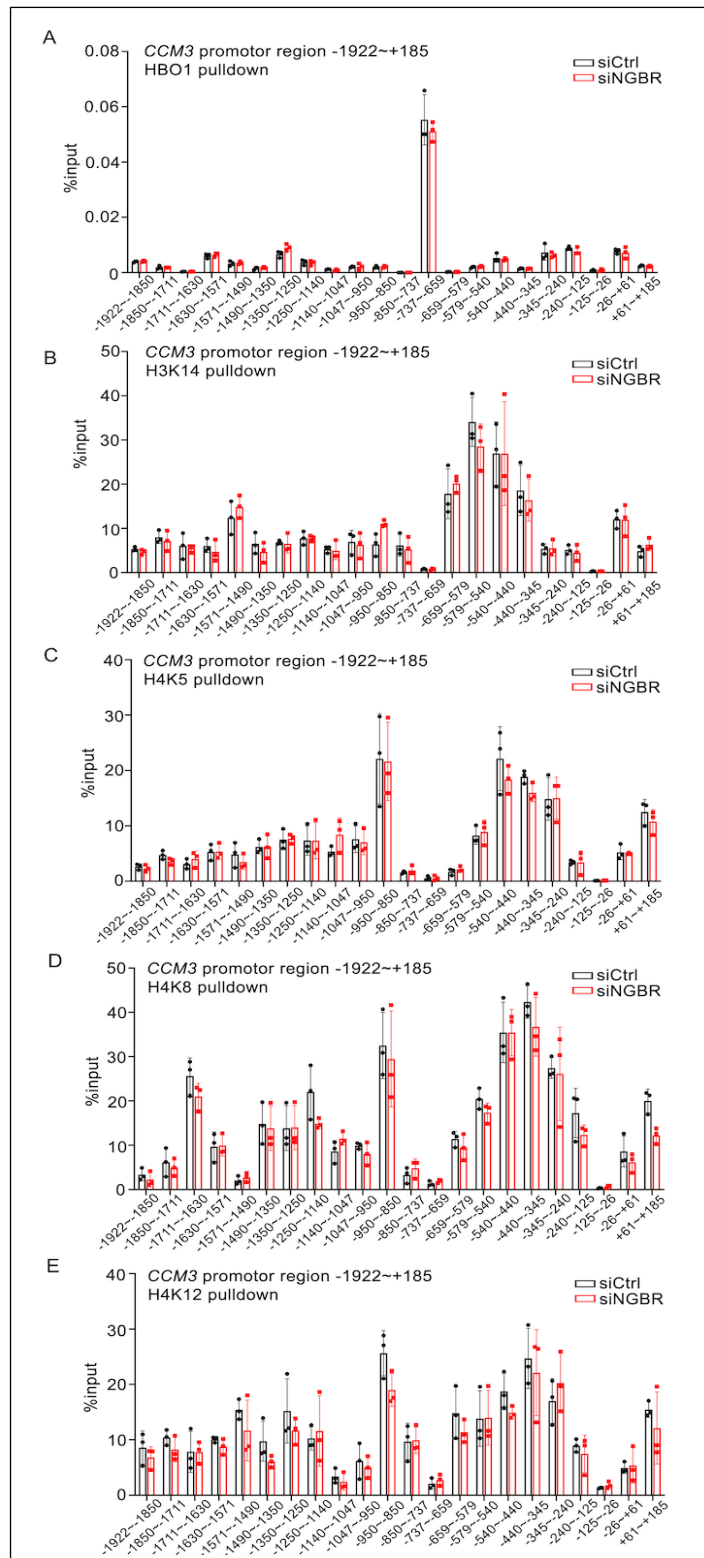


**Figure S8** A-C. HDACs, SIRTs, and HATs expression in RNA-seq data (siNGBR group vs. siCtrl group) were confirmed by RT-qPCR. The results showed that the mRNA level of *HBO1* decreased dramatically, and the mRNA level of *GCN5* also decreased. Data are presented as mean  $\pm$  SD. Significance was tested by 2-tailed unpaired student's t-test. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .  $n = 3$  samples per group. D-E. Western blot results showed *GCN5* decreased in *NGBR* deficient HBMVECs while *GCN5* siRNA treatment didn't show any influence on *CCM1* and *CCM2* expression in HBMVECs even though the knockdown efficiency was sufficient.

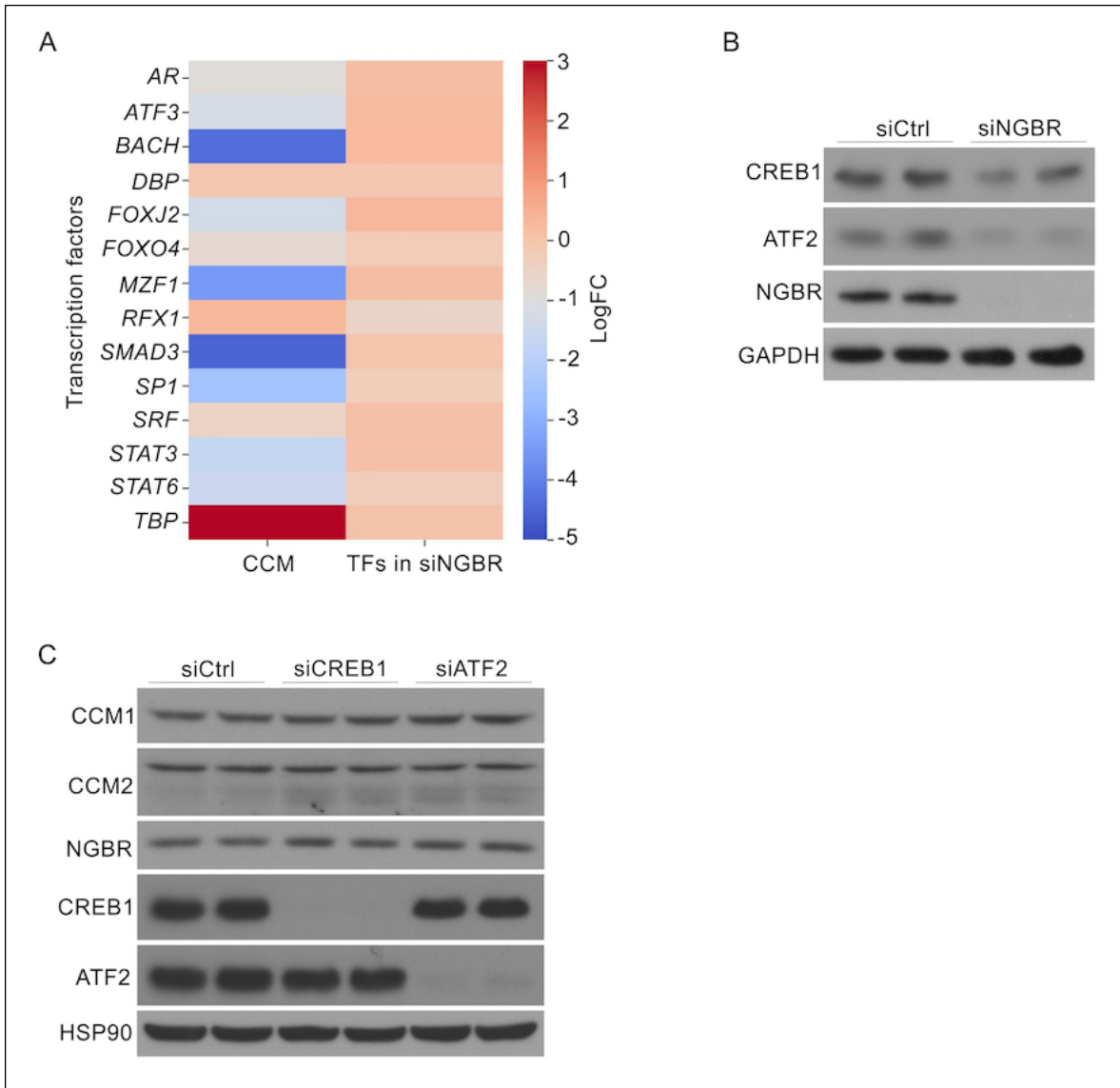


**Figure S9** A-B. Both protein and mRNA levels of SREBP-1c and HBO1 were decreased in *NGBR* knockdown HBMVECs. Data are presented as mean  $\pm$  SD. Significance was tested by 2-tailed unpaired student's t-test.  $*P < 0.05$ ,  $n = 3$  samples per group. C-D. HBO1 protein and mRNA levels were reduced in *SREBP-1c* knockdown HBMVECs. As noted herein, *NGBR* expression did not change. Data are presented as mean  $\pm$  SD. Significance was tested by 2-tailed unpaired student's t-test.  $**P < 0.01$ ,  $***P < 0.001$ ,  $n = 3$  samples per group. E. The binding site of SREBP-1c (SRE-1) on the *HBO1* gene promoter region. F. ChIP-qPCR assays were performed using HBMVECs treated with either control siRNA or *NGBR* siRNA. Chromatin DNA was pulled down using an antibody of SREBP-1c. Results showed that the binding of SREBP-1c on the *HBO1* promoter region (-725~-716) was significantly decreased in *NGBR* deficient HBMVECs. Data are presented as mean  $\pm$  SD. Significance was tested by 2-tailed unpaired student's t-test.  $***P < 0.001$ ,  $n = 3$  samples per group.





**Figure S10 A-E.** ChIP-qPCR assays were performed using HBMVECs treated with either control siRNA or *NGBR* siRNA. Chromatin DNA was pulled down using antibodies of HBO1, acetylated H3K14, H4K5, H4K8, and H4K12. Chromatin DNA pulled down using IgG and H3 was performed as the negative and positive control (data not shown). Results showed that *NGBR* knockdown did not affect the binding of HBO1, acetylated H3K14, and acetylated H4K5/K8/K12 on the promoter region of the *CCM3* gene. Data are presented as mean  $\pm$  SD. Significance was tested by 2-tailed unpaired student's t-test.



**Figure S11** A. GSEA analysis of siNGBR RNA sequencing data utilizing transcription factor geneset generated report was performed. Log fold change was compared among significantly enriched transcription factors ( $P < 0.05$ , FDR  $q$ -value $<0.25$ ). Comparison between the CCM group and the transcription factors enriched in the NGBR knockdown experimental group does not show a direct correlation. B. Transcription factors CREB1 and ATF2 downregulated in *NGBR* deficient HBMVECs. C. The knockdown of either *CREB1* or *ATF2* does not affect CCM1 and CCM2 expression.

**Supplementary Table 1: Chemicals, plasmids, antibodies and siRNAs used in the study**

<b>Name</b>	<b>Cat#</b>	<b>Company</b>
Tamoxifen	T5648	Sigma-Aldrich
Evans Blue	E2129	Sigma-Aldrich
FITC-conjugated dextran	FD2000S	Sigma-Aldrich
FITC-conjugated dextran	FD40S	Sigma-Aldrich
Hematoxylin	MHS16	Sigma-Aldrich
alcoholic eosin Y	1024390500	Sigma-Aldrich
VECTASTAIN® Elite ABC-HRP Kit, Peroxidase (Mouse IgG)	PK-6102	Vector Laboratory
Iron Stain Kit	HT20-1KT	Sigma-Aldrich
TritonX-100	10789704001	Sigma-Aldrich
Tween-20	P1379	Sigma-Aldrich
Paraformaldehyde	158127	Sigma-Aldrich
collagenase II	17101015	Thermo Fisher Scientific
DNase I	10104159001	Sigma-Aldrich
Percoll	P1644	Sigma-Aldrich
collagenase/dispase	SCR139	Sigma-Aldrich
SimpleChIP (R) Plus Kits	9004&9005	Cell Signaling
<b>plasmids</b>		
psPAX2	#12260	Addgene
pVSV-G	#12259	Addgene
pWPXLD	#12258	Addgene
<b>antibodies</b>		
Isolectin B4	I21412	Thermo Fisher Scientific
DAPI	D9542	Sigma-Aldrich
CD31 antibody	AP436PU-N	Acris
CD31 antibody	550274	BD pharmacy
phos-MLC antibody	3671S	Cell Signaling
VE-cadherin antibody	550548	BD pharmacy
VE-cadherin antibody	2500S	Cell Signaling
Claudin-5 antibody	34-1600	Thermo Fisher Scientific
ZO-1 antibody	40-2200	Thermo Fisher Scientific
GFP antibody	50430-2-AP	Proteintech
GFP antibody	GFP-1010	Aves
RhoA antibody	2117S	Cell Signaling
NGBR antibody	ab168351	Abcam
GAPDH antibody	60004-1-Ig	Proteintech
ACTIN antibody	66009-1-Ig	Proteintech
CCM1 antibody	ab196025	Abcam



CCM2 antibody	26270-1-AP	Proteintech
CCM3 antibody	66440-1-Ig	Proteintech
HBO1 antibody	Ab70183	Abcam
HBO1 antibody	58418	Cell Signaling
GCN5 antibody	3305S	Cell Signaling
Histone H3 (D1H2) XP® Rabbit mAb	4499	Cell Signaling
Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb	9649	Cell Signaling
Acetyl-Histone H3 (Lys14) (D4B9) Rabbit mAb	7627	Cell Signaling
Acetyl-Histone H3 (Lys18) (D8Z5H) Rabbit mAb	13998	Cell Signaling
Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb	8173	Cell Signaling
Histone H4 (L64C1) Mouse mAb	2935	Cell Signaling
Acetyl-Histone H4 (Lys5) (D12B3) Rabbit mAb	8647	Cell Signaling
Acetyl-Histone H4 (Lys8) Antibody	2594	Cell Signaling
Acetyl-Histone H4 (Lys12) (D2W6O) Rabbit mAb	13944	Cell Signaling
SREBP-1c antibody	ab28481	Abcam
<b>siRNAs</b>		
<i>CCM1</i> siRNA	sc-43884	Santa Cruz Biotechnology
<i>CCM2</i> siRNA	sc-62594	Santa Cruz Biotechnology
<i>HBO1</i> siRNA	forward sequence: GGCUAAGCCAGAGUUCUCA; reverse sequence: UGAGAACUCUGGCUUAGCC	Generated from IDT
<i>GCN5</i> siRNA	sc-37946	Santa Cruz Biotechnology
<i>NGBR</i> siRNA	forward sequence: GGAAAUACAUAGACCUACA; reverse sequence: UGUAGGUCUAUGUAUUUCC	Generated from IDT

<i>SREBP-1c</i> siRNA	sc-36557	Santa Cruz Biotechnology
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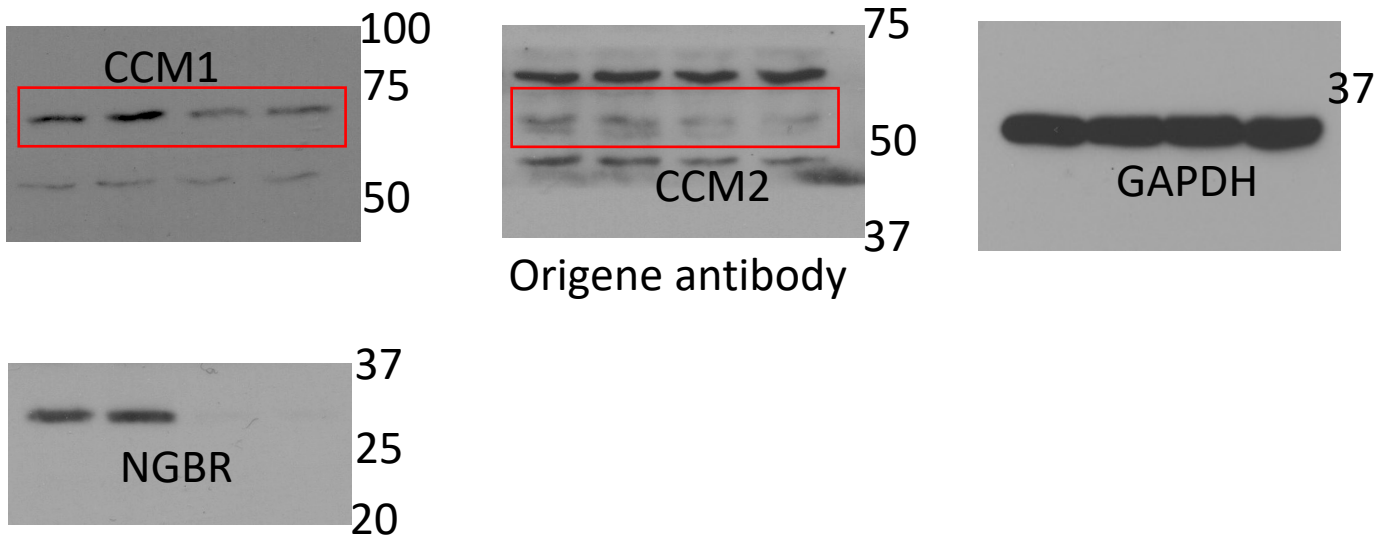
**Supplementary Table 2: Oligonucleotide sequences of primers used**

<b>RT-PCR primers</b>	Forward (5'-3')	Reverse (5'-3')
<i>Ngbr</i> (mouse)	TCCTACATTAGCGTCTACGACC	GCTCTCACAAATATCCGCTTTTCC
<i>NGBR</i> (human)	TGCCAGTGAGATGCCCAGAAGCAA	TGATGTGCCAGGGAAGAAAGCCTA
<i>Ccm1</i> (mouse)	GAAAGACGCCATTAACAAGCC	CCGCATTCCCTCCATTATCTG
<i>Ccm2</i> (mouse)	AGAAAGCCCATGAGAAGGTG	CGGGAATGGATGTGAACTGACC
<i>CCM1</i> (human)	CTGTAAGAACATGCGCTGAAG	TCCATCGTACCTGTTACCAAAC
<i>CCM2</i> (human)	AGAAAGCCCATGAGAAGGTG	CCTGGTATGGACGTTAACTGAC
<i>Hbo1</i> (mouse)	CCCGCTGTATCATAACCTCTC	AGCCACCTTTTCCTTATACCG
<i>HBO1</i> (human)	AGCCCTTCCTGTTCTATGTTATG	CATAGCCCTGTCTCATGTACTG
<i>GAPDH</i> (human)	TGGACAGTCAGCCGCATCTTCTTT	ACCAAATCCGTTGACTCCGACCTT
<i>Gapdh</i> (mouse)	CTGGAGAAACCTGCCAAGTA	TGTTGCTGTAGCCGTATTCA
<i>ACTIN</i> (human)	TTCTACAATGAGCTGCGTGTGGCT	TAGCACAGCCTGGATAGCAACGTA
<i>Actin</i> (mouse)	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
<i>HDAC1</i> (human)	GAGATGACCAAGTACCACAGC	TGACAGAACTCAAACAGGCC
<i>HDAC2</i> (human)	TGACAAACCAGAACACTCCAG	CTTCTCCATCTTCATCTCCACTG
<i>HDAC3</i> (human)	GGACTTCTACCAACCCACG	CAGCACGAGTAGAGGGATATTG
<i>HDAC4</i> (human)	ACAAGGAGAAGGGCAAAGAG	GCGTTTTCCCGTACCAGTAG
<i>HDAC5</i> (human)	TCACCGCAAACTCCTACAG	AGTTCCCGTTGTCATAGCG
<i>HDAC6</i> (human)	TTCAACTCTGTGGCTGTGG	GCAGGGACACATATAGCACAC
<i>HDAC7</i> (human)	GCAGATCATTCAACAGCCATG	TTGGTAGAAGGTTTGCTGGG
<i>HDAC8</i> (human)	AATTAActGGTCTGGAGGTG	TGCAGATCCAATCCACGTAG
<i>HDAC9</i> (human)	ACACATTACCAGGAGCACAAG	CAACATTTCCATCCTTCCGC
<i>HDAC10</i> (human)	AGAAACACGGGCTACACAG	GGTGCCAGGAGAAGTAAAGG
<i>HDAC11</i> (human)	GTTTCTGTTTGAGCGTGTGG	GGTAGATGTGGCGGTTGTAG
<i>SIRT1</i> (human)	CCCTCAAAGTAAGACCAGTAGC	CACAGTCTCCAAGAAGCTCTAC
<i>SIRT2</i> (human)	ACCTTCTACACATCACACTGC	GACGATATCAGGCTTCACCAG
<i>SIRT3</i> (human)	TCATGGAACCTTTGCCTCTG	GCTCCCCAAAGAACACAATG
<i>SIRT4</i> (human)	TCGGAAAGCTGTAActGGTTG	TCTGTTCCCACAATCCAAG
<i>SIRT5</i> (human)	GCCTCCCGCAGAATTGGTA	AGAGGTCGCATCAGGGTTTG
<i>SIRT6</i> (human)	AGGATGTCGGTGAATTACGC	GAAGACTGCCAGACCAGC
<i>SIRT7</i> (human)	AGAAAGGGAGAAGCGTTAGTG	GAGCCCGTCACAGTTCTG
<i>KAT1</i> (human)	ACTCCATTTCAAGGTCAAGGC	CTTCACAAGCACAAAGTCTCG
<i>GCN5</i> (human)	TCTCTACTTCCCTCACCTACGC	ATTCAGCTCACACTCCATCAG
<i>PCAF</i> (human)	GAAGAGAACAGAAGCTCCAGG	GCAATTGGTAAAGACTCGCTG
<i>CREBBP</i> (human)	CAACCCCAAAAGAGCCAAAC	GGTTCCCACTGTTTAAAGGC
<i>EP300</i> (human)	GACCAGACTACAGAAGCAGAAC	ACTGCCACGGATCATACTTG
<i>KAT5</i> (human)	CATCGTGGGCTACTTCTCC	CCTGTTTTCCCTTCCACTTTG
<i>KAT6A</i> (human)	ACATCACTTCCACACTCCAC	CATCTACAGGTCGCAAATTCAG
<i>KAT6B</i> (human)	GACAAACAGAGGAAGAGGAAGG	TCGGGATTGTCTTTACTGCC
<i>KAT8</i> (human)	CAAGATCACTCGCAACCAAAAG	TGTCCACATACTTCACCTTGG
<i>SREBP-1c</i> (human)	CAACACAGCAACCAGAACTC	CTCCACCTCAGTCTTCACG
<b>ChIP assay primers</b>	Forward (5'-3')	Reverse (5'-3')
<i>CCM1</i> promotor	ATACAGGGGAGCGCTCCATTC	TAAAAGTGCTCTGCAGGGCTG
<i>CCM2</i> promotor	CTTGcAGTGAGCCGAGATC	GCACAGCTAGAATGTAAACTGTG

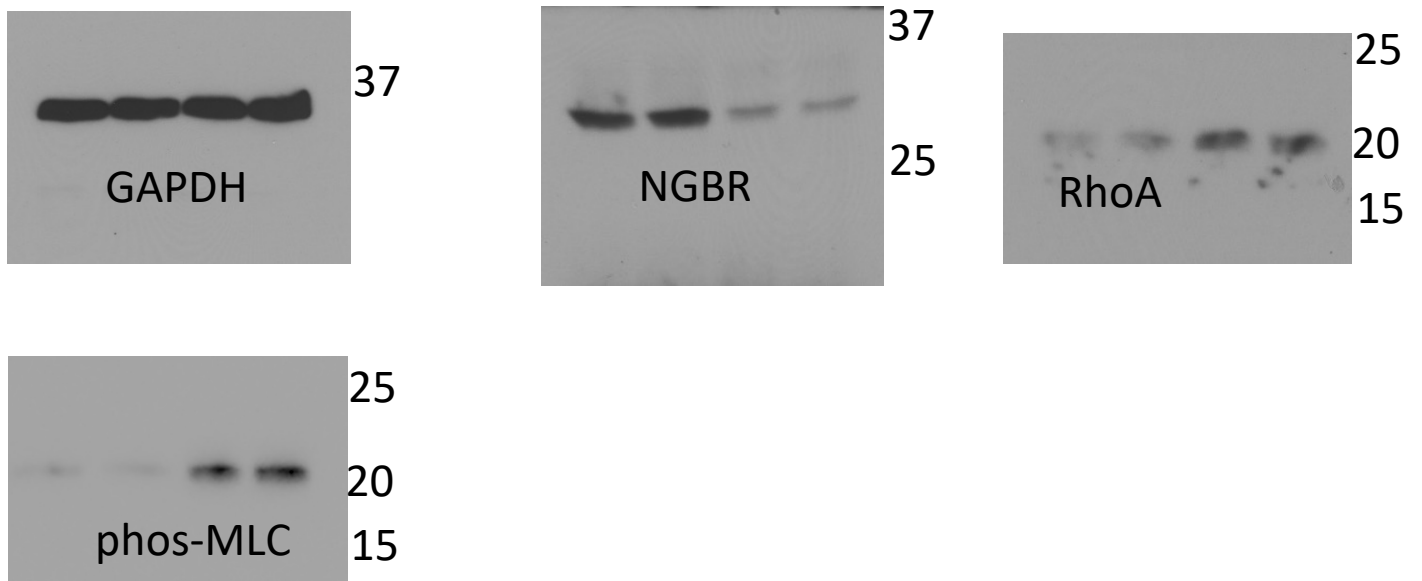


<i>HBO1</i> promotor	GTACTGCATTCCCCACTTC	CCATTCTCCAGAAGCTGCAG
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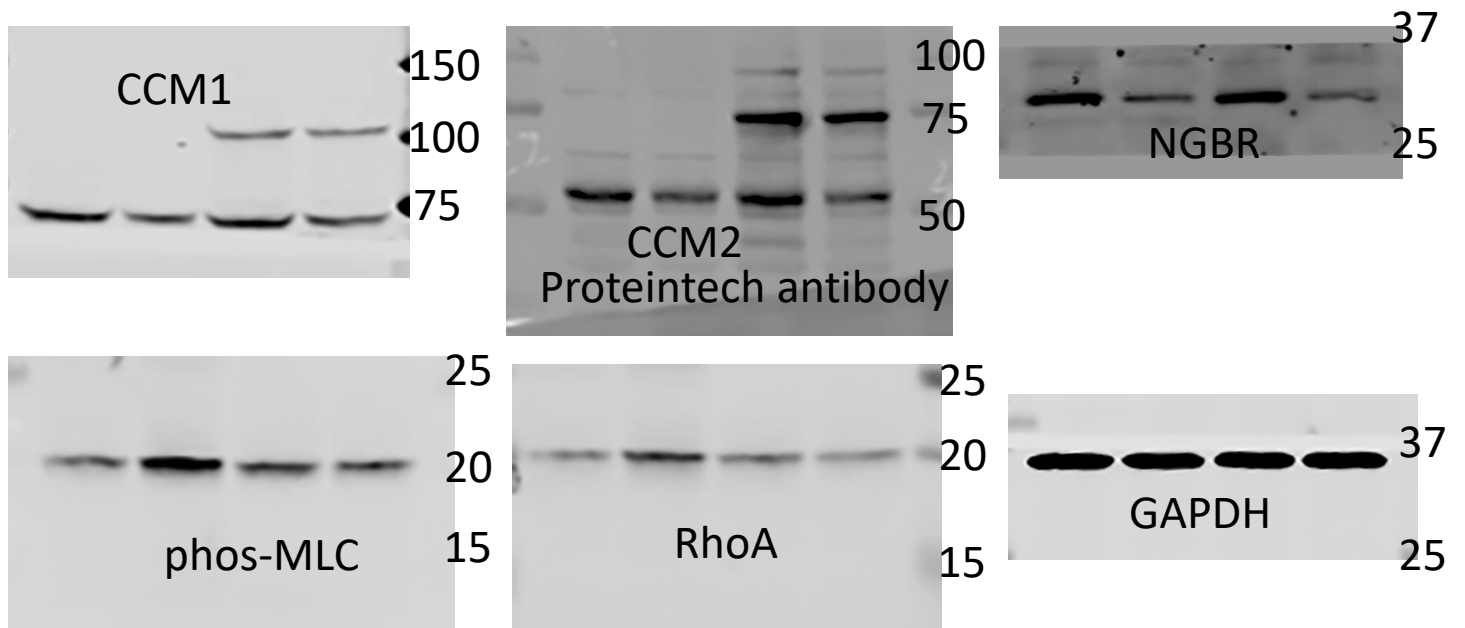
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Full unedited gel for Figure 3H

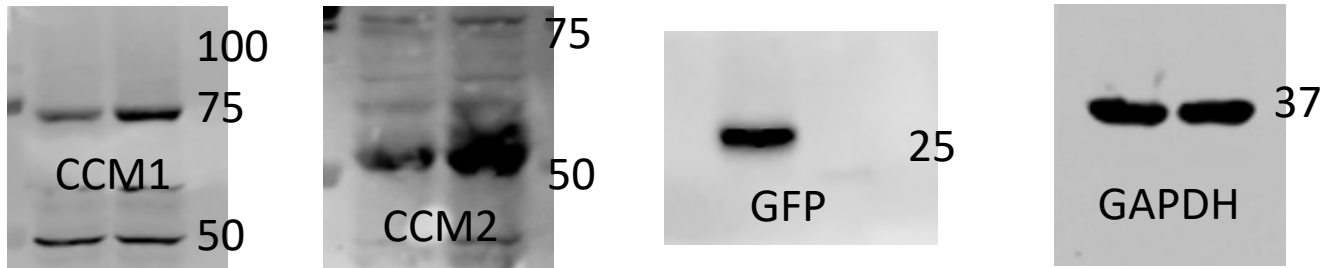


Full unedited gel for Figure 4D

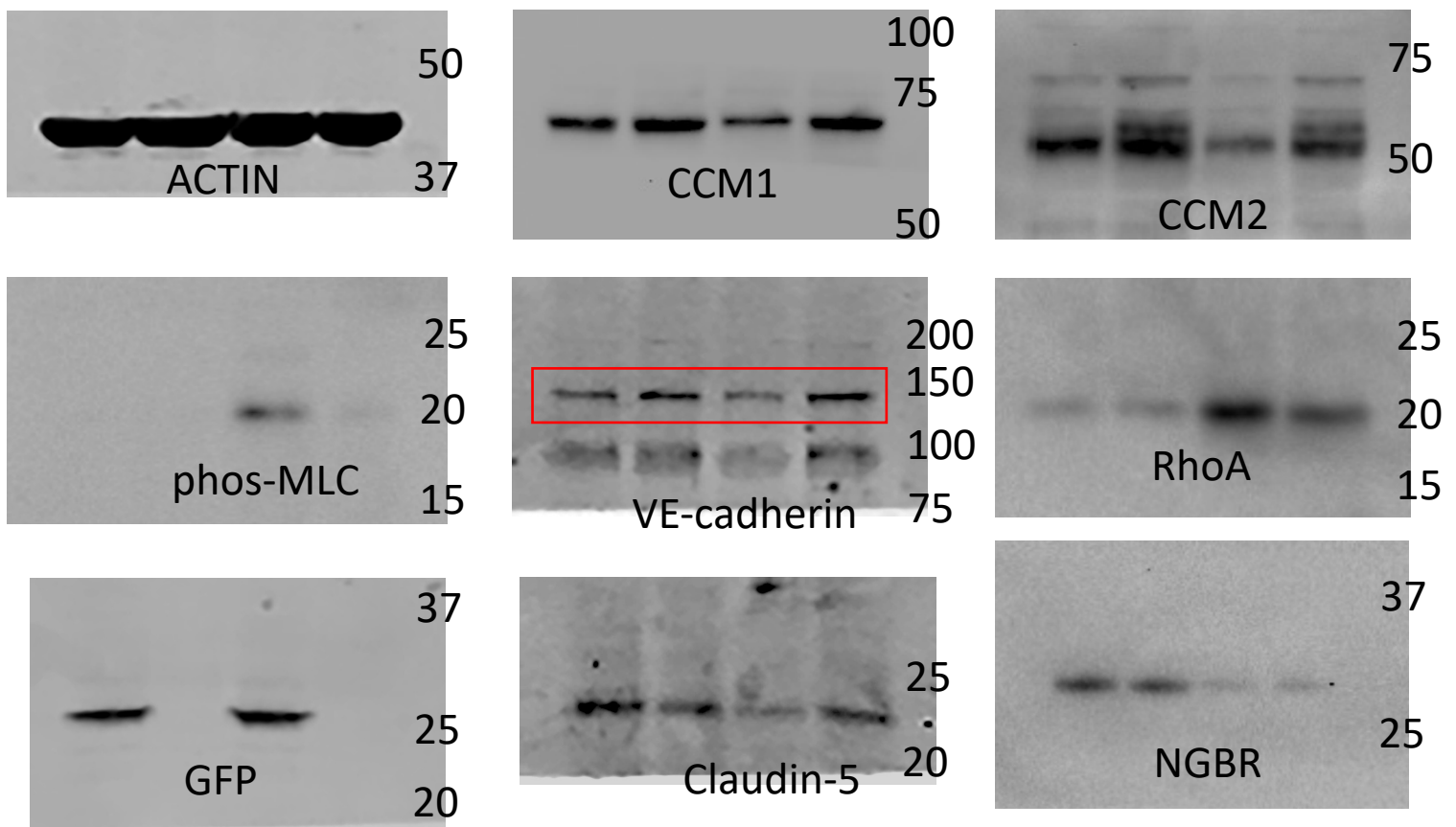




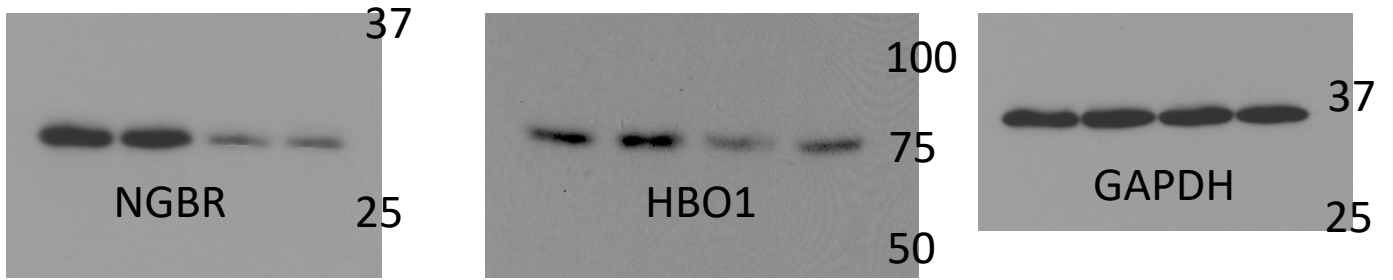
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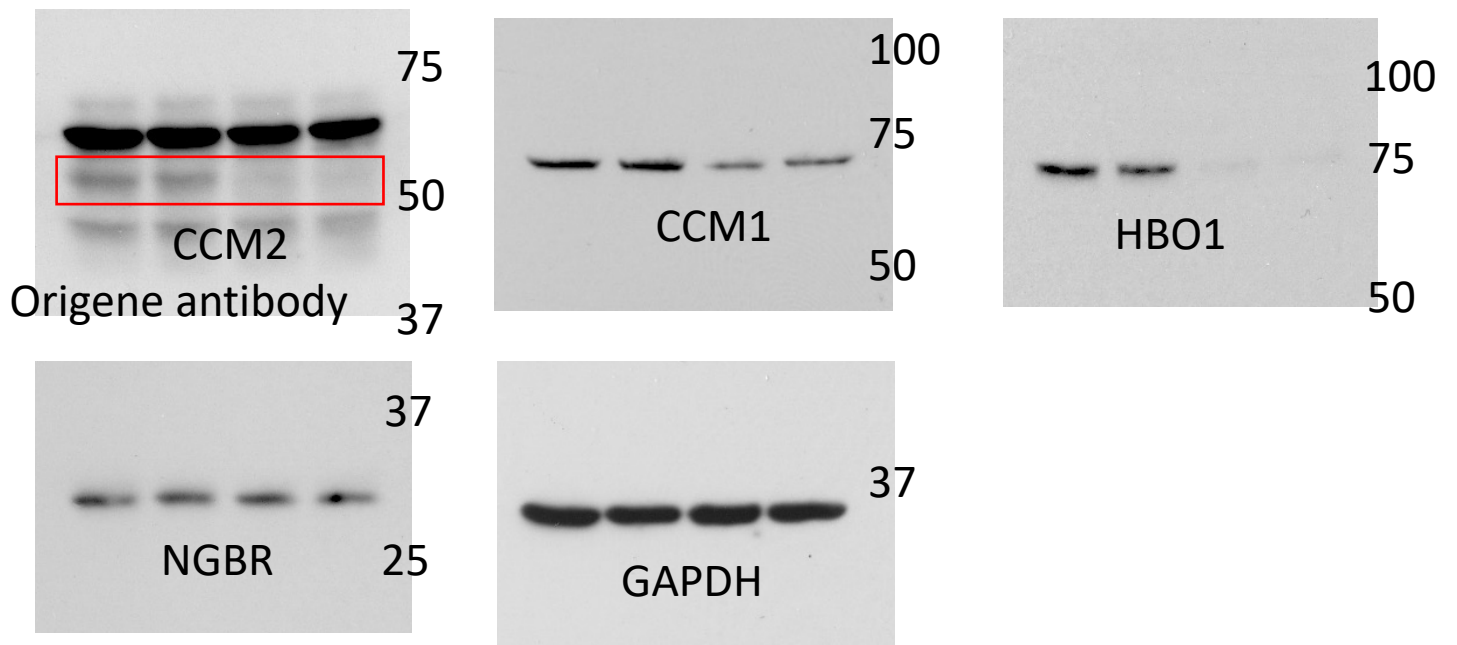
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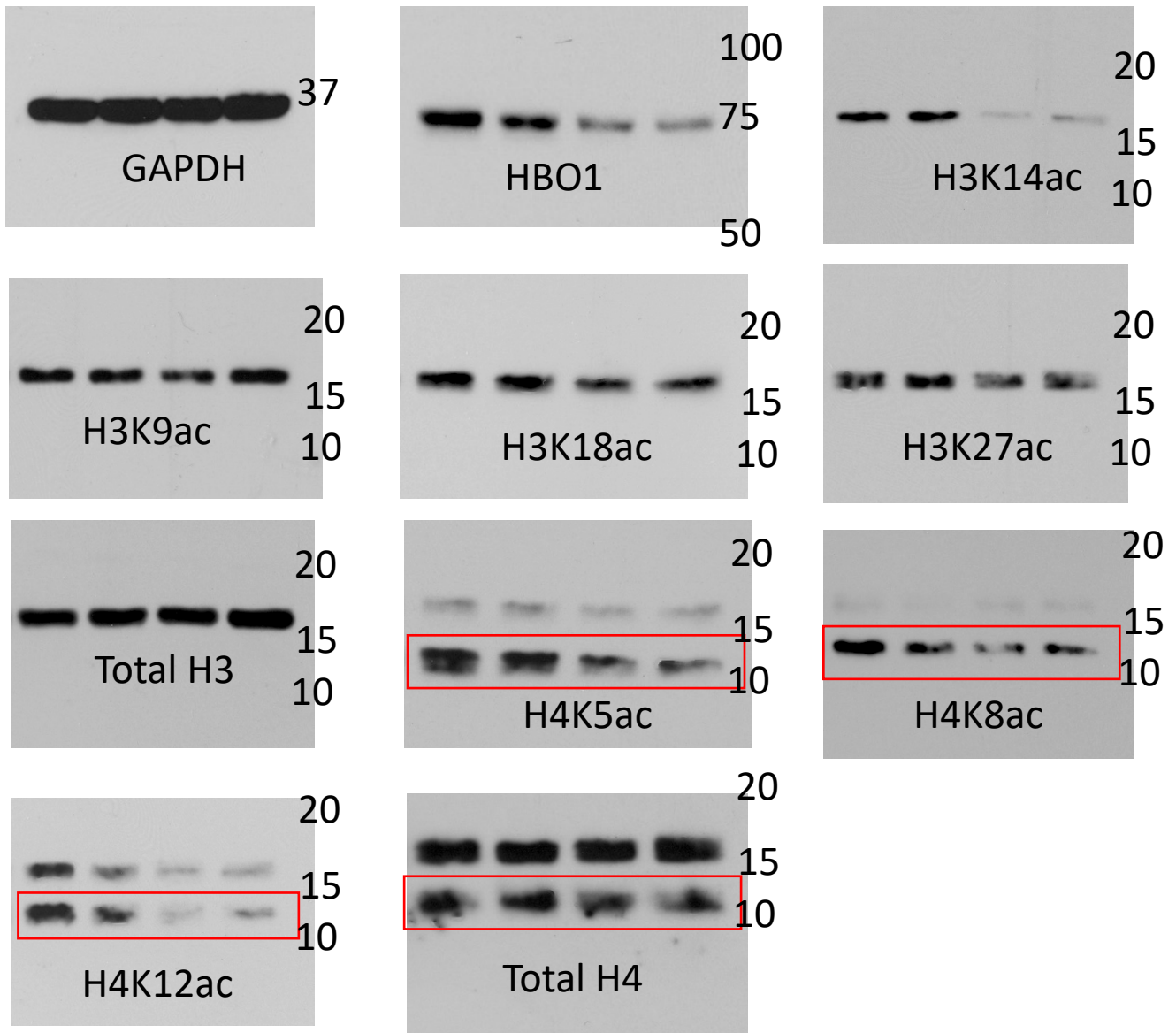
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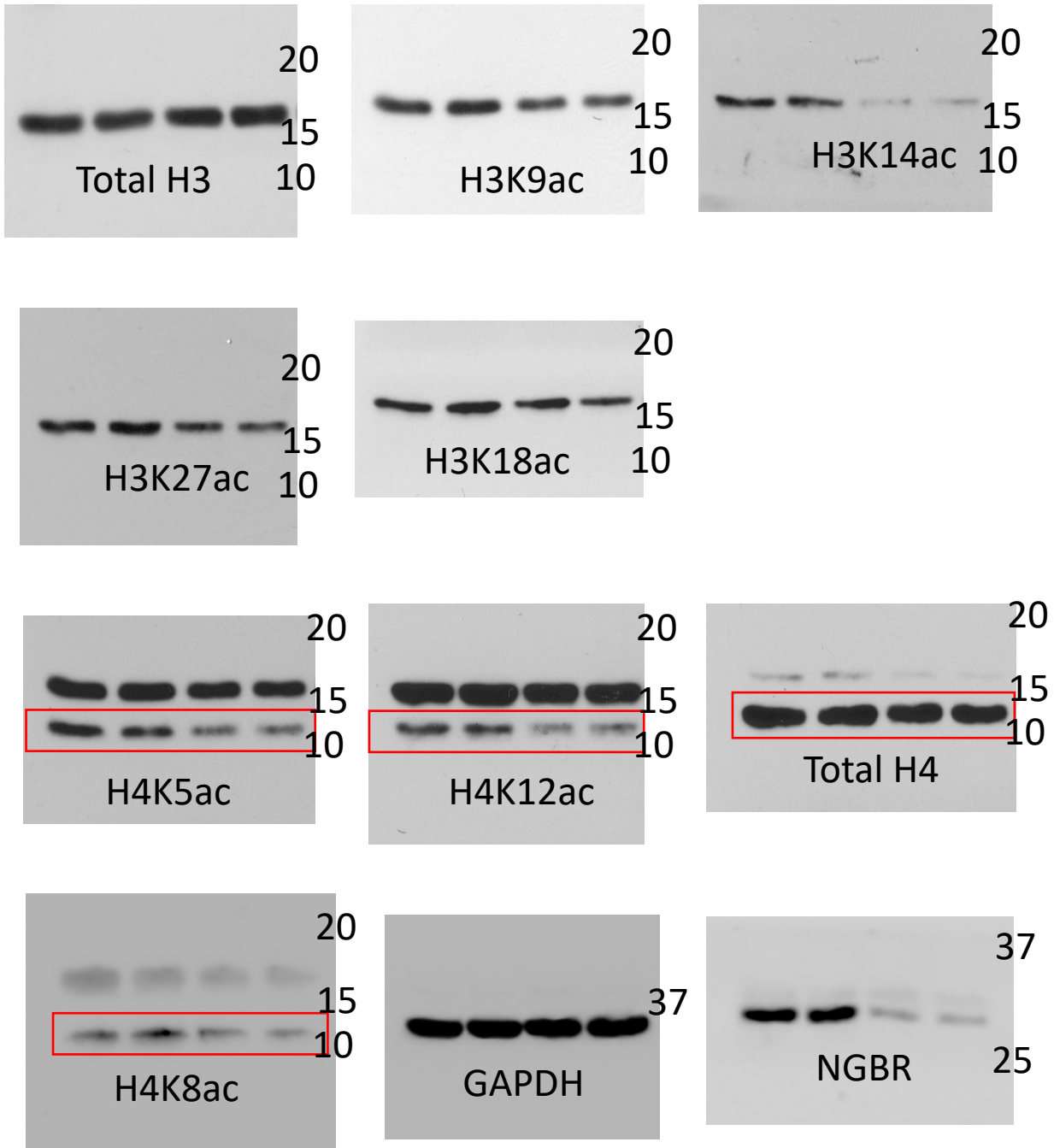
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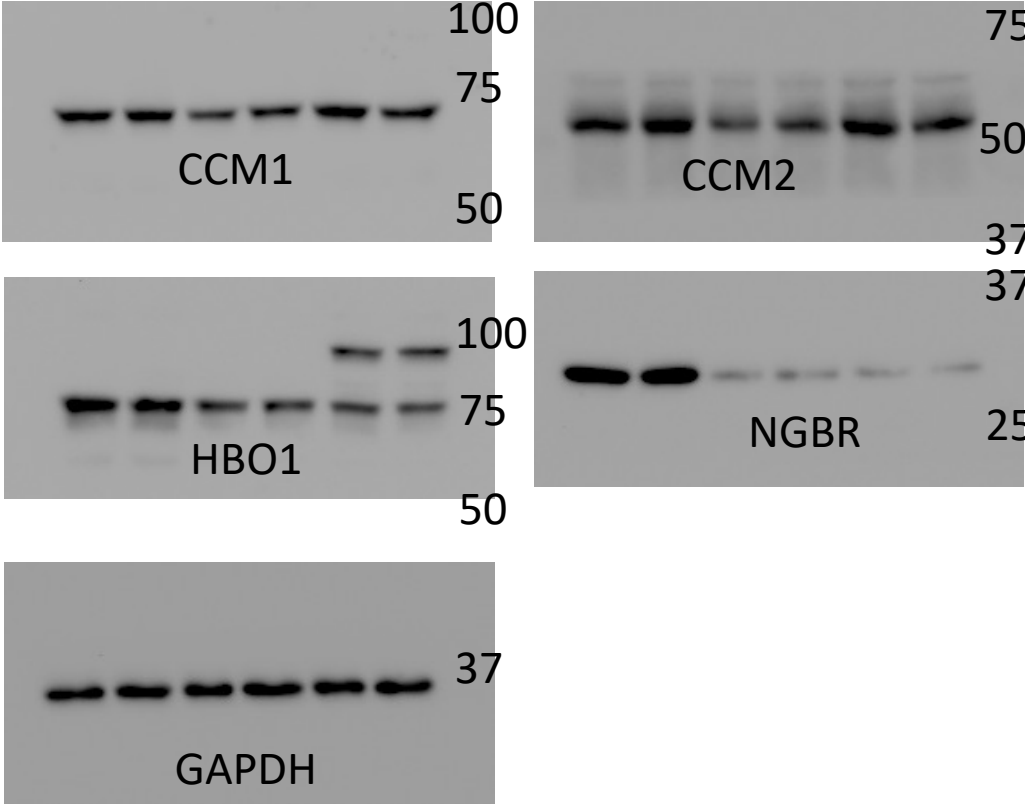
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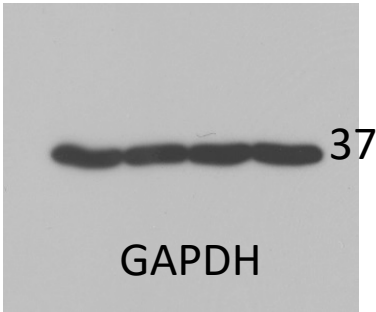
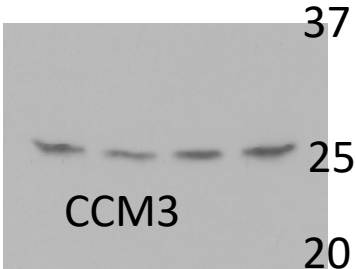
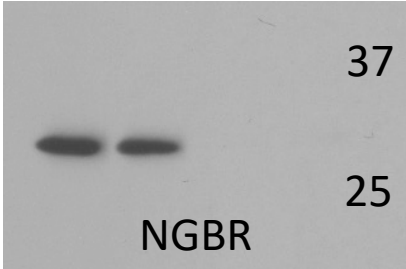


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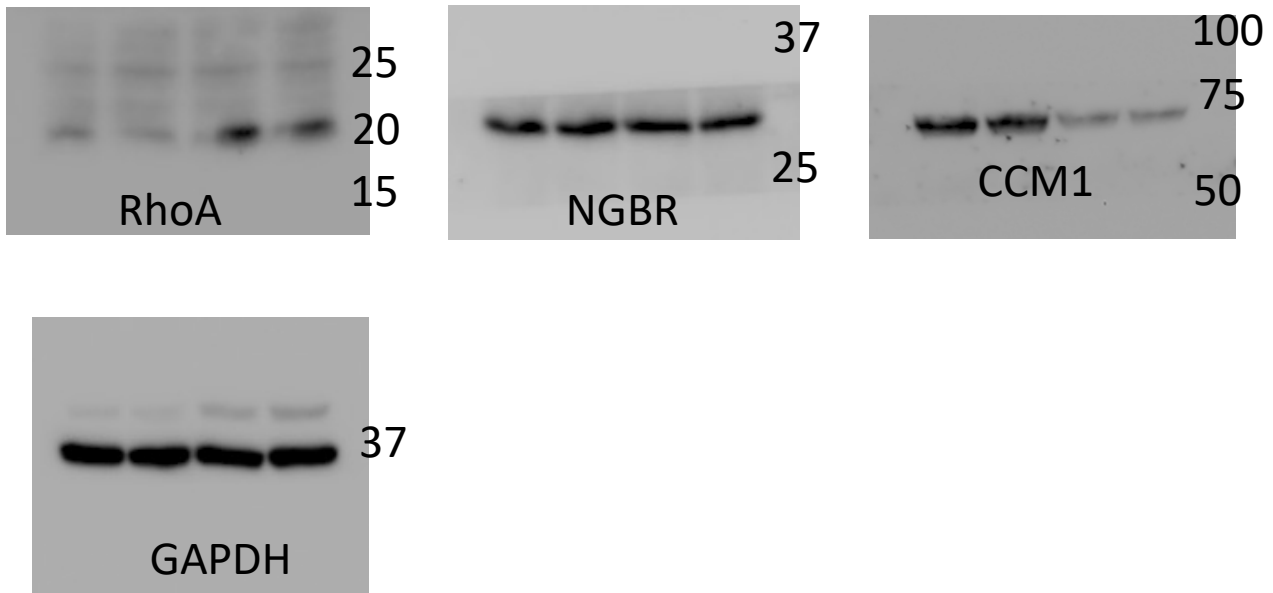




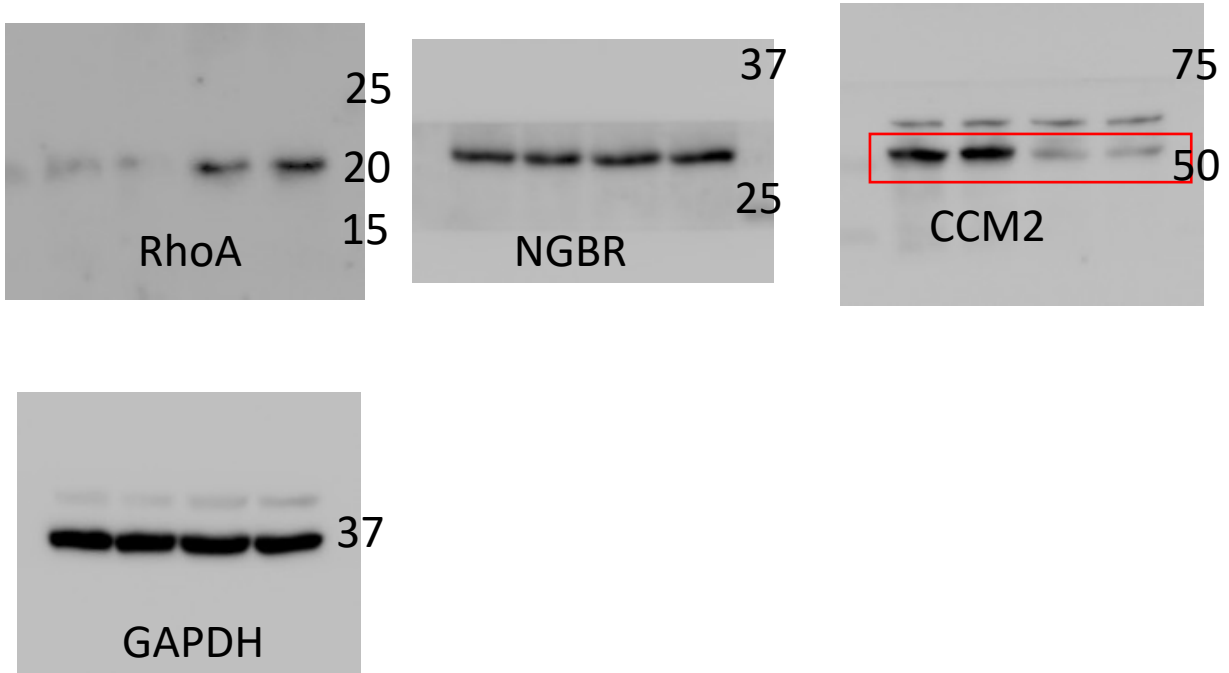
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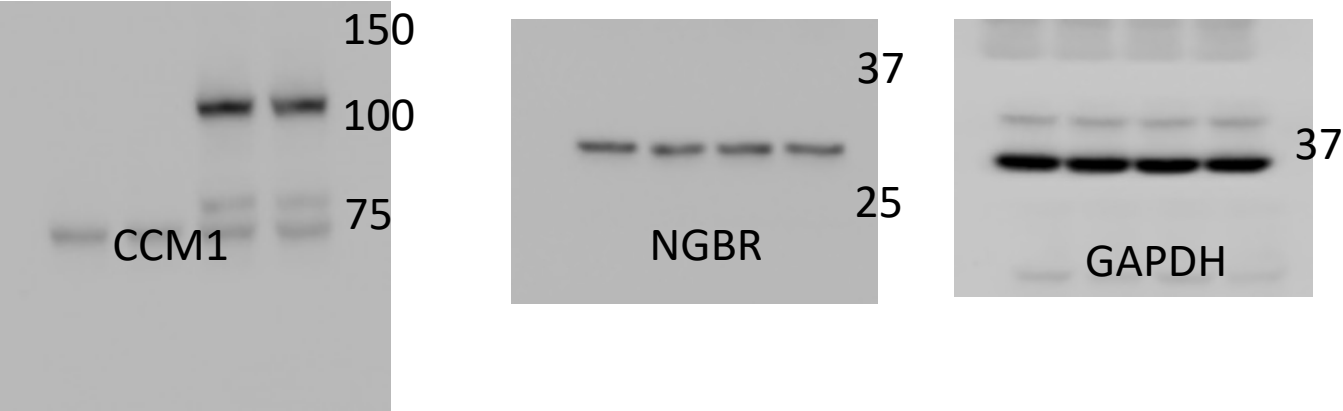
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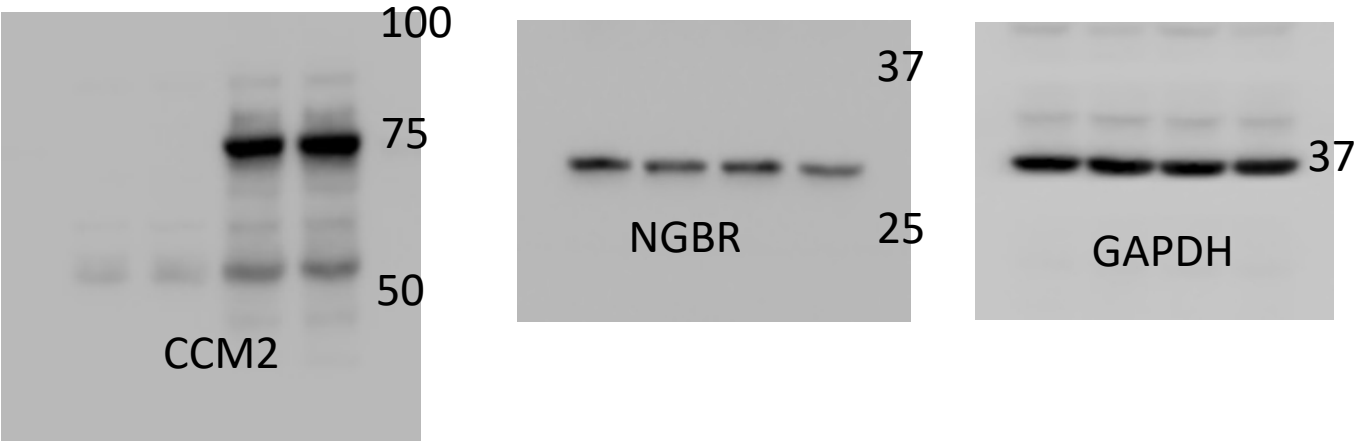
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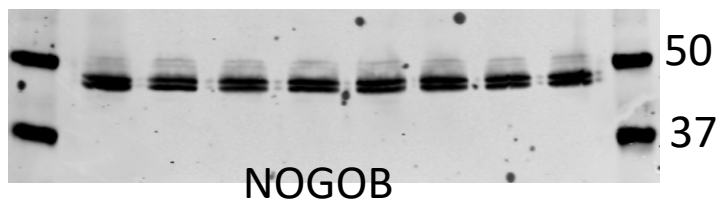
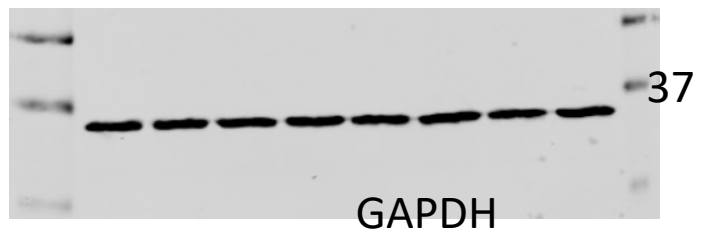
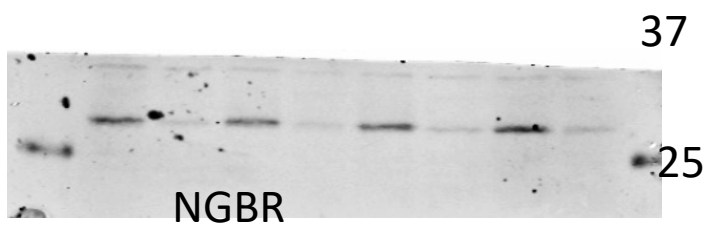
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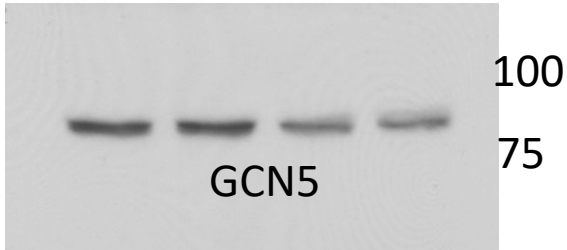
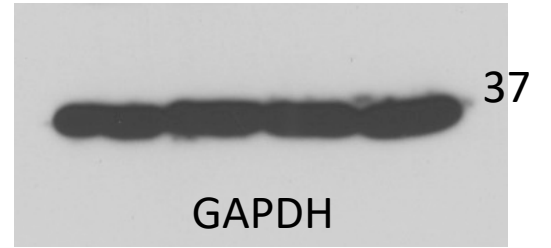
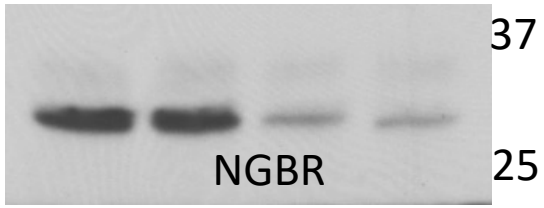
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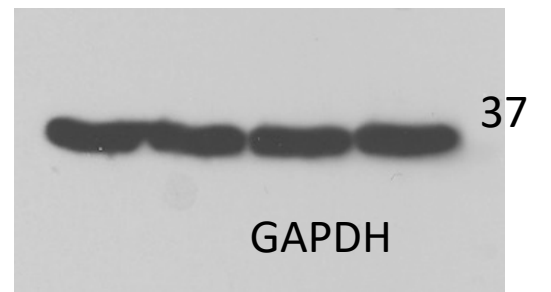
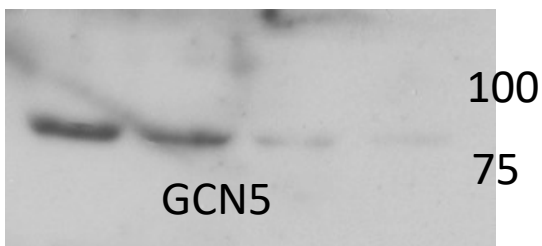
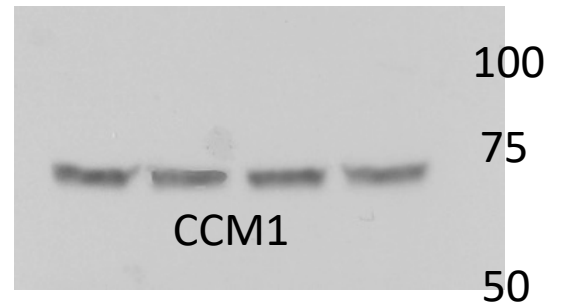
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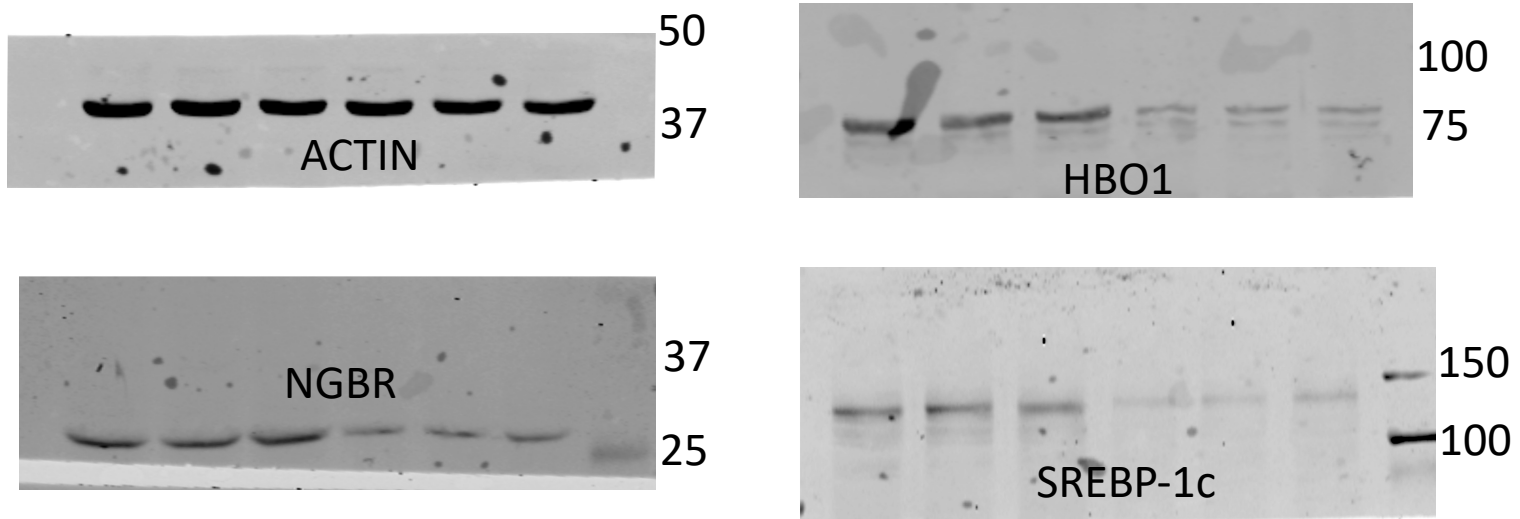
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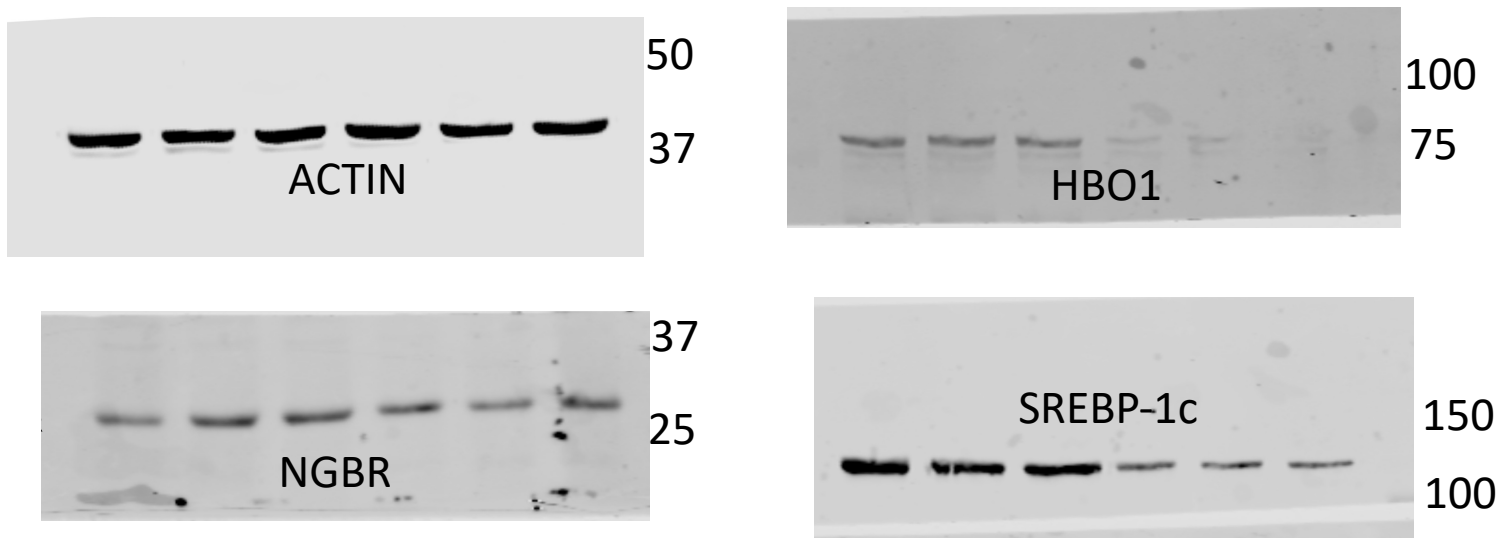
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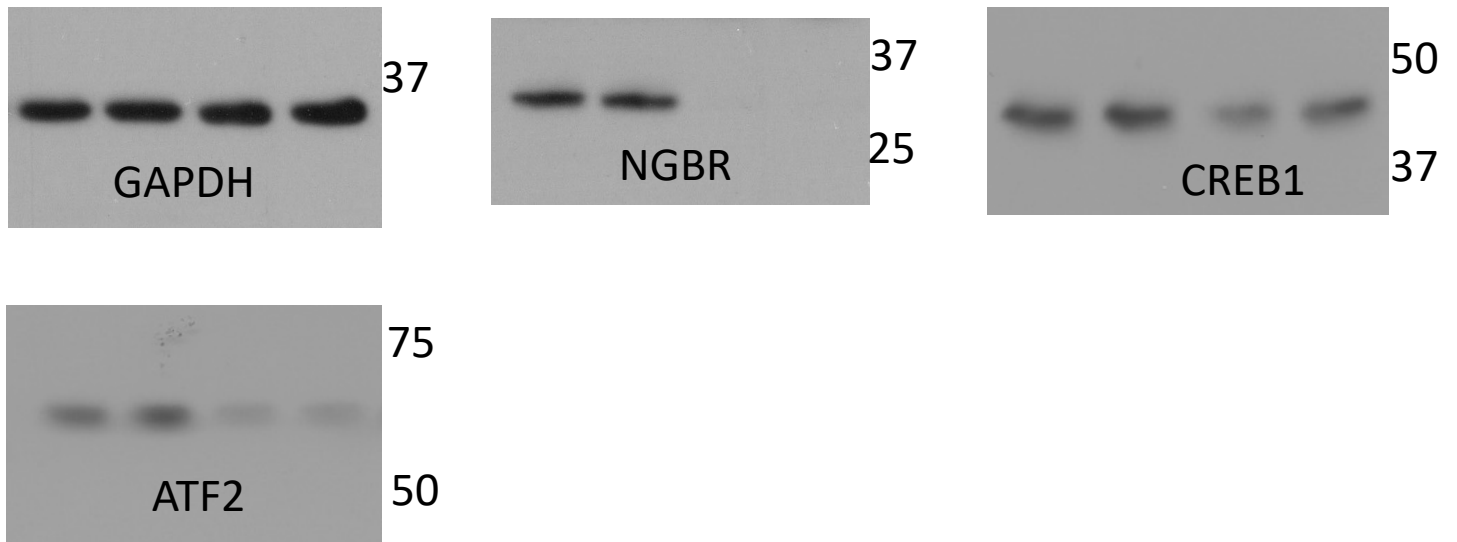
Full unedited gel for Supplementary Figure 9A



Full unedited gel for Supplementary Figure 9C



Full unedited gel for Supplementary Figure 11B



Full unedited gel for Supplementary Figure 11C

