Electronic Supplementary Material (ESI) for Nanoscale.

Supplemental Figures

Figure S1. Stability of lentivirus transduced DU145 cells. Lentivirally transduced cells were maintained in continuous culture with two-passages per week. At 6 weeks, or 15 weeks, TaqMan™ gene expression assays performed for the specified targets. Bars represent triplicate measures ±S.D., and compare relative target expression in non mammalian control (NM Ctrl) cells to targeted shRNA knockdown cells.

Figure S2. Confirmation of shRNA targeting of RAB11B and RAB35. Secretion of nano-vesicle was tested for an additional shRNA sequence targeting RAB11B or RAB35. NTA analysis revealed a similar, small mostly <200nm particulate population (A) with no significant difference in histogram mode (B). Examining particles per cell confirmed perturbation of secretion with RAB11B (shRNA sequence #558) or RAB34 (sequence #796) of 26% or 21% respectively. Bars represent mean±S.D of 6 replicates (*P<0.05, 1-way Anova with Tukey's post test).

Figure S3. Perturbations in late but not early endosomes. Du145 cells (NM Ctrl, RAB11B or RAB35 knockdowns) were seeded identically onto glass coverslip chamber slides, and at 48h were fixed and stained for markers of endosomes CD63 (A), LAMP1 (B), CD63 (C) or a marker of early/recycling endosomes EEA1 (D). Images were taken using structured illumination, using a 63x/1.4 numerical aperture oil-immersion objective. Shown are representative figures from z-axis sections overlaid to generate maximum projection images (Zeiss Z1 Observer, Cambridge, UK, running Zen Pro Software).

Figure S4. Protein Profiling Array. Samples were examined for differences in protein expression levels using a 92-plex protein array covering an assortment of growth factors, chemokines and other components associated with inflammation (Proseek Inflammation Panel, Olink). In cell conditioned media 46 analytes reported with signal below the assay limits of detection (LOD), and a further 8 fell below LOD for the vesicle concentrates. The Venn diagram, and array coverage lists identify those detected and not detected in each category.

Fig S1

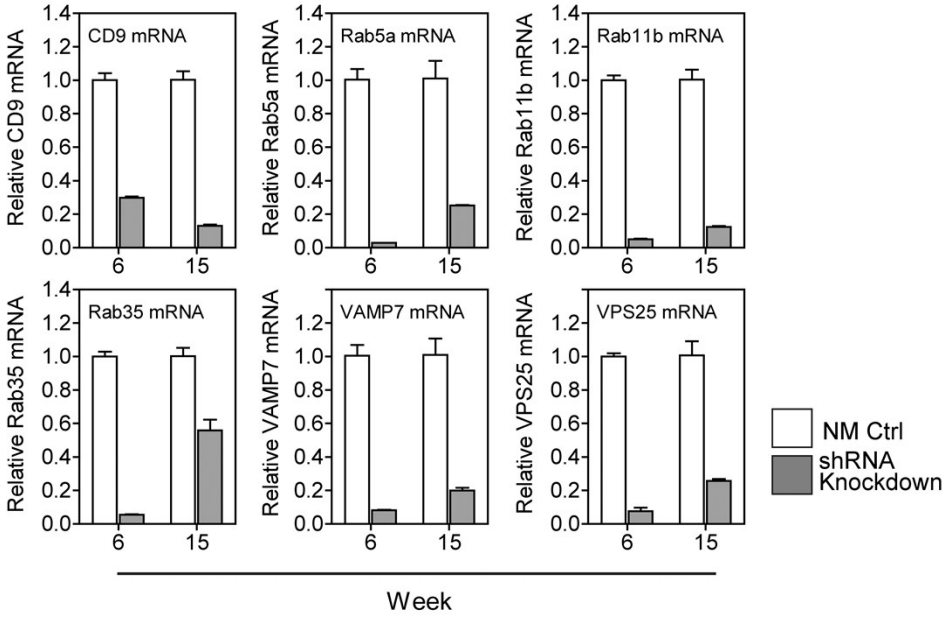


Fig S2.

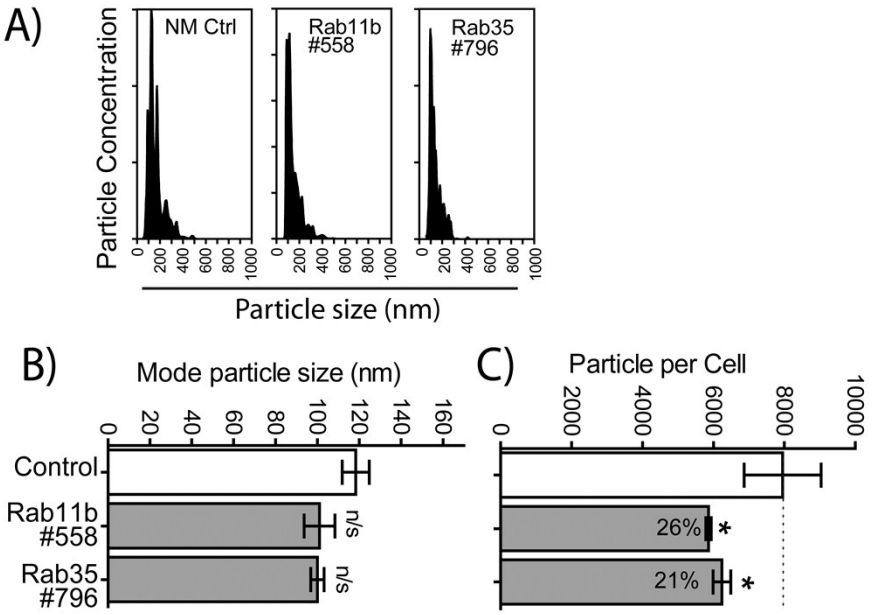

A)
NM Ctrl
Rab11b #558
Rab35 #796
Particle Concentration
0
200
400
600
800
1000
Particle size (nm)
B)
Mode particle size (nm)
0
20
40
60
80
100
120
140
160
Control
Rab11b #558
Rab35 #796
n/s
n/s
C)
Particle per Cell
0
2000
4000
6000
8000
10000
26%
*
21%
*

Fig S3

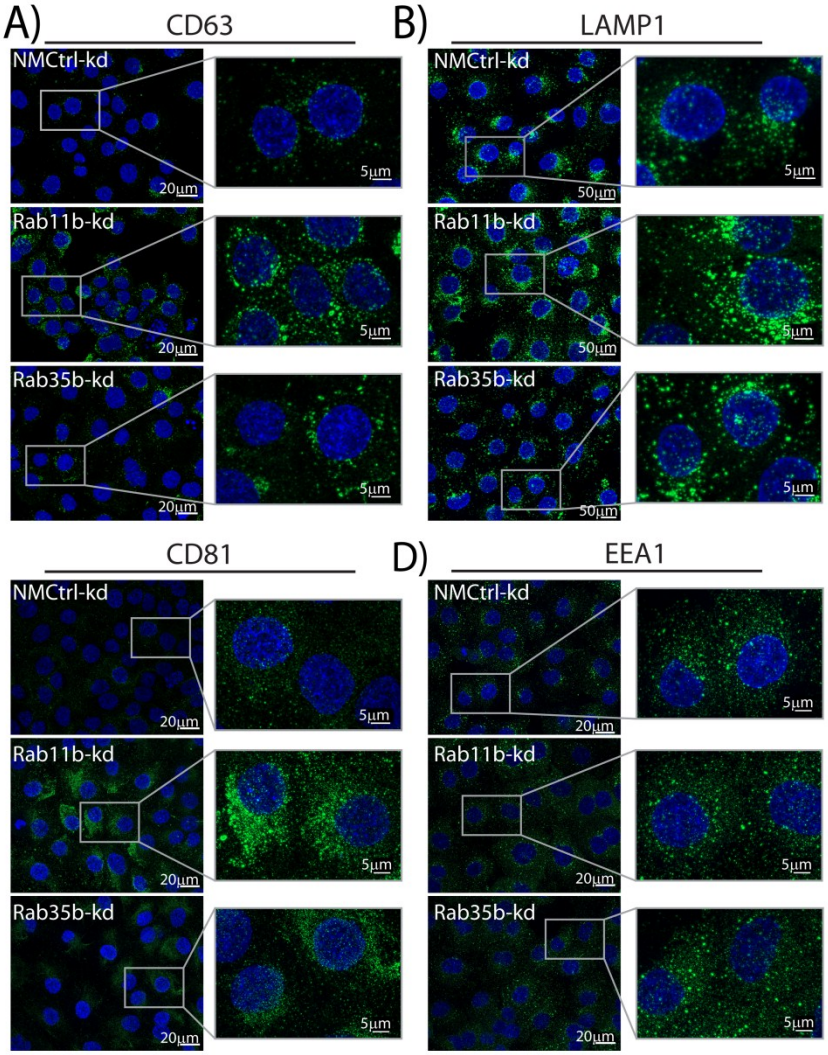
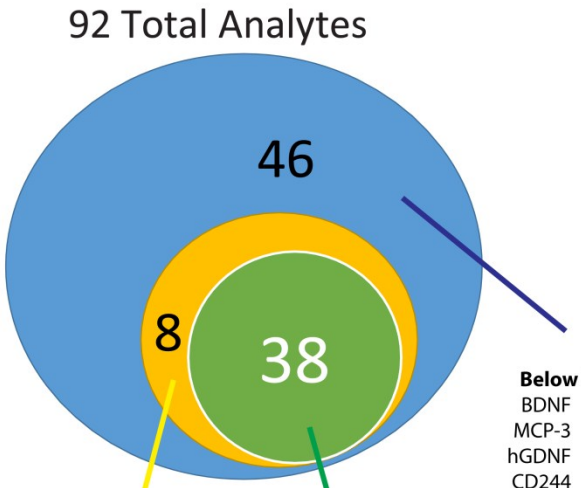

A)
CD63
NMCtrl-kd
Rab11b-kd
Rab35b-kd
20μm
5μm
B)
LAMP1
NMCtrl-kd
Rab11b-kd
Rab35b-kd
50μm
5μm
CD81
NMCtrl-kd
Rab11b-kd
Rab35b-kd
20μm
5μm
D)
EEA1
NMCtrl-kd
Rab11b-kd
Rab35b-kd
20μm
5μm

Fig S4.



In CM but below LOD in Pellet

IL-7
MCP-1
CCL4
FGF-21
IL-15RA
HGF
IL-12B
Flt3L

Proseek Multiplex Inflammation Panel

In CM and Pellet

IL-8	PD-L1
VEGF-A	Beta-NGF
CDCP1	CXCL5
OPG	IL-24
LAP TGF-beta-1	MMP-10
uPA	CXCL6
IL-6	CXCL10
CXCL11	4E-BP1
AXIN1	SIRT2
TRAIL	DNER
CST5	CD40
CXCL1	FGF-19
SCF	LIF
IL-18	CASP-8
GFA	TNFRSF9
MMP-1	TWEAK
IL-10RB	CCL20
IL-18R1	STAMPB
	ADA
	CSF-1

Below LOD

BDNF	TRANCE
MCP-3	IL-13
hGDNF	ARTN
CD244	IL-10
IL-17C	TNF
IL-17A	CCL23
IL-20RA	CD5
CXCL9	CCL3
IL-2RB	IL-20
IL-1 alpha	CCL28
OSM	EN-RAGE
IL-2	IL-33
TSLP	IFN-gamma
CD6	IL-4
SLAMF1	NRTN
MCP-4	MCP-2
CCL11	CCL25
TNFSF14	CX3CL1
FGF-23	NT-3
IL-10RA	ST1A1
FGF-5	IL-5
LIF-R	TNFB
CCL19	
IL-22 RA1	

Table S1

Target	TRC Number	Sequence	Clone ID
CD9	TRCN0000296953 (#953)	CCGGTTCTACACAGGAGTCTATATTCTCGAGAATATAGACTCCTGTGTAGAATTTTTG	NM_001769.2-277s21c1
	TRCN0000296958 (#958)	CCGGCCTGCAATGAAAGGTACTATACTCGAGTATAGTACCTTTCATTGCAGGTTTTTG	NM_001769.2-1072s21c1
	TRCN0000291711 (#711)	CCGGGCTGTTCGGATTTAACTTCATCTCGAGATGAAGTTAAATCCGAACAGCTTTTTG	NM_001769.2-150s21c1
	TRCN0000057469 (#469)	CCGGCATTGGACTATGGCTCCGATTCTCGAGAATCGGAGCCATAGTCCAATGTTTTTG	NM_001769.2-201s1c1
	TRCN0000057470 (#470)	**CCGGGCTGTTCGGATTTAACTTCATCTCGAGATGAAGTTAAATCCGAACAGCTTTTTG**	**NM_001769.2-150s1c1**
Rab5a	TRCN0000380597 (#597)	GTACCGGGAGAGTCCGCTGTTGGCAAATCTCGAGATTTGCCAACAGCGGACTCTCTTTTTTG	NM_004162.4-615s21c1
	TRCN0000380466 (#466)	**GTACCGGCAAGGCCGACCTAGCAAATAACTCGAGTTATTTGCTAGGTCGGCCTTGTTTTTTG**	**NM_004162.4-934s21c1**
	TRCN0000273641 (#641)	CCGGGCAGCCTTCCTTTCCAAAGTTCTCGAGAACTTTGGAAAGGAAGGCTGCTTTTTG	NM_004162.4-2298s21c1
	TRCN0000011215 (#215)	CCGGGCAGCCTTCCTTTCCAAAGTTCTCGAGAACTTTGGAAAGGAAGGCTGCTTTTT	NM_004162.3-2139s1c1
	TRCN0000007974 (#974)	CCGGCCAGGAATCAGTGTTGTAGTACTCGAGTACTACAACACTGATTCCTGGTTTTT	NM_004162.3-999s1c1
Rab11b	**TRCN0000381919 (#919)**	**GTACCGGTTTGCTGCACCCATGAAACTCCTCGAGGAGTTTCATGGGTGCAGCAAATTTTTTG**	**NM_004218.3-951s21c1**
	TRCN0000381558 (#558)	GTACCGGAGACAGCAACATCGTCATCATCTCGAGATGATGACGATGTTGCTGTCTTTTTTTG	NM_004218.3-435s21c1
	TRCN0000380618 (#618)	GTACCGGCATTCAAGAACATCCTCACAGCTCGAGCTGTGAGGATGTTCTTGAATGTTTTTTG	NM_004218.3-587s21c1
	TRCN0000029185 (#185)	CCGGCCTATTCAAAGTGGTGCTCATCTCGAGATGAGCACCACTTTGAATAGGTTTTT	NM_004218.1-36s1c1
	TRCN0000029188 (#188)	CCGGCAAGCACCTGACCTATGAGAACTCGAGTTCTCATAGGTCAGGTGCTTGTTTTT	NM_004218.1-288s1c1
Rab35	**TRCN0000380335 (#335)**	**GTACCGGTGATGATGTGTGCCGAATATTCTCGAGAATATTCGGCACACATCATCATTTTTTG**	**NM_006861.4-475s21c1**
	TRCN0000380080 (#080)	GTACCGGTTCACGAAATCAACCAGAACTCTCGAGAGTTCTGGTTGATTTCGTGAATTTTTTG	NM_006861.4-453s21c1
	TRCN0000380003 (#003)	GTACCGGGGAGAATGTCAACGTGGAAGACTCGAGTCTTCCACGTTGACATTCTCCTTTTTTG	NM_006861.4-601s21c1
	TRCN0000047796 (#796)	CCGGAGAGCAGTTTACTGTTGCGTTCTCGAGAACGCAACAGTAAACTGCTCTTTTTTG	NM_006861.4-207s1c1
	TRCN0000047794 (#794)	CCGGCCTCCGAGCAAAGAAAGACAACTCGAGTTGTCTTTCTTTGCTCGGAGGTTTTTG	NM_006861.4-649s1c1
VAMP7	TRCN0000379810 (#810)	GTACCGGATGAGAGAACAAGGAGTTAAACTCGAGTTTAACTCCTTGTTCTCTCATTTTTTTG	NM_005638.4-867s21c1
	TRCN0000298636 (#636)	CCGGGCGAGTTCTCAAGTGTCTTAGCTCGAGCTAAGACACTTGAGAACTCGCTTTTTG	NM_005638.4-485s21c1
	TRCN0000298637 (#637)	CCGGTCTTATGAGCTATCTACTAAACTCGAGTTTAGTAGATAGCTCATAAGATTTTTG	NM_005638.4-1157s21c1
	TRCN0000293928 (#928)	**CCGGGGAAAGAAGAAGTTACCATTACTCGAGTAATGGTAACTTCTTCTTTCCTTTTTG**	**NM_005638.4-837s21c1**
	TRCN0000059888 (#888)	CCGGGCGAGGAGAAAGATTGGAATTCTCGAGAATTCCAATCTTTCTCCTCGCTTTTTG	NM_005638.3-561s1c1
VPS25	TRCN0000381980 (#980)	GTACCGGAGTCCAGCTTCCTGATCATGTCTCGAGACATGATCAGGAAGCTGGACTTTTTTTG	NM_032353.2-321s21c1
	TRCN0000322763 (#763)	CCGGCCCTTTACTTCTTACCTCCCACTCGAGTGGGAGGTAAGAAGTAAAGGGTTTTTG	NM_032353.2-585s21c1
	TRCN0000322707 (#707)	CCGGAGTCGATCCAGATTGTATTAGCTCGAGCTAATACAATCTGGATCGACTTTTTTG	NM_032353.2-255s21c1
	TRCN0000144757 (#757)	**CCGGGAGTCGATCCAGATTGTATTACTCGAGTAATACAATCTGGATCGACTCTTTTTTG**	**NM_032353.2-254s1c1**
	TRCN0000143764 (#764)	CCGGCCTGTCTCCCTTTACTTCTTACTCGAGTAAGAAGTAAAGGGAGACAGGTTTTTTG	NM_032353.2-578s1c1