Supplemental Data for

CD63, MHC class 1, and CD47 identify subsets of extracellular vesicles containing distinct populations of noncoding RNAs

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Supplementary Figure Legends

Figure S1: (a, b) Capturing efficiency of CD63 conjugated MNPs. **(c-d)** Extracted EVs were prepared using the Exo-Quick kit, and vesicle size and concentration were measured using Nanosight.

Figure S2: (a) Side scattering for calibrating beads of the indicated sizes. **(b)** Bodipy-FL labeled EVs released from Jurkat cells were captured with anti-CD63-MNPs and analyzed by flow cytometry. Volumetric control was used to estimate the concentration of captured EVs

Figure S3: (a) Bodipy labeled EVs released from Jurkat or JinB8 cells where captured with anti-MHC1-MNPs and analyzed using flow cytometry. Volumetric control was used to estimate the concentration of captured EVs

Figure S4: RNA profiles were obtained using a RNA Bioanalyzer for the indicated capture vs uncaptured CD47, CD63 and MHC1-EVs for RNA sequencing samples.

Figure S5: Experimental design and work flow for the RNA-sequencing data analysis of selected small RNAs.

Figure S6: (a-c) RNA profiles were obtained using RPKM normalization comparing captured and uncaptured CD47, CD63 and MHC1 EVs with absolute value of the fold change greater that 2.0 using scale log base 2. The number of upregulated RNAs in captured CD47, CD63 and MHC1 EVs are shown as pie charts.

Figure S7: (a) Scatter graph of biological replicates comparing $CD47^+$ and $CD63^+$ EVs showing 24 transcripts ($p < 0.05$ and absolute value of the fold change greater that 2.0) using scale log base 2 of total RPM, (CD47_CAP and CD63_CAP, Student's- t-test). 627 transcripts pass the fold change cutoff before the application of the statistical test. **(b)** A Scatter graph of biological replicates (n=2) between $CD47^+$/$MHC1^+$ EVs showing 682 transcripts at 2-fold change before application of the statistical test and 41 (White) at $p < 0.05$ using log base 2 of total RPM, X-axis (CD47_CAP and MHC1_CAP). **(c)** A Scatter graph of biological replicates comparing $CD63^+$ and $MHC1^+$ EVs showing 483 transcripts at 2-fold change before application of the statistical test and 9 highlighted (White) at $p < 0.05$ using scale log base 2 of total RPM, (CD63_CAP and MHC_CAP). **(d)** Heat map of hierarchical clustering of the 24 transcripts at $p<0.05$ comparing $CD47^+$ and $CD63^+$ EVs. (**e)** Heat map of hierarchical clustering of the 38 transcripts at $p<0.05$ comparing $CD63^+$

and $CD63^-$ EVs. **(f)** Heat map of hierarchical clustering of the 9 transcripts at $p<0.05$ comparing $CD63^+$ and $MHC1^+$ EVs.

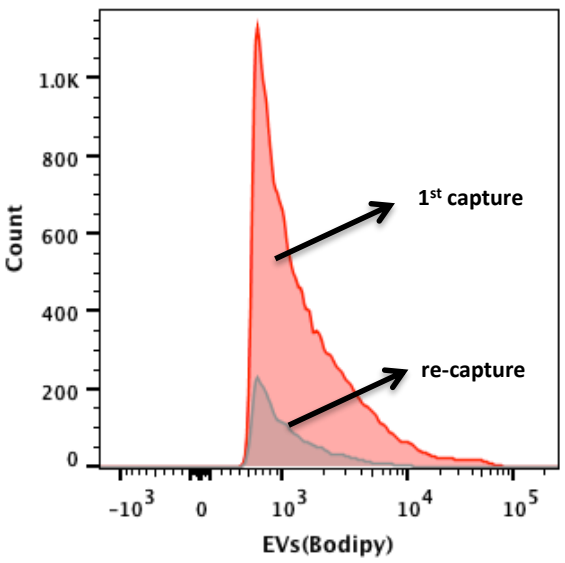
FigureS8: (a) A Scatter graph of biological replicates (n=3) between $CD47^+/CD47^-$ EVs showing 109 miRNA (All species) highlighted (White) at $p<0.05$ using scale log base 2 of total RPM, (CD47_CAP and CD47_UnCap). **(b)** A Scatter graph of biological replicates (n=2) comparing $CD63^+$ and $CD63^-$ EVs showing 208 miRNAs (All species) highlighted (White) at $p<0.05$ using scale log base 2 of total RPM, (CD63_CAP and CD63_UnCap). (**c)** A Scatter graph of biological replicates (n=2) between $MHC1^+/MHC1^-$ EVs showing 135 miRNAs highlighted (White) at $p<0.05$ using scale log base 2 of total RPM, X-axis (MHC1_CAP and MHC_UnCap).

Figure S9: (a) Hierarchical clustering of 135 miRNAs (All species) that were significant at $p<0.05$ (Student-t test) comparing $MHC1^+$ AND MHC^- EVs. **(b)** Out of 135 miRNAs, 7 hsa-miRNA were enriched in $MHC1^+$ EVs. A comparison of $MHC1^+$ and $CD47^+$ EVs showed differential expression of 5 hsa-miRNAs. **(c)** Microarray ratio files were imported into miRNA analysis Array Star project, and fold change was calculated by comparing $CD47^+$ and $CD63^+$ EVs and $CD47^{+b}$ and $CD63^{+b}$ EVs. Using the 1.5 fold change cutoff and P-value ≤ 0.05, 60 miRNA were found common between the RNA sequencing and microarray analyses. The pie chart shows UP (upregulated in $CD47^+$ and $CD63^+$ EVs), Down (in both), and UP/DOWN (not consistent). **(d)** Venn diagram of Captured $CD63^+$, $CD47^+$ and $MHC1^+$ EV miRNAs identified by sequencing analysis aligned with the non-coding genome using DNA Array Star 14.

Figure S1

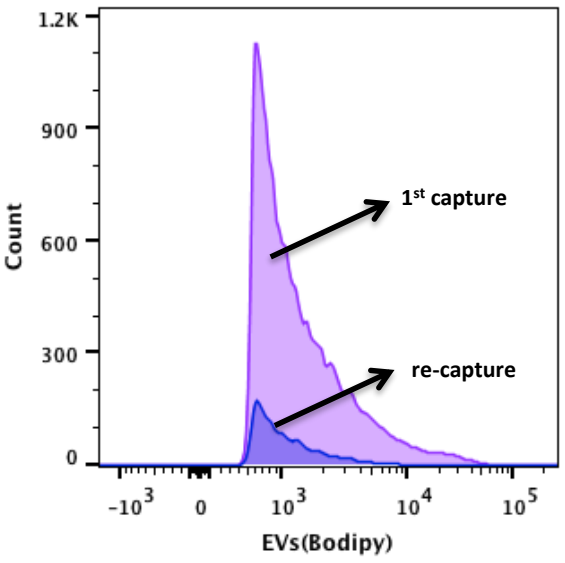
CD63 capture efficiency

a



83.1%

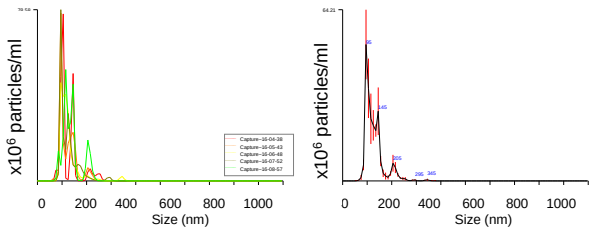
b



87.8%

c

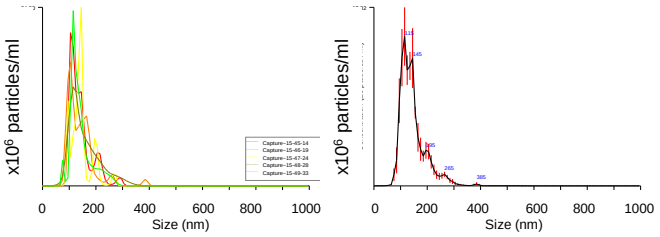
Jurkat



Mean: 122+/-3.0nm
Mode: 101.6 +/-3.7nm

d

JinB8



Mean: 140.1+/-2.8nm
Mode: 117.7 +/-6.8nm

Figure S2

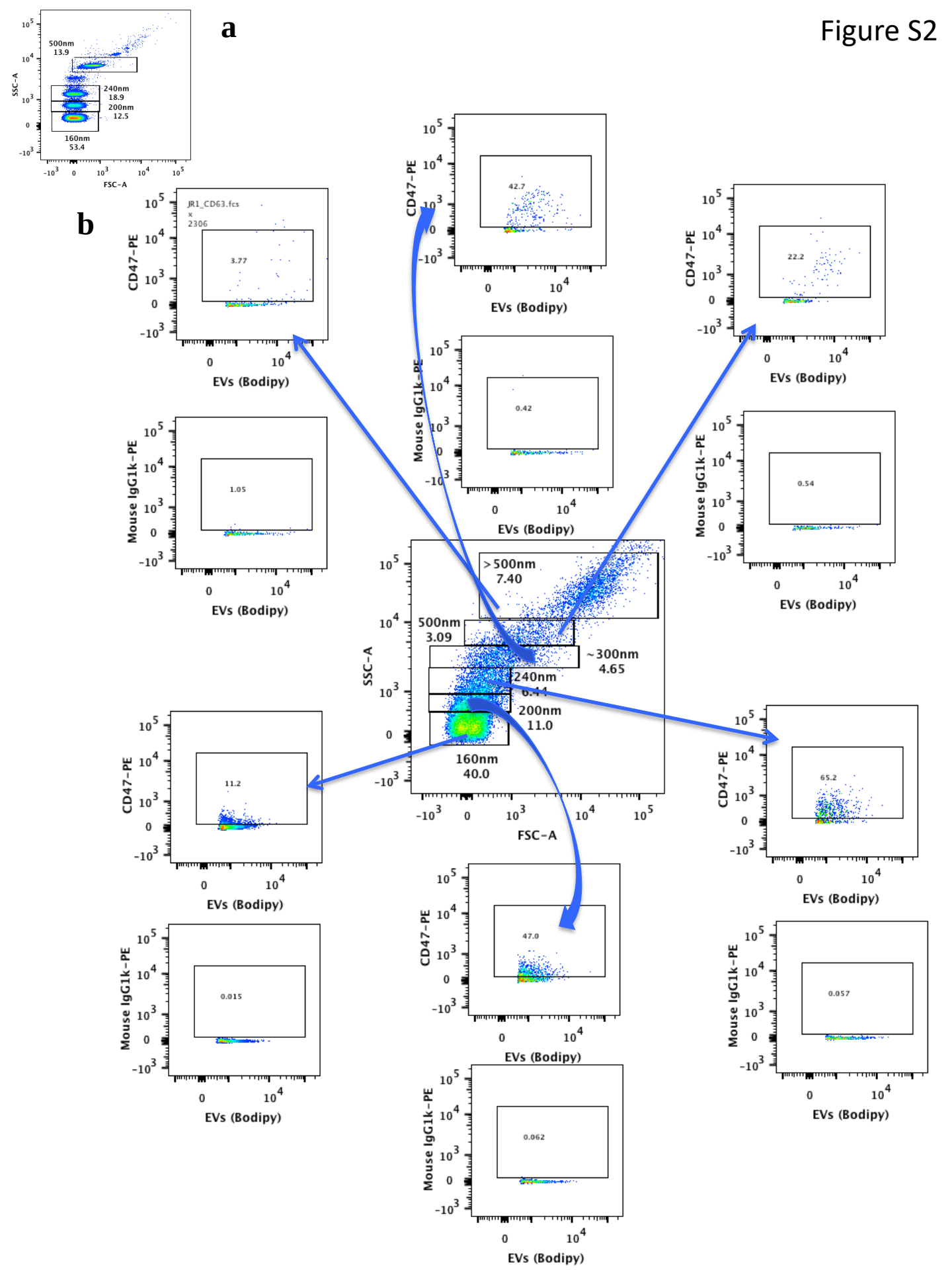

a
500nm
13.9
240nm
18.9
200nm
12.5
160nm
53.4
SSC-A
FSC-A
b
JR1_CD63.fcs
x
2306
3.77
42.7
22.2
0.42
1.05
0.54
11.2
65.2
47.0
0.015
0.057
0.062
CD47-PE
Mouse IgG1k-PE
EVs (Bodipy)
>500nm
7.40
500nm
3.09
~300nm
4.65
240nm
200nm
11.0
160nm
40.0
SSC-A
FSC-A

Figure S3

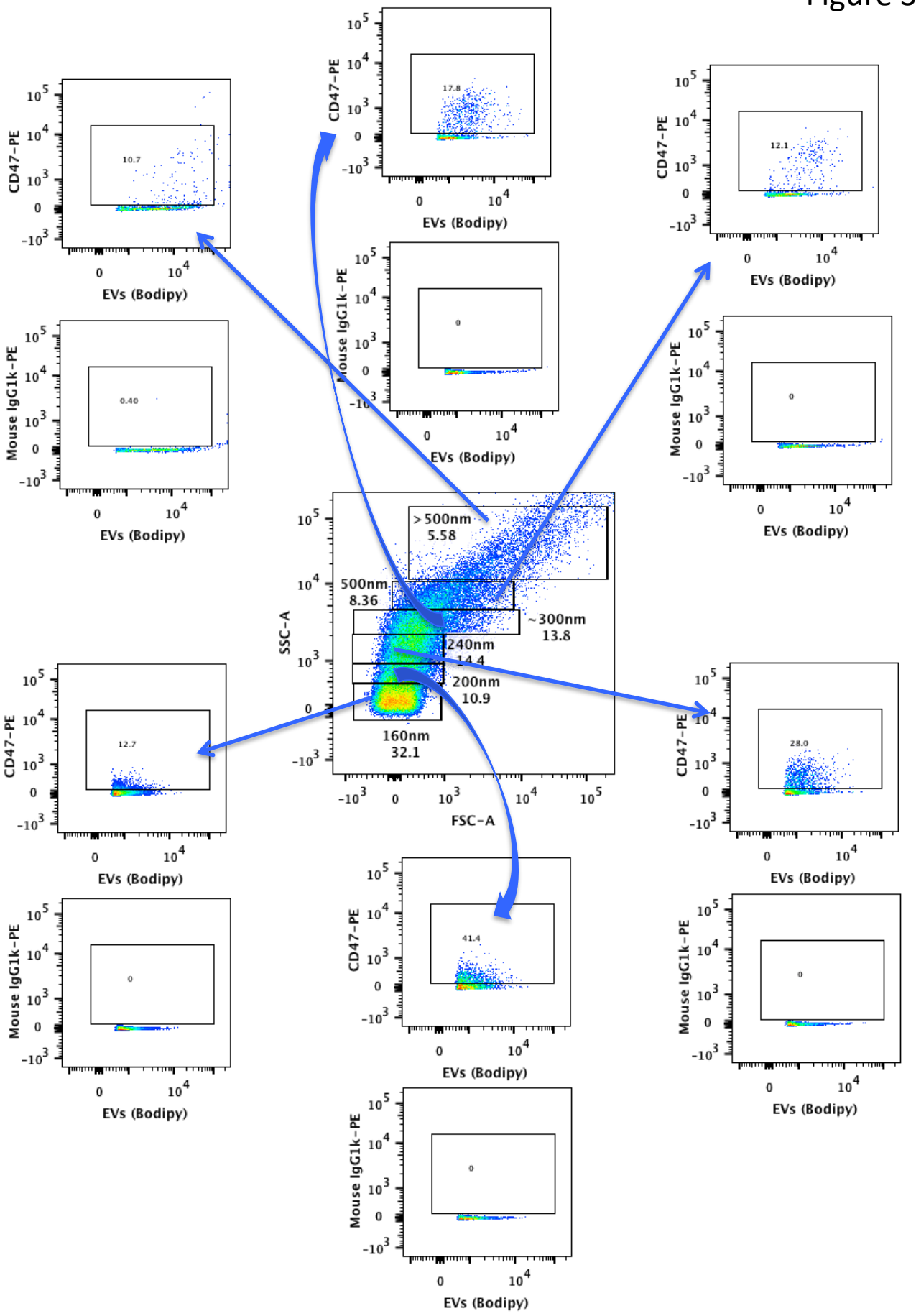
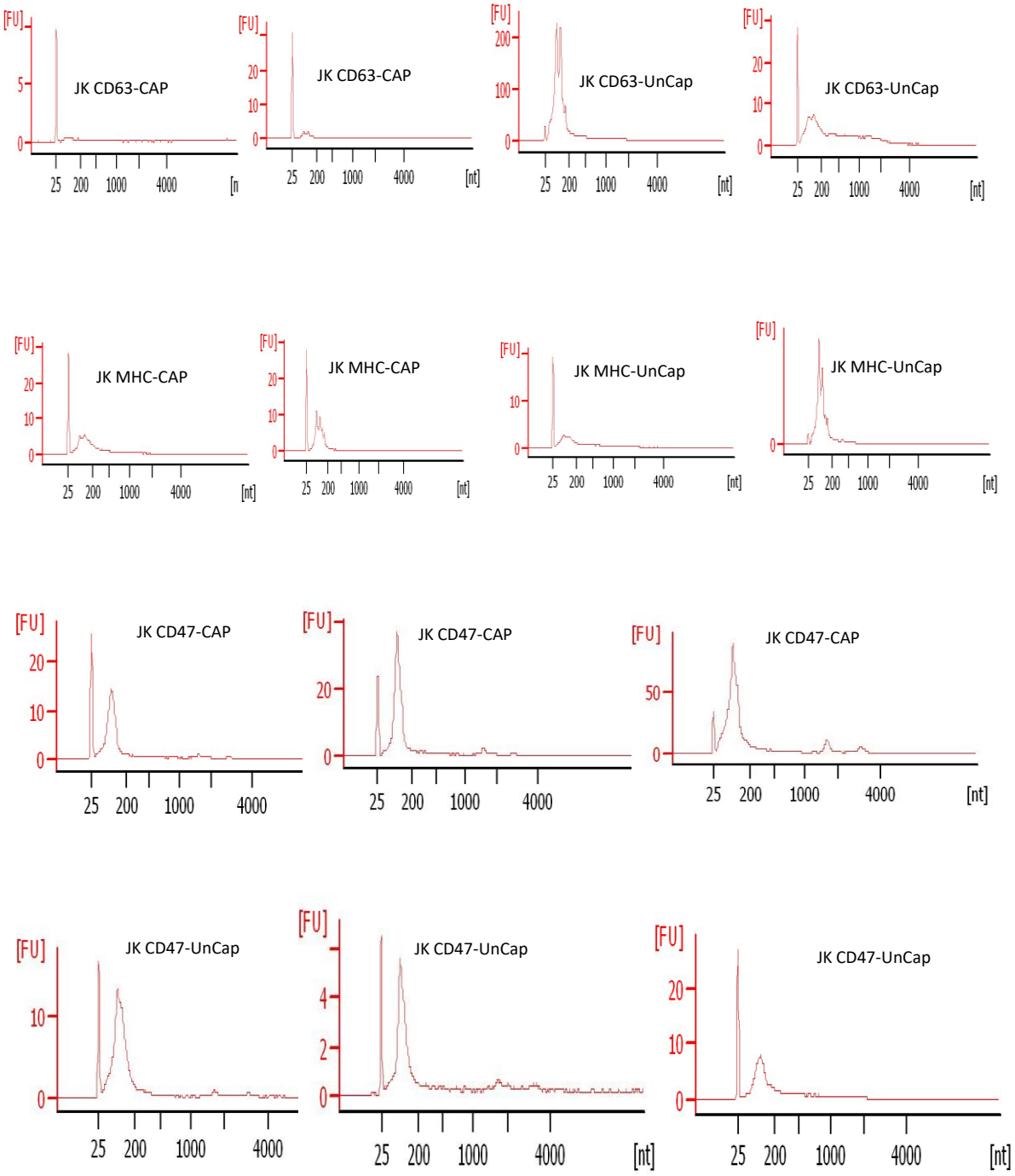

SSC-A
FSC-A
>500nm
5.58
500nm
8.36
~300nm
13.8
240nm
14.4
200nm
10.9
160nm
32.1
CD47-PE
Mouse IgG1k-PE
EVs (Bodipy)
17.8
10.7
12.1
0.40
0
12.7
41.4
28.0

Figure S4


[FU]
JK CD63-CAP
JK CD63-CAP
JK CD63-UnCap
JK CD63-UnCap
JK MHC-CAP
JK MHC-CAP
JK MHC-UnCap
JK MHC-UnCap
JK CD47-CAP
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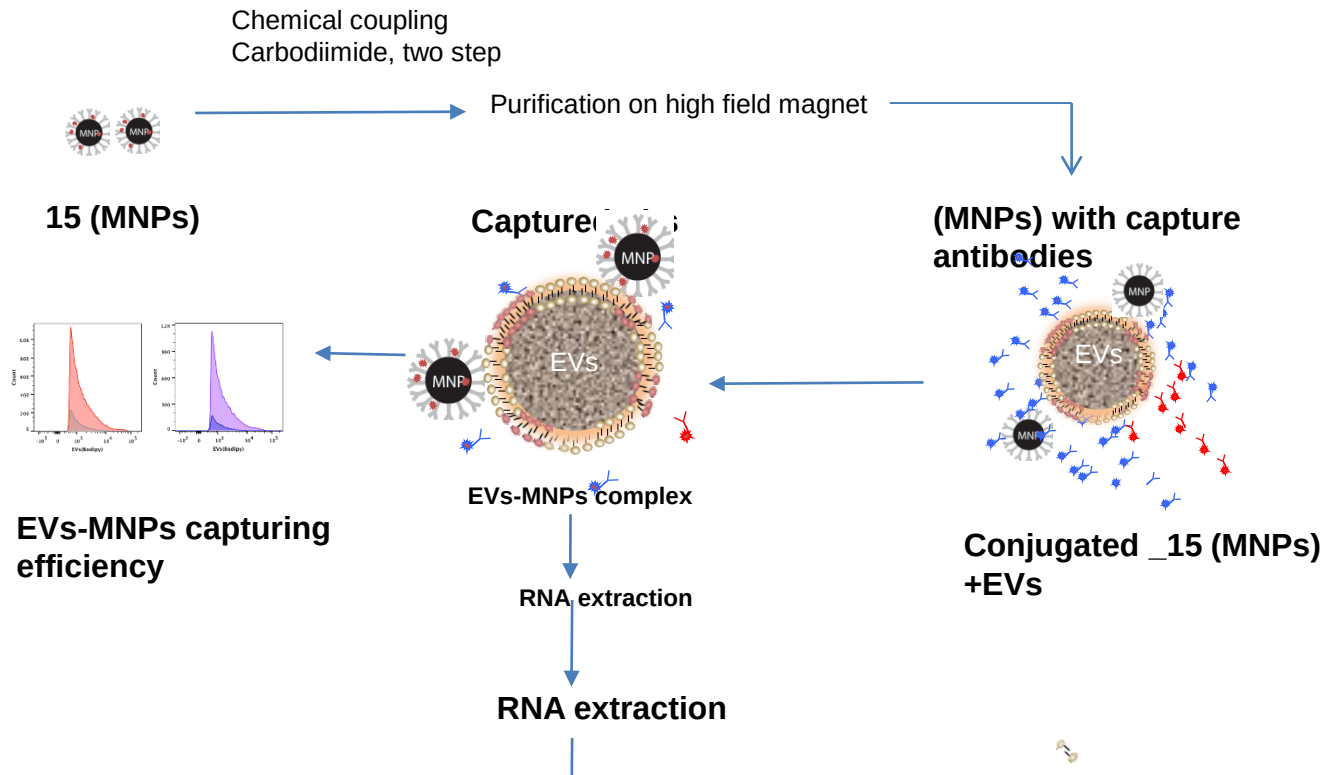
JK CD47-UnCap
JK CD47-UnCap
25 200 1000 4000
[nt]

Figure S5



RNA Sequencing

(12.5 million reads per sample with 75 bp reads and SMARTer small RNA-seq kit for Illumina NextSeq 500 platform

RNA assembly Work Flow

(I)

Global using human genome

mRNA
Non-coding

(II)

Small RNAs

lncRNA
tRNA
rRNA
SnoRNA& miscellaneous

(III)

miRNA sequencing using miRbase

Pre , pri and
Mature miRNA

Normalization methods

RPKM RPM RPM

Figure S6

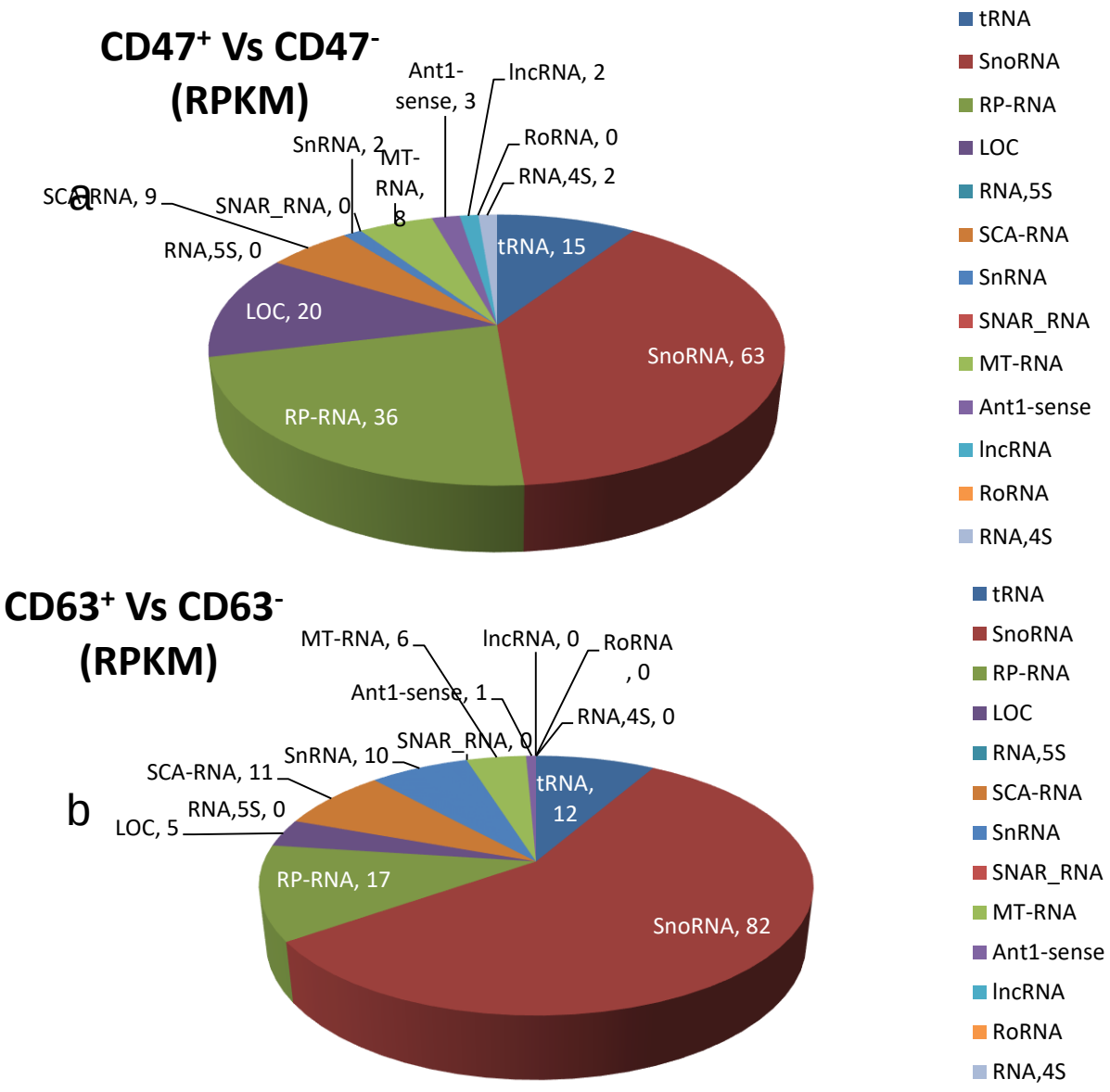

CD47+ Vs CD47-
(RPKM)
a
Ant1-sense, 3
lncRNA, 2
RoRNA, 0
RNA,4S, 2
SnRNA, 2
MT-RNA, 8
SCA-RNA, 9
SNAR_RNA, 0
RNA,5S, 0
tRNA, 15
LOC, 20
SnoRNA, 63
RP-RNA, 36
tRNA
SnoRNA
RP-RNA
LOC
RNA,5S
SCA-RNA
SnRNA
SNAR_RNA
MT-RNA
Ant1-sense
lncRNA
RoRNA
RNA,4S
CD63+ Vs CD63-
(RPKM)
b
MT-RNA, 6
lncRNA, 0
RoRNA, 0
Ant1-sense, 1
RNA,4S, 0
SNAR_RNA, 0
SnRNA, 10
SCA-RNA, 11
tRNA, 12
RNA,5S, 0
LOC, 5
RP-RNA, 17
SnoRNA, 82
tRNA
SnoRNA
RP-RNA
LOC
RNA,5S
SCA-RNA
SnRNA
SNAR_RNA
MT-RNA
Ant1-sense
lncRNA
RoRNA
RNA,4S
MHC1+ Vs MHC1-
(RPKM)
c
Ant1-sense, 11
RoRNA, 2
MT-RNA, 1
RNA,4S, 0
lncRNA, 8
SNAR_RNA, 1
SCA-RNA, 4
SnRNA, 1
tRNA, 22
RNA,5S, 0
SnoRNA, 44
RP-RNA, 18
LOC, 92
tRNA
SnoRNA
RP-RNA
LOC
RNA,5S
SCA-RNA
SnRNA
SNAR_RNA
MT-RNA
Ant1-sense
lncRNA
RoRNA
RNA,4S

Figure S7

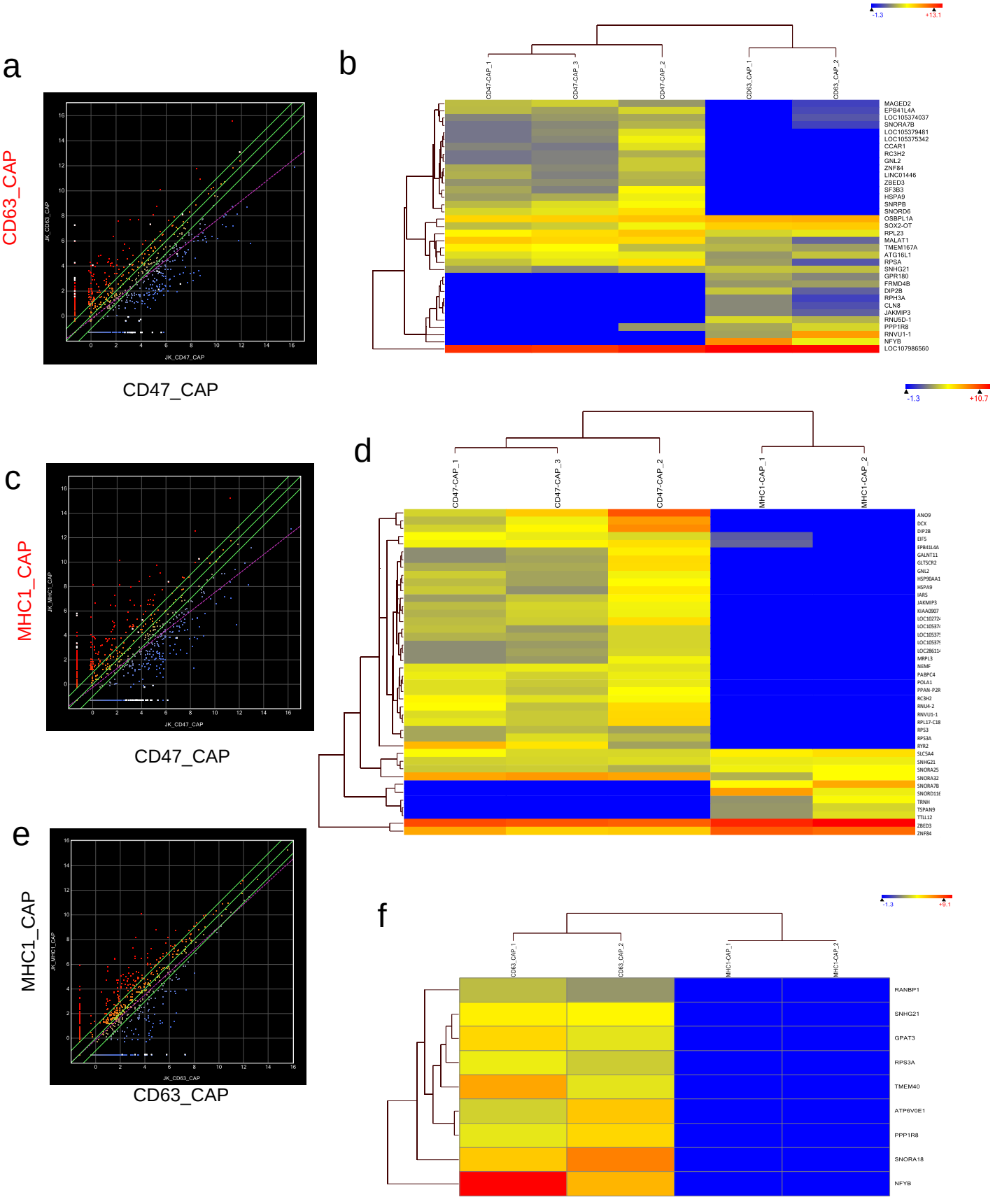
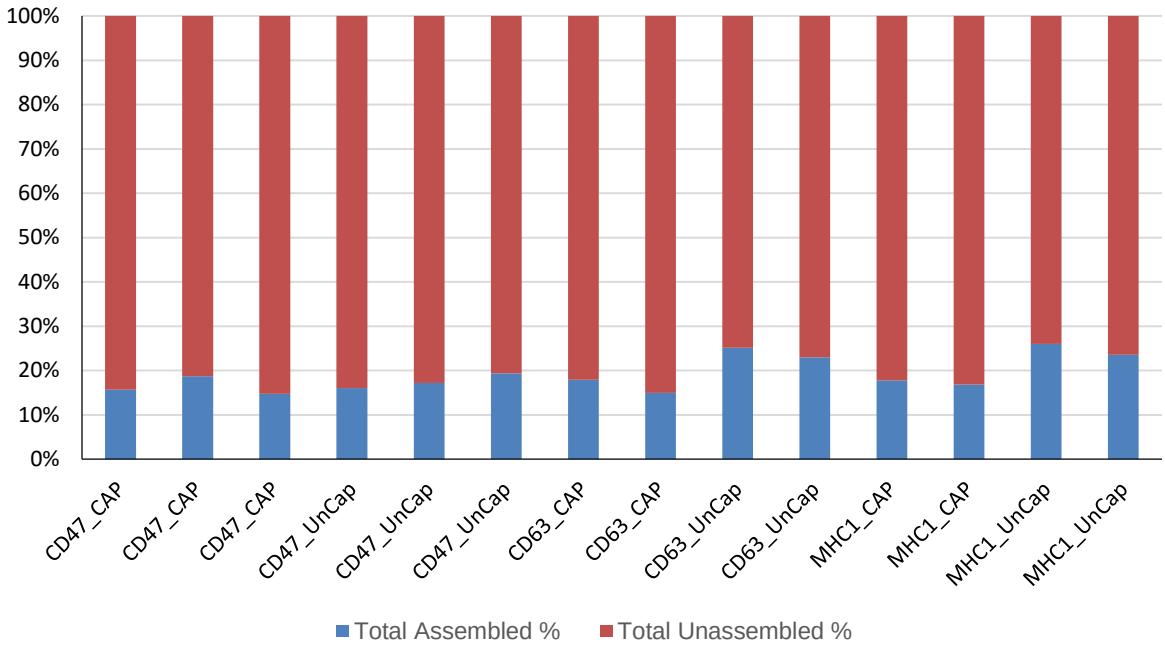

a
CD63_CAP
CD47_CAP
b
CD47-CAP_1
CD47-CAP_3
CD47-CAP_2
CD63_CAP_1
CD63_CAP_2
-1.3
+13.1
MAGED2
EPB41L4A
LOC105374037
SNORA7B
LOC105379481
LOC105375342
CCAR1
RC3H2
GNL2
ZNF84
LINC01446
ZBED3
SF3B3
HSPA9
SNRPB
SNORD6
OSBPL1A
SOX2-OT
RPL23
MALAT1
TMEM167A
ATG16L1
RPSA
SNHG21
GPR180
FRMD4B
DIP2B
RPH3A
CLN8
JAKMIP3
RNU5D-1
PPP1R8
RNVU1-1
NFYB
LOC107986560
c
MHC1_CAP
CD47_CAP
d
CD47-CAP_1
CD47-CAP_3
CD47-CAP_2
MHC1-CAP_1
MHC1-CAP_2
-1.3
+10.7
e
MHC1_CAP
CD63_CAP
f
CD63_CAP_1
CD63_CAP_2
MHC1-CAP_1
MHC1-CAP_2
-1.3
RANBP1
SNHG21
GPAT3
RPS3A
TMEM40
ATP6V0E1
PPP1R8
SNORA18
NFYB

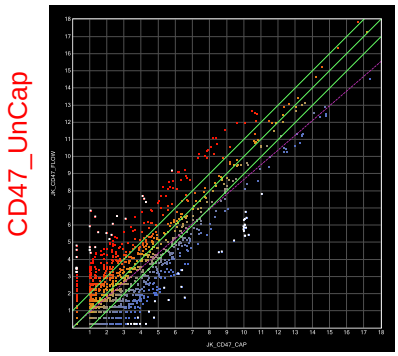
Figure S8

Method III

a

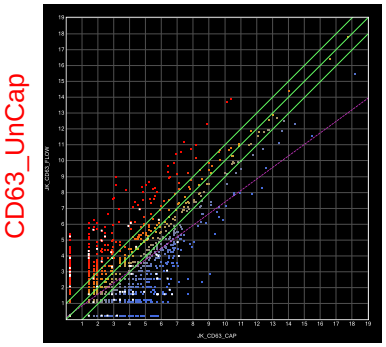


b



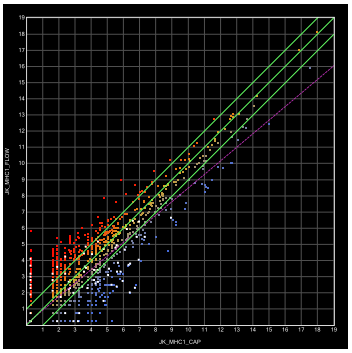
66miRNA @8-fold
1491miRNAs@2-fold

c



240miRNAs @8-fold
1971miRNAs@2-fold

d



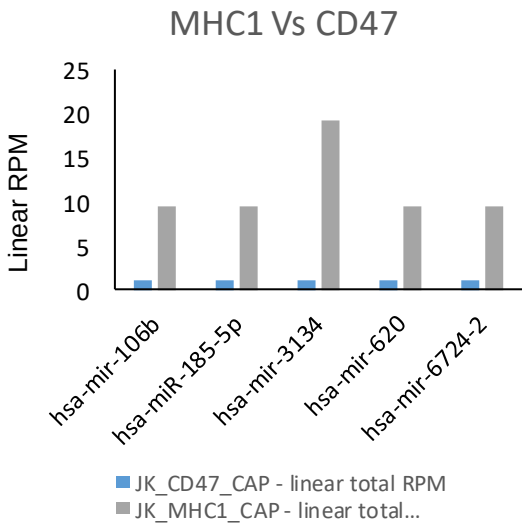
97miRNAs @8-fold
1768miRNAs@2-fold

Figure S9

a

	CD47_CAP - linear total RPM	CD63_CAP - linear total RPM	MHC1_CAP - linear total RPM
hsa-mir-106b	1.179	1.179	9.571
hsa-miR-142-3p	2.619	10.64	9.571
hsa-miR-185-5p	1.179	1.179	9.571
hsa-mir-3610	2.238	1.179	9.571
hsa-mir-620	1.179	1.179	9.571
hsa-mir-663b	7.304	2.718	9.571
hsa-mir-664b	21.369	4.616	9.571

b



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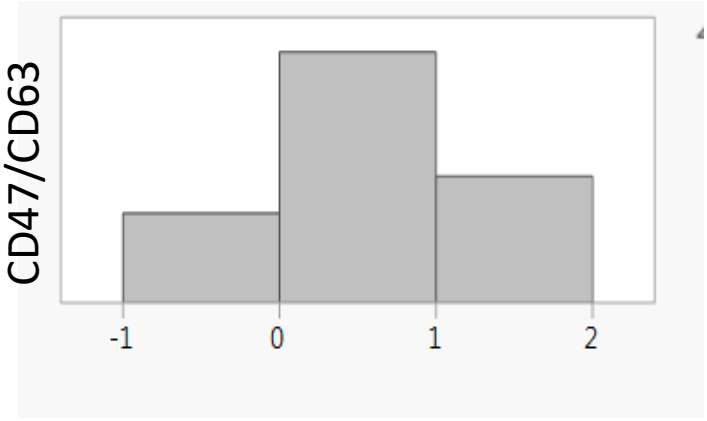

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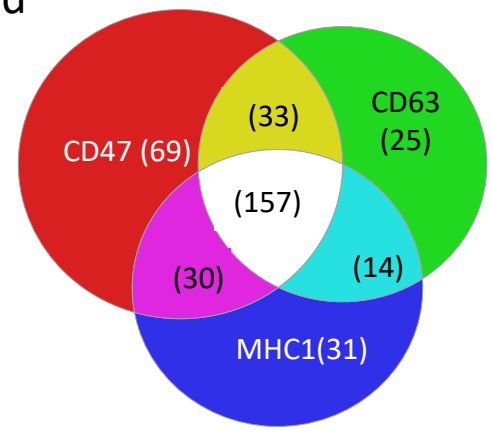

Long non-coding +small RNAs

Supplementary Excel file notes for Kaur et al

File Name: Table A _B_new Fig2_ Method I_RPKM

Contents

1. Data for Figure 2 pie charts (21 excel sheets) -Table-A
2. Data for FDR analysis with RPKM_Method (I)-Table B
3. Table S3 (Mapped and unmapped reads using Method (1)
4. Fig S6 2-fold change Captured vs uncaptured

File Name: Table S4_ Raw data

Contents

1. Table S4 (Mapped and unmapped reads using Method (III)