```
wtCD39            1 MEDTKESNVKTFCSKNILAILGFSSIIAVIALLAVGLTQNKALPENVKYG     50
                                                  ||||||||||||
CD39deltaMIL      1 -----------------------------------TQNKALPENVKYG     13

wtCD39           51 IVLDAGSSHTSLYIYKWPAEKENDTGVVHQVEECRVKGPGISKFVQKVNE    100
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL     14 IVLDAGSSHTSLYIYKWPAEKENDTGVVHQVEECRVKGPGISKFVQKVNE     63

wtCD39          101 IGIYLTDCMERAREVIPRSQHQETPVYLGATAGMRLLRMESEELADRVLD    150
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL     64 IGIYLTDCMERAREVIPRSQHQETPVYLGATAGMRLLRMESEELADRVLD    113

wtCD39          151 VVERSLSNYPFDFQGARIITGQEEGAYGWITINYLLGKFSQKTRWFSIVP    200
                    |||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL    114 VVERSLSNYPFDFQGARIITGQEEGAYGWITINYLLGKFSQK--------    155

wtCD39          201 YETNNQETFGALDLGGASTQVTFVPQNQTIESPDNALQFRLYGKDYNVYT    250
                        ||||||||||||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL    156 ----NQETFGALDLGGASTQVTFVPQNQTIESPDNALQFRLYGKDYNVYT    201

wtCD39          251 HSFLCYGKDQALWQKLAKDIQVASNEILRDPCFHPGYKKVVNVSDLYKTP    300
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL    202 HSFLCYGKDQALWQKLAKDIQVASNEILRDPCFHPGYKKVVNVSDLYKTP    251

wtCD39          301 CTKRFEMTLPFQQFEIQGIGNYQQCHQSILELFNTSYCPYSQCAFNGIFL    350
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL    252 CTKRFEMTLPFQQFEIQGIGNYQQCHQSILELFNTSYCPYSQCAFNGIFL    301

wtCD39          351 PPLQGDFGAFSAFYFVMKFLNLTSEKVSQEKVTEMMKKFCAQPWEEIKTS    400
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL    302 PPLQGDFGAFSAFYFVMKFLNLTSEKVSQEKVTEMMKKFCAQPWEEIKTS    351

wtCD39          401 YAGVKEKYLSEYCFSGTYILSLLLQGYHFTADSWEHIHFIGKIQGSDAGW    450
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
CD39deltaMIL    352 YAGVKEKYLSEYCFSGTYILSLLLQGYHFTADSWEHIHFIGKIQGSDAGW    401

wtCD39          451 TLGYMLNLTNMIPAEQPLSTPLSHSTYVFLMVLFSLVLFTVAIIGLLIFH    500
                    ||||||||||||||||||||||||||
CD39deltaMIL    402 TLGYMLNLTNMIPAEQPLSTPLSHST-------------------------    427

wtCD39          501 KPSYFWKDMV    510

CD39deltaMIL    427 ----------    427
```

FIG 1

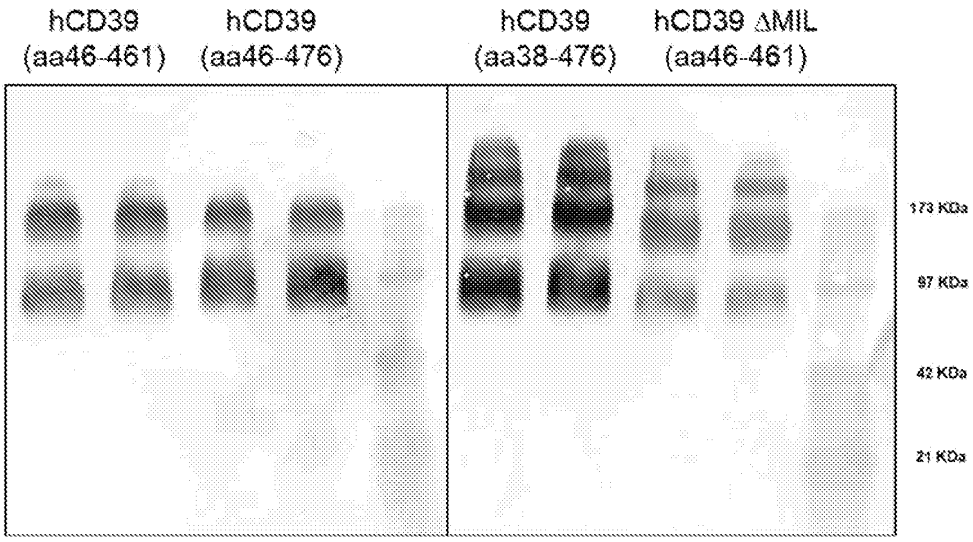

hCD39
(aa46-461)
hCD39
(aa46-476)
hCD39
(aa38-476)
hCD39 ΔMIL
(aa46-461)
173 KDa
97 KDa
42 KDa
21 KDa

FIG 2A

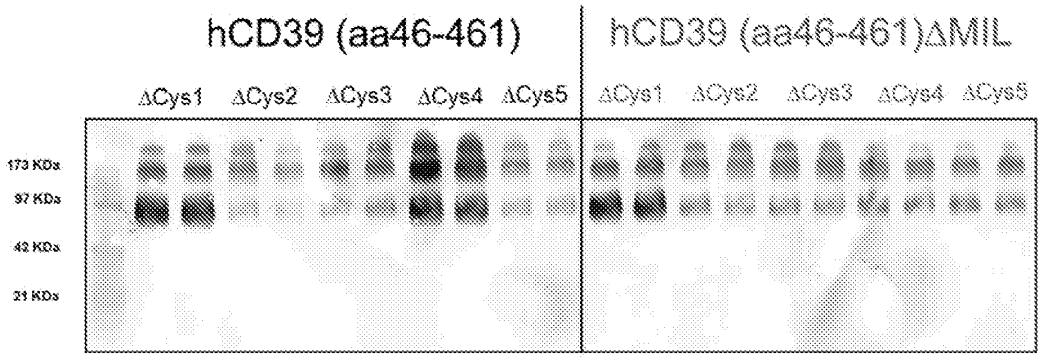

hCD39 (aa46-461)
hCD39 (aa46-461)ΔMIL
ΔCys1
ΔCys2
ΔCys3
ΔCys4
ΔCys5
ΔCys1
ΔCys2
ΔCys3
ΔCys4
ΔCys5
173 KDa
97 KDa
42 KDa
21 KDa

FIG 2B

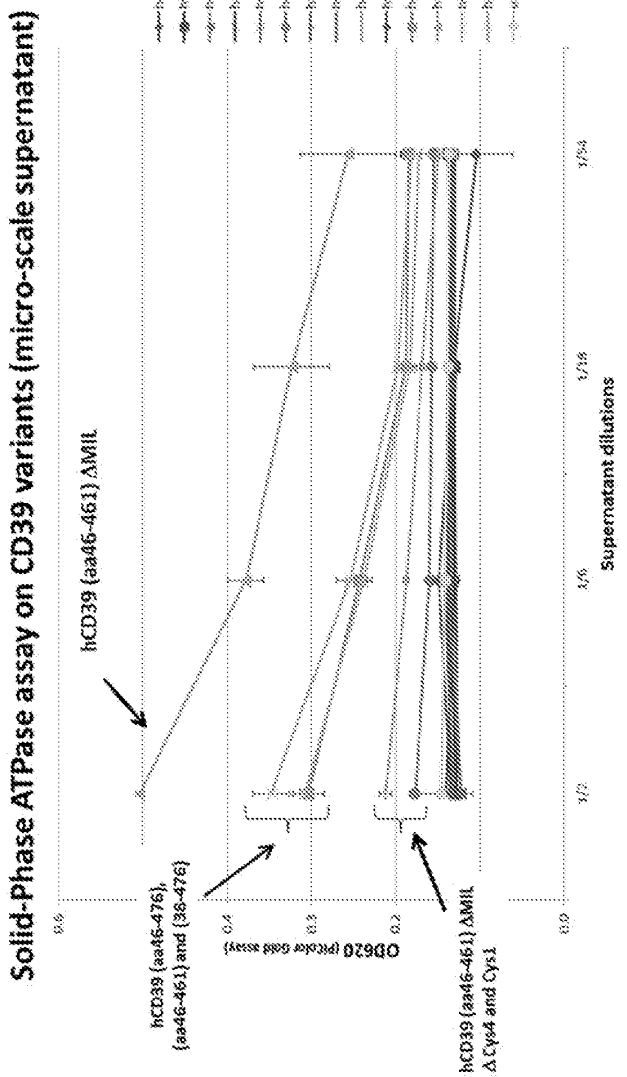

Solid-Phase ATPase assay on CD39 variants (micro-scale supernatant)
hCD39 (aa46-461) ΔMIL
hCD39 (aa46-476), (aa46-461) and (36-476)
hCD39 (aa46-461) ΔMIL Δ Cys4 and Cys1
OD620 (Pi color cold assay)
Supernatant dilutions
1/2
1/6
1/18
1/54
0.6
0.4
0.2
0.0

FIG 3

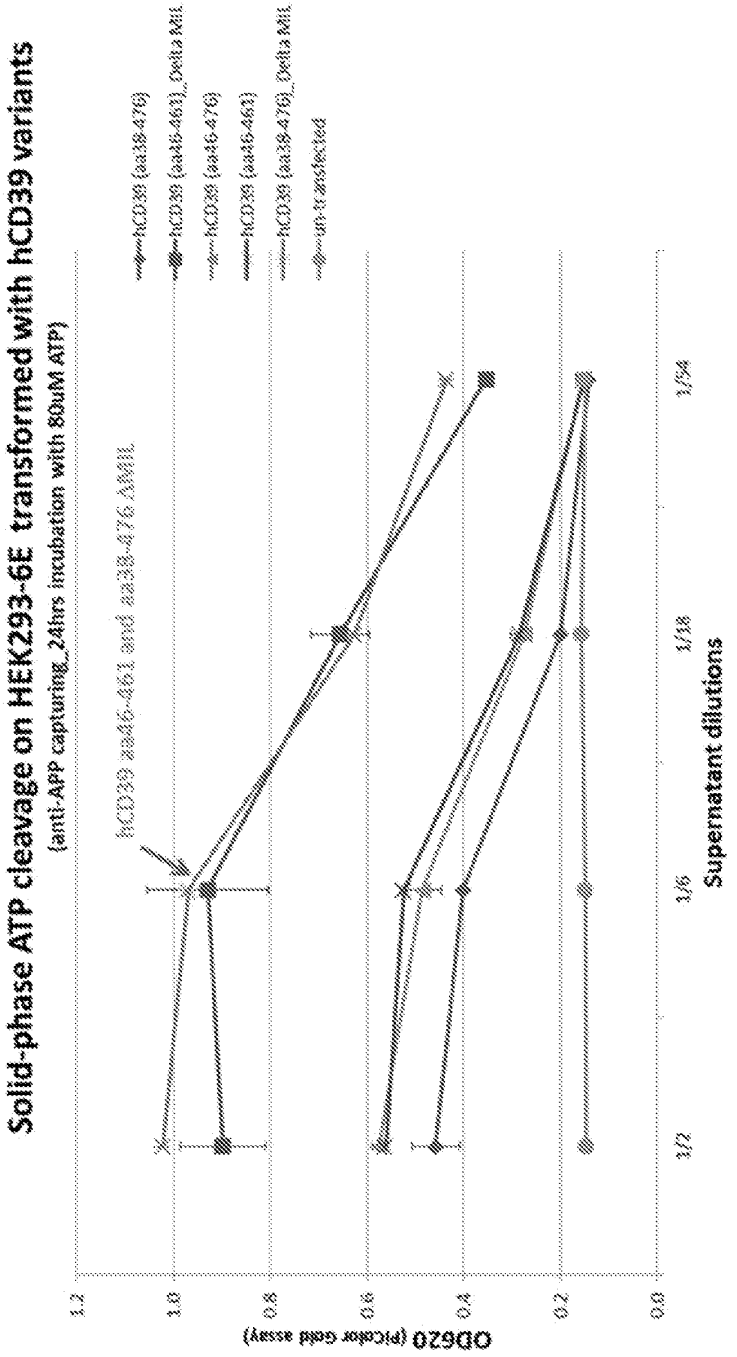

Solid-phase ATP cleavage on HEK293-6E transformed with hCD39 variants
(anti-APP capturing_24hrs incubation with 80uM ATP)
hCD39 aa46-461 and aa38-476 ΔMIL
hCD39 (aa38-476)
hCD39 (aa46-461)_Delta MIL
hCD39 (aa46-476)
hCD39 (aa46-461)
hCD39 (aa38-476)_Delta MIL
un-transfected
OD620 (Pierce Gold assay)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1/2
1/6
1/18
1/54
Supernatant dilutions

FIG 4

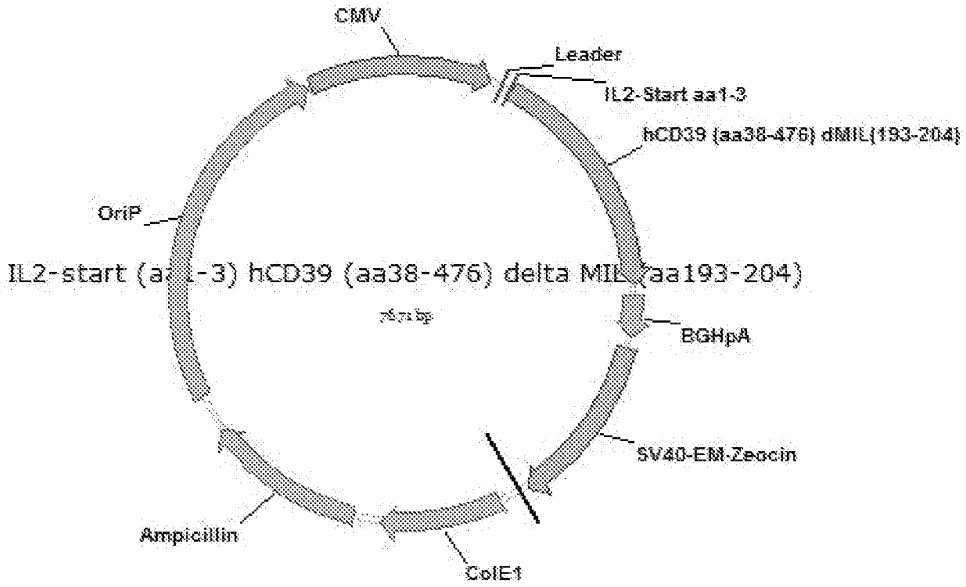

CMV
Leader
IL2-Start aa1-3
hCD39 (aa38-476) dMIL(193-204)
OriP
IL2-start (aa1-3) hCD39 (aa38-476) delta MIL (aa193-204)
BGHpA
SV40-EM-Zeocin
Ampicillin
ColE1

FIG 5

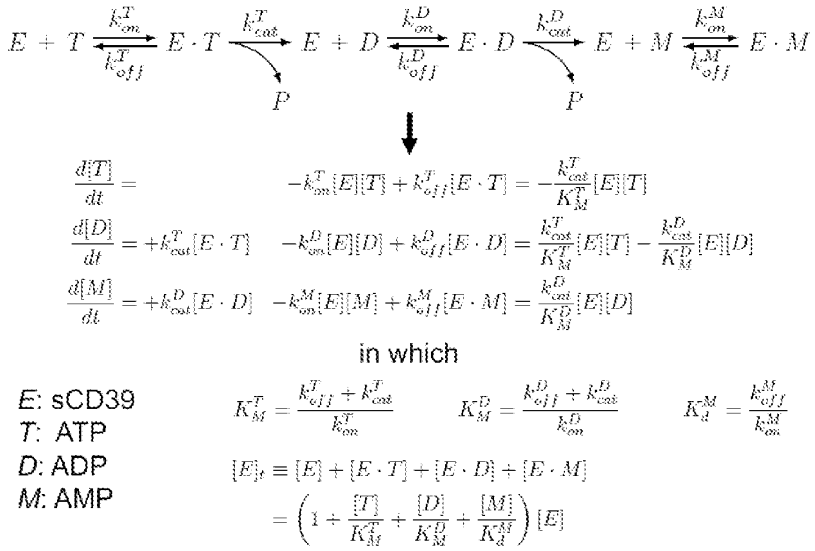
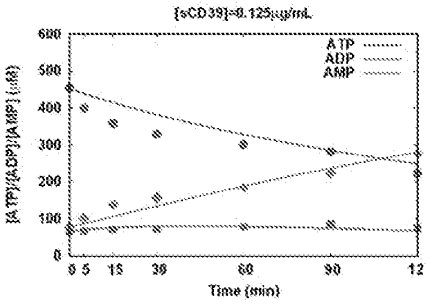
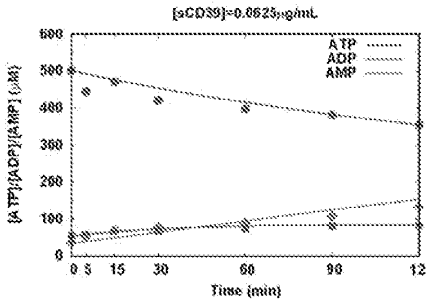
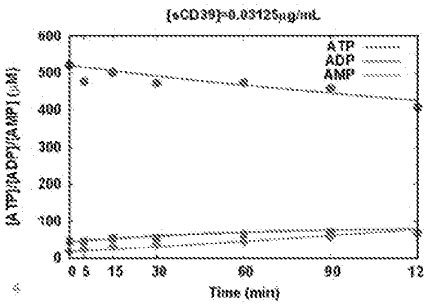

$E + T \underset{k^T_{off}}{\overset{k^T_{on}}{\rightleftharpoons}} E \cdot T \xrightarrow{k^T_{cat}} E + P \quad E + D \underset{k^D_{off}}{\overset{k^D_{on}}{\rightleftharpoons}} E \cdot D \xrightarrow{k^D_{cat}} E + P \quad E + M \underset{k^M_{off}}{\overset{k^M_{on}}{\rightleftharpoons}} E \cdot M$
$\frac{d[T]}{dt} = -k^T_{on}[E][T] + k^T_{off}[E \cdot T] = -\frac{k^T_{cat}}{K^T_M}[E][T]$
$\frac{d[D]}{dt} = +k^T_{cat}[E \cdot T] - k^D_{on}[E][D] + k^D_{off}[E \cdot D] = \frac{k^T_{cat}}{K^T_M}[E][T] - \frac{k^D_{cat}}{K^D_M}[E][D]$
$\frac{d[M]}{dt} = +k^D_{cat}[E \cdot D] - k^M_{on}[E][M] + k^M_{off}[E \cdot M] = \frac{k^D_{cat}}{K^D_M}[E][D]$
in which
E: sCD39
T: ATP
D: ADP
M: AMP
$K^T_M = \frac{k^T_{off} + k^T_{cat}}{k^T_{on}} \quad K^D_M = \frac{k^D_{off} + k^D_{cat}}{k^D_{on}} \quad K^M_d = \frac{k^M_{off}}{k^M_{on}}$
$[E]_t \equiv [E] + [E \cdot T] + [E \cdot D] + [E \cdot M]$
$= \left(1 + \frac{[T]}{K^T_M} + \frac{[D]}{K^D_M} + \frac{[M]}{K^M_d}\right)[E]$

FIG 6

The fitted enzymatic activity data of the wild type sCD39 (EP28)



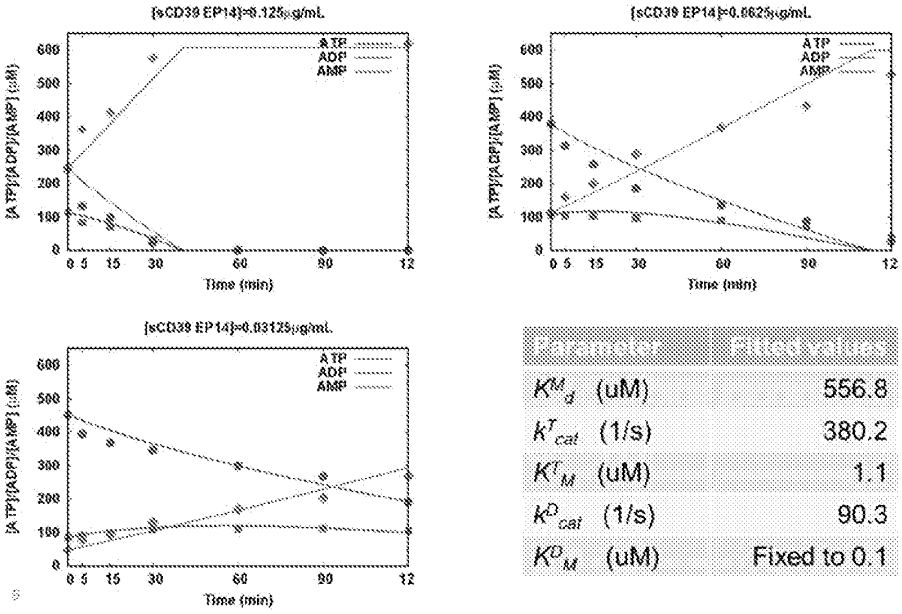




Parameter	Fitted values
K^{M}_{d} (uM)	0.2
k^{T}_{cat} (1/s)	65.3
K^{T}_{M} (uM)	0.7
k^{D}_{cat} (1/s)	40.0
K^{D}_{M} (uM)	0.1

FIG 7

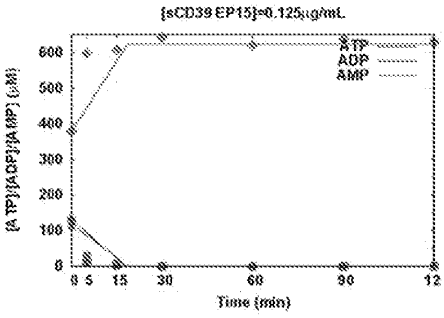
The fitted enzymatic activity data of the sCD39 mutant EP14

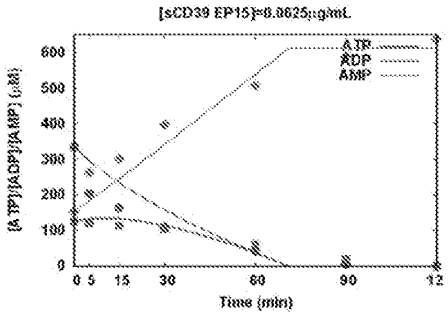


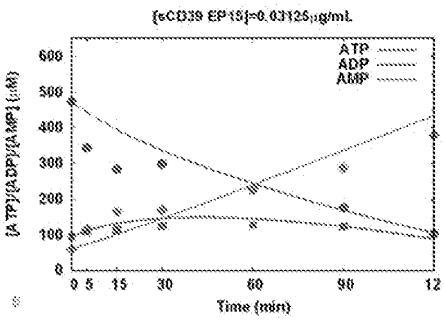
Parameter	Fitted values
K^M_d (uM)	556.8
k^T_{cat} (1/s)	380.2
K^T_M (uM)	1.1
k^D_{cat} (1/s)	90.3
K^D_M (uM)	Fixed to 0.1

FIG 8

The fitted enzymatic activity data of the sCD39 mutant EP15







Parameters	Fitted values
K^M_d (uM)	584.9
k^T_{cat} (1/s)	734.9
K^T_M (uM)	1.1
k^D_{cat} (1/s)	128.7
K^D_M (uM)	0.1

FIG 9

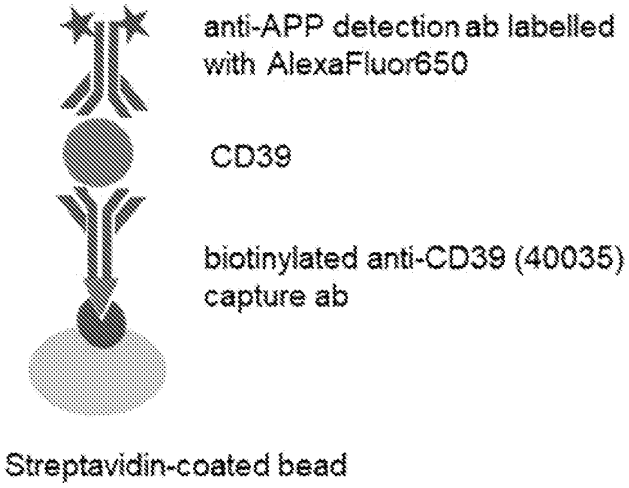


FIG 10A

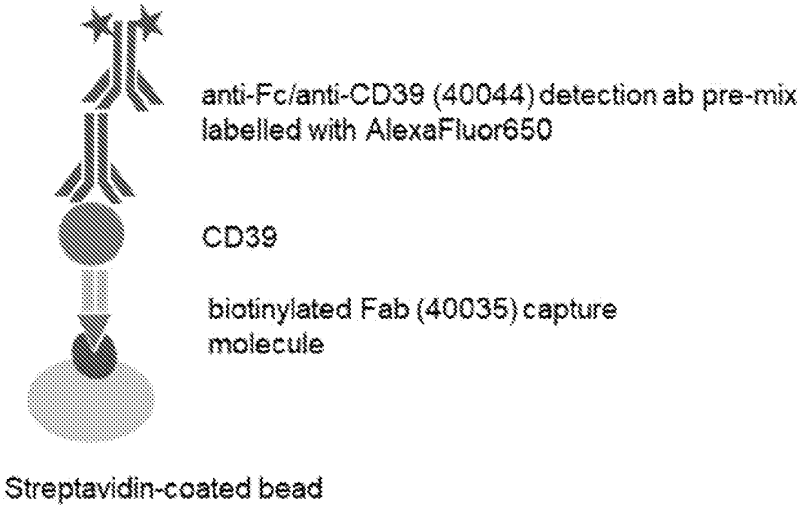


FIG 10B

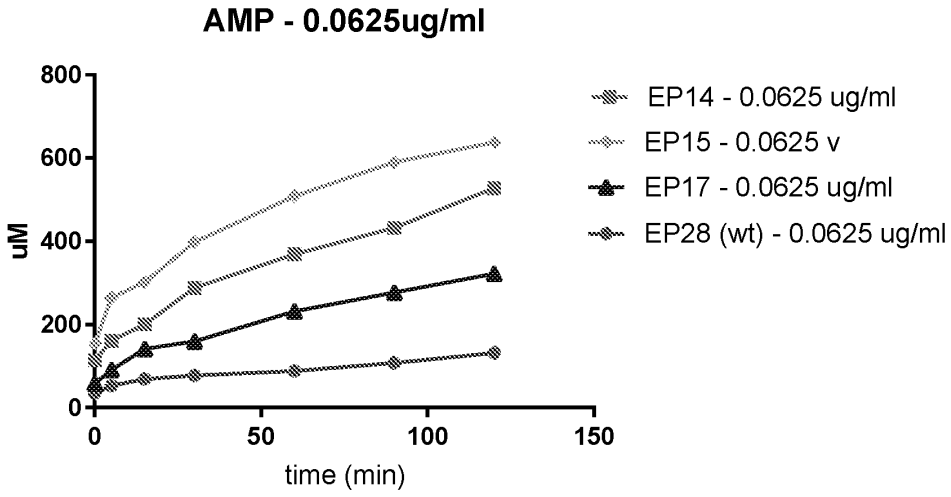

AMP - 0.0625ug/ml
800
600
400
200
0
uM
0
50
100
150
time (min)
EP14 - 0.0625 ug/ml
EP15 - 0.0625 v
EP17 - 0.0625 ug/ml
EP28 (wt) - 0.0625 ug/ml

FIG 11

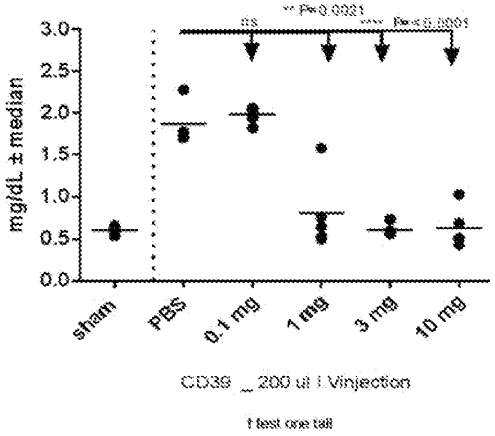


FIG 12A

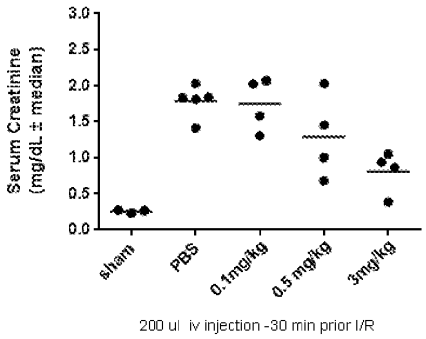


FIG 12B

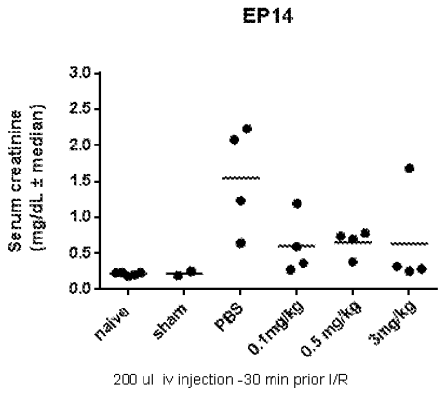


FIG 12C