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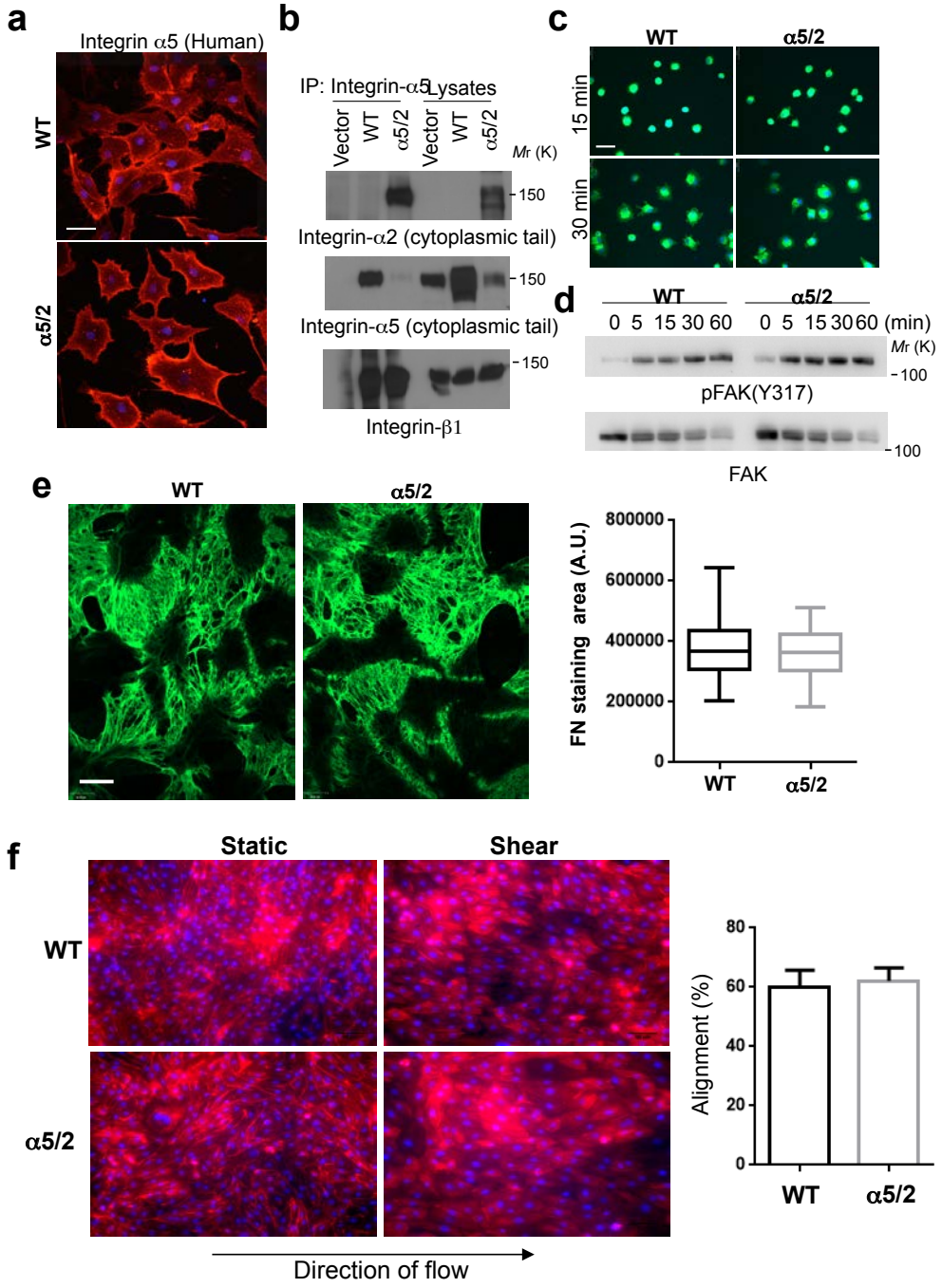
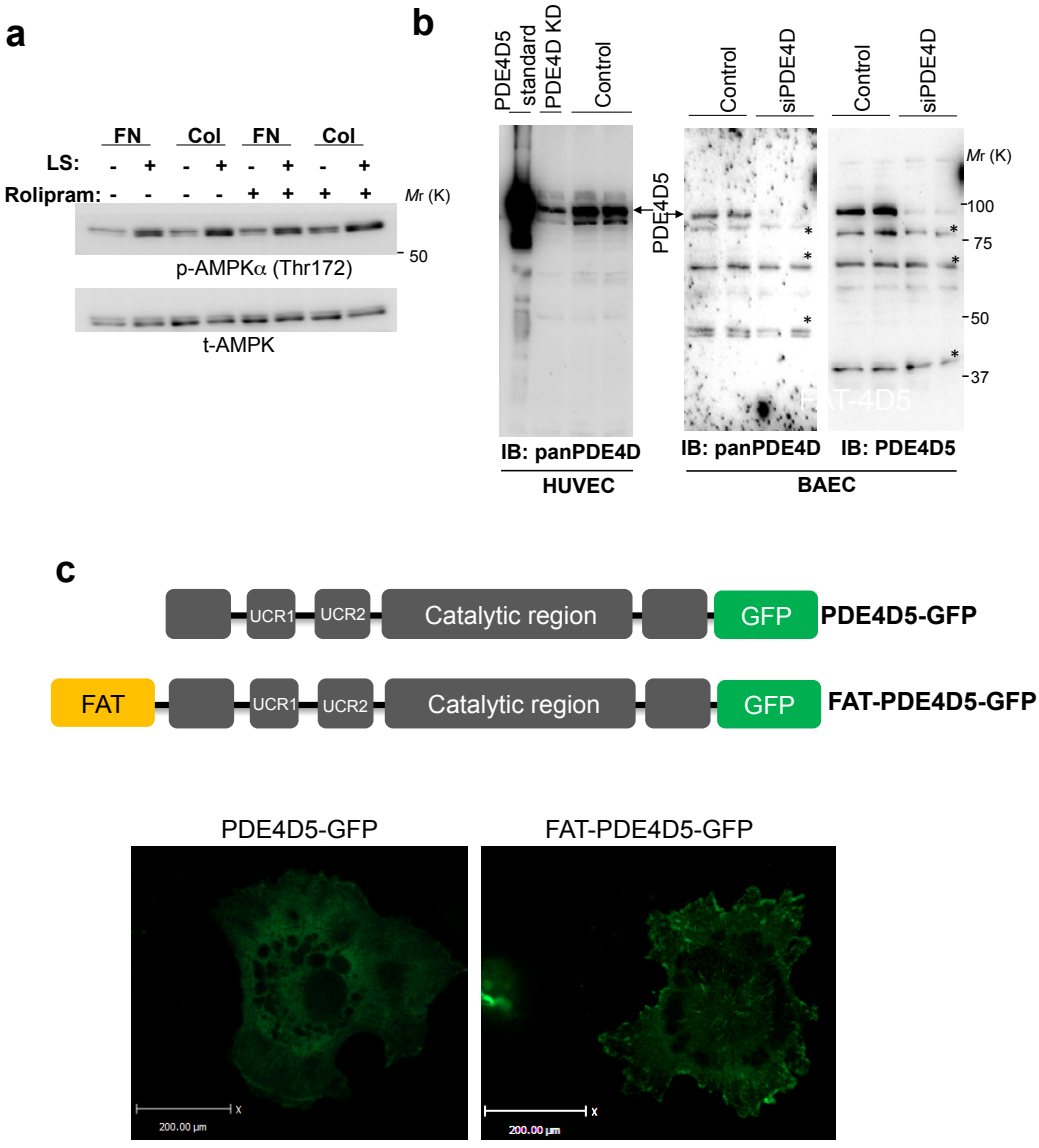
nature cell biology



Supplementary Figure 1 Characterization of α5/2 chimera endothelial cells. **(a)** BAECs expressing human integrin wild type α5 or the α5/2 chimera were stained with mAb16 that recognizes human integrin α5 extracellular domain. **(b)** Wild type α5 or the α5/2 chimera cells were lysed and immunoprecipitated with mAb16 to isolate exogenous human integrin α proteins. Western blots with antibodies against the α5 and α2 cytoplasmic tails confirm the sequences (upper and middle panels), and show similar pairing with the β1 subunit (lower panel). **(c, d)** Wild type α5 and α5/2 chimera cells spreading on fibronectin. BAECs expressing wild type integrin α5 or the chimera were detached and replated on dishes coated with fibronectin (10 μg/ml) for the indicated times. The cells were either fixed and stained with wheat germ agglutinin (c) or lysed and subjected to immunoblotting for FAK phosphorylation (Y397) (d). **(e)** Wild type α5 or α5/2 chimera cells were plated on fibronectin and grown to monolayer and then kept in low-serum media (1% FBS) for two days to induce fibronectin fibrillogenesis. The cells were fixed and stained for fibronectin. For each condition, n=30 images pooled across three independent experiments were averaged. The box plot shows the median, with upper and lower percentiles, and the bars show maxima and minima values. **(f)** Wild type α5 or α5/2 chimera cells were plated on fibronectin and sheared for 36 hrs (20dynes/cm^2). Cells were stained with phalloidin and Hoechst and alignment in the direction of flow was quantified (±30). Scale bars: 50 μm. n=10 images (60-100 cells/field) were used for quantification for each condition. Error bars are SEM.



Supplementary Figure 2 PDE4D5 is responsible for ECM-dependent inflammation in endothelial cells. **(a)** BAECs on FN or collagen were treated with rolipram (1 μM) and assayed for AMPK activation. **(b)** Immunoblotting HUVEC lysate with pan PDE4D antibody detects the major band co-migrating with reference PDE4D5 (left panel). The band was diminished by transfection with PDE4D siRNA, demonstrating specificity. Similarly, PDE4D5 was the only isoform detectable in BAEC lysate with immunobotting using pan PDE4D antibody or PDE4D5 specific antibody (right two panels). * indicates non-specific bands. **(c)** BAECs expressing wild type PDE4D5 or FAT-PDE4D5 were plated on MG for 1hr to monitor GFP signal.

a

LCMS Peptides

Protein ID	PP2AA_BOVIN
Protein Name	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform OS=Bos taurus GN=PPP2CA PE=1 SV=1
Percent Coverage	3.6

One peptide identified with score greater than identity score

Score	Expectation	Peptide Sequence	Start	End	M/Z	Ion Mass	Ion Mass(calc)	Delta	ppm	Charge
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mdekvftkeldqwieqlneckqlsesqvkslcekakeiltkesnvqevrcpvtvcgdvhgqfhdlmelfriggkspdtnylfmgdyvdrgyysvet
vtllvalkvryreritilrgnhesrqitqvygfydeclrkygnanvwkyftdlfdylplttalvdgqifclhgglspsidtldhiraldrlqevphegpmcdllws
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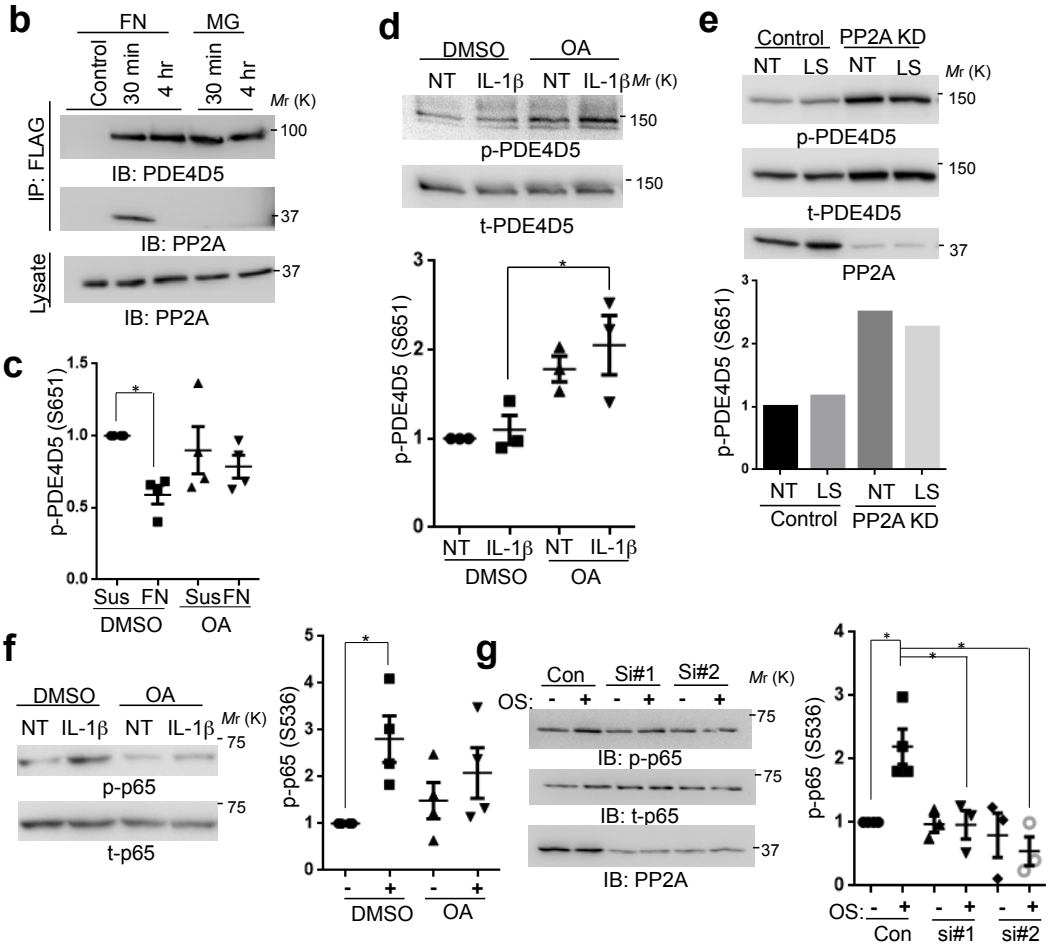
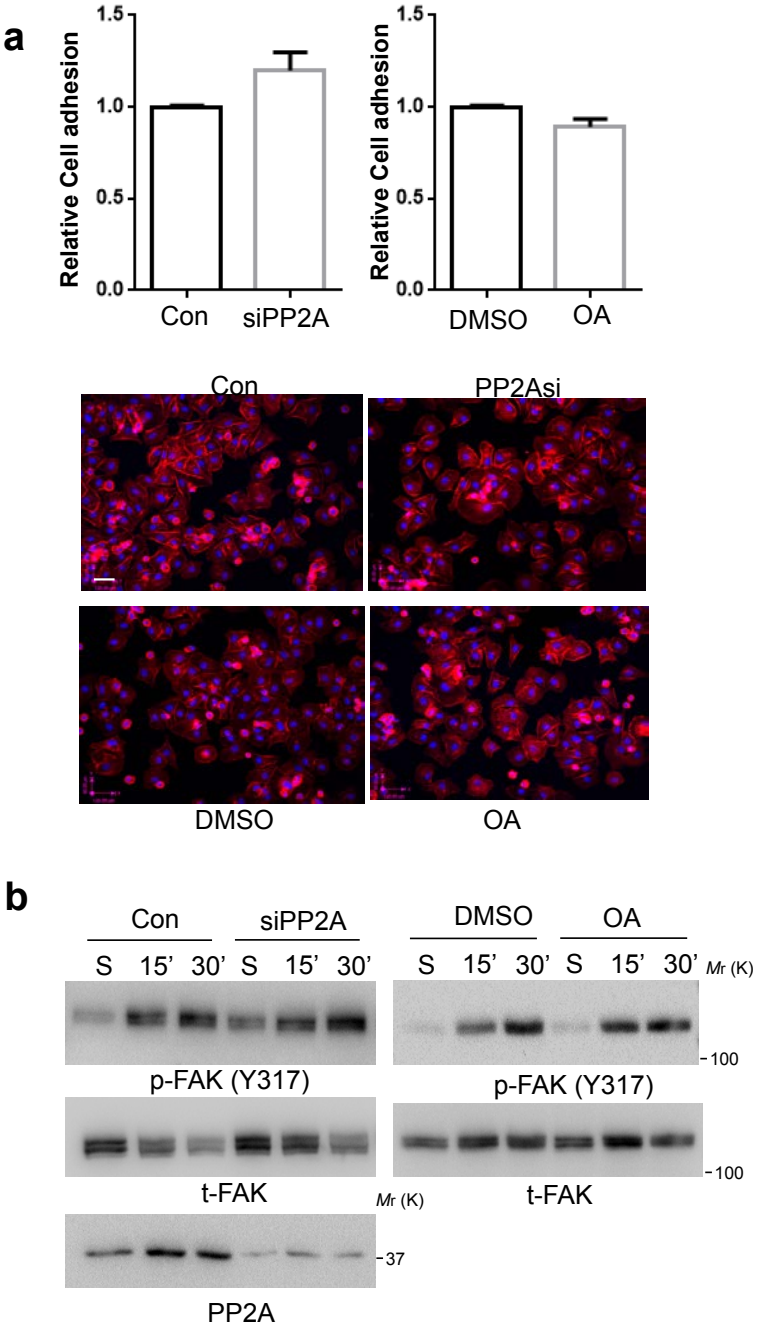
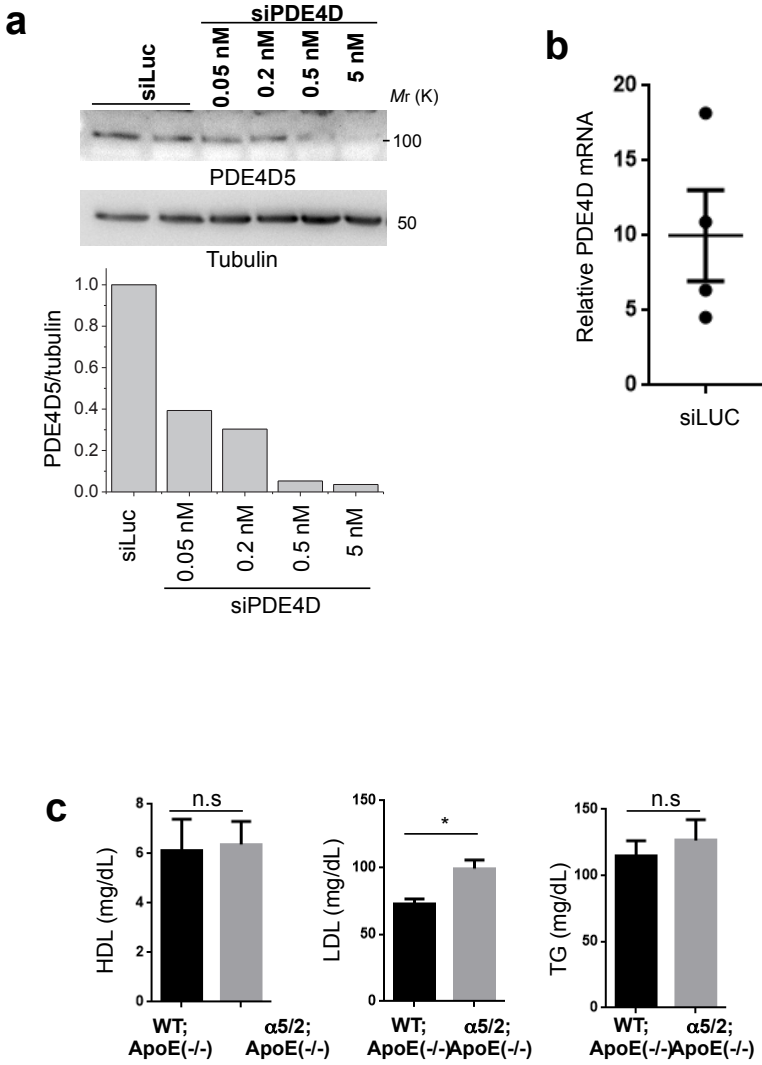


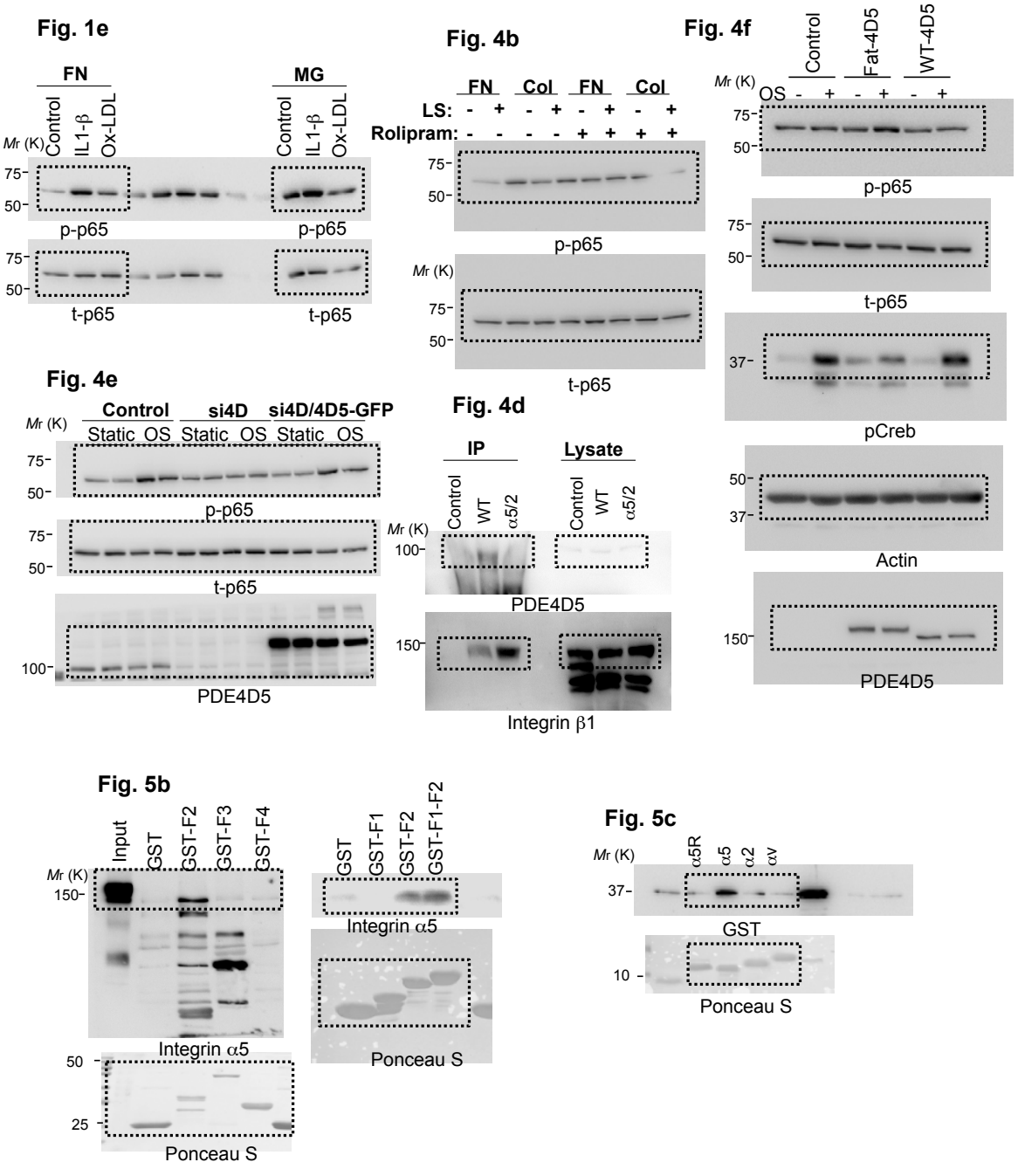
Supplementary Figure 3 PP2A regulates PDE4D5 phosphorylation and inflammation. **(a)** Identification of PP2A as a PDE4D5 binding protein. FLAG-PDE4D5 expressing BAECs on FN were lysed and immunoprecipitated with FLAG antibody. Bound protein with size of 35 kDa was submitted for mass analysis and identified as PP2A catalytic subunit. **(b)** BAECs expressing FLAG-tagged PDE4D5 were plated on either FN or matrigel for indicated times. PDE4D5 was immunoprecipitated with FLAG antibody and eluted with FLAG peptides. Similar results were obtained in 3 experiments. **(c)** BAECs expressing PDE4D5 wild type were kept in suspension for 90 min then replated on FN-coated dishes for the indicated times. For okadaic acid treatment, cells in suspension were added with 5 nM OA for last 20 min before replating on FN. S651 phosphorylation was assayed by Western blotting (n=4 independent experiments). **(d)** BAECs expressing PDE4D5 were plated on FN for 5 hr then pretreated with DMSO or okadaic acid (OA, 5 nM) and then stimulated with IL1β for 30 min. S651 phosphorylation was assayed by Western blotting (n=3 independent experiments). **(e)** BAECs expressing PDE4D5 were transfected with a siRNAs targeting PP2A catalytic subunit. The cells were replated on FN then subject to laminar shear for 30 min. **(f)** BAECs were plated on FN for 5 hr then pretreated with DMSO or okadaic acid (OA, 5 nM) and then stimulated with IL1β for 30 min. NFκB activity was assayed by Western blotting (n=3 independent experiments). **(g)** BAECs were transfected with two different siRNAs targeting the PP2A catalytic subunit. The cells were replated on FN then subject to oscillatory shear for 2 hrs (n=3 independent experiments). Data are represented as means ± SEM. *p<0.05 by one way ANOVA (d, g) or two-tailed t-test (c, f). Source data are provided in Supplementary Table 1.



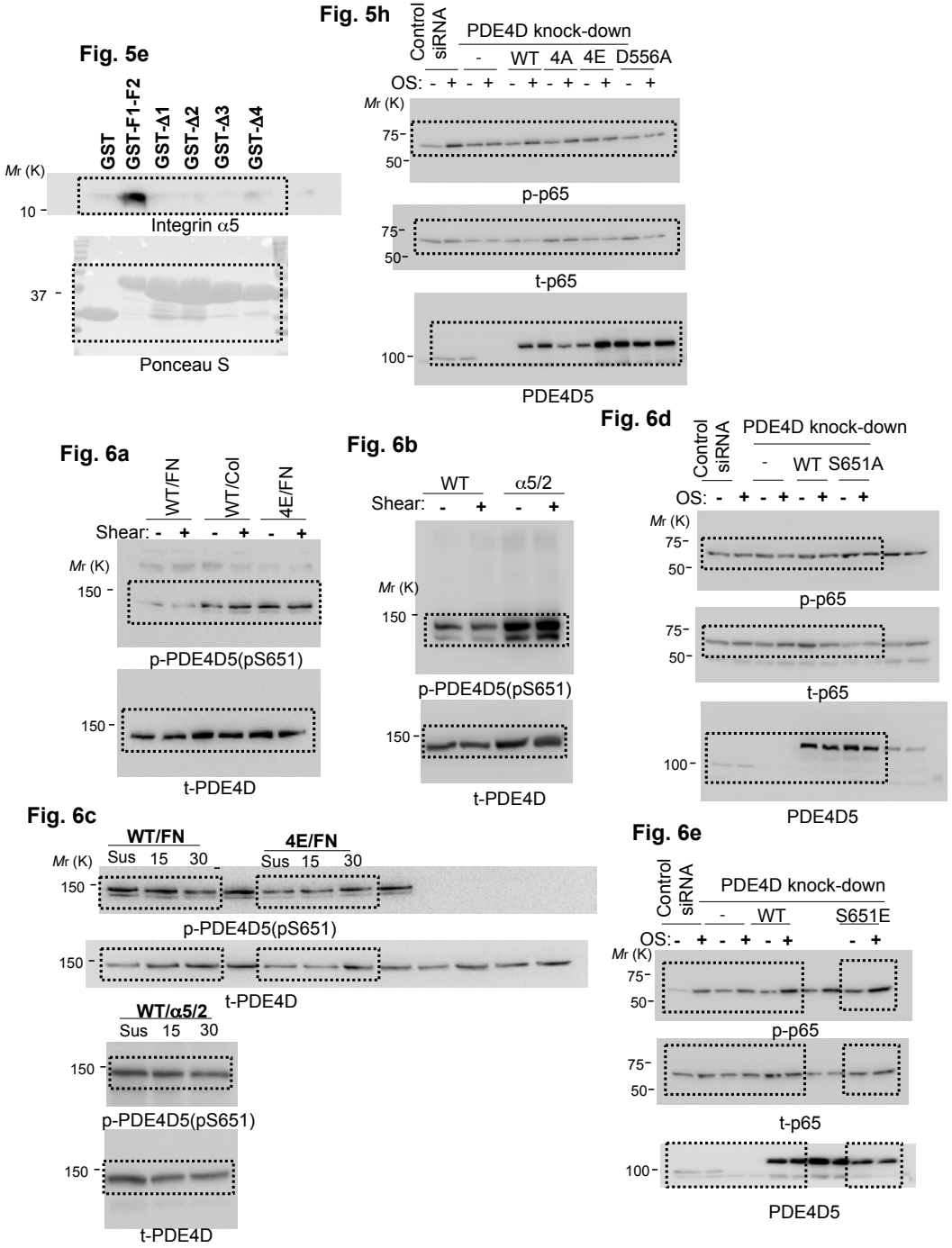
Supplementary Figure 4 Effect of PP2A inhibition on endothelial cell adhesion. **(a)** BAECs were transfected with PP2A siRNA or treated with okadaic acid (OA, 5 nM, 1hr) and plated on FN (10 μg/ml) for 1hr. Cell adhesion was quantified as described in Supplementary Fig. 2 (n=3 independent experiments) and cell morphology was examined after fixation and phalloidin and nuclear staining. Error bars are SEM. Scale bar: 100 μm. **(b)** BAECs treated as (a) were plated on FN (10 μg/ml) for indicated times and FAK phosphorylation was measured by Western blotting.



Supplementary Figure 5 *In vivo* knock-down of endothelial PDE4D. **(a)** NIH3T3 cells were transfected with either luciferase siRNA or mouse PDE4D siRNA used for *in vivo* knock-down. After 72h, PDE4D5 was assayed by Western blotting with tubulin as a loading control. **(b)** Nano-particles containing PDE4D siRNA or luciferase siRNA (1 mg/kg) were injected intravenously. After two weeks, mouse aortas were isolated, and endothelial expression of PDE4D was assayed by qPCR (n=4). (**c**) Serum lipid profile of α5/2; ApoE(-/-) mice after high fat diet (n=6 mice). Data are represented as means ± SEM. *p<0.05 by two-tailed t-test. Source data are provided in Supplementary Table 1.



Supplementary Figure 6 Unprocessed scans of key blots. For the FN and MG samples in Fig. 1e; the IP and lysate samples in Fig. 4d; the WT/FN and 4E/FN samples in Fig. 6c; and the S651E samples with the rest of the samples in Fig. 6e; the samples that were probed with the same antibody were run on the same gel and processed as a single blot, but intervening lanes were cropped from the blots presented in the main figure. The cropped images are marked on the unprocessed scan of each blot by boxes.



Supplementary Figure 6 continued

Supplementary Table 1

Statistics Source Data
Individual data-sets for Figure 1-7, Supplementary Figure 3, 5.