nature genetics

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41588-018-0149-1

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Gorab is a Golgi protein required for structure and duplication of *Drosophila* centrioles

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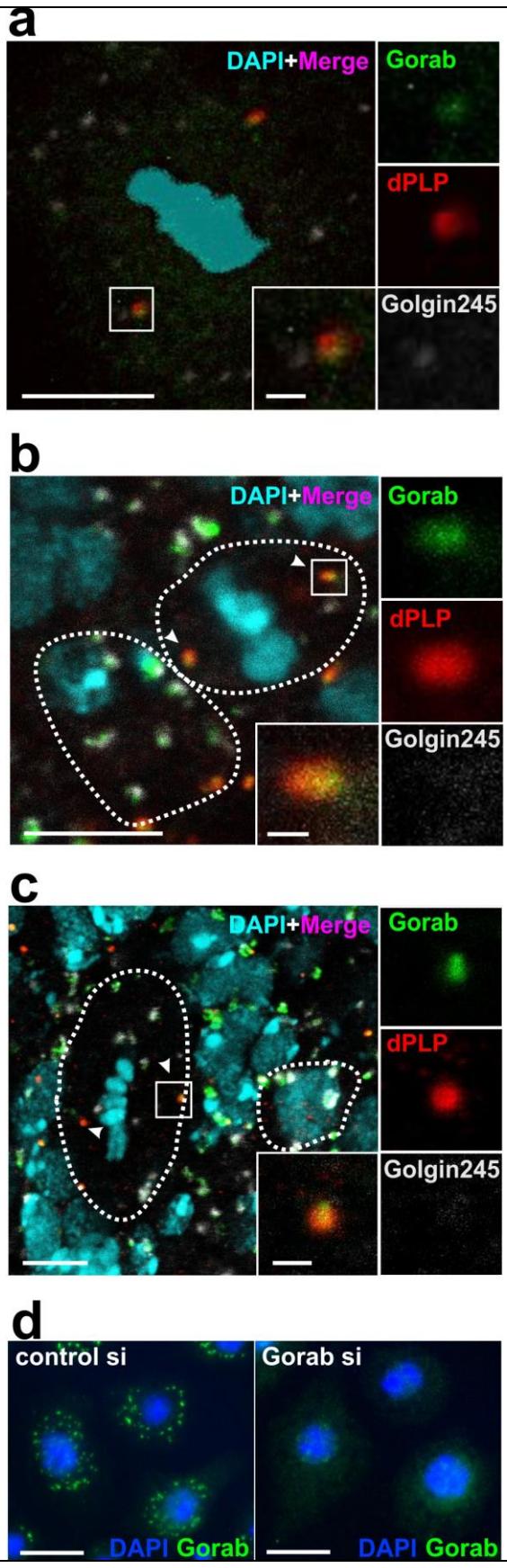
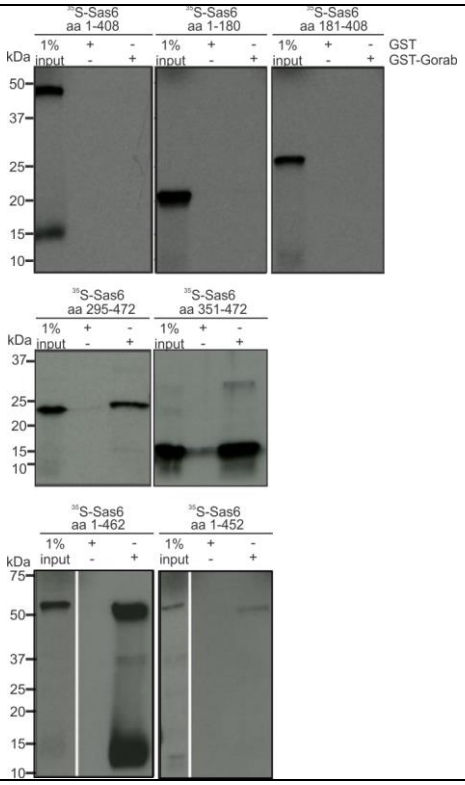

a
DAPI+Merge
Gorab
dPLP
Golgin245
b
DAPI+Merge
Gorab
dPLP
Golgin245
c
DAPI+Merge
Gorab
dPLP
Golgin245
d
control si
Gorab si
DAPI Gorab
DAPI Gorab

Supplementary Figure 1

Localization of Gorab in mitotic cells

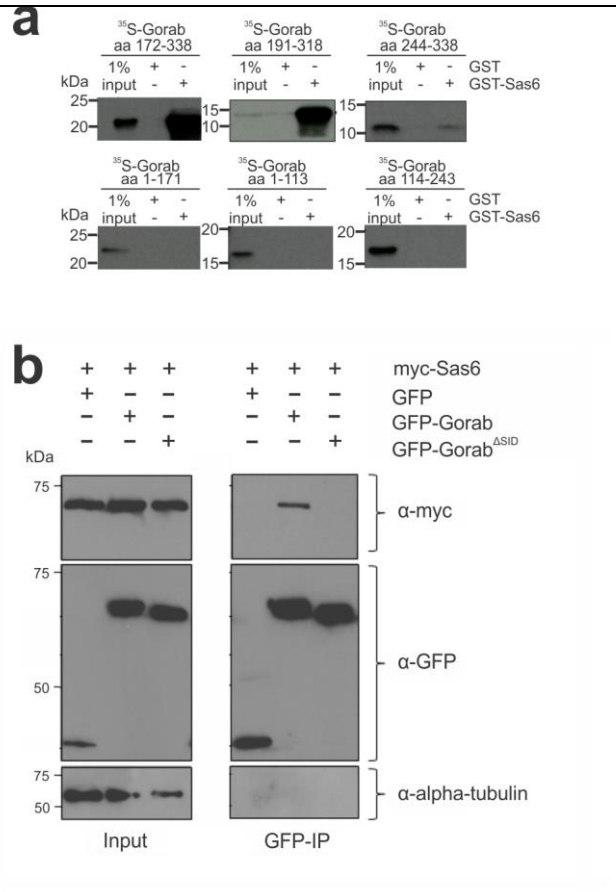
(**a**) D-Mel2 cells in metaphase were immunostained to reveal Gorab, dPLP (centrosome), and Golgin245 (trans-Golgi). Arrowheads indicate centrosomes. Experiment repeated 3 times with similar results.Main scale bar, 5 µm; inset scale bar, 0.5 µm.(**b**) Wing discs from larvae expressing N-terminally GFP-tagged Gorab were immunostained for dPLP (red) and Golgin245 (grey). Dashed lines highlight an interphase (left) and a mitotic cell (right). Arrowheads indicate centrosomes of mitotic cells. Experiement repeated 3 times with similar results.Main scale bar,5 µm.Inset scale bar,0.5µm.(**c**) Brains from larvae expressing N-terminally GFP-tagged Gorab immunostained for dPLP (red) and Golgin245 (grey). Dashed lines highlight an interphase (left) and a mitotic cell (right). Arrowheads indicate centrosomes of a mitotic neuroblast. Experiment repeated with similar result. Main scale bar,5 µm, Inset scale bar, 1µm. (**d**) Fluorescent micrographs of control and Gorab depleted D-Mel2 cells stained by DAPI (blue) and anti-Gorab (green) to demonstrate the specificity of Gorab antibody. Cells subjected to 4 times 4 days siRNA treatment with control or Gorab specific siRNAs followed by fixation in methanol and immunostaning. Experiment independently repeated twice with similar results. Scale bar: 10 µm.



Supplementary Figure 2

Gorab interaction with Sas6

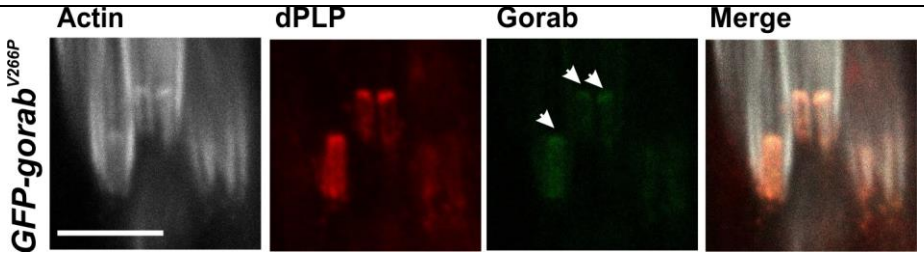
Autoradiograms of GST-tagged Gorab's interaction with the ^{35}S-Methionine-labelled Sas6 protein fragments schematically illustrated in Fig2B. The length of the Sas6 fragments are indicated. Experiments were repeated twice by different investigators with similar results.



Supplementary Figure 3

Sas6 interaction with Gorab

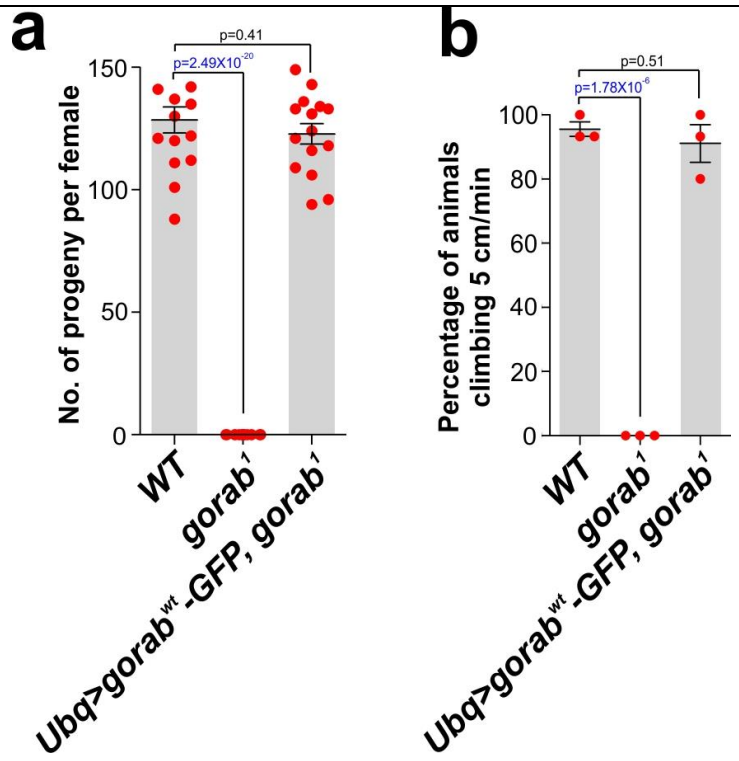
(**a**)Autoradiogram of GST-Sas6 interaction with ^{35}S-Methionine-labelled Gorab protein fragments, schematically illustrated in Fig5B. The experiments were repeated once by a different investigator with similar result. (**b**) Sas6 and Gorab interaction *in vivo*. D-Mel2 cells were transiently transfected with myc-tagged Sas6 and GFP-tagged wild type or ΔSID Gorab. Samples were subjected to GFP-trap immunoprecipitation and western blot using anti-myc and anti-GFP antibodies. Experiments were repeated once by a different investigator with similar result. Anti-alpha tubulin is loading control.



Supplementary Figure 4

Localization of V266P mutant Gorab to basal bodies.

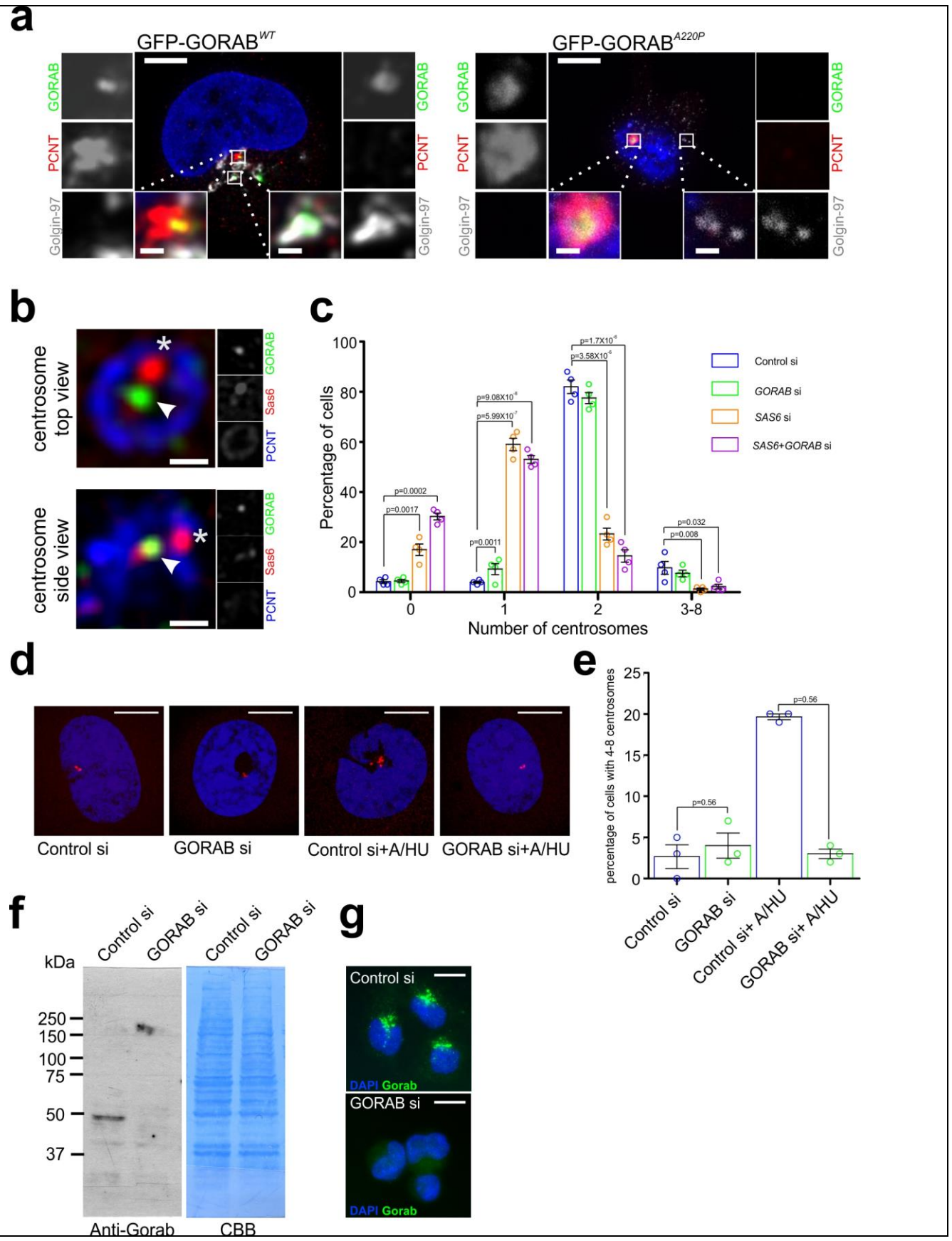
Ciliated neurons from femoral chordotonal organs of flies expressing *GFP-gorab*^{V266P} in a *gorab*^{1} mutant background immunostained with dPLP to reveal basal bodies and with phalloidin to visualize actin in scolopale rods. Experiment was repeated once with similar result. Arrowheads, GFP-Gorab at basal bodies. Scale bar, 10 µm.



Supplementary Figure 5

C-terminally GFP-tagged wild-type Gorab rescues *gorab*^{1} mutant phenotypes

(**a**) Fertility of wild type, *gorab*^{1}mutant and rescued females. Wild type C-terminally GFP tagged *gorab* cDNA was expressed under the control of the ubiqutin promoter in *gorab*^{1} to rescue (*Ubq-gorab*^{wt}*-GFP, gorab*^{1}). Individual females were mated with wild type males and allowed to lay eggs at 25 ^{o}C for 6 days.Data points represent the number of progeny of individual females. Means ± s.e.m are shown for n=15 females per genotype. p values of two tailed unpaired t-tests are shown. p value in blue indicates significant difference (99% confidence interval). Experiment repeated once with similar result.(**b**) Climbing ability of wild type, *gorab*^{1}mutant and rescued flies. Wild type C-terminally GFP tagged *gorab* cDNA was expressed under the control of the ubiqutin promoter in *gorab*^{1} to give rescue (*Ubq-gorab*^{wt}*-GFP, gorab*^{1}). Flies were raised at 29 ^{o}C and subjected to a climbing assay. Each data point represents the percentage of flies from a group of 15 flies. Means ± s.e.m are shown for N=3 independent experiments, n= 15 flies/genotype were investigated in each experiment. p values of two tailed unpaired t-tests are shown. p value in blue indicates significant difference (99% confidence interval).


a
GFP-GORAB^WT
GFP-GORAB^A220P
GORAB
PCNT
Golgin-97
b
centrosome top view
centrosome side view
GORAB
Sas6
PCNT
c
Percentage of cells
Number of centrosomes
Control si
GORAB si
SAS6 si
SAS6+GORAB si
p=0.0002
p=0.0017
p=0.0011
p=9.08X10^-8
p=5.99X10^-7
p=1.7X10^-6
p=3.58X10^-5
p=0.032
p=0.008
d
Control si
GORAB si
Control si+A/HU
GORAB si+A/HU
e
percentage of cells with 4-8 centrosomes
p=0.56
p=0.56
Control si
GORAB si
Control si+ A/HU
GORAB si+ A/HU
f
kDa
Control si
GORAB si
Control si
GORAB si
250
150
100
75
50
37
Anti-Gorab
CBB
g
Control si
GORAB si
DAPI Gorab

Supplementary Figure 6

Centrosomal localization of human GORAB

(a) Centrosomal localization of wild type and A220P mutant GORAB. U-2 OS cells transiently transfected with constructs constitutively expressing N terminally GFP-tagged wild type (left panel) or A220P mutant human GORAB. Pericentrin (PCNT, red) stains centrosomes, Golgin-97 (grey) highlights trans-Golgi. Main scale bar,2 µm. Inset scale bar, 0.2 µm. Experiments repeated twice with similar results.**(b)**GORAB localization inside the centrioles.3D-SIM micrographs of U-2 OS centrosomes immunostained for Pericentrin (blue), SAS6 (red) and GORAB (green). Centrosomes of top (upper panel) and side view (lower panel) are not identical. Experiments repeated twice with similar results. Asterisk, site of procentriole formation where SAS6 is recruited. Arrowheads indicate SAS6, which is initially recruited to the proximal lumen of the cartwheel-less mother centriole and which moves to the site of procentriole formation[45]. Scale bar, 200 nm. **(c)** Synergistic effect of SAS6 and GORAB on loss of centrosomes. U-2OS^{p53DD}cells subjected to 3 times 72h siRNA treatments against the indicated genes. Fixed cells were immunostained with a combination of CENP-J and gamma-Tubulin antibodies to reveal centrioles and centrosomes..Each data point represent the percentage of cells with the given centrosome number from an independent experiment (n=100 cells). Means ± s.e.m are shown for N=4 independent experiments (n=100 cells/experiment)p values of two tailed unpaired t-tests are shown(99% confidence interval). **(d)**Representative images of U-2OS cells subjected to siRNAs against GFP (control) or GORAB and treated with a combination of 4 µM aphidicolin and 1.5 mM hydroxyurea (A/HU). Anti-gamma-tubulin immunostaning reveals centrosomes (red) and DAPI staining (blue) reveals nuclei. Experiment repeated twice with similar results. Scale bar, 5 µm. (**e**) Quantification of centrosome numbers in U-2OS cell shown on **d**. Each data point represents the percentage of cells with a given number from an independent experiment (n=100 cells). Mean ± s.e.m are shown for N=3 independent experiments (n=100 cells/experiment).p values of two tailed unpaired t-tests are shown (99% confidence interval). (**f**) Anti-Gorab Western blot on GORAB depleted U-2 OS^{p53DD} cell lysates. Cells were subjected to3 siRNA treatments, each for 72h, with control or GORAB specific siRNAs to check the specificity of the GORAB antibody (Atlas, #HPA027250). Subsequent Coomassie staining of the membrane (CBB) proves a loading control. Experiment independently repeated once with similar results.(**g**) Fluorescent micrographs of control and GORAB-depleted U-2 OS^{p53DD} cells stained with DAPI (blue) and anti-GORAB (green) to demonstrate the specificity of GORAB antibody (Atlas, #HPA027250). Cells were subjected to3 siRNA treatments, each for 72h, with control or GORAB specific siRNAs followed by fixation in 4% formaldehyde and immunostaning. Experiment independently repeated once with similar results. Scale bar,5 µm.