

SUPPLEMENTARY FILE

Supplementary Table S1. Plasmids (accession number and amino acids sequence of our constructs)

NAME	Plasmid	Accession number
GFP	pET28a	P42212
GFP-ApoAI	pET28a	P42212 NP_000030.1
GFP-EmrE-ApoAI	pET28a	P42212 P23895 NP_000030.1
GFP-ApoaI-EmrE-CPP	pET28a	P42212 NP_000030.1 P23895
TatA	pET28a	P69698
TatAx1	pET28a	P69698
TatAx3	pET28a	P69698
OspA-TatAx3-ApoAI	pET28a	WP_259023821.1 P69698 NP_000030.1
MBP-TatAx3-ApoAI	pET28a	P0AEX9 P69698 NP_000030.1
GFP-ApoAI-TatAx3-CPP	pET28a	P42212 NP_000030.1 P69698
OspA-ApoAI-cahFZD7-CPP	pET28a	WP_259023821.1 NP_000030.1 O75084
OspA-ApoAI-cahGPR56-CPP	pET28a	WP_259023821.1 NP_000030.1 Q9Y653
OspA-ApoAI-capFZD4-CPP	pET28a	WP_259023821.1 NP_000030.1 ABW97516.1
OspA-ApoAI-capFZD1/2/7-CPP	pET28a	WP_259023821.1 NP_000030.1 AQT19762.1
OspA-ApoAI-MthK-CPP	pET28a	WP_259023821.1 NP_000030.1 O27564

TATAx1

SKHMGGISIW QLLIIAVIVV LLFGTKKLGS IGSDLGASIK GFKKAMSEQK LISEEDL

TATAx3

SKHMGGISIW QLLIIAVIVV LLFGTKKLGS IGSDLGASIK GFKKAMSEQK LISEEDL
MGG ISIWQLLIIA VIVVLLFGTK KLGSIGSDLG ASIKGFKKAM SEQKLISEED L
MGGISIWQL LIIAVIVVLL FGTKKLGSIG SDLGASIKGF KKAMSDDEPK QDKEF

OspA-TatAx3-ApoAI

10	20	30	40	50	60
MGNSVSVDDL	GSMKVLVSKS	SNADGKYDLI	ATVDALELSG	TSDKNNGSGV	LEGVKADASK
70	80	90	100	110	120
VKLTISDDL	QTTLLEVFKSD	GSTLVSKKVT	SKHMGGISIW	QLLIIAVIVV	LLFGTKKLGS
130	140	150	160	170	180
IGSDLGASIK	GFKKAMSEQK	LISEEDLMGG	ISIWQLLIIA	VIVVLLFGTK	KLGSIGSDLG
190	200	210	220	230	240
ASIKGFKKAM	SEQKLISEED	LMGGISIWQL	LIIAVIVVLL	FGTKKLGSIG	SDLGASIKGF
250	260	270	280	290	300
KKAMSDDEPK	QDKEFHTLKL	LDNWDVTST	FSKLREQLGP	VTQEFWDNLE	KETEGLRQEM
310	320	330	340	350	360
SKDLEEVKAK	VQPYLDDFQK	KWQEEMELYS	QKVEPLRAEL	QEGARQKLHE	LQEKLSPLGE
370	380	390	400	410	420
EMRDRARAHV	DALRTHLAPY	SDELRQLAA	RLEALKENGG	ARLAEYHAKA	TEHLSTLSEK
430	440	450	460		
AKPALEDLRQ	GLLPVLESFK	VSFLSALEEY	TKKLNTQAAA	LEHHHHHH	

OspA-ApoAI-TatAx3-CPP

10	20	30	40	50	60
MGSSHHHHHH	GSGNSVSVDL	PGSMKVLVSK	SSNADGKYDL	IATVDALELS	GTSDKNNGSG
70	80	90	100	110	120
VLEGVKADAS	KVKLTISDDL	GQTTLLEVFKS	DGSTLVSKKV	TSKHTLKLDD	NWDSVTSTFS
130	140	150	160	170	180
KLREQLGVPVT	QEFWDNLEKE	TEGLRQEMSK	DLEEVKAKVQ	PYLDDFQKKW	QEEMELYSRQ
190	200	210	220	230	240
VEPLRAELQE	GARQKLHELQ	EKLSPLGEEM	RDRARAHVDA	LRTHLAPYSD	ELRQLAARL
250	260	270	280	290	300
EALKENGGAR	LAEYHAKATE	HLSTLSEKAK	PALEDLRQGL	LPVLESFKVS	FLSALEEYTK
310	320	330	340	350	360
KLNTQAAAHM	GGISIWQLLI	IAVIVVLLFG	TKKLGSIGSD	LGASIKGFKK	AMSEQKLISE
370	380	390	400	410	420
EDLMGGISIW	QLLIIAVIVV	LLFGTKKLGS	IGSDLGASIK	GFKKAMSEQK	LISEEDLMGG
430	440	450	460	470	480
ISIWQLLIIA	VIVVLLFGTK	KLGSIGSDLG	ASIKGFKKAM	SDDEPKQDKG	SGKKGSGRRG

SGKK

OspA-ApoAI-cahFZD7-CPP

10	20	30	40	50	60
MGSSHHHHHH	SSGNSVSVDL	PGSMKVLVSK	SSNADGKYDL	IATVDALELS	GTSDKNNGSG
70	80	90	100	110	120
VLEGVKADAS	KVKLTISDDL	GQTTLEVFKS	DGSTLVSKKV	TSKAAAHTLK	LLDNWDSVTS
130	140	150	160	170	180
TFSKLRQQLG	PVTQEFWDNL	EKETEGLRQE	MSKDLEEVKA	KVQPYLDDFQ	KKWQEEMELY
190	200	210	220	230	240
RQKVEPLRAE	LQEGARQKLH	ELQEKLSPLG	EEMRDRARAH	VDALRTHLAP	YSDELQRQLA
250	260	270	280	290	300
ARLEALKENG	GARLAEYHAK	ATEHLSTLSE	KAKPALEDLR	QGLLPVLESF	KVSFLSALEE
310	320	330	340	350	360
YTKKLNTQSG	RFARLVWGVW	SVLCCASTLF	TVLTYLVDMR	RFSYPERPII	FLSGCYFMVA
370	380	390	400	410	420
VAHVAGFLLF	DRAVCVERFS	DDGYRTVAQG	TKKEGCTILF	MVLYFFGMAS	SIWWVILSLT
430	440	450	460	470	480
WFLAAGMKWG	HEAIEANSQY	FHLAAWAVPA	VKTITILAMG	QVDGDLLSGV	CYVGLSSVDA
490	500	510	520	530	540
LRGFVLAPLF	VYLFIGTSFL	LAGFVSLFRI	RTIMKHAGTK	TEKLEALMVR	IGVFSVLYTV
550	560	570	580	590	600
PATIVLACYF	YEQAFREHWE	RTWLLQTCKS	YAVPCPPGHF	PPMSPDFTVF	MIKYLMTMIV
610	620	630	640		
GITTGFWIWS	GKTLQSWRRF	YHRLSHSSKG	ETAVGSGKKG	SGRRGSGKK	

OspA-ApoAI-cahGPR56-CPP

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MGSSHHHHHH	SAAAGGNSVS	VDLPGSMKVL	VSKSSNADGK	YDLIATVDAL	ELSGTSDKNN
70	80	90	100	110	120
GGSVLEGVKA	DASKVKLTIS	DDLQTTLEV	FKSDGSTLVS	KKVTSKAAAH	TLKLLDNWDS
130	140	150	160	170	180
VTSTFSKLRE	QLGPVTQEFW	DNLEKETEGL	RQEMSKDLEE	VKAKVQPYLD	DFQKKWQEEM
190	200	210	220	230	240
ELYRQKVEPL	RAELQEGARQ	KLHELQEKLS	PLGEEMRDRA	RAHVDALRTH	LAPYSDELRO
250	260	270	280	290	300
RLAARLEALK	ENGGARLAEY	HAKATEHLST	LSEKAKPALE	DLRQGGLLPVL	ESFKVSFLSA
310	320	330	340	350	360
LEEYTKKLNT	QSGGPKNVTL	QCVFWVEDPT	LSSPGHWSSA	GCETVRRETQ	TSCFCNHLTY
370	380	390	400	410	420
FAVLMVSSVE	VDAPHKHLYS	LLSYVGCVVS	ALACLVTIAA	YLCSRVPLPC	RRKPRDYTIK
430	440	450	460	470	480
VHMNLLAVF	LLDTSFLLSE	PVALTGSEAG	CRASAIPLHF	SLLTCLSWMG	LEGYNLYRLV
490	500	510	520	530	540
VEVFGTYVPG	YLLKLSAMGW	GFPIFLVTLV	ALVDVDNYGP	IILAVHRTPE	GVIYPSMCWI
550	560	570	580	590	600

RDSLVSYITN LGLFSLVFLF NMAMLATMVV QILRLRPHTQ KWSHVLTLTG LSLVLGLPWA

610 620 630 640 650 660
LIFFSFASGT FQLVVLYLFS IITSFQGFLI FIWYWSMRLQ ARRGSQKKGS GKKGSRRGS

GKK

OspA-ApoAI-capFZD4-CPP

10 20 30 40 50 60
MGSSHHHHH SSGNSVSVDL PGSMKVLVSK SSNADGKYDL IATVDALELS GTSDKNNGSG

70 80 90 100 110 120
VLEGVKADAS KVKLTISDDL GQTTLEVFKS DGSTLVSKKV TSKAAHTLK LLDNWDSVTS

130 140 150 160 170 180
TFSKLRQLG PVTQEFWDNL EKETEGLRQE MSKDLEEVKA KVQPYLDDFQ KKWQEEMELY

190 200 210 220 230 240
RQKVEPLRAE LQEGARQKIH ELQEKLSPLG EEMRDRARAH VDALRTHLAP YSDELRQRLA

250 260 270 280 290 300
ARLEALKENG GARLAEYHAK ATEHLSTLSE KAKPALEDLR QGLLPVLESF KVSFLSALEE

310 320 330 340 350 360
YTKKLNTQSG HDKVFANIWL LGWSVLCFFS CLLTIIVFSC NTNRFlyPEK PIVFLSICYF

370 380 390 400 410 420
FYASGNLFGA ILGRNVVACR AFNDKTDFIV GSGRETTWCK INFLMIYFFG SASALWWVVL

430 440 450 460 470 480
TITWFLSASR HWGYEAIESI SSMLHLVSWA IPALKSIFIL ILHKIDADEL TGQCFVGNSN

490 500 510 520 530 540
NKVLWGFVIV PNMIYLFIGI VFMTMGYISL LKVRRSFLQR PDCIATNNLK RLAKLMAKIG

550 560 570 580 590 600
VFSILYVLPV LCTIVSYIVD AYKMTNFDIT LKALFRFSPN CIGRNGINWS SIKCVKILQP

610 620 630 640 650 660
VLPSVEMRML RIFMNLVIGI TSGIWIWGNK KTVKSCISTL RFWEKKKEEE PTPTNQCDSS

670 680 690 700 710
TIQNNIPLSQ TKQKETLLPY HDWKLNSSYP VVVEYGLPTV SVPGSGKKGS GRRGSGKK

OspA-ApoAI-capFZD1/2/7

10 20 30 40 50 60
MGSSHHHHH SSGNSVSVDL PGSMKVLVSK SSNADGKYDL IATVDALELS GTSDKNNGSG

70 80 90 100 110 120
VLEGVKADAS KVKLTISDDL GQTTLEVFKS DGSTLVSKKV TSKAAHTLK LLDNWDSVTS

130 140 150 160 170 180
TFSKLRQLG PVTQEFWDNL EKETEGLRQE MSKDLEEVKA KVQPYLDDFQ KKWQEEMELY

190 200 210 220 230 240
RQKVEPLRAE LQEGARQKIH ELQEKLSPLG EEMRDRARAH VDALRTHLAP YSDELRQRLA

250 260 270 280 290 300
ARLEALKENG GARLAEYHAK ATEHLSTLSE KAKPALEDLR QGLLPVLESF KVSFLSALEE

310 320 330 340 350 360
YTKKLNTQSG RKFARVWVAL WSFMCVGSTL FTVLTFLIDM KRFQYPERPI IFLSACYLVV

370 380 390 400 410 420

GLTYVAGFFL NDKVACAGPF TNKDSTGKTP KIVVQGTKFE SCIILFMLLY FFSMASAIWW

430 440 450 460 470 480
VVLTTITWYLA AKCHWAHESI GRNSQYFHFA AWAIPAGKTI GILALSKVDG DPLTGVCFTG

490 500 510 520 530 540
LSDPSTLRGF LIAPLCIYLL IGTGFLIAGF VSMFEIRTII KTAGSKTDKL AKLITRIGIF

550 560 570 580 590 600
SVLYVVPADV VIACYFHESM NLYKWMQRWY LIDICRSAEY KDECKKIHDH KPINPGGNAL

610 620 630 640 650 660
IFSQKPEFEL FMIKYLMSLI VGITSGIWIW SGKTLLSWKY FFSRLCGRLN PGDPWTRSDW

670 680 690 700 710 720
LEKNNGYYQR HFVGPTANKM IIQGCDDKQA IPLQYQFPQQ TQQTIGTQEL QKPLLSNVPE

730 740 750 760
SILNAQAQNG NLLPPSQQIG DSKTQKIGFM MSGGKKGSGR RGSQKK

OspA-ApoAI-MthK-CPP

10 20 30 40 50 60

MGSSHHHHHH SSGNSVSVDL PGSMKVLVSK SSNADGKYDL IATVDALELS GTSDKNNGSG

70 80 90 100 110 120
VLEGVKADAS KVKLTISDDL GQTTLEVFKS DGSTLVSKKV TSKAAAHTLK LLDNWDVSTS

130 140 150 160 170 180
TFSKLRQLG PVTQEFWDNL EKETEGLRQE MSKDLEEVKA KVQPYLDDFQ KKWQEEMELY

190 200 210 220 230 240
RQKVEPLRAE LQEGARQKLH ELQEKLSPLG EEMRDRARAH VDALRTHLAP YSDELRQRLA

250 260 270 280 290 300
ARLEALKENG GARLAEYHAK ATEHLSTLSE KAKPALEDLR QGLLPVLESF KVSFLSALEE

310 320 330 340 350 360
YTKKLNTQSG VLVIEIIRKH LPRVLKVPAT RILLVLAVI IYGTAGFHF I EGESWTVSLY

370 380 390 400 410 420
WTFVTIATVG YGDYSPSTPL GMYFTVTLIV LGIGTFAVAV ERLLEFLINR EQMKLMGLID

430 440 450 460 470 480
VAKSRHVVIC GWSESTLECL RELRGSEVFV LAEDENVRKK VLRSGANFVH GDPTRVSDLE

490 500 510 520 530 540
KANVRGARAV IVDLESSET IHCILGIRKI DESVRIIAEA ERYENIEQLR MAGADQVISP

550 560 570 580 590 600
FVISGRLMSR SIDDGYEAMF VQDVLAEEST RRMVEVPIPE GSKLEGVSVL DADIHDTVGV

610 620 630 640 650 660
IIIGVGRGDE LIIDPPRDYS FRAGDIILGI GKPEETIERLK NYISAGSGKK GSGRRGSGKK

Figure SI1. Epigenetics Sample metadata

Path	Sample_Name	Sample_Type	Treatment	Slide	Array
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./Data/207096530008_R02C01	207096530008_R02C01	HEK	Control (RA)	Aston_MethylationEPICv2_20230426	EPICv2
./Data/207096530008_R03C01	207096530008_R03C01	HEK	CHIR99021+RA	Aston_MethylationEPICv2_20230426	EPICv2
./Data/207096530008_R04C01	207096530008_R04C01	HEK	CHIR99021+RA	Aston_MethylationEPICv2_20230426	EPICv2
./Data/207096530008_R05C01	207096530008_R05C01	HEK	iDRIVEcapFZD7+RA	Aston_MethylationEPICv2_20230426	EPICv2
./Data/207096530008_R06C01	207096530008_R06C01	HEK	iDRIVEcapFZD7+RA	Aston_MethylationEPICv2_20230426	EPICv2

R Session information

R version 4.5.1 (2025-06-13)

Platform: aarch64-apple-darwin20

Running under: macOS Tahoe 26.2

Matrix products: default

BLAS:

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LAPACK: /Library/Frameworks/R.framework/Versions/4.5-arm64/Resources/lib/libRlapack.dylib; LAPACK version 3.12.1

locale:

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time zone: America/Denver

tzcode source: internal

attached base packages:

[1] grid parallel stats4 stats graphics grDevices utils datasets methods base

other attached packages:

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[19] locfit_1.5-9.12	iterators_1.0.14
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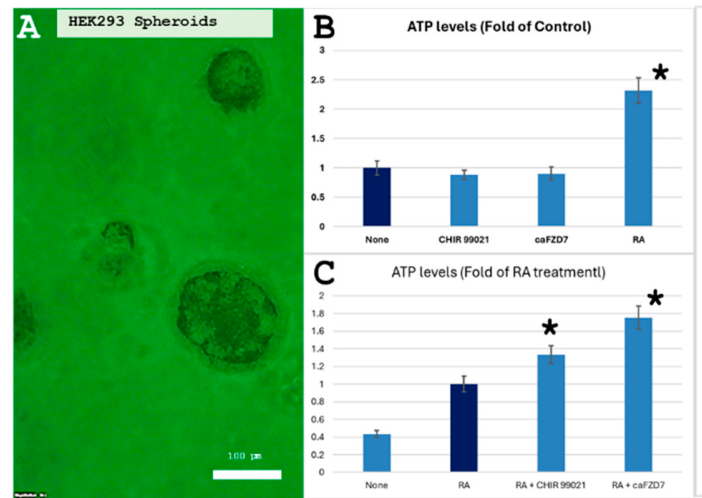


Figure SI2. iDRIVE-Frizzled + RA stimulate HEK 293 embryoid body bioenergetics.

A) Imaging HEK 293 cells cultured as Embryoid Bodies (EBs). **B)** ATP assay of HEK 293 cells in culture. Cells are exposed to CHIR99021, iDRIVE (caFZD7), or Retinoic Acid (RA). Dark blue is used for None (no treatment) and is the control over which ATP levels are considered. **C)** Combinations of CHIR99021 and iDRIVE with RA were tested. Dark blue is used for RA only and is the control over which ATP levels are considered. Asterisks represent statistically significant differences against the control ($p < 0.04$). $n = 48$ per treatment group.

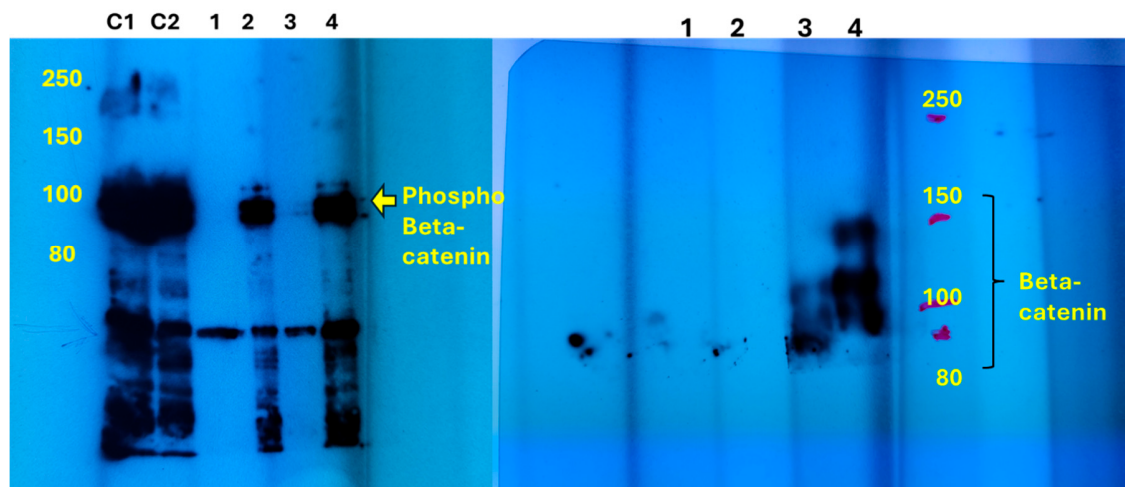


Figure SI3. Phospho- β -catenin and β -catenin western blot. Santa Cruz Biotechnologies, Left panel, Phosphorylated-beta-catenin Antibody (1B11): sc-57533; C1 and C2 - HEK293 cells untreated, 1-Planaria tail+iDRIVE capFRZD1/2/7; 2- Planaria tail+control protein; 3-Planaria head+Control protein; 4-Planaria tail+control protein. **Right panel, Beta-catenin Antibody (12F7): sc-59737. Antibodies are used 1:250 dilution. Incubated at 4 C overnight. Secondary antibodies are diluted 1:3,000. 1 and 2-Planaria tail+control protein; 2- Planaria tail+iDRIVE capFRZD1/2/7 protein; 3-Planaria head+control protein. Numbers correspond to Size Markers (kDa).**

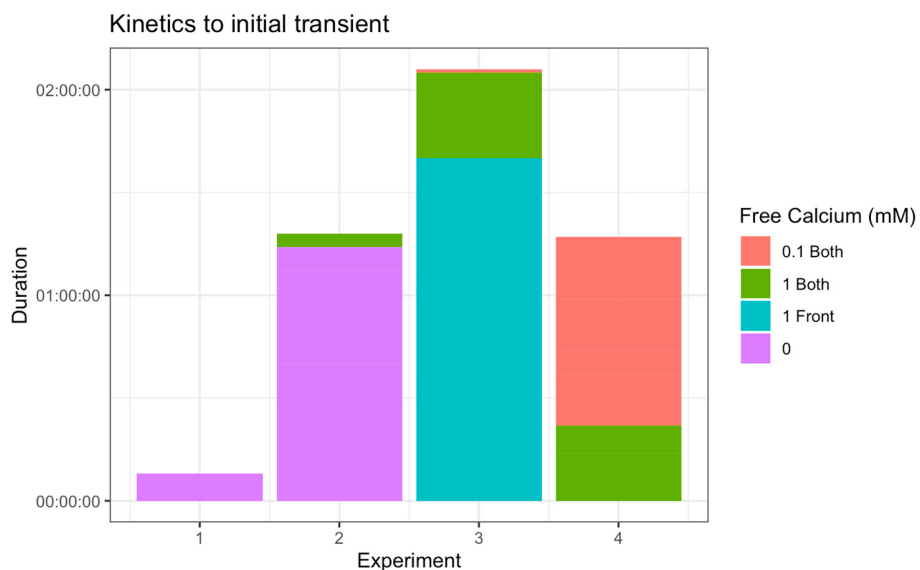


Figure SI4. MthK incubation and free calcium concentration.

Free calcium concentration in PLB experiments. The first conductance event for experiment 3 occurred immediately after the application of 2 mM EDTA (cis and trans chambers). This may suggest an RCK conformational change that is not conducive to plasma membrane insertion.

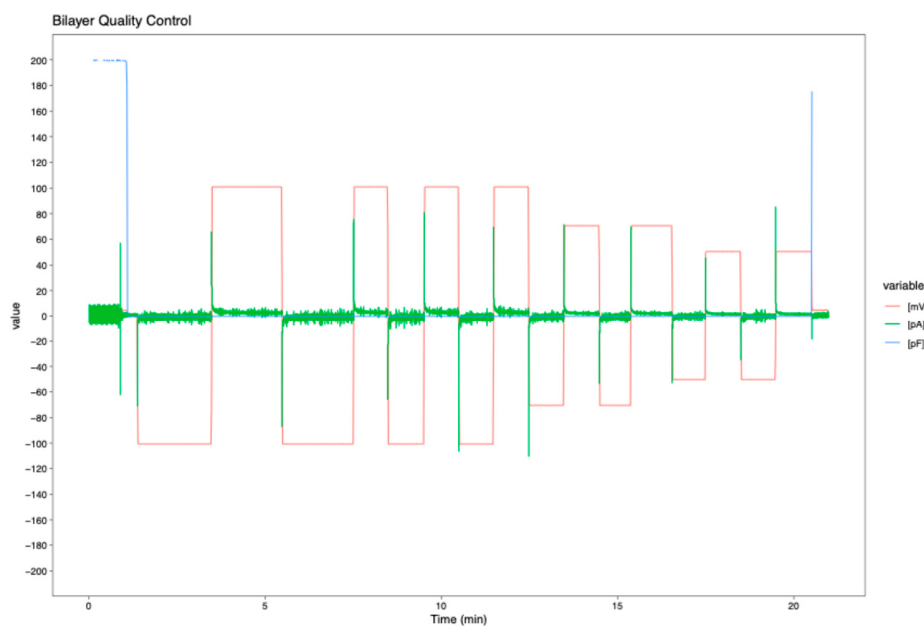


Figure SI5. Planar bilayer conductance quality control.

Alternating voltages between 100 mV and -100 mV applied intermittently every 1-2 minutes. Lipid bilayer integrity assessed through capacitance (pF) measurements.

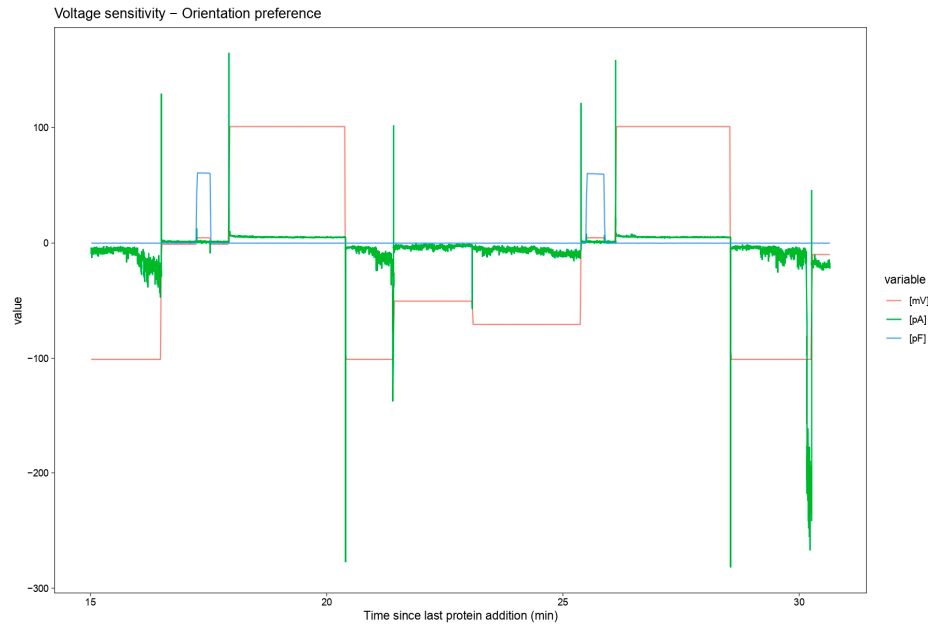


Figure SI6. iDRIVE-MthK re-inserts the lipid bilayer via its C-terminus.

Extension of Figure 5C. Conductance is only triggered with negative voltages, suggesting iDRIVE mediates the insertion into plasma membranes in a uniform orientation. See § 3.4 in the main article for further discussion.

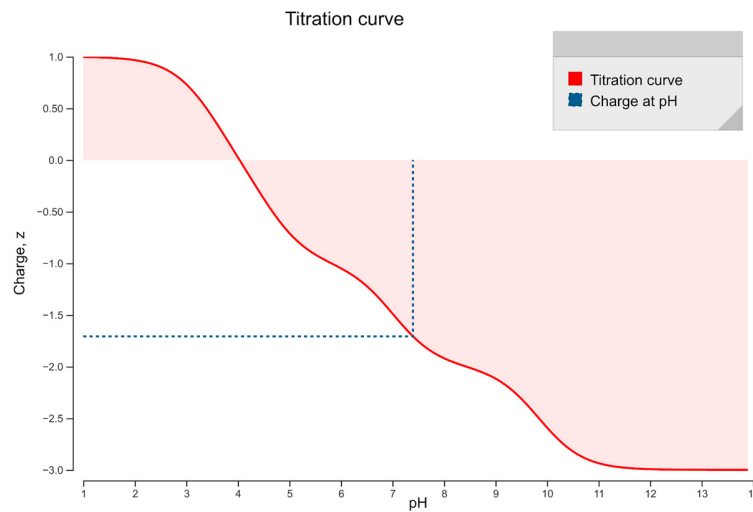


Figure SI7. MthK selectivity filter Titration Curve.

Selectivity filter (TVGYGD) for each MthK subunit was predicted to have a charge of ~ -1.9 at pH 8.02. Thus, we rule out anion currents through MthK.

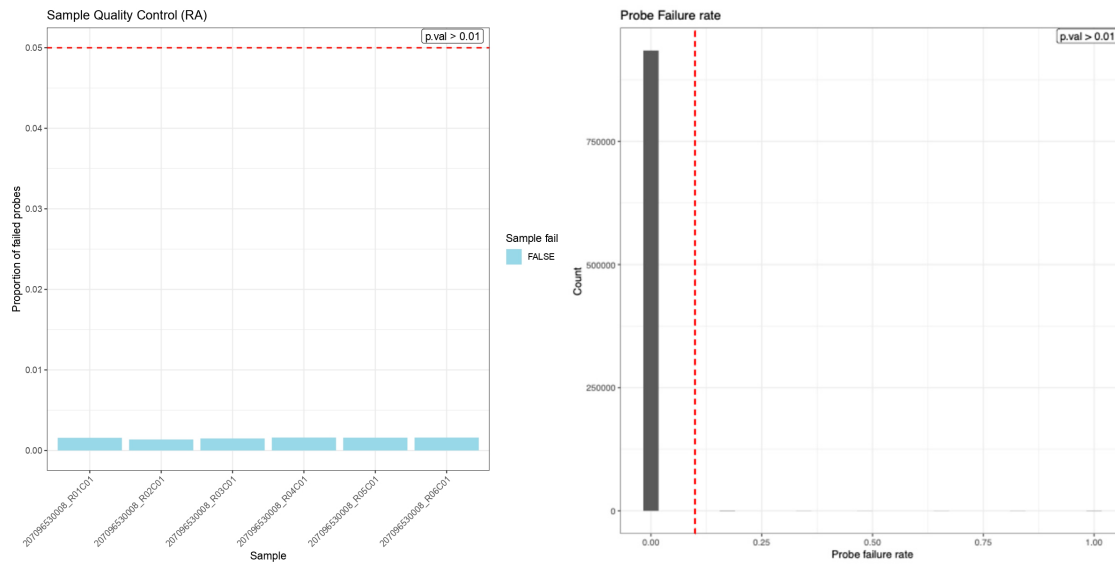
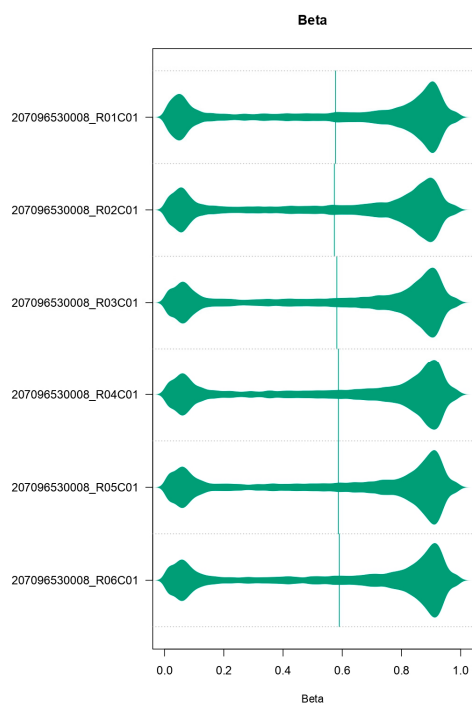
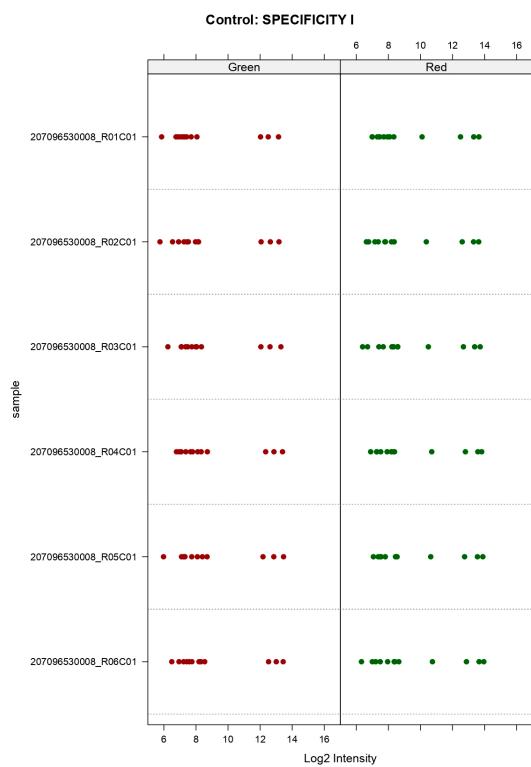
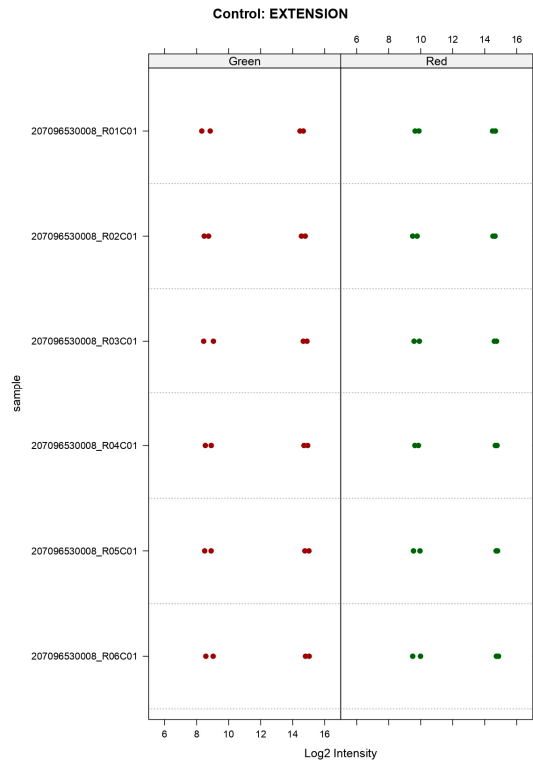
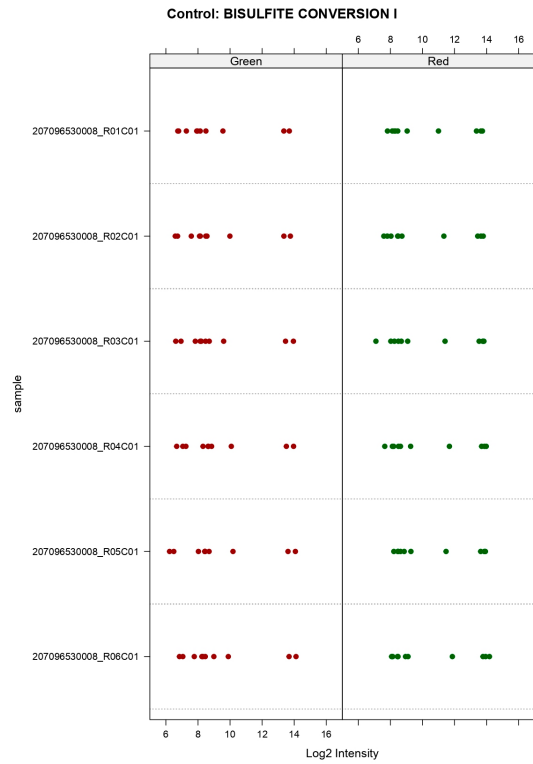
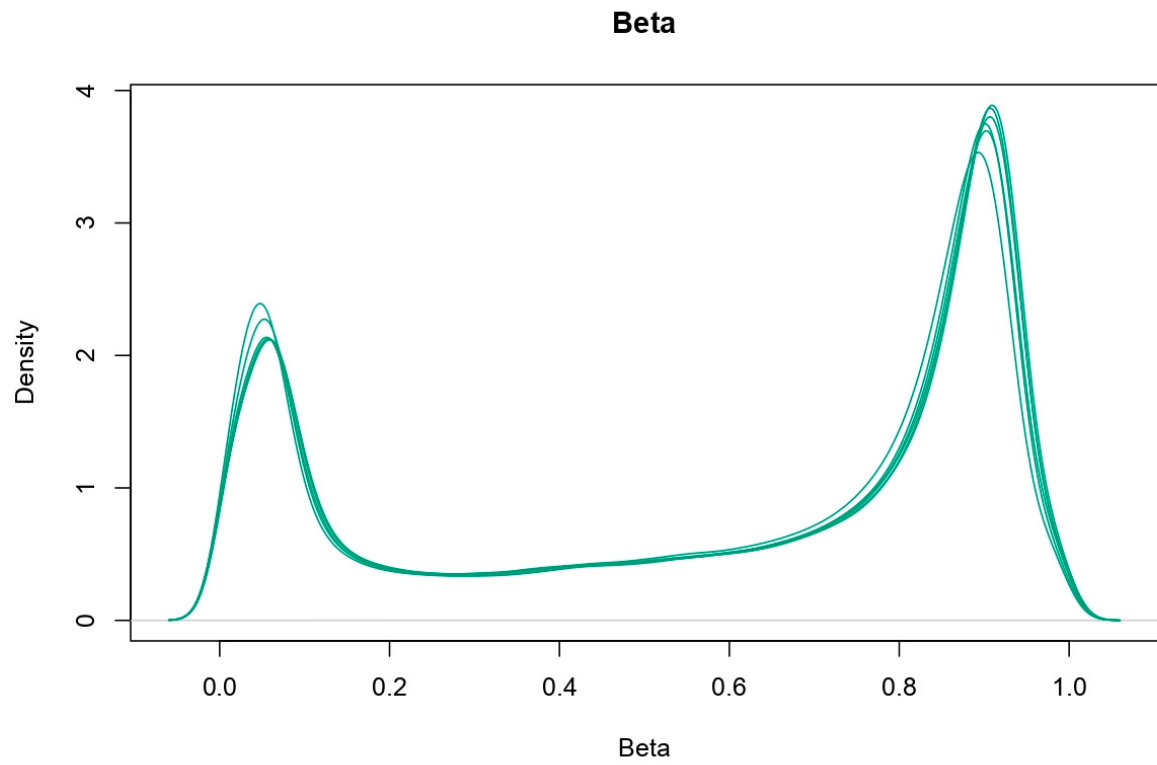


Figure SI8. EPICv2 array sample and probe quality control.

Probes with detection p-val > 0.01 were considered “failed.” Sample quality control was performed by filtering samples that had over 5% “failed” probes. Similarly, probe filtering was performed by removing probes that “failed” in over 10% of the samples.





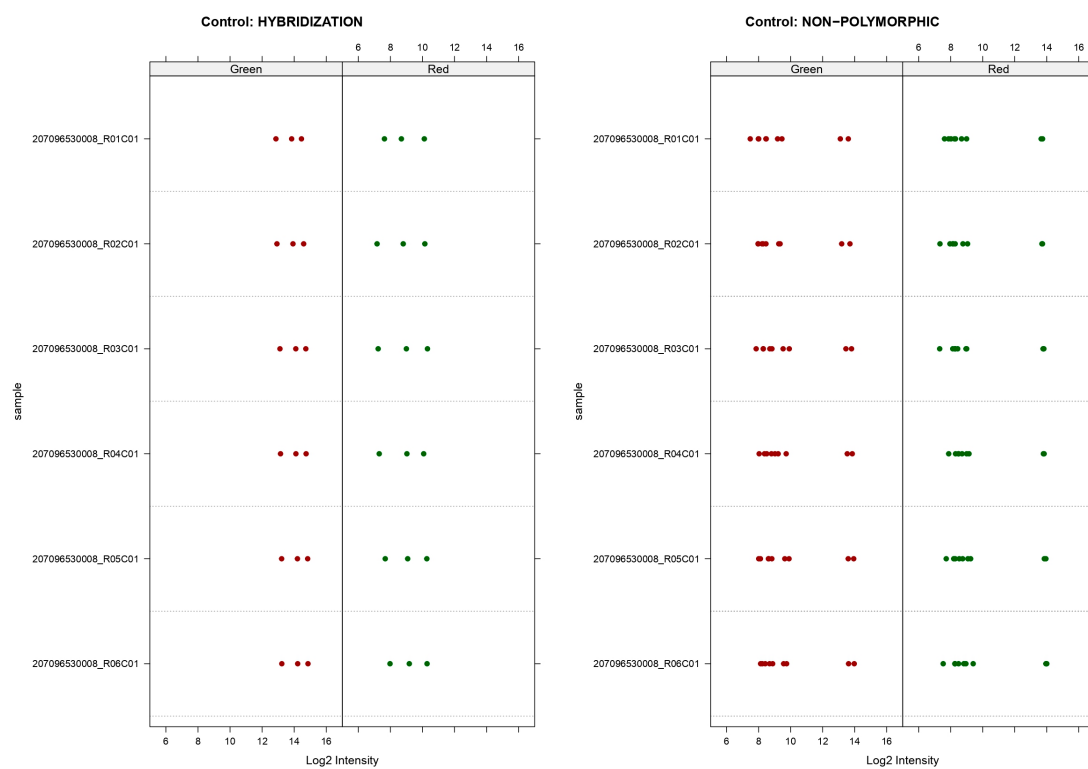


Figure SI9. Comprehensive EPICv2 array methylation quality control.

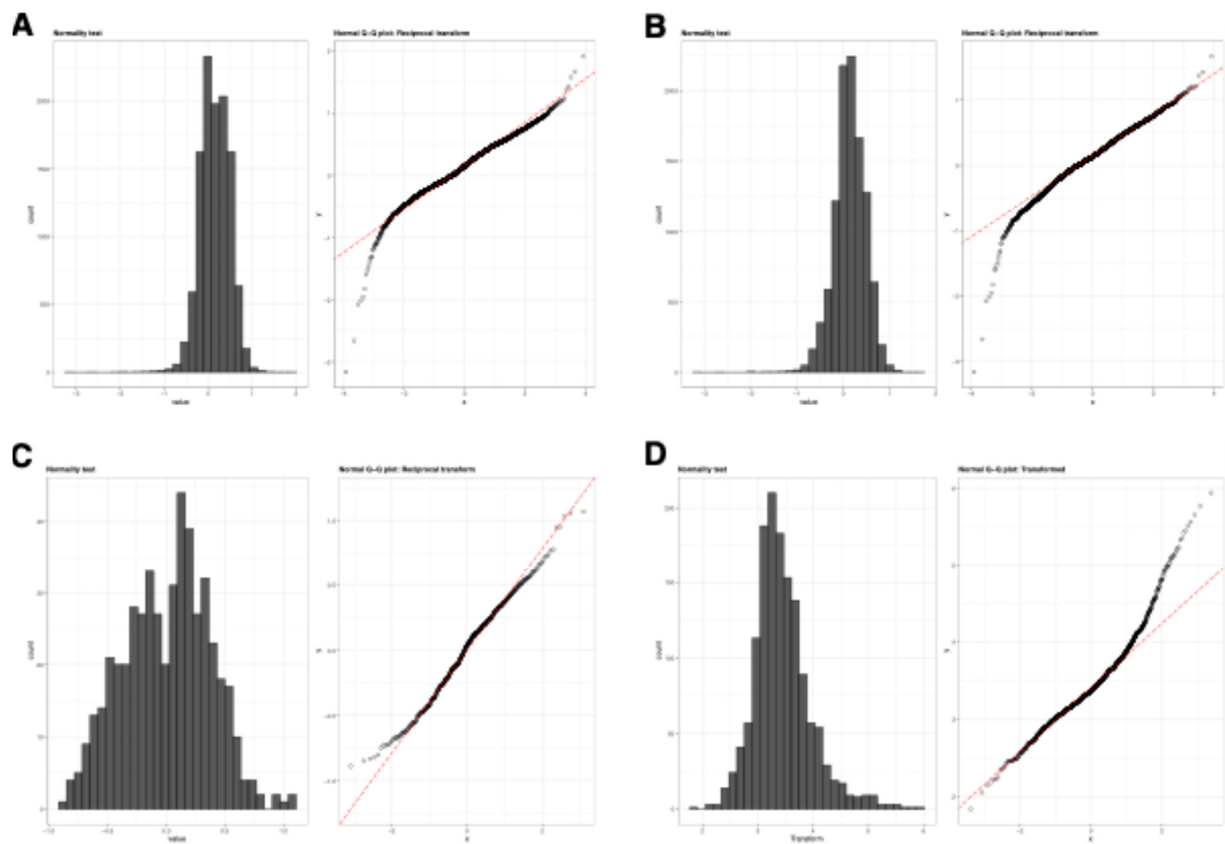


Figure SI10. Normality assessments.

A-C) Average M-value differences across DMRs. **A)** DMRs identified in iDRIVE-cahFZD7 + RA vs RA. **B)** DMRs identified in CHIR99021 + RA vs RA. **C)** DMRs identified in CHIR99021+RA vs iDRIVE-cahFZD7+RA. **D)** Log transformed myotube diameters (Hypertrophy assay)

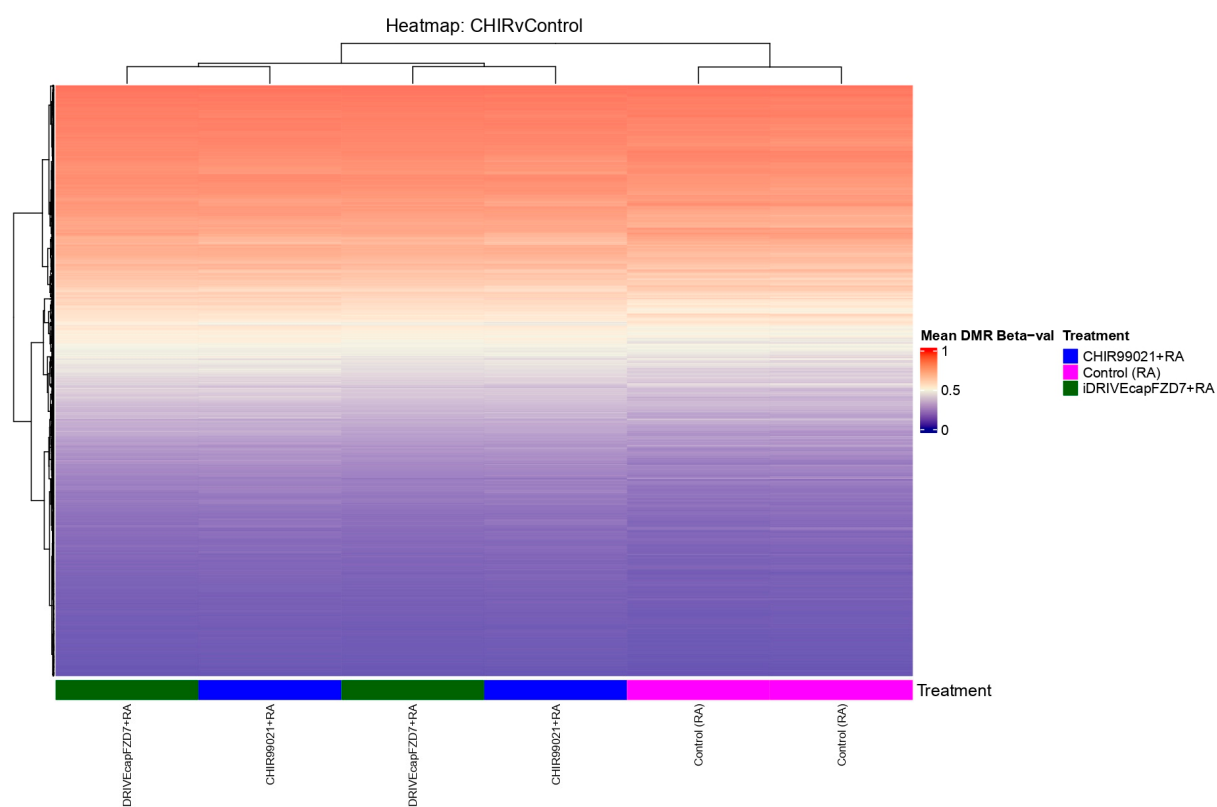


Figure SI11. Average DMR Beta values for significant DMRs in the CHIR99021+RA vs RA contrast.

We note that CHIR99021+RA and iDRIVE-cahFZD7+RA cluster better with each other than with their replicates, L1 distance – average linkage hierarchical clustering.

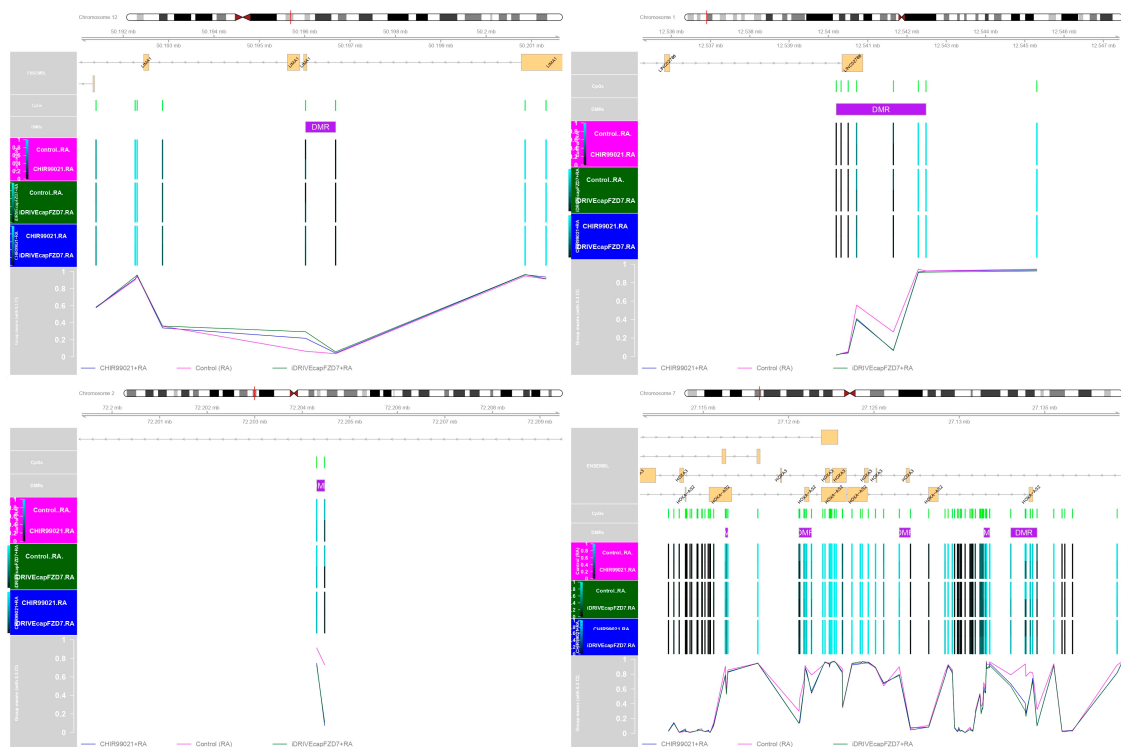


Figure SI12. DMR region plots: iDRIVE-cahFZD7+RA vs Control (RA).
 Top 4 most significant DMRs found in the contrast. We note the close trends between iDRIVE-cahFZD7+RA and CHIR99021+RA methylation patterns.

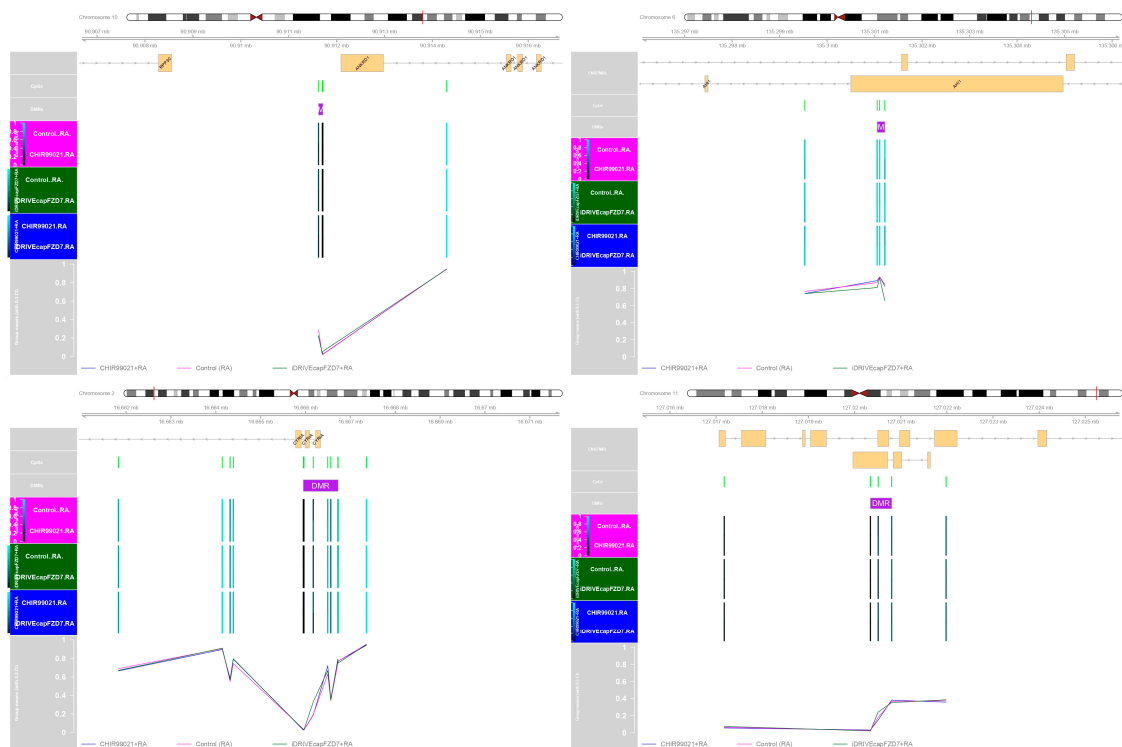
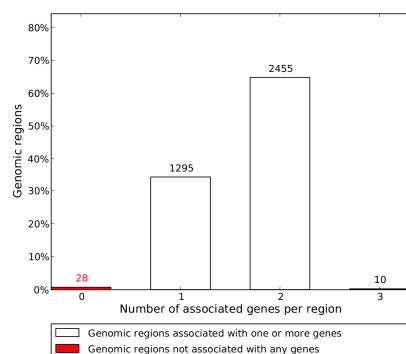


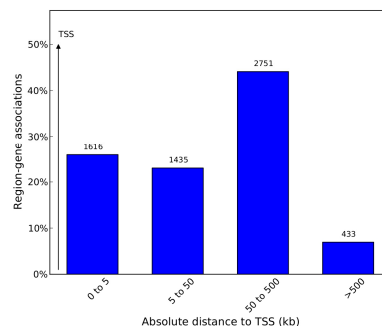
Figure SI13. DMR region plots: iDRIVE-cahFZD7+RA vs CHIR99021+RA.

We note the unique epigenetic patterns elicited by iDRIVE-cahFZD7+RA and not through CHIR99021+RA.

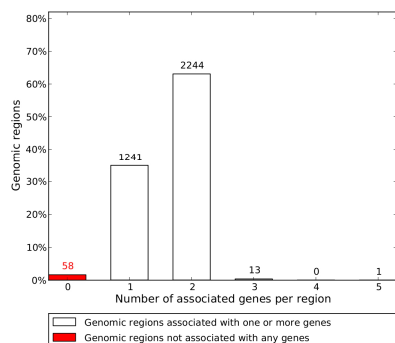
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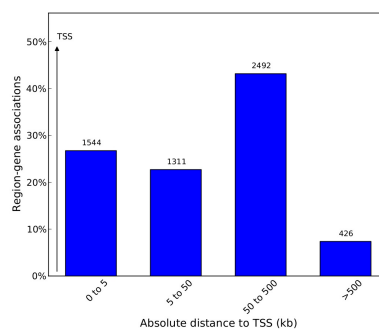
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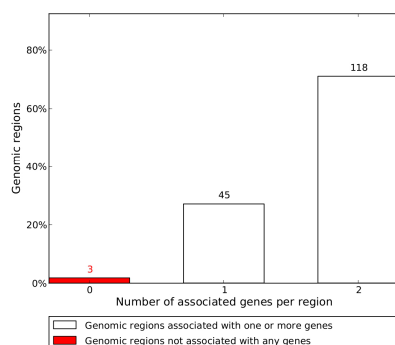
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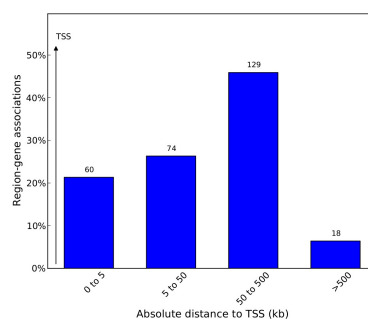


Figure SI14. Associated gene count per DMR and absolute distance to TSS.

We note that iDRIVE and CHIR had a similar number of associated genes with DMRs, but iDRIVE tended to result in higher number of enriched associations. Similar results were observed with absolute distances and distances (data not shown) to TSS.