

**Supplementary Table 1:** related to Supplementary Figure 3a, intensity of EGFR bands from cell fractionations

Fractions	PM	Cyto	NM	NP	Total	Cytoplasmic / Total EGFR (%)
Control	68.40	28.36	5.93	2.67	105.6	
EGF	63.54	63.87	9.32	3.44	140.2	45.6
Noc+EGF	54.67	64.82	3.36	3.04	125.9	

**Supplementary Table 2:** related to Supplementary Figure 1b, intensity of EGFR bands from density gradient centrifugation of cytoplasmic fraction

#	0 mim	10 min	20 min	90 min
5	5.57	0.62	1.42	0.19
6	10.08	1.35	1.66	0.05
7	4.46	6.96	4.27	0.02
8	6.67	22.79	9.53	0.53
9	4.56	26.50	28.71	1.06
10	2.09	19.57	20.85	13.73
11	4.17	6.53	6.38	13.19
12	1.76	3.36	10.56	-0.11
13	0.00	0.00	8.33	-0.14
14	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00
Total intensity of cytoplasmic EGFR	39.35	87.68	91.72	28.51
Fraction 9 / Cytoplasmic EGFR (%)	12	30	31	4
EGF-induced EGFR on Golgi (%)			19	

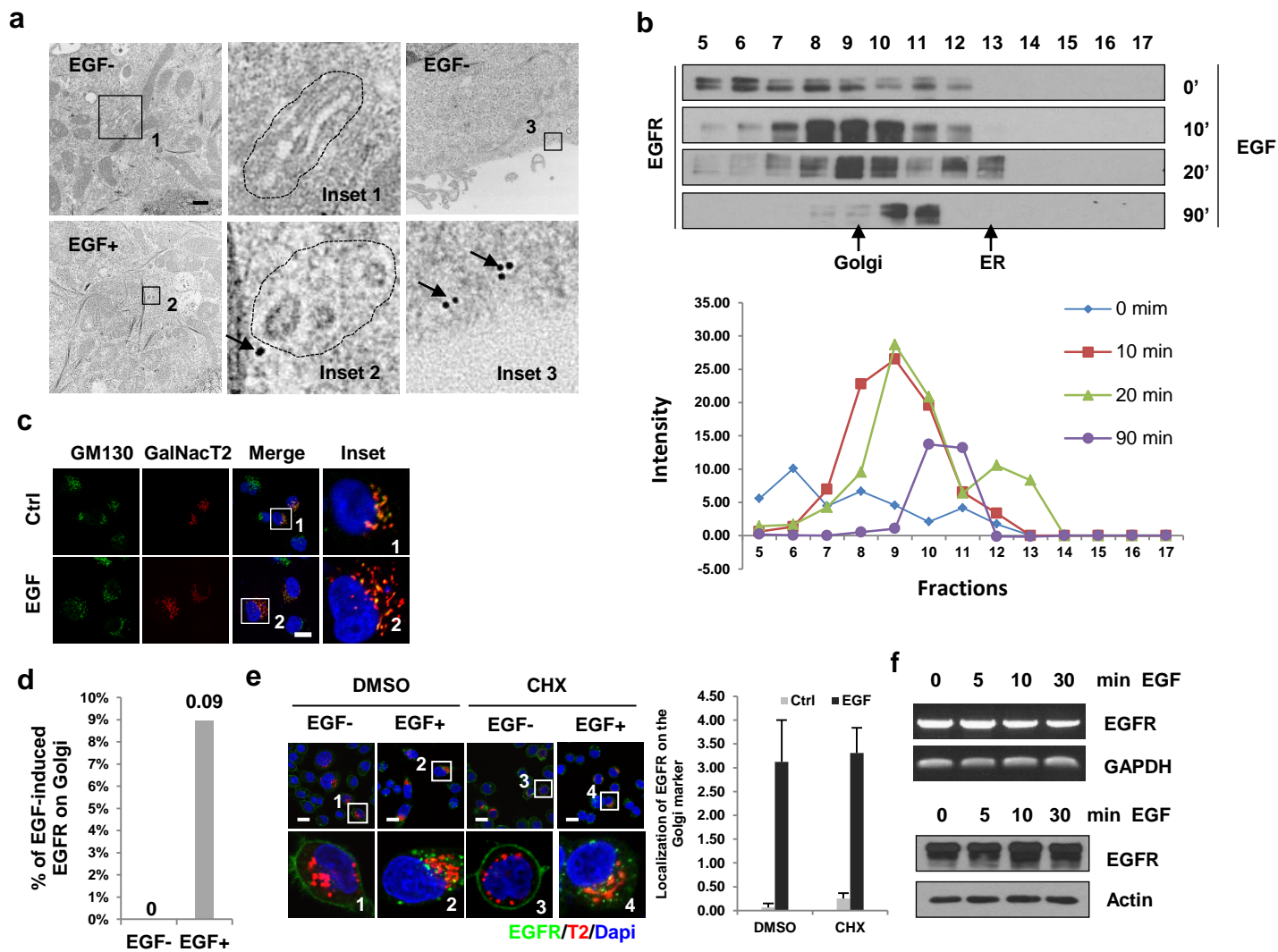


Figure S1 related to Figure 1 (a) HeLa cells were maintained in serum-free media overnight and treated with or without EGF for 30 min and subjected to immuno-EM as described in methods. Scale bars, 500 nm. Arrows indicate EGFR and circled area by the dot line indicate the Golgi. The boxed areas are shown in the insets. (b) Serum-starved HeLa cells were treated with EGF for indicated time. Cytoplasmic fractions were loaded onto the 0-30% OptiPrep density gradient medium and subjected to ultracentrifugation, and fractions were separated using the Gradient Station. Fractions 5-17 were analyzed by SDS-PAGE and western blot to exam the EGFR level. The quantitation of EGFR protein level was shown in the bottom of the panel. (c) Serum-starved HeLa cells were stimulated with EGF and assayed using confocal microscopy with indicated antibodies. Scale bars, 20  $\mu$ m. (d) The quantitative results of EGF-induced EGFR localized on the Golgi. (e) HeLa cells were transfected with EGFP-GalNac T2, and cells were exposed to serum-free media overnight following treatment with EGF (50 ng/ml) for 20 min with and without treatment with CHX. Scale bar, 20  $\mu$ m. The boxed areas are shown in the insets. Quantitation of colocalization of EGFR and Golgi is shown in the right panel. DMSO, dimethyl sulfoxide. (f) HeLa cells were treated with EGF at different times. Total lysates were examined by immunoblot analysis with indicated antibodies. Total mRNA isolated from cells was reversed transcribed to cDNA. mRNA level of EGFR and GAPDH by RT-PCR.

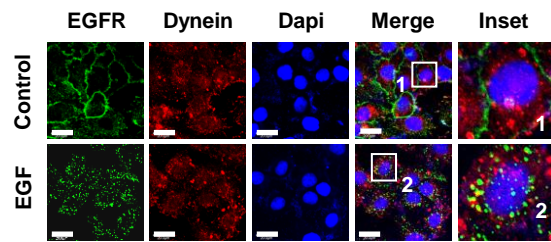


Figure S2 related to Figure 4 (a) Serum-starved HeLa cells were stimulated with EGF and assayed using confocal microscopy with indicated antibodies. Scale bars, 20  $\mu\text{m}$ .

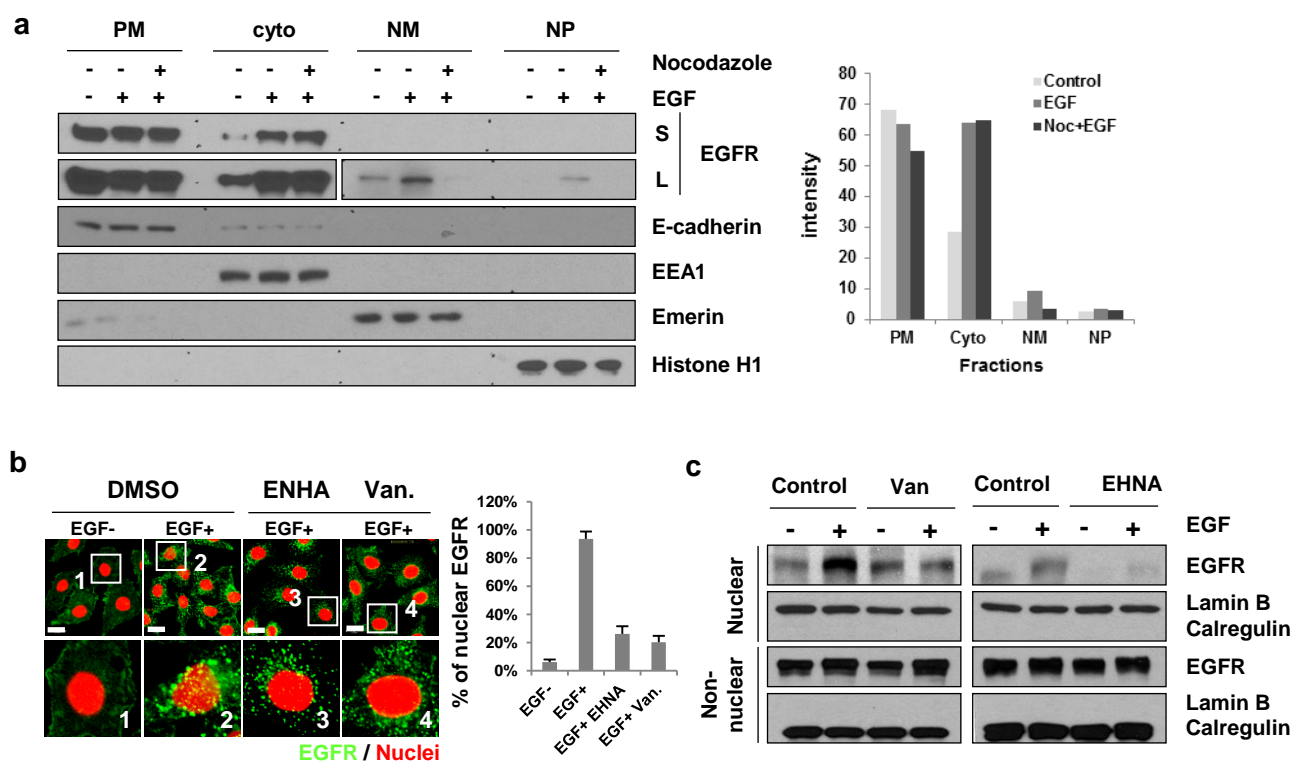


Figure S3 related to Figure 6 (a) Serum-starved HeLa cells were treated with EGF or combined with pre-treatment of nocodazole. Cells were subjected to cell fractionation to isolate the plasma membrane (PM), cytoplasm (cyto), nuclear membrane (NM) and nuclear plasma (NP). Fractions were analyzed by SDS-PAGE and western blot to exam the EGFR level. Quantitation of EGFR protein level is shown on the left. (b) HeLa cells maintained in serum-free media overnight were treated with dynein inhibitors, stimulated with EGF and then analyzed by confocal microscopy. Scale bars, 20 μm. (c) Cells were treated as described in (b). Non-nuclear and nuclear fractions were separated using cellular fractionation and then subjected to immunoblotting with the indicated antibodies.

## Movie Legends

**Movie S1**, related to Figure 1e. EGF induces the colocalization of EGFR with GalNac T2. EGFP-labeled GalNac T2 was transfected into HeLa cells. After serum starvation overnight cells were stimulated with EGF (50 ng/ml) for 20min and performed z-stack confocal image assay. Multiple planes of z-stack images were used to generate a 3-D movie rotating around the y-axis.

**Movie S2**, related to Figure 3e. EGF induces the colocalization of EGFR with syntaxin6. EGFP-labeled EGFR and DsRed-labeled syntaxin6 expression plasmids were transfected into HeLa cells for 48 hr. After serum starvation overnight cells were stimulated with EGF (50 ng/ml). Images were collected at 30-sec intervals and time-lapse covers a period of 40 min.

**Movie S3**, related to Figure 3e. Nocodazole blocks EGF-induced colocalization of EGFR with syntaxin6. EGFP-labeled EGFR and DsRed-labeled syntaxin6 expression plasmids were transfected into HeLa cells for 48hr and then maintained in serum free media overnight. Cells were treated with nocodazole for 30 min and then stimulated with EGF (50 ng/ml). images were collected at 30-sec intervals and time-lapse covers a period of 40 min.

**Movie S4**, related to Figure 4f. EGF induces parallel movement of EGFR to microtubules. EGFP-labeled EGFR and RFP- labeled  $\alpha$ -tubulin expression plasmids were transfected into HeLa cells for 48 hr. After serum starvation overnight cells were stimulated with EGF (50 ng/ml). Images were collected at 20 sec intervals and time-lapse covers a period of 40 min.

**Movie S5**, related to Figure 4f. EGF induces parallel movement of EGFR to microtubules. EGFP-labeled EGFR and RFP- labeled  $\alpha$ -tubulin expression plasmids were transfected into HeLa cells for 48 hr. and then maintained in serum free media overnight. Cells were treated with nocodazole for 30 min and were stimulated with EGF (50 ng/ml). Images were collected at 20 sec intervals and time-lapse covers a period of 40 min.

**Movie S6**, related to Figure 6e. EGF induces nuclear translocation of EGFR. EGFP-labeled EGFR and and RFP-LaminB expression plasmids were transfected into HeLa cells for 48hr. After serum starvation overnight cells were stimulated with EGF (50 ng/ml). Images were collected at 30 sec intervals and time-lapse covers a period of 40 min.

**Movie S7**, related to Figure 6e. Nocodazole blocks EGF-induced nuclear translocation of EGFR. EGFP-labeled EGFR and and RFP-LaminB expression plasmids were transfected into HeLa cells for 48hr. and then maintained in serum free media overnight. Cells were treated with nocodazole for 30 min and then stimulated with. EGF (50 ng/ml). Images were collected at 30 sec intervals and time-lapse covers a period of 40 min.