Supplementary movie legends

Included movies are separate files relevant to the study:
Movies S1-S4 show the advantage of mNeonGreen-Rab11a / mCherry-Rab11b double DExCon (A2780) cells for fluorescence live-cell imaging microscopy (Spinning Disc 3i). Movies S5-S7 show spatiotemporal control of mNeonGreen-Rab25 LUXon cells (A2780) with silenced Rab25 re-activated from genome using blue light (Spinning Disc 3i). Movies S8-S12 show triple knock in A2780 cells (mNeonGreen-Rab11a; mCherry-Rab11b; mTagBFP2-Rab25 DExCon) treated by dox (>94 h); D-F) recycling Alexa-647 labelled Transferrin (Tr647; 30 minutes). Imaging by AiryScan LSM880 or by 3i Lattice LightSheet microscope. Movies S13-S15 and S17 show protein expression kinetics of Rab11s DExCons and degradation kinetics of Rab11s DExogrons (Incucyte® S3 system). Movie S16 shows wound healing experiment automatically imaged and analysed in real time by Incucyte® S3 system of cells as indicated (Rab11s DExCons / DExogrons; shRNA anti-Rab11a or b; A2780).

Supplementary movie S1

Timelapse (Spinning disc 3i, 63x) of mNeonGreen-Rab11a / mCherry-Rab11b double DExCon cells (A2780, dox for 24h) treated by siR-Tubulin (400 nM). Timelapse covers total 5 min with frame taken every 2.67s (approximately 10.3s elapsed time per second of the movie); µ-Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated; Opti-Klear™ Live Cell Imaging Buffer). Selected frame from this movie is shown in S3D.

Supplementary movie S2

Timelapse (Spinning disc 3i, 63x) of mNeonGreen-Rab11a / mCherry-Rab11b double DExCon cells (A2780, dox for 24h) stably expressing LifeActin-iRFP670. Timelapse covers total 7 min with frame taken every 2s (approximately 14.5s elapsed time per second of the movie); µ-Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated; Opti-Klear™ Live Cell Imaging Buffer). Selected frame from this movie is shown in S3D.

Supplementary movie S3

Timelapse (Spinning disc 3i, 63x) of mNeonGreen-Rab11a / mCherry-Rab11b double DExCon cells (A2780, dox for 24h) recycling ALEXA-647 labelled Transferrin (25 ug/ml). Timelapse covers total 5 min with frame taken every 2.67s (approximately 16.2s elapsed time per second of the movie); µ-Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated; Opti-Klear™ Live Cell Imaging Buffer). Selected frame from this movie is shown in S3E.

Supplementary movie S4

Timelapse (Spinning disc 3i, 63x) of mNeonGreen-Rab11a / mCherry-Rab11b double knock in A2780 cells ± TRE3GS promoter (DExCon; ±dox 24h) migrating in Cell Derived Matrix (3D). Timelapse covers total 2.25 min (left) or 4.4 min (right) with frame taken every ~1.5s for both (approximately 10.1s elapsed time per second of the movie); µ-Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated; Opti-Klear™ Live Cell Imaging Buffer). Selected frames from this movie are shown in S3F-G.

Supplementary movie S5

Timelapse (Spinning disc 3i, 63x) of mNeonGreen-Rab25 LUXon cells (Rab25 as green) expressing PA-Tet-OFF with mCherry-NLS reporter (red) 18h after being illuminated by blue light (10 hours). Timelapse covers total 1 min with frame taken every 1s (approximately 6.7s elapsed time per second of the movie); µ-Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated; Opti-Klear™ Live Cell Imaging Buffer). Selected frame from this movie is shown in 3H.

Supplementary movie S6

3D projection (Spinning disc 3i, 63x) of mNeonGreen-Rab25 LUXon cells (Rab25 as green) expressing PA-Tet-OFF with mCherry-NLS reporter (red) 18h after being illuminated by blue light (10 hours). µ-

Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated). Selected frame from this movie is shown in 3H.

Supplementary movie S7

3D projection (Spinning disc 3i, 63x) of mNeonGreen-Rab25 LUXon cells (Rab25 as blue) expressing PA-Tet-OFF with mCherry-NLS reporter (red) 20h after being spatiotemporally illuminated by blue light (10 hours). Cells are invading to cell-free collagen matrix labelled by FN-647 (green) while being illuminated by blue light of varying intensity (see experimental set-up, right corner). µ-Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated) partly covered by black plasticine. Selected frame from this movie is shown in 3J.

Supplementary movie S8

3D projection of Triple knock in A2780 cells (mNeonGreen-Rab11a; mCherry-Rab11b; mTagBFP2-Rab25 DExCon) treated by dox (>94 h). µ-Plate 96 Well Black (Ibidi cat.No 89626; #1.5 polymer coverslip, tissue culture treated; Opti-Klear™ Live Cell Imaging Buffer). AiryScan LSM880 (63x). Selected frames from this movie are shown in S5B.

Supplementary movie S9

3D rendered model (ZEN black software) of Triple knock in A2780 cells (mNeonGreen-Rab11a; mCherry-Rab11b; mTagBFP2-Rab25 DExCon) treated by dox (>94 h). Cells were imaged live while recycling Alexa-647 labelled Transferrin (30 minutes) on glass-bottom dish (MatTek, Ashland, MA, USA; Opti-Klear™ Live Cell Imaging Buffer) coated with 10 µg/ml FN using AiryScan LSM880 (63x). Selected frames from this movie, raw un-rendered, are shown in 4C.

Supplementary movie S10

3D projection of Triple knock in A2780 cells (mNeonGreen-Rab11a; mCherry-Rab11b; mTagBFP2-Rab25 DExCon) treated by dox (>94 h). Cells were imaged live while recycling Alexa-647 labelled Transferrin (30 minutes) and migrating in Cell Derived Matrix (3D). AiryScan LSM880 (63x); Glass-bottom dish (MatTek, Ashland, MA, USA); Opti-Klear™ Live Cell Imaging Buffer). Selected frames from this movie are shown in 4C.

Supplementary movie S11

Triple knock in A2780 cells (mNeonGreen-Rab11a; mCherry-Rab11b; mTagBFP2-Rab25 DExCon) treated by dox (>94 h). Cells were imaged live while recycling Alexa-647 labelled Transferrin (30-60 min) on FN-coated 5 mm coverslip. 3D projections and optical sections are shown. Opti-Klear™ Live Cell Imaging Buffer; 3i Lattice LightSheet microscope. Selected frames from this movie are shown in 4F and S5E.

Supplementary movie S12

Triple knock in A2780 cells (mNeonGreen-Rab11a; mCherry-Rab11b; mTagBFP2-Rab25 DExCon) treated by dox (>94 h) with Alexa-647 labelled Transferrin recycled for 30 min. Cells were imaged fixed on FN-coated 5 mm coverslip. 3D projection animation generated using Imaris Cell Imaging Software. Opti-Klear™ Live Cell Imaging Buffer; 3i Lattice LightSheet microscope. Selected frames from this animation are shown in S5F.

Supplementary movie S13

Timelapse (Incucyte® S3 system, 20x) of protein expression kinetics of mNeonGreen-Rab11a / mCherry-Rab11b or mNeonGreen-Rab11b / mCherry-Rab11b double DExCon cells (A2780) treated by dox (from time 0). Timelapse covers total 49 h with frame taken every 30 min (approximately 30 min elapsed time per second of the movie); 96 well tissue culture plates (Corning); RPMI fenol-free media. Selected frames from this movie are shown in 5G.

Supplementary movie S14

Timelapse (Incucyte® S3 system, 20x) of expression and degradation kinetics of miniIAA7-mCherry-Rab11b DExogron cells (A2780, comparison with the classical endogenous mCherry-Rab11b tagging) treated by ±dox ±IAA (from time 0 or cells pre-treated 24h with dox). Timelapse (mCherry channel is shown) covers total 49 h with frame taken every 30 min (approximately 30 min elapsed time per second of the movie); 96 well tissue culture plates (Corning); RPMI fenol-free media. Selected frames from this movie are shown in 6J.

Supplementary movie S15

Timelapse (Incucyte® S3 system, 20x) of expression and degradation kinetics of miniIAA7-mCherry-Rab11b DExogron / mNeonGreen-Rab11a DExCon cells (A2780, comparison with the classical double endogenous mCherry/mNeonGreen-Rab11b/Rab11a tagging) treated by ±dox ±IAA (from time 0 or cells pre-treated 24h with dox). Timelapse (merge of mCherry/mNeonGreen channel is shown) covers total 49 h with frame taken every 30 min (approximately 30 min elapsed time per second of the movie); 96 well tissue culture plates (Corning); RPMI fenol-free media. Selected frames from this movie are shown in S10A.

Supplementary movie S16

A2780 (Ctrl; stably expressing AtAFBP2); Rab11s DExCons / DExogrons cells or cells with shRNA anti-Rab11a or b as indicated. Wound healing experiment automatically imaged and analysed in real time by Incucyte® S3 system (blue mask determined based on brightfield image taken at time 0 and every other frame taken after the scratch). Confluent cells were pre-treated for 24 ± dox (250 ng/ml) ± IAA (100 µg/ml), scratch by wound the WoundMaker™ and imaged in RPMI fenol free media ± dox/IAA as indicated. Timelapse (merge of brightfield with highlighted blue mask and true mNeonGreen/mCherry fluorescence) covers total 24 h with frame taken every 1h (approximately 1h elapsed time per second of the movie); ImageLock 96-well Plates; RPMI fenol-free media. Selected frames from this movie are shown in S9A.

Supplementary movie S17

Timelapse (Incucyte® S3 system, 20x) of expression and degradation kinetics of miniIAA7-mNeonGreen-Rab11a DExogron / mCherry-Rab11b DExCon cells (clone of A2780, comparison with the classical double endogenous mCherry/mNeonGreen-Rab11b/Rab11a tagging) treated by ±dox ±IAA (from time 0 or cells pre-treated 24h with dox). Timelapse (merge of mCherry/mNeonGreen channel is shown) covers total 49 h with frame taken every 30 min (approximately 30 min elapsed time per second of the movie); 96 well tissue culture plates (Corning); RPMI fenol-free media. Selected frames from this movie are shown in S11B.